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# Photosynthesis and calcification in the articulated coralline alga *Ellisolandia elongata* (Corallinales, Rhodophyta) from intertidal rock pools

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► **To cite this version:**

Hronn Egilsdottir, Jon Olafsson, Sophie Martin. Photosynthesis and calcification in the articulated coralline alga *Ellisolandia elongata* (Corallinales, Rhodophyta) from intertidal rock pools. *European Journal of Phycology*, 2015, 51 (1), pp.59-70. 10.1080/09670262.2015.1101165 . hal-01281671

**HAL Id: hal-01281671**

**<https://hal.sorbonne-universite.fr/hal-01281671>**

Submitted on 2 Mar 2016

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1 **Title:** Photosynthesis and calcification in the articulated coralline alga *Ellisolandia elongata*  
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3

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13

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

15

16

17 **Abstract**

18

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

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

40

41

42 **Introduction**

43

44 Climate change and ocean acidification are advancing as a result of anthropogenic  
45 increase in carbon dioxide concentrations in the Earth's atmosphere (IPCC, 2013).  
46 Investigating organism physiology in relation to environmental variability provides useful  
47 information for predicting acclimation and adaptation to future conditions (Kelly &  
48 Hofmann, 2013). Calcifying benthic algae are generally considered highly sensitive to  
49 climate change and ocean acidification (Kroeker *et al.*, 2013) but many species are also  
50 common in habitats where pH and temperature gradients are steep. Among calcifying  
51 benthic algae, red coralline algae (Corallinales, Rhodophyta) are the most important taxa in  
52 terms of global carbon cycling and ecosystem engineering (Nelson, 2009). They can be  
53 found over a wide latitudinal range and within all depths of the photic zone (Adey &  
54 Macintyre, 1973; Steneck, 1986) where they are known to provide ecosystem services such  
55 as by sheltering high biodiversity and favour larval recruitment and settlement (Adey,  
56 1998). Coralline algae are also of importance with respect to the carbon and carbonate cycle  
57 in shallow coastal waters through high community calcium carbonate production (Martin *et*  
58 *al.*, 2007). Articulated coralline algae (also referred to as geniculate coralline algae) have  
59 non-calcified segments between calcified intergenicula that provide flexibility to the erect  
60 algal thallus (Martone & Denny, 2008). They have been reported to support diverse  
61 assemblages of macrofauna and are important providers of three dimensional frameworks  
62 (Kelaher *et al.*, 2001), being considered as ecosystem engineers (Dayton, 1972; Benedetti-  
63 Cecchi, 2006; Noël *et al.*, 2009). Their unpalatable calcareous structure acts as a barrier  
64 against grazing, thus protecting diverse algal epiphyte assemblages (Pfister & Hay, 1988).

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

81 The equilibrium between inorganic carbon species in an aquatic system is an important  
82 environmental factor affecting the processes that underlay  $CaCO_3$  bioprecipitation  
83 (Borowitzka, 1982; Borowitzka & Larkum, 1987; Raven, 2011; Basso, 2012). For example,  
84 supersaturation of calcium carbonate is generally required for calcification to take place in  
85 algae (Borowitzka, 1989). The calcium carbonate saturation state ( $\Omega_{CaCO_3}$ ) in seawater and  
86 at the calcification site is affected by photosynthesis and respiration through the removal or  
87 addition of  $CO_2$  from or to the extracellular environment (Borowitzka, 1989). In this way,  
88 calcification is induced by photosynthesis as is evident by the strong relationship between

89 the metabolic rates of these processes in all calcifying algae (Borowitzka & Larkum, 1976;  
90 Pentecost, 1978; Borowitzka, 1989; Martin *et al.*, 2013a).

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

108 Articulated coralline algae frequently inhabit the intertidal environment and in particular  
109 tidal pools (Johansen, 1981). Tidal pools are habitats where water exchange is limited or  
110 temporally non-existent as a result of local tidal activity (i.e. amplitude and tidal cycles) and  
111 pool locality (i.e. high or low within the intertidal zone) (Morris & Taylor, 1983). Because  
112 of biological processes and physico-chemical fluxes, the carbonate chemistry within a tidal

113 pool may exhibit great periodical variations which is generally reflected by large pH  
114 fluctuations (Morris & Taylor, 1983; Huggett & Griffiths, 1986).

115 *Ellisolandia elongata* (previously known as *Corallina elongata*) inhabits intertidal rock  
116 pools where large fluctuations in physico-chemical variables occur diurnally (Egilsdottir *et*  
117 *al.*, 2013). It has been suggested that organisms inhabiting environments where large  
118 fluctuations in CO<sub>2</sub>/pH and temperature are common will have a relatively good capacity to  
119 acclimate or adapt to future ocean acidification and warming (Kelly & Hofmann, 2013;  
120 Hofmann *et al.*, 2014). Indeed, studies have indicated a greater tolerance to ocean  
121 acidification in *E. elongata* previously inhabiting a rock-pool environment when compared  
122 to other coralline algae whose distribution is limited to more stable environments  
123 (Egilsdottir *et al.*, 2013; Noisette *et al.*, 2013).

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

132

## 133 **Material and Methods**

134

### 135 *1. Collection of algae*

136

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

152

## 153 *2. Incubation procedure and physiological measurements*

154

155 *E. elongata* were incubated under natural sunlight from 13:00 to 16:00. In summer, algae  
156 were transferred directly to incubation chambers and incubated *in situ* in a large  
157 neighbouring pool exposed to direct sunlight. In winter the algae were transported to the  
158 *Roscoff Marine Station* in a thermostatic tank within 1 h of collection and incubated outside  
159 under natural sunlight in large baths filled with natural seawater pumped at 10 m depth in  
160 front of the station at ambient temperature. Temperature during incubation varied from 17  
161 to 24°C in summer and from 6 to 9°C in winter (Table 2), being representative of changes in



162 the pool during emersion at these seasons (Table 1). Five sets of individual algae ( $N = 5$ )  
163 were incubated per irradiance level in 220 and 460 ml clear and black glass bottles.  
164 Incubations at all irradiance levels were performed simultaneously in each season for 1 to 3  
165 hours in summer and 2 to 4 hours in winter, depending on the expected algal metabolic  
166 response. Mean DW of the sets of algae was  $0.41 \pm \text{SE } 0.03$  g in summer and  $0.81 \pm \text{SE}$   
167  $0.05$  g in winter. Black plastic covers were used to assess respiration and calcification rates  
168 in the dark. Four neutral density filters were used to assess net production and calcification  
169 in algae simultaneously exposed to 5 different mean irradiance (PAR) levels (I1, I2, I3, I4,  
170 and I5; Table 2) of 91, 158, 235, 614, and 1118  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  in summer and 30, 75,  
171 135, 290, and 500  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  in winter, with the highest irradiances  
172 corresponding to natural full light. Irradiance was measured with a Li-Cor quantum sensor  
173 (LiCor Li-190SA, LI-COR Biosciences, USA). Reference incubations without algae were  
174 included for full light and dark conditions to assess and correct the metabolism by any  
175 biological activity in the seawater. Oxygen fluxes were estimated by measuring the oxygen  
176 concentration at the beginning and the end of each incubation with an oxygen probe  
177 (Oxymeter HQ40D, Hach Lange, Ltd portable LDO<sup>TM</sup>, Loveland USA). pH was measured  
178 using a pH meter (HQ40D, Hach Lange, Ltd portable LDO<sup>TM</sup>, Loveland USA) calibrated on  
179 the total scale ( $\text{pH}_T$ , expressed on the total hydrogen ion concentration scale) using Tris/HCl  
180 and 2-aminopyridine/HCl buffer solutions with a salinity of 35 (Dickson *et al.*, 2007). For  
181 measurements of total alkalinity ( $A_T$ ), seawater samples were filtered through 0.7  $\mu\text{m}$   
182 Whatman GF/F filters into 100 mL glass bottles, immediately poisoned with mercuric  
183 chloride and stored in a cool dark place pending analyses.  $A_T$  was determined  
184 potentiometrically using an automatic titrator (Titroline alpha, Schott SI Analytics,  
185 Germany) calibrated on the National Bureau of Standards scale and calculated using a Gran  
186 function applied to pH values ranging from 3.5 to 3.0 (Dickson *et al.*, 2007). Measurements

187 of  $A_T$  were corrected by comparison with standard reference material provided by Andrew  
188 G. Dickson (Batch 111). Other parameters of the carbonate chemistry were calculated from  
189  $pH_T$ ,  $A_T$ , temperature and salinity (35) using the software *CO2Sys*, *EXCEL Macro version*  
190 *2.1* (originally designed by Lewis & Wallace, 1998). Calculations were based on a set of  
191 constants  $K_1$  and  $K_2$  from Mehrbach *et al.* (1973), refit by Dickson and Millero (1987)  
192 (Table 1; Table 2).

193

### 194 *3. Data treatment and statistical analysis*

195

196 Primary production was estimated directly from oxygen fluxes. Net production ( $P_n$ ) and  
197 respiration ( $R$ ) rates ( $\mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ ) were calculated as follows:

$$198 \quad P_n \text{ or } R (\text{O}_2) = \left( \frac{\Delta O_2}{\Delta t \times V} \right) / DW \quad (1)$$

199 where  $\Delta O_2$  is the change in dissolved oxygen ( $\mu\text{mol l}^{-1}$ ),  $\Delta t$  is the incubation time (h),  $DW$  is  
200 the dry weight of the algae and  $V$  is the volume of the incubation chamber (L).

201 The variations of dissolved inorganic carbon (DIC) in the incubations integrate both  
202 organic carbon metabolism (photosynthesis and respiration) and inorganic carbon  
203 metabolism (calcification and dissolution) (Zeebe & Wolf-Gladrow, 2001). Calcification  
204 rates were estimated using the alkalinity anomaly technique (Smith & Key, 1975; Chisholm  
205 & Gattuso, 1991) which is based on the relationship:  $\text{Ca}^{2+} + 2\text{HCO}_3^- \rightarrow \text{CaCO}_3 + \text{CO}_2 +$   
206  $\text{H}_2\text{O}$ , for which total alkalinity decreases by 2 equivalents for each mol of  $\text{CaCO}_3$   
207 precipitated.

208 Calcification rates ( $G$ ,  $\mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ DW h}^{-1}$ ) was calculated as follows:

$$209 \quad G = - \left( \frac{\Delta A_T}{2 \times \Delta t \times V} \right) / DW \quad (2)$$

210 where  $\Delta A_T$  is the change in total alkalinity ( $\mu\text{eq l}^{-1}$ ).

211  $P_n$  and  $R$  expressed in terms of carbon ( $\mu\text{mol CO}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ ) were calculated by the  
 212 difference between the initial and final DIC concentrations (calculated by using the software  
 213 *CO2Sys*) as follows:

$$214 \quad P_n \text{ or } R (\text{CO}_2) = \left( \frac{\Delta\text{DIC} - (0.5 \times \Delta\text{AT})}{\Delta t \times V} \right) / \text{DW} \quad (3)$$

215 where  $\Delta\text{DIC}$  is the change in dissolved inorganic carbon ( $\text{mmol l}^{-1}$ ).

216 The relationship between irradiance ( $E$ ,  $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ ) and  $P_n$  (or  $G$ ) was  
 217 established using  $P_n$  (or  $G$ ) versus  $E$  curves following the function of Platt *et al.* (1980)  
 218 modified by the addition of a respiration ( $R$ ) or dark calcification term ( $G_d$ ,  $\mu\text{mol CaCO}_3 \text{ g}^{-1}$   
 219  $\text{DW h}^{-1}$ ):

$$220 \quad P_n = P_s \times (1 - e^{-\alpha \times \frac{E}{P_s}}) \times e^{-\beta \times \frac{E}{P_s}} + R \quad (4)$$

$$221 \quad G = G_s \times (1 - e^{-\alpha \times \frac{E}{G_s}}) \times e^{-\beta \times \frac{E}{G_s}} + G_d \quad (5)$$

222

223 where  $P_s$  and  $G_s$  are scaling parameters defined as the maximum rate of photosynthesis or  
 224 calcification respectively in the absence of photoinhibition (or decrease in calcification rate  
 225 under high irradiance;  $\mu\text{mol O}_2$ , DIC or  $\text{CaCO}_3 \text{ g}^{-1} \text{ h}^{-1}$ ),  $\alpha$  is the initial slope of the  
 226 relationship ( $\mu\text{mol g}^{-1} \text{ h}^{-1} (\mu\text{mol m}^{-2} \text{ s}^{-1})^{-1}$ ),  $\beta$  is the photoinhibition coefficient ( $\mu\text{mol g}^{-1} \text{ h}^{-1}$   
 227 ( $\mu\text{mol m}^{-2} \text{ s}^{-1})^{-1}$ ). In the absence of evident photoinhibition, the  $\beta$  value was set to zero.

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

$$230 \quad P_g^{\text{max}} \text{ (or } G_g^{\text{max}}) = P_s \text{ (or } G_s) \times \left( \frac{\alpha}{\alpha + \beta} \right) \times \left( \frac{\beta}{\alpha + \beta} \right)^{\frac{\beta}{\alpha}} \quad (6)$$

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

$$232 \quad P_n^{\text{max}} = P_g^{\text{max}} + R \quad (7)$$

233  $G^{max} = G_g^{max} + G_d$  (8)

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

235  $E_k = \frac{P_g^{max}}{\alpha}$  or  $\frac{G_g^{max}}{\alpha}$  (9)

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

238  $E_c = \frac{R}{\alpha}$  (10)

239  $P_n$ - and  $G$ - $E$  curves were fitted to the data using the statistical software STATGRAPHICS  
240 *Plus, version 5.0 for Windows* (StatPoint Technologies Inc., 2013), establishing 5 individual  
241 curves for O<sub>2</sub> flux, CO<sub>2</sub> flux and calcification per season. The statistical software *R 2.15.0*  
242 (The R Foundation for Statistical Computing, 2013) was used when examining differences  
243 between seasons. Normality of data was estimated via the Shapiro-Wilk normality test. The  
244 Welch two sample t-test was used to identify differences between seasons where data was  
245 normally distributed or where log or square-root transformation of data yielded normal  
246 distribution. When normality was not achieved, a Wilcoxon Rank Sum test was used.  
247 Results are expressed as means ± standard error (SE).

248

## 249 **Results**

250

### 251 *Respiration and photosynthesis*

252

253 The mean respiration rate ( $R$ ) was higher in summer than in winter (O<sub>2</sub> fluxes:  $t = -6.63$ ,  $p <$   
254  $0.01$ ; CO<sub>2</sub> fluxes:  $t = 3.97$ ,  $p < 0.05$ ; Table 3). In both seasons primary production measured  
255 under various irradiance levels showed a strong relationship with irradiance in terms of both

256 oxygen production ( $r^2 = 0.88 - 0.98$  in summer;  $r^2 = 0.78 - 0.99$  in winter) and net CO<sub>2</sub>  
257 uptake ( $r^2 = 0.87 - 0.98$  in summer;  $r^2 = 0.87 - 0.98$  in winter; Fig. 1). Photoinhibition was  
258 recorded for primary production at irradiance levels above about 400  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$   
259 in summer and a negligible photoinhibition above about 200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  was  
260 observed in winter (Table 3; Fig. 1). In summer, maximum gross O<sub>2</sub> production and CO<sub>2</sub>  
261 uptake at light saturation ( $P_g^{max}$ ) were about three times those recorded for winter (O<sub>2</sub>  
262 fluxes: W=25,  $p < 0.01$ ; CO<sub>2</sub> fluxes: W=25,  $p < 0.01$ ; Table 2). In winter the maximum net  
263 production ( $P_n^{max}$ ) was about 40% that of summer (O<sub>2</sub> fluxes: W=25,  $p < 0.01$ ; CO<sub>2</sub> fluxes:  
264 W=25,  $p < 0.01$ ; Table 3). The saturating irradiances ( $E_k$ ) for primary production were about  
265 three times higher in summer, relative to winter (O<sub>2</sub> fluxes:  $t = 5.42$ ,  $p < 0.05$ ; CO<sub>2</sub> fluxes:  $t$   
266  $= 5.26$ ,  $p < 0.05$ ; Table 3) and the compensation irradiances ( $E_c$ ) were higher in summer  
267 than in winter (O<sub>2</sub> fluxes:  $t = 4.66$ ,  $p < 0.01$ ; CO<sub>2</sub> fluxes:  $t = 3.32$ ,  $p < 0.05$ ; Table 3).  
268 Coefficient  $\alpha$  (the initial slope of the curve) did not differ between seasons for both O<sub>2</sub> and  
269 CO<sub>2</sub> fluxes. Coefficient  $\beta$  (photoinhibition coefficient) did not differ between seasons for O<sub>2</sub>  
270 fluxes. No statistical comparison between seasons was conducted for  $\beta$  considering CO<sub>2</sub>  
271 fluxes since photoinhibition was not indicated by all five  $P-E$  curves calculated for each  
272 season. The net production rates under 30  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and 15  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$   
273 (*in situ* midday irradiance in summer and winter respectively) were estimated from the  $P-E$   
274 curves to  $8.4 \pm 3.1 \mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$  and  $-5.6 \pm 4.5 \mu\text{mol C g}^{-1} \text{ DW h}^{-1}$  in summer and  $7.4$   
275  $\pm 1.0 \mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$  and  $-11.3 \pm 1.5 \mu\text{mol C g}^{-1} \text{ DW h}^{-1}$  in winter. Gross production  
276 ( $P_g = P_n + R$ ) was thus estimated to  $20.4 \pm 3.1 \mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$  and  $-22.5 \pm 1.9 \mu\text{mol C}$   
277  $\text{g}^{-1} \text{ DW h}^{-1}$  in summer and  $8.4 \pm 1.0 \mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$  and  $-11.8 \pm 1.6 \mu\text{mol C g}^{-1} \text{ DW h}^{-1}$   
278 in winter.

279

280 *Calcification*

281  
282 Calcification rates in the dark ( $G_d$ ) differed between seasons ( $t = 3.07, p < 0.05$ ; Table 3).  $G_d$   
283 was positive in summer but negative in winter, indicating net dissolution of calcium  
284 carbonate. A strong relationship was observed between irradiance and calcification in  
285 summer ( $r^2 = 0.72 - 1.00$ ; Fig 2a) and winter ( $r^2 = 0.93 - 0.99$ ; Fig. 2b). At high irradiances  
286 in summer the relationship between irradiance and calcification is negligible with a high  
287 variance observed for calcification at high irradiances (Fig 2a). Calcification was inhibited  
288 at irradiances higher than about  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$  in both summer and winter. Maximum net  
289 calcification ( $G_n^{max}$ ) differed between seasons ( $t = 3.07, p < 0.05$ ; Table 3) and was about  
290 two times higher in summer relative to winter.  $E_k$  was approximately two times higher in  
291 summer than in winter ( $t = 2.50, p < .005$ ; Table 3). Since calcification in the dark was  
292 positive in summer, an estimate for  $E_c$  in summer was unavailable and consequently a  
293 statistical comparison between seasons was irrelevant. For calcification, coefficients  $\alpha$  and  $\beta$   
294 did not differ between seasons (Table 3). Calcification at midday *in situ* irradiance  
295 estimated from the  $G-E$  curves was  $3.3 \pm 0.3 \mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ DW h}^{-1}$  in summer at  $30 \mu\text{mol}$   
296  $\text{photons m}^{-2} \text{s}^{-1}$  and  $1.1 \pm \text{SE } 0.1 \mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ DW h}^{-1}$  in winter at  $15 \mu\text{mol photons m}^{-2}$   
297  $\text{s}^{-1}$ .

298

## 299 **Discussion**

300

### 301 *Respiration*

302

303 Respiration rates observed here varied seasonally in terms of both  $\text{O}_2$  and  $\text{CO}_2$  fluxes, being  
304 more than 10-fold higher in summer in comparison to winter. The temperature dependence  
305 of respiration is well known for seaweeds (Hurd *et al.*, 2014) and has already been reported

306 for several species of coralline algae with a trend of increasing respiration with increasing  
307 temperature until temperature reaches beyond the thermal tolerance limit of the alga (see  
308 Martin *et al.*, 2013b for a review). For example, respiration rate in the temperate crustose  
309 coralline alga, *Lithophyllum yessoense* increased by ten-fold, from 3 to 29  $\mu\text{g O}_2 \text{ cm}^{-2} \text{ h}^{-1}$   
310 between 5 and 25°C (Ichiki *et al.*, 2001). Changes in respiration rates observed between  
311 winter and summer in *E. elongata* are high in comparison with the threefold increase  
312 measured in the temperate unattached coralline alga (maerl) *Lithothamnion corallioides*  
313 between 10°C (winter) and 16.5°C (summer) (Martin *et al.*, 2006) or in the temperate  
314 crustose coralline alga *Lithophyllum cabiochae* between 13°C (winter) and 22°C (summer)  
315 (Martin *et al.*, 2013b). The large differences observed between winter and summer in *E.*  
316 *elongata* is mainly due to the high respiration rates recorded in summer. A three- to four-  
317 fold lower respiration rate was recorded for this species at a temperature range of 13 to  
318 17°C in a Mediterranean subtidal environment ( $3 \mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ ; El Haïkali *et al.*,  
319 2004) and at a constant temperature of 18°C in a laboratory ( $4 \mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ ;  
320 Egilsdottir *et al.*, 2013). Temperature was considerably higher in summer in the rock pool at  
321 the time of measurements, reaching 23°C at the end of the incubation explaining the high  
322 summer respiration rates in *E. elongata* observed in this study. Higher respiration rates in  
323 summer in comparison to winter may also reflect high rates of primary production shortly  
324 before dark measurements took place as light-enhanced dark respiration has been observed  
325 in plants and algae (Raghavendra *et al.*, 1994; Martin *et al.*, 2013a). The large seasonal  
326 difference observed for respiration could partly be attributed to the low temperature during  
327 winter measurements (reaching below 6°C). In winter, the algae were carefully transported  
328 to a different location and incubated in water-baths. Therefore, it is conceivable that  
329 different handling and transportation of algae prior to incubations contributed seasonal  
330 differences in respiration and other metabolic activity.

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

337

### 338 *Photosynthesis*

339

340 The photosynthesis of *E. elongata* was significantly influenced by the season. The gross  
341 production,  $P_g$ , under ambient irradiance was two- to three-fold higher in summer than in  
342 winter. These seasonal fluctuations of  $P_g$  are related to the changes in both temperature and  
343 irradiance which are the fundamental parameters in the controlling algal photosynthesis  
344 (Hurd *et al.*, 2014). Photosynthetic characteristics of the  $P$ - $E$  curves reflect this with values  
345 of  $P_g^{max}$  threefold higher in summer than in winter. Similar seasonal influence of  
346 temperature and irradiance on photosynthesis has previously been reported in other  
347 temperate coralline algal species such as *L. corallioides* or *L. cabiochae*, which exhibited  
348 values of  $P_g^{max}$  two- to three-fold higher in summer compared to winter (Martin *et al.*, 2006;  
349 Martin *et al.*, 2013b). A strong seasonal difference was observed in the photosynthetic  
350 parameters  $E_k$  and  $E_c$  with both being considerably lower in the winter season, reflecting a  
351 greater degree of low-light acclimation in winter than in summer.  $E_k$  was 2-3 fold higher in  
352 summer compared to winter and is in agreement with  $E_k$  previously reported for low light  
353 acclimated coralline algae, in the range of 7-32  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Kühl *et al.*, 2001; Payri *et al.*,  
354 2001; Roberts *et al.*, 2002; Martin *et al.*, 2013a).  $E_c$  was more than ten fold higher in  
355 summer than in winter. The value of  $E_c$  in summer is consistent with that previously



356 reported for *E. elongata* acclimated to low irradiance ( $E_c = 20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ; Algarra  
357 et al., 1991), while the value  $E_c$  in winter is similar to that reported for other coralline algae  
358 living in dim light condition such as *L. cabiochae* in winter under very low irradiance ( $< 10$   
359  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ; Martin et al. 2013b). Such low  $E_c$  reflects the ability of *E. elongata* to  
360 make a net gain from photosynthesis at very low light levels.

361 Photoinhibition was observed at high irradiances as has previously been reported for low  
362 light acclimated algae (Kain, 1987; Sagert et al., 1997; Kühl et al., 2001; Roberts et al.,  
363 2002; Martin et al., 2013b). Recently, photoinhibition was also observed in *E. elongata*  
364 under rapid light curves (RLCs) (Korbee et al., 2014) giving further evidence to *E. elongata*  
365 being a “shade plant” which Häder et al. (2003) has suggested characterized all geniculate  
366 coralline algal species. Coralline algae have been reported to exhibit dynamic  
367 photoinhibition strategies which likely enable the algae to tolerate high irradiance levels  
368 rather than undergoing photodamage (Burdett et al., 2014). For example, Burdett et al.  
369 (2014) reported varying reflectance of the thallus at different times of the day, and varying  
370 intracellular concentrations of the antioxidant compound DMSP  
371 (dimethylsulphoniopropionate) in crustose coralline algae.

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

374 Temperature directly affects the biochemistry occurring at the algal cellular level. For  
375 example, temperature may influence the activity of the enzyme carbonic anhydrase, active  
376 transport of  $\text{CO}_2$  and  $\text{HCO}_3^-$  and diffusion rates by which photosynthesis may be affected  
377 through the supply of substrate to carbon fixation pathways (Hurd et al., 2014). In this  
378 study, temperature exhibited similar variability at all irradiance levels for each season but is  
379 likely to contribute to the difference in *P-E* curves between seasons.

380 Irradiance affects seawater in tidal pools through regulation of photosynthesis. As observed  
381 in this study, and is well known for photosynthetic organisms, a positive relationship exists  
382 between photosynthetic rates and irradiance up to a certain irradiance level, i.e. near the  
383 saturating irradiance  $E_k$  (Lüning, 1990). In this way, irradiance influences parameters of the  
384 seawater chemistry in tidal pools, such as carbonate chemistry and nutrient concentrations  
385 (Morris & Taylor, 1983; Hurd *et al.*, 2014). Contrary to respiration, algal photosynthesis  
386 removes  $\text{CO}_2$  from seawater and subsequently leads to an elevation in pH and a decrease in  
387 total inorganic carbon concentrations (Hurd *et al.*, 2014). High pH is known to negatively  
388 affect photosynthesis in some macroalgal species (Borowitzka, 1981; Middelboe & Hansen,  
389 2007). For example, Björk *et al.* (2004) found certain algal species to be absent in tidal  
390 pools where high pH values and low inorganic carbon concentration occurred temporarily.  
391 Effect of pH on photosynthesis in *E. elongata* has only been investigated for a pH range of  
392 7.7 to 8.1 with no significant effects observed (Egilsdottir *et al.*, 2013; Noisette *et al.*, 2013)  
393 but the effects of higher pH levels have not yet been investigated for this species. Like most  
394 macroalgae, coralline algae have  $\text{CO}_2$  concentrating mechanisms (CCMs) that allow the  
395 algae to utilize low concentrations of inorganic carbon (Borowitzka, 1981; Gao *et al.*,  
396 1993). These mechanisms are diverse in their exact functionality but for example promote  
397 active transport of inorganic carbon across membranes and/or may facilitate the conversion  
398 of  $\text{HCO}_3^-$  to  $\text{CO}_2$  for photosynthetic metabolism (Raven *et al.*, 2012). Effective CCMs  
399 explain why some algae, including many species of coralline algae, are commonly found in  
400 rock pools where pH is often elevated and inorganic carbon concentrations low (Raven,  
401 2011).

402

403 *Calcification*

404

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

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

413 In winter,  $G_d$  was below zero indicating a net dissolution of calcium carbonate.  
414 Dissolution of calcium carbonate at night in winter has previously been observed within a  
415 shallow infralittoral ecosystem dominated by *E. elongata* in the Mediterranean (Bensoussan  
416 & Gattuso, 2007). Borowitzka (1981) suggested a positive relationship between  $\Omega_{\text{CaCO}_3}$  of  
417 the surrounding seawater and calcification rates in dark in coralline algae. In this study, the  
418 seawater within the incubation chambers was supersaturated with respect to aragonite  
419 throughout all incubations and, based on calculations by Ries (2011), is unlikely to have  
420 become undersaturated with respect to the high-Mg calcite produced by the algae (Mg/Ca  
421 mol %  $\approx$  17-19; Egilsdottir *et al.* 2013). However, chemical properties of seawater in micro-  
422 environments within the diffusion boundary layer differ from the surrounding seawater as a  
423 result of algal metabolism (De Beer & Larkum, 2001; Roberts *et al.*, 2002; Cornwall *et al.*,  
424 2014). Thus, it is conceivable that the dissolution observed in dark in winter is indicative of  
425 undersaturation of the high-Mg calcite within the diffusion boundary layer as a result of  
426 respiration.  $G_d$  in summer was significantly different from  $G_d$  in winter but was about two-  
427 times lower than previously described for the *E. elongata*,  $2.5 \mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ DW h}^{-1}$  (El  
428 Haïkali *et al.*, 2004). However, El Haïkali *et al.* (2004) attributed this relatively high  
429 calcification rate in dark to “residual light metabolism” which has also been suggested for

430 high calcification rates in dark measured in other coralline algae (Chisholm, 2000; Martin *et*  
431 *al.*, 2006). Similarly, the seasonal difference in  $G_d$  observed in this study may result from  
432 different rates of “residual light metabolism”, affected by temperature and irradiance prior  
433 to and during incubations and/or reflect different  $\Omega_{\text{CaCO}_3}$  between seasons.

434 The net calcification rate,  $G_n$  estimated for ambient irradiance around midday was three-  
435 fold higher in summer relative to winter and  $G^{max}$  was twofold higher in summer relative to  
436 winter.  $G^{max}$  in summer was consistent with the mean calcification of *E. elongata* ( $9 \mu\text{mol}$   
437  $\text{CaCO}_3 \text{ g}^{-1} \text{ DW h}^{-1}$ ) measured under higher irradiances in the Mediterranean (El Haïkali *et*  
438 *al.*, 2004).

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

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

455        Photosynthesis promotes calcification through two major ways: by elevation of  $\Omega_{\text{CaCO}_3}$   
456 and by production of organic tissue or compounds. Photosynthesis (and respiration) regulate  
457 pH and  $\Omega_{\text{CaCO}_3}$  within the diffusion boundary layer and at the calcification site via uptake  
458 (and production) of  $\text{CO}_2$  (Smith & Roth, 1979; Gao *et al.*, 1993; Hurd *et al.*, 2011). As  
459 reviewed by Borowitzka (1989), a certain degree of  $\Omega_{\text{CaCO}_3}$  is required to induce nucleation  
460 and for the growth of the algal calcium carbonate crystals.  $\Omega_{\text{CaCO}_3}$  may also be relevant for  
461 structure, morphology and size of the skeleton. Nucleation and calcification can also be  
462 induced by photosynthesis via production of a fibrous organic matrix in the cell walls that is  
463 required for forming calcite crystals (Borowitzka, 1989). In coralline algae the organic  
464 matrix produced also affects the crystal polymorph, i.e. induces the formation of calcite  
465 instead of aragonite (Borowitzka, 1989). The enzyme carbonic anhydrase may be involved  
466 in the interplay between photosynthesis and calcification as originally suggested by Digby  
467 (1977) and later by Hofmann *et al.* (2012) and Martin *et al.* (2013b), by catalyzing the  
468 conversion of  $\text{CO}_2$  into  $\text{HCO}_3^-$  and then  $\text{CO}_3^{2-}$ . Indeed, the presence of carbonic anhydrase  
469 has been reported in *E. elongata* (Mercado *et al.*, 1997). However, carbonic anhydrase is  
470 also used in photosynthesis to convert  $\text{HCO}_3^-$  to  $\text{CO}_2$  which could then lower  $\Omega_{\text{CaCO}_3}$ .  
471 Therefore, it is conceivable that calcification may be reduced at the same time as  
472 photosynthetic rates are high.

473        Results presented here for *E. elongata* suggest a direct relationship between  
474 photosynthetic production and calcification. However this relationship was found to be  
475 limited to low irradiance levels since calcification and photosynthetic processes seem to  
476 evolve differently under high irradiances. In summer, calcification rates varied greatly at  
477 high irradiances. This could reflect different acclimations to irradiance in the selected algae,  
478 resulting from algae inhabiting distinct localities in the rock pool in terms of irradiance  
479 regimes (Häder *et al.*, 1997). Anatomical differences between algae may also explain

480 variable responses. For example, Borowitzka (1979) demonstrated that the uppermost  
481 segment calcified at a much higher rate than the segments below and that they also had a  
482 much higher carbon fixation rate into organic material than the lower segments. In addition,  
483 Borowitzka and Vesk (1979) observed more chloroplasts in young segments compared to  
484 older segments. Furthermore, it is conceivable that the equation used to convert wet weight  
485 of algae into dry weight may have contributed to the variability observed in the data.

486 Calcification rates in coralline algae are affected by temperature and irradiance, which  
487 both exhibit strong diurnal fluctuations in intertidal rock pools. Temperature is positively  
488 correlated with  $\Omega_{\text{CaCO}_3}$ , potentially explaining how elevated temperature may promote  
489 calcification. This is perhaps especially relevant for dark calcification in the absence of  
490 photosynthetic metabolism when the relative importance of abiotic environmental factors  
491 increases. Temperature also directly affects diffusion rates and metabolic processes that may  
492 play a role in calcification (Hurd *et al.*, 2014). For example, temperature affects carbonic  
493 anhydrase activity and active transport of  $\text{HCO}_3^-$  and  $\text{CO}_2$ , both of which are also important  
494 for photosynthesis (Hurd *et al.*, 2014). As discussed above, much of the observed response  
495 in calcification may be directly associated with photosynthesis and respiration conducted by  
496 the algae themselves. In addition, the biological metabolism of all the species residing in a  
497 tidal pool can influence the chemical properties of the seawater and subsequently affect  
498 physiological processes in coralline algae inhabiting the pool.

499 Since calcifying algae in intertidal rock pools experience large fluctuations in the physico-  
500 chemical environment, it has been suggested that they have physiological advantages as  
501 ocean acidification and warming advances (Egilsdottir *et al.*, 2013). However, ocean  
502 acidification and global warming may affect organisms synergistically in the future. For  
503 example, the species investigated in this study inhabited a low-lit tidal pool and respiration  
504 increased significantly over the temperature range observed in the study. Thus, the

505 minimum pH and  $\Omega_{\text{CaCO}_3}$  in rock pools might be lowered in the future as a result of  
506 increased temperatures leading to elevated respiration rates, but this effect may be  
507 somewhat offset due to the positive correlation between temperature and  $\Omega_{\text{CaCO}_3}$ . Coralline  
508 algae inhabiting low light environments may be especially at risk by a rise in temperature  
509 since higher irradiance levels could be required to accomplish a sustainable net  
510 photosynthetic production in the algae.

511

### 512 *Conclusions*

513

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

522

### 523 **Acknowledgements**

524

525 We thank Erwan Ar Gall for his help with species determination and Laure Noël for  
526 assistance in the field. We also thank three anonymous reviewers for their constructive  
527 comments. This work was supported by the CALCAO project, which received funding from  
528 the Region Bretagne and by the “European Project on Ocean Acidification” (EPOCA, Grant  
529 agreement 211384) through the Marine Research Institute, Iceland.

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543 **(Figure captions)**

544

545 **Fig. 1.** Relationship between irradiance and *Ellisolandia elongata* net primary production

546 (in O<sub>2</sub> and CO<sub>2</sub> fluxes) in (a) summer and (b) winter

547

548 **Fig. 2.** Relationship between irradiance and calcification rates in *Ellisolandia elongata* in

549 (a) summer and (b) winter

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554 **References**



- 556 Adey, W.H. (1998). Coral reefs: algal structured and mediated ecosystems in shallow,  
557 turbulent, alkaline waters. *Journal of Phycology*, **34**: 393-406.
- 558 Adey, W.H. & Macintyre, I.G. (1973). Crustose coralline algae: A re-evaluation in the  
559 geological sciences. *Geological Society of America Bulletin*, **84**: 883-904.
- 560 Algarra, P., Viña, G.D.L., & Neill, J. (1991). Effects of light quality and irradiance level  
561 interactions on short-term pigment response of the red alga *Corallina elongata*. *Marine*  
562 *Ecology Progress Series*, **74**: 27-32.
- 563 Andersson, A., Mackenzie, F., & Bates, N. (2008). Life on the margin: implications of ocean  
564 acidification on Mg-calcite, high latitude and cold-water marine calcifiers. *Marine Ecology*  
565 *Progress Series*, **373**: 265-273.
- 566 Basso, D. (2012). Carbonate production by calcareous red algae and global change.  
567 *Geodiversitas*, **34**: 13-33.
- 568 Benedetti-Cecchi, L. (2006). Understanding the consequences of changing biodiversity on  
569 rocky shores: How much have we learned from past experiments? *Journal of Experimental*  
570 *Marine Biology and Ecology*, **338**: 193-204.
- 571 Bensoussan, N. & Gattuso, J.-P. (2007). Community primary production and calcification in a  
572 NW Mediterranean ecosystem dominated by calcareous macroalgae. *Marine Ecology*  
573 *Progress Series*, **334**: 37-45.
- 574 Björk, M., Axelsson, L., & Beer, S. (2004). Why is *Ulva intestinalis* the only macroalga  
575 inhabiting isolated rockpools along the Swedish Atlantic coast? *Marine Ecology Progress*  
576 *Series*, **284**: 109-116.
- 577 Borowitzka, M.A. (1979). Calcium exchange and the measurement of calcification rates in the  
578 calcareous coralline red alga *Amphiroa foliacea*. *Marine Biology*, **50**: 339-347.
- 579 Borowitzka, M.A. (1981). Photosynthesis and calcification in the articulated coralline red  
580 algae *Amphiroa anceps* and *A. foliacea*. *Marine Biology*, **62**: 17-23.
- 581 Borowitzka, M.A. (1982). Mechanisms in algal calcification. *Progress in Phycological*  
582 *Research*, **1**: 137-178.
- 583 Borowitzka, M.A. (1989). Carbonate Calcification in Algae - Inhibition and Control. In  
584 *Biom mineralization: Chemical and Biochemical Perspectives* (Mann, S., Webb, J., &  
585 Williams, R.J.P., editors), 65-94. VCH Verlagsgesellschaft, Germany.
- 586 Borowitzka, M.A. & Larkum, A.W.D. (1976). Calcification in the green alga *Halimeda* III.  
587 The sources of inorganic carbon for photosynthesis and calcification and a model of the  
588 mechanism of calcification. *Journal of Experimental Botany*, **27**: 879-893.
- 589 Borowitzka, M.A. & Larkum, A.W.D. (1987). Calcification in algae: Mechanisms and the  
590 role of metabolism. *Critical Reviews in Plant Sciences*, **6**: 1-45.
- 591 Borowitzka, M.A. & Vesik, M. (1979). Ultrastructure of the Corallinaceae (Rhodophyta) II.  
592 vegetative cells of *Lithothrix aspergillum*. *Journal of Phycology*, **15**: 146-153.
- 593 Burdett, H., Keddie, V., Macarthur, N., McDowall, L., Mcleish, J., Spielvogel, E., Hatton, A.,  
594 & Kamenos, N. (2014). Dynamic photoinhibition exhibited by red coralline algae in the red  
595 sea. *BMC Plant Biology*, **14**: 139.
- 596 Chave, K.E. (1954). Aspects of the Biogeochemistry of Magnesium 1. Calcareous Marine  
597 Organisms. *The Journal of Geology*, **62**: 266-283.
- 598 Chisholm, J.R.M. (2000). Calcification by crustose coralline algae on the northern Great  
599 Barrier Reef, Australia. *Limnol. Oceanogr.*, **45**: 1476-1484.
- 600 Chisholm, J.R.M. & Gattuso, J.P. (1991). Validation of the Alkalinity Anomaly Technique for  
601 investigating calcification and photosynthesis in coral-reef communities. *Limnol. Oceanogr.*,  
602 **36**: 1232-1239.

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

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

610 De Beer, D. & Larkum, A.W.D. (2001). Photosynthesis and calcification in the calcifying  
611 algae *Halimeda discoidea* studied with microsensors. *Plant, Cell & Environment*, **24**: 1209-  
612 1217.

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

615 Dickson, A.G., Sabine, C.L., & Christian, J.R. (2007). *Guide to best practices for ocean CO<sub>2</sub>*  
616 *measurements*. PICES Special Publication 3, 191 pp.

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

620 Egilsdottir, H., Noisette, F., Noël, L., Olafsson, J., & Martin, S. (2013). Effects of *p*CO<sub>2</sub> on  
621 physiology and skeletal mineralogy in a tidal pool coralline alga *Corallina elongata*. *Marine*  
622 *Biology*, **160**: 2103-2112.

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

626 Fisher, K. & Martone, P.T. (2014). Field study of growth and calcification rates of three  
627 species of articulated coralline algae in British Columbia, Canada. *The Biological Bulletin*,  
628 **226**: 121-130.

629 Gao, K., Aruga, Y., Asada, K., Ishihara, T., Akano, T., & Kiyohara, M. (1993). Calcification  
630 in the articulated coralline alga *Corallina pilulifera*, with special reference to the effect of  
631 elevated CO<sub>2</sub> concentration. *Marine Biology*, **117**: 129-132.

632 Häder, D.-P., Lebert, M., Flores-Moya, A., Jiménez, C., Mercado, J., Salles, S., Aguilera, J.,  
633 & Figuero, F.L. (1997). Effects of solar radiation on the photosynthetic activity of the red alga  
634 *Corallina elongata* Ellis et Soland. *Journal of Photochemistry and Photobiology B: Biology*,  
635 **37**: 196-202.

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

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

643 Hofmann, L.C., Yildiz, G., Hanelt, D., & Bischof, K. (2012). Physiological responses of the  
644 calcifying rhodophyte, *Corallina officinalis* (L.), to future CO<sub>2</sub> levels. *Marine Biology*, **159**:  
645 783-792.

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

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

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

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

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

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

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

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

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

669 Korbee, N., Navarro, N.P., García-Sánchez, M., Celis-Plá, P.S.M., Quintano, E., Copertino,  
670 M.S., Pedersen, A., Mariath, R., Mangaiyarkarasi, N., Pérez-Ruzafa, Á., Figueroa, F.L., &  
671 Martínez, B. (2014). A novel in situ system to evaluate the effect of high CO<sub>2</sub> on  
672 photosynthesis and biochemistry of seaweeds. *Aquatic Biology*, **22**: 245-259.

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

676 Köhl, M., Glud, R.N., Borum, J., Roberts, R., & Rysgaard, S. (2001). Photosynthetic  
677 performance of surface-associated algae below sea ice as measured with a pulse-amplitude-  
678 modulated (PAM) fluorometer and O<sub>2</sub> microensors. *Marine Ecology Progress Series*, **223**:  
679 1-14.

680 Lewis, E. & Wallace, D. (1998). *Program Developed for CO<sub>2</sub> System Calculations*. Carbon  
681 Dioxide Information Analysis Center, Oak Ridge National Laboratory.

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

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

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

690 Martin, S., Clavier, J., Chauvaud, L., & Thouzeau, G. (2007). Community metabolism in  
691 temperate maerl beds. I. Carbon and carbonate fluxes. *Marine Ecology Progress Series*, **335**:  
692 19-29.

693 Martin, S., Cohu, S., Vignot, C., Zimmerman, G., & Gattuso, J.-P. (2013b). One-year  
694 experiment on the physiological response of the Mediterranean crustose coralline alga,  
695 *Lithophyllum cabiochae*, to elevated pCO<sub>2</sub> and temperature. *Ecology and Evolution*, **3**: 676-  
696 693.

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

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

703 Mercado, J.M., Figueroa, F.L., Xauier Niell, F., & Axelsson, L. (1997). A new method for  
704 estimating external carbonic anhydrase activity in macroalgae. *Journal of Phycology*, **33**: 999-  
705 1006.

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

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

710 Morse, J.W., Andersson, A.J., & Mackenzie, F.T. (2006). Initial responses of carbonate-rich  
711 shelf sediments to rising atmospheric pCO<sub>2</sub> and "ocean acidification": Role of high Mg-  
712 calcites. *Geochimica Et Cosmochimica Acta*, **70**: 5814-5830.

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

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

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

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

724 Pentecost, A. (1978). Calcification and photosynthesis in *Corallina officinalis* L. using the  
725 <sup>14</sup>CO<sub>2</sub> method. *British Phycological Journal*, **13**: 383 - 390.

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

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

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

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

735 Raven, J.A. (2011). Effects on marine algae of changed seawater chemistry with increasing  
736 atmospheric CO<sub>2</sub>. *Biology and Environment: Proceedings of the Royal Irish Academy*, **111B**:  
737 1-17.

738 Raven, J.A., Giordano, M., Beardall, J., & Maberly, S.C. (2012). Algal evolution in relation to  
739 atmospheric CO<sub>2</sub>: carboxylases, carbon-concentrating mechanisms and carbon oxidation  
740 cycles. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **367**: 493-507.

741 Ries, J.B. (2011). Skeletal mineralogy in a high-CO<sub>2</sub> world. *Journal of Experimental Marine  
742 Biology and Ecology*, **403**: 54-64.

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

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

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

- 750 Smith, S.V. & Key, G.S. (1975). Carbon-Dioxide and Metabolism in Marine Environments.  
751 *Limnol. Oceanogr.*, **20**: 493-495.
- 752 Steneck, R.S. (1986). The Ecology of Coralline Algal Crusts: Convergent Patterns and  
753 Adaptive Strategies. *Annual Review of Ecology and Systematics*, **17**: 273-303.
- 754 Zeebe, R.E. & Wolf-Gladrow, D.A. (2001). *CO<sub>2</sub> in seawater: equilibriums, kinetics, isotopes*.  
755 Elsevier Oceanography Series.  
756

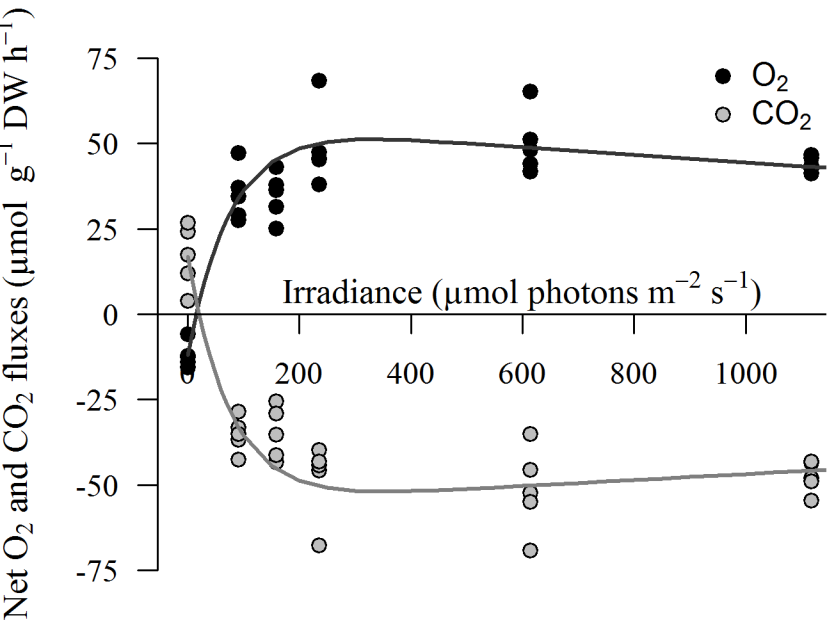


Figure 1a

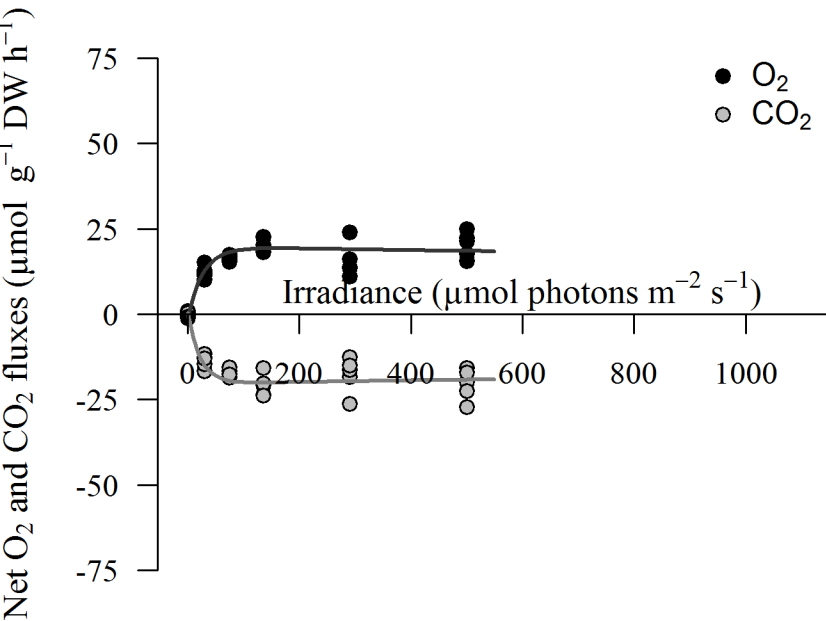


Figure 1b

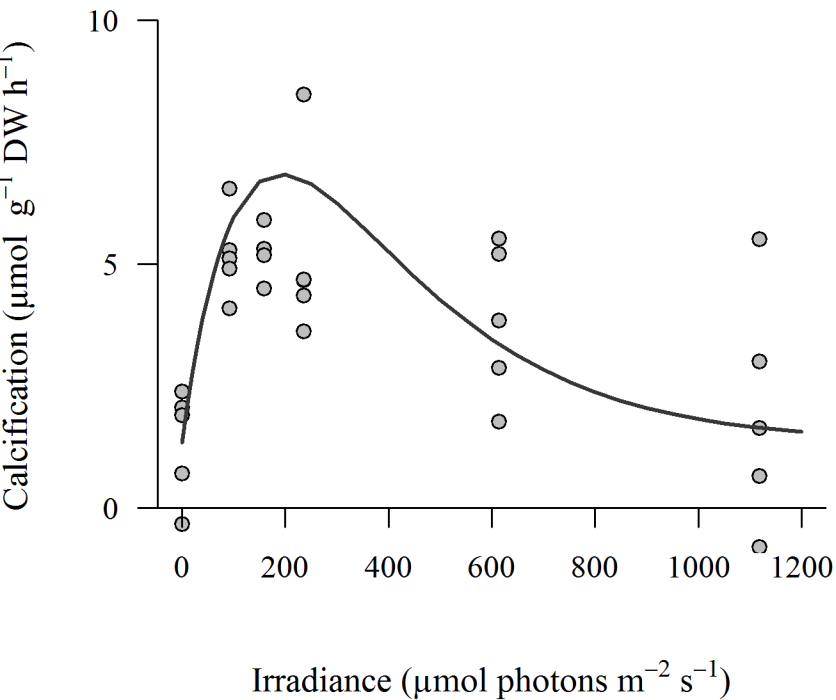


Figure 2a



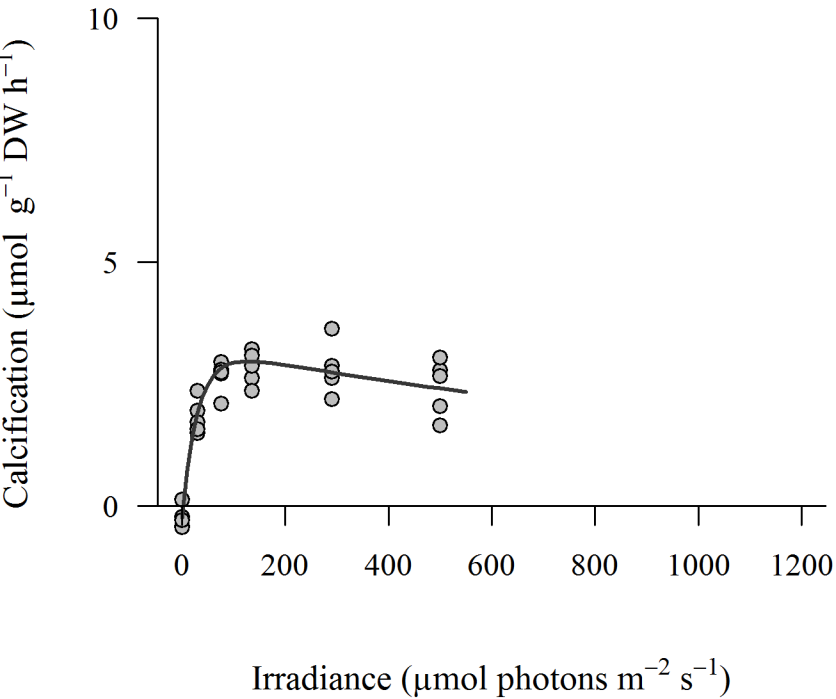


Figure 2b