

1990

Relative effects of nutrient enrichment and grazing on epiphyton-macrophyte (*Zostera marina* L.) dynamics

Hilary A. Neckles

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**Relative effects of nutrient enrichment and grazing on
epiphyton-macrophyte (*Zostera marina* L.) dynamics**

Neckles, Hilary Alison, Ph.D.

The College of William and Mary, 1990

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RELATIVE EFFECTS OF NUTRIENT ENRICHMENT AND GRAZING ON
EPIPHYTON-MACROPHYTE (ZOSTERA MARINA L.) DYNAMICS

A Dissertation

Presented to

The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Doctor of Philosophy

by

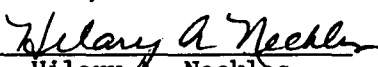
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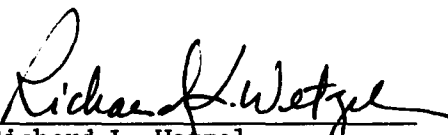
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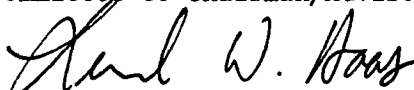
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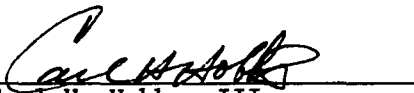

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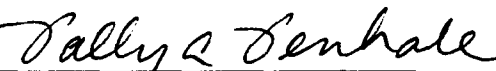
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ABSTRACT

Dissolved nutrient concentrations and invertebrate grazing activity regulate epiphytic biomass. Because epiphyton may limit light and carbon at leaf surfaces and the consequent productivity of submerged macrophytes, factors which influence epiphytic biomass may indirectly affect macrophyte abundance. I measured the simultaneous effects of water column nutrients (ambient or 3x ambient concentrations of nitrogen and phosphorus) and grazing (presence or absence of epifaunal community) on epiphyton and macrophytes seasonally in eelgrass (*Zostera marina* L.) microcosms on lower Chesapeake Bay. Grazing was more important than nutrients in controlling accrual of total epiphytic biomass, although effects on epiphytic components varied; numbers of diatoms responded to grazing, whereas numbers of cyanobacteria responded to nutrients. Numbers of heterotrophic microflagellates mimicked those of bacteria. The indirect effects of nutrients and grazing on macrophytes depended upon the relative magnitude of each factor and the physiological demands of the macrophyte. Under low grazer₁ densities of early summer, macrophyte production ($\text{g m}^{-2} \text{ d}^{-1}$) was reduced with grazer removal and nutrient enrichment independently. In contrast, under high densities of late summer, production was reduced by enrichment with grazers absent only. There were no macrophyte responses to treatment during the spring and fall, regardless of differences in epiphytic biomass; this may have been related to comparatively low light requirements of eelgrass at low temperatures.

I used a simulation model to extrapolate microcosm results to predictions for community persistence. The model included ranges of environmental variables specific to lower Chesapeake Bay, where declines in eelgrass abundance in recent decades were correlated with nutrient enrichment, reduced grazer populations, and increased turbidity. Simulations indicated that neither nutrient enrichment nor loss of grazers alone would limit eelgrass survival, but together would cause community instability. Simulations indicated further that with grazers present, nutrient enrichment with a slight decrease in submarine irradiance would cause macrophyte loss. Measured rates of epiphytic accrual on artificial substrata in situ suggested that with grazers present, light reduction actually reduced the absolute rates of biomass accumulation despite nutrient enrichment. Predictions for macrophyte community stability must thus consider the relative effects of both direct (acting on macrophytes) and indirect (acting via epiphyton) environmental controls.

Chapter 1
INTRODUCTION

The importance of communities of submerged vascular plants to the function of aquatic ecosystems is undisputed. The productivity of these communities rivals that of the world's most productive natural and agricultural systems (Westlake 1963, McRoy and McMillan 1977, Zieman and Wetzel 1980). Submerged macrophytes provide substrata for intricate associations of algae, bacteria, fungi, protozoans, and detritus. The contribution by this epiphyton to the total production of submerged macrophyte communities is widely recognized (e.g. Penhale 1977, Cattaneo and Kalff 1980, Mazzella and Alberte 1986, Libes 1986). The influences of specific physical-chemical and biological controls on production and biomass of macrophytes (reviewed by Barko et al. 1986, Hillman et al. 1989) and attached epiphyton (reviewed by Harlin 1975, Borowitzka and Lethbridge 1989) are well documented. There have been comparatively few experimental studies of the effects of simultaneous changes in diverse environmental variables on either macrophyte or epiphyton dynamics. Similarly, the complex interactions among multiple environmental factors, epiphytic biomass, and macrophyte production remain poorly understood (Lodge et al. 1988).

High densities of epiphyton may limit light transmittance and carbon diffusion to macrophyte surfaces, and consequently reduce macrophyte productivity (Sand-Jensen 1977, Bulthuis and Woelkerling 1983, Sand-Jensen and Borum 1984, Sand-Jensen and Revsbech 1987). Therefore, factors which influence epiphytic biomass may have indirect effects

on macrophyte abundance. Two factors which exert strong control on epiphytic productivity and biomass are dissolved nutrient concentrations and grazing activity: the accrual of epiphyton is enhanced by nutrient enrichment (Orth and van Montfrans 1984, van Montfrans et al. 1984) and diminished by invertebrate grazing (van Montfrans et al. 1982, Howard 1982, Cattaneo 1983). Widespread declines in abundance of submerged macrophytes with cultural eutrophication frequently are attributed in part to reduced productivity caused by epiphytic fouling (e.g. Phillips et al. 1978, Orth and Moore 1983, Twilley et al. 1985, Silberstein et al. 1986). However, few hypotheses concerning the interactive effects of nutrient enrichment and other factors on epiphytic biomass, or, ultimately, on macrophyte production, have been tested.

This dissertation describes the effects of nutrient concentration and grazing activity on the dynamics of eelgrass (Zostera marina L.) and its epiphyton in Chesapeake Bay. I studied eelgrass-epiphyton associations at various levels of organization and over various time scales. I conducted seasonal microcosm experiments to determine the relative effects of nutrient concentration and grazing activity on epiphytic biomass and macrophyte growth and production (Chapter 2), and used simulation model studies to extrapolate these results to predictions for long-term community survival (Chapter 3). I also examined responses by specific components of the epiphytic community to these factors (Chapter 4), and described the growth of epiphyton

in relation to macrophyte distribution and multiple environmental variables in a natural setting (Chapter 5). Results of these studies underscore the importance of complex interactions among vascular plants, epiphyton, and environmental variables to the function of submerged macrophyte communities.

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Chapter 2
RELATIVE EFFECTS OF NUTRIENT ENRICHMENT AND GRAZING ON
EPIPHYTON-MACROPHYTE (ZOSTERA MARINA L.) DYNAMICS
I. SEASONAL COMMUNITY RESPONSES

SUMMARY

The simultaneous effects of nutrient concentration and epiphytic grazers on epiphytic biomass and macrophyte growth and production were tested in eelgrass (Zostera marina L.) microcosms. Experiments were conducted during early summer, late summer, fall, and spring in a greenhouse on the York River estuary of Chesapeake Bay. Nutrient treatments consisted of ambient or enriched (3x ambient) concentrations of inorganic nitrogen and phosphorus, and grazer treatments consisted of the presence or absence of natural densities of isopods, amphipods, and gastropods. During the summer and spring experiments, epiphytic biomass increased with both grazer removal and nutrient enrichment; the effect of grazing was greater than that of nutrient concentration, and there was no interaction between the two factors. There were few differences in epiphytic biomass among treatments during the fall, a result possibly of high ambient nutrient concentrations. Under low grazer densities of early summer, macrophyte production ($\text{g m}^{-2} \text{ d}^{-1}$) was reduced with grazer removal and nutrient enrichment independently. In contrast, under high densities of late summer, production was reduced by enrichment with grazers absent only. During spring and fall there were no macrophyte responses to treatment. The relative effect of epiphytic light attenuation on macrophyte production may have depended upon water temperature and consequent macrophyte light requirements.

INTRODUCTION

The productivity and biomass of submerged macrophytes are governed by a variety of abiotic and biotic variables. Many investigations have explored the effects of individual environmental factors on submerged macrophyte dynamics (reviewed by Barko et al. 1986, Hillman et al. 1989). The combined effects of specific controls on macrophyte growth and production, however, are comparatively little studied. Furthermore, although complex interactions among physical-chemical factors and biological components at various trophic levels are recognized as paramount to the function of diverse other aquatic systems (Kerfoot and Sih 1987, Carpenter 1988), the direct and indirect effects of such interactions within submerged macrophyte communities remain poorly understood (Lodge et al. 1988).

Submerged macrophytes provide substrata for intricate associations of attached algae, bacteria, fungi, protozoans, and organic and inorganic debris. This epiphytic periphyton (i.e. epiphyton) attenuates light and limits carbon exchange at leaf surfaces, and may thereby exert strong controls on macrophyte productivity (Sand-Jensen 1977, Sand-Jensen and Borum 1984, Twilley et al. 1985, Sand-Jensen and Revsbech 1987). Elevated nutrient concentrations enhance epiphytic accrual through the stimulation of algal growth (Orth and van Montfrans 1984, van Montfrans et al. 1984). Declines in macrophyte abundance thus are frequently attributed in part to nutrient enrichment from cultural eutrophication and

consequent increases in epiphytic fouling (e.g. Phillips et al. 1978, Twilley et al. 1985, Silberstein et al. 1986). There is little information, however, on the interactions between elevated nutrient concentrations and other factors that influence macrophyte growth. For example, grazing by invertebrates may control the accumulation of periphyton on both biotic and abiotic substrata (e.g. Nicotri 1977, Howard 1982, van Montfrans et al. 1982, Sumner and McIntire 1982, Cattaneo 1983, Lamberti and Resh 1983, Kairesalo and Koskimies 1987). Grazing on epiphyton therefore enhances macrophyte production indirectly (Brönmark 1985, Hootsmans and Vermaat 1985, Howard and Short 1986), and has been implicated as vital to macrophyte survival (Rogers and Breen 1983, Orth and van Montfrans 1984, Wetzel and Neckles 1986, Borum 1987). Recent studies in freshwater systems indicate that nutrient enrichment and grazing act in concert to regulate periphyton biomass on abiotic substrata (Stewart 1987, Marks and Lowe 1989, Mazumder et al. 1989). However, studies relating the simultaneous effects of these factors to macrophyte dynamics are lacking.

I measured the independent and interactive effects of nutrient concentration and epiphytic grazing on eelgrass (Zostera marina L.)-epiphyton associations in lower Chesapeake Bay. Based upon the individual effects of these factors, I predicted that the highest macrophyte production would occur under low nutrient concentrations with grazers present, and that the lowest would occur under high nutrient concentrations with grazers absent. If the effects of

nutrients and grazers are independent, then either enrichment or grazer removal would be expected to reduce macrophyte production consistently. Conversely, if they are interactive, then the effects of enrichment could be mediated by grazing activity.

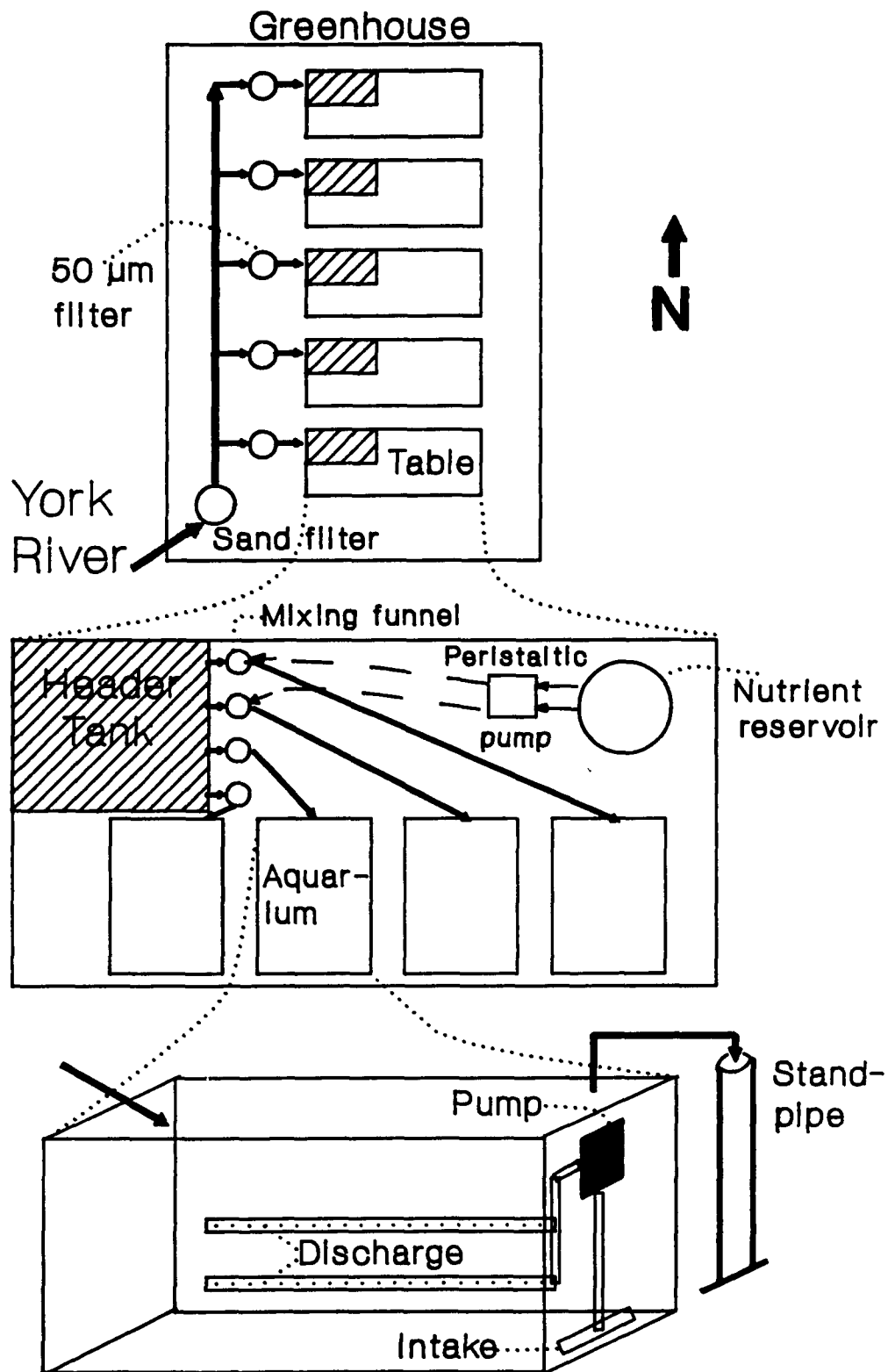
METHODS

Experimental Design

I tested the effects of nutrient enrichment and grazing activity on eelgrass and its epiphyton collected from the York River estuary, Chesapeake Bay ($37^{\circ}15'N$, $76^{\circ}30'W$). Experiments were conducted seasonally in 110 l glass microcosms located in a greenhouse (Fig. 1). Seawater from the York River was pumped continuously into five header tanks through sand- and 50 μm bag-filters. Salinity of the incoming water ranged from 19 to 23 o/oo. Each header tank supplied four aquaria to maintain a constant water volume with a residence time of 1.5 hr. The water in each aquarium was aerated continuously and circulated during daylight hours with a submersed pump (Rule 450 gph) to provide a low to moderate current ($2-9 \text{ cm s}^{-1}$; Marsh-McBirney model 201 electromagnetic current meter).

The microcosms were illuminated with sunlight only. I removed the periphyton regularly from the aquarium walls using a mesh-covered sponge. Preliminary measurements indicated little difference in submarine irradiance or water

Figure 1. Experimental system. Arrows indicate direction of flow (solid=seawater, dashed=concentrated nutrient stocks).



temperature among aquaria. Therefore, within a single aquarium I measured photosynthetically active radiation (PAR: 400-700nm) semiweekly to weekly using a 2-pi cosine corrected quantum sensor (Li-Cor model 185B), and the maximum and minimum water temperatures daily using a mercury thermometer. I measured concentrations of suspended chlorophyll a periodically from all aquaria supplied by three randomly selected header tanks. Determinations were made fluorometrically on DMSO-acetone extracts (Shoaf and Lium 1976).

I conducted four experiments during 1987 and 1988 based on the seasonal pattern of eelgrass growth in Chesapeake Bay (Wetzel and Penhale 1983; Table 1). The experiments initiated in June and August (1987) represented the respective beginning and end of a summer period of low growth, and those initiated in October (1987) and April (1988) coincided with periods of high growth in fall and spring. Each experiment lasted 1 to 2 months. Experiments were terminated when average daily water temperatures reached predetermined endpoints for seasonal periods of eelgrass growth (beginning and ending temperature limits: spring, 9-23°C; summer, 23-25°C, with a mid-range maximum of 30°C; fall, 25-13°C; K. A. Moore, Virginia Institute of Marine Science, unpublished) or treatment-induced mortality left an experimental treatment with few plants.

Experimental material was standardized by selecting only eelgrass shoots with at least 4 leaves and by cutting the rhizomes distal to the fifth internode. I collected

Table 1. Timing of microcosm experiments and densities of invertebrates ($\# \text{ m}^{-2}$ of pot surface) applied to grazer treatments.

	<u>Early Summer</u>	<u>Late Summer</u>	<u>Fall</u>	<u>Spring</u>
Grazer	8 June - 9 July 1987	11 Aug - 16 Sept 1987	12 Oct - 24 Nov 1987	7 April - 8 June 1988
Gastropoda				
<u>Bittium</u> <u>varium</u>	4000	3600	0	0
<u>Mitrella</u> <u>lunata</u>	0	0	1500	0
Isopoda				
<u>Idotea</u> <u>baltica</u>	800	100	100	300
<u>Erichsonella</u> <u>attenuata</u>	0	6000	1000	0
Amphipoda ¹	0	1700	1300	600

Total	4800	11400	3900	900

¹Primarily Gammarus sp. and Amphithoe sp.

sediments for each experiment from unvegetated patches within a York River eelgrass bed. Shoots were planted in homogenized sediments in plastic pots (11.4cm diameter) at reported average annual field densities for lower Chesapeake Bay (1500m^{-2} ; Orth and Moore 1986). The potted plants were acclimated in a large, common tank for two weeks prior to each experiment.

Nutrient-grazer treatments were applied to aquaria following a 2 x 2 factorial design in a randomized complete block pattern. Aquaria supplied by a single header tank represented experimental blocks. Nutrient treatments were applied at ambient or enriched levels. Enrichments were made with ammonium nitrate and disodium phosphate combined to increase the ambient concentrations of dissolved inorganic nitrogen (DIN) and phosphorus 3-fold. This magnitude of increase reflected the average difference in nutrient concentrations between sites in the York River which presently supported eelgrass and sites from which eelgrass disappeared in the early 1970s (K. A. Moore, unpublished). This level of enrichment thus was postulated to have contributed to local eelgrass declines (cf. Orth and Moore 1983). Peristaltic pumps metered nutrients directly to the inflow from concentrated stocks (Fig. 1). I measured concentrations of DIN (as the sum of nitrate, nitrite, and ammonium) and phosphorus (as orthophosphate) biweekly from the inflowing water and the microcosms. Concentrations were determined spectrophotometrically (nitrate, nitrite, and orthophosphate: USEPA 1979; ammonium: Parsons et al. 1984)

and nutrient additions were adjusted as necessary to maintain a 3-fold enrichment.

Grazer treatments were designated as either present or absent. I determined seasonal invertebrate densities on a shoot-specific basis by collecting and quantifying six samples from eelgrass habitat in the York River at the beginning of each experiment. Treatments with grazers present included epifauna collected from a natural grass bed and applied at field densities (Table 1). Populations were controlled during experiments by flushing aquaria with fresh water for 10 minutes as necessary to remove new recruits (approximately biweekly) and then restocking with known densities.

During the second week of the spring experiment a small oil spill occurred near the greenhouse pump intake. The water supply to the microcosms was turned off for 48 h to allow the spill to dissipate. Although a slight oil film was evident on the microcosm water surfaces during this period, evidence indicated that impacts to experimental comparisons were minimal: all microcosms were similarly disturbed, daily temperature extremes were within the range of seasonal measurements, grazers remained active, and epiphytic biota appeared unaffected under observation with epipfluorescence microscopy. The water lines were washed with detergent and flushed thoroughly before recommencing delivery.

Determination of Epiphyton and Macrophyte Responses

At the beginning of each experiment I randomly assigned six pots to each aquarium. At approximately biweekly sampling dates I measured epiphytic biomass from one randomly selected pot per microcosm. Eelgrass grows basally by the sequential formation of individual leaves, resulting in a series of leaves of increasing ages within a shoot. Samples for epiphytic determinations consisted of four to ten leaves per pot from the same relative position within different shoots. The epiphyton was scraped with the edge of a glass slide into filtered seawater and collected by filtration onto precombusted and preweighed filters (Gelman A/E glass fiber filters). Epiphytic dry weight (DW) was determined after drying at 60°C (2-5 d) and ash-free dry weight (AFDW) after combusting at 500°C (5 h). All measurements were normalized to macrophyte leaf area and mass. Leaf area was determined using an area meter (Licor model 3100) and leaf mass (DW and AFDW) was determined as described for epiphyton samples.

The effect of epiphyton on macrophyte photosynthesis may depend upon the spectral selectivity of the epiphytic material (cf. Mazzella and Alberte 1986). Therefore, I estimated the epiphytic attenuation of light both as PAR and at nine discrete, evenly spaced 10 nm bands across the range of PAR from subsamples of leaves during the late summer (n=23), fall (n=73), and spring (n=137) experiments. I used a spectroradiometer (Biospherical MER-1000) to measure the

proportion of light from an artificial source (combined fluorescent and tungsten flood light bulbs) passing through suspensions of epiphyton, following the technique of Sand-Jensen and Søndergaard (1981).

I measured macrophyte growth in one randomly selected pot per microcosm during successive two week sampling intervals using the leaf marking technique of Sand-Jensen (1975). Growth was measured as the length and width of all leaf material produced during a measurement interval. Linear regressions of dry weight on area derived from leaves processed for epiphytic samples ($R^2 > .97$) were used to calculate leaf biomass. Macrophyte growth, production, and population attributes were calculated as shown in Table 2.

Statistical Analysis

Responses to treatment within each experiment were assessed using 3-way analysis of variance with main effects of nutrient concentration, grazer abundance, and sampling period. Epiphytic responses were analyzed within leaf age classes (i.e. relative position within a shoot). Analyses were performed on age classes 1 (youngest) through 4 only, because variable retention of older leaves resulted in highly unbalanced data sets. Data were transformed as $\log(x)$ where necessary and residual analysis was used to verify that assumptions of analysis of variance were satisfied. Factor level means were compared using Bonferroni multiple comparisons (Neter and Wasserman 1974).

Table 2. Macrophyte response variables measured from individual pots sampled.

Variable	Units	Calculation
Relative leaf growth rate	$\text{mg g}^{-1} \text{d}^{-1}$	New leaf biomass/(initial leaf biomass*days growth)
Shoot-specific growth rate	$\text{mg shoot}^{-1} \text{d}^{-1}$	New leaf biomass/(initial # shoots*days growth)
Leaf formation rate	$\text{lvs shoot}^{-1} \text{d}^{-1}$	# new leaves/(initial # shoots*days growth)
Shoot formation rate	$\text{shoots m}^{-2} \text{d}^{-1}$	# new shoots/(pot area* days growth)
Areal leaf production rate	$\text{g m}^{-2} \text{d}^{-1}$	New leaf biomass/(pot area* days growth)
Shoot density	shoots m^{-2}	# shoots/pot area
Leaf density	lvs shoot^{-1}	# leaves/# shoots

When significant interactions included sampling period, effects of nutrients and grazing were determined within individual periods only.

RESULTS

Experimental Conditions

Ambient concentrations of DIN and phosphate supplied to the microcosms remained stable throughout most of the experiments (Table 3). In the fall, however, ambient DIN rose to higher concentrations. Although nutrient additions were intended to yield 3-fold increases, the actual average enrichment ranged from 2- to 4-fold. Nutrient uptake within the microcosms frequently resulted in concentrations of DIN and phosphate that were 20-45% lower than those of the seawater input.

PAR measured at mid-depth in the microcosms varied seasonally; daily maxima ($\mu\text{E m}^{-2} \text{ s}^{-1}$) were approximately 375 during the early summer experiment, 225 during late summer, 175 during fall, and 350 during spring. Suspended chlorophyll *a* concentrations ranged from 5-15 $\mu\text{g l}^{-1}$, and did not differ significantly among microcosm treatments. Water temperatures within the microcosms fluctuated approximately 5°C daily. The average daily temperature rose from 25°C to 28°C during early summer, fluctuated between 27°C and 29°C during late summer, dropped from 18°C to 12°C during fall, and rose from 13°C to 22°C during spring.

Table 3. Nutrient concentrations ($\bar{X} \pm (SD)$ in μM) of seawater inflow to microcosms. SD calculated between sample date means within experiments.

Experiment	No. of Dates	DIN		PO ₄	
		Ambient	Enriched	Ambient	Enriched
Early Summer	3	4.2 (0.2)	16.4 (0.5)	1.0 (0.4)	2.3 (0.5)
Late Summer	3	4.0 (1.9)	10.6 (1.9)	1.6 (1.2)	3.4 (0.5)
Fall	3	10.8 (3.0)	37.8 (6.6)	0.7 ($<.1$)	3.3 (1.0)
Spring	6	4.0 (2.9)	10.8 (3.7)	0.8 (0.2)	1.8 (0.6)

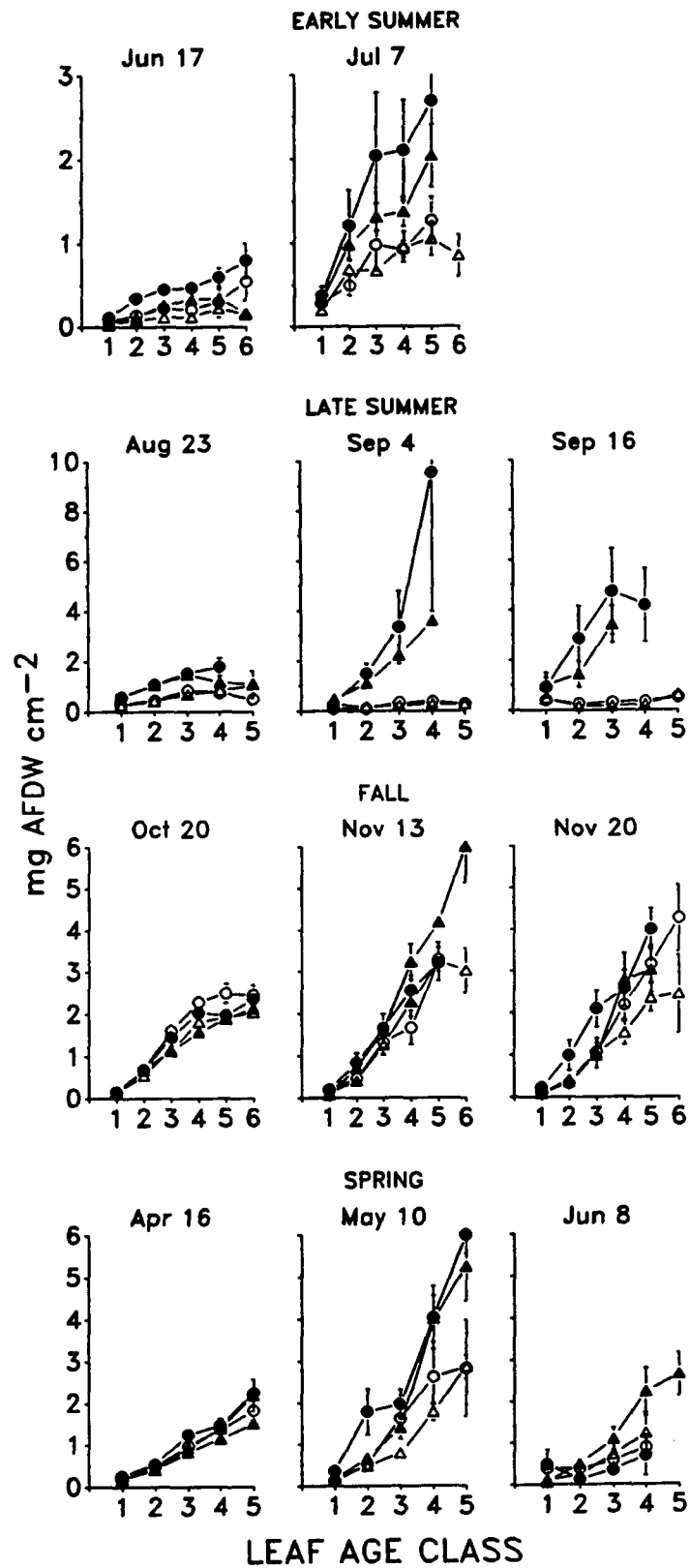
Response by Epiphyton

Epiphytic AFDW constituted from 15% to 40% of total DW during the experiments. Because patterns of accrual were nearly identical ($R > .98$ between masses of AFDW and DW during each experiment), only AFDW measurements are presented here. Similarly, because there were few differences in leaf area-specific and mass-specific responses, results here are confined to area-specific measurements.

Epiphytic responses to treatment were similar between the early and late summer experiments (Fig. 2). Epiphytic biomass increased in the absence of grazers ($P < .01$) similarly across nutrient treatments ($P > .10$). The effect of grazer removal increased over time within an experiment ($P < .01$) and was most pronounced during late summer. During both experiments, epiphytic biomass increased slightly with nutrient enrichment on all leaves but the youngest (early summer: $P < .05$; late summer: $P < .10$) similarly across levels of grazing and sample date ($P > .10$). By the middle of both summer experiments, plants in the enriched, ungrazed aquaria were enshrouded with free floating filamentous algae. In addition, many leaves supported dense tunicate populations. No other treatment combinations were thus affected.

In contrast to the summer experiments, grazing had no effect on epiphytic AFDW during the fall ($P > .10$; Fig. 2). By the third sampling date, biomass on older leaves (age

Figure 2. Epiphyton response ($\bar{X} \pm SE$) to microcosm treatments. Triangles=ambient nutrient concentrations, circles=enriched nutrient concentrations; solid symbols=grazers absent, open symbols=grazers present. All statistical analyses were done on log transformed data.



classes 3 and 5) was increased under enriched conditions ($P < .05$) similarly across grazing levels ($P > .10$).

By the second sample date of the spring experiment (Fig. 2), epiphytic biomass was increased with grazer removal on all but the youngest leaf ($P < .05$) and with nutrient enrichment on intermediate aged leaves ($P < .05$). By the third sampling period, dense growth of the macroalga Enteromorpha sp. (attached and free floating) covered plants in the enriched, ungrazed aquaria, and the amount of microepiphytic material exposed to that treatment combination declined (Fig. 2).

Light was attenuated by the epiphytic matrix following a negative exponential function at all wave bands tested. The epiphytic light attenuation was similar among experiments. Light at short wavelengths was attenuated most rapidly: mean attenuation coefficients among experiments ($\text{cm}^2 \text{ mg DW}^{-1}$, calculated as the negative exponential decay coefficient for light passing through a suspension of epiphyton) declined from 0.48 at 410 nm to 0.25 at 694 nm. The average attenuation coefficient for PAR was 0.24.

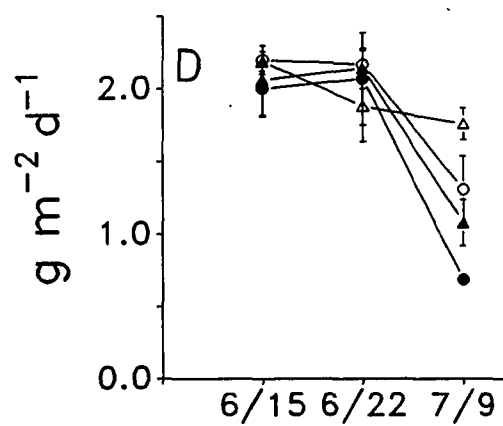
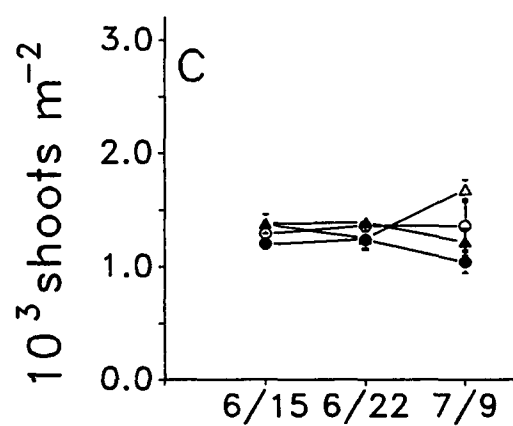
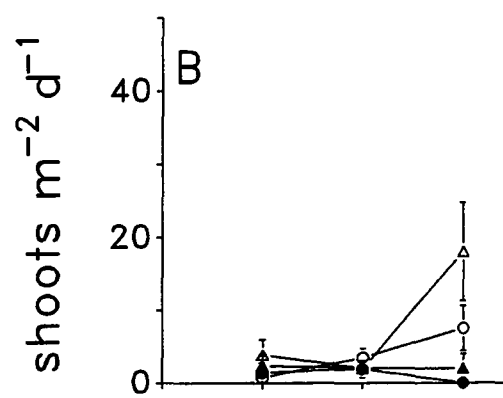
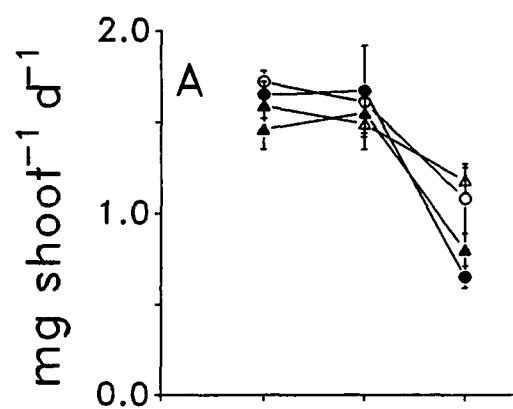
Response by Macrophytes

Macrophyte responses to treatment also varied seasonally. During the early summer experiment, the effects of grazer abundance and nutrient enrichment on all parameters measured were additive; i.e., responses to one factor were proportionally similar across levels of the

second ($P > .10$). By the final sampling period, the shoot-specific growth rate (Fig. 3A), the shoot formation rate (Fig. 3B), and the shoot density (Fig. 3C) decreased in the absence of grazers ($P < .05$). Grazer removal also decreased the mean relative leaf growth during this period from 17.0 to 11.0 $\text{mg g}^{-1} \text{d}^{-1}$ ($P < .01$). During the same period, the mean leaf formation rate decreased from 0.08 to 0.06 leaves shoot $^{-1} \text{d}^{-1}$ under nutrient enriched conditions ($P < .01$). There were no other significant effects of enrichment on macrophyte growth (Fig. 3A,B). However, shoot densities declined under enriched conditions ($P < .05$, Fig. 3C) and leaf density also decreased by 11%, indicating that shoot mortality and leaf loss were greater under enriched conditions. Consequently, although nutrient enrichment did not affect shoot biomass accumulation, it affected areal biomass production (Fig. 3D). By the last sampling period of the early summer experiment, production decreased with both grazer removal ($P < .01$) and nutrient enrichment ($P < .05$; Fig. 3D).

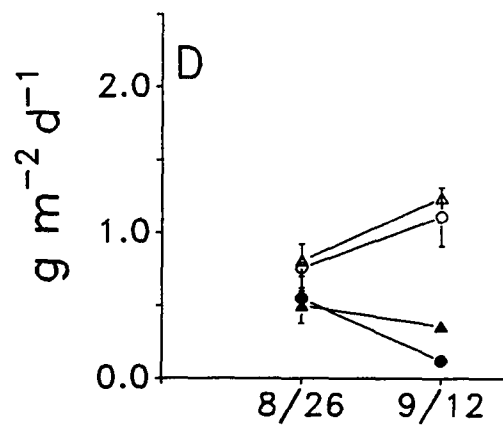
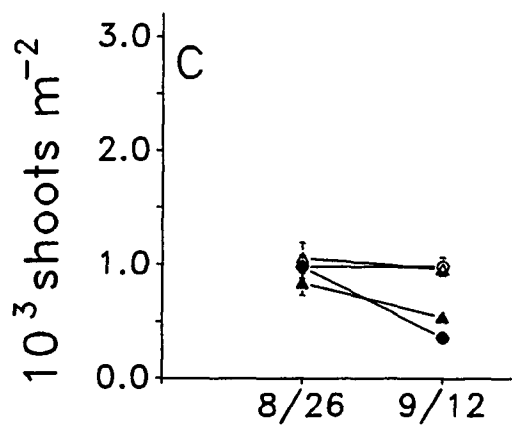
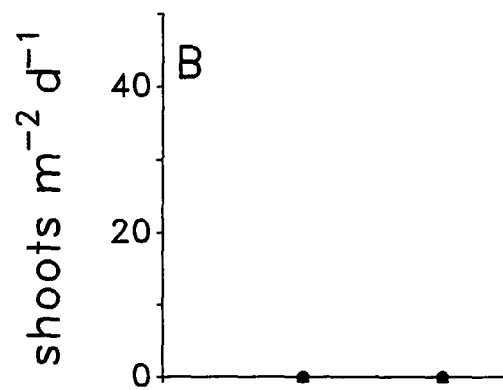
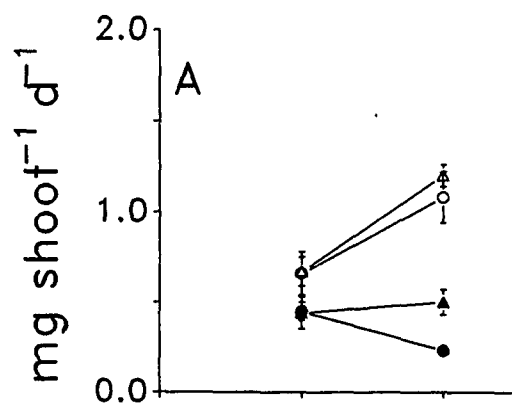
Trends among responses to grazing during the late summer experiment were similar to those of early summer: leaf growth rate (Fig. 4A) and shoot density (Fig. 4C) decreased with grazer removal ($P < .01$). By the last sampling date, the mean relative leaf growth rate also decreased from 25.6 to 12.8 $\text{mg g}^{-1} \text{d}^{-1}$ in the absence of grazers. The leaf growth rate (Fig. 4A) decreased under enriched conditions on the last sample date ($P < .05$). Enrichment did not affect leaf formation rate or leaf density. Although no new shoots

Figure 3. Macrophyte responses ($\bar{X} \pm SE$) to microcosm treatments during early summer. Triangles=ambient nutrient concentrations, circles=enriched nutrient concentrations; solid symbols=grazers absent, open symbols=grazers present. A. Leaf growth rate; B. Shoot formation rate; C. Shoot density; D. Areal leaf production rate. All mass measurements based on DW. Statistical analyses of A, B, and D were done on log transformed data.



SAMPLE DATE (MONTH/DAY)

Figure 4. Macrophyte responses ($\bar{X} \pm SE$) to microcosm treatments during late summer. Triangles=ambient nutrient concentrations, circles=enriched nutrient concentrations; solid symbols=grazers absent, open symbols=grazers present. A. Leaf growth rate; B. Shoot formation rate; C. Shoot density; D. Areal leaf production rate. All mass measurements based on DW. Statistical analyses of A, C, and D were done on log transformed data.



SAMPLE DATE (MONTH/DAY)

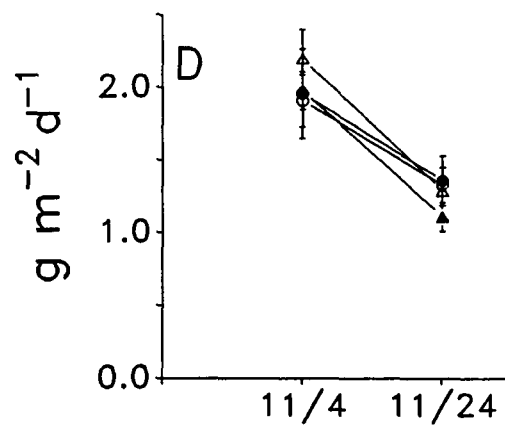
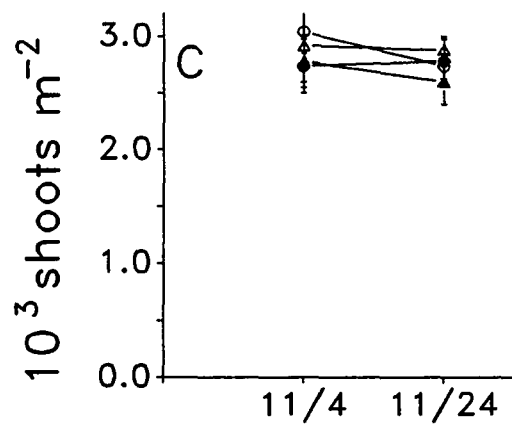
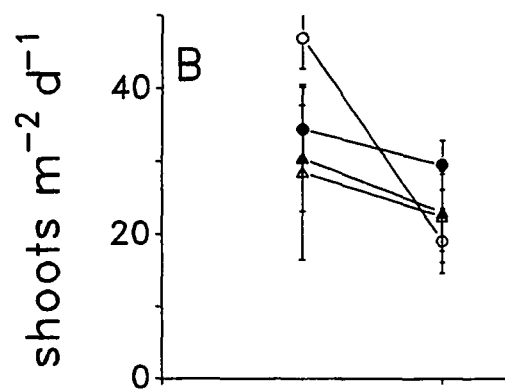
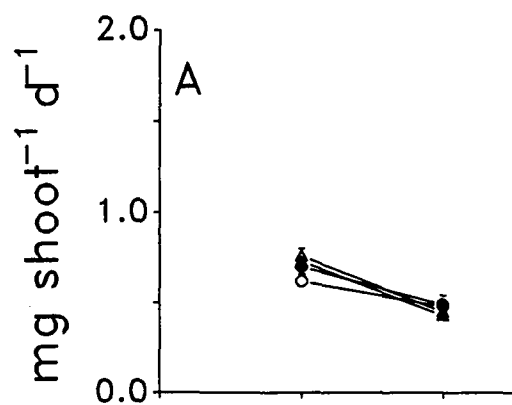
were produced within any treatment combination (Fig. 4B), shoot densities on the final date were lower under enriched than ambient conditions in the ungrazed microcosms ($P < .05$, Fig. 4C), indicating differential effects of treatment on shoot mortality. The combined responses of shoot growth and density resulted in considerable differences in areal biomass production by the end of the experiment (Fig. 4D). Nutrient-grazer interactions were significant for the final sampling period ($P < .05$). Although grazer removal decreased macrophyte production under both nutrient regimes, the magnitude of reduction was greater under enriched conditions, and enrichment reduced production with grazers absent only.

In contrast to the summer experiments, macrophytes exhibited no significant responses to microcosm treatments during the fall (Fig. 5) and spring (Fig. 6) experiments. Although rates of areal biomass production were similar between experiments ($1.3\text{--}2.0 \text{ g m}^{-2} \text{ d}^{-1}$, Figs. 5D, 6D), there were distinct seasonal differences in patterns of population growth. Production in the fall depended more on new shoot formation (Fig. 5B) than shoot-specific growth (Fig. 5A), whereas in the spring the pattern was reversed (Figs. 6A, 6B).

DISCUSSION

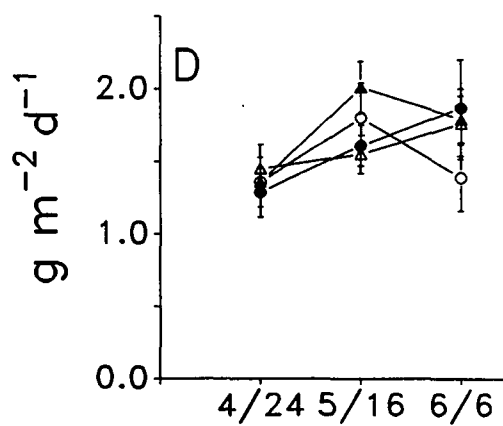
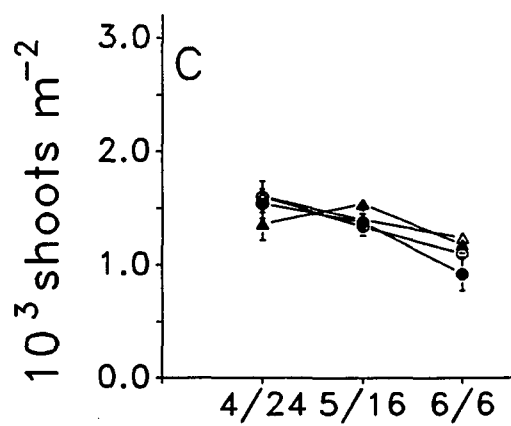
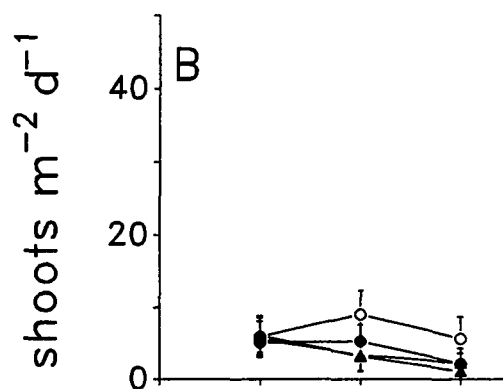
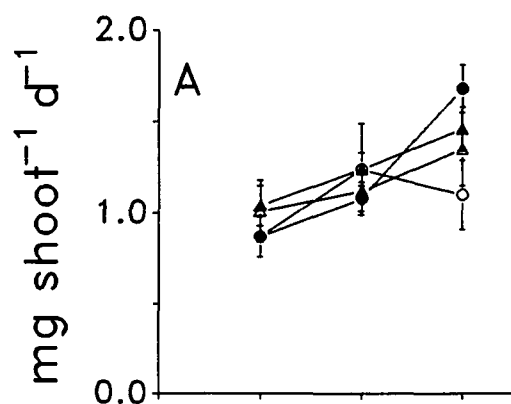
Previous studies have shown that nutrient concentration and grazing activity exert strong control on production of submerged macrophytes. Results of my experiments indicate

Figure 5. Macrophyte responses ($\bar{X} \pm SE$) to microcosm treatments during fall. Triangles=ambient nutrient concentrations, circles=enriched nutrient concentrations; solid symbols=grazers absent, open symbols=grazers present. A. Leaf growth rate; B. Shoot formation rate; C. Shoot density; D. Areal leaf production rate. All mass measurements based on DW. Statistical analyses of A and D were done on log transformed data.



SAMPLE DATE (MONTH/DAY)

Figure 6. Macrophyte responses ($\bar{X} \pm SE$) to microcosm treatments during spring. Triangles=ambient nutrient concentrations, circles=enriched nutrient concentrations; solid symbols=grazers absent, open symbols=grazers present. A. Leaf growth rate; B. Shoot formation rate; C. Shoot density; D. Areal leaf production rate. All mass measurements based on DW. Statistical analyses of A and D were done on log transformed data.



SAMPLE DATE (MONTH/DAY)

that the combined effects of these factors on dynamics of epiphyton-macrophyte associations change seasonally (Table 4). Only during the early summer were predictions based upon the individual effects of nutrient levels and grazer abundance confirmed experimentally; i.e., both enrichment and grazer removal increased epiphytic biomass and decreased macrophyte production consistently. During late summer, although nutrient concentration and grazing activity controlled epiphytic biomass independently, they interacted to influence macrophyte production: enrichment reduced production only when grazers were absent, and grazer removal reduced production of enriched greater than ambient treatments. I measured no macrophyte responses to treatment during the fall or spring, regardless of intermediate effects on epiphyton. This seasonal component of response underscores the importance of replicating microcosm experiments in time to extend their generality (cf. Kemp et al. 1980).

Microcosms must be validated as true analogues of natural systems before their results are extended to those systems (cf. Giesy and Odum 1980). Within the spatial constraints of these microcosms, environmental conditions simulated those of natural eelgrass communities. For example, seasonal irradiances were similar to those reported for lower Chesapeake Bay eelgrass habitat (Murray and Wetzel 1987), as were concentrations of suspended chlorophyll *a* (K. A. Moore, unpublished). The short residence time of water in the aquaria ensured that water chemistry and temperatures

Table 4. Effects of microcosm treatments at the end of each experiment. Symbols indicate direction of response by average epiphytic biomass (E) and areal macrophyte production (M) to major column heading (nutrient enrichment or grazer removal) relative to alternative level of same factor; number of symbols indicates magnitude of response within column sub-heading relative to alternative level of same factor in adjacent column; +=increase, -=decrease, 0=no effect. Using this notation, independent treatment effects for each row are indicated by like entries across column sub-headings within a major column heading, whereas interactive effects are indicated by unlike entries.

Experiment	Response Variable	Nutrient Enrichment		Grazer Removal	
		Grazed	Ungrazed	Ambient	Enriched
Early Summer	E	+	+	+	+
	M	-	-	-	-
Late Summer	E	+	+	+	+
	M	0	-	-	- -
Fall	E	+	+	0	0
	M	0	0	0	0
Spring	E	0	-	+	0
	M	0	0	0	0

deviated little from conditions in the estuary. Current velocities were within the wide range reported from eelgrass beds (e.g. 0 cm s^{-1} reported by Harlin and Thorne-Miller 1981; 110 cm s^{-1} reported by Fonseca et al. 1983). The epiphytic biomass in experimental treatments with grazers present agreed with measurements from marine macrophytes in natural habitats (e.g. Borum and Wium-Andersen 1980, Bulthuis and Woelkerling 1983, Heijs 1984, Borum et al. 1984), including eelgrass in Chesapeake Bay (K. A. Moore, unpublished). Rates of relative leaf growth were within the range of those published from widespread natural eelgrass communities (Dennison and Alberte 1982, Kentula and McIntire 1986), and measurements of shoot-specific leaf growth agreed with data from Chesapeake Bay (K. A. Moore, unpublished). The depressed production I measured during high summer temperatures has been similarly documented in the field (Penhale 1977, Wetzel and Penhale 1983, Thayer et al. 1984, Murray and Wetzel 1987). Results of these microcosm experiments should thus be applicable to natural systems.

Results of this study suggest that during most of the year, grazing activity is more important than nutrient concentration in controlling epiphytic abundance on eelgrass leaves in Chesapeake Bay: during the early summer, late summer, and spring experiments, grazer removal increased the biomass of epiphyton to a greater extent than did nutrient enrichment. As epiphytic AFDW in this region is highly correlated with chlorophyll *a* (Chapter 5), the increase in epiphytic biomass with enrichment presumably represented

enhanced algal growth, and indicated nutrient limitation under ambient conditions. However, enrichment did not increase accumulation rates sufficiently to overcome the effect of natural grazer densities. The growth of filamentous and macrophytic algae observed in the enriched, ungrazed microcosms during the summer and spring experiments is a common response to eutrophied conditions (Harlin and Thorne-Miller 1981, Cattaneo 1987). The low epiphytic biomass in these microcosms at the end of the spring experiment may have been due to inhibitory effects of dense Enteromorpha growth. As evidenced by their absence in the grazed microcosms, grazing activity effectively reduces these algal forms under certain levels of enrichment ($\text{DIN} \leq 16 \text{ uM}$, $\text{PO}_4 \leq 3.4 \text{ uM}$; Table 3).

Other studies of the combined effects of grazing and nutrient enrichment on periphyton show conflicting results. Stewart (1987) demonstrated that grazing limited periphyton biomass despite nutrient additions, whereas Marks and Lowe (1989) found little effect of grazing on nutrient enriched substrata. The relative effect of grazing on biomass accrual depends simultaneously upon grazer characteristics (e.g. density: Cuker 1983, Colletti et al. 1987, Lowe and Hunter 1988; species and associated feeding behavior: Hill and Knight 1988, Lamberti et al. 1987, Steinman et al. 1987; ingestion rates: Jacoby 1987) and the combined effects of nutrient concentration and other abiotic factors regulating growth of periphytic organisms (reviewed by Sand-Jensen 1983). The strong effect of grazing on epiphytic biomass

during the late summer experiment was correlated with high grazer densities and water temperatures (thus presumed high grazer metabolic and ingestion rates), whereas the contrasting lack of effects in the fall was correlated with high ambient nutrient concentrations, low water temperatures, moderate grazer densities, and a switch in taxon of the dominant gastropod grazer (from B. varium to M. lunata). Measurements of grazing and epiphytic growth rates are necessary to clarify the specific mechanisms of interaction.

The influence of epiphyton on macrophyte production in these experiments depended upon interactions with factors which changed seasonally. The amount of light reaching leaf surfaces is regulated by irradiance at the water surface and subsequent water column and epiphytic attenuation. Therefore, the relative effect of epiphyton on macrophyte photosynthesis will vary with incident solar irradiance, water turbidity, and epiphytic density and spectral selectivity. Seasonal correspondences between epiphyton and macrophyte responses in this study were not correlated with incident PAR; for example, although levels of PAR in the microcosms were similarly high during the early summer and spring experiments, only during the early summer was increased epiphytic biomass associated with reduced macrophyte production. The microcosms received water from the same source, and there were no differences in suspended chlorophyll a concentrations among treatments. Therefore, presumably neither were there differences in water column

light attenuation. Seasonal differences in macrophyte responses did not correspond to seasonal patterns of epiphytic densities. Finally, although Mazzella and Alberte (1986) suggested that epiphyton in Massachusetts absorbed wavelengths unused by eelgrass photosynthesis, the epiphytic attenuation of radiation throughout the photosynthetically active range in my microcosms indicated the potential to reduce light for macrophyte use. There were no seasonal differences in epiphytic attenuation, however, corresponding to macrophyte responses.

Although the effects of epiphyton on macrophyte production in this study do not appear related to absolute amounts of PAR reaching leaf surfaces, they may be explained by seasonal variability in macrophyte light requirements. As respiration of eelgrass increases with temperature, higher irradiances become necessary to maintain positive net photosynthesis and longer periods of light-saturated photosynthesis are required to maintain a net daily carbon gain (Marsh et al. 1986). Thus, at the high temperatures of the two summer experiments, macrophyte productivity would have been particularly sensitive to light reductions by epiphytic accumulations. By the end of each summer experiment, the two lowest estimates of macrophyte production (both nutrient regimes with grazers absent; Figs. 3D, 4D) coincided with the two highest epiphytic accumulations (Fig. 2). At the low grazer density of early summer, the epiphytic biomass of the grazed treatments showed an average (across leaf age class) increase of 17%

with nutrient enrichment (Fig. 2), which corresponded to a decrease in macrophyte production of 25% (Fig. 3D). During late summer, high grazer densities reduced the epiphytic biomass of both nutrient treatments to low levels (Fig. 2), and macrophyte production was correspondingly high. The contrasting lack of macrophyte response to treatment during the fall and spring experiments regardless of differences in epiphytic biomass indicates that factors affected by epiphyton were not limiting to macrophyte production, and may be related to the comparatively low light requirements of eelgrass at low temperatures.

These inferences are based upon the presumption that the indirect effects of dissolved nutrient concentrations and grazing activity on macrophyte production are via their direct effects on epiphytic biomass. However, macrophyte growth and production are controlled by factors other than photosynthesis which may also have been influenced by treatment. In particular, because nutrient uptake by roots is concentration-dependent (Penhale and Thayer 1980, Short and McRoy 1984), grazer fecal production has been suggested to enhance macrophyte growth by increasing sediment nutrient concentrations (van Montfrans et al. 1984). If sediment fertilization were the primary mechanism by which epiphytic grazing increased macrophyte growth in these experiments, there would be no clear explanation for the observed seasonal differences in response; i.e., grazing on epiphyton and consequent fecal deposition would have been expected to increase macrophyte production in the spring as well.

Although I can not rule out nutrient deposition as a partial explanation for increases in macrophyte growth in grazed microcosms, it does not appear to have been a dominant mechanism.

This study suggests that submerged macrophyte production is controlled by complex interactions with both dissolved nutrient concentrations and epiphytic grazers. Furthermore, the indirect effects of these factors change seasonally, and can not always be predicted from their individual influences on macrophyte growth. The seasonal differences in response preclude generalizations regarding the relative importance of nutrients and grazing on macrophyte survival. Short-term, seasonal measurements of macrophyte photosynthesis and carbon balance integrated with simulation models of annual production would elucidate the roles of these interactions in the long-term stability of submerged macrophyte communities.

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Chapter 3

RELATIVE EFFECTS OF NUTRIENT ENRICHMENT AND GRAZING ON EPIPHYTON-MACROPHYTE (ZOSTERA MARINA L.) DYNAMICS

II. SIMULATION MODEL PREDICTIONS FOR LONG-TERM COMMUNITY STABILITY

SUMMARY

A computer model of eelgrass (Zostera marina L.) production was used to test the potential long-term effects of nutrient enrichment and epiphytic grazing on macrophyte community stability. Carbon flows in the model were derived as realistic, non-linear feedback controlled functions of the biological compartments and environmental controls. The model included ranges of environmental variables specific to lower Chesapeake Bay, where declines in eelgrass abundance have been correlated with anthropogenic nutrient enrichment, reduced grazer populations, and increased turbidity. Photosynthesis by eelgrass in the model depended upon amounts of light and carbon at leaf surfaces, both of which were reduced by an epiphytic layer. Epiphytic biomass accrual depended upon dissolved nutrient concentrations and grazing activity. A series of 10-year model simulations indicated that the loss of eelgrass in Chesapeake Bay was the result of exposure to a combination of stresses. Either nutrient enrichment or a loss of grazers alone would reduce annual eelgrass standing stocks but would not limit survival. However, these factors in concert would cause long-term instability of eelgrass communities. With grazers present, nutrient enrichment with a slight decrease in submarine irradiance would cause loss of the community. Model results can be combined with environmental measurements to guide conservation and restoration of eelgrass habitats.

INTRODUCTION

Much recent scientific and public interest in the ecology of submerged macrophyte communities was precipitated by losses of vegetation from inland, estuarine, and marine waters worldwide (e.g. Lind and Cottam 1969, den Hartog and Polderman 1975, Moss 1983, Orth and Moore 1983, Cambridge and McComb 1984). Declines in abundance of submerged macrophytes frequently are correlated with anthropogenic nutrient enrichment and consequent increases in epiphytic fouling, which limits light transmittance and carbon diffusion to leaf surfaces and thereby reduces macrophyte productivity (e.g. Phillips et al. 1978, Twilley et al. 1985, Silberstein et al. 1986). The effects of increased epiphytic biomass are further influenced by other environmental variables, such as light attenuation through the water column (Twilley et al. 1985), water temperature (Chapter 2), and population densities of epiphyton-grazers (Chapter 2). These complex interactions hinder predictions for the long-term stability of submerged macrophyte communities (cf. Lodge et al. 1988).

Simulation models incorporating mechanistic relationships among biotic system components and environmental factors may be used to relate results of short-term studies of macrophyte production to long-term community behavior (cf. Hill and Wiegert 1980). I describe here the adaptation of an existing computer model (Wetzel and Neckles 1986) to study the effects of epiphyton on

growth and survival of eelgrass (Zostera marina L.) in Chesapeake Bay. Declines in eelgrass abundance occurred throughout much of Chesapeake Bay in the early 1970s, corresponding to zones of anthropogenic nutrient enrichment (Orth and Moore 1983), reduced water clarity (USEPA 1982), and the elimination of a dominant epiphyton-grazer from many areas following a severe tropical storm (van Montfrans et al. 1982). Previous model simulations suggested that any increase in epiphytic densities would be detrimental to eelgrass growth in this region (Wetzel and Neckles 1986). I thus fit this model to results from short-term studies of enrichment and epiphytic grazing in eelgrass microcosms (Chapter 2) to explore the relative effects of these factors on the long term stability of eelgrass communities.

METHODS

The conceptual and mathematical structure of the model used in this study was described in detail by Wetzel and Neckles (1986). The model simulated the transfer of carbon among major components of an eelgrass community. In general, eelgrass photosynthesis was a function of the amount of light (as photosynthetically active radiation or PAR) and carbon at leaf surfaces, both of which were limited by a layer of epiphyton. Epiphytic biomass was accumulated through the photosynthesis of microalgae, and was diminished by grazing activity. Grazing invertebrates were aggregated into a single biological compartment. Immigration,

emigration, and predation caused seasonal fluctuations in grazer densities. All flows were derived as realistic, non-linear feedback controlled functions of the biological compartments and environmental controls. The model was calibrated for lower Chesapeake Bay by incorporating measured ranges of environmental variables (solar irradiance, photoperiod, water column PAR attenuation, water depth, and water temperature) specific to the region. Simulated standing stocks of biological compartments agreed with field estimates from natural eelgrass communities.

The photosynthetic rate of epiphytic algae was derived in the model as a hyperbolic function of light intensity, the light saturated rate of photosynthesis (P_{\max}), and the half-saturation light intensity. Dissolved inorganic nutrients were not modeled explicitly; rather, their effects were incorporated as limits inherent in growth calculations. Much evidence indicates that phytoplankton respond to nutrient enrichment by an increase in P_{\max} (Parsons et al. 1984). Therefore, I modeled the effects of nutrient enrichment implicitly in this study by increasing epiphytic P_{\max} .

I determined the magnitude of increase in P_{\max} representing enriched conditions by fitting the model to data derived from eelgrass microcosms described previously (Chapter 2). In brief, eelgrass was grown in microcosms for 1-2 months during spring, early and late summer, and fall, under environmental conditions simulating Chesapeake Bay habitats. Experimental treatments included the presence or

absence of invertebrate grazers on epiphyton, and ambient or enriched nutrient concentrations. Ambient nutrient concentrations represented levels of dissolved inorganic nitrogen (DIN) and phosphorus (PO_4) found where eelgrass occurred currently in lower Chesapeake Bay (DIN=4uM in spring and summer, 11uM in fall; PO_4 =0.8-1.6 annual range). Nutrient enrichments (3x ambient) approximated concentrations that have been correlated with local eelgrass declines. I increased epiphytic P_{max} in the model until predicted epiphytic densities (expressed as epiphyton:macrophyte biomass ratios) agreed with final observations from the microcosm experiments. Because leaf age structure was not incorporated in the model, the epiphytic community was simulated as distributed evenly over leaf surfaces. In reality, however, epiphytic density increases with leaf age (e.g. Borum and Wium-Andersen 1980). I thus selected the average aged leaf within a shoot (the third leaf produced within a consecutive sequence of 5-6 leaves) from the microcosm results for comparison with model predictions. The model was then simulated for 10 years to investigate the potential effects of various conditions on community persistence.

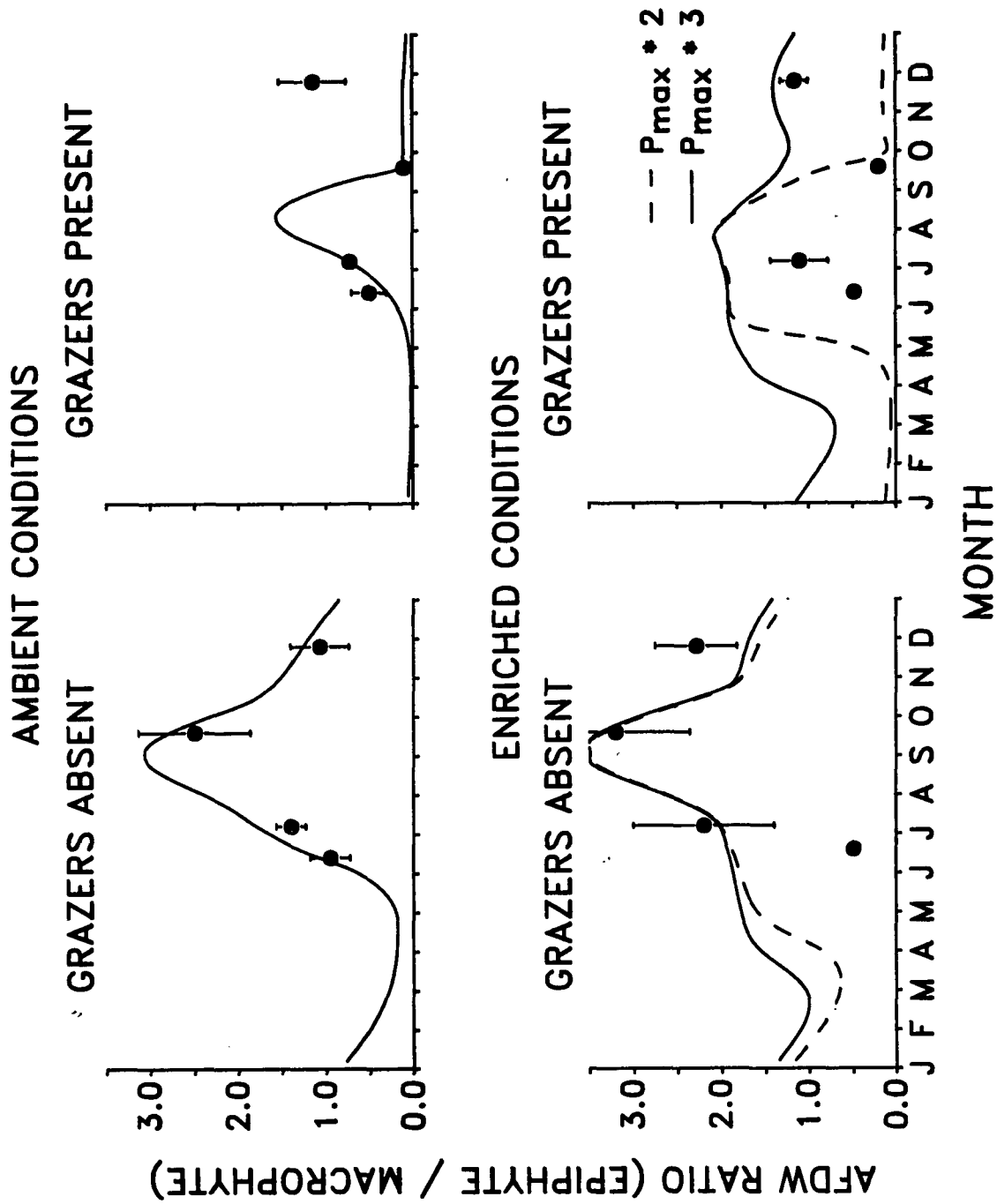
RESULTS

The simulated epiphyton to macrophyte mass ratios under ambient nutrient concentrations agreed closely with observations from the microcosms exposed to both grazer

treatments (Fig. 1). The outlying observation for grazed conditions occurred in the fall, when ambient nutrient concentrations rose to levels which approximated enriched concentrations at other times of the year. Increasing epiphytic P_{\max} in the model by a factor of 2-3 resulted in predicted ratios which were similar to most observations from the enriched microcosms with grazers absent (Fig. 1). The low, outlying measurement was accompanied by anomalous growth of dense macroalgae which may have limited microalgal growth (Chapter 2). Both simulated enrichment levels ($P_{\max} \times 2$ or 3) with grazers present predicted higher epiphyton to macrophyte ratios in the spring and early summer than were observed within the corresponding microcosms. The late summer observation under these conditions agreed closely with a 2-fold increase in P_{\max} , whereas the fall observation agreed with a 3-fold increase. Because ambient nutrient concentrations in the microcosms were high in the fall, enriched levels were higher than occurred at other times of the year as well. To investigate the potential extreme effects of nutrient enrichment on macrophyte survival, enrichment was set as a 3-fold increase in epiphytic P_{\max} for 10 year model simulations.

Initial model simulations incorporated a water column PAR attenuation coefficient of 1 m^{-1} , based upon long-term averages for Chesapeake Bay eelgrass communities (Wetzel and Neckles 1986). Simulation of the model under ambient dissolved nutrient concentrations indicated that a loss of grazers would lower maximum annual leaf biomass of eelgrass

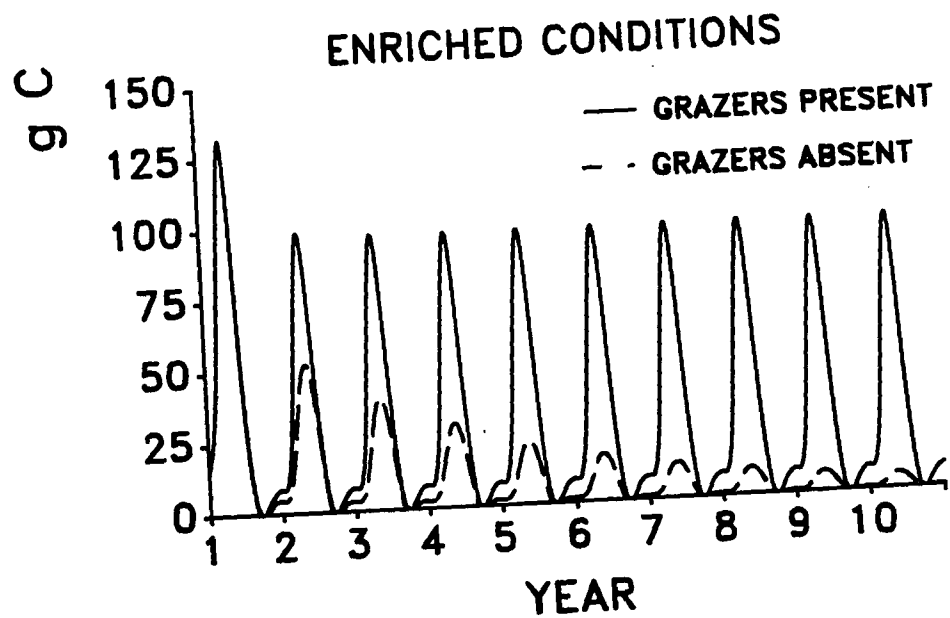
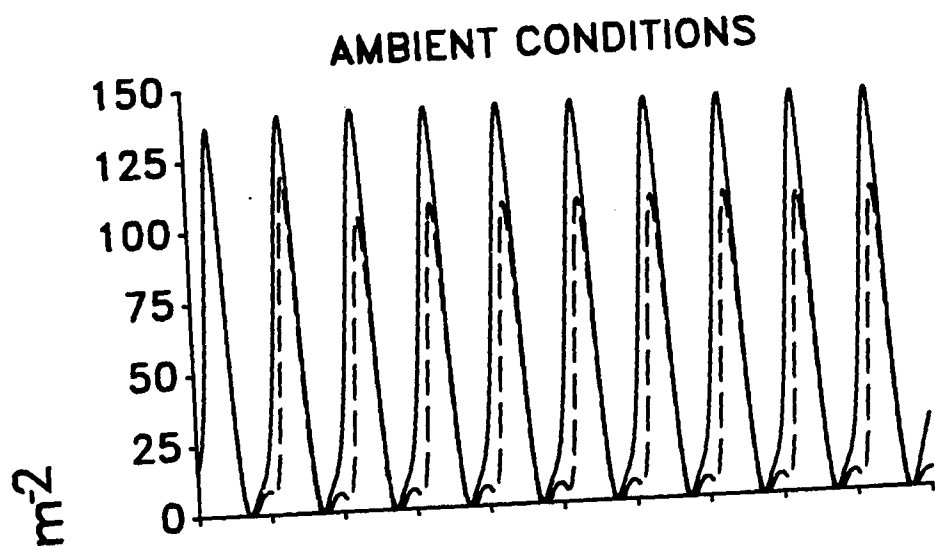
Figure 1. Ratios of epiphyton:macrophyte biomass (as ash-free dry weight, AFDW) as predicted by simulation model (lines) and measured from microcosms (points, $\bar{X} \pm \text{SE}$ from 5 replicates).



but would not limit long-term survival (Fig. 2; see also Wetzel and Neckles 1986). Similarly, as long as grazers were present, nutrient enrichment reduced predicted standing stocks but did not affect community persistence (Fig. 2). Under ambient conditions with grazers present, model parameters revealed the annual maximum eelgrass biomass to be limited by density dependent controls (e.g. sediment nutrient availability). As epiphytic densities increased with either grazer removal or nutrient enrichment, the maximum eelgrass biomass became limited rather by the amount of PAR reaching leaf surfaces. Under enriched conditions with grazers absent, model simulations predicted eventual loss of the eelgrass community due to epiphytic fouling (Fig. 2).

Increasing the water column attenuation of PAR in the model from 1.0 to 1.5 m^{-1} increased the ultimate effects of nutrient enrichment. Such a simulated increase in turbidity under enriched, grazed conditions did not affect the predicted annual maximum density of epiphyton on eelgrass leaves. However, the consequent reduction in PAR reaching the macrophyte canopy increased the relative effect of epiphytic attenuation of PAR on macrophyte survival: the model predicted the loss of the community under these conditions.

Figure 2. Model-predicted eelgrass leaf biomass under various conditions.



DISCUSSION

The simulation model used here was validated previously for stable eelgrass communities in Chesapeake Bay (Wetzel and Neckles 1986), and the predicted epiphyton:macrophyte biomass ratios agreed with observations from microcosms exposed to ambient concentrations of dissolved nutrients typical of Chesapeake Bay eelgrass habitats. Therefore, use of microcosm observations to calibrate the model to enriched conditions should result in equally realistic simulated system behavior. There is little information on photosynthetic rates of epiphyton at different nutrient concentrations. Data reported by Twilley et al. (1985) showed a 6- to 15-fold increase in specific rates of epiphytic photosynthesis in upper Chesapeake Bay with a 2- to 4-fold enrichment of nitrogen and phosphorus. This is considerably higher than the 3-fold increase in P_{max} which forced model predictions to fit observations from enriched microcosms (Fig. 1), and indicates the need for more experimental data on the effects of varying nutrient concentrations on epiphytic photosynthesis. Similarly, the lack of fit between model predictions and microcosm measurements of spring and summer epiphytic densities under enriched, grazed conditions (Fig. 1) suggests the need for more information on the mechanisms of interaction between nutrient enrichment and grazing activity. As model predictions during these periods were higher than microcosm

observations, long-term simulations provided conservative estimates of macrophyte survival.

Previous model simulations indicated that an increase in the PAR attenuation coefficient alone from 1.0 to 1.75 m^{-1} would cause long-term instability of eelgrass communities in Chesapeake Bay (Wetzel and Neckles 1986). The results presented here suggested that under enriched conditions, an increase in the PAR attenuation coefficient to only 1.5 m^{-1} would result in eelgrass loss. Thus the increased epiphytic attenuation of PAR caused by nutrient enrichment increased the relative effect of turbidity on macrophyte survival. Twilley et al. (1985) suggested that epiphytic and water column attenuation of PAR similarly interacted to limit distribution of submerged macrophytes in upper Chesapeake Bay. The model simulations showed further that although nutrient enrichment alone would not limit eelgrass survival, the combined effects of enrichment and loss of grazer populations would cause rapid depletion of macrophyte biomass and ultimate loss of the community. These simulation studies support the hypothesis that epiphytic grazing is essential to macrophyte survival in eutrophic systems (cf. Orth and van Montfrans 1984, Borum 1987).

Declines in abundance of eelgrass in Chesapeake Bay were correlated with increased nutrient concentrations, increased turbidity, and decreased grazer populations. Simulation model studies (Wetzel and Neckles 1986, this paper) indicated that none of these environmental changes

alone was the immediate cause of widespread eelgrass loss; rather, these factors acted in concert to reduce eelgrass production and limit survival. Much of the area from which eelgrass disappeared in Chesapeake Bay remains unvegetated (Orth et al. 1989). Results of these simulations can be combined with measurements of environmental variables to guide conservation and restoration of eelgrass meadows. The results of these simulation studies are specific to lower Chesapeake Bay. However, the relative consequences of environmental changes in other regions can be predicted based upon hypothesized mechanisms of interaction. For example, in areas such as upper Chesapeake Bay where inorganic nutrient concentrations exceed those of lower Chesapeake Bay by an order of magnitude (cf. USEPA 1982), grazing may have little influence on epiphytic densities and consequent effects of macrophyte production. The roles of such interactions remain central to questions addressing the stability of submerged macrophyte communities.

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Chapter 4

RELATIVE RESPONSES OF EPIPHYTIC BIOTA TO NUTRIENT ENRICHMENT AND MACROHETEROTROPHIC GRAZING

SUMMARY

The simultaneous effects of nutrient enrichment and macroheterotrophic grazing on abundances of epiphytic biota (diatoms, cyanobacteria, heterotrophic flagellates, and heterotrophic bacteria) were tested in eelgrass (Zostera marina L.) microcosms. The epiphytic community was examined using epifluorescence microscopy after one and two months of treatment. In general, numbers of diatoms decreased in the presence of grazers and showed little response to nutrient enrichment, whereas numbers of cyanobacteria increased with nutrient enrichment and showed little response to grazing. Thus, proportions of cyanobacteria increased with both enrichment and grazing. Following two months of treatment, dense macroalgal growth under nutrient enriched conditions with grazers absent appeared to limit populations of both autotrophs. Patterns of abundance of heterotrophic bacteria suggested that populations were limited by nutrient supply during the first month of treatment, and by organic carbon excreted by diatoms during the second month. Fluctuations in numbers of heterotrophic flagellates mimicked those of bacteria. Results suggest that microflagellates serve as a heretofore overlooked link between bacterial production and higher trophic levels in submerged macrophyte communities.

INTRODUCTION

Epiphytic communities are complex associations of algae, bacteria, fungi, and detritus attached to plant surfaces. Epiphyton may contribute from 20-60% to the total production of submerged macrophyte communities (e.g. Penhale 1977, Cattaneo and Kalff 1980, Heijs 1985a, Mazzella and Alberte 1986). Previous studies have demonstrated the importance of both external environmental conditions and characteristics of the macrophyte substratum in regulating epiphytic productivity and abundance (reviewed by Harlin 1975, Borowitzka and Lethbridge 1989). Internal processes such as senescence and biotic interactions may also exert strong control on epiphytic community dynamics during late developmental stages (Sand-Jensen 1983). In particular, the widely acknowledged importance of microheterotrophs in planktonic foodwebs (Azam et al. 1983, Fenchel 1988) suggests that similar microbial interactions may be equally important in epiphytic communities. Although the intricate composition of epiphytic communities has been described (e.g. Kita and Harada 1962, Sieburth and Thomas 1973, Novak 1984, Heijs 1985a,b), there remains a paucity of information concerning the relative effects of environmental influences and internal community processes on the abundance of various components of the epiphytic biota.

Nutrient enrichment has been shown frequently to enhance the accumulation of epiphytic biomass (e.g. Eminson and Phillips 1978, Borum 1985, Twilley et al. 1985), whereas

grazing by macroheterotrophs (e.g. isopods, amphipods, snails) has been shown to limit biomass accumulation (Howard 1982, Cattaneo 1983, Hootsmans and Vermaat 1985). Recent studies have examined the interactive effects of nutrients and grazing on the dynamics of periphyton on inert substrata (Stewart 1987, Marks and Lowe 1989, Mazumder et al. 1989). The combined effects of these factors on epiphytic abundance, however, have not been addressed. Furthermore, although many investigators have documented the influence of these external environmental factors on the diversity of algal species (reviewed by Orth and van Montfrans 1984, van Montfrans et al. 1984), the relative responses by other components of the epiphyton have been rarely studied. As part of a larger study of environmental controls on epiphyton-macrophyte associations (Chapter 2), I examined the short-term responses by epiphytic biota to nutrient enrichment and macroheterotrophic grazing in eelgrass (Zostera marina L.) microcosms. I describe here the differential effects of these external environmental controls on abundances of epiphytic diatoms, cyanobacteria, heterotrophic microflagellates, and heterotrophic bacteria, and discuss the potential changes in internal community dynamics associated with these effects.

MATERIALS AND METHODS

Eelgrass was grown in 20 glass aquaria from April-June, 1988, under conditions described in Chapter 2. In brief,

the aquaria (110 l) were housed in a greenhouse on the shore of the York River estuary of Chesapeake Bay, and were equipped with flow-through 50 μM -filtered sea water, aeration, and internal water circulation. The midday irradiance within the aquaria during the experiment was approximately $350 \text{ uE m}^{-2} \text{ s}^{-1}$ (photosynthetically active radiation, 400-700nm). The average daily water temperature increased from 14°C to 24°C during the experiment, and the salinity was approximately $19^{\circ}/\text{oo}$ throughout.

I applied nutrient-grazer treatment combinations to 5 replicate aquaria each, following a 2×2 factorial design (Chapter 2). Experimental nutrient concentrations were either ambient or enriched, and macroheterotrophic grazers were either present or absent. Enrichments of ammonium-nitrate and disodium phosphate were applied continuously to aquaria using peristaltic pumps. The average ambient concentrations of dissolved inorganic nitrogen ($\text{DIN} = (\text{NH}_4\text{-N}) + (\text{NO}_2\text{-N}) + (\text{NO}_3\text{-N})$) and phosphorus (PO_4) were 4 uM and 0.8 uM , respectively, and the average enriched concentrations were 11 uM and 1.8 uM . Treatments with grazers included epifaunal isopods (Idotea baltica) and amphipods (primarily Gammarus sp. and Amphithoe sp.) collected from a natural grassbed and applied and maintained at field densities (isopods: 300 m^{-2} ; amphipods: 600 m^{-2}).

The experiment began on 7 April and the epiphytic community was sampled on 10 May and 8 June. A sample consisted of the epiphyton from one leaf from each aquarium. Eelgrass shoots grow by the successive replacement

individual leaves. Because the plants used in this experiment consisted of 4-6 leaves, the age of the leaf substratum and the epiphytic community varied within a shoot. To standardize the epiphytic developmental stage as much as possible among replicates, I collected the leaf in the third relative position from each shoot. I used the edge of a glass slide to scrape the epiphyton into 100 mls of 0.2 μ m-filtered sea water. Each sample was homogenized by shaking vigorously, diluted with 0.2 μ m-filtered sea water as necessary for efficient microscopic examination, and preserved with 1% glutaraldehyde.

I used epifluorescence microscopy (Zeiss standard microscope) to differentiate and count the epiphytic biota within dominant categories. An aliquot of each sample was stained with proflavine (Haas 1982) and DAPI (Porter and Fieg 1980) and collected on a 0.2 μ m membrane filter. Diatoms, cyanobacteria, and heterotrophic microflagellates were identified by the characteristic presence or absence of autofluorescence under excitation with blue (450-490 nm) and green (510-560 nm) wavelengths (see also Ray et al. 1989). All of the organisms within approximately 50 microscope fields (1 transect across a slide) were counted using 12.5 ocular and 63x objective lenses. Heterotrophic bacteria were counted under ultraviolet excitation using 12.5 ocular and 100x objective lenses. Bacteria were counted until either 400 organisms or 40 ocular grids had been tabulated. Collectively, these categories included the majority of the

epiphytic biota. All counts were normalized to leaf area, which was measured using an area meter (Licor model 3100).

At each sampling date, responses to treatment by each epiphytic category were determined using a 2-way analysis of variance with main effects of nutrients and grazing. Log transformations were applied to stabilize error variances, and residual analysis was used to ensure the appropriateness of the statistical models. Mean abundances were compared using Tukey multiple comparisons (Neter and Wasserman 1974).

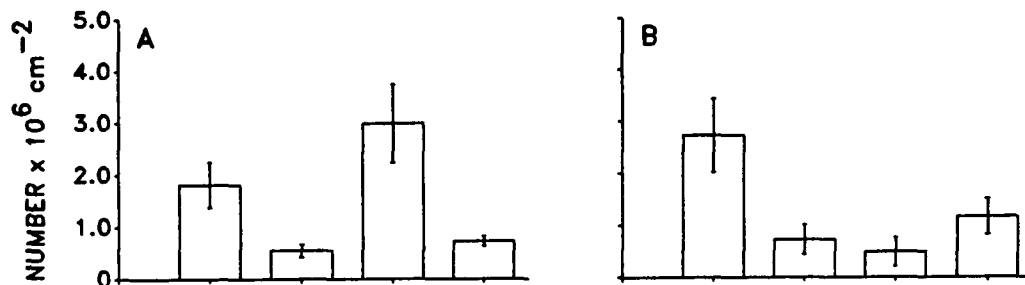
RESULTS AND DISCUSSION

There were no observable differences in the aquarium environments on the first sample date other than those imposed by experimental treatments. By the end of the experiment, however, dense accumulations of the macroalga Enteromorpha sp., both free-floating and attached, enshrouded the eelgrass under enriched conditions without grazers. Harlin and Thorne-Miller (1981) reported a similar response by Enteromorpha to enrichment within a natural eelgrass community. This confounding factor must be considered in addition to the applied treatments as potentially affecting the microscopic epiphyton.

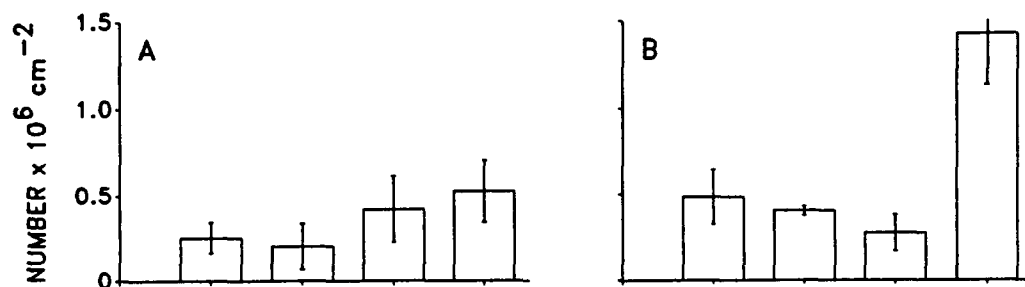
The autotrophic components of the microscopic epiphytic community responded oppositely to the experimental treatments (Fig. 1). Frequently encountered diatoms included Nitzschia sp. (30-50 um long), Licmophora sp. (30-

Figure 1. Responses by epiphytic biota ($\bar{X} \pm SE$) to microcosm treatments. A=sample date 1, B=sample date 2; -G=grazers absent, +G=grazers present.

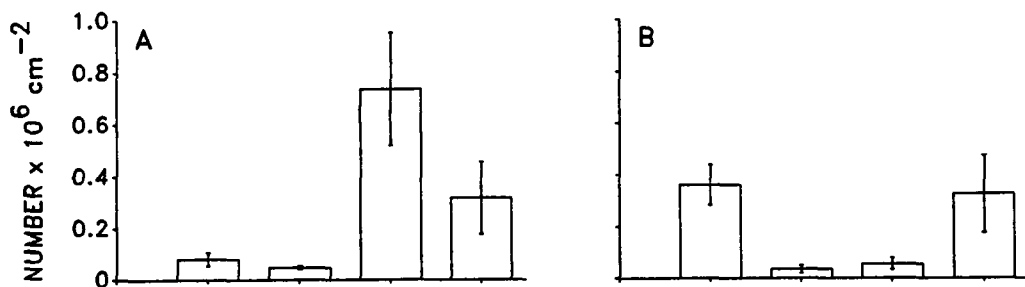
DIATOMS



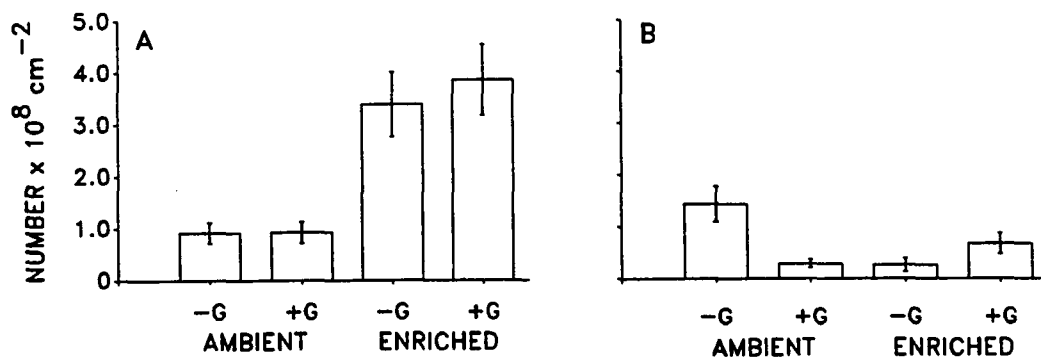
CYANOBACTERIA



HETEROTROPHIC FLAGELLATES



HETEROTROPHIC BACTERIA



65 μm long), and unidentified fusiform and ellipsoidal cells (25-65 μm long) and spherical cells (5-12 μm diameter). On the first sample date, the number of diatoms was reduced in the presence of grazers ($P < .05$) under both ambient and enriched conditions. Although there was a tendency toward increased numbers with enrichment when grazers were absent, there was no overall effect of enrichment ($P > .05$). By the second date, the number of diatoms was similarly reduced by grazing under ambient nutrient levels ($P < .05$). Under enriched conditions, however, diatoms showed a slightly increased abundance with grazers present ($P < .10$), presumably due to inhibitory effects of the dense macroalgal growth with grazers absent. Diatom numbers thus appeared to be regulated more by grazing activity than nutrient enrichment throughout the experiment. In contrast, cyanobacterial numbers appeared to be regulated more by inorganic nutrient supplies. The cyanobacteria included primarily filamentous forms, although rare coccoid cells were counted. There were no differences in mean cyanobacterial numbers among treatments on the first sample date ($P > .05$), but by the second date, numbers were elevated within the enriched, grazed treatment ($P < .05$). Again, the low abundance under enriched, ungrazed conditions was probably due to macroalgal limitation.

Previous studies of the responses by attached algal forms to environmental controls have shown varied results, dependent presumably upon the magnitude and types of external controls and the species of algae considered.

Results from this study are consistent with those of Cattaneo (1983), who showed selective grazing by various macroheterotrophs to reduce the importance of diatoms relative to cyanobacteria in lake epiphytic communities. Cattaneo and Kalff (1986) similarly found the relative dominance of epiphytic cyanobacteria to increase in the presence of large snails. However, Steinman et al. (1987) showed stream grazers to reduce the relative proportion of cyanobacteria in algal communities on tile substrata. Shifts in attached algal communities to dominance by filamentous forms are common in nutrient enriched systems (Cattaneo 1987). My results indicated that the effects of grazing and nutrient supply on epiphytic algal community structure were additive: i.e., the proportion of cyanobacteria was increased by both grazing and enrichment, such that the greatest proportion of cyanobacteria occurred under grazed, enriched conditions (Fig. 1).

Responses by microheterotrophs to experimental treatments differed between sample dates, but were similar between microflagellates and bacteria (Fig. 1). Most microflagellates were 3-6 μm in size. The majority of the bacteria were rod and coccoid forms; occasional bacterial filaments were counted as entire units. On the first sample date, numbers of both heterotrophic components were increased in the enriched treatments ($P < .05$). There were no overall effects of macroheterotrophic grazing on either component ($P > .05$), although the flagellates showed a trend toward reduced numbers under enriched conditions with

grazers present. On the second sample date, however, microflagellates and bacteria did not increase in number with enrichment; rather, patterns of abundance were indential to that shown by diatoms.

Heterotrophic microflagellates are recognized as effective grazers of bacteria in planktonic ecosystems, where their numbers are coupled closely to prey stocks (Azam et al. 1983, Fenchel 1988). The nearly identical patterns of abundance between heterotrophic microflagellates and bacteria throughout the experiment suggested that similar processes occur within epiphytic microbial systems. The relative numerical abundances of bacteria and microflagellates in the eelgrass epiphyton were also the same order of magnitude as observed in planktonic systems (bacteria:flagellate ratios of 10^2 - 10^3 ; Azam et al. 1983). The tendency toward suppression of high densities of epiphytic microflagellates by macroheterotrophs (Fig. 1) may be analogous to their control by micro-zooplankton in planktonic systems (cf. Azam et al. 1983, Sanders et al. 1989).

The densities of bacteria from the microcosms under ambient nutrient conditions were similar to those reported by Newell (1981) from a natural Chesapeake Bay eelgrass community during midsummer. The results indicated a switch in factors controlling bacterial densities throughout the experiment. The most parsimonious explanation for the pattern of bacterial abundance on the first sample date (Fig. 1) was a limitation by inorganic nutrients. This

interpretation is supported by recent evidence that much of the ammonium uptake by marine plankton is due to heterotrophic bacteria (Wheeler and Kirchman 1986). By the end of the experiment, the lack of correspondence between bacterial numbers and nutrient enrichment and the correlation between bacterial and diatom numbers suggested that bacteria were limited by organic carbon supplied by diatom exudate. This interpretation conflicts with previous evidence that epiphytic heterotrophs take up dissolved organic carbon excreted by eelgrass (Penhale and Smith 1977, Kirchman et al. 1984). However, there were no differences in eelgrass production (Chapter 2) or biomass (personal observation) among experimental treatments which corresponded to patterns of diatom or bacterial abundance, and the bacterial uptake of algal excretions is well established for planktonic systems (Jones and Cannon 1986). Smith and Penhale (1980) showed that epiphytic bacteria could take up various organic compounds; the immediate source probably depends upon the relative excretion rates of the juxtaposed macrophyte substratum and epiphytic algae.

The relative influences of top-down vs. bottom-up effects on food web interactions are central to many current questions in aquatic ecology (Crowder et al. 1988). Epiphytic communities may serve as a useful model for investigations of aquatic food webs: they offer complex associations of organisms at various trophic levels, in close proximity to one another and a biologically active substratum. My results indicated that the relative effects

of environmental factors varied over time and between the dominant algal components of eelgrass epiphyton; whereas numbers of diatoms appeared to be regulated by macroheterotrophic grazing, numbers of cyanobacteria appeared to be regulated by inorganic nutrient concentrations. Patterns of abundance of heterotrophic bacteria suggested that numbers were controlled variously by inorganic nutrients supplied external to the epiphytic community and by internally supplied organic carbon. Microheterotrophs, which are ordinarily overlooked in studies of epiphytic communities, may serve as important links between bacterial production and higher trophic levels in submerged macrophyte systems. I have drawn conclusions regarding epiphytic community dynamics from abundances of dominant organisms; future studies of production and transfer rates among components of the epiphyton at different trophic levels are necessary to test these inferences.

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Chapter 5

GROWTH OF EPIPHYTON IN LOWER CHESAPEAKE BAY: RELATIONSHIP TO SUBMERGED MACROPHYTE DISTRIBUTION AND ENVIRONMENTAL VARIABLES

SUMMARY

The growth of epiphyton in relation to environmental characteristics was studied in the York River estuary of Chesapeake Bay. Artificial substrata were deployed at three sites representing present and former limits of submerged macrophyte distribution. Epiphytic accrual was measured over five 30-day growth periods during one year. Multiple regression analysis suggested that net epiphytic growth rate was dependent most strongly upon water temperature and submarine irradiance. Correlations of growth with salinity and ammonium appeared to reflect interrelationships among environmental variables rather than epiphytic limitation by these factors. Site differences in epiphytic growth rates did not reflect patterns of macrophyte abundance, and suggested that epiphyton per se was not limiting macrophyte distribution in this region.

INTRODUCTION

The accrual of epiphytic biomass is regulated by many potential physical, chemical, and biological variables, including nutrient supply, temperature, irradiance, flow velocity, and grazing rates (reviewed by Sand-Jensen 1983, Borowitzka and Lethbridge 1989). The importance of specific environmental parameters varies both temporally and spatially. In various nutrient-enriched systems, declines in production of submerged macrophytes have been related in part to increased accumulation of epiphyton and consequent reduction of light and carbon at leaf surfaces (e.g. Phillips et al. 1978, Twilley et al. 1985, Silberstein et al. 1986, Hough et al. 1989). The development of such epiphytic densities detrimental to macrophyte production depends upon the relative influence of growth-enhancing factors, growth-limiting factors, and removal processes.

Eelgrass (Zostera marina L.) disappeared from much of its former range in lower Chesapeake Bay and its tributaries during the early 1970's (Orth and Moore 1981, 1984). Because areas of macrophyte loss corresponded to zones of nutrient enriched waters, detrimental effects of epiphytic accumulations were postulated to have contributed to the macrophyte decline (Orth and Moore 1983). The purpose of the study described here was to determine whether environmental differences reflected by current patterns of eelgrass distribution resulted in corresponding differences in epiphytic growth. My specific objectives were to compare

epiphytic biomass between presently and formerly vegetated sites in one southwestern tributary of Chesapeake Bay and to relate growth rates at these sites to in situ physical-chemical environmental conditions.

MATERIALS AND METHODS

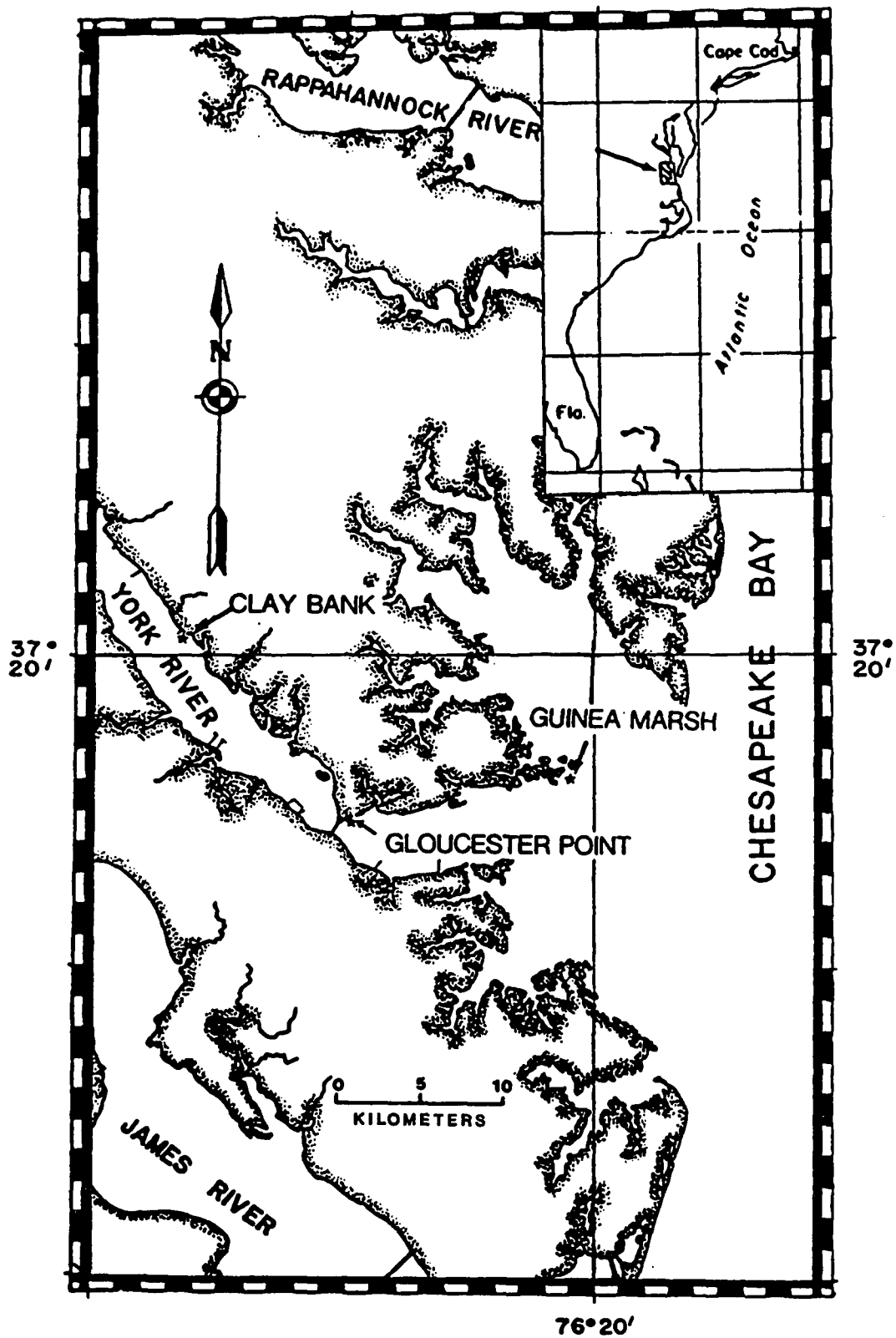
Study Sites

I measured epiphytic growth at three sites in the York River (Fig. 1) which reflected present and former patterns of eelgrass distribution (Orth et al. 1989). An extensive grass bed which persisted during the decline of eelgrass in Chesapeake Bay existed at the mouth of the river, known locally as Guinea Marsh. The present upriver limit of eelgrass distribution was at Gloucester Point; although the abundance of vegetation declined at this site in the early 1970's, considerable regrowth has occurred since that time. The former upriver limit of eelgrass distribution was at Claybank, where no regrowth has occurred.

Epiphytic Biomass and Growth Rates

The accumulation of epiphyton was measured using artificial eelgrass. Although the use of artificial substrata precludes any potential influences of the macrophyte host on epiphytic composition, biomass, and productivity, the benefits of standardization and

Figure 1. Study sites in the York River, Virginia.



replication make this technique valuable for relative site comparisons (Robinson 1983). Artificial plants consisted of four strips of polypropylene ribbon 5mm wide connected at the base. The length of the ribbon varied seasonally from 14cm to 35cm based upon seasonal changes in the morphology of eelgrass in lower Chesapeake Bay (Orth and Moore 1986). The artificial plants were attached to square mats (15cm x 15cm) at average annual field densities.

Five periods for measuring epiphytic growth were initiated from January to November 1987. Rates of biomass accumulation of attached algal populations are most strongly correlated with external physical-chemical conditions during the early stages of development, when losses due to grazing, senescence, and mechanical detachment are lowest (Sand-Jensen 1983). Changes in biomass are dependent largely upon gross growth rates during this stage. In order to limit the effects of losses on biomass accumulation, growth periods were restricted to approximately 30d. At the beginning of each period, 4-6 mats of artificial plants were deployed at each site. The mats were anchored at a depth of approximately 70cm (relative to mean low water). Samples were collected at short intervals (2-4d) during the first week of each period and at longer intervals (8-20d) thereafter. The final sample to be collected from Guinea Marsh during the June-July growth period was lost, presumably due to storm activity.

Samples consisted of 4 replicate artificial plants clipped underwater from the middle of a mat. I collected

separate samples for biomass and chlorophyll determinations. I scraped the epiphyton from each artificial plant into filtered sea water using the edge of a glass slide, and collected it by filtration (Gelman GF/C glass fiber filters). Epiphytic dry weight (DW) and ash-free dry weight (AFDW) were determined following drying at 60°C (2-5d) and combusting at 500°C (5h). Chlorophyll *a* was determined following extraction in a 90% solution of acetone and dimethyl sulfoxide (Ray et al. 1989). Chlorophyll concentrations were ordinarily determined spectrophotometrically (Parsons et al. 1984); extremely low concentrations (e.g. following 2d exposure) were determined fluorometrically (Parsons et al. 1984). Chlorophyll concentrations were not corrected for degradation products. All measurements were standardized by substratum surface area. An autotrophic index of each sample was calculated as the ratio of total AFDW:chlorophyll *a* to indicate the composition of the epiphytic community (Weber 1973). High index values (>400) suggest a predominance of heterotrophs or detritus, whereas low values (<100) suggest a predominance of algal autotrophs (Biggs and Close 1989).

Epiphytic densities were compared among sites during each period using a 2-way analysis of variance with main effects of site and sample date. $\ln(x)$ transformations were used to stabilize sample variances. Means were compared using Bonferroni multiple comparisons with a family confidence coefficient of 0.95 (Neter and Wasserman 1974). I calculated the net specific growth rate during each period

as $(\ln B_f - \ln B_i)/t$, where B_f and B_i represented the biomass measured at the final and initial sample dates over the entire growth period of t days. This value is equivalent numerically to the mean of specific growth rates calculated similarly for each sampling interval, weighted by the number of days in each interval. When analysis of variance revealed no significant differences in biomass between sites for specific dates, biomass estimates were averaged across sites for net growth calculations. Net epiphytic growth rates within each period were related to environmental measurements (see below) using simple linear correlation and multiple regression analyses. I used the Shapiro-Wilk test (Minitab 1988) to ensure the normal distribution of observations intended for correlation analysis, and used residual analysis in conjunction with all analyses of variance and regressions to ensure that assumptions of the models were met.

Environmental Variables

Selected physical and chemical characteristics of the study sites were measured biweekly during epiphytic growth periods. Three subsurface water samples were collected at each site. Total suspended solids (TSS) were determined gravimetrically following filtration onto precombusted, preweighed glass fiber filters (Gelman GF/C). Concentrations of dissolved inorganic nutrients were determined using standard colorimetric methods (nitrate, nitrite,

and orthophosphate: USEPA 1979; ammonium: Parsons et al. 1984). In addition, photosynthetically active radiation (PAR, 400-700nm) was measured with depth at each site using a cosine-corrected quantum sensor (Licor LI-185B), from which calculated light attenuation was calculated as the decay coefficient (K) of a negative exponential function. I estimated daily PAR (PAR_D , $E\ m^{-2}\ d^{-1}$) and instantaneous maxima (PAR_I , $\mu E\ m^{-2}\ s^{-1}$ at solar noon) reaching the artificial substrata as negative exponential functions of water depth and solar irradiance. Daily and instantaneous solar irradiances were predicted from the average insolation and photoperiod for the region (Wetzel and Neckles 1986). Environmental measurements at each site were averaged across date within each period for relating to epiphytic growth rates.

RESULTS

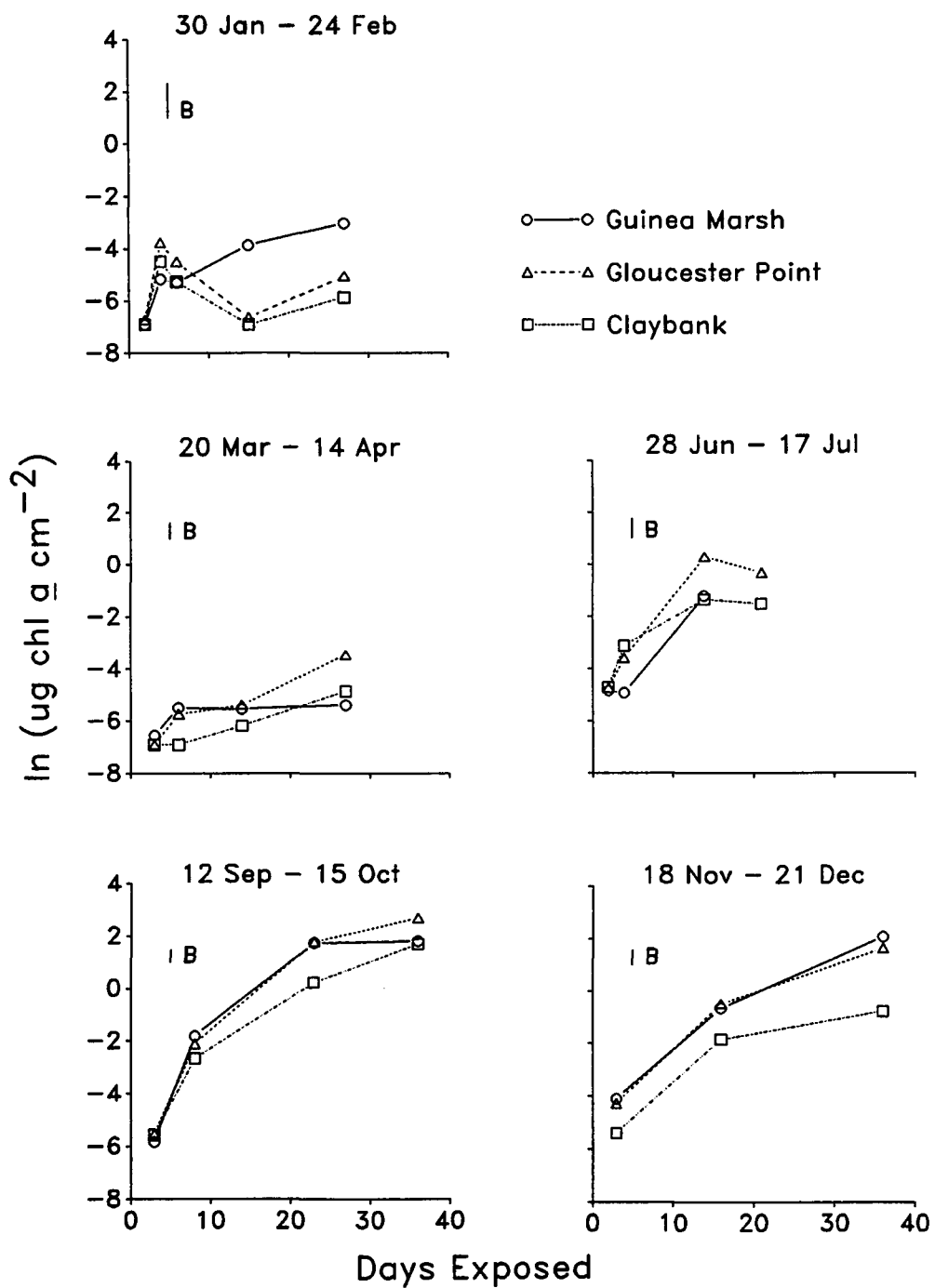
Sample measurements of epiphytic DW, AFDW, and chlorophyll a were highly correlated for each period ($r=.70-.96$, $P<.001$). The autotrophic index generally declined over time within a measurement period. Values were higher during the growth periods initiated in January and March (500-5000) than in June, September, and November (50-400 following the first sampling date). Because patterns of accrual were similar among biomass components, only results for chlorophyll a are presented here.

Seasonal epiphytic accumulations were lowest at all sites during the growth periods initiated in January and March (Fig. 2). Differences in epiphytic densities among sites varied over time within a growth period and among periods (Fig. 2). During the January-February period, densities at Gloucester Point and Claybank fluctuated considerably over time, and did not differ between sites ($P > .05$); by the middle of this period, densities at Guinea Marsh were higher than at the other two sites ($P < .05$). During the remaining four periods, however, consistent differences between Gloucester Point and Claybank emerged: following the first or second sampling dates, epiphytic densities were higher at Gloucester Point ($P < .05$). The relationship of epiphytic responses at Guinea Marsh to those of the other sites varied seasonally; the pattern of biomass accrual at Guinea Marsh was more similar to that at Claybank than at Gloucester Point during the March-April and June-July periods, and more similar to that at Gloucester Point during the final two growth periods.

The net specific growth rate of epiphytic algae generally changed over time within a growth period; i.e., the relationship between \ln (biomass of chlorophyll *a*) and time was not linear (Fig. 2). Therefore, the determination of mean net epiphytic growth was dependent upon the length of the growth period. Because of a shortened growth period, epiphytic accrual at Guinea Marsh in June-July was excluded from analyses of net growth rates.

Average epiphytic growth rates and environmental

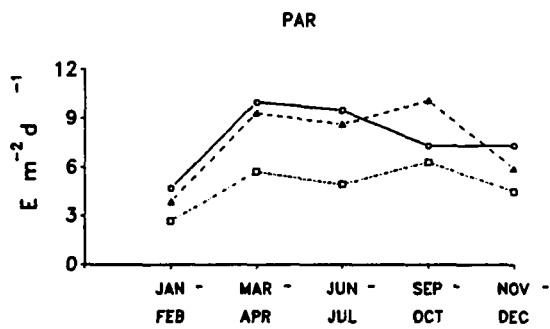
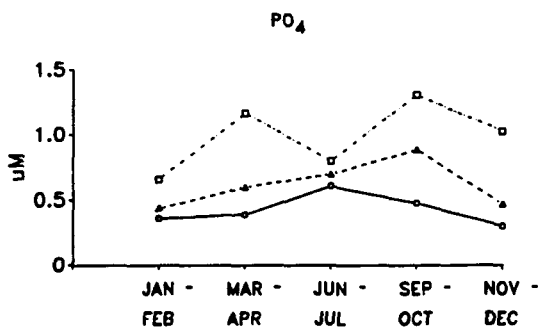
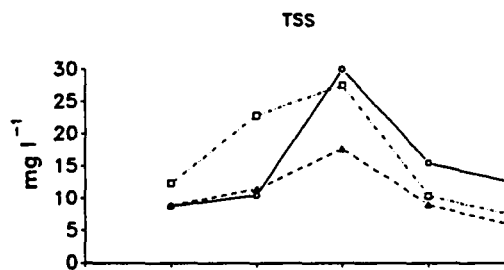
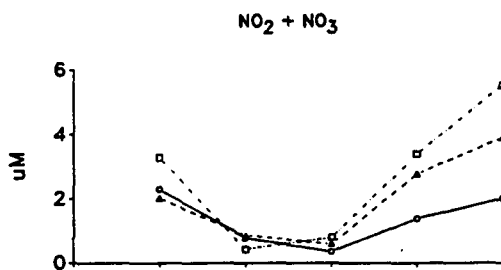
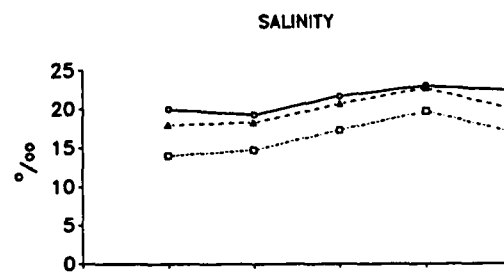
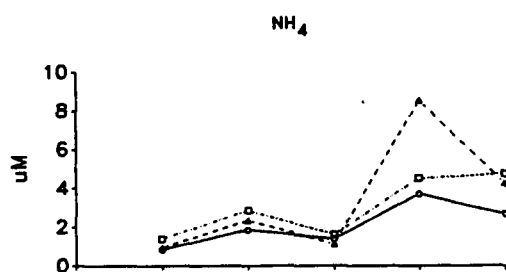
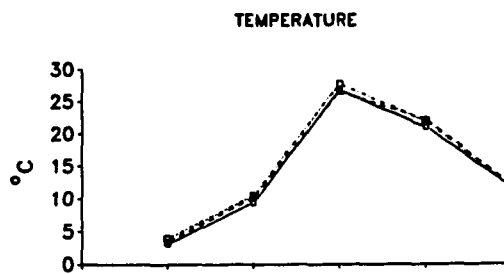
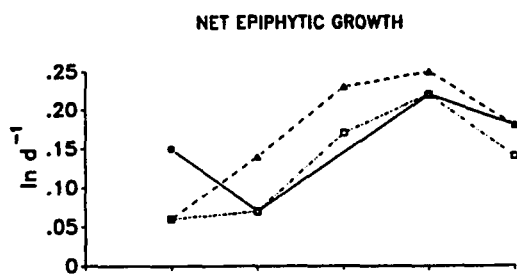
Figure 2. Seasonal patterns of epiphytic biomass at York River study sites. B=significant differences (Bonferroni multiple comparisons, $P<.05$) between 2 means at any date.



parameters showed distinct seasonal and site differences during the study (Fig. 3). The net epiphytic growth rates reflected the differences among sites in epiphytic biomass. Water temperatures were similar among sites and salinity decreased approximately 5‰ from Guinea Marsh to Claybank. Concentrations of inorganic nutrients generally increased with distance upriver, although concentrations of ammonium were highest at Gloucester Point during the September-October period. The TSS included primarily inorganic constituents. Although concentrations of TSS were variable among sites, predicted levels of PAR were consistently lowest at Claybank.

Net epiphytic growth was positively correlated with temperature, salinity, and ammonium during the study (Table 1). However, the addition of ammonium as an explanatory variable to regressions of net growth on either temperature or salinity did not improve the relationship significantly (Table 2). The best predictive model for net growth included temperature and salinity (Table 2). The salinity gradient among sites reflected the decrease in bottom irradiance with distance upriver (correlation with K , PAR_I , and PAR_D , Table 1), and light attenuation in turn reflected the suspended solid load. The low net epiphytic growth at Guinea Marsh during the March-April period did not correspond to the seasonal pattern of any measured environmental variable (Fig. 3), resulting in a low conformity of this observation to the regression models (e.g. standardized residual = 2.36 for the model including

Figure 3. Net specific growth rates of epiphyton (as chlorophyll a) and mean levels of water column characteristics during epiphytic growth periods. Symbols as in Fig. 2: circles=Guinea Marsh, triangles=Gloucester Point, squares=Claybank.



GROWTH PERIOD

Table 1. Correlation matrix of net epiphytic growth (u) with environmental parameters (TEMP=water temperature, SAL=salinity, $\text{NO}_x=\text{NO}_2+\text{NO}_3$, TSS=total suspended solids (DW), K=light attenuation coefficient, PAR_0 =daily PAR, PAR_1 =instantaneous PAR at solar noon). * $P<.10$, ** $P<.05$, *** $P<.01$, $n=14$.

	u	TEMP	SAL	NH_4	NO_x	PO_4	TSS	K	PAR_0
TEMP	0.77***								
SAL	0.77***	0.41							
NH_4	0.55**	0.37	0.39						
NO_x	0.10	-0.21	-0.06	0.45					
PO_4	0.15	0.40	-0.40	0.44	0.22				
TSS	-0.01	0.49	-0.28	-0.31	-0.66**	0.27			
K	-0.14	0.34	-0.52*	-0.37	-0.33	0.40	0.73***		
PAR_0	0.45	0.41	0.61**	0.37	-0.42	-0.09	-0.05	-0.40	
PAR_1	0.40	0.22	0.66**	0.44	-0.28	-0.21	-0.26	-0.64**	0.95***

Table 2. Regression models of net epiphytic growth on environmental parameters. Each row indicates a separate model fitted; entries are coefficients (probabilities from tests of individual coefficients=0) from models including all variables indicated within a row. Models with n=14 fitted to all observations; models with n=13 fitted to matrix eliminating observations from Guinea Marsh, March-April period.

n	Const.	Explanatory Variables					r^2
		TEMP	SAL	NH ₄	PAR _I	PAR _D	
14	.0517 (.057)	.0052 (.005)		.0097 (.133)			.67
14	-.1760 (.060)		.0158 (.006)	.0093 (.156)			.66
14	-.1570 (.021)	.0043 (.002)	.0131 (.002)				.83
13	.0606 (.034)	.0050 (.006)		.0089 (.157)			.67
13	-.1790 (.032)		.0166 (.002)	.0072 (.204)			.74
13	.0283 (.509)			.0073 (.342)		.0174 (.036)	.55
13	-.1600 (.004)	.0039 (.001)	.0139 (.001)				.91
13	.0008 (.980)	.0047 (.002)			.0004 (.017)		.78

temperature and salinity). Removing this observation from the data matrix did not change the relationship among environmental variables, but strengthened the correlation between net growth and irradiance (correlation coefficient of net growth with $PAR_D = .71$ and with $PAR_I = .65$; both $P < .01$). The addition of ammonium to regressions of net growth on temperature, salinity, or PAR_D remained nonsignificant, and the best models for predicting growth included temperature and either salinity or PAR_I as explanatory variables (Table 2).

DISCUSSION

If the accumulation of epiphyton was the dominant factor limiting the distribution of eelgrass in lower Chesapeake Bay, epiphytic biomass would be inversely associated with eelgrass abundance. I did not, however, observe such differences during this study: epiphytic densities on the artificial substrata were generally lowest at Claybank, where eelgrass no longer occurred. Therefore, unlike some other nutrient enriched systems (Sand-Jensen and S ndergaard 1981, Twilley et al. 1985, Silberstein et al. 1986), epiphytic growth in lower Chesapeake Bay does not appear to be the primary factor maintaining patterns of submerged vegetation.

Net epiphytic growth rate in this study was predicted most strongly by water temperature combined with either salinity or submarine irradiance. The dependence of

epiphytic growth on temperature and irradiance is well documented (e.g. Penhale 1977, Borum and Wium-Andersen 1980, Libes 1986). It is unlikely that growth was affected by salinity per se, however. Jacobs and Noten (1980) classified the majority of diatoms in eelgrass epiphyton from the north coast of France as tolerating a much broader range of salinity than was observed in the York River. The annual pattern of salinity along the York River axis was correlated with both predicted seasonal changes in solar irradiance and measured site differences in water column light attenuation (Fig. 3, Table 1). McCauley et al. (1988) found no relationship between epiphytic biomass and salinity in the northern Gulf of Mexico, where seasonal salinity differences of 9‰ were not correlated with irradiance. The relationship between net epiphytic growth and salinity in my study probably reflected the dependence of growth on irradiance.

The highest net epiphytic growth rates at each York River study site occurred when the epiphyton was dominated by algal autotrophs (i.e. the autotrophic index was <400). Atomic ratios of dissolved inorganic N:P throughout the study were generally <16 (Redfield ratio), suggesting that growth of epiphytic algae was nitrogen-limited (cf. Howarth 1988). Other studies have found epiphytic growth and biomass to be dependent upon nutrient availability (e.g. Eminson and Phillips 1978, Borum 1985, Twilley et al. 1985). Although net epiphytic growth was correlated with the concentration of ammonium in my study (Table 1), the addition of ammonium as an explanatory variable did not significantly

improve the predictive relationship between net growth and other physical variables (Table 2). The most parsimonious explanation for this result is that gross growth of epiphytic algae was limited more by temperature and irradiance than by nitrogen supply. Experimental studies of the combined effects of these physical-chemical variables on the biomass accumulation of attached algal communities show varied results. For example, Murray (1983) found epiphytic growth in the York River to be affected more by nutrient enrichment than light reduction; however, the enrichments applied (30 and 70 fold increases in ambient nitrogen concentration) were greater than the differences I observed among study sites (2-3 fold). In contrast, Lowe et al. (1986) found benthic algae to respond to nutrient enrichment only in streams where light limitation had been reduced by clearcutting.

Rates of net epiphytic growth depend upon rates of accrual and loss. During the early stages of development of epiphytic algal populations, increases in biomass are often exponential or linear and losses due to grazing or mechanical detachment are generally low (Sand-Jensen 19983, Borum 1987). As the community becomes more complex, internal and external processes controlling losses become more important and the biomass may remain stable or fluctuate widely. I attempted to reduce the contributions of epiphytic losses to net growth during this study by limiting the duration of epiphytic growth periods. However, assuming that rates of gross growth remained stable over these periods, the decline

in rates of net growth between sampling intervals (Fig. 2) indicated that losses did occur. Therefore, seasonal or site differences in processes controlling losses may have partly controlled the variability among observations of net growth.

Grazing by invertebrates exerts dominant control on the accumulation of epiphytic biomass (e.g. van Montfrans et al. 1982, Howard 1982, Cattaneo 1983). I did not measure grazing rates in this study, but I saw no differences in grazer abundance among sites. Upon collection the artificial plants at all sites harbored amphipods, isopods, and snails, at densities which changed seasonally and reflected local patterns (Marsh 1973). Although grazing undoubtedly contributed to the declining algal growth rate over time (cf. Borum 1987) and may have combined with irradiance to limit effects of nutrient enrichment (cf. Stewart 1987), it is unlikely that differences in grazing pressure among sites were sufficient to cause the observed differences in net growth rates. The processes controlling mechanical detachment, however, may have differed along the York River axis. The relationship between net epiphytic growth and environmental characteristics improved following removal of the observation from Guinea Marsh in March-April (Table 2), indicating that an unmeasured variable influenced biomass accumulation at that site during that period. The early stabilization of epiphytic biomass at Guinea Marsh during March-April suggested that losses contributed strongly to net growth (Fig. 2). Physical disturbance may limit the

development of epiphytic communities (Luttenton and Rada 1986), and even brief periods of strong currents may reduce epiphytic biomass considerably (Kairesalo 1983). Because of the close proximity of Guinea Marsh to the main stem of Chesapeake Bay (Fig. 1), the artificial substrata at that site were the least protected from strong currents associated with periodic storms. Physical disturbance may have contributed to the seasonal variability in net growth rates at Guinea Marsh.

The accumulation of epiphytic biomass is affected by many potentially interacting environmental variables. In areas where seasonal and spatial patterns of physical-chemical factors change simultaneously, it is important that their effects on epiphyton be considered in concert. Irradiance appeared to be more important than nutrient supply in controlling gross epiphytic growth in lower Chesapeake Bay, and both grazing and physical disturbance appeared to contribute variously to epiphytic removal. My results suggested that epiphytic growth was not a primary factor influencing eelgrass distribution in this region. However, the interactive effects of epiphytic biomass and other variables on macrophyte production may be considerable. For example, the relative photosynthetic responses of epiphyton and eelgrass has been shown to result in higher epiphytic densities at reduced irradiance (Murray 1983). Such complex interactions can be best understood through ecosystem-level studies incorporating the responses

by epiphyton and vascular plants to combined environmental variables (e.g. Kemp et al. 1983).

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Chapter 6
CONCLUSIONS

Production and biomass of submerged macrophytes and attached epiphyton are regulated by complex interactions with diverse physical-chemical and biological variables. Dissolved nutrient concentrations and epiphytic grazing previously have been shown to exert strong independent controls on epiphytic biomass. I have shown that at nutrient concentrations typical of Chesapeake Bay eelgrass communities, grazing is the more important factor limiting epiphyton accrual (Chapter 2). Future research addressing epiphytic responses to a range of nutrient concentrations and grazer densities are necessary to test the applicability of these results to other systems. In temperate estuaries such as Chesapeake Bay, the combined effects of nutrient supply and grazing activity on epiphytic abundance appear to vary seasonally (Chapter 2) and among constituents of the community (Chapter 4). Future studies of grazing rates and food selectivity by various macroinvertebrates are necessary to elucidate the patterns of abundance observed in this study. My results show microflagellates to be an important component of the epiphyton, and indicate the need for further research on internal processes controlling epiphytic community structure and function. The effects of the regulatory factors examined experimentally in this study are further influenced by other environmental variables. For example, in estuaries such as the York River where grazing activity and light reduction accompany nutrient enrichment, nutrient supply may little affect the accumulation or biomass of epiphyton (Chapter 5). Thus, experimental

studies of higher order interactions than addressed here will further advance our understanding of in situ processes.

Results of this study suggest that the indirect effects of nutrient enrichment and grazing activity on macrophyte growth and production depend upon the relative magnitude of each independent factor (Chapter 2). For example, whereas production may be depressed under nutrient enriched conditions at low grazer densities, high grazer densities may mediate these effects. Furthermore, macrophyte responses to these environmental variables appear to be dictated by physiological demands which change seasonally. Thus, epiphytic densities resulting from nutrient enrichment or grazer removal appear to be most detrimental to eelgrass production at high temperatures, when macrophyte light requirements are greatest. Simulation model studies show that short-term reductions in macrophyte production do not necessarily indicate long-term impacts on community stability (Chapter 3). Model simulations suggest that nutrient concentration, grazing activity, and water clarity interact strongly to regulate eelgrass survival. Slight changes in the level of one controlling factor (e.g. nutrient enrichment) may simply affect eelgrass standing stocks; however, the same environmental change may provoke loss of the community when combined with other stressors (e.g. loss of grazers or increased turbidity). The effects of multiple environmental changes and their mechanisms of interaction are central to questions concerning the stability of submerged macrophyte communities.

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