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## Biochemical composition of particles and dissolved organic matter along an estuarine gradient: Sources and implications for DOM reactivity

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### Abstract

The chemical composition of high molecular weight dissolved organic matter (DOM) and particulate organic matter (POM) was examined along the salinity gradient of the Delaware Estuary. DOM was collected and fractionated by tangential-flow ultrafiltration into 1–30 kDa (HDOM; high molecular weight) and 30 kDa to 0.2  $\mu\text{m}$  (VHDOM; very high molecular weight) and compared to particles collected in parallel. Polysaccharides comprised 12–43% of particulate organic carbon (POC), 30–56% of VHDOM carbon, and 7.5–19% of HDOM carbon. Hydrolyzable amino acids comprised 17–38% of POC, 5.4–12% of VHDOM carbon, and 1.5–4.2% of HDOM carbon. Only 7–43% of dissolved organic nitrogen in VHDOM and HDOM consisted of amino acids, indicating that organic nitrogen is highly modified within the dissolved pool or an unidentified pool of dissolved organic nitrogen exists. The composition of amino acids and distribution of polysaccharides are consistent with enrichment of structural biopolymers from algae and vascular plants within DOM. Proteinaceous matter released during the growth of an axenic diatom culture contains similar amino acid distributions across size fractions as in Delaware Bay samples. The source of organic matter appears to be as important as microbial processing in determining amino acid content and composition of DOM. Shifts in amino acid composition point to contrasting sources and extent of degradation for organic matter along the estuarine gradient and among size fractions. The lower amino acid and carbohydrate content and higher  $\beta$ -alanine content in HDOM suggests that this fraction is more highly degraded relative to POM and VHDOM and provides geochemical evidence in support of the size-reactivity continuum hypothesis. Spatial patterns in reactivity of organic constituents were also evident with more degraded organic matter in the turbid middle estuary and the release of fresh DOM from diatoms in the lower estuary.

Dissolved organic matter (DOM) is the largest reservoir of organic carbon in the ocean and is comparable to the amount of  $\text{CO}_2$  in the atmosphere ( $0.66 \times 10^{18}$  g C; Hedges 1992). The sources and cycling of DOM is thus a key component of the global carbon cycle, but its dilute concentration within an ion rich solution and chemical heterogeneity have resulted in much of the DOM (46–87%) to be unidentified at the molecular level (e.g., Buffle 1988). Radiocarbon measurements appear to reflect this chemical complexity. Surface ocean DOM is on average >1,000 yr old (56% <30 yr and 44% >6,000 yr), and deep ocean DOM is extremely old, ~6,000 yr old, and thus appears highly refractory (Williams and Druffel 1987). Results from estuarine, continental shelf, and slope waters suggest molecular size is an important property with >10 kDa DOM being contemporary in age (post-1950) with residence times of 1–30 d, whereas the 1–10 kDa fraction is much older (>1 kDa is 380–4,500 yr old; Santschi et al. 1995). These observations suggest that macromolecular DOM cycles rapidly within estuarine and

coastal regions, with the most refractory DOM persisting in the ocean for thousands of years. To understand the role of terrestrial and autochthonous DOM as sources of energy and nutrients in coastal systems and as contributors of recalcitrant DOM to the ocean requires detailed information on its chemical composition through the estuarine interface.

Primary production is ultimately the dominant source of DOM to aquatic systems. Sources include direct algal release via auto-lysis and extracellular excretions or indirectly through sloppy feeding and egestion by grazers, microbial degradation of detritus, viral or bacterial lysis of phytoplankton cells, and the continual release and physical transport of terrestrial material. Approximately 40–60% of autochthonous primary production is cycled through bacteria (Cole et al. 1988; Hoch and Kirchman 1993), which undoubtedly leaves an imprint on the molecular distribution and chemical composition of particles and DOM. Incubations of bacteria with size-fractionated DOM from diverse environments reveal consistently greater utilization, growth, and respiration rates for >1 kDa DOM (Amon and Benner 1996). In the Mississippi River plume, Gardner et al. (1996) found that ammonium and dissolved free amino acids stimulated bacterial utilization of high molecular weight (>1 kDa) DOM more than lower molecular weight material, indicating that the higher weight fraction contained the preferred carbon source. These experimental results provide indirect evidence for compositional differences among size fractions. They also lend support for the size-reactivity continuum hypothesis, which suggests that the diagenetic state of organic matter (seen as the extent of degradation or alteration) decreases with increasing size (Amon and Benner 1996). Although

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these results suggest that compositional differences do exist, detailed chemical data that might explain such differences remain sparse (e.g., Hedges et al. 1994; Skoog and Benner 1997).

As zones that receive significant terrestrial input and maintain high levels of primary production, estuaries and coastal regions can arguably be considered the most complex environments to examine the dynamic nature of particle-DOM interactions. Yet the importance of estuaries in terms of multiple sources of organic matter, the effect of physical interactions, and biological processing on the chemical dynamics of DOM cannot be ignored. The principal goal of this study is to begin to examine the sources and fates of high molecular weight DOM and its link to particulate organic matter (POM) along the salinity gradient of the Delaware Estuary. Three questions were addressed. First, are compositional differences between molecular weight classes evident in the estuary, and do they support the size-reactivity hypothesis? Second, do zones of high phytoplankton production leave a chemical imprint on the composition and distribution of DOM? Third, do the enhanced physical interactions that occur at the estuarine turbidity maximum of the Delaware and elsewhere influence the chemical composition and distribution of DOM? In this system, organic matter enters from the Delaware River as its major source of freshwater, from tidal exchange with the Atlantic Ocean and from in situ primary production. Algal blooms are found primarily in the lower estuary and are isolated from the turbidity zone due to light limitations imposed by the high particle load (Pennock and Sharp 1986). Here we focus on carbohydrates and amino acids because these biochemical components comprise substantial portions of living biomass (80% of algal carbon and 65% of terrestrial plant carbon) and are important components of DOM in freshwater (e.g., Tranvik and Jorgensen 1995), in estuaries (e.g., Sigleo et al. 1983; Coffin 1989; Sigleo 1996), and in the ocean (e.g., Benner et al. 1992; McCarthy et al. 1996). A range of sites spanning the entire salinity gradient was studied to see how river discharge, physical interactions, and primary production influence the partitioning and chemical composition of these biochemical classes between particles and molecular weight classes of DOM.

## Methods

**Sampling and bulk analyses**—Seven stations were sampled between 6 and 9 June 1996 along a transect of the Delaware Estuary corresponding to riverine, turbidity maximum, downstream of turbidity maximum, chlorophyll *a* maximum, downstream of chlorophyll *a* maximum, and two stations within the estuarine plume on the inner continental shelf (Fig. 1). Sample collection and filtration were described previously (Mannino and Harvey 1999). Briefly, large-volume water samples (13–104 liters) for analysis of DOM were collected at 1 m depth, and particles were removed by sequential passage through cartridge filters of 3  $\mu\text{m}$  and 0.2  $\mu\text{m}$  pore size. The filtrate was then separated into three nominal size fractions: 30 kDa to 0.2  $\mu\text{m}$  (VHDOM; very high molecular weight), 1–30 kDa (HDOM; high molecular

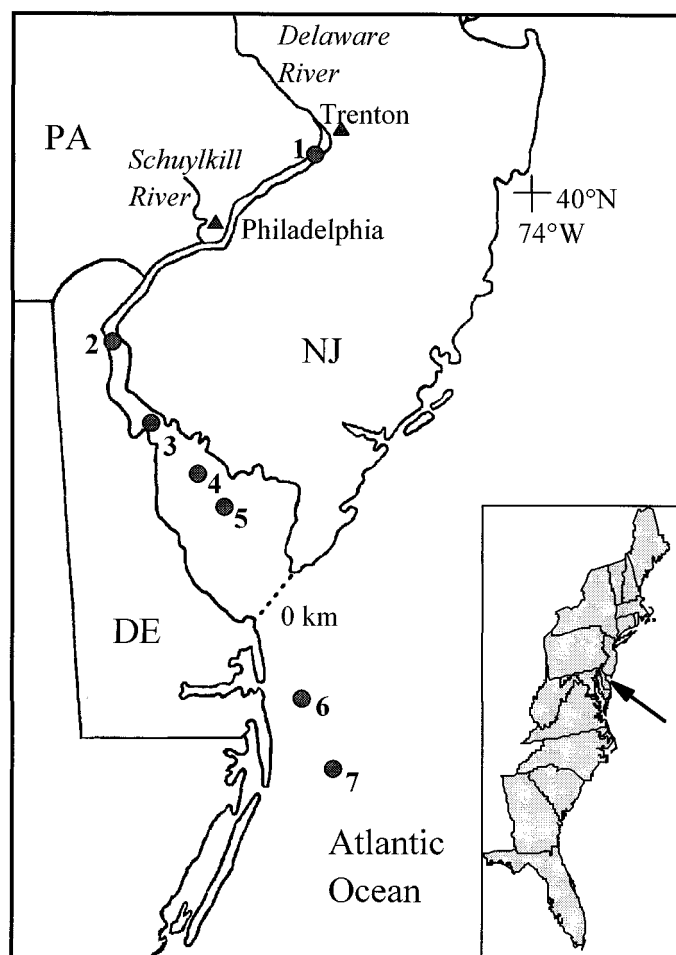


Fig. 1. Map of the Delaware Estuary with station locations. Inset map shows location of the Delaware Estuary in relation to the U.S. Atlantic coast.

weight) and <1 kDa (LDOM; low molecular weight) with an Amicon DC-10L tangential-flow ultrafiltration unit with the S10Y30 and the S10N1 ultrafilters. Because of the high particle load at the turbidity maximum (Sta. 2), only 13 liters were filtered for ultrafiltration and only the 1 kDa to 0.2  $\mu\text{m}$  fraction retained. Immediately following initial fractionation and concentration, the two high molecular weight fractions were desalted with the Amicon unit with 6–9 liters of low organic deionized water. Samples for DOC analysis were collected from the <0.2  $\mu\text{m}$  filtrate and each DOM size fraction (stored frozen) and analyzed by high temperature combustion in triplicate (S.D.  $\leq 5\%$ ) with a Shimadzu TOC 5000 (Benner and Strom 1993). Remaining sample retentates were stored frozen, concentrated further by rotary evaporation and lyophilized to dry powders. LDOM carbon was analyzed for mass balance purposes, but no further characterization was made. For analysis of particles, additional whole water was filtered through precombusted (4–6 h at 450°C) GF/F filters by vacuum filtration. Organic carbon and total nitrogen content were measured with a CHN elemental analyzer for POM and DOM samples.

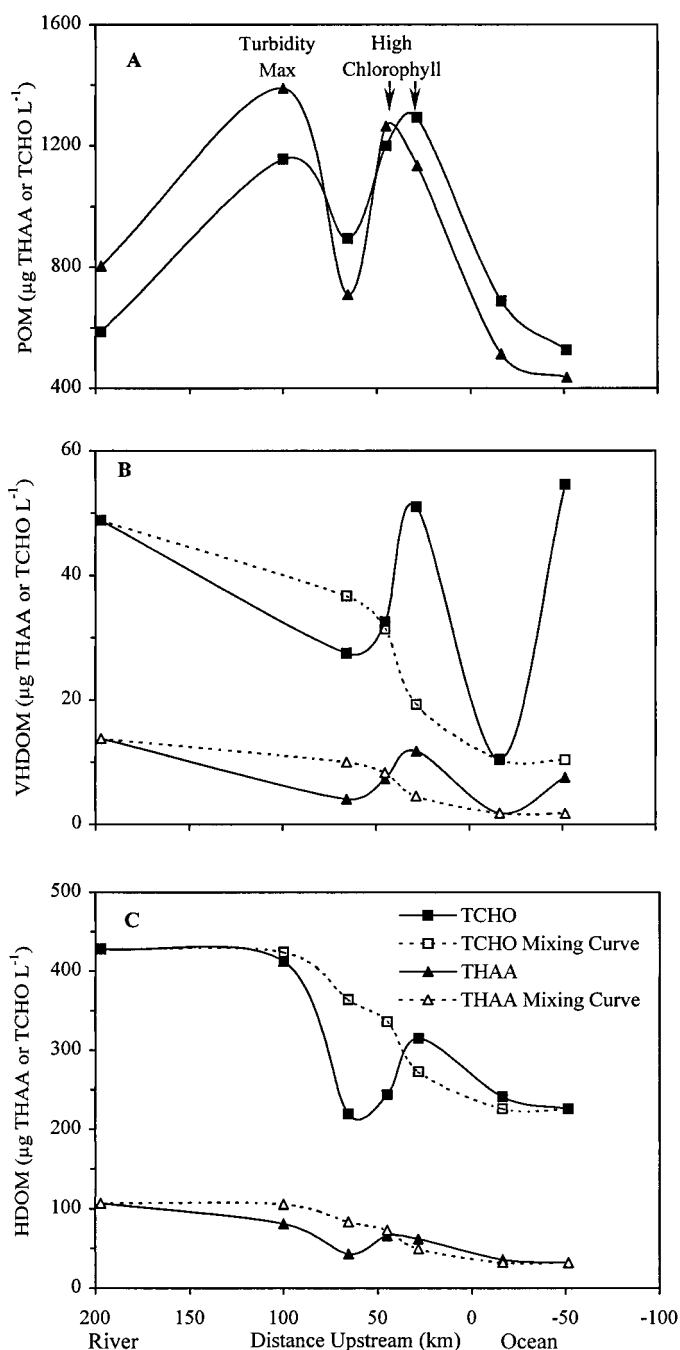


Fig. 2. Polysaccharide (TCHO) and total hydrolyzable amino acid (THAA) concentrations in (A) particles, (B) VHDOM, and (C) HDOM in the Delaware Estuary. Open symbols with dashed lines indicate conservative mixing values based on salinity end-members (Sta. 1 and 7 for HDOM; Sta. 1 and 6 for VHDOM) for polysaccharides and THAA. Data for VHDOM at Sta. 2 are not available, only the  $>1$  kDa fraction was isolated and was designated as HDOM.

**Axenic diatom culture**—An axenic culture of the diatom *Skeletonema costatum* (CCMP1332) was grown to late-log phase to examine the biochemical composition of DOM released during a diatom bloom in the absence of actively growing bacteria. Delaware Bay is generally dominated by

diatoms, and *S. costatum* is the dominant bloom forming species (Pennock and Sharp 1986). The axenic culture was grown over a 14-d period in a 42-liter glass vessel containing f/2 nutrient media plus Si and diluted seawater of  $<1$  kDa nominal size. The sterile estuarine media was prepared with filtered ( $0.2 \mu\text{m}$  filter) coastal seawater (30 psu), which was then diluted to 15 psu with ultraviolet (UV)-oxidized Nanopure water, ultrafiltered to remove the  $>1$  kDa nominal fraction and autoclaved. The culture was grown under a 12 h light: 12 h dark cycle over 14 d to a cell density of  $7.6 \times 10^5$  cells  $\text{ml}^{-1}$ . The culture was aerated under aseptic conditions and maintained in suspension by gentle mixing with a magnetic stirrer. Bacteria were monitored by epifluorescence microscopy with acridine orange (Hobbie et al. 1977). Extremely low abundances of bacteria ( $<4,000$  cells  $\text{ml}^{-1}$ ) were observed on day 14 when the culture was sampled.

**Carbohydrates**—Polysaccharide content was measured with a modification of the MBTH method (Pakulski and Benner 1992). Duplicate subsamples of lyophilized DOM or dried filters for POM (select duplicates) were acidified in 50-ml serum vials with 1 ml of 12 M  $\text{H}_2\text{SO}_4$  for 2 h at room temperature, diluted with 9 ml of Nanopure water and hydrolyzed at  $100^\circ\text{C}$  for 3 h. After cooling, the pH of the hydrolysis solution was neutralized with NaOH. Triplicate aliquots of hydrolysis products (and triplicate blanks) were placed in test tubes, and the aldehydes were reduced to their respective alditols, oxidized to formaldehyde and reacted with MBTH. Glucose standard curves were generated concurrently, and total polysaccharide concentration was calculated as glucose equivalents.

**Total hydrolyzable amino acids**—Select duplicates of lyophilized DOM, dried POM and a standard amino acid mix were each placed in 4-ml vials and hydrolyzed with 1 ml of 6 N sequal-grade HCl at  $150^\circ\text{C}$  for 2 h (modified from Cowie and Hedges 1992a). L- $\gamma$ -Methyl-leucine was added to samples prior to hydrolysis and served as an internal standard. After cooling, hydrolyzates were centrifuged at  $1,200 \times g$  for 5 min and the supernatants transferred and dried by vacuum centrifugation (Speed-Vac). Hydrolyzed amino acids were derivatized to form their respective trifluoroacetyl isopropyl esters following the protocol described by Silfer et al. (1991). Briefly, the carboxyl groups were esterified with acidified isopropanol (4:1 mixture of high performance liquid chromatography (HPLC) grade isopropanol and 99+% acetyl chloride) at  $110^\circ\text{C}$  for 1 h. Amino, hydroxyl, and thiol groups were derivatized with trifluoroacetic anhydride at  $110^\circ\text{C}$  for 10 min. After repeated drying under a gentle stream of  $\text{N}_2$ , samples were dissolved in  $\text{CH}_2\text{Cl}_2$  and analyzed by capillary gas chromatography with flame ionization detection (GC-FID; HP-5890II) with a 60 m DB-5MS column ( $0.32 \text{ mm}$  I.D.,  $0.25 \mu\text{m}$  film thickness). Hydrogen served as the carrier gas ( $2 \text{ ml min}^{-1}$ ), and a temperature program of  $10^\circ\text{C min}^{-1}$  from  $50^\circ\text{C}$  to  $85^\circ\text{C}$  followed by  $3.5^\circ\text{C min}^{-1}$  to  $200^\circ\text{C}$  and  $10^\circ\text{C min}^{-1}$  to  $280^\circ\text{C}$  was used. Reagent blanks processed simultaneously with each sample group indicated no contamination from reagents or handling. Individual amino acid standards and mixtures of amino acids were derivatized as described above and analyzed by GC-



Table 1. Station locations, physical parameters and chemical characteristics for dissolved and particulate fractions through the Delaware Estuary. Station numbers are illustrated in Fig. 1. Distance, distance upstream from bay mouth; TSP, total suspended particles; Chl *a*, chlorophyll *a* (Kirchman unpubl. data); POC, particulate organic carbon; PN, particulate nitrogen; DOC, total dissolved organic carbon; LDOM, <1 kDa DOM; HDOM, 1–30 kDa DOM; VHDOM, 30 kDa to 0.2  $\mu\text{m}$  DOM; ND, not determined.

Station	Site descriptor	Distance (km)	Salinity (psu)	Chl <i>a</i> ( $\mu\text{g L}^{-1}$ )	TSP ( $\text{mg L}^{-1}$ )	POC ( $\mu\text{M}$ )	PN ( $\mu\text{M}$ )	DOC ( $\mu\text{M}$ )	LDOM ( $\mu\text{M C}$ )	HDOM ( $\mu\text{M C}$ )	VHDOM ( $\mu\text{M C}$ )
1	Riverine	197	0.11	4.1	10.7	95.8	10.9	218	112	89	4.5
2	Turbidity maximum	100	0.67	10.8	92.0	318	30.6	218	81	133*	ND
3	Turbid	66.4	9.07	9.7	42.3	147	15.9	330	262	98	1.9
4	Chl <i>a</i> maximum	45	13.19	21.6	8.5	162	24.8	224	177	65	2.4
5	High Chl <i>a</i>	28.4	22.58	21.0	4.3	152	21.7	205	112	60	3.4
6	Coastal ocean	-16.3	29.42	1.7	1.5	53.4	7.5	136	101	42	1.2
7	Coastal ocean	-51.5	29.48	2.1	1.4	54.4	7.4	170	120	43	3.3

\* HDOM and VHDOM combined and designated as HDOM; only the >1 kDa fraction retained, since high particle density precluded filtration of the large volume of water required for isolation of the VHDOM fraction.

FID and GC-MS (HP-5890II GC coupled to a HP-5970B MSD) in parallel for confirmation of amino acid identity in DOM and POM samples. Helium served as the carrier gas for GC-MS, and the temperature program above was used. The MSD was operated in electron impact mode at 70 eV with acquisition over 50–600 a.m.u. range.

Individual amino acids were quantified on the basis of the internal standard and corrected for individual responses from a standard amino acid mix. Hydrolyzed amino acid standards demonstrated that individual amino acids have different responses relative to methyl-leucine from 73% to 134% (mean of 100%) for serine and phenylalanine, respectively, except for arginine (48%) and methionine (57%). Amino acids with both low concentration and low response were difficult to detect. Histidine could not be quantified because of its low concentration and low response. Glutamine and asparagine are deaminated during hydrolysis and were quantified as glutamic acid and aspartic acid, respectively. Although cysteine and  $\gamma$ -aminobutyric acid have high responses and thus low detection limits with this procedure, neither were detected in DOM or POM samples. In contrast to the more common HPLC method with *o*-phthalaldehyde derivatization, proline and hydroxyproline could be quantified. Detection limits for individual amino acids was typically at the low nanogram level. Coefficients of variation from duplicate analyses were on the order of <6% for individual amino acids for DOM samples (<2% for total amino acids) and <10% for POM (except for serine at 12%; <5% for total amino acids).

## Results

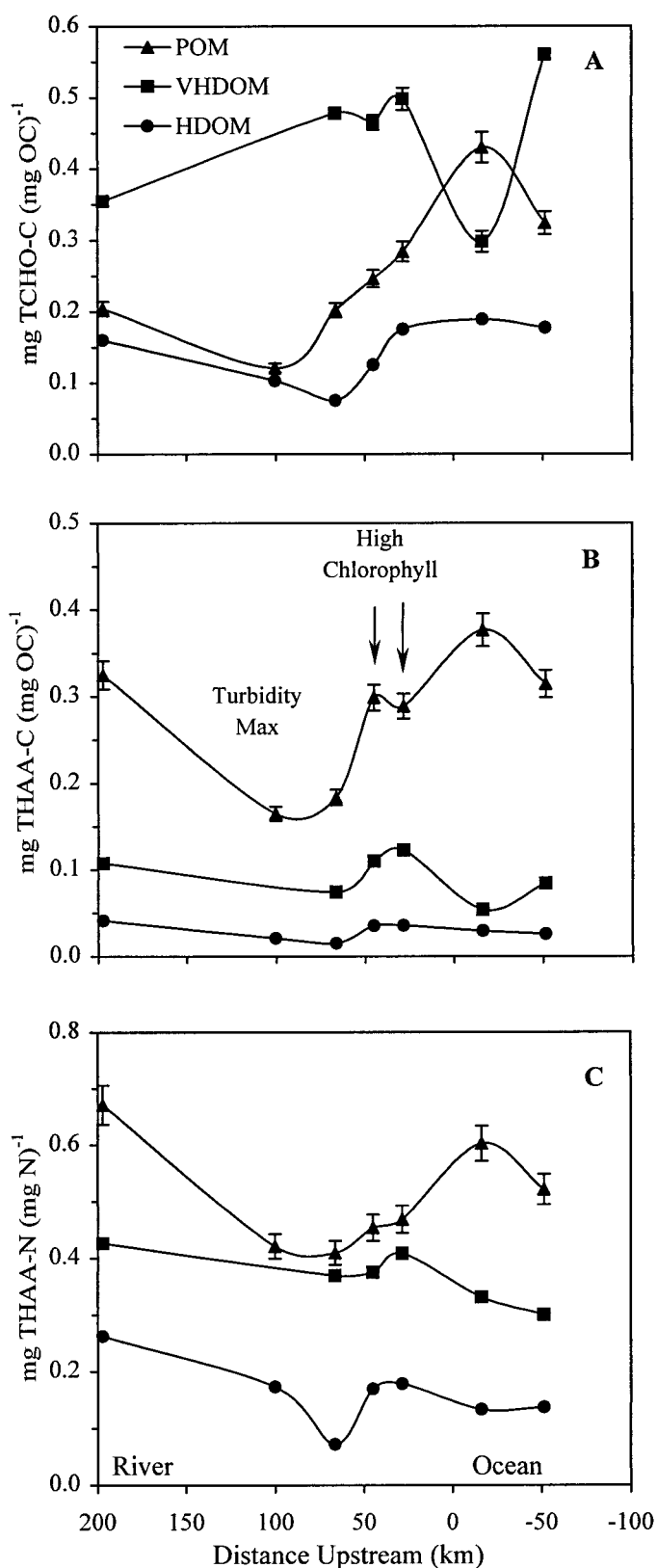
**Bulk measurements**—Maximal POC and DOC concentrations were observed at or just downstream of the turbidity maximum (Table 1). POC peaked at the turbidity maximum (Sta. 2; 318  $\mu\text{M}$  POC) with lower concentrations in riverine and coastal ocean stations. A significant DOC peak occurred at Sta. 3 (330  $\mu\text{M}$  DOC; Table 1). POC and DOC concentrations remained elevated in the lower estuary (Sta. 4 and 5), where substantial algal biomass was present, with the lowest concentrations for both observed at the coastal ocean stations. LDOM comprised the bulk of DOC except at the turbidity maximum where the concentration was 81  $\mu\text{M C}$ , but increased to its maximum at Sta. 3 (262  $\mu\text{M C}$ ). In

contrast, HDOM carbon concentration was highest at the turbidity maximum (133  $\mu\text{M C}$ ) and lowest in the coastal ocean (42  $\mu\text{M C}$ ). The general trend of declining DOC with increasing salinity downstream of Sta. 3 was observed for total DOC, LDOM, and HDOM carbon but not for VHDOM carbon. VHDOM comprised only a small portion of DOC (<3%) with the highest concentration at the riverine station and lowest at Sta. 6.

**Polysaccharides and total hydrolyzable amino acids**—On a volumetric basis, polysaccharide and total hydrolyzable amino acid (THAA) concentrations covaried for each size fraction along the estuary (Fig. 2). Particles contained consistently higher polysaccharide and amino acid concentrations than either VHDOM or HDOM. The highest polysaccharide concentrations for POM were found in the lower estuary (1,199  $\mu\text{g L}^{-1}$  at the chlorophyll maximum and 1292  $\mu\text{g L}^{-1}$  at Sta. 5) and at the turbidity maximum (1,156  $\mu\text{g L}^{-1}$ ; Fig. 2A). The distribution of THAA in particles generally followed the distribution of POC, with the highest concentration occurring at the turbidity maximum (1,389  $\mu\text{g L}^{-1}$  THAA) followed by the chlorophyll maximum (1,265  $\mu\text{g L}^{-1}$ ) and immediately downstream (Sta. 5; 1,134  $\mu\text{g L}^{-1}$ ). Although POC was 50% higher at the turbid site (Sta. 3) than at the riverine station, THAA concentration was higher at the riverine station.

Salinity-based conservative mixing curves suggest that fluctuations in dissolved polysaccharide and THAA concentrations were not due to estuarine mixing, particularly between Sta. 2 and 5 (Figs. 2B, C). Sources of polysaccharides and THAA were observed for VHDOM and HDOM at Sta. 4 and 5 and for VHDOM at Sta. 7. In VHDOM, polysaccharides ranged from 10.4  $\mu\text{g L}^{-1}$  at Sta. 6 to 54.6  $\mu\text{g L}^{-1}$  at Sta. 7 (Fig. 2B). THAA concentrations in VHDOM ranged from 1.8 to 13.8  $\mu\text{g L}^{-1}$  at Sta. 6 and riverine site (Sta. 1), respectively. For HDOM, polysaccharide concentrations were maximal at the riverine site (428  $\mu\text{g L}^{-1}$ ) and lowest at Sta. 3 (219  $\mu\text{g L}^{-1}$ ) and 7 (226  $\mu\text{g L}^{-1}$ ; Fig. 2C). The concentration of THAA in HDOM was highest at the riverine station (107  $\mu\text{g L}^{-1}$ ) and lowest in the coastal ocean (Sta. 7; 32  $\mu\text{g L}^{-1}$ ).

Polysaccharides comprised a substantial portion of organic matter in particles and macromolecular DOM. Along the



estuarine gradient from the turbidity maximum into the lower estuary polysaccharide carbon generally increased (Fig. 3A). Particulate polysaccharides increased substantially through the chlorophyll maximum to 43% of POC in the coastal ocean. The VHDOM fraction was rich in polysaccharides, particularly in the lower estuary and coastal ocean. HDOM polysaccharides decreased from 16% of HDOM carbon at the riverine station to 7.5% at the turbid site (Sta. 3), but returned to 19% of HDOM by Sta. 6. Similar or higher carbohydrate measurements have been observed for HDOM in a range of environments, including 17–40% in the Potomac River (5 kDa to 0.4  $\mu$ m; Sigleo 1996), 73–84% in the Mid-Atlantic Bight (Aluwihare et al. 1997) and 54% at ALOHA station near Hawaii (Benner et al. 1992).

The amount of carbon as THAA was highest for POM (17% to 38%), decreasing with the molecular size of DOM (5.4% to 12.3% for VHDOM and 1.5 to 4.2% for HDOM; Fig. 3B). For POM, the highest amino acid carbon was found in the coastal ocean; whereas in DOM, THAA was generally higher at the riverine station and in the region of the chlorophyll maximum. THAA nitrogen concentrations exhibited similar patterns as for carbon with the lowest proportion of THAA nitrogen observed for HDOM (7 to 26%; Fig. 3C). The diatom culture demonstrated higher THAA-N with increasing size as observed throughout the Delaware Estuary (Fig. 4). For each size fraction, THAA comprised a higher fraction of organic matter in the diatom culture than in Delaware Bay samples.

Distributions of several individual amino acids varied along the estuarine gradient. On a mole % basis, glycine and alanine were the dominant amino acids for POM and HDOM throughout the estuary, except at the chlorophyll maximum where threonine comprised 16% of POM-THAA, but <8.2% elsewhere (Table 2). Although alanine and glycine contributed 15–22% and 10–14% of VHDOM-THAA, respectively, serine also comprised a substantial percentage of VHDOM-THAA (11–19%), especially in the lower estuary. At the riverine station, HDOM and VHDOM were enriched in threonine and hydroxyproline relative to other stations. Within the turbid region (Sta. 2 and 3), POM and DOM fractions were enriched in glycine and  $\beta$ -alanine (DOM only) and depleted in tyrosine and lysine. Aspartic acid comprised 11% of VHDOM-THAA at the chlorophyll maximum and <9% elsewhere.

By averaging amino acid content throughout the estuary by size fraction, differences in amino acid composition were apparent between size fractions and in comparison to the pure diatom culture. In general, the narrow deviations observed for most of the amino acids reveal that THAA com-

Fig. 3. Distribution of biochemical components in the Delaware Estuary on a carbon and nitrogen normalized basis. (A) Polysaccharide content (mean  $\pm$  1 SD), (B) total hydrolyzable amino acids on a carbon normalized basis, and (C) THAA on a nitrogen normalized basis. Error bars indicate the analytical variability of the measurement. For symbols without error bars, the standard deviation is smaller than the size of the symbol. Data for VHDOM at Sta. 2 is not available.

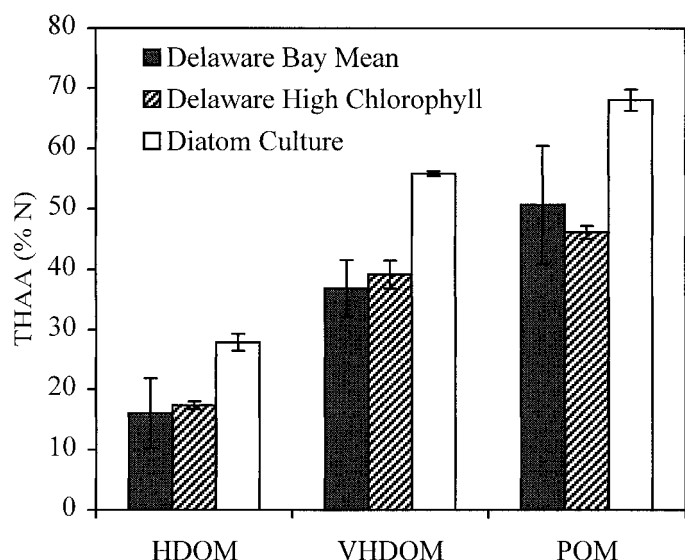


Fig. 4. THAA nitrogen content for HDOM, VHDOM, and POM size fractions for the axenic diatom culture (average  $\pm 1$  SD of duplicate analyses), Delaware High Chlorophyll (Sta. 4 and 5; average  $\pm 1$  SD) and the Delaware Bay average (mean  $\pm 1$ SD).

position among the size fractions varied slightly through the estuary (Table 2; Fig. 5A). POM was enriched in leucine (10–12 mole%), but HDOM was depleted in leucine (3.4–3.9%; Fig. 5A). POM was also enriched in isoleucine and phenylalanine, but depleted in aspartic acid and glutamic acid compared to DOM fractions. VHDOM was enriched in serine, but depleted in glycine and proline. HDOM was enriched in  $\beta$ -alanine ( $\beta$ -ala), aspartic acid, and glutamic acid and depleted in valine, leucine, and isoleucine. On a mole% basis, the *S. costatum* culture contained no  $\beta$ -Ala and lower amounts of alanine and glycine for both cells and dissolved fractions than seen in Delaware Bay (Fig. 5). However, aspartic acid and glutamic acid contributions were much greater in *S. costatum* HDOM than in field samples. For particles, phenylalanine was enriched in Delaware Bay compared to the *S. costatum* culture. Hydroxyproline was observed in all three size fractions of the diatom culture, but was highest in VHDOM.

Amino acids were also categorized on the basis of side-chain functionality and compared across size fractions and to the diatom culture (Fig. 6). Although absolute concentrations of each amino acid group varied substantially along the estuary, the mole% values remained similar with the exception of the chlorophyll maximum. Neutral amino acids comprised the majority of THAA in particles and dissolved fractions for both field and culture samples (Fig. 6). Aromatic amino acids were more abundant in POM than in dissolved fractions. At the chlorophyll maximum, hydroxyl amino acids showed a strong peak in particles. Acidic amino acids comprised a greater portion of THAA in HDOM than in either VHDOM or POM. For both HDOM and VHDOM, acidic amino acids comprised a greater portion of THAA at the chlorophyll maximum than upstream. Amino acid functional group distributions in the *S. costatum* culture showed similar patterns between size fractions as observed for the

Delaware Bay (Fig. 6). Nevertheless, diatoms and the released DOM contained lower amounts of neutral and hydroxyl amino acids and higher amounts of acidic and basic amino acids than observed in the Delaware Estuary.

## Discussion

Only a handful of studies have examined the chemical composition of macromolecular DOM and particles concurrently, especially in estuarine systems where multiple sources and processes result in inherently complex organic matter. However, within the high chlorophyll region of the lower estuary (Sta. 4 and 5) concomitant increases in polysaccharide and amino acid concentrations (relative to upstream concentrations) for all three size fractions clearly indicate algal production as the primary source of polysaccharides and amino acids (Fig. 2). The release of polysaccharides from particles, including extracellular polysaccharides from phytoplankton and bacterial cells (Decho 1990), may contribute to the carbohydrate enrichment seen in VHDOM (Fig. 3A). Copepod grazing may be the source of the higher polysaccharide and amino acid concentrations at Sta. 7 (compared to Sta. 6). Copepods were abundant at Sta. 7, and grazing activity has been shown to be a source of DOM (Strom et al. 1997). In addition, a shift in species composition was observed in the coastal ocean versus the lower estuary with fewer diatoms and a greater relative abundance of dinoflagellates and other flagellates (Mannino and Harvey 1999). Low concentrations of inorganic nutrients were also observed at the coastal ocean sites (Sta. 7: DIN = 0.47  $\mu$ M and PO<sub>4</sub> = 0.15  $\mu$ M; Kirchman unpubl.) where carbohydrate yields were high for HDOM, VHDOM, and POM (Fig. 3). Either nutrient limitation, which can enhance biosynthesis of carbohydrates over proteins, or shifts in plankton community composition to species with higher carbohydrate content (e.g., Brown 1991) would serve to elevate the polysaccharide carbon content in POM in the coastal ocean versus the lower estuary.

Although the application of tangential-flow ultrafiltration in aquatic environments is now widespread, uncertainty regarding the consistency in molecular weight-based fractionation and possible discrimination of organic constituents warrants cautious interpretation. Potential problems including contamination, breakthrough of high molecular weight compounds, retention of lower molecular weight molecules, physico-chemical interactions between macromolecules, and adsorptive losses to the membrane itself, could all affect the fidelity of size-based fractionations (e.g., Bauer et al. 1996; Buesseler et al. 1996; Guo and Santschi 1996; Dai et al. 1998). Guo and Santschi (1996) obtained excellent reproducibility in isolation of high molecular weight DOC by using a similar Amicon system, and found retention exceeding 93% for macromolecules >6 kDa (proteins). In our own test with the S10N1 cartridge (1 kDa nominal size), the retention coefficient for a 4.4 kDa fluorescein-labeled dextran (64  $\mu$ g L<sup>-1</sup> in 8.5 ‰ estuarine water) was >99% with 12% of the labeled dextran unaccounted for in the retentate or filtrate. In this study, the importance of ionic strength on the molecular weight distributions of DOM and various organic con-

Table 2. Distribution of hydrolyzable amino acids in particulate (POM) and high molecular weight dissolved organic matter (HDOM; VHDOM) through the Delaware Estuary. Values for individual amino acids are listed as mole % of total hydrolyzable amino acids ( $\pm 1$  SD). Means for individual amino acids ( $\pm 1$  SD) are also shown for the entire estuary.\*

Station	Fraction	Neutral										Hydroxyl					Acidic				Aromatic			Basic			THAA	
		Ala*	Gly	Val	Leu	Ile	Met	Pro	H-Pro	Thr	Ser	Asp	Glu	Phe	Tyr	Lys	Arg	$\beta$ -Ala	THAA	$\mu$ M								
1	POM	21.4	19.3	7.30	10.4	5.01	ND	4.87	ND	6.02	7.28	3.76	3.62	8.42	1.67	0.88	ND	ND	7.21									
	VHDOM	14.7	13.5	6.77	6.10	4.07	1.51	3.20	1.61	13.3	14.2	8.67	5.64	2.55	0.46	1.85	1.62	0.35	0.117									
	HDOM	15.5	17.1	5.39	3.77	2.60	2.11	4.25	2.48	11.0	11.9	9.66	6.89	1.87	1.15	1.61	0.20	2.55	0.927									
2	POM	18.2	23.1	7.63	10.3	5.34	ND	4.52	ND	6.58	8.88	4.24	2.74	6.50	1.62	0.34	ND	ND	12.7									
	HDOM	16.1	21.4	4.99	3.54	2.45	1.04	3.47	1.26	9.17	9.89	9.78	7.26	2.39	1.24	1.11	1.19	3.77	0.716									
	POM	17.6	21.3	7.58	10.7	5.30	ND	4.69	ND	6.57	9.28	4.86	3.14	7.36	1.46	0.25	ND	ND	6.40									
3	VHDOM	22.2	14.1	7.05	5.20	3.10	0.51	2.43	0.70	12.3	14.2	7.21	5.59	2.20	1.26	0.99	ND	0.97	0.036									
	HDOM	14.2	24.1	4.17	3.46	2.29	ND	4.22	1.31	7.79	8.78	11.7	8.54	1.72	1.31	1.93	ND	4.50	0.382									
	POM	15.9	15.7	6.74	10.0	5.04	ND	3.65	ND	15.6	8.47	5.05	3.87	7.73	1.95	0.43	ND	ND	11.0									
4	VHDOM	18.4	10.0	6.16	5.87	3.37	1.11	2.42	0.55	10.6	18.3	11.0	6.41	2.43	1.76	1.24	ND	0.37	0.063									
	HDOM	15.2	20.7	5.08	3.86	2.69	1.27	4.29	1.06	7.72	8.13	11.1	9.39	2.08	1.43	2.02	0.92	3.11	0.57									
	POM	17.9	17.4	7.23	10.6	5.38	ND	4.29	ND	7.12	9.35	5.51	4.40	8.28	2.04	0.48	ND	ND	9.98									
5	VHDOM	20.1	11.9	6.40	5.73	3.82	0.46	2.29	0.71	10.6	19.1	7.61	5.84	2.50	1.78	1.11	ND	0.16	0.103									
	HDOM	16.2	21.0	4.80	3.55	2.75	ND	4.19	1.26	8.40	8.91	11.6	9.30	1.98	1.19	1.83	ND	3.06	0.540									
	POM	16.2	15.1	7.73	11.7	5.87	ND	3.87	ND	7.96	10.4	5.05	4.03	8.91	2.81	0.31	ND	ND	4.44									
6	VHDOM	20.8	13.2	6.81	5.73	3.77	ND	2.56	1.45	12.1	17.1	7.44	5.37	2.21	1.43	ND	ND	0.016										
	HDOM	18.8	20.8	4.51	3.40	2.53	1.21	3.94	1.32	8.45	9.14	10.5	8.41	2.19	1.04	1.31	ND	2.53	0.317									
	POM	16.5	15.1	8.36	12.2	6.23	ND	4.13	ND	7.71	9.47	4.76	4.04	8.37	2.72	0.37	ND	ND	3.77									
7	VHDOM	18.0	11.8	6.88	6.75	4.16	1.16	2.86	2.29	12.5	10.5	8.74	6.61	3.31	2.01	1.46	0.69	0.33	0.064									
	HDOM	17.8	22.3	4.47	3.69	0.75	1.12	4.28	1.18	7.41	8.60	11.7	9.37	2.09	0.92	1.64	ND	2.78	0.282									
	POM	17.7	18.1	7.51	10.8	5.45	ND	4.29	ND	8.22	9.02	4.75	3.69	7.94	2.04	0.44	ND	ND	7.94									
SD			1.88	3.18	0.50	0.80	0.44			3.31	0.98	0.58	0.57	0.81	0.53	0.21			3.40									
Mean			19.0	12.4	6.68	5.90	3.71	2.62	1.22	11.9	15.6	8.45	5.91	2.53	1.45	1.11	0.39	0.36	0.066									
SD			2.63	1.49	0.33	0.51	0.42	0.56	0.68	1.09	3.22	1.41	0.49	0.41	0.55	0.62	0.66	0.33	0.038									
Mean			16.2	21.0	4.77	3.61	2.29	0.97	1.41	8.56	9.35	10.85	8.45	2.05	1.18	1.63	0.33	3.18	0.534									
SD			1.58	2.12	0.42	0.17	0.70	0.30	0.48	1.22	1.26	0.89	1.03	0.22	1.69	0.33	0.51	0.72	0.232									

\* Ala, Alanine; Gly, Glycine; Val, Valine; Leu, Leucine; Ile, Isoleucine; Met, Methionine; Pro, Proline; H-Pro, Hydroxyproline; Thr, Threonine; Ser, Serine; Asp, Aspartic acid; Glu, Glutamic acid; Phe, Phenylalanine; Tyr, Tyrosine; Lys, Lysine; Arg, Arginine;  $\beta$ -Ala,  $\beta$ -alanine; THAA, total hydrolyzable amino acids; ND, not detected; SD, standard deviation.



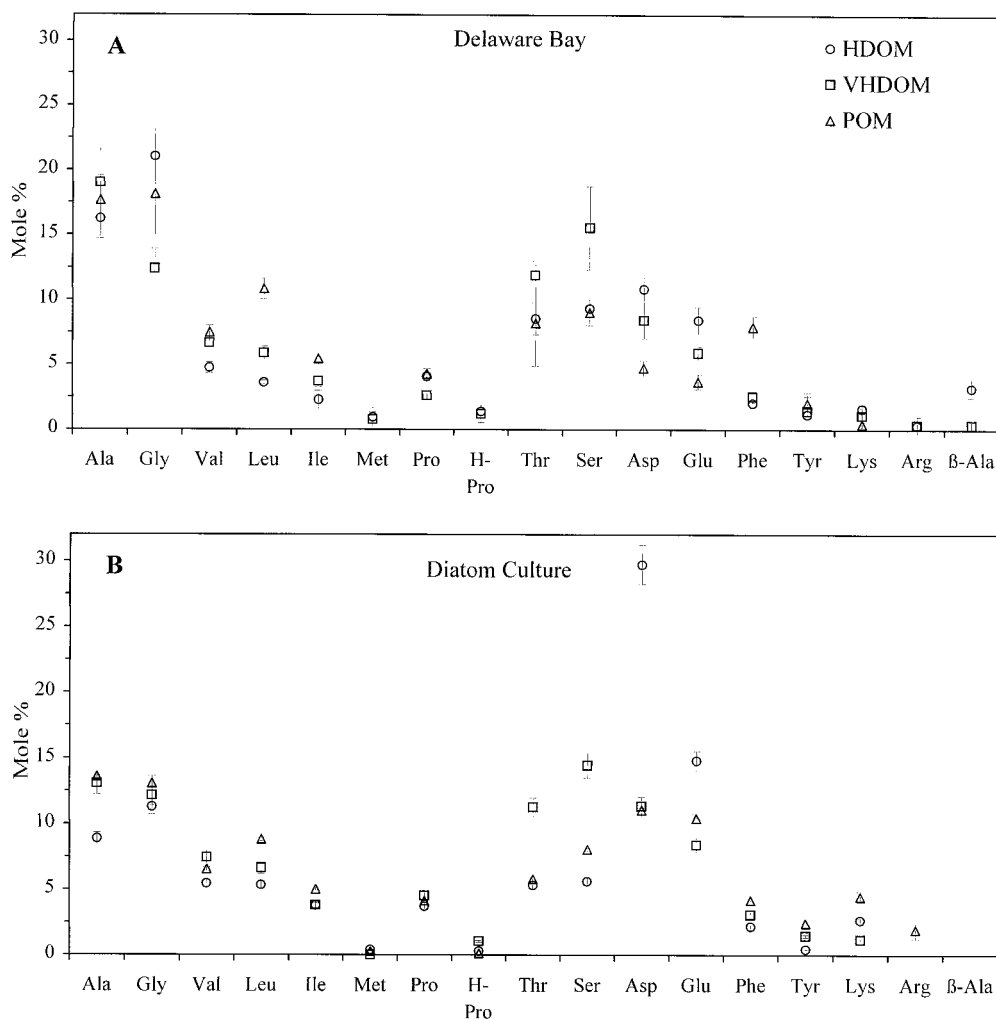


Fig. 5. Mole percentage distributions of individual amino acids for particulate and dissolved size fractions (A) across the entire Delaware Estuary (mean  $\pm$  1 SD) and (B) from the axenic diatom culture (average  $\pm$  1 SD;  $N = 2$ ).

stituents must be considered. Although, Guo and Santschi (1996) found no change in retention characteristics for Vitamin B-12 (1.33 kDa) between deionized water and a solution of seawater, the retention of raffinose (0.595 kDa) decreased at the high ionic strength. However, in the Delaware Estuary shifts in the organic composition (polysaccharide and THAA content; Fig. 3) among particles and dissolved components along the salinity gradient appear to be site specific and unrelated to ionic strength. In addition, the amino acid composition within each of the dissolved fractions was very similar along the estuary as shown by the narrow deviations for each amino acid, with several notable exceptions (Table 2; Fig. 5A). Such evidence suggests that discrimination of organic constituents by ultrafiltration due to changes in ionic strength is of minor importance in this study.

*Physico-chemical processing*—Substantial variations in organic content and composition were observed between the riverine site and the turbid middle estuary, presumably due to physico-chemical processes. Microscopic examinations of

particles revealed a high mineral grain content for the turbidity maximum and Sta. 3 (4.2% organic carbon by weight). The nonconservative behavior of total DOC and each DOM fraction within the high turbidity zone illustrates the importance of physical processes, i.e., sorption and flocculation, and perhaps additional inputs (Mannino and Harvey 1999). Under experimental conditions of particle free seawater, sorption of several monomers including amino acids and glucose onto macromolecular DOM has been observed (Carlson et al. 1985). Wang and Lee (1993) demonstrated that sorption and desorption of small organic molecules (free amino acids and methylamines) to organic-free minerals and organic-rich sediments could occur within a few hours. Keil and Kirchman (1993) proposed that decreases they observed in dissolved free amino acids at the Delaware Bay turbidity maximum and corresponding increases in dissolved combined amino acids resulted from the adsorption of free amino acids onto  $<0.2 \mu\text{m}$  clay minerals, DOM, or both. Although our limited sampling in this region do not allow small shifts in THAA concentrations to be fol-

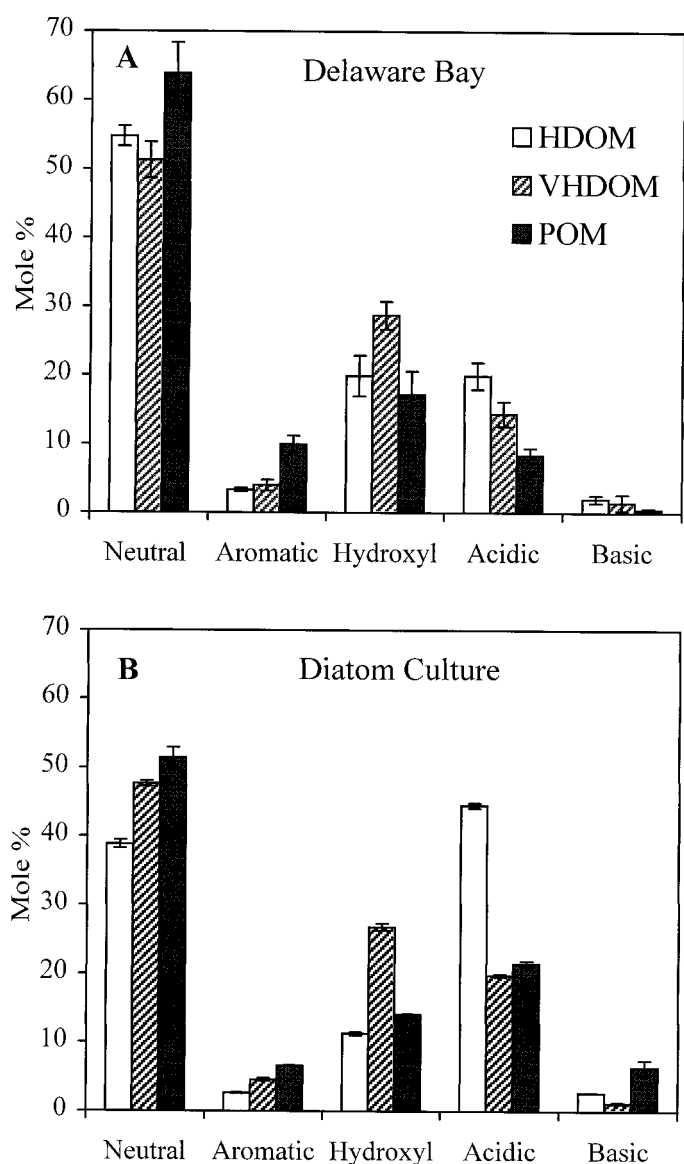


Fig. 6. Distributions of amino acid side-chain functional groups in the (A) Delaware Estuary and the (B) Axenic diatom culture on a mole percentage basis (mean  $\pm$  1SD). Neutral amino acids, Ala, Gly, Val, Leu, Ile, Pro, and Met; Hydroxyl, Ser, Thr and H-Pro; Acidic, Asp, and Glu; Aromatic, Phe, and Tyr; Basic, Lys and Arg.

lowed, it is apparent that sorption/desorption processes occur at sufficiently short time scales to affect the molecular weight distribution of DOC within turbid regions.

Sorption may have also influenced the composition of amino acids within macromolecular DOM. It has been observed that basic amino acids become enriched on particles by adsorption onto clay minerals through ionic interactions (Henrichs and Sugai 1993; Hedges et al. 1994). Yet in the Delaware Estuary, we observed that HDOM was enriched in lysine throughout the estuary while POM was depleted in lysine (Table 2; Fig. 5). Furthermore, at the turbid sites (Sta. 2 and 3) where mineral content was high, particles were relatively depleted in lysine (0.34 and 0.25 mole%), but HDOM was relatively more enriched in lysine (1.1 and 1.9

mole%). The polyelectrolytic nature of humics, major components of riverine and estuarine DOM, may also mediate adsorption of basic amino acids such as lysine as well as arginine, which comprised 1.2 mole% of HDOM-THAA at the turbidity maximum and <1 mole% elsewhere. Fine clay minerals also include constituents of <0.2  $\mu$ m material and have the potential to transfer basic amino acids into the macromolecular dissolved pool. It seems that adsorption or enrichment of basic amino acids may not be simply a particle versus dissolved issue, perhaps due to differences in macromolecular DOM composition as well as clay mineral content and composition.

In addition to changes in dissolved constituents, particle composition was also affected, with a shift from dominance by amino acids to polysaccharides between the turbidity maximum (Sta. 2) and Sta. 3 (Fig. 2). The composition of amino acids and  $\delta^{13}\text{C}$  values (Mannino and Harvey 1999) for POM remained similar at the two turbid stations, implying similar or related organic sources. Settling of particles and sorption of dissolved polysaccharides to particles could account for the declines in particulate THAA and dissolved polysaccharide concentrations, respectively, and also the compositional shift to higher polysaccharide content in particles between the turbidity maximum and Sta. 3. In addition to sorption, formation of polymer gels through aggregation of small molecules including carbohydrates (Chin et al. 1998), could explain the transfer of dissolved carbohydrates to the particulate size fraction. Additional work within turbid environments is needed to understand the processes that affect the distribution of organic compounds among particulate and dissolved size fractions.

*Amino acid composition as indicator of source*—The composition of certain individual amino acids changed along the estuarine gradient for each size fraction. Greater divergence in THAA composition was observed among the size classes in the riverine and turbid regions of the estuary (Table 2). Previous work in Delaware Bay revealed no such divergences in THAA composition among low and high molecular weight dissolved fractions (Coffin 1989; Keil and Kirchman 1993). In contrast, Hedges et al. (1994) found HDOM from the Amazon River to be enriched in aspartic acid, glycine, threonine, and all nonprotein amino acids ( $\beta$ -ala,  $\gamma$ -aminobutyric acid,  $\alpha$ -aminobutyric acid, and ornithine) relative to particles that were enriched in leucine and tyrosine plus the basic amino acids, lysine and arginine. Compared to phytoplankton, dissolved THAA from various marine waters contained higher amounts of serine and glycine and lower amounts of neutral and aromatic amino acids (Buffle 1988 and refs. therein). Differences in amino acid composition were also observed between size fractions in Delaware Bay, shifting from relatively higher proportions of neutral and aromatic amino acids to relatively more acidic and hydroxyl amino acids with decreasing size (Figs. 5A, 6A). Similar THAA distributions among size fractions between Delaware Bay samples and the *S. costatum* culture revealed that algal exudation could contribute to differences in amino acid distributions between size fractions (Fig. 5).

Shifts in amino acid composition along the estuarine gradient and among size fractions suggest changing organic

sources (Fig. 5A). Within the high chlorophyll region of Delaware Bay (Sta. 4 and 5), VHDOM was enriched in serine, which suggests enrichment of cell wall material in VHDOM (Table 2). Microscopic examination of particles revealed that diatoms dominated the plankton community in the lower estuary (Sta. 4 and 5) but were also present in the turbid middle estuary (Sta. 2 and 3) and coastal ocean (Sta. 6 and 7). Diatom cell walls are enriched in serine and glycine versus cytoplasmic amino acids (Hecky et al. 1973; Swift and Wheeler 1992). The association of serine and glycine rich proteins within the cell wall silica-protein matrix has been proposed as a mechanism for preservation of serine and glycine (Siezen and Mague 1978). Selective preservation (or enrichment) of serine and glycine was found in coastal marine sediments (Cowie and Hedges 1992b), in particles from the Pacific (Siezen and Mague 1978) and in POM from diatom decay experiments (Nguyen and Harvey 1997), but not in high molecular weight DOM from the oligotrophic ocean (McCarthy et al. 1996) where diatom production is minor. The THAA composition from Delaware Bay, especially in the lower estuary where primary production peaks, together with the high polysaccharide content and observed serine enrichment of VHDOM in the *S. costatum* culture are consistent with enrichment of diatom cell wall material within VHDOM (Table 2; Figs. 3, 5). The lower glycine content for VHDOM in the lower estuary may be due to additional sources of glycine at other sites.

Hydroxyproline, an atypical protein amino acid primarily found in glycosylated cell wall proteins of higher plants (Brett and Waldron 1990), certain algae (McConville 1982) and within the collagen of animals, was detected in Delaware Bay HDOM and VHDOM but not in particles. Distributions of hydroxyproline in macromolecular DOM indicated three source regions: (1) riverine site, (2) lower estuary (Sta. 4 and 5), and (3) coastal ocean Sta. 7 for VHDOM only (Fig. 7). Extensin, a hydroxyproline rich glycoprotein found in the cell walls of higher plants (40% of amino acids as hydroxyproline; 50–60% carbohydrate by weight; Sommer-Knudsen et al. 1998), may have contributed to the elevated hydroxyproline concentrations in HDOM (23 nM) and VHDOM (1.9 nM) at the riverine site (Fig. 7). Stable carbon isotopic signatures and lipid composition were consistent with a predominantly terrestrial origin for HDOM and VHDOM at the riverine site (Mannino and Harvey 1999). The association of extensin with cellulose and lignin within vascular plant cell walls (Brett and Waldron 1990) may enhance its preservation within the dissolved pool. Furthermore, the high degree of glycosylation in extensin and other hydroxyproline rich glycoproteins (Sommer-Knudsen et al. 1998) may confer greater resistance to microbial attack than nonglycosylated proteins (Keil and Kirchman 1993), permitting enrichment of these proteins in the macromolecular dissolved pool. However, at this time, other potential sources of hydroxyproline at the riverine site, which include collagen from animals and hydroxyproline rich cell wall glycoproteins from freshwater algae such as chlorophytes (Voigt et al. 1994), cannot be excluded.

Increasing concentrations of hydroxyproline in DOM from the lower estuary (Sta. 4 and 5) likely originate from diatoms. Hydroxyproline containing glycoproteins comprise

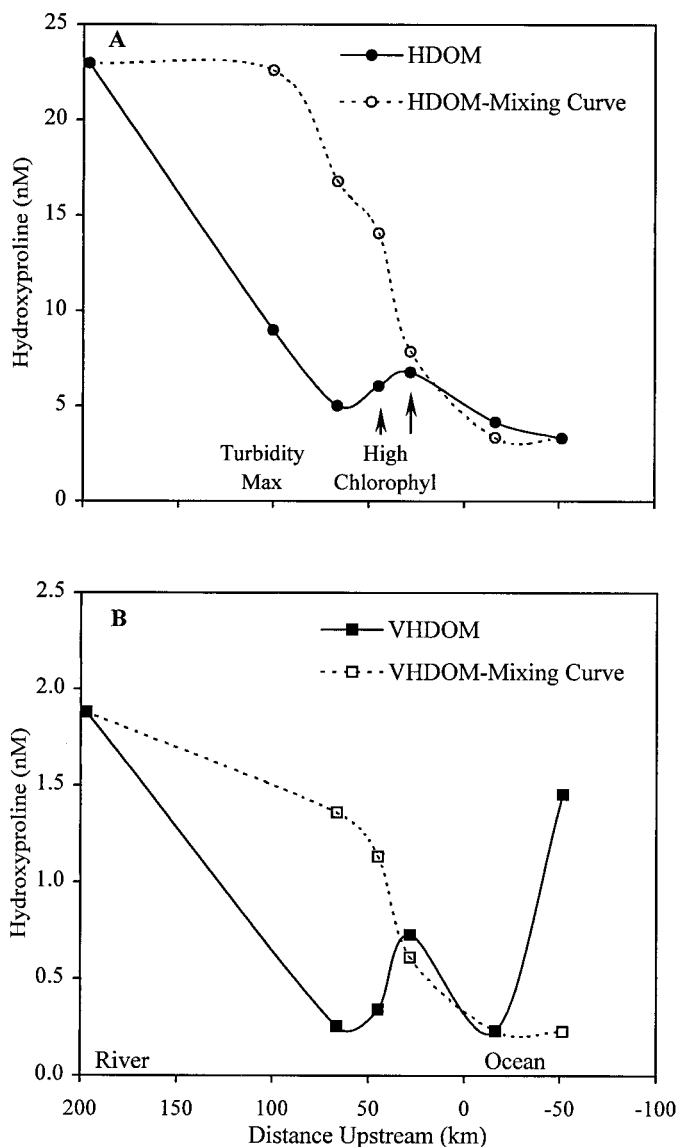


Fig. 7. Hydroxyproline concentrations for high molecular weight DOM fractions in Delaware Bay, (A) HDOM and (B) VHDOM. Open symbols and dashed lines indicate estimated hydroxyproline concentrations that are based on conservative mixing with salinity end-members (see Fig. 2 for details).

a substantial component of the cell wall proteins in diatoms (Kroger et al. 1994). In addition, hydroxyproline was found in *S. costatum* (0.12 mole%) and the macromolecular DOM released during its growth (Fig. 5B). The serine enrichment in VHDOM at Sta. 5 paralleled the higher concentration of hydroxyproline in VHDOM at this station, providing further support for enrichment of diatom cell wall material in VHDOM. The much higher concentration of hydroxyproline observed in VHDOM at coastal ocean Sta. 7 (1.5 nM) compared to Sta. 6 (0.23 nM) indicated additional sources. Various phytoplankton taxa and also copepods were observed at station 7, and potential sources of hydroxyproline include both collagen from copepods and algal derived proteins. To our knowledge, these results are the first evidence of hy-

droxyproline in high molecular weight DOM. Due to its limited distribution in proteins, hydroxyproline is a potential biomarker for distinguishing algal, terrestrial plant, or metazoan sources of proteinaceous matter in DOM when used in combination with compound specific stable isotope analysis.

**Reactivity patterns**—THAA and polysaccharide concentrations were similar in particles, whereas, in both DOM fractions polysaccharide concentrations were much higher than amino acids with similar patterns for carbon normalized values (Figs. 2, 8). This compositional shift from POM to dissolved fractions suggests that as particles are hydrolyzed to DOM and metabolized by microbial consumers, a greater portion of dissolved polysaccharides are inherently more resistant to bacterial degradation. However, in the diatom culture the chemical composition and THAA distributions for particles and dissolved fractions (Fig. 8) revealed that compositional differences between size fractions in natural waters are in part related to different sources of organic matter in the size classes rather than solely due to microbial decomposition of particles. Polysaccharides and THAA comprised similar amounts of VHDOM-C and HDOM-C in the *S. costatum* culture, with slightly higher amounts of both organic components in VHDOM (Fig. 8). For the diatoms, lipids and THAA comprised a substantially higher proportion of carbon than found in the dissolved fractions. The substantial difference in composition between HDOM from the diatom culture and Delaware Bay suggests that a portion of algal exudates within the HDOM fraction is utilized too rapidly by the microbial community to accumulate in the estuary and also that an uncharacterized refractory pool of HDOM persists throughout the estuarine gradient, as indicated by small shifts in HDOM- $\delta^{13}\text{C}$  between Sta. 2 and 5 (Mannino and Harvey 1999). Similarities in VHDOM composition between the culture and estuarine waters may be related to the low concentration of VHDOM (<3% of DOC), which could hinder its utilization. Statistical analyses revealed no significant correlations between the biochemical composition of POM and DOM in our Delaware Bay samples and in situ bacterial production rates or turnover rates of glucose and free amino acids conducted at the time of sample collection (Kirchman et al. unpubl. data). However, leucine-based bacterial production measurements were correlated with VHDOM carbon ( $r = 0.87$ ;  $P < 0.03$ ;  $n = 6$ ) and nitrogen ( $r = 0.90$ ;  $P < 0.02$ ;  $N = 6$ ). Over short time scales bacteria might consume only the most labile portion of organic matter, which may not reflect the polysaccharide or THAA content of each size fraction.

The most labile components of DOM are monosaccharides, primarily glucose, and dissolved free amino acids (DFAA). Low background concentrations of monosaccharides and DFAA are typically observed in natural waters because these compounds are utilized too rapidly to accumulate. Despite low concentrations, the turnover of glucose and DFAA provide substantial contributions to bacterial production, 5–33% from glucose (Rich et al. 1996; Skoog et al. 1999) and 14–100% from DFAA (Fuhrman 1987; Middelboe et al. 1995). Recent evidence indicates that uptake measurements of free glucose include glucose released by enzymatic hydrolysis of dissolved polymers (Skoog et al.

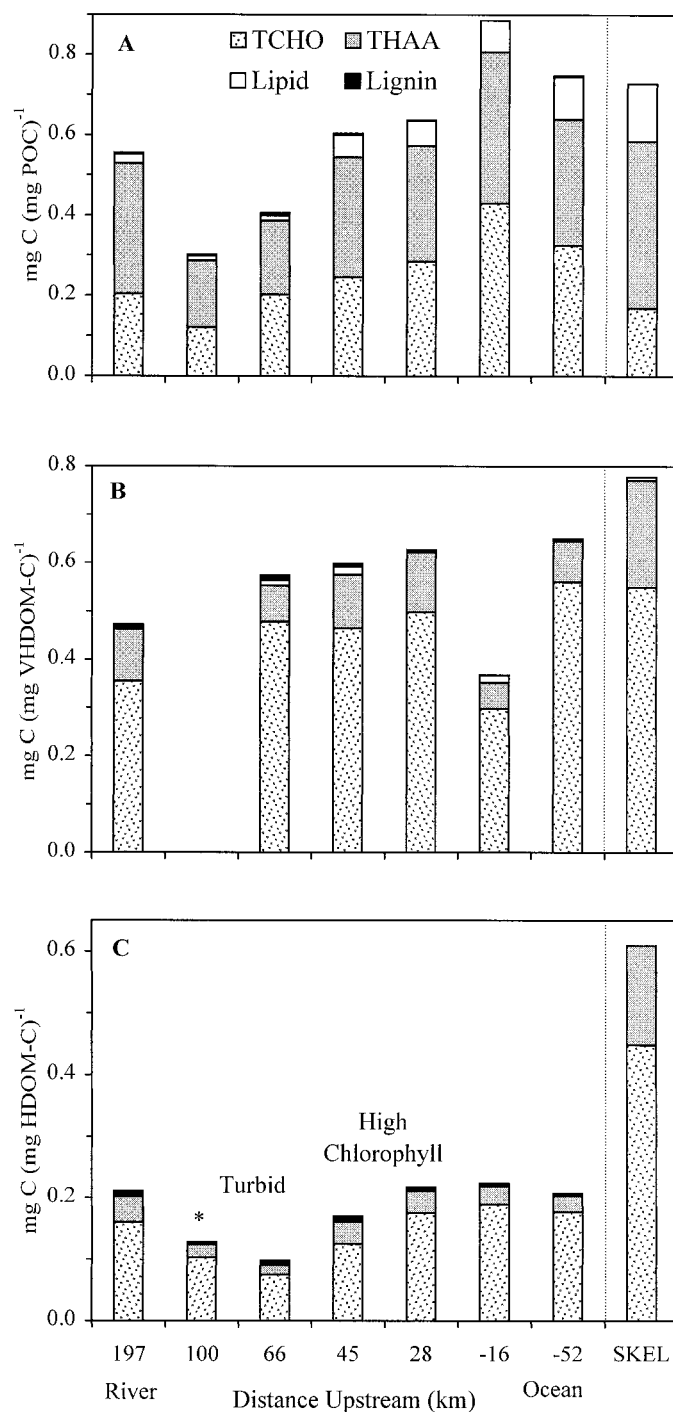


Fig. 8. Distribution of polysaccharides and amino acids along the Delaware Estuary and the *S. costatum* culture (SKEL). (A) POM, (B) VHDOM, and (C) HDOM. Figure includes lipids (Mannino and Harvey 1999) and lignin phenols (Mannino and Harvey, in review) from identical fractions. \* -1 kDa to 0.2  $\mu\text{m}$  DOM.

1999). A similar mechanism for the release of DFAA and subsequent assimilation by bacteria would provide a pathway for the rapid turnover of dissolved labile proteins and polysaccharides. The observed correlations between bacterial production and VHDOM carbon and nitrogen in the Del-



aware Estuary would be consistent with such a mechanism and can also explain the low concentrations of VHDOM. The rate limiting step for the consumption of glucose or DFAA would thus be the hydrolysis of polysaccharides or proteins.

Lipids and stable carbon isotopes reveal a changing pattern of sources along the estuary with a mixture of algal and terrestrial material in the river and turbid middle estuary and a predominantly algal/planktonic signal in the lower estuary and coastal ocean (Mannino and Harvey 1999). The biochemical composition of particles and high molecular weight DOM fractions in the Delaware Estuary suggest that POM and VHDOM more closely resemble living biomass (primarily plankton) than HDOM.  $\delta^{13}\text{C}$  signatures of POM and VHDOM are more similar in the plankton dominated regions of the lower estuary and coastal ocean, indicating similar sources (Mannino and Harvey 1999). Moreover, HDOM accounted for a lower fraction of organic matter as polysaccharide or THAA throughout the estuary (Figs. 3, 8). Yields and distributions of aldoses (neutral carbohydrates) in POM, >1 kDa DOM and LDOM from surface and deep waters of the Gulf of Mexico and equatorial Pacific Ocean indicated that POM was the most reactive fraction (i.e., contained the highest aldose yields and an aldose composition most similar to plankton), and LDOM the most recalcitrant (Skoog and Benner 1997). Polysaccharide and amino acid content as well as amino acid distributional patterns of POM and DOM in Delaware Bay and from the *S. costatum* culture also concur with the size-reactivity continuum model of Amon and Benner (1996), which postulates that the lability of organic matter generally declines along a size spectrum from POM to LDOM, with the exception of highly labile compounds such as glucose and DFAA. Other low molecular weight molecules may also cycle too rapidly to be measured by geochemical techniques. Within the turbid region of the estuary where photosynthesis is limited by light, polysaccharides and THAA accounted for the smallest fraction of HDOM carbon, whereas lignin comprised a significant portion of characterized HDOM (6.2% of characterized HDOM-C at Sta. 3; Mannino and Harvey, in review), suggesting a more highly degraded pool of material (Fig. 8). Since proteins and carbohydrates typically comprise 65% of terrestrial plant carbon ( $\leq 10\%$  as THAA; Cowie and Hedges 1992b) and 80% of algal carbon (19–42% as THAA; Cowie and Hedges 1992b; Nguyen and Harvey 1997) with lipids contributing an additional 10%, the majority of HDOM carbon does not resemble its original biological precursor, neither algae or terrestrial plants.

As seen for carbon, the bulk of organic nitrogen in HDOM and VHDOM does not consist of THAA, even though proteins comprise 30–88% of living algal/vascular plant nitrogen (Cowie and Hedges 1992b). Although the low amounts of dissolved THAA-N can result from the composition of molecules released by algae (Fig. 4), these results illustrate that much of the high molecular weight dissolved organic nitrogen (DON), especially for HDOM, is not in the form of hydrolyzable amino acids. In waters off the coast of Georgia, dissolved THAA comprised only 20% of total DON (Gardner and Stephens 1978). McCarthy et al. (1997) observed that THAA comprised only 8–9% of macromolecular

DON in the oligotrophic ocean but other amide nitrogen (protein or chitin like polymers) distinguishable by  $^{15}\text{N}$ -NMR constituted 65–86% of the remaining nitrogen. Chitin originating from diatoms such as *S. costatum* or amino sugars from bacterial cell walls may contribute to some of this unidentified nitrogen. Lara et al. (1997) observed that in addition to excreting amino acids, diatoms also excrete significant amounts of other hydrophilic nitrogenous molecules. Yet, the higher release rates of amino acids from vapor-phase hydrolysis of dissolved combined amino acids (DCAA) from Delaware Bay compared to liquid-phase hydrolysis suggest the presence of modified amino acids (up to 50% of DCAA) not recognizable as proteins by standard methods (Keil and Kirchman 1993). Since nonmodified proteins comprised only 1–10% of DCAA, Keil and Kirchman (1993) concluded that most DCAA in Delaware Bay was material similar in lability to glycosylated proteins. Modified proteins or those sorbed to clays or to macromolecular DOM may account for a portion of the unidentifiable nitrogen component we observed in VHDOM and HDOM (Fig. 3C). DOM fractions from the *S. costatum* culture contained much lower amounts of THAA than particles, implying that protein modification for the dissolved pool may occur prior to microbial attack. The low THAA-N content in HDOM may explain why bacteria utilize DFAA and ammonium as their primary source of nitrogen in Delaware Bay (primarily DFAA; Middelboe et al. 1995 and refs. therein) and in other systems (Kirchman 1994 and refs. therein).

It has been suggested that increases in the mole% of  $\beta$ -ala plus  $\gamma$ -aminobutyric acid ( $\gamma$ -aba) can be used to gauge the extent of degradation for specific fractions of particulate and dissolved organic matter (Lee and Cronin 1982; Cowie and Hedges 1992b; Hedges et al. 1994). Although  $\beta$ -ala plus  $\gamma$ -aba mole% increased within POM-THAA during decay sequences of diatom and cyanobacteria laboratory bloom simulations, no increase was observed for a similar dinoflagellate decay sequence (Nguyen and Harvey 1997). Nguyen and Harvey (1997) hypothesized that enrichment patterns for  $\beta$ -ala and  $\gamma$ -aba within algal detritus and sediments may be due to sorptive processes related to its surrounding matrix rather than preferential preservation. In the Delaware Estuary, HDOM contained higher mole% values of  $\beta$ -ala and lower amino acid nitrogen content than either VHDOM or POM (Figs. 3C, 5A) consistent with this fraction containing more degraded material. In addition,  $\beta$ -ala mole% contributions to HDOM-THAA and VHDOM-THAA were highest at the turbidity maximum and Sta. 3 (Table 2) where relatively high amounts of lignin and lower polysaccharide and THAA content also suggest a greater proportion of degraded material in HDOM and VHDOM (Fig. 8). Fresh algae are an unlikely source of  $\beta$ -ala as none was detected in particles or dissolved fractions from the *S. costatum* culture. Although the effects of sorption on  $\beta$ -ala distributions remain unclear,  $\beta$ -ala may be a useful diagenetic biomarker for high molecular weight DOM.

## Conclusions

Estuarine systems such as the Delaware represent complex systems where multiple organic sources, physical interac-

tions, and biological processes collide. The release of DOC through physical and biological processes was evident along the estuarine gradient. Amino acid composition reveals contrasting sources and extent of organic degradation along the estuarine gradient and among size fractions. HDOM contained lower amounts of polysaccharides and amino acids and higher mole% content of  $\beta$ -ala than VHDOM or POM, suggesting that HDOM was more degraded. Consequently, the geochemical evidence from the Delaware Estuary shows general agreement with the size-reactivity continuum hypothesis proposed by Amon and Benner (1996). Nevertheless, a portion of dissolved molecules released by algae, which cycle too rapidly for geochemical analysis, may not conform to the size-reactivity continuum. Phytoplankton production within the lower estuary generates a chemical signature in high molecular weight DOM with higher polysaccharide and amino acid concentrations and discernible changes in amino acid composition. Within the turbid region of Delaware Bay, where tidal flow begins to influence salinity, physical processes such as sorption/desorption alter the concentration and molecular weight distribution of DOC and biochemical components. Our results also suggest that structural biopolymers that are inherently more resistant to microbial degradation become enriched in macromolecular DOM, some of which may be released directly by algae. Thus, in addition to modifications of biopolymers during microbial and/or abiotic hydrolysis, the structural nature of the organic matter released by phytoplankton may confer a degree of recalcitrance to HDOM.

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