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PRIMARY PRODUCTIVITY AND NITROGEN FIXATION IN TWO MACROALGAE-CYANOBACTERIA ASSOCIATIONS

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ABSTRACT

Primary productivity and nitrogen fixation (acetylene reduction) were estimated in two macroalgae-cyanobacteria associations from coral reef communities. *Microdictyon* sp., collected at 20 m on the reef proper and *Laurencia* sp., from 1–2 m on an inshore sand flat, showed high productivity rates: 1.15 and 1.90 mg C g^{-1} h^{-1}, respectively. Dissolved organic carbon excretion for both associations was less than 0.5% of the total carbon fixed. Nitrogenase activity was much higher in the *Laurencia* association than in the *Microdictyon* association (9.61 vs. 1.04 μg N g^{-1} h^{-1}); this probably was due to differences in the cyanobacterial epiflora. The effect of light on the rates of photosynthesis and nitrogen fixation was evaluated for the *Microdictyon* association. Nitrogen fixation supplied approximately 2% of the nitrogen demand of the *Microdictyon* complex and 19% of the demand on the *Laurencia* complex. In a nutrient poor environment, any *de novo* nutrient input is of value; for the *Laurencia* association, nitrogen fixation could supply a substantial portion of the nitrogen required for photosynthesis.

Macrophyte-epiphyte interrelationships have received considerable attention in recent years. Experiments have been designed to determine the role of each component in primary productivity and nutrient cycles in marine systems. For example, the relationship between *Zostera marina* and its epiphytes in the processes of primary productivity and dissolved carbon excretion has been investigated (Penhale, 1977; Penhale and Smith, 1977; Sand-Jensen, 1977). The transfer of photosynthetic products between a macroalga and its epiphytes was reported by Harlin and Craigie (1975). The transfer of carbon, nitrogen and phosphorus within a macrophyte-epiphyte association has been demonstrated by Harlin (1973), McRoy and Goering (1974), Wetzel and Penhale (1979), and Penhale and Thayer (1980). Nitrogen fixation by *Thalassia testudinum* epiphytes may supply a considerable portion of the nitrogen required for production (Capone et al., 1979).

Nitrogen fixation also has been shown to occur in several macroalgae-epiphyte complexes; this process may provide a substantial part of the nitrogen requirement of the association, thereby contributing to their success in oligotrophic environments. Carpenter (1972) reported significant nitrogen fixation activity by *Dichothrix,* a cyanobacterial epiphyte attached to pelagic *Sargassum.* Hanson (1977) estimated that nitrogen fixation in a *Sargassum*-epiphyte complex could supply about 40% of the nitrogen required. Head and Carpenter (1975) detected nitrogen fixation by the heterotrophic bacteria attached to *Codium.* High rates of nitrogen fixation by cyanophytes associated with several species of tropical macroalgae were noted by Capone (1977) and Capone et al. (1977).

This paper reports the results of a primary productivity and nitrogen fixation study of two macroalgae-epiphyte associations from coral reef communities. The aim was to determine the rates of the two processes and to estimate the contribution of nitrogen fixation to the nitrogen requirement of the complex.

MATERIALS AND METHODS

This study was carried out during October 1976, at Churchill Beach, Grand Bahama Island (26°31′N, 78°34′W; R. V. CALANUS C7534). Two macroalgae-epiphyte complexes from a coral reef community

were chosen for primary productivity and nitrogenase activity estimates. *Microdictyon* sp. was collected from a fringing coral reef at a depth of approximately 20 m. *Laurencia* sp. was collected from an inshore sand flat at a depth of 1–2 m. The plants were maintained at ambient temperature in a flowing seawater system for up to 3 h prior to experimentation. Care was taken to keep the plants shaded to avoid adverse physiological responses to surface light.

The macroalgae were assayed for nitrogenase activity by the acetylene reduction method (Hardy et al., 1968). Samples of macroalgae and associated epiphytes were placed in 21.5 ml serum bottles (0.05–0.15 g dry wt per bottle) with 5 ml of filtered seawater (Millipore 0.45 μm pore size). After sealing with serum stoppers and preincubating for 10 min, C_2H_2 was added to a final concentration of 10% v/v in air. Previous experimentation has demonstrated the linearity of C_2H_4 production in these assays for up to 12 h (Capone et al., 1977; Capone, 1977). Incubations were carried out for 4 h onboard ship in a 28 ± 1°C water bath illuminated with fluorescent lighting (approximately 250 μE m^{-2} sec^{-1}) or at depth under in situ conditions. For certain experiments using *Microdictyon*, samples were incubated on deck in a running seawater system under neutral density screens which allowed penetration of 30, 10, 3 and 0% of ambient light. Gas samples were assayed for C_2H_4 production by gas chromatography using a Varian model 1700 gas chromatograph equipped with a Porapak R (100–120 mesh) column (10′ × ⅛″). A molar ratio of 3:1 was used to convert C_2H_4 evolved to N_2 fixed (Hardy et al., 1968).

Primary productivity was estimated by the ^{14}C technique adapted for macrophytes by Wetzel (1964). The macroalgae and associated epiphytes were placed in either 50 or 900 ml glass bottles filled with filtered seawater (Millipore 0.45 μm pore size) and incubated for 4 h with [^{14}C] $NaHCO_3$, either in situ or on deck as described above. After incubation, samples were quick frozen with dry ice. Samples were later lyophilized, weighed and combusted in a Packard model 306 tritium-carbon oxidizer. For certain experiments using *Microdictyon*, samples were incubated for 4 h with [^{14}C] $NaHCO_3$ in Millipore filtered seawater (0.22 μm pore size) under neutral density screens which allowed for the penetration of 50, 30, 15, 5 and 1% of ambient light. The excretion of dissolved organic carbon was estimated by the method described by Smith et al. (1977). All samples were counted in a Nuclear Chicago Mark II liquid scintillation counter. Corrections were made for background, counting efficiency and combustion recovery efficiency. Productivity and excretion were calculated according to Penhale (1977) and Penhale and Smith (1977).

Photosynthetically active radiation (400–700 nm) was measured with a Lambda meter with submersible quantum sensor. Lyophilized plant samples were analyzed for cellular nitrogen and carbon with a Perkin Elmer model 240 elemental analyzer using acetanilide as a reference standard. Standing stocks of *Microdictyon* and *Laurencia* were estimated by collecting material within a 0.2 m^2 aluminum frame. Nitrogen demands of the macroalgae-epiphyte associations were calculated by dividing the rate of particulate carbon production by the C:N ratio of the associations.

Results and Discussion

During the study, water temperatures ranged from 28–30°C at the inshore site and from 27–39°C on the reef proper. Surface light intensity at midday averaged 2,000 μE m^{-2} sec^{-1}. Standing stocks for the macroalgal associations averaged 234 ± 22 g dry wt m^{-2} (±SE, n = 3) for *Laurencia* and 64 g dry wt m^{-2} (n = 2) for *Microdictyon*. The *Laurencia* association has a nitrogen content of 0.94% while *Microdictyon* association has an average nitrogen content of 1.05%.

High rates of in situ primary productivity were found for both macroalgae-epiphyte associations (Table 1). The rates of dissolved organic carbon excretion (DOC) were low, in both cases less than 0.5% of the total carbon fixed. These low excretion rates are consistent with the macroalgal values of 1–4% reported by Brylinsky (1977) and 0.3–1.5% reported by Harlin and Craigie (1975) but lower than the 30–40% rates reported by Khailov and Burlakova (1969), Sieburth (1969) and Hanson (1977). The wide variation in DOC excretion estimates may be due to differences, methodologies, the macroalgal species tested, or may be a function of the physiological state of algae at the time of experimentation.

Nitrogenase activity was nearly ten-fold greater in the *Laurencia* association than in the *Microdictyon* association (Table 1). This activity has been previously attributed to epiphytic cyanobacteria (Capone, 1977; Capone et al., 1977). The differences in activity noted for the two associations may be attributed to qualitative and quantitative differences in the cyanobacterial epiflora. We were not

Table 1. In situ carbon fixation in the particulate and excreted fraction (mg C g dry wt^{-1} h^{-1}) and nitrogen fixation (μg N g dry wt^{-1} h^{-1}) associated with two macroalgae-epiphyte complexes. Light levels were approximately 300 and 1,800 μE m^{-2} sec^{-1} for *Microdictyon* and *Laurencia*, respectively. Results are mean values ± SE with n in parentheses

	Carbon Fixation		Nitrogen Fixation
	Particulate	Excreted	
Microdictyon	1.150 ± 0.218 (4)	0.004 ± 0.001 (4)	1.04 ± 0.13 (9)
Laurencia	1.180 ± 0.371 (4)	0.003 ± 0.001 (4)	9.61 ± 1.03 (4)

able to separate the macrophyte-epiphyte complex for biomass estimates of each component. *Calothrix* sp. occurred on both macroalgae but was in greater abundance on the surface of *Laurencia*. Additionally, an extensive mat of non-heterocystous species (*Oscillatoria* sp., *Plectonema* sp.) was evident on *Microdictyon*. Heterocystous cyanobacteria, such as *Calothrix* sp., have generally been implicated as the most important agents of nitrogen fixation in aerobic, photic environments (Webb et al., 1975; Potts and Whitton, 1977; Capone and Taylor, 1977). However, recent evidence indicates that non-heterocystous forms may fix significant amounts of nitrogen in some environments (Carpenter and Price, 1977; Potts and Whitton, 1977; Pearson et al., 1979). The distinct environmental regimes experienced by each association also could contribute to the differences in nitrogenase activity. The *Laurencia* association which occurs in a shallow, high light intensity area, may be adapted to sustain higher rates of light dependent N_2 fixation.

The effect of light on the photosynthetic rate of *Microdictyon* showed the usual pattern observed in algae, with photosynthesis increasing with increasing light to an asymptotic value where the system becomes light saturated; this occurred at 1,000 μE m^{-2} sec^{-1} or approximately 50% of surface light (Fig. 1). No photoinhibitory effect on photosynthesis was observed despite the fact that *Microdictyon* was collected at 20 m where light penetration is generally 15–20% of surface light. The DOC excretion rates were low at all light levels and were generally less than 1% of the total carbon fixed. Wanders (1976a) measured the productivity of several types of shallow coral reef algae at different light intensities. In contrast to *Microdictyon*, these algae grew in a high light environment at 1.5 m where they reached the compensation point at 30 min past sunrise and were light-saturated during most of the midday. Similar to *Microdictyon*, they generally did not show effects of photoinhibition.

Nitrogenase activity associated with *Microdictyon* showed inhibition above the 30% surface light intensity (Fig. 1). Nitrogen fixation increased with increasing light to a peak at 220 μE m^{-2} sec^{-1} (the in situ light level) beyond which the rates decreased. This could reflect a low light adaptation of the cyanobacteria with photoinhibition occurring at high light intensities. In addition, a wavelength adaptation has been suggested in previous work (Capone, 1977). After a preincubation in white light (125 μE m^{-2} sec^{-1}), nitrogenase activity proceeded at higher rates under blue light (450–550 nm) than red light (>600 nm) (Capone, 1977). Nitrogenase activity by cyanobacteria is dependent ultimately on photosynthesis for energy. Cyanobacteria are capable of adapting to changes in light quality by shifts in their array of photosynthetic accessory pigments. Changes in the ratios of phycobiliproteins (phycocyanin and phycoerythrin) in response to spectral shifts have been documented for some cyanobacteria (Fujita and Hattori, 1962); this phenomenon has been termed "complementary chromatic adaptation" by Bo-

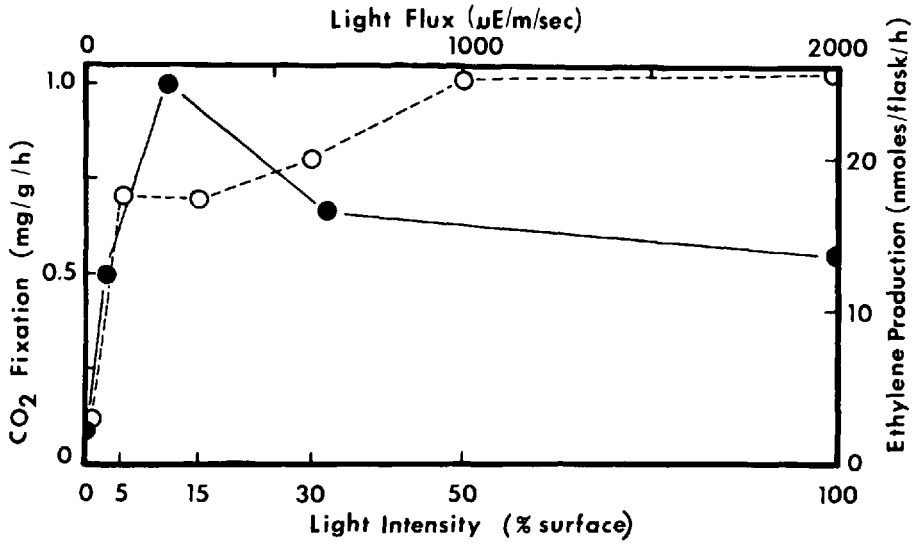

Figure 1. Carbon fixation (O) and nitrogenase activity (●) associated with a *Microdictyon*-epiphyte complex at 29°C at various light levels.

gorad (1975). Future research on light adaptation of these cyanophytes should detail responses of both processes to differences in light quality and quantity as a function of pigment content.

The role of benthic algae in the carbon and nitrogen cycles of coral reef communities has been the subject of several recent investigations. Primary productivity rates are generally high; for example, Wanders (1976a) estimated net productivity of three algal types on a coral reef at Curaçao to be about 77 g C horizontal m^{-2} yr^{-1}. For a *Sargassum* community inshore from a coral reef on the northeast coast of Curaçao, annual primary productivity was estimated at 1,550 g C m^{-2} (Wanders, 1976b). Smith (1973) estimated net productivity rates of two reef flat transects at Eniwetok as 0.72 and 0.25 g C m^{-2} h^{-1} for an algal transect and a coral algal transect, respectively.

These high primary productivity rates occur in tropical marine waters which are generally nutrient poor. Thus, attention has focused on nutrient cycles, particularly the nitrogen cycle, of coral reef communities. Webb et al. (1975) reported a net export of combined nitrogen in water flowing over coral reefs and adjacent reef flats near Eniwetok Atoll. Further investigations (Webb and Wiebe, 1975; Wiebe et al., 1975) suggested that nitrogen fixation and bacterial nitrification resulted in the nitrate enrichment.

Nitrogen fixation is of particular interest as it represents a *de novo* nitrogen source. It is not limited to one component of the coral reef community. Nitrogen

Table 2. Estimates of nitrogen demand and supply to support the production of an inshore *Laurencia*-epiphyte association and a coral reef *Microdictyon*-epiphyte association

	C:N	N-Demand μg N g dry wt^{-1} h^{-1}	N_2 Fixation % Demand
Microdictyon	21	54.8	1.9
Laurencia	25	47.2	19.2

fixation has been reported to occur in macroalgae-epiphyte associations on the reef proper and on inshore flats (Capone, 1977; Capone et al., 1977), in cyanobacterial mats of inshore reef and lagoon flats (Wiebe et al., 1975; Potts and Whitton, 1977), in coral reef flat sediments (Hanson and Gundersen, 1976), and on coral rubble sediments (Crossland and Barnes, 1976). In addition, nitrogen fixation has been reported by cyanobacterial tufts on the reef proper (Mague and Holm-Hansen, 1975) and by symbiotic cyanobacteria in coral reef sponges (Wilkinson and Fay, 1979).

In the present study, we estimated the contribution of nitrogen fixation to the nitrogen requirement of the two macroalgal-cyanobacterial associations from C:N ratios and productivity rates (Table 2). Nitrogen fixation supplied approximately 2% of the nitrogen demand of the *Microdictyon* complex while 19% was supplied to the *Laurencia* complex. These estimates probably represent minimum values since any degree of nutrient retention and recycling by the association would lessen the actual nitrogen requirement, and thereby increase the significance of the input by nitrogen fixation. In nutrient poor waters, any *de novo* nutrient input is of value to the community; in the case of the *Laurencia* association, nitrogen fixation supplied a substantial portion of the nitrogen required for photosynthesis. The relationship between primary productivity and nitrogen fixation, as well as other nitrogen transformations in the coral reef community, deserves further study.

Acknowledgments

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