AED Peer Review '07

Stable Isotope Research at AED: Collaborative Research to Assess the Source and Fate of Anthropogenic Nitrogen in Coastal Ecosystems

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Agency Problem

orative stable isotope research at AED benefits the National and Environmental Effects Laboratory's (NHEERL's) efforts, h Aquatic Stressors research, to develop and improve ecological and diagnostic capabilities for managers and to develop options tection and remediation efforts. Collaborative research projects in isotope ecology at AED also compliment the long term goals of s Multi-Year Plan for Water Quality by:

ding basic research to support the development of tools to assess ces of nitrogen impairment to coastal ecosystems

ding tools for monitoring the progress of ecosystem restoration

loping and assessing approaches to long term monitoring of ent inputs to coastal systems

Research Goals

he past several years AED has been collaborating with academic, l, and international researchers on stable isotope research projects e major areas of emphasis: 1) determining the source and fate of pogenic nitrogen inputs to coastal ecosystems, 2) resource tion and habitat use by migrating and breeding songbirds, and 3) ring food web changes and eutrophication in coastal salt marshes

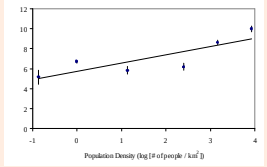
Methods and Approach

we describe two projects to develop tools to monitor and assess the and fate of anthropogenic nitrogen in coastal systems, and two oles of the application of stable isotopes to identify sources of en to aquatic systems. The general approach of these studies es the use of stable nitrogen isotope ratios to track anthropogenic to coastal systems. Nitrogen isotope ratios are generally reported as ference (δ) between the $^{15}N/^{14}N$ ratio of a sample and the ratio of an pheric nitrogen standard. Different types of nitrogen sources tend to ociated with different ranges of $\delta^{15}N$ values; for example, dwater containing nitrate derived solely from atmospheric tion generally has $\delta^{15}N$ values ranging from 2 to 8‰, groundwater from human and animal wastes has δ15N values of 10 to 20‰, and values greater than 30‰ have been reported in wastewater treatment ffluent. Synthetic fertilizer is isotopically light, resulting in dwater $\delta^{15}N$ values of -3 to 3‰. These differences in stable isotope can be used to track the influence of nitrogen from different s on aquatic food webs.

1) Using stable isotopes to monitor anthropogenic nitrogen inputs to estuaries

(Rebecca Bannon and Charles Roman)

To further develop the role of stable isotopes as indicators of anthropogenic nutrient enrichment in long-term ecosystem monitoring, resident producer and consumer species were collected from *Spartina alterniflora* dominated marshes subject to a range of anthropogenic impact in Cape Cod, Massachusetts; Great South Bay, New York; and Jamaica Bay, New York. Tissue isotope ratios of *Spartina alterniflora, Ulva lactuca, Fundulus heteroclitus,* and *Geukensia demissa* were analyzed in order to determine which organisms are the most sensitive indicators of changes in anthropogenic nitrogen source and loading.



$\delta^{15}N$ and Watershed Development

Figure 1. $\delta^{15}N$ values for *Spartina alterniflora* vs. increasing human population in the watershed ($y = 0.8x + 5.7$, $R^2 = 0.7$, $p = 0.04$). Error bars are standard error.

Spartina $\delta^{15}N$ values increased, although not significantly, along a gradient of increasing human population density

Using stable isotopes to monitor anthropogenic nitrogen inputs to estuaries

Variability of $\delta^{15}N$ Values within Marsh Sites

Table 1. Mean $\delta^{15}N$ values (‰) for biota collected in the six study marshes. N=5 for each species. Numbers in parentheses are standard deviations. Letters indicate significantly different $\delta^{15}N$ values within each species (LSM, $p < 0.05$); values labeled with the same letter are not different.

Study Marshes	*Spartina*	Juvenile *Fundulus*	Adult *Fundulus*	*Geukensia*	*Ulva*
Hatches Harbor, MA	5.1 (1.6)^a	8.5 (0.3)^a	9.1 (0.2)^a	7.2 (0.2)^a	-
Fire Island, NY	6.7 (0.2)^b	9.2 (0.6)^b	10.7 (0.4)^b	-	-
Nasset Bay, MA	5.8 (0.9)^{ab}	9.9 (0.3)^c	11.5 (0.5)^c	7.7 (0.2)^b	7.1 (0.5)^a
Town Cove, MA	6.2 (0.8)^b	10.1 (0.2)^c	11.6 (0.2)^c	7.4 (0.2)^{ab}	-
West Sayville, NY	8.6 (0.5)^c	11.2 (0.5)^d	12.7 (0.6)^d	-	-
Jamaica Bay, NY	10.0 (0.6)^c	14.3 (0.3)^e	15.3 (0.3)^e	10.7 (0.7)^c	11.6 (1.5)^b

- **Isotope ratios of consumer species were less variable within each marsh than those of primary producer species**
- **This suggests that consumers might be more reliable indicators of nutrient source than producers**

Sample Size Analysis

Table 2. Sample sizes necessary to detect changes in $\delta^{15}N$ value of producer and consumer species. Sample sizes represent the number of samples per marsh or sampling interval that would be necessary to detect differences with 80% power at a significance level of 0.05.

Difference in $\delta^{15}N$ value (‰)	Sample size				
	Spartina	*Geukensia*	Juvenile *Fundulus*	Adult *Fundulus*	*Ulva*
0.5	37	9	9	10	66
0.6	26	7	7	8	46
0.7	20	6	5	6	34
0.8	15	5	5	5	27
0.9	12	4	4	4	21
1.0	10	4	4	4	18

- **It is desirable to design a monitoring protocol that could detect at least a 1.0‰ difference in Spartina isotope values**
- **Results showed that a 1.0‰ difference could be detected using a sample size of 10**

2) Use of stable isotopes to assess nitrogen sources and fate in Narragansett Bay

(Joaquin Chaves and Scott Nixon)

Anthropogenic nitrogen (N) inputs have been identified as the main source of N to Narragansett Bay (RI, USA). However, high levels of primary production during summer cannot be supported by land-based sources alone, and inputs from adjacent Rhode Island Sound have been invoked as a potential source to account for the Bay's summer production. Using stable isotope tracers, we estimated the input of nitrogen from the Rhode Island Sound entering the Bay during one summer season.

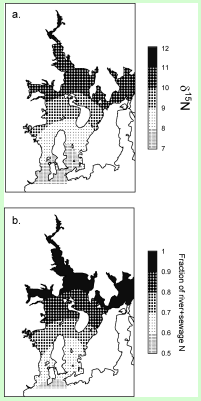


Figure 2a. Modeled $\delta^{15}N$ of dissolved inorganic nitrogen (δMIX). **b**. Estimated fraction of the total dissolved inorganic nitrogen derived from sewage and river inputs.

- **Results suggest that the $\delta^{15}N$ of phytoplankton in the Bay is in great part controlled by the $\delta^{15}N$-DIN along the salinity gradient, which in turn would be largely the product of the mixture of two isotopically different end members.**
- **We estimated a total of 35 x 10^6 moles of nitrogen coming from the R.I. Sound into Narragansett Bay from June through September of 2000, corresponding to an average monthly DIN input of 27 mmoles m^{-2}, not enough to satisfy the nitrogen deficit for primary production in Narragansett Bay.**
- **This suggests that recycling within the water column by bacteria and micro-zooplankton may support most of the carbon fixation measured during the summer.**
- **When fully developed, this approach could provide a powerful tool for tracing the sources and magnitudes of nitrogen inputs into east coast estuaries.**

Future studies are looking at describing the nitrogen isotopic gradient in biota in Narraganset Bay, particularly in the context of proposed changes brought about by tertiary treatment upgrades to reduce nitrogen loads to the Bay (Autumn Oczkowski and Scott Nixon). Preliminary results have suggested that approximately half of the nitrogen commercially harvested clams in Narragansett Bay may be from anthropogenic sources.

Additional Stable Isotope Collaborations

Dissertations and theses:

Chaves, J. 2004. Potential use of 15N to assess nitrogen sources and fate in Narragansett Bay. Ph. D. Dissertation, University of Rhode Island Graduate School of Oceanography.

Podlesak, D. 2004. Metabolic routing of macronutrients in migratory songbirds : effects of diet quality and macronutrient composition revealed using stable isotopes. Ph. D. Dissertation, University of Rhode Island.

Wozniak, A. 2004. Monitoring salt marsh restoration by evaluating trophic relationships : a multiple stable isotope approach. University of Rhode Island Graduate School of Oceanography.

DiMilla, P.A. 2006. Using stable nitrogen isotopes to characterize and evaluate nitrogen sources to Greenwich Bay, RI and their influence on isotopic signatures in estuarine organisms. M.S. Thesis, University of Rhode Island Graduate School of Oceanography.

Bannon, R. Assessing the use of stable isotopes to monitor anthropogenic nitrogen inputs to estuarine systems. M.S. Thesis, University of Rhode Island Graduate School of Oceanography.

Winiarski, K. 2007. Use of multiple stable isotopes to assess the effects of herbivory by nesting snow geese on coastal salt marshes. M.S. Thesis, University of Rhode Island.

Additional collaborative stable isotope projects at AED :

- Investigations into resource allocation and habitat use by migrating songbirds:

Podlesak, D.W., and McWilliams, S.R. 2006. Metabolic routing of dietary nutrients in birds: Effects of diet quality and macronutrient composition revealed using stable isotopes. Physiological and Biochemical Zoology 79(3): 534-549.

Podlesak, D.W., McWilliams, S.R., and Hatch, K.A. 2005. Stable isotopes in breath, blood, feces and feathers can indicate intra-individual changes in the diet of migratory songbirds. Oecologia 142(4): 501-510.

- Using multiple stable isotopes to monitor food web changes in coastal marshes:

Wozniak, A.S., Roman, C.T., Wainright, S.C., McKinney, R.A., and James-Pirri, M.J. 2006. Monitoring food web changes in tide-restored salt marshes: a carbon stable isotope approach. Estuaries and Coasts 29: 568-578.

- Investigations into the fate of nitrogen in the Gulf of Maine:

Sherwood, O.A., Heikoop, J.M., Scott, D.B., Risk, M.J., Guilderson, T.J., and McKinney, R.A. 2005. Stable isotopic composition of deep sea gorgonian corals Primnoa spp.: a new archive of surface processes. Marine Ecology Progress Series 301: 135-148.

- Regional application of nitrogen stable isotopes to identify the source of anthropogenic nitrogen to coastal ecosystems:

Cole, M.L., Valiela, I., Kroeger, K.D., Tomasky, G.L., Cebrian, J., Wigand C., McKinney, R.A., Grady, S.P., and Silva, M.E.C. 2004. Assessment of a ^{15}N isotopic method to indicate anthropogenic eutrophication in aquatic ecosystems. Journal of Environmental Quality 33: 124-143.

Anthropogenic enrichment and nutrients in some tropical lagoons of Ghana, West Africa

(Scott Nixon and M. Entsua-Mensah)

As part of a larger study of demographic change in coastal Ghana, we measured the concentrations of major plant nutrients and phytoplankton chlorophyll in eight coastal lagoons with different land use and human population density. The purpose of our study was to relate human activities to water quality in coastal receiving waters. We also carried out preliminary measurements of stable nitrogen isotopes to quantify the contribution of sewage and fertilizer to fish production in the lagoons.

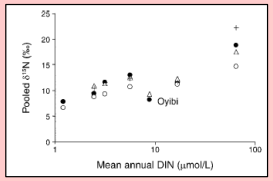


Figure 3. Stable nitrogen isotope ratios in pooled samples of each of the four species collected as a function of the log of the annual mean concentration of dissolved inorganic nitrogen (DIN, log scale) in each lagoon. Solid circles are blackchin tilapia, open circles are mullet, open triangles are Callinectes crabs, and crosses are shrimp.

- **We found a strong correlation between human population density and mean annual dissolved inorganic nitrogen (DIN) concentrations**
- **Stable nitrogen isotope ratios in biota varied consistently among the lagoons, with higher $\delta^{15}N$ values indicative of human sewage in lagoons downstream of more densely populated watersheds**
- **There appears to be a general trend for the more nitrogen-enriched systems to also reflect more human or livestock waste**

Spatial variability of nitrogen isotope ratios of particulate material from northwest Atlantic continental shelf waters

(Richard McKinney and Jerry Prezioso)

Human encroachment on the coastal zone has led to a rise in the delivery of nitrogen to estuarine and near-shore waters. Potential routes of anthropogenic nitrogen inputs include atmospheric deposition and dissolved N inputs from groundwater outflow and export from estuaries. Stable nitrogen isotope ratios provide a means to assess the source of N to shelf waters, particularly since dissolved anthropogenic nitrogen has elevated $\delta^{15}N$ values (range: 7 – 30 ‰). We collected particulate matter from surface waters off the US east coast during spring, summer, and fall from 2000 – 2005.

INSHORE vs. OFFSHORE: all sites, by year by season

Year	Season	Near-shore	Offshore	df	F	p
2000	Fall	6.42	3.49	1	23.2	<0.001
2001	Spring	5.92	5.46	1	0.26	0.28
	Summer	3.41	3.71	1	0.37	0.56
	Fall	6.13	3.62	1	17.9	<0.001
2002	Winter	4.83	4.56	1	0.14	0.71
	Spring	6.85	5.25	1	5.38	0.04
	Fall	6.45	4.59	1	8.76	0.01
2003	Summer	5.30	4.15	1	0.8	0.4
2004	Spring	6.70	4.73	1	10	0.009
	Summer	5.21	3.29	1	3.22	0.11
	Fall	7.32	5.20	1	21.6	0.001
2005	Fall	7.55	3.65	1	25.4	0.002

Table 3. Near-shore (mean 33.7 km from estuary mouth) $\delta^{15}N$ values ranged from 5.5 – 7.7 ‰ and increased from north to south. Offshore values (mean 92.4 km from estuary mouth) were consistently lower than near-shore sites (average 4.7 ± 1.0 ‰ versus 6.8 ± 1.1 ‰).

- **We are currently applying a linear, concentration-dependent isotope mixing model to investigate the proportion of dissolved anthropogenic nitrogen that would result in measured $\delta^{15}N$ ratios at our near-shore sites**
- **Models of this sort can be used to gain insight on the source of nitrogen to shelf waters.**

Impacts and Outcomes

In addition to supporting the long term goals of ORD's Multi-Year Plan for Water Quality by providing sound basic science to support indicator development, our collaborative research projects also provide important insights into the ecology of estuarine ecosystems. For example, the finding that most of the carbon fixed in an east coast estuary during the summer is probably supported by recycling by bacteria and micro-zooplankton within the water column is an important element of the ecology of shallow estuaries that needs to be considered during indicator development. Similar studies carried out with the support of our facility have suggested that differences in pH from nitrogen-stimulated photosynthesis and related shifts in predominance of dissolved carbon species may exert control on the observed $\delta^{13}C$ signatures in coastal systems, and that nitrogen from anthropogenic sources may be an important component in maintaining populations of a commercially important shellfish species.

Collaboration with academic and other federal partners allows us access to the basic scientific research needed to support the development develop tools to assess sources of nitrogen impairment to coastal ecosystems and for monitoring the progress of ecosystem restoration. Often this type of research may be beyond the scope of our scope of work here at AED, but nonetheless is a critical step in developing these types of indicators. Collaborative projects provide a situation where academic researchers and their students are allowed access to an important analytical resource, and EPA benefits from the resulting science.

Future Directions

Several collaborative stable isotope projects are currently taking place that include collaboration of AED with outside researchers:

- A study is underway to test the hypothesis that the dramatic recovery of the fishery off the Mediterranean Coast of Egypt during the past two decades is due to a stimulation of primary production from anthropogenic nutrients that have replaced those carried by the historic flood of the Nile River before it was virtually eliminated by the Aswan High Dam in 1965. Stable isotopes will be used as a tool to trace the influence of anthropogenic nitrogen (and by association, phosphorous) through the Nile River's delta ecosystem and onto the adjacent Levantine Shelf. This research may provide a compelling case study of the utility of nitrogen stable isotopes in detecting pollution signatures in large and diverse ecosystems. (Autumn Oczkowski and Scott Nixon)
- A collaborative effort between AED, researchers at the University of Rhode Island, and biologist with the Delaware Department of Environmental Protection is looking at resource allocation and habitat use by migrating and wintering brant along the east coast of the U.S.

EPA Atlantic Ecology Division Narragansett, Rhode Island