Journal of Environmental Management 131 (2013) 129–137

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Relationship between nitrogen concentration, light, and *Zostera marina* habitat quality and survival in southeastern Massachusetts estuaries

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A R T I C L E I N F O

Article history:
Received 21 March 2013
Received in revised form
15 September 2013
Accepted 20 September 2013
Available online 23 October 2013

Keywords:
Eelgrass
Eutrophication
Nitrogen threshold
Light intensity
Light threshold
Transplant survival

A B S T R A C T

The relationship of eelgrass survival and habitat quality to water column nitrogen level, phytoplankton biomass, particulate matter, bottom light intensity, and light attenuation was quantified at 70 sites within 19 Massachusetts estuaries through 4 growing seasons (2007–2009, 2011). Sites included a range of eelgrass habitat quality, from stable productive eelgrass beds, to degraded beds, to areas that have lost all eelgrass coverage. Survival of transplanted eelgrass culms was used as a bio-indicator of habitat quality. Habitat quality based upon both changes in stability of eelgrass coverage and transplant survival was positively related to light intensity and percent transmittance. Transplant survival was consistent with habitat designations based upon long-term changes in eelgrass coverage, with lowest light coinciding with areas that lost eelgrass in earlier decades. Bottom light declined in proportion to increases in total nitrogen levels, phytoplankton biomass, and water column particulates determined from long-term water quality data. Field surveys indicated that eelgrass survival required bottom light $\geq$100 $\mu E/m^2/s$ and healthy eelgrass existed where tidally-averaged total nitrogen was less than 0.34 mg/L, equivalent to a mid-ebb tide water-column total nitrogen of $<$0.37 mg/L. Traditional sampling of water column nitrogen at mid-ebb tide was found to slightly overestimate the average nitrogen level over a complete tidal cycle. However, since long-term, ebb-tide and tidally-averaged total nitrogen are correlated, it is possible to use the monitoring average to guide management until tidally-averaged TN becomes available. Nitrogen thresholds that support eelgrass communities provide a fundamental tool for managing this habitat and for selection of transplant sites aimed at accelerating restoration of this resource under increasing nitrogen loading of the coastal zone.

1. Introduction

Management and protection of coastal water bodies has become an area of intense focus on local, state, and federal levels. The Clean Water Act aims to set water quality standards and then regulate pollutants entering surface waters to meet those standards. The primary pollutant of concern for estuaries and bays in southeastern Massachusetts, as well as around the world, is nitrogen; and enrichment of this nutrient in coastal waters is increasing as populations grow within the coastal zone (Bricker et al., 1999). Quantifying the ability of estuaries to assimilate nitrogen without loss of habitat quality and setting realistic targets to restore degraded habitat in eutrophic waters is fundamental for managing eutrophication and protecting estuarine resources as watershed nitrogen inputs increase.

Seagrasses are sites of high productivity and provide essential habitat for many ecologically and economically important species (Thayer et al., 1984; Wood et al., 1969; Heck et al., 1995). Seagrasses are sensitive to nitrogen enrichment of coastal waters primarily because of their high light requirements of 15–25% of surface irradiance (Duarte, 1991; Dennison et al., 1993). Increasing nutrient availability causes increased production of phytoplankton (Ryther and Dunston, 1971), epiphytic algae and macroalgae (Hauxwell et al., 2003), resulting in light limitation (Cambridge and McComb, 1984) and seagrass decline (Harlin, 1993). At higher levels, depletion of oxygen in bottom water results due to increased water-column respiration and sediment oxygen demand associated with higher levels of organic matter production (Costa et al., 1992; Pickney et al., 2001 for review) and seagrass root growth and nutrient uptake is negatively impacted by tissue anoxia (Smith

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0301-4797/$ – see front matter
http://dx.doi.org/10.1016/j.jenvman.2013.09.033

et al., 1988; Zimmerman and Alberte, 1996). Seagrass has been lost in many areas such as Chesapeake Bay (Orth and Moore, 1983), Massachusetts (Costa, 1988; Short and Burdick, 1996; Costello and Kenworthy, 2011), and globally where losses have been estimated at 110 km^2 yr^{-1} and accelerating (Waycott et al., 2009). With the loss of seagrass is the associated decline in animal habitats (Lee and Olsen, 1985; Nixon, 1995; Taylor et al., 1995; Deegan, 2002) and economic losses (e.g. tourism, fisheries).

While the links between eutrophication and eelgrass decline have been well documented (Orth and Moore, 1983; Short and Wyllie-Echeverria, 1996; Cloern, 2001; Orth et al., 2006), quantitative nitrogen-related water quality metrics have been more difficult to determine. It is these quantitative habitat metrics that are now needed to support the expanding efforts to restore and protect this biologically diverse and economically important resource.

Investigations have used seagrasses to designate healthy and productive habitats with high water quality (Zieman, 1982; Thayer et al., 1984; Fonseca et al., 1990) and as specific indicators of water quality (Dennison et al., 1993; Lee et al., 2004). Similarly, a direct linkage of eelgrass habitat quality to watershed nitrogen loads has been attempted to investigate changes in seagrass biomass and productivity (Tomasko et al., 1996; Short and Burdick, 1996; Hauxwell et al., 2003). However, the generalizability of the approach is uncertain, since the same watershed nitrogen load will generate widely different eutrophying effects depending on the hydrodynamics of the receiving waters (i.e. the volume and flushing rate). Since eelgrass response in these systems is related to the nitrogen concentration that develops within specific eelgrass beds, dissolved inorganic nitrogen (DIN) levels have been used (Dennison et al., 1993; Stevenson et al., 1993). However, DIN is rapidly transformed to organic forms in many estuaries, with only 1–3% of the nitrogen remaining as DIN (Collos and Slawyk, 1976; Wheeler et al., 1982). In contrast, total nitrogen represents the total fixed nitrogen pool, as dissolved and particulate organic forms as well as DIN, and obviates the need to address the rapid transformations of nitrogen in estuarine waters. In the present investigation, we use total nitrogen (TN) levels instead of DIN, as a more robust indicator of the level of eutrophication.

Multiple parameter models, developed to assess the habitat requirements supportive of healthy seagrass by determining the critical water quality and light associated with submerged aquatic vegetation (Dennison et al., 1993; Stevenson et al., 1993) have had mixed success. Sites exceeding the designated thresholds (light attenuation, total suspended solids (TSS), chlorophyll *a*, dissolved inorganic nitrogen (DIN), and dissolved inorganic phosphorus (DIP)) have significant seagrass survival (Moore et al., 1996). In addition, many previous models have generally focused on large riverine estuaries like Chesapeake Bay (Dennison et al., 1993; Kemp et al., 2004) and the North River in North Carolina (Biber et al., 2008), where TSS, DIP, or dissolved color may predominate, compared to small shallow temperate estuaries. The shallow estuaries of Massachusetts tend to be groundwater dominated with little inorganic sediment loading and TSS, and have light attenuation primarily from nutrient inputs resulting *in situ* phytoplankton productivity rather than riverine transport.

Both increased nitrogen concentration and decreased light have been correlated with inhibition of eelgrass growth and there has been extensive research on the response of eelgrass to nitrogen enrichment (Burkholder et al., 1992; Short et al., 1995). However, the sequence of linked ecological processes initiated by changes in nitrogen inputs and resulting in changes in eelgrass habitat quality has not been extensively studied within the estuarine environment. Here we track increased water column nitrogen to light attenuation and decreased bottom light, and determine their effect on natural coverage and transplant survival of *Zostera marina* across southeastern Massachusetts estuaries.

Using measured water quality parameters, we examine the cascading effects of nitrogen enrichment on water-column constituents resulting in reduced bottom light intensity, and how these coupled factors negatively affect eelgrass habitat and transplant survival. Seventy sites within nineteen estuaries in southeastern Massachusetts with varying degrees of eelgrass health ranging from present stable beds, to areas which historically supported eelgrass (Costello and Kenworthy, 2011), both recently (1995) and decades ago (1951), were analyzed with respect to trends in nitrogen related water quality parameters and bottom light intensity. Eelgrass was transplanted at a sub-set of sites to assess survival relative to total nitrogen concentration and bottom light intensity. The relationships between TN levels, phytoplankton biomass, light level and eelgrass survival and temporal stability of beds were used to determine the existence of potential management thresholds for predicting eelgrass survival and habitat quality.

2. Methodology

2.1. Study systems

Nineteen shallow estuaries throughout southeastern Massachusetts showing ranges of eelgrass coverage and nitrogen enrichment were evaluated (Fig. 1). Bottom light, light attenuation and percent surface transmittance, and associated water quality parameters were measured at 70 locations distributed among nineteen estuaries where long-term changes in eelgrass coverage and water quality had been previously determined. In addition, 52 of the 70 sites with a range of nutrient related water quality and light regimes were selected for determination of eelgrass transplant survival. The field studies were conducted throughout the growing season in 2007, 2008, 2009 and 2011.

Each estuarine location selected for the present study had more than 10 years of summertime water quality measurements associated with the School for Marine Science and Technology, University of Massachusetts Dartmouth (Massachusetts Estuaries Project (MEP) www.oceanscience.net/estuaries); as well as multi-year eelgrass coverage surveys by the Massachusetts Department of Environmental Protection (Costello and Kenworthy, 2011). Locations were selected to include a variety of eelgrass habitat quality based upon the persistence of high quality beds and ranging from healthy (currently have healthy eelgrass beds, degraded (beds that are thinning and/or have extensive epiphytes), lost recently (lost historic beds between 1995 and 2001) and lost eelgrass coverage between 1951 and 1995.

2.2. Water quality sampling

Water quality parameters were measured during the summer period (mid-June to mid-September), when eutrophication related stresses are most pronounced in temperate estuaries. Measurements included Secchi and total depth, dissolved oxygen, temperature and light profiles. Bottom water samples were collected by Niskin bottle and analyzed for particulate and dissolved organic nitrogen, ammonium and nitrate + nitrite, as well as orthophosphate, chlorophyll-*a* and pheophytin-*a*, and salinity. Samples were transported to the laboratory in 1-L acid washed HDPE bottles in dark coolers maintained at 4 °C. Upon return to the laboratory, water was filtered (pre-combusted 25 mm Whatman glass microfiber (GF/F)), dried (at 60 °C to a constant weight), and analyzed on a Perkin Elmer Series II CHN for particulate nitrogen and carbon (Kirsten, 1983). Pigment samples were transported in dark 1-L bottles. Upon return to the laboratory ~300 mL sub-

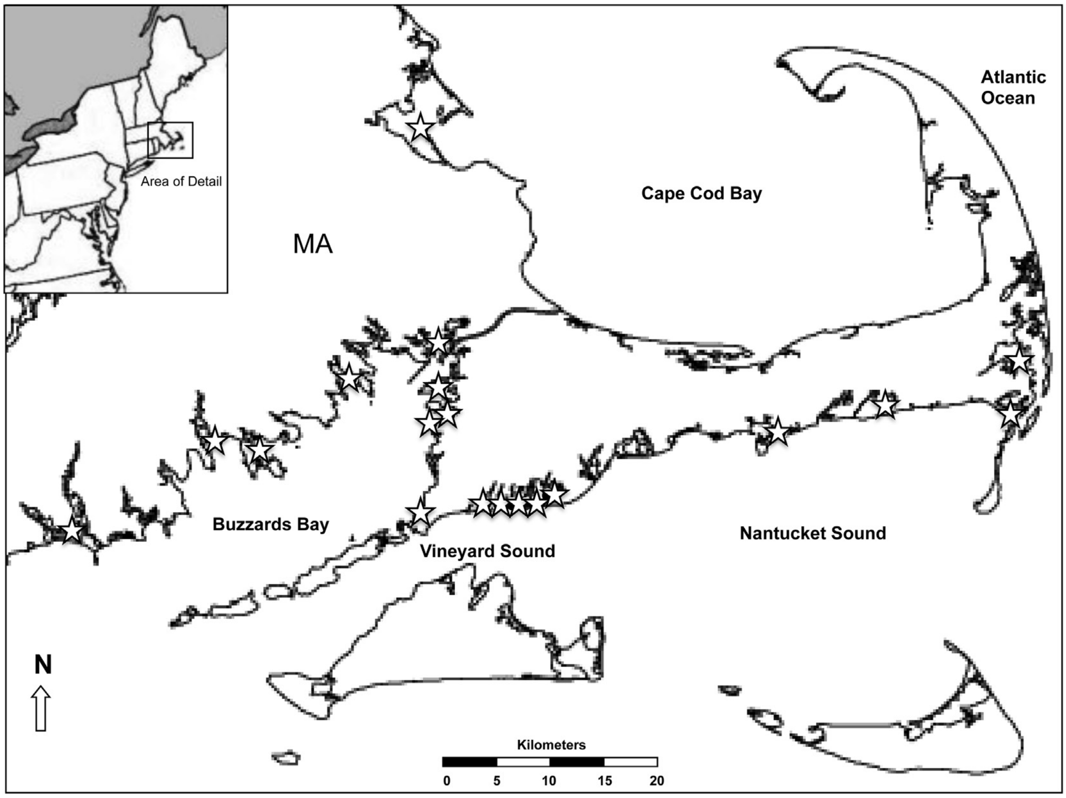

Fig. 1. Location of 19 southeastern Massachusetts estuaries wherein 70 sites were examined (stars).

samples were filtered (0.22 μm Millipore membrane) for chlorophyll-*a* and pheophytin-*a* analysis using a 90% acetone dark extraction and fluormetric analysis (Turner Designs 10-AU Fluorometer; Parsons et al., 1989). Dissolved nutrients were filtered in the field (low nitrogen 0.2 μm cellulose acetate Geofilter) and transported in 60 mL acid-washed polyethylene bottles on ice and assayed for ammonium by the indophenol/hypochlorite method (Scheiner, 1976), ortho-phosphate by the molybdate/ascorbic acid method (Murphy and Riley, 1962), and nitrate + nitrite as N by cadmium reduction (QuikChem 8000 Lachat auto analyzer; Wood et al., 1967). Field profiles of bottom water dissolved oxygen and temperature were made using a YSI 55 dissolved oxygen meter calibrated daily. The Coastal Systems Analytical Facility of the University of Massachusetts Dartmouth School for Marine Science and Technology assayed all water quality data used in this study, either specifically for this thesis or for the Massachusetts Estuaries Project, in support of various municipal and state estuarine monitoring programs.

2.3. Light measurements

Incident photosynthetically active radiation (PAR) was recorded every 15 min from June until October of each sampling season using Smart sensors (S-LIA-M003) and HOBO® Weather Station loggers positioned on docks or piers. Water-column light (total irradiance) and temperature were recorded ~0.3 m above the bottom every 15 min from May/June through October of each sampling season at each water quality site (HOBO® Temperature/Light Pendant Data Logger, UA-002-64). Light sensors were cleaned and downloaded at biweekly intervals and a profile of PAR measured with a Li-cor ® LI-250A light meter with a LI-193 Spherical Quantum Sensor (Photosynthetic Photon Flux Fluence Rate (PPFFR) or Quantum Scalar Irradiance) was recorded in the 2008 and 2009 field seasons.

The PAR sensors measure light intensity over the spectral range of 400–700 nm, expressed in μmols/m^2/s. Since the bottom water sensors, HOBO temperature/light pendants, measure total irradiance in lux from 150 to 1200 nm, it was necessary to develop an empirical relationship between the two types of sensors. Sensors of both types were compared in test basins using ambient light over several diurnal cycles. Each HOBO temperature/light pendant was matched with a PAR sensor to obtain pendant specific relationships to the PAR sensors. A polynomial curve fit was used to allow an estimate of PAR from the irradiance readings ($n = 26$; r^2 range 0.95–1.00). With both the bottom light intensity and the surface PAR measurement in the same units of μE/m^2/s, these light measurements were used to determine the percent surface irradiance within the water column and to calculate extinction coefficients according to the Beer–Lambert Law.

2.4. Eelgrass transplants

Eelgrass transplantation followed the TERF method (transplanting eelgrass remotely using frames) (Short et al., 2002) and a modification of TERF (Lesche et al., 2006). The modified TERF approach anchors the frame to the sediment surface with corner stakes and uses biodegradable jute mesh to hold 50 eelgrass shoots within the 0.25 m^2 frame. Large established eelgrass beds with low incidence of epiphytes, in close proximity to the 52 transplant locations, were used to collect culms for the TERF assays. Less than ~50 shoots were collected from a 1 m^2 area to prevent damage to the donor bed. Eelgrass was held in seawater, affixed to the frames and deployed within 24 h of removal.

Based upon a comparison of eelgrass survival using the TERF and modified TERF methods at 11 sites in 2007, which showed no significant difference in eelgrass transplant success, the modified TERF approach was used for all the stations during the 2008, 2009, and

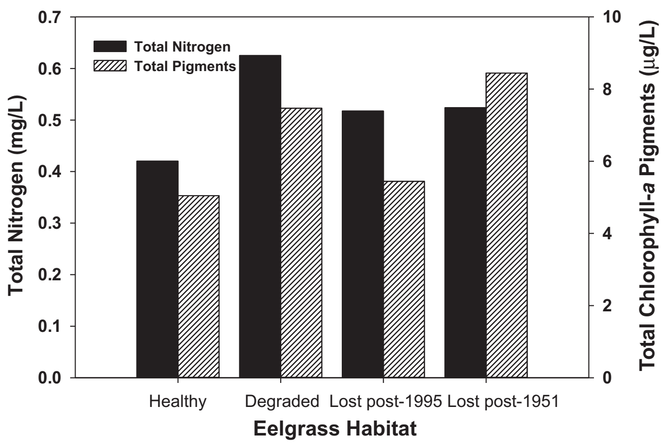

Fig. 2. Long-term total nitrogen and chlorophyll-*a* average concentrations (2000–10) in eelgrass areas that currently support healthy/stable beds, are degraded, or have lost coverage post-1995, or between 1951 and 1995 in southeastern Massachusetts.

2011. The transplants were proximate to existing water quality stations and historical eelgrass habitat. Eelgrass was initially transplanted in May–June of each year and shoots counted biweekly in concert with other monitoring tasks. Transplant success was determined by both the number of living shoots in October and the rate of shoot loss or gain as determined from the linear regression of the biweekly time-series measurements ($r^2 > 0.90$).

2.5. Modified light field and eelgrass survival

To evaluate the response of *Z. marina* density and growth to decreased bottom light, a field experiment was conducted within a natural eelgrass bed in Clarks Cove, New Bedford, MA. Light levels within the bed were manipulated to create replicate plots (2 m $\times$ 2 m) with 100%, and approximately 70%, 40% and 10% of ambient light over a growing season. Light reductions were created using monofilament nets and polyethylene shade cloths held above the eelgrass canopy on PVC pipe frames; controls had frames without shades.

Recording light sensors were placed in the center of each plot and a designated 0.5 m^2 area was staked off for tracking changes in the number of live eelgrass shoots. To confirm that the light treatments were uniform within the plots, light sensors were fixed at each corner of the 0.5 m^2 sample area. Biweekly sampling was conducted by SCUBA, which included the maintenance of the light sensors and shades. The time-series shoot density data was used to determine a rate of shoot loss for each treatment noted above. Parameters related to light followed the methods stated above, including incident PAR recorded on a nearby dock and biweekly measurements of PAR at 0.1 m water depth and below each shade to

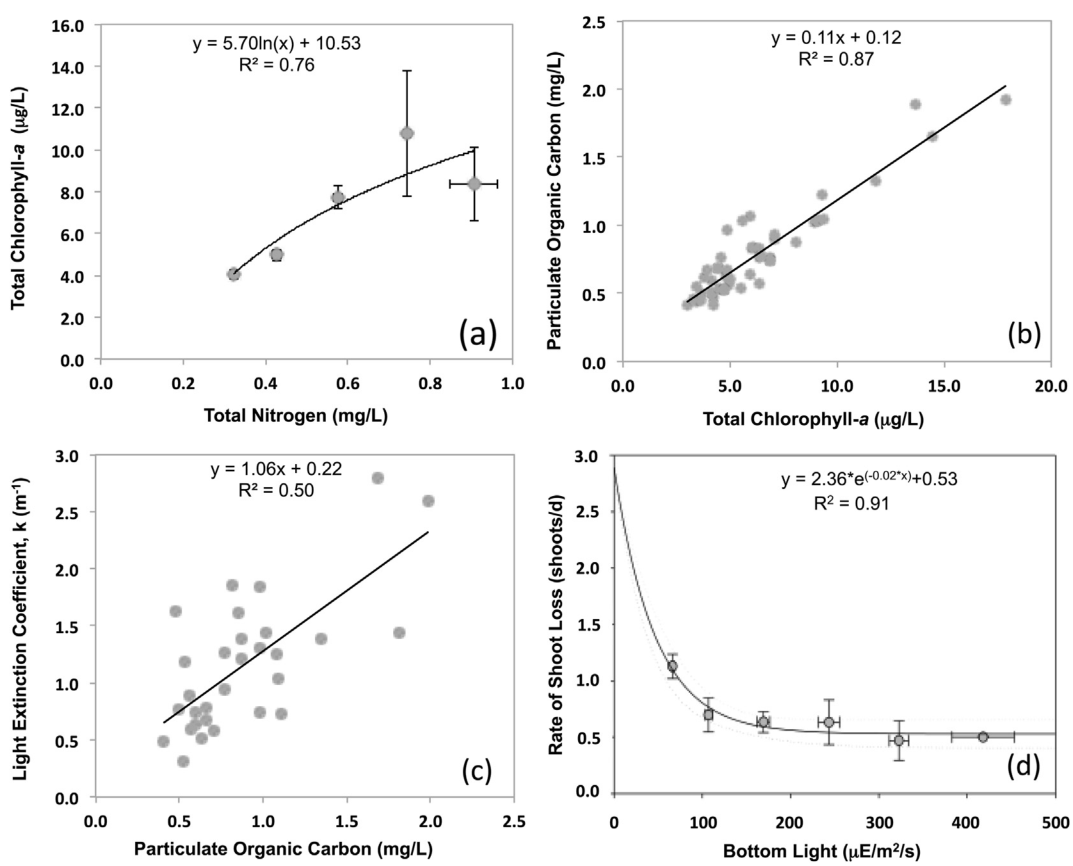

Fig. 3. a–d. Causal mechanism of eutrophication: a) Exponential relationship between total nitrogen and total chlorophyll-*a*concentrations. Data were grouped into ranges of total nitrogen Mean (2000–2010) ± standard error ($r^2 = 0.76$). b) Linear relationship between the total chlorophyll-a and particulate organic carbon in the water column. Mean (2000–2010) ($r^2 = 0.87$). c) Relationship between particulate organic carbon and the light extinction coefficient, *k* compiled from 2008 to 2009. d) Relationship between bottom light and the rate of eelgrass transplant shoot loss in 45 sites compiled from 2007 to 2009 in southeastern Massachusetts. The data were grouped into ranges of bottom light and fit an exponential equation $Y = A^{*}e^{(-k^{*}x)} + B$ ($r^2 = 0.91$). Bars show standard error and dashed lines the 95% confidence limits.

determine the light attenuation and allow a comparison to other studies that used percent of surface irradiance as a key metric related to eelgrass distribution.

3. Results

Water quality monitoring results (2000–2010) from each of the 70 sites within the 19 estuaries were compared to the associated designated eelgrass habitat quality (healthy, degraded, lost post-1995, lost 1951–1995). Healthy eelgrass sites had the lowest total nitrogen and total chlorophyll-*a* pigment concentration of the four groups, 0.42 mg/L and 5.1 μg/L respectively (Fig. 2). The highest total chlorophyll levels were found at sites that had lost historical eelgrass beds prior to 1995, while the highest total nitrogen levels were associated with degraded eelgrass beds. These observations support the contention that in these estuaries, increased nitrogen loading and resulting eutrophication plays a key role in the persistence and loss of eelgrass beds.

The process by which nitrogen enrichment affects eelgrass by decreasing light penetration was examined using the long-term water quality data (2000–2010) collected at the eelgrass sites (Fig. 3). In these estuaries where production is nitrogen-limited, there was a direct positive relationship between total nitrogen and total chlorophyll-*a* levels (Fig. 3a). Following the causal mechanism of eutrophication leading to seagrass loss, a positive linear relationship between total chlorophyll-*a* concentration and particulate organic carbon concentrations existed (Fig. 3b). Phytoplankton biomass is represented as total chlorophyll-*a*, with phytoplankton being the primary source of organic carbon to the waters and sediments of these estuaries, due to the absence of riverine inputs. Particulate organic carbon in estuaries can be represented by bacteria, phytoplankton, microalgae, zooplankton, and organic detritus and are correlated with chlorophyll concentrations (Fisher et al., 1998). As the limiting nutrient to a system is added (nitrogen, shown here as TN Fig. 3a), the rate of phytoplankton production increases, which then in turn contributes to the autochthonous addition of particulate organic carbon (Fig. 3b).

It appears that the primary source of light attenuation associated with nitrogen enrichment is the increase of organic particulate matter by increased primary production. This is supported by the positive relationship between particulate organic matter and light attenuation, in the parallel measurements made at each field site during 2008 and 2009 (Fig. 3c). Light attenuation is based upon the light extinction coefficient, *k*, using field light profiles and the Beer–Lambert Law equation. The net result is less light to support eelgrass production, leading to decline in coverage. This effect is consistent with the observed exponential relationship between measured bottom light and the rate of eelgrass transplant loss (Fig. 3d) in a cross-section of southeastern Massachusetts estuaries. The data also indicate a distinct increase in rate of shoot loss when the mean daily bottom light intensity is less than 100 μE/m^2/s.

A similar result was found when comparing bottom light to eelgrass habitat from the eelgrass sites designated as healthy, degraded, lost post-1995, and lost from 1951 to 1995 based upon the multi-year eelgrass coverage surveys (Costello and Kenworthy, 2011). Resulting 2007–2009 field light data shows the healthy sites had the highest bottom light with a mean of 186 μE/m^2/s, whereas areas where eelgrass beds were declining or had been lost had sequentially lower light (153, 144, 106 μE/m^2/s respectively). Percent of surface irradiance reaching the bottom at sites within each of the habitat classifications showed parallel results with healthy beds having the highest percent light penetration (23.7%) and then tapering light penetration for degraded/declining beds (21.0%), and areas that historically supported eelgrass (post-1995, 19.2%; 1951–1994, 18.0%). However, the percent surface irradiance for all categories was within the minimum light requirements for *Z. marina* determined by Duarte of 10–25% (1991).

In addition to the light records from the designated habitat sites ($N = 70$), a sub-set of these sites ($N = 52$) was assessed using the survival of transplanted eelgrass. Transplant survival was predicted by the habitat designation and bottom light (Fig. 4). The data were compiled into the respective eelgrass habitat designation and the areas with stable existing beds (healthy) had the highest bottom light (186 μE/m^2/s) and highest transplant survival (35%), while sites which have lost beds 1951–1995 had about half the light level, (106 μE/m^2/s) and a 0% transplant success. The reduced bottom light also was related to an increase rate of shoot loss ($r^2 = 0.91$ vs. 0.94). The data fit a logarithmic equation consistent with the behavior of light attenuation. Transplants into healthy eelgrass habitats have a much smaller loss of shoots, compared to the other areas, 0.33 vs. 0.55–1.5 shoots/day.

In addition to the field surveys, the light manipulation experiment, conducted in Clarks Cove, also indicated a direct relationship between bottom light and eelgrass survival. While the results support the contention that nitrogen enrichment resulting in decreased light penetration is a primary mechanism in eelgrass loss in estuaries, the secondary impacts of eutrophication (e.g. epiphytes, low oxygen) did not play a role in the experimental results, as only light penetration was altered. *Z. marina* density decreased as bottom light decreased. Even the least shaded plots (low shade), which received approximately 70% of ambient light or 71 μE/m^2/s (average of light shade treatment) had increased shoot loss (50%)

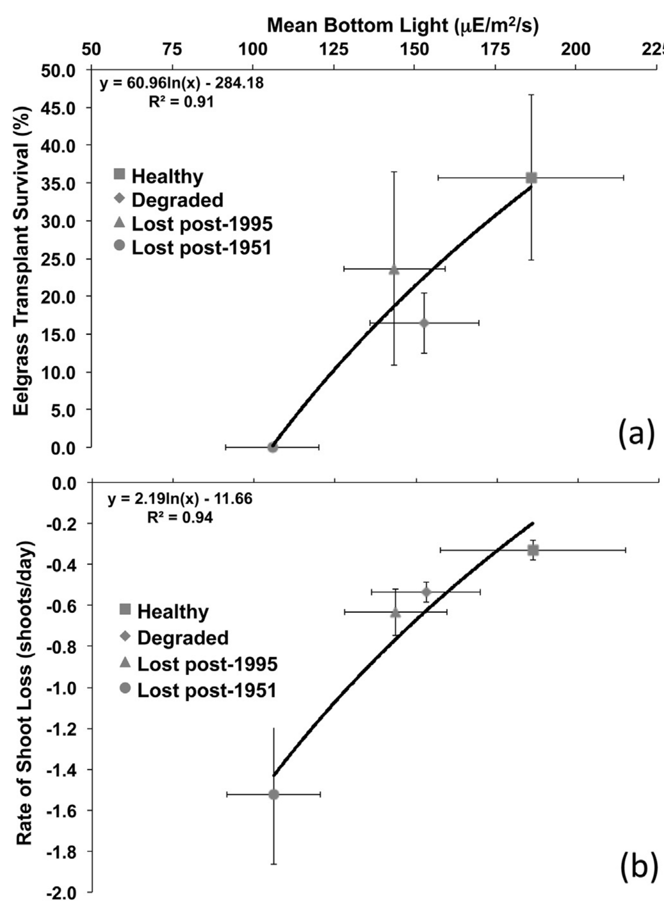

Fig. 4. Relationship between the mean bottom light and (a) eelgrass transplant survival and (b) the rate of shoot loss for 45 sites in 19 southeastern Massachusetts estuaries in the 4 eelgrass habitat categories. The solid line is the logarithmic regression ($r^2 = 0.91$ & 0.94 respectively). Site means ± standard error.

Table 1
Light regimes of each shading treatment over the experimental time range of June through October 2009 conducted in Clarks Cove New Bedford, MA. Mean percentages of surface light penetration, bottom light, and the corresponding rate of eelgrass shoot loss and the percent of density lost of each treatment are shown.

Treatment	n	Surface light penetration (%)				Bottom light (μE/m^2/s)				Rate of shoot loss (shoots/day)		Eelgrass loss (%)
		Mean	Standard deviation	Min	Max	Mean	Standard deviation	Min	Max	Mean	Standard error	
Control	6	44.84	10.69	17.98	87.90	105.86	52.14	7.46	239.24	−0.41	0.06	23.86
Control-2	4	33.63	9.07	8.41	49.38	90.52	39.42	7.93	189.94	−0.39	0.20	−8.06
Low shade	5	26.92	8.90	17.11	62.47	65.29	53.72	0.36	231.82	−0.53	0.23	53.33
LS-2	4	34.14	13.07	6.69	69.67	76.04	48.87	6.26	203.85	−0.42	0.10	47.14
Moderate shade	5	16.40	4.79	3.39	28.77	38.92	35.75	0.53	153.50	−0.96	0.06	96.67
MS-2	4	21.36	4.71	9.46	32.12	38.27	34.16	0.48	125.91	−0.85	0.02	75.61
High shade	5	5.76	1.93	1.11	12.28	7.61	7.97	0.25	39.99	−1.25	0.28	100.00
HS-2	4	3.47	1.31	1.20	7.05	6.40	4.78	0.00	20.06	−1.13	0.22	100.00

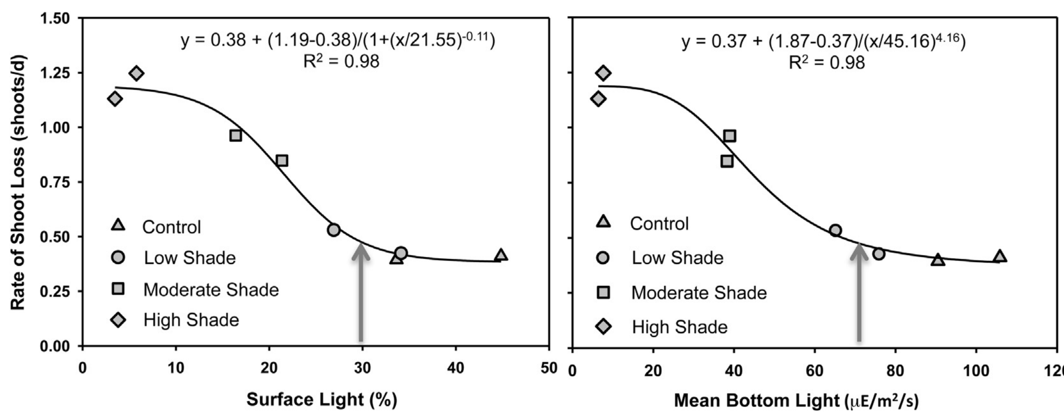

Fig. 5. The relationship between percent surface light and mean bottom light vs. rate of eelgrass shoot loss for each eelgrass shading treatment (shown in legend) from June to October 2009 in Clarks Cove New Bedford. (r^2 = 0.98 and 0.98 respectively) Arrows represents approximate minimum threshold light level.

compared to the unshaded controls (16%), while the most shaded plots (7% of ambient; 7 μE/m^2/s) showed 100% lost of eelgrass (Table 1).

Closer examination of the individual plot data indicates a non-linear effect of light level on eelgrass shoot loss. The sigmoidal relationship between light and eelgrass indicates that a threshold light level exists, below which shoot loss occurs rapidly (Fig. 5). Since the experiment was conducted in a shallow persistent eelgrass bed, it can be assumed that ambient light was sufficient to support the bed (106 μE/m^2/s). This is consistent with the lack of response in the rate of shoot loss as light levels were reduced from 106 μE/m^2/s to 76 μE/m^2/s, with only a small effect seen at 65 μE/m^2/s (Fig. 5). However, when light levels declined further to 40 μE/m^2/s a large increase in the rate loss was observed, which continued with further light reductions. It appears from the light manipulation experiment that a threshold light level of ~70 μE/m^2/s may exist in this system, where lower light levels results in significant eelgrass loss. A corollary may be that mean bottom light levels below 10 μE/m^2/s lead to a rapid loss of shoots (>1 d^{-1}) and complete loss of coverage.

Similar to the investigation of a light threshold for eelgrass survival, the relationship between eelgrass survival and water column nitrogen was investigated to determine if a critical concentration or threshold exists in southeastern Massachusetts estuaries, beyond which eelgrass coverage declines. Combining all of the transplant survival data, the percent eelgrass survival was inversely related to the level of total nitrogen (TN) at the transplant location, such that as TN concentration increases, the eelgrass transplant survival decreases (Table 2). Sites with >75% transplant success had average TN levels of 0.39 mg/L.

4. Discussion

The increase in total nitrogen concentration of estuarine waters, which has been used as a measure of the extent of eutrophication, has been linked to declining water quality and eelgrass loss. Although estuarine eutrophication has been well studied, the full sequence of increasing nitrogen causing increased chlorophyll concentration and decreased light penetration resulting in seagrass loss is rarely demonstrated (Latimer and Rego, 2010). One issue has been the need for comparable analysis across multiple estuaries to discern and quantify key parameters. In the present investigation of 19 southeastern Massachusetts estuaries, without significant riverine inputs, a clear positive relationship was observed between total nitrogen concentrations, phytoplankton biomass and particulate organic matter in the water column (Fig. 3a–c). The result of high production was decreased light penetration and bottom light available to support eelgrass. Decreased bottom light was directly related to losses in eelgrass coverage and lower survival of transplanted eelgrass shoots (Fig. 3d). Nitrogen related water quality in areas supporting stable healthy eelgrass beds were found to have lower total nitrogen and chlorophyll-*a*, and higher bottom light levels than areas that have lost eelgrass coverage, with declining beds having intermediate levels of these key water column constituents.

Light was the proximal cause of eelgrass transplant success and therefore likely the cause of the eelgrass bed loss observed in available multi-year surveys. Seasonally averaged bottom light intensity appears to be a good indicator of eelgrass habitat quality and transplant success. Bottom light was highest at sites supporting stable healthy eelgrass beds, lower in the declining beds, and lower

Table 2
Relationship between eelgrass transplant survival and total nitrogen concentration. The data were sorted into ranges of transplant survival from 2007 to 2009, 2011 and corresponding long-term total nitrogen concentration. Mean ± standard error.

Eelgrass transplant survival (%)	Total nitrogen (mg/L)
<25	0.68 ± 0.11
25–50	0.67 ± 0.11
50–75	0.49 ± 0.12
>75	0.39 ± 0.03

still in areas where eelgrass beds have been lost. The bottom light ranged from 153 to 186 $\mu E/m^2/s$ in areas where eelgrass beds were present, either as stable or declining beds. Sites which lost eelgrass coverage between 1951 and 1995 had a low mean bottom light of approximately 100 $\mu E/m^2/s$ and were unable to support transplants (100% mortality), suggesting that a bottom light level >100 $\mu E/m^2/s$ should exist before bed restoration via transplants is attempted (Fig. 4). In addition, as bottom light increases, the transplant survival was found to increase exponentially, emphasizing the importance of bottom light to eelgrass success (Fig. 4).

The light level required to support eelgrass can be further refined based upon light manipulations of natural eelgrass beds. Surface light irradiance is an important measure for determining the presence of seagrass and its transplant survival because of its high light requirements ranging from 10% to 37% of surface light irradiance (Duarte, 1991; Olesen and Sand-Jensen, 1993; Kenworthy and Fonseca, 1996). In this experiment eelgrass loss can occur when <20% surface light reaches the bed, but at levels >25% surface light irradiance eelgrass persisted. Surface light penetrations <20% lead to mean bottom light averages of 10–40 $\mu E/m^2/s$ and resulted in a loss of about 4 shoots $d^{-1}\ m^{-2}$ (Fig. 5). The light levels to sustain the beds (70 $\mu E/m^2/s$) are higher than lab estimates of the light compensation point for eelgrass, 15–47.3 $\mu E/m^2/s$ (Dennison and Alberte, 1985; Olesen and Sand-Jensen, 1993). The compensation point describes the light intensity required to balance photosynthesis and respiration, while the light threshold determined in this investigation represents a real world bottom light concentration (70 $\mu E/m^2/s$) that support whole plants *in situ* (Fig. 5).

It appears that improved water clarity can be achieved by reducing the nitrogen loads entering the coastal systems and thus aid in the restoration process of *Z. marina*, through increasing bottom light levels. However, the level of total nitrogen that will provide sufficient water clarity in these shallow estuaries has been difficult to quantify, even though water depths are relatively uniform. One problem has been that sampling of water column nitrogen levels needs to be during a consistent time in the tidal cycle, as concentrations change from flood to ebb. The highest concentration at a single point in an estuary is at ebb slack tide, when water has been held the longest and the lowest concentration is at flood slack tide, when the dilution by offshore floodwaters is greatest. To reduce variation due to the tidal cycle, most sampling programs in southeastern Massachusetts collect water at mid-ebb tide or have developed water quality models that determine the average concentration over the tidal cycle based upon these measurements. In the present investigation, the critical total nitrogen level associated with stable eelgrass beds was examined using both methods.

The Massachusetts Estuaries Project (MEP) reports have collected and studied the comprehensive water quality, nutrient loading, eelgrass coverage, benthic community, and hydrodynamics for many estuaries in southeastern Massachusetts to determine ecosystem-wide health. These studies have shown elevated total nitrogen concentrations focused in the upper reaches of estuaries and loss of historic eelgrass coverage (Howes et al., 1999, 2003, 2006; Costello and Kenworthy, 2011). The MEP reports are created by a collaborative effort between the Coastal Systems Program (University of Massachusetts-Dartmouth), Applied Coastal, the Cape Cod Commission, and the Department of Environmental Protection. These reports used a water quality and hydrodynamic model (RMA-2, RMA-4) that include tidal information, bathymetry, nitrogen and flow inputs and outputs, as well as long-term, ebb-tide water quality data to calculate site-specific, tidally-averaged total nitrogen concentrations throughout each embayment (King, 1990). As noted above, tidally-averaged nitrogen represents the best estimate of the TN level associated with a location, such as an eelgrass bed, as it accounts for the natural variation in concentration due to tidal exchanges.

Of the 19 estuaries included in the present study, the MEP estimates of tidally-averaged total nitrogen levels associated with the water quality sampling locations were available for 12 systems (http://www.oceanscience.net/estuaries). All of the stations used in the reports showed the same pattern as results from the current study, which only used sites that supported eelgrass at one time. The lowest tidally-averaged and ebb-tide total nitrogen was found in healthy eelgrass sites, while the highest was found in sites with degraded eelgrass (Fig. 6). Sites that have not supported eelgrass in the past 60 years had the highest total nitrogen concentrations in both the tidally averaged and long-term total nitrogen concentration averages. Sites with healthy eelgrass had a tidally-averaged total nitrogen concentration of 0.34 mg/L and ebb tide TN of 0.37 mg/L. However, a more conservative tool for establishing acceptable TN levels for management of eelgrass habitat and restoration would be the 75th percentile of data. In this case the 75th percentile of tidally-averaged TN was 0.36 mg/L or a long-term, ebb-tide TN of 0.38 mg/L in sites of healthy eelgrass. However, since long-term, ebb-tide nitrogen is correlated with the tidally-averaged TN, it is possible to use the monitoring average to guide management until the tidally-averaged TN value becomes available.

In this study ebb-tide, tidally-averaged, and 75th percentile total nitrogen concentrations were very similar, but the 75th percentile TN concentration would be more perceivable in larger or longer estuaries where volumes and residence time were greater. Remediation of nitrogen enrichment for the purposes of restoring impaired habitat needs to set TN targets that are supportive of benthic infauna, high dissolved oxygen and low to moderate chlorophyll levels. Using eelgrass alone to set nitrogen

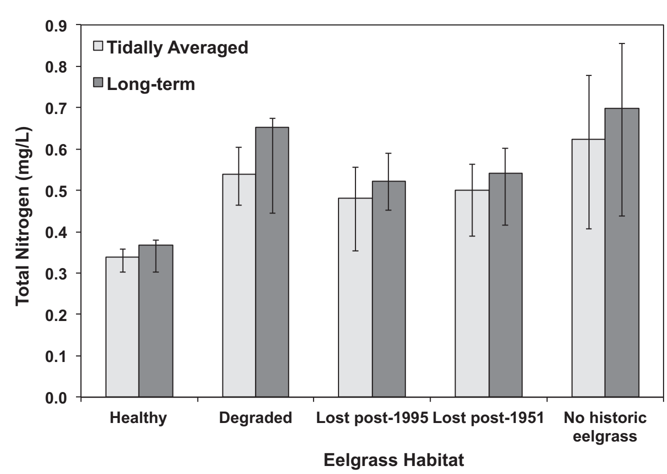

Fig. 6. Comparison of tidally averaged and long-term, ebb-tide total nitrogen concentrations (mg/L) between eelgrass habitats for all sites in twelve Massachusetts Estuaries Project (MEP) reports. The bars represent the upper 75th and lower 25th percentile of TN data. The long-term TN in the MEP reports use data compiled for ≥ 3years.

management targets, for example under the Clean Water Act, will result in the most restrictive and inclusive target concentration since eelgrass is such a sensitive indicator of water quality.

The sites with degraded eelgrass were found to have the highest total nitrogen concentrations, yet the healthy eelgrass areas clearly are associated with low TN and high bottom light. These results indicated that the presence of eelgrass is not descriptive enough to determine target total nitrogen concentrations and grouping all eelgrass sites regardless of if they are stable or in decline obscures the relationship. The resolution gained by using specific eelgrass health increases the predictive power of using total nitrogen as a gauge and determines areas with high priority for restoration. Sites deemed as degraded had one or all of the following characteristics: large amounts epiphytic algae, eelgrass that was being smothered by macroalgae, and sparse thinning beds. These characteristics were noted at sites specifically studied in this field research. Many estuaries are losing eelgrass coverage rapidly and presence and condition of this resource needs to be surveyed to determine the level and rate of degradation occurring.

5. Conclusions

Management actions to restore eelgrass should employ long-term water quality monitoring data to determine when TN reaches levels supportive of eelgrass. These water quality studies should be supplemented with bottom water light records before transplanting is attempted on a large scale. Total water column nitrogen is a common environmental parameter collected in water quality monitoring and can be used to refine the target levels developed for southeastern Massachusetts estuaries and for application to other estuaries, as site-specific targets provide the most efficient management approach. The combination of TN and bottom light provides a more robust approach, as TN alone does not take into account water depth. The strong relationship seen between TN and eelgrass habitat and survival point to the efficacy of using TN as a critical metric in predicting eelgrass restoration success in shallow estuaries. Although the depths were relatively shallow in this study, the underlying process relating TN and bottom light intensity remain true for deeper estuaries in the absence of other sources of turbidity Many restoration projects are currently underway in Massachusetts embayments to lower estuarine nitrogen levels to the TN targets indicated by this study, and continued water quality monitoring and periodic transplant experiments and light measurements are being used to assess habitat restoration.

Acknowledgments

We would like to sincerely thank Dr. Craig Taylor from Woods Hole Oceanographic Institute for his scientific expertise, technical review, and assistance with curve fitting. In addition, we thank John Ramsey and Roland Samimy from the Massachusetts Estuaries Project for providing the tidally averaged total nitrogen results. Field and laboratory assistance was provided by the researchers at the Coastal Systems Program: Michael Bartlett, Sara Sampieri Horvet, Dahlia Medeiros, Kaitlyn Shaw, Camille David, and the Coastal Systems summer interns. We also thank Three Bays Preservation, Falmouth Pond Watchers, Chatham Water Quality Monitoring Program, Yarmouth Water Quality Monitoring Program and the Harwich Water Quality Program for providing water quality information and note the dedication of their water quality monitoring volunteers, without whom this work would not have been possible. We would also like to thank Ross Kessler and Steve Aubrey for their assistance with the eelgrass transplanting method. This research was funded through the Coastal Systems Program, SMAST-UMASS Dartmouth.

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