



Nitrogen loading to Pleasant Bay, Cape Cod: application of models and stable isotopes to detect incipient nutrient enrichment of estuaries

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Abstract

To test and refine methods to detect nutrient enrichment and resulting eutrophication, we applied the Waquoit Bay nitrogen loading model (NLM) and Estuarine loading model (ELM) to estuaries of Pleasant Bay that receive increasing but low N loads (25–199 kg N ha⁻¹ yr⁻¹) from land. Contributions of wastewater to these estuaries increased from 7% to 63% as N loads increased, and modeled estimates of dissolved inorganic nitrogen in the water were within ~27% of measured values. N isotopic signatures in suspended and benthic organic matter and in tissue of quahogs increased as wastewater contributions to N loads increased, with clams ~4‰ heavier than organic matter, indicating that even at these low N loads, N from land-derived sources moved detectably up the food web. These results extend the application of NLM and ELM to detect incipient levels of N enrichment and demonstrate that these models can be used in conjunction with isotope measurements as the basis for food web analyses in a system exposed to relatively lower N loads than previously studied.

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1. Introduction

Increased anthropogenic nitrogen (N) addition to coastal waters is a worldwide major agent of change for coastal ecosystems (GESAMP, 1990; Goldberg, 1995; NRC, 2000). In New England and elsewhere land-derived N loads have increased during the 20th century (Valiela et al., 1992; Smith et al., 1999; Bowen and Valiela, 2001). These increased deliveries of N have prompted eutrophication in many estuaries (Nixon et al., 1986; Nixon, 1992; Valiela et al., 1997; Caraco and Cole, 1999; Valiela and Bowen, 2002) that, in turn, has altered features of the receiving estuarine ecosystems. Awareness of lowered water quality, loss of critical habitats, and reduction of fin- and shellfish stocks has created pressure for management initiatives that define the sources of these problems and provide remediation (NRC, 2000; Valiela et al., 1992; Valiela and Bowen, 2002; Fluharty, 2000).

To manage nutrient enrichment, we have to define and quantify sources of N to coastal waters and identify estuarine responses to N loading. What seems most useful is to develop methods that effectively detect incipient eutrophication before it has substantially altered the estuarine system. To assess whether we can detect such early symptoms of enrichment, we applied two models, the Waquoit Bay nitrogen loading model (NLM) and Estuarine loading model (ELM), along with isotopic methods.

NLM (Valiela et al., 1997, 2000) predicts total dissolved N load (TDN) to estuaries by considering N inputs from three major land-derived sources— atmospheric deposition, fertilizer, and wastewater. NLM calculates the N load to an estuary from these three sources based on the number of people on the watershed and the area of different land cover types (natural vegetation, agriculture, fertilizer, impervious surfaces, wetlands, ponds, and so on). NLM then estimates land-derived TDN to the estuary by accounting for losses of N from the three sources as N moves through the watershed via soil, vadose zone, and aquifer to the estuary.

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ELM (Valiela et al., submitted for publication) considers how dissolved inorganic nitrogen (DIN) inputs from land are altered by biological and physical processes within an estuary, to estimate the concentration of DIN available to producers and consumers in the waterbody. For any estuary of interest, ELM uses the TDN value predicted by NLM and inputs for direct atmospheric deposition to the waterbody to calculate how processes such as denitrification, N_2 fixation, and N burial affect DIN concentrations in the water column. ELM estimates are based on features of the estuary, including depth, flushing rate, and area of the estuary covered by salt marshes, seagrass meadows, and open water.

We have verified NLM and ELM by comparisons to direct measurements in other Cape Cod estuaries (Valiela et al., 2000; Valiela et al., submitted for publication). Coefficients of variation based on standard error for measured versus modeled comparisons across several estuaries was 12% for NLM and 8.1% for ELM (Valiela et al., submitted for publication). Most of the estuaries involved in these verifications, however, are subject to relatively high land-derived N loads (Valiela et al., 2000; Costa, 1994). A useful challenge to our models is to test them in estuaries that are undergoing lower, incipient rates of N loading. Such tests would assess whether we can use this approach for early detection of eutrophication and will provide evidence about the transferability of these models to less extensively studied systems (NRC, 2000).

Pleasant Bay (Fig. 1), the largest groundwater-fed embayment on Cape Cod, MA, provides a good loca-

tion to test the performance of N loading and DIN concentration models and to trace land-derived N in estuarine food webs. Like the majority of Cape Cod, wastewater is delivered to subestuaries of Pleasant Bay primarily through septic systems. Subwatersheds of Pleasant Bay include golf courses, residential lawns, and cranberry bogs that receive fertilizer. There are also several intercepting freshwater ponds and fresh and saltwater wetlands in the Pleasant Bay system. Pleasant Bay is different, however, from other Cape Cod embayments previously modeled in that it has subestuaries with smaller watersheds, somewhat lower flushing times (Table 1), and relatively low levels of urbanization of watersheds compared to estuary size.

We have also established that we can use N stable isotope ratios to determine how coupled estuaries might be to their watersheds and to provide a means to trace N inputs from land to producers and consumers within specific estuaries. These linkages are possible because different combinations of land uses convey different N isotopic signatures to receiving estuaries (McClelland et al., 1997; McClelland and Valiela, 1998a), with N isotopic signatures of biota generally increasing as the % contributions of wastewater increase (McClelland et al., 1997; Voss and Struck, 1997; McClelland and Valiela, 1998b; Waldron et al., 2001; Evgenidou and Valiela, 2002; Weiss et al., 2002; Shriver et al., 2002; Mayer et al., 2002; Cole et al., submitted for publication). The % contributions of wastewater to N loads are a significant predictor of N isotopic signatures of primary producers across a wide geographic range (Cole et al., submitted for publication). N isotopic signatures can

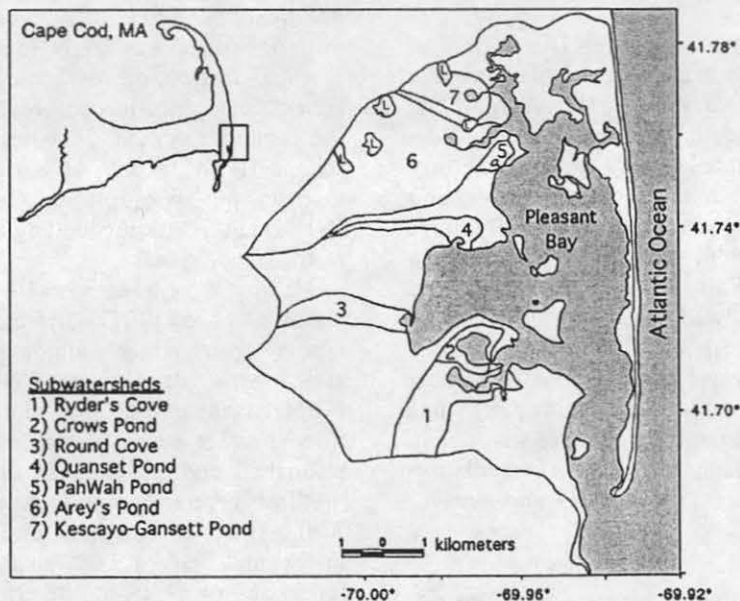


Fig. 1. Location, subwatershed delineations, and associated subestuaries of Pleasant Bay. Subwatershed delineations were similar to those previously reported (Eichner et al., 1998). L = intercepting lakes.

Table 1
Watershed areas, flushing times, embayment areas, and estimated N loading rates for the entire Pleasant Bay system and its subwatersheds (cf. Fig. 1) and for other Cape Cod water bodies

	Watershed area (ha)	Flushing time (d)	Embayment area (ha)	N load (kg yr ⁻¹)	N load (kg ha ⁻¹ yr ⁻¹)
Pleasant Bay	8622	1.0 ^a	2752	67443	25
Crows Pond	74	1.8 ^a	47	1450	31
Kescayo-Gansett Pond	97	1.1 ^a	8	519	62
Pahwah Pond	56	0.7 ^a	3	195	75
Quanset Pond	110	0.7 ^a	5	498	108
Ryder's Cove	627	0.9 ^a	43	4701	109
Arey's Pond	926	2.0 ^a	24	3301	138
Round Cove	116	0.8 ^a	5	1076	199
<i>Other Cape Cod water bodies^b</i>					
Sage Lot Pond	134	1.5	32	844	26
Jehu Pond	427	2.7	43	4059	45
Hamblin Pond	260	2.3	39	2246	58
West Falmouth Harbor	912	1.8	70	5285	76
Green Pond	1658	2.2	60	7430	124
Quashnet River	2084	1.7	25	8671	347
Childs River	875	2.3	13	5697	438

N loading rates were estimated using NLM (Valiela et al., 1997) and adding direct deposition to the water body. Embayment areas do not include salt marsh.

^a Data from Hamilton (1999).

^b Data from Valiela et al. (2000) and Valiela et al. (submitted for publication).

then be traced up the food web because the ratio of each stable isotope becomes heavier by approximately 3–5‰ with each trophic transfer (Peterson and Fry, 1987; Cabana and Rasmussen, 1994). Hence, in the Pleasant Bay estuaries, we examined whether isotopic signatures of producers and clams were related to % wastewater contributions even at low loads.

In this study we tested the ability of our models to reasonably estimate N loads and DIN in water in a series of subwatersheds and subestuaries of the Pleasant Bay estuarine system, subject to different but relatively low N loads. We verified our results in two independent ways, by comparing measured versus modeled DIN concentrations and by examining whether N isotopic signatures of primary producers and a primary consumer were consistent with the pattern of % wastewater contributions predicted by NLM.

2. Methods

2.1. Nitrogen loading calculations

To determine loading rates of TDN to Pleasant Bay and associated estuaries, we applied the Waquoit Bay nitrogen loading model (Valiela et al., 1997). NLM is based on N inputs from specific land-uses on each watershed. We modeled land-derived loading rates from sources on land to seven subestuaries and to the entire Pleasant Bay system (Fig. 1). We calculated the N load in kg ha⁻¹ yr⁻¹ by dividing the load per year estimated by NLM by the embayment area of the estuary of interest (Table 1).

To determine the area of different land uses and number of buildings on the Pleasant Bay watersheds, we examined color aerial orthophotos, at a scale of 1/12,000 (Massachusetts Department of Environmental Protection, Wetlands Conservation Program, 1993).

2.2. Estimating water column DIN

We did not have measured N loads to Pleasant Bay with which to verify estimates obtained using NLM. Instead, we simultaneously verified estimates for NLM and ELM by comparing modeled estimates of mean annual DIN concentrations versus available measurements of DIN (RMA, 2001). Data on surface DIN concentrations in each estuary were available for 1–2 sampling dates each month from May through October during 2000 and 2001 for a total of 12–19 samples per estuary.

To predict the concentrations of DIN available to producers in each subestuary and in Pleasant Bay as a whole, we applied ELM (Valiela et al., submitted for publication). To use ELM we input required data on annual N loads from land (from NLM), the perimeter of each estuary, areas of open water, salt marsh, and eelgrass meadow (from aerial photos and Ridley, 1998), flushing rates (Hamilton, 1999), and some data also used by NLM, including area of watershed and number of houses on the watershed.

2.3. Stable isotope sample collection and preparation

To corroborate the relationship between land use and DIN, as well as to test whether N stable isotopic

measurements detected the low levels of land-derived N loads entering the Pleasant Bay system, we measured isotopic signatures of suspended and benthic producers and in the tissue of a primary consumer from each sub-estuary. We sampled these same biota from other Cape Cod estuaries to compare results from the Pleasant Bay system to those from more highly loaded areas (Table 1).

To test whether land-derived N moves up the estuarine food web, we chose the trophic linkage between shellfish and particulate organic matter. We sampled the quahog, *Mercenaria mercenaria*, which feeds on phytoplankton and benthic microalgae (Kamermans, 1994) and was ubiquitous in Pleasant Bay, by pooling tissues of five or more clams from each location. To sample particulate organic matter (POM) that could be ingested by quahogs, we collected suspended POM from the water column by filtering 2–1 l samples of water onto a pre-ashed 0.7 μm Whatman glass fiber filter, and we sampled benthic POM from the sediment by aggregating 3 cores collected at each site using a 1 cm \times 3 cm modified syringe corer.

Samples were collected from May–July 2001 and June–July 2002. Samples were dried at 60 $^{\circ}\text{C}$, tissues and sediments were ground, and all samples were sent to the University of California—Davis Stable Isotope Facility to determine $\delta^{15}\text{N}$ signatures by mass spectrometry.

3. Results and discussion

3.1. N loads to Pleasant Bay

N loads delivered from groundwater to the subestuaries of Pleasant Bay were lower but somewhat overlapped the range reported for other Cape Cod water bodies (Table 1 and also Valiela et al., 2000; Costa, 1994), and as we suspected, these loads were substantially lower than N loads reported worldwide (Nixon, 1992). The different arrays of land use on watersheds of Pleasant Bay resulted in distinct N loading rates to the different subestuaries (Table 1). Total annual N loads, as a whole, were lower among subestuaries of Pleasant Bay than among previously studied Cape Cod estuaries ($t = -2.63$, $P = 0.02$) (Table 1). Land-derived N loading rates normalized to estuary area ranged from 25 to 199 $\text{kg N ha}^{-1} \text{yr}^{-1}$ (Table 1). These results seemed suitable to assess whether the models and isotopic techniques detected incipient enrichment of estuaries.

The estimates of land-derived N loads plus estimates of direct atmospheric deposition on the surface of the subestuaries of Pleasant Bay can be put together to discern the relative contribution of the different N sources as N loads increased (Fig. 2, top). As urbanization increased, both the resulting N loads from estuaries and the % contributions from wastewater

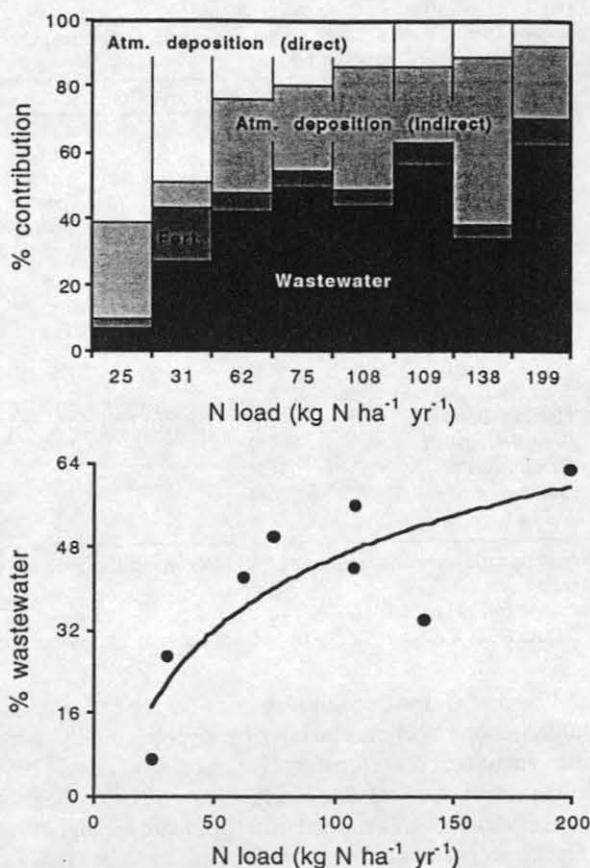


Fig. 2. Percent contribution of different N sources, including atmospheric deposition directly to the water body and indirectly to the watershed, fertilizer, and wastewater (top) and % contribution of wastewater to total N loads ($Y = 20.25 \ln(x) - 47.38$, $R^2 = 0.69$, $F_{\text{reg}} = 13.05$, $P < 0.01$) (bottom) across eight locations in Pleasant Bay having different N loading rates.

increased (Fig. 2, bottom). The standard error associated with the regression in Fig. 2 (bottom) was 10.74, hence, the value for % wastewater predicted by the regression when N loads are zero was not significantly different from zero. The relative contribution by wastewater increased steeply at low N loads, as watersheds became increasingly urbanized and N loads were higher (Fig. 2, top). On Cape Cod there is little agriculture (Valiela and Bowen, 2002) so fertilizer inputs were small (Fig. 2, top). The proportion of total N load contributed by atmospheric deposition on the watershed (defined as "indirect" in Fig. 2, top) did not change very much with increasing N load, while direct atmospheric deposition to the estuary decreased. These data are consistent with previous studies indicating that increases in land-derived N loads are often related to increased wastewater inputs (Valiela et al., 1997; Valiela et al., 2000; Bowen and Valiela, 2001) and point out that remediation of incipient enrichment in these estuaries ought to focus on control of wastewater inputs.

3.2. Comparing measured and modeled DIN concentrations

The relationship between measured and modeled estimates of DIN in the Pleasant Bay estuaries was similar to comparisons of these estimates for other estuaries (Fig. 3). The regression line fit to the combination of Pleasant Bay points and points from other Cape Cod estuaries was significant and had a slope and intercept that did not differ from the 1:1 line of perfect fit (test for homogeneity of slopes: $F = 0.63$, $P = 0.43$, ANCOVA: $F = 1.16$, $P = 0.29$, Fig. 3). The range of both measured and modeled DIN concentrations for locations in Pleasant Bay was lower than that of other Cape Cod water bodies (Fig. 3). This finding is consistent with the generally lower N loads predicted by NLM for subestuaries of Pleasant Bay (Table 1).

To assess the uncertainty that might be connected to each data point in Fig. 3 individually, we plotted the frequency distribution of residuals from each data point to the 1:1 line of perfect fit and normalized each residual to its corresponding modeled DIN concentration. We normalized deviation by dividing each residual by the estimated standard deviation to obtain a standardized residual (Sokal and Rohlf, 1981), divided the standardized residual by the modeled DIN concentration, and multiplied by 100 to derive the % normalized residual to account for differences in magnitude of DIN concentrations among sampling sites (Fig. 3, inset). The uncertainty associated with ELM predictions for Pleasant

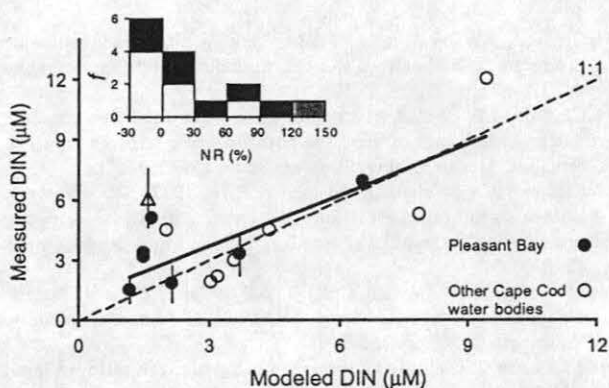


Fig. 3. Comparison of measured and ELM modeled DIN concentrations for Pleasant Bay and other Cape Cod water bodies described in Table 1 ($Y = 0.85x + 1.04$, $R^2 = 0.63$, $F_{reg} = 20.69$, $P < 0.001$). Inset shows the frequency (f) of normalized residuals (NR) (standardized residuals normalized to the % of modeled concentrations) for Pleasant Bay (black) and other Cape Cod water bodies (white). The standardized residual for one Pleasant Bay data point (open triangle and inset, grey bar) emerged as an outlier (Sokal and Rohlf, 1981) and was not included in calculations. Data for other Cape Cod water bodies are from Valiela et al. (submitted for publication). Measured DIN values for Pleasant Bay represent summer mean \pm standard error (RMA, 2001).

Bay estuaries alone was 34% with a median of 13% (Fig. 3, inset). The frequency distribution of these normalized residuals, however, did not differ significantly between Pleasant Bay and other Cape Cod water bodies ($t = -1.59$, $P = 0.17$) and shows that, on average, uncertainty assignable to a specific estuary was $27 \pm 13\%$ (median = 3%) (Fig. 3, inset).

The results of the comparison to measured DIN concentration and the estimate of mean uncertainty per estuary suggest that the combination of NLM and ELM provided a reasonably robust method for estimating TDN loads and DIN concentrations in estuarine systems with relatively small watersheds, shorter flushing times, and lower total N loads.

3.3. Linking land-derived N loads to isotopic signatures

The $\delta^{15}\text{N}$ of suspended and benthic POM that may be food for shellfish (Fig. 4, top and middle) and in tissue of quahogs, *M. mercenaria* (Fig. 4, bottom), increased significantly as % contribution of wastewater increased. The $\delta^{15}\text{N}$ in these biota from Pleasant Bay had the same

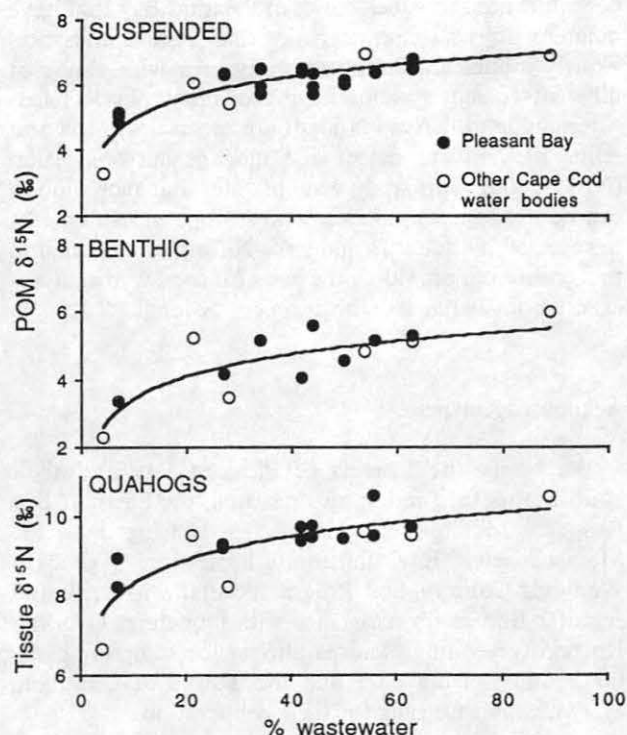


Fig. 4. $\delta^{15}\text{N}$ in suspended and benthic POM and in tissue of quahogs, *M. mercenaria*, compared to % contribution of wastewater to subestuaries of Pleasant Bay and other Cape Cod estuaries (suspended POM: $Y = 0.94 \ln(x) + 2.81$, $R^2 = 0.83$, $F_{reg} = 75.71$; benthic POM: $Y = 0.94 \ln(x) + 1.28$, $R^2 = 0.69$, $F_{reg} = 35.42$; quahogs: $Y = 0.86 \ln(x) + 6.33$, $R^2 = 0.70$, $F_{reg} = 37.22$; all $P_{reg} < 0.0001$). POM = particulate organic matter. No data were available for Hamblin Pond.

magnitude of response to increasing wastewater contributions as biota in other Cape Cod estuaries (Fig. 4). Hence, in Pleasant Bay, an estuarine system with lower N loads and shorter flushing times, land-derived nitrogen loads can be predictably linked to N in biota by means of $\delta^{15}\text{N}$ values.

The slopes of the regressions comparing $\delta^{15}\text{N}$ to % wastewater were the same for suspended and benthic POM and quahogs ($F = 0.12$, $P < 0.89$) (Fig. 4). Mean $\delta^{15}\text{N}$ signatures, however, differed between groups (ANOVA: $F = 137.27$, $P < 0.0001$) (Fig. 4), with $\delta^{15}\text{N}$ values of POM lighter than that of quahogs by approximately 4‰ (Fig. 4). This difference is consistent with expected fractionation from food source to consumer (Peterson and Fry, 1987; Cabana and Rasmussen, 1994), indicating quahogs in Pleasant Bay were likely feeding on POM from one or a combination of these sources. Hence, these results unambiguously show that N derived from land in the specific estuary where we collected the shellfish was transferred up the food web to this consumer.

These analyses have tested and expanded the application of two models that when used together reasonably predicted N loads and available DIN concentrations in subestuaries of Pleasant Bay that have different physical characteristics than subestuaries previously modeled. Continued study of a wide range of different coastal systems having different physical features and land-derived N loads are necessary to test and refine methods to detect and manage eutrophication (NRC, 2000). This study demonstrates that such models can be used in conjunction with isotope measurements to successfully quantify and trace N from inputs on land to a consumer, providing the basis for food web analyses even among estuaries with incipient N loads.

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