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

1 **<u>Rapid multi-generational acclimation of coralline algal reproductive structures to ocean</u>**

2 **<u>acidification</u>**

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31   **Abstract**

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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

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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].

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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_2 grew larger and developed faster when their parents also experienced elevated pCO_2 [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.

92

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

106

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 pH_T of 8.00, selected to represent the present day mean surface seawater

141 pH_T detailed in ocean acidification studies best practices guide [44], and two treatments had a mean

142 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 pH_T, mean daily range of 0.14), (2) present-day

147 high variability (8.00 mean pH_T, mean daily range of 0.58), (3) ocean acidification low variability

148 (7.70 mean pH_T, mean daily range of 0.20) and (4) ocean acidification high variability (7.70 mean

149 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 × 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 × 5.0 cm (length ×

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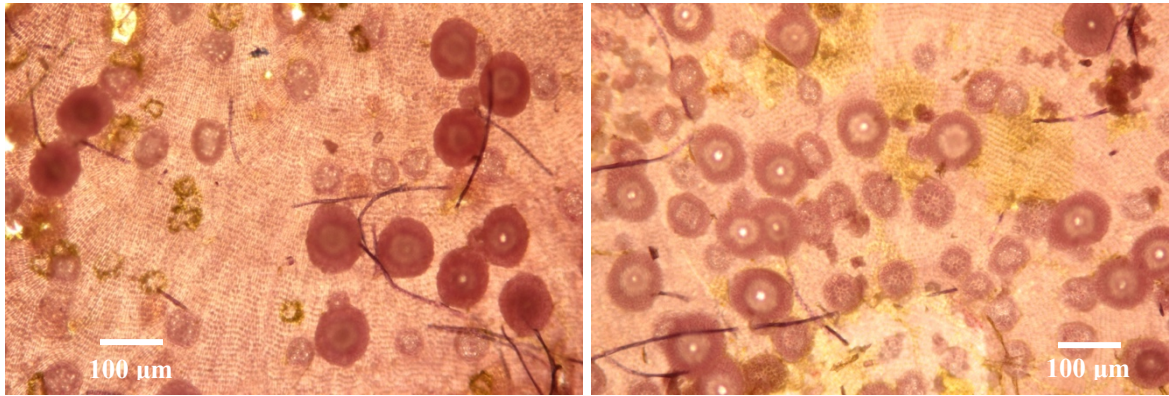
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Figure 1. Images of the *Hydrolithon reinboldii* conceptacles quantified in this study. Shown are examples of the microscopic images used to quantify conceptacle abundance and diameter.

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.

216

217 To allow for comparison with growth and recruitment data, mean conceptacle abundances per plate

218 were averaged for each experiment tank (total of 48 per generation). The effect of conceptacle

219 abundance on the total recruit area of the following generation, as well as the influence of treatment

220 mean pH was assessed using generalised linear mixed models with conceptacle abundance and mean

221 treatment pH as fixed factors. A quasi-Poisson model was used to account for non-normality of data

222 and overdispersion greater than 1. The same model was used to investigate whether changes in growth

223 rates impacted the abundance of conceptacles, however in this model growth and mean pH were

224 specified as fixed factors. Assumptions of all models were checked via visual inspection of standard

225 model diagnostic plots. All analyses were performed in R version 3.6.1.

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Results

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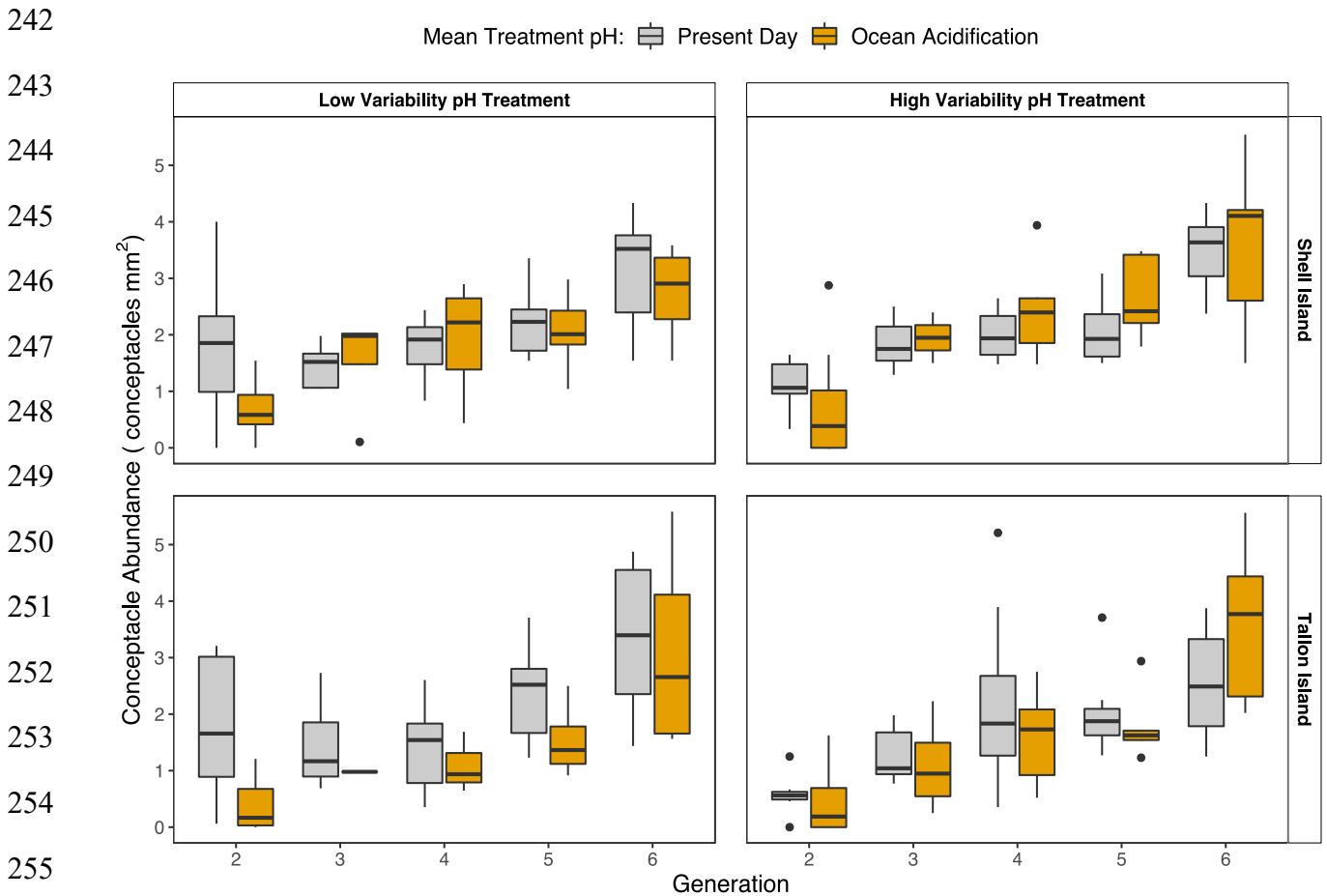
Figure 2. *H. reinboldii* conceptacle abundance after exposure to present-day (mean pH 8.00) and ocean acidification (mean pH 7.70) treatments for between two and six generations. Figures are split by site of origin (Shell or Tallon Island) and variability of pH treatment (low or high). Median, 25% and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

The abundance of conceptacles varied with generation (F = 206.882, p < 0.001), treatment mean pH (F = 8.241, p = 0.004) and coralline algae site of origin (F = 5.354, p = 0.022) (Figure 2). No interactive effects between site of origin, mean treatment pH and level of pH variability were observed, therefore abundance and size data from different populations and pH variability treatments were grouped for subsequent analyses of mean treatment pH and generational effects (Figure S5). 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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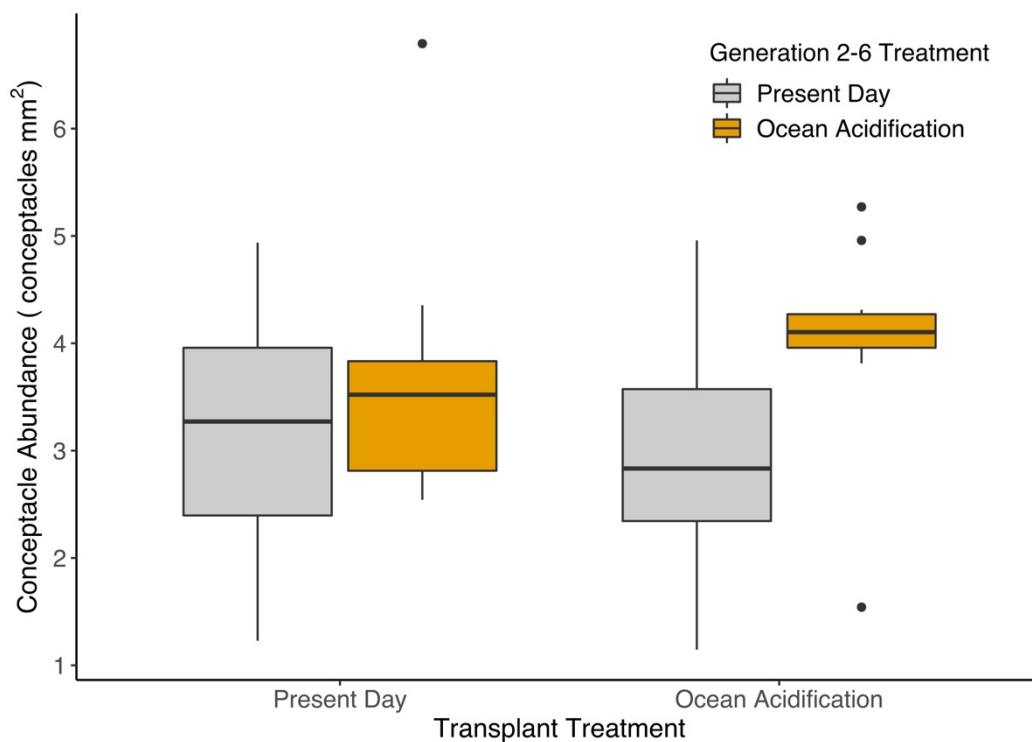
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286   **Figure 3.** Results from reciprocal transplant experiment. *H. reinboldii* conceptacle abundance

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 mm^2, whereas those in

298 present day treatments throughout generations 2–6, had a mean conceptacle abundance of 3.06

299 conceptacles 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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Conceptacle Abundance vs Growth and Recruitment

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

(b) Conceptacle Size

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

<u>Discussion</u>

Here, we demonstrate that coralline algal conceptacle abundance is initially impacted by ocean acidification, but conceptacle size is not. This result indicates that the initial formation of conceptacles, rather than their later development is impaired by altered carbonate chemistry. However, as the first study to assess the impact of ocean acidification on conceptacles across multiple generations, this study also provides evidence that coralline algae may be more robust to the effects of ocean acidification than widely predicted, as from the third generation onwards there is no impact of ocean acidification on conceptacles (Figure 2). Such an observation indicates that coralline algal 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_2, 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

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

498

499 **Conflict of Interest**

500 The authors declare no conflicts of interest.

501

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