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Influence of enhanced CO_2 on growth and photosynthesis of the red algae *Gracilaria* sp. and *G. chilensis*

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Abstract

The influence of elevated CO_2 concentrations on growth and photosynthesis of *Gracilaria* sp. and *G. chilensis* was investigated in order to procure information on the effective utilization of CO_2. Growth of both was enhanced by CO_2 enrichment (air + 650 ppm CO_2, air + 1250 ppm CO_2), the enhancement being greater in *Gracilaria* sp. Both species increased uptake of NO_3^- with CO_2 enrichment. Photosynthetic inorganic carbon uptake was depressed in *G. chilensis* by pre-culture (15 days) with CO_2 enrichment, but little affected in *Gracilaria* sp. Mass spectrometric analysis showed that O_2 uptake was higher in the light than in the dark for both species and in both cases was higher in *Gracilaria* sp. The higher growth enhancement in *Gracilaria* sp. was attributed to greater depression of photorespiration by the enrichment of CO_2 in culture.

Introduction

The role of the oceans in the global carbon cycle is of great concern, particularly in the light of evidence of increased global warming. The ocean pool of carbon contains an amount of dissolved inorganic carbon ($CO_2 + HCO_3^- + CO_3^{2-}$) more than fifty times that in the atmosphere as CO_2. By examining the ratio of various isotopes of carbon, Quay *et al.* (1992) have demonstrated that CO_2 from the combustion of fossil fuels has been taken up by the ocean over the past 20 years. Thus oceanic processes, including physical, chemical and biological ones, play an important role in controlling the atmospheric CO_2 level and global warming.

Marine macroalgae play an important role in carbon cycle in coastal ecosystems adjacent to populated areas. It therefore is important to examine how marine macroalgae respond to elevated CO_2 concentrations. Is the dissolved inorganic carbon (DIC) concentration in seawater high enough to bring about maximum photosynthetic rates for macroalgae? Photosynthesis by most macroalgae is probably limited by inorganic carbon sources in natural seawater (Surif & Raven, 1989; Maberly, 1990; Gao *et al.*, 1991; Levavasseur *et al.*, 1991).

Photosynthetic characteristics are affected by high CO_2 (0.5–5%) in microalgae (see Coleman, 1991; Raven, 1991). Cells that are adapted to a low CO_2 environment typically have higher affinity for CO_2, a lower CO_2 compensation point and a higher pH compensation point compared to those adapted to high CO_2 concentrations. This indicates the presence of a CO_2-concentrating mechanism when cells are grown in low CO_2 environment. It has only been in the last few years that scientists have begun to examine the response of macroalgae to high CO_2 concentrations. Johnston and Raven (1990) reported that *Fucus serratus* survived in 5% CO_2 for three weeks and its photosynthetic physiology was affected in much the same way that had been reported for microalgae. Gao *et al.* (1991) demonstrated that elevated CO_2 concentrations, up to 5 times ambient levels, enhanced the growth of the red alga *Porphyra yezoensis* under optimal light and nutrient conditions. The ecology and physiology of *Gracilaria*, being a commercially important agar-producing macroalga, have been well characterized (e.g. Lapointe, 1987; Lignell & Pedersen, 1989; Hanelt, 1992).

By examining the response of growth and photosynthesis of *Gracilaria* sp. and *G. chilensis* to CO_2 enrichment, this study provides new and useful information for evaluation of the ecological impacts of increased atmospheric CO_2, as well as information about biomass production and CO_2 remediation via macroalgae.

Material and methods

Gracilaria sp. was collected on 16 October 1991 from a depth of 1 m below mean lower low water in Tosa Bay, Shikoku, Japan, and was maintained in a cooler and transported to the laboratory within 3 h. *G. chilensis* (Bird & McLachlan, 1986) was collected in October 1991 from a cultivation site (water depth 2–6 m) off Coquimbo, Chile by M. Ohno, who provided the plant. *G. chilensis* was maintained in flowing seawater under natural sunlight at Usa Marine Biological Institute, Kochi University, Shikoku, Japan, before being transported to our laboratory. Plants were washed in filtered seawater, examined and ones free from epiphytes and diseased tissue were selected for experimentation.

Cultures of both species were initiated on 16 October, 1991. Six cylindrical vessels (15 cm high, 14 cm diameter, with screw-cap and air inlet and outlet) were placed in three incubators under 12:12 LD cycle at 20 °C (light period 0800–2000 at 300 μmol photon m^{-2} s^{-1}). Cultures were subjected to aeration (500 ml min^{-1}) with air (350 ppm CO_2), air + 650 ppm CO_2 and air + 1250 ppm CO_2. The system for CO_2 supply and monitoring remained the same as previously reported (Gao *et al.*, 1991). Variation in CO_2 concentrations in the air was less than 5%. For culture medium, seawater (2.1 μM inorganic N, 0.2 μM inorganic P) collected from the sea off Miyazu facing the Sea of Japan was filtered (Whatman GF/C) and enriched with PES medium (Provasoli, 1966) (20 ml l^{-1} seawater). Ten segments (apical, 20–30 cm long, fresh weight about 0.5 g) from 10 plants were placed in each vessel with 1 l culture medium. The culture medium was renewed every other day throughout the experiment. Fresh weight was measured after blotting water drops off the thalli with tissue paper just before the renewal of culture medium. Relative growth rate (RGR, % day^{-1}) was calculated as:

$$\mathrm{RGR} = \frac{100\ \ln(N_t/N_0)}{t},$$

where N_0 is the initial fresh weight and N_t the fresh weight after t days.

Dissolved inorganic carbon (DIC) concentrations in culture medium were measured by infrared analysis using a Shimadzu total organic carbon analysis unit (TOC-5000). CO_2 concentrations in air at inlets and outlets of the culture vessels were monitored by infrared gas analyzer (IRGA, Shimadzu URA-107, including a continuous measuring unit, IRA-107). Changes in pH of cultures were measured and recorded using a Hanna pH meter (HI8418).

Nitrate uptake was estimated from the decrease

in concentration of NO_3^- in the culture medium. NO_3^- concentration was determined by the brucine color development method (Iwamoto, 1985) modified for seawater analysis.

Photosynthesis was determined as the rate of decreased DIC in filtered (Whatman GF/C) seawater (50 ml) in sealed tubes with thalli (0.5–0.6 g fresh weight), which were incubated for 20 min (incubation longer than 30 min lowered the rate of inorganic carbon uptake) at 20 °C and 300 μmol photon m^{-2} s^{-1} (photosynthesis of both species saturates at 300 μmol photon m^{-2} s^{-1}; Gao, 1992). During the incubation, the tubes with seawater and thalli and those with only seawater were shaken on a shaker, and the decrease in DIC was determined from the difference in DIC concentration between the tubes with and without the thalli.

The evolution of $^{16}O_2$ and uptake of $^{18}O_2$ in the light during photosynthesis were determined simultaneously at 10-s intervals by using a gas-permeable membrane-mass spectrometer system. A silicon membrane (125 μm thick) probe was inserted into a cylindrical reaction vessel (35 ml, 3 cm in diameter) to separate the medium (filtered seawater) from the high-vacuum inlet to a quadrupole mass spectrometer (Ametek M200). The seawater was bubbled with N_2 for 30 minutes to expel $^{16}O_2$ before dissolving $^{18}O_2$ into it. Correction was made for the amount of $^{16}O_2$ consumed by respiration according to Radmer and Ollinger (1980). The reaction vessel was equipped with a water jacket and the temperature within it was controlled at 20 °C.

DIC concentrations of seawater for photosynthesis measurement were modified by first removing DIC by acidification below pH 3 with HCl, and then bubbling CO_2-free air through the water for 5 h to remove gaseous CO_2. The pH was elevated to 8.2–8.4 with addition of NaOH under the CO_2-free air. Inorganic carbon was added to the jars in the form of $NaHCO_3$ and the pH was finally adjusted to 8.22 ± 0.01 with HCl and NaOH. The calculated DIC concentrations were confirmed by measuring the DIC with the total organic analysis unit.

Results

Growth of *Gracilaria* sp. and *G. chilensis* was enhanced by the treatments air + 650 ppm CO_2 and air + 1250 ppm CO_2 compared to controls (Fig. 1). The greatest growth was found in air + 1250 ppm CO_2 treatment. The growth rates per day with + 650 ppm CO_2 and + 1250 ppm CO_2 averaged 11.4% and 14.2% in *Gracilaria* sp. and 10.2% and 14.3% in *G. chilensis*, respectively. Despite similar growth rates under CO_2 enrichment, the growth rate for the *Gracilaria* sp. control was only 4.9%, while that for *G. chilensis* was 8.8%, with the latter approximately 1.8 times that of the former. Compared to the controls, relative enhancement of growth for treatments at + 650 ppm CO_2 and + 1250 ppm CO_2 was 130% and 190% greater for *Gracilaria* sp., but only 20% and 60% greater for *G. chilensis*.

Daily variations of pH for both *Gracilaria* sp. and *G. chilensis* cultures (Fig. 2) showed similar patterns independent of CO_2 concentrations in that pH decreased to the lowest values during dark periods, but rose to the highest values in the light.

Aeration with additional CO_2 resulted in a faster drop of pH in the dark. Daily variation in DIC concentration (Fig. 2) showed the opposite pattern, i.e., DIC increased to reach its highest values during the dark period and decreased to reach its lowest values during the light period. DIC concentrations at the middle of the light period were as low as 1 mM in controls (+ 0) and averaged in 1.5 mM and 2.0 mM in + 1250 ppm CO_2 cultures for *Gracilaria* sp. and *G. chilensis*, respectively.

Net CO_2 losses due to photosynthesis and dissolution of CO_2 were measurable in all treatments (air, air + 650 and + 1250 ppm CO_2). CO_2 loss was greater in the treatments with enriched CO_2 concentrations (Table 1).

Enrichment by CO_2 resulted in greater decreases in NO_3^- concentrations in both species (Fig. 3). NO_3^- concentrations decreased sharply at the beginning of the light period in all cultures, indicating that the uptake of NO_3^- is an energy-linked process. The rate of NO_3^- uptake was 0.5,

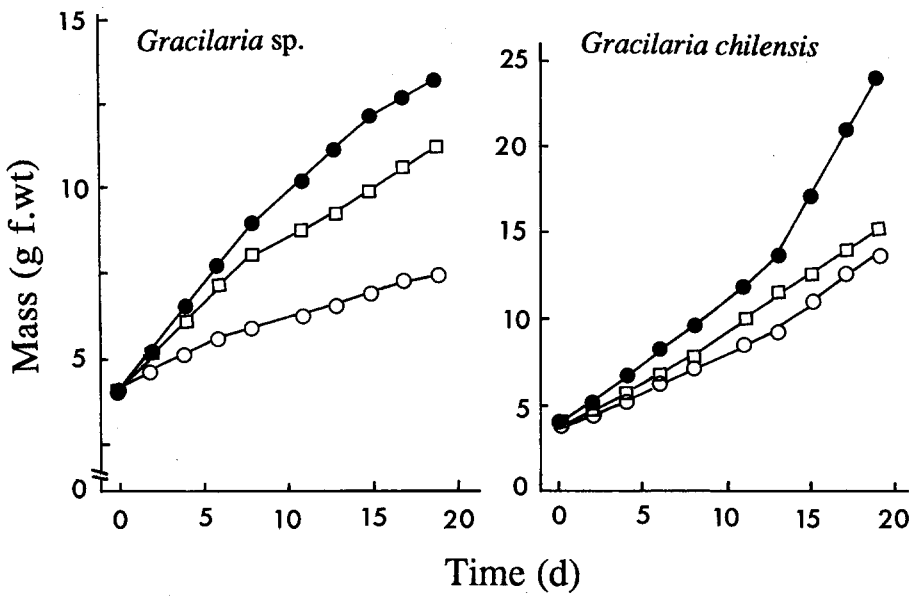

Fig. 1. Growth as change in fresh weight of *Gracilaria* sp. and *G. chilensis* in cultures with N- and P-enriched media aerated by air + 0 ppm CO_2 (○), air + 650 ppm CO_2 (□) and air + 1250 ppm CO_2 (●).

0.8 and 2.2 μmol g (f.w.)$^{-1}$ h^{-1} in *Gracilaria* sp. and 0.9, 2.5 and 2.0 μmol g (f.wt)$^{-1}$ h^{-1} in *G. chilensis* under the air (350 ppm CO_2), air + 650 ppm CO_2 and + 1250 ppm CO_2, respectively. Both CO_2 additions resulted in increased NO_3^- uptake rate by 60% and 340% in *Gracilaria* sp. and by 180% and 120% in *G. chilensis*, respectively.

Photosynthetic inorganic carbon uptake (Fig. 4) increased with increase of DIC concentration of seawater in both *Gracilaria* sp. and *G. chilensis* pre-cultured in air (low-CO_2) and air + 1250 ppm CO_2 (high-CO_2). The rate of inorganic carbon uptake was not saturated up to 10 mM DIC in both species, and the rate was higher in *Gracilaria* sp. than in *G. chilensis*. The response of photosynthetic inorganic carbon uptake to DIC concentrations was slightly affected in *Gracilaria* sp. but remarkably affected in *G. chilensis* by the pre-culture (15 days) in high-CO_2. High-CO_2-grown thalli of *G. chilensis* showed a depressed inorganic carbon uptake rate.

Oxygen uptake was higher in the light than in the dark by 67% in *Gracilaria* sp. and 58% in *G. chilensis* (Table 2). The difference was significant ($P < 0.05$) in each species. Oxygen uptake in

Table 1. CO_2 concentrations (ppm) of the input and output air for cultures of *Gracilaria* sp. and *G. chilensis*. Measured in the light during the time of 15:00–16:00 hrs. The influx and efflux CO_2 concentrations were monitored by infrared gas analysis unit. Values in parentheses indicate biomass (fresh weight, g) in culture vessel.

Treatment	Air	+ 650 ppm	+ 1250 ppm
CO_2 in	345	1050	1616
CO_2 out			
Gracilaria sp.	320	891	1420
	(4.4)	(4.7)	(4.6)
G. chilensis	292	883	1280
	(4.2)	(4.2)	(4.5)

Table 2. Oxygen uptake [μmol g(f.w.)$^{-1}$min^{-1}] by *Gracilaria* sp. and *G. chilensis* in the light (300 μmol photons m^{-2}s^{-1}) or in the dark at 20°C.

Gracilaria sp.		*G. chilensis*	
Light	Dark	Light	Dark
1.52 ± 0.7 ($n = 4$)	0.91 ± 0.42 ($n = 5$)	0.84 ± 0.08 ($n = 4$)	0.53 ± 0.13 ($n = 5$)

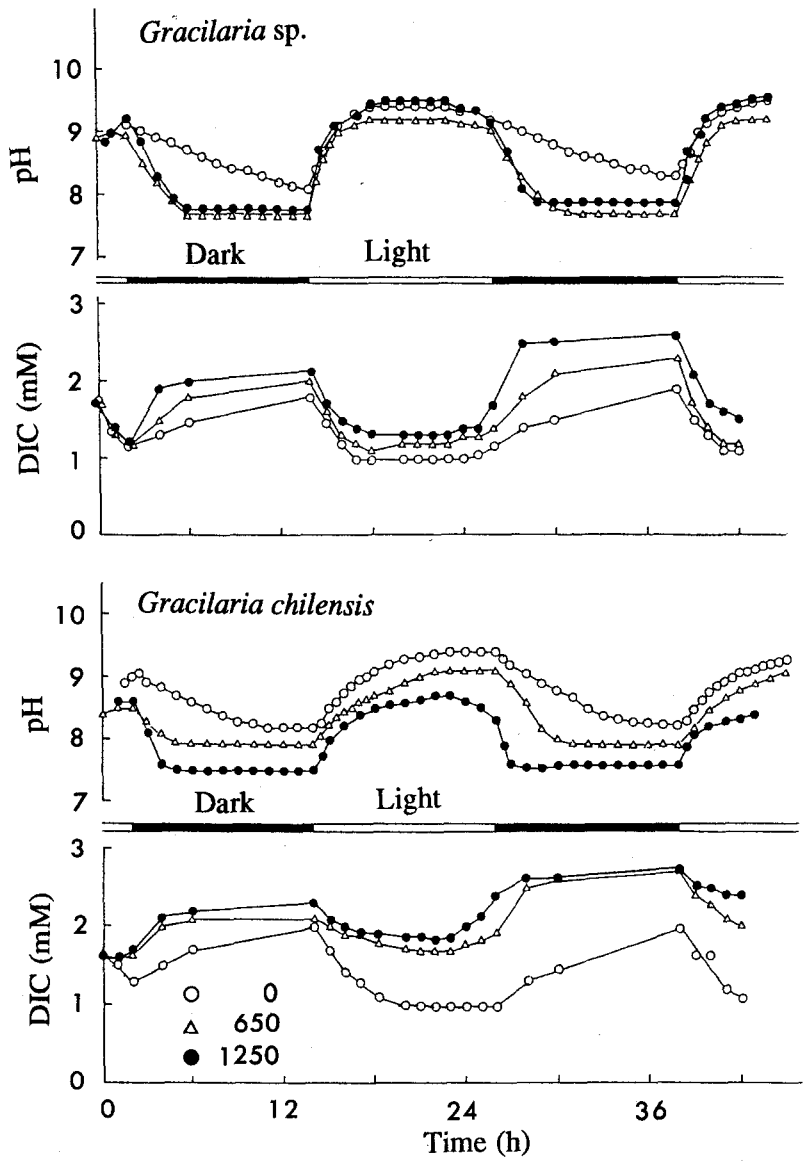

Fig. 2. Diurnal variations of pH and dissolved inorganic carbon (DIC) in N- and P-enriched culture media with *Gracilaria* sp. and *G. chilensis* aerated by air + 0 ppm CO_2, air + 650 ppm CO_2 and air + 1250 ppm CO_2. Measured from the 6th day after the initiation of the experiment.

the light was 81% higher in *Gracilaria* sp. than that in *G. chilensis* ($P<0.01$); that in the dark was 71% higher in *Gracilaria* sp. than that in *G. chilensis* ($P<0.1$).

Discussion

DIC decrease at the beginning of the light period is a result of the photosynthetic removal of inorganic carbon exceeding the rate of CO_2 dissolution. DIC increase at the beginning of the dark period is a result of the continuing influx of CO_2 (including CO_2 from respiration) to restore equilibrium. From linear gradient increase of DIC concentration in the dark, the rate of CO_2 dissolution in the bubbling system was estimated. The DIC decrease rate due to photosynthesis was determined from the linear slope of DIC concentra-

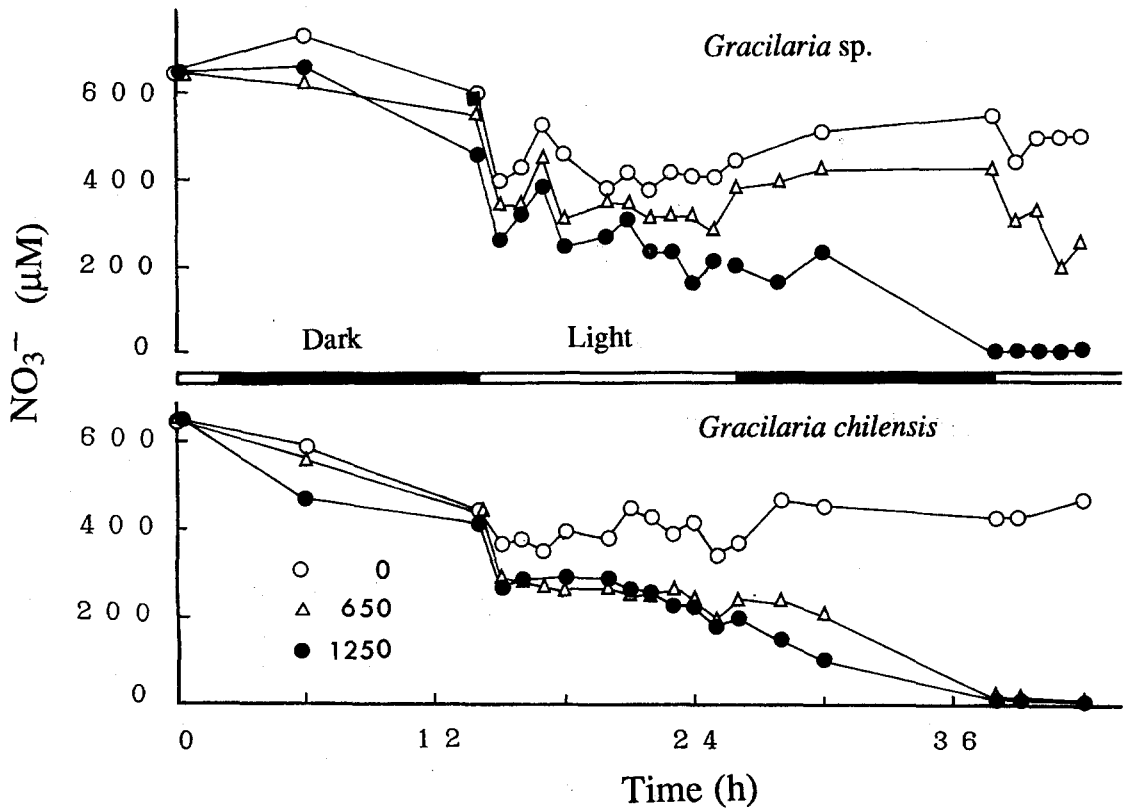

Fig. 3. NO_3^- concentrations in N- and P-enriched cultures of *Gracilaria* sp. and *G. chilensis* aerated with air + 0 ppm CO_2, air + 650 ppm CO_2 and air + 1250 ppm CO_2. Measured from the 6th day after the initiation of the experiment.

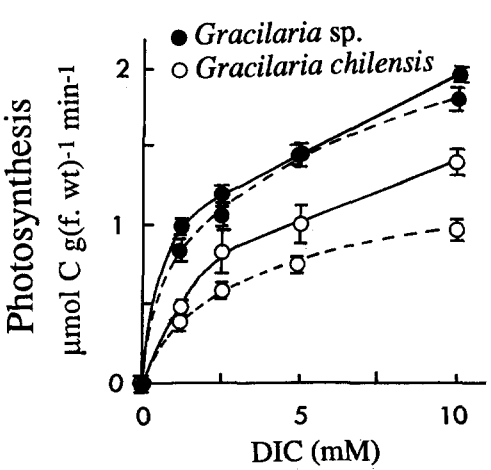

Fig. 4. Photosynthetic inorganic carbon uptake as a function of dissolved inorganic carbon (DIC) concentration of seawater in *Gracilaria* sp. and *G. chilensis* pre-cultured for 15 days with air (solid line) and air + 1250 ppm CO_2 (dotted line). Measured at 20 °C, 300 μmol photon m^{-2} s^{-1}. Means ± SD ($n = 3$).

tion at the beginning of the light period. The integrated total of the rate of CO_2 dissolution and the rate of DIC decrease should yield the rate of photosynthetic carbon removal in the light. The photosynthetic rates estimated in such a way on the basis of values shown in Fig. 2 were 1.3–1.6 μmol g (f.wt)$^{-1}$ min^{-1} or 78–96 μmol g (f.wt)$^{-1}$ h^{-1} in *Gracilaria* sp. and 0.6–1.2 μmol g (f.wt)$^{-1}$ min^{-1} or 36–72 μmol g (f.wt)$^{-1}$ h^{-1} in *G. chilensis*, respectively. These rates, being obtained in the open system (continuous aeration), are higher compared to the rates determined by photosynthesis measurement in closed system (the sealed tubes) at equivalent DIC level (2 mM). For the gas flow-rate of 500 ml min^{-1} and a fractional CO_2 content 350–1600 ppm, the rate of CO_2 supply should have been 8–36 μmol min^{-1}. For a biomass in the vessel of 5 g (f.wt) l^{-1}, the DIC removal by photosynthesis would be 6.5–

8.0 μmol min^{-1} for *Gracilaria* sp. and 3.0–6.0 μmol min^{-1} for *Gracilaria chilensis*, respectively. This is 81–22% and 38–17% of the input rate for *Gracilaria* sp. and *G. chilensis*, respectively. For the 'CO_2' removal to match the input, for example, with the treatment of air + 1250 ppm CO_2, 23 g (f.w.) of *Gracilaria* sp. and 30 g (f.w.) of *Gracilaria chilensis* are needed to be in the vessel with 1 litre medium. However, the biomass at the end of culture did not exceed such values.

Although pH rise in light can occur from removal of CO_2 alone, this change should follow the rate of dehydration of HCO_3^- (about 0.2 μM min^{-1} at 15–20 °C). In the present study and in spite of a continuous influx of CO_2, DIC was reduced at the beginning of light period at a rates of 2–8 μM min^{-1}. These values exceed up to 40 times the dehydration rate for HCO_3^-. The removal of NO_3^- by algae could also raise pH by increasing the alkalinity (Stumm & Morgan, 1981). By comparison, the rate of photosynthetic carbon fixation was 43–156 times greater in *Gracilaria* sp. and 29–72 times greater in *G. chilensis* than for the uptake of nitrate. The effect of nitrate removal on pH is therefore likely negligible and the pH rise in the light is attributable to photosynthetic use of bicarbonate. Nearly constant levels of pH and DIC in the center of the light period indicates a balance between the photosynthetic removal of inorganic carbon and the dissolution of CO_2 from aeration.

The present study has clearly demonstrated that CO_2 additions increased the growth rates of *Gracilaria* sp. and *G. chilensis* in N- and P-enriched media. Whether the growth rates can be raised in N- and P-limited media has yet to be addressed. The answers to that question would be of ecological significance, since N- and P-limited photosynthesis in the sea has been reported for macroalgae (Gao & Nakahara, 1990).

Photosynthetic inorganic carbon uptake was considerably suppressed in the thalli pre-cultured with CO_2 enrichment in *G. chilensis* but was little affected in *Gracilaria* sp. Such specific difference may be associated with different abilities to or to be adapted to use various forms of inorganic carbon, since addition of CO_2 to culture media affects pH and gives rise to varied concentrations of different forms of inorganic carbon. *Gracilaria tikvahiae*, *G. foliifera* and *G. secundata* have been reported to be capable of using bicarbonate (Bidwell & McLachlan, 1985; Lignell & Pedersen, 1989). On the other hand, the existence of intracellular CA has been reported in a great number of seaweeds (Graham & Smillie, 1976; Giordano & Maberly, 1989). Besides, some seaweeds have been reported to possess extracellular surface-bound carbonic anhydrase (CA) (Smith & Bidwell, 1987; Surif & Raven, 1989; Israel & Beer, 1992). Since CA catalyzes the interconversion of HCO_3^- and CO_2, suppression of its synthesis by high CO_2, which has been reported in micro- and macro-algae (Tsuzuki & Miyachi, 1989; Haglund & Pedersen, 1992; Bjok *et al.*, 1993), could result in a reduced capacity in using HCO_3^-. Supposing *G. chilensis* were capable of using bicarbonate, acclimation in the pre-culture with enrichment of CO_2 could result in a decreased capacity to use HCO_3^- and an increased capacity to use CO_2. As a result, the acclimated thalli showed suppressed photosynthesis when incubated in the sealed tubes with limited amount of CO_2, but showed enhanced growth while cultured in the open vessels with continuously supply of CO_2.

By using $^{18}O_2$ to detect the uptake of oxygen in the light, it was found that oxygen uptake was higher in the light than in the dark for both *Gracilaria* sp. and *G. chilensis*. In *Porphyra yezoensis*, the oxygen uptake in the light was also higher than that in the dark; it increased with increased light intensity (Gao *et al.*, 1992). Photorespiration in *Gracilaria* sp. could be higher than that in *G. chilensis*, since oxygen uptake in the light was greater in the former than in the latter. In addition to photorespiration, dark respiration was also more active in *Gracilaria* sp. than in *G. chilensis*. Higher dark respiration and photorespiration give rise to lower productivity of plants (Asada, 1981). Consequently, the productivity of *G. chilensis* could be higher than that of *Gracilaria* sp., although its photosynthetic rate was lower compared to *Gracilaria* sp. *G. chilensis* did grow faster than *Gracilaria* sp. in the control treatment.

Because aeration with addition of CO_2 to the culture raises DIC and aqueous CO_2 partial pressure and disperses photosynthetically-evolved O_2 it serves to elevate the CO_2/O_2 ratio in the culture medium and subsequently at the active site of Rubisco (ribulose-1,5-bisphosphate (RuBP). Elevation of the CO_2/O_2 ratio suppresses photorespiration. Photorespiratory suppression increases photosynthetic efficiency and enhance growth. Growth enhancement of *Gracilaria* sp. and *G. chilensis* under elevated CO_2 concentrations could therefore be a result of accelerated assimilation of carbon and nutrients as well as simultaneously suppressed photorespiration. Greater enhancement of growth in *Gracilaria* sp. may stem from greater depression of photorespiration by the enrichment of CO_2.

Photosynthesis increased with increased DIC in both *Gracilaria* sp. and in *G. chilensis*. This indicates that photosynthetic inorganic carbon uptake in these species is limited by inorganic carbon source (about 2 mM DIC in air-equilibrated seawater). When atmospheric CO_2 concentration increase, part of the increased CO_2 dissolves in seawater, forming carbonic acid, which in turn dissociates to form bicarbonate and then carbonate, reaching a new equilibrium. Elevated CO_2 partial pressure and increased DIC in seawater can increase the photosynthesis and productivity of *Gracilaria* species.

The proportion of algal biomass to water volume in the present study ranged from 0.2 to 0.7 kg m^{-3} for *Gracilaria* sp. and from 0.2 to 1.4 kg m^{-3} for *G. chilensis*. Maximum standing stocks of *Gracilaria* species have been reported from 1–13 kg m^{-2} from April to May in Tosa Bay, when the longest plants reach 1 m (Oresso, 1989). The proportion of the algal biomass to water volume within natural stands of *Gracilaria* can be as high as 13 kg m^{-3}. On the other hand, the initial density for *Gracilaria* species using the scattering culture method is 0.5–2.0 kg m^{-2} (FAO 1990). The present study suggests that, in their natural habitats or cultivation sites, photosynthesis and growth of *Gracilaria* species are likely to be 'CO_2'-limited, especially when the population density is high and water movement is slow. In dense stands diel variation in pH and DIC is likely to show similar patterns to those in Fig. 2. The results indicate that sparging CO_2-enriched air into cultivated *Gracilaria* populations could increase their yield on an areal basis.

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