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STRATEGIES FOR PULSED NUTRIENT SUPPLY TO *GRACILARIA* CULTURES IN THE FLORIDA KEYS: INTERACTIONS BETWEEN CONCENTRATION AND FREQUENCY OF NUTRIENT PULSES[1]

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Abstract: A factorial design experiment, using in situ cage cultures, was used to investigate the effects of frequency and concentration of nutrient pulses on growth, nutrient uptake, and chemical composition (C, N, P) of *Gracilaria tikvahiae* McLachlan in nearshore waters of the Florida Keys. Both frequency and concentration of the nutrient pulses affected growth and chemical composition of *G. tikvahiae*, indicating nutrient limitation occurred during the study. Growth of *G. tikvahiae* increased with increasing pulse frequency up to the highest level used ($2 \cdot wk^{-1}$) at all pulse concentrations; in contrast, growth increased with increasing pulse concentration to the highest concentration at the low pulse frequency but not at the higher pulse frequencies. Although the frequency of nutrient pulses appeared more important in regulating growth and pre-pulse levels of chemical constituents than pulse concentration, the effects of frequency were due to its effects on total nutrient loading (i.e. flux) and not to the effects of frequency of nutrient enrichment per se. Greater variation in percent P compared to percent N in *G. tikvahiae* tissue between pulses and an increased PO_4^{3-} uptake rate in nutrient-limited *G. tikvahiae* suggests that P rather than N was the primary limiting nutrient during the study; however, N was an important secondary limiting nutrient indicating dual nutrient-limitation occurred. While the pulse medium used had a N : P ratio of 18 : 1, much higher uptake ratios, ranging from 27 : 1 to 80 : 1, actually occurred, supporting the contention of P-limitation. Thus, nutrient pulse strategies with *G. tikvahiae* in P-limited systems need to utilize excessively low N : P ratios in the pulse medium to offset the differential uptake rates of NH_4^+ and PO_4^{3-} at the high concentrations typically used in pulse-feeding strategies.

Key words: *Gracilaria*; cage culture; carbon; nitrogen; phosphorus; growth

INTRODUCTION

Nutrient supply is an important operating parameter in the management of seaweed cultivation systems. N is a potential growth-limiting nutrient for *Gracilaria tikvahiae* in a variety of culture systems (Lapointe & Ryther, 1979; Ryther & Hanisak, 1981), supporting the contention that this element can be a primary limiting nutrient in coastal marine systems. Perhaps as an ecological adaptation to growth in a potentially N-limited environment, *G. tikvahiae* also has a great capacity for storage of N that can be used to sustain growth for extended periods of time (2–3 wk) when dissolved inorganic N in its growth medium is at low to undetectable concentrations (Lapointe & Ryther, 1979; Ryther *et al.*, 1981). Nitrogen storage pools in *Gracilaria* include the

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light-harvesting phycobiliprotein pigments (Gantt, 1981; Lapointe, 1981) proteins and amino acids (Bird *et al.*, 1982) and to a lesser degree, inorganic tissue NO_3^- (Lapointe & Duke, 1984). N-limited *G. tikvahiae* also has a greater capacity for NH_4^+ uptake than N-replete *G. tikvahiae* (D'Elia & DeBoer, 1978), a phenomenon common among algae.

The ability for rapid N uptake and storage provides the physiological and biochemical basis for pulse-feeding N to *G. tikvahiae* McLachlan. Ryther *et al.* (1981) demonstrated that N-limited *G. tikvahiae* soaked for as little as 6 h in a full nutrient medium grew at non-nutrient limited rates in enriched flowing sea water for as long as 2 wk before the plants became N deficient and their growth rate declined. While pulse-feeding *G. tikvahiae* at 2-wk intervals appears adequate in N-limited systems, little is known of the storage capacity for P by *G. tikvahiae* or how potential P limitation might affect the utility of the pulse-feeding strategy.

The present study was designed to address the dynamics of N and P in *G. tikvahiae* as a function of two important operating variables that regulate nutrient loading (i.e. flux) in a pulse-fed seaweed culture system: (1) concentration of nutrients during the pulse, and (2) the frequency of nutrient pulses. Whereas much of the previous cultivation research concerning nutrient-growth relationships with *G. tikvahiae* has been performed in land-based systems (culture tanks, ponds, etc.) that received eutrophic estuarine sea water, this study was performed in situ in the lower Florida Keys to allow inferences regarding nutrient limitation under oligotrophic field conditions.

Materials and Methods

Cultures of *G. tikvahiae* were transferred from tank cultures at the Harbor Branch Foundation in Ft. Pierce, Florida to in situ cage cultures in South Pine Channel adjacent to Big Pine Key, 18 km east of Key West, Florida. The cages were constructed of Vexar (2 cm mesh; 0.6 m^2 surface area), attached to PVC flotation collars, and tethered to the bottom in shallow water. Tidal and wind-driven currents within the cages ranged from 2–25 $cm \cdot s^{-1}$ (measured with TSK flow meters) and frequent observations indicated that loss of plant material from the cages was negligible. The experiment was conducted between 14 March 1983 and 12 May 1983 during which irradiance ranged from 20–57 $E \cdot m^{-2} \cdot day^{-1}$, temperature ranged from 20–28 °C, and salinity ranged from 36–37‰.

A 3 × 3 factorial design experiment, utilizing three levels of nutrient concentrations and three levels of pulse frequencies (nine treatments with two replicate cages per treatment = 18 total cages), was used to test for main effects and interaction of nutrient concentration and pulse frequency on growth, nutrient uptake, and chemical composition of *G. tikvahiae*. Experimental protocol consisted of inoculating 300 g wet wt of *G. tikvahiae* into each cage and, at three different frequencies (low = one per 2 wk; medium = one per week; high = two per week), removing and soaking the plants in 14-l Nalgene containers for 6 h at the appropriate nutrient concentrations (low = $f/2$

concentrations of NH_4^+ and PO_4^{3-}; medium = 4 × *f*/2 concentrations; high = 8 × *f*/2 concentrations) where *f*/2 ≃ 700 μM NH_4^+ (NH_4^+ without the NO_3^- usually used in *f* medium) and 40 μM PO_4^{3-}, respectively (Guillard & Ryther, 1962). Preliminary studies indicated that increases in concentration above this "high" level did not result in increased growth of *G. tikvahiae* at any of the frequencies used. During the pulses, the plants were gently aerated to facilitate nutrient uptake. At biweekly intervals, the *G. tikvahiae* populations were harvested back to the initial weight and growth rates calculated as doublings · day^{-1}. The growth data reported herein represent three 2-wk growth periods (1 April 1983–12 May 1983) that followed 2-wk acclimation to this experimental protocol.

To assess NH_4^+ and PO_4^{3-} uptake rates by *G. tikvahiae* during the pulse, initial ($T = 0$) and final ($T = 5$ h) samples of the various pulse media were taken, filtered through a 0.45-μm Gelman filter, and frozen for later analysis. Concentrations of NH_4^+ and PO_4^{3-} were determined on a Technicon II Auto-analyzer by methods described by Zimmerman *et al.* (1977). Compared to NH_4^+, NO_2^- and NO_3^- supplied negligible amounts of N to *G. tikvahiae*.

At the end of the experimental growth period, tissue samples of the various experimental treatments were also taken for determination of molar C : N, C : P, and N : P ratios. Algal samples were rinsed briefly in deionized water (1- to 2-s rinse) and dried to constant weight at 60 °C. C and N were determined using a Perkin–Elmer 240 Elemental Analyzer and total P was measured using a modified persulfate digestion method (Menzel & Corwin, 1965).

Main effects and interaction of concentration and frequency of the nutrient pulses on the growth, nutrient uptake, and plant chemistry were assessed by two-way ANOVA. Significance reported in the results below implies the probability of the null hypothesis is < 0.05.

RESULTS

Both the concentration and frequency of the nutrient pulses, as well as their interaction, significantly affected growth of *G. tikvahiae* during this study; however, frequency alone accounted for most of the variation in growth, 55%, compared to 36% for concentration (Table I). Growth of *G. tikvahiae* increased with increasing pulse frequency up to the highest levels used (two per week) at all pulse concentrations; in contrast, growth increased with increasing pulse concentration to the highest concentration used at the low pulse frequency but not at the higher pulse frequencies (Fig. 1). Growth rates ranged from 0.02 to 0.12 doublings · day^{-1} over the experimental design (Fig. 1).

In the statistical analysis of the growth data given above, frequency of the pulses is confounded within total nutrient flux because treatments receiving more frequent pulses generally received a greater flux of nutrients. To test the effects of frequency per se, the means of the following three treatments were statistically compared: (1) high concen-

TABLE I

Summary of two-way ANOVA for the measured variables as a function of frequency (F) and concentration (C) of nutrient pulses.

Variable	Factor	% Variation explained by factor	F
Growth rate	F	55	82.7**
	C	36	58.4**
	FXC	3	2.2 (ns)
% N^a	F	51	47.3**
	C	16	15.1**
	FXC	29	13.3**
% C^a	F	10	1.21 ns
	C	18	2.10 ns
	FXC	33	1.90 ns
% P^a	F	70	48.2**
	C	9	6.3*
	FXC	15	5.2*
C:N^a	F	54	25.9**
	C	11	5.3*
	FXC	25	6.0*
C:P^a	F	74	14.50
	C	5	2.30
	FXC	8	2.15
N:P^a	F	21	2.1 ns
	C	3	0.3 ns
	FXC	30	1.5 ns
% N^b	F	33	28.6**
	C	44	37.6**
	FXC	18	7.4*
% C^b	F	13	2.4 ns
	C	51	9.3**
	FXC	12	1.1 ns
% P^b	F	40	8.5**
	C	30	6.5*
	FXC	9	0.9 ns
C:N^b	F	22	13.7**
	C	61	38.8**
	FXC	10	3.2 ns
C:P^b	F	24	12.42
	C	72	16.80
	FXC	10	1.90
N:P^b	F	4	0.39 ns
	C	15	1.46 ns
	FXC	33	1.55 ns
NH_4^+ uptake	F	±	0.5 ns
	C	99	395.8**
	FXC	0	0.1 ns
PO_4^{3-} uptake	F	23	5.0**
	C	49	11.0**
	FXC	9	1.0 ns
NH_4^+ : PO_4^{3-} uptake	F	35	6.12**
	C	48	18.21**
	FXC	10	2.91 ns

TABLE I, continued

Variable	Factor	% Variation explained by factor	*F*
% increase in N quota	F	35	6.12*
	C	8	1.31 ns
	FXC	33	2.91 ns
%increase in P quota	F	52	6.43*
	C	3	0.36 ns
	FXC	8	0.50 ns

[a] Tissue constituents sampled before pulse.
[b] Tissue constituents sampled after pulse.
** $P < 0.01$.
* $P < 0.05$.
ns = $P > 0.05$ = not significant.

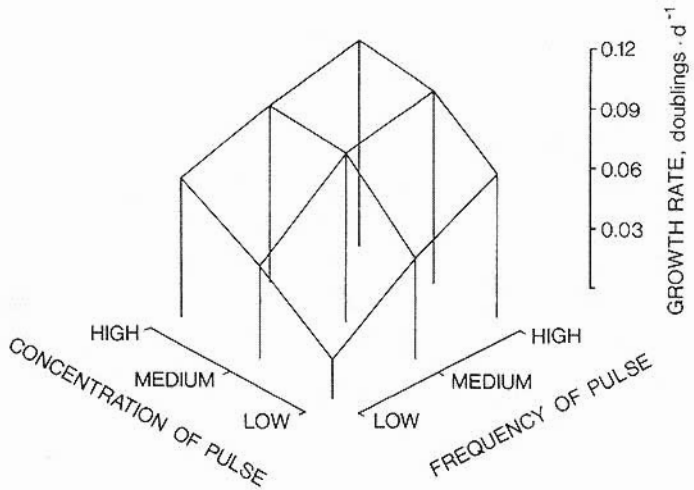

Fig. 1. Growth rate (doublings · day^{-1}) of *Gracilaria tikvahiae* as a function of frequency and concentration of nutrient pulses: values represent means ± 1 SD ($n = 3$).

tration, low frequency, (2) medium concentration, medium frequency, and (3) low concentration, high frequency. Because frequency and concentration of pulses in this experimental design were evenly spaced (i.e. doubled at each level), these three treatments received equal fluxes of nitrogen and phosphorus over the 2-wk growth period (Fig. 1). Differences among these means were insignificant ($P > 0.05$; Duncan's new multiple range test), suggesting that frequency of nutrient enrichment per se did not affect the growth of *G. tikvahiae* during this study.

Both frequency and concentration of the pulses, as well as their interaction, affected nutrient-depleted *G. tikvahiae* tissue (sampled before the pulses). Frequency accounted for the bulk of variation in these parameters, especially P. For example, frequency alone accounted for 51 and 70% of total variation in percent N and P, compared to 16 and 9%, respectively, for concentration (Table I). Percent tissue N and P ranged from 1.15

and 0.046% (low frequency, low concentration) to 3.43 and 0.156% (high frequency, high concentration) of dry weight, respectively (Table II).

Frequency and concentration of the pulses also affected percent N and P in nutrient-enriched (sampled after the pulse) *G. tikvahiae*, but in this case, their effects were more equitable. For example, frequency accounted for 33 and 40% of the variation in N and P, compared to 44 and 30% for concentration (Table I). Percent tissue N and P ranged from 1.50 and 0.091% (low frequency, low concentration) to 3.61 and 0.154% (high frequency, high concentration), respectively (Table II).

Frequency of nutrient pulses was the only significant factor affecting the percentage increase in N and P of *G. tikvahiae* due to the pulses; the effects of concentration and concentration × frequency interaction were both insignificant (Table I). Also, frequency of the pulses was more important to increases in percent P compared to percent N. For example, frequency accounted for 52% of the variation in increases in percent P compared to 35% for percent N (Table I). The greater importance of frequency in regulating increases in percent P compared to percent N is also evident in the magnitude of changes in the tissue N and P values. Increases in percent P resulting from pulses at the low and medium frequencies were consistently greater than the corresponding increase in percent N (Table II). Increases in both percent P and N were also greater at the low frequencies compared to the medium and high frequencies (Table II).

Frequency of the pulse was also the more important factor affecting variation in C : N, C : P, and N : P ratios of nutrient-limited *G. tikvahiae* before the pulses. Frequency accounted for 54, 74, and 21% of the variation in these ratios, respectively, before the pulse, compared to 11, 5, and 3% for concentration (Table I). C : N ratios of *G. tikvahiae*

TABLE II

Levels of C, N, and P in *Gracilaria tikvahiae* grown in situ in South Pine Channel, Florida Keys, and sampled before and after various treatments of nutrient pulses: values represent means ± 1 SD (n = 2).

Pulse treatment		Before pulse					
		% N	% P	% C	C : N[c]	C : P[c]	N : P[c]
Frequency[a]	Concentration[b]						
low	low	1.15 ± 0.31	0.046 ± 0.04	26.1 ± 4.7	26.4 ± 1.4	1460 ± 450	55.4 ± 19.3
low	medium	1.10 ± 0.16	0.059 ± 0.017	26.5 ± 1.0	28.1 ± 5.0	1163 ± 390	41.4 ± 6.7
low	high	1.13 ± 0.11	0.050 ± 0.009	23.5 ± 0.9	24.3 ± 1.4	1220 ± 180	50.1 ± 4.3
medium	low	1.42 ± 0.02	0.080 ± 0.009	23.4 ± 1.3	19.1 ± 1.3	756 ± 46	39.5 ± 4.9
medium	medium	1.54 ± 0.35	0.072 ± 0.015	24.2 ± 0.2	18.4 ± 4.4	871 ± 190	47.4 ± 0.9
medium	high	1.53 ± 0.30	0.083 ± 0.017	20.5 ± 1.0	15.7 ± 2.4	638 ± 100	40.7 ± 0.0
high	low	1.30 ± 0.30	0.084 ± 0.001	23.8 ± 1.1	21.3 ± 3.9	731 ± 47	34.3 ± 8.8
high	medium	2.50 ± 0.0	0.143 ± 0.025	21.7 ± 1.2	10.1 ± 0.5	392 ± 91	38.7 ± 6.8
high	high	3.43 ± 0.23	0.156 ± 0.007	21.9 ± 0.9	7.4 ± 0.3	362 ± 3	48.7 ± 0.0

[a] Low = one pulse per 2 wk; medium = one pulse per week; high = two pulses per week.

[b] Low = initial concentrations of ≈700 μM N_4^+, 40 μM PO_4^{3-} in pulse; medium = initial concentrations of 2.8 mM NH_4^+, 160 μM PO_4^{3-} in pulse; high = initial concentrations of 5.6 mM NH_4^+, 320 μM PO_4^{3-} in pulse.

[c] Molar ratios.

before the pulse ranged from 7.4 to 28.1; C : P ratios ranged from 362 to 1460, and N : P ratios ranged from 34.3 to 55.4 (Table II).

In contrast, concentration rather than frequency had more dominant effects on variation among these molar ratios after the pulse. For example, concentration accounted for 61, 72, and 15% of the variation in C : N, C : P, and N : P ratios after the pulse compared to 22, 24, and 4% for frequency, respectively. All these ratios were lower after the pulses, indicating N and P enrichment during the pulses. C : N ratios of *G. tikvahiae* after the pulse ranged from 6.9 to 20.5; C : P ratios ranged from 362 to 745, and N : P ratios ranged from 36.1 to 51.9. That the variation in percent C among the experimental treatments was much lower than that for percent N and P indicates that the observed changes in these ratios (i.e. decreasing during the pulse) was due mostly to changes in percent N and P (i.e. N and P assimilated during pulse) and not to variations in carbon metabolism.

The uptake rate of NH_4^+ by *G. tikvahiae* measured during the pulse was affected primarily by concentration of the pulse; the effects of frequency were insignificant (Table I). Uptake of NH_4^+ by *G. tikvahiae* ranged from ≈ 6 to 23 μmol · g dry wt^{-1} · h^{-1} and increased linearly with concentration (Fig. 3).

The uptake rate of PO_4^{3-}, however, was affected differently. Both frequency and concentration of the pulse significantly affected PO_4^{3-} uptake, although concentration was the more important factor (Table I). PO_4^{3-} uptake ranged from 0.2 to 0.6 μmol · g dry wt^{-1} · h^{-1} and increased at low and medium frequencies at corresponding medium and high concentrations (Fig. 3).

The ratio of NH_4^+ / PO_4^{3-} uptake was also affected by both frequency and concen-

TABLE II, continued

After pulse						% increase in N quota from pulse	% increase in P quota from pulse
% N	% P	% C	C : N[e]	C : P[e]	N : P[e]		
1.50 ± 0.04	0.091 ± 0.038	26.3 ± 1.4	20.5 ± 1.5	745 ± 280	36.4 ± 16.4	35	102
1.59 ± 0.08	0.097 ± 0.002	24.6 ± 0.3	18.0 ± 0.7	655 ± 26	36.4 ± 2.5	45	74
2.49 ± 0.40	0.118 ± 0.013	24.4 ± 0.8	11.4 ± 2.1	533 ± 7	46.7 ± 2.0	110	145
1.55 ± 0.18	0.092 ± 0.001	25.1 ± 0.2	18.8 ± 2.3	704 ± 13	37.4 ± 3.9	10	16
1.92 ± 0.05	0.098 ± 0.002	21.9 ± 1.8	13.3 ± 0.8	576 ± 33	43.4 ± 0.0	27	40
1.90 ± 0.14	0.117 ± 0.0	21.9 ± 0.3	13.4 ± 6.8	483 ± 7	36.1 ± 2.5	26	44
1.77 ± 0.06	0.105 ± 0.020	27.5 ± 4.0	18.2 ± 3.4	676 ± 95	37.2 ± 7.2	44	42
2.72 ± 0.20	0.150 ± 0.007	21.4 ± 1.4	9.2 ± 0.0	368 ± 7	40.1 ± 1.1	4	9
3.61 ± 0.41	0.154 ± 0.013	21.6 ± 0.4	6.9 ± 0.7	362 ± 20	51.9 ± 2.0	5	1

tration of the pulse although concentration was the more important factor (Table I). The NH_4^+/PO_4^{3-} uptake ratio ranged from 27 to 80 and increased with increasing concentration and frequency (Fig. 4).

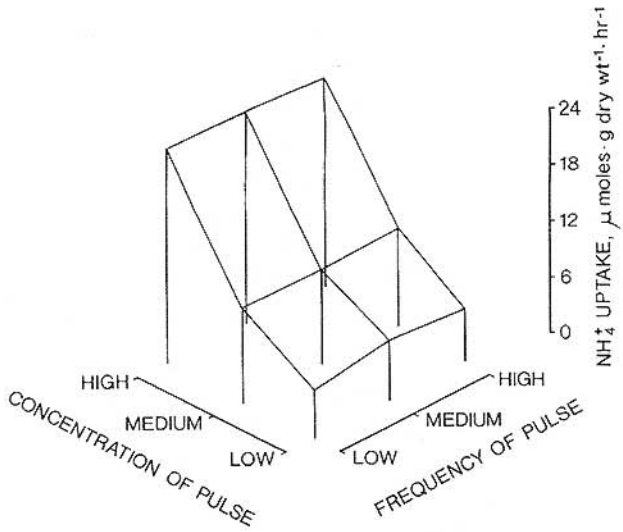

Fig. 2. Uptake of NH_4^+ (μmol · g dry wt^{-1} · h^{-1}) as a function of frequency and concentration of nutrient pulses: values represent means ± 1 SD (n = 2); low frequency = 1 · 2 wk^{-1}; medium frequency = 1 · wk^{-1}; high frequency = 2 · wk^{-1}; low concentration = 700 μM NH_4^+, 40 μM PO_4^{3-}; medium concentration = 2.8 mM NH_4^+, 160 μM PO_4^{3-}; high concentration = 5.6 mM NH_4^+, 320 μM PO_4^{3-}.

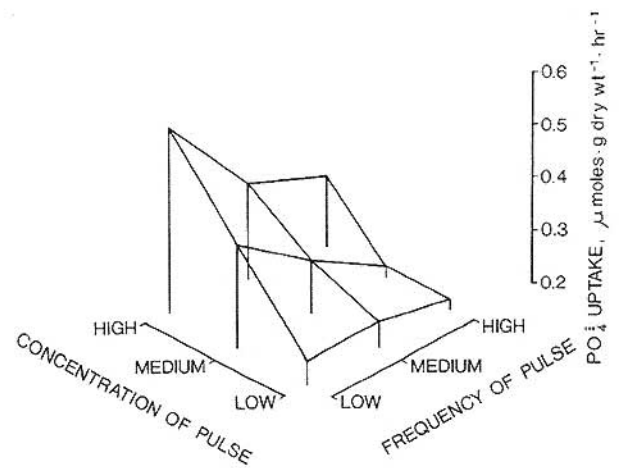

Fig. 3. Uptake of PO_4^{3-} (μmol · g dry wt^{-1} · h^{-1}) as a function of frequency and concentration of nutrient pulses: values represent means + 1 SD (n = 2); low frequency = 1 · 2 wk^{-1}; medium frequency = 1 · wk^{-1}; high frequency = 2 · wk^{-1}; low concentration = 700 μM NH_4^+, 40 μM PO_4^{3-}; medium concentration = 2.8 mM NH_4^+, 160 μM PO_4^{3-}; high concentration = 5.6 mM NH_4^+, 320 μM PO_4^{3-}.

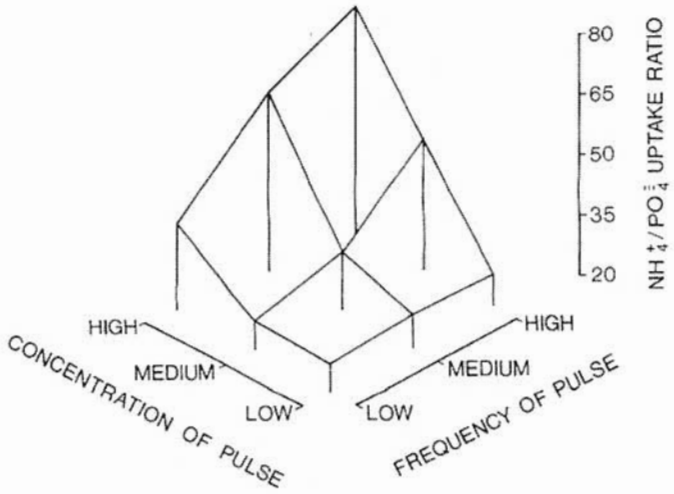

Fig. 4. NH_4^+/PO_4^{3-} uptake ratio by *Gracilaria tikvahiae* during a nutrient pulse as a function of frequency and concentration of nutrient pulses: values represent means ± 1 SD ($n = 2$); low frequency = $1 \cdot 2\ wk^{-1}$; medium frequency = $1 \cdot wk^{-1}$; high frequency = $2 \cdot wk^{-1}$; low concentration = 700 μM NH_4^+, 40 μM PO_4^{3-}; medium concentration = 2.8 mM NH_4^+, 160 μM PO_4^{3-}; high concentration = 5.6 mM NH_4^+, 320 μM PO_4^{3-}.

Discussion

Five-fold differences in growth of *G. tikvahiae* during this study illustrate the importance of frequency and concentration of nutrient pulses for maximizing algal growth in pulse-fed seaweed cultivation systems. Nutrient concentration during the pulse affects growth of *G. tikvahiae* largely through its relationship to nutrient uptake rate, which appeared linear within the high range of NH_4^+ and PO_4^{3-} concentrations used in this study (see Figs. 2 and 3). This supports earlier studies with *Gracilaria* (D'Elia & DeBoer, 1978) which found that NH_4^+ uptake at high concentrations (10–50 μM) did not follow the typical, saturation-type kinetics often reported for phytoplankton (Caperon & Meyer, 1972) and seaweeds (Hanisak & Harlin, 1978; Topinka, 1978).

In contrast, frequency of nutrient pulses, which was quantitatively more important than concentration in regulating growth of *G. tikvahiae* during this study, affects growth through its control of total nutrient flux over time scales that are similar to the doubling times of *G. tikvahiae* – i.e., days to weeks in this study. A comparison of experimental treatments having different pulse frequencies but equal nutrient flux indicated that effects of frequency of nutrient pulses per se is insignificant or, at least, minor. Consequently, the enhancement of growth of *G. tikvahiae* due to increasing pulse frequency is due primarily to the increased nutrient flux associated with increased pulse frequency. Temporal limiting nutrient patchiness (at equal nutrient flux) is an important ecological factor that can control the species composition of phytoplankton communities (Turpin & Harrison, 1979), but its autecological effects on *G. tikvahiae* during this study appeared to be related primarily to its regulation of total nutrient availability.

Two lines of evidence support the contention that phosphorus was equally important, if not more important, than nitrogen as a limiting nutrient in waters of the Florida Keys during this study. First, at the low and medium pulse frequencies where nutrient limitation was most severe, the nutrient pulses consistently increased the P quotas to a greater extent than N quotas (Table II). Second, the uptake rate of PO_4^{3-} during the pulses increased dramatically with decreasing frequency (i.e. increasing nutrient limitation) at both the medium and high pulse concentrations; in contrast, such did not occur for NH_4^+ (compare Figs. 1 and 2). In comparison with previous studies that reported increased uptake of NH_4^+ in N-limited *G. tikvahiae* (D'Elia & DeBoer, 1978), the increased uptake rate of PO_4^{3-} by nutrient-limited *G. tikvahiae* in this study suggests that a similar enhanced uptake ability for PO_4^{3-} occurs under P limitation. This evidence, suggesting P was equally, if not more important than N in regulating growth of *G. tikvahiae*, contrasts with the general view that N is the major growth-limiting nutrient in subtropical and tropical waters (Parsons *et al.*, 1977); however, it supports recent geochemical models (Smith, 1984) and other algal enrichment bioassays in Florida (Myers & Iverson, 1981; Lapointe & Miller, unpubl. data) which also suggest that P can be more important than N in limiting algal growth in marine waters.

That growth of *G. tikvahiae* increased with increasing frequency up to the highest level used in this study (i.e. one to two per week) contrasts with earlier studies where *G. tikvahiae* could be maintained at maximum growth rates for 2 wk between nutrient pulses (Ryther *et al.*, 1981). This disparity may be due, in part, to the predominance of P limitation during the present study in the oligotrophic waters of the Florida Keys compared to N limitation during the previous pulse-feeding experiments with *G. tikvahiae* in the relatively nutrient-enriched estuarine water from the Indian River near Ft. Pierce, Fla. (Ryther *et al.*, 1981). The requirement for more frequent nutrient pulses to maintain maximal growth of *G. tikvahiae* under P limitation compared to N limitation suggests that the storage capacity for P may not be as great as that for N. This idea is supported by our findings that both pulse frequency and concentration affected PO_4^{3-} uptake whereas only concentration affected NH_4^+ uptake. Previous studies have shown phosphate uptake is not a one-way movement into algal cells (influx) but is accompanied by an efflux such that long-term net PO_4^{3-} uptake can be very low at times. Lean & Nalewajko (1976) investigated influx and efflux of phosphate in phytoplankton batch cultures and found both processes were very rapid. Bieleski (1973) also found efflux of phosphate in *Nitella* and suggested the balance between phosphate influx and efflux is critical to plant growth. Considering the important limiting role of P to growth of *G. tikvahiae*, further studies are needed to determine the influx/efflux kinetics of PO_4^{3-} as well as biochemical mechanisms for storage and utilization of P.

The severe P-limitation observed in *G. tikvahiae* may also exacerbate N limitation by negatively affecting its physiological state. P-limited *G. tikvahiae* in the nutrient-limited low and medium frequency treatments was accompanied by parallel low N levels (1.15–1.50%) and elevated C : N ratios (15.7–28.1), indicating that N limitation was also occurring (Lapointe & Ryther, 1979; Lapointe & Duke, 1984). Thus, dual nutrient

limitation occurred in *G. tikvahiae* during this study, in agreement with previous studies (Lapointe & Miller, unpubl. data). Despite these parallel low N levels, the N : P ratios of nutrient-limited *G. tikvahiae* (Table II), which ranged from 34.3–55.4, appear similar to algae which are severely P-limited (sensu Redfield, 1958); this suggests that the N : P ratio of algal tissue may not be a sensitive index of the type or degree of nutrient limitation when both N and P are limiting growth. However, previous studies indicated that increasing P availability at constant levels of N availability led to increased N levels in P-limited *G. tikvahiae*, suggesting severe P limitation can actually result in N limitation of *G. tikvahiae* (Lapointe & Miller, unpubl. data). This is not surprising, considering the importance of P-containing molecules to cellular energetics (ATP, ADP) and membrane structure (phospholipids) – two critical components of the N uptake system in algae. Thus, if a minimum cellular quota of phosphorus (≈ 0.12–0.14% P of dry wt) is not maintained, P limitation may exacerbate N limitation, and possibly other nutrient limitations (e.g., trace metals?) as well, by negatively affecting physiological state of *G. tikvahiae*.

One factor that appears important in pulse-feeding strategies for *G. tikvahiae* in P-limited water types such as the Florida Keys is the N : P ratio of the pulse medium. Although the medium used in this study had an N : P ratio of $\approx 18 : 1$, much higher N : P uptake ratios, ranging from 27 : 1 to 80 : 1, actually occurred (Fig. 4). That the N : P uptake ratio also increased with increasing concentration (Fig. 4) suggests that as NH_4^+ and PO_4^{3-} concentrations are increased at a constant N : P ratio, NH_4^+ uptake increases much faster than PO_4^{3-} uptake. This may relate to a high diffusive component for NH_4^+ uptake at high NH_4^+ concentrations in *G. tikvahiae* (D'Elia & DeBoer, 1978) as well as possible losses of volatilized NH_4^+ from the pulse medium. Clearly, such a pattern of nutrient uptake will serve to exacerbate any background P limitation. Thus, nutrient pulse strategies in P-limited systems need to use excessively low (e.g. 1 : 1–10 : 1) N : P ratios in the pulse medium to offset the differential uptake rates of NH_4^+ and PO_4^{3-} at high concentrations.

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