Macrophytes as indicators of land-derived wastewater: Application of a $\delta^{15}N$ method in aquatic systems

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[1] We measured $\delta^{15}N$ signatures of macrophytes and particulate organic matter (POM) in six estuaries and three freshwater ponds of Massachusetts to assess whether the signatures could be used as indicators of the magnitude of land-derived nitrogen loads, concentration of dissolved inorganic nitrogen in the water column, and percentage of N loads contributed by wastewater disposal. The study focused specifically on sites on Cape Cod and Nantucket Island, in the northeastern United States. There was no evidence of seasonal changes in $\delta^{15}N$ values of macrophytes or POM. The $\delta^{15}N$ values of macrophytes and POM increased as water column dissolved inorganic nitrogen concentrations increased. We found that $\delta^{15}N$ of macrophytes, but not of POM, increased as N load increased. The $\delta^{15}N$ values of macrophytes and groundwater NO_3 tracked the percent of wastewater contribution linearly. This research confirms that $\delta^{15}N$ values of macrophytes and NO_3 can be excellent indicators of anthropogenic N in aquatic systems.

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1. Introduction

[2] Increased nitrogen loading to coastal systems is changing aquatic environments worldwide [*National Research Council*, 1994]. Eutrophication due to increased N loading can lead to increases in phytoplankton and macroalgae [*Duarte*, 1995; *Hauxwell et al.*, 1998], the loss of important estuarine habitats like sea grasses, and the loss of important commercial shellfish and finfish species, such as cod [*Tveite*, 1984], bay scallops [*Pohle et al.*, 1991], and blue crabs [*Heck and Orth*, 1980]. Eutrophied estuaries suffer from anoxia [*Zimmerman and Canuel*, 2000] and harmful algal blooms, including brown tides [*Hodgkiss and Ho*, 1997].

[3] The increases in N loads derive from increases in atmospheric deposition [*Paerl and Whitall*, 1999], the use of fertilizer [*Jordan et al.*, 1997], and the disposal of wastewater [*Galloway*, 1998; *Caraco and Cole*, 1999]. The evident effects of increasing eutrophication have prompted searches for adequate indicators of N sources and the magnitude of N loads [*Costanzo et al.*, 2001; *Schallenberg and Burns*, 2001]. One approach that seems promising is to measure $\delta^{15}N$ values in macrophytes. The ratio of ^{15}N to ^{14}N can be used to identify different sources of N, elucidate food web structure [*Peterson and Fry*, 1987; *McClelland and Valiela*, 1998a], and describe biogeochemical processes such as denitrification, nitrification, and N_2 fixation [*Lund et al.*, 2000; W. Pabich, personal communication, 2002]. The ratio is expressed as $\delta^{15}N$ (‰) = $[(R_{sample} - R_{reference})/R_{reference}] \times 1000$, where R is $^{15}N/^{14}N$ and the reference is atmospheric N_2 [*Peterson and Fry*, 1987].

[4] Nitrogen in untreated wastewater typically has $\delta^{15}N$ values of +5 to +9‰ [*Aravena et al.*, 1993; *Waldron et al.*, 2001], while $\delta^{15}N$ values of nitrogen in atmospheric deposition range from about −12 to +5‰ [*Russell et al.*, 1998] and $\delta^{15}N$ values of nitrogen in fertilizers range from about −3 to +3‰ [*Kreitler and Browning*, 1983]. Physical and biological processes can increase the $\delta^{15}N$ values of all three sources. However, ^{15}N enrichment is most pronounced when excess nitrogen is coupled with a ready carbon source. Thus nitrogen from wastewater, dominated by nitrate, and fertilizers tends to become more ^{15}N enriched than nitrogen from atmospheric deposition. Nitrogen 15 enrichment of wastewater nitrogen has been particularly well documented: Denitrification within septic systems and wastewater treatment plants typically leaves the remaining nitrate pool with $\delta^{15}N$ values between +10 and +22‰ [*Kreitler and Browning*, 1983; *Aravena et al.*, 1993]. Although less well defined, similarly high values for fertilizer-derived nitrate in stream water have also been documented [*Fry et al.*, 2003]. These values are significantly higher than the $\delta^{15}N$ values between −2 and +8‰ that have been measured in groundwater nitrate derived from atmospheric deposition [*Kreitler and Browning*, 1983; *McClelland and Valiela*, 1998b].

[5] The high $\delta^{15}N$ value of NO_3 derived from wastewater and the strong gradient from river to ocean made it possible for *McClelland and Valiela* [1998b], *Heikoop et al.* [2000], and *Wigand et al.* [2001] to use $\delta^{15}N$ to identify entry of wastewater N to coastal waters by examining the signatures of N in water, macrophytes, and fauna within receiving waters. Macrophytes are excellent potential indicators because they are widely distributed, abundant, and long-lived.

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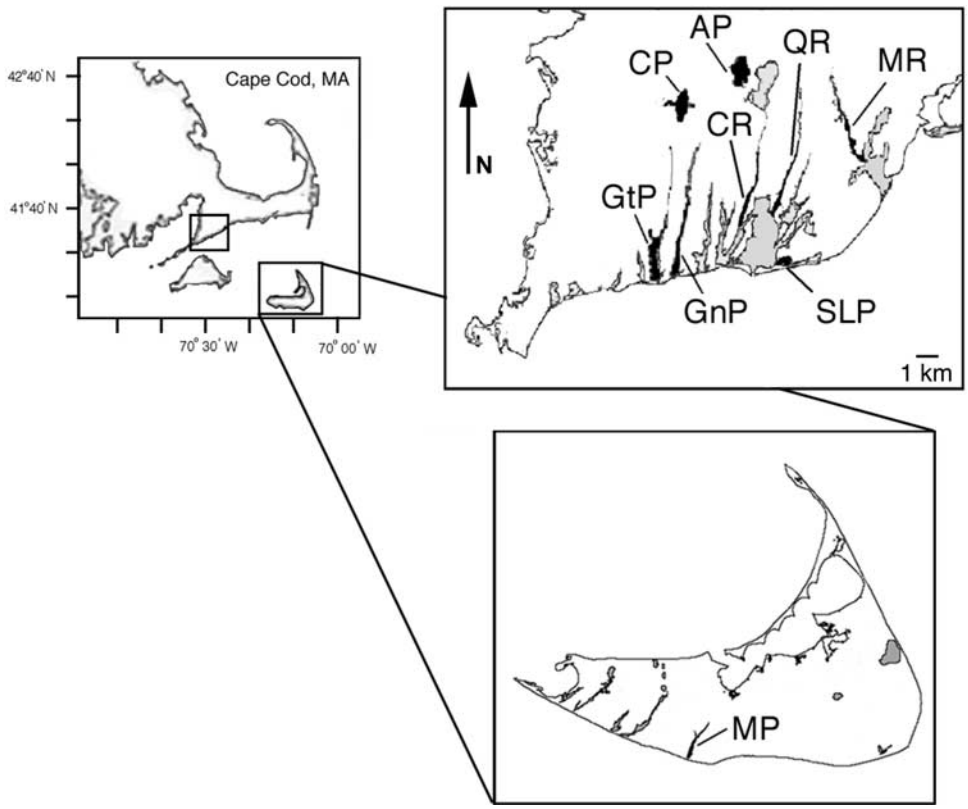

Figure 1. Map of upper Cape Cod and the island of Nantucket, Massachusetts, showing study sites including six sites of present study (Ashumet Pond, AP; Coonamessett Pond, CP; Miacomet Pond, MP; Green Pond, GnP; Great Pond, GtP; Mashpee River, MR) and three sites of *McClelland et al.* [1997] and *McClelland and Valiela* [1998b] (Childs River, CR; Quashnet River, QR; Sage Lot Pond, SLP).

[6] In this paper we further explore the application of isotopic signatures in macrophytes as indicators of N loading from wastewater. Our study focused on sites in the Cape and Islands region of Massachusetts. Freshwater inputs and nutrient loads to estuaries and ponds on the Cape and Islands are delivered predominantly by groundwater. Land cover in the region is primarily forested, and land-use is dominated by residential development. Within this context we specifically examined the relationships between isotopic signatures in macrophytes and the magnitude of land-derived N loads, water column dissolved inorganic nitrogen (DIN) concentrations, and wastewater inputs. We also examined whether particulate organic matter was useful to indicate wastewater N contribution and N loads [*McClelland et al.*, 1997; *McClelland and Valiela*, 1998a], and whether seasonal variation may mask the relationship of primary producer $\delta^{15}N$ to N loads [*Roelke et al.*, 1999]. This study differs from previous work by testing a large number of ponds and estuaries across a range of wastewater loading rates. In addition, this study also compares several types of samples (POM, algae, macroalgae, vascular plants, groundwater nitrate) to assess which sample type provides the best indicator of land-derived wastewater.

2. Methods

2.1. Site Selection

[7] We sampled three estuaries (Mashpee River, Great Pond, and Green Pond) and three freshwater ponds (Coonamessett Pond and Ashumet Pond) in southwestern Cape Cod and on Nantucket Island, Massachusetts (Miacomet Pond) (Figure 1). In addition, we used data for Sage Lot Pond, Quashnet River, and Childs River, all on Cape Cod, previously published by *McClelland et al.* [1997] and *McClelland and Valiela* [1998b]. All nine sites are underlain by sandy, unconsolidated sediments, with an average groundwater travel time of 146 m yr^{-1} [*LeBlanc et al.*, 1991]. Groundwater discharge through seepage faces into water bodies accounts for, on average, 93% of land-derived freshwater inputs to Cape Cod estuaries [*Valiela et al.*, 2002]. Groundwater samples collected at the seepage face, where fresh groundwater meets estuarine or pond water, represent the bulk of groundwater flow into receiving waters [*Valiela et al.*, 2002].

2.2. The $\delta^{15}N$ Measurements in Producers

[8] To assess whether or not plant tissue $\delta^{15}N$ values were a good proxy for groundwater $\delta^{15}NO_3$ values, approximately 500 g of rooted macrophytes, floating macrophytes, and floating macroalgae were collected from 10 widely spaced locations with comparable depths within the freshwater ponds, and in estuaries where salinity was 25–30‰ (Table 1). Subsamples from each location were combined into one sample per estuary. In most cases, whole plants and macroalgae were sampled, with the exception of *Spartina alterniflora* and *Typha latifolia*, where only leaves were collected. The producer tissues were then dried at 60°C for 3 days, ground to a fine powder with a mortar and

Table 1. Macrophyte Species and Particulate Organic Matter (POM) Collected From Each Water Body, Type of Vegetation, $\delta^{15}N$ Value, and Season Collected, Modeled N Load, Wastewater as a Percent of Total N Load, and Water Column DIN Concentration[a]

Estuarine or Freshwater	Species	Nonrooted (NR) or Rooted (R) Vegetation	$\delta^{15}N$, ‰	Season Collected (Summer, Fall, or Winter)	Modeled N Load, Kg N ha^{-1} yr^{-1}	Wastewater, %	DIN, μM
			Mashpee River (MP)[b]				
SW	*Enteromorpha sp.*	NR	7.7	S, F, W	250	44	12.6
	Gracilaria tikvahiae	NR	7.6	S			
	Spartina alterniflora	R	6.8	S, F			
	Ulva lactuca	NR	8.1	S			
	Sargassum filipendula	NR	8.7	S			
	POM		7.7	S, F, W			
			Great Pond[b] *(Gt)*				
SW	*Enteromorpha sp.*	NR	9.9	S, F, W	126	66	...
	Gracilaria tikvahiae	NR	8.3	S, F			
	Spartina alterniflora	R	7.7	S, F			
	POM		7.6	S, F, W			
			Green Pond[b] *(Gn)*				
SW	*Enteromorpha sp.*	NR	7.3	F, W	137	54	4.6
	Gracilaria tikvahiae	NR	8.5	S, F			
	Spartina alterniflora	R	8.1	F			
	Ulva lactuca	NR	8.2	S, F, W			
	Sargassum filipendula	NR	7.7	F			
	POM		7.2	S, F, W			
			Ashumet Pond[b] *(AP)*				
FW	*Callitriche palustris*	R	6.4	S	56	80	...
	Elatine americana	R	11.3	S, F			
	Eleocharis sp.	R	7.4	S			
	Gratiola lutea	R	9.5	S, F, W			
	Hypnum sp.	NR	11.7	S, F			
	Ludwigia sp.	R	12.3	S, F			
	Potomogeton sp.	NR	13.8	S			
	POM		10.8	S, F, W			
			Coonamessett Pond[b] *(CP)*				
FW	*Callitriche palustris*	R	4.7	F	24	17	...
	Elatine americana	R	6.8	S, F			
	Eleocharis sp.	R	5.3	S			
	Eriocaulon sp.	R	5.6	S, F, W			
	Gratiola lutea	R	4.2	S, F, W			
	Polygonum sp.	R	0.5	S			
	POM		7.2	S, F, W			
			Miacomet Pond[b] *(MP)*				
FW	*Callitriche palustris*	R	7.3	F	108	27	...
	Ceratophyllum sp.	NR	8.1	F			
	Elatine americana	R	6.3	F			
	Eriocaulon sp.	R	6.2	F			
	Najas sp.	R	6.2	F			
	Potomogeton perfoliatus	R	5.5	F			
	Ruppia maritima	R	2.9	F			
	Vallisneria Americana	R	5.0	F			
	Typha latifolia	R	5.6	F			
	POM		3.9	F			
			Childs River[c] *(CR)*				
SW	*Enteromorpha sp.*	NR	8.2	S, F	410	65	3.5
	Gracilaria tikvahiae	NR	7.6	S, F			
	Spartina alterniflora	R	7.6	S, F			
	POM		5.7	S, F			
			Quashnet River[c] *(QR)*				
SW	*Enteromorpha sp.*	NR	6.6	S, F	300	30	1.8
	Gracilaria tikvahiae	NR	5.9	S, F			
	Spartina alterniflora	R	6.0	S, F			
	POM		4.7	S, F			

Table 1. (continued)

Estuarine or Freshwater	Species	Nonrooted (NR) or Rooted (R) Vegetation	$\delta^{15}N$, ‰	Season Collected (Summer, Fall, or Winter)	Modeled N Load, Kg N ha^{-1} yr^{-1}	Wastewater, %	DIN, μM
			Sage Lot Pond[c] *(SLP)*				
SW	*Enteromorpha sp.*	NR	4.9	S, F	5	5	1.9
	Gracilaria tikvahiae	NR	5.1	S, F			
	Spartina alterniflora	R	4.4	S, F			
	POM		4.2	S, F			

[a]Estuarine samples (SW) are taken in waters with a salinity between 25 and 30‰.
[b]This study.
[c]*McClelland et al.* [1997] and *Valiela et al.* [1997, 2000].

pestle, and stored in a scintillation vial in a dessicator until analysis.

[9] Particulate organic matter (POM) was collected in 2-L bottles from three locations in each water body, filtered onto ashed glass fiber filters, dried at 60°C for 3 days, and stored in a scintillation vial in a dessicator until analysis. The $\delta^{15}N$ values in the macrophyte tissue and POM were determined by the Boston University Stable Isotope Laboratory and the University of California, Davis, Stable Isotope Facility with a Finnigan Delta-S isotope-ratio mass spectrometer coupled to a Heraeus element analyzer.

2.3. Groundwater Sampling

[10] To link the isotopic signature imparted by watershed land-use patterns to the signature of NO_3 of groundwater about to enter an estuary or pond, we measured $\delta^{15}N$ of the NO_3 in samples of groundwater collected from each watershed. We measured isotopic values of nitrate because it is the dominant inorganic nitrogen source available to producers in these systems [*Valiela et al.*, 2000]. Groundwater was collected at the seepage face along the perimeter of each estuary or pond, using drive-point piezometers [*Valiela et al.*, 2000]. Solute concentrations in groundwater samples are highly variable, particularly in urbanized areas that contain numerous small plumes emanating from septic systems [*Cole*, 2002]. We collected 250 mL of groundwater approximately every 50 m along the perimeter of each estuary or pond. We composited each set of four consecutive groundwater samples, each composite thus representing approximately 200 m of shoreline. For stable isotope analysis, we combined two adjacent 200-m composites, so that each isotope value represented about 400 m of shoreline. In Cape Cod, freshwater ponds commonly receive flowing groundwater on the up-gradient side, and discharge pond water into the aquifer on their down-gradient side [*Strahler*, 1966]. Groundwater was therefore only sampled along the up-gradient portion of a pond margin. After collection, water samples were filtered through 0.7-μm ashed glass fiber filters (Whatman GF/F) and either acidified and stored at 4°C (summer) or frozen (fall and winter).

2.4. Water Column Sampling

[11] To assess the relationship of producer $\delta^{15}N$ to water column DIN concentrations, we measured NO_3 and NH_4 in the water column. We collected water column samples from Mashpee River and Green Pond in September of 1997 and 1998. Water column samples for Sage Lot Pond, Quashnet River, and Childs River were collected monthly from 1991 to 1996 as part of the Waquoit Bay Long-term Monitoring of Ecosystems Research (WBLMER) project (WBLMER database). Water was collected at 0.5 m below the surface and 0.5 m above the sediment, in transects down each estuary. After collection, water samples were filtered through 0.7-μm ashed glass fiber filters (Whatman GF/F) and frozen until nutrient analysis.

2.5. Nutrient Analyses

[12] We measured NH_4 concentrations colorimetrically by the phenol/hypochlorite method [*Strickland and Parsons*, 1972] or fluorometrically [*Holmes et al.*, 1999]. Nitrate concentrations were measured colorimetrically after cadmium reduction to NO_2 using either a manual method [*Jones*, 1984] or using a Lachat autoanalyzer. The values arrived at by this method are actually NO_3 + NO_2, but because NO_2 concentrations were typically an order of magnitude lower than NO_3, we will refer to this value as the NO_3 concentration. In spite of using two methods, the range of concentrations was very large relative to any error associated with these analyses. Concentrations measured by two different methods should certainly be within a few micromoles of each other, and error is assessed based on standards.

2.6. Nitrogen Loading Calculation

[13] The two primary sources of nitrogen to the watersheds of the Cape and Islands come from wastewater disposal and atmospheric deposition [*Valiela et al.*, 1990]. Although contributing considerably less, fertilizers also make a significant contribution to N loads in some locations. To determine the contributions from wastewater disposal, fertilizer use, and atmospheric deposition to the total land-derived nitrogen load to each water body, we used NLM, a nitrogen loading model [*Valiela et al.*, 1997, 2000]. We first identified watershed boundaries using water table contours from the U.S. Geological Survey [*Savoie*, 1995]. We then compiled land uses for each watershed and subwatershed from aerial photos and Geographic Information System databases. The land use data were then entered into NLM, to calculate nitrogen loads. The loads are reported as kg N yr^{-1} per hectare of receiving water. Some assumptions associated with the model were that groundwater is the major N source, that coastal upwelling is negligible, that the watersheds were urbanized with little agriculture, that there was a lack of N_2 fixing crops, and that the sediments were

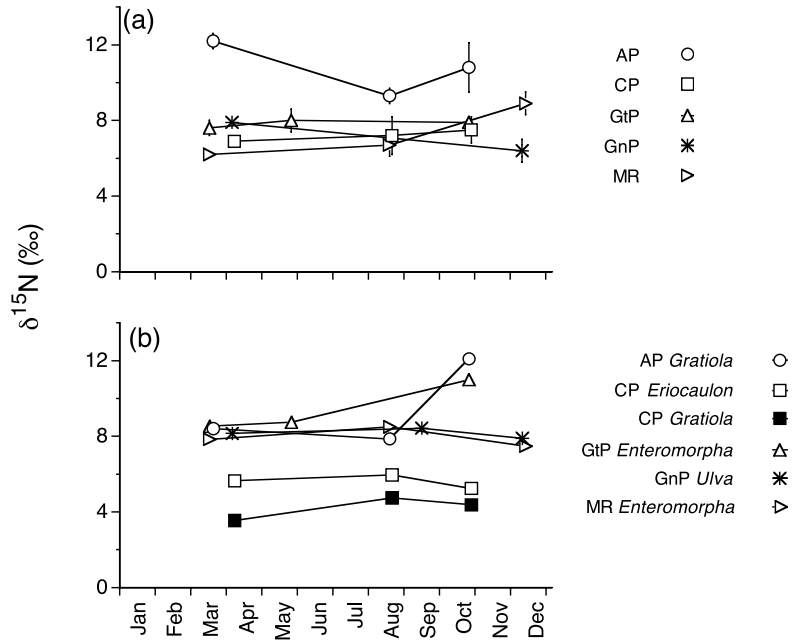

Figure 2. (a) Date of collection versus particulate organic matter $\delta^{15}N$. Error bars are standard error of an average of three samples. (b) Date of collection versus macrophytes for species sampled over three seasons. Each season represents one composite macrophyte sample, and thus standard error could not be calculated. Abbreviations for sites are AP, Ashumet Pond; CP, Coonamessett Pond; GtP, Great Pond; GnP, Green Pond; and MR, Mashpee River.

permeable. Consequently, this nitrogen loading model may not be applicable to other regions where these assumptions are invalid. Modeled loads have previously been published for Childs River, Quashnet River, and Sage Lot Pond [*Valiela et al.*, 2000].

2.7. Stable Isotope Measurements in Groundwater

[14] To assess the relationship of groundwater $\delta^{15}N$ signatures to wastewater, we measured $\delta^{15}N$ of NO_3. Nitrate was isolated from groundwater for $\delta^{15}NO_3$ analysis following the methods of *Sigman et al.* [1997]. MgO was added to raise sample pH to 9.7 to convert NH_4 to NH_3; NH_3 at this pH volatilizes out of the sample. Samples were then boiled down to concentrate NO_3 and to volatilize NH_3. We added ashed reagent-grade NaCl to samples before boiling to compensate for the lack of NaCl in the fresh groundwater, since the method was developed for salt water. An acid trap (a glass fiber filter with H_2SO_4 sandwiched between two Teflon filters) was added to the sample along with Devarda's alloy. Devarda's alloy converts NO_3 to NH_3, which volatilized out of the sample and was trapped on the filter. To allow the diffusion to reach completion, the samples were shaken for 1 week at 40°C [*Sigman et al.*, 1997]. These conditions were shown by *Sigman et al.* [1997] to be sufficient to remove all of the N from the sample. After the diffusion process the acid traps were placed in scintillation vials, dried, and stored in a dessicator until analysis. The N collected on the acid traps was analyzed on a Europa Scientific Hydra 20/20 mass spectrometer at the Boston University Stable Isotope Laboratory and the University of California, Davis, Stable Isotope Facility. Although samples were not intercalibrated between the two labs, both labs use the same standards and precision for replicate analyses is reported as ±0.2 or 0.3‰, which is small compared to the range of measured values.

2.8. Statistics

[15] Relationships between macrophyte $\delta^{15}N$ values and nitrogen load, wastewater, and DIN concentrations and POM $\delta^{15}N$ and wastewater and DIN concentrations, were explored using linear regressions. Nonlinear data for the relationships between macrophyte $\delta^{15}N$ values and nitrogen load and DIN concentrations and between POM $\delta^{15}N$ and wastewater, the nitrogen load, and DIN concentrations were transformed prior to analysis.

[16] To test whether the $\delta^{15}N$ values of the different types of samples measured related differently to nitrogen load, DIN concentration, and wastewater, we tested for homogeneity of slopes. If the slopes were not significantly different, we used an analysis of covariance (ANCOVA) (Statview 5.0.1) with nitrogen load, DIN concentration, and wastewater as covariates to test if the *y*-intercepts of each regression line were significantly different.

3. Results and Discussion

[17] To test how well stable isotopes in producers reflect anthropogenic N input to aquatic systems, we first assessed whether there was seasonal variation in macrophyte and POM $\delta^{15}N$ signatures. We then related aquatic macrophyte and POM $\delta^{15}N$ signatures to three variables that reflect anthropogenic activities: land-derived N loads, water column DIN concentrations, and proportion of the N load derived from wastewater.

[18] The dominant macrophyte taxa we found in the water bodies, including rooted vascular plants, nonrooted vascular plants, and nonrooted macroalgae, differed from

Table 2. Comparison of Slopes and Intercepts for Regressions (ANCOVA) of Macrophyte, Freshwater (FW), Saltwater (SW), Rooted (R), Nonrooted (NR), and Groundwater NO_3 Stable Isotope Values (‰), Versus Land-Derived N Load, and Relative Wastewater N Load, by NLM [*Valiela et al.*, 1997] and Water Column (WC) DIN Concentrations

			F		*F*	
x	y_1	y_2	Degrees of Freedom	Slope	Degrees of Freedom	*y*-Intercept
N load (kg N ha^{-1} yr^{-1})	all R	all NR	11	0.13 ns[a]	12	3.23 ns
Percent wastewater	FW, R	SW, R	5	5.47 ns	6	0.03 ns
Percent wastewater	all R	all NR	13	0.04 ns	14	6.16[b]
Percent wastewater	GW, $\delta^{15}NO_3$	all R	17	0.49 ns	18	6.60[b]
Percent wastewater	GW, $\delta^{15}NO_3$	all NR	16	0.21 ns	17	13.30[c]
WC DIN, μM	SW, R	SW, NR	6	0.04 ns	7	0.33 ns

[a]Nonsignificant.
[b]$F < 0.05$.
[c]$F < 0.01$.

one pond or estuary to another (Table 1). The range of $\delta^{15}N$ encompassed by the values in the producers was considerable (0.5–13.8‰), as was the estimated range of wastewater as a percent of the total land-derived N load (5–80%), and land derived N loads (5–410 kg N ha^{-1} yr^{-1}). Water column DIN concentrations were less variable (1.8–12.6 μM). All data presented can be found in Table 1.

[19] Despite concerns of some authors [*Gu et al.*, 1996; *Brenner et al.*, 1999], in the water bodies in which we were able to evaluate seasonality there seemed to be little seasonal change in the signatures of POM and macrophytes (Figure 2). No significant or consistent trends across time were evident in the data set. *Kwak and Zedler* [1997] also found no seasonal differences in $\delta^{15}N$ values of *Spartina foliosa*. Although there may be differences across seasons in water column DIN $\delta^{15}N$, these variations are apparently not pronounced enough to show up in the time-integrated signatures of macrophytes in these systems. In other systems where freshwater inputs and N loads vary greatly over the course of a year, macrophyte $\delta^{15}N$ values may differ more dramatically between seasons.

[20] There was no difference between the relationships of rooted and nonrooted macrophytes to land-derived N load (Table 2), so regressions were calculated using all the macrophyte data. The $\delta^{15}N$ values of macrophytes increased significantly as N load increased (Figure 3a). There was considerable scatter, as can be seen in the low value for R^2 (0.47). *Lake et al.* [2001] found a similar relationship between water column DIN concentrations and the $\delta^{15}N$ values of sediments, primary and secondary consumers in freshwater ponds. The shape of the curves suggest that the relationship is more sensitive at lower land-derived N loads, above a nitrogen load of approximately 150 kg N ha^{-1} yr^{-1}, macrophyte $\delta^{15}N$ values level off. There was no response of $\delta^{15}N$ of POM to N load (Figure 3b), although points seemed to cluster around 4 and 7‰. The cause of this clustering is not apparent and may need further study.

[21] The $\delta^{15}N$ values in macrophytes (Figure 4a) and in POM (Figure 4b) also increased as DIN concentrations in the water column increased, although with low R^2 values (Figure 4b). The predictive capacity of these relationships seems hampered by the dearth of points, but they suggest the promise of the approach, even in relation to highly variable annual average DIN concentrations. The shape of these responses match results by *Lake et al.* [2001] for sediments and consumers, in spite of the differences in trophic levels between the two studies.

[22] Macrophyte $\delta^{15}N$ signatures became significantly heavier as the percent wastewater of N loads entering the water bodies increased (Figure 5a). We assessed rooted and nonrooted macrophytes separately because their regressions

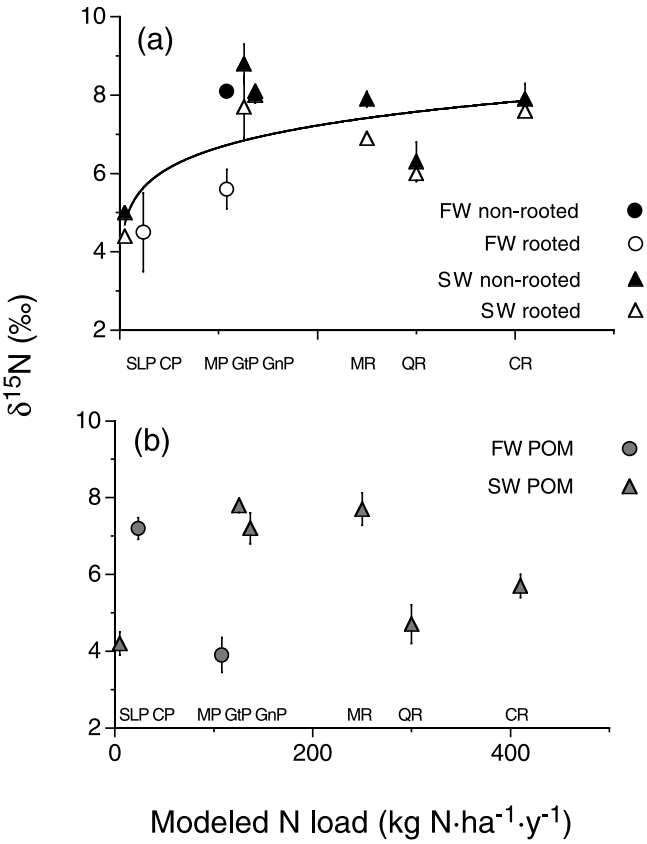

Figure 3. (a) Modeled land-derived N load (using NLM [*Valiela et al.*, 1997]) versus rooted and nonrooted macrophyte $\delta^{15}N$ from freshwater (FW) and estuarine (SW) receiving waters ($y = 3.89x^{0.12}$, $R^2 = 0.47$**), and (b) modeled land-derived N load versus particulate organic matter (POM) $\delta^{15}N$ for five sites of present study and three sites of *McClelland et al.* [1997] and *McClelland and Valiela* [1998b]. Isotope values are means of all seasons collected. Error bars represent standard error. Abbreviations for sites are SLP, Sage Lot Pond; CP, Coonamessett Pond; MP, Miacomet Pond; GtP, Great Pond; GnP, Green Pond; MR, Mashpee River; QR, Quashnet River; and CR, Childs River.

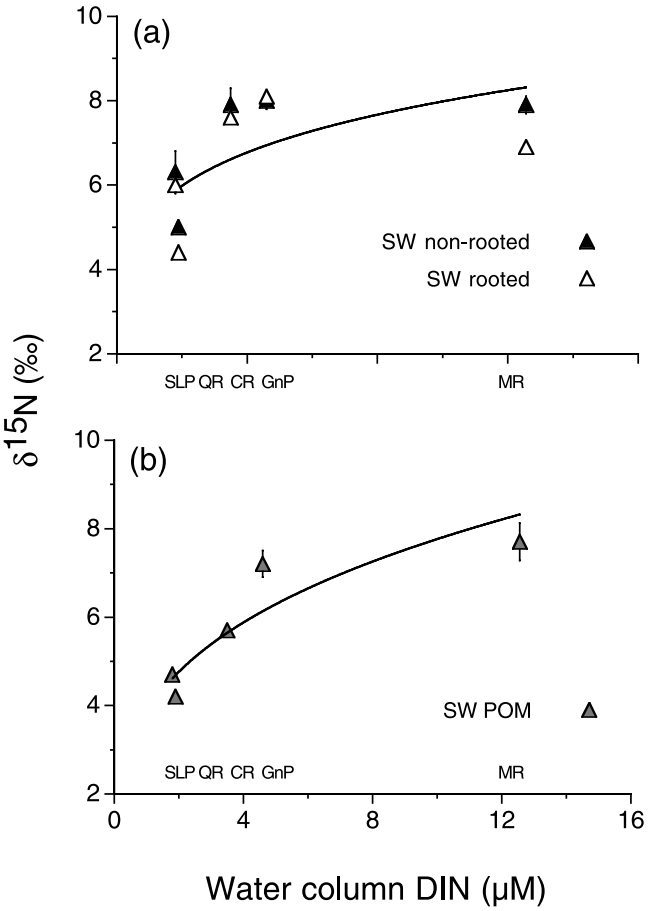

Figure 4. (a) Mean annual water column dissolved inorganic nitrogen (DIN) concentrations versus freshwater (FW) and estuarine (SW) rooted and nonrooted estuarine macrophytes ($y = 5.28x^{0.18}$, $R^2 = 0.40$*) and (b) particulate organic matter (POM) $\delta^{15}N$ ($y = 4.86x^{0.30}$, $R^2 = 0.87$*) for six sites of present study, and three sites of *McClelland et al.* [1997] and *McClelland and Valiela* [1998b]. Isotope values are means of all seasons collected. Error bars represent standard error. Abbreviations for sites are SLP, Sage Lot Pond; GnP, Green Pond; MR, Mashpee River; QR, Quashnet River; and CR, Childs River.

differed significantly. For both rooted and nonrooted macrophytes we combined data for both freshwater and estuarine species, because the regressions did not differ significantly (Table 2). We also plotted the relationship between percent of wastewater and groundwater $\delta^{15}NO_3$ (Figure 5a). The three regression lines shown have the same slope, but are offset from one another. These aquatic systems are acting in a remarkably uniform manner to enrich $\delta^{15}N$ values in the water column. Some similar processes must be occurring in all systems. This interesting phenomenon requires further research. In this study, all producers responded similarly to wastewater inputs. In a previous study of three of the same estuaries, *McClelland and Valiela* [1998b] found that the slope of this relationship for eelgrass (not sampled in the present study) was different from the slope for macroalgae and *S. alterniflora*.

[23] The offset in regressions for the different groups of producers may derive from differences in nutrient sources. Rooted plants have direct access to sediment and groundwater-borne N [*Tobias et al.*, 2001], and had $\delta^{15}N$ values closer to the $\delta^{15}N$ of those sources than nonrooted macrophytes, which take up only water column N. We lack information about water column $\delta^{15}N$ values in relationship to percent wastewater in our sites, but we speculate that they may have a similar slope as macrophytes and groundwater in Figure 5a, but with values heavier than nonrooted macrophytes. When plants assimilate N, there is generally a fractionation of −1‰ to −10‰ (product − substrate [*Peterson and Fry*, 1987; *Fogel and Cifuentes*, 1993]). The fact that both rooted and nonrooted macrophytes had higher $\delta^{15}N$ values than those of groundwater nitrate in spite

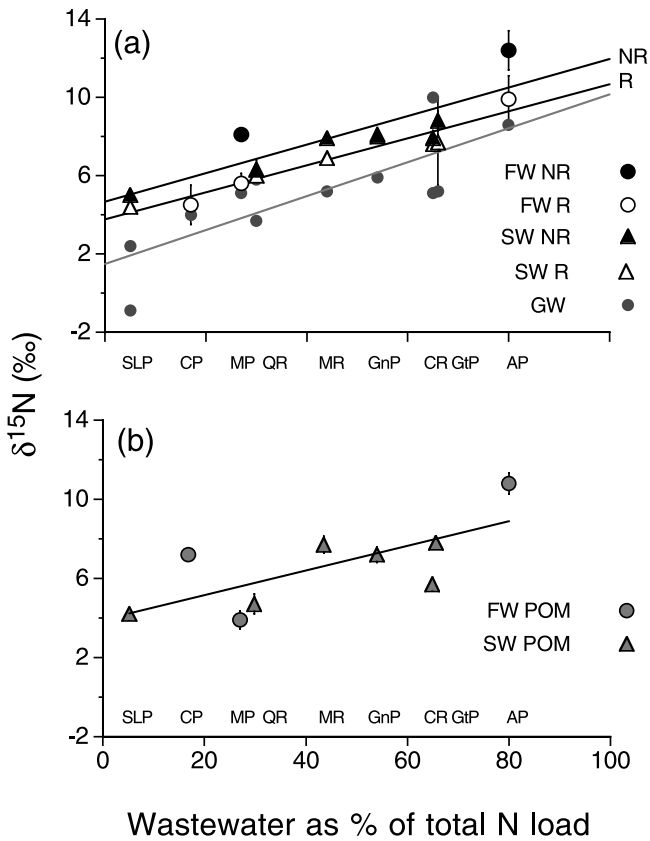

Figure 5. (a) Wastewater as a percent of total N load as calculated using a nitrogen loading model (NLM [*Valiela et al.*, 1997, 2000]) versus freshwater (FW) and estuarine (SW) macrophyte and groundwater $\delta^{15}N$ for six sites of present study, three sites of *McClelland et al.* [1997] and *McClelland and Valiela* [1998b]. Species in each category listed in Table 1. (All nonrooted (NR): $y = 0.07x + 4.67$, $R^2 = 0.71$**; all rooted (R): :$y = 0.07x + 3.88$, $R^2 = 0.93$ ($F < 0.001$); groundwater $\delta^{15}NO_3$ (GW): $y = 0.09x + 1.48$, $R^2 = 0.64$ ($F < 0.001$)). (b) Wastewater as a percent of total N load as calculated using NLM [*Valiela et al.*, 1997, 2000] versus freshwater (FW) and estuarine (SW) particulate organic matter (POM) $\delta^{15}N$ ($y = 0.06x + 3.90$, $R^2 = 0.51$ ($F < 0.05$)). Isotope values are means of all seasons collected. Error bars represent standard error. Abbreviations for sites are SLP, Sage Lot Pond; CP, Coonamessett Pond; MP, Miacomet Pond; GtP, Great Pond; GnP, Green Pond; MR, Mashpee River; QR, Quashnet River; CR, Childs River; and AP, Ashumet Pond.

of any fractionation effect during uptake implies that both rooted and nonrooted macrophytes must have taken up some of their N from the water column. Rooted plants use a mixture of N sources [*Pedersen et al.*, 1997; *Dudley et al.*, 2001], giving them $\delta^{15}N$ values intermediate between those of groundwater and those of nonrooted vegetation. Rooted macrophytes may be better suited as indicators of land-derived wastewater since there is seems to be less processing and fractionation of N than in nonrooted macrophytes. Linear relationships between $\delta^{15}N$ values of macrophytes, sediment, and consumers, and the degree of residential development on watersheds were also reported by *McKinney et al.* [2001] and *Wigand et al.* [2001] in coastal marshes and by *Lake et al.* [2001] in freshwater lakes.

[24] These results broadly support the idea that $\delta^{15}N$ values in producers in estuaries and freshwater ponds of the Cape and Islands reliably indicate entry of wastewater-derived N into these aquatic ecosystems. The high R^2 values make for a highly predictive relationship (Figure 5a), one that is sensitive to even low levels of wastewater enrichment. With a range of $\delta^{15}N$ values of 5‰ and errors of 0.5‰, we think we can estimate percent wastewater inputs to within ±10% of actual inputs. This relationship emerges in the groundwater-fed estuaries and ponds of Cape Cod because the $\delta^{15}N$ signatures of wastewater-derived NO_3 (+10‰ to +22‰) are distinct from nitrate derived from atmospheric/soil (+2‰ to +8‰) and fertilizer (0 to +6‰; W. Pabich, personal communication, 2002) sources. The water column $\delta^{15}N$ values are dominated by the land-derived N sources; in other sites, marine N loading may be a more dominant source. Therefore this relationship exists due to the presence of two land-derived N sources, wastewater with high $\delta^{15}N$ values, and background low $\delta^{15}N$ values. The relationship may be less clear in systems with major differences in the behavior of N within watersheds, in the route of N delivery from the watershed to receiving waters, or in the amount of seasonal coastal upwelling of nitrogen. Nitrogen loading models to calculate percent wastewater need to be appropriate for the geographic region. For instance, in areas with large fertilizer N inputs and extensive denitrification of that source, fertilizer $\delta^{15}N$ values may not differ from those of wastewater [*Fry et al.*, 2003]. In this study, macroalgae and vascular plant $\delta^{15}N$ values responded similarly to wastewater in spite of marked differences in their structures. There may be other species that do not respond in such a manner. For instance, plants that have nitrogen fixing bacteria in their rhizospheres would have a very different $\delta^{15}N$ value than those that do not. Additionally, receiving waters fed by streams or rivers with long residence times may lose the land-derived signal during more extensive cycling of N in route to the receiving water. In this case, a broad geographic study to address these issues is warranted.

[25] Particulate organic matter $\delta^{15}N$ values were also significantly related to modeled percent wastewater contribution to N loads (Figure 5b), but the scatter was greater than for macrophytes and the coefficient of determination was less predictive [*Prairie*, 1996]. Similar variation in the range of POM $\delta^{15}N$ values has been found elsewhere [*Gu et al.*, 1996].

[26] In conclusion, in this study, N stable isotopes were good tracers of land-derived wastewater, regardless of the type of water body or season. It is clear from the broad consistency among sites that this isotopic method can provide managers and policy makers with a simple and cost-effective tool for determining how dominant wastewater is as an N source to ponds and estuaries of the Cape and Islands. The relationship of macrophyte $\delta^{15}N$ to the percent of the N contributed by wastewater is good enough to estimate the percent of wastewater N load in water bodies to within 10% of the actual load where such information is lacking. The method can be taken one step further to calculate a quantity of wastewater load, not just a percentage of the total load, if the total N load is known. This method can be used as a long-term monitoring tool to assess increases in wastewater over time in a particular water body. It can also be used at a regional scale to compare wastewater inputs to a number of ponds and estuaries. Further examination of the relationships between nitrogen sources and stable N isotope ratios of aquatic macrophytes in regions with other hydrologic regimes and land-use patterns will be needed to define the full extent and limitations of using stable N isotopes as a monitoring tool. Nonetheless, the robust relationships demonstrated by this study show that use of macrophyte $\delta^{15}N$ values to evaluate N inputs to aquatic systems can be a very effective approach.

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References

Aravena, R., M. L. Evans, and J. A. Cherry (1993), Stable isotopes of oxygen and nitrogen in source identification of nitrate from septic systems, *Ground Water*, *31*, 180–186.

Brenner, M., T. J. Whitmore, J. H. Curtis, D. A. Hodell, and C. L. Schelske (1999), Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) signatures of sedimented organic matter as indicators of historic lake trophic state, *J. Paleolimnol.*, *22*, 205–221.

Caraco, N. F., and J. J. Cole (1999), Human impact on nitrate export: An analysis using major world rivers, *Ambio*, *28*, 167–170.

Cole, M. L. (2002), Detection of eutrophication in aquatic ecosystems: Nitrogen stable isotopes in macrophytes and groundwater, Ph.D. dissertation, Boston Univ., Boston, Mass.

Costanzo, S. D., M. J. O'Donohue, W. C. Dennison, N. R. Loneragan, and M. Thomas (2001), A new approach for detecting and mapping sewage impacts, *Mar. Pollut. Bull.*, *42*, 149–156.

Duarte, C. M. (1995), Submerged aquatic vegetation in relation to different nutrient regimes, *Ophelia*, *41*, 87–112.

Dudley, B. J., A. M. E. Gahnstrom, and D. I. Walker (2001), The role of benthic vegetation as a sink for elevated inputs of ammonium and nitrate in a mesotrophic estuary, *Mar. Ecol. Prog. Ser.*, *219*, 99–107.

Fogel, M. L., and L. A. Cifuentes (1993), Isotopic fractionation during primary production, in *Organic Geochemistry*, edited by M. H. Engel and S. A. Macko, pp. 73–98, Plenum, New York.

Fry, B., A. Gace, and J. W. McClelland (2003), Chemical indicators of anthropogenic nitrogen loading in four Pacific estuaries, *Pac. Sci.*, *57*, 77–101.

Galloway, J. N. (1998), The global nitrogen cycle: Changes and consequences, *Environ. Pollut.*, *102*, 15–24.

Gu, B., C. L. Schelske, and M. Brenner (1996), Relationships between sediment and plankton isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and primary productivity in Florida lakes, *Can. J. Fish. Aquat. Sci.*, *53*, 875–883.

Hauxwell, J., J. McClelland, P. J. Behr, and I. Valiela (1998), Relative importance of grazing and nutrient controls of macroalgal biomass in three temperate shallow estuaries, *Estuaries*, *21*, 347–360.

Heck, K. L., and R. J. Orth (1980), Structural components of eelgrass (*Zostera marina*) meadows in the lower Chesapeake Bay—Decapod crustacea, *Estuaries*, *3*, 285–289.

Heikoop, J. M., M. J. Risk, A. V. Lazier, E. N. Edinger, J. Jompa, G. V. Limmon, J. J. Dunn, D. R. Browne, and H. P. Schwarcz (2000), Nitrogen-15 signals of anthropogenic nutrient loading in reef corals, *Mar. Pollut. Bull.*, *40*, 628–636.

Hodgkiss, I. J., and K. C. Ho (1997), Are changes in N:P ratios in coastal waters the key to increased red tide blooms?, *Hydrobiologia*, *352*, 141–147.

Holmes, R. M., A. Aminot, R. Kerouel, B. A. Hooker, and B. J. Peterson (1999), A simple and precise method for measuring ammonium in marine and freshwater ecosystems, *Can. J. Fish. Aquat. Sci.*, *56*, 1801–1808.

Jones, M. N. (1984), Nitrate reduction by shaking with cadmium: Alternative to cadmium columns, *Water Res.*, *18*, 643–646.

Jordan, M. J., K. J. Nadelhoffer, and B. Fry (1997), Nitrogen cycling in forest and grass ecosystems irrigated with ^{15}N-enriched wastewater, *Ecol. Appl.*, *7*, 864–881.

Kreitler, C. W., and L. A. Browning (1983), Nitrogen-isotope analysis of groundwater nitrate in carbonate aquifers: Natural sources versus human pollution, *J. Hydrol.*, *61*, 285–301.

Kwak, T. J., and J. B. Zedler (1997), Food web analysis of southern California coastal wetlands using multiple stable isotopes, *Oecologia*, *110*, 262–277.

Lake, J. L., R. A. McKinney, F. A. Osterman, R. J. Pruell, J. Kiddon, S. A. Ryba, and A. D. Libby (2001), Stable nitrogen isotopes as indicators of anthropogenic activities in small freshwater systems, *Can. J. Fish. Aquat. Sci.*, *58*, 870–878.

LeBlanc, D. R., S. P. Garabedian, K. M. Hess, L. W. Gelhar, and R. D. Quadri (1991), Large-scale natural gradient tracer test in sand and gravel, Cape Cod, Massachusetts: 1. Experimental design and observed tracer movement, *Water Resour. Res.*, *27*, 895–910.

Lund, L. J., A. J. Horne, and A. E. Williams (2000), Estimating denitrification in a large constructed wetland using stable nitrogen isotope ratios, *Ecol. Eng.*, *14*, 67–76.

McClelland, J. W., and I. Valiela (1998a), Changes in food web structure under the influence of increased anthropogenic nitrogen inputs to estuaries, *Mar. Ecol. Prog. Ser.*, *168*, 259–271.

McClelland, J. W., and I. Valiela (1998b), Linking nitrogen in estuarine producers to land-derived sources, *Limnol. Oceanogr.*, *43*, 577–585.

McClelland, J. W., I. Valiela, and R. H. Michener (1997), Nitrogen-stable isotope signatures in estuarine food webs: A record of increasing urbanization in coastal watersheds, *Limnol. Oceanogr.*, *42*, 930–937.

McKinney, R. A., W. G. Nelson, C. Wigand, and M. A. Charpentier (2001), Ribbed mussel nitrogen isotope signatures reflect nitrogen sources in coastal salt marshes, *Ecol. Appl.*, *11*, 203–214.

National Research Council (1994), *Priorities for Coastal Science*, Natl. Acad. Press, Washington, D. C.

Paerl, H. W., and D. R. Whitall (1999), Anthropogenically-derived atmospheric nitrogen deposition, marine eutrophication and harmful algal bloom expansion: Is there a link?, *Ambio*, *28*, 307–311.

Pedersen, M. F., E. I. Paling, and D. I. Walker (1997), Nitrogen uptake and allocation in the seagrass *Amphibolis Antarctica*, *Aquat. Bot.*, *56*, 105–117.

Peterson, B. J., and B. Fry (1987), Stable isotopes in ecosystem studies, *Annu. Rev. Ecol. Syst.*, *18*, 293–320.

Pohle, D. G., V. M. Bricelj, and Z. Garcia-Esquivel (1991), The eelgrass canopy: An above-bottom refuge from benthic predators for juvenile bay scallops *Argopecten irradiens*, *Mar. Ecol. Prog. Ser.*, *74*, 47–59.

Prairie, Y. T. (1996), Evaluating the power of regression models, *Can. J. Fish. Aquat. Sci.*, *53*, 490–492.

Roelke, D. L., P. M. Eldridge, and L. A. Cifuentes (1999), A model of phytoplankton competition for limiting and nonlimiting nutrients: Implications for development of estuarine and nearshore management schemes, *Estuaries*, *22*, 92–104.

Russell, K. A., J. N. Galloway, S. A. Macko, J. L. Moody, and J. R. Scudlark (1998), Sources of nitrogen in wet deposition in the Chesapeake Bay region, *Atmos. Environ.*, *32*, 2453–2465.

Savoie, J. (1995), Altitude and configuration of the water table, western Cape Cod aquifer, Massachusetts, March 1993, map, U.S. Geol. Surv.

Schallenberg, M., and C. W. Burns (2001), Tests of autotrophic picoplankton as early indicators of nutrient enrichment in an ultra-oligotrophic lake, *Freshwater Biol.*, *46*, 27–37.

Sigman, D. M., M. A. Altabet, R. Michener, D. C. McCorkle, B. Fry, and R. M. Holmes (1997), Natural abundance-level measurement of the nitrogen isotopic composition of oceanic nitrate: An adaptation of the ammonia diffusion method, *Mar. Chem.*, *57*, 227–242.

Strahler, A. N. (1966), *A Geologist's View of Cape Cod*, Doubleday, New York.

Strickland, J. D. H., and T. R. Parsons (1972), *A Practical Handbook of Seawater Analysis*, Fish. Res. Board of Can., Ottawa, Ont., Canada.

Tobias, C. R., J. W. Harvey, and I. C. Anderson (2001), Quantifying groundwater discharge through fringing wetlands to estuaries: Seasonal variability, methods comparison, and implications for wetland-estuary exchange, *Limnol. Oceanogr.*, *46*, 604–615.

Tveite, S. (1984), 0-Group cod investigations on the Norwegian Skagerrak coast, in *The Propagation of Cod*, *Gadus Morhua*, edited by E. Dahl et al., pp. 581–590, Inst. of Mar. Res., Flødivigen Biol. Stn., Arendal, Norway.

Valiela, I., J. Costa, K. Foreman, J. M. Teal, B. L. Howes, and D. Aubrey (1990), Transport of groundwater-borne nutrients from watersheds and their effects on coastal waters, *Biogeochemistry*, *10*, 177–197.

Valiela, I., G. Collins, J. Kremer, K. Lajtha, M. Geist, B. Seely, J. Brawley, and C. H. Sham (1997), Nitrogen loading from coastal watersheds to receiving estuaries: New method and application, *Ecol. Appl.*, *7*, 358–380.

Valiela, I., M. Geist, J. McClelland, and G. Tomasky (2000), Nitrogen loading from watersheds to estuaries: Verification of the Waquoit Bay nitrogen loading model, *Biogeochemistry*, *49*, 277–293.

Valiela, I., J. L. Bowen, and K. D. Kroeger (2002), Assessment of models for estimation of land-derived nitrogen loads to shallow estuaries, *Appl. Geochem.*, *17*, 935–953.

Waldron, S., P. Tatner, I. Jack, and C. Arnott (2001), The impact of sewage discharge in a marine embayment: A stable isotope reconnaissance, *Estuarine Coastal Shelf Sci.*, *52*, 111–115.

Wigand, C., R. Comeleo, R. A. McKinney, G. Thursby, M. Chintala, and M. A. Charpentier (2001), Outline of a new approach to evaluate ecological integrity of salt marshes, *Human Ecol. Risk Assess.*, *7*, 1541–1554.

Zimmerman, A. R., and E. A. Canuel (2000), A geochemical record of eutrophication and anoxia in Chesapeake Bay sediments: Anthropogenic influence on organic matter composition, *Mar. Chem.*, *69*, 117–137.

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