

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

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4 Francesco Rendina^{1,2,*}, Phil J. Bouchet^{3,4}, Luca Appolloni^{1,2}, Giovanni Fulvio Russo^{1,2},
5 Roberto Sandulli^{1,2}, Regina Kolzenburg⁵, Aditya Putra⁵, Federica Ragazzola⁵

6 ¹Department of Science and Technology, University of Naples “Parthenope”, Centro Direzionale,
7 Is. C4, 80143 Naples, Italy

8 ²National Interuniversity Consortium for Marine Sciences (CoNISMa), 00196 Rome, Italy

9 ³Centre for Research into Ecological & Environmental Modelling, University of St Andrews, St
10 Andrews, UK

11 ⁴School of Mathematics and Statistics, University of St Andrews, St Andrews, Scotland, UK

12 ⁵Institute of Marine Sciences, University of Portsmouth, Ferry Road, PO4 9LY Portsmouth, UK

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14 *Corresponding author: Current address: Department of Science and Technology, University of
15 Naples “Parthenope”, Centro Direzionale, Is. C4, 80143 Naples, Italy

16 E-mail address: francesco.rendina@uniparthenope.it (F. Rendina).

17 **ABSTRACT**

18 Climate change is leading to an increase of mean sea surface temperatures and extreme heat events.
19 There is an urgent need to better understand the capabilities of marine macroalgae to adapt to these
20 rapid changes. In this study, the responses of photosynthesis, respiration, and calcification to elevated
21 temperature in a global warming scenario were investigated in the coralline alga *Corallina officinalis*.
22 Algae were cultured for 7 weeks under 4 temperature treatments: (1) control under ambient-summer
23 conditions (C, ~20°C), (2) simulating a one-week heatwave of 1°C (HW, $T_{\text{control}}+1^{\circ}\text{C}$), (3
24) elevated temperature (+3, $T_{\text{control}}+3^{\circ}\text{C}$), (4) combination of the two previous treatments (HW+3,
25 $T_{+3}+1^{\circ}\text{C}$). After exposure at T_{+3} (up to a T_{max} of ~23°C), respiration and photosynthesis increased
26 significantly. After 5 weeks, calcification rates were higher at elevated temperatures (T_{+3} and $T_{\text{HW}+3}$)
27 compared to T_{control} , but at the end of the experiment (7 weeks) calcification decreased significantly
28 at those temperatures beyond the thermal optimum (six-fold at T_{+3} , and three-fold at $T_{\text{HW}+3}$,
29 respectively). The same trend was noted for all the physiological processes, suggesting that a
30 prolonged exposure to high temperatures (7 weeks up to T_{+3}) negatively affect the physiology of *C.*
31 *officinalis*, as a possible consequence of thermal stress. A one-week heatwave of +1°C with respect
32 to T_{control} (at T_{HW}) did not affect respiration, photosynthesis, or calcification rates. Conversely, a
33 heatwave of 1°C, when combined with the 3°C increase predicted by the end of the century (at $T_{\text{HW}+3}$),
34 induced a reduction of physiological rates. Continued increases in both the intensity and frequency
35 of heatwaves under anthropogenic climate change may lead to reduced growth and survival of
36 primary producers such as *C. officinalis*.

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

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1. Introduction

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Climate change is occurring at a faster rate than in the past, due to increasing concentrations of greenhouse gases in the Earth's atmosphere caused by human combustion of fossil fuels and deforestation (IPCC, 2014). This results in increasing seawater temperatures, rising sea levels, and ocean acidification (IPCC, 2014). The International Panel on Climate Change (IPCC) indicated that global mean surface temperatures have already risen by approximately 0.87°C in the last one and a half centuries (over the period 1850-2015), and will likely increase further (by ca. 3°C by the end of this century, according to the pathways reflecting present nationally stated mitigation goal by 2030; Masson-Delmotte et al., 2018). In addition to long-term warming, extreme events (i.e., storms, droughts, floods and heatwaves) are also becoming more frequent and more intense (Coumou and Rahmstorf, 2012; Perkins et al., 2012; Oliver et al., 2018, Frölicher et al., 2018; Darmaraki et al., 2019). Specifically, marine heatwaves (MHWs) can strongly influence ecosystem structure and functioning by causing widespread mortality, species range shifts and community changes (Jentsch et al., 2007; Hobday et al., 2016, Smale et al., 2019).

Increasing sea surface temperature (SST) is among the main impacts affecting marine ecosystems (Stenseth et al., 2002), which can influence the abundance and distribution of marine organisms, and lead to poleward range shifts or extinctions of populations located at the edge of their thermal tolerance (Perry et al., 2005; Wernberg et al., 2011; Yara et al., 2012; Jueterbock et al., 2013; Sanford et al., 2016; Collin et al., 2018; Kolzenburg et al., 2019). Elevated temperatures can lead to severe ecological impacts, including widespread mortality of benthic communities (Garrabou et al., 2009), loss of seagrass habitats (Marbà and Duarte, 2010), and impacts on fisheries, due to changes in primary productivity and shifts in distribution or mass mortality events of species of commercial interest (Sumaila et al., 2011;

65 Mills et al., 2013; Caputi et al., 2015). In particular, water temperature is a major factor
66 controlling the survival, growth and reproduction of macroalgae, and thus plays an important
67 role in governing both the small-scale vertical and the large-scale geographical distribution
68 of macroalgal species, in addition to their abundance (Breeman, 1988; Lüning, 1990; Nannini
69 et al., 2015). For this reason, it is worth understanding the biological responses of climate-
70 sensitive organisms to short-term extreme events, in concurrence with long-term changes
71 (Jentsch et al., 2007).

72 Coralline red algae (Rhodophyta) are fundamental calcifying primary producers and
73 important habitat-forming species present in most coastal ecosystems, such as coralligenous
74 bioconstructions (Johansen, 1981; Ferrigno et al., 2017; Ingrosso et al., 2018). The species
75 *Corallina officinalis* (Linnaeus 1758) is an erect calcifying alga with a wide distribution that
76 dominates North Atlantic rocky shores and rock pools (Williamson et al., 2015). Due to its
77 complex morphological structure, it represents an important substratum for the settlement of
78 other macroalgae and microalgae, and supports a high biodiversity of marine invertebrates
79 (Akioka et al., 1999; Kelaher, 2003). Despite the importance of coralline algae, their
80 sensitivity to increasing temperatures is still unclear, as different studies have yielded
81 conflicting results (Martin et al., 2013; Comeau et al., 2014; Vásquez-Elizondo and Enríquez,
82 2016). There is further uncertainty around the response of *C. officinalis* in rock pool habitats,
83 as the species must adapt to multiple stressors, including highly variable water temperatures
84 across seasonal, diurnal and tidal cycles (Williamson et al., 2017).

85 In this study, we describe the physiological responses of *C. officinalis* to temperature variation
86 in an ocean warming scenario (RCP 8.5; IPCC, 2014), taking into account natural thermal
87 fluctuations experienced within rock pools across periods of low and high tides (i.e., $\Delta T =$
88 $\sim 3.5^{\circ}\text{C}$, recorded in the field; see Fig. 2). Specifically, we analysed photosynthesis,

89 respiration, and dark/light calcification rates of a South-East UK population exposed to both
90 a temperature increase of +3°C (i.e., simulating the warming expected by the end of this
91 century; Solomon et al., 2007), and a marine heatwave (similar to those registered over the
92 last century, and attributed to anthropogenic climate change; Oliver et al., 2018).

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2. Materials and methods

2.1. Biological material

96 Specimens of the articulated coralline alga *Corallina officinalis* were collected during low
97 tide in intertidal rock pools at ± 0.3 m depth of St. Margarets Bay (Kent, UK; 51°08'52.9"N,
98 1°23'06.9"E) in September 2017. Seawater temperature measured at the time of sampling
99 with a HQ30D flexi multi-meter (Hach Environmental, Loveland, CO, USA) was 15.7 ± 0.2 °C.
100 Algae were immediately transported (~3 hours) in temperature-insulating containers to the
101 Institute of Marine Sciences, University of Portsmouth, UK, where the experiment was
102 carried out. Healthy thalli in the size range of 3-10 cm² were selected for the experiment, and
103 were carefully cleaned of epiphytic organisms, avoiding any damage. Algae were fixed on
104 small stones, in order to simulate natural conditions and keep them upright, and guarantee the
105 same light conditions to each branch as much as possible (~3 g fresh weight for each stone),
106 see Fig. 1.

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2.2. Experimental design

109 Temperature and irradiance during the experiment were set according to ambient summer
110 conditions recorded daily in the field in July-August 2017 by a HOBO pendant
111 temperature/light data logger (Onset Computer Corp., Bourne, MA, USA) placed in a rock
112 pool of the collection site. Algae were acclimated in 8 15-L aquaria in a closed seawater

113 system for 2 weeks before the gradual increase of water temperature. During acclimatization,
114 algae were maintained at a 14:10 light:dark photoperiod, with UV light oscillating in the range
115 $20\text{-}30\ \mu\text{mol m}^{-2}\text{s}^{-1}$ (measured at the position of the submerged algal fronds in the experimental
116 tanks, mimicking sun set and sun rise and with controlled dimming during the day as cloud
117 effect), while the temperature (T) changed during 24h from a T_{\min} of $\sim 16.5\pm 0.1^\circ\text{C}$ to a T_{\max}
118 of $\sim 20\pm 0.1^\circ\text{C}$, around a mean temperature value of $18.5\pm 1.2^\circ\text{C}$, reflecting the T oscillations
119 of daily tides recorded in the field. After acclimatization, specimens were assigned to 16 x
120 11-L glass aquaria (4 tanks per treatment) under 4 temperature conditions. Each aquarium
121 contained three algae-stones, for a total of 12 algae/stones per temperature treatment (Fig. 1).
122 Aquaria were kept in a closed system with seawater sourced directly from the sea off the
123 Institute of Marine Sciences (University of Portsmouth, UK; $50^\circ 47' 40.7''\text{N}$, $1^\circ 01' 50.1''\text{W}$)
124 and processed via a settlement system with glass media filtration (salinity ranging from 34.4
125 to 35.2). Ten percent of the aquaria water was exchanged every other day, in order to keep
126 nutrient levels and alkalinity constant. The four temperature treatments (Fig. 2) were: (1)
127 control treatment (C), kept at the *in situ* acclimatization temperature, with a temperature T_C
128 oscillating according to a thermal range reflective of the daily tides recorded in the field
129 ($16.5^\circ\text{C}\leq T_C\leq 20^\circ\text{C}$); (2) heatwave treatment (HW), where a heatwave was simulated by
130 inducing a temperature increase of $+1^\circ\text{C}$ for a period of 1 week ($T_{\text{HW}}= T_C+1^\circ\text{C}$;
131 $17.5^\circ\text{C}\leq T_{\text{HW}}\leq 21^\circ\text{C}$); (3) elevated temperature treatment (+3), where the temperature was
132 increased by $+3^\circ\text{C}$ according to the predicted temperature increase due to climate change by
133 the year 2100 ($T_{+3}=T_C+3^\circ\text{C}$; $19.5^\circ\text{C}\leq T_{+3}\leq 23^\circ\text{C}$; Solomon et al., 2007); (4) treatment obtained
134 by the combination of the two previous treatments (HW+3), with a $+4^\circ\text{C}$ temperature increase
135 ($T_{\text{HW}+3}=T_C+3^\circ\text{C}+1^\circ\text{C}$; $20.5^\circ\text{C}\leq T_{\text{HW}+3}\leq 24^\circ\text{C}$). Temperature was increased at a rate of 0.5°C
136 per day (over a period of 6 days) to reach the $+3^\circ\text{C}$ temperature change, and of 0.5°C per hour

137 (over a period of 1 hour) when simulating the MHW. Water temperature in all tanks was
138 monitored daily with a HQ30D flexi multi-meter (Hach Environmental, Loveland, CO, USA),
139 and had a continual logging every 15 min with a HOBO pendant data logger (Onset Computer
140 Corp., Bourne, MA, USA). Irradiance levels were monitored throughout the experiment with
141 a Quantitherm light-meter (QRT-1, Hansatech Instruments, Norfolk, UK). pH and salinity
142 were measured using the HQ30D flexi multi-meter by pH and salinity probes (Hach
143 Environmental, Loveland, CO, USA). Total Alkalinity was measured by potentiometric
144 titration (TitroLine 7000, Schott SI Analytics, Mainz, German) following the SOP6 protocol
145 (Dickson et al., 2007). Measurements were validated against Dickson standard (batch #154).
146 Other parameters of the carbonate chemistry were calculated using the software CO2Sys,
147 EXCEL Macro version 2.1 (Lewis et al., 1998). Water motion and filtration in the aquaria
148 was ensured by a submersible pump (V²PowerPump 800, TMC, London, UK).



149

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

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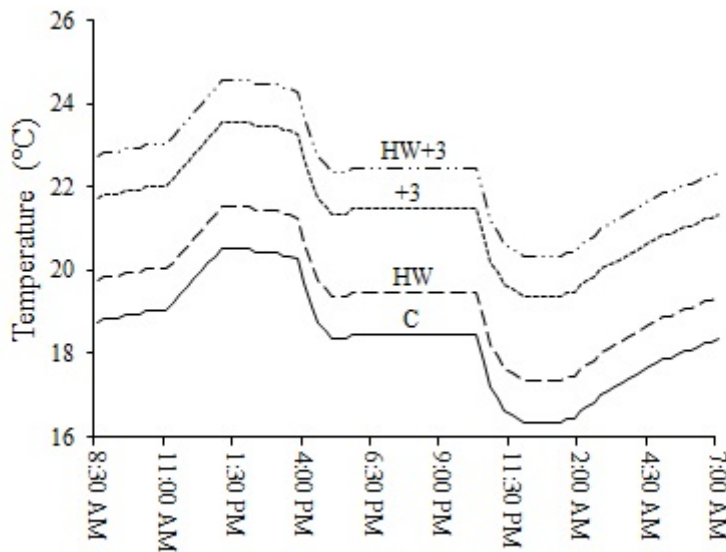
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157 *2.3. Marine heatwave calculation*

158 A one-week MHW of 1°C was calculated in accordance to the definition provided by Hobday
159 et al. (2016), i.e., referring to the temperature values exceeding the 90th percentile threshold
160 of the SST measured for at least five consecutive days in the same 30-day-period window
161 over the last 30 years. SST *in situ* data were obtained from the closest NOAA buoy to the
162 collection site, located off the South UK Coast (about 40 km) along the Greenwich meridian
163 (Station 50°24'0" N 0°0'0" E; National Data Buoy Center, National Oceanic and Atmospheric
164 administration; www.ndbc.noaa.gov/station_page.php?station=62305).

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167 **Fig. 2.** Daily planned temperature changes in the experimental tanks for the 4 treatments (C,
168 HW, +3, HW+3). The temperature fluctuation in the control (C) was performed simulating
169 the environmental thermal excursion due to the daily tides, as recorded in the field. The MHW
170 was performed in the treatments HW and HW+3, and lasted for one week (for more details
171 see Fig. 3).

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174 2.4. *Physiological measurements*

175 Algal thalli (1 g/fresh weight from each tank at each time point) were incubated in 50-ml
176 closed oxygen chambers filled with bubble-free seawater from the aquaria. One hour
177 incubations were conducted under saturating light condition ($300 \mu\text{mol m}^{-2}\text{s}^{-1}$; Ralph and
178 Gademann, 2005) and in the dark. The irradiance levels were controlled with a Quantitherm
179 Light Meter (QRT-1, Hansatech Instruments, Norfolk, UK). The chambers were used to
180 assess net photosynthesis (P_n) and calcification in the light (G_l), while chambers covered with
181 aluminium foils were used to assess dark respiration (R_d) and calcification in the dark (G_d).
182 The concentration of dissolved oxygen (O_2 , $\mu\text{mol l}^{-1}$) was measured inside the chambers
183 before and after incubations using a HQ30D flexi oxygen meter (Hach Environmental). Water
184 samples were taken at the beginning and at the end of the incubations for measurements of
185 pHT (pH on the total scale) and total alkalinity (A_T).
186 P_n and R_d , expressed in terms of O_2 production and consumption (in $\mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$), were
187 calculated after Williamson et al. (2017):

191
$$P_n(\text{or } R_d) = \frac{\Delta O_2 v}{fw \Delta t}$$

188 where ΔO_2 is the difference in O_2 concentration before and after incubation ($\mu\text{mol l}^{-1} \text{ h}^{-1}$), v is
189 the volume of the incubation tubes (l), fw is the fresh weight of the algae incubated (g) and Δt
190 is the incubation time (h).

192 Gross photosynthesis (P_g) was calculated as:

193
$$P_g = |P_n| + |R_d|$$

194 G_l and G_d ($\mu\text{mol CaCO}_3 \text{ gFW}^{-1} \text{ h}^{-1}$) were calculated using the alkalinity anomaly technique
195 (Smith and Key, 1975) as:

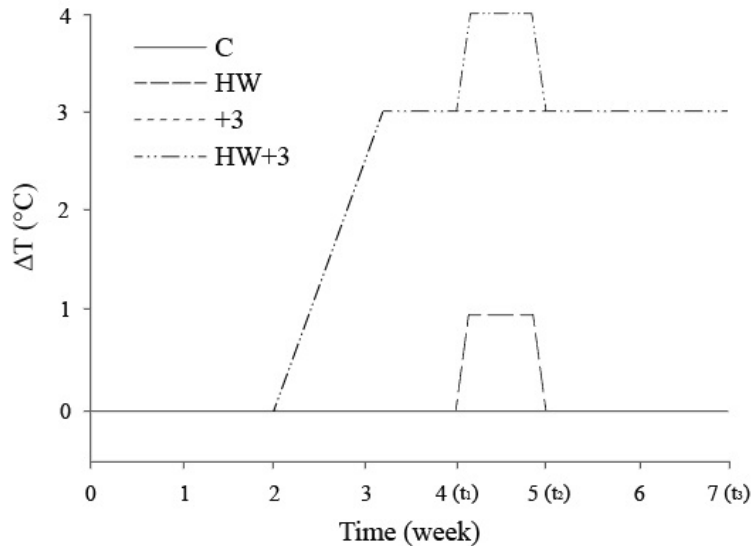
$$G_l(\text{or } G_d) = \frac{\Delta A_T v}{2(f_w \Delta t)}$$

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197 where ΔA_T is the difference between initial and final A_T values ($\mu\text{eq l}^{-1}$).

198 Physiological measurements were taken in all treatments at 3 different times during the
 199 experiment (Fig. 3):

- 200 • t_1 = before the MHW start (4-weeks); i.e., after 2 weeks of acclimatization at the initial
- 201 temperature T_{control} , 1 week of gradual heating up to T_{+3} , and 1 week of acclimatization at T_{+3}
- 202 (the heating up to T_{+3} was induced only in treatments +3 and HW+3);
- 203 • t_2 = right after the MHW end (5-weeks); i.e., 1 week of HW at T_{HW} after t_1 (the HW was
- 204 induced only in treatments HW and HW+3);
- 205 • t_3 = after a recovery period from the MHW end (7-weeks); i.e., 2 weeks of recovery from the
- 206 heatwave-end after t_2 .



207

208 **Fig. 3.** Planned temperature changes during the experiment in the 4 treatments (C, HW, +3,
 209 HW+3). Physiological measurements were taken in all treatments at 3 different times: t_1 (4-
 210 weeks), before the MHW start; t_2 (5-weeks), right after the MHW end; t_3 (7-weeks), after a
 211 recovery period from the MHW end.

212

213 2.5. Data analysis

214 We used linear-mixed effects models (LMMs) to examine whether temperature treatments
215 influenced photosynthesis, respiration, and calcification rates. Models were developed in the
216 *nlme* package in R v3.6.0 (Bates et al., 2015; Pinheiro et al., 2019) with both time and
217 temperature (as well as their interaction) treated as fixed effects, and tank ID included as a
218 random effect to account for autocorrelated errors among algae grown in the same tanks
219 (Speights et al., 2017). Model residuals were visually inspected using QQ plots and residual
220 plots and formally checked for normality and homoskedasticity via Shapiro-Wilks and
221 Levene's tests, respectively. Heterogeneity in residual variance was only identified in the
222 calcification models, and was addressed with an appropriate structure (*varIdent*), allowing
223 residuals to differ in spread between temperature treatments across time without the need to
224 transform the data (Pinheiro and Bates, 2000; Harrison et al., 2018). Results are expressed as
225 mean \pm standard error of the mean (SE). n is the sample size and p_s are Tukey-adjusted p -
226 values, evaluated against a significance threshold of $\alpha = 0.05$.

227 3. Results

228 3.1. Respiration

229 LMMs explained over 76% of the variation in dark respiration (R_d) [conditional pseudo- $R^2 =$
230 0.763; Nakagawa and Schielzeth, 2013]. At time t_1 , before mimicking the marine heatwave,
231 no significant differences were found between R_d rates in the control (C) and the treatment
232 HW, and between the treatments +3 and HW+3 (Fig. 4; Table 1). This trend was expected
233 because, at time t_1 , the treatments C and HW were kept at the same temperature T_c
234 ($16.5^\circ\text{C} \leq T_c \leq 20^\circ\text{C}$); while, +3 and HW+3 were both at T_{+3} ($T_{+3} = T_c + 3^\circ\text{C}$; $19.5^\circ\text{C} \leq T_{+3} \leq 23^\circ\text{C}$;
235 see fig. 3). However at t_1 , C and HW were both different from +3 and HW+3, with lower R_d
236 values measured in +3 and HW+3 (0.88 ± 0.08 and $0.81 \pm 0.09 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$,

237 respectively), and higher values in C and HW (1.27 ± 0.15 and $1.38\pm 0.12 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$,
238 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 ,
239 immediately after the MHW, the lowest R_d rates were measured in the HW treatment
240 ($0.53\pm 0.06 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$), while the highest rates were found in the treatment +3
241 ($1.22\pm 0.04 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$) [$p_{HW,+3} = 0.001$]; intermediate values were measured in C
242 ($0.90\pm 0.07 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$). At time t_3 , in HW+3, the lowest R_d values were registered
243 ($0.45\pm 0.10 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$) [$p_{C,HW+3} = 0.026$].
244 R_d in the control group did not change significantly over time (Fig. 4). In the treatment +3, R_d
245 rates increased from t_1 to t_2 (0.88 ± 0.10 and $1.22\pm 0.04 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$, respectively) [p_{t_1,t_2}
246 = 0.023], and decreased from t_2 to t_3 (1.22 ± 0.04 and $0.88\pm 0.07 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$,
247 respectively) [$p_{t_2,t_3} = 0.027$]. In HW, R_d rates decreased from t_1 to t_2 , going from 1.38 ± 0.12
248 to $0.53\pm 0.04 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$ [$p_{t_1,t_2} < 0.001$]. In HW+3, R_d rates decreased from t_1 to t_3
249 (0.81 ± 0.09 to $0.45\pm 0.10 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$) [$p_{t_1,t_2} = 0.015$]. While, no changes between t_2
250 (right after the MHW end) and t_3 (after the recovery period from the MHW) were observed in
251 both the heatwave conditions HW and HW+3.

252

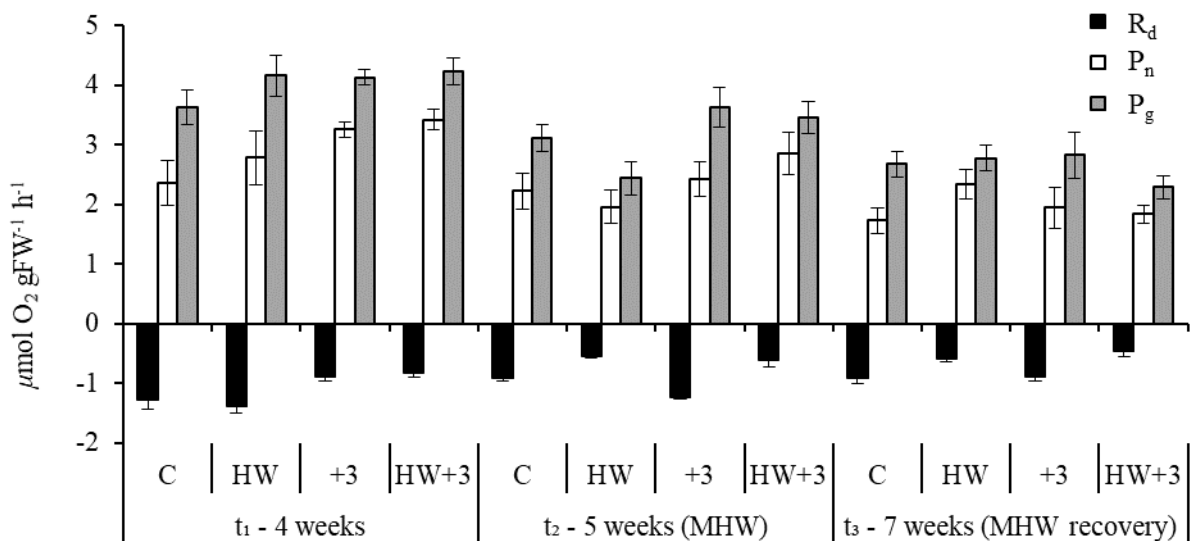
253 3.2. Photosynthesis

254 LMMs explained over 58% of the variation in net photosynthesis (P_n) and 67% of variation
255 in gross photosynthesis (P_g) [conditional pseudo- $R^2 = 0.581$ and 0.672 , P_n and P_g
256 respectively]. No differences in P_n rates were found among temperature treatments at each
257 experimental time (t_1 , t_2 , t_3 ; Fig. 4; Table 1). Likewise, no differences in P_g rates were apparent
258 at time t_1 between the control (C) and the treatment at $+3^\circ\text{C}$ (3.64 ± 0.29 and $4.13\pm 0.12 \mu\text{mol}$
259 $\text{O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$, respectively), or between HW and HW+3 (4.16 ± 0.35 and $4.23\pm 0.23 \mu\text{mol O}_2$

260 gFW⁻¹ h⁻¹, respectively). At time t₂, the lowest P_g values were measured in the treatment HW
 261 (2.44±0.28 μmol O₂ gFW⁻¹ h⁻¹) and the highest in +3 (3.64±0.33 μmol O₂ gFW⁻¹ h⁻¹), with
 262 these being significantly different from each other [*p*_{HW,+3} = 0.036]. No significant differences
 263 were found among all temperature treatments at time t₃, after the recovery period from the
 264 MHW end.

265 In C, P_n rates did not vary among experimental time points (t₁, t₂ and t₃), while P_g decreased
 266 from t₁ to t₃ [*p*_{t₁,t₃} = 0.028]. In the treatment +3, both P_n (from 3.26±0.13 to 1.94±0.35 μmol
 267 O₂ gFW⁻¹ h⁻¹) and P_g (from 4.13±0.12 to 2.83±0.38 μmol O₂ gFW⁻¹ h⁻¹) decreased from t₁ to
 268 t₃ [*p*_{t₁,t₃} = 0.004 and 0.003, P_n and P_g respectively]. In the treatment HW, P_n did not change
 269 significantly overtime, while P_g decreased from t₁ to t₂ (4.16±0.35 and 2.78±0.22 μmol O₂
 270 gFW⁻¹ h⁻¹, respectively) [*p*_{t₁,t₂} < 0.001], as well as from t₁ to t₃ [*p*_{t₁,t₃} = 0.001]. In HW+3, the
 271 lowest P_n and P_g rates were found after the recovery period from the MHW end (t₃; 1.84±0.15
 272 and 2.29±0.20 μmol O₂ gFW⁻¹ h⁻¹, respectively), with t₃ differing from both t₁ and t₂ [*p*_{t₁,t₃} <
 273 0.001 and 0.001, *p*_{t₂,t₃} = 0.025 and 0.007, P_n and P_g respectively].

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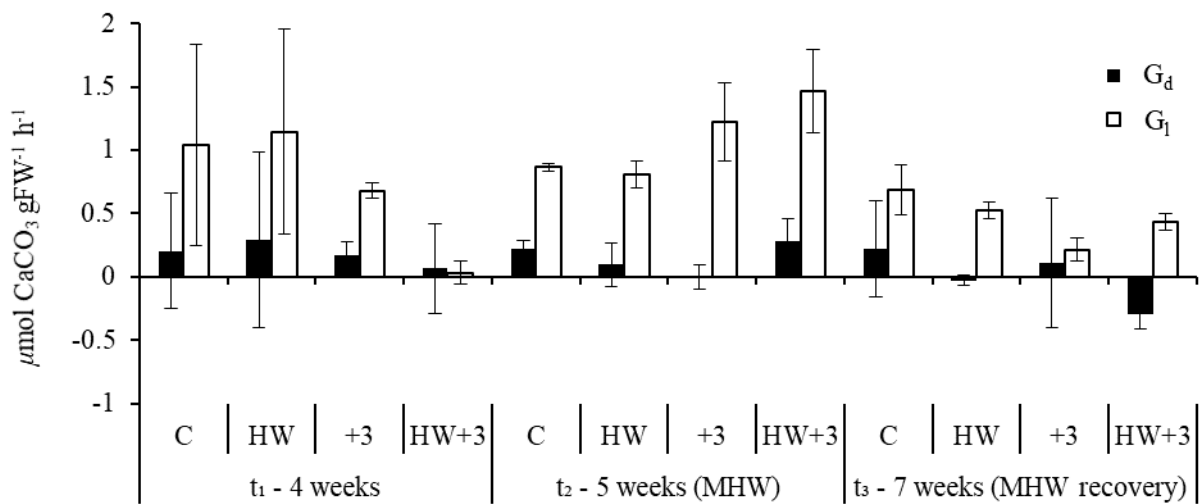
276 **Fig. 4.** Net and gross photosynthesis (P_n and P_g) rates at the experimental irradiance, and
277 respiration (R_d) rates in the dark in the 4 temperature treatments (C, HW, +3, HW+3) for the
278 3 incubation times (t_1 , before the MHW start; t_2 , right after the MHW end; t_3 , after a recovery
279 period from the MHW end). Data are means \pm SE, and are expressed in terms of O_2 release
280 (negative values for respiration correspond to O_2 consumption); $n = 4$ for each treatment.

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282 3.3. Calcification

283 There was only weak evidence for an effect of temperature on calcification rates measured in
284 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 the
286 low percentage of variance explained by the LMMs [G_d conditional pseudo- $R^2 = 0.034$].
287 Variability in G_1 rates was inherently high, especially at the start of the experiment (i.e., time
288 t_1), leading to LMMs with poor explanatory power [conditional pseudo- $R^2 = 0.0601$]. G_1 rates
289 were affected by temperature at time t_1 , with +3 and HW+3 being significantly different from
290 each other [$p_{+3,HW+3} < 0.001$], despite being at the same temperature T_{+3} . At time t_2 , +3 and
291 HW+3 (1.22 ± 0.31 and $1.47 \pm 0.33 \mu\text{mol CaCO}_3 \text{ gFW}^{-1} \text{ h}^{-1}$) exhibited (non-significant) higher
292 G_1 rates than C and HW (0.87 ± 0.03 and $0.81 \pm 0.10 \mu\text{mol CaCO}_3 \text{ gFW}^{-1} \text{ h}^{-1}$, respectively). After
293 2-weeks-recovery from the MHW (t_3), an opposite trend was observed, with +3 and HW+3
294 characterized by the lowest G_1 rates (0.21 ± 0.09 and $0.43 \pm 0.07 \mu\text{mol CaCO}_3 \text{ gFW}^{-1} \text{ h}^{-1}$), and
295 C and HW by the highest rates (0.69 ± 0.20 and $0.52 \pm 0.07 \mu\text{mol CaCO}_3 \text{ gFW}^{-1} \text{ h}^{-1}$,
296 respectively). Although non-significant, at time t_3 the lowest G_d rates were measured in the
297 treatment HW+3, with negative values corresponding to a net dissolution of the algae (-
298 $0.30 \pm 0.11 \mu\text{mol CaCO}_3 \text{ gFW}^{-1} \text{ h}^{-1}$), and the highest ones were found in C ($0.22 \pm 0.38 \mu\text{mol}$
299 $\text{CaCO}_3 \text{ gFW}^{-1} \text{ h}^{-1}$, with one sample having experienced dissolution).

300 G_d rates did not change significantly over time (Table 1). In C and HW, no differences in G_1
 301 rates were observed among experimental time points (t_1 , t_2 and t_3). G_1 rates decreased from t_2
 302 to t_3 in the +3°C condition, with rates varying from 1.22 ± 0.31 to $0.21 \pm 0.09 \mu\text{mol CaCO}_3$
 303 $\text{gFW}^{-1} \text{h}^{-1}$ [$p_{t_2, t_3} = 0.001$]; and in the HW+3 treatment, from 1.47 ± 0.33 to $0.43 \pm 0.07 \mu\text{mol}$
 304 $\text{CaCO}_3 \text{gFW}^{-1} \text{h}^{-1}$ [$p_{t_2, t_3} = 0.015$].



305
 306 **Fig. 5.** Calcification rates in the dark (G_d) and at the experimental irradiance (G_1) in the 4
 307 treatments (C, HW, +3, HW+3) for the 3 incubation times (t_1 , before the MHW start; t_2 , right
 308 after the MHW end; t_3 , after a recovery period from the MHW end). Negative values for algal
 309 calcification correspond to the decalcification activity quantified as increase in total
 310 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 .

Factor	df	Dark			Light						
		R_d		G_d	P_n		P_g		G_l		
		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

4. Discussion

344
345 Macroalgae occurring in intertidal habitats (such as *C. officinalis*) are exposed to strong daily
346 and seasonal temperature fluctuations. As such, they may have a greater ability to acclimate
347 to higher temperature regimes than macroalgae found in more thermally stable conditions.
348 However, little is known of how extreme changes in water temperature, e.g. heatwaves, affect
349 algae physiology in coastal rock pools.

350 Temperature has a fundamental effect on chemical reaction rates, and a general dependence
351 of respiration and photosynthesis to temperature is well known in macroalgae (Lüning, 1990).
352 Our results for *C. officinalis* confirmed this dependence by showing, initially at time t_1 , a
353 decrease in respiration rates at elevated temperatures, followed by an opposite trend after the
354 MHW simulation, at t_2 and t_3 , with an increase in respiration rates with higher temperatures.
355 This increase in respiration rates at elevated temperatures is in line with other studies carried
356 out on different species of coralline algae (Adey, 1973; Digby, 1977; Ichiki et al., 2001;
357 Martin et al., 2006; Steller et al., 2007, Williamson et al., 2017). In particular, when the MHW
358 was simulated, we observed an increase in respiration rates at temperatures raised by +3°C
359 relative to measured summer values (i.e., up to a T_{\max} of about 23°C; see Fig. 2). However, a
360 further 1°C increase (mimicking a MHW) in the HW+3 treatment (i.e., up to a T_{\max} of about
361 24°C) led to respiration rate reductions. This shows that a decline in respiration occurs beyond
362 a thermal optimum that is close to the SST registered in the summer season (i.e., as simulated
363 in our experiment with T_c daily oscillating in the range 16.5-20°C). The same trend is
364 confirmed after 2 weeks of recovery from the MHW end, at time t_3 . These results are in
365 accordance with those reported by Martin et al. (2013) on the temperate coralline alga
366 *Lithophyllum cabiochae*, demonstrating a positive effect on respiration rates at higher
367 temperatures during the colder months, and either a negative or a nil response during the

368 summer when temperatures are closer to a thermal optimum (Anthony et al., 2008). We also
369 observed that a prolonged exposure to high temperatures (i.e., t_3 , 7 weeks up to a T_{\max} of about
370 23°C in daily temperature variation) negatively affected respiration rates, with the lowest
371 respiration values registered in HW+3 ($0.45 \pm 0.10 \mu\text{mol O}_2 \text{ gFW}^{-1} \text{ h}^{-1}$).

372 There was limited evidence for an effect of temperature on photosynthesis of *C. officinalis*,
373 despite a significant increase of P_g rates with temperature, reaching a maximum of $+3^{\circ}\text{C}$
374 variation with respect to the control, was observed after 5 weeks (at t_2). Although non-
375 significant, the same increase is observed in P_n . This general trend mirrors the results reported
376 for *C. officinalis* (Digby, 1977; Williamson et al., 2017) and other coralline algae (Digby,
377 1977; Ichiki et al., 2001; Martin et al., 2006; Steller et al., 2007; Martin et al., 2013), which
378 indicate higher photosynthesis variation as a consequence of elevated temperature (c.a. 10°C)
379 and irradiance changes between winter and summer (Martin et al., 2013; Williamson et al.,
380 2017). By simulating summer conditions of irradiance and SST in our experiment, we
381 recorded small positive variations in photosynthesis between the control and the elevated
382 temperature treatments. This might occur at temperatures already close to the thermal
383 optimum (Anthony et al., 2008), and partially agrees with the observation of Martin et al.
384 (2013) in *L. cabiochae* where significant effects of the 3°C warming were detected on P_g in
385 colder seasons but not in the summer. Importantly, as already noted for respiration, a
386 prolonged exposure to high temperatures (i.e., t_3 , 7 weeks up to a T_{\max} of about 23°C)
387 negatively affected P_n rates, as a possible effect of thermal stress.

388 In general, dark calcification showed high variability in all treatments at all experimental time
389 points. This reflects the findings of Kolzenburg et al. (2019) on *Corallina officinalis*, and
390 suggests that the already small amount of calcification in the dark is easily influenced by
391 environmental factors such as temperature. However, we observed lower rates of calcium

392 carbonate precipitation in the dark with respect to experimental irradiance. This is in line with
393 the results reported for *Amphiroa anceps* and *A. foliacea* by Borowitzka (1981), for *Corallina*
394 *frondescens* and *C. vancouveriensis* by McCoy et al. (2016), and for *C. officinalis* by
395 Kolzenburg et al. (2019), due to the strict connection between algal photosynthetic activity,
396 providing the greatest contribute to CO₂ fixation, and calcification. The rates of calcification
397 under experimental irradiance and in the dark did not exhibit significant responses to
398 temperature within each time point. Previous studies on coralline algae showed similar
399 results, with high variations in calcification rates reported for coralline algae under high
400 changes of both irradiance and temperature (Martin et al., 2013; McCoy et al., 2016;
401 Williamson et al., 2017; Kolzenburg et al. 2019). In the present study we only considered
402 lower temperature regimes and constant values of irradiance compared to previous studies.
403 However, a general (but non-significant) calcification increase under summer irradiance
404 conditions was observed in the first 5 weeks of the experiment for a +3°C (and also +4°C
405 after 4 weeks) variation with respect to the control temperature. This agrees with the lowering
406 of calcification rates at cooler temperatures seen in other *Corallina* species (*C. frondescens*
407 and *C. vancouveriensis*) by McCoy et al. (2016). Critically, the significant decrease measured
408 in G_l rates at elevated temperatures (six fold for the +3°C condition, and threefold for the
409 HW+3 condition, respectively) at the end of the recovery time (from t₂ to t₃) may possibly be
410 related to thermal stress. These results reflect findings of Vásquez-Elizondo and Enríquez
411 (2016) on the coralline algae *Amphiroa tribulusform*, *Neogoniolithon sp.* and *Lithothamnion*
412 *sp.*, indicating losses in algal calcification after exposure to elevated temperature (+ 2°C above
413 the local maximum monthly mean temperature). This decreasing trend is similar in the case
414 of dark calcification, with a significant decrease of G_d in the +4°C condition at the end of the
415 recovery time, consistent with the effect of a stress induced by a prolonged warming.

416 Furthermore, it has to be considered that under anthropogenic climate change, MHWs will
417 likely increase in intensity and frequency (Hobday et al., 2016), and that coralline algae will
418 possibly be affected by other stresses such as ocean acidification (Hall-Spencer et al., 2008;
419 Kuffner et al., 2008; Martin and Gattuso, 2009; Ragazzola et al., 2012; Donnarumma et al.,
420 2014). Our findings indicated that MHWs could be more harmful when combined to the long-
421 term temperature increase predicted by the end of the century. This overall increase in
422 temperature and the increase in frequency and intensity of the heatwaves could thus have
423 severe effects on the species' distribution, creating a range shift northwards (Araújo et al.,
424 2005). Therefore, southern margin populations of *C. officinalis*, together with the high
425 densities of macrofaunal organisms living within their fronds, may risk disappearing in their
426 original environment as temperatures warm in future oceans (Kolzenburg et al., 2019).
427 However, at higher latitudes, aragonite saturation state and ocean pH will reach critically low
428 levels first (Steinacher et al., 2009), potentially leading to a shift of calcifying species
429 distribution southwards (Orr et al., 2005; Yara et al., 2012; Lenton et al., 2015). This might
430 result in a contraction of the natural distribution of *C. officinalis*, yet more studies considering
431 the combined effects of warming and acidification are needed to predict how this species'
432 distribution and abundance may be affected by anthropogenic climate change.

433

434

5. Conclusion

435 By combining the effects of thermal stress induced by tides, gradual ocean warming, and
436 marine heatwaves, we have shown how important insights can be obtained on the likely
437 physiological responses of coralline algae to climate-change induced temperature variations.
438 Our experiment was designed to simulate summer conditions, in order to understand how this
439 species will respond to temperature variations stemming from climate warming when the

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

460

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