

Phosphorus- and nitrogen-limited photosynthesis and growth of *Gracilaria tikvahiae* (Rhodophyceae) in the Florida Keys: an experimental field study

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Abstract

The relative effects of NH_4^+ (N) and PO_4^{3-} (P) on growth rate, photosynthetic capacity (P_{max}), and levels of chemical constituents of the red macroalga *Gracilaria tikvahiae* McLachlan were assayed during winter and summer, 1983 in inshore waters of the Florida Keys by using *in-situ* cage cultures. During winter, both N and P enrichment enhanced growth over that of ambient seawater; however, P rather than N accounted for more (60%) of the increased winter growth. During summer, P, but not N, enhanced growth over ambient seawater and accounted for 80% of increased growth. Similarly, P_{max} was enhanced by both P and N during winter (but mostly by P) and only by P during summer. Elevated C:P, C:N and N:P ratios of *G. tikvahiae* tissue during winter, but only C:P and N:P ratios during summer, support the pattern of winter N and P limitation and summer P-limitation. This seasonal pattern of N vs P limited growth of *G. tikvahiae* appears to be a response to seasonally variable dissolved inorganic N (twofold greater concentrations of NH_4^+ and NO_3^- during summer compared to winter) and constantly low to undetectable concentrations of PO_4^{3-} . Mean C:P and N:P ratios of *G. tikvahiae* tissue during the study were 1 818 and 124, respectively, values among the highest reported for macroalgae.

Introduction

Since the experiments of Ketchum (1939 a, b) describing nitrogen (N) and phosphorus (P) limited growth of *Nitzschia closterium*, a central question in biological oceanography has been to what extent these nutrients control algal growth in marine waters. Low levels of dissolved inorganic N and P are especially common in tropical marine waters (Parsons *et al.*, 1977), yet little is known of their relative and quantitative importance in limiting growth and pro-

ductivity of marine macroalgae. N is considered the primary limiting nutrient for macroalgal growth in North Temperate coastal marine waters as shown both by *in-situ* (Chapman and Craigie, 1977) and *in-vitro* (Topinka and Robbins, 1976; Hanisak, 1979) studies. This is in general agreement with the view that N rather than P is the major nutrient limiting phytoplankton productivity in the oceans (Thomas, 1970; Morris *et al.*, 1971; Ryther and Dunstan, 1971; Vince and Valiela, 1973; Goldman *et al.*, 1979). However, evidence suggests that P may commonly be the primary limiting nutrient to algal growth in certain marine waters. For example, P is frequently more important than N in limiting phytoplankton productivity in nearshore northeastern Gulf of Mexico (Myers and Iverson, 1981) and in an estuary in Western Australia during winter (McComb *et al.*, 1981). Elevated molar N:P ratios in macroalgal tissue from a variety of geographic locations further suggest P could be a primary limiting nutrient to macroalgal growth (Atkinson and Smith, 1983).

One physiological characteristic of nutrient limitation in algae that might be used to gauge the type and degree of nutrient limitation of macroalgae *in situ* is the degree of nutrient enhancement of photosynthetic capacity, P_{max} , at light-saturation. P-limited continuous culture studies with freshwater *Scenedesmus* sp. indicate that P_{max} is a hyperbolic function of the cellular level of P and analogous to the Droop model of nutrient-limited algal growth (Smith, 1983). A similar relationship appears to be true for N (see Smith, 1983) and supports a cell quota model for phytoplankton photosynthesis. If such is equally true for macroalgae, then experimental pulsing of N and P, followed by measurement of P_{max} , could be used as a quantitative diagnostic tool in field studies to determine the degree and type of nutrient limitation experienced by macroalgae.

The purpose of this study was to investigate the relative importance of *in-situ* N and P limitation on productivity, measured both as growth (weight increase) and photosynthetic capacity (P vs I curve and P_{max}), and chemical composition of the red seaweed *Gracilaria tikvahiae*

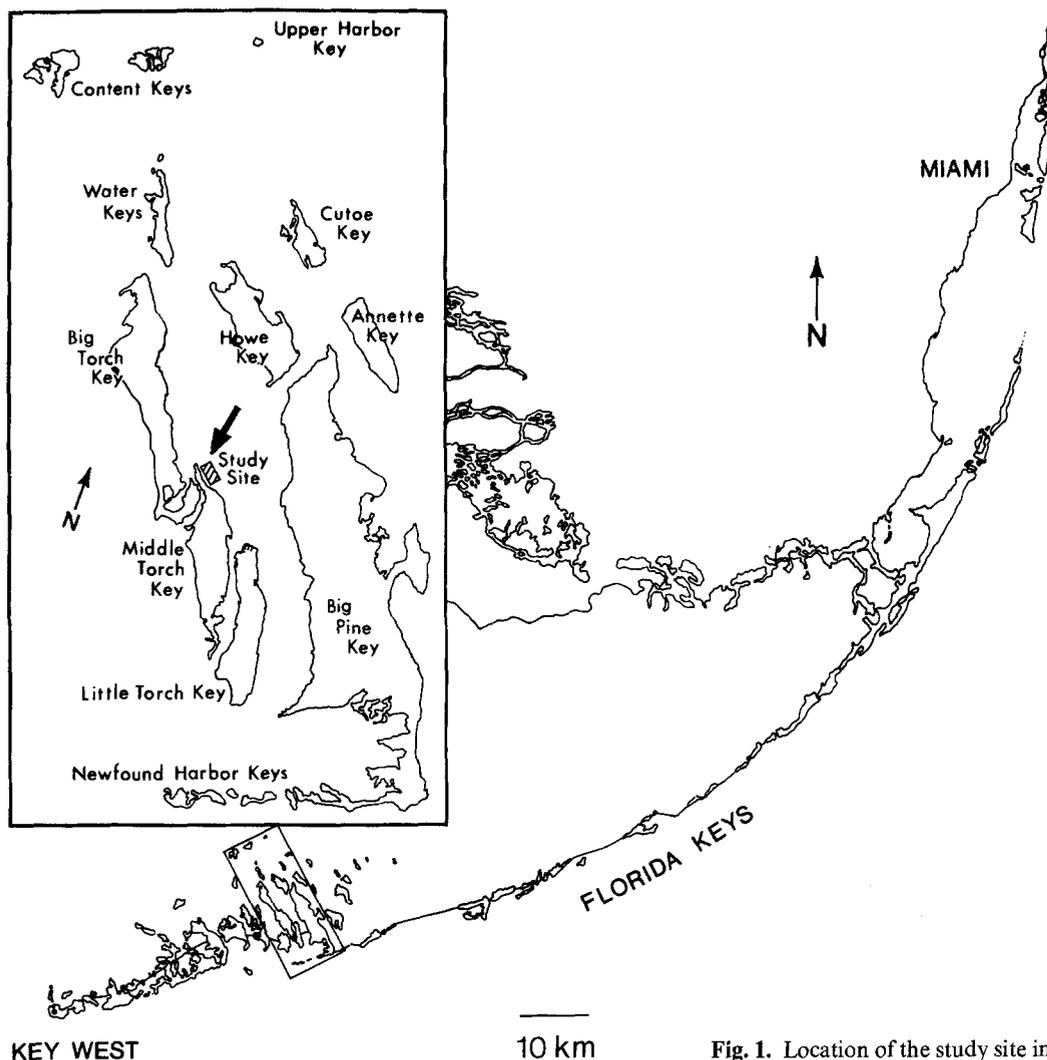


Fig. 1. Location of the study site in the Florida Keys

(previously referred to as *G. foliifera* v. *angustissima*) in the lower Florida Keys, USA. These studies involved experimental pulsing of N and P during both winter and summer to allow inferences regarding seasonality of N and P limited growth of *G. tikvahiae*.

Materials and methods

In-situ culture of *Gracilaria tikvahiae*: experimental design and operation

Cultures of the red seaweed *Gracilaria tikvahiae* McLachlan were transplanted from mass cultures maintained at the Harbor Branch Oceanographic Institution in Ft. Pierce, Florida to *in-situ* vexar cage cultures (2 cm mesh; 0.3 m² surface area) in South Pine Channel, 38 km east of Key West, Florida, USA (Fig. 1). The cages were attached to PVC pipe floatation frames that were tethered to the bottom in shallow water (1.5 m depth). Wind driven and tidal water movement within the cages was adequate for growth

and ranged from 5 to 25 cm s⁻¹ during the studies (measured with a TSK flow meter).

Main effects and interaction of N and P on growth, P vs I curves and P_{max}, and chemical composition of *Gracilaria tikvahiae* during winter (January–February) and summer (June–July) 1983 were assessed by using a 3×3 factorial design enrichment experiment. Three levels of N and P in the experimental design (9 treatments×2 replicate cages treatment⁻¹ = 18 total cages) were achieved by experimentally administering weekly pulses of nutrients. During both seasons, 300 g wet weight of *G. tikvahiae* were placed in each cage. At weekly intervals, nutrients were administered by removing and soaking plants from the various treatment in seawater/nutrient solutions in 14-liter Nalgene containers for 5 h. During the pulses, the plants were gently aerated to facilitate nutrient uptake by preventing excessive diffusion transport limitation. Preliminary experiments were conducted to determine appropriate nutrient concentrations and frequency of pulses to insure adequate nutrient flux to support growth of *G. tikvahiae* in the oligotrophic waters of the Keys (Lapointe,

1986). The "low" level of N and P in the factorial design was ambient seawater; "medium" and "high" levels were seawater spiked with NH_4Cl and/or NaH_2PO_4 . During winter, "medium" levels of NH_4^+ and PO_4^{3-} were 350 and 20 μM , respectively; "high" levels were 700 and 40 μM , respectively. During summer, two-fold higher levels were used and "medium" levels of N and P were 700 and 40 μM , respectively; "high" levels were 1.4 mM and 80 μM , respectively. Although these nutrient concentrations were much higher than concentrations encountered in the Keys marine environment, short-term pulsing with elevated concentrations of N and P are used routinely to maintain mass cultures of *G. tikvahiae* (Ryther *et al.*, 1981; Lapointe, 1986). At 14-d intervals and just prior to every second pulse, the plants in each cage were weighed and harvested back to the initial weight, and growth rates calculated as doublings d^{-1} . The reported growth data represents two weeks of growth following four weeks of acclimation to this experimental protocol.

Physico-chemical parameters

Concentrations of dissolved inorganic N (NH_4^+ and NO_3^-) and P (PO_4^{3-}) in South Pine Channel seawater were determined weekly during the study periods at maximum tide levels by gently filtering seawater samples through a 0.45- μm Gelman filter, and storing frozen in a Nalgene bottle. Subsequently, NO_3^- , NH_4^+ , PO_4^{3-} were analyzed on a Technicon Autoanalyzer II by methods described in Zimmerman *et al.* (1977). Temperature was measured continuously during the experiments with Taylor Max-Min thermometers and salinity was determined with an American Optical hand-held refractometer. These physico-chemical data are summarized in Table 1.

Determination of P vs I curves and P_{max}

To determine the effect of N and P limitation on the P vs I curve and, in particular, photosynthetic capacity (P_{max}) of *Gracilaria tikvahiae*, steady-state photosynthetic rates (mg O_2 g dry wt $^{-1}$ h $^{-1}$) of four of the nine total experimental treatments (low N–low P; low N–high P; high N–low P; high N–high P) were measured at the end of the experimental growth period (end of sixth week) during both winter and summer using the light-dark bottle O_2 method.

A shipboard flowing-seawater Plexiglas incubation chamber provided temperature control (to ambient seawater temperatures) and mixing via water-driven magnetic stirrers. All measurements were made between 10:00 and 14:00 hrs under clear, sunny skies ($\sim 2\,200 \mu\text{E m}^{-2} \text{s}^{-1}$ as measured with a 2π Li-Cor quantum sensor coupled to a Li-Cor Model 185 quantum meter). Small apical thalli (~ 0.1 g dry wt) were incubated in 0.5-liter Wheaton wide mouth reagent bottles (mixed via magnetic stir-bars) filled with 0.45- μm filtered seawater. To minimize photo-inhibition associated with high O_2 tensions during the photosynthetic assays, the seawater was bubbled slightly with N_2 to reduce O_2 levels to ~ 3 ppm. Different layers of fiberglass screening were placed over the bottles to result in four different light levels (I_0 , incident light; $0.57 I_0$; $0.33 I_0$; $0.07 I_0$); four replicate bottles per light level (total of 16 bottles) were used for the incubations which lasted 2 h. Preliminary measurement of O_2 concentrations at 5-min intervals indicated that photosynthetic rates were linear during this period. O_2 levels were measured with a YSI model 57 O_2 analyzer. P_{max} values used for statistical comparisons represented the mean of the four greatest photosynthetic rates obtained in a particular P vs I curve (total of 16 bottles).

Tissue analysis for C:N:P

At the end of the experimental growth periods, algal samples were rinsed in deionized water to remove debris and dried at 60°C for 48 h. Carbon and nitrogen were determined using a Perkin Elmer 240 Elemental Analyzer and total phosphorus was measured by persulfate digestion of dried algal tissue followed by PO_4^{3-} analysis (Menzel and Corwin, 1965). This method for measuring total P recovered >95% of reported P in National Bureau of Standards citrus leaves.

Statistical analysis

N and P enrichment effects on growth rate and levels of chemical constituents were assessed by two-way ANOVA. Duncan's New Multiple Range Test and Student's *t*-tests (Steel and Torrie, 1980) were used to test *a-priori* comparisons of treatment means and differences between P_{max} values. Significance reported in the results below implies that the probability of the null hypothesis was < 0.05.

Table 1. Temperature (°C) and salinity (‰) range and concentration of dissolved inorganic nitrogen (NH_4^+ + NO_3^- , μM) and phosphorus (PO_4^{3-} , μM) in South Pine Channel during the experimental periods in winter (January-February) and summer (June-July), 1983. Values for nutrient concentrations represent means \pm 1 SD ($n = 15$)

Season	Temperature (°C)	Salinity	NH_4^+ + NO_3^-	PO_4^{3-}	N:P
Winter	18–25	31–36	1.14 ± 0.64	0.14 ± 0.07	8.1
Summer	30–34	34–37	2.42 ± 0.43	0.08 ± 0.04	30.3

Results

Winter

During winter 1983, both N and P enrichment as well as their interaction significantly enhanced growth of *Gracilaria tikvahiae*, although P accounted for most of the total variation in growth, 60%, compared to 25% for N and 13% for the N×P interaction (Table 2). Growth rates ranged between 0.005 and 0.082 doublings d^{-1} (Fig. 2).

Percentages of tissue C, N, and P were affected by P but not N enrichment (Table 2). Levels of C ranged from 25.3 to 30.3% of dry weight; N ranged from 1.80 to 2.50% of dry weight; P ranged from 0.040 to 0.076% of dry weight (Table 3). Although both N and P enrichment significantly affected the C:N and N:P molar ratios, P accounted for most of the variation in these ratios, i.e. $\cong 52\%$ (Table 2). Only P enrichment affected the C:P

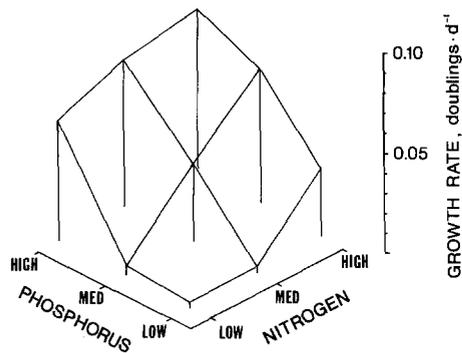


Fig. 2. *Gracilaria tikvahiae*. Growth rate in South Pine Channel, Florida Keys, during winter 1983, in response to different levels of NH_4^+ and PO_4^{3-} enrichment. "Low" represents ambient seawater levels only; "medium" represents weekly 5-h exposure to $350 \mu\text{M}$ NH_4^+ and/or $20 \mu\text{M}$ PO_4^{3-} ; "high" represents weekly 5-h exposure to $700 \mu\text{M}$ NH_4^+ and/or $40 \mu\text{M}$ PO_4^{3-} . Values represent means ($n=6$); coefficient of variation < 0.12

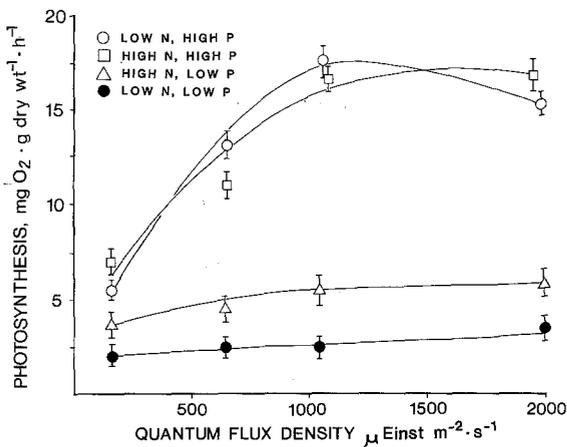


Fig. 3. *Gracilaria tikvahiae*. Photosynthesis vs irradiance (in quantum flux density) curve of *G. tikvahiae* in South Pine Channel, Florida Keys, during winter 1983, in response to different levels of NH_4^+ and PO_4^{3-} enrichment. "Low" represents ambient seawater levels only; "high" represents weekly 5-h exposure to $700 \mu\text{M}$ NH_4^+ and/or $40 \mu\text{M}$ PO_4^{3-} . Values represent means ± 1 SD ($n=4$)

ratio, accounting for 82% of the variation in this ratio (Table 2). C:N ratios ranged from 12.3 to 19.5, C:P ratios ranged from 892 to 2 694, and N:P ratios ranged from 73 to 157 (Table 3).

Although both N and P enrichment enhanced photosynthetic capacity of *Gracilaria tikvahiae* compared to non-enriched plants (i.e. low N, low P treatment), P enrichment resulted in much greater enhancement of P_{max} compared to N enrichment during winter (Fig. 3). Values of P_{max} for P-enriched plants were $\sim 17.2 \pm 1.7 \text{ mg O}_2 \text{ g}$

Table 2. *Gracilaria tikvahiae*. Summary of two-way ANOVA of measured variables as a function of enrichment by nitrogen (NH_4^+), phosphorus (PO_4^{3-}) and N×P interaction

Season	Variable	Factor	% Variation	F
Winter	Growth rate	N	25	67.8*
		P	60	160.3*
		N×P	13	16.7*
	% N	N	12	3.47**
		P	64	18.05*
		N×P	7	1.04**
	% C	N	9	2.20**
		P	54	12.74*
		N×P	17	2.06**
	% P	N	11	3.47**
		P	62	17.09*
		N×P	6	1.24**
	C:N	N	12	4.51**
		P	71	27.51*
		N×P	6	1.14**
C:P	N	10	3.40**	
	P	82	35.30*	
	N×P	7	2.56**	
N:P	N	20	11.88***	
	P	52	30.77*	
	N×P	21	6.11**	
Summer	Growth rate	N	5	3.2**
		P	82	213.6*
		N×P	1	0.71**
	% N	N	10	1.58**
		P	56	8.47*
		N×P	5	0.35**
	% C	N	6	1.54**
		P	66	17.20*
		N×P	11	1.40**
	% P	N	9	3.39**
		P	75	28.80*
		N×P	4	0.80**
	C:N	N	6	0.34**
		P	5	0.29**
		N×P	10	0.29**
C:P	N	8	3.69**	
	P	79	25.40*	
	N×P	5	1.20**	
N:P	N	11	8.34*	
	P	70	50.24*	
	N×P	13	4.58***	

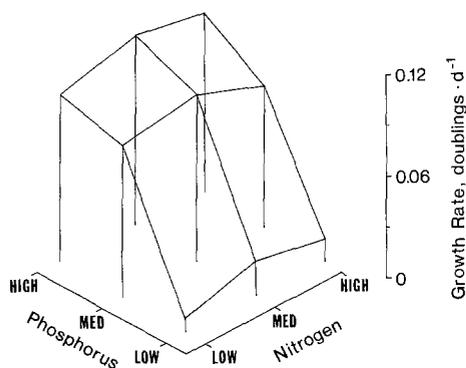
* $P < 0.01$, ** $P > 0.05$ (not significant), *** $P < 0.05$

Table 3. *Gracilaria tikvahiae*. Levels of chemical constituents (in percent of dry weight) and their molar ratios in *G. tikvahiae* grown under different treatments of NH_4^+ (N) and PO_4^{3-} (P) enrichment in South Pine Channel, Florida Keys, during winter 1983. Values represent means \pm SD (in parentheses, $n=2$)

Treatment	% C	% N	% P	C:N	C:P	N:P
Low N, Low P	30.3 (0.5)	1.80 (0.00)	0.040 (0.000)	19.5 (1.5)	1 939 (124)	100 (1)
Low N, Med P	28.7 (0.6)	2.30 (0.16)	0.050 (0.006)	14.5 (1.2)	1 469 (75)	102 (8)
Low N, High P	27.7 (1.1)	2.36 (0.26)	0.065 (0.017)	13.6 (0.6)	1 091 (37)	81 (11)
Med N, Low P	28.7 (0.3)	1.98 (0.03)	0.028 (0.002)	16.8 (0.4)	2 694 (105)	157 (9)
Med N, Med P	29.2 (0.7)	2.25 (0.16)	0.039 (0.000)	15.0 (0.7)	1 916 (81)	128 (8)
Med N, High P	25.3 (0.3)	2.18 (0.09)	0.064 (0.011)	13.5 (0.40)	1 012 (59)	76 (8)
High N, Low P	27.8 (0.8)	2.03 (0.04)	0.041 (0.001)	15.9 (0.9)	1 736 (84)	109 (7)
High N, Med P	29.6 (0.5)	2.43 (0.13)	0.056 (0.004)	14.1 (1.0)	1 353 (63)	96 (11)
High N, High P	26.5 (0.5)	2.50 (0.13)	0.076 (0.016)	12.3 (0.4)	892 (39)	73 (15)

Table 4. *Gracilaria tikvahiae*. Levels of chemical constituents (in percent of dry weight) and their molar ratios in *G. tikvahiae* grown under different treatments of NH_4^+ (N) and PO_4^{3-} (P) enrichment in South Pine Channel, Florida Keys, during summer 1983. Values represent means \pm SD (in parentheses, $n=2$)

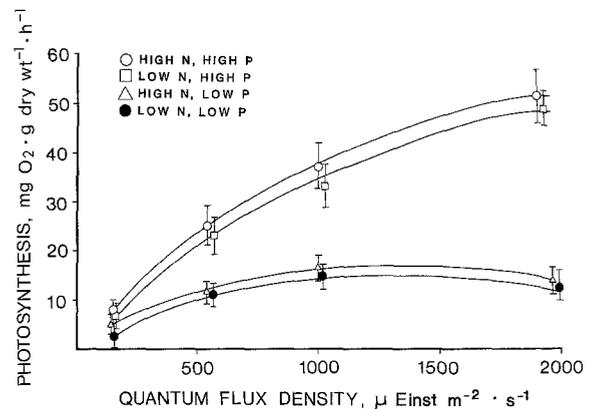
Treatment	% C	% N	% P	C:N	C:P	N:P
Low N, Low P	26.5 (1.5)	2.65 (0.05)	0.040 (0.002)	11.6 (0.40)	1 696 (102)	147 (5)
Low N, Med P	23.4 (0.8)	2.40 (0.04)	0.047 (0.007)	11.3 (0.3)	1 274 (56)	113 (15)
Low N, High P	24.0 (1.5)	2.37 (0.24)	0.057 (0.001)	11.7 (0.4)	1 077 (44)	93 (5)
Med N, Low P	26.0 (0.9)	2.72 (0.16)	0.032 (0.002)	11.1 (0.3)	2 080 (107)	191 (5)
Med N, Med P	24.7 (0.0)	2.43 (0.05)	0.038 (0.008)	11.8 (0.3)	1 664 (126)	142 (9)
Med N, High P	23.0 (0.5)	2.25 (0.35)	0.052 (0.008)	11.9 (1.0)	1 132 (61)	98 (6)
High N, Low P	28.6 (1.5)	2.97 (0.10)	0.026 (0.001)	11.2 (0.9)	2 816 (131)	250 (21)
High N, Med P	24.5 (1.5)	2.50 (0.29)	0.043 (0.004)	11.4 (0.7)	1 458 (72)	127 (19)
High N, High P	23.6 (0.3)	2.44 (0.17)	0.055 (0.004)	11.2 (0.9)	1 098 (49)	98 (14)

**Fig. 4.** *Gracilaria tikvahiae*. Growth rate in South Pine Channel, Florida Keys, during summer 1983, in response to different levels of NH_4^+ and PO_4^{3-} enrichment. "Low" represents ambient seawater levels only; "medium" represents weekly 5-h exposure to $700 \mu\text{M}$ NH_4^+ and/or $40 \mu\text{M}$ PO_4^{3-} ; "high" represents weekly 5-h exposure to $1.4 \mu\text{M}$ NH_4^+ and/or $80 \mu\text{M}$ PO_4^{3-} . Values represent means; coefficient of variation < 0.11

dry $\text{wt}^{-1} \text{h}^{-1}$ ($n=4$) compared to $\sim 5.0 \pm 0.6 \text{ mg O}_2 \text{ g dry wt}^{-1} \text{h}^{-1}$ ($n=4$) for N-enriched plants and $3.0 \pm 0.5 \text{ mg O}_2 \text{ g dry wt}^{-1} \text{h}^{-1}$ ($n=4$) for non-enriched plants (Fig. 3).

Summer

During summer 1983, only P enrichment significantly enhanced growth of *Gracilaria tikvahiae*, accounting for 82% of the total experimental variation in growth. Effects

**Fig. 5.** *Gracilaria tikvahiae*. Photosynthesis vs irradiance (in quantum flux density) curve of *G. tikvahiae* in South Pine Channel, Florida Keys, during summer 1983, in response to different levels of NH_4^+ and PO_4^{3-} enrichment. "Low" represents ambient seawater levels only; "high" represents weekly 5-h exposure to $1.4 \mu\text{M}$ NH_4^+ and/or $80 \mu\text{M}$ PO_4^{3-} . Values represent means \pm 1 SD ($n=4$)

of N and N \times P interaction were both insignificant (Table 2). Growth rates ranged from 0.005 to 0.113 doublings d^{-1} (Fig. 4).

As in winter, percentages of tissue C, N, and P during the summer studies were affected by P but not N enrichment (Table 2). Levels of C ranged from 23.0 to 28.6% of dry weight; N ranged from 2.25 to 2.97% of dry weight; P ranged from 0.032 to 0.057% of dry weight (Table 4). P, N, and N \times P interaction affected the N:P ratio, but only P enrichment affected the C:P ratio, and none of the factors

affected the C:N ratio (Table 2). C:N ratios ranged from 11.1 to 11.9, C:P ratios ranged from 1 098 to 2 816, and N:P ratios ranged from 93 to 250 (Table 4).

P but not N enrichment enhanced P_{\max} values of *Gracilaria tikvahiae* compared to the non-enriched treatments during summer (Fig. 5). Values of P_{\max} for P-enriched plants were $\sim 52.1 \pm 4.2 \text{ mg O}_2 \text{ g dry wt}^{-1} \text{ h}^{-1}$ ($n=4$) compared to $16.1 \pm 2.1 \text{ mg O}_2 \text{ g dry wt}^{-1} \text{ h}^{-1}$ ($n=4$) for N-enriched and $14.8 \pm 1.8 \text{ mg O}_2 \text{ g dry wt}^{-1} \text{ h}^{-1}$ ($n=4$) for non-enriched control plants (Fig. 5).

Discussion

The dominant effects of P-enrichment compared to N-enrichment on growth, photosynthetic capacity, and tissue levels of C, N, and P in *Gracilaria tikvahiae* during both winter and summer in this study demonstrates that P is the more important limiting nutrient for productivity of this alga in inshore waters of the Florida Keys. This demonstration of a P-limited nutrient regime for macroalgae contrasts with studies in the coastal marine environment along eastern North America where N was considered the primary nutrient limiting growth of both phytoplankton (Ryther and Dunstan, 1971; Vince and Valiela, 1973) and macroalgae (Topinka and Robbins, 1976; Chapman and Craigie, 1977; Hanisak, 1979). However, nutrient bioassays along Florida's northern Gulf coast also showed that P was frequently more important than N in regulating phytoplankton productivity (Myers and Iverson, 1981). This supports the opinion held by geochemists (Broecker and Peng, 1982; Smith, 1984) and in particular, Redfield (1958) that the oceans as a whole are P-limited ecosystems.

Elevated C:N, C:P and N:P ratios in the non-enriched cultures of *Gracilaria tikvahiae* support the contention of severe year-round P-limitation and secondary N-limitation during winter. Studies with *G. tikvahiae* have shown that N limits growth above a molar C:N ratio of ~ 15.0 (Lapointe and Duke, 1984). The occurrence of C:N ratios above this level during winter but not summer agrees with the growth rate and photosynthetic data, which also indicate N-limitation only in winter. The C:P and N:P ratios of *G. tikvahiae* during this study were also quite high compared to other reported values. For example, the mean C:P ratio of unenriched *G. tikvahiae* in this study was $> 1 800$, a value three-fold greater than the median value of 550 reported in a survey of C:N:P ratios of benthic marine plants (Atkinson and Smith, 1983). The mean N:P ratio of unenriched *G. tikvahiae* in this study averaged > 120 , also three-fold greater than the median value of 30 reported by Atkinson and Smith (1983). Although critical N:P ratios where N-limitation changes to P-limitation have not been determined for macroalgae, this occurs at a N:P ratio of 30 in freshwater *Scenedesmus* sp. (Rhee, 1978). Thus, on a comparative basis with other marine plants and phytoplankton, the highly elevated C:P and N:P ratios of unenriched *G. tikvahiae* during this study indicate severe and primary limitation of annual

productivity by P, although elevated ratios indicated N may be a significant secondary limiting nutrient during winter.

The persistent P-limitation and seasonal N-limitation of *Gracilaria tikvahiae* during this study reflect seasonal variation in N:P availability in local waters of the Keys. Constantly low PO_4^{3-} concentrations observed in South Pine Channel seawater during this study result not only from the already low PO_4^{3-} in Caribbean blue waters (e.g. see Pilson and Betzer, 1979; Sander and Moore, 1979) that are source waters for the Keys, but also to low rates of P regeneration in the well oxygenated calcium carbonate rich sediments typical of the Keys (Berner, 1974; Morse and Cook, 1978). In contrast, N-fixation is a significant source of "new" N in nearshore waters of the Florida Keys, particularly during summer months when temperatures are maximum (Capone and Taylor, 1980). Seasonal input of groundwater N (as NO_3^-) is also maximum in the Keys during summer when seasonal rainfall is maximum (unpublished data). The potential for shelf-break upwelling, which could also introduce significant N to nearshore waters of the Keys, would also be maximum during summer as based on current concepts of upwelling along Florida's east coast (Smith, 1982; Atkinson *et al.*, 1984; Lapointe and Smith, in review). Such a pattern of seasonal N and P availability would result in seasonal variation in N:P availability, P being constantly low and N increasing from winter to summer. This pattern agrees with the experimental bioassays with *G. tikvahiae* and illustrates how seasonal variability can regulate N and P availability and consequently, ecosystem productivity. McComb *et al.* (1981) have also suggested that an estuarine system in Western Australia may shift seasonally between N and P limitation, with N limiting production during summer and autumn and P limiting production during winter and spring. Thus, the degree and type of nutrient limitation of benthic primary productivity in inshore waters of the Keys, as well as other geographic areas, may not be constant throughout the year and, in fact, may change seasonally. This contrasts with concepts of nutrient limitation in the marine environment that consider an "either/or" situation for a particular water type with respect to N versus P limitation (e.g. see Smith, 1984).

That both P and N simultaneously limited growth and photosynthetic capacity of *Gracilaria tikvahiae* during winter suggests that dual nutrient limitation occurs in a physiological sense in this species under field conditions. The concept that a single limiting nutrient controls growth, which originated from Liebig's "law of the minimum", has been challenged by investigators (Verduin, 1964; Droop, 1973) who have suggested that the effect of a limiting nutrient on algal growth can be varied by the simultaneous effects of other limiting nutrients. The simultaneous occurrence of elevated C:N, C:P, and N:P ratios above critical levels in my unenriched *G. tikvahiae* cultures and growth enhancement by both N and P enrichment during winter supports the concept of dual nutrient limitation. This suggests multiple nutrient limitation may be the rule

rather than the exception during winter in the oligotrophic waters of the Florida Keys where several potentially limiting nutrients may be at or below critical concentrations. Chemostat culture studies with the phytoplankters *Monochrysis lutheri* (Droop, 1974) and *Scenedesmus* sp. (Rhee, 1974, 1978), however, suggest that algal growth is controlled by a single limiting nutrient (i.e. a threshold model), and without the added effects of other limiting nutrients (i.e. a multiplicative model). The disparity between these laboratory studies and the present study is difficult to reconcile considering the differences in environmental conditions that exist between the *in-situ* conditions of the present study and those of a chemostat. Jannasch (1974) pointed out that chemostat studies may not accurately describe *in-situ* events because the criteria for establishing steady-state in natural waters are seldom met.

N-limitation of *Gracilaria tikvahiae* during winter may be related not only to low availability of dissolved inorganic N, but also to physiological stress induced by intense P-limitation at relatively low winter temperatures ($\sim 20^\circ\text{C}$). At all levels of N enrichment, increasing P elevated levels of tissue N (and decreased the C:N ratio) – indicating the basis for the significant interaction between P and N enrichment on tissue N and P levels as well as on growth of *G. tikvahiae*. A similar interaction was observed by Ketchum (1939a) with *Nitzschia closterium*, where N enrichment increased P uptake, although P enrichment apparently had no effect on N uptake. The severe P-limitation during my study could conceivably interact with the N nutrition of *G. tikvahiae* because of the importance of P-containing molecules to cellular energetics (ATP, ADP) and membrane structure (phospholipids) – two critical components of the N uptake process in algae. The lack of an increase in tissue N with P enrichment during summer ($\sim 32^\circ\text{C}$) further suggests that this interaction is affected by a seasonal factor, possibly temperature. An interaction between temperature and N-limitation occurs in phytoplankton (Rhee and Gotham, 1981), but studies of N-limitation in macroalgae have not been related to physiological stress induced by severe P-limitation.

Measurement of the photosynthetic response of macroalgae previously exposed to different types of nutrient enrichment provides a quantitative index for determining the degree and type of nutrient deficiency in natural waters. This method is based on the hyperbolic relationship between photosynthesis and the cellular level of a limiting nutrient as demonstrated for P in freshwater *Scenedesmus* sp. (Smith, 1983). For *Gracilaria tikvahiae*, the photosynthetic response is in close agreement with the growth response, suggesting that short-term productivity measurements can be accurately extrapolated to longer-term rates of organic production. One drawback of the technique, however, is that 12 to 24 h are required after enrichment before photosynthetic assays can be performed because of the time required for nutrient-related physiological changes. However, the technique does not appear to suffer from the ambiguity involved in the short-term

^{14}C nutrient enrichment bioassay (Healey, 1979; Lean and Pick, 1981).

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