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DOC cycling in a temperate estuary: A mass balance approach using natural ^{14}C and ^{13}C isotopes

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Abstract

We measured dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), and their corresponding $\Delta^{14}C$ and $\delta^{13}C$ values in order to study the sources and fates of DOC in the York River Estuary (Virginia, U.S.A.). The $\Delta^{14}C$ and $\delta^{13}C$ values of DOC and DIC at the freshwater end-member indicate that during periods of moderate to high flow, riverine DOC entering the York was composed of decadal-aged terrestrially organic matter. In nearly all cases, DOC concentrations exceeded conservative mixing lines and were therefore indicative of a net DOC input flux from within the estuary that averaged 1.2 $\mu M\ L^{-1}\ d^{-1}$.

The nonconservative behavior of DOC in the York River Estuary was also apparent in carbon isotopic mixing curves and the application of an isotopic mixing model. The model predicted that 20–38% of the DOC at the mouth of the estuary was of riverine (terrestrial + freshwater) origin, while 38–56% was added internally, depending on the isotopic values assigned to the internally added DOC. Measurements of $\Delta^{14}C$ and $\delta^{13}C$ of DOC and DIC and marsh organic matter suggest that the internal sources originated from estuarine phytoplankton and marshes. The isotopic mixing model also indicates a significant concomitant loss (27–45%) of riverine DOC within the estuary.

Changes in DOC concentration, $\Delta^{14}C$-DOC, and $\delta^{13}C$-DOC were also measured during incubation experiments designed to quantify the amounts, sources, and ages of DOC supporting the carbon demands of estuarine bacteria. Results of these experiments were consistent with an estuarine source of phytoplankton and marsh DOC and the preferential utilization of young (^{14}C-enriched) DOC in the low-salinity reaches of the York. However, the average removal of riverine DOC by bacteria accounts for only ~4–19% of the riverine pool; therefore, other significant sinks for DOC exist within the estuary.

Estuaries link terrestrial and continental systems with the coastal ocean and receive large inputs of allochthonous and autochthonous organic matter and nutrients. The balance between these inputs determines estuarine net metabolism (Hopkinson and Vallino 1995). The delivery of allochthonous organic matter and nutrients from land to estuaries has increased dramatically with land use change and urbanization (Howarth et al. 1991; Cole et al. 1993). Consequently, estuaries have some of the highest areal rates of primary and bacterial secondary production (Smith and Hollibaugh 1993) and CO_2 evasion among aquatic and marine systems (Raymond et al. 1997; Cai et al. 1999; Frankignoulle et al. 1998). An understanding of both the degree of coupling between, and overall cycling of, organic matter and nutrients in estuaries is therefore critical for constraining local carbon and nutrient budgets and for evaluating the role of estuaries in regulating carbon and nutrient fluxes between the continents and oceans.

Dissolved organic carbon (DOC) is a major component (~60%) of the riverine organic matter imported to estuaries from rivers and the surrounding watershed (Spitzy and Ittekkot 1991). Estuaries may also receive internal additions of autochthonous DOC (Aminot et al. 1990; Peterson et al. 1994; Fisher et al. 1998; this study) from phytoplankton (Cole et al. 1982) and marshes (Teal 1962; Odum 1980). Estuarine DOC has three main potential fates: (1) it may be oxidized directly to CO_2 through bacterial respiration (Findlay et al. 1992; Coffin et al. 1993; Moran et al. 1999) or photo-oxidation (Amon and Benner 1996); (2) it may undergo physicochemical transformations (e.g., flocculate to form less soluble colloids and particles [Sholkovitz et al. 1978; Fox 1983; Hedges and Keil 1999]); or (3) it may be exported to adjacent coastal and continental shelf waters (Mantoura and Woodward 1983; Moran et al. 1991; Raymond and Bauer 2000).

The quantification of DOC sources and sinks in estuaries is challenging, and it is often hindered by a large number of complex and overlapping interactions between organic matter sources and sinks and relatively short residence times for water. As a consequence, DOC concentrations and mixing curves alone often do not constrain potential sources and sinks in estuaries. Stable carbon isotopes ($\delta^{13}C$) of DOC have been used successfully to quantify the sources of DOC to estuaries and estuarine bacteria (Coffin et al. 1989; Peterson et al. 1994; Meredith et al. 1996; Cifuentes and Eldridge 1998; Kelley and Coffin 1998; Coffin and Cifuentes 1999). However, interpretation of $\delta^{13}C$ measurements in rivers and estuaries can be equivocal due to a significant degree

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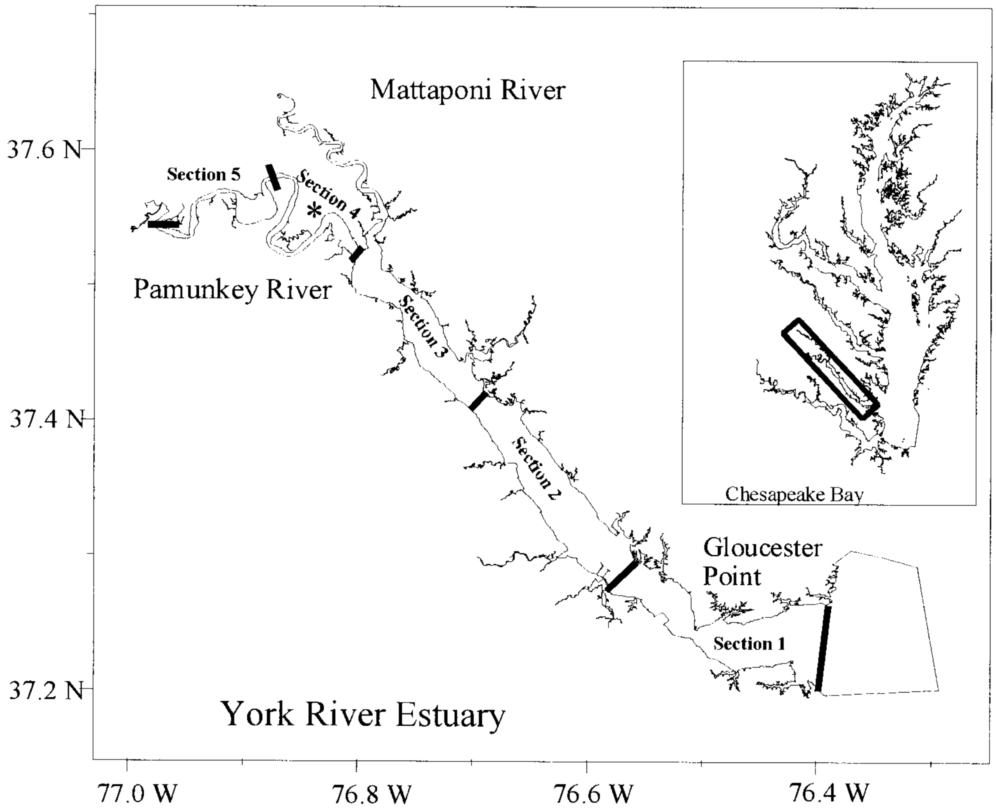

Fig. 1. The York River Estuary system, showing its location relative to Chesapeake Bay. The star shows the location of Sweet Hall Marsh.

of overlap in the isotopic signatures for the presumed major DOC sources (i.e., terrestrial, estuarine phytoplankton, riverine phytoplankton, marsh, and marine organic matter).

The natural radioisotope of carbon (^{14}C) can provide additional and unique information on the sources, ages, and residence times of organic matter in estuaries (Raymond and Bauer 2001; Spiker and Rubin 1975; Cherrier et al. 1999) that is complementary both to DOC concentrations and $\delta^{13}C$. Natural ^{14}C also has the advantage of a greater dynamic range ($\Delta^{14}C$ = around $-1,000$ to $+250$‰) than ^{13}C ($\delta^{13}C$ = around -32 to -12‰) in aquatic and marine organic matter, allowing for an added degree of sensitivity.

Our study site was the York River Estuary, a subestuary of the Chesapeake Bay. Previous work on phytoplankton and bacteria populations (Ducklow 1982; Koepfler 1989; Schultz 1999; Sin et al. 1999; Raymond and Bauer 2000) and carbon cycling (Neubauer et al. 2000; Raymond et al. 2000) in the York River Estuary was invaluable for the work presented here. Based on these earlier studies, we hypothesized that the DOC and DIC isotopic distributions would be influenced by tidal marshes in the upper York and by phytoplankton in the middle and lower York. We further hypothesized that significant quantities of DOC would be remineralized by bacteria in the York River and that the preferential utilization of DOC of different sources and ages would cause isotopic shifts in both DOC and DIC.

To test these hypotheses and to evaluate the magnitude of DOC sources and sinks in the York River Estuary, we combined DOC concentrations and distributions of $\Delta^{14}C$-DOC and $\delta^{13}C$-DOC. We also measured the $\Delta^{14}C$ and $\delta^{13}C$ of the dissolved inorganic carbon (DIC) pool in order to constrain the isotopic values of estuarine phytoplankton. The information from the DOC and DIC concentration measurements and isotopic mixing curves was used to model both the removal of terrestrial and riverine DOC and the simultaneous addition of autochthonous DOC during estuarine mixing and transport. From the collective data, we speculate on the sources and ages of DOC ultimately removed and added within and subsequently exported from a model temperate estuary to the coastal ocean.

Materials and methods

Sample collection—The York River Estuary has an average flow rate of 70 $m^3\ s^{-1}$ and a watershed size of ~4,350 km^2 and is formed by the confluence of the Mattaponi and Pamunkey Rivers ~50 km from its mouth (Fig. 1). During our study, the Mattaponi contributed ~33%, while the Pamunkey contributed ~66% of the freshwater flow to the York (flow data from www.water.usgs.gov). Sampling transects for this study began at the mouth of the estuary and continued up the Pamunkey River until freshwater was encountered (Fig. 1). For this study, large differences in DOC concentrations between the two major tributaries could produce anomalous results. However, data available on the Environmental Protection Agencies (EPA) Chesapeake Bay program website (http:

//www.chesapeakebay.net/bayprogram) indicate that the two rivers have similar ranges in dissolved constituents. For the 1994 water year, average (with SD) monthly DOC concentrations in the Mattaponi and Pamunkey were 485 ± 85 and 448 ± 102 μM, respectively. This high degree of similarity is not surprising considering the Mattaponi and Pamunkey drain watersheds of similar land use and vegetation cover.

Ten transect cruises were conducted along the main stem of the York from July 1996 to September 1997. Transects stretched from the freshwater Pamunkey to the mouth of the York Estuary (typically ~100 km) and had an average salinity range of 0–18. The locations of stations were not fixed in order to ensure that the salinity range was well covered during each sampling. The York's tidal marshes are located in the upper estuary and were within the boundaries of this study. They are flooded with freshwater for the majority of the year yet receive low-salinity (<4) estuarine water during periods of low flow. For this study, the freshwater end-member samples were collected above the tidal freshwater marshes. Surface water (<0.5-m depth) was collected for DOC analysis on all transect cruises. For three of the cruises, samples were collected for DOC and DIC isotopic analysis (Δ^{14}C and δ^{13}C).

Samples for surface-water DOC and isotopic analysis were filtered in the field through baked (~550°C) 142-mm-diameter GF/D (nominal pore size of 3.9 μm) and GF/F glass-fiber filters (nominal pore size of 0.7 μm). Filters were changed frequently to avoid cell lysis and occlusion of filters by particulates. Samples for isotopes were collected in duplicate baked (>550°C) 500-ml amber glass bottles, placed on ice in the dark while in the field, and frozen upon return to the laboratory.

DOC and DIC concentration measurements—For DOC analysis, 4 ml of water was filtered, in duplicate, into baked 7-ml vials and acidified with 25 μl of high-purity 10% HCl. Caps for the vials were acid soaked, rinsed, and lined with baked aluminum foil. Samples were sparged for 4 min with ultrahigh-purity (UHP) nitrogen, and DOC was measured on a Shimadzu TOC-5000A high-temperature analyzer, using a four-point calibration curve with glucose as a standard. High-purity water blanks were run after every 10 samples to ensure that carryover between samples was negligible. The average coefficient of variation for duplicate DOC analysis was 1.2% of the mean.

Duplicate samples for DIC analyses were collected in 7-ml gas-tight test tubes. Samples were stored on ice and in the dark while in the field and were analyzed in the laboratory within 12 h of sampling. Analysis was performed in duplicate on a Shimadzu TOC 5000A in total inorganic carbon mode, using a five-point calibration curve with sodium bicarbonate standards. The average coefficient of variation for this procedure was 4% of the mean for duplicates.

Δ^{14}C and δ^{13}C analysis of DOC and DIC—The method used for isotopic analysis of DOC and DIC is described in detail elsewhere (Williams and Gordon 1970; Bauer et al. 1992; Druffel et al. 1992). Briefly, for DOC, 100 ml of estuarine water was placed in a quartz reaction vessel that interfaced directly with a vacuum extraction line. The sample was acidified to pH ~2.5 with high-purity 85% H_3PO_4 and sparged with UHP nitrogen to remove all DIC. The sample was then saturated with UHP oxygen and irradiated for 2 h with a 2,400-W medium-pressure mercury arc ultraviolet (UV) lamp (Conrad-Hanovia). Following irradiation, the CO_2 produced during DOC oxidation was purged from the reaction vessel with UHP nitrogen. The gas stream was passed through a KIO_3 trap to remove any chlorine and bromine gas produced from seawater salts, and the CO_2 was purified cryogenically on the vacuum extraction line. The purified CO_2 sample was split ~1 : 10 and collected in Pyrex™ break-seal tubes for isotopic analysis. The smaller aliquot was used for δ^{13}C analysis, while the larger aliquot was used for Δ^{14}C.

For DIC, 45–100 ml of sample was placed in a glass-sparging chamber that interfaced directly with the vacuum extraction line (the KIO_3^- trap was removed). The sample was acidified to pH 2–3 with 10% HCl and sparged with UHP nitrogen gas. The evolved CO_2 gas was purified on the vacuum line, split, and collected in Pyrex® break-seal tubes for isotopic analysis. Similar to DOC samples, the smaller aliquot was used for δ^{13}C analysis, while the second, larger aliquot was analyzed for Δ^{14}C.

Δ^{14}C is defined as the parts per thousand (per mil) deviation of a sample from the ^{14}C activity of nineteenth-century wood. For Δ^{14}C analysis (both DOC and DIC), the CO_2 samples were converted to graphite targets in an atmosphere of H_2 over a cobalt catalyst (Vogel et al. 1987). Targets were analyzed at the Center for Accelerator Mass Spectrometry at Lawrence Livermore National Laboratory. All reported Δ^{14}C values were corrected for fractionation using the δ^{13}C values of the samples, according to the conventions of Stuiver and Polach (1977). δ^{13}C (defined as $\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 10^3$ where R is the ratio of ^{13}C to ^{12}C, $R_{standard}$ is the Pee Dee Belemnite standard) was analyzed using a VG 602 isotopic ratio mass spectrometer.

Fifteen in situ samples and 12 incubation end-point samples (*see* below) were processed for DOC isotopes. Because of the difficulty and expense of natural ^{14}C measurements, only one of the in situ samples had duplicate analyses performed on it. Standard deviations of ±0.03‰ for δ^{13}C and ±2‰ for Δ^{14}C were obtained for duplicate analyses of this sample and were within the analytical measurement errors. A total of 13 samples were processed for DIC isotopes. Similarly, replicate analyses were performed for only one in situ DIC sample, and standard deviations of ±0.2‰ for δ^{13}C and ±7‰ for Δ^{14}C were obtained.

Bacterial DOC utilization experiments—Incubations of 2 months' duration were conducted to elucidate the isotopic signatures of DOC utilized by bacteria. This timescale is similar to the 1–2-month residence times for water in the York River Estuary (Sin et al. 1999). To minimize the time elapsed between water collection and the start of a given experiment, incubations were initiated in the field. Approximately 5 liters of water from various salinities was filtered through baked (~550°C) 142-mm-diameter GF/D and GF/F glass-fiber filters to remove particulate organic matter and algae. Filters were changed frequently to avoid cell lysis and occlusion of filters by the high particulate organic matter load. At each site, four 500-ml baked glass amber bottles were filled with ~300 ml of filtered water. Two bottles were immediately placed on ice in the dark for a time-zero sample and immediately frozen upon return to the lab. The remaining two incubation bottles were placed in the dark in a bath of York River water to maintain in situ temperatures. Once in the lab, the bottles were placed in an incubation chamber and incubated in the dark at in situ temperatures for 2 months, after which time they were frozen. At the conclusion of the experiment, Δ^{14}C-DOC and δ^{13}C-DOC isotopes were analyzed for initial and final time points according to the methods outlined above.

Marsh sediment organic matter—Two 30-cm-long cores were collected from the tidal freshwater Sweet Hall Marsh (indicated in Fig. 1) in May 1998, in order to measure Δ^{14}C and δ^{13}C and marsh sediment organic matter. The cores were sectioned in the lab, and sediment organic matter was sampled from depths of 5–6, 7–8, and 9–10 cm. Samples (~5 g dry sediment) were HCl fumed and then sealed in Pyrex™ tubes with CuO and Ag metal and combusted to CO_2 (>900°C) for isotopic analysis (Sofer 1980). The CO_2 gas produced was then purified on a vacuum extraction line and analyzed for Δ^{14}C and δ^{13}C according to methods outlined above.

DOC mixing curves—We estimated the net source of DOC to York River Estuary waters using DOC concentration mixing curves. Mixing curves are commonly used for interpreting source/sink dynamics of estuarine constituents (Officer 1976). When observed concentrations of a solute are distributed linearly relative to salinity (a conservative tracer), it is generally interpreted that the constituent of interest mixes conservatively (i.e., has no significant sources or sinks) with respect to the system's residence time. In contrast, measured values lying above the conservative mixing line indicate a net estuarine source, while values falling below the conservative mixing line indicate a net estuarine sink for DOC.

Kaul and Froelich (1984) presented an equation to estimate the net flux of a dissolved constituent in estuaries. When mixing curves are continuous and can be described using simple quadratic equations, the input flux of a dissolved constituent within an estuary is defined as

$$\text{Input flux} = Q(\text{Cs} - \text{Co}) \quad (1)$$

where Q is freshwater flow (in $m^3\ s^{-1}$), Co (μM) is the concentration where the quadratic equation intersects the y-intercept (i.e., the concentration at zero salinity), and Cs (μM) is the concentration of the constituent where the tangent at the seawater end-member for the equation intersects the y-intercept. For present purposes, when measured values were greater than the conservative mixing line (i.e., an estuarine source of DOC was indicated), we modeled each mixing curve using quadratic equations. We used the quadratic equation to calculate the y-intercept (Co) and the y-intercept for the tangent at the seawater end-member (Cs). According to Kaul and Froelich (1984), the total export flux of a dissolved constituent from an estuary is the product of Cs and Q, the internal flux is the product of Cs − Co and Q, and the riverine flux is the product of Co and Q.

Table 1. DOC distributions and net fluxes in the York River Estuary.

Date	Discharge Q ($m^3\ s^{-1}$)	Freshwater DOC (μM)	Mouth DOC (μM)	Co (μM)	Cs (μM)	Cs − Co (μM)	Internal flux 10^8 mmol d^{-1}
Jul 96	16.6	458	299	462	858	396	5.7
8 Sep 96	15.6	474	272	465	848	383	5.2
9 Sep 96	181.7	713	323	670	*	*	*
Nov 96	80.9	444	303	461	744	282	20
Jan 97	64.3	334	278	366	677	311	17
Mar 97	93.3	390	254	302	301	0	0
Apr 97	52.4	314	266	331	635	304	14
May 97	29.9	436	241	392	749	357	9.2
Jun 97	34.9	354	220	362	608	246	7.4
Jul 97	9.5	378	258	384	910	525	4.3
Sep 97	6.5	450	241	402	894	491	2.8
Avg.	53.2	431	268	431	733	330	8.1
SD	49.3	103	29	101	151	138	6.1

The flux of internally added DOC is defined as Q(Cs − Co) (*see* Materials and Methods for complete description). Freshwater discharge was obtained from http://water.usgs.gov. Flux data were not computed for the 20 Sep 96 transect because this transect was performed after a storm that produced freshwater end-member DOC concentrations that were greater than 2 standard deviations from the mean. This is indicative of nonsteady-state behavior; therefore, the net flux for this transect was not included.

Δ^{14}C-DOC and δ^{13}C-DOC mixing curves—Isotopic mixing curves are dependent on the contributions of two-component end-members. We constructed isotopic mixing curves according to the conventions of Spiker (1980). Using this method, Δ^{14}C or δ^{13}C mixing curves depend on both the total DOC concentration and isotopic composition (I) of the riverine (r) and high-salinity/marine (m) end-members. The conservative isotopic value for a sample at a known salinity is then calculated according to the following equation:

$$I_s = \frac{(f I_r DOC_r + (1 - f) I_m DOC_m)}{DOC_{mix}} \quad (2)$$

where the riverine fraction, f, is calculated from salinity, and DOC_{mix} is the amount of DOC expected due to conservative mixing of the freshwater and marine end-members.

Results and discussion

End-Member DOC concentrations and ages—The mean riverine DOC concentration for all transects averaged 431 ± 103 μM at the riverine end-member and 269 ± 29 μM at the mouth station (Table 1). The Δ^{14}C and δ^{13}C values of DOC in the York are reported in Table 2. Riverine Δ^{14}C-DOC and δ^{13}C-DOC had average values of 229.2 ± 20.6 and −28.2 ± 0.3‰, respectively. The riverine Δ^{14}C-DOC and δ^{13}C-DOC values are both consistent with values of contemporary terrestrial soil organic matter (Schiff et al. 1990; Trumbore et al. 1992) and forest floor organic matter (Rich-

Table 2. DOC concentrations, Δ^{14}C-DOC and δ^{13}C-DOC for the York River Estuary.

Date	Salinity	DOC (μM)	Δ^{14}C-DOC (‰)	δ^{13}C-DOC (‰)
Nov 96	0	443	208	−27.9
Sep 96	0	713	222	−28.0
Mar 97	0.1	390	257	−28.8
Sep 96	1.6	575	219	−28.7
Mar 97	4.5	356	178	−27.3
Sep 96	7.1	457	149	−26.4
Mar 97	8.5	288	130	−25.5
Nov 96	9.0	438	121	−26.0
Sep 96	11.9	354	93	−25.4
Mar 97	12.0	298	92	−24.7
Mar 97	14.5	254	40	−24.2
Nov 96	15.0	347	42	−25.4
Sep 96	15.2	326	50	−24.3
Nov 96	16.6	303	60	−24.0

ter et al. 1999). Based on chlorophyll *a* (Chl *a*) values and DIC isotopes, the contribution from riverine phytoplankton at the riverine end-member for these samples is believed to be minimal (*see* section on DIC isotopes below).

The riverine Δ^{14}C-DOC values were enriched (by as much as 150‰) compared to modern atmospheric $\Delta^{14}C$-CO_2 values of around +100‰ (value obtained from measuring the Δ^{14}C of a leaf from the York River watershed). Atmospheric $\Delta^{14}C$-CO_2 was last +229‰ in the early 1980s (Levin and Kromer 1997). Therefore, we propose that a significant percentage of the riverine DOC was derived from terrestrial sources and was photosynthetically fixed on land at least 15–20 yr ago. At increasing salinities in the York River Estuary, DOC becomes ^{14}C-depleted and ^{13}C-enriched (Table 2). Samples from the mouth of the York had average Δ^{14}C-DOC and δ^{13}C-DOC values of 48 ± 12 and −24.6 ± 0.6‰, respectively (average salinity = ~18).

Nonconservative DOC behavior—In the present study, we used two independent approaches to interpret DOC dynamics in the York River Estuary: DOC mixing curves and isotopic mass balances. The information obtained from each of these is unique in that the DOC mixing curves provide information on net DOC sources or sinks, while the isotopic mixing curves provide information on gross DOC dynamics.

DOC mixing curves: For 8 of the 10 transects, the majority of the measured DOC concentrations were greater than the conservative mixing lines (Fig. 2), indicating a net internal flux of DOC within the estuary (Table 1). This flux was calculated using quadratic equations (represented by the dotted lines in Fig. 2) and Eq. 1, and the results are reported in Table 1. The average r^2 values of the quadratic equations used to estimate Cs in Eq. 1 were 0.88 and ranged from 0.78 to 0.99. The measured and calculated (i.e., point where the dotted line passes through the y-intercept) values of riverine end-member DOC were identical, with the measured values averaging 431 ± 103 μM and the calculated values averaging 431 ± 101 μM. The large r^2 values and close agreement between predicted and measured riverine DOC concentrations support the use of quadratics for calculating the net internal DOC flux in the York River Estuary.

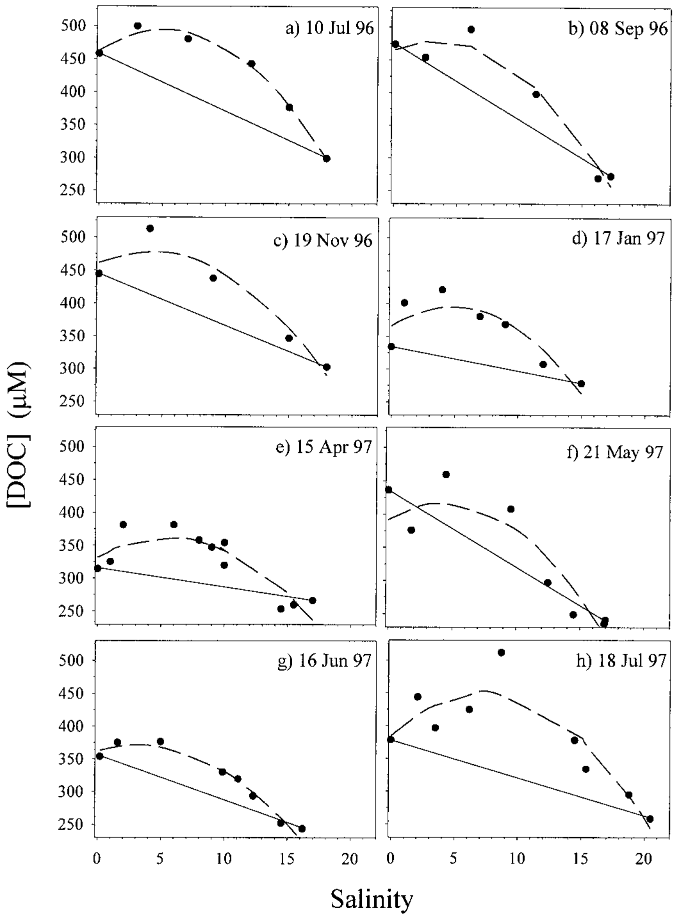

Fig. 2. DOC concentrations (circles), conservative mixing lines (solid lines), and the quadratic equations (dashed lines) used to estimate the net internal flux of DOC in the York River Estuary. DOC values generally fall above the conservative mixing curves, indicating a net estuarine source of DOC. Each point represents the mean of two samples, and the average coefficient of variation for the duplicate samples averaged 1.2% of the mean.

For the 10 transects, the accumulation of DOC within the estuary (Cs − Co) averaged 266 ± 240 μM, which equates to a net flux of internally added DOC into the entire estuary of 8.5 ± 6.1 × 10^8mmol d^{-1} (Table 1). Using a surface area of 134.8 × $10^6 m^2$ and average depth of 5.7 m (Cronin 1971) for the York River Estuary, this equates to an average net daily input of 1.2 μM L^{-1} d^{-1}.

The influence of internal sources on the distribution of nonconservative solutes is modulated by river flow and flushing time (Officer 1976). Over the course of this study, a pattern emerged between the observed accumulation of DOC (Cs − Co) and freshwater discharge (Fig. 3). When discharge is high, residence times are short, and the accumulation of DOC in the estuary is low (Fig. 3). Based on DOC measurements and mixing curves, it appears that there is a relatively constant internal flux of DOC within the York River Estuary. During periods of low to moderate discharge, this flux causes DOC to accumulate in the water column and

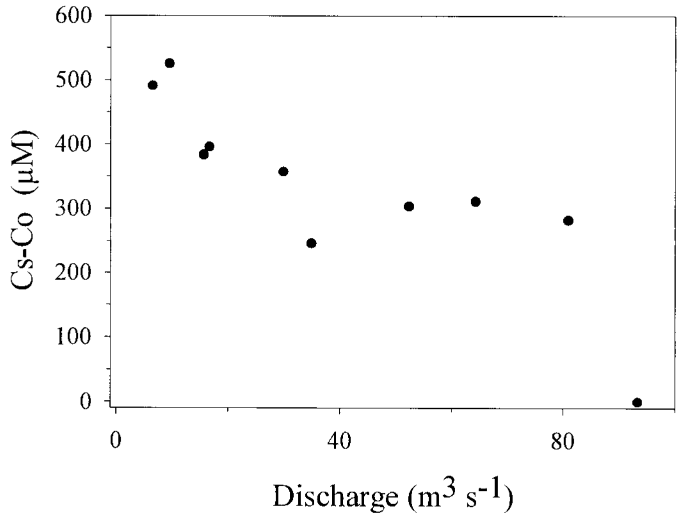

Fig. 3. The net accumulation of DOC (Cs − Co) as a function of discharge (m^3 s^{-1}). The net accumulation of DOC was calculated using Eq. 1.

to be differentiable from the conservative mixing line. However, this does not mean that there is no internal flux during periods of high flow, particularly if high flow periods elevate riverine end-member DOC concentrations.

Δ^{14}C-DOC and δ^{13}C-DOC mixing curves: The Δ^{14}C-DOC and δ^{13}C-DOC mixing curves also indicate nonconservative behavior in the York River Estuary (Fig. 4). In general, Δ^{14}C-DOC values were depleted and δ^{13}C-DOC values were enriched with respect to their expected conservative distributions (Fig. 4). Unlike DOC concentration mixing curves that are linear, isotopic mixing curves are constructed using Δ^{14}C and δ^{13}C values weighted by DOC end-member concentrations (Eq. 2). For this reason, there are two possible explanations for the differences between observed and predicted values in each (^{14}C or ^{13}C) mixing curve: (1) the removal of ^{14}C-enriched and ^{13}C-depleted DOC, and/or (2) the input of ^{14}C-depleted and ^{13}C-enriched DOC. To quantify the relative importance of these two possible fluxes, we employed an isotopic mixing model.

Isotopic mixing model: The isotopic model employed here may be used to estimate the fraction of riverine DOC removed and the total amount of DOC added within the estuary during estuarine mixing. Conceptually, the model is shown in Fig. 5. The model delineates DOC distributions at any point within the estuary into three pools: an internally added pool, a riverine pool, and a marine pool. Then, assuming conservative transport of marine DOC (we examine this assumption below), the marine DOC pool and isotopic signatures were removed in order to estimate the fraction of internally added versus riverine DOC. One strength of the model is that it does not rely on the concentration of riverine DOC, which exhibited temporally variable, nonconservative behavior (Table 1). Furthermore, all equations can be calculated separately for Δ^{14}C and δ^{13}C, which allows for independent verification of the assumptions and estimates of accuracy.

According to the model, estuarine (e) DOC is comprised of marine (m) DOC, riverine (r) DOC, and DOC added (a) within the estuary, each with its respective isotopic (^{14}C or ^{13}C) signature (I).

$$\mathrm{DOC_eI_e = DOC_mI_m + DOC_rI_r + DOC_aI_a} \qquad (3)$$

We can rewrite Eq. 3 so that DOC_e is comprised of only two components: a marine (m) component and nonmarine (nm) component (which itself is comprised of riverine DOC plus DOC added within the estuary).

$$\mathrm{DOC_eI_e} = f_\mathrm{m}\mathrm{DOC_eI_m} + (1 - f_\mathrm{m})\mathrm{DOC_eI_{nm}} \qquad (4)$$

where $(1 - f_m)DOC_e$ is the concentration of nonmarine DOC (DOC_{nm}), and the marine fraction (f_m) is calculated from salinity assuming conservative mixing.

Equation 4 is then rewritten to calculate the isotopic value of nonmarine DOC.

$$\mathrm{I_{nm}} = \frac{(\mathrm{DOC_eI_e - DOC_mI_m})}{\mathrm{DOC_{nm}}} \qquad (5)$$

If DOC in the York River Estuary mixed conservatively, we would expect 100% of the nonmarine DOC to be of riverine origin; therefore, I_{nm} would have isotopic signatures similar to riverine DOC (i.e., around 220‰ for Δ^{14}C and around −28‰ for δ^{13}C).

However, if I_{nm} is not equal to the isotopic signature of the riverine end-member, we can estimate the percentage of riverine (f) and internally added DOC present in the nonmarine pool through a two-component mass balance (Fig. 5), such that

$$\mathrm{I_{nm}} = f\mathrm{I_r} + (1 - f)\mathrm{I_a} \qquad (6)$$

where the percentage of internally added DOC is equal to $(1 - f)$. To solve for f, the isotopic signatures of the internally produced DOC were chosen based on direct measurements of DIC and marsh organic matter in the York. Finally, combining Eq. 4–6 allows for the calculation of riverine or internally added DOC in the total DOC pool.

$$\text{Fraction of riverine DOC} = \frac{(f \cdot \mathrm{DOC_{nm}})}{\mathrm{DOC_e}} \qquad (7)$$

Sensitivity analysis of the isotopic mixing model: The major sources of variation in the isotopic mixing model lie in the choice of a marine end-member for Eq. 5, the assumption that marine DOC mixes conservatively, and the choice of the isotopic signature for the internally produced DOC in Eq. 6. To test the sensitivity of this approach to the choice of the marine end-member, we used three separate and directly measured values of DOC_m and I_m in Eq. 4 (Table 3). When averaging the output for Eq. 5 (Table 4) for the three marine end-members, the average standard deviations for the calculated isotopic values of the nonmarine fraction were 2.5‰, and 0.04‰ for Δ^{14}C and δ^{13}C, respectively. This source of variation is smaller than the analytical errors and would result in negligible error in Eq. 6 and 7.

The isotopic mixing model also assumes the conservative

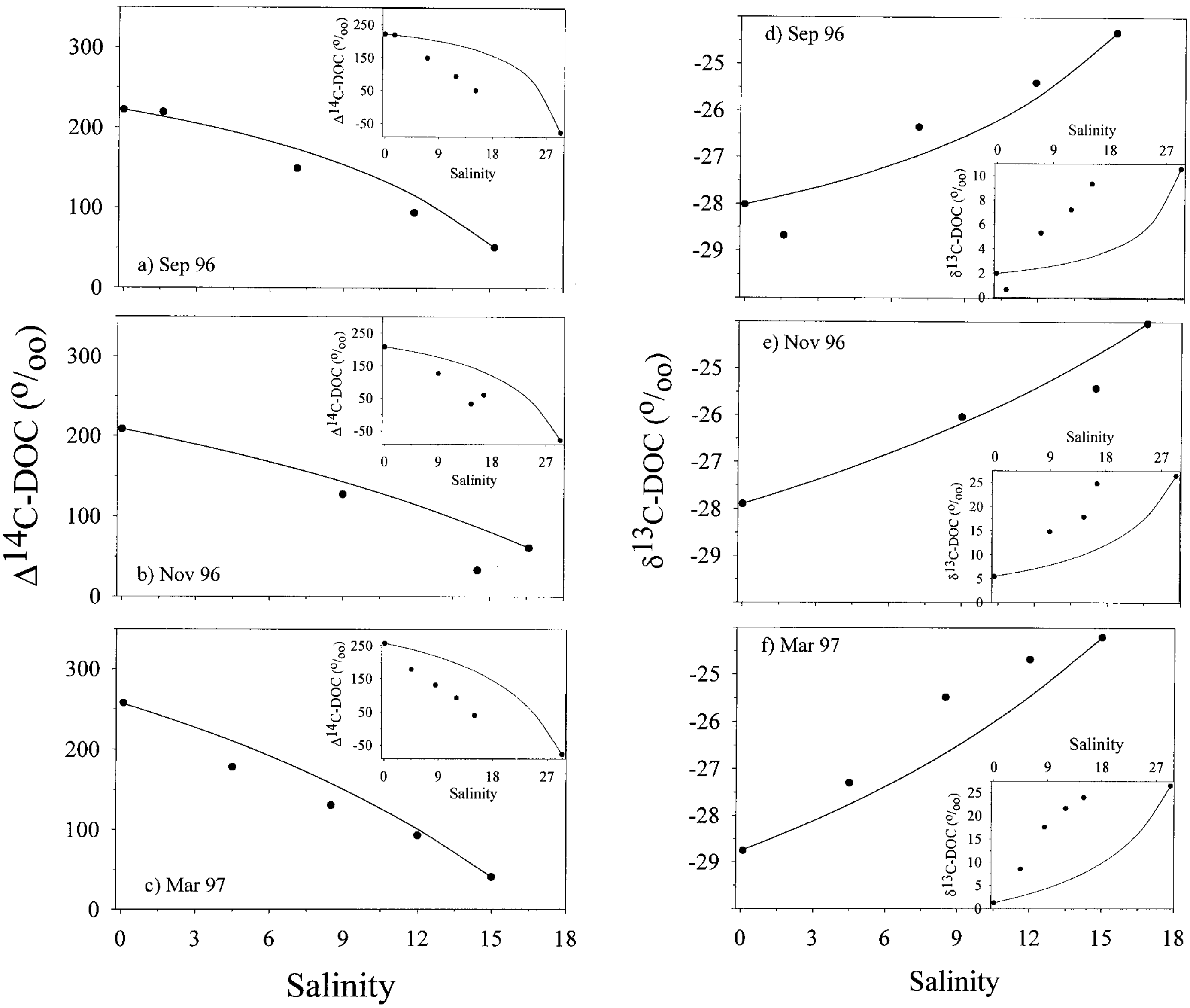

Fig. 4. (a–c) Δ^{14}C-DOC, and (d–f) δ^{13}C-DOC distributions and conservative mixing curves for the three isotopic transects in the York River Estuary. The solid line is the conservative isotopic mixing curve as calculated using Eq. 2. The insets are Δ^{14}C-DOC (a–c) and δ^{13}C-DOC (d–f) distributions and conservative mixing curves for the three isotopic transects using a higher salinity marine end-member. The higher salinity marine end-member was taken in surface waters immediately outside the mouth of the Chesapeake Bay (Table 5).

transport of marine DOC, even though it is probable that a percentage of this marine DOC is removed within the estuary. However, due to the comparatively small amounts of marine DOC found in a York River Estuary sample, the removal of marine DOC produces small changes to our model. As an example, there would be a negligible effect on our isotopic interpretation (<1% change in the estimated percentage of riverine DOC present in a sample) if we ran the model assuming a 10% removal of marine DOC within the estuary, even if we assume that the marine DOC being removed has a modern Δ^{14}C signature.

The final source of variation in the model lies in the choice of the isotopic signature assigned to the internally added DOC (I_a in Eq. 6). To provide a robust range for the output for Eq. 6, the value used for I_a was varied, and Eq. 6 was applied using both Δ^{14}C-DOC and δ^{13}C-DOC. For Δ^{14}C-DOC, we used estimates of +50 and +0‰ for I_a, while for δ^{13}C-DOC, we used estimates of −23 and −20‰. The first set of estimates (+50 and −23‰ for δ^{13}C and Δ^{14}C, respectively) is based on direct isotopic measurements of DIC and marsh organic matter in the York River Estuary. The second set of numbers was chosen to produce a conservative estimate for the fraction of riverine DOC removed during estuarine mixing. By using these different values for I_a in Eq. 6, the average output for the percentage of riverine DOC present in a sample changed by only 12%.

A major strength of this approach is that the assumptions are tested through the dual-isotopic approach. That is, the

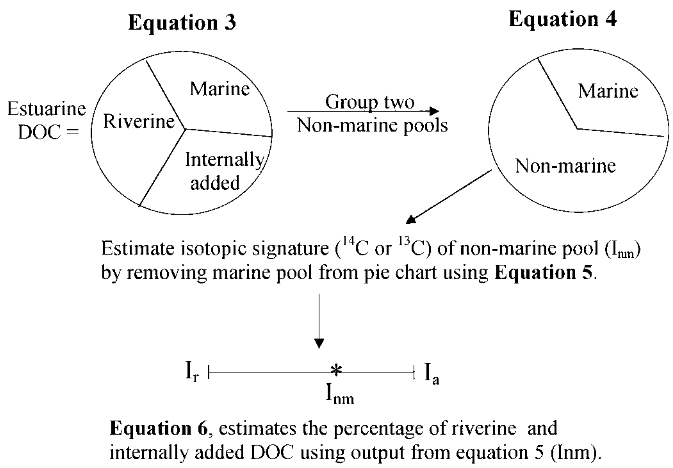

Fig. 5. Conceptual diagram of the isotopic mixing model used in this study. According to the diagram, the fraction of riverine DOC present in a sample (f in Eq. 6) is represented by the fraction of the line to the right of the star on the two-component line diagram representing Eq. 6.

Table 3. Marine end-members used in Eq. 5.

Location	Study	Salinity	DOC (μM)	Δ^{14}C-DOC (‰)	δ^{13}C-DOC (‰)
Chesapeake Bay mouth (CBM$_a$) in 60 m water	Bauer (unpubl. data)	29.40	126	−77	−23.7
Chesapeake Bay plume (CBM$_b$) in 2,000 m water	Bauer (unpubl. data)	33.88	73	−189	−22.8
Deep Sargasso Sea (DS)	Bauer et al. (1992)	34.92	43	−398	−20.9

The two Chesapeake Bay plume samples were collected in Apr 96. The deep Sargasso Sea sample is a composite average for six samples measured at depths of 2,216–4,454 m by Bauer et al. (1992).

two isotopes serve as an independent check on one another because there is no a priori reason why the model should predict similar percentages for the separate end-members using Δ^{14}C versus δ^{13}C. Interestingly, Δ^{14}C and δ^{13}C calculations yielded very similar percentages of riverine and internally added DOC. This is apparent in the low standard deviations for the average estimate shown in Table 5 and attests to the strength of the assumptions used in this study.

Isotopic mixing model results: In all but two cases, the DOC$_{nm}$ fraction was ^{13}C-enriched, and in all cases was ^{14}C-depleted in comparison to average riverine Δ^{14}C-DOC and δ^{13}C-DOC signals of +229 and −28.2‰, respectively (Table 2). As salinity increased, the Δ^{14}C and δ^{13}C values of the DOC$_{nm}$ fraction became more ^{14}C-depleted and ^{13}C-enriched.

Using Eq. 7, we estimated the percentages of riverine and internally added DOC present in a DOC$_{nm}$ sample using both isotopes (Table 5). According to these independent solutions, there is a gradual decrease in the percentage of riverine DOC with increasing salinity. This decrease is balanced by a concomitant increase in the percentage of internally added DOC (Table 5). At the mouth of the estuary, depending on what value was used for I$_a$ in Eq. 6, 21–38% of the DOC was estimated to be of terrestrial/riverine origin, while 38–56% was added internally (Table 5). Below, we examine the importance of phytoplankton, marshes, and bacteria to DOC dynamics in the York River Estuary.

Sources and sinks of DOC in the York River Estuary—Inputs of phytoplankton DOC: The measured Δ^{14}C-DIC and δ^{13}C-DIC values for York River Estuary samples were used to constrain the predicted isotopic signatures of phytoplankton carbon within the York, assuming kinetic fractionations of 20‰ for δ^{13}C (Chanton and Lewis 1999). All Δ^{14}C-values are normalized to a δ^{13}C value of −25‰; therefore, no corrections have to be made for fractionation (Stuiver and Polach 1977). Based on the DIC isotopic measurements, phytoplankton in the low-salinity (≤4) regions of the York would have average Δ^{14}C and δ^{13}C signatures of +148 and −30.9‰, respectively (Table 6). The depleted δ^{13}C signatures are similar to the δ^{13}C values of terrestrial DOC signals and attest to the difficulties associated with relying on δ^{13}C

Table 4. Isotopic values for the nonmarine (NM) fraction of DOC in York River Estuary samples using different marine end-members.

		NM Δ^{14}C-DOC (‰)			NM δ^{13}C-DOC (‰)			Averages (‰)	
Transect	Salinity	CBM$_a$	CBP$_b$	DS	CBP$_a$	CBP$_b$	DS	δ^{13}C	Δ^{14}C
Sep 96	1.6	258	258	258	−28.7	−28.7	−28.7	258	−28.7
Mar 97	4.5	193	188	187	−27.5	−27.4	−27.4	189	−27.4
Sep 96	7.1	166	162	161	−26.6	−26.5	−26.5	163	−26.5
Mar 97	8.5	160	152	150	−25.8	−25.7	−25.7	154	−25.7
Nov 96	9.0	146	142	141	−28.3	−28.1	−28.1	143	−28.2
Sep 96	11.9	126	119	118	−25.7	−25.6	−25.6	121	−25.7
Mar 97	12.0	133	123	122	−24.9	−24.9	−24.9	126	−24.9
Mar 97	15.0	78	72	73	−24.4	−24.4	−24.5	74	−24.4
Nov 96	15.0	59	56	58	−25.8	−25.7	−25.7	57	−25.7
Sep 96	15.2	88	82	83	−24.5	−24.5	−24.6	85	−24.5
Nov 96	16.6	103	95	94	−24.1	−24.2	−24.3	97	−24.2

The calculated isotopic values were obtained using Eq. 5. Isotopic signatures and DOC concentrations of the three different marine end-members used to estimate the isotopic signatures of any nonmarine DOC present in an estuarine sample are given in Table 5. CBM, Chesapeake Bay mouth; CBP, Chesapeake Bay plume.

Table 5. A range of estimated percentages of riverine and internally added DOC in York River Estuary DOC.

Date	Salinity	% riverine DOC: Conservative	% riverine DOC: Field measurement	% internally added DOC: Conservative	% internally added DOC: Field measurement
Mar 97	4.5	91 ± 4	89 ± 7	4 ± 4	6 ± 7
Sep 96	7.1	78 ± 3	70 ± 7	15 ± 3	23 ± 7
Mar 97	8.5	67 ± 6	57 ± 11	20 ± 6	30 ± 11
Nov 96	9.0	81 ± 11	78 ± 15	10 ± 11	13 ± 15
Sep 96	11.9	57 ± 1	45 ± 1	27 ± 1	39 ± 1
Mar 97	12.0	52 ± 4	39 ± 9	28 ± 4	42 ± 9
Sep 97*	14.5	36 ± 16	17 ± 20	41 ± 17	59 ± 20
Nov 96	15.0	41 ± 5	24 ± 3	40 ± 5	57 ± 3
Sep 96*	15.2	39 ± 3	22 ± 1	38 ± 3	55 ± 1
Nov 96*	16.6	40 ± 1	23 ± 6	37 ± 1	54 ± 6

* York River Estuary mouth.

Averages and standard deviations of riverine and internally added DOC present in estuarine samples were calculated using Eq. 7. Averages and standard deviations were obtained by applying Eq. 7 for $\Delta^{14}C$ and $\delta^{13}C$. We used the nearshore Chesapeake Bay mouth sample (Table 5) as the marine end-member. To establish a range of predicted values, the calculation was performed using isotopic signatures of riverine and internally produced DOC based on direct field measurements in the York (+50‰ for $\Delta^{14}C$ and −23‰ for $\delta^{13}C$) and using isotopic signatures for I_a that would produce a conservative estimate for the amount of riverine DOC removed (0‰ for $\Delta^{14}C$ and −20‰ for $\delta^{13}C$).

Table 6. DIC concentrations, $\Delta^{14}C$-DIC, and $\delta^{13}C$-DIC for the York River Estuary.

Date	Salinity	DIC (μM)	$\Delta^{14}C$-DIC (‰)	$\delta^{13}C$-DIC (‰)
Nov 96	0	454	110	−7.5
Sep 96	0	458	164	−14.6
Nov 96	1.7	677	155	−9.4
Sep 96	4	756	160	−12.3
Sep 96	7	1143	92	−5.7
Mar 97	8.5	915	72	−1.8
Nov 96	9.0	1102	59	−4.2
Nov 96	14.5	1348	56	−2.2
Mar 97	15.0	1424	47	−0.2
Sep 96	17	1604	62	−2.7
Nov 96	16.6	1538	53	−2.8

measurements to differentiate between DOC of terrestrial versus riverine algal origin. However, predicted phytoplankton $\Delta^{14}C$ values of +148‰ are ~50‰ lower in $\Delta^{14}C$ than in observed riverine $\Delta^{14}C$-DOC (Tables 2, 6). This suggests that the majority of the riverine DOC entering the estuary for these transects was not of algal origin and is consistent with our hypothesis that this riverine DOC is primarily comprised of decadal-aged terrestrially derived material. Two other supporting pieces of evidence for a relatively small algal contribution to the riverine DOC include (1) riverine Chl *a* values were only 2.4 and 6.5 $\mu g\ L^{-1}$ for the November 1996 and March 1997 samples, respectively (Chl *a* was not measured for the September 1996 transect), and (2) Sin et al. (1999) concluded that phytoplankton in the upper York are limited by low light and high flow rates.

At higher salinities (>14), DIC became increasingly ^{14}C-depleted and ^{13}C-enriched (Table 6). In these samples, phytoplankton would have predicted $\Delta^{14}C$ and $\delta^{13}C$ signatures of around +45 and −22.0‰, respectively. These values are ^{14}C-depleted and ^{13}C-enriched with respect to DOC pools and riverine DOC (Table 2), and both the isotopic mixing curves and the mixing model require a ^{14}C-depleted and ^{13}C-enriched source of DOC. Therefore, inputs of phytoplankton DOC at these reaches are consistent with the anomalies in the isotopic mixing curves and mixing model. Previous findings in the York confirm the input of phytoplankton DOC to the lower York. Sin et al. (1999) concluded that in the middle and lower York, phytoplankton are not limited by light and high flushing rates, resulting in higher rates of growth. Blooms in the middle and lower York consistently produce Chl *a* values in the 30–70 $\mu g\ L^{-1}$ range (Sin et al. 1999). For the November 1996 and March 1997 isotopic transects, Chl *a* values increased from 2.4 to 23.8 and from 6.5 to 92.8 $\mu g\ L^{-1}$ (Raymond and Bauer unpubl. data) between the riverine end-member and the middle York, respectively. Furthermore, the middle and lower portions of the estuary have large areas where the depth is <1 m. Light penetrates to the bottom of these shallow environments facilitating benthic primary production (Anderson pers. comm.).

Marsh $\Delta^{14}C$ and $\delta^{13}C$ measurements and inputs of marsh organic matter: It has long been recognized that marshes are a potentially significant source of organic matter to estuaries (Teal 1962) and to the diets of estuarine secondary consumers (Peterson and Howarth 1987). However, the relative importance of ''outwelling'' of organic matter from marshes is still an intense area of research (Cai et al. 1999). Marshes located in the low-salinity York are sites of significant organic matter decomposition, and they export DOC to the main stem (Neubauer et al. 2000). The water column of the low-salinity York is also the site of high rates of organic matter recycling, which is evident in high rates of bacterial production (Schultz 1999) and pCO_2 supersaturation (Raymond et al. 2000). Therefore, in the low-salinity York, DOC cycling should be strongly influenced by marsh DOC input and organic matter turnover.

Neubauer et al. (2000) estimate that the freshwater marshes of the upper York export ~60 g C $m^{-2}\ yr^{-1}$. These marshes encompass an area of ~$2.0 \times 10^7 m^{-2}$ (Doumelele 1979; Silberhorn and Zacherle 1987). This equates to a flux of 1.2×10^9 g C yr^{-1}, which is ~30% of the average net DOC source estimated by DOC mixing curves (Table 1). Therefore, freshwater marshes appear to be a quantitatively significant term in the DOC cycle of the York River Estuary. Preliminary $\Delta^{14}C$ and $\delta^{13}C$ measurements of organic matter in the top 10 cm of a large freshwater marsh located in the Pamunkey River (Fig. 1) indicate that marsh organic matter is ^{14}C-depleted and ^{13}C-enriched relative to York River Estuary DOC (Table 7). Therefore, the isotopic data and the work of Neubauer et al. (2000) are consistent with an input of marsh DOC to the York River Estuary.

Table 7. $\Delta^{14}C$ and $\delta^{13}C$ values of organic matter for the tidal freshwater Sweet Hall Marsh, Virginia. The location of the Sweet Hall Marsh is shown in Fig. 1.

Depth below marsh surface (cm)	Core A $\Delta^{14}C$ (‰)	Core B $\Delta^{14}C$ (‰)	Core A $\delta^{13}C$ (‰)	Core B $\delta^{13}C$ (‰)
5–6	46	50	−26.3	−26.4
7–8	45	58	−22.3	−25.9
9–10	−1	ND*	−25.8	ND

* ND, not done.

Bacterial DOC utilization experiments: Changes in DOC concentrations, $\Delta^{14}C$-DOC, and $\delta^{13}C$-DOC during incubation experiments are reported in Table 8. The $\delta^{13}C$ values of DOC utilized by bacteria during incubations exhibited a shift from ^{13}C-depleted values at low salinities to ^{13}C-enriched values at higher salinities (Table 8). Assuming end-member values of around −28‰ for terrestrial/riverine DOC (Table 2), −25‰ for freshwater marsh DOC (Table 7), and −24 to −20‰ for estuarine phytoplankton DOC (Table 6), the ^{13}C values are consistent with bacterial utilization of terrestrial and freshwater marsh DOC in the low-salinity York; a mixture of phytoplankton, terrestrial, and marsh DOC in the transitional York; and algal DOC in the high-salinity York. Raymond and Bauer (2000) also predict an internal source of labile DOC to the York River Estuary.

The majority of the $\Delta^{14}C$ values calculated for the utilized DOC fraction support the above interpretations based on the $\delta^{13}C$ values. Calculated $\Delta^{14}C$ values of the utilized fraction were +385 to +698‰ in the upper, low-salinity (<4) York, suggesting utilization of terrestrial DOC (Table 8). These values (+385 to +698‰) correspond to DOC that was fixed during the height of bomb testing ~40–50 yr ago. In the transitional York (salinity = 4–12), three out of four of the calculated $\Delta^{14}C$ values for the utilized DOC ranged between +108 and +163‰, which is consistent with a mixture of phytoplankton, terrestrial, and marsh DOC.

Two of the three $\Delta^{14}C$ values for DOC utilized in the lower York are more difficult to explain (Table 8; March 1997 salinity 12 sample and September 1996 salinity 15.2 sample). These incubations indicate that bacteria utilized DOC with $\Delta^{14}C$ values greater than +450‰. As mentioned above, the $\delta^{13}C$ values for these same samples are consistent with DOC of algal origin, yet because there is no evidence that $\Delta^{14}C$-DIC becomes this enriched at these salinities, it is difficult to invoke a mechanism for obtaining algal material with $\Delta^{14}C$ values greater than +450‰. The high calculated $\Delta^{14}C$ values for the lower York samples could be partly due to the limitations of the incubation experiments and the two end-member mass balance calculations used to model the $\Delta^{14}C$ value of the fraction utilized. For these two incubations (in particular, the September 1996 salinity 17 sample), very small quantities of DOC were utilized over the course of the incubation (Table 8). Therefore, any errors would have a large impact on the mass balance equation. However, similar highly enriched $\Delta^{14}C$ values were observed during incubation experiments performed on open-ocean waters (Bauer in prep.), and we therefore cannot rule out the preferential utilization of a highly ^{14}C-enriched bomb-carbon fraction. The $\Delta^{14}C$ value for the DOC utilized in the March 1997 (salinity 14.5) sample was −76 and is more consistent with $\Delta^{14}C$-DOC values found just outside the Chesapeake Bay mouth (Table 3).

Sources and sinks of DOC in the York River Estuary—The higher concentrations of DOC in riverine waters compared to coastal waters suggest that at the mouth of the York River Estuary, isotopic values should resemble riverine DOC more closely than marine DOC (Fig. 4). However, the mouth of the York River Estuary consistently had $\Delta^{14}C$-DOC and $\delta^{13}C$-DOC values that were closer to coastal marine DOC (Table 2; Fig. 4). This suggests that estuarine DOC cycling significantly alters the isotopic signature of the DOC pool

Table 8. Results of bacterial DOC utilization experiments.

Date	Salinity	Concentration of DOC utilized (μM)	Beginning isotopic values		Ending isotopic values		Calculated isotopic values of DOC utilized	
			$\Delta^{14}C$	$\delta^{13}C$	$\Delta^{14}C$	$\delta^{13}C$	$\Delta^{14}C$	$\delta^{13}C$
Sep 96	0	128	222	−28.0	137	−27.8	698	−29.5
Mar 97	0.1	35	257	−28.8	245	−28.7	411	−28.9
Sep 96	1.6	82	219	−28.7	236	−28.3	108	−31.1
Mar 97	3.4	20	178	−27.3	165	−26.6	385	−38.4
Sep 96	7.1	29	149	−26.4	149	−26.3	146	−27.8
Mar 97	8.5	26	130	−25.5	127	−25.6	164	−24.8
Sep 96	11.9	25	94	−25.4	51	−25.3	589	−26.0
Mar 97	12	25	92	−24.7	54	−25.3	447	−19.0
Mar 97	14.5	44	40	−24.2	63	−24.7	−76	−22.0
Sep 96	15.2	11	51	−24.3	32	−24.5	525	−19.3

Filtered (0.7 μm) York River Estuary water was incubated for 2 months in the dark at in situ temperatures. DOC, $\Delta^{14}C$-DOC, and $\delta^{13}C$-DOC were measured at the start and end of the incubations. The isotopic values of the utilized fraction were calculated by mass balance. On two occasions, duplicate second time points from separate bottles were analyzed in order to report standard deviations on the calculated isotopic values of the utilized fraction. Average standard deviations of 0.3 and 1.4‰ for $\delta^{13}C$ and 13 and 21‰ for $\Delta^{14}C$ were found for the utilized fraction of DOC in these replicate analyses.

that is ultimately exported from this temperate estuary. Evidence for this nonconservative behavior was found in the isotopic mixing curves (Fig. 4), the results from the mixing model (Tables 4, 5), and the bacterial utilization experiments (Table 8).

Results from Eq. 7 indicate that at the mouth of the estuary, 20–38% of DOC was of riverine origin, depending on the values used for I_a (Table 5). Assuming no inputs or removals (i.e., conservative mixing of riverine and coastal end-members), we estimate that 65% of the DOC present at the mouth of the York River Estuary should be of riverine/terrestrial origin. This represents a loss of ~27–45% of riverine DOC within the estuary. However, the DOC concentration mixing curves do not indicate a net loss of DOC in the York River Estuary (Fig. 2). In fact, according to these mixing curves, the loss of riverine DOC is balanced by substantial DOC additions, which is also substantiated by the isotopic mixing model. According to the model, 38–56% of DOC present at the mouth was added internally (Table 5).

The removal of 27–45% of riverine DOC cannot be balanced by bacterial degradation alone. Raymond and Bauer (2000) estimated that only 4–19% (average = 10.8%, n = 7) of riverine DOC is removed on timescales relevant to York River Estuary residence times of 1–2 months (Sin et al. 1999). Photo-oxidation or flocculation could be responsible for the removal of lost riverine DOC. The oxidation of DOC to CO_2 may occur through the absorbance of UV and visible light by organic chromophores (Mopper et al. 1991; Amon and Benner 1996; Moran et al. 1999). In a study in the Amazon River, Amon and Benner (1996) reported average rates of photochemical DOC consumption of 4 μM h^{-1}. These workers estimated that this photolysis occurs only in the top 10 cm of the water column, which is probably a conservative estimate for the turbid York. Therefore, in the York, assuming average depths of 5.7 m (Cronin 1971) and residence times of 1 month (Sin et al. 1999), this equates to a loss of only ~1% of the total DOC pool.

Precipitation of riverine humic materials can remove significant quantities (60–100%) of humic DOC in estuaries (Sholkovitz et al. 1978; Fox 1983). However, because the humic pool is typically a small component of the total DOC pool, this amounts to a relatively small (3–11%) removal of total riverine DOC (Sholkovitz et al. 1978; Fox 1983). In the York, the contribution of humic matter to the total DOC pool is unknown. However, we have proposed that the majority of York riverine DOC originates from terrestrial soils. These soils contain a significant amount of humic organic matter (Hedges and Oades 1997). It therefore seems plausible that flocculation of terrestrial humic DOC may account for a significant percentage (i.e., >10%) of the total riverine DOC pool removed during estuarine transport and mixing in our system. Yet another possibility is the scavenging of DOC by particles within the estuary; once associated with particles, the DOC may then be deposited in estuarine sediments, where it is subject to burial or local remineralization (Hedges and Keil 1999).

Conclusion

In the York River Estuary, a carbon isotopic study using only δ^{13}C would have been constrained by the small dynamic range (~10‰) and overlap in the δ^{13}C signatures of coastal DOC (around −23‰) and DOC produced within the York (−20 to −25‰). By utilizing Δ^{14}C measurements in conjunction with δ^{13}C, the present study benefited from the greater dynamic range (~200‰) and the lack of overlap between coastal Δ^{14}C-DOC (around −80‰) and DOC produced within the estuary (0–100‰). We conclude that riverine DOC entering the York during periods of moderate to high flow is comprised predominantly of decadal-aged terrestrially organic matter. Moreover, as much as half of this riverine DOC is removed during estuarine mixing and transport. Interestingly, this large removal of riverine material is not evident in DOC concentration mixing curves because it is often balanced by an equally large (or greater) input of autochthonous DOC. This autochthonous source is present year-round, originates from phytoplankton and marshes, and supports a percentage of bacterial carbon demand in the higher salinity sections of the York. If the York is representative of other temperate estuaries worldwide, it suggests that estuaries may play an important role in the global carbon budget. Estuaries may remove terrestrial and riverine DOC and simultaneously add and export DOC having a unique estuarine character that potentially has very different isotopic signatures, chemical characteristics, and biological lability.

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