Phosphorus-limited photosynthesis and growth of Sargassum natans and Sargassum fluitans (Phaeophyceae) in the...

1 author:

Brian E. Lapointe
Florida Atlantic University

95 PUBLICATIONS   **4,687** CITATIONS

Phosphorus-limited photosynthesis and growth of *Sargassum natans* and *Sargassum fluitans* (Phaeophyceae) in the western North Atlantic

Brian E. Lapointe*

Abstract—Growth enrichment studies utilizing *in situ* cage cultures and a shipboard flowing seawater culture system were conducted with whole-plant populations of pelagic *Sargassum*—*S. natans* and *S. fluitans* (Phaeophyceae)—in the western Sargasso Sea and at Looe Key Marine Sanctuary adjacent to the Straits of Florida. Growth rates of both species ranged from 0.03 to 0.04 doublings d^{-1} in control cultures receiving no enrichment and in cultures receiving either NO_3^- or NH_4^+ enrichment; in contrast, growth rates ranged from 0.05 to 0.08 doublings d^{-1} in cultures receiving PO_4^{3-} enrichment. Midday photosynthetic rates of *S. natans* and *S. fluitans* were also two-fold higher with PO_4^{3-} enrichment, *ca.* 2.3 mgC g dry wt^{-1} h^{-1} compared to 1.0 to 1.5 mgC g dry wt^{-1} h^{-1} in the NH_4^+, NO_3^- and control treatments. These data suggest that pelagic *Sargassum* may grow faster than previously thought and that phosphorus, rather than nitrogen, may be the primary nutrient limiting growth and productivity of these plants.

INTRODUCTION

THE TWO species of pelagic *Sagassum* indigenous to the Sargasso Sea—*S. fluitans* Borgesen and *S. natans* (Linnaeus) J. Meyen—are important key industry species (*sensu* Elton) to pelagic food webs of the North Atlantic. Pelagic *Sargassum* provides habitat and nutrition for over 100 species of oceanic organisms associated with the *Sargassum* community (MORRIS and MOGELBERG, 1973; BUTLER *et al.*, 1983) as well as 50 species of fish of great commercial and sport fishing value (DOOLEY, 1972). Pelagic *Sargassum* may also be important to detrital food webs over broad areas of the North Atlantic as evidenced by its presence in deep sea photographs and gut contents of the deep sea brittlestar *Amphiophiura bullata* (SCHOENER and ROWE, 1970).

Despite the importance of these plants, our knowledge of their productivity remains quite limited. PARR's (1939) early model of abundance and growth of pelagic *Sargassum* proposed a total standing crop of 7.3 million tons which he speculated grew quite slowly—doubling on the order of every 10 to 100 years. Subsequent indirect (^{14}C, O_2) productivity estimates (CARPENTER and COX, 1974; HOWARD and MENZIES, 1969; BLAKE and JOHNSON, 1976) also suggested low productivity of these plants. Consequently, pelagic *Sargassum* is considered unproductive (e.g. MANN *et al.*, 1980) and therefore does not figure significantly in current models of oceanic productivity in the North Atlantic (e.g. JENKINS, 1982; KERR, 1983). However, recent evidence that traditional indirect algal productivity methods may underestimate *in situ* productivity due to a variety of bottle

* Harbor Branch Foundation Rt3, Box 297A, Big Pine Key, FL 33043, U.S.A.

effects (HARRIS, 1978; GIESKES *et al.*, 1979) suggests the need for direct growth rate measurements of pelagic *Sargassum* populations cultured under natural conditions.

Nutrient limitation is often cited as the reason for the apparent low productivity of pelagic *Sargassum* (e.g. MANN *et al.*, 1980). CARPENTER and COX (1974) found O_2 evolution of *Sargassum* was consistently higher north of 30°N latitude compared to more southerly latitudes, presumably because of a higher degree of winter mixing and nutrient resupply to surface waters in the northern Sargasso Sea compared to the southern Sargasso Sea. Although N and P are generally thought to be primary limiting nutrients in surface waters of the North Atlantic Ocean gyre (PARSONS *et al.*, 1977), the relative importance of these elements in limiting organic production in the oceans is still poorly known. For example, whereas phytoplankton ecologists regard the oceans to be N-limited systems (RYTHER and DUNSTAN, 1971; GOLDMAN *et al.*, 1979; PERRY and EPPLEY, 1981), geochemists maintain that the oceans as a whole are P-limited systems (SMITH, 1984). Considering the current uncertainties of the relative importance of N vs P limitation in the marine environment, studies to determine which nutrient element, if either, limits growth rate of *Sargassum* under natural conditions needs to be resolved.

The present study sought to determine: (1) the growth rate of populations of *S. natans* and *S. fluitans* grown under natural conditions in surface waters of the Sargasso Sea and Gulf Stream systems, and (2) the relative importance of N and P in regulating growth of these plants under ambient nutrient conditions.

MATERIALS AND METHODS

To assess growth of *S. natans* and *S. fluitans* over an approximation of their wide geographic range, growth enrichment studies were performed at two different locations, one adjacent to the Straits of Florida in Looe Key Marine Sanctuary in the lower Florida Keys, and the other during a cruise on the R.V. *Columbus Iselin* (cruise No. CI8311) to the western Sargasso Sea approximately 50 miles east of Eleuthra, Bahamas (Fig. 1). At both locations, whole plants of *S. natans* and *S. fluitans* were collected by dip netting and cleaned of macroepiphytes and macroepizoa before exposing the plants to four experimental treatments: (1) NO_3^- enriched, (2) NH_4^+ enriched, (3) PO_4^{3-} enriched and (4) control (no enrichment). Growth rates were determined as a function of these treatments as doublings d^{-1} by measuring changes in wet weight over approximately a 7- to 10-day growth interval.

Different culture techniques were used at the different geographic locations. The growth experiments in Looe Key Marine Sanctuary, performed between 20 July and 2 August 1983, utilized Vexar cage cultures (23 cm diameter × 41 cm long) in which replicate ($N = 2$) 100 g wet weight populations of *S. natans* and *S. fluitans* were grown. Enrichments consisted of soaking the *Sargassum* populations twice weekly in acid-washed polyethylene plastic bags containing seawater spiked to either 0.2 mM NO_3^- (NaNO_3), 0.2 mM NH_4^+ (NH_4Cl) or 0.2 mM PO_4^{3-} (NaH_2PO_4). After a 5-h exposure to these nutrients, the *Sargassum* was returned to the cages where growth was monitored. Frequent observations by divers indicated that loss of plants from the cages was negligible. During these experiments, irradiance ranged from 39 to 60 Ein m^{-2} d^{-1}, temperature ranged from 28 to 30°C, and salinity was constant at about 36‰.

The growth experiments in the western Sargasso Sea utilized a shipboard continuous-flow seawater system equipped with plexiglass growth tubes (10 cm diameter × 74 cm

Fig. 1.   Location of study areas in the western Sargasso Sea and Looe Key Marine Sanctuary
adjacent to the Straits of Florida.

long; volume = 2.5 l). Within each growth tube replicate ($N = 2$) populations of 50 g
wet weight each of *S. natans* and *S. fluitans* were grown. Seawater flow rates to each
growth tube were monitored and maintained at 200 culture volumes d^{-1} to insure that
seawater flow rate itself was not growth limiting (LAPOINTE and RYTHER, 1979). Nutrient
enrichments were achieved by stopping the seawater flow to the growth tubes and
injecting either NO_3^-, NH_4^+ or PO_4^{3-} to result in identical enrichment concentrations as in
Looe Key experiments. Seawater flow to the control tubes was also stopped but no
nutrients were administered. These nutrient pulses lasted for 5 h during which seawater
circulated around the growth tubes within the seawater system to maintain temperatures
to ambient surface temperatures in the Sargasso Sea. During these experiments,
irradiance ranged from 48 to 60 Ein m^{-2} d^{-1}, temperature ranged from 28 to 30°C, and
salinity was constant at 36‰.

Daily photosynthetic patterns of *S. fluitans* and *S. natans* in response to the nutrient
enrichments were also measured in the western Sargasso Sea by measuring inflow and
outflow O_2 concentrations from the growth tubes with a YSI model 57 O_2 meter.
Differences between inflow and outflow O_2 concentrations, combined with seawater flow
rate estimates and population weights, were used to calculate population photosynthetic
rates as mg O_2 g dry wt^{-1} h^{-1} for each experimental treatment. These rates were
converted to mgC g dry wt^{-1} h^{-1} by assuming a photosynthetic quotient of 1.0.

Surface (1 to 2 m) seawater samples were collected at 3-day intervals during both the
Sargasso Sea and Straits of Florida experiments to determine concentrations of dissolved
inorganic nutrients. After collection of triplicate samples with a Niskin bottle, the
samples were filtered through a 0.45 µm Gelman filter and frozen in a Nalgene bottle.

Within 1 month, the samples were thawed and analyzed for NH_4^+, NO_3^-, NO_2^-, and PO_4^{3-} according to methods described by ZIMMERMAN *et al.* (1977).

<div align="center">RESULTS</div>

Growth rates of *S. natans* and *S. fluitans* ranged from *ca.* 0.03 to 0.08 doublings d^{-1} during the growth enrichment experiments at both geographic locations (Table 1). Lower growth rates, ranging from 0.03 to 0.04 doublings d^{-1}, occurred in the control and NO_3^- and NH_4^+ enriched treatments, compared to almost two-fold higher rates, 0.05 to 0.08 doublings d^{-1}, in the PO_4^{3-} enriched treatments. Two-tailed *t*-tests indicate that PO_4^{3-} enrichment significantly ($P < 0.05$) enhanced growth over the controls whereas NO_3^- and NH_4^+ enrichment did not ($P > 0.05$).

Photosynthetic responses of *S. natans* and *S. fluitans* exposed to PO_4^{3-} enrichment also increased two-fold over the controls and those exposed to N enrichment. Midday (1100 to 1300 h) photosynthetic rates of PO_4^{3-} enriched treatments were *ca.* 2.0 mgC g dry wt^{-1} h^{-1} compared to lower rates of *ca.* 1.0 to 1.5 mgC dry wt^{-1} h^{-1} in the control, NH_4^+ and NO_3^- enriched treatments for both *S. natans* (Fig. 2) and *S. fluitans* (Fig. 3). Pairwise *t*-tests of

Table 1. Growth rates (doublings d^{-1}) of S. natans and S. fluitans in the western Sargasso Sea and Straits of Florida (Looe Key Marine Sanctuary) in response to different types of nutrient enrichment. Values represent means ± 1 S.D. (N = 2)

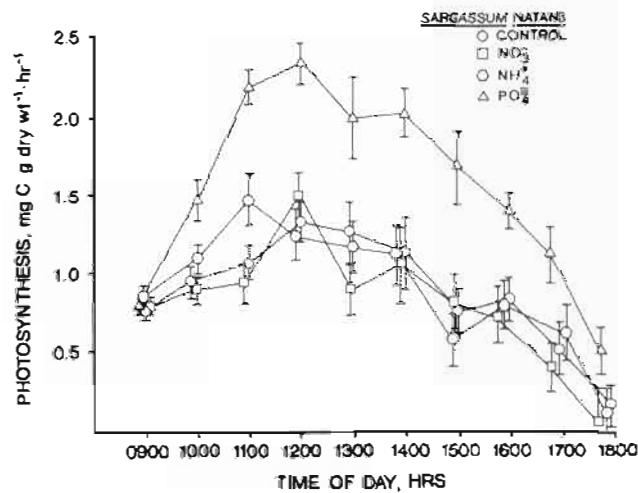| Species	Control	Treatment NO_3^-	NH_4^+	PO_4^{3-}
S. natans				
W. Sargasso Sea	0.035 ± 0.006	0.029 ± 0.005	0.032 ± 0.004	0.055 ± 0.004
Straits of Florida	0.045 ± 0.002	0.048 ± 0.007	0.043 ± 0.009	0.072 ± 0.083
S. fluitans				
W. Sargasso Sea	0.031 ± 0.006	0.039 ± 0.005	0.030 ± 0.006	0.056 ± 0.007
Straits of Florida	0.036 ± 0.007	0.041 ± 0.005	0.042 ± 0.006	0.061 ± 0.007

Fig. 2. Daily photosynthetic patterns of *S. natans* in response to various types of nutrient enrichment and to no enrichment (controls) in the western Sargasso Sea. Values represent means ± 1 S.D. (N = 2).
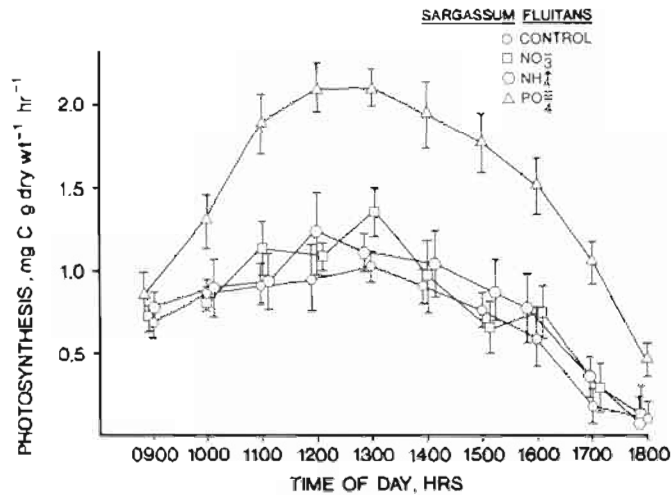
Fig. 3. Daily photosynthetic patterns of *S. fluitans* in response to various types of nutrient enrichment and to no enrichment (controls) in the western Sargasso Sea. Values represent means ± 1 S.D. (N = 2).

Table 2. *Concentrations of dissolved nutrients in surface seawater of the western Sargasso Sea and Straits of Florida during the growth enrichment studies. Values represent means* ±1 S.D. (N = 9)

Location	NH_4^+	NO_3	NO_2	PO_4^{3-}
W. Sargasso Sea	0.24 ± 0.09	U.D.*	U.D.†	U.D.‡
Straits of Florida	0.19 ± 0.05	0.10 ± 0.03	U.D.	U.D.

* <0.10 µM, † <0.02 µM, ‡ <0.03 µM.

the midday photosynthetic rates for both species indicate that PO_4^{3-} enrichment significantly ($P < 0.05$) enhanced photosynthesis over that of the controls, whereas NH_4^+ and NO_3^- enrichment did not ($P > 0.05$).

Concentrations of dissolved inorganic nutrients in surface seawater of the western Sargasso Sea and Straits of Florida were low to undetectable during these experiments. Concentrations of NH_4^+ ranged from 0.19 to 0.25 µM, while concentrations of NO_3^-, NO_2^-, and PO_4^{3-} were at low to undetectable levels (Table 2).

DISCUSSION

Compared to previous estimates of *Sargassum* growth rate as based on speculation and indirect methods, direct growth rate measurements during this study suggests that pelagic *Sargassum* may be growing faster than previously thought. Assuming the doubling times of the controls (approximately 0.03 doublings d^{-1}) to be representative of natural *Sargassum* populations, pelagic *Sargassum* populations may be turning over up to 10 times per year. These biomass turnover times agree with previous ^{14}C-based turnover times of *ca.* 40 days (CARPENTER and COX, 1974) but are much shorter than the doubling times on the order of 10 years often reported for pelagic *Sargassum* in the literature (e.g. PARR, 1939; MICHANEK, 1975; MANN *et al.*, 1980). That pelagic *Sargassum* appears to be

growing faster than previously thought supports the idea that growth must be relatively rapid to offset continuous loss of plants by sinking. stranding. herbivory. etc. Considering the tremendous capacity of these removal mechanisms (e.g. PARR. 1939) and that the abundance of pelagic *Sargassum* appears not to have changed in the last 50 years in the Sargasso Sea (BUTLER and STONER. 1984). a relatively rapid growth rate would appear to be required to maintain dynamic equilibrium in these populations.

Because productivity of *Sargassum* in the Sargasso Sea is the product of standing crop and growth rate. the relatively short doubling times of pelagic *Sargassum* also suggests *Sargassum* may be more productive than previously thought. The doubling times of the control cultures combined with PARR's (1939) estimate for total standing crop of pelagic *Sargassum* (7.3 million tons) suggest that pelagic *Sargassum* could account for up to 10% of the total phytoplankton productivity in the Sargasso Sea (as compared to phytoplankton productivity data of RYTHER and MENZEL, 1960). This estimate is greater than previous estimates of 0.5% (CARPENTER and COX, 1974) and 4.0% (HOWARD and MENZIES, 1969). Clearly. standing crop data are critical to these productivity estimates and although Parr's standing crop data are 50 years old they still represent the best estimate for total *Sargassum* abundance (e.g. BUTLER and STONER, 1984). However. the significance of pelagic *Sargassum* may lie not so much in its contribution to the productivity of the Sargasso Sea as a whole. but rather in its contribution to a unique and highly productive community that serves as a nursery ground for commercially valuable fish as well as sea turtles and pelagic birds. The relatively minor fraction of total productivity of *Sargassum* in the Sargasso Sea does not preclude its importance to overall higher food chain production; established herbivory (BUTLER *et al.*. 1983) combined with relatively short food chains associated with the pelagic *Sargassum* community suggests that *Sargassum* patches should be properly perceived as 'floating reefs' and need to be considered for accurate modeling of C and N dynamics in the North Atlantic.

Furthermore. estimates for doubling times of pelagic *Sargassum* based on the P-limited control cultures may. to some extent. underestimate true productivity of *Sargassum*. Depending on sea state. oceanic frontal convergences can produce *Sargassum* patches that range from 0.5 m^2 to several km^2 in size (PARR, 1939). Although open water nutrient concentrations during this study were low and typical of nutrient impoverised tropical blue surface waters. CULLINEY (1970) found that concentrations of PO_4^{3-} were two- or three-fold higher within *Sargassum* patches than in surrounding Sargasso Sea seawater— due presumably to metabolism within the patches. i.e. bacterial mineralization of dying *Sargassum* and excretion by resident animals. Considering the high degree of P limitation observed in the present study. association of pelagic *Sargassum* with a patch enriched in PO_4^{3-} may be ecologically important in supplying a major growth-limiting nutrient. For example. pelagic *Sargassum* having a recent association with a patch containing elevated PO_4^{3-} may have midday photosynthetic rates comparable to our P-enriched rates. i.e. 2.1 to 2.4 mgC g dry wt h^{-1}. or almost twice the photosynthetic rate observed in the N-enriched or control treatments. This finding suggests that much of the variability in previously reported photosynthetic rates of *Sargassum* may be due to variability in degree of P limitation alone and not solely to methodological differences among studies [see BLAKE and JOHNSON (1976) for a discussion; Table 3]. Because growth rates were also two-fold higher in the P-enriched treatments (about 0.08 doublings d^{-1}). the effects of P enrichment are also translated to greater rates of organic productivity in pelagic *Sargassum*. Thus, the temporal and spatial variability of *Sargassum* patches as well as

Table 3. *A comparison of photosynthetic rates in pelagic* Sargassum *as measured in previous studies and the present study*

Net photosynthesis (mgC g dry wt^{-1} h^{-1})	Method	Reference
0.14 – 0.76	O_2, flowing seawater system, entire *Sargassum* community	BLAKE and JOHNSON (1976)
0.13 – 1.04	O_2, light and dark bottle, whole plants	CARPENTER and COX (1974)
0.44 – 5.50	O_2, light and dark bottle apical meristems	HOWARD and MENZIES (1969)
2.10 – 2.40	O_2, flowing seawater system, whole plants, PO_4^{3-} enriched	This study
1.00 – 1.50	O_2, flowing seawater system, whole plants, NO_3 and NH_4^+ enriched and controls receiving no enrichment	This study

their degree of community development (e.g. relative abundance of fish, crabs, etc. that would affect PO_4^{3-} cycling) in the Sargasso Sea may be important ecological factors regulating growth and productivity in these populations.

These data, suggesting P-limited growth and productivity of *Sargassum*, are particularly interesting as they support the view held by most geochemists (LERMAN *et al.*, 1975; MEYBECK, 1982; BROECKER and PENG, 1982; SMITH, 1984) and particularly REDFIELD (1958) that P availability limits net organic production in the sea. REDFIELD (1958) pointed out that N deficiency can be made up by N fixation in that N compounds can accumulate until the available P is utilized. Accordingly, enhancement of *Sargassum* growth by P enrichment may be due in part to its effects on N fixation; the occurrence of a N-fixing blue-green algal flora on the surface of pelagic *Sargassum* is well known (CARPENTER, 1972; HANSON, 1977) and other evidence exists that N-fixing phytoplankton in the oceans are indeed P limited (DOREMUS, 1977; MAGUE *et al.*, 1977). However, research with phytoplankton bioassays (e.g. THOMAS, 1970; RYTHER and DUNSTAN, 1971; PERRY and EPPLEY, 1981) has resulted in a paradigm among marine biologists that N, not P, is the primary limiting nutrient for marine algal productivity. On the basis of phytoplankton grown experimentally under N and P limitation, GOLDMAN *et al.* (1979) recently concluded that "severe phosphorus limitation probably does not occur in the world's oceans". The data of this study suggest this is unlikely in the case of pelagic *Sargassum* although further studies are needed to determine the spatial and temporal extent of P limitation in *Sargassum* populations in the Sargasso Sea and its boundary currents before any accurate generalizations can be made.

REFERENCES

BLAKE N. J. and D. L. JOHNSON (1976) Oxygen production–consumption of the pelagic *Sargassum* community in a flow-through system with arsenic addition. *Deep-Sea Research*, **23**, 773–778.

BROECKER W. S. and T.-H. PENG (1982) *Tracers in the sea*. Eldigio Press, Palisades, New York, 960 pp.

BUTLER J. N. and A. W. STONER (1984) Pelagic *Sargassum*: has its biomass changed in the last 50 years? *Deep-Sea Research*, **29**, 953–965.

BUTLER J. N., B. F. MORRIS, J. CADWALLADER and A. W. STONER (1983) Studies of *Sargassum* and the *Sargassum* community. Bermuda Biological Station Special Publ. No. 22, 307 pp.

CARPENTER E. J. (1972) Nitrogen fixation by a blue-green epiphyte on pelagic *Sargassum*. *Science, Wash.*, **178**, 1207–1209.

CARPENTER E. J. and J. L. COX (1974) Production of pelagic *Sargassum* and a blue green epiphyte in the Western Sargasso Sea. *Limnology and Oceanography*, **19**, 429–436.

CULLINEY J. L. (1970) Measurement of reactive phosphorus associated with pelagic *Sargassum* in the northwestern Sargasso Sea. *Limnology and Oceanography*, **15**, 304–306.

DOOLEY J. K. (1972) Fishes associated with the pelagic *Sargassum* complex, with a discussion of the *Sargassum* community. *Contributions in Marine Science, University of Texas*, **16**, 1–32.

DOREMUS C. (1982) Geochemical control of dinitrogen fixation in the open ocean. *Biol. Oceanography*, **1**, 429–436.

GIESKES W. W. C., G. W. KRAAY and M. A. BAARS (1979) *Netherlands Journal of Sea Research*, **13**, 58.

GOLDMAN J. C., J. J. MCCARTHY and D. G. PEAVY (1979) Growth rate influence on the chemical composition of phytoplankton in oceanic waters. *Nature, London*, **279**, 210–215.

HANSON R. B. (1977) Pelagic *Sargassum* community metabolism: carbon and nitrogen. *Journal of Experimental Marine Biology and Ecology*, **29**, 107–118.

HARRIS G. P. (1978) Photosynthesis, productivity and growth: the physiological ecology of phytoplankton. *Ergebnisse der Limnologie*, **10**, 1–171.

HOWARD K. L. and R. J. MENZIES (1969) Distribution and production of *Sargassum* in the waters off the Carolina coast. *Botanica Marina*, **12**, 244–254.

JENKINS W. J. (1982) Oxygen utilization rates in North Atlantic subtropical gyre and primary production in oligotrophic systems. *Nature, London*, **300**, 246–248.

KERR R. A. (1983) Are the ocean's deserts blooming? *Nature, London*, **220**, 397–398.

LAPOINTE B. E. and J. H. RYTHER (1979) The effects of nitrogen and seawater flow rate on growth and biochemical composition of *Gracilaria foliifer* v. *angustissima* in mass outdoor cultures. *Botanica Marina*, **22**, 529–537.

LERMAN A., F. T. MACKENZIE and R. M. GARRELS (1975) Modeling geochemical cycles: phosphorus as an example. *Geological Society of America, Memoir*, **142**, 205–210.

MAGUE T. H., F. C. MAGUE and O. HOLM-HANSEN (1977) Physiology and chemical composition of nitrogen-fixing phytoplankton in the central North Pacific ocean. *Marine Biology*, **41**, 213–227.

MANN K. H., A. R. O. CHAPMAN and J. A. GAGNE (1980) Productivity of seaweed: the potential and the reality. In: *Primary productivity in the sea*, P. G. FOLKOWSKI, editor, Plenum Press, New York, pp. 363–380.

MCCARTHY J. J. and E. H. CARPENTER (1979) *Oscillatoria* (Trichodesmiun) *thiebautii* (Cyanophyta) in the central North Atlantic ocean. *Journal of Phycology*, **15**, 75–82.

MEYBECK M. (1982) Carbon, nitrogen, and phosphorus transport by world rivers. *American Journal of Science*, **282**, 410–450.

MICHANEK G. (1975) *Seaweed resources of the ocean*. Food and Agricultural Organization, Rome.

MORRIS B. F. and D. D. MOGELBERG (1973) Identification manual to pelagic *Sargassum* fauna. Special Publication No. 11, Bermuda Biological Station, 63 pp.

PARR A. E. (1939) Quantitative observations on the pelagic *Sargassum* vegetation of the Western North Atlantic. *Bulletin of the Bingham Oceanographic Collection*, **6**, 1–94.

PARSONS T. R., M. TAKAHASHI and B. HARGRAVE (1977) *Biological oceanographic processes*. Pergamon Press, New York, 332 pp.

PERRY M. J. and R. W. EPPLEY (1981) Phosphate uptake by phytoplankton in the central North Pacific Ocean. *Deep-Sea Research*, **28**, 39–49.

REDFIELD A. C. (1958) The biological control of chemical factors in the environment. *American Scientist*, **46**, 205–222.

RYTHER J. and W. M. DUNSTAN (1971) Nitrogen, phosphorus, and eutrophication in the coastal marine environment. *Science*, **171**, 1008–1013.

RYTHER J. H. and D. W. MENZEL (1960) The seasonal and geographic range of primary production in the western Sargasso Sea. *Deep-Sea Research*, **6**, 235–238.

SCHOENER A. and G. T. ROWE (1970) Pelagic *Sargassum* and its presence among the deep-sea benthos. *Deep-Sea Research*, **17**, 923–925.

SMITH S. V. (1984) Phosphorus versus nitrogen limitation in the marine environment. *Limnology and Oceanography*, **29**, 1149–1160.

THOMAS W. H. (1970) On nitrogen deficiency in tropical Pacific Ocean phytoplankton, *Limnology and Oceanography*, **15**, 380–385.

ZIMMERMAN G., M. PRICE and J. MONTGOMERY (1977) Operation methods and quality control of Technicon Autoanalyzer II systems for nutrient determinations in seawater. Harbor Branch Foundation Technical Report No. 11.