Assessment of macroalgal nitrogen limitation in a seasonal upwelling region

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ABSTRACT: The relationship between nitrogen (N) availability and the growth of macroalgae in a seasonal upwelling region (Yaquina Head, Oregon, USA) was investigated. Water column nutrient concentrations were relatively high and stable during the winter, decreased in early spring, and were highly variable in the summer and early fall. Periods of high nutrient availability due to upwelling commenced in May and alternated with periods of low nutrient availability until September. Dissolved inorganic nitrogen (DIN) concentration was a poor predictor of N limitation of macroalgal growth due to the ability of macroalgae to store N for continued growth when ambient DIN concentrations were low. The relationship between growth and internal N content for *Pelvetiopsis limitata* (Phaeophyta : Fucaceae) and *Ulva rigida* (Chlorophyta : Ulvales) depended on the N source and culture conditions. Results of an in situ N enrichment experiment indicated that *P. limitata* growth may have been N-limited in April and May. A nitrogen budget for *P. limitata* indicated that NH_4^+ was the major N source for growth at Yaquina Head, due to its rapid uptake, despite the greater abundance of NO_3^- The critical N value (tissue N level during the transition from N limiting to non-N limiting conditions) for growth of *U. rigida* on NO_3^- (≤ 2.4 % dry wt) was much lower than that for growth on NH_4^+ (3.0 % dry wt). Ammonium was the major N source for growth of *U. rigida* at Yaquina Head, and growth was probably N-limited in April and May, as indicated by the critical N level for growth on NH_4^+ Growth of *U. rigida* may also have been intermittently N-limited between upwelling events. Problems with the use of cirtical N levels and nutrient monitoring to assess N limitation are discussed. Use of critical tissue N levels is complicated by variations in this parameter with the N source used for growth.

INTRODUCTION

The seasonality of macroalgal productivity is often controlled by light and nutrient availability. In temperate regions, irradiance and growth are usually low during the winter and early spring, while nutrient levels are high (e.g. Gagne et al. 1982). As light conditions improve, nutrients are utilized by both macroalgae and phytoplankton and often become depleted in the euphotic zone. Low nutrient levels may limit primary production during the summer and fall when adequate light is still available.

Most studies of the relationship between the availability of dissolved inorganic nitrogen (DIN) and macroalgal productivity have been carried out in Atlantic coastal waters (e.g. Chapman & Craigie 1977, Hanisak 1979, Harlin & Thorne-Miller 1981, Davison et al. 1984)

where winter mixing provides the dominant seasonal input of DIN to the euphotic zone. A contrasting situation occurs along the eastern boundaries of the oceans where coastal upwelling occurs. Upwelling is particularly strong off the coast of Oregon and the upwelling season usually spans 6 mo (May to October). During this period upwelling is episodic, with 4 to 6 major upwelling events and the same number of weaker events (Small & Menzies 1981).

Other natural environments (such as salt ponds and estuaries) and cultivation systems also have periodic episodes of high DIN availability ('pulses') superimposed on a background of low DIN availability. In these environments, macroalgae take up DIN rapidly when available and store it to support growth between pulses (Rosenberg & Ramus 1982, Rosenberg et al. 1984, Fujita 1985a, Lapointe 1985). Gerard (1982) has suggested that the giant kelp *Macrocystis pyrifera* is able to use transient pulses of NO_3^- supplied by upwelling, thermocline excursions (Zimmerman & Kremer 1984), and runoff to alleviate N starvation in southern Califor-

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nia waters. However, some species have a limited capacity to store N perhaps due to their high growth rates (Fujita 1985a). Thus, the frequency with which DIN pulses occur in the environment may determine whether or not a particular species is N-limited. For example, growth of the red alga *Gracilaria tikvahiae* continued unabated in a salt pond when enriched with DIN only once every 5 d, while abundance of the fast-growing green algae *Ulva lactuca* and *Enteromorpha* spp. greatly decreased (Fujita 1985b). Nutrient storage uncouples growth from nutrient availability, complicating the assessment of nutrient limitation. To determine and predict the effects of nutrient availability on macroalgal growth in the field, it is necessary to measure nutrient concentrations on an ecologically relevant scale, assess the kinetics of nutrient uptake, determine which nutrient species are being utilized, and measure the length of time that growth can be supported in the absence of nutrients (i.e. the nutrient storage capacity).

The relationship between internal nutrient levels and growth rates has been analysed extensively for both higher plants and for phytoplankton. For microalgae, a well-defined hyperbolic relationship between cell nutrient content and growth rate is found in continuous cultures (Droop 1974). The relationship for mineral content of plant tissue, on the other hand, is more complex and is divided into three or more 'zones' of nutrient status (Smith 1962), with the 'critical level' usually being defined as the level separating the zone of deficiency and the zone of adequacy (Ulrich 1952). Here, we follow Hanisak's (1979) definition of the 'critical nutrient' content as the internal concentration that just limits maximal growth for macroalgae.

In this paper, we describe the temporal variability of nutrients in an intertidal macroalgal community at Yaquina Head (Oregon, USA) over a seasonal cycle. We determined critical tissue N levels by growing macroalgae in continuous culture systems with varying levels of N supply, then used these values to predict the onset of N limitation in situ. We also report 'subsistence' levels, tissue nutrient levels at zero growth rate, as used in the phytoplankton literature (Eppley & Strickland 1968), and conducted an in situ N enrichment experiment to evaluate N limitation.

MATERIALS AND METHODS

Site description. Water samples and algal specimens were collected from Yaquina Head, Oregon, USA (44° 41′ N, 124° 05′ W). Yaquina Head is a rocky headland extending about 2 km into the Pacific Ocean. The field experiment was conducted on a relatively flat rock platform in the midst of a dense bed of *Pelvetiopsis limitata* (Gardn.) (Phaeophyta Fucaceae) at the south

end of Yaquina Head. *P. limitata*, with *Fucus distichus* (L.) (Powell) (Phaeophyta : Fucaceae), dominates the high intertidal zone and is present year-round. All *P. limitata* thalli were collected from the vicinity of this site. *Ulva rigida* C. Ag. (Chlorophyta : Ulvales) was present lower in the intertidal at the same site, and abundance was highly variable, but was generally greater during the summer than during spring and fall. *U. rigida* was scarce during the winter. *U. rigida* thalli were collected from the vicinity of the field experiment and nutrient monitoring site for tissue N analysis; thalli for growth experiments were collected in nearby Yaquina Bay, where they were present in much greater abundance. Other *Ulva* species also occurred at Yaquina Head, but were not as abundant.

Nutrient concentrations and tissue N content at the field site. We collected water samples from Yaquina Head in polypropylene bottles over various temporal scales. In August 1985, we sampled surface water hourly for 24 h to characterize tidal variations in nutrient concentrations during an upwelling event. For high-tide samples, we used a garden hose fitted with a perforated bottle to minimize intake of debris. For low tide samples debris was not evident. Water samples were taken 6 m seaward from the edge of the rock ledge at a depth of ca 1 m below the surface through hosing connected to a hand-held bilge pump. All samples were packed in ice and filtered through Whatman GF/C glass-fiber filters within 4 h of collection. They were analysed for NH_4^+, NO_3^-, NO_2^-, and PO_4^{3-} within 6 h of collection on a Technicon Autoanalyzer II using the methods of Grasshoff et al. (1983) with minor modifications. Nitrite concentrations were always less than 5 % of NO_3^- concentrations and are not reported here. Coefficients of variation for replicates in each nutrient analysis were typically <1 %.

We sampled water in the same manner at both high and low tides every 1 to 3 d during June, July and August. Samples were taken throughout the rest of the year at intervals of 1 to 30 d, usually at or near low tide. These samples were filtered and frozen within 1 h of collection and stored for later analysis. The differences in nutrient concentrations observed between fresh and frozen samples were small for NO_3^- (1.7 % greater in fresh samples) but rather high for NH_4^+ and PO_4^{3-} (10.3 and 10.5 % greater in fresh samples, respectively).

We collected whole specimens of *Pelvetiopsis limitata* and *Ulva rigida* periodically from the general vicinity of our experimental site, taking care not to damage the thalli. Debris was removed by hand and the thalli rinsed in nutrient-free seawater (see 'Critical N content'). Excess water was removed by shaking or spinning in a salad spinner for 15 s. The thalli were frozen at −70 °C for at least 2 h, freeze-dried for at least 24 h, and ground to a fine powder in a Spex Industries

mixermill. Carbon and N contents were measured with a Perkin-Elmer 240B elemental analyzer. For some samples, N content was measured as NO_3^- after persulfate digestion (Grasshoff et al. 1983), which gave results comparable to those obtained using the elemental analyzer.

Outdoor culture experiments. *Pelvetiopsis limitata* and *Ulva rigida* were grown in outdoor flow-through tanks at a density of 10 g wet wt per tank (0.9 g l^{-1}). The outdoor culture system was developed to provide high turnover rates to simulate natural conditions more closely than was possible using an indoor culture system. The tanks were injection-molded ABS plastic half-cylinders 30 cm in diameter and 43 cm in length, arranged in sets of 4. Outflow pipes were fitted with perforated plastic bottles to prevent clogging, resulting in a working volume of 11.5 l. Agitation was provided with compressed air; nylon needle valves were used to equalize flow rates. Plexiglas covers kept out rain and debris. Biomass was cropped weekly to 10 g and growth rate (% d^{-1}) was calculated as ln $(W_t W_o^{-1})t^{-1} \times 100$, where W_t is the wet weight at the end of the growth period, W_o is the weight at the start of the growth period, and t is the duration of the period. *P. limitata* was cultured from 20 April to 20 May 1986 to coincide with the in situ enrichment experiment. *U. rigida* was cultured from 5 August to 27 August 1986.

Salinity-adjusted (30 to 32 ‰) seawater from Yaquina Bay was sand-filtered and pumped through 10- and 1-μm cartridge filters into a 4-tiered, air-agitated, flow-through nutrient stripping tank stocked with *Ulva* and *Enteromorpha* spp. Flow rates were adjusted to maximize the residence time of the water and thus increase the efficiency of nutrient removal. The water was then pumped to a 600 l head tank provided with an overflow to maintain a constant head, and directed through a carefully leveled manifold to the tanks. Each tank was provided with a nylon needle valve for control of flow rates.

Two 2000 l reservoir tanks were filled with nutrient-stripped water every 3 to 4 d and enriched to 150 μM NH_4^+ or NO_3^-. Phosphate (15 μM) was provided in each case to prevent P limitation (N:P=10:1). The enriched seawater was then pumped to separate 600 l head tanks with overflows and allowed to flow into the tanks through nylon needle valves. The 2000 l reservoir tanks were operated in tandem with two 600 l tanks to provide nutrient-enriched water continuously.

Nominal influent N concentrations of 0, 10, 20, 30, 40, and 50 μM were achieved by adjusting the relative flow rates of enriched and unenriched seawater. The total flow rate to each tank was held constant at 216 l d^{-1} (18.8 turnovers d^{-1}). The flow rates were checked daily and adjusted as necessary. The treatments were assigned to tanks randomly to avoid systematic effects

of shading or other gradients in the outdoor courtyard where the system was located. Inorganic N and P concentrations in the seawater prior to nutrient enrichment were always less than 3.4 and 0.41 μM, respectively. Dissolved organic N (DON) and P were not measured. The possible presence of DON may have contributed to the fast growth of *Ulva rigida* in outdoor cultures, but the use of DON by *Pelvetiopsis limitata* is not likely (see 'Discussion'). Salinity ranged between 30 and 32 ‰, while temperature (11 to 20 °C) and irradiance were highly variable.

Indoor culture experiments. The critical N content was determined by measuring tissue N content for algae grown over a range of growth rates controlled by nitrogen supply. We grew *Pelvetiopsis limitata* from 23 October to 23 December 1986 in 1.2 l plastic containers with water input and output fittings and filtered air agitation. Small, non-reproductive thalli (2 to 3 cm in length) with intact holdfasts were collected from the field site and cultured in enriched seawater in a constant temperature room. The seawater was obtained from Yaquina Bay and mixed with high salinity seawater as needed to maintain salinity above 30 ‰. Nutrients were stripped from the water in 2000 l outdoor tanks by dense, air-agitated batch cultures of *Ulva* spp. for at least 4 d. The water was then pumped through 10- and 1-μm cartridge filters, and a Millipore Pellicon® high-volume tangential flow filter system was used to remove > 0.2 μm particulate material. Residual concentrations of dissolved inorganic N and P were always less than 1.9 and 0.15 μM, respectively.

Separate polypropylene carboys were filled with seawater enriched with f/2 vitamins and minerals (Guillard 1975) and varying amounts of NH_4^+ or NO_3^- plus sufficient PO_4^{3-} to ensure N limitation (N:P = 2:1). Medium was pumped to the cultures at 1 ± 0.1 ml min^{-1} (0.5 l d^{-1}), light was maintained at 370 μE $m^{-2} s^{-1}$ (measured in culture medium) with cool white GE fluorescent tubes, and temperature was held constant at 13 °C. Photosynthesis should have been saturated at the light intensity used (Oakes & Murray 1983). However, we found in preliminary studies that *Pelvetiopsis limitata* became necrotic and died after about 1 wk of constant submergence under these conditions. We subsequently simulated natural conditions in the high intertidal by providing a 14:10 L:D cycle (lights on between 07:00 and 21:00 h), with 8 h of submergence in the light, 6 h of emersion in the light, and 10 h of emersion in the dark. Thalli were simply removed from the medium and left in plastic weighing dishes for the emersion period. Natural populations of *P. limitata* experienced a similar cycle of photoperiod and submergence/emergence at Yaquina Head during the spring.

Five to 7 thalli of ca 1.5 g wet wt each were cultured in each container, resulting in 7.5 to 8.5 g wet wt total

biomass and a biomass:volume ratio of about 8 g l^{-1}. Growth was measured weekly immediately after removal from the medium at 17:00 h by spinning in a salad spinner for 15 s and weighing. We found that this resulted in consistent weights after repeated periods of submergence. No systematic effects of desiccation during weighing (about 1 h) were observed. Tissue was cropped at each weighing to maintain the biomass at about 8 g. Growth rate (% d^{-1}) was calculated as $\ln(W_t\ W_o^{-1})\ t^{-1} \times 100$, where t is the number of days between weighings, W_o is the initial weight, and W_t is the final weight. The cropped tissue was frozen at $-70\,°C$ and freeze-dried in preparation for CHN analysis.

Ulva rigida was grown from 16 September to 20 October 1986 under the same conditions of irradiance, temperature, and nutrient loading. Photoperiod was set at 13:11 (L:D) and the algae were continuously submerged. Biomass was cropped to 6.5–8.5 g wet wt weekly at every weighing. Growth rate was calculated as described above.

In situ nutrient enrichment. Naturally occurring clumps of *Pelvetiopsis limitata* attached to barnacles were removed from the field site and attached to 10 × 20 × 1 cm black, UV-resistant plastic plates with Marine-Tex marine epoxy cement. Cement was applied between the barnacles and plates and allowed to cure for 24 h. Plates were inverted over a tank with flowing seawater to keep the thalli submerged while the cement dried. Initial weights were determined after the algae were allowed to dry for 4.5 h at 20 °C. We attached the plates directly to black basalt at the field site with stainless steel bolts and expansion nuts. The treatments were distributed randomly over a level platform in the midst of a dense bed of *P. limitata* at Yaquina Head.

Daily at lowest low tides (Oregon's semidiurnal tides are asymmetrical) between 25 April and 13 May 1986 we enriched the algae by unbolting the plates and placing them in translucent buckets filled with 7 l of freshly collected seawater for at least 2 h. We enriched one bucket to 50 μM NH$_4^+$ and another was left unenriched to serve as a control. The buckets were swirled every 10 to 15 min to reduce diffusion resistance. Enrichment was continued for 18 d; at the end of the experiment the plates were collected and allowed to dry in the laboratory for 4.5 h at 20 °C before the final weighing. Growth was calculated as the difference between the initial and final weights. Attempts to conduct this experiment with *Ulva rigida* failed due to excessive tissue loss.

In situ growth rates. We measured the growth rates of *Pelvetiopsis limitata* thalli which were cemented onto nylon bolts that were threaded into plastic plates attached to the rock during March and early April of

1986. We found that the product of the longest frond length and the number of fronds was linearly related to wet weight ($r^2 = 0.9$). Therefore, weight gain could be extrapolated from frond length. We measured frond lengths and numbers weekly during the experimental period. We also established a permanent transect line with markers adjacent to individual thalli. Frond length and number of thalli along the transect were measured weekly as an independant estimate of growth rate under undisturbed conditions.

RESULTS

Nutrient concentrations and tissue N content at the field site

We characterized the temporal distribution of macronutrients at Yaquina Head on time scales ranging from hours to months. Hourly sampling, over a tidal cycle in August (during upwelling) revealed that NO$_3^-$ concentrations ranged between 15 and 22 μM with highest concentrations generally occurring at high tides (Fig. 1). Ammonium and PO$_4^{3-}$ concentrations were low (<1 and 3 μM, respectively) and did not vary significantly over the tidal cycle (Fig. 1). For sampling dates between mid-June and mid-August, low-tide NO$_3^-$ concentrations were an average of 72 % of high-tide concentrations.

Upwelling events were detected and characterized by sampling every 2 to 4 d without regard to the tidal cycle (Fig. 2). Upwelling commenced in mid-May 1985 and resulted in pulses of elevated nutrient concentrations (henceforth to be called pulses) (7 to 28 μM NO$_3^-$ and 1 to
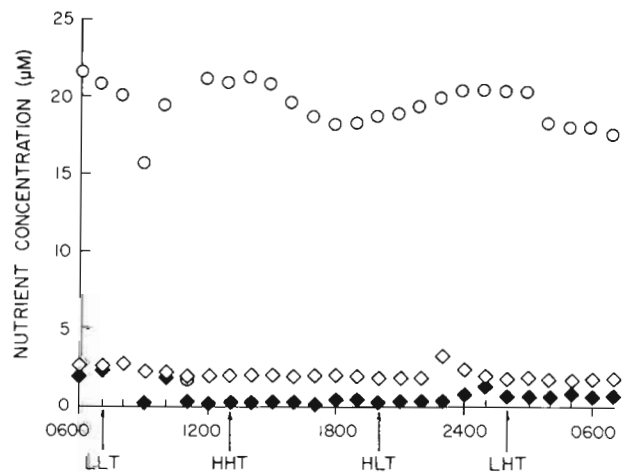

Fig. 1. Tidal cycle of nutrient abundance in surface water (0 to 3 m depth) at Yaquina Head, 29 to 30 Aug 1985. Nitrate (○), PO$_4^{3-}$ (◇), and NH$_4^+$ (◆) concentrations are shown. LLT: lowest low tide; HHT: highest high tide; HLT: highest low tide; LHT: lowest high tide
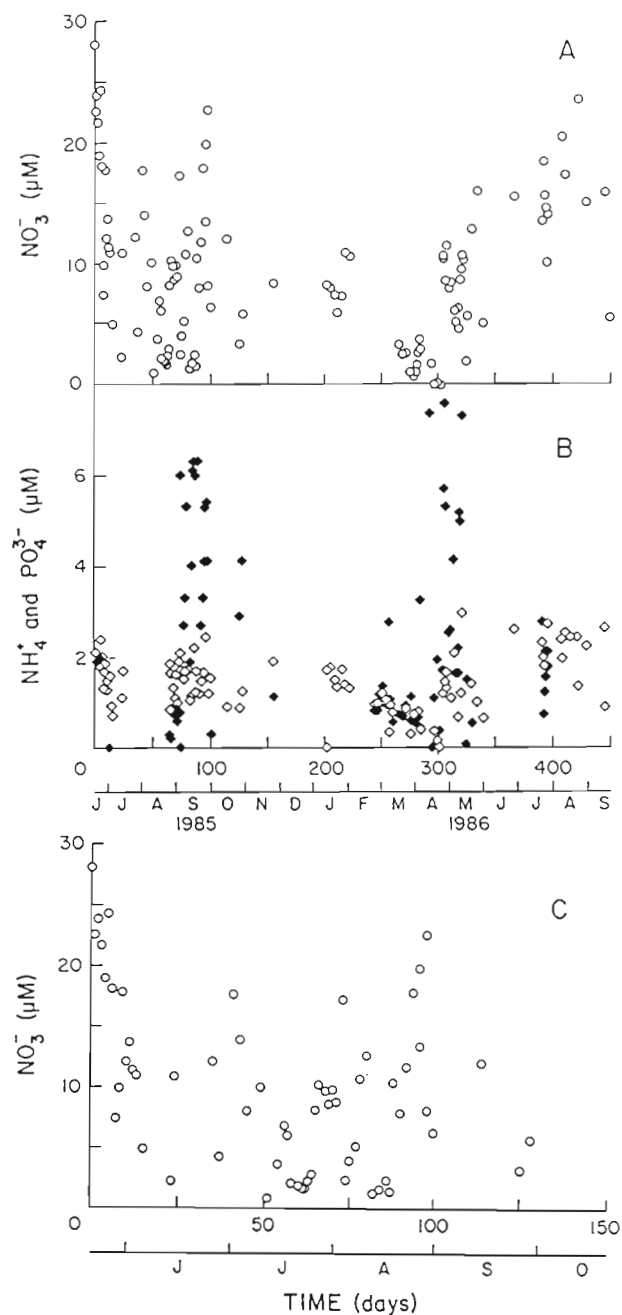
Fig. 2. (A) Seasonal cycle of NO_3^- concentration and (B) NH_4^+ (♦) and PO_4^{3-} (◊) concentration at Yaquina Head. Note the scale change. (C) Nitrate concentration during the upwelling season, 1985

October, high nutrient concentrations occurred sporadically, ranging from 1.3 to 10.5 μM NO_3^-, 0.9 to 2.2 μM PO_4^{3-}, and 0 to 6.3 μM NH_4^+. Satellite and ship-based thermal analysis indicated that the 1985 upwelling season ceased abruptly during the last few days of September (NOAA 1985); the moderately high NO_3^- concentrations observed during October were therefore probably residual (Fig. 2A). Nutrient concentrations were more stable during the winter (6 to 12 $\mu M NO_3^-$ and 1 to 2 μM PO_4^{3-}) and decreased in March to 0 to 4 μM NO_3^- and 0.2 to 1 μM PO_4^{3-}. The decrease in nutrients may have resulted from a spring phytoplankton and macroalgal bloom (Stefánsson & Richards 1964). In 1987, nutrient levels were also relatively low during March and early April (0.8 to 5.7 $\mu M NO_3^-$ and 0.6 to 1.9 $\mu M NH_4^+$), increasing in late April (15 to 23 $\mu M NO_3^-$ and 1.9 to 4.4 μM NH_4^+ (data not shown).

Tissue N content of naturally occurring algae

Tissue N level of *Pelvetiopsis limitata* thalli collected from Yaquina Head ranged from 1.5 to 2.2 % dry wt but showed no significant seasonal variation (Fig. 3). The tissue N content of *Ulva rigida* thalli collected from Yaquina Head was more variable (2.1 to 4.2 % dry wt), but also showed no distinct seasonal trend (Fig. 3).
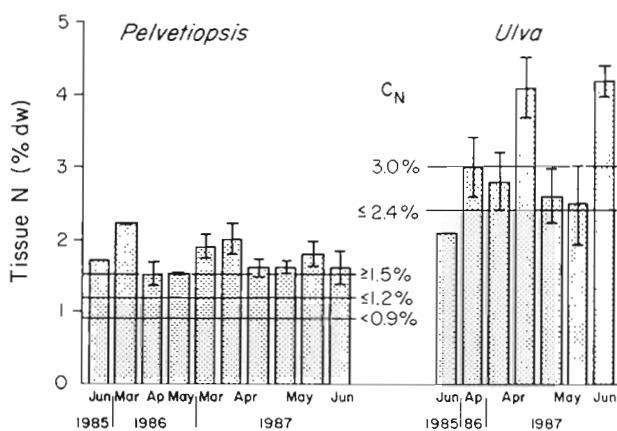

Fig. 3. *Pelvetiopsis limitata* and *Ulva rigida*. Tissue N content of specimens collected at Yaquina Head. Two collections were made for each of April and May 1987. Error bars indicate 1 standard deviation. C_N (and horizontal lines) = critical N level (see text)

3 μM PO_4^{3-}) separated by periods of low nutrient availability during July and August (0.7 to 3 $\mu M NO_3^-$ and 1 to 1.3 μM PO_4^{3-}) (Fig. 2B, C). In June 1986, NO_3^- concentrations were usually above 7 μM. Ammonium concentrations ranged between 0 and 8 μM (Fig. 2B); these unusually high levels may have been due to the large deposits of guano in the area. In September and

Growth kinetics

Growth of *Pelvetiopsis limitata* was hyperbolically related to influent NH_4^+ loading (concentration × culture turnover rate) and was a linear function of influent NO_3^- loading in outdoor culture after 10 to 17 d

of acclimation (Fig. 4A). Growth was faster when NH_4^+ was the N source. Growth at nominally zero N loadings probably resulted from residual inorganic N ($\leq 3.3\ \mu M$) in the incoming medium which is equivalent to a loading of ≤ 0.7 mmol d^{-1}. In indoor culture growth was a hyperbolic function of NH_4^+ and NO_3^- loading after 64 and 54 d of acclimation, respectively, but growth rates were much lower than for outdoor culture (Fig. 4B).

Growth of *Ulva rigida* in outdoor culture after 10 d of acclimation was independent of NH_4^+ loading at low levels and of NO_3^- loadings at high levels. At higher NH_4^+ loading and lower NO_3^- loading, growth rates were positively correlated with N loading (Fig. 4C). Growth at nominally zero N loading probably resulted from residual inorganic N in the incoming medium as noted above for *Pelvetiopsis limitata*. *U. rigida* growth in indoor culture was a linear function of NO_3^- loading, but showed a weaker positive correlation with NH_4^+ loading after a 19 d acclimation period (Fig. 4D).

Critical N content

Growth rates of *Pelvetiopsis limitata* were relatively high and constant at tissue N levels between 0.9 and 2.2 % dry wt for algae grown outdoors on NH_4^+ (Fig. 5A). For algae grown outdoors on NO_3^-, growth rate was nearly a linear function of tissue N. For algae grown indoors, growth rate was slow and independent of tissue N for NH_4^+ and linearly related to tissue N for

NO_3^- (Fig. 5A). No single critical N value can be determined for *P. limitata*; the relationship between growth and tissue N content depends on N source and culture conditions such as irradiance, temperature, and culture turnover rate. All of these factors differed between the indoor and outdoor culture experiments. The critical N value for growth indoors on NO_3^- was ≥ 1.2 % dry wt, but no value could be determined for growth on NH_4^+. Critical N values for growth on NH_4^+ and NO_3^- were < 0.9 and ≥ 1.5 % dry wt, respectively. Subsistence tissue N level (the level at which no growth occurs) was ca 0.5 % dry wt.

The relationship between *Ulva rigida* growth and tissue N also appears to vary with N source and culture conditions (Fig. 5B). Thalli grown outdoors on NH_4^+ exhibited N limitation at tissue N levels less than 3.0 % dry wt, while outdoor growth on NO_3^- resulted in a critical N level ≤ 2.4 % dry wt. Indoor culture results did not yield critical N levels because growth did not saturate at the tissue N contents achieved (Fig. 5B). Growth rates of *U. rigida* at the lowest N loadings attainable in the indoor cultures indicate that the subsistence N level is ca 1.2 % dry wt (Fig. 5B).

In situ growth rates and effects of nutrient enrichment

Growth rates of *Pelvetiopsis limitata* determined by outplanting (3.7 ± 1.5 % d^{-1}) and transect methods (3.9 ± 3.6 % d^{-1}) had high variance but agreed well

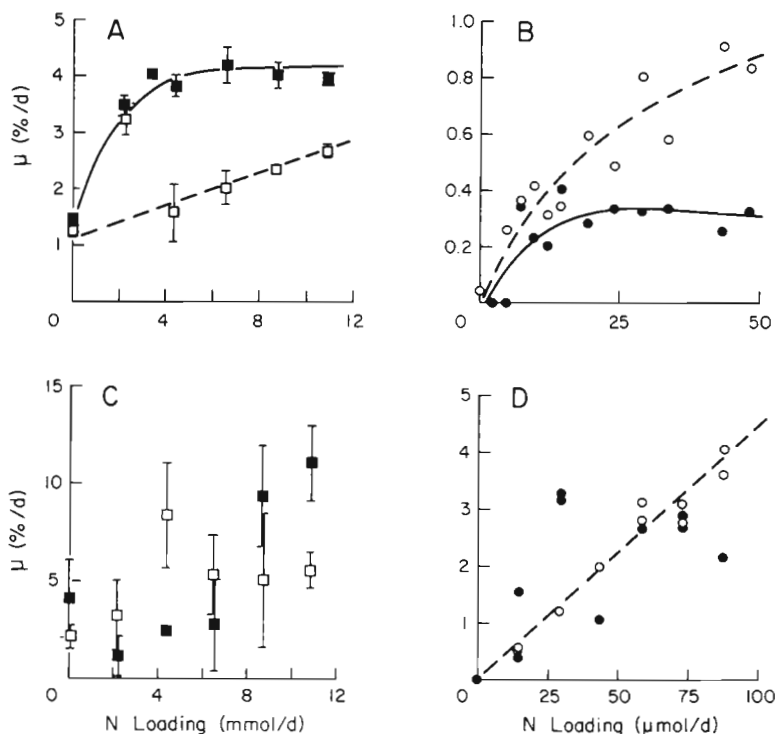Fig. 4. *Pelvetiopsis limitata* and *Ulva rigida*. Mean growth rates on NH_4^+ (■, ●) and NO_3^- (○, □) plotted as functions of influent N loading for flow-through cultures. Error bars for outdoor cultures indicate 1 standard deviation. No replicates were run for indoor cultures. (A) *P. limitata*, outdoor culture. (B) *P. limitata*, indoor culture. Note scale change. (C) *U. rigida*, outdoor culture. (D) *U. rigida*, indoor culture. Note scale changes
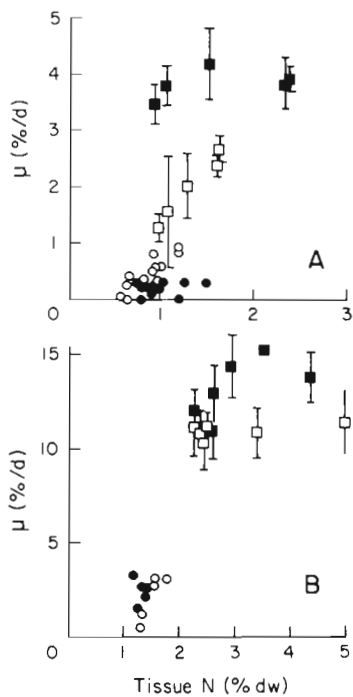
Fig. 5. *Pelvetiopsis limitata* and *Ulva rigida*. Relationship between growth rate and tissue N content for the indoor culture on NO_3^- (○) and NH_4^+ (●) and outdoor culture on NO_3^- (□) and NH_4^+ (■). Error bars indicate 1 standard deviation. No replicates were run for indoor cultures. (A) *P. limitata.* (B) *U. rigida*

Table 1. *Pelvetiopsis limitata*. In situ nitrogen enrichment. Experiment conducted at Yaquina Head from 25 April to 11 May 1986. Means ± 1 standard deviation are presented

Treatment	Number of replicates	Weight loss (g wet wt)	N content (% dry wt)
Control	3	1.8 ±0.26	1.5±0.02
N-enriched (50 µM NH_4^+)	3	0.11±0.15	1.9±0.10

with each other and with maximum growth rates observed in culture (3.9 ± 0.44 % d^{-1}).

Pelvetiopsis limitata in both the control and the N-enriched treatments showed a decrease in weight over the experimental period (Table 1). Since few grazers were observed on the *P. limitata* beds adjacent to the experimental site, and since no obvious grazer-induced damage was observed, we suggest that the weight loss was due to the senescence of individual fronds with subsequent breakage and loss. We observed senescent fronds and frequent signs of breakage within both naturally occurring and outplanted clumps of thalli. Similar numbers of thalli were lost from control and N-enriched treatments (5 and 6, respectively; the treat-

ments initially had 17 and 13 thalli, respectively). Since biomass loss was relatively uniform between the treatments, a comparison of growth rates (with less loss indicating more rapid growth) can be made. Algae enriched with N lost significantly ($p < 0.05$, Mann-Whitney U-test) less weight (i.e. grew more rapidly) and had higher tissue N levels than control (unenriched) algae (Table 1).

DISCUSSION

Our studies of N availability, critical N content, and the effects of in situ N enrichment allow us to integrate the results from 3 methods of assessing N limitation of macroalgal growth. The first method predicts N limitation during periods of low N availability as measured by water column concentrations. High temporal resolution is required, because some algae may have limited capacities to store N for growth (Fujita 1985a) and could become N-limited between intermittent upwelling events. The second method predicts N limitation when tissue N levels of algae from natural communities are less than a critical value established in culture experiments. The third method predicts N limitation when enrichment with N results in increased growth.

We have assumed in this study that dissolved organic N is not an important N source for rocky intertidal macroalgae. *Ulva* spp. can use urea and amino acids as nitrogen sources (Nasr et al. 1968, Mohsen et al. 1974). However, in general the capacity of macroalgae to utilize organic forms of N is very limited (Stephens 1981). Furthermore, concentrations of both urea (Kokkinakis & Wheeler 1988) and primary amines (measured by fluorescamine, Wheeler unpubl.) are not particularly high in the Oregon coastal waters, and we feel justified in excluding these possible N sources in the present discussion.

Based on water column N concentrations, we would predict that *Pelvetiopsis limitata* growth would be N-limited only in March and April, when inorganic N levels were consistently low. To apply the critical N concept to field populations of *P. limitata*, it is necessary to determine the major N source for growth because the N source affects the critical N relationship (Fig. 5A). We can make a rough estimation of the relative contributions of NH_4^+ and NO_3^- to total N demand by constructing a N budget (Table 2). In our calculations, we estimated mean exposure time at low tides by direct observation throughout the year (A. Olsen pers. comm.) and assumed that the low tides were symmetrical (resulting in an overestimation of exposure time, since all observations were made at the lowest-low tides). We further assumed that NO_3^- uptake is light dependent while NH_4^+ uptake is not

Table 2. *Pelvetiopsis limitata.* Nitrogen budget for in situ growth at Yaquina Head, Oregon. Uptake rates were measured as a function of N concentration in short-term laboratory experiments and used in conjunction with ambient nutrient concentrations to calculate in situ uptake rates

Month	Time submersed per day (h)	Estimated in situ uptake rate (μmol[g dry wt]$^{-1}$h^{-1}) NO$_3^-$	NH$_4^+$	% N required for maximum growth potentially supplied NO$_3^-$	NH$_4^+$
Jan	9	1.7	3.7	26	113
Feb	5	1.6	3.7	30	143
Mar	7	0.8	3.7	14	132
Apr	8	1.0	5.3	16	174
May	8	1.7	5.3	28	174
Jun	9	4.6	5.3	71	163
Jul	11	2.2	3.7	28	96
Aug	9	1.6	3.7	24	113
Sep	8	2.0	3.7	33	122
Oct	12	1.6	5.3	19	130
Nov	9[a]	1.6	3.7	25	116
Dec	9[a]	1.6	3.7	25	116

[a] No data available; means for entire year used

(Hanisak 1983), that growth rate was constant at 4 % d^{-1} (growth rates in outdoor cultures varied between 2 and 4 % d^{-1} and in situ growth rates averaged 3.9 % d^{-1}), and that tissue N levels averaged 1.7 % dry wt throughout the year (mean of all tissue N values in Fig. 3; standard deviation = 0.23). The budget indicates that NO$_3^-$ could account for 14 to 33 % of the N demand, while NH$_4^+$ could potentially supply 96 to 174 % of the total N demand (Table 2). The uptake rates used in the calculation of the N budget were measured in short-term experiments and may overestimate long-term or steady-state uptake (Probyn & Chapman 1982), resulting in percentages of the total N demand supplied by NH$_4^+$ in excess of 100 % (Table 2). Nonetheless, it is unlikely that in situ growth is N-limited. The in situ enrichment experiment, however, suggests that, despite high N concentrations present during early May, *P. limitata* growth may have been N-limited (Table 2). The algae in the control treatment had a tissue N level of 1.5 % dry wt while N-enriched thalli had a tissue N content of 1.9 % dry wt. Since the critical N level for growth in outdoor cultures was $\geq$ 1.5 % dry wt, these results suggest that *P. limitata* was growing on NO$_3^-$ in situ.

The 3 methods for the assessment of N limitation used in this study (water column DIN concentrations, critical tissue N level, and in situ enrichment) do not yield the same conclusion for *Pelvetiopsis limitata.* Water column DIN concentrations indicate that *P. limitata* may be N-limited in March and April (Fig. 2A). The critical N levels obtained in outdoor culture

coupled with the results of the N budget (i.e. that NH$_4^+$ is the major N source in the field) indicate that this species is never likely to be N-limited. The results of the in situ enrichment experiment indicate that *P. limitata* may be N-limited in April and May only if NO$_3^-$ is the major N source for growth in the field, contrary to the conclusion drawn from N budget analysis (Table 2).

Based on water column N concentrations, *Ulva rigida* would also only exhibit N limitation in April and May. The relationships between growth and critical N content depended on N source in outdoor culture (3.0 % dry wt on NH$_4^+$, $\leq$2.4 % dry wt on NO$_3^-$). A N budget was constructed to determine which N source was more important in situ. We assumed that thalli were always submerged, experienced 10 h of light during the spring and 12 h during the summer, and that N content and growth rate varied from 3 % dry wt and 20 % d^{-1} to 4 % dry wt and 30 % d^{-1} in the spring and summer, respectively. The budget (Table 3) indicates that NH$_4^+$ may supply most of the N requirements for growth despite much higher concentrations of NO$_3^-$. Using the critical N level derived from the outdoor cultures of *U. rigida* on NH$_4^+$ (3.0 % dry wt), growth is just on the verge of N limitation most of the spring (Fig. 3).

We were unable to conduct an in situ N enrichment study with *Ulva rigida* due to excessive tissue loss. Similarly, we were unable to measure the growth rate of *Ulva* spp. in situ. However, our outdoor cultivation studies indicate that the growth rate of these algae can be relatively high (up to 12 % d^{-1}). Many other workers have also reported high growth rates for *Ulva* spp. (e.g., Lapointe & Tenore 1981, Fujita 1985a, Duke et al. 1986). *Ulva* and *Enteromorpha* spp. are capable of extremely rapid N uptake when N availability is high, and continued growth for up to 5 d after N is depleted (Fujita 1985a, O'Brien 1987). However, because N pulses sometimes occur as much as 6 d apart at Yaquina Head, ulvoid algae may be intermittently N-limited if growth rates are high enough to deplete stored tissue N and ambient N is too low to maintain growth rates.

When N was provided only once every 5 d in a field experiment, ulvoids decreased in abundance while the red alga *Gracilaria tikvahiae* (which can grow for 14 d in the absence of N; Ryther et al. 1981, Fujita 1985a), dominated mixed beds (Fujita 1985b). Nitrogen uptake by *Ulva rigida* is rapid (ca 12 μmol N g dry wt^{-1} h^{-1}) even at low concentrations (2 μM; Fujita unpubl.). Uptake at 2 μM N would provide sufficient N to support growth at 16 % d^{-1} of *U. rigida* containing 2.5 % dry wt N. Very high growth rates (ca 27 % d^{-1}, Davis 1981) would result in greater N demand and possible N limitation. More measurements of growth rates and tissue N levels in naturally occurring thalli and an in situ enrichment experiment are needed to test these possibilities.

Table 3. *Ulva rigida*. Nitrogen budget for in situ growth at Yaquina Head, Oregon. Uptake rates were measured as a function of N concentration in short-term laboratory experiments and used in conjunction with ambient N concentrations to calculate in situ uptake rates

Month	Hours daylight per day	Mean N concentration (μM)		Estimated uptake rate ($\mu mol[g\ dry\ wt]^{-1}h^{-1}$)		% N required for maximum growth potentially supplied	
		NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+
Jan	8	8.4	1.00	23.4	6.0	134	103
Feb	8	8.2	1.07	22.2	6.0	127	103
Mar	10	3.0	1.14	10.2	6.0	32	46
Apr	10	3.4	2.50	27.6	32.4	64	181
May	10	8.7	2.31	72.0	26.4	168	148
Jun	12	33.2	2.71	16.8	24.6	23	69
Jul	12	14.6	1.51	19.2	18.0	27	50
Aug	12	19.8	1.24	18.6	18.0	26	50
Sep	10	10.8	1.22	13.8	18.0	32	101
Oct	10	7.1	3.20	21.0	12.6	150	216
Nov	10	8.4	1.14	24.0	5.4	171	93
Dec	8	8.3	1.00	23.4	6.0	134	103

The critical N concept appears to be a useful tool for diagnosing N limitation of *Ulva rigida* growth because it provides a relationship between an index of N status (tissue N content) and growth rate. Nitrogen source has a strong effect on the relationship; growth on NH_4^+ yielded a higher critical N level (3.0 % dry wt) than growth on NO_3^- (≤ 2.4 % dry wt). Similarly, the relationship between tissue N and growth of *Pelvetiopsis limitata* is strongly dependent upon N source and other culture conditions. The low N loadings used in the indoor cultures should have resulted in lower N content and low growth rates, as seen for *U. rigida* (Fig. 5B). Even though the growth rates of *P. limitata* were much lower in indoor culture, N content varied over a wide range (0.6 to 1.6 % dry wt) which included N contents of algae grown outdoors (Fig. 5A). This indicates that, in spite of provision of micronutrients and adequate light, some factor other than N availabilty limited

growth of *P. limitata* indoors. The elevated growth rates obtained with NH_4^+ as the N source (in comparison with NO_3^-) at equivalent tissue N levels suggest that it is energetically more favorable to assimilate NH_4^+ than NO_3^- for both species (Fig. 5A, B). The critical N levels established in this study for *P. limitata* fall within the range of critical N levels established for other species; however, critical N levels for *U. rigida* exceed this range (Table 4).

The relationship between *Pelvetiopsis limitata* growth rate and influent NH_4^+ concentration indicates that *P. limitata* growth was N-limited at influent NH_4^+ loadings less than 4.3 mmol d^{-1} (20 μM) in outdoor culture, while growth did not saturate at the highest NO_3^- loading tested (10.8 mmol d^{-1}; 50 μM) (Fig. 4A). Growth at nominally zero N concentration or loading was probably supported by N (both DIN and DON) remaining after nutrient stripping (0 to 3 μM total DIN).

Table 4. Critical N levels as % dry wt for marine macroalgae

Species	Critical N (% dry wt)	Source
Cladophora albida	2.1	Gordon et al. (1981)
Cladophora spp.	1.5	Wong & Clark (1976)
Codium fragile	2.1	Hanisak (1979)
Chordaria flagelliformis	0.7	Rosenberg et al. (1984)
	1.2	Probyn & Chapman (1983)
Pelvetiopsis limitata		
outdoors on NH_4^+	<0.9	This study
outdoors on NO_3^-	≥1.5	This study
indoors on NH_4^+	Not determined	
indoors on NO_3^-	≤1.2	This study
Ulva rigida		
outdoors on NH_4^+	3.0	This study
outdoors on NO_3^-	≤2.4	This study

The potential effects of organic compounds produced in the high-density nutrient stripping tanks are unknown. Indoor cultures did not grow at zero N-loading due to the more effective nutrient stripping possible with batch cultures (Fig. 4B, D).

Growth rate in indoor culture was not saturated at the highest NO_3^- loading (48 µmol d^{-1}; 100 µM), but was N-limited at NH_4^+ loadings less than 15 µmol d^{-1}) (30 µM), (Fig. 4B). Growth of Ulva rigida in outdoor culture appeared to be N-limited at influent NO_3^- loadings less than 6.48 mmol d^{-1} (30 µM), but was not saturated with NH_4^+ (Fig. 4C). U. rigida growth appears to be N-limited in indoor culture at influent NH_4^+ loadings less than 25 µmol d^{-1} (20 µM), but growth was not saturated at any of the NO_3^- loadings tested (Fig. 4D). These relationships cannot be used to predict N-limited growth in nature because of the strong dependence of growth rate on water flow rate and biomass (e.g., Lapointe & Ryther 1979, Fujita & Goldman 1985). At higher flow rates, perhaps more characteristic of the natural habitat, growth saturation would probably occur at much lower N concentrations than those indicated in the present study (> 20 µM) due to the increased flux of N to the algae (Fujita & Goldman 1985). In addition, the relationships shown in Fig. 4 apply only under relatively steady-state conditions of flow rate, N concentration, and biomass level; these conditions probably seldom occur in nature.

In conclusion, we have shown that N availability based on water column N concentrations is a poor indicator of N limitation because N storage by macroalgae introduces time lags between low water column N concentrations and the onset of N-limited growth. Critical tissue N levels for Ulva rigida are different for growth on NH_4^+ and NO_3^-, but can be used as an index of the N status if the identity of the major N source is known. A greater range of tissue N levels and growth rates attained in outdoor culture (possibly by using lower flow rates) is needed for the establishment of more definitive critical N levels for U. rigida and Pelvetiopsis limitata. The critical N content for U. rigida was found to be ≤ 2.4 % dry wt for growth on NO_3^- and 3.0 % dry wt for growth on NH_4^+ in this study. The critical N levels for P. limitata growth in outdoor culture on NH_4^+ and NO_3^- are < 0.9 and ≥ 1.5 % dry wt, respectively. Nitrogen budgets constructed for P. limitata and U. rigida indicate that the major N source for growth is NH_4^+ despite the greater abundance of NO_3^- at Yaquina Head. Therefore, the critical N level for predicting N limitation in the field was based on the relationship between tissue N content and growth on NH_4^+ in culture. The critical N levels for P. limitata growth on NH_4^+ in outdoor culture (< 0.9 % dry wt) is not consistent with the results of a field enrichment experiment, in which thalli with tissue N content of 1.5 % dry

wt appeared to be N-limited. The critical N level for U. rigida growth on NH_4^+ in outdoor culture (3.0 % dry wt) indicates that growth was at times N-limited in the spring. Nitrogen budgets that assess the relative contributions of NH_4^+ and NO_3^- to total N demand must be used in conjunction with critical N levels for both N species to assess N limitation for algal species that exhibit relationships between growth and N content that depend on the N source.

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