

Effects of elevated CO₂ on the red seaweed *Gracilaria lemaneiformis* (Gigartinales, Rhodophyta) grown at different irradiance levels

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The red seaweed *Gracilaria lemaneiformis* (Bory) Weber-van Bosse (Gigartinales, Rhodophyta) from Nanao Island, Shantou, China, was cultured at 370 and 700 $\mu\text{l l}^{-1}$ CO₂ in aeration and at intermediate (160 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and low (30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) irradiance levels in order to examine the influences of the elevated atmospheric CO₂ concentrations on growth, photosynthetic performance and some biochemical components in this commercially important species. Relative growth rate (RGR) was significantly higher in *G. lemaneiformis* thalli grown using CO₂-enriched air with respect to nonenriched air when the algae were subjected to intermediate irradiance. However, RGR was similar between these two CO₂ treatments when the algae were grown under the low-irradiance condition. Extra CO₂ in the culture decreased phycobiliprotein (PB, including phycoerythrin, PE, and phycocyanin, PC) contents of *G. lemaneiformis* thalli at the higher growth irradiance. However, chlorophyll *a* (Chl *a*) and soluble protein contents were unchanged by the CO₂ levels in culture. Both PB and Chl *a* contents were higher in *G. lemaneiformis* thalli grown at the lower irradiance than at the higher irradiance, regardless of the CO₂ levels in culture. The parameters for photosynthetic responses to irradiance and inorganic carbon were mostly not altered with the increase of CO₂ concentrations in culture. However, light-saturated photosynthetic rates (P_{max}) and apparent carboxylating efficiencies (ACE), expressed per unit Chl *a*, were significantly higher in algae grown at the intermediate irradiance compared to the low irradiance. Photosynthetic rate was reduced by an increase in pH of seawater from 8.2 to 9.1, and it was also strongly inhibited by the external carbonic anhydrase inhibitor acetazolamide (AZ) in *G. lemaneiformis* thalli grown at each CO₂ and irradiance condition. Moreover, pH compensation points were not affected by the growth conditions. These results suggested that *G. lemaneiformis* under both growth conditions had a similar capacity of the photosynthetic utilization of external HCO₃⁻ pool in seawater. However, ACE decreased in *G. lemaneiformis* thalli grown at the low irradiance with respect to the higher irradiance implied that the transport of Ci towards Rubisco within the cell was weakened. Taken together, the data showed that an increase of CO₂ was less effective on *G. lemaneiformis* than the irradiance levels. We concluded that CO₂ affected photosynthesis and growth performance when light was not the limiting factor.

KEY WORDS: CO₂, *Gracilaria lemaneiformis*, Growth, Irradiance, Photosynthesis, Seaweeds

INTRODUCTION

Burning of fossil fuels and deforestation has been causing atmospheric CO₂ concentration to rise since the onset of the Industrial Revolution. The trend of such an increase of CO₂ in the atmosphere suggests that the present level will double within this century (Houghton *et al.* 2001), which can also be predicted to result in a proportional increase of the dissolved CO₂ and a concomitant reduction of pH in nearshore areas (Stumm & Morgan 1996). Much interest has been provoked in the influences of CO₂ enrichment on plants and ecosystems (e.g. reviews by Bowes 1993; Short & Neckles 1999; Nowak *et al.* 2004).

Seaweeds are a major component of coastal primary productivity and play a key role in the coastal carbon cycle (Reiskind *et al.* 1989). The effects of increased CO₂ concentrations on seaweeds depend largely on the degree of carbon limitation present in natural systems. Photo-

synthesis of seaweeds would be severely limited under current atmospheric and oceanic conditions if it were dependent only on the diffusional entry of CO₂ from the medium to the site of fixation via the carbon assimilating enzyme Rubisco (Beardall *et al.* 1998). However, the photosynthesis in many of the seaweeds would be fully or nearly saturated with the current ambient dissolved inorganic carbon (Ci) composition, mainly because of their efficient utilization of the high HCO₃⁻ concentration available in seawater as the principal bulk source of Ci for photosynthesis (Beer 1994; Beer & Koch 1996; Raven 1997). Use of HCO₃⁻ could allow an increase in the CO₂ concentration around Rubisco and a decrease in the photorespiration (Beer 1994; Raven 1997) and thereby act as carbon-concentrating mechanisms (CCM). Seaweeds show wide-ranging capacities to extract HCO₃⁻ from the pool in seawater. Therefore, they would show a heterogeneous, often species-specific response to elevated CO₂.

It was demonstrated that acclimation to elevated CO₂ not only reduced the carbonic anhydrase activity (CA, EC 4.2.1.1; an enzyme that is linked to the ability of algae to use

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HCO₃⁻ by catalysing the conversion of HCO₃⁻ to CO₂) in *Fucus serratus* (Johnston & Raven 1990), *Ulva* sp. (Björk *et al.* 1993), *Gracilaria tenuistipitata* (García-Sánchez *et al.* 1994) and *Porphyra leucosticta* (Mercado *et al.* 1997) but also decreased the pigments (such as chlorophyll and phycobiliprotein) contents in *G. tenuistipitata* and *P. leucosticta* (García-Sánchez *et al.* 1994; Mercado *et al.* 1999), which would affect the acquisition of Ci and light energy. The responses of seaweeds to elevated CO₂ also depend on the environmental conditions under which CO₂ enrichment is imposed. Since the metabolic pathway of carbon and nitrogen are highly coordinated and coupled, a few authors have focused on the interactive effect of different Ci and nitrogen levels on seaweeds. For example, Levavasseur *et al.* (1991) observed that maximum photosynthetic response to dissolved Ci enrichment was related to thallus N and Rubisco levels in *Ulva rotundata*. It was also found that only under conditions of N sufficiency were the photosynthesis and growth enhanced by Ci enrichment in *Cladophora vagabunda* and *Gracilaria tikvahiae* (Rivers & Peckol 1995) and *G. gaditana* (Andría *et al.* 1999). Gordillo *et al.* (2001) reported that the growth enhancement in *Ulva rigida* by increased levels of CO₂ was entirely dependent on the enhancement effect of CO₂ on N assimilation. However, to our knowledge, no attention has been paid to the responses to CO₂ enrichment of seaweeds grown at varied irradiance levels.

This study focused on the red seaweed *Gracilaria lemaneiformis* (Bory) Weber-van Bosse (Gigartinales, Rhodophyta). This species is an important species for seaweed cultivation in China and is commonly used for a high-quality raw material for the agar industry and as a good food source in abalone aquaculture (Tseng 2001). Moreover, the cultivation of this species can be an effective bioremediation measure for eutrophication control in coastal waters (Fei 2004). Therefore, *G. lemaneiformis* is now being considered for even further development of large-scale cultivation in China. However, little has been documented on the ecophysiology in this species. Our previous investigation demonstrated that its photosynthesis depends on the external CA activity that mediates the dehydration of HCO₃⁻ to CO₂ extracellularly, and the formed CO₂ is then taken up into the cells (Zou *et al.* 2004). The present study examined the responses of growth, photosynthetic properties and some biochemical compositions to atmospheric CO₂ enrichment in *G. lemaneiformis* grown at two different irradiance levels. Two specific questions were asked: (1) Is the ability of *G. lemaneiformis* to acclimate to CO₂ enrichment irradiance dependent? (2) How is the inorganic carbon acquisition by this alga during photosynthesis affected by the growth conditions? In its farming field at Nanao Island, Shantou, China, the thalli of *G. lemaneiformis* were cultivated by ropes hung horizontally, usually at various depths and sometimes vertically in the water column. Thalli of *G. lemaneiformis* are subjected to large fluctuation of growth irradiance availability because of the water depths, daytime and self-shading. Therefore, it is important to examine the combined effects of elevated CO₂ and irradiance levels in this maricultured crop.

MATERIALS AND METHODS

Plant materials

Gracilaria lemaneiformis (Bory) Weber-van Bosse was collected from a cultivation field at Nanao Island, Shantou, China (23°20'N, 116°55'E). The plants were gently rinsed and cleared of visible epiphytes and of any accumulated sediments and then placed in a cooler containing some fresh seawater during the transportation to the laboratory (about 3 h). The samples were maintained in filtered natural seawater (salinity 33‰) supplemented with 100 µM NO₃⁻ and 10 µM H₂ PO₄⁻ (final concentrations) at 20 ± 1°C for 4–6 d prior to further treatment. The seawater medium was aerated vigorously and changed every other day. Florescent illumination provided an irradiance of 100 µmol photons m⁻² s⁻¹ for a light:dark period of 12:12. The irradiance was quantified by means of a quantum sensor (SKP200, ELE International). For practical reasons, the 12-h light cycle was set from 0900 to 2100 (local time).

Experimental treatments

Gracilaria lemaneiformis thalli were cultured under two levels of CO₂ conditions—regular air (actual CO₂ atmospheric levels, *c.* 370 µl l⁻¹) and CO₂-enriched air (700 µl l⁻¹ CO₂ in air)—and two irradiance levels—30 µmol photons m⁻² s⁻¹ (low irradiance, LI) and 160 µmol photons m⁻² s⁻¹ (intermediate irradiance, II). There were three replicates for each treatment. Cultures started when 2.5 g fresh weight (FW) algae were transferred to each of 12 Erlenmeyer flasks containing 3 litres of filtered seawater. Six flasks were placed into each of two CO₂ chambers (Conviron, EFT). One of the CO₂ chambers was programmed to supply a CO₂ concentration of 370 µl l⁻¹, and the other was programmed to supply 700 µl l⁻¹ CO₂. In each of the CO₂ chambers, three of the flasks received incident light of 160 µmol photons m⁻² s⁻¹, and the other three were illuminated with 30 µmol photons m⁻² s⁻¹. The nutrients in seawater medium, temperature and photoperiod conditions were the same as indicated previously. The water motion resulting from the aeration allowed the algae to move gently without tumbling. The measured concentrations of the total dissolved Ci, using the Total Organic Carbon Analyzer (TOC-5000A, Shimadzu), were 2.01 and 2.09 mM, and the pH values were 8.21 and 7.92 in the seawater in equilibrium with regular air and CO₂-enriched air, respectively. The measured total alkalinity (*c.* 2226 µeq l⁻¹) was similar between the seawater equilibrated with regular air and CO₂-enriched air. The estimated concentrations of dissolved CO₂ and HCO₃⁻, according to Strickland & Parsons (1972), were 12.1 and 1795.3 µM in normal seawater, and 25.6 and 1951.5 µM in CO₂-enriched seawater, respectively. Since the seawater media used for culture of *G. lemaneiformis* were not buffered, the pH values of the seawater varied with the daytime mainly because of photosynthetic utilization of dissolved Ci and the aeration. There was a general pattern that pH increased in the light period and decreased in the dark period. The fluctuations of pH values in seawater media were larger in cultures with regular air

and/or intermediate growth irradiance than CO₂-enriched air and/or lower growth irradiance. An increase of CO₂ levels in aeration lowered the pH value of medium approximately 0.3–0.9 units. The algae were harvested and used for experimental measurement after 7–8 d of cultures, a period that would be enough for acclimation in seaweeds (Björk *et al.* 1993; Mercado *et al.* 1999; Andria *et al.* 2001; Zou *et al.* 2003; Zou 2005).

Growth rates and biochemical components

Biomass was measured in each culture to estimate growth. The relative growth rate (RGR), expressed as percentage of increase in FW biomass per day (% d⁻¹), was estimated assuming exponential growth during the culture period according to the formula $RGR = [(\ln W_t - \ln W_o)/t] \times 100$, where W_o represents the initial and W_t the final FW of the algae, and t is the time of culture in days. Excess water was removed by blotting softly on filter paper before FW determination.

To determine chlorophyll *a* (Chl *a*), about 0.2 g FW per sample were extracted in 100% acetone. The concentration of Chl *a* was calculated spectrophotometrically using the equation given by Jensen (1978). For phycobiliprotein (PB) determination, samples of about 0.2 g FW of algal biomass were placed in 5 ml of 0.1 M phosphate buffer (pH 6.8), homogenized at 4°C using a mortar and pestle (with a little acid-washed sand) and rinsed with a further 5 ml of buffer. The extracts were then centrifuged at 5000 × *g* for 20 min. The concentrations of phycoerythrin (PE) and phycocyanin (PC) in the supernatant were determined spectrophotometrically using the chromatic equations of Beer and Eshel (1985). The supernatants were also used to estimate the contents of soluble protein (SP) by the Coomassie Blue G-250 method (Bradford 1976).

The response of photosynthesis to irradiance

Photosynthetic rates were measured as O₂ evolution by using a Clark-type oxygen electrode (YSI Model 5300) at 20°C, which was maintained with a circulating water bath (Cole Parmer). The illumination was provided by a halogen lamp. It had been shown that proton buffers (such as TRIS) could disturb photosynthetic utilization of Ci in the brown macroalga *Laminaria* spp. (Axelsson *et al.* 2000; Klenell *et al.* 2004; Mercado *et al.* 2006) and other macrophytes (Hellblom *et al.* 2001; Beer *et al.* 2002; Uku *et al.* 2005) because these buffers would eliminate proton extrusion, forming low pH (acid zones) in the external HCO₃⁻ dehydration on the thalli surface. However, in the case of the red seaweed *G. lemaneiformis*, we had previously shown that TRIS had no inhibitory effects on carbon acquisition by this alga (Zou *et al.* 2004). Therefore, photosynthetic rates in responses to irradiance, amount of Ci, pH and acetazolamide, as described below, were determined using TRIS buffer.

About 0.15 g FW of segments of the algal samples were introduced into the electrode chamber with 8 ml of filtered TRIS (25 mM)-buffered (pH 8.2) natural seawater, which was magnetically stirred. The algal samples were allowed to equilibrate in the darkness until the rate of oxygen

consumption was constant, usually for approximately 4–6 min, and respiratory rate (R_d) was monitored. The sample was exposed to a series of increasing irradiance from 0 to 600 μmol photons m⁻² s⁻¹, which was adjusted by changing the distance between the light source and the assimilation chamber. Parameters for the photosynthetic response to irradiance (P-E curve) were analyzed. The light-saturated photosynthetic rate (P_{max}), which was normalized to biomass (FW) and Chl *a*, respectively, was calculated from the mean values in the asymptote region of the P-E curve. Apparent photosynthetic efficiency (α) was estimated as the ascending slope at limiting irradiance levels. The light saturation (E_k) and compensation points (E_c) were calculated as (R_d + P_{max})/α and R_d/α, respectively, according to Henley (1993).

The response of photosynthesis to inorganic carbon

The response of net maximal photosynthesis to Ci concentration (P-C curve) was measured at 20°C and saturating irradiance of 400 μmol photons m⁻² s⁻¹ using the oxygen electrode described above. The Ci-free seawater was prepared by acidifying the filtered seawater to pH less than 4.0 with 0.5 M HCl and sparging for at least 2 h with high-purity N₂ gas to remove the Ci in seawater. TRIS buffer was added to give a final concentration of 25 mM, and the pH was adjusted to 8.0 with freshly prepared 0.5 M NaOH. About 0.2 g of segments of *G. lemaneiformis* samples were transferred to the assimilation chamber containing 8 ml of Ci-free seawater. Samples were left to photosynthesize to consume the possible remaining Ci of the buffer and the intracellular and intercellular pools of Ci until zero net oxygen evolution was reached. Different amounts of 200 mM NaHCO₃ stock solution were then injected into the chamber, and photosynthesis was measured over a range of Ci concentrations up to 6.6 mM. The apparent carboxylating efficiency (ACE), that is, the initial slope of P-C curve, was calculated by linear regression over the range of 0–0.55 mM Ci. This value was used to indicate how effectively algae use low concentration of Ci, as discussed by Johnston *et al.* (1992). The half-saturated constant of photosynthesis for total Ci (K_{m(Ci)}) was estimated using a double-reciprocal plot of the rates of O₂ evolution and the Ci concentrations.

The effects of pH and acetazolamide on photosynthesis

Photosynthetic rates were measured in natural filtered seawater buffered at pH values of 8.2 and 9.1 with 25 mM (final concentration) TRIS buffer. The light-temperature condition was the same as the measurement of P-C curve. After a constant oxygen evolution rate was reached, acetazolamide (AZ) solution was injected into the chamber to a final concentration of 200 μM, and then the oxygen evolution was recorded again within 3–6 min. Stock solution of AZ was prepared with 0.05 M NaOH to a concentration of 50 mM. It is generally assumed that AZ inhibits the extracellular CA activity (Haglund *et al.* 1992; Axelsson *et al.* 1995).

pH compensation point

In order to obtain the pH compensation point (an indicator of the algae's ability to utilize HCO₃⁻; Johnston & Raven 1990; Maberly 1990; Johnston *et al.* 1992) of *G. lemaneiformis* grown under different conditions, pH-drift experiments were conducted in sealed glass vials containing 0.8 g fresh algae and 20 ml unbuffered natural seawater at the same light-temperature condition adopted in the measurement of P-C curve. The final pH values were determined until there were no further increases (after 5–8 h).

Statistics

The data were expressed as the mean values ± standard deviation (*s*). The significance of the data was tested with statistical analysis using SPSS for Windows version 10, including the analysis of variance and *t* test. The significance level was set at *P* < 0.05.

RESULTS

The growth of *G. lemaneiformis* thalli remained positive in each culture. Additional CO₂ concentration in the culture enhanced the growth rate by 78% (*P* < 0.01) relative to control at the intermediate growth irradiance. However, growth rate in the culture with extra CO₂ was only marginally (*P* > 0.05) higher than normal CO₂ when *G. lemaneiformis* thalli were subjected to a low growth irradiance level (Fig. 1).

Raising the CO₂ levels in aeration did not affect the Chl *a* content of *G. lemaneiformis* thalli. However, Chl *a* content increased significantly (*P* < 0.01) in the culture under the lower growth irradiance with respect to the higher growth irradiance (Table 1). Both PE and PC contents were remarkably (*P* < 0.01) greater in *G. lemaneiformis* thalli grown at the low irradiance than the intermediate irradiance, regardless of the CO₂ levels in culture. Moreover, an enrichment of CO₂ in the culture significantly (*P* < 0.01) decreased both PE and PC contents in thalli grown at the higher irradiance, but that caused only a slight (*P* > 0.05) decrease of BP contents under the low irradiance condition. The soluble protein content was insensitive to the culture conditions. The growth condition affected the ratio of Chl *a* to BP content, with the highest value being obtained in the culture at high CO₂ and intermediate

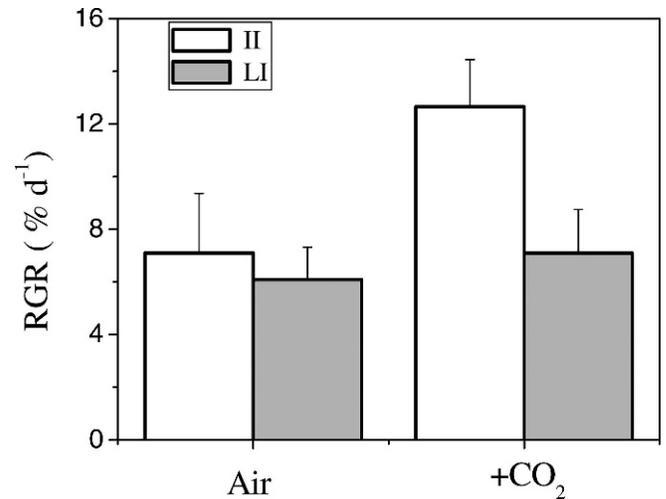


Fig. 1. Relative growth rate (RGR) of *Gracilaria lemaneiformis* cultured under regular (370 μl l⁻¹ CO₂, air) and CO₂-enriched (700 μl l⁻¹ CO₂, +CO₂) air and under intermediate irradiance (160 μmol photons m⁻² s⁻¹, II) and low irradiance (30 μmol photons m⁻² s⁻¹, LI). Vertical bars represent ± *s* of the means (*n* = 4).

irradiance and the lowest value being found in the treatment with regular CO₂ and low irradiance (Table 1).

The photosynthetic response of *G. lemaneiformis* thalli to irradiance is illustrated in Fig. 2. No photoinhibition was observed over the irradiance range tested (0–600 μmol photons m⁻² s⁻¹). *Gracilaria lemaneiformis* thalli exhibited a light-saturated net photosynthetic rate (*P*_{max}) of about 51.8–60.3 μmol O₂ g⁻¹ FW h⁻¹ or 315.4–454.3 μmol O₂ mg⁻¹ Chl *a* h⁻¹. *Gracilaria lemaneiformis* grown in normal CO₂ showed similar *P*_{max}, dark respiration (*R*_d), apparent photosynthetic efficiency (*α*), light saturation (*E*_k) and compensation points (*E*_c) than the algae grown in additional CO₂. However, *P*_{max} normalized to Chl *a* was higher (*P* < 0.05) in thalli grown at the higher irradiance than at the lower irradiance. Additionally, *R*_d, *E*_c and *E*_k tended to decrease, but *α* expressed per unit FW tended to increase, with the lower growth irradiance relative to the higher growth irradiance (Table 2).

The photosynthetic responses to *C*_i concentration are shown in Fig. 3. Photosynthetic rate was fully or nearly saturated at 2.2 mM of *C*_i, the *C*_i concentration representative of that in normal natural seawater. Low growth irradiance tended to increase the values of *K*_{m(*C*_i)} (the *C*_i

Table 1. Chlorophyll *a* (Chl *a*), phycoerythrin (PE), phycocyanin (PC), and soluble protein (SP) contents in *Gracilaria lemaneiformis* cultured at regular (370 μl l⁻¹ CO₂, air) and CO₂-enriched (700 μl l⁻¹ CO₂, +CO₂) air and at intermediate (160 μmol photons m⁻² s⁻¹, II) and low (30 μmol photons m⁻² s⁻¹, LI) irradiance levels for 6–8 d. Values are means ± *s* (*n* = 4); different superscripts indicate significant difference (*P* < 0.05).

	Air		+CO ₂	
	II	LI	II	LI
Chl <i>a</i> (mg g ⁻¹ FW)	0.131 ± 0.01 ^a	0.168 ± 0.011 ^b	0.114 ± 0.013 ^a	0.175 ± 0.062 ^b
PE (mg g ⁻¹ FW)	0.510 ± 0.004 ^a	1.068 ± 0.066 ^b	0.440 ± 0.011 ^c	1.009 ± 0.061 ^b
PC (mg g ⁻¹ FW)	0.162 ± 0.001 ^a	0.210 ± 0.008 ^b	0.128 ± 0.009 ^c	0.199 ± 0.004 ^b
SP (mg g ⁻¹ FW)	16.56 ± 2.72 ^a	17.01 ± 3.59 ^a	14.23 ± 2.55 ^a	17.01 ± 2.09 ^a
Chl <i>a</i> /(PE + PC)	0.195	0.131	0.201	0.145

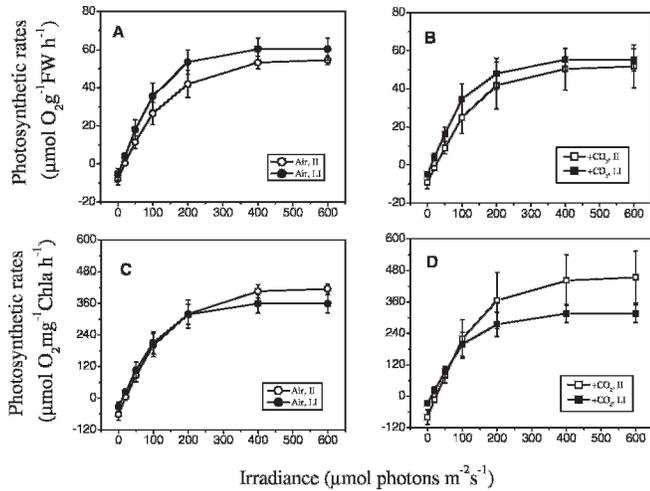


Fig. 2. Net photosynthetic O₂ evolution rates versus irradiance curves for *Gracilaria lemaneiformis* cultured under different conditions. Photosynthetic rates were normalized to biomass (FW; A, B) and Chl *a* (C, D), respectively. Vertical bars represent $\pm s$ of the means ($n = 4$). Aeration with regular air and intermediate growth irradiance (370 $\mu\text{l l}^{-1}$ CO₂ + 160 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, air, II). Aeration with regular air and low growth irradiance (370 $\mu\text{l l}^{-1}$ CO₂ + 30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, air, LI). Aeration with CO₂-enriched air and intermediate growth irradiance (700 $\mu\text{l l}^{-1}$ CO₂ + 160 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, +CO₂, II). Aeration with CO₂-enriched air and low growth irradiance (700 $\mu\text{l l}^{-1}$ CO₂ + 30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, +CO₂, LI).

concentration to give the half-saturated rate of photosynthesis); although, there were no statistically significant differences for values of $K_{m(\text{Ci})}$ due to the large standard deviation. Extra CO₂ in culture had also hardly effect on the $K_{m(\text{Ci})}$ values. The initial slope of the P-C curves (apparent carboxylating efficiency, ACE), expressed per unit Chl *a*, was significantly ($P < 0.01$) reduced with the lower growth irradiance with respect to the higher growth irradiance, regardless of the CO₂ levels in culture (Table 3). Additionally, CO₂ enrichment in culture decreased the FW-normalized ACE compared to normal CO₂ when the algae were grown at intermediate irradiance.

Table 2. Photosynthetic parameters of the P-E curves in *Gracilaria lemaneiformis* cultured under regular (370 $\mu\text{l l}^{-1}$ CO₂, air) and CO₂-enriched (700 $\mu\text{l l}^{-1}$ CO₂, +CO₂) air and under intermediate irradiance (160 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, II) and low irradiance (30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, LI). Values were derived from Fig. 2. Photosynthetic rates were normalized to FW (A) and Chl *a* (B). Values are means $\pm s$ ($n = 4$); different superscripts indicate significant difference ($P < 0.05$).

	Air		+CO ₂	
	II	LI	II	LI
A				
P _{max} ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ FW h}^{-1}$)	54.5 \pm 2.3 ^a	60.3 \pm 5.8 ^a	51.8 \pm 11.3 ^a	55.2 \pm 5.9 ^a
R _d ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ FW h}^{-1}$)	-8.1 \pm 2.8 ^{ab}	-5.5 \pm 3.1 ^a	-9.2 \pm 3.2 ^b	-4.9 \pm 1.1 ^a
α [$(\mu\text{mol O}_2 \text{ g}^{-1} \text{ FW h}^{-1})/(\mu\text{mol photons m}^{-2} \text{ s}^{-1})$]	0.390 \pm 0.045 ^a	0.470 \pm 0.082 ^a	0.362 \pm 0.049 ^a	0.420 \pm 0.067 ^a
E _c ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)	20.9 \pm 7.9 ^{ab}	12.2 \pm 6.4 ^a	25.5 \pm 9.2 ^b	11.9 \pm 3.6 ^a
E _k ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)	161.8 \pm 16.3 ^{ab}	143.3 \pm 25.9 ^{ab}	168.2 \pm 12.8 ^b	144.2 \pm 9.3 ^a
B				
P _{max} ($\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$)	415.8 \pm 17.6 ^a	358.8 \pm 34.6 ^b	454.3 \pm 99.3 ^a	315.4 \pm 33.6 ^b
R _d ($\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$)	-62.1 \pm 21.7 ^{ab}	-33.0 \pm 18.2 ^a	-80.8 \pm 28.5 ^b	-27.8 \pm 6.5 ^a
α [$(\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1})/(\mu\text{mol photons m}^{-2} \text{ s}^{-1})$]	2.98 \pm 0.34 ^{ab}	2.80 \pm 0.49 ^{ab}	3.17 \pm 0.43 ^a	2.40 \pm 0.38 ^b
E _c ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)	20.9 \pm 7.9 ^{ab}	12.2 \pm 6.4 ^a	25.5 \pm 9.2 ^b	11.9 \pm 3.6 ^a
E _k ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)	161.8 \pm 16.3 ^{ab}	143.3 \pm 25.9 ^{ab}	168.2 \pm 12.8 ^b	144.2 \pm 9.3 ^a

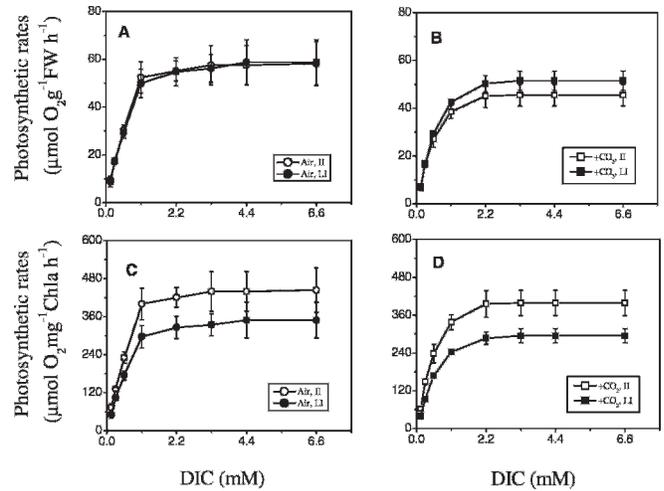


Fig. 3. Net photosynthetic O₂ evolution rates versus inorganic carbon concentrations curves for *Gracilaria lemaneiformis* cultured under different conditions. Photosynthetic rates were normalized to biomass (FW; A, B) and Chl *a* (C, D), respectively. Vertical bars represent $\pm s$ of the means ($n = 3$). Symbols as in Fig. 2.

Figure 4 shows the photosynthetic rates of *G. lemaneiformis* thalli at two pH values: 8.2 and 9.1, and with the inhibitor of the external CA, acetazolamide (AZ). At both pH 8.2 and 9.1, *G. lemaneiformis* thalli grown at the intermediate irradiance tended to have higher Chl-normalized photosynthetic rates relative to the thalli grown at the low irradiance. The rates of photosynthesis measured at pH 8.2 or 9.1 were similar between the normal CO₂ and extra CO₂ in cultures. For all the culture treatments, the increase of pH from 8.2 to 9.1 caused a significant ($P < 0.01$) reduction in photosynthetic rates. Moreover, at both pH 8.2 and 9.1, AZ strongly inhibited the photosynthetic rates in all the growth treatments, with the inhibition percentage being higher at pH 9.1 (c. 70%) than at pH 8.2 (c. 30%).

The pH compensation point of *G. lemaneiformis* thalli was around 9.6, and it was not affected ($P > 0.05$) by the growth conditions (Fig. 5).

Table 3. Parameters for photosynthetic responses to inorganic carbon (P-C curves) in *Gracilaria lemaneiformis* cultured under regular (370 $\mu\text{l l}^{-1}$ CO₂, air) and CO₂-enriched (700 $\mu\text{l l}^{-1}$ CO₂, +CO₂) air and under intermediate irradiance (160 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, II) and low irradiance (30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, LI). Values were derived from Fig. 3. Values are means \pm s ($n = 3$); different superscripts indicate significant difference ($P < 0.05$).

	Air		+CO ₂	
	II	LI	II	LI
ACE [($\mu\text{mol O}_2 \text{ g}^{-1} \text{ FW h}^{-1}$)/(mM)]	45.5 \pm 5.5 ^a	44.3 \pm 6.6 ^{ab}	34.2 \pm 2.8 ^c	38.6 \pm 1.5 ^{bc}
ACE [($\mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl } a \text{ h}^{-1}$)/(mM)]	354.9 \pm 41.9 ^a	263.8 \pm 39.4 ^b	300.3 \pm 24.9 ^a	220.6 \pm 8.3 ^b
K _{m(Ci)} (mM)	1.04 \pm 0.27 ^a	1.48 \pm 0.78 ^a	1.18 \pm 0.29 ^a	1.85 \pm 0.64 ^a

DISCUSSION

The present results showed that the growth response of *G. lemaneiformis* to high levels of CO₂ in seawater was irradiance dependent, with enhanced growth occurring under the intermediate irradiance condition but no response under the low irradiance condition. Similar results were noted in *Ulva rigida* (Gordillo *et al.* 2001), in which high CO₂ in culture increased markedly the growth rate under nitrogen sufficiency and was only slightly changed with nitrogen limitation. These responses contrast with the colimitation of growth in freshwater macrophytes by light and CO₂, which demonstrated that marked growth enhancement by elevated Ci occurred at both high and low irradiance levels (Madsen & Sand-Jensen 1994; Andersen & Pedersen 2002).

Our previous work (Zou *et al.* 2004) showed that *G. lemaneiformis* possessed the ability of using the bulk HCO₃⁻ pool in seawater as a major source of Ci for photosynthesis, which involved CO₂ formation extracellularly from HCO₃⁻ dehydration catalyzed by external CA activity. In the present study, the aeration with an increase in atmospheric CO₂ would cause a proportional increase in the concentration of dissolved CO₂, which consequently would cause a slight shift in the dissolved Ci equilibrium and a concom-

itant decrease of pH value in the culture medium. During the light period, the pH value in the culture medium rose, which could be attributed to the photosynthetic utilization of HCO₃⁻ by *G. lemaneiformis* thalli from the surrounding medium, producing OH⁻ and CO₂ from the HCO₃⁻ dehydration via the mediation of external CA. It was evident that in the light period, the production rate of OH⁻ ions resulting from photosynthesis was faster than that of H⁺ ions from dissociation of hydrated CO₂ stemming from the aeration. It has been demonstrated that the current ambient Ci composition in natural seawater (pH 8.2, 2.2 mM Ci) could fully saturate photosynthesis of *G. lemaneiformis*; however, when pH in seawater rose, the affinity for Ci was dramatically reduced, and photosynthesis became Ci limited (Zou *et al.* 2004). Therefore, photosynthesis of *G. lemaneiformis* in the culture with the intermediate irradiance might be Ci limited because of the pH rise in the medium, and additional CO₂ in the culture would consequently enhance *in situ* photosynthesis and thereby growth. In contrast, the pH rise in the medium under the low irradiance might not reach the extent to cause the Ci limitation of photosynthesis, and the main factor limiting photosynthesis was irradiance instead of CO₂. Therefore, the photosynthesis and growth of *G. lemaneiformis* under low irradiance showed no conspicuous response to the extra CO₂ in the culture.

Photosynthetic acclimation in seaweeds to a high level of Ci generally resembles the responses to higher irradiance, resulting in a decrease of pigment contents. For example, the

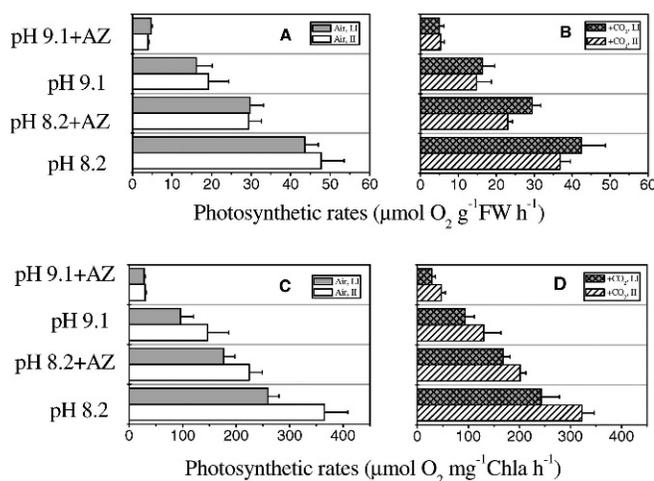


Fig. 4. Net photosynthetic O₂ evolution rates of *Gracilaria lemaneiformis* cultured under different conditions. O₂ evolution was measured at two different pH values: 8.2 and 9.1. Acetazolamide (AZ), an inhibitor of the external CA, was added at 200- μM final concentration. Photosynthetic rates were normalized to biomass (FW; A, B) and Chl *a* (C, D), respectively. Vertical bars represent \pm s of the means ($n = 3$). Symbols as in Fig. 2.

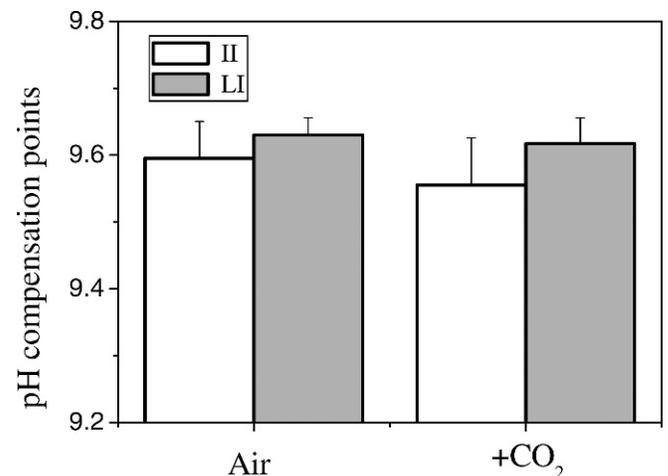


Fig. 5. pH compensation points of *Gracilaria lemaneiformis* cultured under different conditions. Vertical bars represent \pm s of the means ($n = 4$). Symbols as in Fig. 1.

PB (PE and PC) and Chl *a* contents were reduced in *Gracilaria sp.* (Andr a *et al.* 1999, 2001), *G. tenuistipitata* (Garc a-Sanchez *et al.* 1994) and *P. leucosticta* (Mercado *et al.* 1999) cultured at high levels of Ci (up to 5% CO₂ in air) with respect to those at normal Ci levels. The present results also showed that PB (PE and PC) contents in *G. lemaneiformis* decreased significantly with aeration of CO₂-enriched air compared to normal air when the algae were exposed to the higher growth irradiance. In the culture with elevated CO₂ and higher irradiance, it was quite possible that PB were being used as an N reservoir. Enhanced growth would require increased N availability, and BP was then used as an N source in case of a need for N. This gave a physiological explanation for the decreased PB contents in *G. lemaneiformis* grown at elevated CO₂ and higher irradiance. However, the PB contents were not changed by an increase of CO₂ levels in culture at the lower growth irradiance. Additionally, the Chl *a* contents in *G. lemaneiformis* were unaffected by the CO₂ levels in culture, regardless of the growth irradiance levels. This agreed with the results found in *Fucus serratus* (Johnston & Raven 1990) and *Enteromorpha intestinalis* (Andr a *et al.* 2001). Low growth irradiance increased the photosynthetic pigment contents of *G. lemaneiformis* thalli with respect to the higher growth irradiance, regardless of the background CO₂ levels in culture. It was noted that the relative increase of PE caused by low growth irradiance was larger compared to that of other pigments, implying an acclimatory response of *G. lemaneiformis* grown under low irradiance to a possibly green-enriched light spectrum in field.

The elevation of CO₂ levels in culture had little effect on the parameters of photosynthetic responses to irradiance measured in natural seawater, regardless of the units (either FW or Chl *a* basis) for expressing photosynthesis (Table 2). This suggested that the Rubisco carboxylating capacity and the efficiencies of light energy harvesting and conversion were not changed in *G. lemaneiformis* following the culture at high CO₂. These results were in many ways parallel to those found in *Gracilaria sp.* (Gao *et al.* 1993) and *P. leucosticta* (Mercado *et al.* 1999). In contrast, *F. serratus* (Johnston & Raven 1990), *G. tenuistipitata* (Garc a-Sanchez *et al.* 1994) and *U. rigida* (Gordillo *et al.* 2003) grown at high CO₂ exhibited decreased P_{max} and photosynthetic efficiencies. It appeared that a general pattern for photosynthetic acclimation in seaweeds to elevated CO₂ was not evident. On the other hand, *G. lemaneiformis* thalli exhibited higher P_{max} on the basis of Chl *a* when grown at the higher irradiance relative to the lower irradiance level. This response was in accord with that described in the literature regarding the algae acclimated to varied irradiance levels (e.g. Falkowski & LaRoche 1991; Mercado *et al.* 2000).

The data obtained from the inhibition of photosynthesis to AZ and the increase of pH from 8.2 to 9.1, as well as the pH-drift experiments, indicated that *G. lemaneiformis* was capable of using HCO₃⁻ as its Ci source to drive photosynthetic carbon fixation, as shown in our previous work (Zou *et al.* 2004). The present work further demonstrated that the way in which Ci was utilised in this alga was not altered with the growth conditions, the CO₂ and/or irradiance levels in culture. In addition, the pH compensation points and the sensitivity to pH and AZ were

not changed by the different treatments in culture, suggesting that *G. lemaneiformis* exhibited the similar capability of using the external HCO₃⁻, regardless of the background CO₂ and/or irradiance levels in culture. However, the apparent carboxylating efficiency (ACE), that is, the initial slope of the P-C curve, was significantly higher in the intermediate irradiance-grown *G. lemaneiformis* thalli than the low irradiance-grown thalli. It was also reported that the photosynthetic conductance for Ci (i.e. the effectiveness of Ci acquisition) was reduced at low growth irradiance compared to high irradiance in *Palmaria palmata* (K bler & Raven 1995) and *G. tenuistipitata* (Mercado *et al.* 2000). The increase of the photosynthetic conductance was suggested to be related to the activation of the Ci-uptake mechanism. There were two major steps in *G. lemaneiformis* involved in the supply of CO₂ to Rubisco from the external pool of HCO₃⁻. First, the external conversion of HCO₃⁻ into CO₂ by external CA and, second, the transport of Ci within the cell close to the carboxylating site of Rubisco for final photosynthetic CO₂ fixation. Therefore, the differences of ACE between *G. lemaneiformis* grown at the higher and lower irradiance could not be attributed to the changes in the capacity to use the external HCO₃⁻ but might be attributable to the changes in the transport of Ci toward Rubisco within the cell, as suggested by Mercado *et al.* (2000). Whether the Rubisco carboxylating capacity and/or the electron transport capacity were also enhanced in the intermediate irradiance-grown *G. lemaneiformis* thalli relative to low irradiance-grown thalli is yet to be addressed.

In conclusion, the results from our investigation have shown that there was a interaction between elevated CO₂ and irradiance levels on the red seaweed *G. lemaneiformis* in that the growth rate responded positively but only marginally to the CO₂ enrichment in seawater when the algae were subjected to the intermediate irradiance or low irradiance, respectively. It was evident that CO₂ concentrations in culture had a weaker effect than the irradiance levels on the photosynthetic performance and biochemical components in this maricultured species.

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