PARTICLE SIZE DISTRIBUTION AND $^{13}$C CONTENT OF DISSOLVED ORGANIC MATTER IN A SALT MARSH

John A. Calder and Fie Kearsley
Department of Oceanography, Florida State University
Tallahassee, Florida 32308

ABSTRACT

The dissolved organic carbon (DOC) in a stream flowing through a Florida coastal salt marsh, populated by Spartina and Juncus, undergoes continuous modification as it is transported over the salinity gradient. High molecular weight organics of terrestrial origin are lost from the dissolved fraction at mid-salinity and large quantities of low molecular weight (less than 1,000 MW) dissolved organics are added to the water. The water leaving the salt marsh contains about the same concentration of total DOC as the water entering the marsh, but the effluent waters contain a much greater proportion of low molecular weight material. This material may have greater biological activity than the high molecular weight material that it replaced, and therefore be an important input to the estuarine food chain. The implications of the changing composition for processes of peat accumulation are noted.

INTRODUCTION

The types of marsh or swamp that have or may in the future give rise to coal seams are likely to have an input of dissolved matter in streams flowing through or around the area. There will be also an output of dissolved matter as the streams leave the marsh or swamp, as shown by Reuter and Beck (this volume) for the Okefenokee Swamp. These inputs and outputs obviously affect the ecology of the area and the nature of the organic matter that accumulates and may ultimately form coal. The input/output situation in a coastal marsh is examined in this paper. The marsh selected for study is not a peat swamp. The growth medium for the plant cover is an organic-rich sand, but the results should be relevant to other areas where the same plant cover is giving rise to peat accumulations.

The dissolved organic matter in natural waters is derived from a variety of sources and is subject to many processes in solution. The result is a complex and variable composition. At the present time, analysis of the substances present is not only difficult but the data are difficult to interpret, except for dissolved hydrocarbons (Barbier and others, 1973; Iliffe and Calder, 1974), which, however, are present only in low concentrations in waters. Analyses for properties that are characteristic of the bulk organic matter in solution are easier and should allow one to observe some of the major processes acting on the pool of dissolved organic matter. Parameters to be considered here are concentration of dissolved organic carbon (DOC), molecular weight distribution, and $^{13}/^{12}$C ratio, as $\delta^{13}$C, where

$$\delta^{13} = \frac{\left(\frac{^{13}C}{^{12}C}\right)_{\text{sample}} - 1}{\left(\frac{^{13}C}{^{12}C}\right)_{\text{standard}}} \times 10^{3}$$
The concentration of DOC in fresh water ranges from 1 to greater than 10 mg C/l (Calder, 1969; Dreyer, 1973). In the open ocean, DOC concentrations are generally less than 1.5 mg C/l with higher values in coastal waters (Fredericks and Sackett, 1970; Maurer and Parker, 1972). The concentration and $\delta^{13}C$ of DOC in a river-bay system was studied as a function of time by Parker and Calder (1970). No clear seasonal trend was observed. The $\delta^{13}C$ values ranged from -12 to -30% (vs. NBS 20 standard). In the open ocean, $\delta^{13}C$ values are more constant, being about $-20\% \pm 2\%$ (Williams, 1968 and 1971; Parker and Calder, 1970).

The molecular weight distribution of marine DOC from the Gulf of Mexico was investigated by Maurer (1971) using an ultrafiltration technique. In general, more than half of the total DOC had an apparent molecular weight (MW) of less than 10,000. The apparent molecular weights of dissolved humic compounds in river water, swamps and coastal waters are said rarely to exceed 5000 (Prakash, 1971).

The concentration of DOC in a salt marsh ranges from 5 to 20 mg C/l (Dreyer, 1973). This high concentration level, together with the presence of a salinity gradient, the effects of tidal oscillations and the relatively high biological activity make a salt marsh an attractive area in which to study the concentration, molecular weight distribution and $\delta^{13}C$ of DOC.

**METHODS**

Samples were collected in August and November, 1972, at three locations over the salinity gradient in a stream flowing through a salt marsh at Turkey Point, Florida. Both sets of collections were made on an incoming tide. The marsh surrounding the stream was populated with *Spartina* and *Juncus* species and surrounded by a pine-palmetto community. The fresh water input drained a larger pine forest. The stream bed and surrounding tidal flats were composed of organic-rich sand with a surface layer of finely divided plant litter. Pyrex 9 liter glass bottles were filled by immersion just below the surface. Samples were poisoned with mercuric chloride immediately after collection. After returning to the laboratory, the samples were filtered through Gelman Type A glass fiber filters to remove large particulates. An aliquot of the filtrate was saved for determining concentration and $\delta^{13}C$ of total DOC and the remainder was subjected to molecular weight fractionation.

The sizes of molecules that will or will not pass through a given membrane, or will or will not be excluded from a given gel, are determined by the hydrodynamic volume, which depends on the shape and flexibility of molecules and their interactions with the solvent as well as on their mass. Therefore the materials separated by ultrafiltration will differ chiefly in some characteristic dimension of the molecules, and a molecular weight range cannot be assigned on the basis of the results. Thus the procedures adopted here provided an arbitrary fractionation into materials certainly differing in size, but the nature of the size parameter is obscure. In what follows it should be understood that the mention of molecular weight ranges is intended only as a means of identifying fractions.

Fractionation into various molecular weight ranges was accomplished by use of ultrafiltration cells and membranes obtained from the Amicon Corporation. Specifically, we used model 402 teflon lined stainless steel
filtration cells and 20 liter stainless steel reservoirs. The filters utilized and their cutoff limits, as stated by the manufacturer in terms of molecular weight units, were:

<table>
<thead>
<tr>
<th>Filter</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM-2</td>
<td>1000</td>
</tr>
<tr>
<td>PM-10</td>
<td>10,000</td>
</tr>
<tr>
<td>XM-50</td>
<td>50,000</td>
</tr>
<tr>
<td>XM-100A</td>
<td>100,000</td>
</tr>
</tbody>
</table>

The UM and PM series come packed with glycerol and about 10 liters of doubly distilled water must be passed through these filters before the last traces of glycerol are removed. Soaking for a few hours as recommended by the manufacturer is not sufficient. The water sample was placed in the reservoir and cell, and pressurized with prepurified nitrogen according to manufacturer's instructions. After the sample had passed through the membrane (it is best not to let the filter go completely dry), the system was slowly depressurized (the stirrer is turned off during depressurization to avoid damaging the membrane), and 50 ml of doubly distilled water were added. After about 3 hours of stirring the liquid was removed from the cell and set aside. The filter membrane was then placed in a beaker with 100 ml of 0.05N NaOH and exposed to ultrasonics overnight to remove adhering organic matter. The total sample was then combined for analysis. Water samples were filtered sequentially through all four filters, beginning with the highest molecular weight cut-off.

Analyses of the samples for DOC and $\delta^{13}\text{C}$ were conducted by slight modifications of the persulfate oxidation method of Calder (1969). Details of the modifications will be published later (Schultz, 1974). All $\delta^{13}\text{C}$ values are reported relative to National Bureau of Standards Isotope Reference Material, NBS-20. The standard PDB is $+1.06\%$ on this scale. The totalDOC and the less than 1 K fraction were always analyzed in duplicate: data for other fractions represent single analyses. The average deviation about the mean for duplicates was $\pm 0.27$ mg C/l for concentrations and $\pm 0.48\%$ for $\delta^{13}\text{C}$. The $\delta^{13}\text{C}$ values have been corrected for machine factors, but not for $\delta^{17}\text{O}$. This latter correction is small relative to analytical error.

In several cases, the removal of molecular weight fractions from the ultrafiltration membranes was not complete and the membranes retained a brownish coloration. In these cases, the sum of the DOC in the fractions is less than the total DOC. In fact, it will be noted from the figures to be presented in Table 1 that the sum of DOC in the fractions is generally in poor agreement with the total DOC determined on the unfractionated water. Nevertheless, some interesting conclusions can be drawn from the data, as will be seen; these depend on major differences in the numbers, well outside the experimental error. It should be recalled that the <1 K fraction passes through the finest filter and so is not subject to recovery errors.

**RESULTS**

The concentrations of total DOC and the various MW fractions are presented in Table 1 (next frame). The concentration of total DOC ranged from 9.45 to
## Table 1. Concentration of Dissolved Organic Carbon in Salt Marsh Samples

<table>
<thead>
<tr>
<th>Particle Size range</th>
<th>Salinity, %</th>
<th>August Samples</th>
<th>November Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>14</td>
<td>26</td>
</tr>
<tr>
<td>Total DOC</td>
<td>9.45</td>
<td>8.57</td>
<td>3.75</td>
</tr>
<tr>
<td>&gt;100 K</td>
<td>2.69</td>
<td>0.45</td>
<td>0.55</td>
</tr>
<tr>
<td>50 K - 100 K</td>
<td>2.71</td>
<td>0.40</td>
<td>0.12</td>
</tr>
<tr>
<td>10 K - 50 K</td>
<td>0.15</td>
<td>1.60</td>
<td>0.14</td>
</tr>
<tr>
<td>1 K - 10 K</td>
<td>3.52</td>
<td>1.60</td>
<td>0.43</td>
</tr>
<tr>
<td>&lt; 1 K</td>
<td>0.60</td>
<td>3.83</td>
<td>0.87</td>
</tr>
</tbody>
</table>

### (b) November Samples

<table>
<thead>
<tr>
<th>Particle Size range</th>
<th>Salinity, %</th>
<th>0</th>
<th>7</th>
<th>23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total DOC</td>
<td>6.17</td>
<td>8.27</td>
<td>5.41</td>
<td></td>
</tr>
<tr>
<td>&gt;100 K</td>
<td>2.37</td>
<td>0.26</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>50 K - 100 K</td>
<td>1.23</td>
<td>0.81</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>10 K - 50 K</td>
<td>0.53</td>
<td>0.25</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>1 K - 10 K</td>
<td>0.62</td>
<td>1.05</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>&lt;1 K</td>
<td>2.58</td>
<td>5.13</td>
<td>4.16</td>
<td></td>
</tr>
</tbody>
</table>
3.75 mg C/l. A general tendency is evident for the DOC values to be less at the most saline site, and for the relatively coarse material (>10 K) to decrease sharply with increasing salinity. In Figures 1 and 2 (next frames) the concentrations of total DOC and of the greater than 100 K and less than 1 K fractions are plotted versus salinity. In both sample sets, the less than 1 K fraction has its maximum concentration at mid-salinity and the greater than 100 K fraction has its maximum concentration at zero salinity, the other two values being nearly equal.

The $\delta^{13}C$ values for the total DOC, greater than 100 K fraction and the less than 1 K fraction are shown in Table 2 (next frame). Data from the two collecting periods have been averaged, and the spread about the mean is shown. In most cases the spread about the mean is fairly small, indicating that the fluctuation of $\delta^{13}C$ with the season is not large. The one case where the fluctuation was large was in the <1 K fraction at the zero salinity site. The values at the zero and mid-salinity sites do not differ greatly, but those at the high-salinity site are quite appreciably less negative. It is the >100 K fraction that shows the greatest differences for all samples. The differences in $\delta^{13}C$ values for the zero and high salinity sites are shown in Table 2 as $\Delta \delta^{13}C$; the $\Delta$ value is significantly greater for the >100 K fraction than for either of the other samples.

If the $\delta^{13}C$ value of terrestrial organic carbon can be taken as $-26\%_o$ and that of marine organic carbon as $-20.7\%_o$, then the fraction of terrestrial carbon in a given fraction can be computed from the following equation:

$$F_t = \frac{\delta^{13}C_{t}^{o} - \delta^{13}C_{m}^{o}}{\delta^{13}C_{t}^{m} - \delta^{13}C_{m}^{m}}$$

where

- $F_t$ = fraction of terrestrial carbon
- $\delta^{13}C_{t}^{o}$ = $-26\%_o$
- $\delta^{13}C_{m}^{o}$ = $-20.7\%_o$
- $\delta^{13}C_{t}^{m}$
- $\delta^{13}C_{m}^{m}$ = observed value for any given fraction

A value of $-26\%_o$ for $\delta^{13}C$ of terrestrial carbon was chosen because this is the average value found for total DOC and the MW fractions at zero salinity in this marsh, that is, in the purely terrestrial input to the system. This value represents the result of all sources of DOC and all processes acting on this DOC. Plant type, CO$_2$ fixation mechanism, $\delta^{13}C$ of source CO$_2$ are all accounted for in the observed value of $-26.0\%_o$. For marine carbon, a value of $-20.7\%_o$ was chosen, because this was the most positive value observed in any fraction at high salinity and also because it is a typical value for $\delta^{13}C$ of open ocean DOC. The values for $\delta^{13}C_{t}^{m}$ and $\delta^{13}C_{m}^{m}$ may change slightly with time and the calculated values of $F_t$ would vary accordingly. The absolute magnitude of an $F_t$ value is less important than are the differences in $F_t$ values for various fractions.

The values of $F_t$ are given for every fraction in Table 2. On the criteria given above, the total DOC appears purely terrestrial at zero and mid-salinity but only 30% terrestrial at high salinity. The greater than 100 K fraction on the same basis is purely terrestrial at low salinity, 70% terrestrial at mid-salinity and purely marine at high salinity. In contrast the less than 1 K fraction is purely terrestrial at both low and mid-
August

Dissolved Organic Carbon, mg/l vs Salinity of Site, o‰

- Total DOC
- <1K
- >100K
TABLE 2. VALUES OF $\delta^{13}C$ AND $F_t$ FOR SALT MARCH DOC

(for August and November samples mixed)

<table>
<thead>
<tr>
<th>Fraction</th>
<th>low salinity site $\delta^{13}C$</th>
<th>low salinity site $F_t$</th>
<th>mid salinity site $\delta^{13}C$</th>
<th>mid salinity site $F_t$</th>
<th>high salinity site $\delta^{13}C$</th>
<th>high salinity site $F_t$</th>
<th>(high) - (low) salinity $\Delta\delta^{13}C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total DOC</td>
<td>-25.5±0</td>
<td>1.0</td>
<td>-25.9±0.5</td>
<td>1.0</td>
<td>-22.1±0.4</td>
<td>0.3</td>
<td>3.4</td>
</tr>
<tr>
<td>&gt;100 K</td>
<td>-26.0±0.3</td>
<td>1.0</td>
<td>-24.3±0.5</td>
<td>0.7</td>
<td>-20.7±0.5</td>
<td>0.0</td>
<td>5.3</td>
</tr>
<tr>
<td>&lt;1 K</td>
<td>-26.4±1.2</td>
<td>1.0</td>
<td>-25.9±0.2</td>
<td>1.0</td>
<td>-24.6±0.5</td>
<td>0.7</td>
<td>1.8</td>
</tr>
</tbody>
</table>

$\delta^{13}C$ values are relative to NBS 20, with range about the mean.

$F_t$ is estimated fraction of terrestrial organic carbon.
DISCUSSION

The dissolved organic carbon in the water flowing through this salt marsh undergoes changes in both concentration and composition. At mid-salinity there is a significant input of organic matter of less than 1000 MW and a simultaneous loss of high molecular weight DOC (>100 K MW). The loss of high molecular weight material may well result from precipitation of terrestrially-derived humic materials, when the water becomes of higher ionic strength in the mid and high salinity reaches of the stream, as suggested by Prakash, 1971 (see also below). The $\delta^{13}C$ values of the greater than 100 K fraction support this suggestion, since the shift to less negative $\delta^{13}C$ values with increasing salinity is particularly marked with this fraction (Table 2).

The input of low molecular weight (less than 1 K MW) DOC at mid-salinity must be derived from the marsh itself. The most obvious source is primary production within the marsh. The source of this low molecular weight material needs careful consideration. Tidal scour of the marsh will tend to remove into the stream discarded organs or parts of organs of the vascular plants. In water these will be attacked by fungi and animals, the net increase in nitrogen content being presumably due to selective loss of carbon and conservation of nitrogen (Fell, this volume). Any soluble organic compounds formed as a result of this attack will not necessarily have the same isotope ratio as the original bulk plant material.

At the base of the marine food chain are the algae, the photosynthetic lower plants. In marine waters, bicarbonate ion is the source of their carbon rather than dissolved CO$_2$. This is 7% heavier in C$^{13}$ than CO$_2$ because of an isotope shift in the reaction:

$$\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{H}^+$$

This is why all organic constituents of sea water (e.g. the lipids of algae) tend to be isotopically heavier than corresponding constituents of the fresh water biota and associated dissolved organic matter. However, in the environment under consideration, the situation is further complicated by the presence in the marsh of Spartina, which is one of the relatively few vascular plants that, because of an alternative photosynthetic pathway, show less negative values of $\delta^{13}C$. Johnson and Calder (1973) found that bulked tissues of the fresh plant, Spartina alternifolia, from a Florida salt marsh had a mean $\delta^{13}C$ of -12.5%, while tissues of Juncus roemerianus from the same habitat had a $\delta^{13}C$ of -23.2%. Thus the significance of a mean "terrestrial" $\delta^{13}C$ value within the marsh becomes blurred. Also, at first sight, Spartina can be ruled out as an important source of DOC of any molecular weight range at the mid-salinity sampling site.

Inasmuch as vast piles of plant debris do not accumulate in salt marshes (or anywhere else), decomposition obviously does occur. The point is, are the decomposition processes such that soluble or particulate organic matter will be washed into streams by tidal scour? Odum and de la Cruz (1967) stated that Juncus is resistant to degradation in the sense that it appeared to be...
of minor importance in supplying particulate organic carbon to streams. Dreyer (1973) found the rate of release of DOC from decomposing *Juncus* to be appreciable, but only one half that from decomposing *Spartina* and about a third that of decomposing marine grasses. The finding by Exarchos and Given (this volume) that cellulose partly immersed in peat decomposes at different rates in different locations in the marshes and swamps of the Everglades, even when the surface vegetation is the same, suggests that conclusions such as those of Odum and de la Cruz, and of Dreyer, should not be generalized too widely.

In the greater part of the marsh under study, the dominant plants are *Juncus* (70% coverage) and *Spartina* (30%), though *Spartina* is predominant near the seaward margin. It is difficult to think of feasible sources of the <1 K material added in the mid-salinity region other than the two vascular plants (could there be a big enough algal population?--probably not). Therefore on the evidence so far available, *Juncus* seems the most likely source of much of the low MW DOC in the stream.

Swanson and others (1972) while studying a similar salt marsh environment in Florida observed flocculated humic acids in tidal marsh sediments. From both direct observation and the chemical composition of the flocculated material, they concluded that 80% of it was derived from the surrounding forest and 20% from marsh grass, primarily *Juncus*. In the present study a similar layer of humic material was observed on the stream bottom and on the exposed tidal flats adjacent to the stream. Samples of the stream bottom sediment and tidal flat were analyzed and found to contain 22% and 26% organic carbon respectively (dry wt. basis) with δC¹³ values of -16.3‰ and -17.5‰ respectively. The most likely source of this material is particulate or colloidal matter derived from a mixture of *Spartina* and *Juncus*.

It is interesting to compare the molecular weight distribution of the DOC in the high salinity marsh samples with that of marine waters and sediments. In the high salinity marsh sample, from 23 to 77% of the total recovered DOC was in the less than 1 K fraction. These samples represent water exposed to a recent, relatively high level of biologic activity. In the marine waters of the Yucatan Channel, a depth profile taken by Maurer (1971) showed that at 50 m 64% of the total recovered DOC was less than 1000 MW and none was greater than 100 K while at 500 m 82% was less than 1000 MW and 7% was greater than 100 K MW. However, at 1000 m the recovered DOC was almost evenly divided between the less than 1000 MW fraction and the greater than 100 K MW fraction, leading the author to conclude that, with time, the DOC may polymerize, thus rendering it more refractory to oxidative processes. Reuter and Perdue (1972) also postulate continuous polymerization of dissolved organics in an oxidizing environment, leading ultimately to water-insoluble high molecular weight materials which precipitate in the sediments.

However, in the Atlantic Ocean, Kearsley (1973) reported that at least 90% of the total recovered DOC was in the less than 1 K fraction at both 100 m and 2000 m. On the basis of these limited data, it appears that the bulk of marine DOC is of low molecular weight. In marked contrast, the soluble humic materials from marine sediments are primarily of high molecular weight, usually greater than 10 K and ranging to greater than 2 KK (Rashid and King, 1969; Prakash, 1971). These high molecular weight humics are apparently generated within the sediment rather than being deposited from the water column. Nissenbaum and others (1971) report substantial quantities of non-dialyzable
high molecular weight DOC in the interstitial waters of the mud at the bottom of a reducing fjord, an observation consistent with the generation of high molecular weight polymers within the sediment.

CONCLUSIONS

The data presented here indicate that in this salt marsh the dissolved organic matter is undergoing continuous modification in response to biologic and/or physico-chemical processes. The principal results of these modifications are:

1. Loss of terrestrially-derived high molecular weight (>100 K), isotopically negative dissolved organic matter.

2. Production in the marsh and dissolution of low molecular weight (<1 K) isotopically negative dissolved organic material, which is carried out of the marsh. The source of this material and the process of generation are not clear at this time, though it is argued that Juncus is probably an important contributor.

3. Deposition of humic matter, probably derived from Spartina and Juncus decomposition, on the stream bottom.

It seems a reasonable inference that similar additions and removals of material will occur in peat-accumulating environments. Thus although in terms of recognizable plant tissues a peat may be an essentially autochthonous deposit, in chemical terms it may be partly allochthonous.

The amount of DOC leaving the marsh is probably not greater than the amount being supplied to the marsh from terrestrial sources. However, the DOC leaving the marsh is significantly enriched in low MW components relative to that which enters it. The effects of marsh DOC on marine ecology may be very significant inasmuch as the low molecular weight dissolved organics leaving the marsh may be more biologically active than unmodified terrestrially-derived dissolved organics. For example, Prakash and Rashid (1968) found that humic substances exerted stimulatory effects on marine dinoflagellates. They observed an enhanced response in yield, growth rate and C14 uptake upon the addition of low, rather than high, MW humic materials. Such possible biologic roles for marsh-derived low MW DOC provide another strong argument in favor of the protection and preservation of coastal marshes.

ACKNOWLEDGMENT

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Figure 1. CONCENTRATIONS OF TOTAL DOC AND SELECTED MOLECULAR WEIGHT FRACTIONS AS A FUNCTION OF SALINITY (AUGUST SAMPLES).

Figure 2. CONCENTRATIONS OF TOTAL DOC AND SELECTED MOLECULAR WEIGHT FRACTIONS AS A FUNCTION OF SALINITY (NOVEMBER SAMPLES).