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The Effects of Nitrogen and Seawater Flow Rate on the Growth and Biochemical Composition of *Gracilaria foliifera* var. *angustissima* in Mass Outdoor Cultures*

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Abstract

The effects of nitrogen species ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$), loading (m moles of N/day) and seawater flow rate on the growth of the red seaweed *Gracilaria foliifera* var. *angustissima* (Harvey) Taylor were investigated in outdoor culture tanks. In one experiment, cultures received seawater enriched with either NH_4^+ , NO_3^- or secondarily treated wastewater (with N as NO_3^-) but the same daily nitrogen load at four seawater turnover rates (1, 7.5, 15 and 30 culture volume exchanges/day). In another experiment, cultures were maintained at four turnover rates (1, 7.5, 15 and 30 culture volume exchanges/day) with NH_4^+ or NO_3^- at a given concentration so that nitrogen loading was proportional to turnover rate.

Below 15 m moles of N/day, algal growth was N – limited and the plants had high C/N values and appeared bleached; above 15 m moles/day, the plants had C/N values of ≤ 10 and were a dark brown color. Above this minimal daily N loading, algal growth was highly dependant upon flow rate *per se*. Yield of *Gracilaria* was greater with NH_4^+ than NO_3^- at nitrogen loadings just adequate to support exponential growth; however, at higher levels of nitrogen loading the maximum recorded yields (up to 44 g dry weight/m² · day⁻¹) occurred with NO_3^- rather than NH_4^+ .

Water, ash and caloric content of *Gracilaria* was positively correlated with C/N values in N – limited plants. Absolute levels of both phycoerythrin and chlorophyll increased proportionally with decreasing C/N values of the plants. Thus the observed nitrogen-related pigment changes in *Gracilaria* are light intensity and not necessarily light quality adaptations.

Introduction

Recently, the increasing demand for seaweed hydrocolloids, especially carrageenan and agar, spurred interest in the cultivation of commercially-important species (Silverthorne and Sorenson 1971). To date, rope and pond culture techniques in developing countries have been quite successful and are supplying an increasing fraction of the raw material used by the hydrocolloid industries (Doty 1973, Shang 1976).

Although nitrogen can limit the growth of seaweeds in coastal waters (Chapman and Craigie 1977), its role in regulating algal growth and biochemical composition is not fully understood. For example, there are seasonal variations in caloric content, percent dry weight, percent

ash (Baardseth 1970, Himmelman and Carefoot 1975, Mann 1972) and pigment content (Moon and Dawes 1976) of seaweeds but these changes have not been related to seasonal nitrogen availability or growth rate *per se*. Also, although C/N values might correlate with growth rates in seaweeds (Mann 1972, Niell 1976), their relationship is not fully understood. Much recent work has examined the effects of nutrient concentration on the growth of red (DeBoer *et al.* 1978), green (Waite and Mitchell 1972) and brown algae (Topinka and Robbins 1976) but there are little data available relating nitrogen loading (flow rate X nutrient concentration) and/or flow rate *per se* to algal yields in long term mass cultures. Because nitrogen availability to an alga is a function of both concentration and flow rate near the plant thallus, the N-loading parameter is perhaps the single most useful tool for budgeting N-algal yield relationships. Also, although algal growth-flow rate interactions have not been studied quantitatively, natural seaweed populations are often described as more luxuriant in turbulent areas (Conover 1968). Such data are useful for optimizing

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yields in commercial seaweed ponds without wasting fertilizer or contributing to epiphyte growth.

To study these factors we designed an intermediate-sized system to culture whole plants under natural sunlight in flowing seawater with controlled nutrient addition. We report here the effects of N-loading and seawater flow rate on yield (i.e. growth), caloric content, percent water, percent ash, C/N ratio and levels of phycoerythrin and chlorophyll in the agar-producing red alga *Gracilaria foliifera* var. *angustissima* (Harvey) Taylor.

Materials and Methods

Experimental Culture System: Design and Operation

PVC pipes (0.4 m diameter) were sectioned longitudinally and divided by fibreglassed plywood partitions into 0.75 m long (50 l; 0.23 m² surface area) chambers. Compressed air (5 psi) entered along the bottom of each chamber through small drilled holes from an airline – a half section of a one inch PVC pipe cemented to the out-

side bottom of the chamber. Each compartment also had a non-clogging overflow drain. The 16 growth chambers were located out-of-doors in full sunlight (Fig. 1). Total radiant energy was measured with an Epply pyroheliometer.

Seawater was pumped from the Harbor Branch Foundation ship channel which connects to the Indian River, a shallow lagoon on the Atlantic Ocean near Fort Pierce, Fla. and gravity fed to the sixteen culture chambers at the desired flow rates – four chambers receiving each of the desired 1, 7.5, 15 and 30 turnovers per day. Ambient water temperature ranged from 23 to 34 °C and salinity from 26 to 33 ‰ during the experimental period (11 May to 24 June 1977).

The different media from 4000 L reservoirs were gravity fed to the appropriate chambers in Experiment I. The three media (NH₄⁺-N, NO₃⁻-N, and NO₃⁻-N enriched secondary-treated sewage effluent – all three enriched with PO₄³⁻-P) at concentrations of 300 μM of ΣIN (ΣIN = NH₄⁺-N+NO₂⁻-N+NO₃⁻-N) and 30 μM of PO₄³⁻-P were added at different flow rates to the twelve chambers such that the chambers receiving the three different media types

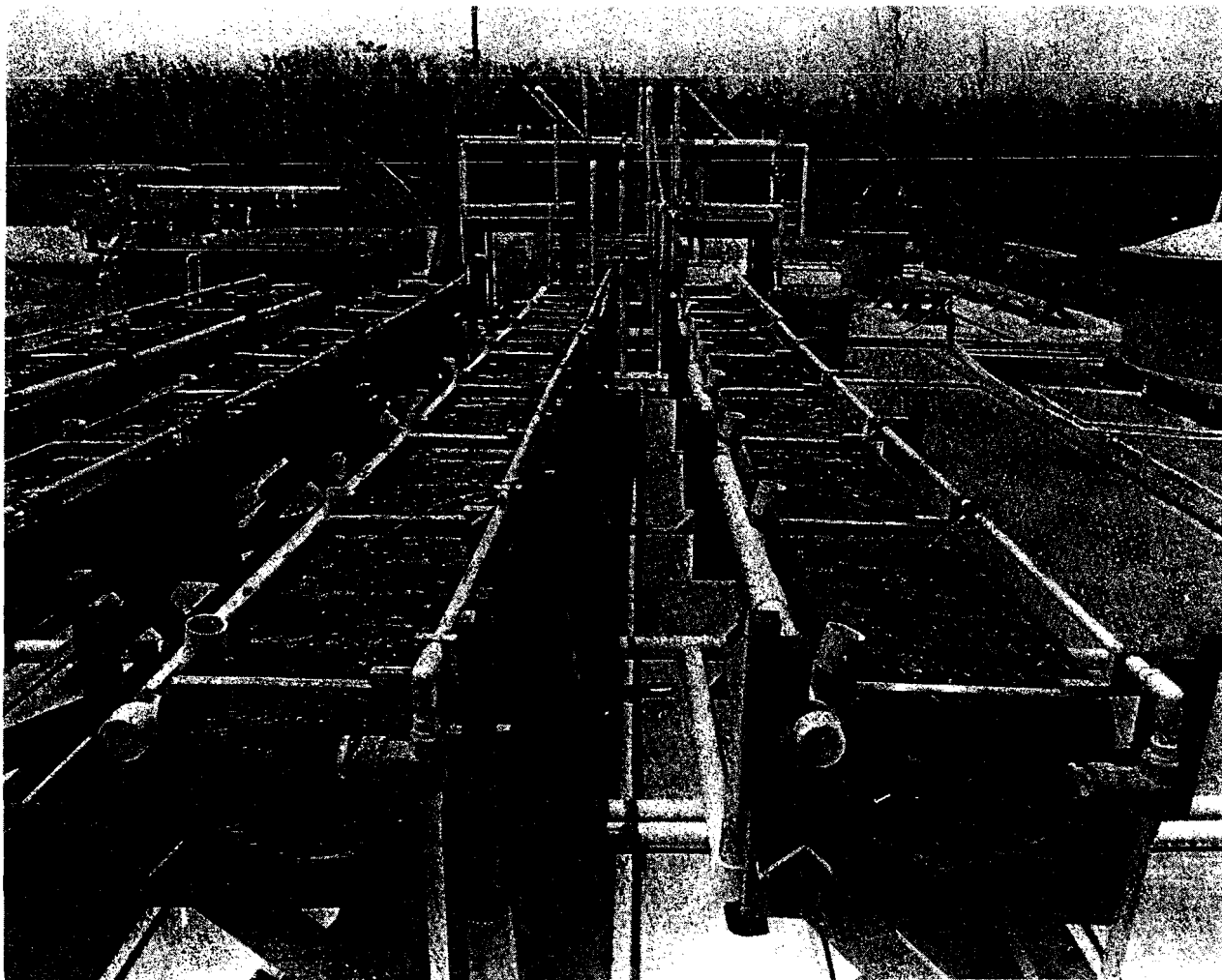


Fig. 1. Photo of experimental seaweed culture system showing 0.23 m² chambers receiving seawater and nutrients from headboxes.

at each of the 1, 7.5, 15 and 30 turnovers per day (seawater flow rate/culture volume) all maintained the same daily total nitrogen loading. In effect, the instantaneous influent nutrient concentration was inversely proportional to turnover rate, and resulted in influent nutrient concentrations of 10, 20, 40 and 300 μM of ΣIN with decreasing turnover rates. The four remaining "control" chambers were given seawater without nutrient enrichment at the 1, 7.5, 15 and 30 turnovers per day.

In Experiment II, two media ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$, both enriched with $\text{PO}_4^{3-}\text{-P}$) were each maintained at a concentration of 50 μM ΣIN and 5 μM $\text{PO}_4^{3-}\text{-P}$. These media were distributed to eight culture chambers at 1, 7.5, 15 and 30 turnovers per day in such a way as to maintain equal influent nutrient concentrations. In this case, the daily nitrogen loading was directly proportional to turnover rate.

The *Gracilaria* stock used for these experiments had been growing rapidly in nitrogen-enriched media (NO_3^-) and all of the plants used had a C/N ratio of 10 at the beginning of the experiments. An initial biomass of 280 g wet wt. of *Gracilaria* per chamber gave a starting density of 1.2 kg wet wt./ m^2 a somewhat lower than optimal density for maximum *Gracilaria* yield in this culture system (Lapointe and Ryther 1978). We purposely selected relatively low biomass levels to reduce high plant density effects of self-shading, metabolite inhibition and possible CO_2 limitation.

At intervals of 4 to 6 days, the plants were removed from the chambers, shaken vigorously in the air to remove excess water and weighed. Plant biomass was removed from each culture to return to initial densities. This biomass increase was used to determine yield per given time for each treatment. A dry weight conversion factor (see Table I) was used to calculate growth in grams of dry weight $\cdot \text{m}^{-2} \cdot \text{day}^{-1}$.

Water Analysis

Water samples were taken at midday for chemical analyses of $\text{PO}_4^{3-}\text{-P}$ (Murphy and Riley 1962), $\text{NH}_4^+\text{-N}$ (Solarzano 1969) and $\text{NO}_3^-\text{-N}$ (Wood *et al.* 1967). Midday pH values were recorded at 4-h intervals twice during the experimental period. Culture temperatures and salinities

Tab. 1. Caloric value per gram dry weight and per gram ash-free dry weight, carbon/nitrogen ratio, percent dry weight and percent ash weight of *Gracilaria* cultured in enriched (A) and un-enriched (B) media. Values are given as mean \pm 1 standard deviation based on 3 observations.

	Calories/g dry weight	Calories/g ash-free dry weight	C/N	% Dry Weight	% Ash Weight
<i>Gracilaria</i> A	2656 \pm 52	4618 \pm 96	9.9	10.2	48
<i>Gracilaria</i> A	2619 \pm 24	4595 \pm 42	11.0	10.3	47
<i>Gracilaria</i> B	2855 \pm 08	4326 \pm 12	35.0	7.8	34
<i>Gracilaria</i> B	3106 \pm 37	4314 \pm 51	39.0	7.5	31

were measured daily at each turnover rate with Taylor maximum-minimum thermometers and a Beckman induction type salinometer.

Tissue Analysis

During Experiment I and II, C/N values were determined from plant samples taken at each weigh interval. The *Gracilaria* was dried at 60 $^\circ\text{C}$ for 48 hours and total nitrogen and carbon determined on a Perkin-Elmer 240 elemental analyzer.

Upon termination of Experiment I, samples of *Gracilaria* from the various treatments were analyzed for percent water, percent ash, pigments, and caloric content. Percent water (of fresh weight) was determined by oven-drying the seaweed at 90 $^\circ\text{C}$ for 48 hours. Ash content was determined by combusting dried samples at 475 $^\circ\text{C}$ for 8 hours. Pigment analysis was carried out by extraction and spectrophotometric techniques, using cold aqueous phosphate buffer (pH 7.0) for phycoerythrin and 80% spectro-analyzed acetone for chlorophyll (Moon and Dawes 1976). Caloric content of dried *Gracilaria* (90 $^\circ\text{C}$) was determined using a Parr microbomb calorimeter.

Results

Experiment I

In this experiment, we looked at the effects of nitrogen species, nitrogen loading and seawater turnover rate on the growth and composition of *Gracilaria*. Daily nitrogen loading was constant in the three enriched regimes (15 m moles/day of $\text{NO}_3^-\text{-N}$, $\text{NH}_4^+\text{-N}$ or sewage $\text{NO}_3^-\text{-N}$) while the daily nitrogen loading in the non-enriched seawater controls was dependent upon the available ΣIN in the seawater and thus a function of turnover rate.

At each turnover rate, algal yields of the non-enriched seawater controls were lower than that of the nitrogen enriched treatments (Fig. 2). In the first 6 days, non-enriched and enriched yields at given turnovers were similar, ranging from 8.0 at 1 turnover/day to 24.2 g dry wt. $\text{m}^{-2} \text{day}^{-1}$ at 30 turnovers/day. After day 6, yields of the non-enriched controls continually decreased until day 16 when there was some indication of leveling off. These final yields increased with increasing turnover rate and ranged from 0.8 at the 1 turnover/day to 18.0 g dry wt. $\text{m}^{-2} \text{day}^{-1}$ at the 30 turnover/day treatment.

Carbon/nitrogen values of the non-enriched seawater controls increased dramatically from the initial ratio of 10 after day 6 until at day 22 they were 31, 26, 20 and 16 in the 1, 7.5, 15 and 30 turnover/day treatments, respectively (Fig. 3). All the enriched treatments maintained C/N values of 10 ± 1 during the experiment.

Yields in both the controls and the three enriched treatments consistently increased with increasing turnover rate (Fig. 2). For example, in the first six days, yields of

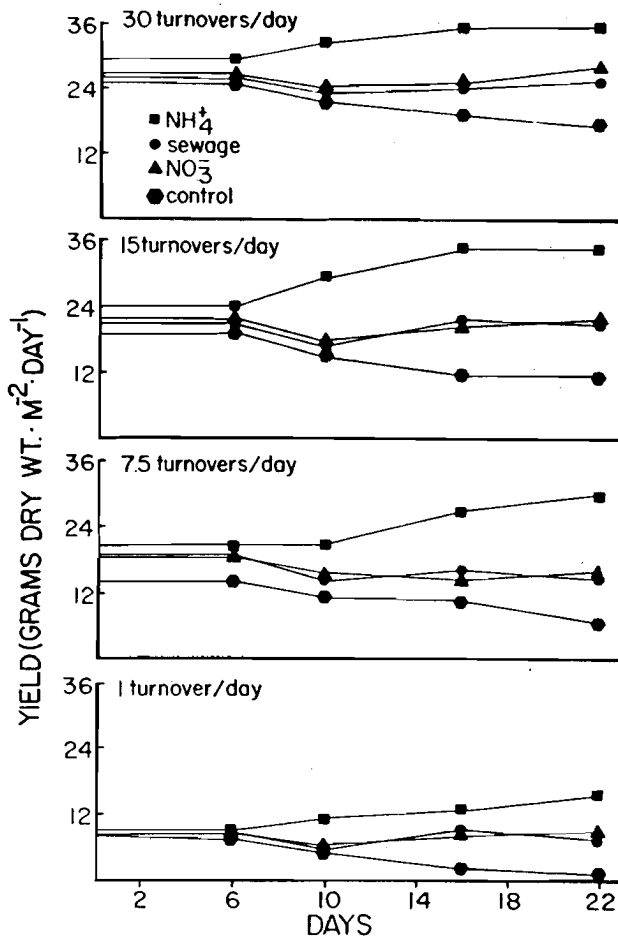


Fig. 2. In Experiment I, yield of *Gracilaria* cultured with three N-enriched media ($\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-enriched sewage}$) and unenriched seawater at four turnover rates. The three enriched cultures received equal daily loadings of nitrogen whereas the seawater controls received only that nitrogen present in the seawater.

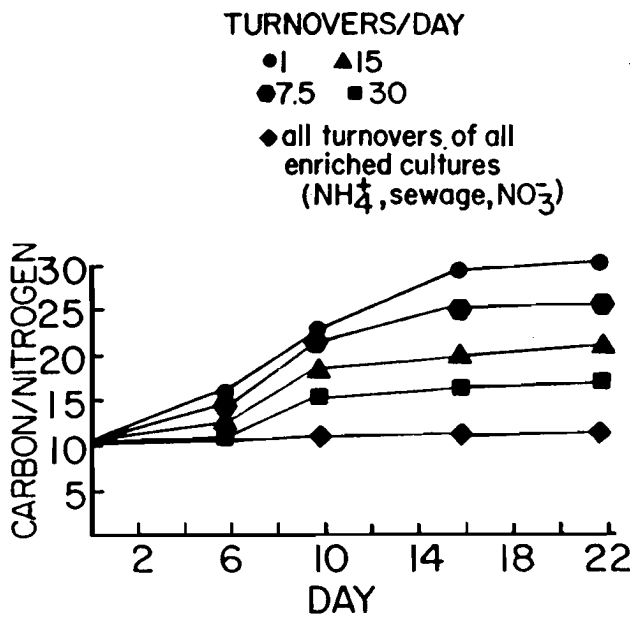


Fig. 3. In Experiment I, changes with time of carbon/nitrogen values of *Gracilaria* cultured at different turnover rates and nutrient enrichment.

the controls increased from 8.0 at 1 turnover to 24.1 g dry wt. $\text{m}^{-2} \text{day}^{-1}$ at 30 turnovers/day. Similarly yields in the NH_4^+ enriched cultures increased during the last weigh interval from 16.0 at 1 turnover/day to 35.9 g dry wt. $\text{m}^{-2} \text{day}^{-1}$ at 30 turnovers/day.

Diurnal changes in pH were greater in the enriched treatments than the controls and ranged from 8.2 (0400 hours) to 9.3 (1600 hours) (Fig. 4). Midday pH values decreased with increasing turnover rate.

Yield of *Gracilaria* in the NH_4^+ treatments were consistently higher than the NO_3^- and sewage cultures at all turnover rates and increased over the duration of the experiment (Fig. 2). Maximum yields in the enriched cultures occurred during the last weigh interval at 30 turnovers/day and varied from 35.9 with NH_4^+ , 28.0 with sewage and 25.2 g dry wt. $\text{m}^{-2} \text{day}^{-1}$ with NO_3^- cultures. Yields and their change during the course of the experiment for NO_3^- and sewage treatments were particularly similar.

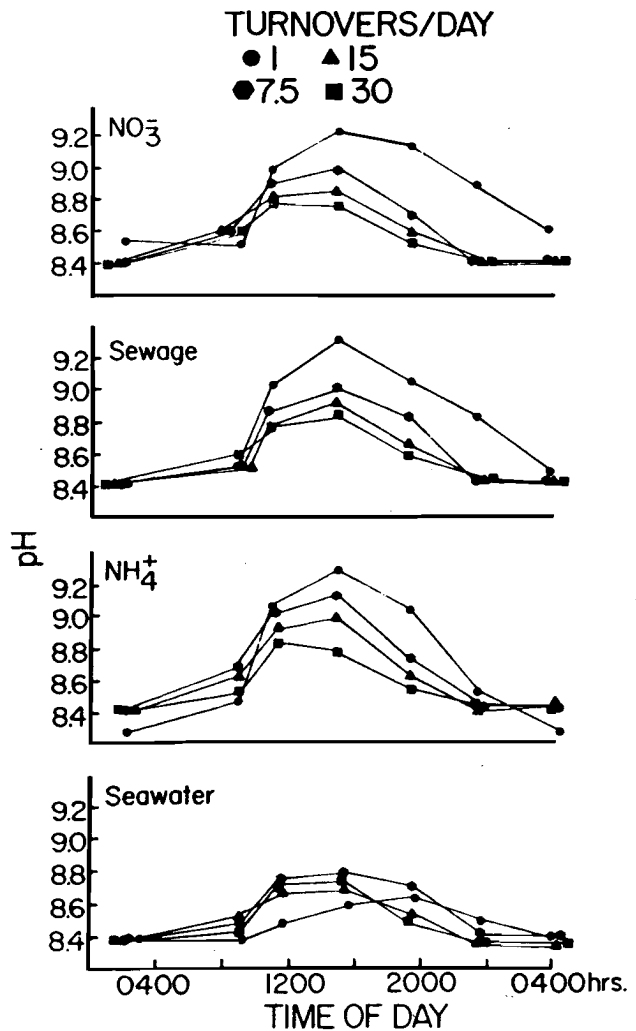


Fig. 4. In Experiment I, differences in diurnal change of pH of culture water of *Gracilaria* grown at different turnover rates and nutrient enrichment.

The N/P assimilation ratios averaged 26.1 ± 1.1 in all the enriched cultures, whereas they decreased linearly from 19.0 ± 1.2 at 30 turnovers to 6.6 ± 1.7 at 1 turnover/day in the controls (Fig. 5). These values are based on three influent and effluent samplings.

At the end of Experiment I, dry weight of *Gracilaria* varied from 6.9 to 10% of wet weight over a C/N range of 31 to 10. Percent dry weight of the four control cultures (C/N values of 31, 26, 20 and 16) and the three 30 turnover/day enriched cultures (C/N \approx 10) are plotted in Figure 6. A regression of these data with C/N values shows good correlation ($r^2 = 0.96$). Percent ash of these same cultures are also illustrated in Figure 6, and show

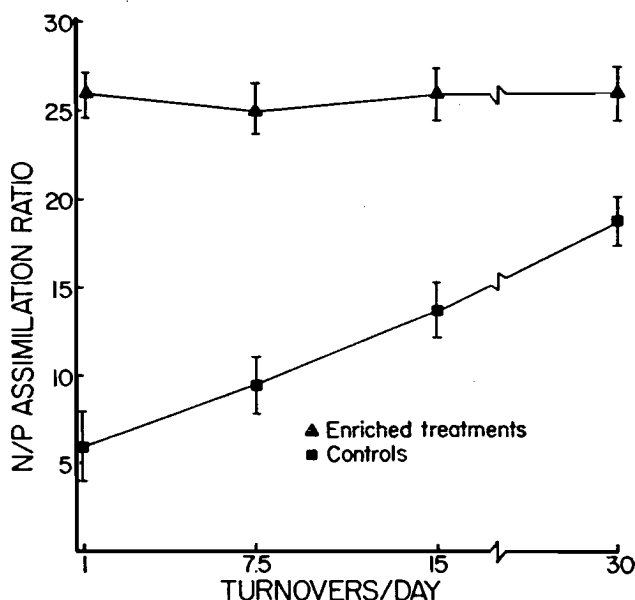


Fig. 5. In Experiment I, changes of nitrogen/phosphorus assimilation values due to turnover rates and nutrient enrichment.

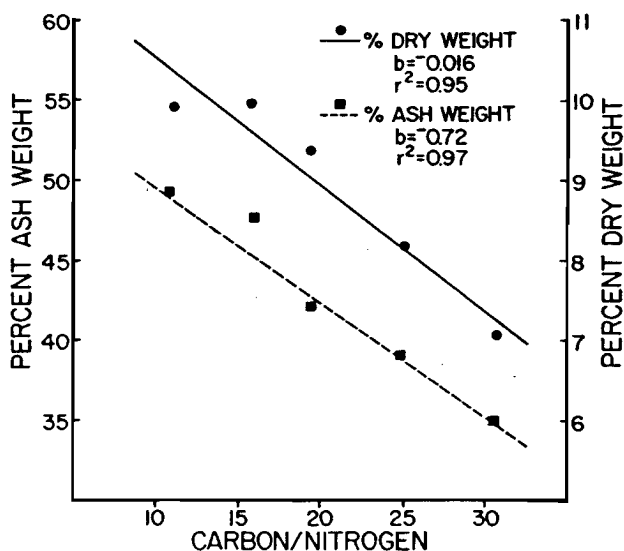


Fig. 6. Percent ash and dry weight of *Gracilaria* as a function of carbon/nitrogen ratio.

good correlation with C/N ratio over the range of 10 to 31 ($r^2 = 0.99$). Not shown in this ash regression are the data points for the 1, 7.5 and 15 turnover(s)/day enriched treatments which ranged from 35 to 50 percent ash and in all cases increased their ash content with increasing turnover rate. Because all these algae had C/N values of 10, they did not fit into the regression of Figure 7 and were omitted. Similarly, caloric content of the organic fraction of these plants correlated with C/N ($r^2 = .98$) and ranged from 4130 to 4500 calories/g ash-free dry wt. (Fig. 7).

To illustrate how caloric, ash and dry weight content change with nitrogen content of *Gracilaria*, plants representing the extreme of enriched and unenriched culture conditions were assayed (Table I). This *Gracilaria* was derived from stock cultures grown separately from the experimental treatments. Caloric content of unenriched *Gracilaria* is significantly greater than that of enriched material when expressed in g/dry wt.; however, the opposite trend is seen when expressed as calories/g ash-free dry wt. Percent dry weight and percent ash follow the same trend with C/N values as that reported above for *Gracilaria* derived from Experiment I.

Chlorophyll and phycoerythrin content were also measured at the end of Experiment I. Both pigments increased their absolute levels with decreasing C/N values and were highly correlated, ($r^2 = .85$ for chlorophyll; $r^2 = .88$ for phycoerythrin) (Fig. 8). Phycoerythrin/chlorophyll (P/C) ratios, based on Figure 8, varied from 7.2 to 8.5 for the range of C/N values observed.

Experiment II

In this experiment we maintained a constant influent nutrient concentration ($50 \mu\text{M}$) at the four turnover rates

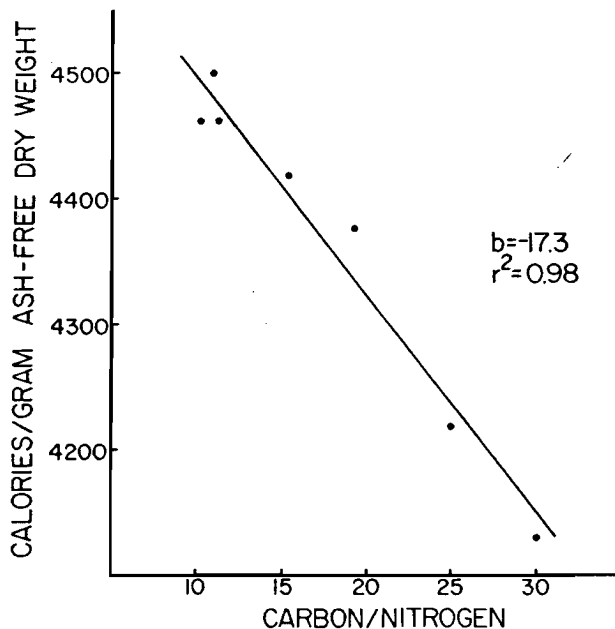


Fig. 7. Caloric value of *Gracilaria* as a function of carbon/nitrogen ratio.

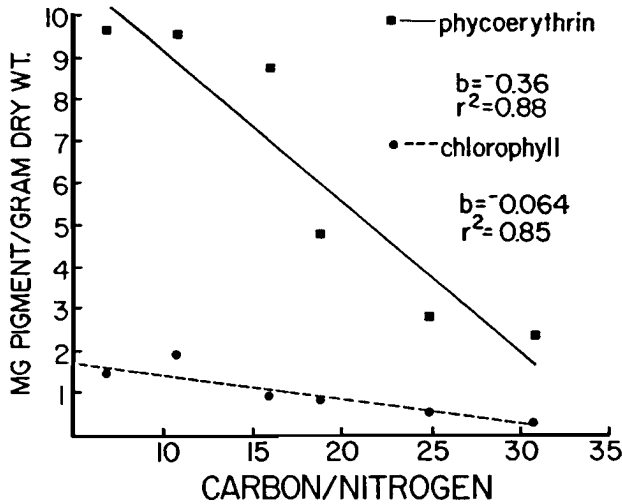


Fig. 8. Pigment content (phycoerythrin and chlorophyll) of *Gracilaria* as a function of carbon/nitrogen ratio.

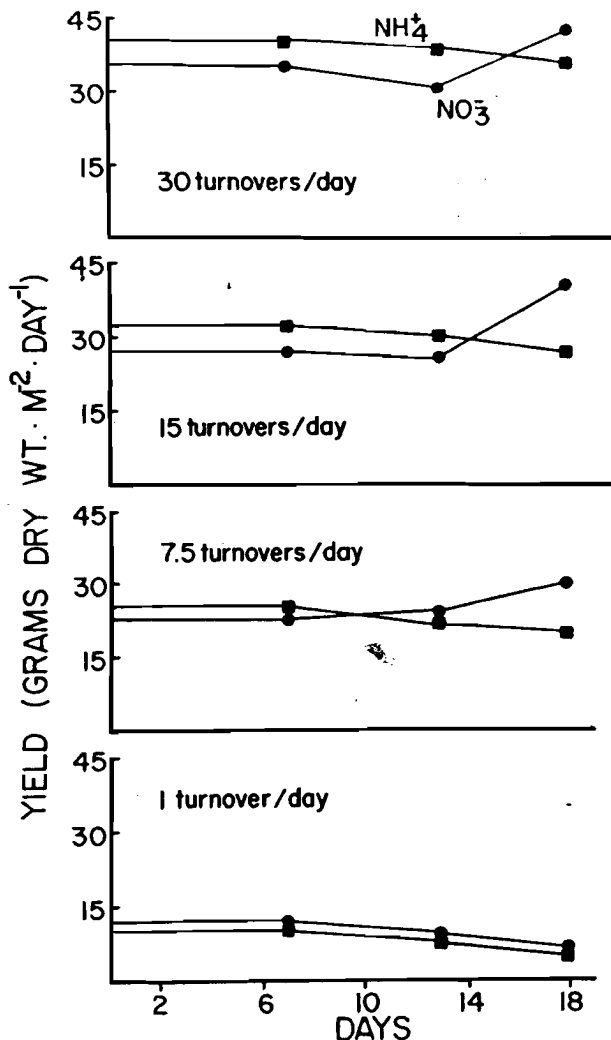


Fig. 9. In Experiment II, yield of *Gracilaria* cultured with two N-enriched media ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) at four turnover rates. Influent nutrient concentrations were held constant so that daily nitrogen loading was directly proportional to turnover rate.

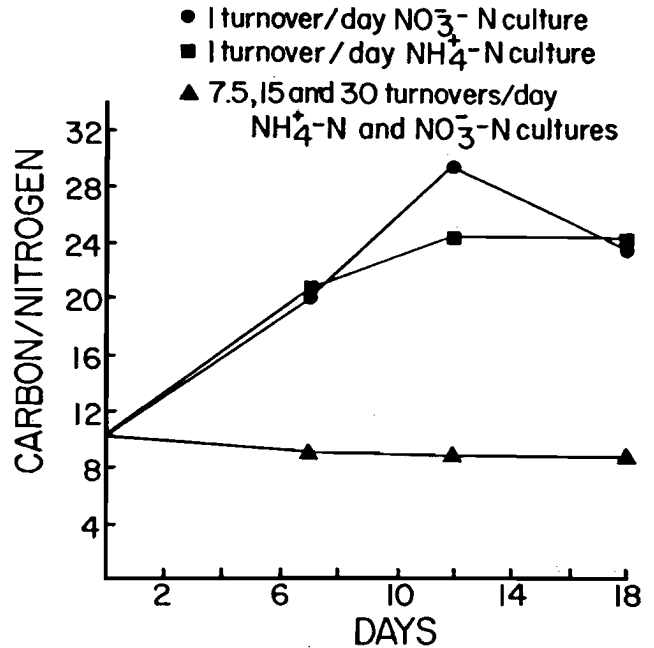


Fig. 10. In Experiment II, changes with time in carbon/nitrogen values of *Gracilaria* grown at different turnover rates and nutrient enrichment.

(1, 7.5, 15 and 30) with both NH_4^+ and NO_3^- treatments. In contrast to Experiment I, daily nitrogen loading to these eight cultures was directly proportional to turnover rate.

During this experiment, yield of *Gracilaria* consistently increased with increasing turnover rate (Fig. 9). In the 1 turnover/day treatment, algal yields decreased after the first 7 days of the experiment in both the NH_4^+ and NO_3^- treatments. At the higher turnover rates, however, yields of the NH_4^+ culture decreased slightly over the duration of the experiment, whereas the NO_3^- culture yields increased. Only at the end of this experiment (19th day) were large differences in yield between NH_4^+ and NO_3^- treatments observed (i. e. 7.5, 15 and 30 turnovers/day on day 18).

The initial C/N value of 10 of the *Gracilaria* increased to 24 in the NH_4^+ and NO_3^- cultures maintained at 1 turnover/day. All other treatments maintained a C/N value of ≈ 9 (Fig. 10).

Discussion

Changes in algal growth and C/N values

Decreased yields, increased C/N values and decreased N/P assimilation ratios in the seawater controls of Experiment I support the hypothesis that the maximum yield of *Gracilaria* was N-limited below a daily loading of ≈ 15 m moles/day of N. A C/N ratio greater than 10 indicated N-limitation in these experiments. Even the highest turnover seawater control (30/day) in Experiment I received ca. 8.6 m moles/day of N yet was N-

limited (C/N = 16). Additionally, in Experiment II, the low turnover cultures received only 2.5 m moles/day of N and were also N-limited (C/N = 24).

That the yield and C/N of the N-limited control cultures took up to 16 days to "level off" shows that relatively long term experiments are required to study seaweed-N relationships. Initially the *Gracilaria* probably utilized stored inorganic and amino acid N to support its relatively rapid growth in the unenriched seawater. Eventually, these reserves became depleted and algal yields reflected N-availability or flow rate. *Laminaria* can store nutrients in winter when inorganic nitrogen is available to support rapid growth in spring when nutrients are low but light is more favorable (Chapman and Craigie 1977). The ability of seaweeds to utilize stored N makes preconditioning, or at least adequate length of culture studies a necessity in algal-nutritional research. Otherwise, the resulting data could reflect the nutrient history of the plant rather than experimental treatments.

In addition to N, flow rate *per se* also limited algal growth. Although increasing growth with increasing flow rate in the unenriched controls is clearly a function of increased N availability, this was not the case in the enriched cultures of Experiment I and II. In these, all treatments maintained C/N values of ≤ 10 independent of turnover rate but yields increased with increasing turnover rate.

This growth-enhancing effect of flow rate, although not resolved in the present study, could be due to concomitant changes in CO₂ availability, pH, diurnal temperature variation, metabolite inhibition and micronutrient availability. Availability of CO₂ increases with increasing turnover rate and diurnal changes in pH from 8.4 at 0900 hrs to 9.3 at 1500 hrs in the enriched cultures at the lowest turnover (1/day) may reflect CO₂ limitation. If CO₂ assimilation (photosynthesis) outstrips CO₂ addition to the cultures, the aqueous bicarbonate reservoir will be depleted and the pH will rise accordingly. Additionally, the optimal pH for growth of *Gracilaria* may be nearer that achieved at high turnover rates (lower pH) than at low rates (higher pH). For example, Shacklock *et al.* (1973) found the growth of *Chondrus crispus* was greater at pH values of 7.3 to 7.8 than at 6.7 or 9.4. But the pH control in this experiment was achieved by CO₂ addition to the cultures. Consequently, either pH or the increased carbon (or both) actually stimulated the plants. However, addition of sodium bicarbonate, increasing total carbonate but not affecting pH, also stimulated the growth of *Chondrus*. Such CO₂ limitations are more common in freshwater natural systems and mass cultures (Goldman *et al.* 1972), but may also affect the growth of dense natural populations of seaweeds (Jackson 1977). Also, diurnal temperature variation was slightly greater at low turnover rates and may have contributed to lower growth. Micronutrient and vitamin limitation (Fries 1973) as well as autoinhibition by algal metabolites (Fogg 1966) would be minimized at high turnover rates

and could also be a significant factor. Lastly, culture mixing (i. e. shear), important in breaking down "diffusion transport limitation" of essential mineral nutrients in intense cultures (Gavis 1976), could also be affected by flow rate. But this was unlikely in view of the vigorous aeration that was provided to all cultures. Because the cost of pumping seawater will be a major operating expense in any large-scale seaweed culture (Huguenin 1976, Jackson 1977), further studies of maximizing algal yield under cost-saving culture conditions but minimizing adverse affects associated with decreased seawater flow rates should be a major avenue of research in the future.

Values of C/N provide an index of growth only when the plant is growing in log phase in N-limited or N-enriched conditions. For example, all enriched cultures in Experiment I had C/n values of ≈ 10 , whereas growth was extremely variable – a direct function of flow rate. In nature, algal growth could be limited by many other factors, especially genetic constraints (Chapman and Craigie 1977) and similarly uncouple correlation of C/N with growth rate. However, C/N values do provide an estimate of nitrogen limitation and in this study C/N values greater than 10 denoted N-limitation. Because seaweeds are usually attached, the C/N value is a useful "integrator" of past N levels in the water column and helps in assessing growth limitation in field studies. Yet, C/N values in general vary among red, green and brown algal phyla (Niell 1976) and within a phyla due to tissue differentiation. Thus a C/N value of 10 may not be a good estimate of N-limitation of macroalgae in general, but should be determined for specific algae through culture studies.

Nitrogen availability, by regulating growth rate, affects levels of protein and carbohydrate in seaweeds and is partially reflected by the N-limited C/N changes. When N limits growth, carbohydrate synthesis predominates and accounts for increased polysaccharide levels (Neish and Shacklock 1971, Dawes *et al.* 1974) and related high C/N values. During N-enriched exponential growth, protein synthesis predominates and is reflected by the high protein pigment levels (phycoerythrin) and chlorophyll in *Gracilaria*. These N-related growth phenomena are general biochemical principles (Fogg 1964) that should be considered in compromising between maximum algal yields and polysaccharide content for optimizing extract yield.

Although NH₄⁺ supported greater growth in these experiments, there was some indication of NH₄⁺ toxicity at the end of Experiment II. Conflicting data in the literature show both NO₃⁻ and NH₄⁺ support 'optimal' growth in various species of red algae (DeBoer *et al.* 1978, Fries 1963, Neish and Shacklock 1971, Yamada 1961). Ammonia was toxic at 60 μ M for *Ulva* (Waite and Mitchell, 1972) and 30 μ M for juvenile *Macrocystis* sporophytes (Haines and Wheeler, 1978). Even though influent concentrations of NH₄⁺ during Experiment II were constant

at ca. 50 μM , large daily loads in well mixed cultures, particularly if a significant fraction of the assimilated NH_4^+ was stored in the inorganic form could have caused toxicity.

Changes in Plant Composition

Percent dry weight and ash content correlated with C/N values, suggesting control by nitrogen uptake. However, these regressions were based on data from the N-limited control cultures with C/N values ranging from 16 to 31, and from the fast growing, enriched high turnover cultures with C/N values of ≈ 10 . Because the other enriched cultures (1, 7.5 and 15 turnovers/day) all had C/N values of 10 and also had ash contents ranging from 35 to 50% of dry weight, they do not fit the regression. This implies that ash content is a function of growth rate *per se*, and correlates well with N only when N limits growth.

Ash content influences the organic and caloric content of *Gracilaria*. N-limited and/or slow growing *Gracilaria* contains less ash and thus greater caloric content per unit dry weight. However, caloric content of the organic matter *per se* (on an ash-free dry weight basis) is higher in N-enriched plants, indicating a difference in organic storage products. Since N can limit the growth of seaweeds in coastal areas (Chapman and Craigie 1977), N may be a major factor controlling both temporal and spatial variations in growth of seaweeds and thus, variable ash and caloric content observed in natural populations of seaweeds (Himmelman and Carefoot 1977).

Changes in nitrogen availability can cause pigment alterations in *Gracilaria* (Lapointe *et al.* 1976) that are similar to pigment adaptations due to light intensity found in land plants. Changes in the ratio of accessory pigments i. e. phycoerythrin to chlorophyll (P/C), is used as an

indicator of complimentary chromatic adaptation for algae growing at different water depths and exposed to light of different spectral composition (Ramus *et al.* 1976, Moon and Dawes 1976). In contrast, increases in actual pigment concentrations is used to signify intensity-related pigment changes analogous to the "sun-shade" adaptation of land plants (Bjorkman 1973). Visually, N-starved plants from the controls in Experiment I (e. g. 1 turnover/day, C/N = 31) appeared straw colored whereas N-enriched plants (C/N = 10) appeared dark brown. The range and magnitude of P/C values over all the treatments in Experiment I is quite small (7.2 to 8.5) compared to the P/C values reported for *Eucheuma isiforme* chromatically adapting seasonally (Moon and Dawes 1976). Thus, the N-related pigment alterations in *Gracilaria* during Experiment I were increases in absolute levels of both phycoerythrin and chlorophyll – an adaptation of seaweeds first described by Oltmann (1891). Functionally, increased pigment levels in N-enriched *Gracilaria* increases the photon gathering "antennae", allowing these plants to achieve some of the highest yields and solar conversion efficiencies reported for outdoor algal cultures or fast growing food crops such as sugar and rice (Lapointe *et al.* 1976).

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