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Sources of estuarine dissolved and particulate organic matter: A multi-tracer approach

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Abstract

Assessing the fate of organic matter (OM) in estuaries is challenging due to its numerous sources, diagenetic processing, and physical and biogeochemical sinks. In order to evaluate differences in the sources and potential transformations of OM, particulate organic matter (POM) and high molecular weight dissolved organic matter (HMW DOM ($\geqslant$3 kDa)) were characterized along the York River estuary (Virginia, USA) using lipid biomarker, elemental ratios, and stable isotope (δ^{13}C and δ^{15}N) analyses. Isotopic signatures of HMW DOM from the freshwater site were distinct from both its POM counterpart and HMW DOM from higher salinity regions of the estuary. Lipid compound classes were depleted in HMW DOM relative to POM. Saturated fatty acids (FA) dominated the dissolved organic pool while monounsaturated and polyunsaturated FA were the primary FA classes in POM. Concentrations of sterols diagnostic of allochthonous (i.e., plant/freshwater algae) sources were enriched in HMW DOM relative to the POM at higher salinities. Ternary plots based on FA biomarkers show that POM is characterized by contributions from polyunsaturated FA, labile compounds representing contributions from ''fresh'' phytoplankton/zooplankton sources. In contrast, FA in HMW DOM reflect bacterial and vascular plant signatures. Disparities in the composition of HMW DOM and POM likely reflect differences in the dominant biological sources, different susceptibilities to transformation and/or differential influences from abiotic processes (sorption–desorption, flocculation, etc.). Thus, the physical associations of OM (particulate vs. dissolved) may be a fundamental control on both the distribution and biogeochemical processing of OM whereby terrigenous DOM may be selectively exported while terrigenous POM is retained within the estuary. These observations have implications for the selective processing of different sources and forms of OM in rivers and estuaries prior to export to the coastal ocean.

1. Introduction

Although rivers have been termed ''arteries of the continent'' (Degens et al., 1991) they do not necessarily transport organic matter (OM) passively from land to sea. Instead rivers and their associated estuaries are vital hydrologic and biogeochemical links between the continents and oceans. Bulk OM pools entering an estuary are potentially modified in several ways: (1) chemically through photolytic reactions (Amon and Benner, 1996; Miller and Moran, 1997); (2) biologically through autochthonous production (Peterson et al., 1994; Raymond and Bauer, 2001), heterotrophic degradation (McCallister et al.,

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2004) and respiration (Findlay et al., 1992; Moran et al., 1999); and (3) physically through homogenization of spatially distinct sources (Findlay et al., 1998; Cloern et al., 2002), flocculation (Sholkovitz et al., 1978; Fox, 1983), sorptive fractionation (Aufdenkampe et al., 2001) and sedimentation (Hedges, 1992; Prahl et al., 1994). Consequently estuaries are among the most challenging aquatic systems in which to trace the origins, transformations and fates of OM (Hedges and Keil, 1999).

The molecular and isotopic characteristics of dissolved and particulate OM (DOM and POM) fractions may also be influenced by abiotic (Aufdenkampe et al., 2001; Mopper and Kieber, 2002) and biotic (Sobczak et al., 2002; McCallister et al., 2006) exchange and removal processes. For example, although the generally low levels of algal biomarkers in ultrafiltered DOM relative to POM have been attributed to limited exchange between these pools (Zou et al., 2004), alternative processes potentially contributing to these observations include photochemical removal (Mopper and Kieber, 2002), sorptive losses (Aufdenkampe et al., 2001) and bacterial modification (Sobczak et al., 2002; McCallister et al., 2006). As a consequence, geochemical compositional patterns may provide a more accurate picture when considered in the context of simultaneous biogeochemical and physicochemical processes.

The efficiency of estuaries in retaining and transforming terrigenous OM is critical for constraining global C budgets (Stallard, 1998; Hedges et al., 1997). Despite reports that estuarine dissolved organic carbon (DOC) is transported conservatively in certain systems (e.g., Sharp et al., 1982; Mantoura and Woodward, 1983; Ittekot, 1989), the lack of identifiable terrigenous compounds in open ocean dissolved OM suggests that this material does not persist in the oceans (Hedges et al., 1997; Opsahl and Benner, 1997). The apparent contradiction in these findings underscores the shortcomings inherent in tracing selected constituents of OM (e.g., terrigenous-derived OM) strictly through bulk OM concentrations.

Stable isotopes have proven useful as a tool for inferring sources and following pathways of OM cycling in aquatic systems (Hedges et al., 1988; Canuel et al., 1995; Currin et al., 1995) and are frequently coupled with elemental information (C/N ratios) to provide additional resolution. Because estuaries span freshwater to marine conditions, a correspondingly large range of $\delta^{13}C$ values in dissolved inorganic carbon (DIC) as well as both living and non-living OM is often observed (Coffin et al., 1994; Cloern et al., 2002). Consequently, isotope-based source assignments for OM are frequently compromised by overlapping isotopic signatures and other complicating factors (Canuel et al., 1995; Cloern et al., 2002).

The application of lipid biomarker compounds can further differentiate sources of OM within complex aquatic ecosystems by distinguishing: (1) autochthonous primary producers (e.g., diatoms, dinoflagellates), (2) OM of primary (e.g., algal) and secondary (e.g., zooplankton, bacteria) autochthonous origin, and (3) the relative importance of terrigenous sources along the estuarine continuum. Previous studies have utilized biomarkers to examine sources of dissolved and particulate matter along estuarine salinity gradients. For example, Mannino and Harvey (1999, 2000) examined the composition of high molecular weight (HMW) DOM and POM along the highly urbanized Delaware Estuary. Similarly, Jaffé et al. (1995) compared the composition of lipid biomarker compounds in DOM and POM in the tropical Orinoco River, which has both pristine and anthropogenically influenced segments. Other estuarine studies have focused on the use of biomarkers associated with dissolved (Zou et al., 2004; McCallister et al., 2006) or particulate phases (Canuel et al., 1995; Canuel, 2001) alone. Collectively, these studies have provided source-specific information about allochthonous and autochthonous inputs and the diagenetic states of DOM and POM in coastal systems.

The primary objective of this study was to assess the relative contributions of allochthonous (i.e., terrigenous) and autochthonous (i.e., estuarine plankton) sources of organic material to both POM and ultrafiltered DOM throughout a relatively pristine, temperate estuary by employing a suite of bulk OM parameters (C:N ratios, $\delta^{13}C$, $\delta^{15}N$) concurrently with two classes of lipid biomarker compounds (fatty acids and sterols). The York River was selected for its relatively pristine condition, providing a useful comparison with the nearby, but more heavily impacted, Chesapeake Bay. Additionally, we interpret biomarker findings from this study with supporting data either collected simultaneously at the same locations (McCallister et al., 2004; McCallister et al., 2006) or at the same locations but the previous year (McCallister et al., 2005), to better constrain the roles of bacterial and photo-

chemical processes in regulating the observed trends in HMW DOM biomarker distributions.

2. Methods

2.1. Study sites and sampling locations

The York River estuary is a moderately stratified sub-estuary of the Chesapeake Bay encompassed by a watershed of ~4350 km^2, with an average (50 years) annual mean flow rate (Pamunkey River) of 28.5 $m^3 s^{-1}$ (Fig. 1). The York is formed by the convergence of the Pamunkey and Mattaponi Rivers, which account for ~80% and 20% of the riverine inputs, respectively. Extensive tidal freshwater marshes, encompassing an area of $\sim 2.0 \times 10^7$ m^2 (Neubauer et al., 2000), provide an important source of organic matter to the Pamunkey River. Phytoplankton biomass in the York displays large seasonal and temporal fluctuations, with maximum chlorophyll *a* (Chl *a*) concentrations attaining ~50 $\mu g\, l^{-1}$ in the spring at the low salinity transitional area of the estuary (Sin et al., 1999). Previous studies in the York suggested that biogeochemical processing along the estuary significantly alters the isotopic character and reactivity of terrigenous DOM during its seaward transport (Raymond and Bauer, 2001; McCallister et al., 2004). Relative to other estuaries, the York is characterized by DOM and POM that is relatively "young" in age as determined by radiocarbon isotopes (McCallister et al., 2004; Raymond et al., 2004).

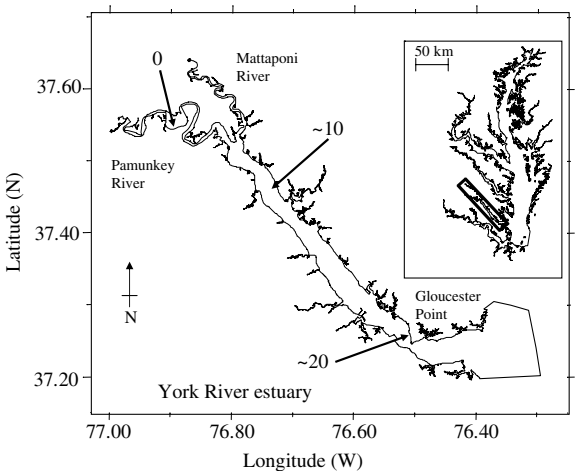

Fig. 1. The York River estuary. Inset shows the York's location relative to the Chesapeake Bay proper. Sampling locations are designated by arrows and approximate salinities.

2.2. Sample collection

Surface water (57–125 l) was collected in March 2000 (mean river flow 30.5 $m^3 s^{-1}$; Table 1) and in October 2000 (3.5 $m^3 s^{-1}$) from three sites (Fig. 1): (1) the mouth of the estuary where it enters the Chesapeake Bay ($S = 21.5$); (2) the Pamunkey River ($S = 0$); and (3) an intermediate site that approximated an equal mixture of the two end-members ($S = 11.5$). Water samples were collected using a peristaltic pump and filtered through combusted (525 °C for 4 h) Whatman GF/F filters (0.7 μm nominal pore size) to concentrate particles for isotopic and lipid analyses. The 0.7-μm filtrate was subsequently passed through an in-line Gelman filter capsule (0.2 μm) to remove bacteria, and the HMW DOM ($\geqslant$3 kDa) was concentrated to a 1-l volume with an Amicon DC-10L tangential flow ultrafiltration unit equipped with two spiral-wound polysulfone cartridges. The >3 kDa HMW fraction comprised from 18% to 33% of the total DOC pool (Table 1). The sample was further reduced to a final volume of ~50 ml by turbo-evaporation (Zymark Turbo Vap 500) and subsequently lyophilized. Both POM and DOM samples were stored at −80 °C until further analysis.

2.3. Lipid extraction and analysis

All glassware for lipid processing was combusted at 450 °C for 4.5 h. Prior to extraction, filters were shredded with solvent-rinsed forceps, and a lipid standard mixture (a C_{19} fatty acid methyl ester (FAME), a C_{19} alcohol, and myristyl arachidate, a wax ester that yielded a C_{14} alcohol and a C_{20} FAME following saponification) was added to both POM and HMW DOM fractions. Lipids were extracted from POM and HMW DOM with $MeCl_2$/MeOH (2:1) using accelerated solvent extraction (Dionex ASE 200). The extraction was repeated twice, solvents were combined, and the lipid fraction partitioned following a modification of the procedures of Bligh and Dyer (1959) as described in Canuel and Martens (1993). Lipid extracts were concentrated to 1 ml using turbo-evaporation (Zymark Turbo Vap 500), and total lipid contents were determined gravimetrically. The entire HMW DOM and a portion of the particulate (30–70%) lipid extract was saponified with 3 ml of 1 N KOH, in 15 ml glass tubes at 110 °C for 2 h and analyzed following previously published methods (Canuel and Martens, 1993).

Fatty acids (FA, as methyl esters) and sterols (as trimethylsilyl ethers) were analyzed by gas chroma-

Table 1
Site and water characteristics of the York River estuary

Sampling date	Streamflow ($m^3 s^{-1}$)[a]	Salinity	Water temperature (°C)	Chl *a* ($\mu g l^{-1}$)	Bacterial abundance ($cells\ l^{-1} \times 10^9$)	POC (μM)	TDOC (μM)	C:N		>3 kDa (%TDOC)[d]	PhytoOC[e]
								POM	DOM[c]		
Mar 2000	30.5	0	14.9	3.5 (0.4)[b]	2.4 (0.4)	168 (11)	424 (6)	9	17.9	33	0.10
		10	12.2	34.4 (1.6)	2.5 (0.2)	210 (16)	352 (7)	5.8	10.8	20	0.74
		21	11.3	8.0 (1.0)	3.4 (0.4)	82 (7)	263 (4)	7.3	7.4	18	0.44
Oct 2000	3.5	0	19.0	3.7 (0.5)	3.7 (0.3)	111 (10)	422 (7)	6.9	16.5	31	0.16
		13	19.0	12.3 (2.2)	3.8 (0.4)	118 (8)	362 (5)	7.7	13.2	25	0.48
		22	20.0	7.2 (0.6)	5.8 (0.5)	61 (5)	233 (5)	5.5	9.8	22	0.54

[a] Data obtained from US Geological Survey (http://waterdata.usgs.gov). Pamunkey River freshwater flow reported for the York River estuary.
[b] Numbers in parentheses are ±1 SD of mean.
[c] HMW DOM (>3 kDa) fraction.
[d] % of total dissolved organic carbon (TDOC) recovered in HMW.
[e] Phytoplankton organic carbon (PhytoOC) was calculated as the sum of Chl *a* + phaeophytin (assuming a C:pigment ratio of 35:1) normalized to POC concentrations.

tography (Hewlett–Packard 5890 Series II) and injected directly onto a 30 m × 0.32 mm i.d. DB-5 fused silica capillary column (J&W Scientific) according to the conditions outlined in Arzayus and Canuel (2005). 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 FA and 5α(H)-cholestane for sterols). Identifications of selected compounds were confirmed by gas chromatography–mass spectrometry (Hewlett Packard 6890 Series Gas Chromatograph–Mass Selective Detector) operating at 70 eV with mass acquisition range of 50–550 Da. Because FA recoveries were lower for HMW DOM than POM, concentrations for the HMW DOM were corrected based on the recovery of C_{19} and C_{20} FAME standards. No corrections were made to the FA extracted from the POM.

2.4. Elemental and isotopic analyses

Separate filters for stable isotope analyses of POM (see above for collection details) were thawed, dried at 60 °C, acid fumed overnight with HCl and subsequently dried again at 60 °C. Aliquots of lyophilized DOM were reconstituted in ~10 ml of deionized water and desalted overnight in Pierce 3.5 kDa slide-a-lyzers according to manufacturer's specifications. Desalted HMW DOM was transferred to baked (500 °C) 2 ml vials, acidified with 10% HCl, and dried overnight at low heat on a hotplate in an effort to minimize the potential loss of volatile and low molecular weight compounds. Organic carbon and total nitrogen contents of particulate and lyophilized DOM fractions were measured using a Fisons Instruments Model EA1108 CHNS-O analyzer using acetanilide and sulfanilimide as external standards. Stable carbon and nitrogen isotope ratios were measured as CO_2 and N_2, respectively, on an isotope ratio mass spectrometer using a continuous flow system with on-line sample combustion (Europa Scientific Integra, University of California, Davis-Stable Isotope Facility). Selected samples were run in duplicate to assess analytical precision (standard deviation <0.4‰). Results for stable isotope values are reported in standard δ notation as:

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

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

2.5. Other analyses

Samples for bacterial abundance were preserved with 0.2 μm filtered 25% glutaraldehyde diluted to a final concentration of 2%. Preserved samples were stored at 4 °C until slide preparation. Bacteria were enumerated by acridine orange epifluorescence microscopy (Hobbie et al., 1977) with a Zeiss Axiophot microscope.

For Chl *a* analysis, ~100 ml of surface water was collected from each sampling location, in an amber polycarbonate bottle, and stored on ice until analysis. Aliquots (8 ml) were filtered through Whatman GF/F filters ($n = 3$). Filters were placed in 8 ml test tubes filled with DMSO/acetone/water solution (45:45:10) sealed and kept in the dark for a minimum of 48 h (Burnison, 1980). Sample fluorescence was read on a Turner fluorometer (model 10-AU).

DOC was determined using a Shimadzu TOC-5000 with an analytical precision of ±7 μM for the range of concentrations found in the York River (McCallister, 2002).

2.6. Data analysis

Data were imported into MiniTab and analyzed statistically when possible given the limited replication in the lipid biomarker dataset. Unless otherwise noted, all confidence intervals are expressed at the 95% ($p = 0.05$) level. Relationships between variables were examined by Model I linear regressions. Analysis of variance (ANOVA) was used to examine variability in space and time and between dissolved and particulate organic fractions for grouped samples. March and October 2000 samples were combined to assess spatial variability. Tukey's multiple comparison test was used to conduct pairwise comparisons between sites. The influence of high vs. low flow was assessed by grouping the POM and HMW DOM samples collected at each salinity. Compositional differences were evaluated by pooling POM and HMW DOM samples across time (March and October 2000) and/or salinity.

3. Results

3.1. Water column and bulk OM characteristics

The C:N of HMW DOM was higher and more variable than that of the corresponding POM with values ranging from 7.4 to 17.9 and 5.5 to 9.0, respectively (Table 1). The C:N of HMW DOM decreased with increasing salinity ($r^2 = 0.86$; $p < 0.01$) while no discernible trend with salinity was observed for the POM (Table 1). The $\delta^{13}C$ of both HMW DOM and POM showed similar patterns through the estuary with the most depleted values found in the riverine end-member (−28.1‰) and the most enriched $\delta^{13}C$ values at the mouth (−21.9‰, Fig. 2). The $\delta^{13}C$ of HMW DOM was depleted on average by ~1‰ relative to the corresponding POM, with the exception of the October sampling at the York mouth where POM was depleted by 0.6‰ relative to HMW DOM (Fig. 2).

Similar to $\delta^{13}C$, $\delta^{15}N$ was most enriched at the mouth of the York estuary (+10.4‰) and most depleted at the riverine end-member (+4.5‰, Fig. 2). On average, particulate $\delta^{15}N$ values from the mid-estuary and mouth were enriched in March by ~2.2 ± 0.2‰ relative to October. Conversely, $\delta^{15}N$ of riverine POM was enriched by ~1.5‰ in October relative to March. On average, POM was enriched by 1.8 ± 1.2‰ relative to HMW DOM, with the exception of the October sampling at the mouth of the estuary (Fig. 2).

3.2. Fatty acids

Total FA concentrations, normalized to organic carbon (OC), ranged from 11.1 μg mg_{oc}^{-1} to 97.3 μg mg_{oc}^{-1} for POM and 0.4 μg mg_{oc}^{-1} to 2.9 μg mg_{oc}^{-1} for HMW DOM (Table 2). Total FA concentrations were significantly lower ($p < 0.005$) in HMW DOM (mean ± SD = 1.1 ± 0.9 μg mg_{oc}^{-1}) relative to POM (mean ± SD = 43.2 ± 32.3 μg mg_{oc}^{-1}). In addition

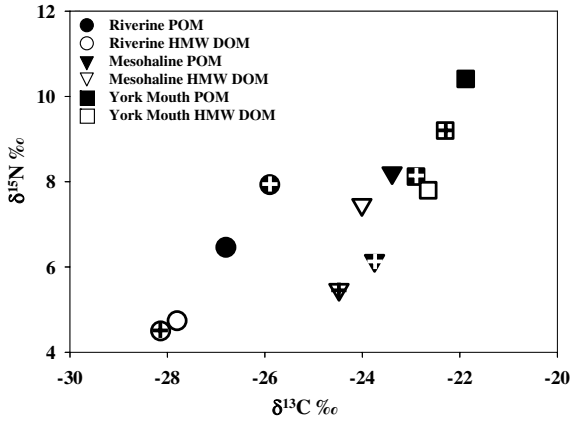

Fig. 2. $\delta^{15}N$ vs. $\delta^{13}C$ for POM and HMW DOM from the York River estuary. Solid symbols indicate March sampling and symbols with cross bars indicate October sampling, respectively.

Table 2
Fatty acid distributions in POM and HMW DOM from the York River estuary

Component (%)	March 2000						October 2000					
	$S = 0$		$S = 10$		$S = 21$		$S = 0$		$S = 13$		$S = 22$	
	POM	DOM	POM	DOM	POM	DOM	POM	DOM	POM	DOM	POM	DOM
12:0	–[a]	tr[b]	tr	3.0	tr	3.7	tr	2.2	tr	1.8	tr	1.1
i13	–	tr	–	tr	–	–	–	–	–	–	–	–
a13	–	tr	–	–	–	–	–	–	tr	–	tr	–
13:0	–	tr	tr	–	tr	–	–	–	tr	–	tr	–
i14	tr	tr	tr	tr	tr	tr	tr	–	tr	1.6	tr	–
14:1	–	tr	–	–	tr	–	–	–	tr	–	tr	–
14:0	**6.4**[c]	**4.9**	**11.6**	–	**10.7**	**8.1**	**7.5**	**7.0**	**10.3**	–	**9.5**	**9.8**
15:1	tr	1.1	tr	tr	1.1	–	tr	–	tr	–	tr	–
i15	1.4	2.7	tr	3.1	tr	1.7	1.9	**6.7**	tr	**6.1**	tr	**6.2**
a15	tr	1.7	tr	1.8	tr	1.2	tr	1.6	tr	1.1	tr	1.2
15:0	tr	1.4	tr	1.6	tr	1.5	tr	2.3	1.3	3.7	1.0	3.3
16br	–	1.0	–	tr	tr	tr	–	2.1	–	2.1	–	1.3
16:3	1.9	–	4.8	–	2.3	–	1.2	–	1.6	–	1.7	–
16:2	**7.8**	–	4.1	–	2.5	–	3.6	–	4.7	–	1.9	–
16:1ω7	**19.1**	**17.7**	**25.9**	**12.4**	**26.0**	**10.2**	**22.1**	**13.8**	**22.8**	**11.2**	**15.5**	**13.4**
16:1ω9	1.3	1.2	tr	1.0	tr	tr	1.2	–	tr	2.0	tr	–
16:0	**15.9**	**24.4**	**16.0**	**30.5**	**16.8**	**32.3**	**20.0**	**31.0**	**20.6**	**38.1**	**20.0**	**32.7**
10Me17br	–	tr	–	tr	–	–	–	–	tr	–	tr	–
i17	tr	tr	tr	tr	tr	tr	–	–	tr	–	tr	2.8
a17	tr	tr	tr	tr	–	tr	–	–	tr	–	tr	tr
17:1	tr	1.0	tr	tr	–	–	–	–	–	1.9	tr	–
17:0	tr	1.6	tr	1.6	tr	2.0	tr	**8.4**	tr	**6.1**	tr	**6.0**
18:4	4.7	tr	**5.0**	tr	**6.8**	tr	**5.0**	–	**4.9**	–	**7.6**	–
18:2	2.1	tr	1.8	**3.3**	2.8	1.7	3.0	2.3	1.9	2.2	2.3	2.4
18:1ω9c	**8.7**	**4.2**	2.7	**4.8**	5.8	**5.8**	**13.2**	3.2	**6.4**	4.1	**8.5**	3.0
18:1ω9t	2.9	**4.5**	2.6	**6.5**	2.9	**4.4**	3.9	4.5	3.4	**4.3**	5.7	4.5
18:0	2.6	**13.5**	1.5	**16.7**	1.1	**18.8**	2.9	**10.6**	1.8	**13.5**	2.2	**7.6**
20:4ω6	1.3	tr	tr	–	tr	–	tr	–	tr	–	tr	–
20:5ω3	**11.8**	tr	**10.4**	tr	**7.4**	tr	**4.7**	–	**7.3**	–	**8.3**	1.4
20:3	–	–	tr	–	tr	–	–	–	–	–	–	–
20:4	tr	–	tr	–	tr	–	–	–	tr	–	tr	–
20:2	tr	tr	tr	–	tr	–	–	–	tr	–	tr	–
20:1	tr	tr	tr	tr	tr	–	–	–	tr	–	tr	–
22:6ω3	2.8	tr	**5.9**	tr	**6.7**	–	1.1	–	2.3	–	6.1	–
22:4ω3	–	–	tr	–	tr	–	–	–	tr	–	tr	–
22:2	–	–	–	–	tr	–	–	–	–	–	tr	1.1
22:1ω9	tr	1.3	tr	tr	tr	tr	–	–	tr	–	tr	–
22:0	tr	1.7	tr	1.3	tr	tr	1.0	2.2	tr	–	tr	1.1
23:0	–	tr	–	tr	tr	–	–	–	–	–	–	–
24:1	–	–	tr	–	tr	–	–	–	1.0	–	tr	–
24:0	1.0	2.5	tr	1.7	tr	1.6	1.3	2.1	1.0	–	tr	tr
25:0	–	1.3	–	tr	–	–	–	–	–	–	–	–
26:0	tr	1.5	tr	1.1	–	tr	tr	–	tr	–	tr	–
28:0	tr	1.2	tr	tr	–	tr	tr	–	tr	–	–	–
30:0	tr	1.0	–	tr	–	–	–	–	–	–	–	–
Total FA (μg mg_{oc}^{-1})	11.2	0.4	56.0	0.7	97.4	0.6	11.4	1.1	35.2	0.9	48.2	2.9

[a] –, Denotes below detection limits.
[b] tr, denotes trace amounts (<1% of total FA).
[c] Boldface indicates FA is amongst the six most abundant FA (on a % basis) comprising the total distribution.

the depletions of FA in HMW DOM relative to POM increased with salinity and during the March sampling (Table 2).

Saturated FA, and C_{14}, C_{16}, and C_{18} in particular, generally comprised the dominant (55–70%) FA in the HMW DOM pool, with monounsatu-

rated FA contributing 22–32% (Table 2). Long-chain saturated FA made up a small fraction (<2%) of the total FA associated with HMW DOM. Of the monounsaturated FA, 16:1ω7 and 18:1ω9 comprised 83–100% of the total monounsaturated pool (Table 2). Contributions of polyunsaturated FA (PUFA) to HMW DOM were nominal and accounted for <6% of total FA (Fig. 3A). In contrast, PUFA made up a substantial portion (19–34%) of total FA in POM collected at both sampling times (Fig. 3A). Particulate FA were approximately evenly distributed between the three main classes (saturated, monounsaturated and polyunsaturated), with minor contributions (1.8–3.5%) from *iso* and *anteiso* branched FA (Br–FA; Table 2, Fig. 3A and B). Despite considerable spatial and temporal variation of Chl *a* (Table 1), relative concentrations of PUFA (% of Total FA) associated with POM remained relatively constant with only slight decreases ($\lesssim$10%) in October at the riverine end-member (Fig. 3A).

Overall, POM contained a greater number of individual FA (30–40) than HMW DOM (15–25) (Table 2). However, six compounds comprised the majority of FA in POM (>70%) (Table 2). Four FA dominated the POM across all sites and during both seasons: 14:0, 16:1ω7, 16:0 and 20:5ω3. While 18:4 was not among the six most abundant FA in POM at the freshwater river site in March, it comprised 5–8% of total FA at all other sites in both seasons (Table 2). During the period of elevated Chl *a* in the mouth and mid-salinity stations (March), 22:6ω3 comprised ~6% of the total FA pool in POM. 18:1ω9 accounted for 6.4–13.0% of total FA in POM across all salinities in October, and at the riverine location in March (Table 2).

Similar to POM, 16:0 and 16:1ω7 were dominant FA in all HMW DOM samples (Table 2). Stearic acid (18:0) was common to all DOM samples comprising on average 7.5–20.0% of total FA. Oleic acid, 18:1ω9, was prominent only in HMW DOM collected in March (Table 2). Although 14:0 was absent from HMW DOM FA at the mid-salinity station, it accounted for 5–10% of FA at the two end-member sites. In contrast to POM, two FA generally ascribed to bacterial sources, i15:0 and 17:0, were among the six most abundant FA in HMW DOM collected during October (Table 2).

3.3. Neutral lipids

Total sterol concentrations were an order of magnitude lower than FA in both POM and HMW DOM (Tables 2 and 3). Particulate sterols

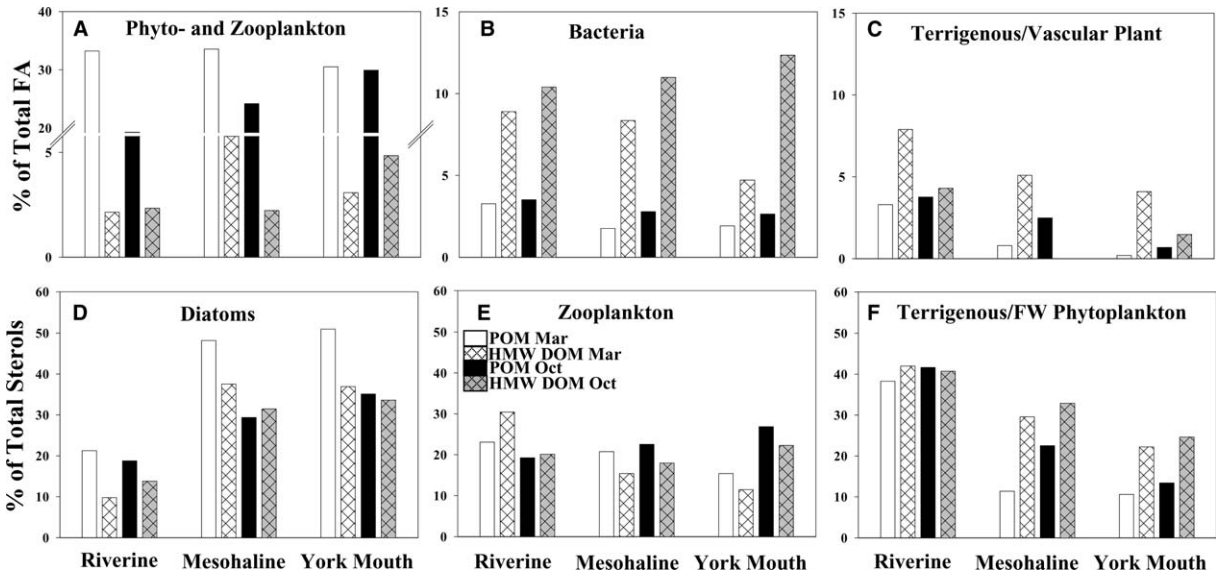

Fig. 3. Temporal and spatial variations in fatty acids (FA, in %) diagnostic for: (A) phytoplankton and zooplankton (polyunsaturated C_{18}, C_{20} and C_{22} FA), (B) bacterial (odd numbered C_{13}–C_{17}, branched and normal FA), and (C) terrigenous/vascular plant (even long chain FA (LCFA) C_{24}–C_{30}) sources; and sterols (%) diagnostic for (D) diatom (24-nor-cholesta-5,22*E*-dien-3β-ol, 24-methylcholesta-5,24(28)-dien-3β-ol, 24-methylcholesta-5,22*E*-dien-3β-ol), (E) zooplankton (cholest-5-en-3β-ol), and (F) terrigenous OM and freshwater (FW) phytoplankton (24-ethylcholesta-5,22*E*-dien-3β-ol, 24-methylcholest-5-en-3β-ol, 24-ethylcholest-5-en-3β-ol) sources in the York River estuary.

Table 3
Neutral lipid distributions in POM and HMW DOM from the York River estuary

Component (%)	March 2000						October 2000					
	$S = 0$		$S = 10$		$S = 21$		$S = 0$		$S = 13$		$S = 22$	
	POM	DOM	POM	DOM	POM	DOM	POM	DOM	POM	DOM	POM	DOM
24-Nor-cholesta-5,22*E*-dien-3β-ol	–[a]	–	**13.0**[b]	**19.1**	**9.0**	**9.6**	–	–	4.0	5.1	6.9	6.1
24-Nor-5α-cholest-22*E*-en-3β-ol	–	–	–	–	tr[c]	–	–	–	–	–	–	–
5β-Cholestan-3β-ol	–	–	–	–	–	–	–	5.4	–	–	–	–
Cholesta-5,22*E*-dien-3β-ol	4.7	5.0	4.8	6.5	5.6	8.1	4.7	6.4	5.2	7.7	7.7	**11.0**
Cholest-5-en-3β-ol	**23.1**	**30.4**	**20.8**	**15.4**	**11.4**	9.3	**19.3**	**20.2**	**22.4**	**18.0**	**26.4**	**22.3**
5α-Cholestan-3β-ol	2.9	4.7	1.3	–	tr	–	5.1	–	2.5	–	1.3	–
24-Methylcholesta-5,22*E*-dien-3β-ol	**12.8**	**6.9**	**15.0**	5.7	**15.9**	**10.1**	**13.2**	**9.2**	**12.9**	**16.5**	**16.3**	**16.2**
24-Methyl-5α-cholest-22*E*-en-3β-ol	–	–	–	–	tr	–	–	–	tr	–	1.9	–
4α-Methylcholest-8(14)-en-3β-ol	–	–	1.3	–	–	–	–	–	1.1	–	1.1	–
24-Methylcholesta-5,24(28)-dien-3β-ol	**8.5**	2.9	**20.3**	**12.8**	**26.0**	**17.3**	5.5	4.6	**12.3**	**9.9**	**11.3**	**11.4**
24-Methylcholest-5-en-3β-ol	8.1	**7.1**	2.2	4.8	1.7	3.2	**6.8**	6.4	5.8	5.5	2.8	4.8
23,24-Dimethylcholesta-5,22*E*-dien-3β-ol	–	–	1.3	–	2.3	–	–	–	1.4	–	1.8	–
24-Ethylcholesta-5,22*E*-dien-3β-ol	**13.3**	**16.0**	3.0	**7.4**	2.1	4.3	**14.6**	**15.8**	3.7	**10.9**	2.6	6.2
4α, 24-Dimethylcholest-22*E*-en-3β-ol	–	–	–	–	tr	–	–	–	–	–	–	–
23,24-Dimethylcholest-5-en-3β-ol	–	–	–	–	tr	–	–	–	tr	–	–	–
24-Ethylcholest-5-en-3β-ol	**16.8**	**19.0**	**6.2**	**17.4**	6.8	**14.6**	**20.2**	**18.5**	**12.9**	**16.5**	**7.8**	**13.7**
24-Ethylcholesta-5,24(28)-dien-3β-ol	3.7	6.0	2.7	4.7	**10.5**	**18.2**	5.2	**9.8**	2.3	7.5	**9.7**	5.1
24-Propylcholesta-5,24(28)-dien-3β-ol	2.4	–	2.5	–	1.9	–	3.7	–	**7.7**	–	tr	–
4α,23,24-Trimethylcholest-22*E*-en-3β-ol	1.7	tr	5.7	6.3	3.6	5.3	1.7	3.6	3.1	2.5	1.6	3.4
4α,23R,24R-Trimethylcholestan-3β-ol	–	–	–	–	tr	–	–	–	tr	–	–	–
Total sterols (μg mg_{oc}^{-1})	1.7	0.2	4.9	0.2	8.0	0.1	1.2	0.2	4.5	0.3	6.5	0.4

[a] –, Denotes below detection limits.

[b] tr, denotes trace amounts (<1% of total FA).

[c] Boldface indicates sterol is amongst the five most abundant sterols (on a % basis) comprising the total distribution.

ranged from an average of 1.5 μg mg_{oc}^{-1} at the riverine end-member to 7.3 μg mg_{oc}^{-1} at the mouth of the York (Table 3). Sterol concentrations in the particulate fraction were 10–30% higher in March relative to October. Concentrations in the dissolved fraction were an order of magnitude lower than those measured in POM and ranged from 0.2 μg mg_{oc}^{-1} to 0.4 μg mg_{oc}^{-1}, with no consistent trends with salinity or between sampling times (Table 3).

Approximately 68–80% of the total sterol concentrations in both POM and HMW DOM were explained by five individual sterols characteristic of both autochthonous and allochthonous sources (Table 3). Two sterols indicative of autochthonous estuarine OM sources, cholest-5-en-3β-ol and 24-methylcholesta-5,22*E*-dien-3β-ol, dominated the POM across all sites and sampling periods. Additionally, 24-methylcholesta-5,24(28)-dien-3β-ol was a significant component of POM, except at the freshwater site in October (Table 3). 24-Nor-cholesta-5,22*E*-dien-3β-ol was prominent in POM at the mid-salinity and estuary mouth sites in March. In contrast to the mid and lower York, three sterols (24-ethylcholesta-5,22*E*-dien-3β-ol, 24-methylcholest-5-en-3β-ol and 24-ethylcholest-5-en-3β-ol) dominated riverine POM (Table 3).

The dominant sterols associated with HMW DOM showed both some general similarities as well as some significant differences relative to the POM (Table 3). The spatial and temporal patterns in the contribution of 24-nor-cholesta-5,22*E*-dien-3β-ol were similar in HMW DOM and POM. Overall, cholest-5-en-3β-ol, 24-methylcholesta-5,22*E*-dien-3β-ol and 24-methylcholesta-5,24(28)-dien-3β-ol were dominant, and of similar abundance, in most HMW DOM samples (Table 3). 24-Ethylcholesta-5,22*E*-dien-3β-ol, 24-methylcholest-5-en-3β-ol and 24-ethylcholest-5-en-3β-ol were enriched ($p < 0.005$) in the HMW DOM relative to POM, with the most pronounced differences at the mid and high salinity sites (Fig. 3F).

4. Discussion

4.1. Bulk parameters: Identification of OM sources

Organic matter in the York was differentiated using property–property plots, with riverine

($S = 0$) HMW DOM and estuarine ($S = 21$) POM representing the end-members (Fig. 2). Riverine HMW DOM was resolved from both POM and from other HMW DOM samples along the estuarine salinity gradient by its higher C:N ratio and seaward increase in $\delta^{13}C$ values, respectively (Table 1, Fig. 2). Furthermore, riverine POM and HMW DOM were distinguishable from other sites in the York River estuary by their significantly ($p < 0.001$) depleted $\delta^{13}C$ signatures, averaging $-26.4 \pm 0.6‰$ and $-28.0 \pm 0.2‰$, respectively (Fig. 2).

Concurrent measurements of $\delta^{15}N$ and C:N ratios helped to further resolve potential allochthonous and autochthonous sources to POM and HMW DOM (Table 1, Fig. 2). The C:N of fresh phytoplankton is generally ~6.7 (Redfield, 1958) while vascular plants are N-depleted (C/N = 20–500; Hedges et al., 1997). C:N showed a fairly strong, though not significant, inverse relationship to PhytoOC ($r^2 = 0.60$; $p < 0.1$) indicating that as the fraction of algal-derived OM to the total POM pool increased, C:N decreased, consistent with a greater relative contribution of algal-derived OM. C:N values in riverine HMW DOM (~17.4) were significantly higher than either mean C:N ratios for POM or HMW DOM collected in the mid and lower York. These N-depleted values may reflect either a greater allochthonous (vascular plant) signature or increased microbial reworking of the HMW DOM relative to POM. Microbially mediated diagenesis of OM generally results in increased C:N ratios due to preferential remineralization of N relative to C (Goldman et al., 1987; Hopkinson et al., 1997; McCallister, 2002). Alternatively, differences in the C:N of HMW DOM versus POM could reflect abiotic processes such as selective sorption of N to POM (Aufdenkampe et al., 2001).

In general, C:N and stable isotopic compositions are useful for distinguishing riverine POM and HMW DOM from estuarine and marine OM throughout the York River estuary. These findings suggest a greater terrigenous signature for HMW DOM than POM, consistent with previous findings in other temperate coastal systems (Moran et al., 1991; Mannino and Harvey, 2000). The $\delta^{13}C$ and $\delta^{15}N$ evidence, along with C:N, also suggest a possible small contribution of terrigenous vascular plant material to the POM and HMW DOM in the middle to lower York River estuary. This is perhaps not surprising given the relatively narrow dynamic ranges of these stable isotopes and overlapping $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures of potential sources. As a result, bulk stable isotope ratios provide limited ability to resolve potential source contributions compared with more sensitive indices (e.g., specific biomarker compounds; Canuel et al., 1995; Cloern et al., 2002).

4.2. Spatial and seasonal variations in OM sources using lipid and bulk tracers

Fatty acid and sterol biomarkers may allow for a more detailed examination of spatial and temporal variations in the sources comprising OM along an estuarine salinity continuum. Though several of the most abundant FA in this study (e.g., 14:0, 16:0, 16:1ω7 (Table 2)) may be ascribed to a mixed planktonic source (i.e., phytoplankton, zooplankton, bacteria), other less abundant FA provide greater source-specificity. For example, monounsaturated FA such as 18:1ω9 and C_{20} and C_{22} PUFA, are primarily derived from phytoplankton (Cranwell, 1982; Volkman, 1986; Killops and Killops, 1993).

The sum of C_{18}, C_{20} and C_{22} PUFA (% of total) associated with POM showed little variation along the estuarine salinity gradient in March (Fig. 3A) despite substantial changes in Chl *a* concentrations (Table 1). Although stable isotope values (Fig. 2), Chl *a* concentrations (Table 1) and previous bulk $\Delta^{14}C$-DOC (Raymond and Bauer, 2001) measurements suggest minimal algal inputs at the freshwater end-member of this estuary, ~30% of the total FA associated with POM were comprised of PUFA diagnostic for algal sources (Fig. 3A). The presence of algal biomarkers in the freshwater regions of the York (Figs. 3 and 4A) suggest that lipid biomarkers may be more sensitive than bulk OM characteristics that provide a weighted average measure of organic matter composition (e.g., stable and radio-isotopes, Chl *a*, C:N). The % PUFA distributions (Fig. 3A) suggest contributions from algal FA along the salinity continuum. However, when normalized to OC, abundances of PUFA were low indicating that the autochthonous signal at the freshwater end of the estuary is likely diluted by contributions from terrigenous (e.g., plant/soil/marsh) OM (Fig. 4A).

Consistent with the above interpretation, long-chain FA (LCFA; $\geqslant C_{24}$), predominately derived from terrigenous vascular plant inputs (Kolattukudy, 1980; Canuel and Martens, 1993), were higher at the riverine end-member (Fig. 3C) and were inversely related to salinity ($r^2 = 0.50$,

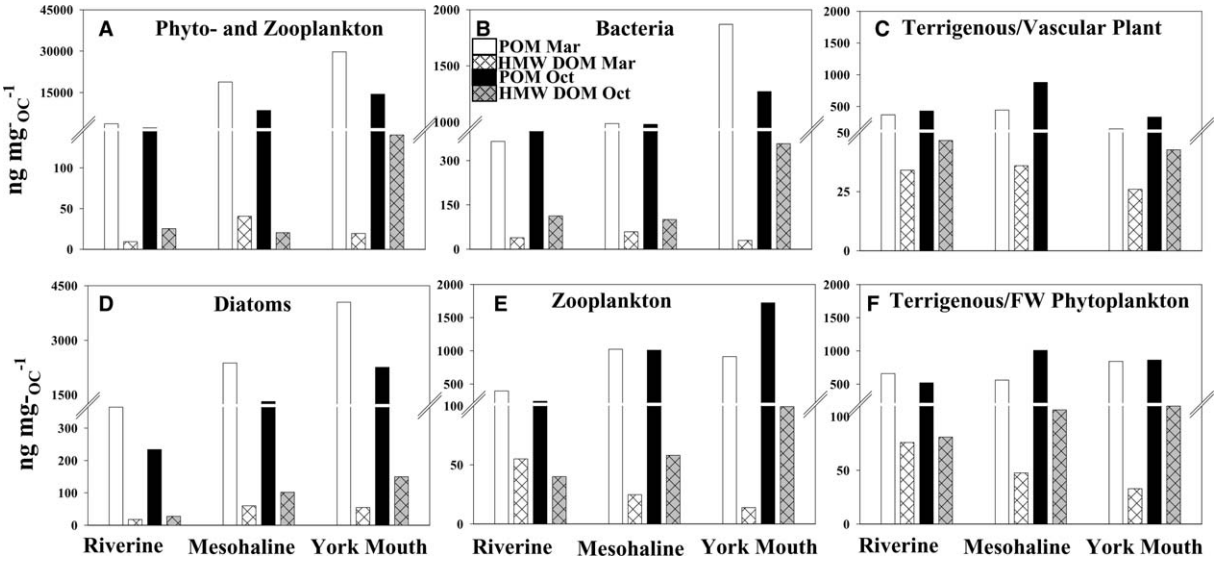

Fig. 4. Temporal and spatial variations in FA (ng mg_{oc}^{-1}) diagnostic for: (A) phytoplankton and zooplankton (polyunsaturated C_{18}, C_{20} and C_{22} FA), (B) bacterial (odd numbered C_{13}–C_{17}, branched and normal FA), and (C) terrigenous/vascular plant (even long chain FA (LCFA) C_{24}–C_{30}) sources; and sterols (ng mg_{oc}^{-1}) diagnostic for (D) diatom (24-nor-cholesta-5,22*E*-dien-3β-ol, 24-methylcholesta-5,24(28)-dien-3β-ol, 24-methylcholesta-5,22*E*-dien-3β-ol), (E) zooplankton (cholest-5-en-3β-ol), and (F) terrigenous OM and freshwater (FW) phytoplankton (24-ethylcholesta-5,22*E*-dien-3β-ol, 24-methylcholest-5-en-3β-ol, 24-ethylcholest-5-en-3β-ol) sources in the York River estuary.

$p < 0.01$). Flocculation and sedimentation likely provide a depositional sink for allochthonous OM in the upper estuary (Mannino and Harvey, 2000; Shi et al., 2001), thereby depleting the terrigenous signature in downstream OM (Fig. 3C). However, on a carbon-normalized basis, there was no consistent pattern with salinity, suggesting additional biological and/or physico-chemical controls on terrigenous OM distributions (Fig. 4C). For example, the mid-salinity region of the York River is characterized by seabed mixing and high levels of resuspension of bed sediments (Dellapenna et al., 1998; Arzayus and Canuel, 2005), providing potential sources of terrigenous OM to the overlying water column (Fig. 4C).

Sterols derived mainly from diatoms (24-nor-cholesta-5,22*E*-dien-3β-ol, 24-methylcholesta-5,22*E*-dien-3β-ol and 24-methylcholesta-5,24(28)-dien-3β-ol (Kates et al., 1978; Volkman, 1986)) comprised 30–50% of total sterols in the mid-salinity region and mouth of the York with maximum values coincident with the chlorophyll maximum (Fig. 3D). Conversely, concentrations of these algal sterols in riverine OM were significantly lower than at the more saline sites ($p < 0.005$) by approximately 50% (Fig. 3D). As a group, diatom sterols (%) in both particulate and dissolved OM showed a significant positive correlation with salinity ($r^2 = 0.58$, $p < 0.005$; Fig. 3D), consistent with higher phytoplankton production in these regions of the estuary (Sin and Wetzel, 2002).

Three sterols normally associated with vascular plants, (24-ethylcholesta-5,22*E*-dien-3β-ol (stigmasterol), 24-methylcholest-5-en-3β-ol (campesterol) and 24-ethylcholest-5-en-3β-ol (Nichols et al., 1982; Volkman, 1986)) were used as tracers for terrigenous plant OM. However, assignment of these sterols exclusively to terrigenous vascular plant material is compromised by their presence in some, particularly freshwater, phytoplankton (Volkman, 1986; Volkman et al., 1998). Thus, the sum of stigmasterol, campesterol and 24-ethylcholest-5-en-3β-ol is attributed to a combined vascular plant/riverine algal source, which although not strictly terrigenous, is nonetheless unique to riverine OM. The combined inventories of these sterols accounted for ~40% of total sterols at the head of the estuary and were significantly and negatively correlated with salinity ($r^2 = 0.66$; $p < 0.001$; Fig. 3F) with pronounced enrichments in the Pamunkey River compared to the mid and lower estuary. Similar to other FA diagnostic of terrigenous biomarkers (LCFA ($\geqslant C_{24}$); Fig. 4C), terrigenous sterols when normalized to OC do not change along the estuarine salinity continuum in a consistent manner (Fig. 4F). Instead, positive excursions from conservative mix-

ing suggest an additional source of terrigneous OM to POM and HMW DOM pools (Fig. 4F).

One plausible source of this terrigneously derived OM to the water column is the resuspension of sediments which likely have a higher concentration of terrestrial OM relative to water column POC (Shi et al., 2001; Arzayus and Canuel, 2005). Further, physical resuspension of sediments may produce abrupt physico-chemical gradients and thus initiate extensive exchange between POM and DOM (Aufdenkampe et al., 2001; Komada and Reimers, 2001) with terrigenous sources preferentially solubilized (Bianchi et al., 1997; Mitra et al., 2000; Mannino and Harvey, 2000). Although resuspension may promote bacterial decomposition of terrigenous OM, previous studies in the York suggest little contribution of terrigenously derived OM to bacterial biomass production in the mid and lower York (McCallister et al., 2004). Thus, sediment-derived subsidies from resuspension may help to preserve the observed allochthonous signature in the HMW DOM throughout estuarine transit (Fig. 4F).

FA typically ascribed to bacterial sources include *iso*- and *anteiso*-branched FA and odd numbered saturated FA (C_{15}–C_{17}) (Parkes and Taylor, 1983; Kaneda, 1991). Higher concentrations of Br–FA in the dissolved phase were measured in October and corresponded to warmer (~7 °C) water column temperatures (Table 1, Figs. 3 and 4B). In the POM, bacterial FA normalized to OC showed a strong positive correlation with salinity ($r^2 = 0.85$; $p < 0.01$; Fig. 4B) consistent with previous studies showing an increase in bacterial biomass down-estuary (Schultz et al., 2003). Br–FA in the HMW DOM fraction showed no relationships with salinity (Fig. 4B) despite evidence for strong correlations between salinity and bacterial abundance and production, in previous studies (Schultz et al., 2003). The refractory nature of bacterial membrane components (Mannino and Harvey, 1999; Wakeham et al., 2003; Zou et al., 2004) may contribute to the accumulation of Br–FA in the dissolved phase (Figs. 3B, 4B) and thus reflect patterns in DOC processing that do not necessarily vary as a function of salinity (McCallister et al., 2006).

The sterol composition of crustaceans, and zooplankton in particular, is usually dominated by cholesterol (cholest-5-en-3β-ol) (Volkman, 1986; Killops and Killops, 1993). However, cholesterol can be present in low levels in microalgae as well as aquatic and terrigenous plants (Volkman, 1986; Canuel and Martens, 1993). Cholesterol may also have an anthropogenic source but this is unlikely in the York River given the absence, or low abundances, of sewage sterols such as coprostanol (5β-cholestan-3β-ol) (Table 3). In this study, cholesterol comprised a substantial (~20 ± 5.8%) portion of total sterols in both POM and HMW DOM at all locations (Fig. 3E). Cholesterol concentrations normalized to OC in both the HMW DOM and POM had a positive relationship with salinity ($r^2 = 0.72$; $p < 0.05$; Fig. 4E). A significant increase in relative cholesterol concentrations (% of total) was observed in October at the high salinity site indicating a greater impact on OM composition by zooplankton, thereby suggesting the potential importance of heterotrophic processing of OM in this region of the estuary during summer/fall (Fig. 3E).

In summary, the contributions of diatom and higher plant lipids varied inversely along the estuarine salinity gradient. Based on stable isotope analysis, FA, and sterol distributions, OM in the mid- and high-salinity regions of the estuary displayed a more pronounced algal (diatom) signature while the riverine end-member reflected the importance of both higher plants and algal sources other than diatoms. In addition, the mid-estuary exhibited a strong autochthonous signature with contributions from a mixed assemblage of phytoplankton and heterotrophs such as bacteria and zooplankton.

4.3. Contrasting composition of estuarine POM and DOM

Previous studies have compared the lipid compositions of dissolved (variable MW cut-offs) versus particulate OM pools using liquid:liquid extraction of dissolved lipids into an organic solvent (Kattner et al., 1983; Jaffé et al., 1995). In general, a predominance of saturated C_{16} and C_{18} was found in the dissolved phase using both ultrafiltration (Table 2 and Mannino and Harvey, 1999) and liquid:liquid extraction (Berdie et al., 1995; Jaffé et al., 1995) methodologies. However, some studies have reported a greater proportion of unsaturated FA (Jaffé et al., 1995; Thoumelin et al., 1997) in certain systems. Despite dramatic changes in ionic strength in estuarine environments, Harvey and Mannino (2001) found that FA composition of DOM was similar across the size distribution of HMW DOM isolated by tangential-flow ultrafiltration. Nevertheless, given that physical processes likely influence the partitioning of organic constituents between the HMW and truly dissolved phases, future studies

examining the molecular composition of different size classes would benefit from a direct comparison of the ultrafiltration and liquid:liquid extraction methods.

As a means of illustrating the principal sources comprising POM and HMW DOM in a temperate coastal plain estuary such as the York, ternary plots were constructed for POM and HMW DOM, with each normalized to the ranges (minimum and maximum) in biomarker concentrations representing potential sources (e.g., PUFA for "fresh" phytoplankton/zooplankton; branched FA for bacteria; and LCFA ($\geqslant C_{24}$) for terrigenous sources) (Fig. 5). Compositional dissimilarities between POM and HMW DOM components (displayed as distance along the sides of the triangles in Fig. 5) reflect the dominance of different OM sources in each.

The POM was characterized by PUFA derived primarily from a "fresh" autochthonous source (phytoplankton/zooplankton) with a stronger signal during March, coincident with elevated Chl a concentrations (Table 1), and the spring bloom (Fig. 5A). In contrast, HMW DOM was comprised of FA derived from mixed bacterial and terrigenous origin (Fig. 5A and B) and had, at most, trace levels of PUFA. Riverine POM and HMW DOM consistently displayed a greater contribution of LCFA (terrigenous; plant), with contributions decreasing seaward. The distinct separations in the sources of both POM and HMW DOM along the estuarine transect were retained during low flow conditions in October 2000 (Fig. 5B). However, increased flushing of the estuary in March may have homogenized the composition of terrigenous POM and HMW DOM (Fig. 5A). In March, HMW DOM had a ~50:50 mix of bacterial and terrigenous (plant) FA while POM was influenced by biomarkers representing "fresh" plankton (i.e., PUFA) (Fig. 5A) likely reflecting the effects of flow on HMW DOM and the spring bloom on POM.

The ternary plots reveal two distinct features of the HMW DOM relative to POM: (1) a highly depleted signature of a "fresh" algal component (PUFA) in HMW DOM and (2) an enrichment of bacterial and terrigenous compounds in HMW DOM. These biochemical compositional differences (Fig. 5) between the size fractions reflect not only variations in source inputs but also subsequent abiotic and biotic processing (Loh et al., in press).

The autochthonous signature of HMW DOM is distinct from POM, with PUFA enriched in the POM and low or absent from the HMW DOM (Figs. 4, 5). Zou et al. (2004) attributed differences in the composition of POM and DOM to limited exchange between the pools. An alternative interpretation, supported by work conducted concurrently with this study (McCallister et al., 2004, 2006), is that bacterial processing is an important removal term for this autochthonous DOM, thereby placing a strong regulatory constraint on its accumulation. The radiocarbon ($\Delta^{14}C$) and stable isotopic ($\delta^{13}C$) values of bacterial nucleic acids (extracted from the 0.2–0.7 μm size collected in this study) suggest that autochthonous algal-derived OM comprised up to 25–90% of the OM fueling bacterial biomass production along the estuarine salinity gradient (McCallister et al., 2004). Further, McCallister et al. (2006) concluded that while the geochemical signature of "fresh" algal-derived OM in the dissolved phase (i.e., PUFA) may appear quantitatively insignificant

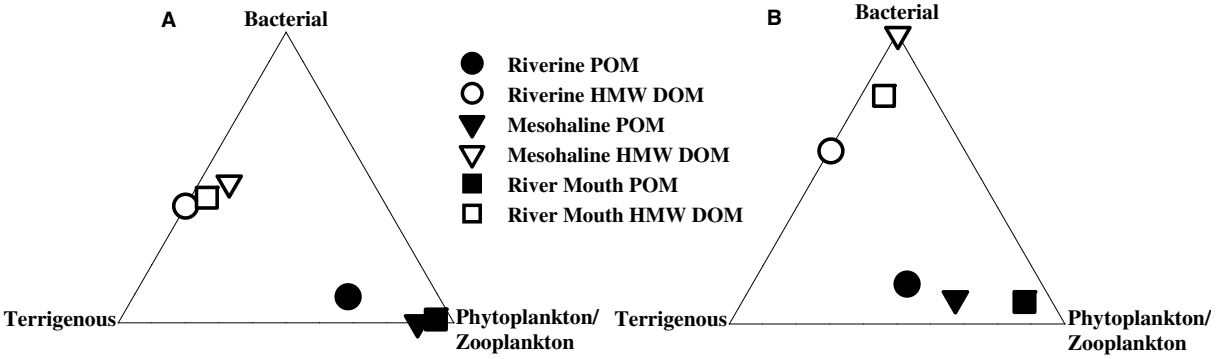

Fig. 5. Source classifications of FA (% of total) for POM and HMW DOM in the York River estuary in March (A) and October (B) 2000. Sources are classified as follows: phytoplankton and zooplankton (polyunsaturated C_{18}, C_{20} and C_{22} FA); bacterial (odd numbered C_{13}–C_{17}, branched and normal FA) and terrigenous/vascular plant (even long chain FA (LCFA) C_{24}–C_{30}). Distances calculated are relative to the maximum and minimum concentration for each OM source.

(Figs. 3A and 5), these compounds may represent a principal source of bioreactive OM to heterotrophic bacteria in estuarine waters.

The predominance of short-chain (C_{14}–C_{18}) saturated and Br–FA in the HMW DOM (Fig. 5) suggests OM processing by bacteria may influence the lipid signature in the HMW DOM through either bacterially repackaged (i.e., refractory) OM (Brophy and Carlson, 1989; Ogawa et al., 2001) or the direct transfer of bacterial membrane components (Mannino and Harvey, 1999; Wakeham et al., 2003; Zou et al., 2004) to the dissolved pool. Thus while bacteria may be a strong sink for algal-derived OM on one hand, they may simultaneously be a significant source of less labile, more microbially reworked DOM on the other and, as a result, exert a biological control on the compositional nature of the HMW DOM.

Photolytic processing provides both a significant direct (photomineralization) and/or indirect (increased bioreactivity) sink for terrigenous DOM in many nearshore aquatic systems (Moran and Covert, 2003). In the York, however, the direct abiotic loss of DOC (presumably terrigenous) as a result of photolysis was undetectable (McCallister et al., 2005). Further, although photochemical modifications may have enhanced the bacterial consumption of DOC (McCallister et al., 2005), terrigenously derived DOM remained of nominal importance to bacterial biomass production in the mid and lower York River estuary (McCallister et al., 2004).

Collectively our data suggest that the preferential transformation and utilization of algal DOM by bacteria may explain the absence of PUFA and the high levels of short-chain saturated FA in the HMW DOM and the persistence of a terrigenous signature in this OM fraction along the estuarine salinity gradient (Fig. 5). Thus, varying degrees of susceptibility of terrigenous POM and HMW DOM to physico-chemical and biogeochemical processes may ultimately regulate the persistence of terrigenous-derived OM within an estuary and consequently determine the relocation of river and/or estuarine OM to regions of net heterotrophy (Smith and Hollibaugh, 1993; del Giorgio and Duarte, 2002; Ducklow and McCallister, 2004). Furthermore, we suggest that the signature of bacterial processing of DOM along the estuarine salinity gradient is reflected both by the removal of biomarkers of ''fresh'' algal OM as well as contributions from bacterial biomarker compounds.

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