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

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► **To cite this version:**

S. Comeau, C. Cornwall, T. Decarlo, S. Doo, C. Carpenter, et al.. Resistance to ocean acidification in coral reef taxa is not gained by acclimatization. *Nature Climate Change*, 2019, 9 (6), pp.477-483. <10.1038/s41558-019-0486-9>. <hal-02178762>

HAL Id: hal-02178762

<https://hal.sorbonne-universite.fr/hal-02178762v1>

Submitted on 10 Jul 2019

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

3
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20

21 **Abstract**

22

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

35 Changes in seawater carbonate chemistry caused by OA generally lead to decreasing
36 calcification of marine organisms¹ as well as increased dissolution of existing calcium
37 carbonate structures². The magnitude of responses to OA are species-specific and can
38 even be intra-specific in reef taxa, although some species exhibit significant resistance
39 to OA³⁻⁵. The physiological mechanisms responsible for declines in calcification in
40 corals and calcifying algae due to OA are, however, still unclear. Short-term
41 experiments on small numbers of species have limited potential to assess whether
42 calcifying organisms can acclimatize to changes in seawater carbonate chemistry, or
43 alternatively, whether species-specific differences in calcification physiology dictates
44 the overall magnitude of responses.

45 Insights into changes in calcification mechanisms can be gained by
46 investigating the response of carbonate chemistry in the calcifying fluid (CF) from
47 which the calcium carbonate is precipitated⁶. Corals exert a strong control on their CF
48 chemistry, with pH in the CF (pH_{cf}) maintained above seawater pH^{7-9} . Similarly, the
49 dissolved inorganic carbon (DIC) is concentrated in the CF by $\sim 1.5\text{--}2$ -fold above
50 seawater DIC¹⁰⁻¹³. As a result, the saturation state within the CF is $\sim 3\text{--}4$ times greater
51 than the surrounding seawater, which, in association with the production of organic
52 matrices, favours the precipitation of calcium carbonate in the CF. While less data on
53 the calcification physiology of tropical calcifying algae are available¹⁴, the few studies
54 on coralline algae also show that the response of their CF chemistry to seawater
55 carbonate chemistry is similar to that of corals^{10,15,16}.

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

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

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

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

94 *Experimental conditions*

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

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

108

109 *Calcification and calcifying fluid chemistry*

110 At the end of the 1-year incubation, calcification of *Pocillopora* and *Porites*
111 was not affected by the treatments (Fig. 1), while conversely the calcification of
112 *Psammocora* and *Acropora* decreased linearly with pCO_2 (slope p-value = 0.022 and
113 0.039, respectively, Table S1). Calcification of *Lithophyllum* also decreased linearly
114 with pCO_2 (slope p-value = 0.032, Table S1).

115 pH_{cf} of the four corals exhibited contrasting responses to the treatment after 1
116 year. There was no significant linear relationship between pH_{cf} or DIC_{cf} and pCO_2 for
117 *Porites*, whereas in the three other corals (*Psammocora*, *Pocillopora*, and *Acropora*)
118 there were significant linear relationships between pH_{cf} and the pCO_2 (slope p-value <
119 0.01, Table S1, Fig. 2). DIC_{cf} responded in the opposite direction of pH_{cf} , with higher
120 DIC_{cf} in the high pCO_2 treatments in these three corals (Fig. 3) (slope p-values =
121 0.008, 0.030, and 0.007; Table S1).

122 For both algae, pH_{cf} decreased linearly with increasing pCO_2 (slope p-value =
123 0.012 and < 0.001; for *Lithophyllum* and *Halimeda*, respectively; Table S1, Fig. 2).

124 There were no consistent linear relationships between the aragonite Ω_{cf} and
125 pCO_2 in three of the four corals (*Pocillopora*, *Acropora*, and *Porites*, Fig. S1, Table
126 S1). However, there was a decrease of Ω_{cf} with increasing pCO_2 in *Psammocora*
127 (Table S1, Fig. S1). $\text{Ca}_{\text{cf}}^{2+}/\text{Ca}_{\text{sw}}^{2+}$ was only affected by the pCO_2 in *Pocillopora* (Fig.
128 4) (slope p-value = 0.009, Table S1) with maximum $\text{Ca}_{\text{cf}}^{2+}/\text{Ca}_{\text{sw}}^{2+}$ measured in the
129 1053 μatm treatment.

130

131 *Seasonal variation in calcifying fluid composition*

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

134 contrasting morphologies and different potential sensitivities to ocean acidification. In
135 *Acropora*, pH_{cf} was consistently higher in the 356 µatm treatment (Fig. 5), with a
136 significant effect of the treatment (ANOVA, p < 0.001). There was also a significant
137 effect of treatment (ANOVA, p = 0.045) and time (p = 0.023) on Ω_{cf} that was driven
138 by slightly higher Ω_{cf} in the lowest pCO₂ treatment, particularly at the end of the
139 incubation (Fig. S2). In contrast, DIC_{cf} (ANOVA, p = 0.096) and Ca²⁺_{cf} / Ca²⁺_{sw} (p =
140 0.107) were not affected by the treatment, but were affected by time (both p < 0.001)
141 (Fig. 5 and S2). Seawater temperature and the mean daily light did not affect pH_{cf} and
142 DIC_{cf}, while the mean daily light had a positive effect on Ca²⁺_{cf} / Ca²⁺_{sw} and Ω_{cf}
143 (Table S2).

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

149

150 *Discussion*

151

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

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

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

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

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

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

207 pH_{cf} homeostasis²⁴ is not the only mechanism of resistance to OA. Here,
208 calcification of *Pocillopora* was unaffected by the treatments despite a decrease in
209 pH_{cf} with seawater pH. Similarly, pH_{cf} of *Pocillopora damicornis* decreased with
210 seawater pH during a two-month laboratory study but calcification remained
211 constant¹⁸. The lack of an effect of seawater pH on the calcification of *Pocillopora*
212 *damicornis* was explained by the increase in calcium concentration in the calcifying
213 fluid that enabled the corals to maintain constant precipitation of calcium carbonate
214 despite decreasing pH_{cf} ³³. Here, we observed a similar increase in the calcium
215 concentration of *Pocillopora verrucosa* when seawater pH decreased, which favoured
216 the maintenance and even the slight (but not statistically significant) increase of Ω_{arag}
217 $_{\text{cf}}$ with pCO_2 . This result suggests that increasing $[\text{Ca}^{2+}]$ in response to decreasing pH
218 is a common feature for corals of the genus *Pocillopora* and could explain the
219 resistance of some *Pocilloporidae* to OA reported previously^{34–36}. The mechanism
220 responsible for increases in $[\text{Ca}^{2+}]$ still need to be confirmed. However, previous
221 evidence points toward an active transport of calcium by various intracellular
222 pathways to the site of calcification³⁷.

223 It has been suggested that short-term laboratory experimental results can be
224 misleading because they do not consider seasonal variations in biotic and abiotic
225 factors. For example, seasonal cycles of pH_{cf} and DIC_{cf} , have been documented on
226 massive *Porites* sp. from the Great Barrier Reef and Ningaloo Reef, Australia¹¹, and
227 *Acropora youngae* and *Pocillopora damicornis* from Rottneest Island, Western
228 Australia³⁰. Here, the seasonal cycles in *Porites* pH_{cf} and DIC_{cf} was conserved across
229 pH treatments, suggesting that such cycles are not driven by seawater pH (which were
230 constant across seasons) but rather by other abiotic (light, temperature, nutrient) or
231 biotic parameters (photosynthetic rates, tissue thickness, reproduction cycles, growth
232 rates). Ross et al.³⁰ attributed the seasonal variations in pH_{cf} and DIC_{cf} to temperature.
233 We found similar effects of temperature on pH_{cf} and DIC_{cf} of *Porites*. Contrary to
234 conclusions of previous studies¹¹, the presence (*Porites*) and absence (*Acropora*) of
235 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.

257

258

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261 **Acknowledgments**

262 We thank A.-M. Comeau-Nisumaa, J. D’Olivo, P. Edmunds for support for
263 this study. Funding support was as follows: MTM by an ARC Laureate Fellowship
264 (LF120100049) and ARC Centre of Excellence for Coral Reef Studies
265 (CE140100020), SC by an ARC DECRA (DE160100668), CEC by ARC Centre of
266 Excellence for Coral Reef Studies (CE140100020), and a Rutherford Discovery
267 Fellowship (Royal Society of New Zealand; RDF-VUW1701), RC by a National
268 Science Foundation grant OCE 14- 15268, the Moorea Coral Reef LTER program
269 (NSF OCE 12-36905) and from the Gordon and Betty Moore Foundation. The
270 University of Western Australia’s Centre for Microscopy, Characterisation &
271 Analysis (CMCA). Research was completed under permits issued by the Haut-
272 commissariat de la République en Polynésie Française (DRRT) (Protocole d’Accueil
273 2015–2016). This is contribution number 286 of the CSUN Marine Biology Program.
274

275 **Author contributions**

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

281

282 **Data availability**

283 All data are available on the public data repositories BCO-DMO ([https://www.bco-](https://www.bco-dmo.org/dataset/756211)
284 [dmo.org/dataset/756211](https://www.bco-dmo.org/dataset/756211)).

285

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391

392 **Methods**

393 *Sample collection and preparation*

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

418

419 *Experimental set-up*

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

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

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

439 Seawater was circulated in the flumes at 0.1 m s^{-1} to match the average yearly
440 seawater velocity in the back reef of Moorea⁴¹. Freshly pumped sand filtered seawater
441 (corresponding to a mesh size of 500 μm) was continuously delivered to the flumes at
442 $\sim 5 \text{ L min}^{-1}$ to maintain constant A_T and dissolved oxygen levels within the flumes.
443 The corals were not fed but small particles (i.e. $< 500 \mu\text{m}$) were not filtered and
444 served as potential food source. Light levels were adjusted with shade cloths to match
445 maximum intensities typically ranging between 1000 and 2000 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ in
446 the back reef of Moorea. Because the flumes were exposed to natural sunlight, light
447 varied strongly seasonally and daily. Temperature was controlled to approximate the
448 mean monthly seawater temperature in the back reef of Moorea, which ranged from
449 29.3°C in April to 25.8°C in September³⁸.

450

451 *Calcification*

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

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

462

463 pH_{cf} and DIC_{cf}

464 Skeletons were stained by placing the organisms for 48 hours in seawater
465 enriched with the fluorescent dye calcein at 50 mg L⁻¹ with a pH adjusted to ~8.1 by
466 the addition of NaOH prior to the start of the experiment. The stain line was used to
467 identify the part of the skeleton that grew during the experiment (Fig. S3). The $\delta^{11}B$
468 proxy method was used to estimate pH in the calcifying fluid of all taxa (pH_{cf})⁷ and
469 the $\delta^{11}B$ and B/Ca method was used to estimate the dissolved inorganic carbon
470 concentration in the calcifying fluid (DIC_{cf}) in the aragonitic taxa^{11,43}. Measurements
471 of geochemistry were conducted on the dead skeleton after the experimental period.
472 Skeleton was sampled from the tip of the branches or the top 1–2 mm of the skeleton
473 for the 6 *Pocillopora* and *Psammocora* individuals, and 3 *Lithophyllum* per treatment.
474 This portion of the skeleton was selected to sample material deposited during the last
475 months of the experiment to detect any potential acclimatization to the experimental
476 conditions. Because *Halimeda* degraded in the flumes, they were replaced several
477 times during the experiment. The sampling for geochemistry was conducted on 3
478 *Halimeda* per treatment that were in the flumes for ~ 2 months. Only the top segments
479 were used to ensure that they developed under the experimental conditions. Because
480 the branches of *Acropora* extend linearly and quickly (i.e., 5-10 cm y⁻¹) it was
481 possible to sample from four individuals per treatment skeleton parts that
482 corresponded to different time points of the year of incubation (Fig. S3). The first
483 section corresponded to the start of the experiment (i.e. the stain line). The rest of the
484 12 months linear extension was divided in four and was assumed to represent the
485 growth after 0-3, 3-6, 6-9, and 9-12 months in the different treatments. Likewise, for
486 three *Porites* individuals per treatment, four portions of the skeleton were sampled.
487 One sample of the skeleton was taken below the stain line to represent the conditions
488 prior to the experiment and the skeleton grown above the stain line was divided in
489 three equal parts that were assumed to roughly represent the growth during the period
490 0–4, 4–8, and 8–12 months. Seasonal changes in temperature were confirmed using
491 the inverse relationship between Sr/Ca and temperature for *Porites*. The sections
492 prior to the start of the experiment and the one just below the tissue (section month 8-
493 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 in³⁸). 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 HNO₃, and the $\delta^{11}\text{B}$ was quantitatively separated on ion exchange columns.
510 $\delta^{11}\text{B}$ 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 \pm SE, n = 12), which is similar to the 24.33 ± 0.11 ‰
513 reported previously⁴⁴. Calculations of pH_{cf} based on $\delta^{11}\text{B}$ were made in R using the
514 calculations of⁴⁵:

$$515 \quad \text{pH}_{\text{cf}} = \text{pK}_{\text{B}} - \log \left[\frac{(\delta^{11}\text{B}_{\text{SW}} - \delta^{11}\text{B}_{\text{carb}})}{(\alpha_{\text{B3-B4}} \delta^{11}\text{B}_{\text{carb}} - \delta^{11}\text{B}_{\text{SW}} + 1000 (\alpha_{\text{B3-B4}} - 1))} \right] \quad (1)$$

516 where pK_{B} is the dissociation constant dependent on temperature and salinity,
517 $\delta^{11}\text{B}_{\text{sw}} = 39.61$, and $\alpha_{\text{B3-B4}}$ is the boron isotopic fractionation factor for the pH
518 dependent equilibrium of the borate ($\text{B}(\text{OH})_4^-$) relative to the boric acid ($\text{B}(\text{OH})_3$)
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 $\delta^{11}\text{B}$, were utilized to
523 determine $[\text{CO}_3^{2-}]$ and $[\text{DIC}]$ at the site of calcification $[\text{DIC}]_{\text{cf}}$ following¹¹ 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:

527
$$[\text{CO}_3^{2-}]_{\text{cf}} = K_{\text{D}}[\text{B}(\text{OH})_4^-]_{\text{cf}} / (\text{B}/\text{Ca})_{\text{CaCO}_3} \quad (2)$$

528 Where $K_{\text{D}} = K_{\text{D},0} \exp(-k_{\text{KD}}[\text{H}^+]_{\text{T}})$ with $K_{\text{D},0} = 2.97 \pm 0.17 \times 10^{-3}$ ($\pm 95\%$ CI), k_{KD}
529 $= 0.0202 \pm 0.042$. The concentration of DIC_{cf} was then calculated from estimates of
530 pH_{cf} and $[\text{CO}_3^{2-}]_{\text{cf}}$. It was not possible to determine correct DIC_{cf} values for *Halimeda*
531 likely because of dissolution and precipitation of calcium carbonate between the day
532 and the night. DIC_{cf} was not determined on *Lithophyllum* because their skeleton is
533 made of high Mg Calcite for which no inorganic precipitation experiment linking
534 B/Ca and $[\text{CO}_3^{2-}]_{\text{cf}}$ have been made.

535

536 *Raman spectroscopy*

537 Confocal Raman spectroscopy was used to determine for the corals estimates
538 of calcifying fluid aragonite saturation state $\Omega_{\text{arag cf}}^{47}$. Measurements were conducted
539 on a WITec Alpha300RA+ using a 785 nm infrared laser, a 20x objective with 0.5
540 numerical aperture, and a 1200 mm^{-1} grating to achieve a spectral resolution of
541 approximately 1.3 cm^{-1} . Skeleton powders were placed on glass slides and topography
542 maps were made with the TrueSurface module. The automated stage followed the
543 topography while conducting Raman measurements so that the optics were always in
544 focus on the sample surfaces. 100 spectra were collected per sample in a 300 μm by
545 300 μm grid using 1 s integrations. Spectra with poor signal (< 50 intensity units) or
546 contaminated by cosmic rays were excluded.

547 The widths of the ν_1 peaks were used as proxy measures of $\Omega_{\text{arag cf}}^{47}$. CaCO_3
548 minerals precipitating from more supersaturated solutions incorporate more impurities
549 and are more disordered, which causes Raman peak broadening due to greater
550 distributions of C-O bond lengths⁴⁷. We used the abiogenic aragonite calibration
551 equation of⁴⁷ to calculate $\Omega_{\text{arag cf}}$ for the coral species from the ν_1 full width at half
552 maximum intensity (FWHM). The mean and median of the standard errors for each
553 individual $\Omega_{\text{arag cf}}$ ($n = 193$) were 0.19 and 0.16 units, respectively, where these
554 uncertainties are based on the 100 replicate spectra collected for each individual.
555 Repeated measurement of JCp-1 were used for instrument drift correction based on its
556 $\Omega_{\text{arag cf}}$ of 12.3 (DeCarlo et al., 2017). The largest of these $\Omega_{\text{arag cf}}$ corrections, which
557 were applied systematically to all samples measured in different analytical sessions,
558 was 2 units. Analyses of *Porites*, *Psammocora*, and *Pocillopora* were all conducted
559 within single analytical sessions, and therefore comparisons among treatments are not

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

564

565 *[Ca²⁺]_{cf} determination*

566 $[Ca^{2+}]_{cf}$ of corals was calculated as:

567
$$[Ca^{2+}]_{cf} = \frac{\Omega_{Ar} * K_{Sp}}{[CO_3^{2-}]_{cf}} \quad (1)$$

568 where $[CO_3^{2-}]_{cf}$ and $\Omega_{arag\ cf}$ are derived from boron systematics and Raman
569 spectroscopy, respectively⁴⁷. $Ca_{cf}^{2+}/Ca_{sw}^{2+}$ ratios were calculated by normalizing to
570 $[Ca^{2+}]_{sw}$, which was estimated from seawater salinity (average = 35.5). Estimates of
571 $[Ca^{2+}]_{sw}$ were made based on the assumptions that metabolic activity (calcification)
572 did not change the relationship seawater salinity $[Ca^{2+}]_{sw}$. This assumption was
573 supported by the fact that seawater was pumped from Cook's Bay where there is no
574 reef and only very few corals.

575

576 *Statistical analyses*

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

588

589 **References (Methods section)**

590

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616

617 **Legends**

618

619 **Fig. 1.** Effects of four pCO₂ treatments (~360, 550, 700 and 1050 μatm) on the
620 surface area-normalized net calcification rates. Calcification over the 1-year long
621 experiment was measured on the corals *Psammocora profundacella* (A), *Pocillopora*
622 *verrucosa* (B), *Acropora pulchra* (C), massive *Porites* sp. (D), and the coralline alga
623 *Lithophyllum kotschyianum* (E). Individual rates are shown as dots and the squares
624 represent the mean ± SE (n = 5 for *Psammocora*; n = 6 or 5 for *Pocillopora* and
625 *Acropora*; n = 5 for *Porites* and n = 4 for *Lithophyllum*). Linear regressions and the
626 95% confidence intervals are shown when the regressions had a statistically
627 significant slope (p < 0.05).

628

629 **Fig. 2.** Estimates of pH in the calcifying fluid (pH_{cf}) at the end of the 1-year
630 experiment. The geochemical proxy δ¹¹B was used to estimate pH_{cf} of the corals
631 *Psammocora profundacella* (A), *Pocillopora verrucosa* (B), *Acropora pulchra* (C),
632 and massive *Porites* sp. (D) and the algae *Lithophyllum kotschyianum* (E) and
633 *Halimeda minima* (F). Individual rates are shown as dots and the squares represent the
634 mean ± SE (n = 6 or 5 for *Psammocora* and *Pocillopora*; n = 4 for *Acropora*; n = 3
635 for *Porites* and *Halimeda*, and n = 4 for *Lithophyllum*). Linear regressions and the
636 95% confidence intervals are shown when the regressions had a statistically
637 significant slope (p < 0.05).

638

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

647

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

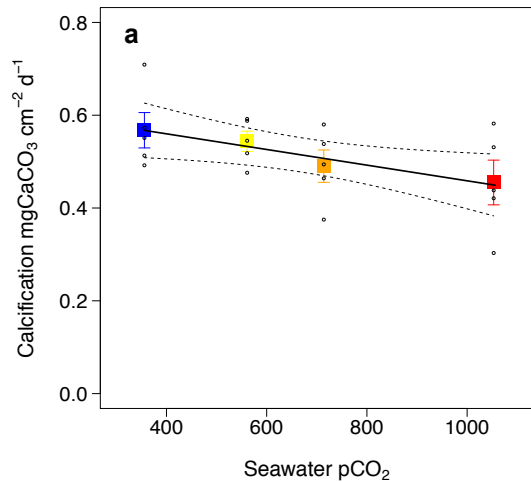
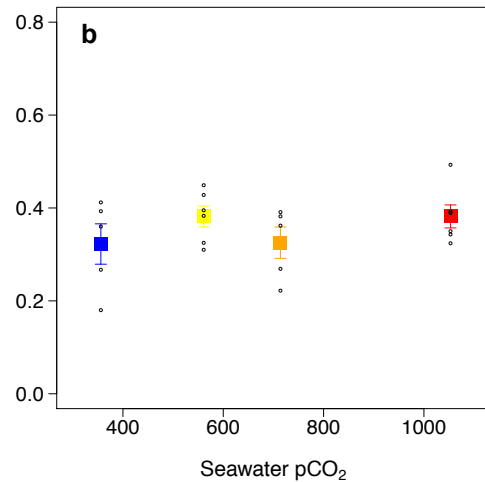
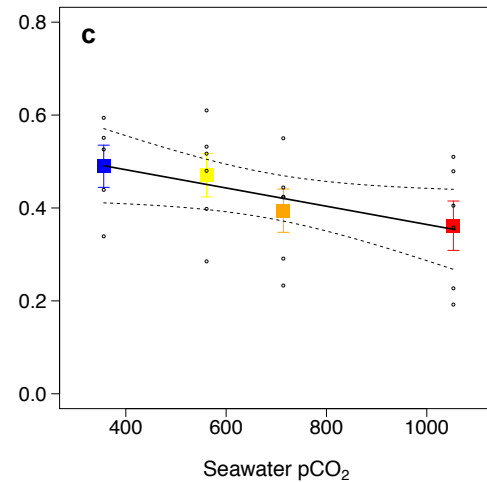
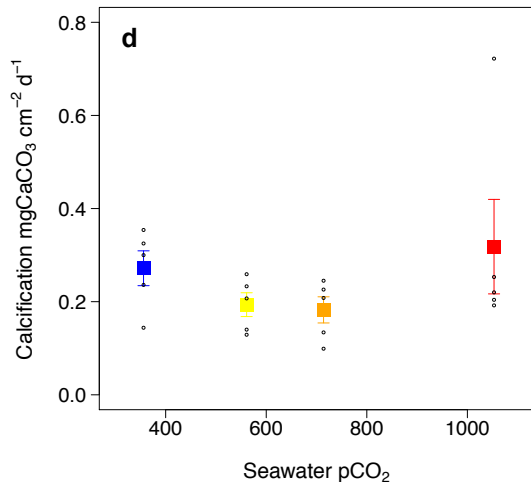
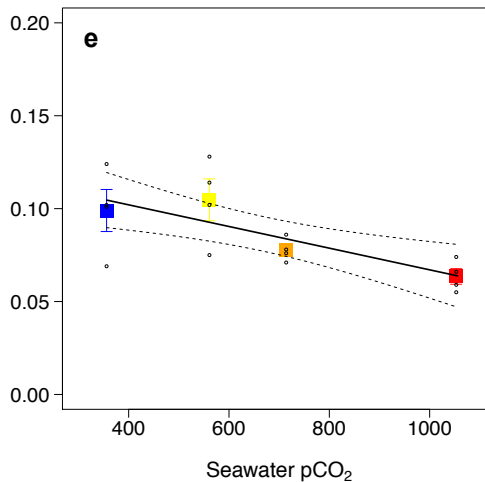
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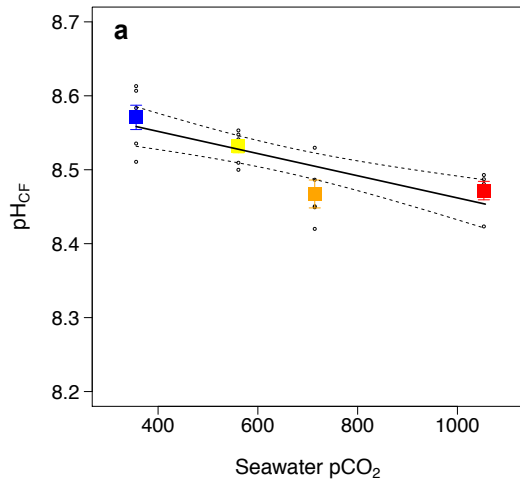
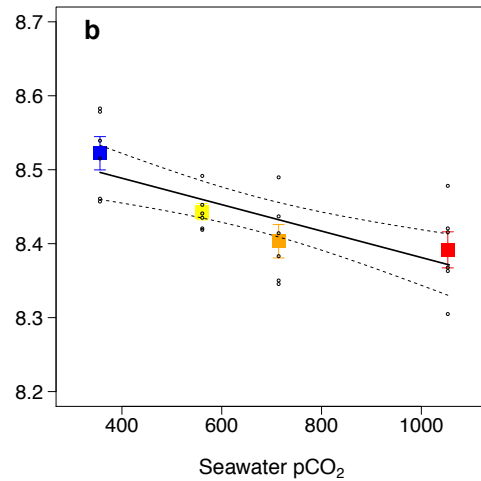
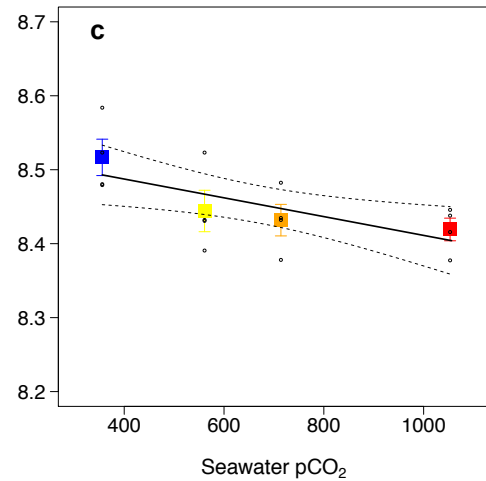
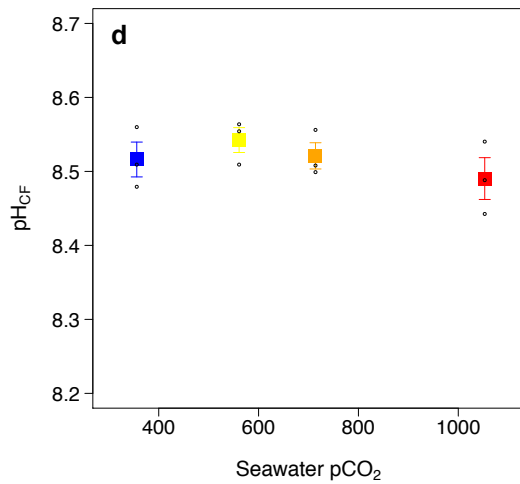
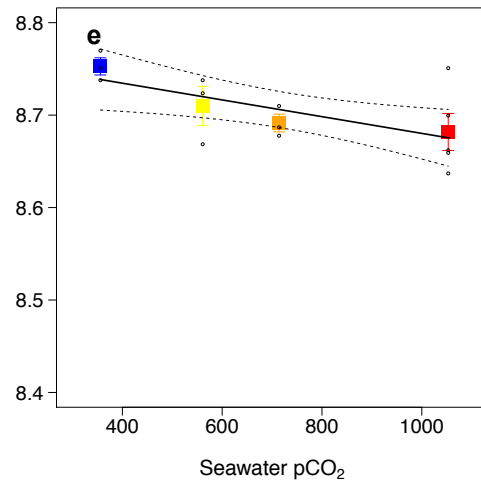
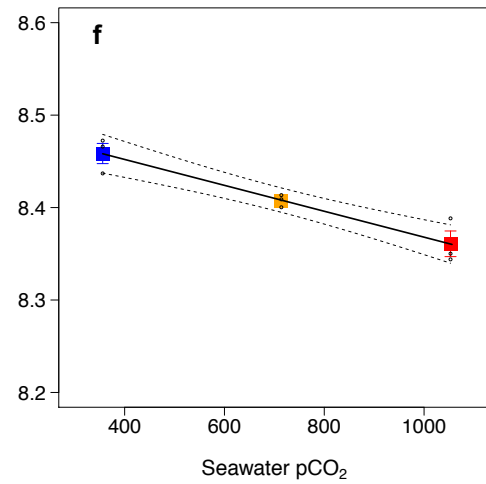
655 **Fig. 5.** Temporal variations of *A. pulchra* and massive *Porites* sp. calcifying fluid pH
656 and DIC. The skeleton of *A. pulchra* was sampled to select sections grown at the start
657 of the experiment, and after ~ 0-3, 3-6, 6-9 and 9-12 months of experiment (panels A
658 and B), while the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

662

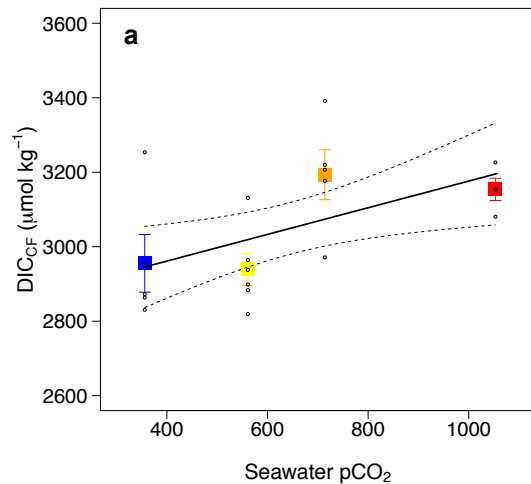
663 **Fig. 6.** Schematic summary of the responses to ocean acidification measured in the
664 corals *Acropora pulchra*, *Psammocora profundacella*, massive *Porites* sp., and
665 *Pocillopora verruca*. The calcifying fluid chemistry of the two corals whose
666 calcification was affected by OA exhibited 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*.

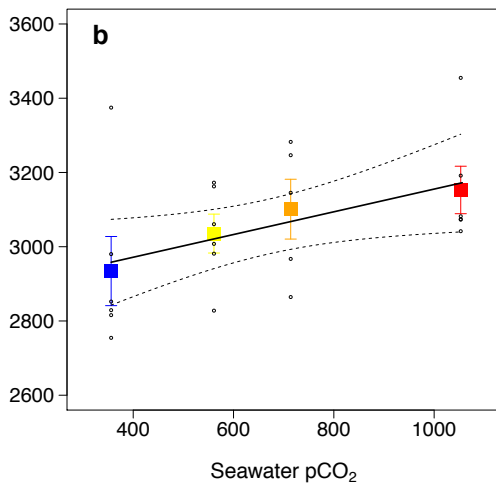
Psammocora*Pocillopora**Acropora**Porites**Lithophyllum*

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

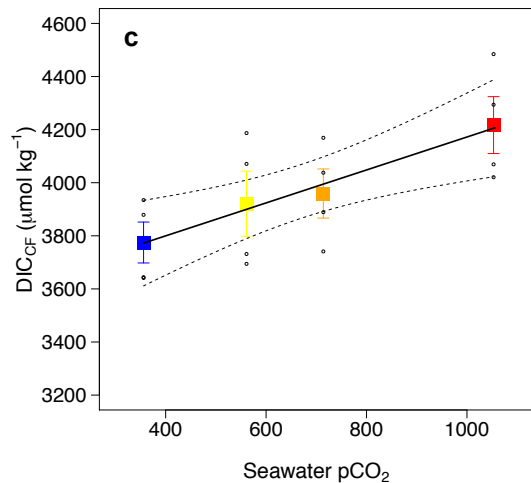
Psammocora



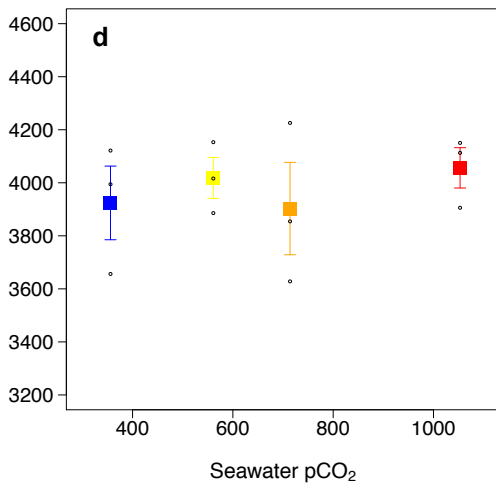
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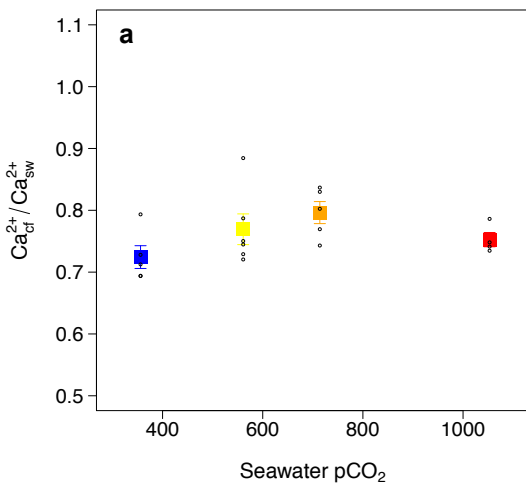
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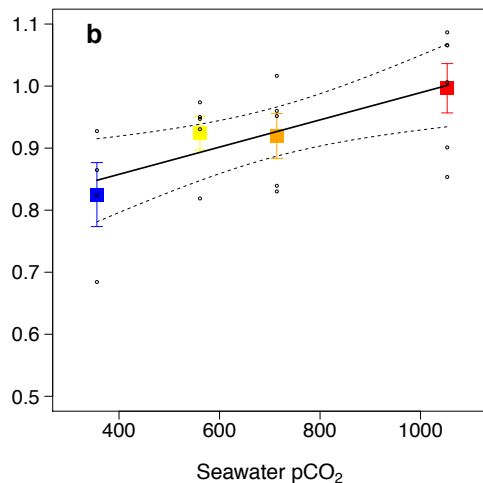
Porites



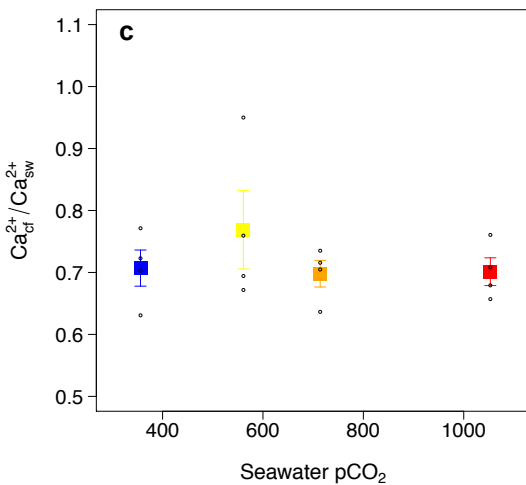
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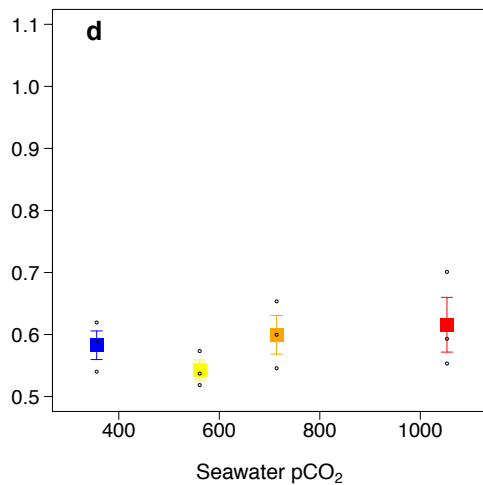
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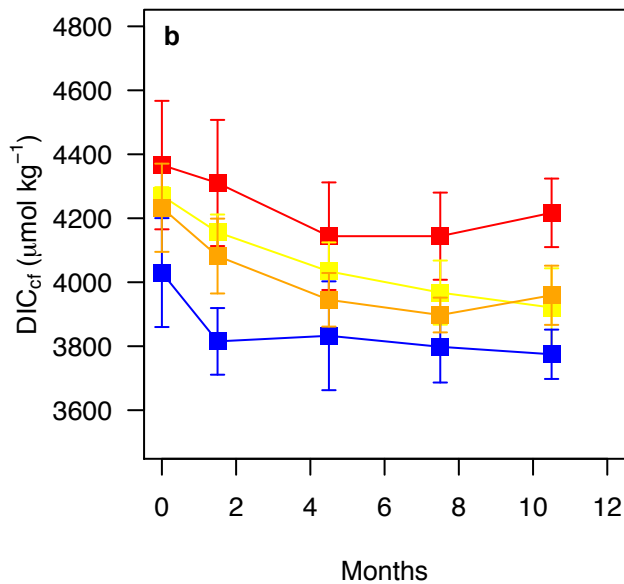
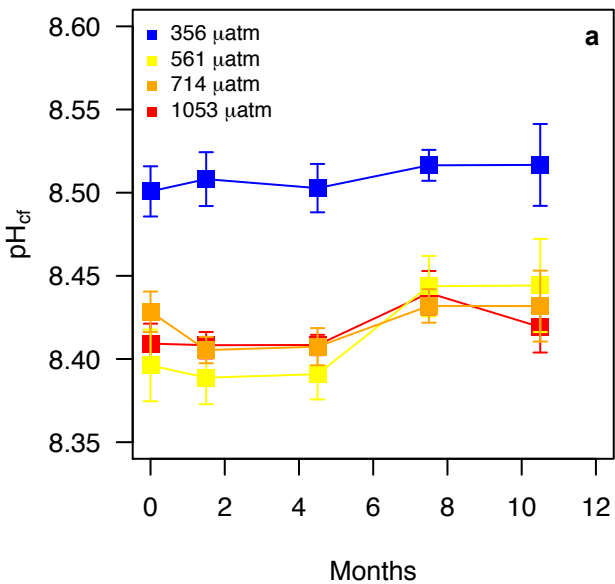
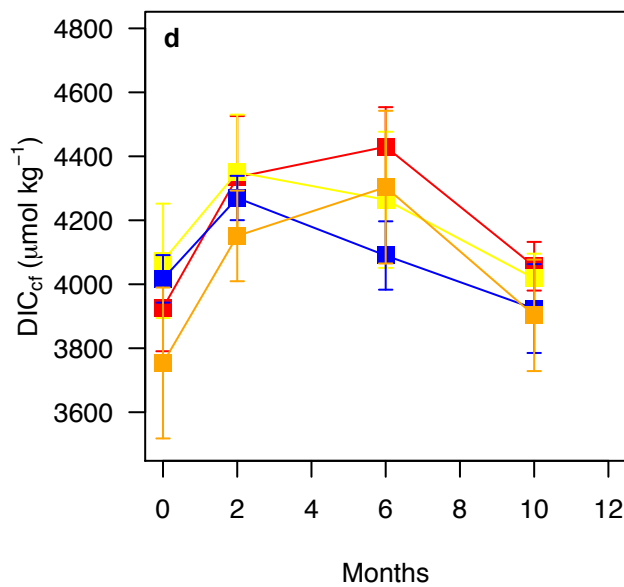
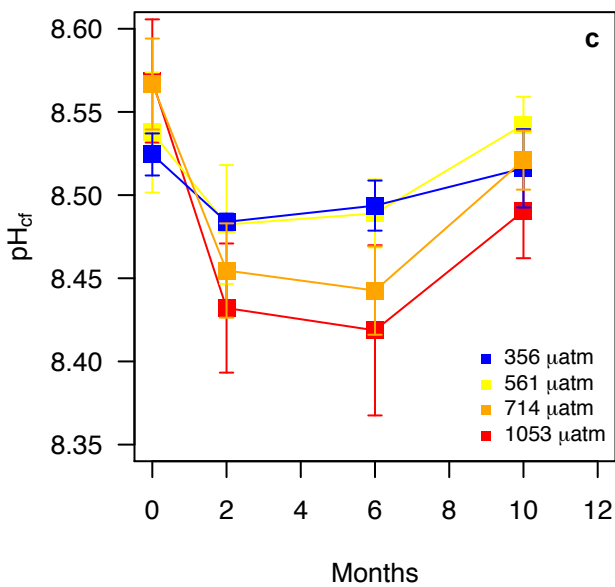


Acropora



Porites



Acropora***Porites***

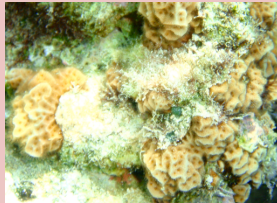
Species-specific response of calcification to OA

Affected

Acropora



Psammocora



Calcifying fluid

↓ pH ↑ DIC = Ca²⁺

Not affected

Porites



Pocillopora



Homeostasis



Calcifying fluid

= pH
= DIC = Ca²⁺



Ca²⁺_{cf} increase



Calcifying fluid

↓ pH
↑ DIC ↑ Ca²⁺

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).

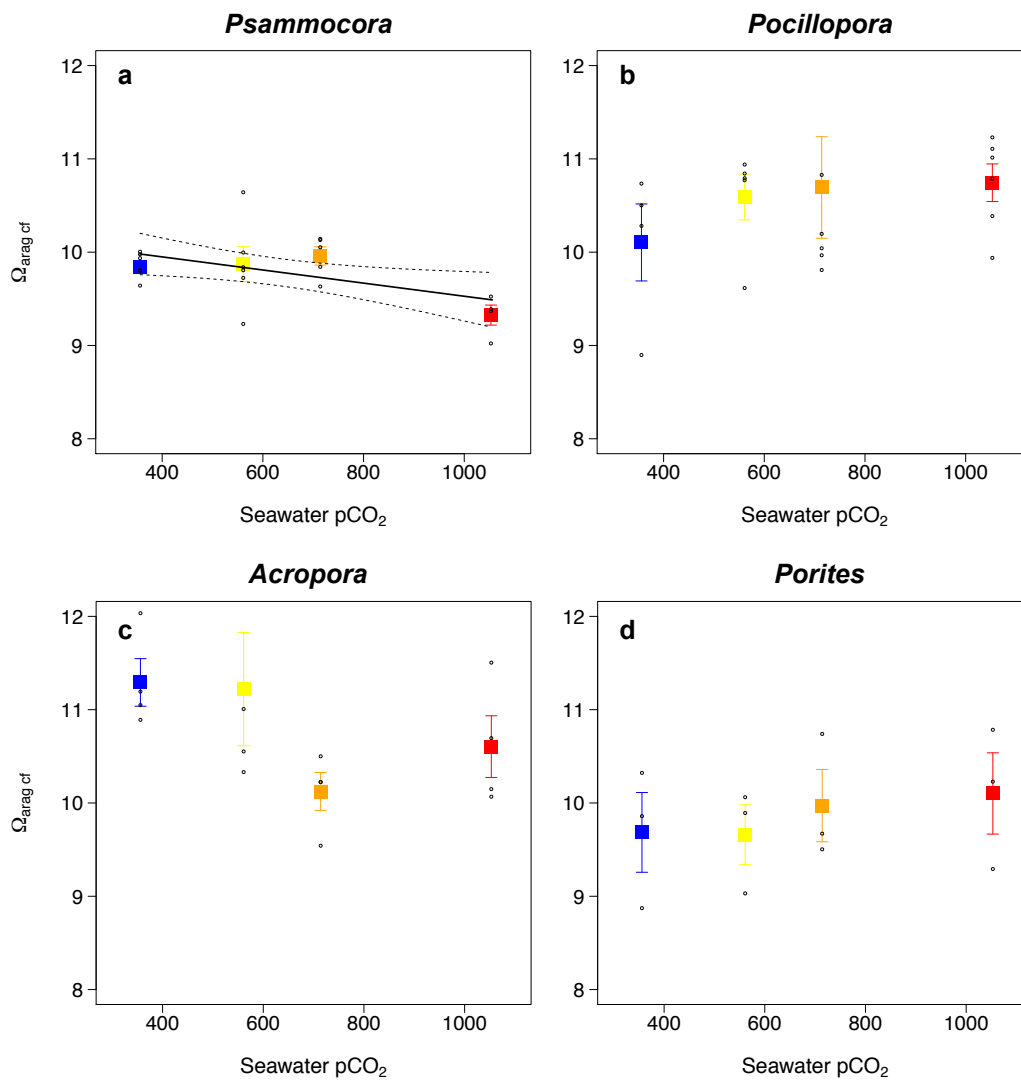


Fig S2. Temporal variations of *A. pulchra* and massive *Porites* sp. calcifying fluid Ω and $\text{Ca}^{2+}_{\text{cf}} / \text{Ca}^{2+}_{\text{sw}}$. The skeleton of *A. pulchra* was sampled to select sections grown at the start of the experiment, and after \sim 0-3, 3-6, 6-9 and 9-12 months of experiment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

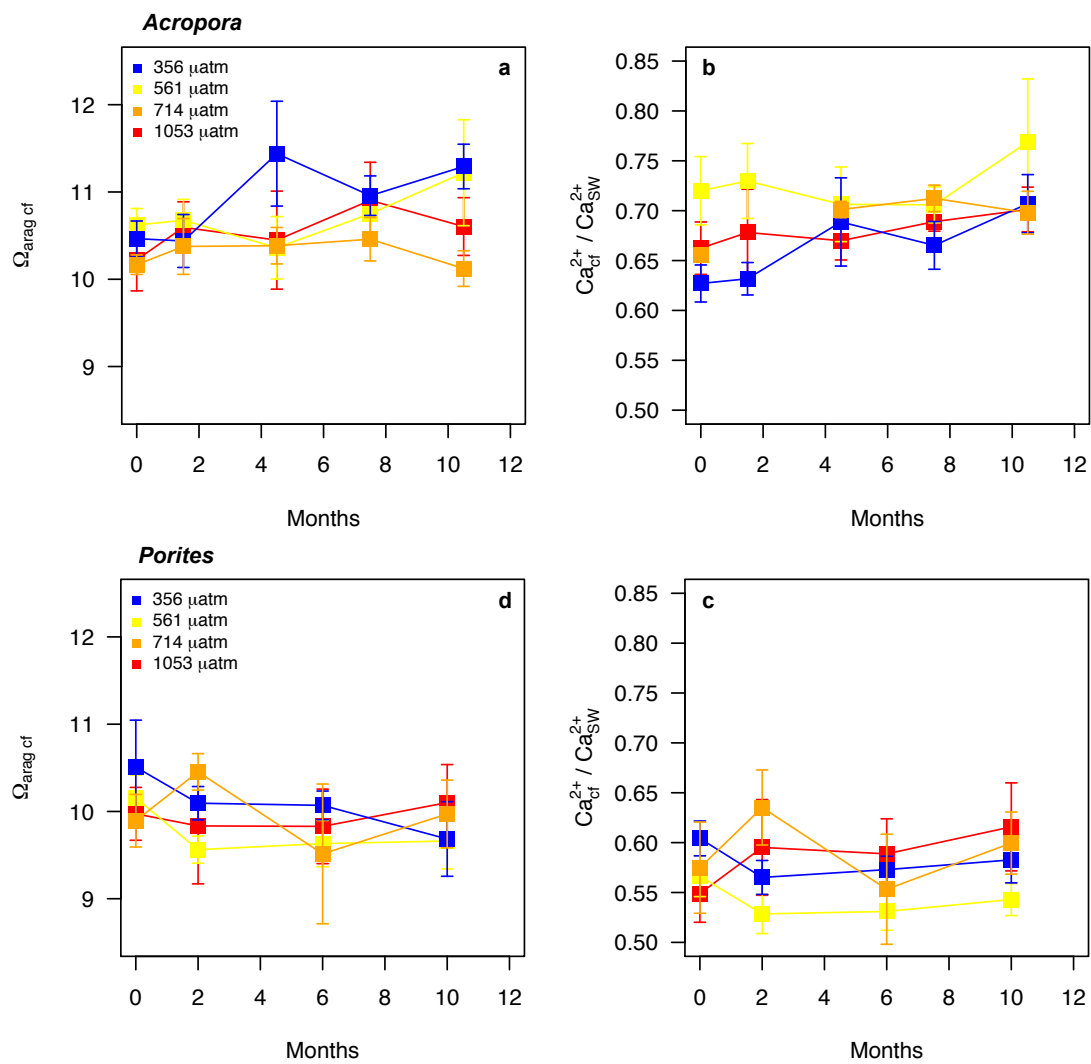


Fig. S3. Schematic showing the portions of the skeleton of *Acropora pulchra* that were selected to represent the conditions in the calcifying fluid at the start of the experiment (section 0), and after ~ 0-3 (section 1), 3-6 (section2), 6-9 (section 3), and 9-12 (section 4) months of experiment. The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

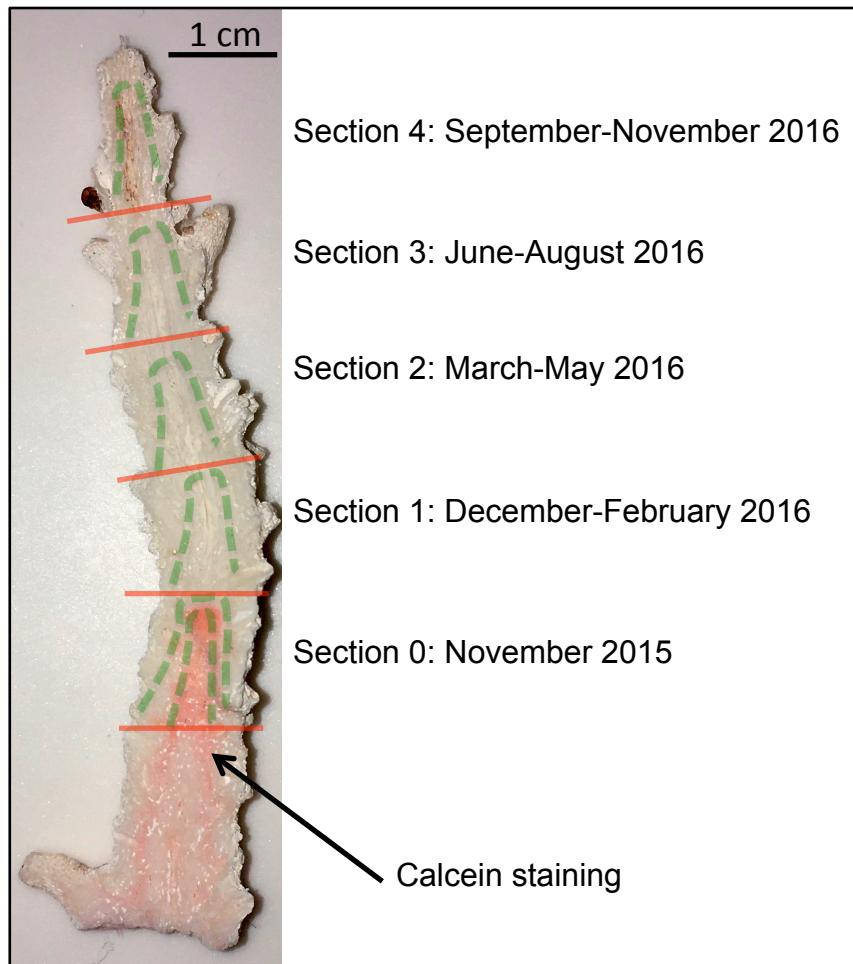


Table S1: Parameters of the linear estimates of the relationship between calcification, pH_{cf} , DIC_{cf} , $\text{Ca}^{2+}_{\text{cf}}$, and Ω_{cf} , and seawater pCO_2 (μatm).

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
	$\text{Ca}^{2+}_{\text{cf}}$	$0.73 + 1.10 \cdot 10^{-5} \cdot \text{pCO}_2$	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
	$\text{Ca}^{2+}_{\text{cf}}$	$0.77 + 2.19 \cdot 10^{-4} \cdot \text{pCO}_2$	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
	$\text{Ca}^{2+}_{\text{cf}}$	$0.74 - 3.63 \cdot 10^{-5} \cdot \text{pCO}_2$	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
	$\text{Ca}^{2+}_{\text{cf}}$	$0.54 + 6.8 \cdot 10^{-5} \cdot \text{pCO}_2$	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