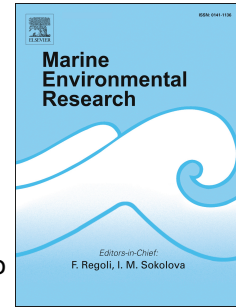# Accepted Manuscript

Physiological response of the coralline alga *Corallina officinalis* L. to both predicted long-term increases in temperature and short-term heatwave events

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1   **Physiological response of the coralline alga *Corallina officinalis* L. to both**

2   **predicted long-term increases in temperature and short-term heatwave events**

3

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ABSTRACT

Climate change is leading to an increase of mean sea surface temperatures and extreme heat events. There is an urgent need to better understand the capabilities of marine macroalgae to adapt to these rapid changes. In this study, the responses of photosynthesis, respiration, and calcification to elevated temperature in a global warming scenario were investigated in the coralline alga *Corallina officinalis*. Algae were cultured for 7 weeks under 4 temperature treatments: (1) control under ambient-summer conditions (C, ~20°C), (2) simulating a one-week heatwave of 1°C (HW, $T_{control}+1°C$), (3) elevated temperature (+3, $T_{control}+3°C$), (4) combination of the two previous treatments (HW+3, $T_{+3}+1°C$). After exposure at T_{+3} (up to a T_{max} of ~23°C), respiration and photosynthesis increased significantly. After 5 weeks, calcification rates were higher at elevated temperatures (T_{+3} and T_{HW+3}) compared to $T_{control}$, but at the end of the experiment (7 weeks) calcification decreased significantly at those temperatures beyond the thermal optimum (six-fold at T_{+3}, and three-fold at T_{HW+3}, respectively). The same trend was noted for all the physiological processes, suggesting that a prolonged exposure to high temperatures (7 weeks up to T_{+3}) negatively affect the physiology of *C. officinalis*, as a possible consequence of thermal stress. A one-week heatwave of +1°C with respect to $T_{control}$ (at T_{HW}) did not affect respiration, photosynthesis, or calcification rates. Conversely, a heatwave of 1°C, when combined with the 3°C increase predicted by the end of the century (at T_{HW+3}), induced a reduction of physiological rates. Continued increases in both the intensity and frequency of heatwaves under anthropogenic climate change may lead to reduced growth and survival of primary producers such as *C. officinalis*.

Keywords: *algae, climate change, ocean warming, temperature, heatwaves, thermal stress, calcification, photosynthesis, respiration.*

1. Introduction

42

43 Climate change is occurring at a faster rate than in the past, due to increasing concentrations

44 of greenhouse gases in the Earth's atmosphere caused by human combustion of fossil fuels

45 and deforestation (IPCC, 2014). This results in increasing seawater temperatures, rising sea

46 levels, and ocean acidification (IPCC, 2014). The International Panel on Climate Change

47 (IPCC) indicated that global mean surface temperatures have already risen by

48 approximately 0.87°C in the last one and a half centuries (over the period 1850-2015), and

49 will likely increase further (by ca. 3°C by the end of this century, according to the pathways

50 reflecting present nationally stated mitigation goal by 2030; Masson-Delmotte et al., 2018).

51 In addition to long-term warming, extreme events (i.e., storms, droughts, floods and

52 heatwaves) are also becoming more frequent and more intense (Coumou and Rahmstorf,

53 2012; Perkins et al., 2012; Oliver et al., 2018, Frölicher et al., 2018; Darmaraki et al., 2019).

54 Specifically, marine heatwaves (MHWs) can strongly influence ecosystem structure and

55 functioning by causing widespread mortality, species range shifts and community changes

56 (Jentsch et al., 2007; Hobday et al., 2016, Smale et al., 2019).

57 Increasing sea surface temperature (SST) is among the main impacts affecting marine

58 ecosystems (Stenseth et al., 2002), which can influence the abundance and distribution of

59 marine organisms, and lead to poleward range shifts or extinctions of populations located at

60 the edge of their thermal tolerance (Perry et al., 2005; Wernberg et al., 2011; Yara et al.,

61 2012; Jueterbock et al., 2013; Sanford et al., 2016; Collin et al., 2018; Kolzenburg et al.,

62 2019). Elevated temperatures can lead to severe ecological impacts, including widespread

63 mortality of benthic communities (Garrabou et al., 2009), loss of seagrass habitats (Marbà

64 and Duarte, 2010), and impacts on fisheries, due to changes in primary productivity and

65 shifts in distribution or mass mortality events of species of commercial interest (Sumaila et

3

66 al., 2011; Mills et al., 2013; Caputi et al., 2015). In particular, water temperature is a major

67 factor controlling the survival, growth and reproduction of macroalgae, and thus plays an

68 important role in governing both the small☐scale vertical and the large☐scale geographical

69 distribution of macroalgal species, in addition to their abundance (Breeman, 1988; Lüning,

70 1990; Nannini et al., 2015). For this reason, it is worth understanding the biological

71 responses of climate-sensitive organisms to short-term extreme events, in concurrence with

72 long-term changes (Jentsch et al., 2007).

73 Coralline red algae (Rhodophyta) are fundamental calcifying primary producers and

74 important habitat-forming species present in most coastal ecosystems, such as coralligenous

75 bioconstructions (Johansen, 1981; Ferrigno et al., 2017; Ingrosso et al., 2018). The species

76 *Corallina officinalis* (Linnaeus 1758) is an erect calcifying alga with a wide distribution that

77 dominates North Atlantic rocky shores and rock pools (Williamson et al., 2015). Due to its

78 complex morphological structure, it represents an important substratum for the settlement of

79 other macroalgae and microalgae, and supports a high biodiversity of marine invertebrates

80 (Akioka et al., 1999; Kelaher, 2003). Despite the importance of coralline algae, their

81 sensitivity to increasing temperatures is still unclear, as different studies have yielded

82 conflicting results (Martin et al., 2013; Comeau et al., 2014; Vásquez-Elizondo and

83 Enríquez, 2016). There is further uncertainty around the response of *C. officinalis* in rock

84 pool habitats, as the species must adapt to multiple stressors, including highly variable water

85 temperatures across seasonal, diurnal and tidal cycles (Williamson et al., 2017).

86 In this study, we describe the physiological responses of *C. officinalis* to temperature

87 variation in an ocean warming scenario (RCP 8.5; IPCC, 2014), taking into account natural

88 thermal fluctuations experienced within rock pools across periods of low and high tides (i.e.,

89 $\Delta T = \sim 3.5°C$, recorded in the field; see Fig. 2). Specifically, we analysed photosynthesis,

90 respiration, and dark/light calcification rates of a South-East UK population exposed to both

91 a temperature increase of +3°C (i.e., simulating the warming expected by the end of this

92 century; Solomon et al., 2007), and a marine heatwave (similar to those registered over the

93 last century, and attributed to anthropogenic climate change; Oliver et al., 2018).

94

95 **2. Materials and methods**

96 *2.1. Biological material*

97 Specimens of the articulated coralline alga *Corallina officinalis* were collected during low

98 tide in intertidal rock pools at ± 0.3 m depth of St. Margarets Bay (Kent, UK;

99 51°08'52.9"N, 1°23'06.9"E) in September 2017. Seawater temperature measured at the time

100 of sampling with a HQ30D flexi multi-meter (Hach Environmental, Loveland, CO, USA)

101 was 15.7±0.2°C. Algae were immediately transported (~3 hours) in temperature☐insulating

102 containers to the Institute of Marine Sciences, University of Portsmouth, UK, where the

103 experiment was carried out. Healthy thalli in the size range of 3-10 cm^2 were selected for

104 the experiment, and were carefully cleaned of epiphytic organisms, avoiding any damage.

105 Algae were fixed on small stones, in order to simulate natural conditions and keep them

106 upright, and guarantee the same light conditions to each branch as much as possible (~3 g

107 fresh weight for each stone), see Fig. 1.

108

109 *2.2. Experimental design*

110 Temperature and irradiance during the experiment were set according to ambient summer

111 conditions recorded daily in the field in July-August 2017 by a HOBO pendant

112 temperature/light data logger (Onset Computer Corp., Bourne, MA, USA) placed in a rock

113 pool of the collection site. Algae were acclimated in 8 15-L aquaria in a closed seawater

114   system for 2 weeks before the gradual increase of water temperature. During

115   acclimatization, algae were maintained at a 14:10 light:dark photoperiod, with UV light

116   oscillating in the range 20-30 μmol m^{-2}s^{-1} (measured at the position of the submerged algal

117   fronds in the experimental tanks, mimicking sun set and sun rise and with controlled

118   dimming during the day as cloud effect), while the temperature (T) changed during 24h

119   from a T_{min} of ~16.5±0.1°C to a T_{max} of ~20±0.1°C, around a mean temperature value of

120   18.5±1.2°C, reflecting the T oscillations of daily tides recorded in the field. After

121   acclimatization, specimens were assigned to 16 x 11-L glass aquaria (4 tanks per treatment)

122   under 4 temperature conditions. Each aquarium contained three algae-stones, for a total of

123   12 algae/stones per temperature treatment (Fig. 1). Aquaria were kept in a closed system

124   with seawater sourced directly from the sea off the Institute of Marine Sciences (University

125   of Portsmouth, UK; 50°47'40.7''N, 1°01'50.1''W) and processed via a settlement system

126   with glass media filtration (salinity ranging from 34.4 to 35.2). Ten percent of the aquaria

127   water was exchanged every other day, in order to keep nutrient levels and alkalinity

128   constant. The four temperature treatments (Fig. 2) were: (1) control treatment (C), kept at

129   the *in situ* acclimatization temperature, with a temperature T_C oscillating according to a

130   thermal range reflective of the daily tides recorded in the field (16.5°C≤T_C≤20°C); (2)

131   heatwave treatment (HW), where a heatwave was simulated by inducing a temperature

132   increase of +1°C for a period of 1 week (T_{HW}= T_C+1°C; 17.5°C≤T_{HW}≤21°C); (3) elevated

133   temperature treatment (+3), where the temperature was increased by +3°C according to the

134   predicted temperature increase due to climate change by the year 2100 (T_{+3}=T_C+3°C;

135   19.5°C≤T_{+3}≤23°C; Solomon et al., 2007); (4) treatment obtained by the combination of the

136   two previous treatments (HW+3), with a +4°C temperature increase (T_{HW+3}=T_C+3°C+1°C;

137   20.5°C≤T_{HW+3}≤24°C). Temperature was increased at a rate of 0.5°C per day (over a period

of 6 days) to reach the +3°C temperature change, and of 0.5°C per hour (over a period of 1

hour) when simulating the MHW. Water temperature in all tanks was monitored daily with

a HQ30D flexi multi-meter (Hach Environmental, Loveland, CO, USA), and had a

continual logging every 15 min with a HOBO pendant data logger (Onset Computer Corp.,

Bourne, MA, USA). Irradiance levels were monitored throughout the experiment with a

Quantitherm light-meter (QRT-1, Hansatech Instruments, Norfolk, UK). pH and salinity

were measured using the HQ30D flexi multi-meter by pH and salinity probes (Hach

Environmental, Loveland, CO, USA). Total Alkalinity was measured by potentiometric

titration (TitroLine 7000, Schott SI Analytics, Mainz, German) following the SOP6 protocol

(Dickson et al., 2007). Measurements were validated against Dickson standard (batch #154).

Other parameters of the carbonate chemistry were calculated using the software CO2Sys,

EXCEL Macro version 2.1 (Lewis et al., 1998). Water motion and filtration in the aquaria

was ensured by a submersible pump (V^2PowerPump 800, TMC, London, UK).

Fig. 1. Experimental set-up with four temperature treatments (C, HW, +3, HW+3). Each treatment was performed in a large tank, acting as a water bath, in which four 11-L glass aquaria were immersed (a total of 16 aquaria). Every aquarium contained three algae-stones (as showed in the detail, top right).

157

158

2.3. Marine heatwave calculation

160 A one-week MHW of 1°C was calculated in accordance to the definition provided by

161 Hobday et al. (2016), i.e., referring to the temperature values exceeding the 90th percentile

162 threshold of the SST measured for at least five consecutive days in the same 30-day-period

163 window over the last 30 years. SST *in situ* data were obtained from the closest NOAA buoy

164 to the collection site, located off the South UK Coast (about 40 km) along the Greenwich

165 meridian (Station 50°24'0" N 0°0'0" E; National Data Buoy Center, National Oceanic and

166 Atmospheric administration; www.ndbc.noaa.gov/station_page.php?station=62305).

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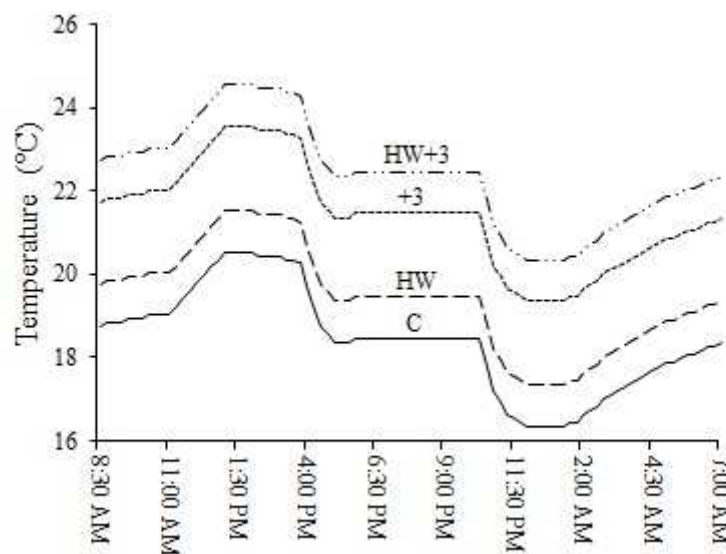
168

169 **Fig. 2.** Daily planned temperature changes in the experimental tanks for the 4 treatments (C,

170 HW, +3, HW+3). The temperature fluctuation in the control (C) was performed simulating

171 the environmental thermal excursion due to the daily tides, as recorded in the field. The

172 MHW was performed in the treatments HW and HW+3, and lasted for one week (for more

173 details see Fig. 3).

174

175

2.4. Physiological measurements

177 Algal thalli (1 g/fresh weight from each tank at each time point) were incubated in 50-ml

178 closed oxygen chambers filled with bubble-free seawater from the aquaria. One hour

179 incubations were conducted under saturating light condition (300 μmol m^{-2}s^{-1}; Ralph and

180 Gademann, 2005) and in the dark. The irradiance levels were controlled with a Quantitherm

181 Light Meter (QRT-1, Hansatech Instruments, Norfolk, UK). The chambers were used to

182 assess net photosynthesis (P_n) and calcification in the light (G_l), while chambers covered

183 with aluminium foils were used to assess dark respiration (R_d) and calcification in the dark

184 (G_d). The concentration of dissolved oxygen (O_2, μmol l^{-1}) was measured inside the

185 chambers before and after incubations using a HQ30D flexi oxygen meter (Hach

186 Environmental). Water samples were taken at the beginning and at the end of the

187 incubations for measurements of pHT (pH on the total scale) and total alkalinity (A_T).

188 P_n and R_d, expressed in terms of O_2 production and consumption (in μmol O_2 gFW^{-1} h^{-1}),

189 were calculated after Williamson et al. (2017):

$$P_n(or\ R_d) = \frac{\Delta O_2\ v}{fw\ \Delta t}$$

190 where ΔO_2 is the difference in O_2 concentration before and after incubation (μmol l^{-1} h^{-1}), v

191 is the volume of the incubation tubes (l), fw is the fresh weight of the algae incubated (g)

192 and Δt is the incubation time (h).

193 Gross photosynthesis (P_g) was calculated as:

$$P_g = |P_n| + |R_d|$$

194 G_l and G_d (μmol CaCO$_3$ gFW^{-1} h^{-1}) were calculated using the alkalinity anomaly technique

195 (Smith and Key, 1975) as:

9

$$G_l(or\ G_d) = \frac{\Delta A_T\ v}{2(fw\ \Delta t)}$$

196 where ΔA_T is the difference between initial and final A_T values (μeq l^{-1}).

197 Physiological measurements were taken in all treatments at 3 different times during the

198 experiment (Fig. 3):

199 • t_1= before the MHW start (4-weeks); i.e., after 2 weeks of acclimatization at the initial

200 temperature $T_{control}$, 1 week of gradual heating up to T_{+3}, and 1 week of acclimatization at

201 T_{+3} (the heating up to T_{+3} was induced only in treatments +3 and HW+3);

202 • t_2= right after the MHW end (5-weeks); i.e., 1 week of HW at T_{HW} after t_1 (the HW was

203 induced only in treatments HW and HW+3);

204 • t_3= after a recovery period from the MHW end (7-weeks); i.e., 2 weeks of recovery from

205 the heatwave-end after t_2.
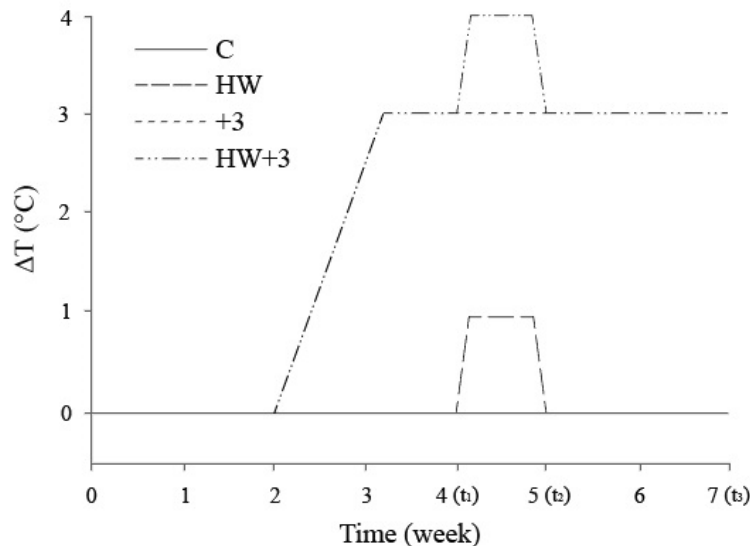
206

207 **Fig. 3.** Planned temperature changes during the experiment in the 4 treatments (C, HW, +3,

208 HW+3). Physiological measurements were taken in all treatments at 3 different times: t_1 (4-

209 weeks), before the MHW start; t_2 (5-weeks), right after the MHW end; t_3 (7-weeks), after a

210 recovery period from the MHW end.

211

10

212   *2.5. Data analysis*

213   We used linear-mixed effects models (LMMs) to examine whether temperature treatments

214   influenced photosynthesis, respiration, and calcification rates. Models were developed in the

215   *nlme* package in R v3.6.0 (Bates et al., 2015; Pinheiro et al., 2019) with both time and

216   temperature (as well as their interaction) treated as fixed effects, and tank ID included as a

217   random effect to account for autocorrelated errors among algae grown in the same tanks

218   (Speights et al., 2017). Model residuals were visually inspected using QQ plots and residual

219   plots and formally checked for normality and homoskedasticity via Shapiro-Wilks and

220   Levene's tests, respectively. Heterogeneity in residual variance was only identified in the

221   calcification models, and was addressed with an appropriate structure (varIdent), allowing

222   residuals to differ in spread between temperature treatments across time without the need to

223   transform the data (Pinheiro and Bates, 2000; Harrison et al., 2018). Results are expressed

224   as mean ± standard error of the mean (SE). n is the sample size and p_s are Tukey-adjusted *p*-

225   values, evaluated against a significance threshold of $\alpha = 0.05$.

3. Results

227   *3.1. Respiration*

228   LMMs explained over 76% of the variation in dark respiration (R_d) [conditional pseudo-R^2

229   = 0.763; Nakagawa and Schielzeth, 2013]. At time t_1, before mimicking the marine

230   heatwave, no significant differences were found between R_d rates in the control (C) and the

231   treatment HW, and between the treatments +3 and HW+3 (Fig. 4; Table 1). This trend was

232   expected because, at time t_1, the treatments C and HW were kept at the same temperature Tc

233   ($16.5°C \leq T_C \leq 20°C$); while, +3 and HW+3 were both at T_{+3} ($T_{+3}=T_C+3°C$; $19.5°C \leq T_{+3} \leq 23°C$;

234   see fig. 3). However at t_1, C and HW were both different from +3 and HW+3, with lower R_d

235   values measured in +3 and HW+3 (0.88 ± 0.08 and 0.81 ± 0.09 μmol O_2 gFW^{-1} h^{-1},

236   respectively), and higher values in C and HW (1.27 ± 0.15 and 1.38 ± 0.12 μmol O_2 gFW^{-1} h^{-1},

237   respectively) [$p_{C,+3} = 0.049$, $p_{C,HW+3} = 0.021$, $p_{HW,+3} = 0.012$, $p_{HW,HW+3} = 0.005$]. At time t_2,

238   immediately after the MHW, the lowest R_d rates were measured in the HW treatment

239   (0.53 ± 0.06 μmol O_2 gFW^{-1} h^{-1}), while the highest rates were found in the treatment +3

240   (1.22 ± 0.04 μmol O_2 gFW^{-1} h^{-1}) [$p_{HW,+3} = 0.001$]; intermediate values were measured in C

241   (0.90 ± 0.07 μmol O_2 gFW^{-1} h^{-1}). At time t_3, in HW+3, the lowest R_d values were registered

242   (0.45 ± 0.10 μmol O_2 gFW^{-1} h^{-1}) [$p_{C,HW+3} = 0.026$].

243   R_d in the control group did not change significantly over time (Fig. 4). In the treatment +3,

244   R_d rates increased from t_1 to t_2 (0.88 ± 0.10 and 1.22 ± 0.04 μmol O_2 gFW^{-1} h^{-1}, respectively)

245   [$p_{t_1,t_2} = 0.023$], and decreased from t_2 to t_3 (1.22 ± 0.04 and 0.88 ± 0.07 μmol O_2 gFW^{-1} h^{-1},

246   respectively) [$p_{t_2,t_3} = 0.027$]. In HW, Rd rates decreased from t_1 to t_2, going from 1.38 ± 0.12

247   to 0.53 ± 0.04 μmol O_2 gFW^{-1} h^{-1} [$p_{t_1,t_2} < 0.001$]. In HW+3, Rd rates decreased from t_1 to t_3

248   (0.81 ± 0.09 to 0.45 ± 0.10 μmol O_2 gFW^{-1} h^{-1}) [$p_{t_1,t_2} = 0.015$]. While, no changes between t_2

249   (right after the MHW end) and t_3 (after the recovery period from the MHW) were observed

250   in both the heatwave conditions HW and HW+3.

251

252   *3.2. Photosynthesis*

253   LMMs explained over 58% of the variation in net photosynthesis (P_n) and 67% of variation

254   in gross photosynthesis (P_g) [conditional pseudo-R^2 = 0.581 and 0.672, P_n and P_g

255   respectively]. No differences in P_n rates were found among temperature treatments at each

256   experimental time (t_1, t_2, t_3; Fig. 4; Table 1). Likewise, no differences in P_g rates were

257   apparent at time t_1 between the control (C) and the treatment at +3°C (3.64 ± 0.29 and

258   4.13 ± 0.12 μmol O_2 gFW^{-1} h^{-1}, respectively), or between HW and HW+3 (4.16 ± 0.35 and

12

259   4.23 ± 0.23 μmol O_2 gFW^{-1} h^{-1}, respectively). At time t_2, the lowest P_g values were measured

260   in the treatment HW (2.44 ± 0.28 μmol O_2 gFW^{-1} h^{-1}) and the highest in +3 (3.64 ± 0.33 and

261   μmol O_2 gFW^{-1} h^{-1}), with these being significantly different from each other [$p_{HW,+3}$ =

262   0.036]. No significant differences were found among all temperature treatments at time t_3,

263   after the recovery period from the MHW end.

264   In C, P_n rates did not vary among experimental time points (t_1, t_2 and t_3), while P_g decreased

265   from t_1 to t_3 [p_{t_1,t_3} = 0.028]. In the treatment +3, both P_n (from 3.26 ± 0.13 to 1.94 ± 0.35 μmol

266   O_2 gFW^{-1} h^{-1}) and P_g (from 4.13 ± 0.12 to 2.83 ± 0.38 μmol O_2 gFW^{-1} h^{-1}) decreased from t_1 to

267   t_3 [p_{t_1,t_3} = 0.004 and 0.003, P_n and P_g respectively]. In the treatment HW, P_n did not change

268   significantly overtime, while P_g decreased from t_1 to t_2 (4.16 ± 0.35 and 2.78 ± 0.22 μmol O_2

269   gFW^{-1} h^{-1}, respectively) [p_{t_1,t_2} < 0.001], as well as from t_1 to t_3 [p_{t_1,t_3} = 0.001]. In HW+3, the

270   lowest P_n and P_g rates were found after the recovery period from the MHW end (t_3;

271   1.84 ± 0.15 and 2.29 ± 0.20 μmol O_2 gFW^{-1} h^{-1}, respectively), with t_3 differing from both t_1 and

272   t_2 [p_{t_1,t_3} < 0.001 and 0.001, p_{t_2,t_3} = 0.025 and 0.007, P_n and P_g respectively].

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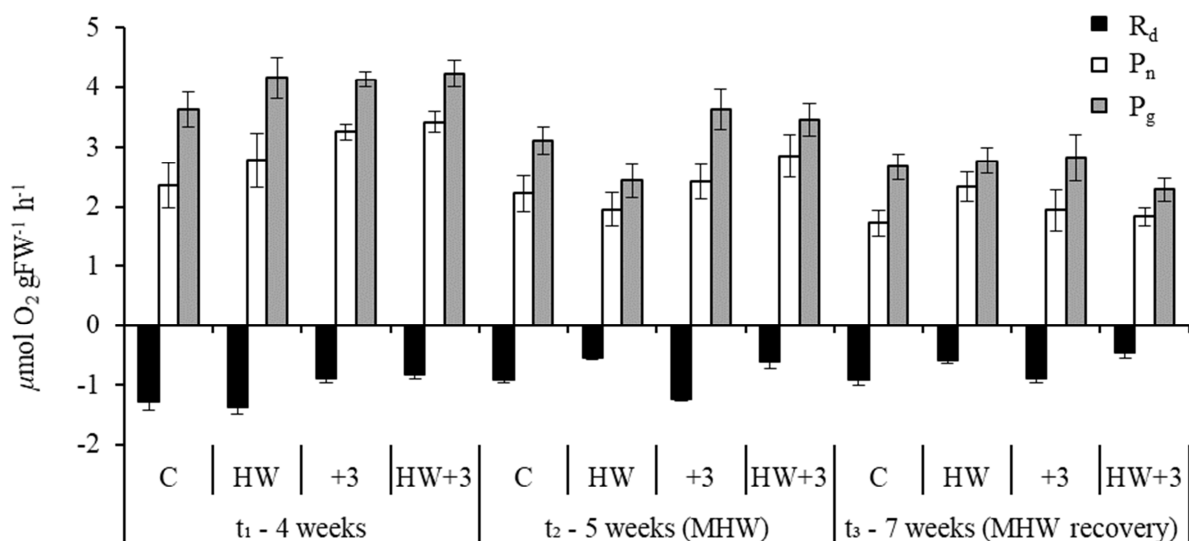
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275   **Fig. 4.** Net and gross photosynthesis (P_n and P_g) rates at the experimental irradiance, and

276   respiration (R_d) rates in the dark in the 4 temperature treatments (C, HW, +3, HW+3) for the

277   3 incubation times (t_1, before the MHW start; t_2, right after the MHW end; t_3, after a

278   recovery period from the MHW end). Data are means ± SE, and are expressed in terms of

279   O_2 release (negative values for respiration correspond to O_2 consumption); n = 4 for each
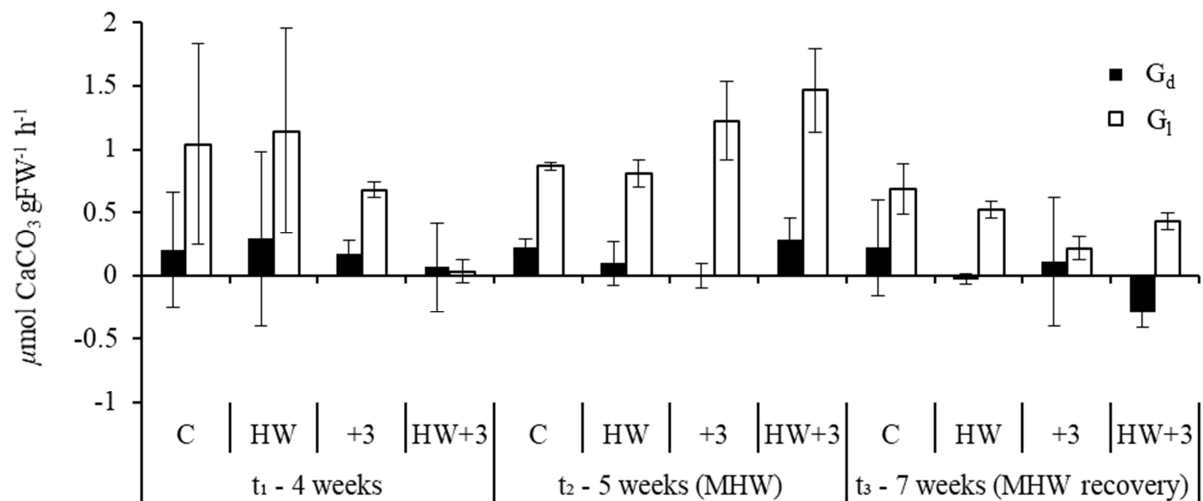
280   treatment.

281

282   *3.3. Calcification*

283   There was only weak evidence for an effect of temperature on calcification rates measured

284   in the dark (G_d; Table 1), with no post-hoc differences found among temperature treatments,

285   highlighting uncertainty about how temperature affects G_d rates. This is in alignment with

286   the low percentage of variance explained by the LMMs [G_d conditional pseudo-R^2 = 0.034].

287   Variability in G_l rates was inherently high, especially at the start of the experiment (i.e.,

288   time t_1), leading to LMMs with poor explanatory power [conditional pseudo-R^2 = 0.0601].

289   G_l rates were affected by temperature at time t_1, with +3 and HW+3 being significantly

290   different from each other [$p_{+3,HW+3}$ < 0.001], despite being at the same temperature T_{+3}. At

291   time t_2, +3 and HW+3 (1.22±0.31 and 1.47±0.33 μmol $CaCO_3$ gFW^{-1} h^{-1}) exhibited (non-

292   significant) higher G_l rates than C and HW (0.87±0.03 and 0.81±0.10 μmol $CaCO_3$ gFW^{-1} h^{-1}

293    $^{-1}$, respectively). After 2-weeks-recovery from the MHW (t_3), an opposite trend was

294   observed, with +3 and HW+3 characterized by the lowest G_l rates (0.21±0.09 and 0.43±0.07

295   μmol $CaCO_3$ gFW^{-1} h^{-1}), and C and HW by the highest rates (0.69±0.20 and 0.52±0.07 μmol

296    $CaCO_3$ gFW^{-1} h^{-1}, respectively). Although non-significant, at time t_3 the lowest G_d rates

297   were measured in the treatment HW+3, with negative values corresponding to a net

298   dissolution of the algae (-0.30±0.11 μmol $CaCO_3$ gFW^{-1} h^{-1}), and the highest ones were

299   found in C (0.22 ± 0.38 μmol CaCO$_3$ gFW^{-1} h^{-1}, with one sample having experienced

300   dissolution).

301   G$_d$ rates did not change significantly over time (Table 1). In C and HW, no differences in G$_l$

302   rates were observed among experimental time points (t$_1$, t$_2$ and t$_3$). G$_l$ rates decreased from t$_2$

303   to t$_3$ in the +3°C condition, with rates varying from 1.22 ± 0.31 to 0.21 ± 0.09 μmol CaCO$_3$

304   gFW^{-1} h^{-1} [$p_{t_2,t_3} = 0.001$]; and in the HW+3 treatment, from 1.47 ± 0.33 to 0.43 ± 0.07 μmol

305   CaCO$_3$ gFW^{-1} h^{-1} [$p_{t_2,t_3} = 0.015$].

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307   **Fig. 5.** Calcification rates in the dark (G$_d$) and at the experimental irradiance (G$_l$) in the 4

308   treatments (C, HW, +3, HW+3) for the 3 incubation times (t$_1$, before the MHW start; t$_2$,

309   right after the MHW end; t$_3$, after a recovery period from the MHW end). Negative values

310   for algal calcification correspond to the decalcification activity quantified as increase in

311   total alkalinity. Data are expressed as means $\pm$ SE; $n = 4$ for each treatment.

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Table 1

Summary of linear mixed effects models testing the effect of temperature and experimental time on *C. officinalis* metabolism in the dark and at the incubation irradiance. R_d, dark respiration; P_n, net production; P_g, gross production; G_d, net calcification in the dark; G_l, net calcification in the light. Bolded values indicate *p*-values < 0.05.

		Dark				Light					
		R_d		G_d		P_n		P_g		G_l	
Factor	df	F-value	*p*-value	F-value	*p*-value	F-value	*p*-value	F-value	*p*-value	F-value	*p*-value
Temperature	3	8.374	**0.003**	4.962	**0.018**	1.568	0.248	1.124	0.378	32.424	**<.001**
Time	2	21.963	**<.001**	3.030	0.068	15.261	**<.001**	33.143	**<.001**	7.393	**0.004**
Interaction	6	8.836	**<.001**	0.833	0.557	1.873	0.127	2.536	**0.048**	6.733	**<.001**

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4. Discussion

Macroalgae occurring in intertidal habitats (such as *C. officinalis*) are exposed to strong daily and seasonal temperature fluctuations. As such, they may have a greater ability to acclimate to higher temperature regimes than macroalgae found in more thermally stable conditions. However, little is known of how extreme changes in water temperature, e.g. heatwaves, affect algae physiology in coastal rock pools.

Temperature has a fundamental effect on chemical reaction rates, and a general dependence of respiration and photosynthesis to temperature is well known in macroalgae (Lüning, 1990).

Our results for *C. officinalis* confirmed this dependence by showing, initially at time t_1, a decrease in respiration rates at elevated temperatures, followed by an opposite trend after the MHW simulation, at t_2 and t_3, with an increase in respiration rates with higher temperatures. This increase in respiration rates at elevated temperatures is in line with other studies carried out on different species of coralline algae (Adey, 1973; Digby, 1977; Ichiki et al., 2001; Martin et al., 2006; Steller et al., 2007, Williamson et al., 2017). In particular, when the MHW was simulated, we observed an increase in respiration rates at temperatures raised by +3°C relative to measured summer values (i.e., up to a T_{max} of about 23°C; see Fig. 2). However, a further 1°C increase (mimicking a MHW) in the HW+3 treatment (i.e., up to a T_{max} of about 24°C) led to respiration rate reductions. This shows that a decline in respiration occurs beyond a thermal optimum that is close to the SST registered in the summer season (i.e., as simulated in our experiment with T_c daily oscillating in the range 16.5-20°C). The same trend is confirmed after 2 weeks of recovery from the MHW end, at time t_3. These results are in accordance with those reported by Martin et al. (2013) on the temperate coralline alga *Lithophyllum cabiochae*, demonstrating a positive effect on

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369 respiration rates at higher temperatures during the colder months, and either a negative or a

370 nil response during the summer when temperatures are closer to a thermal optimum

371 (Anthony et al., 2008). We also observed that a prolonged exposure to high temperatures

372 (i.e., t_3, 7 weeks up to a T_{max} of about 23°C in daily temperature variation) negatively

373 affected respiration rates, with the lowest respiration values registered in HW+3 (0.45±0.10

374 μmol O_2 gFW^{-1} h^{-1}).

375 There was limited evidence for an effect of temperature on photosynthesis of *C. officinalis*,

376 despite a significant increase of P_g rates with temperature, reaching a maximum of +3°C

377 variation with respect to the control, was observed after 5 weeks (at t_2). Although non-

378 significant, the same increase is observed in P_n. This general trend mirrors the results

379 reported for *C. officinalis* (Digby, 1977; Williamson et al., 2017) and other coralline algae

380 (Digby, 1977; Ichiki et al., 2001; Martin et al., 2006; Steller et al., 2007; Martin et al.,

381 2013), which indicate higher photosynthesis variation as a consequence of elevated

382 temperature (c.a. 10°C) and irradiance changes between winter and summer (Martin et al.,

383 2013; Williamson et al., 2017). By simulating summer conditions of irradiance and SST in

384 our experiment, we recorded small positive variations in photosynthesis between the control

385 and the elevated temperature treatments. This might occur at temperatures already close to

386 the thermal optimum (Anthony et al., 2008), and partially agrees with the observation of

387 Martin et al. (2013) in *L. cabiochae* where significant effects of the 3°C warming were

388 detected on P_g in colder seasons but not in the summer. Importantly, as already noted for

389 respiration, a prolonged exposure to high temperatures (i.e., t_3, 7 weeks up to a T_{max} of about

390 23°C) negatively affected P_n rates, as a possible effect of thermal stress.

391 In general, dark calcification showed high variability in all treatments at all experimental

392 time points. This reflects the findings of Kolzenburg et al. (2019) on *Corallina officinalis*,

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393 and suggests that the already small amount of calcification in the dark is easily influenced

394 by environmental factors such as temperature. However, we observed lower rates of calcium

395 carbonate precipitation in the dark with respect to experimental irradiance. This is in line

396 with the results reported for *Amphiroa anceps* and *A. foliacea* by Borowitzka (1981), for

397 *Corallina frondescens* and *C. vancouveriensis* by McCoy et al. (2016), and for *C. officinalis*

398 by Kolzenburg et al. (2019), due to the strict connection between algal photosynthetic

399 activity, providing the greatest contribute to CO_2 fixation, and calcification. The rates of

400 calcification under experimental irradiance and in the dark did not exhibit significant

401 responses to temperature within each time point. Previous studies on coralline algae showed

402 similar results, with high variations in calcification rates reported for coralline algae under

403 high changes of both irradiance and temperature (Martin et al., 2013; McCoy et al., 2016;

404 Williamson et al., 2017; Kolzenburg et al. 2019). In the present study we only considered

405 lower temperature regimes and constant values of irradiance compared to previous studies.

406 However, a general (but non-significant) calcification increase under summer irradiance

407 conditions was observed in the first 5 weeks of the experiment for a +3°C (and also +4°C

408 after 4 weeks) variation with respect to the control temperature. This agrees with the

409 lowering of calcification rates at cooler temperatures seen in other *Corallina* species (*C.*

410 *frondescens* and *C. vancouveriensis*) by McCoy et al. (2016). Critically, the significant

411 decrease measured in G_l rates at elevated temperatures (six fold for the +3°C condition, and

412 threefold for the HW+3 condition, respectively) at the end of the recovery time (from t_2 to

413 t_3) may possibly be related to thermal stress. These results reflect findings of Vásquez-

414 Elizondo and Enríquez (2016) on the coralline algae *Amphiroa tribulusfrom,*

415 *Neogoniolithon sp.* and *Lithothamnion sp.*, indicating losses in algal calcification after

416 exposure to elevated temperature (+ 2°C above the local maximum monthly mean

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417 temperature). This decreasing trend is similar in the case of dark calcification, with a

418 significant decrease of G_d in the +4°C condition at the end of the recovery time, consistent

419 with the effect of a stress induced by a prolonged warming.

420 Furthermore, it has to be considered that under anthropogenic climate change, MHWs will

421 likely increase in intensity and frequency (Hobday et al., 2016), and that coralline algae will

422 possibly be affected by other stresses such as ocean acidification (Hall-Spencer et al., 2008;

423 Kuffner et al., 2008; Martin and Gattuso, 2009; Ragazzola et al., 2012; Donnarumma et al.,

424 2014). Our findings indicated that MHWs could be more harmful when combined to the

425 long-term temperature increase predicted by the end of the century. This overall increase in

426 temperature and the increase in frequency and intensity of the heatwaves could thus have

427 severe effects on the species' distribution, creating a range shift northwards (Araújo et al.,

428 2005). Therefore, southern margin populations of *C. officinalis,* together with the high

429 densities of macrofaunal organisms living within their fronds, may risk disappearing in their

430 original environment as temperatures warm in future oceans (Kolzenburg et al., 2019).

431 However, at higher latitudes, aragonite saturation state and ocean pH will reach critically

432 low levels first (Steinacher et al., 2009), potentially leading to a shift of calcifying species

433 distribution southwards (Orr et al., 2005; Yara et al., 2012; Lenton et al., 2015). This might

434 result in a contraction of the natural distribution of *C. officinalis*, yet more studies

435 considering the combined effects of warming and acidification are needed to predict how

436 this species' distribution and abundance may be affected by anthropogenic climate change.

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438 **5. Conclusion**

439 By combining the effects of thermal stress induced by tides, gradual ocean warming, and

440 marine heatwaves, we have shown how important insights can be obtained on the likely

physiological responses of coralline algae to climate-change induced temperature variations. Our experiment was designed to simulate summer conditions, in order to understand how this species will respond to temperature variations stemming from climate warming when the algae already experience conditions that are near their thermal optimum. Our results indicated that temperature has a significant effect on *C. officinalis* physiology. After exposure to +3°C from the field temperature (up to a T_{max} of about 23°C), both respiration and photosynthesis increased. This trend has already been reported for several species of coralline algae (Martin et al., 2013). After 5 weeks, calcification seemed to be enhanced at higher temperatures (up to a T_{max} of about 23 and 24°C), but at the end of the experiment calcification rates decreased at those temperatures beyond the thermal optimum. The same trend was noted for all the physiological processes, suggesting that a prolonged exposure to high temperatures (i.e., 7 weeks up to a T_{max} of about 23°C) negatively affects the physiology of *C. officinalis*, as a possible effect of thermal stress. A one-week heatwave of +1°C with respect to the control temperature did not significantly affect respiration, photosynthesis, or calcification rates. This might be explained by the good adaptation of *C. officinalis* to both seasonal and tidal temperature variability (Williamson et al., 2017). Conversely, a further increase of 1°C (due to the MHW) to the 3°C increase predicted by the end of the century, often induced physiological rate reductions, underlining that MHWs may have a negative impact on this species in the near future. Given the fundamental ecological role of *C. officinalis* and other coralline algae as habitat-forming species, stronger and more frequent temperature extremes over the next decades could result in a decrease in coralline algal abundance or a shift in the species' distribution, with potentially major consequences for biodiversity in coastal ecosystems.

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Highlights

- After a 7-weeks exposure, physiological rates decreased at elevated temperatures.

- A one-week heatwave of 1°C did not affect algal metabolism.

- Heatwave of 1°C added to the 3°C increase induced a reduction of algal metabolisms.