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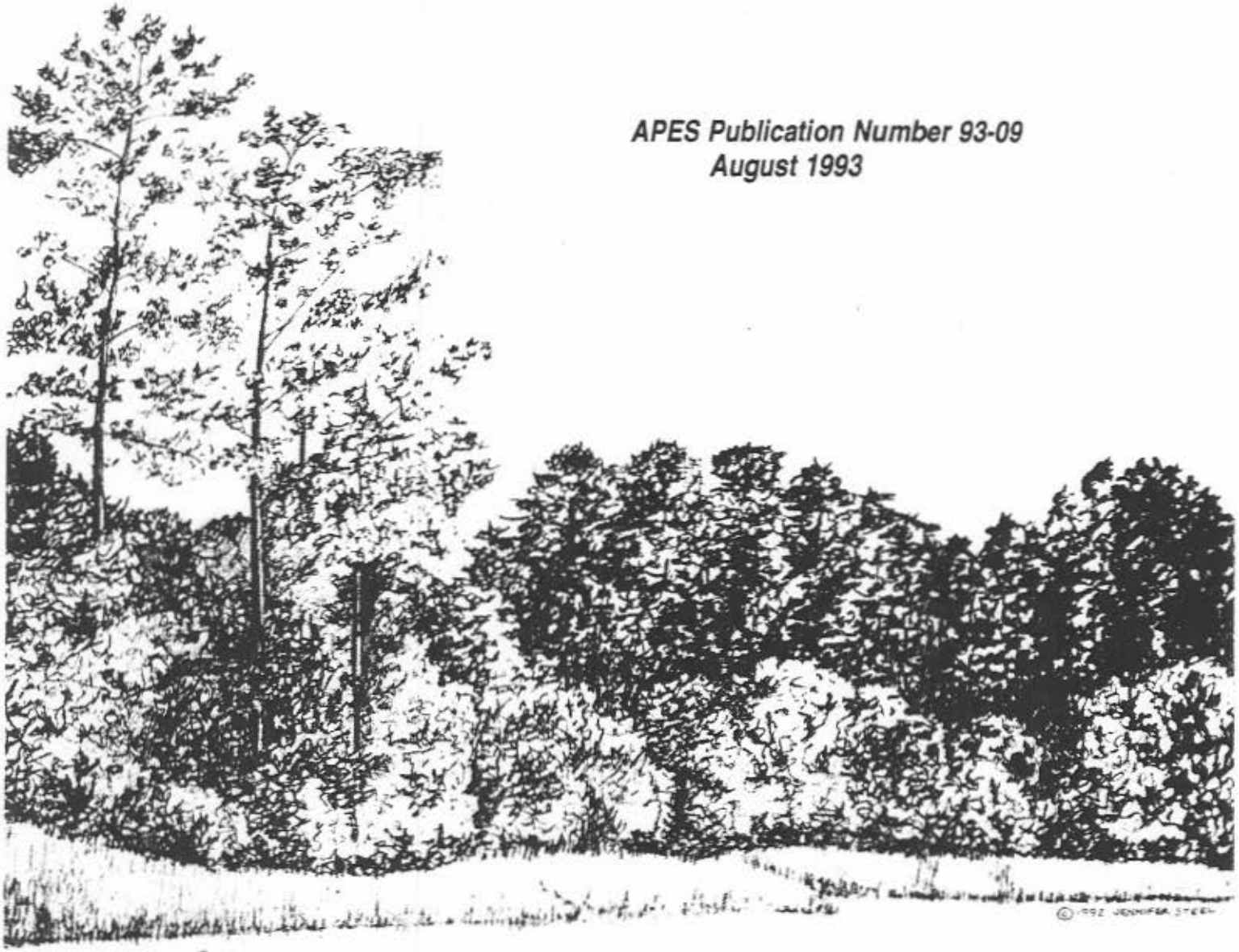

COMPARATIVE EFFECTS OF WATER-COLUMN NITRATE ENRICHMENT ON EELGRASS, SHOAL GRASS AND WIDGEON GRASS

ALBEMARLE-PAMLICO ESTUARINE STUDY

NC Department of Environment, Health, and Natural Resources

Environmental Protection Agency National Estuary Program

COMPARATIVE EFFECTS OF WATER-COLUMN NITRATE ENRICHMENT ON EELGRASS, SHOAL GRASS AND WIDGEON GRASS

by

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To continue setup, Howard Glasgow completely restructured the electrical system after digging about 50 m of trenches to uncover inadequately insulated wiring. With hours of volunteer consultation and hands-on assistance from Tommy Williams, Howard then managed to set up an available but inadequate chiller system so that it sufficiently cooled the mesocosms to track bay-water temperatures throughout the spring experiment. In early

summer I received additional funding that enabled us, again with help from Tommy Williams, to replace the chiller system with more powerful, dependable units prior to initiating the late summer - fall experiments. Howard was assisted in these tasks by Paul Bates, Therman Brister, Jeff Compton, Jake Cooke, Doug Lewis, Fagan Johnson, and Jack Russell. In completing the setup effort, Mark Fonseca is gratefully acknowledged for his assistance in *Zostera* collection efforts prior to the spring experiment, and in the transplanting prior to mid-summer. Therman Brister, Ginny Coleman, Jeff Compton, Jake Cooke, Howard Glasgow, Sister Gretchen, Mindy McAuley, Gene Morgan, Leslie Taggett, and Debbie Tan helped pull plants and redistribute them, and also helped transplant freshly collected shoots including several hundred marked plants for growth measurements.

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EXECUTIVE SUMMARY

The objectives of this study were to work toward determining the threshold range of water-column nitrate enrichment that promotes destruction of eelgrass (*Zostera marina* L.), and to examine whether nitrate enrichment is toxic to two other submersed aquatic marine / estuarine plants, shoal grass (the seagrass *Halodule wrightii* Ascher) and widgeon grass (*Ruppia maritima* L.). These species are of interest for potential use in re-establishing beds of submersed aquatic vegetation within areas where eelgrass habitat loss has been correlated with nutrient enrichment or other factors associated with cultural eutrophication and coastal development.

In an experimental mesocosm system during the spring 1992 growing season (late March - late June), we compared growth of established eelgrass populations in replicated controls without nitrate additions (< 2 μM ambient NO_3^-N) to growth by populations in treated mesocosms with additions of ca. 5 μM NO_3^-N (or about 70 $\mu g\ l^{-1}$) added as pulsed daily additions. Low water exchange (10% d^{-1}) was used to simulate conditions in sheltered embayments or lagoons, and light reduction from high tide was simulated by covering the mesocosms with neutral-density screens for 3 h d^{-1}. Robust plants that had overwintered in the mesocosms under flow-through, running seawater were collected, cleaned, sorted and bundled for use in this experiment, while the mesocosm walls were scraped and the sediments vacuumed to reset the systems with similar macroinvertebrate densities and remove most macroalgae. In replanting, the bundled shoots from each mesocosm were subdivided for equal distribution among duplicate control and N-enriched mesocosms. Plants were acclimated for nearly 4 wk prior to initiating the experiment.

The spring was unusually cold (third coldest in a 50-year record, based on National Oceanic & Atmospheric Administration data for mean daily bay water temperatures). Bay water temperatures were at 24°C by late June, in contrast to a more typical condition of bay water temperatures at 28-30°C by mid-May. Growth of *Zostera marina* is favored under colder conditions, and after 12 wk shoot densities in the enriched and unenriched mesocosms were comparable, although both control and nitrate-enriched replicates showed high variability. The data indicate that, under the cool spring temperature regime, 5 μM NO_3^-N added as a daily spike over a 12-week period was sufficiently low to protect eelgrass. Results from

this unusually cold spring should be considered together with data from a similar experiment completed during an unusually warm spring in 1990. In that experiment, low pulsed daily spikes of 3 μM of nitrate resulted in death of 75% of the plants after 8 weeks. Collectively, these data strongly suggest that high temperatures act synergistically with nitrate to adversely affect eelgrass survival.

From late summer through fall 1992 (Sept. - early Dec.), we assessed the response of both the long-term-mesocosm-acclimated (LTMA) eelgrass with prehistory of nitrate exposure (spring), and recent field transplants of eelgrass, shoal grass, and widgeon grass to moderate water-column nitrate enrichment (10 μM NO_3^-N, or ~ 140 $\mu g\ l^{-1}\ d^{-1}$), added as in spring. This period included a second, smaller growing season within the annual cycle for *Zostera marina* and also encompassed the main growing season for *Halodule wrightii* and *Ruppia maritima*.

Autumn was characterized by cool temperatures that generally were comparable to or lower than 10-year monthly averages (National Oceanic & Atmospheric Administration, unpubl. data). Within-treatment eelgrass and macroalgal growth were more consistent than during the spring experiment. Recent field transplants of *Zostera marina* showed a trend for decreased growth under moderate nitrate enrichment, but shoot production of control and N-enriched plants was not significantly different. The LTMA control *Z. marina* from previously unenriched mesocosms in spring produced significantly more shoots than did control recent field transplants. Further, among the LTMA plants, shoot production was significantly higher by control plants with no former history of nitrate enrichment than by previously enriched eelgrass (spring) under moderate nitrate enrichment (fall). These results indicate that the recent field transplants may not have been as well acclimated to the enclosure conditions as the LTMA plants that had been grown in the mesocosms for a longer period, although the trend of decreased growth under nitrate enrichment was similar for both. Interestingly, the data also suggest that plants with prehistory of nitrate enrichment were not similarly benefited by long-term acclimation to the enclosures; previous nitrate loading apparently had weakened these plants so that their growth was considerably less than that of long term-acclimated but unenriched controls.

In contrast to the negative effect of moderate water-column nitrate enrichment on *Zostera marina*, *Halodule wrightii* was mildly stimulated by nitrate. This species showed a

small decline in shoot production of unenriched controls, and a slight increase of enriched shoots. *Z. marina, H. wrightii* and unenriched *Ruppia maritima* increased shoot production by, at most, ca. 50%, throughout the experiment. *R. maritima,* however, was highly stimulated by the pulsed moderate nitrate enrichment, and increased shoot production by more than 300% over experimental period.

This research indicates that *Halodule wrightii* or *Ruppia maritima* could be established by transplanting efforts as a management strategy in nitrate-enriched waters where eelgrass meadows have disappeared. Unlike *Zostera marina*, these plants apparently have physiological mechanisms to more efficiently process and control consumed nitrate; indeed, *R. maritima* seems to have derived a competitive advantage in nitrate-enriched conditions. *Z. marina* likely evolved in nitrogen-limited waters, and it is highly proficient at taking up water-column nitrate with no apparent "shut-off" mechanism. As anthropogenic inputs have increased and the coastal environment has become progressively more eutrophic, this formerly advantageous physiological strategy in maximizing nitrate uptake may have become a major underlying factor in the disappearance of *Z. marina* from many quiet upper embayments and poorly flushed coastal lagoons throughout the world.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

I. INTRODUCTION

A. The Discovery of Eelgrass Inhibition by Nitrate

The high productivity, habitat value, substrate stabilization, and other beneficial effects of seagrass meadows prompted ecologists McRoy & McMillan (1977) to write, "We know enough to understand in general what we would lose if catastrophe took seagrass ecosystems from the world oceans. We would lose options. More than ever, that is a loss beyond affording." Seagrass meadows provide enormous surface area for colonization by algae and animals used as food by valuable finfish, shellfish and waterfowl that spawn and nest in the habitat (Thayer *et al.* 1984). Within the past few decades, catastrophic losses of thousands of hectares of seagrass habitat have occurred throughout the world (Cambridge & McComb 1984, Orth & Moore 1983). Among the factors most frequently correlated with the disappearance of seagrass meadows are nitrate enrichment from sewage and agricultural drainage, and reduction in available light from shading by floating algae that are stimulated by nutrient loading (Borum 1985, Twilley *et al.* 1985, Orth *et al.* 1986). For example, along a nitrate gradient in an estuary in Denmark with influent sewage and agricultural drainage, epiphyte biomass on eelgrass increased 100-fold in the most enriched locations during summer, and this algal growth was implicated in the decline of the host plants (Borum 1985).

The effects of cultural eutrophication on aquatic plant survival would be expected to change with season, depending on the growth period for the species and the duration of perturbations. Under sustained nutrient enrichment and moderate or high water exchange, herbivores can effectively control epiphyte biomass (Orth & van Montfrans 1984, Neckles 1993). But in quiet embayments, epiphytes and macroalgae can respond so quickly to water-column enrichment that they may seasonally outgrow grazing pressure, leading to severe light reduction and decline of the underlying host macrophyte (Harlin & Thorne-Miller 1981, van Montfrans *et al.* 1984, Borum 1985). Eutrophication effects on seagrass meadows are most severe in sheltered habitats with reduced tidal flushing, where nutrient loadings are both concentrated and frequent, and where temperatures fluctuate more widely than in areas with greater water exchange (Stevenson 1988, Maier & Pregnall 1990). Increasing temperatures can act synergistically with light reduction to increase respiration, adversely affect enzymes and other proteins involved in general metabolism, and sufficiently weaken the seagrass plants for invasion by disease organisms (Short *et al.* 1988, Zimmerman *et al.* 1989), while

also stimulating rapid growth of epiphytes and thick mats of floating macroalgae under nutrient enrichment (Borum 1985, Harlin & Thorne-Miller 1981).

Nearly all of the phosphate and ammonium from anthropogenic sources adsorbs to particulates (Alberts & Moldenhauer 1981, Grobbelaär 1983, Simon 1989). The nutrient present in greatest abundance and readily available for plant uptake is the highly soluble nitrate (Craig & Kuenzler 1983, Jacobs & Gilliam 1985, Stanley 1988). If this inorganic N_i enrichment (as ammonium and nitrate) did not stimulate nuisance algae, the increasing N_i availability likely would be regarded as beneficial to seagrass growth. Most seagrasses obtain N_i from nutrient-rich sediments (McRoy & McMillan 1977, Thursby & Harlin 1982). They are also able to take advantage of N_i when it becomes available in the overlying water, and apparently do so especially if growing in sandy habitat where sediment concentrations of ammonia and nitrate are low (Short & McRoy 1984). Eelgrass (*Zostera marina* L.) collected from sandy habitat in New England, for example, has been reported to exhibit sustained high nitrate uptake under water-column enrichments with no apparent "shut-off" mechanism (Roth & Pregnall 1988).

Along the North Carolina coast, the dominant seagrass, *Zostera marina*, lies at the southernmost extension of its range (Thayer *et al.* 1984) and grows stunted from high-temperature stress (Den Hartog 1970). In a previous experiment, we set out to examine the influence of cultural eutrophication on survival of eelgrass in this inherently "high-stress" region (Burkholder *et al.* 1992). We constructed an experimental mesocosm system and modified it until we obtained excellent growth of *Z. marina* (> 2.5 cm d^{-1} with ambient flowing seawater). In 1990 we completed our first successful spring / fall-comparison experiment, in which a replicated gradient of water-column nitrate enrichment (3.5, 7.0, and 35.0 μM in "low," "moderate" and "high" treatments, respectively, added each day as a pulsed enrichment) was imposed under low water exchange (5-10% new water d^{-1}). Within 3-4 h following nitrate additions, concentrations were reduced to ambient levels (< 1 μM) in the low treatment, and to ca. 2 μM under moderate enrichment, indicating uptake by the algae and eelgrass plants. Substantial accumulation of nitrate was maintained only in the high treatment, where concentrations remained well below levels reported in septic effluent leachate draining into eelgrass beds (250 μM in the high treatment vs. 450 μM in areas receiving septic effluent leachate; Maier & Pregnall 1990). We assessed the response of

epiphytic algae, floating macroalgae, phytoplankton, macroinvertebrate algal herbivores, and eelgrass to these levels of water-column nitrate enrichment.

After 4 wk under unusually warm spring temperatures (water temperatures 27 - 31°C from mid-April through early May) in the highest imposed nitrate regime, the *Zostera marina* shoots were dark green in color and appeared robust. But when attempts were made to gently remove plants, the meristematic region (shoot base) disintegrated. Between weeks 5 - 7 nearly all the plants from the high treatment died, regardless of abundances of algae or macroinvertebrate algal herbivores (Burkholder *et al.* 1992). Morever, after 8 wk under the above-average temperature conditions, most plants under the low and moderate nitrate enrichments died, as well.

Although autumn 1990 was also unusually warm, conditions were cooler than in the previous spring. Low or moderate nitrate enrichment (3.5 and 7.0 μM NO_3^-N d^{-1}, respectively) depressed eelgrass C/N ratios especially in belowground tissue during warming periods, indicating a potential effect on carbon storage. Many plants survived and appeared structurally intact, but production of new shoots significantly declined under moderate enrichment, and was also less in the low nitrate treatment (Burkholder *et al.* 1992). The severity of nitrate inhibition during warm spring conditions suggested that the innate seasonality of *Zostera marina* growth could influence its response to water-column nitrate enrichment. Further, high temperatures could act synergistically with nitrate to adversely affect eelgrass survival. During fall we also maintained the former "high" treatment with nitrate accumulation in the bottom sediments (ca. 2 μM, vs. < 0.5 μM in the controls and other treatments) but without further nitrate additions, to determine the influence of the sediment "nutrient memory" on *Zostera marina* growth. Both leaf growth and production of new shoots in the nitrate-enriched sediment were comparable to growth of control plants in unenriched sediment. The results indicated that if water-column NO_3^-N enrichment were reduced, perhaps residual sediment N_i could be microbially converted over time so that *Z. marina* might be re-established by transplanting.

B. Potential Mechanisms for the Inhibitory Effects of Nitrate

One possible explanation for the effect of nitrate on eelgrass survival is that, given the fact that eelgrass lacks a "shut-off" mechanism for leaf uptake of nitrate (Roth & Pregnall 1988), under increased nitrate availability the shoots may have been forced to shunt

a high proportion of their carbon and/or phosphorus supplies into amino acid production in order to avoid ammonia toxicity following nitrate uptake and conversion (Turpin 1991). That is, despite non-limiting light and non-limiting carbon in the medium, the plants may not have been able to photosynthesize rapidly enough to keep pace with their high nitrate uptake. Such conditions, if sustained, would lead to self-imposed internal carbon or other nutrient limitation. A likely location for severe effects of carbon or phosphorus "starvation" would be the actively growing meristem region -- hence, the disintegrated condition of the meristem that we observed in nitrate-fertilized plants.

This hypothesized mechanism could account for the disappearance of eelgrass from poorly flushed lagoons and embayments that can receive much higher loading than was imposed in our low or moderate enrichments (Maier & Pregnall 1990). Under nitrate loading carbon stress may be exacerbated, especially with increasing temperatures that adversely affect translocation enzymes involved in shunting stored carbon from the rhizome area to the meristem during dehiscence (ideas in part from Zimmerman *et al.* 1989). Nitrate toxicity has been reported anecdotally in one other study of *Zostera marina* (Harlin & Thorne-Miller 1981); it has also been known to occur in macroalgae (Steffensen 1976), and could be operative in a wide array of other plants (Osborne 1987, Turpin 1991).

C. Objectives of This Research: Two Experiments

Results from the former study demonstrated that under warm spring conditions with simulated reduced tidal flushing, water-column nitrate enrichment can cause death to eelgrass as a direct physiological effect (Burkholder *et al.* 1992). One surprising aspect of the data was that pulsed daily additions of even low levels of water-column nitrate enrichment (3.5 - 7.0 μM NO_3^-N, or ca. 50-100 $\mu g\ l^{-1}$, significantly enriched over ambient control concentrations) were detrimental to the eelgrass plants. For this reason, we were unable to establish a permissible nitrate concentration that would allow long-term survival of *Zostera marina*. It is reasonable to assume that the threshold level which did not adversely affect eelgrass would change depending on interactions with other variables such as temperature, past history of exposure to nitrate, duration of exposure to elevated nitrate, and age / general physiological condition of the plants.

The two major objectives of the present research were to (1) work toward determining the permissible seasonal level(s) of nitrate that does not suppress growth of *Zostera*

marina, and (2) compare the response of *Z. marina* and two other co-inhabitant perennial angiosperms, shoal grass (*Halodule wrightii* Ascher) and widgeon grass (*Ruppia maritima* L.), to water-column nitrate enrichment. Although *R. maritima* and *H. wrightii* generally are considered to be of lower habitat value than *Z. marina*, the two species provide habitat and food for commercially important finfish, shellfish, and waterfowl (Kikuchi & Peres 1977; Thayer *et al.* 1979, 1984). They are also of potential interest for re-establishing beds of submersed aquatic vegetation in areas where habitat loss has been linked to coastal development (Kemp *et al.* 1983, Orth *et al.* 1986, Thorhaug 1986).

The two objectives were addressed in two separate experiments. The first experiment, to examine permissible levels of nitrate for eelgrass survival, was conducted during the spring season of maximal eelgrass sensitivity to water-column nitrate (Burkholder *et al.* 1992). As mentioned, previous experiments had suggested that warm temperatures act synergistically with nitrate in adversely affecting eelgrass. In consideration of predictions for an unusually cold spring, a nitrate enrichment level was selected (5 μM d^{-1}, or 70 $\mu g\ l^{-1}\ d^{-1}$) which actually was higher than the low nitrate treatment (3.5 μM $NO_3^-N\ d^{-1}$) d^{-1}) that had caused a 75% loss of plants in the previous experiment that had been completed during an unusually warm spring. If *Zostera marina* survived this higher level of nitrate loading under cold spring conditions, it was reasoned that the data could be considered as evidence for variable permissible levels in controlling plant survival under nitrate enrichment, with potential synergism between water-column nitrate and increasing temperatures in eelgrass inhibition.

The second experiment, comparing the response of three seagrass species to moderate nitrate enrichment, was conducted from late summer through late fall. Whereas *Zostera marina* has both spring and fall growing seasons, *Halodule wrightii* and *Ruppia maritima* are more warm-optimal plants with growth that begins in late spring and extends into mid / late fall (Muenscher 1964, Penhale 1977, Thayer *et al.* 1984). Hence, the selected experimental period encompassed seasonal maxima for growth by all three species. Both experiments contribute information toward the goal of predicting the success of transplanting efforts involving *Z. marina, H. wrightii* and/or *R. maritima* in locations affected by anthropogenic water-column nitrate enrichment.

PROJECT PROCEDURES

A. The Study Area

This research was completed in North Carolina, U.S.A., representing the southernmost extension for *Zostera marina* and the northernmost extension for *Halodule wrightii* on the western Atlantic Coast (Thayer *et al.* 1984). The three macrophyte species are perennials, with *Z. marina* attaining maximal growth in early spring, versus late summer - fall maxima for *H. wrightii* and *Ruppia maritima* (Muenscher 1964, Thayer *et al.* 1984). In this high-temperature area, the strap-shaped leaves of *Z. marina* shoots average only ca. 40 cm in length (as compared to lengths of 1 - 3 m in more northern climates; McRoy & McMillan 1977). *H. wrightii* and *R. maritima* can attain similar height but have much thinner, more delicate leaves. Shallow bay waters typically reach 33°C from late spring through early autumn (National Oceanographic & Atmospheric Administration [NOAA], unpubl. data; Thayer *et al.* 1984). Temperatures above 30°C have been shown to be detrimental to eelgrass by increasing respiration and impairing enzyme activities (Lambers 1985, Marsh *et al.* 1986, Zimmerman *et al.* 1989). Agriculture, industry and accelerated coastal development in North Carolina have been associated with increased nutrient loading (Jacobs & Gilliam 1985, Stanley 1988), and loss of eelgrass habitat in upper embayments has been reported anecdotally in the absence of long-term vegetation maps (Ferguson *et al.* 1988, Mather 1988).

B. The Experimental System

The experimental seagrass mesocosm system was located outside on the north shore of Beaufort Inlet at the Southeast Laboratory of the National Marine Fisheries Service (NMFS) on Pivers Island, North Carolina. The system consisted of fiberglass mesocosms coated with non-toxic white gelcoat resin, and each mesocosm was 2 m in diameter x 1 m in height. The working depth of each mesocosm was established at 80 cm by creating a raised bottom that was designed to accommodate the small plants (mean length 40 cm) while reducing the edge shading effects encountered at a deeper working depth. Water depth and sediment thickness in the mesocosms during experiments were 0.5 m and 12 cm, respectively. Running seawater was pumped into the system from a depth of 4 m (at high tide), from a location upstream from the dock area at NMFS. Ambient nitrate, temperature and salinity of the bay water were $< 1\ \mu M\ NO_3^-$-N, 17-24°C, and 28-36‰, respectively, during

the spring experimental period. A chilling system (consisting of 2 custom-designed, 4.5-metric ton condensing units each capable of a maximum of 60,000 BTUs h^{-1} cooling capacity) was used to maintain bay temperatures and mixing, with current velocities set at 5-10 cm sec^{-1}. The sediment consisted of clean dredge sand and salt marsh mud in a 3:1 ratio by volume. To minimize heterogeneity, ca. 8 metric tons of sediment were homogenized with a cement mixer and distributed equally to a depth of about 15 cm within the mesocosms. For nearly 1 yr before beginning the experiments, the sediment was maintained with transplanted eelgrass shoots (initially ~ 500 plants m^{-2}) in continuously exchanging bay water (one complete water exchange each 20 min). After 6 months the sediment had become well-colonized by algae and invertebrates (as described in Burkholder *et al.* 1992).

Maximum temperatures during experiments were maintained at $\leq$ 27°C on warmest days. Light reduction from high tide was simulated using neutral density shades that reduced photosynthetically active radiation (PAR) by about 30% while maintaining light above saturation for eelgrass photosynthesis. Diurnal temperature extremes in the mesocosms were at $\leq$ 5°C during both seasons, as compared to $\leq$ 4°C in the embayment. Maximum temperatures reached 27-28°C in both late spring and early fall, and were comparable to maxima shown by the ambient bay water.

C. Biological Characteristics of the Mesocosm System

In the 11-month system acclimation period following sediment exchange, eelgrass shoots that were transplanted into the mesocosms during May 1991 (from an unenriched field site, Middle Marsh near Beaufort) had attained dense growth of > 1,500 shoots m^{-2} in most mesocosms, with thick masses of roots and rhizomes that were difficult to dislodge. In an attempt to reset the system with homogeneous populations of eelgrass, algae, and macroinvertebrates prior to beginning the spring 1992 nitrate experiment, we removed all eelgrass plants (including attached belowground tissue) from the mesocosms, separated the plants by mesocosm number, and floated them in running seawater. Mesocosm walls were scraped clean of most macroalgae and repeatedly drained to flush residual algal suspensions. The sediments and walls were also vacuumed.

Plants were cleaned of most epiphytes, sorted into loose bundles of 5-8 shoots, and maintained in running seawater at ambient baywater temperature (14°C) until they were replanted (within 3 d). Bundled plants collected from a given mesocosm were divided into 12 equal subgroupings, with 1 subgrouping replanted into each mesocosm. This procedure

ensured that any long-term effects of maintenance in specific enclosures were normalized among the mesocosm populations. Shoots were transplanted to attain initial densities of $\geq$ 850 plants m^{-2}, and were allowed to acclimate for 3 wk before beginning nitrate additions in treated mesocosms. One day before initiating the spring experiment, enclosure walls and macrophytes were again cleared of most periphyton and macroalgae. Macroalgal wall growth was removed (and quantified) periodically as necessary to minimize confounding enclosure surface-area effects. To help control herbivore densities and to include a higher trophic level, we added 3 small Atlantic croaker (*Micropogonias undulatus* L.; total length of each ca. 10 cm) to each mesocosm. Dead fish were removed and replaced with live fish throughout the experiments.

The spring control and nitrate enrichment regimes were replicated in triplicate mesocosms from late March through mid-June 1992. For more than 3 wk following termination, all mesocosms were maintained at 10% water exchange d^{-1} and the N-treated mesocosms received no further enrichment. Nitrate-enriched plants from treated mesocosms were then collected and segregated by mesocosm number. Over a 4-d period (12 - 15 July), the plants were cleaned of most epiphytes and bundled for replanting, with bundles separated into three equal subgroupings. In the formerly enriched mesocosms, 1/4 of the surface area was replanted with 50% bundles each from the N-1 and N-2 replicates, whereas the relatively sparse population of N-3 plants was discarded. A similar process was followed for eelgrass shoots that were collected from / replanted into the spring control mesocosms. Hence, these "long-term mesocosm-acclimated" (LTMA) eelgrass plants from the spring experiment subsequently were used as a tested plant category in the late summer - fall experiment.

To compare macrophyte species response to water-column nitrate, *Zostera marina, Halodule wrightii* and *Ruppia maritima* were freshly transplanted into the mesocosms from the same unenriched field site where the LTMA eelgrass had been collected. Each plant type occupied one delineated quarter of each mesocosm. From 12 - 14 July these plants were collected from field habitat, held in shallow tanks of running seawater, cleaned of most epiphytes and animals, bundled, and transplanted following procedures of Burkholder *et al.* (1992). The section area designated for each of the four plant categories (LTMA *Z. marina* and recent field transplants of *Z. marina, H. wrightii,* and *R. maritima*) was randomly selected

within each mesocosm. Since eelgrass is considerably larger than the other two species, it was planted at an approximate density of 800 shoots m^{-2}, whereas *H. wrightii* and *R. maritima* were each planted at an approximate density of 1,200 shoots m^{-2}. The plants were acclimated in the mesocosms for a 6-wk period prior to initiation of the late summer - fall experiment (30 Aug.). Fungal attack occurred in one control and one enriched mesocosm between experiments, and led to omission of the two mesocosms for consideration in the fall experiment so that treatments were imposed in duplicate. During the first 2 wk of the acclimation period, the mesocosms were flushed with running seawater (1 full exchange per 20 min); for the remaining 4 wk, water exchange was imposed at 10% d^{-1}. Water temperatures were maintained at $\leq$ 31°C throughout the 6-wk acclimation period.

D. Sampling and Experimental Design

During the spring season of maximal eelgrass growth (Thayer *et al.* 1984) and maximal sensitivity to water-column nitrate (Burkholder *et al.* 1992), plants were subjected to low nitrate additions under ambient baywater temperatures. The spring nitrate exposure and late summer-fall experiment were completed from 26 March - 22 June and from 30 Aug. - 6 Dec. 1992, respectively. Replicate mesocosms were randomly selected as controls or N-treated, with each mesocosm maintained as either a control or an N-enriched replicate throughout. Nitrate was added daily between 0800 - 1000 h when high plant uptake was expected, whereas low water exchange (10% d^{-1}, simulating conditions in poorly flushed coastal embayments and lagoons) was completed between 1600 - 1800 h during late afternoon. Each mesocosm was independent of the others in plumbing of intake and outflow lines, so that there was no treatment cross-contamination. Nitrate-contaminated water was pumped to a small salt marsh ca. 300 m from the mesocosm water intake site, for effective treatment and to prevent contamination of mesocosms during water exchange.

The experimental design in spring included controls (ambient baywater NO_3^-N at < 2 μM or ca. 30 $\mu g\ l^{-1}$) and an imposed "low" nitrate treatment with pulsed additions of ca. 5 $\mu M\ NO_3^-N\ d^{-1}$. At approximately 30 minutes after adding nitrate to enriched enclosures, nitrate averaged 5.2 $\pm$ 1.3 μM (or ca. 73 $\pm$ 18 $\mu g\ NO_3^-N\ l^{-1}$); at 24 h after addition, mean nitrate was only slightly higher than ambient baywater concentrations (2.5 $\pm$ 0.5 $\mu M\ NO_3^-N$; n = 3 trials at monthly intervals). In comparison, NO_3^-N typically is reported at ca. 1.0 - 1.5 μM in North Carolina estuarine habitat such as the lower Neuse River, except for increases

up to ca. 12 μM (170 μg l^{-1}) during precipitation / runoff events (Paerl *et al.* 1990, Christian *et al.* 1991, Mallin *et al.* 1993) that can extend for several or more weeks in spring during major periods of crop fertilization and frequent storms.

During autumn under conditions of decreasing temperature (and photoperiod), eelgrass previously was shown to be less sensitive to water-column nitrate additions than in spring (Burkholder *et al.* 1992). That is, "moderate" nitrate additions (ca. 7 μM NO_3^-N as pulsed daily additions for 8 wk) during an unusually warm spring had killed more than 90% of the treated plants. Similar nitrate loading for 12 wk during an unusually warm autumn had reduced growth, but initial plant densities were not significantly affected (Burkholder *et al.* 1992). The moderate concentration of 10 μM NO_3^-N (140 μg l^{-1}), known to adversely affect eelgrass growth in autumn based on the previous study, was selected for testing compara-tive effects on LTMA and recently transplanted *Zostera marina*, *Halodule wrightii* and *Ruppia maritima* in the 1992 late summer - fall experiment. As in spring, control nitrate levels were generally < 2 μM NO_3^-N, whereas nitrate accumulated in the water column of enriched enclosures. At ca. 30 min after treatment, nitrate averaged 22.0 $\pm$ 1.5 μM NO_3^-N versus concentrations of 6.3 $\pm$ 4.5 μM NO_3^-N measured 24 h later prior to the next pulsed nitrate addition (n = 4 trials at biweekly intervals).

E. Physical, Chemical, and Community Variables

Environmental variables that were measured routinely included temperature, salinity, photosynthetically active radiation (PAR), and water-column nitrate (NO_3^--N), ammonium (NH_4^+N), and total phosphorus (TP). Temperature was monitored daily by an automated digitized system that consisted of 12 Johnson control model SET189A-600 temperature sensors that were mounted inside the mesocosms within immersion wells (WEL11A-601R). Salinity was measured daily with a Reichert refractometer. Light (PAR) in the upper eelgrass canopy was recorded daily using a LiCor data logger (model 1000) connected to a submersible 4π PAR quantum sensor (model LI-193SA). Samples for water-column nutrient analyses were collected weekly in the early morning prior to addition of nitrate in treatments. Nitrate was determined on a Technicon autoanalyzer (model II), using the copper-cadmium reduc-tion procedure of Parsons *et al.* (1985). Ammonium was measured with the Solórzano method (Parsons *et al.* 1985) using modifications of Burkholder & Sheath (1985) for immediate preservation with phenol. TP was analyzed after acid persulfate digestion (Parsons *et al.* 1985).

Macroalgal abundance was estimated from harvests of three 0.1 m^2 quadrats haphazardly positioned among *Z. marina* in spring. During the fall experiment, one quadrat was haphazardly placed among growth of each of the three macrophyte species, and macroalgae were collected from all surfaces within the quadrat (seagrass leaves, sediment, shells, etc.). In both experiments, the three quadrats and collections of wall growth were combined to obtain an estimate of macroalgal abundance for each mesocosm on the basis of surface area. Macroalgal taxa were separated, cleaned and oven-dried at 60°C. Periodic checks indicated that phytoplankton abundances were low (< 400 cells/mL) throughout both experiments. Based on monthly estimates as in Burkholder *et al.* (1992), the biomass of microalgal epiphytes was negligible $\pm$ nitrate enrichment, in comparison to that of floating and benthic macroalgae.

Herbivory can control algal biomass seasonally in some seagrass habitats (Zimmerman *et al.* 1979, Neckles 1993), and may indirectly influence eelgrass survival under nitrate enrichment. Hence, on 3 dates (initial, midpoint, and final) macroinvertebrate algal herbivores were quantified from 3 cores (4-cm diameter) that were collected from haphazardly selected locations; each core included one or more seagrass shoots. The animals were preserved in 10% formalin stained with Rose Bengal solution; they were cleaned and stored in 70% ethanol until they were quantified (within 4 months) at 20x using a Wild M5-51475 dissecting microscope.

F. Macrophyte Variables

Aboveground eelgrass productivity ("longitudinal" growth) during spring was measured by the leaf puncture method of Zieman & Wetzel (1980). Shoots were marked at 3-wk intervals, with randomly selected subsets of 8-10 marked plants (or tags from dead plants) harvested weekly. This method is flawed since only shoots that are sufficiently large and robust to enable marking are selected; hence, the data overestimate mean growth and more closely approximate maximum estimates. Leaf growth measurements were taken under low nitrate loading in spring, when within-shoot growth may be the primary growth strategy for *Zostera marina* (Kenworthy & Fonseca 1992). A more reliable indicator of plant response was obtained in both spring and fall (when lateral spread or increase in shoot number may be more important for *Z. marina* populations; Kenworthy & Fonseca 1992) by considering shoot production (lateral growth) as a population-level index. Change in shoot density of

the four plant categories (LTMA *Z. marina* and recent field transplants of *Z. marina, H. wrightii* and *R. maritima*) from each mesocosm over the experimental period was determined by quantifying the total shoots within a marked 0.1 m^2 quadrat.

Eelgrass and other angiosperms are known to decrease production of anti-microbial phenolics in N_i-enriched, light-replete conditions (Bazzaz *et al.* 1987, Buchsbaum *et al.* 1990). To assess whether water-column nitrate enrichment affected seagrass susceptibility to pathogenic organisms, the proportion of aboveground *Zostera marina* tissue (percentage of brown and blackened areas) infected by the opportunistic slime mold-like pathogen, *Labyrinthula zosterae* (Muehlstein *et al.* 1991) was estimated from plants harvested for growth measurements at 7-to 9-d intervals during spring, and in the first and last 2 wk of the late summer - fall experiment.

Above- and belowground tissue content of carbon, nitrogen, and phosphorus was determined for *Zostera marina* at the beginning, mid-point and end of both the spring and late summer - fall experiments. Tissue C, N, and P were also measured for *Halodule wrightii* and *Ruppia maritima* near the end of the late summer - fall experiment (11 wk). For *Z. marina,* 12-15 shoots were collected from each mesocosm, whereas sufficient material of the two smaller species was collected to obtain 250 mg of dried tissue for above- and belowground analyses (approximately 50 shoots per species). Whole plants with leaves, rhizomes, and roots were harvested between 1000 and 1300 h, carefully cleaned of algae and sediment in running seawater (< 10 min), and frozen on dry ice for transport to the laboratory. They were held at -70°C prior to separation of aboveground from belowground tissue. The tissue was thawed, oven-dried at 60°C for 12 h, finely ground, and analyzed for C, N and P content by the Department of Forestry and the Analytical Laboratory in the Department of Soil Science at North Carolina State University.

Two tissue components, total protein content and total acid-soluble carbohydrates, were measured separately in above- and belowground tissue of *Zostera marina* near the end of the late summer - fall experiment (Dec., wk 12). Plants were harvested between 1500 - 1800 h, carefully cleaned of epiphytes and sediment in running seawater, and transported to the laboratory in darkness on ice. The three youngest leaves and the living rhizomes (with beige-colored interior) and roots were used in the tissue analyses. For each mesocosm, total protein was determined from a pooled sample of three randomly selected plants,

following a modification of the Coomassie brilliant blue procedure (Dawes & Kenworthy 1990). Acid-soluble or dissolved carbohydrates (including simple sugars, oligosaccharides, polysaccharides, and derivatives having a free or potentially free radical group) were measured following Dawes & Kenworthy (1990).

On similar tissue as for the total protein and acid-soluble carbohydrate assays, the activity of the primary enzyme involved in nitrate uptake, nitrate reductase (NRase), was assayed from *Zostera marina* after 3 wk in the spring experiment, and after 3 and 14 wk in the late summer - fall experiments. From each mesocosm six plants were harvested, cleaned, and transported to the laboratory, and NRase was assayed using the technique of Roth & Pregnall (1988). The activity of a key enzyme involved in N_i assimilation, glutamine synthetase (GS), was also assayed for *Z. marina* after 3 wk and 14 wk in the late summer - fall experiment, following the procedure of Pregnall *et al.* (1987).

G. Statistical Analyses

Separate data analyses were completed for the two seasonal experiments. Correlation analysis was performed initially by date to examine relationships between eelgrass above-ground ("longitudinal" or leaf) growth (spring), macroalgal abundances, PAR, temperature, lag-effect temperature, and salinity (SAS Institute, Inc. 1987). Treatment means were compared using the student "*t*" test (Gill 1978). The LTMA *Zostera marina* plants used in the spring - fall manipulations had been maintained in the mesocosms for longer duration than plants included in the three-species comparison during late summer - fall. Hence, direct statistical comparison of the effects of water-column nitrate on LTMA *Z. marina* and the recent field transplants of *Z. marina* was not possible, although inferences were formed from trends in the data.

III. DATA SUMMARY

A. The Spring Experiment: Eelgrass and Low Nitrate Exposure

The spring season was the third coldest in 50 yr, with air and bay-water temperatures 7°C and 4°C lower than average, respectively (10-yr means; from NOAA 1980 - 1990). Mesocosm water temperatures gradually increased over the spring season as expected, but with exception of 1 date, temperatures remained below 25°C until the second week of June (Fig. 1). Salinity was relatively constant (30-35‰) except toward the end of the experiment when

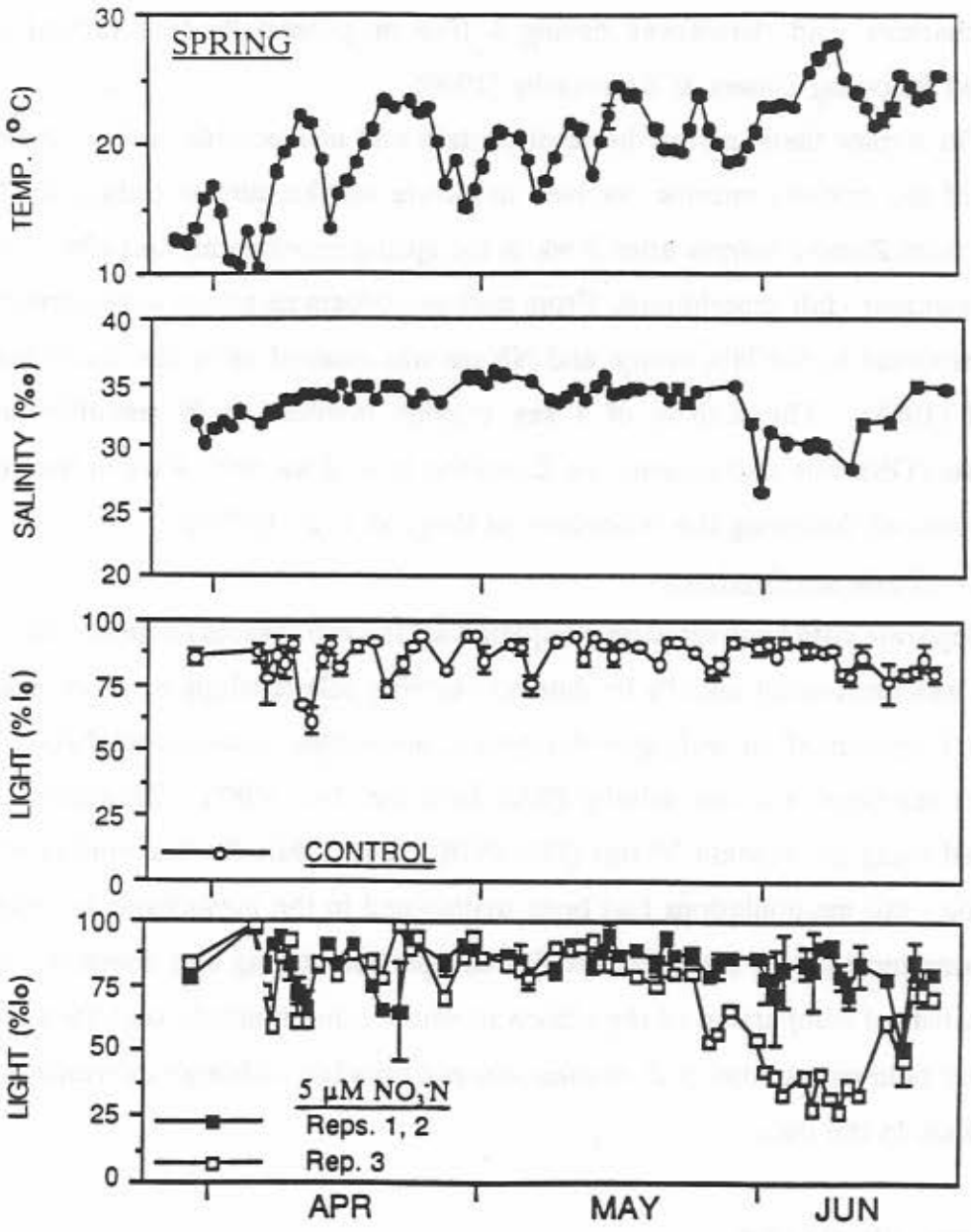

Figure 1. Temperature, salinity and light (as the percent of total PAR just below the surface) at the base of the eelgrass canopy during the spring 1992 experiment (means $\pm$ 1 standard error [SE]), considering control and 5 μM NO_3^-N-enriched mesocosms collectively for temperature and salinity, and plotting light separately for control and N-enriched mesocosms to show the variability imposed by crab activity in replicate N-3).

high precipitation reduced salinity to 26‰ on one date, and held salinity below 30‰ for 8 d (Fig. 1). On each sampling date, water temperatures varied by $\leq$ 2°C among all mesocosms, and salinities varied by $\leq$ 3‰. Light (PAR) was above saturation for eelgrass photosynthesis (> 350 μEinst m^{-2} sec^{-1}; Dennison & Alberte 1982, Marsh *et al.* 1986, Dennison 1987, but see Zimmerman *et al.* 1991). PAR was comparable among the mesocosms except for the last 3 wk, when a blue crab stirred the sediments in N-enriched replicate 3 (N-3) prior to its capture and removal (Fig. 1).

As expected, nutrient concentrations in control and enriched mesocosms were comparable throughout the spring experiment. Mean NO_3^-N levels in controls remained at 2 μM or less except for increases up to ca. 7 μM during two precipitation events (Fig. 2). Samples collected from enriched mesocosms 24 h after the previous pulsed nitrate addition showed nitrate concentrations that were comparable to those in controls. The onset of warmer conditions toward the end of the spring experiment was associated with qualitatively higher mean levels of ammonium and total phosphorus in the enriched treatment, but concentrations of both nutrients also did not differ significantly from those in controls (Fig. 2).

Growth of macroalgae and *Zostera marina* was highly variable among replicates of both controls and enriched mesocosms. The green macroalga *Chaetomorpha linum* (O.F. Muller) Kutz. (Chlorophyceae) bloomed in control replicate CON-1, so that the highest macroalgal biomass in unenriched mesocosms occurred in that replicate except during April, when the brown macroalga *Ectocarpus siliculosus* Sauvageau (Phaeophyceae) increased in replicate CON-3 (Fig. 3A). Macroalgal abundance was comparable among controls and enriched mesocosms (< 80 g m^{-2}, with mean abundance $\leq$ 35 g m^{-2}) except for enriched replicate N-3, where the green macroalgae *Enteromorpha* spp. and *Cladophora montagneana* Kutz. were dominant with maxima during late April (235 g dry wt m^{-2}) and June (485 g dry wt m^{-2}), respectively (Fig. 3). In the N-3 replicate, *Enteromorpha* spp. produced most of the macroalgal biomass during April, whereas *C. montagneana* and *E. siliculosus* were co-dominant in June. Macroinvertebrate densities were comparable in controls and N-enriched mesocosms throughout most of the experiment, except that amphipods were ca. 5-fold more abundant in controls during the first 2 wk ($p < 0.05$; Fig. 4). There was no significant correlation between macroinvertebrate densities and macroalgal abundances, with or without nitrate enrichment.

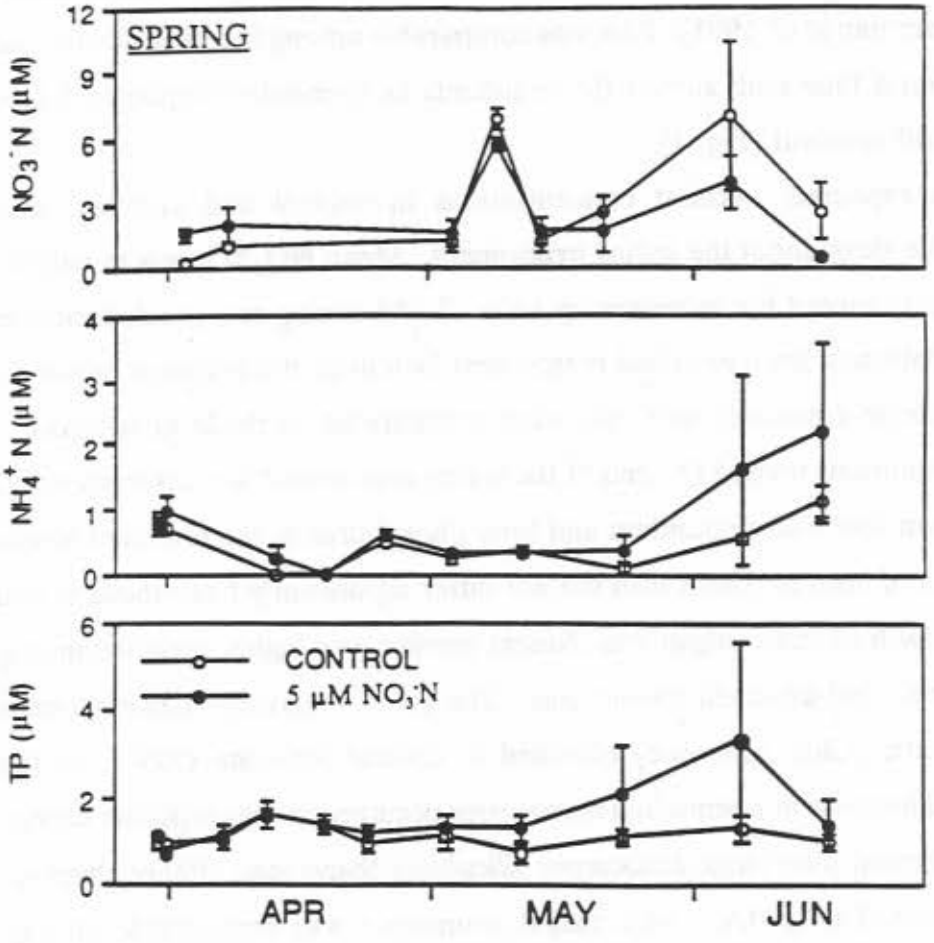

Figure 2. Nutrient concentrations NO_3^-N, NH_4^+N and TP during spring 1992, testing the response of *Zostera marina* to 5 µM NO_3^-N enrichment (means ± 1 SE).

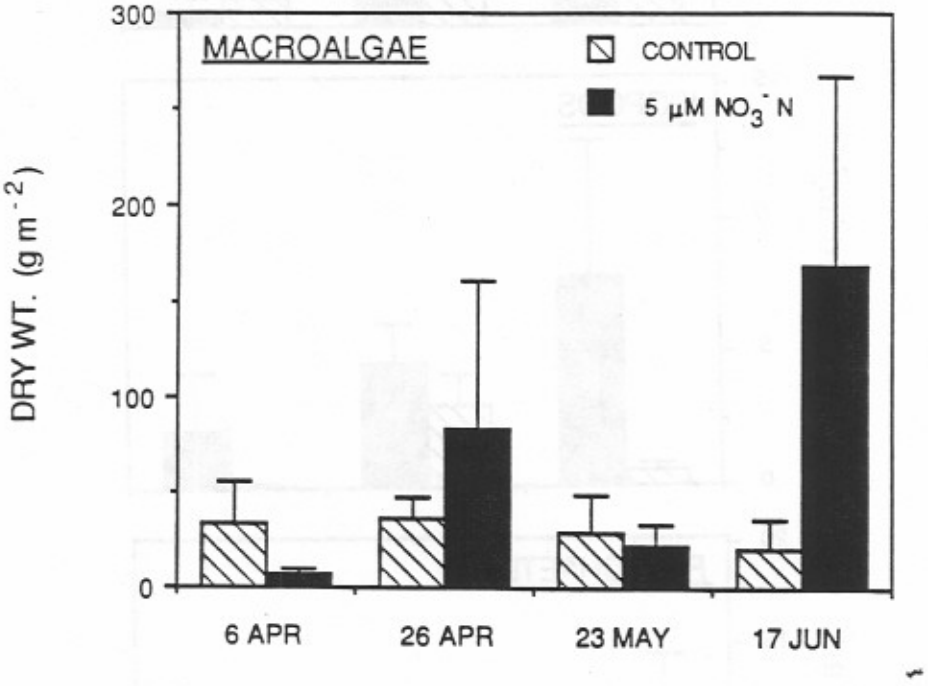

Figure 3. Total abundance of macroalgae during spring 1992, as harvested dry weight per unit surface area (means $\pm$ 1 SE).

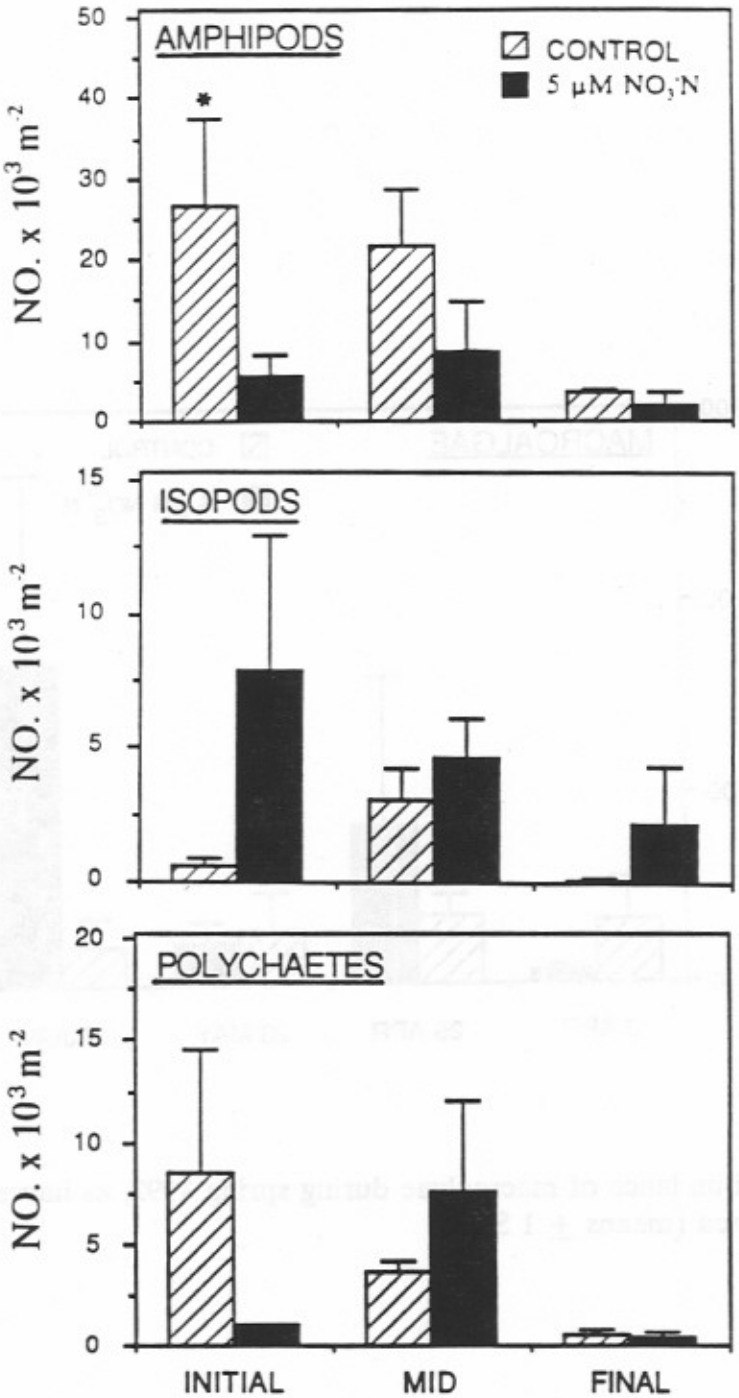

Figure 4. Densities of the three most abundant components of the macroinvertebrate community during spring 1992, as individuals per unit sediment surface area (means $\pm$ 1 SE). Asterisks (*) indicate significantly higher abundance ($p < 0.05$).

Zostera marina leaf growth on marked shoots was comparable among control and enriched mesocosms throughout the experiment, except for significantly higher growth by control plants at wk 12 (early June; $p < 0.05$; Fig. 5). Attack of leaf tissue by the pathogen *Labyrinthula zosterae* was similar in enriched plants and controls throughout the experiment ($8 \pm 4\%$ versus $5 \pm 3\%$ invasion of control and enriched shoots, respectively; $n = 12$ dates), indicating that the low level of water-column nitrate enrichment did not increase eelgrass susceptibility to this opportunistic disease vector. By the end of the experiment, N-enriched eelgrass had significantly higher aboveground tissue nitrogen content than controls ($p < 0.05$; Table 1), indicating that a portion of the water-column nitrate source had been taken up by the plants. Further, belowground tissue carbon content was significantly lower in enriched than in control shoots (Table 1). NRase activity was highly variable among the nitrate-enriched plants; mean NRase activity after 3 wk was not significantly different between control and enriched shoots, although there was a trend for higher NRase activity under nitrate enrichment (leaf NRase 18 ± 7 versus 57 ± 52 μM NO_2^- g^{-1} h^{-1}, respectively, in control and N-enriched eelgrass; root NRase activity 17 ± 4 versus 28 ± 8 μM NO_2^- g^{-1} h^{-1}, respectively, in control and N-enriched shoots). Total C/N ratios in aboveground tissue were comparable in control and N-enriched plants, but belowground tissue C/N ratios were significantly higher in controls after 4 wk and 12 wk ($p < 0.05$; qualitatively higher belowground tissue C/N ratios in controls after 8 wk, also; Table 2). Throughout the experiment, there was a qualitative trend for higher N/P ratios in aboveground tissue of N-enriched plants relative to controls. Further, by wk 12 the N/P ratio of belowground tissue was significantly higher in N-enriched plants than in controls ($p < 0.01$; Table 2).

The population-level index of shoot density was variable in spring, with negligible eelgrass shoot production in 2 of 3 control replicates and a 24% decline in shoot numbers from replicate CON-3 by the end of the experiment (Fig. 6). This decline apparently was unrelated to macroalgal abundance, which remained low in the CON-3 mesocosm even during the *Ectocarpus siliculosus* increase (maximum at 58 g dry weight m^{-2} in April, then 6-8 g dry weight m^{-2} during May - June; Fig. 3). Among nitrate-enriched mesocosms, shoot numbers increased slightly in replicates N-1 and N-2, but there was a 44% decline in shoots of N-3. This decline coincided with both high macroalgal growth in N-3 (485 g dry weight m^{-2}) and reduction in available light from both macroalgae and sediment dispersal associated

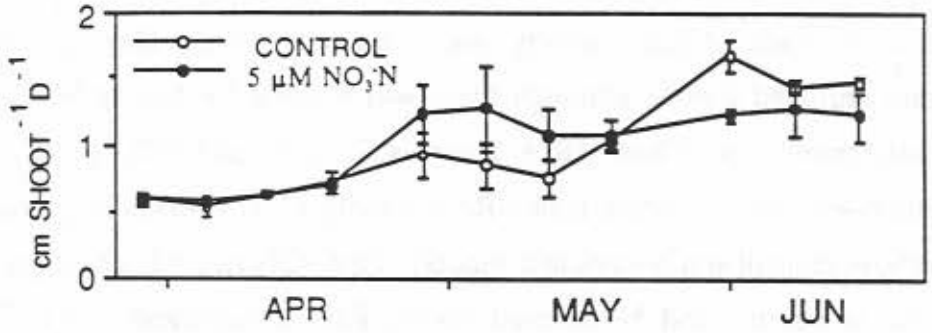

Figure 5. Leaf growth of *Zostera marina* populations in control and 5 μM NO_3^-N-enriched mesocosms during spring 1992 (means $\pm$ 1 SE).

Table 1. *Zostera marina.* Tissue content (% dry weight) of C, N, and P in aboveground (above) and belowground (below) tissue of eelgrass during the spring 1992 experiment. Data are given as means ± 1 SE for the replicate control and enriched (5 μM NO_3^-N) mesocosms, using pooled values of 12 to 15 shoots for each mesocosm. Asterisks (*) indicate significant differences from shoot content in controls ($p < 0.05$).

Date	Tissue	Control	5 μM NO_3^-N
Carbon			
24 Apr	Above	35.52 ± 0.33	34.94 ± 0.25
	Below	32.15 ± 0.52	31.67 ± 0.49
17 May	Above	35.41 ± 0.55	34.77 ± 0.36
	Below	31.08 ± 0.87	30.10 ± 0.98
17 Jun	Above	35.33 ± 0.20	35.54 ± 0.16
	Below	31.28 ± 0.74	* 29.46 ± 0.56
Nitrogen			
24 Apr	Above	1.44 ± 0.04	1.62 ± 0.14
	Below	0.74 ± 0.00	* 0.81 ± 0.02
17 May	Above	1.42 ± 0.09	1.60 ± 0.08
	Below	0.75 ± 0.03	0.76 ± 0.05
17 Jun	Above	1.45 ± 0.07	* 1.78 ± 0.04
	Below	0.78 ± 0.05	0.87 ± 0.02
Phosphorus			
24 Apr	Above	0.21 ± 0.01	0.21 ± 0.01
	Below	0.20 ± 0.01	0.19 ± 0.02
17 May	Above	0.18 ± 0.01	0.19 ± 0.01
	Below	0.17 ± 0.01	0.17 ± 0.03
17 Jun	Above	0.18 ± 0.01	0.19 ± 0.03
	Below	0.15 ± 0.02	0.11 ± 0.01

Table 2. *Zostera marina.* Tissue C/N and N/P ratios of eelgrass during the spring 1992 experiment. Data are given as means ± 1 SE for aboveground (above) and belowground (below) tissue. Asterisks (*) indicate significant differences from shoot content in controls ($p < 0.05$).

Date	Tissue	Control	5 μM NO_3^-N
C/N			
24 Apr	Above	24.6 ± 1.1	21.9 ± 3.2
	Below	43.7 ± 0.7	* 39.1 ± 1.6
17 May	Above	25.2 ± 2.9	21.8 ± 1.8
	Below	41.4 ± 2.7	40.0 ± 2.2
17 Jun	Above	24.5 ± 3.2	20.0 ± 0.6
	Below	40.1 ± 2.0	* 33.8 ± 2.5
N/P			
24 Apr	Above	6.9 ± 0.3	7.7 ± 0.7
	Below	3.6 ± 0.3	4.4 ± 0.6
17 May	Above	8.0 ± 0.8	8.5 ± 0.5
	Below	4.4 ± 0.6	4.8 ± 2.7
17 Jun	Above	8.3 ± 1.1	9.6 ± 1.6
	Below	5.2 ± 0.7	* 7.9 ± 0.6

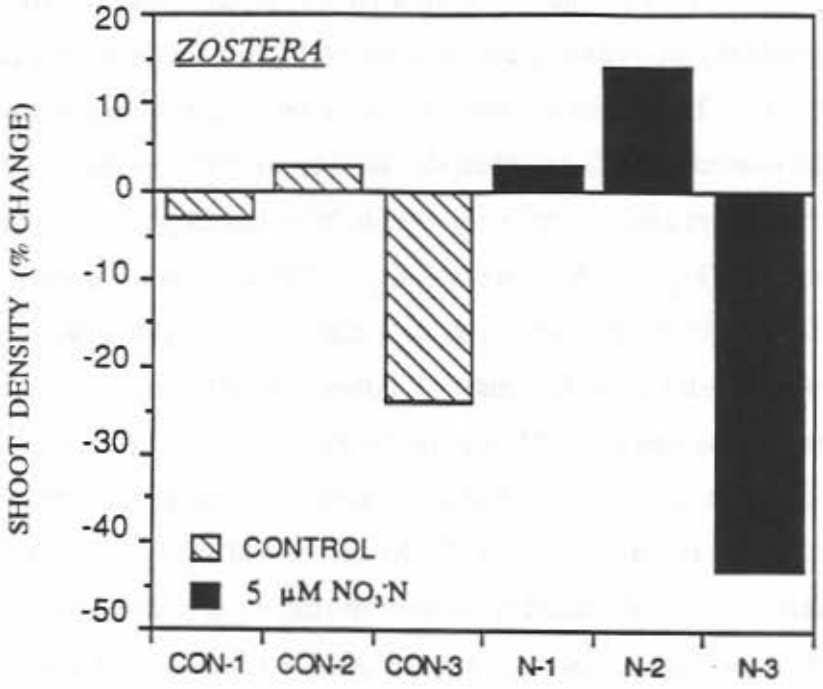

Figure 6. Change in shoot densities of *Zostera marina* populations in control and 5 μM NO_3^-N- enriched mesocosms during spring 1992 (initial and final dates compared as percent change in shoot numbers). Individual replicates are plotted, given high variation in response within each treatment. Note: The lowest *Z. marina* shoot production replicate CON-3 also coincided with low macroalgal production, whereas lowest *Z. marina* shoot production in replicate N-3 coincided with highest macroalgal growth.

with blue crab activity (Figs. 1,3,6). Significant influences on eelgrass growth by temperature, salinity, PAR, macroalgal abundances, or macroinvertebrate densities were not detected in statistical analyses. Hence, the data, although highly variable, indicated comparable growth by eelgrass under control and low nitrate-enriched regimes during this unusually cold spring.

B. The Late Summer-Fall Experiment: Seagrass Response to Water-Column Nitrate

Autumn was characterized by cool temperatures that were comparable to or slightly lower (1-2°C) than 10-year monthly averages (from NOAA 1980 - 1990). Mesocosm water temperatures gradually decreased from maxima at 27-29°C in Sept. to a minimum of 9°C in mid-Nov. (Fig. 7). This minimum was followed by a temporary warming period when temperatures increased to 18°C and then declined to ca. 16°C by the end of the experiment. A series of overcast days and precipitation events from late Sept. through mid-Oct. coincided with reduced salinity (Fig. 7). Minimal salinity of 23‰ occurred during early Oct.; by the third week in Oct., salinity had increased to a similar range (30-35‰) as before the rain-storms. Light was non-limiting (Dennison & Alberte 1982); more than 75% of the PAR just below the water surface was available within the *Zostera marina* canopy, decreasing to > ca. 50% when plants were shaded for 3 h daily to simulate light reduction from high tide (Fig. 7). Nitrate generally remained below 2 μM in control mesocosms, with concentrations comparable to those in the enriched treatment during most of the first 7 wk (Fig. 8). In the latter part of the experiment, however, nitrate usually was higher in the N-enriched regime, indicating water-column accumulation over time (range up to ca. 21 μM NO_3^-N; at 24 h after nitrate additions, significantly higher in N-treated than in control mesocosms on 3 dates; $p < 0.05$; Fig. 8). As during spring, NH_4^+N and TP generally were low and comparable among control and enriched mesocosms (Fig. 8).

Macroalgal abundance was low (ca. 50 g dry weight m^{-2}) in controls and in 1 nitrate-enriched replicate (N-1; Fig. 9). The second enriched mesocosm (N-2) developed dense growth of the red macroalga *Polysiphonia* sp., with maximal accumulation by mid-Oct. (ca. 330 g dry weight m^{-2}). This alga covered the sediment and walls, but mostly occurred as an epiphyte on all three macrophyte species. *Polysiphonia* sp. declined to ca. 75 g dry weight m^{-2} in the second enriched replicate by mid-Nov. As for macroalgae, macroinvertebrate densities were, again, highly variable (Fig. 10). The N-2 replicate initially supported abundant isopods (134.1×10^3 individuals m^{-2} sediment surface, versus 8.7×10^3 m^{-2} in the

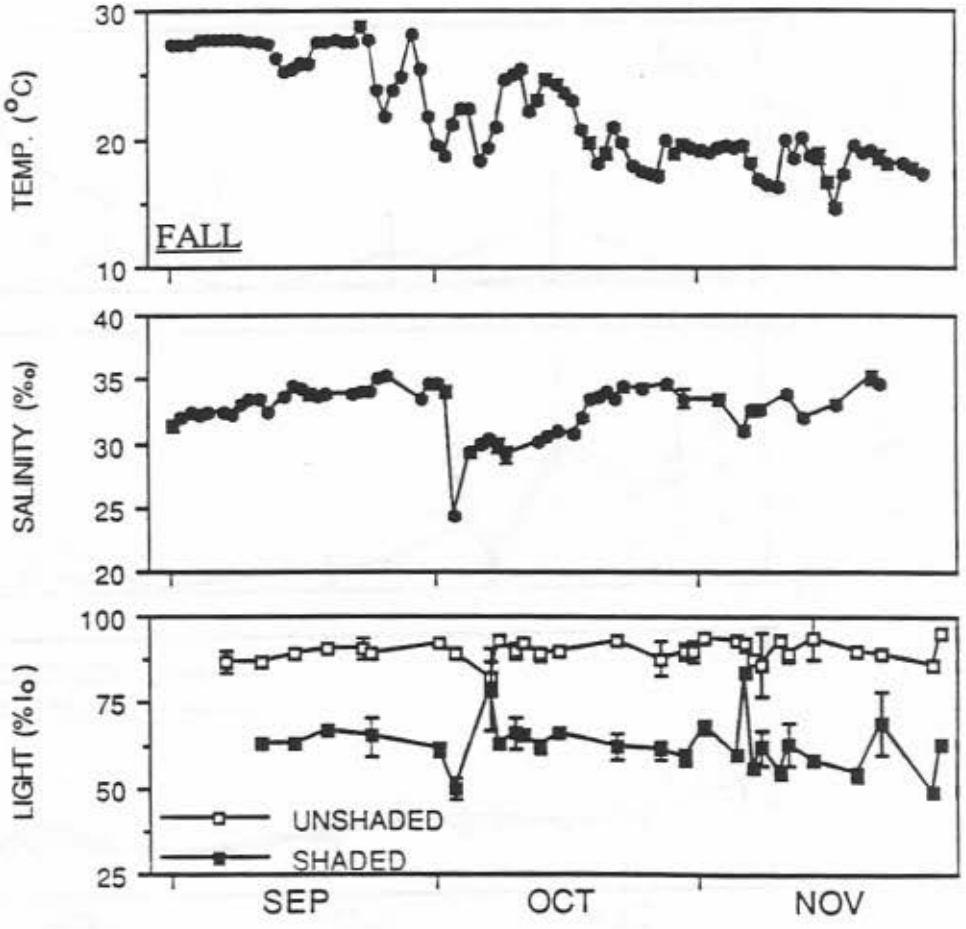

Figure 7. Temperature, salinity, and light at the base of the canopy during the late summer - fall 1992 experiment (means $\pm$ 1 SE for control and 10 μM NO_3^-N-enriched mesocosms considered collectively in all plots). The panel depicting PAR includes both shaded and unshaded data to indicate the decrease in PAR that occurred when plants were shaded for 3 h d^{-1} to simulate light reduction from high tide.

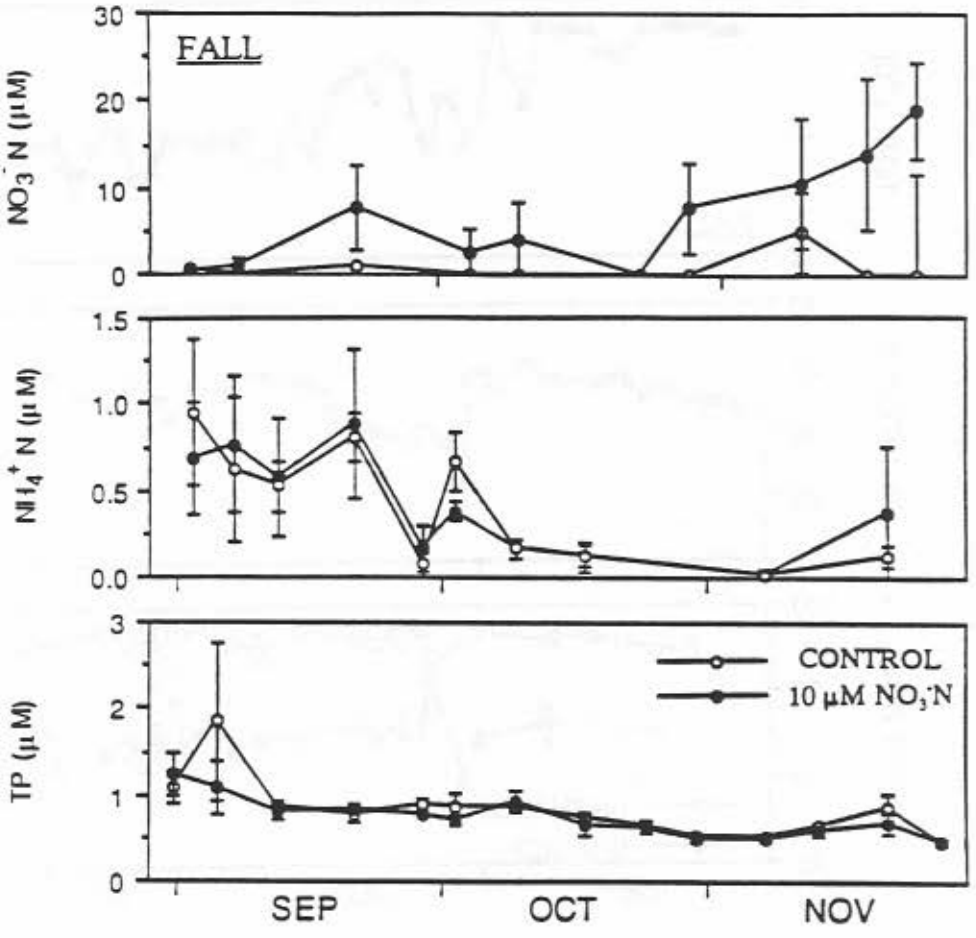

Figure 8. Concentrations of NO_3^-N, NH_4^+N and TP during late summer - fall 1992, testing the response of three macrophyte species to 10 μM NO_3^-N enrichment (means $\pm$ 1 SE).

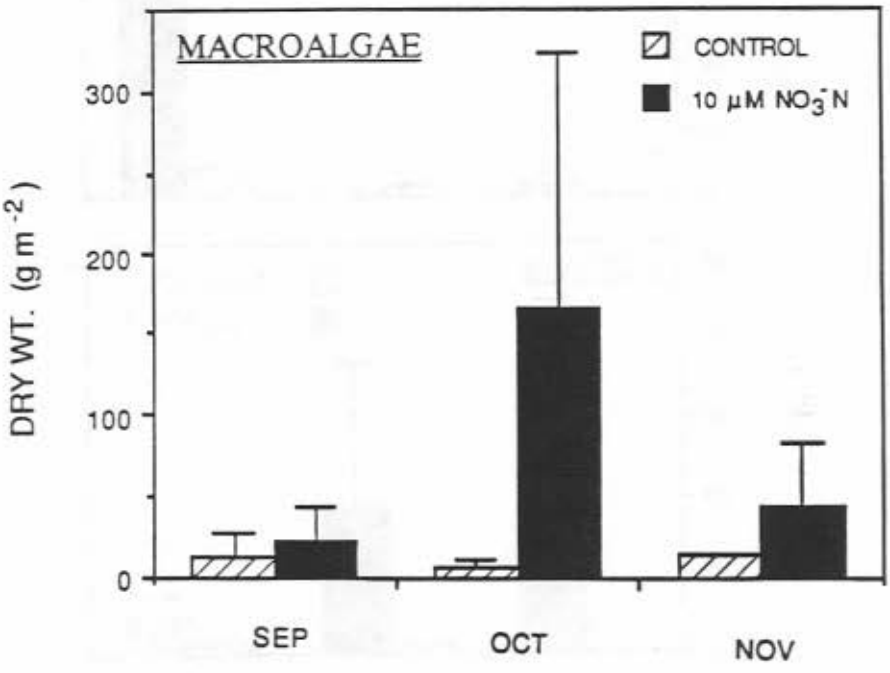

Figure 9. Response of macroalgae to 10 μM NO_3^-N enrichment in late summer - fall 1992, as harvested dry weight per unit sediment surface area (means $\pm$ 1 SE). Note that although there was a trend for higher macroalgal biomass under N enrichment, the high variability in macroalgal response resulted in production that was not significantly different in control versus enriched mesocosms.

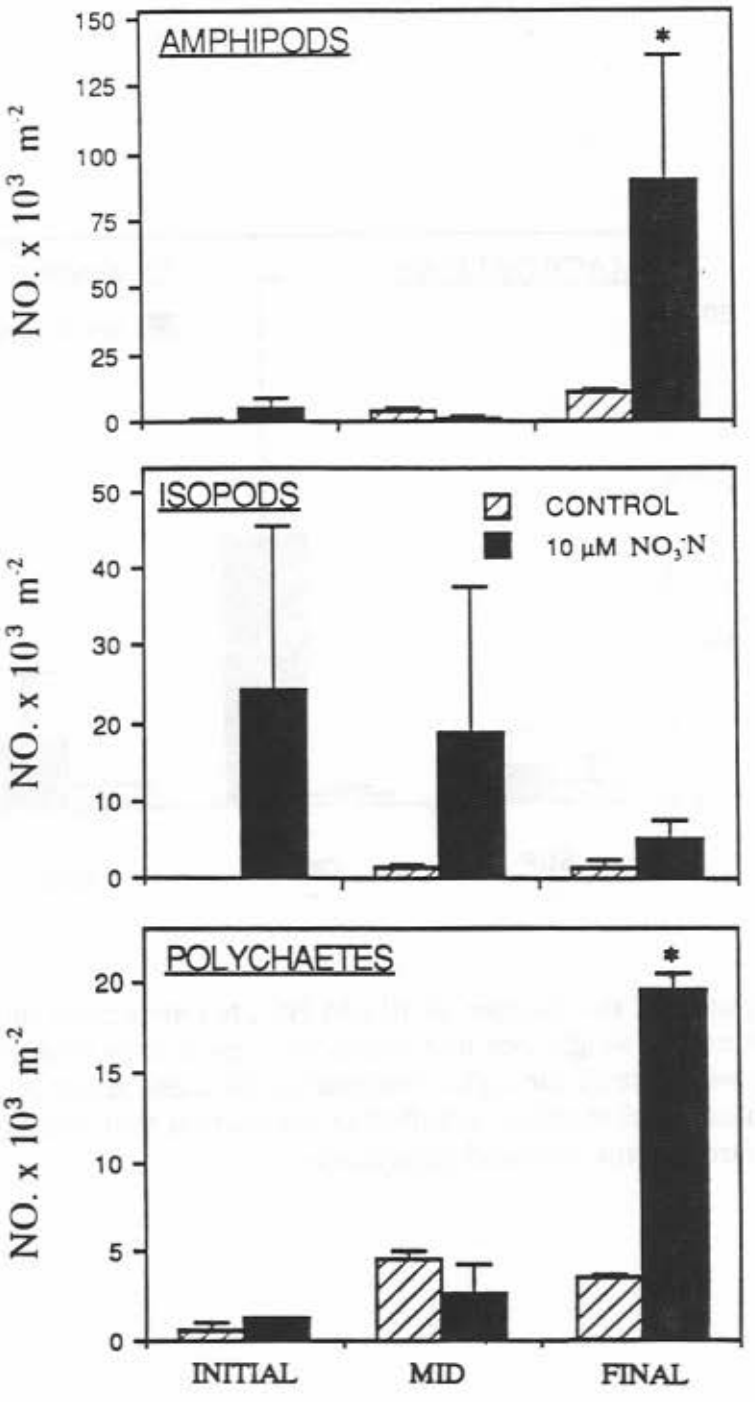

Figure 10. Densities of the three most abundant components of the macroinvertebrate community during late summer - fall 1992, as individuals per unit sediment surface area (means $\pm$ 1 SE). Asterisks (*) indicate significantly higher abundance ($p < 0.05$).

other mesocosms). By Dec. amphipod densities were significantly higher in N-enriched mesocosms than in controls ($p < 0.05$; Fig. 10). Polychaetes were significantly more abundant among nitrate-enriched mesocosms than in controls ($p < 0.05$), indicating a positive (indirect) effect of water-column nitrate in enhancing organic detritus and other food resources.

Growth of *Zostera marina* in replicated mesocosms was less variable in fall than during spring. As in spring, similar although 2-fold higher invasion by the pathogen *Labyrinthula zosterae* was observed in the leaf tissue of LTMA and recent field transplants in control and N-enriched plants ($18 \pm 4\%$ versus $25 \pm 8\%$ infection, respectively; $n = 3$ dates in early, mid and late fall). Higher NRase activity (410 ± 120 nM NO_2^- g^{-1} h^{-1} and 820 ± 50 nM NO_2^- g^{-1} h^{-1} in control and enriched recent field transplants, respectively, after 3 wk), higher N tissue content, higher C/N ratios, and lower N/P ratios in above- and belowground tissue of N-enriched plants relative to controls indicated that the enriched plants had taken up a portion of the nitrate ($p < 0.05$; Figs. 11, 12, 13). Total protein content was similar among control and N-enriched shoots, whereas GS activity was lower in N-enriched plants (GS at 42 ± 1 versus 31 ± 6 mmol - glutamyl hydroxamate g^{-1} tissue in controls and N-enriched plants, respectively, after 3 wk; $p < 0.06$; Figs. 11, 14).

In enriched *Zostera marina*, however, the carbon content of belowground tissue and the total dissolved carbohydrates of both above- and belowground tissue were significantly lower than in controls by the end of the experiment ($p < 0.05$; Fig. 12). Plants in the N-1 replicate apparently were most adversely affected by water-column nitrate; after 14 wk NRase activity of N-1 plants was much lower than that of plants from other N-enriched or control mesocosms (in 2-h assays, 250 nM NO_2^- / g tissue fresh weight in the N-1 replicate, versus > 2,000 nM NO_2^- / g fresh weight in the other N-enriched replicate and 430 ± 10 nM NO_2^- / g fresh weight in controls). The P content of aboveground tissue in the N-1 eelgrass was low and suggestive of P limitation (Duarte 1990), in comparison to plants from the other enriched and control mesocosms (< 0.08% in N-1 shoots, versus > 0.20% in plants from the other N-enriched replicates and controls). These data indicated that internal nutrient imbalances of the N-1 plants, in particular, were severe. Further, whereas nitrate-enriched plants generally became weakened and easily fragmented in the shoot meristem region, the "crumbling meristem" condition was visibly most pronounced in the N-1 replicate and

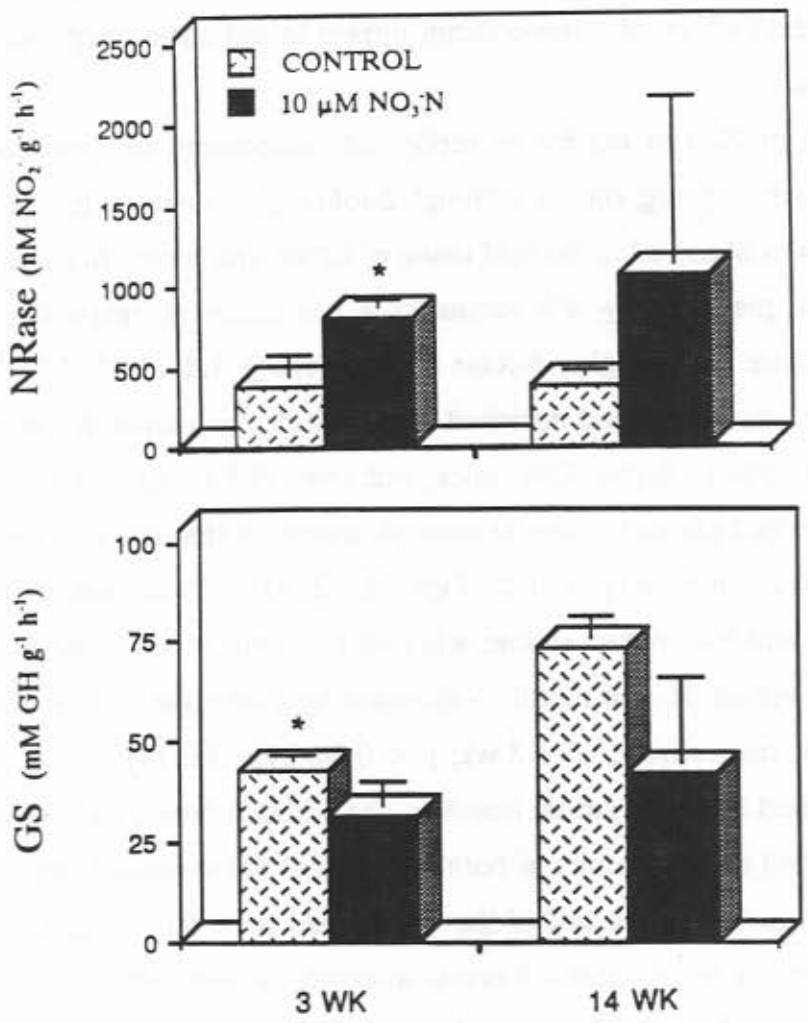

Figure 11. Nitrate reductase activity and glutamine synthetase activity (upper and lower panels, respectively) of control and N-enriched (10 μM NO_3^-N) recently transplanted *Zostera marina* after 3 wk and 14 wk in late summer - fall 1992 (means $\pm$ 1 SE). Asterisks (*) indicate significantly higher enzyme activity ($p < 0.05$).

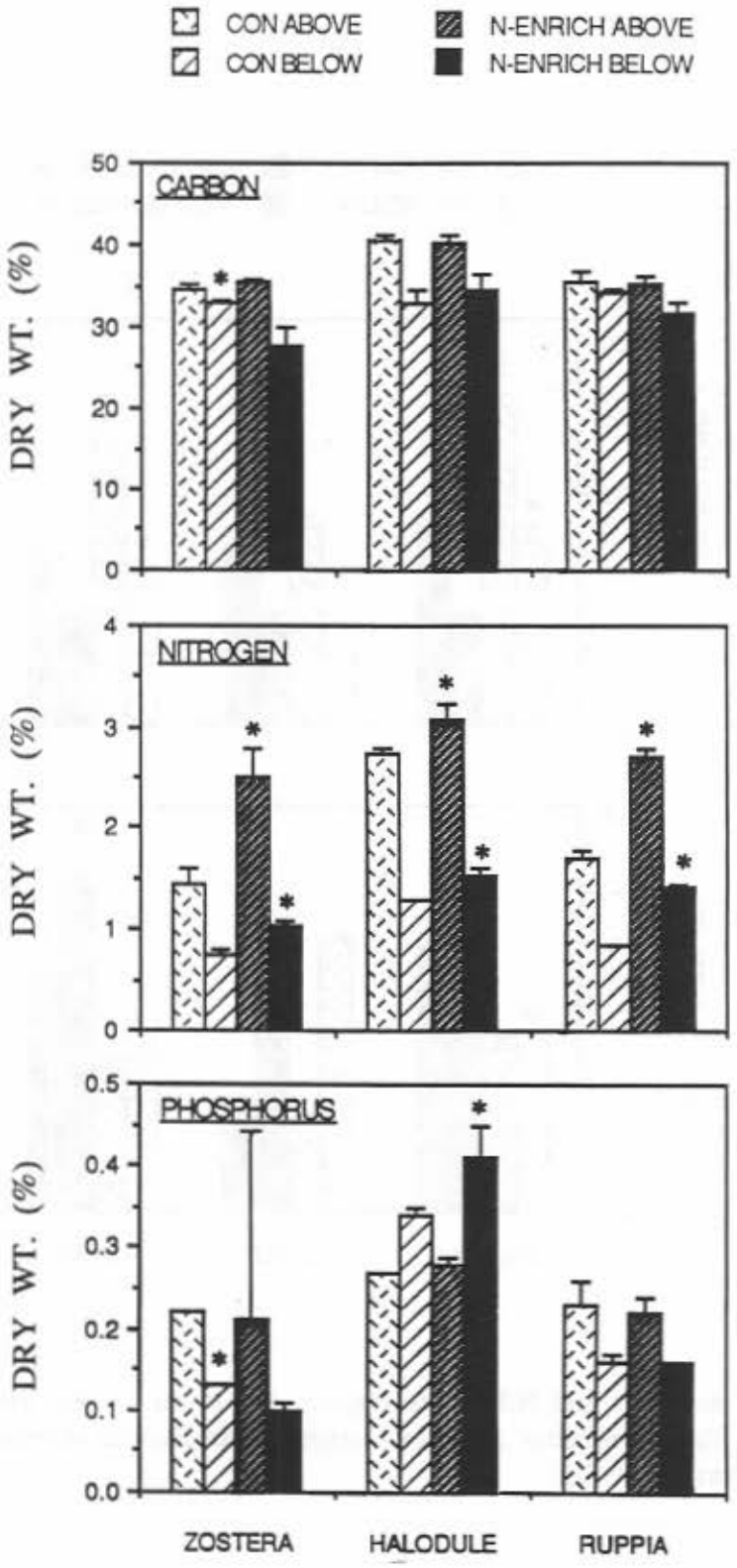

Figure 12. Tissue content (percent dry weight) of C, N and P in aboveground (above) and belowground tissue of recently transplanted *Zostera marina, Halodule wrightii*, and *Ruppia maritima* during late summer - fall 1992 (means + 1 SE).

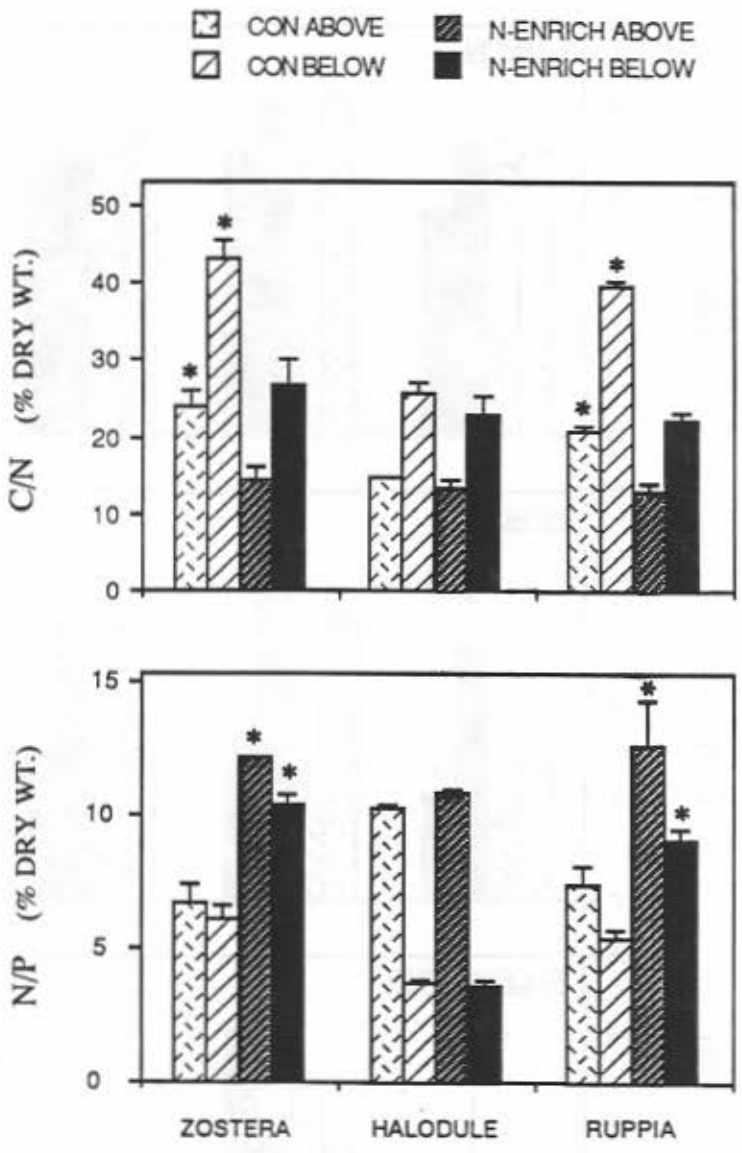

Figure 13. Tissue C/N and N/P ratios (upper and lower panels, respectively) of recent transplants of *Zostera marina, Halodule wrightii*, and *Ruppia maritima* during late summer - fall 1992 (means $\pm$ 1 SE).

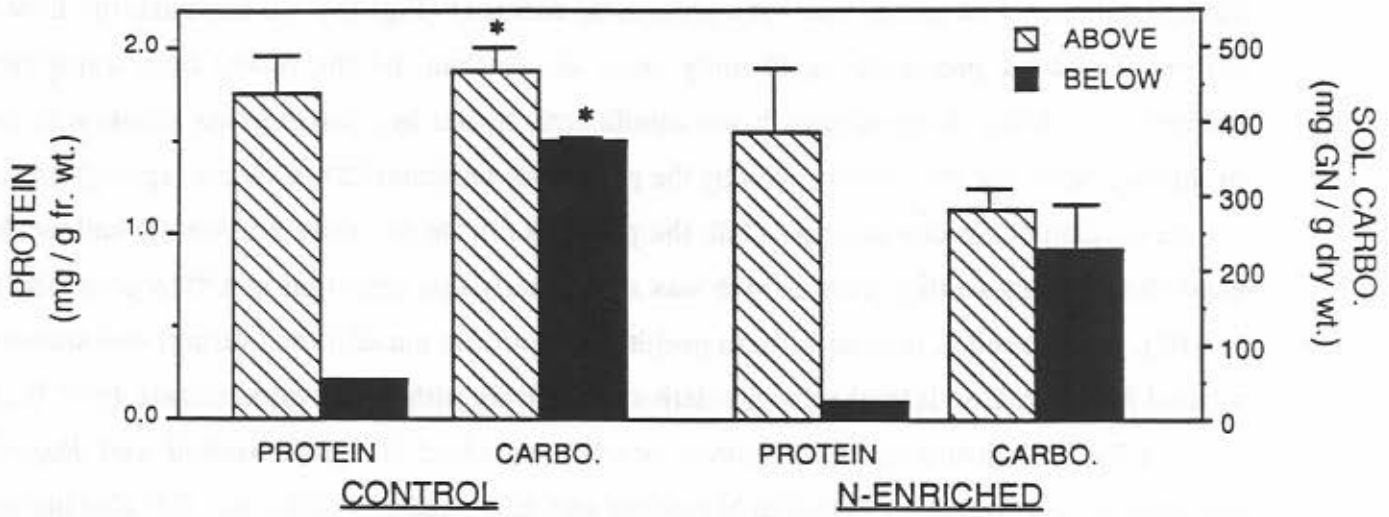

Figure 14. Total protein and acid-soluble (dissolved) carbohydrate content of recently transplanted *Zostera marina* after 14 wk in late summer - fall 1992 (means $\pm$ 1 SE). Asterisks (*) indicate significantly higher tissue content ($p < 0.05$). Note that for acid-soluble carbohydrates, a standard curve was produced using D-glycogen (data given as mg glycogen g^{-1} dried plant powder).

coincided with higher water-column phosphorus than measured in previous weeks (65 μg TP/L in N-1 at 14 wk [Dec.], versus 20 μg TP/L at wk 13, based on duplicate subsamples). Similar increases in water-column TP were not observed in other N-enriched or control mesocosms.

Zostera marina without nitrate enrichment prehistory showed a trend for decreased lateral growth under moderate nitrate enrichment in fall, although shoot production of control and enriched plants was not significantly different (Fig. 15). In contrast, the LTMA control *Z. marina* produced significantly more shoots than did the recent field transplant controls ($p < 0.05$). Lateral growth was significantly higher by control LTMA plants with no prehistory of nitrate enrichment than by the previously enriched LTMA shoots (spring) under nitrate enrichment in late summer - fall; the previous nitrate exposure apparently had weakened those plants so that their growth was significantly less than that of LTMA controls ($p < 0.05$). N-enriched *Z. marina* without prehistory of nitrate enrichment (spring) also showed a trend for decreased lateral growth relative to controls without elevated nitrate ($p < 0.2$).

Like the nitrate-enriched *Zostera marina,* enriched *Halodule wrightii* and *Ruppia maritima* were significantly higher in N content and, consequently, had lower C/N and higher N/P ratios than did unenriched controls by late fall, indicating elevated nitrate uptake and tissue N accumulation ($p < 0.05$; Figs. 12, 13). However, *H. wrightii* and *R. maritima* appeared to have innately higher N content, especially *H. wrightii*; control plants of both species were significantly higher in N than *Z. marina* (above- and belowground tissue; $p < 0.05$; Fig. 12). In both control and N-enriched regimes, tissue C and P content were significantly higher in *H. wrightii* than in *Z. marina* or *R. maritima* (both above- and belowground tissue; $p < 0.05$; Fig. 12). Further, belowground tissue C content was significantly depressed in N-enriched *R. maritima* and *Z. marina* relative to that of unenriched controls ($p < 0.05$), whereas *H. wrightii* maintained comparable belowground C with and without water-column nitrate enrichment.

In contrast to the apparent negative effect of moderate water-column nitrate enrichment on *Zostera marina* shoot growth, at the population level *Haldule wrightii* was mildly stimulated by nitrate (Fig. 15). This species showed a small decline in shoot production by unenriched controls, and an increase in numbers of enriched shoots. *Z. marina, H. wrightii* and unenriched *Ruppia maritima* increased shoot production by < 50%

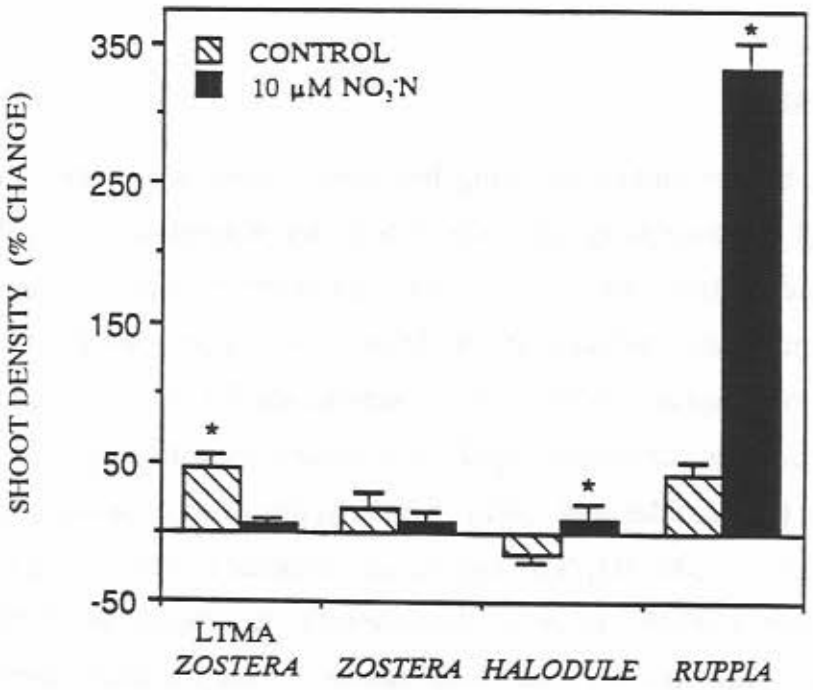

Figure 15. Shoot production (lateral growth) of the three macrophyte species over the duration of the late summer / fall 1992 experiment testing response to 10 μM NO_3^-N enrichment (initial and final dates compared as percent change in shoot numbers; means $\pm$ 1 SE). Comparisons include long-term-mesocosm-acclimated (LTMA) *Zostera marina* with prehistory of water-column nitrate enrichment (in spring), and recently transplanted *Zostera marina, Halodule wrightii,* and *Ruppia maritima* without enrichment prehistory (initial and final dates compared as percent change in shoot numbers; means $\pm$ 1 SE). Asterisks (*) indicate significantly higher shoot production of control or N-enriched plants, considering each of the four plant types separately ($p < 0.05$).

during the experiment. *R. maritima,* however, was highly stimulated by pulsed moderate nitrate additions and increased shoot production by more than 300% over the course of the experiment (significantly greater lateral growth than shown by controls, enriched *Z. marina,* or enriched *H. wrightii*; $p < 0.05$).

IV. DISCUSSION

During the unusually cold spring, low water-column nitrate enrichment (5 μM NO_3^-N d^{-1}, 12 wk) did not discernibly affect shoot densities of eelgrass, although by the end of the experiment internal balances in N-enriched plant nutrient supplies had shifted relative to the nutrient content of unenriched controls. In previous research we demonstrated that during an unusually warm spring (1990) under otherwise-similar experimental conditions, *Zostera marina* shoot densities decreased significantly after 8 wk with only 3.5 μM NO_3^-N in pulsed daily additions (Burkholder *et al.* 1992). Similarly, during an unusually warm autumn (1990), nitrate additions at 7 μM NO_3^-N d^{-1} significantly reduced lateral growth of recent field transplants of *Z. marina* relative to unenriched controls (Burkholder *et al.* 1992). Under cooler temperatures in late summer - fall 1992, however, recently transplanted eelgrass did not significantly decline over a comparable experimental duration at 10 μM NO_3^-N d^{-1}. Collectively, these findings point to potential synergisism between warm temperatures and water-column nitrate enrichment in promoting eelgrass decline (e.g., Salisbury & Ross 1978, North & Zimmerman 1984), an hypothesis that remains to be tested.

The enriched plants in the N-3 replicate from the cold-temperature spring experiment likely were influenced by the decrease in light availability that extended for a 3-wk period, as well as by the elevated water-column nitrate. Small error bars in the tissue data for N-enriched plants indicate that the three replicates remained similar in total C, N, and P content following increased light availability in the N-3 mesocosm; for example, belowground carbon content, reflecting carbon storage, was comparably depressed among the plants from all 3 enriched mesocosms relative to controls. Nonetheless, the N-3 plants were discarded after the spring nitrate exposure to avoid the potential confounding factor of the 3-wk difference in former light regime when interpreting spring N-enriched plant response to subsequent nitrate enrichment. In the subsequent experiment, eelgrass with this

prehistory of exposure to water-column nitrate additions was adversely affected by moderate nitrate enrichment during late summer - fall, relative to the unenriched controls.

In contrast, N-enriched eelgrass without prior exposure to elevated nitrate maintained a qualitative but non-significant decrease in shoot density in comparison to controls. Differences between plant duration in the mesocosms prevented statistical comparison of the LTMA populations from the spring - fall manipulation with the more recently transplanted eelgrass. However, the higher shoot growth shown by LTMA control plants relative to more recent field-transplanted controls suggests that the mesocosm environment did not negatively affect eelgrass, and that prehistory of water-column nitrate enrichment may weaken *Zostera marina* for survival under subsequent nitrate loading. Pre-exposed plants would be at a disadvantage if, for example, the high carbon demand resulting from sustained nitrate uptake and conversion to amino acids by leaf tissue (Turpin 1991) interfered with carbohydrate synthesis or storage, or with production of anti-microbial phenolics (Bazzaz *et al.* 1990). Although we observed no apparent increase in the common pathogen *Labyrinthula zosterae* on N-enriched eelgrass relative to controls, this effect has been reported by other researchers (Buchsbaum *et al.* 1990).

Our data indicated that nitrate enrichment can impair carbohydrate metabolism in *Zostera marina*. During the late summer - fall growing season, enriched and control plants were comparable in total protein content, but other N-containing constituents were not measured; for example, excess nitrogen in the enriched eelgrass could have accumulated as free amino acids or other N-containing, non-proteinaceous components. The low NRase, high tissue N, extremely low aboveground tissue P, and meristem condition of the N-1 eelgrass in late summer - fall collectively were considered to provide evidence that these plants likely were moribund after 14 wk of water-column nitrate additions. In previous experiments (Burkholder *et al.* 1992) and in the late summer - fall N-1 replicate, the "crumbling meristem" condition of nitrate-enriched shoots preceded plant death by 1-2 wk, and coincided with significantly higher water-column total phosphorus concentrations than in previous weeks. One possible explanation is that the enriched eelgrass shoots lost membrane structural integrity prior to death, resulting in substantial phosphorus leakage (reported, for example, in stressed terrestrial plants prior to death; Levitt 1980). In future

work, determination of the fate of incorporated excess nitrate, including associated phosphorus demands and carbon "sinks," will provide insights about the physiological mechanisms underlying inhibition of *Z. marina* by water-column nitrate enrichment.

The mild stimulation of *Halodule wrightii* and the strong stimulation of *Ruppia maritima* under moderate nitrate enrichment suggest that unlike *Zostera marina,* these species have developed more advantageous physiological mechanisms for controlling uptake and assimilation of water-column nitrate. *H. wrightii* and *R. maritima* are considered to co-exist with eelgrass on the North Carolina coast; their innate seasonality is believed to preclude sustained competitive interactions with the more cold-optimal *Z. marina* (Thayer *et al.* 1984). In natural habitat under nitrate enrichment from septic effluent leachate and other sources, however, *R. maritima* and/or *H. wrightii* have been observed to gradually replace eelgrass without reappearance of *Z. marina* in colder seasons (Harlin & Thorne-Miller 1981, Orth & Moore 1983, Batiuk *et al.* 1992, Burkholder unpubl. data).

The results from this research have important implications for management of submersed aquatic vegetation in coastal habitat. *Zostera marina* is more sensitive to simulated cultural eutrophication than either of the two other seagrass species, and likely would be outcompeted by *Halodule wrightii* or *Ruppia maritima* in mixed beds impacted by progressive nitrate enrichment. In nitrate-enriched locations such as coastal lagoons with loading from septic effluent leachate where eelgrass has declined, *H. wrightii* or *R. maritima* might be successfully transplanted to re-establish vegetated coastal habitat. Eelgrass is considered the most valuable habitat for maintenance of some commercially important fisheries, however (McRoy & Helfferich 1980, Thayer *et al.* 1984), since these species apparently do not utilize meadows of *H. wrightii* or *R. maritima* when *Z. marina* is no longer available. For *Z. marina,* confronted by projected increases in nitrate loading within many coastal waters throughout the world (Miller 1992, World Resources Institute 1992), suitable area for colonization is projected to decrease. Collectively, our findings from 1990 and 1992 suggest that efforts to maintain or re-establish eelgrass in warm, poorly flushed eutrophic embayments and lagoons with seasonal anthropogenic nitrate loading will have low probability for long-term success.

V. RECOMMENDATIONS

Previous research has demonstrated that during an unusually warm spring (with water temperatures 4°C above the monthly average over 10 yr) under otherwise-similar experimental conditions, *Zostera marina* declined significantly after 8 wk with only 3.5 μM NO_3^-N in pulsed daily additions (Burkholder *et al.* 1992). In contrast, during the unusually cold 1992 spring, 5 μM d^{-1} enrichment for 12 wk caused no discernable adverse effects for eelgrass, although plants apparently were weakened for survival under additional nitrate loading in fall. Based on data from the 1990 and 1992 experiments considered collectively, the threshold concentration of nitrate that permits eelgrass survival under pulsed loading should be viewed as a range that lies between 3 and < 10 μM NO_3^-N. Caution should be used by regulatory agencies, however, in adopting a concentration of ca. 3 μM NO_3^-N (pulsed daily spikes, 8 wk = loading of ca. 5 g m^{-2} over that period) or other value as a permissible level of nitrate addition to eelgrass habitat, until the synergistic relationship between water-column nitrate enrichment and increasing temperature in promoting eelgrass decline can be experimentally evaluated.

The upper level of < 10 μM NO_3^-N in this suggested threshold range coincides with the permissible maximum average concentration of dissolved inorganic nitrogen (i.e., nitrate + ammonium) indicated by Batiuk *et al.* (1992) to sustain beds of submersed angiosperms in the Chesapeake Bay, although for a different reason -- the authors believed that phytoplankton blooms and associated biogenic turbidity would be enhanced at higher dissolved inorganic nitrogen levels. Ambient nitrate concentrations generally range from 3 to 5 μM NO_3^-N or higher in the Chesapeake Bay (Batiuk *et al.* 1992, Neckles 1993), where loss of eelgrass habitat has been correlated with increasing water-column turbidity. In such habitats, nitrate enrichment and warming temperatures likely are synergistic in reducing eelgrass viability.

Experiments that are critically needed to resolve the potential interaction between nitrate and temperature in controlling eelgrass survival will require imposing a gradient of NO_3^-N (e.g., 2, 4, 6, 8 and 10 μM) across carefully controlled gradients of temperature and light, and comparing the seasonal response of the eelgrass populations. These experiments should be completed in northern, mid and southern regions of the geographic range for *Zostera marina,* since plant response is expected to vary depending on the physiological race and previous "prehistory" of water-column nitrate exposure. Regulatory agencies in areas

with pulsed nitrate enrichment at ca. 3 μM NO_3^-N d^{-1} or higher for a sustained period of 2-3 months -- a situation that increasingly describes many coastal areas affected by agricultural runoff during spring, or year-round inputs of sewage, or spring / summer tourism -- should consider replacement of lost eelgrass habitat by *Ruppia maritima* or, in more southern locations, by *Halodule wrightii*.

The results of these and previous experiments in the North Carolina experimental mesocosm system have ominous implications for efforts to protect endangered eelgrass habitat. From the perspective of nitrate loading, *Zostera marina* is an oligotrophic plant; in North Carolina during high-temperature spring conditions, it is highly sensitive to nitrate at concentrations above minimal enrichment levels. We intend to continue research to understand the physiological strategies that impart great success to *R. maritima*, versus inhibition of *Z. marina*, under water-column nitrate enrichment. Once the physiological mechanisms for control of nitrate uptake and incorporation are better understood from this comparative standpoint, perhaps genetic engineering to impart greater resistance in *Z. marina* to adverse levels of nitrate enrichment may be feasible as a long-term goal. In many developed coastal areas, it may not be feasible to reduce nitrate to permissible levels for long-term eelgrass survival. Nitrate-containing fertilizer usage in North Carolina, alone, increased about 400% from 1945 - 1983 (Jacobs & Gilliam 1985). Acid precipitation now contributes up to 25% of the nitrate inputs to North Carolina and Chesapeake estuaries (Magnien *et al.* 1992; H. Paerl, pers. comm.), and this source is projected to increase (Paerl 1985, Miller 1992, World Resources Institute 1992).

These nonpoint sources that are difficult, at best, to control, coupled with increasing nitrate loading in wastewater point sources from exponential population expansion along our coasts (Smayda 1989) -- and additional eelgrass losses from turbidity caused by dredging, shoreline erosion, and other activities related to coastal development (Giesen *et al.* 1990) -- point to increasing loss of eelgrass habitat. In many regions, eelgrass meadows have already declined to ca. 10-50% or less of the areal extent shown by historic records. On the basis of the North Carolina mesocosm data, there is little hope of long-term re-establishment of *Zostera marina* by transplanting efforts in poorly flushed, eutrophied upper embayments and coastal lagoons until, perhaps, the physiological mechanisms for this plant's reaction to nitrate might be corrected by molecular techniques. For the present, further declines in eelgrass meadows appear inevitable as long as nitrate enrichment is perpetuated.

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APPENDIX: ABBREVIATIONS AND TERMS

Abbreviations

A. Units of Measurement

°C	Degrees Celsius (or degrees Centigrade)
%	Parts per hundred (percent)
‰	Parts per thoudand (salinity units)
$\mu g\ l^{-1}$	Micrograms per liter (concentration unit)
$mg\ l^{-1}$	Milligrams per liter (concentration unit)
nM	Nanomolar (10^{-9} molar, or 10^{-9} moles l^{-1}; concentration unit)
μM	Micromolar (10^{-6} molar, or 10^{-6} moles l^{-1}; concentration unit)
$nM\ g^{-1}\ h^{-1}$	Nanomolar per gram per hour (assay of enzyme activity)
$mmol\ g^{-1}$	Millimoles per gram (concentration unit)
sec	Second
h	Hour
d	Day
wk	Week
yr	Year
$\mu Einst\ m^{-2}\ sec^{-1}$	MicroEinsteins per square meter per second (light quantity)
BTU	British thermal unit (cooling capacity units)
cm^2	Square centimeter (area unit)
m	Meter(s) (length unit)
m^2	Square meters (area unit)
km	Kilometer(s) (length unit)
km^2	Square kilometer (area unit)
ha	Hectare ($10^4\ m^2$) (area unit)
$cm\ d^{-1}$	Centimeters per day (leaf growth)

B. Parameters

pH	$-\log(H^+)$, or minus the log of the hydrogen ion concentration; measure of the acidity of the water.
DO	Dissolved oxygen concentration.
NH_4^+N	The nutrient ammonia, as ammonium-nitrogen concentration (N content of that form), which is the preferred inorganic nitrogen source for many phytoplankton and submersed macrophytes; generally low in concentration but added by precipitation and high in sewage.

NO_3^-N The nutrient nitrate (as nitrate-nitrogen concentration), which is the second form of inorganic nitrogen used by plants; also added by precipitation.

NO_2^-N Nitrite, as nitrite-nitrogen concentration, substrate used in assay of NRase activity.

TP Total phosphorus, including dissolved and particulate, organic and inorganic forms.

SRP Soluble reactive phosphate; refers here to $PO_4^{-3}P$ (phosphate-phosphorus), the form of the nutrient, phosphorus which is immediately available for uptake by plant cells.

Terms

Aerobic: Referring to an environment or process in which dissolved oxygen is present.

Algae (plural; alga, singular): Primitive plants that may photosynthesize like higher plants, but mostly lack vascular tissue (and, therefore, have no flowers, roots, stems or leaves).

Amphipod (order Amphipoda within one of two main lines of subclass Mandibulata, the Crustacea, in Phylum Arthropoda): Small macroinvertebrates, mostly marine with a few freshwater and amphibious representatives. The body is usually bilaterally flattened (i.e., flattened from side to side); they have no carapace (hard chitinous exoskeleton covering on head and thorax), with gills occurring in the mid-region (thorax) of the body. May filter-feed through specialized appendages (Meglitsch 1972).

Angiosperm: Flowering plant.

Anaerobic: Referring to an environment or process in which dissolved oxygen is absent.

Anoxia: Status wherein the water (e.g., the hypolimnion) is depleted of dissolved oxygen. Anoxia typically develops in seagrass beds in darkness, resulting from high respiration of abundant plants and animals.

Autotrophic: Requiring only inorganic compounds for nutrition, along with energy provided by light.

Benthic: Bottom-dwelling; growing on or within the sediment, or growing attached to a substratum that is in contact with the sediment (e.g., on rocks, seagrasses, etc.).

Biomass: The total living particulate organic matter (generally reported as fresh or dry weight) present per unit volume of water, or per unit of mud-substratum surface.

Chlorophyll: Green pigment found in all plants that undergo photosynthesis (including, for example, blue-green algae, other algae, and angiosperms).

Community: A group of interacting populations within a given habitat; e.g., the *Zostera* community refers to all bacteria, fungi, plants and animals inhabiting an eelgrass meadow.

Concentration: Quantity of a chemical (for example, quantity of nitrate) added per unit volume (e.g., micrograms of nitrate per liter).

Cultural Eutrophication: Enriching of aquatic systems by anthropogenic nutrient sources; somtimes referred to as "accelerated" eutrophication.

Epiphyte: Algae growing on other plants; in this report, the term refers to microalgae and small macroalgae (mostly red algae, Division Rhodophyta) growing on the submersed angiosperms.

Eutrophic: High in nutrients and high in organic (biological) production (original meaning -- nutrient-rich). Eutrophic estuarine and marine coastal waters typically are shallow with limited light transparency from algal blooms and suspended sediments, and abundant plant nutrients in both the water column and the sediment. Late summer algal blooms by phytoplankton or macroalgae may be common.

Extinction Coefficient: An expression of the exponential attenuation of irradiance at depth in relation to that at the surface. The total extinction of natural waters is the sum of absorption by the water itself, dissolved compounds, and suspended particles.

Isopod (order Isopoda within one of two main lines of subclass Mandibulata, the Crustacea, in Phylum Arthropoda): Small aquatic, amphibious or terrestrial macroinvertebrates with dorsoventrally flattened bodies (i.e., flattened from upper surface to lower surface), no carapace (hard chitinous exoskeleton covering on head and thorax), and abdominal gills; feed on particulate matter (note -- filter-feeding through specialized appendages is not involved in obtaining food; Meglitsch 1972).

"Light" (Photosynthetic Photon Flux Density):

The number of photons (quanta) in the 400- to 700-nanometer (10^{-9} meters) wave band incident per unit time on a known amount (unit) of surface area (i.e., the the photon flux density of photosynthetically active radiation in μEinst m^{-2} sec^{-1}) (Wetzel 1983).

Light Absorption: The diminution of light energy with depth by transformation to heat (Wetzel 1983).

Loading: Total quantity of nutrient added per unit surface area of a system, per unit time. In our 1992 spring experiment, for example, sufficient nitrate was added to effect an initial concentration of ca. 5 μM NO_3^-N d^{-1} in the water of enriched mesocosms immediately after treatment. Given a mean volume of ca. 1,250 l per mesocosm,

and a mean surface area of 2 m^2, this would translate into a loading of ca. 38 mg m^{-2} d^{-1}, or a total of ca. 3.2 g m^{-2} over the 12-week treatment period (spring season). Nutrient loadings to an estuary are most frequently calculated as kilograms of nutrient added per square kilometer per year (e.g., Magnien *et al.* 1992). While such data are valuable from the standpoint of comparing nutrient enrichment among estuaries, *seasonal loadings -- especially in the spring period of crop fertilization and frequent precipitation / runoff events* -- would be more useful in interpreting the potential for nitrate inhibition of eelgrass. Regulatory agencies involved in seagrass habitat protection should consider seasonal mean nutrient concentrations, and seasonal nutrient loadings, in strategies to optimally manage remaining seagrass habitat.

Macroalga: A macroscopic alga that is clearly discerned without aid of a microscope.

Macroinvertebrate: A macroscopic invertebrate.

Macrophyte: Although technically this term means "macroscopic plant," it is usually restricted to refer to vascular plants.

Mesocosm: A mid-sized enclosure (1-3 m in diameter and > 100 L in volume).

Mesotrophic: Moderate in production; intermediate between eutrophic and oligotrophic.

Microalgae: Microscopic algae that cannot be clearly discerned without aid of a microscope.

Neutral Density Screen: A screen that removes all light qualities (colors) or wavelengths equally.

Oligotrophic: Low in nutrients and low in plankton production. Oligotrophic waters generally are clear with high light penetration, low in plant nutrients especially in the water column, abundant in water-column DO at all depths at all times, and high in species diversity. In estuarine or coastal habitat, the term usually has a more restricted meaning and refers to waters that are low in nutrients.

Photosynthetically Active Radiation:
Radiant energy (from the sun) in the 400- (blue) to 700- (far-red) nanometer waveband of the visible spectrum (Wetzel 1983).

Phytoplankton: Microscopic algae which are suspended in the water column. Most phytoplankton have only limited ability to control their location, and tend to be distributed by water currents and wind mixing.

Polychaete: Type of worm related to earthworms (Phylum Annelida) that burrows into marine sediments.

Population: A defined assemblage of individuals of one species.

Production: The increase in biomass (weight, volume) formed over a known period of time

(an accumulation over relatively long periods, usually on an annual basis).

Productivity: The increase in growth (weight, leaf length, carbon content) over a known period (a **rate**, usually on the basis of hours or 1 d).

Salinity: The saltiness of water, usually expressed as grams of dissolved salts per kilogram of seawater or as parts per thousand (‰ or ppt). Salinity is defined as the weight of solids obtained by drying 1 kg of water under standard conditions (Dawes 1981).

Seagrass: An ecological grouping of marine monocotyledonous plants. Throughout the world there are only ~50 species of submersed flowering plants in marine habitat. These plants are not true grasses (i.e., not in the family Poaceae); rather, they are more closely related to the lily family and are included in the families Potamogetonaceae (*Zostera*, *Halodule*) and Hydrocharitaceae. These plants have well-developed rhizomes (horizontal stems just below the sediment surface) and alternating leaves in two ranks that usually arise from small erect side stems (shoots) or from the rhizomes (Den Hartog 1970, Dawes 1981). They are hydrophilous (i.e., they complete flowering and sexual reproduction while completely submersed). The leaves are flat, ribbon-shaped or cylindrical in cross section, with a well-developed cuticle and closed stomates (residual feature from terrestrial ancesters, as passageways for entrance of gaseous atmospheric CO_2). Supporting vascular tissue is greatly reduced, but the phloem is well developed for transport of nutrients from the sediments to the roots and the aboveground tissue. While most nutrients (except CO_2) are are obtained from the sediments in undisturbed (unenriched) habitat, seagrass leaves are also capable of taking up nutrients such as nitrate and phosphate from the water.

Seaweed: A marine macroalga; originally referred to the typically abundant marine brown macroalgae (division Phaeophyta) from the mid / lower rocky intertidal habitat in temperate regions. The term now refers to marine macroalgae from divisions Chlorophyta, Phaeophyta, and Rhodophyta (green, brown, and red algae, respectively).

Trophic Level: Functionally similar organisms within a biological community. E.g., all primary producers generally are considered to comprise the lowest or primary trophic level that supports the remainder of the food web.

Trophic Structure (of a community): Refers to the pathways by which energy is transferred and nutrients are cycled through the community trophic levels. All primary producers (phytoplankton, benthic microalgae, and submersed or floating plants such as *Zostera, Halodule, Ruppia*, and macroalgae such as *Enteromorpha* and *Ectocarpus* represent the first level of the trophic structure. The primary producers are eaten by primary consumers or herbivores (second trophic level), which are successively consumed by secondary, tertiary, etc. consumers (third, fourth trophic levels, etc.), up to the "top" carnivores of the food web.

Vascular Plant: Plant having vascular tissue (xylem, phloem); includes all angiosperms.

Vegetative (as in vegetative reproduction): Asexually produced.

Figure 9. Response of macroalgae to 10 μM NO_3^-N enrichment in the late summer - fall experiment, as harvested dry weight per unit sediment surface area (means $\pm$ 1 SE). Note that although there was a trend for higher macroalgal biomass under nitrate enrichment, the high variability in macroalgal response resulted in production that was not significantly different in control versus enriched mesocosms.

Figure 10. Densities of macroinvertebrate herbivores and other abundant macroinvertebrates from control and nitrate-enriched mesocosms during the late summer - fall experiment (10^3 individuals m^{-2} sediment surface; means $\pm$ 1 SE).

Figure 11.

Figure 12. Tissue C/N and N/P ratios (upper and lower panels, respectively) of *Zostera marina* without nitrate enrichment prehistory, during the late summer - fall 1992 experiment. Data are given as in Fig. 5.

Figure 13. Total protein and acid-soluble (dissolved) carbohydrate content of *Zostera* without nitrate enrichment prehistory after 14 wk in the late summer - fall 1992 experiment (means $\pm$ 1 SE for above- and belowground tissue). Note that for acid-soluble carbohydrates, a standard curve was produced using D-glycogen and data were reported as mg glycogen g^{-1} dried plant powder.

Figure 14. Lateral growth of the three macrophyte species during the late summer - fall 1992 experiment including long-term mesocosm-acclimated (LTMA) *Zostera marina* with prehistory of water-column nitrate enrichment (in spring), and recently transplanted *Z. marina, Halodule wrightii* and *Ruppia maritima* without nitrate enrichment prehistory (initial and final dates compared as percent change in shoot numbers; means $\pm$ 1 SE).