Limnol. Oceanogr., 42(5), 1997, 930–937


# Nitrogen-stable isotope signatures in estuarine food webs: A record of increasing urbanization in coastal watersheds

*James W. McClelland and Ivan Valiela*
Boston University Marine Program, Marine Biological Laboratory, Woods Hole, Massachusetts 02543

*Robert H. Michener*
Department of Biology, Boston University, 5 Cummington Street, Boston, Massachusetts 02215

*Abstract*

Nutrient enrichment as a result of anthropogenic activity concentrated along the land–sea margin is increasing eutrophication of near-shore waters across the globe. Management of eutrophication in the coastal zone has been hampered by the lack of a direct method to trace nitrogen sources from land into coastal food webs. Stable isotope data from a series of estuaries receiving nitrogen loads from 2 to 467 kg N $ha^{-1}$ $yr^{-1}$ from the Waquoit Bay watershed, Cape Cod, Massachusetts, indicate that producer and consumer $^{15}N$-to-$^{14}N$ ratios record increases in wastewater nitrogen inputs. Nitrate from groundwater-borne wastewater introduces a $^{15}N$-enriched tracer to estuaries. This study explicitly links anthropogenically derived nitrogen from watersheds to nitrogen in estuarine plants and animals, and suggests that wastewater nitrogen may be detectable in estuarine biota at relatively low loading rates, before eutrophication leads to major changes in species composition and abundance within estuarine food webs.

Eutrophication caused by increased anthropogenic nitrogen inputs is a major mechanism altering coastal habitats worldwide (Sand-Jensen and Borum 1991; Duarte 1995; GESAMP 1990; NAS 1994). A variety of indexes have been developed to quantify the extent of eutrophication brought about by N loading (Schmitt and Osenberg 1995). Most of these indexes make use of taxonomic shifts and changes in abundance of producers and consumers resulting from eutrophication. Such indicators, although useful, provide an a posteriori assessment of eutrophication; they depend on changes that have already taken place in the biota. Restoration of habitats already altered by eutrophication is difficult, so methods that detect increases in nutrient loads while they are still relatively low would be welcome tools for coastal habitat management. In addition, identification of N sources would make management responses more effective. The use of N stable isotope ratios to track anthropogenic N directly into estuarine food webs may, for the first time, provide a method to detect incipient eutrophication, as well as identify the responsible source.

The stable isotopes $^{14}N$ and $^{15}N$ occur overall on earth in a fixed proportion of approximately 273 $^{14}N$ atoms for each $^{15}N$ atom, while the ratio of $^{15}N$ to $^{14}N$ differs among specific N pools in the environment (Peterson and Fry 1987). Although the absolute magnitude of natural variation in N-stable isotope ratios in the environment is small, N isotopic signatures from different pools are often quit distinct, making N sources identifiable and traceable within an ecosystem. N-stable isotope data are typically normalized relative to the $^{15}N/^{14}N$ of atmospheric $N_2$ as $\delta^{15}N$ (‰) $= [(R_{sample}/R_{atm}) - 1] \times 10^3$ (Peterson and Fry 1987).

In areas with sandy unconsolidated aquifers such as Cape Cod, Massachusetts, watershed sources of N are delivered to estuaries almost exclusively via groundwater flow (Valiela et al. 1992). Groundwater studies have used natural abundance stable isotope signatures of $NO_3^-$ to help identify the major sources of N (wastewater, fertilizer, and atmospheric deposition) to aquifers (Kreitler et al. 1978; Gormly and Spalding 1979; Kreitler 1979; Aravena et al. 1993). Groundwater influenced only by atmospheric deposition typically bears $NO_3^-$-$\delta^{15}N$ values ranging from +2 to +8‰, while $NO_3^-$ derived from human and animal wastes is more enriched in $^{15}N$ (+10 to +20‰), and nitrate from synthetic fertilizers is more depleted in $^{15}N$ (−3 to +3‰). Elevated $\delta^{15}N$ values in groundwater generated from human and animal wastes are attributed to volatilization of $^{14}N$-rich ammonia during early stages of wastewater degradation, as well as microbial processes acting on wastewater N before it reaches the aquifer (Macko and Ostrom 1994). The low $\delta^{15}N$ values associated with synthetic fertilizers are due to the conversion of atmospheric $N_2$ during manufacturing (Freyer and Aly 1974; Gormly and Spalding 1979).

Physical and biological processes that fractionate N can make it difficult to precisely quantify N contributions from different sources (Hauck et al. 1972; Bremner and Tabatabai 1973; Hauck 1973; Mariotti et al. 1988), particularly for studies that rely only on absolute stable isotope values to assess source contributions. Comparisons of $\delta^{15}N$ values in groundwater from aquifers with potential anthropogenic N loads to groundwater $\delta^{15}N$ from relatively pristine locations have, however, produced valuable information on source contributions (Kreitler et al. 1978; Gormly and Spalding 1979; Kreitler 1979). With this comparative approach, dif-

*Acknowledgments*

Grants from the NSF Land Margin Ecosystems Research, EPA Region 1, NOAA Sanctuaries and Reserves, NOAA Coastal Oceans, and WHOI Sea Grant programs supported this work. We thank staff of the Waquoit Bay National Estuarine Research Reserve and L. Soucy for technical support, B. Peterson and A. Giblin for their comments on earlier versions of this manuscript, and B. Lapointe and B. Fry for critical review of this manuscript. We are grateful to Trish and David Palmer for providing graduate student support through the Waquoit Bay Fellowship.

ferences in $\delta^{15}N$ values between sampling locations, in addition to absolute $\delta^{15}N$ values in groundwater, can be used to assess N contributions from different sources.

Food web studies have used stable isotope ratios to study the flow of N through marine communities (Peterson et al. 1985; Sholto-Douglas et al. 1991; Hobson and Welch 1992; Wainright et al. 1993). Primary producers have distinctive N stable isotope signatures that reflect the $\delta^{15}N$ of their inorganic N sources plus a variable amount of fractionation (differential use $^{15}N$ vs. $^{14}N$) during N uptake (Fogel and Cifuentes 1993; Lajtha and Marshall 1994). The amount of fractionation that takes place depends on the concentration of N available (Pennock et al. 1996) and the enzymatic processes acting during N assimilation (Wada and Hattori 1978; Wada 1980; Mariotti et al. 1982). Fractionation is small or absent when dissolved inorganic nitrogen (DIN) uptake is the rate-limiting step (Wada and Hattori 1978; Wada 1980; Mariotti et al. 1982; Pennock et al. 1996). Consumers typically show a 2–4‰ increase in $\delta^{15}N$ relative to their food source (Minagawa and Wada 1984), a fractionation caused by kinetic differences between light and heavy isotopes during metabolism.

The largest single source of N to many coastal regions is freshwater-borne wastewater (Cole et al. 1993). When conveyed by groundwater, this N exists largely as $NO_3^-$ (Valiela et al. 1992). Increased $NO_3^-$ contributions to coastal aquifers as a result of wastewater loading should elevate the overall N stable isotope signature of groundwater entering estuaries. In this paper we present evidence that groundwater N with an elevated $\delta^{15}N$ signature as a result of wastewater input is directly identifiable in estuarine producers, and that this signal is passed on to consumers throughout the estuarine food web. This study marks the first time that sources of N from watersheds have been linked to N in food webs of adjoining estuaries using stable isotope signatures, and suggests that wastewater N may be detectable in estuarine biota before major changes in species composition and abundance resulting from eutrophication take place.

## Methods

*Cross-estuary comparisons as a space-for-time substitution*—To define the relationship between major sources of N from watersheds and N stable isotope signatures in estuarine biota, we measured $^{15}N/^{14}N$ in producers and consumers from a set of five estuaries that enter Waquoit Bay, Massachusetts (Fig. 1). Each estuary is associated with a distinct subwatershed. The three major sources of N loading to Waquoit Bay are atmospheric deposition, wastewater, and fertilizer (Valiela et al. 1992, 1997). The total amount of N contributed by atmospheric deposition to each estuary is to a great extent determined by the size of the subwatershed associated with each estuary (Table 1, compare 1st and 3rd columns). The relative amounts of N contributed to each estuary by atmospheric deposition, wastewater, and fertilizer (Table 1, last three columns), however, are strongly influenced by land use (Valiela et al. 1997). As urbanization increases across watersheds, there is a shift from N inputs dominated by atmospheric deposition to N inputs increasingly contributed

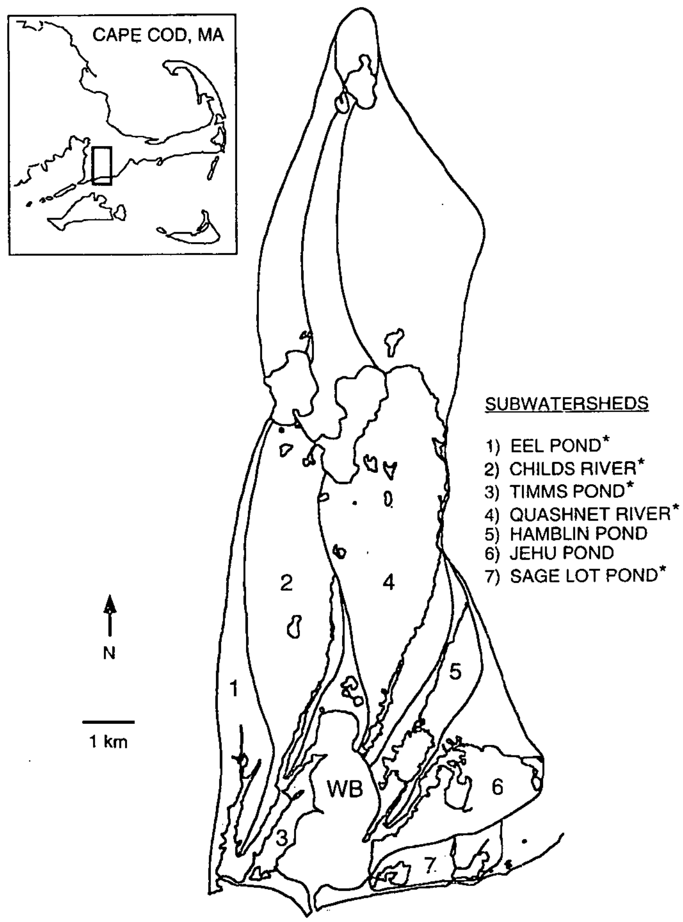


Fig. 1. Watershed of Waquoit Bay (WB), Massachusetts, with delineated subwatersheds and associated estuaries. Asterisks mark subwatersheds associated with estuaries where biota was sampled for stable isotope analysis.

by wastewater and to a lesser extent by fertilizer use (Table 1). Sham et al. (1995) made back-cast model predictions that show that N inputs from wastewater have been increasing since the 1930s, with accelerated N loads related to more intense urban development in the 1960s and 1970s.

The Quashnet River provides an example of an estuary with a high total N load but with a relatively low proportion of its N coming from wastewater (Table 1). This is because atmospheric deposition makes an exceptionally large contribution to the total N load at Quashnet River (because of the size of its subwatershed), not because wastewater inputs are particularly low. In fact, the Quashnet River receives the second highest wastewater input of all the sites (Table 1, 5th column).

Nitrogen loading rates to the different estuaries of the Waquoit Bay system range from 2 to 467 kg N $ha^{-1}$ $yr^{-1}$ (Table 1). This range of N loading rates spans the lower 10% of the range of N loading rates compiled by Nixon (1992) for marine ecosystems worldwide. Two thirds of the systems included in this compilation by Nixon (1992), however, have N loading rates that fall within the range of loading rates received by the different estuaries of Waquoit Bay. Thus, the N loads received by the different estuaries of Waquoit Bay

Table 1. Nitrogen loading rates and relative contributions to total N load by atmospheric deposition (atm.), wastewater, and fertilizer to five estuaries adjoining separate subwatersheds within the Waquoit Bay watershed. Loading rates were calculated using the Waquoit Bay LMER nitrogen loading model (Valiela et al. 1997).

| | | N load per estuary area ($kg\ N\ ha^{-1}\ yr^{-1}$) | | | | Percent of total N load | | |
|---|---|---|---|---|---|---|---|---|
| Estuary | Subwatershed area (ha) | Total | Atm. | Fertilizer | Wastewater | Atm. | Fertilizer | Waste-water |
| Timms Pond | 85 | 2 | 2 | 0 | 0 | 100 | 0 | 0 |
| Sage Lot Pond | 119 | 9 | 7.3 | 0.3 | 1.4 | 81 | 3 | 16 |
| Eel Pond | 356 | 88 | 21.1 | 9.7 | 57.2 | 24 | 11 | 65 |
| Quashnet River | 2,084 | 390 | 175.5 | 101.4 | 109.2 | 45 | 26 | 28 |
| Childs River | 875 | 467 | 121.4 | 56.0 | 289.5 | 26 | 12 | 62 |

are typical of many marine systems but are substantially lower than the N loads received by some systems around the world. Comparisons of different estuaries within the Waquoit Bay system serve as a surrogate to follow effects of increasing nutrient enrichment over time on estuarine ecosystems. This space-for-time substitution (Pickett 1989) allows us to examine N stable isotope ratios in estuarine biota exposed to increasing N loads from groundwater, while N loads are still relatively low (as viewed from a worldwide perspective).

*Nitrogen loading calculation*—Loading rates of total dissolved N to estuaries were calculated using the Waquoit Bay Land Margin Ecosystems Research nitrogen loading model (NLM) (Valiela et al. 1997), which is based on N inputs to the specific land-use mosaics on each watershed. NLM considers N inputs via wet and dry atmospheric deposition, use of fertilizer, and wastewater disposal, and estimates losses of N from each of these sources as the N moves through vegetation, soil, vadose zone, and aquifer. The model considers total N transport, including inorganic and organic components. The modeled N loading values have standard errors of 12–14% and standard deviations of 37–38% of each loading value (Valiela et al. 1997). These estimates of uncertainty in N loading values generated by NLM were determined by error propagation methods (Meyer 1975) as well as by a bootstrap calculation (Efron and Tibshirani 1991). The two different approaches gave standard error estimates that differ by only 2% and standard deviation estimates that differ by only 1%.

*Sample collection*—Biota from the Waquoit Bay estuaries were sampled for stable isotope analysis in November 1993, July 1994, May 1995, and July 1995. Regions sampled within each estuary were characterized by salinities of 25–30‰. Eelgrass (*Zostera marina*) and the macroalga *Gracilaria tikvahiae* were sampled from Timms Pond, Sage Lot Pond, Quashnet River, Childs River, and Eel Pond (Fig. 1). Two other species of macroalgae (*Cladophora vagabunda,* and *Enteromorpha* sp.), plankton (suspended particulate organic matter), and salt marsh cordgrass (*Spartina alterniflora*) were sampled from Sage Lot Pond, Quashnet River, and Childs River. Consumers were sampled from Childs River and Sage Lot Pond.

Plankton was sampled by collecting 2-liter bottles of seawater from a depth of 0.5 m below the surface at three locations within each estuary. Benthic primary producers and invertebrates were sampled from 15 locations within each estuary, and cordgrass was sampled from 15 locations along the saltmarsh bank at the water's edge. Primary producers were collected by hand, and invertebrates were collected with an Ekman dredge. Dredge contents were rinsed through a 1-mm sieve, and invertebrate species retained on the sieve were sorted by species. Fish were collected by seine.

*Sample preparation*—Plankton were removed from seawater samples onto an ashed Gelman A/E glass fiber filter with a low-pressure vacuum pump. Animals were held in filtered seawater for 24 h to allow their guts to clear. All samples were dried at 60°C, ground into a homogeneous powder (excepting plankton on filters), and combined to make single composite samples of each species per estuary per sampling date. Separate composites consisting of 15–250 individuals (depending on the size of the species collected) were prepared for stable isotope analysis from each estuary for each season. Replicate composites from a single sampling date were also prepared for eelgrass and *G. tikvahiae.* Whole organisms were used in all cases except for *Mya arenaria,* for which the shell was removed.

*Nitrogen stable isotope analysis*—Samples were analyzed in the Boston University Stable Isotope Laboratory using a Finnigan Delta-S isotope ratio mass spectrometer. All samples but filters were weighed and loaded into tin capsules and combusted in a Heraeus element analyzer. The resulting combustion gasses were cryogenically separated and purified in a Finnigan CT-CN trapping box before introduction into the mass spectrometer. Filters were acidified with $PtCl_2$, dried, then combusted using the Dumas combustion technique. Combustion gasses were again separated and purified before analysis (Lajtha and Michener 1994). Precision of replicate analyses was ±0.2‰.

## Results and discussion

We first examine the relationship between N load and $\delta^{15}N$, and the potential roles of the three major sources of N entering Waquoit Bay in determining stable isotope values in eelgrass and *G. tikvahiae.* We then compare and contrast the change in N stable isotope values of a variety of different

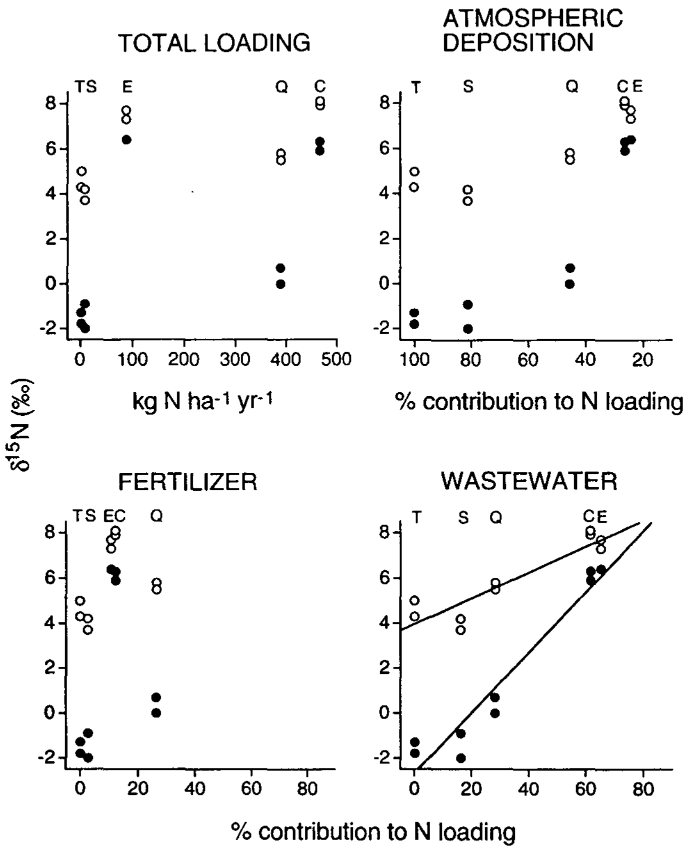


Fig. 2. Total nitrogen loading and percent contribution to N loading by atmospheric deposition, fertilizer, and wastewater to the Timms Pond (T), Sage Lot Pond (S), Eel Pond (E), Quashnet River (Q), and Childs River (C) estuaries of Waquoit Bay versus $\delta^{15}$N in eelgrass, *Zostera marina* (●), and the macroalgae *Gracilaria tikvahiae* (○) in these estuaries. Eelgrass and *G. tikvahiae* were sampled from 15 different sites within each estuary, and each plotted point represents a composite of 15 samples. Two separate composites were made from each estuary, except for eelgrass from Eel Pond, for which only a single composite was made. Eelgrass was collected May 1995, and *G. tikvahiae* was collected July 1995. Linear regression analysis of the data in the total loading vs. $\delta^{15}$N graph generates $P = 0.09$, $r^2 = 0.36$ for eelgrass and $P = 0.06$, $r^2 = 0.37$ for *G. tikvahiae.* Linear regression analysis of the data in the percent wastewater vs. $\delta^{15}$N graph generates $P = 0.0001$, $r^2 = 0.93$ for eelgrass and $P = 0.0001$, $r^2 = 0.87$ for *G. tikvahiae.*

producers across estuaries and propose a general response of $\delta^{15}$N in producers to wastewater loading. Lastly, we demonstrate that interestuary differences in the $\delta^{15}$N of producer are maintained in consumers throughout the food webs of different estuaries.

*Change in $\delta^{15}N$ of eelgrass and* G. tikvahiae *across estuaries*—Eelgrass and *G. tikvahiae* $\delta^{15}$N values increase with total N loading across five estuaries of Waquoit Bay (Fig. 2, top left), but there is considerable variability in this relationship. This variability indicates that factors other than total N loading are primarily responsible for the differences in $\delta^{15}$N of eelgrass and *G. tikvahiae* among estuaries. Because N from atmospheric deposition, wastewater, and fertilizer imparts different $\delta^{15}$N signatures to groundwater, differences in $\delta^{15}$N of eelgrass and *G. tikvahiae* among estuaries may be due to variation in the mix of these N sources in groundwater entering the different estuaries (Table 1).

To distinguish the relative influences of atmospheric deposition, wastewater, and fertilizer N entering Waquoit Bay on the $\delta^{15}$N signatures of eelgrass and *G. tikvahiae,* we plot $\delta^{15}$N vs. percent N contributed by each nitrogen source to the five estuaries (Fig. 2). As atmospheric contributions to total N loading decline, $\delta^{15}$N values for eelgrass and *G. tikvahiae* increase (Fig. 2, top right). The decreasing percent contribution of atmospheric deposition N across estuaries, however, is not likely to be the direct cause of changes in $\delta^{15}$N in eelgrass and *G. tikvahiae* but rather reflects increases in fertilizer and wastewater contributions as urbanization increases. There is no trend evident in eelgrass or *G. tikvahiae* stable isotope values in relation to percent loading by fertilizer (Fig. 2, bottom left). In contrast, the proportion of wastewater contributions to total loading is well correlated with N stable isotope values in eelgrass and *G. tikvahiae* (Fig. 2, bottom right).

Even though N stable isotope ratios in eelgrass and *G. tikvahiae* increase linearly as wastewater contributions to total N loading increase, the $\delta^{15}$N values for these two producers do not change as wastewater inputs increase initially from 0 to 16% between estuaries (Fig. 2, bottom left). The lack of change in $\delta^{15}$N of eelgrass and *G. tikvahiae* as the proportion of N loading by wastewater increases from 0 to 16% may be attributed to the low total N loads received by both of these estuaries (Fig. 2, top left, eight points along y-axis).

After N loading reaches 90 kg N ha$^{-1}$ yr$^{-1}$ $\delta^{15}$N, values in eelgrass and *G. tikvahiae* are elevated (Fig. 2, top left). There seems to be a threshold between 0 and 90 kg N ha$^{-1}$ yr$^{-1}$ where N stable isotope signatures delivered by groundwater to estuaries become detectable in the primary producers. There should also be an upper threshold of N loading where $\delta^{15}$N values of estuarine primary producers begin to decrease due to fractionation because N is no longer a limiting nutrient. Such fractionation has been demonstrated in phytoplankton under N-replete conditions (Pennock et al. 1996). Increasing phytoplankton and macroalgal production in Waquoit Bay with increasing N loads (Valiela et al. 1992; Lyons et al. 1995; Callaway et al. 1995; Hersh 1996) has maintained water column concentrations of DIN at relatively low levels (Table 2), so that N availability continues to limit primary producer growth rates (Peckol et al. 1994). Even in Childs River, the site with the greatest total N load, batch fertilization experiments indicate N limitation of phytoplankton growth in waters of 20‰ and greater salinity (Tomasky and Valiela 1995).

*Comparison of changes in $\delta^{15}N$ of plankton, macroalgae, eelgrass, and cordgrass across estuaries*—$\delta^{15}$N signatures of plankton, the macroalgae species *Cladophora vagabunda,* and *Enteromorpha* sp., and cordgrass from three estuaries entering Waquoit Bay increase with greater proportions of wastewater loading (Fig. 3) as seen in eelgrass and *G. tikvahiae* from five estuaries within the Waquoit Bay system (Fig. 2). The mix of organic matter contributing to plankton

Table 2. Water column nitrate and ammonium concentrations (annual average ± SD) for the reaches under investigation in the present study at Childs River, Quashnet River, and Sage Lot Pond. Data are from WBLMER data base 1991–1996. Concentrations were determined using a Lachat Instruments Quik Chem AE automated ion analyzer.

| Estuary | Concn ($\mu$M) | |
|---|---|---|
| | $NO_3^-$ | $NH_4^+$ |
| Childs River | 2.0±2.9 | 1.5±2.6 |
| Quashnet River | 0.7±1.1 | 1.1±1.5 |
| Sage Lot Pond | 0.4±1.1 | 1.5±2.1 |

differs among the estuaries of Waquoit Bay, but the $\delta^{15}N$ of plankton is predominantly influenced by phytoplankton at all of the estuaries (Yelenik et al. 1996). Plankton and macroalgae show similar responses in $\delta^{15}N$ relative to increased percent wastewater loading. The change in $\delta^{15}N$ of plankton, however, is less pronounced than the change in $\delta^{15}N$ of the other algal species across estuaries (Fig. 3). This is likely due to mixing of plankton from different estuaries within Waquoit Bay proper during tidal exchange.

The change in $\delta^{15}N$ of eelgrass recorded through the range of wastewater N inputs to the estuaries of Waquoit Bay is nearly three times larger than that observed for the other producers (Fig. 4). A switch from sediment to water column N uptake by eelgrass (a rooted vascular plant) with increasing water column N availability (Izumi and Hattori 1982; Short and McRoy 1984) may account for the unique response of eelgrass to increased N loads from wastewater. The low $\delta^{15}N$ signature of eelgrass relative to macroalgae and plankton at Sage Lot Pond (Fig. 4) suggests that eelgrass obtains most of its N from sediments, while macroalgae and plankton use water column N in this estuary. In contrast, the $\delta^{15}N$ of eelgrass in Childs River has an elevated $\delta^{15}N$ value that lies within the range of $\delta^{15}N$ values observed for macroalgae and plankton (Fig. 3). The similar $\delta^{15}N$ signatures of eelgrass, macroalgae and plankton at Childs River suggest that they all take up the majority of their N from the water column in this estuary. It is also possible, however, that the $\delta^{15}N$ of the water column and sediment N pools at Childs River have become indistinguishable, and that eelgrass still primarily relies on uptake of N through roots as it does under low N loading conditions. Measurement of $\delta^{15}N$ in porewater and water column DIN will help elucidate the mechanism by which N loads from groundwater-borne wastewater influence $\delta^{15}N$ in eelgrass.

Though salt marsh cordgrass is a rooted vascular plant as is eelgrass, it shows an increase in $\delta^{15}N$ across estuaries similar to that observed for macroalgae and plankton (Fig. 4). It is unlikely that cordgrass relies heavily on direct uptake of N from the water column as is the case for plankton and macroalgae, but increases in water column $\delta^{15}N$ as a result of increased N loads from groundwater-borne wastewater may influence the $\delta^{15}N$ of the DIN pool in salt marsh bank sediments. Groundwater may also influence the $\delta^{15}N$ of the DIN pool in salt marsh sediments directly, as salt marshes are interposed between land and the open water of estuaries.

We did not detect substantial changes in $\delta^{15}N$ of producers between summer and fall in the Waquoit Bay estuaries (Table 3). This finding is contrary to seasonal shifts in phytoplankton N stable isotope values of 3–6‰ between summer and fall that have been demonstrated in some freshwater

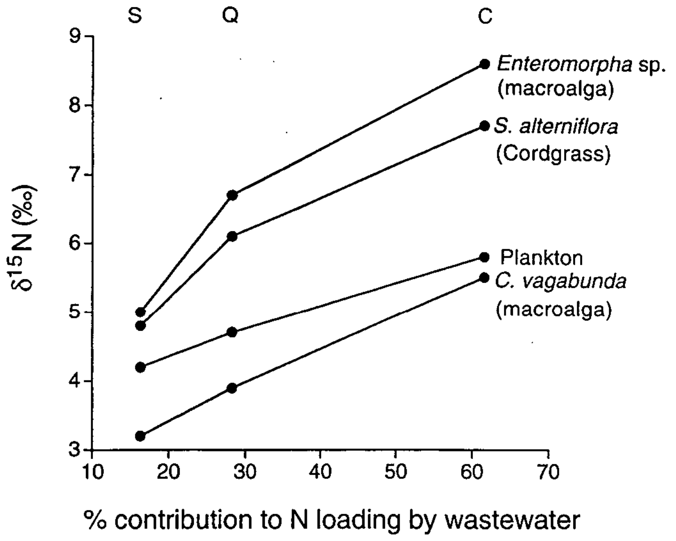


Fig. 3. Percent contribution to nitrogen loading by wastewater to the Sage Lot Pond (S), Quashnet River (Q), and Childs River (C) estuaries of Waquoit Bay versus $\delta^{15}N$ in various primary producers from the estuaries. Samples were collected during July 1994 from 15 different sites within each estuary, and each plotted point represents a composite of 15 samples. All *Spartina alterniflora* samples were collected from banks adjacent to the open water of the estuaries.

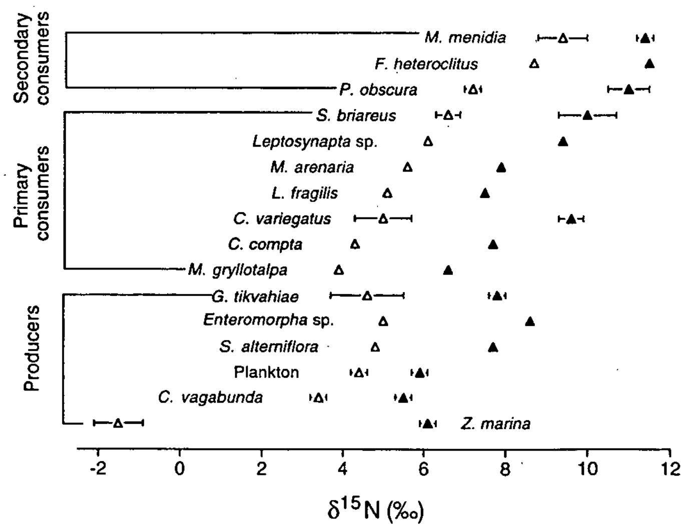


Fig. 4. Nitrogen stable isotope signatures of producers and consumers from the Sage Lot Pond (16% wastewater, △) and Childs River (61% wastewater, ▲) estuaries of Waquoit Bay. Data without error bars are from composite samples (15–250 individuals) taken during July 1994. Average ± standard error are presented for biota sampled during multiple seasons. Common names of consumers from top to bottom are: silverside, mummichog, polychaete worm, sea cucumber, sea cucumber, soft shell clam, polychaete worm, sheepshead minnow, amphipod, and amphipod.

Table 3. Comparison of $\delta^{15}N$ (‰) in plankton, two macroalgal species (*Cladophora vagabunda* and *Gracilaria tikvahiae*), and eelgrass (*Zostera marina*) collected in fall 1993 and summer 1994 from the Childs River and Sage Lot Pond estuaries of Waquoit Bay. Plankton values are from whole seawater filtrate, and macrophyte values are from composites of 15 samples.

| | Childs River $\delta^{15}N$ | | Sage Lot Pond $\delta^{15}N$ | |
|---|---|---|---|---|
| Producer | November 1993 | July 1994 | November 1993 | July 1994 |
| Plankton | 5.9 | 5.8 | 4.5 | 4.2 |
| *C. vagabunda* | 5.4 | 5.5 | 3.5 | 3.2 |
| *G. tikvahiae* | 7.9 | 7.7 | 6.7 | 5.4 |
| *Z. marina* | — | — | 1.3 | 0.3 |

(Yoshioka et al. 1994) and marine systems (Wainright and Fry 1994). These seasonal shifts have been attributed to changes in fractionation during DIN uptake by phytoplankton with changes in N availability (Pennock et al. 1996) or changes in the $\delta^{15}N$ signature of the DIN pool between summer and fall (Horrigan et al. 1990; Mariotti et al. 1984). It may be that relatively constant N inputs from groundwater to Waquoit Bay (Valiela et al. 1992) and low water column nitrogen concentrations as a result of N uptake by macroalgae throughout the year (Peckol et al. 1994; Hersh 1996) promote consistency in $\delta^{15}N$ of producers in Waquoit Bay between summer and fall.

*General response of N stable isotopes signatures in primary producer to wastewater loading*—The increase in N stable isotope ratios in all primary producers across estuaries suggests that wastewater, with its distinctly high $NO_3^-$-$\delta^{15}N$ signature, elevates the overall $\delta^{15}N$ signature of groundwater entering the estuaries as urbanization within the Waquoit Bay watershed increases. Although atmospheric deposition and fertilizer N also must influence groundwater stable isotope signatures in Waquoit Bay to some degree, increases in wastewater loading appear to drive the change in $\delta^{15}N$ among estuaries.

Primary producer $\delta^{15}N$ signatures record changes in wastewater inputs to Waquoit Bay even though nitrification, denitrification, microbial remineralization, and algal uptake fractionate N in estuaries (Mariotti et al. 1984; Cifuentes et al. 1989; Horrigan et al. 1990). The differences in $\delta^{15}N$ among producer species within individual estuaries of Waquoit Bay indicate different amounts of fractionation during N uptake and assimilation, or use of N generated by different transformations within an estuary. The strong correlation between percent wastewater loading and $\delta^{15}N$ in producers, and the lack of a strong correlation between total N load and producer $\delta^{15}N$, however, suggest that changes in $\delta^{15}N$ in producers across estuaries are primarily influenced by differences in the groundwater source signal entering each estuary and not due to differences in the processes at work within each estuary as a result of increased total N loads.

*Wastewater nitrogen in estuarine consumers*—The increase in $\delta^{15}N$ signatures of primary producers with increasing wastewater loading to the Waquoit Bay estuaries is passed on to consumers in the food webs of these estuaries; consumers from Childs River all have higher $\delta^{15}N$ signatures than their counterparts in Sage Lot Pond (Fig. 4). There are no discernible correlations between feeding mode and the magnitude of difference in $\delta^{15}N$ signatures between estuaries. The average difference in $\delta^{15}N$ between Childs River and Sage Lot Pond consumers is 3.0 ± 0.3‰. This difference is similar to the average difference of 3.4 ± 0.9‰ observed for primary producers, suggesting that the $\delta^{15}N$ signal imparted by wastewater to primary producers is maintained as the N moves through the estuarine food web.

The major pathway of groundwater N into the upper levels of the food webs in Waquoit Bay is most likely through consumption of plankton and macroalgae, and not through consumption of eelgrass. Nutrient loading has resulted in nearly complete loss of eelgrass from Childs River (Lyons et al. 1995), so eelgrass cannot make a significant contribution to the food web of this estuary. Even in Sage Lot Pond, however, where eelgrass is abundant, the N stable isotope data suggest that eelgrass makes little or no contribution to the diet of consumers. If eelgrass made a major dietary contribution to consumers at Sage Lot Pond, we would see a difference in $\delta^{15}N$ approaching 8‰ between Sage Lot Pond and Childs River consumers, reflecting the loss of eelgrass as a dietary source. In fact, consumer $\delta^{15}N$ increases by only 2–5‰ between these two estuaries, a shift similar to the 2–4‰ increase found for plankton and macroalgae (Fig. 4).

A stable isotope study using C and N together to investigate the relationship between particulate organic matter and the diet of the ribbed mussel (*Geukensia demissa*) in Waquoit Bay also identifies eelgrass as an unimportant dietary component (Yelenik et al. 1996). The insignificant role of eelgrass in the diet of consumers at Sage Lot Pond is consistent with the findings of Stephenson et al. (1986) for an eelgrass community in Nova Scotia, Canada. Eelgrass does, however, make a major contribution to the diet of consumers in some locations (Thayer et al. 1978; McConnaughey and McRoy 1979).

*Wastewater provides a regional-scale $^{15}N$-enriched tracer*—Manipulative experiments that use $^{15}N$ additions to track N have been suggested as an alternative to natural abundance stable isotope studies in the environment for understanding nitrogen cycling (Hauck 1973). An experimental approach has the advantage of introducing a well-defined, identifiable N pool to a system, but $^{15}N$ additions must, for practical reasons, be carried out over relatively brief intervals. Wastewater inputs, on the other hand, provide a sustained, longer-term addition of $^{15}N$-enriched N. Thus, the regional-scale $^{15}N$-enriched tracer provided by wastewater inputs may be an excellent complement to short-term experimental $^{15}N$-enrichment studies for better understanding the coupling between terrestrial and marine environments and estuarine N cycling.

## Conclusions

The stable isotope data from Waquoit Bay provide evidence that increases in wastewater N loads to nearshore waters can be identified, even at relatively low loading rates

(<90 kg N $ha^{-1}$ $yr^{-1}$), in primary producers and consumers. Groundwater with an elevated N isotope signature as a result of wastewater input appears to act as a $^{15}$N-enriched tracer introduced to estuaries. The ability to detect wastewater N in estuarine food webs provides the basis for developing a new way to assess wastewater-driven eutrophication in coastal environments. Comparisons of $\delta^{15}$N values of biota between N-loaded and "pristine" estuaries or monitoring N isotope signatures over time at the same estuary would identify increasing wastewater inputs while loading rates were still relatively low. For the first time, it may be possible to identify incipient wastewater-driven eutrophication before the profound changes in nearshore communities presently used as indicators of eutrophication become evident. Restoration of habitats altered by nutrient enrichment is difficult; early detection and prevention of nutrient enrichment are more feasible and desirable management options.

## 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.

BREMNER, J. M., AND M. A. TABATABAI. 1973. Nitrogen-15 enrichment of soils and soil-derived nitrate. J. Environ. Qual. **2:** 363–365.

CALLAWAY, D. W., I. VALIELA, D. FOREMAN, AND L. A. SOUCY. 1995. Effects of nitrogen loading and salt marsh habitat on gross primary production and chlorophyll *a* in estuaries of Waquoit Bay. Biol. Bull. **189:** 254–255.

CIFUENTES, L. A., M. L. FOGEL, J. R. PENNOCK, AND J. H. SHARP. 1989. Biogeochemical factors that influence the stable nitrogen isotope ratio of dissolved ammonium in the Delaware Estuary. Geochim. Cosmochim. Acta **53:** 2713–2721.

COLE, J. J., B. L. PEIERLS, N. F. CARACO, AND M. L. PACE. 1993. Nitrogen loading of rivers as a human-driven process, p. 141–157. *In* M. J. McDonell and S. T. A. Pickett [eds.], Humans as components of ecosystems: The ecology of subtle human effects and populated areas. Springer-Verlag.

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

EFRON, B., AND R. TIBSHIRANI. 1991. Statistical analysis in the computer age. Science **253:** 390–395.

FOGEL, M. A., AND L. A. CIFUENTES. 1993. Isotopic fractionation during primary production p. 73–98. *In* M. H. Engel and S. A. Macko [eds.], Organic geochemistry. Plenum.

FREYER, H. D., AND A. I. M. ALY. 1974. Nitrogen-15 variations in fertilizer nitrogen. J. Environ. Qual. **3:** 405–406.

GESAMP. 1990. The state of the marine environment. Joint Group of Experts on the Scientific Aspects of Marine Pollution. Rep. and Stud. 39. United Nations Environmental Programme.

GORMLY, J. R., AND R. F. SPALDING. 1979. Sources and concentrations of nitrate-nitrogen in ground water of the central Platte region, Nebraska. Ground Water **17:** 291–301.

HAUCK, R. D. 1973. Nitrogen tracers in nitrogen cycle studies—past use and future needs. J. Environ. Qual. **2:** 317–326.

———, AND OTHERS. 1972. Use of variations in natural nitrogen isotope abundance for environmental studies: A questionable approach. Science **177:** 453–454.

HERSH, D. A. 1996. Abundance and distribution of intertidal and subtidal macrophytes in Cape Cod: The role of nutrient supply and other controls. Ph.D. dissertation, Boston Univ. 143 p.

HOBSON, K. A., AND H. E. WELCH. 1992. Determination of trophic relationships within a high arctic marine food web using $\delta^{13}$C and $\delta^{15}$N analysis. Mar. Ecol. Prog. Ser. **84:** 9–18.

HORRIGAN, S. G., J. P. MONTOYA, J. L. NEVINS, AND J. J. MCCARTHY. 1990. Natural isotopic composition of dissolved inorganic nitrogen in the Chesapeake Bay. Estuar. Coast. Shelf Sci. **30:** 393–410.

IIZUMI, H., AND A. HATTORI. 1982. Growth and organic production of eelgrass (*Zostera marina* L.) in temperate waters of the Pacific coast of Japan. III. The kinetics of nitrogen uptake. Aquat. Bot. **12:** 245–256.

KREITLER, C. W. 1979. Nitrogen-isotope ratio study of soils and groundwater nitrate from alluvial fan aquifers in Texas. J. Hydrol. **42:** 147–170.

———, S. RAGONE, AND B. G. KATZ. 1978. $N^{15}/N^{14}$ ratios of ground-water nitrate, Long island, New York. Ground Water **16:** 404–409.

LAJTHA, K., AND J. D. MARSHALL. 1994. Sources of variation in the stable isotopic composition of plants, p. 45–62. *In* K. Lajtha and R. H. Michener [eds.], Stable isotopes in ecology. Blackwell Scientific.

———, AND R. H. MICHENER. 1994. Stable isotopes in ecology. Blackwell Scientific.

LYONS, J., J. AHERN, J. MCCLELLAND, AND I. VALIELA. 1995. Macrophyte abundances in Waquoit Bay estuaries subject to different nutrient loads and the potential role of fringing salt marsh in groundwater nitrogen interception. Biol. Bull. **198:** 255–256.

MACKO, S. A., AND N. E. OSTROM. 1994. Pollution studies using stable isotopes, p. 45–62. *In* K. Lajtha and R. H. Michener [eds.], Stable isotopes in ecology. Blackwell Scientific.

MARIOTTI, A., C. LANCELOT, AND G. BILLEN. 1984. Natural isotopic composition of nitrogen as a tracer of origin for suspended organic matter in the Sheldt estuary. Geochim. Cosmochim. Acta **48:** 549–555.

———, A. LANDREAU, AND B. SIMON. 1988. $^{15}$N isotope biogeochemistry and natural denitrification process in groundwater: Application to the chalk aquifer of north France. Geochim. Cosmochim. Acta **52:** 1869–1878.

———, F. MARIOTTI, M. L. CHAMPIGNY, N. AMARGER, AND A. MOYSE. 1982. Nitrogen isotope fractionation associated with nitrate reductase activity and uptake of $NO_3^-$ by Pearl Millet. Plant Physiol. **69:** 880–884.

MCCONNAUGHEY, T., AND C. P. MCROY. 1979. $^{13}$C label identifies eelgrass (*Zostera marina*) carbon in an Alaskan estuarine food web. Mar. Biol. **53:** 263–269.

MEYER, S. L. 1975. Data analysis for scientists and engineers. Wiley and Sons. 513 p.

MINAGAWA, M., AND E. WADA. 1984. Stepwise enrichment of $^{15}$N along food chains: Further evidence and the relation between $\delta^{15}$N and animal age. Geochim. Cosmochim. Acta **48:** 1135–1140.

NATIONAL ACADEMY OF SCIENCES. 1994. Priorities for coastal science. National Academy.

NIXON, S. W. 1992. Quantifying the relationship between nitrogen input and the productivity of marine ecosystems. Proc. Adv. Mar. Tech. Conf. **5:** 57–83.

PECKOL, P., B. DEMEO-ANDERSON, J. RIVERS, I. VALIELA, M. MALDONADO, AND J. YATES. 1994. Growth, nutrient uptake capacities and tissue constituents of the macroalgae, *Cladophora vagabunda* and *Gracilaria tikvahiae,* related to site-specific nitrogen loading rates. Mar. Biol. **121:** 175–185.

PENNOCK, J. R., D. J. VELINSKY, J. M. LUDLAM, AND J. H. SHARP. 1996. Isotopic fractionation of ammonium and nitrate during uptake by *Skeletonema costatum:* Implications for $\delta^{15}$N dynamics under bloom conditions. Limnol. Oceanogr. **41:** 451–459.

PETERSON, B. J., AND B. FRY. 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. **18:** 293–320.

———, R. W. HOWARTH, AND R. H. GARRITT. 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. Science **227:** 1361–1363.

PICKETT, S. T. A. 1991. Space for time substitution as an alternative to long-term studies, p. 110–135. *In* G. E. Likens [ed.], Long-term studies in ecology: Approaches and alternatives. Springer-Verlag.

SAND-JENSEN, K., AND J. BORUM. 1991. Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. Aquat. Bot. **41:** 137–175.

SCHMITT, R. J., AND C. W. OSENBERG. 1995. Detecting ecological impacts; concepts and applications in coastal habitats. Academic.

SHAM, C. H., J. W. BRAWLEY, AND M. MORITA. 1995. Quantifying septic nitrogen loading to receiving waters: Waquoit Bay, Mass. Int. J. Geogr. Info. Syst. **9:** 463–473.

SHOLTO-DOUGLAS, A. D., J. G. FIELD, A. G. JAMES, AND N. J. VAN DER MERWE. 1991. $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotope ratios in the Southern Benguela ecosystem: Indicators of food web relationships among different size-classes of plankton and pelagic fish; differences between fish muscle and bone collagen tissues. Mar. Ecol. Prog. Ser. **78:** 23–31.

SHORT, F. T., AND C. P. MCROY. 1984. Nitrogen uptake by leaves and roots of the seagrass *Zostera marina* L. Bot. Mar. **27:** 547–555.

STEPHENSON, R. L., F. C. TAN, AND K. H. MANN. 1986. Use of stable carbon isotope ratios to compare plant material and potential consumers in a seagrass bed and a kelp bed in Nova Scotia, Canada. Mar. Ecol. Prog. Ser. **30:** 1–7.

THAYER, G. W., P. L. PARKER, M. W. LACROIX, AND B. FRY. 1978. The stable carbon isotope ratio of some components of an eelgrass, *Zostera marina,* bed. Oecologia **35:** 1–12.

TOMASKY, G., AND I. VALIELA. 1995. Nutrient limitation of phytoplankton growth in Waquoit Bay, Massachusetts. Biol. Bull. **189:** 257–258.

VALIELA, I., AND OTHERS. 1992. Couplings of watersheds and coastal waters: Sources and consequences of nutrient enrichment in Waquoit Bay, Massachusetts. Estuaries **15:** 443–457.

———, AND OTHERS. 1997. Nitrogen loading from coastal watersheds to receiving estuaries: New method and application. Ecol. Appl. **7:** 358–380.

WADA, E. 1980. Nitrogen isotope fractionation and its significance in biogeochemical processes occurring in marine environments, p. 375–398. *In* E. D. Goldberg and Y. Horibe [eds.], Isotope marine chemistry. Uchida-Rokakuho.

———, AND A. HATTORI. 1978. Nitrogen isotope effects in the assimilation of inorganic nitrogenous compounds by marine diatoms. Geomicrobiol. J. **1:** 85–101.

WAINRIGHT, S. C., M. J. FOGARTY, R. C. GREENFIELD, AND B. FRY. 1993. Long-term changes in the Georges Bank food web: Trends in stable isotopic compositions of fish scales. Mar. Biol. **115:** 481–493.

———, AND B. FRY. 1994. Seasonal variation of the stable isotopic compositions of coastal marine plankton from Woods Hole, Massachusetts and Georges Bank. Estuaries **17:** 552–560.

YELENIK, S., J. MCCLELLAND, N. FEINSTEIN, AND I. VALIELA. 1996. Changes in N and C stable isotope signatures of particulate organic matter and ribbed mussels in estuaries subject to different nutrient loading. Biol. Bull. **191:** 329–330.

YOSHIOKA, T., E. WADA, AND H. HAYASHI. 1994. A stable isotope study of seasonal food web dynamics in a eutrophic lake. Ecology **75:** 835–846.

*Submitted: 25 April 1996*
*Accepted: 16 January 1997*