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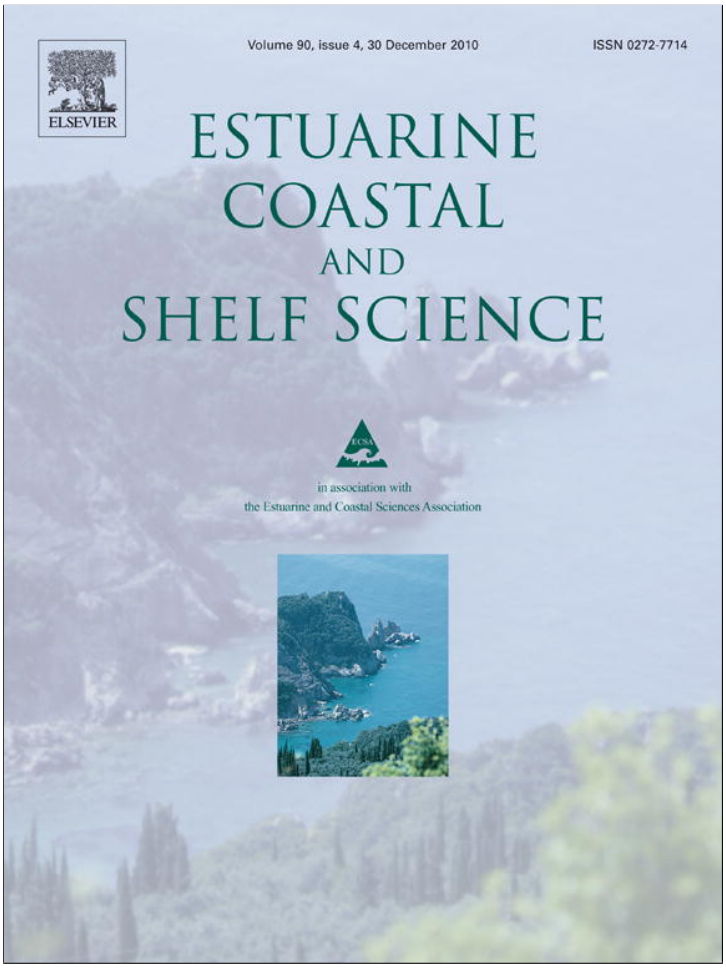

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Empirical relationship between eelgrass extent and predicted watershed-derived nitrogen loading for shallow New England estuaries

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ABSTRACT

Seagrasses provide important ecological services that directly or indirectly benefit human well-being and the environment. Excess nitrogen inputs are a major cause of eelgrass loss in the marine environment. Here we describe the results of a study aimed at quantifying the extent of eelgrass as a function of predicted watershed-derived nitrogen loading for small-to-medium-sized shallow estuaries in New England. Findings confirm that reduced extent of eelgrass corresponds to increased loading of nitrogen to this class of estuary. At lower levels of nitrogen loading ($\leq$50 Kg ha^{-1} yr^{-1}), eelgrass extent is variable and is likely controlled by other ecosystem factors unrelated to water quality. At higher loading rates, eelgrass coverage decreases markedly, with essentially no eelgrass at loading levels $\geq$100 Kg ha^{-1} yr^{-1}.

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1. Introduction

1.1. Excess nutrients as ecosystem Pressures

Human activities have dramatically changed the amounts, distributions, and movement of major nutrient elements (nitrogen and phosphorus) and have increased nutrient loading to receiving waters (Howarth et al., 2002). Some of these changes affect use of the nation's aquatic resources and pose risks to human health and the environment (NRC, 2000). EPA is in the process of developing guidelines that the states and tribes can use to set nutrient criteria for our nation's waters. For waters assessed as impaired, nutrient loading target plans, called total maximum daily loads (TMDLs), are needed to eliminate the cause(s) of the impairment. However, our current understanding of marine ecosystems is inadequate to extrapolate from nutrient load-ecological effects models developed for estuaries with extensive data (e.g., Chesapeake Bay and Long Island Sound) to those estuaries or, classes of estuaries, across regions with more limited data. To fill the gap in understanding this paper describes how seagrass extent varies with predicted nitrogen load for small-shallow estuaries in New England.

1.2. Ecological effects of excess nutrients

Ecological responses to excess nutrients generally fall into two categories: primary and secondary (Cloern, 2001). The primary response is an increase in algal production or carbon supply as defined by Nixon, (1995) and/or shifts in the algal community composition at the base of the food web. Secondary responses include an increase in extent and duration of hypoxia, loss of submerged aquatic vegetation (SAV) including eelgrass, and change and loss of biodiversity, including change in fish abundance and species composition (Cloern, 2001). There is a consensus among estuarine ecologists that excess nitrogen, not excess phosphorus, is the main cause of eutrophication in most estuaries (Howarth and Marino, 2006).

Seagrasses provide important ecological services including fish and shellfish habitat, and shore-bird feeding habitats, nutrient and carbon cycling, sediment stabilization, and biodiversity in tropical and temperate regions throughout the world (Orth et al., 2006; Duarte et al., 2008). Excess nitrogen loading has long been implicated in the loss of eelgrass (Short et al., 1995). The major mechanism of decline is considered to be nitrogen-fueled eutrophication through light limitation via planktonic, macro-algal, or epiphytic shading (Neckles et al., 1993; Duarte, 1995; Hauxwell et al., 2001, 2003). Vegetation in the shallow water marine environment compete with each other for nutrients and light in such as way that some forms thrive while others languish (Sand-Jensen and Borum, 1991; Havens et al., 2001). Also factors such as waves, currents, and

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tides affect the distribution of seagrasses (Koch, 2001). High organic loading derived from excess nitrogen may cause water column hypoxia and sediment sulfide production which also has been shown to affect seagrass health (Sand-Jensen and Borum, 1991; Koch, 2001; Eldridge et al., 2004; Vaudrey, 2008a). Thus, while light limitation is considered the major proximate cause of eelgrass decline, the ultimate cause is generally considered to be excess nutrient loading (Hauxwell et al., 2001; Leschen et al., 2010).

SAV abundance in subestuaries of the Chesapeake Bay has been shown to exhibit a strong threshold response to point source nitrogen inputs (Li et al., 2007). Moreover, using data from a limited number of estuaries ($n \leq 10$), others have described a threshold relationship between watershed-derived nitrogen loading and seagrass coverage in the northeast US (Short and Burdick, 1996; Bowen and Valiela, 2001; Hauxwell et al., 2003). The present study of over sixty (60) estuaries in New England is tailored to confirm and further define eelgrass coverage threshold behavior with respect to watershed-derived nitrogen loading.

2. Methods

The development of relationships between predicted nitrogen load and ecosystem response is based on a comparative systems approach in which loading and ecological responses are determined for a number of study embayments along a nitrogen load gradient. The research is divided into two components: 1) determination of nitrogen loading rates to coastal embayments from the watershed and atmosphere (Latimer and Charpentier, 2010); and 2) assessment of eelgrass extent, using aerially-derived digital images. As noted above, there is considerable science that describes the causal mechanisms between excess nitrogen, eutrophication, and seagrass effects (Conley et al., 2009; Waycott et al., 2009) typically following the paradigm: nitrogen loading => nitrogen concentration => chlorophyll-a concentration (or epiphyte magnitude) => light attenuation => seagrass light requirements => seagrass coverage. However, in this study we use a simplified approach that involves nitrogen loading => seagrass coverage using a comparative systems approach.

2.1. Study systems

Sixty-two (62) estuarine embayments, reflecting a gradient of watershed-derived + atmospheric nitrogen loading in southern New England, were evaluated in this study (Fig. 1). The study estuaries were classified as semi-enclosed coastal water bodies that are influenced by fresh water input that reduces salinity to below 30 psu during at least two months of the year (Madden et al., 2005).

Table 1 contains the descriptors and the range of values applicable for the study systems in this research summary. Table 2 provides summary statistics for some of the physical characteristics of the study embayments as well as loading estimates and eelgrass extent. To compare the results of this study to an estuary beyond those evaluated, one should keep in mind the components of the study systems noted in Tables 1 and 2. The closer the match

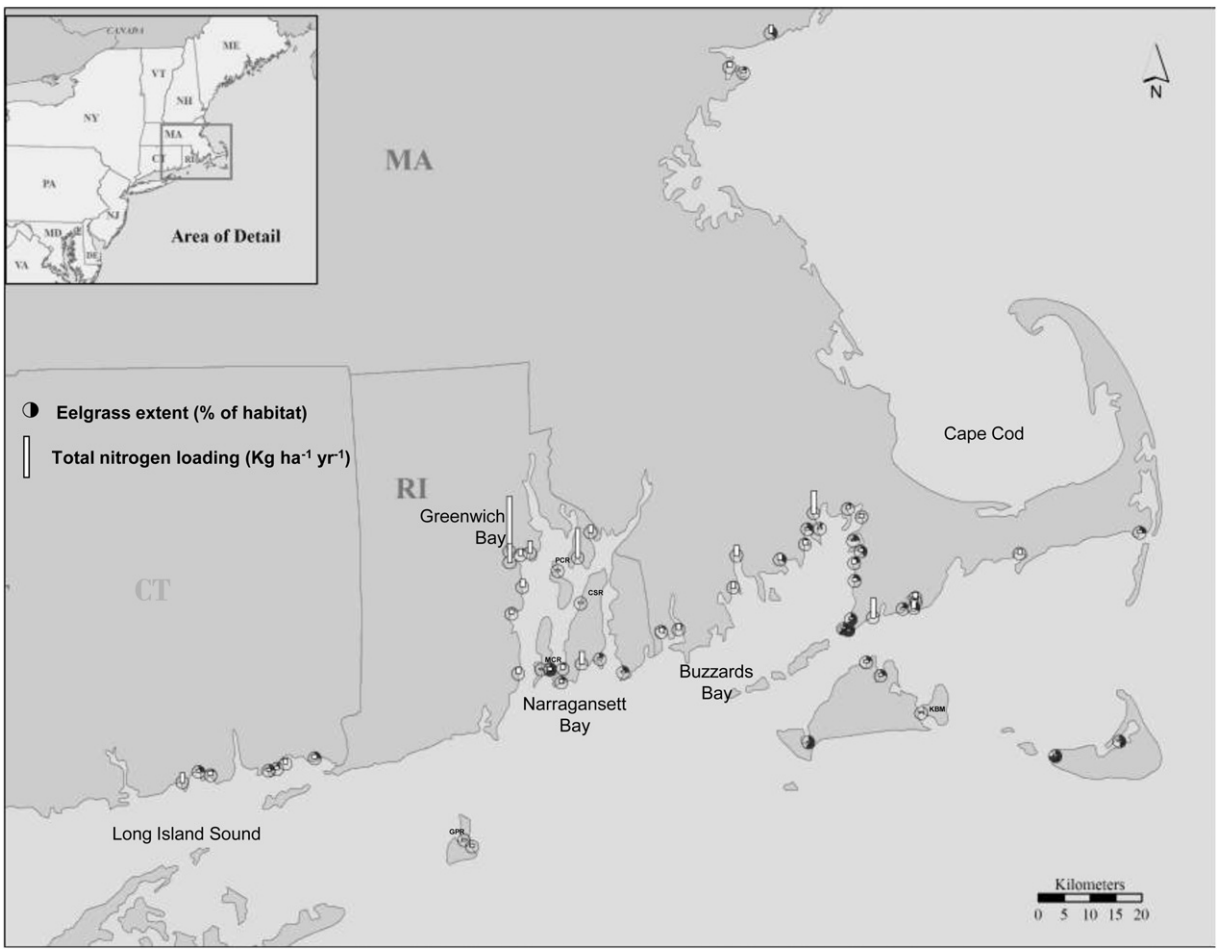

Fig. 1. Map of abundance of eelgrass in (percent of available habitat), and nitrogen loading to (Kg N ha^{-1} yr^{-1}), the study embayments.

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Table 1
Descriptors to define the class of estuarine embayment for the study systems.

Descriptors	Magnitudes
Temperature Class:	Cold (0–10 C) to temperate (10–20 C)
Salinity Class:	Mesohaline (5–18 psu) to euhaline (30–40 psu)
Oxygen Class:	Variable (anoxic, hypoxic, normoxic, saturated, supersaturated)
Turbidity Class:	Moderately turbid (2–4 m)
Turbidity Type:	Mixed (chlorophyll, mineral, colloidal, dissolved color, detrital)
Turbidity Provenance:	Mixed (allochthonous, autochthonous, resuspended, terrigenous, marine)
Energy Type:	Wind/tide/current
Energy Intensity:	Moderate (moderate currents and wave action, 2–4 kn)
Energy Direction:	Mixed
Depth Class:	Very shallow (0.3–8 m)
Tide Class:	Micro (<0.8 m), meso (0.8–1.8 m), macro (>1.8 m) tidal range (Bricker et al., 1999)
Primary Water Source:	Watershed, local estuary, local marine (non - river dominated)
Enclosure Status:	Partially - enclosed (<1–55% area encircled by land)
Vertical Stratification Type:	Homogeneous to minor stratification (Bricker et al., 1999)
Freshwater Influence Type:	Small ($\leq 10^{-3}$), moderate ($\geq 10^{-2} - < 10^{-1}$), large ($\geq 10^{-1}$) (Bricker et al., 1999)
Flushing Potential Type:	Low (micro and meso tide classes, small freshwater influence); moderate (micro and meso tide classes, moderate freshwater influence; macro tide class and small freshwater influence); high (micro and meso tide classes and large freshwater influence; macro tide class and moderate to large freshwater influence) (Bricker et al., 1999)
Dilution Potential Type:	High ($\leq \sim 2 \times 10^{-7}$), moderate ($> \sim 2 < \sim 8 \times 10^{-7}$) and low ($\geq \sim 8 \times 10^{-7}$) adjusted from (Bricker et al., 1999)
Trophic Status:	Oligotrophic (<5 ug Chl-a L^{-1}) to eutrophic (>50 ug Chl-a L^{-1})
Region:	Seven (7) Acadian Atlantic and Eight (8); Virginian Atlantic Region (Madden et al., 2005)
Embayment Size:	Small (<0.1 km^2) to medium (19 km^2)
Watershed Size:	Small (<0.1 km^2) to medium (240 km^2)
Terrestrial Ecoregions:	Northeastern Coastal Zone and Atlantic Coast Pine Barrens (Shirazi et al., 2003)
Geographic:	New England Region (CT, RI, and MA coastal)

of that estuary with those used to develop the seagrass relationships, the more likely that the conclusions would apply.

2.2. Estimation of nitrogen loading rate

Watershed and atmospherically derived nitrogen loading rates were estimated for each of the study embayments using a modification of a published and verified nitrogen loading model (NLM) (Valiela et al., 1997, 2000; Bowen et al., 2007a; Bowen et al., 2007b; Latimer and Charpentier, 2010). As originally constructed, the model estimates total dissolved nitrogen loads to shallow embayments for rural and suburban watersheds where the watersheds are underlain by unconsolidated sands and groundwater flow is the dominant method of transport. The NLM includes nitrogen inputs from wastewater (via septic systems, using values for per capita contributions of nitrogen), fertilizer use on turf and agriculture, and atmospheric deposition (estimated from regional data). We have modified the model to include inputs from wastewater treatment facilities (WWTFs) when present, as well as atmospheric deposition to the estuary surface (Latimer and Charpentier, 2010). Equations in the NLM describe attenuation of nonpoint source nitrogen during passage through different types of land uses within a watershed (i.e., natural vegetation, turf, agriculture, and impervious surfaces) and losses during travel through the soils, vadose zone, and the aquifer. WWTF inputs to the embayments are not attenuated and were computed from point source effluent monitoring data. The attenuation rates used in each component of the watersheds were derived from published empirical measurements (Valiela et al., 1997). The estimates derived from the NLM reflect the sum of the attenuated nitrogen loading from each source (wastewater, fertilizer, and atmospheric deposition) to produce an estimate of the total dissolved nitrogen entering the receiving embayment; these estimates are consistent with published values and from other loading models. For a more detailed description of the NLM application for these estuaries, see Latimer and Charpentier (2010).

2.3. Determination of eelgrass extent

Due to the broad geographic range of the study estuaries, we relied on data collected and validated from different but comparable sources along the coasts of Connecticut, Rhode Island and Massachusetts. Our goal was to utilize the most recent full coverage survey for each state. Below we briefly describe how these data were collected based on reported metadata.

2.3.1. Data acquisition

2.3.1.1. Connecticut. Seagrass data for Connecticut estuaries were obtained from US Fish and Wildlife Service (USFWS), Hadley MA. In spring of 2006, the USFWS National Wetlands Inventory Program (Region 5) funded by EPA Region 2, collected true color aerial

Table 2
Physical characteristics, nitrogen loading rates, and the results of the eelgrass analyses for the study ($n = 62$ estuaries).

Statistic	Estuarine Physical characteristics			Nitrogen Loading Rates			Eelgrass Extent	
	Volume (m^3)	Surface Area (km^2)	Mean Depth (m)	(Kg N yr^{-1})	Volume – normalized (g N m^{-3} yr^{-1})	Area[a] – normalized (Kg N ha^{-1} yr^{-1})	Areal Extent (ha)	% of Available Habitat[b]
Mean	5,900,000	2.41	2.53	19,900	7.12	106	31.7	16.8
Median	3,000,000	1.53	2.15	10,300	3.01	57.5	4.9	10.1
± SD	9,300,000	3.33	1.56	26,800	13.1	140	90.5	19.7
Minimum	65,500	0.02	0.25	43.1	0.31	24.2	0.0	0.0
Maximum	49,900,000	19.5	7.75	137,000	74.3	928	667	75.1

[a] Estuary area-normalized.
[b] Area of estuary with water depth ≤3 m.

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imagery (TCI) along the Connecticut coastline from the RI border to Clinton Harbor, CT. The imagery was collected at a scale of 1:20,000 and followed the remote sensing standard developed by NOAA: C-CAP protocols (Dobson et al., 1995; Tiner et al., 2007). Field verification work (*in situ* ground-truthing) was conducted by the USFWS during the summer at 290 field sites (Tiner et al., 2007). These images were scanned and rectified using a backdrop of ½ meter TCI derived from an airborne digital scanner to produce the final product.

2.3.1.2. Massachusetts. Seagrass data for Massachusetts estuaries were obtained from the Massachusetts Department of Environmental Protection website and are publically available (MAGIS, 2006). These data contained seagrass delineated polygons from two years, 1995 and 2001. For the purposes of our study only the 2001 data were utilized. Image acquisition occurred starting in 1999 and continued to 2001 between May and August using Aerocolor 2448 color positive film collected at a scale of 1:20,000. General C-CAP flight requirements were followed (Dobson et al., 1995). Fieldwork (*in situ* ground-truthing) was conducted to verify interpreted photosignatures using visual techniques and GPS. Orthorectified imagery was produced as the final product (resolution 1 m; accuracy 3 m) (MAGIS, 2006).

2.3.1.3. Rhode Island. Seagrass data for Rhode Island estuaries were obtained from the University of Rhode Island. Aerial photography was collected by a consortium in Narragansett Bay and Block Island Sound on August 5th, 2006 (Bradley et al., 2007). C-CAP flight protocols were followed during the collection event (Dobson et al., 1995). Polygon delineations were made and adjustments were done after field verification (*in situ* ground-truthing) activities concluded.

2.4. Data analysis

Estuarine (embayment) boundaries for the research sites were overlaid onto coastline coverages. Boundaries of each coverage were visually inspected to ensure overlay accuracy. After this initial review procedure, acquired seagrass data were added to the mapping project in ArcMap 9.3. Some seagrass coverages required reprojection to units that allowed for geoprocessing and map calculation. Seagrass coverages were then clipped using the estuary boundary. After geoprocessing, a subset of 10% of systems, chosen at random, from each dataset, was submitted for QA review. This involved visual inspection of post-processed GIS data and manual checks of calculated values.

Maximum seagrass depth was calculated using the Beer–Lambert equation and appropriate regional coefficients obtained from the literature. The Beer–Lambert optical law (Eq. (1)) is commonly used to evaluate various aspects of the light and depth requirements for seagrasses:

$$Z_{\max} = \frac{-\ln\left(\frac{I_z}{I_o}\right)}{K_d} \tag{1}$$

where: $Z_{\max}$ = depth of light penetration –considered maximum depth of eelgrass; K_d = light extinction coefficient (1/z); I_z = light irradiance at depth z–considered SAV light requirement; I_o = light irradiance at surface.

Using a light extinction coefficient of 0.7 and a light requirement of 15% ($I_z/I_o = 0.15$), eelgrass was expected to grow in the study region where the water depth is less than ~3 m. These values were obtained from a recently published report on water quality characteristics protective of eelgrass habitat for the state of CT (Vaudrey, 2008a), and are consistent with published depth limits for the region (EPA, 1988; Koch and Beer, 1996).

We evaluated the threshold response of eelgrass coverage with watershed nitrogen loading by using nonparametric change-point analysis (Qian et al., 2003). This method allows for the determination of a point along a gradient where the characteristics of the distribution abruptly change. In the case of the specific load-response relationship, this analysis provides a point along the loading axis where the eelgrass coverage changes, implying a shift in the character of the load-response relationship. We conducted the threshold analysis by identifying the first split of the regression tree in each of 500 bootstrap samples of the original data. The analysis used the rpurt and bootstrap functions in R. (R Development Core Team. 2008; Vienna Austria) (J. Grear, personal communication, EPA, Narragansett RI).

Direct determination of eelgrass loss was not possible because quantitative data on historical eelgrass extent were not available for any of our estuaries. We calculated eelgrass loss as the difference between potential eelgrass habitat area (i.e., area of estuary with water depth ≤3 m – from optical considerations alone, see above) and actual eelgrass extent. This is numerically equivalent to transforming the extent data using the equation: eelgrass loss (%) = 100 - habitat-normalized eelgrass extent (%). Others have reported eelgrass loss directly from historical data as well as loss using the same transformation as we did, and consider the data derived by both methods comparable (Vaudrey, 2008b).

The ecological response to nitrogen loading to an estuary will be modulated by its physical characteristics. Response to external nitrogen inputs will initially be a function of those processes that affect the nitrogen supply to autotrophs. These processes include additional internal nitrogen sources and sinks, as well as mixing (dilution) and exchange (flushing). The current study will interpret the results by generally accounting for dilution and flushing; future work, using the Estuarine Loading Model, would provide a more comprehensive evaluation of those processes that mediate between watershed nitrogen inputs and ecosystem response (Valiela et al., 2004). We derived estuarine susceptibility to nitrogen inputs using procedures outlined in Bricker et al. (1999) and Scavia and Liu (2009). The dilution potential is based on the idea that the water column is available to dilute nitrogen inputs and thus will be some function of estuarine volume. The flushing potential is the exchange capacity of an estuary and will be a function of tidal range and freshwater influence. The overall susceptibility to nitrogen loading will thus be a combination of dilution and flushing. Table 1 provides information on the data used to calculate estuarine susceptibility (vertical stratification type, freshwater influence, etc.). Additional information on the specifics of the computations may be found in the supplemental material for this manuscript (see Supplementary material).

3. Results and discussion

3.1. Spatial patterns of nitrogen and eelgrass

The underlying hypothesis tested in this study is that ecological response should exhibit some relationship with nitrogen loading, all other factors being equal. The loading gradient for the study is significant. Raw annual loading ranged from <50 to 1.4×10^5 Kg yr^{-1}, a gradient of ~4 orders of magnitude. A further refinement of the hypothesis is that the same nitrogen loading rate would have a greater effect on smaller estuaries than on larger ones. As noted earlier, estuaries are classified as small-to-medium (Table 1); however, they differed by nearly 3 orders of magnitude in surface area, from less than 0.1 to nearly 20 km^2 (mean 2.4 ± 3.3 km^2, Table 2). Therefore, on this more appropriate areal loading basis, the

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range in the nitrogen loading was a factor of 40 (Table 2). The estuaries with the greatest loading were located in upper/northern Narragansett Bay and inner/western Buzzards Bay (Fig. 1).

In the study systems, overall eelgrass extent ranged from zero to 667 ha (Table 2). Expressed as a percentage of total available habitat (i.e., area of estuary with water depth ≤3 m – from optical considerations alone, see methods section), eelgrass extent ranged from 0% to 75%. Fig. 1 shows the geographic extent of the eelgrass coverage for our study estuaries. When the three large aggregate estuaries (i.e., Long Island Sound, Narragansett Bay, and Buzzards Bay) are compared, there are clear differences. Except for three estuaries at the mouth of Narragansett Bay, the remaining sub-estuaries are nearly devoid of eelgrass. Narragansett Bay sub-estuaries had a mean of less than 1 ha of eelgrass which represents 7% of the total available habitat. In contrast, based on our estuarine boundaries, the mean area of eelgrass in the subestuaries of Long Island Sound was 27 ha, or, 14% of available habitat; for Buzzards Bay subestuaries, these values were 52 ha and 24%, respectively.

3.2. Relationship of nitrogen loading rates to extent of eelgrass

The associations between nitrogen input rates and eelgrass extent in the study estuaries fell into three categories: (1) low eelgrass extent when nitrogen loads are large, (2) high eelgrass extent when nitrogen loads are small, and (3) low eelgrass extent when nitrogen loads are small. Categories 1 and 2 are consistent with the accepted paradigm of water quality induced eelgrass response; category 3 represents those estuaries that do not seem to follow this water quality paradigm.

Categories 1 and 2. It is clear from a spatial evaluation of the data that, when nitrogen loads are large, eelgrass levels are very low or not existent (Figs. 1 and 2). This is particularly evident for estuaries in upper Narragansett Bay and a few estuaries in the inner area of Buzzards Bay and on the southern region of Cape Cod (Fig. 1). Estuaries in the outer portion of Buzzards Bay (and the islands), Long Island Sound and the mouth of Narragansett Bay fall into category 2; i.e., have relatively large abundances of eelgrass when nitrogen loading rates are low (Fig. 1). In summary, eelgrass and nitrogen loading trends in both category 1 and 2 estuaries (nearly all of the estuaries) are consistent with known causal mechanisms (Neckles et al., 1993; Duarte, 1995; Short et al., 1995; Hauxwell et al., 2001, 2003).

Category 3. There were five embayments that are extreme examples of estuaries with low watershed-derived nitrogen inputs and essentially no eelgrass (Table 3; labeled in Fig. 1 and designated as "anomalous systems" in Figs. 2 and 3). The explanation for low eelgrass levels with very low nitrogen inputs is difficult to specify but may involve either (a) an underestimation of actual nitrogen loads (and thus they would fall into category 1) or (b) other factors that affect eelgrass. All of these estuaries have limited watershed/atmospheric nitrogen inputs, and therefore it seems likely that water quality conditions should support eelgrass. However, nutrients from transient and moored boating may be an additional source for Coggeshall Point Harbor (CPR) and the Great Salt Pond (GPR) since these have been identified as having seasonally densely occupied marinas (RIDEM 2004) suggesting that water quality indeed may indeed not be sufficient to support eelgrass.

For Potter Cove (PCR), and to a lesser extent, Coggeshall Point Harbor (CSR), significant nitrogen loading from the larger Narragansett Bay system, may cause water quality to be insufficient for eelgrass (Fig. 1). As noted above, the NLM estimates nitrogen loading derived from the specific watershed surrounding each embayment plus direct atmospheric deposition to the estuarine surface, and does not account for nitrogen entering from the seaward boundary of the subestuary. We suggest that for many of the Narragansett Bay subestuaries, particularly those in the upper and middle Bay and Providence River (not just PCR and CSR) (Fig. 1), nitrogen from the larger estuary may play a significant role (beyond

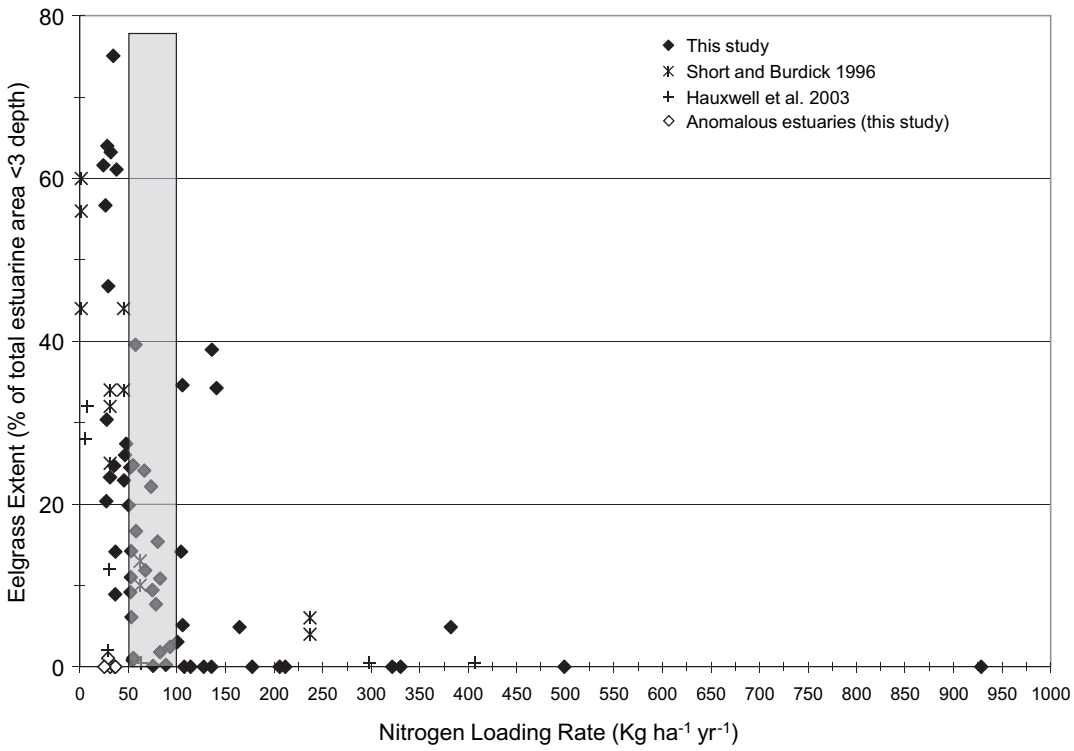

Fig. 2. Plot of eelgrass extent (percent of available habitat) vs. nitrogen loading rate (Kg N ha^{-1} yr^{-1}) (including other published values); gray bar is the nitrogen loading threshold range from the literature 50 - 100 Kg ha^{-1} yr^{-1}).

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Table 3
Estuaries with low calculated nitrogen input and low eelgrass levels (these have been designated as anomalous in Figs. 2 and 3).

Embayment	Aggregate Estuary	Kg N ha^{-1} yr^{-1}	Eelgrass % of Available Habitat	Comments
Katama Bay (KBM)	Atlantic Ocean	31	0	≻Historical presence (Wilcox, 2008) ≻Storm susceptibility (Wilcox, 2008) ≻303d[a] for bacteria (Connors, 2003)
Coggeshall Point Harbor (CSR)	Narragansett Bay	36	0	≻Historical presence 1945–1960 (Kopp et al., 1995) ≻Dense boat marina N input ≻Narragansett Bay N input
Great Salt Pond (GPR)	Block Island/RI Sound	36	0	≻Historical presence 1999 (C. Pesch, US EPA, personal communication) ≻Dense boat marina N input (RIDEM, 2000)
Mackerel Cove (MCR)	Narragansett Bay	29	<1	≻Historical presence 1975–1995 (Kopp et al., 1995) ≻storm susceptibility
Potter Cove (PCR)	Narragansett Bay	25	0	≻Historical presence 1933–1941; 1970s (Kopp et al., 1995) ≻Narragansett Bay N input

[a] Clean Water Act section that signifies that the water body assessment unit is impaired or "water quality limited", in this case for bacteria.

watershed loading) in causing poor water quality within the embayments. The major sources of nitrogen to Narragansett Bay are the rivers and wastewater treatment facilities that discharge into the northern and northeastern sectors of the estuary (Nixon et al., 2005). Moreover, published data support the notion that the upper and middle Bay has the highest nitrogen loading and water concentrations, decreasing with distance from this area (Oviatt, 2008; Vadeboncoeur et al., 2010). Therefore, the simplifying assumption that the "effective" nitrogen input from the seaward boundary is negligible compared to the input from the watershed of the embayment may not hold for some of the subestuaries of Narragansett Bay. According to recently published data (Whitall et al., 2007), Narragansett Bay receives nearly twelve times as much nitrogen on an area basis as Buzzards Bay. Thus, in terms of effective nitrogen input, we hypothesize that upper and middle Narragansett Bay would likely have a much larger effect on its subestuaries than would Buzzards Bay. In fact, this "seaward" source of nitrogen has been quantified for Greenwich Bay, a sub-estuary in the middle region of Narragansett Bay (see Fig. 1), to be from 37% to 56% of the total watershed inputs (Granger et al., 2000) and for Long Island Sound this "boundary load" has been estimated to be about 34% of the total in basin watershed load (NYSDEC and CTDEP, 2000). Thus, in contrast to a recently published paper (Nixon et al., 2009), current and future nitrogen reductions may help restore water quality and increase the likelihood of a return of eelgrass for at least the subestuaries in the middle portion of Narragansett Bay. An estimation of the effective nitrogen inputs from the seaward boundaries of our study systems would be required to test this hypothesis.

The remaining estuaries in category 3, Katama Bay (KBM) and Mackerel Cove (MCR), may reflect the effects of periodic storm-related wave and current conditions that make the systems less habitable to eelgrass. These estuaries may represent "no-grow zones" (EPA, 2003) and thus would be insensitive to water quality improvements caused by nitrogen loading reductions.

Finally, for anomalous category 3 estuaries, in addition to uncharacterized nitrogen inputs and hydrodynamic effects, factors such as substrate characteristics, non-algal particle water clarity

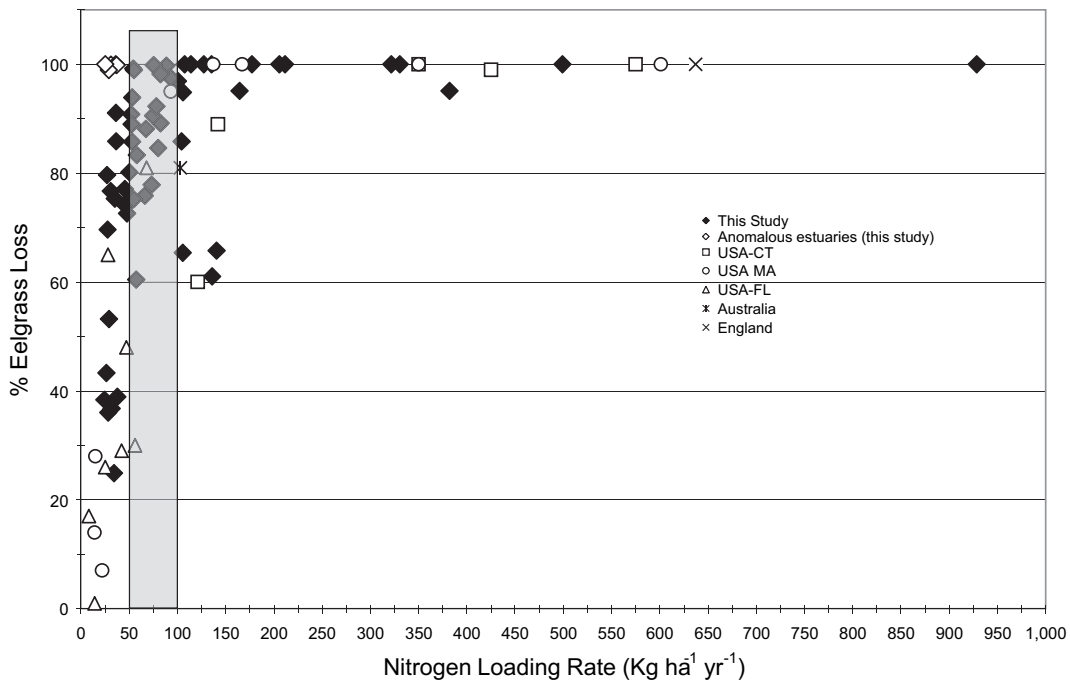

Fig. 3. Plot of eelgrass loss (percent) vs. nitrogen loading rate (Kg N ha^{-1} yr^{-1}) (including other published values). Data sources: (Valiela and Cole, 2002; Steward and Green, 2007; Vaudrey, 2008b); note not all of the data from the literature are for *Zostera Marina*. Gray bar is the loading threshold range from the literature 50 - 100 Kg ha^{-1} yr^{-1}).

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Table 4
Nitrogen loading thresholds vs. eelgrass loss; literature values and results from this study.

Loading Threshold Kg ha^{-1} yr^{-1}	Description				Reference
>20 100	• Areal cover of eelgrass sharply reduced; • Meadows disappeared (Cape Cod estuaries, $n = 10$)				Bowen and Valiela, 2001
~30 ≥60	• Substantial eelgrass loss (80–96% of bed area); • Total disappearance (Cape Cod estuaries, $n = 7$)				Hauxwell et al., 2003
≥64[a]	Threshold based on nonparametric change-point analysis (95% probability of change) (Chesapeake Bay estuaries, $n = 101$)				Li et al., 2007
≥52	Threshold based on nonparametric change-point analysis (95% probability of change) (New England, estuaries, $n = 57$)				This Study
Consensus of literature:	% eelgrass area loss (Fig. 4, $n = 57$):				
	Mean	Median	25th percentile	75th percentile	
≤50	62%	73%	39%	78%	This Study
51–99	88%	89%	82%	98%	
≥100	93%	100%	95%	100%	

[a] This only includes point source inputs.

effects, availability of seed stock for reproduction, predator activity, and even boat propellers, docks and moorings, can all reduce the viability of eelgrass, even when nitrogen-derived water quality may be good or improving (Fonseca et al., 1998; Burdick and Short, 1999; Bell et al., 2002). If all of these factors could be incorporated into the analysis it is likely that they would no longer be anomalous.

The results of this large study confirm that habitat-normalized eelgrass extent is inversely proportional to area-normalized nitrogen loading in these estuaries and that the data exhibit threshold behavior (Fig. 2). Our results are in agreement with published values for similar estuaries in New England (Short and Burdick, 1996; Hauxwell et al., 2003) (Fig. 2) and Chesapeake Bay (Li et al., 2007). At low nitrogen loading rates, eelgrass levels varied markedly, signaling that other variables such as extent of fringing marshes (which mitigate nitrogen from watershed), non-algal particles, colored dissolved organic matter (Vaudrey, 2008b) and substrate type, which are not considered, are likely to have an effect on eelgrass. Also, as noted above, one cannot completely rule out the possibility that, at least for estuaries in upper-mid Narragansett Bay, nitrogen from the larger estuary caused poor water quality. Nevertheless, eelgrass extent consistently decreased with increasing nitrogen levels.

In order to compare our results with those of other estuaries around the world, we transformed our data into what can be

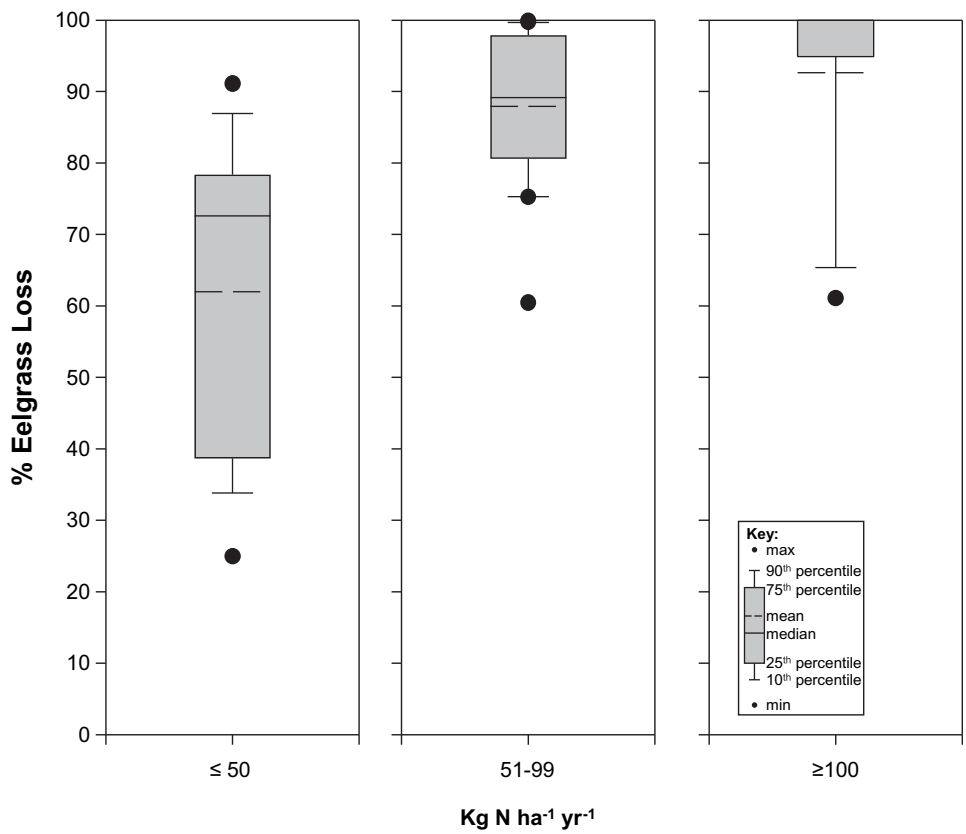

Fig. 4. Box and whisker plot of eelgrass loss (percent) by loading category (Kg N ha^{-1} yr^{-1}).

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considered a proxy for eelgrass loss (Fig. 3). Our results are in good agreement with other published values for estuaries from many regions of the U.S., including the Indian River and Banana River, FL (Steward and Green, 2007), Sarasota Bay, FL, Tampa Bay, FL, Waquoit Bay, MA (Bowen and Valiela, 2001; Hauxwell et al., 2003), and Great South Bay, NY (Valiela and Cole, 2002), as well as from England and Australia (note that not all of the data are specifically for *Zostera marina*). The data show significant variability, but it is clear that area-normalized nitrogen inputs are proportional to eelgrass loss and that the data exhibit threshold behavior (Fig. 3).

The eelgrass loss comparison with other studies across the globe suggests that a critical nitrogen loading threshold may be applicable beyond the small-to-medium sized southern New England estuaries. Although limited to seven study estuaries in New England, Hauxwell concluded that total loss of eelgrass was evident at nitrogen loading rates $\geq$60 Kg ha^{-1} yr^{-1}. Others, for estuaries around the country, have shown upper thresholds of 50 (Boynton et al., 1996; Vaudrey, 2008b) to 100 (Valiela and Cole, 2002) Kg ha^{-1} yr^{-1}. In a study of subestuaries of Chesapeake Bay a similar threshold was observed but with only point source nitrogen computed (Li et al., 2007). In the current study nonparametric change-point analysis detected a change point in eelgrass extent with watershed nitrogen loading. The value, where there is a 95% probability of a change point, 52 Kg ha^{-1} yr^{-1}, is similar to what other studies have observed (Table 4). This threshold behavior suggests that estuaries can change from eelgrass habitable to uninhabitable over a relatively narrow change in nitrogen loading (Fig. 1).

Using the calculated threshold from this study as well as literature threshold values, we grouped the data for the New England estuaries into three categories based on loading: $\leq$50, 51 to 99 and $\geq$100 Kg ha^{-1} yr^{-1} (Table 4 and Fig. 4). The mean eelgrass loss in New England estuaries with nitrogen loading up to 50 Kg ha^{-1} yr^{-1} was statistically lower (62%) than that in both the higher estuarine loading classes (51–99 Kg ha^{-1} yr^{-1}, 88%; $\geq$100, 93%) (*T*-test, $P < 0.05$). The results are consistent with the change-point analysis and suggest that water quality degrades to such an extent in estuaries with nitrogen loading rates greater than 50 Kg ha^{-1} yr^{-1} that the ability of eelgrass to thrive diminishes markedly. Moreover, at loading rates greater than 100 Kg ha^{-1} yr^{-1} eelgrass is essentially absent (this threshold range is shown in Figs. 2 and 3 as a gray bar) (Fig. 4).

This paper provides significant new evidence of threshold behavior of seagrass to nitrogen loading inputs from watersheds. Ecological effects, however, are not simply derived from the magnitude of nitrogen that comes from the watershed (and direct atmospheric deposition), but rather, include the mitigating or magnifying factors such as the flushing and dilution. To assess estuarine nitrogen susceptibility both dilution and flushing potentials were calculated for each of the 62 estuaries (see supplemental material for computation details). From this analysis (and excluding the anomalous estuaries), we determined that 22 estuaries (39%) are classified in the low susceptibility category, 16 (28%) in the moderate, and 19 (33%) in the category of high susceptibility to nitrogen inputs. Therefore, 33% of the study estuaries should be highly susceptible to the effects of nitrogen inputs. By combining observed eelgrass losses with the estimated nitrogen loading thresholds, it is possible to determine whether the derived susceptibility categories make sense. As a test we assumed that estuaries classified as highly susceptible should experience higher eelgrass loss for a given nitrogen load. In addition, those estuaries in the low susceptibility category should exhibit the least eelgrass loss. Indeed from 64% to 100% of those estuaries classified as highly susceptible showed large eelgrass losses, that is, were correctly classified (Table 5). This means that 64–100% of the estuaries that are most at risk actually show the effects of nitrogen. However, there are estuaries that are classified in the low susceptibility category that exhibit high eelgrass loss. Table 5 indicates that between 67% and 80% of the estuaries in the low susceptibility category show large eelgrass loss, that is, were incorrectly classified. This is likely due to an inadequate susceptibility assessment or that other factors besides excess nitrogen, are causing eelgrass loss. Nevertheless, it is possible to use this schema to aid in prioritizing estuaries according to their susceptibility to the effects of nitrogen inputs.

Table 5
Comparison of estuarine susceptibility, nitrogen loading, and eelgrass loss for study estuaries.

High Susceptibility	Low Susceptibility
N loading Rate threshold: >50 Kg ha^{-1} yr^{-1}	
64% correctly classified	67% incorrectly classified
36% incorrectly classified	33% correctly classified
N loading Rate threshold: $\geq$100 Kg ha^{-1} yr^{-1}	
100% correctly classified	80% incorrectly classified
0% incorrectly classified	20% correctly classified

4. Conclusions

Our analysis of 62 watershed-estuary systems in New England provides the most comprehensive study to date on the relationship between eelgrass coverage and watershed-derived nitrogen loading inputs. Analysis of the data suggest that nitrogen input loading rates greater than 50 Kg ha^{-1} yr^{-1} are likely to have a significant deleterious effect on eelgrass habitat extent in these types of estuaries. The findings provide insights relevant to state and national efforts to derive critical nitrogen load limits for shallow estuaries in the New England region and may be applicable to shallow estuaries over larger regions of the country:

- Eelgrass extent for shallow embayments along the southern New England coast is sensitive to land-derived and atmospheric inputs of nitrogen and the data show threshold behavior.
- Data for system-level ecological response variables (e.g., eelgrass extent) can yield useful relationships with nitrogen loading rates through an empirical comparative approach.

To our knowledge, this is the largest comparative ecological study of eelgrass extent and total watershed-derived nitrogen loading for US estuaries. The results of this study compare favorably to smaller studies for other estuaries around the US. As such, nitrogen loading rate and eelgrass loss patterns appear to be generalizable and could be used in the context of resource-based management, similar to what has been used for Tampa Bay, FL (Lewis and Devereux, 2009). The results are based on a system-level metric of eelgrass coverage in relation to predicted watershed nitrogen inputs. Eelgrass health and extent require good water quality commensurate with appropriate levels of nitrogen inputs, but they require additional factors such as, for example, favorable substrate, wave and current regime, water depth, non-algal particle concentration, and availability of seed stock for reproduction.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ecss.2010.09.004.

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