



**HAL**  
open science

## **A coralline alga gains tolerance to ocean acidification over multiple generations of exposure**

C. E Cornwall, S. Comeau, Q. D'alexis, T. M Decarlo, E. Larcombe, K. Giltrow, B. Moore, F. Puerzer, M. T Mcculloch

### ► To cite this version:

C. E Cornwall, S. Comeau, Q. D'alexis, T. M Decarlo, E. Larcombe, et al.. A coralline alga gains tolerance to ocean acidification over multiple generations of exposure. *Nature Climate Change*, 2020, 10 (2), pp.143-146. 10.1038/s41558-019-0681-8 . hal-03007123

**HAL Id: hal-03007123**

**<https://hal.science/hal-03007123>**

Submitted on 16 Nov 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 A coralline alga gains tolerance to ocean acidification over multiple generations of exposure

2 C. E. Cornwall<sup>1,2,3,\*†</sup>, S. Comeau<sup>1,2,4,†</sup>, T. M. DeCarlo<sup>1,2</sup>, E. Larcombe<sup>1</sup>, B. Moore<sup>1</sup>, K.

3 Giltrow<sup>1</sup>, F. Puerzer<sup>1</sup>, Q. D’Alexis<sup>1</sup>, M. T. McCulloch<sup>1,2</sup>

4 <sup>1</sup>Oceans Institute and Oceans Graduate School, The University of Western Australia, 35

5 Stirling Hwy, Crawley 6009, Australia.

6 <sup>2</sup>ARC Centre of Excellence for Coral Reef Studies, The University of Western Australia, 35

7 Stirling Hwy, Crawley 6009, Australia.

8 <sup>3</sup>School of Biological Sciences, Victoria University of Wellington, Wellington, New Zealand

9 <sup>4</sup>Sorbonne Université, CNRS-INSU, Laboratoire d’Océanographie de Villefranche, 181

10 chemin du Lazaret, F-06230 Villefranche-sur-mer, France

11 \*For correspondence email: [Christopher.cornwall@vuw.ac.nz](mailto:Christopher.cornwall@vuw.ac.nz)

12 †These authors contributed equally

13

14 Crustose coralline algae (CCA) play a crucial role in the building of reefs in the photic zones  
15 of nearshore ecosystems globally and are highly susceptible to ocean acidification<sup>1-3</sup>. Yet the  
16 extent to which CCA can gain tolerance to ocean acidification over multiple generations of  
17 exposure is unknown. We show that while calcification of juvenile CCA is initially highly  
18 sensitive to ocean acidification, after 6 generations of exposure the effects of ocean  
19 acidification disappears. A reciprocal transplant experiment conducted on the 7<sup>th</sup> generation  
20 where half of all replicates were interchanged across treatments confirmed that they had  
21 acquired tolerance to low pH and not simply to laboratory conditions. Neither exposure to  
22 greater pH variability, nor chemical conditions within the micro-scale calcifying fluid  
23 internally, appeared to play a role in fostering this capacity. Our results demonstrate that reef-  
24 accreting taxa can gain tolerance to ocean acidification over multiple generations of  
25 exposure, suggesting that this cosmopolitan species could maintain its critical ecological role  
26 in reef-formation.

27

## 28 **Introduction:**

29 A growing body of evidence suggests ocean acidification will greatly reduce the capacity of  
30 major reef-forming species to continue calcifying and hence to accrete the calcium carbonate  
31 building blocks of reefs<sup>2</sup>. CCA are ecologically important calcareous red macroalgae that  
32 help create and cement reefs from the tropics to the poles and provide a settlement substrate  
33 for marine invertebrate larvae such as corals, abalones and sea urchins. Coralline algae  
34 precipitate high magnesium calcite, one of the most soluble forms of calcium carbonate, and  
35 are therefore expected to be highly susceptible to increased dissolution rates under ocean  
36 acidification<sup>4</sup>. Juvenile coralline algae are at particular risk, with extremely reduced  
37 abundance under ocean acidification<sup>5,6</sup>. Ocean acidification decreases both linear extension  
38 and calcification (hereafter termed growth) of CCA by altering the calcifying fluid chemistry  
39 of adult CCA to become less favorable for precipitation of calcium carbonate<sup>7-9</sup>. Adult  
40 coralline algae that are most resistant to ocean acidification tend to be those that can alter  
41 their mineralogy<sup>10</sup> or resist changes to their calcifying fluid<sup>7-9</sup>.

42 It is currently unknown whether benthic calcifying organisms gain tolerance to ocean  
43 acidification after exposure for multiple generations. Our understanding of these processes in  
44 marine species is limited to either evolutionary responses of short-lived, non-reef-building  
45 species such as phytoplankton and non-calcareous polychaete worms over multiple (five to  
46 hundreds) generations<sup>11-13</sup>, or acclimatization responses of longer-lived species over 1 or 2  
47 generations<sup>14,15</sup>. Most reef-forming species have slower growth rates and generation times  
48 than microalgae, which has limited our ability to test whether they can gain tolerance to  
49 ocean acidification. However, some tropical reef-building and cementing CCA grow  
50 extremely fast to colonize available space after disturbance<sup>16</sup>, occupying a broad range of  
51 habitats that requires high phenotypic plasticity<sup>17</sup>. Some CCA also reproduce sexually or  
52 asexually within weeks of settlement, even while still microscopic<sup>18</sup>. These traits make CCA  
53 valuable model organisms to test whether reef-accreting calcifying species can potentially  
54 gain tolerance to ocean acidification over multiple generations of exposure.

55 In many shallow-water reef environments, night-time declines in pH due to local  
56 metabolic activity often exceed the changes in open-ocean pH predicted to occur by 2100  
57 under business-as-usual CO<sub>2</sub> emissions scenarios (e.g. Representative Concentration Pathway  
58 8.5, 0.4 pH units)<sup>7,20,21</sup>. It has been proposed that this natural diurnal variability may impart  
59 tolerance to low pH for resident calcifying species via selection for individuals that are better  
60 adapted to survive in these environments<sup>20,22</sup>. Additionally, transgenerational acclimation can  
61 occur via a variety of epigenetic or maternal effects after exposure of subsequent generations

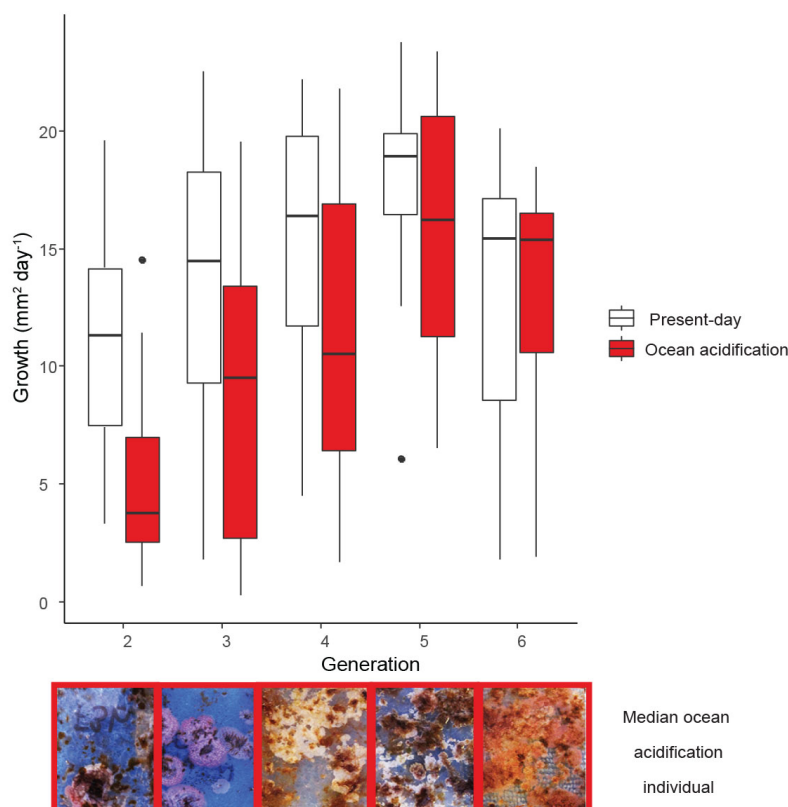
62 to stressors<sup>14,23</sup>, and could be promoted by prior exposure of individuals to greater variability  
63 in that stressor<sup>24</sup>.

64 We investigate whether the cosmopolitan reef-accreting and cementing species  
65 *Hydrolithon reinboldii* (synonymous with *Hydrolithon boergesenii*) can increase its tolerance  
66 to ocean acidification after multiple generations of exposure via mineralogical changes in  
67 skeletal formation or conversely the ability to resist seawater induced chemical changes to  
68 their calcifying fluid. We also test whether this tolerance can be gained faster in individuals  
69 with prior or regular exposure to low pH. We grew CCA for 8 generations under present-day  
70 (pH 8.00) and future ocean acidification treatments (pH 7.70), with both high ( $\pm 0.37$ ) and  
71 low ( $\pm 0.07$ ) pH variability, using populations from sites with either naturally low or high pH  
72 variability (see materials and methods and Table S1) and tracked complex changes in their  
73 calcification mechanisms. For the first time, we demonstrate that a species of coralline algae  
74 can gain tolerance to ocean acidification after 6 generations of exposure, but neither exposure  
75 to greater pH variability, nor changes in their calcification physiology related to mineralogy  
76 or chemistry in the calcifying fluid influence this capacity.

77

#### 78 **Changes in sensitivity to ocean acidification**

79 The resistance of juvenile CCA to ocean acidification increased incrementally over  
80 each generation during this 423-day experiment. Second generation CCA grew 56.1% slower  
81 across all ocean acidification treatments relative to the present-day treatments (Fig. 1). This  
82 sensitivity to lower mean pH declined over subsequent generations to 33.1, 29.7, 12.8, and  
83 2.0% between generation 2 to 6, supported by the significant interaction between mean pH  
84 and generation in the general linear model (Table S2,  $F_{1,224} = 8.60$ ,  $p = 0.004$ ). The difference  
85 further decreased to 0.6% when compared to the generation 7 populations kept in their  
86 original treatments (Fig. 2).



87

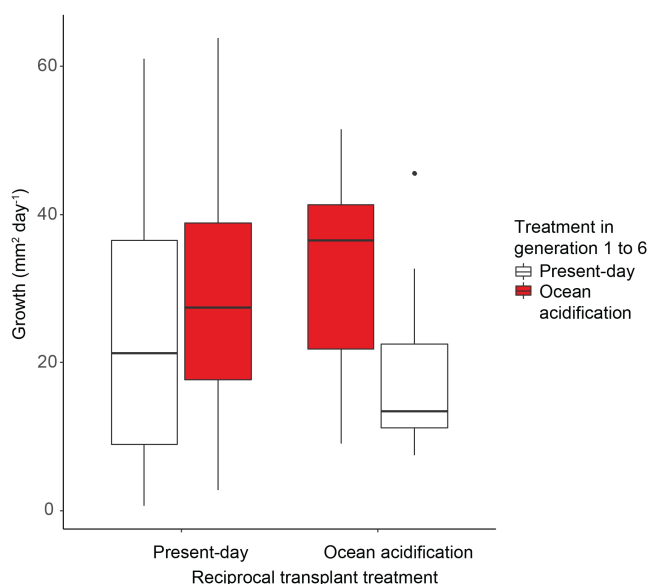
88 Figure 1: Growth of CCA populations after 2 to 6 generations of exposure to present-day (pH  
 89 8.00) and ocean acidification (pH 7.70) treatments. Also shown are the median individual  
 90 coralline algal plates for each generation under the ocean acidification treatments (out of 24).  
 91 Scale 50 by 45 mm of images. Note here responses to constant and variable pH regimes  
 92 combined. See supplementary figure Fig. S1 for individual treatment responses. Median and  
 93 75% quartiles, mean  $n = 24$  per treatment and generation combination.

94

## 95 Reciprocal transplant experiment

96 A reciprocal transplant experiment was also undertaken to test whether the CCA  
 97 gained tolerance to ocean acidification rather than just the aquaria settings. This was  
 98 undertaken at generation 7 where half of all replicates were interchanged with replicates of  
 99 treatments with the opposite mean pH treatment, while keeping the variability the same as the  
 100 previous generation (see methods). Growth was 40.3% lower in generation 7 populations  
 101 transferred from the present-day into the ocean acidification treatments compared to those  
 102 grown there for 6 generations, and this was 22.2% lower than the extension of control  
 103 populations kept within the control treatments (Fig. 2, significant interaction between mean  
 104 pH treatment and the treatment of origin: Table S3,  $F_{1,79} = 4.6$ ,  $p = 0.035$ ). The negative  
 105 effects of low pH on growth were larger in populations originally from the more variable pH

106 site compared to the stable pH site in early generations over the course of the experiment  
 107 (Fig. S1,  $F_{1,224} = 4.05$ ,  $p = 0.045$ ). This is contrary to the hypothesis that individuals from  
 108 more variable environments are more tolerant to ocean acidification, or that they gain this  
 109 tolerance more rapidly over multiple generations. Trends in total recruit area typically  
 110 mirrored those of growth rates, but were inherently more variable (Fig. S2, S3, Table S3–4).  
 111



112  
 113 Figure 2: Growth of CCA after 7 generations grown in present-day (pH 8.00) and ocean  
 114 acidification (pH 7.70) treatments (controls), versus those transferred into these novel  
 115 treatments after being in the treatment with the other mean pH for 6 generations. Responses  
 116 to constant and variable pH regimes are combined. Median and 75% quartiles,  $n = 12$  per  
 117 treatment and generation combination.

118

### 119 **Implications for future nearshore reefs**

120 This is the first demonstration that over multiple generations of exposure benthic  
 121 calcifying species can increase their tolerance to ocean acidification, indicating that at least  
 122 this species of CCA has the potential to gain tolerance to ocean acidification well within the  
 123 timeframe of when its effects will manifest. Although this finding is by-itself critical to more  
 124 accurately forecasting reef futures, outstanding questions remain, including the generality of  
 125 this finding amongst other coralline algal species and in combination with other stressors  
 126 such as ocean warming or in the presence of competitors that could be unaffected by ocean  
 127 acidification. However, if coralline alga can develop resistance to ocean acidification over  
 128 multiple generations of exposure, it is possible that other calcifying benthic species with

129 similar calcification mechanisms<sup>9</sup> could have the same capability. This information is crucial  
130 for coral reefs, for example, where many coral species are long-lived and experimentally  
131 testing their capacity to gain tolerance over multiple generations is logistically challenging,  
132 requiring years or decades of exposure to ocean acidification in controlled laboratory settings.  
133

#### 134 **Evolutionary and physiological mechanisms promoting tolerance**

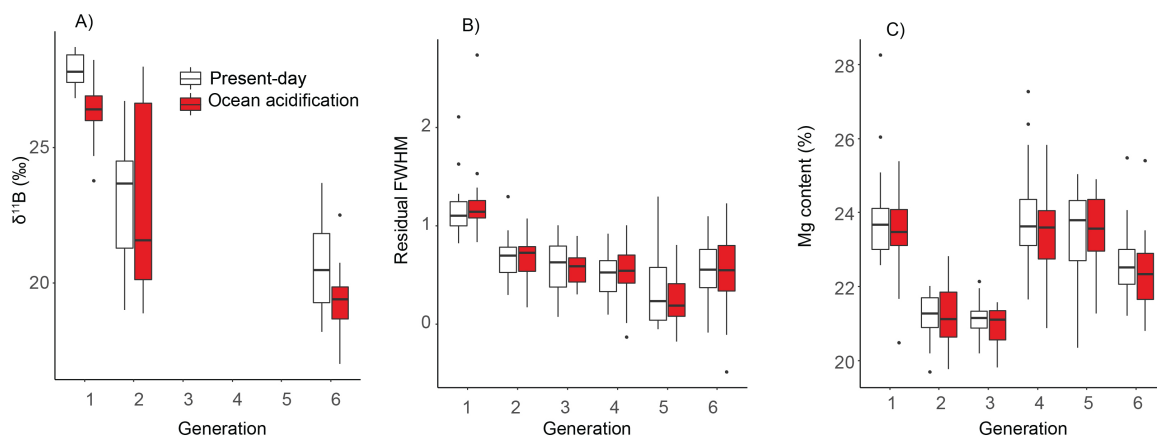
135         The diminishing effect of ocean acidification on growth over each subsequent  
136 generation supports the premise that successive cohorts of CCA are subject to strong  
137 selection for resistance to ocean acidification, or that transgenerational acclimation occurred  
138 on individuals within each tank. We did not aim to determine the underlying evolutionary  
139 processes that could alter tolerance to ocean acidification. Previous research indicates that  
140 species can gain complete tolerance after a full generation of exposure to climate change  
141 stressors<sup>14</sup>. Our evidence indicates slower, more incremental increases in tolerance to climate  
142 change stressors could also occur in select taxa in the future. However, the trends observed  
143 here were also rapid in the context of evolutionary adaptation. The rapid (less than two years),  
144 yet incremental, nature of these changes, and the predominately asexual reproduction,  
145 indicate that transgenerational acclimatory processes could have played a large role in  
146 inducing the changes observed. However, the effects of ocean acidification were particularly  
147 strong on the recruitment of the first generations of CCA, and because selective pressure is a  
148 combination of the effect size of the stressor selected against and the heritability of the trait,  
149 we cannot rule out that selection for individuals better able to calcify under these treatments  
150 occurred. While not our aim here, the next step is to further understand the contributions of  
151 genetic versus epigenetic processes that contribute to changing tolerance under climate  
152 change in reef-building and accreting species.

153         Regardless of the eco-evolutionary processes involved, exposure to greater variability  
154 in pH did not foster the capacity to gain tolerance to ocean acidification faster<sup>24</sup>. This is  
155 surprising given that organisms from these habitats tend to respond more positively to ocean  
156 acidification<sup>22,25</sup>, and that phenotypic or transgenerational plasticity is predicted to be higher  
157 on these populations<sup>17,24</sup>. Therefore, transplanting organisms from highly variable pH  
158 environments into habitats with constant pH regimes, as has been suggested regarding ocean  
159 warming and temperature variability<sup>26</sup>, might not be a successful strategy to combat against  
160 ocean acidification. The CCA also did not alter their calcifying fluid toward conditions



161 traditionally considered favorable for precipitation<sup>8</sup>. Hence, physiological mechanisms that  
 162 allow for tolerance in adult CCA could have no bearing on their persistence in future reefs.

163 The physiological mechanisms responsible for reduced sensitivity under the ocean  
 164 acidification treatments are complex. Raman spectroscopy analyses indicated that changes in  
 165 the mineralogy from high magnesium calcite into less readily dissolved polymorphs such as  
 166 dolomite did not occur here (Table S5, Figure 3). Similarly, neither  $\delta^{11}\text{B}$  ( $\text{pH}_{\text{cf}}$  proxy) or  
 167 Raman full width at half maximum (FWHM: proxy of the calcite saturation state in the  
 168 calcifying fluid [ $\Omega_{\text{cf}}$ ] when precipitation occurs) were elevated in individuals kept for 8  
 169 generations under mean low pH compared to generation 2 individuals (Table S6, S7, Figure  
 170 3). In fact, both  $\delta^{11}\text{B}$  and FWHM decreased between generations 1, 2 and then the  
 171 experimental endpoint, and they tended to be lower in ocean acidification treatments  
 172 generally. The physiological ability to maintain elevated  $\text{pH}_{\text{cf}}$  or  $\Omega_{\text{cf}}$  under low pH in adult  
 173 coralline algae and corals are traits related to resistance to ocean acidification, and we  
 174 hypothesized *a priori* that any CCA here that gained resistance to ocean acidification would  
 175 have done so via improving these traits. However, changes in these calcification-related  
 176 parameters ( $\delta^{11}\text{B}$ , FWHM, Mg) do not match changes in growth rates over successive  
 177 generations under ocean acidification. The inverse relationship between FWHM and growth  
 178 throughout the course of the experiment (Figure S5), together with lack of changes in other  
 179 geochemical parameters, indicates that CCA tolerant to ocean acidification here were those  
 180 which could calcify when carbonate chemistry is suboptimal for precipitation within the  
 181 calcifying fluid, when compared to that of adults or controls. This is likely caused by both the  
 182 rapid use of calcium and carbonate during calcification in the semi-enclosed space<sup>28</sup>, and  
 183 declining  $\text{pH}_{\text{cf}}$  via ocean acidification<sup>8</sup>. Additionally, traits such as increasing organic matrix  
 184 proteins involved in calcification<sup>29</sup> could also contribute to trends observed here.



186 Figure 3: a)  $\delta^{11}\text{B}$  (proxy for pH in the calcifying fluid), b) FWHM (proxy for calcite  
187 saturation state in the calcifying fluid), c) and Mg content of CCA over 6 generations under  
188 present-day or ocean acidification treatments. Median and 75% quartiles, mean  $n = 24$  per  
189 treatment and generation combination.

190

## 191 **Conclusions**

192 These results highlight that we cannot predict which species will be winners or losers  
193 under climate change based solely on their responses to short-term exposure to acute  
194 stressors. We demonstrate for the first time that some coralline algae are capable of rapidly  
195 gaining tolerance to ocean acidification over six generations. If this capacity is present in  
196 other select species of coralline algae, then as a group they could be the largest contributors  
197 to global reef accretion in future oceans, given their already high abundance in temperate  
198 nearshore reefs, tolerance to ocean warming<sup>30</sup>, and the recent large-scale declines in the  
199 abundance of the longer-lived tropical scleractinian corals<sup>31</sup>.

200

## 201 **Methods**

### 202 **Collection and treatment design**

203 We collected adult coralline algae, *Hydrolithon reinboldii* (synonymous with *boergesenii*<sup>32</sup>),  
204 rhodoliths from Western Australia's northern Kimberley locations of Tallon (16°40S,  
205 123°14E) and Shell Islands (16°48S, 123°04E) in October 2016. These two sites are  
206 characterised by similar thermal regimes, but different pH regimes. Tallon Island is a  
207 seaweed and seagrass dominated reef with strong diel pH variability (up to 1.06 units daily).  
208 Shell Island is dominated by *Acropora* spp. corals and experiences a low range in pH  
209 variability (typically < 0.10 units daily). This species was chosen because it is the most  
210 common species at both sites, and because it was the only species that lives at both sites that  
211 can be identified using morpho-anatomical methods, hence retaining intact and undamaged  
212 individuals that would not occur if molecular sequencing was required prior to the  
213 experiment starting. This species is common in the Indo-Pacific region<sup>33</sup>, and was identified  
214 with assistance by staff at the Western Australian Museum. Samples are lodged both at  
215 Victoria University of Wellington and the University of Western Australia. The collection,  
216 environmental variables at the collection sites, and experimental design and monitoring are  
217 described in detail within the initial segment of the research on the adults in Cornwall et al.<sup>7</sup>.  
218 Briefly, we created 4 treatments, within which each of the two source population were  
219 placed: 1) control means (8.00) and low pH variability (daily range of 0.14), or 2) high pH

220 variability (0.58 daily range); 3) ocean acidification means (7.70) and low pH variability  
221 (0.20 range daily), or 4) high pH variability (0.89 daily range). See Table S1 for full details,  
222 or Figure 1 from Cornwall et al.<sup>7</sup> for schematic of details. There were 6 independent  
223 experimental tanks per treatment combination per population, for a total of 48 experimental  
224 tanks per generation. This allows us to determine the interactive effects of changes in both  
225 mean and variability in pH. As recommended by all best practices guides<sup>34-37</sup>, we used CO<sub>2</sub>  
226 gas to lower pH/elevate CO<sub>2</sub> concentrations, and CO<sub>2</sub>-free air to increase pH/lower CO<sub>2</sub>.  
227 Seawater was pumped from an area of soft-sediment bottom 100m offshore at Watermans  
228 Bay, Perth, then filtered to 25 µm using a series of sand filters, and then subsequently ran into  
229 a header tank where pH conditions were modified before moving into each experimental  
230 tank. There were 3 header tanks per treatment. Temperature was maintained in a series of  
231 waterbaths at 26 to 27 °C, 4°C warmer than the summer maximum outside of our  
232 experimental facility. No recruitment of coralline algae occurred within this header tank,  
233 allowing us to be certain that coralline algal recruits throughout our experiment were not  
234 sourced from local populations. This temperature (26 to 27 °C) roughly matches that of the  
235 collections site in the Kimberley region in Winter<sup>38</sup> or is slightly higher, and was chosen to  
236 limit the stress that the CCA here would be exposed to. We chose to hold temperature  
237 constant during the duration of the experiment to avoid any confounding effects of  
238 temperature variations between generations.

239

### 240 **Multigenerational experiment**

241 On collection, only healthy adult coralline algae were selected that possessed extensive  
242 numbers of conceptacles (i.e. reproductive structures where spores are formed). On day 0 (i.e.  
243 the collection date), adult coralline algae were extensively cleaned of epiphytes and scrubbed  
244 to limit carry-over of other coralline algal spores from the field into the experimental set up.  
245 On day 78, coralline algal recruits were visible and on day 128 the adults were removed and  
246 the process of creating the generation 3 and subsequent generations began. This involved  
247 cutting out two 4.5 by 5 cm (L × W) sections of the tank containing the highest cover of  
248 coralline algae recruits and placing these into completely new tanks, attached vertically (Fig.  
249 S7). Four of the generation 2 ocean acidification (both high and low variability) replicate  
250 tanks contained no visible recruits. For these tanks, populations from different tanks were  
251 used to create the third and subsequent generations. Therefore, we used 44 distinct  
252 populations of CCA to source the subsequent generations. We repeated this process once  
253 CCA recruits in the control treatment obtained the same estimated size as the 2<sup>nd</sup> generation,

254 roughly every 41 to 51 days. Once visible, CCA grew rapidly, approaching 1 cm diameter per  
255 week. The CCA on plates would then develop conceptacles and release spores which disperse  
256 into the water column around 2 weeks after being placed into brand new experimental tanks  
257 not previously used. A standardised size was used rather than a standardised time because of  
258 slight seasonal differences in the duration required to reproduce during each generation. This  
259 was process was continued until generation 7. This resulted in using 336 separate  
260 experimental tanks (7 ×48) throughout the course of this study.

261 We acknowledge that it is possible that reproduction could be occurring in recruits even when  
262 microscopic (e.g.<sup>18</sup>), so in theory each generation could possibly be comprised of small  
263 amounts of differing generations. This would result in the number of generations here being  
264 more than the 8 recorded. However, we consider that extremely unlikely. This is because the  
265 experimental tanks were monitored daily, and we did not observe more rapid recruitment than  
266 would be expected if the recruits were reproducing before they became visible (i.e. weeks), or  
267 if time to reproductive maturity was variable, as recruits became visible in all tanks within the  
268 same weekly time frame when recruitment occurred. This consistent time to visible  
269 recruitment also means it was highly unlikely that recruitment occurred in generation 2 from  
270 spores of other species carried-over from the field. All coralline algal recruits possessed  
271 similar morphoanatomical features, including similarly sized and shaped conceptacles that  
272 aligned with the adults' identification<sup>39</sup>. Thousands of conceptacles were produced in each  
273 generation, and all that we analyzed contained multiple spores indicating that they were  
274 asexually reproducing<sup>40</sup>. Damaged conceptacles were not analyzed. In other words, the algae  
275 were asexually reproducing which is most common in coralline algae<sup>41</sup>, especially for this  
276 species for which only these life history stages have been noted<sup>39</sup>. The probability of skipping  
277 sexual reproduction in many species has previously been suggested<sup>41</sup>. A proportion of  
278 conceptacles were not fully formed at the end of each generation or their contents were  
279 already emptied or they were damaged, for these we cannot fully confirm the life stage or  
280 nature of reproduction.

281 We measured the linear extension of CCA on the created replicate plates using images  
282 taken every two weeks and analysed in ImageJ. This step involved analyzing 2496 images.  
283 We also assessed the total cover of recruits at the end of every generation. The trends in total  
284 area occupied by coralline algae in the tanks, and the growth on monitored plates were  
285 similar. However, it became apparent that the total area of recruitment was affected by both  
286 the treatment effect (mean pH) on growth and random variability in the number of recruits.  
287 Therefore, we used the change in total area of the individuals of created plates as the focal

288 measurement of interest, as it contained one response variable and not two (i.e. growth and  
289 recruitment success). See supplementary Figure 2 for these data, which follow similar trends  
290 but are inherently more variable. This step involved analyzing 1440 images. A random  
291 selection of more than 10% of all images were analyzed twice to check for consistency  
292 between multiple assessors.

293

294

295

### 296 **Reciprocal transplant experiment**

297 The process of exposing subsequent generations to their original treatment was continued in  
298 the 48 populations until generation 7, whereupon we conducted a reciprocal transplant to  
299 determine whether improved growth in ocean acidification treatments over time were  
300 representative of altered response to the treatments themselves, or an interaction between lab  
301 effects and altered response to treatments<sup>23</sup>. We exchanged half of the ocean acidification  
302 replicates with half of the ambient replicates within the same variability treatments, i.e. pH  
303 7.70 low pH variability replicates interchanged with pH 8.00 low pH variability replicates  
304 and vice versa. We did this because of the observed changing effects of mean pH over  
305 subsequent generations and lack of effects of pH variability. Therefore, we were not  
306 interested in the effects of switching individuals' treatments with differences in pH  
307 variability, only in how switching them between different mean pH treatments would  
308 influence fitness. When we create the additional novel or original treatments (i.e.  
309 "transferred" into novel treatments or placed in the same treatment as "controls") we have 16  
310 different treatment combinations. We assessed growth over the first 2 weeks after transferring  
311 the generation 7 individuals into novel and control treatments, before they became limited for  
312 further space. We also measured total recruitment of their offspring after 61 days. We  
313 removed all standardised coralline algal plates from their tanks for the same length of time  
314 irrespective of whether they were transferred into a new treatment or placed into a control  
315 treatment.

316

### 317 **Measurement metrics**

318 We also measured proxies to aid in understanding changes in calcification physiology of the  
319 coralline algae at the completion of the experiment.  $\delta^{11}\text{B}$ , a proxy for pH at the site of  
320 calcification, was measured on generations 1, 2, 6, 7 and 8 to determine trends over  
321 generations and due to treatments using previously published methods<sup>7-9</sup>, with the only

322 difference being bleaching durations of 3 times as long. We also measured Raman  
323 spectroscopy on every generation as a proxy of the saturation state of calcium carbonate  
324 (based on  $\nu_1$  Full Width at Half Maximum; FWHM) and magnesium content (based on  $\nu_1$   
325 wavenumber), following previously described protocols<sup>7,9</sup>.

326

327

328

### 329 **Statistical analysis**

330 We used general linear models in R to determine differences in growth, recruit area, and  
331 geochemical proxies across all available generations assessing the full interaction between  
332 mean pH (control or ocean acidification), pH variability (low or high), generations (2, 3, 4, 5,  
333 6), and site of origin (Shell or Tallon). Waterbath was used a random factor in models to  
334 account for this potential source of variability. For generations 7 and 8, we used the same  
335 model structure, except the generation factor was replaced with the transfer (novel treatment  
336 or original control treatment) treatment and each generation was assessed separately, and  
337 waterbath was removed from the model. We used a log-fit mixed model for recruit area of  
338 generations 2 to 7, due to non-normally distributed data.

339

### 340 **Data availability**

341 Data is available at dryad: <https://doi.org/10.5061/dryad.pzgmsbcfq>.

342

### 343 **Code availability**

344 All R code will be available upon request.

345

### 346 **Acknowledgments**

347 We thank A.-M. Nisumaa-Comeau and J.P. D'Olivo for laboratory assistance. V. Schoepf and  
348 S. McCoy for comments on a previous version. R. Townsend from the Western Australian  
349 Museum for training in species' identification. Funding: MTM was supported by an  
350 Australian Research Council (ARC) Laureate Fellowship. CEC, TMD and CEC by the ARC  
351 Centre of Excellence for Coral Reef Studies (CE140100020). SC was supported by an ARC  
352 Discovery Early Career Researcher Award (DE160100668). CEC was also supported by a  
353 Rutherford Discovery Fellowship from The Royal Society of New Zealand Te Apārangi  
354 (RDF-VUW1701).

355

356 **Author contributions**

357 CEC and SC conceived and ran the experiment and wrote the manuscript. CEC ran the  
 358 statistical analysis and conducted the boron isotope measurements. MTM conceived the  
 359 experiment, provided vital laboratory equipment, facilities and resources. TMD conducted  
 360 the Raman spectroscopy. EL, BM, KG, FP, and QD all ran the experiment and measured  
 361 seawater carbonate chemistry. All authors edited the manuscript, provided intellectual input,  
 362 and agreed to its submission.

363

364 **Conflicting interests**

365 We have no conflicts of interest.

366

367 **References:**

- 368 1 Nelson, W. A. Calcified macroalgae - critical to coastal ecosystems and vulnerable to  
 369 change: A review. *Marine and Freshwater Research* **60**, 787-801 (2009).
- 370 2 Kroeker, K. J. *et al.* Impacts of ocean acidification on marine organisms: quantifying  
 371 sensitivities and interaction with warming. *Global Change Biology* **19**, 1884-1896  
 372 (2013).
- 373 3 Heyward, A. & Negri, A. Natural inducers for coral larval metamorphosis. *Coral*  
 374 *Reefs* **18**, 273-279 (1999).
- 375 4 Ries, J. B. Skeletal mineralogy in a high-CO<sub>2</sub> world. *Journal of Experimental Marine*  
 376 *Biology and Ecology* **403**, 54-64 (2011).
- 377 5 Fabricius, K., Kluebenschedl, A., Harrington, L., Noonan, S. & De'Ath, G. In situ  
 378 changes of tropical crustose coralline algae along carbon dioxide gradients. *Scientific*  
 379 *reports* **5**, 9537, doi:10.1038/srep09537 (2015).
- 380 6 Ordoñez, A., Kennedy, E. V. & Diaz-Pulido, G. Reduced spore germination explains  
 381 sensitivity of reef-building algae to climate change stressors. *PLOS ONE* **12**,  
 382 e0189122, doi:10.1371/journal.pone.0189122 (2017).
- 383 7 Cornwall, C. E. *et al.* Resistance of corals and coralline algae to ocean acidification:  
 384 physiological control of calcification under natural pH variability. *Proceedings of the*  
 385 *Royal Society B: Biological Sciences* **285**, doi:10.1098/rspb.2018.1168 (2018).
- 386 8 Cornwall, C. E., Comeau, S. & McCulloch, M. T. Coralline algae elevate pH at the  
 387 site of calcification under ocean acidification. *Global Change Biology* **23**, 4245-4256  
 388 (2017).
- 389 9 Comeau, S., Cornwall, C. E., DeCarlo, T. M., Krieger, E. & McCulloch, M. T.  
 390 Similar controls on calcification under ocean acidification across unrelated coral reef  
 391 taxa. *Global Change Biology* **24**, 4857-4868, doi:10.1111/gcb.14379 (2018).
- 392 10 Nash, M. C. *et al.* Dolomite-rich coralline algae in reefs resist dissolution in acidified  
 393 conditions. *Nature Climate Change* **3**, 268-272 (2013).
- 394 11 Lohbeck, K. T., Riebesell, U. & Reusch, T. B. H. Adaptive evolution of a key  
 395 phytoplankton species to ocean acidification. *Nature Geoscience* **5**, 346,  
 396 doi:10.1038/ngeo1441 (2012).

- 397 12 Schlüter, L. *et al.* Adaptation of a globally important coccolithophore to ocean  
398 warming and acidification. *Nature Climate Change* **4**, 1024,  
399 doi:10.1038/nclimate2379 (2014).
- 400 13 Gibbin, E. M., Massamba N’Siala, G., Chakravarti, L. J., Jarrold, M. D. & Calosi, P.  
401 The evolution of phenotypic plasticity under global change. *Scientific Reports* **7**,  
402 17253, doi:10.1038/s41598-017-17554-0 (2017).
- 403 14 Donelson, J. M., Munday, P. L., McCormick, M. I. & Pitcher, C. R. Rapid  
404 transgenerational acclimation of a tropical reef fish to climate change. *Nature Climate*  
405 *Change* **2**, 30-32 (2012).
- 406 15 Putnam, H. M. & Gates, R. D. Preconditioning in the reef-building coral *Pocillopora*  
407 *damicornis* and the potential for trans-generational acclimatization in coral larvae  
408 under future climate change conditions. *Journal of experimental Biology* **218**, 2365-  
409 2372, doi:10.1242/jeb.123018 (2015).
- 410 16 Smale, D. A. & Wernberg, T. Ecological observations associated with an anomalous  
411 warming event at the Houtman Abrolhos Islands, Western Australia. *Coral Reefs* **31**,  
412 441-441 (2012).
- 413 17 Boyd, P. W. *et al.* Biological responses to environmental heterogeneity under future  
414 ocean conditions. *Global Change Biology* **22**, 2633-2650,  
415 doi:DOI:10.1111/gcb.13287 (2016).
- 416 18 Roleda, M. Y. *et al.* Effect of ocean acidification and pH fluctuations on the growth  
417 and development of coralline algal recruits, and an associated benthic algal  
418 assemblage. *PLoS ONE* **10**, e0140394, doi:DOI:10.1371/journal.pone.0140394  
419 (2015).
- 420 19 Rengefors, K., Kremp, A., Reusch, T. B. H. & Wood, M. Genetic diversity and  
421 evolution in eukaryotic phytoplankton: revelations from population genetic studies.  
422 *Journal of Plankton Research* (2017).
- 423 20 Rivest, E. B., Comeau, S. & Cornwall, C. E. The role of natural variability in shaping  
424 the response of coral reef organisms to climate change. *Current Climate Change*  
425 *Reports* **3**, 271-281 (2017).
- 426 21 Anthony, K. R. N., A. Kleypas, J. & Gattuso, J.-P. Coral reefs modify their seawater  
427 carbon chemistry – implications for impacts of ocean acidification. *Global Change*  
428 *Biology* **17**, 3655-3666, doi:10.1111/j.1365-2486.2011.02510.x (2011).
- 429 22 Kapsenberg, L. & Cyronak, T. Ocean acidification refugia in variable environments.  
430 *Global Change Biology* **0**, doi:10.1111/gcb.14730 (2019).
- 431 23 Torda, G. *et al.* Rapid adaptive responses to climate change in corals. *Nature Climate*  
432 *Change* **7**, 627, doi:10.1038/nclimate3374 (2017).
- 433 24 Donelson, J. M., Salinas, S., Munday, P. L. & Shama, L. N. S. Transgenerational  
434 plasticity and climate change experiments: Where do we go from here? *Global*  
435 *Change Biology* **24**, 13-34, doi:10.1111/gcb.13903 (2018).
- 436 25 Vargas, C. A. *et al.* Species-specific responses to ocean acidification should account  
437 for local adaptation and adaptive plasticity. *Nature Ecology & Evolution* **1**, 0084  
438 (2017).
- 439 26 van Oppen, M. J. H., Oliver, J. K., Putnam, H. M. & Gates, R. D. Building coral reef  
440 resilience through assisted evolution. *Proceedings of the National Academy of*  
441 *Sciences* **112**, 2307-2313 (2015).
- 442 27 Gruber, R. K., Lowe, R. J. & Falter, J. L. Metabolism of a tide-dominated reef  
443 platform subject to extreme diel temperature and oxygen variations. *Limnology and*  
444 *Oceanography* (2017).



- 445 28 Ross, C. L., DeCarlo, T. M. & McCulloch, M. T. Environmental and physiochemical  
446 controls on coral calcification along a latitudinal temperature gradient in Western  
447 Australia. *Global Change Biology*, doi:doi:10.1111/gcb.14488 (2018).
- 448 29 Mass, T. *et al.* Cloning and characterization of four novel coral acid-rich proteins that  
449 precipitate carbonates in vitro. *Current Biology* **23**, 1126-1131 (2013).
- 450 30 Cornwall, C. E., Diaz-Pulido, G. & Comeau, S. Impacts of Ocean Warming on  
451 Coralline Algal Calcification: Meta-Analysis, Knowledge Gaps, and Key  
452 Recommendations for Future Research. *Frontiers in Marine Science* **6**,  
453 doi:10.3389/fmars.2019.00186 (2019).
- 454 31 Hughes, T. P. *et al.* Global warming and recurrent mass bleaching of corals. *Nature*  
455 **543**, 373, doi:10.1038/nature21707 (2017).
- 456 32 Rösler, A., Perfectti, F., Peña, V. & Braga, J. C. Phylogenetic relationships of  
457 corallinaceae (Corallinales, Rhodophyta): taxonomic implications for reef-building  
458 corallines. *Journal of Phycology* **52**, 412-431, doi:10.1111/jpy.12404 (2016).
- 459 33 Comeau, S. *et al.* Pacific-wide contrast highlights resistance of reef calcifiers to ocean  
460 acidification. *Proceedings of the Royal Society B-Biological Sciences* **281**,  
461 doi:10.1098/rspb.2014.1339 (2014).
- 462 34 Gattuso, J. P., Gao, K., Lee, K., Rost, B. & Schulz, K. G. in *Guide to best practices*  
463 *for ocean acidification research and data reporting* (eds U. Riebesell, V. J. Fabry, L.  
464 Hansson, & J. P. Gattuso) Ch. 2, 41-51 (Publications Office of the European Union,  
465 2010).
- 466 35 Hurd, C. L., Hepburn, C. D., Currie, K. I., Raven, J. A. & Hunter, K. A. Testing  
467 methods of ocean acidification on algal metabolism: consideration for experimental  
468 designs. *Journal of Phycology* **45**, 1236-1251 (2009).
- 469 36 Cornwall, C. E. & Hurd, C. L. Experimental design in ocean acidification research:  
470 problems and solutions. *ICES Journal of Marine Science* **73**, 572-581,  
471 doi:10.1093/icesjms/fsv118 (2015).
- 472 37 Barry, J. P., Hall-Spencer, J. M. & Tyrrell, T. in *Guide to best practices for ocean*  
473 *acidification research and data reporting* (eds U. Riebesell, V. J. Fabry, L. Hansson,  
474 & J. P. Gattuso) (Publications Office of the European Union, 2010).
- 475 38 Schoepf, V. *et al.* Stress-resistant corals may not acclimatize to ocean warming but  
476 maintain heat tolerance under cooler temperatures. *Nature Communications* **10**, 4031,  
477 doi:10.1038/s41467-019-12065-0 (2019).
- 478 39 Townsend, R. A. & Huisman, J. M. in *Algae of Australia: Marine benthic algae of*  
479 *North-Western Australia* Vol. 2 (ed J.M. Huisman) (CSIRO Publishing, 2018).
- 480 40 Harvey, A., Woelkerling, W., Farr, T., Neill, K. & Nelson, W. Vol. 57 145 p  
481 (NIWA Information Series, 2005).
- 482 41 Miklasz, K. A. *Physical constraints on the size and shape on microalgae* PhD thesis,  
483 Stanford University, (2012).