

Functional variation among polysaccharide-hydrolyzing microbial communities in the Gulf of Mexico

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ABSTRACT

Marine polysaccharides are structurally diverse, as are the microbial communities capable of remineralizing them. Variations in the diversity, richness, and metagenome content of pelagic microbial communities are well documented, but variation in the spectrum of substrates accessible to those communities is less well understood. Here we investigate variability in the abilities of microbial communities to access specific polysaccharides along lateral and depth gradients in the Gulf of Mexico. Patterns of polysaccharide degradation during long-term incubations varied in ways that could not be predicted from bulk community measurements of bacterial production and glucose metabolism. There was greater diversity of function among epipelagic communities than among mesopelagic communities, and the communities in the two deepest water samples (700 m and 905 m) were more specialized in their abilities to access specific polysaccharide structures than were shallower-water communities. Timecourses of polysaccharide hydrolysis suggest that the capacity of communities to access specific polysaccharides may be influenced in part by variability in the composition or activity of the rare biosphere.

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1. Introduction

Polysaccharides constitute an abundant, reactive (Benner, 2002), and potentially structurally diverse (Painter, 1983) fraction of marine dissolved organic matter (DOM). Microbial degradation of polysaccharides first requires extracellular enzymatic hydrolysis, followed by cross-membrane transport of suitably small hydrolysis products. Efficient microbial metabolism of a diverse range of polysaccharides in DOM therefore requires members of the heterotrophic community to produce a diverse set of extracellular enzymes, membrane transporters, and metabolic pathways, matching the specific polysaccharide structures present.

Individual microbial species which specialize in metabolizing polysaccharides typically express a wide range of polysaccharide hydrolases, which act in concert to break down complex molecular structures (Bauer et al., 2006; Hutcheson et al., 2011). The specific set of polysaccharides accessible to individual microorganisms varies among species, however the extent of variation in the spectrum of polysaccharides available to an entire pelagic microbial community is only beginning to be explored (Arnosti et al., 2005, 2011; Steen et al., 2008; Ziervogel et al., 2010). Substantial variation exists in the composition, richness, and metagenome content of pelagic microbial communities (DeLong and Karl, 2005; Fuhrman et al., 2008; Konstantinidis et al., 2009),

as well as the abundance of genes encoding specific classes of polysaccharide hydrolases (Elifantz et al., 2008) as a function of location, depth, and time. Variability in the capacity for bulk DOM metabolism has also been observed among microbial communities as a function of water column depth: DOM that persists for months in stratified surface water may rapidly be mineralized by microbial communities inhabiting vertically adjacent mesopelagic water (Carlson et al., 2010; Morris et al., 2005). These variations suggest the possibility that the spectrum of polysaccharides available to whole microbial communities may differ among communities, in ways that would be difficult to predict from bulk geochemical measurements.

The goal of this study was to assess variation in the capacity of microbial communities to metabolize specific polysaccharides, along gradients of space and depth in the Gulf of Mexico. We compared rates and timecourses of enzymatic hydrolysis of six structurally defined polysaccharides with bacterial protein production and glucose metabolism to determine the relationship between the initial step in utilization of specific substrates and measures of bulk microbial community activity.

Dissolved polysaccharides are a particularly relevant lens through which to examine variations in heterotrophic community function. Polysaccharides represent a large fraction of high molecular weight DOM in the Gulf of Mexico and elsewhere (Pakulski and Benner, 1994), and are on average more rapidly degraded than bulk DOM (Amon and Benner, 2003; Wang et al., 2010). Understanding microbial community interactions with polysaccharides is therefore useful also in determining factors that may control the behavior of semi-labile DOM (Kirchman et al., 2001), and may also provide a model

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for the manner in which microbial communities interact with other classes of DOM that are more difficult to characterize chemically and study directly.

2. Methods

2.1. Study sites and sampling

Water samples were collected along depth profiles at three sites in the northern Gulf of Mexico on 27 and 28 September 2007, using Niskin bottles mounted on the conductivity-temperature-depth (CTD) rosette from the R/V *Cape Hatteras* (Table 1). Station 1 (28° 51.131' N, 89° 29.810' W; depth 41 m) was most influenced by the Mississippi River plume, while station 2 (28° 32.041' N, 89° 21.503' W; depth 285 m), and Station 3 (28° 16.369' N, 89° 21.772' W, depth 925 m) were further offshore. Concurrent with sample collection, vertical profiles of temperature, salinity and photosynthetically-active radiation (PAR) were obtained (PAR was not available at Station 1). Stations 2 and 3 both were characterized by photic zone depths of approximately 40 and 50 m, respectively. All samples were processed immediately aboard ship after collection.

2.2. Polysaccharide hydrolysis rates

Hydrolysis rates of fluorescently-labeled (fl-) arabinogalactan, chondroitin sulfate, fucoidan, laminarin, pullulan, and xylan were determined according to the method of Arnosti (1996, 2003). All six of these polysaccharides are relevant to carbon degradation in marine systems, since they are marine-derived, and/or enzymes hydrolyzing these polysaccharides have been identified in marine bacteria (e.g. Alderkamp et al., 1997; Araki et al., 2000; Arnosti and Repeta, 1994) and sequences corresponding to genes that code for these enzymes have been identified in the genomes of fully-sequenced marine bacteria (Bauer et al., 2006; Glöckner et al., 2003; Weiner et al., 2008).

Briefly, polysaccharides were fluorescently labeled according to the method of Glabe et al. (1983) as modified by Arnosti (1996, 2003). Fl-polysaccharides were added to 50 mL seawater to a concentration of 3.5 $\mu\text{mol monomer L}^{-1}$ (2.8 $\mu\text{mol L}^{-1}$ for xylan), which was then divided into three, 16.6 mL replicate samples. Additionally, sample water was autoclaved to serve as a killed control, and prepared in the same way except that only a single killed control was used. One, approximately 2 mL subsample was immediately withdrawn from each subsample, filtered using 0.2 μm pore-size surfactant free cellulose acetate (SFCA) syringe filters into microcentrifuge tubes and frozen. Incubations were carried out in the dark at temperatures listed in Table 1. Subsamples were withdrawn after 2, 4, 6, 11, 14, and 26 days. Fl-polysaccharide size distributions were determined using gel permeation chromatography. Size distributions and hydrolysis rates were calculated as per Arnosti (1996, 2003). Note that the rates determined

here are potential rates, since they are measured using an externally added substrate in competition with natural substrate of unknown concentration for enzyme active sites. Furthermore, the incubation times are sufficiently long that enzyme production and microbial growth may occur during the incubation. Substrate addition levels of 2.8–3.5 μM -monomer-equivalent polysaccharide (14–21 $\mu\text{M C}$) were comparable to likely total dissolved carbohydrate concentrations, so concentrations of added fl-polysaccharides were large relative to *in situ* concentrations of structurally homologous polysaccharides (Benner, 2002). These measurements therefore reflect the capacity of the microbial community to react to a relatively large input of fresh organic matter, over timescales consistent with changes in microbial populations. Maximum hydrolysis rates at 2 m depth at stations 1 and 2 have been reported previously (Arnosti et al., 2011) as part of a global compilation of polysaccharide hydrolysis patterns.

2.3. Polysaccharide hydrolysis data analysis

Polysaccharide hydrolysis rates were calculated for each timepoint of each incubation as the extent of hydrolysis determined chromatographically, corrected for the extent of hydrolysis observed in sterile controls, divided by total incubation time (Arnosti, 1996, 2003). The maximum hydrolysis rate among all timepoints in a sample is taken as a measure of the maximum rate at which the microbial community could access a specific polysaccharide. Hydrolysis timecourses were visualized by plotting the extent of hydrolysis as a function of incubation time.

In order to compare patterns of maximum hydrolysis rates at different depths and stations, cluster analysis was performed. Euclidian distances between sites (plotted by maximum hydrolysis rate, with each polysaccharide representing an orthogonal dimension) were calculated using the *pdist* function in MATLAB, and a dendrogram was calculated to express the resulting distance matrix. Confidence values at each node, expressing the likelihood that dendrogram topology is robust to hydrolysis rate variation among replicates, were calculated as described below.

Hydrolysis rate evenness, or the degree to which the summed hydrolysis rates were dominated by a single polysaccharide, was calculated using the Shannon index as described previously (Steen et al., 2010). For this experiment, the maximum possible evenness score was 1.79.

All other statistical analyses were performed using the *R* statistical environment. All *p*-values are corrected for multiple comparisons according to the Bonferroni–Holm adjustment.

2.4. Calculation of node confidence values for site relatedness dendrogram

Node confidence values were calculated using a custom Monte Carlo algorithm implemented in MATLAB R2008b. The algorithm constructed an ensemble of 10,000 synthetic data sets, in which each synthetic hydrolysis rate $r_{\text{stn,subs}}^*$ was given by

$$r_{\text{stn,subs}}^* = r_{\text{stn,subs}} + \varepsilon(0, \sigma_{\text{stn,subs}})$$

where $r_{\text{stn,subs}}$ is the measured rate (average of three replicates) for a given substrate at a given station and $\varepsilon(0, \sigma_{\text{stn,subs}})$ is a random number given by a Gaussian distribution with mean of zero and standard deviation equal to the standard deviation of the three replicates. A dendrogram was created for each synthetic data set. The confidence value for each node is equal to the fraction of synthetic dendrograms containing equivalent nodes (i.e., nodes containing exactly the same set of branches).

Table 1
Sample depths and characteristics.

Stn	Depth	Notable features	Salinity	In-situ temp, °C	Incubation temp, °C
1	surface	Surface	26	28	28
	35 m	Bottom	35.3	28	28
2	surface	Surface	34.5	29	28
	17 m	Just above pycnocline	35.5	29	28
	150 m		36	16	16
	285 m	Bottom	35.4	11	11
3	surface	Surface	35.5	29	28
	47 m	Chlorophyll maximum	36.5	25	28
	125 m		36.3	18	16
	350 m	Oxygen minimum	35.2	9.8	11
	700 m		34.9	6.4	6
	905 m	Bottom	34.9	5.4	6

2.5. Radiotracer measurements

Radiotracer rates were measured immediately after sampling, and therefore reflect *in situ* rates rather than responses to multi-day incubations. Leucine incorporation rates were determined using ^3H -labeled leucine (Sigma L-5897, 92.6 Ci mmol^{-1} , diluted by a factor of 5 with unlabeled L-leucine) according to Kirchman (1993), with the exception that precipitated protein was collected on $0.2\text{ }\mu\text{m}$ cellulose acetate filters. Final leucine concentration was 20 nmol L^{-1} in a 10 mL sample. Incubations were carried out at the same temperature as the fl-polysaccharide incubations. Incubation times were approximately 3 hr (approximately 6 hr for the 125 m, 250 m, 700 m, and 900 m depths at station 3).

Glucose incorporation and respiration rate constants were measured using methods modified from Rich et al. (1996) and Skoog et al. (1999) as well as techniques and equipment developed by D. Albert (pers. comm.). Prior to sample collection, $5.0\text{ pmol }^{14}\text{C}$ -labeled glucose (Sigma G-5020, 262 mCi mmol^{-1}) was added to 20 mL scintillation vials with plastic caps (for PO^{14}C measurement) or PTFE septum caps (for DI^{14}C measurement). Vials serving as killed controls were also amended with $526\text{ }\mu\text{L}$ 100% trichloroacetic acid (TCA) for a final concentration (including sample) of 5% (v/v) TCA. Within ten minutes of sample collection, 10 mL water sample were dispensed into each of six scintillation vials to serve as live incubations, and two vials containing TCA to serve as killed controls. Samples were mixed vigorously and incubated for approximately 1 hr at the same temperature as the fl-polysaccharide incubations; precise incubation times were used to calculate rates.

To measure respiration of ^{14}C -labeled glucose, incubations were stopped by adding 1 mL of 1 M NaOH and chilling on ice. CO_2 was then trapped using a sparging apparatus, in which each sample vial was connected to a 20 mL scintillation vial containing 15 mL modified Woeller's solution (50% ScintiVerse II scintillation cocktail, 25% methanol, 20% β -phenethylamine) via a trap containing 10 mL , 1 M H_2SO_4 . All vials had caps with PTFE septa and were connected using syringe needles such that gas evolved from one vial was bubbled through the next. Samples were connected to this system and then acidified with H_2SO_4 introduced via syringe. N_2 was bubbled through the system at a rate of several mL min^{-1} for 20 min, and radioactivity was measured in the trapping solution. Trapping efficiency was determined to be 100% by adding $\text{NaH}^{14}\text{CO}_3$ to 10 mL artificial seawater, pH 8.2, and treating this standard in the same way that samples were treated.

To measure ^{14}C -labeled glucose incorporated into biomass, the incubation was stopped by adding 1 mL 1 M NaOH and chilling on ice, after re-acidification to dissolve CaCO_3 using 1 mL 100% TCA. Samples were then filtered onto $0.2\text{ }\mu\text{m}$ mixed cellulose ester filters. Filters were dissolved in 15 mL modified Woeller's solution and radioactivity was measured as described above.

Glucose respiration and assimilation (collectively, glucose metabolism) rate constants k were calculated assuming first-order kinetics,

$$k = \frac{1}{t} \ln \left(\frac{a_{\text{glu},t0}}{a_{\text{glu},t0} - a_t} \right)$$

where t is incubation time, $a_{\text{glu},t0}$ is the blank-corrected activity of the initial glucose added, and a_t is activity at time t of PO^{14}C or DI^{14}C . The rate constant for total glucose uptake was calculated as the sum of the glucose respiration and assimilation rate constants. Error in k was propagated from the standard deviation among replicates for each measurement. Glucose utilization efficiency, the fraction of glucose assimilated into biomass, was calculated as $\text{GUE} = k_{\text{assim}} / (k_{\text{assim}} + k_{\text{resp}})$.

3. Results

3.1. Maximum hydrolysis rates

Hydrolysis rates of the six fluorescently-labeled polysaccharides were assessed during incubations of 2–26 days, using previously described methods (Arnosti, 1996, 2003). For each polysaccharide, maximum hydrolysis rates in the epipelagic ($\leq 47\text{ m}$ depth), taken as a group, were greater than rates in the mesopelagic ($\geq 125\text{ m}$; Student's t test, $n = 36$ for each substrate, $p < 0.05$; Fig. 1). Chondroitin sulfate, laminarin and xylan hydrolysis rates were faster in epipelagic samples than in mesopelagic samples by factors of 4.5, 4.8, and 4.1, respectively. Arabinogalactan, fucoidin and pullulan hydrolysis rates were also faster in the epipelagic than in the mesopelagic, but by smaller factors: 3.1, 2.4, and 2.8 respectively. The summed maximum hydrolysis rates in the epipelagic were on average 4.0 times faster than those in the mesopelagic (Student's t test, $p < 0.001$, $n = 36$).

The relative contribution of each substrate to the summed hydrolysis rate also differed significantly between samples (Figs. 2 and S1; one-way ANOVA, $p < 0.01$ for each substrate, with Tukey HSD post-hoc analysis, $n = 36$ for each substrate; QQ plots [not shown] indicate the data were approximately normally-distributed). When considered individually by polysaccharide, these differences were not related to depth zone (epipelagic vs mesopelagic, Student's t test, $p > 0.05$,

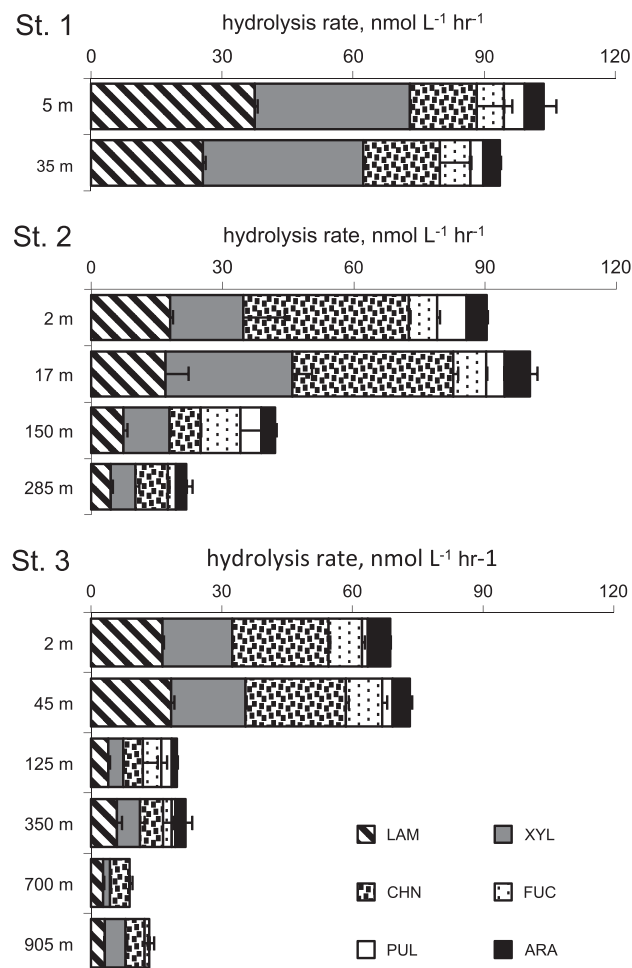


Fig. 1. Maximum hydrolysis rates for each substrate, depth, and station. Error bars indicate the standard deviation of three replicates, and point only to the right for clarity. Lam = laminarin, xyl = xylan, chn = chondroitin, fuc = fucoidin, pull = pullulan, ara = arabinogalactan.

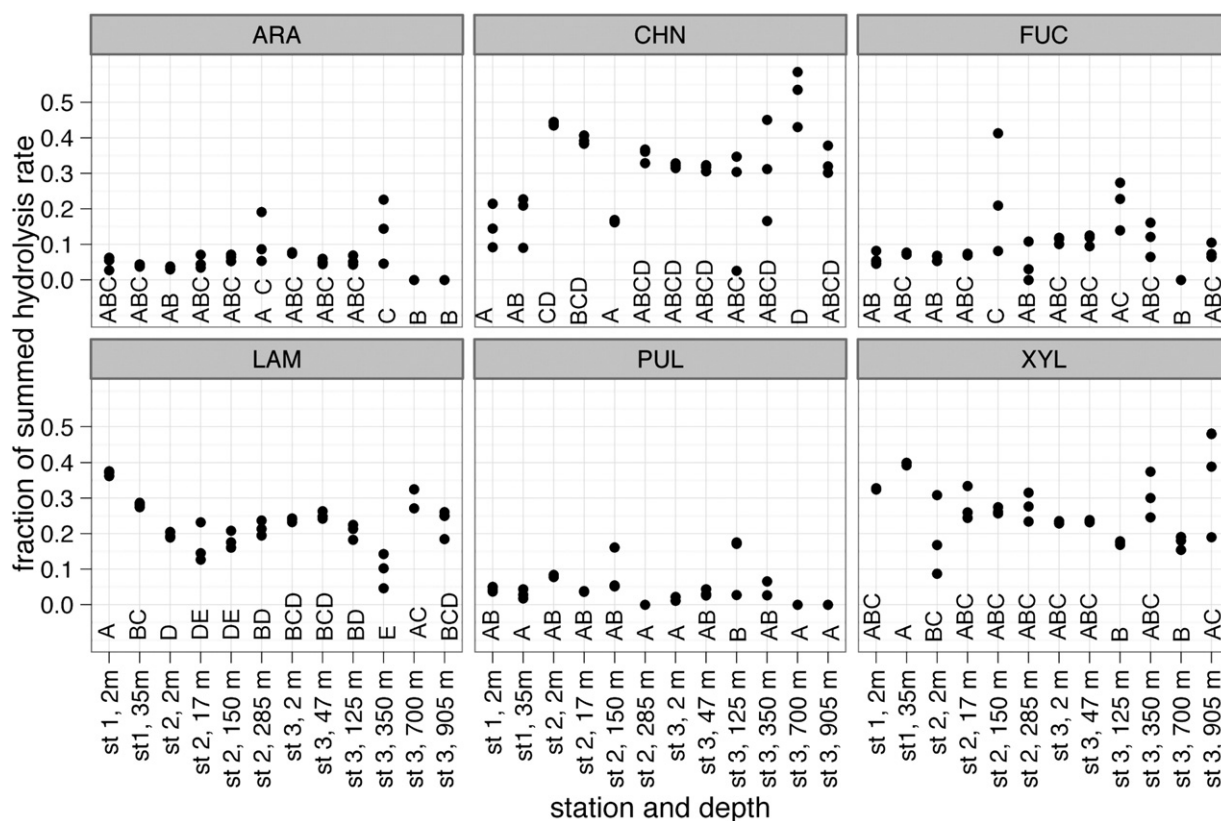


Fig. 2. Contribution of the hydrolysis rate of each polysaccharide in a water sample relative to the summed hydrolysis rates for all polysaccharides in that water sample. Samples sharing a letter have statistically indistinguishable relative rates (adjusted p -value > 0.05), when compared to the same polysaccharide in different water samples.

$n=36$ for each substrate) or any other obvious environmental feature. However, when all polysaccharide hydrolysis rates for a sample were considered together, cluster analysis revealed that patterns of hydrolysis rates were related to depth: all mesopelagic samples clustered together (Fig. 3). All epipelagic samples also clustered together with the exception of station 1 (2 m depth) which is the most deeply-branching of all samples. The dendrogram indicates much greater variation in hydrolysis rate patterns among epipelagic samples than among mesopelagic samples. In addition, hydrolysis rates at 700 m and 905 m depth at station 3 were substantially less even (i.e., communities were more specialized to access specific polysaccharides) than in warmer, shallower water samples (Table 2).

3.2. Timecourses of hydrolysis

The timecourse over which a polysaccharide is hydrolyzed provides information about the reaction of a microbial community to the input of a specific substrate. These timecourses varied considerably by polysaccharide, by sample, and by depth, showing patterns that are best visualized by plotting the total extent of substrate hydrolysis versus elapsed time (Figs. S2–S7). In this study, timecourses fit one of three patterns: i) roughly constant hydrolysis rates, beginning at time zero and continuing throughout the incubation or until the substrate was completely hydrolyzed; ii) hydrolysis that began or became considerably more rapid after a lag of two or more days, and iii) no detectable hydrolysis throughout the 26-day incubation. In deeper water samples, incubated at colder temperatures, lag times were typically longer and cases in which the polysaccharide was not detectably hydrolyzed were more common. However, the patterns were not solely determined by substrate, sample location, or depth. For instance, in station 1 surface water, arabinogalactan was hydrolyzed at an approximately constant rate throughout the first 14 days of incubation, whereas chondroitin hydrolysis was slow for the first two days and then much faster

between 2 and 4 days (Fig. 4). That pattern was reversed at station 2 in surface and 17 m water samples: arabinogalactan hydrolysis was slow during an initial lag phase of 4–14 days, whereas chondroitin was hydrolyzed rapidly with no measurable lag. Lag times could be as long as 14 days in some cases (Fig. 5). Like maximum hydrolysis

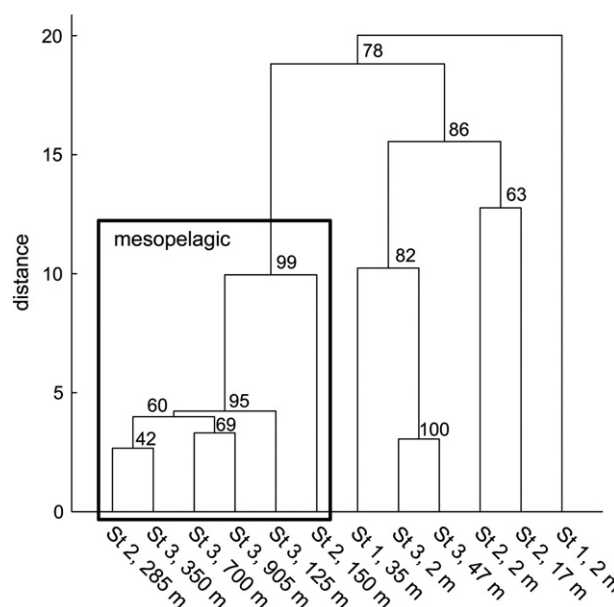


Fig. 3. Dendrogram showing relationships between hydrolysis rate patterns by station and depth. Numbers on nodes signify robustness of node to variation among replicate measurements as a percentage.

Table 2

Summed maximum hydrolysis rates, hydrolysis rate evenness, and radiotracer measurements. Glucose growth efficiency indicates the fraction of incorporated glucose that is assimilated as biomass; see equation. n.d. indicates below detection limit of 0.2 pM hr⁻¹.

Stn.	Depth, m	Total glucose metabolism rate constant, day ⁻¹	Glucose incorp. rate constant, day ⁻¹	Glucose respiration rate constant, day ⁻¹	Glucose growth efficiency, %	Leucine incorp., pM hr ⁻¹	Sum hydrolysis rate, nM hr ⁻¹	Evenness index
1	2	2.6 ± 0.1	1.22 ± 0.08	1.37 ± 0.07	47 ± 2	320 ± 30	101 ± 7.2	1.44 ± 0.07
	35	2.0 ± 0.1	0.773 ± 0.053	1.27 ± 0.09	38 ± 2	69 ± 18	74 ± 6.9	1.53 ± 0.03
2	2	2.1 ± 0.1	1.15 ± 0.08	0.975 ± 0.061	54 ± 2	76 ± 63	90 ± 1.3	1.53 ± 0.01
	17	1.48 ± 0.07	0.704 ± 0.049	0.775 ± 0.047	48 ± 2	39 ± 50	101 ± 7.2	1.51 ± 0.03
	150	0.0930 ± 0.007	0.0501 ± 0.003	0.0429 ± 0.0064	52 ± 4	0.7 ± 0.2	42 ± 7.0	1.66 ± 0.08
	285	0.0708 ± 0.008	0.0298 ± 0.002	0.0410 ± 0.0073	42 ± 5	n.d.	22 ± 1.8	1.47 ± 0.07
3	2	0.84 ± 0.04	0.343 ± 0.023	0.493 ± 0.030	41 ± 2	22 ± 10	62 ± 1.1	1.56 ± 0.02
	47	0.25 ± 0.01	0.102 ± 0.007	0.146 ± 0.0078	41 ± 2	7.3 ± 2.4	66 ± 1.7	1.56 ± 0.02
	125	0.081 ± 0.06	0.0315 ± 0.0021	0.0493 ± 0.0053	39 ± 3	0.8 ± 0.2	17 ± 6.8	1.62 ± 0.09
	350	0.050 ± 0.003	0.0142 ± 0.0010	0.0361 ± 0.0031	28 ± 2	n.d.	21 ± 2.4	1.63 ± 0.09
	700	0.055 ± 0.004	0.0144 ± 0.0010	0.0406 ± 0.0035	26 ± 2	n.d.	9.0 ± 0.33	1.02 ± 0.03
	905	0.048 ± 0.03	0.0161 ± 0.0011	0.0322 ± 0.0028	33 ± 2	n.d.	13 ± 0.5	1.27 ± 0.03

rates, however, lag times were not determined solely by the kinetic effect of temperature: in some cases (e.g. laminarin) lag times for a specific substrate were shorter for samples from deeper water, incubated at colder temperatures, than for samples from shallower water incubated at warmer temperatures (Fig. 6).

3.3. Bacterial production and glucose metabolism

Rate constants for glucose assimilation into biomass (PO¹⁴C), respiration (DI¹⁴C), and rates of bacterial production were significantly slower in the mesopelagic zone than in the epipelagic zone (Table 2; Student's *t* test, *n* = 36, *p* < 0.001). Daily turnover of glucose in the epipelagic zone of stations 1 and 2 ranged between 1.45 ± 0.1 day⁻¹ to 2.5 ± 0.2 day⁻¹, decreasing by nearly two orders of magnitude in the mesopelagic samples of station 3. Glucose concentrations in the northern Gulf of Mexico decrease with depth (Skoog et al., 1999), indicating

that the depth-related decreases in glucose metabolic rate constants measured here likely cause matching decreases in absolute rates of glucose metabolism. Glucose utilization efficiency was also lower in the mesopelagic than in the epipelagic (Student's *t* test, *n* = 36, *p* < 0.001).

Mean summed maximum hydrolysis rates were significantly correlated to both mean bacterial production and mean total glucose metabolic rate constant ($k_{\text{assim}} + k_{\text{resp}}$) (*p* < 0.001, Spearman's rank correlation, *n* = 12 for each). However, these relationships appeared saturating rather than linear (Fig. 7). The saturating relationship is partly due to the fact that, at the most active stations, some polysaccharides were nearly fully hydrolyzed by the first timepoint (e.g. xylan at station 1). It may also indicate that highly active microbial communities are more reliant on simple substrates than relatively inactive communities.

4. Discussion

Patterns of maximum polysaccharide hydrolysis rates (Fig. 1), cluster analysis of maximum hydrolysis rates (Fig. 2), maximum hydrolysis rate evenness (Table 2) and timecourses of hydrolysis (Figs S2–S7) all demonstrate variation among sites and depths in the ability of heterotrophic microbial communities to access specific polysaccharides. Previous work comparing sites along estuarine gradients (Keith and Arnosti, 2001; Steen et al., 2008) and among globally-distributed marine sites (Arnosti et al., 2005, 2011; Steen et al., 2010) have shown variations in patterns of potential polysaccharide hydrolysis rates in surface water; here we demonstrate the existence of such patterns as a function of depth in the Gulf of Mexico.

Polysaccharide hydrolysis rates, bacterial production, glucose metabolic rate constants and glucose utilization efficiency all decreased with depth, with the most notable decrease between samples from the epipelagic zone and samples from the mesopelagic zone. Depth-related changes in microbial community composition and decreases in broad

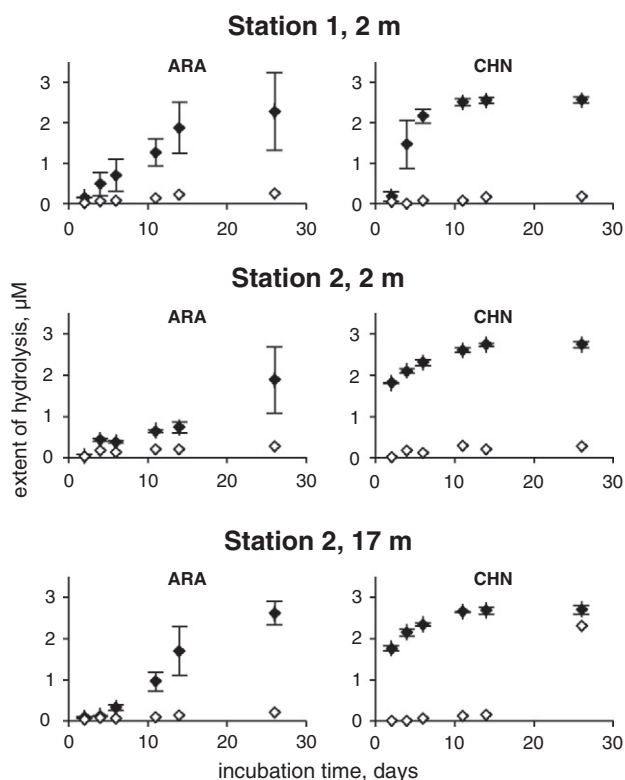


Fig. 4. Examples of variations in lag times prior to the onset of rapid polysaccharide hydrolysis; ara = arabinogalactan, chn = chondroitin. Filled symbols represent live samples, open symbols represent killed controls. Error bars represent the standard deviation of three replicates.

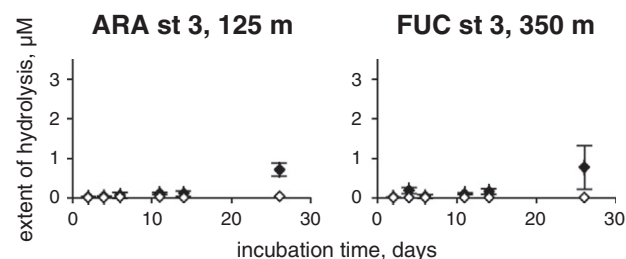


Fig. 5. Examples of samples in which the lag time prior to detectable levels of polysaccharide hydrolysis was at least 14 days; ara = arabinogalactan, fuc = fucoidan. Filled symbols represent live samples, open symbols represent killed controls. Error bars represent the standard deviation of three replicates.

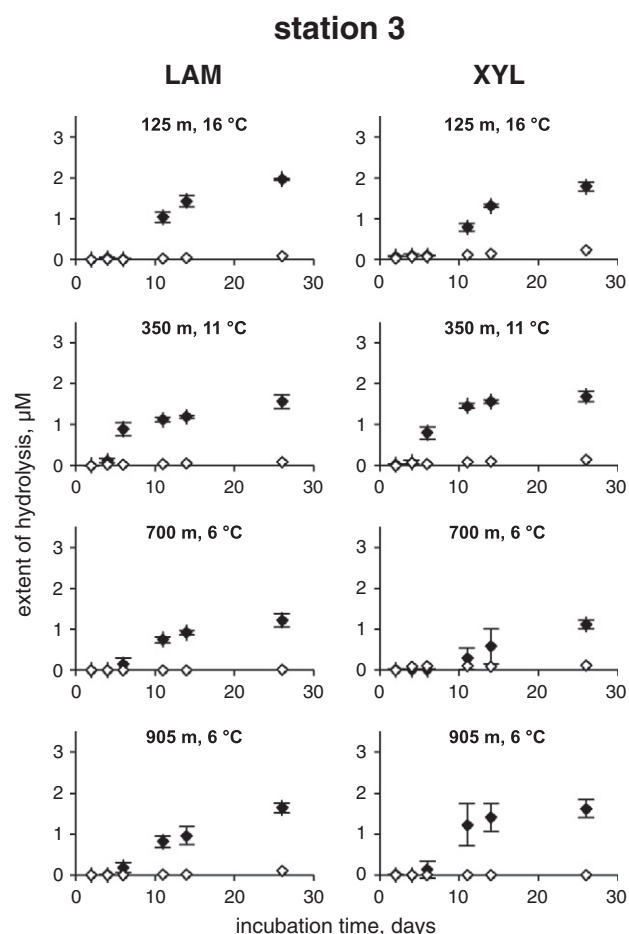


Fig. 6. Examples of polysaccharide hydrolysis timecourses, indicating that lag times were not solely a function of incubation temperature. Filled symbols represent live samples, open symbols represent killed controls. Error bars represent the standard deviation of three replicates.

measures of heterotrophic microbial activity, growth efficiency, and cell counts are common features of the marine water column (e.g. Herndl et al., 2008). Here, the relative depth-related decreases in potential polysaccharide hydrolysis rates were far smaller than decreases in bacterial production and glucose metabolism. Glucose metabolism rate constants decreased 30-fold and 20-fold, and leucine incorporation rates decreased by more than 100-fold, between the most and least active depths of stations 2 and 3 respectively (Table 3). Summed potential hydrolysis rates, however, decreased by factors of just 4.5 and 7.1 at stations 2 and 3, indicating that microbial communities retain the

potential to respond to the input of fresh polysaccharides even when *in situ* activities are low. However, the potential response varies widely depending on the specific polysaccharide.

All incubations were performed at near *in situ* temperature, which decreased with increasing depth. However, temperature differences are not sufficient to explain the observations described above. Relative potential hydrolysis rates within a single sample (Fig. 2, S1) are not likely to be influenced by temperature, since all enzyme activities should be influenced roughly equally by temperature changes. Moreover, in some cases (e.g., laminarin, Fig. 6) potential hydrolysis rates were faster, and lag times were shorter, in samples incubated at colder temperatures relative to samples incubated at warmer temperatures, indicating that microbial metabolic effects were sometimes large enough to override the kinetic effect of temperature.

Extracellular enzyme activities in the bathypelagic have been observed to be higher than in surface water when expressed on a per-cell basis (Baltar et al., 2009), and a higher fraction of bathypelagic enzyme activity appears in the $<0.2 \mu\text{m}$ size fraction (Baltar et al., 2010). Those results, obtained using fluorogenic small-substrate analogs with relatively short incubation times, indicate *in situ* levels of potential enzyme activity and suggest that high molecular weight substrates are important to deep-sea heterotrophic metabolism. The 26-day incubations with labeled polysaccharides reported here confirm that microbial communities can increase enzyme activities upon introduction of polysaccharide substrates, but to an extent and on a timescale that varies by specific substrate and location.

Cluster analysis indicates two major patterns of microbial community function with respect to polysaccharide metabolism (Fig. 2). First, substantial functional diversity exists among the epipelagic samples; hydrolysis rates do not necessarily scale with bacterial production or with each other. For instance, in the epipelagic zone, potential laminarin hydrolysis was considerably faster at station 1 (38 and 26 nM hr^{-1} ; Fig. 1) than at station 2 (18 and 17 nM hr^{-1}), whereas chondroitin hydrolysis was considerably slower (15 and 16 nM hr^{-1} at station 1 versus 38 and 37 nM hr^{-1} at station 2). Second, diversity among mesopelagic samples was consistently lower than among epipelagic samples, and all mesopelagic samples clustered together, separately from epipelagic samples. This pattern indicates the existence of a characteristic mesopelagic community function with respect to polysaccharide hydrolysis, which is relatively homogenous and distinct from epipelagic patterns. The relatively consistent pattern of hydrolysis rates in the mesopelagic might be related to the fact that aldose composition in deep water is more homogenous than in surface water (McCarthy et al., 1996; Skoog and Benner, 1997).

Hydrolysis rate evenness was lower in the 700 and 905 m depths of station 3 than in any other station (Table 2). We have interpreted hydrolysis rate evenness as an index of microbial community specialization with respect to polysaccharide remineralization (Steen et al., 2010). A low evenness score indicates that one or several polysaccharides are hydrolyzed much faster than the others, which we interpret

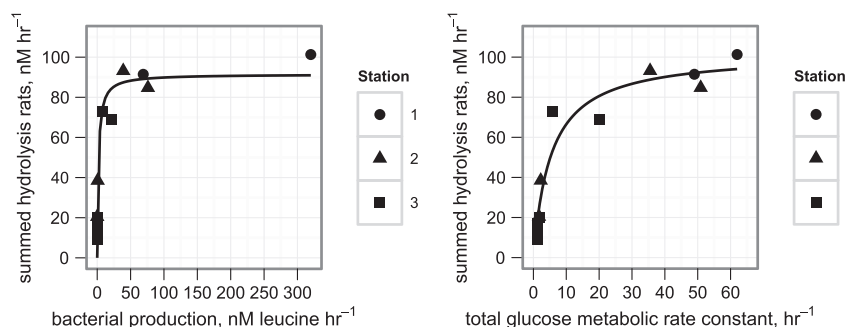


Fig. 7. Summed hydrolysis rates versus bacterial production (left) and total glucose metabolic rate constant (right). The line is a nonlinear least-squares fit to a rectangular hyperbolic function (i.e., Michaelis–Menten form).

Table 3

Minimum values of potential hydrolysis rates, glucose metabolism, and bacterial production in sub-surface waters at each station, demonstrating a larger decrease with depth in glucose metabolism and leucine incorporation than in polysaccharide hydrolysis. These values are calculated by expressing the smallest value measured in the depth profile as a percentage of the value measured in surface waters. The mesopelagic communities retained potential for relatively rapid polysaccharide hydrolysis even when bulk measures of *in situ* activity were low. Note that values for leucine incorporation are a minimum value, assuming a limit of detection of 0.2 pM day^{-1} , because the smallest values were below detection limit.

Station	Summed polysaccharide hydrolysis	Glucose incorporation	Glucose respiration	Total glucose metabolism	Leucine incorporation
St. 2	22%	2.6%	4.2%	3.3%	0.2%
St. 3	14%	4.1%	6.5%	4.9%	0.9%

as an indication of community specialized for specific polysaccharides. A high evenness score indicates a generalist community capable of accessing a wide range of polysaccharides at similar rates. Evenness scores indicate a specialized community at station 3, 700 and 905 m, but do not indicate that communities at 125–350 m at Stations 1 and 2 were more specialized than epipelagic communities. This indicates that overall patterns of hydrolysis were similar in the mesopelagic, but the two deepest stations were distinguished by the degree to which hydrolysis rates of chondroitin sulfate, laminarin, and xylan exceeded those of arabinogalactan, fucoidan and pullulan. Such changes in community metabolic potential and specialization would not be evident from bulk measurements such as bacterial production or small substrate metabolism rates.

The question of whether DOM composition is best seen as driving microbial community composition, or vice versa, has received considerable attention in the literature (Kirchman et al., 2004). Microbial community composition varies in the Gulf of Mexico on a horizontal scale of 5 km or less (Hewson et al., 2006) and (elsewhere) on short vertical scales across pycnoclines (Morris et al., 2005). The composition of polysaccharides within DOM is more spatially homogenous, particularly in deep water (McCarthy et al., 1996; Skoog and Benner, 1997), although polysaccharide composition has been measured only as the concentration of individual combined sugars in DOM, since current analytical techniques do not allow measurements of which sugars are bonded to each other in marine DOM, or the position or orientation of the bonds by which they are linked (Arnosti, 2011). The varying patterns of polysaccharide hydrolysis are therefore likely related to variation in microbial community composition rather than differences in DOM composition.

The extent to which DOM in the ocean is considered to be labile, semi-labile, or refractory is often considered in terms of its chemical composition (e.g. Benner, 2002). The data presented here indicate that variations in microbial community composition may also affect the relative reactivity of DOM. The communities sampled here were sometimes, but not always, capable of rapidly metabolizing a large input of fresh polysaccharides. To the extent that the polysaccharides used here represent polysaccharides that enter the DOM pool, the apparent reactivity of individual polysaccharides will vary by location and depth. Consequentially, specific polysaccharides that would have been rapidly degraded in surface water may be more recalcitrant if exported to the mesopelagic zone.

One factor influencing the metabolic potential of marine microbial communities may be the composition of the rare biosphere (Sogin et al., 2006). The composition of the pelagic rare biosphere varies geographically and temporally, suggesting active community dynamics and therefore a potential biogeochemical function (Campbell et al., 2011; Galand et al., 2009). The timecourses observed here (Fig S2–S7) also suggest that rare species may have been involved in degradation of specific polysaccharides. Lag times prior to polysaccharide hydrolysis may be a result of the time required to induce production of extracellular enzymes, or by the time required for growth of rare microbes that

need to multiply in order to degrade a polysaccharide at a detectable rate. The specific factors underlying the lag times observed here cannot be determined definitively without more extensive data on the biomass, metabolic capabilities, composition, and activity of specific microbes during the long-term polysaccharide incubations, information that in part awaits future technical developments. However, the lag times of 2–14 days (or longer) observed here (Figs. 4, 5) are more consistent with timescales of population growth than of timescales for enzyme expression, which may occur in minutes or hours. Genomic analysis suggests that rare taxa are adapted to a 'stop-and-start' or opportunistic lifestyle (Yooseph et al., 2010), which is also consistent with longer lag times. If long lag times here are due to growth of rare microbial species, remineralization of episodically-produced polysaccharides (for instance, those produced during a phytoplankton bloom) may be driven more by rare specialist species growing in response to environmental change, rather than by metabolically flexible generalists (Mou et al., 2008).

A major focus of recent work in marine microbial ecology has been the identification of variability in pelagic microbial community composition over a broad range of spatial scales (Fuhrman et al., 2008; Hewson et al., 2006; Long and Azam, 2001). However, the consequences of this variation for the carbon cycle are not well understood. Previous investigations suggest that the fraction of DOM that can be degraded depends to some extent on the microbial community present. For instance, DOM extracted from surface water at the Bermuda Atlantic Time Series station was mineralized more efficiently by mesopelagic microbial communities than by surface water communities, indicating differences in metabolic capabilities between these communities (Carlson et al., 2004). The variability in polysaccharide hydrolysis rates and patterns presented here provides direct evidence that microbial communities vary in their ability to access specific polysaccharides that on the basis of solubility and monomer composition would otherwise be classified as labile. Such information that cannot be detected using bulk measures of community activity or low-molecular weight enzyme substrate proxies. The timecourses of hydrolysis imply that rare organisms may be responsible for some of those differences. These results indicate that polysaccharide reactivity is a function of microbial community composition as well as polysaccharide structure, and that more diverse microbial communities may be capable of mineralizing a wider range of high-molecular weight compounds.

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