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Linking nitrogen in estuarine producers to land-derived sources

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Abstract

It is clear that anthropogenic nitrogen inputs from watersheds to estuaries stimulate eutrophication. It has been difficult, however, to explicitly link anthropogenic N entering estuaries to N found in estuarine producers. To explore this link, we compared stable isotope ratios of N in groundwater and producers from the Waquoit Bay watershed–estuary system, Cape Cod, Massachusetts. The $\delta^{15}N$ values of groundwater nitrate within the Waquoit Bay watershed increase from −0.9‰ to +14.9‰ as wastewater contributions increase from 4 to 86% of the total N pool. As a result, the average $\delta^{15}N$ of dissolved inorganic nitrogen (DIN, nitrate + ammonium) received by different estuaries around Waquoit Bay increases from +0.5‰ to +9.5‰. This increase is strongly correlated to increases in $\delta^{15}N$ of eelgrass, macroalgae, cordgrass, and suspended particulate organic matter. The increase of all producers examined in Waquoit Bay with increasing $\delta^{15}N$ of DIN in groundwater demonstrates a tight coupling between N contributed to coastal watersheds and N used by primary producers in estuaries. The ability to identify effects of increasing wastewater N loads on $\delta^{15}N$ of estuarine producers may provide a means to reliably identify incipient eutrophication in coastal waters.

Nitrogen enrichment as a result of anthropogenic activity along the land–sea margin is increasing eutrophication of coastal waters across the globe (GESAMP 1990; Natl. Acad. Sci. 1994). As coastal eutrophication progresses, overall increases in primary production are accompanied by major shifts in the dominant flora (Duarte 1995; Valiela et al. 1997*b*) and fauna (Heip 1995) living in estuaries. Although defining the "health" of an estuary is difficult, and still more difficult to determine is the value of maintaining an estuary in its natural state, some of the changes that accompany eutrophication of coastal waters have a clear negative socioeconomic impact. For example, along the northeast coast of the United States commercially important species, such as the bay scallop (*Argopecten irradians*), winter flounder (*Pseudopleuronectes americanus*), and Atlantic menhaden (*Brevoortia tyrannus*), depend on seagrass habitat as a nursery and feeding ground (Curley et al. 1971; Valiela et al. 1992). This habitat rapidly disappears as shallow estuaries become eutrophied (Duarte 1995; Valiela et al. 1997*b*; Short and Burdick 1996).

Methods to identify incipient eutrophication brought about by increased N loading would greatly help efforts by environmental managers to preserve critical coastal habitats. The use of stable N isotope ratios (expressed as $\delta^{15}N$ in per mil units) in order to track wastewater N from coastal watersheds into estuarine food webs may provide environmental managers with one such method (McClelland et al. 1997). Theoretically, direct detection of wastewater N in estuarine biota should provide a means to identify increasing contributions of wastewater N to estuarine food webs before increased N availability leads to visible changes of population and community structure.

Transformations and losses of wastewater N, as it travels from a septic tank into an aquifer, leave wastewater-derived nitrate enriched in ^{15}N relative to nitrate from natural soils (Kreitler et al. 1978; Kreitler and Browning 1983; Aravena et al. 1993; Macko and Ostrom 1994). Inorganic N leaves septic tanks predominantly in the form of NH_4^+ (Valiela et al. 1997*a*), with $\delta^{15}N$ values ~6‰ (J. McClelland unpubl. data). As ammonium moves through the effluent leaching field, some N is lost due to volatilization of NH_3, while the remaining ammonium is converted to nitrate by autotrophic bacteria. More N is then lost to denitrification, i.e. the conversion of nitrate to N_2 gas by anaerobic heterotrophic bacteria, as it travels along the effluent plume (Valiela et al. 1997*a*). Both volatilization of ammonia and denitrification remove ^{14}N at a faster rate than ^{15}N, so that the remaining nitrate from wastewater that enters the aquifer typically has $\delta^{15}N$ values between +10 and +20‰ (Kreitler et al. 1978; Kreitler and Browning 1983; Aravena et al. 1993; Macko and Ostrom 1994). The $\delta^{15}N$ values for nitrate in groundwater from natural soils fall below this range, often between +2 and +8‰ (Macko and Ostrom 1994).

In coastal watersheds, nitrate derived from septic systems makes its way into estuaries via groundwater (Valiela et al. 1992). Because this nitrate is enriched in ^{15}N, we hypothesize that increased wastewater input to coastal watersheds leads to increased $\delta^{15}N$ of the total dissolved inorganic nitrogen (DIN) pool delivered to estuaries, and that increased $\delta^{15}N$ of groundwater DIN delivered to estuaries leads to increased $\delta^{15}N$ of producers taking up N within estuaries. McClelland et al. (1997) presented evidence that the $\delta^{15}N$ of estuarine biota increase as wastewater N loads to estuaries increase. Here, we first present data that link the degree of urbanization of coastal watersheds to stable N isotope ratios of un-

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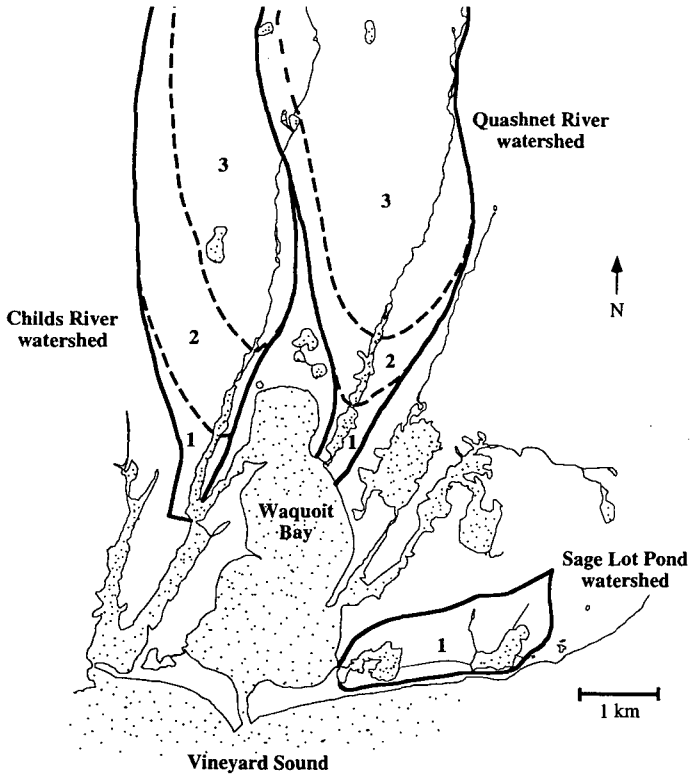

Fig. 1. The Childs River, Quashnet River, and Sage Lot Pond estuaries of Waquoit Bay, Massachusetts, and the watersheds adjoined to them. The Childs River and Quashnet River watersheds are divided into three recharge areas (delineated by dashed lines) that deliver groundwater to specific reaches of each estuary.

derlying groundwater. Subsequently, we examine how N load and groundwater $\delta^{15}N$ entering estuaries relate to the $\delta^{15}N$ of primary producers taking up N within estuaries.

Methods

Cross-site comparisons of natural abundance stable N isotope ratios—To examine how urbanization in coastal watersheds affects $\delta^{15}N$ in underlying groundwater, we have made comparisons among three watersheds that adjoin separate estuaries (Sage Lot Pond, Quashnet River, and Childs River) around Waquoit Bay, Cape Cod, Massachusetts (Fig. 1). Each of these watersheds has a different level of urban land use. The Sage Lot Pond watershed is primarily forested and has a low building density; the Quashnet River watershed has an intermediate building density; the Childs River watershed has the highest building density (Table 1). With increased urbanization, wastewater N inputs to each watershed increase (Table 1, compare columns 1 and 2). This nitrogen, minus losses as it travels through the watershed, is then delivered to the estuaries of Waquoit Bay (Table 1) via groundwater flow (Valiela et al. 1992). Although atmospheric deposition, fertilizer use, and wastewater disposal all contribute N to Waquoit Bay, wastewater is the dominant source, contributing ~50% of the total N load and 75% of the anthropogenic (wastewater + fertilizer) N load to the bay (Valiela et al. 1997*a*).

Table 1. Building densities on the Childs River, Quashnet River and Sage Lot Pond watersheds, and nitrogen loads from wastewater (WW) to these watersheds and their adjoining estuaries. Loading rates were calculated using the Waquoit Bay LMER nitrogen loading model (Valiela et al. 1997*a*). This model estimates N inputs to each watershed from WW disposal by using building density and an average occupancy rate of two people per building within the Waquoit Bay watershed. Nitrogen losses as WW moves through the Vadose zone, and aquifer are then subtracted from the N inputs to each watershed to estimate N inputs to each estuary.

	Watershed		Estuary	
Site	Building density (buildings ha^{-1})	WW N load (kg N ha^{-1} yr^{-1})	WW N load (kg N ha^{-1} yr^{-1})	% WW in total N load
Childs River	1.6	13.7	268.3	63
Quashnet River	0.4	3.0	118.1	28
Sage Lot Pond	0.1	1.0	0.6	4

We also made cross-site comparisons within the Waquoit Bay system to examine the coupling between groundwater $\delta^{15}N$ signatures received by estuaries and $\delta^{15}N$ of producers living within them. Physical and biological processes that fractionate N within aquifers (Mariotti et al. 1988) and estuaries (Mariotti et al. 1984; Cifuentes et al. 1989; Horrigan et al. 1990) and during uptake by producers (Fogel and Cifuentes 1993; Pennock et al. 1996), can make it difficult to use stable N isotope ratios to trace N through ecosystems (Hauck et al. 1972; Bremner and Tabatabai 1973; Hauck 1973). Cross-site comparisons, however, make data interpretation clearer because we can use magnitude and direction of change in $\delta^{15}N$ values among sites to help identify the influence of different N sources (McClelland et al. 1997).

Groundwater collection and preparation—Groundwater about to enter Sage Lot Pond, Quashnet River, and Childs River was initially sampled during July 1994. Additional groundwater was sampled during September 1995 and May 1996 at Childs River, and during August 1996 at Sage Lot Pond. Samples from different months were considered comparable, because groundwater in the Waquoit Bay watershed has a constant temperature throughout the year and flows slow enough to integrate seasonal variaton in N inputs to the watershed surface (Valiela et al. 1997*a*). Sampling of Childs River and Quashnet River groundwater was partitioned to include inputs from three separate recharge areas associated with each estuary (Fig. 1). There was no need to partition groundwater sampling at Sage Lot Pond because of the small size of its watershed. Samples were collected along the shoreline of each estuary (10–20 m apart) using a well-point piezometer, attached to a negative-pressure pump. The well-point piezometer was driven ~1.5 m into the soil on the landward-side of the seepage face. All groundwater samples were tested using an Atago hand-held refractometer (analytical error of ±0.3 ppt) to ensure that they had a salinity of 0 ppt. Samples were filtered through 0.45-μm Gelman Metricel membranes, acidified with HCl to pH 3, and stored at 6°C. DIN concentrations were determined using a Lachat Instruments Quik Chem AE automated ion analyzer.

The number of samples required to estimate reasonable average $\delta^{15}N$ values in groundwater leaving a given recharge area was largely determined by shoreline length; more samples were needed from recharge areas with long shorelines than with short shorelines in order to achieve a similar level of confidence in estimates of averages. Samples collected during July 1994 were combined into composite samples for each estuary, by mixing equal volumes of individual samples together. Samples collected at Childs River and Sage Lot Pond during subsequent months were kept as individuals so that we could assess within-site variability in $\delta^{15}N$ values. Sample sizes for composites and for averages from individual samples are noted in the figures and tables where data are presented.

Nitrate was isolated from groundwater samples in preparation for stable isotope analysis using the method developed by Sigman et al. (1997) for oceanic nitrate. Because this method was developed for seawater, we had to add NaCl (precombusted at 490°C for 4 h) to our groundwater samples prior to extracting nitrate. Nitrate extractions using this method involved boiling samples to reduce volume, converting nitrate to ammonium with Devarda's alloy, and collecting volatilized ammonia by gas-phase diffusion onto an acid trap. Boiling samples down to a small volume concentrated the nitrate, which aided nitrate reduction as well as recovery of ammonia during the extraction process. MgO was added during the boiling step to stabilize sample pH at 9.7. At this pH, NH_4^+ is converted to NH_3, which volatilizes from the sample. Thus, any ammonium in the sample, including that from the decomposition of labile organic N, was removed. After boiling, acid traps (acidified glass-fiber filter enclosed in a teflon membrane) and Devarda's alloy were added to the samples, and they were incubated until essentially 100% of the N was recovered. After incubation, acid traps were removed, dried, and stored individually in sealed vials until they were analyzed by mass spectrometry.

To ensure that N was not lost or gained during the extraction procedure, we compared the quantity of N measured by mass spectrometry to the quantity of nitrate originally in each sample. Mass balance calculations included a correction for a small blank associated with Devarda's alloy. Only extractions with N recovery between 95 and 105% were considered successful.

Ammonium was isolated from groundwater samples in preparation for stable isotope analysis using the method of Holmes et al. (1998). This method was developed for fresh, estuarine, and marine waters with low ammonium concentrations. Ammonium extractions using this method involved gas-phase diffusion onto an acid trap, as described for the final step of the nitrate extraction method above, but with volumes that varied according to the concentration of NH_4^+ in the sample. Volume was increased because samples could not be boiled down prior to diffusion (dissolved organic N breakdown during boiling contaminates the NH_4^+ pool). MgO was added to samples according to their volume, and samples were incubated and gently shaken at 40°C for 14 d. The proportion of ammonium recovered from groundwater samples after a 14-day incubation/shaking period at 40°C decreased with increasing diffusion volume, and the $\delta^{15}N$ value of NH_4^+ extracted from the samples decreased correspondingly. As discussed in Holmes et al. (1998), the regression equation that describeds this relationship was used to calculate the $\delta^{15}N$ of ammonium.

Primary producer collection and preparation—Macrophytes and suspended particulate organic matter (POM) were sampled from Childs River, Quashnet River, and Sage Lot Pond in November 1993, July 1994, May 1995, July 1995, and May 1996. We refer to suspended POM as a "producer" because it is made up largely of organic matter from producers and because the $\delta^{15}N$ of POM in the Waquoit Bay estuaries is primarily influenced by phytoplankton (Yelenik et al. 1996). POM was sampled by collecting 2-liter bottles of seawater (25–30 ppt salinity) from a depth of 0.5 m below the surface at three locations within each estuary. The POM was concentrated on an ashed Gelman A/E glass-fiber filter with a low-pressure vacuum pump. Benthic producers were collected individually from 15 locations within each estuary. After collection, samples were dried at 60°C, ground into a homogeneous powder (filters with POM were kept whole), and combined to make single composite samples of each species per estuary per sampling date.

Stable isotope analysis—Groundwater N samples were analyzed using a Europa Scientific 20/20 mass spectrometer, coupled to a Europa Scientific Roboprep element analyzer. Producer samples were analyzed using a Finnigan Delta-S isotope-ratio mass spectrometer, coupled to a Heraeus element analyzer. Precision of replicate analyses was ±0.3‰ for groundwater N samples and ±0.2‰ for producer samples.

Results and discussion

To examine the coupling between N inputs to coastal watersheds and the N found in primary producers living in nearshore waters, we first discuss the frequency distribution of nitrate and ammonium $\delta^{15}N$ values in groundwater from watersheds with low vs. high urbanization. We then calculate average $\delta^{15}N$ values for groundwater from different recharge areas in the Waquoit Bay watershed, and discuss how these $\delta^{15}N$ values change as the proportion of N contributed by wastewater to each recharge area changes. Finally, we calculate average $\delta^{15}N$ signatures for groundwater delivered to the Sage Lot Pond, Quashnet River, and Childs River estuaries of Waquoit Bay, and discuss how these groundwater $\delta^{15}N$ signatures relate to the $\delta^{15}N$ signatures of primary producers living within the estuaries.

Frequency distribution of nitrate and ammonium $\delta^{15}N$ values in groundwater about to enter Sage Lot Pond vs. Childs River—The $\delta^{15}N$ values for nitrate and ammonium in groundwater about to enter Sage Lot Pond range from −1.5‰ to +4.5‰ (Fig. 2). Because the watershed surrounding Sage Lot Pond is largely forested, this range of $\delta^{15}N$ values can be considered representative of uncontaminated groundwater. Nitrate and ammonium in rainwater typically have $\delta^{15}N$ values below zero (Hoering 1957; Moore 1977; Heaton 1987). However, losses and transformations of rainwater N (for example uptake by vegetation and denitrifica-

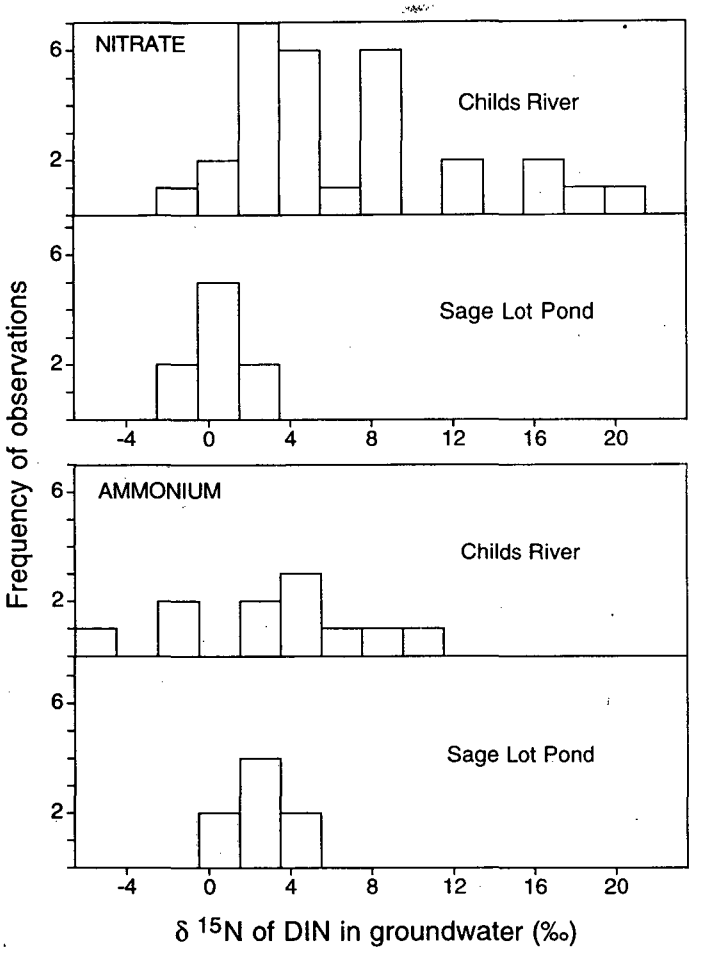

Fig. 2. Frequency distributions of nitrate and ammonium $\delta^{15}N$ in groundwater entering the Childs River and Sage Lot Pond estuaries of Waquoit Bay. Kolmogorov–Smirnov test of nitrate $\delta^{15}N$ in groundwater entering Childs River vs. Sage Lot Pond generates $P = 0.03$. Kolmogorov–Smirnov test of ammonium $\delta^{15}N$ in groundwater entering Childs River vs. Sage Lot Pond generates $P = 0.52$.

tion) as it passes through vegetation and soil cause the isotopic signature of rainwater N to become heavier by the time it reaches the aquifer (Macko and Ostrom 1994). Thus, the $\delta^{15}N$ values for nitrate and ammonium in groundwater about to enter Sage Lot Pond reflect the $\delta^{15}N$ of rainwater N plus any enrichment during transport across the watershed.

Relative to the range of $\delta^{15}N$ values for nitrate in groundwater about to enter Sage Lot Pond, much of the groundwater nitrate about to enter Childs River is enriched in ^{15}N (Fig. 2, top panel). This ^{15}N enrichment is indicative of wastewater inputs from septic systems. We arrive at this conclusion because nitrate derived from wastewater typically has a $\delta^{15}N$ of 10–20‰ (Kreitler et al. 1978; Kreitler and Browning 1983; Aravena et al. 1993), and because building density— and therefore the number of septic systems—is 16 times greater in the watershed surrounding Childs River than in the watershed surrounding Sag Lot Pond (Table 1). As wastewater contributions to the nitrate pool in groundwater increase, the $\delta^{15}N$ values of nitrate in groundwater become enriched.

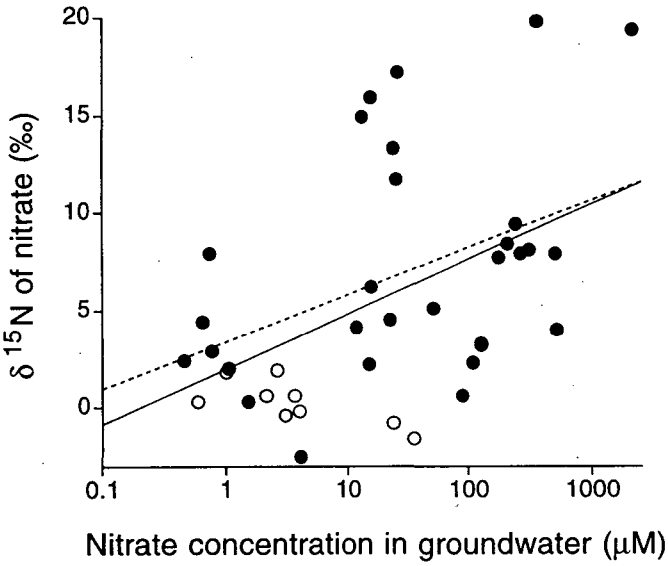

Fig. 3. $\delta^{15}N$ of nitrate vs. nitrate concentration in groundwater entering the Childs River (●) and Sage Lot Pond (○) estuaries of Waquoit Bay. Data are from individual samples collected along the seepage face of each estuary. Regression analysis using all of the data (——) generates $P = 0.002$, $r^2 = 0.23$. Regression analysis using Childs River data (– – –) alone generates $P = 0.03$, $r^2 = 0.18$.

In contrast to the $\delta^{15}N$ values of nitrate, no obvious unidirectional shift occurs in the distribution of ammonium $\delta^{15}N$ values about to enter Childs River, relative to the distribution of ammonium $\delta^{15}N$ values about to enter Sage Lot Pond (Fig. 2, bottom panel). The ammonium $\delta^{15}N$ values from Childs River do, however, cover a wider range than those from Sage Lot Pond. This could be related to differences in nutrient inputs between sites, but we suspect that the larger sample size at Childs River simply captured more natural variation in ammonium $\delta^{15}N$ values than at Sage Lot Pond. In any case, our comparison of ammonium $\delta^{15}N$ values in groundwater between sites suggests that increased wastewater inputs from septic tanks do not change the average $\delta^{15}N$ of ammonium in groundwater.

The $\delta^{15}N$ of nitrate is positively correlated with the concentration of nitrate in groundwater (Fig. 3). This correlation

Table 2. Nitrate and ammonium $\delta^{15}N$ estimates using concentration-weighted averages from individual samples, and using composite samples of groundwater entering the Childs River and Sage Lot Pond estuaries of Waquoit Bay.

	Groundwater $\delta^{15}N$ (‰)	
Watershed	Concentration-weighted average ± SE	Composite
Childs River (NO_3^-)	12.9±4.2 (n = 29)	10.8 (n = 16)
Sage Lot Pond (NO_3^-)	−0.9±0.5 (n = 9)	0.6 (n = 22)
Sage Lot Pond (NH_4^+)	3.2±1.0 (n = 8)	2.0 (n = 22)

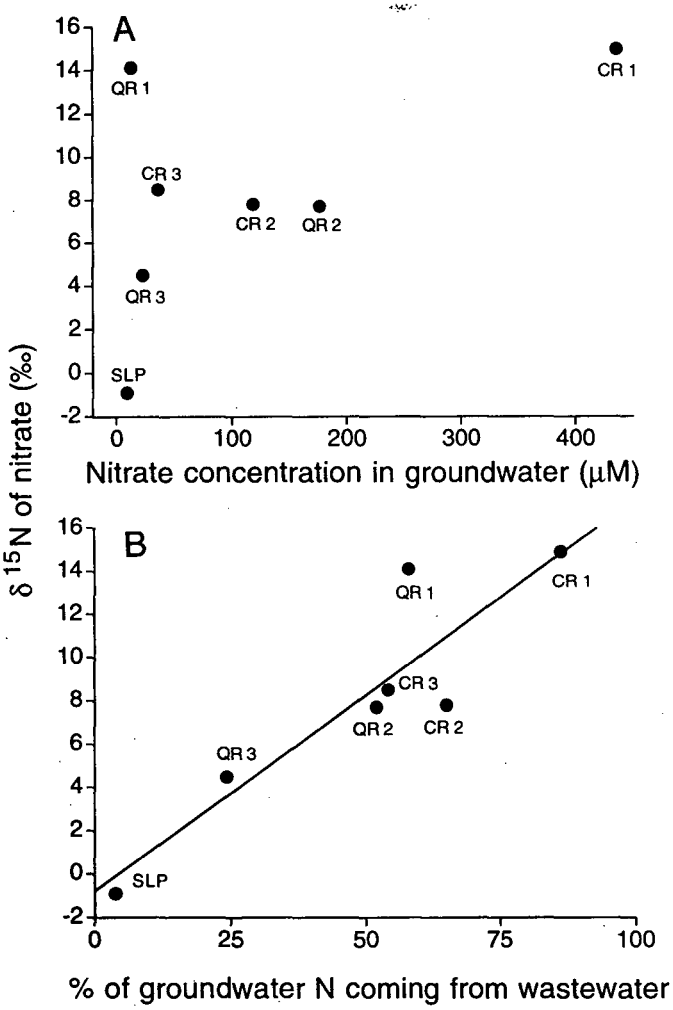

Fig. 4. Concentration of nitrate in groundwater (A) and percentage of groundwater N contributed by wastewater (B) vs. $\delta^{15}N$ of nitrate in groundwater about to enter the Childs River (CR), Quashnet River (QR), and Sage Lot Pond (SLP) estuaries of Waquoit Bay. The three points for Childs River and Quashnet River correspond to the three recharge areas within each of their watersheds (*see Fig. 1*). Wastewater contributions to groundwater were estimated using the Waquoit Bay Land Margin Ecosystems Research N loading model (Valiela et al. 1997*a*). Regression analysis of the data in the concentration vs. $\delta^{15}N$ graph generates $P = 0.20$, $r^2 = 0.31$. Regression analysis of the data in the percentage of wastewater vs. $\delta^{15}N$ graph generates $P = 0.005$, $r^2 = 0.82$.

is not surprising, given that nitrate loads from wastewater to groundwater are enriched in ^{15}N. However, a large amount of variability exists in the relationship between $\delta^{15}N$ and concentration of nitrate in groundwater—heavy $\delta^{15}N$ values can be found at relatively low nitrate concentrations, and light $\delta^{15}N$ values can be found at relatively high nitrate concentrations (Fig. 3). Heavy $\delta^{15}N$ values at low nitrate concentrations probably come from wastewater that has been diluted in the aquifer. Even after dilution, wastewater-derived nitrate can make up a large proportion of the nitrate pool in groundwater because background concentrations of nitrate are often <1 μM (McDonnell et al. 1994). Light $\delta^{15}N$ values at high nitrate concentrations may result from fertilizer inputs, which typically have $\delta^{15}N$ values between -2 and 2‰ (Kreitler 1979; Gormly and Spalding 1979).

Quantifying the relationship between wastewater inputs and $\delta^{15}N$ of nitrate in groundwater—To more closely examine the influence of wastewater N inputs on the $\delta^{15}N$ of nitrate in groundwater, we compare average concentrations of nitrate, and model estimates of the percentage of groundwater N coming from wastewater (Valiela et al. 1997*a*) to the $\delta^{15}N$ of nitrate in groundwater from three recharge areas within the Childs River watershed, three recharge areas within the Quashnet River watershed, and one recharge area within the Sage Lot Pond watershed (Fig. 1).

We estimated average $\delta^{15}N$ of NO_3^- about to enter the estuaries of Waquoit Bay from each recharge area using two different approaches. As a first approach, we multiplied the $\delta^{15}N$ value of each groundwater sample within a recharge area by the concentration of nitrate in the sample, and then calculated a concentration weighted average for the recharge area as a whole. This weighting procedure was necessary because of the general increase in $\delta^{15}N$ of nitrate with increasing nitrate concentration in groundwater, as discussed above (Fig. 3). The higher the nitrate concentration of a sample, the more N it contributes to the $\delta^{15}N$ signal of the nitrate pool within a recharge area. The second approach for estimating the average $\delta^{15}N$ of groundwater nitrate within a recharge area used composite samples. Composites were made by combining equal volumes of groundwater from each sample collected within a recharge area to make a single sample. Our two approaches for determining the average $\delta^{15}N$ of NO_3^- in groundwater give similar estimates (Table 2). We can therefore compare concentration-weighted averages from Sage Lot Pond and Childs River to composite values from Quashnet River.

The average $\delta^{15}N$ of nitrate in groundwater that enters the estuaries of Waquoit Bay from different recharge areas increases from -0.9‰ to $+14.9$‰ as the proportion of groundwater nitrogen contributed by wastewater increases from 4% to 86% (Fig. 4B). This relationship is evident among watersheds, as well as within them. Average $\delta^{15}N$ values of nitrate in groundwater are not well correlated with average concentrations of nitrate in different recharge area within the Waquoit Bay watershed (Fig. 4A). Thus, it is not the quantity of nitrate in groundwater, but an increase in the proportion of wastewater-derived nitrate contributing to the total N pool in groundwater that leads to increased $\delta^{15}N$ of nitrate delivered to estuaries.

The assumption that N source, rather than total quantity of N, determines nitrate $\delta^{15}N$ values in groundwater has been the basis for past studies using $\delta^{15}N$ to identify N sources to groundwater (e.g. Kreitler et al. 1978; Aravena et al. 1993). To the best of our knowledge, however, we are the first to quantitatively demonstrate that this assumption is valid. The generality of the relationship between the percentage of groundwater N coming from wastewater and $\delta^{15}N$ of nitrate in groundwater needs to be tested for other systems.

Estimating $\delta^{15}N$ values of nitrate, ammonium, and total DIN delivered to whole estuaries via groundwater flow—To arrive at estimates of the $\delta^{15}N$ values of nitrate and ammo-

Table 3. Nitrate and ammonium loads and associated $\delta^{15}N$ in groundwater entering the Childs River, Quashnet River, and Sage Lot Pond estuaries of Waquoit Bay. Inputs to Childs River and Quashnet River have been calculated for three separate recharge areas in their adjoining watersheds. N loads were calculated by multiplying annual recharge by average N concentration of groundwater samples collected along the shoreline associated with each recharge area. $\delta^{15}N$ data for each section are concentration-weighted averages ±1 SE. Quashnet River data are from composite samples. N load-weighted averages for each estuary as a whole are presented in bold type. Numbers in parentheses are sample sizes.

Site	NO_3^- load (kg N yr^{-1})	δ^{15} N of NO_3^- (‰)	NH_4^+ load (kg N yr^{-1})	$\delta^{15}N$ of NH_4^+ (‰)	$\delta^{15}N$ of DIN (‰)
Childs River					
Recharge area 1	1,842	14.9±7.6 (10)	44	3.1 (1)	
Recharge area 2	2,873	7.8±4.2 (10)	144	−3.5±1.3 (6)	
Recharge area 3	1,786	8.5±5.4 (9)	338	6.0±3.4 (4)	
N load-weighted average		**10.0**		**3.2**	**9.5**
Quashnet River					
Recharge area 1	30	14.1 (13)	4	—	
Recharge area 2	2,799	7.7 (11)	11	—	
Recharge area 3	4,062	4.5 (17)	343	—	
N load-weighted average		**5.8**			**~5.8**
Sage Lot Pond	85	−0.9±0.5 (9)	44	3.2±1.0 (8)	
N load-weighted average		**−0.9**		**3.2**	**0.5**

nium delivered to each of the Waquoit Bay estuaries as a whole via groundwater flow, we had to account for differences in the rate of N input from the different recharge areas adjoining each estuary (Table 3). The N loading rate from a recharge area depends on the volume of water passing through it, which is largely a function of catchmant surface area, and the concentration of N in the groundwater. The $\delta^{15}N$ signature of N delivered to an estuary was calculated by multiplying average $\delta^{15}N$ values of N from the different recharge areas adjoining the estuary (Table 3, $\delta^{15}N$ columns) by the N load specifically coming from each recharge area (Table 3, N load columns), then taking an average of these values (Table 3, N load-weighted average).

The $\delta^{15}N$ of nitrate delivered to the Waquoit Bay estuaries via groundwater differs among sites, whereas the $\delta^{15}N$ of ammonium remains constant (Table 3, N-load weighted average). Nitrate delivered to Childs River has an average $\delta^{15}N$ that is nearly 11‰ higher than the average $\delta^{15}N$ of nitrate delivered to Sage Lot Pond. The average $\delta^{15}N$ of nitrate delivered to Quashnet River falls between the $\delta^{15}N$ of nitrate delivered to the other two estuaries. This increase in the $\delta^{15}N$ of nitrate tracks an increase in the proportion of total N input contributed by wastewater to the estuaries (Table 1).

The $\delta^{15}N$ of the total DIN pool delivered to the Waquoit Bay estuaries increases as the $\delta^{15}N$ of nitrate in groundwater increases among sites (Table 3). However, $\delta^{15}N$ of the total DIN pool is also influenced by the relative contributions of nitrate and ammonium loading to the estuaries. At Childs River, where N loading is high, ammonium contributes only a small proportion to the total DIN load, so that the $\delta^{15}N$ of total DIN in groundwater is essentially the same as the $\delta^{15}N$ of nitrate alone (Table 3). Ammonium also makes up only a small proportion of the total N load at Quashnet River and, thus, cannot have a strong influence on the $\delta^{15}N$ of total DIN at this estuary. At Sage Lot Pond, however, 34% of the DIN load comes from ammonium, so that the $\delta^{15}N$ of DIN in groundwater actually reflects the influence of both nitrate and ammonium (Table 3).

Coupling groundwater N with $\delta^{15}N$ of producers in estuaries—The $\delta^{15}N$ values of producers in the Waquoit Bay estuaries become heavier as $\delta^{15}N$ values of DIN in groundwater delivered to the estuaries become heavier (Fig. 5). The producers examined include two rooted vascular plants (eelgrass, *Zostera marina,* and salt-marsh cordgrass, *Spartina alterniflora*), three species of macroalgae (*Enteromorpha* sp., *Gracilaria tikvahiae,* and *Cladophora vagabunda*), and suspended POM. The increase in $\delta^{15}N$ of all producers suggests that the N isotopic composition of groundwater delivered to estuaries has a pervasive influence on $\delta^{15}N$ of producers living within estuaries.

The magnitude of change in $\delta^{15}N$ of eelgrass is similar to the change in $\delta^{15}N$ of DIN in groundwater among estuaries (Fig. 5; compare the change in $\delta^{15}N$ of eelgrass to the slope of the 1 : 1 line). The simplest explanation for this relationship is that eelgrass takes up groundwater N directly as groundwater enters each of the estuaries. Eelgrass has been shown to take up groundwater N directly at other locations along the shoreline of Cape Cod (Maier and Pregnall 1990).

The lower $\delta^{15}N$ values of eelgrass relative to $\delta^{15}N$ values of groundwater N delivered to the estuaries (Fig. 5) can be explained by preferential use of ^{14}N over ^{15}N (fractionation) during uptake. Fractionation leads to lower $\delta^{15}N$ values in producers relative to their N source when excess N is available for uptake (Pennock et al. 1996). Eelgrass probably fractionates N similarly during uptake in all of the estuaries of Waquoit Bay, because light availability limits eelgrass growth, even in low-N environments (Zimmerman et al. 1987; Dennison and Alberte 1985; Duarte 1995).

The magnitude of change in $\delta^{15}N$ of cordgrass, macroalgae, and suspended POM is approximately one third the size of the change in eelgrass $\delta^{15}N$ among estuaries (Fig. 5). This

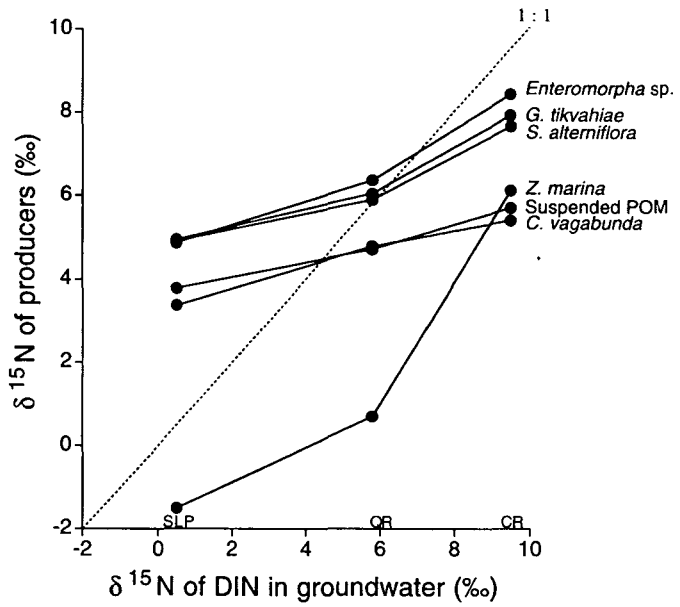

Fig. 5. $\delta^{15}N$ of DIN in groundwater delivered to Childs River, Quashnet River, and Sage Lot Pond vs. $\delta^{15}N$ of macroalgae (*Enteromorpha* sp., *Gracilaria tikvahiae,* and *Cladophora vagabunda*), salt-marsh cordgrass (*Spartina alterniflora*), eelgrass (*Zostera marina*), and suspended POM in these estuaries. Producer $\delta^{15}N$ was measured on composite samples collected November 1993, July 1994, May 1995, July 1995, and May 1996, and each point represents an average over these dates. Standard deviations of producer $\delta^{15}N$ averages are presented in Table 4.

may be due to increased fractionation of N by cordgrass, macroalgae, and phytoplankton with increased N availability. Unlike eelgrass, growth of cordgrass, macroalgae, and phytoplankton is N limited under natural conditions (for algae, *see* Duarte 1995; for cordgrass, *see* Valiela and Teal 1979). Primary producers have $^{15}N:^{14}N$ ratios that are similar to their source when N is in limited supply, but as more N becomes available, preferential use of ^{14}N over ^{15}N leads to producer $\delta^{15}N$ values that are lower than the $\delta^{15}N$ of their N source (Wada and Hattori 1978; Wada 1980; Mariotti et al. 1982; Pennock et al 1996).

The relatively small change in macroalgae, cordgrass, and POM $\delta^{15}N$ among estuaries (Fig. 5) may also be attributed to a shift from using predominantly recycled N to using predominantly new N as N loads increase. We define "recycled" N broadly to include N recycled within the estuaries, as well as any N delivered to the estuaries from Waquoit Bay proper during tidal exchange. We define "new" N as DIN delivered to the estuaries in groundwater that has not yet undergone transformations in estuarine waters. The original source of N to an estuary is largely from its surrounding watershed, but processes such as denitrification and burial of refractory organic matter leave the remaining N recycled within an estuary enriched in ^{15}N (Mariotti et al. 1984; Cifuentes et al 1989; Horrigan et al. 1990). Because recycled nitrogen is heavier than the new nitrogen from which it is derived, switching from recycled N to new N uptake would result in a smaller change in producer $\delta^{15}N$ than groundwater $\delta^{15}N$ among the estuaries of Waquoit Bay.

Table 4. Comparison of standard deviations of average $\delta^{15}N$ values for primary producers within estuaries to changes in average producer $\delta^{15}N$ values among estuaries. Standard deviations of averages reflect differences among composite samples collected during November 1993, July 1994, May 1995, July 1995, and April 1996 (SLP, Sage Lot Pond; QR, Quashnet River; CR, Childs River).

	SD of avg $\delta^{15}N$ within estuaries			Change in avg $\delta^{15}N$ among estuaries		
	SLP	QR	CR	QR–SLP	CR–QR	CR–SLP
POM	0.3	0.5	0.3	0.9	1.0	1.9
Enteromorpha sp.	0.1	0.3	0.2	1.5	2.1	3.6
Cladophora vagabunda	0.1	0.1	0.1	1.4	0.6	2.0
Gracilaria tikvahiae	0.4	0.3	0.4	1.0	1.8	2.8
Spartina alterniflora	0.6	0.1	0.1	1.1	1.9	3.0
Zostera marina	0.6	0.4	0.2	2.2	5.4	7.6

In practical terms, the relationships between groundwater $\delta^{15}N$ values and producer $\delta^{15}N$ values that we observe in the Waquoit Bay system suggest that monitoring of $\delta^{15}N$ values of estuarine producers may help coastal zone managers identify incipient eutrophication. The standard deviations of average producer $\delta^{15}N$ values within estuaries are small relative to differences in producer $\delta^{15}N$ values among estuaries (Table 4). *Cladophora vagabunda* has a particularly high signal-to-noise ratio, even though it shows the second smallest change of any of the producers among estuaries. This level of sensitivity may make it possible to detect increasing wastewater N in the tissues of estuarine producers before changes in production (as well as second-order effects) stimulated by increased N availability are measurable. To define the utility of $\delta^{15}N$ signatures in producers as indicators of incipient eutrophication, tests in other systems and, ultimately, development of a general model that can predict wastewater inputs based on the $\delta^{15}N$ signatures of producers will be necessary.

Conclusions

The consistent increase in producer $\delta^{15}N$ with increasing groundwater $\delta^{15}N$ in Waquoit Bay system suggests that the isotopic signature of N delivered to an estuary from its surrounding watershed has a pervasive influence on the nitrogen isotope signatures of producers living in the estuary. More specifically, our stable N isotope data demonstrate the close coupling between urbanization in coastal watersheds and N use by primary producers in nearshore waters. As urban land use in the Waquoit Bay watershed increases, and therefore the number of septic systems increases, the $\delta^{15}N$ of groundwater flowing into the estuaries of Waquoit Bay increases. Producers within the estuaries appear to integrate the stable N isotope signatures from groundwater, which vary considerably along the shoreline, to create a composite record that reflects the proportion of N delivered to the estuary from wastewater inputs.

The relationships between producer $\delta^{15}N$ and groundwater $\delta^{15}N$ within the Waquoit Bay system fall into two categories. Changes in the $\delta^{15}N$ values of eelgrass parallel changes in

the $\delta^{15}N$ of groundwater among estuaries. Changes in the $\delta^{15}N$ values of macroalgae, cordgrass, and suspended POM are approximately one-third as large as changes in groundwater $\delta^{15}N$ among estuaries. This relatively small change suggests that, in addition to the isotopic signature of groundwater N, the $\delta^{15}N$ values of macroalgae, cordgrass, and suspended POM also record processes going on in estuaries between the time groundwater N enters estuaries and the time it is incorporated into the tissues of primary producers.

The ability to identify the effects of increasing wastewater N loads on the $\delta^{15}N$ of estuarine producers may allow coastal zone managers to reliably identify incipient eutrophication, before changes in population and community indicators that are now commonly used to identify eutrophication are measurable.

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