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# *Zostera marina* (eelgrass) growth and survival along a gradient of nutrients and turbidity in the lower Chesapeake Bay

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ABSTRACT: Survival of transplanted *Zostera marina* L. (eelgrass), *Z. marina* growth, and environmental conditions were studied concurrently at a number of sites in a southwestern tributary of the Chesapeake Bay to elucidate the factors limiting macrophyte distribution in this region. Consistent differences in survival of the transplants were observed, with no long-term survival at any of the sites that were formerly vegetated with this species but that currently remain unvegetated. Therefore, the current distribution of *Z. marina* likely represents the extent of suitable environmental conditions in the region, and the lack of recovery into historically vegetated sites is not solely due to lack of propagules. Poor long-term survival was related to seasonally high levels of water column light attenuation. Fall transplants died by the end of summer following exposure to levels of high spring turbidity ($K_d > 3.0$). Accumulation of an epiphyte matrix during the late spring (0.36 to 1.14 g $g^{-1}$ dry wt) may also have contributed to this stress. Differences in water column nutrient levels among sites during the fall and winter (10 to 15 µM dissolved inorganic nitrogen and 1 µM dissolved inorganic phosphates) had no observable effect on epiphyte accumulation or macrophyte growth. Salinity effects were minor and there were no symptoms of disease. Although summertime conditions resulted in depressions in growth, they did not alone limit long-term survival. It is suggested that water quality conditions enhancing adequate seagrass growth during the spring may be key to long-term *Z. marina* survival and successful recolonization in this region.

KEY WORDS: Chesapeake Bay · *Zostera marina* · Seagrass · Growth · Survival · Epiphytes · Water quality · Inorganic nutrients · Turbidity

## INTRODUCTION

Declines in submersed macrophyte populations have been documented at many locations worldwide during the past several decades. Frequently, potential causes are identified by comparing the existing environmental conditions of formerly vegetated sites either to nearby areas that have remained vegetated or to historical records. In this manner, significant losses of vegetation have often been attributed to excessive anthropogenic inputs of suspended particulate material, dissolved nutrients, or both (e.g. den Hartog & Polderman 1975, Phillips et al. 1978, Davis & Carey 1981, Kemp et al. 1983, Orth & Moore 1983, Giesen et al. 1990, Stevenson et al. 1993).

In order to relate persistent lack of vegetation to unsuitable habitat, environmental conditions and *in situ* plant growth and survival must be studied concurrently. For example, Jupp & Spence (1977) used reciprocal transplants to determine the importance of wave action and sediment nutrient concentrations in limiting macrophyte recolonization and growth in a eutrophic lake. Similarly, Cambridge et al. (1986) concluded from transplant experiments that the conditions initially causing the loss of seagrasses from an Australian sound still existed in that region. Without such information, poor recruitment because of an insufficient supply of propagules remains an alternative hypothesis to explain persistent lack of vegetation.

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*Zostera marina* is the dominant submersed macrophyte in the mesohaline and polyhaline regions of Chesapeake Bay. Historically, extensive seagrass beds covered the shoal areas of less than 2 m depth along the bay and the eastern and western shore tributaries. Declines in abundance of *Z. marina* occurred throughout the bay in the early 1970s (Orth & Moore 1983, 1984). Losses were greatest in the upriver sections of the western tributaries and the deeper, channelward areas of macrophyte distribution. Many areas of lower Chesapeake Bay that once supported dense seagrass beds currently remain unvegetated.

Here we describe a series of studies designed to elucidate the factors limiting submersed macrophyte distribution in one southwestern tributary of Chesapeake Bay, the York River. *Zostera marina* populations declined precipitously from the upriver and deeper areas of the York River by 1974, and many areas remain devoid of vegetation (Batiuk et al. 1992). We used both field manipulations and observations to explore the relationships between macrophyte distribution and environmental conditions in the York River: (1) we tested the hypothesis that environmental quality, rather than macrophyte recruitment, restricts macrophyte distribution to a subset of its former range; (2) we experimentally evaluated the potential for differences in macrophyte growth at currently and formerly vegetated sites; and (3) we quantified differences in water quality between currently and formerly vegetated sites that may be influencing patterns of *Z. marina* abundance. Our results demonstrate environmental control of plant distribution and suggest those variables contributing to persistent lack of vegetation in the region.

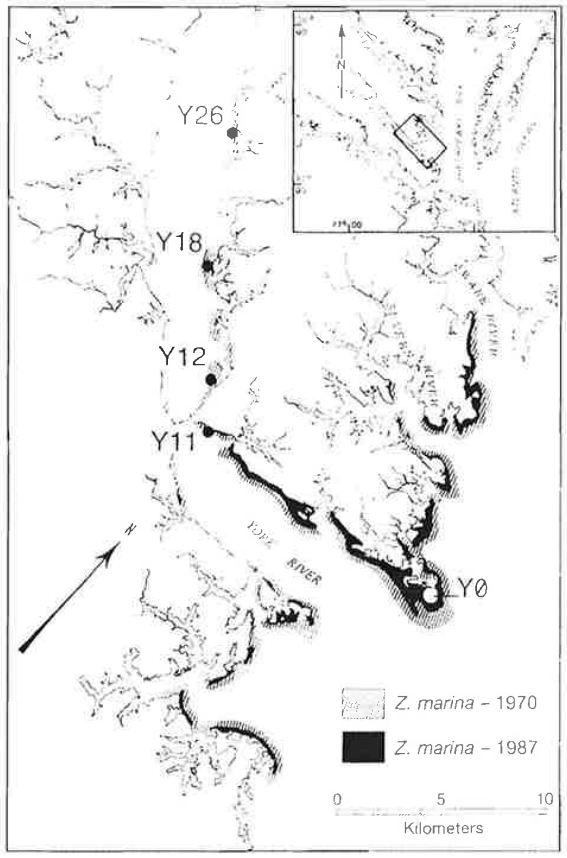


Fig. 1 York River, Virginia, USA, study area showing study sites and submersed macrophyte distributions in 1970 and 1987

## STUDY SITES

Study sites were established in the York River, Virginia, USA, extending from the mouth of the tributary to the historic upriver limits of macrophyte distribution (Fig. 1). We selected sites in areas that had been or are currently vegetated with *Zostera marina* (Marsh 1970, 1973, Orth 1973, Orth et al. 1979). In this region *Z. marina* is most abundant at depths of 80 to 110 cm below mean sea level (MSL) and *Ruppia maritima* L. (sensu lato) occurs at shallower depths (Orth & Moore 1988). All stations were therefore located at approximately 80 cm below MSL to permit our conclusions to be related to the majority of potential *Z. marina* habitat in this region.

The first station in this York River estuarine transect, Y0, (Guinea Marsh; 0 km) is located at the mouth of the tributary and supports *Zostera marina* beds that have decreased only moderately in area since 1937 (Orth et al. 1979). The second station, Y11, (Gloucester Point; 11 km) is located approximately 11 km upriver and is at the upriver limit of the current *Z. marina* distribution. Populations disappeared from this area by 1974, and have since regrown slightly from both transplanting and natural recruitment. The last 3 stations, Y12 (Mumfort Island; 12 km), Y18 (Catlett Island; 18 km), and Y26 (Claybank; 26 km) lie successively upriver. Extensive beds of *Z. marina* disappeared completely from these 3 sites by 1974. All sites are characterized by shallow flats (<2 m below MSL) extending landward from a narrow but much deeper (>10 m below MSL) mid-channel region. Sediments in the shoal areas are principally fine sands.

## METHODS

**Transplant experiments.** We used transplant 'gardens' to test the hypothesis that environmental conditions ultimately limit distribution of *Zostera marina* in

the York River. We transplanted *Z. marina* to currently and formerly vegetated sites to determine the present capacity of various sites to support macrophyte growth. Previous transplanting efforts in this region have determined that fall is the best season to ensure transplant success (Fonseca et al. 1985, K. Moore & R. Onth unpubl. data), therefore transplanting was undertaken in September and October of 1984, 1985, and 1986. Plants were collected from the established bed at Y0, transferred to transplant sites, and responses measured; the designs of the transplant experiments are summarized in Table 1. In 1984, planting units consisted of sods (20 cm × 20 cm) with intact sediments. During subsequent years the shoots were washed free of sediments, and planting units consisted of 10 to 15 shoots bundled together with a metal twist tie similar to methods of Fonseca et al. (1982, 1985) for ease of transplanting. No apparent differences have been observed in the survival rate of transplants in this region using these 2 methods (Fonseca et al. 1985, K. Moore & R. Onth unpubl. data). All vegetation was transplanted within 24 h of removal from the donor site. Planting units were spaced at 2 m or 0.5 m centers (Table 1) in 3 to 4 replicate 5 × 5 arrays of 25 planting units at each site. Survivorship was monitored each year (Table 2) at monthly to bimonthly intervals until either no plants remained at a site or the planting units had coalesced. Survivorship was calculated as the percent of original planting units remaining in individual replicate arrays.

During 1984 and 1985, 4 similar arrays of planting units were established adjacent to the survivorship plots at each transplant site to provide material for destructive sampling. The additional macrophyte responses measured are summarized in Table 1. Plants transplanted in 1984 were sampled in November 1984 and January, March, May, and July 1985. On each sample date, 3 to 5 core samples of 0.33 $m^2$ were taken from the natural seagrass bed at Y0 and 5 arbitrarily selected planting units were excavated from the destructive sampling arrays at each transplant site for macrophyte biomass determination. The plants were washed gently in the field to remove sediment and transported immediately to the laboratory. Leaves were separated from roots and rhizomes and all plant material was dried at 55°C. Five separate samples consisting of 5 large terminal shoots each were collected at each site for epiphyte sampling to quantify differences in epiphyte loads between presently and formerly vegetated sites that may be affecting macrophyte survival. Shoots, which consisted of all leaf material above the meristematic region (Sand-Jensen 1975), were separated from the remainder of the plant and swirled several times in a beaker of filtered seawater to remove loosely adhering material. The leaves in each sample were separated into leaf age classes, and the epiphytic material was scraped into filtered seawater with the edge of a glass microscope slide. Mobile epifauna were discarded. Epiphytic material was collected on pre-combusted glass fiber filters (Gelman, Type A/E), dried at 55°C, and combusted at 500°C for 5 h. The area of leaf substrate for each sample was determined using a Li-Cor Model 31 area meter and leaf dry weight and ash-free weight were determined.

Plants transplanted in 1985 were sampled in March, May, June, and July 1986. At each site, 5 to 7 planting units were arbitrarily collected, from which 5 subsamples containing 5 large terminal shoots each were formed. Epiphytic mass was determined as described previously. The areas of leaves were measured and dry weight and ash-free weight were determined. The biomass of remaining leaves was then calculated from the linear regression of leaf weight on leaf area. Belowground biomass was determined from 3 of the samples. The rhizomes were separated into individual internodes for dry weight and ash-free weight measurements. The roots from all internodes in a sample were combined for analyses.

**Growth experiments.** Although the transplant experiments yielded information on patterns of macrophyte survival and biomass allocation, ambient turbidity prevented us from measuring actual macrophyte growth *in situ*. Therefore, to evaluate the effect of water quality on macrophyte growth at currently and formerly vegetated sites, we relocated turfs of *Zostera*

Table 1. Design of transplant experiments

| Time of transplanting | Method (spacing) | Transplant sites | Response measured |
|---|---|---|---|
| Fall 1984 | Sods (2 m) | Y11, Y26 | Transplant survivorship<br>Entire sods collected for macrophyte biomass[a]<br>Individual shoots collected for epiphytic material[a] |
| Fall 1985 | Bundles (0.5 m) | Y0, Y11, Y12, Y18, Y26 | Transplant survivorship<br>Individual shoots collected for macrophyte biomass and epiphytic material |
| Fall 1986 | Bundles (0.5 m) | Y11, Y12, Y18, Y26 | Transplant survivorship |

[a]Because no plants were transplanted to Y0, samples were taken from natural *Zostera marina* bed

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Table 2. *Zostera marina*. Percent survival at transplant sites. Values are back-transformed means of arcsine square root transformed data. Unlike letters denote significant differences ($p < 0.05$) among sites on each sample date. bd: transplanted planting units coalesced with one another or new recruits beyond determination. E: water column turbidity precluded survivorship determination

| Transplant period | Site | Sample date | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Nov 1984 | Mar 1985 | | May 1985 | | Jul 1985 | Aug 1985 | Oct 1985 |
| Fall 1984 | Y11 | 100 [a] | 82 [a] | | 82 [a] | | 64 [a] | 64 [a] | bd |
| | Y26 | 91 [b] | 36 [b] | | 9 [b] | | 0 [b] | 0 [b] | 0 |
| | | Oct 1985 | Mar 1986 | | May 1986 | Jun 1986 | Jul 1986 | Aug 1986 | Oct 1986 |
| Fall 1985 | Y0 | 100 [a] | 100 [a] | | bd | bd | bd | bd | bd |
| | Y11 | 100 [a] | 60 [b] | | 60 [a] | 60 [a] | 60 [a] | 60 [a] | bd |
| | Y12 | 100 [a] | 64 [b] | | 64 [a] | 64 [a] | 12 [a] | 0 [b] | 0 |
| | Y18 | 100 [a] | 62 [b] | | 56 [a] | 44 [b] | 8 [b] | 0 [b] | 0 |
| | Y26 | 100 [a] | 60 [b] | | 60 [a] | 34 [c] | 0 [c] | 0 [b] | 0 |
| | | Oct 1986 | | Apr 1987 | May 1987 | | Jul 1987 | Aug 1987 | Oct 1987 |
| Fall 1986 | Y11 | 100 [a] | | 80 [a] | 80 [a] | | 41 | 41 [a] | bd |
| | Y12 | 100 [a] | | 87 [b] | 87 [b] | | E | 0 [b] | 0 |
| | Y18 | 100 [a] | | 91 [b,c] | 91 [b,c] | | E | 0 [b] | 0 |
| | Y26 | 100 [a] | | 95 [c] | 95 [c] | | E | 0 [b] | 0 |

*marina* from the stable grassbed at Y0 to sites Y11 and Y26. We measured *in situ* macrophyte growth from April 1985 to July 1986 using a modified leaf marking technique (Sand-Jensen 1975). Whole turfs of *Z. marina*, including roots, rhizomes, and undisturbed sediments to a depth of 20 cm, were obtained from the grass bed at Y0, placed in polyethylene boxes (40 × 60 × 20 cm), and 1 box placed at Y11 and 1 at Y26. After a 2 wk acclimation period, three 15 cm diameter rings were arbitrarily located within each box. Each shoot within each circular quadrat was tagged with a numbered, monel metal band placed around its base. The youngest leaf was marked with a small notch and the leaf lengths and widths were recorded. At approximately weekly intervals the boxes were retrieved, placed in a seawater bath, and the length and width of all leaves on tagged shoots recorded. The number of new leaves on each shoot was recorded, any new shoots within the quadrats were tagged, and the youngest leaf on all shoots was marked. Thus, individual leaves could be uniquely identified and measured from formation through loss. Leaf growth was determined as changes in leaf length. Dry weight and ash-free weights at each sampling period were derived using leaf weight to area relationships determined from the experimental transplants for each period. Specific rates of biomass change were calculated for each marking interval as leaf production or loss divided by initial biomass. Boxes at the sites were disturbed periodically, generally through the burrowing action of crabs or fish. Therefore, when excavation occurred in a box at either site, boxes at both sites were replaced with others that had been acclimating at the respective sites for identical periods of time, generally ranging from 3 to 4 wk. Plants in boxes were not used for survivorship measurements.

Using growth information derived from the marked plants, rhizome production rates of the plants transplanted to Y11 and Y26 in the fall of 1985 were estimated. It was assumed that on average, the individual rhizome internodes were formed at the same rate as leaves (Sand-Jensen 1975, Jacobs 1979, Aioi et al. 1981). Using the calculated leaf formation rates, the ages of individual internodes were thus determined for each of the transplant samples obtained in March, May, June, and July 1986. Rhizome production was then calculated by summing the biomass of rhizome internodes (including roots) produced between sample dates.

**Environmental monitoring.** Worldwide declines of submersed macrophyte populations have been variously attributed to increases in water column turbidity and to increases in dissolved nutrient concentrations and consequent epiphyte accumulation. Therefore, to determine whether water quality differences may be influencing patterns of *Zostera marina* abundance in the York River, we monitored water quality at the transplant sites from January 1985 through December 1987. We collected triplicate subsurface water samples approximately every 14 d at each of the sites. All samples were obtained sequentially on the same day over a 2 to 4 h period beginning with the most downriver site; samples were stored in the dark on ice for up to 4 h while being transported to the laboratory and were analyzed immediately on arrival. Nitrite, nitrate, and ammonium were determined spectrophotometrically

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following the methods of Parsons et al. (1984) and inorganic phosphorus following the methods of USEPA (1979). Suspended matter was collected on precombusted, Gelman Type A/E glass fiber filters, dried to constant weight at 55°C and combusted at 500°C for 5 h. Chlorophyll *a* (chl *a*) was collected on Whatman GF/F glass fiber filters, extracted in a solvent mixture of acetone, dimethyl sulfoxide and 1% diethylamine (45:45:10 by volume) and determined fluorometrically (Shoaf & Lium 1976). Chlorophyll concentrations were uncorrected for phaeopigments. Salinity was measured with a refractometer.

We measured diffuse downwelling photosynthetically active radiation (PAR) from triplicate, water column profiles of photosynthetic photon flux density (PPFD) using an underwater $2\pi$, cosine-corrected sensor (LI-COR, Inc., LI-192SA). These data were obtained concurrently with the water samples. Measurements of PPFD on each sample date were summarized as the attenuation of downwelling PAR. The downwelling attenuation coefficient ($K_d$) was calculated according to Beer's Law.

**Statistical analysis.** Macrophyte and epiphyte response variables and environmental measurements were analyzed using 2-way analysis of variance with main effects of site and date (SPSSx subprogram MANOVA, SPSS, Inc. 1986). Experimental units were replicate arrays for survivorship measurements, samples for macrophyte and epiphyte biomass measurements, quadrats for growth measurements, and water samples or light profiles for environmental measurements. Residual analysis was used to check model assumptions and log transformations were applied where necessary (Neter & Wasserman 1974). Means were compared among sites within sample dates using Tukey or Bonferroni Multiple comparisons with a family confidence coefficient of 0.95.

Table 3. *Zostera marina*. Shoot biomass, 1984 to 1985. Biomass values are back-transformed from means of log transformed data. Unlike letters denote significant differences ($p < 0.05$) among means on each sample date. S/R: shoot to root-rhizome ratio. ns: no survival at Y26 by Jul 1985

| Date | Site | n | Shoot (mg dry mass $sh^{-1}$) | Root-rhizome (mg dry mass $sh^{-1}$) | S/R |
|---|---|---|---|---|---|
| Nov 1984 | Y0 | 5 | 38.80 [a] | 28.23 [a] | 1.37 [a] |
| | Y11 | 5 | 26.14 [a] | 40.39 [a] | 0.65 [a] |
| | Y26 | 5 | 38.13 [a] | 39.78 [a] | 0.95 [a] |
| Jan 1985 | Y0 | 5 | 15.90 [a] | 50.35 [a] | 0.32 [a] |
| | Y11 | 5 | 12.75 [a] | 68.80 [a] | 0.19 [a] |
| | Y26 | 5 | 14.58 [a] | 15.93 [b] | 0.92 [b] |
| Mar 1985 | Y0 | 3 | 18.79 [a,b] | 28.90 [a] | 0.65 [a,b] |
| | Y11 | 5 | 13.31 [a] | 41.18 [a] | 0.32 [a] |
| | Y26 | 5 | 27.40 [b] | 24.85 [a] | 1.10 [b] |
| May 1985 | Y0 | 3 | 105.03 [a] | 61.66 [a] | 1.70 [a] |
| | Y11 | 5 | 39.08 [a] | 45.30 [a] | 0.86 [a] |
| | Y26 | 5 | 53.20 [a] | 35.36 [a] | 1.50 [a] |
| Jul 1985 | Y0 | 3 | 119.65 [a] | 75.49 [a] | 1.58 [a] |
| | Y11 | 5 | 42.48 [b] | 36.58 [a] | 1.14 [a] |
| | Y26 | ns | ns | ns | ns |

## RESULTS

### Transplant experiments

Survival of *Zostera marina* transplants differed consistently between sites upriver and downriver of Y11 during all 3 yr of transplanting (Table 2). At Y11 and Y0, after some initial losses during the winter, the transplants became well established and persistent. At Y26, loss of transplants occurred during the spring and early summer, so that by August no vegetation remained. At Y12 and Y18, although the plants survived for a longer period through the summer than Y26 they also died out completely by the end of August.

Initially no significant differences in shoot biomass measurements of 1984 transplants were observed among sites (Table 3). By January, however, Y26 shoots had lower below-ground biomass, resulting in a significantly higher shoot to root/rhizome (S/R) ratio. In March, S/R ratios of plants at Y26 remained higher than of those at Y11. By May, increases in growth were evident at all sites. The greatest leaf biomass occurred at Y0. No biomass differences occurred between Y11 and Y26. By July, no living plants remained at Y26, although dead, blackened rhizomes provided evidence of recent, viable plants.

Sampling of the 1985 transplants revealed a similar pattern of S/R ratios along the river axis (Table 4). In March 1986, only the S/R ratios at Y26 were significantly higher than at Y0; by June, the S/R ratio increased with distance upriver. By July all plants at Y26 were gone.

Various measures of epiphytic density (dry or ash-free mass of epiphytes per unit area or mass of leaf tissue) yielded similar patterns among sites, and responses to sites were similar among leaf age classes. Therefore, results are expressed only as dry weight ratios calculated on a whole shoot basis (Table 5). The epiphytic material included diatoms such as *Nitzschia* sp. and *Licmophora* sp. as well as heterotrophic flagellates and bacteria, and attached debris (Neckles et al. 1994). Macroalgae (e.g. *Enteromorpha* sp.) formed a small proportion (<5%) of the total mass and were excluded from analysis. The highest epiphyte mass

Table 4. *Zostera marina*. Shoot biomass, 1985 to 1986. Biomass values are back-transformed from means of log transformed data. Unlike letters denote significant differences ($p < 0.05$) among means on each sample date. S/R: shoot to root-rhizome ratio. ns: no survival at Y26 by July 1986

| Date | Site | n | Shoot (mg dry mass $sh^{-1}$) | Root-rhizome (mg dry mass $sh^{-1}$) | S/R |
|---|---|---|---|---|---|
| Mar 1986 | Y0 | 5 | 22.03 [a] | 30.41 [a] | 0.72 [a] |
| | Y11 | 5 | 29.85 [a] | 33.81 [a] | 0.88 [a,b] |
| | Y12 | 5 | 25.76 [a] | 26.00 [a] | 0.99 [a,b] |
| | Y18 | 5 | 46.56 [b] | 42.27 [a] | 1.10 [a,b] |
| | Y26 | 5 | 47.42 [b] | 30.97 [a] | 1.54 [b] |
| May 1986 | Y0 | 5 | 66.37 [a] | 85.70 [a] | 0.77 [a,b] |
| | Y11 | 5 | 71.28 [b] | 91.62 [a,b] | 0.78 [a,b] |
| | Y12 | 5 | 90.99 [a,b] | 182.39 [b] | 0.50 [a] |
| | Y18 | 5 | 151.71 [b] | 185.35 [b] | 0.82 [a,b] |
| | Y26 | 5 | 104.47 [b] | 95.06 [b] | 1.10 [b] |
| Jun 1986 | Y0 | 5 | 68.08 [a] | 131.52 [a,b] | 0.52 [a] |
| | Y11 | 5 | 117.49 [b] | 195.88 [b] | 0.60 [a,b] |
| | Y12 | 5 | 102.56 [a,b] | 104.95 [a,b] | 0.98 [b] |
| | Y18 | 5 | 168.65 [b] | 172.58 [b] | 0.98 [b] |
| | Y26 | 5 | 136.14 [b] | 63.09 [c] | 2.16 [c] |
| Jul 1986 | Y0 | 5 | 69.34 [a,b] | 95.50 [a] | 0.72 [a] |
| | Y11 | 5 | 210.38 [c] | 234.42 [b] | 0.90 [a] |
| | Y12 | 5 | 95.50 [b] | 106.91 [a,b] | 0.89 [a] |
| | Y18 | 5 | 66.07 [a] | 68.23 [a] | 0.97 [a] |
| | Y26 | ns | ns | ns | ns |

occurred on the Y11 transplants in November 1984. Each year, densities were significantly higher at Y26 than at the other 2 sites immediately before the Y26 transplants disappeared.

Although no formal measures of the incidence of disease were taken, the plants were observed throughout the study for evidence of infection such as might be caused by *Labyrinthula* sp. associated with the eelgrass wasting disease (Muehlstein et al. 1988). Typically, the older leaves on the plants had occasional dark patches of damaged tissue which covered no more than 5% of the leaf tissue as recently described by Burdick et al. (1993). There was no evidence of necrosis on the younger leaves however, and no evidence of the characteristic infection of younger leaves from adjacent older leaves as has been documented (cf. Short et al. 1988, Burdick et al. 1993). As the production of new leaves slowed during the summer, especially at sites upriver of Y11, older leaves were gradually lost and the numbers of leaves per shoot decreased. Eventually, many shoots were composed of only several small leaves that had ceased elongating, with no evidence of infected spots or patches.

## Growth experiments

At both Y11 and Y26 highest growth rates occurred each spring and a second period of increased growth occurred in the fall (Fig. 2A). Leaf growth was low during the summer and winter (Fig. 2A). Significant differences between the sites were observed only during the spring and fall periods of rapid growth. The rate of leaf formation (Fig. 2C) was significantly greater at Y11 than at Y26 during early September 1985 and during April and May 1986. Rates of leaf loss were highest at both sites during late summer (Fig. 2D). However, leaf loss increased earlier in the season at Y26 than at Y11 (Fig. 2D), resulting in a significantly greater rate upriver, from April through July 1986. The rate of leaf growth was greater at Y11 throughout the spring and fall periods (Fig. 2A). Differences in leaf replacement and growth resulted in considerable seasonal differences in shoot size between sites. For example, the mean shoot biomass at Y11 in May 1986 was 45 mg compared to 11 mg at Y26. Similar site differences of lesser magnitude occurred in the fall

Table 5. *Zostera marina*. Epiphytic density (g $g^{-1}$ dry mass $^{-1}$) for 1984 and 1985. Data are back-transformed from means of log transformed data. Unlike letters denote significant differences ($p < 0.05$) among sites on each sample date. ns: no survival at Y26 by July 1985 and July 1986

| Transplant period | Site | Sample date | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Nov 1984 | Jan 1985 | Mar 1985 | May 1985 | | Jul 1985 | Oct 1985 |
| Fall 1984 | Y0 | 0.67 [a] | 0.85 [a] | 1.17 [a] | 0.06 [a] | | 0.29 [a] | 1.15 [a] |
| | Y11 | 7.03 [b] | 0.58 [a] | 0.74 [a] | 0.18 [b] | | 0.54 [b] | 1.11 [a] |
| | Y26 | 1.82 [c] | 1.06 [a] | 0.06 [b] | 1.14 [c] | | ns | |
| | | | | Mar 1986 | May 1986 | Jun 1986 | Jul 1986 | |
| Fall 1985 | Y0 | | | 0.07 [a] | 0.07 [a] | 0.13 [a] | 0.70 [a] | |
| | Y11 | | | 1.02 [b] | 0.28 [b] | 0.08 [a] | 0.65 [a] | |
| | Y26 | | | 0.27 [a] | 0.34 [b] | 0.36 [b] | ns | |

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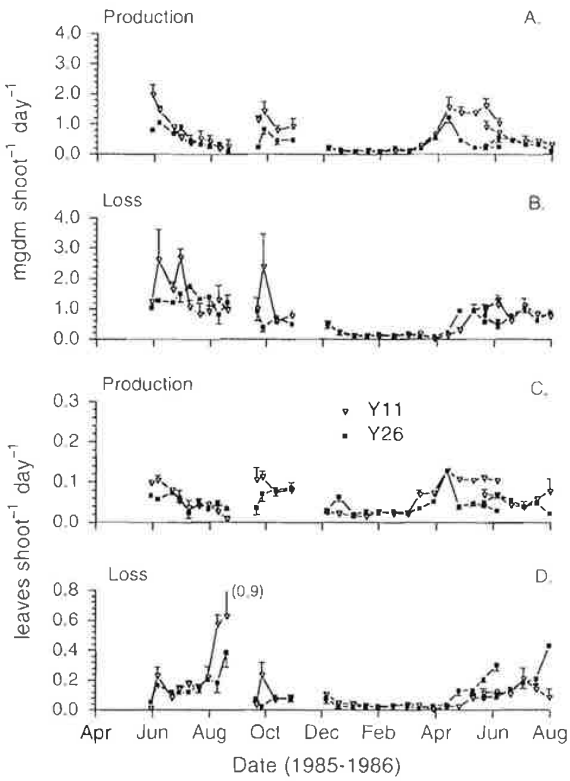


Fig. 2. *Zostera marina*. Results of growth experiments. Mean rates of (A) shoot production, (B) shoot loss, (C) leaf production, and (D) leaf loss for *Z. marina* turfs contained in boxes at sites Y11 and Y26. Bars are 1 SE

as well. This difference in shoot size contributed to a greater rate of total biomass loss at Y11 during the spring and fall (Fig. 2B), although the mean daily net change in biomass remained higher at this site during these periods. Mass specific rates of leaf biomass accumulation and loss at each site followed the same general patterns as did shoot-specific leaf growth.

Below-ground rhizome production (Table 6) was similar at Y11 and Y26 from November to March, during which time rates at both sites were quite low. From March until the die-off of vegetation at Y26 in July, rates were significantly greater at Y11. Maximum production occurred at both sites between March and May.

## Environmental monitoring

Environmental variables were compared among sites within each sampling date. The spatial and temporal distribution of water quality parameters were consistent from year to year, so data are presented graphically as monthly means from 1985 to 1987. For clarity, only data from Y0, Y11, and Y26 are included. Levels of environmental parameters at Y12 and Y18 were generally intermediate between Y11 and Y26.

Water temperatures were similar at all sites with annual minima approaching 0°C in late January and maxima near 30°C in August (Fig. 3A). Salinity decreased approximately 5‰ from Y0 to Y26 (Fig. 3B). Minima and maxima were during January and August, respectively, and paralleled river inflow into the bay system.

Concentrations of total suspended solids (TSS) were variable among sites but usually increased with distance upriver (Fig. 3C). Consistently, each spring (April to June) concentrations at Y26 were significantly greater than at downriver sites. The suspended load consisted principally of inorganic particles; organic content of the seston was usually less than 30%. This percentage decreased with distance upriver.

Patterns of increasing light attenuation ($K_d$) with distance upriver paralleled those observed for the suspended particles (Fig. 3D). Step-wise, multiple regression of $K_d$ on the principal measured components of attenuation [filterable inorganic matter (FIM), filterable organic matter (FOM), and chl *a*] revealed

Table 6. *Zostera marina*. Belowground production for 1985 to 1986. Production data are back-transformed from means of log transformed data. Unlike letters denote significant differences ($p < 0.05$) between sites during each period. na: data not available due to complete mortality at Y26 by 21 July 1986

| Site | Period | Days | n | Mean no. of segments formed | Production (mg dry mass sh$^{-1}$ d$^{-1}$) |
|---|---|---|---|---|---|
| Y11 | 15 Nov 1985 to 18 Mar 1986 | 124 | 25 | 4 | 0.06 [a] |
| Y26 | 4 Nov 1985 to 9 Mar 1986 | 124 | 25 | 5 | 0.07 [a] |
| Y11 | 24 Mar 1986 to 9 May 1986 | 47 | 25 | 8 | 2.05 [a] |
| Y26 | 20 Mar 1986 to 13 May 1986 | 47 | 25 | 3 | 0.63 [b] |
| Y11 | 8 May 1986 to 9 Jun 1986 | 33 | 25 | 3 | 1.18 [a] |
| Y26 | 8 May 1986 to 10 Jun 1986 | 34 | 25 | 2 | 0.26 [b] |
| Y11 | 10 Jun 1986 to 21 Jul 1986 | 42 | 25 | 3 | 0.65 |
| Y26 | 10 Jun 1986 to 21 Jul 1986 | 42 | na | na | na |

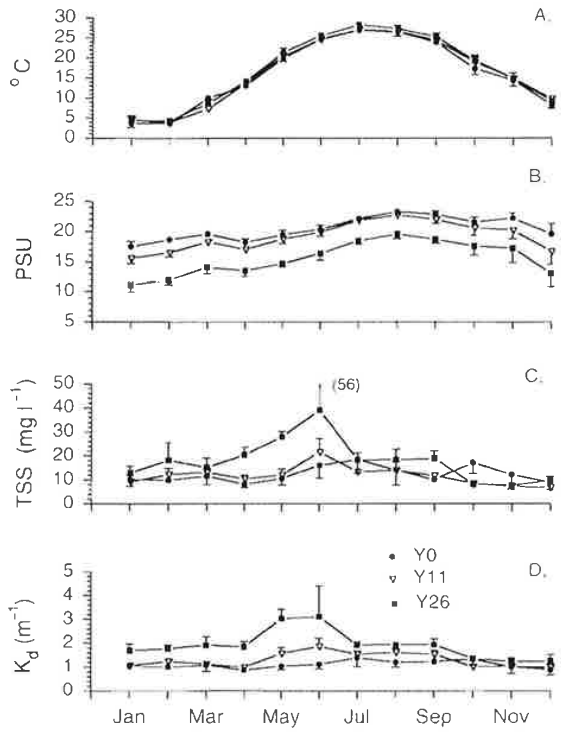


Fig. 3. Mean monthly (A) temperature, (B) salinity, (C) total suspended solids (TSS) and (D) light attenuation ($K_d$) at Y0, Y11, and Y26 for the period of January 1985 to December 1987. Bars are 1 SE

significant effects of FIM and chl *a* on $K_d$, but no effect of FOM (Table 7). Therefore a regression equation using FIM and chl *a* as independent variables explained 46% of the variation in $K_d$. There were no consistent differences in chl *a* levels between the 2 upriver sites (Y11 and Y26; Fig. 4D). However, chl *a* concentrations were significantly lower at Y0 than at all upriver sites during the early spring bloom (Fig. 4D). This seasonal, marked increase in chl *a* during February and March had little apparent effect on total, water column light attenuation during that period (Fig. 3D)

Table 7. Stepwise multiple linear regression of water quality variables on light attenuation ($K_d$). FIM: filterable inorganic matter. Chl *a*: chlorophyll *a*. FOM: filterable organic matter. *b*: estimate of regression coefficient β

| | $r^2$ | *b* | SE *b* | p |
|---|---|---|---|---|
| FIM | 0.39 | 0.040 | 0.005 | 0.000 |
| Chl *a* | 0.46 | 0.014 | 0.004 | 0.001 |
| FOM | 0.46 | 0.013 | 0.033 | 0.690 |
| Constant | | 0.636 | 0.078 | 0.000 |

Highest levels of dissolved inorganic nitrogen (DIN) occurred during the fall and winter periods (September to February; Fig. 4A). At this time, DIN species consisted principally of ammonium although nitrite comprised approximately 50% of DIN by December, especially at Y26. Concentrations of DIN were significantly higher at Y26 than at the downriver sites during the fall and winter. During the summer (June to August; Fig. 4A) ammonium accounted for greater than 80% of DIN and there were generally no differences in DIN levels among the stations. Nitrate accounted for approximately 5 to 15% of DIN at all stations throughout the year.

Dissolved inorganic phosphate (DIP) levels showed little annual variability (Fig. 4B). Increasing levels with distance upriver were observed during much of the year. The highest DIP levels occurred at Y26 during the fall with intermediate levels at Y11.

N:P ratios for dissolved inorganic nutrients (Fig. 4C) generally followed the patterns for DIN availability. Ratios usually exceeded 15 from October through January and were less than 15 from February through September. A marked increase in N:P was observed in

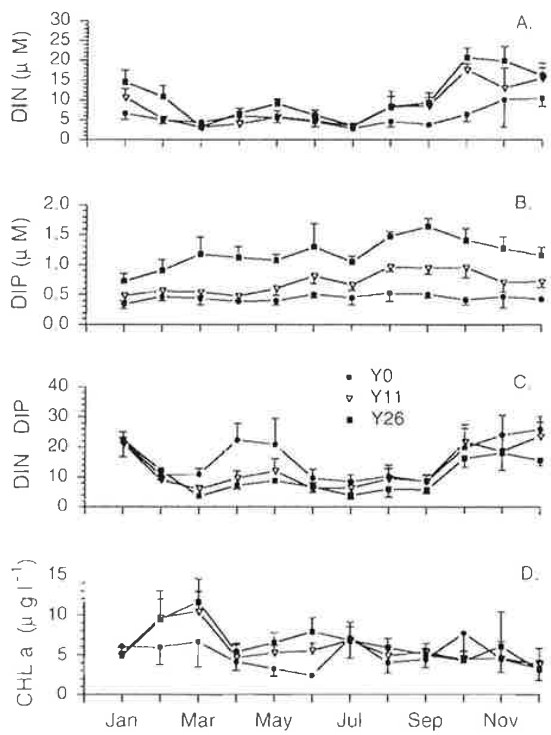


Fig. 4. Mean monthly (A) dissolved inorganic nitrogen (DIN), (B) dissolved inorganic phosphorus (DIP), (C) DIN:DIP ratios, (D) chlorophyll *a* at Y0, Y11, and Y26 for the period of January 1985 to December 1987. Bars are 1 SE

April and May at Y0. This was principally due to an interval of elevated nitrate (ranging from 5 to 8 µM) that was observed in 1986 at this site, with no concomitant change in DIP.

## DISCUSSION

### Distribution of *Zostera marina*: propagule supply or habitat suitability?

Distinct differences in the survival of transplants along the York River indicate there are differences among sites that are limiting re-colonization. Plants did not survive at any of the historically vegetated sites upriver of Y11. Therefore, the lack of macrophyte regrowth into formerly vegetated areas of this estuary has not been due simply to a lack of propagule recruitment. The distribution of *Zostera marina* in the lower Chesapeake Bay at this time likely represented the extent of suitable environmental conditions in the region. Current surveys (Orth et al. 1993) of submersed macrophyte distribution in the York region show a continued lack of plants upriver of Y11.

Transplant failure in these experiments was not attributable simply to the absence of existing vegetation which might modify the local environment and provide improved conditions for growth (Orth 1977, Fonseca et al. 1982, Kenworthy et al. 1982). At Y11, for example, where transplants were successfully established, the littoral was largely unvegetated before transplanting. Differences in environmental conditions among study sites with varying degrees of transplant success should, therefore, be related to causes of the reduced level of macrophyte populations found in lower Chesapeake Bay.

Transplant mortality along the river axis in the fall and winter immediately following planting was similar among sites and appeared related to physical disturbance. Shoot biomass was low at all sites during this winter period and all plants looked healthy and vigorous. At many locations where planting units were missing, wire anchors were found protruding out of the sediment and there was no evidence of below-ground or other material remaining. It thus appeared that overwinter transplant loss was mainly due to scouring activity of storms which occurred before the planting units were additionally anchored by new root/rhizome growth. The lower initial loss of planting units at Y0 may have been related to the attenuation of wave energies by adjacent vegetation (Ward et al. 1984).

Transplant mortality during the summer, in contrast, appeared related to enviromental conditions. Although a variety of organisms can result in great destruction to seagrass beds (Orth 1975), we found little evidence of disruption of the transplants by burrowing activities of crustaceans or fish during the growing season. At transplant sites upriver of Y11 where all the transplants eventually died, dead rhizomes could usually be found in the sediment at the locations of the individual planting units. This confirmed that the plants died *in situ*, and were not simply uprooted or physically removed. Also, a decrease in the size and shoot abundance of the individual planting units preceded their complete loss.

Results of growth experiments at Y11 and Y26 suggest seasonal differences in water quality between upriver and downriver sites that may have influenced transplant success. The similarity in growth between sites during the winter provides further evidence that transplant loss during this period was unrelated to water quality. In contrast, differences in growth in the spring indicate that differences in environmental suitability occurred during that period.

### Patterns of plant response

Patterns of *Zostera marina* growth and biomass allocation along the York River suggest potential mechanisms of plant response to environmental conditions. The greatest differences in plant growth between upriver and downriver study sites occurred during April and May, when growth rates were at their annual maxima; no differences were evident during the summer months of June and July when growth rates were low at both sites (Fig. 2A). Mortality of experimental transplants at Y26 occurred throughout the spring and summer, so that no plants remained by August each year. Transplant mortality may be attributable to inadequate production and ensuing carbohydrate storage during the spring. There is evidence that seasonal accumulation of carbohydrates in seagrass rhizomes during favorable growth periods can provide a source of energy for structural and respiratory requirements during periods of unfavorable, growth-limiting conditions such as high temperature or low light (Dawes & Lawrence 1979, Titus & Adams 1979, Ott 1980, Wittman & Ott 1982, Bulthuis 1983, Drew 1983, Pirc 1985, Dawes et al. 1987). In the present study, transplants were characterized by increasing S/R biomass ratios (Tables 3 & 4) and reduced below-ground production (Table 6) with distance upriver, suggesting that carbohydrate storage of upriver plants may have been insufficient to meet metabolic demands during the summer. Chesapeake Bay is near the southern limit of *Z. marina* distribution, where high water temperatures result in high respiratory demands during summer months (Evans et al. 1986). The storage and subsequent mobilization of photosynthate may be an important mechanism for summertime survival of *Z. marina* in this region (Burke et al. 1996).

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## Influence of environmental conditions

### Salinity stress

Although *Zostera* sp. can tolerate a wide range of salinities, photosynthesis and respiration are inhibited in waters where salinities are either hypo- or hypertonic (Ogata & Matsui 1965, Bieble & McRoy 1971, Kerr & Strother 1985). Although all sites used in this study had historically supported *Zostera marina* beds prior to die back in the 1970s, salinities do decrease with distance upriver, suggesting a possible effect contributing to the decreased growth and survival observed here. Evidence suggests, however, that the salinity effect was minor. Salinity decreased on average approximately 4 to 5‰ between Y11 and Y26. Using a linear relationship between shoot production and salinity determined by Pinnerup (1980) for *Z. marina* transplants in Danish waters during the summer, we estimate an approximate 10% decrease in shoot production due to lower salinities between sites Y11 and Y26. This compares to the approximately 85% difference in shoot production measured between Y11 and Y26 during May and June in the growth experiments.

### Disease

Evidence has led investigators to suggest that environmental stress may result in a weakened eelgrass host that would allow a pathogen such as the marine slime mold *Labyrinthula* sp. to decimate the populations (Rasmussen 1977, Short et al. 1988, Burdick et al. 1993). Although this is a possible explanation for results documented in this study, there was no evidence of widespread disease symptoms in the transplants here. The pattern of die-off in this study also suggests an alternative explanation. Die-off here occurred in the upriver stations where salinities were generally below 22‰ (Fig. 4B). In general, *Labyrinthula* sp. tends to be most infective at salinities higher than these (Burdick et al. 1993).

### Water column light attenuation

The precipitous drop in shoot growth in April at Y26 when plant growth rates were at their annual maxima (Fig. 2A) coincided with a period of high suspended load and reduced light (Fig. 3C, D). During May to June at sites Y0 and Y11 PAR at transplant depth was approximately 25 to 50% of sub-surface irradiance ($I_0$) as determined from $K_d$ measured during that period. However for the May to June period at Y26, PAR at transplant depth was only 12% of $I_0$. This would only be marginally sufficient for growth (Duarte 1991, Dennison et al. 1993) even given no other stressors such as epiphytes. Thus, low light availability was probably a dominant factor causing the low growth and ultimate mortality of plants at Y26. Similar relations have been observed previously, where reductions in total daily light availability in June resulted in complete loss of *Zostera marina* plants by the end of summer (Dennison & Alberte 1985). Zimmerman et al. (1991) have suggested that extended periods of high turbidity in spring may be responsible for the limited depth distribution of *Z. marina* in San Francisco Bay.

## Dissolved nutrient concentrations

Declines of submersed macrophytes in some systems has been attributed in part to nutrient enrichment and consequent increases in epiphytic accumulation that limits light and carbon available for leaf photosynthesis (e.g. Phillips et al. 1978, Twilley et al. 1985, Silberstein et al. 1986, Hough et al. 1989). During fall periods when elevated nutrient concentrations were measured in the formerly vegetated, upriver sections of the York River, however, concomitantly higher epiphytic biomass was not observed. Thus, in this study factors other than nutrient supply, such as invertebrate grazing activity (Howard 1982, van Montfrans et al. 1982, Cattaneo 1983, Borum 1987, Neckles et al. 1993) or temperature (Penhale 1977, Borum & Wium-Andersen 1980, Libes 1986), limited epiphyte growth during the fall. Periodically higher epiphyte loads at downriver stations (Y0 and Y11) than upriver (Y26) during the fall and winter (Table 5) did not appear to affect transplant survival. Since light at the macrophyte leaf surface is a function of both water column and epiphytic attenuation, lower water column turbidities (Fig. 3) at these downriver stations during this period may have mitigated the effects of higher epiphyte loads.

In the late spring (May to June) epiphytic biomass was significantly higher at Y26 than at other sites; this was immediately before the transplants disappeared. Atomic ratios of dissolved inorganic N:P (<10:1) indicated that algal growth was likely limited by nitrogen rather than phosphorus at this time. March to April concentrations of DIN were similar among sites upriver of Y0 (Fig. 4A), although DIN concentrations were observed to be significantly higher at Y26 than downriver sites in May. DIP concentrations remained consistently higher at Y26 than downriver sites throughout the year (Fig. 4B). Although epiphytic growth may have been dependent upon rapid recycling of N rather than absolute concentrations, other factors may have also contributed to increased epiphytic densities upriver at Y26 in late spring. In turbid estuaries, considerable amounts of inorganic and organic debris may be en-

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trapped by the epiphyte matrix (Kemp et al. 1983). Higher concentrations of this fouling material at Y26 may thus reflect high springtime concentrations of suspended particles at that site. In addition, Murray (1983) found the relative photosynthetic efficiencies of epiphytic algae and *Zostera marina* to result in increasing epiphyte:macrophyte ratios with decreasing light intensity. Differences in the mass of this epiphytic material along the York River axis in the spring may thus reflect responses to light availability. Small increases in accumulation of this material may limit macrophyte survival at high levels of $K_d$ (Wetzel & Neckles 1986), and *Z. marina* appears most sensitive to epiphyte light limitation at high water temperatures (Neckles et al. 1993). Therefore, epiphyte biomass may have contributed to reduced macrophyte growth upriver during the spring turbidity peak.

Chronic water column nitrate enrichment has been related to eelgrass declines in some mesocosm enrichment experiments (Burkholder et al. 1992, 1994). Although the mechanism is not understood, it is hypothesized that chronic water column nitrate enrichment may promote internal nutrient imbalances that lead to plant death. In our study, differences in nitrate concentrations between Y0 and Y26 were generally less than 1 µM, especially during the spring and summer. This level of enrichment suggests that nitrate toxicity was not a significant contributor to eelgrass declines in the York River

## Conclusions

The lack of regrowth of *Zostera marina* into formerly vegetated sites in a lower Chesapeake Bay tributary is not simply due to lack of propagules but can be related to environmental conditions, especially high levels of turbidity during spring periods of potentially maximum growth and carbohydrate storage. Prolonged periods of nitrogen enrichment during the fall and winter had no observable effect on epiphytic accumulations or macrophyte growth, presumably because of overriding control by other factors. However, the accumulation of an epiphytic matrix on the leaves during the spring may contribute to an initiation of the seagrass decline. Symptoms of *Labyrinthula* infection were not observed. We suggest that insufficient growth during the spring limits *Z. marina* survival through the summer. Although summertime conditions may stress eelgrass populations in this region, they do not alone limit long-term survival. Relatively short-term stresses during certain critical periods can therefore have lasting effects on seagrass populations. Water quality conditions enhancing adequate seagrass growth during the spring may be key to long-term *Z. marina* survival and successful recolonization in this region.

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## LITERATURE CITED

Ajoi K, Mukai H, Koike I, Ohtsu M, Hattori A (1981) Growth and organic production of eelgrass (*Zostera marina* L.) in temperate waters of the Pacific coast of Japan. II. Growth analysis in winter. Aquat Bot 10:175–182

Batiuk RA, Orth RJ, Moore KA, Dennison WC, Stevenson JC, Staver LW, Carter V, Rybicki NB, Hickman RE, Kollar S, Bieber S, Heasly P (1992) Chesapeake Bay submerged aquatic vegetation habitat requirements and restoration goals: a technical synthesis. USEPA CBP/TRS83/92, Chesapeake Bay Program, Annapolis, MD

Biebl R, McRoy CP (1971) Plasmatic resistance and rate of respiration and photosynthesis of *Zostera marina* at different salinities and temperatures. Mar Biol 8:48–56

Borum J (1987) Dynamics of epiphyton on eelgrass (*Zostera marina* L.) leaves: relative roles of algal growth, herbivory, and substratum turnover. Limnol Oceanogr 32:986–992

Borum J, Wium-Andersen S (1980) Biomass and production of epiphytes on eelgrass (*Zostera marina* L.) in the Øresund, Denmark. Ophelia Suppl 1:57–64

Bulthuis DA (1983) Effects of *in situ* light reduction on density and growth of the seagrass *Heterozostera tasmanica* (Martens ex Aschers.) den Hartog in Western Port, Victoria, Australia. J Exp Mar Biol Ecol 67:91–103

Burdick DM, Short FT, Wolf J (1993) An index to assess and monitor the progression of wasting disease in eelgrass *Zostera marina*. Mar Ecol Prog Ser 94:83–90

Burke MK, Dennison WC, Moore KA (1996) Non-structural carbohydrate reserves of eelgrass *Zostera marina*. Mar Ecol Prog Ser 137:195–201

Burkholder JM, Mason KM, Glasgow HB Jr (1992) Water-column nitrate enrichment promotes decline of eelgrass *Zostera marina* L.: evidence from seasonal mesocosm experiments. Mar Ecol Prog Ser 61:163–178

Burkholder JM, Glasgow HB Jr, Cooke JE (1994) Comparative effects of water-column nitrate enrichment on eelgrass *Zostera marina*, shoalgrass *Halodule wrightii*, and widgeongrass *Ruppia maritima*. Mar Ecol Prog Ser 105: 121–138

Cambridge ML, Chiffings AW, Brittan C, Moore L, McComb AJ (1986) The loss of seagrass in Cockburn Sound, Western Australia. II. Possible causes of seagrass decline. Aquat Bot 24:269–285

Cattaneo A (1983) Grazing on epiphytes. Limnol Oceanogr 28:124–132

Davis GJ, Cary DF (1981) Trends in submerged macrophyte communities of the Currituck Sound: 1977–1979. J Aquat Plant Manage 19:3–8

Dawes C, Chan M, Chinn R, Koch EW, Lazar A, Tomasko D (1987) Proximate composition, photosynthetic and respiratory responses of the seagrass *Halophila engelmannii* from Florida. Aquat Bot 27:195–201

Dawes CJ, Lawrence JM (1979) Effects of blade removal on the proximate composition of the rhizome of the seagrass *Thalassia testundinum* Banks ex Konig. Aquat Bot 7:255–266

Dennison WC, Orth RJ, Moore KA, Stevenson JC, Carter V, Kollar S, Bergstrom PW, Batiuk RA (1993) Assessing

water quality with submersed aquatic vegetation. BioSci 43:86-94
Dennison WC, Alberte RS (1985) Role of daily light period in the depth distribution of *Zostera marina* (eelgrass). Mar Ecol Prog Ser 25:51-61
den Hartog D, Polderman PJG (1975) Changes in the seagrass populations of the Dutch Waddenzee. Aquat Bot 1: 141-147
Drew EA (1983) Sugars, cyclitols, and seagrass phylogeny. Aquat Bot 15:387-408
Duarte CM (1991) Seagrass depth limits. Aquat Bot 40: 363-377
Evans AS, Webb KL, Penhale PA (1986) Photosynthetic temperature acclimation in two coexisting seagrasses, *Zostera marina* L. and *Ruppia maritima* L. Aquat Bot 24:185-197
Fonseca MS, Fisher JS, Zieman JC, Thayer GW (1982) Influence of the seagrass, *Zostera marina* L., on current flow. Estuar Coast Shelf Sci 15:351-364
Fonseca MS, Kenworthy WJ, Thayer GW, Heller DY, Cheap KM (1985) Transplanting of the seagrasses *Zostera marina* and *Halodule wrightii* for sediment stabilization and habitat development on the east coast of the United States. Technical Report EL-85-9, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS
Giesen WBJT, van Katwijk MM, den Hartog C (1990) Eelgrass condition and turbidity in the Dutch Wadden Sea. Aquat Bot 37:71-85
Hough RA, Fornwall MD, Negele BJ, Thompson RL, Putt DA (1989) Plant community dynamics in a chain of lakes: principal factors in the decline of rooted macrophytes with eutrophication. Hydrobiologia 173:199-217
Howard RK (1982) Impact of feeding activities of epibenthic amphipods on surface-fouling of eelgrass leaves. Aquat Bot 14:91-97
Jacobs RPWM (1979) Distribution and aspects of the production and biomass of eelgrass, *Zostera marina* L., at Roscoff, France. Aquat Bot 7:151-172
Jupp BP, Spence DHN (1977) Limitations on macrophytes in a eutrophic lake, Loch Leven. J Ecol 65:175-186
Kemp WM, Boynton WR, Twilley RR, Stevenson JC, Means JC (1983) The decline of submerged vascular plants in upper Chesapeake Bay: summary of results concerning possible causes. Mar Technol Soc J 17:78-89
Kenworthy WJ, Zieman JC, Thayer GW (1982) Evidence for the influence of seagrasses on the benthic nitrogen in a coastal plain estuary near Beaufort, North Carolina (USA). Oecologia 54:152-158
Kerr EA, Strother S (1985) Effects of irradiance, temperature and salinity on photosynthesis of *Zostera muelleri*. Aquat Bot 23:177-183
Libes M (1986) Productivity-irradiance relationship of *Posidonia oceanica* and its epiphytes. Aquat Bot 26:285-306
Marsh GA (1970) A seasonal study of *Zostera* epibiota in the York River, Virginia. PhD dissertation, College of William and Mary, Williamsburg, VA
Marsh GA (1973) The *Zostera* epifaunal community in the York River, Virginia. Chesapeake Sci 14:87-97
Muehlstein LK, Porter D, Short FT (1988) *Labyrinthula* sp., a marine slime mold producing the symptoms of wasting disease in eelgrass, *Zostera marina*. Mar Biol 99:465-472
Murray L (1983) Metabolic and structural studies of several temperate seagrass communities, with emphasis on microalgal components. PhD dissertation, College of William and Mary, Williamsburg, VA
Neckles HA, Wetzel RL, Orth RJ (1993) Relative effects of nutrient enrichment and grazing on epiphyte-macrophyte (*Z. marina* L.) dynamics. Oecologia 93:285-295
Neckles HA, Koepfler ET, Haas LW, Wetzel RL, Orth RJ (1994) Dynamics of epiphytic photoautotrophs and heterotrophs in *Zostera marina* (eelgrass) microcosm: responses to nutrient enrichment and grazing. Estuaries 17: 597-605
Neter J, Wasserman W (1974) Applied linear statistical models. Richard D. Irwin, Inc, Homewood
Ogata E, Matsui T (1965) Photosynthesis in several marine plants of Japan as affected by salinity, drying and pH, with attention to their growth habitats. Bot Mar 8:199-217
Orth RJ (1973) Benthic infauna of eelgrass, *Zostera marina*, beds. Chesapeake Sci 14:258-269
Orth RJ (1975) Destruction of eelgrass, *Zostera marina*, by the cownose ray, *Rhinoptera bonasus*, in the Chesapeake Bay. Chesapeake Sci 16:205-208
Orth RJ (1977) The importance of sediment stability in seagrass communities. In: Coull BC (ed) Ecology of marine benthos. University of South Carolina Press, Columbia, p 281-300
Orth RJ, Moore KA (1983) Chesapeake Bay: an unprecedented decline in submerged aquatic vegetation. Science 222:51-53
Orth RJ, Moore KA (1984) Distribution and abundance of submerged aquatic vegetation in Chesapeake Bay: an historical perspective. Estuaries 7:531-540
Orth RJ, Moore KA (1988) Distribution of *Zostera marina* L. and *Ruppia maritima* L. sensu lato along depth gradients in the lower Chesapeake Bay, USA. Aquat Bot 32:291-305
Orth RJ, Moore KA, Gordon HH (1979) Distribution and abundance of submerged aquatic vegetation in the lower Chesapeake Bay, Virginia. Final Report. 600/8-79-029/ SAV1 US EPA, Annapolis, MD
Orth RJ, Nowak JF, Anderson GA, Whiting JR (1993) Distribution of submerged aquatic vegetation in the Chesapeake Bay and tributaries and Chincoteague Bay—1992. Final Report. USEPA, Chesapeake Bay Program, Annapolis, MD
Ott JA (1980) Growth and production in *Posidonia oceanica* (L.) Delile. PSZN I: Mar Ecol 1:47-64
Parsons TR, Miata Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, New York
Penhale PA (1977) Macrophyte-epiphyte biomass and productivity in an eelgrass (*Zostera marina* L.) community. J Exp Mar Biol Ecol 26:211-224
Phillips GL, Eminson D, Moss B (1978) A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. Aquat Bot 4:103-126
Pinnerup SP (1980) Leaf production of *Zostera marina* L. at different salinities. Ophelia Suppl 1:219-224
Pirc H (1985) Growth dynamics in *Posidonia oceanica* (L.) Delile. I. Seasonal changes of soluble carbohydrates, starch, free amino acids, nitrogen and organic anions in different parts of the plant. PSZN I: Mar Ecol 6:141-165
Rasmussen E (1977) The wasting disease of eelgrass (*Zostera marina*) and its effects on environmantal factors and fauna. In: McRoy CP, Helfferich C (eds) Seagrass ecosystems: a scientific perspective. Marcel Dekker, New York
Sand-Jensen K (1975) Biomass, net production and growth dynamics in an eelgrass (*Zostera marina* L.) population in Vellerup Vig, Denmark. Ophelia 14:185-201
Shoaf WT, Lium BW (1976) Improved extraction of chlorophyll *a* and *b* from algae using dimethyl sulfoxide. Limnol Oceanogr 21:926-928
Short FT, Ibelings BW, den Hartog C (1988) Comparison of a current eelgrass disease to the wasting disease in the 1930s. Aquat Bot 30:295-304

Silberstein K, Chiffings AW, McComb AJ (1986) The loss of seagrass in Cockburn Sound, Western Australia. III. The effect of epiphytes on productivity of *Posidonia australis* Hook. f. Aquat Bot 24:355–371

SPSS Inc (1986) SPSSx users' guide, 2nd edn. Chicago

Stevenson JC, Staver LW, Staver KW (1993) Water quality associated with survival of submersed aquatic vegetation along an estuarine gradient. Estuaries 16:346–361

Titus JE, Adams MS (1979) Comparative carbohydrate storage and utilization patterns in the submersed macrophytes, *Myriophyllum spicatum* and *Vallisneria americana*. Am Midl Nat 102:263–271

Twilley RR, Kemp WM, Staver KW, Stevenson JC, Boynton WR (1985) Nutrient enrichment of estuarine submersed vascular plant communities. I. Algal growth and effects on production of plants and associated communities. Mar Ecol Prog Ser 23:179–191

USEPA (1979) Manual of methods for chemical analysis of water and wastes. EPA-600-4-79-020. Environmental Monitoring and Support Laboratory, Cincinnati, OH

van Montfrans J, Orth RJ, Vay SA (1982) Preliminary studies of grazing by *Bittium varium* on eelgrass periphyton. Aquat Bot 14:75–89

Ward LG, Kemp WM, Boyton WR (1984) The influence of waves and seagrass communities on suspended particlates in an estuarine embayment. Mar Geol 59: 85–103

Wetzel RL, Neckles HA (1986) A model of *Zostera marina* L. photosynthesis and growth: simulated effects of selected physical-chemical variables and biological interactions. Aquat Bot 26:307–323

Wittmann KJ, Ott JA (1982) Effects of cropping on growth in the Mediterranean seagrass *Posidonia oceanica* (L.) Delile. PSZN I: Mar Ecol 3:151–159

Zimmerman RC, Reguzzone JL, Wyllie-Echeverria S, Josselyn M, Alberte RS (1991) Assessment of environmental suitability for growth of *Zostera marina* L. (eelgrass) in San Francisco Bay. Aquat Bot 39:353–366

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