J. exp. mar. Biol. Ecol., 1981, Vol. 53, pp. 135–152
Elsevier/North-Holland Biomedical Press

EXPERIMENTAL OUTDOOR STUDIES WITH *ULVA FASCIATA* Delile. I. INTERACTION OF LIGHT AND NITROGEN ON NUTRIENT UPTAKE, GROWTH, AND BIOCHEMICAL COMPOSITION

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Abstract: The effects of algal density, light adaptation, and interaction of light and nitrogen on nutrient uptake, growth, and biochemical composition of *Ulva fasciata* Delile were investigated in outdoor continuous cultures.

With increasing plant densities, specific growth rates decreased exponentially from 0.36 to 0.02 doublings $\cdot$ day^{-1}. In contrast, yields increased to a maximum of 4.7 g C $\cdot$ m^{-2} $\cdot$ day^{-1} at the intermediate density and then decreased.

Plants grown under low (62 ly $\cdot$ day^{-1}) and high (324 ly $\cdot$ day^{-1}) light conditions were subsequently exposed to medium light (282 ly $\cdot$ day^{-1}). Initially, plants from low light conditions had twice the chlorophyll *a* content and yield than plants from high light conditions. Within 1 wk, all plants showed no difference with regard to chlorophyll *a* and yield.

Growth of *U. fasciata* did not saturate up to the highest light intensities used (255 ly $\cdot$ day^{-1}). Nitrogen (NO_3^-) additions at low light did not affect growth but at high light there was increasing growth with increasing nitrogen. This interaction between light and nitrogen was apparent when growth was measured as specific growth rate (doublings $\cdot$ day^{-1}), activity coefficient (mg C $\cdot$ g C^{-1} $\cdot$ day^{-1}) and yield (g C $\cdot$ m^{-2} $\cdot$ day^{-1}). These growth measures all significantly correlated with %C and ash content but not with %N, the C : N or C : Chl *a* ratios. Photosynthetic rate (g C $\cdot$ g Chl *a*$^{-1}$ $\cdot$ day^{-1}) showed no interaction between light and nitrogen because it corrected for increases in chlorophyll *a* content caused by nitrogen additions. This growth measure correlated with %N and the C : N and C : Chl *a* ratios as well as %C and ash content. Photosynthetic efficiency of *U. fasciata* was positively correlated with %N and chlorophyll *a* content and was affected by light intensity only at low nitrogen levels. Uptake of NO_3^- by *U. fasciata* depended mostly on the daily loadings of this nutrient, but was also inversely proportional to light intensity.

INTRODUCTION

Compared to phytoplankton, little work has focused on the effects of environmental factors on growth and biochemical composition of seaweeds. As with phytoplankton, factors that limit growth of seaweeds include nitrogen (Chapman & Craigie, 1977; Hanisak, 1979; Lapointe & Ryther, 1979) and light (Ryther, 1956; Kanwisher, 1966; Brinkhuis & Jones, 1974). Although both factors interact in affecting growth (Maddux & Jones, 1964) and nutrient uptake (MacIsaac & Dugdale, 1972) of phytoplankton, virtually no data exist that relate growth, nutrient uptake, and biochemical composition of seaweeds to interaction of nitrogen and light at natural levels. Similarly, effects of light adaptation (Ryther & Menzel, 1959) and algal density (Tamiya, 1957) have not been well studied with seaweeds.

0022-0981/81/0000-0000/$ 2.50

Tissue contents of carbon, nitrogen, and chlorophyll *a* are particularly important to the growth and utilization of seaweeds. For example, the chlorophyll content may be an index of photosynthetic capacity (Ramus *et al.*, 1976a) whereas nitrogen content ultimately determines the nutritive value of seaweed detritus to deposit-feeding food chains (Tenore & Rice, 1980). Ratios of carbon : nitrogen (C : N) and carbon : chlorophyll *a* (C : Chl *a*) have been used widely in phytoplankton growth studies (Myers & Graham, 1971; Thomas & Dodson, 1972; Caperon & Meyer, 1972; Yoder, 1979) and could be useful in understanding growth of seaweeds in culture or field studies.

Therefore, we investigated the effects of: (1) algal density on growth, (2) light adaptation on growth and chlorophyll *a* content, and (3) interaction of light and nitrogen on nutrient uptake, growth, and biochemical composition of *Ulva fasciata* Delile in medium-size outdoor continuous cultures.

MATERIALS AND METHODS

CULTURE SYSTEM: DESIGN AND OPERATION

Outdoor culture chambers (481, 0.22 m^2; Fig. 1) were modified from those described by Lapointe & Ryther (1979) and used in all experiments. Each chamber received 5-μm filtered, temperature-regulated sea water (Tenore & Huguenin, 1973) metered by PVC chemcock valves (500 ml $\cdot$ min^{-1}; retention time of 15 chamber volumes/day) and mixed by oil-free compressed air.

Concentrated medium containing nitrogen ($NaNO_3$) and phosphorus ($Na_2H_2PO_4$) at an atomic ratio of 6.5 : 1 of N : P was metered at different flow rates by single-channel peristaltic pumps to provide different levels of nitrogen loading (N loading),

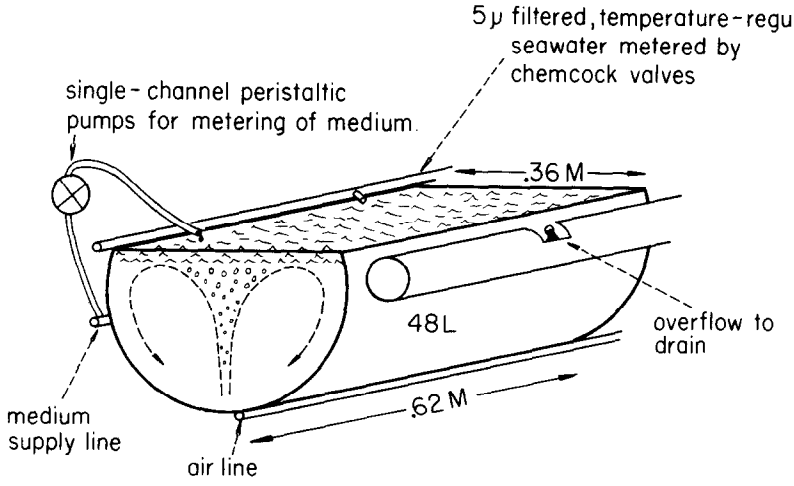

Fig. 1. Diagram of seaweed culture chambers.

i.e. (NO_3^-) × flow rate. Because nitrogen limits growth of seaweeds (Chapman & Craigie, 1977; Hanisak, 1979; Lapointe & Ryther, 1979), we were interested primarily in the effects of this nutrient. Phosphorus was added only to maintain an atomic N:P ratio similar to that of the incoming sea water. Light levels were obtained with different layers of neutral-density screening. Daily solar radiation was determined with a weather measure star R413 pyranometer. Salinity was measured daily with an optical refractometer and ranged from 16 to 20‰ during all experiments.

To measure growth and maintain biomass of *Ulva* at desired levels during these experiments, the cultures were periodically (e.g. 3- to 4-day intervals) weighed and harvested back to the initial plant density. Based on an initial culture density experiment, a 75 g (wet wt) inoculum was used in the subsequent experiments as

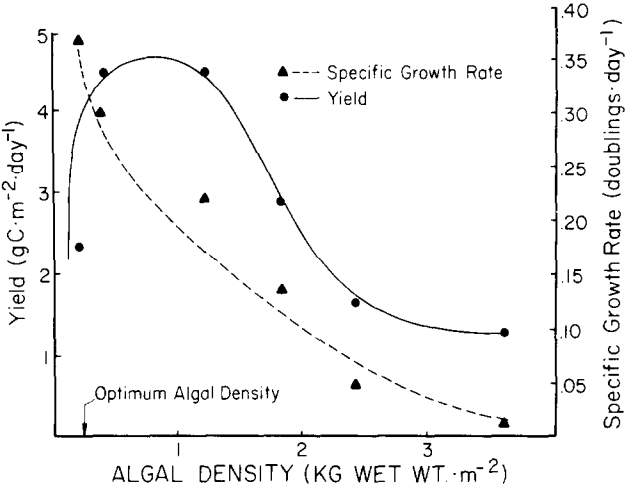

Fig. 2. Specific growth rate and yield of *Ulva lactuca* as a function of algal density.

this density maximized growth of *Ulva* (see Fig. 2). Increases in damp-dried wet weight over time were used to calculate specific growth rates as:

$$\mu = 1/t \cdot \log_2 (N_0 + \Delta N/N_0) ,$$

where N_0 is the initial biomass and ΔN is the change in biomass with time (t). These biomass changes over time were also used with results of tissue analyses (e.g. % dry weight, %C, chlorophyll *a*) to express growth as yield (g C · m^{-2} · day^{-1}), activity coefficient (mg C · g C^{-1} · day^{-1}) and phytosynthetic rate (g C · g Chl a^{-1} · day^{-1}) (Vollenweider, 1974). Photosynthetic efficiency was determined using an empirical factor of 11.4 g · cal · mg C^{-1} to convert carbon to calories (Platt & Irwin, 1973):

$$PE = \frac{11.4\ (g \cdot cal \cdot mg\ C^{-1}) \times \text{photosynthetic production}\ (mg\ C \cdot m^{-2} \cdot day^{-1})}{\text{total incident solar radiation}\ (g \cdot cal \cdot m^{-2} \cdot day^{-1})} .$$

TISSUE AND SEA-WATER ANALYSES

Tissue analyses for nitrogen, carbon, and C:N ratio were determined using a Perkin-Elmer 240 Elemental Analyzer. Fresh *Ulva* was dried at 90 °C for 48 h to determine dry weight and combusted at 475 °C for 8 h to determine ash content. Chlorophyll *a* was determined on fresh *Ulva* by extraction techniques described previously (Lapointe & Ryther, 1979). Concentrations of NO_3^- (Wood *et al.*, 1967), NH_4^+ (Solorzano, 1969), and PO_4^{3-} (Murphy & Riley, 1962) were determined on incoming sea water, medium, and culture effluents (i.e. residual concentrations).

EFFECT OF ALGAL DENSITY

Ulva lactuca Linnaeus was grown at plant densities ranging from 0.13 to 4.5 kg wet wt $\cdot m^{-2}$. The high sea-water turnover rates used in these experiments minimized the possibility of metabolite inhibition that may be associated with dense seaweed cultures (Lapointe & Ryther, 1979). Because of similar morphology, chloroplast structure, and photophysiology, we assumed that growth results obtained with *U. lactuca* are applicable to *U. fasciata*. Specific growth rates and yields were measured over a 14-day period. All cultures received enriched sea water that maintained non-limiting residual concentrations of NO_3^- and PO_4^{3-}. Incident light averaged 283 ly $\cdot$ day^{-1} and temperature was maintained at 17 $\pm$ 1 °C.

In situ light levels were calculated as a function of algal density using the equation:

$$\bar{I} = \frac{I_0}{kz}(1 - e^{-kz}),$$

where $\bar{I}$ is the light intensity at the average depth of the culture, I_0 the incident light intensity, k the extinction coefficient for chlorophyll *a* (Dubinsky & Berman, 1979) and z the average depth of the culture chambers.

EFFECT OF LIGHT ADAPTATION

Cultures (two replicate chambers per treatment) were maintained on unenriched sea water and grown under low (62 ly $\cdot$ day^{-1}) and high (324 ly $\cdot$ day^{-1}) light for a period of 10 days; subsequently, both were exposed to medium light levels (282 ly $\cdot$ day^{-1}) for 11 days. Yield, chlorophyll *a*, and incident light were monitored during the experiment. Temperature was maintained at 14 $\pm$ 1 °C.

INTERACTIVE EFFECTS OF LIGHT AND NITROGEN

A factorial design (3 light levels × 3 nitrogen levels × 2 replicate chambers) was used to study the interactive effects of light and N loading. *U. fasciata* was grown under different levels of light (255, 137 and 49 ly $\cdot$ day^{-1} i.e., 100, 54, and 19% of incident light, respectively) and N loading (10.1, 5.5 and 1.0 mmol $\cdot$ day^{-1}). The low

N loading was unenriched sea water. Temperature was maintained at 14 ± 1 °C. Water samples of the incoming sea water, medium and culture effluents were taken at midday twice during the experiment for determination of residual nutrient concentrations and uptake of NO_3^-. Because of the short generation time of *Ulva* (Smith, 1947) and the possibility of induced sporulation by increased nitrogen levels (Boney, 1966), the plants were removed and weighed after 7 days. Upon termination, *Ulva* from the initial stock and from the treatments was assayed for chlorophyll *a*, dry wt, ash, carbon, and nitrogen. Simple analysis of variance and orthogonal single degree of freedom tests of significance were used to detect main effects and interaction of light and nitrogen on the yield and chlorophyll *a* content. Simple linear regression and tests for significance of slopes were used to correlate growth and biochemical composition.

RESULTS

EFFECTS OF ALGAL DENSITY ON SPECIFIC GROWTH RATE AND YIELD

Specific growth rate of *U. lactuca* decreased from 0.36 to 0.02 doublings · day^{-1} in cultures when density increased from 0.2 to 3.6 kg wet wt · m^{-2} (Fig. 2). Expressed in terms of light intensity, specific growth rates increased linearly ($r = 0.98$, $b = 0.186$) up to the highest in situ light level (203 ly · day^{-1}) that occurred in cultures with the lowest plant density (Fig. 3). In contrast, yields increased with increasing density up to a maximum of 4.6 g C · m^{-2} · day^{-1} at a density of 0.8 kg wet wt · m^{-2}. Above that plant density, yields decreased with increasing biomass (Fig. 2).

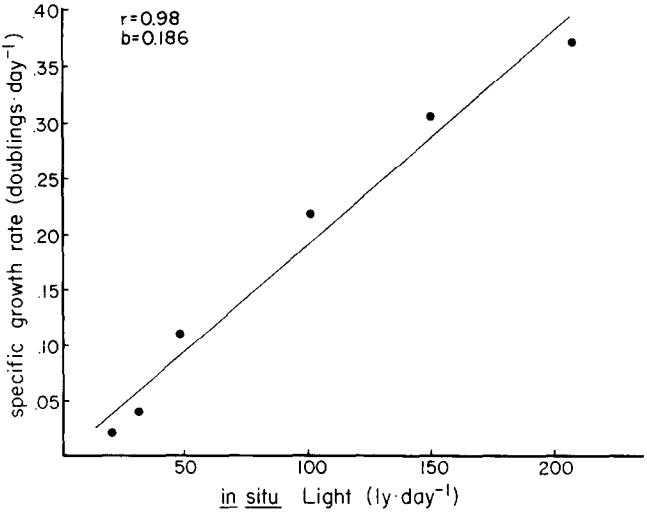

Fig. 3. Specific growth rate of *Ulva lactuca* as a function of in situ light intensity.

EFFECT OF LIGHT ADAPTATION ON YIELD AND CHLOROPHYLL *a* CONTENT

When grown under low light (62 ly · day^{-1}) for 10 days, *U. fasciata* contained twice the chlorophyll *a* than that grown under high light (324 ly · day^{-1}) (Fig. 4).

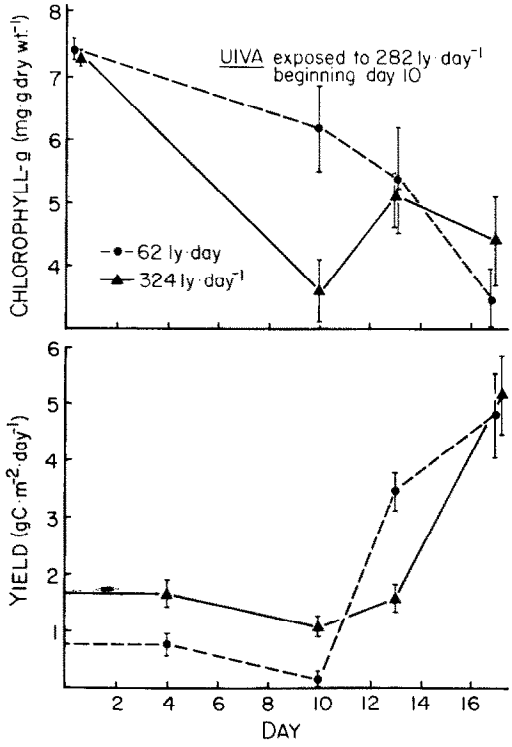

Fig. 4. Effect of light adaptation on yield and chlorophyll *a* content of *Ulva fasciata*: values are given as means ± 1 SD based on two observations.

When exposed to medium light, the yield of plants preadapted to low light was twice the yield of plants preadapted to high light for the 3 days following the adaptation period. After 7 days, the chlorophyll *a* content and yields of both treatments were similar.

INTERACTIVE EFFECTS OF LIGHT AND NITROGEN ON GROWTH, BIOCHEMICAL COMPOSITION AND NUTRIENT UPTAKE

There was significant interaction ($P < 0.01$) of light and nitrogen on the yield of *U. fasciata* (Table I). Overall, yield increased mostly due to increasing light (Fig. 5). At low light (49 ly · day^{-1}) there was no increase in yield with increasing N loading from 1.0 to 10.1 mmol · day^{-1}. At the medium light level (137 ly · day^{-1}) yield

increased linearly with increasing nitrogen; but at the highest light level (255 ly · day^{-1}) yield did not increase above a N loading of 5.5 mmol N · day^{-1}.

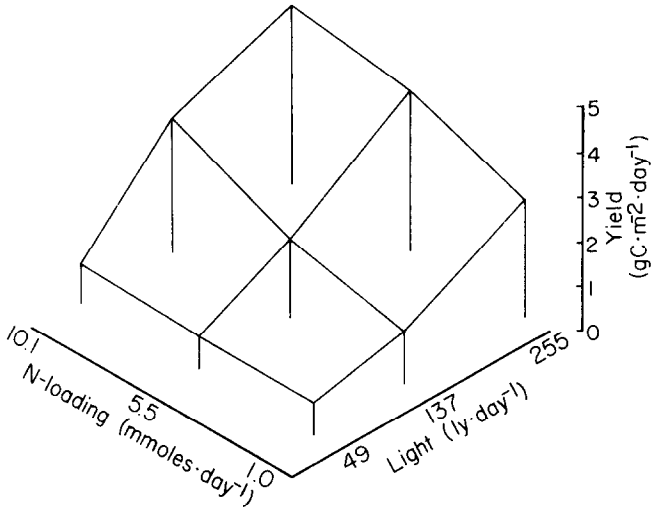

Fig. 5. Yield of *Ulva fasciata* cultured under different light and N loading conditions.

Light and nitrogen had similar interactive effects on the biochemical composition of *U. fasciata* (Table I). Chlorophyll *a* content decreased with increasing light but within each light level increased with increasing N loading (Fig. 6). Consequently, chlorophyll *a* correlated positively with %N ($r = 0.89$); and negatively with C : N ratio ($r = -0.93$). C : N and C : Chl *a* ratios showed a similar interaction, decreasing

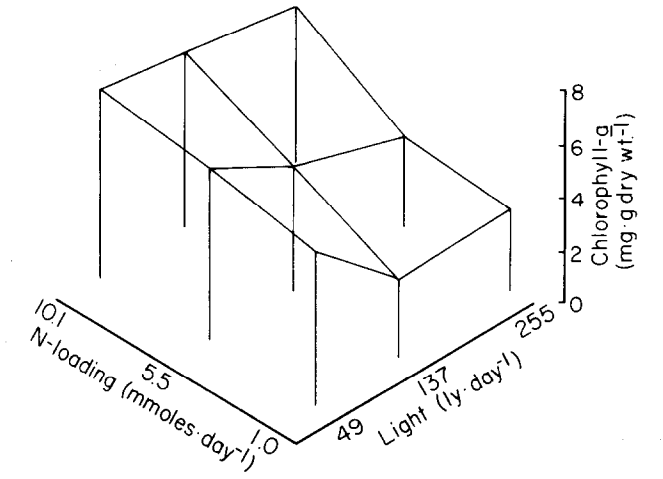

Fig. 6. Chlorophyll *a* content of *Ulva fasciata* cultured under different light and N loading conditions.

TABLE I

Biochemical composition and growth measurements of *Ulva fasciata* cultured under different light and N loading

Treatments[a]	%N[b]	%C[b]	C : N[b]	%Ash[b,c]	Chl *a* (mg · g dry wt^{-1})	Carbon : Chl *a*
Initial	4.16 ± 0.12	31.3 ± 0.5	7.5 ± 0.1	25	6.5 ± 0.4	48 ± 0
49 – 1.0	3.93 ± 0.04	33.4 ± 1.1	8.5 ± 0.2	26	5.8 ± 0.2	58 ± 4
49 – 5.5	4.32 ± 0.09	33.6 ± 0.4	7.8 ± 0.1	26	6.5 ± 0.2	52 ± 3
49 – 10.1	4.44 ± 0.13	33.9 ± 0.4	7.7 ± 0.2	25	6.9 ± 0.3	49 ± 2
137 – 1.0	3.40 ± 0.02	35.5 ± 0.4	10.4 ± 0.2	23	2.9 ± 0.3	124 ± 12
137 – 5.5	4.10 ± 0.00	35.7 ± 0.3	8.7 ± 0.1	23	4.7 ± 0.4	77 ± 7
137 – 10.1	4.47 ± 0.03	35.2 ± 0.2	7.9 ± 0.1	24	6.4 ± 0.4	55 ± 5
255 – 1.0	2.99 ± 0.03	35.5 ± 0.7	11.9 ± 0.0	22	3.0 ± 0.1	116 ± 2
255 – 5.5	3.54 ± 0.22	35.8 ± 1.4	10.1 ± 0.2	22	3.4 ± 0.1	107 ± 4
255 – 10.1	4.65 ± 0.22	37.3 ± 0.1	8.0 ± 0.4	21	5.7 ± 0.1	65 ± 0

[a] Light (ly · day^{-1}) – N loading (mmol · day^{-1}).
[b] Dry weight basis.
[c] SD < 0.8.

with increasing N loading within each light level and increasing with increasing light.

Yield (g C · m^{-2} · day^{-1}), activity coefficient (mg C · g C^{-1} · day^{-1}) and specific growth rate (μ, doublings · day^{-1}) all correlated positively with %C ($0.82 \leqslant r \leqslant 0.85$), negatively with %ash ($-0.76 \leqslant r \leqslant -0.78$) and were themselves highly correlated ($r \geqslant 0.98$) (Table I). These growth parameters also correlated with the %N of *U. fasciata* as a function of light level (Fig. 7). For example, yield was not significantly correlated with %N at low light levels ($r = 0.31$) but showed a significant correlation at medium and high light levels ($r = 0.98$ and 0.75, respectively).

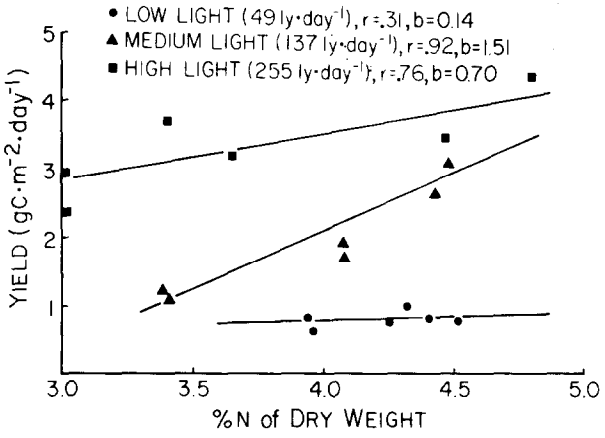

Fig. 7. Relationship of yield to %N of *Ulva fasciata* at different levels of light.

conditions: values are given as means ±1 SD based on two observations.

Specific growth rate, μ (doublings · day^{-1})	Activity coefficient (mg C · g C^{-1} · day^{-1})	Photosynthetic rate (g C · g Chl a^{-1} · day^{-1})	Yield (g C · m^{-2} · day^{-1})	Photosynthetic efficiency
–	–	–	–	–
0.06 ± 0.01	41 ± 4	2.4 ± 0.1	0.7 ± 0.1	1.7 ± 0.1
0.06 ± 0.00	41 ± 1	2.2 ± 0.2	0.8 ± 0.1	1.8 ± 0.1
0.07 ± 0.01	44 ± 6	2.2 ± 0.4	0.9 ± 0.4	2.1 ± 0.4
0.09 ± 0.01	58 ± 6	7.1 ± 0.1	1.2 ± 0.1	1.0 ± 0.0
0.11 ± 0.01	73 ± 7	5.6 ± 1.0	1.8 ± 0.3	1.5 ± 0.2
0.15 ± 0.00	101 ± 2	5.6 ± 0.5	2.9 ± 0.3	2.4 ± 0.3
0.15 ± 0.01	106 ± 5	12.0 ± 1.3	2.7 ± 0.5	1.2 ± 0.2
0.18 ± 0.02	115 ± 12	12.3 ± 0.9	3.5 ± 0.4	1.6 ± 0.2
0.18 ± 0.02	117 ± 10	7.6 ± 0.6	3.9 ± 0.6	1.8 ± 0.4

The slopes of the medium and high light regressions (Fig. 7) are significant ($P < 0.05$), but the low light regression is not ($P > 0.05$). Correlations between yield and C : N ratio are similar to that of yield and %N because of the strong correlation between %N and C : N ratio ($r = 0.96$).

Photosynthetic rate (g C · g Chl a^{-1} · day^{-1}) of *U. fasciata* followed a slightly different trend than the other three growth measures (Table I). This growth measure correlated with nitrogen-related variables such as C : N ratio ($r = 0.79$), C : Chl a ratio ($r = 0.80$), and Chl a ($r = 0.80$) as well as %C ($r = 0.69$) and the other three growth measures ($0.80 \leqslant r \leqslant 0.83$). Interactive effects of light and nitrogen were insignificant ($P \geqslant 0.05$) on photosynthetic rate (Fig. 8).

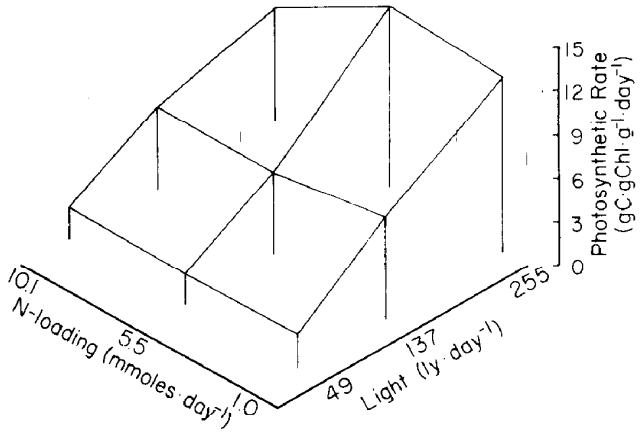

Fig. 8. Photosynthetic rate of *Ulva fasciata* cultured under different light and N loading conditions.

Photosynthetic efficiency, a measure of the conversion of light energy into chemical energy (see Table I) correlated positively with chlorophyll *a* ($r = 0.87$) and %N ($r = 0.81$). Photosynthetic efficiency decreased with increasing light at low levels of N loading, but was unaffected by light at medium and high nitrogen levels.

TABLE II

Concentrations (μM) and uptake (μmol · g dry wt^{-1} · day^{-1}) of NO_3^--N during the growth of *Ulva fasciata* under different light and N loading conditions: values are given as means ± 1 SD based on four midday observations.

	Light		
	Low (49 ly · day^{-1})	Medium (137 ly · day^{-1})	High (255 ly · day^{-1})
Low (1.0 mmol · day^{-1})	1.4 ± 0.4[a] 0.6 ± 0.4[b] 51.0 ± 5.7[c]	1.4 ± 0.4 0.3 ± 0.1 60.0 ± 1.4	1.4 ± 0.4 0.3 ± 0.1 50.0 ± 4.2
Medium (5.5 mmol · day^{-1})	7.9 ± 0.0 0.8 ± 0.2 416.7 ± 38.9	7.9 ± 0.0 0.9 ± 0.3 336.0 ± 22.6	7.9 ± 0.0 0.6 ± 0.1 279.0 ± 14.1
High (10.1 mmol · day^{-1})	14.2 ± 0.0 2.9 ± 0.7 640.0 ± 29.7	14.2 ± 0.0 2.7 ± 0.9 457.5 ± 60.1	14.2 ± 0.0 2.4 ± 0.9 439.0 ± 38.2

[a] Influent.
[b] Residual.
[c] Uptake rate.

As illustrated in Table II, the level of N loading was the most important factor affecting uptake of NO_3^- and residual NO_3^- concentrations. Both uptake rate and residual concentrations consistently increased with N loading up to maximum values in the high N loading treatments. Yield (or growth) of *Ulva* showed no overall correlation with NO_3^- residual concentrations although there was correlation within certain light levels (Fig. 9). For example, at low light levels, there was no increase in yield up to NO_3^- concentrations of ≈ 3 μM. At medium light levels, however, yield increased linearly with increasing NO_3^- concentrations up to ≈ 3 μM. At high light, yields increased up to NO_3^- concentrations of ≈ 1 μM. Above 1 μM, further increases in NO_3^- concentration did not affect yield. Similarly, yield showed no overall correlation with uptake rate of NO_3^- (Fig. 10). There was significant correlation at medium and high light levels ($r = 0.85$ and 0.76, respectively) but not at low light ($r = 0.49$). The slope of the low light regression was not significant ($P > 0.05$).

However, at medium and high levels of N loading, the uptake rate of NO_3^- was inversely proportional to light level (Table II). For example, the rate of uptake at low light, high N loading (640.1 ± 29.7 μmol · g dry wt^{-1} · day^{-1}) was significantly

greater ($P < 0.05$) than that at high light, high N loading (439.0 ± 38.2 μmol · g dry wt^{-1} · day^{-1}).

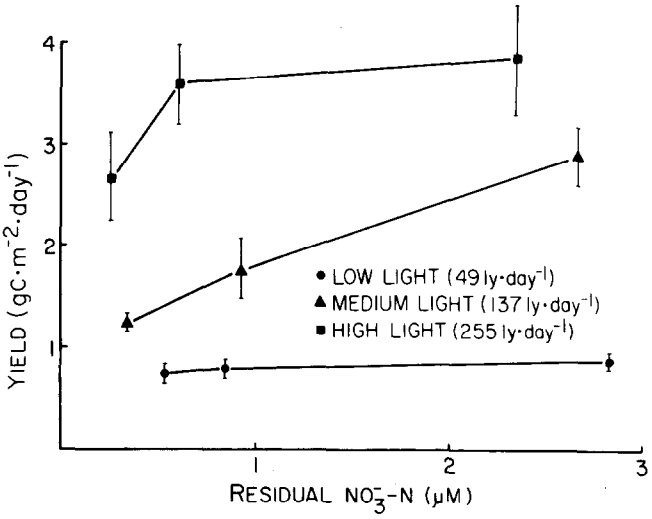

Fig. 9. Relationship of yield of *Ulva fasciata* to residual NO_3^- concentration at different levels of light.

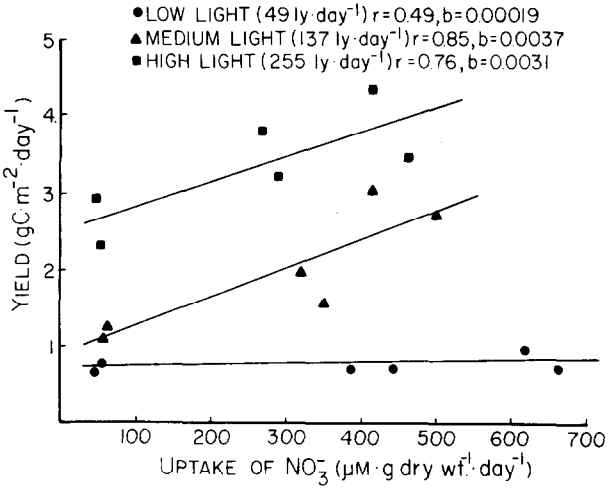

Fig. 10. Relationship of yield to uptake rate of NO_3^- by *Ulva fasciata* at different levels of light.

DISCUSSION

This study is one of the few that addresses population growth of seaweeds, measured by biomass change over time, under controlled but relatively natural conditions. Most seaweed studies in the past have been relatively short term and have used small algal samples in standing flask cultures with artificial light. Typically, such studies measure "apparent photosynthesis" using O_2-evolution techniques. Because algal growth is a summation of all metabolic processes, O_2 evolution cannot always be equated to growth (Merril & Waaland, 1979). In addition, several species of seaweeds including *Ulva lactuca* have diurnal rhythms in photosynthetic activity (Mishkind *et al.*, 1979; Ramus & Rosenberg, 1980) that introduce error when one extrapolates short-term O_2 measurements to long-term growth rates. Also, nutrients, whose growth effects on seaweeds are concentration dependent, cannot be studied adequately in batch cultures where nutrient concentrations continuously change (Caperon & Meyer, 1972).

Measuring growth of seaweeds by biomass change in outdoor continuous cultures eliminates many of these problems and, like phytoplankton continuous culture studies, allows more quantitative results. A major difference between phytoplankton and seaweed continuous cultures is that true steady-state conditions cannot be achieved with seaweeds. This is because seaweed biomass is always retained in the culture chamber whereas the dilution rate of phytoplankton cultures can be adjusted to give steady-state conditions where cell density remains constant. Thus, in addition to the direct effects of light intensity and nitrogen concentration, the effects of algal density and light adaptation on growth, both of which change with time in cultured and natural populations of seaweeds, needs to be considered.

EFFECTS OF ALGAL DENSITY ON GROWTH RATE AND YIELD

Algal density, by controlling light levels, strongly affects growth of *Ulva* but its effects are dependent on the measure of growth used. Specific growth rate or activity coefficient, measures of population growth, decrease with increasing density due to self-shading that reduces light levels. This is especially obvious in algae with high photosynthetic efficiencies such as *Ulva* or *Chorella* (Tamiya, 1957). In contrast, yield initially increases to a maximum value at intermediate densities where the algal biomass is sufficient to fully exploit incoming light. For example, maximum yields with *Ulva* occurred at algal densities that had sufficient chlorophyll ($\approx 500\ mg \cdot m^{-2}$) for maximum light utilization in aquatic systems (Margalef, 1974). At lower plant densities, incoming light is not completely exploited; at higher plant densities, self-shading reduces yields. The dense cultures of *Ulva*, although grown under non-nutrient limiting conditions and bright sunlight, stopped growing not because photosynthesis had ceased but because of the high "respiratory loss" of the total population. Light penetrates less and is selectively absorbed so that

photosynthesis does not offset total respiration, thus lowering the photosynthesis: respiration ratio and net growth. In natural populations of *Ulva*, growth rate would be greatest during the early stages of development when algal densities are low whereas yield would become maximum only after sufficient biomass has accumulated. Although specific growth rate and activity coefficient are convenient for measuring growth of small populations of plants in physiological studies, yield is the best growth measure for studying energetics in aquacultural or field studies. Because all these variables are highly density dependent, activity coefficient, which normalizes growth per unit biomass, is obviously the best for comparative purposes when different plant densities are used.

EFFECT OF LIGHT ADAPTATION ON CHLOROPHYLL *a* AND YIELD

Results presented here and by others (Beale & Appleman, 1971; Durbin, 1974; Ramus *et al.*, 1976b) show that algae grown under low light increase their chlorophyll content. In the present study, both chlorophyll *a* content and yield varied by a factor of two between the high- and low-light preadapted plants. Our results support those of Ramus *et al.* (1976a) that the growth capacity of *Ulva* is regulated by its chlorophyll content, and chlorophyll, even at different concentrations, functions with similar efficiencies. Because this light-adaptation in *Ulva* is time dependent (≈ 3 days), an adequate acclimation period for this plant would help eliminate pigment differences that affect its growth capacity.

EFFECTS OF LIGHT AND NITROGEN ON GROWTH AND BIOCHEMICAL COMPOSITION

The high light-saturation intensity (I_s) of *Ulva* combined with secondary growth effects of nitrogen enrichment suggests that light may often be the major growth-limiting factor for this alga. In the present study, growth of *U. fasciata* did not saturate up to the highest incident light intensity of 255 ly $\cdot$ day^{-1} (in situ light intensity of 203 ly $\cdot$ day^{-1}); however, this maximum represented full winter light and it was impossible to look at higher intensities to determine the true I_s for *U. fasciata*. The growth of *U. lactuca* saturates at light levels equivalent to ≈ 432 ly $\cdot$ day^{-1} (assuming a 12:12 light:dark cycle; Ramus, 1978). Such light levels represent nearly the maximum values of solar radiation that could be available to *Ulva* growing subtidally. The biochemical composition (%N, %C, C:Chl *a*) of the initial sample of *U. fasciata*, collected in the Indian River near Titusville, Florida, U.S.A., is comparable to that of our low light, high N loading treatment (see Table I). This supports the idea of severe light-limitation for the Indian River population of *U. fasciata* during the winter.

That the photosynthetic efficiency of *Ulva* decreased with increasing light at low nitrogen levels is probably due to the low chlorophyll *a* levels of these plants. Low chlorophyll *a* levels reduce the efficiency of the light reactions in *Ulva*, one of two major components that determines photosynthetic efficiency of an alga

(Goldman, 1979). At higher nitrogen levels, adequate chlorophyll *a* levels allow for efficient light reactions so that photosynthetic efficiency of these plants is then controlled by I_s, the other major component of photosynthetic efficiency. The photosynthetic efficiencies of *U. fasciata* in this study under high N loading are comparable to maximum values for primary production in coastal waters (Ryther, 1962) and explain the blooms that occur in high light, high nitrogen environments (Sawyer, 1965).

Two factors cause strong interaction of light and nitrogen on the growth of *U. fasciata*. First, nitrogen additions are more growth enhancing in high light, fast growing treatments due to increased demand for nitrogen as compared to the lower light, slower growing plants. Second, chlorophyll content, which increased with increasing nitrogen loading, affects the growth capacity of *Ulva* so that nitrogen additions are more growth enhancing under high than low light conditions. For example, the interaction is obvious only when growth is expressed as specific growth rate, activity coefficient or yield. Use of photosynthetic rate largely eliminates this interaction because it normalizes growth on a unit chlorophyll basis.

The interactive effects of light and nitrogen on algal growth are probably common in natural situations. In general, N limitation is not as important under low light as it is during high light periods. Variations in light due to self-shading, water transparency, and local weather patterns will continuously change the nature of these interactions over short time periods (e.g., days, weeks) and is a notable problem in modeling the growth of seaweeds (Seip *et al.*, 1979). However, long-term average values for light and nutrients vary seasonally and may regulate seasonal growth, and more importantly, production. For example, in situ N limitation has been reported for red (Lapointe & Ryther, 1979), green (Hanisak, 1979) and brown (Topinka & Robbins, 1976; Chapman & Craigie, 1977) algae only during summer when seasonal light is maximum and nitrogen levels are low.

Light and nitrogen also had interactive effects on the biochemical composition of *U. fasciata*. Increasing light increased whereas increasing N loading decreased the C : N and C : Chl *a* ratios in *U. fasciata*. A similar increase in the C : Chl *a* ratio due to increasing light was found for *Chlorella* (Myers & Graham, 1971) and several studies have reported decreasing C : N ratios with increasing nitrogen for seaweeds (D'Elia and DeBoer, 1978; Hanisak, 1979; Lapointe & Ryther, 1979). Increased carbohydrate production is associated with high light intensities (Cook, 1963) as are reduced chlorophyll levels (Durbin, 1974), both of which increase the C : Chl *a* and C : N ratios. Although these ratios are related to algal growth (Tett *et al.*, 1975), they correlated with growth in our study as a function of light level, showing good correlation only when nitrogen limited growth in medium and high light treatments. This agrees with earlier studies that show C : N ratios of seaweeds correlate with growth only under N limited conditions (Lapointe & Ryther, 1979). Because nitrogen can often limit seaweed growth in coastal areas (Topinka & Robbins,

1976; Chapman & Craigie, 1977; Hanisak, 1979), these ratios could be used to study seasonal growth patterns, but only when nitrogen limits growth. However, our results suggest that %C and ash content have a good overall correlation with growth rate and are better than the C:N and C:Chl *a* ratios in reflecting the physiological activity of *Ulva*, especially in an environment where growth-limiting factors continuously change. This may be because %C and ash content of *Ulva* are controlled primarily by light intensity, which appears to be the most important environmental variable affecting growth rate. In other words, %C and ash content are physiologically controlled (i.e., light), whereas the C:N and C:Chl *a* ratios are affected by the aqueous chemical environment (i.e., nitrogen) of *Ulva* as well as its physiology.

The primary role of light in regulating growth and biochemical composition of *Ulva* may also bear important considerations for growth studies with phytoplankton. Recent studies concerning the influence of growth rate on chemical composition of phytoplankton (Caperon & Meyer, 1972; Goldman *et al.*, 1979) have stressed mostly nutrient effects. Typically, these studies are conducted in continuous cultures maintained under growth-saturating irradiation. Based on our results, such studies at high light may unduly overemphasize the importance of nutrients in controlling phytoplankton growth and composition in nature, where light is most often the limiting factor (Ryther, 1956). Besides controlling growth rates, light also strongly affects the cellular content of major nutrients (e.g., nitrogen; Laws & Wong, 1978; Laws & Bannister, 1980), which are often used to predict growth rate in mathematical expressions (e.g., Droop equation). Also, photosynthetic pigment content, which is largely controlled by light, must also affect the photosynthetic efficiency of a phytoplankton cell as it did our "optically thin" *Ulva*. Thus, light intensity appears to play an important and virtually inseparable role from major nutrients in algal growth and nutrition studies. In view of the few phytoplankton studies concerning algal nutrition under low light slow growth conditions, there is a clear need for more information on this subject.

LIGHT, GROWTH AND UPTAKE OF NO_3^-

The NO_3^- uptake characteristics of an alga are often coupled with light (MacIsaac & Dugdale, 1972) and growth rate (Eppley & Thomas, 1969). Although NO_3^- uptake in our study was dependent primarily on the daily loadings of this nutrient, light and growth rate were inversely proportional to NO_3^- uptake at medium and high levels of N loading. This inverse relationship between light and NO_3^- uptake is supported by the higher chlorophyll *a* and nitrogen content of low light plants compared to high light plants. In contrast, uptake of NO_3^- by *Codium fragile* (Hanisak & Harlin, 1978) and natural phytoplankton populations (MacIsaac & Dugdale, 1972) have been reported to vary positively with light intensity.

The inverse relationship between light and uptake of NO_3^- by *Ulva* may be

related to competition between uptake processes of NO_3^- and inorganic carbon fixation for ATP. For example, carbon fixation by *Skeletonema costatum* was temporarily suppressed after enrichment with NO_3^-, due presumably to nutrient uptake processes competing for high energy nucleotides with dark reactions (Falkowski & Stone, 1975). High carbon–low nitrogen levels of *Ulva* associated with high light versus low carbon–high nitrogen levels associated with low light (Table I) support this hypothesis. Such a growth strategy may be energetically advantageous for *Ulva*. As pointed out by Falkowski & Stone (1975), a green plant cell can make up to 30 times as much ATP in the light by photophosphorylation as in the dark by oxidative phosphorylation. Thus, for "optically thin" *Ulva*, whose ability to harvest light is strongly related to chlorophyll content, increases in chlorophyll content via increased nutrient uptake at low light levels may represent a net gain in energy through increased photophosphorylation. Obviously, our results do not conclusively show this but can only offer this hypothesis as an interesting avenue of research for the future.

In addition, maximum growth rates of *Ulva* occurred with residual NO_3^- concentrations of ≈ 1 μM. This suggests a high affinity (low k_s) for this nutrient. This high affinity for NO_3^- together with the ability to increase NO_3^- uptake under light limited growth may partially account for the competitive success of *Ulva* in nature where both nitrogen and light can often limit growth.

Acknowledgements

This research was supported by NSF grant No. OCE77-20244. We wish to thank Mssrs. R. Walker, M. Fleetwood, E. Chesney, L. Bryant and B. Dornseif for technical assistance during the course of this research, and Drs. D. W. Menzel, J. A. Yoder, and C. J. Dawes for their reviews of the manuscript.

References

Beale, S. I. & D. Appleman, 1971. Chlorophyll synthesis in *Chlorella*. *Pl. Physiol.*, Vol. 47, pp. 230–235.

Boney, A. D., 1966. *A biology of marine algae*. Anchor Press, Essex, England, 216 pp.

Brinkhuis, B. H. & R. F. Jones, 1974. Photosynthesis in whole plants of *Chondrus crispus*. *Mar. Biol.*, Vol. 27, pp. 137–144.

Caperon, J. & J. Meyer, 1972. Nitrogen-limited growth of marine phytoplankton. 1. Changes in population characteristics with steady-state growth rate. *Deep-Sea Res.*, Vol. 19, pp. 601–618.

Chapman, A. R. O. & J. S. Craigie, 1977. Seasonal growth in *Laminaria longicruris*: relations with dissolved inorganic nutrients and internal reserves of nitrogen. *Mar. Biol.*, Vol. 40, pp. 107–205.

Cook, J. R., 1963. Adaptations in growth and division in *Euglena* affected by energy supply. *J. Protozool.*, Vol. 10, pp. 436–444.

D'Elia, C. & J. DeBoer, 1978. Nutritional studies of two red algae. II. Kinetics of ammonium and nitrate uptake. *J. Phycol.*, Vol. 14, pp. 266–272.

Dubinsky, F. & T. Berman, 1979. Seasonal changes in the spectral composition of downwelling irradiance in Lake Kinneret (Israel). *Limnol. Oceanogr.*, Vol. 24, pp. 652–663.

DURBIN, E. G., 1974. Studies on the autecology of the marine diatom *Thalassiosira nordenskiöldii* Clev. 1. The influence of daylength, light intensity and temperature on growth. *J. Phycol.*, Vol. 10, pp. 220–225.

EPPLEY, R. W. & W. H. THOMAS, 1969. Comparison of half-saturation constants for growth and nitrate uptake of marine phytoplankton. *J. Phycol.*, Vol. 5, pp. 375–379.

FALKOWSKI, P. G. & D. P. STONE, 1975. Nitrate uptake in marine phytoplankton: Energy sources and the interaction with carbon fixation. *Mar. Biol.*, Vol. 32, pp. 77–84.

GOLDMAN, J. C., 1979. Outdoor algal mass cultures. II. Photosynthetic yield limitations. *Wat. Res.*, Vol. 13, pp. 119–136.

GOLDMAN, J. C., J. J. MCCARTHY & D. G. PEAVY, 1979. Growth rate influence on the chemical composition of phytoplankton in oceanic waters. *Nature, Lond.*, Vol. 279, pp. 210–215.

HANISAK, M. D., 1979. Nitrogen limitation of *Codium fragile* ssp. *tomentosoides* as determined by tissue analysis. *Mar. Biol.*, Vol. 50, pp. 333–337.

HANISAK, M. D. & M. M. HARLIN, 1978. Uptake of inorganic nitrogen by *Codium fragile* subsp. *tomentosoides* (Chlorophyta). *J. Phycol.*, Vol. 14, pp. 450–454.

KANWISHER, J. W., 1966. Photosynthesis and respiration in some seaweeds. In, *Some contemporary studies in marine science*, edited by H. Barnes, Allen & Unwin Ltd., London, pp. 407–420.

LAPOINTE, B. E. & J. H. RYTHER, 1979. The effects of nitrogen and seawater flow rate on the growth and biochemical composition of *Gracilaria foliifera* v. *angustissima* in mass outdoor cultures. *Bot. Mar.*, Vol. 22, pp. 529–537.

LAWS, E. A. & T. T. BANNISTER, 1980. Nutrient- and light-limited growth of *Thalassiosira fluviatilis* in continuous culture, with implications for phytoplankton growth in the ocean. *Limnol. Oceanogr.*, Vol. 25, pp. 457–473.

LAWS, E. A. & D. C. WONG, 1978. Studies of carbon and nitrogen metabolism by three marine phytoplankton species in nitrate-limited continuous culture. *J. Phycol.*, Vol. 14, pp. 406–416.

MACISAAC, J. J. & DUGDALE, R. C., 1972. Interactions of light and inorganic nitrogen in controlling nitrogen uptake in the sea. *Deep-Sea Res.*, Vol. 19, pp. 209–232.

MADDUX, W. & R. JONES, 1964. Some interactions of temperature, light intensity and nutrient concentration during the continuous culture of *Nitzschia closterium* and *Tetraselmis* sp. *Limnol. Oceanogr.*, Vol. 9, pp. 79–86.

MARGALEF, R., 1974. *Ecologia*. Ometa, Barcelona, 955 pp.

MERRILL, J. E. & J. R. WAALAND, 1979. Photosynthesis and respiration in a fast growing strain of *Gigartina exasperata* (Harvey and Bailey). *J. exp. mar. Biol. Ecol.*, Vol. 39, pp. 281–290.

MISHKIND, M., D. MAUZERALL & S. I. BEALE, 1979. Diurnal variation *in situ* of photosynthetic capacity in *Ulva* is caused by a dark reaction. *Pl. Physiol.*, Vol. 64, pp. 896–899.

MURPHY, J. & J. P. RILEY, 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica chim. acta*, Vol. 26, pp. 31–36.

MYERS, J. & J. GRAHAM, 1971. The photosynthetic unit in *Chlorella* measured by repetitive short flashes. *Pl. Physiol.*, Vol. 48, pp. 282–286.

PLATT, T. & B. IRWIN, 1973. Caloric content of phytoplankton. *Limnol. Oceanogr.*, Vol. 18, pp. 306–310.

RAMUS, J., 1978. Seaweed anatomy and photosynthetic performance: the ecological significance of light guides, heterogeneous absorption and multiple scatter. *J. Phycol.*, Vol. 14, pp. 352–363.

RAMUS, J., S. I. BEALE & D. MAUZERALL, 1976a. Correlation of changes in pigment content with photosynthetic capacity of seaweeds as a function of water depth. *Mar. Biol.*, Vol. 37, pp. 231–238.

RAMUS, J., S. I. BEALE, D. MAUZERALL & K. L. HOWARD, 1976b. Changes in photosynthetic pigment concentration in seaweeds as a function of water depth. *Mar. Biol.*, Vol. 37, pp. 223–229.

RAMUS, J. & G. ROSENBERG, 1980. Diurnal photosynthetic performance of seaweeds measured under natural conditions. *Mar. Biol.*, Vol. 56, pp. 21–28.

RYTHER, J. H., 1956. Photosynthesis in the ocean as a function of light intensity. *Limnol. Oceanogr.*, Vol. 1, pp. 61–70.

RYTHER, J. H., 1962. On the efficiency of primary production in the oceans. *Proc. 9th Pacif. Sci. Congr.*, Vol. 4, pp. 188–200.

RYTHER, J. H. & D. W. MENZEL, 1959. Light adaptation by marine phytoplankton. *Limnol. Oceanogr.*, Vol. 4, pp. 492–497.

SAWYER, C., 1965. The sea lettuce problem in Boston Harbor. *J. Wat. Pollut. Control Fed.*, Vol. 37, p. 8.

SEIP, K. L., G. LUNDE, S. MELSOM, E. MEHLUM, A. MELHUUS & H. M. SEIP, 1979. A mathematical

model for the distribution and abundance of benthic algae in a Norwegian Fjord. *Ecol. Modeling*, Vol. 6, pp. 133–166.

SMITH, G. M., 1947. On the reproduction of some Pacific coast species of *Ulva*. *Am. J. Bot.*, Vol. 34, pp. 80–87.

SOLORZANO, L., 1969. Determination of ammonia in natural waters by the phenol-hypochlorite method. *Limnol. Oceanogr.*, Vol. 14, pp. 799–801.

TAMIYA, H., 1957. Mass culture of algae. *A. Rev. Microbiol.*, Vol. 8, pp. 309–334.

TENORE, K. R. & J. E. HUGUENIN, 1973. A flowing experimental system with filtered and temperature-regulated seawater. *Chesapeake Sci.*, Vol. 14, pp. 280–282.

TENORE, K. R. & D. L. RICE, 1980. A review of trophic factors affecting secondary production of deposit feeders. In, *Marine benthic dynamics*, edited by K. R. Tenore & B. C. Coull, University of South Carolina Press, Columbia, S. C., pp. 325–340.

TETT, P., J. COTTRELL, D. TREW & B. WOOD, 1975. Phosphorus quota and the chlorophyll : carbon ratio in marine phytoplankton. *Limnol. Oceanogr.*, Vol. 20, pp. 587–603.

THOMAS, W. & A. DODSON, 1972. On nitrogen deficiency in tropical Pacific oceanic phytoplankton. 2. Photosynthesis and cellular characteristics of a chemostat grown diatom. *Limnol. Oceanogr.*, Vol. 17, pp. 515–523.

TOPINKA, J. A. & J. V. ROBBINS, 1976. Effect of nitrate and ammonium enrichment on growth and nitrogen physiology in *Fucus spiralis*. *Limnol. Oceanogr.*, Vol. 21, pp. 659–664.

VOLLENWEIDER, J. A., 1974. *A manual on methods for measuring primary production in aquatic environments*. Blackwell Scientific Publications, London.

WOOD, E. D., F. A. J. ARMSTRONG & R. A. RICHARDS, 1967. Determination of nitrate in seawater by cadmium-copper reduction to nitrite. *J. mar. biol. Ass. U.K.*, Vol. 47, pp. 23–31.

YODER, J., 1979. Effect of temperature on light-limited growth and chemical composition of *Skeletonema costatum* (Bacillariophyceae). *J. Phycol.*, Vol. 15, pp. 362–370.