

Photosynthesis might be limited by light, not inorganic carbon availability, in three intertidal Gelidiales species

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Summary

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- The interaction between incident photon fluence density (PFD) and dissolved inorganic carbon (DIC) availability on photosynthesis is reported for three species of Gelidiales (Rhodophyta) from the Canary Islands.

- Photosynthetic O₂ evolution in response to both DIC concentration and PFD, and external carbonic anhydrase activity were measured in thalli of *Gelidium canariensis*, *Gelidium arbuscula* and *Pterocladia capillacea* maintained in a controlled-environment room.

- No detectable external carbonic anhydrase activity, high sensitivity to alkaline pH and moderate values of photosynthetic conductance for DIC indicated that the three species had a low capacity for using the external pool of HCO₃⁻; therefore photosynthetic rates were dependent on CO₂ availability. The seawater concentration of DIC was insufficient to saturate photosynthesis at high PFD; photosynthesis vs PFD was not affected by DIC concentration at 0.07–0.04 mol O₂ mol⁻¹ photon. ANOVA revealed that incident PFD had a greater effect than DIC availability on photosynthesis rates in *G. canariensis*.

- Photosynthesis is probably limited by incident light rather than DIC concentration despite the apparent low affinity for HCO₃⁻ in *G. canariensis*, *G. arbuscula* and *P. capillacea* in their natural habitats.

Key words: carbonic anhydrase, Gelidiales, inorganic carbon, intertidal, macroalgae, photosynthesis, quantum yield.

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Abbreviations

α , ascending slope at limiting PFDs of photosynthesis vs PFD curves; DIC, dissolved inorganic carbon; g_p , photosynthetic conductance for DIC; I_k , light saturation parameter; I_m , PFD at which photosynthesis is maximal; $K_{1/2}$, half-saturation constant for DIC; LST, lowest spring tide level; PFD, photon fluence density; P_m , maximal photosynthetic rates at saturating PFD.

Introduction

The macroalgae that inhabit upper-zones on the shore are daily subjected to emersion. Such environmental fluctuations often lead to changes in the availability of CO₂ for photosynthesis (Axelsson *et al.*, 1995; Larsson *et al.*, 1997), the importance of which is related to the position of the alga on the shore. As an example, some reports have focused on the

possible correlation between the height on shore and the capacity to use the external pool of HCO₃⁻ (Giordano & Maberly, 1989; Surif & Raven, 1989, 1990; Maberly, 1990). It has been demonstrated that the affinity for inorganic carbon (DIC) varies widely among intertidal macroalgae (Sand-Jensen & Gordon, 1984; Axelsson & Uusitalo, 1988; Mercado *et al.*, 1998b). According to Maberly (1990), the most efficient HCO₃⁻ users are macroalgae located at the

highest position of the intertidal system. These macroalgae have developed energy-dependent mechanisms to accumulate DIC internally and their photosynthesis rates are saturated at the concentration of DIC in seawater. Other species rely on diffusive entry of CO₂ and, at saturating light, their rates of photosynthesis are restricted by the concentration of CO₂ in seawater (Colman & Cook, 1985; Holbrook *et al.*, 1988; Mercado *et al.*, 1997, 1998b). Some authors have suggested that reliance on CO₂ is more widely spread among macroalgae inhabiting zones with a low incident light. The probable interaction between use of light and DIC acquisition has been studied in terms of benefit-cost (Beardall, 1991; Johnston *et al.*, 1992; Kübler & Raven, 1994, 1995; Madsen & Sand-Jensen, 1994). According to Raven (1991), a DIC-saturated plant relying on diffusive entry of CO₂ has a lower cost of photosynthesis than plants with a mechanism of HCO₃⁻ uptake. However, when the concentration of CO₂ in seawater is low (e.g., at its usual concentration of 15 mmol m⁻³), the photon cost of DIC fixation could be higher in organisms with diffusive CO₂ entry because the air-equilibrium concentrations of O₂ and CO₂ would result in significant photorespiratory activity (Raven, 1984; Kerby & Raven, 1985; Beardall, 1989).

In the present study, the interaction between inorganic carbon acquisition and the use of light was investigated using three species of Gelidiales. This group of red macroalgae is widely distributed in tropical and temperate waters and its members frequently occur in habitats with strong water movement (Santelices, 1988, 1991). The Gelidiales become dominant in shaded habitats receiving 25–50% of incident light (Santelices, 1974). Different aspects of the photosynthetic physiology of the Gelidiales have been studied (Macler, 1986; Rico, 1991; Torres *et al.*, 1991; Carmona *et al.*, 1996). Previous reports based on three species of *Gelidium* (Tseng & Sweeney, 1946; Mercado *et al.*, 1998a,b) indicate that photosynthesis is dependent on diffusive CO₂ entry. The objective of the experiments described in this paper was to determine whether DIC availability or incident photon fluence density could be more relevant in determining the rates of photosynthesis in three other species of Gelidiales. The use of light as a function of DIC concentration in the medium was analysed and the possible ecological implications of the results are discussed.

Materials and Methods

Algal material

Gelidium canariensis (Grunow) Seoane-Camba, *Gelidium arbuscula* Bory ex Boergesen and *Pterocladia capillacea* (Gmelin) Santelices and Hommersand were collected at Puerto de la Cruz (Tenerife, Canary Islands, Spain) in July 1998. These species inhabited lower-intertidal zones. They occurred as successive horizons along a vertical elevation at shaded lower intertidal zone.

Pterocladia capillacea grows at the uppermost zone and *G. canariensis* occurs at the lowest zone. The three species studied inhabit a zone from 20 to 90 cm above the lowest spring tide level (LST). *G. canariensis* grows at 20 cm above LST and forms a layer of 40–50 cm in thickness. *G. arbuscula* grows at 60 cm above the LST and *P. capillacea* grows at 80 cm above LST. The tidal range at the collection site is 2.8 m. Therefore, the thalli are emerged at low tide, although they do not become desiccated because they are kept hydrated by continuous wave splashes.

Photon fluence density (PFD) at the sampling site was determined in air using a spherical sensor (193 SB, Li-Cor) connected to a data logger (Li-Cor 1000, Lincoln, NB, USA). PFD was measured in air every 2 h over 10 d before collecting the algae. The mean PFD was 700 μmol photons m⁻² s⁻¹ whilst the daily maximal PFD was approx. 3000 μmol photons m⁻² s⁻¹. Incident light during the period of low tide at the exact place where the plants grew was between 40 to 60% lower than the PFD measured in air. Mean seawater temperature was 20°C, as measured with a marine thermometer.

After collection, thalli were maintained in a controlled temperature room (20°C) at 12 h light c⁻¹ d⁻¹ for not more than 10–12 d. The thalli were stored in Plexiglas cylinders containing 3 dm⁻³ of natural seawater at 35 salinity. The seawater was enriched with Provasoli medium (Starr & Zeikus, 1987) and filtered through Millipore discs (GS, 0.22 μm pore size). The medium was vigorously aerated (c. 3 dm⁻³ air min⁻¹). A constant PFD of 100 μmol photons m⁻² s⁻¹ (measured as described earlier in this section) was provided with daylight fluorescent lamps (F20W/DL Osram, Munich, Germany).

Fresh mass of the samples used for experiments was determined after blotting off surface water with absorbent paper. Dry mass of samples of known fresh mass was determined after drying at 80°C overnight. Surface area was determined by image analysis using a video system connected to a computer as described by Lüning (1992). The thallus of known fresh mass was put between two slides and pressed to give a flat image (Mercado *et al.*, 1998b).

Photosynthetic O₂ evolution

O₂ evolution was measured in 8 cm⁻³ chambers at 20°C with Clark-type oxygen electrodes (Yellow Spring Instruments 5221 OH, USA). A temperature was maintained constant a Haake Fison DC1 (Haake Mess-Technik GmbH u. Co., Karlsruhe, Germany). The signal from the O₂ electrode was recorded on a strip chart recorder. A using PFD of 600 μmol photons m⁻² s⁻¹ was provided by a halogen lamp (Xenophot HLX, Osram, Munich, Germany). PFD inside the chamber was measured with a spherical quantum sensor (20HM33CM12KG, Zemoko, Holland). About 50 mg of alga were transferred to the O₂ evolution chamber containing filtered natural seawater at 35 practical salinity units. Then, the seawater pH was adjusted with buffers. Three different values

of pH were tested using independent samples of algae: 5.6, 8.1 and 8.7. Mes was used for buffering at pH 5.6 and Tris was used at pH 8.1 and 8.7. The buffers were used at a final concentration of 50 mol m^{-3} (2 kmol m^{-3} in stock). Suitable agitation of the medium in the chamber was obtained by a magnetic stirrer.

Determination of $K_{1/2}$ and conductance (g_p) for DIC

Response of photosynthesis to increasing DIC concentration was measured at two different PFDs, 50 and $600 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. A sample (50 mg) of alga was transferred to the O_2 evolution chambers containing DIC-free synthetic seawater ($450 \text{ mol m}^{-3} \text{ NaCl}$, $30 \text{ mol m}^{-3} \text{ MgSO}_4$, $10 \text{ mol m}^{-3} \text{ KCl}$, $10 \text{ mol m}^{-3} \text{ CaCl}_2$) as described by Beer *et al.* (1990). The synthetic seawater was filtered through Millipore filters (GS, $0.22 \mu\text{m}$ pore size) before the experiments. The pH was adjusted to 8.1 with Tris as already described. After approx. 10 min, a zero net O_2 exchange rate had been attained. Then, small amounts of $200 \text{ mol m}^{-3} \text{ NaHCO}_3$ were injected into the chamber in order to create different DIC concentrations ranging from 50 to 5600 mmol m^{-3} . After addition of HCO_3^- the concentration of CO_2 in the chamber was estimated by using the apparent dissociation constants of carbonic acid in seawater (Riley & Chester, 1977). O_2 evolution was recorded during 10–15 min after each addition of NaHCO_3 . The half-saturation point ($K_{1/2}$) was estimated from the Michaelis–Menten equation. The goodness of fit was tested by using least-square regression analysis. Four independent curves (each based on different samples) were conducted at 50 and $600 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and analysed separately. The conductance for DIC was obtained from the initial slope of these curves by expressing the rates of photosynthesis on an area basis. The initial slopes of the curves were calculated by linear regression over the range of 0.0 – 1.1 mol m^{-3} DIC (with the exception of some curves performed at $50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for which the range of 0.0 – 0.5 mol m^{-3} was used). The average R^2 value obtained from the least-squares regression analysis was 0.95.

Curves of O_2 evolution vs PFD

Response of the rate of O_2 evolution to changes in PFD was determined at three different inorganic carbon concentrations: 1.1, 2.2 and 5.6 mol m^{-3} . These concentrations were achieved by injection of small amounts of $200 \text{ mol m}^{-3} \text{ NaHCO}_3$ into chambers containing synthetic seawater, without inorganic carbon, and buffered at pH 8.1. Ten different PFDs ranging from 12 to $1000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ were obtained using glass neutral density filters. Respiration was measured in darkness before the first illuminated measurement. O_2 evolution was recorded for 10–15 min. Maximal photosynthetic rates (P_m) and ascending slope at limiting PFDs (α) were obtained from the fit of the curves to the equation provided by Henley (1993):

$$P = P_m [\alpha \text{ PFD} / (P_m + \alpha \text{ PFD})] + R_d$$

(P , the rate of photosynthesis; R_d , the rate of respiration in darkness.) The light saturation parameter (I_k) was estimated as P_m/α . Optimum PFD (PFD at which photosynthesis was maximal; I_m) was determined by direct examination of the experimental data. Four independent curves (using different samples) were conducted at the three DIC concentrations tested. Each curve was analysed separately. The goodness of fit to the curve model was tested by least-squares regression analysis. The average R^2 value obtained from the least-squares regression analysis was 0.99.

Assay of external carbonic anhydrase

External carbonic anhydrase activity was assayed using the potentiometric method. The time required for a drop of 0.4 pH units within the pH range 8.4–7.4 was measured at 0 – 2°C using a vessel containing 3 cm^3 of 100 mol m^{-3} Tris, 5 mol m^{-3} EDTA-Na and 25 mol m^{-3} ascorbic acid (Haglund *et al.*, 1992). Fragments of 50–200 mg fresh mass were transferred from seawater to the vessel and then washed three times with buffer before assay. The reaction was started by rapidly introducing 1 cm^3 of ice-cold CO_2 -saturated distilled water. One unit of enzyme activity (REA) was defined as $(t_o/t_c) - 1$ where t_o and t_c are the times for a drop of 0.4 pH units in the noncatalysed and catalysed reactions, respectively. The activity was calculated on a fresh mass and surface area basis. Total carbonic anhydrase activity (internal plus external) was determined by measuring the activity in crude extracts, obtained by grinding 30–50 mg of alga in 2 cm^3 of the buffer used for the activity assay.

Statistics

The results were expressed as the mean values \pm standard deviation (SD). The effects of PFD on the parameters of photosynthesis vs DIC curves and the effects of DIC concentration on photosynthesis vs PFD curve parameters were analysed in each species using Model I one-way ANOVA. Comparisons among the three species were conducted using the same analysis. Where appropriate, subsequent multiple comparisons were done using a multirange test by Fisher's protected least significant difference (Sokal & Rohlf, 1981). Effects of DIC concentration and PFD on the rates of photosynthesis were tested with Model I two-way ANOVA. The P -value obtained from each comparison is indicated. Interaction significance was tested before performing the analysis. When interaction was not significant, the main effects (DIC and PFD) were analysed (Nie *et al.*, 1975). The percentage of variance explained by DIC and PFD was calculated by dividing the total square sum by the factor square sum.

Table 1 Morphometric characteristics of the thalli of three intertidal Gelidiales species

	<i>Gelidium canariensis</i>	<i>Gelidium arbuscula</i>	<i>Pterocladia capillacea</i>	<i>P</i>
FW : DW (g g ⁻¹)	3.16 ± 0.49 ^a (6)	3.03 ± 0.19 ^a (12)	2.96 ± 0.27 ^a (9)	0.136
FW : Area (g m ⁻²)	185.5 ± 43.4 ^a (16)	154.7 ± 31.8 ^{a,b} (16)	128.4 ± 38.8 ^b (16)	0.038
DW : Area (g m ⁻²)	58.7 ± 16.5 ^a (16)	51.1 ± 11.0 ^{a,b} (16)	43.4 ± 13.7 ^b (16)	0.019

FW, fresh mass; DW, dry mass. Data are means ± SD. The number of samples is given into brackets. The values of *P* are from one-way ANOVA. Means with different superscripts were different significantly (*P* < 0.05).

Carbaicc anhyrase activity	<i>Gelidium canariensis</i>	<i>Gelidium arbuscula</i>	<i>Pterocladia capillacea</i>	<i>P</i>
External activity (REA g ⁻¹)	-0.91 ± 0.68 (5)	-0.13 ± 0.48 (5)	0.03 ± 0.84 (8)	0.165
Total activity (REA g ⁻¹)	20.12 ± 6.86 (5)	12.34 ± 8.52 (7)	11.60 ± 1.50 (5)	0.105
(REA cm ⁻²)	0.31 ± 0.14	0.19 ± 0.14	0.15 ± 0.02	0.305

Data are means ± SD. The values of *P* are from one-way ANOVA (number of samples indicated in brackets).

Table 2 External and total carbonic anhydrase activity expressed as relative enzymatic activity units (REA). External carbonic anhydrase was determined using intact thalli whilst total carbonic anhydrase activity was obtained using a crude extract of the alga

Results

Table 1 shows the morphometric characteristics of the thalli. No significant differences were found among the fresh : dry mass ratios obtained from the three species studied. However, a higher value of fresh mass per unit area was found in *G. canariensis*. The lowest fresh mass per unit area was found in *P. capillacea*. These values are comparable to those reported by Mercado *et al.* (1998b) for *Gelidium sesquipedale* and *G. pusillum*.

External carbonic anhydrase was not detectable by means of the potentiometric method (Table 2). In fact, negative values of external activity in *G. canariensis* and *G. arbuscula* were obtained. These negative values indicated that the time taken for a pH change of 0.4 units in the presence of the samples was greater than that in the control. Although a slightly positive mean value of external activity was found in *P. capillacea*, it cannot be taken as an actual indication of external activity since it was detected in only two out of eight samples. By contrast, activity was detected in thallus homogenates. This total carbonic anhydrase activity expressed on an area basis was greater, though not different statistically (*P* > 0.05), in *G. canariensis*. These results suggested that most of the carbonic anhydrase activity was located internally in the three species studied.

Fig. 1 shows the rates of photosynthesis expressed on the basis of surface area, which were obtained at three different pH values. Changing the pH of the medium results in different ratios of free CO₂ : HCO₃⁻ without any change in total DIC concentration. At pH 5.6, 75% of DIC is in the form of CO₂; by contrast, 75% of DIC is in the form of HCO₃⁻ at pH 8.7. Therefore, a decrease in photosynthesis rates with increasing pH can be attributed to changes in the availability

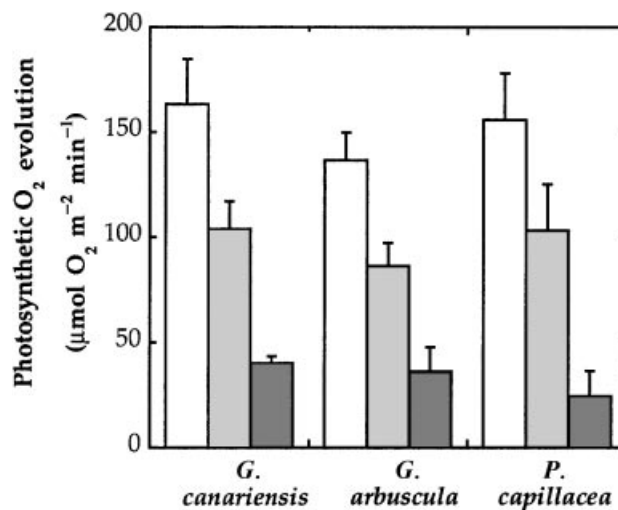


Fig. 1 Photosynthetic rates, expressed per area of *Gelidium canariensis*, *G. arbuscula* or *Pterocladia capillacea*, and measured at 600 µmol photons m⁻² s⁻¹. The pH of seawater was adjusted with buffers to give three different values: 5.6 (open bars), 8.1 (light grey bars) and 8.7 (dark grey bars). Mes was used for buffering at pH 5.6 and Tris was used at pH 8.1 and 8.7. Values are means + SD, independent samples *n* = 8.

of the preferred DIC source for photosynthesis. In our experiments, greater O₂ evolution rates were reached at pH 5.6 in the three species (*n* = 8, *P* < 0.005). An increase in the pH above 8.1 produced a decrease in the rate of photosynthesis by *c.* 80% (with respect to the maximum rate in the three species studied) indicating a high sensitivity to alkaline pH. When the three species were compared, the differences in the maximum photosynthesis rates were not statistically significant (*n* = 8, *P* = 0.213).

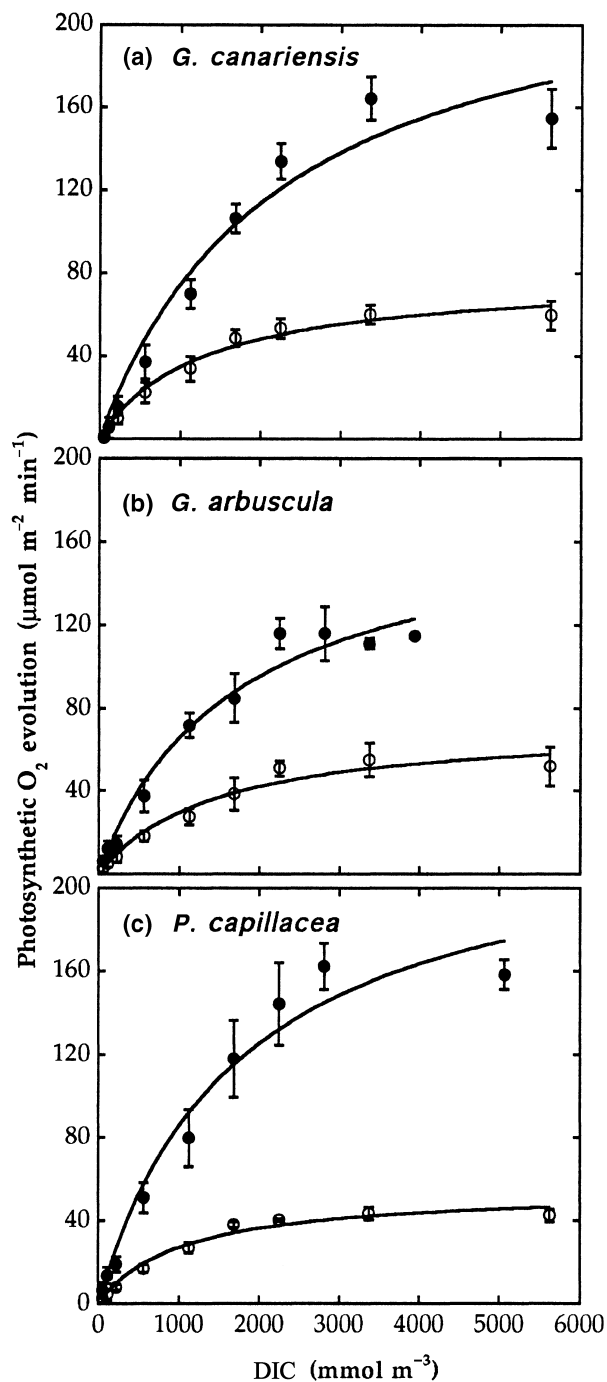


Fig. 2 Response of O_2 evolution to increasing dissolved inorganic carbon (DIC) concentration at $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (open circles) and $600 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (closed circles) for (a) *Gelidium canariensis* (b) *Gelidium arbuscula* and (c) *Pterocladia capillacea*. Curves fitted hyperbolic functions ($P < 0.001$). Individual points are means \pm SD, $n = 4$.

Curves of photosynthesis vs DIC concentration were hyperbolic (Fig. 2). The response of photosynthesis to increasing DIC concentration was basically similar in the three species. In fact, no significant differences were found when

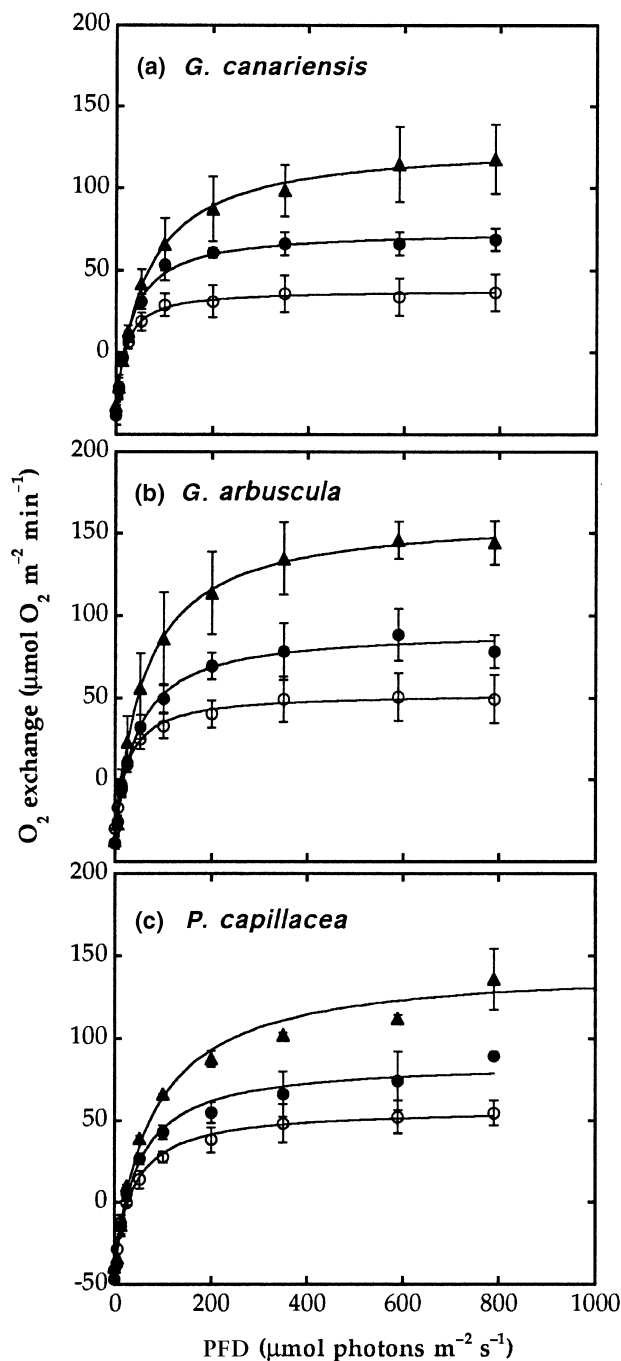
the values of $K_{1/2}$ and g_p obtained from the three species were compared (Table 3). It has to be taken into account that $K_{1/2}$ -values obtained at high-light are only approximate since few data points near saturation were obtained (Ritchie & Prvan, 1996). At $600 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, photosynthesis was not fully saturated with 2 mol m^{-3} of DIC since an increase of DIC concentration above this figure produced an increase in the rate of O_2 evolution. The $K_{1/2}$ obtained for the three species ranged from 2.25 to 1.04 mol m^{-3} (Table 3) and the values of g_p ranged from 0.59 to $1.25 \times 10^{-6} \text{ m s}^{-1}$. It is interesting to note that g_p decreased approx. twofold at $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ compared with at $600 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ($n = 12$, $P = 0.034$), although $K_{1/2}$ was unaffected ($n = 12$, $P = 0.262$).

The response of photosynthesis to increasing light was determined in synthetic seawater medium containing different DIC concentrations (Fig. 3). Respiration rates were similar ($n = 12$, $P = 0.254$) in the three species (35.9 ± 12.1 , 35.3 ± 7.4 and $42.5 \pm 7.9 \mu\text{mol O}_2 \text{ m}^{-2} \text{ min}^{-1}$ for *G. canariensis*, *G. arbuscula* and *P. capillacea*, respectively) and were unaffected by DIC concentration ($n = 12$, $P = 0.271$). By contrast, optimum PFD (I_m) varied slightly among the three species studied. At 5.6 mol m^{-3} of DIC, the values of I_m were approx. 500, 400 and $600 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for *G. canariensis*, *G. arbuscula* and *P. capillacea*, respectively. When DIC was decreased to 1.1 mol m^{-3} , I_m for *G. canariensis*, *G. arbuscula* and *P. capillacea* decreased to 240, 180 and $350 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, respectively. It has to be noted that photoinhibition was not detected. Among the three species, the differences in I_k and α values were not significant statistically (Table 4), with the exception of the highest I_k value and the lowest α value obtained for *P. capillacea* at 1.1 mol m^{-3} . The light saturation parameter (I_k , Table 4) and maximum photosynthesis rates (P_m) were affected by DIC concentration within each species ($n = 4$, $P < 0.025$). Both increased when the DIC concentration was increased from 1.1 to 5.6 mol m^{-3} . By contrast, the initial slope of the photosynthesis vs PFD curves (α , $\text{mol O}_2 \text{ mol}^{-1} \text{ photons}$) did not vary with DIC concentration ($n = 4$, $P > 0.025$).

In order to test whether, PFD or DIC concentration had more of an effect in determining rates of photosynthesis, the data in Figs 2 and 3 were combined and analysed by Model I two-way ANOVA (Table 5). Photosynthetic rates at DIC concentrations of 1.1 mol m^{-3} (close to $K_{1/2}$ for DIC), 2.2 mol m^{-3} (approximately the actual DIC concentration in seawater) and 5.6 mol m^{-3} (a saturating DIC concentration for photosynthesis) were compared at 50 and $600 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ of PFD. A PFD of $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ was considered to be low enough (with respect to I_m values) to determine that photosynthesis was controlled by light-reactions. By contrast, it was considered that photosynthesis was saturated by PFD at $600 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. The interaction was not significant for *G. canariensis*. For this species, the proportion of the total variance attributable to PFD was approx. 0.4. The percent of

Photon fluence density (PFD) $\mu\text{mol photons m}^{-2} \text{s}^{-1}$	<i>Gelidium canariensis</i>	<i>Gelidium arbuscula</i>	<i>Pterocliadiella capillacea</i>	<i>P</i>
50				
g_p ($\mu\text{m s}^{-1}$)	0.59 ± 0.19	0.44 ± 0.08	0.48 ± 0.20	0.560
$K_{1/2}$ (mol m^{-3})	1.28 ± 0.24	1.46 ± 0.40	1.04 ± 0.21	0.953
600				
g_p ($\mu\text{m s}^{-1}$)	1.13 ± 0.19	1.15 ± 0.15	1.25 ± 0.19	0.239
$K_{1/2}$ (mol m^{-3})	2.25 ± 0.71	1.66 ± 0.43	1.76 ± 0.48	0.529

Table 3 Photosynthetic conductance (g_p) and half-saturation points ($K_{1/2}$) for dissolved inorganic carbon (DIC) obtained from photosynthesis vs DIC curves. The results show the mean of four different curves. The values of *P* are from one-way ANOVA ($n = 4$) conducted to compare the parameter values obtained from the three species



variance explained by DIC concentration was lower, at 0.18. The interaction was significant in *G. arbuscula* and *P. capillacea*, indicating that the effect of one variable (PFD or DIC) was not uniform across the different categories of the other variable.

Discussion

DIC acquisition and carbonic anhydrase activity

Few data regarding inorganic carbon acquisition in *Gelidium* spp. can be found in the literature. The three species that have been previously studied, *G. cartilagineum* (Tseng & Sweeney, 1946), *G. sesquipedale* and *G. pusillum* (Mercado *et al.*, 1998b), have a low capacity for using external HCO_3^- for photosynthesis. The results obtained in the present study indicate that the new species examined behave similarly. Thus, the photosynthetic rates decreased in parallel with an increase in pH over the range 8.1–8.7 (which produces an 80% decrease in CO_2 concentration; Prins & Elzenga, 1989), whilst the conductance values were not high enough to indicate a high ability to extract inorganic C from natural seawater at alkaline pH when most of DIC is in the form of HCO_3^- (Johnston *et al.*, 1992; Mercado *et al.*, 1998b). Therefore CO_2 must be the major source of DIC for the species studied. It should be noted that our data do not rule out a limited capacity to use HCO_3^- since the values of $K_{1/2}$ for CO_2 (calculated by multiplying $K_{1/2}$ to CO_2 : DIC ratio) are lower than the values of K_m reported for Rubisco in red algae and the values of g_p obtained are higher than that proposed by Johnston *et al.* (1992) and Mercado *et al.* (1997) as compatible with carbon uptake solely relying on diffusion of CO_2 through the boundary layer.

The low capacity to use HCO_3^- is consistent with the apparent absence of external carbonic anhydrase activity. However, the presence of external carbonic anhydrase cannot

Fig. 3 Photosynthesis vs photon fluence density (PFD) curves for (a) *Gelidium canariensis* (b) *Gelidium arbuscula* and (c) *Pterocliadiella capillacea*. Measurements were made at dissolved inorganic carbon (DIC) values of 1.1 mol m^{-3} (open circles), 2.2 mol m^{-3} (closed circles) and 5.6 mol m^{-3} (closed triangles). Negative values of O_2 exchange indicate net consumption of O_2 whilst positive values indicate net production of O_2 . Points are means \pm SD, $n = 4$.

Table 4 Ascending slope at limiting photon fluence density (PFDs) (α , mol O₂ per mol photon) and light saturation parameter (I_k , $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) obtained from photosynthesis vs PFD curves. The curves were obtained at three different dissolved inorganic carbon (DIC) concentrations (1.1, 2.2 and 5.6 mol m⁻³) in filtered natural seawater, buffered at pH 8.1 with 50 mol m⁻³ Tris. Values of P are from one-way ANOVA ($n = 4$) conducted to compare the parameter values obtained from the three species. Means with different superscripts were significantly different ($P < 0.05$)

DIC	<i>Gelidium canariensis</i>	<i>Gelidium arbuscula</i>	<i>Pterocladia capillacea</i>	P
1.1 mol m ⁻³				
α	0.07 ± 0.02 ^a	0.05 ± 0.01 ^a	0.02 ± 0.002 ^b	0.025
I_k	19.1 ± 3.4 ^a	28.9 ± 11.4 ^a	77.3 ± 8.6 ^b	0.007
2.2 mol m ⁻³				
α	0.06 ± 0.001	0.05 ± 0.01	0.03 ± 0.006	0.076
I_k	33.2 ± 7.6	47.6 ± 11.3	84.9 ± 27.1	0.057
5.6 mol m ⁻³				
α	0.04 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.127
I_k	64.8 ± 14.5	93.3 ± 10.3	122.1 ± 3.6	0.171

Table 5 Model I two-way ANOVA on photosynthetic rates measured at three different dissolved inorganic carbon (DIC) concentrations (1.1, 2.2 and 5.6 mol m⁻³) and two photon fluence density (PFD) (50 and 600 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) for *Gelidium canariensis*. Interaction was significant for *Gelidium arbuscula* and *Pterocladia capillacea*

Source of variation	ANOVA df	MS	F	Variance percentage
<i>(G. canariensis)</i>				
PFD	1	6526.5	39.89*	0.39
DIC concentration	2	1515.4	9.26*	0.18
Interaction	2	282.2	1.72	0.03
Error	39	163.6		

*mean squares (MS) are significant with respect to error MS ($P < 0.05$).

be totally eliminated on the basis of results from the potentiometric method since Mercado *et al.* (1997) showed that a low activity (but significant from a functional viewpoint) can remain undetected by this method. In addition, Mercado *et al.* (1998b) showed that photosynthesis of *G. sesquipedale* and *G. pusillum* was inhibited with acetazolamide (an inhibitor of external carbonic anhydrase) even though external carbonic anhydrase activity was not detected. The absence of external carbonic anhydrase detectable by the potentiometric method appears to be characteristic of the Gelidiales. Absence of external carbonic anhydrase has also been previously reported for other groups of Rhodophyceae, in particular within members of the orders Nemaliales and Gigartinales (Cook *et al.*, 1986; Giordano & Maberly, 1989; Johnston *et al.*, 1992). Currently, internal carbonic anhydrase activity has been detected in all the red macroalgae studied: the Gelidiales do not appear to be an exception. Indeed, the values obtained in our experiments are comparable to those reported for other macroalgae (Giordano & Maberly, 1989).

Another possibility to explain the (low) capacity for use of HCO₃⁻ in the three species studied is the presence of a mechanism for its direct uptake. The occurrence of these mechanisms in green and brown macroalgae has been described by various authors (Surif & Raven, 1989; Drechsler & Beer, 1991; Axelsson *et al.*, 1995). However, the values of g_p for DIC at alkaline pH reported for these species are much higher (*c.* three–four fold) than the values obtained for the three Gelidiales studied. In addition, according to Axelsson *et al.* (1995), absent (or very low) sensitivity to changes of pH in

the range from 8.1 to 8.7 should be expected if HCO₃⁻ uptake mechanism is operating. Therefore, direct HCO₃⁻ uptake in the studied species is not supported by our data.

Interaction between use of light and DIC acquisition

Inorganic carbon concentration is not usually considered as a relevant factor in determining the response of algae to increasing light (Henley, 1993). By contrast, our results demonstrate that changes in DIC concentration have a relevant effect on the parameters of photosynthesis vs PFD curves. An increase in the DIC concentration above its value in normal seawater produced greater rates of photosynthesis at saturating-PFD (*i.e.* higher P_m) and changes in the light saturation parameter (I_k) which was almost doubled at 5.6 mol m⁻³ with respect to 2.2 mol m⁻³ of DIC. According to Henley (1993), I_k qualitatively designates the region of transition from control of photosynthesis by electron transport to control by DIC assimilation. Therefore, a lower value of I_k indicates a less efficient use of high PFDs by the thalli incubated at low DIC concentration. It can be speculated that the effects of DIC concentration on the parameters of the photosynthesis vs PFD curves were due to changes in the rates of photorespiration. Since photosynthesis was mainly dependent on availability of CO₂ in the three species studied, it is possible that the air-equilibrium concentrations of O₂ and CO₂ result in significant photorespiratory activity (Raven, 1984, 1997; Kerby & Raven, 1985; Beardall, 1989). In our experiments, the CO₂ : O₂ ratio in the medium was modified by changing

the DIC concentration (Mercado & Niell, 1999). It varied from 0.03 to 0.16 mol O₂ mol⁻¹ CO₂ at 1.1 and 5.6 mol m⁻³ of DIC, respectively. A decreased rate of photosynthesis at light saturation, due to changes in the CO₂ : O₂ ratio, has been demonstrated for various algae that possess a C₃-like photosynthetic gas exchange physiology, as is described for the Gelidiales species studied (Surbeck *et al.*, 1962; Mercado & Niell, 1999). By contrast, low sensitivity to O₂ has been demonstrated for algae with a C₄-like gas exchange physiology, due to the presence of a carbon concentration mechanism based on active uptake of HCO₃⁻. It has to be noted that the hypothetical loss of energy due to photorespiration at low DIC concentration was not a relevant factor in decreasing the photosynthetic efficiency at low PFD since α was unaffected by the concentration of DIC.

Acquisition of DIC by the three species studied was affected by the availability of light. Thus, g_p was doubled when photosynthesis vs DIC curves were performed at saturating PFD (600 μ mol photons m⁻² s⁻¹). Johnston *et al.* (1992) reported similar results for four red macroalgae with quite different affinities for DIC. Taking into account the suggestion that photosynthesis in the species studied was mainly dependent on diffusive entry of CO₂, the dependence of g_p on incident PFD is not easy to justify and requires further investigation. However, it could explain the significant interactive effect observed between DIC concentration and PFD.

Ecological implications

Raven (1997) suggested that the limitation of photosynthesis by CO₂ in seawater at light saturation could mean that CO₂ was saturating at the low photon flux density at which these algae grow. Data presented in this study support this suggestion. They indicate that PFD might become a more important factor than availability of DIC in determining the rates of photosynthesis in the species studied. Availability of CO₂ and light vary at different places on shore. At the uppermost intertidal level, PFD is high but an active photosynthesis could lead to important decreases in the availability of CO₂. This is the case in high-intertidal rock pools during neap tides where they may not be flushed by fresh seawater for days at a time (Larsson *et al.*, 1997). By contrast, continuous replenishment of the medium in subtidal or lower intertidal zones permits maintenance of constant CO₂ concentration although incident light is lower. The studied species were collected at the lowest part of the intertidal zone where the medium is continually replenished by the effect of wave splashes. The results obtained accord with the suggestion by Maberly (1990) that species inhabiting the low intertidal shaded regions have a low capacity to extract DIC from seawater at alkaline pH. Furthermore, our data demonstrate that saturation of photosynthesis by DIC is possible at low incident light. Therefore it is probable that, in their natural

habitats, photosynthesis in the three species studied is limited by incident light rather than by DIC concentration in spite of their apparent low affinity for HCO₃⁻. To test this hypothesis, however, the photosynthetic performance must be studied in the field.

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