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Nutrient Limitation of the Macroalga, *Penicillus capitatus*, Associated with Subtropical Seagrass Meadows in Bermuda

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ABSTRACT: Nutrient limitation of the rhizophytic macroalga *Penicillus capitatus* found associated with subtropical seagrass meadows in Bermuda was determined from enrichment assays and subsequent tissue analyses. The photosynthetic response of *P. capitatus* to additions of inorganic nitrogen (N) or phosphorus (P), measured as oxygen evolution in closed incubation chambers, increased significantly in both the 16 h and 6 d experiments only with nitrogen enrichment. The average photosynthetic response for all treatments was virtually identical in the two experiments, indicating that there was not a significant time lag in nutrient uptake and that the short term (16 h) assay accurately reflected the longer term (6 d) photosynthetic response to nutrient enrichment. Average tissue nitrogen levels for the nitrogen-treated algae were 29% higher than the phosphorus-treated algae and 18% greater than the controls, corroborating the results from the photosynthesis assay. *P. capitatus* may acquire nutrients directly from sediment sources via rhizoid holdfasts. Ratios of total dissolved nitrogen (TN) to total dissolved phosphorus (TP) in pore water at 10 and 20 cm depths (6.1 and 4.5, respectively) indicate a nitrogen-limited nutrient pool. These low pore water TN:TP ratios may be a function of a limited sorptive capacity of the calcium carbonate sediments for phosphate, anthropogenic nutrient inputs, or high rates of denitrification, all of which would induce N rather than P limitation in these carbonate-rich sediments.

Introduction

Nutrient limitation of primary productivity in marine ecosystems is largely a function of the relative availabilities of the primary limiting nutrients, nitrogen (N) and phosphorus (P). In a recent review, Howarth (1988) suggested that differences in external nutrient inputs and in internal biogeochemical processes may explain why net primary production of tropical estuaries and lagoons may be phosphorus limited while temperate estuaries may be nitrogen limited. The extent to which the biogeochemical processes of nitrogen fixation and denitrification affect the availability of N relative to P may be influenced by water residence time (Smith 1984). In this paper, we are concerned specifically with nutrient limitation of benthic macroalgae, defined as limitation on the growth of current populations (sensu Howarth 1988). Results from short-term enrichment bioassays indicate that, despite the generalization of P-limited tropical marine ecosystems, tropical macroalgae do not show the same consistent response to N and/or P additions as do macroalgae from temperate estuaries. Nitrogen generally is considered to be the primary limiting nutrient for temperate macroalgae (Lapointe et al. 1976; Lapointe and Ryther 1979). For macroalgae in tropical and subtropical habitats, however, evidence suggests that some species may be simultaneously limited by nitrogen and phosphorus (Steffensen 1976; Gordon et al. 1981; Lapointe et al. 1987; Lapointe and O'Connell 1989), some may switch seasonally between limiting nutrients (Lapointe 1987), and some may be limited by either nitrogen (Lapointe et al. 1987; Littler et al. 1988) or phosphorus (Birch et al. 1981; Littler et al. 1988). Lapointe et al. (1987) further suggest that nutrient limitation may be both species- and habitat-specific, depending in part on ambient nutrient supplies.

High rates of benthic nitrogen fixation in shallow tropical waters (see review by Howarth et al. 1988) and carbonate geochemical processes (Short et al. 1985) may explain why benthic macrophytes are often phosphorus limited in tropical marine ecosystems. Numerous studies have reported the occurrence of nitrogen fixers associated with the root zone of tropical seagrasses (Patriquin and Knowles 1972; Capone and Taylor 1977) and as

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epiphytes on macroalgae and seagrasses (Carpenter 1972; Head and Carpenter 1975; Mague and Holm-Hansen 1975; Capone et al. 1977; Hanson 1977; Penhale and Capone 1981; see review by Howarth et al. 1988). In the carbonate-rich sediments typical of tropical lagoons, available dissolved phosphate pools in the pore water may be depleted by the high affinity of phosphate ions for calcium carbonate (Berner 1974; Kitano et al. 1978; Morse et al. 1987), further reducing the availability of phosphorus relative to nitrogen. However, Krom and Berner (1980) suggest that the accumulation of organic matter in some anoxic marine sediments may inhibit phosphate adsorption to calcium carbonate.

The strategy of nutrient uptake also may be important in determining which nutrient is primarily limiting to growth. Nutrient limitation may differ accordingly between those species capable of acquiring pore water nutrients and those for which the water column is the sole source. It has been suggested that certain rhizophytic species, attached to the substratum by means of rhizoid holdfasts, have the ability to utilize the high concentrations of pore water nutrients. Littler et al. (1988) compared nutrient limitation of four rhizophytic *Halimeda* species. They attributed the difference between nitrogen limitation in two epilithic (rock-dwelling) species and phosphorus limitation in two psammophytic (sand-dwelling) species to the ability of the latter to utilize pore water sources presumably having high N:P ratios. Williams (1984) showed directly that a related species, the siphonous green alga *Caulerpa cupressoides* could take up $^{15}NH_4^+$ from the substratum and translocate it throughout the thallus. Nutrient limitation of macroalgae, thus, may differ between species with extensive rhizoid holdfasts in sandy or muddy substrata and reef-dwelling or unattached, free-floating species, depending on the relative concentrations of nitrogen and phosphorus in the sediments and the water column.

In this study, we tested the general hypothesis that phosphorus limits primary productivity of tropical macroalgae for a rhizophytic species, *Penicillus capitatus,* found associated with subtropical seagrass meadows in Bermuda. This species is in the order Caulerpales and is composed of fused and branching filaments or strands lacking true cross walls. *P. capitatus* is attached to the substratum by a modified holdfast and typically occurs in sandy or muddy substrata associated with seagrasses throughout Bermuda's inshore waters (Sterrer 1986). This work is part of a broader study on the factors regulating growth and nutrient limitation of macroalgae associated with nearshore seagrass meadows in Bermuda, designed partly in response to growing concerns about the effects of eutrophication on the seagrass-macroalgal community. Understanding the mechanisms by which nutrient availability alters the competitive advantages of macroalgae may aid in predicting the response of seagrass-macroalgal communities to nutrient enrichment.

Site Description and Methods

Nutrient limitation of *P. capitatus* associated with *Thalassia testudinum* meadows in Bailey's Bay, Bermuda was determined using a nutrient enrichment assay in outdoor seawater tanks in August 1988. Bailey's Bay is a shallow (1–3 m), approximately 2×10^4 m^2, lagoon on the north shore of the island and is protected by a string of limestone islands (Fig. 1). Within the bay there was a steep (200 m) nutrient gradient, where the dissolved organic and inorganic nitrogen concentrations in the water column were 2 to 10 times higher in the inner part of the bay than the outer portion adjacent to the islands, presumably resulting from groundwater nutrient inputs from domestic cesspits. Dissolved organic and inorganic phosphorus concentrations were not significantly different between the two sites. At the study location in the outer, low nutrient, portion of Bailey's Bay, dissolved inorganic nitrogen concentrations ($NH_4^+ + NO_3^-$) in the water column averaged 1.5 μM, and dissolved inorganic phosphorus concentrations averaged <0.05 μM (McGlathery 1992). The sediments in this area consisted of coarse sands and shell fragments and smelled strongly of hydrogen sulfide.

Algal specimens were collected from the outer portion of Bailey's Bay on the morning of the experiment, cleaned of obvious epiphytes and loose sediments, and allowed to acclimate for approximately 6 h in the outdoor 20-l tanks. Seawater was collected from the same location on the same day and used in the experiments to ensure that the control accurately reflected the ambient low nutrient conditions in the field. We randomly assigned four replicate algal samples to one of three nutrient treatments, +N, +P, and control, and added nutrients in a batch design as 20 μM $(NH_4)_2SO_4$ and 1.25 μM KH_2PO_4. These concentrations represented approximately 10 times the ambient nutrient concentrations in the water column, but were within the range to which this species is typically exposed in Bermuda's inshore waters (A. Knap, personal communication). Each 20-l treatment tank was placed within a 110-l flowing seawater tank to maintain temperatures at levels typical of field conditions (26–30°C). Light was regulated at field levels of 800–1900 μE m^{-2} s^{-1} using shadecloth; these levels are well within the

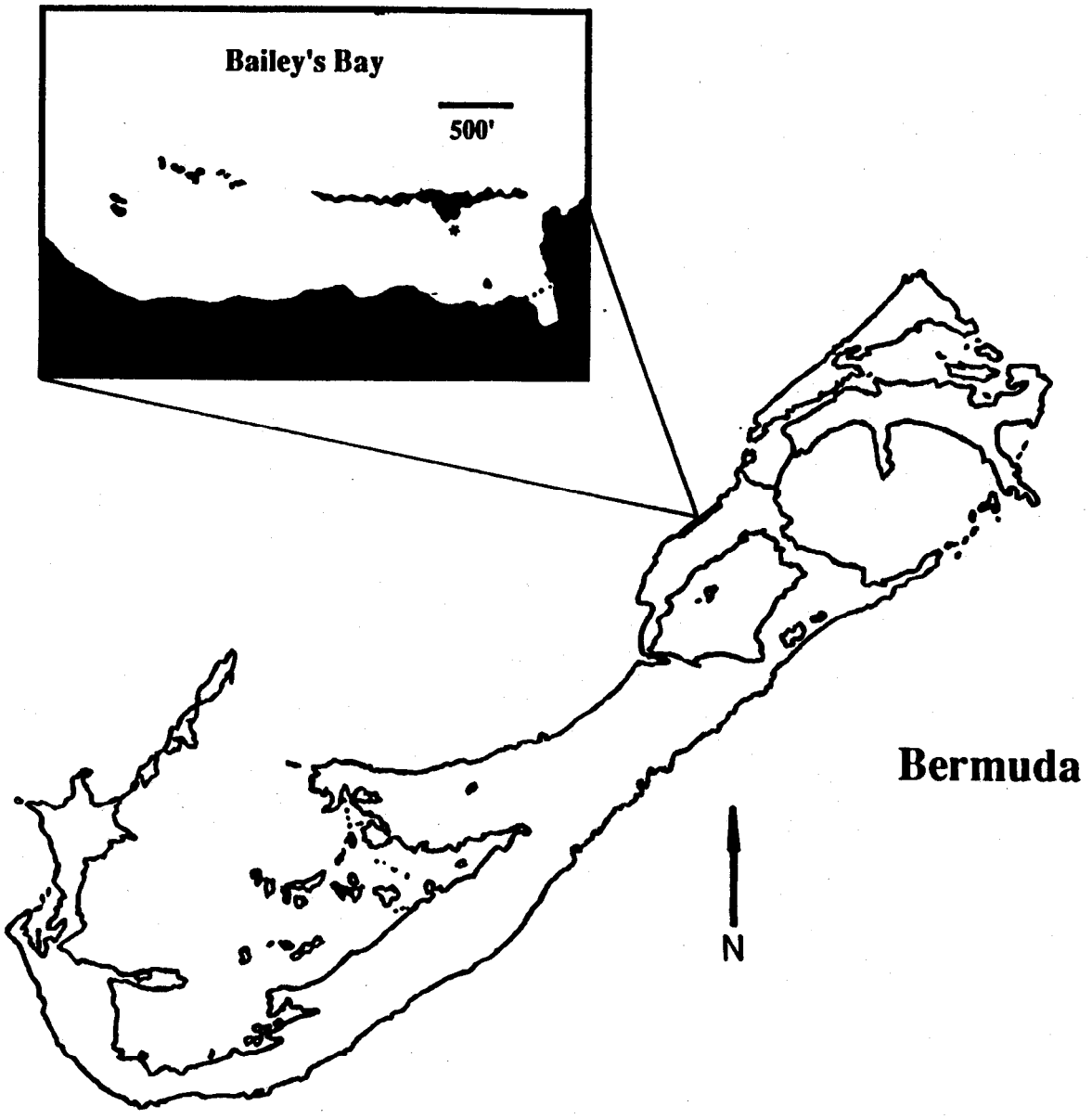

Fig. 1. Study location in Bailey's Bay, Bermuda. Algal samples and treatment water for nutrient enrichment experiment and porewater nutrients were taken from the outer, low nutrient, portion of the bay adjacent to the island, indicated by the *. Dark portions of insert are land masses.

range of light intensities known to saturate photosynthesis for several shallow, tropical macroalgae (Lapointe 1987; Littler et al. 1988; Lapointe and O'Connell 1989). There were no significant differences in either light or temperature conditions between the treatment tanks, and both the 16-h and 6-d experiments were carried out under similar conditions. Filtered compressed air provided circulation within the tanks during the treatment periods. Salinity was constant between 35‰ and 36‰.

We determined a short-term (16 h) and longer term (6 d) response of *P. capitatus* to nutrient enrichment. In the 6-d experiment nutrients and water were replenished every 2 d. We understand that these experimental conditions may not perfectly reflect field conditions; however, we are interested in the relative photosynthetic rates in order to determine nutrient limitation, and we expect any experimental artifacts to affect all treatments equally. After the enrichment period, we evaluated nutrient limitation as the photosynthetic response, de-

termined by oxygen production of whole thalli in separate, closed 1-l glass chambers which were incubated under field light and temperature conditions (Lapointe et al. 1987). Oxygen saturation at the end of a 4-h incubation period was measured and compared with initial saturation using a YSI model 53 oxygen meter equipped with a BOD probe which fit into the neck of the incubation chamber. The chambers were stirred by hand at 15-min intervals during the incubation period and continuously during each oxygen measurement to reduce boundary layer effects. We ran three replicate seawater blanks for each treatment to determine oxygen production or consumption associated with phytoplankton or bacteria in the water and corrected sample values accordingly. Oxygen production was normalized to grams dry weight, determined by drying samples (at 60°C) to a constant weight.

Tissue concentrations of nitrogen and phosphorus at the end of the experiment were measured and compared between treated and control algae to determine if differences in tissue nutrient levels corroborated the results of the photosynthetic responses to nutrient enrichment. Algal samples were dried at 60°C to a constant weight, ground to a powder with mortar and pestle, and analyzed for nitrogen using a Carlo-Erba NA 1500 CNS analyzer. Tissue phosphorus was determined by dry-ashing at 550°C for 6 h, acid dissolution at 104°C, and subsequent colorimetric PO_4^{3-} analysis (modified from Stainton et al. 1974). The recovery and analytical precision of the phosphorus technique was determined from replicate National Bureau of Standards citrus leaves (0.13 dry wt. %P) to be 102% ± 6% (n = 4) for dry weights of standards containing between 6.4 mg P and 7.0 mg P. Tissue nitrogen and phosphorus content are reported as percent of sample dry weight.

We measured pore water nutrient concentrations at 10 cm and 20 cm depths in the vicinity of macroalgal sample collection. Pore water at each depth was sampled using lysimeters constructed of PVC and nylon, with a sampling chamber fitted with 20-μm polyethylene frits. The lysimeters were filled with filtered Sargasso seawater, degassed to reduce oxygen levels to approximately 10% saturation, and placed in the field at the desired depth 24 h before sampling to minimize disturbance effects. Pore water was extracted by applying suction through a sample port; the first 10 ml were discarded and 40 ml subsequently were taken for analysis of total dissolved nitrogen and phosphorus. Water samples were immediately pressure filtered through 0.45-μm Magna nylon filters into vacutainers and kept on ice in the dark while in the field. Samples were acidified with 1.2 N HCl and

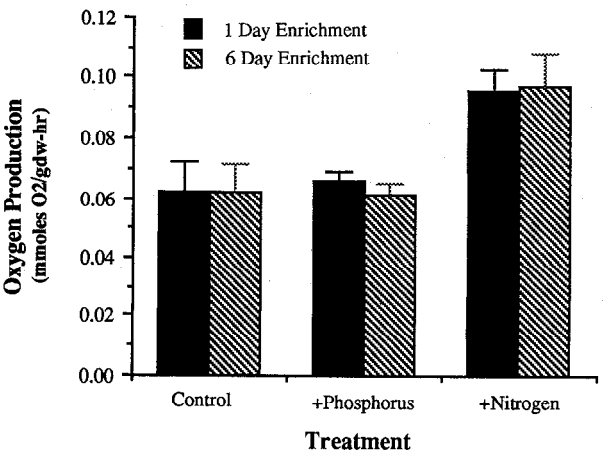

Fig. 2. Mean photosynthetic response (n = 4) of *Penicillus capitatus* from Bailey's Bay to enrichment of inorganic nitrogen ($(NH_4)_2SO_4$) or phosphorus (KH_2PO_4), measured as oxygen production after a 4-h incubation in closed chambers. Vertical bars are ±1 standard error.

degassed with N_2 for 10 min to avoid interference by H_2S in the subsequent colorimetric assays. The pH was readjusted with HCO_3^- to its original level and the samples were then frozen for later analysis. After persulfate digestion, total dissolved nitrogen (TN) was assayed spectrophotometrically using an autoanalyzer equipped with a cadmium reduction column (D'Elia et al.1977). We recognize that this method may underestimate DON levels in oligotrophic waters (Sugimura and Suziki 1988). Total dissolved phosphorus (TP) was determined after persulfate oxidation (American Public Health Association 1975) and assayed according to the molybdate-antimony method of Stainton et al. (1974).

Results

Photosynthesis measurements from the nutrient enrichment assay showed that the rhizophytic macroalga, *P. capitatus*, was nitrogen limited in the outer, low nutrient portion of Bailey's Bay (Fig. 2). Only the nitrogen-treated algae had significantly higher oxygen production rates during the 4-h incubation period than the control algae for both the overnight and 6-d experiments, with average photosynthetic rates (n = 4) of 0.96 mmoles O_2 gdw^{-1} h^{-1} and 0.98 mmoles O_2 gdw^{-1} h^{-1}, respectively ($p < 0.001$ ANOVA). The oxygen production rates for the phosphorus-treated plants were not significantly different from the control algae in either the overnight (0.66 vs. 0.62 mmoles O_2 gdw^{-1} h^{-1}) or 6-d (0.61 vs. 0.62 mmoles O_2 gdw^{-1} h^{-1}) experiments ($p < 0.05$). The average photosynthetic response for all treatments was virtually identical in the two experiments, indicating that there was not

TABLE 1. Tissue concentrations of nitrogen and phosphorus for *Penicillus capitatus* nutrient enrichment experiment. Values represent mean (n = 4) percentage of dry weight and standard errors (in parentheses). Mean nitrogen : phosphorus (N:P) ratio calculated as the average of the N:P ratios determined for each sample.

Treatment	% N	% P	N:P (Molar)
+Nitrogen	1.4 (0.16)	0.033 (0.004)	93:1
+Phosphorus	1.1 (0.07)	0.045 (0.004)	54:1
Control	1.2 (0.19)	0.034 (0.002)	75:1

TABLE 2. Total nitrogen and total phosphorus porewater concentrations in Bailey's Bay. Values represent mean concentrations (n = 4) and standard errors (in parentheses). Mean ratio of total dissolved nitrogen to total dissolved phosphorus (TN:TP) at each depth calculated as the average of TN:TP ratios determined for each sample.

Depth	TN (μM)	TP (μM)	TN:TP
10 cm	107 (26.4)	17.9 (4.9)	6.1:1
20 cm	60.4 (27.6)	23.8 (8.9)	4.5:1

a significant time lag in nutrient uptake and that the short-term assay accurately reflected the longer term photosynthetic response.

The nutrient content of dried algal tissues following the experiments showed significant differences in the concentrations of nitrogen and phosphorus in nutrient-treated algae compared to the controls (Table 1). The average nitrogen concentration of nitrogen-treated algae was 29% higher than the phosphorus-treated algae and 18% greater than the controls ($p < 0.05$), corroborating the results of the productivity assay that this species was nitrogen limited in the low nutrient portion of Bailey's Bay. For the phosphorus-treated algae, the tissue phosphorus concentration increased significantly ($p < 0.05$), but was not associated with an increase in photosynthetic rates. The phosphorus content of the P-enriched algae was 36% higher than the N-enriched algae and 32% greater than the controls. The differences in the tissue concentrations of nitrogen and phosphorus between the treatments can be seen in the average molar N:P ratios in Table 1. The high N:P ratio of 93:1 for the nitrogen-treated algae and the low ratio of 54:1 for the phosphorus-treated algae relative to the control ratio of 75:1 reflect the uptake of nitrogen and phosphorus, respectively.

The pore water TN:TP ratios, ranging from 4.5 to 6.1, reflect the high concentration of phosphorus relative to nitrogen in these calcium carbonate sediments (Table 2). The average TN concentration at 10 cm was 77% greater than that at 20 cm, although there was considerable variability at both depths, with values ranging from 49.7μM to 172.9 μM and 27.1 μM to 142.9 μM, respectively. Average TP concentrations were more consistent with depth, but also were variable, with concentrations ranging from 10.7 μM to 32.0 μM at 10 cm and 11.0 μM to 49.6 μM at 20 cm. In all cases, pore water nutrient concentrations were significantly higher than in the overlying water ($p < 0.05$).

Discussion

Nutrient limitation generally is the most important factor controlling macroalgal photosynthesis in environments where light is not limiting (Lapointe and Tenore 1981). In the low nutrient portion of Bailey's Bay, light levels at the sediment-water interface typically exceed 1,500 μE m^{-2} s^{-1}, well above the saturation level for many shallow, tropical macroalgae (Lapointe 1987; Littler et al. 1988; Lapointe and O'Connell 1989). The nutrient enrichment bioassay and tissue analysis clearly indicate that nitrogen limited the photosynthetic capacity of *P. capitatus* at this site. We used considerably lower concentrations of nitrogen and phosphorus in the enrichment assay than those reported by other authors, and hence, our results represent a more conservative estimate of nutrient limitation. The elevated phosphorus tissue concentration for the P-enriched algae without a concomitant increase in photosynthetic rate suggests that this species may be capable of luxury uptake for phosphorus. Luxury uptake is known or inferred for several tropical and temperate macroalgae (Chapman and Craigie 1977; Birch et al. 1981; Wheeler 1983; Rosenberg et al. 1984; Rosenberg and Ramus 1984; Fujita 1985; Fujita et al. 1989). The tissue N:P ratios reported in this study are higher than the mean N:P ratio of 35:1 calculated by Atkinson and Smith (1983) for over 90 benthic seagrasses and macroalgae from several locations worldwide, but our control ratio (75:1) is within the range of values (33:1 to 78:1) calculated by these authors for other siphonous species in the order Caulerpales.

The results from the photosynthesis assay and tissue analysis are consistent with N limitation found in a similar assay for a related sand-dwelling species, *Halimeda opuntia,* from a mangrove site in Belize (Lapointe et al. 1987), but are in contrast to P limitation reported by Littler et al. (1988) for two sand-dwelling species, *H. tuna* and *H. simulans,* from a back reef habitat in the Bahamas. The difference in nutrient limitation of these rhizophytic

species may be in part a function of differences in the organic content of the sediments. Organic matter in anoxic sediments may increase available pore water phosphorus pools because organic coatings on the surface of calcium carbonate may inhibit phosphate adsorption, as suggested by Krom and Berner (1980). Sediments surrounding mangrove sites are typically high in organic content from decomposing material, and the sediments in Bailey's Bay smelled strongly of hydrogen sulfide, indicating reducing conditions from heterotrophic activity. In the back reef site of the Bahamas studied by Littler et al (1988), the algae were collected from bare sediments where both organic content and pore water phosphorous concentrations were probably lower than those in the vegetated substrata in Bermuda and from the mangrove site in Belize.

Certain siphonous species in the order Caulerpales may be capable of acquiring nutrients directly from the sediments, where concentrations of nutrients are typically one to several orders of magnitude higher than those in the water column (Williams 1984). The mechanism for nutrient uptake and translocation by these species has not been developed, although it has been suggested that siphonous species, lacking true cross walls, can translocate nutrients via rapid turnover and cytoplasmic streaming (Littler et al. 1988). The ability to use the high sediment-nutrient concentrations may confer a competitive advantage to these species in oligotrophic environments where water column nutrients are extremely low and episodic. Assuming that pore water nutrients are an important source for the siphonous, rhizophytic alga, *P. capitatus*, the low TN:TP ratios of sediment pore water in outer Bailey's Bay suggest a N-limited nutrient pool. TN:TP ratios below the Redfield ratio, and the faster recycling rate of dissolved organic P relative to that of dissolved organic N (Jackson and Williams 1985; Smith et al. 1985), make it highly likely that nitrogen availability limits benthic primary productivity at this site. External nitrogen inputs from groundwater nitrates are low compared to the inner, eutrophic portion of the bay, and nitrogen stable isotopic ratios indicate that there is little contribution of fixed nitrogen to the overall nitrogen requirement of *P. capitatus* at this location (K. McGlathery unpublished data). The low TN:TP ratio of the sediment pore waters, therefore, is most likely a function of high phosphorus solubility and regeneration and/or of high rates of denitrification. Significant losses of nitrogen via denitrification, which would favor nitrogen limitation, have been reported in clastic sediments in temperate estuaries (Knowles 1982; Seitzinger 1988). There are fewer data on potential denitrification rates in tropical carbonate-rich sediments, but rates can be high (Capone and Taylor 1980; Seitzinger and D'Elia 1985). The sulfidic Bailey's Bay sediments indicate significant heterotrophic metabolism, which would be expected to lead to high denitrification rates.

The pore water phosphorus concentrations in Bailey's Bay are high compared to similar carbonate-rich sediments in the Caribbean (Morse et al. 1985; Short et al. 1985; Morse et al. 1987), but fall well within the range reported for Bermuda sediments (Morris et al. 1977; Hines and Lyons 1982). These differences may represent anthropogenic inputs, as the Bermuda sites are all in closer proximity to groundwater input and surface runoff than the Caribbean sites, or may represent different sorptive capacities of the carbonate sediments between the locations. Unlike the sediments in Bailey's Bay, the Bahamian sediments studied by Morse et al. (1985, 1987) were not reducing and presumably had lower levels of heterotrophic activity and organic matter accumulation. The low organic matter content would presumably favor adsorption of phosphate to calcium carbonate (Krom and Berner 1980). Hines and Lyons (1982) measured similar PO_4^{3-} pore water concentrations to those we found in a small bay several kilometers east of Bailey's Bay on Bermuda's north shore.

Standing stocks of organic and inorganic nutrients are imperfect measures of potential nutrient limitation because turnover of dissolved nutrients is rapid (Jackson and Williams 1985; Smith et al. 1985) and short-term pulses of nutrients from groundwater discharge or surface runoff may be more important than standing stock nutrient concentrations in oligotrophic tropical waters. Nonetheless, the enrichment bioasssay and tissue analysis clearly show that nitrogen rather than phosphorus was limiting the photosynthetic capacity of *P. capitatus* in the low nutrient portion of Bailey's Bay. These results, coupled with the nutrient pore water data, indicate that not all carbonate-rich tropical sediments induce phosphorus limitation of benthic macrophytes, suggesting that site-specific controls on relative nitrogen and phosphorus availabilities are important in determining nutrient limitation (Howarth 1988). Several factors should be considered in studies of nutrient limitation of tropical marine macrophytes, namely: (1) phosphate sorption and regeneration in calcium carbonate sediments containing different amounts of organic matter; (2) magnitude and frequency of external nutrient inputs; and (3) rates of denitrification and benthic nitrogen fixation.

The results reported here represent the physi-

ological response of *P. capitatus* to nutrient enrichment on the time scale of 1 d to 1 wk, and do not necessarily reflect the longer term ecosystem-level or community-level effects of nutrient enrichment. Presumably those species, such as *P. capitatus,* found in oligotrophic water are adapted to low nutrient conditions in the water column either through the ability to acquire nutrients directly from the substratum and/or through luxury uptake. Nutrient enrichment via groundwater inputs or surface runoff is more likely to cause a change in species composition, favoring rapidly-growing, opportunistic species, an effect similar to the change observed in temperate phytoplankton community composition following nutrient enrichment (Sanders et al. 1987).

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Literature Cited

American Public Health Association, American Water Works Association, and Water Pollution Control Federation (APHA, AWWA, WCPA). 1975. Standard Methods for the Examination of Water and Waste-water. 14th Ed. American Public Health Association, Washington, DC.

Atkinson, M. J. and S. Smith. 1983. C:N:P ratios of benthic marine plants. *Limnology and Oceanography* 28:568–574.

Berner, R. A. 1974. Kinetic models for the early diagenesis of nitrogen, sulfur, phosphorus, and silicon in anoxic marine sediments, p. 427–450. *In* E. D. Goldberg (ed.), The Sea, Vol. 5. John Wiley and Sons, New York.

Birch, P. B., D. M. Gordon and A. J. McComb. 1981. Nitrogen and phosphorus nutrition of *Cladophora* in the Peel-Harvey Estuarine system, Western Australia. *Botanica Marina* 24: 381–387.

Capone, D. G. and B. F. Taylor. 1977. Nitrogen fixation (acetylene reduction) in the phyllosphere of *Thalassia testudinum. Marine Biology* 40:19–28.

Capone, D. G. and B. F. Taylor. 1980. Microbial nitrogen cyling in a seagrass community, p. 153–161. *In* V. S. Kennedy (ed.), Estuarine Perspectives. Academy Press, New York.

Capone, D. G., D. L. Taylor, and B. F. Taylor. 1977. Nitrogen fixation (acetylene reduction) associated with macroalgae in a coral-reef community in the Bahamas. *Marine Biology* 40:29–32.

Carpenter, E. J. 1972. Nitrogen fixation by a blue-green epiphyte on pelagic *Sargassum. Science* 178:1207–1209.

Chapman, A. R. O. and J. S. Craigie. 1977. Seasonal growth in *Laminaria longicruris*: Relations with dissolved inorganic nutrients and internal reserves of nitrogen. *Marine Biology* 40: 197–205.

D'Elia, C. F., P. A. Steudler, and N. Corwin. 1977. Determination of total nitrogen in aqueous samples using persulfate digestion. *Limnology and Oceanography* 22:760–764.

Fujita, R. M. 1985. The role of nitrogen status in regulating transient ammonium uptake and nitrogen storage by macroalgae. *Journal of Experimental Marine Biology and Ecology* 92: 283–301.

Fujita, R. M., P. A. Wheeler, and R. L. Edwards. 1989. Assessment of macroalgal nitrogen limitation in a seasonal upwelling region. *Marine Ecology Progress Series* 53:293–303.

Gordon, D. M., P. B. Birch, and A. J. McComb. 1981. Effects of inorganic phosphorus and nitrogen on the growth of an estuarine *Cladophora* in culture. *Botanica Marina* 24:93–106.

Hanson, R. B. 1977. Pelagic *Sargassum* community metabolism: Carbon and nitrogen. *Journal of Experimental Marine Biology and Ecology* 29:107–119.

Head, W. D. and E. J. Carpenter. 1975. Nitrogen fixation associated with the marine macroalga *Codium fragile. Limnology and Oceanography* 20:815–823.

Hines, M. E. and W. B. Lyons. 1982. Biogeochemistry of nearshore Bermuda sediments. I. Sulfate reduction rates and nutrient regeneration. *Marine Ecology Progress Series* 8:87–94.

Howarth, R. W. 1988. Nutrient limitation of net primary production in marine ecosystems. *Annual Review of Ecology and Systematics* 19:89–110.

Howarth, R. W., R. Marino, and J. J. Cole. 1988. Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 2. Biogeochemical controls. *Limnology and Oceanography* 33:688–701.

Jackson, G. A. and P. M. Williams. 1985. Importance of dissolved organic nitrogen and phosphorus to biological nutrient cycling. *Deep Sea Research* 32:223–235.

Kitano, T., M. Okumura, and M. Idogak. 1978. Uptake of phosphate ion by calcium carbonate. *Geochemical Journal* 12: 29–37.

Knowles, R. 1982. Denitrification. *Microbiology Review* 46:43–70.

Krom, M. D. and R. A. Berner. 1980. Adsorption of phosphate in anoxic marine sediments. *Limnology and Oceanography* 25:797–806.

Lapointe, B. E. 1987. Phosphorus- and nitrogen-limited photosynthesis and growth of *Gracilaria tikvahiae* (Rhodophyceae) in the Florida Keys: An experimental field study. *Marine Biology* 93:561–568.

Lapointe, B. E., M. M. Littler, and D. S. Littler. 1987. A comparison of nutrient-limited productivity in macroalgae from a Caribbean barrier reef and from a mangrove ecosystem. *Aquatic Botany* 28:243–255.

Lapointe, B. E. and J. O'Connell. 1989. Nutrient-enhanced growth of *Cladophora prolifera* in Harrington Sound, Bermuda: Eutrophication of a confined, phosphorus-limited marine ecosystem. *Estuarine and Coastal Shelf Science* 28:347–360.

Lapointe, B. E., and J. H. Ryther. 1979. The effects of nitrogen and seawater flow rate on the growth and biochemical composition of *Gracilaria foliifera* var. *angustissima* in mass outdoor cultures. *Botanica Marina* 22:529–537.

Lapointe, B. E. and K. R. Tenore. 1981. Experimental outdoor studies with *Ulva fasciata* Delile. I. Interaction of light and nitrogen on nutrient uptake, growth, and biochemical composition. *Journal of Experimental Marine Biology and Ecology* 53:135–152.

Lapointe, B. E., L. D. Williams, J. C. Goldman, and J. H. Ryther. 1976. The mass outdoor culture of macroscopic marine algae. *Aquaculture* 8:9–21.

Littler, M. M., D. S. Littler, and B. E. Lapointe. 1988. A comparison of nutrient- and light-limited photosynthesis in psammophytic versus epilithic forms of *Halimeda* (Caulerpales, Halimedaceae) from the Bahamas. *Coral Reefs* 6:219–225.

Mague, T. H. and O. Holm-Hansen. 1975. Nitrogen fixation on a coral reef. *Phycologia* 14:87–92.

McGlathery, K. J. 1992. Nutrient and herbivore influences on seagrass community dynamics. PhD Dissertation. Cornell University, Ithaca, New York.

MORRIS, B., J. BARNES, F. BROWN, AND J. MARKHAM. 1977. The Bermuda Marine Environment: A report of the Bermuda Inshore Waters Investigations 1976–1977. Bermuda Biological Station Special Publication number 15.

MORSE, J. W., J. J. ZULLIG, L. D. BERNSTEIN, F. J. MILLERO, P. MILNE, A. MUCCI, AND G. R. CHOPPIN. 1985. Chemistry of calcium carbonate-rich shallow water sediments in the Bahamas. *American Journal of Science* 285:147–185.

MORSE, J. W., J. J. ZULLIG. R. L. IVERSON, G. R. CHOPPIN, A. MUCCI, AND F. J. MILLERO. 1987. The influence of seagrass beds on the chemistry of coarse-grained sediments in the Bahamas. *Marine Chemistry* 22:71–83.

PATRIQUIN, D. G. AND R. KNOWLES. 1972. Nitrogen fixation in the rhizosphere of marine angiosperms. *Marine Biology* 16: 49–58.

PENHALE, P. A. AND D. G. CAPONE. 1981. Primary productivity and nitrogen fixation in two macroalgae-cyanobacteria associations. *Bulletin of Marine Science* 31:164–169.

ROSENBERG, G., T. A. PROBYN, AND K. H. MANN. 1984. Nutrient uptake and growth kinetics of brown seaweeds: Response to continuous and single additions of ammonium. *Journal of Experimental Marine Biology and Ecology* 80:125–146.

ROSENBERG, G. AND J. RAMUS. 1984. Uptake of inorganic nitrogen and seaweed surface : volume ratios. *Aquatic Botany* 19: 65–72.

SANDERS, J. G., S. J. CIBIK, C. F. D'ELIA, AND W. R. BOYNTON. 1987. Nutrient enrichment studies in a coastal plain estuary: Changes in phytoplankton species composition. *Canadian Journal of Fisheries and Aquatic Sciences* 47:83–90.

SEITZINGER, S. P. 1988. Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnology and Oceanography* 88:702–724.

SEITZINGER, S. P. AND C. F. D'ELIA. 1985. Preliminary studies of denitrification on a coral reef, p. 119–208. *In* M. L. Reaka (ed.), The Ecology of Coral Reefs. NOAA, Department of Commerce, Washington, DC.

SHORT, F. T., M. W. DAVIS, R. A. GIBSON, AND C. F. ZIMMERMANN. 1985. Evidence for phosphorus limitation in carbonate sediments of the seagrass *Syringodium filiforme. Estuarine and Coastal Shelf Science* 20:419–430.

SMITH, R., E. H. HARRISON, AND W. G. HARRIS. 1985. Phosphorus exchange in marine microplankton communities near Hawaii. *Marine Biology* 86:5–84.

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

STAINTON, M. P., M. J. CAPEL, AND F. A. ARMSTRONG. 1974. The chemical analysis of freshwater. Miscellaneous Special Publication 25. Department of the Environment, Freshwater Institute, Research and Development Directorate. Winnipeg, Manitoba, Canada

STEFFENSEN, D. A. 1976. The effect of nutrient enrichment and temperature on the growth in culture of *Ulva lactuca* L. *Aquatic Botany* 2:337–351.

STERRER, W. 1986. Marine Fauna and Flora of Bermuda. John Wiley and Sons, New York.

SUGIMURA, Y. AND Y. SUZIKI. 1988. A high temperature catalytic oxidation method for nonvolatile dissolved organic carbon in seawater by direct injection of liquid samples. *Marine Chemistry* 24:105–131.

WHEELER, P. A. 1983. Phytoplankton nitrogen metabolism, p. 309–346. *In* E. J. Carpenter and D. G. Capone (eds.), Nitrogen in the Marine Environment. Academic Press, New York.

WILLIAMS, S. L. 1984. Uptake of sediment ammonium and translocation in a marine green macroalga *Caulerpa cupressoides. Limnology and Oceanography* 29:374–379.

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