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Interactive effects of nitrogen and dissolved inorganic carbon on photosynthesis, growth, and ammonium uptake of the macroalgae *Cladophora vagabunda* and *Gracilaria tikvahiae*

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Abstract Dissolved inorganic carbon (DIC) is rarely considered limiting for macroalgae, but some research suggests that under conditions of N sufficiency, photosynthetic capacity is enhanced with DIC enrichment. During spring (April–May) and summer (July–August) 1993, we investigated the interactive effects of nitrogen (N) and DIC on photosynthetic capacity, growth, and nutrient uptake rates of the macroalgae, *Cladophora vagabunda* (L.) van den Hoek and *Gracilaria tikvahiae* (McLachlan), dominant species in a temperate eutrophic estuary (Cape Cod, Massachusetts, USA). Water-column CO₂ concentrations showed significant diurnal fluctuations, ranging from a morning CO₂ peak (21 μM) to an afternoon low (13 μM) during summer, probably associated with metabolic activities in a thick algal mat. Results from instantaneous photosynthesis measurements and microcosm experiments indicate that DIC limits photosynthetic capacity and growth rates of *C. vagabunda* during summer, perhaps related to tissue N sufficiency and low water-column CO₂ concentrations. For example, this species showed enhanced growth ($F=8.69$, $P<0.02$) under DIC but not N enrichment. *G. tikvahiae* showed marginal DIC enhancement of maximum photosynthetic rate, while growth was significantly stimulated by addition of N. Reduced thallus N of this species during the summer further identifies N as the primary factor limiting growth. In addition, *G. tikvahiae* has the ability to use DIC in its several forms, while *C. vagabunda* primarily uses dissolved CO₂. DIC enrichment resulted in

a depression of NH₄⁺ uptake rates for both species, particularly during summer at saturating (60 μM) ammonium levels, suggesting competition between NH₄⁺ uptake and DIC acquisition under conditions of N sufficiency. Dominance of *C. vagabunda* and *G. tikvahiae* in areas undergoing eutrophication has been attributed to their successful procurement and storage of N as well as to high growth rates. The present study revealed that under conditions of N sufficiency during summer, DIC may control rates of production of these opportunistic macroalgae.

Introduction

Urbanization of coastal areas has greatly increased the inputs of nitrogen (N) and phosphorus (P) into many estuarine systems, with resultant impacts at the population and ecosystem level (Nixon et al. 1986; Valiela et al. 1990). Increased abundances of nuisance macroalgae are among the direct consequences of nutrient loading (Harlin and Thorne-Miller 1981; Valiela et al. 1992; Peckol et al. 1994). The macroalgae that proliferate under these circumstances may become important primary producers in coastal embayments or may outcompete preferred species. Additionally, these opportunistic algae are capable of uptake, assimilation, and storage of large amounts of nitrogen in areas of high N loading, resulting in low water-column concentrations of nutrients (Valiela et al. 1992; Peckol et al. 1994). Metabolic processes of macrophytes may also affect concentrations of dissolved gasses, including dissolved inorganic carbon (DIC), sometimes affecting the DIC equilibrium in certain coastal areas (Bidwell and McLachlan 1985; Pregnall and Rudy 1985; Axelsson and Uusitalo 1988; Johnston et al. 1992).

Due to the high solubility of CO₂ in seawater, DIC is rarely considered limiting for macroalgae. However, dissolved CO₂ can be depleted during the day in dense vegetation and in brackish or isolated waters (Kremer 1981; Pregnall and Rudy 1985), suggesting that DIC may become

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a major limiting factor affecting photosynthesis and growth in many marine systems (Bowes and Reiskind 1987). Beer and Eschel (1983) found that the reduced rates of carbon fixation measured for algae in dense stands probably results from a combination of self-shading, increased stability of boundary layers, and severe DIC depletion.

Results from studies on DIC limitation in marine macrophytes have been somewhat conflicting, in part related to distinctive species' responses and habitat variations. For example, while some species rely on only dissolved CO_2 as a DIC source, other macroalgae are capable of using HCO_3^- as well (Kremer 1981; Sand-Jensen and Gordon 1984). Total DIC concentration in seawater ranges from ca. 2.2 to 2.5 mM, but those species relying solely on CO_2 may have only 10 μM available (at ambient pH of 8.1, summer water temperatures). Further, photosynthesis is inhibited in some species as carbonate levels increase associated with a rise in water-column pH (Dromgoole 1978; Beer and Eschel 1983; Lignell and Pedersen 1989; Frost-Christensen and Sand-Jensen 1990). Thus, macroalgae able to use various forms of DIC should be at an advantage under conditions of limited dissolved CO_2 levels.

Some studies have identified an interactive relationship between N uptake and DIC acquisition. For example, Rigano et al. (1986) found that CO_2 is required for NH_4^+ assimilation. They suggested that an internal buildup of nitrogen intermediates, or a lack of carbon skeletons under CO_2 deprivation, may inhibit NH_4^+ uptake. Nitrogen status of algae may also affect physiological response to DIC availability. Levassasseur et al. (1991) found that maximum photosynthetic response to DIC enrichment was related to thallus N and RUBISCO levels in the green alga *Ulva rotundata*. Thus, there is likely a complex relationship between DIC acquisition and NH_4^+ uptake, particularly under conditions where CO_2 shows diurnal fluctuations or algae show high thallus N, such as under eutrophic conditions. Under conditions of N sufficiency in macroalgae, we predict that DIC levels will become limiting to photosynthesis and growth.

In recent decades, the flora of a New England embayment (Waquoit Bay, Massachusetts) has become dominated by two macroalgae, *Cladophora* spp. and *Gracilaria tikvahiae*, species often found in areas undergoing eutrophication (Thorne-Miller et al. 1983; Lee and Olsen 1985; Peckol et al. 1994). In an N-loaded area of this embayment, tissue N in *C. vagabunda* is well above the estimated 2% necessary for optimal growth (Gordon et al. 1981; Hanisak 1983; Lavery and McComb 1991) and falls below 2% for *G. tikvahiae* only during peak summer growth (Peckol et al. 1994). This system enabled us to test the predictions of Levassasseur et al. (1991), i.e., greater response to DIC enrichment associated with higher tissue N, and to determine if success of *C. vagabunda* and *G. tikvahiae* in eutrophic systems is in part related to an ability to use various forms of DIC. We focused on the interactive effects of N and DIC on photosynthetic performance, nutrient uptake, and growth rates of these opportunistic macroalgal species.

Materials and methods

Study area

The Waquoit Bay system is a NOAA National Estuarine Research Reserve, located on Cape Cod, Massachusetts, USA (Valiela et al. 1992; Peckol et al. 1994). The study area within this system is a shallow (1 to 2 m depth) estuary, Childs River, with relatively high concentrations (average 133 μM NO_3^-) of N entering as groundwater due to high housing density. Such groundwater inputs of N translate into relatively high water-column N levels, often exceeding 5 μM , except during summer (Valiela et al. 1992; Peckol et al. 1994). Total water-column DIC levels range from 1 to 2 mM throughout the growth season (Rivers 1994). During summertime, macroalgal biomass accumulates in mats >75 cm thick, with *Cladophora vagabunda* (L.) van den Hoek and *Gracilaria tikvahiae* (McLachlan) accounting for the highest biomass.

During spring (April–May) and summer (July–August) 1993, we collected *Cladophora vagabunda* and *Gracilaria tikvahiae* from Childs River and held them aerated under ambient temperatures and nearly saturating photosynthetically active radiation (PAR) photon flux densities (350 $\mu\text{E m}^{-2} \text{s}^{-1}$) until use. We selected healthy fronds for laboratory experiments. If it was not possible to perform measurements immediately, we enriched the water with NH_4Cl (5 μM NH_4^+) in order to replicate the actual environment in the field and thus avoid an artifact (nutrient-starved conditions) from being introduced. Experiments were run within a few hours or 1 d of collection.

Photosynthesis vs irradiance measurements

We measured photosynthetic performance using the 18.5-ml water-jacketed and thermostat-equipped Plexiglas chamber described by Henley et al. (1991). This was fitted with a Hansatech polarographic O_2 electrode at the base (Walker 1989) and controlled by an IBM personal computer through an interface (controller CB1-D, interface IF-1; Hansatech Ltd., Kings Lynn, Norfolk, UK). A cosine-corrected underwater quantum sensor (Li-Cor Inc.) was mounted against the back wall of the chamber to calibrate the light source. Calibration of the instrument is detailed by Rivers (1994). Following calibration, the chamber was filled with 0.8- μm filtered seawater, and dissolved O_2 concentrations were reduced to 40% saturation by purging with N_2 . Algal samples (approximately 0.01 g dry wt or 1 cm^2), placed in the chamber containing filtered seawater, were secured in a small mesh bag. After enclosing the experimental system, the algae were held in darkness for approximately 2 to 5 min, and respiration rates were monitored. After assuring no 'leakage' in the system, we ran a sequence of photon flux densities automatically by the computer at a predetermined number of 180-s time intervals (0 to 1400 $\mu\text{E m}^{-2} \text{s}^{-1}$). Response curves were generated during April–May [water temperature (WT)=15°C] and July–August (WT=25°C). Photosynthetic measurements were made under ambient (approximately 1.5 mM in spring) and enriched (10 mM NaHCO_3) DIC. We measured pH at the beginning and end of these experiments and found negligible variation from pH 8.1. In order to test for a preference for various forms of DIC, we also measured photosynthetic response to ambient and DIC enriched conditions at pH 6.0 ($\approx 50\%$ CO_2 , $\approx 50\%$ HCO_3^-), pH 8.1 ($\approx 1\%$ CO_2 , $\approx 75\%$ HCO_3^- , $\approx 24\%$ CO_3^{2-}), and pH 9.3 ($\approx 40\%$ HCO_3^- , $\approx 60\%$ CO_3^{2-}) using 12 N NaOH and 12 N HCl to alter the pH. At the end of each run, a photosynthesis response curve was generated by the computer. Photosynthetic response curve data were recorded with Hansatech software, and all P vs. I (photosynthesis vs irradiance) figures (3 to 5 curves treatment⁻¹) were generated with Microsoft® Excel version 4.0. We determined photosynthetic maximum (P_{max}) by averaging points on the light-saturated portion of each P vs I curve (e.g. as the slope approached zero).

Microcosm growth experiment

To investigate the interactive effects of N and DIC, we determined growth rates of *Cladophora vagabunda* and *Gracilaria tikvahiae* dur-

ing July 1993 in outdoor, continuous-flow growth tanks (Peckol and Ramus 1985). Each microcosm, independently fed seawater pumped from the north end of Waquoit Bay, held 30 liters and had a turnover rate of >40 volumes d^{-1} . Irradiance was held at saturating photon flux densities (0.7 incident irradiance I_0) with neutral density screens. Algae were collected from Childs River, cleaned of macroscopic epiphytes, pre-weighed (fresh wt) following removal of superficial water with a lettuce spinner, and held in the tanks as loose, two-species mats containing 30 g fresh wt of each species, similar to their distribution in the field (Peckol et al. 1994). A comparison of growth rates of *C. vagabunda* and *G. tikvahiae* held in single- and two-species mats in these tanks indicated that growth was not reduced (t -tests, $P>0.05$) when the species were grown together at these initial weights (Peckol unpublished data). Tanks were enriched with slow-release ammonium (+N) fertilizer (Sierra Chemical Co.) introduced from the bottom of the tanks (15 to $20 \mu M$). $NaHCO_3$ was added at 2-h intervals from 08:00 to 20:00 hrs, bringing tank concentrations initially up to $20 \mu M$. This added DIC was flushed out of the tanks over the 2-h period (Rivers 1994). Control tanks received no enrichment, and tank N and DIC remained similar to ambient conditions (Rivers unpublished data). We investigated effects of N and DIC using a design that included the following treatments: ambient N, ambient DIC; +N, ambient DIC; ambient N, +DIC; and +N, +DIC. Growth rates were calculated as doublings d^{-1} (Rhee 1980) after 7 d.

Ammonium uptake rates

Vegetative fronds were collected during May ($WT=15^\circ C$) and July ($WT=25^\circ C$) 1993 from Childs River, cleaned of macroscopic epiphytes and kept aerated overnight in filtered seawater at ambient water temperatures. Algal samples (0.04 to 0.08 g dry wt) were placed in acid-washed glass chambers containing 350 ml of $0.8\text{-}\mu m$ filtered seawater which had been spiked up to initial N concentrations (25 and $60 \mu M$) using NH_4Cl . During spring, ammonium uptake rates are saturated at $25 \mu M$ for both species; during summer, uptake saturates at approximately $50 \mu M N$ (Peckol et al. 1994). DIC was maintained at ambient levels (approximately 1.5 mM) or enriched to 10 mM with $NaHCO_3$. Chambers ($n=4$, each treatment) were continuously shaken throughout the incubation period. Uptake rates were measured at ambient temperatures and a photon flux density of $350 \mu E m^{-2} s^{-1}$. Uptake was determined by disappearance of ammonium from the medium after 1 h. Initial and final ammonium concentrations were determined by the salicylate method (Bower and Holm-Hansen 1980).

Tissue constituents

We collected *Cladophora vagabunda* and *Gracilaria tikvahiae* from Childs River during spring (April–May) and summer (July–August) 1993 for tissue N analysis. Collections were made by divers swimming over the algal mat, taking samples in a systematic fashion, e.g. every five fin kicks. Samples were dried, homogenized, and combusted in a Perkin-Elmer 240 Elemental Analyzer.

Diurnal variations of water-column DIC above and within the mat

Throughout spring and summer 1993, we made several measurements of DIC in Childs River during morning, mid-day, and afternoon to detect diurnal fluctuations. We collected water 2 to 3 cm above and 18 to 20 cm within a thick (>0.5 m) algal mat using either snorkel or SCUBA gear, filtered it through a type A/E Gelman glass fiber filter ($0.8 \mu m$) using a Nalgene vacuum hand-pump apparatus, and immediately recorded the temperature and salinity. If it was not possible to run these samples immediately, we froze them in plastic bottles until analysis for carbonate alkalinity and total DIC (all forms) (Parsons et al. 1984). Using equations of Drever (1982) we calculated the concentration of dissolved CO_2 in the water samples.

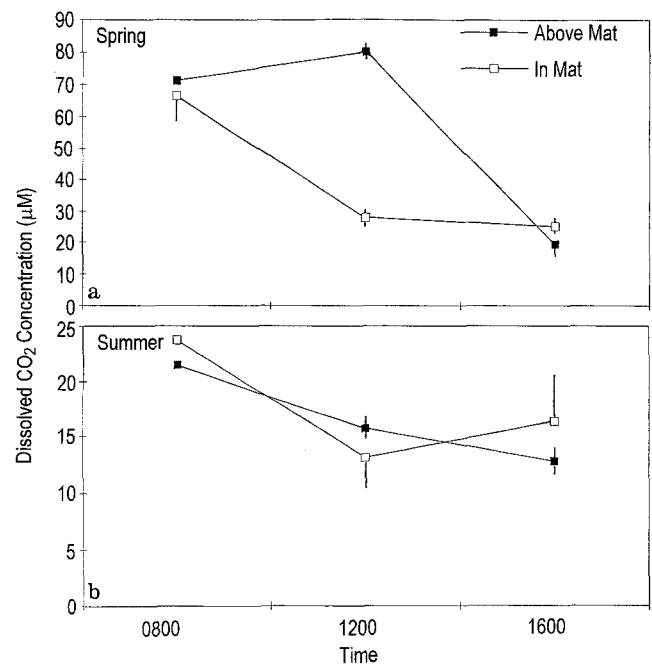


Fig. 1 Mean (\pm SD) dissolved CO_2 concentrations (μM) taken 2 to 3 cm above and within an algal mat at Childs River throughout the day during **a** spring (May 1993, $n=3$ for each of four sampling dates) and **b** summer (July–August 1993, $n=3$ for each of six sampling dates) (note axes differences)

Statistical analysis

Two-way analysis of variance (ANOVA) was used to analyze for significant differences among means of growth rates for the microcosm enrichment experiment (DIC \times N); t -tests were used to compare tissue N levels, N uptake rates and P_{max} values from photosynthesis response curves. One-way ANOVA was used to compare diurnal measurements of water-column CO_2 concentrations. Tukey's test was used to make multiple comparisons among treatment means from significant ANOVA tests (Day and Quinn 1989). Homogeneity of variance was determined using the F_{max} test (Sokal and Rohlf 1981).

Results

Water-column CO_2 levels

While total water-column DIC was generally 1 to 1.5 mM both above and within the algal mat (Rivers 1994), dissolved CO_2 concentrations varied throughout the day during spring (April–May) and summer (July–August) (Fig. 1). During spring, dissolved CO_2 ranged from a morning peak of approximately $70 \mu M$ to an afternoon low of $22 \mu M$. This diurnal variation was presumably related to an active algal mat, and CO_2 levels fell rapidly (Tukey's, $P<0.02$) from morning to noon. During summer, CO_2 levels were again highest in the morning both above ($F=12.1$, $P<0.001$), and within ($F=8.0$, $P<0.01$), the algal mat, and morning values were not different (t -test, $P>0.05$) between the two depths. By late afternoon, dissolved CO_2 had fallen to approximately $10 \mu M$, presumably due to photosyn-

thetic activities, and mat waters had significantly ($t=2.2$, $P<0.05$) higher concentrations than levels measured above the mat. Dissolved CO_2 concentrations were five to ten times higher during spring ($\text{WT}=15^\circ\text{C}$) than summer ($\text{WT}=25^\circ\text{C}$).

Tissue constituents

Tissue-N levels of *Cladophora vagabunda* and *Gracilaria tikvahiae* varied between spring and summer (Table 1). While tissue N remained above 4% for *C. vagabunda*, this constituent showed a significant reduction ($t=7.47$, $P<0.001$) in *G. tikvahiae* from spring to summer but remained above the estimated 2% necessary for optimal growth.

Photosynthesis vs irradiance measurements

We investigated the effect of DIC enrichment on photosynthetic capacity during spring (15°C) and summer (25°C) for *Cladophora vagabunda* and *Gracilaria tikvahiae* (Figs. 2 and 3, Table 2). At 15°C , neither species exhibited a DIC enhancement effect (t -tests on P_{max} values, $P>0.05$). In contrast, during the summer growth peak, both *C. vagabunda* ($t=5.76$, $P<0.01$) and *G. tikvahiae* ($t=3.16$, $P<0.05$) showed enhanced photosynthetic capacity under DIC enrichment. For example, P_{max} was nearly twice as high under DIC enrichment compared with control conditions for *C. vagabunda*. Thus, the DIC enhancement effect occurred during the season when ambient water-column CO_2 concentrations were quite low (Fig. 1).

Shown in Table 3 are relative P_{max} values calculated for *Cladophora vagabunda* and *Gracilaria tikvahiae* under ambient and enriched DIC and varying pH conditions (6.0, 8.1, 9.0). Under ambient DIC, *C. vagabunda* showed enhanced photosynthetic capacity at pH 6.0 relative to the ambient pH treatment, suggesting a propensity for acquisition of dissolved CO_2 over bicarbonate. This response was eliminated with DIC enrichment, presumably due to adequate absolute levels of CO_2 in this treatment. In contrast, *G. tikvahiae* had similar P_{max} across the three pH treatments, suggesting a greater ability to use various forms of DIC.

DIC- and N-enriched microcosm growth experiment

Cladophora vagabunda and *Gracilaria tikvahiae* showed different growth responses to +N or +DIC enrichment (Table 4). While growth in the +N treatment was marginally higher than controls ($F=3.72$, $P=0.08$) for *C. vagabunda*, the +DIC treatment resulted in a significant growth enhancement ($F=8.69$, $P<0.02$). This response mirrored the DIC effect on P_{max} measured at 25°C (Table 2) for this species. In contrast, two-way ANOVA revealed a significant +N enrichment effect for *G. tikvahiae* ($F=4.92$, $P<0.05$), but DIC-enriched growth rates did not vary significantly

Table 1 *Cladophora vagabunda* and *Gracilaria tikvahiae*. Mean (± 1 SD) tissue N levels (mg g^{-1} dry wt) during spring and summer ($n=9$). * indicates a significant (t -tests, $P<0.001$) difference between means

Species	Tissue nitrogen	
	Spring	Summer
<i>C. vagabunda</i>	40.92 \pm 0.73*	46.93 \pm 3.25
<i>G. tikvahiae</i>	30.72 \pm 2.71*	21.00 \pm 2.08

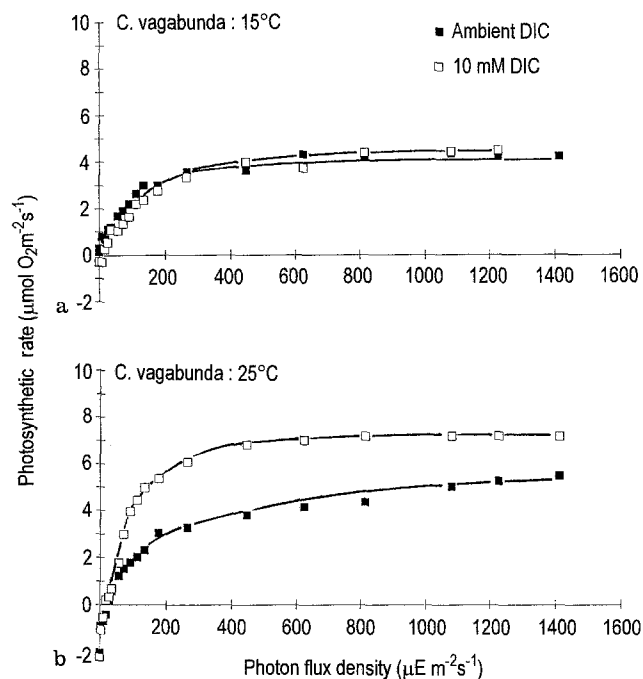


Fig. 2 *Cladophora vagabunda*. Photosynthetic rates ($\mu\text{mol O}_2 \text{ m}^{-2} \text{ s}^{-1}$) at varying photon flux rate densities ($\mu\text{E m}^{-2} \text{ s}^{-1}$) at **a** 15°C and **b** 25°C under ambient and enriched (10 mM) dissolved inorganic carbon (DIC) ($n=3$ to 5, for each curve)

($F<1.0$, $P=0.84$) from values measured in the control treatment.

Ammonium uptake rates

Shown in Figs. 4 and 5 are ammonium uptake rates for *Cladophora vagabunda* and *Gracilaria tikvahiae* under ambient and enriched (10 mM) DIC during spring ($\text{WT}=15^\circ\text{C}$) and summer ($\text{WT}=25^\circ\text{C}$). At 15°C , *C. vagabunda* showed a slight (t -tests, $P>0.05$) but consistent depression in uptake rates under enriched (10 mM) DIC compared with ambient DIC at both ammonium concentrations. In contrast, DIC level had little effect on N uptake of *G. tikvahiae* at this temperature. During summer, N uptake rates were depressed for both species (for *C. vagabunda*, $t=2.64$, $P<0.05$; for *G. tikvahiae*, $t=11.34$, $P<0.001$) under

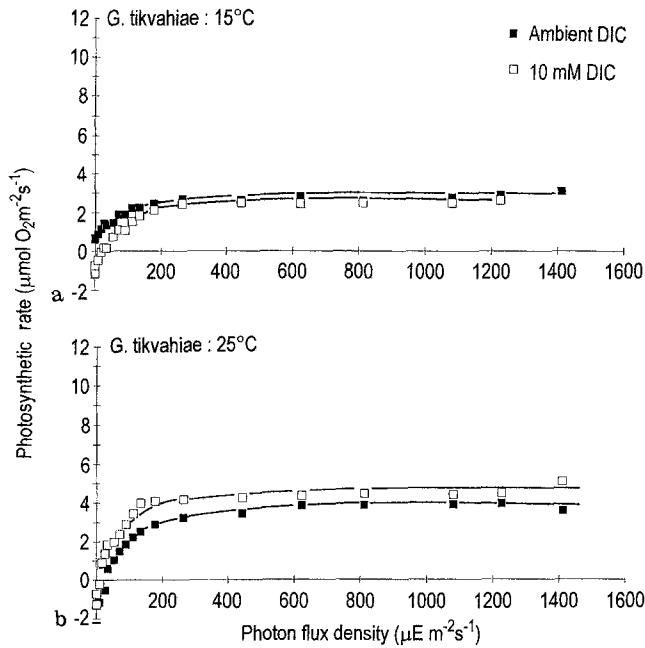


Fig. 3 *Gracilaria tikvahiae*. Photosynthetic rates (μmol O₂ m⁻² s⁻¹) at varying photon flux rate densities (μE m⁻² s⁻¹) at **a** 15°C and **b** 25°C under ambient and enriched (10 mM) dissolved inorganic carbon (DIC) (n=3 to 4, for each curve)

Table 2 *Cladophora vagabunda* and *Gracilaria tikvahiae*. Mean (n=3 to 5) (±1 SD) maximum photosynthetic rate (P_{max}) values (μmol O₂ m⁻² s⁻¹) at 15 and 25°C under ambient and dissolved inorganic carbon (DIC)-enriched (10 mM) conditions

Species Conditions	P _{max}	
	15°C	25°C
<i>C. vagabunda</i>		
Ambient DIC	4.26 ± 0.70	4.58 ± 1.43
10 mM DIC	4.41 ± 0.70	7.17 ± 0.10
<i>G. tikvahiae</i>		
Ambient DIC	2.71 ± 0.35	3.70 ± 0.15
10 mM DIC	2.31 ± 0.15	4.49 ± 0.42

Table 3 *Cladophora vagabunda* and *Gracilaria tikvahiae*. Relative maximum photosynthetic rate (P_{max}) (shown as a proportional difference relative to P_{max} at pH 8.1) under ambient and enriched (10 mM) dissolved inorganic carbon (DIC) at 25°C

Species Conditions	Relative P _{max} (at varying pH)		
	6.0	8.1	9.3
<i>C. vagabunda</i>			
Ambient DIC	1.72	1.00	0.71
10 mM DIC	0.99	1.00	0.46
<i>G. tikvahiae</i>			
Ambient DIC	1.37	1.00	1.21
10 mM DIC	1.25	1.00	0.98

Table 4 *Cladophora vagabunda* and *Gracilaria tikvahiae*. Mean (±1 SD) growth rates (doublings d⁻¹) in microcosms under ambient or enrichment [+N=15 μM NH₄⁺; + dissolved inorganic carbon (DIC)=20 mM NaHCO₃] conditions (n=4, each treatment) and saturating irradiances (0.7 I₀) during July (water temperature=25°C)

Species	N level	DIC level	
		Ambient	10 mM DIC
<i>C. vagabunda</i>	Ambient	0.08±0.02	0.10±0.01
	+N	0.09±0.01	0.12±0.01
<i>G. tikvahiae</i>	Ambient	0.10±0.01	0.10±0.02
	+N	0.11±0.02	0.12±0.01

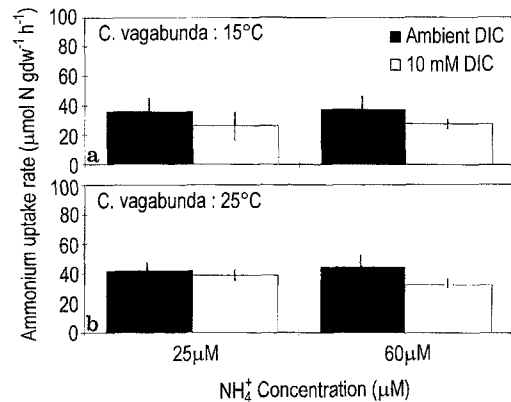


Fig. 4 *Cladophora vagabunda*. Mean (±1 SD) ammonium uptake rates (μmol N g⁻¹ dry wt h⁻¹) under varying NH₄⁺ concentrations (25 and 60 μM) and ambient vs enriched (10 mM) dissolved inorganic carbon (DIC) at **a** 15°C and **b** 25°C (n=4, each treatment)

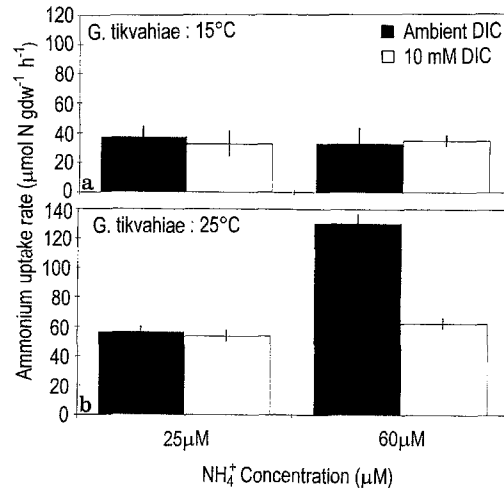


Fig. 5 *Gracilaria tikvahiae*. Mean (±1 SD) ammonium uptake rates (μmol N g⁻¹ dry wt h⁻¹) under varying NH₄⁺ concentrations (25 and 60 μM) and ambient vs enriched (10 mM) dissolved inorganic carbon (DIC) at **a** 15°C and **b** 25°C (n=4, each treatment)

DIC enrichment compared with ambient conditions at saturating ($60 \mu\text{M N}$) ammonium levels. For example, NH_4^+ uptake at saturating N concentrations was more than twice as high under the ambient compared with the enriched DIC treatment for *G. tikvahiae*.

Discussion

The results of the present study coupled with others from Waquoit Bay (Valiela et al. 1990; Valiela et al. 1992; Peckol et al. 1994) indicate that several factors are interacting to affect growth rates and relative abundances of algae present in eutrophic systems. Nutrient enrichment, due to high N inputs via groundwater and regenerative processes, has contributed to enhanced productivity and growth of opportunistic macroalgae, including *Cladophora* spp. and *Gracilaria tikvahiae* (Lee and Olsen 1985). Under these conditions of N sufficiency, we found that maximum photosynthetic rate (P_{max}) was increased under DIC enrichment for *C. vagabunda* and *G. tikvahiae* during the summer period of peak growth and low ambient dissolved CO_2 concentrations. We documented increased growth for *C. vagabunda* under DIC enrichment, while *G. tikvahiae* showed enhanced growth under N enrichment. However, we also measured a depression in ammonium uptake rates associated with DIC enrichment. These results thus reveal an interaction between NH_4^+ uptake and DIC acquisition under conditions of N sufficiency.

Physiological response to DIC and N enrichment was related to season and thallus N for both species. Under elevated DIC, *Cladophora vagabunda* showed a clear enhancement of both photosynthetic capacity and growth during the summer. Thallus N of this species remained high throughout the growth season, explaining the lack of a significant growth increase under N enrichment in the microcosm experiment. In contrast, Peckol et al. (1994) measured enhanced growth under N enrichment for *C. vagabunda* populations with lower (2 to 2.5%) thallus-N levels. Although *Gracilaria tikvahiae* showed DIC enhanced P_{max} during summer, growth rates increased only under the +N treatment. The marginal response to DIC enrichment, the significant decrease in thallus N from spring to summer, and the significant growth response to N enrichment emphasize that for this species, N is the primary limiting factor during summer, as indicated in other studies (LaPointe and Duke 1984; Peckol et al. 1994).

Different species' responses to DIC enrichment may also be related to varying abilities to use the available forms of DIC. As in several other algae (Bidwell and McLachlan 1985; Johnston 1990; Johnston et al. 1992), our results suggest that *Gracilaria tikvahiae* can successfully use various forms of DIC, while *Cladophora vagabunda* shows a clear preference for dissolved CO_2 . Because the pH of Waquoit Bay water was ≈ 8.1 throughout the growth season, DIC is predominantly in the form of HCO_3^- ($\approx 1\%$ dissolved CO_2 , $\approx 75\%$ HCO_3^- , $\approx 24\%$ CO_3^{2-}) (Drever 1982), perhaps providing *G. tikvahiae* with an ample supply of

DIC for photosynthetic processes. This ability to use various forms of DIC would enhance photosynthetic capacity of *G. tikvahiae* under conditions of saturating photon flux densities and high algal biomass, as occurs in shallow areas of eutrophic systems. It is under these environmental conditions that this species proliferates in Waquoit Bay (Valiela et al. 1992; Peckol et al. 1994).

Previous work addressing the relationship between NH_4^+ uptake and DIC acquisition may offer an explanation for the depressed N uptake rates measured at enriched DIC in our study. For example, Rigano et al. (1986) found that CO_2 is necessary for uptake of NH_4^+ and suggested that a high internal level of carbon skeletons is required as a signal to stimulate uptake of NH_4^+ across the cell membrane. In addition, Bird et al. (1982) found that a carbon supply is necessary for increased amino acid and protein synthesis, further evidence of a positive relationship between NH_4^+ uptake and DIC levels. While we measured no decrease of N uptake under enriched, relative to ambient DIC, at subsaturating NH_4^+ concentrations (present study; unpublished data), the depression in uptake under saturating NH_4^+ and DIC suggests a competition between DIC acquisition and NH_4^+ uptake for both *Cladophora vagabunda* and *Gracilaria tikvahiae*. Lobban et al. (1985) suggested that photosynthetic gas exchange is shut down during rapid nutrient uptake, characteristic of algae grown under low N availability (D'Elia and DeBoer 1978; Fujita 1985). We hypothesize that under summer conditions of N sufficiency and low ambient CO_2 levels, DIC acquisition takes precedence over N uptake, particularly under enriched DIC levels. Our findings also expand the work of Levvasseur et al. (1991) regarding the interplay between thallus N and DIC acquisition. While high thallus N permits maximum photosynthetic response to DIC enrichment due, in part, to higher RUBISCO levels, such an N status may actually allow an alga to take advantage of higher available DIC, by shutting down N uptake.

Results of the present study emphasize the complex interactions among environmental factors, e.g. temperature, N availability, irradiance, DIC levels, that likely determine species dominance in eutrophic systems. For example, with N loading from anthropogenic inputs, higher N availability results in increased macroalgal biomass. High water-column temperatures and algal metabolic activities drive dissolved CO_2 concentrations down during summer, resulting in a system that becomes DIC limited. Macroalgae relying predominantly on dissolved CO_2 as a DIC source will be at a disadvantage relative to species able to use various forms of DIC. This scenario would have far-reaching effects for systems like Waquoit Bay where *Cladophora vagabunda* is currently the dominant species (Valiela et al. 1992). Eutrophication may eventually result in a shift toward dominance by species like *Gracilaria tikvahiae*, particularly in shallow areas. Such a pattern already seems evident as the contribution of *G. tikvahiae* to total algal biomass increases throughout the summer (Peckol et al. 1994). Productivity of opportunistic species in systems like Waquoit Bay is likely controlled by available DIC during peak summer growth.

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