Application and assessment of a nutrient pollution indicator using eelgrass (Zostera marina L.) in Barnegat Bay–Little Egg Harbor estuary, New Jersey

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**A B S T R A C T**

Eutrophication degrades numerous estuaries worldwide and a myriad of assessment metrics have been developed. Here, we apply an example of a previously developed metric (Lee et al., 2004) designed to indicate incipient estuarine eutrophication to validate this technique in an already eutrophic estuary end-member, Barnegat Bay–Little Egg Harbor, New Jersey. The metric, termed ‘Nutrient Pollution Indicator’ (NPI) uses eelgrass (Zostera marina L.) as a bioindicator and is calculated as the ratio of leaf nitrogen content (SN) to area normalized leaf mass (mg dry wt cm⁻²). Eelgrass samples were collected along the entire length of the Barnegat Bay–Little Egg Harbor from June to October 2008 to determine if leaf chemistry and morphology reflect eutrophication status and a north–south gradient of nitrogen loading from the Barnegat Bay watershed. Nitrogen content, area normalized leaf mass, and NPI values all significantly (p < 0.05) varied temporally but not spatially. NPI values did not significantly correspond to the north–south gradient of nitrogen loading from the Barnegat Bay watershed. The NPI metric is therefore not deemed to reliably indicate estuarine eutrophic status. Differences between sampling effort (number of stations) and replication did not bias the overall conclusions.

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1. **Introduction**

Barnegat Bay–Little Egg Harbor, New Jersey, is a coastal lagoon affected by multiple anthropogenic stressors and drivers of seagrass habitat change. Among the most notable factors impacting seagrass beds are nutrient over-enrichment, nuisance and toxic algal blooms, high epiphytic abundance, elevated turbidity, prop scarring, and dredging (Kennish et al., 2010). With continued population growth and development in the Barnegat Bay–Little Egg Harbor watershed (Fig. 1), future impacts on seagrass and other essential estuarine habitat are likely to increase. Cultural eutrophication poses the most serious threat to the long-term health and integrity of this lagoonal estuary (Kennish et al., 2007a,b).

Overt symptoms of eutrophication in the Barnegat Bay–Little Egg Harbor estuary include heavily degraded seagrass habitat, low dissolved oxygen (in the northern segment), and marked reduction of fisheries (e.g., hard clams, Mercenaria mercenaria) (Olsen and Mahoney, 2001; Kennish et al., 2007a,b, 2008). Since 2004, system eutrophication has worsened, and the condition of the seagrass habitat has markedly declined. For example, seagrass biomass in the estuary decreased by 50–88% over the 2004–2006 period, and by 2010 it had dropped to 8 g dry wt m⁻² (aboveground) and 27 g dry wt m⁻² (belowground), which were the lowest levels ever recorded (Kennish et al., in revision). Seagrass shoot density, blade length and percent area cover have also generally declined since 2004, eliminating habitat for hard clams, bay scallops (Argopecten irradians), and other benthic and demersal organisms. Explosions of sea nettles (Chrysaora quinquecirrha), concurrent with escalating eutrophy, have occurred in the estuary in recent years.

Barnegat Bay–Little Egg Harbor is classified as a highly eutrophic estuary, susceptible to nutrient loading because it is shallow, poorly flushed, and bordered by highly developed watershed areas (Bricker et al., 2007; Kennish et al., 2007a,b). Most of the estimated 650,000 kg N year⁻¹ load entering the estuary derives from surface runoff (66%; 431,000 kg N year⁻¹), but substantial fractions also originate from atmospheric deposition (22%; 141,000 kg N year⁻¹) and direct groundwater discharges (12%; 78,000 kg N year⁻¹) (Wieben and Baker, 2009). Though the Barnegat Bay watershed can be divided into 12 subwatersheds (Fig. 1) with total nitrogen loadings ranging from 1700 to 170,000 kg year⁻¹ (Wieben and Baker, 2009), data was unavailable for some subwatersheds. Wastewater treatment plants located within the Barnegat Bay watershed discharge effluent to the Atlantic Ocean (Fig. 1), and therefore only a minimal, diluted fraction of these nutrient inputs would re-enter the estuary via inlet exchange and would no longer be considered ‘point source’, thus no point source inputs of nitrogen exist in the Barnegat Bay watershed. Confined animal feeding operations (52 total) comprise a minimal fraction of the watershed area and...
it is necessary to determine detection limits and scope in order to ensure applicability to a wide variety of ecosystems.

Specifically, we address the following questions, (1) Does the ratio of Zostera marina leaf nitrogen content to area normalized leaf mass (NPI) reflect eutrophication status? (2) Do spatial patterns of NPI values in Barnegat Bay–Little Egg Harbor estuary correspond to either the north–south gradient of nitrogen loading in its watershed or the spatial patterns of total nitrogen concentration in the water column?

We hypothesize that if the NPI metric does indeed reflect eutrophication status, then NPI values in Barnegat Bay–Little Egg Harbor should be significantly greater than in areas of incipient eutrophication (as investigated by Lee et al. (2004)). Further, if the NPI metric is robust within eutrophic ecosystems, NPI values should correspond to either nitrogen loading or nitrogen availability. This study thus aims to test a metric previously identified as an indicator of important ecosystem processes (Lee et al., 2004), and is part of a long-term effort to assess and remediate eutrophic impacts in the Barnegat Bay–Little Egg Harbor estuary (Kennish et al., 2010).

2. Study area

Barnegat Bay–Little Egg Harbor is located along the central New Jersey coastline (Fig. 1). It forms an irregular tidal basin ~70 km long, 2–6 km wide, and 1.5 m deep. The surface area is 280 km², and the volume, 3.54 × 10⁸ m³ (Kennish, 2001). Toms River (39°56′58″N, 74°12′28″W) provides 3.66 m³ s⁻¹ (low streamflow) to 7.33 m³ s⁻¹ (high streamflow) of freshwater inputs (Wieben and Baker, 2009), resulting in lower salinities in the northern segment compared to central and southern segments. The location of the barrier island complex (Island Beach and Long Beach Island) restricts exchange of water with the coastal ocean. Water residence time for the entire Barnegat Bay–Little Egg Harbor estuarine complex is protracted, up to 74 days in summer (Guo et al., 2004). Exchange of bay and ocean water occurs through Barnegat Inlet, Little Egg Inlet, and the Pt. Pleasant Canal, and the locations of these inlets geologically separate Little Egg Harbor (stations 1–5 in the south) from Barnegat Bay (stations 6–10 in the north). Stations 1–5 and 6–10 are therefore grouped for comparisons of eelgrass and NPI values between these geomorphological components of the estuary.

The Barnegat Bay watershed covers 1730 km², and thus the watershed: estuary area ratio is 6.5:1. Nearly 575,000 people (332 people km⁻²) live in the surrounding watershed year-round, but the population is greater than 1,200,000 people (694 people km⁻²) during the summer tourist season. A north-to-south gradient of decreasing developed watershed area (Fig. 1) and associated total nitrogen loading occurs in the watershed (Hunchak-Kariouk and Nicholson, 2001; Seitzinger et al., 2001), such that the Toms and Metedeconk River basins in the north account for >60% of the nitrogen load from surface water discharge (Wieben and Baker, 2009).

3. Materials and methods

We established 10 sampling stations from lower Little Egg Harbor to upper Barnegat Bay (Fig. 1). Station locations were selected from within previously mapped eelgrass beds (Lathrop et al., 2001) prior to fieldwork to ensure sampling across all habitats along a roughly south to north transect in this small but heterogeneous system. At these stations, we attempted to collect 3 Z. marina samples on sampling dates in June (period 1), August (period 2) and October (period 3). These time periods were selected based upon the growing season for Z. marina in Barnegat Bay–Little Egg Harbor. Although an effort was made to collect 90 Z. marina samples at these stations over the course of the study, only 77 of them were actually...
obtained because some stations were devoid of seagrass. For example, no Z. marina was found at station 9 during sampling period 2 or 3. In Barnegat Bay as in other estuaries, light, salinity, and habitat (sediment type, depth) generally control the spatial extent of Z. marina (Kemp et al., 2004). Thus, Z. marina is dominant in the central and southern segments of the estuary, but is rarer in the less saline northern segment, where Ruppia maritima dominates.

Water quality parameters (temperature, salinity, dissolved oxygen, pH, Secchi depth, total depth) were measured at the 10 sampling stations using a handheld YSI 600 XL datasonde coupled with a handheld YSI 650 MDS display unit and an automated YSI 6600 unit (equipped with a turbidity probe). Water quality data other than Secchi depth were collected at a uniform depth (~10 cm) above the sediment–water interface prior to collecting the seagrass samples. Dissolved nutrient (NO₃⁻, NH₄⁺, PO₄⁻) concentrations were measured. Collected water quality data were used to create a distance matrix (Proc Distance, SAS Inc.), standardized by standard deviation (to minimize differences between variances), which was then used as input for non-parametric multidimensional scaling analysis (Proc MDS, SAS Inc.; similar to principal components analysis) and data in the resulting unitless two-dimensional plot was identified by time period and station. Non-parametric techniques were utilized because data were verified as non-normally distributed (Proc Univariate, SAS Inc.). Long-term nutrient monitoring data was obtained from the New Jersey Department of Environmental Protection, Bureau of Marine Water Monitoring for comparison to physico-chemical, eelgrass, and watershed data.

Eelgrass samples were collected by a diver using a 10-cm diameter PVC corer. The diver-deployed corer extended deep enough in the bottom sediments to extract all aboveground and belowground fractions (roots and rhizomes). Each core sample was placed in a 3 mm × 5 mm mesh bag and rinsed to separate plant material from sediment. The seagrass sample was then removed from the mesh, placed in a labeled bag, and stored on ice in a closed container prior to transport back to the Rutgers University Marine Field Station (RUMFS) in Tuckerton.

In the laboratory, the samples were carefully sorted and separated into aboveground (shoots) and belowground (roots and rhizomes) components. To ensure a standardized sampling technique, the shortest leaf from each core sample was selected and its length and width measured to the nearest millimeter. Epiphytes on leaves, common and unavoidable in Barnegat Bay–Little Egg Harbor given a representative sampling design, were removed by carefully scraping them off using a razor blade. The leaf was subsequently cut into three segments and oven dried at 50–60 °C for 48 h. The area normalized leaf mass (mg dry wt cm⁻²) of the blade was measured to the third decimal place. Each leaf segment was then run for carbon and nitrogen content on a Carlo Erba NA 1500 series 2 Elemental Analyzer and the nitrogen content (N%) was then calculated for each eelgrass leaf.

The ratio of the leaf nitrogen content (N%) to area normalized leaf mass (mg dry wt cm⁻²) was used to calculate a ‘Nutrient Pollution Indicator’ value, as developed by Lee et al. (2004):

\[
\text{NPI} = \frac{\text{Leaf nitrogen content (N\%)} \times \text{Area normalized leaf mass (mg dry wt cm}^{-2}\text{)}}{
\]

Leaf nitrogen content, area normalized leaf mass, and NPI values were analyzed statistically using one-way non-parametric Kruskal–Wallis test (Proc Npar1way, SAS Inc.) due to non-normal data distribution (verified by Shapiro–Wilks statistic W > 0.07, stem-leaf plot, box-plot, normal probability plot; Proc Univariate, SAS Inc.). Chi-square (χ²) and p-values are reported for Kruskal–Wallis tests conducted separately for class variables: site (1 through 10), bay (stations 1–5 = Little Egg Harbor or stations 6–10 = Barnegat Bay), and time period (June–July, August–September, or October–November). Least squares means (Proc Mixed, SAS Inc.) were tested for variables with significant differences to confirm differences between all ordinals.

4. Results

4.1. Water quality metrics

Multivariate correlations of physico-chemical water quality measurements (temperature, salinity, dissolved oxygen, pH, Secchi depth and station depth) are summarized in a multidimensional scaling analysis (MDS, Fig. 2a), with the corresponding variable vectors (Fig. 2b). The x-axis positively correlated with dissolved oxygen: % saturation (r = 0.78, p < 0.01) and concentration (r = 0.60, p < 0.01) and also negatively correlated with depth: both Secchi depth (r = −0.52, p < 0.01) and total depth (r = −0.49, p < 0.01) (Fig. 2b). The y-axis positively correlated with temperature (r = 0.63, p < 0.01) and also negatively correlated with both Secchi depth (r = −0.73, p < 0.01) and total depth (r = −0.81, p < 0.01) (Fig. 2b). Neither salinity nor pH explained a significant amount of the variation of the water quality data.

In a post hoc analysis, the data clustered by time period but generally not spatially. Temperatures were highest and dissolved oxygen (both % saturation and concentration) was lowest in June–July, each was intermediate in August–September, while in October–November temperature was lowest and dissolved oxygen was greatest (Fig. 2a and b). No spatial gradient (which would have been represented by a sequential clustering) along any water quality parameter was detected, though in general data varied most along the Secchi and total depth axis. Since no latitudinal gradient was observed, there was likely no spatial correlation of these metrics with urban area (Fig. 1). Nevertheless, a group of stations in the north (stations 7, 8, and 9) and a pair of stations in the south (stations 2 and 4) formed two small clusters that co-varied. Over the three time periods, the northern group varied substantially in temperature and dissolved oxygen while these metrics varied minimally in the southern pair of stations (Fig. 2a and b).

4.2. Leaf nitrogen content

Mean leaf nitrogen content (%N) of Z. marina in the Barnegat Bay–Little Egg Harbor estuary ranged from 1.05 ± 0.14% N (station 6 in June–July) to 3.94 ± 0.84% N (station 3 in October–November) (Fig. 3a). Spatially, no significant differences were found either between individual stations (χ² > 9.07, p > 0.43) or between Barnegat Bay (stations 6–10) and Little Egg Harbor (stations 1–5; χ² > 0.58, p > 0.44) using one-way non-parametric Kruskal–Wallis
test. Seasonally, however, nitrogen content significantly varied ($\chi^2 > 32.27, p < 0.01$; Kruskal–Wallis test, Proc Npar1way, SAS Inc.) and least squares means (Proc Mixed, SAS Inc.) indicated that successive increases between time periods were significant ($p < 0.05$; Fig. 3a).

### 4.3. Area normalized leaf mass

Mean area normalized leaf mass varied from 1.05 ± 0.92 (station 2 in August–September) to 110.95 ± 161.27 (station 1 in June–July) mg dry wt cm$^{-2}$ though all but 8 observations were under 6.0 mg dry wt cm$^{-2}$, and half of the mean values (14 of 28) were under 3.0 mg dry wt cm$^{-2}$ (Fig. 3b). As with nitrogen content, no significant spatial patterns were identified for area normalized leaf mass either between individual stations ($\chi^2 > 9.64, p > 0.037$; Kruskal–Wallis test, Proc Npar1way, SAS Inc.) or between Barnegat Bay (stations 6–10) and Little Egg Harbor (stations 1–5; $\chi^2 > 1.93, p > 0.16$; Kruskal–Wallis test, Proc Npar1way, SAS Inc.). Area normalized leaf mass significantly differed between time periods ($\chi^2 > 9.24, p < 0.01$; Kruskal–Wallis test, Proc Npar1way, SAS Inc.), with significantly greater values in June–July than later in the year, either in August–September (Proc Mixed, SAS Inc. least squares means $p < 0.01$) or October–November (Proc Mixed, SAS Inc. least squares means $p < 0.01$). There was no difference between these last two time periods (Fig. 3b).

### 4.4. Nutrient pollution indicator

Regression analysis (Proc Reg, SAS Inc.) revealed no significant relationship between leaf nitrogen content and area normalized leaf mass in Barnegat Bay–Little Egg Harbor during any time period ($p > 0.98$ in June–July, $p > 0.45$ in August–September, and $p > 0.29$ in October–November) or overall on all 77 observations ($p > 0.08$). No pattern was visible in a plot of area normalized leaf mass vs. leaf nitrogen content (Fig. 4).

NPI values ranged from 0.13 ± 0.20 (station 5, June–July) to 4.09 ± 3.15 (station 2, August–September) (Fig. 3c). As with both nitrogen content and area normalized leaf mass, the NPI values did not vary spatially either by individual station ($\chi^2 > 7.87, p > 0.54$) or between Barnegat and Little Egg Harbor bays ($\chi^2 > 2.52, p > 0.11$; Kruskal–Wallis test, Proc Npar1way, SAS Inc.). NPI values significantly varied seasonally ($\chi^2 > 18.73, p < 0.01$; Kruskal–Wallis test, Proc Npar1way, SAS Inc.) with means in June–July significantly lower than in August–September ($p < 0.01$) or October–November ($p < 0.05$) but the latter two did not significantly differ ($p > 0.67$, Proc Mixed, SAS Inc.).

### 5. Discussion

Seagrasses are important indicators of water and sediment quality in estuaries, and their tissue nitrogen content serves as a good indicator of environmental nitrogen history (Duarte, 1990; Bortone, 2000; Larkum et al., 2006). Seagrass leaf nitrogen content in $Z$. marina provides an integrated measure of nitrogen availability in the estuarine environment (Fourqueuran et al., 1997). Lee et al. (2004), working in Great Bay Estuary (NH), Narragansett Bay (RI), and Waquoit Bay (MA) demonstrated that the ratio of leaf nitrogen content (N%) to area normalized leaf mass (mg dry wt cm$^{-2}$) in $Z$. marina (coined as a nutrient pollution indicator, NPI) could potentially serve as an indicator of incipient eutrophication in these ecosystems. Lee et al. (2004) reported an inverse relationship between the area normalized leaf mass and leaf tissue nitrogen content (%) NPI for each of the three estuaries studied by these authors (2004) varied spatially along a gradient of eutrophication, leading to their conclusion that NPI values effectively indicate incipient eutrophication in those estuaries. If widely applicable, high NPI values would provide a valuable tool for environmental managers and decision-makers to identify ecosystems at risk of or
Table 1
Comparison of the effect of replication on standard error, grand mean, and standard error of the mean (SEM) of the nutrient pollution indicator (NPI) as reported by Lee et al. (2004) (10 replicates per site per season) and this study (3 replicates per site per season).

<table>
<thead>
<tr>
<th>Season</th>
<th>Date</th>
<th>Location</th>
<th># Sites</th>
<th>Replication</th>
<th>Minimum SE</th>
<th>Maximum SE</th>
<th>Grand mean</th>
<th>SEM</th>
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<td>0.1</td>
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<tr>
<td></td>
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<td>7</td>
<td>10</td>
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<td>0.1</td>
<td>2.1</td>
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</tr>
<tr>
<td></td>
<td>Summer</td>
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<td>5</td>
<td>10</td>
<td>0.0</td>
<td>0.1</td>
<td>2.0</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>Jun-08</td>
<td>Barnegat Bay–Little Egg Harbor, NJ</td>
<td>10</td>
<td>3</td>
<td>0.1</td>
<td>0.8</td>
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<tr>
<td></td>
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<td>0.0</td>
<td>0.9</td>
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<tr>
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<td></td>
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Regardless of the balance between error and effort, comparing the outcomes of the NPI in a ‘highly eutrophic’ end-member ecosystem such as Barnegat Bay–Little Egg Harbor can verify its ability to positively identify eutrophic state and thus verify the applicability of this technique across a variety of ecosystems. The ranges of NPI values in both studies were similar, which also served as further evidence that the difference in sampling effort minimally affected overall outcomes. Mean station values of NPI ranged from 0.43–2.20 in Great Bay, 0.29–0.59 in Narragansett Bay, and 0.32–0.84 in Waquoit Bay (Lee et al., 2004) and in Barnegat Bay–Little Egg Harbor from 0.13–1.00 in June–July, 0.44–4.09 in August–September, and 0.57–1.75 in October–November. Therefore, we feel justified and confident in our comparison and assessment of the utility of the NPI in Barnegat Bay–Little Egg Harbor and do not find evidence that lower replication substantively biased our conclusions. Because mean NPI values were not statistically different (Table 2, Tukey adjusted p<0.05) in ecosystems experiencing incipient eutrophication (Lee et al., 2004) to those in a ‘highly eutrophic’ (Bricker et al., 2007) end-member such as Barnegat Bay–Little Egg Harbor, we maintain that the NPI metric does not reflect eutrophication status and is thus not a useful metric.

The range of Z. marina leaf nitrogen content values in the Barnegat Bay–Little Egg Harbor estuary (0.94–3.84% dry weight) was greater than that recorded by Lee et al. (2004) in the Great Bay Estuary, NH (2.1%–3.5% dry weight), Narragansett Bay, RI (2.0%–2.3%), and Waquoit Bay, MA (1.6%–2.4% dry weight). Variation of Z. marina leaf nitrogen content along nitrogen loading gradients were evident in the Great Bay Estuary, Narragansett Bay, and Waquoit Bay, but not in Barnegat Bay–Little Egg Harbor estuary (Figs. 1 and 3a; Table 3).

The spatial distribution of area normalized leaf mass values in the Barnegat Bay–Little Egg Harbor estuary also differed from that observed in the Great Bay Estuary, Narragansett Bay, and Waquoit...
Bay. While the area normalized leaf mass was significantly higher in a seaward direction in the latter three estuaries, the area normalized leaf mass in the Barnegat Bay–Little Egg Harbor estuary was significantly higher at up-estuary (northern) locations. The range of area normalized leaf mass values in the Barnegat Bay–Little Egg Harbor estuary (0.73–5.45 mg dry wt cm$^{-2}$) exceeded that in the other three estuaries.

Inorganic nitrogen concentrations in the water column vary substantially both temporally and spatially due to fluxes in nutrient loading, uptake by plants, removal to bottom sediments, ammonification, circulation patterns, flushing to the nearshore ocean, and other factors. In addition, Z. marina assimilates nitrogen from both the water column and bottom sediments, and thus the concentration of nitrogen in eelgrass blades reflects its availability in both media. Inorganic nitrogen levels in the water column of the estuary during the warmer months of the year are typically very low, often less than 1 µM, largely due to plant uptake (Seitzinger et al., 2001; Kennish et al., 2007a, 2010). Potentially, dissolved organic nitrogen may substantially contribute to chronic problems including harmful algal blooms (HABs) and other phytoplankton, as is the case in similar coastal lagoons in Maryland (Gilbert et al., 2007; Wazniak et al., 2007). As a consequence, total nitrogen concentration in the water column may be a better indicator of eutrophic condition than dissolved inorganic nitrogen. Indeed, there is a significant positive relationship ($R^2 = 0.68$, $p < 0.01$) between water column total nitrogen concentration and total nitrogen loading by subsurface waters (Fig. 5a). Spatial patterns of total nitrogen concentrations match the variation of median NPI values, with higher values in two areas of elevated long-term total nitrogen concentration in the water column (stations 9 in the north and 3 in the south (Figs. 3c and 6a–c)) and lower values closer to the two oceanic inlets (stations 1 in the south and 6 in the central area (Figs. 3c and 6a–c)). Biotic responses to nutrient loading thus provide an integrated measure of nitrogen over-enrichment in this system (Bricker et al., 2007; Kennish et al., 2007a, 2010).
Fig. 5. Simple linear regression of (a) water column total nitrogen (μgNL⁻¹), (b) Z. marina nitrogen content (%N), and (c) nutrient pollution indicator vs. areal normalized subwatershed total nitrogen loading (kg TN km⁻² year⁻¹) by subwatershed within the Barnegat Bay–Little Egg Harbor estuary watershed.

2007a) but can be indirectly influenced by physical processes and geomorphology.

In the Barnegat Bay–Little Egg Harbor estuary, higher nitrogen loading and water column nitrogen concentrations have been reported in northern reaches of Barnegat Bay; the highest coastal watershed development also exists in this area (Seitzinger et al., 2001; Kennish et al., 2009). Wieben and Baker (2009) determined that total nitrogen concentrations were greatest in highly developed land areas (median values = 0.39–1.2 mg l⁻¹), with highest yields in the Toms River, Metedeconk, and Wrangle Brook basins draining the northern part of the watershed. These areas are situated in the northern portion of the watershed and also contain all but one of the S2 confined animal feeding operations in the Barnegat Bay watershed (Fig. 1), and thus inputs from these sources may combine with non-point inputs from development given their spatial juxtaposition, while direct inputs from wastewater treatment plants are minimal because outfalls discharge to the Atlantic Ocean rather than this lagoonal estuary.

As delineated in this study, NPI values do not significantly increase along the north–south nitrogen loading gradient (Figs. 1 and 5c) associated with developed parts of the estuary watershed (Figs. 1 and 3c), differing from that reported for the three New England estuaries (Lee et al., 2004). The relationship between nitrogen content and area normalized area normalized leaf mass similarly differed between these two studies. Possibly, differences in temporal patterns of salinity gradients, tissue nitrogen content, or plant growth could account for the differences observed between studies. Salinity gradients in Great Bay, Waquoit Bay, and Narragansett Bay are stronger than the gradients in the Barnegat Bay–Little Egg Harbor estuary, where salinities did not significantly contribute to variation in water quality physicochemical measurements (Fig. 2a and b). Temporally, NPI values varied but were largely driven by variations in nitrogen content, which is further driven by nitrogen availability and growth patterns (Fig. 4).

Marked changes in NPI values in the estuary were noted in proximity to Barnegat Inlet, where flushing is greatest and removal of nitrogen from the system can be rapid. Barnegat Inlet provides strong flushing of waters in the central bay yet the overall residence time for Barnegat Bay–Little Egg Harbor estuary is nearly 75 days (Guo et al., 2004) and though a robust hydrodynamic model for this estuary is not available, water likely remains in areas at increasing distances north and south of the inlet than close to it due to oceanic exchange. Gradients of residence times therefore likely lead to the observed long-term accumulation of nitrogen and associated elevated concentrations in northern and southern areas of the estuary, furthest away from these inlets (Fig. 6). Potentially, the influence

Fig. 6. Total nitrogen concentrations measured at fixed stations by New Jersey Department of Environmental Protection Bureau of Marine Water Monitoring and interpolated (by kriging) throughout Barnegat Bay–Little Egg Harbor estuary. Means of measurements over a twenty-one year period (1989 through 2009) reported for (a) June–July, (b) August–September, and (c) October–November.
of water circulation patterns and nitrogen accumulation could partially explain the lack of spatially linear response of eelgrass and NPI values.

6. Conclusions

Indicators of eutrophication require application in ecosystems already considered eutrophic as end-members to validate their ability to yield true-positive indication of eutrophication status. Barnegat Bay–Little Egg Harbor served as a eutrophic end-member estuary to validate a previously developed eutrophication indicator coined as a ‘Nutrient Pollution Indicator’ (NPI; Lee et al., 2004). As initially developed, NPI values were calculated as the ratio of leaf nitrogen content (%) to area normalized leaf mass (mg dry wt cm⁻²) measured in Zostera marina tissues and would thus link plant nitrogen chemistry and morphology with ecosystem nutrient availability. However, NPI values were not significantly different between a eutrophic ecosystem and three ecosystems experiencing incipient eutrophication and thus do not provide a consistent and widely applicable metric of eutrophication status. Further, NPI values did not reflect eutrophication status, nitrogen availability, or total nitrogen loading for Barnegat Bay–Little Egg Harbor, as they did not significantly vary between sampling stations or between Barnegat Bay and Little Egg Harbor and neither did Z. marina nitrogen content, area normalized leaf mass, or NPI values. Further, these variables did not significantly correlate to water column total nitrogen concentrations or a north–south gradient of total nitrogen loading from the watershed, though total nitrogen loading and estuarine total nitrogen concentrations positively correlated (p < 0.05). Differences in sampling effort and replication did not account for the NPI’s failure in Barnegat Bay–Little Egg Harbor. Additionally, NPI values and constituent measurements varied seasonally (June to October, p < 0.05) in Barnegat Bay–Little Egg Harbor. Thus, utilization of NPI values must account for temporal variations to accurately identify eutrophication. Finally, future studies applying eelgrass metrics as indicators of ecosystem eutrophication should acknowledge the influence of estuarine residence times and water circulation because proximity to oceanic exchange affects spatial patterns of nitrogen availability and can thus indirectly affect bioindicator responses.

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