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Aspects of nitrogen fixation in *Sargassum* communities off the coast of Florida

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Abstract: The magnitude and character of nitrogen fixation in *Sargassum* communities was studied for three different species, *S. fluitans* Borgesen, *S. natans* J. Meyen and *S. filipendula* C. Agardh. Nitrogen fixation activity was measured using the acetylene reduction technique. The character of epiphytic populations on the surface of *Sargassum* was investigated by scanning electron microscopy. All three species of *Sargassum* exhibited the potential for high levels of acetylene reduction. Mean rates of up to 7.1 μmol C_2H_4 produced $\cdot g^{-1}$ (*Sargassum* dry wt.) $\cdot h^{-1}$ were observed at one location. Nitrogen fixation activity was strongly light dependent. Saturation light intensity for nitrogen fixation was low, i.e. $<100\ \mu E \cdot m^{-2} \cdot s^{-1}$, and no photoinhibition was observed under full sunlight intensity (i.e. photon flux of $\approx 2000\ \mu E \cdot m^{-2} \cdot s^{-1}$). Results indicated that cyanobacteria were responsible for nitrogen fixation. Both *Calothrix* and LPP type cyanobacteria were commonly represented on the surface of the *Sargassum*. Activity associated with the benthic species *S. filipendula* exhibited significant seasonal variability. Nitrogen fixation activity in pelagic samples was variable but high throughout the year. The contribution of nitrogen fixation to the nitrogen budget of *Sargassum* communities appears to be particularly pronounced in the pelagic environment.

Key words: N_2 fixation; *Sargassum*; Cyanobacteria; Epiphytes

Introduction

The process of nitrogen fixation (i.e. conversion of $N_2 \rightarrow NH_3$) by microorganisms is recognized as an important mechanism for the introduction of usable nitrogen into both terrestrial and aquatic ecosystems. It has been hypothesized that nitrogen fixation has a particularly significant impact on the nitrogen budget of areas where other external inputs are small, like the Sargasso Sea and many other open ocean environments (Stewart, 1971; Soderlund & Svensson, 1976; Fogg, 1978, 1982; Martinez *et al.*, 1983). In a recent review Capone & Carpenter (1982) designated three major sources of fixation activity in the ocean; the pelagic cyanobacterium *Trichodesmium* (i.e. *Oscillatoria* sp.) (Dugdale *et al.*, 1961; Bunt *et al.*, 1970; Taylor *et al.*, 1973; Carpenter & McCarthy, 1975; Carpenter & Price, 1977; Mague *et al.*, 1977; Saino & Hattori, 1978; McCarthy & Carpenter, 1979; Bryceson & Fay, 1981), epiphytes on the surface of the pelagic brown macroalgae *Sargassum* (Carpenter, 1972; Hanson, 1977) and symbiotic cyanobacteria within pelagic *Rhizosolenia* mats (diatom) (Venrick, 1974;

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Martinez *et al.*, 1983). The contributions of these groups were estimated to be 4.8×10^{12}, 0.018×10^{9} and $< 10^{9}$ g nitrogen $\cdot$ yr^{-1}, respectively. There are, however, factors which could alter these figures. In general, the spatial and temporal patchiness of nitrogen fixation activity, combined with the vastness of the oceanic habitat, make it difficult to estimate total world-wide nitrogen fixation. New investigations are continuously providing better insight into the character of nitrogen fixation activity. For example, there are species of cyanobacteria whose quantitative importance, or even existence was not recognized until the 1970s. Unicellular cyanobacteria, for instance, have recently been shown to be prominent in the euphotic zone of oceanic habitats (Johnson & Sieburth, 1979; Waterbury *et al.*, 1979; Glover & Morris, 1981; Guillard & Murphy, 1985; Murphy & Haugen, 1985). It is suspected that some of these unicellular forms may be capable of aerobic nitrogen fixation. Similar forms of single-celled marine cyanobacteria from coastal habitats have been shown to exhibit exceptionally high rates of aerobic nitrogen fixation (Duerr & Mitsui, 1980; Reddy, 1984).

Another area of continued interest is nitrogen fixation in the pelagic *Sargassum* community. Recent studies of nitrogen fixation activity associated with pelagic *Sargassum* from the Sargasso Sea have yielded varying results. Carpenter (1972) reported rates of nitrogen fixation of 0.02 to 1.08 μg N $\cdot$ m^{-2} $\cdot$ h^{-1} for epiphytes from *Sargassum* collected at seven stations in the western Sargasso Sea in May and July. In contrast, Hanson (1977) obtained an average rate of 4.5 μg N $\cdot$ m^{-2} $\cdot$ h^{-1} for eight stations made in the western Sargasso Sea during the month of June.

The purpose of this study is to examine further nitrogen fixation activity in *Sargassum* communities with three goals in mind; (1) reconcile the substantial divergence in previously reported rates, (2) compare nitrogen fixation of *Sargassum* communities from environments of differing external nitrogen input potential, and (3) provide a picture of seasonal variability in activity. With these goals in mind a seasonal comparison was made of nitrogen fixation in estuarine (benthic) and oceanic (pelagic) *Sargassum* communities off the coast of Florida. It was hypothesized that nitrogen fixation would represent a significant input to the nitrogen budget of pelagic *Sargassum* communities, but that the role in benthic near shore populations would be considerably smaller.

Materials and Methods

Collection of samples

Samples of pelagic *Sargassum*, i.e., *S. fluitans* Borgesen, and *S. natans* J. Meyen (Taylor, 1979) were collected with a dip net from the Western edge of the Gulf Stream off Miami, FL (25°43′N : 80°2′W). Samples were transferred to cooler chests containing Gulf Stream water. Samples were taken between 0700 and 0900 and immediately transferred to a laboratory at the Rosentiel School of Marine and Atmospheric Science in Miami. Gulf Stream water was collected in 4-l sterile containers.

Samples of pelagic *Sargassum* were also collected from the western edge of the Gulf Stream off of St. Augustine, FL (30°0′N : 80°50′W).

Samples of the benthic *Sargassum* species, *S. filipendula* C. Agardh (Taylor, 1979), were collected between 0800 and 0900 from the mouth of the Homosassa River in the Gulf of Mexico (28°46.5′N : 82°42.5′W). Samples were immediately transferred to experimental flasks and conveyed to a laboratory at the University of Florida.

NITROGEN FIXATION ASSAYS

Activity was tested using the acetylene reduction technique. This technique has been reviewed in detail elsewhere (Burris, 1972; Taylor, 1983). Parts of the *Sargassum* were excised and put into 25-ml Erlenmeyer flasks. *Sargassum* not used in the assay was put in plastic bags for subsequent dry-weight determination. Approximately 0.2 g (equivalent dry weight) of *Sargassum* were put into each flask. The samples contained the blades along with their respective flotation vesicles and stalks. The flasks were then filled to 15 ml with filtered (0.20 μm) Gulf Stream water and closed with rubber stoppers. Incubation was begun by injecting 1.5 ml of acetylene into each flask (i.e., 10% of the gas phase).

Experimental preparations were completed within 2 h of sampling to minimize the exposure of the algae to artificial conditions. Standard incubations were performed in illuminated water baths at a light intensity of 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ (i.e. fluorescent cool-white). The temperature of the baths was adjusted to that recorded in the sampling site during the time of collection. The flasks were shaken gently at approximately 30 rpm. After $\approx$4–6 h an aliquot of the gas phase was injected into a gas chromatograph for analysis. Preliminary experiments indicated that rates of acetylene reduction were stable for at least 12 h. In addition, there was no lag phase before the initiation of acetylene reduction activity. A Carle gas chromatograph with a poropak R column was used for the separation of gases, along with a flame ionization detector. Estimates of nitrogen fixation used in the discussion section were based on an assumed 3 : 1 ratio between moles C_2H_2 reduced: moles N_2 fixed.

In outdoor (in situ) experiments, flasks were attached to a floating framework. Flasks were suspended at $\approx$7.5 cm below the surface. At various times during the day samples of gas were withdrawn to test ethylene formation.

Data on nitrogen fixation activity were analyzed statistically. Comparisons of data sets were carried out with analysis of variance. In order to allow realistic comparisons of data from a range of sampling times, activity figures were log transformed before analysis of variance.

PHOTOSYNTHETIC O_2 EVOLUTION

Oxygen evolution by *Sargassum* blades was measured using a Clark type oxygen electrode (YSI Model 53). Experiments were performed with freshly excised blades of *Sargassum*. The incubation solution for the photosynthetic experiments contained 3 ml

of minimal media (3.2 $g \cdot l^{-1}$ $MgCl_2 \cdot 6\ H_2O$ and 0.5 $g \cdot l^{-1}$ KCl), 0.6 ml buffer solution (200 mM HEPES at pH 7.8), and 1 ml H_2O. Salt concentration was adjusted to that found at the sampling site with NaCl. Choice of the incubation media was made after consultation with Dr. S. Izawa (Wayne State University), who suggested this formula to reduce the chance of chemical interference with electrode function. The reaction volume was 5 ml. Temperature was maintained at 30 °C. Light was provided by a projector (tungsten–halogen bulb) equipped with an infra-red filter. Light intensity was adjusted with neutral density filters. At the start of each experiment 0.2 ml of 150 mM $NaHCO_3$ was injected into the incubation vessel as a source of CO_2.

MEASUREMENT OF LIGHT INTENSITY

Light intensity was measured as photon flux with a LiCor 185B quantum radiometer. Light intensity values represent PAR light (400–700 nm).

DRY WEIGHT ANALYSIS

Dry weight was determined by drying the *Sargassum* samples in an oven at 90 °C, as described by Sorokin (1973).

SCANNING ELECTRON MICROSCOPY

Selected samples of *Sargassum* were taken from acetylene reduction assay flasks of known activity. These samples were cut into sizes suitable for mounting on SEM stubs. Each sample was taken through an alcohol series and critical point dried. Pieces of blade, flotation vesicles and stalk were then mounted, gold coated and examined with the scanning electron microscope. In addition to these samples, freshly collected *Sargassum* was preserved in 2.5% glutaraldehyde and later compared with those taken from the assay flasks. In order to determine whether epiphytes were lost from the algal surface during acetylene reduction assays, the residual medium was filtered and examined in the SEM.

Conclusions about the distribution of epiphytes were based upon a systematic survey of the algal fragments in question. Each sample (i.e. stub) was scanned and at least 10 photographs taken covering all sectors of the sample grid. The relationship between nitrogen fixation activity and cyanobacterial density was examined using a counting grid method. A 1/4″ square transparent grid was placed over 8″ × 10″ scanning electron micrographs. Wherever a line on the grid intercepted a cyanobacterium, a count was registered. In the case of pelagic *Sargassum*, 15 micrographs per sample were analyzed, i.e. five sections of a new leaf, five sections of an older leaf associated with epifauna, and five sections of an older leaf not associated with epifauna.

RESULTS

NITROGEN FIXATION ACTIVITY IN THE DARK

Nitrogen fixation, under aerobic conditions, exhibited strong light dependency. All samples assayed for acetylene reduction immediately after collection showed very low or no activity in the dark. Mean rates of acetylene reduction in the dark were 3.60 (range 0.00–19.86; SD 5.39), 0.79 (range 0.00–5.51; SD 1.93), and 25.0 nmol C_2H_4 produced $\cdot g^{-1}$ (*Sargassum* dry wt.)$^{-1} \cdot h^{-1}$ (range 1.26–97.9; SD 23.9), for *S. fluitans*, *S. natans* and *S. filipendula*, respectively.

NITROGEN FIXATION ACTIVITY IN THE LIGHT

Significant levels of aerobic nitrogen fixation activity were observed in the light with all three species of *Sargassum* (Fig. 1).

In the Miami area the yearly mean rates of acetylene reduction were 0.61 and 0.53 μmol C_2H_4 produced $\cdot g^{-1}$ (*Sargassum* dry wt.) $\cdot h^{-1}$ for *S. fluitans* and *S. natans*, respectively. Both species were represented throughout the time period included in this study, although the relative biomass of each varied.

At Homosassa Springs the benthic population of *S. filipendula* averaged 0.60 μmol C_2H_4 produced $\cdot g^{-1}$ (*Sargassum* dry wt.) $\cdot h^{-1}$ during the spring and summer. However, in fall and winter the mean rates of acetylene reduction dropped to nominal levels (Fig. 1).

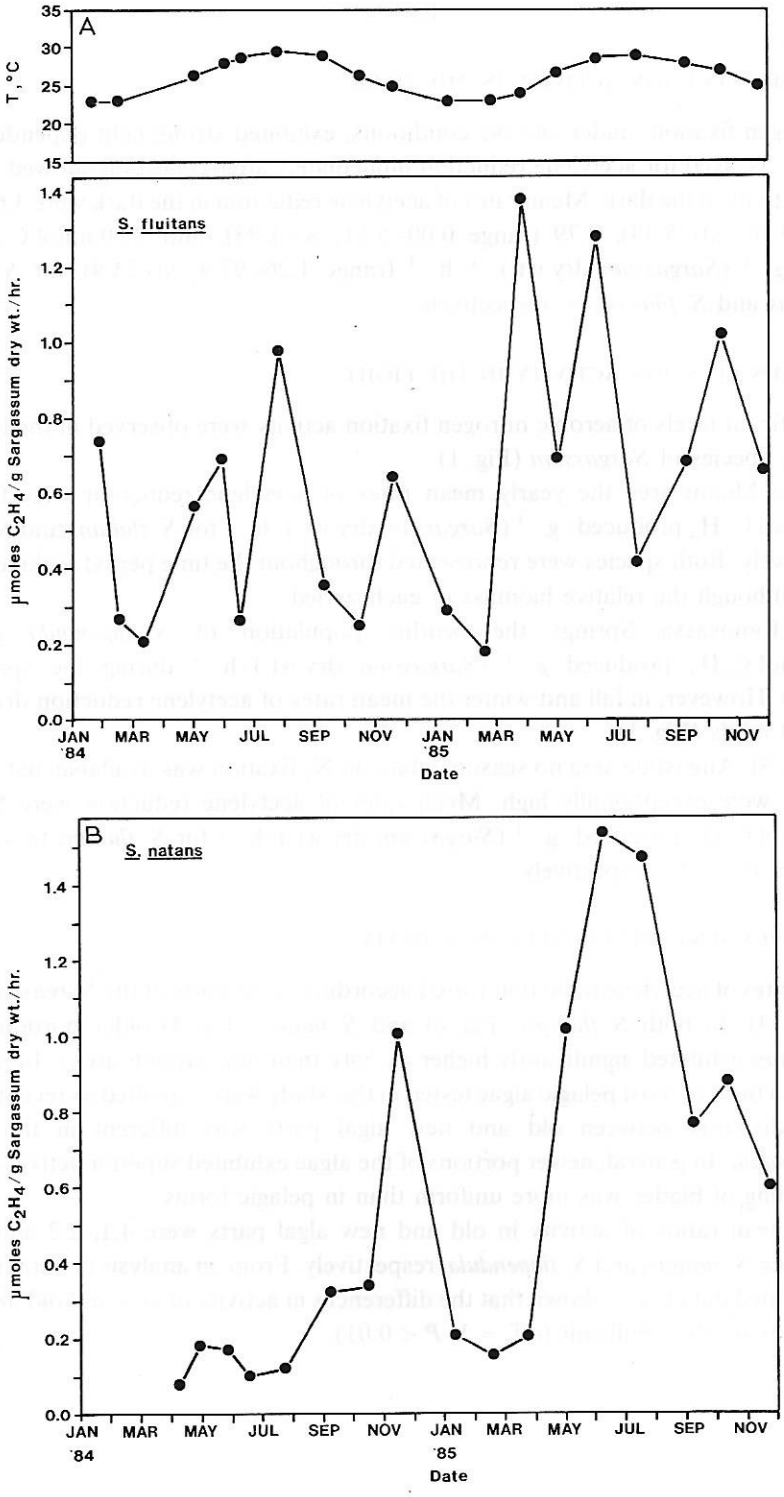
In the St. Augustine area no seasonal data on N_2 fixation was available, but the rates for July were exceptionally high. Mean rates of acetylene reduction were 5.22 and 7.10 μmol C_2H_4 produced $\cdot g^{-1}$ (*Sargassum* dry wt.) $\cdot h^{-1}$ for *S. fluitans* ($n = 32$) and *S. natans* ($n = 32$), respectively.

AGE DEPENDENT DIFFERENCES IN ACTIVITY

The rates of acetylene reduction varied according to the parts of the *Sargassum* tested (Figs. 2–4). In both *S. fluitans* (Fig. 2) and *S. natans* (Fig. 3) older portions of the *Sargassum* exhibited significantly higher activity than new growth areas. In general a tenth to a third of most pelagic algae tested in this study were classified as recent growth.

The disparity between old and new algal parts was different in the benthic *S. filipendula*. In general, newer portions of the algae exhibited superior activity (Fig. 4), and fouling of blades was more uniform than in pelagic forms.

The mean ratios of activity in old and new algal parts were 4.1, 2.2 and 0.4 for *S. fluitans*, *S. natans*, and *S. filipendula*, respectively. From an analysis of variance of log transformed data it was shown that the differences in activity of new and old algal parts were statistically significant (d.f. = 1, $P < 0.01$).


A
T, °C
35
30
25
20
15
S. fluitans
µmoles C_2H_4/g Sargassum dry wt./hr.
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
JAN '84
MAR
MAY
JUL
SEP
NOV
JAN '85
MAR
MAY
JUL
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NOV
Date
B
S. natans
µmoles C_2H_4/g Sargassum dry wt./hr.
1.4
1.2
1.0
0.8
0.6
0.4
0.2
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JAN '84
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EFFECT OF LIGHT INTENSITY ON NITROGEN FIXATION ACTIVITY AND PHOTOSYNTHETIC OXYGEN EVOLUTION

The response of nitrogen fixation to light intensity (i.e. photon flux) was studied in both indoor and outdoor experiments using *S. fluitans*. In laboratory experiments, the saturation light intensity was found to be low, i.e. between 25 and 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Table I). No inhibition was observed at a light intensity of 500 $\mu E \cdot m^{-2} \cdot s^{-1}$ (i.e. tungsten light). In outdoor experiments it was shown that even midday sunlight (i.e. 2000–2500 $\mu E \cdot m^{-2} \cdot s^{-1}$) did not significantly inhibit nitrogen fixation activity. In a comparison of outdoor (midday) and indoor samples (100 $\mu E \cdot m^{-2} \cdot s^{-1}$ of fluorescent light) the mean rates of acetylene reduction were 0.75 (n = 8, SD 0.22) and 0.76 μmol C_2H_4 produced $\cdot g^{-1}$ (*Sargassum* dry wt.) $\cdot h^{-1}$ (n = 8, SD 0.25), respectively.

The response of photosynthetic oxygen evolution to light intensity was determined for *S. filipendula* and *S. fluitans* (Fig. 5). Saturation light intensities for both species were between 600 and 800 $\mu E \cdot m^{-2} \cdot s^{-1}$. No apparent photoinhibition of photosynthesis

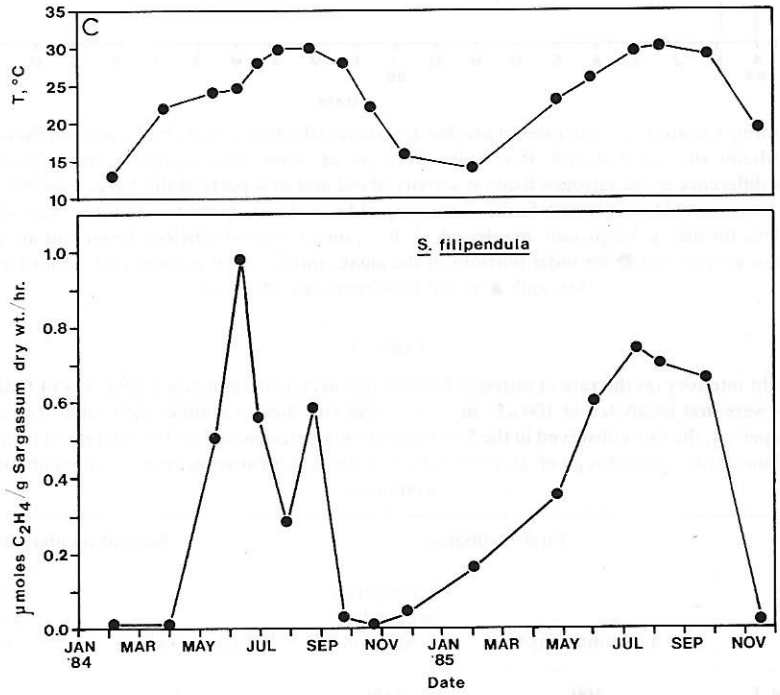


Fig. 1. Summary of monthly variation in aerobic, light, acetylene reduction activity associated with *Sargassum fluitans* (A), *S. natans* (B), and *S. filipendula* (C): the mean rates of ethylene formation shown were calculated by combining data shown in Figs. 2–4 with data on the dry wt. composition of new and older algal portions of all the *Sargassum* collected at each site; monthly temperature variation at the Miami and Homosassa River sites are shown above acetylene reduction data for *S. fluitans* (Miami) and *S. filipendula* (Homosassa).

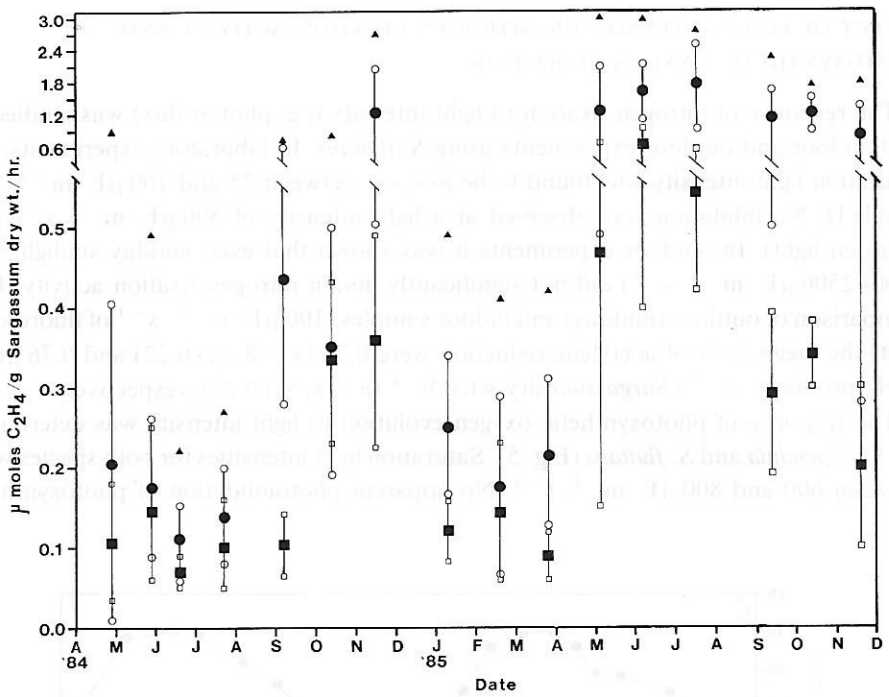


Fig. 2. Monthly variation in mean rates of aerobic acetylene reduction activity by *Sargassum fluitans* samples from the Miami site: each month 48 samples were set up from three sampling sites; because of the substantial difference in the nitrogen fixation activity of old and new parts of the *Sargassum* the sample set from each site was split evenly into flasks containing older and new portions of algae; rates are given as μmol ethylene formed · g *Sargassum* · mg dry $wt^{-1} \cdot h^{-1}$; mean rates of ethylene formation are designated by ■ for new growth and ● for older portions of the algae; smaller open squares and circles represent the SD, and ▲ is the maximum rate observed.

TABLE I

Effect of light intensity on the rate of nitrogen fixation (i.e. acetylene reduction): four sets (4 flasks per set) of samples were first incubated at 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ and switched to another light intensity for a second incubation period; the rates observed in the first incubation are designated as 1.0; the rate of fixation during the second incubation period is given as a percentage of the first incubation period; 3-h incubation periods were used.

	First incubation		Second incubation	
	Light intensity[a]	Relative rate of acetylene reduction	Light intensity[a]	Relative rate of acetylene reduction
Sample set 1	100	1.00	25	0.86
Sample set 2	100	1.00	100	1.04
Sample set 3	100	1.00	500	1.02
Sample set 4	100	1.00	2000[b]	0.98

[a] $\mu E \cdot m^{-2} \cdot s^{-1}$.
[b] Sunlight.

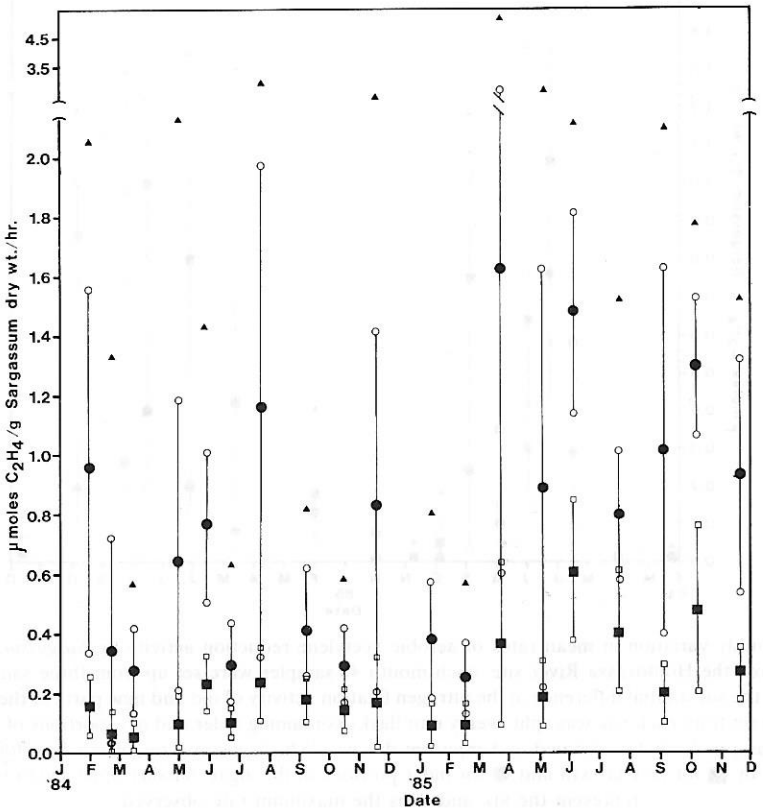


Fig. 3. Monthly variation in mean rates of aerobic acetylene reduction activity by *Sargassum natans* sample from the Miami site: each month 48 samples were set up from three sampling sites; because of the substantial difference in the nitrogen fixation activity of old and new parts of the *Sargassum* the sample set from each site was split evenly into flasks containing older and new portions of algae; rates are given as μmol ethylene formed · g *Sargassum* dry $wt^{-1} \cdot h^{-1}$; mean rates of ethylene formation are designated by ■ for new growth and ● for older portions of the algae; smaller open squares and circles represent the SD, and ▲ is the maximum rate observed.

was observed at 2000 $\mu E \cdot m^{-2} \cdot s^{-1}$, at least over the 10-min incubation period used in these experiments.

EFFECT OF TEMPERATURE ON NITROGEN FIXATION ACTIVITY

The effect of temperature on nitrogen fixation was examined in two species of *Sargassum*, the pelagic form *S. fluitans* and the benthic species *S. filipendula*. The optimal temperature for samples of both species taken in July, 1984, was 30 °C (Fig. 6). The upper limit for nitrogen fixation was dependent on the length of incubation. For short incubation periods (i.e. 3 h) nitrogen fixation was observed up to 45 °C. However, extended exposure (i.e. 9 h) to temperatures above 40 °C stopped all activity. Similar series of temperature experiments were attempted with *S. filipendula* samples from

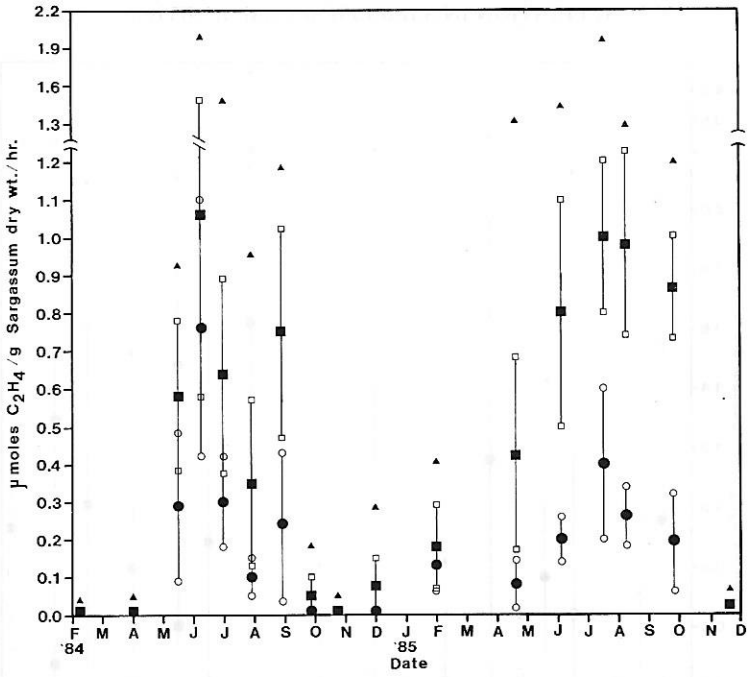


Fig. 4. Monthly variation in mean rates of aerobic acetylene reduction activity by *Sargassum filipendula* samples from the Homosassa River site: each month 48 samples were set up from three sampling sites; because of the substantial difference in the nitrogen fixation activity of old and new parts of the *Sargassum* the sample set from each site was split evenly into flasks containing older and new portions of algae; rates are given as μmol ethylene formed · g *Sargassum* dry $wt^{-1} \cdot h^{-1}$; mean rates of ethylene formation are designated by ■ for new growth and ● for older portions of the algae; smaller open squares and circles represent the SD, and ▲ is the maximum rate observed.

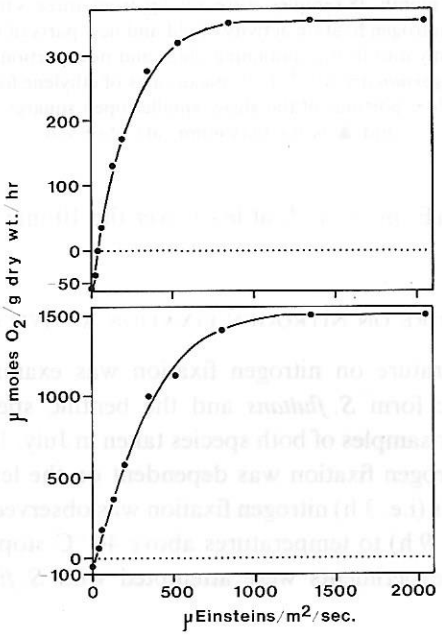


Fig. 5. Response of photosynthetic oxygen evolution by *Sargassum fluitans* (top) and *S. filipendula* (bottom) to light intensity; temperature was 30 °C; light intensity is given as PAR light.

November (1984), however, nitrogen fixation activity was nominal and similar throughout the temperature range tested.

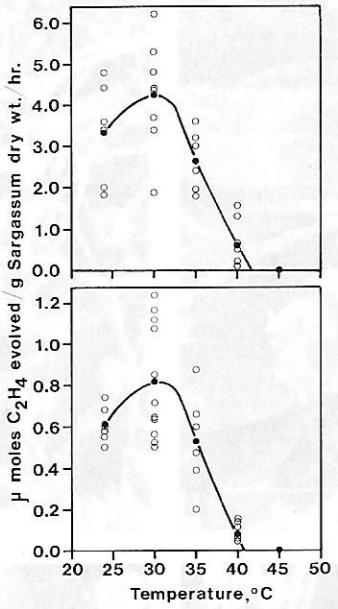


Fig. 6. The response of acetylene reduction to temperature in *Sargassum fluitans* (top) and *S. filipendula* (bottom); ● indicates the mean value determined from the observed rates of acetylene reduction (○).

CHARACTER OF THE EPIPHYTIC POPULATIONS ASSOCIATED WITH *S. FLUITANS*, *S. NATANS*, AND *S. FILIPENDULA*

Scanning electron microscopy of the *Sargassum* samples revealed that the level of acetylene reduction activity was related to the abundance of epiphytes on the algal surfaces. Samples with high activity exhibited heavy colonization by epiphytes (e.g. Fig. 7). This is exemplified by the relationship between the density of cyanobacteria and nitrogen fixation activity (Table II). Within the same patch of pelagic *Sargassum* older blades exhibited extensive fouling by microorganisms (Fig. 8A), while new parts of the algae were often relatively devoid of epiphytes (Fig. 8B) and accordingly showed very little acetylene reduction activity. Flotation vesicles and stalks of the algae exhibited patterns similar to those found on the blades. The pattern of fouling was reversed in benthic *Sargassum* (e.g. Figs. 8C and D).

In the case of *S. fluitans* and *S. natans*, the highest concentration of epiphytes was associated with the surface of epifaunal hydroids, rather than the *Sargassum* itself (Fig. 8A). The distribution of cyanobacterial tufts (i.e. mostly bundles of *Calothrix* trichomes) was closely related to the distribution epifaunal hydroids (Fig. 9).

A variety of different epiphytes were observed, including several types of cyano-

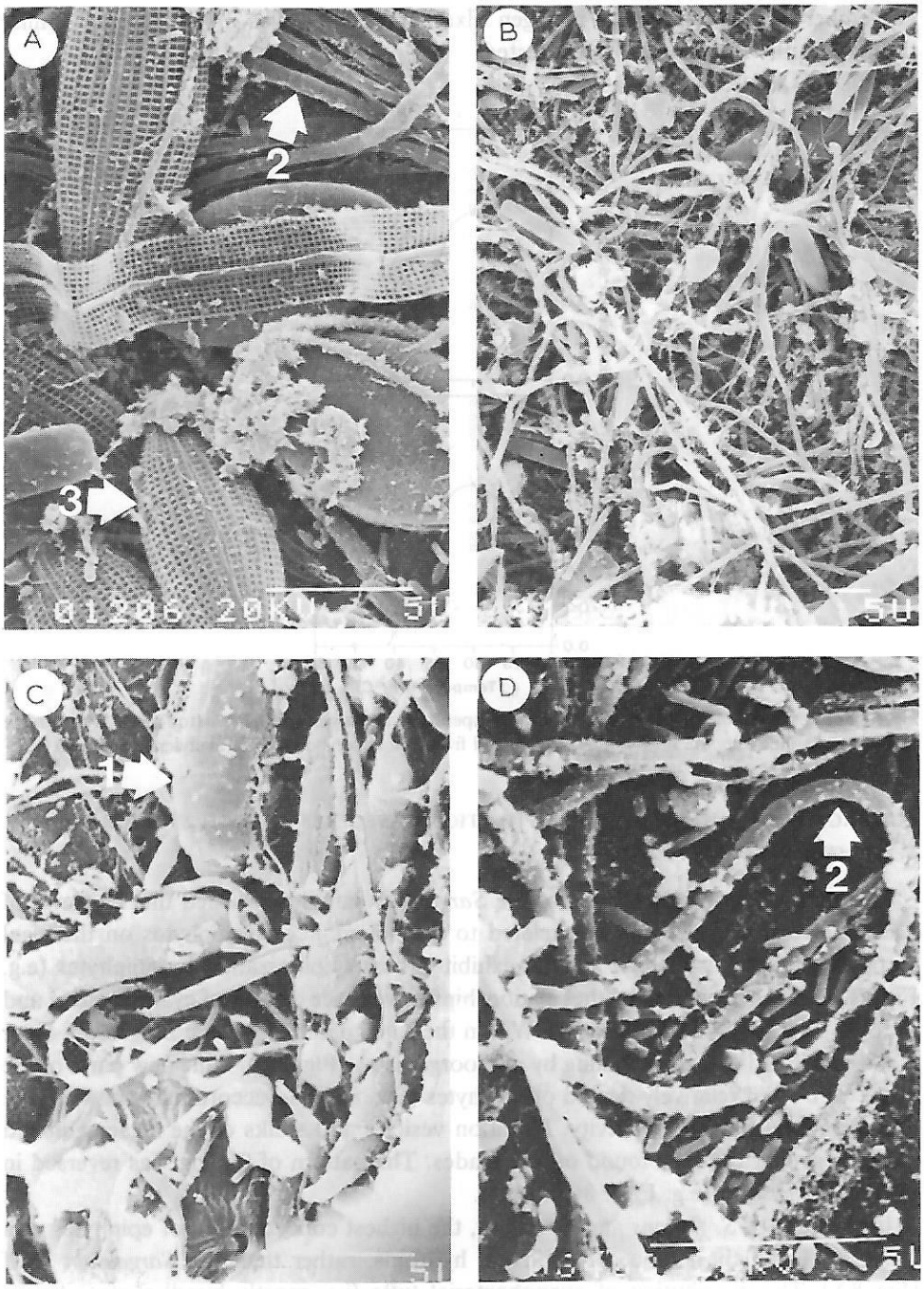


Fig. 7. Examples of heavy epiphytic colonization of *Sargassum* leaves of *S. fluitans* (A and B) and *S. natans* (C and D): arrows indicate representative examples of *Calothrix* (arrows with no. 1) and LPP (arrows with no. 2) type cyanobacteria, which were commonly found on the surface of these two pelagic species; numerous other species of microorganisms were observed, like the very apparent diatoms indicated by arrow no. 3; line at the bottom of each photomicrograph indicates the scale; i.e. U, μm.

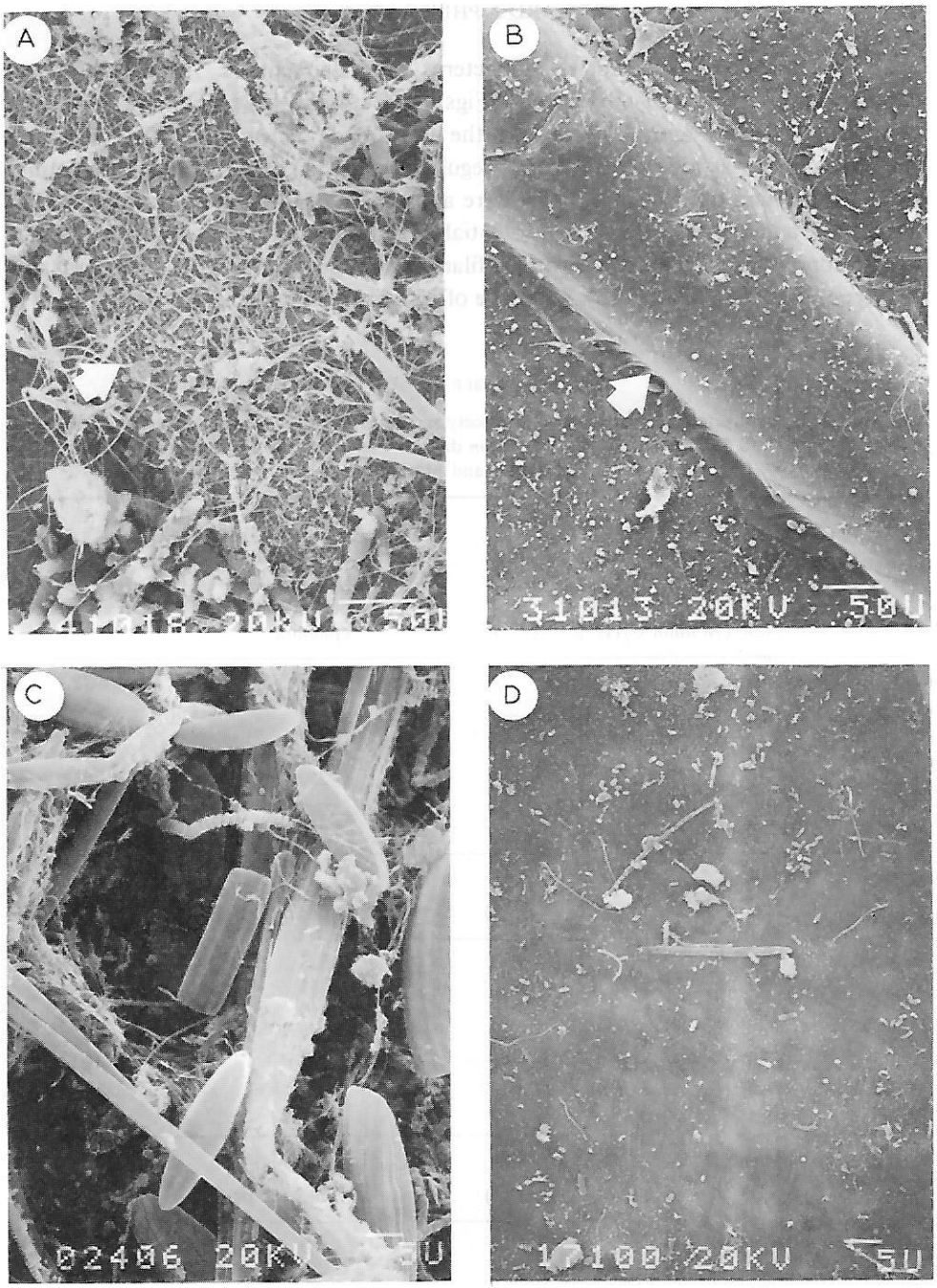


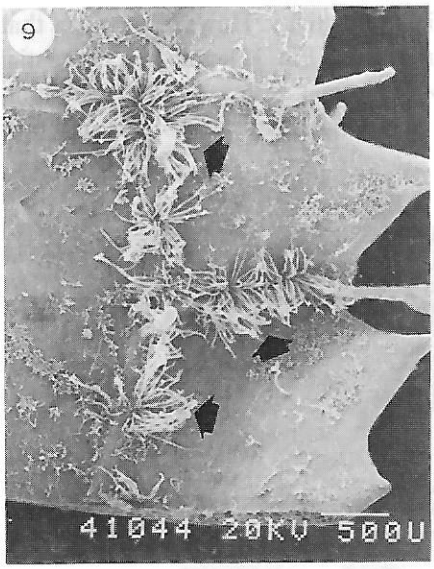
Fig. 8. Epiphytic colonization of new (B) and older (A) blades of the pelagic *Sargassum fluitans*, and new (C) and old (D) blades of benthic *S. filipendula*: white arrows indicate the presence of an epifaunal hydroid; the hydroid polyps are connected by tubular bodies which form a matrix on the surface of the *Sargassum*; the body of the hydroid in "B" is ≈ 150 μm in diameter; the hydroid in "A" is almost completely obscured by the heavy epiphytic colonization; these hydroids can also be seen on two different scales in Figs. 9 and 10; the presence of epiphytic *Calothrix* spp. is shown by black arrows; SEM photomicrograph; U, μm.

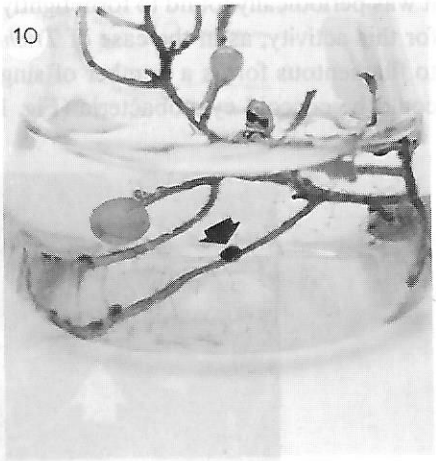
bacteria. The most prominent cyanobacteria were filamentous. Among the most abundant were tapered *Calothrix* species (Figs. 7C, 8A, 9 and 10) which were commonly found on *S. fluitans* and *S. natans*. In the case of *S. filipendula*, a *Nostoc* sp. of cyanobacterium was also observed with regularity (Fig. 11) and the conspicuous tufts of *Calothrix* found on pelagic samples were absent. All of these cyanobacteria exhibit heterocysts and therefore have the potential of carrying out nitrogen fixation under aerobic conditions. The most widespread filamentous species was a phycoerythrin rich LPP type (Figs. 7A, 7D and 12). This type of cyanobacterium lacks heterocysts and is

TABLE II

Comparison of cyanobacterial density and rates of acetylene reduction for new and old blades of *Sargassum fluitans*, *S. natans* and *S. filipendula*: g = g *Sargassum* dry wt.; methods used for counting are described in Materials and Methods.

Sample 1: *S. natans*			
		Old (809 nmol $C_2H_4 \cdot g^{-1} \cdot h^{-1}$)	
	New (96 nmol $C_2H_4 \cdot g^{-1} \cdot h^{-1}$)	Associated with epifauna	Not associated
Counts	64	195	72
	6	219	55
	34	229	131
	56	356	88
	19	250	96
Mean	36	250	88
Sample 2: *S. fluitans*			
	New (211 nmol $C_2H_4 \cdot g^{-1} \cdot h^{-1}$)	Old (1374 nmol $C_2H_4 \cdot g^{-1} \cdot h^{-1}$)	
Counts	61	322	209
	67	251	166
	39	419	75
	101	388	99
	15	401	192
Mean	57	357	148
Sample 3: *S. filipendula*			
	New (489 nmol $C_2H_4 \cdot g^{-1} \cdot h^{-1}$)	Old (56 nmol $C_2H_4 \cdot g^{-1} \cdot h^{-1}$)	
Counts	221	16	
	332	12	
	191	28	
	184	33	
	252	22	
Mean	236	22	





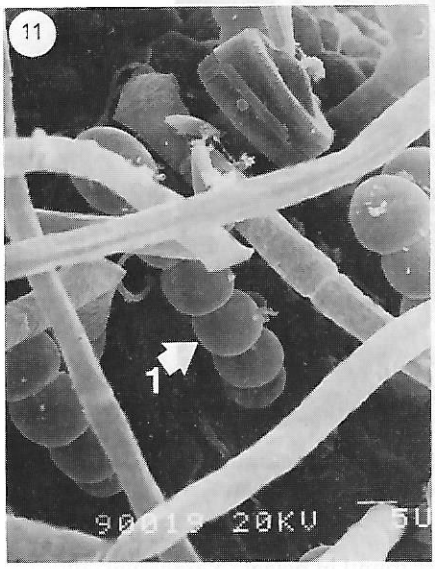


Fig. 9. Tufts of cyanobacterial filaments (black arrows) associated with epifaunal hydroids on the surface of a *Sargassum fluitans* blade: the hydroid bodies form a connecting matrix on the surface of the *Sargassum*, with polyps protruding perpendicularly from the surface as indicated by the arrow; SEM photomicrograph; U, μm.

Fig. 10. Tuft of *Calothrix* type cyanobacteria (arrow) on a blade of *Sargassum natans* from the Miami area: this type of formation was commonly found on floats of *S. fluitans* and *S. natans* which exhibited high nitrogen fixation activity; close-up photograph of algae in 25-ml flask; also discernable are the epifaunal hydroids, e.g. arrow.

Fig. 11. Epiphytic colonization of *Sargassum filipendula*: arrow 1 indicates the presence of a *Nostoc* type cyanobacterium; SEM photomicrograph; U, μm.

Fig. 12. Tightly wound bundle of LPP type cyanobacterial filaments: SEM photomicrograph; U, μm.

not normally associated with aerobic nitrogen fixation activity. However, the fact that it was periodically found to form tightly woven trichomes (Fig. 12) indicates a potential for this activity, as in the case of *Trichodesmium* (Bryceson & Fay, 1981). In addition to filamentous forms a number of single-celled microorganisms were observed which could be coccoid cyanobacteria (Fig. 13).

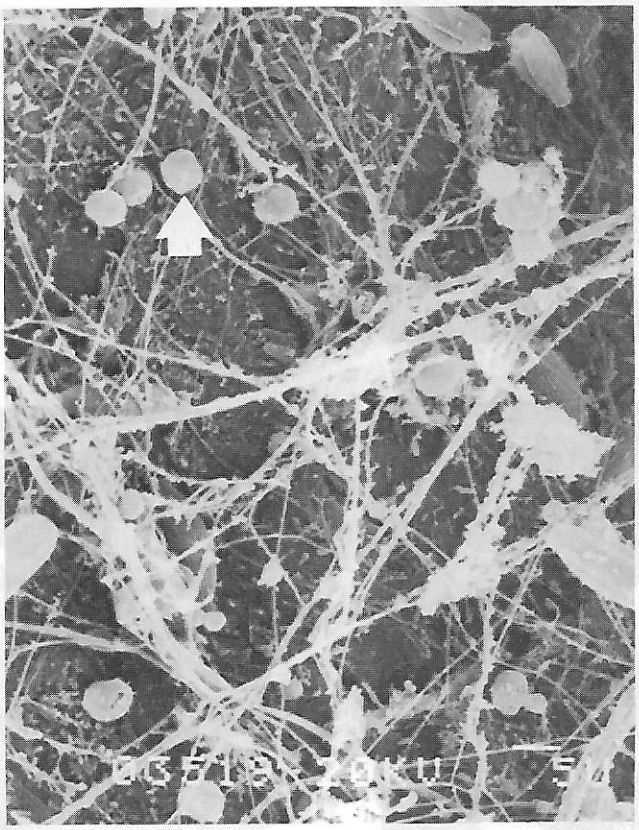


Fig. 13. Spherical microorganisms (white arrow) found on the surface of *S. fluitans* and *S. natans*: these may be coccoid cyanobacteria; SEM photomicrograph; U, μm.

By far the highest rates of nitrogen fixation were found in pelagic *Sargassum* collected from the St. Augustine area in July. These *Sargassum* samples exhibited heavy epiphytic populations including exceptionally large quantities of cyanobacterial tufts (i.e. *Calothrix* spp.).

Discussion

EFFECT OF ENVIRONMENTAL FACTORS ON NITROGEN FIXATION WITHIN THE *SARGASSUM* COMMUNITY

Aerobic nitrogen fixation in the *Sargassum* community is strongly light dependent which indicates that cyanobacteria (i.e. blue–green algae) are responsible for most of the activity. This reaffirms the importance of cyanobacteria in nitrogen fixation within the marine environment (Fogg, 1978, 1982; Capone & Carpenter, 1982).

The response of nitrogen fixation to light intensity reveals a surprisingly low light requirement for saturation. The experimental results indicate that the saturation light intensity for nitrogen fixation is 25–100 $\mu E \cdot m^{-2} \cdot s^{-1}$. This is approximately one tenth of the value observed for saturation of photosynthesis by the host *Sargassum* (Fig. 5). This low saturation intensity probably enhances the ability of cyanobacterial epiphytes to maintain high rates of nitrogen fixation below the surface canopy of *Sargassum* blades or during periods of low light availability (e.g. dawn and dusk). Similarly low values for saturation light intensity have been observed for nitrogenase activity in other species of tropical marine cyanobacteria (Phlips & Mitsui, 1983).

At the other end of the light intensity scale full sunlight (i.e. 2000 to 2500 $\mu E \cdot m^{-2} \cdot s^{-1}$) does not inhibit nitrogen fixation (Table I). This observation matches the lack of high light intensity (i.e. 2000 $\mu E \cdot m^{-2} \cdot s^{-1}$) inhibition of photosynthesis in both pelagic and benthic *Sargassum* (Fig. 5). Apparently, these cyanobacterial epiphytes and their macroalgal hosts are well adapted to strong sunlight, as might be expected for organisms which inhabit the surface layers of tropical marine environments (Phlips & Mitsui, 1982a and b).

RELATIONSHIP BETWEEN NITROGEN FIXATION ACTIVITY AND MICROBIAL EPIPHYTES

Notwithstanding the physical and chemical factors which can regulate nitrogen fixation activity in the *Sargassum* community, the most important determinant of that potential is the population size of the epiphytes themselves. The results of this study indicate that there is a relationship between the quantity of epiphytes on *Sargassum* and nitrogen fixation activity (Table II). However, the distribution and character of the epiphytic communities are not the same in benthic and pelagic species, or even within individual *Sargassum* floats.

Flotation vesicles of pelagic *Sargassum* keep the entire alga at the surface of the water column. In this sense pelagic *Sargassum* may best be classified as hyponeustonic. Consequently all parts of the algae, new or old, are exposed to similar environmental conditions (e.g. light and temperature). In addition, the relatively small seasonal variation of conditions in the tropical (or subtropical) Atlantic, and apparent lack of a reproduction phase in pelagic *Sargassum*, contribute to environmental stability. The development of epiphytic communities on *Sargassum* is certainly time dependent but

also appears to be retarded at early stages of plant development. Newer portions of the algae (i.e. recent growth) have few epiphytes, while older portions of the same patch may be heavily colonized (Fig. 8). If algae are subdivided into old and new fractions, the rates of nitrogen fixation activity are significantly different (Figs. 2–4), reflecting disparities in the size and quality of the epiphytic community. There are various factors which may affect the rate of fouling. Conover & Seiburth (1964) have suggested that *Sargassum* releases phenolic compounds which inhibit fouling. It is possible that younger blades release more of these compounds than older blades, thereby delaying colonization by epiphytes. The fact that epiphytes seem to prefer colonizing the surface of epifaunal hydroids rather than the *Sargassum* surface itself supports this hypothesis (Fig. 8). It is also possible, however, that the hydroids release compounds which stimulate the attachment and growth of epiphytes, or that the surface characteristics of hydroids are more suitable for attachment. Considering the limited amount of free nutrients available in the open ocean, it is possible that growth-stimulating substances (e.g. CO_2, phosphate) released by hydroids stimulate the development of epiphytes. Alternatively photosynthetic oxygen evolution by *Sargassum*, may partially inhibit the development of bacterial populations (including cyanobacteria).

The key to the occurrence of very high N_2 fixation activity appears to be the existence of cyanobacterial tufts (Figs. 9 and 10). This is exemplified by the exceptionally high levels of activity observed during periods of pronounced tuft development. Both species of pelagic *Sargassum* exhibited these clusters of *Calothrix* trichomes, but not necessarily at the same time.

In the case of the benthic *S. filipendula*, the pattern is substantially different. *S. filipendula* grows attached to rocky substrata of shallow estuarine environments. Flotation vesicles keep the algae vertical in the water column. Most of the biomass is located towards the upper portion of the thallus, where more light is available. Nitrogen fixation activity is also higher in this newer portion of the algae, perhaps for the same reason, or because the photosynthetically more active blades release more nutritional compounds used by epiphytic cyanobacteria (e.g. as electron donors for nitrogen fixation, or growth stimulating substances). The fact that newer portions of these algae demonstrate high activity may indicate a basic difference in bacterial colonization of estuarine and pelagic *Sargassum* species.

SEASONAL CHANGES IN ACTIVITY

The benthic *Sargassum* community also differs from the pelagic in the sense that nitrogen fixation is subject to seasonal variability. Activity is low during the winter and jumps dramatically in the spring along with a marked increase in biomass. From an analysis of variance it was shown that this variation in activity was statistically significant (d.f. = 23, $P < 0.01$). The low activity in winter may be related to the reproductive cycle of this species. During the fall and winter rates of photosynthesis in *S. filipendula* are very low (C. Dawes, pers. comm.) and much of the standing crop

produced during the spring and summer is lost. Winter is also characterized by substantially lower temperatures and changes in river runoff, all of which may play a role in determining the nature of nitrogen fixation. There is an apparent coincidence of reduced water temperature (Fig. 1) and low nitrogen fixation activity in the estuarine environment, but these parameters may be autocorrelated. It was noted that incubation of winter *Sargassum* samples at 30 °C yielded little enhancement of the very low levels of activity observed at ambient temperatures.

In the case of pelagic *Sargassum*, high rates of nitrogen fixation were observed year around, despite the existence of significant monthly variability. The pattern of variability was, however, quite different from that observed in benthic *Sargassum*, lacking a distinct seasonality. Peaks of activity were observed throughout the year. This is analogous to the year around fluctuations of productivity observed in warm oceanic environments by numerous researchers (Parsons *et al.*, 1977) and could be related to pulsing of other limiting elements, like phosphate.

ROLE OF NITROGEN FIXATION IN THE NITROGEN CYCLE OF THE *SARGASSUM* COMMUNITY

The final and broadest question which must be asked is how it affects present views of the quantitative impact of nitrogen fixation on the nitrogen budget of the *Sargassum* community. Capone & Carpenter (1982) estimated that the average rate of nitrogen fixation associated with *Sargassum* is 1.5 $\mu g\ N \cdot g^{-1}$ (*Sargassum* dry wt.) $\cdot\ h^{-1}$. The observations presented in this paper indicate that this figure may be on the low side. For example, it is 39 times less than the average rate of fixation observed at the St. Augustine sampling site in July (i.e., 57.91 $\mu g\ N \cdot g^{-1}$ (*Sargassum* dry wt.) $\cdot\ h^{-1}$). This is the most directly comparable sample because of its proximity in time and space to the areas studied by Hanson (1977) and Carpenter (1972). It should not, however, be concluded that this is representative of all pelagic *Sargassum* communities. The monthly averages for nitrogen fixation in the Miami area ranged from 1.9 to 13, and 0.9 to 14.5 $\mu g\ N \cdot g^{-1}$ (*Sargassum* dry wt.) $\cdot\ h^{-1}$, for *S. fluitans* and *S. natans*, respectively. The average rate for benthic *Sargassum* (*S. filipendula*) was 5.06 $\mu g\ N \cdot g^{-1}$ (*Sargassum* dry wt.) $\cdot\ h^{-1}$ during the spring and summer (April to September) and 0.43 $\mu g\ N \cdot g^{-1}$ (*Sargassum* dry wt.) $\cdot\ h^{-1}$ during the fall and winter (October to March). As a relative measure of the significance of these rates, it is possible to compare the monthly means of nitrogen fixation activity with production figures for *Sargassum*. Two preliminary assumptions must be made: (1) the C : N ratio of *Sargassum* is 20 : 1 (Hanson, 1977), and (2) gross production of pelagic *Sargassum* is $\approx 930\ \mu g\ C \cdot g^{-1}$ (*Sargassum* dry wt.) $\cdot\ h^{-1}$ (Hanson, 1977), while that of the benthic species is perhaps five-fold higher during the spring as indicated by the P_{max} values in Figs. 5 and 6. Based on a combination of these two assumptions, the mean monthly rates of nitrogen fixation by *S. fluitans* in the Miami area represent 4–29% of the nitrogen requirement for maximum rates of production. For *S. natans* the values range from 2–32%. The high rates of

nitrogen fixation observed at the St. Augustine site yield even higher percentages, i.e. 106% for *S. fluitans* and 143% for *S. natans*. In contrast the rates observed for the benthic *Sargassum* community would represent only 2.2% of the estimated nitrogen requirement. Unfortunately, the actual efficiency and mechanism by which fixed nitrogen is transferred to *Sargassum* is not known. Seasonal data on the productivity of pelagic *Sargassum* is also absent. These limit the practical usefulness of these types of comparisons, but do not detract from their heuristic value.

It is clear that nitrogen fixation may be subject to regional and seasonal variability. The results for the St. Augustine site indicate that *Sargassum* communities can have very high rates of activity. At these rates the contribution of nitrogen fixation to the formation of combined nitrogen is highly significant (i.e. $\sim 100\%$ of estimated demand for community production). A more definitive statement about the specific level of contribution will require further investigation of regional variation in N_2 fixation activity and seasonal variability of *Sargassum* productivity, as well as a more thorough understanding of the overall nitrogen cycle within these communities.

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