The regulation of coralline algal physiology, an *in-situ* study of *Corallina officinalis* (Corallinales, Rhodophyta)

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Abstract

Calcified macroalgae are critical components of marine ecosystems worldwide, but face considerable threat both from climate change (increasing water temperatures) and ocean acidification (decreasing ocean pH and carbonate saturation). It is thus fundamental to constrain the relationships between key abiotic stressors and the physiological processes that govern coralline algal growth and survival. Here we characterize the complex relationships between the abiotic environment of rock pool habitats, and the physiology of the geniculate red coralline alga, *Corallina officinalis* (Corallinales, Rhodophyta). Paired assessment of irradiance, water temperature and carbonate chemistry, with *C. officinalis* net production (*NP*), respiration (*R*) and net calcification (*NG*) was performed in a south-west UK field site, at multiple temporal scales (seasonal, diurnal and tidal). Strong seasonality was observed in *NP* and nighttime *R*, with a *P*_{max} of 22.35 µmol DIC gDW^{-1} h^{-1}, *E*_{k} of 300 µmol photons m^{-2} s^{-1} and *R* of 3.29 µmol DIC gDW^{-1} h^{-1} determined across the complete annual cycle. *NP* showed a significant exponential relationship with irradiance (*R*^{2} = 0.67), although was temperature dependent given ambient irradiance > *E*_{k} for the majority of the annual cycle. Over tidal emersion periods, dynamics in *NP* highlighted the ability of *C. officinalis* to acquire inorganic carbon despite significant fluctuations in carbonate chemistry. Across all data, *NG* was highly predictable (*R*^{2} = 0.80) by irradiance, water temperature and carbonate chemistry, providing a *NG*_{max} of 3.94 µmol CaCO_{3} gDW^{-1} h^{-1}, and *E*_{k} of 113 µmol photons m^{-2} s^{-1}. Light-*NG* showed strong seasonality and significant coupling to *NP* (*R*^{2} = 0.65), as opposed to rock pool water carbonate saturation. In contrast, the direction of dark-*NG* (dissolution vs. precipitation) was strongly related to carbonate saturation, mimicking abiotic precipitation dynamics.
Data demonstrated that *C. officinalis* is adapted to both long-term (seasonal) and short-term (tidal) variability in environmental stressors, although the balance between metabolic processes and the external environment may be significantly impacted by future climate change.

1. Introduction

Calcified macroalgae are critical components of marine ecosystems from polar to tropical regions (Littler et al., 1985, McCoy and Kamenos, 2015), constituting one of the most important structural elements in many coastal zones (van der Heijden and Kamenos, 2015). In shallow temperate areas, heavily calcified ‘coralline’ red macroalgae (Corallinales, Rhodophyta) act as autogenic ecosystem engineers (Johansen, 1981; Jones et al., 1994; Nelson, 2009), providing habitat for numerous small invertebrates, shelter from the stresses of intertidal life via their physical structure, and surfaces for the settlement of epifauna and microalgal epiphytes (Nelson, 2009; Perkins et al., 2016). Temperate corallines are also of significant importance in the carbon and carbonate cycles of shallow coastal ecosystems, due to their relatively high productivity and calcium carbonate precipitation and dissolution (Martin and Gattuso, 2009; van der Heijden and Kamenos, 2015).

Species of the geniculate (jointed) coralline genus *Corallina* form extensive turfs across large areas of NE Atlantic intertidal regions, providing substratum, habitat and refugia for a number of important organisms (Coull and Wells, 1983; Kelaher, 2002; 2003; Hofmann et al., 2012a; Brodie et al., 2016; Perkins et al., 2016). Within rock pool habitats, *Corallina* must maintain productivity and growth under the influence of a myriad of highly variable stressors, including irradiance, water temperature and
carbonate chemistry, which fluctuate on seasonal, diurnal and tidal time scales (Egilsdottir et al., 2013; Williamson et al., 2014a). During summer, high irradiance, water temperature, pH and carbonate saturation ($\Omega_{\text{CO}_3^{2-}}$) dominate, whilst winter is associated with limiting irradiance and temperature, and decreased water pH (i.e. increased acidity) and $\Omega_{\text{CO}_3^{2-}}$ (Ganning, 1971; Morris and Taylor, 1983; Williamson et al., 2014a). Across daytime tidal emersion periods, rock pool water temperatures generally increase and community photosynthetic activity serves to strip CO$_2$ and HCO$_3^-$ from the water, with concomitant increases in pH and $\Omega_{\text{CO}_3^{2-}}$ (Williamson et al., 2014a). In contrast, night-time emersion is dominated by respiration processes within rock pools, with CO$_2$ production driving down water pH and $\Omega_{\text{CO}_3^{2-}}$ (Morris and Taylor, 1983). In order to sustain their dominance of temperate coastlines, *Corallina* must balance this environmental variability with their requirements for key physiological processes, including photosynthesis, respiration and calcification.

The interactions between *Corallina* physiology and environmental variability is likely to be significantly impacted by on-going climate change (increasing temperatures) and ocean acidification (decreasing pH and $\Omega_{\text{CO}_3^{2-}}$). It is therefore critical to constrain *Corallina* ecophysiology under current environmental conditions to aid projections under future climate scenarios (Nelson, 2009; Koch et al., 2013; Brodie et al., 2014; Hofmann and Bischof, 2014). It is also important to understand the present-day role of these dominant community members in coastal carbon cycles and how this may change into the future (van der Heijden and Kamenos, 2015).

This study focuses on *Corallina officinalis*, a species that dominates North Atlantic turfing assemblages (Williamson et al., 2015) and has been the focus of recent studies
aiming to understand coralline algal physiology and future fate (Hofmann et al., 2012a, b; Williamson et al., 2014a, b; Williamson et al., 2015; Perkins et al., 2016).

Whilst the skeletal mineralogy (Williamson et al., 2014b), photophysiology (Williamson et al., 2014a; Perkins et al., 2016), and phylogenetics of *C. officinalis* (Williamson et al., 2015) have been examined, information on *in-situ* physiology in relation to key environmental stressors is currently lacking. We therefore performed the first high-resolution *in-situ* assessment of *C. officinalis* physiology (production, respiration and calcification) in relation to key environment stressors (irradiance, temperature and carbonate chemistry) over both daytime and night-time tidal emersion periods, across multiple seasons. By characterizing the influence of abiotic stressors on key physiological processes, this study significantly advances efforts to understand the ecology and fate of coralline algae in a changing world.

2. Methods

This study was conducted at Combe Martin (CM), north Devon, UK (51°12’13N, 4°2’19W, Fig. 1), a north-west facing rocky intertidal site, positioned within a sheltered bay. *Corallina officinalis* dominates intertidal rock pools at CM, including large (ca. 40 m³, 0.5 m depth) upper shore (Chart Datum + 5.5 m) rock pools created by a man-made walkway (Fig. 1b and 1c).

To assess *C. officinalis* net production, respiration and calcification, incubation experiments were performed at CM during daytime tidal emersion in December 2013, and March, July and September 2014, and night-time tidal emersion during the latter three sampling months (sampling dates and tidal timings are presented in Table 1). Two sets of approximately 1 h timed incubations were performed per emersion period, at
both the start (initiated within 30 mins of tidal emersion) and end (over the final 1.5 h) of emersion. Irradiance and rock pool water salinity, temperature and carbonate chemistry were monitored in parallel throughout.

2.1. Physiology incubations

Net production \((NP)\) and respiration \((R)\) \(\text{(DIC flux, } \mu\text{mol g dry weight (DW)}^{-1} \text{ h}^{-1})\), and net light and dark calcification rates \((NG)\) \(\text{(} \mu\text{mol CaCO}_3 \text{ gDW}^{-1} \text{ h}^{-1})\) were determined using closed chamber incubations. Ten discrete \(C. \text{ officinalis}\) fronds were collected randomly from upper shore CM rock pools and placed individually into 0.5 l clear glass chambers filled with rock pool water. Final dry weight of incubated \(C. \text{ officinalis}\) averaged \(4.0 \pm 0.15 \text{ g}\) across incubations. Two additional chambers were filled only with rock pool water to serve as controls for non-\(Corallina\) biological activity. At the beginning of incubations, five 100 ml initial rock pool water samples were collected for pH and total alkalinity (TA) determination (see below), and poisoned with saturated mercuric chloride solution to prevent biological activity. Incubation chambers were then sealed, and six chambers (5 \(Corallina\), 1 control) positioned in an upper shore rock pool to maintain ambient irradiance and temperature conditions. The remaining six chambers (5 \(Corallina\), 1 control) were placed in opaque bags to create dark conditions during daytime incubations (or shield from moonlight during night-time) and placed within the same rock pool to maintain ambient temperature. After incubating for ca. 1 h, chambers were removed from the rock pool and a final 100 ml water sample was collected from each chamber for pH and TA measurements. In parallel to all incubations, ambient irradiance \((\text{PAR } \mu\text{mol photons } m^{-2} s^{-1})\), rock pool water temperature \(\text{(} ^\circ\text{C})\), and salinity \((S)\), were monitored every 30 min using a 2-pi LI-COR cosine-corrected quantum sensor positioned ca. 5 cm above the surface of the rock pool.
(15 s average irradiance measurements were taken using an in-built function of the sensor), a digital thermometer, and a hand-held refractometer, respectively. Cumulative photodose (PAR, mol photons m\(^{-2}\)) was calculated from irradiance measurements by integrating PAR over time from the start of tidal emersion of rock pools. Following incubations, _C. officinalis_ fronds were collected from incubation chambers for weighing after drying at 100\(^\circ\)C for 24 h.

The pH (total scale) of water samples was measured immediately using a Mettler Toledo Inlab-expertpro pH probe calibrated using Tris-buffers (pH 4, 7, and 10) prepared in artificial seawater. TA of water samples was measured by the potentiometric method using Gran titration with a Mettler Toledo DL50 Graphix automatic titrator. Reference material measurements of Na\(_2\)CO\(_3\) standards (0.5 and 1 mmol kg\(^{-1}\)) prepared in 0.6 mol kg\(^{-1}\) NaCl background medium were used to correct sample measurement for accuracy. The relative error of TA measurements was 4.6 ± 0.24 \%, with a relative standard deviation of 3.35 ± 1.5 \%. pH, TA, water temperature and salinity were subsequently input into CO2SYS v2.1 (Pierrot et al., 2016) to determine all carbonate chemistry parameters (DIC, \(p\)CO\(_2\), HCO\(_3^−\), CO\(_3^{2−}\)) and the saturation states of aragonite \([\Omega_{\text{arg}}]\) and calcite \([\Omega_{\text{calc}}]\), allowing both calculation of _C. officinalis_ NP/R (\(\Delta\text{DIC}\)) and NG (\(\Delta\text{TA}\)) during incubations, and the monitoring of ambient rock pool water carbonate chemistry. CO2SYS was run using the constants of Mehrbach et al. (1973) refitted by Dickson and Millero (1987). The carbonate chemistry of rock pool water was represented by initial water samples (n = 5) collected at the beginning of each incubation experiment, providing an assessment of water chemistry at both the start and end of tidal emersion periods, matching productivity analyses. _C. officinalis_ NP (assessed from daytime light treatment incubations) and _R_...
(assessed from daytime dark treatment and all night-time incubations) were calculated from the difference between initial and final incubation DIC concentrations, as:

\[ NP \text{ or } R_{\text{DAY/NIGHT}} = \left( \frac{\Delta \text{DIC}}{\Delta t} \right) \frac{v}{dw} - NG \]

where \( NP \) and \( R_{\text{DAY/NIGHT}} \) are net production and respiration during the day or night, respectively (\( \mu \text{mol DIC gDW}^{-1} \text{ h}^{-1} \)); \( \Delta \text{DIC} \) is the change in dissolved inorganic carbon concentration during the incubation (\( \mu \text{mol DIC kg}^{-1} \text{ seawater} \)); \( v \) is the incubation chamber volume (l); \( dw \) is the dry weight of \( C. \ officinalis \) incubated (g); \( \Delta t \) is the incubation time (h); and \( NG \) is the net calcification rate (\( \mu \text{mol CaCO}_3 \text{ gDW}^{-1} \text{ h}^{-1} \)). \( NG \) was estimated using the alkalinity anomaly technique (Smith and Key, 1975; Chisholm and Gattuso, 1991), whereby TA decreases by 2 equivalents for each mol of CaCO\(_3\) precipitated. Light calcification (assessed from daytime light treatment incubations) and dark calcification (assessed from daytime dark and all night-time incubations) were thus calculated as:

\[ NG_{\text{DAY}} \text{ (or } NG_{\text{NIGHT}})_{-\text{LIGHT/DARK}} = \frac{\Delta \text{TA} \times v}{2(dw \times \Delta t)} \]

where \( NG_{\text{DAY-LIGHT/DARK}} \) and \( NG_{\text{NIGHT-LIGHT/DARK}} \) are net calcification during daytime or night-time tidal emersion periods, determined from light or dark treatment incubations (\( \mu \text{mol CaCO}_3 \text{ gDW}^{-1} \text{ h}^{-1} \)); \( \Delta \text{TA} \) is the change in total alkalinity during the incubation (\( \mu \text{mol kg}^{-1} \text{ seawater} \)); \( v \) is the incubation chamber volume (l); \( dw \) is the dry weight of \( C. \ officinalis \) incubated (g); and \( \Delta t \) is the incubation time (h).

2.2. Data analysis
All statistical analyses and plotting of data were performed using R v.3.0.2 (R Core Team, 2014). Prior to all analyses, normality of data was tested using the Shapiro-Wilk test and examination of frequency histograms. If data were not normally distributed, Box-Cox power transformation was applied using the boxcox function of the MASS package (Venables and Ripley, 2002), and normality re-checked. Following the application of models to data, model assumptions were checked by examination of model criticism plots. Whilst sampling for determination of NP, R and NG was performed in the same rock pools over a number of dates at each site, measurements were performed on different individuals during each sampling date and thus repeated measures analysis of variance (ANOVA) was not utilized during the present study.

**Abiotic Environment:** Differences in irradiance and rock pool water temperature between sampling months and tidal emersion periods were examined using 2-way ANOVA with interaction. Post hoc Tukey honest significant differences analysis was performed on all significant ANOVA results. To facilitate comparison of rock pool water carbonate chemistry between months and tidal emersion periods, all variables were summarized using principal components analysis (PCA) with scaled variables, allowing for transformation of the highly correlated carbonate chemistry variables into uncorrelated PCs for comparison between independent variables (month and tide). Differences in carbonate chemistry were thus examined by ANOVA analysis of principal component one (PC1) separately for daytime and night-time data, as above. Least squares multiple linear regression was used to examine relationships between daytime PC1 and irradiance (analysed separately as both irradiance measured and calculated cumulative photodose) and rock pool water temperature. The relative importance of predictor variables was calculated using the relaimpo package with type
‘lmg’ (Grömping, 2006). Least squares linear regression was used to examine relationships between night-time PC1 and rock pool water temperature.

Net production, respiration and calcification: NP, \( R_{\text{DAY/NGHT}} \) and NG rates were analyzed separately for daytime and night-time data using 3-way ANOVA with the factors month, tide and light-treatment, with all interactions. All \( C. \text{ officinalis} \) NP/R and NG data were plotted as an exponential function \( P-E \) of ambient irradiance \( E \) (\( \mu \text{mol photons m}^{-2} \text{s}^{-1} \)), as:

\[
NP/R (NG) = P_{\text{max}} \left(1 - e^{-E/E_k}\right) + c
\]

where \( P_{\text{max}} \) is the rate of maximum net production (or calcification) (\( \mu \text{mol DIC gDW}^{-1} \text{h}^{-1} \), or \( \mu \text{mol CaCO}_3 \text{ gDW}^{-1} \text{h}^{-1} \)); \( E_k \) is the minimum saturating irradiance (\( \mu \text{mol m}^{-2} \text{s}^{-1} \)); and \( c \) is the dark respiration rate (or calcification rate) (\( \mu \text{mol DIC/CaCO}_3 \text{ gDW}^{-1} \text{h}^{-1} \)).

To examine relationships between NP, R and NG with water temperature and carbonate chemistry (PC1\text{day/night}), temperature and PC1 were added individually into the above model as linear terms, in addition to construction of a ‘global model’ containing irradiance as an exponential function, and both water temperature and PC1 as linear terms. The goodness-of-fit of the respective models was compared using estimated \( R^2 \) and Akaike Information Criterion (AIC), and ANOVA comparisons were performed to test the significance of the inclusion of respective terms into each model. The relationship between \( C. \text{ officinalis} \) NG and NP/R was modeled using non-linear regression as detailed above.

3. Results
3.1. Abiotic environment

Irradiance varied between all sampling months \((F_{3,32} = 193.385, P < 0.0001)\), being maximal in July and minimal in December (Fig. 2), with significant change in irradiance over tidal emersion only apparent in July \((F_{1,32} = 8.114, P < 0.01\), TukeyHSD \(P < 0.05)\). Warmest daytime rock pool water temperatures were observed in July, with the coldest in March, and a significant difference apparent between all sampling months \((F_{3,32} = 760.94, P < 0.0001)\) (Fig. 2). Water temperature significantly increased over daytime tidal emersion during July and September \((F_{1,32} = 97.48, P < 0.0001\), TukeyHSD \(P < 0.05\) in both cases), whereas no change occurred in December or March, as supported by significant interaction between month and tide \((F_{3,32} = 37.01, P < 0.0001)\). Night-time rock pool water temperatures were greatest in September and lowest in March, with a significant difference between all sampling months \((F_{2,13} = 168.534, P < 0.0001)\). Over night-time tidal emersion, a significant decrease in water temperature was apparent during July \((15.6 \pm 0.16 \text{ to } 14.7 \pm 0.14^\circ C)\) and September \((16.8 \pm 0.45 \text{ to } 15.7 \pm 0.15^\circ C)\) \((F_{1,13} = 20.049, P < 0.01\), TukeyHSD \(P < 0.05\) in all cases).

Changes in rock pool water carbonate chemistry were observed over daytime and night-time tidal emersion periods during each sampling month (Supplementary Figures 1 & 2). Over daytime emersion, \(pCO_2\) and \(HCO_3^-\) decreased, with concomitant increases in pH, \(CO_3^{2-}\), \(\Omega_{\text{arg}}\) and \(\Omega_{\text{cal}}\). From the start to end of night-time emersion, the opposite trends were observed, with increases in \(pCO_2\) and \(HCO_3^-\) paralleled by decreases in pH and \(\Omega_{CO_3^{2-}}\). Principal components analysis (PCA) served to summarize daytime and night-time carbonate chemistry parameters for subsequent analyses (Table 2 & Fig. 3), with PC1\(_{\text{day}}\) and PC1\(_{\text{night}}\) describing 84 % and 83 % of the variance in carbonate
chemistry observed over seasonal and tidal time-scales, respectively. For all subsequent analyses, PC1\textsubscript{day} and PC1\textsubscript{night} were taken as representative of carbonate chemistry dynamics.

PC1\textsubscript{day} and PC1\textsubscript{night} were significantly different between sampling months ($F_{3,67} = 27.528$ and $F_{2,47} = 39.73$, respectively, $P < 0.0001$ in both cases, Fig. 4), with higher PC1\textsubscript{day} observed in July and September in comparison to December and March, and significantly different PC1\textsubscript{night} observed between all night-time sampling months (March, July and September; TukeyHSD, $P < 0.05$ in all cases). PC1\textsubscript{day} significantly increased over daytime tidal emersion, representing decreased DIC, $p$CO$_2$ and HCO$_3^-$, and increased pH and $\Omega$CO$_3^{2-}$ parameters, during all sampling months but December ($F_{1,67} = 1.912$, $P < 0.0001$, TukeyHSD $P < 0.05$ in all cases). Over night-time tidal emersion the opposite trends were observed, with significant decrease in PC1\textsubscript{night} apparent during every sampling month, representing increased DIC, $p$CO$_2$ and HCO$_3^-$ and consequent decreases in pH and $\Omega$CO$_3^{2-}$ ($F_{1,47} = 810.90$, $P < 0.0001$, TukeyHSD $P < 0.05$ in all cases). The magnitude of change in rock pool water carbonate chemistry over night-time tidal emersion increased from March to September, as evidenced by significant interaction between month and tide ($F_{2,47} = 73.31$, $P < 0.0001$).

Least squares multiple linear regression (Table 3) revealed significant relationships between PC1\textsubscript{day}, irradiance (28% relative importance) and water temperature (71% relative importance) ($R^2 = 0.63$, $P < 0.0001$) (Table 3), and between PC1\textsubscript{day}, calculated cumulative photodose (58% relative importance) and water temperature (41% relative importance) ($R^2 = 0.69$, $P < 0.0001$). PC1\textsubscript{night} showed a minimal relationship to water temperature ($R^2 = 0.08$, $P < 0.05$).
3.2. Net production and respiration

*Corallina officinalis* demonstrated maximal NP (negative DIC flux) in July (start of emersion = 25.80 ± 0.94 µmol DIC gDW⁻¹ h⁻¹), with lowest values recorded during December and March (end of March emersion = 1.56 ± 0.74 µmol DIC gDW⁻¹ h⁻¹) ($F_{3,69} = 6.838, P < 0.001$) (Fig. 5). In contrast, no significant difference in *C. officinalis* $R_{\text{DAY}}$ was observed between sampling months (Fig. 5a). Whilst significant changes in NP and $R_{\text{DAY}}$ were recorded in relation to the factor tide ($F_{1,69} = 8.684, P < 0.01$), post-hoc TukeyHSD did not recover significant differences in either parameter between the start and end of tidal emersion, within any sampling month. Over night-time tidal emersion, no significant difference was apparent in $R_{\text{NIGHT}}$ between light treatment or the start and end of tidal emersion periods, and thus data are pooled for presentation (Fig. 6a). Across sampling months, a significant increase in *C. officinalis* $R_{\text{NIGHT}}$ was apparent from March to July and September ($F_{2,52} = 22.170, P < 0.0001$), with ca. 4.5-fold greater $R_{\text{NIGHT}}$ observed during September as compared to March.

Across all data, NP showed a significant relationship with irradiance ($R^2 = 0.67, P < 0.0001$ for all parameters, AIC = 885.64), giving a $P_{\text{max}}$ of 22.35 µmol DIC gDW⁻¹ h⁻¹, $E_\text{k}$ of 301 µmol photons m⁻² s⁻¹ and estimated overall respiration rate of 3.29 µmol DIC gDW⁻¹ h⁻¹ (Fig. 7a, Table 4). Addition of water temperature and carbonate chemistry (both individually and together) into the model did not significantly improve the goodness-of-fit (Table 4). This may be due to correlations between irradiance and water temperature ($r = 0.42, P < 0.0001$), irradiance and PC1 ($r = 0.19, P < 0.05$) and temperature and PC1 ($r = 0.59, P < 0.0001$) (data not shown).
3.3. Calcification

*Corallina officinalis NG$_{\text{DAY}}$* was greatest during July and September as compared to December and March ($F_{3,69} = 16.814$, $P < 0.0001$, TukeyHSD $P < 0.05$ in all cases), with a significant difference between $NG_{\text{DAY-LIGHT}}$ and $NG_{\text{DAY-DARK}}$ apparent in all sampling months ($F_{1,69} = 290.075$, $P < 0.0001$) (Fig. 5b). Highest $NG_{\text{DAY-LIGHT}}$ (4.62 ± 0.45 µmol CaCO$_3$ gDW$^{-1}$ h$^{-1}$) was recorded at the end of daytime tidal emersion during July, with lowest $NG_{\text{DAY-LIGHT}}$ (1.70 ± 0.08 µmol CaCO$_3$ gDW$^{-1}$ h$^{-1}$) recorded at the end of tidal emersion during December. Both negative (indicating CaCO$_3$ dissolution) and positive (indicating CaCO$_3$ precipitation) $NG_{\text{DAY-DARK}}$ values were observed, with maximal CaCO$_3$ dissolution in the dark (-0.53 ± 0.20 µmol CaCO$_3$ gDW$^{-1}$ h$^{-1}$) at the start of March daytime tidal emersion and maximal precipitation in the dark (2.01 ± 0.35 µmol CaCO$_3$ gDW$^{-1}$ h$^{-1}$) at the end of September daytime tidal emersion (Figure 5b). Significant differences in $NG_{\text{DAY}}$ observed in relation to tide ($F_{1,69} = 5.028$, $P < 0.05$) were confined to increases in $NG_{\text{DAY-DARK}}$ from the start to end of July and September tidal emersion periods (TukeyHSD $P < 0.05$ in both cases), with significant interaction between month and tide ($F_{3,69} = 5.104$, $P < 0.01$). No significant differences in $NG_{\text{DAY-LIGHT}}$ were observed between the start and end of tidal emersion periods despite concomitant increases in rock pool water Ω$_{\text{CO}_3^{2-}}$.

During night-time tidal emersion, there was no significant difference between $NG_{\text{NIGHT-LIGHT}}$ and $NG_{\text{NIGHT-DARK}}$, or between the start and end of tidal emersion within any sampling month, and thus data are pooled for presentation (Fig. 6b). Whilst net CaCO$_3$ dissolution was observed during both March and September night-time tidal emersion, with maximal dissolution in the latter month (monthly average of -0.83 ± 0.11 µmol CaCO$_3$ gDW$^{-1}$ h$^{-1}$), net CaCO$_3$ precipitation was apparent across the duration of July.
night-time emersion (monthly average of 0.46 ± 0.14 µmol CaCO$_3$ gDW$^{-1}$ h$^{-1}$); rates being significantly different between all sampling months ($F_{2,52} = 25.50$, $P < 0.0001$, TukeyHSD $P < 0.05$ in all cases) (Fig. 6b).

Across all data, $NG$ showed a significant exponential relationship with ambient irradiance (estimated $R^2 = 0.76$, $P < 0.0001$ for all parameters, AIC = 383.17), providing a $NG_{max}$ of 4.41 µmol CaCO$_3$ gDW$^{-1}$ h$^{-1}$, and an $E_k$ of 201 µmol photons m$^{-2}$ s$^{-1}$ (Fig. 7b, Table 4). Addition of water temperature and/or carbonate chemistry (as PC1) increased the goodness-of-fit (estimated $R^2$ and AIC) of the models to $NG$ data (Table 4). The best representation of $NG$ was provided by the ‘global model’ including irradiance as exponential term, and both water temperature and carbonate chemistry as linear terms (estimated $R^2 = 0.80$, $P < 0.05$ for all parameters, AIC = 360.57), providing a $NG_{max}$ of 3.94 µmol CaCO$_3$ gDW$^{-1}$ h$^{-1}$, and an $E_k$ of 113 µmol photons m$^{-2}$ s$^{-1}$ (Table 4). ANOVA comparison demonstrated all $NG$ models to be significantly different to one another (data not shown). Across all data, a significant relationship was also identified between $NG$ and $NP/R$ ($R^2 = 0.65$, $P < 0.05$ for all parameters, n = 140) (Fig. 8).

4. Discussion
Through the pairing of physiological and environmental monitoring, this study has constrained the regulation of key physiological processes of a coralline alga by irradiance, water temperature and carbonate chemistry. It is fundamental to understand the interactions of coralline algae with their environment, given the continuing perturbation of key abiotic stressors by climate change and ocean acidification. The knowledge presented here significantly advances our understanding of the
ecophysiology of *Corallina officinalis*, which will be vital when making future projections for the fate of this ecosystem engineer.

4.1. Production and respiration

This study highlights significant seasonality in *C. officinalis* net production that follows dynamics in irradiance, water temperature and carbonate chemistry. In marine macrophytes, photosynthetic capacity is generally greatest during months when irradiance and temperature are highest (Lüning, 1990; Cabello-Pasini and Alberte, 1997). Consistent with previous accounts for other calcifying macroalgae (e.g. Martin et al., 2006; 2007; Egilsdottir et al., 2015), *C. officinalis* net production was maximal during July and minimal in December, showing a significant exponential relationship with irradiance ($R^2 = 0.67$). Whilst inclusion of water temperature and carbonate chemistry into models did not improve predictive ability, co-variance between predictors may have hindered interpretation of their influence. At saturating levels of irradiance, the enzymatic reactions that limit photosynthesis are temperature dependent (Lüning, 1990). The light-saturation coefficient ($E_k$) determined by the present study (ca. 300 µmol photons m$^{-2}$ s$^{-1}$ ambient irradiance) highlighted that *C. officinalis* photosynthesis was light-saturated for the majority of the annual cycle; ambient irradiance $> E_k$ was recorded in every sampling month other than December, consistent with the findings of Williamson et al. (2014a). Thus maximal rates of *C. officinalis* production were likely temperature-dependent, as is known for other intertidal macroalgae (Kanwisher, 1966).

Strong seasonality was also identified in *C. officinalis* dark respiration determined during night-time incubations, in line with accounts for other coralline algae (e.g.
Martin et al., 2006; Egilsdottir et al., 2015). The ca. 4.5-fold increase observed in night-time respiration from March to September is within the range reported for the maerl-forming species, *Lithothamnion coralloides*, which demonstrated a 3-fold increase in respiration during summer months (Martin et al., 2006), and the closely related geniculate species, *Ellisolandia elongata*, which demonstrated a 10-fold summer increase in respiration (Egilsdottir et al., 2015). Whilst night-time respiration rates determined here for *C. officinalis* (ca 1–4.5 µmol DIC gDW⁻¹ h⁻¹) fall within the lower end of the range reported for *E. elongata* from similar habitats (ca. 0.4 - 17 µmol CO₂ gDW⁻¹ h⁻¹), Egilsdottir et al. (2015) note that their high summer rates were likely driven by high water temperatures during summer measurements (23°C as compared to 16°C during the present study).

Consistent with observations made in *E. elongata* dominated habitats (Bensoussan and Gattuso, 2007), *C. officinalis* demonstrated increased rates of daytime respiration as compared to night-time, with 6-fold greater daytime rates during March, and 1.1-times greater rates during July and September. Previously, Bensoussan and Gattuso (2007) observed large variations in winter respiratory activity under both daylight and dark conditions in assemblages dominated by *E. elongata*, with significantly higher respiration during the afternoon and first part of the night. Such diurnal variations are reflected by our findings, with maximal daytime respiration decreasing to lower levels across night-time emersion. Our data further demonstrated that seasonality in respiration was better reflected by night-time incubations, whereas no seasonal patterns were apparent in daytime rates. This is likely due to the influence of residual biological activity after passage from light to dark conditions, given differences in the photo-history of day and night incubated *C. officinalis*. Daytime samples were collected from
100% ambient irradiance and immediately transferred to complete darkness, whereas
night-time samples had been in darkness for a number of hours prior to incubations.
Future assessments may benefit from use of, for example, the Kok method for
determination of light respiration rates, as applied by Zou et al. (2011) to several
macroalgal species.

Differences between light and dark respiration rates have direct consequences for the
conventional calculation of gross production \( GP = \text{net production} + \text{respiration} \)
\cite{BensoussanGattuso2007}, although estimates can be made for \textit{C. officinalis}
using our data. Net production recorded at the start of tidal emersion ranged seasonally
from ca. 11 (December) to 26 (July) µmol DIC gDW\(^{-1}\) \(h^{-1}\). Assuming our lower,
seasonally variable night-time rates of respiration to be representative, \textit{C. officinalis} \(GP\)
is estimated as ranging 15.9 (March) to 27.7 (July) µmol DIC gDW\(^{-1}\) \(h^{-1}\); though
December data are omitted due to the absence of night-time incubations. Similarly,
correcting net production with daytime respiration rates reveals a \(GP\) range of 16.7
(December) to 27.8 (July) µmol DIC gDW\(^{-1}\) \(h^{-1}\) for \textit{C. officinalis}. These estimates are
highly comparable to \(GP\) reported for \textit{E. elongata} from NW France during winter (11.8
\(±\) 1.6 µmol C gDW\(^{-1}\) \(h^{-1}\)) and summer (22.5 \(±\) 1.9 µmol C gDW\(^{-1}\) \(h^{-1}\)) \cite{Egilsdottir2015},
and serve to highlight the high productivity of geniculate corallines in
comparison to other calcified algal groups. For example, Martin et al. (2006) reported
a seasonal range of 0.68 to 1.48 µmol C gDW\(^{-1}\) \(h^{-1}\) for the maerl forming \textit{Lithothamnion
coralloides} off NW France. Currently, the contribution of coralline algae to global
carbon cycles is not well constrained, particularly that of geniculate turfing species \cite{ElHaïkali2004,VanderHeijdenKamenos2015}. Given their comparatively
high production identified here, our data indicate that geniculate corallines likely play
a significant role in coastal carbon cycling, despite their presumably reduced overall
benthic coverage as compared to maerl-forming or crustose coralline algal species.
Inclusion of geniculate corallines into future estimates of coastal carbon cycles is
therefore essential.

Over tidal emersion periods, patterns in *C. officinalis* production demonstrate the
inorganic carbon (*Ci*) acquisition ability of this calcified alga over a range of CO₂ and
HCO₃⁻ concentrations, however findings indicate potential vulnerability to periods of
low irradiance e.g. winter. Maintenance of net production over July and September
daytime tidal emersion, despite decreases in rock pool *pCO₂* of 84% and 39%,
respectively, highlight the ability of *C. officinalis* to effectively utilize both CO₂ and
HCO₃⁻ as substrates for photosynthesis, as previously noted (Cornwall et al., 2012).
This allows access to the relatively high HCO₃⁻ concentrations in seawater when CO₂
diffusion is limiting (Koch et al., 2013). During December and March, however, when
overall minimal irradiance prevailed, a decrease in *C. officinalis* net production was
observed. Estimation of GP/R ratios for these emersion periods (using daytime
respiration data) revealed decreases from 3.45 to 1.9 over December-, and 3.93 to 1.2
over March- daytime emersion. Thus decreases in net production were driven by
decreases in photosynthesis relative to respiration, which approached unity by the end
of emersion in winter months. This reflects ecosystem wide GP/R ratios for
assemblages dominated by *E. elongata* in the NW Mediterranean, which remained close
to 1 (1.1 ± 0.1) over 24 h periods during winter (Bensoussan and Gattuso, 2007).
Although water temperature, nor irradiance, showed significant change over December
or March tidal emersion, reductions in photosynthesis may have been driven by
inorganic carbon limitation due to seasonal minima in irradiance. Under low light
conditions, the ability to utilize \( \text{HCO}_3^- \) can be energetically limited, increasing reliance on \( \text{CO}_2 \) diffusion (Koch et al., 2013). \( C. \text{ officinalis} \) photosynthesis may thus have been sensitive to the relatively small decrease in rock pool \( p\text{CO}_2 \) (ca. 30%) that occurred over December and March emersion periods.

4.2. Calcification

This study demonstrates that \( C. \text{ officinalis} \) calcification is highly influenced by seasonal and diurnal variability in other metabolic processes (photosynthesis and respiration), in addition to the external carbonate chemistry environment. Across the entire annual cycle, \( C. \text{ officinalis} \) calcification was highly predictable (\( R^2 = 0.80 \)) by irradiance, water temperature and carbonate chemistry, providing a calculated \( \text{NG}_{\text{max}} \) of 3.94 \( \mu \text{mol CaCO}_3 \text{ gDW}^{-1} \text{ h}^{-1} \) and an \( E_k \) of 113.45 \( \mu \text{mol photons m}^{-2} \text{ s}^{-1} \). Irradiance was the greatest predictor of calcification (accounting for 76% of variability), reflecting photosynthetic enhancement of CaCO₃ precipitation (see below), although by contrasting light and dark calcification dynamics, the variable influences of physiology and external environment have been determined.

Light-enhanced calcification, i.e. CaCO₃ precipitation, was observed across the entire seasonal cycle, with maximal light-calcification rates during July and September in comparison to December and March. The seasonal range of net light-calcification was significantly higher than reported for the maerl species \( L. \text{ corallioides} \) (Martin et al., 2006), comparable to \( E. \text{ elongata} \) from NW France (Egilsdottir et al., 2015), and lower than reported for \( E. \text{ elongata} \) from the Mediterranean (El Haïkali et al., 2004). Light-enhanced calcification is typical for calcifying macroalgae, and is a product of light-dependent increase in carbonate saturation (\( \Omega_{\text{CO}_3^{2-}} \)) at the sites of calcification, due to
photosynthetic activity (Littler, 1976; Koch et al., 2013). In the Corallinales, calcification takes place in the cell wall, from which CO$_2$ (and potentially HCO$_3^-$) uptake by adjacent cells for photosynthesis increases the pH, shifting the carbonate equilibrium in favour of ΩCO$_3^{2-}$ and CaCO$_3$ precipitation (Littler, 1976; Borowitzka, 1982; Koch et al., 2013). Photosynthetic enhancement of *C. officinalis* calcification during the present study is strongly supported by the significant relationship identified between the two processes ($R^2 = 0.65$), as was also observed by Pentecost (1978).

Interestingly, our data further demonstrated that internal enhancement of ΩCO$_3^{2-}$ at the site of calcification, as opposed to external ΩCO$_3^{2-}$, was the dominant control on light-calcification rates. This was evidenced by a lack of increase in light calcification rates over summer tidal emersion periods, despite significant increases in rock pool pH and ΩCO$_3^{2-}$. With decreases in net production over daytime tidal emersion, e.g. during March, minimal levels of production were sufficient to maintain increased internal ΩCO$_3^{2-}$, permitting maintenance of calcification. This is supported by the overall lower $E_6$ determined for calcification (ca. 110 µmol photons m$^{-2}$ s$^{-1}$) as compared to net production (ca. 300 µmol photons m$^{-2}$ s$^{-1}$).

In contrast to light calcification, the direction of *C. officinalis* dark calcification (dissolution vs. precipitation) was strongly related to rock pool water ΩCO$_3^{2-}$, mimicking abiotic CaCO$_3$ precipitation dynamics (Millero, 2007; Ries 2009). During seasonal minima of ΩCO$_3^{2-}$, net dissolution of CaCO$_3$ was apparent across dark daytime (December) and night-time (March) incubations, as observed during winter for *E. elongata* (Egilsdottir et al., 2015). With increases in pH and ΩCO$_3^{2-}$ over March, July and September daytime tidal emersion, initially negative (indicating net dissolution) or low positive dark calcification rates increased significantly, indicating net CaCO$_3$
precipitation at levels 40 – 46 % of light calcification. Additionally, net CaCO₃ precipitation was recorded across all dark daytime and night-time incubations during July, coinciding with seasonal maxima in ΩCO₃²⁻. CaCO₃ precipitation in the dark has previously been documented for calcifying macroalgae (e.g. Pentecost, 1978; Borowitzka, 1981; Gao et al., 1993; Lee and Carpenter, 2001; de Beer and Larkum, 2001; Martin et al., 2006), typically at lower rates (e.g. 10 – 40 %) than light calcification (Pentecost, 1978; Borowitzka, 1981), and has been attributed to belated biological activity after a passage from light to dark conditions (Pentecost, 1978; Martin et al., 2006). Our findings demonstrate that dark calcification is possible over complete diurnal cycles for *C. officinalis*, and can be significantly exaggerated under conditions of rock pool water CO₃²⁻-super-saturation. This mechanism can, however, be overridden by enhanced respiration. At the level of the organism, respiration can promote CaCO₃ dissolution via internal generation of CO₂ (Koch et al., 2013). During September, when maximal night-time respiration was observed, net CaCO₃ dissolution was apparent over the duration of night-time emersion, despite seasonal highs in ΩCO₃²⁻. Dissolution pressures can thus be exacerbated by high rates of respiration, mitigating the positive impacts of maxima in external ΩCO₃²⁻. This may have significant ramifications for the future fate of coralline algae if increases in water temperature drive corresponding increases in respiration.

**Conclusions**

Our findings indicate that *Corallina* species are highly tolerant to environmental stress, and are well-adapted to intertidal habitats, in agreement with previous studies (Williamson et al., 2014; Guenther and Martone, 2014). Photosynthesis, respiration and calcification varied significantly with abiotic stressors, and strongly interacted with one
another to produce predominantly beneficial outcomes at the level of the organism. With predicted acidification and warming of the world’s oceans, the balance between these processes and the external environment may be perturbed. Whilst acidification may relieve putative CO$_2$ limitation in rock pools during low irradiance winter months, increases in night-time dissolution are predicted given the strong coupling between carbonate chemistry and dark calcification dynamics identified here. Similarly, whilst increasing temperatures may facilitate increases in gross productivity, temperature driven increases in night-time respiration could further exacerbate dark dissolution by reducing carbonate saturation at the sites of calcification. *Corallina officinalis* will be most vulnerable to future change during winter months, and monitoring to assess impacts should be focused on such periods. This study adds to the growing understanding of coralline algal physiology, and provides a baseline against which to monitor future change.

**Acknowledgements**

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**References**


Coull, B.C. and Wells, J.B.J.: Refuges from fish predation - experiments with phytal meiofauna from the New Zealand rocky intertidal, Ecology, 64, 1599–1609, 1983.


R Core Team: R: A Language and Environment for Statistical Computing, 2014.


Figures

Figure 1. Sampling site and habitat, showing location of Combe Martin (a), and an example upper-shore rock pool (b) dominated by turfing assemblages of Corallina officinalis (c).
Figure 2: Irradiance (a) and rock pool water temperature (b) recorded at the start (grey bars) and end (white bars) of daytime tidal emersion periods during December 2013 (Dec ’13), and March (Mar ’14), July (Jul’14) and September (Sep ’14) 2014 (Average ± SE). Upper-case and lower-case letters denote TukeyHSD homogenous subsets in relation to the factors ‘month’ and ‘tide’, respectively.
Figure 3: Principal components analysis of (a) daytime and (b) night-time carbonate chemistry parameters, showing principal component one in relation to principal component two. Upper-case letters indicate sampling month (D = December, M = March, J = July, S = September) and lower-case letters indicate start (s) or end (e) tidal emersion.
Figure 4: Boxplots showing the median, minimum, maximum and first and third quartiles of PC1\textsubscript{day} (a) and PC1\textsubscript{night} (b) in relation to sampling month (Dec = December, Mar = March, Jul = July, Sep = September) and tidal emersion period (S = start, E = End). Upper-case and lower-case letters denote TukeyHSD homogenous subsets in relation to the factors 'month' and 'tide', respectively.
Figure 5: Average daytime (a) NP (-ve DIC flux) and R$_{DAY}$ (+ve DIC flux), and (b) NG$_{DAY}$ as determined from light (L – white bars) and dark (D – black bars) treatment incubations conducted at the start (s) and end (e) of daytime tidal emersion periods during December 2013 and March, July and September 2014 (Average ± SE, $n = 5$). Upper-case and lower-case letters denote TukeyHSD homogenous subsets in relation to the factors ‘month’ and ‘tide’, respectively.
Figure 6: Average night-time (a) $R_{\text{NIGHT}}$ and (b) $NG_{\text{NIGHT}}$ as determined across both light/dark treatment incubations and the start/end of tidal emersion periods (Average ± SE, $n = 20$). Upper-case letters denote TukeyHSD homogenous subsets in relation to the factor ‘month’.
Figure 7: Relationship of (a) net production/respiration ($NP$ and $R$) and (b) net calcification ($NG_{D\text{AY/NIGHT}}$) with irradiance (Model 1, Table 4), showing regression line (solid red line) and 95 % confidence intervals (dashed red lines).
Figure 8: Relationship between calcification (NG) and production / respiration (NP/R), showing regression line (solid red line) and 95 % confidence intervals (dashed red lines).
Table 1: Sampling dates and tidal details. All times are expressed in GMT.

<table>
<thead>
<tr>
<th>Sampling Date</th>
<th>Dec 4/5th 2013</th>
<th>Mar 16/17th 2014</th>
<th>Jul 1st/2nd 2014</th>
<th>Sep 9/10th 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time</strong></td>
<td><strong>Height (m)</strong></td>
<td><strong>Time</strong></td>
<td><strong>Height (m)</strong></td>
<td><strong>Time</strong></td>
</tr>
<tr>
<td>06:30</td>
<td>9.6</td>
<td>05:50</td>
<td>8.8</td>
<td>08:12</td>
</tr>
<tr>
<td>12:30</td>
<td>0.7</td>
<td>11:51</td>
<td>1.2</td>
<td>13:59</td>
</tr>
<tr>
<td>18:50</td>
<td>9.5</td>
<td>18:09</td>
<td>8.9</td>
<td>20:23</td>
</tr>
<tr>
<td>00:55</td>
<td>0.8</td>
<td>00:02</td>
<td>1</td>
<td>02:20</td>
</tr>
<tr>
<td>07:15</td>
<td>9.7</td>
<td>06:23</td>
<td>9</td>
<td>08:45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>06:31</td>
</tr>
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Table 2: Component loadings of principal components analysis of daytime and nighttime carbonate chemistry parameters (TA, DIC, pH, $pCO_2$, HCO$_3^-$, CO$_3^{2-}$, $\Omega_{arg}$ and $\Omega_{cal}$)

<table>
<thead>
<tr>
<th>Variable</th>
<th>PC$_{1\text{DAY}}$ (%)</th>
<th>PC$_{2\text{DAY}}$ (%)</th>
<th>PC$_{1\text{NIGHT}}$ (%)</th>
<th>PC$_{2\text{NIGHT}}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of variance</td>
<td>84.3</td>
<td>13.2</td>
<td>83.6</td>
<td>16.0</td>
</tr>
<tr>
<td>Cumulative proportion</td>
<td>84.3</td>
<td>97.6</td>
<td>83.6</td>
<td>99.7</td>
</tr>
<tr>
<td>Component Loadings</td>
<td>PC$_{1\text{DAY}}$</td>
<td>PC$_{2\text{DAY}}$</td>
<td>PC$_{1\text{NIGHT}}$</td>
<td>PC$_{2\text{NIGHT}}$</td>
</tr>
<tr>
<td>TA</td>
<td>-0.07</td>
<td>0.94</td>
<td>-0.18</td>
<td>-0.77</td>
</tr>
<tr>
<td>DIC</td>
<td>-0.36</td>
<td>0.17</td>
<td>-0.35</td>
<td>-0.36</td>
</tr>
<tr>
<td>pH</td>
<td>0.38</td>
<td>0.04</td>
<td>0.37</td>
<td>-0.16</td>
</tr>
<tr>
<td>$pCO_2$</td>
<td>-0.36</td>
<td>0.01</td>
<td>-0.38</td>
<td>0.05</td>
</tr>
<tr>
<td>HCO$_3^-$</td>
<td>-0.38</td>
<td>0.09</td>
<td>-0.37</td>
<td>-0.23</td>
</tr>
<tr>
<td>CO$_3^{2-}$</td>
<td>0.37</td>
<td>0.14</td>
<td>0.37</td>
<td>-0.24</td>
</tr>
<tr>
<td>$\Omega_{arg}$</td>
<td>0.37</td>
<td>0.14</td>
<td>0.37</td>
<td>-0.24</td>
</tr>
<tr>
<td>$\Omega_{cal}$</td>
<td>0.37</td>
<td>0.14</td>
<td>0.37</td>
<td>-0.24</td>
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</table>
Table 3: Multiple linear regression analysis of $PC_{1DAY}$ in relation to irradiance (Irrad.) or cumulative photodose (Photo.) plus water temperature (Temp.), and linear regression analysis of $PC_{1NIGHT}$ in relation to water temperature (Temp.), showing associated standard error (SE) of coefficients, the significance of predictor variables (Pred. sig.) within the model, the percent relative importance of predictor variables (Rel. Imp.), the proportion of variance explained by the regression ($R^2$), the overall model significance ($P$), and the number of observations ($n$).

<table>
<thead>
<tr>
<th>Relationship ($y = a + b_1X_1 + b_2X_2$)</th>
<th>Coefficient $SE$</th>
<th>Pred. sig.</th>
<th>Rel. Imp. (%)</th>
<th>$R^2$</th>
<th>$P$</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$PC_{1DAY} = -7.03 + 0.002<em>Irrad. + 0.61</em>Temp.$</td>
<td>0.73</td>
<td>0.00</td>
<td>0.07</td>
<td>&lt;0.001</td>
<td>28</td>
<td>71</td>
</tr>
<tr>
<td>$PC_{1DAY} = -2.52 + 1.41<em>Photo. + 9.10</em>Temp.$</td>
<td>0.72</td>
<td>2.72</td>
<td>6.38</td>
<td>&lt;0.001</td>
<td>58</td>
<td>41</td>
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<tr>
<td>$PC_{1NIGHT} = -2.89 + 0.22*Temp.$</td>
<td>1.40</td>
<td>0.10</td>
<td>-</td>
<td>&lt;0.05</td>
<td>0.08</td>
<td>72</td>
</tr>
</tbody>
</table>
Table 4: Values of parameters (SE in parentheses) calculated by non-linear regression of net production (NP, µmol DIC gDW\(^{-1}\) h\(^{-1}\)) and net calcification (NG, µmol CaCO\(_3\) gDW\(^{-1}\) h\(^{-1}\)): in relation to (Model 1) irradiance (E, µmol photons m\(^{-2}\) s\(^{-1}\)), where c is estimated dark respiration or calcification; and in relation to (Model 2) irradiance and temperature (T, °C), where f is a constant; and in relation to (Model 3) irradiance and carbonate chemistry (PC1); and in relation to (Model 4) irradiance, temperature and carbonate chemistry. Asterisks denote coefficient significance in models (P <0.05*, P <0.01**, P <0.001***). Estimation of overall model fit is presented as the proportion of variance explained by the regression (R\(^2\)) and as Akaike Information Criterion (AIC). n denotes the number of observations.

<table>
<thead>
<tr>
<th>Model</th>
<th>Formula</th>
<th>(P(G)_{max})</th>
<th>(E_{k})</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>R(^2)</th>
<th>AIC</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1: NP (NG) = (P(G)<em>{max}) (1 - e(^{-E/E</em>{k}})) + c</td>
<td></td>
<td>4.41(0.22)***</td>
<td>300(65)***</td>
<td>3.29(0.56)***</td>
<td>0.67</td>
<td>885</td>
<td>140</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 2: NP (NG) = (P(G)<em>{max}) (1 - e(^{-E/E</em>{k}})) + dT + f</td>
<td></td>
<td>-23.3(1.48)***</td>
<td>377(99)***</td>
<td>0.15(0.12)</td>
<td>1.07(1.82)</td>
<td>0.68</td>
<td>886</td>
<td>140</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 3: NP (NG) = (P(G)<em>{max}) (1 - e(^{-E/E</em>{k}})) + ePC1 + f</td>
<td></td>
<td>3.92(0.21)***</td>
<td>115(24)***</td>
<td>0.08(0.01)***</td>
<td>-1.28(0.26)***</td>
<td>0.80</td>
<td>363</td>
<td>140</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 4: NP (NG) = (P(G)<em>{max}) (1 - e(^{-E/E</em>{k}})) + dT + ePC1 + f</td>
<td></td>
<td>-23.6(1.96)***</td>
<td>375(99)***</td>
<td>0.07(0.14)</td>
<td>0.22(0.23)</td>
<td>2.12(2.12)</td>
<td>0.68</td>
<td>887</td>
<td>140</td>
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</table>