ABSTRACT

Title of Document:	INTACT BACTERIAL HOPANOIDS AS SPECIFIC TRACERS OF BACTERIAL CARBON IN MARINE AND ESTUARINE ENVIRONMENTS
	Karen Ann Taylor, M.S., 2009
Directed By:	Professor H. Rodger Harvey, Marine Estuarine Environmental Sciences

Intact bacteriohopanepolyols (BHPs) and their degrative products were investigated in surface sediments and particulate organic matter from the Bering Sea, Western Arctic Ocean and Chesapeake Bay to trace the inputs of bacterial carbon sources and the dominant microbial processes operative during organic matter recycling. Despite cold temperatures and the dominance of diatoms, cyanobacteria are ubiquitous and inhabit the deeper layers of the euphotic zone in the Bering Sea, where their contributions to sediments were directly traced. As a small but important contribution to the total system chlorophyll, cyanobacteria represent a previously undocumented fraction of the organic carbon pool in this region. In the Western Arctic, soil derived bacterial sources were abundant and include a fraction that likely degraded on land prior to being transported into the Arctic Ocean. Bacterial signatures in Chesapeake Bay transition along the salinity gradient with intact hopanoids reflecting a diverse range of potential bacterial sources.

INTACT BACTERIAL HOPANOIDS AS SPECIFIC TRACERS OF BACTERIAL
CARBON IN MARINE AND ESTUARINE ENVIRONMENTS

By

Karen Ann Taylor

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Advisory Committee:
Professor H. Rodger Harvey, Chair
Assistant Professor Byron Crump
Assistant Professor Cindy Palinkas

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Chapter 1: Introduction and Overview

Background

Bacteria are ubiquitous and account for the vast majority of living biomass in aquatic systems (Sherr and Sherr 2000; Whitman et al. 1998). They play a key role in the biogeochemical cycling of organic matter through a diversity of metabolic pathways (Kirchman 2000) and mediate earth's major elements through processes such as fermentation, nitrification, nitrogen-fixation, sulfate reduction and methane oxidation among others (González et al. 1999; Sherr and Sherr 2000; Zehr and Ward 2002). In their role as primary producers, bacteria can also transfer energy to higher trophic levels and provide a crucial link in aquatic food webs (Sherr and Sherr 2000). While carbon calculations based on abundance suggest bacterial contributions to organic matter reservoirs in the ocean are substantial (Whitman et al. 1998); quantifying this fraction is constrained by the lack of specific biomarkers and the recognized abundance of marine microbes that cannot be grown in laboratory cultures (Rappé and Giovannoni 2003).

Many potential tracers of bacterial carbon have been suggested and include muramic acid, which was applied to estimate bacterial biomass in marine and terrestrial environments (Millar and Casida 1970; King and White 1977) as well as in pure cultures (Rönkkö et al. 1994). Muramic acid was also used to detect for the presence of bacteria in indoor air (Fox and Rosario 1994), clinical samples (Gilbart et al. 1986) and extraterrestrial samples (Kozar et al. 2001); yet its strong association with dead bacterial biomass has recently been revealed, and thus muramic acid is not an appropriate tracer of

living bacterial carbon (Guggenberger et al. 1999; Benner and Kaiser 2003; Glaser et al. 2004). Though not exclusive to bacteria (Brückner et al. 1994; Lee and Bada 1977), D isomers of amino acids were proposed as markers of bacterial derived organic matter (Ueda et al. 1989; Caspo et al. 2001); however both D-amino acids and peptidoglycan are not suitable modes for measuring bacterial biomass as cellular concentrations vary depending upon cell type (Quigley and Schwab 1988; Schleifer and Kandler 1967; Fukuhara et al. 1983). Specific membrane lipids such as odd- and branched-chain fatty acids (i.e. C_{15} and C_{17}) have often been suggested as proxies of bacterial biomass (Guckert et al. 1985), yet recently strong correlations between fatty acid synthesis and differing carbon substrates have been shown, implying their limitations as a universal measure of bacterial carbon inputs to marine environments (Harvey et al. 2006). Other membrane lipids have been found as signatures of specific metabolic processes such as ladderane lipids that are tracers of the anaerobic oxidation of ammonium to N_2 (i.e. anammox; Kuypers et al. 2003) and some forms of phospholipids that are markers of heterotrophic recycling, particularly in phosphorus-limited regions like the Sargasso Sea (Van Mooy et al. 2008). The ubiquitous marine archaea also have diagnostic lipid signatures (i.e. glycerol dialkyl glycerol tetraether lipids, GDGTs) that appear to reflect both autotrophic and heterotrophic metabolisms (Ingalls et al. 2006). In contrast, eukaryotic lipids have shown to have a large suite of defined structures that provide both source and process markers in marine and terrestrial environments (Bianchi et al. 1989; Fahl and Stein 1997, 1999). Sterols and related membrane stabilizing compounds (Gennis 1989) have been important tracers of organic carbon sources to the dissolved

(Mannino and Harvey 2000), and particulate (including sedimentary) organic matrices of the global ocean (Volkman 1986).

Prokaryotes, however, do not synthesize sterols (with few exceptions) but many members instead produce a group of membrane lipids known as intact bacteriohopanepolyols (BHPs, Fig. 1.1). Intact BHPs are triterpenoids biosynthesized by a variety of prokaryotes as structurally diverse cell membrane lipids, containing a C_{30} pentacyclic skeleton and highly functionalized side chain structure (Fig. 1.1; Ourisson and Rohmer 1982; Rohmer et al. 1984). Intact BHPs are hypothesized to stabilize bacterial cell membranes, regulate fluidity and induce order to the phospholipid layer(s), helping to protect the organism from external stresses such as temperature, pH and desiccation (Kannenberg and Poralla 1999). Membrane lipids are one mechanism by which microorganisms have adapted to wide temperature ranges in aquatic systems, and examples of significant correlations among hopanoid concentration and growth temperature have been reported (Poralla et al. 1984). In the case of *Bacillus acidocaldarius,* BHP concentration increased from 4 to 16% of the total lipids when temperature was raised from 50 to 65 degrees Celsius (Ourisson et al. 1987). Tolerance towards high ethanol concentrations of *Zymomonas mobilis* and *Gluconacetobacter* sp. is suspected to be modulated by intact BHPs, which may also protect these organisms from ethanol toxicity (Hermans et al. 1991; Rohmer et al. 1992). Hopanoids appear to be absent in symbiotic and parasitic bacteria, perhaps due to the protected environment in which they live (Rohmer et al. 1992); therefore it is possible that these membrane components are only biosynthesized under specific developmental or environmental conditions.

The history of the bacterial hopanoids is unique, as they have been widely observed and used among the petroleum geochemical community even prior to the establishment of their bacterial linkages (Ourisson et al. 1979). Their diagenetic products, the geohopanoids, are found ubiquitously throughout the geological record and have been observed in modern and ancient organic-rich sediments and oils dating back 2500 million years (Summons and Walter 1990; Ourisson and Albrecht 1992; Summons et al. 1999; Brocks et al. 2003). Geohopanoids comprise simple C_{29} (**i;** refer to structures in Fig. 1.1) and C_{30} (**j**, Fig. 1.1) structures and include hopanoic acids, hopanes, hopenes, hopanoidal aldehydes and ketones. They have been found in taxa of higher plants such as ferns, mosses and fungi, and were first isolated from the dammar resin produced by tropical trees of the genus *Hopea* (named after the eighteenth century British botanist John Hope; Ourisson et al. 1987; Kannenberg and Poralla 1999); therefore, hopanoids were initially suggested as tracers of terrestrial or freshwater bacteria. Degraded hopanoid products are useful in petroleum studies as proxies of maturity (Rohmer et al. 2002), and more notably as potential indicators of the timing of oxygenic photosynthesis on Earth as 2-methylhopanes are the most ancient compounds produced by cyanobacteria (Summons et al. 1999). Geohopanoids have been observed in the environment for some time (Dastillung et al. 1980a, b; Rohmer et al. 1980), yet only recently has the origin of their complex biological precursors been revealed (Ourisson and Albrecht 1992). The oxidation of intact hopanoids to their simpler, molecular fossils is often recognized (Mycke et al. 1987; Farrimond et al. 2003); however BHPs have been observed in ancient sediments (Bednarczyk et al. 2005; van Dongen et al. 2006), demonstrating their potential to remain intact for much longer periods of time. Therefore, the recalcitrance of

both intact BHPs and their degrative products allows their consideration as ideal biomarkers for tracing microbial derived organic carbon in aquatic systems.

Assignment of BHP structures to specific bacterial sources is complicated by the lack of information on the breadth of bacteria which produce the structures and the synthesis patterns of those which inhabit natural environments. Interpretations are generally limited to cultured organisms that have been examined for the production of bacterial hopanoids (Rohmer et al. 1992; Farrimond et al. 1998). Intact BHPs are observed across a wide range of prokaryotes and have been detected in approximately 57% of those tested (>270 strains), although studies are biased towards members of the alpha-, beta-, and gamma-proteobacteria and cyanobacteria (Farrimond et al. 1998; Talbot and Farrimond 2007). While their biosynthesis does not appear to require the presence of free oxygen, hopanoids are typically considered as markers of aerobic bacteria (Ourisson and Rohmer 1982); however, facultative anaerobes including *Rhodomicrobium vannielii* (Neunlist et al. 1985), *Rhodopseudomonas* spp. (Neunlist and Rohmer 1985; Neunlist et al. 1988), *Rhodospirillum rubrum* (Llopiz et al. 1992) and the fermentative *Zymomonas mobilis* (Bringer et al. 1985) have been found as producers of hopanoids. Hopanoid biosynthesis has also been observed in anaerobic cultures of *Geobacter* sp. (Fischer et al. 2005; Härtner et al. 2005) as well as in enrichment cultures of the anaerobic Planctomycetes (Sinninghe Damsté et al. 2004).

Intact BHP structures have a C-30 pentacyclic skeleton and an extended side chain containing four, five or six functional groups. Tetrafunctionalized BHPs are most common, and typically have three hydroxyl groups (-OH) at C-32, 33 and 34 with the terminal group at C-35 occupied by a hydroxyl (-OH; bacteriohopanetetrol, BHT, **1a**;

numbers and letters refer to structures in Fig. 1.1) or amino (-NH$_2$; aminotriol, **1b**, Fig. 1.1) functionality. More complex structures containing composite moieties such as sugar (**1f**, Fig. 1.1; Talbot et al. 2008), amino-sugar (**1c**, Fig. 1.1; Rohmer 1993) and nucleoside (**1g**, Fig. 1.1; Talbot et al. 2007c) derivatives can also be linked at the terminal position on the side chain. Other commonly known structural modifications include an additional methyl (-CH$_3$) group at C-2 or C-3 and/or unsaturation at C-6 and/or C-11 in the pentacyclic skeleton. Any combination of the above is a potential BHP structure, yet very little is known about their biosynthesis and specific function in bacterial cell membranes. Moreover, new compounds are continuously being discovered as additional bacteria are cultured and a broader range of environmental settings are tested for the occurrence of intact hopanoids.

BHT (**1a**, Fig. 1.1), aminotriol (**1b**, Fig. 1.1) and BHT cyclitol ether (**1c**, Fig. 1.1) are among the most commonly reported BHPs isolated in cultured bacteria as well as in samples from various sedimentary environments (Fox et al. 1998; Talbot et al. 2003b, c; Blumenberg et al. 2006; Talbot and Farrimond 2007). BHT (**1a**, Fig. 1.1) has been observed in a diverse suite of organisms including cyanobacteria, purple non-sulfur bacteria, methylotrophs and some gram-negative and gram-positive bacteria (i.e. Rohmer et al. 1984); whereas aminotriol (**1b**, Fig. 1.1) is commonly found in Type II methanotrophs (Neunlist and Rohmer 1985; Talbot et al. 2001) and has also been seen in purple non-sulfur bacteria (Flesch and Rohmer 1988), cyanobacteria including various *Microcystis* sp. (Rohmer 1993; Talbot et al. 2008), and nitrogen fixing bacteria (Vilcheze et al. 1994). Furthermore, production of BHT cyclitol ether (**1c**, Fig. 1.1) has been reported in the cyanobacteria *Chrocosphaera watsonii, Anacystis montana* and

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Trichodesmium erythraeum (Talbot et al. 2008), the gram-negative bacterium

Zymomonas mobilis (Talbot et al. 2003a), and several species of methylotrophs (Talbot

and Farrimond 2007). While these compounds appear to be widespread among

hopanoid-producing bacteria, they have the potential to be useful indicators of bacterial

carbon contributions to organic matter reservoirs and the subject of this thesis. Other

BHPs offer more source-specific information as cultured organisms have revealed

differences in taxonomic expression, such that particular bacterial groups have been

shown to produce distinct hopanoid signatures (Farrimond et al. 2000).

Cyanobacteria have been shown to generate a characteristic suite of intact

hopanoids. Pentafunctionalized BHP side chains with an additional hydroxyl (-OH)

functionality linked at the terminal position (pentol, **1d**, Fig. 1.1) have been most

commonly reported in the cyanobacteria *Nostoc muscorum* and *Nostoc* sp. (Bisseret et al.

1985; Zhao et al. 1996), but have also been identified in certain acetic acid bacteria

(Zundel and Rohmer 1985). Talbot et al. 2008 described novel tetrafunctionalized BHPs

including saturated (BHT pentose, **1f**, Fig. 1.1), unsaturated (**2f**, Fig. 1.1) and C-2

methylated (**3f**, Fig. 1.1) compounds with a fully hydroxylated terminal sugar that have

only been observed in the cyanobacteria *Gloeocapsa* sp. and *Chroococcidiopsis* sp.

While not exclusive to these organisms, methylation at C-2 is most notably recognized in

cyanobacteria; therefore many Spanish (i.e. Laguna Grande, Chica, Salina de la Muerte)

and Antarctic (i.e. Sombre and Druzhby) lakes that are known to contain cyanobacterial

mats or planktonic cyanobacterial blooms appear to be well established sources of 2-

methylhopanoids (Talbot et al. 2003c). Minor abundances have also been observed in

some methylotrophs (Renoux and Rohmer 1985; Knani et al. 1994) and nitrogen-fixing bacteria (Vilcheze et al. 1994).

Intact BHPs with unsaturation at C-6 and/or C-11 (Fig. 1.1) are most abundantly produced by acetic acid bacteria, including *Acetobacter pasteurianus*, *Gluconacetobacter europaeus, Gluconacetobacter xylinus*, etc. (Talbot et al. 2007b); however, unsaturated compounds have also been observed as minor components in cyanobacteria (Rohmer et al. 1984). An additional methyl group ($-CH_3$) at C-3 (Fig. 1.1) in the pentacyclic skeleton is also an important structural modification known from acetic acid bacteria (Zundel and Rohmer 1985; Simonin et al. 1994) as well as certain methanotrophs *Methylococcus capsulatus* and *Methylomonas methanica* (Zundel and Rohmer 1985; Neunlist and Rohmer 1985), with the former also typically containing unsaturation at C-6 and/or C-11 (Rohmer and Ourisson 1986; Simonin et al. 1994; Talbot et al. 2007b).

Type I methane oxidizing bacteria *Methylococcus* and *Methylocaldum* spp. are the only known sources of 35-aminobacteriohopane-30, 31, 32, 33, 34-pentol (**1h**, Fig. 1.1), a hexafunctionalized structure containing five hydroxyl groups and a NH_2 functionality at C-35 (Neunlist and Rohmer 1985; Talbot and Farrimond 2007). High concentrations of this compound were distinctively observed in cores from two anoxic lacustrine settings, Lake Pollen (Norway; 3-103 µg g^{-1} dry sediment; Innes et al. 1998) and Priest Pot (UK; 290-750 µg g^{-1} dry sediment; Innes et al. 1997), where the prevalence of methane oxidation is known in the water column. Methanotrophs are known producers of the pentafunctionalized aminotetrol (**1e**, Fig. 1.1; Talbot and Farrimond 2007), which has also been reported in trace amounts in some species of sulfate-reducing bacteria (Blumenberg et al. 2006).

Other complex composite BHPs including adenosylhopanes (**1g, 1g'**, Fig. 1.1) have been suggested as markers of as yet uncultured soil bacteria that could be used to trace terrestrial microbes transported to marine sediments (Talbot and Farrimond 2007; Cooke et al. 2008 a, b). Adenosylhopanes have been reported in various cultured organisms including purple non-sulfur bacteria (Neunlist and Rohmer 1985; Neunlist et al. 1988), ammonia oxidizers (Seemann et al. 1999) and nitrogen fixing bacteria (Bravo et al. 2001).

Research goals and overview

The overall goal of this thesis was to investigate the bacterial hopanoids as potential tracers for sources and transport of bacterial carbon. Given their longevity in the environment and diversity of structures, this allows the potential to quantify the 'living' and 'degraded' contributions to particulate and sedimentary organic carbon pools using these specific molecular proxies (i.e. intact bacteriohopanepolyols, BHPs). Three diverse regions were examined including the Bering Sea, Western Arctic Ocean and Chesapeake Bay.

The main objectives of this study were:

1) To identify and determine the diversity and abundance of individual intact hopanoids in organic matter substrates from the Bering Sea, western Arctic and Chesapeake Bay.

2) To quantify ($\mu g\ g^{-1}$ OC) the total intact and degraded hopanoid fractions in samples from each geographic location and estimate contributions of specific

bacterial groups based on the distribution of intact hopanoids determined from objective #1.

3) To exploit hopanoid signatures to trace the direct input of bacterial carbon from particulate material to the underlying surface sediments.

4) To use intact BHPs to infer and compare the bacterial sources and processes occurring among each geographic location based on chlorophyll biomass, terrigenous inputs and flow patterns and the transport of bacterial carbon signatures along land-sea gradients.

In this thesis, Chapter 3 focuses on tracing the abundance and distribution of cyanobacterial carbon contributions to ice, particles and surface sediments from the Bering Sea, which is a diatom dominated system. Bacterial carbon inputs to surface sediments from this region are then contrasted with those observed in the Western Arctic Ocean, which receives significant inputs of both marine and terrestrial derived organic carbon sources.

In Chapter 4 bacterial carbon contributions to western Arctic sediments are further discussed with an emphasis on tracing soil specific signatures across two shelf to basin transects and in two Arctic rivers where unidirectional flow patterns dominate. Bacterial carbon signatures in samples from the western Arctic are then compared to those observed in particles and surface sediments along the salinity gradient of Chesapeake Bay and the coastal Atlantic Ocean. Chesapeake Bay is a temperate estuary that experiences eutrophication and seasonal hypoxia/anoxia due to the vast inputs received from numerous rivers and the adjacent watershed. The multiple bacterial sources

and processes reflected by intact BHPs and the dominance of their degraded products in surface sediments are discussed.

The varying sources and abundance of organic matter inputs to these marine and estuarine environments impact the bacterial communities that reside in each region, making the Bering Sea, western Arctic and Chesapeake Bay ideal for the investigation of specific molecular bacterial proxies. The ubiquity of bacteria in the environment suggests their potential as important contributors to organic carbon pools, and this fraction of organic matter can now be traced using intact bacterial hopanoids. Intact BHPs not only provide quantitative measures of bacterial carbon, but also offer clues to the bacterial sources and processes occurring in an environment as well as how bacterial communities might respond to warming ocean temperatures.
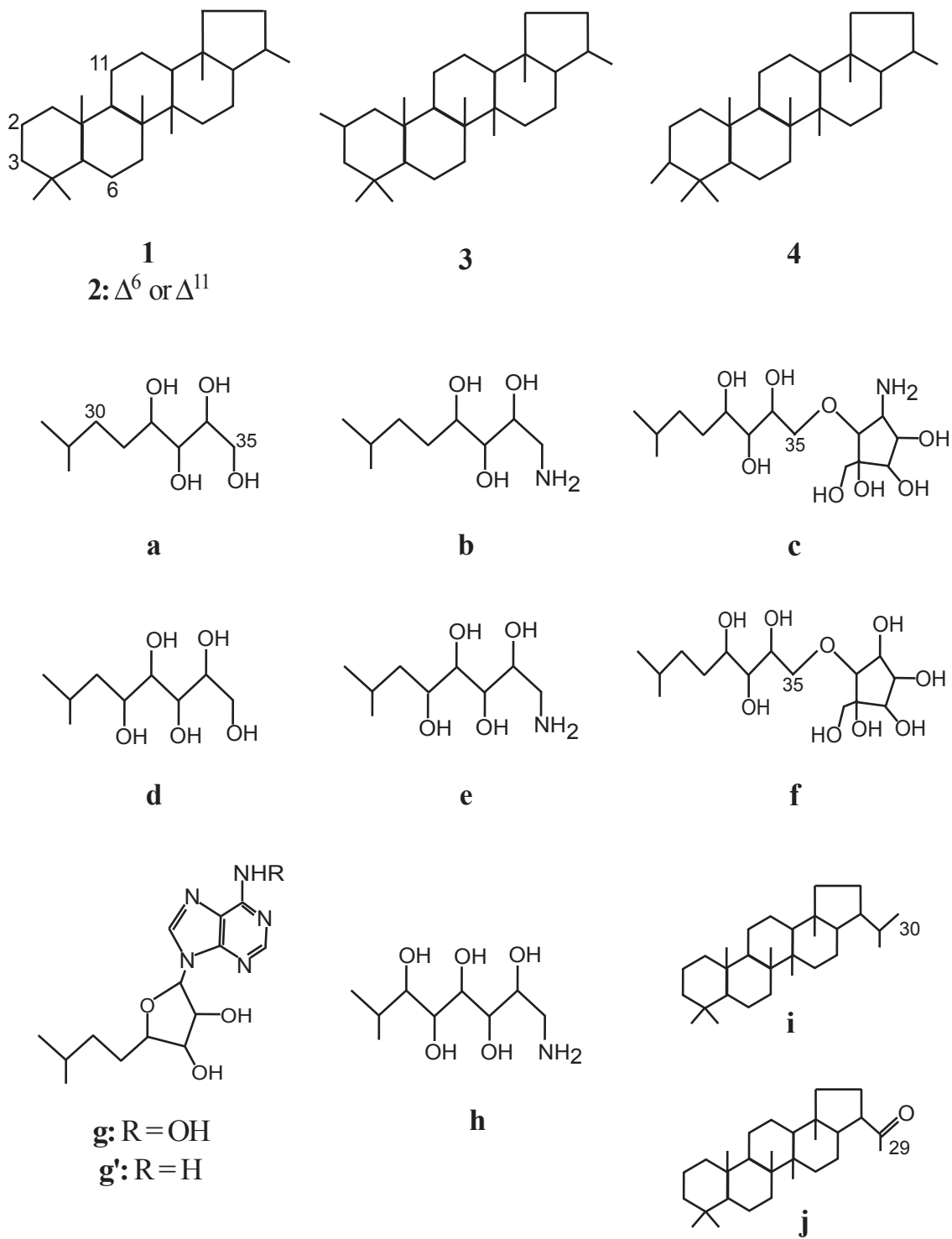
Figure 1.1. Bacterial hopanoid structures referred to in text.

Chapter 2: Analytical Framework

The analytical schemes of this study were designed to identify and quantify both intact BHPs as well as their degradative products in samples from the Bering Sea, western Arctic and Chesapeake Bay. Recent advances in atmospheric pressure chemical ionization-liquid chromatography-tandem ion-trap mass spectrometry (APCI-LC/MS^n) allow for the examination of individual intact hopanoids in great detail, and thus the number and nature of functional groups associated with each side chain structure can now be determined (Talbot et al. 2001, 2003a, b, 2007a, b, 2008; Talbot and Farrimond 2007). Prior to these analytical advances, hopanoid products were generally restricted to analysis by gas chromatography (GC), and thus the breadth of information available on their biological precursor compounds could not be fully utilized. Nevertheless the ability to now examine individual BHPs by APCI-LC/MS^n remains a challenge due to the lack of a pure BHP standard and the quantitative limitations of ion-trap mass spectrometry; therefore, relative abundance (%) of intact hopanoids are typically reported with few exceptions (Cooke et al. 2008a, b). In this study, an external calibration curve was constructed in an attempt to quantify intact BHPs by APCI-LC/MS^n and to calibrate between LC-MS and GC based on a pure standard of lupeol (i.e. lup-20(29)-en-3β-ol), which is a C$_{30}$ pentacyclic triterpene that is amenable to both LC-MS and GC analyses (Figs. 2.1 & 2.2; Sunitha et al. 2001). Thirteen standard solutions ranging in concentration from 0 to 0.165 µg µl^{-1} were analyzed in triplicate by APCI-LC/MS^n and linear regression analysis was used to obtain the best-fit line (y = 6 x 10^{-6} x; R^2 = 0.9944;

Fig. 2.1). In order to calibrate between instruments, the same thirteen standard solutions were also analyzed in triplicate by GC (y = 0.2236x; R^2 = 0.9817; Fig. 2.2). By comparison, slope and base peak area response values of LC-MS versus GC to similar concentrations of lupeol were quite different (Figs. 2.1 & 2.2) and suggest significant analytical and quantification differences among these instruments.

To link the measures made here with previous work, GC with flame ionization detection was the principal method for quantization in this study because in general it is considered more quantitative than the MS ion-trap responses which vary with molecular charge state of the structure as well as other variables. Conventional lipid analyses utilize 5α-cholestane as an internal standard for quantification by GC; therefore to compare the response efficiency of lupeol versus 5α-cholestane, similar concentrations of each standard were examined in triplicate by GC. The average peak area response ratio of 5α-cholestane: lupeol versus the amount of standard (μg) analyzed is plotted in Fig. 2.3 with the equation of the best-fit line determined using Matlab (y = $a^{(bx)}$ + $c^{(dx)}$, where a = 3.255, b = -14.93, c = 2.056 and d = 0.02836). The 5α-cholestane signal was always greater than that of lupeol when the same amount of each standard was analyzed; however, the differences in their responses varied depending upon concentration. The 5α-cholestane response was almost six times greater than that of lupeol at very low detection amounts, but was nearly double at more optimal detection amounts (Fig. 2.3). While at greater concentrations, the differences among 5α-cholestane and lupeol signals were reduced and fairly consistent, the majority of the samples described herein fall within the first data two points (0.001, 5.7; 0.00425, 4.6) where the response efficiencies of lupeol are the lowest (Fig. 2.3). GC analyses revealed that lupeol does not respond as

efficiently as 5α-cholestane, particularly at very low concentrations; therefore it was

determined that lupeol is not an adequate standard to use for quantifying hopanoids by

GC and thus LC-MS.

Because lupeol was not ideally suited as an external standard for hopanoid

calibration, the abundance and distribution of bacterial hopanoids was determined using

two analytical approaches. The first was APCI-LC/MS^n which was applied to identify

and determine the origin and relative abundance (%) of individual BHPs. Intact lipids

must first be converted to their acetate derivatives (OAc) with acetic anhydride: pyridine

(1:1) and heat (50^oC, 1hr) prior to analysis for ease of separation by reversed phase-high

performance liquid chromatography (RP-HPLC; Fig. 2.4). The HPLC system is

interfaced to an LC/MS ion-trap (Agilent 1100 Series) equipped with an APCI source

interface. After separation by HPLC, intact hopanoids become ionized in the source

under atmospheric pressure with high temperature (i.e. 400^oC) and nitrogen flow rates

(i.e. 60 psi) to obtain a +1 charge. Ions are focused into a mass analyzer where they are

sorted by their mass-to-charge ratio (m/z) and then detected according to their m/z. The

detector measures and provides data in the form of chromatographic peaks (each

representing an individual BHP or other compound of interest) for determining the

abundance of each parent ion (base peak) present by integration of the peak area

response. To obtain further structural information, tandem mass spectrometry (MS^n)

allows for the fragmentation of parent ions in the mass analyzer; this causes intact BHPs

to break apart into product ions that can be used to identify the number and nature of

functional groups linked to a BHP side chain. Fig. 2.5 illustrates the fragmentation

pathways of the common bacteriohopanetetrol (BHT), showing its full (+MS) and

tandem (+MS2) mass spectra. Specific ions represent the loss (or fragmentation) of each acetylated hydroxyl group (OAc) having an m/z of 60 kDa with fragmentation of the pentacyclic skeleton producing ions with m/z 191 and m/z 463. It should be noted that the parent ion (base peak) for BHT (as well as other intact hopanoids) is m/z 655 [MS-OAc]$^{+}$ and not its protonated molecular mass (i.e. m/z 715 [MS]$^{+}$) as an OAc group is typically lost prior to its detection. By knowing the protonated molecular mass and/or the parent ion (base peak) of your compound of interest (i.e. m/z 655 for BHT), by subtraction you can determine the product ions to be m/z 595 [+MS-2OAc], m/z 535 [+MS-3OAc], m/z 475 [+MS-4OAc] and m/z 463 [+MS-192], thus confirming the presence of BHT (Fig. 2.5).

The second approach for total hopanoids and their degraded products relied on their analysis by GC and GC-MS with concentration (μg g^{-1}OC) determined using 5α-cholestane, as an internal standard. Since intact BHPs are amphiphilic and highly functionalized, they are not amendable to conventional GC and GC-MS techniques. This obstacle is overcome in part through treatment with periodic acid (H_5IO_6) to oxidize vicinal diols and form aldehyde products, which are then reduced to more readily analyzable primary alcohols with sodium borohydride ($NaBH_4$; Fig. 2.6; Talbot et al. 2001). While all source-specific information associated with the side chain is lost, the resulting alcohols are directly related to the number of functional groups originally linked to the side chain structure; therefore total tetra-, penta- and hexafunctionalized hopanoids and their degraded products can be quantified by this method (Fig. 2.7; Talbot et al. 2001). BHP side chain structures, however, are not cleaved upon conventional lipid hydrolysis techniques; therefore hopanols detected after hydrolysis and GC/GC-MS

analyses are suggested as degraded products and were quantified as such. This combined

approach allows for the quantification of total, intact and degraded hopanoid fractions.
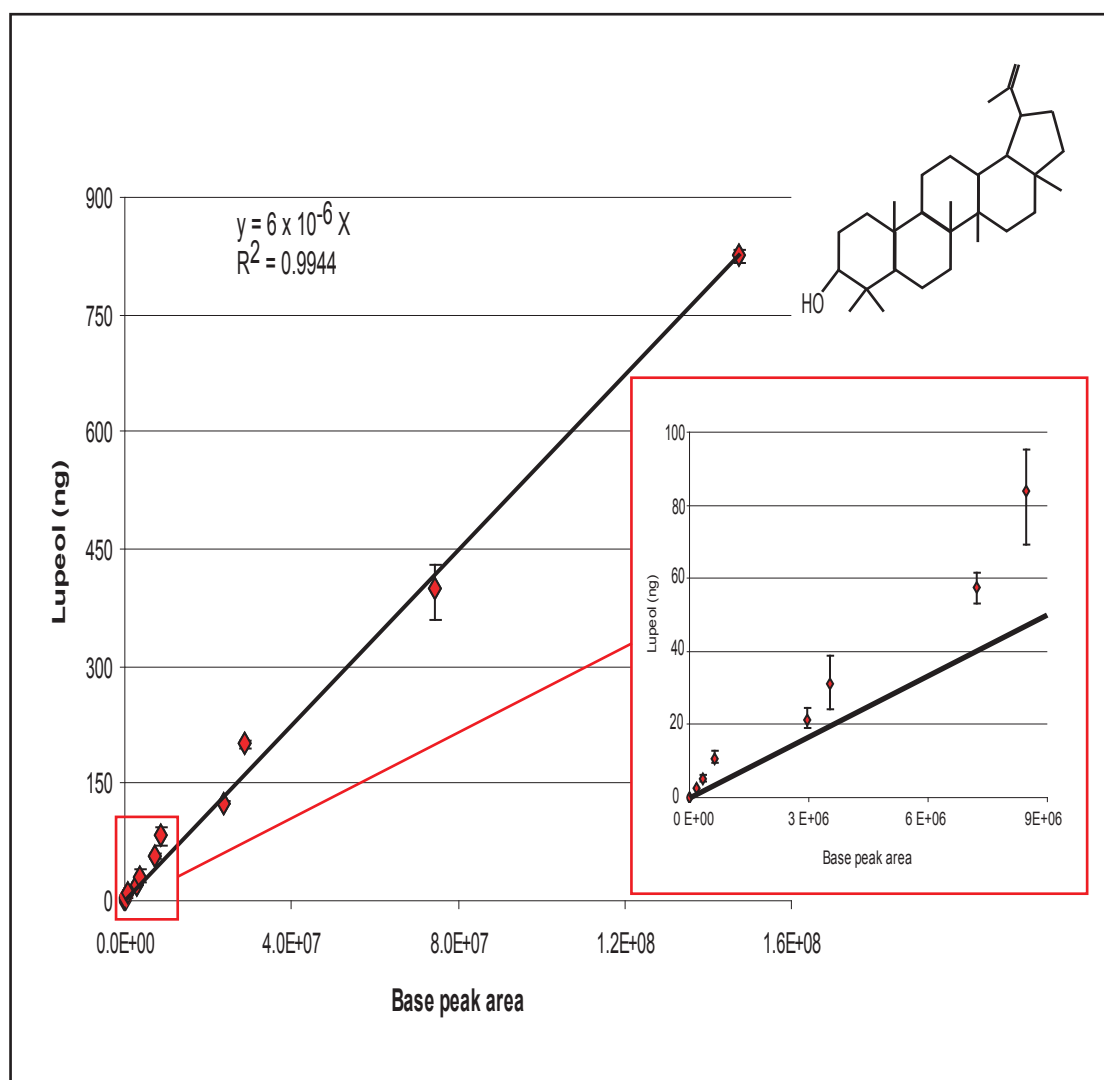
Figure 2.1. External calibration curve of thirteen lupeol standard solutions ranging from 0 - 0.165 μg μl^{-1} and analyzed by APCI-LC-MS^n. The inset shows data points of lower detection amounts. In order to calibrate between instruments, the same lupeol standard solutions were analyzed by GC.
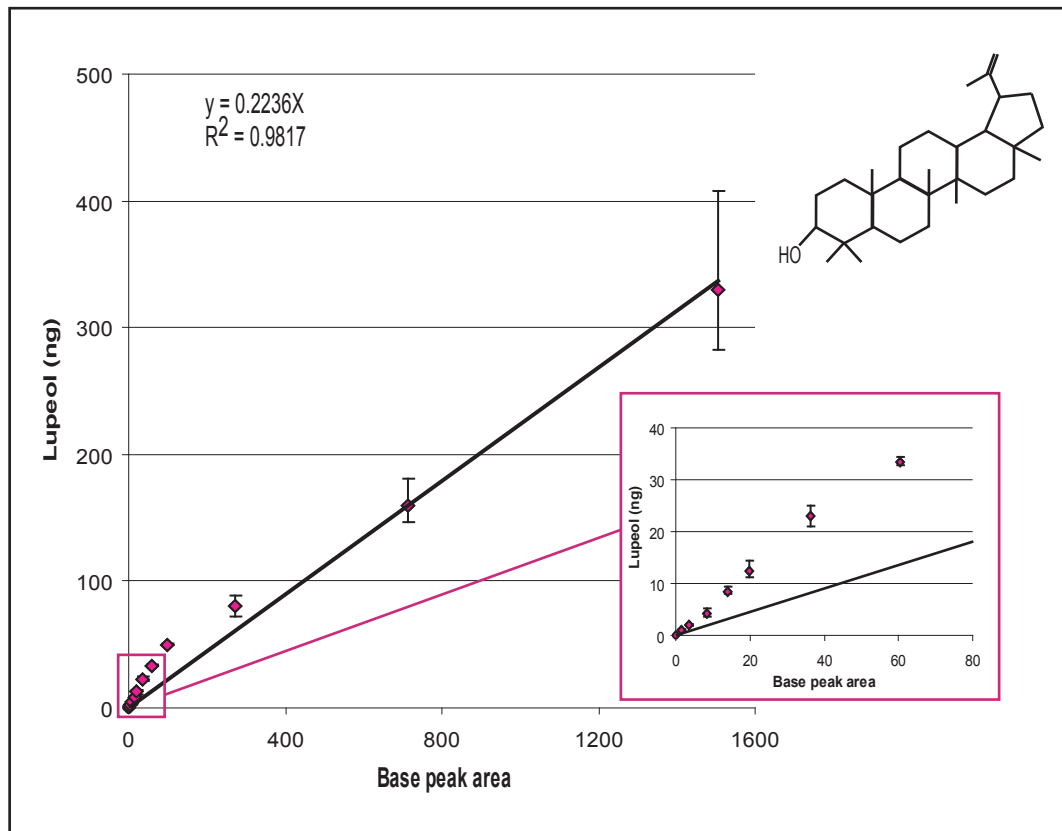
Figure 2.2. External calibration curve of thirteen lupeol standard solutions ranging from 0 - 0.165 µg µl^{-1} and analyzed by GC. The inset shows data points of lower detection amounts. Slope and base peak area response values are very different compared to those determined by APCI-LC/MS^{n} (Fig. 2.1), implying significant analytical and quantification discrepancies among instruments.

5α-cholestane and lupeol (μg)

5*α*-cholestane:lupeol avg. peak area calibration

$y = a^{(b*x)} + c^{(d*x)}$

a = 3.255
b = -14.93
c = 2.056
d = 0.02836
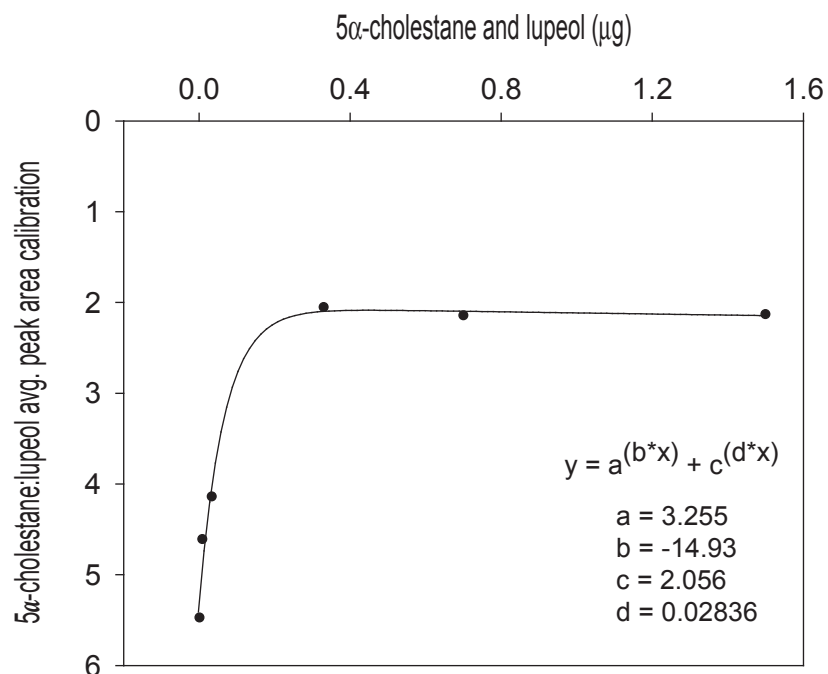
Figure 2.3. Average peak area response ratio of 5α-cholestane:lupeol versus the quantity of standards analyzed by GC. The response of 5α-cholestane was always greater than that of lupeol when the same amount of each standard was analyzed, but how much greater depended upon the concentration. Lupeol does not response as efficiently as 5α-cholestane, particularly as low detection amounts.
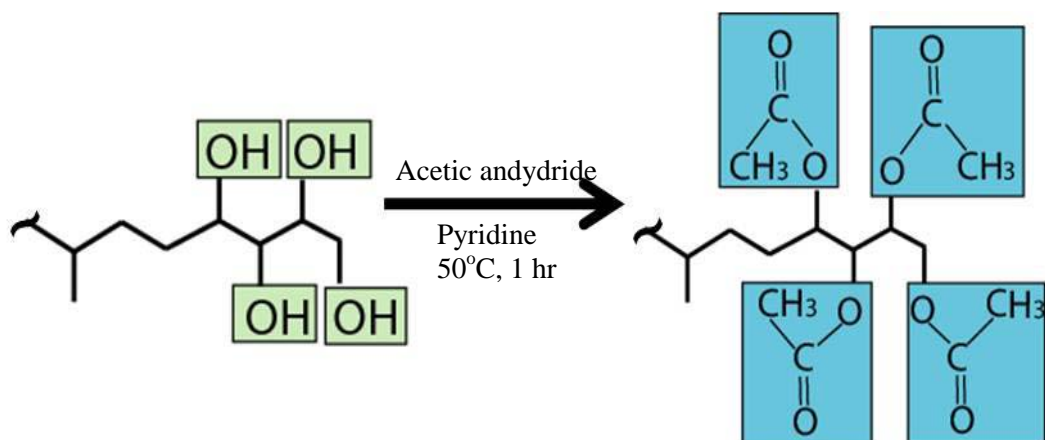
Figure. 2.4. Conversion of hydroxyl groups (OH) of bacteriohopanetetrol (BHT) to their acetate derivatives (OAc) with acetic anhydride: pyridine (1:1) and heat (50°C, 1hr) prior to APCI-LC/MS^{n} analysis for ease of separation by reversed phase-high performance liquid chromatography.

(a) Characteristic fragmentation pathways of bacteriohopanetetrol (BHT)
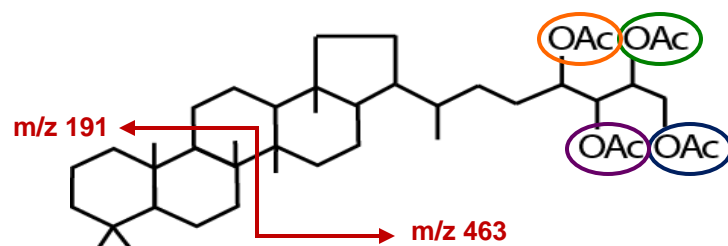
(b) Mass spectra of BHT
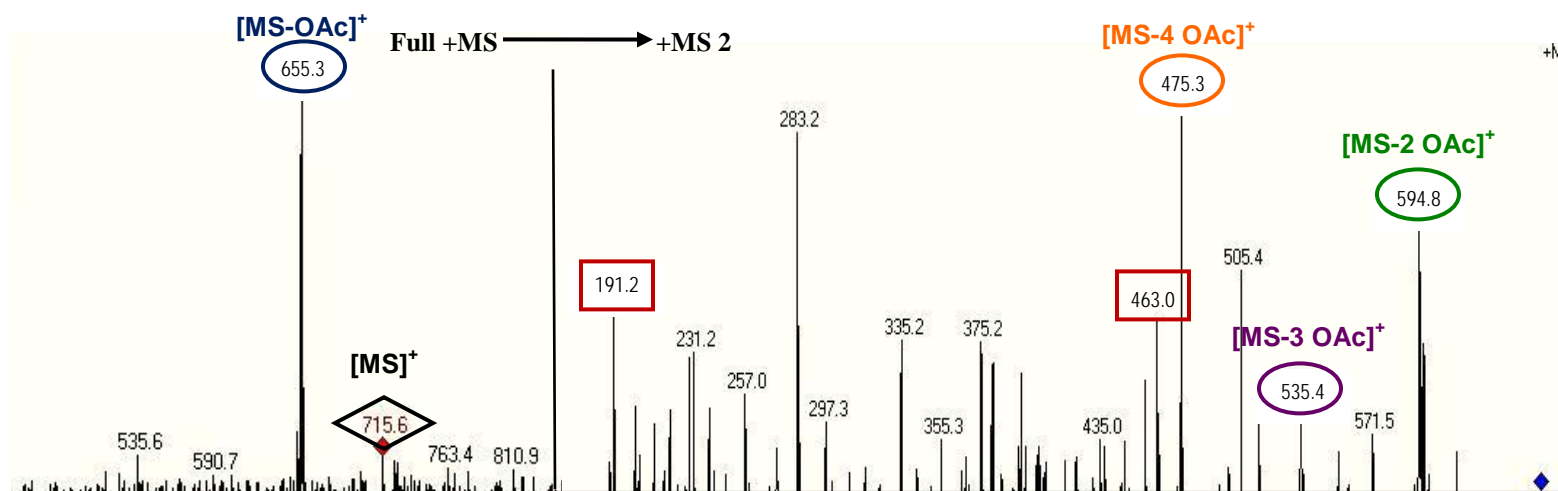
Figure 2.5. (a) Fragmentation pathways and (b) full MS+ and MS2+ APCI-LC/MS^n spectra of bacteriohopanetetrol (BHT). Hydroxyl groups (OH) are converted to their acetate derivates prior to analysis for ease of separation by HPLC. The loss (or fragmentation) of each acetylated hydroxyl group (OAc) represents 60 kDa. Fragmentation of the pentacyclic skeleton produces ions with m/z 191 and m/z 463. Tandem MS provides additional structural information to confirm the presence of individual BHPs in your sample.
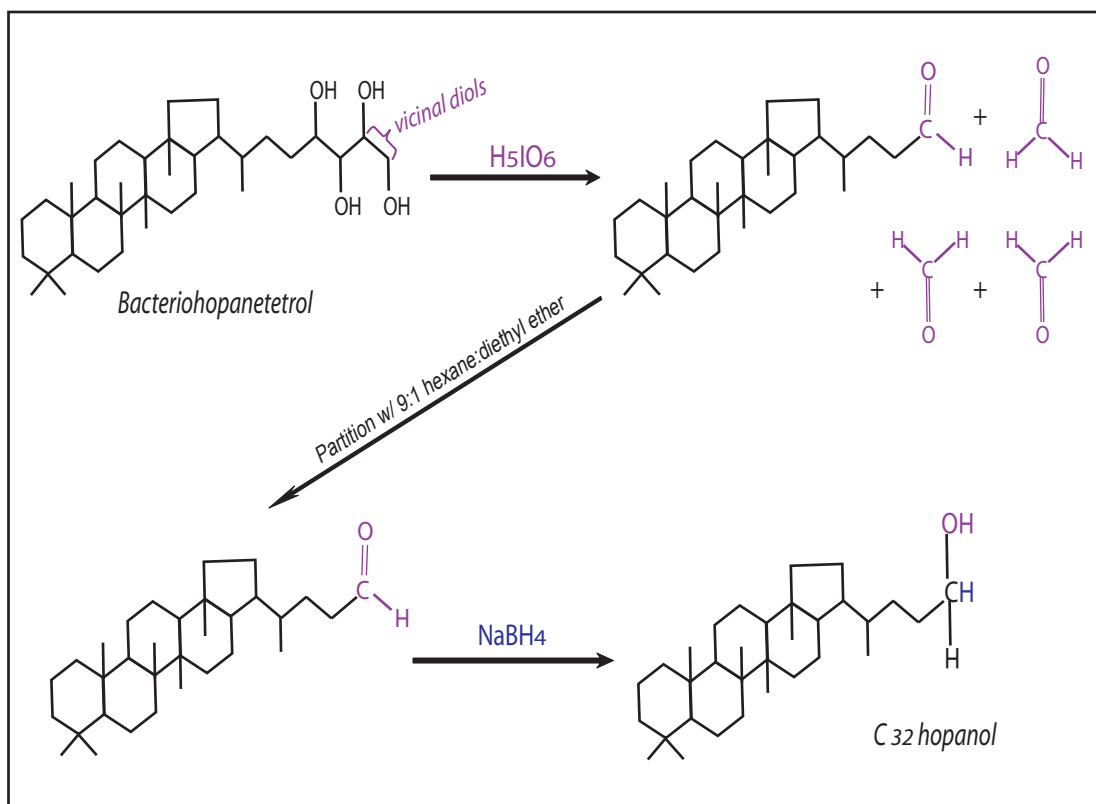
Figure 2.6. Periodate method to convert intact BHPs to more readily analyzable primary alcohols by GC and GC-MS. The conversion of bacteriohopanetetrol (BHT) to a C_{32} hopanol is shown in this example. Periodic acid is applied during an oxidation reaction to cleave viscinal diols and subsequently form aldehyde products. After partitioning the oxidized BHT with a neutral solvent mixture of 9:1 hexane:diethyl ether, sodium borohydride is used to reduce the aldehyde and yield a C_{32} hopanol.

TETRA
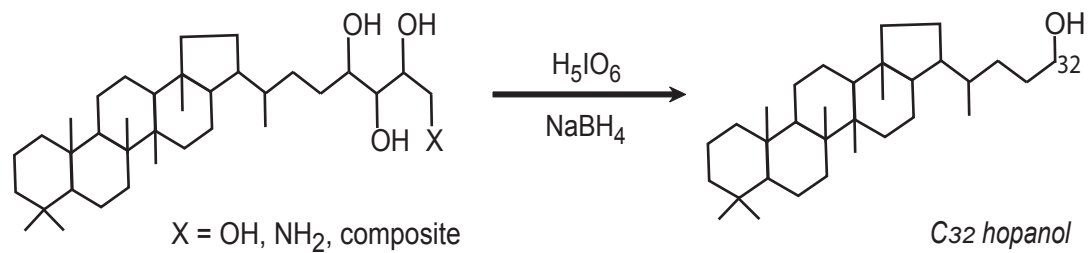
PENTA
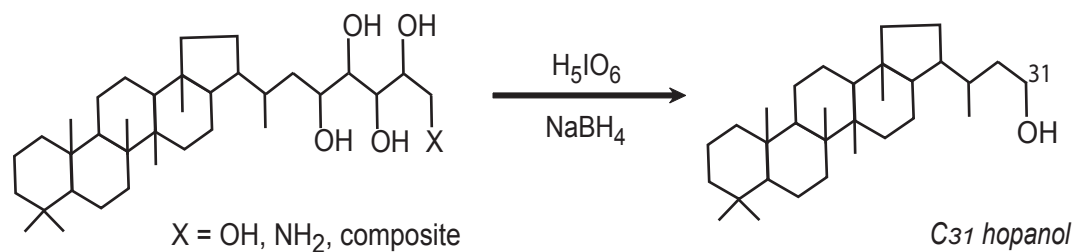
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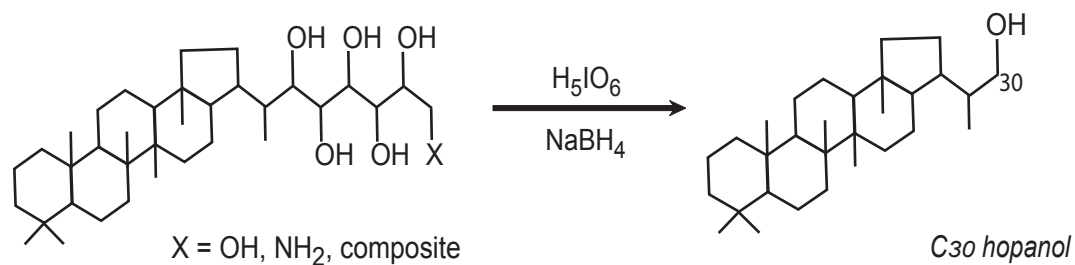
Figure 2.7. Oxidation-reduction reactions with periodic acid and sodium borohydride to convert intact BHPs to primary alcohols for GC and GC-MS analyses. The resulting alcohols are directly related the number of functional groups originally linked to BHP side chain structures.

Chapter 3: Bacterial hopanoids as explicit markers of bacterial carbon: Tracing the contribution of cyanobacteria in the Bering Sea

This chapter has been submitted and is in review to *Limnology and Oceanography* as Taylor, K. A. and H. R. Harvey, "Bacterial hopanoids as explicit markers of bacterial carbon: Tracing the contribution of cyanobacteria in the Bering Sea," 2009.

Abstract

The importance of microbial carbon to marine particles and sediments has been limited by the lack of specific markers for their presence and the general dominance of primary production by phytoplankton as a major source of organic material. We applied recent advances in cellular structural analysis to determine the amount and origin of intact bacteriohopanepolyols (BHPs) as tracers of bacterial carbon inputs in the Bering Sea and Arctic Ocean. BHPs specific to cyanobacteria and their degraded products were found to be widespread in sea-ice, surface sediments and particles from the Bering Sea and suggest their low abundances year-round despite cold temperatures. Comparative analysis of intact and degraded hopanoid fractions in sediments revealed that degradation of intact membranes begins in the water column with at least a fraction of the degraded material originating from cyanobacteria. A broad suite of bacterial membrane components were observed in surface sediments, reflecting a range of bacterial sources and the intense microbial recycling of organic material on the productive Bering Sea shelf. In contrast, intact BHPs analyzed across a shelf to basin transect of surface

sediment from the Western Arctic Ocean showed no evidence of cyanobacterial signatures; however hopanoids specific to soil derived material were in abundance. Though neighboring high-latitude environments receive different inputs of organic matter and thus different sources of intact bacterial hopanoids, both systems show significant bacterial organic carbon contributions to sediments.

Introduction

It is well established that bacteria play a key role in the biogeochemical cycling of organic matter in aquatic systems (Kirchman 2000) and thus have potential as important contributors of organic carbon to sediments and soils (Whitman et al. 1998). Yet the lack of specific tracers of bacterial carbon and the acknowledged gap among bacteria which cannot be cultured (Suzuki et al. 1997; Rappé and Giovannoni 2003) have been major hurdles in defining bacterial carbon contributions. Membrane lipids such as odd- and branched-chain fatty acids have often been suggested as proxies of bacterial biomass (Guckert et al. 1985), yet more recent work has shown that fatty acid synthesis is dependent upon the available carbon source; thus allowing insight into bacterial community structure in natural settings, but with limited information on their quantitative contributions (Harvey et al. 2006). This contrasts with the rich history of various lipids as markers for eukaryotic carbon in both marine and terrestrial systems and their use as both process and source markers (Bianchi et al. 1989; Fahl and Stein 1997, 1999). Recent exceptions have been notable and include the use of archael specific markers (i.e. glycerol dialkyl glycerol tetraether lipids, GDGTs) to determine the presence of the ubiquitous autotrophic and heterotrophic marine archaea (Ingalls et al. 2006) and the use

of selective intact membrane lipids for some members of the eubacteria (Van Mooy et al. 2008).

Intact bacteriohopanepolyols (BHPs) are cell membrane lipids biosynthesized by a variety of bacteria including cyanobacteria (Ourisson and Rohmer 1982; Rohmer et al. 1984). Their diagenetic products (i.e. geohopanoids) have been observed in sediments for some time (Dastillung et al. 1980a, b; Rohmer et al. 1980), yet recently have their complex biosynthetic origins been unraveled (Ourisson and Albrecht 1992). The oxidation of the structurally complex intact hopanoids to their simpler, molecular fossils is often recognized in sediments and ancient materials (Mycke et al. 1987), yet there are recent examples of the intact BHPs in sediments of geological age (Bednarczyk et al. 2005; van Dongen et al. 2006). The most ancient products of cyanobacterial (i.e. 2-methylhopanes) origin have been suggested as potential indicators of the timing of oxygenic photosynthesis on Earth (Summons et al. 1999). This recalcitrance of both the intact BHPs and their identifiable products provides a means of tracing bacterial specific organic carbon inputs to aquatic systems. The ubiquity of bacterial carbon is reflected in the reports of these compounds in various environmental settings that include marine and lacustrine sediments (Rohmer et al. 1984; Innes et al. 1997, 1998), soils (Cooke et al. 2008a, b), peat (Quirk et al. 1984; Crossman et al. 2001), microbial mats (Jahnke et al. 2004; Talbot et al. 2008), and particulate organic matter from the Black Sea (Blumenberg et al. 2007).

Reflecting a range of bacterial sources, certain intact BHPs (i.e. BHT **1a**; aminotriol **1b**; BHT cyclitol ether **1c**, Fig. 3.7) are widespread among many aerobic bacteria while others represent specific biomarkers of particular bacterial groups and

processes. Talbot et al. (2008) described novel tetrafunctionalized BHPs including saturated (BHT pentose **1f**, Fig. 3.7) and C-2 methylated (**3f**, Fig. 3.7) structures with a fully hydroxylated terminal sugar, having only been observed in both environmental samples and enrichment cultures of the cyanobacteria *Gloeocapsa* sp. and *Chroococcidiopsis* sp. A related unsaturated, non-methylated compound (**2f**, Fig. 3.7) was also identified in the environment, but has yet to be grown in culture (Talbot et al. 2008). Cyanobacteria are widely reported as the most abundant producers of C-2 methylated hopanoids and are also observed as sources of BHPentol (**1d**, Fig. 3.7; Bisseret et al. 1985). Additionally, the pentafunctionalized alcohol (**1d**, Fig. 3.7) is known from acetic acid bacteria, the most prolific source of unsaturated BHPs to date (Zundel and Rohmer 1985). Both acetic acid bacteria and certain methane-oxidizing bacteria are shown to produce C-3 methyl hopanoids with the latter also observed as a source of aminotetrol (**1e**, Fig. 3.7; Talbot and Farrimond 2007); a compound known from sulfate-reducing bacteria as well (Blumenberg et al. 2006). Other complex BHPs that include adenosylhopanes (**1g**, **1g'**,**1h**, **3h**, Fig. 3.7) are hypothesized to be derived only from as yet uncultured soil bacteria, and have been suggested as tracers of terrestrial organic matter transported to marine sediments (Cooke et al. 2008a, b).

Here, we investigate the abundance and distribution of bacterial hopanoids associated with particulate material and their potential to trace the direct input of bacterial carbon to the underlying sediments in the diatom dominated Bering Sea. To investigate offshore trends, particulate organic matter (POM) and surface sediment samples were taken from shelf, slope and basin regions in the Bering Sea. To contrast this marine-dominated system with one that receives substantial terrestrial inputs, surface sediments

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were investigated across a shelf to basin transect in the Chukchi Sea of the Western Arctic Ocean. The differing effects of heightened primary productivity and significant inputs of terrestrial organic matter impact the bacterial communities that reside in the Bering Sea and western Arctic, respectively, making these regions ideal for the comparison of molecular bacterial proxies.

Methods

Sample collection and culture

To characterize POM and maximize the capture of bacterial versus algal material, large-volume particulate samples were collected from below the chlorophyll maximum during the April and July 2008 Bering Sea Ecosystem Study (BEST) cruises (refer to Fig. 3.1 and Table 3.1 for station locations). An ice core was also retrieved and melted for the examination of POM entrained during seasonal freezing. Whole water and melted sea-ice was vacuum filtered through pre-combusted ($450^{o}C$, 4-6 h) Whatman GF/F filters for biomarker and particulate organic carbon (POC) analyses. All filters were immediately frozen ($-80^{o}C$) and stored ($-70^{o}C$) until chemical analysis. Surface sediments (0-2 cm) were obtained using a MC-800 multicorer (10 cm cores) to examine bacterial inputs to surface sediments. Sediments were collected from the northern shelf just south of St. Lawrence Island (station SL-8), mid-shelf southeast of St. Matthew Island (station MN-5A, B), and from the shelf break/upper slope and basin west of the Pribilof Islands (stations NP-14 and NP-15), respectively (Fig. 3.1; Table 3.1). Station MN-5 was sampled on 9 July 2008 (A) and then reoccupied 23 July 2008 (B) with POM only collected during the first visit. Surface sediment (0-1 cm) was also previously taken from another deep location (station DBS) near international waters (Fig. 3.1; Table 3.1).

Surface sediments from the Western Arctic Ocean were collected across the West Hanna

Shoal transect in the Chukchi Sea (Fig. 3.1; Table 3.1). An additional three samples were

retrieved from undisturbed cores with a Pouliot 0.06 m^2 box corer during the May 2002

Shelf-Basin Interactions Program process cruise. All sediments were stored in glass or

plastic I-Chem jars with Teflon lined lids and immediately frozen aboard the ship (-80°C)

and in the laboratory (-70°C). Each sample was thoroughly homogenized after thawing

prior to chemical analysis. *Synechococcus* sp. culture #46 originally isolated from an

upper 50 m integrated water column sample taken from the Gulf of Alaska (59°51' N,

148°52' W) was used to compare lipid signatures and grown axenically at 13°C in a

12/12 hr light/dark cycle and harvested at late log phase of growth. The organic carbon

content of POM, sediment (after removal of $CaCO_3$) and *Synechococcus* sp. culture were

measured with an Exeter Analytical, Inc. CE-440 Elemental Analyzer with chlorophyll

concentration of cultured *Synechococcus* sp. measured by standard methods.

Cyanobacterial cell abundances were calculated based on the hopanoid concentrations

(μg g^{-1} dry wt) in *Synechococcus* cultures reported by Summons et al. 1999 and assuming

that 48% of the dry mass is organic carbon (Jørgensen 1979; Mahlmann et al. 2008).

Analytical approach

The analysis schemes were designed to identify and quantify both intact BHPs as

well as their degradative products. Total lipids containing both fractions were extracted

from particles, surface sediments and cultured *Synechococcus* similar to that described by

Mannino and Harvey (1999). Briefly, samples were sonicated (bath) in a mixture of

methylene chloride:methanol (CH_2Cl_2:MeOH; 2:1) and lipids were partitioned three

times and combined. Solvent was removed by rotary evaporation and the total lipid

extract was split into fractions for the analysis of specific hopanoid classes. Total

extracts and primary hopanols (described below) were converted into their acetylated

derivatives using acetic anhydride and pyridine (4 ml, 1:1) similar to that previously

described (Innes et al. 1997). Acetylated total lipids were dissolved in a mixture of

methanol:propanol (3:2) prior to reversed-phase high-performance liquid

chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry

(RP-HPLC-APCI-MS^n) analysis of intact BHPs with methods modified from Talbot et al.

2001. LC-MS^n was conducted with an Agilent 1100 Series LC/MS ion trap linked to an

APCI source interface operated in positive ion mode. Instrument settings were as

follows: nebulizer gas pressure 60 psi; dry gas flow 6 L min^{-1}; dry gas temperature

200°C; APCI vaporizer temperature 400°C; and corona discharge current +3200 nA. The

source and mass spectrometer were tuned by direct infusions of an isoprenoid isolated

from *Gluconacetobacter xylinus*. Relative abundances of individual intact hopanoids

were calculated based on the peak area response of the parent ions (base peak). Structures

were assigned based on comparison to published +MS and +MS2 spectra when possible

(Talbot et al. 2007a, b; Talbot et al. 2008). The total hopanoid content of each sample

was determined by GC and GC-MS after intact BHPs were chemically degraded to

primary hopanols and derivatized similar to methods reported by Innes et al. 1997. To

examine degraded hopanoid products, a fraction of the lipid extracts was subject to

alkaline hydrolysis followed by derivitization to form trimethylsilyl ethers and analyzed

by GC and GC-MS.

Primary hopanols and degraded BHP products were quantified using an Agilent

6890N Network GC system with flame ionization detection and hydrogen as the carrier

gas. Structural identification was performed with a coupled GC-MS system (Agilent 5973) operated in electron ionization mode with helium as the carrier gas. Column conditions and instrument methods are similar to those reported in Belicka et al. 2002. The intact BHP fraction was calculated based on the increase seen in total hopanoid content before and after reduction of BHP products.

Results

Distribution of individual intact hopanoids

Particle samples and their corresponding surface sediments taken from the Bering Sea are shown in Fig. 3.2. Up to six extended side chain structures were observed in the POM with cyanobacteria markers representing important contributors as five of the BHPs. The relative contributions of BHPs varied widely among stations with BHT (**1a**, Fig. 3.7), 2-methyl BHT (**3a**, Fig. 3.7) and BHT pentose (**1f**, Fig. 3.7) as the dominating structures and only minor amounts of BHpentol (**1d**, Fig. 3.7), unsaturated BHT pentose (**2f**, Fig. 3.7), and 2-methyl BHT pentose (**3f**, Fig. 3.7) were generally found (Fig. 3.2a). In contrast, surface sediment samples in the Bering Sea yielded a greater diversity of BHPs. Cyanobacteria specific markers were present, but accompanied by a suite of additional bacterial products. Shelf sediments showed the broadest range of compounds (Fig. 3.2b). Relative contributions were similar among stations, and approximately 85% of all intact hopanoids were observed as the common BHT (**1a**; Figs. 3.2b & 3.7). Aminotriol (**1b**, Fig. 3.7) followed and averaged nearly 5% of the total (Fig. 3.2b). Samples also contained 2-methyl BHT (**3a**, Fig. 3.7), which accounted for more than 4% of the total. BHT pentose (**1f**, Fig. 3.7) and related compounds (**2f, 3f**, Fig. 3.7) were observed in all Bering Sea shelf and slope sediments, but were absent in the basin (Fig.

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3.2b). The distribution of intact hopanoids in particle and ice core samples taken during

spring is shown in Fig. 3.3. Markers of cyanobacteria (**3a**, **1f**, **3f**, Fig. 3.7) were dominant

in the suspended particles and accounted for approximately 72% of the total (Fig. 3.3).

Sea ice revealed BHT (**1a**, Fig. 3.7) as the most abundant compound with cyanobacteria

specific signatures contributing nearly 13% of the total (Fig. 3.3).

Surface sediments collected across the shelf to basin transect in the Chukchi Sea

are shown in Fig. 3.4. Shelf and slope samples yielded a greater diversity of compounds

than found in the Chukchi basin with up to 10 structures identified (Fig. 3.4).

Cyanobacterial signatures were absent, but markers specific to soil derived material (**1g**,

1h, **3h**, Fig. 3.7) were present in minor amounts (Fig. 3.4). BHT (**1a**, Fig. 3.7) was the

dominant intact BHP observed, and accounted for approximately 84% of the total (Fig.

3.4). Aminotriol (**1b**, Fig. 3.7) followed and averaged more than 5% of all intact

hopanoids (Fig. 3.4). Samples also contained 3-methyl unsaturated BHpentol (**4d**, Fig.

3.7), which contributed nearly 4% of the total. Intact BHPs identified in cultured

Synechococcus are shown in Fig. 3.5. The cyanobacteria specific BHT pentose (**1f**, Fig.

3.7) was the dominant compound and accounted for almost 77% of all intact BHPs. The

unsaturated-BHT pentose (**2f**, Fig. 3.7) and unsaturated BHT (**2a**, Fig. 3.7) were also

found and averaged 12% and 11% of the total, respectively (Fig. 3.5).

Total intact and degraded hopanoid fractions

Total intact BHPs plus their degradation products in samples of POM and surface

sediment are shown in Fig. 3.6. Only intact structures were found in the POM samples;

whereas both intact and degraded hopanoid products were observed and identified in all

surface sediments (Fig. 3.6). The resistance of these structures to rapid hydrolysis

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compared to other membrane components was apparent with intact BHPs representing 71-87% of the total hopanoid content in all sediment samples (Fig. 3.6). The single exception was station NP-15, where the concentrations of intact and degraded material were nearly equal. The amount of degraded hopanoids as a fraction of sediment organic carbon was remarkably uniform across Bering Sea shelf and slope samples, averaging nearly 11 µg g^{-1} OC (Fig. 3.6). This fraction doubled in the basin sediments with over 23 µg g^{-1} OC on average (Fig. 3.6). This pattern contrasted with that in Arctic sediments with degraded material contributing nearly 13 µg g^{-1} OC to the shelf; while slope and basin samples were uniform and averaged 2.4 µg g^{-1} OC (Fig. 3.6).

Integration of cultured *Synechococcus* and environmental samples

We used hopanoid amounts reported by Summons et al. 1999 to estimate upper and lower limits of cyanobacterial abundances in samples of POM and surface sediments are listed in Table 3.3. Based on intact hopanoid concentrations and analysis of cultured isolates, our estimates of average cyanobacterial abundance in the POM ($2.2 \times 10^7 – 1.7 \times 10^8$ cells L^{-1}) were greater than that observed in the melted ice core ($3.6 \times 10^6 – 2.8 \times 10^7$ cells L^{-1}; Table 3.3). Average cell abundances based on chlorophyll content and varied between $0.063 – 0.48$ µg Chl *a* L^{-1} in the POM and $0.01 – 0.08$ µg Chl *a* L^{-1} in the melted ice core (Table 3.3). Assuming the total chlorophyll content in the Bering Sea is approximately 8 µg Chl *a* L^{-1}, we estimate that cyanobacteria account for $0.76 – 6.1\%$ of the total in the POM on average and $0.13 - 0.98\%$ in the ice core (Table 3.3). In surface sediments, average cyanobacterial cell abundances ranged from $1.4 \times 10^8 – 1.1 \times 10^9$ cell g^{-1} or $0.38 – 2.9$ µg Chl *a* g^{-1} (Table 3.3).

Discussion

This is the first report to demonstrate direct inputs of cyanobacterial carbon as a contributor to sediments of high-latitude environments. While cyanobacteria are often regarded as minute, ubiquitous components of the global ocean as well as ecologically important N_2-fixers in oligotrophic waters of tropical and subtropical regions (Waterbury et al. 1979), they have rarely been observed in high-latitude oceans. There has been report of the terrestrial epilithic N_2-fixer *Gloeocapsa* sp. (Ohki et al. 2008) in the Canadian High Arctic (Talbot et al. 2008), but low temperatures are generally believed to limit free-living marine cyanobacteria as important components of polar oceans. The importance of cyanobacteria and their contribution to sediments have previously gone unrecognized in the Bering Sea. This diatom dominated region shows intense blooming periods during the spring freshet when the ice edge retreats and sunlight no longer limits primary productivity (Grebmeier et al. 1988). Primary production can exceed 5.0 g C m^{-2} d^{-1} during these times (Goering and Iverson, 1978), with the rapid deposition of ungrazed material which fuels areas of intense benthic activity. Cyanobacteria have been reported, but are considered minor contributors (Liu et al. 2002a, b; Suzuki et al. 2002). Chlorophyll concentrations typically peak at 6-10 μg L^{-1} during spring diatom blooms (Sakshaug 2004) and cyanobacteria appear to represent a minor (0.063 – 0.48 μg Chl *a* L^{-1}) but significant contributor (Table 3.3). While this is a small component of the total system chlorophyll, the presence of cyanobacteria in the Bering Sea also appears as a ubiquitous resident of the system as membrane components of living cells can also be detected in sea-ice and traced to the surface sediment. Calculating chlorophyll flux to sediments is constrained as the recalcitrance and diagenetic rates of intact hopanoids are

not well understood; however we conservatively estimate cyanobacterial abundance to average $0.38 - 2.9$ µg Chl a g^{-1} in Bering Sea sediments (Table 3.3), which is a minor percentage of the annual total carbon flux ($2 - 24$ mg C $m^{-2} d^{-1}$; Maita et al. 1999). Examination of water column samples (Sukhanova et al. 1999) and HPLC analyses of chemotaxonomic pigment markers (Suzuki et al. 2002) have observed *Synechococcus* sp. as the dominant cyanobacteria species accounting for only a minor fraction of photosynthetic communities. These and other small autotrophs become vastly out-numbered by larger diatoms that are more efficient in the uptake and assimilation of nutrients from the water column. Although their limited presence in spring might be expected, the significant contribution of cyanobacterial carbon to both particles and more importantly sediments in this region suggests that bacterial carbon and cyanobacterial carbon in particular represents a previously undocumented component of the organic carbon reservoir of the Bering Sea.

A general pattern of decreasing cyanobacterial abundance with increasing latitude has been observed in the waters of the North Atlantic (Murphy and Haugen 1985) and Greenland Sea (Legendre et al. 1993) as well as in the North and South Pacific Oceans (Church et al. 2008). A somewhat similar distribution is seen in particles from the Bering Sea with a greater abundance of cyanobacteria-specific BHPs in the south and limited presence in northern locations. Ratios of cyanobacteria derived BHPs to chlorophyll *a* concentration in POM samples suggest photosynthetic bacteria constitute a greater fraction of the chlorophyll *a* signal beyond the southeastern shelf-edge (station NP-15) relative to the other locations (Table 3.2). This deep station also reflects a greater diversity of intact compounds (Fig. 3.2a) and an overall enhanced hopanoid signal (Fig.

3.6) which might result from off-shelf particle advection caused by the formation of mesoscale eddies near the continental slope. Similar processes were suggested to account for the presence of neritic phytoplankton species observed offshore in the Aleutian Basin (Sapozhnikov and Naletova 1995). Cyanobacterial markers contributed less to the chlorophyll *a* signal at station NP-14 (Table 3.2); however this sample was taken from within the thermocline and at a shallower water depth where sunlight was more available. Coccoid cyanobacteria like *Synechococcus* sp. often prefer much lower light intensities than larger phytoplankters (Glover and Morris 1981) and have the ability to utilize 1% light levels for growth (Jerlov 1976). In addition, *Synechococcus* sp. have tested positive for the production of bacterial hopanoids (Llopiz et al. 1996; Summons et al. 1999; Jahnke et al. 2004) while others were reported not to synthesize any BHPs (Rohmer et al. 1984, Talbot et al. 2008). Therefore it is probable that cyanobacteria are occupying a selective niche deeper in the water column where they have a competitive advantage over diatoms for nutrients and sunlight, and perhaps their detection might have gone overlooked if POM was not sampled from below the chlorophyll maximum. Similarly if our sample at station NP-14 was collected deeper in the water column, the ratio of cyanobacteria derived BHPs to chlorophyll *a* concentration could have been more comparable to that of its deep neighbor. POM from station UP-3 was also taken from a shallow water depth; however due to the proximity of the Unimak Pass, physical processes and particle transport are likely diluting the cyanobacterial signal as well. In northern locations (stations SL-8 and MN-5A), ratios of cyanobacteria derived BHPs to chlorophyll *a* concentrations were reduced by an order of magnitude relative to southern sites (Table 3.2). Here, the causes are two-fold with near-freezing water temperatures

reducing the growth of cyanobacteria and nutrient-laden deep water stimulating phytoplankton growth and thus chlorophyll *a* concentrations on the northern shelf.

A caveat for preferring reduced light levels and residing deeper in the water column is the ability to survive extreme low temperatures. Cyanobacterial mats have been found growing along the perimeter of small melt-water pools (Talbot et al. 2008) and beneath perennial ice-cover in the McMurdo Dry Valley lakes of Antarctica (Hawes and Schwarz 2000). The occurrence of cyanobacteria might thus be expected in association with first-year sea-ice in the Bering Sea, and the confirmation of cyanobacteria in ice provides a mechanism for inoculation of surface waters in spring. Intact cyanobacterial hopanoids comprised approximately 4.9 μg g^{-1} OC in a core taken from the shelf (station NP-7; Fig. 3.3). This together with their occurrence (4.7 μg g^{-1} OC) in POM collected near the ice-edge during April (station ZZ-18) suggest their retention in surface waters after thawing. There have been several studies (Hunt and Stabeno 2002; Hunt et al. 2002; Jin et al. 2007) documenting the timing of sea-ice algae release during melting and the subsequent ice-associated blooms that result in the open, low-salinity stratified waters of the Bering Sea. Yet these reports focus on diatoms, and the results observed here suggest that a similar mechanism is likely for cyanobacteria as well.

Not only can we observe linkages among ice-associated cyanobacteria and their presence in open water during spring (Fig. 3.3), but more importantly the specificity of membrane components which allow direct tracing of their input from POM to surface sediment (Fig. 3.2). Given their small size (0.2-20 μm), single cell sedimentation seems unlikely but intact BHPs suggest that cyanobacteria are delivered to the benthos.

Particulate aggregates such as marine snow or fecal pellets produced by zooplankton are possible transport mechanisms (Alldredge 1987) and only limited information is available in the Bering Sea on grazing preference by resident zooplankton. While cyanobacteria contributed minimally to the chlorophyll *a* signal in POM from the northern shelf (stations SL-8 and MN-5A discussed above), concentrations in northern surface sediments were an order of magnitude greater than that observed in the southern locations (Table 3.2). Warming water temperatures observed during summer in these shallow zones may allow for increased cyanobacterial production and their subsequent input to sedimentary organic carbon pools. We might speculate that cyanobacteria populations could propagate in a northward direction as marine ecosystems have already begun responding to a shift in the subarctic-Arctic temperature boundary (Grebmeier et al. 2006).

Observing direct inputs of cyanobacterial markers from POM to Bering Sea sediments demonstrates the utility of sedimentary BHPs as markers of bacterioplankton and agrees with findings by Blumenberg et al. 2009 who have suggested that zones where effective transport mechanisms (i.e. fecal pellets) exist allow for the accumulation of bacterial remnants in sediments. Early diagenetic transformation of sedimentary BHPs has previously been described (Talbot et al. 2003c; Innes et al. 1997) as well as their potential to represent living bacteria deep (200 m) within the sediment (Saito and Suzuki 2007). Based on the observed distribution in the water column and sediments seen here, it appears that hydrolysis of intact BHPs begins in the water column as almost every particle sample (29 – 101 μg g^{-1} OC), show far greater abundances of total intact BHPs relative to their corresponding surface sediments (20– 52 μg g^{-1} OC; Fig. 3.6). Station

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MN-5 is an exception since both surface sediment samples revealed greater abundances of intact hopanoids compared to the overlying water (Fig. 3.6). Its reoccupation 14 days later suggests the active input of bacterial carbon to the shallow underlying sediments with increased amounts of intact BHPs seen in sediments and amounts of degraded material remaining unaltered (Fig. 3.6). While sediment heterogeneity and bioturbation have to be considered, the increase seen in intact cyanobacterial carbon and consistent concentrations of its degradative product are highly suggestive of direct cellular input. Degradation and remineralization of sinking organic material enhances through extended exposure to oxygen in deep waters (Meyers 1997); therefore considerably larger fractions of degraded material were observed in Bering Sea basin sites than shallow water sediments (Fig. 3.6). Despite the degradative loss of all source-informative functionalities, we can assume that at least a fraction of degraded hopanoid products is derived from cyanobacteria as these were dominant sources of intact BHPs (45 – 85%) in the water column (Fig. 3.2a). The ability to distinguish between intact and degraded components in sediments provides important hints for understanding the diagenetic transformations that convert BHPs to their molecular fossils.

Cyanobacterial sources as well as a diverse bacterial contribution are reflected by the broad suite of intact BHPs observed in surface sediments (Fig. 3.2b). While bacterial communities in sediments appear more diverse than those seen in POM, BHPs provide a direct measure of bacterial OC (Fig. 3.2). Other compounds offer useful hints to help elucidate bacterial groups and processes in sediments, but complete linkages between intact BHPs and their specific sources are unknown. This is due in part to a bias among cultured organisms with growth conditions often affecting whether an organism produces

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BHPs and the types of BHPs being produced. Intact hopanoids were first isolated from the acetic acid bacterium *Gluconacetobacter xylinus* (Yamada et al. 1997). Although this and many similar species have been found as abundant producers of unsaturated BHPs (Rohmer et al. 1984; Talbot et al. 2007b), acetic acid bacteria are unlikely sources in the Bering Sea. Grebmeier and McRoy (1989) observed sulfate reduction in northern sediments, which could be a potential process reflected by intact BHPs as some species have been found to biosynthesize hopanoids (Blumenberg et al. 2006). Measurements of 1-4 μmol L^{-1} NH_4 in bottom waters from our two shelf sites could imply benthic production by hopanoid-producing bacteria and the movement of remineralized products across the sediment-water interface. The flux of oxygen and nitrogenous compounds out of sediments is enhanced through benthic faunal bioturbation and irrigation (Sorensen et al. 1979). Shallow shelf sediments host a diverse suite of organisms with biomass "hotspots" occurring where high-quality, recent organic matter accumulates rapidly (Grebmeier et al. 1988). Enhanced phytodetritus and bioturbation promote intense microbial recycling with up to three-times more intact BHPs identified in shelf sediments than in the basin site (Fig. 3.2b). Deep-water sediments experience less microbial recycling as organic matter deposition is slower and heightened benthic biomass cannot be supported.

While the Bering Sea shows specific hopanoid sources, cyanobacterial signatures were absent in a shelf to basin transect of surface sediment from the Western Arctic Ocean, yet the diversity of intact BHPs observed suggests many possible bacterial sources and particularly those originating from soils. A contrasting distribution of intact hopanoids might thus be expected as a lack of free-living marine cyanobacteria and

significant inputs of terrestrial derived organic carbon (Goñi et al. 2000) delivered by

major rivers (Gordeev et al. 1996) and vastly eroding coastline (Rachold et al. 2004) set

this region apart from its sub-Arctic neighbor. Previous studies of bacterioplankton

assemblages in the Arctic Ocean have reported cloned fragments of 16S rRNA genes

(Bano and Hollibaugh 2000, 2002) as well as measurements of both bacterial secondary

production and community respiration rates (Cota et al. 1996). In the productive Arctic

waters, in situ production is rapidly utilized by the heterotrophic food web as the primary

source of OC (Cota et al. 1996) and thus remnants of these bacterial communities are

potentially reflected in surface sediments by intact BHPs. Significant bacterial recycling

is suggested over the Chukchi slope (station WHS-5) as a greater diversity of intact

hopanoids is observed in the surface sediment relative to shelf and basin samples (Fig.

3.4). This agrees with findings by Cota et al., 1996 who reported this region to have

elevated rates of primary productivity and subsequent heterotrophic respiration, due to

intense vertical mixing and the advection of nutrients by currents and edge waves

(Johnson 1989; Aagaard and Roach 1990). Bacterial contributions were highest (56 μg g^{-1} OC) in Chukchi shelf (station WHS-2) sediment relative to slope (19 μg g^{-1} OC) and

basin (12 μg g^{-1} OC) samples (Fig. 3.6), again implying intense bacterial recycling and

potential heterotrophic activity as primary production is typically greatest near the

continental margin. While low estimated rates of primary productivity have been reported

in the central Arctic basin (Wallace et al. 1987), this region appears to be net

heterotrophic (Wheeler et al. 1996, 1997) as most of the organic matter is likely supplied

from the continental shelf and delivered along isopycnals (Walsh et al. 1989). The

limited diversity (Fig. 3.4) and decreased abundance (Fig. 3.6) of intact BHPs in the

Chukchi basin (station WHS-12) thus reflect a reduction of in situ production and bacterial respiration. Bano and Hollibaugh (2000) suggest that ammonium released upon heterotrophic activity is subsequently oxidized by ammonia oxidizing bacteria as the dominant presence of marine *Nitrosospira*-like and *Nitrosomonas*-like organisms were discovered by PCR amplification of 16S rRNA genes. *Nitrosospira* sp. has been found in abundance from natural environments whereas *Nitrosomonas* sp. are more commonly detected in enrichment cultures (Bano and Hollibaugh 2000), and thus observations of intact BHPs might be expected in the latter. Several intact hopanoids were isolated from cultures of the β-proteobacterium *Nitrosomonas europaea* and include aminotriol (**1b**, Fig. 3.7) and related di- (**1g'**, Fig. 3.7) and triacetylated (**1g**, Fig. 3.7) adenosylhopanes among others (Seemann et al. 1999; Talbot et al. 2007a). Additionally, Bano and Hollibaugh (2002) report Arctic bacterioplankton communities consisting of α- and γ-proteobacteria groups from cloned fragments of 16S rRNA genes. Investigations of intact BHPs among these groups reveal many possible sources that include purple non-sulfur bacteria (α-proteobacteria, Neunlist et al. 1985), N_2-fixing bacteria (α-proteobacteria, Bravo et al. 2001), acetic acid bacteria (α-proteobacteria, Forster et al. 1973), and Type I methanotrophs (γ-proteobacteria, Rohmer et al. 1984).

Adenosylhopanes (**1g**, **1g'**, Fig. 3.7) identified from the ammonia oxidizer *Nitrosomonas europaea* and a related compounds (**1h**, **3h**, Fig. 3.7) are suggested to be markers of soil input to aquatic systems (Talbot and Farrimond 2007; Cooke et al. 2008a, b). The presence and distribution of these compounds in surface sediments across the West Hanna Shoal transect suggest the movement of terrestrial material into the Chukchi Sea (Fig. 3.2). Previous studies show significant contributions of other terrigenous lipid

biomarkers (i.e. long-chain *n*-alkanes and *n*-alcohols, α-amyrin and mono- and dicarboxylic acids) and emphasize the potential response of Arctic carbon cycling to allochthonous inputs (Belicka et al. 2002, 2004). In West Hanna Shoal surface sediments, soil-derived BHPs were most abundant near-shore (2.1 μg g^{-1} OC) and decreased by an order of magnitude in slope (0.46 μg g^{-1} OC) and basin (0.25 μg g^{-1} OC) sediments. While the Chukchi Sea is relatively far from a direct land source, intact BHPs are likely delivered via the Beaufort Gyre, by sea-ice entrained with significant amounts of terrigenous material (Eicken et al. 2005). Upon thawing, sea-ice rafted debris is released into the water column where deposition occurs rapidly (~3 month timescales) on the shelf (Cooper et al. 2005), and thus the greatest contributions of both intact and degraded hopanoid products were detected (Fig. 3.6). It is possible that a portion of this allochthonous material is transported along isopycnals into deeper waters as the diversity (Fig. 3.4) and abundance (Fig. 3.6) of soil derived BHPs appears diluted offshore; however drifting sea-ice and the direct release of terrestrial material is also a potential mode of organic matter delivery to deeper waters. Given the shallow depth and fast accumulation rates of the Chukchi shelf, it is doubtful that the abundance of degraded hopanoids results solely from the breakdown of intact BHPs in the water column (as discussed above), but it is possible that sea-ice entrains and then deposits older bacterial carbon that had degraded on land prior to being transported into the Arctic Ocean.

Summary

Despite extreme low temperatures, cyanobacteria inhabit the deeper layers of the euphotic zone of the Bering Sea and contribute directly to the organic matter of surface sediments in this shallow system. These organisms are present year-round and account

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for a small fraction of the total chlorophyll relative to diatoms, with ice-associated

organisms inoculating the surface waters upon spring thawing. Cyanobacterial together

with additional bacterial contributions are reflected by the diverse range of intact

hopanoids observed in surface sediments as well as the significant present of their

degraded products. In contrast, cyanobacterial signatures were absent across a transect of

surface sediment from the Chukchi Sea; however intact BHPs reveal an abundance of

terrestrial derived sources including a portion that likely degraded on land prior to being

transported into the Arctic Ocean. Bacteria and particularly cyanobacterial carbon

contributions to sediments of high-latitude systems are more important than previously

recognized and may provide clues as to how bacterial communities might respond to

warming ocean temperatures.

Table 3.1. Locations and bulk measures for particulate and sediment samples in the Bering Sea and western Arctic Ocean

Station name	Station number [b]	Sample location	Latitude (N)	Longitude (W)	Bottom depth (m)	Sediment depth (cm)	Sediment TOC (%)
SL-8	HLY0803-046	Bering Shelf	62°13'	172°43'	58	0-2	0.66
MN-5A[a]	HLY0803-031	Bering Shelf	59°54'	170°24'	62	0-2	0.63
MN-5B [a]	HLY0803-099	Bering Shelf	59°53'	170°24'	62	0-2	0.79
NP-7	HLY0802-067	Bering Shelf	57°54'	169°12'	69	n/a	n/a
UP-3	HLY0803-004	Bering Slope	54°26'	165°09'	135	n/a	n/a
ZZ-18	HLY0802-097	Bering Slope	58°51'	177°28'	137	n/a	n/a
NP-14	HLY0803-079	Bering Slope	56°16'	171°05'	140	0-2	0.40
NP-15	HLY0803-068	Bering Basin	56°04'	171°19'	2755	0-2	1.59
DBS	HLY0702-172	Bering Basin	60°04'	179°40'	2360	0-1	1.06
WHS-2	HLY0201-006	Chukchi Shelf	72°51'	160°34'	58	0-1	0.93
WHS-5	HLY0201-009	Chukchi Slope	73°17'	160°07'	1198	0-1	0.96
WHS-12	HLY0201-052	Chukchi Basin	72°11'	157°24'	3748	0-1	0.93

[a] Station MN-5 was occupied and sampled twice during the cruise; MN-5A on 9 July 2008 and MN-5B on 23 July 2008
[b] Additional inforrmation on each station is avaialble through the BEST-BSIERP data management portal

Table 3.2. Water column parameters and cyanobacteria-specific BHPs observed in particles and sediments from Bering Sea stations

Station Name	POC $(\mu g\ C\ L^{-1})$	Temperature (^oC)	Chlorophyll α max (m)	Chlorophyll α max $(\mu g\ L^{-1})$	Chlorophyll α POM (m)	Chlorophyll α POM $(\mu g\ L^{-1})$	Cyano-BHPs [b]: Chlorophyll α POM	Cyano-BHPs [b] in sediment $(\mu g\ g^{-1}\ OC)$ [c]
SL-8 [a]	146	-1.59	35	3.8	41.5	0.61	28.8	2.09
MN-5A [a]	135	-0.87	mixed	< 0.5	49.7	0.57	56.1	2.93
UP-3 [a]	74.4	5.33	mixed	< 0.4	20.8	0.29	29.7	n/a
NP-14 [a]	67.2	5.15	surface	0.8	30.2	0.27	110.4	0.68
NP-15 [a]	31.7	3.91	surface	6.0	60.3	0.06	751.7	0.99

[a] See Table 3.1 for station locations
[b] Intact BHPs derived by cyanobacterial sources include the following: **3a**, **1d**, **1f**, **2f**, **3f** (refer to Fig. 3.7 for structural details)
[c] See Table 3.1 for %TOC in surface sediment samples

Table 3.3. Estimated abundance of cyanobacterial cellular contribution to POM and surface sediments from the Bering Sea based on hopanoid concentrations verses measured Chlorophyll α of a cultured isolate

Station	POM [abc]						0 - 2 cm [abc]			
	Lower limit cells L^{-1}	Upper limit cells L^{-1}	Lower limit µg Chlα L^{-1}	Upper limit µg Chl α L^{-1}	Lower limit % Cyano Chl α [d]	Upper limit % Cyano Chl α [d]	Lower limit cells g^{-1}	Upper limit cells g^{-1}	Lower limit µg Chl α g^{-1}	Upper limit µg Chl α g^{-1}
MN-5A	4.6×10^{7}	3.5×10^{8}	0.13	0.98	1.58	12.28	2.0×10^{8}	1.5×10^{9}	0.55	4.23
SL-8	2.7×10^{7}	2.0×10^{8}	0.07	0.58	0.93	7.24	1.4×10^{8}	1.1×10^{9}	0.40	3.07
NP-14	2.1×10^{7}	1.6×10^{8}	0.06	0.45	0.73	5.67	2.9×10^{7}	2.2×10^{8}	0.08	0.62
NP-15	1.5×10^{7}	1.2×10^{8}	0.04	0.33	0.53	4.07	1.7×10^{8}	1.3×10^{9}	0.46	3.58
UP-3	7.1×10^{6}	5.5×10^{7}	0.02	0.15	0.25	1.92	n/a	n/a	n/a	n/a
ZZ-18	1.9×10^{7}	1.5×10^{8}	0.05	0.41	0.67	5.18	n/a	n/a	n/a	n/a
NP-7	3.6×10^{6}	2.8×10^{7}	0.01	0.08	0.13	0.98	n/a	n/a	n/a	n/a
DBS	n/a	n/a	n/a	n/a	n/a	n/a	1.5×10^{8}	1.1×10^{9}	0.41	3.15

[a] Upper and lower limits were determined based on concentrations (2.0×10^{-5} and 1.55×10^{-4} g g^{-1} dry wt) of hopanoids in cultured *Synechococcus* (Summons et al. 1999) and that 48% of the dry mass is organic carbon (Jørgensen 1979; Mahlmann et al. 2008)
[b] Calculations based on organic carbon content (294 fgC cell^{-1}) of *Synechococcus* sp. culture (Gulf of Alaska), which is within the range (103 - 400 fgC cell^{-1}) reported by others (Burkhill et al. 1993; Zubkov et al. 1998; Liu et al. 2002)
[c] Estimates calculated with measured chlorophyll α content (2.8×10^{-9} g cell^{-1}) of *Synechococcus* culture (Gulf of Alaska)
[d] Calculated based on average diatom abundance during spring blooming periods in the Bering Sea (8 g Chl α L^{-1})
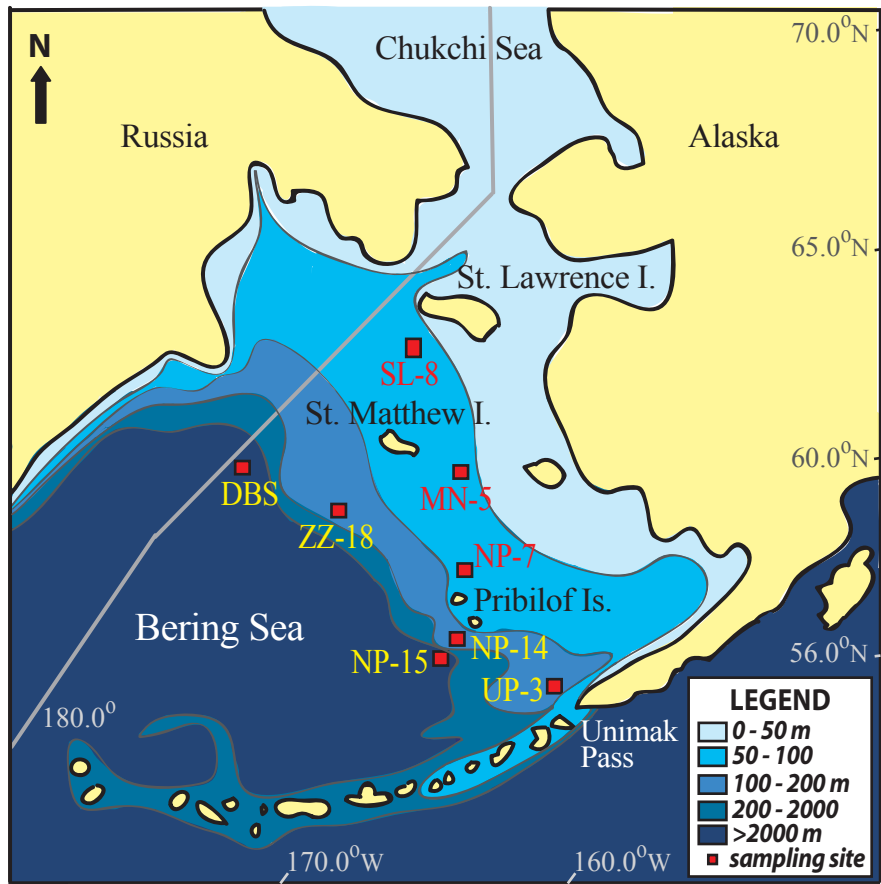
Figure 3.1. Map of the Bering Sea, showing locations and approximate depths of sampling stations.
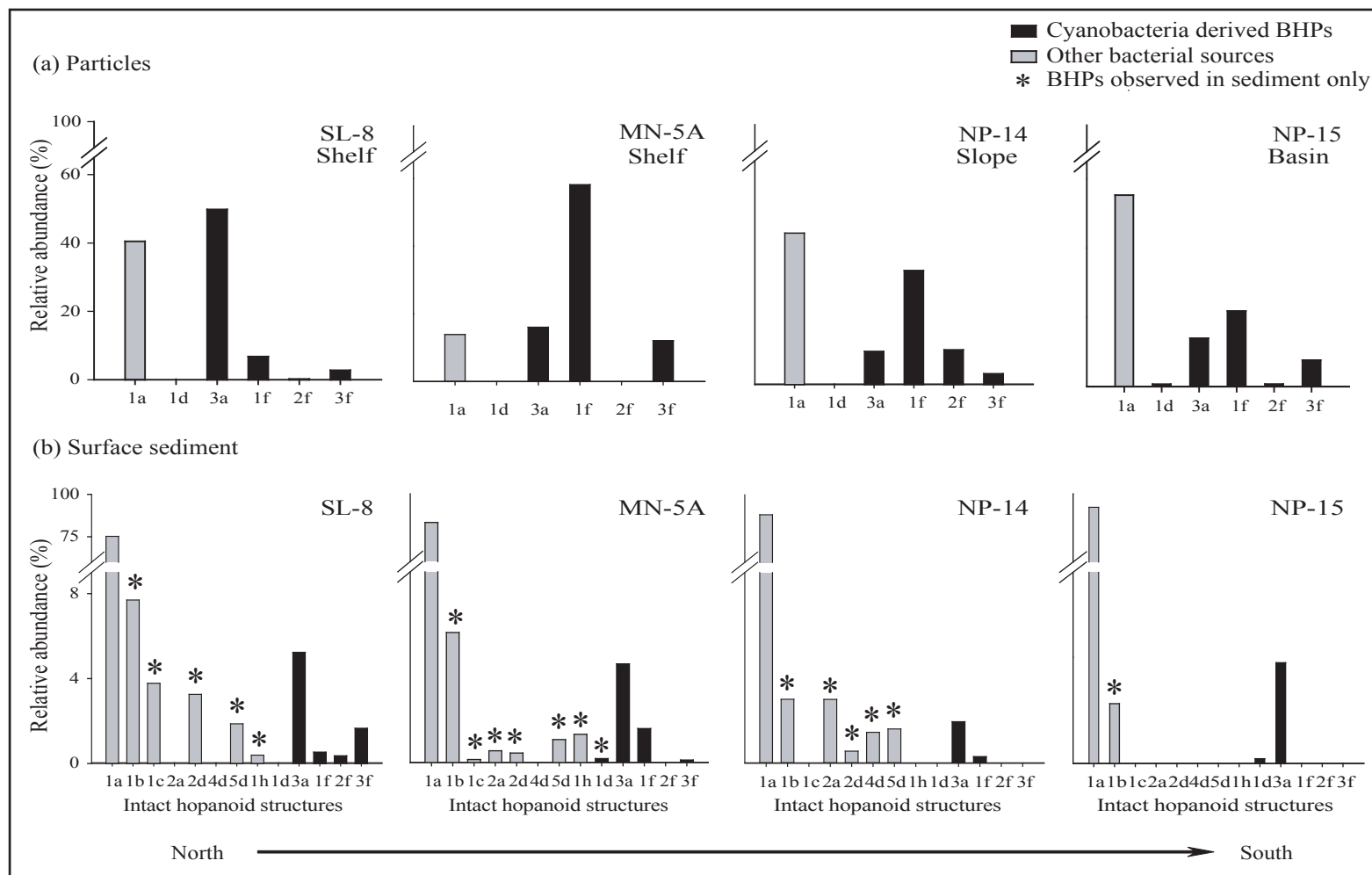
Figure 3.2. The relative abundance (%) of individual intact hopanoids in (a) particles and (b) surface sediment collected during summer from four stations investigated in the Bering Sea. Station numbers refer to locations shown in Table 3.1 and Fig. 3.1.
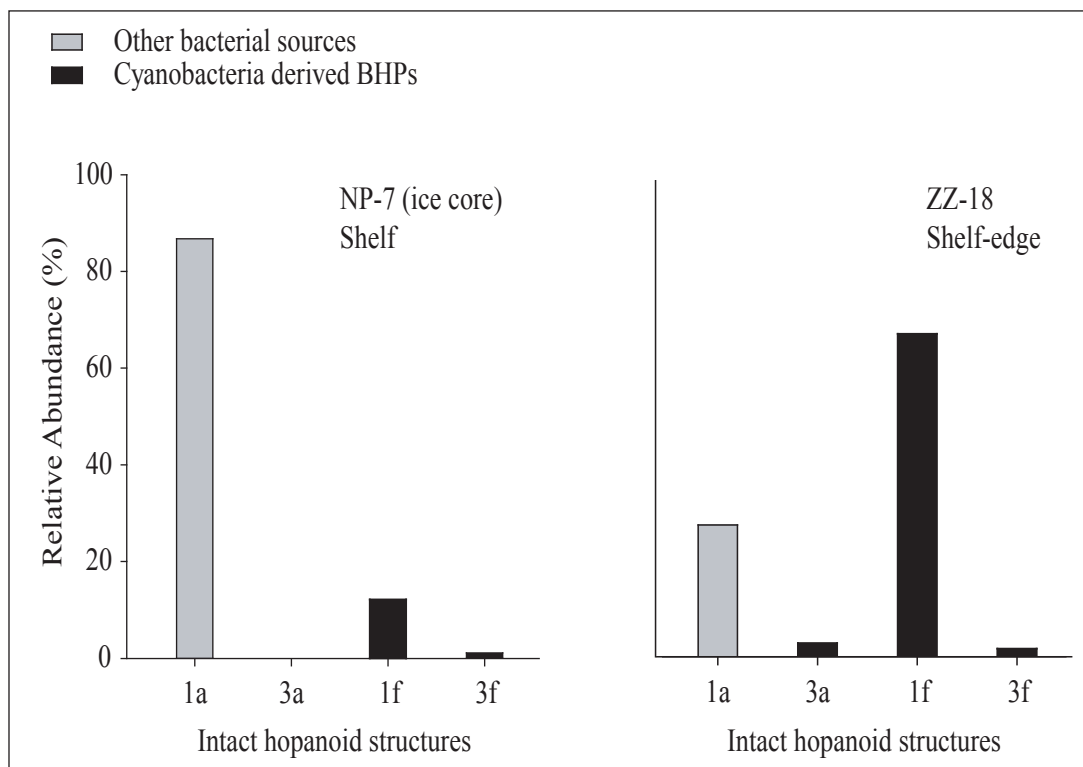
Figure 3.3. The relative abundance (%) of individual intact hopanoids in particles from an ice core and open water collected during spring from two stations examined in the Bering Sea. The station numbers refer to locations shown in Table 3.1 and Fig. 3.1.
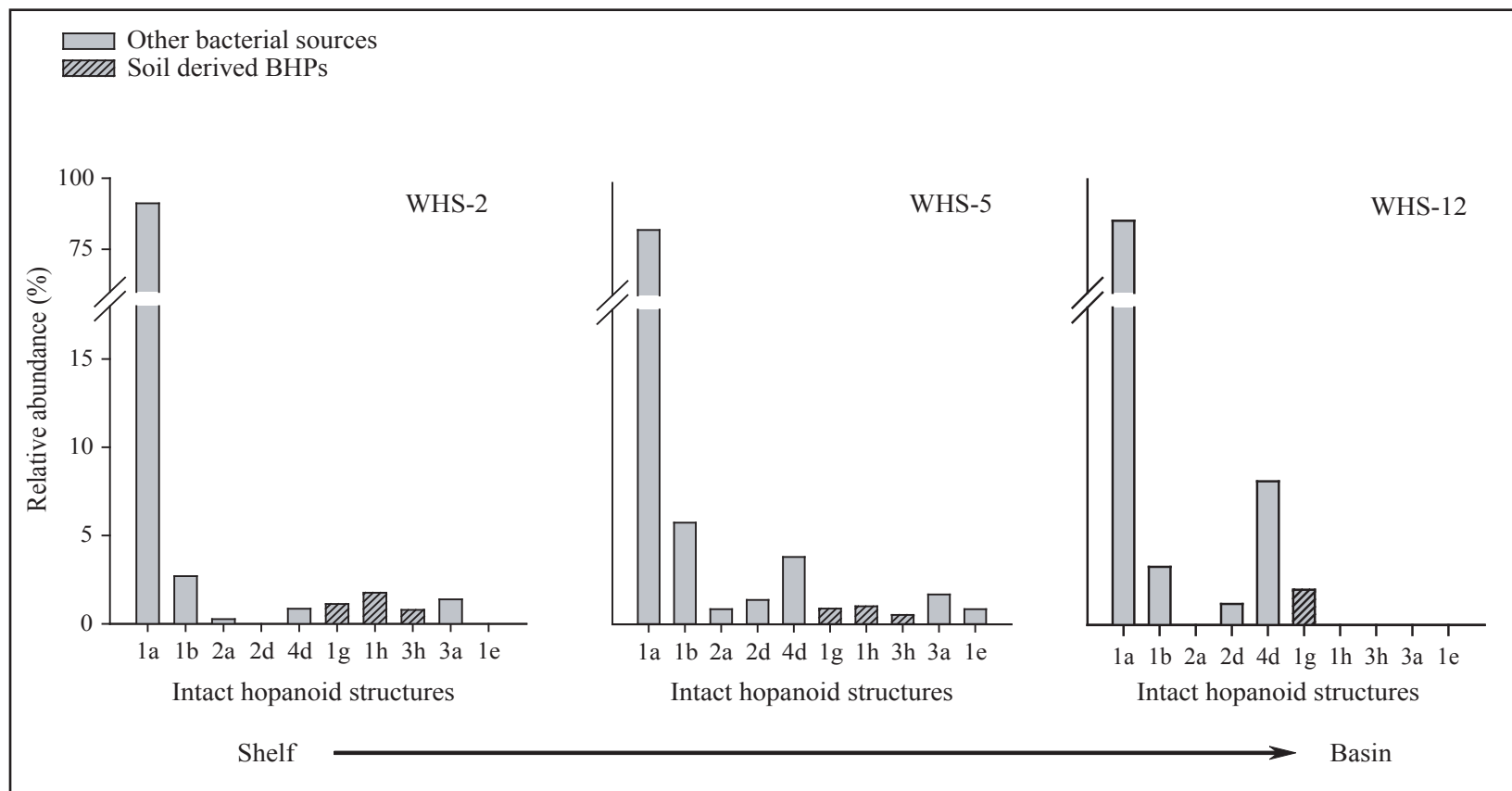
Figure 3.4. The relative abundance (%) of individual intact hopanoids investigated in surface sediments across the West Hanna Shoal (WHS) shelf to basin transect in the western Arctic Ocean. Station numbers refer to locations shown in Table 3.1.
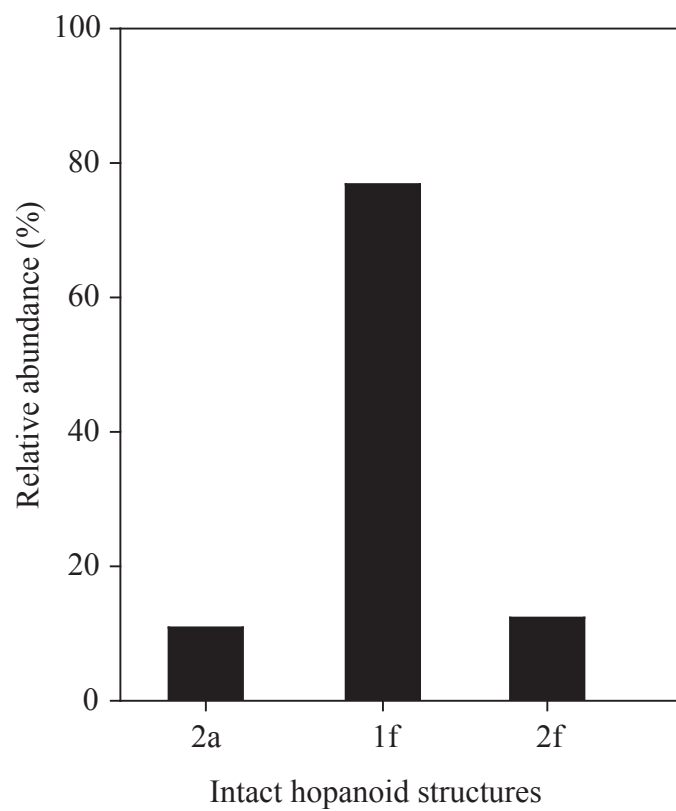
Figure 3.5. The relative abundance (%) of cyanobacteria specific hopanoids (**1f**, **2f**, **3f**; refer to Fig. 3.7 for structural details) isolated in a culture of *Synechococcus* originating from the Gulf of Alaska.
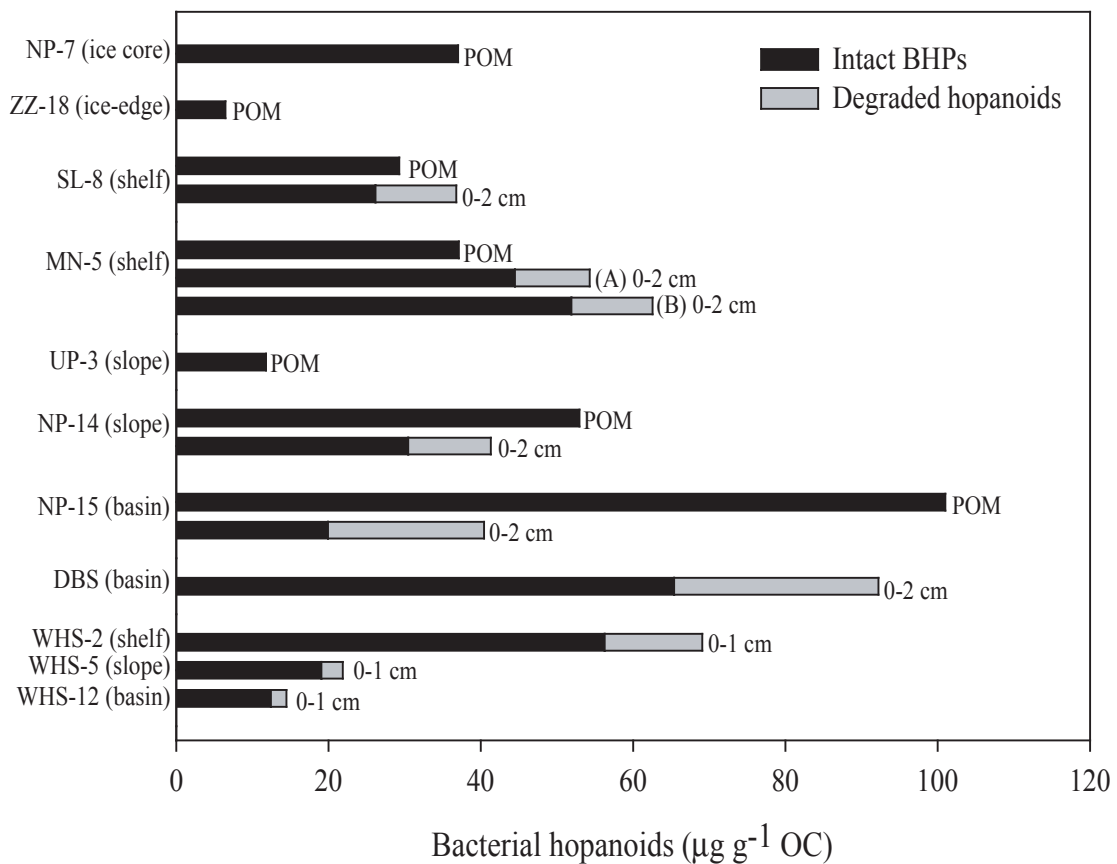
Figure 3.6. Analysis of total hopanoids in particulate and surface sediment samples from eight locations in the Bering Sea, including NP-7, ZZ-18, UP-3 and DBS not shown in Fig. 3.1, and three locations in the western Arctic (WHS-2, 5 and 12). Total concentrations are shown as the sum of the intact and degraded material. Intact BHPs include all individual compounds observed at each station.
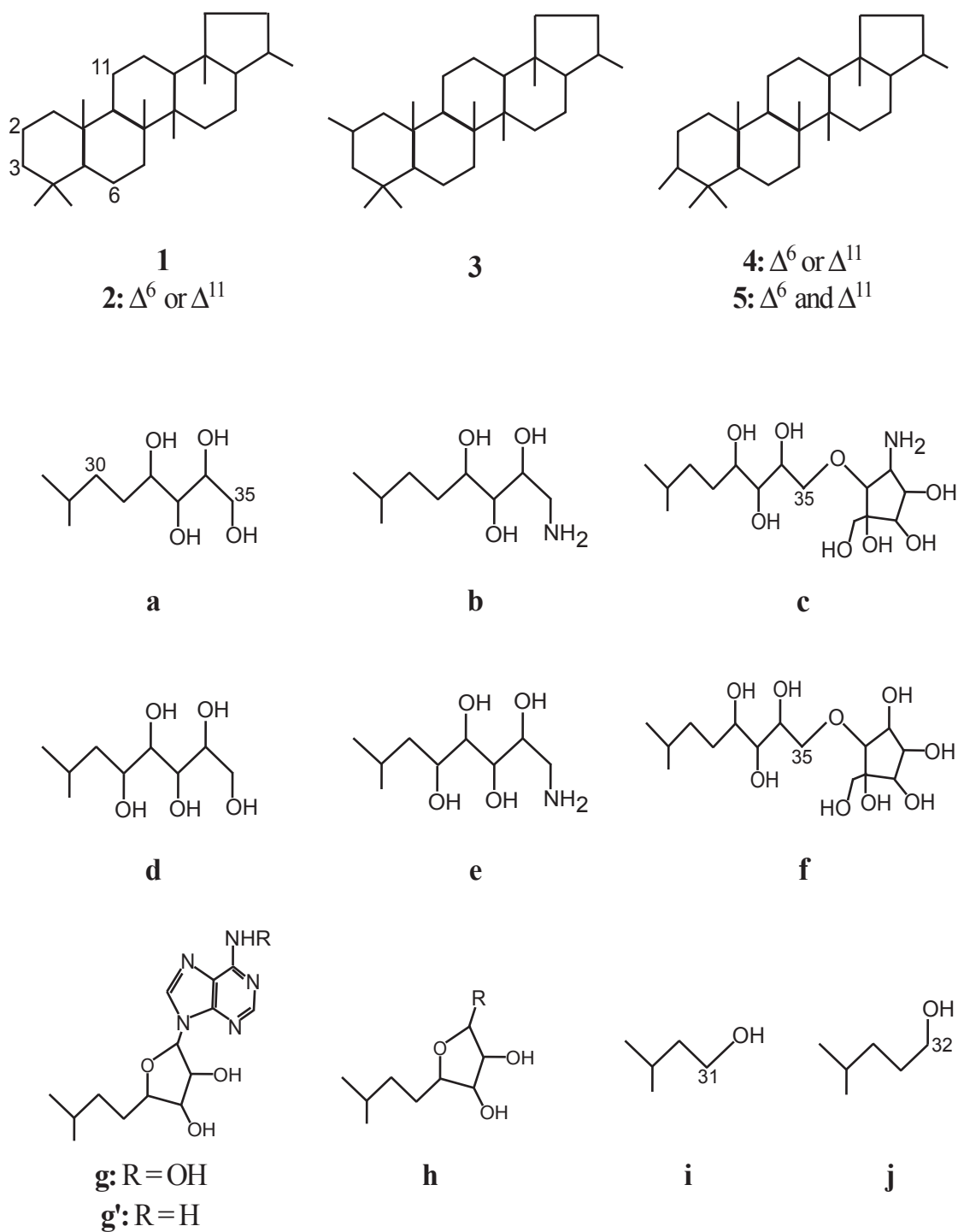
Figure 3.7. Bacteriohopanepolyol structures and their degrative products analyzed in particles, surface sediments and cultured *Synechococcus*.

Chapter 4: Intact bacterial hopanoids as tracers of organic carbon sources and processing in estuarine and marine sediments

Abstract

Accurate measures of bacterial organic carbon contributions to marine systems have been constrained by their lack of distinguishing geochemical tracers and limited cellular abundance in sediments compared to other sources of organic matter. Intact bacteriohopanepolyols (BHPs) provide a direct means to identify bacterial carbon in aquatic systems and we applied and compared recent analytical approaches to quantify and identify bacterial contributions in samples from the Western Arctic Ocean and Chesapeake Bay. In western Arctic sediments, intact hopanoids specific to soil derived material reflect offshore trends similar to that of other terrestrial derived lipids with at least a fraction representing their degrative products. Long-term preservation of primary production and subsequent heterotrophic recycling is reflected in the western Arctic as patterns of elevated chlorophyll biomass in the water column corresponded with increased inputs of bacterial organic carbon in the surface sediment. Intense microbial degradation of peat material was evident in Arctic river samples with differing sources of bacterial carbon contributing downstream to riverine surface sediments. In contrast, bacterial signatures transition along the salinity gradient of Chesapeake Bay with intact membrane components in particles and surface sediments reflecting a diverse range of possible bacterial sources. Markers of methanotrophs and cyanobacteria were directly traced from the water column to the underlying surface sediment and imply the transport

of organic material away from its region of production. Limited contributions of soil specific BHPs in sediments suggest terrigenous sources are represented among the substantial inputs of degraded hopanoid products. Chesapeake Bay outflow influences production and inputs to its neighboring shelf sediments with heterotrophic organisms and cyanobacteria as the main contributors of bacterial organic carbon in the coastal Atlantic Ocean.

Introduction

Microbes are widely recognized as the primary catalysts of organic matter recycling in aquatic ecosystems and through their collective biomass account for a major component of the world's living carbon (Whitman et al. 1998). Their cells are subjected to degradation and consumption by microheterotrophs in the water column (Arndt et al. 1990); therefore only a small fraction of cellular material likely reaches the benthos and contributes to the organic carbon of marine sediments. A number of possible tracers of bacterial carbon have been proposed including muramic acid, which has been used to estimate bacterial biomass in pure cultures (Rönkkö et al. 1994) as well as in marine and terrestrial environments (Millar and Casida 1970; King and White 1977), and has been applied to trace the presence of bacteria in various settings such as indoor air (Fox and Rosario 1994), clinical samples (Gilbart et al. 1986) and extraterrestrial samples (Kozar et al. 2001). However, recently muramic acid was found to be more associated with dead bacterial biomass, and thus is no longer considered a marker of living bacterial carbon (Guggenberger et al. 1999; Benner and Kaiser 2003; Glaser et al. 2004). Ueda et al. (1989) and Caspo et al. (2001) suggested D isomers of amino acids as appropriate

tracers of bacterial biomass, yet these compounds have also been observed in algae and fungi (Brückner et al. 1994; Lee and Bada 1977) and are thus not exclusive markers of bacteria. In addition, their concentration in cells varies depending upon cell type and the content of peptidoglycan in the cell (Quigley and Schwab 1988). The amount of peptidoglycan also varies according to cell type (Schleifer and Kandler 1967; Fukuhara et al. 1983), and thus is not useful for quantifying bacterial inputs to organic matter pools. Branched-chain fatty acids (i.e. C_{15} and C_{17}) were suggested as bacterial cell membrane components and used as biomarkers of organic carbon inputs (Guckert et al. 1985); however recent studies have found strong linkages among available carbon sources and fatty acid synthesis (Harvey et al. 2006). All these methods have both advantages and drawbacks and their application as proxies of bacterial carbon is constrained by their lack of specificity and the recognized diversity of uncultured microbes in the marine environment (Rappé and Giovannoni 2003). In contrast, the use of lipids as eukaryotic markers have been notable and include sources derived from both marine and terrestrial environments (Bianchi et al. 1989; Fahl and Stein 1997, 1999). Recent advances use branched and isoprenoid tetra ether lipids as markers of soil-derived bacteria and archaea, respectively (Ingalls et al. 2006) as well as intact phospholipids as signatures of some species of the eubacteria (Van Mooy et al. 2008).

Intact bacteriohopanepolyols (BHPs) are pentacyclic triterpenoids biosynthesized by variety prokaryotes as cell membrane components (Ourisson and Rohmer 1982; Rohmer et al. 1984). Their pentacyclic carbon skeleton is relatively resistant to degradation, thus allowing the preservation of their diagenetic products (i.e. geohopanoids) in sediments and soils over geological time scales (Dastillung et al. 1980a,

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b; Rohmer et al. 1980). Indeed their diagenetic products, the geohopanoids, have been observed in the environment over geological time scales (Dastillung et al. 1980a, b; Rohmer et al. 1980), but only recently has the existence of their complex biological precursors been discovered (Ourisson and Albrecht 1992). While the diagenetic processes converting intact hopanoids to their simpler molecular fossils remains unknown, BHPs have been found in sediments nearly 50 million years old (van Dongen et al., 2006) and in samples from the Upper Jurassic period (Bednarczyk et al., 2005), demonstrating the potential of intact structures to resist degradation for long periods of time. Therefore both intact BHPs and their degrative products are considered as ideal biomarkers for tracing bacterial specific organic carbon contributions to marine settings.

Intact hopanoid structures appear to represent a broad suite of bacterial sources. While some compounds are commonly observed among many organisms, including BHT (**1a**, Fig. 4.8), aminotriol (**1b**, Fig. 4.8) and BHT cyclitol ether (**1c**, Fig. 4.8; Talbot et al. 2003b, c; Blumenberg et al. 2006; Talbot and Farrimond 2007), others are reflective of particular bacterial groups and processes. Talbot and Farrimond (2007) first suggested triacetylated adenosylhopane (**1j**, Fig. 4.8) and two related compounds (**1i**, **3i**, Fig. 4.8) as potential markers of as yet uncultured soil bacteria that could be used to trace terrestrial microbes transported to marine sediments. Purple non-sulfur bacteria (Neunlist and Rohmer 1985; Neunlist et al. 1988), ammonia-oxidizers (Seemann et al. 1999) and nitrogen-fixing bacteria (Bravo et al. 2001) have also been shown as producers of adenosylhopanes. To date the only known source of the hexafunctionalized aminopentol (**1g**, Fig. 4.8) is that of methane-oxidizing bacteria, which have also been found as sources of the pentafunctionalized aminotetrol (**1e**, Fig. 4.8; Neunlist and Rohmer 1985;

Cvejic et al. 2000; Talbot et al. 2001); a compound also known from some sulfate reducing bacteria (Blumenberg et al. 2006). Both methane-oxidizers and acetic acid bacteria are sources of the C-3 methylated hopanoids; however unsaturation in the carbon skeleton likely accompanies the latter as these fermentative organisms are recognized as the most abundant sources of unsaturated BHPs thus far (Zundel and Rohmer 1985). BHPentol (**1d**, Fig. 4.8) is known from both cyanobacteria and acetic acid bacteria with the former reported as the most widespread source of C-2 methylated hopanoids (Bisseret et al. 1985). Talbot et al. (2008) also described novel tetrafunctionalized BHPs with a fully hydroxylated terminal sugar known only from the cyanobacteria *Gloeocapsa* sp. and *Chroococcidiopsis* sp. that include BHT pentose (**1f**, Fig. 4.8) and related unsaturated (**2f**, Fig. 4.8) and C-2 methylated (**3f**, Fig. 4.8) homologues.

In this study, we examine the abundance and distribution of intact bacterial hopanoids in particles and surface sediments of river-dominated regions that receive varied and significant inputs of organic material from the adjacent watershed. Offshore patterns were investigated in surface sediments from two shelf to basin transects collected from the Western Arctic Ocean. To compare the shelf to basin transport of this remote polar system to a temperate estuary with multiple riverine inputs, particles and surface sediments were taken along the salinity gradient of Chesapeake Bay and the coastal Atlantic Ocean. The regional differences and flow regimes of the western Arctic and Chesapeake Bay provide unique locations to examine the potential for bacterial specific proxies to trace the inputs of organic carbon sources and the dominant microbial processes operative during its recycling.

Methods

Sample collection

Bacterial contributions to surface (0-1 cm) sediments were examined in samples taken from the Western Arctic Ocean across the West Hanna Shoal and East of Barrow Canyon transects in the Chukchi and Alaskan Beaufort Seas, respectively (Fig. 4.1a). Eight surface sediment samples were collected from undisturbed cores with a Pouliot 0.06 m^2 box corer during the May to August 2002 Shelf-Basin Interactions Program process cruises aboard the USCGC *Healy* (refer to Table 4.1 and Fig. 4.1 for station locations). Samples of surface sediment and peat were also obtained using helicopter landings near the Ikpikpuk and Kokolik Rivers. Sediments were also taken from river mouths and along the salinity gradient of Chesapeake Bay and the coastal Atlantic Ocean (Fig. 4.1b; refer to Table 4.1 for station locations). Twelve surface (0-2 cm) sediment samples were collected with a Van Veen grab during July and October 2007 cruises aboard the R/V Hugh Sharp. All sediments were stored in glass or plastic I-Chem jars with Teflon lined lids and immediately frozen aboard the ship (-80°C) and stored (-70°C). Each sample was thawed and thoroughly homogenized prior to chemical analysis.

To characterize particulate organic matter (POM), six large-volume particulate samples were collected from Chesapeake Bay and the coastal ocean (Fig. 4.1b; refer to Table 4.1 for station locations). To capture the particle maximum and maximize the bacterial versus algal fraction of organic matter, samples from stations BR-858 and LB-724 were taken from below the chlorophyll maximum (Table 4.2) and pre-filtered through a 3μm filter cartridge. Two particulate samples were obtained from station ATL-6 (refer to Tables 4.1 & 4.2 for details) prior to pre-filtration to ≤3 μm for the comparison

of bacterial contributions in surface waters to those at depth. Two surface water samples

(stations MB-2 & YRK-4) were also collected and the bacterial fraction concentrated

using an Amicon DC-10 tangential-flow ultrafiltration unit equipped with a 30 kDa filter

after prefiltering through a 3 μm filter cartridge. Pre-filtered and concentrated POM

samples were subsequently vacuum filtered through pre-combusted (450°C, 4-6 h)

Whatman GF/F filters and frozen (-70°C) until analyses. The organic carbon content of

sediment (after removal of $CaCO_3$) and POM were measured with an Exeter Analytical,

Inc. CE-440 Elemental Analyzer.

Hopanoid class analysis

Both intact BHPs and their degraded products were identified and quantified in

surface sediments; however only the distribution of intact hopanoids was examined in

particulate samples. Total lipids containing both intact and degraded fractions were

extracted from surface sediments and particles similar to that previously described

(Mannino and Harvey 1999). In brief, lipids were extracted in a mixture of methylene

chloride:methanol (CH_2Cl_2:MeOH; 2:1) with sonication (bath), partitioned three times

and combined. Solvent was removed by rotary evaporation and total lipid extracts were

split into three fractions for the analysis of specific hopanoid classes. Total extracts and

primary hopanols (described below) were analyzed as their acetate derivatives formed by

acetic anhydride and pyridine (4 ml, 1:1) similar to methods described by Innes et al.,

1997. Acetylated total lipids were dissolved in a mixture of methanol:propanol (3:2) for

the analysis of intact BHPs by reversed phase-high performance liquid chromatography

(RP-HPLC)-APCI-MS^{n} methods modified from Talbot et al., 2001. LC-MS^{n} was

conducted with an Agilent 1100 Series LC/MS ion trap linked to an APCI source

interface operated in positive ion mode. Instrument settings were as follows: nebulizer gas pressure 60 psi; dry gas flow 6 L min^{-1}; dry gas temperature $200^{o}C$; APCI vaporizer temperature $400^{o}C$; and corona discharge current +3200 nA. Direct infusions of an isoprenoid isolated from *Gluconacetobacter xylinus* were applied for tuning the source and mass spectrometer calibration. Relative abundances of individual intact hopanoids were determined based on the peak area response of the extracted parent ions (base peak). Structures were assigned based on comparison to published +MS and +MS2 spectra when applicable (Talbot et al. 2001; Talbot et al. 2007a, b; Talbot et al. 2008).

The total hopanoid content of each sediment sample was determined by GC-GC/MS analysis of primary hopanols formed by periodic acid/sodium borohydride treatment and derivatization of intact BHPs similar to methods previously reported (Innes et al. 1997). Degraded hopanoid products were also analyzed by GC-GC/MS after lipid extracts were hydrolyzed with a solution of 0.5 N KOH in methanol and deriviatized with bis(trimethylsilyl)trifluoroacetamide amended with 25% pyridine. To reduce complexity of the East Barrow mixtures, extracts were further fractionated by normal phase-HPLC prior to GC/GC-MS analysis as described by Tolosa and de Mora, 2004. Primary hopanols and degraded hopanoid products were quantified using an Agilent 6890N Network GC system with flame ionization detection and hydrogen as the carrier gas. Structural identification was performed with a coupled GC-MS system (Agilent 5973N) operated in electron ionization mode with helium as the carrier gas. Column parameters and instrument methods are similar to those described by Belicka et al., 2002. The intact BHP fraction was calculated based on the difference between the total hopanoid content and degraded products.

Distribution of intact BHPs in the Western Arctic Ocean

Relative contributions of individual intact BHPs in surface sediments across the

West Hanna Shoal (WHS) and East of Barrow Canyon (EB) shelf to basin transects are

shown in Fig. 4.2. Up to ten intact hopanoids were observed in West Hanna Shoal

sediments and reveal a suite of bacterial sources. Slope sediments (stations WHS-5, 6)

showed the broadest range of compounds. Relative contributions were variable among

stations with approximately 84% of all intact hopanoids observed as the common BHT

(**1a**, Figs. 4.2a & 4.8). Aminotriol (**1b**, Fig. 4.8) followed, contributing nearly 7% to the

total hopanoid content. Samples also contained the methyl-unsaturated BHPentol (**5d**,

Fig. 4.8), which accounted for almost 4% of all BHPs. Soil derived (**1j**, **1i**, **3i**, Fig. 4.8)

sources were present in all samples with reduced contributions to basin sediments and a

greater diversity of adensylhopane and adenosylhopane-like compounds in the shelf and

slope samples (Fig. 4.2a). Up to eight intact BHPs were found in East Barrow sediments

with the greatest diversity of compounds observed in the slope (station EB-4) sample

(Fig. 4.2b). Relative contributions were similar among stations and nearly 93% of all

intact hopanoids were observed as BHT (**1a**, Figs. 4.2b & 4.8). Soil derived (**1j**, **1i**, **3i**,

Fig. 4.8) sources accounted for more than 3% of the total on average with contributions

greatest in the slope (station EB-4) sediment (Fig. 4.2b).

BHPs present in Ikpikpuk and Kokolik River peat and surface sediments are

shown in Fig. 4.3. Both show a suite of intact BHPs with sediments containing a greater

diversity of structures relative to the peat samples. The widespread BHT (**1a**, Fig. 4.8)

was the dominant compound among the Ikpikpuk samples and averaged nearly 40% of

the total hopanoid content (Fig. 4.3a). Soil derived (**1j**, **1i**, **3i**, Fig. 4.8) sources followed

and accounted for approximately 30% of all hopanoids (Fig. 4.3a). There were also four

unsaturated BHPs (**2a**, **2d**, **5d**, **6a**, Fig. 4.8) with contributions over 17% of the total

amount seen (Fig. 4.3a). The distribution of intact BHPs was most variable among

Kokolik River samples. While the 2-methyl adenosylhopane-type compound (**3i**, Fig. 4.8)

was most the abundant in the peat, BHT (**1a**, Fig. 4.8) dominated surface sediments (Fig.

4.3b). Adenosylhopanes (**1j**, **1i**, **3i**, Fig. 4.8) were also important contributors to Kokolik

peat and sediment samples, averaging 40% and 29% of the total, respectively (Fig. 4.3b).

The unsaturated-BHPentol pentose (**2h**, Fig. 4.8) was present in both Kokolik samples

(Fig. 4.3b) as well as the Ikpikpuk River peat (Fig. 4.3a). Contributions of BHPs

suggested from as yet uncultured soil bacteria (**1j**, **1i**, **3i**, Fig. 4.8) seen in western Arctic

samples are shown in Fig. 4.4. Ikpikpuk and Kokolik Rivers reflect the highest

abundances of adenosylhopanes (**1j**, **1i**, **3i**, Fig. 4.8), followed by the West Hanna Shoal

and East Barrow transects, respectively (Fig. 4.4).

Distribution of intact BHPs in Chesapeake Bay and the coastal Atlantic Ocean

The relative abundance of individual intact hopanoids in surface sediments along

the salinity gradient of Chesapeake Bay and the coastal Atlantic Ocean are shown in Fig.

4.5. Up to sixteen intact BHPs were observed across the estuarine interface, with the

greatest diversity of structures seen in the northern half of Chesapeake Bay (Fig. 4.5).

With only one exception, the common BHT (**1a**, Fig. 4.8) dominated in sediments and

accounted for over 68% of the total hopanoids among all stations (Fig. 4.5). At station

MB-2, unsaturated-BHT (**2a**, Fig. 4.8), unsaturated-BHPentol (**2d**, Fig. 4.8) and 3-methyl

BHT (**4a**, Fig. 4.8) were the greatest contributors to the overall BHP signal (Fig. 4.5). A

BHP known to date only in methane-oxidizing bacteria, the hexafunctionalized aminopentol (**1g**, Fig. 4.8) was most abundant at station UB-1 and averaged 5.3% of the total (Fig. 4.5). Stations MB-2 and MB-3 followed and contributed approximately 5% and 3%, respectively. Aminopentol (**1g**, Fig. 4.8) also observed in minor amounts from stations BR-858, PAX-818, POT-804 and RAP-61 (Fig. 4.5). Markers specific to cyanobacteria (**3a**, **1f**, **2f**, **3f**, Fig. 4.8) were found as minor components in eight of the surface sediment samples. Cyanobacterial contributions were greatest in sediment from station MB-2, accounting for over 8% of the total (Fig. 4.5). Stations UB-1 and MB-3 followed, averaging over 6% and 5%, respectively (Fig. 4.5). Soil derived (**1i**, **1j**, Fig. 4.8) sources were only seen in four of the sediments with highest abundances at stations RAP-61 (3.2%) and MB-2 (2.9%), respectively (Fig. 4.5). The contribution of intact hopanoids to Chesapeake Bay POM is shown in Fig. 4.6. Up to ten BHPs were found and reflect a range of possible bacterial sources. Approximately 55% of all intact hopanoids at Station BR-858 were observed as specific tracers (**1g**, Fig. 4.8) of methane-oxidizing bacteria (Table 4.2; Fig. 4.6). Cyanobacterial sources (**1f**, **3f**, Fig. 4.8) were present in four of the six POM samples with inputs representing nearly 27% and 17% of all hopanoids at stations LB-724 and YRK-4, respectively (Table 4.2; Fig. 4.6). Unsaturated BHPentol pentose (**2h**, Fig. 4.8) was the most abundant compound in both samples of POM from station ATL-6 and averaged over 80% of the total hopanoid content (Fig. 4.6). Its saturated homologue (**1h**, Fig. 4.8) was also present in five of the six POM samples and accounted for 15% of all BHPs on average (Fig. 4.6).

Intact BHPs and their degrative products

Total intact hopanoids and their degradation products in surface sediment from the western Arctic, Chesapeake Bay and coastal Atlantic Ocean are shown in Fig. 4.7. Both intact and degraded hopanoid products were present in all samples with variable distributions among the geographic locations. Intact BHPs represented 77-87% of the total hopanoid content in West Hanna Shoal surface sediments with no obvious trend offshore (Fig. 4.7). In surface sediments across the East Barrow transect the relative contribution of intact hopanoids decreased offshore (shelf: 96%, slope: 86%, basin: 69% of the total), while contributions of degraded products increased with water depth (shelf: 4%, slope: 13%, basin: 30% of the total; Fig. 4.7). The majority of bacterial products present in Ikpikpuk and Kokolik River peat and sediment samples were seen as intact structures and ranged from 77-95% of all hopanoids (Fig. 4.7). A changing pattern of intact and degraded hopanoid contributions is evident along the salinity gradient of Chesapeake Bay and the coastal ocean (Fig. 4.7). Degraded products averaged over 78% of all hopanoids observed in surface sediments from northern and mid-Bay stations (UB-1, BR-858, MB-2, MB-3 and PAX-818) with inputs decreasing seaward (Fig. 4.7). In portions of the mid and southern Bay (stations POT-804, RAP-61 and LB-724) nearly equal contributions of intact BHPs and their degrative products were observed (Fig. 4.7). However, intact membrane lipids dominated (71 – 92% of all hopanoids) in sediments from the southernmost station in Chesapeake Bay (YRK-4) and the coastal Atlantic Ocean (stations ATL-6, 7, 9) showing similar contributions to those shown in western Arctic sediments (Fig. 4.7).

Discussion

The distribution and diversity of intact bacterial hopanoids in surface sediments of polar and temperate oceans reflect the importance of bacteria as both contributors to the organic carbon compartment and as essential catalysts to carbon recycling. Despite extreme low temperatures, Wheeler et al. 1996 discuss high bacterial production in the central Arctic basin and others (Sherr et al. 1997; Rich et al. 1997) suggest the majority of this activity is heterotrophic and show significant correlations among bacterial and phytoplankton production (Rich et al. 1997). The abundance of intact BHPs in WHS shelf (station WHS-2) sediment (Fig. 4.7) potentially reflects this relationship as measurements of subsurface (~25 m) chlorophyll biomass (40 mg m^{-3}) and primary productivity (3 g C m^{-2} d^{-1}) were found to be the highest near this shallow site relative to other WHS stations during summer (Hill and Cota 2005) with annual production rates exceeding 300 g C m^{-2} y^{-1} over the Chukchi shelf (Sambrotto et al. 1984; Springer and McRoy 1993; Springer et al. 1996; Sakshaug 2004; Hill and Cota 2005). Similarly, the diversity (Fig. 4.2b) and abundance (Fig. 4.7) of intact BHPs observed in the Beaufort Sea slope (station EB-4) sediment mimic the enhanced subsurface (~40 m) chlorophyll biomass (3 mg m^{-3}) measured during summer (Hill et al. 2005). While phytoplankton and bacterial production in the water column are indicative of short-term processes, the occurrence of bacterial cell membrane components in Arctic sediments potentially reflects long-term preservation of primary production and subsequent organic matter recycling. Bacteria may be the primary recyclers of organic matter in the marine water column, but only a small fraction of bacterial carbon reaches the underlying sediment as these small cells provide an important link among primary productivity and heterotrophic

food webs through their consumption by microheterotrophs (Sherr et al. 1997). Tight

pelagic-benthic coupling has been described for western Arctic shelves (Grebmeier 1993;

Moran et al. 2005; Belicka and Harvey, *in press*), and the presence of effective transport

mechanisms (i.e. marine aggregates, fecal pellets) allow for the accumulation of bacterial

remnants in sediments (Alldredge 1987). Therefore, the pulse of intact BHPs to Chukchi

Sea shelf sediment (station WHS-2) suggests that a significant fraction of bacterial

biomass is exported to the benthos (Fig. 4.7). Heightened inputs of membrane

components derived from living bacterial cells may also suggest intense benthic recycling

of rapidly accumulating labile organic material on the productive Chukchi Sea shelf. The

lower amounts of intact BHPs across WHS surface sediments (Fig. 4.7) also support the

possible relationship between primary production and subsequent heterotrophic activity

as modest amounts of chlorophyll biomass (<0.5 mg m^{-3}) over the Chukchi slope and

basin have been reported (Hill et al. 2005). Concentrations of suspended particles are far

lower in the central basin than those observed in shallow regions (Lepore et al. *in press*)

and reflect a dilution of shelf material that is being laterally advected offshore. Moran et

al. 2005 suggest up to 20% of summer production (6 ± 7 mmol C m^{-2} d^{-1}) is laterally

exported and contributes to observations of a pronounced subsurface (~100 m) POM

maximum extending off-shelf. Mesoscale eddies that form along the continental slope

are important mechanisms of organic matter transport off-shelf, and thus observations of

both intact and degraded hopanoid products in WHS slope and basin surface sediments

(Figs. 4.2a & 4.7) likely reflect the advection of bacterial organic carbon originating in

shallower waters (Walsh et al. 2005). Longer accumulation times in deeper water

columns expose sinking bacterial cells to oxic conditions for extended time periods, thus

enhancing their likelihood of degradation and assimilation by bactivores (Meyers 1997) and reducing their contributions to surface sediments. Similarly, the diversity (Fig. 4.2b) and abundance (Fig. 4.7) of intact BHPs observed in the Beaufort Sea slope (station EB-4) sediment mimic the enhanced subsurface (~40 m) chlorophyll biomass (3 mg m^{-3}) measured during summer (Hill et al. 2005) and further reflect the long-term preservation of primary production and subsequent heterotrophic recycling in this region. The Beaufort Sea slope (station EB-4) appears as a focusing zone of nutrient-rich water and organic material flowing through Barrow Canyon, and thus high concentrations of suspended organic matter extend off-shelf (Bates et al. 2005). While degradation and grazing in the water column limits bacterial carbon contributions to Beaufort Sea sediments, elevated organic carbon concentrations reported in both particles (Bates et al. 2005) and sediments (Belicka and Harvey, *in press*) from this region suggest coupling between pelagic and benthic realms and the input of bacterial remnants to surface sediments.

At least a fraction of ammonium released upon the regeneration of organic material is suggested to be assimilated by ammonia oxidizing bacteria in the western Arctic (Nishino et al. 2005). Cloned fragments of 16S rRNA genes show the dominance of marine *Nitrosospira*-like and *Nitrosomonas*-like organisms in Arctic waters (Bano and Hollibaugh 2000), which suggests potential sources of intact bacterial hopanoids to WHS and EB surface sediments. While marine derived clones are phylogenetically distant from those originating in soils (Stephen et al. 1996), investigations of intact BHPs isolated from the soil derived *Nitrosomonas europaea* reveal the common aminotriol (**1b**, Fig. 4.8) as well as di- (**1j'**, Fig. 4.8) and triacetylated (**1j**, Fig. 4.8) adenosylhopanes

(Seemann et al. 1999; Talbot et al. 2007c), which have recently been suggested as specific tracers of soil bacterial organic carbon transported to marine sediments (Cooke et al. 2008a, b). The presence and distribution of adenosylhopanes across the WHS and EB transects (Fig. 4.2) suggest the movement of terrestrial material into the Chukchi and Alaskan Beaufort Seas, respectively. Many studies have attempted to quantify the movement of land-derived material using lipid biomarkers (e.g. Goñi et al. 2000; Schubert and Calvert 2001; Stein et al. 1994, 1999; Boucsein and Stein 2000; Belicka et al. 2002) since allochthonous sources provide important inputs of organic carbon to this region. Major rivers deliver considerable amounts of terrigenous material to the Arctic each year with total organic carbon fluxes reaching 40×10^6 t yr^{-1} for the entire region (Rachold et al. 2004). Erosion of the Alaskan Beaufort Sea coastline also provides significant quantities of terrestrial organic carbon with rates on the order of 0.5 to 6 m yr^{-1} (Are et al. 2008; Lantuit and Pollard 2008). While the WHS transect is distant from a direct land source, intact BHPs are likely delivered by melting sea-ice that forms along the northern Alaskan coast. Upon freezing terrestrial derived material becomes entrained in sea-ice after resuspension at shallow (<20 m) water depths where coastal erosion rates are high (Eicken et al. 2005). Sea-ice rafted debris is subsequently transported in an east-west direction via the Beaufort Gyre (Eicken et al. 2005) and released upon thawing. A greater input of soil derived BHPs to WHS shelf sediment and reduced contributions offshore (Fig. 4.4) are in agreement with the occurrence of other lipid biomarkers of terrestrial origin (i.e. C_{23}-C_{31} saturated alkanes, C_{24}-C_{28} saturated fatty acids and alcohols, α-amyrin, and $C_{29}\Delta^5$; Table 4.3; Hopmans et al. 2004; Belicka and Harvey *in press*). The distribution of degraded hopanoids follows a similar pattern with inputs averaging 13 μg

g^{-1} OC in Chukchi shelf sediment and 2.9 µg g^{-1} OC in slope and basin samples (Fig.

4.7). The diversity and increased inputs of adenosylhopanes (Figs. 4.2a & 4.4) and

degraded hopanoids (Fig. 4.7) near-shore reflect the rapid accumulations rates (~3 month

timescales) of sea-ice rafted POM to Chukchi shelf sediments estimated by Cooper at el.

2005. Sea-ice likely entrains and then releases older bacterial carbon that had degraded

on land prior to being transported into the Arctic Ocean as reflected by the abundance of

geohopanoids at station WHS-2 (Fig. 4.7). The direct deposition of sea-ice rafted material

extends into deeper waters, and degraded hopanoid signatures in slope and basin

sediment may also result from longer accumulation times and exposure to oxygen during

the transit of intact BHPs through deeper water columns. Lateral advection and the

formation of eddies (as discussed above) are also effective mechanisms for transporting

soil bacterial organic carbon and degraded hopanoid products into the Arctic basin

(Moran et al. 2005; Pickart et al. 2005).

In contrast to the WHS transect, EB sediments are geographically closer to direct

terrestrial sources that include the eroding, peat-enriched Alaskan coastline (Rachold et

al. 2000) as well as small rivers (including the Colville) that discharge short, intense

pulses of organic matter (MacDonald et al. 2004). It might be expected that EB shelf

sediment receives the greatest amounts of terrigenous material due to its shallow depth

and closeness to land; however contributions of soil derived BHPs were enriched (0.48

µg g^{-1} OC) in the slope (EB-4) by an order of magnitude relative to shelf (EB-2; 0.09 µg

g^{-1} OC) and basin (EB-7; 0.06 µg g^{-1} OC) inputs (Fig. 4.4). Again this is consistent with

other terrestrial derived signatures reported from this region (Table 4.3; Belicka and

Harvey *in press*). The continental slope (station EB-4) appears to be a favorable site of

sediment focusing as indicated by the diversity and abundance of adenosylhopanes (**1j**, **1i**, **3i**; Figs. 4.2b, 4.4, 4.8) and degraded hopanoid products (Figs. 4.7). Ice rafting (Arnborg et al. 1967; Naidu and Mowatt 1975) as well as a near-surface (~10-15 m) POM maximum extending northward from the Beaufort shelf (Bates et al. 2005) are responsible for the delivery of riverine material and thus adenosylhopanes and geohopanoids to the EB slope. In addition, episodic sediment gravity flows along the shelf and slope move significant quantities of terrigenous and potentially degraded material into deeper waters (MacDonald et al. 2004). Despite the greater abundance of geohopanoids in slope sediment (1.5 μg g^{-1} OC), degraded bacterial carbon was found to increase proportionately offshore and contribute over 30% to the total hopanoid content in the Beaufort Basin (Fig. 4.7). Apart from water column and sediment transport, intact compounds might also be breaking down in the water column prior to their deposition at deep sites (as discussed above).

Despite the proximity to land, concentrations of adenosylhopanes and geohopanoids were at least an order of magnitude less in EB sediments relative to those found in the pelagic WHS transect (Figs. 4.4 & 4.7) and suggest the retention of soil bacterial organic carbon and degraded hopanoid products in deltaic sediments. While concentrations of other terrestrial derived biomarkers showed the opposite pattern, total lipids (including marine markers) across the EB transect were found to contain proportionately less terrigenous sources than those in WHS sediments (Table 4.3) due to enhanced phytoplankton contributions (Belicka and Harvey, *in press*). Enhanced contributions of soil derived BHPs were observed in samples of peat and surface sediments collected from two relatively small rivers on the northern slope of Alaska (Fig.

4.4). The inherent heterogeneity of organic-rich peat material is reflected by the varying

contributions and diversity of individual intact BHPs observed in Ikpikpuk and Kokolik

River samples (Fig. 4.3). The abundance of intact hopanoids (Fig. 4.7) and particularly

adenosylhopanes (Fig. 4.4) in peat from the high bank of the Ikpikpuk River are

significantly greater than that observed in surface sediment taken from the river mouth,

suggesting active microbial degradation of peat and a dilution of soil bacterial organic

carbon downstream. Similarly, a greater contribution of degraded hopanoid products was

found in Ikpikpuk peat (11.5 μg g^{-1} OC) relative to riverine (0.57 μg g^{-1} OC) sediment

(Fig. 4.7). In contrast, the concentration of intact BHPs and thus adenosylhopanes were

reduced in the Kokolik peat sample collected near the water's edge (4.0 μg g^{-1} OC)

relative to surface sediment (4.7 μg g^{-1} OC) taken from the river main stem (Figs. 4.3 &

4.4). Contributions of degraded hopanoid products in peat (1.9 μg g^{-1} OC) were also less

than those observed in riverine sediment (3.0 μg g^{-1} OC). Therefore it is possible that our

sample was not 100% peat, but contained a mixture of peat and sediment as the %TOC in

the Kokolik River peat was nearly half (7.36%; Table 4.1) that measured in the Ikpikpuk

River sample (14.23%; Table 4.1).

  While the western Arctic reveals patterns of soil derived bacterial carbon

consistent with inputs of other terrigenous markers, bacterial hopanoids in Chesapeake

Bay reflect multiple bacterial sources and processes that transition along the land-sea

gradient with methane oxidation, intense bacterial recycling and a dominance of degraded

products observed in the northern Bay to reduced bacterial activity and a dominance of

intact compounds seen in the southern Bay and coastal ocean (Figs. 4.5 and 4.6). Other

potential sources of bacterial carbon such as cyanobacteria appear consistently along the

salinity gradient (Figs. 4.5 & 4.6). Changes in bacterial community structure down the

estuary are driven by strong hydrological gradients and diverse habitats that are

influenced by many organic matter inputs from both natural and anthropogenic sources.

Chesapeake Bay is the largest and most productive estuary in the United States that has

been the focus of intense research on how eutrophication causes seasonal hypoxia/anoxia,

loss of submerged aquatic vegetation and the alteration of food webs. Similarly,

Zimmerman and Canuel (2000) applied lipid biomarkers as proxies of phytoplankton and

bacterial carbon inputs with dramatic increases in production suggested within the last

half of the 20th century; therefore Chesapeake Bay is shifting from a benthic-dominated

to a pelagic-dominated, light-limited system. Intact hopanoids provide a direct measure

of bacterial organic carbon with communities in surface sediments appearing more

diverse than those in the POM (Figs. 4.5 & 4.6). In particular, a greater diversity of intact

BHPs in northern and mid-Bay sediments (Fig. 4.5) suggest intense bacterial recycling at

the sediment interface driven by high rates of organic and inorganic nutrient loadings

delivered by the Susquehanna River (Meybeck 1982; Kempe 1984). Influencing nearly

42% of the Chesapeake Bay drainage basin (Hobbs et al. 1992), average fluxes from the

Susquehanna River reach 151 x 10^9 g C y^{-1}, contributing approximately 53% of the total

organic carbon input to the estuary (Boynton et al. 1995; Kemp et al. 1997) with

suspended sediment loads reaching 2.0 million t y^{-1} (Gross et al. 1978). While a fraction

of riverine-derived material is refractory (Meybeck 1982), considerable amounts of waste

water effluents provide a source of labile organic matter to the upper Bay (Van Es and

Laane 1982). Susquehanna River flow and the adjacent watershed also supply significant

inputs of inorganic inputs (i.e. agricultural runoff, surface and ground water inputs) that

fuel primary productivity and subsequent respiration by resident heterotrophic organisms (Boynton et al. 1995). Similar to the western Arctic, strong correlations among primary productivity and heterotrophic respiration are found in Chesapeake Bay during the summer season (Malone at el. 1991; Smith and Kemp 1995) with reports of bacteria assimilating 30 to >100% of the phytoplankton production (Ducklow et al. 1988; Jonas 1992), which often leads to annual hypoxic/anoxic conditions in bottom waters. High turbidity conditions and thus greater contributions of particle-associated bacteria cause enhanced heterotrophic recycling and limited photosynthesis in the northern region. The importance of riverine-derived carbon decreases along the salinity gradient (Van Es and Laane 1982; Matson and Brinson 1990; Cifuentes et al. 1988), and reduced but substantial contributions of suspended sediments and organic matter are delivered to the lower Bay with the ocean, tributary estuaries and coastal erosion as the main sources (Officer et al. 1984). Reports of a net heterotrophic upper Bay and net autotrophic lower Bay suggest heterotrophic production is supplemented by riverine inputs in the north (Kemp et al. 1997); therefore the diversity of intact BHPs in surface sediments (Fig. 4.5) likely reflects a dilution of terrigenous inputs and thus heterotrophic recycling along the land-sea gradient.

Observations of the hexafunctionalized aminopentol (**1g**, Fig. 4.8) in POM and surface sediments from the northern Chesapeake Bay suggest the presence of methane oxidizing bacteria (Neunlist and Rohmer 1985; Talbot et al. 2001) and demonstrate the specificity of intact BHPs that allow their inputs to be directly traced from the water column to the underlying surface sediment. The enhanced signal in subsurface (12 m) POM collected from turbid, hypoxic (1.2 mg O_2 L^{-1}) water at station BR-858 potentially

reflects the occurrence of reduced microniches due to oxygen-consuming bacterial consorts. Experimentally Sieburth et al. 1993 found methylotrophs to be responsible for initiating the formation of such microzones in Chesapeake Bay by oxidizing methylamine produced by algae, which could subsequently support methane-producing (i.e. methanogens) and methane-oxidizing (i.e. methanotrophs) bacteria. Strict methylotrophs have been reported to produce intact BHPs; however only hexafunctionalized structures are known as yet from those that can oxidize methane (Neunlist and Rohmer 1985; Talbot et al. 2001). While it remains unknown whether the accumulation of methane produced by methanogens in the water column is enough to sustain a viable population of methanotrophs, enrichment cultures from in and around the pycnocline near stations UB-1 and BR-858 revealed unique estuarine methane-oxidizers in Chesapeake Bay (Sieburth et al. 1993) that may be sources of aminopentol (**1g**, Fig. 4.8) in the POM (Fig. 4.6). Methane production is also known to occur in the deep sediment layers at station BR-858 through anaerobic oxidation of organic matter (Reeburgh 1969). The potential formation and release of gas bubbles from deep sediments into the water column (Reeburgh 1969) might also support oxidizing bacteria and thus the occurrence of the hexafunctionalized BHP (**1g**, Fig. 4.8) in POM. Production and oxidation of methane in surface sediments is limited by the availability of sulfate as a terminal electron acceptor; therefore aminopentol (**1g**, Fig. 4.8) in northern Bay sediments may indicate direct inputs from the overlying water column or the oxidation of methane bubbles prior to their release from the benthos. In addition, accumulation of aminopentol (**1g**, Fig. 4.8) in sediment from station MB-2 and its absence in POM (Figs. 4.5 & 4.6) likely reflects surface water transport down-estuary and deposition in the deep mid-Bay channel (as discussed above,

Biggs and Flemer 1972). Although not seen in POM, aminotetrol (**1e**, Fig. 4.8) was observed in sediments from three (stations UB-1, BR-858, PAX-818) of the five locations also containing the hexafunctionalized BHP (**1g**, Fig. 4.8) and has been found in minor amounts in methanotrophs (Neunlist and Rohmer 1985; Talbot et al. 2001) as well as some species of sulfate reducing bacteria (Blumenberg et al. 2006). Sulfate reduction is widely recognized as a primary pathway of organic matter diagenesis in estuarine sediments and accounts for 60-80% of the total sediment carbon metabolism in the mid-Bay region (Roden et al. 1995). Low rates were measured seasonally in northern Bay surface sediments (0.2 - 0.7 mmol m^{-2} d^{-1}) relative to middle (8.2 - 16.2 mmol m^{-2} d^{-1}) and southern (2.3 - 6.3 mmol m^{-2} d^{-1}) regions (Marvin-DiPasquale et al. 2003), suggesting aminotetrol (**1e**, Fig. 4.8) is more likely to reflect methanotrophy at stations UB-1 and BR-858 and sulfate reduction at stations PAX-818 and YRK-4 (Fig. 4.5).

The majority of organic material accumulating in surface sediments is composed of polymeric compounds, and thus sulfate reducers must rely on the hydrolytic abilities of fermentative bacteria to produce substrates that they are able to metabolize (Widdel 1988; Jørgensen and Bak 1991). Acetic acid is recognized as the principle substrate for sulfate reduction in marine sediments (Parkes et al. 1989; Sørensen et al. 1981) and rapid turnover (≥ 2 hr^{-1}) of acetate pools in the mid-Bay region have been measured (Roden and Tuttle 1996). Unsaturated BHPs are bi-products of bacterial cells that rely on fermentation (Talbot et al. 2007b), and thus their occurrence in Chesapeake Bay sediments (**2a**, **2d**, **5d**, **6a**, Fig. 4.8) may potentially reflect these specialized microbial populations. Hydrolytic activities are known from fermentative bacteria belonging to the genera *Bacillus* and *Clostridium* (Ahmed et al. 1984), and while *Bacillus acidocaldarius*

is known to produce hopanoids, *Bacillus subtilus* and *Clostridium* sp. have been shown

not to produce any BHPs (Rohmer et al. 1984). Relative abundances of unsaturated

hopanoids (Fig. 4.5) were generally higher (~ 23%) in mid-Bay sediments, which agrees

with sulfate reduction rates reported by Marvin-DiPasquale et al. 2003 (discussed above).

Fermentation end-products might also be important beneath the pycnocline in

Chesapeake Bay since tremendously high rates of acetate turnover (>70% of the acetate

pool used per hour) were measured (Bell et al. 1988); however unsaturated BHPs (**2a**, **2d**,

Fig. 4.8) in the POM (Fig. 4.6) likely do not represent fermenting organisms as samples

from stations MB-2 and LB-724 were taken from oxygenated waters (Table 4.2).

Instead, this signal may reflect heterotrophic bacterial activity as discussed above.

Other potential sources of intact BHPs to Chesapeake Bay surface sediments

include bacteria that live attached to the leaves or roots of sea grasses (Crump and Koch

2008). Methylotrophic bacteria that utilize by-products of plant metabolism including

methanol and methylamine produced by the stomata (MacDonald and Fall 1993;

Nemecek-Marshall et al. 1995) tested positive for the production of intact hopanoids and

have been shown as sources of the common BHT (**1a**, Fig. 4.8), BHT cyclitol ether (**1c**,

Fig. 4.8) and 2-methyl BHT (**3a**, Fig. 4.8; Renoux and Rohmer 1985; Knani et al. 1994).

Methylation at C-2 is also an important structural characteristic of cyanobacteria

(Summons et al. 1999) and thus its presence in sediments along with BHT pentose (**1f**,

Fig. 4.8) and related unsaturated (**2f**, Fig. 4.8) and C-2 methylated (**3f**, Fig. 4.8)

homologues reflect the potential importance of cyanobacterial carbon inputs.

Distributions of cyanobacteria specific BHPs in particles also imply that these signatures

can be directly traced to surface sediments (Fig. 4.5) as particulate aggregates such as

marine snow or fecal pellets produced by zooplankton are potential transport mechanisms (Alldredge 1987). Marine *Synechococcus* dominate the picophytoplankton in Chesapeake Bay (Chen et al. 2006), contributing 10-20% of the total primary production during summer (Ray et al. 1989; Malone et al. 1991; Affronti and Marshell 1993). While some *Synechococcus* strains were reported to produce bacterial hopanoids (Llopiz et al. 1996; Summons et al. 1999; Jahnke et al. 2004), others were shown not to synthesize any BHPs (Rohmer et al. 1984, Talbot et al. 2008). The relative abundance of cyanobacteria specific BHPs (**1f**, **3f**, Fig. 4.8) in surface water from station LB-724 were greater (17% of the total hopanoid content) than those in the northern sites (0-6%; Fig.4. 6) and agree with findings by Chen et al. 2006 who reported *Synechococcus* to account for over 14% of the bacterial fraction (10^6 cells ml^{-1}) in surface waters from the lower Bay and represent 10% and 3% of the bacterial fraction in the mid and upper Bay regions, respectively. Observations of cyanobacterial specific BHPs in POM from the euphotic zone and their absence in bottom waters from the offshore site (ATL-6) might thus be expected; however relative contributions were greatest in POM collected at depth (10 m) from station YRK-4 (27%; Fig. 4.6) and may imply resident populations sinking through the water column (Jerlov 1976). The absence of cyanobacterial signatures in sediments from lower Bay stations (RAP-61, LB-724, YRK-4; Fig. 4.6) suggests organic matter was transported away from its region of production, either up-estuary in high salinity bottom waters or offshore in the surface layer (Schubel and Pritchard 1986). The cyanobacterial signal in surface sediment from station ATL-7 (Fig. 4.5) may have been influenced by the nutrient-rich outflow from Chesapeake Bay, and could have also resulted from in situ production as *Synechococcus* sp. have been observed in surface waters along the mid-

Atlantic coastline (Cottrell et al. 2006). Similarly, cyanobacteria specific BHPs likely reflect in situ production near the mid-Atlantic shelf break (station ATL-9; Fig. 4.5) with *Synechococcus* sp. abundances reported to be near 1.5 x 10^5 cells ml^{-1} (Cottrell et al. 2006). Relative contributions of cyanobacterial markers ranged from 0.2 - 8.6% of the total intact hopanoids in surface sediments from the upper, mid-Bay and offshore regions, and likely reflect diverse strains of *Synechococcus* as cloned fragments of 16S rRNA genes revealed isolates belonging to phylogenetically distinct clusters with estuarine, marine and mixed strains dominating the northern, southern and mid-Bay water columns, respectively (Chen et al. 2006). Two hopanoids from as yet unknown sources (i.e. BHPentol pentose, **1h** and unsaturated BHPentol pentose, **2h**; Fig. 4.8), contributed to nearly 90% of the total hopanoids in particles from both sample depths at station ALT-6 (Fig. 4.6),and the absence of these compounds in the underlying surface sediment (Fig. 4.5) might suggest grazing by bacteriovores.

Considerable amounts of terrigenous material enter Chesapeake Bay each year and yet adenosylhopanes (**1i**, **1j**, Fig. 4.8) were absent in POM (Fig. 4.6) and only minor contributors (1.6 - 3.2% of all intact BHPs) to surface sediments (Fig. 4.5). Perhaps most of the terrestrial inputs from rivers and coastal erosion are reflected by degraded hopanoid products in surface sediments (Fig. 4.7) as a significant fraction of allochthonous sources consist of old, refractory carbon (Meybeck 1982; Loh et al. 2006). Over half of the total hopanoid content in surface sediments from Chesapeake Bay reflect geohopanoids, with only few exceptions, and contributions in some locations reach >90% (Fig. 4.7). Station BR-858 in particular is located just south of the estuary turbidity maximum (ETM; typically found between latitudes $39^o10'N$ and $39^o28'N$); a zone of

high suspended sediment concentrations near the freshwater/saltwater interface driven by estuarine circulation, tidal resuspension and transport that traps the majority of terrigenous, refractory material supplied by the Susquehanna River (Sanford et al. 2001). The significant amount (57 µg g^{-1} OC) of degraded hopanoid products in surface sediment from station BR-858 (Fig. 4.7) may result from sedimenting terrestrial material that bypassed the ETM due to a shift in the salinity structure of the northern Bay caused by large flood events related to storms and hurricanes (Sanford et al. 2001). Additionally, sedimentation rates near this site (1.23 g cm^{-1}y^{-1}; Officer et al. 1984) are an order of magnitude greater than that of the adjacent shoals where terrigenous inputs are dominated by coastal erosion; therefore a focusing of older bacterial carbon into the deep channel could also be reflected by geohopanoids in surface sediment. Similarly, high contributions of degraded hopanoid products in sediment from station MB-3 (74%) imply considerable shoreline erosion as this site is located on the western shoal of the main channel. Sediment from within the ETM (station UB-1) shows approximately equal contributions of both intact and degraded hopanoid products (Fig. 4.7) with geohopanoids potentially indicative of terrigenous inputs delivered by the Susquehanna River and retained in the sediment; whereas intact BHPs suggest heightened bacterial activity and recycling within the ETM resulting from particle-associated bacteria and heterotrophic organisms whose growth and respiration are supported by primary producers also trapped within this region (Smith and Kemp 1995).

The greatest amount of total hopanoids might be expected in surface sediment from station MB-2 as this is a focal point for sediment accumulation Bay-wide (as discussed above); however hopanoid contributions are likely limited due to seasonal

anoxia and grazing my microheterotrophs (Shiah and Ducklow 1994). Slower sedimentation in the mid-Bay (as discussed above) could also result in greater contributions of degraded hopanoid products. In contrast, extremely high sedimentation rates on the order of 5.5 g cm^{-2} y^{-1} are likely near the mouth of the York River due to slumping or rapid infilling of sediment as this is a popular site of dredging in the lower Chesapeake Bay (Nichols et al. 2005). The enhanced signal of intact compounds (i.e. 78% of the total hopanoid content; Fig. 4.7) suggests recent inputs of bacterial OC that could represent heterotrophic recycling as labile, autochthonous material dominates near the Bay mouth. Discharge from Chesapeake Bay likely contributes to the greater amount of intact BHPs in surface sediment from station ATL-7 relative to ATL-6 as outflow is forced south along the Atlantic coastline due to Coriolis effects. Enhanced bacterial contributions to surface sediment from the mid-Atlantic slope (station ATL-9; Fig. 4.7) are likely fueled by nutrient-rich currents and warm core rings that support autochthonous production and thus cyanobacterial growth and heterotrophic respiration (Glover et al. 1985). Degradation and remineralization of sinking organic material increases during prolonged exposure to oxygen in deep waters (Meyers 1997); therefore greater contributions of degraded hopanoid products were found in slope sediment (station ATL-9, 12 μg g^{-1} OC) relative to samples from stations ATL-6 (4.3 μg g^{-1} OC) and ATL-7 (1.9 μg g^{-1} OC) on the shelf.

Summary

The abundance and distribution of intact BHPs in Arctic surface sediments generally parallel the decreasing abundance of chlorophyll biomass offshore, suggesting their origin among heterotrophic organisms and long-term preservation of phytoplankton

production and subsequent recycling. Contributions of adenosylhopanes to WHS and EB sediments agree with inputs of other terrestrial derived lipids and imply the transport of terrigenous bacterial carbon sources into the Arctic Ocean. Intense microbial degradation of peat material was evident in samples from the Ikpikpuk and Kokolik Rivers with differing sources of bacterial organic carbon contributing to riverine surface sediments downstream. In contrast, bacterial membrane components in Chesapeake Bay transition along the land-sea gradient and reflect the occurrence of many possible bacterial sources and processes. The abundance and distribution of intact BHPs in surface sediments suggest a dilution of riverine inputs and thus heterotrophic recycling along the land-sea gradient as contributions of soil derived bacterial carbon are likely represented among the significant presence of degraded hopanoid products. Specific contributions of methane-oxidizing bacteria and cyanobacteria in particles were directly traced to surface sediments and suggest the movement of organic material away from its region of production. Discharge from Chesapeake Bay likely has some affect on production and inputs to Atlantic coastal shelf sediments, where heterotrophs and cyanobacteria appear as important contributors of bacterial derived organic carbon.

Table 4.1. Station locations and bulk parameters for particle and surface sediment samples in the western Arctic Ocean and Chesapeake Bay

Station name	Station number	Sample location	Latitude (N)	Longitude (W)	Bottom depth (m)	Sediment depth (cm)	Sediment TOC (%)
EB-2	HLY0203-023	Beaufort Shelf	71°27'	152°33'	89	0-1	0.92
EB-4	HLY0203-021	Beaufort Slope	71°39'	152°25'	498	0-1	1.87
EB-7	HLY0203-018	Beaufort Basin	72°19'	151°59'	3264	0-1	1.59
WHS-2	HLY0201-006	Chukchi Shelf	72°51'	160°34'	58	0-1	0.93
WHS-5	HLY0201-009	Chukchi Slope	73°17'	160°07'	1198	0-1	0.96
WHS-6	HLY0201-010	Chukchi Slope	73°27'	159°50'	1855	0-1	0.95
WHS-7	HLY0201-011	Chukchi Basin	73°37'	159°33'	2443	0-1	1.04
WHS-12	HLY0201-052	Chukchi Basin	73°54'	157°51'	3748	0-1	0.93
IKP-P [a]	HLY0403	Ikpikpuk R. high bank	-----	-----	< 1	0-2	14.23
IKP-S [b]	HLY0403	Ikpikpuk R. mouth	-----	-----	< 1	0-2	0.38
KOK-P [a]	HLY0203	Kokolik R. water's edge	-----	-----	< 1	0-2	7.36
KOK-S [b]	HLY0203	Kokolik R. main stem	-----	-----	< 1	0-2	0.36
UB-1	CB07B-001	Upper Ches. Bay	39°21'	76°11'	11	0-2	2.25
BR-858	CB07B-858	Ches. Bay Bridge	38°58'	76°22'	24	0-2	3.29
MB-2	CB07A-002	Mid Bay channel	38°28'	76°23'	28	0-2	3.28
MB-3	CB07B-003	Mid Bay shoal	38°34'	76°29'	10	0-2	3.48
PAX-818	CB07A-818	Paxtuxent R. mouth	38°16'	76°16'	28	0-2	2.51
POT-804	CB07A-804	Potomac R. mouth	38°04'	76°13'	28	0-2	2.83
RAP-61	CB07A-061	Rapp. R. mouth	37°58'	76°13'	13	0-2	2.63
LB-724	CB07A-724	Lower Ches. Bay	37°23'	76°04'	19	0-2	0.98
YRK-4	CB07A-004	York R. mouth	37°16'	76°09'	12	0-2	0.58
ATL-6	CB07B-006	Atlantic shelf N of Bay	37°06'	75°43'	16	0-2	0.43
ATL-7	CB07B-007	Atlantic shelf S of Bay	36°42'	75°41'	15	0-2	0.06
ATL-9	CB07B-009	Atlantic slope	36°34'	74°48'	>600	0-2	3.72

[a] Arctic peat sample
[b] Arctic River surface sediment sample

Table 4.2. Sample depths, particulate carbon values and relative abundance (%) of intact BHPs among five bacterial groups in six samples of POM collected from Chesapeake Bay

Station name	Sample depth (m)	PC (mg C L^{-1})	Widespread [d] (%)	Fermenter-like [d] (%)	Methanotroph [d] (%)	Cyanobacteria [d] (%)	Unknown [d] (%)
BR-858[a]	12	0.07	45	-----	55	-----	-----
MB-2[a]	surface[c]	38.7	32	29	-----	6	33
LB-724[a]	10	0.218	37	15	-----	27	21
YRK-4[a]	surface[c]	13.7	46	-----	-----	17	38
ATL-6[ab]	4	0.0858	6	-----	-----	4	91
ATL-6[ab]	13	0.198	11	-----	-----	-----	89

[a] See Table 4.1 for station locations

[b] Replicate station

[c] Concentrated bacterial fraction

[d] Widespread: **1a**, **1b**, **1c**; Fermenter-like: **2a**, **2d**; Methanotrophs: **1g**; Cyanobacteria: **1f**, **3f**; Unknown: **1h**, **2h**
  (see Fig. 4.8 for structural details)

Table 4.3. Concentrations of known terrestrial and marine derived lipids ($\mu g\ g^{-1}$ OC) in surface sediments from the western Arctic Ocean compiled from Belicka and Harvey, *in press*.

Compounds	Stations							
	WHS-2	WHS-5	WHS-6	WHS-7	WHS-12	EB-2	EB-4	EB-7
Terrestrial derived lipids								
Long chain (C_{23}-C_{31}) *n*-alkanes	242	128	333	47	111	72	205	139
Long chain (C_{24}-C_{28}) fatty acids	466	191	141	31	162	101	229	133
Long chain (C_{24}-C_{28}) *n*-alcohols	403	157	77	12	154	117	302	189
Sterols (α-amyrin & C29Δ5) [a]	137	50	19	13	40	215	476	160
Total terrestrial lipids ($\mu g\ g^{-1}$ OC)	1248	526	571	103	468	505	1212	621
Marine derived lipids								
Polyunsaturated fatty acids [b]	979	15	25	1	37	3480	4216	294
Phytol	435	43	27	6	13	409	609	84
Sterols (C28Δ5,22, C28Δ5,24(28)) [c]	270	39	17	8	17	333	435	84
Total marine lipids ($\mu g\ g^{-1}$ OC)	1684	97	69	15	67	4222	5260	462
Marine: Terrestrial ($\mu g\ g^{-1}$ OC)	1.35	0.18	0.12	0.14	0.14	8.35	4.34	0.74

[a] C29Δ5 = 24-ethylcholest-5-en-3β-ol
[b] Polyunsaturated fatty acids concentrations consist of 16:2, 18:2, 18:2, 20:4, 20:5
[c] C28Δ5,22 = 24-methylcholesta-5,22-dien-3β-ol; C28Δ5,24(28) = 24-methylcholesta-5,24(28)-dien-3β-ol

(a) Western Arctic Ocean
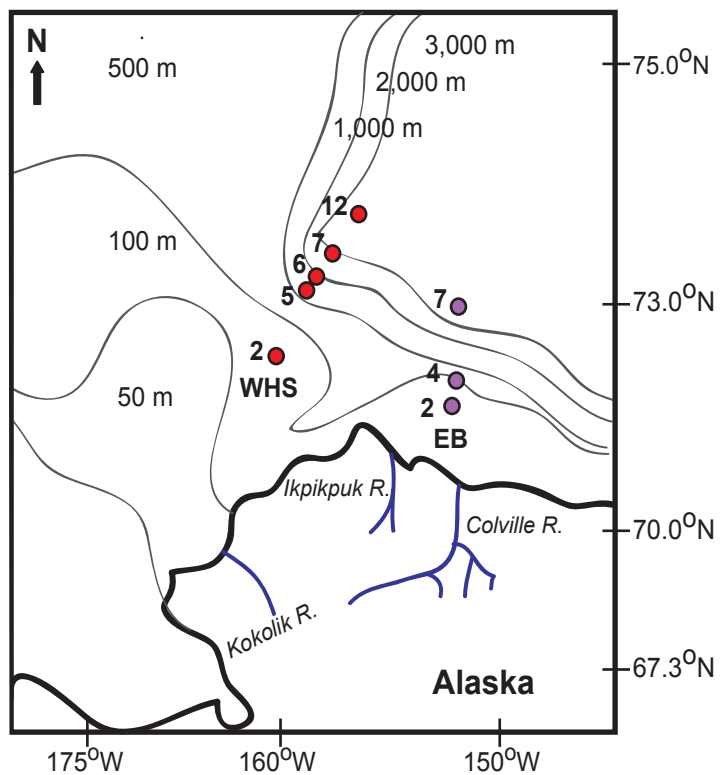
(b) Chesapeake Bay
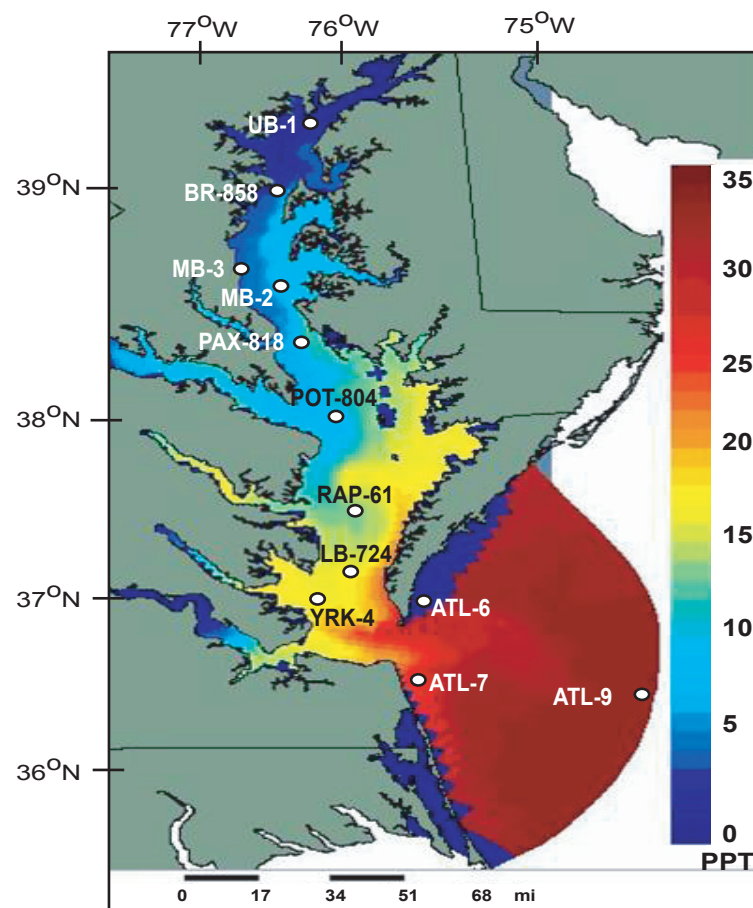
Figure 4.1. (a) Map of the western Arctic Ocean showing locations of surface sediment samples analyzed in this study. (b) Map of Chesapeake Bay showing locations of particulate and surface sediment samples analyzed in this study.
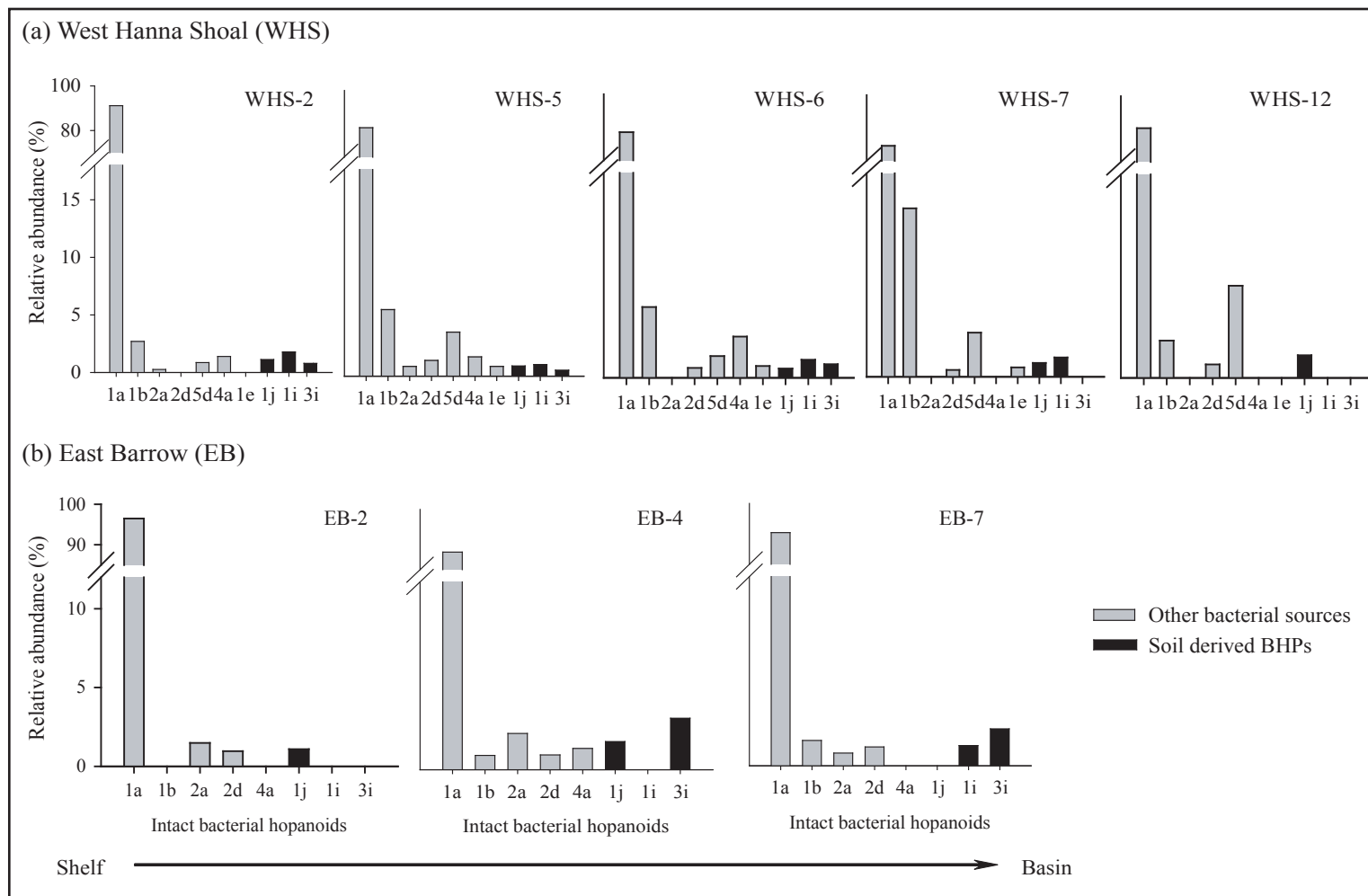
Figure 4.2. The relative abundance (%) of individual intact hopanoids investigated in surface sediments across the (a) West Hanna Shoal (WHS) and (b) East of Barrow Canyon (EB) transects in the western Arctic Ocean. Station numbers refer to the locations in Table 4.1 and Fig. 4.1.
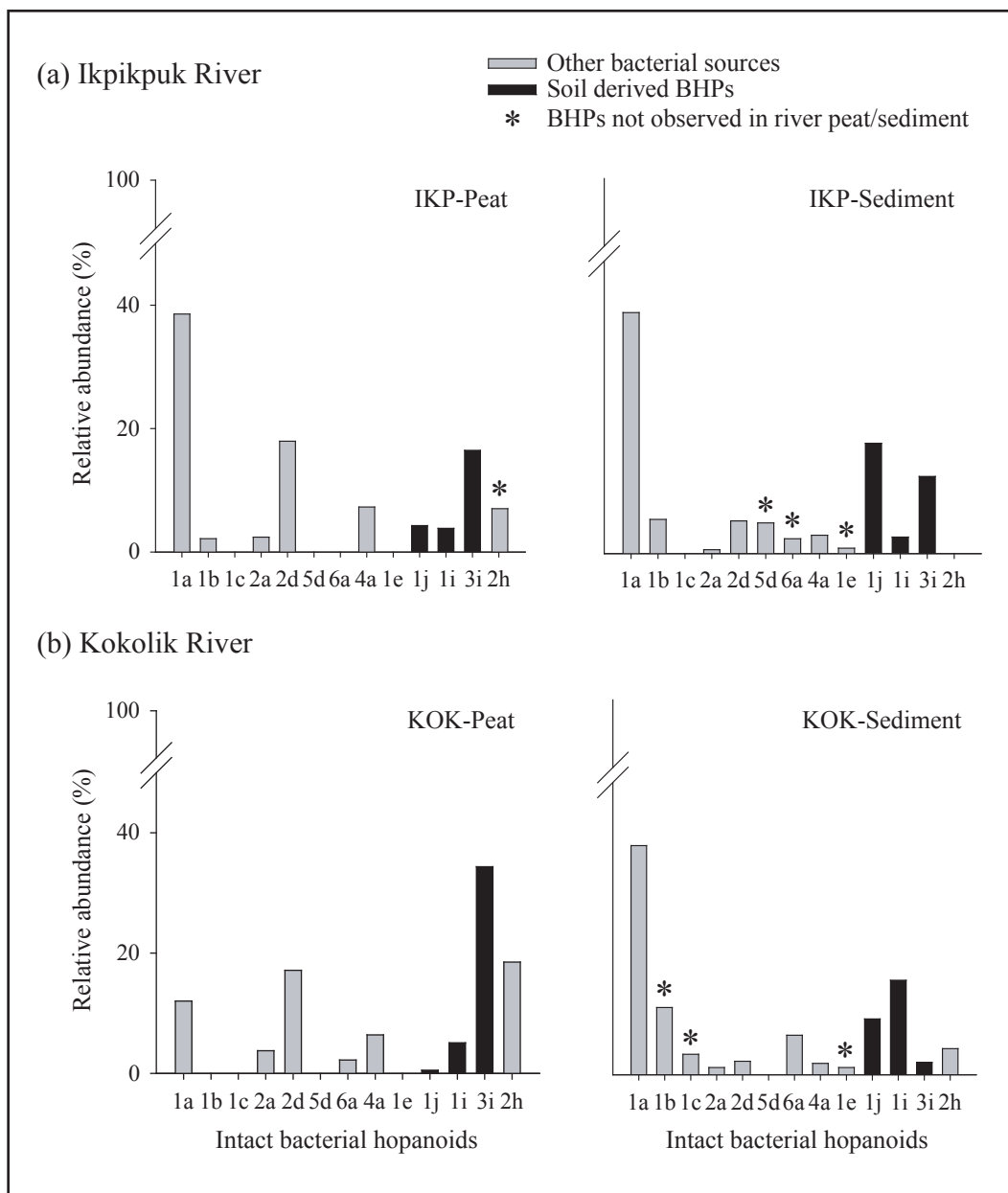
Figure 4.3. (a) The relative abundance (%) of individual intact hopanoids investigated in peat from the high bank of the Ikpikpuk River and in surface sediment from the river mouth. (b) The relative abundance (%) of individual intact hopanoids investigated in peat from the Kokolik River water's edge and in surface sediment from the river main stem. Refer to Fig. 4.1 for the locations of Arctic rivers.
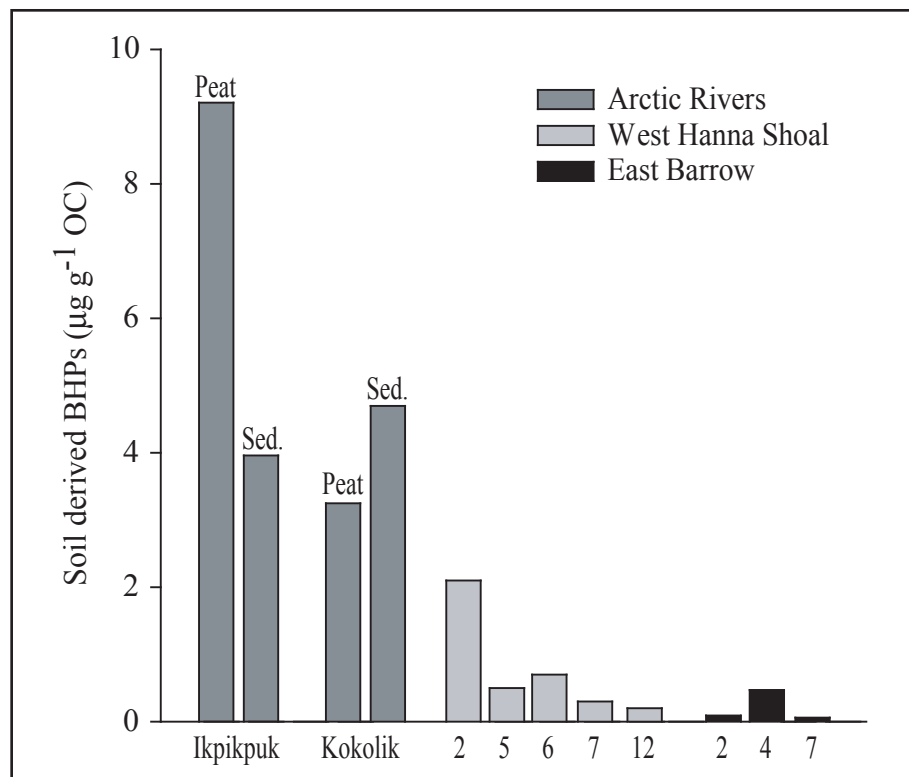
Figure 4.4. Analysis of soil derived (**1j**, **1i**, **3i**; see Figure 4.8 for structural details) BHPs in peat and surface sediment samples from the Ikpikpuk and Kokolik Rivers, and in eight surface sediments across the WHS and EB shelf to basin transects in the western Arctic Ocean. Station numbers refer to locations in Table 4.1 and Fig. 4.1.
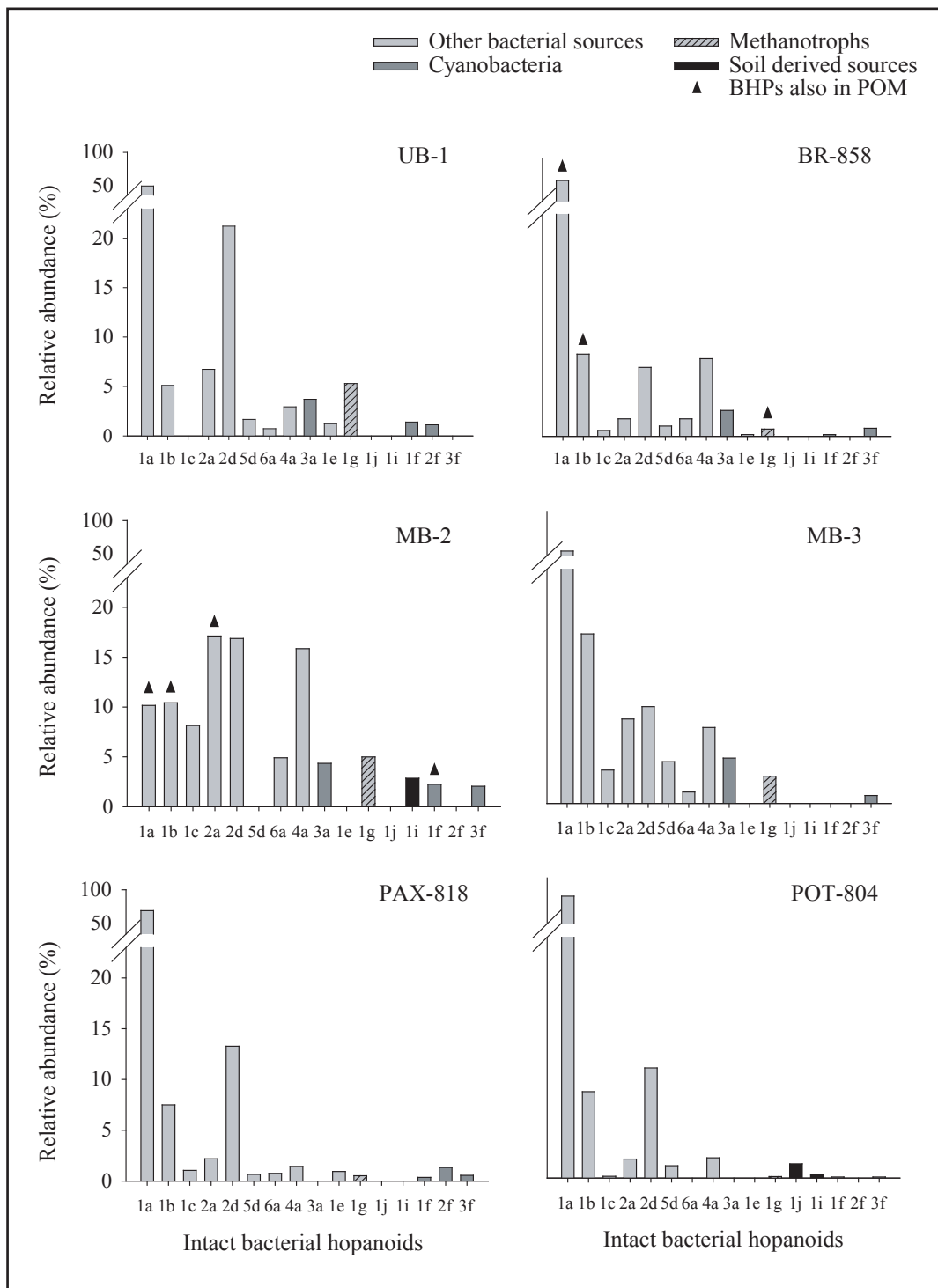
Figure 4.5. The relative abundance (%) of individual intact hopanoids investigated in surface sediments from nine locations along the salinity gradient of Chesapeake Bay and three locations in the coastal Atlantic Ocean with the northern Bay (stations UB-1 and BR-858) and mid-Bay (stations MB-2, MB-3, PAX-818, POT-804) shown here. Refer to Table 4.1 and Fig. 4.1 for station locations.
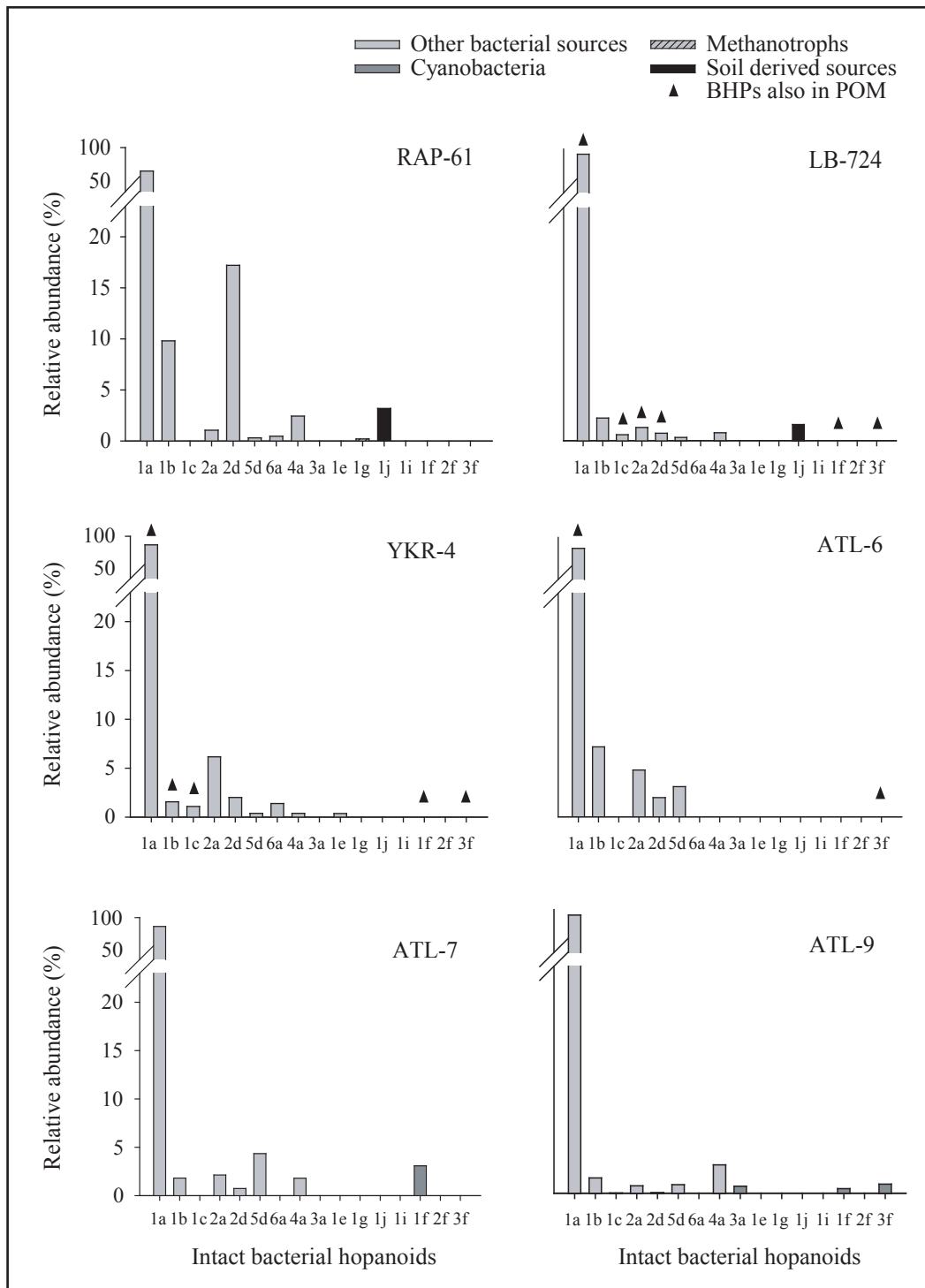
Figure 4.5 (continued). The relative abundance (%) of individual intact hopanoids investigated in surface sediments from nine locations along the salinity gradient of Chesapeake Bay and three locations in the coastal Atlantic Ocean with the southern Bay (stations RAP-61, LB-724 and YRK-4) and coastal ocean (stations ATL-6, 7, 9) shown here. Refer to Table 4.1 and Fig. 4.1 for station locations.
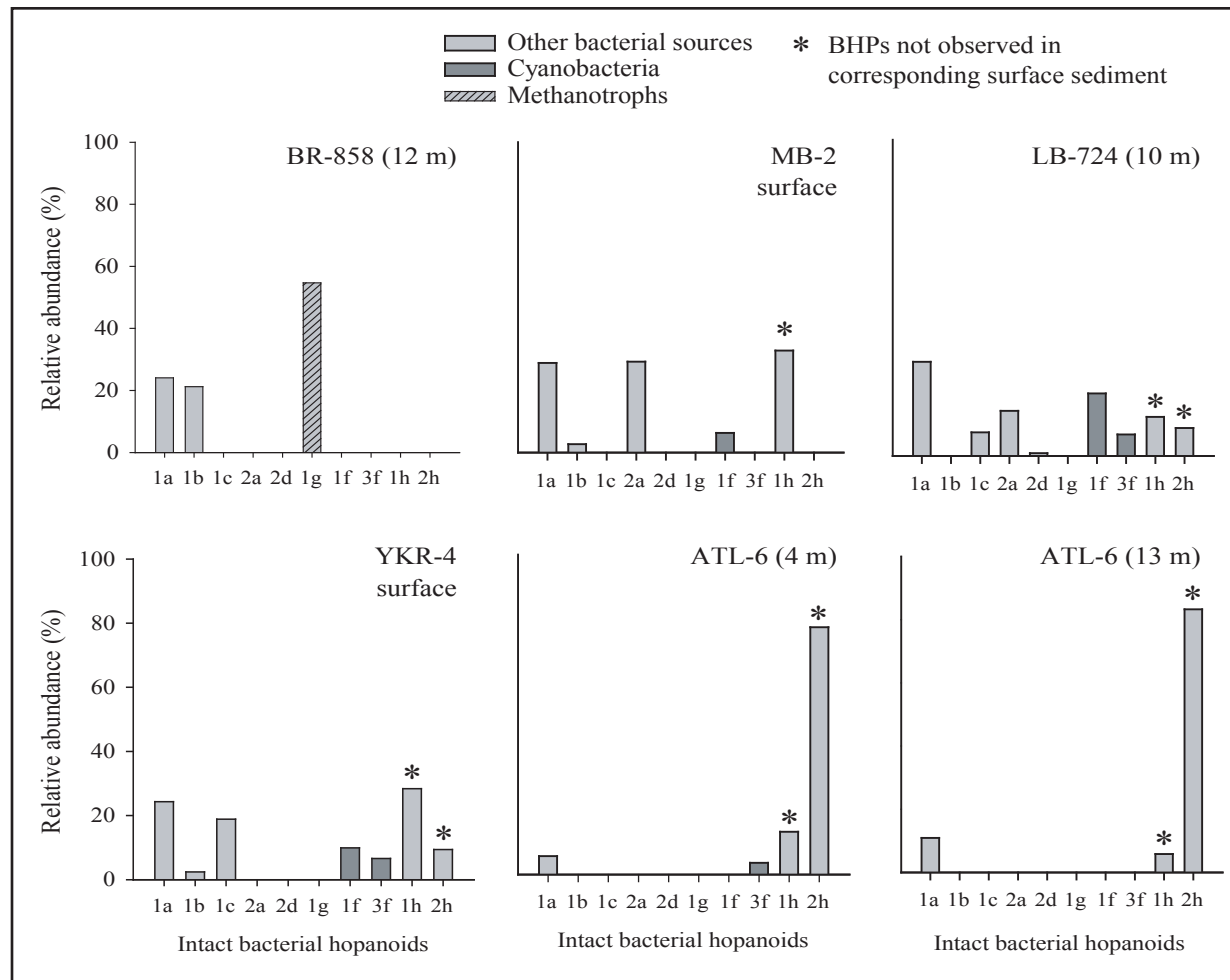
Figure 4.6. The relative abundance (%) of individual intact hopanoids investigated in particles from five locations in Chesapeake Bay. Station numbers refer to locations in Tables 4.1 & 4.2 and Fig. 4.1.
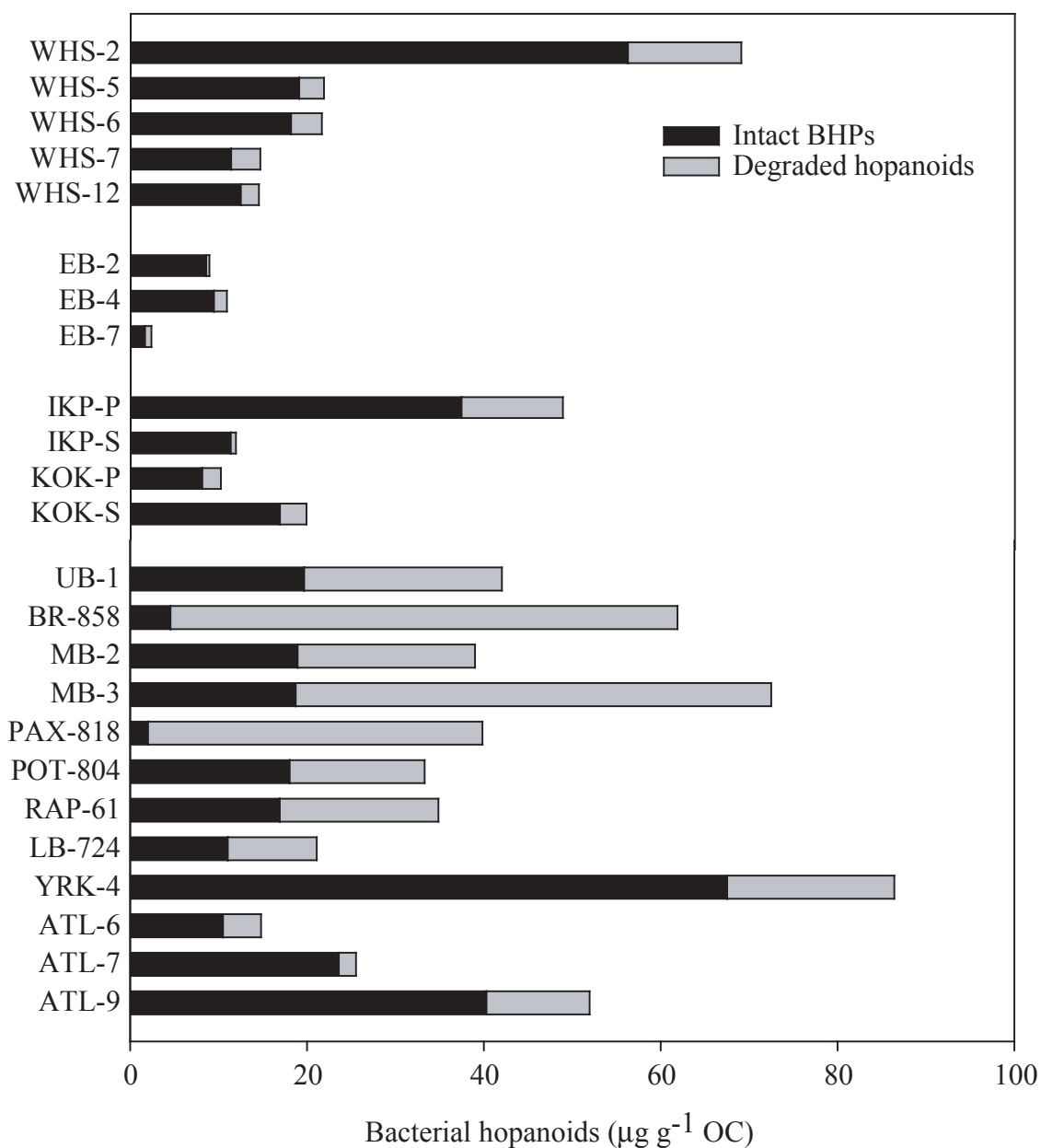
Figure 4.7. Analysis of total hopanoids in surface sediments across two shelf to basin transects and in samples of peat and surface sediment from two rivers in the western Arctic Ocean. Total hopanoids were also analyzed in nine surface sediments along the salinity gradient of Chesapeake Bay and in three surface sediments from the coastal Atlantic Ocean. Total concentrations are shown as the sum of the intact and degraded material. Intact BHPs include all individual compounds observed at each station.

95
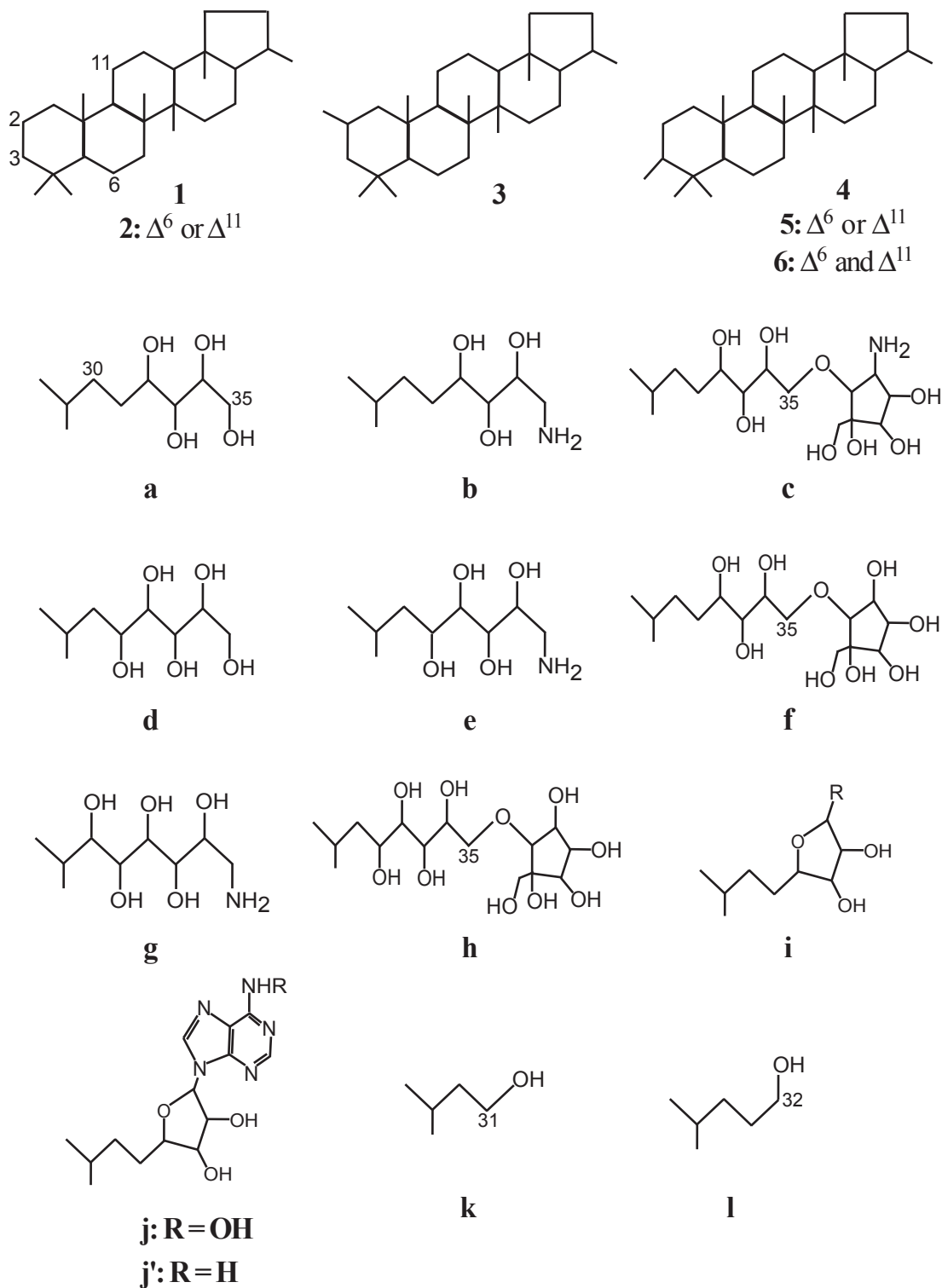
Figure 4.8. Bacteriohopanepolyol structures and their degrative products analyzed in samples of particles and surface sediments from the western Arctic, Chesapeake Bay and coastal Atlantic Ocean.

Chapter 5: Conclusions

Bacteria are ubiquitous and known to represent a significant pool of organic carbon in aquatic systems; however the lack of information on the contribution of bacterial carbon to the organic signal and the importance of its preservation in the geological record have prompted the work documented in this thesis which sought to address four main objectives that are discussed below.

My results in Chapters 3 and 4 discuss the broad suite of intact bacterial hopanoids in organic matter substrates from the Bering Sea, western Arctic and Chesapeake Bay that reflect the diversity and abundance of bacterial organic carbon contributions to environments that receive different inputs of organic matter. In Chapter 3 I found that cyanobacteria are ubiquitous in the Bering Sea and represent a small but important contribution to the total system chlorophyll (i.e. contributing $0.04 – 0.98$ μg Chl α L^{-1} or $0.25 – 12.3\%$ in particles and $0.08 – 4.2$ μg Chl α g^{-1} in sediments) and organic carbon reservoir of the Bering Sea, where diatoms dominate. While cyanobacteria are vastly out-numbered by larger phytoplankton in the Bering Sea system, observations of membrane components from living cyanobacterial cells in ice, particles and surface sediments represent a previously unappreciated and undocumented fraction of the organic carbon pool in this region. Cyanobacteria as well as other bacterial sources are reflected by the diverse suite of intact hopanoids observed in surface sediments and imply intense microbial recycling of organic matter on the productive Bering Sea shelf. In contrast, intact BHPs analyzed across two shelf to basin transects of surface sediment

from the Western Arctic Ocean showed no indication of cyanobacterial inputs; however, I found hopanoids specific to soil derived material (i.e. adenosylhopanes) in abundance. The presence of adenosylhopanes implies the movement of terrigenous organic carbon into the Chukchi and Alaskan Beaufort Seas and distributions corresponded to those of other terrestrial derived lipid signatures. Intense microbial recycling of Arctic peat and the occurrence of additional bacterial carbon sources downstream in riverine surface sediments were evident in samples from the Ikpikpuk and Kokolik Rivers. In Chapter 4, I showed a broad range of bacterial cell membrane components in both particles and surface sediments from Chesapeake Bay which reflect multiple bacterial sources and processes that transition along the land-sea gradient. The northern Bay hosts a broader diversity of intact BHPs and a greater abundance of total hopanoids (including both intact and degraded fractions), implying more intense bacterial recycling relative to the lower Bay and coastal ocean due to elevated inputs of organic matter delivered by the Susquehanna River. I found an abundance of intact membrane components from living bacterial cells in southern Chesapeake Bay that are indicative of cyanobacteria and potentially heterotrophic organisms which recycle the labile, autochthonous material that dominates at the Bay mouth. A combination of strong hydrological gradients, diverse habitats and inputs from many organic matter sources cause bacterial community structures to shift along the salinity gradient of Chesapeake Bay.

Analyses of the total intact and degraded hopanoid fractions in particles and surface sediments from the Bering Sea are discussed in Chapter 3 and show that deciphering between these two components provides clues to the diagenetic processes that convert intact BHPs to their simpler molecular fossils. Only intact membrane lipids

were found in particles from the Bering Sea with the majority (45 – 85%) appearing to be derived by cyanobacteria. While specific cyanobacterial signatures were also reported in the corresponding surface sediments, these samples showed contributions of both intact and degraded hopanoid fractions. Therefore my results imply that hydrolysis of intact membrane lipids beings in the water column as a reduction of total intact BHPs (20– 52 µg g^{-1} OC) and an increase of degraded products (29 – 101 µg g^{-1} OC) were observed in Bering Sea sediments. It also seems likely that a significant and perhaps large fraction of degraded hopanoid products in sediments are of cyanobacterial origin as these were the dominant sources of cell membrane components in the water column. My results in Chapter 4 describe the total intact and degraded hopanoid fractions in surface sediments from the Western Arctic and Chesapeake Bay and reveal that degraded products represent older bacterial carbon that had degraded on land prior to being transported these marine and estuarine systems. In the western Arctic, the pulse of both intact and degraded products in surface sediment from Chukchi Sea shelf reflect intense microbial recycling and the rapid deposition of organic material, including soil derived sources. Therefore given the shallow depth and fast accumulation rates of the Chukchi Sea shelf, the abundance of degraded products observed were likely delivered via melting sea-ice that entrained older bacterial carbon supplied by rivers and coastal erosion upon freezing near the Alaskan coastline. In Chesapeake Bay contributions of soil derived BHPs were limited; however degraded products were shown to dominate the total hopanoid content in the upper Bay, where sedimentation rates are high and outflow from the Susquehanna River supplies substantial amounts of organic material to this region. In addition, contributions of degraded hopanoids decrease seaward as they become diluted with

marine organic sources; further suggesting that soil derived bacterial carbon is represented among the abundance of degraded hopanoid products in Chesapeake Bay. For future work, determination of carbon isotopic composition in surface sediments along the salinity gradient of Chesapeake Bay would significantly improve the ability to distinguish organic carbon inputs and thus the substrates available for bacterial recycling.

My results in Chapter 3 document the direct input of bacterial organic carbon from particulate material to the underlying surface sediments in the Bering Sea. Despite extreme low temperatures, linkages among ice-associated cyanobacteria and their presence in open water during summer provide evidence that sea-ice melting initiates the inoculation of surface waters and that cyanobacteria grow and thrive within the water column. While their contributions to sea-ice (4.9 μg g^{-1} OC) and particles (4.7 μg g^{-1} OC) during spring are limited, signatures of cyanobacterial carbon are abundant in POM collected from below the chlorophyll maximum during summer (8.6 – 45.1 μg g^{-1} OC), and suggests that these small organisms preferentially reside deeper in the euphotic zone where they are not competing with diatoms for nutrients and sunlight. While single-cell sedimentation seems unlikely, the specificity of intact BHPs as a direct and specific component of bacterial membranes shows that cyanobacteria are delivered to the benthos. My results show substantial inputs of cyanobacterial carbon to surface sediments on the northern Bering Sea shelf where warming water temperatures observed during summer in these shallow zones may allow for increased cyanobacterial production and their subsequent input to sedimentary organic carbon pools. I speculate that cyanobacteria populations could propagate in a northward direction as marine ecosystems have already begun responding to a shift in the subarctic-Arctic temperature boundary (Grebmeier et

al. 2006). For future work, detailed analysis of both intact and degraded hopanoid fractions in surface and down-core sediments along a north-south transect of the Bering Sea shelf may provide clues as to how bacterial communities and specifically cyanobacteria might response to warming ocean temperatures.

The long-term preservation of primary production and subsequent organic matter recycling is discussed in Chapter 4 as elevated bacterial carbon contributions to Arctic sediments correspond to increased amounts of chlorophyll in the water column. While bacterial cells are subject to degradation and consumption by bactivores in open water, tight pelagic-benthic coupling and effective transport mechanisms deliver a portion of suspended bacterial organic carbon to the benthos. The ability to distinguish and quantify the bacterial fraction of organic carbon reaching Arctic surface sediments is novel and future work detailing both the intact and degraded hopanoid components in down-core sediment samples would provide further information regarding the preservation of bacterial organic carbon in polar environments. My results in Chapter 4 document the transport of bacterial carbon signatures along land-sea gradients of the Western Arctic Ocean and Chesapeake Bay. In surface sediments across the West Hanna Shoal and East Barrow shelf to basin transects, dilution of bacterial carbon contributions offshore reflect the lateral advection of organic material originating in shallower waters. Offshore transport is facilitated by the formation of mesoscale eddies along the continental slope and the focusing of nutrient-rich water and organic material through Barrow Canyon to deeper waters (Jonas and Anderson 1990; Walsh et al. 1990, 2005). Episodic sediment gravity flows are also responsible for the movement of soil derived and degraded bacterial carbon offshore, particularly to the East Barrow slope which appears to be a

favorable site of sediment focusing in the Beaufort Sea (MacDonald et al. 2004). Ice rafting also delivers soil derived bacterial inputs to the Chukchi and Alaskan Beaufort Seas with a fraction of material representing older bacterial carbon that had degraded on land prior to being transported into the Arctic Ocean. Flow regimes are quite different in Chesapeake Bay where specific signatures indicative of methane oxidizing bacteria and cyanobacteria are shown in particles and directly traced to surface sediments; however, their distribution suggests the transport of material away from its region of production, either down-estuary in fresher surface waters or up-estuary in high salinity bottom waters. As mentioned above, my results show increasing concentrations of intact BHPs and decreasing abundance of degraded products in sediments along the salinity gradient of Chesapeake Bay which implies the progressive dilution of soil derived bacteria along the land-sea gradient. Outflow of nutrient-rich water and organic material from Chesapeake Bay likely influences bacterial production and inputs to Atlantic coastal shelf sediments and vice versa as my results show a similar distribution of intact BHPs in particles and surface sediment among the lower Bay and coastal ocean.

The results of this thesis conclude that bacteria represent a small but important contribution to earth's organic matter reservoirs than previously recognized. The utility of bacterial cell membrane components as both source and process tracers now allow for the characterization and quantification of both living and degraded bacterial fractions of organic matrices. The ability to distinguish and directly measure the contributions of bacterial carbon to particulate and sedimentary organic matter pools is novel, and thus demonstrates the importance of bacterial carbon preservation over geologic time scales

and provides useful insight into how bacterial communities may respond to future climate

conditions and warming ocean temperatures.

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