Oecologia (1993) 93:285–295

Oecologia

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

Hilary A. Neckles*, Richard L. Wetzel, Robert J. Orth

Virginia Institute of Marine Science, College of William and Mary School of Marine Science, Gloucester Point, VA 23062, USA

Received: 4 December 1991 / Accepted: 23 September 1992

Abstract. The independent and interactive effects of nutrient concentration and epiphyte grazers on epiphyte biomass and macrophyte growth and production were examined in *Zostera marina* L. (eelgrass) 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 (3 × ambient) concentrations of inorganic nitrogen (ammonium nitrate) and phosphate. Grazer treatments consisted of the presence or absence of field densities of isopods, amphipods, and gastropods. Epiphyte biomass increased with both grazer removal and nutrient enrichment during summer and spring experiments. The effect of grazers was stronger than that of nutrients. There was little epiphyte response to treatment during the fall, a result possibly of high ambient nutrient concentrations and low grazing pressure. Under low grazer densities of early summer, macrophyte production (g m^{-2} d^{-1}) was reduced by grazer removal and nutrient enrichment independently. Under high grazer densities of late summer, macrophyte production was reduced by enrichment only with grazers absent. During spring and fall there were no macrophyte responses to treatment. The relative influence of epiphytes on macrophyte production may have been related to seasonally changing water temperature and macrophyte requirements for light and inorganic carbon.

Key words: Epiphytes – Grazing – Nutrient enrichment – Submersed macrophytes – *Zostera marina*

The structure of aquatic communities is a function of multiple interactions occurring within and across trophic boundaries (Kerfoot and Sih 1987; Carpenter 1988). Controlling factors may have direct effects on specific components and indirect repercussions can be transmitted through ecosystem linkages. Accurate predictions regarding the ultimate consequences of environmental changes are, thus, contingent on an understanding of the complex interactions underlying community structure.

The productivity and biomass of submersed macrophytes are regulated by a variety of abiotic and biotic factors. Although the direct effects of individual controls on macrophyte growth and production are well documented (reviewed by Barko et al. 1986; Hillman et al. 1989), the importance of indirect effects in structuring submersed macrophyte communities is poorly understood (Lodge et al. 1988). The combined influences of multiple controlling factors are also comparatively little studied.

Submersed macrophytes provide substrata for an epiphytic matrix of algae, bacteria, fungi, protozoans, and organic and inorganic debris. The epiphytic periphyton, or epiphytes, attenuates light and reduces carbon exchange at leaf surfaces, and may thereby exert strong controls on macrophyte productivity (Sand-Jensen 1977; Sand-Jensen and Borum 1984; Sand-Jensen and Revsbech 1987; Sand-Jensen and Borum 1991). Any variable with direct effects on epiphytes may, therefore, have indirect effects on submersed macrophytes. A primary control of epiphyte biomass accrual is nutrient supply (Eminson and Phillips 1978; Orth and van Montfrans 1984; Twilley et al. 1985). By increasing epiphyte accumulations, elevated nutrient concentrations may reduce submersed macrophyte productivity (Twilley et al. 1985; Silberstein et al. 1986; Sand-Jensen and Borum 1991; Tomasko and Lapointe 1991). Worldwide declines in abundance of submersed macrophytes have been attributed in part to such indirect control by anthropogenic nutrient enrichment (e.g. Phillips et al. 1978; Carpenter 1980; Kemp et al. 1983; Orth and Moore 1983; Cambridge et al. 1986; Hough et al. 1989).

The direct and indirect effects of nutrient supply in submersed macrophyte communities depend on the concomitant influences of other environmental variables.

* *Present address:* U.S. Fish and Wildlife Service, National Wetlands Research Center, 700 Cajundome Blvd, Lafayette, LA 70506, USA

Correspondence to: H.A. Neckles

Little is known, however, about the interactions of nutrients with other potential controlling factors. In particular, grazing by invertebrates is widely recognized as regulating periphyton biomass (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). Epiphyte removal by grazers has been shown to enhance 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). Although recent studies suggest that nutrient supply and grazing interact to control periphyton biomass on abiotic substrata (Stewart 1987; Marks and Lowe 1989; Mazumder et al. 1989; Mulholland et al. 1991), an examination of the simultaneous effects of these factors on epiphytes or macrophytes is lacking. It is unknown, for example, whether grazing activity mitigates the effects of increased nutrient supplies or, conversely, whether nutrient enrichment invariably signals macrophyte decline.

We measured the independent and interactive effects of nutrient concentration and epiphyte grazing on *Zostera marina* L. (eelgrass)-epiphyte associations in lower Chesapeake Bay. Losses of *Z. marina* in the early 1970s in this region were correlated with, among other potential causes, anthropogenic nutrient enrichment (Orth and Moore 1983) and loss of a dominant epiphyte grazer following a severe tropical storm (van Montfrans et al. 1982). We examined the relative importance of these controls on the structure of *Z. marina* communities in experimental microcosms. Specifically, we tested the direct effects of nutrient enrichment and grazer removal on epiphyte biomass accrual, and their indirect effects on macrophyte production. If the effects of nutrients and grazers are independent, then either nutrient enrichment or grazer removal would be predicted to increase epiphyte biomass and reduce macrophyte production consistently. If they are interactive, however, then their combined effects could not be predicted from knowledge of either factor alone.

Methods

Experimental design

We measured the effects of nutrient concentration and grazing activity on *Z. marina* and its epiphytes collected from the York River estuary, Chesapeake Bay (37° 15′ N, 76° 30′ W). Nutrient-grazer treatments were applied to microcosms following a 2 × 2 factorial design in a randomized complete block pattern. Microcosms consisted of 110-l glass aquaria located in a greenhouse and supplied with flow-through seawater. Aquarium dimensions were 60 cm (l) × 30 cm (w) × 60 cm (h). Seawater from the York River was pumped continuously into the greenhouse, through a sand filter and 50-μm bag filters, and into 5 header tanks. Each header tank delivered water to 4 aquaria to maintain a constant volume with a total exchange time of 1.5 h. Aquaria supplied by a single header tank represented an experimental block, so that the experimental design yielded 5 replicates of each treatment combination.

We conducted 4 experiments during 1987 and 1988, timed to reflect the seasonal pattern of *Z. marina* growth in Chesapeake Bay (Wetzel and Penhale 1983, Murray and Wetzel 1987; Table 1). The experiments initiated in June and August 1987 represented the

Table 1. Experimental conditions

	Early Summer	Late Summer	Fall	Spring
Date	8 Jun–9 Jul, 1987	11 Aug–16 Sep, 1987	12 Oct–24 Nov, 1987	7 Apr–8 Jun, 1988
Midday PAR (μE m^{-2} s^{-1})[a]	375	225	175	350
Water temperature (°C)				
Seasonal range[b]	25–29	26–30	19–9	12–24
Average[a,b]	27	28	15	18
DIN (μM) of inflow[a]				
Ambient treatment	4.2	4.0	10.8	4.0
Enriched treatment	16.4	10.6	37.8	10.8
Phosphate (μM) of inflow[a]				
Ambient treatment	1.0	1.6	0.7	0.8
Enriched treatment	2.3	3.4	3.3	1.8
Invertebrates applied to grazer treatment (# m^{-2} pot)				
Total	4800	11400	3900	900
Gastropoda				
Bittium varium	4000	3600	0	0
Mitrella lunata	0	0	1500	0
Isopoda				
Idotea baltica	800	100	100	300
Erichsonella attenuata	0	6000	1000	0
Amphipoda[c]	0	1700	1300	600

[a] Data are averages over experimental periods
[b] Diurnal temperature range was 5° C
[c] Primarily *Gammarus* sp. and *Ampithoe* sp

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 1–2 months. Experiments were terminated when average daily water temperatures reached predetermined endpoints for seasonal periods of *Z. marina* growth (Batiuk et al. 1992), or when treatment-induced mortality left an experimental treatment with few plants.

Conditions were established within the spatial constraints of the microcosms to simulate the natural environment of *Z. marina*. Salinity varied seasonally with that of the estuary (19–23‰). The water in the microcosms was aerated continuously. To avoid limitation of photosynthesis by development of a thick diffusion boundary layer at leaf surfaces, water was circulated during daylight hours with submersed pumps. Current velocities in the microcosms (2–9 cm s^{-1}) were within the wide range reported for *Z. marina* communities (e.g. <1 cm s^{-1} reported by Harlin and Thorne-Miller 1981, 110 cm s^{-1} reported by Fonseca et al. 1983). The microcosms were illuminated only with sunlight passing through the greenhouse. Periphyton growing on the microcosm walls was removed regularly. Because preliminary measurements indicated little difference in submarine irradiance or water temperature among microcosms, we monitored photosynthetically active radiation (PAR, 400–700 nm, semiweekly measurements at midday) and water temperatures (daily maximum and minimum) at mid water depth within a single microcosm only. Seasonal irradiances and temperatures in the microcosms (Table 1) were typical of *Z. marina* beds in Chesapeake Bay (Murray and Wetzel 1987). Daily average microcosm temperatures were within 1° C of ambient bay temperatures.

The similarity of environmental conditions among microcosms was supported by data for water column chlorophyll content. We measured concentrations of suspended chlorophyll *a* periodically from all aquaria within three randomly selected experimental blocks. Determinations were made fluorometrically on DMSO-acetone extracts (Shoaf and Lium 1976). Concentrations did not differ significantly among treatment combinations and approximated those found in the natural *Z. marina* communities (5–20 μg l^{-1}; Batiuk et al. 1992).

We collected plants and sediments for each experiment from local *Z. marina* beds. Experimental material was standardized by selecting only shoots with at least 4 leaves and by cutting the rhizomes distal to the fifth internode. Sediments were primarily fine sands of low organic content (ca. 1–2%). Shoots were planted in homogenized sediments in plastic pots (11.4 cm diameter) at reported average annual field densities for Chesapeake Bay (1500 m^{-2}; Orth and Moore 1986). The potted plants were acclimated in a large, common tank for 2 weeks prior to each experiment.

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 surface sheen occurred in the microcosms during this period, evidence indicated that impacts to experimental comparisons were minimal: all microcosms were similarly disturbed, grazers remained active, daily temperature extremes were within the range of seasonal measurements, and epiphytic biota appeared unaffected under observation with epifluorescence microscopy. The water lines were washed with detergent and flushed thoroughly before resuming delivery.

Treatment application

Nutrient treatments were applied at ambient or enriched concentrations. Ambient concentrations were characteristic of sites in the York River currently vegetated with *Z. marina*. Continuous enrichments were made with ammonium nitrate and disodium phosphate combined to increase the ambient concentrations of dissolved inorganic nitrogen (DIN) and phosphate threefold. These levels were characteristic of formerly vegetated sites in the York River, thereby representing nutrient concentrations that have been correlated with regional *Z. marina* declines (Batiuk et al. 1992). Peristaltic pumps metered nutrients directly to the inflow from concentrated stocks. We measured concentrations of DIN (as the sum of nitrate, nitrite, and ammonium) and phosphate (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 treatment levels. Average ambient nutrient concentrations of the seawater inflow were similar throughout all experiments except for fall, when DIN reached higher concentrations (Table 1). Fall peaks of DIN are typical of the region (Batiuk et al. 1992). Due to lags between variation in ambient nutrient levels during experiments and adjustment of nutrient additions, average enrichments ranged from twofold to fourfold (Table 1). Nutrient uptake resulted in reductions of DIN and phosphate of 20–45% within the microcosms.

Grazer treatments were designated as either present or absent. We determined seasonal, shoot-specific densities of epifaunal invertebrates by collecting and quantifying 6 samples from *Z. marina* habitat in Chesapeake Bay at the beginning of each experiment. Treatments with grazers present included epifauna collected from a natural eelgrass bed and applied at field densities (Table 1). Seasonal changes in treatment densities were characteristic of epifauna communities associated with *Z. marina* in Chesapeake Bay (cf. Marsh 1973). Densities were kept constant by flushing aquaria with fresh water for 5–10 min approximately biweekly to remove the grazer population (cf. Duffy 1990), which may have been augmented by new recruits, and then restocking with treatment densities. The regular removal of periphyton from aquaria surfaces ensured that epifauna in the microcosms were distributed primarily on the plants.

Determination of epiphyte and macrophyte responses

At the beginning of an experiment each microcosm contained 6 randomly assigned pots. We measured epiphyte biomass from one randomly selected pot per microcosm on approximately biweekly sampling dates. *Zostera marina* grows basally by the sequential formation of individual leaves, resulting in a series of leaves of increasing age outward within a shoot. Shoots generally consist of 4–6 leaves. Samples for epiphyte determinations consisted of 4–10 leaves of the same relative age. All leaf age classes present within a pot were sampled separately. Epiphytes were 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). Microscopic leaf examination showed that this scraping technique was not sufficiently abrasive to scrape cells from the macrophyte tissue. Epiphyte dry weight (DW) was determined after drying at 60° C (2–5 d), and ash-free dry weight (AFDW) was determined after combusting at 500° C (5 h). All measurements were normalized to macrophyte leaf area and mass. Leaf area was estimated using an area meter (Licor Model 3100), and leaf mass (DW and AFDW) was determined as described for epiphyte samples. Patterns of accrual of epiphyte DW and AFDW were nearly identical ($R > 0.98$ between DW and AFDW within each experiment), as were responses normalized to leaf area and mass. Therefore, statistical analyses were confined to area-specific measurements of epiphyte AFDW.

The effect of epiphytes on macrophyte photosynthesis may depend on the spectral selectivity of the epiphytic material (cf. Losee and Wetzel 1983, Mazzella and Alberte 1986). Therefore, we estimated epiphyte attenuation of light at 9 discrete, evenly spaced 10-nm bands across the range of PAR from subsamples of leaves during late summer ($n = 23$), fall ($n = 73$), and spring ($n = 137$) experiments. We used a spectroradiometer (Biospherical MER–1000) to measure the proportion of light from an artificial source (combined fluorescent and tungsten floodlight bulbs) passing through epiphyte suspensions, following the technique of Sand-Jensen and Søndergaard (1981).

We measured macrophyte growth in one randomly selected pot per microcosm during successive 2-week sampling intervals using a modification of 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 epiphyte samples ($R^2 > 0.97$) were used to calculate leaf biomass. Leaf growth was calculated as new leaf biomass, or the biomass produced during a measurement interval, divided by initial number of shoots. Specific growth was calculated as new leaf biomass divided by initial leaf biomass, and areal leaf production was calculated as new leaf biomass divided by pot area. Leaf formation was calculated as number of leaves produced during a measurement interval divided by initial number of shoots, and shoot formation as number of shoots produced divided by pot area. All macrophyte growth and production parameters were divided by the measurement interval to yield daily rates.

Statistical analysis

Responses within each experiment were assessed using repeated-measures analysis of variance, with microcosms as the subjects of repeated sampling (Winer 1971). The analysis incorporated between-subjects main effects of nutrients and grazing, and within-subjects main effects of time. As recommended by Potvin et al. (1990), Mauchly's criterion was used to test whether the probabilities from univariate F tests were correct (i.e., whether the covariance structure of repeated measures indicated equal correlations among pairs of observations on the same microcosm). If this test indicated mild violation of assumptions of univariate analysis, then conclusions were based on adjusted significance levels. In no case did the preceding tests reveal the form of the covariance matrix to require multivariate analysis (cf. Potvin et al. 1990). Residual analysis was used to diagnose departures from basic assumptions of analysis of variance, and log transformations were applied as necessary to correct for nonnormality and nonconstancy of error variance (Neter et al. 1990). Factor level means were compared *a posteriori* using Bonferroni multiple comparisons (Neter et al. 1990). Analyses were performed using the GLM procedure of the Statistical Analysis System (SAS) for personal computers (SAS 1987).

Results

Epiphyte responses

Observations under epifluorescence microscopy revealed the epiphytes to consist primarily of diatoms, cyanobacteria (blue-green algae), heterotrophic protozoans and bacteria, and inorganic and organic debris (Neckles 1990). Epiphyte AFDW formed from 15% to 40% of epiphyte DW. Within a pot, epiphyte biomass increased on average 15-fold from youngest to oldest leaves. Although the magnitude of epiphyte response to individual treatment combinations frequently increased with leaf age, the direction of response was consistent across leaf age classes. Results here are thus represented by responses on intermediate aged leaves only (middle position in leaf sequence).

Epiphyte responses were similar between the early and late summer experiments. Biomass increased significantly with nutrient enrichment and grazer removal (Fig. 1, Table 2) and the effects of treatments were additive (i.e., responses to one factor were proportionally similar across levels of the other; Table 2). During both summer experiments, the average percent increase in AFDW was greater with grazer removal than with nutrient enrichment, but the difference in treatment effects was most pronounced during late summer (early summer: 58% biomass increase with nutrient enrichment, 106% with grazer removal; late summer: 25% with nutrient enrichment, 592% with grazer removal). The effect of grazing increased significantly over time during late summer (Fig. 1, Table 2). By the middle of both summer experiments, plants in the enriched, ungrazed aquaria were covered with unattached algal filaments (primarily tube-dwelling diatoms). Also, many leaves supported dense tunicate populations. No other treatment combinations were thus affected.

In contrast to the summer experiments, grazing had no significant effect on epiphyte AFDW during the fall

Table 2. Analysis of variance of epiphyte responses on intermediate-aged leaves (middle position in leaf sequence) with repeated measurements on microcosms. Between-subjects sources of variation are Nutrients (N), Grazing (G), and interactions, and within-subjects sources are Time (T) and interactions. Block effects were nonsignificant ($P > 0.10$) and are not shown. Probabilities are those provided by univariate F tests (see text) from analyses of log (X) transformed data; entries of 0 represent $P < 0.001$

Source	Early summer			Late summer			Fall			Spring		
	DF	F	*P*	DF	F	*P*	DF	F	*P*	DF	F	*P*
Between subjects												
N	1	8.47	0.013	1	6.95	0.030	1	4.55	0.054	1	0.14	0.714
G	1	20.87	0.000	1	128.43	0.000	1	2.27	0.158	1	0.19	0.670
NG	1	0.08	0.784	1	0.84	0.385	1	0.43	0.525	1	2.73	0.130
Error	12			8			12			10		
Within subjects												
T	1	146.74	0.000	2	0.78	0.474	2	1.29	0.293	2	15.08	0.000
TN	1	3.10	0.104	2	0.28	0.762	2	1.97	0.161	2	6.11	0.009
TG	1	0.14	0.718	2	15.63	0.000	2	1.02	0.377	2	1.53	0.242
TNG	1	0.04	0.849	2	1.19	0.330	2	0.57	0.572	2	4.43	0.026
Error	12			16			24			20		

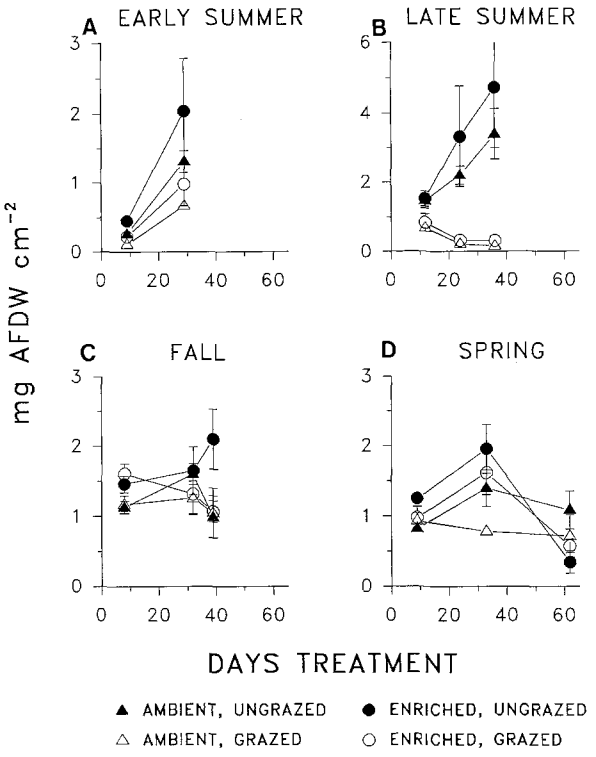

Fig. 1A–D. Epiphyte responses ($\bar{X} \pm SE$, intermediate-aged leaves) to microcosm treatments. Dates of experiments are listed in Table 1

(Fig. 1, Table 2). The effect of nutrients was marginally significant, and was independent of grazer treatment and consistent over time (Table 2). The average AFDW increase with enrichment was 28%.

During the spring experiment, the interaction of nutrient and grazer treatments over time rendered their average effects nonsignificant (Table 2), such that conclusions were based on comparisons of treatment means at each date. By the second sample date of spring, epiphyte biomass increased with grazer removal under both nutrient treatments ($P < 0.05$, Fig. 1) and increased with nutrient enrichment under both grazer treatments ($P < 0.05$, Fig. 1). At that time, the average percent biomass increase with enrichment (72%) was greater than with grazer removal (40%). By the end of the experiment, however, a decline in epiphyte biomass eliminated effects of enrichment under grazed conditions ($P > 0.10$, Fig. 1). The effect of grazer removal persisted to the end of the experiment in microcosms at ambient nutrient concentrations (Fig. 1). During the second half of the experiment, dense growth of the macroalga *Enteromorpha* sp. replaced epiphyte biomass in enriched microcosms with grazers absent. The consequent low epiphyte biomass under these conditions at the end of the experiment (Fig. 1) resulted in nonsignificant grazer effects under enriched conditions and a decrease in biomass with enrichment under nongrazed conditions.

Light was attenuated by the epiphyte matrix following a negative exponential function at all wavebands tested. Epiphyte light attenuation was similar among experiments. Light at low wavelengths was attenuated most rapidly: mean attenuation coefficients among experiments (cm^2 mg DW^{-1}, calculated as the negative exponential decay coefficient for light passing through an epiphyte suspension) declined from 0.48 at 410 nm to 0.25 at 694 nm.

Macrophyte responses

Macrophyte responses to treatment also varied seasonally. During the early summer experiment, the effects of grazer abundance and nutrient enrichment on all measured parameters were additive (NG and TNG terms, Table 3), indicating independent treatment effects. By the final sample date, leaf growth rate (Fig. 2A), shoot formation rate (Fig. 2B), and shoot density (Fig. 2C) decreased significantly in the absence of grazers ($P < 0.05$). Grazer removal also decreased the mean specific growth rate at the end of the experiment from 17.0 to 11.0 mg g^{-1} d^{-1} ($P < 0.01$). At the same time, mean leaf formation rate decreased from 0.08 to 0.06 leaves shoot^{-1} d^{-1} under nutrient-enriched conditions ($P < 0.01$). There were no other significant effects of enrichment on macrophyte growth (Fig. 2A, B; Table 3). However, average shoot densities decreased significantly with enrichment (Table 3) by 10%, and average number of leaves per shoot (i.e. leaf density) decreased by 11% ($P < 0.05$), indicating that enrichment increased shoot mortality and leaf loss. Consequently, although nutrient enrichment did not affect shoot biomass accumulation, it affected areal biomass production (Fig. 2D). By the last sampling period of the early summer experiment, areal leaf production decreased independently with grazer removal ($P < 0.01$) and nutrient enrichment ($P < 0.05$; Fig. 2D).

Rates of macrophyte growth and production were lowest during late summer. By the last sample date, mean specific growth rate decreased from 25.6 to 12.8 mg g^{-1} d^{-1} ($P < 0.01$) in the absence of grazers and from 21.6 to 16.8 mg g^{-1} d^{-1} ($P < 0.05$) with enrichment. Effects of nutrient and grazer treatments on other measured parameters varied not only with time (TN and TG terms, Table 3) but also interacted significantly over time (TNG terms, Table 3). Thus, concurrent information on levels of nutrient and grazer treatments was required to predict macrophyte responses during late summer. Although no new shoots were produced (Fig. 3B), final shoot densities differed among treatment combinations, indicating variable effects of treatment on shoot mortality. At the end of the experiment, grazer removal resulted in reduced leaf growth rate (Fig. 3A), shoot density (Fig. 3C), and areal leaf production (Fig. 3D) under both nutrient regimes ($P < 0.05$), but the magnitude of reduction was greater under enriched conditions, while enrichment reduced these macrophyte responses only with grazers absent.

In contrast to the summer experiments, macrophytes showed no significant responses to nutrient or grazer treatments during the fall (Fig. 4) and spring (Fig. 5) experiments (Table 3). Although rates of areal leaf production were similar between experiments (1.3–2.0 g m^{-2} d^{-1}, Figs. 4D, 5D), there were distinct seasonal differences in patterns of population growth. Production in the

Table 3. Analysis of variance of macrophyte responses with repeated measurements on microcosms. Between-subjects sources of variation are Nutrients (N), Grazing (G), and interactions, and within-subjects sources are Time (T) and interactions. Block effects were nonsignificant ($P > 0.20$) and are not shown. Probabilities are those provided by univariate F tests unless otherwise indicated (see text); entries of 0 represent $P < 0.001$

Source	DF	Leaf growth (mg shoot^{-1} d^{-1})		Shoot formation (shoots m^{-2} d^{-1})		Shoot density (shoots m^{-2})		Areal leaf production (g m^{-2} d^{-1})	
		F	*P*	F	*P*	F	*P*	F	*P*
Early summer									
Between subjects									
N	1	0.01	0.918[a]	2.18	0.166[b]	5.77	0.033	3.60	0.082[a]
G	1	12.00	0.005	8.39	0.013	6.35	0.027	9.89	0.009
NG	1	0.12	0.736	0.04	0.845	0.39	0.545	0.32	0.582
Error	12								
Within subjects									
T	2	41.63	0.000	1.19	0.321	0.03	0.974	29.26	0.000
TN	2	1.94	0.165	1.09	0.352	1.33	0.283	3.36	0.051
TG	2	3.98	0.032	4.41	0.023	4.73	0.018	4.44	0.023
TNG	2	0.15	0.861	0.49	0.618	1.01	0.378	0.50	0.615
Error	24								
Late summer									
Between subjects									
N	1	3.32	0.094[a]	–		0.72	0.412[a]	3.64	0.081[a]
G	1	45.31	0.000	–		25.57	0.000	48.86	0.000
NG	1	1.12	0.311	–		0.32	0.583	1.04	0.328
Error	12								
Within subjects									
T	1	3.29	0.095	–		66.73	0.000	4.46	0.056
TN	1	7.25	0.020	–		8.68	0.012	9.65	0.009
TG	1	22.61	0.000	–		55.48	0.000	32.79	0.000
TNG	1	5.15	0.043	–		14.06	0.003	7.98	0.015
Error	12								
Fall									
Between subjects									
N	1	0.13	0.723[a]	2.65	0.130	0.05	0.824	0.00	0.970[a]
G	1	0.00	0.984	0.00	0.978	1.65	0.223	1.58	0.232
NG	1	1.77	0.208	0.08	0.777	0.09	0.767	1.78	0.207
Error	12								
Within subjects									
T	1	42.34	0.000	5.21	0.041	0.60	0.455	36.08	0.000
TN	1	2.45	0.143	0.90	0.363	0.00	0.950	1.64	0.224
TG	1	0.17	0.684	1.14	0.307	0.10	0.753	0.04	0.855
TNG	1	0.21	0.659	1.44	0.254	0.60	0.455	0.08	0.789
Error	12								
Spring									
Between subjects									
N	1	1.28	0.280[a]	2.40	0.147	2.82	0.119	1.03	0.330[a]
G	1	2.48	0.141	1.46	0.251	1.89	0.194	0.36	0.561
NG	1	0.03	0.858	0.98	0.342	0.02	0.881	0.12	0.734
Error	12								
Within subjects									
T	2	7.88	0.002	1.80	0.187*	14.99	0.000	3.42	0.049
TN	2	0.17	0.843	0.84	0.442	1.79	0.189	0.15	0.862
TG	2	1.41	0.263	0.25	0.780	1.39	0.269	0.80	0.461
TNG	2	1.23	0.309	0.05	0.950	0.57	0.573	1.03	0.371
Error	24								

[a] Analysis of log (X) transformed data

[b] Analysis of log (X+1) transformed data

* Huynh–Feldt adjustment used to correct *P*-values of within-subjects effects for unequal correlations among pairs of repeated measures (Potvin et al. 1990)

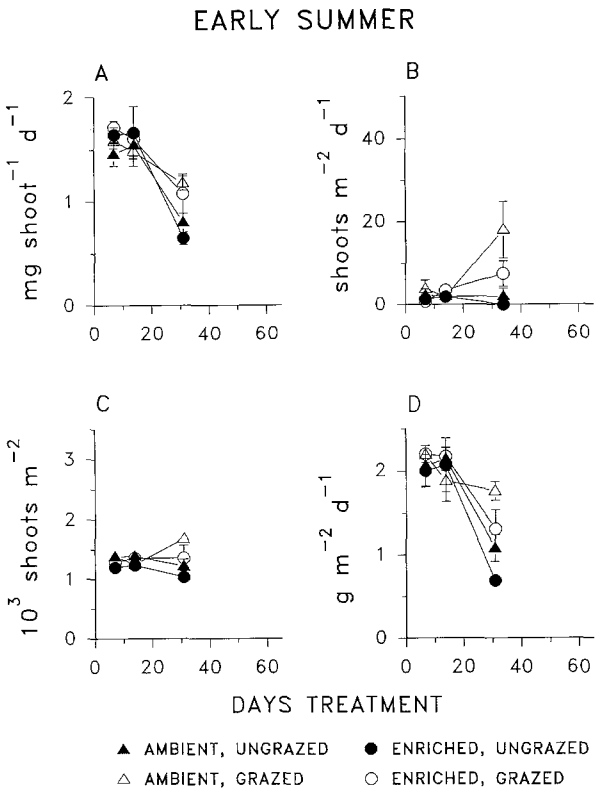

Fig. 2A–D. Macrophyte responses ($\bar{X} \pm SE$) to microcosm treatments during early summer (8 June–9 July). **A.** Leaf growth rate; **B.** Shoot formation rate; **C.** Shoot density; **D.** Areal leaf production rate

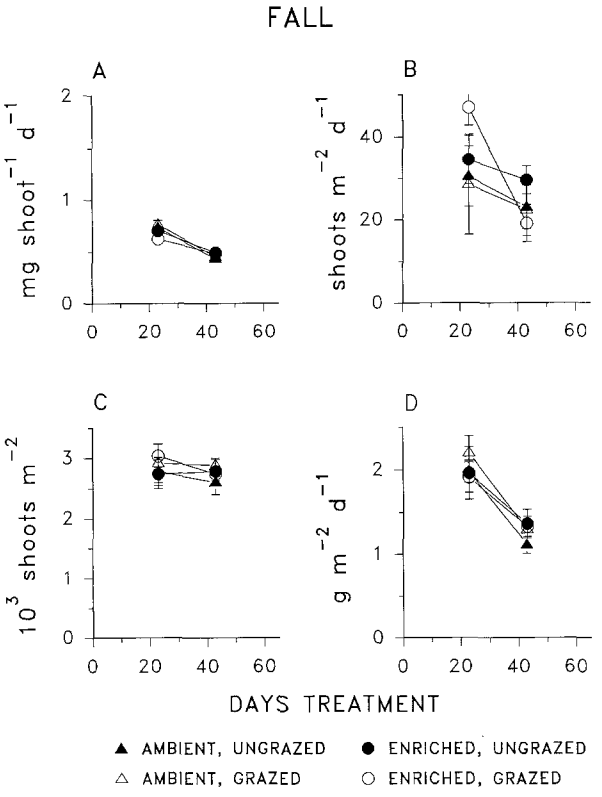

Fig. 4A–D. Macrophyte responses ($\bar{X} \pm SE$) to microcosm treatments during fall (12 October–24 November). Panel titles as in Fig. 2

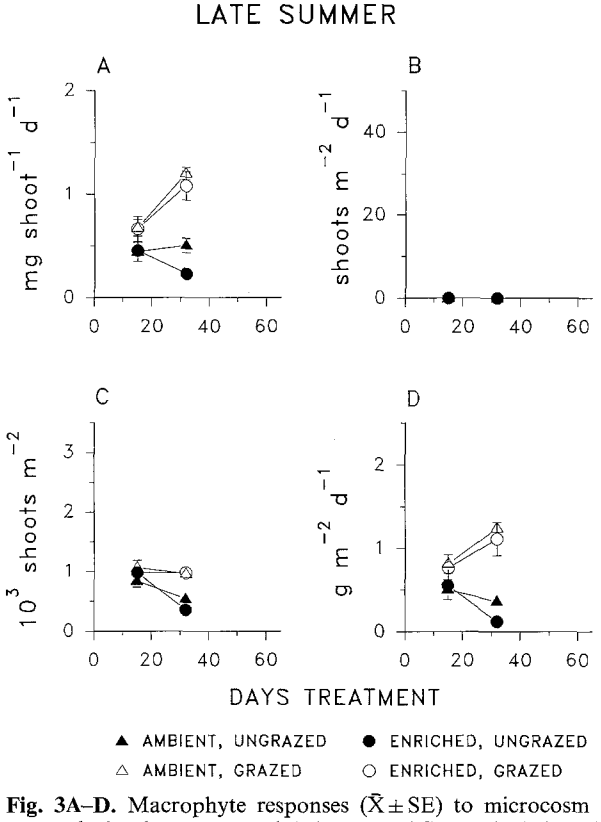

Fig. 3A–D. Macrophyte responses ($\bar{X} \pm SE$) to microcosm treatments during late summer (11 August–16 September). Panel titles as in Fig. 2

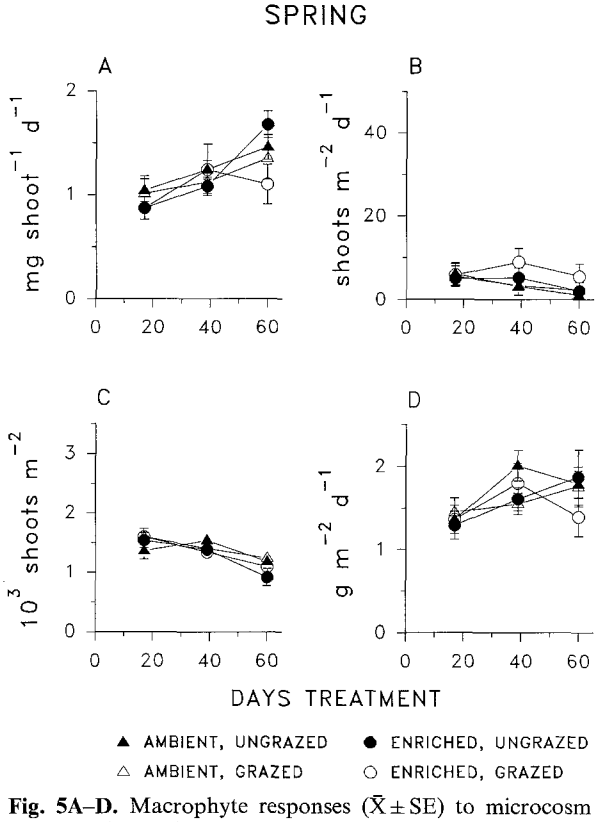

Fig. 5A–D. Macrophyte responses ($\bar{X} \pm SE$) to microcosm treatments during spring (7 April–8 June). Panel titles as in Fig. 2

fall depended more on new shoot formation (Fig. 4B) than leaf growth (Fig. 4A), whereas in the spring the pattern was reversed (Figs. 5A, 5B).

Discussion

Previous studies have shown that nutrients and grazing exert strong individual controls on accrual of epiphytes and production of submersed macrophytes. Our experiments indicate that the combined direct and indirect effects of these factors in Chesapeake Bay can change seasonally. Only during early summer were predictions based on the independent effects of nutrients and grazers confirmed experimentally; i.e. both enrichment and grazer removal increased epiphyte biomass and decreased macrophyte production consistently. During late summer, although nutrient concentration and grazing activity controlled epiphytes independently, they interacted to influence macrophyte production: enrichment reduced production only with grazers absent, and grazer removal reduced production of enriched treatments more than ambient treatments. Macrophytes did not respond to treatment during fall or spring, regardless of intermediate responses by epiphytes. Stewart (1987) advised that nutrient and grazing factors must be examined simultaneously to accurately interpret the dynamics of stream periphyton communities. Results of our experiments extend this caution to the interpretation of estuarine epiphyte dynamics. Our results suggest that indirect effects of these factors depend, further, on macrophyte characteristics, and that ecosystem-level consequences of nutrient concentration and grazing pressure cannot necessarily be predicted from single-season experiments.

Epiphyte biomass levels in microcosms with grazers present were within ranges reported for natural *Z. marina* beds in Chesapeake Bay (Batiuk et al. 1992) and for other marine macrophytes worldwide (e.g. Borum and Wium-Andersen 1980; Bulthuis and Woelkerling 1983; Heijs 1984; Borum et al. 1984). Our measurements of macrophyte growth rates also agree with reports for *Z. marina* in Chesapeake Bay (Batiuk et al. 1992) and elsewhere (Dennison and Alberte 1982; Kentula and McIntire 1986). The depressed production during high summer temperatures has been documented in the field (Penhale 1977; Wetzel and Penhale 1983; Thayer et al. 1984; Murray and Wetzel 1987). The general similarity between these experimental measurements and various field measurements indicates that the microcosm results can be used to elucidate the dynamics of natural systems (cf. Giesy and Odum 1980).

Epiphyte biomass increased with nutrient enrichment during all experiments. As epiphyte AFDW in this region is strongly correlated with concentration of chlorophyll *a,* (Neckles 1990), the increase in biomass with enrichment presumably represented enhanced growth of algal epiphytes. This is consistent with widespread evidence of periphyton accrual as nutrient-limited (Eminson and Phillips 1978; Fairchild et al. 1985; Twilley et al. 1985; Pringle 1987; Fairchild and Everett 1988; Carrick and Lowe 1989; Hough et al. 1989; Hart and Robinson 1990). However, enrichment did not increase epiphyte growth rates sufficiently to overcome grazing pressure; i.e., by the end of each experiment, average biomass of the ambient, ungrazed treatments was still greater than or equal to that of the enriched, grazed treatments (Fig. 1). Growth of filamentous algae as observed in the enriched, ungrazed microcosms is a common response to eutrophication (Harlin and Thorne-Miller 1981; Cattaneo 1987). Grazing prevented accumulation of filamentous algae in our microcosms, even under enriched conditions.

Studies of the relative importance of grazing and nutrients in controlling biomass accrual show conflicting results, probably because of the potential complexity of interactions between these two factors. For example, Stewart (1987) and Mulholland et al. (1991) found the effect of grazing to be stronger than that of nutrient supply in determining biomass of stream periphyton, whereas Marks and Lowe (1989) found the reverse. Effects of grazing on periphyton biomass depend simultaneously on grazer characteristics such as density (Cuker 1983; Colletti et al. 1987; Lowe and Hunter 1988; Power 1990), size (Cattaneo and Kalff 1986), species and associated feeding behavior (Hill and Knight 1988; Lamberti et al. 1987; Steinman et al. 1987; DeNicola et al. 1990; Duffy 1990), and ingestion rates (Jacoby 1987). Effects of nutrients depend similarly on various factors, including external nutrient supply rate, degree of internal nutrient cycling (Mulholland et al. 1991), and levels of other environmental controls such as light, temperature, and current (Sand-Jensen 1983). During our early summer, later summer, and spring experiments, grazer removal increased epiphyte biomass to a greater extent than did nutrient enrichment. At the level of enrichment found in the York River estuary of Chesapeake Bay, the presence of grazers thus appears to be more important than nutrient concentration in determining epiphyte biomass on *Z. marina* leaves. However, our results cannot be attributed unequivocally to treatments. In an attempt to maximize realism by seasonally varying both nutrient concentration and grazer populations, our experimental approach sacrificed the ability to test specific mechanisms. The strong effect of grazing during late summer was correlated with high grazer densities, high water temperatures (thus presumed high grazer metabolic and ingestion rates), and relatively low nutrient concentrations (Table 1). The contrasting lack of effects in fall was correlated with moderate grazer densities, low water temperatures, high nutrient concentrations, and a switch in taxon of the dominant gastropod grazer (Table 1). Systematic variation of nutrient concentration and grazer density is needed to help clarify causal relationships.

Treatment-induced reductions in macrophyte areal production were related to decreased leaf growth and increased leaf mortality. Such indirect effects of nutrients and grazing could be explained by epiphyte-mediated reductions in light and carbon availability for macrophyte photosynthesis. However, seasonal changes in effects of treatment on macrophyte production did not appear solely related to amounts of light and carbon reaching leaf surfaces. The relative influence of epiphytes

on the macrophyte light environment varies with incident solar irradiance, water transparency, epiphyte density, and epiphyte spectral selectivity (Losee and Wetzel 1983; Sand-Jensen and Borum 1984). First, seasonal differences in macrophyte responses in this study did not correspond to incident PAR. For example, production of ungrazed treatments by the end of early summer was depressed, despite levels of PAR as high as those in spring (Table 1). Second, the microcosms received water from the same source, and there were no differences in suspended chlorophyll *a* concentrations among treatments. Therefore, presumably there were also no differences in water-column light attenuation. Third, the differences in macrophyte responses among treatments did not always correspond to patterns of epiphyte densities; i.e. only by the end of the summer experiments was epiphyte biomass inversely correlated with macrophyte production (Figs. 1, 2D, 3D). Finally, epiphyte attenuation of light throughout the photosynthetically active spectral range was consistent among seasons. Although the relative influence of epiphytes on the amount of inorganic carbon diffusing to leaf surfaces may vary with dissolved carbon concentration (Sand-Jensen 1977) and water current (Sand-Jensen and Borum 1991), there were no differences in water source or motion among treatments that would suggest differences in ambient carbon availability.

Macrophyte responses in this study might be explained in part by seasonal variability in photosynthetic requirements. Reduced productivity of *Z. marina* at high temperatures (e.g. $\geq 30°$ C) has been attributed to rapid increases in respiration with temperature (Marsh et al. 1986) and lethal effects of high temperature on carbohydrate metabolism with consequent leaf loss (Zimmerman et al. 1989). Either mechanism would cause an increase in whole-plant requirements for light and inorganic carbon at extreme summer temperatures. At the high temperatures of the two summer experiments, macrophyte photosynthesis could have been particularly sensitive to epiphyte limitation. By the end of each summer experiment, macrophyte production was lowest in both nutrient regimes with grazers absent (Figs. 2D, 3D); these treatments supported the highest epiphyte biomass (Fig. 1). At the lower grazer density of early summer (Table 1), epiphytes on the grazed treatments showed an average (across leaf age class) increase of 17% with nutrient enrichment, while macrophyte production decreased 25% (Fig. 2D). During late summer, high grazer densities reduced the epiphytes of both nutrient treatments to low levels (Fig. 1), and macrophyte production was correspondingly high. The contrasting lack of macrophyte response to treatment during the fall and spring experiments suggests that factors affected by epiphytes were not limiting to macrophyte production, and may be related to the comparatively low light and inorganic carbon requirements of *Z. marina* at low temperatures.

This study indicates that production of *Z. marina* can be partly controlled by indirect effects of both dissolved nutrient concentrations and epiphyte grazers. The combined influence of these factors depends on their relative magnitudes and macrophyte characteristics. Summertime macrophyte production in Chesapeake Bay may be depressed by nutrient enrichment at low grazer densities, whereas high grazer densities may mitigate these effects. The seasonal differences in response measured here preclude generalizations regarding the relative importance of nutrients and grazing on macrophyte survival. The roles of such complex interactions remain central to questions addressing the long-term stability of submersed macrophyte communities.

Acknowledgements. We thank B. Berry, G. Lapointe, and K. Moore for assisting all aspects of this study. S. Leyland, S. Loonam, N. Morse, S. Knur, and S. Robertson provided greenhouse assistance. This manuscript was improved by the comments of L. Haas, M. Harlin, W. Hobbs, C. Onuf, P. Penhale, and J. van Montfrans. The comments of three anonymous reviewers were also very helpful. Support for portions of this study was received from Allied-Signal Inc., the Virginia General Assembly through the Chesapeake Bay Initiatives, the Houston Underwater Club, and the U.S. Fish and Wildlife Service. This is contribution number 1775 from the Virginia Institute of Marine Science.

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