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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

Abstract—High-molecular-weight dissolved organic matter (HMW-DOM, > 1,000 Daltons) is actively involved in the global biogeochemical cycling of many elements, but its carbon sources and detailed formation pathways are still not well understood. In this study, we measured bulk stable carbon and nitrogen isotopic ratios, lipid composition, and compound-specific carbon isotopic ratios of HMW-DOM samples collected from four U.S. estuaries (Boston Harbor/Massachusetts Bay, Delaware/Chesapeake Bay, San Diego Bay, and San Francisco Bay). Analytical results show (1) a fraction of HMW-DOM (lipid associated) in estuarine and coastal waters is derived from bacteria and phytoplankton; (2) this fraction of HMW-DOM is formed by various release processes of bacterial membrane components and bacterial reworking of phytoplankton-derived material; (3) this fraction of HMW-DOM is generally present in all samples from different coastal systems despite variable organic matter inputs and environmental conditions, suggesting an important bacterial role in HMW-DOM formation.

Dissolved organic matter (DOM) represents one of the largest reservoirs of organic carbon in natural waters, but its composition is the least well characterized (Ogawa et al. 2001). It is widely recognized that DOM plays an important role in the global carbon cycle; however, the bioreactivity of DOM depends on its molecular size and origin (Amon and Benner 1992). In aquatic systems, high-molecular-weight DOM (HMW-DOM) accounts for a significant fraction (20–35%) of the total DOM pool and controls the cycling of many particle-reactive elements such as Th and Cu due to its high specific surface area and complexation capacity (Benner et al. 1992; Guo and Santschi 1997). Previous studies conducted in the open ocean suggest that HMW-DOM appears to be derived mainly from direct exudation by phytoplankton (Biddanda and Benner 1997). A great deal of controversy still exists concerning the carbon sources and formation pathways of HMW-DOM in coastal waters, where organic carbon inputs come from multiple sources (Mannino and Harvey 1999; Mitra et al. 2000; Benner and Opsahl 2001; Repeta et al. 2002).

Organic carbon sources in estuarine and coastal waters include in situ phytoplankton production and grazer input, terrestrial organic matter from river and groundwater inputs, runoff from land, atmospheric deposition, direct anthropogenic input such as sewage, and organic material from the ocean. HMW-DOM represents a unique organic carbon pool and its chemical composition differs distinctly from those of

particulate organic matter (POM) and low-molecular-weight dissolved organic matter (LMW-DOM, <1,000 Daltons) (Harvey and Mannino 2001). Lipids have been used widely as source biomarkers of organic matter in various aquatic and sedimentary environments (Wakeham and Beier 1991; Volkman et al. 1998) and likewise have the potential to provide information concerning sources and formation pathways of HMW-DOM when their isotopic compositions are combined.

Materials and methods—To explore the organic carbon source and formation pathway of HMW-DOM, bulk stable carbon and nitrogen isotopic ratios, lipid composition, and compound-specific stable isotopic ratios ($\delta^{13}\text{C}$) were determined for samples collected from four U.S. estuarine and coastal areas, including Boston Harbor/Massachusetts Bay, Delaware/Chesapeake Bay, San Diego Bay, and San Francisco Bay (Fig. 1, Table 1). Sampling was conducted during July and August 1998 on the east coast of the United States and during January and June 1999 on the west coast. Samples from 10 sampling locations representing a wide range of salinity (0.2–33.9 psu) were chosen for this study (Table 1). Surface water (100–200 liters; 2–5 m) was collected through a Teflon tube using a Grundfos stainless-steel submersible pump and filtered inline through prebaked GF/F (0.7 μm) and acid-cleaned 0.2- μm Gelman polycarbonate filters to remove particles and bacteria. HMW-DOM was isolated and concentrated through tangential-flow ultrafiltration using an Amicon DC-10L system fitted with two 1-kD, polysulfone, spiral-wound cartridges. Samples were desalted shipboard and frozen immediately for transport.

After subsequent freeze drying, samples were analyzed for bulk isotopic composition and C/N ratio (Table 1). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of bulk HMW-DOM were analyzed using an automated elemental-analyzer coupled to a Finnigan MAT 251 isotope ratio mass spectrometer in continuous-flow mode at University of Massachusetts at Dartmouth. For these analyses, acidified HMW-DOM samples were placed on precombusted (500°C for 6 h) 25 mm GF/F filters. The filters were then vacuum dried and analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Standardization was conducted both by combustion of solid materials of known isotopic composition and by injections of standard gases into the carrier gas (He). Reproducibility for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was better than $\pm 0.15\%$. Total organic carbon (TOC) and total nitrogen (TN) contents of samples were analyzed using a Perkin-Elmer 2400 CHN analyzer.

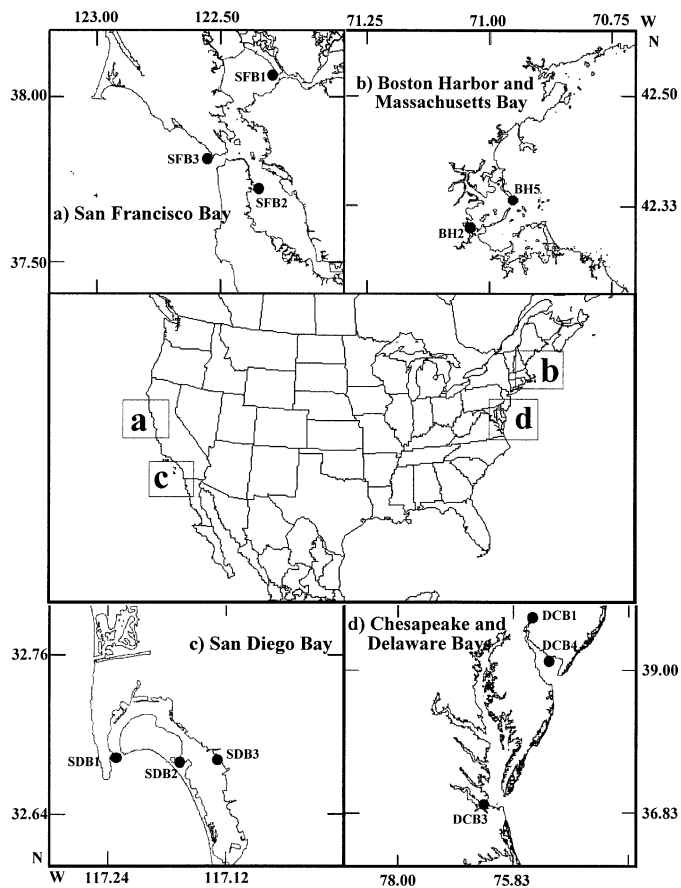


Fig. 1. Map of the sampling locations in U.S. coasts: (a) San Francisco Bay, (b) Boston Harbor/Massachusetts Bay, (c) San Diego Bay, and (d) Chesapeake/Delaware Bays.

Approximately 20–30 mg of dried HMW-DOC sample was ultrasonically extracted with organic solvents (methanol and methylene chloride) for lipid and molecular isotopic ratio measurements. Extracted lipids were saponified and then separated into neutral and acid fractions by further extraction under different pH conditions. Neutral lipids were treated with BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) in acetonitrile to form trimethylsilyl-ethers and fatty acids were methylated with 5% BF₃-MeOH to form fatty acid methyl esters (FAMES). The lipid compounds were analyzed using a Hewlett Packard-6890 capillary gas chromatograph with an on-column injector and a flame ionization detector. Separations of lipids were achieved with a 30-m × 0.25-mm-inner diameter column coated with 5% phenyl methyl silicone (HP-5, Hewlett-Packard). Operation temperature was programmed as 50–150°C at 20°C min⁻¹, followed by 150–310°C at 4°C min⁻¹, and held at 310°C for 5 min. Hydrogen was used as carrier gas and nitrogen as make-up gas. Internal standards (α (H)-cholestane for neutral lipids and nonadecanoic acid methyl ester for FAMES) were added to samples immediately prior to gas chromatography (GC) analysis to aid in quantification. Selected samples were analyzed with a Shimadzu QP5000 gas chromatograph-mass spectrometer for identification. The GC-MS system used a 30-m × 0.25-mm-inner diameter column coated with 5% phenyl methyl silicone (XTI-5, Restek), and helium was used as carrier gas. Operating conditions were as follows: mass range 50–610 amu with a 0.4-s scan interval; 70 eV ionizing energy; GC temperature gradient was the same as that described for GC quantification. Lipid molecular carbon isotopic ratios were determined using a GC-combustion system connected to an isotope ratio mass spectrometer (Finnigan MAT 252 IRMS). Compounds eluting from the GC column were combusted to CO₂ over CuO/Pt wires at 850°C. The mass spectrometer was operated at 10-kV acceleration potential and by magnetic sector mass separation. Carbon isotope ratios were expressed relative to supercritical fluid

Table 1. Sampling locations and bulk geochemical parameters (salinity, C/N ratio, bulk carbon and nitrogen isotopic ratio, and total fatty acid concentration).

Sta.	Date	Latitude (N)	Longitude (W)	Salinity	C/N (mole)	$\delta^{13}\text{C-TOM}$ (‰)	$\delta^{15}\text{N-TOM}$ (‰)	Total fatty acids ($\mu\text{g mg}^{-1}$)
Boston Harbor/Massachusetts Bay								
BH2	Jul 98	42°17.14'	71°02.26'	25.0	13.0	-25.7	3.27	1.27
BH5	Jul 98	42°20.38'	70°57.35'	30.3	10.9	-24.3	2.86	3.79
Delaware/Chesapeake Bay								
DCB1	Aug 98	39°48.21'	75°24.53'	0.2	16.1	-24.8	4.40	1.05
DCB3	Aug 98	36°59.28'	76°19.79'	20.0	11.7	-24.5	8.92	3.62
DCB4	Aug 98	39°04'	75°16'	26.0	11.4	-23.1	5.98	2.81
San Diego Bay								
SDB2	Jan 99	32°40.81'	117°10.39'	33.9	9.1	-22.2	6.01	1.20
SDB3	Jan 99	32°41.27'	117°07.88'	33.9	10.9	-25.1	5.27	1.65
San Francisco Bay								
SFB1	Jun 99	38°06'	122°30'	17.1	15.5	-26.1	5.10	4.82
SFB2	Jun 99	37°73'	122°35'	28.5	12.9	-23.1	6.35	3.68
SFB3	Jun 99	37°81'	122°51'	32.3	7.9	-27.8	4.00	0.94

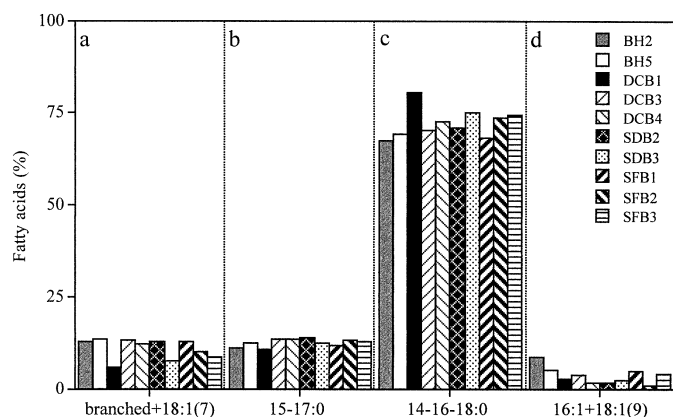


Fig. 2. Fatty acid composition of HMW-DOM samples collected from 10 sites in four estuarine and coastal areas: (a) the sum of odd-number branched 15:0 and 17:0 (iso- and anteiso-) and 18:1 ω 7 fatty acids, (b) the sum of odd-number saturated 15:0 and 17:0 fatty acids, (c) the sum of even-number saturated C₁₄–C₁₈ fatty acids, and (d) the sum of two monounsaturated 16:1 ω 7 and 18:1 ω 9 fatty acids.

chromatography (SFC) CO₂ (99.999%) standard ($\delta^{13}\text{C} = -10.36\text{‰}$).

Results and discussion—Lipid analysis showed that few neutral lipids were present in the HMW-DOM samples. The majority of the lipids measured were fatty acids, dominated by even carbon number, saturated C₁₄–C₁₈ fatty acids (68–80% of total), followed by bacteria-specific, odd carbon number, C₁₅–C₁₇ (branched and normal) plus 18:1 ω 7 fatty acids (17–27%). Monounsaturated, 16:1 ω 7 and 18:1 ω 9 fatty acids accounted for 2–8%, and neither polyunsaturated nor long-chain (>C₂₀) saturated fatty acids were found in these HMW-DOM samples (Fig. 2). Fatty acid compositions of the samples in different locations appeared to be quite similar. Total fatty acid concentrations in these samples were in the range of 1–5 $\mu\text{g mg}^{-1}$ dry weight HMW-DOM (Table 1), which accounted for less than 2% of TOC. Stable carbon isotopic ratios of bacteria-specific fatty acids (branched, odd-number saturated C₁₅–C₁₇ plus 18:1 ω 7) in these samples averaged $-24.88 \pm 1.45\text{‰}$ (solid lines in Fig. 3a,b). For saturated even-number fatty acids, the stable carbon isotopic ratios of 14:0 were $-24.95 \pm 0.4\text{‰}$, while 16:0 and 18:0 had slightly heavier isotopic ratios of $-22.97 \pm 0.4\text{‰}$ and $23.43 \pm 0.65\text{‰}$ in all HMW-DOM samples (Fig. 3c). The isotopic ratios of the monounsaturated 16:1 ω 7 and 18:1 ω 9 fatty acids were more variable, with values ranging from -20‰ to -28‰ (Fig. 3d).

There are similarities and differences between our lipid composition results and previously reported data (Mannino and Harvey 1999) for the samples taken from the same area (Delaware Estuary) but at different times. A major similarity is the predominance of short-chain even-number fatty acids (16:0 and 18:0) in the lipid composition. By contrast, small amounts of long-chain (>C₂₀) saturated and polyunsaturated fatty acids (absent in our samples) were observed in their HMW-DOM samples but only in a few sites. Bacteria-specific fatty acids (branched and normal, odd number) were

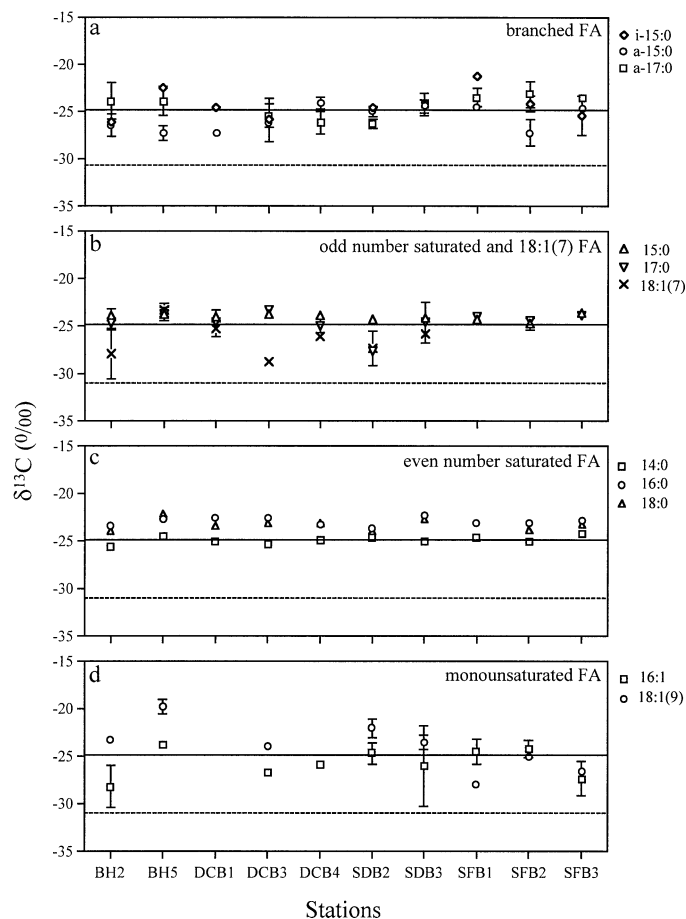


Fig. 3. Lipid molecular isotopic ratios of HMW-DOM samples: (a) isotopic ratios for branched odd number fatty acids (iso- and anteiso-15:0 and anteiso-17:0), (b) isotopic ratios for odd-number saturated fatty acids (15:0 and 17:0) and 18:1 ω 7, (c) isotopic ratios for even-number saturated C₁₄–C₁₈ fatty acids, and (d) isotopic ratios for monounsaturated 16:1 ω 7 and 18:1 ω 9 fatty acids. The solid line (-24.88‰) is the average isotopic ratios of all bacteria-specific fatty acids, which is close to the assumed isotopic ratios (-25‰) of bacteria-specific fatty acids utilizing marine substrates (-20‰). The dashed line (-31‰) represents the assumed isotopic ratios of bacteria-specific fatty acids utilizing terrestrial substrates (-26‰). The average fractionation between substrates and bacteria-specific fatty acids is assumed to be -5‰ . Duplicate measurements of isotopic ratios indicated that the error range was dependent on the relative concentration of individual fatty acids. More abundant even-number saturated C₁₄–C₁₈ fatty acids had very small error ranges (error bars generally less than the symbols) while other fatty acids with low concentration had relatively larger error ranges.

abundantly present in all of our samples but these fatty acids were found in significant amounts only at their sites where chlorophyll *a* was at a maximum. It is not clear what factors cause these discrepancies in lipid composition of the samples from the same area. Variability is possibly related to sampling location and time, sampling method, river discharge rate, local primary production, and the interaction between phytoplankton and bacterial community.

In general, long-chain (>C₂₀) saturated fatty acids are associated with vascular plants and are thought to originate

from terrestrial sources (Cranwell 1982). Our fatty acid compositional analysis for all samples showed no occurrence of long-chain ($>C_{20}$) fatty acids in the HMW-DOM (detection limit $< 0.01 \mu\text{g mg}^{-1}$), providing no evidence for the direct transfer of vascular plant material into the HMW-DOM pool. However, lignin-derived phenols (biomarkers of terrestrial sources) have been observed at $\sim 0.3\text{--}1.7 \mu\text{g g}^{-1}$ OC in HMW-DOM samples collected from Chesapeake Bay and Middle Atlantic Bight waters (Mitra et al. 2000). Concentrations of lignin phenol in HMW-DOM were observed to dramatically decrease from river to coastal waters (Mannino and Harvey 2000). Two processes are responsible for the depletion of terrestrially derived lignin phenols in HMW-DOM during transport from land to ocean: (1) loss due to flocculation in low salinity waters and (2) removal due to photooxidation in higher salinity waters (Benner and Opsahl 2001). The low levels and rapid removal of lignin-derived phenols in coastal waters, along with the absence of long-chain fatty acids in the HMW-DOM, imply that vascular plant contribution to the HMW-DOM pool is greatly reduced from river to coast (Mannino and Harvey 2000).

The absence of polyunsaturated fatty acids (PUFAs) in our HMW-DOM samples also suggests that phytoplankton material was not directly transferred into this pool. It is well known that various phytoplankton species produce a variety of PUFAs, such as 18:4, 20:4, 20:5, and 22:6 in their biomass (Volkman et al. 1989), and abundant PUFAs have been widely observed in large sinking and small suspended particles (Wakeham and Beier 1991). Phytoplankton also produce abundant monounsaturated fatty acids (MUFAs), and the relatively high ratio of 16:1 ω 7 to 16:0 is often used as an algal input index (Volkman et al. 1989). A small portion of MUFAs was found in our HMW-DOC samples relative to that normally found in phytoplankton biomass. Previous studies have demonstrated that unsaturated fatty acids, especially PUFAs, are rapidly and preferentially degraded compared with their saturated counterparts (Sun and Wakeham 1994). Based on the absence of PUFAs and low concentrations of MUFAs in the current HMW-DOM samples, it appears that part of HMW-DOM is derived from largely degraded phytoplanktonic organic matter. Consistent with this hypothesis, recent studies have confirmed that HMW-DOM is less labile than particulate organic matter (POM) but more reactive than LMW-DOM (Amon and Benner 1992; Mannino and Harvey 1999; Guo et al. 2003).

A remarkable feature of the fatty acid composition in our HMW-DOM samples is the abundant occurrence of bacteria-specific fatty acids. Bacteria specifically synthesize a series of odd-number $C_{15}\text{--}C_{17}$ (branched and normal) and also 18:1 ω 7 fatty acids, which are associated with membrane phospholipid material (Kaneda 1991). It has been recognized that bacteria are an important contributor to the DOM pool (Azam 1998). Bacteria rapidly utilize labile compounds and produce refractory DOM that resists further degradation (Ogawa et al. 2001). Likewise, a substantial fraction of dissolved organic nitrogen in the sea is derived from bacteria (McCarthy et al. 1998). Bacteria release their cellular components into the DOM pool through several different path-

ways: direct release from bacterial capsular material (Stoderegger and Herndl 1998), viral lysis of free-living and particle-associated bacteria (Fuhrman 1999), and heterotrophic grazing of flagellates on bacteria (Nagata and Kirchman 1992). The released DOM may contain bacterial membrane components, which enrich lipid macromolecules and form liposome structures (Borch and Kirchman 1999). It seems likely that these membrane materials and liposome structures readily enter the HMW-DOM pool. It was found that phospholipids were an important component of the HMW-DOM (10,000 Daltons to $0.45 \mu\text{m}$) lipid fraction (Liu et al. 1998). Hydroxy fatty acids were observed in HMW-DOM samples collected from the equatorial Pacific Ocean, Gulf of Mexico, and North Sea, indicating that bacterial membrane-derived lipid material was a contributor of organic carbon to the DOM pool (Wakeham et al. 2003). Our analytical results also provide a direct evidence that bacteria-derived organic carbon represents a unique fraction of HMW-DOM in coastal waters.

Based on the above discussion, it appears that a fraction of the HMW-DOM (lipid associated) is derived from bacterial and degraded phytoplankton materials. Analysis of lipid molecular isotopic ratios further supports this conclusion and also provides some insight into the formation pathway of this HMW-DOM fraction in coastal waters. Terrestrial vascular plants and marine phytoplankton produce organic carbon with distinct isotopic ratios, which is the basis for distinguishing organic carbon sources in coastal systems. Bulk $\delta^{13}\text{C}$ of DOM derived from marine phytoplankton is generally heavier ($\sim -20\text{‰}$) than that of terrestrial origin ($\sim -26\text{‰}$) (Peterson et al. 1994). Laboratory and field experiments have shown that $\delta^{13}\text{C}$ ratios of bacteria-specific fatty acids are depleted by approximately 3–6‰ relative to that of the substrate used (Canuel et al. 1997; Boschker et al. 1999). In our study, most $\delta^{13}\text{C}$ ratios measured for bacteria-specific fatty acids in the HMW-DOM were close to -25‰ (Fig. 3a,b), clearly indicating that phytoplankton-derived (marine) organic compounds ($\sim -20\text{‰}$) are the most preferred substrates for bacterial growth, although terrestrial organic substrates are also available in coastal waters.

Short-chain saturated fatty acids, especially 16:0 (most abundant lipid component), have a universal origin so that the 16:0 fatty acids in HMW-DOM may come from any of the various potential sources in coastal waters. However, terrestrial vascular plants have been found to have a depleted $\delta^{13}\text{C}$ ratio ($< -30\text{‰}$) for their 16:0 fatty acid (Canuel et al. 1997). The $\delta^{13}\text{C}$ ratios of 16:0 fatty acids in our samples were in the range of $-22.97 \pm 0.4\text{‰}$, which is most likely a consequence of mixing isotopic signals from bacteria and phytoplankton detritus, but clearly cannot be derived from a significant contribution from vascular plants. Therefore, analytical results of lipid composition and compound-specific stable carbon isotopic ratios suggest two specific formation pathways of HMW-DOM in coastal waters: (1) the transfer of largely degraded phytoplankton detritus, reworked by bacteria where labile PUFA is thus completely degraded and MUFA is greatly reduced during these reworking processes; and (2) the transfer of bacterial membrane components,

which are released directly by various biochemical processes mentioned above. However, it should be noted that these two pathways are only responsible for the formation of one fraction of HMW-DOM (lipid associated).

Because total lipids accounted for less than 2% of the total carbon in these HMW-DOM samples, the significance of the formation pathways needs to be clarified by analyses of stable isotopic compositions of bulk carbon and nitrogen. Values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measured for the bulk HMW-DOM ranged from -22.2‰ to -27.8‰ and 2.86‰ to 8.92‰ , respectively (Table 1). In general, these $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are lighter than those reported for the HMW-DOM collected from the open ocean sites such as Pacific and Atlantic surface waters ($\delta^{13}\text{C} = -21.3$ to -22.2‰ ; $\delta^{15}\text{N} = 6.6$ to 9.7‰) (Benner et al. 1997) but are quite comparable with the values measured for HMW-DOM collected from many coastal regions such as Chesapeake Bay and Middle Atlantic Bight (Guo et al. 2003), the Mississippi River plume (Benner et al. 1992), and Galveston Bay in the Gulf of Mexico (Guo et al. 2003). The lighter bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values measured in this study likely reflect the influence of organic carbon sources from terrestrial and local organic inputs. The incorporation of organic carbon and nitrogen into the HMW-DOM pool is generally considered to be through two major pathways: (1) entrance of dissolved organic matter, including terrestrial and anthropogenic inputs, by adsorption, condensation, humification, and abiotical aggregation (Sigleo and Means 1990; Opsahl and Benner 1997; Mitra et al. 2000; Kerner et al. 2003); and (2) transfer of labile particulate organic matter by microbial reworking and release (Rochelle-Newall and Fisher 2002). These two processes represent two very different formation pathways and could regulate isotopic signatures of bulk HMW-DOM by their relative roles. At the molecular level, our lipid isotopic analysis confirms the second pathway while the bulk isotope measurement implies that the first pathway is also important for formation of HMW-DOM in estuarine and coastal waters.

High similarity in chemical composition of HMW-DOM has been observed in surface seawaters collected from geographically diverse sites (the Atlantic and Pacific Oceans) (Aluwihare et al. 1997) and even in a suite of lakes, rivers, seawater, and marine sediment interstitial waters (Repeta et al. 2002). Despite the diversity of coastal regions studied and the wide range of salinities in different sampling locations, the lipid composition and associated molecular isotopic ratios in these HMW-DOM samples were remarkably consistent. These observations suggest that part of HMW-DOM (lipid associated) in all these systems is formed through similar pathways: release of bacterial membrane components and reworking of phytoplankton material. These findings also support one idea that bacteria play an important role in formation of HMW-DOM from labile particulate organic matter in diverse coastal waters, although other formation pathways such as aggregation from various dissolved components may also be important. Increased knowledge concerning carbon sources and formation pathways of HMW-DOM in natural waters may help us understand the active role of this unique carbon pool in carbon cycling.

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References

- ALUWIHARE, L. I., D. J. REPETA, AND R. F. CHEN. 1997. A major biopolymeric component to dissolved organic carbon in surface seawater. *Nature* **387**: 166–169.
- AMON, R. M. W., AND R. BENNER. 1992. Rapid cycling of high-molecular-weight dissolved organic matter in the ocean. *Nature* **369**: 549–552.

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- AZAM, F. 1998. Microbial control of ocean carbon flux: The lot thickens. *Science* **280**: 694–696.
- BENNER, R., B. BIDDANDA, B. BLACK, AND M. MCCARTHY. 1997. Abundance, size distribution, and stable carbon and nitrogen isotopic compositions of marine organic matter isolated by tangential-flow ultrafiltration. *Mar. Chem.* **57**: 243–263.
- , AND S. OPSAHL. 2001. Molecular indicators of the sources and transformations of dissolved organic matter in the Mississippi river plume. *Org. Geochem.* **32**: 597–611.
- , J. D. PAKULSKI, M. MCCARTHY, J. I. HEDGES, AND P. G. HATCHER. 1992. Bulk chemical characteristics of dissolved organic matter in the ocean. *Science* **255**: 1561–1564.
- BIDDANDA, B., AND R. BENNER. 1997. Carbon, nitrogen, and carbohydrate fluxes during the production of particulate and dissolved organic matter by marine phytoplankton. *Limnol. Oceanogr.* **42**: 506–518.
- BORCH, N. H., AND D. L. KIRCHMAN. 1999. Protection of protein from bacteria degradation by sub-micron particles. *Aquat. Microbial Ecol.* **16**: 265–272.
- BOSCHKER, H. T. S., J. F. 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**: 306–319.
- CANUEL, E. A., K. H. FREEMAN, AND S. G. WAKEHAM. 1997. Isotopic compositions of lipid biomarker compounds in estuarine plants and surface sediments. *Limnol. Oceanogr.* **42**: 1570–1583.
- CRANWELL, P. A. 1982. Lipids of aquatic sediments and sedimenting particulates. *Prog. Lipid Res.* **21**: 271–308.
- FUHRMAN, J. A. 1999. Marine viruses and their biogeochemical and ecological effects. *Nature* **399**: 541–548.
- GUO, L. D., AND P. H. SANTSCHI. 1997. Composition and cycling of colloids in marine environments. *Rev. Geophys.* **35**: 17–40.
- , N. TANAKA, D. M. SCHELL, AND P. H. SANTSCHI. 2003. Nitrogen and carbon isotopic composition of high-molecular-weight dissolved organic matter in marine environments. *Mar. Ecol. Prog. Ser.* **252**: 51–60.
- HARVEY, H. R., AND A. MANNINO. 2001. The chemical composition and cycling of particulate and macromolecular dissolved organic matter in temperate estuaries as revealed by molecular organic tracers. *Org. Geochem.* **32**: 527–542.
- KANEDA, T. 1991. Iso- and anteiso-fatty acids in bacteria: Biosynthesis, function, and taxonomic significance. *Microbiol. Rev.* **55**: 288–302.
- KERNER, M., H. HOHENBERG, S. ERTL, M. RECKERMANN, AND A. SPITZY. 2003. Self-organization of dissolved organic matter to micelle-like microparticles in river water. *Nature* **422**: 150–154.
- LIU, Q., C. C. PARRISH, AND R. HELLEUR. 1998. Lipid class and carbohydrate concentrations in marine colloids. *Mar. Chem.* **60**: 177–188.
- MANNINO, A., AND H. R. HARVEY. 1999. Lipid composition in particulate and dissolved organic matter in the Delaware Estuary: Sources and diagenetic patterns. *Geochim. Cosmochim. Acta* **63**: 2219–2235.
- , 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.
- MCCARTHY, M. D., J. I. HEDGES, AND R. BENNER. 1998. Major bacterial contribution to marine dissolved organic nitrogen. *Science* **281**: 231–234.
- MITRA, S., T. S. BIANCHI, L. D. GUO, AND P. H. SANTSCHI. 2000. Terrestrially derived dissolved organic matter in the Chesapeake Bay and the Middle Atlantic Bight. *Geochim. Cosmochim. Acta* **64**: 3547–3557.
- NAGATA, T., AND D. L. KIRCHMAN. 1992. Release of macromolecular organic complexes by heterotrophic marine flagellates. *Mar. Ecol. Prog. Ser.* **83**: 233–240.
- OGAWA, H., Y. AMAGAI, I. KOIKE, K. KAISER, AND R. BENNER. 2001. Production of refractory dissolved organic matter by bacteria. *Science* **292**: 917–920.
- OPSAHL, S., AND R. BENNER. 1997. Distribution and cycling of terrigenous dissolved organic matter in the ocean. *Nature* **386**: 480–482.
- PETERSON, B., B. FRY, M. HULLAR, S. SAUPE, AND R. WRIGHT. 1994. The distribution and stable carbon isotopic composition of dissolved organic carbon in estuaries. *Estuaries* **17**: 111–121.
- REPETA, D. J., T. M. QUAN, L. I. ALUWIHARE, AND A. M. ACCARDI. 2002. Chemical characterization of high molecular weight dissolved organic matter in fresh and marine waters. *Geochim. Cosmochim. Acta* **66**: 955–962.
- ROCHELLE-NEWALL, E. J., AND T. R. FISHER. 2002. Production of chromophoric dissolved organic matter fluorescence in marine and estuarine environments: An investigation into the role of phytoplankton. *Mar. Chem.* **77**: 7–21.
- SIGLEO, A. C., AND J. C. MEANS. 1990. Organic and inorganic components in estuarine colloids: Implications for sorption and transport of pollutants. *Rev. Environ. Contam. Technol.* **112**: 123–147.
- STODEREGGER, K., AND G. J. HERNDL. 1998. Production and release of bacterial capsular material and its subsequent utilization by marine bacterioplankton. *Limnol. Oceanogr.* **43**: 877–884.
- SUN, M.-Y., AND S. G. WAKEHAM. 1994. Molecular evidence for degradation and preservation of organic matter in the anoxic Black Sea Basin. *Geochim. Cosmochim. Acta* **58**: 3395–3406.
- VOLKMAN, J. K., S. M. BARRETT, S. I. BLACKBURN, M. P. MANSOUR, E. L. SKIES, AND F. GELIN. 1998. Microalgal biomarkers: A review of recent research developments. *Org. Geochem.* **29**: 1163–1179.
- , S. W. JEFFREY, P. D. NICHOLS, G. I. ROGERS, AND C. D. GARLAND. 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *J. Exp. Mar. Biol. Ecol.* **128**: 219–240.
- WAKEHAM, S. G., AND J. A. BEIER. 1991. Fatty acid and sterol biomarkers as indicators of particulate matter source and alteration processes in the Black Sea. *Deep-Sea Res.* **38**: S943–S968.
- , T. K. PEASE, AND R. BENNER. 2003. Hydroxy fatty acids in marine dissolved organic matter as indicators of bacterial membrane material. *Org. Geochem.* **34**: 857–868.

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