1-2006

Bioreactivity of estuarine dissolved organic matter: A combined geochemical and microbiological approach

SL McCallister
Virginia Institute of Marine Science

JE Bauer
Virginia Institute of Marine Science

Elizabeth A. Canuel
Virginia Institute of Marine Science

Recommended Citation

McCallister, SL; Bauer, JE; and Canuel, Elizabeth A., Bioreactivity of estuarine dissolved organic matter: A combined geochemical and microbiological approach (2006). *Limnology and Oceanography*, 51(1), 94-100.
10.4319/lo.2006.51.1.0094

Limnol. Oceanogr., 51(1), 2006, 94–100

Bioreactivity of estuarine dissolved organic matter: A combined geochemical and microbiological approach

S. Leigh McCallister,[1] *James E. Bauer, and Elizabeth A. Canuel*
School of Marine Science, College of William and Mary, Gloucester Point, Virginia 23062

Abstract

An integrated multidisciplinary study utilizing geochemical and microbial ecological approaches was conducted to characterize the origins, chemical nature, and quantities of dissolved and particulate organic matter (OM) utilized by heterotrophic bacteria in a temperate estuary. C : N, stable isotope ($\delta^{13}C$), and lipid biomarker analyses revealed differences in the inferred reactivity of autochthonous versus allochthonous OM sources. Isotopic comparison of OM size fractions and bacterial nucleic acids suggests that high-molecular-weight dissolved OM (DOM) is consistently linked to bacterial biomass synthesis along the estuarine salinity gradient. Polyunsaturated fatty acids (as percent of total fatty acids, FA) were a reliable predictor of DOM decomposition in bioassays, thus providing an indicator directly linking DOM reactivity to its composition. Significant positive correlations between FA diagnostic of bacterial sources and lipid biomarker compounds diagnostic of planktonic origin indicate a systematic bacterial response to autochthonous DOM sources along the estuarine continuum. These findings further suggest that, although the geochemical signature of algal-derived OM in the dissolved phase may appear quantitatively insignificant, this fraction may nevertheless represent a principal source of bioreactive OM to heterotrophic bacteria in estuarine waters.

Introduction

Delineating the sources and biological fate of aquatic organic matter (OM) is an ongoing challenge to ecologists and geochemists alike. While recent methodological advances have enhanced our ability to characterize dissolved OM (DOM) (Hansell and Carlson 2002 and references therein; Seitzinger et al. 2005), such information only allows biologically reactive versus refractory components to be inferred across different, and often uncoupled, time and space scales. In contrast, microbial ecological studies have traditionally ignored DOM characterization at the molecular and substrate levels and relied instead on bioassays to predict the reactivity and availability of heterogeneous pools such as bulk OM to natural microbial populations (Benner 2003 and references therein; Sobczak et al. 2002). More recent approaches have coupled incubation studies with changes in OC composition and character, including measures of neutral sugars and amino acids, C : N ratios, and relative aliphatic versus aromatic carbon content as indices of diagenesis and predictors of reactivity (Benner 2003). Although these and other parameters have been used to estimate the bioreactivity of bulk DOM, the geochemical fingerprints they provide frequently do not distinguish the actual sources of bioreactive OM.

Previous studies have suggested that allochthonous OM sources may fuel significant fractions of both microbial (Cole et al. 2002; Kritzberg et al. 2004; McCallister et al. 2004) and higher food web production (Cole et al. 2002; Pace et al. 2004) in aquatic systems. Estuaries are especially challenging systems in which to assess the multiple sources (e.g., algae, higher plant) and potential fates (e.g., transfer to higher trophic levels, export) of OM. Lipid biomarker compounds and stable isotopes are examples of complementary approaches for simultaneously tracing both the sources (Mannino and Harvey 1999, McCallister 2002; Zou et al. 2004) and biological fates (Canuel et al. 1995, Boschker et al. 1999, 2005) of OM in estuarine systems. For example, Canuel et al. (1995) employed lipid biomarker compounds and stable isotopic tracers to establish the contributions of algal versus higher plant-derived particulate OM (POM) sources to a suspension feeding bivalve in sediments across broad space and time scales in San Francisco Bay. However, resolving sources of labile OM to the pelagic microbial food web has proven more challenging, in part, because of difficulties in isolating bacteria from other sources of OM (e.g., seston, detritus). Coupled biomarker and natural abundance isotopic approaches may thus provide the sensitivity to begin elucidating the OM sources fueling pelagic bacterial communities (Coffin et al. 1990; McCallister et al. 2004; Boschker et al. 2005).

To address the general question of OM bioreactivity and the sources of aquatic OM supporting heterotrophic bacteria in estuarine waters, we used individual and grouped lipid biomarker compounds of known sources concurrently with $\delta^{13}C$ isotopic signatures and microbial bioassays to (1) provide an index of DOM bioreactivity and (2) assess the biological fates of DOM of various autochthonous and allochthonous origins in a temperate coastal plain estuary.

[1] To whom correspondence should be addressed. Present address: Institute of Marine and Coastal Science, Rutgers University, 71 Dudley Road, New Brunswick, New Jersey 08901.

Acknowledgments

We thank Melissa Ederington-Hagy and Beth Waterson for their invaluable help with lipid analyses. We thank Ai Ning Loh for her help and advice with ultrafiltration, Eva Bailey for field assistance, and François Guillemette for statistical advice. This work was greatly improved by the insightful comments of two anonymous reviewers. This is VIMS contribution number 2700.

This work was supported by NSF grants OCE-9810669, OCE-0327423, and EAR-0403949 to J.E.B., OCE-9521170 to E.A.C., and DEB-0073243 to S.L.M., and U.S. Department of Energy Ocean Margins Program grant FG05-94ER61833 to J.E.B.

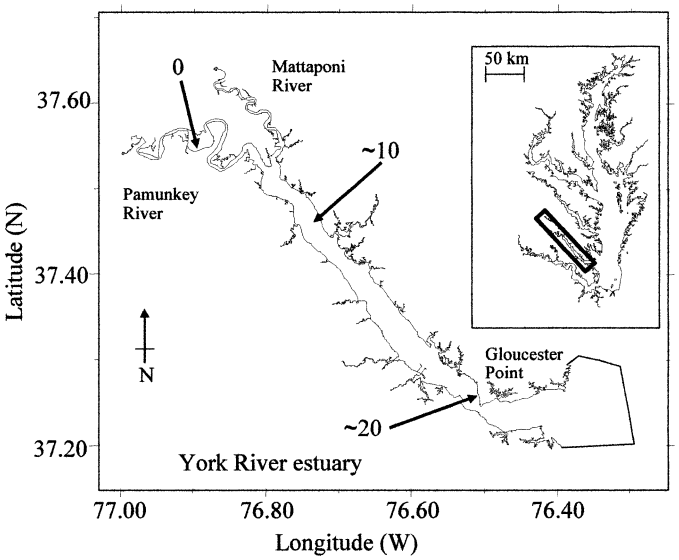

Fig. 1. The York River estuary. Inset shows the York's location relative to the Chesapeake Bay proper. Sampling locations and their approximate salinities are designated by arrows.

Methods

Sample collection and processing—Surface water samples (57–125 liters) were collected in acid-leached (10% HCl) Nanopure-rinsed polycarbonate bottles (~20 liters) from three sites along the salinity gradient (S = ~0, 10, 20) of the York River estuary (Fig. 1) during both high-flow (March 2000) and low-flow (October 2000) regimes. Samples were filtered through combusted (525°C for 4 h) Whatman GF/F filters (0.7-μm nominal pore size) using a peristaltic pump to concentrate particles for isotopic analyses. The 0.7-μm filtrate was subsequently passed through an in-line Gelman filter capsule (0.2 μm) to remove bacteria, and the high-molecular-weight (HMW) DOM was subsequently concentrated to a final volume of 1 liter using an Amicon DC-10 L tangential flow ultrafiltration unit equipped with two spiral-wound polysulfone cartridges (3 kDa nominal cutoff). The sample was concentrated to ~50 mL by low-temperature (30°C) evaporation (Zymark TurboVap 500) and was then lyophilized. Both POM and HMW DOM samples were stored at −80°C until analysis.

Lipid extraction and analysis—Detailed lipid extraction procedures are provided in McCallister (2002). In brief, lipids were extracted from HMW DOM samples by accelerated solvent extraction (Dionex ASE 200) using $MeCl_2$: MeOH (2 : 1) and partitioned following the procedures of Bligh and Dyer (1959). The aqueous fraction was subsequently re-extracted with hexane and the organic fractions were combined. The combined organic phases were refrigerated overnight over predried (450°C, 4.5 h) anhydrous Na_2SO_4. The lipid extracts were concentrated to 1 mL, saponified with 3 mL of 1 mol L^{-1} KOH at 110°C for 2 h and analyzed following previously published methods (Canuel and Martens 1993).

Fatty acids (as methyl esters) and sterols (as trimethylsilyl ethers) were analyzed using gas chromatography (Hewlett Packard 5890 Series II) by injecting directly onto a 30 m × 0.32 mm inside diameter (i.d.) DB-5 fused silica capillary column (J&W Scientific). Individual peaks were identified on the basis of retention times of known standards, and peak areas were quantified relative to internal standards (methyl heneicosanoate for fatty acids and 5α(H)-cholestane for sterols). Identifications of selected compounds were confirmed by gas chromatography-mass selective spectrometry (GC-MSD; Hewlett Packard 6890 Series Gas Chromatograph-Mass Selective Detector). Fatty acid concentrations in the HMW DOM fraction were corrected based on the recovery of a surrogate added initially. No corrections were applied to the sterol fraction.

Elemental and isotopic analyses—Detailed methodology for bacterial nucleic acid collection and extraction is described in McCallister et al. (2004). In brief, water samples were prefiltered through Whatman 0.7-μm combusted GF/F filters. Filters were exchanged frequently to reduce particulate loading which may result in a decrease in the nominal pore size and an increase in bacterial retention. Filtrates (<0.7 μm) contained approximately 80% of the cell counts relative to unfiltered samples (Schultz 1999). Although GF/F filters may retain the attached fraction of bacteria, this accounted for less than 10% of the total bacterial cell counts and both fractions (attached and free) have been found to have comparable cell-specific activities, suggesting similar metabolic capabilities (Schultz 1999).

Bacteria were concentrated from the filtrate onto acid (10% HCl) and Nanopure water-cleaned Gelman microculture capsules (0.2-μm pore size). Bacterial cells collected in the microcapsules were lysed by adding a detergent/buffer solution (20 mmol L^{-1} TRIS, 2 nmol L^{-1} ethylenediaminetetraacetate [EDTA], and 2% sodium dodecyl sulfate [SDS]) and heating the sealed capsule in a 100°C water bath for 15 min (McCallister et al. 2004). Following precipitation and removal of the SDS from the lysate, nucleic acids were isolated and purified by dialysis, followed by ethanol, phenol, and isoamyl alcohol/chloroform precipitations. Nucleic acids were subsequently acidified overnight with 1 mL of 3% $H_3PO_4^-$ and evaporated under vacuum until all water was removed.

Although the specificity to bacteria of nucleic acids in the 0.2- to 0.7-μm fraction has been demonstrated previously with 16S RNA analysis (Coffin et al. 1990), the potential for an isotopic bias by the inclusion of picoplankton may exist in different systems. The contribution from picoplankton (e.g., cyanobacteria) in the York River estuary, however, is believed to be limited based on findings that they comprise 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).

POM and lyophilized HMW DOM were acidified with 10% HCl and dried overnight prior to organic carbon and nitrogen analysis using an elemental analyzer (Fisons Instruments Model EA1108 CHNS-O analyzer). Stable carbon isotope ratios for POM, HMW DOM, and bacterial nucleic acids were measured using a continuous-flow isotope ratio mass spectrometer with on-line sample combustion (Europa

Scientific Integra, University of California, Davis–Stable Isotope Facility). Selected samples were assessed for analytical precision and run in duplicate (relative standard deviation <0.4‰). Results for stable isotope values are reported in standard δ notation as:

$$\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 10^3 \quad (1)$$

where R is $^{13}C/^{12}C$.

DOM bioavailability incubations—To assess the bioavailability of bulk DOM, approximately 3 liters of water was retained after the 0.2-μm filtration from each sampling site at both sampling times (March and October 2000). The bacteria-free filtrate was dispensed into acid cleaned (10% HCl) 1-liter polycarbonate bottles and incubations were initiated by inoculating with a 1% (vol : vol) bacterial inoculum by using the 0.7-μm filtrate. Samples were incubated in the dark at room temperature (22°C) and monitored for losses of dissolved organic carbon (DOC) over a 28-day period. Samples were stored frozen (−20°C) until analysis by high-temper ature combustion on a Shimadzu TOC-5000. All samples were run in triplicate. Apparent first-order decay constants (k') for DOC decomposition were calculated following the equation:

$$k' = \frac{\ln\left(\frac{C_t}{C_{t_0}}\right)}{(t - t_0)} \quad (2)$$

where C_t and C_{t_0} were the concentrations of DOC at the start (t_0) and termination (t) of the incubation period. Standard deviations for k' were estimated from duplicate and triplicate incubations and ranged from 5% to 30% of the mean.

Results and discussion

Evaluation of OM size fraction utilization—Microbial uptake and metabolism of OM may be limited by the size (<600 Da), quality, and geometry of available substrates (Arnosti 2004). Thus, different components of the size and molecular weight spectrum of OM (i.e., POM, HMW DOM, low-molecular-weight [LMW] DOM) may support bacterial biomass production to varying degrees (Benner 2003). Molecular level comparisons to discern the sources and diagenetic relationships between POM and HMW DOM have yielded conflicting results. Previous studies have found that the organic characteristics of the particulate and dissolved OM pools (Mannino and Harvey 1999; McCallister 2002; Ducklow and McCallister 2004) differ significantly, whereas Hopkinson et al. (1998) suggest that both pools have common sources and dynamic links. Based on similarities in the $\delta^{13}C$ values of estuarine bacterial lipids and POM, Boschker et al. (2005) reasoned that either POM was the dominant carbon pool assimilated by bacteria or, alternatively, the isotopic composition of the particulate and dissolved pools was similar (however, these workers did not measure $\delta^{13}C$ in DOM). In the present study we are able to compare the $\delta^{13}C$ signatures between various OM pools and bacterial nucleic acids (McCallister et al. 2004) to assess potential contributions of different OM size classes and potential sources supporting bacterial biomass production.

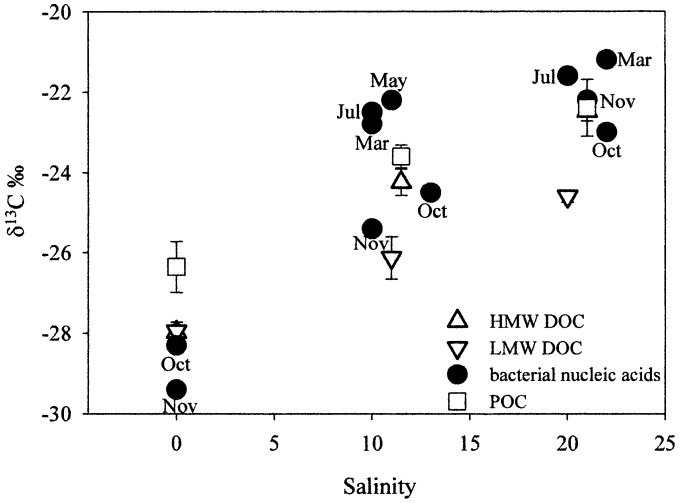

Fig. 2. $\delta^{13}C$ signatures of bacterial nucleic acids (McCallister et al. 2004), HMW DOC, LMW DOC, and POC as a function of salinity in the York River estuary. $\delta^{13}C$ of LMW DOC was calculated from the equation: $\delta^{13}C_{(DOC)} = \delta^{13}C_{(HMW)}(x) + \delta^{13}C_{(LMW)}(y)$, where DOC $\delta^{13}C$ values are averages for each sampling location taken from Raymond and Bauer (2001), and x and y are the relative contributions of HMW and LMW DOC, respectively, to total DOC (Table 1). Error bars denote the average ($n = 2$) ± the range of values for samples collected in March and October 2000 for POC, HMW DOC, and LMW DOC. The $\delta^{13}C$ values of bacterial nucleic acids from McCallister et al. (2004) are presented as single data points and identified by the month in which they were collected in 2000.

In general, $\delta^{13}C$ values were directly related to the "size" of OM (i.e., POM > HMW DOM > LMW DOM [<3 kDa]) with the most pronounced patterns in the mid- and lower York River estuary where the LMW pool was depleted in $\delta^{13}C$ relative to the HMW fraction by 1.9 ± 0.2‰ and 2.1 ± 0.1‰, respectively (Fig. 2). Bacterial nucleic acids have been previously found to reflect the $\delta^{13}C$ of OM assimilated with minor $\delta^{13}C$ (<1–2 ‰) fractionation by heterotrophic metabolism (Coffin et al. 1989). The $\delta^{13}C$ signatures of bacterial nucleic acids, HMW DOM, and POM (Fig. 2) from the York River estuary mouth and midsalinity stations were not significantly different from each other ($p > 0.05$; 2-way analysis of variance [ANOVA]). In contrast, the $\delta^{13}C$ values of the LMW DOM pool and bacterial nucleic acids differed significantly ($p < 0.05$; 2-way ANOVA) at those stations. This suggests that bacteria may preferentially assimilate an isotopically enriched $\delta^{13}C$ organic component (presumably derived from $\delta^{13}C$-enriched algal material) instead of $\delta^{13}C$-depleted allochthonous OM (Coffin et al. 1990; McCallister et al. 2004).

The freshwater site is a notable exception to the interpretation above for several possible reasons. First, there is a distinct isotopic offset between freshwater bacterial nucleic acids (−28.9‰ ± 0.8‰) and POM (−26.4‰ ± 0.6‰; Fig. 2). Second, in contrast to the two higher salinity sites, the HMW DOM and the LMW DOM pools are isotopically indistinguishable. Two factors that are potentially responsible

Table 1. Site and water characteristics of the York River estuary.

Sampling date	Salinity	Chl *a** (μg L^{-1})	POC (μmol L^{-1} C)	DOC (μmol L^{-1} C)	% HMW† DOC‡	C:N	
						POM	DOM
Mar 2000	0	3.5(0.4)§	168(11)	424(6)	33	9.0	17.9
	10	34.4(1.6)	210(16)	352(7)	20	5.8	10.8
	21	8.0(1.0)	82(7)	263(4)	18	7.3	7.4
Oct 2000	0	3.7(0.5)	111(10)	422(7)	31	6.9	16.5
	13	12.3(2.2)	118(8)	362(5)	25	7.7	13.2
	22	7.2(0.6)	61(5)	233(5)	22	5.5	9.8

* Chl *a* was determined by dimethyl sulfoxide/acetone extraction according to Burnison (1980).
† High-molecular weight (HMW) (>3 kDa) fraction.
‡ % of total DOC recovered in HMW (0.3 kDa) fraction.
§ Number in parentheses are ±1 SD of mean.

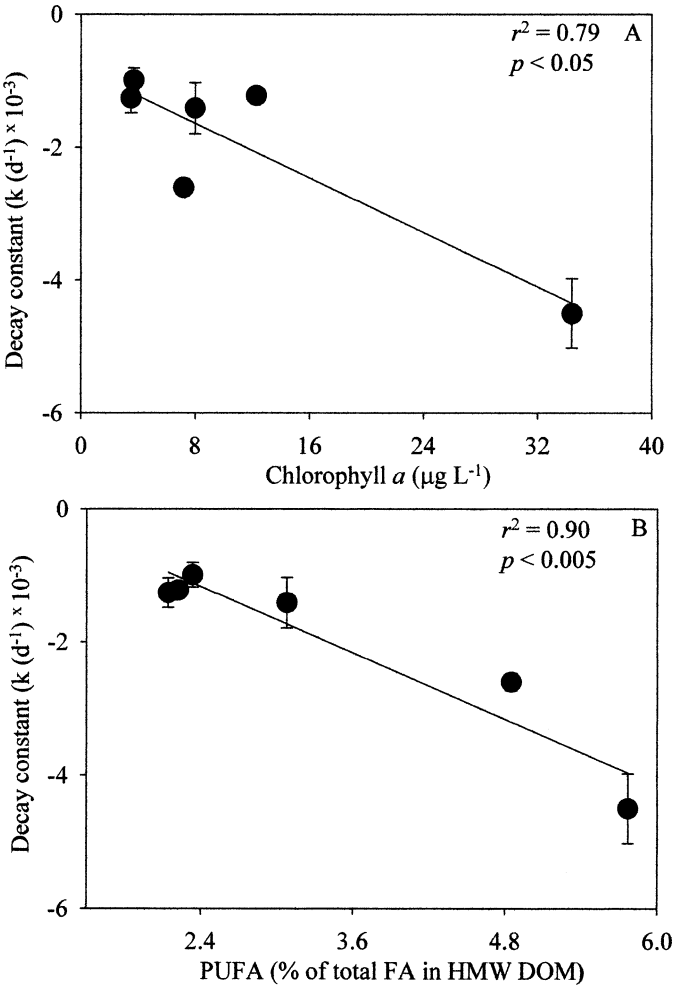

Fig. 3. Regressions for calculated decay constant (k') of DOC decomposition and (A) Chl *a* (Table 1) and (B) polyunsaturated fatty acids (PUFA; as percent of total FA in HMW DOC). PUFA include 18:2, 18:3, 18:4, 20:2, 20:4ω6, 20:5ω3, 22:2, and 22:6ω3 FA. Data were tested for normality and log transformed when appropriate.

for these observations are that the bulk POC pool is dominated by terrigenous sources (Raymond and Bauer 2001; Sobczak et al. 2005), and the predicted isotopic signature of freshwater algal-derived OM (−30.5‰ ± 3.7‰; Raymond and Bauer 2001) is lighter than its terrigenous counterpart (~−26‰; McCallister et al. 2004 and references therein). Accordingly, we suggest that POM has little direct contribution to riverine bacterial biomass production (McCallister et al. 2004) despite the paradox that lipid biomarker signatures imply this fraction has greater reactivity than the dissolved (McCallister 2002). Thus, an initial isotopic (δ^{13}C) comparison between OM size fractions (POM, HMW DOM, and LMW DOM) and bacterial nucleic acids shows that the δ^{13}C values for bacterial nucleic acids most consistently track those of HMW DOM, consistent with the previous finding (Benner 2003) that this fraction is the primary pool linked to bacterial biomass synthesis along the estuarine salinity gradient (Fig. 2). We next evaluate the potential contribution of POM as a labile OM source to bacteria using a more sensitive lipid biomarker approach.

Indicators of DOM bioreactivity—The potential of OM to support bacterial production is related in part to its source and diagenetic state (i.e., the extent of its prior heterotrophic processing). This study builds on previous work by coupling bulk geochemical characteristics (Table 1) and biomarker composition (Figs. 3 and 4) with bulk DOC utilization along the estuarine continuum (Table 2). Decay constants (Table 2) were calculated from 28-day incubations and examined for their relationships to various indices of source and diagenesis (C:N and lipid biomarkers) in the dissolved and particulate pools as a means of identifying an indicator of bulk OM reactivity (Table 1, Fig. 3).

Bacteria metabolized between ~10 and 44 μmol L^{-1} or 3–12% of the total DOC (Table 2) across the three stations in the York River estuary. Although previous studies have noted a positive correlation between C:N ratios and DOC bioavailability (estimated by bacterial biomass production), a significant relationship was not found in the York River estuary. In contrast to the high humic characteristics and predominant terrigenous origin of OM in previously studied river/estuarine environments, OM from the York River estuary is primarily derived from terrigenous sources at its freshwater end member and from a mix of marsh, algal, and ad-

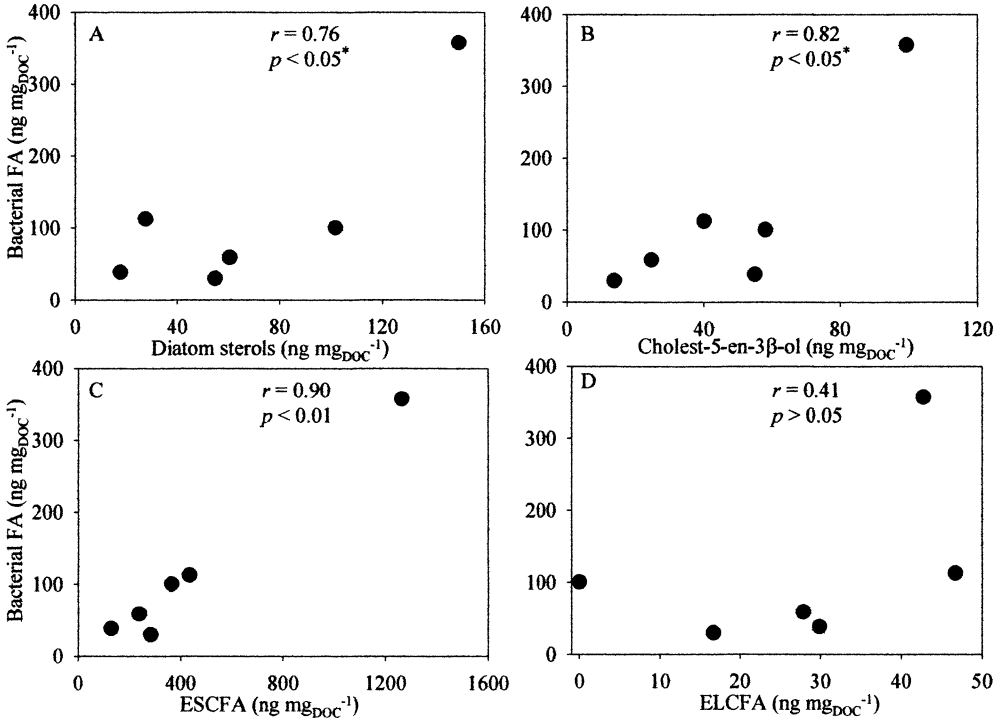

Fig. 4. Correlations between bacterial FA (odd-numbered C_{13}–C_{17}, branched and normal) and the sum of diatom sterols (24-norcholesta-5,22-dien-3β-ol, 24-methylcholesta-5,24(28)-dien-3β-ol, 24-methylcholesta-5,22-dien-3β-ol) (A), cholest-5-en-3β-ol (B), even short-chain FA (ESCFA; C_{12}–C_{16}) (C), and even long-chain FA (ELCFA; C_{22}-C_{28}) (D) in the HMW DOC fraction. Data were tested for normality and log transformed when appropriate. Values with asterisks indicate that the correlations were not significant when the highest point was removed.

vected riverine OM at its mid- and high-salinity regions (Raymond and Bauer 2001; McCallister et al. 2004). Thus, variations in OM sources and lability between different river/estuarine systems, coupled with the ability of heterotrophic bacteria to efficiently utilize inorganic N sources (Kirchman 1994), may in part explain between-system differences.

Both the decay constant (k') and the percent total DOC utilized were explained by indices of planktonic/algal sources (Fig. 3, data for percent DOC not shown). Decay constants depended on bulk indicators of algal OM (chlorophyll *a* [Chl *a*]) in the particulate phase ($r^2 = 0.79$, $p < 0.05$; Fig. 3A); however, this dependency was largely driven by a single high Chl *a* data point. Lipid biomarker compounds may provide a more sensitive assessment of algal contributions to both the dissolved and particulate OC pools than indices such as Chl *a*. Polyunsaturated fatty acids (PUFA), typically ascribed to mixed planktonic sources, are also indicators for ''fresher,'' more reactive material (Canuel and Martens 1996). Although these bioreactive components accounted for between ~ 20% and 35% of the total FA in the particulate phase, there was no relationship between them and the decay constant (data not shown) suggesting additional biogeochemical constraints (i.e., solubilization, enzyme accessibility) on the availability of POM to free-living bacteria (McCallister 2002). In contrast, contributions of PUFA (C_{18}, C_{20}, and C_{22} FA) to HMW DOM were nominal and accounted for at most ~ 6% of total FA (Fig. 3B) and <0.1% of the DOC pool. Nonetheless, percent PUFA (i.e., relative to total FA) was found to be the strongest predictor of both the percent of DOC decomposed over a 28-day period (the water residence time of the York River estuary) and the decay constant ($r^2 = 0.90$, $p < 0.005$; Fig. 3B). Although di- and tri-C_{18} unsaturated FA (e.g., 18:2ω6, 18:3ω3) may be attributed to higher plant sources, significant regressions between k' and PUFA specific to algal sources (20:5ω3 + 22:6ω3, $r^2 = 0.67$, $p < 0.05$) imply that the labile OC is of algal origin (Volkman 1986). These findings suggest that although the geochemical signature of algal-derived OC in the dissolved phase may appear quantitatively insignificant (McCallister 2002; Ducklow and McCallister 2004), this

Table 2. Amounts, percentages, and decay constants of DOC utilized in the York River estuary.

Date	Salinity	Bulk DOC utilization* (μmol L^{-1} C)	Bulk DOC utilization (% total)	Decay constant (k′ × 10^{-3})† (Δt = 28 d)
Mar 2000	0	14.7(2.5)‡	3.5(0.6)	−1.3(0.2)
	10	44.0(4.6)	11.8(1.3)	−4.5(0.5)
	21	10.1(2.8)	3.9(0.7)	−1.4(0.4)
Oct 2000	0	11.5(2.2)	2.7(0.4)	−1.0(0.2)
	13	12.3(0.8)	3.4(0.2)	−1.2(0.1)
	22	16.2(1.1)	7.0(0.3)	−2.6(0.1)

* Duration of incubations for DOC utilization (28 d).
† Decay constants expressed as d^{-1}.
‡ Numbers in parentheses represent ±1 SD of the mean (n=3).

fraction of OC may nevertheless represent the principal form of bioreactive estuarine DOC.

Biological fate of allochthonous and autochthonous DOM—Several recent studies have examined the relative roles of autochthonous and allochthonous OM sources to estuarine, riverine, and lake foodwebs (Sobczak et al. 2002; Kritzberg et al. 2004; McCallister et al. 2004). The general view arising from these studies is that allochthonous OM supports significant but variable amounts of bacterial and higher trophic level production in freshwater and brackish systems. To evaluate the contributions of allochthonous terrigenous OM supporting bacterial biomass production in a temperate estuary, we examined correlations between biomarkers representing bacterial biomass and algal and higher plant sources.

Significant positive correlations between FA diagnostic of bacterial sources (i.e., odd-numbered C_{13}–C_{17}, branched FA [Br-FA] and normal FA [Kaneda 1991]) and biomarkers diagnostic of planktonic origin (Fig. 4A–C) suggest a systematic bacterial response to autochthonous DOM sources along the estuarine continuum. Even-numbered, short-chain FA (ESCFA C_{12}–C_{16}) derived from aquatic sources (algal, zooplanktonic, and bacterial; Meyers 1997) were highly correlated to Br-FA concentrations ($r = 0.90$, $p < 0.01$; Fig. 4C). Sterols primarily diagnostic of diatom-derived OM (24-norcholesta-5,22-dien-3β-ol, 24-methylcholesta-5,22-dien-3β-ol and 24-methylcholesta-5,24(28)-dien-3β-ol [Volkman 1986]) also support the inferred importance of algal sources to bacterial distributions along the estuarine gradient (Fig. 4B). Further, the relationship ($r = 0.82$, $p < 0.05$) between Br-FA and crustacean (zooplankton) sterols, represented by cholest-5-en-3β-ol (Volkman 1986) suggests a direct response to zooplankton byproducts or an indirect response to algal OM produced via zooplankton sloppy feeding. This correlation could also be the result of responses of both zooplankton and bacteria to the availability of labile OM rather than a link between zooplankton processing and bacteria. The latter two relationships (Fig. 4A,B), driven primarily by an outlying data point, are not as robust as the correlation with ESCFA (Fig. 4C), which integrates several potential autochthonous OM inputs (e.g., algal exudation, zooplankton byproduct, sloppy feeding). These findings thus suggest multiple autochthonous OM sources may simultaneously influence estuarine bacterial distributions. In contrast, concentrations (ng mg_{OC}^{-1}) of even-numbered long-chained fatty acids (ELCFA) ($>C_{22}$), predominantly derived from vascular plant inputs (Canuel and Martens 1993; Meyers 1997) were unrelated to bacterial biomarker distributions ($r = 0.41$, $p > 0.05$; Fig. 4D). Although based on a relatively small dataset, these findings support the preferential selection of algal material by heterotrophic bacteria that has previously been observed for other freshwater (Kritzberg et al. 2004) and estuarine (Sobczak et al. 2002; McCallister et al. 2004) systems despite the quantitative dominance of allochthonous OM to the bulk pool. Collectively, our results underscore the importance of autochthonous (HMW DOM) sources in regulating bacterial distributions (Fig. 4) and as a significant source of bioreactive OM (Fig. 3) in the York River and possibly other estuaries (Sobczak et al. 2002).

We conclude that the sources, physical states and size classes, and quantities of DOM utilized by bacteria may be assessed more fully through the use of combined geochemical and microbial ecological techniques and approaches for characterizing OM cycling. Our findings for the temperate York River estuary emphasize the importance of autochthonous OM sources in supporting microbial processes in systems often dominated quantitatively by allochthonous forms of both DOM and POM. This approach appears to provide a high degree of sensitivity for discerning both the sources and amounts of bioreactive DOM in a complex aquatic system having abundant and diverse sources of OM.

References

ARNOSTI, C. 2004. Speed bumps and barricades in the carbon cycle: Substrate structural effects on carbon cycling. Mar. Chem. **92:** 263–273.

BENNER, R. 2003. Molecular indicators of the bioavailability of dissolved organic matter, p. 121–137. *In* S. E. G. Findlay and R. L. Sinsabaugh [eds.], Aquatic ecosystems: Interactivity of dissolved organic matter. Academic Press.

BLIGH, E. G., and W. J. DYER. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37:** 911–917.

BOSCHKER, H. T. S., J. F. C. DE BROUWER, AND T. E. CAPPENBERG. 1999. The contribution of macrophyte-derived organic matter to microbial biomass in salt-marsh sediments: Stable carbon isotope analysis of microbial biomarkers. Limnol. Oceanogr. **44:** 309–319.

———, J. KROMKAMP, AND J. J. MIDDELBURG. 2005. Biomarker and carbon isotopic constraints on bacterial and algal community structure and functioning in a turbid, tidal estuary. Limnol. Oceanogr. **50:** 70–80.

BURNISON, B. K. 1980. Modified dimethyl sulfoxide (DMSO) extraction for chlorophyll analysis of phytoplankton. Can. J. Fish. Aquat. Sci. **37:** 729–733.

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.

———, AND C. S. MARTENS. 1993. Seasonal variations in the sources and accumulation of organic matter associated with recently-deposited sediments. Org. Geochem. **20:** 563–577.

———, AND ———. 1996. Reactivity of recently deposited organic matter: Degradation of lipid compounds near the sediment-water interface. Geochim. Cosmochim. Acta **60:** 1793–1806.

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

———, 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.

COLE, J. J., S. R. CARPENTER, J. F. KITCHELL, AND M. L. PACE. 2002. Pathways of organic carbon utilization in small lakes: Results from a whole-lake 13C addition and coupled model. Limnol. Oceanogr. **47:** 1664–1675.

DUCKLOW, H. W., AND S. L. MCCALLISTER. 2004. CO_2 exchange and the metabolic balance of the global continental ocean., p. 269–315. *In* A. R. Robinson, J. McCarthy, and B. J. Rothschild [eds.], The global coastal ocean: Multiscale interdisciplinary processes. V. 13 The sea volume. Wiley.

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.

HANSELL, D. A., AND C. A. CARLSON. 2002. Biogeochemistry of marine dissolved organic matter. Academic Press.

HOPKINSON, C. S., I. BUFFAM, J. HOBBIE, J. VALLINO, M. PERDUE, B. EVERSMEYER, J. PRAHL, J. COVERT, R. HODSON, M. A. MORAN, E. SMITH, J. BAROSS, B. CRUMP, S. FINDLAY, AND K. FOREMAN. 1998. Terrestrial inputs of organic matter to coastal ecosystems: An intercomparison of chemical and bioavailabilty. Biogeochemistry **43:** 211–234.

KANEDA, T. 1991. Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. Microbiol. Rev. **55:** 288–302.

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

KRITZBERG, E. S., J. J. COLE, M. L. PACE, W. GRANELI, AND D. L. BADE. 2004. Autochthonous versus allochthonous carbon sources to bacteria: Results from whole-lake 13C addition experiments. Limnol. Oceanogr. **49:** 588–596.

MANNINO, A., AND H. R. HARVEY. 1999. Lipid composition in particulate and dissolved organic matter in the Delaware Estuary: Source and diagenetic patterns. Geochim. Cosmochim. Acta **63:** 2219–2235.

MCCALLISTER, S. L. 2002. Organic matter cycling in the York River estuary, VA: An analysis of potential sources and sinks, p. 220. Ph.D. dissertation. School of Marine Science. The College of William and Mary.

———, J. E. BAUER, J. CHERRIER, AND H. W. DUCKLOW. 2004. Assessing the sources and ages of organic matter supporting estuarine bacterial production: A novel multiple isotope ($\delta^{13}C$, $\delta^{15}N$, $\Delta^{14}C$) approach. Limnol. Oceanogr. **49:** 1687–1702.

MEYERS, P. 1997. Organic geochemical proxies of paleoceanographic, paleolimnologic, and paleoclimatic processes. Org. Geochem. **27:** 213–250.

PACE, M. L., J. J COLE, S. R. CARPENTER, J. F. KITCHELL, J. R. HODGSON, M. C. VAN DE BOGERT, D. L. BADE, E. S. KRITZBERG, AND D. BASTVIKEN. 2004. Whole lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature **427:** 240–243.

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

SCHULTZ, G. E. 1999. Bacterial dynamics and community structure in the York River estuary. Ph.D. dissertation. School of Marine Science. The College of William and Mary.

SEITZINGER, S. P., H. HARTNETT, R. LAUCK, M. MAZUREK, T. MINEGISHI, G. SPYRES, AND R. STYLES. 2005. Molecular-level chemical characterization and bioavailability of dissolved organic matter in stream water using electrospray-ionization mass spectrometry. Limnol. Oceanogr. **50:** 1–12.

SOBCZAK, W. V., J. E. CLOERN, A. D. JASSBY, B. E. COLE, T. S. SCHRAGA, AND A. ARNSBERG. 2005. Detritus fuels ecosystem metabolism but not metazoan food webs in San Francisco estuary's freshwater delta. Estuaries **28:** 124–137.

———, ———, ———, AND A. B. MUELLER-SOLGER. 2002. Bioavailability of organic matter in a highly disturbed estuary: The role of detrital and algal resources. Proc. Natl. Acad. Sci. USA **99:** 8101–8105.

VOLKMAN, J. K. 1986. A review of sterol markers for marine and terrigenous organic matter. Org. Geochem. **9:** 83–99.

ZOU, L. X.-C. WANG, J. CALLAHAN, R. A. CULP, R. F. CHEN, M. A. ALTABET, AND M.-Y SUN. 2004. Bacterial roles in the formation of high-molecular-weight dissolved organic matter in estuarine and coastal waters: Evidence from lipids and the compound-specific isotopic ratios. Limnol. Oceanogr. **49:** 297–302.

Received: 8 April 2005
Accepted: 29 September 2005
Amended: 29 September 2005