Ecological Applications, 11(1), 2001, pp. 203–214

RIBBED MUSSEL NITROGEN ISOTOPE SIGNATURES REFLECT NITROGEN SOURCES IN COASTAL SALT MARSHES

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Abstract. The stable nitrogen isotope ratio in tissue of the ribbed mussel (*Geukensia demissa*) was investigated as an indicator of the source of nitrogen inputs to coastal salt marshes. Initially, mussels were fed a diet of ^{15}N-enriched algae in the laboratory to determine how the tissue nitrogen isotope ratio (δ^{15}N) changed with time. Steady-state times were calculated and found to be size dependent, ranging from 206 to 397 d. This indicated that mussels are long-term integrators of δ^{15}N from their diet and may reflect nitrogen inputs to a marsh. Next, indigenous mussels were collected from 10 marshes with similar hydrology and geomorphology in Narragansett Bay, Rhode Island, USA, and mussel δ^{15}N values were evaluated as indicators of nitrogen source. Significant positive correlations were observed between δ^{15}N in mussels and the fraction of residential development in the marsh watersheds. In contrast, mussel isotope ratios showed significant negative correlations with the fraction of combined agricultural and recreational land use. These correlations suggested that the mussel nitrogen isotope signature is influenced by nitrogen derived from human activities in the adjoining marsh watershed. A more detailed examination of these relationships indicated that land use practices in close proximity to marshes and estuarine characteristics may also influence the observed nitrogen isotope signature. A simple, empirical model based on the 10 watersheds was developed to predict mussel δ^{15}N from land use characteristics. The predictive ability of the model was tested with data from 12 additional marshes having similar geomorphology as the original 10, but differing in hydrology and mode of nutrient input. The model showed that ribbed mussel nitrogen isotope signatures may provide information on the source of nitrogen to coastal areas. This could be of use in developing general policies or strategies for monitoring and assessing coastal eutrophication. In addition, the isotopic ratio of mussels is useful as a proxy for watershed land use practices when assessing ecological responses to nutrient enrichment in coastal marshes.

Key words: Geukensia demissa; *land use characteristics; mussel; nitrogen input indicator; nitrogen isotope ratio; ribbed mussel; salt marsh.*

Introduction

Widespread increases in the input of anthropogenic nitrogen to coastal systems have resulted in an overall increase in the number of ecosystems altered by eutrophication, with effects that include changes in biodiversity, habitat alterations, and toxic algal blooms (Erisman et al. 1998). Non-point nutrient sources, especially nitrogen, contribute significantly to the nutrient enrichment of coastal ecosystems, and identification and control of these diffuse inputs is generally more difficult than point sources (Carpenter et al. 1998). In addition to ongoing efforts to evaluate the overall extent of nitrogen enrichment in coastal systems, there is a recognized need for the development of indicators of nitrogen source (Cowling et al. 1998, Erisman et al. 1998, Bricker et al. 1999). Stable nitrogen isotope measurements can be used to trace nitrogen from diffuse, land-based sources to coastal marshes (McClelland et al. 1997, McClelland and Valiela 1998).

Manuscript received 9 June 1999; revised 2 February 2000; accepted 10 February 2000.

Linking nitrogen sources from watersheds to coastal ecosystems would provide important information for managing eutrophication at both local and regional scales. Because the nitrogen isotope ratio increases in a fairly predictable fashion as it passes through the food chain (Minigawa and Wada 1984, Fry 1988), anthropogenic nitrogen inputs can influence the isotopic composition of organisms in an ecosystem. Therefore, the nitrogen isotope ratio, or signature, of one component or several biotic components of an ecosystem could be used to characterize inputs to that system.

The best reflection of nitrogen source to an ecosystem may be the isotope ratio of biotic components at or near the base of the food chain, described by the δ^{15}N of an organism common to the systems being compared or studied. However, measuring isotopic ratios of these organisms can be problematic. Seasonal fluctuations in isotope ratios, as a result of changes in source and species-dependent fractionation make it difficult to determine an accurate nitrogen isotopic composition for primary producers (Cifuentes et al. 1989, Cabana and Rasmussen 1996, Roelke et al. 1999). For example, Hoch et al. (1996) report significant variability in the distribution of $\delta^{15}N$ among different size classes of particulate organic nitrogen in aquatic environments, and there are several reports of variability in the $\delta^{15}N$ of primary producers in estuaries (Cifuentes et al. 1996, Fourqurean et al. 1997), with differences ranging up to 3‰. These seasonal fluctuations in base level isotope ratio make it difficult to determine an accurate isotopic composition. Difficulties may also arise when trying to discern nitrogen source from the isotope ratio of higher level consumers, since food chains vary in length and complexity (Kling and Fry 1992, Cabana and Rasmussen 1994). Ideally, an organism low in the food chain which integrates the highly variable $\delta^{15}N$ of primary producers would best reflect the base level nitrogen isotope signal of an ecosystem.

The ribbed mussel, *Geukensia demissa*, is a filter-feeding bivalve (i.e., primary consumer) that occupies a position near the base of the food chain in coastal ecosystems. Mussels feed on available particulate organic material (POM) which may be comprised of algae, detritus, and bacteria (Gosner 1971). They are also important in nutrient flow and cycling within a marsh. In a typical New England salt marsh, mussels were found to filter 1.8× as much particulate nitrogen as was exported from the marsh by tidal processes, and ~25% of that nitrogen was retained by the mussels and incorporated into somatic growth or gamete production (Jordan and Valiela 1982). By processing large amounts of nitrogen, and because of their relatively low tissue turnover rates (McMahon 1991), the $\delta^{15}N$ of ribbed mussels may be a good indicator of the source of nitrogen to an ecosystem.

By retaining nitrogen from primary producers in their tissues, mussels reflect the stable isotopic composition of source nitrogen. The $\delta^{15}N$ in primary producers is determined by the $\delta^{15}N$ of dissolved inorganic and organic nitrogen assimilated by those organisms and subsequent alteration of the isotopic composition by fractionation (Fogel and Cifuentes 1993, Waser et al. 1998). McClelland and Valiela (1998) have demonstrated that the $\delta^{15}N$ of particulate organic material at a given location reflects that of dissolved inorganic nitrogen (DIN) input to that location. The isotope ratio of dissolved nitrogen, while determined in part by biological processes such as denitrification (Fourqurean et al. 1997, Corbett et al. 1999), can reflect isotopic variation as a result of anthropogenic activity. Source nitrogen to a marsh may be enriched or depleted in ^{15}N by dilution with nitrogen altered by anthropogenic processes. For example, dissolved nitrogen derived from synthetic fertilizers is relatively depleted in ^{15}N, which results in $\delta^{15}N$ values in the range of −3‰ to +3‰. In contrast, nitrogen derived from wastewater is relatively enriched in ^{15}N, which results in $\delta^{15}N$ values in the range of 10‰ to 20‰ (Freyer and Aly 1974, Kreitler et al. 1978, Gormley and Spalding 1979, Aravena et al. 1993).

We propose that the isotope ratio of ribbed mussels could provide information about the source of nitrogen to coastal ecosystems which would be of value to coastal zone managers and regulators in developing general policies or strategies regarding monitoring and assessment of coastal eutrophication. Assessment and monitoring strategies in the United States have identified the need to develop indicators of nutrient source to estuaries (U.S. Environmental Protection Agency 1998, Bricker et al. 1999). Additionally, isotope signatures may be useful as a proxy for land use practices when assessing relationships between ecological effects and nutrient enrichment in coastal marshes.

In the present study, we investigate the use of ribbed mussel $\delta^{15}N$ values as site-specific indicators of the source of nitrogen input to coastal salt marshes. The study was conducted in three phases. First, mussels were exposed to a ^{15}N-enriched diet in a laboratory feeding experiment to quantify the extent and rate at which the $\delta^{15}N$ tissue values of mussels reflected that of their diet. Secondly, indigenous mussel populations were examined at 10 salt marsh locations of similar geomorphology and hydrology to determine if $\delta^{15}N$ values vary with mussel size and site, and whether the values changed over time during seasonal sampling. Sample locations were chosen along a gradient of anthropogenic stress described by watershed land use practices and headwater nitrogen concentrations, and correlations between marsh watershed land use and isotope ratios were investigated. Finally, we developed a simple, empirical model using data from the 10 marsh sites to predict mussel isotope ratio based on watershed land use characteristics, and tested the model with data from 12 additional marshes of similar geomorphology but differing in their hydrology and other physical characteristics.

METHODS

Nitrogen uptake experiment

Mussels collected in late January 1998 from a reference site in East Sandwich, Massachusetts, were returned to the laboratory and acclimated over a 2-wk period to 15°C. Mussels were separated into two size classes: ''30 mm'' with shell lengths ranging from 28 to 32 mm (initial $\delta^{15}N$ [mean ± 1 SE] = 8.0 ± 0.4‰); and ''50 mm'' with shell lengths ranging from 47 to 55 mm (initial $\delta^{15}N$ = 8.6 ± 0.2‰). The alga *Tetraselmis* (spp.) was cultured under two sets of conditions: ambient conditions, resulting in a control diet with $\delta^{15}N$ values ranging from 0‰ to 3‰; and ^{15}N-enriched conditions, resulting in an enriched diet with an average $\delta^{15}N$ value of 43.4 ± 0.6‰. Three replicates of each diet, each consisting of six mussels from each size class, were placed in gallon jars and exposed to flowing seawater containing either the control or enriched algae. Algal density was maintained at 20 × 10^6 cells/L and added continuously to the sample jars for 16 h out

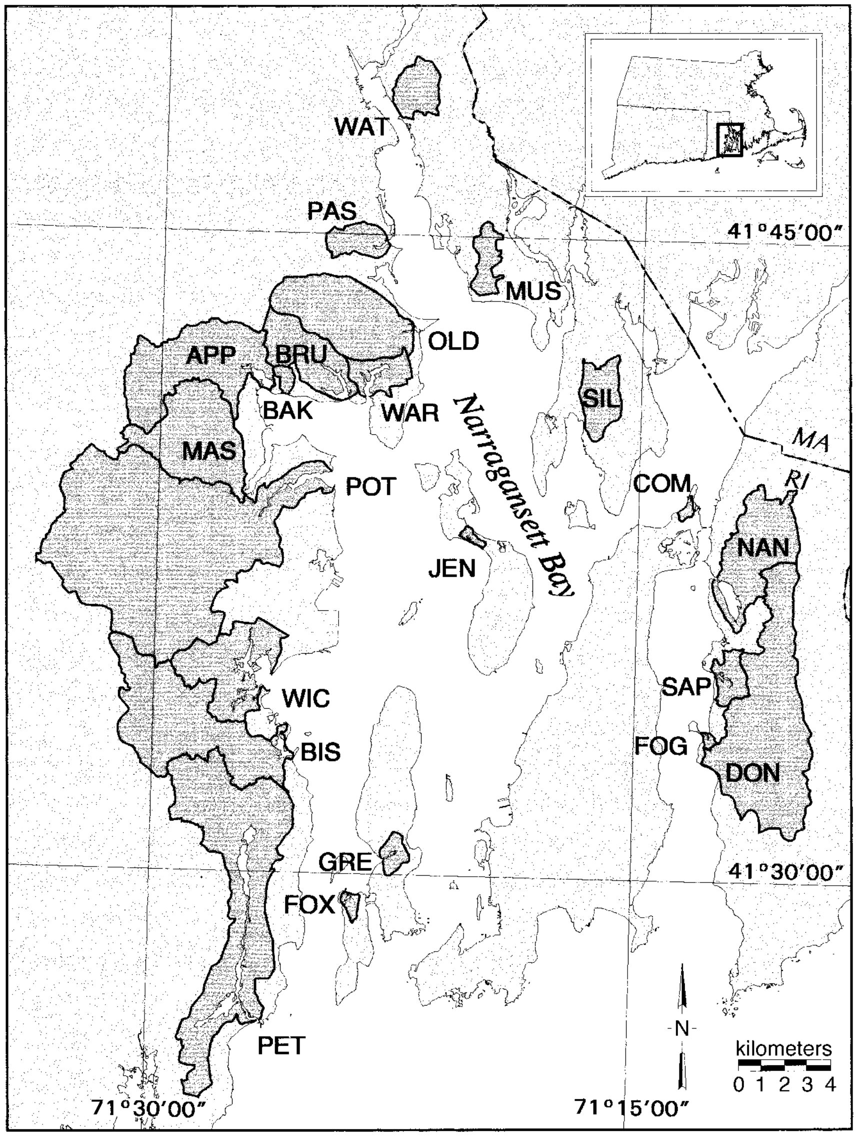

FIG. 1. Marsh sampling locations in Narragansett Bay, Rhode Island, USA. For sample site descriptions see Tables 1 and 3.

of each 24-hour period at a rate of 400 × 10^6 cells/h. During sampling (0, 3, 7, 14, 28, 42, and 56 d), one mussel from each size class was removed from each replicate without replacement. A control treatment for mussel density was maintained with the same number of mussels as the treatment jars; however, this was sampled only at 0, 28, and 56 d. Comparison of results between the control and treatment mussels showed no differences due to the decrease in mussel density in the sample jars as the experiment progressed. Mussels were depurated in filtered seawater for 24 h to clear their gut contents prior to dissection. Mussel tissue was removed from the shell, dried at 104°C for four hours, and homogenized using a mortar and pestle. Tissue nitrogen isotopic composition and percent nitrogen were determined by continuous flow isotope ratio mass spectrometry (CF-IRMS), using a Carlo Erba NA 1500 Series II Elemental Analyzer (Carlo Erba Instruments, Milan, Italy) interfaced to a Micromass Optima Mass Spectrometer (Micromass, Manchester, UK). The stable nitrogen isotope ratio ($\delta^{15}N$) is generally expressed as the ratio of the ^{15}N isotope to ^{14}N in a sample to that in a reference material ($\delta^{15}N = (^{15}N/^{14}N_{sample})/(^{15}N/^{14}N_{standard}) - 1 \times 1000‰$) and is reported in parts per thousand or per mille (‰) (Mariotti 1983). All samples were analyzed in duplicate with a typical difference of ~0.1‰. Sample material was re-analyzed periodically over a period of several months, and exhibited a precision of ±0.30‰, calculated as a single sigma standard deviation of all replicate values. This latter estimate of precision is appropriate for $\delta^{15}N$ values determined in this study.

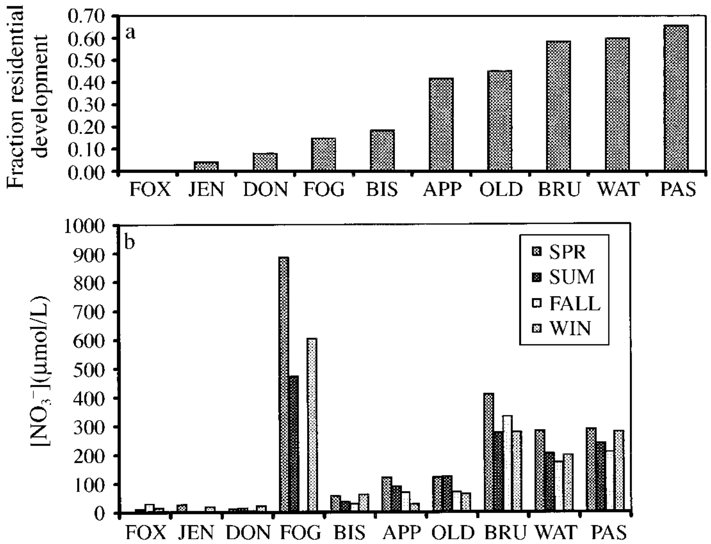

FIG. 2. (a) Fraction residential land use in marsh watersheds. (b) Headwater nitrate concentrations measured during the spring, summer, and fall of 1998, and winter of 1999 at the marsh sample sites. See Fig. 1 for locations, and Table 3 for marsh site abbreviations.

Site selections for marsh sampling

Semi-enclosed sub-estuaries of Narragansett Bay, Rhode Island, USA, which contained emergent coastal wetlands and their adjoining watersheds were identified using geographic information system (GIS) topographic databases (Fig. 1). The estuaries and their respective watersheds were delineated using 15 minute (1:24 000) scale United States Geological Survey (USGS) topographic maps. The watershed information was processed using the Environmental Systems Research Institute (ESRI) ARC/INFO GIS software package. Additional geographic information system (GIS) data layers (e.g., land use and land cover) were obtained from the Rhode Island Geographic Information System (RIGIS). We minimized confounding natural variability among salt marshes by choosing sub-estuaries in the main basin with the same geological bedrock (i.e., sedimentary rock), and similar hydrology as characterized by average depth, mouth diameter, tidal range, and volume determined from NOAA National Ocean Service charts and USGS topographic maps. Each marsh also had stream input, with measurable nitrate concentrations (Fig. 2b), which we termed stream nitrogen input.

To choose salt marshes along a gradient of low to high anthropogenic stress, 1995 GIS land use and land cover data were used to describe land use practices in the watersheds, and headwater nitrate concentrations were measured in 1998 and 1999. We were interested in diffuse nutrient sources so any sub-estuaries in the group with active point source pollution were eliminated. To narrow down the possible sites to a manageable group, 10 sites were chosen which were accessible for sampling and showed a gradient of low to high impact based on our results of the watershed land use (fraction residential development) and headwater nitrate concentrations (Figs. 1 and 2). According to 1990 GIS data and information obtained from town public works departments, three of the 10 marsh watersheds (OLD, PAS, APP) have nominal (<20%) sewer coverage; in the balance of these and the other seven watersheds, residential waste is treated in domestic septic systems. Twelve additional marshes in Narragansett Bay with similar geological bedrock and absence of point source nutrient inputs were sampled in order to test the proposed predictive model (Table 1). Four of the 12 additional sites (MUS, PET, SIL, WAR) had

TABLE 1. Nitrogen isotope values of mussels (5.5–7.5 cm, $n = 6$) from the 12 marsh sites in Narragansett Bay, Rhode Island, USA, used to test the predictive model.

Marsh site	δ^{15} N, ‰	Similar hydrology?	Stream input?
BAK	10.7 ± 0.4	yes	yes
COM	11.5 ± 0.2	yes	no
GRE	9.0 ± 0.3	yes	no
MAS	10.9 ± 0.3	no	yes
MUS	10.4 ± 0.2	no	yes
NAN	10.5 ± 0.5	no	yes
PET	9.8 ± 0.3	no	yes
POT	10.6 ± 0.4	no	yes
SAP	9.5 ± 0.2	yes	no
SIL	10.8 ± 0.3	yes	yes
WAR	11.5 ± 0.4	yes	no
WIC	10.8 ± 0.2	no	yes

Notes: Whether or not the marsh sites have similar hydrology (embayment dimensions within the range of the initial 10 marshes) and similar mode of nitrogen input (stream input) is also indicated.

Marsh sites are sub-embayments of Narragansett Bay, Rhode Island: BAK = Bakers Creek; COM = Common Fence Point; GRE = Great Creek; MAS = Maschachawege Brook; MUS = Mussachuck Creek; NAN = Nannaquaket Pond; PET = Pettaquamscutt Cove; POT = Potowomut River; SAP = Sapaeowet Marsh; SIL = Silver Creek; WAR = Warwick Cove; WIC = Wickford Cove.

sewer development within their watershed boundaries, but none exceeded the range of sewer development reported for the original 10 marshes (<20%).

Sample collections

Eighteen mussels between 10 mm and 110 mm were collected from each site at the same tidal height (in the *Spartina alterniflora* zone) and were transported to the laboratory on ice where they were depurated in filtered seawater for 24 h. All 10 marsh sites were sampled at least twice, during May and August 1998; additionally the marshes BRU, FOX, PAS, and WAT were sampled four additional times, during July and October 1998, and January and April 1999, to assess temporal changes in nitrogen isotope ratio. Mussels were collected by hand within a 10 m radius along the marsh bank facing open water in the *Spartina alterniflora* zone. Salinity at the sample sites at the time of sample collection ranged from 27 to 30 g solid per kilograms seawater, except for the PAS marsh site which showed a salinity of 21 g solid per kilograms seawater. Following depuration, mussels were frozen pending dissection and analysis.

Marsh plants and plant litter (*Spartina alterniflora*) were collected by hand in March 1999 in close proximity to the mussel sample sites, and prepared for isotopic analysis by washing with deionized water and drying at 65°C. Samples were then homogenized in a Wiley mill prior to analysis for carbon isotopic composition by continuous flow isotope ratio mass spectrometry (CF-IRMS), using a Carlo-Erba NA 1500 Series II Elemental Analyzer interfaced to a Micromass Optima Mass Spectrometer (CE Instruments, Milan, Italy). Carbon isotopic composition is reported as parts per thousand deviation (δ^{13}C‰) from the reference standard PDB (the fossil belemnite, *Belemnitella americana*, from the Peedee formation in South Carolina). Mussel tissue samples from the 10 marsh sites were also analyzed for δ^{13}C‰.

Nitrogen isotope ratio of dissolved inorganic nitrogen (DIN) was measured for seven of the 10 marsh sites by collecting water (0.2–0.4 L) from the headwater inputs to the marsh sites at the upper edge of the marsh during March 1999, and extracting the DIN using a modification of the method of Sigman et al. (1997). The modification, which is outlined in the original method (Sigman et al. 1997), involves adding sodium chloride to adjust the water samples to the proper salinity prior to extraction. The isotope ratio was then determined on the extracted nitrogen.

Marsh sample sites

Land use correlations.—Correlations between mussel isotope ratios and land use practices were examined using land use and land cover data classified using a modified USGS system (Anderson 1976). The original data was classified into more than 35 categories and, for the purposes of this study, aggregated into five broad subcategories: residential; commercial and industrial; agricultural and recreational; forested; and wetlands. The GIS data was used to generate the fraction of each category within each watershed.

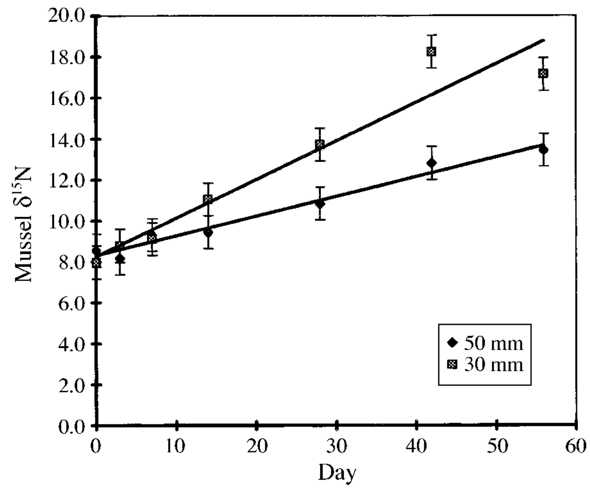

FIG. 3. Uptake of ^{15}N-enriched diet by two size classes of mussels in the laboratory feeding experiment.

Predictive model.—A simple, empirical model to predict mussel isotope ratio based on watershed land use characteristics was developed from a multiple regression using the fraction residential development and the fraction of combined agricultural and recreational land as the independent variables, and mussel nitrogen isotope ratio as the dependent variable. The model incorporated data from the 10 original marsh sites that were selected based upon similar geomorphology, hydrology, and presence of stream nitrogen input. The resulting model was then tested with mussel isotope data from 12 additional marsh sites. These sites all had similar geomorphology to the original 10, but 10 of these 12 differed either in hydrology or by the absence of stream input.

Data analysis

Differences in mussel size class nitrogen isotope ratio was tested using a single factor ANOVA with shell length as the independent variable and isotope ratio as the dependent variable. Significant differences between nitrogen isotope ratios from the marsh sampling sites were determined using a single factor ANOVA with marsh site as the independent variable and isotope ratio as the dependent variable. Correlations between isotope ratio and land use characteristics were examined using multiple regressions between fraction land use (residential, combined agricultural and recreational) or fraction nitrogen source (wastewater, fertilizer, atmospheric) and isotope ratio. Significant differences were reported at $P < 0.05$.

RESULTS

Nitrogen uptake experiment

Mussels fed the ^{15}N-enriched algal diet showed an increase in their nitrogen isotope ratio over time (Fig.

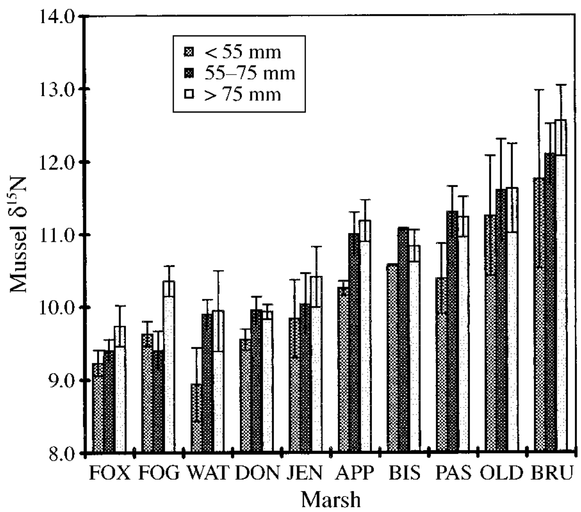

FIG. 4. Mean mussel $\delta^{15}N$ of three size classes of mussels from 10 marsh sites sampled in summer 1998.

3). Those in the 30 mm size class reflected the increased $\delta^{15}N$ diet more rapidly and to a greater extent, increasing from 8.0‰ to 17.1‰, than those in 50 mm size class, which increased from 8.6‰ to 13.4‰ (ANCOVA, $P = 0.004$). The standard deviation associated with $\delta^{15}N$ measurements averaged 0.9‰ and 1.0‰, respectively; however, the standard deviation generally increased over the course of the experiment for both size classes. The average rates of nitrogen assimilation can be described by relating nitrogen isotope ratio to days of exposure to an altered diet: for ''30 mm,''

$$\delta^{15}N = 0.19 \times (\text{days of exposure}) + 8.25‰$$

($r^2 = 0.93$, $P = 4.78 \times 10^{-4}$); and for ''50 mm,''

$$\delta^{15}N = 0.096 \times (\text{days of exposure}) + 8.31‰$$

($r^2 = 0.97$, $P = 4.11 \times 10^{-5}$). Standard errors in the 30 mm estimates are for slope ±0.023‰ and for intercept ±0.68‰; for the 50 mm estimates standard errors were ±0.0071‰ for slope and ±0.21‰ for intercept; r^2 values are from the data in Fig. 3.

Marsh sample sites

Size considerations.—Mean $\delta^{15}N$ values of mussels collected at the 10 marsh sites during the summer of 1998 are summarized in Fig. 4. Mussels were grouped into three size classes; shell lengths 0–55 mm, 55–75 mm, and >75 mm. Six of the 10 marsh sites (BRU, FOX, FOG, JEN, PAS, and WAT) showed a significant relationship of $\delta^{15}N$ with shell length (Table 2). The nitrogen isotope ratio increased at an average rate of 0.02‰ per mm shell length at these six sites. These results were consistent with those seen at other sampling times in the marshes.

Nitrogen isotope values.—Mussel stable nitrogen isotope values from the 10 marsh sites, derived from mussels in the 55–75 mm size class, ranged from 9.4‰ for the FOX and FOG sites to 12.1‰ for the BRU site (Table 3). Significant differences were found in average $\delta^{15}N$ values among sites (ANOVA, $P < 0.001$). At six of the 10 sites, $\delta^{15}N$ values are averages from two sampling events, spring and summer 1998. The other four sites were sampled more extensively to determine any seasonal changes. Isotope ratios measured at the BRU, FOX, PAS, and WAT sites showed no significant within-site differences among the six sampling events (May, July, August, and October 1998, and January and April 1999; ANOVA, $P < 0.001$). Nitrogen isotope values of mussels from the additional 12 marsh sites, used to test the proposed model, ranged from 9.0‰ to 11.5‰ (Table 1). There was no significant difference in mean percent nitrogen in mussel tissue (9.2 ± 0.1%, with a range of 4.0%).

Nitrogen isotope ratio values of headwater dissolved inorganic nitrogen measured at seven of the 10 marsh sites ranged from 4.1‰ at JEN to 10.8‰ at OLD (Table 3).

Carbon isotope values.—The $\delta^{13}C$ values of mussels from the 10 marsh sites had a mean value of −17.6 ± 1.5‰ with a range of 2.1‰. The mean $\delta^{13}C$ values for marsh plants (*Spartina alterniflora*) collected from the 10 sites was −12.9 ± 0.3‰ with a range of 1.3‰, and for plant litter was −16.1 ± 1.3‰ with a range of 1.3‰.

Estuarine dimensions.—The mean depth of the embayments at mean low water was 0.73 ± 0.14 m with a range of 0.35 m, the mean mouth diameter of the embayments was 183 ± 27 m with a range of 65 m, and the mean tidal range across all sites was 1.28 ± 0.11 m with a range of 0.24 m. The estimated volumes of the embayments, based upon available depth data and embayment areas determined from GIS data, had a range of 0.20–4.79 × 10^5 cubic meters, with a mean value of 1.92 × 10^5 cubic meters.

TABLE 2. Statistics from the relationship between tissue $\delta^{15}N$ and shell length in mussels collected from the 10 marsh sites during the summer of 1998.

Statistic	APP	BIS	BRU	DON	FOG	FOX	JEN	OLD	PAS	WAT
r^2	0.16	0.21	0.58	0.11	0.35	0.21	0.47	0.19	0.30	0.43
P	0.11	0.07	<0.001	0.16	0.01	<0.001	<0.001	0.07	<0.001	<0.001
m	0.012	0.007	0.014	0.006	0.017	0.010	0.012	0.006	0.018	0.022
b	9.9	10.4	11.2	9.4	8.9	8.9	9.4	11.1	9.8	8.2

Notes: The table reports the coefficient of determination (r^2), and associated probability (P) from the analysis; m and b are the coefficients of the linear relationship described by the equation $\delta^{15}N = m(\text{length}) + b$.

TABLE 3. Nitrogen isotope values of mussels (5.5–7.5 cm, $n = 7$–31) and headwater DIN δ^{15}N ($n = 1$) from 10 marsh sites in Narragansett Bay, Rhode Island, USA.

Marsh site	Mussel δ^{15}N (‰)	Headwater DIN δ^{15}N (‰)
APP	11.0 ± 0.3	7.6
BIS	11.1 ± 0.3	7.3
BRU	12.1 ± 0.3	···
DON	10.0 ± 0.2	6.2
FOG	9.4 ± 0.4	···
FOX	9.4 ± 0.4	7.7
JEN	10.0 ± 0.5	4.1
OLD	11.6 ± 0.2	10.8
PAS	11.3 ± 0.4	···
BRU	9.9 ± 0.5	9.1

Note: Marsh site descriptions: APP = Apponaug Cove; BIS = Bissel Cove; BRU = Brush Neck Cove; DON = Mary Donovan Marsh; FOG = Fogland Marsh; FOX = Fox Hill Salt Marsh; JEN = Jenny Pond; OLD = Old Mill Creek; PAS = Passeonkquis Cove; WAT = Watchemoket Cove.

Land use correlations.—The relative importance of land use categories varied among watersheds (Table 4). These areas are used to derive fractions of each land use type in the watershed. The average δ^{15}N values from mussels from the 10 marsh sites (Fig. 5) showed a significant positive correlation with the fraction of residential land use in the marsh watershed ($r = 0.72$, $P = 0.03$), and a significant negative correlation with the fraction of combined agricultural and recreational land use ($r = -0.75$, $P = 0.02$). Adding the fraction of combined agricultural and recreational land use to the fraction of residential land use regression resulted in a significant increase in the correlation ($P = 0.05$); the average δ^{15}N values from mussels showed a significant ($r = 0.84$, $P = 0.01$) relationship with both the fraction of residential and fraction of combined agricultural and recreational land use in the marsh watershed.

Additionally, we were able to obtain several samples of fertilizers commonly used in agriculture within the study area. Nitrogen isotope ratios ranged from 0.38‰ to 3.8‰ for these materials.

Predictive model.—A simple, empirical model was developed from multiple regression analysis using the GIS land use characteristics fraction residential development (frac res) and fraction combined agricultural and recreational (frac ag/rec) land use in the marsh watershed as independent variables, and marsh ribbed mussel δ^{15}N as the dependent variable. Mussel isotope data from the initial 10 marsh sites (Table 3) resulted in the relation:

$$\delta^{15}N_{pred} = 1.8 \times (\text{frac res}) - 3.4 \times (\text{frac ag/rec}) + 10.4‰$$

($r^2 = 0.71$, $P = 0.01$). The measured δ^{15}N values of mussels from the 12 additional sites (Table 1) is plotted against the predicted isotope ratio (Fig. 6).

DISCUSSION

Nitrogen uptake experiment

Results of the laboratory feeding experiment indicate that ribbed mussels do reflect the δ^{15}N of their diet, albeit at different incorporation rates depending on size class. This observation is probably the result of a difference in tissue turnover and nutrient assimilation rates between the faster growing younger mussels and slower growing older mussels. The time period required for the laboratory mussels to reach equilibrium δ^{15}N values (i.e., reflecting that of their food) can be estimated if it is assumed that the mussels continue to assimilate nitrogen at the calculated experimental rate indicated. The mean algae δ^{15}N used in the feeding experiment was 43.4‰. We expect that the mussels would eventually reach an isotopic composition of 46.8‰, which is the isotopic value of the food source (43.4‰) plus a fractionation effect of 3.4‰ which represents a one trophic level enrichment of the food source (Minigawa and Wada 1984). Using the 46.8‰ target value, we estimated that mussels in the "30 mm" size class would reach an equilibrium δ^{15}N value in 206 d, while those in the "50 mm" size class would reach an equilibrium value in 397 d. These results differ from tissue turnover times under natural conditions, which have been reported to be on the order of three years for six-year-old mussels (Hawkins 1985, McMahon 1991). However, it is clear from this experiment that mussels do indeed reflect the nitrogen isotopic ratio of their food, and that the time period required to fully reflect a change in δ^{15}N is greater than seasonal changes in the δ^{15}N of primary producers which comprise their diet. From this information we concluded that mussels act as long-term integrators of the nitrogen isotopic ratio of their food source, thus providing an overall average value of short-term intermittent changes (i.e., daily, weekly, seasonal) often seen in the δ^{15}N values of primary producers.

Marsh sample sites

Size considerations.—Data from indigenous mussels collected from the marsh sites showed a positive linear

TABLE 4. Watershed areas associated with selected GIS land use categories for 10 marsh sites in Narragansett Bay, Rhode Island, USA.

Marsh site	Total watershed area (m^2) (× 10^{-5})	Residential area† (m^2) (× 10^{-5})	Agricultural and recreational area‡ (m^2) (× 10^{-5})
APP	178	74.6	11.3
BIS	229	42.3	10.5
BRU	83.1	48.6	2.79
DON	318	25.7	59.8
FOG	2.96	0.44	1.37
FOX	6.16	0.02	1.73
JEN	4.15	0.17	0.00
OLD	151	68.0	6.57
PAS	31.4	20.5	1.18
WAT	40.1	22.5	5.60

Note: For marsh site definitions, see *Note* of Table 3.

† Includes the following GIS land use categories: high density, medium high density, medium density, medium low density, and low density residential.

‡ Includes pasture, tillable cropland, and recreational development.

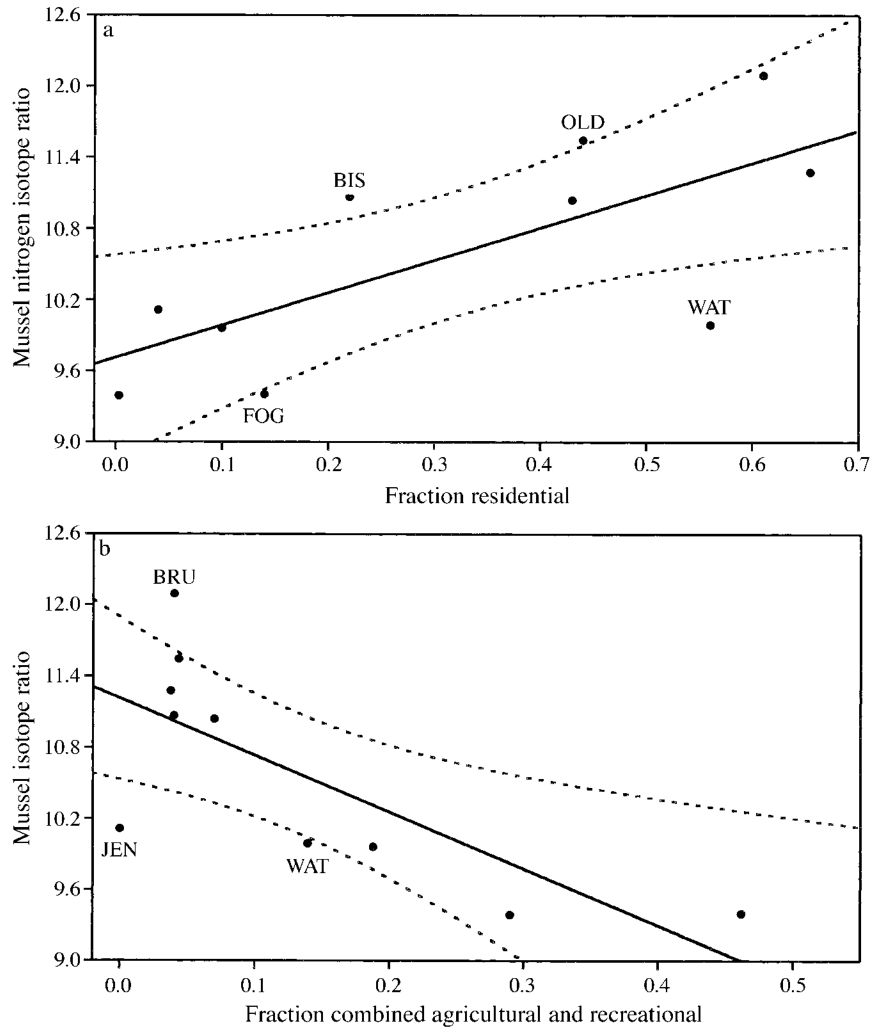

FIG. 5. Correlation of (a) fraction residential and (b) fraction combined agricultural and recreational land use with mussel nitrogen isotope ratio for the 10 marsh sites. The 95% CI is drawn for each regression. Marsh site abbreviations: BIS = Bissel Cove, BRU = Brush Neck Cove, FOG = Fogland Marsh, JEN = Jenny Pond, OLD = Old Mill Creek, and WAT = Watchemoket Cove (see Fig. 1).

relationship between $\delta^{15}N$ and shell length, and hence age. This is most likely a result of some difference in the manner in which nitrogen is incorporated in mussel tissue, or perhaps of repeated tissue turnover processes which result, over time, in the retention of the heavier isotope of nitrogen in the tissue of mature mussels. Alternatively, if the smaller, younger mussels were reflecting the periodic seasonal fluctuations in the $\delta^{15}N$ of primary producers, their isotope values would not be expected to vary linearly with shell length, but rather would show both positive and negative variations from the site average. This type of behavior would be reflected in the standard deviation of the $\delta^{15}N$ values of the smaller mussels; however, the standard deviations of the smallest size class mussels were not significantly larger than those of other size classes (ANOVA, P = 0.62). This result indicates that care must be taken to sample mussels within a certain range of shell lengths when comparing isotope ratios between sites.

Nitrogen isotope values.—Nitrogen isotope values in mussels measured from the 10 marsh sites were significantly different and increased along the watershed gradient from low to high anthropogenic stress upon the marshes. We suggest that the mussel nitrogen isotope ratio is influenced by the degree of anthropogenic impact as measured by land use practices and headwater nutrient concentrations. In addition, the mussel nitrogen isotope ratio at a marsh is influenced by biota and biogeochemical processes (e.g., nutrient cycling) at a particular marsh site. The question arises as to whether the mussel nitrogen isotope ratio reflects that of the primary producers which incorporate marsh-derived nutrients, or that of primary producers which incorporate marine-derived nutrients, or some portion of

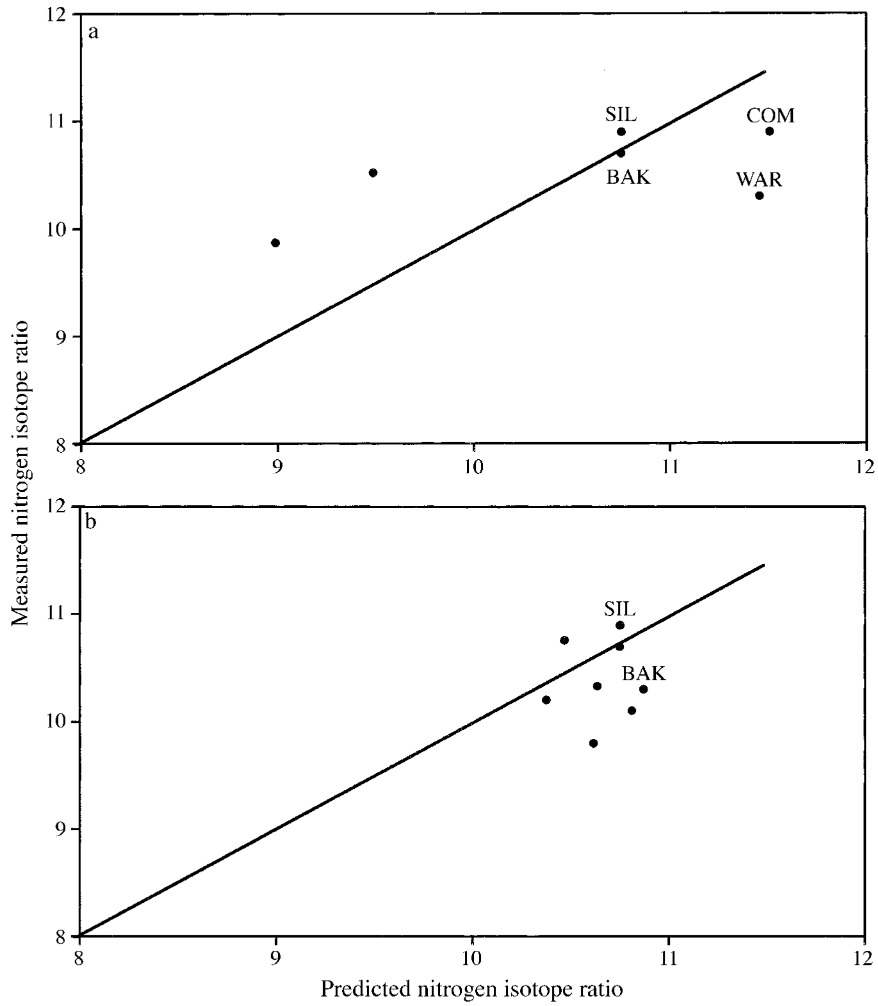

FIG. 6. Measured vs. predicted mussel $\delta^{15}N$ values for marsh sites exhibiting (a) similar marsh hydrology and (b) similar mode of nitrogen input (i.e., stream input) as the original 10 marsh sample sites. Marsh site abbreviations: BAK = Bakers Creek, COM = Common Fence Point, SIL = Silver Creek, and WAR = Warwick Cove (see Fig. 1).

both sources. The carbon isotope ratio ($\delta^{13}C$) may provide insight into the food source of mussels.

Several studies have linked $\delta^{13}C$ with carbon source and carbon flow in marine ecosystems (Fry 1984, Fry and Sherr 1984, Boutton 1991). Specifically, differences in $\delta^{13}C$ have been noted in biota and sediments from terrestrial, estuarine, and marine environments (Simenstad and Wissmar 1985, Giorgio and France 1996), and general trends in carbon isotope values have been described. In the Great Sippewisset Marsh, Massachusetts, Peterson et al. (1985) report differences in the isotope ratios of mussels that have ready access to *Spartina* detritus and those with access to marine plankton. Measured $\delta^{13}C$ values of mussels from our study fall within the range of salt marsh mussels deriving carbon primarily from marsh plant sources as described in Peterson et al. (1985). Our mussel $\delta^{13}C$ values also fall within the range of $\delta^{13}C$ of marsh derived food sources from our sites, including marsh plants and plant litter. These values differ, however, from the $\delta^{13}C$ of mussels we collected from a marine location (rocky/sandy beach area in Buzzards Bay, Massachusetts, −22.0‰), and which presumably would have access to marine plankton but limited access to marsh-derived food (Peterson et al. 1985).

These data suggest that the source of food of mussels in this study has a land-based or marsh component. Nutrients and particulate organic material (POM) from marine waters delivered via tidal exchange undoubtedly contribute to some extent to the total nitrogen available to the mussels (Nixon et al. 1986, Nixon et al. 1995). However, because of similar estuarine dimensions, we assume that tidal exchange is similar for the 10 Narragansett Bay sites and that water turnover rates do not vary significantly among the marshes. Therefore, the marine source of nitrogen in the diet of mussels can be considered to be similar across the study sites.

Land use correlations.—Watershed land use data was used to derive the fraction residential and the fraction of combined agricultural and recreational land use within the watersheds. The fractions of these two land use categories correlate significantly with the mussel nitrogen isotope ratios from the marshes. Other studies have indicated that nitrogen derived from these sources can have distinct isotope compositions. For example, nitrogen derived from wastewater and altered in isotopic composition during transport via groundwater and surface waters by volatilization and denitrification processes results in $\delta^{15}N$ values in the range of 10‰ to 20‰ (Kreitler et al. 1978, Gormley and Spalding 1979, Aravena et al. 1993, Macko and Ostrum 1994), while nitrogen from fertilizer is generally in the range of −3‰ to +3‰ (Freyer and Aly 1974). These ranges were confirmed in the present study by analyzing various fertilizers.

Based on studies linking nitrogen in primary producers to land-derived sources, nitrogen derived from wastewater would result in a more positive isotopic ratio, while that from fertilizer would result in a less positive isotope ratio (McClelland et al. 1997, McClelland and Valiela 1998, Aguilar et al. 1999). Our results confirm this effect, and agree with recent studies which report a similar increase in filter feeder $\delta^{15}N$ with increasing influx of wastewater-derived nitrogen in estuarine (Yelenik et al. 1996, McClelland et al. 1997, Fry 1999), and freshwater environments (McKinney et al. 1999). Correlations with land use practices in the present study suggest that the nitrogen isotope value in mussels in a marsh is influenced by nitrogen derived from anthropogenic activities within the marsh watershed.

The nitrogen isotope ratio of dissolved inorganic nitrogen (DIN) from marsh headwaters does not significantly correlate with mussel $\delta^{15}N$. For this study, $\delta^{15}N$ of DIN was a single measurement in the spring, and although care was taken to sample at least 72 h after any recorded rainfall, stream flow is generally increased in the spring. This may result in a measured $\delta^{15}N$ that does not reflect solely groundwater nitrogen, but also other contributions such as atmospheric deposition. Also, mussel $\delta^{15}N$ reflects that of marsh primary consumers, which to some extent fractionate nitrogen from DIN. This fractionation has been demonstrated to be highly variable and seasonally dependent (Cabana and Rasmussen 1996, Cifuentes et al. 1996, Roelke et al. 1999). Repeated measurements of DIN as well as primary producer isotope ratios over several seasons may be required to establish a direct relationship between DIN and mussel $\delta^{15}N$.

The $\delta^{15}N$ values for mussels at the marsh sites Bissel Cove (BIS) and Old Mill Cove (OLD) are higher, and those at Fogland Marsh (FOG) and Watchemoket Cove (WAT) are lower than predicted by the relationship of $\delta^{15}N$ with fraction residential land use (Fig. 5a). Both of these observations suggest that local effects and marsh characteristics may influence the nitrogen isotope signature. The marsh at FOG borders a large farm that is actively involved in potato production, and surface water flow characteristics show that runoff from this farm impacts the marsh site. The marsh watershed also contains significant residential development; however, the farm runoff, presumably influenced by nitrogen from fertilizer with its less positive isotopic ratio, could offset the contribution of the more positive influence of wastewater-derived nitrogen. Similarly, the marsh at WAT borders a large municipal golf course and although the watershed contains extensive residential development, the marsh is separated from developed areas by a road and a grass buffer area. The direct contribution of fertilizer nitrogen from the golf course coupled with the relatively long distance with concomitant losses (Valiela et al. 1997) that wastewater nitrogen would travel before reaching the marsh may result in a predominance of fertilizer-derived nitrogen being reflected in the isotopic signature. The marsh at BIS is surrounded by a relatively large watershed which shows overall moderate residential development (Fig. 2). However, there is dense residential development within a 200 m buffer zone immediately adjacent to the marsh. Previous studies have shown that wastewater nitrogen derived from septic systems within a 200 m zone can significantly influence estuarine and marsh environments (McClelland et al. 1997, Valiela et al. 1997). In this case, wastewater-derived nitrogen from the immediate proximity of the marsh may be driving the isotope ratio higher than that predicted by the overall fraction of residential development in the watershed. The marsh at OLD is surrounded by older, high density residential developments, and again shows dense development within a 200 m buffer of the marsh. Also, the marsh at OLD is highly impacted by development that physically encroaches on the marsh. This has resulted in a narrow, fringe marsh consisting primarily of low marsh dominated by tall *Spartina alterniflora* with very little high or upland marsh. The lack of an expansive marsh area and its nitrogen filtering capacity may result in anthropogenic nitrogen having a greater influence on the mussel isotopic ratio.

Predictive model.—The model developed with data from the original 10 sample sites showed varying degrees of agreement between predicted and measured mussel $\delta^{15}N$ values when tested with data from 12 additional marshes (Fig. 6). The two sites (BAK, SIL) which had similar marsh hydrology and stream nitrogen input to the original 10 gave the best agreement with the model as demonstrated by close proximity to the line with slope = 1 in the plots (Fig. 6). A trend towards actual values being lower than predicted for marshes which did not exhibit similar hydrology may be discerned (Fig. 6b). This may result from the effect of marsh hydrology on nitrogen residence time. Nitrogen entering better flushed embayments may be diluted or removed with the result that less nitrogen is available to affect marsh processes and be incorporated into the food chain. These comparisons indicate that more detailed information on marsh hydrology may have to be incorporated in order to further refine the model.

Conclusions

Based on initial laboratory experiments, this study demonstrates that ribbed mussels act as long-term integrators of the nitrogen isotopic ratios in their diet, and therefore, may be used as an indicator of watershed nitrogen sources to a salt marsh. For comparative purposes, mussels should be sampled at a consistent size range (i.e., shell length). Based on carbon isotope analysis, the mussels from our marshes appear to derive a significant portion of their food from land-based rather than marine sources. Mussel isotope ratios correlated with land use practices such as fraction residential development and fraction combined agricultural and recreational land, suggesting that the nitrogen isotope signature is influenced by nitrogen derived from anthropogenic sources such as wastewater and fertilizer. These correlations provide further evidence that the marsh resource base (i.e., primary producers) is to some extent processing nitrogen from land-based sources. We cannot infer the amount of anthropogenic nitrogen influencing biota at a marsh site, or the extent to which that nitrogen is utilized, from mussel isotope values. However, this study demonstrates that the nitrogen isotope values of mussels from a marsh reflect differences in the sources of nitrogen to the marsh, information which may be useful to coastal zone managers in assessing and monitoring coastal eutrophication. Nitrogen isotope data may need to be interpreted with caution, as our results suggest that land use practices in close proximity to marshes, and characteristics such as embayment hydrology and nitrogen residence time may influence the observed isotope values. A simple empirical model demonstrated the potential for predicting mussel isotope values from land use data for marsh areas in embayments with similar hydrology, geomorphology, and mode of nutrient input. However, further study is needed to determine if mussel isotope values and land use data can be effectively incorporated into a more robust model which can address differences in marsh hydrology.

Acknowledgments

The authors thank the internal technical reviewers Dan Campbell, Giancarlo Chiccetti, Marty Chintala, and Jim Lake for their comments, and Wayne Munns for critical review of the manuscript. Many thanks to Jonathan Garber for helpful discussions. Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the U.S. Environmental Protection Agency. This report has been reviewed by the U.S. EPA's National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, Narragansett, Rhode Island, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency. This is NHEERL-NARR Contribution 2124.

Literature Cited

Aguilar, C., M. L. Fogel, and H. W. Paerl. 1999. Dynamics of atmospheric combined inorganic nitrogen utilization in the coastal waters off North Carolina. Marine Ecology Progress Series **180**:65–79.

Anderson, J. R., E. E. Hardy, J. T. Roach, and R. W. Whitmer. 1976. A land use and land cover classification system for use with remote sensor data. Geological Survey Professional Paper 964. U.S. Geological Survey, Washington, D.C., USA.

Aravena, R., 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.

Boutton, T. W. 1991. Stable carbon isotope ratios of natural materials: II. Atmospheric, terrestrial, marine, and freshwater environments. Pages 173–185 *in* D. C. Coleman and B. Fry, editors. Carbon Isotope Techniques. Academic Press, San Diego, California, USA.

Bricker, S. B., D. E. Pirhalla, S. P. Orlando, and D. R. G. Farrow. 1999. National estuarine eutrophication assessment: effects of nutrient enrichment in the Nation's estuaries. NOAA, National Ocean Service, Special Projects Office and the National Center for Coastal Ocean Science, Silver Springs, Maryland, USA.

Cabana, G., and J. B. Rasmussen. 1994. Modeling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature **372**:255–257.

Cabana, G., and J. B. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proceedings of the National Academy of Sciences USA **93**:10844–10847.

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorous and nitrogen. Ecological Applications **8**:559–568.

Cifuentes, L. A., R. B. Coffin, W. C. Solorzano, J. Espinoza, and R. R. Twilley. 1996. Isotopic and elemental variations of carbon and nitrogen in a mangrove estuary. Estuarine, Coastal and Shelf Science **43**:781–800.

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. Geochimica Cosmochimica Acta **53**:2713–2721.

Corbett, D. R., J. Chanton, W. Burnett, K. Dillon, C. Rutkowski, and J. W. Fourqurean. 1999. Patterns of groundwater discharge into Florida Bay. Limnology and Oceanography **44**:1045–1055.

Cowling, E. B., J. W. Erisman, S. Smeulders, S. C. Holman, and B. M. Nicholson. 1998. Optimizing air quality management in Europe and North America: justification for integrated management of both oxidized and reduced forms of nitrogen. Environmental Pollution **102**:S1599–608.

Erisman, J. W., T. Brydges, K. Bull, E. Cowling, P. Grennfelt, L. Nordberg, K. Satake, T. Schneider, S. Smeulders, K. W. Van der Hock, J. R. Wisniewski, and J. Wisniewski. 1998. Summary statement. Environmental Pollution **102**:S13–12.

Fogel, M. L., and L. A. Cifuentes. 1993. Isotopic fractionation during primary production. Pages 73–98 *in* M. H. Engel and S. Macko, editors. Organic geochemistry. Kluwer Academic/Plenum, New York, New York, USA.

Fourqurean, J.W., T. O. Moore, B. Fry, and J. T. Hollibaugh. 1997. Spatial and temporal variation in C:N:P ratios, $\delta^{15}N$ and $\delta^{13}C$ of eelgrass *Zostera marina* as indicators of ecosystem processes, Tomales Bay, California. Marine Ecology Progress Series **157**:147–157.

Freyer, H. D., and A. I. M. Aly. 1974. Nitrogen—15 variations in fertilizer nitrogen. Journal of Environmental Quality **3**:405–406.

Fry, B. 1984. $^{13}C/^{12}C$ ratios and the trophic importance of algae in Florida *Syringodium* seagrass meadows. Marine Biology **79**:11–19.

Fry, B. 1988. Food web structure on Georges Bank from stable C, N, and S isotopic compositions. Limnology and Oceanography **33**:1182–1190.

Fry, B. 1999. Using stable isotopes to monitor watershed influences on aquatic trophodynamics. Canadian Journal of Fisheries and Aquatic Sciences **56**:2167–2171.

Fry, B., and E. B. Sherr. 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Marine Science **27**:13–47.

Giorgio, P. A., and R. L. France. 1996. Ecosystem-specific patterns in the relationship between zooplankton and POM or microplankton $\delta^{13}C$. Limnology and Oceanography **41**: 359–365.

Gormly, J. R., and R. F. Spalding. 1979. Sources and concentrations of nitrate-nitrogen in groundwater of the central Platte region, Nebraska. Ground Water **17**:291–301.

Gosner, K. L. 1971. Guide to identification of marine and estuarine invertebrates; Cape Hatteras to the Bay of Fundy. Wiley Interscience, New York, New York, USA.

Hawkins, A. J. S. 1985. Relationships between the synthesis and breakdown of protein, dietary absorption, and turnover of N and C in the blue mussel, *Mytilus edulis* L. Oecologia **66**:42–49.

Hoch, M. P., R. S. Snyder, L. A. Cifuentes, and R. B. Coffin. 1996. Stable isotope dynamics of nitrogen recycled during interactions among marine bacteria and protists. Marine Ecology Progress Series **132**:229–239.

Jordan, T. E., and I. Valiela. 1982. A nitrogen budget of the ribbed mussel, *Geukensia demissa*, and its significance in nitrogen flow in a New England salt marsh. Limnology and Oceanography **27**:75–90.

Kling, G. W., and B. Fry. 1992. Stable isotopes and planktonic trophic structure in arctic lakes. Ecology **73**:561–566.

Kreitler, C. W., S. Ragone, and B. G. Katz. 1978. $^{15}N/^{14}N$ ratios of ground water nitrate, Long Island, New York. Ground Water **16**:404–409.

Macko, S. A., and N. E. Ostrum. 1994. Sources of variation in the stable isotopic composition of plants. Pages 45–62 *in* K. Lajtha and R. H. Michener, editors. Stable isotopes in ecology and environmental science. Blackwell Scientific, Boston, Massachusetts, USA.

Mariotti, A. 1983. Atmospheric nitrogen is a reliable standard for natural ^{15}N abundance measurements. Nature **303**:685–687.

McClelland, J. W., and I. Valiela. 1998. Linking nitrogen in estuarine producers to land-derived sources. Limnology and Oceanography **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. Limnology and Oceanography **42**:930–937.

McKinney, R. A., J. L. Lake, M. Allen, and S. Ryba. 1999. Spatial variability in mussels used to assess base level nitrogen isotope ratio in freshwater ecosystems. Hydrobiologia **412**:17–24.

McMahon, R. F. 1991. Mollusca: Bivalva. Page 367 *in* J. H. Thorp and A. P. Covich, editors. Ecology and classification of North American freshwater invertebrates. Academic Press, Harcourt Brace Janovich, San Diego, California, USA.

Minigawa, 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.

Nixon, S. W., S. L. Granger, and B. L. Nowicki. 1995. An assessment of the annual mass balance of carbon, nitrogen and phosphorous in Narragansett Bay. Biogeochemistry **31**: 15–61.

Nixon, S. W., C. A. Oviatt, J. Frithsen, and B. Sullivan. 1986. Nutrients and the productivity of estuarine and coastal ecosystems. Journal of the Limnological Society of South Africa **12**:43–71.

Peterson, B. J., 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**:1361–3.

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.

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 nitrogen: an adaption of the ammonia diffusion method. Marine Chemistry **57**:227–242.

Simenstad, C. A., and R. C. Wissmer. 1985. $\delta^{13}C$ evidence of the origins and fates of organic carbon in estuarine and nearshore food webs. Marine Ecology Progress Series **22**: 141–152.

U.S. Environmental Protection Agency. 1998. National Strategy for the Development of Regional Nutrient Criteria. EPA 822-R-98-002, U.S. Environmental Protection Agency Office of Water, Washington, D.C., USA.

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. Ecological Applications **7**:358–380.

Waser, N. A. D., P. J. Harrison, B. Nielsen, S. E. Calvert, and D. H. Turpin. 1998. Nitrogen isotope fractionation during the uptake and assimilation of nitrate, nitrite, ammonium, and urea by a marine diatom. Limnology and Oceanography **43**:215–224.

Yelenik, S., J. W. 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. Biological Bulletin **191**:329–330.