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# Development of a nutrient pollution indicator using the seagrass, *Zostera marina*, along nutrient gradients in three New England estuaries

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## Abstract

Worldwide, seagrasses provide important habitats in coastal ecosystems, but seagrass meadows are often degraded or destroyed by cultural eutrophication. Presently, there are no available tools for early assessment of nutrient over-enrichment; direct measurements of water column nutrients are ineffective since the nutrients typical of early enrichment are rapidly taken up by plants within the ecosystem. We investigated whether, in a gradient of nutrient availability but prior to actual habitat loss, eelgrass (*Zostera marina* L.) plant morphology and tissue nutrients might reflect environmental nutrient availability. Eelgrass responses to nitrogen along estuarine gradients were assessed; two of these plant responses were combined to create an early indicator of nutrient over-enrichment. Eelgrass plant morphology and leaf tissue nitrogen (N) were measured along nutrient gradients in three New England estuaries: Great Bay Estuary (NH), Narragansett Bay (RI) and Waquoit Bay (MA). Eelgrass leaf N was significantly higher in up-estuary sampling stations than stations down-estuary, reflecting environmental nitrogen gradients. Leaf N content showed high variance, however, limiting its ability to discriminate the early stages of eutrophication. To find a stronger indicator, plant morphological characteristics such as number of leaves per shoot, blade width, and leaf and sheath length were examined, but they only weakly correlated with leaf tissue N. Area normalized leaf mass (mg dry weight cm<sup>-2</sup>), however, exhibited a strong and consistently negative relationship with leaf tissue N and a significant response to the estuarine nutrient gradients. We found the ratio of leaf N to leaf mass to be a more sensitive and consistent indicator of early

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eutrophication than either characteristic alone. We suggest the use of this ratio as a nutrient pollution indicator (NPI).

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**Keywords:** Seagrass; *Zostera marina*; Eelgrass; Nutrients; Nitrogen; Eutrophication; Indicator; Estuary; New England

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## 1. Introduction

Significant declines in seagrass coverage have been reported from many coastal areas (Short and Wyllie-Echeverria, 1996), and the declines are usually related to human activities (Kemp et al., 1983; Cambridge and McComb, 1984; Short and Burdick, 1996; Burdick and Short, 1999; Udy et al., 1999). Estuarine and coastal ecosystems receive increasing amounts of nutrients as a consequence of anthropogenic loading (Valiela et al., 1992; Short and Burdick, 1996; Tomasko et al., 1996; McMahon and Walker, 1998). Increased nutrient loading is widely acknowledged to impact the structure and function of coastal ecosystems (Valiela et al., 1990; Lapointe et al., 1994).

Nutrient over-enrichment leads to nuisance algal blooms, reduced dissolved oxygen in the water column, and decreased fish stocks (Nixon et al., 1986; Taylor et al., 1999; Deegan et al., 2002). Increased nutrient inputs to the water column can also adversely affect seagrass survival and production through stimulation of growth in phytoplankton, epiphyte and macroalgal communities (Harlin and Thorne-Miller, 1981; Short, 1987; Short et al., 1995). Stimulation of competing primary producers caused by water column nutrient enrichment leads to reduction of light available to seagrasses and often to their demise (Harlin and Thorne-Miller, 1981; Van Montfrans et al., 1984; Borum, 1985; Tomasko and Lapointe, 1991; Van Lent et al., 1995).

Because of the harmful effects of nutrient over-enrichment on estuarine and coastal ecosystems, early detection of eutrophication is critical for management. In New England, eutrophication results from nitrogen over-enrichment, as estuarine systems in this region are nitrogen limited (Ryther and Dunstan, 1971); detection of eutrophication requires focus on nitrogen. Direct measurement of in situ nitrogen concentrations to estimate eutrophication is ineffective, however, as estuarine conditions both dilute and dissipate nitrogen loading through tidal and current action as well as microbial and plant uptake. Since phytoplankton and submerged macrophytes can remove nitrogen from the water column rapidly (Morgan and Simpson, 1981; Short and McRoy, 1984; Stapel et al., 1996; Terrados and Williams, 1997; Lee and Dunton, 1999b), over-enrichment of coastal ecosystems rarely can be detected by direct measurements of water column nitrogen concentrations. One of the general indicators of nitrogen over-enrichment in temperate estuarine and coastal ecosystems has been eelgrass (*Zostera marina* L.) die-off and a concomitant increase in algal competitors, but by the time this occurs, ecosystem function has been severely disrupted (Kemp et al., 1983; Orth and Moore, 1983; Short et al., 1995; Short and Burdick, 1996; Short et al., 1996). Managers of coastal environments would benefit from an early indicator of over-enrichment and eutrophication.

Seagrasses respond to nitrogen enrichment both physiologically and morphologically (Burkholder et al., 1992, 1994; Short et al., 1995; Taylor et al., 1995; Tomasko et al., 1996; Udy and Dennison, 1997; Udy et al., 1999). Increased tissue N as a result of N enrichment has been reported for seagrasses (Bulthuis and Woelkerling, 1981; Harlin and Thorne-Miller, 1981; Short, 1987; Duarte, 1990; Bulthuis et al., 1992; Erftemeijer et al., 1994; Alcoverro et al., 1997; Udy and Dennison, 1997; Lee and Dunton, 1999a). Additionally, seagrasses from low-nutrient environments have significantly higher C:N and C:P ratios than plants growing in high nutrient conditions (Atkinson and Smith, 1983; Duarte, 1990; Short et al., 1990; Lee and Dunton, 1999a). Since increased external nutrient concentrations result in increased seagrass tissue nutrient content, we hypothesized that the tissue nitrogen content of eelgrass could contribute to an indicator of early over-enrichment.

Seagrass morphology and growth are strongly linked to available nutrient resources. Short (1983) reported a strong correlation between sediment N and eelgrass leaf morphology. Plants characterized by short and narrow leaves grew in low nitrogen sediment, while plants exhibiting long, wide leaves were found in high nitrogen sediments. Seagrass morphological characteristics such as shoot height and blade width also respond to changes in nutrient loading (Short, 1987; Udy and Dennison, 1997; Lee and Dunton, 2000), but they are influenced by other environmental factors such as light availability, current and wave strength, and tidal exposure.

Eelgrass leaf tissue N and plant morphology were measured along nitrogen gradients in Great Bay Estuary (NH), Narragansett Bay (RI) and Waquoit Bay (MA), USA to correlate eelgrass responses to levels of N exposure. To evaluate the geographic consistency of our results, eelgrass physiological and morphological responses were compared among the three estuarine systems. Many eelgrass morphological characteristics were then evaluated to identify a measure that, in combination with leaf N, would form a robust nutrient pollution indicator (NPI).

## 2. Materials and methods

### 2.1. Study sites

The study was conducted in Great Bay Estuary (New Hampshire; 43°05'N, 70°50'W), Narragansett Bay (Rhode Island; 41°35'N, 71°20'W), and Waquoit Bay (Massachusetts; 41°35'N, 70°30'W) along the New England coast, USA (Fig. 1). Twenty sampling stations in the Great Bay Estuary, seven in Narragansett Bay, and five in Waquoit Bay were located from the mouth of each estuary to upper estuarine embayments. Water column dissolved inorganic nitrogen (DIN) concentrations in Great Bay Estuary and Narragansett Bay were low in down-estuary locations and high up-estuary (Short et al., 1993). Pore water ammonium concentrations were generally greatest in Great Bay Estuary, though the ammonium regeneration rate was highest in Narragansett Bay sediments. Up-estuary, both Waquoit Bay and Narragansett Bay showed poor water clarity.

Of the three, Great Bay Estuary had the greatest tidal range (>3 m). Water in the upper part of Great Bay Estuary was well mixed and had consistently lower salinities and higher summer temperatures than down-estuary in Little Bay and the Piscataqua River (Swift

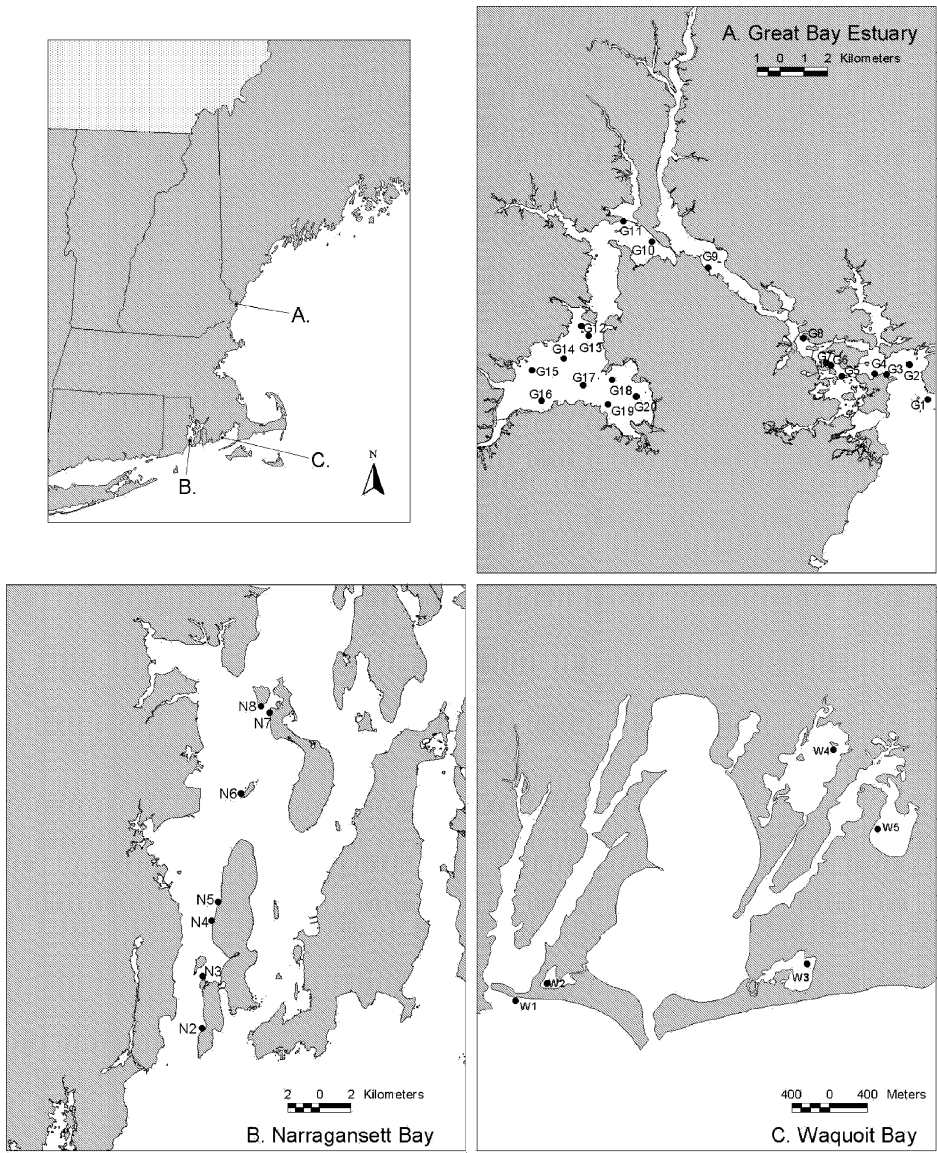


Fig. 1. Location of the three New England (USA) estuaries investigated. Study sites in Great Bay Estuary, New Hampshire (A), Narragansett Bay, Rhode Island (B) and Waquoit Bay, Massachusetts (C). Twenty sampling stations in Great Bay Estuary, seven in Narragansett Bay and five in Waquoit Bay were located from the mouth of each estuary to up-estuary.

and Brown, 1983; Short et al., 1986). Narragansett Bay had a greater tidal range than Waquoit Bay (1.0 m versus 0.3 m), with a small diurnal component (Short et al., 1993). Salinities were less variable in Narragansett Bay (29–31‰) than in the other two estuaries. Major oscillations of eelgrass populations in the Great Bay Estuary have been linked not to pollution or increased nutrient loading but to outbreaks of wasting disease (Short et al., 1986; Burdick et al., 1993). Eelgrass meadows in Narragansett Bay have exhibited relatively high indices of wasting disease in the past, while Waquoit Bay eelgrass populations have had consistently low levels of infection by wasting disease. However, epiphytes and macroalgal and phytoplankton blooms have covered and eliminated most of the eelgrass beds that once existed in Waquoit Bay; the gradient measured in Waquoit Bay ranged from oligotrophic outside the Bay to elevated levels of nutrient loading in various sub-estuaries (Short and Burdick, 1996). The upper parts of Narragansett Bay have been exposed to very high nutrient loads for decades (Nixon and Pilson, 1983); no eelgrass exists in the uppermost reaches of the bay.

## 2.2. *Plant collection and morphological measurements*

Ten mature terminal eelgrass shoots were collected individually from a boat using a sampling hook at each sampling station during June 1998 in Waquoit Bay and September 1999 in Great Bay Estuary and Narragansett Bay. Sheath length was measured to the nearest 1.0 mm from the meristem to the top of the outermost intact sheath. Shoot height was measured to the nearest 1.0 mm and the width of the longest leaf was measured to the nearest 0.2 mm. The number of leaves per shoot was counted. All epiphytes were carefully scraped from the longest intact leaf using a razor blade, placed onto a pre-weighed glass fiber filter, and dried at 60 °C to a constant weight. Epiphyte biomass was quantified on a leaf area basis (mg dry weight epiphyte cm<sup>-2</sup> leaf). Wasting Index was measured as a percentage of diseased area for each leaf and then averaged for each shoot (Burdick et al., 1993).

We measured area normalized leaf mass, which we refer to simply as “leaf mass” (mg dry weight cm<sup>-2</sup> leaf area), a weight per area of plant tissue similar to the “leaf weight per leaf area” of Olesen and Sand-Jensen (1993) and the inverse of specific leaf area (SLA) used by Olesen et al. (2002). Leaf mass was determined on the second and third youngest leaves of each shoot. All epiphytes were removed from these leaves; six 10 cm long sections of constant width were cut from the leaves to obtain samples of mature leaf tissue. The cleaned leaf sections were dried at 60 °C to constant weight and leaf mass was quantified.

### 2.2.1. *Leaf C and N content*

Leaf tissue C and N content were determined from tissues of the second and third youngest leaves for each shoot. Dried leaf material was ground to pass through a 40 mesh screen in a Wiley mill, and 2–3 mg of ground tissue was used to determine leaf C and N content using an elemental analyzer (Carlo Erba Nitrogen Analyzer 1500); molar C:N was calculated.

### 2.2.2. *Statistics*

All values are reported as means  $\pm$  1 standard error (S.E.). Data were tested for normality and homogeneity of variance to meet the assumptions of parametric statistics. Signifi-

cant differences in seagrass morphological parameters and tissue nutrient content among sampling stations within an estuary were tested using 1-way ANOVA, as were significant differences in parameters among the three estuarine systems. When a significant difference was observed, the means were analyzed by Tukey's multiple comparison test to determine where the significant differences occurred within and among estuarine systems. Slopes of linear regressions between variables were tested for significance.

### 3. Results

#### 3.1. Leaf N content

Eelgrass leaf N content in the Great Bay Estuary ranged from 2.1 to 3.5% dry weight, and was significantly ( $P < 0.001$ ) higher in up-estuary stations than in those seaward (Fig. 2A). Sampling stations at the mouth of the estuary, where water is well mixed with ocean water, had the lowest plant tissue N content. Eelgrass leaf C content in the Great Bay Estuary also differed significantly ( $P < 0.001$ ) among sampling stations, but there was no discernable pattern (Table 1). The C:N in eelgrass leaf tissue exhibited an inverse trend to leaf N content and was higher in down-estuary stations and lower in up-estuary stations (Fig. 3A).

In Narragansett Bay, eelgrass leaf N content varied little, from 2.0 to 2.3% (Fig. 2B), and differences between stations were not significant ( $P = 0.072$ ). Leaf C content was lower in stations down-estuary than up-estuary and was significantly ( $P < 0.001$ ) different among sampling stations (Table 1). The C:N was not significantly ( $P = 0.41$ ) different among the Narragansett Bay stations (Fig. 3B).

In Waquoit Bay, eelgrass leaf N content ranged from 1.6 to 2.4% and was significantly ( $P < 0.001$ ) lower at the two stations located outside (W1) and just inside (W2) the mouth of the estuary (Fig. 2C). The C:N decreased significantly ( $P < 0.001$ ) along the gradient in Waquoit Bay (Fig. 3C), showing a similar, but inverse, trend to leaf N content. Leaf C content showed significant differences between sampling stations but no clear trend (Table 1).

Comparing the three estuaries, mean values of eelgrass leaf N content (Fig. 2) were significantly ( $P < 0.001$ ) higher in the Great Bay Estuary (2.8% N) than in Narragansett Bay (2.1% N) and Waquoit Bay (2.0% N). Mean C content (Table 1) was significantly ( $P < 0.001$ ) lower in Narragansett Bay (35.5% C) than in the Great Bay Estuary (37.9% C) and Waquoit Bay (37.8% C). The C:N (Fig. 3) was highest in Waquoit Bay (22.6), intermediate in Narragansett Bay (19.5), and lowest in the Great Bay Estuary (16.4).

#### 3.2. Leaf mass and plant morphology

Leaf mass in the Great Bay Estuary varied between 1.7 and 4.8 mg dry wt. cm<sup>-2</sup> leaf area, and was significantly ( $P < 0.001$ ) higher seaward than at up-estuary stations (Fig. 4A). In Narragansett Bay and Waquoit Bay, leaf mass was also significantly ( $P < 0.001$ ) higher seaward (Fig. 4B and C). Mean leaf mass varied among the three estuaries and was highest in Narragansett Bay (5.1 mg cm<sup>-2</sup>) and lowest in Great Bay Estuary (3.0 mg cm<sup>-2</sup>; Fig. 4A and B).

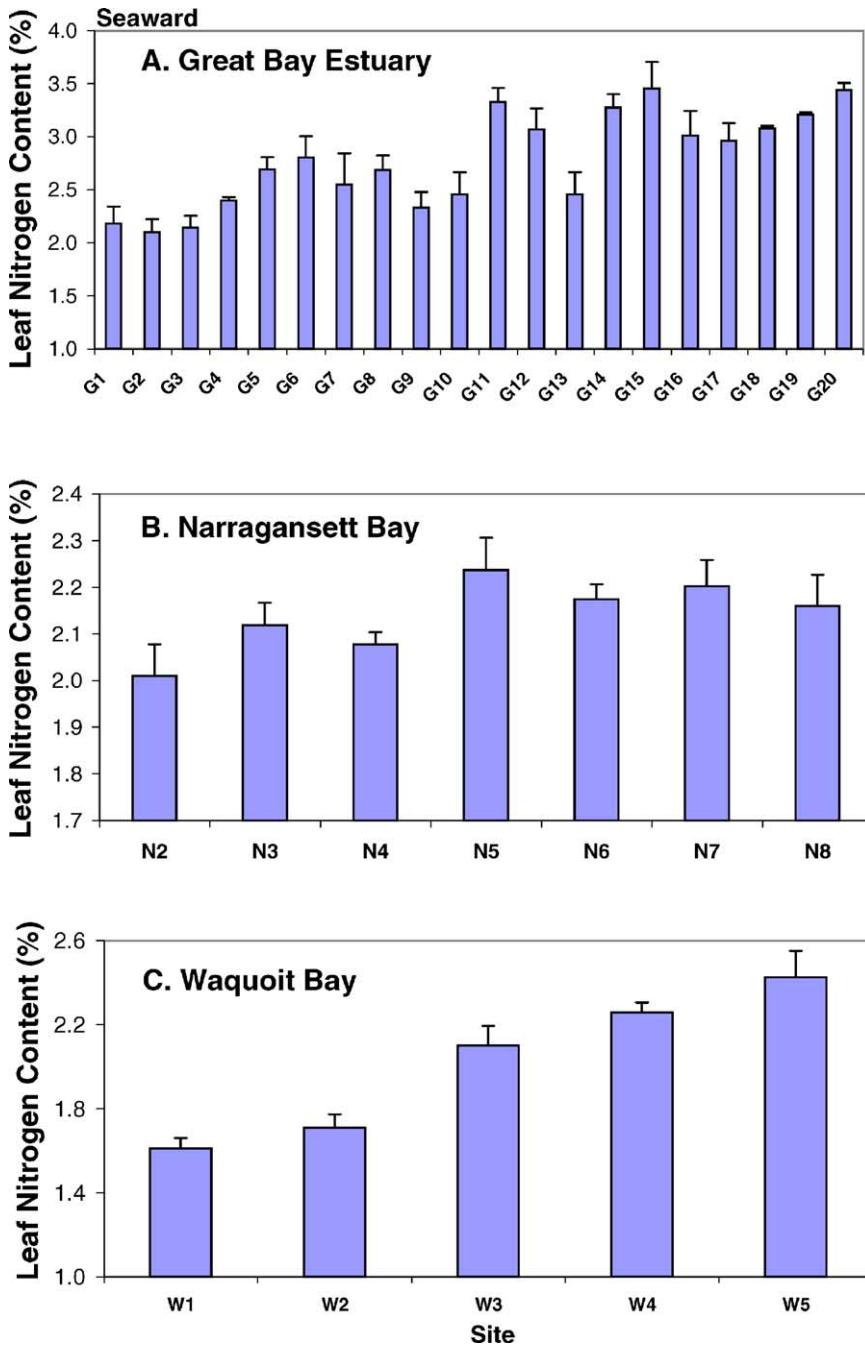


Fig. 2. Leaf nitrogen content of eelgrass along estuarine gradients in Great Bay Estuary (A), Narragansett Bay (B), and Waquoit Bay (C).

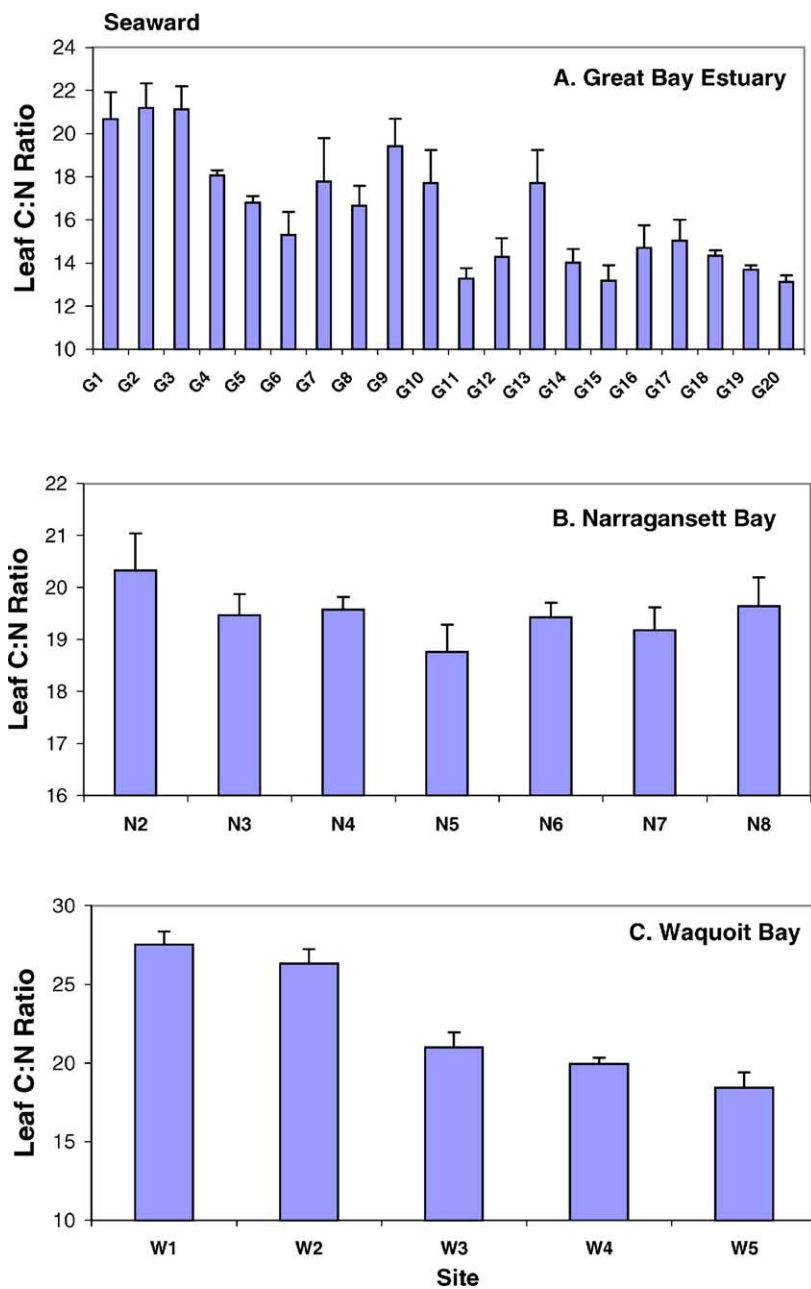


Fig. 3. Ratios of carbon to nitrogen content of eelgrass leaves along estuarine gradients in Great Bay Estuary (A), Narragansett Bay (B), and Waquoit Bay (C).



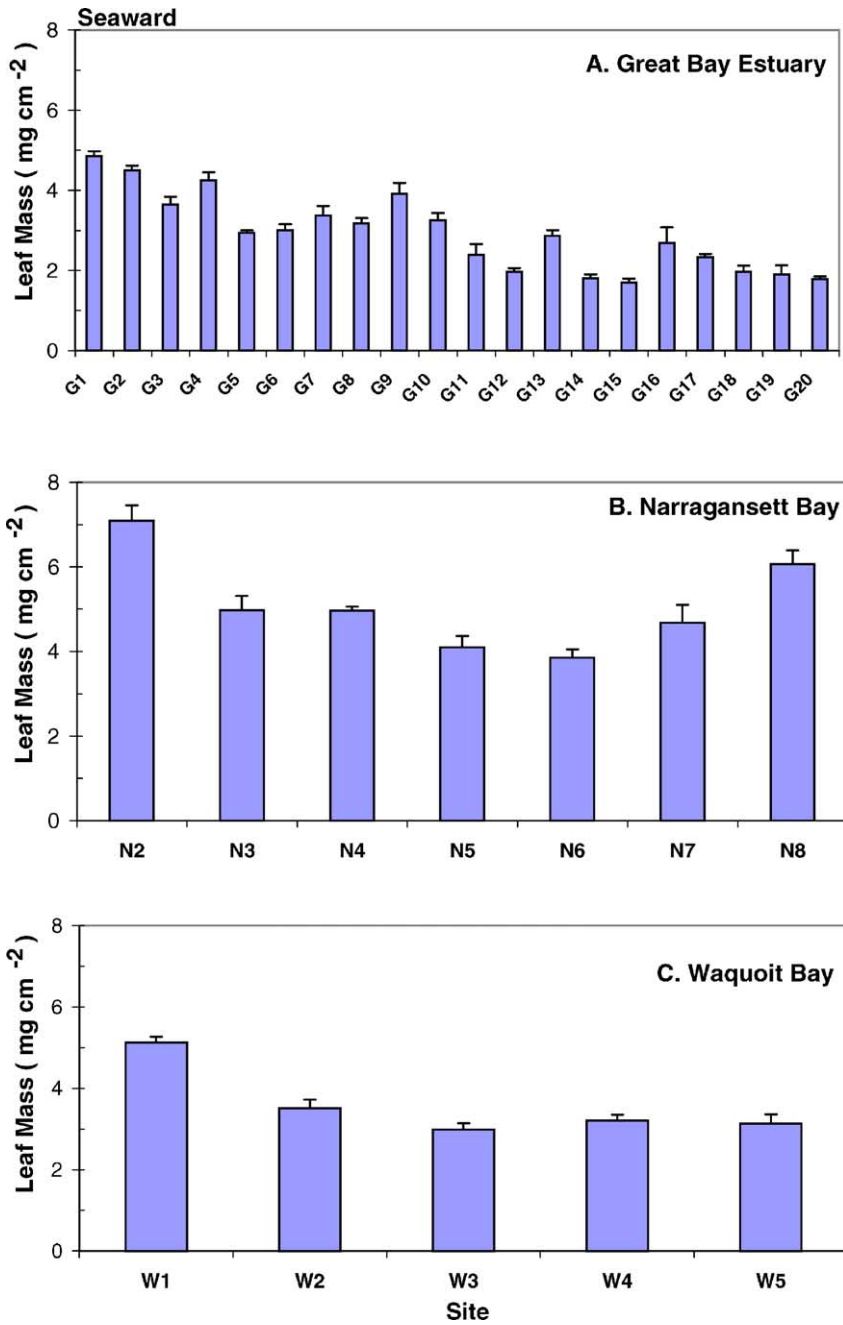


Fig. 4. Leaf mass from the mouth of the estuary to up-estuary in Great Bay Estuary (A), Narragansett Bay (B), and Waquoit Bay (C).

Table 1

Morphological parameters, epiphytes, Wasting Index and leaf tissue nutrient content of *Zostera marina* along nutrient gradients in Great Bay Estuary, Narragansett Bay and Waquoit Bay

| Site             | No. of leaves<br>(Leaves shoot <sup>-1</sup> ) | Sheath<br>length<br>(cm) | Shoot height<br>(cm) | Leaf width<br>(mm) | Epiphytes<br>(mg cm <sup>-2</sup> ) | Wasting<br>Index (%) | Leaf C content<br>(%) |
|------------------|--|--------------------------|----------------------|--------------------|-------------------------------------|----------------------|-----------------------|
| Great Bay        |  |                          |                      |                    |                                     |                      |                       |
| G1               | 4.5 ± 0.2                                      | 14.4 ± 1.0               | 59.9 ± 3.9           | 4.3 ± 0.2          | 0.013                               | 1.6 ± 0.5            | 38.3 ± 0.4            |
| G2               | 4.2 ± 0.2                                      | 28.2 ± 1.6               | 113.1 ± 7.8          | 4.8 ± 0.1          |                                     | 10.2 ± 1.9           | 37.9 ± 0.3            |
| G3               | 3.9 ± 0.3                                      | 17.4 ± 1.3               | 78.1 ± 5.1           | 4.5 ± 0.1          | 0.013                               | 10.4 ± 2.6           | 38.6 ± 0.2            |
| G4               | 4.6 ± 0.2                                      | 23.2 ± 1.9               | 106.5 ± 8.3          | 5.7 ± 0.2          | 0.003                               | 15.1 ± 2.9           | 37.1 ± 0.4            |
| G5               | 4.6 ± 0.2                                      | 16.7 ± 1.1               | 75.3 ± 5.4           | 4.6 ± 0.2          | 0.010                               | 6.6 ± 1.5            | 38.7 ± 0.9            |
| G6               | 4.5 ± 0.2                                      | 19.5 ± 0.7               | 90.6 ± 3.4           | 5.8 ± 0.1          | 0.679                               | 7.7 ± 2.4            | 36.4 ± 0.2            |
| G7               | 3.9 ± 0.2                                      | 17.5 ± 2.1               | 73.6 ± 9.5           | 4.2 ± 0.3          | 0.004                               | 9.1 ± 2.5            | 37.8 ± 0.5            |
| G8               | 4.6 ± 0.3                                      | 15.0 ± 1.9               | 76.8 ± 9.9           | 4.1 ± 0.2          | 0.192                               | 6.7 ± 2.8            | 38.1 ± 0.2            |
| G9               | 3.5 ± 0.2                                      | 11.6 ± 0.9               | 46.2 ± 4.4           | 3.4 ± 0.2          | 0.165                               | 4.9 ± 1.9            | 38.5 ± 0.3            |
| G10              | 3.5 ± 0.2                                      | 12.6 ± 0.7               | 58.6 ± 4.9           | 4.5 ± 0.2          | 0.008                               | 1.0 ± 0.4            | 36.7 ± 0.2            |
| G11              | 4.6 ± 0.2                                      | 15.5 ± 1.0               | 80.2 ± 4.7           | 4.9 ± 0.2          | 0.066                               | 0.3 ± 0.3            | 37.8 ± 0.2            |
| G12              | 3.1 ± 0.4                                      | 17.8 ± 1.1               | 72.8 ± 5.6           | 4.3 ± 0.2          | 0.009                               | 6.2 ± 0.9            | 37.2 ± 0.0            |
| G13              | 3.9 ± 0.2                                      | 15.2 ± 2.3               | 64.1 ± 9.6           | 3.8 ± 0.3          | 0.580                               | 1.7 ± 1.5            | 36.7 ± 0.2            |
| G14              | 4.7 ± 0.3                                      | 12.0 ± 0.8               | 58.6 ± 3.7           | 4.4 ± 0.3          | 0.017                               | 5.4 ± 1.7            | 39.2 ± 0.4            |
| G15              | 4.0 ± 0.3                                      | 14.2 ± 1.0               | 69.8 ± 6.8           | 3.9 ± 0.2          | 0.355                               | 3.8 ± 1.0            | 38.8 ± 0.9            |
| G16              | 4.3 ± 0.2                                      | 20.7 ± 0.8               | 86.3 ± 4.5           | 3.8 ± 0.1          | 0.013                               | 1.5 ± 0.5            | 37.5 ± 0.3            |
| G17              | 4.9 ± 0.1                                      | 18.6 ± 1.3               | 87.0 ± 6.1           | 4.8 ± 0.2          | 0.016                               | 1.2 ± 0.6            | 37.9 ± 0.5            |
| G18              | 4.5 ± 0.2                                      | 13.9 ± 0.6               | 66.7 ± 4.9           | 4.3 ± 0.2          | 0.035                               | 0.7 ± 0.3            | 37.8 ± 0.4            |
| G19              | 3.8 ± 0.2                                      | 15.3 ± 1.3               | 79.3 ± 6.7           | 4.2 ± 0.1          | 0.007                               | 1.3 ± 0.8            | 37.6 ± 0.8            |
| G20              | 4.1 ± 0.2                                      | 9.8 ± 0.5                | 50.5 ± 3.7           | 4.2 ± 0.2          | 0.000                               | 10.5 ± 2.9           | 38.7 ± 0.2            |
| Narragansett Bay |  |                          |                      |                    |                                     |                      |                       |
| N2               | 3.3 ± 0.2                                      | 22.9 ± 2.5               | 112.0 ± 5.3          | 3.7 ± 0.2          | 0.031                               |                      | 34.7 ± 0.3            |
| N3               | 3.3 ± 0.2                                      | 22.0 ± 1.3               | 74.3 ± 3.3           | 5.2 ± 0.2          | 1.285                               |                      | 35.2 ± 0.3            |
| N4               | 2.7 ± 0.2                                      | 21.5 ± 1.0               | 84.0 ± 5.5           | 4.7 ± 0.2          | 0.567                               |                      | 34.8 ± 0.1            |
| N5               | 3.1 ± 0.2                                      | 12.7 ± 1.4               | 54.7 ± 4.5           | 4.4 ± 0.2          | 0.292                               |                      | 35.7 ± 0.2            |
| N6               | 2.9 ± 0.2                                      | 13.3 ± 0.9               | 63.7 ± 2.8           | 4.4 ± 0.2          | 0.124                               |                      | 36.1 ± 0.1            |
| N7               | 3.3 ± 0.2                                      | 14.1 ± 1.5               | 71.2 ± 7.3           | 4.3 ± 0.2          |                                     |                      | 36.1 ± 0.3            |
| N8               | 2.7 ± 0.2                                      | 8.9 ± 0.9                | 36.4 ± 3.7           | 3.0 ± 0.1          | 1.266                               |                      | 36.1 ± 0.2            |
| Waquoit Bay      |  |                          |                      |                    |                                     |                      |                       |
| W1               | 3.9 ± 0.2                                      |                          | 63.8 ± 4.6           | 4.3 ± 0.1          | 0.000                               |                      | 37.8 ± 0.1            |
| W2               | 4.0 ± 0.0                                      |                          | 49.0 ± 4.4           | 4.9 ± 0.2          | 0.000                               |                      | 38.2 ± 0.2            |
| W3               | 4.2 ± 0.3                                      |                          | 48.2 ± 2.6           | 5.3 ± 0.4          | 0.007                               |                      | 37.2 ± 0.2            |
| W4               | 4.2 ± 0.3                                      |                          | 78.0 ± 3.8           | 4.7 ± 0.2          | 0.035                               |                      | 38.4 ± 0.2            |
| W5               | 4.2 ± 0.3                                      |                          | 31.6 ± 2.0           | 4.8 ± 0.3          | 0.052                               |                      | 37.4 ± 0.3            |

Values are mean ± S.E.

Number of leaves per shoot was significantly ( $P < 0.001$ ) different among sampling stations in Great Bay Estuary, but there was no trend along the estuarine gradient (Table 1). Numbers of leaves per shoot were not significantly different among the sampling stations in either Narragansett Bay or Waquoit Bay ( $P = 0.06$  and  $0.77$ , respectively; Table 1). Mean leaf number per shoot was lowest in Narragansett Bay (3.0), and was not significantly different between Great Bay Estuary (4.2) and Waquoit Bay (4.1; Table 1). Sheath lengths were significantly ( $P < 0.001$ ) longer in down-estuary than up-estuary stations in both

Great Bay Estuary and Narragansett Bay, and were not significantly ( $P = 0.58$ ) different between these two estuaries (Table 1). Sheath lengths were not measured in Waquoit Bay. Shoot heights varied throughout Great Bay Estuary, with no clear pattern along the gradient (Table 1), although taller plants were associated with deeper water. In Narragansett Bay, shoot height was greatest at the mouth of the estuary and least in the upper estuary. In Waquoit Bay, shoots were significantly ( $P < 0.001$ ) shorter at the mouth of the estuary. Shoot heights varied in the three estuaries: the plants were significantly taller in Great Bay Estuary (74.7 cm) and Narragansett Bay (70.9 cm) than in Waquoit Bay (54.1 cm; Table 1).

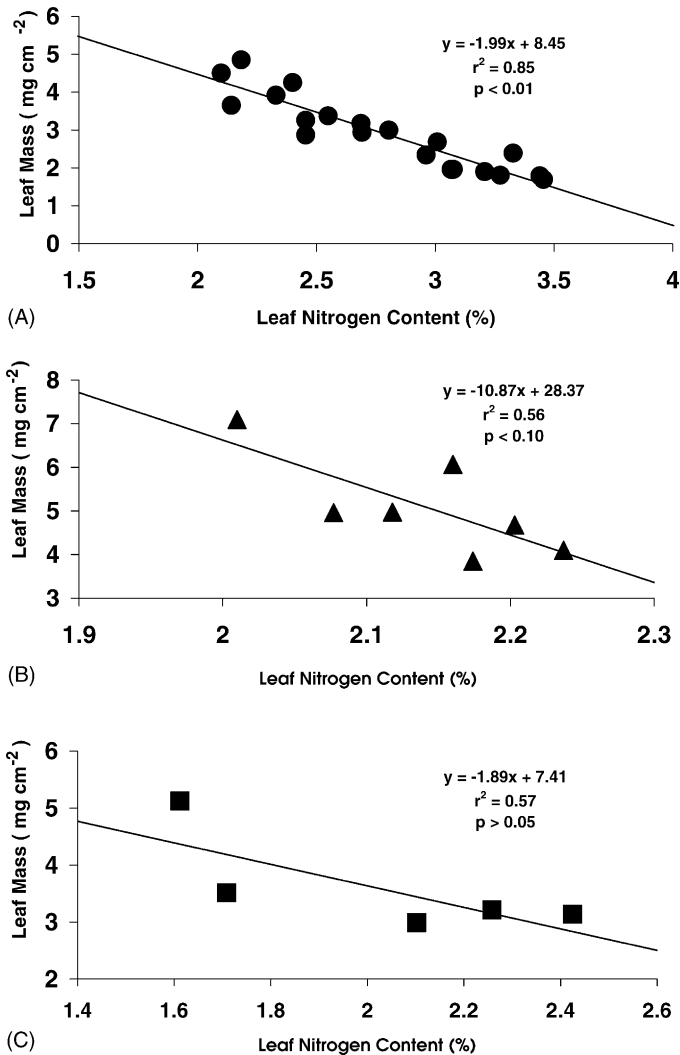


Fig. 5. Relationships between leaf nitrogen content and leaf mass in Great Bay Estuary (A), Narragansett Bay (B), and Waquoit Bay (C).

Leaf widths varied significantly ( $P < 0.001$ ) between stations in Great Bay Estuary and between stations in Narragansett Bay but were not significantly different ( $P = 0.07$ ) in Waquoit Bay. Leaves were significantly ( $P < 0.01$ ) wider in Waquoit Bay (4.8 mm) than in the Great Bay Estuary (4.4 mm) and Narragansett Bay (4.2 mm; Table 1). Leaf widths showed no significant trends within each estuarine system.

### 3.3. Epiphytes and Wasting Index

Epiphyte biomass did not show any clear trends along the estuarine gradient in Great Bay Estuary or Narragansett Bay. In Waquoit Bay, eelgrass leaves had more epiphytes up-estuary than seaward (Table 1). Mean epiphyte biomass was highest in Narragansett Bay ( $0.59 \text{ mg cm}^{-2}$  leaf area) and lowest in Waquoit Bay ( $0.02 \text{ mg cm}^{-2}$  leaf area; Table 1).

The extent of the wasting disease on eelgrass shoots was assessed using the Wasting Index (Burdick et al., 1993) for Great Bay only because very low levels of wasting disease were observed in Waquoit and Narragansett Bays during the study. Wasting Index in Great Bay Estuary was significantly ( $P < 0.001$ ) higher in seaward than in up-estuary stations, although stations 1 and 21 were exceptions (Table 1).

### 3.4. Relationships between leaf N content and plant characteristics

Eelgrass leaf N content exhibited negative relationships with leaf mass in all three estuarine systems (Fig. 5). Slopes of regression lines for these two parameters were similar for Great Bay Estuary and Waquoit Bay, but the slope was steeper for Narragansett Bay. The correlation between tissue N content and leaf mass was significant when values of the three estuaries were combined (Fig. 6).

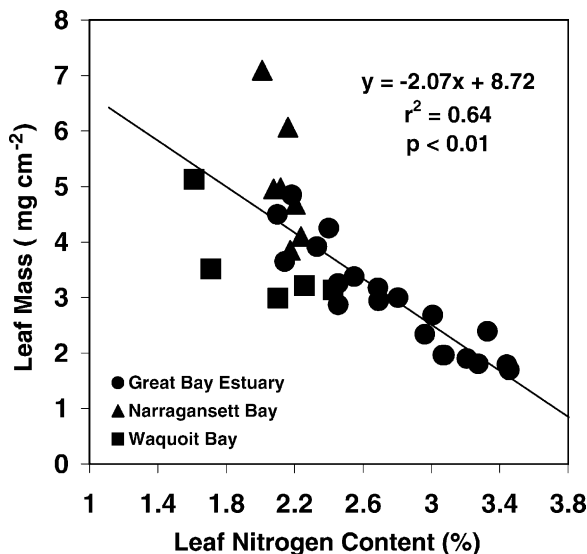


Fig. 6. Relationship between leaf nitrogen content and leaf mass for the three estuarine systems.

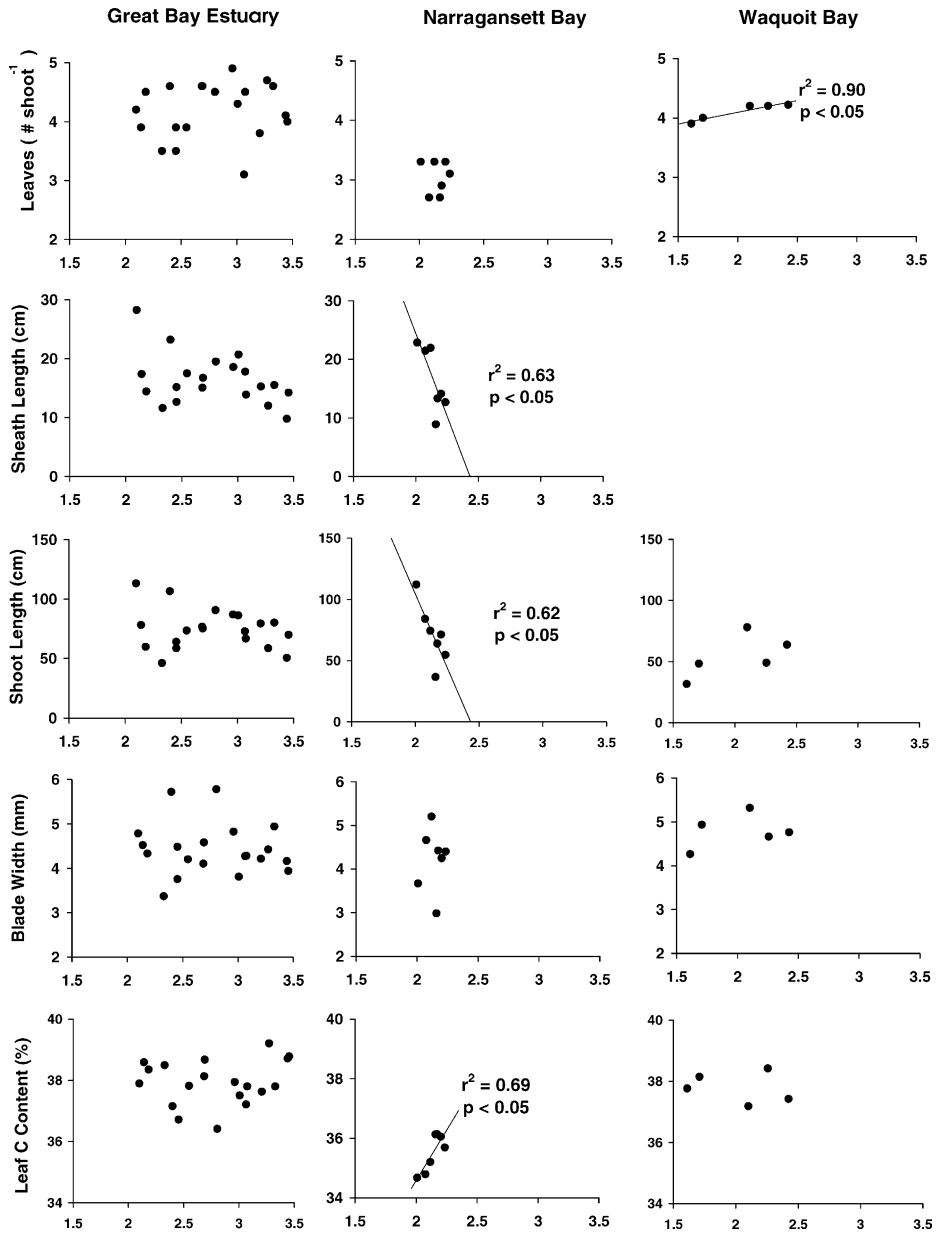


Fig. 7. Leaf nitrogen content vs. morphological parameters, leaf carbon content, epiphyte biomass, and Wasting Index in Great Bay Estuary, Narragansett Bay, and Waquoit Bay.

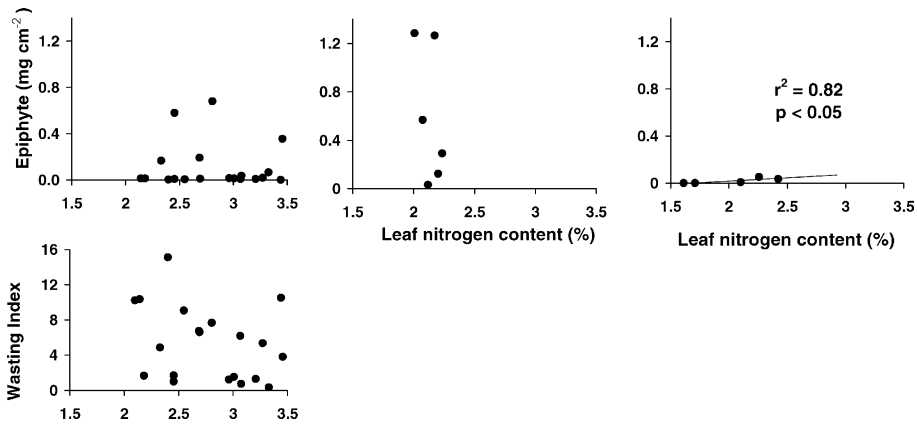


Fig. 7. (Continued).

In Great Bay Estuary, leaf N content did not significantly correlate with any other plant morphological characteristic, with epiphyte biomass, nor with Wasting Index (Fig. 7). In Narragansett Bay, leaf N content negatively correlated with sheath length and shoot height and positively correlated with leaf tissue C content (Fig. 7). Leaf tissue N content showed positive correlations with number of leaves per shoot and epiphyte biomass in Waquoit Bay (Fig. 7).

### 3.5. Ratios of leaf N content to leaf mass

Mean ratios of eelgrass leaf N content to leaf mass (NPI) ranged from 0.4 to 2.2 in the Great Bay Estuary, and were significantly ( $P < 0.001$ ) higher up-estuary than seaward (Fig. 8A). In Narragansett Bay, the mean ratios varied from 0.3 to 0.6, and also showed significant differences ( $P < 0.001$ ) among sampling stations (Fig. 8B). The mean ratios in Waquoit Bay, which ranged from 0.3 to 0.8, were lowest at the seaward station (W1) and were significantly ( $P < 0.001$ ) higher in the upper parts of the estuary (Fig. 8C).

## 4. Discussion

Dissolved inorganic nutrient concentrations in the water column can be measured directly, but such measures represent only the instantaneous nutrient status after rapid uptake by primary producers and dilution. The nutrient content of marine plants responds to the nutrient availability and motion of the surrounding waters (Fonseca and Kenworthy, 1987; Carpenter et al., 1991; Fong et al., 1994b; Hurd et al., 1996; Stevens and Hurd, 1997). Marine plant tissue nutrient content has thus been suggested as an indicator of environmental nutrient history (Atkinson and Smith, 1983; Duarte, 1990; Short et al., 1990; Fong et al., 1994a; Fourqurean et al., 1997). To derive a robust indicator of nutrient overenrichment, we conducted a space-for-time substitution using nutrient gradients to evaluate eelgrass response to nitrogen availability in three estuaries.

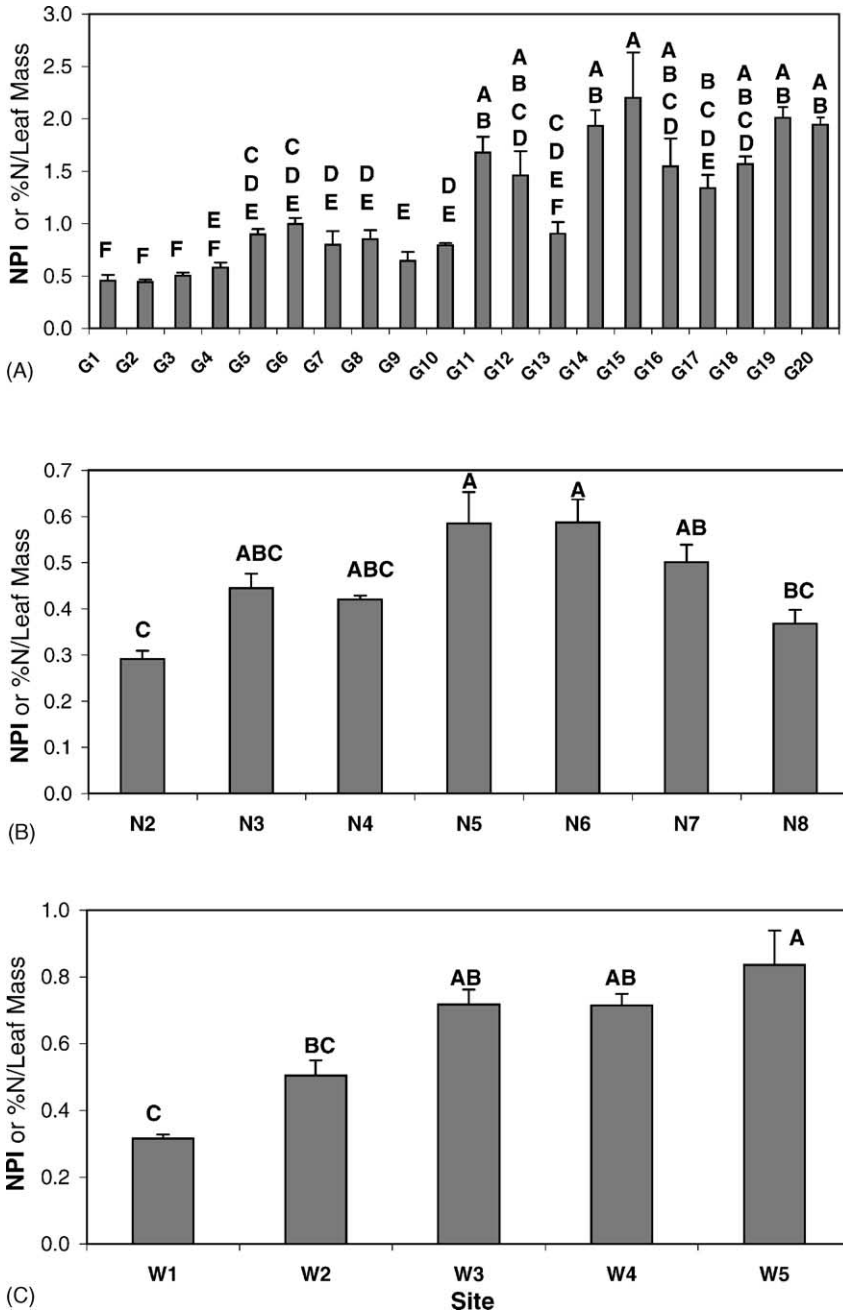


Fig. 8. The Nutrient Pollution Indicator (NPI), defined as the ratio of leaf nitrogen content (% N) to leaf mass, along nutrient gradients in Great Bay Estuary (A), Narragansett Bay (B), and Waquoit Bay (C). Values with same letter (listed above each bar) are not significantly ( $P < 0.05$ ) different among sampling stations.

For eelgrass, we found leaf N content provides an integrated measure of environmental nitrogen experienced by the plants. Unfortunately, similar to previous investigation (Fourqurean et al., 1997), we found that eelgrass N content alone was often too variable to clearly demonstrate significant differences in nitrogen availability. Eelgrass morphological characteristics such as number of leaves per shoot, blade width, sheath length and shoot height correlated only weakly with eelgrass leaf tissue N content, and these relationships of plant morphology to N content were not consistent for the three estuarine systems (Fig. 7). After analyzing many plant characteristics, a second variable, leaf mass (area normalized, i.e., weight of leaf tissue per unit leaf area), was found to vary consistently with changes in nitrogen availability across a range of enrichment, and the relationship was significant when values of the three estuaries were combined (Fig. 6). Further, its response was highly correlated to, and inverse to, that of eelgrass leaf N content; as nitrogen available to the plants increased, leaf mass decreased.

We found that leaf N content alone could not significantly separate sampling stations located along the nitrogen gradient in Narragansett Bay (Fig. 2B), but ratios of leaf N content to leaf mass were significantly different along the gradient (Fig. 8B). In Great Bay Estuary, the highest leaf N content (3.5%) was 1.7-fold greater than the lowest (2.1%), but the highest ratio of leaf N content to leaf mass (2.2) was 5.5-fold that of the lowest ratio (0.4). These differences in scale between measures of leaf N content alone and ratios of leaf N content to leaf mass indicate the greater sensitivity of the ratio to changes in environmental nitrogen. By combining leaf N content and leaf mass in a ratio (leaf N (%):leaf mass), changes in both plant nitrogen chemistry and plant morphology are captured by the NPI.

Because seagrasses can take up inorganic nitrogen from both the sediment and the water column (Iizumi and Hattori, 1982; Thursby and Harlin, 1982, 1984; Short and McRoy, 1984; Stapel et al., 1996; Pedersen et al., 1997; Terrados and Williams, 1997; Lee and Dunton, 1999b), eelgrass leaf N content reflects the nitrogen availability in both, which are not separated by using rooted plants for the NPI. Although the NPI is useful as applied here for detecting changes in nutrient gradients within an estuarine system, testing of hydroponically deployed eelgrass is necessary for direct comparison between estuaries (Lee et al., in preparation).

Primary producers compete for inorganic nutrients in the water column (Fong et al., 1994b). Since phytoplankton (in the water column) and epiphytes (on the seagrass blade surface) encounter nutrients released to the water before these nutrients reach seagrasses, they out-compete seagrasses for nutrients (Short et al., 1995). Excessive nutrient loading in an estuary can convert the seagrass-dominated community to a plankton-dominated or macroalgal-dominated ecosystem, or a system with excessive amounts of epiphytic algal growth on the seagrass (Short et al., 1993). Narragansett and Waquoit Bays already have converted to phytoplankton and macroalgal dominance, respectively, and have lost large amounts of their seagrass habitat (Valiela et al., 1992; Short et al., 1993). Nutrients entering Narragansett Bay and Waquoit Bay are likely taken up by various algal communities first, with eelgrass leaves exposed to the remaining nutrient concentrations. Nutrient competition among primary producers may be a cause of the lower leaf N content of eelgrass growing in Narragansett Bay and Waquoit Bay (Fig. 8B and C). Eelgrass leaf tissue N content is likely not fully representative of the nitrogen regime of estuarine systems which have large



populations of phytoplankton or macroalgae, making the NPI useful primarily at early stages of overenrichment.

In conclusion, eelgrass leaf N content reflected the environmental nitrogen exposure which plants experienced. Plant morphological characteristics such as number of leaves per shoot, blade width, sheath length and shoot height showed inconsistent trends along the three estuarine gradients and correlated only weakly with eelgrass leaf N content. Leaf mass showed strong and consistent negative correlation with all three nitrogen gradients and with eelgrass leaf N content, suggesting that leaf mass also reflects environmental nitrogen. The ratio of eelgrass leaf N content to leaf mass (NPI) provides a robust and sensitive early indicator of overenrichment. Further testing of the NPI will evaluate temporal differences and relate the indicator to absolute nutrient exposures.

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