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7 **Short-term interactive effects of increased temperatures and acidification on the**
8 **calcifying macroalgae *Lithothamnion crispatum* and *Sonderophycus capensis***
9

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32
33 **Abstract**

34 Combined effect of acidification and sea warming derived from future conditions
35 of Climate Change have been little investigated in marine photoautotrophs, especially on
36 sensitive organisms such as calcifying macroalgae. The aim of this investigation was to
37 evaluate the interactive effects of acidification and increased temperatures on the two
38 Brazilian calcifying macroalgae *Lithothamnion crispatum* and *Sonderophycus capensis*.
39 Both species were cultured for 48 h under: (1) current pH (7.8 ± 0.2) and temperature (18
40 $\pm 2^\circ\text{C}$) during winter; (2) future pH (7.4 ± 0.2) and temperature ($30 \pm 2^\circ\text{C}$) during
41 summer; (3) current temperature and future pH; and (4) future temperature and current

42 pH. We evaluated photosynthetic performance (measured $\Delta F/F'_m$), growth rates (weight),
43 relative carbonate content, and total phenolic compounds. Our results showed similar
44 negative effects under decreased pH and increased temperatures in both species, although
45 carbonate content *S. capensis* was less affected than in *L. crispatum*. Total phenolic
46 compounds measured in *S. capensis* showed the highest levels at potential future pH and
47 temperature conditions. Given that stress conditions associated with decreased pH and
48 increased temperatures are important inductors of an oxidative response, it is likely that
49 phenolic compounds are synthesized to fulfil an antioxidant purpose. Even though
50 physiological performance was affected in both calcifying macroalgae under the most
51 likely negative future pH and temperature conditions, their biological viability indicates
52 they may be able to thrive under coming Climate Change scenarios.

53

54 **Keywords:** Climate Change; acidification; warming; macroalgae.

55

56 INTRODUCTION

57 Atmospheric carbon dioxide (CO₂) concentrations have been increasing from 280
58 ppm since the pre-industrial period to above 400 ppm nowadays; moreover, CO₂ levels are
59 expected to increase in up to 970 ppm by the end of the 21st Century (Harley et al. 2006;
60 Harley et al. 2012). Predicted CO₂ rise and accompanied increase in global temperatures
61 is expected to induce greater sea surface temperatures, which could fluctuate between 2
62 and 6°C depending on the strictness of the extrapolation (IPCC 2014). On the other hand,
63 approximately 25% of atmospheric CO₂ is absorbed by the ocean, which in reaction with
64 seawater increases the levels of carbonic acid (H₂CO₃), subsequently diminishing the pH;
65 this process is expected to induce a 0.2-0.4 pH decline by the year 2100 (Feely et al.
66 2004). Ocean acidification produces changes in total dissolved inorganic carbon (DIC),
67 manifested in higher concentration of carbonate ions (CO₃²⁻) and bicarbonate (HCO₃⁻);
68 important molecules that may affect physiological processes, mostly in marine calcifying
69 organisms (Martin and Hall-Spencer 2017; Orr et al. 2005; Koch et al. 2013). As signal
70 of Climate Change, monitoring of coastal marine habitats has revealed a concerning
71 increase in the incidence of mass mortalities in flora and fauna due to marine heat waves
72 as observed in Australia (Wernberg et al. 2011) or in the south Atlantic (Ferreira et al.

73 2015). Elevated temperature, even over a short period, can be lethal due to biochemical
74 damages (Gouvêa et al. 2017). In these coastal and shallow regions, high pH variability is
75 observed, eventually exposing organisms to conditions frequently predicted to the end of
76 this century considering ocean acidification (Duarte et al. 2013). Changes in ocean
77 circulation also provide localized and short variability in ocean pH due
78 upwelling/downwelling zones (Gruber et al. 2012; Findlay et al. 2013), CO₂ enrichment
79 from volcanic vents (Hall-Spencer et al. 2008), and as a function of the metabolism of
80 some marine communities (Anthony et al. 2011) or even in anoxic zones (Melzner et al.
81 2013). Therefore, extreme values of temperature and pH/pCO₂ present in different
82 regions of the world can accentuate or even represent nowadays ocean acidification
83 impacts on the biology of marine biodiversity.

84 Calcifying macroalgae are key ecological components of the Atlantic coast. They
85 are considered ecosystem bioengineers, as create habitat, niche for settlement and nursery
86 for other algae, invertebrates, and fish (Figueiredo et al. 2012; Riul et al. 2009; Amado-
87 Filho et al. 2007). Furthermore, these organisms are major producers of carbonate
88 sediment in the marine environment (Basso 2012). Previous studies have shown that
89 unbalanced DIC mediated by decreased pH reduces the saturation of calcium carbonate
90 (CaCO₃) in calcifying marine organisms, such calcifying macroalgae, corals, foraminifera
91 and coccolithophores; this process can affect calcification rates when producing new
92 skeleton (Basso 2012; Martin and Hall-Spencer 2017; Orr et al. 2005). CaCO₃ occurs in
93 skeletons of calcifying macroalgae as calcite or aragonite; however, magnesium (Mg²⁺)
94 can replace calcium in calcite macroalgae, which has been observed to be more prone to
95 dissolution under acidification than macroalgae with aragonite skeleton (Basso 2012;
96 Jury et al. 2010; Smith et al. 2012).

97 Calcifying macroalgae are capable of performing calcification and photosynthesis
98 at different rates depending on CO₂ concentrations (Hofmann et al. 2012; Hurd et al.
99 2009; Koch et al. 2013). Despite that acidification can produce adverse effects on
100 calcification rates, changing carbonate content, high concentrations of CO₂ have been
101 detected to favor photosynthesis in some cases (Hurd et al. 2009; Koch et al. 2013). For
102 instance, Semesi et al. (2009) showed that a progressive decline in pH induced a 13%
103 increase in photosynthesis rates in coralline algae, although compromising their structure

104 due to 20% less calcification. Tropical and subtropical organisms survive near suboptimal
105 physiological temperature; however, Climate Change-mediated increase in sea
106 temperatures threatens their thermal tolerance thresholds (Latham 2008; Eggert 2012;
107 Harley et al. 2006). Within tolerance ranges, rising temperature can induce an increase in
108 photosynthesis, growth and carbonate deposition in calcifying macroalgae, but when
109 these exceed tolerance thresholds, it can cause bleaching and chlorophyll degradation
110 (Martin and Hall-Spencer 2017; Glynn 1996).

111 Drastic environmental disturbances can generate biological stress in macroalgae
112 through the over-production of reactive oxygen species (ROS) (Bischof and Rautenberger
113 2012). ROS excess can produce damage by oxidizing lipids, proteins and nucleic acids;
114 however, the cell can maintain ROS within homeostatic levels through the production of
115 antioxidant compounds (Moenne et al. 2016). In this context, phenolic compounds have
116 been observed to have strong ROS scavenging properties (Sáez et al. 2015; Flores-
117 Molina et al. 2016). ROS-induced increase in phenolic compounds in macroalgae have
118 been observed subject to several environmental stressors, among which can be mentioned
119 herbivory, changes in temperature, salinity, irradiance and metal excess (Bischof and
120 Rautenberger 2012; Moenne et al. 2016; Sáez et al. 2015; Flores-Molina et al. 2016).

121 Records on the interaction of different stressors associated with Climate Change
122 on marine calcifying macroalgae are scarce; thus, more information on aspect such as the
123 combined effects of acidification and increased temperatures would significantly improve
124 our understanding on their current and future responses to progressive Climate Change.
125 The aim of this research was evaluate the interactive effects of a short acidification and
126 warming on physiological aspects of the calcifying red coralline *Lithothamnion*
127 *crispatum* Hauck and the red aragonite *Sonderophycus capensis* (M) M.J. Wynne. *L. L.*
128 *crispatum* has a wide range of latitudinal distribution; considered as a dominant species
129 in the entire Atlantic coast of Brazil, its temperature tolerance ranges between 15 to 30°C
130 (Pascelli et al. 2013; Riul et al. 2009). On the other hand, *S. capensis* has a limited
131 distribution (southeastern Atlantic of Brazil) with tolerance ranges defined between 17
132 and 20°C (Zavialov et al. 1999). Taking into account limits of temperature tolerance and
133 CaCO₃ types of skeleton in these species, it is expected that *L. crispatum* and *S. capensis*

134 have differential responses to single and combined effects of increased temperatures and
135 acidification.

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138

139 MATERIALS AND METHODS

140 *Collection and experimental design.* Entire individuals of two calcifying red
141 macroalgae species, the coralline *L. crispatum* and the aragonite-based *S. capensis*, were
142 collected at 10 m depth from Reserva Biologica do Arvoredo, in Santa Catarina
143 (27° 15' S, 48° 20' W) and in Xavier Island (27° 36' S, 48° 23' O), respectively, in
144 Brazil. After collection, individuals were stored in plastic bags with seawater, and
145 immediately transported inside coolers to the Laboratory of Phycology at Universidad de
146 Santa Catarina. Prior to experiments, the specimens were acclimated for 24 h with
147 filtered seawater (0.45 µm) under constant aeration, temperature ($15 \pm 2^\circ\text{C}$) and light (50
148 µmol photons $\text{m}^{-2}\text{s}^{-1}$). The experiment was carried out in a microcosm system over a
149 short-term period (48 h). The experimental system consisted in two tanks with 50 L
150 seawater that delivered seawater with a pump to 12 chambers of 1 L. Both macroalgae
151 species were randomly distributed and maintained in four conditions: **(1)** average current
152 pH (7.8 ± 0.2) and temperature ($18 \pm 2^\circ\text{C}$) registered in Florianopolis bay, Brazil, during
153 winter **(2)**; future values of pH (7.4 ± 0.2) and temperature ($30 \pm 2^\circ\text{C}$) that can be
154 extrapolated for Florianopolis bay during summer by the end of the 21st Century
155 according to predictions by IPCC (2014); **(3)** Current temperature ($18 \pm 2^\circ\text{C}$) and future
156 pH (7.4 ± 0.2); and **(4)** Future temperature ($30 \pm 2^\circ\text{C}$) and current pH (7.8 ± 0.2). For
157 each treatment, three independent replicates were used. pH levels considered for control
158 treatments respond to local levels registered in seawater nearby macroalgae collection
159 sites. Certainly, in these locations there are several estuaries, such as Barra de Lagoa, that
160 supply freshwater and inland material that induces a pH drop in coastal waters; in this
161 context, several reports in this area show average pH levels around 7.8 (Martins-Pereira
162 2004; Dalinghaus 2016; Cancellier-Cechinel 2013). Considering the latter, acidification

163 treatments with a 0.4 pH decrease were made according IPCC predictions upon current
164 local pH levels.

165 To achieve low pH conditions, bubbles of CO₂ were injected with a pump the
166 experimental seawater. The added CO₂ were managed by a pH controller, model pH2010
167 (Weipro, China), connected to solenoid valves that regulated the amount of gas pumped
168 into the seawater (Russell et al. 2009). Experimental temperatures were achieved by
169 placing tanks on a homemade temperature-gradient table; this is a horizontal metal-made
170 table, which surface temperature gradually increases from 15 °C to 35 °C, from one
171 extreme to the other. In addition, the table constantly agitates to maintain temperature
172 homogeneity in the seawater.

173 ***Water carbonate chemistry parameters.*** HCO₃⁻² and CO₃⁻² concentrations, pCO₂,
174 calcite and aragonite saturation (Ω), were obtained via CO₂Sys_v2.1.xls program, using
175 K1, K2, as in Millero et al. (1998). These values are summarized in Table 1. Estimations
176 were based in measurements of pH, temperature, salinity and total alkalinity in seawater.
177 Gran titration method was used to measure total alkalinity, using 0.0025 N hydrochloric
178 acid (HCl; Merck) (Carmouze 1994); these measurements were taken after 48 h of
179 experiments.

180 ***Photosynthetic performance.*** Photosynthetic measurements were performed
181 throughout the course of the experiments in all treatments, at 6, 24 and 48 h. Effective
182 quantum yield of photosystem II (PII; $\Delta F/F'_m$) was measured using a portable pulse-
183 amplitude modulated (PAM) chlorophyll α fluorometer (DIVING PAM; Walz GmbH,
184 Effeltrich, Germany). This parameter was measured according to Schreiber and Neubauer
185 (Schreiber and Neubauer 1990): $\Delta F/F'_m \hat{=} (F'_m - F_t)/F'_m$. In the latter, F'_m is the maximal
186 fluorescence of light-acclimated sample induced by a saturating actinic light pulse (9000
187 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 800 ms), and F_t is the intrinsic steady-state fluorescence emission
188 in light-acclimated sample (Baker 2008).

189 ***Growth rate.*** To estimate the growth, the change in fresh weight (FW) biomass of
190 the species was measured, prior and after 48 h of experiments. The mean relative growth
191 was expressed as FW per day (FW d⁻¹), and calculated according to Packard and

192 Boardman (1999): $RG = \frac{W_t - W_0}{t}$. W_0 is the initial weight, W_t is final weight, and t is time
193 in days (d).

194 **Relative carbonate content (RCC).** Biomass was dried at 60°C for 24 h and
195 weighted. Initial weight (DW_i) was obtained and then immersed in 10% HCl (Merck) for
196 24 h to initiate CaCO₃ dissolution, in order to maintain the same weight/volume ratio.
197 After the experiments, samples were dried (60°C) again and were re-weighted (DW_f)
198 (Figueroa et al. 2014). RCC as a proxy of calcification was calculated to the formula: %
199 RCC $((DW_i - DW_f) / DW_f) * 100$.

200 **Content of total phenolic compound.** Due to biomass availability, total phenolic
201 compounds were only determined in *S. capensis*, according to Randir et al. (2002). After
202 48 h experiments, samples were immediately frozen in liquid nitrogen and stored at -
203 80°C. Between 40-50 mg biomass were grounded to powder in a mortar with liquid
204 nitrogen, at 4°C chamber in the darkness. One mL of 80% methanol (Merck) and 5 ml of
205 95% methanol (Merck) were added to each sample in centrifuge tubes. Samples were
206 centrifuged at 10,000 rpm for 5 min at room temperature. One mL of the supernatant was
207 placed in a clean tube and mixed with 5 mL of distilled water, 1 mL of 95% ethanol and
208 500 µL of Folin-Ciocalteu; the mixture was incubated for 5 min in the darkness. Then, 1
209 mL of NaCO₃ (5% w/v) was added and incubated in the dark for 1 h at room temperature.
210 Finally, absorbance was measured in a spectrophotometer, model P-220 (Biospectro,
211 Brazil), at 725 nm. To calculate the content of total phenolic compounds from crude
212 extracts, a standard curve of known concentrations of gallic acid was used (50 to 800 µg
213 mL⁻¹ – $r^2 = 0.99$; $y = 1.254$).

214 **Statistical analyses.** Effects of temperature and pH on both species ($\Delta F / F_m'$,
215 RCC, growth rate and phenolics content), and chemical changes in seawater, were
216 assessed statistically by using a factorial analysis of variance (Factorial-ANOVA). A *post*
217 *hoc* analysis of Student Newman-Keuls tests (SNK) was applied when Cochran's C test
218 and visual inspection of residuals were conducted to test homogeneity of variances
219 (Underwood 1997). A level of significance of 99% confidence interval ($p = 0.01$) was
220 applied. These analyses were performed using the software Statistica version 7 (StatSoft
221 Inc., Tulsa, OK, USA).

222 RESULTS

223 Photosynthetic responses

224 For both species, a significant interaction was found between temperature (18 and 30°C)
225 and pH (7.8 and 7.4) on photosynthetic performance (measured as $\Delta F/Fm'$) (Factorial
226 ANOVA, $p < 0.01$; Figure 1; Table 2). $\Delta F/Fm'$ significantly increased with respect to
227 basal values in *L. crispatum* after 6, 24 and 48 h experiments at 18°C under both 7.4 and
228 7.8 pH conditions (Figure 1A). A similar trend was observed in *L. crispatum* at 30°C
229 under pH 7.4, although without significant differences at 24 h experiments (Figure 1A). Δ
230 F/Fm' significantly decreased under pH 7.8 at 30°C in all experimental times, with lower
231 values at 24 and 48 h experiments. In relation to *S. capensis*, no specific trends compared
232 to basal values were observed at 18°C under both pH conditions and at 30°C under pH
233 7.4 (Figure 1B). However, significantly lower $\Delta F/Fm'$ levels were recorded at 30°C
234 under pH 7.8, especially at 24 and 48 h experiments (Figure 1; Table 2).

235 Responses in calcification

236 For both species a significant interaction was observed between temperature (18 and
237 30°C) and pH (7.8 and 7.4) on RCC (Factorial ANOVA, $p < 0.01$; Figure 2; Table 2).
238 Similar trends were observed in RCC between studied species. When decreasing the pH
239 from 7.8 to 7.4, RCC decreased significantly under both 18 and 30°C (Figure 2). The
240 exception was at 30°C in *L. crispatum*, where no significant differences were detected
241 between both experimental pH conditions (Figure 2). }

242 Relative growth rate

243 No changes were recorded in growth rates in *L. crispatum*, with exception of the
244 treatment at 30°C and pH 7.8, where negative values were observed (Figure 3A). A
245 similar trend of negative growth rates were detected in *S. capensis* at 30°C and pH 7.8,
246 although the rest of the treatments were similarly positive (Factorial ANOVA, $p < 0.01$;
247 Figure 3B; Table 2).

248 Phenolic compound

249 A significant interaction was observed between temperature (18 and 30°C) and
250 pH (7.8 and 7.4) on total phenolic content in *S. capensis* (Factorial ANOVA, $p < 0.01$;
251 Figure 4A). Under both experimental temperatures, total phenolic content increased at pH
252 7.4 with respect to 7.8.

253 **DISCUSSION**

254 In this investigation, it was observed that the two calcareous macroalgae *L.*
255 *crispatum* and *S. capensis* suffered similar negative effects subject to the effects of
256 increased temperatures, from 18 (similar to nowadays winter temperatures) to 30°C
257 (comparable to summer end of the 21st Century temperatures), or decreased pH, from
258 current 7.8 to expected future 7.4 (by the year 2100); this was evidenced in terms of
259 photosynthetic performance ($\Delta F/F_m'$), relative carbon content (RCC) and growth rates.
260 However, when increased temperatures (30°C) were combined with acidification (pH
261 7.4), at least in terms of photosynthesis and growth, both species seemed to be less
262 affected than when both stressors were applied independently. In terms of calcification,
263 acidification at 18°C caused a dramatic decay in RCC in both *L. crispatum* and *S.*
264 *capensis*. Moreover, although RCC decreased under increased temperatures and
265 acidification, compared to 18°C and pH 7.8, levels remained always higher than at 18°C
266 and 7.4 pH; this for both calcareous macroalgae species. Indeed, increased temperatures
267 appeared to diminish the negative effects induced by acidification, especially in relation
268 to calcification, since at increased temperatures of 30°C and low pH of 7.4 both
269 macroalgae species displayed higher RCC if compared to treatments at 18°C and pH 7.4.
270 Total phenolic content measured in *S. capensis* showed the lowest levels at nowadays
271 temperatures and pH. These increased similarly at 18°C and pH 7.4, and at 30°C and pH
272 7.8, and with the highest values at 30°C and pH 7.4.

273 The detrimental effects of increased temperatures on *L. crispatum* and *S. capensis*
274 could be attributed exceeding the tolerance thresholds of both species. Certainly, it has
275 been observed that increased temperatures beyond tolerance ranges can induce an excess
276 in the levels of reactive oxygen species (ROS) and subsequently oxidative stress in other
277 photoautotrophs. For instance, it has been observed that the reaction catalyzed by the

278 enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO), located in the
279 chloroplast and involved in carbon fixation and photorespiration, can cause greater levels
280 of the ROS H_2O_2 under high temperatures due to increased oxygenase reactions (Kim and
281 Portis 2004). Moreover, it has been observed that increased temperatures induce
282 incomplete H_2O oxidation in the PSII electron donor side, forming H_2O_2 , which is then
283 reduced by manganese to the highly oxidizing $\text{HO}\cdot$ through Fenton reaction (Pospíšil
284 2016). In this context, Müller et al. (2012) found a systematic increase in the ROS $\cdot\text{O}^{2-}$ in
285 parallel with greater temperatures of up to 18°C in the gametophytes of the brown
286 macroalgae *Alaria esculenta*, *Laminaria digitata*, and *Saccharina latissima*. An extended
287 oxidative stress in macroalgae is capable of causing damage and degradation to
288 photosynthetic pigments; thus, affecting photosynthesis and development (Moenne et al.
289 2016). The effects of high temperatures have been reported in the rhodolith *Lithophyllum*
290 *margaritae*, which from 10 to 30°C showed optimum oxygenic photosynthetic threshold
291 at 25°C ; however, when values exceeded these temperatures photosynthesis began to
292 decline (Steller et al. 2007). Reports by Vásquez-Elizondo and Enríquez (2016)
293 demonstrated that from 30°C to 32°C , photosynthesis (F_v/F_m) suffered nearly a 45%
294 decline after 3 d of experiments in rhodolith *Neogoniolithon* sp., articulate *Amphiroa*
295 *tribulus* and crustace *Lithothamnion* sp. Despite the latter, similar increased-temperature
296 experiments (from optimal) on other tropical calcareous macroalgae, such as
297 *Tricleocarpa cylindrica*, *Padina gymnospora*, and *Lithothamnion corallioides* have not
298 evidenced negative effects in terms of photosynthetic performance (Schermer et al. 2016;
299 Noisette et al. 2013). Therefore, photosynthetic performance under increased
300 temperatures seems to be a species-specific feature in calcifying macroalgae.

301 In relation to the effects of acidification, our results demonstrated that negative
302 effects of increasing temperatures from 18 to 30°C were softened when decreasing the
303 pH from 7.8 to 7.4 . Our records are similar to those reported by Semesi et al. (2009),
304 which observed that a progressive decrease in pH (from 9.8 to 7.6) were accompanied by
305 an exponential increase in photosynthetic O_2 exchange in the rodolith *Hydrolithon* sp.
306 Recent studies by Scherner et al. (2016) demonstrated the differential effects of low pH
307 on photosynthesis in calcifying macroalgae; while from pH 8.1 to 7.2 F_v/F_m decreased in
308 *Lithophyllum stictaeforme*, *Pneophyllum conicum* and *Porolithon pachydermum*, in

309 *Tricleocarpa cylindrica* it increased. Additionally, Kram et al. (2015) observed no change
310 in photosynthesis in the articulated *Jania adhaerens* and in the incrusting *Lithothamnion*
311 *californicum* from pH 8.1 to 7.6, although growth rates decreased. Even though
312 photosynthesis enhancement has been observed in different macroalgae species subject to
313 acidification, published research suggests that this phenomenon may be more related to
314 CO₂ availability rather than reduction of pH itself. According to Martin and Hall-Spencer
315 (2017), higher concentration of CO₂ in seawater induces macroalgae to rely exclusively
316 of CO₂ diffusion, own-regulating their CO₂-concentrating mechanisms (CCM) to save
317 energy. Certainly, high availability of diffused CO₂ may provide greater substrate for
318 RUBISCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) and carbon fixation,
319 inducing photosynthesis and growth (Martin and Hall-Spencer (2017).

320 The decalcification of calcifying macroalgae induced by acidification has been
321 already well described (Basso 2012; Feely et al. 2004). Likewise, we observed similar
322 decalcification (about 80%) under lower pH from 7.8 to 7.4 at 18°C in both *L. crispatum*
323 and *S. capensis*. Semesi et al. (2009) showed that a progressive decrease in pH values
324 from a 10 to 7.5 led to nearly 100% decalcification in *Hydrolithon* sp. Similarly, Noisette
325 et al. (2013) observed decrease in calcification rates from pH 8.1 to 7.7 in *Lithothamnion*
326 *corallioides*. Interestingly, these authors observed that at an increase in environmental
327 temperatures reduced the impact of pH 7.7 in calcification rates. In this context, other
328 studies on coralline algae have shown higher levels of calcification during summer, when
329 average sea surface temperatures are the highest (Steller et al. 2007; Martin and Gattuso
330 2009). Despite the latter, there are also investigations that account for the combined
331 negative effects of increased temperatures and acidification on calcification rates, as
332 described for the coralline *Porolithon onkodes* (Anthony et al. 2008; Diaz- Pulido et al.
333 2012). The information suggests that although temperature softened the detrimental
334 effects of acidification (and increased CO₂) in *L. crispatum* and *S. capensis*, this seems to
335 be an intraspecific feature that cannot be applied to all calcifying macroalgae.

336 Another feature assessed in *S. capensis* was its phenolic content upon exposure to
337 increased temperatures and decreased pH. Total phenolics increased at nowadays
338 temperatures under acidification, but also at increased temperatures and subject to lower
339 pH. Different investigations have revealed that increased temperatures and acidification

340 are capable of inducing an oxidative stress condition due to de over-production of ROS
341 by different metabolic pathways (e.g. Celis-Plá et al. 2017; Flores- Molina et al. 2016;
342 Pospíšil 2016). It has been well described the role of phenolic compounds as antioxidants
343 to counteract ROS-excess (Moenne et al. 2016); thus, the records may indicate that *S.*
344 *capensis* induces the production of phenolic compounds to inactivate ROS and avoid
345 oxidative damage.

346 Short-term assessment of temperature and acidification conditions on
347 physiological and metabolic features of *L. crispatum* and *S. capensis* reinforce the
348 importance of local stressors to promote local baseline shifts. In addition, similar records
349 on other macroalgae species suggest that short-term responses may be similar to those
350 expressed in a long-term and, thus, may provide a good representation of potential
351 tolerance and survival thresholds under upcoming environmental conditions. For
352 instance, Chen et al. (2017) observed that within 60 min, light-saturated net
353 photosynthetic O₂ evolution rate (NPRm) in the green macroalga *Ulva lactuca* decreased
354 steadily when the pH dropped from 8.2 to 7.5 and, even more, to 6.5. After this time,
355 NPRm decrease stopped and levels were maintained without a trend of change. Indeed,
356 similar NPRm levels were observed in the alga exposed to pHs 7.5 and 6.5 at 60 min, 90
357 min and 8 days of experiments. Similar records on short-term exposure (within 48 h) to
358 future climate change scenarios have recorded in other macroalgae species belonging to
359 different phylogenetic groups (e.g. Celis-Pla et al. 2014; Cruces et al. 2017; Flores-
360 Molina et al. 2016).

361 In conclusion, we observed that both macroalgae species, the coralline *L.*
362 *crispatum* and the aragonite *S. capensis*, displayed similar physiological responses to
363 acidification. Moreover, and considering the interaction of higher sea surface
364 temperatures and acidification, the information suggests that both assessed macroalgae
365 species may be able to cope and develop under expected future marine environmental
366 scenarios mediated by Climate Change.

367

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375 in Ocean Islands: Biodiversity, Chemistry, Ecology and Biotechnology; Rede Coral Vivo
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580 **Figures**

581 **Figure 1.** Changes in maximum fluorescence ($\Delta F/Fm'$) emitted by (A) *Lithothamnium*
582 *crispatum* and (B) *Sonderophycus capensis* exposed to increased temperatures and
583 decreased pH for up to 48 h. Data correspond to mean \pm SD ($n=9$). Letters represent
584 statistical differences at 95 % confidence interval (SNK tests, $p < 0.05$).

585

586 **Figure 2.** Changes in relative carbon content (RCC), as a proxy of calcification rates,
587 observed in (A) *Lithothamnium crispatum* and (B) *Sonderophycus capensis* exposed to
588 increased temperature and decreased pH for up to 48 h. Data correspond to mean \pm SD
589 ($n=3$). Letters represent statistical differences at 95% confidence interval (SNK tests, $p <$
590 0.05).

591

592 **Figure 3.** Change in growth rate measured in (A) *Lithothamnium crispatum* and (B)
593 *Sonderophycus capensis* exposed to increased temperature and decreased for up to 48 h.
594 Data correspond to mean \pm SD ($n=3$). Letters represent statistical differences at 95%
595 confidence interval (SNK tests, $p < 0.05$).

596

597 **Figure 4.** Change in total phenolic compound in *Sonderophycus capensis* exposed to
598 increased temperature and decreased pH for up 48 h. Data correspond to means \pm SD
599 ($n=6$). Letters represent statistical differences at 95% confidence interval (SNK tests, $p <$
600 0.05).

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608 **Table 1** Summary of the chemical characteristics of experimental seawater, including T°,
 609 pH, $p\text{CO}_2$, HCO_3^- and CO_3^{2-} concentration, Ω_{calcite} (Ω_{Ca}) and $\Omega_{\text{aragonite}}$ (Ω_{Ar}).

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T(°C)	pH	$p\text{CO}_2$ (ppm)	HCO_3^- ($\mu\text{mol kg SW}^{-1}$)	CO_3^{2-} ($\mu\text{mol kg SW}^{-1}$)	Ω_{Ca}	Ω_{Ar}
18±2	7.8±0	432±14	1164±51	62.563±3.2	1.491±0.07	0.96±0.04
18±2	7.4±2	2035±19	1387±13	18.816±1.8	0.449±0.04	0.290±0.02
30±2	7.4±2	2504±23	1590±15	33.393±3	0.811±0.07	0.543±0.05
30±2	7.8±0	455±1.10	1148±0.24	95.524±0.6	2.319±0.01	1.553±0.01

614

615 Note: $n = 3$ (means \pm SDs). Abbreviations: T (°C) = Temperature, $p\text{CO}_2$ = partial
 616 pressure of carbon dioxide, HCO_3^- = bicarbonate, CO_3^{2-} = carbonate, Ω_{Ca} = Calcite
 617 saturation, Ω_{Ar} = Aragonite Saturation.

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630 **Table 2** Summary table of Factorial ANOVA of the effects of temperature and pH
 631 treatment on $\Delta F/Fm'$ and calcification percentage in *Lithothamnion crispatum* and
 632 *Sonderophycus capensis*.

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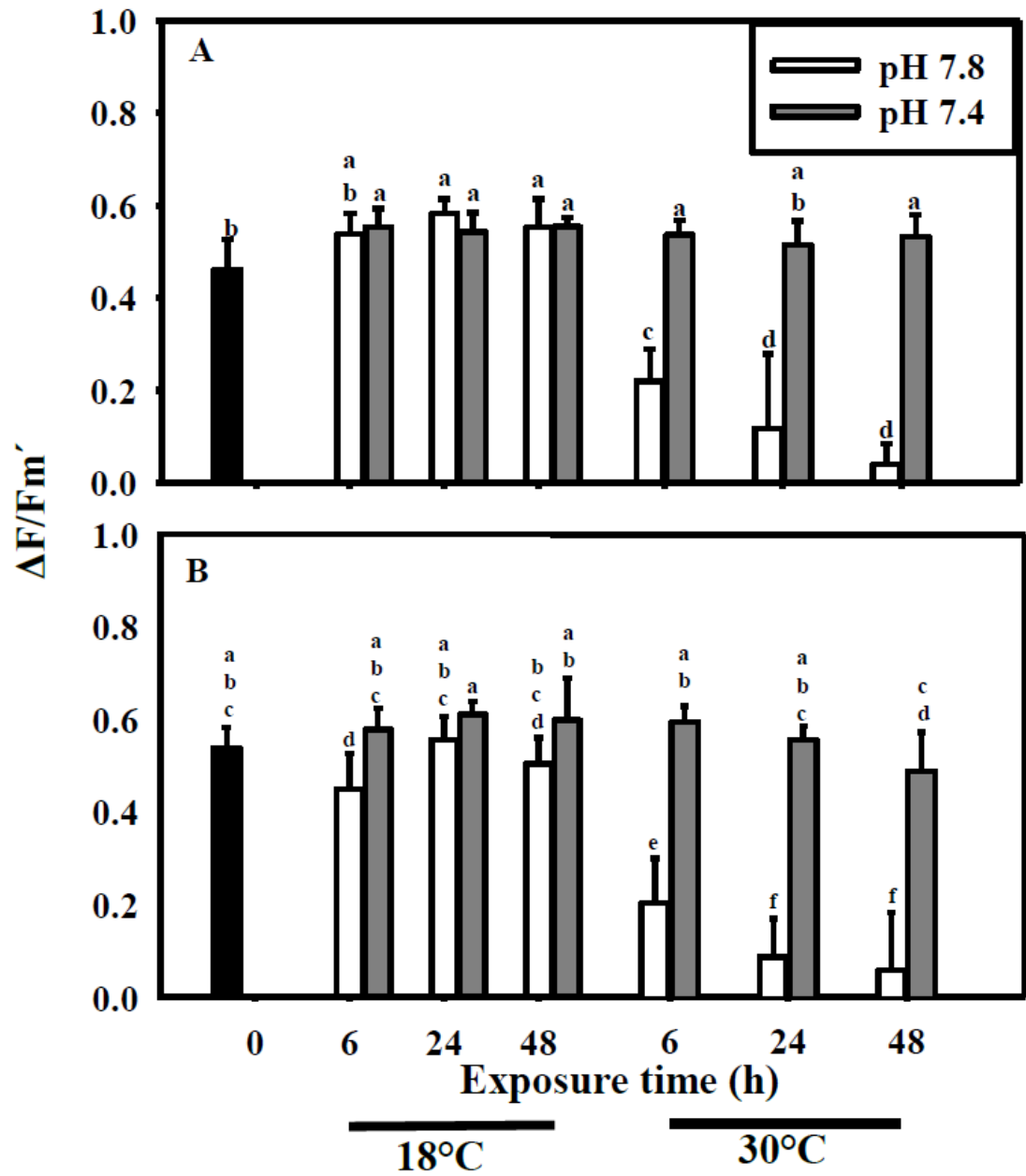
	<i>Lithothamnion crispatum</i>				<i>Sonderophycus capensis</i>			
	<i>df</i>	MS	F	<i>p</i>	<i>df</i>	MS	F	<i>p</i>
$\Delta F/Fm'$								
Temperature (T)	1	0.25	110.53	p<0.01	1	0.11	25.940	p<0.01
pH	1	0.25	108.05	p<0.01	1	0.60	131.84	p<0.01
T*pH	1	0.20	88.84	p<0.01	1	0.15	33.76	p<0.01
Error	32	0.002			32	0.004		
Calcification								
T	1	13.84	0.24	0,63	1	0.49	0.01	0,911
pH	1	4793.10	86.23	p<0.01	1	5159.99	141.58	p<0.01
T*pH	1	3301.41	59.40	p<0.01	1	1474.34	40.45	p<0.01
Error	8	55.58			8	36.45		

Note:

$n = 8$ for $\Delta F/Fm'$; $n = 3$ for calcification percentage. Abbreviations: T = Temperature, *df* = degrees of freedom.

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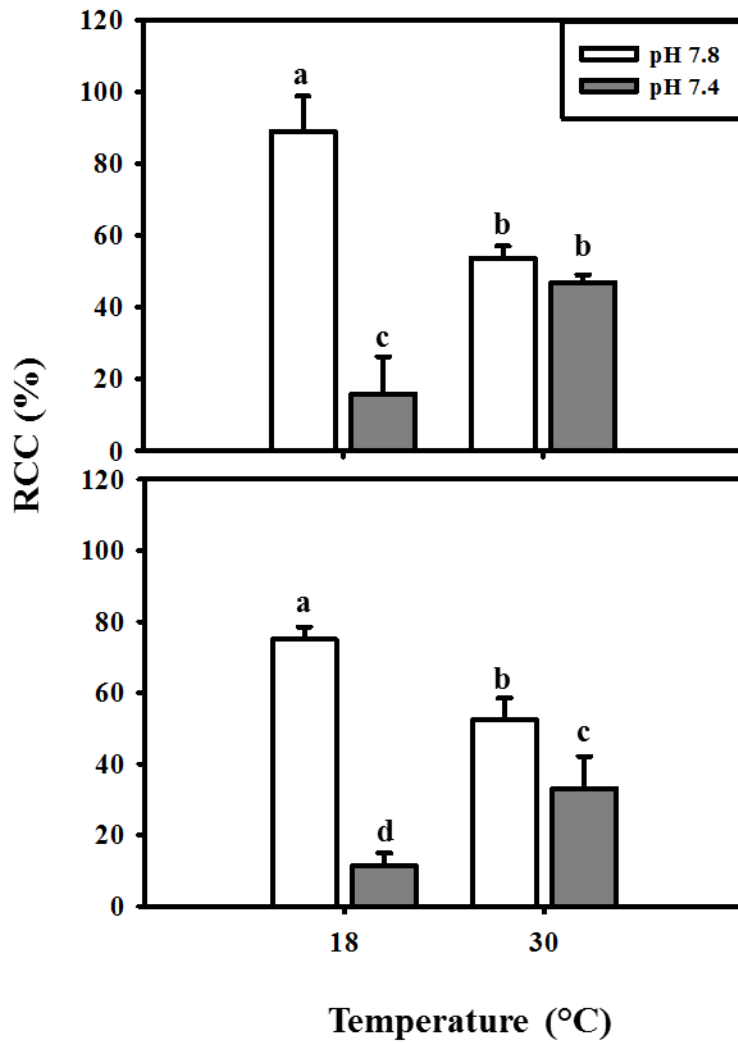
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654 **Figure 1**

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Figure 2

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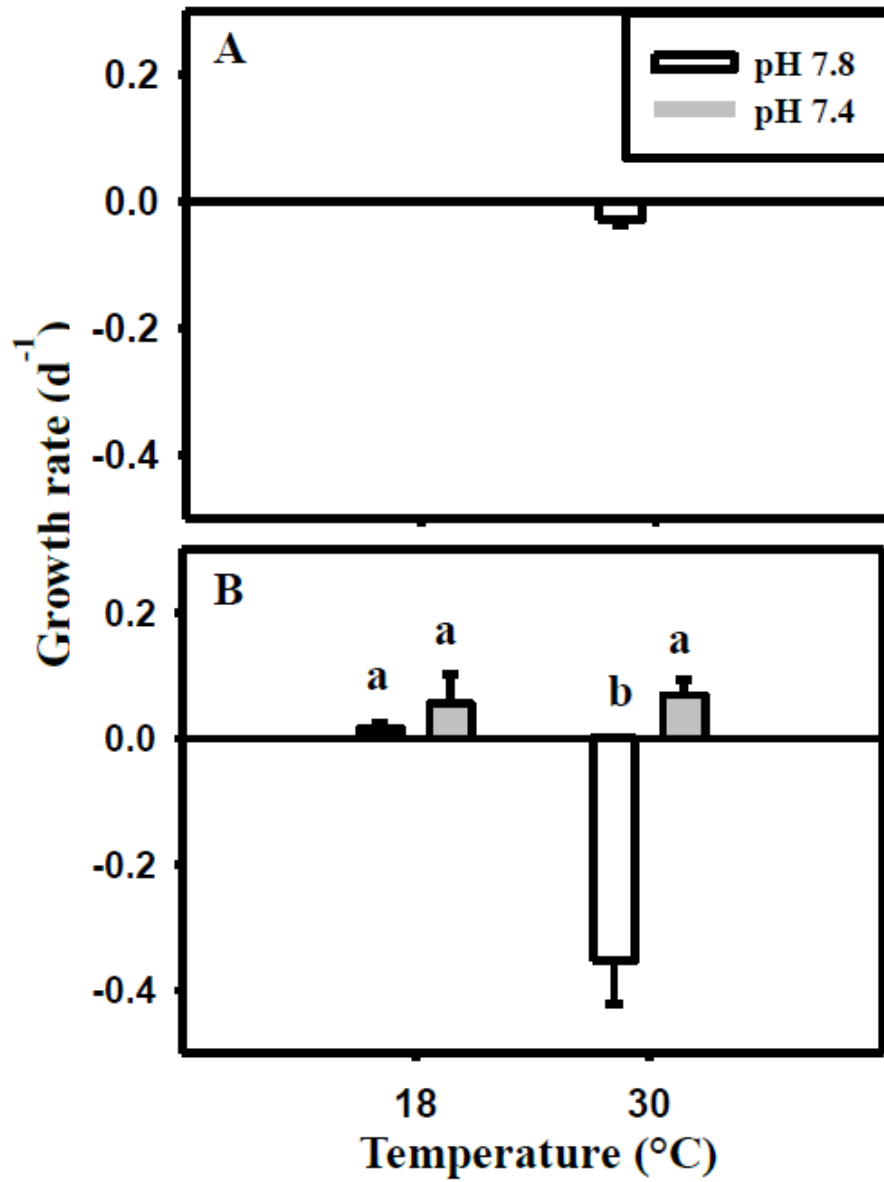
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667 **Figure 3**

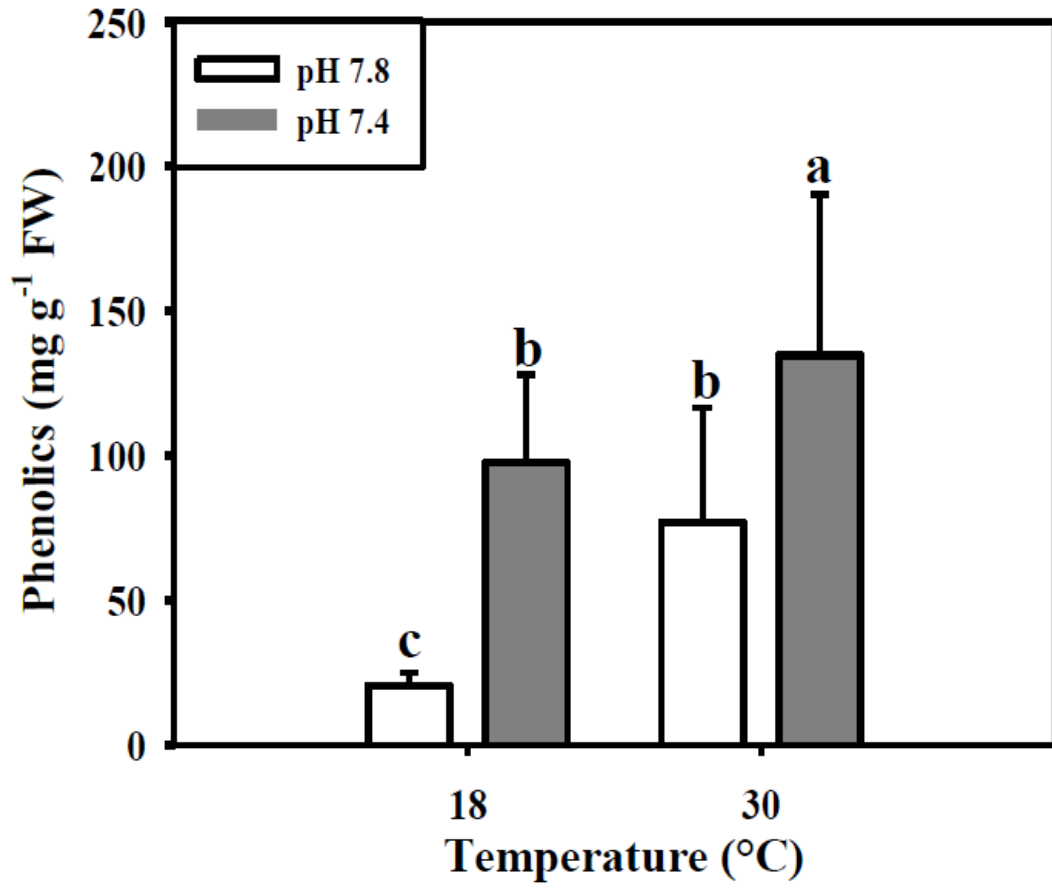
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Figure 4

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