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Dynamics of dissolved carbohydrates in the Chesapeake Bay: Insights from enzyme activities, concentrations, and microbial metabolism

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Abstract

The interactions between heterotrophic microbes and high-molecular-weight (HMW) dissolved organic carbon in estuaries are complex and poorly understood. This study examined the coupling between hydrolysis of HMW carbohydrates (polysaccharides) and uptake of monosaccharides by bacterioplankton along a salinity gradient in the Chesapeake Bay water column and nearby coastal waters in order to evaluate the potential importance of polysaccharides as a carbon source for the estuarine microbial loop. We measured the rates of enzymatic hydrolysis of six polysaccharides (arabinogalactan, chondroitin sulfate, fucoidin, laminarin, pullulan, and xylan) as well as total carbohydrate and monosaccharide concentrations, bacterioplankton abundance, and monosaccharide assimilation rates. Enzymatic hydrolysis rates were sufficiently rapid to produce on a daily basis 40–62% of the monosaccharides present in Chesapeake Bay surface waters but a lower percentage (23%) of monosaccharides present in surface water on the continental shelf. Rates of both monosaccharide assimilation and polysaccharide hydrolysis were markedly lower on the continental shelf than in the Chesapeake Bay. These measurements suggest that at the time of sampling, polysaccharides in the Chesapeake Bay were rapidly recycled, while rates of cycling were considerably slower on the nearby continental shelf. In contrast to the apparently rapid turnover of bulk polysaccharides, hydrolysis of two polysaccharides, pullulan and chondroitin sulfate, was essentially undetectable, implying that those substrates would be unavailable to the microbial communities sampled on a timescale of 10 d.

Estuaries function as complex, dynamic bioreactors processing organic matter. Input of organic matter from terrestrial sources supplements primary production by estuarine phototrophs. The combined dissolved and particulate pools of autochthonous and allochthonous organic carbon are modified and in part remineralized in the estuary prior to export to the ocean. The heterotrophic microbial communities processing this rich mixture of organic compounds are likewise complex, often comprising (depending on river and estuary characteristics) distinct freshwater and marine communities, with estuarine-specific communities evident when water residence times at intermediate salinities are sufficiently long (Crump et al. 2004). The output of organic matter from estuaries into the coastal ocean thus represents the end product of the combined capabilities of these communities extracting carbon and energy from the diverse potential substrates at hand.

High-molecular-weight carbohydrates—polysaccharides—constitute a considerable fraction of estuarine as well as marine dissolved organic matter (DOM). In the Delaware Estuary, for instance, carbohydrates constituted 30–56% of very-high-molecular-weight (30 kDa to 0.2 μm) dissolved organic carbon (DOC) and 7.5–19% of high-molecular-weight (1 kDa to 30 kDa) DOC (Mannino and Harvey 2000*a*). Within the broad classes of high-molecular-weight (HMW) and low-molecular-weight (LMW) carbohydrates, however, dynamic and compositional information remains elusive.

Heterotrophic microbes rely on extracellular enzymes to hydrolyze HMW carbohydrates into fragments small enough for direct uptake (roughly, trisaccharides or smaller; Benz and Bauer 1988). Enzymatic hydrolysis of polysaccharides may be decoupled from uptake of hydrolysate (Smith et al. 1992; Arnosti et al. 1994), so each of these steps represents a potential control on the cycling of dissolved carbohydrates. The complex nature of these transformations, as well as the wide variety of structures

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within the broad class of HMW carbohydrates (and the wide variety of enzymes to hydrolyze them), pose a major challenge in understanding the role of estuarine heterotrophic microbes in processing carbohydrates. The extent to which particulate and dissolved carbohydrates are transformed and remineralized by microbial communities thus depends critically on their structure, and on the activities and nature of the extracellular enzymes present in the system.

The molecular-scale composition of estuarine DOM has been painstakingly documented in a number of studies (Mannino and Harvey 2000*a*; Minor et al. 2006), yet characterizing DOM and particulate organic matter (POM) at a sufficient level of detail to characterize microbial processing of specific organic structures is a major challenge in organic geochemistry (Hedges et al. 2000). The roles of specific microbial clades in processing organic matter have also been challenging to determine. Recent investigations have demonstrated that major microbial lineages differ in their relative proclivity to consume high- versus low-molecular-weight substrates (Kirchman 2002; Malmstrom et al. 2005); however, the underlying factors driving these differences remain to be established (Elifantz et al. 2005).

The goal of this study was to investigate the degradation and microbial consumption of carbohydrates along an axial transect of the Chesapeake Bay. These processes depend on the ability of bacterioplankton communities to access specific polysaccharide structures, and on the coupling between different steps in the hydrolysis and uptake of dissolved carbohydrates. To carry out this investigation, we measured in parallel the concentration of monosaccharides and polysaccharides, rates of monosaccharide uptake, and potential hydrolysis rates of six structurally defined polysaccharides at four stations along the main stem of the Chesapeake Bay and adjacent coastal ocean (Fig. 1). These measurements provide a means of comparing the degradation rates of specific polysaccharides with the microbial uptake of monomers that can be produced by hydrolysis of those polysaccharides. Using these data, rates of production and consumption of monosaccharides, variations in hydrolysis rates of polysaccharides as a function of polysaccharide structure, and potential turnover rates of the monosaccharide and polysaccharide pools were assessed and compared. By comparing these rates, we have estimated the rate of input of polysaccharides into the substrate pool. Although degradation rates of specific polysaccharides have been measured in a range of marine environments (Arnosti et al. 2005), hydrolysis rates along a riverine–estuarine transect have been investigated only in a single study, only a limited subset of substrates were available for that study, and no additional information on carbohydrate concentrations or microbial metabolism was obtained (Keith and Arnosti 2001). In the present study, concurrent measurements of monosaccharide assimilation rates, mono- and polysaccharide concentrations, and potential hydrolysis rates permit a first comparison of the detailed coupling of individual steps in the initial phase of remineralization of polysaccharides in the water column.

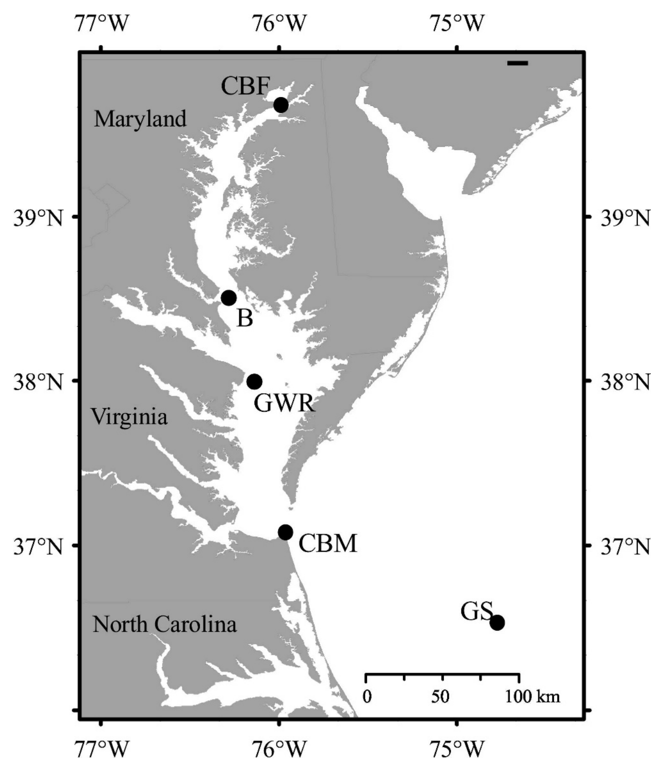

Fig. 1. Locations of sampling stations.

Methods

Study sites and sampling—Water samples were collected during a cruise aboard the R/V *Cape Henlopen*, 22 September 2003–26 September 2003. Samples were taken at one marine site (36.4354°N, 74.7574°W, Sta. GS) and at four estuarine sites in the Chesapeake Bay (Fig. 1), including one at the bay mouth (36.9625°N, 75.9974°W, Sta. CBM), two at mid-bay (37.8342°N, 76.1813°W, Sta. GWR; and 38.3257°N, 76.3308°W, Sta. B), and one at the head of the bay (39.4533°N, 76.0225°W, Sta. CBF). Surface-water samples (~1.5 m) were collected via the shipboard flow-through system at each site. Additionally, two subsurface-water samples, representing near-bottom (146-m water depth, several meters above the seafloor) and the depth of maximum dissolved oxygen (90-m water depth), were collected at the marine site (GS) using a 10-L Niskin bottle mounted on the conductivity-temperature-depth (CTD) probe. Concurrent with sample collection, vertical profiles of temperature and salinity were obtained. All samples were processed immediately, except for the subsurface samples, which were processed within 12 h of collection. Polysaccharide hydrolysis rates were measured at Sta. GS (surface, 90 m, and 146 m), CBM, B, and CBF, while carbohydrate concentration and bacterioplankton activity data were measured at Sta. GS (surface and 90 m), CBM, GWR, and CBF.

Hurricane Isabel passed over the Chesapeake Bay on 18 September 2003–19 September 2003, 3–7 d prior to sampling, causing disturbances that almost certainly affected biogeochemical cycles in the bay. However, at the time of sampling, physical parameters including

salinity, stratification, and total suspended sediment (Boicourt 2005; Matarrese et al. 2005), as well as biological parameters including bacterioplankton abundance and activity (Jonas and Tuttle 1990; Hamdan and Jonas 2006), were within typical ranges for the Chesapeake Bay in late summer/early fall. The fact that this wide range of physical and biological parameters was within normal bounds suggests that the snapshot of carbohydrates and bacterioplankton obtained here was within normal variability for this dynamic system.

Polysaccharide substrates—Arabinogalactan (mixed polymer of galactose and arabinose), chondroitin sulfate (sulfated polymer of *N*-acetylgalactoseamine), fucoidin (sulfated fucose polysaccharide), and xylan (β(1,4)-linked xylose) were obtained from Fluka. Laminarin (β(1,3)-linked glucose) and pullulan (α(1,6)-linked maltotriose [α(1,4)-linked glucose]) were obtained from Sigma-Aldrich. Fluorescent labeling was carried out by the method of Glabe et al. (1983) as modified by Arnosti (1996, 2003). Carbohydrate concentration was determined by the phenol–sulfuric acid method (Chaplin and Kennedy 1986) using the corresponding polysaccharide as a standard.

Substrate addition and sample incubation—Enzyme activity was determined by measuring the rate of degradation of fluorescently labeled (FL)-polysaccharides added to samples. A single concentrated FL-substrate was added to 50 mL of sample such that final substrate concentrations were 3.5 μmol L^{-1} (monomer equivalent) for chondroitin sulfate, laminarin, pullulan, and xylan. Because arabinogalactan and fucoidin tend to be labeled at a lower density than other substrates, they were added to a final concentration of 7.0 μmol L^{-1} (monomer equivalent).

Each 50-mL portion was mixed, divided into three replicates, and was incubated under one of two temperature regimes, depending on the in situ temperature. These replicates provide information about the precision of hydrolysis rate measurements from a single water sample, and about the extent to which rates might vary on subcentimeter spatial scales or due to changes in microbial community activity over the time course of incubation. Surface-water samples, with in situ temperatures of 21–24°C, were incubated in a plastic cooler at approximately 22°C. Due to shipboard logistical constraints, the 90-m and 146-m samples, for which in situ temperatures were 10–12°C, were incubated at 5°C. Subsamples (2 mL each) were removed for analysis after 0 d, 2 d, 4 d, 6 d, and 10 d, filtered through a 0.2-μm pore-size syringe filter, and immediately frozen. Samples were analyzed by gel permeation chromatography (GPC) by mixing 750 μL of sample with 300 μL phosphate buffer (100 mmol L^{-1} NaCl + 50 mmol L^{-1} phosphate, pH 8), and refiltered by 0.2-μm pore-size syringe filter just prior to GPC analysis.

Measurement of extracellular enzyme activity—Extracellular enzymatic activity was calculated as described in Arnosti (1995, 2000). In brief, the molecular-weight distribution of hydrolyzed polysaccharides was determined via GPC with fluorescence detection at excitation and emission maxima of 490 nm and 530 nm, respectively. Chromatographic conditions, column standardization, and rate calculations were as described in Arnosti (2000). Calculated rates are "potential" rates because the added substrates may compete with naturally occurring substrates for an unknown quantity of enzyme active sites.

Measurement of water column carbohydrate concentrations—Dissolved monosaccharides were analyzed according to Johnson and Sieburth (1977). Subsamples (20 mL each) were filtered through ashed (450°C for 4 h) Gelman-type A/E glass-fiber filters (nominal pore size 1.0 μm) into ashed glass scintillation vials and immediately frozen at −20°C until analysis (within 2 weeks). The three-step assay reduced monosaccharides to their respective sugar alcohols, which were then oxidized to aldehyde groups and finally derivatized with 3-methyl-2-benzothirazolione hydrazone hydrochloride (MBTH; Sigma-Aldrich). Absorbance was measured at 635 nm on a Lambda 25 UV/VIS Spectrometer (Perkin-Elmer). Monosaccharide concentrations were calculated using glucose standards (range 0.1–500 μmol L^{-1}), and are reported as μmol C L^{-1} assuming monomers are hexoses (6-carbon sugars).

Total dissolved carbohydrates were measured similarly, using a modification of the method for dissolved monosaccharides (Johnson and Sieburth 1977). Subsamples (20 mL each) were collected, filtered, and preserved as for monosaccharides. The MBTH assay was preceded by an acid hydrolysis step in which 0.5 mL of 1 mol L^{-1} hydrochloric acid was added to 5 mL of sample (in triplicate) in a glass tube with a Teflon-lined, gas-tight cap and allowed to react for 20 h ± 2 h at 100°C. The concentration of polysaccharides was calculated by difference between total dissolved carbohydrates and monosaccharides; reported error was propagated from error associated with the measurements of total carbohydrates and monosaccharides.

Measurement of in situ metabolic activity—Glucose assimilation was measured using ^{14}C-labeled glucose, according to Hamdan and Jonas (2007) using a modification of the methods of Jonas et al. (1988) and Smith and Azam (1992). Prior to sample collection, 2.0-mL capacity screw-cap microcentrifuge tubes were inoculated with 5 μL of a solution containing a final concentration of 1.2 μg L^{-1} D-[U-^{14}C] glucose (ICN Radiochemicals). One of four replicates received 50 μL of 100% trichloroacetic acid (TCA) prior to sample addition (5% final concentration after sample addition) and served as an abiotic control. One milliliter of sample was added to each tube; all tubes were incubated in the dark at in situ temperature for 1 h. To terminate incubations, macromolecules were precipitated with 50 μL 100% TCA and samples were immediately frozen until analysis. Upon return to the lab, samples were thawed, vortexed, centrifuged for 8 min at 16,000 × g to pellet precipitated macromolecules, and aspirated. Pelleted samples were mixed with 1 mL 5% TCA, centrifuged, and pelleted two additional times to remove any unincorporated radiolabel. Radioactivity was

determined on a Beckman-Coulter LS6500 liquid scintillation counter.

The rate constant for glucose assimilation, k_{glu}, was calculated as

$$k_{glu} = {}^{14}C_p / ({}^{14}C_t \times t) \quad (1)$$

where $^{14}C_p$ is the particulate radioactivity due to ^{14}C, $^{14}C_t$ is the total amount of radioactivity due to ^{14}C added, and t is incubation time (all equations and their results are compiled in Table 1). Making the simplifying assumption that glucose assimilation can serve as a proxy for monosaccharide assimilation in general, monosaccharide assimilation rates (MAR) were calculated as

$$MAR = k_{glu} \times [ms] \quad (2)$$

where [ms] is the concentration of monosaccharides in units of mol hexose L^{-1}. Studies comparing rates of uptake of different radiolabeled monosaccharides typically show that glucose is taken up as fast or faster than all other monosaccharides (e.g., Bunte and Simon 1999). Glucose is also typically among the most abundant monosaccharides in estuarine and ocean water (Kirchman and Borch 2003; Grossart et al. 2004). These results therefore likely set an upper limit on the rate of monosaccharide assimilation by the community.

Measurement of bacterioplankton abundance—Bacterioplankton abundance was determined according to Hobbie et al. (1977) as modified by Hamdan and Jonas (2006, 2007). Duplicate subsamples were diluted 1 : 1 with 0.2-μm filtered Chesapeake Bay water, stained with 0.1%, 0.2-μm filtered acridine orange, collected on 0.2-μm black polycarbonate filters, and observed at 1,000× magnification. High-resolution images were captured using a Retiga 1300 (Q-Imaging) high-sensitivity digital color camera and enumerated using Bioquant Nova Prime software (©2001).

Statistical analysis—Statistical comparisons of surface-water polysaccharide hydrolysis rates, monosaccharide and total carbohydrate concentrations, and rate constants for glucose assimilation were made by one-factor analysis of variance (ANOVA) using the fit-y-by-x platform in JMP 5.0.1a. Post hoc comparisons were made for all pairs using Tukey–Kramer Honestly Significant Difference. Statistical differences in bacterioplankton abundance among sites were not significance tested because only duplicate measurements were made. Polysaccharide concentrations and monosaccharide uptake rates were not statistically tested because these quantities are calculated from independent direct measurements, and so do not satisfy the ANOVA criterion of independence of replicates.

Results

Extracellular enzyme activities along a salinity gradient—Hydrolysis rates of polysaccharides varied from very rapid to near-detection limit. The rates reported reflect 4-d incubations for all substrates except pullulan (10-d incubation; Fig. 2). In general, hydrolysis rates were similar (within a factor of two) for all incubation times. Laminarin, fucoidin, and xylan were hydrolyzed more quickly in the bay stations (Sta. CBF, B, and CBM) than on the continental shelf (Sta. GS). Arabinogalactan and chondroitin sulfate showed no statistically significant differences in hydrolysis rate among stations. Pullulan hydrolysis was statistically distinguishable among stations, but considerably slower than all other substrates, and very close to the detection limit. Among the bay stations, xylan, fucoidan, and laminarin showed different patterns of hydrolysis: xylanase activity was high and nearly equal at all three bay sites, while fucoidinase activity was highest at the bay mouth (Sta. CBM), and laminarinase activity was highest at Sta. B.

Overall, xylan hydrolysis was fastest, followed by fucoidan, laminarin, or arabinogalactan, depending on the location, with much lower rates of chondroitin sulfate hydrolysis. Pullulan hydrolysis was close to the detection limit. Differences in hydrolysis rates between substrates were considerably larger than differences between stations for a single substrate. Maximum activity of xylanase (28 nmol monomer L^{-1} h^{-1}) was ~60 times greater than maximum activity of pullulanase (0.48 nmol monomer L^{-1} h^{-1}). This general trend was also apparent for the other time points.

Carbohydrate concentrations and uptake—Concentrations of total dissolved carbohydrates averaged 24–44 μmol C L^{-1} (Fig. 3a). Monosaccharides accounted for 43–74% of the total carbohydrates in surface-water samples, with an average of 61%. Between-station differences in monosaccharide and total carbohydrate concentrations were not statistically significant ($p > 0.1$). Rate constants of glucose assimilation (Fig. 3b) varied systematically along the salinity gradient, with values in excess of 0.01 h^{-1} at the bay head (Sta. CBF) and $<7 \times 10^{-4}$ h^{-1} at Sta. GS. Monosaccharide assimilation rates (Fig. 3c) followed the same pattern due to the relatively constant concentration of monosaccharides among sampling locations.

Cell counts—Bacterioplankton abundance in surface waters ranged from 1.9×10^6 cells mL^{-1} to 11.2×10^6 cells mL^{-1} (Fig. 3d). Bacterioplankton abundance at 90 m at Sta. GS was below the detection limit of the counting protocol used. Statistical differences in bacterioplankton abundance among sites were not significance tested because only duplicate measurements were made.

Extracellular enzyme activities as a function of depth on the continental shelf—Enzyme activities were generally much lower in Sta. GS surface water than in surface samples from Chesapeake Bay (Fig. 4). These activities decreased further with depth at Sta. GS: enzyme activities in the 90-m and 146-m samples were far lower than in any surface-water sample. At 90-m depth, arabinogalactanase was the only enzyme with activity distinguishable from zero, whereas at 146 m, only laminarinase activity was distinguishable from zero. Xylanase had the greatest mean activity at 90 m, but the standard deviation for replicate samples was greater than the mean because only a single

Table 1. Calculation of carbohydrate dynamics: equations and results.

Eq.	Term	Units	Equation	Station			
				CBF	B/GS	CBM	GS
1	Rate constant for glucose assimilation	h^{-1}	$k_{glu} = \frac{^{14}C_p}{^{14}C_t \times t}$	0.010±0.0010	0.0045±0.0006	0.0039±0.0003	0.00067±0.00003
2	Monosaccharide assimilation rate	nmol L^{-1} h^{-1}	$MAR = k_{glu} \times [ms]$	32±3.7	14±1.1	11±5.3	1.6±0.61
3	Daily fractional monosaccharide turnover (due to assimilation)	d^{-1}	$\tau_{ms\text{-}assim} = 24 \times k_{glu}$	0.24±0.014	0.11±0.0085	0.094±0.0048	0.016±0.0042
4	Glucose utilization efficiency	Dimensionless	$GUE = \frac{MAR}{MAR + MRR}$	Assumed 20% or 40%			
5	Daily fractional monosaccharide turnover due to uptake (outflow)	d^{-1}	$\tau_{ms\text{-}uptake} = \frac{24 \times k_{glu}}{GUE}$ (GUE = 20%)	1.20±0.070	0.54±0.042	0.47±0.024	0.080±0.021
			$\tau_{ms\text{-}uptake} = \frac{24 \times k_{glu}}{GUE}$ (GUE = 40%)	0.60±0.035	0.27±0.021	0.23±0.012	0.040±0.011
6	Daily fractional turnover (inflow) of monosaccharides due to hydrolysis of polysaccharides	d^{-1}	$\tau_{ms\text{-}\Sigma EEA} = \frac{24 \times \Sigma EEA}{[ms]}$	0.40±0.029	0.58±0.026	0.62±0.17	0.23±0.057
7	Daily fractional turnover (outflow) of polysaccharides due to hydrolysis	d^{-1}	$\tau_{ps\text{-}\Sigma EEA} = \frac{24 \times \Sigma EEA}{[ps]}$	0.52±0.10	0.45±0.18	1.29±0.76	0.34±0.18

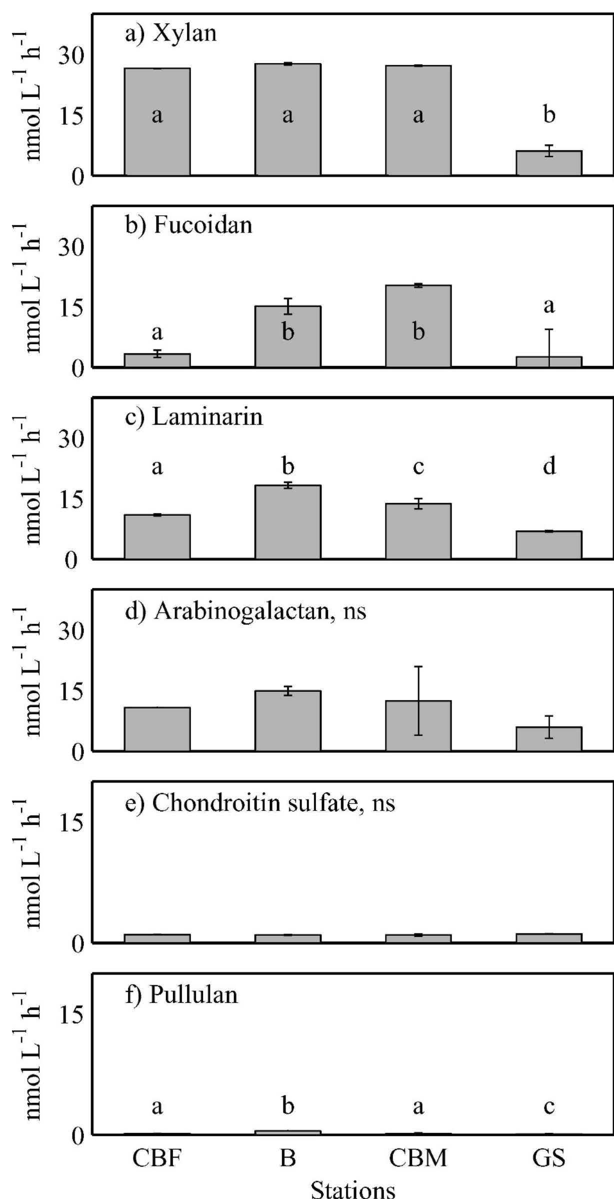

Fig. 2. Polysaccharide hydrolysis rates at the four surface stations. Note that axes for pullulan and chondroitin sulfate are half the scale of those for other substrates. Error bars are ±1 standard deviation. Bars marked with the same letter, within a single plot, are statistically indistinguishable. n.s. indicates that differences among sites were not significant. All data are from 4-d incubations, except pullulan, which was incubated for 10 d.

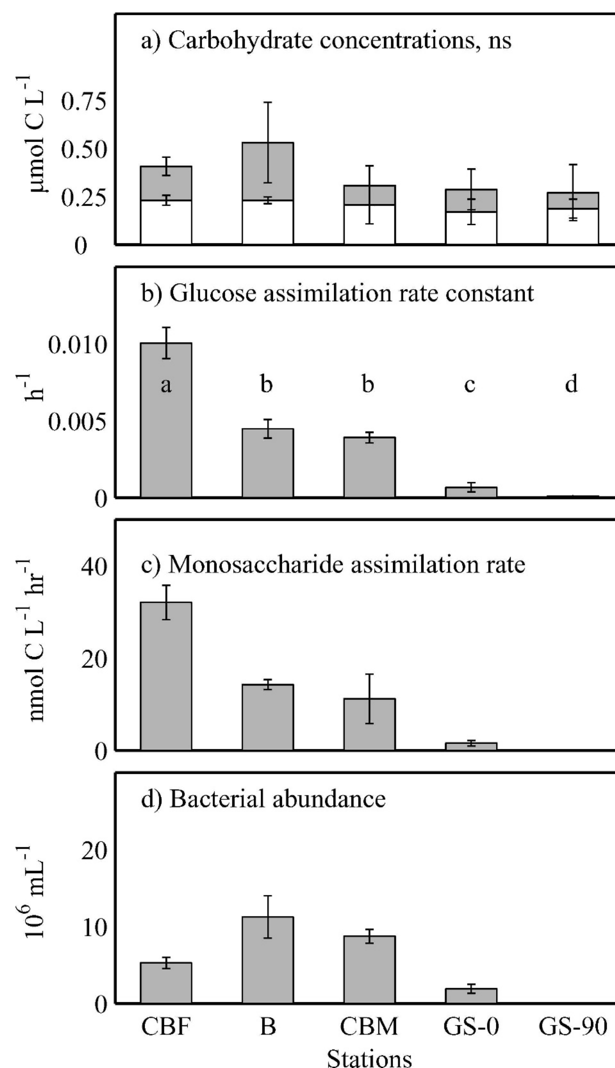

Fig. 3. Carbohydrate concentrations (a), metabolic activity (b, c), and bacterial abundance (d). Error bars are ±1 standard deviation (propagated error in the case of polysaccharides) except for bacterioplankton abundance, for which they indicate the range of duplicate samples. White bars denote polysaccharides, gray bars denote monosaccharides. At 90 m, there were too few bacteria to count, and glucose turnover was 0.010 ± 0.008 μg L^{-1} h^{-1}. Bars marked with the same letter, within a single plot, are statistically indistinguishable. n.s. indicates no significant differences. Bacterial abundance and monosaccharide assimilation rate were not significance-tested (*see* Methods).

incubation vial showed hydrolytic activity. The patterns of hydrolysis rates at depth were qualitatively different from patterns of enzyme activities in the surface water: for instance, laminarinase activity was considerably greater than any other enzyme activity in the 146-m water sample, but it was not the most active enzyme in any of the six surface-water samples.

Discussion

Estimation of carbohydrate cycling rates: a simplified model—Concurrent measurements of polysaccharide hydrolysis and monosaccharide assimilation rates illuminate dynamics in carbohydrate cycling that cannot be determined solely via measurements of carbohydrate concentration and composition. In order to investigate these dynamics, we have employed a simple conceptual model describing estuarine carbohydrate cycling (Fig. 5). Under this scenario, polysaccharide input to estuarine DOM is due to the combination of diverse sources, including dissolved polysaccharides in terrestrial runoff, direct exudation of dissolved polysaccharides by estuarine autotrophs, and liberation of polysaccharides due to degradation of particulate organic matter (including living cells).

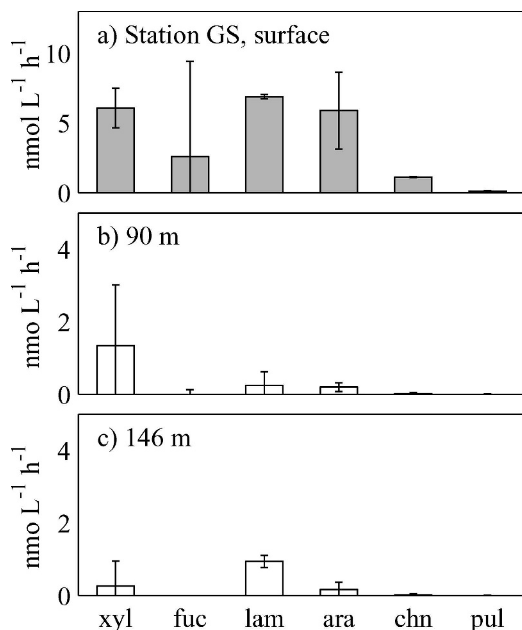

Fig. 4. Rates of hydrolysis of xylan (xyl), fucoidin (fuc), laminarin (lam), arabinogalactan (ara), chondroitin sulfate (chn), and pullulan (pul) at Sta. GS. Error bars are ±1 standard deviation. For the surface sample, all data except pullulan refer to the 4-d incubation. The pullulan data in the surface water, and all data points from the deep water, refer to the 10-d incubation, except chondroitin sulfate, which refers to the 4-d sample. Hydrolysis in these samples was generally not detectable prior to 10 d.

Polysaccharides may be hydrolyzed by extracellular enzymes or exported intact from the estuary. Monosaccharides resulting from polysaccharide hydrolysis may either be taken up by microbial heterotrophs or exported from the estuary; monosaccharides taken up by microbes may be respired or assimilated as biomass. This simple model addresses only biologically mediated processes that may act on estuarine carbohydrates, neglecting direct exudation of monosaccharides by autotrophs as well as abiotic processes, such as sorption of polysaccharides to sinking particles. This model also relies on the assumption that carbohydrate production and consumption are at steady state within each of the study sites.

This simplified framework provides a means to compare rates of degradation of dissolved polysaccharides with microbial uptake rates of carbohydrate monomers, and to constrain the rates at which dissolved carbohydrates are turned over in the estuary. Turnover rate of the monosaccharide pool can be investigated by measuring the rate of monosaccharide uptake by bacterioplankton, or via the potential rate of production of monosaccharides from enzymatic hydrolysis of polysaccharides. Using the measurements of monosaccharide assimilation rates, the fraction of the monosaccharide pool turnover over daily due to assimilation, $\tau_{\text{ms-assim}}$ can be estimated as

$$\tau_{\text{ms-assim}} = 24 \times k_{\text{glu}} \quad (3)$$

where k_{glu} is the glucose assimilation rate constant in units of h^{-1}.

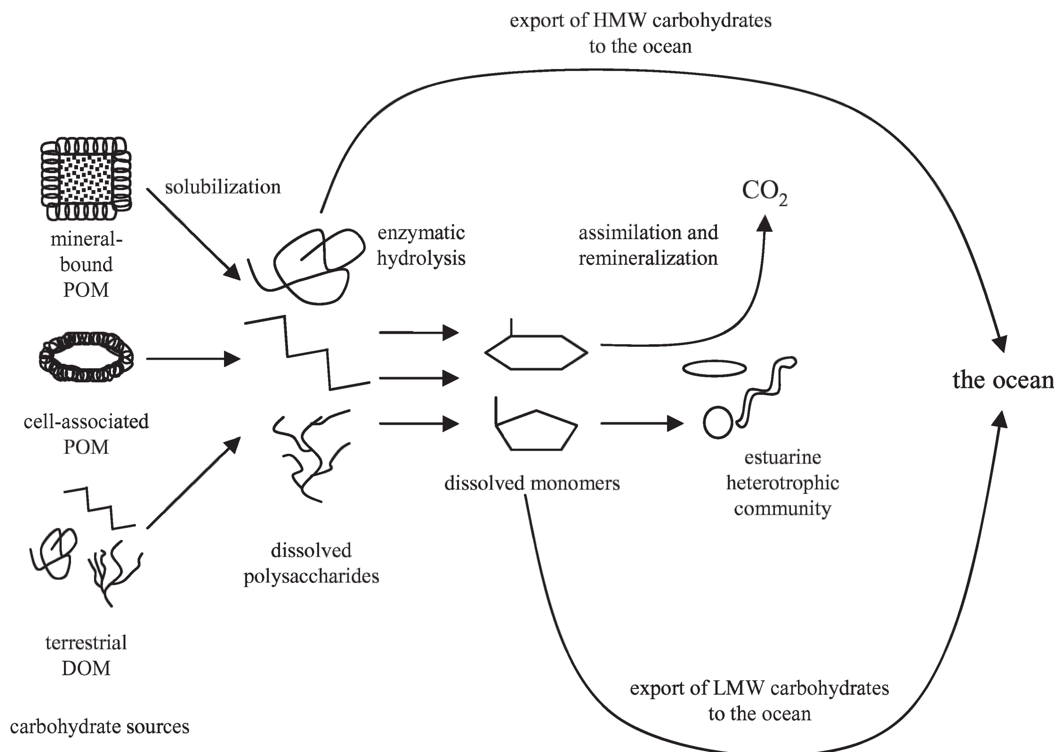

Fig. 5. Conceptual model relating some of the biologically catalyzed reactions of carbohydrates in an estuary.

Monosaccharides taken up by microbial heterotrophs may not all be assimilated, however; some fraction of total monosaccharide uptake is used to fuel respiration. This can be expressed as "glucose utilization efficiency" (GUE, after Kirchman et al. 2001),

$$\mathrm{GUE} = \frac{\mathrm{MAR}}{\mathrm{MAR} + \mathrm{MRR}} \tag{4}$$

where MRR is monosaccharide respiration rate. This quantity is analogous to bacterial growth efficiency as classically defined, except that it addresses only the assimilation and respiration of monosaccharides rather than total organic carbon. Assuming GUE of 20–40% (consistent with typical estuarine bacterial growth efficiencies of 20–40%; Del Giorgio and Cole 1998), then the daily fractional monosaccharide turnover due to microbial uptake, $\tau_{\text{ms-uptake}}$, is calculated as

$$\tau_{\text{ms-uptake}} = \frac{24 \times k_{\text{glu}}}{\mathrm{GUE}} \tag{5}$$

Alternately, turnover of the monosaccharide pool can be calculated using the potential rate of monosaccharide production from polysaccharides due to extracellular enzyme activity. In lieu of direct knowledge of the nature of all polysaccharides and polysaccharide hydrolases present in the water column, total extracellular enzyme activity (ΣEEA) was taken as the sum of hydrolysis rates for all polysaccharides measured. Using this method, $\tau_{\text{ms-}\Sigma\text{EEA}}$ was estimated as

$$\tau_{\text{ms-}\Sigma\text{EEA}} = \frac{24 \times \Sigma\mathrm{EEA}}{[\mathrm{ms}]} \tag{6}$$

where ΣEEA is the sum of activities of all polysaccharide hydrolases in the water column, expressed in units of nmol (monomer-equivalent) L^{-1} h^{-1}, and [ms] is the concentration of monosaccharides, expressed in units of nmol L^{-1}. Although polysaccharide hydrolases other than the ones measured could have contributed to the production of monosaccharides, information about the precise characteristics of enzymes active in aquatic systems cannot be determined with currently available methodologies. Furthermore, the enzyme activities measured here are relevant to this system (*see* below) so these data provide the basis for at least an initial estimate of carbohydrate cycling in the estuary.

The fraction of the polysaccharide pool turned over daily, $\tau_{\text{ps-}\Sigma\text{EEA}}$, can be estimated similarly, as

$$\tau_{\text{ps-}\Sigma\text{EEA}} = \frac{24 \times \Sigma\mathrm{EEA}}{[\mathrm{ps}]} \tag{7}$$

where [ps] is the concentration of polysaccharides, in units of nmol L^{-1} (monomer-equivalent).

Carbohydrate turnover in the estuary and offshore: production and consumption rates and their implications—As estimated using Eq. 1, the fraction of dissolved monosaccharides assimilated on a daily basis was 0.09–0.25 d^{-1} in the estuary and 0.016 d^{-1} at Sta. GS (Table 1).

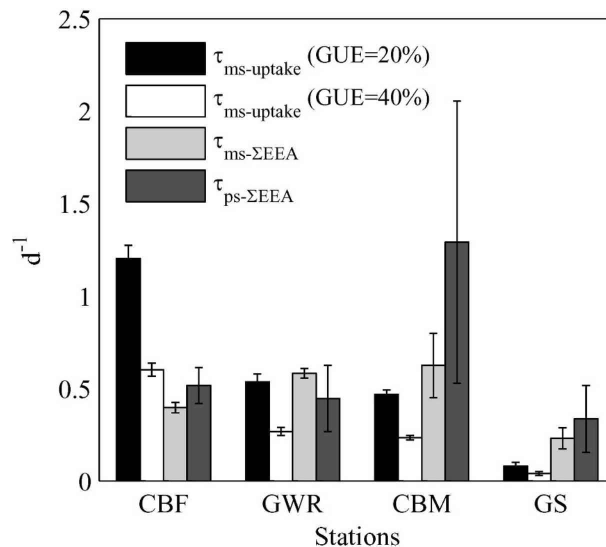

Fig. 6. Carbohydrate turnover and consumption. Monosaccharide uptake (assuming GUE of 20% and 40%) calculated according to Eq. 5. Fractional daily turnover of monosaccharide and polysaccharide pools due to extracellular enzyme activity calculated according to Eq. 6 and Eq. 7, respectively.

However, accounting for the fact that monosaccharides taken up by bacterioplankton may be respired as well as assimilated, a larger fraction of the monosaccharides present at each station is likely turned over daily (Fig. 6). The consumption of monosaccharides by bacterioplankton matches the production rate by enzymatic hydrolysis of polysaccharides much more closely at the two estuarine stations than at the freshwater or marine stations. With the exception of CBF, the bacterioplankton community appears to be capable of hydrolyzing polysaccharides more rapidly than it is able to take up the resulting monomers (Fig. 7). The substantial concentration of monosaccharides relative to polysaccharides at these stations supports this possibility. (Not all extracellular polysaccharide hydrolases are necessarily "intentionally" produced as such by heterotrophic prokaryotes; Karner et al. 1994).

Despite the fact that enzymatic hydrolysis of macromolecules has often been assumed to be the rate-limiting step in the microbial degradation of organic matter (Arrieta and Herndl 2002), hydrolysis of polysaccharides by extracellular enzymes has been observed to outpace consumption of those polysaccharides in enrichment cultures of marine bacteria (Arnosti et al. 1994), sediments (Brüchert and Arnosti 2003), and in sinking marine snow (Smith et al. 1992). The observation that monosaccharide concentrations in the Chesapeake Bay are typically a substantial fraction of total carbohydrates (Hamdan 2003) suggests that it may be typical for hydrolytic rates to outstrip monosaccharide uptake in these locations.

High monosaccharide concentrations relative to polysaccharide concentrations in an environment in which monosaccharide turnover is rapid implies that sufficient polysaccharides are present to fuel monosaccharide production. From Eq. 5, fractional polysaccharide turnover rates are between 0.34 d^{-1} and 1.29 d^{-1}, which are

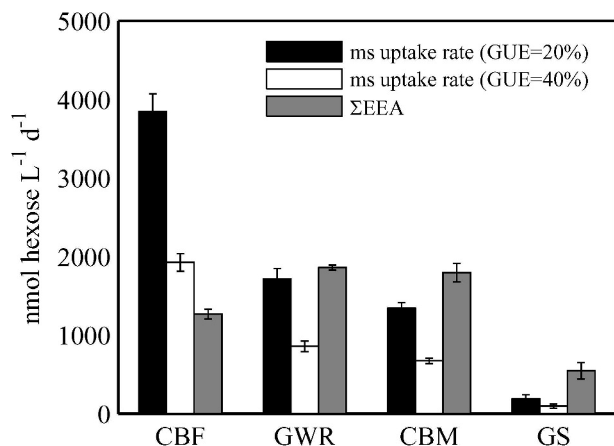

Fig. 7. Consumption of monosaccharides compared to potential production of monosaccharides by extracellular enzyme activity.

somewhat higher than fractional turnover of the much larger monosaccharide pool. These calculations suggest that on average the entire polysaccharide pool turns over on timescales of hours to days, a scenario consistent with suggestions that HMW DOM in general represents a dynamic, rapidly cycling subset of the DOM pool (Amon and Benner 1994).

This rapid turnover implies an equally rapid source of polysaccharides to replace those lost to enzymatic hydrolysis. Potential sources of polysaccharides include solubilization of "dead" POM, direct exudation of dissolved polysaccharides by estuarine phytoplankton, and input of dissolved allochthonous polysaccharides in terrestrial runoff (Bertilsson and Jones 2003). ^{13}C and ^{14}C signatures of HMW (>3 kDa) carbohydrates suggest that terrestrial runoff is a more important source of dissolved polysaccharides in the Susquehanna River (at the head of Chesapeake Bay) than at the bay mouth, where autochthonous polysaccharides were dominant (Loh et al. 2006). A variety of tracers showed a similar pattern in the Delaware Estuary (Mannino and Harvey 2000*b*), and Benner and Opsahl (2001) also observed an algal source of carbohydrates at mid-salinities in the Mississippi River plume. In the York River estuary, a tributary of the Chesapeake, terriginous matter accounted for much of the organic matter feeding bacterial production at lower salinities, while assimilation of organic matter of planktonic and marsh origin became dominant in more saline estuarine waters (McCallister et al. 2004).

The rapid rates of hydrolysis of laminarin suggest that diatom-derived polysaccharides may form a particularly rapidly cycling component of the estuarine DOM. Pelagic diatoms exist in the Chesapeake Bay year-round (Marshall et al. 2005) and may contribute up to ~30% of primary production as DOC (e.g., Biddanda and Benner 1997). If laminarin is representative of diatom-derived polysaccharides generally, these results point to rapid turnover of diatom-derived polysaccharides, particularly in the mid- and lower bay.

A comparison of the carbon-inflow estimates highlights the contrasts between the estuarine and marine stations. The scenario suggested by Fig. 6 shows that daily fractional turnover of carbohydrates is likely a high fraction of total carbohydrates in the freshwater and estuarine stations. At the marine station, however, daily fractional turnover of carbohydrates is only in the range of 3–6% of total carbohydrate concentration. Lower activity and relatively lower rates of carbon flow are consistent with lower bacterial numbers and lower enzymatic hydrolysis rates at Sta. GS relative to the other stations (Figs. 2, 3). Lower carbohydrate turnover at the marine station relative to the river and bay stations is also consistent with the possibility of lesser (in the absolute sense) phytoplankton-derived dissolved carbohydrate production and the decrease in direct input of terrestrial organic matter with increasing distance from the coast (Mannino and Harvey 2000*a*).

The turnover of carbohydrates was far more rapid in the bay stations than at the marine station, as shown independently by monosaccharide assimilation rates (Fig. 3) and by enzyme activities (Fig. 2). These changes may reflect differences in polysaccharide composition and bioavailability, and/or assimilation among different bacterial groups in heterotrophic microbial communities (Elifantz et al. 2005). Site-related variability in hydrolysis rates of specific polysaccharides likewise may be due to specific variations in bacterioplankton community composition or by variability in the presence of inducers or repressors for specific enzymes. With an average residence time of 12 months (Schubel and Pritchard 1987), far longer than the days to tens of days (several times the average doubling time of the microbial community) required for the development of distinct "estuarine" microbial communities (e.g., Crump et al. 2004), the Chesapeake Bay likely has a gradient of distinct microbial communities. Since the ability to consume HMW DOC varies considerably between bacterial phylotypes (Martinez et al. 1996) and between clades (Elifantz et al. 2005; Malmstrom et al. 2005), variation in abundance or activity of specific groups or phylotypes could drive the observed variation in extracellular enzyme activities.

Structural effects on polysaccharide bioavailability—Bulk HMW DOM is typically carbohydrate-rich and highly reactive (Amon and Benner 1994; Pakulski and Benner 1994). Within the class of HMW carbohydrates (polysaccharides), substantial differences in reactivity can exist. For instance, HMW neutral sugars made up ~45% of the semilabile DOM pool remaining after phytoplankton blooms in the Ross Sea, and yet HMW neutral sugars were also evident in the apparently recalcitrant DOM pools in the same study (Kirchman et al. 2001). The reasons for the differences in reactivity among polysaccharides presumably relate to the nature of interactions between the heterotrophic microbial community and the structures of polysaccharides present in DOM, but the factors driving those interactions remain unclear.

The slow hydrolysis of pullulan highlights the extent to which small changes in polysaccharide structure can lead to major changes in reactivity. In contrast to the rapid hydrolysis of laminarin, also a soluble glucose polymer,

none of the bacterioplankton communities sampled were able to hydrolyze pullulan after 10 d. This suggests that any pullulan or pullulan-like structures that entered the Chesapeake Bay would be unavailable to the microbial communities present, at least on timescales comparable to the 10-d duration of this experiment. Pullulan may not be degradable on longer timescales as well, since it remained unhydrolyzed in seawater from a variety of marine provinces after ~15 d of incubation (Arnosti et al. 2005), and in Delaware Estuary water after 40 d (Keith and Arnosti 2001).

It is possible that pullulan does not naturally enter the Chesapeake DOM pool in appreciable concentrations, so there is no selective pressure for Chesapeake bacterioplankton to maintain the ability to hydrolyze pullulan. In that case, the failure of the bacterioplankton communities to hydrolyze pullulan in this experiment would suggest that these communities are only capable of hydrolyzing substrates to which they are exposed on a regular basis. Under such a scenario, substrates that do not frequently constitute part of the Chesapeake DOM pool would be unavailable to the bacterioplankton community on a timescale of days to weeks.

If pullulan were an unrealistic substrate for pelagic bacterioplankton in the Chesapeake Bay, however, this would imply a sharp spatial zonation in the relevance of pullulan as a substrate. Pullulan is very likely a relevant substrate in the marshes bordering the Chesapeake Bay because the bacterium *Sacchrophagus degradans*, a model bacterium used to study the regulation of pullulanase expression, was isolated from a mat of decaying *Spartina alterniflora* in a Chesapeake Bay marsh (Ensor et al. 1999). Furthermore, pullulan hydrolysis has been rapid in every sediment in which it has been measured to date (e.g., Arnosti 1995, 2000) including sediments from the Delaware Estuary (K. Ziervogel pers. comm.). This suggests that pullulan is a relevant (i.e., microbially accessible) substrate in the marshes surrounding the Chesapeake Bay, and in the sediments of the bay, but not in the water column. A similar mismatch has been observed in the case of fucoidin, which is typically hydrolyzed in Svalbard sediment but not in fjord waters (Arnosti 2004), despite the presence of fucoidin-producing *Fucus* species in Svalbard fjords (Hop et al. 2002).

Enzymatic hydrolysis as a function of sampling depth on the continental shelf—The striking, broad decline in enzyme activities from the surface-water sample at Sta. GS to the deeper samples was likely due in part to kinetic factors—the deeper-water samples were incubated at 5°C, whereas the surface-water samples were incubated at ~22°C—and to the less abundant, less active microbial community at depth. However, the pattern of enzyme activities also changed with depth, implying changes in the nature of heterotrophic activity, as well as in the absolute rates. Such changes cannot be explained by temperature or kinetic effects alone but may be related to differences in water mass origin.

A temperature–salinity plot from Sta. GS suggests that the water below 45 m derived from a mixture of Gulf Stream and Cold Pool water, whereas the surface water was a mixture of the Chesapeake and Delaware Bay plumes with mid-Atlantic Bight surface water (A. Aretxabaleta pers. comm.). It is thus likely that the composition of the microbial community, or of the DOM pool, was different in the two deep-water samples than in other samples, perhaps also leading to different spectra of enzyme activities.

Due to logistical constraints, the two subsurface-water samples were incubated at a temperature about 7°C colder than the in situ temperature. As a general rule, a decrease in temperature of 10°C reduces activity of purified enzymes by a factor of two to four (Feller and Gerday 2003). If this relationship holds for the mixture of enzymes in our samples, measured rates of enzymatic hydrolysis were likely decreased by a factor of ~1.5–2.5 by the cooler incubation temperature. Nevertheless, relative differences in potential activities between surface water and deep water point to differing HMW DOM processing capacities of surface versus deep microbial communities.

We have investigated carbohydrate cycling in Chesapeake Bay by combining measurements of enzymatic hydrolysis of specific polysaccharides with measurements of dissolved carbohydrate concentrations and microbial glucose uptake. These measurements in combination yield inferences about the coupling of polysaccharide hydrolysis with monosaccharide uptake (i.e., initial and terminal steps of microbial remineralization of HMW DOM) that are not possible using other techniques. Monosaccharide cycling in the Chesapeake Bay at the time of sampling was rapid, with monosaccharide uptake rates sufficient to cause much or all of the monosaccharide pool to turn over daily. This suggests that dissolved polysaccharides are rapidly hydrolyzed in order to fuel this consumption of monosaccharides. Carbohydrate cycling was much slower on the nearby continental shelf, particularly in subsurface waters. In all surface-water samples except Sta. CBF, extracellular enzymes were capable of generating more than enough monomers to fuel microbial uptake of monosaccharides, providing evidence that extracellular hydrolysis is not always the rate-limiting step in degradation of HMW DOC, as is sometimes assumed (Arrieta and Herndl 2002). Even in the context of this rapid recycling of dissolved carbohydrates, some polysaccharides—which are environmentally realistic, and readily available to microbial communities in other aquatic environments—were essentially unavailable to the microbial communities we sampled over 10 d. Polysaccharides with structures similar to these may be able pass through sections of the Chesapeake Bay "undigested," so that they might fuel microbial metabolism far from their site of production.

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