Effects of Water-Column Enrichment on the Production Dynamics of Three Seagrass Species and Their Epiphytic Algae

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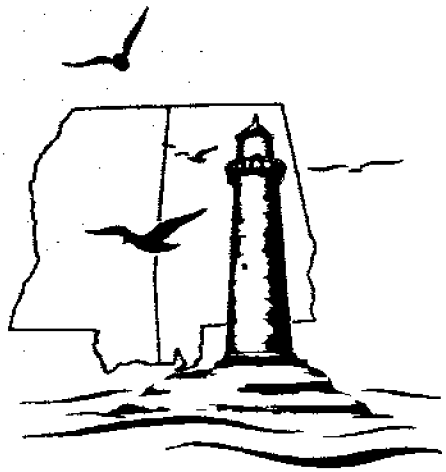

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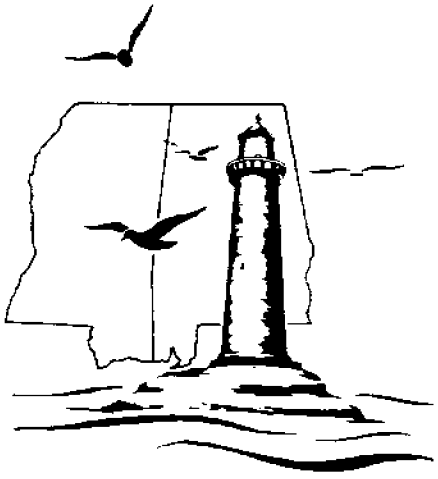

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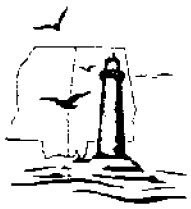

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ABSTRACT

Monospecific beds of the seagrasses *Halodule wrightii*, *Syringodium filiforme*, and *Thalassia testudinum* in Big Lagoon, Perdido Key, Florida, were enriched with a slow-release (3-4 month) Osmocote™ (N-P-K) fertilizer from August 1993 through September 1994. Measurements of primary production (as ^{14}C uptake), biomass (dry weight), and chlorophyll *a* (measured by HPLC) of epiphytes in enriched beds were significantly greater than values obtained for epiphytes in control beds. Based on microscopic observations, the dominant epiphytic algae were diatoms and red and brown algae. Populations of the brown alga *Myriotrichia subcorymbosa* and the red alga *Acrochaetium flexuosum* increased greatly in enriched plots of all three seagrass species. Data from multiple linear regression supported observational data and showed that the pigment signatures selected for the dominant epiphytes (fucoxanthin, zeaxanthin, and violaxanthin) explained 97% of the variation in chlorophyll *a*. A strong correlation between measured and predicted chlorophyll *a* ($r = 0.98$) suggested that chlorophyll *a* is an excellent indicator of epiphytic biomass in this seagrass system. Enrichment had a significantly positive effect on production rates of seagrass blades but not on their biomass.

INTRODUCTION

Seagrasses are submerged, perennial angiosperms which occur in shallow, coastal waters at all latitudes except the polar regions (Short 1987). Although sexual reproduction is common, propagation from rhizomes is probably more important in the formation of seagrass meadows. A diverse assemblage of algae attached to the seagrass leaves includes various species of diatoms and red, brown, green, and blue-green algae (Ballantine & Humm 1975, Humm 1964, Sullivan 1979, Thursby & Davis 1984). This autotrophic seagrass-epiphyte complex constitutes one of the most productive ecosystems in the biosphere (Short 1987).

Trophic interactions within seagrass ecosystems are complex because of the great diversity and abundance of organisms present. Many commercially important finfishes, invertebrates, and migratory waterfowl either use seagrass beds directly for food and/or habitat or prey on species which spend part of their life cycle in seagrass vegetation (Michot & Chadwick 1994, Thayer *et al.* 1975). Most research efforts have focused on the productivity and presumed trophic importance of the macroscopic seagrasses; recent investigations have demonstrated that epiphytic algae are the basis of the food web in many seagrass ecosystems (Dauby 1989, Fry *et al.* 1987, Kitting *et al.* 1984, Morgan & Kitting 1984).

Nutrient availability is one of the most important factors controlling production of the seagrass-epiphyte complex (Short 1987, Smith 1984, Twilley *et al.* 1985, van Lent *et al.* 1995). Natural coastal waters are normally oligotrophic, and growth of macrophytes and epiphytes is often nutrient-limited (Short 1987, Short *et al.* 1990, Twilley *et al.* 1985). Macrophytes obtain nutrients from the sediments via roots (Jackson *et al.* 1994, Short & McRoy 1984, Wium-Anderson 1971), the water column via leaves (Short & McRoy 1984, Thursby & Harlin 1982), and indirectly through epiphytes (Harlin 1973). Nutrient uptake via sediments is usually considered to be most important to macrophytes, but uptake of water-column nutrients via leaves of *Zostera marina* may account for 60-70% of total nitrogen (N) uptake (Hemminga *et al.* 1991).

Epiphytic algae obtain nutrients directly from the water column or from leaves of the host macrophyte (Harlin 1973, McRoy & Goering 1974, but see Jackson *et al.* 1994). Water-column nutrients are more important to epiphytic algae than sediment-derived nutrients because of the relative position of epiphytes within the water column (Williams & Ruckelshaus 1993), the fact that epiphytes lack vascular conducting tissue, and the efficient nutrient-uptake kinetics of microalgae (Nixon & Pilson 1983).

During the last several decades, thousands of hectares of seagrass meadows have been lost throughout the world

(Orth & Moore 1983, Walker & McComb 1992). Increased concentrations of nutrients in the water column caused by cultural eutrophication are considered to be the primary factor leading to seagrass demise (Cambridge & McComb 1984, Duarte 1991, Sand-Jensen & Borum 1991). Nutrient enrichment stimulates the growth of phytoplankton and epiphytic microalgae on seagrass leaves and thus reduces the spectral quality and/or amount of light available to the photosynthetic tissues of the seagrasses (Orth & Moore 1983, Silberstein *et al.* 1986). Light attenuation, therefore, is a secondary effect of nutrient enrichment, and these factors synergistically affect the health and vigor of seagrasses (Sand-Jensen & Borum 1991).

Previous research that has focused on the effects of nutrient enrichment on seagrass systems may be grouped into two broad categories based on whether the sediment or water column was enriched. Studies of sediment enrichment (Bulthuis *et al.* 1992, Erftemeijer *et al.* 1994, Orth 1977, Perez *et al.* 1991, Powell *et al.* 1989, Short *et al.* 1990, van Lent *et al.* 1995, Williams & Ruckelshaus 1993) have been used primarily to determine the limiting nutrient in seagrass environments. These studies do not mimic the effects of cultural eutrophication on the seagrass-epiphyte complex.

Of the 57 recognized species of seagrasses, only a few have been studied from the standpoint of water-column

enrichment, largely because the geographic distribution of seagrasses often makes it difficult to use more than one species in a given study.

Studies of water-column enrichment have involved both laboratory (Burkholder *et al.* 1992, Burkholder *et al.* 1994, Coleman & Burkholder 1994, Lapointe *et al.* 1994, Neckles *et al.* 1993, 1994, Neundorfer & Kemp 1993, Tomasko & Lapointe 1991) and field experiments (Coleman & Burkholder 1995, Fourqurean *et al.* 1995, Harlin & Thorne-miller 1981, Tomasko & Lapointe 1991, Tomasko *et al.* 1994). In these studies, biomass was used most often for assessing the response of epiphytic algae to enrichment. Measurements of biomass may be misleading because epiphytic algae have rapid turnover rates (Borum 1987) and the dry weight of animals, carbonates, sediments, and materials other than epiphytic algae may be included (Burkholder & Wetzel 1989). Actual rates of carbon fixation (i.e. ^{14}C uptake) provide a direct measurement of organic matter assimilation by epiphytic algae (Steeman-Nielson 1952). Although Coleman & Burkholder (1994, 1995) used ^{14}C uptake rates in both their laboratory and field studies, only the epiphytic component of the seagrass-epiphyte complex was examined. Because the effects of enrichment on seagrasses are proposed to be mediated through the epiphytic algae, simultaneous measurement of the responses of both autotrophic components to enrichment is necessary.

I used ^{14}C uptake rates and measurements of biomass of both autotrophic components to assess the response to water-column nutrient enrichment. I tested the null hypothesis that water-column enrichment exerts no effect on the primary production rates of either epiphytic algae or seagrass blades. The use of both uptake rates and biomass allowed me to compare the efficacy of these techniques for detecting enrichment effects. A study site in which monospecific beds of three subtropical seagrass species occur was selected, thereby allowing comparisons among these species and their epiphytic algae.

MATERIALS AND METHODS

Study Area

All field work was conducted within seagrass beds at Big Lagoon, Perdido Key, Escambia County, Florida, USA (Fig. 1). Perdido Bay is a shallow, brackish-water inlet which is influenced by seasonal shifts in prevailing winds and freshwater effluent from the adjacent mainland. This area is unique because monospecific beds of three seagrass species (*Halodule wrightii* Ascherson, *Syringodium filiforme* Kützing, and *Thalassia testudinum* König) are present. These seagrasses are abundant and widely distributed in the Gulf of Mexico, along the east coast of Florida, and in the West Indies.

Experimental Design

Fifty-four sections of 5-cm diameter PVC pipe were cut into 20.5-cm lengths. Six equally-spaced, longitudinal rows of 6, 6.4-mm holes were drilled in each section, which was termed a tube. A plastic cap was glued onto one end of each tube and a screw cap was fitted to the other end. A nylon stocking placed in each tube was filled with 454 g of a slow-release (3-4 month), temperature-sensitive, Osmocote™ fertilizer. This fertilizer contained 19% N (as ammonium nitrate), 6% P (as anhydrous phosphoric acid), and 12% K (as anhydrous potassium hydroxide) by weight. Laboratory experiments at the Dauphin Island Sea Lab, Dauphin Island,

Alabama, demonstrated that this fertilizer releases nutrients to the water column at a constant rate (Dr. Jon Pennock, pers. comm.).

Four experimental plots were selected for each of the three seagrass species. Two of the four plots were designated for water-column enrichment and two served as controls. All plots were located at least 20 m apart. Nine dispensing tubes were anchored vertically in each of the six plots designated for enrichment (two plots for each seagrass species). Eight tubes were spaced equally in a circle (1.5-m diameter) in the middle of the plot and one tube was placed in the center of the circle. During summer, early fall, and late spring, nutrient tubes were cleaned and replaced about every 3 weeks to ensure a constant release of fertilizer. Each time nutrient tubes were changed, 10 tubes were selected at random and the fertilizer from these tubes was dried to a constant weight (60°C) and re-weighed to provide an estimate of fertilizer release per tube per day.

Sampling Strategy

Nutrient enrichment began in August 1993 and continued through September 1994. Sampling was conducted about every two months from October 1993 to September 1994. On the first sampling date (30 October 1993), water-column samples were collected from control and enriched plots to determine the concentration of ammonia, nitrate, and inorganic phosphorus. Analyses were performed by the Mississippi

State University Chemical Laboratory.

On each subsampling date, treatment effects were quantified by measuring *in situ* primary production rates and biomass of both seagrasses and epiphytes in each of the 12 experimental plots. Samples were obtained from each plot for determination of epiphytic algal community structure. Water temperature, salinity, and photosynthetically active radiation (PAR) (400-700 nm) were measured during primary production incubations. PAR measurements were obtained in air and at the sediment surface in seagrass beds using a LiCor Quantum/Radiometer/Photometer, Model LI-185B.

On 3 June and 27 June 1994 two nutrient tubes without fertilizer were placed in the field at distances of 1 and 2 m from a tube containing fertilizer. These experiments were conducted to provide information regarding the effect of current on fertilizer dispersion once nutrients were released into the water column (i.e. fertilizer drift). If fertilizer release was relatively unaffected by water current, a gradient with regard to the colonization of algae on tubes was anticipated, with the most algae occurring on the tube containing fertilizer and the least algae on the tube that was 2 m away. Tubes remained in the field for about 3 weeks and were then removed and photographed.

Primary Production Measurements

Primary production rates were determined separately for seagrasses and their epiphytes by ^{14}C uptake measurements.

The technique was a modification of that of Penhale (1977). Seagrass blades with attached epiphytes were placed in incubation chambers (glass, screw-cap test tubes). Three incubation chambers were used in each of the 12 experimental plots. Each chamber was completely filled with unfiltered seawater and the contents of a 1 ml, 5 μCi ampoule of sodium bicarbonate ($NaH^{14}CO_3$) was injected with a 22-gauge needle and syringe. An 18-gauge needle and syringe was used to inject an aliquot of dichlorophenyl dimethyl urea (DCMU) into one incubation chamber per plot so that the final concentration of DCMU per chamber was 10^{-5} M. The uptake rate determined for the chamber containing DCMU was used to correct for inactive uptake of ^{14}C (Legendre *et al.* 1983). Similarly, determination of the primary production rate of phytoplankton (incubated in BOD bottles) was used to correct for ^{14}C uptake by phytoplankton in each incubation chamber. Samples were incubated *in situ* for 2 h before incubation was stopped with the addition of 4% buffered formalin.

In the laboratory, epiphytes were scraped from seagrass blades with a silicon-rubber stopper (Burkholder & Wetzel 1990) and collected on a 0.45 μm cellulosic membrane filter. Surface adsorbed label was removed from epiphytes with washes of 2% HCl. The surface area of seagrass blades was measured on a LiCor (Model LI-3000) portable surface area meter after placing radio-labelled blades between two acetate sheets to prevent contamination. Blade surface area

was determined so that primary production rates for both seagrass blades and epiphytes could be expressed in units of square centimeters. Samples were burned in a biological material oxidizer (R. J. Harvey, Model OX-500) and a liquid scintillation counter (Beckman LS 3801) determined ^{14}C disintegrations per minute.

The method of Strickland & Parsons (1977) was used to calculate field pH and the total amount of ^{12}C available for uptake in incubation chambers. ^{14}C disintegrations per minute (dpm) were converted to carbon uptake rates using the formula:

$$[((L - D) / R) \times A \times C] / P \times T,$$

where L = dpm of sample, D = dpm of sample containing DCMU, R = efficiency of sample oxidizer, A = total ^{12}C available for uptake (milligrams per milliliter) multiplied by the volume of the incubation chamber (milliliters), C = 1.064 (correction term for difference in ^{12}C and ^{14}C isotopic masses), P = 1.11×10^7 dpm (2.22×10^6 dpm per μCi x 5μCi ^{14}C added per sample), and T = incubation time in hours. These results were expressed in units of milligrams carbon per sample per hour and were divided by the total surface area (square centimeters) of blades in each sample to convert the results to milligrams carbon per square centimeter blade surface per hour.

Biomass Determinations

Aboveground live biomass in control and nutrient-

enriched seagrass beds was estimated by a method similar to that reported by Morgan & Kitting (1984). Two 10 x 10-cm quadrats were selected in each bed. All blades in each quadrat were clipped at the sediment surface and placed in a plastic bag. I subsampled (8-15) blades from each quadrat and these were placed in a separate plastic bag. Subsampling of blades was done immediately after blades were clipped, as epiphytes may fall from blades after being removed from the water. A small amount of distilled water was added to the bags containing the samples before they were placed on ice for transport.

In the laboratory, the total number of blades per quadrat (including the number which was subsampled) was counted. Epiphytes were scraped from the subsamples with a silicon-rubber stopper (Burkholder & Wetzel 1990). Blades and epiphytes were placed in separate aluminum weighing dishes and were dried at 60°C to a constant weight. The total number of blades in each quadrat was divided by the number of blades in each subsample and this number was multiplied by the dry weight of epiphytes and blades to determine the contribution of each to total dry weight. These dry-weight determinations were used to estimate total combined dry weight (per quadrat) for seagrass leaves and epiphytes. Measurements of biomass were not conducted during the January sampling period because of low water temperature.

Epiphytic Algal Community Structure

On each sampling date, approximately 20 seagrass blades with attached epiphytes were clipped from each of the 12 experimental plots. Half of the total number of blades were placed in each of 2 plastic bags. A small amount of seawater was added to each bag. Samples were stored immediately on ice for transport. In the laboratory, one bag from each plot was used for the identification of epiphytic algae, and the other was used for pigment analysis. Non-diatom epiphytic algae were identified to genus or species when possible. A species list of epiphytes by phylogenetic group (division) was recorded for each experimental plot on each sampling date.

Samples used for pigment analysis were processed in a darkened room to minimize pigment degradation. Epiphytes were scraped from blades and filtered onto 0.7 μm glass microfiber filters (Whatman GF/F) described previously (see primary production measurements). Filters were folded in half, patted dry with paper toweling, and frozen at -80°C (Revco Model ULT1786). The surface area of cleaned blades was measured in units of square centimeters as above.

High-Performance Liquid Chromatography (HPLC) was used to identify and quantify phylogenetic groups of algae (at the division or class levels) based on the presence and amounts of pigments which are unique or "diagnostic" of each algal group (Millie *et al.* 1993). Concentrations of

chlorophyll *a*, fucoxanthin, violaxanthin, lutein, and zeaxanthin were determined because they were characteristic of the algal groups observed in live material. Chlorophyll *a* is ubiquitous among oxygenic photosynthesizing organisms and was used to estimate the abundance of all algal taxa. Fucoxanthin is found in brown algae (Phaeophyta) and diatoms (Bacillariophyta) whereas violaxanthin is found in brown algae and does not normally occur in high concentrations in the diatoms (Rowan 1989). Diatoms were the most abundant algal group observed in both control and enriched plots. The concentration of lutein was used to estimate the abundance of green algae (Chlorophyta), although very few genera were observed in live material. The concentration of zeaxanthin was used as an indicator of the abundance of red algae (Rhodophyta). Although blue-green algae (Cyanobacteria) also contain zeaxanthin, microscopic examination of live material indicated that few cyanobacteria were present as epiphytes.

Data Analysis

Statistical Analysis System (SAS 1988) for Windows was used for all data analyses. A modified split-plot design which accounted for sampling over time was used for the analysis of variance of primary production and biomass data. The least significant difference (LSD) test was used for separation of means. Blade production, epiphytic production, and total production (blades + epiphytes) were

used as response variables in the analysis of primary production data. The dry weights of blades and epiphytes and total dry weight (blades + epiphytes) were used as response variables in the analysis of biomass data. Pigment data were also subjected to a modified split-plot design which accounted for sampling over time. The pigments chlorophyll *a*, fucoxanthin, violaxanthin, lutein, and zeaxanthin were used as response variables to distinguish among the abundance of epiphytic algal groups. Because the variables, seagrass species and time, were not significant sources of variation (using the split-plot design), the data were re-analyzed using a completely randomized design and the variables, seagrass species and time, were pooled with the error term to increase error degrees of freedom. Multiple linear regression was used to determine how well the analyzed pigments predicted chlorophyll *a*. Correlation analysis was used for between-treatment comparisons of algal pigments.

RESULTS

Environmental Data

The environmental data collected during the study period are listed in Table 1. Water temperature ranged from 29°C in September 1993 to 7°C in January 1994. The lowest salinity was recorded in August 1994 (13 ppt) and the highest in October 1993 (27 ppt). Low salinity was associated with an increase in the amount of regional rainfall. Photosynthetically active radiation (PAR) was consistently lower in beds of *S. filiforme* and *T. testudinum* than in beds of *H. wrightii*. *H. wrightii* occurs at more shallow depths than the other two species in Big Lagoon, and therefore receives a greater amount of PAR. The average PAR reaching the sediment surface of beds of *H. wrightii* and *S. filiforme/T. testudinum* was 670 and 280 $\mu E\ m^{-2}\ s^{-1}$, respectively.

Analysis of variance showed that there was no difference in the mean concentrations of ammonia (0.10 mg l^{-1}), nitrate (0.25 mg l^{-1}), and inorganic phosphorus (0.26 mg l^{-1}) in the water column between control and enriched plots. Of the three nutrients, only the mean value reported for nitrate slightly exceeded the limit of its detection.

Fertilizer Release

Based on the dry weight measurement of fertilizer from 90 randomly selected nutrient tubes, the mean release of fertilizer was 4.6 g per tube per day. Therefore, about 41.4 g of fertilizer per day were released in each of the 6 enriched plots yielding a total of 248.4 g of fertilizer per day released into the system. In field experiments (May - October 1993) at Port St. Joseph Bay, Florida, USA, 3,178 g of this fertilizer per experimental plot was shown to release nutrients at concentrations 2-4 times that of ambient nutrient concentrations (Dr. Jon Pennock, pers. comm.). In my experiment, 4,086 g of fertilizer were used per experimental plot.

The two experiments designed to test for the effects of current on fertilizer dispersion showed that there was a gradient with regard to colonization by algae. The greatest biomass of algae occurred on the tube containing fertilizer and the least biomass occurred on the tube that was 2 m away. These data supported my observation that flow rates are low in Perdido Bay. Furthermore, because of the distance between plots (≥20 m), these experiments provided evidence of an extremely small possibility of fertilizer drifting into and contaminating control plots.

Primary Production

The combined analysis of variance showed that a two-way interaction term (seagrass species x time) and the main

effect of fertilizer were significant for each of the three response variables (seagrass blade productivity, epiphytic productivity, and total productivity) (Table 2). On the sampling date in August, the average ^{14}C uptake rate of *T. testudinum* blades was significantly less than uptake rates of blades of *S. filiforme* and *H. wrightii* (Fig. 2). On the sampling date in September, the average ^{14}C uptake rate of *H. wrightii* blades was significantly greater than those of the other two species (Fig. 2). Production rates of seagrass blades in enriched plots were about 1.4 times greater than rates of blades in control plots (Fig. 3).

On the sampling date in January, the average ^{14}C uptake rate for epiphytes of *T. testudinum* was significantly less than values measured for epiphytes of *H. wrightii* and *S. filiforme* (Fig. 4). On the sampling date in September, the average ^{14}C uptake rate of epiphytes of *S. filiforme* was greater than that of epiphytes of either of the other two seagrass species (Fig. 4). Production rates of epiphytic algae in enriched plots were about twice those measured for control plots (Fig. 5). Furthermore, production rates of epiphytic algae in enriched plots were greater than corresponding rates for seagrass blades (Figs. 3, 5). Production rates for blades and epiphytes in control plots were similar (Figs. 3, 5).

On the sampling dates in October and January, total production rates (blades + epiphytes) of *T. testudinum* were

significantly less than rates of the seagrass-epiphyte complex of either of the other two species (Fig. 6). On the sampling date in September, total production rates of the *S. filiforme* seagrass-epiphyte complex were significantly greater than rates of the *H. wrightii* and *T. testudinum* seagrass-epiphyte complexes (Fig. 6). Total production in enriched plots was about 1.7 times greater than that in control plots (Fig. 7). The percent contribution of epiphytes to total primary production in enriched plots of *H. wrightii* was greater than that in control plots; the proportional contribution of epiphytes was almost equal in enriched and control plots of *S. filiforme* and *T. testudinum* (Fig. 8). In control plots of *H. wrightii* and *T. testudinum*, blades and epiphytes contributed almost equally to total production (Fig. 8).

Biomass Measurements

The combined analysis of variance showed that the main effects of seagrass species and time were significant for biomass of seagrass blades (Table 3). The average dry weight of *H. wrightii* blades was less than that of *S. filiforme* and *T. testudinum* blades (Fig. 9). The average biomass of blades of the three seagrass species combined was least on the sampling dates in April and September and greatest on the sampling dates in June and August (Fig. 10). Enrichment had no effect on biomass of seagrass blades.

Analysis of variance showed that a two-way interaction

term (seagrass species x time) and the main effect of fertilizer were significant for biomass of epiphytes (Table 2). The dry weight of *H. wrightii* epiphytes was significantly less than that of *T. testudinum* epiphytes on the sampling date in September (Fig. 11). Average biomass of epiphytes among the three species of seagrass on all other sampling dates did not differ significantly (Fig. 11). In contrast, epiphytes in enriched plots had a greater biomass (1.4 times greater) than epiphytes in control plots (Fig. 12).

Significant variation of total biomass (blades + epiphytes) was found for the main effect of seagrass species (Table 3). Total biomass of *H. wrightii* (including its epiphytes) was significantly less than that of either of the other two species (Fig. 13). There was no difference between total biomass of *T. testudinum* and *S. filiforme* (Fig. 13). The percent contribution of epiphytes to total biomass was greater in enriched plots than in control plots for all three seagrass species (Fig. 14). Percent contribution of epiphytes (31-44%) to total biomass was less than that of blades for all species and all treatments except for enriched plots of *H. wrightii* (Fig. 14).

Epiphytic Algal Community Structure

Microscopic examination of epiphytes showed that diatoms (Bacillariophyta) were the most abundant algal group in both control and enriched plots. Species richness of

macroalgae was highest among the red algae (Rhodophyta) (Table 4). The abundance of macroalgae was lower in control than in enriched plots (Table 5). Enriched plots of *H. wrightii* had a greater species richness of epiphytic macroalgae than enriched plots of *S. filiforme* and *T. testudinum* (Table 5). Populations of the brown alga *Myriotrichia subcorymbosa* and the red alga *Acrochaetium flexuosum* increased greatly in enriched plots of all seagrass species.

Analysis of variance of data derived from HPLC determinations of identity and quantity of pigment showed that there was a significant difference in the concentrations of pigments between control and enriched plots for chlorophyll a, fucoxanthin, violaxanthin, and zeaxanthin. No significant difference in the concentration of the pigment lutein was found between control and enriched plots (Tables 6, 7). Concentrations of chlorophyll *a*, fucoxanthin, and zeaxanthin were 3 times greater whereas that of violaxanthin was 6 times greater in enriched plots than in control plots (Table 6).

Multiple linear regression [using selections for Mallow's C(p) statistic, adjusted R-square, and mean square error] showed that the best model for predicting total chlorophyll a was a three-variable model which included the concentrations of the pigments fucoxanthin, violaxanthin, and zeaxanthin. Lutein was found not to contribute

significantly to the predicted concentration of chlorophyll *a*. This model may be expressed as

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_4 X_4 + \epsilon,$$

where X1 = [fucoxanthin], X2 = [violaxanthin], X4 = [zeaxanthin], and Y = predicted [chlorophyll *a*]. This finding was consistent with the observation of very few green algae (Tables 4, 5). Collectively, the concentrations of fucoxanthin, violaxanthin, and zeaxanthin explained 97% (R^2 = 0.9691) of the variation in concentrations of chlorophyll *a* (Fig. 15). As a single variable, fucoxanthin explained 88% (R^2=0.8816) of the variation in chlorophyll *a*. This relationship is consistent with the great abundance of diatoms observed in live material.

Because violaxanthin has been reported in low concentrations in some species of diatoms (Rowan 1989), it was necessary to verify that this pigment is a valid indicator of the abundance of brown macroalgae. Brown algae were observed in high concentration in enriched plots, but their abundance was low in control plots (Table 5). If brown algae were the primary contributors of violaxanthin, then the correlation between this pigment and fucoxanthin should be lower in control than enriched plots because fucoxanthin is dominant in both diatoms and brown algae. Correlation analysis showed that the relationship between fucoxanthin and violaxanthin was significant in enriched plots (r = 0.67) but not significant in control plots (r =

0.30). Furthermore, multicollinearity diagnostics (variance inflation factor, tolerance, and eigenvalues) showed that violaxanthin contributed unique information to the linear regression model for predicting chlorophyll *a* concentration.

DISCUSSION

To clarify the following discussion, we will first present the limitations and advantages of the kinds of techniques used in the collection of data. The remainder of the discussion is presented in sections that pertain to seagrass blades, epiphytes and their community structure, and the consequences of long-term enrichment in Perdido Bay.

Uptake Rates Versus Biomass

Biomass and ^{14}C uptake measure different components of production in the seagrass-epiphyte community and there is no significant correlation ($r = 0.2164$) between measurements of biomass and primary production (^{14}C uptake) in my study. Biomass has been the method of choice for assessing the response of epiphytic algae to enrichment in most studies. This technique is inexpensive and can be performed easily in the field. Biomass is used to measure the long-term accrual of organic matter and is usually expressed in units of grams dry weight per unit area of seagrass bed. With such measurements, the surface area of seagrass blades is usually ignored and less information is provided relative to the density of epiphytes on blades. Biomass is a poor indicator of the abundance of epiphytes because the dry weight of animals, carbonates, sediments, and materials other than epiphytic algae is included (Burkholder & Wetzel 1989). Because much of what is weighed is not alive, the actual contribution of epiphytes to the production of the system

cannot be accurately defined. Epiphytic algae have rapid turnover rates (Borum 1987) which are not reflected in a measure of dry weight. Biomass is a more effective method for estimating macrophyte production because seagrass blades have long turnover times and epiphytic algae and other debris can be removed from the surface of blades.

^{14}C uptake rates provide a nearly instantaneous (2 h) measure of carbon assimilation. This method is expensive compared with that of biomass, and special permits and training are required for use of ^{14}C in the laboratory and field. To understand the effects of enrichment on the seagrass-epiphyte complex, both autotrophic components should be measured simultaneously. It is also important to use a measurement which will accurately express the contribution of each autotrophic component to overall system production exclusive of non-living material. For this reason, ^{14}C uptake rates are better than measurements of biomass. Actual rates of carbon fixation (i.e. ^{14}C uptake) provide a direct measurement of organic matter production by epiphytes and seagrasses and obviate some of the problems inherent in measurements of biomass. Although some non-photosynthetic organisms can take up ^{14}C, this inactive uptake can be taken into account by use of an herbicide. This method prevents underestimation of primary production which can occur with use of a dark bottle (Legendre *et al.* 1983).

HPLC Pigment Analysis

HPLC pigment analysis has been used extensively to identify and quantify phytoplankton groups in marine and estuarine environments (Claustre 1994, Gieskes & Kraay 1983, Millie *et al.* 1993, Tester *et al.* 1995). In our study, this technique was used for the first time to identify and quantify the pigments of epiphytic algae in the marine environment. Because seagrass beds are open systems, concentrations of water-column chlorophyll *a* are better than water-column nutrient concentrations, but are not as accurate as epiphytic algal biomass for indicating trophic status (Borum 1985, Tomasko & Lapointe 1991). Although epiphytes are not static, they are much less motile than phytoplankters. The strong correlation ($r = 0.98$) between predicted and measured chlorophyll *a* shows that chlorophyll *a* is an excellent indicator of epiphytic biomass. This method offers an alternative to estimates of biomass based on measurements of dry weight for epiphytic algae.

Production of Seagrass Blades

The interaction of seagrass species x time was significant for all measurements of primary production (blades, epiphytes, and total production). We suggest that availability of light rather than nutrients was the principal factor influencing primary production rates of seagrass blades and epiphytes among species. *H. wrightii* occurs in shallow water in Big Lagoon, receives the greatest

PAR (Table 1), and in general, was found to have greater production rates than *T. testudinum* and *S. filiforme*. *T. testudinum* occurs in the deepest water in Big Lagoon, receives the least PAR, and its production rates were observed generally to be less than the other two seagrass species. *S. filiforme* occurs in deeper water than *H. wrightii* but in more shallow water than *T. testudinum*. Primary production rates of *S.filiforme* were observed generally to be intermediate between the other two seagrass species. The production rates of epiphytes followed a similar pattern. Fertilizer (nutrients) was only significant as a main effect, thus the importance of light availability on production among species is further supported. If seagrasses had responded differently to increased nutrient levels, then the interaction between fertilizer and seagrass species would have been significant.

Because water-column nutrients are taken up very rapidly (Suttle & Harrison 1988, Suttle *et al*. 1990, Tomasko & Lapointe 1991), accurate measurement of their concentrations is difficult. Accordingly, measurement of nutrients is a poor indicator of the extent of eutrophication. The one measurement of nutrient levels taken at the study site showed that the concentrations of all nutrients were low; only nitrate was above the lower limit of detection. A low rate of nutrient delivery during the experiment likely explains why blade production

increased instead of decreasing as I predicted. Enrichment was sufficient to stimulate growth of epiphytic algae, but the increase in epiphytic biomass was not enough to inhibit production rates of seagrass blades by shading. Ambient nutrient concentrations in this experiment were similar to those measured by Short (1995) in mesocosm experiments with *Zostera marina* L. Short (1995) found that growth of *Z. marina* was inhibited by epiphytic and filamentous algae at concentrations 6 times that of ambient. Assuming that nutrient delivery rates in this experiment were 2-4 times greater than ambient, (Dr. Jon Pennock, pers. comm.), we estimate that nutrient additions 2.0-2.5 times greater than we used would be needed before a detrimental effect on macrophytes would be observed. However, it is difficult to accurately predict the amount of nutrient required to cause a detrimental effect on macrophytes because the community structure of epiphytic algae differs from one system to another. Per unit of chlorophyll *a*, epiphytic algal groups differ in their capacity to attenuate light because of differences in concentrations of accessory pigments. For example, diatoms contain a large amount of fucoxanthin and have a light attenuation coefficient about twice that of green algae, which lack this pigment (Steeman-Nielsen 1962). The natural oligotrophy of the Big Lagoon system is confirmed by the fact that enrichment increased production of blades and epiphytes. In systems which are heavily

grazed, an increase in primary production should also benefit secondary production (i.e. provide more food for consumers). Based on my observations, however, seagrass beds in Big Lagoon are not heavily grazed by macrofauna.

Production of Epiphytes

The prolific growth of chain-forming, epiphytic diatoms [*Grammatophora oceanica* Ehr., *Rhabdonema adriaticum* Kütz., *Striatella unipunctata* (Lyngbye) Ag., *Hyalosira interrupta* (Ehr.) Navarro, and *Licmophora debilis* (Kütz.) Grun.] and filamentous macroalgae indicate that flow rates in Perdido Bay are low. Tidal maxima are 0.6 m and the presence of a sandy beach indicates that wave action is not great. My data (see also Coleman & Burkholder 1994) show that epiphytic production was stimulated by the level of enrichment I used. However, under a high-flow regime, increases in production of epiphytic algae may not occur (Coleman & Burkholder 1995). In low-flow, oligotrophic systems, the autotrophic components are nutrient-limited and act as a sink when nutrient concentrations are increased above what is normal for the system. Enrichment produces a system dominated by autotrophic processes (see mesocosm experiments of Nilsson *et al.* 1991) as primary production rates increase faster than secondary production rates. In systems characterized by a low population of grazers (thus less control on growth of epiphytes), enrichment is more likely to have a negative effect on the growth of

macrophytes (Howard & Short 1986, Philippart 1995). Although few large grazing species were observed in the Big Lagoon system, the level at which we enriched the water column may have been insufficient to produce such an effect. The abundance of small grazing species may have increased in response to enrichment (Nilsson *et al.* 1991), but their abundance was not sufficient to inhibit epiphytic algal production. My data show that Big Lagoon is currently a nutrient-limited system dominated by autotrophic processes, but future enrichment studies need to address the role of grazers in removing epiphytic algal biomass. This information will become more important as commercial development continues in this area.

Biomass of Blades

Primary production rates of blades increased with enrichment but an increase in biomass was not detected. One explanation for this discrepancy is the difference in sensitivity of the two techniques used for assessment. ^{14}C uptake rates provide a nearly instantaneous (2 h) measure of carbon assimilation. Biomass represents a long-term (weeks) accrual of organic matter. We suggest that the resolution of the biomass measurements was not precise enough to detect changes in aboveground biomass of seagrass blades. An alternate explanation is that increases in aboveground biomass of seagrass blades via enrichment were not detected because carbon fixation indicated by ^{14}C uptake was stored

in underground roots and/or rhizomes rather than channeled to blades. Under oligotrophic conditions, Pérez *et al.* (1994) observed that seagrasses allocate a greater proportion of fixed carbon to root development to increase the capacity of roots to remove inorganic nutrients from the sediment pore water. At higher nutrient concentrations, shoot production increases; this is correlated with a reduction in the lifespan of shoots, and the reason for this response is not known (Pérez *et al.* 1994). Thus, under oligotrophic or eutrophic conditions, an increase in aboveground biomass of blades may not be detected.

Biomass of Epiphytes

A significant interaction of seagrass species x time occurred for epiphytic biomass. *T. testudinum*, *S. filiforme*, and *H. wrightii* differ considerably in blade morphology, density, and height, and these species occur at different depths. *T. testudinum* blades provide the greatest surface area per blade for attachment by epiphytic algae, but have fewer blades per unit area. This occurs because *T. testudinum* grows in deeper water than the other two seagrass species and light availability decreases with depth. Blades of *S. filiforme* are circular in cross section and have greater surface area per blade than *H. wrightii*. In general, *S. filiforme* occurs in deeper water than *H. wrightii*, and because of less PAR, has lower blade density per unit area of seagrass bottom. Under these conditions,

greater surface area offers an advantage for attachment, but this advantage is mitigated by a reduction in light availability and blade density. From our data, it is not possible to separate effects of light availability and blade density from the effects of blade morphology because measurements of biomass were based on unit area of seagrass bottom and not on surface area of seagrass blades. The number of old versus young blades in a biomass sample also affects the abundance of epiphytes. Seagrass leaves exhibit basal growth and young blades have allelopathic properties (van Montfrans *et al.* 1984). The greatest abundance of epiphytic algae occurs on the distal (oldest) portions of blades. Biomass samples which have numerous young blades will have less epiphytic growth than samples which contain mostly older blades. Biomass measurements for epiphytic algae should be interpreted with caution (see above for additional discussion).

Community Structure of Epiphytic Algae

Diatoms were the dominant algal group in this and other enrichment studies (Coleman & Burkholder 1994, Sundbäck & Snoeijs 1991). For many years, diatoms have been used to monitor water quality in freshwater systems (Dixit *et al.* 1992), but little research regarding their value as biomonitors in the marine environment has been conducted. Because measurements of nutrient levels in the water column do not indicate the trophic status of aquatic systems, other

methods of assessment need to be established. Diatoms are ideal organisms for this purpose because individual species vary in their ability to tolerate environmental variables (i.e. pH, nutrients, salinity, temperature, etc.) (Dixit *et al.* 1992). As the environment changes, the dominant diatom taxa may also change. The examination of community structure, including the species diversity of dominant epiphytic diatoms, should provide information regarding the usefulness of diatoms for monitoring water quality in the marine environment.

Because diatoms are a highly nutritious and preferred food source for marine grazers (Admiraal 1984, Plante-Cuny & Plante 1986), moderate amounts of enrichment to nutrient-limited systems may increase the quantity and quality of organic matter available to higher trophic levels. This appears to be the case in the present study. Although no measurements of secondary production were made, no obvious increase in the abundance of grazers in enriched plots was noted.

The increase in epiphytic biomass and pigment concentrations in response to enrichment was paralleled by an increase in the abundance of macroalgae in enriched plots (Table 5). In particular, populations of the red alga *Acrochaetium flexuosum* and the brown alga *Myriotrichia subcorymbosa* increased greatly in response to nutrient addition in plots of all three seagrass hosts. *A. flexuosum*

was the dominant non-diatom epiphyte of *H. wrightii* over an annual cycle in Mississippi Sound and *M. subcorymbosa* was abundant at this site in winter (Moncreiff et *al.* 1992). These two taxa remain the dominant non-diatom epiphytes in Mississippi Sound (M. J. Sullivan, pers. obs.), which suggests that these seagrass beds are subject to nutrient enrichment. *A. flexuosum* and *M. subcorymbosa* respond to lower concentrations of enrichment than other macroalgal species (see below). At the concentration of enrichment used in this study, it appears that the distribution and abundance, rather than the presence or absence, of macroalgal species are indicative of enrichment. Pigment data from this study show that the groups of algae comprising the epiphytic community did not change significantly over time and did not differ significantly among seagrass species. We suggest that greater concentrations of nutrients would have resulted in significant changes in the epiphytic community. An increase in the abundance of some epiphytic species may lead to a decrease in diversity of the epiphytic community (Tilman 1982, but see Pringle 1990).

The green macroalga *Enteromorpha* often responds to water-column enrichment in the marine environment (Harlin & Thorne-Miller 1981, Lapointe et *al.* 1994, Neckles et *al.* 1994, Sundbäck & Snoeijs 1991). In the present study, few green algae were observed as epiphytes (Table 4), but

Enteromorpha did occur in high concentrations on nutrient tubes. Apparently, the rates of nutrient delivery in the present study were below the threshold required to stimulate growth of *Enteromorpha* away from the source. In experiments designed to test the effects of current on fertilizer dispersion (see Sampling Strategy), *Enteromorpha* only colonized the tube containing fertilizer, whereas diatoms were the only algae to colonize the two tubes without fertilizer. Therefore, the presence of *Enteromorpha* was in response to the highest nutrient concentration rather than the result of nutrient tubes providing a better substrate for attachment than seagrasses. This suggests that an abundance of *Enteromorpha* in seagrass systems is indicative of high water-column nutrient concentrations.

Consequences of Enrichment

The consequences of long-term, low-level enrichment in Big Lagoon are not known, but several scenarios can be suggested. (1) Grazers will prevent an excessive accumulation of epiphytes on seagrass leaves, and therefore the entire system would benefit from enrichment. (2) The system will continue to be dominated by autotrophic processes and grazers will not prevent an excessive accumulation of epiphytes on seagrass leaves. Primary production of epiphytic algae will increase sufficiently to shade seagrasses. Shading of seagrass blades by epiphytic algae will decrease seagrass production and possibly lead to

the disappearance of one or more species (see below). (3) The climax species *T. testudinum*, which is long-lived, might be replaced by the pioneer species *H. wrightii* and *S. filiforme* (Gallegos et *al.* 1994) which are short-lived. This results from elevated water-column nutrients which cause a sufficient environmental disturbance and change the system from a more stable, K-selected, oligotrophic environment to a less predictable, r-selected, eutrophic environment. Also, over several years, low-level enrichment may continue to stimulate production of seagrass blades but *T. testudinum* will be replaced by *H. wrightii*, which has a greater nutrient demand than the former species (Fourqurean et *al.* 1992, 1995, Powell et *al.* 1991). If there is a negative correlation between longevity in seagrasses and their demand for nutrients, then the pioneer species *S. filiforme* may also replace *T. testudinum*.

Conclusions

All three seagrass species and their epiphytes responded similarly to water-column enrichment. Fertilizer was significant as a main effect for all measured variables (production, biomass, and pigment concentrations). The strong response of epiphytes to enrichment suggests that cultural eutrophication could pose a serious threat to the seagrass beds of Big Lagoon as recreational and agricultural development continue in Perdido Bay and surrounding areas. Negative effects could be manifested as a reduction in the

coverage of shallow, bottom sediments by seagrass beds and/or the elimination of one or two species.

This study addressed the response of the seagrass-epiphyte complex to nutrient enrichment, but an understanding of the effects of enrichment on secondary production is crucial to the development of a workable paradigm for the Big Lagoon system. Future research should incorporate the interaction of top-down and bottom-up trophic processes if a paradigm is to be established. The level of enrichment we used did not show a decrease in production of seagrass blades. Because of this, we suggest an experiment which includes multiple levels of enrichment (ambient, enriched, and 4X enriched). In addition to the response variables we have measured, the species composition and abundance of epi- and macrofauna should be determined.

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Table 1. Environmental data measured on each sampling date from October 1993 to September 1994. °C = Celsius; ppt = parts per thousand; PAR = photosynthetically active radiation; μE = microEinsteins per square meter per second; H = *Halodule wrightii*; S = *Syringodium filiforme*; T = *Thalassia testudinum*.

	Oct.	Jan.	Apr.	Jun.	Aug.	Sep.
Water Temp. (°C)	21	7	17	28	27	29
Salinity (ppt)	27	25	20	19	13	18
Ambient PAR (μE)	1650	350	1600	1700	1500	1800
H PAR (μE)	650	300	1000	1000	350	700
S / T PAR (μE)	425	175	500	275	100	200

Table 2. Summary statistics for the analysis of variance for ^{14}C uptake rates of seagrass blades, epiphytes, and total production (blades + epiphytes) from October 1993 to September 1994 (* indicates significance of F value, α = 0.05).

Source	DF	F Value	Pr >F
Response variable: blades			
Seagrass species	2	9.56	.0499
$Error_{(a)}$	3	2.98	.0368
Fertilizer	1	22.07	*.0182
Fertilizer X species	2	1.01	.4629
$Error_{(b)}$	3	1.38	.2550
Time	5	15.16	.0001
Time X species	10	3.52	*.0036
Time X fertilizer	5	1.76	.1514
Time X species X fertilizer	10	0.70	.7194
$Error_{(c)}$	30	1.42	.1129
Subsampling	72		
Response variable: epiphytes			
Seagrass species	2	11.49	.0392
$Error_{(a)}$	3	4.70	.0048
Fertilizer	1	24.21	*.0161
Fertilizer X species	2	3.02	.1913
$Error_{(b)}$	3	3.41	.0222
Time	5	6.04	.0006
Time X species	10	2.24	*.0430
Time X fertilizer	5	2.03	.1032
Time X species X fertilizer	10	1.59	.1566
$Error_{(c)}$	30	3.33	.0001
Subsampling	72		

Table 2 (continued)

Response variable:	total production		
Seagrass species	2	9.31	.0517
$Error_{(a)}$	3	5.86	.0012
Fertilizer	1	27.63	*.0134
Fertilizer X species	2	2.61	.2203
$Error_{(b)}$	3	3.20	.0283
Time	5	7.97	.0001
Time X species	10	2.69	*.0177
Time X fertilizer	5	1.15	.3540
Time X species X fertilizer	10	1.02	.4467
$Error_{(c)}$	30	2.58	.0006
Subsampling	72		

Table 3. Summary statistics for the analysis of variance for biomass of seagrass blades, epiphytes, and total biomass (blades + epiphytes) from October 1993 to September 1994 (* indicates significance of F value, $\alpha = 0.05$).

Source	DF	F Value	Pr >F
Response variable: blades			
Seagrass species	2	17.66	*.0219
$Error_{(a)}$	3	1.09	.3590
Fertilizer	1	0.08	.7967
Fertilizer X species	2	0.74	.5483
$Error_{(b)}$	3	0.62	.6048
Time	4	4.53	*.0072
Time X species	8	0.54	.8135
Time X fertilizer	4	0.41	.8010
Time X species X fertilizer	8	1.46	.2235
$Error_{(c)}$	24	1.13	.3414
Subsampling	60		
Response variable: epiphytes			
Seagrass species	2	5.34	.1028
$Error_{(a)}$	3	0.88	.4584
Fertilizer	1	30.58	*.0117
Fertilizer X species	2	2.19	.2593
$Error_{(b)}$	3	0.21	.8861
Time	4	2.21	.0983
Time X species	8	3.62	*.0067
Time X fertilizer	4	1.04	.4085
Time X species X fertilizer	8	1.71	.1463
$Error_{(c)}$	24	1.42	.1382
Subsampling	60		

Table 3 (continued)

Response variable: total biomass			
Seagrass species	2	25.99	*.0127
$Error_{(a)}$	3	0.55	.6485
Fertilizer	1	3.53	.1568
Fertilizer X species	2	0.49	.6530
$Error_{(b)}$	3	0.36	.7811
Time	4	1.60	.2059
Time X species	8	1.49	.2117
Time X fertilizer	4	0.77	.5582
Time X species X fertilizer	8	1.50	.2078
$Error_{(c)}$	24	1.28	.2205
Subsampling	60		

Table 4. A list of macroalgae identified as epiphytes of *Halodule wrightii*, *Syringodium filiforme*, and *Thalassia testudinum* in Big Lagoon, Perdido Key, FL, USA (from October 1993 to September 1994).

CHLOROPHYTA
Cladophora sp.
Enteromorpha sp.
Rhizoclonium riparium (Roth) Harvey
PHAEOPHYTA
Hummia onusta (Kütz.) Fiore
Myriotrichia subcorymbosa (Holden) Blomquist = gametophyte
Stictyosiphon subsimplex Holden = sporophyte
RHODOPHYTA
Acrochaetium flexuosum Vickers
Bryocladia sp.
Centroceras clavulatum (C. Ag.) Montagne
Ceramium byssoideum Harvey
Champia parvula (C. Ag.) Harvey
Chondria sp.
Erythrotrichia carnea (Dillwyn) J. Ag.
Griffithsia tenuis C. Ag.
Heteroderma lejolisii (Rosanoff) Fosl.
Laurencia poiteaui (Lamouroux) Howe
Polysiphonia sp.
Sahlingia subintegra (Rosenv.) Kornmann
Spyridia hypnoides (Bory) Papenfuss
CYANOBACTERIA
Calothrix sp.
Lyngbya semiplana Ag.
Lyngbya sp.
Oscillatoria sp.

Table 5. Dominant epiphytic macroalgae in control and enriched plots of *Halodule wrightii*, *Syringodium filiforme*, and *Thalassia testudinum* from October 1993 to September 1994. Macroalgae are listed in order of decreasing abundance for each date and seagrass species; absence of names indicates low abundance of macroalgae. Refer to Table 3 for complete list of scientific names (H = *Halodule*, S = *Syringodium*, T = *Thalassia*).

	October	January	April	June	August	September
			CONTROL			
H	*Lyngbya*					
S		*Champia* *Heteroderma*				
T	*Heteroderma*				*Polysiphonia*	
			ENRICHED			
H	*Erythrotrichia* *Rhizoclonium*	*Erythrotrichia* *Myriotrichia*	*Myriotrichia* *Acrochaetium* *Erythrotrichia* *Stictyosiphon*	*Heteroderma* *Acrochaetium*	*Acrochaetium*	*Acrochaetium*

Table 5 (continued)

October		January	April	June	August	September
S	*Lyngbya*	*Myriotrichia* *Acrochaetium*	*Myriotrichia* *Acrochaetium*			
T	*Heteroderma* *Lyngbya*		*Myriotrichia*	*Myriotrichia* *Acrochaetium*	*Acrochaetium* *Lyngbya*	*Lyngbya*

Table 6. Summary statistics for the analysis of variance for HPLC pigment analysis of chlorophyll a, fucoxanthin, violaxanthin, lutein, and zeaxanthin from January 1993 to September 1994 (* indicates significance of F value, $\alpha = 0.05$).

Source	DF	F Value	Pr >F
Response variable: chlorophyll a			
Fertilizer	1	13.81	*.0005
Error	57		
Response variable: fucoxanthin			
Fertilizer	1	16.00	*.0002
Error	57		
Response variable: violaxanthin			
Fertilizer	1	8.08	*.0062
Error	57		
Response variable: lutein			
Fertilizer	1	1.92	.1714
Error	57		
Response variable: zeaxanthin			
Fertilizer	1	11.45	*.0013
Error	57		

Table 7. Mean pigment concentrations (nanograms per square centimeter of seagrass blade) and standard errors in control and enriched plots from January 1994 to September 1994 (n = 30; S.E. = standard error; * indicates significant difference between control and enriched plots, $\alpha = 0.05$).

Pigment (ng cm^{-2})	Control		Enriched	± S.E.
Chlorophyll *a*	492.9	*	1521.9	194.7
Fucoxanthin	230.7	*	640.0	72.3
Violaxanthin	5.2	*	31.4	6.5
Lutein	10.3		17.9	3.9
Zeaxanthin	5.5	*	14.1	1.8

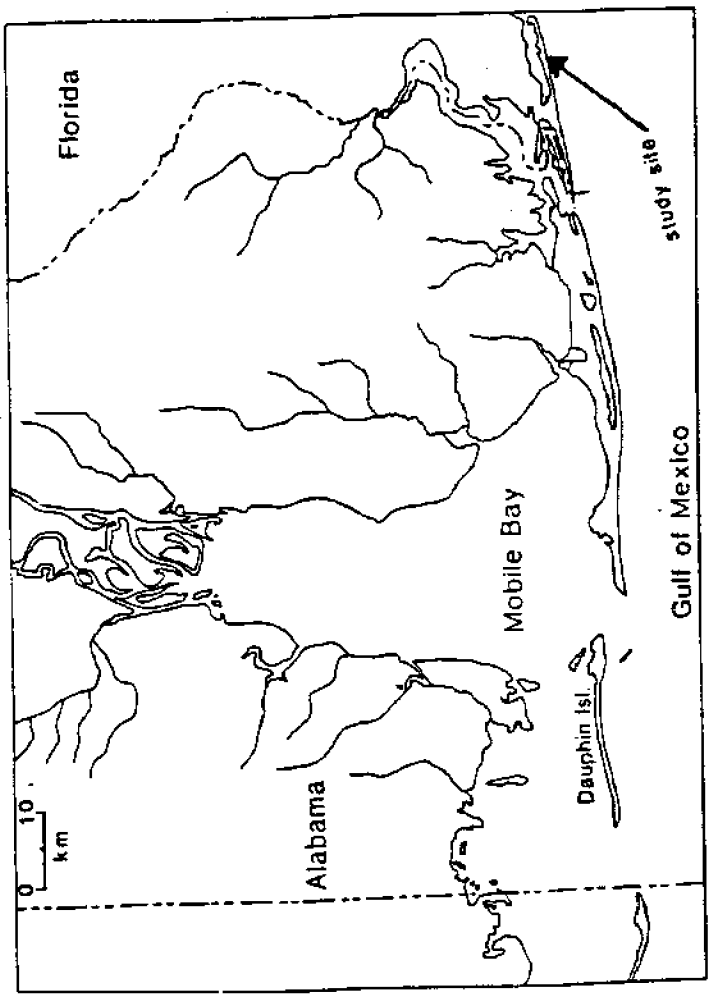

Fig. 1. Map of Big Lagoon, Perdido Key, showing location of study area

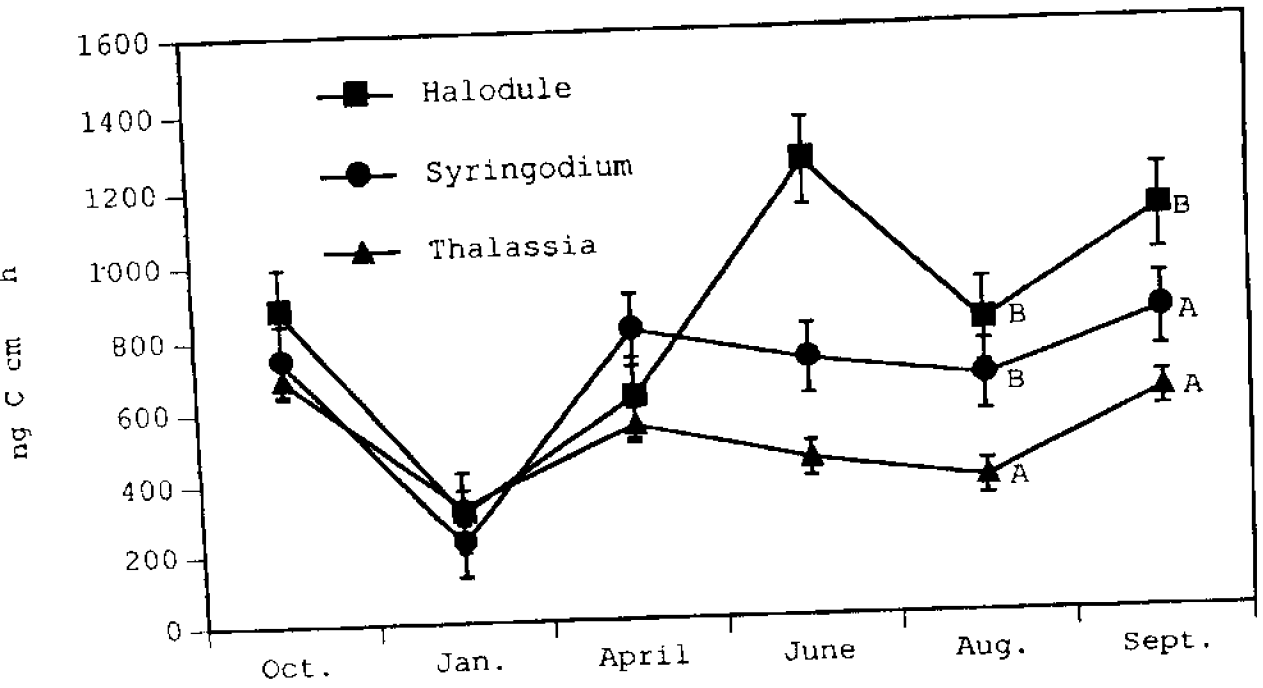

Fig. 2. Average hourly production rates (nanograms of carbon per square centimeter of seagrass blade per hour) and standard error bars of seagrass blades from October 1993 to September 1994. Means with the same letter on a sampling date are not significantly different (n = 8).

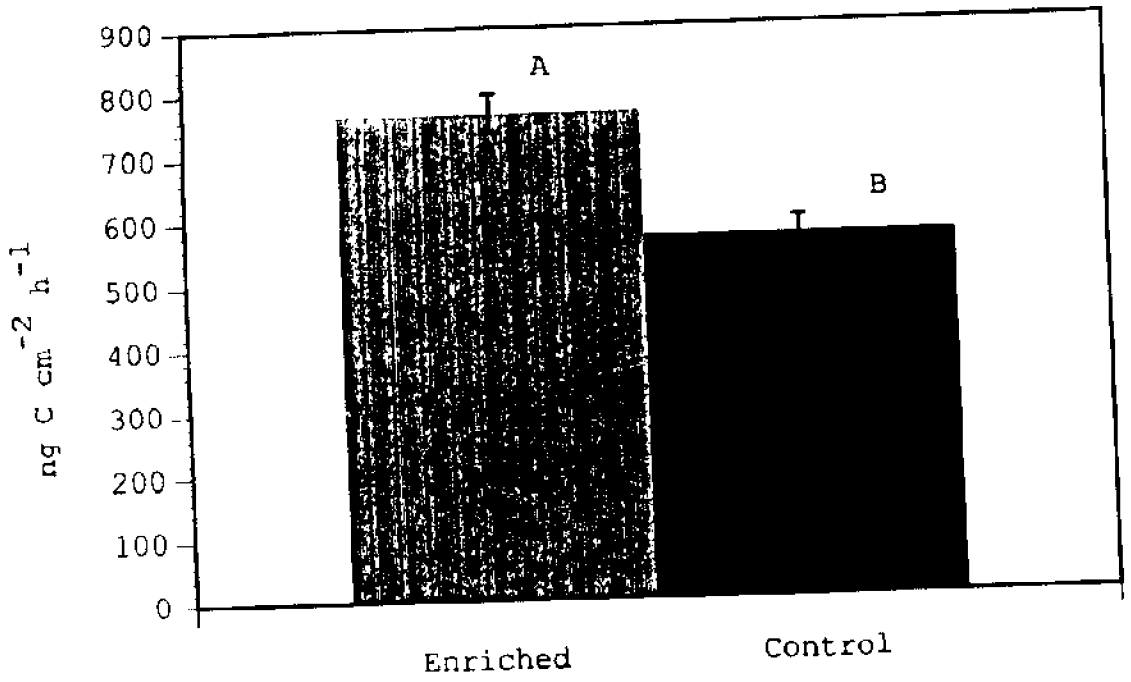

Fig. 3. Average hourly production rates (nanograms of carbon per square centimeter of seagrass blade per hour) and standard error bars for seagrass blades in control and enriched plots from October 1993 to September 1994. Means with the same letter are not significantly different (n = 72).

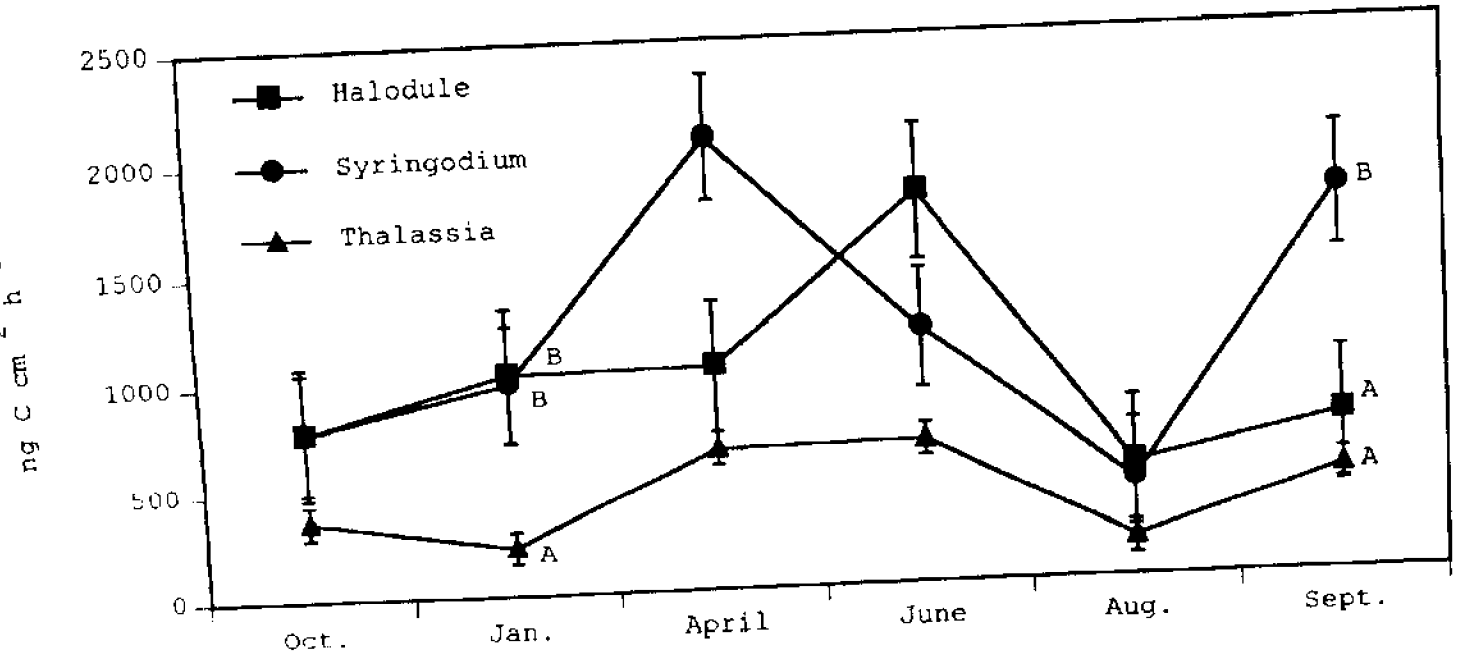

Fig. 4. Average hourly production rates (nanograms of carbon per square centimeter of seagrass blade per hour) and standard error bars for epiphytic algae from October 1993 to September 1994. Means with the same letter on a sampling date are not significantly different (n = 8).

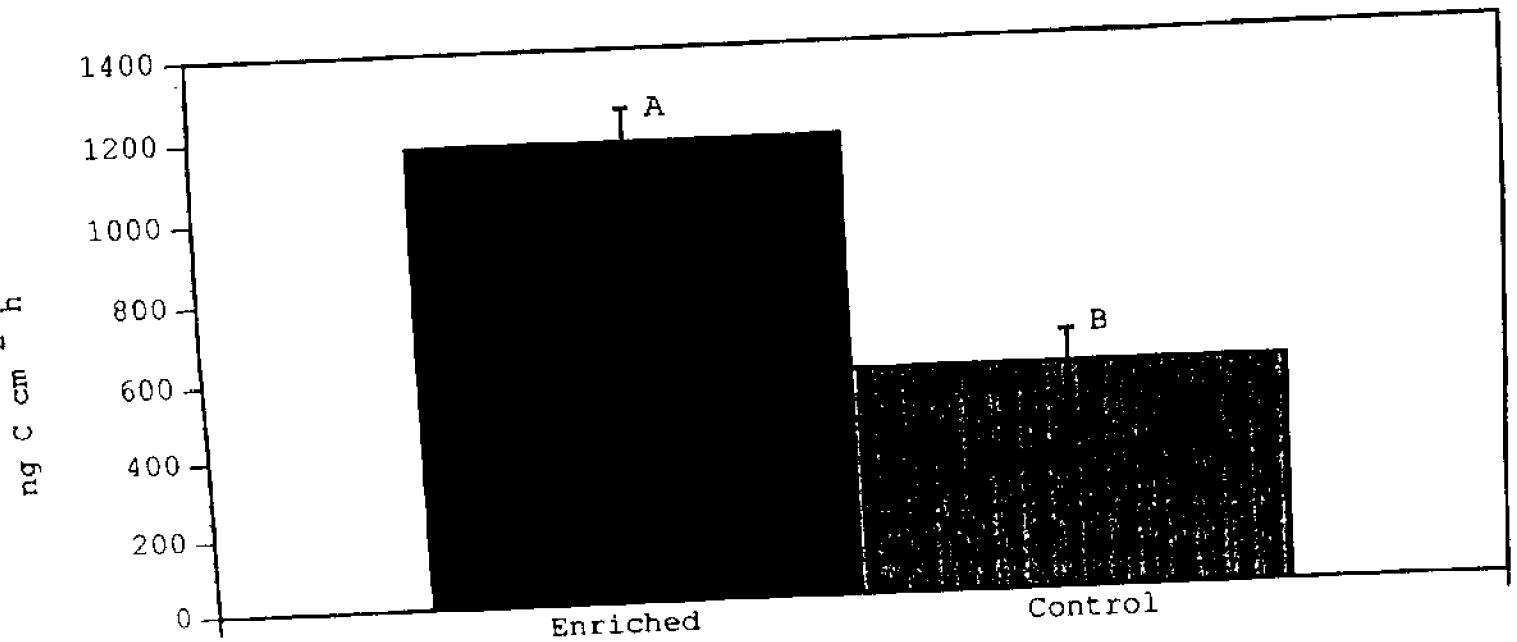

Fig. 5. Average hourly production rates (nanograms of carbon per square centimeter of seagrass blade per hour) for epiphytic algae in control and enriched plots from October 1993 to September 1994. Means with the same letter are not significantly different ($n = 72$).

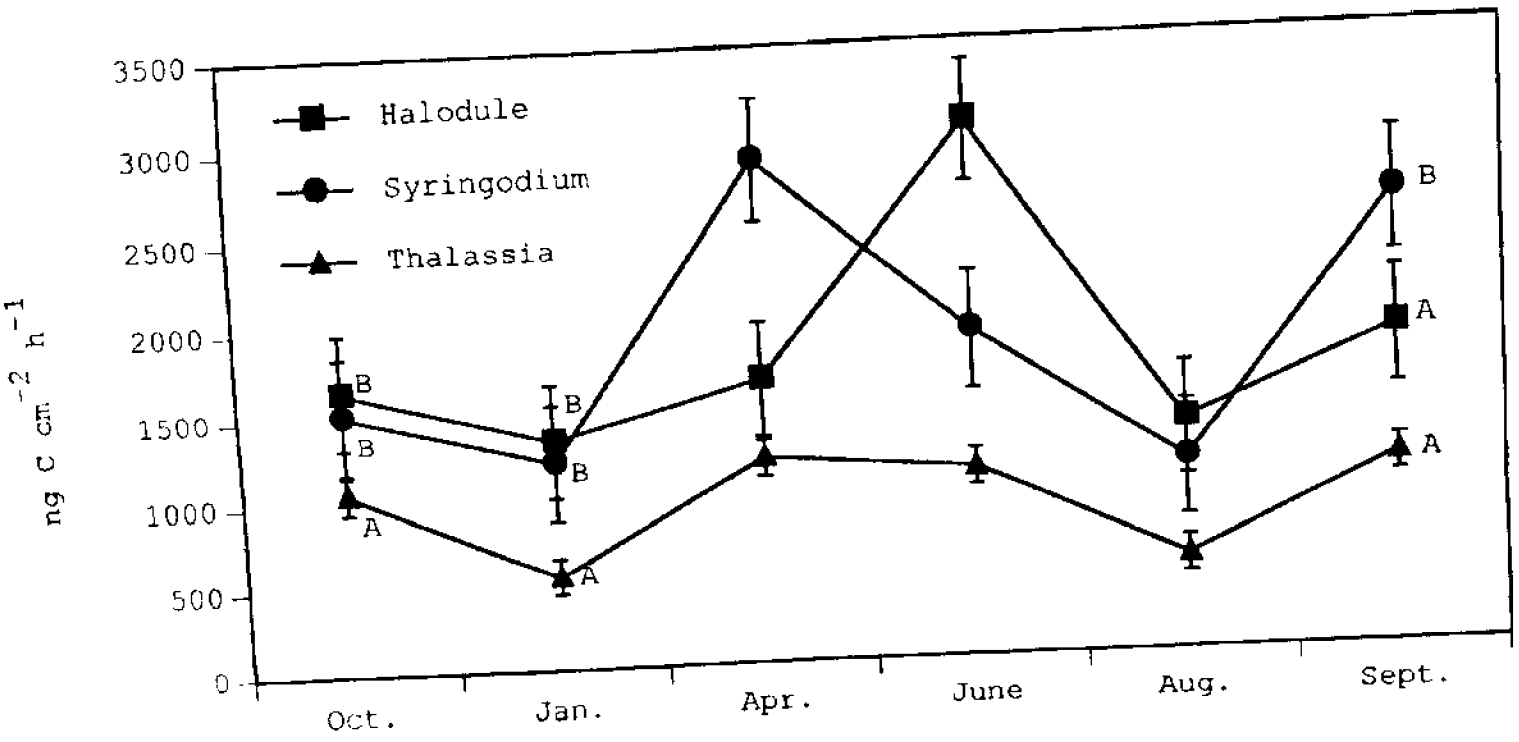

Fig. 6. Average hourly total production rates (nanograms of carbon per square centimeter of seagrass blade per hour) and standard error bars for seagrass blades and epiphytes from October 1993 to September 1994. Means with the same letter on a sampling date are not significantly different (n = 8).

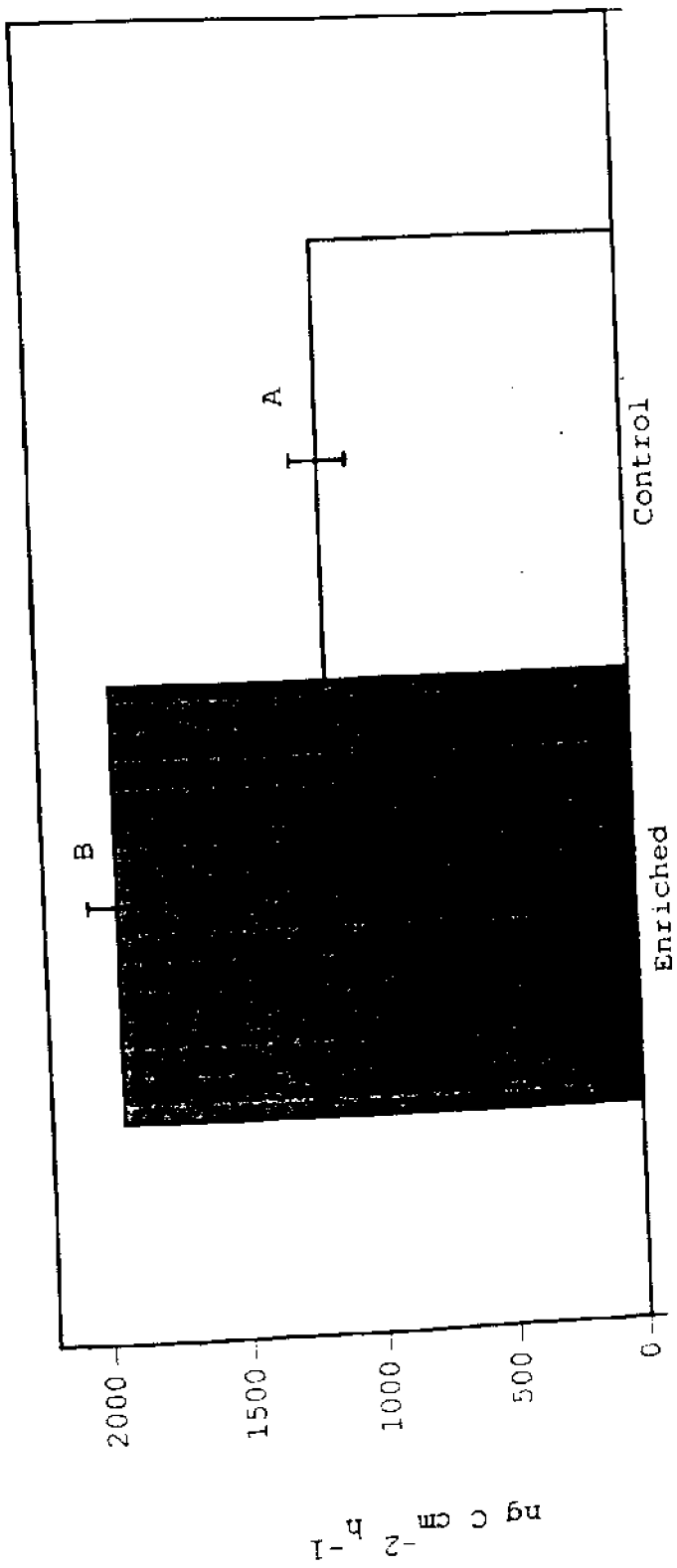

Fig. 7. Average hourly total production rates (nanograms of carbon per square centimeter of seagrass blade per hour) and standard error bars for seagrass blades and epiphytes in control and enriched plots from October 1993 to September 1994. Means with the same letter are not significantly different (n = 72).

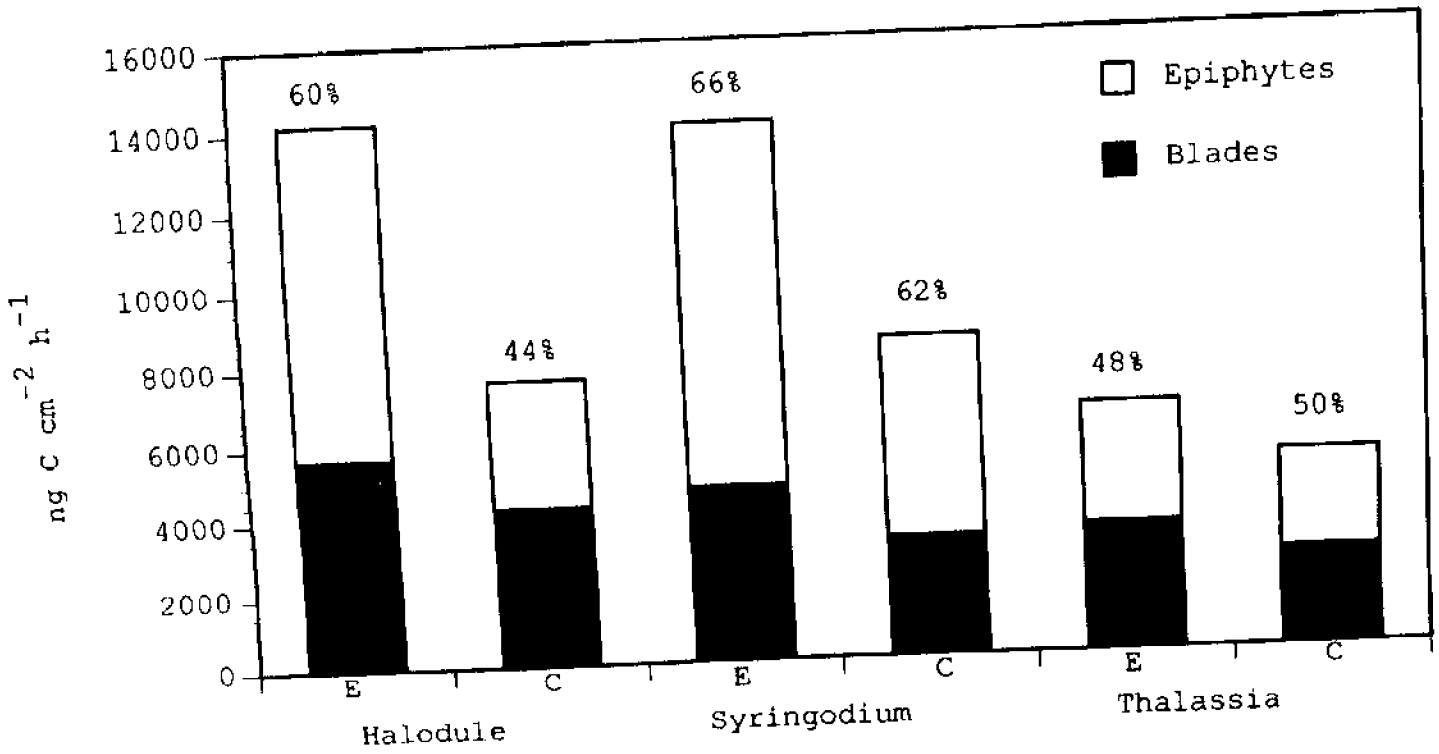

Fig. 8. Average total primary production values (nanograms of carbon per square centimeter of seagrass blade per hour) for blades and epiphytes of each seagrass species from October 1993 to September 1994 (n = 24; % = % contribution of epiphytes; E = enriched; C = control).

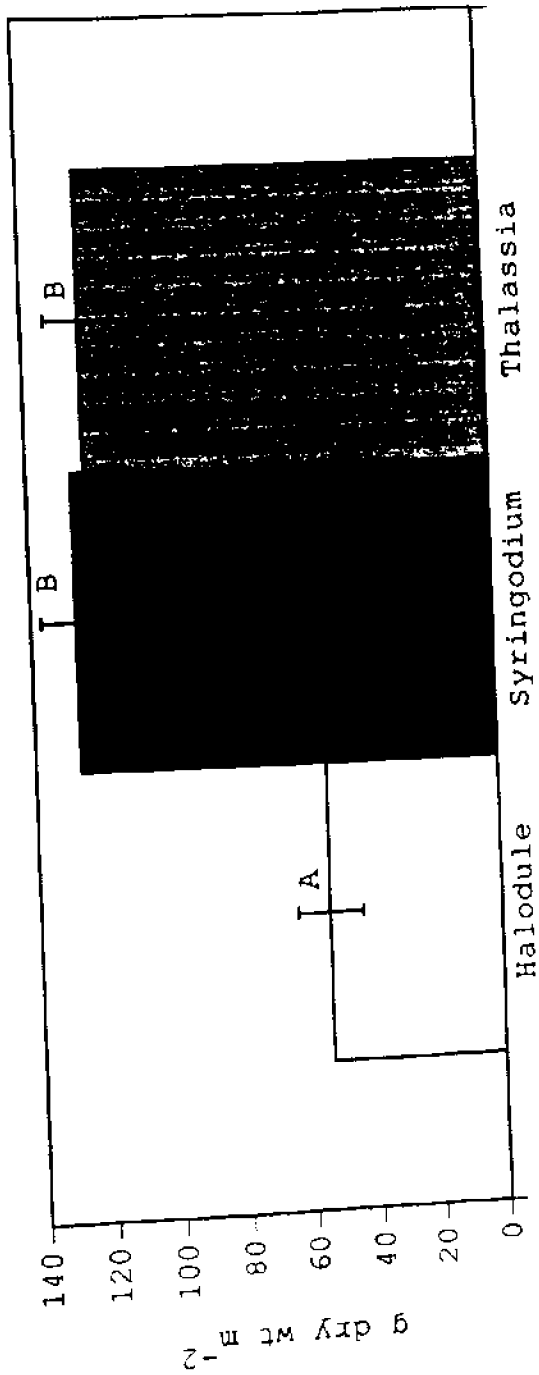

Fig. 9. Average biomass values (grams dry weight of segrass blades per square meter) and standard error bars for blades of each seagrass species from October 1993 to September 1994. Means with the same letter are not significantly different (n = 40).

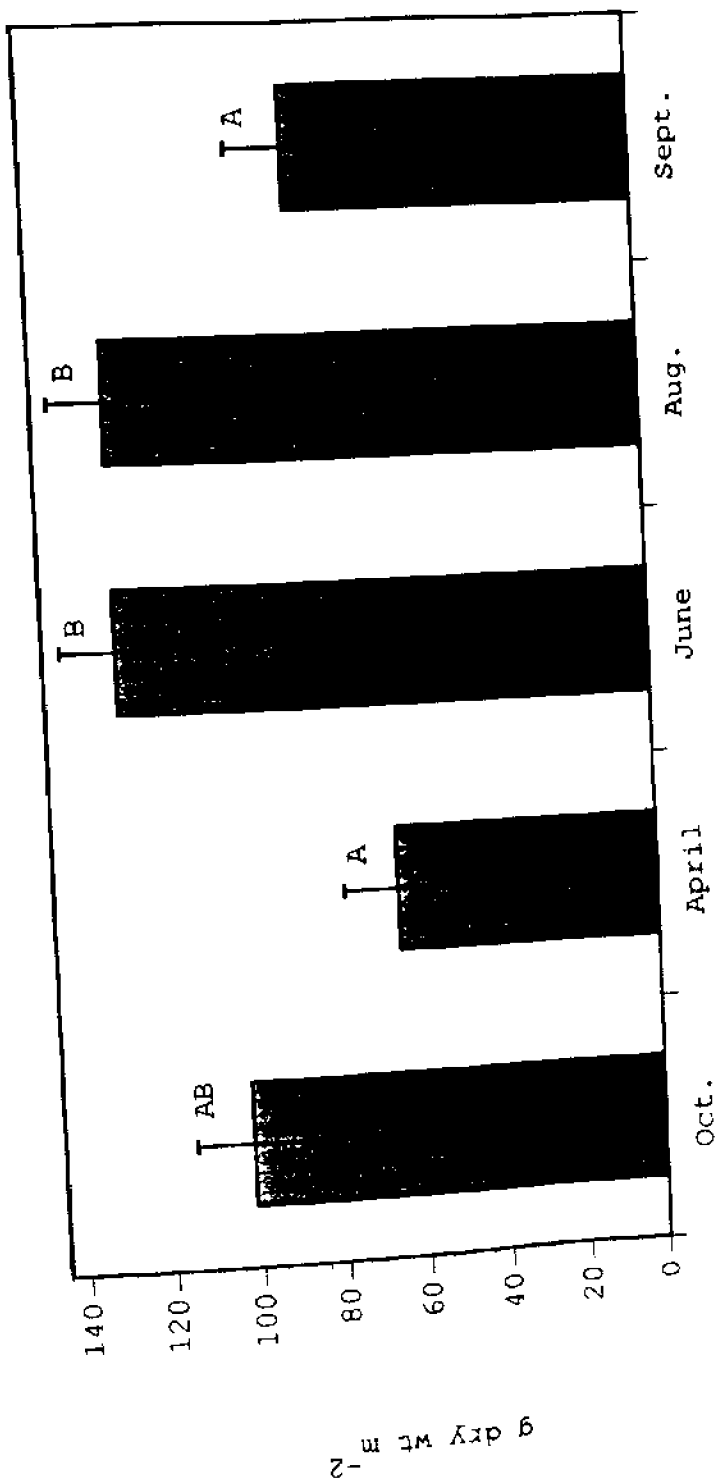

Fig. 10. Average biomass values (grams dry weight of seagrass blades per square meter) and standard error bars for seagrass blades of the three seagrass species combined from October 1993 to September 1994. Means with the same letter on a sampling date are not significantly different (n = 24).

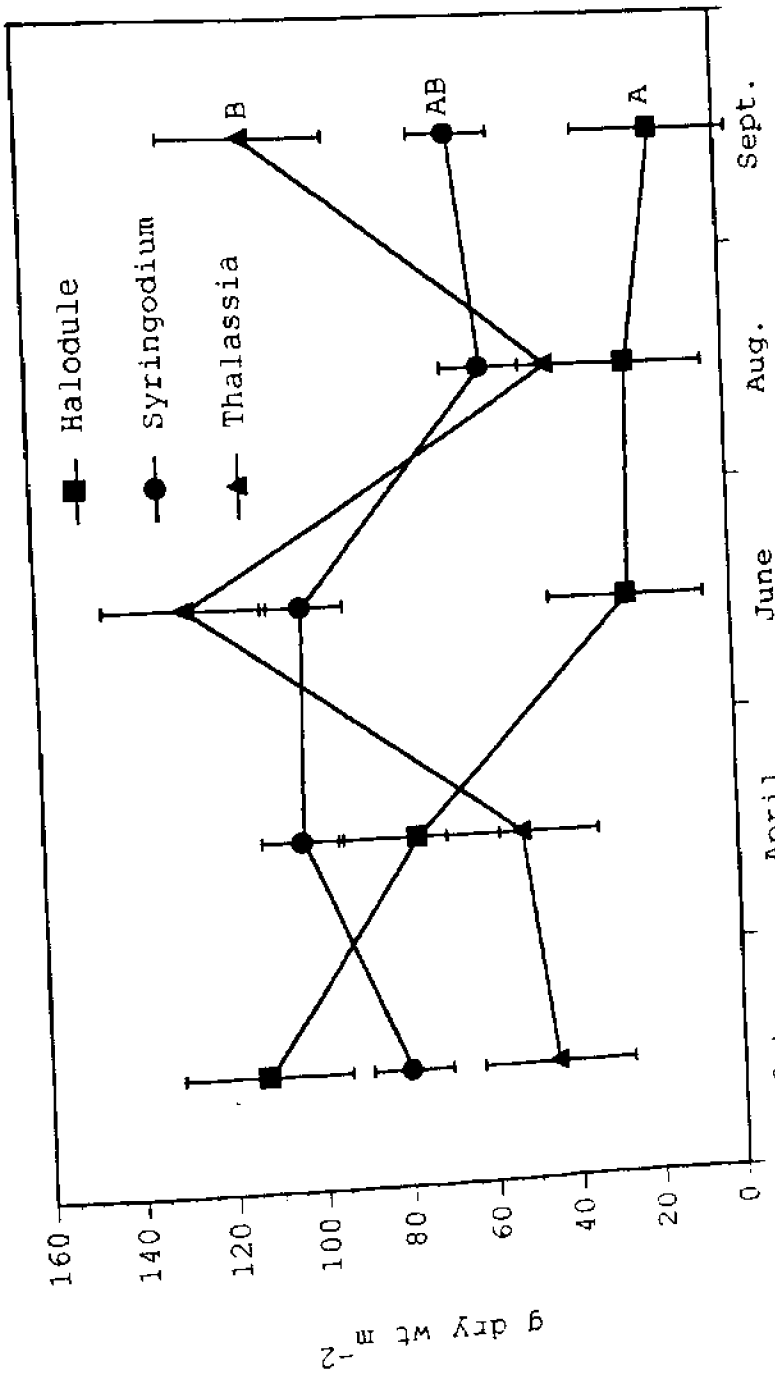

Fig. 11. Average biomass values (grams dry weight of epiphytes per square meter) and standard error bars for epiphytes of each seagrass species from October 1993 to September 1994. Means with the same letter on a sampling date are not significantly different (n = 8).

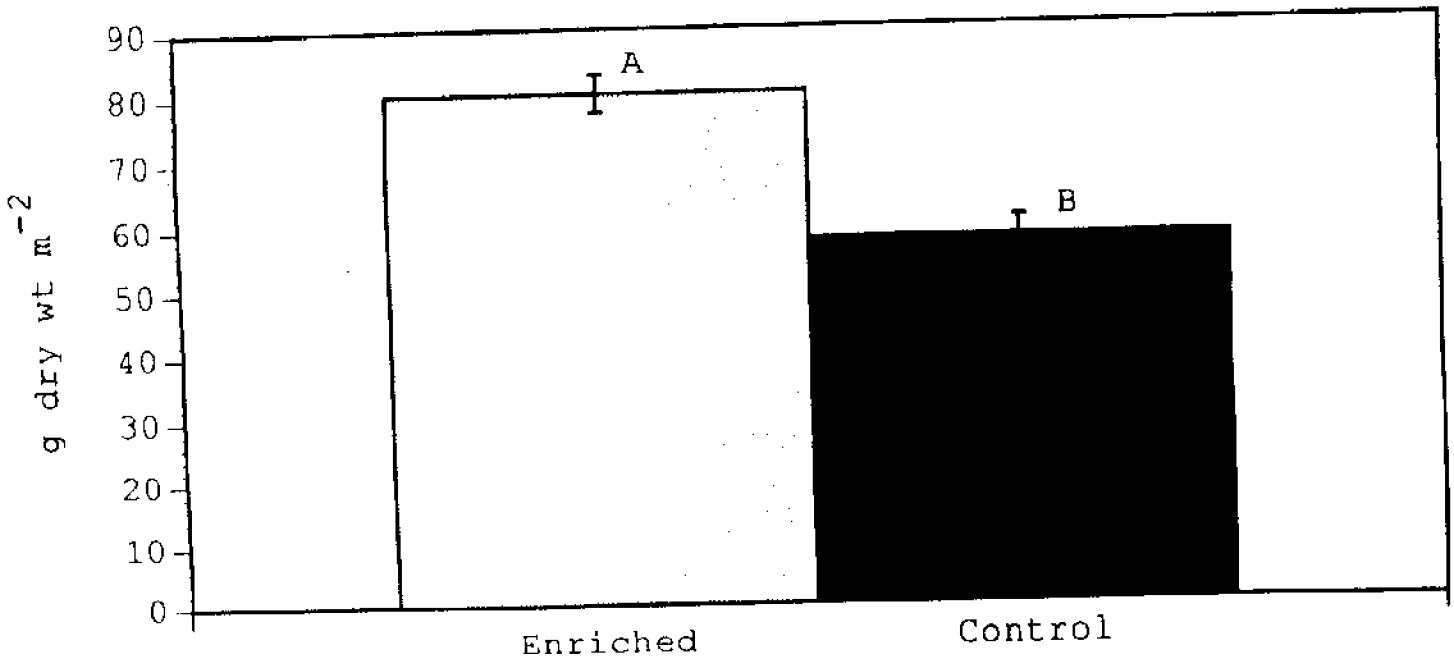

Fig. 12. Average biomass values (grams dry weight of epiphytes per square meter) and standard error bars for epiphytes in control and enriched plots from October 1993 to September 1994. Means with the same letter are not significantly different (n = 60).

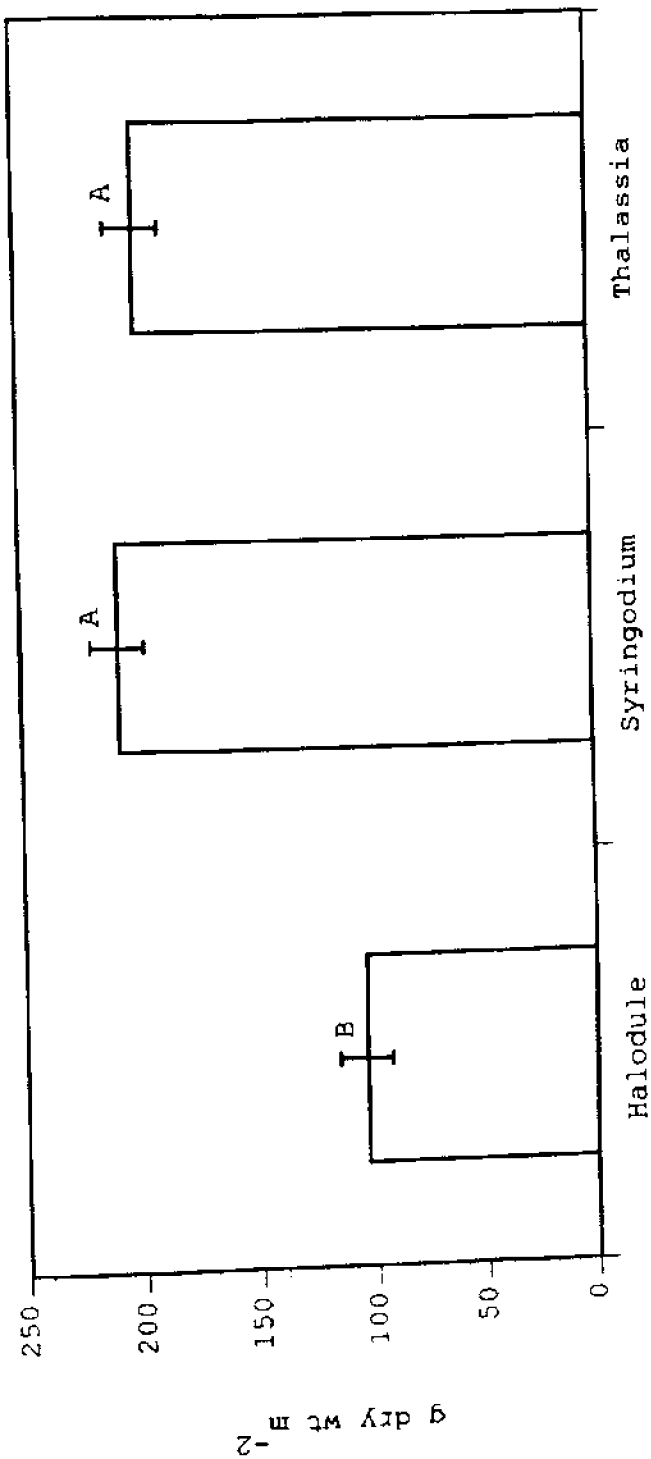

Fig. 13. Average total biomass values (grams dry weight of blades and epiphytes per square meter) and standard error bars for seagrass blades and epiphytes from October 1993 to September 1994. Means with the same letter are not significantly different ($n = 40$).

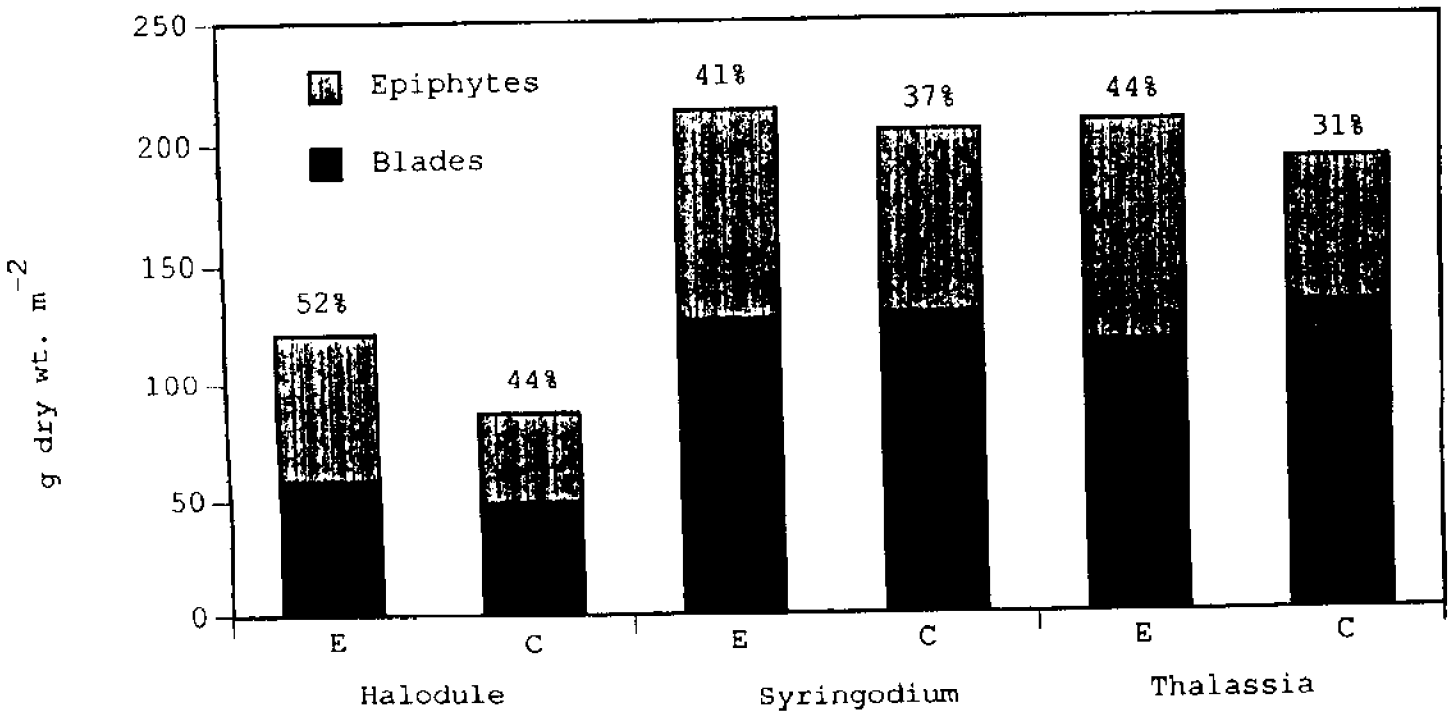

Fig. 14. Average total biomass values (grams dry weight of blades and epiphytes per square meter) for blades and epiphytes of each seagrass species from October 1993 to September 1994 (n = 20; % = % contribution of epiphytes; E = enriched; C = control).

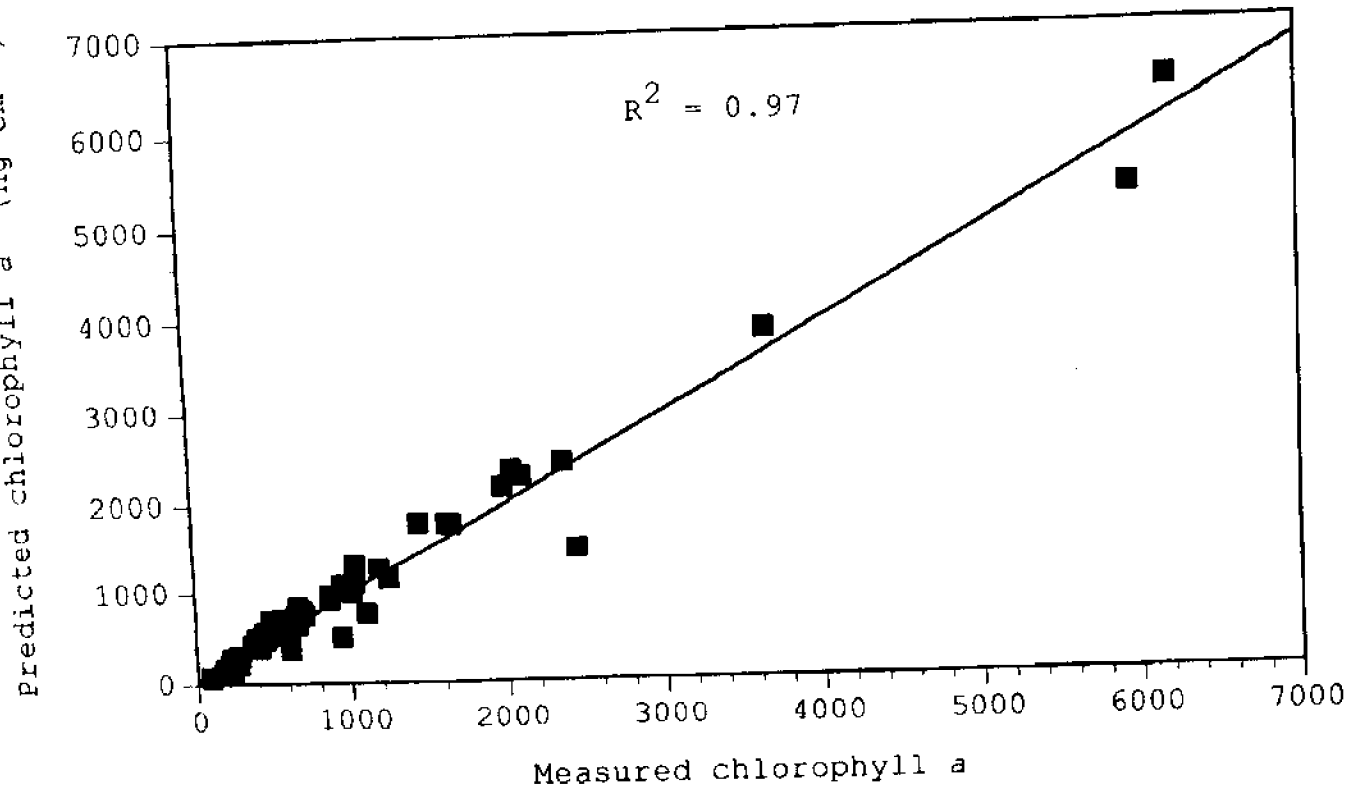

Fig. 15. Regression between measured values of chlorophyll *a* (nanograms per square centimeter of seagrass blade) and values predicted by the model: $Y = X_1\beta_1 + X_2\beta_2 + X_4\beta_4$ where X_1 = fucoxanthin, X_2 = violaxanthin, and X_4 = zeaxanthin.