Multiple Stable Isotopes Used to Trace the Flow of Organic Matter in Estuarine Food Webs

Abstract. *The use of a combination of the stable isotopes of sulfur, carbon, and nitrogen allows the flow of organic matter and trophic relations in salt marshes and estuaries to be traced while eliminating many ambiguities that accompany the use of a single isotopic tracer. Salt-marsh grasses take up the isotopically light sulfides formed during sulfate reduction, and the transfer of this light sulfur through the marsh food web is illustrated with data on the ribbed mussel* (Geukensia demissa) *from various locations in a New England marsh. The multiple isotope approach shows that this filter feeder consumes both marsh grass* (Spartina) *detritus and plankton, with the relative proportions of each determined by the location of the mussels in the marsh.*

Salt-marsh embayments and estuaries serve as important nursery and rearing grounds for many species of fish and shellfish. Analysis of the trophic structure of the salt-marsh–estuarine community is complicated by the detrital character of major parts of the food web, the opportunistic feeding behavior of many species, the migratory or transitory patterns of use by certain species, and most importantly, the variety of potentially important organic matter sources including marsh grasses such as *Spartina alterniflora*, plankton, benthic algae, eelgrass, chemosynthetic and photosynthetic bacteria, and organic matter imported from upland communities.

Stable isotopic tracers such as ^{13}C and ^{15}N have been used to follow the flows of organic matter in marshes and estuaries (*1*). The stable isotope ratios of carbon are different for upland C-3 plants, plankton, and *Spartina alterniflora*, a C-4 plant (Table 1) (*2, 3*). The nitrogen isotope ratio is higher in marine plankton than in terrestrial vegetation (*4*).

The stable sulfur isotope ^{34}S is an additional tool for studying energy flow in salt marshes and estuaries, particularly when used with ^{13}C and ^{15}N. The stable sulfur isotope ratios in the organic matter of marine algae, of upland plants, and of marsh grasses are different because their sources of inorganic sulfur are different. Planktonic algae and seaweeds draw on sea-salt sulfate with $\delta^{34}S$ value of about +20.3 per mil (*5*) and fractionate it little during uptake and assimilation into organic sulfur compounds (*6*). Upland plants in aerobic soils also fractionate sulfate little during uptake and assimilation, but they obtain sulfate ion originating in precipitation with a $\delta^{34}S$ value in the range of +2 to +8 per mil (*7*). Marsh plants such as *Spartina* which are rooted in anoxic sediments apparently use sulfides for at least some of the sulfur needed to produce their organic sulfur compounds (*8*). Since these sulfides are greatly depleted in ^{34}S, the organic matter produced by *Spartina* is also depleted in ^{34}S.

We studied the distribution of sulfur, carbon, and nitrogen isotopes in the biota of Great Sippewissett Marsh, Falmouth, Mass. *Spartina alterniflora* is the dominant primary producer in this and most other salt marshes of the eastern coast of the United States. We have analyzed 14 samples of *Spartina* from Great Sippewissett Marsh and found $\delta^{34}S$ values ranging from −7.7 to +5.5 per mil with a mean value of −2.4 per mil and standard deviation of ±4.4. The crop of organic matter produced by *Spartina* has a sulfur isotopic signature that is quite distinct from the $\delta^{34}S$ value characteristic of both seawater sulfate (+20.3 per mil) and planktonic organisms (+19 per mil) (Table 1).

Planktonic algae are also potentially important as food for consumers in Sippewissett Marsh because the marsh is flooded twice daily with seawater from Buzzards Bay. A plankton sample collected by towing a 300-μm mesh net in Woods Hole passage in November 1981 gave a $\delta^{34}S$ value of +18.6 per mil and a $\delta^{13}C$ value of −20.0 per mil for a catch of mixed diatoms and copepods with smaller amounts of detritus.

For the sulfur isotope to be useful as a tracer in food web studies, we must know whether there are large changes in isotopic composition between an organism's diet and its body tissue. Two feeding studies have shown that the sulfur isotope ratio in an organism reflects the ratio in its food. Gypsy moth caterpillars raised on a commercial baby food had a $\delta^{34}S$ value of +7.6 per mil compared with +6.3 per mil for the food. Small trout fed a diet with a $\delta^{34}S$ value of +8.2 per mil had a $\delta^{34}S$ value of +9.4 per mil, whereas large ones had a value of +9.6 per mil. These trophic shifts are small relative to the range of sulfur isotope values (−10 per mil to +20 per mil) for organisms found in the marsh. The carbon isotope ratio also shifts slightly with consumers that are slightly heavier than their food (*9*). Nitrogen isotope ratios are enriched by as much as 2 to 3 per mil with each trophic transfer (*10*).

A dual isotope or multiple isotope approach provides significantly more power to resolve food web structure than the use of a single stable isotope. An examination of mean values and standard deviations for plankton, *Spartina*, and upland plants shows that some combinations of isotopes are more powerful than others for distinguishing organic matter sources (Table 1). Carbon-13 is very useful for distinguishing upland plants from *Spartina* because the mean values are widely separated and the variation is relatively small. Use of ^{15}N is limited because the overall range is small and the variability in a plant like *Spartina* is quite high. Sulfur-34 is useful for separating plankton from *Spartina* and upland plants from plankton but is not as powerful for distinguishing upland plants from *Spartina*.

We used multiple isotope tracers to study the dominant filter feeder in the marsh, *Geukensia demissa*, the ribbed mussel (*11*). Isotopic compositions of mussels from nine sites along a transect running from the back reaches of the marsh toward the main tidal channel (Fig. 1) were examined and $\delta^{34}S$ values were found to depend on the location where the mussels grew in the marsh. The highest values were in mussels from the banks of the main tidal channels, and the values decreased systematically to the low values characteristic of *Spartina* in the innermost reaches of the marsh along mosquito ditch banks (site 1), on the marsh surface (site 2), and in pannes on the marsh surface (site 3). It seems

Table 1. Mean carbon, nitrogen, and sulfur isotope ratios and standard deviations for plankton, *Spartina*, and upland plants. Units are per mil (*2, 3*). Data on plankton: $\delta^{13}C$ (*14*), $\delta^{15}N$ (*10*), and $\delta^{34}S$ (*5, 15*).

Sample	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Upland C-3 plants	−28.6 ± 1.3 (4)*	−0.6 ± 1.2 (2)	+4.7 ± 0.9 (2)
Spartina C-4	−13.1 ± 0.8 (17)	+3.8 ± 2.6 (7)	−2.4 ± 4.4 (14)
Plankton	−21.3 ± 1.1 (56)	+8.6 ± 1.0 (4)	+18.8 ± 0.6 (4)

*Numbers in parentheses represent the number of samples.

reasonable that mussels remote from the main tidal channels would have ready access to *Spartina* detritus but limited access to plankton and that mussels near Buzzards Bay would have ready access to plankton but limited access to *Spartina* detritus. However, the sulfur isotope data alone do not allow such conclusions to be drawn. It is not possible on the basis of the sulfur isotope data alone to rule out the possibility that organic matter originating from the uplands might contribute to the mussels' food since the $\delta^{34}S$ values for upland plants are intermediate between the values for *Spartina* and plankton. A second tracer such as ^{13}C is needed to prove that these tentative conclusions are valid.

We plotted the distribution of the major foods available in the marsh showing $\delta^{34}S$ values as a function of $\delta^{13}C$ (Fig. 2A). We assumed that there are only three major food resources—plankton, *Spartina*, and upland plants—as would be the case in many riverine estuaries. In fact, Great Sippewissett is a pocket marsh, and organic matter input from the uplands is minor, but the same approach would be of value in a riverine estuary, where organic matter input from rivers is important.

A mixture of organic matter from upland plants and *Spartina* would have a $\delta^{13}C$ value similar to plankton on the $\delta^{13}C$ axis, but these three categories of organic matter are well separated on Fig. 2A. A mixture of *Spartina* and plankton would appear identical to organic matter originating from the uplands on the $\delta^{34}S$ axis, but the addition of the $\delta^{13}C$ data resolves this ambiguity.

The carbon isotope values, although they span a much smaller range than the $\delta^{34}S$ values, support the interpretation that *Spartina* and plankton are the main food sources for the ribbed mussel (Fig. 2A). The relative proportions of *Spartina* and plankton vary from site to site. Mussels in the interior portions of the marsh have an isotopic composition that reflects a diet of 80 percent *Spartina*, whereas the diet of mussels near Buzzards Bay consists of up to 70 percent plankton and only 30 percent *Spartina*. Examination of $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 2B) also suggests a mixture of *Spartina* detritus and plankton in the diet. The mussels from site 3, which were collected in a panne on the marsh surface, had a low $\delta^{15}N$ value, perhaps reflecting a larger input of fixed nitrogen. It appears from each approach (Fig. 2, A and B) that the food resources available to the ribbed mussel are isotopically different at various locations in the marsh.

The idea that the diet of consumers may be qualitatively different at various locations has not received sufficient attention in trophic studies. Consumers are often categorized as detritivores, herbivores, or planktivores. Data on these ribbed mussels show that a filter feeder well adapted to feed on plankton and even on small free-living bacteria (*12*) can also use detrital organic matter with its associated fungi and bacteria when it is available as small particles in the tidal currents.

Ribbed mussels throughout the marsh are located at high elevation on the creek banks rather than on the creek bottoms. A creek bottom organism, such as the hard clam *Mercenaria mercenaria*, collected in the main channel near Buzzards Bay in November 1981, had a low $\delta^{34}S$ value of +2.6 per mil (Fig. 2A), whereas mussels collected on the creek banks near that site had a $\delta^{34}S$ value of +12.1 per mil. Clams in the channel bottom can feed during low ebbing tides when detritus from the marsh is in the channel, whereas the ribbed mussels are out of water for much of the ebbing tide period. Thus, the vertical as well as horizontal location of an organism in the marsh can determine the type of food available and hence the isotopic ratios in its tissues. The blue mussel *Mytilus edulis*, collected in Woods Hole Passage in the fall, had a $\delta^{34}S$ value of +18.6 per mil and a $\delta^{13}C$

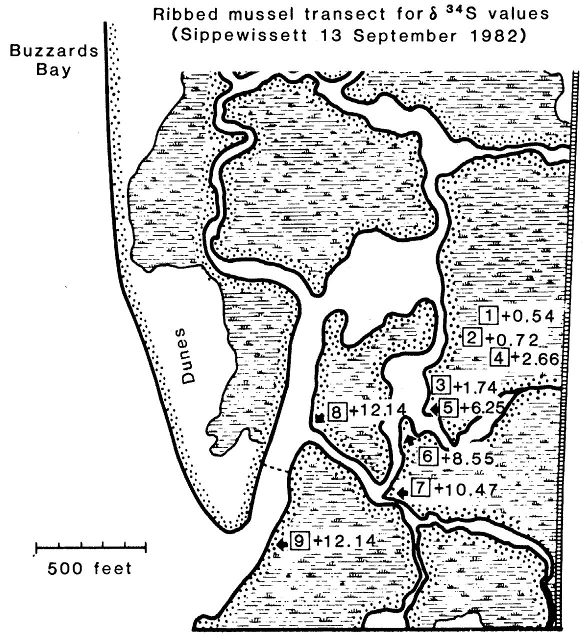

Fig. 1. The collection sites and $\delta^{34}S$ values for the ribbed mussel, *Geukensia demissa*, collected along a transect from the inland portions of Great Sippewissett marsh to Buzzards Bay. Site 1 is a mosquito ditch, site 2 is a low-lying area on the marsh surface, site 3 is a panne, and sites 4 through 9 are creek bank locations in a progression from lower to higher order creeks.

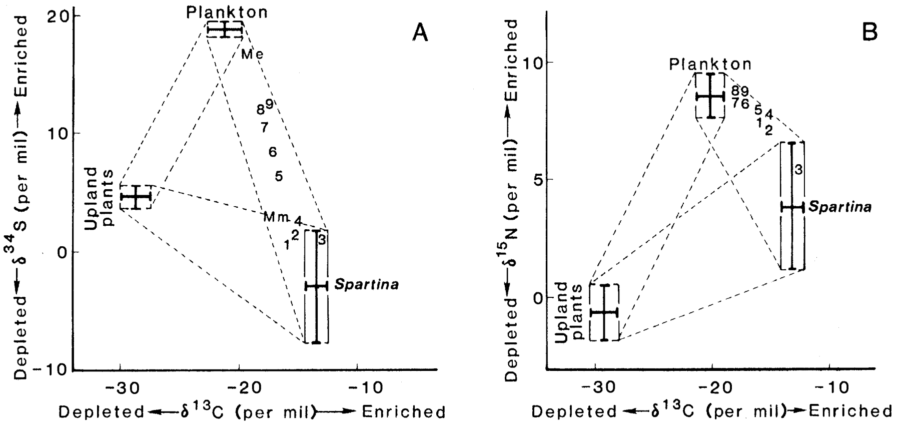

Fig. 2. (A) The position of *Geukensia demissa* from the sites shown in Fig. 1 with $\delta^{34}S$ values plotted as a function of $\delta^{13}C$. Bars are means ± 1 standard deviation for *Spartina* ($n = 11$) and upland plants ($n = 4$ for C and 2 for S). For plankton the values for $\delta^{34}S$ are from (*15*) plus our value of +18.6 per mil; the $\delta^{13}C$ values are the means and standard deviations from (*14*). The points 1 to 9 are isotope ratios for muscle tissue of *Geukensia demissa*. Abbreviations: *Mm* refers to *Mercenaria mercenaria* collected in the channel bottom near the location of sample number 9 of the ribbed mussel collection; *Me* refers to a collection of *Mytilus edulis* taken from Woods Hole Passage several kilometers from the marsh. (B) The mussel transect data showing $\delta^{15}N$ plotted as a function of $\delta^{13}C$.

value of −19.4 per mil (Fig. 2A). Both values indicate a diet predominantly composed of plankton; this is consistent with the site of collection, far from any significant area of marshes.

In summary, the stable isotopes of sulfur offer a valuable tool for tracing the flow of light sulfur produced during sulfate reduction in marine sediments. The sulfur in *Spartina* is isotopically light relative to the sulfur in plankton. The $\delta^{34}S$ values found in animals vary systematically in a manner that appears to reflect diet. The use of multiple isotopes, $\delta^{13}C$, $\delta^{34}S$, and $\delta^{15}N$, allows us to distinguish between three or more potential food resources.

The ribbed mussels in Great Sippewissett Marsh appear to obtain the bulk of their food from a source that is depleted in ^{34}S and enriched in ^{13}C. *Spartina* is the most likely source of this food, but it must be broken down to fine detrital particles before it is available to the mussels. Plankton appears to be the second major food resource, especially for the mussels near the main marsh channels connecting with Buzzards Bay. This finding supports the concept that marsh detritus is distributed along a gradient of maximum availability in the smallest marsh creeks to lower availability near the ocean (*13*), but there may be vertical gradients as well. As expected, there is no evidence for an important input of organic matter from the uplands, since Sippewissett is a pocket marsh with no major riverine input.

Where there is a major river input of organic matter, a combination of carbon, sulfur, and nitrogen isotopes can be used to discriminate between organic matter derived from the plankton, from salt-marsh plants, and from upland plants. The $\delta^{13}C$ and $\delta^{34}S$ values of consumer organisms appear to reflect within about 1 or 2 per mil what they eat. This multiple isotope approach may help resolve long-standing questions about the role of salt marshes in the support of coastal fisheries and, in particular, the role of *Spartina* in providing detritus to marsh and estuarine consumers.

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References and Notes

1. B. Fry, W. L. Jeng, R. S. Scalan, P. L. Parker, J. Baccus, *Geochim. Cosmochim. Acta* **42**, 1289 (1978); B. Fry, A. Joern, P. L. Parker, *Ecology* **59**, 498 (1978); B. Fry and P. L. Parker, *Estuarine Coastal Mar. Sci.* **8**, 499 (1979); B. Fry, R. S. Scalan, P. L. Parker, *Geochim. Cosmochim. Acta* **41**, 1875 (1977); C. T. Hackney and E. B. Haines, *Estuarine Coastal Shelf Sci.* **10**, 703 (1980); E. B. Haines, *Limnol. Oceanogr.* **21**, 880 (1976); *Estuar. Coastal Mar. Sci.* **4**, 609 (1976); ______ and C. L. Montague, *Ecology* **60**, 48 (1979); E. B. Haines, *Oikos* **29**, 254 (1977); C. McMillan, P. L. Parker, B. Fry, *Aquat. Bot.* **9**, 237 (1980); K. E. Peters, R. E. Sweeney, I. R. Kaplan, *Limnol. Oceanogr.* **23**, 598 (1978).
2. The δ notation indicates the depletion (−) or enrichment (+) of the heavy isotope relative to the lighter isotope according to the formula $X = [(R_{\mathrm{sample}} - R_{\mathrm{standard}})/R_{\mathrm{standard}}] \times 10^3$ where X is $\delta^{13}C$, $\delta^{34}S$, or $\delta^{15}N$ and R is $^{13}C/^{12}C$, $^{34}S/^{32}S$, or $^{15}N/^{14}N$. The standards are Peedee Belemnite for carbon, Canyon Diablo troilites for sulfur, and air for nitrogen.
3. Plant tissue samples for $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$ were initially washed free of extraneous mud and debris. The tissues were dried at 70°C, then ground in a Wiley mill (1 mm mesh). Samples were washed in four 1-hour rinses of deionized water (4:1, water to sample). Samples were redried at 70°C. Plankton tow samples for $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$ were collected in a 153-μm mesh nitex net. The sample was rinsed four times, first with tap water and then with deionized water in the 153-μm cod end of the plankton net. The sample was dried at 70°C. Animal tissue samples for $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$ were prepared in a manner preventing gut and bone contamination. Animal tissue was dried at 70°C and then ground with mortar and pestle. Samples were washed in four 1-hour rinses of deionized water (4:1 water to sample). Samples were dried at 70°C. A subsample (>3 g, dry weight) was selected for $\delta^{34}S$ analysis. The remaining sample (>0.1 g for $\delta^{13}C$ and $\delta^{15}N$) was washed in acid (10 percent HCl) for 1.5 hours to remove carbonate contaminants and again washed in deionized water. Samples of plant and animal tissues were examined for $\delta^{13}C$ and $\delta^{34}S$ either at Geochron Labs, Boston, Mass., or at Global Geochemistry Corp., Canoga Park, Calif. All $\delta^{15}N$ determinations were made at Global Geochemistry Corp.
4. K. E. Peters, R. E. Sweeney, I. R. Kaplan, *Limnol. Oceanogr.* **23**, 598 (1978).
5. I. R. Kaplan, K. O. Emery, S. C. Rittenberg, *Geochim. Cosmochim. Acta* **27**, 297 (1963).
6. V. U. Mekhtiyeva and R. G. Pankina, *Geokhimiya* **6**, 719 (1968).
7. J. O. Nriagu and R. D. Coker, *Tellus* **30**, 365 (1978).
8. P. R. Carlson, Jr., and J. Forrest, *Science* **216**, 633 (1982).
9. T. McConnaughy and C. P. McRoy, *Mar. Biol.* **53**, 257 (1979).
10. G. Rau, in *Coastal Water Research Project, Biennial Report, 1981–1982*, W. Bascom, Ed. (Southern California Coastal Water Research Project, Long Beach, Calif., 1982), p. 143.
11. T. E. Jordan and I. Valiela, *Limnol. Oceanogr.* **27**, 75 (1982).
12. R. T. Wright, R. B. Coffin, C. P. Ersing, D. Pearson, *ibid.*, p. 91.
13. W. E. Odum, *Bull. Mar. Sci.* in press.
14. J. N. Gearing, P. J. Gearing, D. T. Rudnick, A. G. Requejo, M. J. Hutchins, *Geochim. Cosmochim. Acta*, in press.
15. M. Hartman and H. Nielsen, *Geol. Rundsch.* **58**, 621 (1969); I. R. Kaplan and S. C. Rittenberg, *J. Gen. Microbiol.* **34**, 195 (1964).
16. We thank S. Beard, R. Marino, and S. Merkel for assistance in collecting and preparing samples. Gypsy moths were provided by the U.S. Department of Agriculture, Otis Methods Development Center, Otis Air Force Base, Massachusetts. Samples of trout chow and trout raised on this food were provided by S. McCormick of the Woods Hole Oceanographic Institution. Supported by NSF grant 81-04701.

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Mutual Restraint in Tree Swallows: A Test of the TIT FOR TAT Model of Reciprocity

Abstract. *The TIT FOR TAT model of reciprocity, which is based on a successful program for the game known as the Prisoner's Dilemma, was experimentally tested on a population of tree swallows (*Tachycineta bicolor*). Parent and nonbreeding tree swallows have conflicts of interests that resemble those in the Prisoner's Dilemma. TIT FOR TAT predicts restraint of conflict before a competitor's act of defection, retaliation after defection, and a resumption of restraint following retaliation. After a simulated act of defection by nonbreeders, parents behaved as predicted by the model.*

Contemporary evolutionary biology has confronted the challenge of explaining the existence of cooperative behavior by invoking kinship (*1*) and reciprocity (*2*) theory. Empirical evidence, particularly from avian communal breeding systems, has generally supported kinship theory (*3*), although some studies (*4*) have revealed that the genetic relatedness of interactants is too low for kin selection to be the ultimate force maintaining cooperative behavior.

Until recently (*4*), reciprocity has received little attention in empirical studies of avian social systems because of the lack of a formal theory of reciprocity to adequately explain how it could first develop among unrelated individuals and then persist in competition with individuals that cheat in reciprocal arrangements (*5*). Axelrod and Hamilton (*5*) used game theory (*6*) and the concept of an evolutionarily stable strategy (ESS) (*7*) to develop a model of the evolution of cooperation based on reciprocity. Their model is a solution to the familiar game Prisoner's Dilemma (*8*).

Prisoner's Dilemma is a symmetrical two-player nonzero sum game in which each player has the option to cooperate or defect during any interaction (*9*). Regardless of what a competitor does, a selfish act of defection receives a higher payoff than an act of cooperation. However, if both players defect, they do less well than if they both had cooperated (*10*).

When the game is played only once, the only ESS is to always defect, and cooperation is not favored (*5, 11*). During an iterated game the strategy called TIT FOR TAT becomes an ESS (*5*). To employ this strategy an individual initially cooperates and thereafter does whatever its competitor did on the previous move (*12*).

Because the TIT FOR TAT model can explain the evolution of cooperation