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FOUR LEVELS OF NUTRIENT ENRICHMENT**

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PHOTOSYNTHESIS VS. IRRADIANCE CURVES FOR SIX SPECIES OF MACROALGAE FROM THE SEYCHELLES ISLANDS UNDER FOUR LEVELS OF NUTRIENT ENRICHMENT

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ABSTRACT

The relative nutritional status, with respect to phosphorus (P) versus nitrogen (N) limitation, and light-limited photosynthesis (P_s) was examined over a broad range of quantum fluxes (I) for four species of Chlorophyta (*Codium* sp., *Avrainvillea amadelpha* f. *montagnei*, *Dictyosphaeria cavernosa*, *Udotea orientalis* and two species of Rhodophyta (*Gracilaria crassa*, *G. multifurcata*) taken from Seychelles coastal waters. The results are consistent with the hypothesis that differences in nutrient status among algal forms are related to antecedent habitat history. Maximum photosynthetic rates (P_{max}) for *Codium* sp., *U. orientalis* and *G. multifurcata* from nutrient-poor habitats increased ($P < 0.05$) following nutrient enrichment. However, the species examined from nutrient-rich waters, *A. amadelpha* f. *montagnei*, *D. cavernosa* and *G. crassa*, showed decreases in the initial slope (α) of the P vs. I curve and no significant ($P > 0.05$) increases in P_{max} following nutrient pulses consistent with their previous nutrient environments. The findings suggest that shallow species of tropical macroalgae are adapted to take advantage of episodic nutrient pulses, and that nutrient exposure history can override differences between the various life forms in determining photosynthetic responses to nutrients.

The six shallow water algal species investigated appear well adapted to variable light regimes, including low light conditions. In natural populations of the green alga *A. amadelpha* f. *montagnei*, light-saturated photosynthesis occurred at quite low irradiances ($I_k = 100 \mu E \cdot m^{-2} \cdot s^{-1}$). Associated with low saturation irradiances were low light requirements for photosynthetic compensation ($I_c = 30 \mu E \cdot m^{-2} \cdot s^{-1}$) in this species and reasonably efficient use of low photon flux densities as indicated by a relatively steep slope (α) of the P_s vs. I curve. Conversely, *Codium* sp., *G. crassa* and *G. multifurcata* showed increased photosynthesis as a function of increasing I, with no indication of photoinhibition and relatively shallow α 's.

INTRODUCTION

Large standing stocks of macroalgae on reefs are usually correlated with elevated nutrient supplies (Adey et al. 1977), an observation consistent with the idea that growth rates of tropical reef macroalgae may often be nutrient limited. However, the question of which macronutrient element -- N, P or their interactions -- might limit macroalgal productivity in reef ecosystems has infrequently been addressed experimentally. Traditionally, N is considered the primary limiting nutrient in

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tropical marine waters (e.g., Parsons et al. 1977), although information from geochemical models (Broecker and Peng 1982, Smith 1984) and recent *in situ* macroalgal bioassays (Lapointe 1985, 1987, 1992, Littler et al. 1991) suggests that P may be the more important growth-limiting nutrient in carbonate-rich tropical marine waters. A knowledge of photosynthetic responses to P and N and light by species of tropical reef algae from contrasting habitats could prove useful to clarify current discrepancies (e.g., see Smith 1984) concerning the role of N versus P limitation to algal stocks in tropical marine environments.

Based upon habitat differences in the Seychelles Islands, and the presumed influence of habitat history on nutrient limitation, we hypothesized that different light and nutrient-related responses would be shown by dominant algae from different island environments. To begin to understand the comparative physiological ecology of Seychelles reef algae, we examined the relative nutritional status (N vs P limitation) for four Chlorophyta and two Rhodophyta species. These studies were conducted with manipulations of ambient light levels to test the role of light as an interacting factor with nutrient limitation.

The following specific questions were addressed:

Do macroalgae from enriched waters differ in their relative levels of nutrient limitation from species growing in nutrient-poor habitats?

Is there interaction between nutrients and irradiance in the photosynthetic responses of different species?

Do dark respiration rates (R) and photosynthetic rates at light saturation (P_{max}) vary among species from different habitats?

Do Seychelles Island reef species differ in their light-limited photosynthetic characteristics (α , I_c , I_k).

METHODS AND MATERIALS

This investigation was performed from the research vessel R/V Alexander A. Nesmeyanov throughout the Seychelles Islands from 1 February to 31 March 1989. The specific study sites included St. Joseph Atoll, Pagoda Island of the Cosmoledo Group (a bird island with Guano), and inner and outer harbor sites off the city of Victoria, Mahé Island (Fig. 1). The specimens were held overnight in metal-free, 106-liter, insulated, plastic containers of ambient seawater aboard ship under shaded conditions. Voucher materials of all species were preserved in 4% buffered Formalin and deposited in the Algal Collection, National Museum of Natural History, Smithsonian Institution, Washington, D.C.

On the day prior to photosynthetic assays (i.e., the day of collection), the macroalgae were transported to the R/V Alexander A. Nesmeyanov where they were cleaned of sediments and epiphytes. Subsequently, replicate whole plants of each species were soaked in one of three treatments, a factorial design enrichment of N (NaNO_3) and P (NaH_2PO_4), that consisted of either +N, +P, +N+P or a control (no enrichment). Initial concentrations of N and P in the enrichment were 160 and 16 μM , respectively. The concentrations used were chosen to saturate the uptake rates and represent the upper range of natural levels encountered in highly enriched environments (e.g., bird islands). Following the overnight (12 h) enrichment, the algae were flushed with 3 changes of fresh seawater under shaded conditions (50% I_0) 4 h prior to incubation. Although this enrichment method is based on concepts developed by Smith (1983) for freshwater algae, it has been field tested

previously (Lapointe 1987, Lapointe et al. 1987, Littler et al. 1988) as a macroalgal photosynthetic bioassay, and the results indicated close correlation with longer-term growth responses.

For all productivity (photosynthesis) measurements that followed the 12-h nutrient enrichment period, six replicate incubations per treatment were run. Experimental levels of 7 irradiances and 4 nutrient-pulsed conditions were set up simultaneously in a 7X4 factorial design at ambient water temperatures (27.5-28.4°C) and run between 1000 and 1530 hrs with an average ambient photon-flux density of $2200 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of photosynthetically active radiation (PAR), ranging from a minimum of 1480 to a maximum of $5000 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Incubation chambers were lined with highly reflective aluminum foil to help attain light levels in excess of saturation. Different layers of neutral density screening were used to produce seven experimental light levels (in approximate percent of available sunlight): 100.0%, 50.0%, 25.0%, 12.5%, 3.2%, 0.8% and dark. Photon-flux densities were measured in the field with two integrating 4π sensors (Li Cor Model LI-1000 Data Logger) throughout the incubation periods. Seven layers of neutral density screening and an insulated lid were placed over the 106-liter insulated chamber containing the six incubation jars at the beginning of each run to exclude all light. Incubations were conducted in one liter glass jars that received continuous cooling. Stirring via stir bars on each of the six oxygen electrodes provided vigorous water motion. At five minute intervals, the lid or appropriate layers of screening were removed and dissolved oxygen was recorded at five minute intervals to $0.01 \text{ mg}\cdot\text{l}^{-1}$ with an Orbisphere Model 2610 oxygen analyzer and converted to carbon fixed utilizing respiratory and photosynthetic quotients of 1.00 to facilitate comparisons with other studies. Photosynthesis and respiration were normalized to organic dry weight, which was determined by drying the samples to constant weight at 70°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).

Photosynthetic light saturation values (I_k) were obtained for each species at each nutrient level by determining the intersection of a line drawn parallel to the abscissa and through the point of maximum photosynthesis (P_{max}) with the slope of the light-limited P_s vs. I curve (α). The initial slope (α) of each P_s vs. I curve was determined from the least squares linear regression of all productivity values obtained for the linear portion of the curve (i.e., below 12.7 to $50.8 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) including dark respiration (8-18 data points). Compensation irradiances (I_c) were calculated as the intersection of the regression line of the initial P_s vs. I response with the abscissa.

P_{max} values used for statistical comparisons represented the mean of the six greatest photosynthetic values obtained in a particular P_s vs. I curve. Nitrogen and P enrichment effects on P_{max} were assessed by two-way ANOVA and Bonferroni t-test of differences. Significance reported in the results below implies that the probability of the null hypothesis was <0.05 .

RESULTS

Maximum net apparent photosynthesis (P_{max}) in *Codium* sp. from the pristine St. Joseph Atoll (Table 1, Fig. 2) was strongly N-limited (N significantly greater than control, $P < 0.05$). Values of samples pulsed with P were consistently higher than the controls but not to a statistically significant degree ($P > 0.05$). The two nutrients combined produced a significant ($P < 0.05$) synergistic increase in net apparent photosynthesis. Net photosynthesis began to show light saturation (I_k) above about $I_k = 250 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the controls and at lower light levels (150 - $170 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in the pulsed samples (Table 1). The nutrient-pulsed material had compensation intensities (I_c , Table 1) that were all less than half that of the controls ($80 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). The α values of the pulsed material were consistently higher than that of the control samples (Table 1).

For *Udotea orientalis* from another pristine environment on St. Joseph Atoll, P_{\max} significantly increased ($P < 0.05$) following pulses of N or P, but no P + N synergism occurred (Fig. 3, Table 1). Net apparent photosynthesis became light saturated at around $170\text{--}280 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (I_k) in the control and N-pulsed samples but was substantially higher ($410 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in the samples pulsed with P alone (Table 1). The nutrient-pulsed samples showed somewhat lower I_c values than the controls, particularly in the case of the N-pulsed material (30 vs. $140 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, Table 1). The α values were similar with the exception of the P-pulsed material which was two to three times lower (Table 1).

In *Avrainvillea amadelpha* f. *montagnei* (Fig. 4, Table 1), from the guano-influenced Pagoda Island, Cosmoledo Atoll, there was no significant ($P > 0.05$) change in P_{\max} for any of the pulsed treatments. Net photosynthesis began to show light saturation at the relatively low I_k of $100 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the controls and at 310 , 354 and $633 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for N-, P- and N+P-pulsed material, respectively (Table 1). I_c values followed this same trend with lower control values and a much higher I_c in the N+P-pulsed samples. The α values of the nutrient enriched materials were substantially lower, particularly in the case of the N+P-pulsed material, than in the controls (Table 1).

Dictyosphaeria cavernosa, the dominant alga from Pagode Island (Fig. 5), Cosmoledo Atoll, under the direct influence of seabird guano, showed no significant ($P > 0.05$) nutrient stimulation of P_{\max} , with substantial inhibition in the P- and N+P-pulsed samples (Table 1). Net photosynthesis tended to become light saturated at $320\text{--}370 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, except in the case of the N-pulsed samples ($I_k = 540 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). The nutrient-pulsed material had slightly elevated I_c values than the controls (Table 1). The α values, conversely, were nearly twice as high in the controls as in the pulsed material.

Gracilaria multifurcata from Cerf Island outside of Victoria Harbor, Mahe (Fig. 6), had P_{\max} stimulated most by pulses of P (significant at $P < 0.05$) and to a lesser degree by N and N+P. Light saturation of the control (Table 1) was somewhat high compared to the other algal species, as reflected by an I_k of $480 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The nutrient-pulsed samples showed quite high I_k values ranging from 510 to $870 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Only the N-pulsed samples had substantially higher I_c values than the controls (120 vs. $70 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, Table 1).

In *Gracilaria crassa* (Fig. 7), a dominant in the highly eutrophic shallow waters adjacent to the shoreline of Victoria Harbor, Mahe, all combinations of additions of N, P, or both, significantly ($P < 0.05$) inhibited P_{\max} . Light saturation began to appear earlier in the controls, with an I_k of $380 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ compared with $1200\text{--}1975 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for the pulsed samples. Correspondingly, the new compensation value was much lower for the controls, which had an I_c of 60 compared with $280\text{--}400 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for the pulsed material. Concomitantly, the pulsed samples showed substantially reduced α values compared with the untreated controls (Table 1).

There was a tendency for respiration (R) to decrease following nutrient pulses in most species. However, statistically significant ($P < 0.05$) declines in R were shown only for the pulsed samples of *Udotea orientalis* from St. Joseph Atoll (Table 1).

DISCUSSION

McRoy and Lloyd (1981) have categorized marine macrophytes as comprising two fundamentally different groups: (1) the macroalgae and (2) the seagrasses. The former group, as characterized by these authors, is analogous to filter-feeding animals in terms of extraction of nutrients from the seawater milieu, while secured to two-dimensional substrata by means of a holdfast. Members of the latter group extract nutrients from both the water column and pore waters of the soft, sedimentary,

