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

Munoz, PT; Saez, CA; Brezo, Martinez-Callejas M; Rosa, Flores-Molina M; Bastos, Eduardo; Fonseca, Alessandra; Gurgel, C. Frederico D.; Barufi, José Bonomi; Roerig, L; Hall-Spencer, Jason M.; Horta, Paulo Antunes

**Published in:**  
Aquatic Botany

**DOI:**  
[10.1016/j.aquabot.2018.04.008](https://doi.org/10.1016/j.aquabot.2018.04.008)

**Publication date:**  
2018

**Link:**  
[Link to publication in PEARL](#)

**Citation for published version (APA):**

Munoz, PT., Saez, CA., Brezo, M.-C. M., Rosa, F.-M. M., Bastos, E., Fonseca, A., Gurgel, C. F. D., Barufi, J. B., Roerig, L., Hall-Spencer, J. M., & Horta, P. A. (2018). Short-term interactive effects of increased temperatures and acidification on the calcifying macroalgae *Lithothamnion crispatum* and *Sonderophycus capensis*. *Aquatic Botany*, 148(0), 46-52. <https://doi.org/10.1016/j.aquabot.2018.04.008>

1 This is the author's accepted manuscript. The final published version of this work (the  
2 version of record) is published by Elsevier in Aquatic Botany made available online on  
3 the 26 April 2018 at: <https://doi.org/10.1016/j.aquabot.2018.04.008>. This work was made  
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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 ( $7.8 \pm 0.2$ ) and temperature ( $18$   
40  $\pm 2^\circ\text{C}$ ) during winter; (2) future pH ( $7.4 \pm 0.2$ ) 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 (CO<sub>2</sub>) concentrations have been increasing from 280  
58 ppm since the pre-industrial period to above 400 ppm nowadays; moreover, CO<sub>2</sub> levels are  
59 expected to increase in up to 970 ppm by the end of the 21<sup>st</sup> Century (Harley et al. 2006;  
60 Harley et al. 2012). Predicted CO<sub>2</sub> 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 CO<sub>2</sub> is absorbed by the ocean, which in reaction with  
64 seawater increases the levels of carbonic acid (H<sub>2</sub>CO<sub>3</sub>), 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 (CO<sub>3</sub><sup>2-</sup>) and bicarbonate (HCO<sub>3</sub><sup>-</sup>);  
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), CO<sub>2</sub> 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/pCO<sub>2</sub> 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 (CaCO<sub>3</sub>) 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). CaCO<sub>3</sub> occurs in  
93 skeletons of calcifying macroalgae as calcite or aragonite; however, magnesium (Mg<sup>2+</sup>)  
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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CaCO<sub>3</sub> 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 ( $7.8 \pm 0.2$ ) and temperature ( $18 \pm 2^\circ\text{C}$ ) registered in Florianopolis bay, Brazil, during  
153 winter **(2)**; future values of pH ( $7.4 \pm 0.2$ ) and temperature ( $30 \pm 2^\circ\text{C}$ ) that can be  
154 extrapolated for Florianopolis bay during summer by the end of the 21<sup>st</sup> Century  
155 according to predictions by IPCC (2014); **(3)** Current temperature ( $18 \pm 2^\circ\text{C}$ ) and future  
156 pH ( $7.4 \pm 0.2$ ); and **(4)** Future temperature ( $30 \pm 2^\circ\text{C}$ ) and current pH ( $7.8 \pm 0.2$ ). 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 CO<sub>2</sub> were injected with a pump the  
166 experimental seawater. The added CO<sub>2</sub> 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.*** HCO<sub>3</sub><sup>-2</sup> and CO<sub>3</sub><sup>-2</sup> concentrations, pCO<sub>2</sub>,  
174 calcite and aragonite saturation ( $\Omega$ ), were obtained via CO<sub>2</sub>Sys\_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  $\alpha$  fluorometer (DIVING PAM; Walz GmbH,  
184 Effeltrich, Germany). This parameter was measured according to Schreiber and Neubauer  
185 (Schreiber and Neubauer 1990):  $\Delta F/F'_m \approx (F'_m - F_t)/F'_m$ . 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<sup>-1</sup>), 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<sub>i</sub>) was obtained and then immersed in 10% HCl (Merck) for  
196 24 h to initiate CaCO<sub>3</sub> 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<sub>f</sub>)  
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 NaCO<sub>3</sub> (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<sup>-1</sup> –  $r^2 = 0.99$ ;  $y = 1.254$ ).

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).  $\Delta$   
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 21<sup>st</sup> 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  $\text{H}_2\text{O}_2$  under high temperatures due to increased oxygenase reactions (Kim and  
281 Portis 2004). Moreover, it has been observed that increased temperatures induce  
282 incomplete  $\text{H}_2\text{O}$  oxidation in the PSII electron donor side, forming  $\text{H}_2\text{O}_2$ , 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  $18^\circ\text{C}$  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  $30^\circ\text{C}$  showed optimum oxygenic photosynthetic threshold  
291 at  $25^\circ\text{C}$ ; 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  $30^\circ\text{C}$  to  $32^\circ\text{C}$ , 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  $30^\circ\text{C}$  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  $\text{O}_2$  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 CO<sub>2</sub> availability rather than reduction of pH itself. According to Martin and Hall-Spencer  
315 (2017), higher concentration of CO<sub>2</sub> in seawater induces macroalgae to rely exclusively  
316 of CO<sub>2</sub> diffusion, own-regulating their CO<sub>2</sub>-concentrating mechanisms (CCM) to save  
317 energy. Certainly, high availability of diffused CO<sub>2</sub> 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 CO<sub>2</sub>) 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 O<sub>2</sub> 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

368 **AWKNOWLEDGEMENTS**

369 We thank financial support for Master studies from Becas Chile Magister 2010  
370 (CONICYT, Chile) to Pamela Muñoz. This work was supported by grants from Boticario  
371 Foundation; FAPESC—Foundation Support Research and Innovation in the State of  
372 Santa Catarina; CNPq—National Council for Scientific and Technological Development;  
373 Capes-Higher Education Personnel Improvement Coordination; REBENTOS—Habitat  
374 Monitoring Network Coastal Benthic and ProspecMar-Islands— Sustainable Prospecting  
375 in Ocean Islands: Biodiversity, Chemistry, Ecology and Biotechnology; Rede Coral Vivo  
376 and REDEALGAS; Brazilian Research Network on Global Climate Change,  
377 FINEP/Rede CLIMA Grant Number 01.13.0353-00 and FAPESP—Foundation Support  
378 Research and Innovation in the State of São Paulo (FAPESP, 2014/00012-1).

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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,  $p\text{CO}_2$ ,  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  concentration,  $\Omega_{\text{calcite}}$  ( $\Omega_{\text{Ca}}$ ) and  $\Omega_{\text{aragonite}}$  ( $\Omega_{\text{Ar}}$ ).

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| T(°C) | pH    | $p\text{CO}_2$<br>(ppm) | $\text{HCO}_3^-$<br>( $\mu\text{mol kg SW}^{-1}$ ) | $\text{CO}_3^{2-}$<br>( $\mu\text{mol kg SW}^{-1}$ ) | $\Omega_{\text{Ca}}$ | $\Omega_{\text{Ar}}$ |
|-------|-------|-------------------------|--|--|----------------------|----------------------|
| 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           |

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615 Note:  $n = 3$  (means  $\pm$  SDs). Abbreviations: T (°C) = Temperature,  $p\text{CO}_2$  = partial  
 616 pressure of carbon dioxide,  $\text{HCO}_3^-$  = bicarbonate,  $\text{CO}_3^{2-}$  = carbonate,  $\Omega_{\text{Ca}}$  = Calcite  
 617 saturation,  $\Omega_{\text{Ar}}$  = 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> |
| <b><math>\Delta F/Fm'</math></b> |                                |         |        |          |                               |         |        |          |
| 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   |        |          |
| <b>Calcification</b>             |                                |         |        |          |                               |         |        |          |
| 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   |        |          |

638 Note:

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

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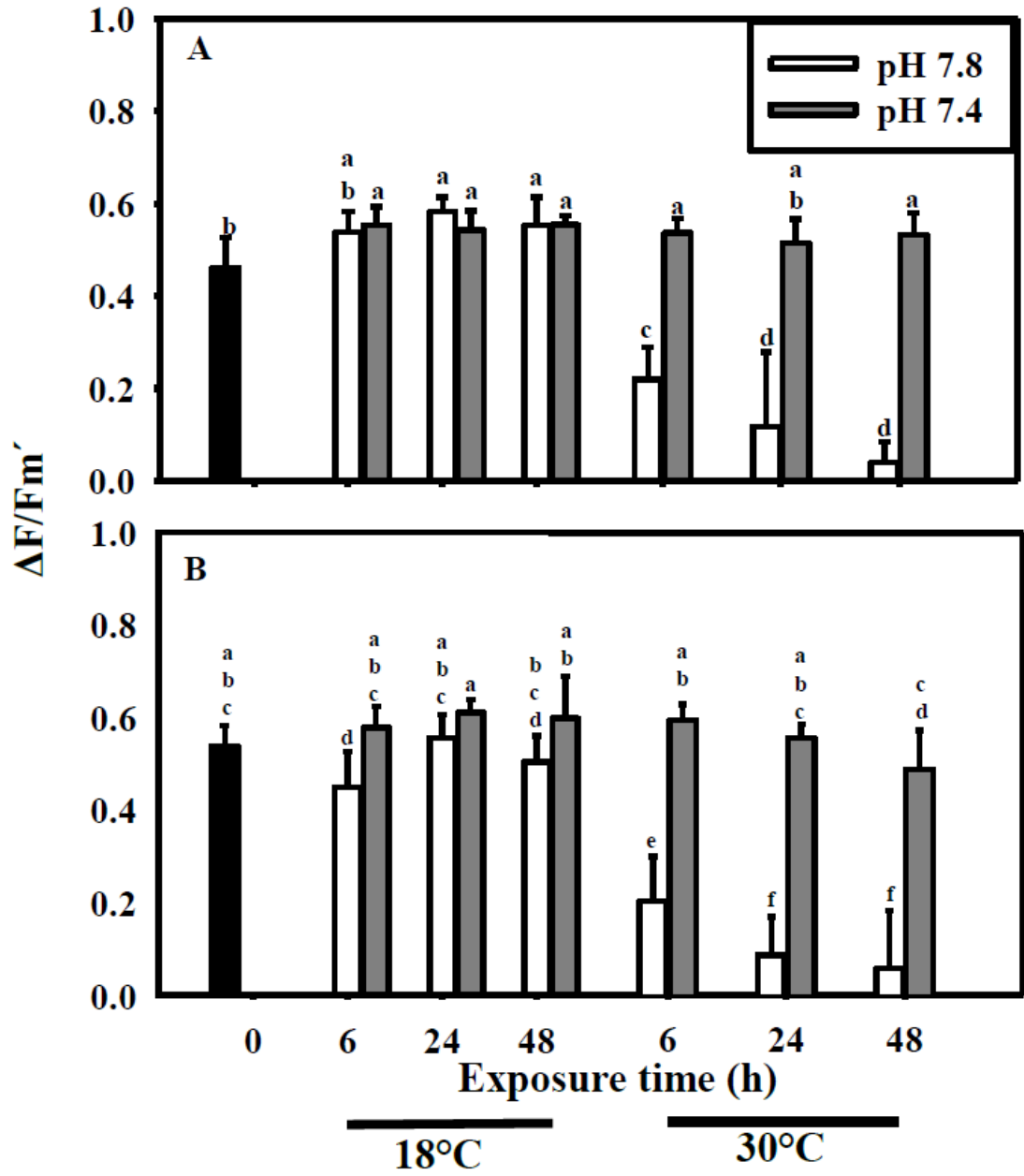
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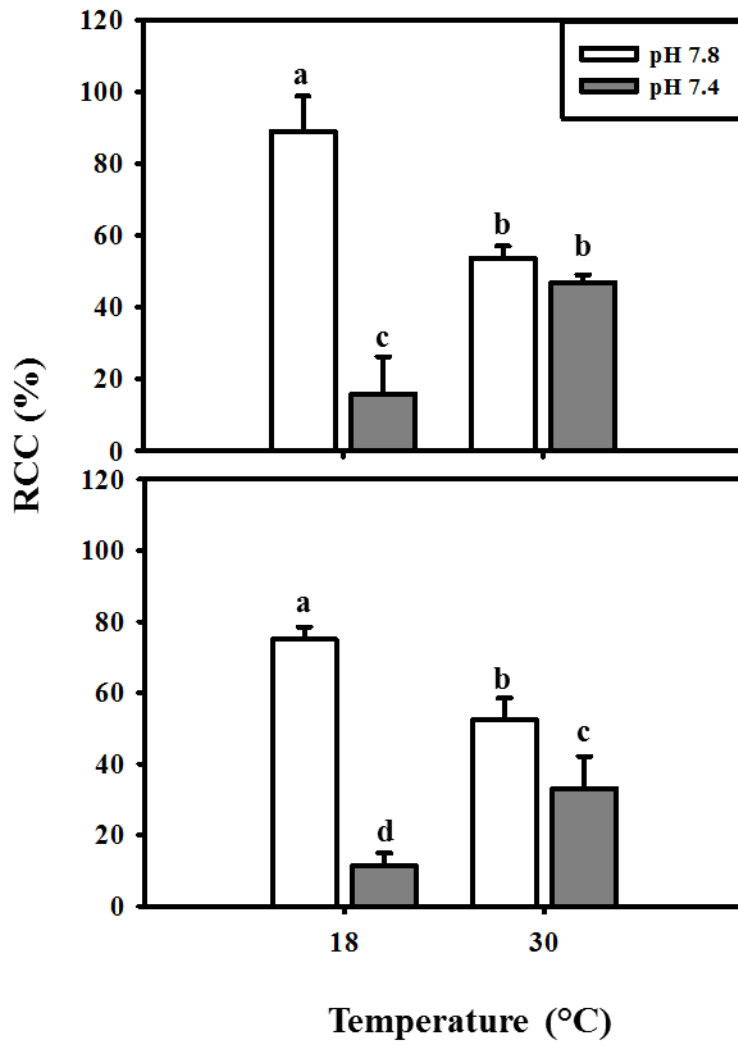
654 **Figure 1**

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Figure 2

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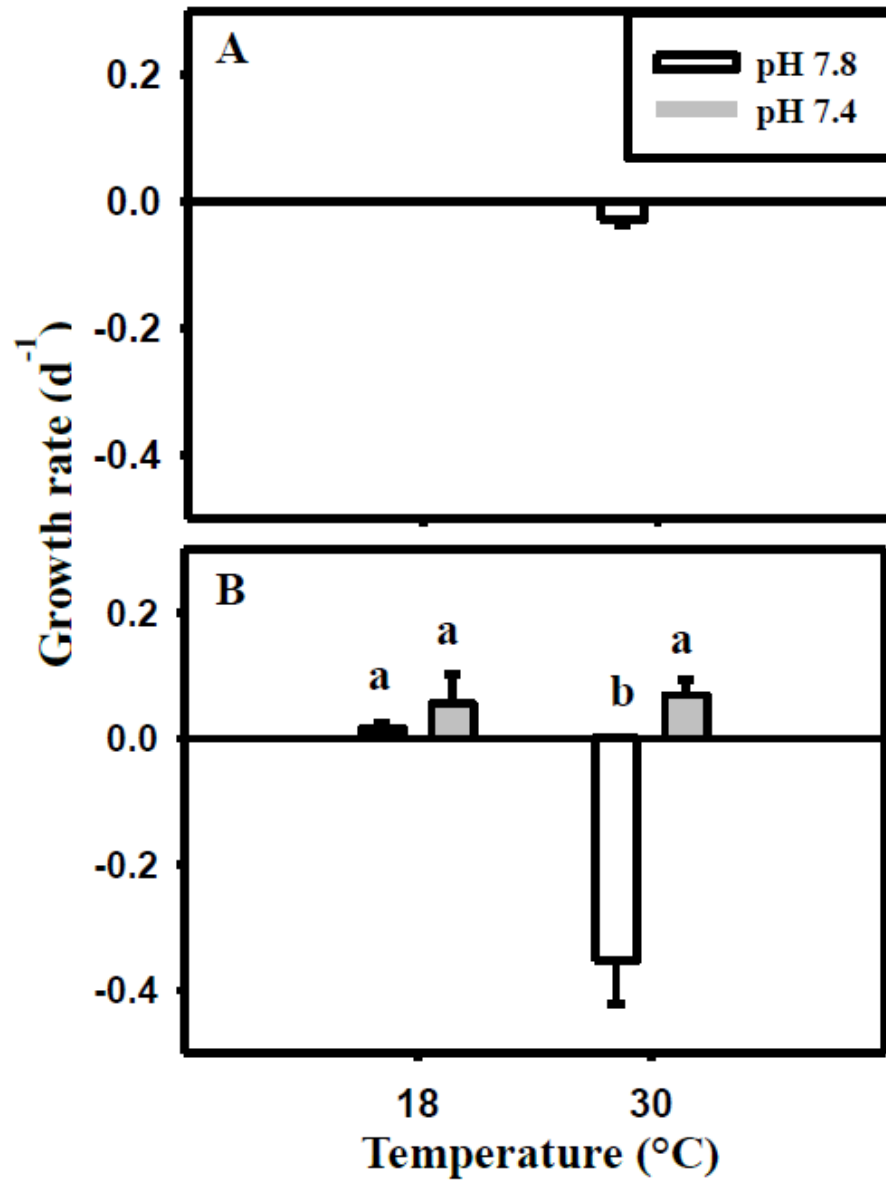
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667 **Figure 3**

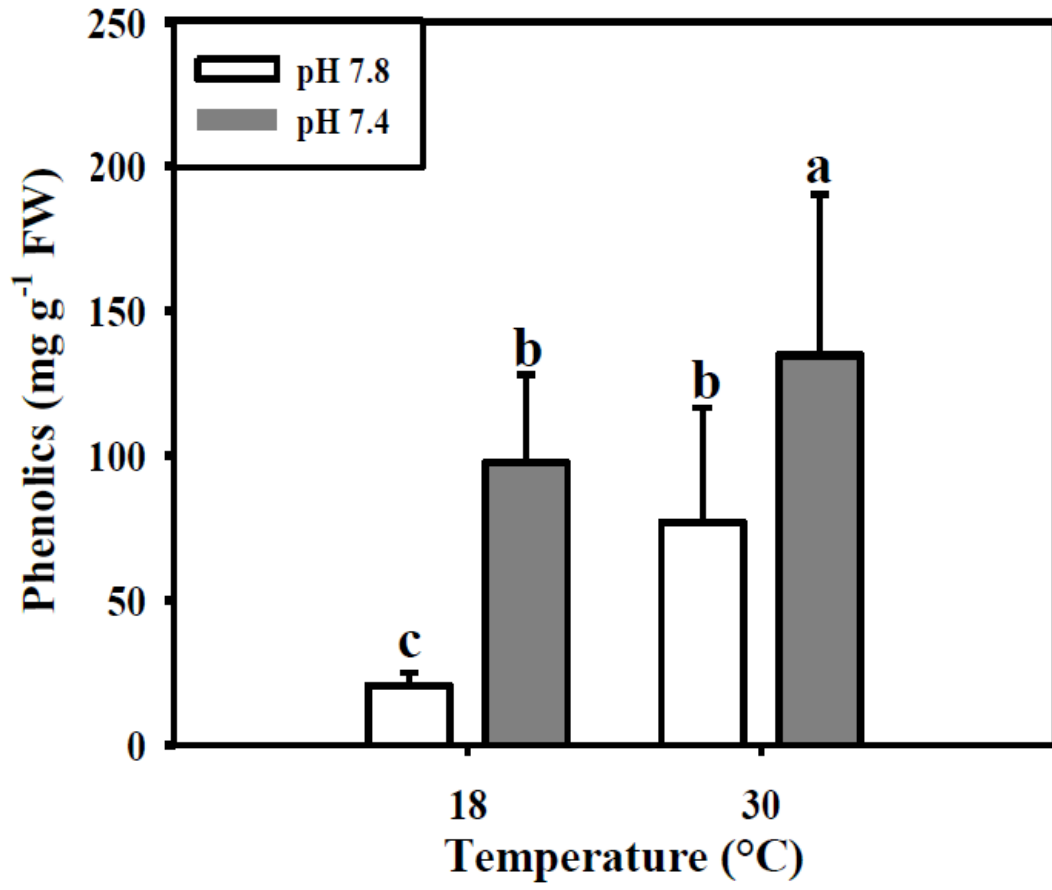
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Figure 4

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