Productivity and nutrient relationships in psammophytic versus epilithic forms of Bryopsidales (Chlorophyta): comparisons based on a short-term physiological assay

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Abstract

Members of the green algal order Bryopsidales (= Caulerpales) are important calcifying agents of tropical reefs and comprise two fundamentally different life-form groups: (1) epilithic species with limited attachment structures and (2) psammophytic forms that have extensive subterranean rhizoidal systems. Because the shallow-water habitats of the former have relatively low nitrogen (N) to phosphorus (P$_i$) ratios compared to the pore waters of the sedimentary carbonate-rich substrata in which the latter are anchored, we hypothesized that epilithic forms should tend to be relatively more limited by N, while psammophytic species should tend to show P$_i$ limitation. In partial support of the hypothesis, light-saturated net photosynthesis (P$_{\text{max}}$) in the epilithic forms, Halimeda opuntia, H. lacrimosa and H. copiosa, tended to be enhanced by N, while P$_i$ was inhibitory or had no effect. In contrast, the psammophytic forms, Udotea sp., U. conglutinata, H. monile, H. tuna and H. simulans, tended to be stimulated more by P$_i$, whereas N had little effect. The utility of a bioassay to assess macroalgal nutrient limitation, based on a physiological response (net P$_{\text{max}}$) to short-term nutrient pulses, is demonstrated.

Introduction

Traditionally, nitrogen is considered (Hatcher & Larkum, 1983) to be the nutrient most often limiting tropical marine algal growth. Phosphorus, due to its effective recycling on reefs, generally has been thought (Pilson & Betzer, 1973) less likely to be in short supply. However, geochemical interpretations (Broecker & Peng, 1982; Smith, 1984; Smith & Atkinson, 1984) and recent in situ macroalgal bioassays (Lapointe, 1985, 1987, 1989) implicate P$_i$ as the more frequent growth-limiting nutrient in carbonate-rich tropical marine waters. In such environments, particularly in interstitial pore waters, dissolved P$_i$ (PO$_4^{3-}$) concentrations become substantially reduced by equilibrium reactions with carbonate fluoroapatite (Gulbrandsen & Roberson, 1973) and P$_i$ is adsorbed readily and bound onto CaCO$_3$, aragonite and calcite crystals (DeKanel & Morse, 1978). Fixation of atmospheric nitrogen by blue-green algae and bacteria associated with sediments (Capone & Taylor, 1980), microalgal turfs (Mague & Holm-Hansen, 1975; Wiebe et al., 1975), macrophyte populations (Capone, 1977; Capone & Taylor, 1977; Capone et al., 1977) and
cals (Crossland & Barnes, 1976) can elevate N : P$_i$ ratios while further accelerating P$_i$ limitation by competing for available orthophosphate (Redfield, 1958; Doremus, 1982).

Members of the green algal order Bryopsidales (sensu Silva, 1982) are recognized (e.g. Chapman & Mawson, 1906; Ginsburg, 1956; Milliman, 1974; Hillis-Colinvaux, 1980, 1986) as major calcifying elements of both tropical Atlantic and Pacific reefs. Bryopsidales can be viewed as composed of two fundamentally different functional groups: (1) epiphytic forms that have only limited attachment structures and (2) psammophytic species that have well-developed bulbous rhizoidal systems. The first group extracts nutrients solely from the water-column milieu while secured to two-dimensional hard substrata by means of small holdfasts. Members of the second group also extract nutrients from the water column, but potentially can obtain nutrients from the pore waters of sedimentary three-dimensional substrata by means of their extensive root-like rhizoidal systems that also serve as anchors. It is of interest that typical pore-water levels of N in the tropical western Atlantic range between 120–200 μM (Williams & Fisher, 1985), whereas associated water-column levels are frequently three orders of magnitude lower (Williams, 1984; Lapointe, 1989). This differential, as well as the stripping action of CaCO$_3$ particles on P$_i$, results in greatly elevated N : P$_i$ ratios in sedimentary environments. Bryopsidales are siphonaceous algae and, because cross walls are minimal, can utilize active transport together with rapid turnover and cytoplasmic streaming to translocate nutrients (Williams, 1984).

Because (1) sedimentary carbonate-rich pore waters have relatively high N : P$_i$ ratios (Berner, 1974; Rosenfeld, 1979) compared with the lower N : P$_i$ ratios characteristic of shallow tropical waters (Lapointe, 1989), and (2) based upon the above morphological divergences and the influence of morphology on nutrient-uptake (e.g. Rosenberg & Ramus, 1984), we predicted that different nutrient-related responses may have developed within the order. As a working hypothesis, psammophytic members should show a tendency to be relatively more limited by P$_i$ whereas epiphytic forms should tend to show N limitation. This study reexamines previous data (Littler et al., 1988) on the photosynthetic responses for psammophytic and epiphytic forms of four Bahamian Bryopsidales under four combinations of nutrient enrichment and tests the above hypothesis for four other Bryopsidales from a different system (i.e. the Florida Keys).

Methods and materials

The field studies reported here were conducted during 20–23 December 1988 from a boat dock in South Pine Channel adjacent to Little Torch Key 38 km east of Key West, Florida, USA. This habitat shows a seasonal trend in dissolved inorganic nitrogen (Lapointe, 1989) with water-column winter minima and summer maxima as follows: NH$_4^+$ = 0.03–1.20 μM, NO$_3^-$ = 0.25–2.10 μM. Concentrations of PO$_4^{3-}$ were almost undetectable throughout the year, generally <0.12 μM.

For the experiments conducted in the Florida Keys, whole plants of the epiphytic form, Halimeda opuntia (L.) Lamour., were contrasted with the psammophytic species H. monile (Ellis & Solander) Lamour., collected from 1 m deep within a few meters of each other on the west side of South Pine Channel next to the site of incubation on Little Torch Key (24°40’00” N, 81°23’30” W). Additionally, two other sympatric psammophytes, Udotea sp. nov. (abbottiorum, Littler & Littler, 1990) and U. conglomerata (Ellis & Solander) Lamour., were collected (1 m deep) from north of South Pine Channel on the largest of the western Content Keys group. All sedimentary substrata consisted primarily of white, sand-sized fragments of Halimeda segments that did not appear to be anaerobic; the epiphytic carbonate substratum consisted of Miami Oolite. Additional comparative data were reassessed from our earlier studies in the Bahamas (Littler et al., 1988) using the same techniques. The relative nutritional status of Bahamian Bryopsidales, with respect to N versus P, limitation at P$_{max}$, was
examined for two psammophytes, *Halimeda tuna* (Ellis & Solander) Lamour, and *H. simulans* Howe, and for two epilithic forms, *H. lacrimosa* Howe and *H. copiosa* Goreau & Graham, taken from clear, shallow (3–7 m deep) waters (Jerlov type I).

On the day of collection in all cases, the macroalgae were cleaned of sediments and epiphytes and replicate whole plants were soaked overnight in one of four treatments that consisted of enrichment with either N (NaNO₃), P₁ (NaH₂PO₄), N + P₁ or a control (no enrichment). Initial enrichment concentrations were 160 µM – N and 16 µM – P₁ in order to saturate (Lapointe, 1989) the uptake rates. These concentrations represent the upper range of natural levels encountered in eutrophic environments (e.g. bird islands) and the mid range found in Caribbean sediment pore waters (i.e. 120–200 µM – N, Williams & Fisher, 1985). Following the 12 h overnight period of enrichment, the algae were flushed with 3 changes of seawater 4 h prior to incubation in full sunlight. This enrichment assay for nutrient limitation has been found (Littler et al., 1986, 1988; Lapointe, 1987, 1989; Lapointe et al., 1987) to correlate closely with longer-term growth responses for macroalgae.

For all net productivity (photosynthesis) measurements that followed the 12 h nutrient-enrichment period in Florida material, 14 replicate incubations per treatment were run for a total of 56 samples species⁻¹ at ambient water temperatures (24.0–24.2 °C) between 0820 and 1700 hrs in full sunlight with a natural photon flux density of 2332 µmol m⁻² s⁻¹ of photosynthetically active radiation (PAR), ranging from a minimum of 1035 to a maximum of 3673 µmol m⁻² s⁻¹. Photon irradiance was measured in the field with an integrating 4 π sensor (LiCor Model LI-550 printing integrator) throughout the incubation periods. Incubations were conducted in one-liter glass jars that received continuous cooling and stirring via water-driven magnetic turbines. Dissolved oxygen was measured to 0.01 mg L⁻¹ with an Orbisphere Model 2610 oxygen analyzer and converted to carbon fixed utilizing a photosynthetic quotient of 1.00 to facilitate compari-

sons. Photosynthesis was normalized to organic dry weight, which was determined by drying the samples to constant weight at 80 °C and ashing them to constant weight at 500 °C. The methods concerning selection of material, handling, incubation and oxygen analysis were within the limits recommended by Littler (1979).

### Results

In the nutrient enrichment treatments (Fig. 1), trends were exhibited by the four Bahamian Bryopsidales that varied according to both life form and species. For those possessing an extensive, subterranean, rhizoidal, root-like system (*Halimeda tuna* and *H. simulans*, Fig. 1), P₁ pulses had significant (*p < 0.05*, two-way ANOVA) enhancement effects on net $P_{\text{max}}$. No significant

![Fig. 1. $P_{\text{max}}$ of the Bahamian psammophytes, *Halimeda simulans* and *H. tuna*, and two epilithic forms, *H. copiosa* and *H. lacrimosa*, in response to enrichment by NO₃⁻ and/or PO₄³⁻. C = control. Values represent means ± 1 SD (N = 4–6). Reanalyzed from Littler et al. (1988). Asterisks indicate values that are significantly different from the controls (*p < 0.05*, two-way ANOVA).](image-url)
responses in $P_{\text{max}}$ ($p > 0.05$) were shown by either of these two Bahamian psammophytes following N pulses. Phosphorus and nitrogen combined stimulated net $P_{\text{max}}$ (significant at $p < 0.05$) for *H. tuna* as well.

In contrast, $P_i$ pulses inhibited net $P_{\text{max}}$ ($p < 0.05$, Fig. 1) for the Bahamian rock dwellers *Halimeda lacrimosa* and *H. copiosa*. Phosphorus in combination with N also inhibited $P_{\text{max}}$ in *H. lacrimosa* (significant at $p < 0.05$). Pulses of N had a significant ($p < 0.05$) enhancement effect on $P_{\text{max}}$ in *H. lacrimosa*, and greatly stimulated (significant at $p < 0.05$) $P_{\text{max}}$ in *H. copiosa*.

The productivity of the two calcareous psammophytes, *Udotea conglutinata* and *Udotea* sp. (abb.), in Florida (Fig. 2) emulated the pattern established for psammophytes in the Bahamas (Fig. 1). There was substantial (significant at $p < 0.05$, two-way ANOVA) stimulation of $P_{\text{max}}$ following pulses of $P_i$, but not N, in both species. In *U. conglutinata*, the combination of $N + P_i$ had no significant effect ($p > 0.05$), while in *Udotea* sp. (abb.), $N + P_i$ significantly ($p < 0.05$) elevated $P_{\text{max}}$. Correspondingly, the psammophyte *Halimeda monile* from Florida (Fig. 2) also showed a significant ($p < 0.05$) increase in $P_{\text{max}}$ following exposure to $P_i$ whereas pulses of N alone and $N + P_i$ together had no significant effects ($p > 0.05$). In the rock dwelling *H. opuntia* from Florida (Fig. 2), there was significant ($p < 0.05$) inhibition of $P_{\text{max}}$ due to enrichment with $P_i$, but no significant ($p > 0.05$) changes in $P_{\text{max}}$ subsequent to pulses of either N or $N + P_i$.

**Discussion**

The photosynthetic response at light-saturation ($P_{\text{max}}$) to nutrient enhancement is a parameter with considerable potential to reveal the type and degree of nutrient limitation in marine macroalgae. Field studies have shown that rates of $P_{\text{max}}$ in a freshwater microalga (Auer & Canale, 1982) and a freshwater macrophyte (Schmitt & Adams, 1981) are correlated with cellular levels of $P_i$. If this correlation applies to macroalgae, then prior pulsing of $N$ and $P_i$ followed by subsequent determination of $P_{\text{max}}$ could be used as a diagnostic field assay to examine the degree of nutrient limitation to primary productivity in coastal environments. Such utility is demonstrated by the present bioassay in addressing the question of whether or not psammophytic forms of Bryopsidales differ in their relative levels of nutrient limitation from members of the order that only attach to hard substrata.

The results for the four Bahamian species of *Halimeda* (Fig. 1) suggested that psammophytic forms tend to differ in nutrient status from epilithic forms. Similar Belizean experiments on epilithic *H. opuntia* also showed significant ($p < 0.05$, two-way ANOVA) stimulation of $P_{\text{max}}$ following pulses of N but not $P_i$, even though the two populations examined came from quite dif-
different dissolved nutrient regimes (i.e. Twin Cays N & P_1 ~ 3 fold > Curlew Cay (Lapointe et al., 1987). Trends from our Bahamian (Littler et al., 1988) and Belizean (Lapointe et al., 1987) studies and the new findings on Halimeda and Udotea from the Florida Keys can be summarized as follows. In partial support of the hypothesis, P_{max} in psammophytes tended to be stimulated by pulses of P_i, while high levels of N consistently had no effect. In contrast, epilithic species tended to be stimulated more by N enrichment, whereas high levels of P_i inhibited P_{max} or had little effect.

The demonstration of a P_i-limited nutrient regime for the sand dwellers Halimeda tenua, H. simulans, H. monile, Udotea sp. (abb.) and U. conglutinata contrasts with studies in temperate coastal environments along eastern North America where N was the primary growth-limiting nutrient of both phytoplankton (Ryther & Dunstan, 1971; Vince & Valiela, 1973) and epilithic macroalgae (Topinka & Robbins, 1976; Chapman & Craigie, 1977; Hanisak, 1979). However, nutrient bioassays using phytoplankton off Florida’s northern Gulf coast (Myers & Iverson, 1981), as well as epilithic macroalgal assays near the site studied here (Lapointe, 1989), have shown that P_i can be more important than N in regulating primary productivity. Lapointe (1989) recorded water-column dissolved inorganic N : P_i ratios of less than 10 during winter in South Pine Channel, which should result in N limitation (Ryther & Dunstan, 1971; Smith, 1984); however, this was not the case in any of the Florida Bryopsidales (i.e. mostly psammophytes) we examined (Fig. 2).

The recent series of studies using epilithic Phaeophyta and Rhodophyta from the Florida Keys (Lapointe, 1985, 1986, 1987, 1989) also documented mostly P_l limitation. This agrees with predictions based on geochemistry (Redfield, 1958; Broecker & Peng, 1982; Smith, 1984) that the productivity of oceanic ecosystems should most often be P_l limited. In contrast, our data for the rock-dwellers Halimeda lacrimosa and H. copiosa agree with those for other epilithic algae (see Topinka & Robbins, 1976; Chapman & Craigie, 1977; Hanisak, 1979; Hatcher & Larkum, 1983) and for the closely related rock-dweller, H. opuntia from Belize (Lapointe et al., 1987), which also demonstrated photosynthetic enhancement primarily by N enrichment.

These findings support the hypothesis that the two life-forms characteristic of Bryopsidales may show differential limitation by inorganic N or P_i, corresponding to the ratios present in their respective environments, while having the ability to take advantage of episodic nutrient pulses. The trend toward P_i-limitation observed in the psammophytic species corresponds with the observed (Berner, 1974; Rosenfeld, 1979) high N : P_i ratios of sediment pore waters in carbonate-rich sediments, which could result in the P_i-limited state we recorded. The lack of inhibitory responses to either nutrient shown by the psammophytic forms might be related to relatively high half-saturation constants (K_s, the nutrient concentration at which half the maximum uptake rate is achieved). Values of K_s as high as 107 μM – N have been documented (Williams, 1984) for psammophytic Bryopsidales, strongly indicating adaptation to the presence of high nutrient levels. Macrophytes adapted to using only water-column nutrients, which occur in concentrations several orders of magnitude lower, should have correspondingly lower K_s values (Williams, 1984). The epilithic forms that must rely on water-column N and P_i availability tended to be more N-limited, possibly because of the much lower N : P_i ratios characteristic of shallow tropical waters.

It is difficult to generalize concerning nutrient limitation in marine macroalgae since previous nutrient-exposure history and life form are among the important parameters. Because episodic nutrient pulses are both sporadic and frequent in nature (Meyer et al., 1983; Zimmerman & Kremer, 1984) and, as shown here, different functional groups respond differently (see also Rosenberg & Ramus, 1984), the degree and type of nutrient limitation in benthic macrophytes is dependent on a spectrum of variables.

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