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Understanding coralline algal responses to ocean acidification: Metaanalysis and synthesis

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9 Understanding coralline algal responses to ocean acidification: meta-analysis and synthesis

10 Running title: Coralline algae and ocean acidification

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27

28

29 **Abstract:**

30 Ocean acidification (OA) is a major threat to the persistence of biogenic reefs throughout the
31 world's oceans. Coralline algae are comprised of high magnesium calcite, and have long been
32 considered one of the most susceptible taxa to the negative impacts of OA. We summarise
33 these impacts and explore causes of variability in coralline algal responses using a
34 review/qualitative assessment of all relevant literature, meta-analysis, quantitative assessment
35 of critical responses, and a discussion of physiological mechanisms and directions for future
36 research. We find that most coralline algae experienced reduced abundance, calcification
37 rates, recruitment rates, and declines in pH within the site of calcification in laboratory
38 experiments simulating ocean acidification or at naturally elevated CO₂ sites. There were no
39 other consistent physiological responses of coralline algae to simulated OA (e.g. photo-
40 physiology, mineralogy and survival). Calcification/growth were the most frequently
41 measured parameters in coralline algal ocean acidification research, and our meta-analyses
42 revealed greater declines in seawater pH were associated with significant decreases in
43 calcification in adults and similar but non-significant trends for juveniles. Adults from the
44 family Mesophyllumaceae also tended to be more robust to OA, though there was insufficient
45 data to test similar trends for juveniles. OA was the dominant driver in the majority of
46 laboratory experiments where other local or global drivers were assessed. The interaction
47 between OA and any other single driver was often additive, though factors that changed pH at
48 the surface of coralline algae (light, water motion, epiphytes) acted antagonistically or
49 synergistically with OA more than any other drivers. With advances in experimental design
50 and methodological techniques, we now understand that the physiology of coralline algal
51 calcification largely dictates their responses to OA. However, significant challenges remain,
52 including improving the geographic and life-history spread of research effort and a need for
53 holistic assessments of physiology.

54 **Keywords:** Climate change, ocean acidification, coralline algae, CCA. Rhodoliths, maerl,
55 calcification, meta-analysis

56 **Introduction:**

57 Ocean acidification (OA) is the absorption of anthropogenically-derived CO₂ by the surface
58 seawaters of the world's oceans (Caldeira and Wickett 2003). OA alters seawater chemistry
59 as follows: hydrogen ions (H⁺) and carbon dioxide (CO₂) increase, dissolved inorganic
60 carbon (DIC) and bicarbonate (HCO₃⁻) increase slightly, and carbonate (CO₃²⁻)
61 concentrations and the calcium carbonate saturation states (Ω) decrease (Feely et al. 2004).
62 These changes in seawater chemistry have physiological repercussions for many marine
63 organisms, particularly those that calcify or photosynthesize (Harvey et al. 2013; Kroeker et
64 al. 2013).

65 Coralline algae are important foundation species in shallow ecosystems from the
66 tropics to the poles, both forming and cementing reefs, acting as settlement substrates and
67 nurseries for marine invertebrates, and providing coastal sediments (Nelson 2009; Milazzo et
68 al. 2014; Cornwall et al. 2021). However, as calcifying species, they are particularly sensitive
69 to ocean acidification (McCoy and Kamenos 2015; Martin and Hall-Spencer 2017). Earlier
70 laboratory experiments indicated that the complex changes in seawater carbonate chemistry
71 occurring under ocean acidification would impact coralline algae through reduced
72 calcification rates, sizes and/or numbers of recruits, and observations along natural gradients
73 showed that coralline algae decline in abundance as CO₂ levels in seawater rise (Anthony et
74 al. 2008; Hall-Spencer et al. 2008; Jokiel et al. 2008; Kuffner et al. 2008; Martin et al. 2008;
75 Martin and Gattuso 2009). While coralline algae are one of the most sensitive taxa to ocean
76 acidification (Kroeker et al. 2013), there is considerable variability between different species'
77 responses (Peña et al. 2021a). This variability could be related to differences in mineralogy,
78 the ability to regulate favourable carbonate chemistry at the site of calcification, and
79 morphology (e.g. thallus thickness or geniculate versus non-geniculate) (Nash et al. 2013;
80 McCoy and Pfister 2014; Cornwall et al. 2017; Barner et al. 2018). Earlier observations were
81 that ocean acidification could increase mortality through bleaching that could be exacerbated
82 by warming (Anthony et al. 2008; Martin and Gattuso 2009), as well as change the photo-
83 physiology (Borowitzka 1981) or mineralogy of these important foundation species (Ries et
84 al. 2009; Ries 2011).

85 To more accurately predict the future of reefs where coralline algae play essential
86 ecological roles, the scientific community needs to better understand the drivers of variability
87 in response to ocean acidification caused by phylogeny, geography and physiology. Earlier

88 reviews on the topic (Hurd et al. 2009; Nelson 2009; Hofmann and Bischof 2014; McCoy and
89 Kamenos 2015) provided good starting points for the field of research but were too premature
90 to assess generalisable results properly, the mechanisms responsible, or get accurate estimates
91 of the magnitude of the effects. Thirteen years from the initial burst of publications and 6
92 years from the last specialised review, we critically re-evaluate the literature. Here, we aim to
93 both collate the knowledge of coralline algal responses to ocean acidification and provide
94 numerical estimates of its effects using meta-analyses and predictive models. The manuscript
95 is split into 1) qualitative review, 2) meta-analyses, 3) projected declines in cover,
96 recruitment and calcification, and 4) coralline algal calcification physiology under ocean
97 acidification and frontiers in future research.

98

99 **Methods:**

100 *Qualitative analysis:*

101 We searched Web of Science with different combinations of coralline algal terms and “ocean
102 acidification”. The different coralline algal terms were “coralline algae”, “calcifying algae”
103 and “CCA”. The list of suitable publications was then cross-checked against the OAICC
104 database on Pangaea and the list used in Kroeker et al. (2013). This search was completed on
105 December 1st, 2020 and resulted in 298 papers (see Table S1). We then compiled the main
106 effects of ocean acidification on the coralline algae from each suitable paper, the main effects
107 of any other assessed driver (e.g. temperature or seasonality), the directionality of the
108 interaction between OA and the other driver (antagonistic, synergistic, additive, or one or
109 both and their interaction are unmeasurable Crain et al. 2008), and which driver was the
110 putative “dominant” driver. We used an approach where four of our author team reviewed the
111 findings of every study to reduce bias and misinterpretation. In some instances, we also
112 assessed each study using an $\alpha = 0.05$, rather than the reduced alpha used in some
113 research as corrections for multiple analyses are not optimal. Concerning the main effects of
114 OA, we classified responses as “net dissolution or complete mortality or lack of
115 recruitment/cover”, statistically significant negative, statistically significant parabolic
116 (including both positive and negative parabolic), no measurable effect, statistically significant
117 positive effect. Hereafter, we refer to these scores as dissolution or complete removal,
118 negative, parabolic, no measurable effect, and positive. By dominant driver, we qualitatively
119 refer to the one with the most considerable effect that can assist future decisions regarding

120 experimental design in multi-driver experiments (sensu Boyd et al. 2015; Boyd et al. 2018).
121 We refer to these (usually) environmental factors as “drivers” because their effects did not
122 always manifest in a negative direction. We found 392 responses to OA that could be scored
123 in this manner. Here, we refer to “responses” as all combined treatments in any one
124 experiment per species.

125

126 *Meta-analysis:*

127 During this process, it became evident that adult calcification/linear extension/growth and
128 juvenile growth/linear extension were the most commonly recorded parameters. Because of
129 their importance as overall indicators of “success” under these OA experiments, we chose to
130 conduct meta-analyses to further explore patterns in these responses. We used the same
131 papers identified above. To obtain the calcification and growth data, we extracted means of
132 calcification rates and their associated error whenever they were listed. When data were not
133 deposited freely online those data were extracted from figures within publications using the
134 software Datathief (<http://www.datathief.org>).

135 Seawater carbonate chemistry (pH_T, total alkalinity, dissolved inorganic carbon),
136 salinities, and temperatures were extracted or calculated along with the calcification data in
137 instances where they were not given. Seawater carbonate chemistry was recalculated in some
138 studies where inconsistencies were found between pH scales. Studies using the NBS scale
139 were converted to the total scale using the excel macro CO2Sys (Pierrot et al. 2006).
140 Research examining the effects of ocean acidification were excluded if they did not present
141 standardized measurements of seawater carbonate chemistry that could allow us to determine
142 pHT values (Dickson et al. 2007) accurately. That rarely occurred (Smith and Roth 1979;
143 Borowitzka 1981; Gao et al. 1993). We found a total of 538 calcification rates measured
144 across different seawater pH treatments that we could include in our analysis.

145 We used the bias-corrected Hedge’s g parameter in our assessment. Hedges g was
146 calculated as treatment – control divided by the pooled standard deviation. The bias
147 correction $\left(1 - \frac{3}{4(df) - 1}\right)$ (Hedges 1981) was used to control for the often-small samples
148 sizes within studies. There were instances where the total alkalinity anomaly was used to
149 measure calcification rates. Because some papers only published light calcification rates,
150 while others also presented dark calcification rates, we excluded all dark calcification
151 measurements in our analyses.

152 We conducted a meta-analysis in R with the *metafor* package that assessed the effects
153 of decreasing pH relative to the control values designated by the authors of each study. This
154 software used a multivariate mixed-effects linear model (function: *rma.mv*), with the unique
155 observation ID nested within the study ID as random effects. This approach meant that the
156 impacts of any one study with multiple pH levels were controlled for. For moderators, we
157 used relative change in pH_T between the control and treatment within the experiment (ΔpH_T ;
158 continuous), the temperature of the experiment (°C; continuous), the duration (in days;
159 continuous), the photosynthetically active radiation level ($\mu\text{mol}\cdot\text{photons m}^{-2}\text{ s}^{-1}$; continuous),
160 and the taxonomic family of the study organism (categorical: Corallinaceae,
161 Lithothamniaceae, Lithophyllaceae, Mesophyllumaceae, and Sporolithaceae). In terms of
162 carbonate chemistry, we choose ΔpH_T because we considered it to be physiologically more
163 robust than any other seawater carbonate chemistry parameter to test against (Comeau et al.
164 2018). We additionally tested the model using ‘climate’ (modified from Nybakken 2001
165 where we also consider the Mediterranean sea as "warm temperate") with tropical, warm-
166 temperate, cold-temperate and polar, and ‘ocean basin’ (Atlantic, Pacific and Indian Oceans)
167 as categorical moderators. However, these were subsequently dropped from the model due to
168 their non-significant effects on the model. Our choice of the taxonomic families for the
169 moderator followed the current systematics of coralline algae, with reference to Algaebase
170 (Guiry and Guiry 2021).

171 Cook’s distance (function: *cooks.distance.rma.mv*) was used to test for extreme
172 outliers, using a conservative cut-off threshold of $2\sqrt{((k + 1)/(n - k - 1))}$. This resulted
173 in a cut-off value of 0.31 for adult responses (removing 12 from 255 observations), and 0.69
174 for juvenile responses (removing 4 from 57 observations). Studies removed for the adult
175 responses were from Bergstrom et al. 2020 (6), Fine et al. 2016 (1), Ragazzola et al. 2013 (3)
176 and Sordo et al. 2018 (2)), and for the juvenile responses were recorded in Page & Diaz-
177 Pulido 2020 (2), Russell et al. 2011 (1) and Padilla-Gamiño et al. 2016 (1).

178 Meta-analysis results are presented using bubble plots (function: *regplot.rma*). The
179 results of the multivariate mixed-effects linear model were extrapolated for each of the RCP
180 2.6, RCP4.5 and RCP8.5 scenarios (function: *predict.rma*) using yearly global mean surface
181 pH_T values from the CanESM2 climate model (Canadian Centre for Climate Modelling and
182 Analysis; CMIP5, ensemble r1i1p1) along with mean values used for the other moderators
183 (temperature, duration, irradiance and taxonomic family).

184

185 *Proportional responses:*

186 Our initial qualitative review revealed that natural cover of coralline algae along pH/pCO₂
187 gradients and the percent cover of juvenile coralline algae on recruitment tiles had been
188 measured in consistent ways that could easily be compared between studies. We fitted non-
189 linear models (negative exponential models) for each location individually and pooled the
190 locations together for an overall model. For both the natural cover and juvenile recruitment,
191 non-linear models were deemed to provide a better fit compared to linear models based on
192 lower Akaike information criterion, Bayesian information criterion and estimated standard
193 error of the residuals. We projected estimates of uncertainty (95 % confidence intervals) in
194 coverage for the overall models by bootstrapping the model fits for 1,000 runs.

195

196 *Qualitative analysis*

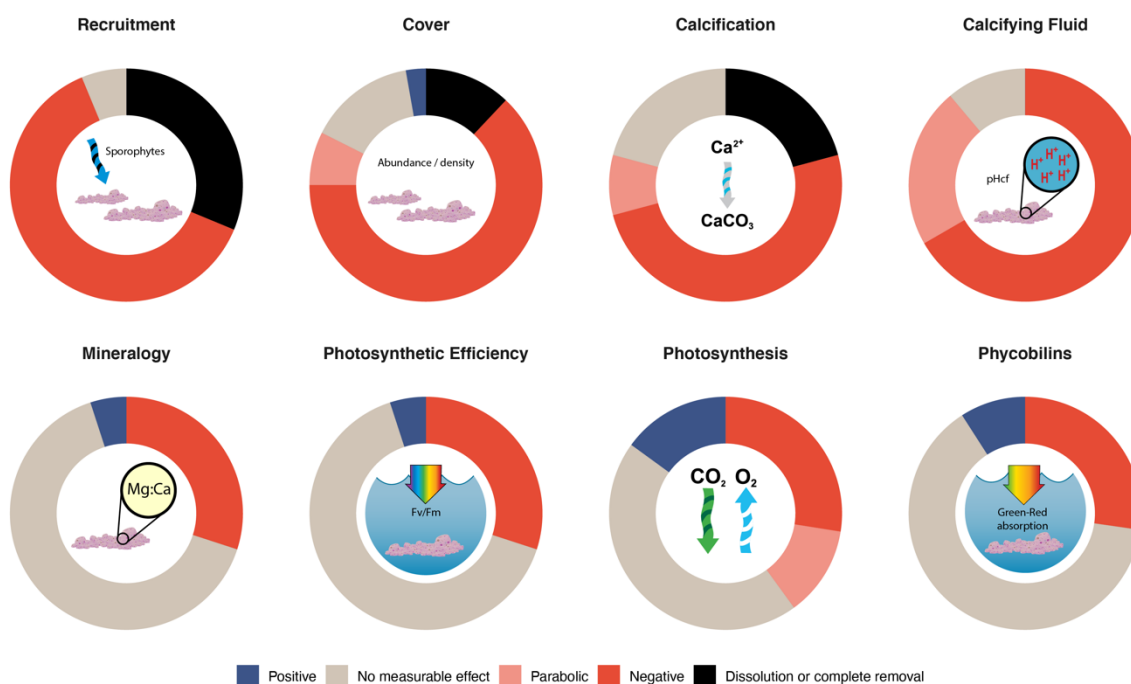
197 Table S1 details the findings of all research we could find on coralline algal responses to OA.
198 This table summarises what we consider are the main findings. While individual researchers
199 may argue of the nuances of each study for such a subjective analysis, we used an approach
200 where four of our author team ultimately reviewed the findings of every study to reduce bias
201 and misinterpretation. In some instance, we also assessed each study using an alpha = 0.05,
202 not reduced values used in some research. Arguments of why are beyond this paper, but
203 corrections for multiple analyses are now considered non-optimal. Especially in this case, i.e.
204 hundreds of analyses were conducted by the collective research papers, should these be
205 counted up and corrected for in each study?

206

207 **Results:**208 *Qualitative analysis of main effects*

209 There were 392 recorded responses of coralline algae to the effects of ocean acidification,
210 within which 108 were responses of calcification/growth. The top 10 most frequently
211 recorded were as follows with numbers of responses in parentheses: photosynthetic rates (40;
212 including 26 with respiration rates), cover (24), Fv/Fm (20), mineralogy (20), chlorophyll *a*
213 content (18), recruitment rates/sizes (16), bleaching/mortality (14), electron transport rates

214 (ETR; 14), phycobilin contents (11) and pH in the calcifying fluid (pH_{cf} ; 9). We focus mostly
 215 on these parameters for the remainder of this review and in Figure 1. Five of these response
 216 types were negative more often than not (recruitment 94%, calcification rates 75%, cover
 217 71% and pH_{cf} 67%). Bleaching/mortality was negative 50% of the time; however, this
 218 response is something visually obvious that was not noted in the majority of laboratory
 219 studies. Therefore, the proportion of times it truly would have been significantly negative is
 220 extremely low (< 5%). Negative effects of OA on Fv/Fm, mineralogy (classified as decrease
 221 in Mg content or increase in aragonite content), photosynthetic rates, chlorophyll *a* content,
 222 ETR and respiration occurred 30% or less in occasions where they were recorded. Positive
 223 responses of photosynthetic rates (15%) and ETR (14%) occurred more frequently for these
 224 top ten measured metrics than any others. Parabolic responses were noted in 21 responses
 225 from all measured 392 responses (i.e. 5% of times).

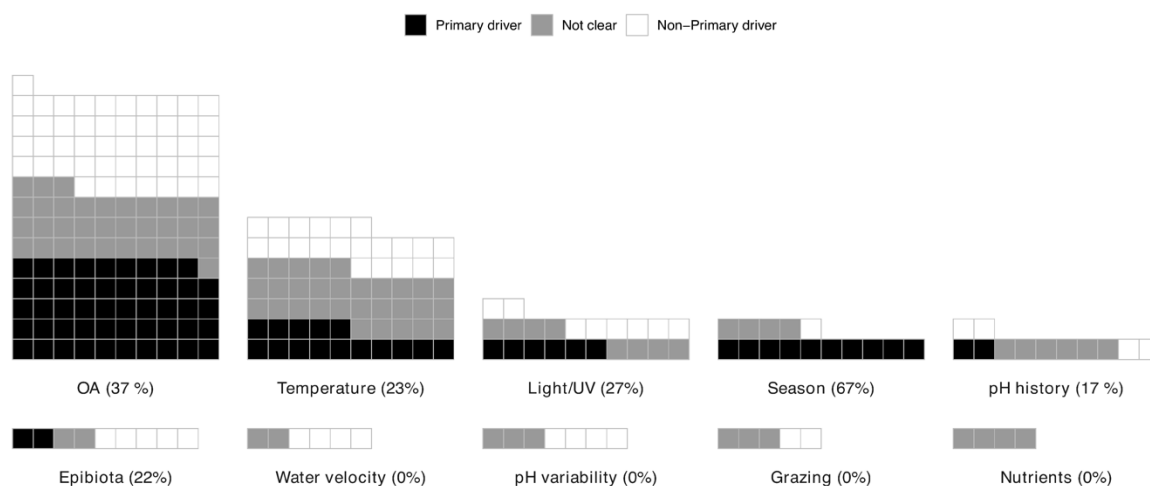


227 Figure 1: Effects of OA on coralline algae recorded in the literature, with the eight most
 228 negatively affected response parameters shown here for those parameters that were measured
 229 at least nine times. Responses are described as either 'positive', 'no measurable effect',
 230 'parabolic', 'negative' or 'dissolution or complete removal', see methods for more details.

231

232 *Dominant driver*

233 Seasonality was the dominant driver more times than any other (67%: Figure 2). OA was the
 234 next, being the putative dominant driver 37% of occasions, then light quality/quantity (27%),
 235 temperature (23%), epiphyte/turf presence (22%), and past pH history of the coralline algae
 236 (17%). Water velocity, pH variability, herbivory/grazing and nutrients were never dominant.



238 Figure 2: Number and proportion of times different drivers were assessed in combination
 239 with OA or another driver in 3+ factorial approaches. In parentheses are the percentage of
 240 times the driver was dominant. See methods for the definition of dominant.

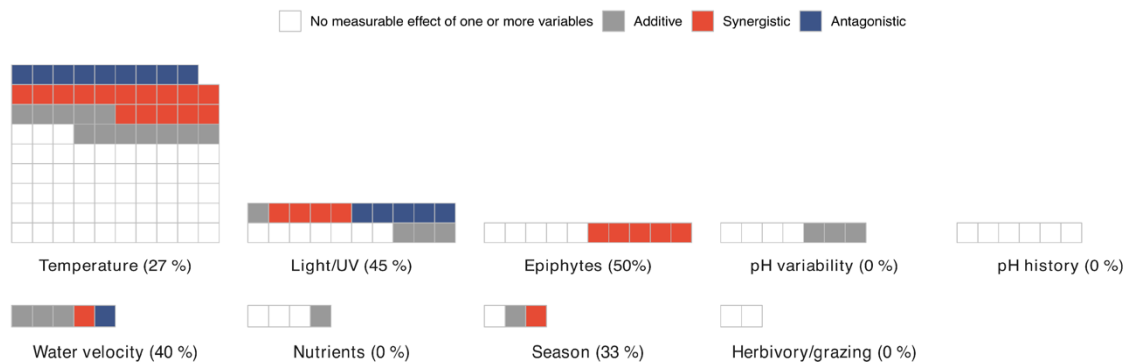
241

242 *Interaction types*

243 Here we split the interactions by the measured response type (e.g. calcification rates, etc.) and
 244 the driver that was tested. We report response types that were measured in multi-driver
 245 experiments 10 or more times. Photosynthetic rates had the highest number of “non-
 246 surprising” interactions (additive or unmeasurable main effects and interactions; 87%),
 247 followed by Fv/Fm (83%), calcification rates (72%) and respiration rates (60%). There was a
 248 total of 21 antagonistic and 18 synergistic response from 153 recorded, 14% and 12%
 249 respectively.

250 There were clear patterns in the driver types that more often resulted in these
 251 antagonist and synergistic interactions. These were mostly drivers that can alter the pH at the
 252 surface of coralline algae or their photo-physiology: epiphyte/turf presence (50% of
 253 antagonistic + synergist interactions), light (45%) and water velocity (40%). Season (33%)
 254 and temperature (27%) also had some antagonistic or synergistic interactions. Interactions

255 with pH variability, nutrients, herbivory and past pH history did not result in synergistic or
 256 antagonistic effects.



258 Figure 3: Number of different interaction types between OA and other drivers within
 259 coralline algal OA research. The proportion of synergistic and antagonistic interactions is
 260 indicated within parentheses. Note here we define “no measurable effect of one or more
 261 variables” as being separate from “additive”, with the former being occasions where one or
 262 both drivers have no measurable effect in either direction, and the addition of the other driver
 263 does not change the effect of OA.

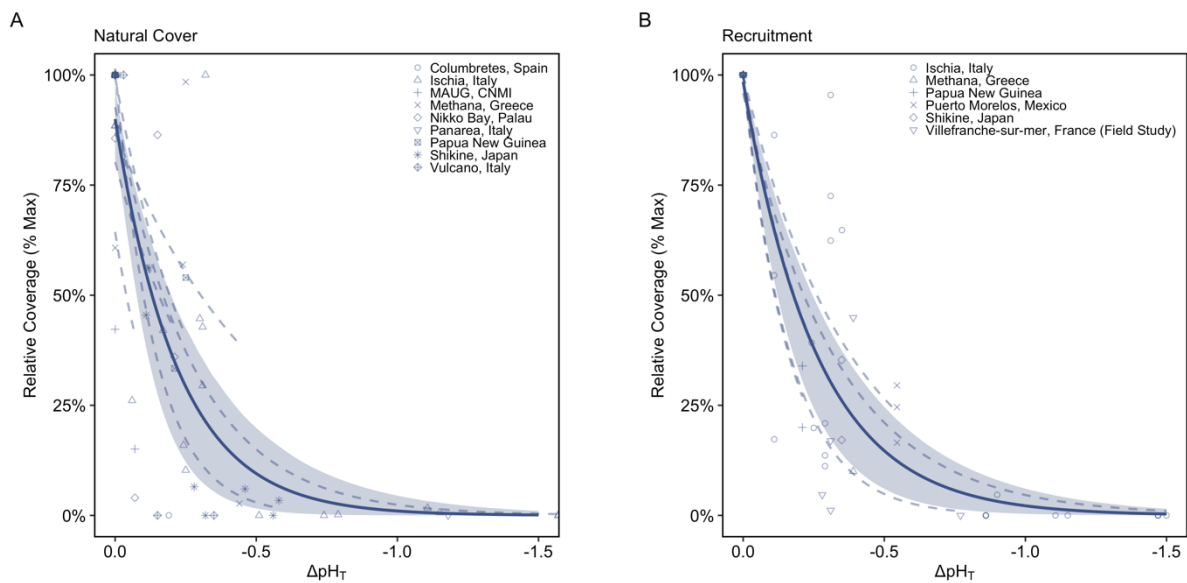
264

265 *Proportional effects*

266 *Field cover and recruitment rates*

267 There were large reductions in coralline algae cover as pH declined, with the non-linear
 268 model predicting that relative coverage may drop to ~ 15 % (2 – 27, 95 % CI) of their
 269 associated reference/control site(s) once pH_T declines below -0.4, and continue to approach
 270 towards zero covers (Figure 4a). Recruitment cover demonstrated a similar decline as pH is
 271 reduced, albeit less steeply, with ~ 21 % (11 – 32, 95 % CI) compared to the

272 reference/control site(s) at a ΔpH_T decline of -0.4 (Figure 4b).



273

274 Figure 4: A) Relative cover change (%) of coralline algae at reduced pH sites in the field
 275 compared to nearby control sites. B) Relative coverage (%) of coralline algal recruits on
 276 settlement tiles at reduced pH locations compared to nearby controls.

277

278 *Meta-analysis*

279 ΔpH_T had a significant relationship with calcification in the multivariate meta-analysis model
 280 for adult coralline algae (Fig. 5), indicating that as pH_T is reduced, the negative impact on
 281 calcification may become greater. Based on the model, after a threshold of $-0.062 \Delta\text{pH}_T$ (at
 282 Hedge's g : -0.53 ± 0.53 95% CI), adult coralline algae had their calcification significantly
 283 reduced, reaching (for example) effect size of -1.68 ± 0.36 (95 % CI) at $-0.4 \Delta\text{pH}_T$ (where
 284 the effect size of Hedge's is deemed to be a 'large effect' at 0.8). Some phylogenetic
 285 differences were found within the adult coralline algae responses, where those coralline algae
 286 from the family Mesophyllumaceae had a significantly different slope than the other families
 287 (Table S2, S1).

288 There were fewer responses available in the literature for juvenile coralline algae, and
 289 those that were available assessed growth/calcification over a smaller range of ΔpH_T , which
 290 likely led to no significant effect of ΔpH_T on the model outcome (Table S3, Fig. S2).

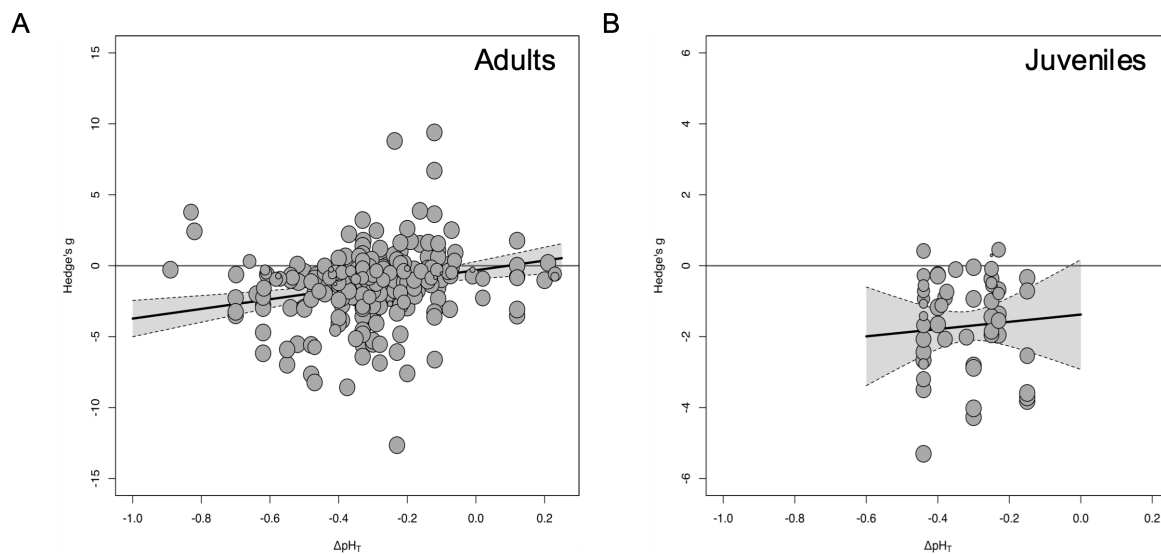
291 Although the calcification response did not significantly change with ΔpH_T , the observations
 292 themselves were still almost entirely negative; for example, at $-0.4 \Delta\text{pH}_T$ an effect size of

293 -1.79 ± 0.57 (95 % CI) was observed, indicating a significant and large negative effect on the
 294 calcification of juvenile coralline algae. In addition, there was a significant difference
 295 between the responses of juveniles from both families Lithophyllaceae and Corallinaceae
 296 compared to other families. This is likely simply because there is sufficient statistical power
 297 to assess differences between these two families but not enough data for the others.

298 When considering our analysis in the context of the RCP scenarios, we found the
 299 effects of altered seawater pH were highly dependent on the RCP scenario being considered.
 300 For the subtle effects of seawater pH under conditions representing RCP2.6, we predict non-
 301 significant declines in adult calcification by 2100 compared to the present-day (Figure 6). We
 302 also predict significant declines in calcification under RCP4.5 and 8.5 by 2100 compared to
 303 present-day, with calcification rates declining drastically in the RCP8.5 scenario as this
 304 scenario deviates from RCP4.5 around the year 2040 (Figure 6).

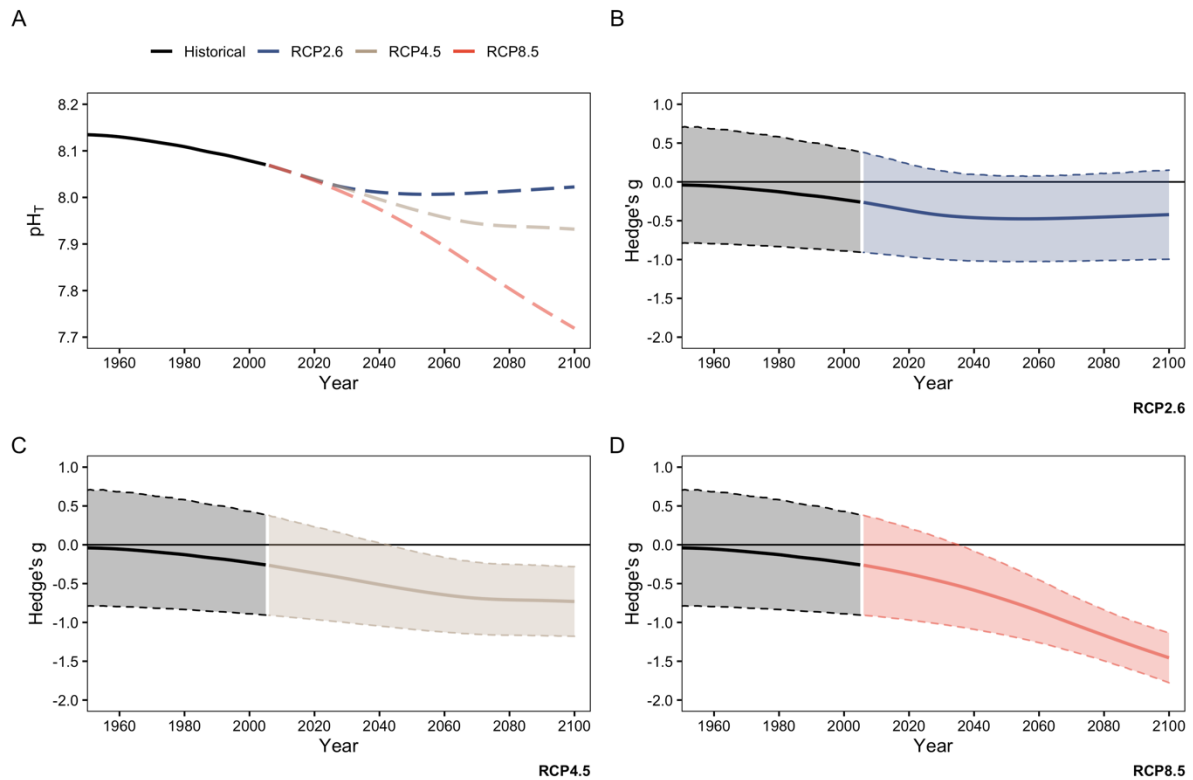
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306



308 Figure 5: (A and B) – Bubble plots for adults and juveniles, respectively, showing how the
 309 effect size on calcification changes with ΔpHT (while still considering the other predictors).
 310 The size of the points indicates the weighting, and the dashed line indicates 95% CI.

311



313 Figure 6: (A) Predicted global mean pH_T level historically, and projected for RCP2.6, 4.5 and
 314 8.5 scenarios up to the year 2100 using the CanESM2 climate model. (B – D) The mean
 315 effect size \pm 95% CI for adults at the predicted global mean pH_T level historically, and for
 316 RCP2.6 (B), 4.5 (C) and 8.5 (D) scenarios up to the year 2100.

317 **Discussion:**

318 We demonstrate here that OA is a major threat to the persistence of coralline algae in the
319 future using multiple lines of evidence. Early studies that reported severe declines in coralline
320 algal cover and recruitment at high CO₂ sites (Hall-Spencer et al. 2008; Martin et al. 2008;
321 Fabricius et al. 2011), and declines in calcification and recruitment (Anthony et al. 2008;
322 Jokieli et al. 2008; Kuffner et al. 2008; Martin and Gattuso 2009; Russell et al. 2009) have
323 been fully corroborated by a host of research demonstrating that such effects are the norm,
324 rather than the exception. Though not explicitly tested, no such “decline effect” (Clements et
325 al. 2020), as has been purported in other taxa, was found here. Indeed, the studies that
326 showed the larger effect sizes, were some of the most recent (Barner et al. 2018; Sordo et al.
327 2018; Qui-Minet et al. 2019; Bergstrom et al. 2020). Declines in calcification, recruitment,
328 coralline cover at naturally high CO₂ analogues (and less commonly, pH within the
329 calcification fluid (pH_{cf})) were the norm, rather than the exception across all published
330 literature.

331 Despite consistent responses of many of these vital ecological and physiological
332 processes, some other physiological processes had inconsistent responses to OA. By
333 understanding such distinctions, we can better assess how coralline algal communities are
334 likely to be altered by future climate change. For example, some Authors showed that OA
335 would cause widespread coralline algal mortality due to bleaching and declines in pigment
336 concentrations and decreases in photosynthetic rates or DIC uptake (Anthony et al. 2008;
337 Martin and Gattuso 2009). These results led to speculation that OA could cause similarly
338 catastrophic impacts for coralline algal dominated reefs as the increased temperature does for
339 corals. Fortunately, we find little evidence for this. However, the declines in calcification
340 observed here still threaten the ability of coralline algae to perform vital ecological services,
341 such as providing settlement substrata or building and cementing biogenic reefs (Cornwall et
342 al. 2021). Additionally, we find inconsistent evidence for other benefits to photosynthesis,
343 photo-physiology or DIC uptake that earlier studies proposed (Cornwall et al. 2012), or
344 changes in mineralogy that could either benefit or impair their physiology (Ries 2011; Diaz-
345 Pulido et al. 2014). That highlights the importance of pooling studies for reanalysis to refine
346 hypotheses. Conversely, it was clear that OA usually impacts pH_{cf} of the coralline algae (e.g.
347 Cornwall et al. 2017; Donald et al. 2017), though other elemental or geochemical differences
348 were unclear. Irrespective of their findings, these earlier studies collectively led and

349 developed the field of coralline algal responses to OA, allowing the current synthesis of the
350 results.

351

352 The relative importance of global and local drivers in determining ecological change
353 is highly dependent on the taxa being assessed. For corallines, OA was the dominant driver
354 more often than any other local or global stressor it was assessed against, with seasonality
355 being the only driver that was dominant more often. Despite the sensitivity of many taxa to
356 changes in seawater temperature, it was rarely the clear dominant driver of coralline algal
357 responses. This observation supports recent work questioning the relative importance of
358 temperature on coralline algal physiology, compared to more temperature-sensitive
359 organisms such as corals or kelps (Cornwall et al. 2019; Anton et al. 2020). The combined
360 effects of ocean warming and OA are better constrained in the literature than the role of other
361 drivers. However, there is still very little known regarding its impacts (Cornwall et al. 2019),
362 and it should be noted that multiple responses recorded here were from the same study (e.g.
363 multiple species or multiple response metrics). While 15 “responses” were recorded where
364 temperature played a dominant role, 6 of these were recorded from the same study (Vásquez-
365 Elizondo and Enríquez 2016). Conversely, there is little understanding of how marine
366 heatwaves will impact coralline algae, and these would be expected to have much greater
367 impacts than slow ongoing ocean warming that was often simulated in most research we
368 assessed here, for example that possessed treatments that were simply 2 or 3 degrees above a
369 control seawater. Likewise, the role of light was often complex and only sometimes (6/22)
370 played a dominant role in determining the physiological state of the coralline algae. It is more
371 difficult to synthesise the effects of light quality and quantity into a single category to assess
372 their role as dominant or not. Exposure to differing spectra or total PAR daily doses (either
373 too low or too high) within the realms of that experienced at any one collection site could
374 elicit stronger responses than changes in light. Multi-stressor experiments need to carefully
375 choose stress levels and combinations to obtain the most informative results (Boyd et al.
376 2018).

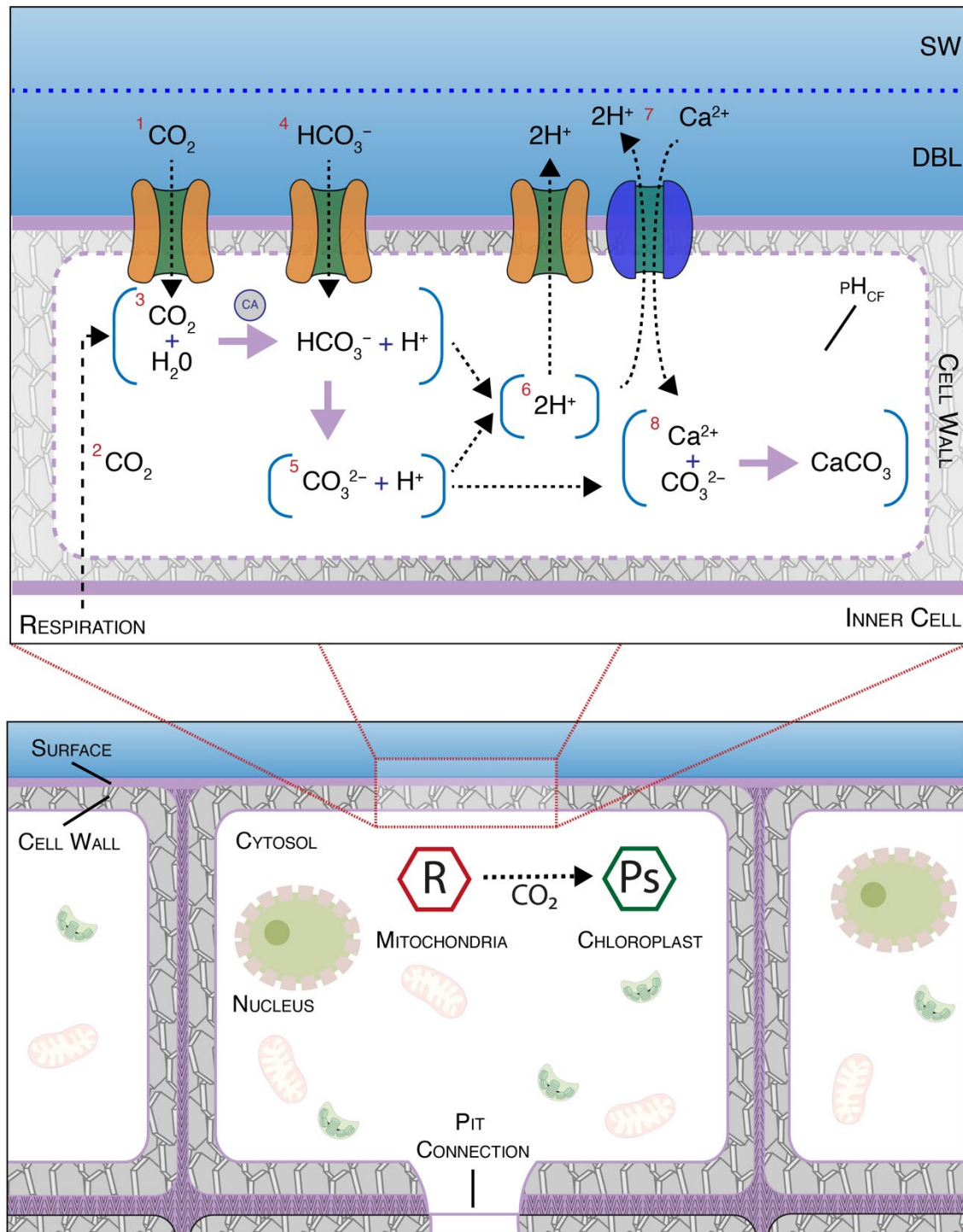
377 Here we found that environmental/ecological factors that were most likely to elicit
378 antagonistic or synergistic effects were those that impacted either photo-physiology or pH at
379 the surface of coralline algae. A possible reason for that may be that the pH experienced by
380 the organism is more important than that of the bulk seawater. At the surface of most

381 photoautotrophs, pH is set by the thickness of the diffusion boundary layer (DBL: a region of
382 seawater where the movement of dissolved substances is primarily by molecular diffusion).
383 The theoretical maximum of the DBL is set by water velocity near the organism, and this
384 near-surface velocity would be reduced by the presence of any epiphytes or canopy-forming
385 species above the coralline algae, hence increasing pH gradients (Cornwall et al. 2015). The
386 pH achieved within the DBL will be dependent on the rate of metabolic activity within it (e.g.
387 photosynthesis or respiration) and the DBL thickness. Therefore, it is not surprising that
388 drivers that modify pH within the DBL (light, epiphytes, and water velocity) often nullify the
389 impacts of OA (Cornwall et al. 2014; Guy-Haim et al. 2020), although not always when
390 nutrient concentrations are low (Comeau et al. 2014; Comeau et al. 2019b). However, the
391 mechanisms responsible for the antagonistic roles of season and temperature are harder to
392 disentangle. Indeed, interpreting many of the seasonal impacts on coralline algae were
393 difficult during the process of constructing Table S1, especially when additional factors such
394 as temperature and light were either modified at each season or tested interactively (e.g. Qui-
395 Minet et al. 2018; Legrand et al. 2019; Qui-Minet et al. 2019).

396 Changes to the ecological role of coralline algae in a future low pH/high CO₂ ocean is
397 uncertain; we demonstrate their ability to calcify, recruit and persist in abundances similar to
398 those today are impacted by OA. We also demonstrate that their calcification rates will depart
399 significantly from that of today's as OA intensifies. The effects noted in our qualitative
400 analysis are mostly linear, with few parabolic or rapid "tipping point" type responses. Our
401 meta-analyses indicate that under RCP8.5 they might be particularly severely impacted,
402 highlighting the urgent need to reduce our CO₂ emissions to allow these critical species
403 continuing their role as reef accrete and preferred settlement substrate for many invertebrate
404 species (Fabricius et al. 2017). Surface seawater pH has been declining since the industrial
405 revolution, meaning that the impacts of OA have likely already manifested in some way
406 across global oceans (McCoy and Pfister 2014), and this is supported by comparing sites with
407 naturally low, pre-industrial levels of seawater CO₂ (Agostini et al. 2018). Select coralline
408 algae can acclimatise over multiple generations to completely resist the effects of OA
409 (Cornwall et al. 2020; Moore et al. 2021). However, whether all species can respond
410 similarly, if the presence of rapidly growing competitors reduces this ability, or whether there
411 are trade-offs in genetic diversity that occur in these populations remains unknown.

412 The negative effects on calcification manifest across all major oceans and across the
413 range from tropical to polar climates. Specific phylogenies appeared more robust than others,

414 particularly the adult Mesophyllumaceae. A scientific effort in monitoring the abundance or
415 ecology of coralline algae beyond simply acknowledging their collective presence or absence
416 is lacking. That is hindered by the only recent understanding that coralline algae diversity is
417 greater than previously believed (Gabrielson et al. 2018; Twist et al. 2019; Peña et al. 2021b),
418 and extends far beyond the previous mostly morphological groupings and has important
419 implications for much of the previous work in this field (Twist et al. 2020). Recent molecular
420 work shows that some groups of coralline algae are more vulnerable to OA than others and
421 that the projected adverse effects on coralline algal biodiversity have been greatly
422 underestimated (Peña et al. 2021a). Likely changes in species abundances, competitive
423 hierarchies, and physiologies (McCoy and Pfister 2014; McCoy and Ragazzola 2014) before
424 the advent of molecular technology make it challenging to establish a baseline abundance for
425 most coralline algae. Future research should focus on pairing physiological and molecular
426 identifications wherever possible.



428 Figure 7: Schematic showing movement of dissolved substances in and out of coralline algal
 429 cells. This gives an overview of the processes that could be affected by ocean acidification, it
 430 is not intended to describe the full calcification process. In some taxa there can be multiple
 431 cells between seawater and the site of calcification hereafter “Calcifying fluid = CF”.
 432 Provision of photosynthesis by 1) movement of CO_2 (or external carbonic anhydrase
 433 mediated conversion of HCO_3^- to CO_2) across aquaporins or through seawater entering the

434 cell wall and 2) produced during respiration. 3) The reaction from CO_2 to HCO_3^- will be
435 catalysed by internal carbonic anhydrase to some extent within the cell, while 4) active
436 transport of incoming HCO_3^- will occur via CO_2 concentrating mechanisms involving influx
437 or efflux to balance the charge (not shown). 6) Increasing pH_{cf} will be due to proton pumps or
438 7) Ca^{2+} ATPases or other mechanisms of H^+ efflux. 8) Elevated saturation state of CaCO_3
439 will increase precipitation rates within the calcifying fluid pockets.

440

441 The physiology of coralline algae is poorly understood, hindering our ability to
442 interpret many of the observed responses to seawater carbonate chemistry. Their calcification
443 is impacted by declining seawater pH via both increased dissolution rates and an inhibition in
444 the precipitation of calcium carbonate. The amount of dead exposed skeletal material within
445 any experiment would therefore strongly influence the rate of dissolution, further impairing
446 our ability to compare experimental results. After decades of models based on eco-
447 physiology, scanning electron microscope images, and some carbon isotope work, new
448 models and techniques have emerged. These have allowed us to determine that calcification
449 in coralline algae is highly controlled by the organism, while at the same time is still dictated
450 by environmental conditions. Recent models have demonstrated that control within the site of
451 calcification, likely μm or smaller sized pockets of instantaneous fluid that we term the
452 “calcifying fluid” (CF), is strongly influenced by ocean acidification. The $\delta^{11}\text{B}$ of calcium
453 carbonate tracks the pH of the solution in which it is formed (Klochko et al. 2006). For
454 coralline algal calcite, experimental work has demonstrated that this proxy tracks pH within
455 the CF, finding that it is highly upregulated, beyond what is possible simply due to
456 photosynthetic activity, in the majority (but not all) of the species measured to date (Cornwall
457 et al. 2017; Donald et al. 2017; Cornwall et al. 2018; Anagnostou et al. 2019; Comeau et al.
458 2019a; Comeau et al. 2019b). Importantly, the species-specific magnitude of its decline under
459 simulated ocean acidification is linked with a greater capacity for calcification to resist ocean
460 acidification for some species (Cornwall et al. 2017). However, it is likely to be more
461 complex than suggested by those initial observations

462 Both DIC and the saturation state of calcite within CF are likely important factors in
463 the regulation of calcification in coralline algae, even if these have not been measured
464 directly with existing technology. Raman spectroscopy (FWHM) and B/Ca ratios track Ω_{cf}
465 and DIC_{cf} in coral aragonite (Holcomb et al. 2016; DeCarlo et al. 2017; McCulloch et al.

2017). However, similar calibrations do not exist for coralline algal high Mg calcite. In both corals and coralline algae these parameters are altered by the same environmental factors (pH, DIC, light and water flow) in the same direction (with some species-specific offsets), leading to the more likely scenario that they also represent Ω_{cf} and DIC_{cf} in coralline algal calcite. There have been some suggestions for corals that B/Ca may even represent a differential uptake of boron under changing DIC (Gagnon et al. 2021). However, this would not account for the remarkably similar DIC_{cf} estimated with electrodes (Cai et al. 2016). Overall, these proxies would indicate that coralline algal calcite saturation state and DIC is likely much higher than that of seawater. This would rule out photosynthetic control over carbonate chemistry within the CF, even if DIC was slightly higher than that of seawater, as DIC would be expected to decrease under higher photosynthetic rates. However, that is not to say that higher photosynthetic rates would not further elevate pH_{cf} or Ω_{cf} if this was in concert with increased pumping of H^+/Ca^{2+} due to increasing energy. This potential Ω_{cf} remains invariant under many conditions, though it is influenced by Mg content and should be adjusted accordingly (Perrin et al. 2016). There are, however, two conditions in which it is altered. Like in corals, it appears that very rapid calcification is associated with lower Ω_{cf} values for some fast growing juveniles (Cornwall et al. 2020); which could be associated with the drawdown of Ca^{2+} and would slow the precipitation of calcium carbonate when combined with lower pH_{cf} under OA. Conversely, this could also represent changes in Ω_{cf} driven by an inability to adequately regulate a combination of DIC_{cf} and pH_{cf} . Ω_{cf} declined in *Neogoniolithon* sp. (but not *Sporolithon durum*) when seawater DIC was reduced experimentally (Comeau et al. 2018). Interestingly, *S. durum* pH_{cf} increased and DIC_{cf} declined in response to the same conditions, with pH_{cf} declining and DIC_{cf} increasing under lower seawater pH, but with no effect on *Neogoniolithon* sp. Low light can reduce Ω_{cf} (Comeau et al. 2019b), likely due to a reduced ability to pump Ca^{2+} , as pH_{cf} and DIC_{cf} remain constant under low light. Further species-specific investigations are required, especially those that assess apparent phylogenetic differences in calcification modes (Auer and Piller 2020) with differential responses of CF chemistry. Though previously identified morphological differences in calcification modes (Auer and Piller 2020) did not match more resistant or susceptible taxa here, it could reveal important information if indeed crystal orientation does impact the CF chemistry or responses to OA and other abiotic factors.

There are still many fundamental questions left unanswered in understanding the responses of coralline algae to OA. Coralline algal CF chemistry is species-specific and has

499 large uncertainties compared to that of aragonitic corals. While small windows of knowledge
500 have been opened by using these proxies, there is still much work to be done in calibrating
501 B/Ca and FWHM to understand the actual values of DIC_{cf} and Ω_{cf} in coralline algae and how
502 they respond to different environmental drivers, particularly OA. Taxonomic variation in
503 responses, and its influence on other factors that we explored here can only be guessed at
504 currently with the existing issues of non-molecular identification in the majority of studies
505 included here (Twist et al. 2020). Better integration of molecular work while assessing their
506 response to OA is needed in the laboratory. How this can be implemented in field settings
507 such as natural CO_2 vents is a more difficult consideration. If plans can be implemented to
508 include molecular identification in CO_2 vent work, this will go a large way in disentangling
509 which species are tolerant there, as has been recently completed at two CO_2 seep sites (Peña
510 et al. 2021a). Additionally, the next steps are to combine “omics” tools with physiological
511 and geochemical tools to better determine the mechanisms responsible for individual species
512 tolerance to OA, and how and why these tolerances can change over multiple lifetimes in
513 some coralline algae (Cornwall et al. 2020). Here, we do not attempt to fully discuss
514 calcification mechanisms, but future inclusions of emerging techniques with morphological
515 knowledge (e.g. Auer and Piller 2020) will also further our ability to understand taxa-specific
516 responses. Only by not ignoring all available information will progress be made in better
517 predicting the future of ecosystems in which coralline algae act as important foundation
518 species.

519

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528 **References**

- 529 Agostini S, Harvey BP, Wada S, Kon K, Milazzo M, Inaba K, Hall-Spencer JM (2018) Ocean acidification drives
530 community shifts towards simplified non-calcified habitats in a subtropical–temperate transition
531 zone. *Scientific Reports* 8:11354
- 532 Anagnostou E, Williams B, Westfield I, Foster GL, Ries JB (2019) Calibration of the pH- $\delta^{11}\text{B}$ and temperature-
533 Mg/Li proxies in the long-lived high-latitude crustose coralline red alga *Clathromorphum compactum*
534 via controlled laboratory experiments. *Geochimica et Cosmochimica Acta* 254:142-155
- 535 Anthony KRN, Kline DI, Diaz-Pulido G, Dove S, Hoegh-Guldberg O (2008) Ocean acidification causes bleaching
536 and productivity loss in coral reef builders. *Proceedings of the National Academy of Sciences*
537 105:17442-17446
- 538 Anton A, Randle JL, Garcia FC, Rossbach S, Ellis JI, Weinzierl M, Duarte CM (2020) Differential thermal tolerance
539 between algae and corals may trigger the proliferation of algae in coral reefs. *Global change biology*
540 26:4316-4327
- 541 Auer G, Piller WE (2020) Nanocrystals as phenotypic expression of genotypes—An example in coralline red
542 algae. *Science Advances* 6:eaay2126
- 543 Barner AK, Chan F, Hettlinger A, Hacker SD, Marshall K, Menge BA (2018) Generality in multispecies responses
544 to ocean acidification revealed through multiple hypothesis testing. *Global Change Biology* 0
- 545 Bergstrom E, Ordoñez A, Ho M, Hurd C, Fry B, Diaz-Pulido G (2020) Inorganic carbon uptake strategies in
546 coralline algae: Plasticity across evolutionary lineages under ocean acidification and warming. *Marine*
547 *Environmental Research*:105107
- 548 Borowitzka MA (1981) Photosynthesis and calcification in the articulated coralline red algae *Amphiroa anceps*
549 and *A. foliacea*. *Mar Biol* 62:17-23
- 550 Boyd PW, Dillingham PW, McGraw CM, Armstrong EA, Cornwall CE, Feng Yy, Hurd CL, Gault-Ringold M, Roleda
551 MY, Timmins-Schiffman E, Nunn BL (2015) Physiological responses of a Southern Ocean diatom to
552 complex future ocean conditions. *Nature Climate Change* 6:207
- 553 Boyd PW, Collins S, Dupont S, Fabricius K, Gattuso J-P, Havenhand J, Hutchins DA, Riebesell U, Rintoul MS, Vichi
554 M, Biswas H, Ciotti A, Gao K, Gehlen M, Hurd CL, Kurihara H, McGraw CM, Navarro JM, Nilsson GE,
555 Passow U, Pörtner H-O (2018) Experimental strategies to assess the biological ramifications of
556 multiple drivers of global ocean change—A review. *Global Change Biology* 24:2239-2261
- 557 Cai W-J, Ma Y, Hopkinson BM, Grottoli AG, Warner ME, Ding Q, Hu X, Yuan X, Schoepf V, Xu H (2016)
558 Microelectrode characterization of coral daytime interior pH and carbonate chemistry. *Nature*
559 *Communications* 7
- 560 Caldeira K, Wickett ME (2003) Anthropogenic carbon and ocean pH. *Nature* 425:365
- 561 Clements J, Sundin J, Clark TD, Jutfelt F (2020) An extreme decline effect in ocean acidification fish ecology.
562 *EcoEvoRxiv*
- 563 Comeau S, Edmunds PJ, Lantz CA, Carpenter RC (2014) Water flow modulates the response of coral reef
564 communities to ocean acidification. *Scientific Reports* 4:6681
- 565 Comeau S, Cornwall CE, DeCarlo TM, Krieger E, McCulloch MT (2018) Similar controls on calcification under
566 ocean acidification across unrelated coral reef taxa. *Global Change Biology* 24:4857-4868
- 567 Comeau S, Cornwall CE, DeCarlo TM, Doo SS, Carpenter RC, McCulloch MT (2019a) Resistance to ocean
568 acidification in coral reef taxa is not gained by acclimatization. *Nature Climate Change* 9:477-483
- 569 Comeau S, Cornwall CE, Pupier CA, DeCarlo TM, Alessi C, Trehern R, McCulloch MT (2019b) Flow-driven micro-
570 scale pH variability affects the physiology of corals and coralline algae under ocean acidification.
571 *Scientific Reports* 9:12829
- 572 Cornwall CE, Comeau S, McCulloch MT (2017) Coralline algae elevate pH at the site of calcification under ocean
573 acidification. *Global Change Biology* 23:4245-4256
- 574 Cornwall CE, Diaz-Pulido G, Comeau S (2019) Impacts of Ocean Warming on Coralline Algal Calcification: Meta-
575 Analysis, Knowledge Gaps, and Key Recommendations for Future Research. *Frontiers in Marine*
576 *Science* 6
- 577 Cornwall CE, Pilditch CA, Hepburn CD, Hurd CL (2015) Canopy macroalgae influence understory corallines'
578 metabolic control of near-surface pH and oxygen concentration. *Marine Ecology Progress Series*
579 525:81-95
- 580 Cornwall CE, Comeau S, DeCarlo TM, Moore B, D'Alexis Q, McCulloch MT (2018) Resistance of corals and
581 coralline algae to ocean acidification: physiological control of calcification under natural pH variability.
582 *Proceedings of the Royal Society B: Biological Sciences* 285
- 583 Cornwall CE, Hepburn CD, Pritchard DW, McGraw CM, Currie KI, Hunter KA, Hurd CL (2012) Carbon-use
584 strategies in macroalgae: differential responses to lowered pH and implications for ocean
585 acidification. *Journal of Phycology* 48:137-144

- 586 Cornwall CE, Boyd PW, McGraw CM, Hepburn CD, Pilditch CA, Morris JN, Smith AM, Hurd CL (2014) Diffusion
587 Boundary Layers Ameliorate the Negative Effects of Ocean Acidification on the Temperate Coralline
588 Macroalga *Arthrocardia corymbosa*. PLOS ONE 9:e97235
- 589 Cornwall CE, Comeau S, DeCarlo TM, Larcombe E, Moore B, Giltrow K, Puerzer F, D'Alexis Q, McCulloch MT
590 (2020) A coralline alga gains tolerance to ocean acidification over multiple generations of exposure.
591 Nature Climate Change
- 592 Cornwall CE, Comeau S, Kornder NA, Perry CT, van Hooidonk R, DeCarlo TM, Pratchett MS, Anderson KD,
593 Browne N, Carpenter R, Diaz-Pulido G, D'Olivo JP, Doo SS, Figueiredo J, Fortunato SAV, Kennedy E,
594 Lantz CA, McCulloch MT, González-Rivero M, Schoepf V, Smithers SG, Lowe RJ (2021) Global declines
595 in coral reef calcium carbonate production under ocean acidification and warming. Proceedings of the
596 National Academy of Sciences 118:e2015265118
- 597 Crain CM, Kroeker KJ, Halpern S (2008) Interactive and cumulative effects of multiple human stressors in
598 marine systems. Ecology Letters 11:1304-1315
- 599 DeCarlo TM, D'Olivo JP, Foster T, Holcomb M, Becker T, McCulloch MT (2017) Coral calcifying fluid aragonite
600 saturation states derived from Raman spectroscopy. Biogeosciences 14:5253-5269
- 601 Diaz-Pulido G, Nash MC, Anthony KRN, Bender D, Opdyke BN, Reyes-Nivia C, Troitzsch U (2014) Greenhouse
602 conditions induce mineralogical changes and dolomite accumulation in coralline algae on tropical
603 reefs. Nature Communications 5:3310
- 604 Dickson AG, Sabine CL, Christian JR (2007) Guide to best practices for Ocean CO₂ measurements. North Pacific
605 Marine Science Organization
- 606 Donald HK, Ries JB, Stewart JA, Fowell SE, Foster GL (2017) Boron isotope sensitivity to seawater pH change in
607 a species of *Neogoniolithon* coralline red alga. Geochimica et Cosmochimica Acta
- 608 Fabricius K, Langdon C, Uthicke S, Humphrey C, Noonan S, De'ath G, Okazaki R, Muehllehner N, Glas MS, Lough
609 JM (2011) Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations.
610 Nature Climate Change 1:165-169
- 611 Fabricius KE, Noonan SHC, Abrego D, Harrington L, De'ath G (2017) Low recruitment due to altered settlement
612 substrata as primary constraint for coral communities under ocean acidification. Proceedings of the
613 Royal Society B: Biological Sciences 284:20171536
- 614 Feely RA, Sabine CL, Lee K, Berelson W, Kleypas JA, Fabry VJ, Millero FJ (2004) Impact of anthropogenic CO₂ on
615 the CaCO₃ system in the oceans. Science 305:362-366
- 616 Gabrielson PW, Hughey JR, Diaz-Pulido G (2018) Genomics reveals abundant speciation in the coral reef
617 building alga *Porolithon onkodes* (Corallinales, Rhodophyta). Journal of Phycology 54:429-434
- 618 Gagnon AC, Gothmann AM, Branson O, Rae JWB, Stewart JA (2021) Controls on boron isotopes in a cold-water
619 coral and the cost of resilience to ocean acidification. Earth and Planetary Science Letters 554:116662
- 620 Gao K, Aruga Y, Asada K, Ishihara T, Akano T, Kiyohara M (1993) Calcification in the articulated coralline alga
621 *Corallina pilulifera*, with special reference to the effect of elevated CO₂ concentration. Marine Biology
622 117:129-132
- 623 Guiry MD, Guiry GM (2021) AlgaeBase, National University of Ireland, Galway
- 624 Guy-Haim T, Silverman J, Wahl M, Aguirre J, Noisette F, Rilov G (2020) Epiphytes provide micro-scale refuge
625 from ocean acidification. Marine Environmental Research 161:105093
- 626 Hall-Spencer JM, Rodolfo-Metalpa R, Martin S, Ransome E, Fine M, Turner SM, Rowley SJ, Tedesco D, Buia MC
627 (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. Nature 454:96-99
- 628 Harvey BP, Gwynn-Jones D, Moore PJ (2013) Meta-analysis reveals complex marine biological responses to the
629 interactive effects of ocean acidification and warming. Ecology and Evolution 3:1016-1030
- 630 Hedges LV (1981) Distribution Theory for Glass's Estimator of Effect Size and Related Estimators. Journal of
631 Educational Statistics 6:107-128
- 632 Hofmann LC, Bischof K (2014) Ocean acidification effects on calcifying macroalgae. Aquatic Biology 22:261-279
- 633 Holcomb M, DeCarlo T, Gaetani G, McCulloch M (2016) Factors affecting B/Ca ratios in synthetic aragonite.
634 Chemical Geology 437:67-76
- 635 Hurd CL, Hepburn CD, Currie KI, Raven JA, Hunter KA (2009) Testing methods of ocean acidification on algal
636 metabolism: consideration for experimental designs. Journal of Phycology 45:1236-1251
- 637 Jokiel PL, Rodgers KS, Kuffner IB, Andersson AJ, Cox EF, MacKenzie FT (2008) Ocean acidification and calcifying
638 reef organisms: a mesocosm investigation. Coral Reefs 27:473-483
- 639 Klochko K, Kaufman AJ, Yao W, Byrne RH, Tossell JA (2006) Experimental measurement of boron isotope
640 fractionation in seawater. Earth and Planetary Science Letters 248:276-285

- 641 Kroeker KJ, Kordas RL, Crim RN, Hendriks IE, Ramajo L, Singh GG, Duarte CM, Gattuso JP (2013) Impacts of
642 ocean acidification on marine organisms: quantifying sensitivities and interaction with warming.
643 *Global Change Biology* 19:1884-1896
- 644 Kuffner IB, Andersson AJ, Jokiel PL, Rodgers KS, MacKenzie FT (2008) Decreased abundance of crustose
645 coralline algae due to ocean acidification. *Nature Geoscience* 1:114-117
- 646 Legrand E, Riera P, Lutier M, Coudret J, Grall J, Martin S (2019) Grazers increase the sensitivity of coralline
647 algae to ocean acidification and warming. *Journal of Sea Research* 148-149:1-7
- 648 Martin S, Gattuso JP (2009) Response of Mediterranean coralline algae to ocean acidification and elevated
649 temperature. *Global Change Biology* 15:2089-2100
- 650 Martin S, Hall-Spencer JM (2017) Effects of Ocean Warming and Acidification on Rhodolith/Maërl Beds. In:
651 Riosmena-Rodríguez R, Nelson W, Aguirre J (eds) *Rhodolith/Maërl Beds: A Global Perspective*.
652 Springer International Publishing, Cham, pp55-85
- 653 Martin S, Rodolfo-Metalpa R, Ransome E, Rowley S, Buia MC, Gattuso JP, Hall-Spencer J (2008) Effects of
654 naturally acidified seawater on seagrass calcareous epibionts. *Biology Letters* 4:689-692
- 655 McCoy SJ, Pfister CA (2014) Historical comparisons reveal altered competitive interactions in a guild of
656 crustose coralline algae. *Ecology Letters* 17:475-483
- 657 McCoy SJ, Ragazzola F (2014) Skeletal trade-offs in coralline algae in response to ocean acidification. *Nature*
658 *Climate Change* 4:719
- 659 McCoy SJ, Kamenos NA (2015) Coralline algae (Rhodophyta) in a changing world: integrating ecological,
660 physiological, and geochemical responses to global change. *Journal of Phycology* 51:6-24
- 661 McCulloch MT, D'Olivo JP, Falter J, Holcomb M, Trotter JA (2017) Coral calcification in a changing world and
662 the interactive dynamics of pH and DIC upregulation. *Nature Communications* 8:15686
- 663 Milazzo M, Rodolfo-Metalpa R, Chan VBS, Fine M, Alessi C, Thiyagarajan V, Hall-Spencer JM, Chemello R (2014)
664 Ocean acidification impairs vermetid reef recruitment. *Scientific Reports* 4:4189
- 665 Moore B, Comeau S, Bekaert M, Cossais A, Purdy A, Larcombe E, Puerzer F, McCulloch MT, Cornwall CE (2021)
666 Rapid multi-generational acclimation of coralline algal reproductive structures to ocean acidification.
667 *Proceedings of the Royal Society B: Biological Sciences* 288:20210130
- 668 Nash MC, Opdyke NN, Troitzsch U, Russell BD, Adey WH, Kato A, Diaz-Pulido G, Brent C, Gardner M, Pritchard
669 J, Kline DI (2013) Dolomite-rich coralline algae in reefs resist dissolution in acidified conditions. *Nature*
670 *Climate Change* 3:268-272
- 671 Nelson WA (2009) Calcified macroalgae - critical to coastal ecosystems and vulnerable to change: A review.
672 *Marine and Freshwater Research* 60:787-801
- 673 Nybakken JW (2001) *Marine biology: An ecological approach*. Benjamin Cummings, San Francisco, CA
- 674 Peña V, Harvey BP, Agostini S, Porzio L, Milazzo M, Horta P, LeGall L, Hall-Spencer J (2021a) Major loss of
675 coralline algal diversity in response to ocean acidification. *Global Change Biology* In press
- 676 Peña V, Bélanger D, Gagnon P, Richards JL, Le Gall L, Hughey JR, Saunders GW, Lindstrom SC, Rinde E, Husa V,
677 Christie H, Fredriksen S, Hall-Spencer JM, Steneck RS, Schoenrock KM, Gitmark J, Grefsrud ES, Anglès
678 d'Auriac MB, Legrand E, Grall J, Mumford TF, Kamenos NA, Gabrielson PW (2021b) *Lithothamnion*
679 (Hapalidiales, Rhodophyta) in the changing Arctic and Subarctic: DNA sequencing of type and recent
680 specimens provides a systematics foundation. *European Journal of Phycology*:1-26
- 681 Perrin J, Vielzeuf D, Laporte D, Ricolleau A, Rossman GR, Floquet N (2016) Raman characterization of synthetic
682 magnesian calcites. *American Mineralogist* 101:2525-2538
- 683 Pierrot D, Lewis E, Wallace DWR (2006) MS Excel Program Developed for CO₂ System Calculations
- 684 Qui-Minet ZN, Coudret J, Davoult D, Grall J, Mendez-Sandin M, Cariou T, Martin S (2019) Combined effects of
685 global climate change and nutrient enrichment on the physiology of three temperate maerl species.
686 *Ecology and Evolution* 9:13787-13807
- 687 Qui-Minet ZN, Delaunay C, Grall J, Six C, Cariou T, Bohner O, Legrand E, Davoult D, Martin S (2018) The role of
688 local environmental changes on maerl and its associated non-calcareous epiphytic flora in the Bay of
689 Brest. *Estuarine, Coastal and Shelf Science* 208:140-152
- 690 Ries JB (2011) Skeletal mineralogy in a high-CO₂ world. *Journal of Experimental Marine Biology and Ecology*
691 403:54-64
- 692 Ries JB, Cohen AL, McCorkle DC (2009) Marine calcifiers exhibit mixed responses to CO₂-induced ocean
693 acidification. *Geology* 37:1131-1134
- 694 Russell BD, Thompson JJ, Falkenberg LJ, Connell SD (2009) Synergistic effects of climate change and local
695 stressors: CO₂ and nutrient-driven change in subtidal rocky habitats. *Global Change Biology* 15:2153-
696 2162

- 697 Smith AD, Roth AA (1979) Effect of carbon dioxide concentration on calcification in the red coralline alga
698 *Bossiella orbisiana*. *Marine Biology* 52:217-225
- 699 Sordo L, Santos R, Barrote I, Silva J (2018) High CO₂ decreases the long-term resilience of the free-living
700 coralline algae *Phymatolithon lusitanicum*. *Ecology and Evolution* 8:4781-4792
- 701 Twist BA, Neill KF, Bilewitch J, Jeong SY, Sutherland JE, Nelson WA (2019) High diversity of coralline algae in
702 New Zealand revealed: Knowledge gaps and implications for future research. *PLoS one* 14:e0225645-
703 e0225645
- 704 Twist BA, Cornwall CE, McCoy SJ, Gabrielson PW, Martone PT, Nelson WA (2020) The need to employ reliable
705 and reproducible species identifications in coralline algal research. *Marine Ecology Progress Series*
706 654:225-231
- 707 Vásquez-Elizondo RM, Enríquez S (2016) Coralline algal physiology is more adversely affected by elevated
708 temperature than reduced pH. *Scientific Reports* 6:19030
- 709