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

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

Introduction:

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

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

In many shallow-water reef environments, night-time declines in pH due to local metabolic activity often exceed the changes in open-ocean pH predicted to occur by 2100 under business-as-usual CO_2 emissions scenarios (e.g. Representative Concentration Pathway 8.5, 0.4 pH units)[7,20,21]. It has been proposed that this natural diurnal variability may impart tolerance to low pH for resident calcifying species via selection for individuals that are better adapted to survive in these envrionments[20,22]. Additionally, transgenerational acclimation can occur via a variety of epigenetic or maternal effects after exposure of subsequent generations

62 to stressors[14,23], and could be promoted by prior exposure of individuals to greater variability

63 in that stressor[24].

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 (± 0.37) and

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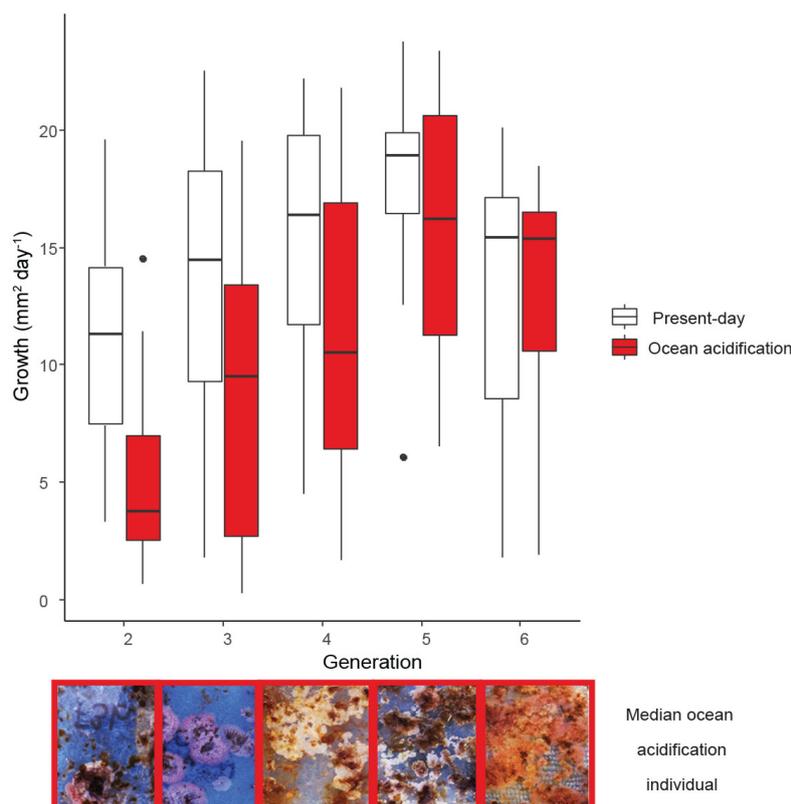
Figure 1: Growth of CCA populations after 2 to 6 generations of exposure to present-day (pH 8.00) and ocean acidification (pH 7.70) treatments. Also shown are the median individual coralline algal plates for each generation under the ocean acidification treatments (out of 24). Scale 50 by 45 mm of images.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

Reciprocal transplant experiment

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

site compared to the stable pH site in early generations over the course of the experiment

(Fig. S1, $F_{1,224} = 4.05$, $p = 0.045$). This is contrary to the hypothesis that individuals from

more variable environments are more tolerant to ocean acidification, or that they gain this

tolerance more rapidly over multiple generations. Trends in total recruit area typically

mirrored those of growth rates, but were inherently more variable (Fig. S2, S3, Table S3–4).
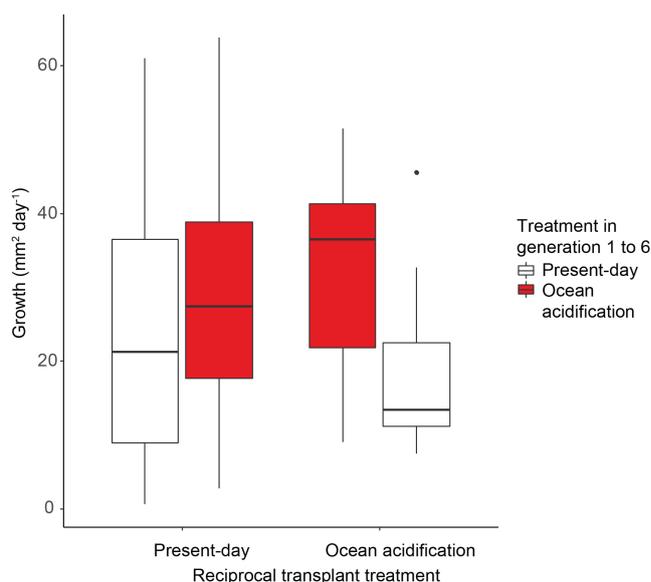
Figure 2: Growth of CCA after 7 generations grown in present-day (pH 8.00) and ocean

acidification (pH 7.70) treatments (controls), versus those transferred into these novel

treatments after being in the treatment with the other mean pH for 6 generations. Responses

to constant and variable pH regimes are combined. Median and 75% quartiles, $n = 12$ per

treatment and generation combination.

Implications for future nearshore reefs

This is the first demonstration that over multiple generations of exposure benthic

calcifying species can increase their tolerance to ocean acidification, indicating that at least

this species of CCA has the potential to gain tolerance to ocean acidification well within the

timeframe of when its effects will manifest. Although this finding is by-itself critical to more

accurately forecasting reef futures, outstanding questions remain, including the generality of

this finding amongst other coralline algal species and in combination with other stressors

such as ocean warming or in the presence of competitors that could be unaffected by ocean

acidification. However, if coralline alga can develop resistance to ocean acidification over

multiple generations of exposure, it is possible that other calcifying benthic species with

129 similar calcification mechanisms[9] 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[14]. 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 adaption. 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[24]. This is

155 surprising given that organisms from these habitats tend to respond more positively to ocean

156 acidification[22,25], and that phenotypic or transgenerational plasticity is predicted to be higher

157 on these populations[17,24]. 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[26], 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[8]. 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 $\delta^{11}B$ (pH$_{cf}$ proxy) or

167 Raman full width at half maximum (FWHM: proxy of the calcite saturation state in the

168 calcifying fluid [Ω_{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 $\delta^{11}B$ 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 pH$_{cf}$ or Ω_{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 ($\delta^{11}B$, 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[28], and

183 declining pH$_{cf}$ via ocean acidification [8]. Additionally, traits such as increasing organic matrix

184 proteins involved in calcification[29] could also contribute to trends observed here.
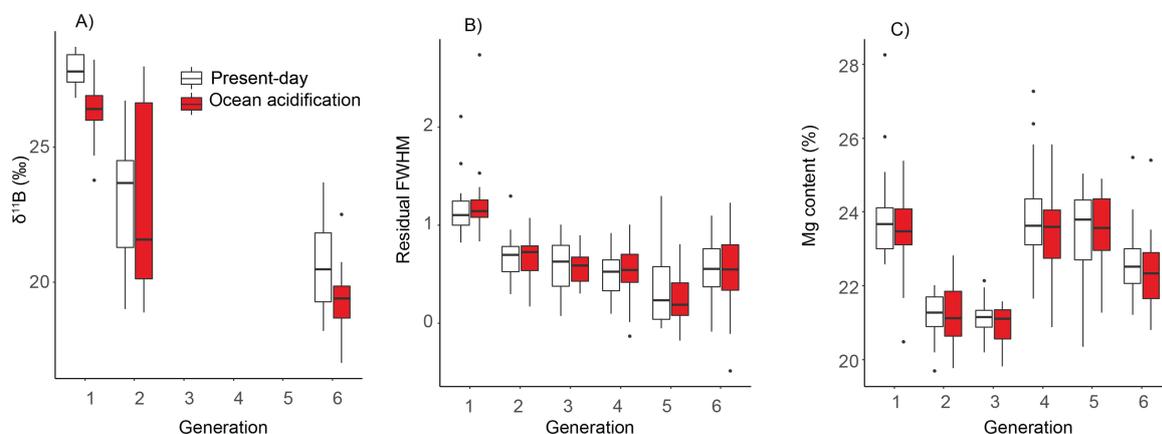
185

186   Figure 3: a) $\delta^{11}B$ (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[30], and the recent large-scale declines in the

199   abundance of the longer-lived tropical scleractinian corals [31].

200

201   **Methods**

202   **Collection and treatment design**

203   We collected adult coralline algae, *Hydrolithon reinboldii* (synonymous with *boergesenii* [32]),

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[33], 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.[7].

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.[7] 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[34-37], we used CO_2

226 gas to lower pH/elevate CO_2 concentrations, and CO_2-free air to increase pH/lower CO_2.

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 where 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[38] 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 2nd 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.[18]), 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[39]. Thousands of conceptacles were produced in each

273 generation, and all that we analyzed contained multiple spores indicating that they were

274 asexually reproducing[40]. Damaged conceptacles were not analyzed. In other words, the algae

275 were asexually reproducing which is most common in coralline algae[41], especially for this

276 species for which only these life history stages have been noted[39]. The probability of skipping

277 sexual reproduction in many species has previously been suggested[41]. 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

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[23]. 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

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. $\delta^{11}B$, 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[7-9], 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 ν_1 Full Width at Half Maximum; FWHM) and magnesium content (based on ν_1

325 wavenumber), following previously described protocols[7,9].

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**

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_2 world. *Journal of Experimental Marine*
376   *Biology and Ecology* **403**, 54-64 (2011).
377 5   Fabricius, K., Kluibenschedl, 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 dissoltuion 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 contrainsts on the size and shape on microalgae* PhD thesis,
483   Stanford University, (2012).

484