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The Biogeochemistry of Marine Polysaccharides: Sources, Inventories, and Bacterial Drivers of the Carbohydrate Cycle

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Abstract

Polysaccharides are major components of macroalgal and phytoplankton biomass and constitute a large fraction of the organic matter produced and degraded in the ocean. Until recently, however, our knowledge of marine polysaccharides was limited due to their great structural complexity, the correspondingly complicated enzymatic machinery used by microbial communities to degrade them, and a lack of readily applied means to isolate and

characterize polysaccharides in detail. Advances in carbohydrate chemistry, bioinformatics, molecular ecology, and microbiology have led to new insights into the structures of polysaccharides, the means by which they are degraded by bacteria, and the ecology of polysaccharide production and decomposition. Here, we survey current knowledge, discuss recent advances, and present a new conceptual model linking polysaccharide structural complexity and abundance to microbially driven mechanisms of polysaccharide processing. We conclude by highlighting specific future research foci that will shed light on this central but poorly characterized component of the marine carbon cycle.

POLYSACCHARIDES IN THE OCEAN: AN OVERVIEW

The oceans account for half of global primary productivity, much of which is rapidly respired back to CO_2 (Falkowski et al. 1998). This rapid cycling is due largely to the activities of heterotrophic prokaryotes that efficiently degrade organic matter. However, our understanding of microbially driven organic carbon cycling is quite sparse compared with our knowledge of specific steps, products, and organisms involved in the marine nitrogen, phosphorus, and sulfur cycles. In part, this disconnect arises because these cycles can be traced by marker genes for specific processes (e.g., Canfield et al. 2010), whereas the transformation of organic carbon is driven by a multitude of processes and manifold associated enzymes, many of which remain to be identified. This problem is particularly acute in the case of polysaccharides—polymers of monosaccharides, generally containing more than 10 sugars—and carbohydrates in general. Although they constitute a large fraction of phytoplankton and macroalgae (Biersmith & Benner 1998, Hedges et al. 2002, Mabeau & Kloareg 1987) as well as dissolved and particulate organic matter (DOM and POM, respectively) (Benner et al. 1992, Hedges et al. 2001), little is known about their biogeochemical processing compared with other major compound classes, such as proteins, lipids, and nucleic acids. Analytical problems associated with polysaccharide solubility, size, and lack of easily tracked spectroscopic characteristics make the separation and analysis of polysaccharides particularly challenging. Recent examination of genes, transcripts, and metagenomes of prokaryotes, however, have highlighted the importance of polysaccharides and their transformations for carbon metabolism in the ocean (e.g., Teeling et al. 2012, 2016). Hence, closer examination of the cycling of marine polysaccharides and of the communities that drive their degradation is necessary.

Polysaccharide: a chain of more than 10 monosaccharides linked together

Monosaccharide: an individual sugar

CAZyme: carbohydrate-active enzyme; this group includes enzymes that are involved in the degradation, synthesis, or binding of complex carbohydrates

Difficulties in characterizing marine polysaccharides and in determining their transformation pathways relate directly to their complex structures, which pose a major challenge for chemists as well as for microbes (here, bacteria and archaea). Unlike other biopolymers, such as proteins and nucleic acids, the monosaccharide constituents of polysaccharides can be connected in multiple ways, creating enormous molecular diversity. Two hexose monosaccharides of the same type (e.g., glucose) can be linked with two possible linkage configurations (α or β) via six possible points ($1\rightarrow1, 1\rightarrow2, 1\rightarrow3\ldots$), yielding 12 different disaccharides, compared with one possible dipeptide or dinucleotide for proteins and DNA, respectively (**Figure 1*a***). With different monosaccharides and increasing polymer length, diversity increases exponentially (Laine 1994) (**Figure 1*b***). Moreover, polysaccharides can also be branched at any of their hydroxyl groups (**Figure 1**) and frequently contain additional functional groups (sulfate, amino, methyl, or acetate groups) at one or more positions on different monosaccharide units. Polysaccharides can also form aggregates and gels of varying size, charge, and physical states (Chin et al. 1998, Passow 2002).

To utilize polysaccharides as substrates, microbes thus require carbohydrate-active enzymes (CAZymes) of the correct structural specificity. CAZymes comprise 167 families of glycoside hydrolases, 40 families of polysaccharide lyases, 17 families of carbohydrate esterases, 110 families

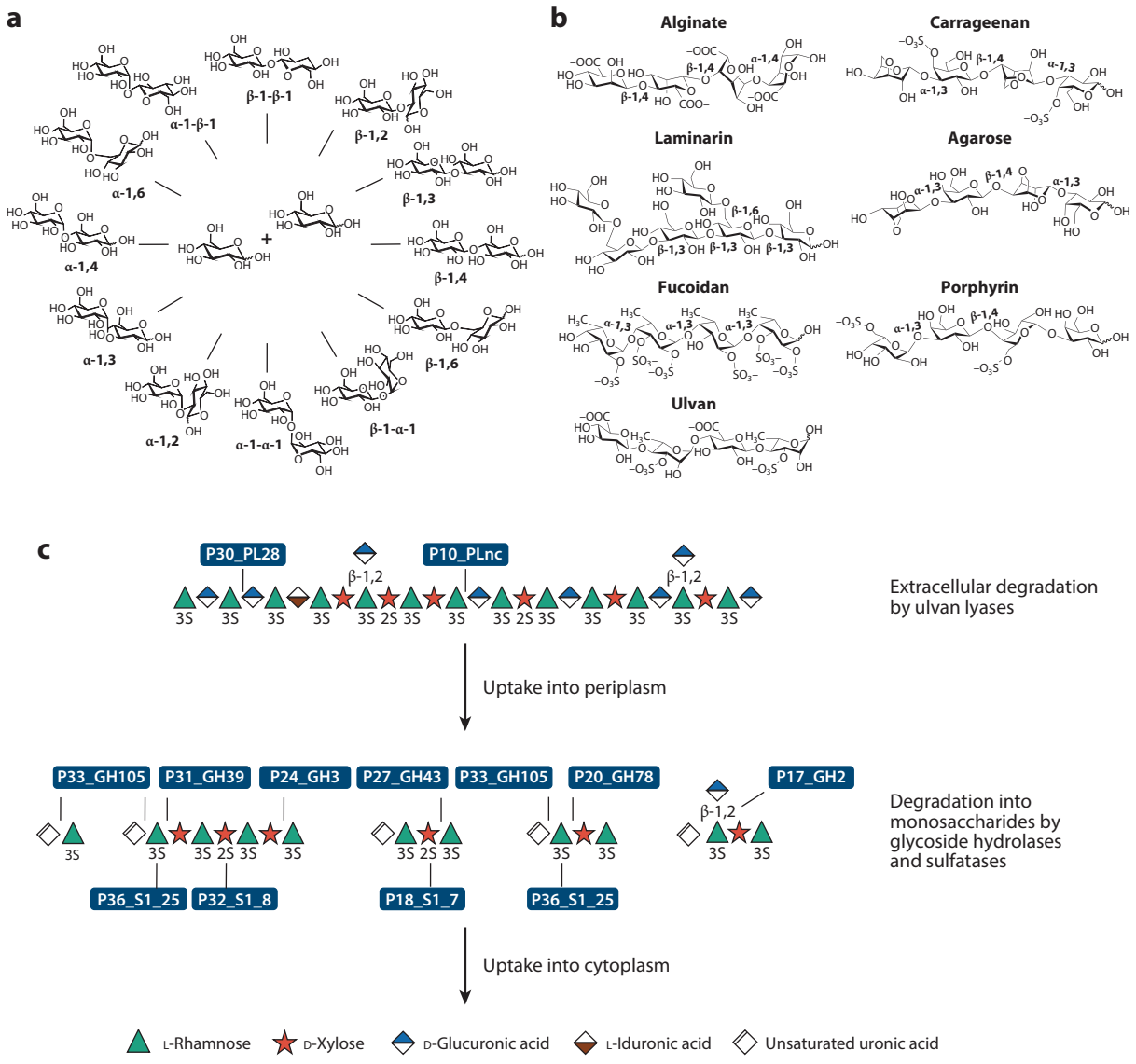


Figure 1

Carbohydrate structures. (*a*) Structural combinations of monosaccharides. Two molecules of glucose could be linked to form 12 different disaccharides. (*b*) Structures of some marine-related polysaccharides. Laminarin is found in macroalgae and phytoplankton; carrageenan, alginate, agarose, fucoidan, porphyrin, and ulvan are common in macroalgae. (*c*) Hydrolysis of highly complex polysaccharides requires a cascade of carefully coordinated enzymes. Here, ulvan is sequentially hydrolyzed into oligosaccharides at the outer membrane, then transported into the periplasmic space, where the oligosaccharides are further hydrolyzed to disaccharides and monosaccharides that can be transported into the cytoplasm. The symbol key shows the monomer constituents of ulvan. Numbers followed by the letter S indicate the positions of sulfate groups on individual sugars. Blue rectangles identify the specific enzyme involved in an individual transformation step. Data replotted from Reisky et al. (2019).

of glycosyltransferases, and 16 families of enzymes with auxiliary activities, plus 86 families of carbohydrate-binding modules [as given in the Carbohydrate-Active Enzymes (CAZy) database (Lombard et al. 2014; **http://www.cazy.org**) as of May 2020]. Since CAZymes are categorized by sequence similarities and common ancestry, irrespective of their substrate specificity, each CAZyme family includes enzymes with different substrate specificities. Moreover, each unique glycosidic bond—and there may be many in a complex polysaccharide—requires a specifically adapted enzyme for effective binding and hydrolysis (**Figure 1*c***). The enzyme must recognize the local geometry of the bond and adjacent sugars, the chemical group modifications on the sugars, and more distant sugars in the macromolecular assembly, all of which add complexity for enzymatic binding, cleavage, and depolymerization. Moreover, a glycosidic linkage deeply buried within the three-dimensional molecular network of a highly branched polysaccharide may not be reached by its complement enzyme due to steric exclusion (Reisky et al. 2019). Therefore, a multitude of debranching enzymes have to act in the correct sequence in order to hydrolyze the polysaccharide backbone. If a necessary enzyme is not present, the polysaccharide may be only partially degraded (**Figure 1*c***).

CAZyme family: an enzyme family in the CAZy database; these families are classified by their structural (not functional) relationships

A complex balance of enzyme production, excretion, and capture of the resulting hydrolysate thus governs the energy calculus of polysaccharide processing by bacteria. This energy calculus is further complicated by the fact that it applies to organisms and communities in a highly diverse array of habitats, with widely differing polysaccharide availability. In the ocean, these organisms and communities include macroalgae-associated communities; planktonic bacteria in the surface ocean, where polysaccharide availability is temporally and spatially highly variable; bacteria in the mesopelagic and deep ocean, where substrate availability is likely even more heterogeneous and probably related to temporal pulses from sinking particles and vertically migrating animals; bacteria associated with different types of particles and aggregates; and bacteria in sediments of different types and redox states and at different depths (**Figure 2**).

The temporal span of polysaccharide production and degradation in the ocean extends over timescales of milliseconds to millennia, covering processes ranging from the transcription of genes by bacteria to the preservation of carbohydrates in sediments over geologic timescales. These processes are influenced by factors such as glycan complexity, enzyme specificity, and physical controls on polysaccharide structure and solubility. The capabilities and limitations of individual microbes and the effects of grazers and viruses on microbial communities also play important roles. Insights obtained across multiple studies and scientific disciplines are beginning to yield a clearer perspective on these processes.

A MULTITUDE OF POLYSACCHARIDE SOURCES, INVENTORIES, AND ANALYTICAL CHALLENGES

Phytoplankton and macroalgae, the foundation of the marine food web, constitute the major sources of polysaccharides in the marine environment (**Figure 2**). Carbohydrate building blocks produced via photosynthesis serve as energy storage products and cell wall constituents, as well as the feedstock for other cellular molecules. Many of the polysaccharide constituents of macroalgae (Mabeau & Kloareg 1987, Painter 1983) and of some phytoplankton (Gugi et al. 2015, Haug & Myklestad 1976, Percival et al. 1980) have been structurally characterized, although a considerable effort will be required to comprehensively characterize the majority of micro- and macroalgal polysaccharides (Deniaud-Bouet et al. 2017). These analyses have helped delineate some important differences between marine and terrestrial polysaccharides. In particular, sulfate is a common constituent of macroalgal as well as other marine polysaccharides (Helbert 2017), while the major polysaccharide of the terrestrial biosphere—cellulose—is comparatively rare in marine systems.

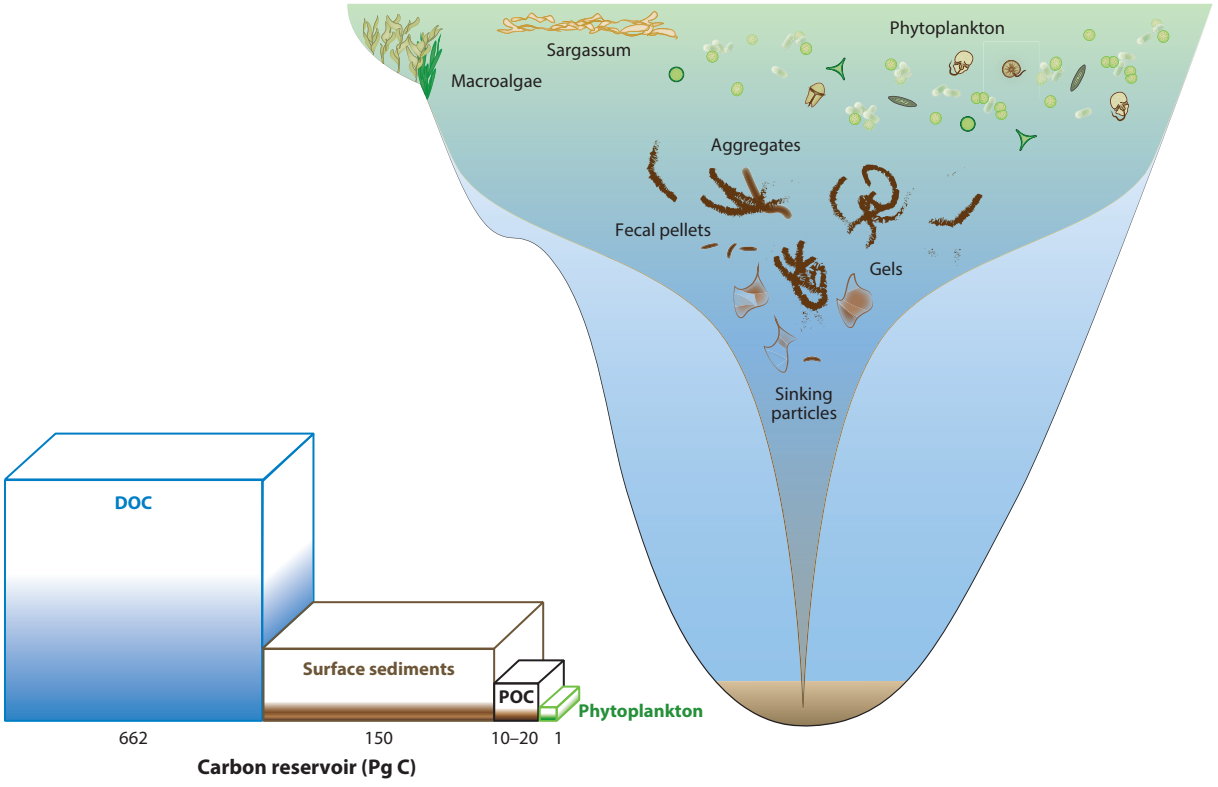


Figure 2

Polysaccharide sources and reservoirs in the ocean. This schematic shows macroalgae and phytoplankton as major polysaccharide sources. Transformation of phytoplankton-derived organic matter through the marine food web yields aggregates, gels, fecal pellets, and particles that contribute to the sinking flux of detrital organic matter; a small percentage of this sinking flux (symbolized by the narrowing funnel from the upper ocean) ultimately reaches the sediments. Note that the largest detrital reservoir of carbohydrates in the ocean occurs as dissolved organic carbon (DOC). The boxes at the bottom of the figure are proportionate to the carbon content of each reservoir; the quantity of each reservoir is shown in petagrams (10^{15} g) of carbon. The shading of these boxes illustrates the contribution of carbohydrates to the carbon content in each reservoir. Sources for carbon content: DOC, Hansell (2013); surface sediments, Hedges (1992); particulate organic carbon (POC), Eglinton & Repeta (2006); phytoplankton, Falkowski et al. (1998). Sources for carbohydrate content are given in the main text.

Phytoplankton carbohydrate contents vary by species and with growth phase; the total carbohydrate content of phytoplankton is considerable, ranging from 21% to 50% (Biersmith & Benner 1998) or more (Becker et al. 2020). In addition to their intracellular stock of carbohydrates, phytoplankton actively excrete carbohydrate-rich DOM (Thornton 2014), with neutral carbohydrates (uncharged sugars) constituting up to half of freshly excreted DOM (Aluwihare & Repeta 1999, Hama & Yanagi 2001, Hama et al. 2004). In macroalgae, polysaccharides such as laminarin, alginate, fucoidan, agarose, and carrageenan serve as structural and storage compounds, constituting more than 50% of dry mass (Mabeau & Kloareg 1987). Exopolysaccharides produced by benthic diatoms (Hofmann et al. 2009), as well as mucus and biofilms produced by a range of organisms, also contribute to the polysaccharide diversity and inventory of the ocean.

Although polysaccharides are major components of living biomass in the ocean, most of the organic matter in the ocean, including carbohydrates, is part of the nonliving (detrital) reservoir (**Figure 2**). This reservoir originates from myriad processes, including predation, excretion, and

viral lysis of algae and bacteria (Jumars et al. 1989, Suttle 2007). Detrital organic matter is operationally defined (typically by filtration) as either DOM or POM. DOM is by far the largest reservoir of organic carbon in the ocean: At 662 Pg C, it contains more than 200 times more carbon than is found in living marine biomass (Hansell 2013). DOM is sometimes further subdivided experimentally based on chemical or physical properties, such as extraction or ultrafiltration (see also Hansell & Carlson 2015). However, the operational distinction between DOM and POM in ocean waters is somewhat arbitrary, since DOM that passes through a 0.2-μm filter may aggregate or assemble into gels (Chin et al. 1998) or transparent exopolymeric particles (Passow 2002).

Neutral sugar: an uncharged monosaccharide (e.g., with no carboxyl or sulfate groups)

Amino sugar: a monosaccharide in which a hydroxyl group is replaced by an amine group

Uronic acid: a monosaccharide in which a hydroxyl group is replaced by a carboxylic acid group

Combined carbohydrates: a general term for carbohydrates that are linked together

Efforts to determine the composition and track the dynamics of detrital carbohydrates are complicated by numerous analytical challenges. Polysaccharides lack distinct spectroscopic signatures and are either highly soluble in water (and therefore very difficult to isolate from the much more abundant sea salts) or solid, and sometimes associated with other fractions of organic matter (and thus difficult to isolate). Ideally, structural information about polysaccharides would include the identity, linkage position, and orientation of monomer constituents, the location of branches, and the presence of additional functional groups (e.g., sulfate, carboxyl, or amino groups; **Figure 1**). However, the carbohydrate composition of detrital organic matter—whether DOM or POM—is usually characterized in one of three ways, none of which provide all of this information. (For a comprehensive review of techniques used to analyze carbohydrates in marine samples, and their limitations, see Panagiotopoulos & Sempere 2005.) Total carbohydrate analyses typically employ acid hydrolysis, then either colorimetric quantification (Dubois et al. 1956, Myklestad et al. 1997, Pakulski & Benner 1992) or (much more rarely) solid-state nuclear magnetic resonance analysis, which provides information about the overall carbohydrate component of a sample (Hedges et al. 2001, 2002; Mao et al. 2012). With some acid hydrolysis procedures, concentrations of monomeric versus polymeric carbohydrates can be determined by comparing the quantity of reducing sugars (sugars with a free anomeric carbon, the one at the #1 position; **Figure 1**) prior to and following acid hydrolysis (Myklestad et al. 1997). Certain types of acid hydrolysis can also be followed by derivatization and analysis of neutral sugars (Quijada et al. 2015, Shen et al. 2016). Acid hydrolysis can also be followed by high-performance anion exchange chromatography with pulsed amperometric detection to separate and quantify the monosaccharide composition of diverse samples (Borch & Kirchman 1997). Specific sample preparation permits analysis of neutral, amino, and acidic sugars in high-molecular-weight DOM and POM (Engel & Handel 2011); anhydrosugars can also be measured using high-performance anion exchange chromatography with pulsed amperometric detection under specific chromatographic conditions (Nouara et al. 2019). For POM analysis, acid hydrolysis of increasing severity (Quijada et al. 2015) and/or a variety of different POM extraction techniques (Miyajima et al. 2001) can provide an indication of the structure and matrix in which carbohydrates are found (Lin & Guo 2015). Furthermore, although marine polysaccharides frequently contain sulfate or uronic acids (Bergamaschi et al. 1999, Hung et al. 2003), analyses of these components are quite rare.

Despite these analytical challenges, some general trends have emerged from carbohydrate analysis of DOM and POM. Most important, carbohydrates constitute a considerable fraction of DOM: Bulk chemical characterizations suggest that total carbohydrates constitute approximately 15–50% of DOM in the ocean (Benner et al. 1992, Hung et al. 2003, Lin & Guo 2015, McCarthy et al. 1996, Pakulski & Benner 1992) (**Figure 2**). Much of the total carbohydrate fraction is combined carbohydrates (oligo- and polysaccharides); monosaccharides are generally a lower fraction (approximately one-third; Lin & Guo 2015, Myklestad & Borsheim 2007) and often a more consistent fraction of DOM, with spatial (Hung et al. 2003) and seasonal changes (Lin & Guo 2015), especially among the polysaccharide fraction (Myklestad & Borsheim 2007). Acid hydrolysis followed by chromatographic separation of individual neutral monosaccharides has demonstrated

that a range of neutral monosaccharides, including glucose, galactose, mannose, xylose, fucose, rhamnose, and arabinose, contribute to DOM (Goldberg et al. 2009), but neutral monosaccharides generally constitute a comparatively small percentage of DOM (approximately 4–5%; Goldberg et al. 2009, Shen et al. 2016).

_O_-methyl sugar: a monosaccharide in which the proton of a hydroxyl group is replaced with a methyl group

Overall, there is a considerable difference between the dissolved carbohydrate content in seawater measured colorimetrically (an approach that integrates a wider range of carbohydrates, which are measured via addition of reagents that yield colored products after acid hydrolysis) and the inventory of individual uncharged sugars (neutral monosaccharides) that can be measured chromatographically. As summarized by Panagiotopoulos & Sempere (2005), in seawater monosaccharides measured as individual neutral sugars contribute 3% to DOM, versus a 3–6% contribution determined colorimetrically; combined carbohydrates contribute 11–24% to DOM as measured colorimetrically but only 3–6% chromatographically.

This difference between concentrations measured via colorimetric and chromatographic techniques likely has many root causes, including problems with acid hydrolysis (e.g., lack of hydrolysis of acid-resistant polysaccharides, as well as destruction of acid-sensitive components; Cao et al. 2017, Lin & Guo 2015). The fact that most chromatographic analyses focus on neutral sugars and do not include methylated sugars (Bergamaschi et al. 1999), amino sugars (Kerherve et al. 1995), uronic acids (Mopper et al. 1995), and *N*-acetyl amino sugars (Aluwihare et al. 2005, Cao et al. 2017), all of which likely contribute to the carbohydrate fraction of DOM, probably adds to these differences. Moreover, some components are likely underestimated, even in colorimetric tests. Hung et al. (2001), for example, noted that sulfated polysaccharides produced only half the signal in colorimetric tests compared with uncharged polysaccharides; in addition, they found that uronic acids—which typically are not quantified—contributed an average of 10% of DOM.

In general, the concentrations of combined carbohydrates in POM are variable, depending in part on the origin and transport history of the particles (Bergamaschi et al. 1999, Cowie & Hedges 1984, Wakeham et al. 1997). In addition to neutral carbohydrates, *O*-methyl sugars and uronic acids contribute to POM, contributing 1–6% and approximately 3–8% of POM, respectively, in sediment trap samples collected on an annual cycle (Bergamaschi et al. 1999). In the Gulf of Mexico, total carbohydrates averaged 18% of POM, with uronic acids averaging approximately 3% and acidic polysaccharides approximately 10% of the total carbohydrates (Hung et al. 2003). A broad survey of data from a range of studies at different locations found that carbohydrates constitute approximately 8–10% of suspended POM and 3–18% of sinking POM (Panagiotopoulos & Sempere 2005).

In comparison with other compound classes, such as lipids and amino acids, neutral carbohydrates are a relatively invariant fraction of POM. Analysis of POM collected in the equatorial Pacific from surface plankton tows, sinking particles collected at three depths over a range of more than 3,500 m, and surficial sediments demonstrated that the concentration of organic carbon decreased by four orders of magnitude between the surface ocean and surficial sediments. Over the same depth range, the fraction of organic matter that could be characterized analytically as amino acids and lipids decreased greatly, concurrent with an increase in the uncharacterizable fraction from 15% to 80% of the total. Neutral carbohydrates, however, constituted a relatively invariant fraction of POM from the surface ocean to surficial sediments, averaging approximately 4–6% of the total (Wakeham et al. 1997).

In sediments, total carbohydrates constitute approximately 14–19% of POM, with neutral carbohydrates (measured by chromatographic techniques) comprising 5–17% of the total (Panagiotopoulos & Sempere 2005). As in DOM and POM, in sediments neutral carbohydrates include a range of neutral sugars (glucose, galactose, mannose, fucose, xylose, rhamnose, and arabinose; Cowie & Hedges 1984). Pore waters of organic-rich surface sediments have a

disproportionately high concentration of carbohydrates, with reports of total carbohydrates comprising 24–85% (Arnosti & Holmer 1999) and 10–55% (Burdige et al. 2000) of DOM; half of the carbohydrates in the study by Burdige et al. (2000) could be identified as neutral aldoses. The contribution of total carbohydrates to total organic carbon in sediments (Miyajima et al. 2001) and the ratio of neutral sugars to total organic carbon are relatively invariant with depth (Burdige et al. 2000). Combined carbohydrates persist to a considerable depth and for a considerable time in sediments (Cowie et al. 1995): At a depth of 50 m (estimated age 120,000 years), neutral sugars were reported to constitute 1.7–3.7% of total organic carbon (Quijada et al. 2015). Despite invariant concentrations of total carbohydrates, increasing concentrations with sediment depth of *O*-methyl sugars and uronic acids suggest that they can also be markers of a bacterial source (Bergamaschi et al. 1999).

In sum, we know that polysaccharides constitute a substantial fraction of algal and phytoplankton biomass, and that these sources fuel marine ecosystems and ultimately contribute to a considerable portion of the not-yet-well-characterized detrital polysaccharide pools (**Figure 2**). We also know that neutral sugar composition by itself does not provide a clear distinction among potential marine sources, nor does it reliably trace carbohydrate degradation pathways (Cowie & Hedges 1984). Despite our difficulties in structurally characterizing polysaccharide-containing organic matter, however, heterotrophic microbial communities are efficient at accessing and recycling much of this organic matter.

MICROBIAL COMMUNITIES SHOW PATTERNS OF POLYSACCHARIDE DEGRADATION ACROSS OCEAN ENVIRONMENTS

CAZymes are the essential tools used by heterotrophic microbes to initiate degradation of marine polysaccharides. The activities and structural specificities of these enzymes determine which organic matter is hydrolyzed to smaller sizes and transported into a cell for further metabolism. They also determine which fractions evade remineralization, to be processed by other organisms under other conditions or in other locations, or potentially leading to preservation in sediments over geologic timescales (Arnosti 2011). Different organisms working in concert may be responsible for degradation of complex organic matter. Since the collective enzyme activities of a heterotrophic community are important determinants of polysaccharide lability, measurements that integrate community enzyme activities in seawater and sediments have long been a focus of research. The most common technique to measure microbial enzyme activities relies on fluorescent substrate proxies (typically methylumbelliferyl-α-glucose and methylumbelliferyl-β-glucose; Hoppe 1993). Over the past decades, measurements made with these substrate proxies have revealed broad-scale patterns in the ocean (see also the summaries in Hoppe et al. 2002). For example, glucosidase activities are considerably higher in the surface ocean than in the deep ocean, although rates decrease less with depth when normalized to cellular abundance (Baltar et al. 2009). On particles and aggregates, glucosidase activities are considerably higher than they are in the surrounding water column (Grossart et al. 2003, Smith et al. 1992), suggesting that rapid enzyme activities on particles can also fuel organisms in the surrounding environment. In deep-sea sediments and trench environments, glucosidase activities are also considerable (e.g., Boetius et al. 1996). Although very few measurements have been made at sediment depths greater than approximately 20 cm, very low but measurable glucosidase activities have been reported (Bird et al. 2019, Coolen & Overmann 2000).

Substrate proxies yield rapid assessment of enzyme activities that can be compared across environments and experiments, but they provide limited insight into the activities and structural specificities of polysaccharide-hydrolyzing enzymes. In particular, low-molecular-weight

substrate proxies do not represent the structural complexity of natural polysaccharides (e.g., Warren 1996), nor do they measure the activities of endo-acting enzymes that cleave polysaccharides midchain (**Figure 1**). Furthermore, analyses of phytoplankton, algae, DOM, and POM have demonstrated that glucose is only one of many monosaccharides contributing to marine polysaccharides. Thus, measurements of exo-acting glucosidase activities cannot provide a comprehensive view of polysaccharide degradation in the ocean.

Fluorescently labeled polysaccharides (Arnosti 1996) provide an alternative means to measure hydrolysis rates and patterns in seawater and sediments, in particles, and in aggregates. With this technique, polysaccharides are labeled with a covalently linked fluorophore, and hydrolysis is measured as the change in polysaccharide molecular weight as it is progressively hydrolyzed to smaller sizes (Arnosti 2003). Measurements made with fluorescently labeled polysaccharides capture activities of endo- as well as exo-acting enzymes, facilitating comparison of the hydrolysis rates of structurally distinct polysaccharides. Investigations using fluorescently labeled polysaccharides have revealed large-scale patterns in the capabilities of entire microbial communities, not just individual organisms, to access polysaccharides (**Figure 3**). Both the rates and the substrate spectrum (the number of different types) of polysaccharides hydrolyzed typically narrow considerably with depth in the ocean (Balmonte et al. 2018, Hoarfrost & Arnosti 2017, Steen et al. 2012) and from estuarine to coastal and offshore waters (D'Ambrosio et al. 2014, Steen et al. 2008). In the water column, formation of aggregates can lead to a broadened spectrum of enzyme activities compared with the same seawater without aggregates (Ziervogel et al. 2010), suggesting that processes such as quorum sensing may also act to regulate enzyme activities (Jatt et al. 2015).

Comparisons across multiple locations have also revealed a latitudinal gradient in CAZyme activities, with a narrower spectrum of enzyme activities measurable in high-latitude waters compared with temperate waters (Arnosti et al. 2011, Balmonte et al. 2018) (**Figure 3**). These distinct patterns in enzyme activities are congruent with differences in microbial community composition in ocean waters (Arnosti et al. 2012) and parallel emergent patterns in microbial biogeography (e.g., Fuhrman et al. 2008, Salazar et al. 2016, Zinger et al. 2011), as well as patterns of spatial differences among CAZyme-encoding genes (Elifantz et al. 2008, Gomez-Pereira et al. 2012, Neumann et al. 2015).

A relationship between microbial community composition and CAZyme function is also supported by the observation that the spectrum of enzyme activities measurable in surface sediments is often considerably broader than that in the overlying seawater (Arnosti 2000) (**Figure 3**). These differences in CAZyme function parallel differences in microbial community composition (Cardman et al. 2014, Teske et al. 2011), suggesting that benthic communities may have access to a much wider range of organic matter than their pelagic counterparts. To date, few investigations have focused on CAZymes deep in the sediment column (Hoarfrost et al. 2017). However, binned genomes of Bathyarchaeota in sediments (Lazar et al. 2016), as well as metatranscriptomes from a deep sediment core, show evidence for secretion of specific CAZymes at sediment depths greater than 150 m (Orsi et al. 2018), suggesting a role for polysaccharides in fueling members of the deep biosphere. In sum, the relative degradability of different polysaccharides is dependent not only on polysaccharide structure but also on the net capabilities of microbial communities, which are not uniform throughout the ocean.

POLYSACCHARIDE-HYDROLYZING BACTERIA AND THEIR CAZYMES

Physiological, genomic, and enzymatic analyses of cultured environmental microbes have provided detailed insights into the polysaccharide-processing capabilities of marine bacteria. Members of the phylum Bacteroidetes, particularly the Flavobacteriales, which are commonly found

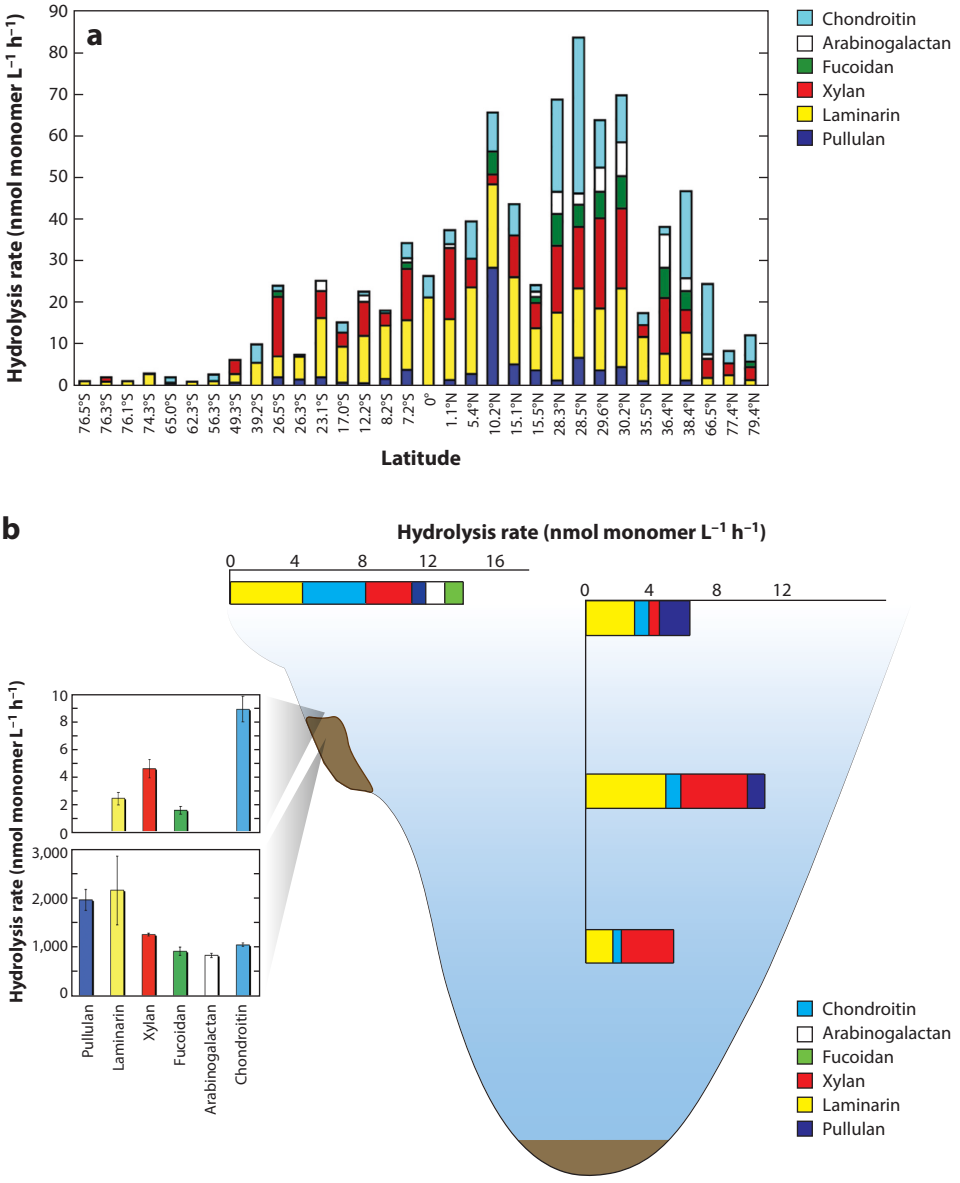


Figure 3

Patterns of polysaccharide hydrolase activities in the ocean. (*a*) Latitudinal gradient in the enzymatic hydrolysis rates of six polysaccharides in surface ocean water. A broader spectrum of substrates is hydrolyzed at temperate latitudes compared with high latitudes. Panel adapted from Arnosti et al. (2011). (*b*) Contrasting patterns of polysaccharide hydrolase activities in bottom water and surface sediments (data replotted from Teske et al. 2011), along with an onshore–offshore gradient and depth gradient in polysaccharide hydrolase activities in the water column (data replotted from D'Ambrosio et al. 2014).

associated with micro- and macroalgae, are considered pivotal polysaccharide-degrading bacteria (Fernandez-Gomez et al. 2013, Kirchman 2002). Other active polysaccharide degraders include members of the Gammaproteobacteria, which are known to respond rapidly to increases in organic matter concentrations (Sarmento et al. 2016), as well as members of the Planctomycetes (Boedeker et al. 2017, Reintjes et al. 2017) and Verrucomicrobia (Cardman et al. 2014, Martinez-Garcia et al. 2012, Sichert et al. 2020). However, broad phylogenetic characterizations yield only initial clues about a potential role in polysaccharide degradation: Phylogenetically related bacterial species may exhibit considerable differences in enzymatic capabilities (e.g., Avcı et al. 2020, Badur et al. 2017, Kappelmann et al. 2018, Xing et al. 2014, Zimmerman et al. 2013), including substantial variability at the strain level (Koch et al. 2019a, 2020). These differences are often attributed to horizontal gene transfer (Gobet et al. 2018, Thomas et al. 2012), which may redefine the ecological niches of microbial species (Hehemann et al. 2017). For example, entire polysaccharide utilization loci (PULs) targeting algal glycans can be transferred between bacteria from the ocean and the human intestine while retaining their functionality (Hehemann et al. 2010, 2012). Moreover, entire plasmids can be dedicated to polysaccharide degradation, likely constituting an important vehicle for specific adaptations (Gobet et al. 2018, Koch et al. 2019b) that seem to be maintained in certain niches over wide geographic scales (Foran et al. 2017).

Polysaccharide utilization locus (PUL): a cluster of colocalized, coregulated genes that encode proteins to bind, hydrolyze, and transport polysaccharides

Oligosaccharide: a chain of up to 10 monosaccharides linked together

The CAZyme complement of bacteria may scale with the complexity of their target polysaccharides: A single CAZyme can be responsible for the hydrolysis of a specific linkage, meaning that a substantial enzymatic arsenal is required to hydrolyze highly complex polysaccharides (Reisky et al. 2019, Sichert et al. 2020). Moreover, the apparent redundancy of CAZymes in some bacteria may relate to the targeting of different substructures of a polysaccharide. This point has been shown for sulfatases specific for distinct residues of carrageenan in the flavobacterium *Zobellia galactanivorans* (Ficko-Bean et al. 2017), as well as for laminarinases in the genus *Formosa* (Unfried et al. 2018).

The clustering of CAZymes in PULs amplifies degradation efficiency by coupling the expression of proteins that sense, bind, transport, and hydrolyze polysaccharides (Martens et al. 2009). Studies of intestinal bacteria have shown that coexpression of these proteins tightly couples uptake and digestion, minimizing substrate loss (Martens et al. 2009). Some components of PULs, however, are also expressed in the absence of substrate; these sentry enzymes and transporters are secreted or surface attached and can hydrolyze polysaccharides, digesting them into smaller oligosaccharides that are taken up to induce the rest of the pathway (Thomas et al. 2012). An absence of sentry proteins thus may render bacteria blind to the presence of target polysaccharides. Polysaccharide degradation can also be independent of dedicated PULs, as is the case for Planctomycetes (Glöckner et al. 2003). In Gammaproteobacteria, moreover, the SusD components of PULs are largely missing (Gobet et al. 2018), suggesting evolutionary boundaries limiting horizontal gene transfer, or reliance on different ecological strategies of polysaccharide degradation. However, even in PUL-rich bacteria, some degradation systems are not exclusively clustered in PULs. For example, carrageenan utilization by *Z. galactanivorans* relies on PULs as well as genes dispersed throughout the genome, despite being coinduced and upregulated in response to carrageenan (Ficko-Bean et al. 2017). In a similar manner, marine vibrios (Gammaproteobacteria) contain enzyme-encoding genes and other genes for alginate degradation within operons, but also as single-enzyme genes throughout the genome (Hehemann et al. 2016).

Regulation of polysaccharide degradation is presumably influenced by genome size, with more refined regulation in larger and more complex genomes than in smaller and streamlined genomes (Cottrell & Kirchman 2016). Bacteria with larger genomes may induce expression of their entire CAZyme repertoire only upon contact with a relevant substrate, as observed in *Polaribacter* with chondroitin sulfate (Xing et al. 2014) and *Z. galactanivorans* with alginate (Thomas et al. 2012). In

Alteromonas macleodii, a low basal expression of alginate lyases was observed in cells growing on glucose, but expression was increased 5–72-fold upon the availability of alginate, demonstrating the importance of the presence of a target substrate (Neumann et al. 2015). *A. macleodii* has also been shown to use substrate prioritization, in which polysaccharides are consumed in preferential order. Catabolite repression resulted in initial laminarin utilization, followed by simultaneous alginate and pectin utilization, coincident with pronounced shifts in gene expression, protein abundance, and metabolite secretion (Koch et al. 2019a). Moreover, *A. macleodii* carries multiple PULs for the same substrate (Koch et al. 2019a), indicating differential regulation dependent on the exact monomer composition, potential decorations, and/or chain length.

Changing substrate chemical composition is thus likely a driving force behind the succession of bacteria and CAZymes that respond to natural phytoplankton blooms, as revealed through metagenomic and metaproteomic analysis of successive spring blooms at Helgoland in the North Sea (Avcı et al. 2020; Teeling et al. 2012, 2016) (**Figure 4**). High expression of different CAZymes and carbohydrate transporters (Kappelmann et al. 2018; Krüger et al. 2019; Teeling et al. 2012, 2016) and differences in the spectrum of polysaccharide hydrolase activities (Reintjes et al.

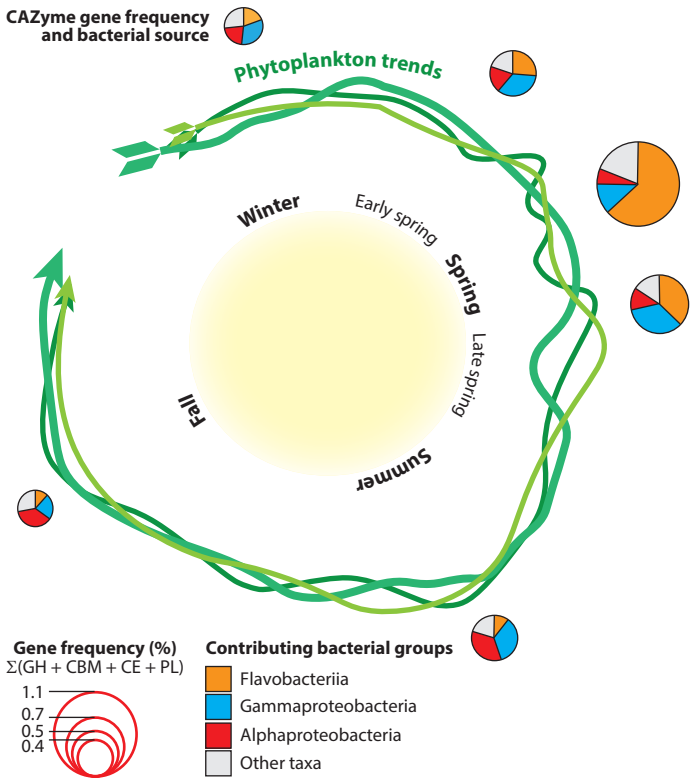


Figure 4

Seasonal cycles of phytoplankton, bacteria, and carbohydrate-active enzymes (CAZymes) at Helgoland in the North Sea. CAZyme gene frequency increased during bloom development, as did the relative contribution of Flavobacteriia. Circle placement corresponds to sample time point, circle size corresponds to CAZyme gene frequency, and colors indicate the bacterial groups contributing these genes. Abbreviations: GH, glycoside hydrolase; CBM, carbohydrate-binding module; CE, carbohydrate esterase; PL, polysaccharide lyase. Data are from samples collected in 2009, replotted from Teeling et al. (2016).

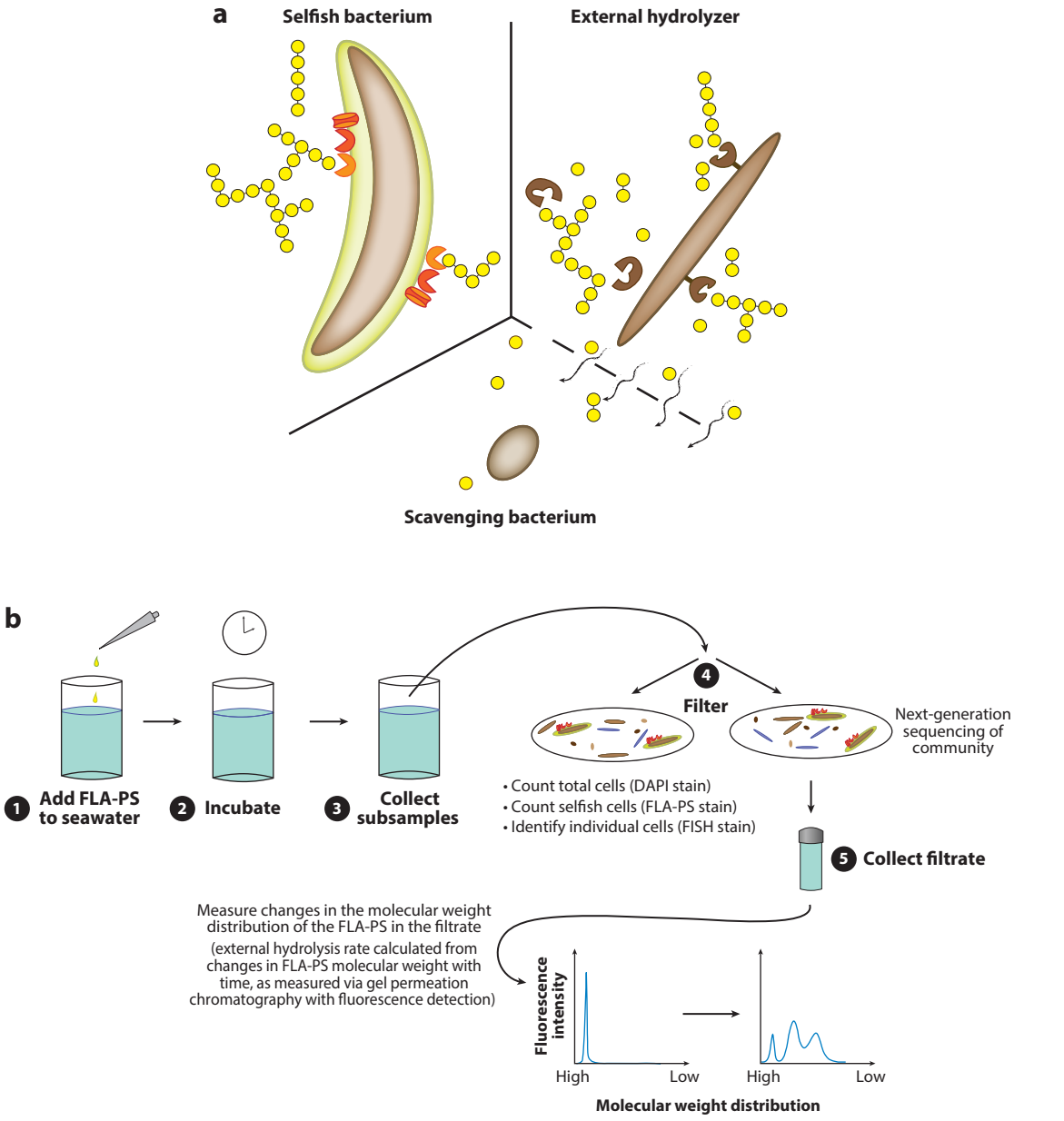
2020) associated with different bloom stages likely reflect the different suites of polysaccharides produced (S. Vidal-Melgosa, A. Sichert, B. Francis, D. Bartosik, P.L. Buttigieg, et al., manuscript in preparation) during bloom stages dominated by different phytoplankton (Reintjes et al. 2020; Teeling et al. 2012, 2016). Tracking PUL composition and abundance and identifying associated CAZymes in environmental samples (Avcı et al. 2020, Kappelmann et al. 2018, Krüger et al. 2019) thus provide indications about the nature and identity of major polysaccharides utilized by heterotrophic bacteria. From these data, we can generate models and testable hypotheses about polysaccharide cycling by heterotrophic bacterial communities.

ECONOMICS OF MICROBIAL POLYSACCHARIDE DEGRADATION: NEW MODELS

Microbial strategies of enzyme production and biopolymer degradation have typically been viewed via a cost/benefit analysis or a public-goods problem of hydrolysate production (Drescher et al. 2014, Traving et al. 2015). Producing extracellular enzymes—whether cell-surface bound or freely released—that are active in the external environment represents a cost to an organism, a cost that would be beneficial only if the resulting hydrolysate obtained by the organism compensates for enzyme production. These costs and benefits have been explored in models that also consider the extent to which organisms that do not produce extracellular enzymes [referred to as cheaters (Allison 2005) or scavengers (Reintjes et al. 2019)] are able to benefit from hydrolysis products produced via enzyme activity (e.g., Allison 2005, Drescher et al. 2014). Cell-associated enzymes may allow for tighter coupling between substrate hydrolysis and product uptake and thus higher retention of substrate by the enzyme-producing cell (Traving et al. 2015). Hydrolysis via enzymes that are not associated with the cell surface, however, might be necessary for the degradation of insoluble substrates, since tight associations with cell surfaces would limit the ability to access substrates that are shielded from the external environment. Likewise, such enzymes would permit foraging for substrates in sediments (Vetter et al. 1998). However, substrate hydrolysis via detached enzymes may also lead to sharing hydrolysis products with competitors (Hehemann et al. 2016).

These two-player models will need to be revisited in light of the recent discovery that a considerable fraction—up to 25%—of bacteria in surface ocean waters are capable of hydrolyzing polysaccharides and taking up the hydrolysis products (Reintjes et al. 2017) with little or no loss of hydrolysate, a mode of substrate acquisition that Cuskin et al. (2015) have characterized as "selfish." Widespread use of selfish uptake (Reintjes et al. 2017, 2019) suggests that a three-player model (Arnosti et al. 2018) (**Figure 5*a***) is required, one that includes selfish bacteria, bacteria that carry out hydrolysis in the external environment and produce externally available hydrolysate (external hydrolyzers), and scavenging bacteria that can take up the hydrolysis products of polysaccharides but either do not or cannot produce the enzymes necessary for initial hydrolysis (**Figure 5*a***). These bacteria can be identified and quantified in samples collected from the environment (**Figure 5*b***). The balance between selfish uptake and external hydrolysis has the potential to affect the size range of dissolved carbohydrates and thus the population sizes of scavenging bacteria (Arnosti et al. 2018).

Clues about the conditions under which these different mechanisms of polysaccharide hydrolysis may dominate are provided by a recent investigation during a phytoplankton bloom at Helgoland in the North Sea. Early in the bloom, selfish uptake (except of laminarin) was low but measurable, and only a few substrates were externally hydrolyzed (Reintjes et al. 2020) (**Figure 6**). Selfish uptake increased considerably as the bloom progressed, as did extracellular hydrolysis rates. The late bloom phase was characterized by declining selfish activity as bacterial cell counts increased, and the substrate spectrum and activities of extracellular enzymes remained



(*Caption appears on following page*)

Figure 5 (*Figure appears on preceding page*)

(*a*) A new model of polysaccharide degradation that includes three types of bacteria: selfish bacteria, which release little or no low-molecular-weight hydrolysis products to the external environment; external hydrolyzers, which use cell-surface-attached and freely released extracellular enzymes to hydrolyze substrates, leading to the production of low-molecular-weight hydrolysis products in the external environment; and scavenging bacteria, which cannot or do not produce extracellular enzymes and therefore are dependent on the activities of external hydrolyzers for low-molecular-weight hydrolysis products (shown by the *arrows*, signifying diffusing mono- and disaccharides). Note that selfish bacteria have green stain in the periplasmic space, signifying uptake of fluorescently labeled polysaccharides. (*b*) Schematic diagram of the experimental procedures to identify and quantify selfish bacteria via the fluorescently labeled polysaccharides (FLA-PS) in their periplasmic space, measure enzymatic hydrolysis rates in seawater, and identify potential sharing and scavenging bacteria using next-generation sequencing. Individual cells are identified by fluorescence in situ hybridization (FISH) using 16S rRNA–targeted oligonucleotide probes. (For further experimental details, see Reintjes et al. 2017, 2019, 2020.) Additional abbreviation: DAPI, 4′,6-diamidino-2-phenylindole.

high (Reintjes et al. 2020). Selfish uptake and external hydrolysis thus followed somewhat different trajectories.

Two additional observations are notable: Laminarin processing did not follow this pattern and instead showed increasing selfish uptake simultaneously with increasing hydrolysis rates, while arabinogalactan was taken up in a selfish manner but not externally hydrolyzed (**Figure 6**). On the one hand, these dynamics likely reflect the abundance of laminarin in the bloom as well as overall in the ocean (estimated annual oceanic production of 5–15 billion metric tons; Alderkamp et al. 2007, Becker et al. 2020), so that selfish uptake and external hydrolysis occurred simultaneously at high rates; on the other hand, they suggest a comparative rarity of arabinogalactan-like polysaccharides during the bloom.

To these observations we recently added a surprising new discovery: Selfish bacteria are present in bottom waters of the western North Atlantic, at water depths of 3,190–5,580 m (G. Giljan, S. Brown, C. Lloyd, S. Ghobrial, C. Arnosti & R. Amann, unpublished data). These bacteria also took up polysaccharides that were not externally hydrolyzed at the same depths (C. Lloyd, S. Brown, S. Ghobrial, G. Giljan, R. Amann & C. Arnosti, unpublished data). Together, these observations have led us to a new conceptual model of the interplay among different mechanisms of carbon processing in the context of the varying availability and structural complexity of marine polysaccharides, one inspired in part by Malik et al.'s (2020) model of bacterial trait-based characteristics in soil. Our model applies to both DOM and POM in the ocean, focusing on differences in mechanisms of polysaccharide processing—selfish uptake and external hydrolysis—and how they relate to polysaccharide quantity and complexity. We divide polysaccharide-processing bacteria among rapidly responding external hydrolyzers (E)—e.g., boom-and-bust specialists, or opportunitrophs (Polz et al. 2006)—that are focused on rapid growth; selfish bacteria (S) that accumulate resources; and bacteria that act as passive oligotrophs (O) (Polz et al. 2006), which survive on low concentrations of structurally less-complex substrates. This three-part array (**Figure 7*a***) is an alternative to distinguishing only between copiotrophs and oligotrophs within marine bacterial communities (Lauro et al. 2009).

These trait-based strategies are placed on a matrix defined specifically to consider polysaccharide structure and inventory in the ocean (**Figure 7*b***). The axes define structural complexity and substrate abundance on scales of high to low, with quadrants dominated by different members of the trait-based triangle. Highly complex polysaccharides present in high abundance could be acquired by either external hydrolyzers or selfish bacteria, highly complex substrates in low abundance will be acquired predominantly by selfish bacteria, abundant substrates that are not complex are predominantly hydrolyzed externally, and passive oligotrophs use structurally less-complex substrates at low concentrations.

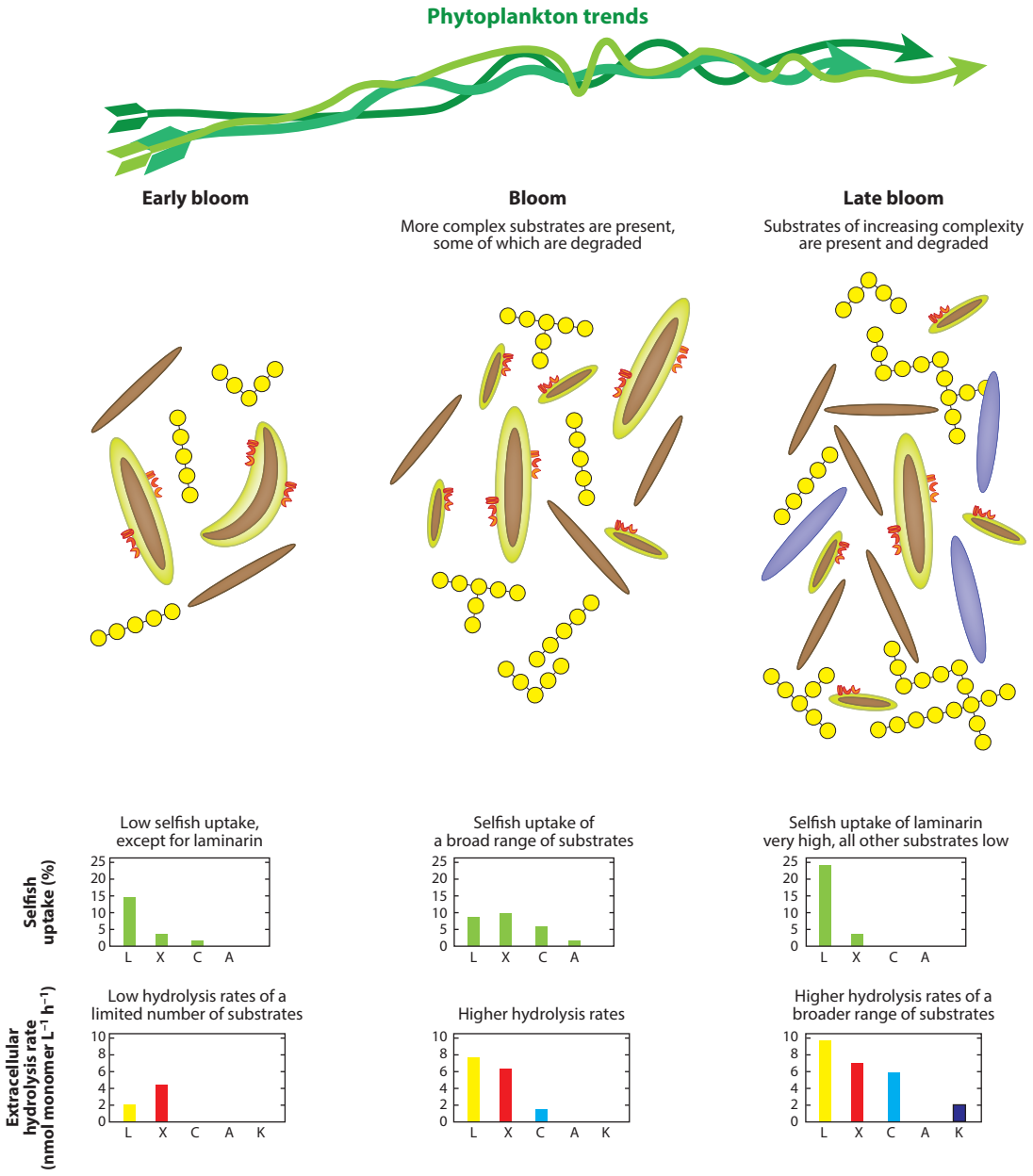


Figure 6

Selfish substrate uptake and rates of extracellular hydrolysis during the course of a spring phytoplankton bloom at Helgoland in the North Sea. In the early bloom phase, selfish uptake of substrates other than laminarin was comparatively low, and few substrates were hydrolyzed externally. During the main bloom phase, a broader range of substrates were taken up selfishly, and external hydrolysis rates increased. In the late bloom phase, selfish uptake decreased for all substrates except laminarin, and the external hydrolysis rates of a broader range of substrates were high. Note that carrageenan uptake could not be measured due to aggregation of the polysaccharide on the filter. Selfish uptake is given as percentage of 4′,6-diamidino-2-phenylindole (DAPI)–stainable cells. Abbreviations: L, laminarin; X, xylan; C, chondroitin; A, arabinogalactan; K, carrageenan. Figure adapted from Reintjes et al. (2020).

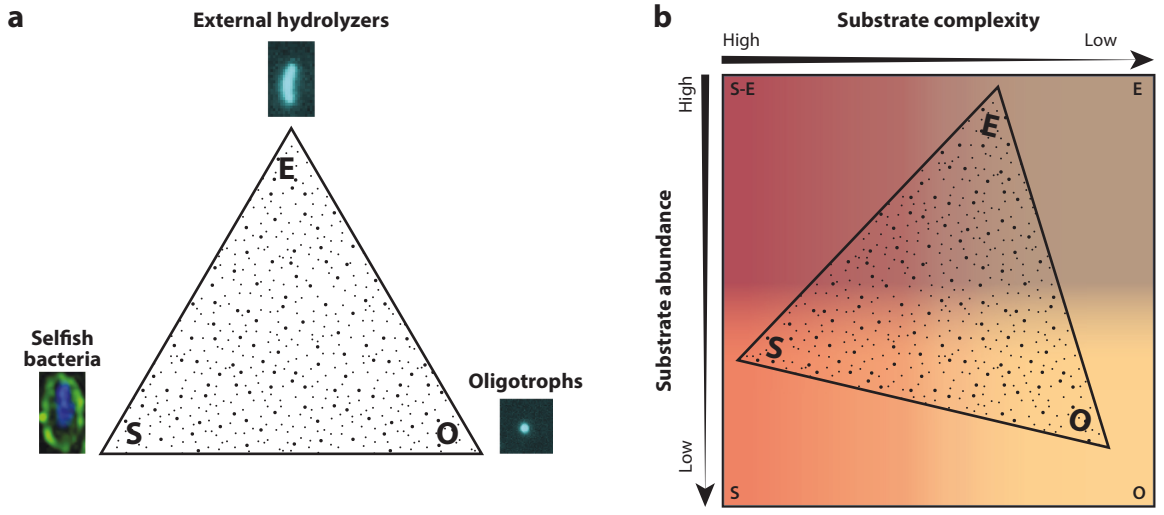


Figure 7

Framework illustrating the relationships among microbial substrate-processing mechanisms in the context of the varying availability and structural complexity of marine organic matter. (*a*) Conceptual model of trait-based microbial life strategies. External hydrolyzers (E) grow rapidly under conditions with moderate to high substrate concentrations, selfish bacteria (S) focus on obtaining targeted substrates at low as well as high substrate concentrations, and passive oligotrophs (O) obtain less-complex substrates at low concentrations. (*b*) Matrix depicting gradients of substrate complexity and abundance. The life strategies triangle on top of the gradient indicates which strategies may prevail under different conditions. Photos courtesy of G. Reintjes and G. Giljan; framework inspired in part by Malik et al. (2020).

This model provides a conceptual lens through which to interpret our recent observations. During the spring bloom at Helgoland in the North Sea, selfish bacteria initially became more abundant and then decreased in importance as external hydrolysis of a wider range of substrate occurred (**Figure 6**). These changes could be represented in **Figure 7*b*** as a transition mostly from quadrant S to quadrants S-E and E as the bloom progressed. Laminarin, the exceptional substrate that was taken up by a large number of selfish bacteria and simultaneously externally hydrolyzed at high rates at the end of the bloom (**Figure 6**), would occupy a point in the S-E quadrant, at intermediate structural complexity and very high abundance. In North Atlantic bottom water, selfish uptake of substrates that were not measurably hydrolyzed in an external manner falls into quadrant S. This quadrant also fits results from an Atlantic transect, where fucoidan was not hydrolyzed externally but was taken up by selfish bacteria (Reintjes et al. 2019), and data from Helgoland showing that arabinogalactan was taken up selfishly but not hydrolyzed externally (Reintjes et al. 2020) (**Figure 6**): Select, highly complex polysaccharides at low concentration may be targeted specifically by selfish bacteria.

To summarize, this conceptual model suggests that complex polysaccharides are bioavailable only in the presence of organisms with the specific enzymatic systems that can hydrolyze them to smaller subunits. These enzyme systems have genetic and energetic costs and are therefore unevenly distributed among bacteria (Krüger et al. 2019, Zimmerman et al. 2013), even among bacteria that are phylogenetically closely related (Avcı et al. 2020, Kappelmann et al. 2018, Koch et al. 2020, Unfried et al. 2018, Xing et al. 2014). Furthermore, the bacteria themselves are unevenly distributed over space and time in the ocean (Neumann et al. 2015; Teeling et al. 2012, 2016; Wietz et al. 2015). The variable reactivity of the polysaccharide components of POM and high-molecular-weight DOM are thus intimately linked to the varying enzymatic capabilities of

bacteria comprising heterotrophic microbial communities. Moreover, bacteria capable of accessing complex organic matter use different strategies: External hydrolyzers function especially well when polysaccharides are abundant (Traving et al. 2015), when they are spatially found in sufficiently dense patches (Ebrahimi et al. 2019), and under conditions in which diffusive loss is hindered (or perhaps when it is sufficiently rapid in biofilms; see Drescher et al. 2014) so that they can obtain sufficient return on their enzymatic investments (i.e., also on particles, aggregates, or in biofilms) (Vetter et al. 1998). Since selfish bacteria capturing organic matter share little or none of the resulting hydrolysate (Cuskin et al. 2015), they are effective especially under circumstances that require the coordination of highly complex enzymatic machinery—whether these polysaccharides are abundant or scarce—or, alternatively, under conditions that may require rapid uptake due to high competition for a specific substrate. The trade-off for these acquisition capabilities for selfish bacteria, however, may be that they grow less rapidly than external hydrolyzers. These considerations suggest that polysaccharides that are not reactive or not microbially accessible require enzymatic capabilities that are absent or not expressed in a given microbial community, have characteristics that make them difficult to transport into the cell, are structurally modified so that they do not fit specific transporters, or are enzymatically inaccessible (e.g., due to complexation, aggregation, or surface interactions). This conceptual model thus also provides a road map of future research needs to understand polysaccharide cycling in the ocean.

KEY ISSUES, NEEDS, AND OPPORTUNITIES

Three key research needs can be crystallized from the above: (*a*) better structural characterization of polysaccharides, especially those in the detrital pool; (*b*) more complete assessment of microbial enzymatic capabilities; and (*c*) a fuller perspective on polysaccharide turnover in the ocean.

New Ways to Characterize and Quantify Polysaccharides in the Ocean

New experimental approaches to more effectively characterize the structure and quantities of marine polysaccharides are an urgent research need. Promising new approaches include using well-characterized CAZymes from marine bacteria as selective tools to quantify specific polysaccharides (Becker et al. 2017). This approach was recently used to measure the laminarin content of marine POM and to assess the contribution of laminarin to POM in the ocean (Becker et al. 2020). POM extracts were treated with select laminarin-hydrolyzing enzymes; the resulting hydrolysis products were then quantified by well-established methods (Becker et al. 2017). In principle, a similar approach could be used for a wide variety of polysaccharides; further application requires the identification and characterization of specific marine-related CAZymes.

Another promising approach focuses on carbohydrate microarrays, which are established tools to profile polysaccharide composition in plants (Moller et al. 2007). Comprehensive microarray polymer profiling consists of performing several sequential extractions from plant material that are then printed by microarrayers. Polysaccharide-specific probes are applied to the resulting microarrays to detect the polysaccharide epitopes present in the different extracts (Moller et al. 2007). Recent application to marine systems has revealed the presence of arabinogalactan proteins and β-glucans in macroalgae (Herve et al. 2016, Salmean et al. 2018), the presence of specific polysaccharides during a microalgae bloom (S. Vidal-Melgosa, A. Sichert, B. Francis, D. Bartosik, P.L. Buttigieg, et al., manuscript in preparation), and the release of polysaccharide mixtures by macroalgae, including *Fucus* and *Saccharina* (Koch et al. 2019a). A related avenue is to target carbohydrate-binding modules and SusD proteins, which can provide structurally relevant information about target polysaccharides (Salmean et al. 2018). A microarray approach that includes a

variety of (newly developed) probes could be used to track changes in polysaccharide inventories in concert with other routine biological and biogeochemical measurements on a recurring basis at long-term marine monitoring sites. A further goal should be to develop polysaccharide detection modules for in situ measurements at sea, so that drifters or autonomous underwater vehicles (Yamahara et al. 2019) could provide high-resolution depth profiles of specific polysaccharides or carbohydrate-binding modules, even in remote oceanic regions.

Integrated Investigations of Organism Capabilities, Gene Expression, and Enzymatic Function

The integration of bacterial functional and enzymatic diversity, combining omic investigations of community-wide patterns during polysaccharide degradation with organism-based research for the identification and characterization of novel CAZymes, should provide a fruitful pathway forward. Bacterial isolates remain an essential resource by providing complete genomes that enable the formulation and investigation of testable hypotheses regarding bacterial physiology and phenotypes (Koch et al. 2020). Polysaccharide-degrading model isolates such as *A. macleodii* (Neumann et al. 2015) and different flavobacteria (Bauer et al. 2006, Kabisch et al. 2014, Mann et al. 2013, Thomas et al. 2012) will continue to provide essential insight into physiological and enzymatic aspects of polysaccharide degradation (Foran et al. 2017, Hehemann et al. 2012, Rebuffet et al. 2011, Thomas et al. 2012). However, other microbial clades that may be involved in hydrolytic processes, including SAR86, SAR202, and marine group II euryarchaea (Dupont et al. 2012, Orsi et al. 2015, Saw et al. 2020), still lack cultured representatives. Targeted cultivation efforts might facilitate the discovery of novel CAZyme families in phyla that have not yet been cultured. Genome-centric metagenomics and the reconstruction of PULs from environmental sequence data (Krüger et al. 2019) will also help to decipher the CAZyme repertoire among bacteria that have yet to be cultured, providing clues about substrate preferences and potentially facilitating cultivation using tailored substrate regimes.

In addition to the isolation and characterization of specific bacteria, further biochemical studies of CAZymes will be necessary, since the identification of CAZyme families is not sufficient to understand function. Posttranscriptional expression data will be needed to know which enzymes are actually produced; the enzymes themselves need to be isolated and characterized to understand their structure and function (Foran et al. 2017, Hehemann et al. 2012, Rebuffet et al. 2011). Moreover, the identification of selfish bacteria requires direct experimental measurements, since this mechanism of substrate utilization cannot be predicted solely from genome-based information. A further need concerns the locations from which we obtain bacterial isolates. Since most of the ocean remains unexplored, we know comparatively little about the polysaccharide-degrading capabilities of microorganisms in the deep ocean and in sediments (Bird et al. 2019, Lazar et al. 2016, Orsi et al. 2018).

Understanding Polysaccharide Turnover and Enzyme Lifetime in the Ocean

Understanding the cycling of marine polysaccharides requires greater insight into the system as a whole, including currently underexplored regions. Several key questions remain to be addressed: For example, what factors control access to polysaccharides in the ocean? Polysaccharides can be rapidly cycled as energy sources, but the presence of carbohydrates in deep-ocean sediments (Cowie et al. 1995) and as part of POM in sinking particles (Wakeham et al. 1997) suggests that microbial or enzymatic access to some polysaccharides is impeded (Enke et al. 2018), at least for substantial periods of time. Also, to what extent does shielding of polysaccharides through

complexation with other organic or inorganic constituents (e.g., clay particles) affect enzyme access or degradability? How might such shielding affect polysaccharide degradation on particles, particularly in sediments? A related issue concerns the composition, structure, and persistence of the carbohydrate-containing fraction of DOM: Why does some fraction of polysaccharides persist in the water column? Explanations from other environments—soils, sediments, and freshwater environments—are not sufficient to answer this question (Arnosti et al. 2014).

These issues also raise key questions about polysaccharide-hydrolyzing enzymes: What is the functional lifetime of an extracellular enzyme in the environment? Are membrane-vesicle-associated enzymes (Li et al. 2016) protected from degradation? Does bacterial attachment to particles affect the lifetime and diffusion of enzymes, and therefore the extent to which hydrolysate is made available to a community (Smith et al. 1992)? How do lifetimes in the water column compare with lifetimes in sediments? These considerations—for which we have few data (Steen & Arnosti 2011)—are also central to better calculations of return on investment for microbes and their enzymes.

Looking Forward

The complexity of polysaccharides and CAZymes provides both opportunities and problems. Given the fine-scale structural fit of CAZymes and their substrates, careful examination and interpretation can provide important clues about the presence and relevance of distinct polysaccharides in marine environments and their importance to specific microbes. Many proteins fall within known families and subfamilies of CAZymes with a defined range of possible substrates. Combined in the context of a PUL, these predicted genes can provide clues about putative structures present within polysaccharides in the environment. In a similar matter, data about polysaccharide hydrolase activities can provide important clues about the role of specific polysaccharides and microbial communities at distinct times and locations in the ocean. For example, the observation that laminarin is hydrolyzed virtually everywhere in the ocean, whereas pullulan hydrolysis is less widespread (Arnosti et al. 2011, Balmonte et al. 2018, Hoarfrost & Arnosti 2017, Steen et al. 2012), doubtless points to important differences in the presence and prevalence of these polysaccharides, which were recently confirmed in part by a new assessment of laminarin inventories in the ocean (Becker et al. 2020). In short, testable hypotheses can be generated from information about specific PULs, CAZymes, and CAZyme activities; this kind of information cannot be derived for other macromolecules.

Polysaccharides thus have the potential to serve as biogeochemical Rosetta stones: They are abundant components of living biomass as well as detrital organic matter, their structural diversity is linked to their origins and processing, and they are widely distributed and transformed in the ocean. The development of new methods that yield greater structural insights, enable more rapid measurements in a wider range of ocean environments, and facilitate our understanding of the links between polysaccharides and the organisms that transform them will constitute a key to understanding a substantial portion of the marine carbon cycle.

SUMMARY POINTS

1. Polysaccharides constitute a major fraction of living biomass as well as detrital (dissolved and particulate) organic matter. Although most polysaccharides are rapidly recycled and fuel the marine food web, they also constitute a notable fraction of dissolved organic matter in ocean waters and particulate organic matter preserved in sediments.

2. Different polysaccharides are cycled at different rates in the water column—some polysaccharides are hydrolyzed almost everywhere, whereas others are hydrolyzed only at select locations or under specific conditions. Sedimentary microbial communities appear to have a broader range of enzymatic capabilities than many of their water column counterparts. The enzymatic capabilities of heterotrophic microbial communities are thus important determinants of polysaccharide bioavailability.
3. Determining the structure of polysaccharides with sufficient resolution is challenging—polysaccharides have a multitude of linkage possibilities and include diverse functional groups, and analyzing them without losing important structural information is difficult.
4. The complex structure of polysaccharides is also an opportunity: Since polysaccharides and their carbohydrate-active enzymes (CAZymes) are finely tuned to one another, omic analyses can be used to develop testable hypotheses about the presence and dynamics of specific polysaccharides.
5. Work with isolated bacteria and individual enzymes, however, is also essential: In silico assignments of target substrates may not be correct, and newly discovered enzymes may have no related counterparts in databases (Foran et al. 2017, Rebuffet et al. 2011).
6. A new conceptual model suggests that mechanisms of polysaccharide cycling in the ocean may be controlled by polysaccharide structural complexity and abundance. From this perspective, a lack of cycling of polysaccharides may be due to specific enzymatic capacities being absent or not expressed, or the polysaccharides themselves may have characteristics that impede transport into the cell, may have structural modifications such that they do not fit transporters, or may be enzymatically inaccessible due to complexation, aggregation, or surface interactions. This model constitutes a testable framework for further investigations.

FUTURE ISSUES

1. Better methods to analyze complex structures of polysaccharides, including detrital components, and separation and analytical techniques that do not destroy structural information are particularly required.
2. Further progress is needed in identifying (and isolating representatives of) major polysaccharide degraders, especially among uncultivated microbial lineages.
3. Further insights are needed into the links between organisms, genes, transcripts, and proteins and their target polysaccharides and into the dynamics of different organisms involved in the degradation of specific polysaccharides.
4. More and better rate measurements of polysaccharide turnover in the ocean are required to accurately quantify rates of organic carbon cycling.
5. A better understanding of the factors facilitating or impeding solubilization of solid polysaccharides is required.
6. Investigations of the factors or variables controlling the effective lifetimes of extracellular enzymes in the water column and sediments—essential knowledge for accurate

calculation of microbial return on investment—would vastly improve our understanding of constraints on carbon cycling.

7. The development of methods to measure polysaccharides and CAZyme genes, transcripts, and proteins that can be automated and deployed in situ—using drifters, remotely operated vehicles, buoys, or landers—will be central to obtaining a clear picture of the polysaccharide seascape of the ocean.

DISCLOSURE STATEMENT

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Errata

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