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## Rapid multi-generational acclimation of coralline algal reproductive structures to ocean acidification

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1 **Rapid multi-generational acclimation of coralline algal reproductive structures to ocean**  
2 **acidification**

3  
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28 Keywords: acclimation, conceptacles, coralline algae, multi-generational, ocean acidification, pH  
29 variability

30

### 31 **Abstract**

32

33 The future of coral reef ecosystems is under threat because vital reef-accreting species such as  
34 coralline algae are highly susceptible to ocean acidification. Although ocean acidification is known to  
35 reduce coralline algal growth rates, its direct effects on the development of coralline algal  
36 reproductive structures (conceptacles) is largely unknown. Furthermore, the long-term, multi-  
37 generational response of coralline algae to ocean acidification is extremely understudied. Here, we  
38 investigate how mean pH, pH variability and the pH regime experienced in their natural habitat affect  
39 coralline algal conceptacle abundance and size across six generations of exposure. We show that  
40 second generation coralline algae exposed to ocean acidification treatments had conceptacle  
41 abundances 60% lower than those kept in present day conditions, suggesting that conceptacle  
42 development is initially highly sensitive to ocean acidification. However, this negative effect of ocean  
43 acidification on conceptacle abundance disappears after three generations of exposure. Moreover, we  
44 show that this transgenerational acclimation of conceptacle development is not facilitated by a trade-  
45 off with reduced investment in growth, as higher conceptacle abundances are associated with crusts  
46 with faster growth rates. These results indicate that the potential reproductive output of coralline algae  
47 may be sustained under future ocean acidification.

48

### 49 **Introduction**

50 Ocean acidification is a threat to many marine organisms, with calcifying taxa and the ecosystems  
51 they support particularly at risk [1,2]. As a hotspot for marine biodiversity [3,4], and an annual source  
52 of billions of dollars to the global economy [5] coral reefs are one of the most valuable ecosystems  
53 threatened by ocean acidification. These ecosystems are vulnerable because framework-building  
54 corals and the coralline algae that cement this framework together may both be impaired by changes  
55 in seawater carbonate chemistry [1,2]. Furthermore, coralline algae are predicted to be highly

56 susceptible to these altered conditions, as they precipitate one of the most soluble calcium carbonate  
57 polymorphs, high magnesium calcite [6,7]. Coralline algae are important to the functioning of coral  
58 reefs as they maintain the structural integrity of reefs by cementing them together, whilst also  
59 providing a number of ecosystem services such as facilitating coral larval settlement [8,9] and  
60 providing food for invertebrates [10]. Therefore, if coralline algal survival, growth or reproductive  
61 output is reduced by future ocean acidification these services that are vital to the persistence of coral  
62 reefs could be lost. Previous research has demonstrated reduced calcification and growth in mature  
63 coralline algae under ocean acidification [11–13], as well as significant reductions in coralline algae at  
64 natural analogues of future ocean acidification [14,15]. Moreover, coralline algal recruitment and  
65 calcification during early life-stages appear to be highly susceptible to reduced seawater pH lower  
66 than 7.91 as demonstrated through decreasing calcification rates and ~90% reduced recruitment rate  
67 (or success) [16–18]. If such large reductions in recruitment are realized under ocean acidification,  
68 coralline algal populations will inevitably experience significant declines, with severe negative  
69 consequences for coral reefs [19].

70

71 When assessing the impacts of environmental change on marine organisms it is important to consider  
72 time scales that match the timespan organisms will have to respond to external changes, as previous  
73 studies have demonstrated differing responses under long and short term exposure [20,21]. Such  
74 considerations are also required as a host of transgenerational processes can act over multiple  
75 generations to improve fitness. For example, tropical reef fish are initially sensitive to ocean warming  
76 but can rapidly acclimate over two generations [22], whilst Rock Oyster larvae exposed to elevated  
77 pCO<sub>2</sub> grew larger and developed faster when their parents also experienced elevated pCO<sub>2</sub> [23]. When  
78 discussing generational improvements in performance it is important to define the use of terms such  
79 as acclimation or adaptation. Here, we use the term acclimation to refer to changes in performance  
80 driven by non-genetic plasticity in responses [22,24,25], and the term adaptation to refer to changes in  
81 performance driven by genetic change [26,27]. A recent study by Cornwall *et al.*, [18] was the first to  
82 assess the response of a reef-accreting calcifier to ocean acidification across multiple (>two)  
83 generations. They report that although coralline algae are initially highly sensitive to ocean

84 acidification, growth was no longer affected by ocean acidification after six generations of exposure.  
85 Yet, this novel result should be interpreted with caution, as increases in growth after multiple  
86 generations under ocean acidification may be at the expense of other processes such as reproduction.  
87 Similar trade-offs have been observed in coralline algae, where maintaining calcification rates under  
88 ocean acidification was coupled with harmful changes in skeletal ultrastructure [28,29], although such  
89 trade-offs are not always observed [30]. Therefore, whilst measuring growth is valuable, a multi-  
90 generational assessment of reproductive structures in response to ocean acidification will provide  
91 further insights into how coralline algae respond to ocean acidification.

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93 The pH variability within any one habitat can dictate the sensitivity of resident calcifying species to  
94 ocean acidification. Exposure to variable pH regimes is common in shallow-water reef ecosystems  
95 where local metabolic activity and long water residence times combine to cause large diurnal  
96 oscillations in seawater chemistry [31,32]. Such variable pH regimes may mitigate the effects of  
97 ocean acidification on coralline algae by facilitating enhanced calcification rates during the day  
98 [13,33], by selecting for individuals with low night pH tolerance due to phenotypic plasticity [34,35]  
99 or natural transgenerational acclimation [25,36]. However, such hypothesised benefits are not always  
100 apparent, with roughly half of past studies indicating coralline algae respond negatively to greater pH  
101 variability [37], and that pre-exposure to low pH due to pH variability does not facilitate acclimation  
102 to ocean acidification [18,38–40]. Contrasting results such as these suggest the effect of pH variability  
103 on coralline algal responses to ocean acidification may be species or population specific. To-date, the  
104 impacts of pH variability on the conceptacles of coralline algae from sites with vastly different pH  
105 regimes has not been assessed

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107 To understand the vulnerability of coralline algal recruitment and early life stages to ocean  
108 acidification, it would be ideal to isolate and investigate the various stages of reproduction and early  
109 life stage development. Coralline algae theoretically have a triphasic life cycle that allows  
110 reproduction via both sexual and asexual pathways. In coralline algae, like in many rhodophytes, the

111 asexual phase may be the only one ever observed [41] and occurs through tetraspore-to-tetraspore-  
112 cycling, in which tetrasporophytes produce self-perpetuating diploid spores [42]. Additionally,  
113 tetrasporophytes have been observed to produce diploid bispores, which can also grow directly into  
114 tetrasporophytes [42]. In coralline algae, many reproductive structures, including the spore producing  
115 sporangia, are housed within reproductive conceptacles found on the surface of intergenicula or crusts  
116 [42]. Thus, the development and abundance of these conceptacles (Figure 1) have a key role in  
117 sustaining coralline algal populations and can be used as a proxy for reproductive output. However, it  
118 is important to note that measurements of conceptacle abundance do not exactly equal fecundity.  
119 Despite their importance, there has been limited research into the direct effects of ocean acidification  
120 on coralline algal conceptacles, with the single study conducted finding no impact of reduced pH  
121 (7.81, 7.60) on *Pneophyllum* sp. conceptacle numbers [43].

122

123 Here, we investigate how reduced mean pH, differing levels of pH variability and the pH regime  
124 experienced by coralline algae in their natural habitat impact the abundance and size of conceptacles  
125 of the reef building coralline algae *Hydrolithon reinboldii*, across six generations of exposure. We  
126 hypothesize that, (1) coralline algal conceptacle abundance and size will decline under ocean  
127 acidification, (2) prior exposure to low pH will not influence *H. reinboldii*'s tolerance to ocean  
128 acidification, (3) diurnal pH variability will not reduce the effect of ocean acidification, and (4) any  
129 impact of ocean acidification on *H. reinboldii* conceptacles will be reduced after multiple generations  
130 of exposure.

131

## 132 **Materials and Methods**

133

### 134 **Treatment Design and Experimental Set-up**

135 Coralline algal rhodoliths of the species *Hydrolithon reinboldii* were collected from two sites with  
136 contrasting pH regimes in the macrotidal Kimberley region of Australia in October 2016 (see figure  
137 S1 for a map of these sites and figure S2 for information on the pH regimes). See supplementary

138 methods for site description and collection information. To investigate the effects of mean pH and  
139 different levels of pH variability on *H. reinboldii* four pH treatments were employed within aquaria.  
140 Two treatments had a mean  $\text{pH}_T$  of 8.00, selected to represent the present day mean surface seawater  
141  $\text{pH}_T$  detailed in ocean acidification studies best practices guide [44], and two treatments had a mean  
142  $\text{pH}_T$  of 7.70, designed to simulate potential future pH under ocean acidification (Representative  
143 Concentration Pathway 8.5; 37). For the two treatments with the same mean pH, one was  
144 characterised by high pH variability and the other low pH variability, designed to emulate the pH  
145 regimes of Tallon and Shell Island respectively. Thus, the four experimental pH treatments were  
146 denoted: (1) present-day low variability (8.00 mean  $\text{pH}_T$ , mean daily range of 0.14), (2) present-day  
147 high variability (8.00 mean  $\text{pH}_T$ , mean daily range of 0.58), (3) ocean acidification low variability  
148 (7.70 mean  $\text{pH}_T$ , mean daily range of 0.20) and (4) ocean acidification high variability (7.70 mean  
149  $\text{pH}_T$ , mean daily range of 0.89). Figure S3 highlights the pH regime of all four treatments and Table  
150 S1 displays the achieved pH for each of the four pH treatments used. Coralline algae collected from  
151 the two sites were divided amongst the four treatments in 48 experimental tanks, resulting in six  
152 independent experimental tanks for each of the eight pH treatment  $\times$  site of origin combinations. See  
153 supplementary methods for full details of experimental set-up and design.

154

### 155 **Multi-generational Experiment**

156 In this study only the conceptacles of coralline algae that recruited during the experiment were  
157 assessed, therefore the generation one mature rhodoliths collected from the wild (F0), were used  
158 solely as source populations. The juvenile coralline algae assessed in this study are the same as those  
159 studied in Cornwall *et al.*, [18]. Whilst in the experimental tanks the generation one wild coralline  
160 algae released spores which went on to form recruits on the inside walls of the tanks. After 78 days  
161 these generation two coralline algal recruits were visible on the walls of the experimental tank.  
162 Mature wild individuals were removed from the tanks on day 128 and two 4.5 cm  $\times$  5.0 cm (length  $\times$   
163 width) sections were cut out of each tank. These two sections of each tank were primarily cut from the  
164 area near the water outflow where possible, as this area contained the highest cover of coralline algal

165 recruits. These plates were then placed into completely new tanks (in the same treatment as the  
166 previous generation), allowing for assessment of this second generation. Within these new  
167 experimental tanks generation two algae formed conceptacles and released spores, which again settled  
168 onto the walls of the tank. Two 4.5 cm × 5.0 cm sections containing generation 3 recruits were cut  
169 from the experimental tank and placed into a new experimental tank (See figure S4 for schematic  
170 representation of this experimental approach). This process was repeated every 41–51 days, allowing  
171 five generations (generation numbers 2–6) of plate-based coralline algal conceptacles to be studied.  
172 Coralline algal recruits across all generations possessed uniporate conceptacles, containing multiple  
173 tetrasporangial spores which are characteristic of *H. reinboldii* [41], and suggests each new generation  
174 was a result of asexual reproduction [46]. Contamination by foreign spores from local environments  
175 was unlikely due to the consistent times to visible recruitment across generations, the absence of  
176 coralline algae recruiting in the header tanks, and the observation that all recruits had similar  
177 anatomical features [41]. To investigate whether any observed differences across generation were due  
178 to an altered response to treatments or an interaction between altered response to treatments and  
179 laboratory effects [47], a reciprocal transplant experiment was conducted. For full details of this  
180 reciprocal transplant experiment see supplementary methods.

181

## 182 **Conceptacle Measurements**

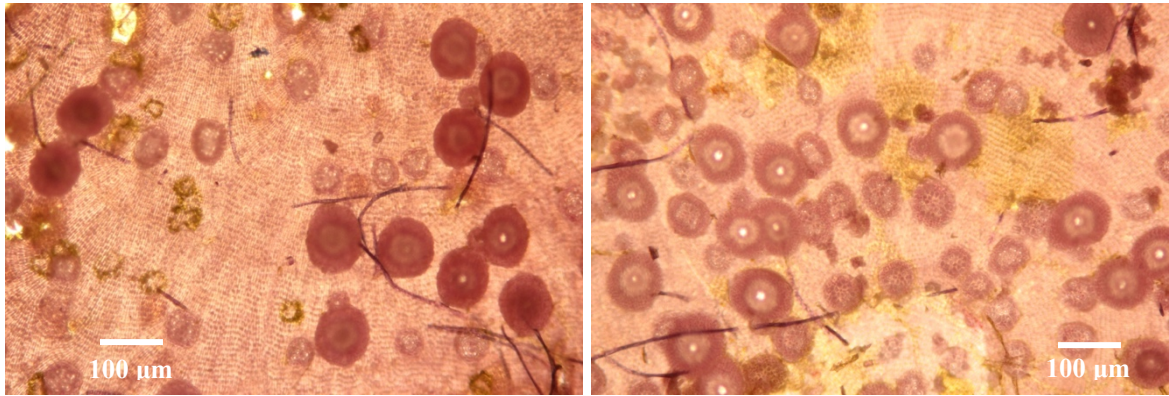
183 At the end of each generation, the coralline algae plates were removed from the tanks and stored in  
184 silica gel for up to three years, before three random locations on each plate were blindly selected and  
185 photographed using a compound microscope for analysis. For each plate conceptacle abundance was  
186 measured by counting all the visible conceptacles in the area of analysis. Individual conceptacle  
187 diameters were measured as the distance between the two most distant points of each conceptacle. For  
188 full details of microscopic conceptacle imaging and analysis see supplementary methods.

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**Figure 1.** Images of the *Hydrolithon reinboldii* conceptacles quantified in this study. Shown are examples of the microscopic images used to quantify conceptacle abundance and diameter.

**Growth and Recruitment Measurements**

Multi-generational growth and total recruit area of the same coralline algae populations were investigated in relation to the conceptacle abundances measured in this study. Full methods and data are reported in Cornwall *et al.*, [18], but see supplementary methods for details of these measurements and their comparison with conceptacle abundances.

**Statistical Analysis**

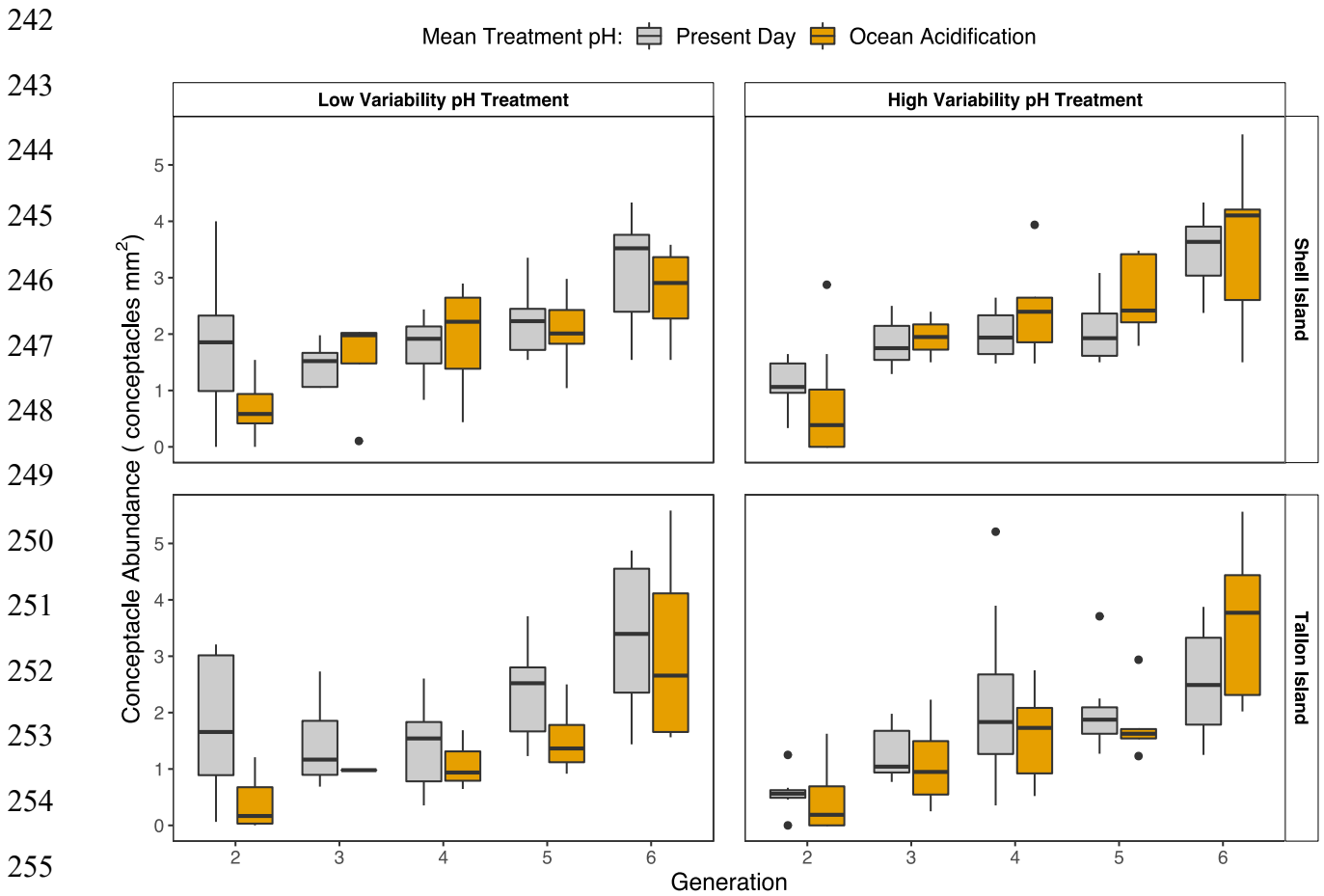
Conceptacle abundance per plate and mean conceptacle diameter per plate was analysed using generalised linear mixed models with generation, mean treatment pH, pH variability and site of origin as fixed factors and header tank and water bath as random effects. As data was not normally distributed a Poisson distribution was specified for conceptacle abundances and a Gamma distribution was specified for conceptacle diameter measurements. For the reciprocal transplant experiment, both conceptacle abundance and diameter were assessed using the same model structure, except that generation and mean treatment pH factors were replaced with the factors; generation 2–6 mean treatment pH and transplant treatment mean pH. Following the reciprocal transplant experiment, Wilcoxon rank sum tests were used to test for pairwise differences in conceptacle abundances between the different generation 2–6 and transplant treatment combinations.

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To allow for comparison with growth and recruitment data, mean conceptacle abundances per plate were averaged for each experiment tank (total of 48 per generation). The effect of conceptacle abundance on the total recruit area of the following generation, as well as the influence of treatment mean pH was assessed using generalised linear mixed models with conceptacle abundance and mean treatment pH as fixed factors. A quasi-Poisson model was used to account for non-normality of data and overdispersion greater than 1. The same model was used to investigate whether changes in growth rates impacted the abundance of conceptacles, however in this model growth and mean pH were specified as fixed factors. Assumptions of all models were checked via visual inspection of standard model diagnostic plots. All analyses were performed in R version 3.6.1.

240 **Results**

241 **(a) Conceptacle Abundance**



256 **Figure 2.** *H. reinboldii* conceptacle abundance after exposure to present-day (mean pH 8.00) and ocean  
257 acidification (mean pH 7.70) treatments for between two and six generations. Figures are split by site of  
258 origin (Shell or Tallon Island) and variability of pH treatment (low or high). Median, 25% and 75%  
259 quartiles are presented.

260

261 The abundance of conceptacles varied with generation ( $F = 206.882, p < 0.001$ ), treatment mean pH  
262 ( $F = 8.241, p = 0.004$ ) and coralline algae site of origin ( $F = 5.354, p = 0.022$ ) (Figure 2). No  
263 interactive effects between site of origin, mean treatment pH and level of pH variability were  
264 observed, therefore abundance and size data from different populations and pH variability treatments  
265 were grouped for subsequent analyses of mean treatment pH and generational effects (Figure S5).  
266 Coralline algae exposed to ocean acidification treatments for two generations had a mean conceptacle

267 abundance that was 60% lower than coralline algae kept in present-day treatments (Figure S5) (Mean  
 268 conceptacle abundances, standard error and n for each generation by treatment combination are  
 269 detailed in Table S2). However, after three generations of exposure the reduction in mean conceptacle  
 270 abundances under ocean acidification declined to 7% (Figure S5). This differing effect of treatment  
 271 mean pH across generations is supported by a significant interaction between generation and mean pH  
 272 ( $F = 8.204, p = 0.005$ ).

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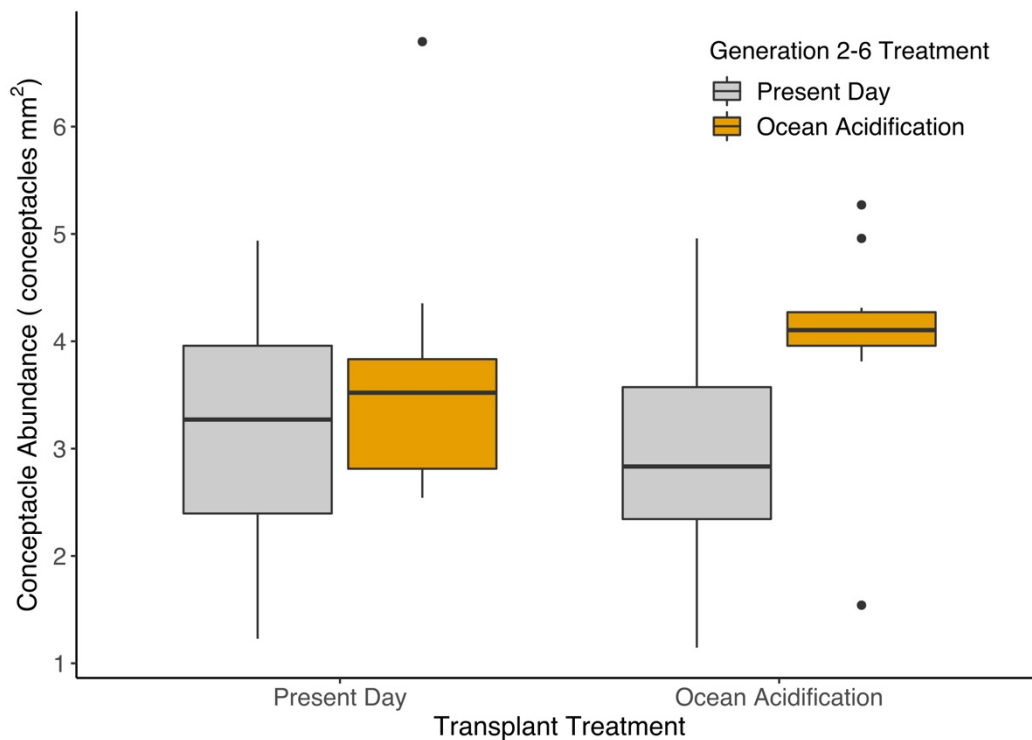
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286 **Figure 3.** Results from reciprocal transplant experiment. *H. reinboldii* conceptacle abundance  
 287 after seven generations of exposure to present-day and ocean acidification treatments (same  
 288 generation 2–6 treatment and transplant treatment), and those transplanted to novel treatments  
 289 in generation seven (different generation 2–6 and transplant treatment). Responses to low and  
 290 high variability pH treatments are combined. Median, 25% and 75% quartiles are displayed.

291

292 During the reciprocal transplant experiment, coralline algae exposed to ocean acidification treatments  
 293 throughout generations 2–6 had higher conceptacle abundances than those in present-day treatments  
 294 in generations 2–6 ( $F = 7.797, p = 0.012$ ) (Figure 3). However, transplant treatment ( $F = 0.059, p =$

295 0.810) and the interaction between generation 2-6 treatment and transplant treatment ( $F = 0.303$ ,  $p =$   
296  $0.589$ ) did not affect conceptacle abundance. Populations exposed to ocean acidification treatments  
297 for seven generations had a mean conceptacle abundance of  $4.04$  conceptacles  $\text{mm}^2$ , whereas those in  
298 present day treatments throughout generations 2–6, had a mean conceptacle abundance of  $3.06$   
299 conceptacles  $\text{mm}^2$  after being transplanted to ocean acidification treatments in generation seven. This  
300 represents a 25% decline in conceptacle abundance in populations transplanted to ocean acidification  
301 treatments compared with those in ocean acidification treatments for seven generations. This  
302 difference is demonstrated by a significant pairwise difference between conceptacle abundances in  
303 these two reciprocal transplant groups ( $W=38.5$ ,  $p = 0.024$ ). Mean conceptacle abundance, standard  
304 error and  $n$  for each generation 2–6 treatment by transplant treatment combination is detailed in Table  
305 S3.

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### 323 **Conceptacle Abundance vs Growth and Recruitment**

324 In each generation an increase in mean coralline algal growth per tank has a positive effect on the  
325 mean conceptacle abundance of the same coralline algae (Figure S6). Increasing conceptacle  
326 abundance with increasing growth rates are evidence of this ( $F = 110.937$   $p < 0.001$ ). The relationship  
327 between growth and conceptacle abundance is not affected by mean treatment pH ( $F = 0.006$ ,  $p =$   
328  $0.937$ ) or generation ( $F = 1.958$ ,  $p = 0.163$ ). As the conceptacle abundance of the plate-based coralline  
329 algae increases, the total recruit area (of the next generation) derived from that population also  
330 increases ( $F = 39.671$ ,  $p < 0.001$ ) (Figure S7). Mean treatment pH ( $F = 18.848$ ,  $p < 0.001$ ) and  
331 generation ( $F = 7.610$ ,  $p = 0.006$ ) have a significant effect on total recruit area ( $F = 18.848$ ,  $p <$   
332  $0.001$ ). The relationship between conceptacle abundance and next generation recruitment is not  
333 affected by mean treatment pH ( $F = 0.006$ ,  $p = 0.937$ ) yet it is affected by generation ( $F = 5.100$ ,  $p =$   
334  $0.0250$ ).

335

### 336 **(b) Conceptacle Size**

337 Conceptacle diameter increased with generation ( $F = 14.099$ ,  $p < 0.001$ ,  $df = 1$ ) (Figure S8), however  
338 diameter did not vary with mean treatment pH, site of origin or level of pH variability (Table S7,  
339 Figure S9). The reciprocal transplant experiment showed no effect of generation 2–6 treatment,  
340 transplant treatment or any interaction between the two on diameter (Figure S10).

341

### 342 **Discussion**

343 Here, we demonstrate that coralline algal conceptacle abundance is initially impacted by ocean  
344 acidification, but conceptacle size is not. This result indicates that the initial formation of  
345 conceptacles, rather than their later development is impaired by altered carbonate chemistry.  
346 However, as the first study to assess the impact of ocean acidification on conceptacles across multiple  
347 generations, this study also provides evidence that coralline algae may be more robust to the effects of  
348 ocean acidification than widely predicted, as from the third generation onwards there is no impact of  
349 ocean acidification on conceptacles (Figure 2). Such an observation indicates that coralline algal  
350 conceptacles will continue to develop in a more acidic ocean. This is important as conceptacles are

351 vital reproductive structures, without which the production of spores and subsequent production of  
352 next-generation recruits would not be possible. Thus, if conceptacles were impacted by future ocean  
353 acidification it would have severe consequences for the persistence and proliferation of coralline algal  
354 populations. This ability to maintain conceptacle abundances and size under ocean acidification will  
355 also be beneficial to other coral reefs organisms that are threatened by environmental change [1,2].  
356 For example, continuing spore production and the formation of new coralline algal crusts will provide  
357 substrate for future coral larval settlement [8,9] and will also aid in binding calcium carbonate reef  
358 structures together, as they are increasingly weakened by ocean acidification [7,28]. Therefore,  
359 through such downstream effects the maintenance of coralline algal conceptacles under ocean  
360 acidification could be vital to the future persistence of coral reefs.

361

362 We consider it likely that the reduced effect of ocean acidification across generations observed in this  
363 study is due to non-genetic acclimation, as coralline algae across all generations possessed features  
364 that are indicative of asexual reproduction [46]. However, we cannot definitively state that the  
365 changes observed here were not driven by selective genetic adaptation across generations. The  
366 transgenerational acclimation of coralline algal conceptacle abundances to ocean acidification  
367 observed here follows the trend observed for growth rates [18]. Moreover, the positive relationship  
368 between conceptacle abundances and growth rates (Figure S6) across all generations and treatments  
369 demonstrates that trade-offs between these two processes are not underlying the multi-generational  
370 acclimation of conceptacle abundance and growth to ocean acidification. For example, if such a trade-  
371 off was driving this acclimation you would expect to see a negative relationship between growth and  
372 conceptacle abundance within the later “acclimated” generations. This observation that multi-  
373 generational acclimation is observed for conceptacle abundance and growth [18] further indicates that  
374 coralline algae are capable of acclimating to ocean acidification. However, despite the overall  
375 similarity in responses, acclimation of growth rates to ocean acidification were stepwise/linear in  
376 nature (reported in Cornwall *et al.*, [18]), and therefore not as rapid as the acclimation of  
377 conceptacles. Whilst the acclimation of conceptacle formation observed here is deemed rapid  
378 compared with growth, similar rapid transgenerational acclimation has been observed in differing

379 marine taxa. For example, the reproductive output of marine invertebrates such as polychaetes [48]  
380 and copepods [49] is initially impaired under ocean acidification, however this effect disappears by  
381 the third generation, as the reproductive output of populations exposed to ocean acidification does not  
382 differ from controls. Additionally, transgenerational acclimation of growth rates to ocean acidification  
383 has been observed within three generations of exposure in calcifying organisms such as oysters [50]  
384 and clams [24], suggesting various biological processes are capable of rapid acclimation to ocean  
385 acidification. However, our results and those of Cornwall *et al.*, [18] suggest that different processes  
386 may exhibit contrasting acclimation times.

387

388 The varying rates of acclimation of different biological processes in coralline algae are likely driven  
389 by the physiological mechanisms underpinning these processes. Here, we propose two mechanisms  
390 that may have driven the faster multi-generational acclimation of conceptacle development, compared  
391 with growth [18]. Firstly, linear extension of coralline algae is dictated by both growth of organic  
392 tissue and calcification of the cell walls. The latter is impacted by factors such as the control of  
393 calcification site chemistry and the production of organic matrices, both of which may be disrupted by  
394 ocean acidification [51,52]. Thus, the complexity of calcification and its dependence on specific  
395 chemical conditions may represent a physiochemical bottleneck to the rate at which calcification, and  
396 therefore growth can acclimatise to ocean acidification. In contrast, the primarily uncalcified [53,54],  
397 conceptacles are not subject to the same chemical constraints, potentially facilitating faster  
398 acclimation. An alternate and not mutually exclusive explanation is that coralline algae prioritise  
399 energetic investment in reproduction over calcification. Energetic trade-offs between reproduction and  
400 other processes such as growth commonly occur in nature [55,56], and are enhanced due to increased  
401 maintenance costs under stressful conditions [57]. Therefore, rapid acclimation of reproductive  
402 conceptacles may come from prioritizing energetic investment in reproduction, as ensuring the  
403 production of offspring under stress is of the greatest benefit to Darwinian fitness. Similar multi-  
404 generational trends in resource allocation towards reproduction under ocean acidification have also  
405 been observed in copepods [58]. Here, our novel comparison of acclimatory responses (growth rates



406 vs conceptacle development), indicates that the persistence of populations under ocean acidification  
407 will be constrained by the biological process that undergoes the slowest rate of acclimation.  
408

409 The reduction in second generation conceptacle abundances under ocean acidification mirror the  
410 widely reported negative impacts of ocean acidification on the calcification, growth and recruitment  
411 of first and second generation coralline algae [11,16,59]. This initial reduction in conceptacle  
412 abundance observed here, highlights reduced conceptacle formation as one potential mechanism  
413 driving these previously reported declines in coralline algae recruitment under ocean acidification  
414 [16,17]. However, in contrast to the initial negative effect observed here, Ordoñez *et al.*, [43] found  
415 that conceptacle numbers in *Pneophyllum* sp. coralline algae were not reduced when exposed to  
416 medium (pH 7.81) or low (pH 7.60) pH treatments. Such a result is unexpected, as these populations  
417 were subject to neither transgenerational nor developmental exposure to low pH conditions, as they  
418 recruited into experimental conditions from the wild. However, species-specific responses to ocean  
419 acidification have been documented in coralline algae [37,60], therefore this opposing result may be  
420 due to differences between the thin crusted *Pneophyllum* sp. studied by Ordoñez *et al.*, [43], and the  
421 thick-crusted *H. reinboldii* assessed here. Evidence of such species effects are displayed in Ordoñez *et*  
422 *al.*, [43] as they observed a significant decline in the relative density of thick crusted *Porolithon*  
423 *onkodes* under high pCO<sub>2</sub>, but not *Pneophyllum* sp., suggesting that morphology can influence  
424 response to ocean acidification. Alternatively, these populations may have had natural resilience to  
425 this magnitude of ocean acidification, and further research is required to elucidate which factors could  
426 be driving such differences.

427

428 The rapid acclimation of reproductive conceptacles observed here is not translated to rapid increases  
429 in final recruit area of the next generation, as recruit area acclimation was slower and stepwise [18].  
430 Therefore, although the trend of increasing recruit area with increasing conceptacle abundance (Figure  
431 S7) suggests that higher conceptacle abundances may aid in buffering the impacts of ocean  
432 acidification on recruitment, it is likely that final recruitment will be determined by the cumulative  
433 impact of ocean acidification on reproductive structure formation, spore settlement, and early

434 development processes. For example, coralline algae display delayed and weakened spore attachment  
435 [61], as well as reduced spore germination [62] in response to ocean acidification, potentially  
436 highlighting the later sporulate stage as a bottleneck in final recruitment.

437

438 Our results also demonstrate that pH variability does not modify the impact of ocean acidification on  
439 coralline algal conceptacles. This is shown as coralline algae grown in both high variability and low  
440 variability pH treatments were not differently affected by the mean pH corresponding to ocean  
441 acidification. This lack of effect of pH variability is at odds with some past literature, where pH  
442 variability has been shown to reduce the impact of ocean acidification [33,38], while it can also  
443 enhance the negative impacts of ocean acidification [37]. These contrasting and inconsistent effects of  
444 pH variability demonstrate that its interaction with future ocean acidification will be complex and can  
445 potentially elicit a full range of effects from negative to positive, with the lack of effect observed here  
446 in between the gamut of responses. As previous studies have highlighted that the influence of pH  
447 variability varies amongst species [20,63], it appears that the effect of ocean acidification on  
448 *H.reinboldii* is not dictated by pH variability as the calcification and photosynthesis of mature  
449 rhodoliths [40], the growth rates and recruitment of juveniles [18] and the size and abundance of  
450 juvenile conceptacles are all unaffected.

451

452 The effect of ocean acidification on coralline algal conceptacles is not influenced by the pH variability  
453 experienced by the population in their natural environment here. This was hypothesized *a priori*, as  
454 similar responses were observed with adult calcification rates [40] and juvenile growth rate [18].  
455 Nevertheless, this result contrasts with the often-posed hypothesis in the literature that environments  
456 with naturally high variations in an abiotic factor, will increase the resistance of resident organisms to  
457 future mean changes in that abiotic factor [37]. Though this trend has recently been observed in corals  
458 from habitats with greater thermal variability [64,65], mean conditions may be as important or more  
459 important than variability [66]. For example, mean pH is higher at Tallon Island, while pH variability  
460 is greater than Shell Island. Therefore, both could be acting against each other in conditioning  
461 coralline algal responses to low pH. Our results suggest a similar priming effect of mean pH, as three

462 generations of exposure to reduced mean pH was enough to eliminate the effects of reduced mean pH  
463 on the conceptacles of later generations.

464

465 Overall, this study demonstrates that the abundance of coralline algal conceptacles is greatly reduced  
466 in second generation coralline algae reared under simulated ocean acidification conditions. However,  
467 in just three generations of exposure to these same ocean acidification conditions the coralline algal  
468 reproductive structures can rapidly acclimate, as we observe no effects of ocean acidification beyond  
469 the second generation. This acclimation is much faster than that previously reported for growth rates,  
470 as growth took six generations to acclimate. Despite differing rates of acclimation, the acclimation of  
471 conceptacle development observed here is not facilitated by reduced growth, as both processes  
472 acclimate together across all generations. Therefore, this study further supports the possibility that  
473 coralline algae could acclimate to the effects of ocean acidification. As multiple studies have reported  
474 the ability of marine organisms to acclimate to environmental change, our observation that  
475 acclimation rates vary for different biological processes introduces a new consideration for studies  
476 assessing how marine organisms will respond to future environmental change.

477

#### 478 **Ethics**

479 All local regulations and permit requirements were followed during this study.

480

#### 481 **Data accessibility**

482 Data is accessible in the Dryad Digital Repository:

483 [https://datadryad.org/stash/share/BVDT9xgRvmfMvs-ruppJEYdEBSB81PoKG8c\\_1UmetTQ](https://datadryad.org/stash/share/BVDT9xgRvmfMvs-ruppJEYdEBSB81PoKG8c_1UmetTQ)

484

#### 485 **Authors Contributions**

486 B.M. wrote the paper. C.E.C and S.C. conceived the research and designed the experiment. C.E.C.,  
487 S.C. and B.M. conducted fieldwork. B.M., C.E.C., S.C., F.P. and E.L. ran the experiment. B.M., A.C.,  
488 M.B. and A.P. conducted photograph analysis. M.T.M. provided essential laboratory equipment,

489 facilities and analyses. B.M. conducted the statistical analyses. All authors edited the manuscript, or  
490 provided intellectual input, and agreed to its submission.

491

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498

#### 499 **Conflict of Interest**

500 The authors declare no conflicts of interest.

501

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