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SULFUR AND CARBON ISOTOPES AS TRACERS OF SALT-MARSH ORGANIC MATTER FLOW[1]

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Abstract. Stable isotopes of sulfur and carbon were used to trace the dominant flows of organic matter from producers to macroconsumers in Great Sippewissett Salt Marsh on Cape Cod. *Spartina alterniflora* and sulfur-oxidizing bacteria were found to assimilate isotopically light sulfides produced via sulfate reduction, and this light sulfur was detected in consumers. In contrast, phytoplankton and upland plants assimilate isotopically heavier SO_4^{2-} with little or no fractionation. A dual-isotope approach using both $\delta^{13}C$ and $\delta^{34}S$ showed that *Ilyanassa obsoleta* and *Fundulus heteroclitus* depend very heavily on *Spartina* detritus, while filter feeders such as *Crassostrea virginica* and *Geukensia demissa* depend on a mixture of plankton and *Spartina* detritus. *Spartina* detritus and plankton were both shown to be much more important as organic matter sources for marsh macroconsumers than either sulfur-oxidizing bacteria or organic matter derived from terrestrial inputs.

Key words: Cape Cod; carbon isotope; food webs; organic matter; salt marsh; sulfur isotope; tracer.

INTRODUCTION

Salt marshes and adjacent estuaries are sites of intense biological activity. These ecosystems draw on the resources of the atmosphere, uplands, sediments, and marine waters to sustain high levels of biological productivity. However, relating secondary production in estuaries to a particular primary producer such as *Spartina alterniflora* is difficult. The variety of pathways available for nutrient, water, organic matter, and energy exchanges makes the quantitative analysis of estuarine trophic relationships very complicated. Questions concerning which organic matter sources contribute food to consumer organisms in the coastal zone are impossible to answer using the whole ecosystem mass balance approach because the total amount of organic matter produced and imported into estuaries greatly exceeds the organic matter requirements of the resident higher organisms, such as crabs, clams, fishes, and waterfowls. Much of the organic matter originating from *Spartina* or from rivers is not immediately suitable for food, and once the organic matter becomes either dissolved or broken down to fine particulate detritus, its origin is not revealed by routine microscopic or chemical analysis. For these reasons studies which demonstrate that marshes export or import organic matter do not tell us whether or not this organic matter is used by consumers either in the marsh or offshore. However, such information can be obtained from measurements of the stable isotopic composition of the organic matter and the consumers.

Teal's (1962) analysis of energy flow in the Georgia salt marsh ecosystem emphasized the dominant role of *Spartina* in supplying detrital organic matter to marsh and estuarine consumers. It appears that the underlying rationale for many of the subsequent marsh import/export studies has been to determine the potential use of detrital carbon by the economically and ecologically important macroconsumers in estuaries (reviewed by Nixon 1980). Many researchers were surprised when Haines (1977) reported that seston in the tidal creeks and rivers at Sapelo Island, Georgia, did not have carbon isotope ratios similar to those of *Spartina*. Seston $\delta^{13}C$ values were similar to the values expected for phytoplankton, or for chemosynthetic bacteria (Peterson et al. 1980), or for a mixture of *Spartina* detritus and organic matter imported via rivers. Carbon isotope ratios alone cannot unambiguously identify the ultimate source of estuarine detrital carbon. We have recently reported that use of a combination of the stable isotopes of nitrogen, sulfur, and carbon can greatly increase the power of the isotopic tracer approach (Peterson et al. 1985). We suggested that this multiple isotope approach could provide important information on the origins of detrital organic matter and on its transfer in the estuarine food web. Isotopic information complements both the ecosystem-level studies of organic matter production, decomposition, and export and also studies of the feeding of estuarine consumers.

The use of stable isotopes to discern trophic connections is not a substitute for direct analysis of stomach contents or feeding studies. It is not possible to determine from isotopic analyses the important species interactions, since a wide variety of species may have similar isotopic values but very distinct diets. On the other hand, the isotopic approach is well suited to answer questions such as what is the ultimate source of organic matter supporting a particular consumer organism? Or, how important is *Spartina* detritus to shellfish? It is a valuable supporting technique for biogeochemical studies that seek to determine the flows of carbon, sulfur, and nitrogen in coastal ecosystems.

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In this paper we present in detail our analysis of the sulfur isotope ratios in waters, sediments, plants, and animals of Great Sippewissett Salt Marsh on Cape Cod, Massachusetts. The work was undertaken with the specific objectives of resolving the problem that carbon isotope ratios by themselves are ambiguous when more than two sources of organic matter are present, and to address questions about the possible importance of sulfur-oxidizing bacteria in salt marsh food webs (Peterson et al. 1980). We have surveyed several major primary producers and macroconsumers in and near Sippewissett Marsh with the objective of determining the degree of dependence of these consumers on *Spartina* detritus or on organic matter from other sources.

Methods

The δ notation indicates the depletion (−) or the enrichment (+) of the heavy isotope relative to the lighter isotope according to the following formula:

$$\delta X = \frac{R\ (\text{sample}) - R\ (\text{standard})}{R\ (\text{standard})} \times 10^3,$$

where $X = {}^{13}C$, ^{34}S, or ^{15}N and $R = {}^{13}C/{}^{12}C$, $^{34}S/{}^{32}S$, or $^{15}N/{}^{14}N$ of the samples and the standards. The standards were Peedee belemnite for C, Canyon Diablo troilites for S, and nitrogen in air for N.

Porewater and sediment samples were taken from a stand of short *Spartina alterniflora* at various seasons throughout the year. The short *Spartina* zone sediments were organic-rich peat (organic matter 50–85% of dry mass) and were of low density with little sand (dry mass density: ≈ 0.20 g/cm^3). The $\delta^{34}S$ samples of sulfides and sulfates in marsh sediment porewaters were collected using a 15 cm diameter polyvinyl chloride core tube and squeezing 5-cm sediment sections anoxically and as rapidly as possible in a large (15 cm diameter) Reeburgh core press (Reeburgh 1967). Sulfide $\delta^{34}S$ samples were collected from acidified (H_3PO_4) porewater using a bubbling gas stripping apparatus, and were trapped anoxically as cadmium sulfide in an aqueous cadmium acetate solution (150 g/L). Sulfate samples were precipitated as barium sulfate in an aqueous barium chloride solution (100 g/L). Dried (70°C) and weighed cadmium sulfide and barium sulfate precipitates were sent to Krueger Enterprises (Cambridge, Massachusetts, USA) for mass spectrometry analyses of $\delta^{34}S$. Samples were not taken from tall *Spartina* sites on creek banks because sulfate depletion is much less in these sites and sulfide concentrations are much lower. These characteristics make it difficult to obtain sufficient sulfide for analysis and difficult to determine the relationship between sulfate depletion and changes in $\delta^{34}S$ values of sulfate and sulfide.

Samples for pyrite (FeS_2) from the short *Spartina* zone were collected as cadmium sulfide precipitates following a chromium reduction acid distillation, after Zhabina and Volkov (1978). Samples for total sulfur in sediments were prepared by digesting samples with Aqua Regia followed by oxidation with bromine to convert the sulfur to sulfate (Howarth and Teal 1979). Plant tissue samples from *Spartina,* upland plants, seaweed, and eelgrass 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°, then ground in a Wiley mill (1 mm mesh). Samples were washed in four 1-h rinses of deionized water, 4:1 ratio by volume, water to sample. Samples were redried at 70°.

Samples of sulfur bacteria were collected in the field at sites where dense aggregations or mats developed. Colorless sulfur oxidizers (*Thiotrix* sp.) and the free-living purples (photosynthetic bacteria) were collected by suction pipetting samples from the surface of creek sediments. Microbial mats on the marsh surface contained an upper zone dominated by cyanobacteria (blue-green algae) and a lower zone of purple sulfur bacteria. These mats were separated into two layers by hand and the layers were analyzed separately. Samples were rinsed in deionized water and dried. Subsamples processed to remove elemental sulfur were extracted with hexane in a Soxhlet extractor for 5 h.

Plankton net tow samples for $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$ were collected in a 300-μm mesh Nitex net. The samples, which contained large diatoms, copepods, and detritus, were rinsed four times, first with tap water and then with deionized water in the 153-μm cod end (terminal collecting jar) of the plankton net, to remove sulfate. The samples were dried at 70°.

Animal tissue samples for $\delta^{34}S$ and $\delta^{13}C$ were prepared in a manner preventing gut and bone contamination. Animals were either held in aquaria to allow gut clearance or the guts were dissected out. Animal tissues were dried at 70°, then ground with mortar and pestle. Samples were washed in four 1-h rinses of deionized water, 4:1 ratio by volume, water to sample. Samples were dried at 70°. A subsample (>3 g dry mass) was selected for $\delta^{34}S$ analysis. The remaining sample (>0.1 g for $\delta^{13}C$) was acid washed in 10% HCl for 1.5 h to remove carbonate contaminants, and again washed in deionized water.

Samples of particulate organic carbon <153 μm, of dissolved organic carbon, of meiofauna, and of other microconsumers were not collected because we did not have adequate procedures for obtaining the large amounts (2–8 g dry mass) of these materials required for $\delta^{34}S$ analyses.

Samples of sulfide, pyrite, and sulfate were run for $\delta^{34}S$ by mass spectrometry at Krueger Enterprises. All batches of samples were accompanied by a sample of sulfate precipitated from "Copenhagen Standard Seawater" which served as an unknown standard. Results of these blind standard analyses varied by only 0.2‰. Samples for plant and animal tissues were run for $\delta^{13}C$ and $\delta^{34}S$ analyses either at Krueger Enterprises or at Global Geochemistry Corporation, Canoga Park, California. All $\delta^{15}N$ determinations were run by Global Geochemistry Corporation.

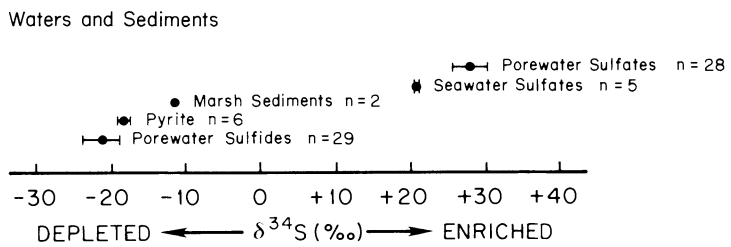

FIG. 1. The $\delta^{34}S$ values (mean ± 1 SD) for samples of seawater sulfate, porewater sulfate, porewater sulfide, pyrite, and total particulate sulfur in Great Sippewissett Marsh. The porewater samples were from a single site in the short *Spartina* zone.

The plant and animal tissue determinations were performed on pooled samples from many (10–200) individuals of the same species at each sampling site. The primary reason for pooling was that the sulfur isotope determinations required a large amount of tissue, usually >3 g dry mass. This pooling probably served to reduce some of the variability that might have been detected if individual organisms were analyzed (Montague et al. 1981). The mean difference between duplicate determinations on the same homogenized sample was usually <0.2‰ for both $\delta^{13}C$ and $\delta^{34}S$. This error is very small relative to the differences of up to 10‰ in $\delta^{34}S$ we see in collections of the same organism from different sites and/or in samples of the same organism throughout a season. It is also very small relative to the range of values of up to 20‰ in $\delta^{34}S$ found for different species of bacteria, plants, and animals in the marsh.

RESULTS AND DISCUSSION

Sulfur isotope ratios in waters and sediments

The sulfur isotope values of porewaters and sediments of the Great Sippewissett Marsh encompass a wide range between the very low mean value for porewater sulfides (−22‰) and the high mean value for porewater sulfates (+28‰) from the short *Spartina* zone (Fig. 1). Pyritic sulfur falls within the range found for porewater sulfides and may reflect the long-term mean sulfide $\delta^{34}S$ value. Marsh sediments from a muddy creek bottom and from the 2–7 cm depth zone in a short *Spartina alterniflora* stand gave values of −11.9‰ and −11.8‰, respectively, when analyzed for total solid-phase sulfur (organic S plus pyrite). Pyrite is the major component of the reduced sulfur pool in these sediments (Howarth 1984).

The high standard deviations for both the porewater sulfate and sulfide values in Fig. 1 reflect more than random sampling or analytical errors. Most of the variation is related to the degree of sulfate depletion in the porewaters (Fig. 2). As sulfate reduction proceeds, the porewater sulfate pool is partially depleted, and the remaining sulfate becomes isotopically enriched in ^{34}S. The sulfide produced via sulfate reduction exhibits a constant fractionation of about −50‰ relative to porewater sulfate as sulfate is consumed.

Sulfur isotope ratios in organic matter producers

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 use seasalt sulfate with a $\delta^{34}S$ value of about +20.3‰ (Kaplan et al. 1963), and fractionate it only slightly during uptake and assimilation into organic sulfur compounds (Mekhtiyeva and Pankina 1968). Upland plants in aerobic soils also fractionate sulfate little during uptake and assimilation, but they obtain sulfate originating in precipitation with a $\delta^{34}S$ value in the range of +2 to +8‰ (Nriagu and Coker 1970). *Spartina alterniflora* is the dominant primary producer in Great Sippewissett and in most other salt marshes of the eastern

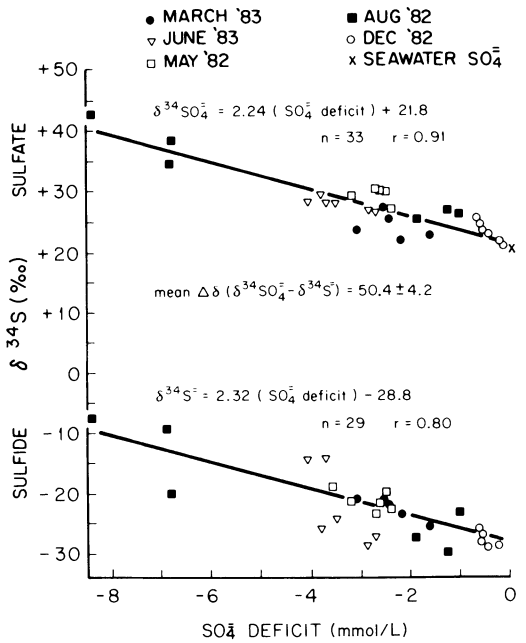

FIG. 2. The relationship between the sulfate deficit and the $\delta^{34}S$ values for sulfides and sulfates in porewaters sampled at different times during the year. The porewaters were taken from three depths (2–7 cm, 12–17 cm, and 22–27 cm) in a stand of short *Spartina alterniflora*. The seawater SO_4^{2-} value (×) represents five samples collected at the times of porewater sampling.

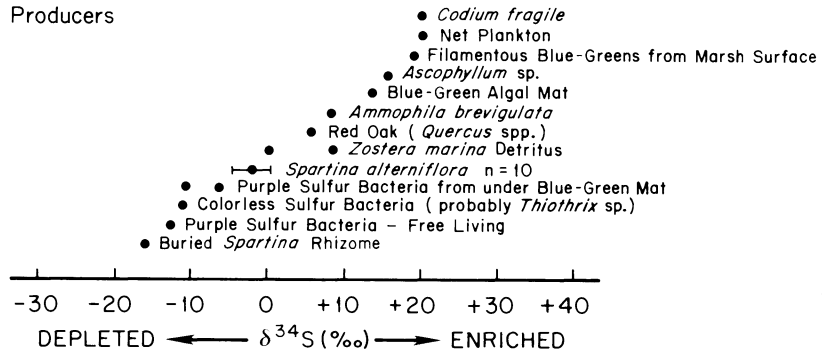

FIG. 3. The $\delta^{34}S$ values for organic sulfur in tissues of organic matter producers. The less negative of the two values for purple sulfur bacteria represents subsamples extracted with hexane to remove elemental sulfur. There were two samples of *Zostera*, with appreciably different $\delta^{34}S$ values. For *Spartina alterniflora*, the error bar indicates ± 1 SD.

coast of the United States. Marsh plants such as *Spartina* that are rooted in anoxic sediments apparently use sulfides or sulfide plus sulfate to produce their organic sulfur compounds (Carlson and Forrest 1982, Fry et al. 1982). Since these porewater sulfides are greatly depleted in ^{34}S (Figs. 1 and 2), the organic matter produced by *Spartina* is also depleted in ^{34}S relative to sulfate in the porewaters or in seawater (Carlson and Forrest 1982).

One should expect some spatial and seasonal variability in the sulfur isotopic composition of *Spartina*, depending partly on the extent of sulfate depletion in the sediments at various sites and at different seasons (Fig. 2). This in turn is affected by interactions among the rates of hydrologic flushing, in-situ sulfide reoxidation, and sulfate reduction. For example, if sulfate were completely depleted, which happens in some marsh sediments, the porewater sulfides could have $\delta^{34}S$ values similar to seawater sulfate. If this happened, even though *Spartina* reflected the $\delta^{34}S$ value of the sedimentary sulfides, the ability to use sulfur isotopes as food web tracers would be lost, since the $\delta^{34}S$ value of the sulfide assimilated by *Spartina* would be similar to the $\delta^{34}S$ value of sulfate taken up from seawater by plankton. We have analyzed 13 samples of short *Spartina* from Great Sippewissett Marsh and found values ranging from -9.5 to $+5.5$‰ with a mean value ($\pm$SD) of -3.6 ± 5.0‰. We have also analyzed six samples of tall *Spartina* from creekbank sites and found a range of $\delta^{34}S$ values from -7.7 to $+2.2$‰ with a mean of -3.3 ± 3.3‰. It is clear that the crop of organic matter produced by *Spartina* has a $\delta^{34}S$ value much lower than the values characteristic of either seawater sulfate (Fig. 1) or planktonic organisms (Kaplan and Rittenberg, 1964, Mekhtiyeva and Pankina 1968).

The spectrum of $\delta^{34}S$ values for various organic matter producers encompasses most of the range of values between porewater sulfides and seawater sulfate (Fig. 3). The lowest $\delta^{34}S$ value found for organic matter was -16.2‰ for a partly decomposed *Spartina* rhizome at a depth of 30 cm in the *Spartina* peat. However, this value may include some pyrite on or in the rhizome as well as the organic sulfur of the rhizome. Purple sulfur bacteria, which are abundant on the marsh surface, especially in waterlogged areas and in marsh pannes, had $\delta^{34}S$ values of -10 to -13‰. These bacteria use sulfide in photosynthesis and also apparently incorporate the isotopically light sulfides in their organic tissue. Colorless sulfur bacteria of the genus *Thiothrix* are commonly found in dense aggregations on marsh creek bottoms where sulfide seeps in from the creekbank sediments. These bacteria also have low $\delta^{34}S$ values (-11‰). The bacterial mat that covers small patches of the marsh surface is composed of two distinct layers. The upper portion of the mat is dominated by filamentous cyanobacteria ($\delta^{34}S$: $+13.1$‰), whereas the lower portion is composed of photosynthetic purple sulfur bacteria with a lower $\delta^{34}S$ value, probably reflecting a greater dependence on sulfide as a source of sulfur for synthesis of organic tissues. These bacteria, after extraction with hexane, had a $\delta^{34}S$ value of -6.5‰. In samples not extracted with hexane, lower values of -11.1‰ were found, presumably due to elemental sulfur. The short *Spartina* zone in Sippewissett is colonized by filamentous epibenthic algae (Van Raalte et al. 1976). These algae had a $\delta^{34}S$ value of $+18.2$‰, which indicates that they use sea-salt sulfate.

There are other potential food sources available to salt marsh consumers, such as plankton and macrophyte detritus. *Zostera marina* (eelgrass) is abundant in Buzzards Bay and is swept into the marsh with the tides, especially in the fall. Two samples of eelgrass leaves collected in the marsh gave $\delta^{34}S$ values of -0.4‰ and $+8.4$‰ in November and June, respectively. A brown alga, *Ascophyllum* sp., commonly found in the marsh had a $\delta^{34}S$ value of $+15.1$‰, reflecting a dependence on predominantly sea-salt sulfate. Another common seaweed, *Codium fragile*, gave a $\delta^{34}S$ value of $+19.9$‰. Planktonic algae are potentially important as food for salt marsh consumers because the marsh floods twice a day with Buzzards Bay seawater. A plankton net tow in Woods Hole Passage in November 1981

TABLE 1. Examples of trophic transfer shifts for $\delta^{34}S$ values. We assume that seawater sulfate is at trophic level 0.

Ecosystem	$\delta^{34}S$ value		Number of trophic levels	Shift per trophic level
	Sample 1	Sample 2		
Woods Hole Passage	seawater SO_4 + 20.3‰	net plankton + 18.6‰	1.5	−1.1‰
Woods Hole Passage	net plankton + 18.6‰	blue mussel + 16.8‰	1.0	−1.8‰
Georges Bank	seawater SO_4 + 20.2‰	swordfish + 18.2‰	4.0	−0.5‰
Cape Cod forests	oak leaves + 5.4‰	gray squirrel + 4.9‰	1.0	−0.5‰

gave a $\delta^{34}S$ value of +18.6‰ for a catch of large diatoms and copepods with smaller amounts of detritus.

Trophic transfer shifts in sulfur isotope ratios

If the sulfur isotopes are to be useful as a tracer in food web studies, one must know if there are large differences is isotopic composition between an organism's diet and its body tissue. The data in Table 1 indicate that the trophic shifts are quite small relative to the range of $\delta^{34}S$ values in the potential foods (−10‰ to +20‰) and to the range in consumers (0‰ to +20‰). Net plankton (a mixture of phytoplankton and zooplankton) from Woods Hole are within 1.1‰, per trophic level transfer, of the Woods Hole seawater sulfate value, and the blue mussel, *Mytilus edulis,* is within 1.8‰ of the net plankton. Swordfish (*Xiphias gladius*) caught on Georges Bank, where we assume that phytoplankton are the major source of organic matter in the ecosystem, were analyzed to give an indication of how big a shift might occur during multiple trophic level transfers. Assuming that the swordfish is at least at trophic level 4, the isotopic shift is only −0.5‰ or less per trophic level. A gray squirrel, *Sciurus carolinensis,* which we assume to be an herbivore, fell within the expected $\delta^{34}S$ range for sulfate in precipitation and was within −0.5‰ of the $\delta^{34}S$ value for oak leaves. These examples indicated slight shifts in the negative (^{34}S depletion) direction. A feeding experiment with gypsy moth caterpillars indicated a shift in the positive direction of +1.3‰, and a second feeding experiment with brook trout gave a similar small shift in the positive (^{34}S enrichment) direction of +1.2 to +1.4‰ (Peterson et al. 1985). We are not sure why the feeding experiments show positive shifts in $\delta^{34}S$ values while the less certain field comparisons show negative shifts. Probably there is a slight fractionation which discriminates against ^{34}S as sulfate is taken up by marine plankton (Chambers and Trudinger 1979). It appears, however, that sulfur isotopes are fractionated only slightly during subsequent feeding, assimilation, and metabolism of organic sulfur. These relatively small fractionations should not unduly bias estimates of the dependence of consumers on plankton as compared to *Spartina* because there is a $\delta^{34}S$ difference of 20‰ between these potential foods at Sippewissett (Fig. 3). This is fortunate since thus far the available data are insufficient to establish a trophic transfer fractionation correction.

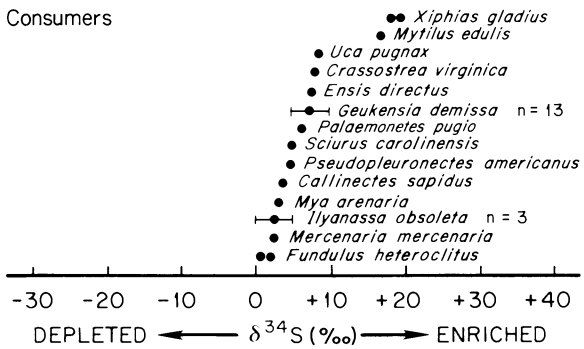

FIG. 4. The $\delta^{34}S$ values for organic sulfur in tissues of consumers in Great Sippewissett Marsh. Error bars indicate ±1 SD.

Sulfur isotope ratios in organic matter consumers

A survey of macroconsumer organisms indicated that all the consumers from the marsh had significant amounts of light sulfur derived from sulfide in their tissues (Fig. 4). All the marsh fauna fell in the $\delta^{34}S$ range of 0 to +10‰, whereas *Mytilus edulis* from Woods Hole Passage and *Xiphias gladius* from Georges Bank, which presumably draw on the plankton-based food web, fell in the range of +16 to +19‰.

The marsh fauna such as *Fundulus heteroclitus* (killifish) and *Ilyanassa obsoleta* (mud snail) had the lowest $\delta^{34}S$ values, indicating that these species are isotopically most similar to *Spartina.* Organisms such as *Uca pugnax* (fiddler crab) and *Crassostrea virginica* (oyster) had higher $\delta^{34}S$ values, indicating that *Spartina* probably contributes much less to their diet. The range of values for *Geukensia demissa* (ribbed mussel) was very wide. The individual values (range: +0.5 to +19.2‰) span the entire range of $\delta^{34}S$ values found for other consumers in the marsh. Most of this variation appears to be due to the location of the mussels in the marsh. In one set of samples taken in September, the mussels collected near the mouth of the marsh had relatively high values of +12.0‰ while values from the innermost reaches of the marsh had low values of +0.5 to +2.7‰ (Peterson et al. 1985).

Seasonal shifts in $\delta^{34}S$ values

We collected both tall and short *Spartina,* killifish, and ribbed mussels at particular sites throughout the

year. *Spartina* might be expected to show a seasonal shift in $\delta^{34}S$ values due to seasonal changes in the degree of sulfate depletion in the sediments (Fig. 2) or to changes in sulfide concentration in the sediments (Howarth et al. 1983). There appears to be little seasonal change in the $\delta^{34}S$ values for tall *Spartina alterniflora* (Fig. 5). In contrast, there is a seasonal increase in the $\delta^{34}S$ values for short *Spartina,* from a low of $-9‰$ in summer to higher values of -1 to $+2‰$ in winter. This difference between tall and short *Spartina* is consistent with the hypothesis that seasonal porewater sulfate depletion may result in a seasonal change in the $\delta^{34}S$ value of *Spartina,* because sulfate depletion would be expected to be greater in the short *Spartina* zone where the rate of porewater renewal is lower than for creekbank sites (Fig. 2). Since *Spartina* is not available to most consumers until it becomes detritus, the fall and winter $\delta^{34}S$ values probably reflect most closely the $\delta^{34}S$ value of detrital *Spartina* from the short *Spartina* zone. The shift from the relatively high $\delta^{34}S$ value of standing dead short *Spartina* in March to the lower δ values during June and July does not represent a shift in isotopic composition within the same tissue, because the summer samples contained only new shoots. The seasonal shifts in the consumers *Fundulus heteroclitus* and *Geukensia demissa* appear to be very slight. In fact, the seasonal data for *Geukensia* show much less change than the variation found among locations in the marsh on one date (Peterson et al. 1985). Of course, the adult consumers used in these comparisons have relatively slow tissue turnover times and perhaps the smaller size classes would show larger shifts either because of more rapid tissue turnover or because of larger changes in diet as they develop.

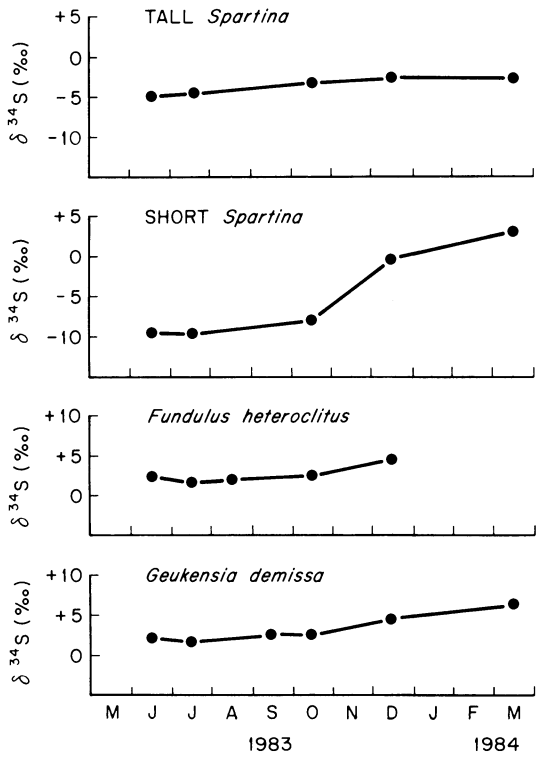

FIG. 5. Seasonal changes in $\delta^{34}S$ values for tall *Spartina alterniflora,* short *Spartina alterniflora, Fundulus heteroclitus,* and *Geukensia demissa* at selected sites in Great Sippewissett Marsh.

A dual isotopic tracer approach using $\delta^{34}S$ and $\delta^{13}C$

A dual isotope or multiple isotope approach provides significantly more power to resolve food web structure than does a single isotope approach (Fry 1983, Peterson et al. 1985). If only one isotope is used and there are three potential organic matter sources, a mixture of the foods with high and low δ values will be indistinguishable from organic matter sources having intermediate values. The use of two isotopes often allows this ambiguity to be resolved.

Table 2 shows the relative power of the carbon and sulfur isotopes for distinguishing between different potential food sources. The potential usefulness of an isotopic tracer depends on both the magnitude of the difference between the mean δ values for two organic matter sources (end members) and the degree of variation found for each potential food source. The large signal (difference) to noise (variation) ratios for the upland-*Spartina* pair for $\delta^{13}C$ and for the upland-plankton pair for $\delta^{34}S$ indicate that a combination of carbon and sulfur isotopes is especially powerful in systems where organic matter inputs from uplands, *Spartina* marshes, and plankton are potentially important. The advantage of using more than one isotopic tracer is that ambiguous carbon isotope values can be resolved by reference to the sulfur isotope values. For example, a mixture of organic matter from *Spartina* and upland vegetation would appear to be plankton if only $\delta^{13}C$ values were available. However, the high $\delta^{34}S$ value of plankton would clearly indicate that a sample of predominantly planktonic origin is not a mixture of organic matter from *Spartina* and upland vegetation. The sulfur values also can serve to distinguish between plankton and sulfur-oxidizing bacteria, which have overlapping $\delta^{13}C$ values (Peterson et al. 1980). Additional resolution can be obtained with other isotopes, such as nitrogen and hydrogen (Peters et al. 1978; Estep and Dabrowski 1980), but our experience is that for estuarine work the sulfur-carbon combination is the most powerful because of the favorable signal-to-noise ratios.

Fig. 6 shows the distribution of major foods and of several marsh macroconsumers on the $\delta^{34}S$ vs. $\delta^{13}C$ diagram. We have assumed for simplicity that the major food resources are four: plankton, *Spartina,* sulfur-oxidizing bacteria, and upland plants. The distribution of animal $\delta^{13}C$ and $\delta^{34}S$ values on this dual isotope plot shows a definite pattern. All of the macroconsumers fall near the band of values expected if they assimilated plankton, *Spartina,* or a mixture of the two. The or-

TABLE 2. Signal-to-noise ratio for discriminating the dominant food sources in estuarine food web studies using ^{13}C and ^{34}S tracers. The data used to derive this table are from Table 1 of Peterson et al. (1985).*

Isotope	Sample 1	vs. 2	Signal range (‰)	Noise (SD_1 + SD_2) (‰)	Signal/ noise ratio
Carbon-13	plankton	*Spartina*	8.2	1.9	4.3
	upland	*Spartina*	15.4	2.1	7.3
	upland	plankton	7.3	2.4	3.0
Sulfur-34	plankton	*Spartina*	21.2	5.1	4.2
	upland	*Spartina*	7.1	5.3	1.3
	upland	plankton	14.1	1.5	9.4

* The signal is the separation or difference of the mean δ values. The noise is the sum of the standard deviations of the mean values chosen for each comparison. The signal-to-noise ratio is the difference between the means divided by the sum of their standard deviations.

ganisms most closely resembling *Spartina,* in isotopic composition include *Ilyanassa obsoleta* (mud snail), *Fundulus heteroclitus* (killifish), *Mya arenaria* (soft clam), *Mercenaria mercenaria* (hard clam), *Palaemonetes pugio* (grass shrimp), *Callinectes sapidus* (blue crab), *Pseudopleuronectes americanus* (flounder), and *Geukensia demissa* (ribbed mussel). This does not mean that these organisms literally eat *Spartina.* It does suggest that the organic matter produced by *Spartina,* which is relatively enriched in ^{13}C and depleted in ^{34}S, makes an important contribution to the growth of these animals, perhaps via a variety of trophic transfers in the detritus food web. The slight tendency for *Ilyanassa obsoleta* and *Fundulus heteroclitus* to be enriched in $\delta^{13}C$ even relative to *Spartina* may be due to the preferential metabolic loss of the lighter carbon isotope (McConnaughey and McRoy 1979). Also, one must bear in mind that detrital eelgrass can have an isotopic signature on both the $\delta^{13}C$ (−10, −11‰) and $\delta^{34}S$ (+8, 0‰) axes that is similar to *Spartina.* This could be very important for mobile species such as the flounder, blue crab, and grass shrimp.

A second group of organisms falls more or less halfway between the mean values for *Spartina* and plankton (Fig. 6). These include *Crassostrea virginica* (oyster), *Uca pugnax* (fiddler crab), *Geukensia demissa,* and *Ensis directus* (razor clam). These organisms derive nourishment either from a mixture of sources including both plankton and *Spartina* detritus or from foods with an intermediate isotopic composition, but we think the former explanation is more likely.

A third group of consumers, including *Geukensia demissa, Mytilus edulis* (blue mussel), and *Xiphias gladius* (swordfish), are located at the planktonic end of the plankton-*Spartina* band. These organisms are presumably dependent upon primary producers which assimilate sulfate from seawater. *Mytilus* was collected in Woods Hole Passage several kilometres from the marsh and it is not surprising that they are isotopically similar to plankton and not similar to *Spartina.* The swordfish were chosen to represent top trophic-level consumers from the offshore pelagic ecosystem. It is clear that trophic transfers have not erased the carbon or sulfur isotopic signals which one associates with the planktonic end on the $\delta^{13}C$ vs. $\delta^{34}S$ plot.

The sulfide-oxidizing bacteria have been hypothesized to play an important role in both the energy flow and trophic relationships in salt marshes (Howarth and Teal 1980, Peterson et al. 1980). The data in Fig. 6 suggest that the marsh macrofauna do not derive a large fraction of their organic matter from the sulfur bacteria. Perhaps smaller organisms such as meiofauna will be shown to feed on the sulfur oxidizers. Recent work on the sulfur cycle at Sippewissett has shown that most of the reduced sulfur is reoxidized within the marsh

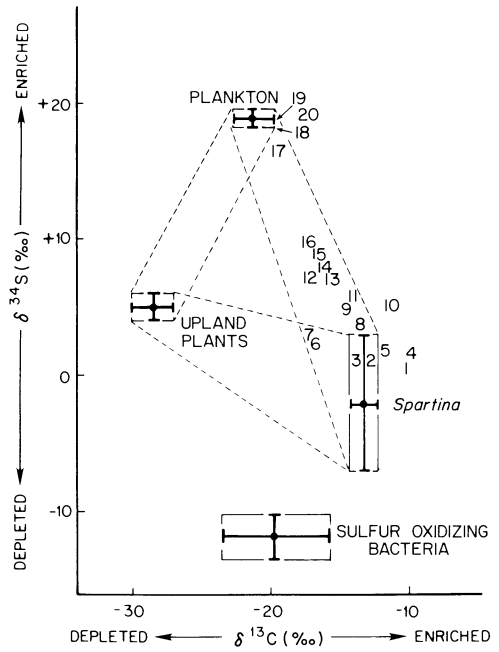

FIG. 6. The location of consumer organisms (numbers) on a plot of $\delta^{13}C$ versus $\delta^{34}S$ values. As an aid in interpretation, the locations of upland plants, marine plankton, and *Spartina* for Great Sippewissett Marsh have been included. Data are means with error bars indicating ±1 SD. For *Spartina,* N = 11; for upland plants, N = 4 for carbon, and N = 2 for sulfur. For plankton, the values for $\delta^{34}S$ are from Hartmann and Nielsen (1969), Kaplan et al. (1963), and Kaplan and Rittenberg (1964), plus our own value of +18.6‰; the $\delta^{13}C$ values are the mean and standard deviations given in Gearing et al. (1984). The values for the consumers represent pooled samples of 10–200 individuals except for *Pseudopleuronectes americanus* and *Xiphias gladius,* where a single individual was used for each sample.
Key: 1, 4, 10 *Ilyanassa obsoleta*; 2, 5 *Fundulus heteroclitus*; 3, 15, 16, 20 *Geukensia demissa*; 6 *Mercenaria mercenaria*; 7 *Mya arenaria*; 8 *Callinectes sapidus*; 9 *Pseudopleuronectes americanus*; 11 *Palaeomonetes pugio*; 12 *Ensis directus*; 13 *Crassostrea virginica*; 14 *Uca pugnax*; *17 Mytilus edulis*; 18, 19 *Xiphias gladius.*

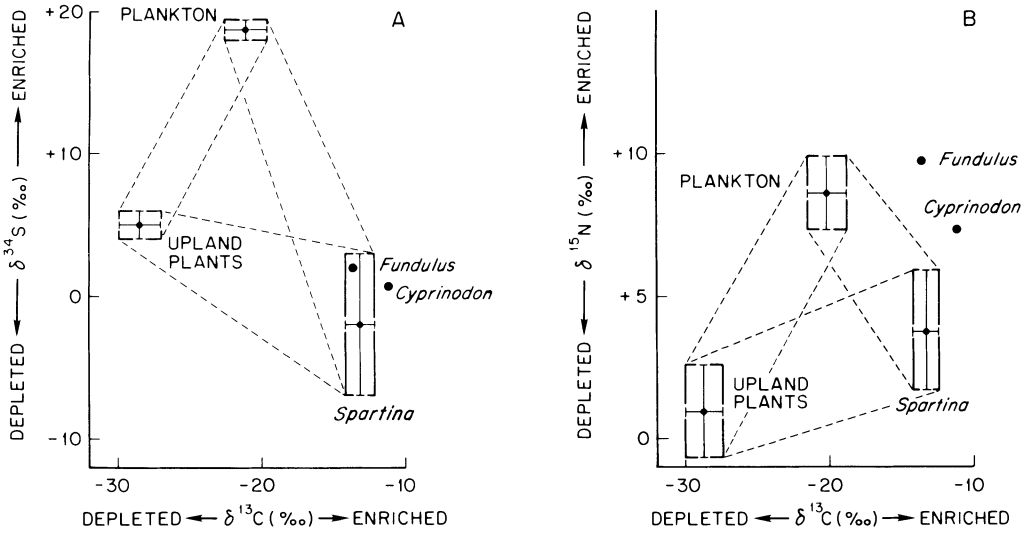

FIG. 7. A comparison of the isotopic compositions of *Fundulus hetroclitus* and *Cyprinodon variegatus*. The values for the producers are from Peterson et al. (1985).

soils, so that the potential for the sulfur-oxidizing bacteria to contribute organic matter to the marsh macrofaunal consumers is smaller than we originally hypothesized (Howarth et al. 1983, Howarth 1984). We collected greenhead fly larvae (*Tabanus* sp.) in a depression on the marsh surface that was filled with purple sulfur bacteria and decaying *Spartina*. The fly larvae had a $\delta^{13}C$ value of $-23.7‰$ whereas the bacteria had a value of $-25.5‰$. This is the only example we have thus far of a marsh consumer that was probably heavily dependent on the sulfur-oxidizing bacteria. Since we did not run $\delta^{34}S$ analyses on this sample because the sample was too small, this is a tentative conclusion.

Comparison of Fundulus heteroclitus *and* Cyprinodon variegatus

There are two abundant killifishes in Great Sippewissett Marsh, *Fundulus heteroclitus* and *Cyprinodon variegatus*, and we did an isotopic comparison of adult fish collected at the same time (August) and at the same location in the marsh. The collections were matched for size as well. The carbon and sulfur isotope ratios for the two species were very similar (Fig. 7A); both appear to depend heavily on *Spartina*. However, the $\delta^{15}N$ values were quite different, although both species were enriched in ^{15}N relative to *Spartina* (Fig. 7B). Previous work has shown that $\delta^{15}N$ values increase by up to 3‰ per trophic transfer (Rau 1982). Thus the $\delta^{15}N$ difference may indicate that *Fundulus* occupies a higher trophic position than *Cyprinodon*. The diet of *Cyprinodon* includes both vegetable and animal matter, whereas *Fundulus* feeds mostly on small invertebrates (Bigelow and Schroeder 1953). *Cyprinodon* has a longer, more convoluted gut (an adaptation which favors digestion of plant material) than does *Fundulus*, and recent experiments indicate that *Cyprinodon* is better able to assimilate nitrogen from ^{15}N-labelled *Spartina* detritus than is *Fundulus* (C. Van Raalte, *personal communication*). A laboratory feeding study has shown that *Fundulus* will ingest detritus collected in marsh creeks but that they cannot grow or survive on detritus (Prinslow et al. 1974). These findings appear to support our interpretation from the $\delta^{15}N$ analyses that *Fundulus* occupies a higher trophic position than *Cyprinodon*, and perhaps these two species reduce interspecific competition in this way.

The relationship between gut content analysis and isotopic analysis of *Fundulus heteroclitus* diet was determined in a study by Kneib et al. (1980) in a North Carolina salt marsh. Throughout the year the fish muscle tissue was 1–3‰ enriched in $\delta^{13}C$ relative to the diet. The stomach contents had carbon isotope values typical of benthic algae (-16 to $-18‰$), whereas the muscle tissue averaged -14 to $-16‰$. The stomach contents included polychaete worms (*Nereis succinea*), small crustaceans (*Leptochelia rapax*), fiddler crabs (*Uca pugnax*), and detritus. While this study supports the ideas that the carbon isotope ratios are a useful indicator of organic matter flow and that *Fundulus* is largely carnivorous, it also illustrates that metabolic fractionation which results in a positive shift in the carbon isotope δ values as organic matter is transformed from food to consumer muscle tissue. In the particular case of salt marsh studies, this tends to make it appear that consumers are more dependent on *Spartina* than is actually the case, unless correction is made for this shift.

CONCLUSIONS

The sulfur stable isotope ratios provide a means of tracing the movement of the light sulfide produced

during sulfate reduction through the marsh food web. *Spartina* assimilates the sulfides, and consequently it is possible to estimate the utilization of detrital *Spartina* by consumers. A combination of sulfur and carbon isotopes provides the basis for estimating the relative importance to consumers of up to three or four isotopically distinct classes of producers in the marsh.

The macrofauna 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 ultimate source of this food, although eelgrass cannot be excluded by isotopic evidence alone. Plankton appears to be the second major food resource, especially for filter feeders in the main marsh channels connecting with Buzzards Bay.

The majority of the marsh macrofauna appear to use a mixture of organic matter derived from both plankton and *Spartina*. It may be that consumers such as the bivalves actually ingest a mixture or "stew" of bacteria, algae, and detritus, with the relative proportions varying according to location and season. Predators such as *Callinectes sapidus* and *Pseudopleuronectes americanus* probably feed on consumers that are already a mixture of several organic matter sources. There is no evidence for an important input of organic matter from the uplands, as expected since Sippewissett is a pocket marsh with no major riverine input. There is also no indication that the sulfur-oxidizing bacteria that we have been able to sample are important to the marsh macroconsumers, although they may well be important for meiofauna or for consumers not yet sampled.

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