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This study was carried out by G. A. Pearson during the course of a Natural Environment Research Council studentship.

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EFFECT OF NITROGEN AND PHOSPHORUS SUPPLY ON GROWTH AND TISSUE COMPOSITION OF *ULVA FENESTRATA* AND *ENTEROMORPHA INTESTINALIS* (ULVALES, CHLOROPHYTA)[1]

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ABSTRACT

The chlorophyte macroalgae Ulva fenestrata *(Postels and Ruprecht) and* Enteromorpha intestinalis *(Linnaeus) Link. were grown under various nutrient regimes in indoor semi-continuous and batch cultures. Tissue nitrogen contents ranged from 1.3–5.4% N (dry wt), whereas tissue P ranged from 0.21–0.56% P (dry wt). Growth in low nitrogen medium resulted in N:P ratios of 5–8, whereas growth in high nitrogen medium resulted in N:P ratios of 21–44. For* U. fenestrata, *tissue N:P < 16 was indicative of N-limitation. Tissue N:P 16–24 was optimal for growth and tissue N:P > 24 was indicative of P-limitation. Growth of* U. fenestrata *was hyperbolically related to tissue N but linearly related to tissue P. Phosphorus-limited* U. fenestrata *maintained high levels of tissue N, but N-limited algae became depleted of P. For* E. intestinalis, *tissue N remained at maximum levels during P-limitation whereas tissue P decreased to about 85% of maximal levels during N-limitation. Growth rates for* U. fenestrata *decreased faster during P-limitation than during N-limitation. Simultaneously, tissue P was depleted faster than tissue N. Our results suggest that comparing tissue N and P of macroalgae grown in batch cultures is useful for monitoring the nutritional status of macroalgae.*

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TABLE 1. *Growth rates (% wet wt·d^{-1}) obtained during high nutrient conditions (HNHP). Average μ is calculated from daily growth during entire experiment. Maximum μ is the highest daily growth observed; n = 4 for* Enteromorpha *Expt. 1, n = 2 for other Expts.*

Algae	Expt. (n)	Average μ ± SD	Max μ ± SD
U. fenestrata	1 (22)	14.1 ± 9.3	34.4 ± 4.9
	2 (20)	17.2 ± 6.4	29.6 ± 2.8
E. intestinalis	1 (21)	12.6 ± 6.8	21.5 ± 2.9
	2 (8)	7.3 ± 4.6	14.1 ± 0.5

Key index words: Chlorophyta; Enteromorpha intestinalis; *growth rates; nutrient limitation; tissue nitrogen; tissue phosphorus;* Ulva fenestrata

The majority of work on nutrient uptake and chemical composition of algae has been done with microalgae (see McCarthy 1981). In recent years, research on the physiology and ecology of marine macroalgae has increased due to potential commercial usage (Bird and Benson 1987) and environmental changes in polluted areas (Wallentinus 1981). Since nitrogen (N) has been considered to be the primary limiting nutrient for macroalgal growth in temperate coastal marine waters (Ryther and Dunstan 1971, Smith 1984), many studies have focused on the uptake and metabolism of N and its effects on growth rates (e.g. Topinka and Robbins 1976, DeBoer et al. 1978, Hanisak 1979a, Morgan and Simpson 1981, Wheeler and Weidner 1983).

Many species among the perennial brown (Phaeophyceae) and red (Rhodophyceae) seaweeds have the ability to store nutrients which can then be used for growth during periods of low ambient nutrient concentrations. The relationship between N uptake, internal N levels and growth has been investigated in a number of studies (e.g. Chapman and Craigie 1977, Chapman et al. 1978, Hanisak 1979b, Wheeler and North 1980, Gerard 1982, Rosenberg and Ramus 1982, Lapointe and Duke 1984, Fujita 1985, Hwang et al. 1987). Similar studies with annual seaweeds have used opportunistic, pollution-related species, especially among the green (Chlorophyceae) macrophytes (e.g. Waite and Mitchell 1972, Harlin 1978, Lapointe and Tenore 1981, Duke et al. 1986, O'Brien 1987, Duke et al. 1989).

Relatively few studies have examined the uptake of both nitrogen and phosphorus (P). Ketchum (1939) investigated N- and P-limited growth of the diatom *Nitzschia closterium*. Other studies with microalgae relate N and P uptake to internal pigment and nutrient composition (e.g. Vince and Valiela 1973, Rhee 1974). The pollution of coastal waters with phosphate and the assumption that P could be a limiting nutrient in certain marine environments (e.g. in estuaries and tropical waters, Smith 1984) have led to investigations of N and P regulation of macroalgal growth. These studies have examined the relationship between N and P supply, chemical composition and its effect on growth rates (Steffensen 1976, Birch et al. 1981, Gordon et al. 1981, Wallentinus 1981, Kautsky 1982, Lapointe 1987).

The purpose of our study was to investigate the effects of N and P supply on tissue nutrient levels and growth rates of *Ulva fenestrata* and *Enteromorpha intestinalis* grown in semi-continuous and batch cultures under controlled laboratory conditions.

MATERIALS AND METHODS

Collection sites. U. fenestrata and *E. intestinalis* (Ulvales, Chlorophyta) were collected from Yaquina Bay (44°41′ N, 124°05′ W) and Boiler Bay (44°50′ N, 124°03′ W) Oregon, respectively. *U. fenestrata* thalli were collected from mudflats in Yaquina Bay estuary in late November 1988. The estuary is well-mixed during summer and fall and partly-mixed during winter and spring (Kulm 1965). The average tidal fluctuations are about 3 m with a semidiurnal tidal cycle. Epiphyte-free, fresh-looking *E. intestinalis* thalli were collected during June and July 1988 from partly exposed tidepools in the upper intertidal of Boiler Bay. This bay has a rocky shore with mudstone benches and conglomerate flat rocks and receives moderate wave action. The inner part of the bay is a sandy beach which receives freshwater runoff and is exposed to waves only during storms.

Culturing system. The algae were cultured in eight acid-washed (10% HCl) 2-L glass containers illuminated with fluorescent light (Philips Cool White 40 W) with a 14:10 h LD cycle. Photon fluence rate ranged from 320–360 μE·m^{-2}·s^{-1}. *U. fenestrata* was cultured at 13 ± 2° C and *E. intestinalis* at 18 ± 2° C. Cultures were aerated by air-bubbling.

Medium. Sand-filtered and UV-treated seawater was filtered through Whatman glass-fiber filters (pore size 1.2 μm) and then filtered through a Gelman MiniCapsule Filter (0.2 μm). A modified version of Guillard's f/2 medium (Guillard 1975) with trace metals and vitamins was used for the nutrient enrichments. When supplied, NO_3^- and PO_4^{3-} ($NaNO_3$ and NaH_2PO_4) were added daily at concentrations of 100–200 and 6–12 μM, respectively. The medium was changed daily for *E. intestinalis*. To conserve seawater in later experiments nutrients were added to medium daily for *U. fenestrata*.

Experimental design. Algal tissue (1.2–3.0 g wet weight) was cultured in 1.5 L of medium. Four nutrient treatments were used; low NO_3^- and PO_4^{3-} (LNLP), high NO_3^- and low PO_4^{3-} (HNLP), low NO_3^- and high PO_4^{3-} (LNHP) and high NO_3^- and high PO_4^{3-} (HNHP). Two growth experiments were conducted for each alga, and within each experiment each treatment was run in duplicate (i.e. two containers per treatment). Background nutrient levels in the seawater were 9–13 μM NO_3^-, 1–2 μM NH_4^+ and 0.8 μM PO_4^{3-}. The daily nutrient additions for HN and HP treatments were sufficient to allow growth of 30%·d^{-1} assuming tissue levels of 5% N and 0.5% P. These levels approximate the calculated daily nutrient use for *Enteromorpha* spp in Yaquina Bay during high nutrient conditions (O'Brien 1987). Tissue samples were taken daily from each culture and kept frozen prior to analysis.

Growth measurements. Growth rates of the algal mass in each container were measured daily as changes in tissue wet weight for a period of 7–12 days. Excess water was either removed by careful blotting on Kimwipes or spinning in a salad spinner for 30 s before weighing. Specific growth rates (μ) were calculated using the equation

$$\mu = [\ln(wt_2/wt_1)]/(t_2 - t_1)$$

where wt_1 and wt_2 are wet weights at times t_1 and t_2, respectively.

Nutrient and tissue analysis. Nutrients were analyzed during the growth experiments to insure that daily additions were adequate for the supplemented cultures and that nutrients were depleted in the low nutrient treatments. Nitrate and PO_4^{3-} were determined as described by Strickland and Parsons (1972). Nitrate and PO_4^{3-} uptake rates were calculated from net changes in nutrient

TABLE 2. *Nitrate and phosphate uptake rates for high nutrient treatments. Rates are means and SE for daily measurements.*

	NO_3^- uptake	NO_3^- uptake	PO_4^{3-} uptake	PO_4^{3-} uptake
	(μmol·g wet wt^{-1}·h^{-1})			
	HNLP	HNHP	LNHP	HNHP
U. fenestrata				
Expt. 1	36.4 ± 6.9	86.5 ± 4.2	1.01 ± 0.36	3.24 ± 0.53
(n)	(14)	(12)	(14)	(12)
Expt. 2	27.1 ± 6.9	133.7 ± 9.3	2.30 ± 0.60	5.04 ± 0.38
(n)	(12)	(16)	(12)	(16)
E. intestinalis				
Expt. 1	—[a]	56.7 ± 2.8	—	3.42 ± 0.18
(n)		(36)		(36)
Expt. 2	105.0 ± 1.6	109.7 ± 2.4	6.34 ± 0.27	6.54 ± 0.12
(n)	(14)	(14)	(14)	(14)

[a] Data not taken.

concentrations in the media normalized to wet wt of the algal tissue.

Total N and P in algal tissue was determined by alkaline persulphate digestion using a modified version of the procedure described by D'Elia et al. (1977). Algal tissue was dried for 24 h at 60°C, ground in a Mixer Mill (Spex 5100), weighed (500–700 μg) on a Perkin-Elmer Microbalance (AD 22) and put into silanized 250 mL glass bottles with screw caps. Five mL of deionized water (DIW) and 30 mL of oxidizing reagent (3.0 g NaOH and 6.7 g low N potassium persulphate dissolved in 1 L of deionized water) were added to each bottle. Samples were autoclaved at 100–110° C (at 15 psi) for 1 h. After cooling to room temperature, the samples were acidified with 3 mL 0.3 M HCl and buffered with 4.0 mL of borate buffer (30.9 g H_3BO_3 and 100 mL 1 M NaOH in 1 L DIW, pH 8.0). The volume was then brought to 50 mL by adding 8 mL of DIW. Nitrate and PO_4^{3-} were measured colorimetrically (Strickland and Parsons 1972). A bovine protein gammaglobulin (BPGG) solution was used as a standard for the N assay. Due to turbidity, the samples were filtered through a Whatman GF/F filter before the PO_4^{3-} analysis. Disodium carbamyl phosphate was used as a standard for the P assay. Persulfate digestions were done in duplicate for each tissue sample, and NO_3^- and PO_4^{3-} were measured in duplicate for each digested sample.

As a cross calibration for tissue P we compared the persulfate method to electron probe elemental analyses for *Porphyra* sp. For tissue P levels of 0.40–0.60% P, we found a mean difference of less than 20% between the two methods. Since the difference was neither consistently high or low, no adjustments have been made to the data presented here.

RESULTS

Growth rates, nutrient uptake rates and residual nutrient levels. Ulva fenestrata and *E. intestinalis* grew well in semi-continuous cultures when N and P were supplied (Table 1). Maximum growth rates were significantly higher (Student's *t*-test, $P < 0.01$) for *U. fenestrata* (32 ± 3%·d^{-1}, mean ± SD) than for *E. intestinalis* (18 ± 5%·d^{-1}, mean ± SD). The low growth rates in the second experiment with *E. intestinalis* were due to a spore release, which caused inaccurate (lower) estimates of algal mass.

Nitrate uptake rates ranged from 57–149 μmol·g^{-1}·d^{-1} for both algae when NO_3^- and PO_4^{3-} were added simultaneously to the medium, whereas PO_4^{3-} uptake ranged from 3.0–6.5 μmol·g^{-1}·d^{-1} (Table 2). For *U. fenestrata*, NO_3^- and PO_4^{3-} uptake rates for *U. fenestrata* were reduced by 53–91% when only one of the two nutrients was supplied. For *E. intestinalis*, however, high uptake of NO_3^- and PO_4^{3-} was maintained even in the absence of PO_4^{3-} and NO_3^- supplements, respectively (Table 2).

Residual NO_3^- and PO_4^{3-} levels in the medium for LN and LP treatments, respectively, were below detection limits within 1–2 days (data not shown) which prevents calculation of uptake rates. When both nutrients were added (HNHP), residual PO_4^{3-} levels ranged from 1–9 μM (Table 3) indicating that excess P was always available. Residual NO_3^- levels were low (<1 μM) for *E. intestinalis* for both the HNLP and HNHP treatments. Nonetheless, the high growth rates achieved in these experiments suggest

TABLE 3. *Residual nutrient levels for high nutrient treatments.*

	Residual nutrients (μM)			
	NO_3^- HNLP	NO_3^- HNHP	PO_4^{3-} LNHP	PO_4^{3-} HNHP
U. fenestrata				
Expt. 1 (n = 14, 12)	238	11.3	20.4	5.8
Expt. 2 (n = 12, 16)	151	28.2	20.4	9.4
E. intestinalis				
Expt. 1 (n = 36)	—[a]	0.27	—	1.03
Expt. 2 (n = 14)	0.92	0.06	3.83	1.29

[a] Data not taken.

TABLE 4. *Nitrogen and phosphorus content of algal tissue. Values are means for low and high nutrient treatments respectively (n = 4).*

	Low nutrient mean (% dry wt) ± SD	High nutrient mean (% dry wt) ± SD
	Nitrogen	
U. fenestrata	1.32 ± 0.22	5.42 ± 0.30
E. intestinalis	1.38 ± 0.34	4.07 ± 0.33
	Phosphorus	
U. fenestrata	0.21 ± 0.05	0.51 ± 0.07
E. intestinalis	0.30 ± 0.05	0.56 ± 0.10
	N:P	
	LNHP mean	HNLP mean
U. fenestrata	7.8 ± 3.9	44.0 ± 15.6
E. intestinalis	4.6 ± 0.9	21.1 ± 0.9

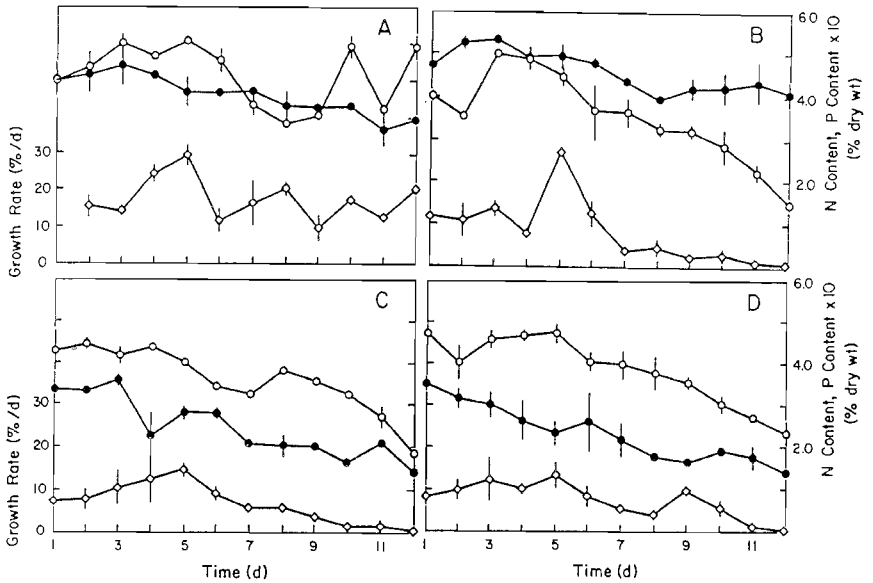

FIG. 1A–D. Changes in growth rate, tissue N and tissue P of *U. fenestrata*. Error bars indicate one standard deviation. Each treatment was run in duplicate. A) HNHP medium. B) HNLP medium. C) LNLP medium. D) LNHP medium. (●) tissue N, (○) tissue P, (◇) growth rate.

that N availability was not limiting in these experiments (see below).

Tissue N and P. The highest tissue N levels were obtained for *U. fenestrata* whereas maximum tissue P levels were similar for both algae (Table 4). The maximum/minimum ratios for tissue N were about 40% higher compared to those for tissue P, suggesting a greater storage capacity for the nitrogenous constituents. Alternatively, there may be a greater tolerance for nitrogen deprivation. Tissue N:P ratios were generally higher during all treatments for *U. fenestrata* (Table 4).

Effect of nutrient deprivation on growth rates. For *U. fenestrata,* there was a 3–4 day lag period with little change in growth rate or tissue composition (Fig. 1). During the HNLP treatment, after an initial transient increase in growth rate to ca. 30%·d^{-1}, growth decreased rapidly from day 5–7, followed by a slower decrease during the five last days, finally ending in a complete cessation of algal growth on day 12 (Fig. 1B). An increase followed by a decrease in growth also occurred during the LNLP treatment, although with less pronounced differences (Fig. 1C). With only N limiting (LNHP), growth rate decreased more slowly, compared to the other treatments (Fig. 1D). In order to assess the effect of tissue composition on growth rate for *U. fenestrata,* we considered the first 7 days as an acclimation period. Means for duplicate cultures for each treatment for the next 5 days were used to establish the relationship between tissue composition and growth rate.

Growth of *U. fenestrata* was hyperbolically related to tissue N, with a maximum growth rate of 16%·d^{-1}, and sustenance level of 1.8% N (Fig. 2A). High N levels but low growth rates were characteristic of P-limited growth (HNLP) (Fig. 2A). In contrast, growth was linearly related to tissue P over the range of 0.2–0.6% P (Fig. 2B). Furthermore, both tissue N and P levels decreased during N-limited growth (LNHP) (Fig. 1D). Growth of *U. fenestrata* could be divided into three regions of nutrient status as a function of N:P (Fig. 2C), i.e. N:P < 16, N-limitation; N:P 16–24, N-sufficient and P-sufficient; N:P > 24, P-limitation. Tissue N:P ≤ 16 resulted from minimum tissue N (about 2% dry wt) and variations in tissue P from 0.25–0.40% dry wt. Tissue N:P in the range 16–24 resulted from high tissue N (4.0–4.5% dry wt) and high tissue P (0.40–0.55% dry wt) (Fig. 3). Tissue N:P greater than 24 resulted from maintenance of maximum tissue N during depletion of tissue P (Fig. 3).

The rate of change in growth rate and tissue nutrients for *U. fenestrata* during either N- or P-limitation (LNHP and HNLP, respectively) was analyzed for the 5 last days of the experiment. Using day 7 as a reference, growth rates were generally higher (30–70%) during N-limitation compared to P-limited growth (10–50%) (Fig. 4). Tissue N decreased about 30% initially but remained constant during the remaining period (Fig. 4A), whereas tissue P decreased at a steady rate (~10%·d^{-1}) (Fig. 4B).

As a consequence of sporulation induced by weighing procedures for *E. intestinalis,* we were unable to obtain accurate estimates of growth rates for the nutrient-deprivation treatments. Nonetheless, the algae did grow well during the 9 days of culturing when both nutrients were provided and accumulation of algal mass was significantly lower in the deprivation treatments. Moreover, large differences

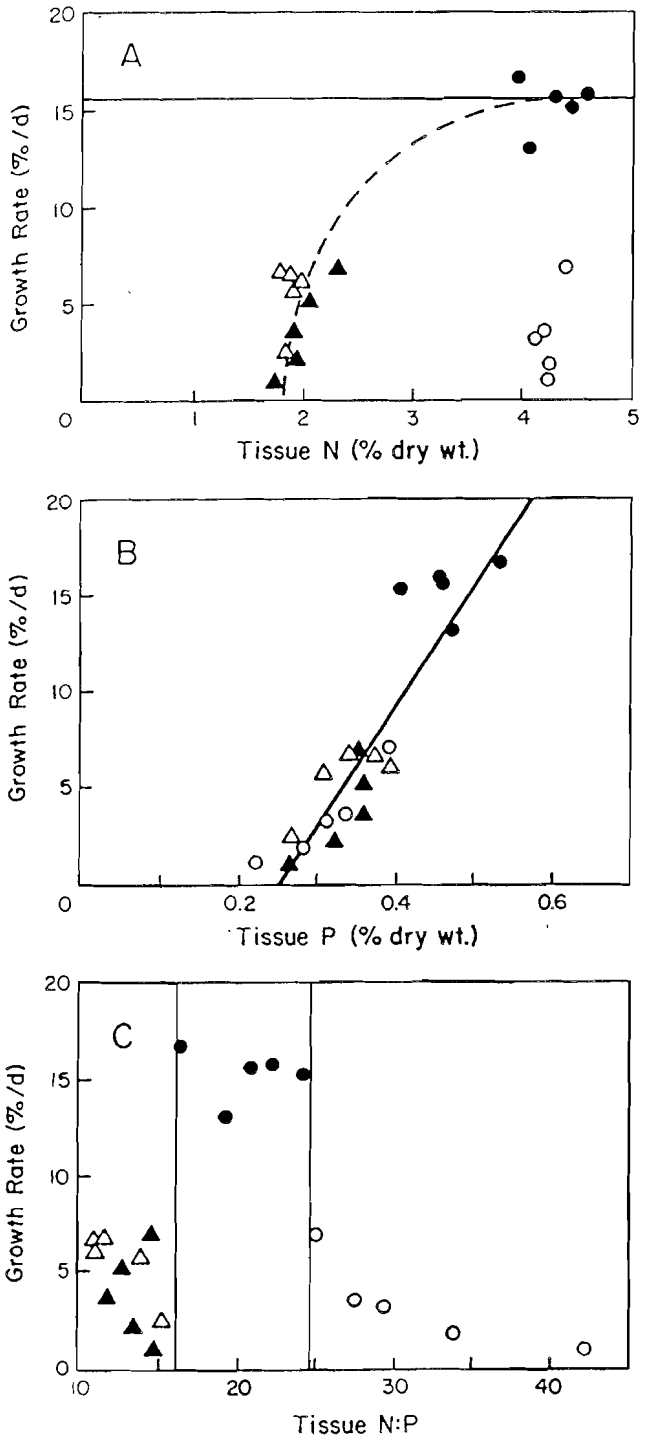

FIG. 2A–C. Growth rate as a function of A) tissue N, B) tissue P and C) N:P for *U. fenestrata*. (●) HNHP medium (○) HNLP medium, (▲) LNLP medium, and (△) LNHP medium.

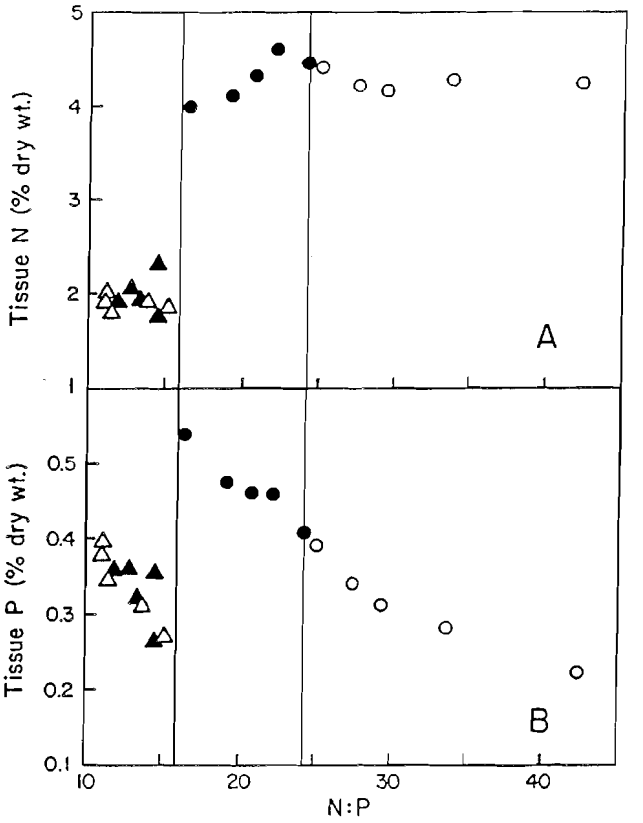

FIG. 3A, B. Tissue nutrient plotted as a function of N:P for *U. fenestrata*. A) Tissue N. B) Tissue P. (●) HNHP medium, (○) HNLP medium, (▲) LNLP medium, and (△) LNHP medium.

in tissue composition resulted from the various treatments. Nitrogen deprivation resulted in tissue N levels of 1.38% N, whereas P deprivation resulted in tissue P levels of 0.30% P (Table 4). Tissue N reached maximum levels during both HNHP and HNLP treatments, whereas tissue P for LNHP treatments accumulated to about 85% of maximum levels for HNHP treatments (data not shown).

Patterns of changes in tissue N and P for *E. intestinalis* were similar to those observed for *U. fenestrata*. Tissue N:P < 12 resulted from low tissue N (1–2% dry wt) and variations in tissue P from 0.3–0.6% dry wt. (Fig. 5). For *E. intestinalis*, accumulation of tissue P did occur during N limitation. In the region of N and P sufficiency (as defined for *U. fenestrata*), tissue N ranged from 3.0–4.5% dry wt, whereas tissue P ranged from 0.4–0.7% dry wt (Fig. 5). Tissue N:P > 16 resulted from high tissue N (~4%) and relatively low (0.4–0.5%) tissue P (Fig. 5).

DISCUSSION

The maximum growth rates for *U. fenestrata* and *E. intestinalis* reported here are lower than growth rates obtained for other ulvoids by Lapointe and Tenore (1981), Ramus and Venable (1987), and Duke et al. (1989) (36, 30 and 52% wet wt·day^{-1}, respectively). This difference is probably due to our use of a lower temperature for cultivation. Growth rate of *Enteromorpha prolifera* in continuous outdoor cultures (O'Brien 1987) was about the same (21% wet wt·day^{-1}) as the maximum for the semi-continuous cultures in this study. Although the temporal pattern of nutrient supply in situ may be variable

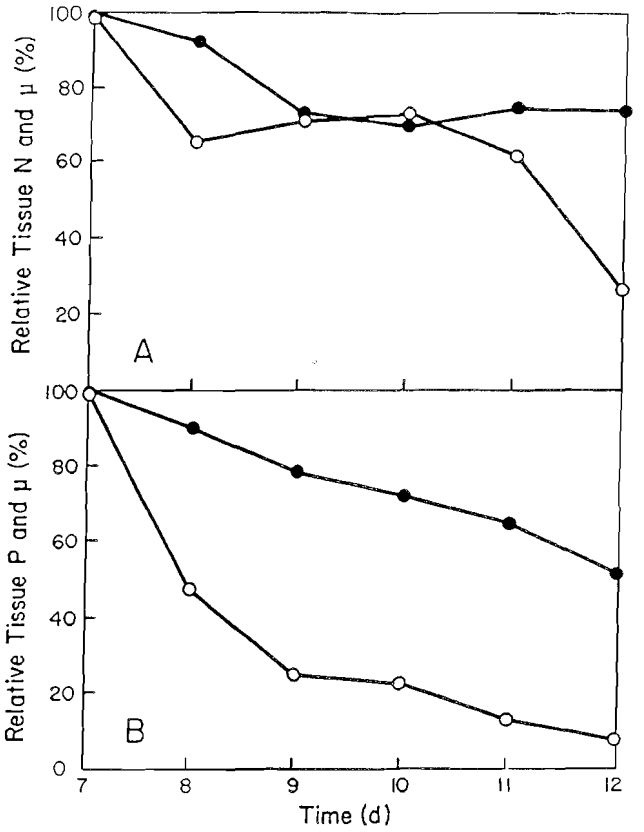

FIG. 4A, B. Relative growth rate, tissue N and tissue P for *U. fenestrata* in N-limited and P-limited media. Values are three-day running means for day 7–12. A) LNHP medium. (●) tissue N, (○) growth rate. B) HNLP medium. (●) tissue P, (○) growth rate.

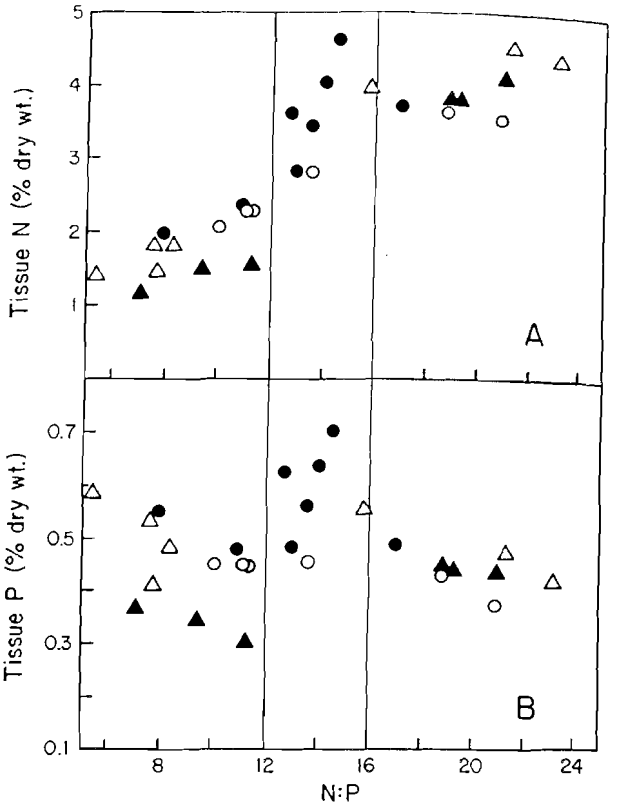

FIG. 5A, B. Tissue nutrient plotted as a function of N:P for *E. intestinalis*. A) Tissue N. B) Tissue P. Data are means for days 1–7. (●) HNHP medium, (○) HNLP medium, (▲) LNLP medium, and (△) LNHP medium.

(Fujita 1985), our results (O'Brien 1987, Fujita et al. 1989, present study) indicate that both continuous and semi-continuous culture regimes can be used to obtain high growth rates with a controlled nutrient supply.

The presence or absence of nutrient supplements in our experiments resulted in large differences in tissue N and P for both species. The range for *U. fenestrata* (1.32–5.42% N) here is greater than that reported earlier for *Ulva* by Fujita (1985) who obtained tissue N levels between 1.02–3.59% N dry wt. The lower maximum/minimum ratio in tissue N for *E. intestinalis* could be a sporulation effect. Algal strands began sporulating during incubation, and this may have influenced tissue N levels. Both Fujita (1985) and O'Brien (1987) reported greater tissue N ranges for *Enteromorpha* (0.57–3.85% N dry wt and 1.78–5.43% N dry wt, respectively) than we observed (1.38–4.07% N).

The ranges of tissue P levels (maximum/minimum) were smaller than those for tissue N for both species, with *U. fenestrata* showing the greater variability in tissue P levels. Tissue P for *E. intestinalis* was greater than found by O'Brien (1987) who reported tissue P levels between 0.10–0.33% dry wt for *E. prolifera*. When exposed to extremely high phosphate concentrations (1000 μM), accumulation of even higher levels of surplus P (~0.84% P dry wt) was found for *Cladophora intestinalis* by Schramm and Booth (1981). Average P content in field samples of *Cladophora* from Bermuda was extremely low, 0.04% P dry wt (Schramm and Booth 1981), while *Cladophora* from the Baltic varied between 0.07–0.54% P dry wt (Wallentinus 1981). The maximum tissue nutrient levels for *Ulva* in our semi-continuous cultures were similar to field data from Boiler Bay, Oregon, where maximum tissue N and P levels during July and November–December 1988 were 5.73% dry wt and 0.62% dry wt, respectively (Wheeler and Björnsäter, unpubl. results). Tissue N and P levels for field samples of *Enteromorpha* collected during spring and early summer were higher (5.34% N dry wt and 0.75% P dry wt) than for our laboratory cultures (Wheeler and Björnsäter, unpubl. results).

The low and high extremes of N:P ratios for the LNHP and HNLP treatments reflect N- and P-limitation respectively. *Enteromorpha linza* cultured in a sewage-seawater mixture had an N:P ratio of 38.7 (Prince 1974), while a lower field value of 16 was reported for *E. flexuosa* (Atkinson and Smith 1983). Tissue N:P ratios ranging from 8–66 have been found in field samples of *Cladophora glomerata* from the Baltic (Wallentinus 1981) and laboratory cultures

with the same species had a N:P range of 4–42 (Gordon et al. 1981).

Information on the relationship between growth rate and internal nutrient concentration of seaweeds generally indicates a threshold response (Hanisak 1979b, Gordon et al. 1981, Manley and North 1984). Nitrate-limited batch and flow-through cultures of *Macrocystis pyrifera* showed a linear relationship between tissue nitrogen and growth rates (Wheeler and North 1980). In our experiments with *U. fenestrata* a hyberbolic relationship was found between growth rate and tissue N (Fig. 2A). In contrast, a linear relationship was found between growth rate and tissue P (Fig. 2B), suggesting that no storage of P occurred over the range examined.

Nitrogen has been considered to be the most limiting nutrient in the marine environment for both phytoplankton (Ryther and Dunstan 1971, Vince and Valiela 1973) and macroalgae (Topinka and Robbins 1976, Chapman and Craigie 1977, Hanisak 1979a, b). However, in certain marine waters P has been shown to be the primary limiting nutrient to algal growth (Birch et al. 1981, Myers and Iverson 1981). Indications of P-limitation are provided by elevated N:P ratios for macroalgae from a variety of geographical locations (Atkinson and Smith 1983). Seasonal shifts between N- and P-limitation have been indicated for phytoplankton growth in both lakes and seawater (Rhee 1978) and for seaweeds (Wallentinus 1981, Lapointe 1987).

The relationship between changes in tissue N and P and growth rate for *U. fenestrata* in this study differed between treatments. The results show that growth decreases faster during P-limitation than during N-limitation. At the end of the experiment, tissue P was decreasing at a steady rate during P-limitation, whereas tissue N remained high. Nitrogen pools in *Ulva*, consisting mainly of chlorophyll-protein complexes and other soluble organic N (Rosenberg and Ramus 1982) are apparently depleted at a slower rate than the P pools, which probably consist of polyphosphates (PoP). This is supported by studies showing that *Enteromorpha* and *Ulva* are capable of hydrolysis of PoP (Eppley 1962). Enzymatic hydrolysis of PoP can be extremely fast (58 nmol $PO_4^{3-} \cdot min^{-1} \cdot mg^{-1}$) for *Cladophora* (Lin 1977).

Phosphorus uptake was highest when N was nonlimiting. When tissue N was being depleted (LNHP), however, a rapid depletion of tissue P was evident. The opposite situation (depletion of tissue N during P-limitation) also occurred, although it was less pronounced. These results are consistent with those of Gordon et al. (1981) for *Cladophora*, where removal of either N or P from the medium, in the presence of the other, resulted in a reduction in growth rates with a corresponding decrease in both tissue N and P. Since the rate of decrease in tissue N (or P) in our experiments was about the same during both N and P limitation, the algae seem to regulate the N or P uptake in order to maintain a "balanced" internal N:P ratio. The effect of P limitation on growth rate was more pronounced than was N-limitation. This could be due to a more rapid depletion of internal phosphorus pools compared to nitrogenous constituents, yielding a faster response in growth rate.

Early attempts to evaluate the nutritional status of macroalgae on the basis of ambient nutrient concentrations were unsuccessful as a result of 1) rapid fluctuations in nutrients and difficulty in assessing the nutrient regime and 2) the ability of macroalgae to store nutrients. The relationship between tissue composition and growth provides a more useful criterion for evaluating nutrient status since tissue composition integrates the available nutrient supply for some period prior to collection of the samples. Steady-state nutrient-limited growth experiments can be used to determine the critical light- and temperature-dependent tissue nutrient level, i.e. the level at which growth rates start to decrease. Such experiments are time-consuming and limited information is available in the literature on critical N and critical P levels for macroalgae. Our results suggest that determination of N:P ratios in batch and semicontinuous cultures may provide a better index for nutritional status of macroalgae. We see clear transitions in the relationship between changes in tissue N and tissue P and the N:P ratio that appear to delineate regions of N-limitation, nutrient sufficiency, and P-limitation.

For phytoplankton, N:P of 10–20 is considered relatively constant and representative of nutrient sufficiency with respect to both N and P. Ratios <10 indicate N-limitation, and ratios >20 indicate P-limitation (Goldman et al. 1979). Our results suggest that, for *U. fenestrata*, an N:P tissue compostion of 16–24 reflects nutrient sufficiency, whereas N:P $<$ 16 reflects N-limitation and N:P $>$ 24 reflects P-limitation. The region of N-limitation is characterized by low tissue N and decreases in N:P which result from the accumulation of tissue P. The region of nutrient sufficiency is characterized by both high tissue N and P. The region of P-limitation is characterized by high tissue N but increases in N:P resulting from decrease in tissue P. For *U. fenestrata*, delineation of these nutrient status regions was supported by the relationship of tissue N and P to measured growth rates. We were unable to determine growth rates for *E. intestinalis*. Nonetheless, the relationship between tissue N and tissue P and the N:P ratio suggest that the same pattern may occur as observed for *U. fenestrata*. Thus, for *E. intestinalis*, 12–16 could reflect nutrient sufficiency, whereas N:P $<$ 12 could reflect N-limitation, and N:P $>$ 16 could indicate P-limitation.

Our results suggest that simultaneous monitoring of tissue N and P is a very useful means of monitoring nutritional sufficiency of macroalgae. Experimental determination of the range of tissue N and

P under nutrient limited growth conditions and comparisons of changes in tissue N and P as a function of tissue N:P appears to provide a clear indication of N and P status at defined light and temperature. Moreover, these relationships can be determined easily in batch culture starvation experiments.

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SEASONAL SUCCESSION OF ALGAL PERIPHYTON FROM A WASTEWATER TREATMENT FACILITY[1]

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ABSTRACT

Attached algal populations were sampled at weekly or biweekly intervals to characterize successional changes in the secondary clarifiers of a wastewater treatment plant. Three communities were compared from areas of slow, medium and rapid current velocities. In general, the algae resembled those reported for other hypereutrophic flowing waters. Of the twenty-three algae recorded, Stigeoclonium, Oedogonium, Oscillatoria, Lyngbya, *and* Pleurocapsa *were dominant at some point in the 15 month sampling period. Nutrient concentrations were consistently high ($N = 1.1–21.4$ mg·L^{-1}; $P = 0.1–10.4$ mg·L^{-1}); therefore, changes in temporal distribution of algae were probably dependent on seasonal changes in light and temperature. Colonization of artificial substrates was also observed. Small unicellular algae were the first autotrophs to attach and these were followed by larger filamentous forms.*

Key index words: periphyton; sewage; succession; tertiary treatment; wastewater

Many sewage treatment processes utilize a step in which particulate matter is removed by settling in secondary clarifying tanks. The nutrient-rich supernatant flows over the weirs of these tanks and is disinfected before returning to the environment (Fig. 1). Approximately 60% of the total influent nitrogen and 70% of the total influent phosphorus remain in the final effluent (Hammer 1977). The clarifying tanks are open and the combination of high nutrient loads, flowing water, and daylight permit the development of extensive periphyton communities. The potential for rapid algal growth in these hypereutrophic systems has promoted recent interest in using periphyton communities for biological treatment of wastewater (Chan et al. 1982, Pretorius and Hensman 1984, Lavoie and de la Noue 1985, Cook et al. 1986, Wood 1987). However, literature on secondary clarifier periphyton is scarce. Two studies compiled a list of algae in secondary clarifiers in the Ukraine (Dogadina et al. 1970, Dogadina and Chukhlebova 1971) but provided quantitative information only for phytoplankton. Ohtake et al. (1978) provide some information on taxonomic composition in experimental troughs receiving effluent discharged from secondary clarifiers in Japan. Traaen (1975, 1978) used outdoor channels to study the effects of different wastewater treatments on periphyton, but the effluents were greatly diluted. Sládečková et al. (1983) used both indoor and in situ stream models to follow nutrient uptake and community composition of attached algae in enriched influents in drinking water reservoirs. In general, previous studies have not provided data on seasonal changes, relative abundance, or population dynamics of algal periphyton.

Here we provide a descriptive analysis of the algae present in a secondary clarifier over a 15 month period. Frequent sampling enabled determination of colonization sequences, taxonomic richness, and correlations of physicochemical parameters with individual taxa. This study should provide a base upon which future investigations of community structure and dynamics may be built. Thus it may enable better application of the periphyton in tertiary wastewater treatment.

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