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

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

15

16

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.

2

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).

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 (Ω_{CaCO3}) 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

5

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_2/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 11th of August 2010 (summer) and on the 21st 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 µmol photons m^{-2} s^{-1} in summer and *ca*. 15 µmol photons m^{-2} 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 $0.41 \pm$ SE 0.03 g in summer and $0.81 \pm$ 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 µmol photons m^{-2} s^{-1} in summer and 30, 75,

171   135, 290, and 500 µmol photons m^{-2} 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^{TM}, Loveland USA). pH was measured

178   using a pH meter (HQ40D, Hach Lange, Ltd portable LDO^{TM}, Loveland USA) calibrated on

179   the total scale (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 µm

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 *K1* and *K2* 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 ($\mu mol\ O_2\ g^{-1}\ DW\ h^{-1}$) were calculated as follows:

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

199   where ΔO_2 is the change in dissolved oxygen ($\mu mol\ l^{-1}$), Δt 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: $Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 +$

206   H_2O, for which total alkalinity decreases by 2 equivalents for each mol of $CaCO_3$

207   precipitated.

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

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

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

211   P_n and R expressed in terms of carbon (µmol CO_2 g^{-1} 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    $$P_n \text{ or } R \text{ } (CO_2) = \left(\frac{\Delta DIC - (0.5 \times \Delta A_T)}{\Delta t \times V} \right) / DW \qquad (3)$$

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

216   The relationship between irradiance (E, µmol photon m^{-2} 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, µmol $CaCO_3$ g^{-1}

219   DW h^{-1}):

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

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

222

223   where Ps and Gs 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; µmol O_2, DIC or $CaCO_3$ g^{-1} h^{-1}), α is the initial slope of the

226   relationship (µmol g^{-1} h^{-1} (µmol m^{-2} s^{-1})$^{-1}$), β is the photoinhibition coefficient (µmol g^{-1} h^{-1}

227   (µmol m^{-2} s^{-1})$^{-1}$). In the absence of evident photoinhibition, the β value was set to zero.

228   The deduced maximal rate of gross photosynthesis (or calcification) at light saturation, P_g^{max}

229   (or G_g^{max}), are calculated as:

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

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

232    $$P_n^{max} = P_g^{max} + R$$

$$(7)$$

233 $$G^{max} = G_g^{\,max} + G_d$$

(8)

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

235 $$E_k = \frac{P_g^{\,max}}{\alpha} \text{ or } \frac{G_g^{\,max}}{\alpha}$$

(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_2 flux, CO_2 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_2 fluxes: $t = -6.63$, $p <$

254 0.01; CO_2 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_2

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 µmol photons m^{-2} s^{-1}

259 in summer and a negligible photoinhibition above about 200 µmol photons m^{-2} s^{-1} was

260 observed in winter (Table 3; Fig. 1). In summer, maximum gross O_2 production and CO_2

261 uptake at light saturation (P_g^{max}) were about three times those recorded for winter (O_2

262 fluxes: W=25, $p < 0.01$; CO_2 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_2 fluxes: W=25, $p < 0.01$; CO_2 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_2 fluxes: $t = 5.42$, $p < 0.05$; CO_2 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_2 fluxes: $t = 4.66$, $p < 0.01$; CO_2 fluxes: $t = 3.32$, $p < 0.05$; Table 3).

268 Coefficient α (the initial slope of the curve) did not differ between seasons for both O_2 and

269 CO_2 fluxes. Coefficient β (photoinhibition coefficient) did not differ between seasons for O_2

270 fluxes. No statistical comparison between seasons was conducted for β considering CO_2

271 fluxes since photoinhibition was not indicated by all five *P-E* curves calculated for each

272 season. The net production rates under 30 µmol photons m^{-2} s^{-1} and 15 µmol photons m^{-2} s^{-1}

273 (*in situ* midday irradiance in summer and winter respectively) were estimated from the *P-E*

274 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

275 ± 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

276 ($P_g = P_n + R$) was thus estimated to 20.4 ± 3.1 µmol O_2 g^{-1} DW h^{-1} and -22.5 ± 1.9 µmol C

277 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}

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 µmol m^{-2} 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 α and β

294   did not differ between seasons (Table 3). Calcification at midday *in situ* irradiance

295   estimated from the *G-E* curves was 3.3 ± 0.3 µmol CaCO$_3$ g^{-1} DW h^{-1} in summer at 30 µmol

296   photons m^{-2} s^{-1} and $1.1 \pm$ SE 0.1 µmol CaCO$_3$ g^{-1} DW h^{-1} in winter at 15 µmol photons m^{-2}

297   s^{-1}.

298

299   **Discussion**

300

301   *Respiration*

302

303 Respiration rates observed here varied seasonally in terms of both O$_2$ and 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

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.

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 µmol m^{-2} 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 μmol photons m^{-2} 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   umol photons m^{-2} 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 CO_2 and 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 though 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 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 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 HCO_3^- to 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 Mediterranian (Bensoussan

416   & Gattuso, 2007). Borowitzka (1981) suggested a positive relationship between Ω_{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 % ≈ 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 μmol $CaCO_3$ g^{-1} DW h^{-1} (El

428   Haïkali *et al.*, 2004). However, El Haïkali *et al.* (2004) attributed this reletively 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 Ω_{CaCO3} 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 µmol

437 $CaCO_3$ g^{-1} 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 µmol photons m^{-2} 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 800-1000 µmol photons m^{-2} 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 µmol photons m^{-2} s^{-1}; Martin *et al.*, 2013b).

455   Photosynthesis promotes calcification through two major ways: by elevation of Ω_{CaCO3}

456   and by production of organic tissue or compounds. Photosynthesis (and respiration) regulate

457   pH and Ω_{CaCO3} within the diffusion boundary layer and at the calcification site via uptake

458   (and production) of CO_2 (Smith & Roth, 1979; Gao *et al.*, 1993; Hurd *et al.*, 2011). As

459   reviewed by Borowitzka (1989), a certain degree of Ω_{CaCO3} is required to induce nucleation

460   and for the growth of the algal calcium carbonate crystals. Ω_{CaCO3} 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 CO_2 into HCO_3^- and then 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 HCO_3^- to CO_2 which could then lower Ω_{CaCO3}.

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 Ω_{CaCO3}, 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 HCO_3^- and 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 Ω_{CaCO3} 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 Ω_{CaCO3}. 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**

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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

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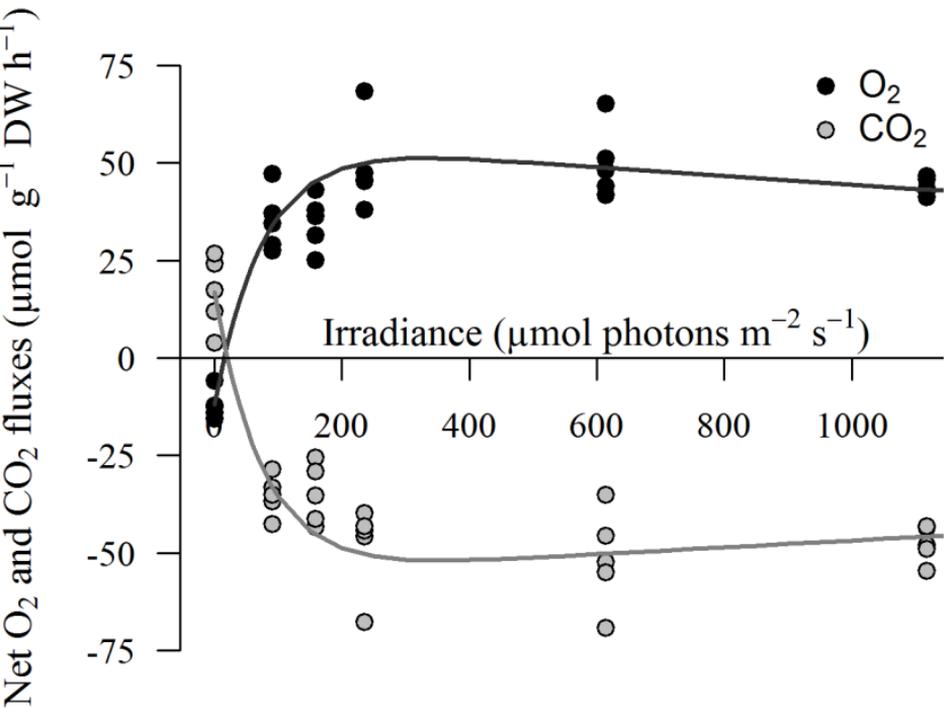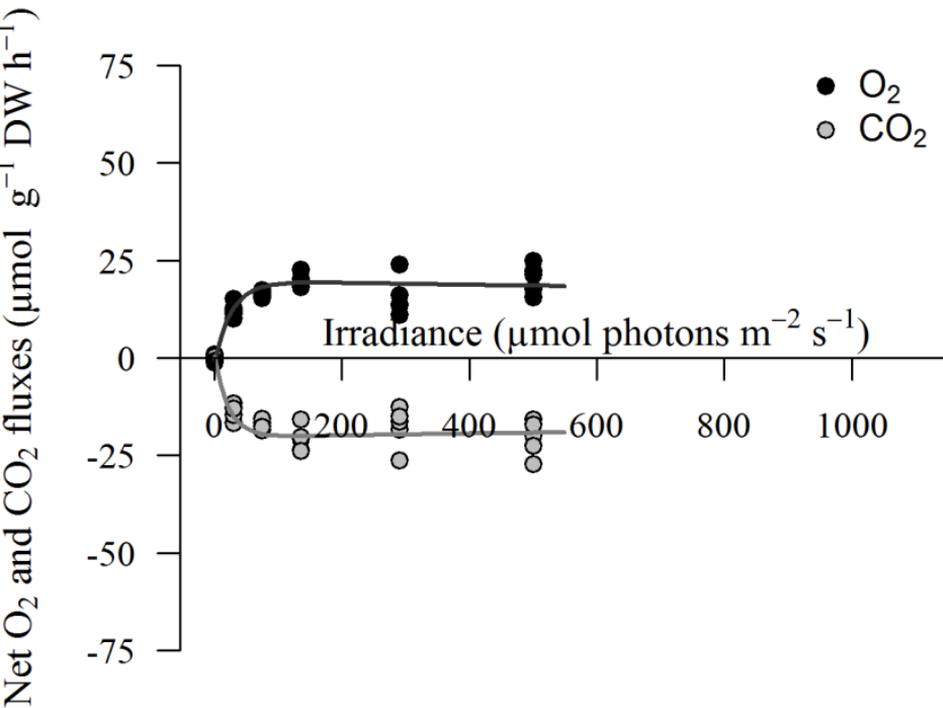755   Elsevier Oceanography Series.
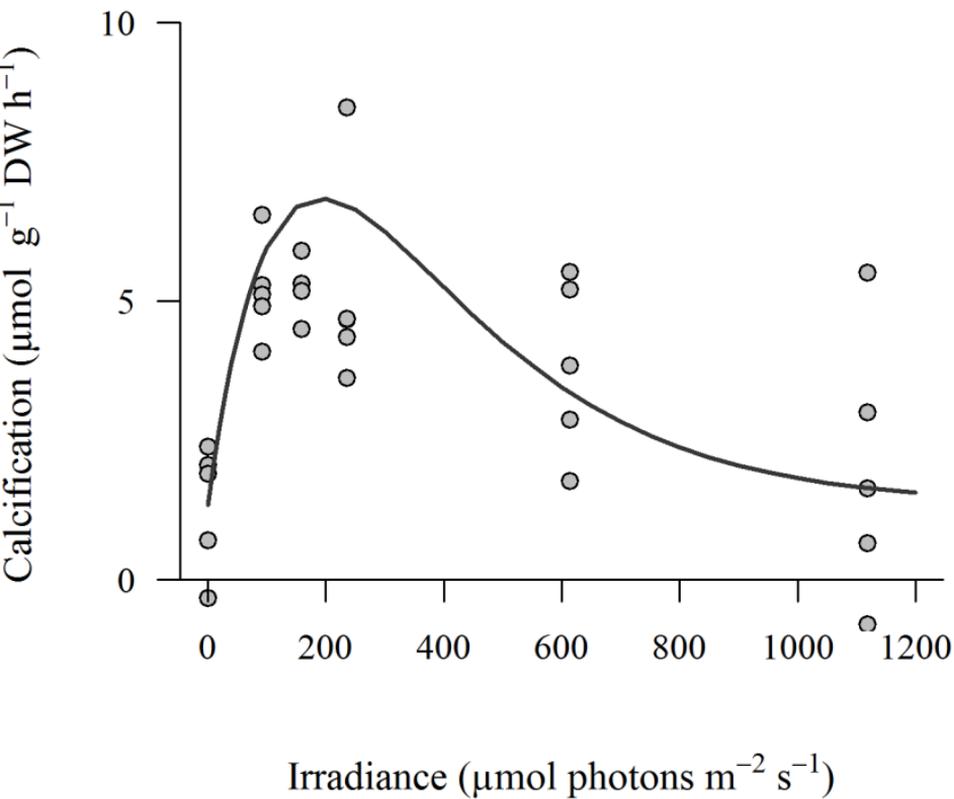756
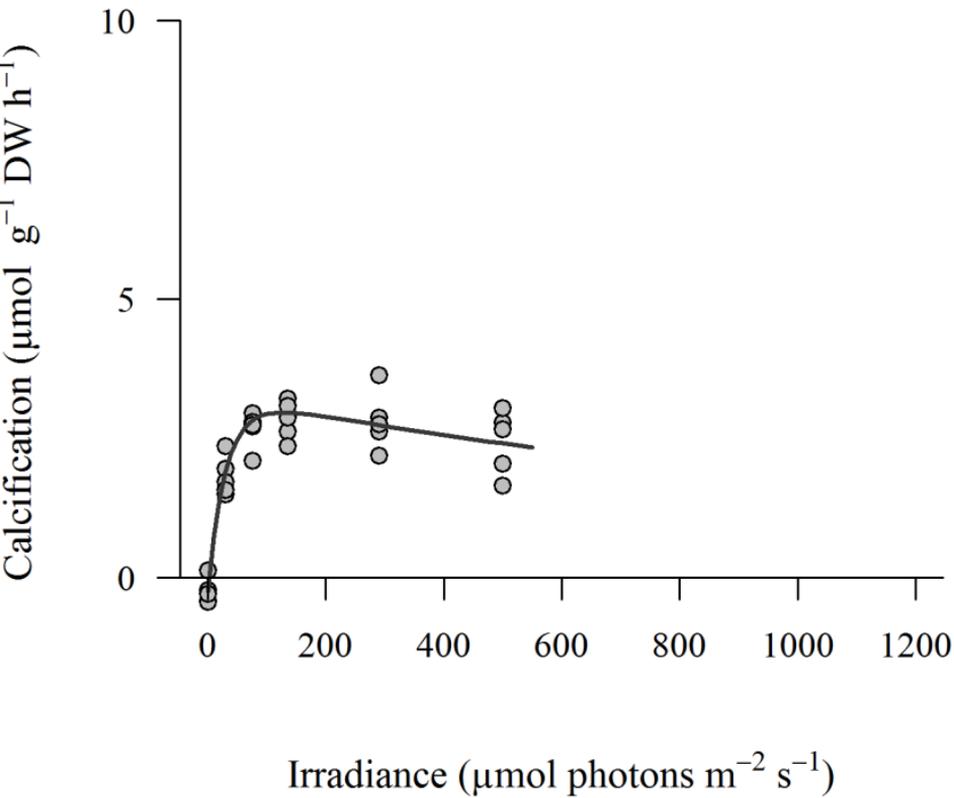
Figure 1a

Figure 1b

Figure 2a

Figure 2b