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

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

In this study, we describe the physiological responses of *C. officinalis* to temperature variation
in an ocean warming scenario (RCP 8.5; IPCC, 2014), taking into account natural thermal
fluctuations experienced within rock pools across periods of low and high tides (i.e., $\Delta T =
\sim 3.5^\circ$C, recorded in the field; see Fig. 2). Specifically, we analysed photosynthesis,
respiration, and dark/light calcification rates of a South-East UK population exposed to both a temperature increase of +3°C (i.e., simulating the warming expected by the end of this century; Solomon et al., 2007), and a marine heatwave (similar to those registered over the last century, and attributed to anthropogenic climate change; Oliver et al., 2018).

2. Materials and methods

2.1. Biological material

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

2.2. Experimental design

Temperature and irradiance during the experiment were set according to ambient summer conditions recorded daily in the field in July-August 2017 by a HOBO pendant temperature/light data logger (Onset Computer Corp., Bourne, MA, USA) placed in a rock pool of the collection site. Algae were acclimated in 8 15-L aquaria in a closed seawater
system for 2 weeks before the gradual increase of water temperature. During acclimatization, algae were maintained at a 14:10 light:dark photoperiod, with UV light oscillating in the range 20-30 μmol m⁻² s⁻¹ (measured at the position of the submerged algal fronds in the experimental tanks, mimicking sun set and sun rise and with controlled dimming during the day as cloud effect), while the temperature (T) changed during 24h 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 18.5±1.2°C, reflecting the T oscillations of daily tides recorded in the field. After acclimatization, specimens were assigned to 16 x 11-L glass aquaria (4 tanks per treatment) under 4 temperature conditions. Each aquarium contained three algae-stones, for a total of 12 algae/stones per temperature treatment (Fig. 1). Aquaria were kept in a closed system with seawater sourced directly from the sea off the Institute of Marine Sciences (University of Portsmouth, UK; 50°47’40.7’’N, 1°01’50.1’’W) and processed via a settlement system with glass media filtration (salinity ranging from 34.4 to 35.2). Ten percent of the aquaria water was exchanged every other day, in order to keep nutrient levels and alkalinity constant. The four temperature treatments (Fig. 2) were: (1) control treatment (C), kept at the in situ acclimatization temperature, with a temperature T_C oscillating according to a thermal range reflective of the daily tides recorded in the field (16.5°C ≤ T_C ≤ 20°C); (2) heatwave treatment (HW), where a heatwave was simulated by inducing a temperature 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 temperature treatment (+3), where the temperature was increased by +3°C according to the predicted temperature increase due to climate change by the year 2100 (T_+3 = T_C + 3°C; 19.5°C ≤ T_+3 ≤ 23°C; Solomon et al., 2007); (4) treatment obtained by the combination of the two previous treatments (HW+3), with a +4°C temperature increase (T_{HW+3} = T_C + 3°C + 1°C; 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 (V²PowerPump 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).
2.3. Marine heatwave calculation

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

![Fig. 2. Daily planned temperature changes in the experimental tanks for the 4 treatments (C, HW, +3, HW+3). The temperature fluctuation in the control (C) was performed simulating the environmental thermal excursion due to the daily tides, as recorded in the field. The MHW was performed in the treatments HW and HW+3, and lasted for one week (for more details see Fig. 3).](image-url)
2.4. Physiological measurements

Algal thalli (1 g/fresh weight from each tank at each time point) were incubated in 50-ml closed oxygen chambers filled with bubble-free seawater from the aquaria. One hour incubations were conducted under saturating light condition (300 μmol m\(^{-2}\)s\(^{-1}\); Ralph and Gademann, 2005) and in the dark. The irradiance levels were controlled with a Quantitherm Light Meter (QRT-1, Hansatech Instruments, Norfolk, UK). The chambers were used to assess net photosynthesis (\(P_n\)) and calcification in the light (\(G_l\)), while chambers covered with aluminium foils were used to assess dark respiration (\(R_d\)) and calcification in the dark (\(G_d\)). The concentration of dissolved oxygen (\(O_2\), μmol l\(^{-1}\)) was measured inside the chambers before and after incubations using a HQ30D flexi oxygen meter (Hach Environmental). Water samples were taken at the beginning and at the end of the incubations for measurements of pH\(T\) (pH on the total scale) and total alkalinity (\(A_T\)).

\(P_n\) and \(R_d\), expressed in terms of \(O_2\) production and consumption (in μmol O\(_2\) gFW\(^{-1}\) h\(^{-1}\)), were calculated after Williamson et al. (2017):

\[
P_n (\text{or } R_d) = \frac{\Delta O_2 \cdot v}{f_w \cdot \Delta t}
\]

where \(\Delta O_2\) is the difference in \(O_2\) concentration before and after incubation (μmol l\(^{-1}\) h\(^{-1}\)), \(v\) is the volume of the incubation tubes (l), \(f_w\) is the fresh weight of the algae incubated (g) and \(\Delta t\) is the incubation time (h).

Gross photosynthesis (\(P_g\)) was calculated as:

\[
P_g = |P_n| + |R_d|
\]

\(G_l\) and \(G_d\) (μmol CaCO\(_3\) gFW\(^{-1}\) h\(^{-1}\)) were calculated using the alkalinity anomaly technique (Smith and Key, 1975) as:
\[
G_t (or \ G_d) = \frac{\Delta A_T \cdot v}{2(f_w \cdot \Delta t)}
\]

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

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

• \(t_1\) = before the MHW start (4-weeks); i.e., after 2 weeks of acclimatization at the initial temperature \(T_{\text{control}}\), 1 week of gradual heating up to \(T_{+3}\), and 1 week of acclimatization at \(T_{+3}\) (the heating up to \(T_{+3}\) was induced only in treatments +3 and HW+3);

• \(t_2\) = right after the MHW end (5-weeks); i.e., 1 week of HW at \(T_{HW}\) after \(t_1\) (the HW was induced only in treatments HW and HW+3);

• \(t_3\) = after a recovery period from the MHW end (7-weeks); i.e., 2 weeks of recovery from the heatwave-end after \(t_2\).

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

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

3. Results

3.1. Respiration

LMMs explained over 76% of the variation in dark respiration ($R_d$) [conditional pseudo-$R^2 = 0.763$; Nakagawa and Schielzeth, 2013]. At time $t_1$, before mimicking the marine heatwave, no significant differences were found between $R_d$ rates in the control (C) and the treatment HW, and between the treatments +3 and HW+3 (Fig. 4; Table 1). This trend was expected because, at time $t_1$, the treatments C and HW were kept at the same temperature $T_c$ ($16.5^\circ C \leq T_c \leq 20^\circ C$); while, +3 and HW+3 were both at $T_{+3}$ ($T_{+3} = T_c + 3^\circ C$; $19.5^\circ C \leq T_{+3} \leq 23^\circ C$; see fig. 3). However at $t_1$, C and HW were both different from +3 and HW+3, with lower $R_d$ values measured in +3 and HW+3 ($0.88 \pm 0.08$ and $0.81 \pm 0.09 \mu$mol O$_2$ gFW$^{-1}$ h$^{-1}$, respectively).
respectively), and higher values in C and HW (1.27±0.15 and 1.38±0.12 μmol O₂ gFW⁻¹ h⁻¹, 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 \(t_2\), immediately after the MHW, the lowest \(R_d\) rates were measured in the HW treatment (0.53±0.06 μmol O₂ gFW⁻¹ h⁻¹), while the highest rates were found in the treatment +3 (1.22±0.04 μmol O₂ gFW⁻¹ h⁻¹) \([p_{HW,+3} = 0.001]\); intermediate values were measured in C (0.90±0.07 μmol O₂ gFW⁻¹ h⁻¹). At time \(t_3\), in HW+3, the lowest \(R_d\) values were registered (0.45±0.10 μmol O₂ gFW⁻¹ h⁻¹) \([p_{C,HW+3} = 0.026]\).

\(R_d\) in the control group did not change significantly over time (Fig. 4). In the treatment +3, \(R_d\) rates increased from \(t_1\) to \(t_2\) (0.88±0.10 and 1.22±0.04 μmol O₂ gFW⁻¹ h⁻¹, respectively) \([p_{t_1,t_2} = 0.023]\), and decreased from \(t_2\) to \(t_3\) (1.22±0.04 and 0.88±0.07 μmol O₂ gFW⁻¹ h⁻¹, 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 to 0.53±0.04 μmol O₂ gFW⁻¹ h⁻¹ \([p_{t_1,t_2} < 0.001]\). In HW+3, \(R_d\) rates decreased from \(t_1\) to \(t_3\) (0.81±0.09 to 0.45±0.10 μmol O₂ gFW⁻¹ h⁻¹) \([p_{t_1,t_2} = 0.015]\). While, no changes between \(t_2\) (right after the MHW end) and \(t_3\) (after the recovery period from the MHW) were observed in both the heatwave conditions HW and HW+3.

### 3.2. Photosynthesis

LMMs explained over 58% of the variation in net photosynthesis (\(P_n\)) and 67% of variation in gross photosynthesis (\(P_g\)) \([\text{conditional pseudo-}R^2 = 0.581 \text{ and } 0.672, P_n \text{ and } P_g\) respectively]. No differences in \(P_n\) rates were found among temperature treatments at each experimental time (\(t_1, t_2, t_3; \text{Fig. 4; Table 1}\)). Likewise, no differences in \(P_g\) rates were apparent at time \(t_1\) between the control (C) and the treatment at +3°C (3.64±0.29 and 4.13±0.12 μmol O₂ gFW⁻¹ h⁻¹, respectively), or between HW and HW+3 (4.16±0.35 and 4.23±0.23 μmol O₂
At time $t_2$, the lowest $P_g$ values were measured in the treatment HW (2.44±0.28 $\mu$mol O$_2$ gFW$^{-1}$ h$^{-1}$) and the highest in +3 (3.64±0.33 and $\mu$mol O$_2$ gFW$^{-1}$ h$^{-1}$), with these being significantly different from each other [$p_{HW,+3} = 0.036$]. No significant differences were found among all temperature treatments at time $t_3$, after the recovery period from the MHW end.

In C, $P_n$ rates did not vary among experimental time points ($t_1$, $t_2$ and $t_3$), while $P_g$ decreased 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 $\mu$mol O$_2$ gFW$^{-1}$ h$^{-1}$) and $P_g$ (from 4.13±0.12 to 2.83±0.38 $\mu$mol O$_2$ gFW$^{-1}$ h$^{-1}$) decreased from $t_1$ to $t_3$ [$p_{t_1,t_3} = 0.004$ and 0.003, $P_n$ and $P_g$ respectively]. In the treatment HW, $P_n$ did not change significantly overtime, while $P_g$ decreased from $t_1$ to $t_2$ (4.16±0.35 and 2.78±0.22 $\mu$mol O$_2$ 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 lowest $P_n$ and $P_g$ rates were found after the recovery period from the MHW end ($t_3$; 1.84±0.15 and 2.29±0.20 $\mu$mol O$_2$ gFW$^{-1}$ h$^{-1}$, respectively), with $t_3$ differing from both $t_1$ and $t_2$ [$p_{t_1,t_3} < 0.001$ and 0.001, $p_{t_2,t_3} = 0.025$ and 0.007, $P_n$ and $P_g$ respectively].

![Graph showing $R_d$, $P_n$, and $P_g$ values over different conditions and time points.](image-url)
Fig. 4. Net and gross photosynthesis (Pn and Pg) rates at the experimental irradiance, and respiration (Rd) rates in the dark in the 4 temperature treatments (C, HW, +3, HW+3) for the 3 incubation times (t1, before the MHW start; t2, right after the MHW end; t3, after a recovery period from the MHW end). Data are means ± SE, and are expressed in terms of O2 release (negative values for respiration correspond to O2 consumption); n = 4 for each treatment.

3.3. Calcification

There was only weak evidence for an effect of temperature on calcification rates measured in the dark (Gd; Table 1), with no post-hoc differences found among temperature treatments, highlighting uncertainty about how temperature affects Gd rates. This is in alignment with the low percentage of variance explained by the LMMs [Gd conditional pseudo-R² = 0.034]. Variability in Gi rates was inherently high, especially at the start of the experiment (i.e., time t₁), leading to LMMs with poor explanatory power [conditional pseudo-R² = 0.0601]. Gi rates were affected by temperature at time t₁, with +3 and HW+3 being significantly different from each other [p⁺3,HW+3 < 0.001], despite being at the same temperature T⁺3. At time t₂, +3 and HW+3 (1.22±0.31 and 1.47±0.33 μmol CaCO₃ gFW⁻¹ h⁻¹) exhibited (non-significant) higher Gi rates than C and HW (0.87±0.03 and 0.81±0.10 μmol CaCO₃ gFW⁻¹ h⁻¹, respectively). After 2-weeks-recovery from the MHW (t₃), an opposite trend was observed, with +3 and HW+3 characterized by the lowest Gi rates (0.21±0.09 and 0.43±0.07 μmol CaCO₃ gFW⁻¹ h⁻¹), and C and HW by the highest rates (0.69±0.20 and 0.52±0.07 μmol CaCO₃ gFW⁻¹ h⁻¹, respectively). Although non-significant, at time t₃ the lowest Gd rates were measured in the treatment HW+3, with negative values corresponding to a net dissolution of the algae (-0.30±0.11 μmol CaCO₃ gFW⁻¹ h⁻¹), and the highest ones were found in C (0.22±0.38 μmol CaCO₃ gFW⁻¹ h⁻¹, with one sample having experienced dissolution).
Gd rates did not change significantly over time (Table 1). In C and HW, no differences in Gg rates were observed among experimental time points (t1, t2 and t3). Gg rates decreased from t2 to t3 in the +3°C condition, with rates varying from 1.22±0.31 to 0.21±0.09 μmol CaCO3 gFW⁻¹ h⁻¹ [p(t2,t3) = 0.001]; and in the HW+3 treatment, from 1.47±0.33 to 0.43±0.07 μmol CaCO3 gFW⁻¹ h⁻¹ [p(t2,t3) = 0.015].

**Fig. 5.** Calcification rates in the dark (Gd) and at the experimental irradiance (Gg) in the 4 treatments (C, HW, +3, HW+3) for the 3 incubation times (t1, before the MHW start; t2, right after the MHW end; t3, after a recovery period from the MHW end). Negative values for algal calcification correspond to the decalcification activity quantified as increase in total alkalinity. Data are expressed as means ± SE; n = 4 for each treatment.
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.

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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 t₁, a decrease in respiration rates at elevated temperatures, followed by an opposite trend after the MHW simulation, at t₂ and t₃, 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 Tₘₐₓ 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 Tₘₐₓ 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 Tₑ 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 t₃. 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 respiration rates at higher temperatures during the colder months, and either a negative or a nil response during the
summer when temperatures are closer to a thermal optimum (Anthony et al., 2008). We also observed that a prolonged exposure to high temperatures (i.e., t3, 7 weeks up to a T_{max} of about 23°C in daily temperature variation) negatively affected respiration rates, with the lowest respiration values registered in HW+3 (0.45±0.10 μmol O₂ gFW⁻¹ h⁻¹).

There was limited evidence for an effect of temperature on photosynthesis of *C. officinalis*, despite a significant increase of Pₘ rates with temperature, reaching a maximum of +3°C variation with respect to the control, was observed after 5 weeks (at t₂). Although non-significant, the same increase is observed in Pₙ. This general trend mirrors the results reported for *C. officinalis* (Digby, 1977; Williamson et al., 2017) and other coralline algae (Digby, 1977; Ichiki et al., 2001; Martin et al., 2006; Steller et al., 2007; Martin et al., 2013), which indicate higher photosynthesis variation as a consequence of elevated temperature (c.a. 10°C) and irradiance changes between winter and summer (Martin et al., 2013; Williamson et al., 2017). By simulating summer conditions of irradiance and SST in our experiment, we recorded small positive variations in photosynthesis between the control and the elevated temperature treatments. This might occur at temperatures already close to the thermal optimum (Anthony et al., 2008), and partially agrees with the observation of Martin et al. (2013) in *L. cabiochae* where significant effects of the 3°C warming were detected on Pₘ in colder seasons but not in the summer. Importantly, as already noted for respiration, a prolonged exposure to high temperatures (i.e., t₃, 7 weeks up to a T_{max} of about 23°C) negatively affected Pₙ rates, as a possible effect of thermal stress.

In general, dark calcification showed high variability in all treatments at all experimental time points. This reflects the findings of Kolzenburg et al. (2019) on *Corallina officinalis*, and suggests that the already small amount of calcification in the dark is easily influenced by environmental factors such as temperature. However, we observed lower rates of calcium
carbonate precipitation in the dark with respect to experimental irradiance. This is in line with the results reported for *Amphiroa anceps* and *A. foliacea* by Borowitzka (1981), for *Corallina frondescens* and *C. vancouveriensis* by McCoy et al. (2016), and for *C. officinalis* by Kolzenburg et al. (2019), due to the strict connection between algal photosynthetic activity, providing the greatest contribute to CO$_2$ fixation, and calcification. The rates of calcification under experimental irradiance and in the dark did not exhibit significant responses to temperature within each time point. Previous studies on coralline algae showed similar results, with high variations in calcification rates reported for coralline algae under high changes of both irradiance and temperature (Martin et al., 2013; McCoy et al., 2016; Williamson et al., 2017; Kolzenburg et al. 2019). In the present study we only considered lower temperature regimes and constant values of irradiance compared to previous studies. However, a general (but non-significant) calcification increase under summer irradiance conditions was observed in the first 5 weeks of the experiment for a +3°C (and also +4°C after 4 weeks) variation with respect to the control temperature. This agrees with the lowering of calcification rates at cooler temperatures seen in other *Corallina* species (*C. frondescens* and *C. vancouveriensis*) by McCoy et al. (2016). Critically, the significant decrease measured in G$_i$ rates at elevated temperatures (six fold for the +3°C condition, and threefold for the HW+3 condition, respectively) at the end of the recovery time (from t$_2$ to t$_3$) may possibly be related to thermal stress. These results reflect findings of Vásquez-Elizondo and Enríquez (2016) on the coralline algae *Amphiroa tribulus* from, *Neogoniolithon sp.* and *Lithothamnion sp.*, indicating losses in algal calcification after exposure to elevated temperature (+2°C above the local maximum monthly mean temperature). This decreasing trend is similar in the case of dark calcification, with a significant decrease of G$_d$ in the +4°C condition at the end of the recovery time, consistent with the effect of a stress induced by a prolonged warming.
Furthermore, it has to be considered that under anthropogenic climate change, MHWs will likely increase in intensity and frequency (Hobday et al., 2016), and that coralline algae will possibly be affected by other stresses such as ocean acidification (Hall- Spencer et al., 2008; Kuffner et al., 2008; Martin and Gattuso, 2009; Ragazzola et al., 2012; Donnarumma et al., 2014). Our findings indicated that MHWs could be more harmful when combined to the long-term temperature increase predicted by the end of the century. This overall increase in temperature and the increase in frequency and intensity of the heatwaves could thus have severe effects on the species’ distribution, creating a range shift northwards (Araújo et al., 2005). Therefore, southern margin populations of *C. officinalis*, together with the high densities of macrofaunal organisms living within their fronds, may risk disappearing in their original environment as temperatures warm in future oceans (Kolzenburg et al., 2019). However, at higher latitudes, aragonite saturation state and ocean pH will reach critically low levels first (Steinacher et al., 2009), potentially leading to a shift of calcifying species distribution southwards (Orr et al., 2005; Yara et al., 2012; Lenton et al., 2015). This might result in a contraction of the natural distribution of *C. officinalis*, yet more studies considering the combined effects of warming and acidification are needed to predict how this species’ distribution and abundance may be affected by anthropogenic climate change.

5. Conclusion

By combining the effects of thermal stress induced by tides, gradual ocean warming, and 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 $T_{\text{max}}$ 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 $T_{\text{max}}$ 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 $T_{\text{max}}$ 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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