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Assessing sources and ages of organic matter supporting river and estuarine bacterial production: A multiple-isotope ($\Delta^{14}C$, $\delta^{13}C$, and $\delta^{15}N$) approach

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Abstract

We used radiocarbon ($\Delta^{14}C$) and stable isotopic ($\delta^{13}C$, $\delta^{15}N$) signatures of bacterial nucleic acids to estimate the sources and ages of organic matter (OM) assimilated by bacteria in the Hudson River and York River estuary. Dual-isotope plots of $\Delta^{14}C$ and $\delta^{13}C$ coupled with a three-source mixing model resolved the major OM sources supporting bacterial biomass production (BBP). However, overlap in the stable isotopic ($\delta^{13}C$ and $\delta^{15}N$) values of potential source end members (i.e., terrestrial, freshwater phytoplankton, and marsh-derived) prohibited unequivocal source assignments for certain samples. In freshwater regions of the York, terrigenous material of relatively recent origin (i.e., decadal in age) accounted for the majority of OM assimilated by bacteria (49–83%). Marsh and freshwater planktonic material made up the other major source of OM, with 5–33% and 6–25% assimilated, respectively. In the mesohaline York, BBP was supported primarily by estuarine phytoplankton–derived OM during spring and summer (53–87%) and by marsh-derived OM during fall (as much as 83%). Isotopic signatures from higher salinity regions of the York suggested that BBP there was fueled predominantly by either estuarine phytoplankton-derived OM (July and November) or by material advected in from the Chesapeake Bay proper (October). In contrast to the York, BBP in the Hudson River estuary was subsidized by a greater portion (up to ~25%) of old (~24,000 yr BP) allochthonous OM, which was presumably derived from soils. These findings collectively suggest that bacterial metabolism and degradation in rivers and estuaries may profoundly alter the mean composition and age of OM during transport within these systems and before its export to the coastal ocean.

The fate of organic matter (OM) in aquatic systems is controlled primarily by heterotrophic bacterial respiration and biomass production (Findlay et al. 1992; Williams 2000). Sources and sinks of OM in river and estuarine systems in particular are often difficult to establish quantitatively because of such factors as spatial and temporal variability in the simultaneous inputs and turnover of autochthonous and allochthonous forms and the subsequent homogenization of OM source signatures (Canuel et al. 1995; Cloern et al. 2002). Although bioassays are frequently used to evaluate the reactivity of bulk pools such as dissolved organic matter (DOM; del Giorgio and Davis 2003), the information they provide about the biochemical composition and age structure of potential sources of OM supporting heterotrophic production is often limited.

Globally, rivers transport ~0.25 Pg of dissolved organic carbon (DOC) per year toward the ocean (Hedges et al. 1997). This typically occurs via estuaries or similar mixing zones, where a number of biogeochemical and physical processes may modify the quantities and characteristics of the OM delivered to the ocean. Some studies have reported conservative transport of DOC through estuaries (e.g., Mantoura and Woodward 1983; Ittekot 1989), which suggests insignificant removal by bacteria. However, others have indicated that DOC processing in estuaries is more complex and may include both internal sources and sinks of DOC (Mannino and Harvey 2000; Raymond and Bauer 2000*a*). Thus, there may be no general pattern governing OM transport through estuaries as a whole. Instead, different river-estuary systems may possess unique physical, hydrological, and biogeochemical features that result in distinct OM dynamics.

Biogeochemical processing in estuaries is a primary control on the transfer of terrigenous OM from land to the coastal sea. Allochthonous OM delivered from watersheds to rivers and estuaries has traditionally been classified as refractory. However, net system heterotrophy in coastal eco-

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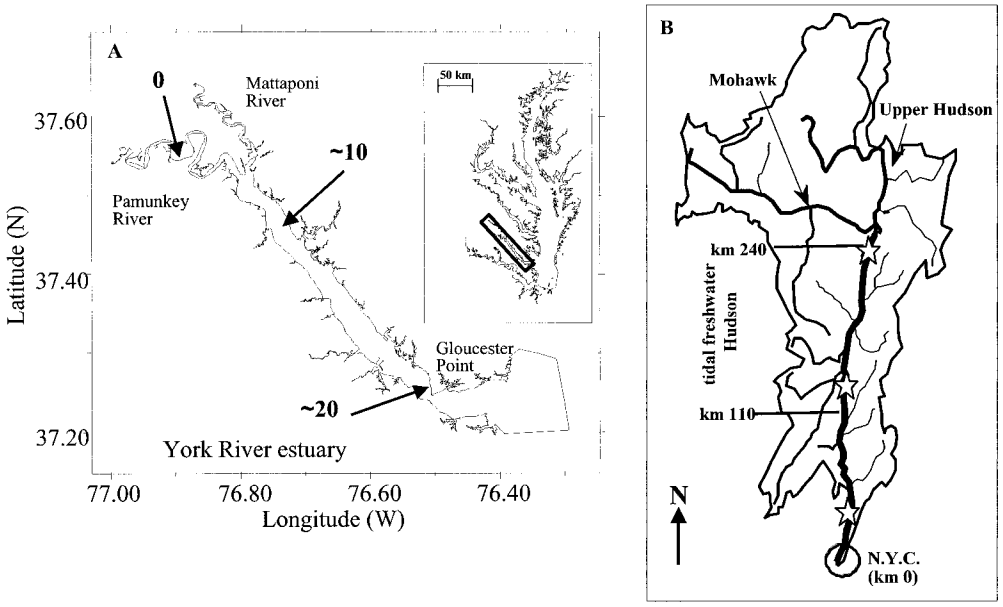

Fig. 1. (A) The York River estuary. Inset shows the York's location relative to the Chesapeake Bay proper. Sampling locations are designated by an arrow and the approximate salinity. (B) The Hudson River and associated watershed. The map shows the tidal Hudson River (heavy line) formed by the confluence of the Upper Hudson River and the Mohawk River and running from river 240 km south to New York City. Stars denote sampling locations.

systems (Smith and Hollibaugh 1993; Frankignoulle et al. 1998) requires the de facto utilization of some portion of this allochthonous material by microheterotrophs. At present, the relative importance of autochthonous versus allochthonous OM to heterotrophic pathways of energy flow in most river-estuary systems remains largely unknown. Furthermore, the relative susceptibility of allochthonous OM sources (e.g., eroded agricultural soils vs. forest runoff) to heterotrophic decomposition is not readily predictable using current approaches. Thus, a better understanding of the quantitative and qualitative processing of OM in estuarine systems may be key to reconciling the biogeochemical fate of terrigenous OM as it is transported to coastal seas.

Stable isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, etc.) have been used previously to infer OM inputs and cycling in freshwater and marine systems (e.g., Lajtha and Michener 1994), although the relative contributions of multiple sources to bulk OM pools and trophic levels can be difficult to ascertain because of overlap in the isotopic signatures of different components (Cloern et al. 2002). The simultaneous use of multiple isotopic tracers may, however, help overcome some of these limitations (Peterson et al. 1985; Bauer et al. 2002). Both $\delta^{13}C$ and $\delta^{15}N$ have been applied, with different degrees of success, for the identification of the sources of OM assimilated by bacteria (Coffin et al. 1989, 1990; Coffin and Cifuentes 1999). Natural abundance ^{14}C measurements also have the potential to provide additional resolution in discerning the relative importance of allochthonous and autochthonous OM sources to bacterial production (Cherrier et al. 1999). The greater sensitivity and potential dynamic range of $\Delta^{14}C$ (approximately −1,000‰ to +435‰) compared with $\delta^{13}C$ sources (approximately −35‰ to −12‰) or $\delta^{15}N$ sources (approximately −2‰ to +40‰) may permit even greater resolution of multiple OM sources in rivers and estuaries. In addition, autochthonous and allochthonous forms of OM may be better differentiated and more accurately quantified by using simultaneous $\Delta^{14}C$ and stable isotope signatures (Raymond and Bauer 2001*a*,*b*; Bauer et al. 2002).

The objectives of the present study were to evaluate the sources and ages of DOM supporting bacterial production in two distinct temperate systems, the Hudson River and York River estuary, using a novel natural radiocarbon ($\Delta^{14}C$) and stable isotopic ($\delta^{13}C$ and $\delta^{15}N$) approach. Previous findings have suggested that a substantial portion of heterotrophic bacterial biomass production (BBP) in both the York and Hudson rivers must be supported by allochthonous (i.e., terrigenous) sources of OM (Findlay et al. 1991; Howarth et al. 1996; Raymond et al. 2000; Schultz et al. 2003). The large difference in the mean DOM ages of these two geochemically distinct river-estuary systems (modern age in the York, 10^2–10^3 yr BP in the Hudson; Raymond and Bauer 2001*b*,*c*) therefore provides a unique opportunity to evaluate these isotopes for tracing the natural sources and ages of OM that fuel bacterial metabolism in both.

Materials and methods

Study sites and sampling locations—The York River estuary is a moderately stratified subestuary of the Chesapeake Bay that is encompassed by a watershed size of ~4,350 km^2 and has an average (50-yr) annual mean flow rate (Pamunkey River) of 28.5 m^3 s^{-1} (Fig. 1A). The York is formed by the convergence of the Pamunkey and Mattaponi Rivers, which account for 80% and 20% of the freshwater inputs, respec-

Table 1. Water characteristics of the York River estuary and Hudson River.

Site and date	Stream flow* (m^3 s^{-1})	Salinity	Water volume (L) (method of concentration)	Water temperature (°C)	Chl *a* (μg L^{-1})
York River Estuary					
Mar 2000	30.5	0	ND†	14.9	3.5
		10	140 (GM)‡	12.2	34.4
		22	180 (GM)	11.3	8.0
May 2000	18.0	0	ND	21.2	5.0
		11	75 (GM)	20.4	15.7
		20	90 (GM)	17.9	7.1
Jul 2000	9.4	0	ND	27.0	5.7
		10	115 (GM)	27.0	23.5
		20	120 (GM)	26.5	17.9
Oct 2000	3.5	0	95 (HF)§	19.0	3.7
		13	125 (GM)	19.0	12.3
		21	85 (GM)	20.0	4.9‖
Nov 2000	5.0	0	215 (HF)	16.0	2.6‖
		10	215 (HF)	15.2	10.0‖
		21	215 (HF)	13.7	10.1‖
Hudson River estuary					
Oct 2000	457.3				
240 km		0	220 (HF)	ND	0.5¶
122 km		0	200 (HF)	ND	6.6
Jun 2001	263.4				
240 km		0	185 (GM)	ND	2.5#
122 km		0	185 (GM)	ND	5.8
25 km		3.2	95 (GM)	ND	ND

* Data obtained from the U.S. Geological Survey (http://waterdata.usgs.gov). The Pamunkey River freshwater flow reported for the York River estuary.

† Not determined.

‡ GM, concentration of bacteria directly onto Gelman microcapsules (0.2 μm).

§ HF, samples concentrated by tangential flow filtration (hollow-fiber cartridge, 0.1 μm) before Gelman microcapsules.

‖ Data obtained from the Chesapeake Bay Program (http://chesapeakebay.net).

¶ Data courtesy of Drs. Nina Caraco and Jon Cole, IES.

June 2001 Chl *a* concentrations are the average of data collected 16 May 2001 and 17 July 2001 (data courtesy of Drs. Nina Caraco and Jon Cole, IES).

tively. Both rivers are considerably narrower than the estuary proper, and the Pamunkey has extensive tidal freshwater marshes that encompass an area of $\sim 2.0 \times 10^7$ m^2 (Neubauer et al. 2000). During our study, the maximal flow (30.5 m^3 s^{-1}) in the Pamunkey occurred during spring (March 2000) and decreased to a low during fall (October 2000; 3.5 m^3 s^{-1}; Table 1). Levels of chlorophyll *a* (Chl *a*) varied in space and time, with maximal concentrations consistently associated with the midsalinity station (Table 1).

The Hudson River basin (33,500 km^2) encompasses parts of Vermont, eastern New York, Massachusetts, Connecticut, and New Jersey and has an average (50 yr) annual mean flow rate of 390 m^3 s^{-1} (Fig. 1B). The tidally influenced, freshwater Hudson extends south from the head of tide at Green Island, New York (240 km) for ~130 km before it encounters saline waters in the Hudson River estuary in the vicinity of Newburgh, New York. We concentrated on the freshwater region of the Hudson; however, seawater intruded into the lower reaches of the study area in June 2001. The phytoplankton biomass in the Hudson River, although historically high, has been decimated in recent years by the invasion of the zebra mussel (*Dreissena polymorpha*), which has drastically reduced the standing stock of phytoplankton (Chl *a* ~30 to <5 mg m^{-3}; Smith et al. 1998). More than 90% of the freshwater inputs are attributed to flow over the Green Island dam (83%) and Roundout Creek (10%; Findlay et al. 1998). Samples were collected from the Hudson River estuary during moderate to high flow periods (260–460 m^3 s^{-1}, Table 1). Chl *a* concentrations (Table 1) were consistently higher at km 122 (Poughkeepsie) compared with the upriver station (km 240, Corning Preserve).

Sampling and experimental design—Water samples for bacterial nucleic acid extractions were collected from three sites along the salinity gradient of the York River estuary (Fig. 1A) during different flow regimes and seasons (Table 1). In the tidal freshwater Hudson River, water samples were collected from Poughkeepsie at 122 km and Corning Preserve at 240 km in October 2000 (Fig. 1B) and from these two sites and an additional downstream site (George Washington Bridge, salinity 3.2 at 25 km) in June 2001. Humic substances were found to coextract with nucleic acids in October and November 2000 at the freshwater York station and during the June 2001 sampling of the Hudson River (Corning Preserve and Poughkeepsie). Therefore, separate water samples were collected at a later date (October 2001 for the York and June 2002 for Hudson km 240) at these sites and extracted for the isotopic determination of the humic materials, which were then used to correct the values for bacterial biomass (see below).

The different methodologies used for collecting samples for the stable and radioisotope signatures of OM assimilated by bacteria are listed in Table 1. On the basis of initial estimates of cell abundances in the two systems (Findlay et al. 1991; Schultz et al. 2003), an assumed nucleic acid content per cell of 6–35 fg $cell^{-1}$ (Coffin and Cifuentes 1993), and the assumed C content of nucleic acids (~45%; Coffin and Cifuentes 1993), it was estimated that 100–200 liters of sample would be necessary for the extraction of sufficient bacterial C for radiocarbon analysis (minimum of 100 μg C). After collection, water samples were returned to the School of Marine Science (York samples) or the Institute of Ecosystem Studies (IES) (Hudson samples) for immediate processing.

Sample collection and concentration of bacteria—Surface water samples were collected in multiple acid-leached (10% HCl) Nanopure-rinsed polycarbonate bottles (~20 liters). Water samples were prefiltered through Whatman 0.7-μm combusted glass-fiber filters to remove macrozooplankton, particulate organic matter (POM), protozoans, and larger phytoplankton (Coffin et al. 1990; Cherrier et al. 1999); bacteria were subsequently concentrated by tangential flow ultrafiltration to a final volume of ~1 liter using an Amicon DC-10 ultrafiltration unit equipped with a single polysulfone

hollow-fiber cartridge (0.1 μm pore size; Table 1). Bacterial concentrates were then filtered onto acid-soaked (10% HCl), Nanopure-rinsed Gelman microculture capsules (0.2 μm pore size). The filter capsule was purged of water and sealed with combusted aluminum foil and stored at −80°C until extraction. An alternative means of sample collection was to concentrate bacteria directly onto the microcapsules (Table 1) after in-line prefiltration through glass-fiber filters. Microcapsules were kept on ice throughout the concentration process and stored as described above until extraction.

Nucleic acid extraction—Bacterial nucleic acid extractions for isotopic analysis were performed according to the modified method of Coffin and Cifuentes (1993) as outlined in Cherrier (1997). In brief, bacterial cells collected in the microcapsules were lysed by adding a detergent/buffer solution (20 mmol L^{-1} Tris, 2 nM ethylene diaminetetraacetic acid, and 2% sodium dodecyl sulfate [SDS]) and heating the sealed capsule in a 100°C water bath for 15 min. After precipitation and the removal of the SDS from the lysate, nucleic acids were isolated and purified by dialysis, followed by ethanol, phenol, and isoamyl alcohol/chloroform precipitations. Two capsules were reextracted using the same protocol, to serve as methodological blanks for assessing potential contamination by solvents and processing. Possible contamination of the extract by protein was assessed by spectrophotometric absorbance ($A_{260}:A_{280}$) ratios (Sambrook et al. 1989). Approximately 10% of the extract was used to verify purity and was then retained for the subsequent $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis. The remainder was used for natural abundance $\Delta^{14}C$ analysis. Extracts were stored at −80°C until isotopic analysis (<1 month).

The specificity to bacteria of nucleic acids in the 0.2–0.7-μm fraction has been demonstrated previously with 16S RNA analysis (Coffin et al. 1990); however, the potential for an isotopic bias by the inclusion of picoplankton may exist in different systems. Picoplankton (e.g., cyanobacteria) in the York make up only ~7% of the total autotrophic biomass (Ray et al. 1989) and contribute an order of magnitude less C than bacteria (Eldridge and Sieracki 1993). Since the establishment of *Dreissena* in the Hudson, cyanobacterial densities have decreased >700-fold, and they are now no more than a few percentage of total cell counts (Smith et al. 1998). Thus, cyanobacterial contributions to the observed nucleic acid isotopic signature should be minimal, and no correction was applied.

Humic materials may coextract with nucleic acids when they are present in high concentrations (Coffin and Cifuentes 1993; Jackson et al. 1997; Edgecomb et al. 1999). The coextraction of humics occurred in October and November 2000 at the freshwater York station and during the June 2001 sampling of Hudson River (Corning Preserve and Poughkeepsie). In an initial attempt to remove humics from the nucleic acid extracts, two different gel separation techniques were used, according to manufacturers' specifications and procedures outlined in Edgecomb et al. (1999) and Jackson et al. (1997). Columns retained a fraction of humic material, but the eluent remained amber colored, despite several passes of sample through columns. Because the complete removal of contaminating humic materials was not possible, the $\delta^{13}C$ and $\Delta^{14}C$ isotopic values of the total extracts were corrected for the contribution of humics as

$$C_{(\text{humics}+\text{NA})} = C_{(\text{humics})}(x) + C_{(\text{NA})}(y) \quad (1)$$

where $C_{(\text{humics}+\text{NA})}$ is the measured isotopic value ($\delta^{13}C$ and $\Delta^{14}C$) for the total extract, $C_{(\text{humics})}$ is the isotopic signature of humics (measured as described below), and x and y are the relative contributions from humic and nucleic acid carbon, respectively ($x + y = 1.0$). The values of x and y were estimated independently from C : N values as

$$C:N_{(\text{humics}+\text{NA})} = C:N_{(\text{humics})}(x) + C:N_{(\text{NA})}(y) \quad (2)$$

The C : N values of the total extract ($C:N_{(\text{humics}+\text{NA})}$) and of the humic materials alone were measured on a FinniganMAT Delta^{plus} dual-inlet continuous flow isotope ratio mass spectrometer. A literature value of ~2.25 was used for C : N values for nucleic acids (Coffin and Cifuentes 1993). Humic compounds from both the York and Hudson were isolated as described in Moran and Hodson (1994). Equation 2 was then solved for the relative contribution of humics and nucleic acids in the total extract value. Values for x and y were subsequently substituted into Eq. 1 and solved for $C_{(\text{NA})}$, the isotopic composition of nucleic acids.

DOM isolation—DOM for stable isotopic ($\delta^{13}C$ and $\delta^{15}N$) and $\Delta^{14}C$ analyses was concentrated by tangential flow ultrafiltration (Amicon DC-10 equipped with a 3-kDa polysulfone spiral-wound cartridge) after filtration through a Gelman capsule (0.2 μm) of York River water (~ 75–125 L) at three sampling locations (0, 10, and 20 salinity in March and October 2000). After initial concentration to ~1 liter, the sample was further reduced to a final volume of ~50 ml by turboevaporation and then lyophilized. Lyophilized DOM was reconstituted in ~10 ml of deionized (DI) water and desalted overnight in Pierce 3.5-kDa slide-a-lyzers according to manufacturer's specifications. The desalted DOM was lyophilized, acidified with 10% HCl, and prepared for isotope analysis as outlined below. Leaves of *Peltandra virginica*, the most prominent freshwater plant in the York, were collected in July 2000, before senescence, for $\delta^{13}C$ analysis. After rinsing with DI water, the leaves were allowed to extract in the dark at laboratory temperature in an aerated acid washed carboy (20 liters) with DI water for 10 d. A subsample (2 liters) was subsequently reduced in volume by turboevaporation to ~50 ml and was then lyophilized to remove all associated water. After acidification with 10% HCl, samples were analyzed for $\delta^{13}C$ (see below).

Sample preparation and isotopic analyses—For stable isotope analyses, aliquots of nucleic acid extracts (approximately one tenth the initial sample) were thawed and transferred quantitatively to combusted (500°C) Pyrex centrifuge tubes. Samples were reduced in volume to ~100 μl by vacuum evaporation (Labconco Centrivap model 78100–00D). Nucleic acids and lyophilized DOM (humics, *Peltandra* leachate, and high-molecular-weight [HMW] DOM) were transferred quantitatively to acetone-rinsed tin foil CHN capsules and dried overnight at 60°C. Nucleic acids and humic OM were analyzed using a FinniganMAT Delta^{plus} dual-inlet continuous flow isotope ratio mass spectrometer (G.G. Hatch

Table 2. Bacterial nucleic acid $\Delta^{14}C$ and $\delta^{13}C$ values, corrected for humic contributions.

Site and date	Salinity or river km	C:N	Uncorrected $\Delta^{14}C$ (‰)	Uncorrected $\delta^{13}C$ (‰)	Corrected $\Delta^{14}C$ (‰)	Corrected $\delta^{13}C$ (‰)
York						
Oct 2000	S=0	8.5	188	−28.7	234	−29.4
Nov 2000	S=0	7.5	168	−28.1	193	−28.3
Humic	S=0	18.5	111	−27.5	NA*	NA
Hudson						
Jun 2001	240 km	7.5	6	−27.2	3	−27.2
Jun 2001	122 km	6.2	16	−25.3	16	−25.0
Humic	240 km	34.7	21	−27.2	NA	NA

* NA, not applicable.

Isotope Laboratories, University of Ottawa). HMW DOM and lyophilized *Peltandra* leachate were analyzed with a Europa Scientific Hydra 20/20 continuous flow isotope ratio mass spectrometer (Stable Isotope Facility, University of California, Davis). Stable isotope values are reported in standard (δ) notation as

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 10^3 \quad (3)$$

where X is ^{13}C or ^{15}N and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The recognized standards are PeeDee Belemnite (NBS-1) and atmospheric N_2 for $\delta^{13}C$ and $\delta^{15}N$, respectively.

For natural abundance $\Delta^{14}C$ measurements, nucleic acid extracts (~1 ml final volume) were thawed, transferred quantitatively (three rinses) to prebaked (500°C) 13-mm-diameter Pyrex tubes, and reduced in volume to ~2 ml by vacuum evaporation. Lyophilized humic OM and HMW DOM (freshwater) were transferred to prebaked (500°C) Pyrex tubes. Samples were subsequently acidified overnight with 1 ml of 3% $H_3PO_4^-$, reduced in volume again by vacuum evaporation, transferred to combusted (500°C) quartz tubes (6 mm diameter), and evaporated under vacuum until all water was removed (minimum 14 h). The tubes were then sealed under vacuum and combusted at 900°C using a CuO/Ag metal catalyst to CO_2 (Sofer 1980). The CO_2 was subsequently reduced to graphite in an atmosphere of H_2 over cobalt catalyst (Vogel et al. 1987). Graphite targets were analyzed at the Center for Accelerator Mass Spectometry at Lawrence Livermore National Laboratory. $\Delta^{14}C$ is defined as the per mil (‰) deviation of a sample from the ^{14}C activity of 19th-century wood. All reported $\Delta^{14}C$ values were corrected for fractionation using the $\delta^{13}C$ values of the samples and the conventions of Stuiver and Pollach (1977). Total measurement uncertainties for $\Delta^{14}C$ analyses of these samples were typically ±5–10‰.

Results

Isotopic signatures of bacterial nucleic acids—Humic correction: Humic materials were discernible from nucleic acids by their greater C:N ratios of 18.5 and 34.7 for the York and Hudson, respectively (Table 2). The mean $\delta^{13}C$ values of humic materials collected from the York (−27.5‰) and Hudson (−27.2‰) river estuaries were similar to those for the uncorrected nucleic acid extracts; thus, $\delta^{13}C$ corrections were <1‰ for both systems (Table 2). In the York River estuary, however, isolated humics were significantly depleted in $\Delta^{14}C$ ($\Delta^{14}C_{humic}$ = 111‰) relative to uncorrected nucleic acid extracts ($\Delta^{14}C_{(humics+NA)}$ = 168–188‰). Corrected nucleic acid extracts were therefore enriched in $\Delta^{14}C$ by ~25–46‰ (Table 2) relative to uncorrected values. Conversely, humic isolates in the Hudson River were slightly enriched in $\Delta^{14}C$ (by 15‰ and 5‰ for Corning Preserve and Poughkeepsie, respectively) relative to uncorrected extracts (Table 2).

Table 3. Stable ($\delta^{13}C$ and $\delta^{15}N$) and radio ($\Delta^{14}C$) isotopic values of extracted nucleic acids.

Location and date	Salinity or river km	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)*	$\Delta^{14}C$ (‰)
York River estuary				
Mar 2000	S=10	−22.8		
	S=22	−21.2		
May 2000	S=11	−22.2		91
	S=20	−27.6		
Jul 2000	S=10	−22.5	12.6	45
	S=20	−21.6	7.6	39
Oct 2000	S=0†	−29.4	5.5	234
	S=13	−24.5		61
	S=21	−23.0		−35
Nov 2000	S=0†	−28.3	5.4	193
	S=10	−25.4	17.3	52
	S=21	−22.2	8.9	41
Hudson River estuary				
Oct 2000	S=0/240 km	−28.4		NA‡
	S=0/122 km	−26.8		−153
Jun 2001	S=0/240 km†	−27.2	0.9	3
	S=0/122 km†	−25.0	8.7	16
	S=3.2/25 km	−25.6		−144

* Sample prioritized for $\delta^{13}C$; $\delta^{15}N$ was obtained when possible.
† Corrected for the contribution of humics. See Table 2 and associated text for a full explanation.
‡ Not applicable, sample lost.

York River estuary: The $\Delta^{14}C$ signatures of bacterial nucleic acids were most enriched at the freshwater end member of the York where values averaged 214 ± 29‰ (Table 3, Fig. 2A). Values became more depleted (i.e., older) with increasing salinity (Fig. 2A) and averaged 62 ± 20‰ and 15 ± 43‰ for the midsalinity and mouth locations, respectively (Table 3). The corresponding $\delta^{13}C$ values were lightest at the freshwater end member (−28.9 ± 0.9‰) and increased seaward with the exception of May 2000, when bacterial nucleic acids at the mouth displayed an anomalously light $\delta^{13}C$ value of −27.6‰ (Fig. 2B).

Dual-isotope ($\Delta^{14}C$ vs. $\delta^{13}C$) plots of bacterial nucleic acids in the York River estuary show distinct separations between the fresh and saltwater regions (Fig. 3). The freshwater region was enriched in $\Delta^{14}C$ and depleted in $\delta^{13}C$, relative to the mouth. A clear differentiation of isotopic values was not always possible for bacterial nucleic acids between the midsalinity station and the mouth, because sig-

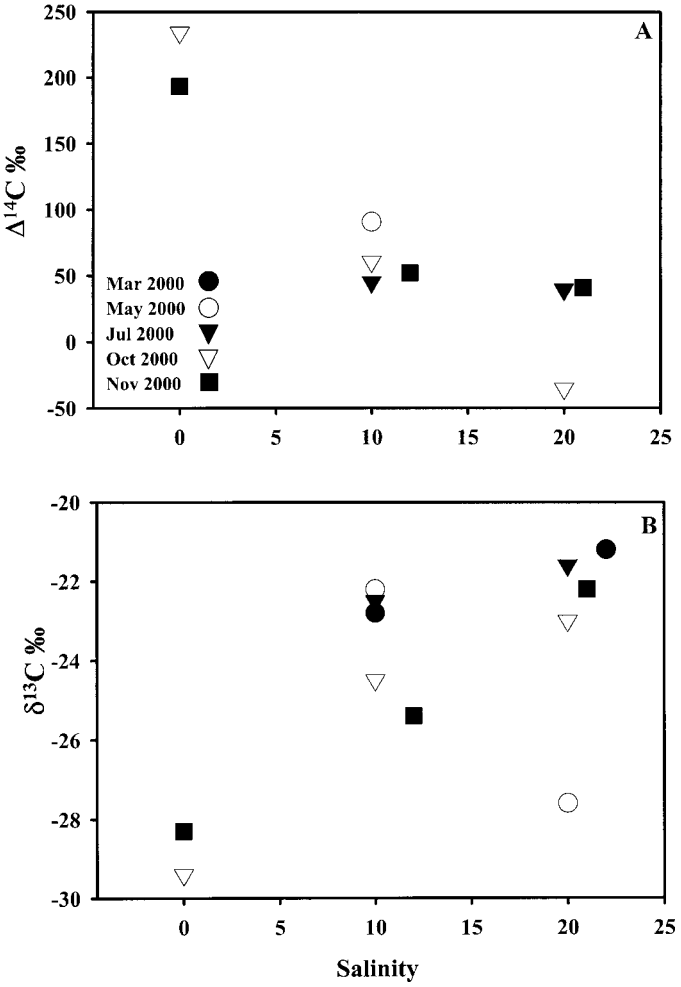

Fig. 2. (A) $\Delta^{14}C$ and (B) $\delta^{13}C$ signatures of bacterial nucleic acids as a function of salinity in the York River estuary.

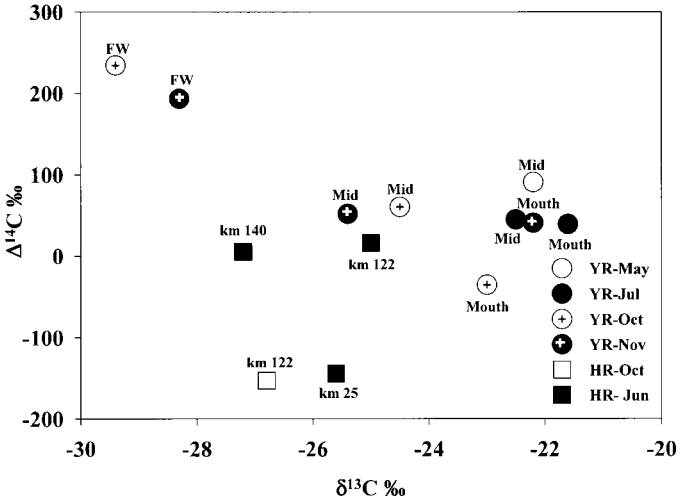

Fig. 3. $\Delta^{14}C$ vs. $\delta^{13}C$ of bacterial nucleic acids from the York River estuary and the Hudson River. Sample locations are as follows. York: freshwater (FW), midsalinity (Mid), and mouth; Hudson: Corning Preserve (140 km), Poughkeepsie (122 km), and Palisades (25 km).

natures overlapped (July 2000; Table 3, Fig. 3). Bacterial nucleic acid $\Delta^{14}C$ signatures in October 2000 at the mouth were the most depleted (−35‰) of all $\Delta^{14}C$ values in the York River estuary.

Hudson River: The $\delta^{13}C$ signatures of bacterial nucleic acids in the Hudson River ranged from −28.2‰ to −25.0‰ and fell within the range of average freshwater (−28.9‰) and midsalinity (23.5‰) values for York River bacterial nucleic acids (Table 3). However, the $\Delta^{14}C$ values of bacterial nucleic acids in the Hudson (16‰ to −153‰) were, in general, much more depleted than those of the York (234‰ to −35‰; Table 3, Fig. 3).

The $\delta^{13}C$ signatures of bacterial nucleic acids in the Hudson River estuary were ~2.7‰ heavier than their freshwater counterparts in the York (Fig. 3), and there were significant differences in the isotopic signatures of bacterial biomass between the two systems (analysis of variance, $p < 0.05$). Bacterial nucleic acids collected during fall 2000 from the freshwater portions of both systems differed by ~370‰ in the $\Delta^{14}C$ of OM assimilated, with Hudson River bacterial $\Delta^{14}C$ being highly depleted (−153‰) and York River bacteria containing post-1950s bomb ^{14}C (+214‰). Nucleic acids from the freshwater (240 and 122 km) Hudson in June 2001 also contained bomb carbon ($\Delta^{14}C$ = +3–16‰) and were most similar isotopically at this time and location to nucleic acids from the mouth of the York (Fig. 3).

DOM and Peltandra isotopic signatures—The $\delta^{13}C$ values of HMW DOM increased by ~6‰ from the head of the York River estuary (−28.1‰) to the mouth (−22.3‰; Table 4). The $\delta^{13}C$ value of *P. virginica* leachate (−29.6‰) was 1.5–5.6‰ more depleted than values of HMW DOM isolated from fresh and midsalinity regions of the estuary. The corresponding $\delta^{15}N$ signature of HMW DOM showed a similar trend of increasing values down-estuary, ranging from 4‰ to 9.2‰ in freshwater and high salinity, respectively (Table 4). Ultrafiltered DOM collected from the head of the York was enriched in $\Delta^{14}C$ (+434‰), compared with the humic material isolated by resins ($\Delta^{14}C$ = +111‰), which may reflect differences in the fractions isolated by each method as well as potential temporal variations in OM sources.

Discussion

Simultaneous measurements of $\delta^{13}C$ and $\delta^{15}N$ in bacterial biomass (Hopkinson et al. 1998; Coffin and Cifuentes 1999) have been used to identify OM sources utilized by bacteria with a greater degree of sensitivity and specificity than either isotope alone. The fractionation of ^{13}C by heterotrophic metabolism is generally thought to be small (~1–2‰; Coffin et al. 1989), whereas ^{15}N fractionation may be significant (as high as ~15‰) when N is abundant (Peterson and Fry 1987). The interpretation of $\delta^{15}N$ values in bacteria is further confounded by the large number of potential N sources (Kirchman 1994). For example, although amino acids are the preferred bacterial N source in estuaries, ammonium may

Table 4. Published ranges of isotope values of potential organic matter sources to estuaries.

Source	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\Delta^{14}C$ (‰)	References
In literature				
Terrigenous (vascular plant)	−26 to −30	−2 to +2		Fry and Sherr 1984; Deegan and Garritt 1997
Terrigenous soils (surface)/forest litter	−23 to −27	2.6 to 6.4	+152 to +310	Cloern et al. 2002; Richter et al. 1999
Freshwater phytoplankton	−24 to −30	5 to 8		Anderson and Arthur 1983; Sigleo and Macko 1985
Marine/estuarine phytoplankton	−18 to −24	6 to 9		Fry and Sherr 1984; Currin et al. 1995
C-4 salt marsh plants	−12 to −14	3 to 7		Fry and Sherr 1984; Currin et al. 1995
Benthic microalgae	−12 to −18	0 to 5		Currin et al. 1995
C-3 Freshwater/Brackish marsh plants	−23 to −26	3.5 to 5.5		Fry and Sherr 1984; Sullivan and Moncreiff 1990
Specific to York River Estuary				
Freshwater grass leachate (*Peltandra virginica*)	−29.6			This study
Marsh OM (0–6 cm)	−22.3 to −26.4		+45 to +58	Raymond and Bauer 2001*a*
Marsh macrophytes	−23.3 to −28.9	5.3 to 11.0		Neubauer 2000
Marsh microalgae (benthic)	−23.7 to −27.7	8.4 to 11.3		Neubauer 2000
Phytoplankton (freshwater end member)*	−27.5 to −34.6		+110 to +164	Raymond and Bauer 2001*a*
Phytoplankton (midsalinity)	−21.8 to −24.2		+56 to +72	Raymond and Bauer 2001*a*
Phytoplankton (York River mouth)	−20.1 to −22.8		+47 to +62	Raymond and Bauer 2001*a*
Chesapeake Bay DOM	−23.7		−77	Raymond and Bauer 2001*a*
Terrigenous (leaf OM)			+100	Raymond and Bauer 2001*a*
HMW DOM (0 salinity)	−27.8 to −28.1	4.0 to 4.7	+434	This study
HMW DOM (10 salinity)	−24.0 to −24.5	5.5 to 7.5		This study
HMW DOM (20 salinity)	−22.3 to −22.7	7.8 to 9.2		This study
FW POM	−28.2 to −30.0	6.4 to 7.9	+24 to −190	Raymond and Bauer 2001*c*; this study
Humics (resin-extracted)	−27.5		+111	This study
Specific to Hudson River				
POM (240 km)	−29.0	6.0	−101 to −156	This study; Raymond and Bauer 2001*c*
POM (122 km)†	−27.1 to −27.4	2.8 to 3.2	−96	This study; Raymond and Bauer 2001*c*
DOC (240 km)	−27.0 to −27.2		−73 to −137	Bauer et al. unpubl. data
DOC (152 km)	−27.0		−110	Bauer et al. unpubl. data
Phytoplankton (240 km)	−30.0 to −31.1	8.0	−44 to −50	Bauer et al. unpubl. data; Caraco et al. 1998
Phytoplankton (165 km)‡	−24.2		−74	Caraco unpubl. data
Phytoplankton (152 km)	−30.5	8.0	−52	Bauer et al. unpubl. data; Caraco et al. 1998
Submerged macrophytes	−21.7 to −22.2	8.0	−37 to −38	Caraco et al. 1998; Caraco unpubl. data
Emergent macrophytes	−26.0	8.0	+90	Caraco et al. 1998; Raymond and Bauer 2001*b*
Terrestrial (leaf OM)	−27.0	−2.0		Caraco et al. 1998
Terrigenous (sedimentary rock)§	−28.6 to −29.8		−866 to −999	Petsch 2000
Humics (resin-extracted)	−27.2		+22	This study

* Phytoplankton isotopic values for the York and Hudson (unless otherwise noted) were predicted from measured $\delta^{13}C$-DIC and $\Delta^{14}C$-DIC values and assumed a kinetic fractionations of 20‰ for $\delta^{13}C$ values (Chanton and Lewis 1999). Because $\Delta^{14}C$ values were normalized to $\delta^{13}C$ according to the principles of Stuiver and Polach (1977), no additional correction was applied.
† $\Delta^{14}C$ from 152 km.
‡ Phytoplankton signature from plankton net tow.
§ OM isotopic values are from weathering profiles of Marcellus Shale (Hudson/Mohawk River valley) at depths of 8, 57, and 170 cm.

also account for a significant fraction (5–60%) of total N uptake by bacteria (Kirchman 1994). The subsidization of N from inorganic sources may therefore limit the use of $\delta^{15}N$ for tracing OM sources.

The natural abundance of ^{14}C may make it a useful alternative tracer for examining the sources of OM supporting BBP (Cherrier et al. 1999). Its much greater dynamic range (>1,000‰), compared with $\delta^{13}C$ (~30‰) and $\delta^{15}N$ (~40‰) in coastal systems, potentially makes it a more sensitive tracer of coastal and estuarine carbon and OM cycling (Raymond and Bauer 2001; Bauer et al. 2002). Radiocarbon also often displays a much broader range of values across potential OM reservoirs than stable isotopes, which thus allows for greater differentiation of the sources of OM to a given pool.

Potential OM sources to York River estuarine bacteria— $\delta^{13}C$-$\delta^{15}N$ signatures of potential OM sources to bacteria: Potential inputs of OM to estuaries may arise from numerous allochthonous and autochthonous sources that span a broad range of $\Delta^{14}C$, $\delta^{13}C$, and $\delta^{15}N$ signatures. For most systems, however, isotopic data with which to constrain potential OM sources are limited or incomplete, especially for $\delta^{15}N$ and $\Delta^{14}C$. Existing literature values (Table 4) were therefore compiled in an attempt to constrain the isotopic signatures of potential allochthonous and autochthonous OM sources supporting BBP.

The ranges for published $\delta^{13}C$ and $\delta^{15}N$ values of potential OM sources to estuaries in general, along with the $\delta^{13}C$ and $\delta^{15}N$ values of bacterial nucleic acids measured in the present study, are displayed in Fig. 4. The comparison of bacterial $\delta^{13}C$ and $\delta^{15}N$ signatures to those of potential OM sources at the mouth of the York suggests that BBP may be supported exclusively by marine/estuarine phytoplankton. In contrast, bacterial isotopic signatures in the midsalinity region fall outside the primary sources of OM because of their elevated $\delta^{15}N$ values. The enriched $\delta^{15}N$ values may be explained in part by N sources that are derived from higher trophic-level metabolism. Overall, $\delta^{15}N$ in heterotrophic organisms is generally believed to reflect an enrichment of ~3–4‰ per trophic level over that of primary producers (Michener and Schell 1994). The bacterial acquisition of N derived from higher trophic levels is supported by elevated concentrations of lipid biomarkers that are diagnostic of zooplankton inputs in the midsalinity region of the York River (McCallister 2002). Hydrodynamic controls (e.g., tidal mixing and advection) also partially regulate the accumulation of phytoplankton in the mesohaline area of the York (Sin and Wetzel 2002). This region of hydraulic retention may therefore promote enhanced N recycling and drive greater algal fractionation of N. Subsequent bacterial incorporation of ^{15}N-enriched inorganic nitrogen, which is potentially derived from a combination of nitrification and algal fractionation (Cifuentes et al. 1989), may have contributed to the anomalously high midsalinity isotopic values ($\delta^{15}N$ = ~17‰; Fig. 4).

In freshwater regions of the York, overlapping $\delta^{13}C$ and $\delta^{15}N$ values of freshwater phytoplankton, terrestrial/soil DOM, freshwater, and brackish waster marsh OM (Fig. 4) make differentiation of the dominant sources supporting BBP at the head of the estuary more difficult. Thus, although dual-isotope plots using $\delta^{15}N$ versus $\delta^{13}C$ may help to delineate the potential OM sources, large overlaps in end-member sources and possible complications by inorganic N assimilation in the York prohibit a direct assessment of the contribution of each potential OM source to BBP.

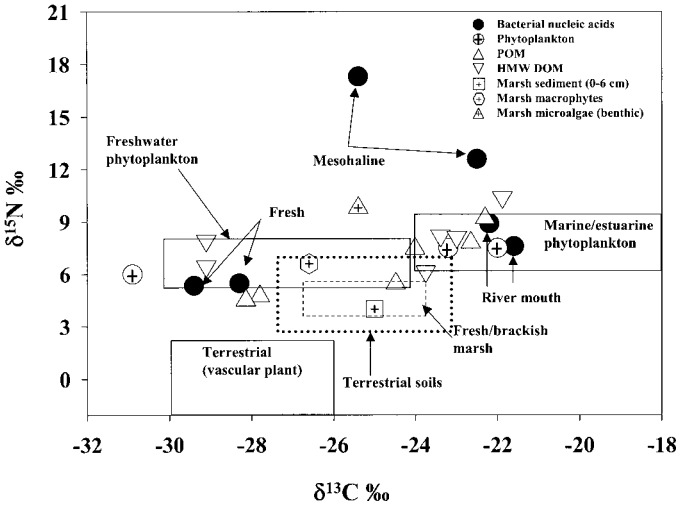

Fig. 4. Comparison of $\delta^{15}N$ vs. $\delta^{13}C$ of bacterial nucleic acids and potential sources of organic matter for bacteria within the York River estuary. Rectangles are published isotopic ranges (*see Table 4*) for each potential estuarine source group and are not necessarily unique to the York River estuary. Measured isotopic values for bacterial nucleic acids, POM, and HMW DOM are also shown. Multiple points represent different sampling sites and times. Symbols with crosshairs are published literature values from previous isotopic studies in the York (for ranges, *see Table 4*). Phytoplankton were assigned an average $\delta^{15}N$ of 6.5‰ and 7.5‰ (median of published range, *see Table 4*) for freshwater and saline regions of the York, respectively. Marsh sediment, macrophyte, and microalgal OM were assigned the median $\delta^{15}N$ of literature values (Table 4).

$\Delta^{14}C$-$\delta^{13}C$ signatures of potential OM sources to bacteria: The natural abundance of ^{14}C provides an additional isotopic dimension for evaluating the relative importance of OM sources to BBP. Compared with $\delta^{13}C$ and $\delta^{15}N$, there are far fewer $\Delta^{14}C$ measurements in most estuarine systems, with the exception of the York and Hudson (Table 4). Previous work in the York using $\Delta^{14}C$ and $\delta^{13}C$ (Raymond and Bauer 2001*a*,*b*) identified the important autochthonous and allochthonous OM sources as riverine/estuarine phytoplankton, marsh-derived material, and terrestrially derived (i.e., soil or forest litter) material. It is difficult to define a unique, discrete terrigenous end member, because the land-derived component is likely a composite of soil-, surface runoff–, and forest litter–derived OM. We therefore chose a $\Delta^{14}C$ range that was defined by contributions from shallow soil-derived OM (229‰; Richter et al. 1999) and the average $\Delta^{14}C$ (272‰) of resin-extracted OM (+111‰) and HMW DOM (+434‰) from the freshwater end member as a likely approximation of terrigenous OM. The ranges in isotopic signatures of these potential end-member sources, along with

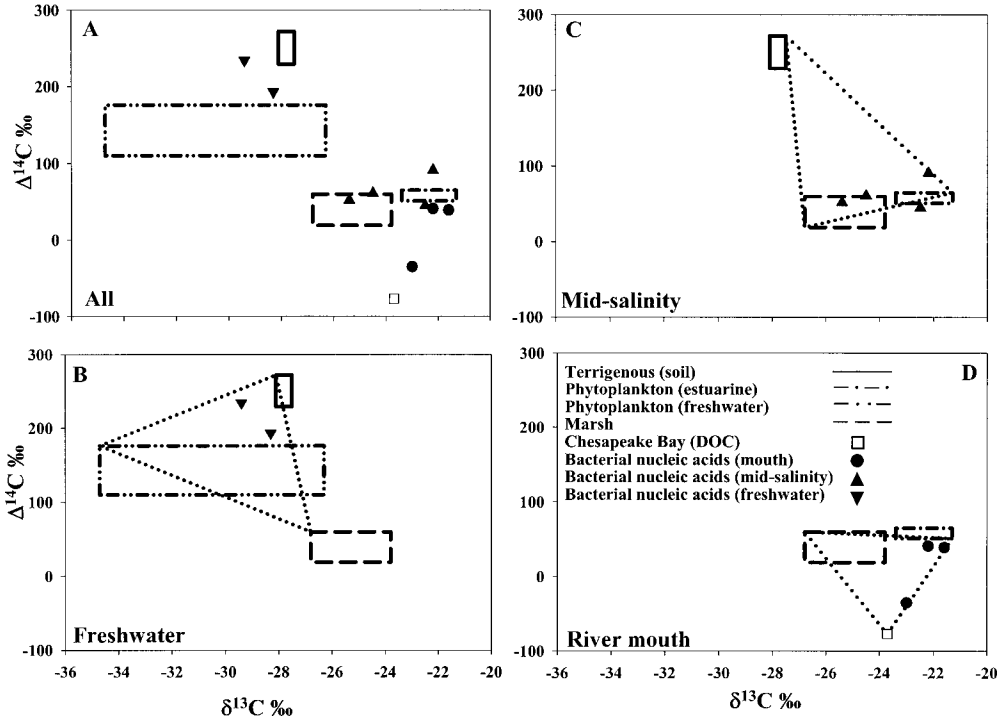

Fig. 5. Comparison of $\Delta^{14}C$ vs. $\delta^{13}C$ of bacterial nucleic acids and potential sources for the (A) entire York River estuary and (B) for the freshwater, (C) midsalinity, and (D) high-salinity mouth sites in the York River estuary. Boxes are the 95% confidence intervals for potential end members in the York, with the exception of the terrigenous and Chesapeake Bay end members (*see text and Table 5 for details*). Dotted lines encompass the solution space from one run of the model. Table 6 lists the complete model output.

values for all bacterial nucleic acids measured in the York during the present study, are shown in Fig. 5A.

In contrast to the $\delta^{15}N$ vs. $\delta^{13}C$ relationships (Fig. 4), $\Delta^{14}C$ versus $\delta^{13}C$ delineates the potential sources of OM specific to the York relative to bacteria in fresh (Fig. 5B), midsalinity (Fig. 5C), and high salinity (Fig. 5D) regions of the system. Terrestrial/soil DOM and freshwater phytoplankton DOM are especially well defined isotopically (Fig. 5A,B). Recently fixed terrestrial OM will reflect present-day atmospheric $\Delta^{14}C$ values of ~100‰ (Raymond and Bauer 2001*a*) and, consequently, the average $\Delta^{14}C$ value of 215‰ for bacterial nucleic acids requires OM from CO_2 fixation at least 15–20 yr ago when atmospheric inventories of $\Delta^{14}C$-CO_2 were ~115‰ greater than they are today (Levin and Kromer 1997). Unlike phytoplankton, which should reflect current $\Delta^{14}C$–dissolved inorganic carbon (DIC) values (143‰), bacterial nucleic acids were more ^{14}C-enriched (~215‰). This is consistent with contemporary terrestrial soil OM (Trumbore et al. 1992) and forest floor OM (Richter et al. 1999) as the primary sources for BBP (*see Table 4*).

Estimates of OM sources supporting estuarine bacterial biomass production—The relative contributions of the various potential OM sources to BBP were estimated using a dual-isotope, three-source mixing model (Fry and Sherr 1984; Bauer et al. 2002). The series of three equations and three unknowns is

$$\delta^{13}C_{NA} = f_1 \cdot \delta^{13}C_{OM1} + f_2 \cdot \delta^{13}C_{OM2} + f_3 \cdot \delta^{13}C_{OM3} \quad (4)$$

$$\Delta^{14}C_{NA} = f_1 \cdot \Delta^{14}C_{OM1} + f_2 \cdot \Delta^{14}C_{OM2} + f_3 \cdot \Delta^{14}C_{OM3} \quad (5)$$

$$f_1 + f_2 + f_3 = 1 \quad (6)$$

where $\delta^{13}C_{NA}$ and $\Delta^{14}C_{NA}$ are the isotopic signatures of the bacterial nucleic acids measured in each region of the York (e.g., freshwater, midsalinity, and high salinity) and the Hudson resulting from utilization of the three most likely major OM sources (*see Table 5*). The variable f is the relative contribution of each of the three assumed potential OM sources to the nucleic acids in each sample. The equations were solved for the values of f and then evaluated in Excel by substituting values for the isotopic signatures of three OM sources (end members) and the bacterial nucleic acids in each region.

To test the sensitivity of the three-source model to the choice of isotopic values for each end member, Eqs. 4–6 were solved with each possible combination of the high and low 95% confidence intervals for $\Delta^{14}C$ and $\delta^{13}C$ for each source (Table 5). For example, in the freshwater York, the model was run 64 times, to test all possible combinations of high and low isotopic source values. Model output values for fractions 1, 2, and 3 that yielded an isotopic solution space (*see triangles in Fig. 5B–D*) that encompassed the measured nucleic acid values for a given salinity (York) or season/location (Hudson; see below) were accepted (Table

Table 5. Isotopic values of potential end-member sources used in mass-balance calculations.

Source	Average		95% confidence interval		95% confidence interval		Reference
	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	$\Delta^{14}C$ (‰)	
York River Estuary							
Riverine phytoplankton	−30.5 (3.7)*	+143 (29)	−26.3	−34.7	176	110	Raymond and Bauer 2001*a*; Caraco unpubl. data
Riverine/Terrigenous†	−27.9 (0.4)	+251 (30)	−27.5‡	−28.1	229	272	Raymond and Bauer 2001*a*; Richter et al. 1999; this study
Estuarine phytoplankton (S=8.5–17)§	−22.3 (1.4)	+58 (9)	−21.3	−23.4	65	51	Raymond and Bauer 2001*a*
Marsh OM	−25.3 (1.7)	+50 (6)	−23.8	−26.8	56	44	Raymond and Bauer 2001*a*
Chesapeake Bay DOM	−23.7	−77	ND‖	ND	ND	ND	Raymond and Bauer 2001*a*
Hudson River							
Terrigenous (sedimentary rock)¶	−29.3 (0.6)	−950 (73)	−28.6	−30.1	−877	−1,000	Petsch 2000
Phytoplankton	−29.0 (3.2)	−55 (13)	−25.8	−32.1	−42	−68	Bauer et al. unpubl. data; Caraco unpubl. data
SAV	−22.0 (0.4)	−38 (<1)	−21.5	−22.4	−37	−38	Caraco unpubl. data
Emergent OM (marsh and forest litter)#	−26.5	90	ND	ND	ND	ND	Raymond and Bauer 2001*b*
Autochthonous OM**	−25.5 (4.9)	−46 (12)	−18.6	−32.3	−29	−63	Bauer et al. unpubl. data; Caraco unpubl. data

* Values in parentheses are ±1 SD of the mean ($n \geq 3$).
† Two separate sources were chosen to approximate the range in terrigenous $\Delta^{14}C$. Contributions from soil OM ($\Delta^{14}C = +229$ ‰; Richter et al. 1999) and the average $\Delta^{14}C$ of humic and HMW DOM from the head of the York (see Table 4 for values).
‡ Range of values shown instead of 95% confidence interval (see above).
§ Isotopic values for estuarine (salinity = 8.5–17) phytoplankton estimated from DIC isotopic values (see text for details).
‖ ND, not determined.
¶ Rock weathering profiles were used to estimate the isotopic signatures of OM released from sedimentary rock (see Table 4 for values).
# Emergent OM encompasses OM derived both from marshes and forest litter.
** Autochthonous OM is a combined phytoplankton- and SAV-derived source.

Table 6. Estimates of the relative contribution of potential OM sources assimilated by bacteria along the York River salinity gradient and the Hudson River. See text for details.

		Relative contribution (%) of				
Date	Salinity	DOC* riverine/ terrigenous	Phyto-plankton freshwater	Phyto-plankton estuarine	OM marsh	OM Chesapeake Bay
York River Estuary						
May 2000	11	13	NA†	83–87	1–5	NA
Jul 2000	10	0–4	NA	53–78	21–46	NA
	20	NA	NA	88–90	1–4	8–10
Oct 2000	0	49–80	6–25	NA	8–33	NA
	13	2–20	NA	0–71	20–83	NA
	21	NA	NA	29–32	0–5	66–70
Nov 2000	0	72–83	7–17	NA	5–15	NA
	10	5–10	NA	27–45	47–65	NA
	21	NA	NA	63–81	9–29	4–17

		Relative contribution (%) of:		
Date	Distance (km)	Sedimentary rock DOC	Phyto-plankton SAV‡	Emergent (forest litter/marsh)
Hudson River				
Oct 2000	122	21–25	3–7	69–76
Jun 2001	240	7–8	7–9	83–85
	122	4–5	20–21	74–76
	25	18–22	17–21	57–64

* Isotopic values for potential sources shown in Table 5.
† NA, not applicable (end member not used in isotopic mass balance).
‡ The isotopic range of autochthonous OM (Table 5) used in isotopic mass balance.

6). A three-source model was selected as a reasonable approximation of a likely more complex situation in nature itself. Although a greater number of sources is possible, the series of equations is cumbersome to solve and has many more possibilities to evaluate ($2^8 = 256$ possibilities for four end members). Given the uncertainties in assigning isotopic values for even three sources, the use of more seems unjustified. Our results illustrate the principle for future applications.

The three potential sources of OM used in our model varied depending on the location of each site. The relative importance of OM derived from estuarine phytoplankton in different parts of the York can be established using salinity as a proxy. Thus, potential OM sources to the freshwater York are limited to freshwater phytoplankton, marsh, and terrigenous OM. The midsalinity region of the York is more complex as potential sources (freshwater and estuarine phytoplankton, marsh, and terrigenous derived OM) are possible. However, when the $\Delta^{14}C$ and $\delta^{13}C$ ranges for freshwater phytoplankton were substituted into Eqs. 4–6, they did not yield a solution space that encompassed the isotopic values for nucleic acids in May 2000. Therefore, end members of estuarine phytoplankton, marsh and terrigenous derived OM were selected as solutions were possible for all sampling periods. The high-salinity $\Delta^{14}C$ values of nucleic acids (41‰ to −35‰) require the assimilation of isotopically depleted OM. We selected a bay-derived OM source (i.e., a mixture of bay phytoplankton, marsh, and terrigenous OM) as the $\Delta^{14}C$ depleted end member.

The solution of Eqs. 4–6 using $\Delta^{14}C$ and $\delta^{13}C$ signatures for terrigenous, freshwater algal, and marsh-derived OM as the major sources for the freshwater York (Fig. 5B) indicates that (1) up to ~83% of the OM assimilated by bacteria may be of terrigenous origin and (2) OM derived from marshes and freshwater phytoplankton made up the balance of OM assimilated (8–33% and 6–25%, respectively; Table 6). Thus, despite relatively low Chl *a* (Table 1) concentrations and the predominant terrigenous signature of DOC (Raymond and Bauer 2001*a*), algal-derived OM may be an important component supporting BBP in freshwater regions of the York. The significance of algal-derived OM to BBP is further corroborated by lipid biomarker analysis of dissolved OM, which linked the presence of labile algal biomarkers (polyunsaturated fatty acids) to the relative bioavailability of bulk DOC (McCallister 2002). The $\Delta^{14}C$ signature of freshwater particulate organic carbon (POC; −68‰) suggests that it contributes little to freshwater BBP, despite the greater lability of the particulate versus dissolved phase, as implied by the lipid signatures of each fraction (McCallister 2002).

The $\Delta^{14}C$ and $\delta^{13}C$ isotopic distributions for bacterial biomass in the midsalinity region of the York lie within the area defined by terrigenous, estuarine phytoplankton, and freshwater marsh inputs, the relative importance of each which may vary temporally (Fig. 5C). In contrast to the freshwater site (Fig. 5B), bacterial biomass appears to be a minor sink for terrigenous OM in the mesohaline regions of the York, with land-derived OM contributing, at most, ~20% of the assimilated OM (Table 6). Bulk OM derived from marshes

was ~2‰ to 4‰ depleted in $\delta^{13}C$ relative to phytoplankton sources, but both sources were similar in their $\Delta^{14}C$ signatures (Table 5, Fig. 5C). During spring and summer (May and July 2000), the bacterial isotopic distribution is best described by OM primarily of phytoplankton origin, which contributed 53–87% of the bacterial nucleic acid signature (Table 6; Fig. 5C). Potential solutions to the three-source model for the midsalinity region in October yielded a broad range of estimated contributions from the two primary sources: algal (0–71%) and marsh-derived (20–83%) OM (Table 6). These ranges are a consequence of the broad ranges in isotopic values for the potential OM sources (large rectangles in Fig. 5) and the multiple solutions to Eqs. 4–6. However, solutions to the model suggest that marsh-derived OM is of greater importance to BBP in the fall (October and November 2000), and accounts for an estimated 20–83% of the OM assimilated by bacteria, compared with in the spring (1–5%; Table 6). Bacterial assimilation of marsh-derived OM coincides with *Peltandra* senescence and a ~90% reduction in above-ground biomass from mean summer values (Neubauer et al. 2000).

These findings are consistent with previous work in the York, which indicated that marshes in the low-salinity reaches are sites of intense OM recycling and export ~60 g C m^{-2} yr^{-1} (Neubauer et al. 2000). Furthermore, marsh-derived OM is thought to subsidize a significant portion of BBP in the York (Raymond and Bauer 2001*a*). The enriched $\delta^{13}C$ signatures of bacterial nucleic acids that appear to correspond almost exclusively with OM of phytoplankton origin may alternatively result from the selective utilization of an isotopically enriched component of marsh-derived OM. In addition, bacteria are expected to preferentially assimilate more highly reactive components of DOM and to discriminate between individual compounds derived from the same source reservoir (Benner 1987; Coffin et al. 1990). For example, Coffin et al. (1990) suggested that bacteria selected cellulose and hemicellulose components of *Spartina alterniflora* and discriminated against the more refractory and isotopically lighter lignin component. Conceivably, the anomalously light $\delta^{13}C$ value (−27.6‰) from the mouth of the York in May 2000 may reflect a preferential assimilation of more bioreactive OM compounds.

Isotopic distributions of bacterial nucleic acids from the high-salinity mouth of the York were best described by primary contributions from in situ algal production and the landward advection of Chesapeake Bay OM and secondary contributions from marsh-derived OM (Fig. 5D; Table 6). Although marsh-derived OM was not a dominant source (<29%) of OM supporting BBP in the high-salinity regions of the York (Fig. 5D, Table 6), its greatest contribution was in November, consistent with the increased importance of marsh-derived OM during fall in the midsalinity region. In July and November, bacteria from the lower York assimilated OM of estuarine phytoplankton origin almost exclusively (63–90%), with nominal contributions from Chesapeake Bay DOC (4–17%; Table 6, Fig. 5D). In contrast, in October 2000, when Chl *a* concentrations declined by 72% and 50% over July and November values (Table 1), respectively, allochthonous (Bay) OM made up the greater portion (66–70%) of OM assimilated by bacteria in the lower York (Table 6). This finding challenges the widely held assumption that OM age and bioavailability are inversely related, because approximately two-thirds of the OM assimilated was older and more depleted of $\Delta^{14}C$ than alternative sources (i.e., phytoplankton and marsh). In contrast to the conclusions of Raymond and Bauer (2001*a*) in the York and Cherrier et al. (1999) from the Santa Rosa Sound (Florida), who found that bacteria were solely supported by OM of modern origin, our results suggest a greater complexity in the age and source of OM assimilated by estuarine bacteria.

Potential OM sources to bacterial production in the Hudson River—In comparison to the York River estuary, fewer stable isotopic and radiocarbon values are available for Hudson River OM. Table 4 lists the available $\delta^{13}C$, $\delta^{15}N$, and $\Delta^{14}C$ values for potential OM sources at stations sampled in the freshwater Hudson River. Corning Preserve, the northward extent of sampling, is primarily influenced by large inputs of allochthonous carbon from the surrounding watershed, which flows over the Green Island Dam at Troy, New York. In contrast, the middle sampling location (Poughkeepsie) may be influenced by OM export from freshwater wetlands (i.e., near Tivoli Bays), release from shoals and fine sediments (i.e., near Kingston), and input from additional tributaries (near Roundout Creek; Table 4; Findlay et al. 1998). Terrestrial inputs to the Hudson River are relatively large in comparison to autochthonous sources and are estimated at ~650 g C m^{-2} yr^{-1} (Howarth et al. 1996). Conversely, combined in situ phytoplankton and macrophyte production in the Hudson accounts for only ~5% of the OM supplied by allochthonous sources (Howarth et al. 1996). It is important to note that human-induced (e.g., urban development and agriculture) and ecological (i.e., introduction of *Dreissena*) changes over the past 200 yr may have fundamentally altered the biogeochemical functioning of the Hudson River ecosystem by artificially inflating the current importance of terrestrial OM inputs to the food web (Howarth et al. 1991, 1996; Smith et al. 1998).

The relative contributions of OM sources to BBP in the Hudson (Fig. 6) were estimated using the three-source mixing model (Eqs. 4–6). Potential OM sources were placed into broad groupings, because the $\Delta^{14}C$ isotopic signatures for end members in the Hudson are not as rigorously constrained as in the York. Isotopic data for submerged aquatic vegetation (SAV) and phytoplankton-derived OM were combined to represent an autochthonous end member (*see Table 5 for values and groupings*). ^{14}C-depleted bacterial nucleic acids ($\Delta^{14}C = -153‰$) and low Chl *a* concentrations (Table 1) from the mid-Hudson (122 km) site suggested a primarily allochthonous origin for the OM assimilated. Possible sources of old OM (>1,280 yr BP) to the Hudson are essentially limited to allochthonous (soil-derived) materials, because phytoplankton and SAV are more enriched with $\Delta^{14}C$ (−55‰ and −38‰, respectively; Fig. 6; Table 5). The desorption or diffusion of OM from sediments may provide an additional mechanism for the introduction of ^{14}C-depleted DOM to be classified as autochthonous in origin; however, compared with the flux of organic C from tributaries, this input would be predicted to be of minor importance (Komada and Reimers 2001). Model results suggest that during

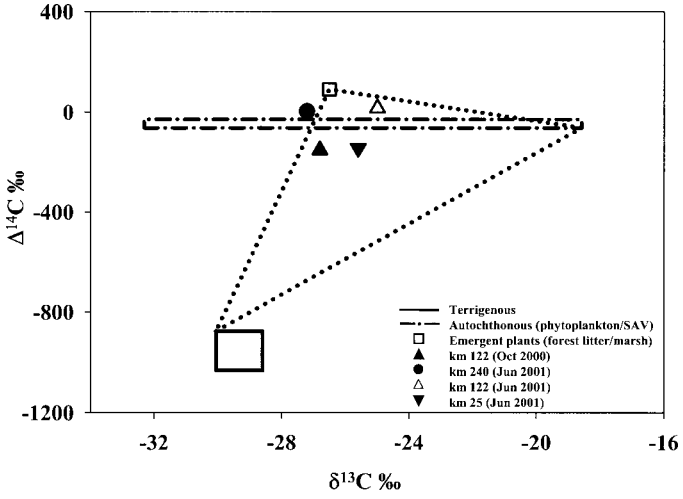

Fig. 6. Comparison of $\Delta^{14}C$ vs. $\delta^{13}C$ of bacterial nucleic acids and potential sources in the Hudson River. Terrigenous OM values are derived from the weathering of ancient sedimentary rock. Phytoplankton and SAV isotopic ranges were combined to generate an autochthonous OM source. Emergent vegetation includes both marsh- and forest litter–derived OM. Boxes are the 95% confidence intervals for potential end members in the Hudson, with the exception of the combined emergent vegetation (marsh)/forest litter end member (*see Table 5 for details*). Dotted lines encompass the solution space from one run of the model. Table 6 lists the complete model output.

fall, the majority (>90%) of BBP (km 122) was supported by allochthonous OM, with an ancient sedimentary source contributing 21–25% and recent emergent plant (marsh and forest litter) accounting for 69–76% (Table 6, Fig. 6).

In June 2001, bacterial nucleic acid $\Delta^{14}C$ values were variable in the Hudson River. Bacteria at both Corning Preserve (240 km) and Poughkeepsie (122 km) were significantly enriched with ^{14}C (+5‰ and +16‰, respectively) in comparison to the nucleic acids collected further downstream (−144‰ at 25 km) as well as those from the previous October 2000 sampling (Table 3, Fig. 6). In contrast to fall, the isotopic mass balance model predicted that ~20% of the OM assimilated in June at the Poughkeepsie site was autochthonous in origin (Table 6), with a concomitant decrease in the importance of soil-derived OM (~5%; Fig. 6). Upriver (240 km) nucleic acid values, however, did not suggest a significant assimilation of autochthonous OM (~8%; Table 6, Fig. 6). Solutions to the isotopic mass-balance model suggest that, in both locations, the majority of OM assimilated was derived from recent vascular plant production (marsh/forest litter), which accounted for ~75% and 85% of the total OM assimilated at 122 and 240 km, respectively.

Bacterial nucleic acids from the Hudson at 25 km were substantially more depleted in ^{14}C ($\Delta^{14}C = -144‰$) than either of the upstream locations (Table 3, Fig. 6). The model predicted that 18–22% of the OM assimilated was of ancient sedimentary rock origin, with significant additional contributions from both autochthonous (17–21%) and vascular plant (57–64%) OM (Table 6, Fig. 6). It should be noted that the intrusion of small amounts of seawater in June at 25 km (salinity = 3.2) suggests a potential, but presumably small, input of other OM sources (e.g., sewage or marine/estuarine phytoplankton) that was not taken into account in the model solutions. Collectively, our findings of the relative proportion of potential sources contributing to BBP in the Hudson (Table 6) are consistent with the conclusions of Findlay et al. (1998), who suggested that the relative importance of OM sources to BBP is determined by quantitative differences in source inputs.

Differences in the OM sources supporting BBP in the York and Hudson—System net heterotrophy in the York River estuary (Raymond et al. 2000) and Hudson River (Findlay et al. 1991; Howarth et al. 1996), and in rivers and estuaries in general (Raymond and Bauer 2001*b*), necessitates the utilization of allochthonous OM within these systems. The York and Hudson appear to be fundamentally similar with respect to both the nominal dependence of bacteria on contemporary phytoplankton production and their implied reliance on allochthonous OM (i.e., both estuaries are strongly net heteotrophic). However, the two systems differ dramatically with regard to the mean ages of reactive, bioavailable OM and the relative importance of autochthonous production to total bioavailable OM concentrations.

Sources were grouped to compare the relative importance of exogenous and internal OM sources to BBP both between and along the York and Hudson (Fig. 7). A limited number of source-specific $\Delta^{14}C$ values for OM from each system required slight differences in the individual sources making up each grouping (*see Fig. 7 legend*). Howarth et al. (1996) considered emergent macrophytes (marsh-derived OM) in the Hudson to be external to the ecosystem, and we therefore applied the same classification to our analyses in the York and Hudson. There is a general trend of increasing autochthonous OM importance to BBP with salinity in the York, although this varies seasonally (Fig. 7A). On average, 13% of BBP was fueled by internal sources at the head of the York, with the majority (~72%) explained by allochthonous inputs of terrigenous (e.g., soil and forest litter) OM. Conversely, autochthonous OM was estimated to account for up to ~75–80% of the OM assimilated by bacteria in the mid- and high-salinity regions of the York, although its importance also varies temporally (Fig. 7A). Nonetheless, the assimilation of allochthonous OM by bacteria was still quantitatively significant in the mesohaline and mouth regions of the York. At times, 55% and 70% of the OM supporting BBP in the mid- and high-salinity regions, respectively, may be derived from exogenous sources in the form of marsh and Bay-derived OM (Fig. 7A).

In contrast to the York, BBP in the ~220-km stretch of the Hudson River estuary sampled in this study was overwhelmingly supported by allochthonous OM (Fig. 7B). Autochthonous OM derived from both algae and SAV appeared to contribute, at most, ~20% of the total OM assimilated by bacteria. The vast majority (60–85%) of OM supporting BBP in the Hudson was a result of allochthonous inputs primarily derived from emergent vegetation and forest litter, although >20% of this allochthonous subsidy may originate from ancient (~24,000 yr BP; Petsch 2000) OM weathered from rocks (Fig. 7B).

There are a number of possible reasons for the observed

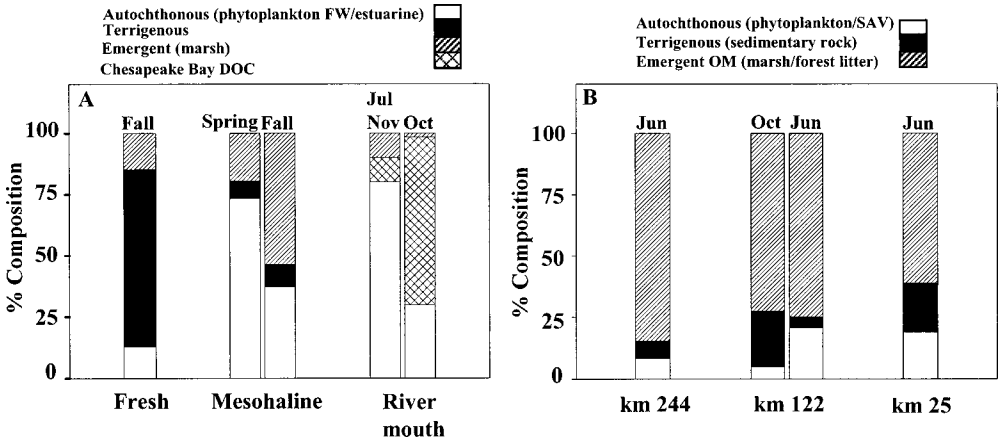

Fig. 7. Comparison of the proportion of allochthonous and autochthonous sources assimilated by bacteria in (A) the York River estuary and (B) the Hudson River. Percentages are the averages of model output given in Table 6. See legend for differences between groupings for the two systems (see text for more details). Autochthonous sources are designated by solid white bars. Allochthonous sources include both solid black and hatched bars. Autochthonous sources to the York were limited to algal-derived OM, whereas the Hudson included both algal and SAV–derived OM. Terrigenous sources in the York encompass inputs from both forest litter and soils, whereas, in the Hudson, sedimentary rocks were considered the primary source. Marsh- and forest litter–derived OM were categorized together in the Hudson.

OM age differences between the two systems in the present study. First, the majority of the DOC in the Hudson River is supplied by inputs from tributaries that drain agricultural and highly developed (urban/residential) land (Howarth et al. 1996; Findlay et al. 1998). Second, many soils in the Hudson River watershed are developed on ancient sedimentary rocks that are rich in OM which, during weathering, may release significant quantities of ^{14}C-depleted OM into overlying soils (Petsch et al. 2001). Conceivably, anthropogenic inputs of petroleum hydrocarbons may provide an additional source of ancient OM. In contrast, the York River estuary drains thicker, younger soils than the Hudson and, thus, may receive smaller contributions from older soil profiles and sedimentary rocks (Raymond and Bauer 2001*c*). In addition, the York maintains approximately three times the standing stock of phytoplankton compared with the Hudson and may be subsidized with DOC from extensive freshwater marshes. The striking differences in radiocarbon ages of bulk DOC and POC in the two systems (Raymond and Bauer 2001*b*,*c*) may result from a combination of disparate sources of allochthonous terrestrial OM (e.g., ancient OM from sedimentary rocks in the Hudson vs. surficial soils/forest litter in the York) and the greater input of recently fixed algal and marsh OM in the York. Whereas bulk DOC at the head of the York is modern in age (i.e., it contains ''bomb'' ^{14}C), Hudson River DOC was formed, on average, ~1,400 yr ago (Raymond and Bauer 2001*b*,*c*). Similarly, POC ages in the York range from modern to 1,690 yr BP, whereas Hudson POC is of considerably older mean age (4,600 yr BP; Raymond and Bauer 2001*b*,*c*).

The Δ^{14}C values of bacterial nucleic acids at the head of the York River estuary (average $\Delta^{14}C = +214‰$; Table 3) indicate that the DOC assimilated there is no more than decadal in age and likely resulted from recently deposited soils and degraded modern vegetation (Richter et al. 1999). Conversely, the significantly lower Δ^{14}C signatures of Hudson River bacteria (Table 3) may result from deeper soil OM thousands of years old. Thus, OM that has resisted terrestrial decomposition for millennia during prior storage appears to be capable of supporting high levels of estuarine heterotrophic BBP on timescales of days to weeks.

Two potential mechanisms have been identified as primary controls on the preservation and reactivity of OM. First, the association of OM with mineral grains in soils and sediments may convey long-term resistance to microbial decomposition (Keil et al. 1994). However, these associations are rarely permanent on geochemically relevant timescales (Thimsen and Keil 1998), and, once OM is dissociated from mineral surfaces through changes in redox, pH, solute concentration, or resuspension, it may be rapidly degraded (Keil et al. 1994). Alternatively, allochthonous OM is often composed of highly condensed humic substances that may be extremely photoreactive. Once released into rivers and estuaries, these materials are exposed to sunlight and may be photochemically transformed into more reactive forms of OM (Mopper and Kieber 2002 and references therein). Therefore, the York and Hudson appear to be quite similar in the context of measurements traditionally used to characterize the metabolic states of rivers and estuaries (e.g., measurements of DIC, bacterial abundance and production, Chl *a*, primary production, and community respiration). However, intensive agriculture and urban/residential land use in the Hudson watershed (Howarth et al. 1991) delivers deeper/older soil horizons to the river and is thus likely to be a principal source of OM supporting net heterotrophy in this and similar systems. The application of multiple natural isotopes, including ^{14}C, may thus be an important emerging tool to allow the underlying differences in organic matter sources and fates to be detected within river and estuarine systems.

A traditional paradigm in biogeochemistry has been that terrigenous OM is refractory and that its age is a reasonable first-order indicator of bioreactivity. Although this paradigm has undergone revision (Moran and Hodson 1994), the term ''old'' still remains synonymous with refractory. However, $\Delta^{14}C$ values of bacterial nucleic acids in the Hudson River estuary suggest that the terms ''refractory'' and ''old'' are not necessarily interchangeable. Additionally, bacteria from both the Hudson and York were shown to incorporate substantial amounts of terrigenous OM into their biomass. The present findings of OM assimilation by bacteria suggest the existence of broad classifications of OM reactivity based on source (e.g., terrigenous) or age are not entirely valid.

References

ANDERSON, T. F., AND M. A. ARTHUR. 1983. Stable isotopes of oxygen and carbon and their application to sedimentologic and paleoenvironmental problems, p. 1–151. *In* M. A. Arthur, T. F. Anderson, I. R. Kaplan, J. Veizer, and L. S. Land [eds.], Stable isotopes in sedimentary geology. Soc. Econ. Paleontol. Mineral.

BAUER, J. E., E. R. M. DRUFFEL, D. M. WOLGAST, AND S. GRIFFIN. 2002. Temporal and regional variability in sources and cycling of DOC and POC in the northwest Atlantic continental shelf and slope. Deep-Sea Res. II **49:** 4387–4419.

BENNER, R., M. L. FOGEL, E. K. SPRAGUE, AND R. E. HODSON. 1987. Depletion of ^{13}C in lignin and its implications for stable carbon isotope studies. Nature **327:** 708–710.

CANUEL, E. A., J. E. CLOERN, D. B. RINGELBERG, J. B. GUCKERT, AND G. H. RAU. 1995. Molecular and isotopic tracers used to examine sources of organic matter and its incorporation into the food webs of San Francisco Bay. Limnol. Oceanogr. **40:** 67–81.

CARACO, N. F., G. LAPMAN, J. J. COLE, K. E. LIMBURG, M. L. PACE, AND D. FISCHER. 1998. Microbial assimilation of DIN in a nitrogen rich estuary: Implications for food quality and isotope studies. Mar. Ecol. Prog. Ser. **167:** 59–71.

CHANTON, J. P., AND F. G. LEWIS. 1999. Plankton and dissolved inorganic carbon isotopic composition in a river-dominated estuary: Apalachicola Bay, Florida. Limnol. Oceanogr. **22:** 575–583.

CHERRIER, J. E. 1997. Carbon flow through bacterioplankton in eastern North Pacific surface waters. Ph.D. dissertation, Florida State Univ.

———, J. E. BAUER, E. M. DRUFFEL, R. B. COFFIN, AND J. P. CHANTON. 1999. Radiocarbon in marine bacteria: Evidence for the ages of assimilated carbon. Limnol. Oceanogr. **44:** 730–736.

CIFUENTES, L. A., M. L. FOGEL, J. R. PENNOCK, AND J. H. SHARP. 1989. Biogeochemical factors that influence the stable nitrogen isotope ratio of dissolved ammonium in the Delaware Estuary. Geochim. Cosmochim. Acta **53:** 2713–2721.

CLOERN, J. E., E. A. CANUEL, AND D. HARRIS. 2002. Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. Limnol. Oceanogr. **47:** 713–729.

COFFIN, R. B., AND L. A. CIFUENTES. 1993. Approaches for measuring stable carbon and nitrogen isotopes in bacteria, p. 663–675. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole [eds.], Handbook of methods in aquatic microbial ecology. Lewis.

———, AND ———. 1999. Stable isotope analysis of carbon cycling in the Perdido Estuary, Florida. Estuaries **22:** 917–926.

COFFIN, R. B., B. FRY, B. J. PETERSON, AND R. T. WRIGHT. 1989. Carbon isotopic composition of estuarine bacteria. Limnol. Oceanogr. **34:** 1305–1310.

COFFIN, R. B., D. J. VELINSKY, R. DEVEREUX, W. A. PRICE, AND L. A. CIFUENTES. 1990. Stable carbon isotope analysis of nucleic acids to trace sources of dissolved substrates used by estuarine bacteria. Appl. Environ. Microbiol. **56:** 2012–2020.

CURRIN, C. A., S. Y. NEWELL, AND H. W. PEARL. 1995. The role of standing dead *Spartina alterniflora* and benthic microalgae in salt marsh food webs: Considerations based on multiple stable isotope analysis. Mar. Ecol. Prog. Ser. **121:** 99–116.

DEEGAN, L. A., AND R. H. GARRITT. 1997. Evidence for spatial variability in estuarine food webs. Mar. Ecol. Prog. Ser. **147:** 31–47.

DEL GIORGIO, P., AND J. DAVIS. 2003. Patterns in dissolved organic matter lability and consumption across aquatic ecosystems, p. 400–424. *In* S. E. G. Findlay and R. L. Sinsabaugh [eds.], Aquatic ecosystems: Interactivity of dissolved organic matter. Academic.

EDGECOMB, V. P., J. H. MCDONALD, R. DEVEREUX, AND D. W. SMITH. 1999. Estimation of bacterial cell numbers in humic acid-rich salt marsh sediments with probes directed to 16S ribosomal DNA. Appl. Environ. Microbiol. **65:** 1516–1523.

ELDRIDGE, P. M., AND M. E. SIERACKI. 1993. Biological and hydrodynamic regulation of the microbial food web in a periodically mixed estuary. Limnol. Oceanogr. **38:** 1666–1679.

FINDLAY, S., M. L. PACE, AND D. LINTS. 1992. Bacterial metabolism of organic carbon in the tidal freshwater Hudson estuary. Mar. Ecol. Prog. Ser. **89:** 147–153.

———, ———, ———, D. LINTS, J. J. COLE, N. CARACO, AND B. PEIERLS. 1991. Weak coupling of bacterial and algal production in a heterotrophic ecosystem, the Hudson River estuary. Limnol. Oceanogr. **36:** 268–278.

———, R. L. SINSABAUGH, D. T. FISHER, AND P. FRANCHINI. 1998. Sources of dissolved organic carbon supporting planktonic bacterial production in the tidal freshwater Hudson River. Ecosystems **1:** 227–239.

FRANKIGNOULLE, M., AND OTHERS. 1998. Carbon dioxide emission from European estuaries. Science **282:** 434–436.

FRY, B., AND E. B. SHERR. 1984. 13C Measurements as indicators of carbon flow in marine and freshwater ecosystems. Contrib. Mar. Sci. **27:** 13–47.

HEDGES, J. I., R. G. KEIL, AND R. BENNER. 1997. What happens to terrestrial organic matter in the ocean? Org. Geochem. **27:** 195–212.

HOPKINSON JR., C. S., J. GIBLIN, R. H. GARRITT, J. TUCKER, AND M. A. J. HULLAR. 1998. Influence of the benthos on growth of planktonic estuarine bacteria. Aquat. Microb. Ecol. **16:** 109–118.

HOWARTH, R. W., J. R. FRUCI, AND D. SHERMAN. 1991. Inputs of sediment and carbon to an estuarine ecosystem: Influence of land use. Ecol. Appl. **1:** 27–39.

———, R. SCHNEIDER, AND D. SWANEY. 1996. Metabolism and organic carbon fluxes in the tidal freshwater Hudson River. Estuaries **19:** 848–865.

ITTEKOT, V. 1989. Global trends in the nature of organic matter in river suspension. Nature **332:** 436–438.

JACKSON, C. R., J. P. HARPER, D. WILLOUGHBY, E. E. RODEN, AND P. F. CHURCHILL. 1997. A simple efficient method for the separation of humic substances and DNA from environmental samples. Appl. Environ. Microbiol. **63:** 4993–4995.

KEIL, R. G., D. B. MONTLUCON, F. G. PRAHL, AND J. I. HEDGES. 1994. Sorptive preservation of labile organic matter in marine sediments. Nature **370:** 549–552.

KIRCHMAN, D. L. 1994. The uptake of inorganic nutrients by heterotrophic bacteria. Microb. Ecol. **28:** 255–271.

KOMADA, T., AND C. E. REIMERS. 2001. Resuspension-induced partitioning of organic carbon between solid and solution phases from a river-ocean transition. Mar. Chem. **76:** 155–174.

LAJTHA, K., AND R. H. MICHENER. 1994. Stable isotopes in ecology and environmental science. Blackwell Scientific.

LEVIN, I., AND B. KROMER. 1997. Twenty years of atmospheric $^{14}CO_2$ observations at Schauinsland station, Germany. Radiocarbon **39:** 205–218.

MANNINO, A., AND H. R. HARVEY. 2000. Terrigenous dissolved organic matter along an estuarine gradient and its flux to the coastal ocean. Org. Geochem. **31:** 1611–1625.

MANTOURA, R. F. C., AND E. M. S. WOODWARD. 1983. Conservative behavior of riverine DOC in the Severn Estuary: Chemical and geochemical implications. Geochim. Cosmochim. Acta **7:** 1293–1309.

MCCALLISTER, S. L. 2002. Organic matter cycling in the York River estuary, VA: An analysis of potential sources and sinks. Ph.D. dissertation, School of Marine Science, The College of William and Mary.

MICHENER, R. H., AND D. M. SCHELL. 1994. Stable isotope ratios as tracers in marine aquatic food webs, p. 138–157. *In* K. Lajtha and R. H. Michener [eds.], Methods in ecology: Stable isotopes in ecology and environmental science. Blackwell Scientific.

MOPPER, K., AND D. J. KIEBER. 2002. Photochemistry and the cycling of carbon, sulfur, nitrogen and phosphorus, p. 455–508. *In* D. A. Hansell and C. A. Carlson [eds.], Biogeochemistry of marine dissolved organic matter. Academic.

MORAN, M. A., AND R. E. HODSON. 1994. Support of bacterioplankton production by dissolved humic substances from three marine environments. Mar. Ecol. Prog. Ser. **110:** 241–247.

NEUBAUER, S. C. 2000. Carbon dynamics in a tidal freshwater marsh. Ph.D. dissertation, School of Marine Science, The College of William and Mary.

———, W. D. MILLER, AND I. C. ANDERSON. 2000. Carbon cycling in a tidal freshwater marsh ecosystem: A carbon gas flux study. Mar. Ecol. Prog. Ser. **199:** 13–30.

PETERSON, B. J., AND B. FRY. 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. **18:** 293–320.

———, R. W. HOWARTH, AND R. H. GARRIT. 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. Science **227:** 1361–1363.

PETSCH, S. T. 2000. A study on the weathering of organic matter in black shales and implications for the geochemical cycles of carbon and oxygen. Ph.D. dissertation, Yale Univ.

———, T. I. EGLINTON, AND K. J. EDWARDS. 2001. 14C-dead living biomass: Evidence for microbial assimilation of ancient organic carbon during shale weathering. Science **292:** 1127–1131.

RAY, R. T., L. W. HAAS, AND M. E. SIERACKI. 1989. Autotrophic picoplankton dynamics in a Chesapeake Bay sub-estuary. Mar. Ecol. Prog. Ser. **52:** 273–285.

RAYMOND, P., AND J. BAUER. 2001*a*. DOC cycling in a temperate estuary: A mass balance approach using natural ^{14}C and ^{13}C isotopes. Limnol. Oceanogr. **46:** 655–667.

———, AND ———. 2001*b*. Use of ^{14}C and ^{13}C natural abundances for evaluating riverine, estuarine, and coastal DOC and POC sources and cycling: A review and synthesis. Org. Geochem. **23:** 469–485.

———, AND ———. 2001*c*. Riverine export of aged terrestrial organic matter to the North Atlantic Ocean. Nature **409:** 497–499.

———, ———, AND J. J. COLE. 2000. Atmospheric CO_2 evasion, dissolved inorganic carbon production, and net heterotrophy in the York River Estuary. Limnol. Oceanogr. **45:** 1707–1717.

RICHTER, D. D., D. MARKEWITZ, S. E. TRUMBORE, AND C. G. WELLS. 1999. Rapid accumulation and turnover of soil carbon in a re-establishing forest. Nature **400:** 56–58.

SAMBROOK, J., E. F. FRITSCH, AND T. MANIATIS. 1989. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory.

SCHULTZ, G. E. J., E. D. I. WHITE, AND H. W. DUCKLOW. 2003. Bacterioplankton dynamics in the York River estuary: Primary influence of temperature and freshwater inputs. Aquat. Microb. Ecol. **30:** 135–148.

SIGLEO, A. C., AND S. A. MACKO. 1985. Stable isotope and amino acid composition of estuarine dissolved colloidal material, p. 29–46. *In* A. C. Sigleo and A. Hattori [eds.], Marine and estuarine geochemistry. Lewis.

SIN, Y., AND R. L. WETZEL. 2002. Ecosystem modeling analysis of size-structured phytoplankton dynamics in the York RIver estuary, Virginia (USA). II. Use of a plankton ecosystem model for investigating controlling factors on phytoplankton and nutrient dynamics. Mar. Ecol. Prog. Ser. **228:** 91–101.

SMITH, S. V., AND J. T. HOLLIBAUGH. 1993. Coastal metabolism and the oceanic organic carbon balance. Rev. Geophys. **31:** 75–89.

SMITH, T. E., R. J. STEVENSON, N. F. CARACO, AND J. J. COLE. 1998. Changes in phytoplankton community structure during the zebra mussel (*Dreissena polymorpha*) invasion of the Hudson River (New York). J. Plankton Res. **20:** 1567–1579.

SOFER, Z. 1980. Preparation of carbon dioxide for stable carbon isotope analysis of petroleum fractions. Anal. Chem. **52:** 1389–1391.

STUIVER, M., AND H. A. POLACH. 1977. Discussion: Reporting of C14 data. Radiocarbon **19:** 355–363.

SULLIVAN, M. J., AND C. A. MONCREIFF. 1990. Edaphic algae are an important component of salt marsh food webs: Evidence from multiple stable isotope analyses. Mar. Ecol. Prog. Ser. **62:** 149–159.

THIMSEN, C. A., AND R. G. KEIL. 1998. Potential interactions between sedimentary dissolved organic matter and mineral surfaces. Mar. Chem. **62:** 65–76.

TRUMBORE, S. E., S. L. SCHIFF, R. ARAVENA, AND R. ELGOOD. 1992. Sources and transformation of dissolved organic carbon in the Harp Lake forested catchment: The role of soils. Radiocarbon **34:** 635–636.

VOGEL, J. S., D. E. NELSON, AND J. R. SOUTHON. 1987. 14C background levels in an accelerator mass spectrometry system. Radiocarbon **29:** 323–333.

WILLIAMS, P. J. LE B. 2000. Heterotrophic bacteria and the dynamics of dissolved organic material, p. 153–200. *In* D. L. Kirchman [ed.], Microbial ecology of the oceans. Wiley-Liss.

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