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Short running title: Photosynthesis and calcification in *E. elongata*

Abstract

Calcifying coralline algae are functionally important in many ecosystems but their existence is now threatened by global climate change. The aim of this study was to improve our understanding of coralline algal metabolic functions and their interactions by assessing the respiration, photosynthesis and calcification rates in an articulated (geniculate) coralline alga, *Ellisolandia elongata*. Algae were collected from an intertidal rock-pool on the coast of Brittany (France). Physiological rates were assessed in summer and in winter by measurements of oxygen, dissolved inorganic carbon and total alkalinity fluxes at five irradiance levels and in the dark using incubation chambers.

Respiration, photosynthetic and calcification rates were strongly affected by season. Respiration increased with temperature, being ten-fold higher in summer than in winter. Photosynthetic parameters of the photosynthesis-irradiance (*P-E*) curve, P_g^{max}, P_n^{max} and E_k, were two- to three-fold higher in summer relative to winter. Photoinhibition was observed under high irradiance levels indicating an acclimation of *E. elongata* to low irradiance. Parameters of the calcification-irradiance (*G-E*) curve, G^{max} and E_k, were approximately two-fold higher in summer, compared to winter. Calcification in summer was more strongly inhibited at high irradiance levels than photosynthesis. By inhabiting intertidal rock pools, *E. elongata* exhibits tolerance to a dynamic physico-chemical environment. Information on respiration, photosynthesis and calcification rates in a calcifying coralline alga inhabiting such dynamic environments in terms of pH and temperature is important for better understanding of how global changes, including ocean acidification and warming, will affect coralline algae in the future.

Introduction

Climate change and ocean acidification are advancing as a result of anthropogenic increase in carbon dioxide concentrations in the Earth's atmosphere (IPCC, 2013). Investigating organism physiology in relation to environmental variability provides useful information for predicting acclimation and adaptation to future conditions (Kelly & Hofmann, 2013). Calcifying benthic algae are generally considered highly sensitive to climate change and ocean acidification (Kroeker *et al.*, 2013) but many species are also common in habitats where pH and temperature gradients are steep. Among calcifying benthic algae, red coralline algae (Corallinales, Rhodophyta) are the most important taxa in terms of global carbon cycling and ecosystem engineering (Nelson, 2009). They can be found over a wide latitudinal range and within all depths of the photic zone (Adey & Macintyre, 1973; Steneck, 1986) where they are known to provide ecosystem services such as by sheltering high biodiversity and favour larval recruitment and settlement (Adey, 1998). Coralline algae are also of importance with respect to the carbon and carbonate cycle in shallow coastal waters through high community calcium carbonate production (Martin *et al.*, 2007). Articulated coralline algae (also referred to as geniculate coralline algae) have non-calcified segments between calcified intergenicula that provide flexibility to the erect algal thallus (Martone & Denny, 2008). They have been reported to support diverse assemblages of macrofauna and are important providers of three dimensional frameworks (Kelaher *et al.*, 2001), being considered as ecosystem engineers (Dayton, 1972; Benedetti-Cecchi, 2006; Noël *et al.*, 2009). Their unpalatable calcareous structure acts as a barrier against grazing, thus protecting diverse algal epiphyte assemblages (Pfister & Hay, 1988).

Coralline algae have been identified as among the most vulnerable calcifying marine taxa threatened by ocean acidification and decreasing calcium carbonate saturation levels (Kroeker *et al.*, 2013). Reliable predictions of coralline algal responses to future environmental changes require knowledge of the processes involved in calcification and their interaction with other metabolic processes and environmental factors. Coralline algae precipitate calcite within the cell wall and can also deposit calcite within intercellular spaces (Borowitzka, 1989). They precipitate high-Mg calcite (Ries, 2011) which becomes more solvable with increased incorporation of Mg^{2+} into the calcite lattice. The solubility of calcite exceeds that of aragonite when the Mg/Ca mol% is above approximately 12 (Morse *et al.*, 2006; Andersson *et al.*, 2008) with higher values often observed in calcite produced by coralline algae (Chave, 1954). An organic matrix within the cell wall is important for the calcification process but the exact chemical and functional properties of the organic matrix are yet not fully understood (Borowitzka, 1989; Rahman & Halfar, 2014). For example, only recently the presence of chitin was discovered in a coralline alga species, a substance which likely serves an important role in nucleation, growth and orientation of the mineral crystals (Rahman & Halfar, 2014).

The equilibrium between inorganic carbon species in an aquatic system is an important environmental factor affecting the processes that underlay $CaCO_3$ bioprecipitation (Borowitzka, 1982; Borowitzka & Larkum, 1987; Raven, 2011; Basso, 2012). For example, supersaturation of calcium carbonate is generally required for calcification to take place in algae (Borowitzka, 1989). The calcium carbonate saturation state (Ω_{CaCO3}) in seawater and at the calcification site is affected by photosynthesis and respiration through the removal or addition of CO_2 from or to the extracellular environment (Borowitzka, 1989). In this way, calcification is induced by photosynthesis as is evident by the strong relationship between the metabolic rates of these processes in all calcifying algae (Borowitzka & Larkum, 1976; Pentecost, 1978; Borowitzka, 1989; Martin *et al.*, 2013a).

Photosynthetic rates in algae are primarily controlled by environmental factors such as irradiance, nutrient concentration, temperature and the availability of inorganic carbon as photosynthetic substrate (Hurd *et al.*, 2014). A useful method for investigating photophysiological characteristics in algae is to look at the relationship between irradiance and photosynthesis through *P* (photosynthesis) versus *E* (irradiance) curves (also termed *P-I* curves) (Lüning, 1990). Photosynthetic rates increase with increasing irradiance until the photosynthetic mechanism of the algae becomes saturated (Lüning, 1990). The irradiance needed to saturate an alga is generally high for algae acclimated or adapted to high levels of irradiance and low for algae acclimated or adapted to dim light conditions (Lüning, 1990). Under higher irradiances algae may either continue at the maximum photosynthetic rate or photosynthetic rates may be negatively affected above a certain irradiance level, i.e. photoinhibition occurs (Lüning, 1990). Due to the close relationship between photosynthesis and calcification an irradiance response curve may also be produced for calcification (then termed *G-E* (or *G-I*) curve) (Chisholm, 2000; Martin *et al.*, 2006; Martin *et al.*, 2013a; Martin *et al.*, 2013b). Through comparing irradiance response curves for photosynthesis and calcification in algae, a better understanding of the relationship between these metabolic processes may be achieved.

Articulated coralline algae frequently inhabit the intertidal environment and in particular tidal pools (Johansen, 1981). Tidal pools are habitats where water exchange is limited or temporally non-existent as a result of local tidal activity (i.e. amplitude and tidal cycles) and pool locality (i.e. high or low within the intertidal zone) (Morris & Taylor, 1983). Because of biological processes and physico-chemical fluxes, the carbonate chemistry within a tidal pool may exhibit great periodical variations which is generally reflected by large pH fluctuations (Morris & Taylor, 1983; Huggett & Griffiths, 1986).

Ellisolandia elongata (previously known as *Corallina elongata*) inhabits intertidal rock pools where large fluctuations in physico-chemical variables occur diurnally (Egilsdottir *et al.*, 2013). It has been suggested that organisms inhabiting environments where large fluctuations in CO_2/pH and temperature are common will have a relatively good capacity to acclimate or adapt to future ocean acidification and warming (Kelly & Hofmann, 2013; Hofmann *et al.*, 2014). Indeed, studies have indicated a greater tolerance to ocean acidification in *E. elongata* previously inhabiting a rock-pool environment when compared to other coralline algae whose distribution is limited to more stable environments (Egilsdottir *et al.*, 2013; Noisette *et al.*, 2013).

Although, *E. elongata* is predicted to exhibit resilience under future conditions, there have been no studies on the relationship between photosynthesis and calcification in *E. elongata* under natural conditions, and there is a lack of information on the seasonal difference in the rates of these processes. Such data is valuable, for example for predicting the response of *E. elongata* to future conditions. The aim of this study was to characterize the metabolic activity of photosynthesis and calcification and how they interact *in situ* in the articulated coralline alga *E. elongata* inhabiting tidal pools where strong physico-chemical variations occur.

Material and Methods

1. Collection of algae

Individuals of *Ellisolandia elongata* (J. Ellis & Solander, 1786) K. R. Hind & G. W. Saunders 2013 were collected in a shaded tidal-pool in the low intertidal level on the rocky beach “Les Amiets” in Cléder on the North coast of Brittany, France (48°41’45 N, 4°7’26 W) on the 11th of August 2010 (summer) and on the 21st of February 2013 (winter). The irradiance in photosynthetic active radiations (PAR) at the surface of the pool was measured using a flat quantum sensor (LiCor, Li-190SA, LI-COR Biosciences, USA). Irradiance was *ca*. 30 µmol photons m^{-2} s^{-1} in summer and *ca.* 15 µmol photons m^{-2} s^{-1} in winter under the shade of rocks overhanging the tidal pool at around midday under sunny conditions. Temperature in the pool at the time of collection of algae was 17 °C in summer and 10 °C in winter. The physico-chemical characteristics of the rock pool are given in Table 1. Individual algae with a frond length of 5-10 cm and free of epiphytic organisms were selected for the experiment. They were carefully separated from their substrate to obtain their encrusting base. After experiments the fresh weight (FW) of the algae was determined and converted to dry weight (DW) using the FW/DW relationship established by Egilsdottir *et al.* (2013).

2. Incubation procedure and physiological measurements

E. elongata were incubated under natural sunlight from 13:00 to 16:00. In summer, algae were transferred directly to incubation chambers and incubated *in situ* in a large neighbouring pool exposed to direct sunlight. In winter the algae were transported to the *Roscoff Marine Station* in a thermostatic tank within 1 h of collection and incubated outside under natural sunlight in large baths filled with natural seawater pumped at 10 m depth in front of the station at ambient temperature. Temperature during incubation varied from 17 to 24°C in summer and from 6 to 9°C in winter (Table 2), being representative of changes in the pool during emersion at these seasons (Table 1). Five sets of individual algae (N = 5) were incubated per irradiance level in 220 and 460 ml clear and black glass bottles. Incubations at all irradiance levels were performed simultaneously in each season for 1 to 3 hours in summer and 2 to 4 hours in winter, depending on the expected algal metabolic response. Mean DW of the sets of algae was 0.41 ± SE 0.03 g in summer and 0.81 ± SE 0.05 g in winter. Black plastic covers were used to assess respiration and calcification rates in the dark. Four neutral density filters were used to assess net production and calcification in algae simultaneously exposed to 5 different mean irradiance (PAR) levels (I1, I2, I3, I4, and I5; Table 2) of 91, 158, 235, 614, and 1118 μmol photons m^{-2} s^{-1} in summer and 30, 75, 135, 290, and 500 μmol photons m^{-2} s^{-1} in winter, with the highest irradiances corresponding to natural full light. Irradiance was measured with a Li-Cor quantum sensor (LiCor Li–190SA, LI-COR Biosciences, USA). Reference incubations without algae were included for full light and dark conditions to assess and correct the metabolism by any biological activity in the seawater. Oxygen fluxes were estimated by measuring the oxygen concentration at the beginning and the end of each incubation with an oxygen probe (Oxymeter HQ40D, Hach Lange, Ltd portable LDO^{TM}, Loveland USA). pH was measured using a pH meter (HQ40D, Hach Lange, Ltd portable LDO^{TM}, Loveland USA) calibrated on the total scale (pH_T, expressed on the total hydrogen ion concentration scale) using Tris/HCl and 2-aminopyridine/HCl buffer solutions with a salinity of 35 (Dickson *et al.*, 2007). For measurements of total alkalinity (A_T), seawater samples were filtered through 0.7 μm Whatman GF/F filters into 100 mL glass bottles, immediately poisoned with mercuric chloride and stored in a cool dark place pending analyses. A_T was determined potentiometrically using an automatic titrator (Titroline alpha, Schott SI Analytics, Germany) calibrated on the National Bureau of Standards scale and calculated using a Gran function applied to pH values ranging from 3.5 to 3.0 (Dickson *et al.*, 2007). Measurements of A_T were corrected by comparison with standard reference material provided by Andrew G. Dickson (Batch 111). Other parameters of the carbonate chemistry were calculated from pH_T, A_T, temperature and salinity (35) using the software *CO2Sys*, *EXCEL Macro version 2.1* (originally designed by Lewis & Wallace, 1998). Calculations were based on a set of constants *K1* and *K2* from Mehrbach *et al.* (1973), refit by Dickson and Millero (1987) (Table 1; Table 2).

3. Data treatment and statistical analysis

Primary production was estimated directly from oxygen fluxes. Net production (P_n) and respiration (R) rates (µmol O_2 g^{-1} DW h^{-1}) were calculated as follows:

$$P_n \text{ or } R\,(O_2) = \left(\frac{\Delta O_2}{\Delta t \times V}\right) / DW \qquad (1)$$

where ΔO_2 is the change in dissolved oxygen (µmol l^{-1}), Δt is the incubation time (h), DW is the dry weight of the algae and V is the volume of the incubation chamber (L).

The variations of dissolved inorganic carbon (DIC) in the incubations integrate both organic carbon metabolism (photosynthesis and respiration) and inorganic carbon metabolism (calcification and dissolution) (Zeebe & Wolf-Gladrow, 2001). Calcification rates were estimated using the alkalinity anomaly technique (Smith & Key, 1975; Chisholm & Gattuso, 1991) which is based on the relationship: $Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O$, for which total alkalinity decreases by 2 equivalents for each mol of $CaCO_3$ precipitated.

Calcification rates (G, µmol $CaCO_3$ g^{-1} DW h^{-1}) was calculated as follows:

$$G = -\left(\frac{\Delta A_T}{2 \times \Delta t \times V}\right) / DW \qquad (2)$$

where ΔA_T is the change in total alkalinity (µeq l^{-1}).

P_n and R expressed in terms of carbon (µmol CO_2 g^{-1} DW h^{-1}) were calculated by the difference between the initial and final DIC concentrations (calculated by using the software *CO2Sys*) as follows:

$$P_n \text{ or } R\,(CO_2) = \left(\frac{\Delta DIC - \left(0.5 \times \Delta A_T\right)}{\Delta t \times V}\right) / DW \qquad (3)$$

where ΔDIC is the change in dissolved inorganic carbon (mmol l^{-1}).

The relationship between irradiance (E, µmol photon m^{-2} s^{-1}) and P_n (or G) was established using P_n (or G) versus E curves following the function of Platt *et al.* (1980) modified by the addition of a respiration (R) or dark calcification term (G_d, µmol $CaCO_3$ g^{-1} DW h^{-1}):

$$P_n = Ps \times (1- e^{-\alpha \times \frac{E}{Ps}}) \times e^{-\beta \times \frac{E}{Ps}} + R \qquad (4)$$

$$G = Gs \times (1- e^{-\alpha \times \frac{E}{Gs}}) \times e^{-\beta \times \frac{E}{Gs}} + G_d \qquad (5)$$

where Ps and Gs are scaling parameters defined as the maximum rate of photosynthesis or calcification respectively in the absence of photoinhibition (or decrease in calcification rate under high irradiance; µmol O_2, DIC or $CaCO_3$ g^{-1} h^{-1}), α is the initial slope of the relationship (µmol g^{-1} h^{-1} (µmol m^{-2} s^{-1})$^{-1}$), β is the photoinhibition coefficient (µmol g^{-1} h^{-1} (µmol m^{-2} s^{-1})$^{-1}$). In the absence of evident photoinhibition, the β value was set to zero.

The deduced maximal rate of gross photosynthesis (or calcification) at light saturation, P_g^{max} (or G_g^{max}), are calculated as:

$$P_g^{max} \text{ (or } G_g^{max}\text{)} = P_s \text{ (or } G_s\text{)} \times \left(\frac{\alpha}{\alpha+\beta}\right) \times \left(\frac{\beta}{\alpha+\beta}\right)^{\frac{\beta}{\alpha}} \qquad (6)$$

The maximal rate of net production (or calcification), $P_{max}{}^{net}$ (or G^{max}) can be estimated as:

$$P_n{}^{max} = P_g{}^{max} + R \qquad (7)$$

$$G^{max} = G_g^{max} + G_d \tag{8}$$

The saturating irradiance (E_k, index of light adaptation) is expressed as:

$$E_k = \frac{P_g^{max}}{\alpha} \text{ or } \frac{G_g^{max}}{\alpha} \tag{9}$$

The compensation irradiance (E_c) where the rate of photosynthesis equals the rate of respiration is expressed as:

$$E_c = \frac{R}{\alpha} \tag{10}$$

P_n- and *G-E* curves were fitted to the data using the statistical software STATGRAPHICS *Plus, version 5.0 for Windows* (StatPoint Technologies Inc., 2013), establishing 5 individual curves for O_2 flux, CO_2 flux and calcification per season. The statistical software *R 2.15.0* (The R Foundation for Statistical Computing, 2013) was used when examining differences between seasons. Normality of data was estimated via the Shapiro-Wilk normality test. The Welch two sample t-test was used to identify differences between seasons where data was normally distributed or where log or square-root transformation of data yielded normal distribution. When normality was not achieved, a Wilcoxon Rank Sum test was used. Results are expressed as means ± standard error (SE).

Results

Respiration and photosynthesis

The mean respiration rate (*R*) was higher in summer than in winter (O_2 fluxes: $t = -6.63$, $p < 0.01$; CO_2 fluxes: $t = 3.97$, $p < 0.05$; Table 3). In both seasons primary production measured under various irradiance levels showed a strong relationship with irradiance in terms of both oxygen production (r^2 = 0.88 - 0.98 in summer; r^2 = 0.78 - 0.99 in winter) and net CO_2 uptake (r^2 = 0.87 - 0.98 in summer; r^2 = 0.87 - 0.98 in winter; Fig. 1). Photoinhibition was recorded for primary production at irradiance levels above about 400 µmol photons m^{-2} s^{-1} in summer and a negligible photoinhibition above about 200 µmol photons m^{-2} s^{-1} was observed in winter (Table 3; Fig. 1). In summer, maximum gross O_2 production and CO_2 uptake at light saturation (P_g^{max}) were about three times those recorded for winter (O_2 fluxes: W=25, $p < 0.01$; CO_2 fluxes: W=25, $p < 0.01$; Table 2). In winter the maximum net production (P_n^{max}) was about 40% that of summer (O_2 fluxes: W=25, $p < 0.01$; CO_2 fluxes: W=25, $p < 0.01$; Table 3). The saturating irradiances (E_k) for primary production were about three times higher in summer, relative to winter (O_2 fluxes: $t = 5.42$, $p < 0.05$; CO_2 fluxes: $t = 5.26$, $p < 0.05$; Table 3) and the compensation irradiances (E_c) were higher in summer than in winter (O_2 fluxes: $t = 4.66$, $p < 0.01$; CO_2 fluxes: $t = 3.32$, $p < 0.05$; Table 3). Coefficient α (the initial slope of the curve) did not differ between seasons for both O_2 and CO_2 fluxes. Coefficient β (photoinhibition coefficient) did not differ between seasons for O_2 fluxes. No statistical comparison between seasons was conducted for β considering CO_2 fluxes since photoinhibition was not indicated by all five *P-E* curves calculated for each season. The net production rates under 30 µmol photons m^{-2} s^{-1} and 15 µmol photons m^{-2} s^{-1} (*in situ* midday irradiance in summer and winter respectively) were estimated from the *P-E* curves to 8.4 ± 3.1 µmol O_2 g^{-1} DW h^{-1} and -5.6 ± 4.5 µmol C g^{-1} DW h^{-1} in summer and 7.4 ± 1.0 µmol O_2 g^{-1} DW h^{-1} and -11.3 ± 1.5 µmol C g^{-1} DW h^{-1} in winter. Gross production ($P_g = P_n + R$) was thus estimated to 20.4 ± 3.1 µmol O_2 g^{-1} DW h^{-1} and -22.5 ± 1.9 µmol C g^{-1} DW h^{-1} in summer and 8.4 ± 1.0 µmol O_2 g^{-1} DW h^{-1} and -11.8 ± 1.6 µmol C g^{-1} DW h^{-1} in winter.

Calcification

Calcification rates in the dark (G_d) differed between seasons ($t = 3.07$, $p < 0.05$; Table 3). G_d was positive in summer but negative in winter, indicating net dissolution of calcium carbonate. A strong relationship was observed between irradiance and calcification in summer ($r^2 = 0.72 - 1.00$; Fig 2a) and winter ($r^2 = 0.93 - 0.99$; Fig. 2b). At high irradiances in summer the relationship between irradiance and calcification is negligible with a high variance observed for calcification at high irradiances (Fig 2a). Calcification was inhibited at irradiances higher than about 200 µmol m^{-2} s^{-1} in both summer and winter. Maximum net calcification (G_n^{max}) differed between seasons ($t = 3.07$, $p < 0.05$; Table 3) and was about two times higher in summer relative to winter. E_k was approximately two times higher in summer than in winter ($t = 2.50$, $p < .005$; Table 3). Since calcification in the dark was positive in summer, an estimate for E_c in summer was unavailable and consequently a statistical comparison between seasons was irrelevant. For calcification, coefficients α and β did not differ between seasons (Table 3). Calcification at midday *in situ* irradiance estimated from the *G*-*E* curves was 3.3 ± 0.3 µmol $CaCO_3$ g^{-1} DW h^{-1} in summer at 30 µmol photons m^{-2} s^{-1} and 1.1 ± SE 0.1 µmol $CaCO_3$ g^{-1} DW h^{-1} in winter at 15 µmol photons m^{-2} s^{-1}.

Discussion

Respiration

Respiration rates observed here varied seasonally in terms of both O_2 and CO_2 fluxes, being more than 10-fold higher in summer in comparison to winter. The temperature dependence of respiration is well known for seaweeds (Hurd *et al.*, 2014) and has already been reported for several species of coralline algae with a trend of increasing respiration with increasing temperature until temperature reaches beyond the thermal tolerance limit of the alga (see Martin *et al.*, 2013b for a review). For example, respiration rate in the temperate crustose coralline alga, *Lithophyllum yessoense* increased by ten-fold, from 3 to 29 µg O_2 cm^{-2} h^{-1} between 5 and 25°C (Ichiki *et al.*, 2001). Changes in respiration rates observed between winter and summer in *E. elongata* are high in comparison with the threefold increase measured in the temperate unattached coralline alga (maerl) *Lithothamnion corallioides* between 10°C (winter) and 16.5°C (summer) (Martin *et al.*, 2006) or in the temperate crustose coralline alga *Lithophyllum cabiochae* between 13°C (winter) and 22°C (summer) (Martin *et al.*, 2013b). The large differences observed between winter and summer in *E. elongata* is mainly due to the high respiration rates recorded in summer. A three- to four-fold lower respiration rate was recorded for this species at a temperature range of 13 to 17°C in a Mediterranean subtidal environment (3 µmol O_2 g^{-1} DW h^{-1}; El Haïkali *et al.*, 2004) and at a constant temperature of 18°C in a laboratory (4 µmol O_2 g^{-1} DW h^{-1}; Egilsdottir *et al.*, 2013). Temperature was considerably higher in summer in the rock pool at the time of measurements, reaching 23°C at the end of the incubation explaining the high summer respiration rates in *E. elongata* observed in this study. Higher respiration rates in summer in comparison to winter may also reflect high rates of primary production shortly before dark measurements took place as light-enhanced dark respiration has been observed in plants and algae (Raghavendra *et al.*, 1994; Martin *et al.*, 2013a). The large seasonal difference observed for respiration could partly be attributed to the low temperature during winter measurements (reaching below 6°C). In winter, the algae were carefully transported to a different location and incubated in water-baths. Therefore, it is conceivable that different handling and transportation of algae prior to incubations contributed seasonal differences in respiration and other metabolic activity.

Although changes between day and night respiration rates occur in subtidal coralline algae in a more stable environment in terms of temperature (Martin *et al.*, 2013a), our results suggest that respiration in coralline algae may vary strongly with temperature changes in tidal pools, even on a very short time scale. Such variations may in turn have consequences for the whole metabolism of algae and in particular for the balance between respiration and photosynthesis.

Photosynthesis

The photosynthesis of *E. elongata* was significantly influenced by the season. The gross production, P_g, under ambient irradiance was two- to three-fold higher in summer than in winter. These seasonal fluctuations of P_g are related to the changes in both temperature and irradiance which are the fundamental parameters in the controlling algal photosynthesis (Hurd *et al.*, 2014). Photosynthetic characteristics of the *P-E* curves reflect this with values of P_g^{max} threefold higher in summer than in winter. Similar seasonal influence of temperature and irradiance on photosynthesis has previously been reported in other temperate coralline algal species such as *L. corallioides* or *L. cabiochae*, which exhibited values of P_g^{max} two- to three-fold higher in summer compared to winter (Martin *et al.*, 2006; Martin *et al.*, 2013b). A strong seasonal difference was observed in the photosynthetic parameters E_k and E_c with both being considerably lower in the winter season, reflecting a greater degree of low-light acclimation in winter than in summer. E_k was 2-3 fold higher in summer compared to winter and is in agreement with E_k previously reported for low light acclimated coralline algae, in the range of 7-32 µmol m^{-2} s^{-1} (Kühl *et al.*, 2001; Payri *et al.*, 2001; Roberts *et al.*, 2002; Martin *et al.*, 2013a). E_c was more than ten fold higher in summer than in winter. The value of E_c in summer is consistent with that previously reported for *E. elongata* acclimated to low irradiance (E_c = 20 µmol photons m^{-2} s^{-1}; Algarra et al., 1991), while the value E_c in winter is similar to that reported for other coralline algae living in dim light condition such as *L. cabiochae* in winter under very low irradiance (< 10 umol photons m^{-2} s^{-1}; Martin *et al.* 2013b). Such low E_c reflects the ability of *E. elongata* to make a net gain from photosynthesis at very low light levels.

Photoinhibition was observed at high irradiances as has previously been reported for low light acclimated algae (Kain, 1987; Sagert *et al.*, 1997; Kühl *et al.*, 2001; Roberts *et al.*, 2002; Martin *et al.*, 2013b). Recently, photoinhibition was also observed in *E. elongata* under rapid light curves (RLCs) (Korbee *et al.*, 2014) giving further evidence to *E. elongata* being a “shade plant” which Häder *et al.* (2003) has suggested characterized all geniculate coralline algal species. Coralline algae have been reported to exhibit dynamic photoinhibition strategies which likely enable the algae to tolerate high irradiance levels rather than undergoing photodamage (Burdett *et al.*, 2014). For example, Burdett *et al.* (2014) reported varying reflectance of the thallus at different times of the day, and varying intracellular concentrations of the antioxidant compound DMSP (dimethylsulphoniopropionate) in crustose coralline algae.

Intertidal rock pools typically exhibit large diel fluctuations in temperature and irradiance levels, both of which affect photosynthesis in algae.

Temperature directly affects the biochemistry occurring at the algal cellular level. For example, temperature may influence the activity of the enzyme carbonic anhydrase, active transport of CO_2 and HCO_3^- and diffusion rates by which photosynthesis may be affected through the supply of substrate to carbon fixation pathways (Hurd *et al.*, 2014). In this study, temperature exhibited similar variability at all irradiance levels for each season but is likely to contribute to the difference in *P-E* curves between seasons.

Irradiance affects seawater in tidal pools though regulation of photosynthesis. As observed in this study, and is well known for photosynthetic organisms, a positive relationship exists between photosynthetic rates and irradiance up to a certain irradiance level, i.e. near the saturating irradiance E_k (Lüning, 1990). In this way, irradiance influences parameters of the seawater chemistry in tidal pools, such as carbonate chemistry and nutrient concentrations (Morris & Taylor, 1983; Hurd *et al.*, 2014). Contrary to respiration, algal photosynthesis removes CO_2 from seawater and subsequently leads to an elevation in pH and a decrease in total inorganic carbon concentrations (Hurd *et al.*, 2014). High pH is known to negatively affect photosynthesis in some macroalgal species (Borowitzka, 1981; Middelboe & Hansen, 2007). For example, Björk *et al.* (2004) found certain algal species to be absent in tidal pools where high pH values and low inorganic carbon concentration occurred temporarily. Effect of pH on photosynthesis in *E. elongata* has only been investigated for a pH range of 7.7 to 8.1 with no significant effects observed (Egilsdottir *et al.*, 2013; Noisette *et al.*, 2013) but the effects of higher pH levels have not yet been investigated for this species. Like most macroalgae, coralline algae have CO_2 concentrating mechanisms (CCMs) that allow the algae to utilize low concentrations of inorganic carbon (Borowitzka, 1981; Gao *et al.*, 1993). These mechanisms are diverse in their exact functionality but for example promote active transport of inorganic carbon across membranes and/or may facilitate the conversion of HCO_3^- to CO_2 for photosynthetic metabolism (Raven *et al.*, 2012). Effective CCMs explain why some algae, including many species of coralline algae, are commonly found in rock pools where pH is often elevated and inorganic carbon concentrations low (Raven, 2011).

Calcification

Although calcification in articulated coralline algal communities may have the potential to significantly influence regional carbon balance (Bensoussan & Gattuso, 2007), very few data are available on calcification rates in articulated coralline algae *in situ* (El Haïkali *et al.*, 2004; Bensoussan & Gattuso, 2007; Fisher & Martone, 2014) and none considered the seasonal response of this process.

Irradiance had a positive effect on calcification both in winter and in summer. These findings are in agreement with previous studies revealing calcification in coralline algae to be greater in light than in dark (Borowitzka, 1981; Gao *et al.*, 1993).

In winter, G_d was below zero indicating a net dissolution of calcium carbonate. Dissolution of calcium carbonate at night in winter has previously been observed within a shallow infralittoral ecosystem dominated by *E. elongata* in the Mediterranian (Bensoussan & Gattuso, 2007). Borowitzka (1981) suggested a positive relationship between Ω_{CaCO3} of the surrounding seawater and calcification rates in dark in coralline algae. In this study, the seawater within the incubation chambers was supersaturated with respect to aragonite throughout all incubations and, based on calculations by Ries (2011), is unlikely to have become undersaturated with respect to the high-Mg calcite produced by the algae (Mg/Ca mol % ≈ 17-19; Egilsdottir *et al*. 2013). However, chemical properties of seawater in micro-environments within the diffusion boundary layer differ from the surrounding seawater as a result of algal metabolism (De Beer & Larkum, 2001; Roberts *et al.*, 2002; Cornwall *et al.*, 2014). Thus, it is conceivable that the dissolution observed in dark in winter is indicative of undersaturation of the high-Mg calcite within the diffusion boundary layer as a result of respiration. G_d in summer was significantly different from G_d in winter but was about two-times lower than previously described for the *E. elongata*, 2.5 μmol $CaCO_3$ g^{-1} DW h^{-1} (El Haïkali *et al.*, 2004). However, El Haïkali *et al.* (2004) attributed this reletively high calcification rate in dark to “residual light metabolism” which has also been suggested for high calcification rates in dark measured in other coralline algae (Chisholm, 2000; Martin *et al.*, 2006). Similarly, the seasonal difference in G_d observed in this study may result from different rates of "residual light metabolism", affected by temperature and irradiance prior to and during incubations and/or reflect different Ω_{CaCO3} between seasons.

The net calcification rate, G_n estimated for ambient irradiance around midday was three-fold higher in summer relative to winter and G^{max} was twofold higher in summer relative to winter. G^{max} in summer was consistent with the mean calcification of *E. elongata* (9 µmol $CaCO_3$ g^{-1} DW h^{-1}) measured under higher irradiances in the Mediterranean (El Haïkali *et al.*, 2004).

In agreement with previous studies (see Borowitzka, 1989 and references therein), a relationship between irradiance and calcification was observed both in summer and in winter. Borowitzka and Larkum (1976) suggested that photosynthetic rates and calcification rates were directly related in calcifying algae and an association there between has also been reported by other studies (Pentecost, 1978; Martin *et al.*, 2006; Martin *et al.*, 2013a; Martin *et al.*, 2013b).

Above approximately 200 µmol photons m^{-2} s^{-1} in summer, calcification rates indicate a stronger inhibition than observed for photosynthetic rates. As with photosynthetic rates, inhibition of calcification processes under high irradiances likely reflects a local acclimation of *E. elongata* to a shaded environment. Calcification inhibition under high irradiances (up to 800-1000 µmol photons m^{-2} s^{-1}) was not observed for 'light-acclimated' tropical coralline algae such as *Hydrolithon onkodes*, *Neogoniolithon brassica-florida*, *Hydrolithon reinboldii* and *Neogoniolithon conicum* (Chisholm, 2000) and other species such as the temperate one *L. corallioides* living in shallow waters (1-8 m depth) (Martin *et al.*, 2006). Conversely, inhibition of calcification was reported under high irradiance levels for *L. cabiochae* living in dim-light conditions ($\leq$ 35 µmol photons m^{-2} s^{-1}; Martin *et al.*, 2013b).

Photosynthesis promotes calcification through two major ways: by elevation of Ω_{CaCO3} and by production of organic tissue or compounds. Photosynthesis (and respiration) regulate pH and Ω_{CaCO3} within the diffusion boundary layer and at the calcification site via uptake (and production) of CO_2 (Smith & Roth, 1979; Gao *et al.*, 1993; Hurd *et al.*, 2011). As reviewed by Borowitzka (1989), a certain degree of Ω_{CaCO3} is required to induce nucleation and for the growth of the algal calcium carbonate crystals. Ω_{CaCO3} may also be relevant for structure, morphology and size of the skeleton. Nucleation and calcification can also be induced by photosynthesis via production of a fibrous organic matrix in the cell walls that is required for forming calcite crystals (Borowitzka, 1989). In coralline algae the organic matrix produced also affects the crystal polymorph, i.e. induces the formation of calcite instead of aragonite (Borowitzka, 1989). The enzyme carbonic anhydrase may be involved in the interplay between photosynthesis and calcification as originally suggested by Digby (1977) and later by Hofmann *et al.* (2012) and Martin *et al.* (2013b), by catalyzing the conversion of CO_2 into HCO_3^- and then CO_3^{2-}. Indeed, the presence of carbonic anhydrase has been reported in *E. elongata* (Mercado *et al.*, 1997). However, carbonic anhydrase is also used in photosynthesis to convert HCO_3^- to CO_2 which could then lower Ω_{CaCO3}. Therefore, it is conceivable that calcification may be reduced at the same time as photosynthetic rates are high.

Results presented here for *E. elongata* suggest a direct relationship between photosynthetic production and calcification. However this relationship was found to be limited to low irradiance levels since calcification and photosynthetic processes seem to evolve differently under high irradiances. In summer, calcification rates varied greatly at high irradiances. This could reflect different acclimations to irradiance in the selected algae, resulting from algae inhabiting distinct localities in the rock pool in terms of irradiance regimes (Häder *et al.*, 1997). Anatomical differences between algae may also explain variable responses. For example, Borowitzka (1979) demonstrated that the uppermost segment calcified at a much higher rate than the segments below and that they also had a much higher carbon fixation rate into organic material than the lower segments. In addition, Borowitzka and Vesk (1979) observed more chloroplasts in young segments compared to older segments. Furthermore, it is conceivable that the equation used to convert wet weight of algae into dry weight may have contributed to the variability observed in the data.

Calcification rates in coralline algae are affected by temperature and irradiance, which both exhibit strong diurnal fluctuations in intertidal rock pools. Temperature is positively correlated with Ω_{CaCO3}, potentially explaining how elevated temperature may promote calcification. This is perhaps especially relevant for dark calcification in the absence of photosynthetic metabolism when the relative importance of abiotic environmental factors increases. Temperature also directly affects diffusion rates and metabolic processes that may play a role in calcification (Hurd *et al.*, 2014). For example, temperature affects carbonic anhydrase activity and active transport of HCO_3^- and CO_2, both of which are also important for photosynthesis (Hurd *et al.*, 2014). As discussed above, much of the observed response in calcification may be directly associated with photosynthesis and respiration conducted by the algae themselves. In addition, the biological metabolism of all the species residing in a tidal pool can influence the chemical properties of the seawater and subsequently affect physiological processes in coralline algae inhabiting the pool.

Since calcifying algae in intertidal rock pools experience large fluctuations in the physico-chemical environment, it has been suggested that they have physiological advantages as ocean acidification and warming advances (Egilsdottir *et al.*, 2013). However, ocean acidification and global warming may affect organisms synergistically in the future. For example, the species investigated in this study inhabited a low-lit tidal pool and respiration increased significantly over the temperature range observed in the study. Thus, the minimum pH and Ω_{CaCO3} in rock pools might be lowered in the future as a result of increased temperatures leading to elevated respiration rates, but this effect may be somewhat offset due to the positive correlation between temperature and Ω_{CaCO3}. Coralline algae inhabiting low light environments may be especially at risk by a rise in temperature since higher irradiance levels could be required to accomplish a sustainable net photosynthetic production in the algae.

Conclusions

Data presented in this study provides valuable information on respiration, photosynthesis and calcification in a coralline alga from an intertidal rock pool and their seasonal differences. This information is relevant in developing an understanding of how climate change will affect coralline algae in the future. This study also highlights the importance of considering different light regimes when studying the response of coralline algae to environmental change. Certainly, the relationship between photosynthesis and calcification metabolism is dynamic and care must be taken when assuming a direct relationship there between.

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(Figure captions)

Fig. 1. *R*elationship between irradiance and *Ellisolandia elongata* net primary production (in O_2 and CO_2 fluxes) in (a) summer and (b) winter

Fig. 2. *R*elationship between irradiance and calcification rates in *Ellisolandia elongata* in (a) summer and (b) winter

References

Adey, W.H. (1998). Coral reefs: algal structured and mediated ecosystems in shallow, turbulent, alkaline waters. *Journal of Phycology,* **34**: 393-406.

Adey, W.H. & Macintyre, I.G. (1973). Crustose coralline algae: A re-evaluation in the geological sciences. *Geological Society of America Bulletin,* **84**: 883-904.

Algarra, P., Viña, G.D.L., & Neill, J. (1991). Effects of light quality and irradiance level interactions on short-term pigment responce of the red alga *Corallina elongata*. *Marine Ecology Progress Series,* **74**: 27-32.

Andersson, A., Mackenzie, F., & Bates, N. (2008). Life on the margin: implications of ocean acidification on Mg-calcite, high latitude and cold-water marine calcifiers. *Marine Ecology Progress Series,* **373**: 265-273.

Basso, D. (2012). Carbonate production by calcareous red algae and global change. *Geodiversitas,* **34**: 13-33.

Benedetti-Cecchi, L. (2006). Understanding the consequences of changing biodiversity on rocky shores: How much have we learned from past experiments? *Journal of Experimental Marine Biology and Ecology,* **338**: 193-204.

Bensoussan, N. & Gattuso, J.-P. (2007). Community primary production and calcification in a NW Mediterranean ecosystem dominated by calcareous macroalgae. *Marine Ecology Progress Series,* **334**: 37-45.

Björk, M., Axelsson, L., & Beer, S. (2004). Why is *Ulva intestinalis* the only macroalga inhabiting isolated rockpools along the Swedish Atlantic coast? *Marine Ecology Progress Series,* **284**: 109-116.

Borowitzka, M.A. (1979). Calcium exchange and the measurement of calcification rates in the calcareous coralline red alga *Amphiroa foliacea*. *Marine Biology,* **50**: 339-347.

Borowitzka, M.A. (1981). Photosynthesis and calcification in the articulated coralline red algae *Amphiroa anceps* and *A. foliacea*. *Marine Biology,* **62**: 17-23.

Borowitzka, M.A. (1982). Mechanisms in algal calcification. *Progress in Phycological Research,* **1**: 137-178.

Borowitzka, M.A. (1989). Carbonate Calcification in Algae - Inhibition and Control. In *Biomineralization: Chemical and Biochemical Perspectives* (Mann, S., Webb, J., & Williams, R.J.P., editors), 65-94. VCH Verlagsgesellschaft, Germany.

Borowitzka, M.A. & Larkum, A.W.D. (1976). Calcification in the green alga Halimeda III. The sources of inorganic carbon for photosynthesis and calcification and a model of the mechanism of calcification. *Journal of Experimental Botany,* **27**: 879-893.

Borowitzka, M.A. & Larkum, A.W.D. (1987). Calcification in algae: Mechanisms and the role of metabolism. *Critical Reviews in Plant Sciences,* **6**: 1-45.

Borowitzka, M.A. & Vesk, M. (1979). Ultrastructure of the Corallinaceae (Rhodophyta) II. vegetative cells of *Lithothrix aspergillum*. *Journal of Phycology,* **15**: 146-153.

Burdett, H., Keddie, V., Macarthur, N., Mcdowall, L., Mcleish, J., Spielvogel, E., Hatton, A., & Kamenos, N. (2014). Dynamic photoinhibition exhibited by red coralline algae in the red sea. *BMC Plant Biology,* **14**: 139.

Chave, K.E. (1954). Aspects of the Biogeochemistry of Magnesium 1. Calcareous Marine Organisms. *The Journal of Geology,* **62**: 266-283.

Chisholm, J.R.M. (2000). Calcification by crustose coralline algae on the northern Great Barrier Reef, Australia. *Limnol. Oceanogr.,* **45**: 1476-1484.

Chisholm, J.R.M. & Gattuso, J.P. (1991). Validation of the Alkalinity Anomaly Technique for investigating calcification and photosynthesis in coral-reef communities. *Limnol. Oceanogr.,* **36**: 1232-1239.

Cornwall, C.E., Boyd, P.W., Mcgraw, C.M., Hepburn, C.D., Pilditch, C.A., Morris, J.N., Smith, A.M., & Hurd, C.L. (2014). Diffusion boundary layers ameliorate the negative effects of ocean acidification on the temperate coralline macroalga *Arthrocardia corymbosa*. *PLoS ONE,* **9**: e97235.

Dayton, P.K. (1972). Toward an understanding of community resilience and potential effects of enrichments to the benthos at McMurdo Sound, Antartica *Antartica Proceedings of the Colloquium Conservation Problems in Antartica*: 81-96.

De Beer, D. & Larkum, A.W.D. (2001). Photosynthesis and calcification in the calcifying algae *Halimeda discoidea* studied with microsensors. *Plant, Cell & Environment,* **24**: 1209-1217.

Dickson, A.G. & Millero, F.J. (1987). A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Sea Research,* **34**: 1733-1743.

Dickson, A.G., Sabine, C.L., & Christian, J.R. (2007). *Guide to best practices for ocean* CO_2 *measurements*. PICES Special Publication 3, 191 pp.

Digby, P.S.B. (1977). Photosynthesis and respiration in the coralline algae, Clathromorphum circumscriptum and Corallina officinalis and the metabolic basis of calcification. *Journal of the Marine Biological Association of the United Kingdom,* **57**: 1111-1124.

Egilsdottir, H., Noisette, F., Noël, L., Olafsson, J., & Martin, S. (2013). Effects of pCO_2 on physiology and skeletal mineralogy in a tidal pool coralline alga *Corallina elongata*. *Marine Biology,* **160**: 2103-2112.

El Haïkali, B., Bensoussan, N., Romano, J.-C., & Bousquet, V. (2004). Estimation of photosynthesis and calcification rates of *Corallina elongata* Ellis and Solander, 1786, by measurements of dissolved oxygen, pH and total alkalinity. *Scientia Marina,* **68**: 45-56.

Fisher, K. & Martone, P.T. (2014). Field study of growth and calcification rates of three species of articulated coralline algae in British Columbia, Canada. *The Biological Bulletin,* **226**: 121-130.

Gao, K., Aruga, Y., Asada, K., Ishihara, T., Akano, T., & Kiyohara, M. (1993). Calcification in the articulated coralline alga *Corallina pilulifera*, with special reference to the effect of elevated CO_2 concentration. *Marine Biology,* **117**: 129-132.

Häder, D.-P., Lebert, M., Flores-Moya, A., Jiménez, C., Mercado, J., Salles, S., Aguilera, J., & Figuero, F.L. (1997). Effects of solar radiation on the photosynthetic activity of the red alga *Corallina elongata* Ellis et Soland. *Journal of Photochemistry and Photobiology B: Biology,* **37**: 196-202.

Häder, D.-P., Lebert, M., & Helbling, E.W. (2003). Effects of Solar Radiation on the Patagonian Rhodophyte *Corallina officinatis* (L.). *Photosynthesis Research,* **78**: 119-132.

Hofmann, G.E., Evans, T.G., Kelly, M.W., Padilla-Gamiño, J.L., Blanchette, C.A., Washburn, L., Chan, F., Mcmanus, M.A., Menge, B.A., Gaylord, B., Hill, T.M., Sanford, E., Lavigne, M., Rose, J.M., Kapsenberg, L., & Dutton, J.M. (2014). Exploring local adaptation and the ocean acidification seascape-studies in the California Current Large Marine Ecosystem. *Biogeosciences,* **11**: 1053-1064.

Hofmann, L.C., Yildiz, G., Hanelt, D., & Bischof, K. (2012). Physiological responses of the calcifying rhodophyte, *Corallina officinalis* (L.), to future CO_2 levels. *Marine Biology,* **159**: 783-792.

Huggett, J. & Griffiths, C.L. (1986). Some relationships between elevation, physico-chemical variables and biota of the intertidal rock pools. *Marine Ecology Progress Series,* **29**: 189-197.

Hurd, C.L., Cornwall, C.E., Currie, K., Hepburn, C.D., Mcgraw, C.M., Hunter, K.A., & Boyd, P.W. (2011). Metabolically-induced pH fluctuations by some coastal calcifiers exceed projected 22nd century ocean acidification: a mechanism for differential susceptibility? *Global Change Biology,* **17**: 3254-3262.

Hurd, C.L., Harrison, P.J., Bischof, K., & Lobban, C.S. (2014). *Seaweed ecology and physiology (second edition)*. Cambridge University Press, United Kingdom.

Ichiki, S., Mizuta, H., Yasui, H., & Yamamoto, H. (2001). Effect of irradiance and water temperature on the photosynthesis and growth of the crustose coralline alga *Lithophyllum yessoense* Foslie (Corallinales, Rhodophyceae). *Bulletin of Fisheries Sciences, Hokkaido University,* **52**: 103-109.

Ipcc (2013). *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Johansen, H.W. (1981). *Coralline Algae, A First Synthesis*. CRC Press.

Kain, J.M. (1987). Seasonal growth and photoinhibition in *Plocamium cartilagineum* (Rhodophyta) off the Isle of Man. *Phycologia,* **26**: 88-99.

Kelaher, B.P., Chapman, M.G., & Underwood, A.J. (2001). Spatial patterns of diverse macrofaunal assemblages in coralline turf and their associations with environmental variables. *Journal of the Marine Biological Association of the United Kingdom,* **81**: 917-930.

Kelly, M.W. & Hofmann, G.E. (2013). Adaptation and the physiology of ocean acidification. *Functional Ecology,* **27**: 980-990.

Korbee, N., Navarro, N.P., García-Sánchez, M., Celis-Plá, P.S.M., Quintano, E., Copertino, M.S., Pedersen, A., Mariath, R., Mangaiyarkarasi, N., Pérez-Ruzafa, Á., Figueroa, F.L., & Martínez, B. (2014). A novel in situ system to evaluate the effect of high CO_2 on photosynthesis and biochemistry of seaweeds. *Aquatic Biology,* **22**: 245-259.

Kroeker, K.J., Kordas, R.L., Crim, R., Hendriks, I.E., Ramajo, L., Singh, G.S., Duarte, C.M., & Gattuso, J.-P. (2013). Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biology,* **19**: 1884-1896.

Kühl, M., Glud, R.N., Borum, J., Roberts, R., & Rysgaard, S. (2001). Photosynthetic performance of surface-associated algae below sea ice as measured with a pulse-amplitude-modulated (PAM) fluorometer and O~ 2 microsensors. *Marine Ecology Progress Series,* **223**: 1-14.

Lewis, E. & Wallace, D. (1998). *Program Developed for CO_2 System Calculations*. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory.

Lüning, K. (1990). *Seaweeds: Their Environment, Biogeography, and Ecophysiology*. John Wiley & Sons, Inc., Cambridge.

Martin, S., Castets, M.-D., & Clavier, J. (2006). Primary production, respiration and calcification of the temperate free-living coralline alga *Lithothamnion corallioides*. *Aquatic Botany,* **85**: 121-128.

Martin, S., Charnoz, A., & Gattuso, J.-P. (2013a). Photosynthesis, respiration and calcification in the Mediterranean crustose coralline alga *Lithophyllum cabiochae* (Corallinales, Rhodophyta). *European Journal of Phycology,* **48**: 163-172.

Martin, S., Clavier, J., Chauvaud, L., & Thouzeau, G. (2007). Community metabolism in temperate maerl beds. I. Carbon and carbonate fluxes. *Marine Ecology Progress Series,* **335**: 19-29.

Martin, S., Cohu, S., Vignot, C., Zimmerman, G., & Gattuso, J.-P. (2013b). One-year experiment on the physiological response of the Mediterranean crustose coralline alga, *Lithophyllum cabiochae*, to elevated pCO_2 and temperature. *Ecology and Evolution,* **3**: 676-693.

Martone, P.T. & Denny, M.W. (2008). To bend a coralline: effect of joint morphology on flexibility and stress amplification in an articulated calcified seaweed. *Journal of Experimental Biology,* **211**: 3421-3432.

Mehrbach, C., Culberson, C.H., Hawley, J.E., & Pytkowicz, R.M. (1973). Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnol. Oceanogr.,* **18**: 897-907.

Mercado, J.M., Figueroa, F.L., Xauier Niell, F., & Axelsson, L. (1997). A new method for estimating external carbonic anhydrase activity in macroalgae. *Journal of Phycology,* **33**: 999-1006.

Middelboe, A.L. & Hansen, J.P. (2007). Direct effects of pH and inorganic carbon on macroalgal photosynthesis and growth. *Marine Biology Research,* **3**: 134-144.

Morris, S. & Taylor, A.C. (1983). Diurnal and seasonal variation in physico-chemical conditions within intertidal rock pools. *Estuarine, Coastal and Shelf Science,* **17**: 339-355.

Morse, J.W., Andersson, A.J., & Mackenzie, F.T. (2006). Initial responses of carbonate-rich shelf sediments to rising atmospheric pCO_2 and "ocean acidification": Role of high Mg-calcites. *Geochimica Et Cosmochimica Acta,* **70**: 5814-5830.

Nelson, W.A. (2009). Calcified macroalgae - critical to coastal ecosystems and vulnerable to change: a review. *Marine and Freshwater Research,* **60**: 787-801.

Noël, L.M.L.J., Hawkins, S.J., Jenkins, S.R., & Thompson, R.C. (2009). Grazing dynamics in intertidal rockpools: Connectivity of microhabitats. *Journal of Experimental Marine Biology and Ecology,* **370**: 9-17.

Noisette, F., Egilsdottir, H., Davoult, D., & Martin, S. (2013). Physiological responses of three temperate coralline algae from contrasting habitats to near-future ocean acidification. *Journal of Experimental Marine Biology and Ecology,* **448**: 179-187.

Payri, C.E., Maritorena, S., Bizeau, C., & Rodière, M. (2001). Photoacclimation in the tropical coralline alga *Hydrolithon onkodes* (Rhodophyta, Corallinaceae) from a Frence Polynesian reef. *Journal of Phycology,* **37**: 223-234.

Pentecost, A. (1978). Calcification and photosynthesis in *Corallina officinalis* L. using the $^{14}CO_2$ method. *British Phycological Journal,* **13**: 383 - 390.

Pfister, C. & Hay, M. (1988). Associational plant refuges: convergent patterns in marine and terrestrial communities result from differing mechanisms. *Oecologia,* **77**: 118-129.

Platt, T., Gallegos, C.L., & Harrison, W.G. (1980). Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *Journal of Marine Research,* **38**: 687-701.

Raghavendra, A.S., Padmasree, K., & Saradadevi, K. (1994). Interdependence of photosynthesis and respiration in plant cells: interactions between chloroplasts and mitochondria. *Plant Science,* **97**: 1-14.

Rahman, M.A. & Halfar, J. (2014). First evidence of chitin in calcified coralline algae: new insights into the calcification process of *Clathromorphum compactum*. *Scientific Reports,* **4**.

Raven, J.A. (2011). Effects on marine algae of changed seawater chemistry with increasing atmospheric CO_2. *Biology and Environment: Proceedings of the Royal Irish Academy,* **111B**: 1-17.

Raven, J.A., Giordano, M., Beardall, J., & Maberly, S.C. (2012). Algal evolution in relation to atmospheric CO_2: carboxylases, carbon-concentrating mechanisms and carbon oxidation cycles. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **367**: 493-507.

Ries, J.B. (2011). Skeletal mineralogy in a high-CO_2 world. *Journal of Experimental Marine Biology and Ecology,* **403**: 54-64.

Roberts, R.D., Kühl, M., Glud, R.N., & Rysgaard, S. (2002). Primary production of crustose coralline red algae in a high Arctic fjord. *Journal of Phycology,* **38**: 273-283.

Sagert, S., Forster, R.M., Feuerpfeil, P., & Schubert, H. (1997). Daily course of photosynthesis and photoinhibition in *Chondrus crispus* (Rhodophyta) from different shore levels. *European Journal of Phycology,* **32**: 363-371.

Smith, A.D. & Roth, A.A. (1979). Effect of carbon dioxide concentration on calcification in the red coralline alga *Bossiella orbigniana*. *Marine Biology,* **52**: 217-225.

Smith, S.V. & Key, G.S. (1975). Carbon-Dioxide and Metabolism in Marine Environments. *Limnol. Oceanogr.*, **20**: 493-495.

Steneck, R.S. (1986). The Ecology of Coralline Algal Crusts: Convergent Patterns and Adaptive Strategies. *Annual Reviewof Ecology and Systematics,* **17**: 273-303.

Zeebe, R.E. & Wolf-Gladrow, D.A. (2001). *CO_2 in seawater: equilibriums, kinetics, isotopes*. Elsevier Oceanography Series.

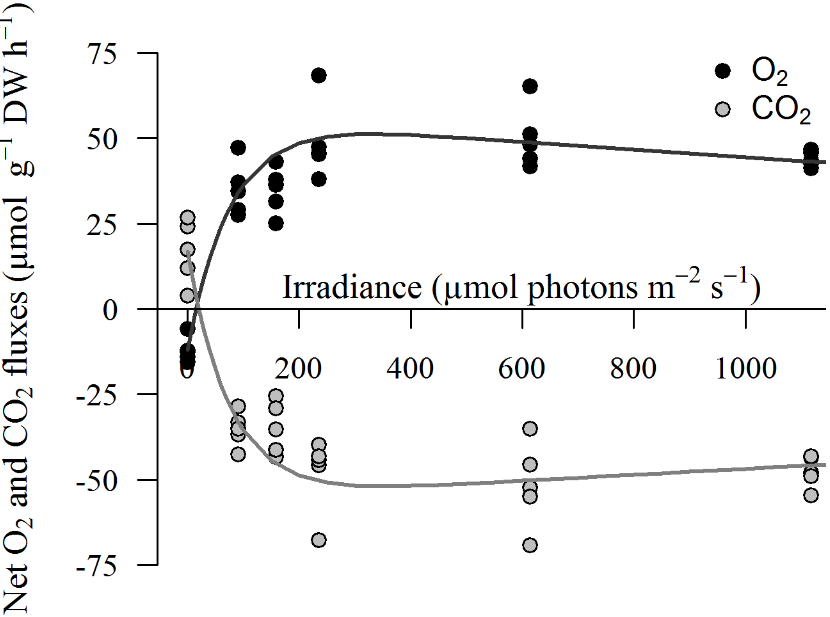
Net O_2 and CO_2 fluxes (µmol g^{-1} DW h^{-1})
75
50
25
0
-25
-50
-75
O_2
CO_2
Irradiance (µmol photons m^{-2} s^{-1})
0
200
400
600
800
1000

Figure 1a

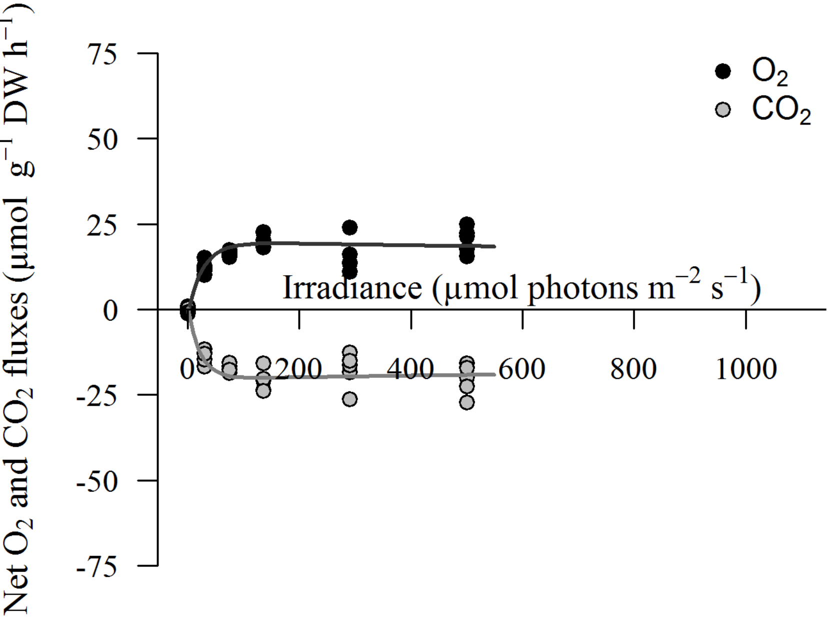
Net O_2 and CO_2 fluxes (µmol g^{-1} DW h^{-1})
75
50
25
0
-25
-50
-75
Irradiance (µmol photons m^{-2} s^{-1})
0
200
400
600
800
1000
● O_2
● CO_2

Figure 1b

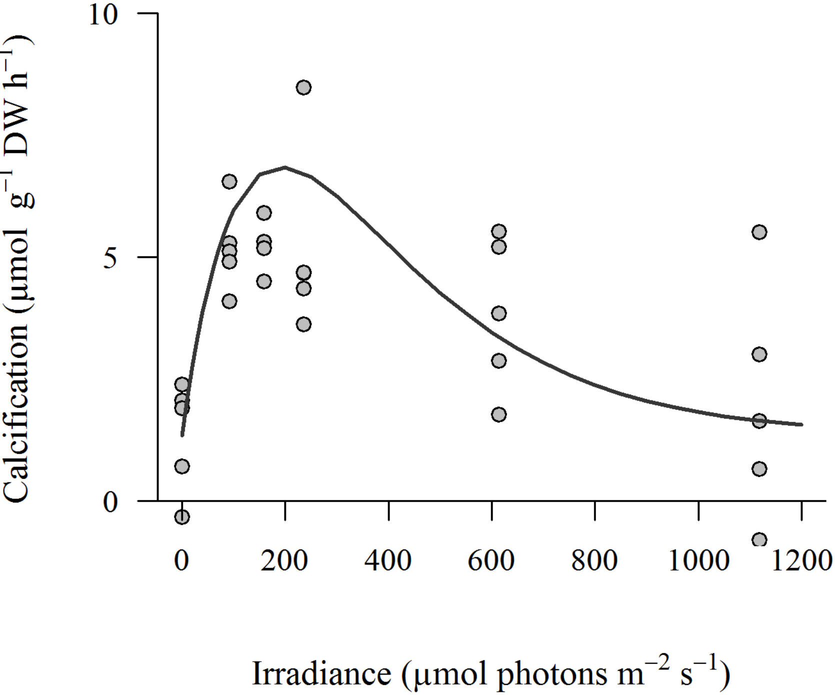
10
5
0
Calcification (µmol g^{-1} DW h^{-1})
0
200
400
600
800
1000
1200
Irradiance (µmol photons m^{-2} s^{-1})

Figure 2a

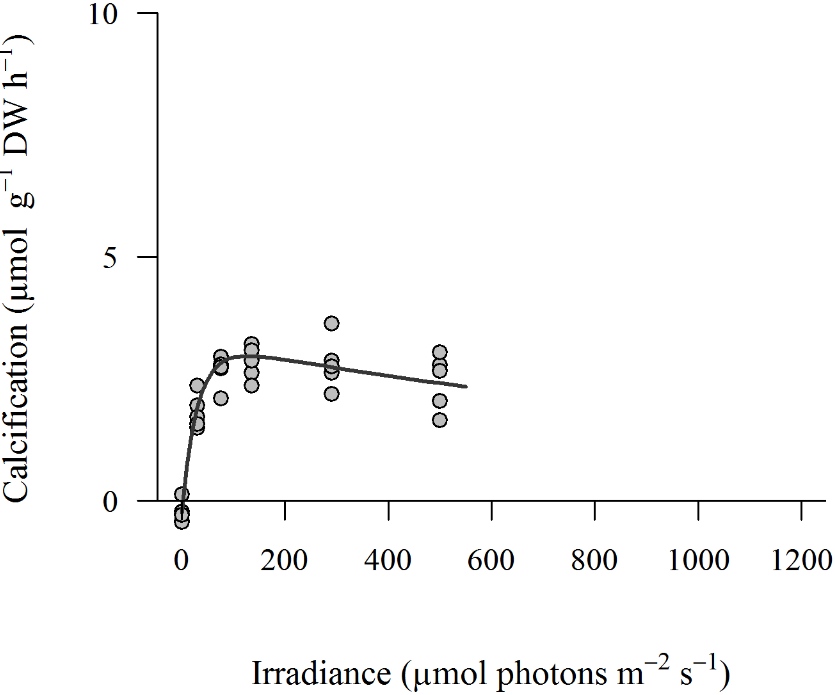
10
5
0
Calcification (µmol g^{-1} DW h^{-1})
0
200
400
600
800
1000
1200
Irradiance (µmol photons m^{-2} s^{-1})

Figure 2b