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Hopanoids and lipid biomarkers as indicators of microbial communities in modern microbialites from Fayetteville Green Lake, NY and Great Salt Lake, UT

Sierra Jech
sjech11@gmail.com

HOPANOID AND LIPID BIOMARKERS AS INDICATORS OF MICROBIAL

COMMUNITIES IN MODERN MICROBIALITES FROM FAYETTEVILLE

GREEN LAKE, NY AND GREAT SALT LAKE, UT

by

Sierra Dawn Jech

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Department of Chemistry

Approved by:
Mark Teece, Major Professor
Sharon Moran, Chair, Examining Committee
Ivan Gitsov, Department Chair
S. Scott Shannon, Dean, The Graduate School

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TABLE OF CONTENTS

List of Tables

List of Figures

Abstract

Jech, S. D. Hopanoid and Lipid Biomarkers as Indicators of Microbial Communities in Modern Microbialites from Fayetteville Green Lake, NY and Great Salt Lake, UT, USA. 154 pages, 12 tables, 24 figures, 2018. Organic Geochemistry style guide used.

Microbialites are composed of a complex community of microbes whose net metabolic activity results in the deposition of carbonate rock. Modern microbialite structures actively grow in a variety of environments and are similar to the oldest preserved form of life on Earth. This research used lipid biomarkers to study the microbial composition of microbialites from a freshwater meromictic lake (Fayetteville Green Lake, NY) and a hypersaline shallow lake (Great Salt Lake, UT). Lipid biomarkers are useful for tracking similarities and differences in the autotrophic and heterotrophic community with variable growth conditions. This work focused on the hopanoid biomarkers, bacterial cellular membrane components preserved in ancient microbial carbonates. Hopanoid biomarkers including diploptene, hop-21-ene, diplopterol, tetrahymanol, bacteriohopanetetrol, and 2-methyl forms were present in microbialite samples, indicating similarities in microbial communities. The microbialites in New York and Utah are valuable for their broad applicability to studying past microbial life.

Key Words:
Fayetteville Green Lake, Great Salt Lake, microbialite, hopanoid, lipid biomarker, microbial ecology

S. D. Jech
Candidate for the degree of Master of Science, May 2018
Mark Teece, Ph.D.
Department of Chemistry
State University of New York College of Environmental Science and Forestry,
Syracuse, New York

Chapter 1: Introduction

1.1 Microbialites- microbial carbonates

Microbialites are composed of a consortium of *Bacteria*, *Archaea*, and *Eukarya* whose net metabolic activity results in the deposition of carbonate rock (Cerqueda-Garcia and Falcon, 2016; Mobberley et al., 2015; Visscher and Stolz, 2005). Modern microbialites are quite rare, currently identified in a handful of lakes, coastal marine and marsh environments around the world (28 thrombolitic microbialites worldwide) (Mobberley et al., 2015; Myshall, 2012; Patterson, 2014; White III et al., 2015). These modern microbialites are the descendants of ancient microbial structures, existing through 80% of Earth's history and the oldest preserved form of life on Earth (3.7 billion years old) (Grotzinger and Knoll, 1999; Nutman et al., 2016).

Because microbialites accrete in particular patterns or layers that can be identified after fossilization processes, scientists can identify these structures as having a presumed biological origin (Allwood et al., 2006; Grotzinger and Knoll, 1999). This would otherwise be impossible because early life as individual cells (prokaryotes) degrade rapidly and are not likely to be preserved through time in the rock record (Newman et al., 2016). Scientists therefore rely on evidence from actively growing microbial carbonates (stromatolites, microbialites, thrombolites, and others) to learn about ancient structures including: carbonate accretion processes, microbial community composition, and degradation or preservation of biological molecules (Burns et al., 2011; Brocks et al., 2005; Cerqueda-Garcia and Falcon, 2016; Edgcomb et al., 2014; Mobberley et al., 2015; Nitti et al., 2012; Pace et al., 2016; Papineau et al., 2005; Patterson, 2014; Talbot et al., 2008; Thompson et al., 1990; Wilhelm and Hewson, 2012). Understanding the differences in these characteristics in a variety of environments is anticipated to benefit the study of the evolution of life on early Earth (Talbot et al., 2008).

1.2 Study sites

The number of reported microbialites in North America is quite limited but they cover a wide range of carbonate morphologies in freshwater lakes (Pavilion Lake, BC and Kelly Lake, BC; Fayetteville Green Lake, NY), inland saline lakes (Great Salt Lake, UT), and one open pit mine (Clinton Creek, Yukon). At Clinton Creek, the microbialites grow in an old asbestos mine (White III et al., 2015). In British Colombia there are massive bottom-dwelling mound microbialites with extensive research on microbial genetics, isotopes, and community composition (Brady et al., 2011; Russell et al., 2014). For their visibility, extent of growth, and accessibility, the microbialites at the Great Salt Lake (GSL) and Fayetteville Green Lake (FGL) microbialites are not well studied, with researchers focused on other biological components of the lake system. FGL microbialites form as giant shelves along the lake shore (Thompson et al., 1990), while GSL structures carpet the shallow benthos in black-green bottom mounds (Pace et al., 2016).

Environmental growth conditions of the North American microbialites are quite variable; it is surprising that functionally similar microbial consortia assemble in them to create carbonate structures. Table 1.1 compares water quality, geology and hydrology, biology, and human use at FGL and GSL, which were selected as the study sites for this research. Both lakes maintain close to neutral pH (pH 6-9) and typical yearly water temperatures range from approximately -5 to 35°C (Brunskill and Ludlam, 1969; Chagas et al., 2016; Post, 1977; Takahashi et al., 1968). Otherwise, these are very different environments. Fayetteville Green Lake is a well-protected 54-m deep meromictic (lacks seasonal mixing) lake that was formed by a glacial waterfall (Chagas et al., 2016; Brunskill and Ludlam, 1969). The lake is rich in dissolved calcium and is oligotrophic (nutrient poor) (Chagas et al., 2016). Cyanobacteria, predominantly *Synechococcus*,

bloom annually in the water column causing whiting events that turn the water milky blue-green (Thompson et al., 1990). Human-use is limited to swimming and boating at the north end of the lake at a well-developed park. Visitors also enjoy fishing and hiking around the lake as well as neighboring Round Lake that is also meromictic.

FGL can be contrasted with hypersaline Great Salt Lake, the remnant of an inland sea. GSL is shallow (average depth of 10 m) and continuous evaporation has caused this lake to be highly saline (Arnow and Stephens, 1990; Post, 1977). It is also hypereutrophic, supporting large populations of brine flies and brine shrimp (Roberts and Conover, 2014; Wurtsbaugh and Gliwicz, 2001). Salt and brine shrimp are harvested for industry (Wurtsbaugh and Gliwicz, 2001). Human use is limited to boating and swimming. The lake does not support a fishery.

Some research has been done to link modern Great Salt Lake microbialites to oil and gas deposits that are highly productive in order to locate more promising petroleum reservoirs in the future (Chidsey et al., 2015). The Great Salt Lake microbialites were studied in terms of the shrimp industry as microbialites happen to be crucial to the brine shrimp life-cycle (Wurtsbaugh and Gliwicz, 2001). Migratory bird research has also incorporated some microbialite work, as they provide crucial habitat and are the base for the food chain (microorganisms-brine flies-birds) (Roberts and Conover, 2014). The carbonate accretion processes of Fayetteville Green Lakes microbialites was studied in 1990 and again in 2014 (Patterson, 2014; Thompson et al., 1990). Thus, there is much room for expanding microbialite knowledge in both of these systems.

The following text summarizes current knowledge of carbonate accretion and the composition of the microbial community of microbialites from both Fayetteville Green Lake and Great Salt Lake. Next, lipidomics, with a focus on hopanoid biomarkers, is employed as a

method for identifying microbial community composition using gas chromatography-mass

spectrometry and standardized protocols from lab-cultured hopanoid-producing microbes.

Table 1.1 Comparison of Fayetteville Green Lake, NY and Great Salt Lake, UT.

	Fayetteville Green Lake, NY	Great Salt Lake, UT	References
Water Quality			
Major Ions	Ca- 24 meq/L, Mg- 5.9 meq/L, Si- 0.05 meq/L	Ca- 8.6 meq/L, Mg- 146 meq/L, Si- <0.2 meq/L	Chagas et al. 2016, Havig et al. 2015, Post 1977
pH	7.5	8.6	Chagas et al. 2016, Havig et al. 2015, Takahashi et al. 1968
Alkalinity	3.2 meq/L	7 meq/L	Chagas et al. 2016
Salinity	2.2 g/L	50.4 g/L or 120 g/mL (southern arm)	Chagas et al. 2016, Post 1977
Water Temperatures	-7 to 21°C	-5 to 35°C	Havig et al, 2015, Post 1977, Brunskill & Ludlam 1969
Nutrient Status	Oligotrophic	Hypereutrophic	Chagas et al. 2016, Belovsky et al. 2011
Geology and Hydrology			
Watershed size	4.33 km^2	97,000 km^2	Brunskill & Ludlam 1969, Post 1977
Lake Area	0.3 km^2	2400 km^2 or 3,900 km^2	Chagas et al. 2016, Post 1977, Brunskill & Ludlam 1969
Hydrochemical types (secondary cations-anions)	Ca-SO$_4$ (Ca, Mg-SO$_4$, HCO$_3$)	Na-Cl (Na, Mg-Cl, SO$_4$)[1]	Chagas et al. 2016, Post 1977
Max Water Depth	54 m	10 m (Average ~10 m)	Chagas et al. 2016, Havig et al 2015, Post 1977, Brunskill & Ludlam 1969
Mixing	Meromictic	Meromictic	Chagas et al. 2016
Hydrological system	Open	Closed	Chagas et al. 2016
Biology and Human Use			
Macro-organisms	zebra mussels, Calanoid copepods, freshwater sponges, shorebirds, bass, sunfish, *Chara*	brine shrimp, brine flies, major stopover site for migrating waterfowl	Arnow & Stephens 1990, Post 1977, Thompson et al. 1990
Human use and impacts	non-motorized watercraft, visitors stand on Dead Man's Point[2], fishing, well developed trail system around entire lake, swimming at north shore	boating, brine shrimp harvesting, visitor recreation (swimming) near shore, salt harvesting, railroad causeway, agriculture	observations at both State Parks

[1] Post (1977) describes a calcium-carbonate type headwater with two large feeder streams highly mineralized with sodium sulfate or sodium chloride types. [2] a large nearshore shelf microbialite

1.3 Aquatic chemistry and carbonate accretion processes

Given the variety of environments that are suitable for carbonate-producing microbial communities, research often starts with the accretion, or carbonate deposition, process. Is the precipitation of carbonate abiotic or biotic? To what extent does microbial metabolism cause carbonate precipitation and is that process temporally or spatially restricted? Microbial metabolism does not always favor precipitation, so how do the microbes interact in a way that results in net precipitation (not dissolution) and the formation of a carbonate structure? These seemingly basic questions are quite complicated to unravel due to interacting processes.

Carbonate accretion starts with CO_2 in the air dissolving into water following Henry's Law constant which changes with temperature (Eq. 1). This aqueous CO_2 is immediately hydrated to carbonic acid (H_2CO_3) (Dupraz et al., 2009) (Eq. 2). The carbonate equilibrium results in mostly bicarbonate given the neutral pH levels of natural systems (Eq. 3). Temperature, concentration and pH are key factors that shift the carbonate equilibrium, resulting in either precipitation of calcium carbonate (Eq. 4) or dissolution of calcium carbonate toward aqueous ions (Eq. 4).

(1)
$$CO_2(g) \leftrightarrow CO_2(aq)$$

(2)
$$CO_2(aq) + H_2O \leftrightarrow H_2CO_3(aq)$$

Carbonate Equilibrium

(3)
$$H_2CO_3(aq) \leftrightarrow H^+ + HCO_3^-(aq) \leftrightarrow H^+ + CO_3^{2-}(aq)$$
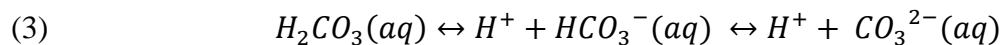
Precipitation and Dissolution of Calcium Carbonate

(4)
$$CO_3^{2-}(aq) + Ca^{2+}(aq) \leftrightarrow \boldsymbol{CaCO_3}(s)$$
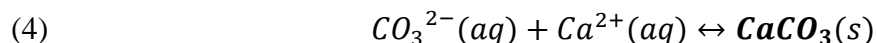
$$dissolution \leftarrow \boldsymbol{CaCO_3}(s) \rightarrow precipitation$$

Microbial metabolism is expected to impact pH, thereby influencing carbonate precipitation and dissolution (Dupraz et al., 2009; Visscher and Stolz, 2005). For instance, the metabolic activities of photoautotrophic microbes may increase the surrounding water pH to about 10 (Visscher and van Gemerden, 1991). This shifts the carbonate equilibrium toward the carbonate ion, thus causing calcium carbonate to precipitate. Meanwhile, aerobic respiration has been shown to decrease pH (Dupraz et al., 2009; Visscher and Stolz, 2005). This activity is expected to cause carbonate dissolution by pulling the carbonate equilibrium toward carbonic acid (H_2CO_3). In natural systems, photosynthesis and respiration are expected to occur together as well as a broad range of other metabolic processes that could contribute to either precipitation or dissolution (Visscher and Stolz, 2005). For microbial systems that rely on a calcium carbonate substrate like the ones in this study from Fayetteville Green Lake and Great Salt Lake, it is important that the balance between the two processes does not result in net dissolution of the substrate.

In addition to the microbial processes, there are abiotic factors that make this more complex. Temperature changes on diurnal and seasonal cycles will influence the carbonate equilibrium as well as ion concentrations. It is no coincidence that microbial carbonate structures form in hard-water lakes where carbonate ions are abundant (constantly added to the system from groundwater, bedrock dissolution, or other sources). Accretion processes for the carbonate structures in FGL (Patterson, 2014) and GSL (Pace et al., 2016) have been studied within the past five years. In both cases, the abiotic conditions as well as the microbial communities play a role in carbonate precipitation.

1.3.1 Current Fayetteville Green Lake carbonate accretion model

Patterson (2014) studied carbonate accretion of FGL microbialites using petrographic thin sections and scanning electron microscopy (SEM) to observe precipitation and dissolution dynamics in microbialite samples from Dead Man's Point (a large shelf microbialite). Patterson (2014) showed seasonal variability in the thickness and structure of the actively forming mat community at the surface of the microbialite, indicating an annual cycle of colonization (Figure 1.1). In early spring, the hard, clotted fabric is colonized by a green-brown microbial layer. An early season whiting event causes small calcium carbonate particles to collect on the surface and in the voids of the microbialite. Filamentous bacteria thrive at the surface and produce exo-polymeric substances (EPS), trapping and binding even more carbonate particles from the water column. Additional carbonate precipitation occurs via microbial metabolism; the microbes can completely encase themselves in carbonate.

As light becomes less available in fall, the filamentous cyanobacteria horizontally orient to optimize light-capturing capacity, creating a micritic (limestone) crust. Winter ice and freeze-thaw cycles destroy this upper loosely associated carbonate layer. In this model, filamentous cyanobacteria are implicated as the key organisms in the accretion process, modifying earlier evidence from Thompson et al. (1990) that implicated a coccoid cyanobacterium, *Synechococcus*, with microbialite accretion.
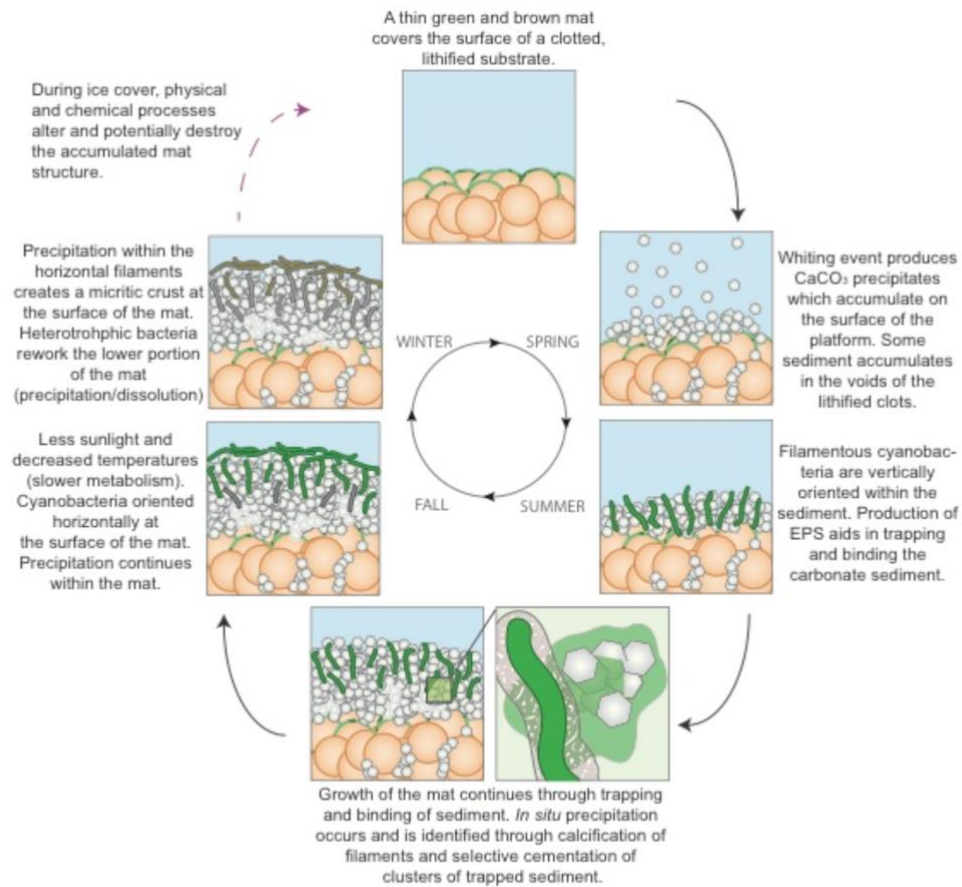
Figure 75. An ecological model of thrombolite formation in Green Lake. During the spring, whiting events provide to help (along with precipitation) build the mat throughout the growing season (spring to fall). Changes in temperature and light supply impact the lithification potential in the mat, influencing the preservation of the fabric.

Figure 1.1 Seasonal succession of microbial growth and carbonate accretion for Green Lakes State Park microbialites (From Patterson, 2014).

1.3.2 Great Salt Lake carbonate accretion model

      Pace et al. (2016) completed similar work for the microbialite structures in the Great Salt Lake. Their conceptual model was built on evidence from microelectrodes and microscopy. First, clusters of coccoid cyanobacteria form a mat on the lake floor. Inside the clusters, magnesium-silica crystals form due to photosynthetic activity that increases the pH to favor magnesium precipitation. Magnesium and silica are available from dissolved ions in the water. Diatoms with silica exoskeletons provide an additional source of silica near the surface of the mat. Next, heterotrophs, like sulfate reducing bacteria (SRB), "rework" the carbonate substrate. Their metabolic activities result in dissolution of the initial crystals and the subsequent formation of aragonite and dolomite at deeper layers (Figure 1.2). Structures form as stable layered carbonate upon oolitic sand grains in the lake benthos.
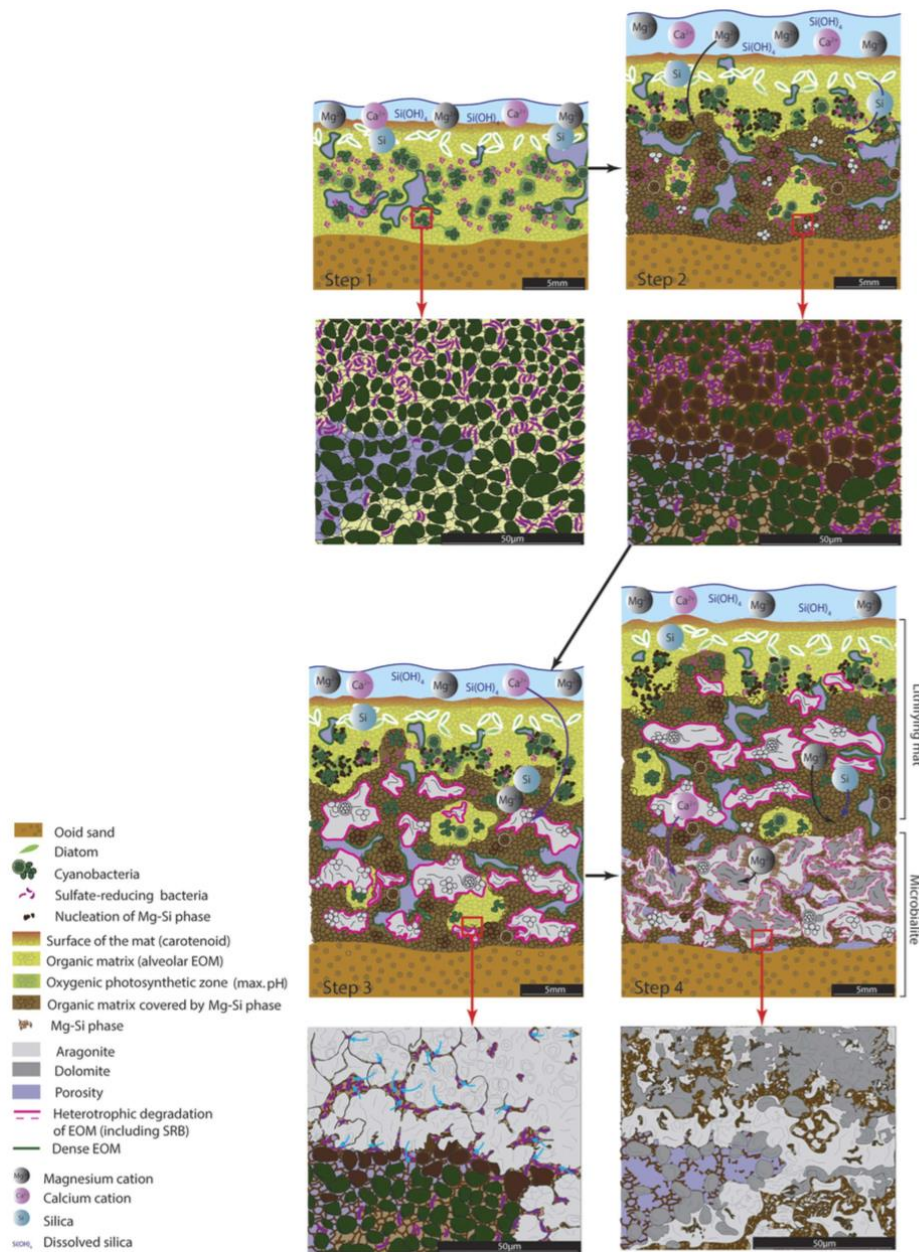
Figure 5. Conceptual model of microbialite mineralisation in GSL (see text for detail). Step 1: Development of a microbial mat on a hard substrate. Step 2: Mg-Si phase nucleation on the EOM in the zone of active oxygenic photosynthesis. Two sources of silica can be identified: lake water-dissolved silica and silica originating from the dissolution of diatom skeletons in the zone of maximum photosynthesis (pH > 10). Step 3: Degradation of EOM by heterotrophs, notably SRB, allowing the nucleation of aragonite. Aragonite precipitates first inside dead coccoid cells and then extends into the EOM (blue arrows). Degradation of EOM creates a zone of low pH in the deepest parts of the mat, inducing a partial dissolution of aragonite. Step 4: Pockets of partially degraded EOM bind Mg^{2+} (from porewater and possibly from Mg-Si phase) and Ca^{2+} (from aragonite dissolution), leading to the nucleation of dolomite at the interface between degraded EOM and previously precipitated aragonite/Mg-Si phase patches.

Figure 1.2 Microbialite accretion processes with depth in the Great Salt Lake microbialites (From Pace et al., 2016).

11

1.4 Microbial communities

Both carbonate accretion models focus on cyanobacteria as the primary carbonate sequestering organism. In Fayetteville Green Lake, the key cyanobacteria are filamentous, while in Great Salt Lake, they are coccoid (Patterson 2014; Pace et al., 2016). The defining feature in both cases is the production of an extracellular ooze (exo-polymeric substance) that serves many roles: trapping and binding cations, providing a sticky substrate for other microorganisms, feeding higher trophic level organisms, or acting as a photo-protective layer (Mobberley et al., 2015).

It is clear that cyanobacteria play a major role in the initiation of microbialite structure development, but there is more to the accretion story. Figure 1.3 provides an example of the genetic diversity of microorganisms in microbialites at Great Salt Lake (Lindsay et al., 2016). Photosynthetic eukaryotes (diatoms) and cyanobacteria are important components of the healthy Great Salt Lake microbialite (16% of rRNA gene sequences). But Lindsay et al. (2016) found almost 60% of all rRNA genes were from non-photosynthetic organisms including aerobic and anaerobic heterotrophic microorganisms. Twenty-two percent of the sequences were designated as "other", or microbes for which we currently do not have enough information to fully identify. This microbial diversity and complexity of the GSL microbialites suggests that non-cyanobacterial organisms also play an important role in the microbial ecology of microbialites (Mobberley et al., 2015).
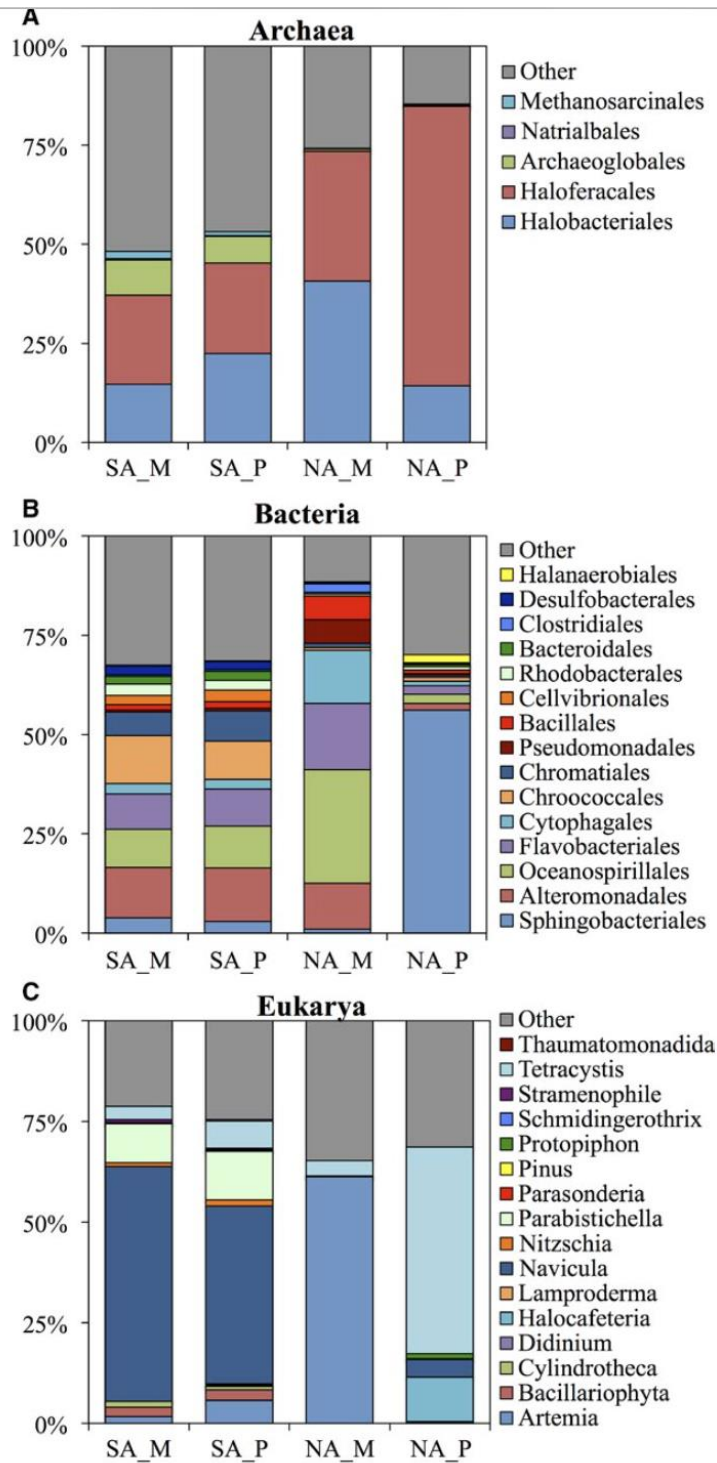
FIGURE 5 Composition of small subunit rRNA genes recovered from microbialite (M) and planktonic (P) communities in the South Arm

Figure 1.3 Archaean, Bacterial, and Eukaryotic genetic composition of microbialites in the North arm (NA) and South arm (SA) of Great Salt Lake (From Lindsay et al., 2016).

The arrangement of microbial metabolisms in microbialites is not expected to differ greatly from similar sessile microbial communities. In fact, researchers have begun referring to the microbial community consortia as the "global microbialite microbiome" (Foster and Green, 2011; Paerl et al., 2000; White III et al., 2015). There is a regular spatial distribution of microbial metabolism that results from resource availability or limitation with depth (Visscher and Stolz, 2005). In general, light and carbon dioxide are available at the surface, so photosynthetic organisms dominate those spaces; organisms compete with one another for resources and space. They have a wide variety of adaptations that allow them to succeed over others within a given environment including diverse body plans, colony morphologies, motility, nitrogen fixation, and production of toxins. Other adaptations allow for great diversity within a single niche; two different organisms can use light of differing wavelengths (photoautotrophs) (Ionescu et al., 2015) or by alternating activity throughout the day or throughout seasons (Paerl and Pickney, 1996).

Gradients of light, oxygen, carbon dioxide, sulfide, and other resources allow a variety of metabolisms to become competitively advantageous over a very small spatial scale (Ionescu et al., 2015; Paerl et al., 2000). The spatial distribution of these metabolisms depends on the reduction-oxidation (redox) ladder (Figure 1.4) that shows how certain electron acceptors result in greater energy production than others. The redox ladder dictates which organisms occur at what depth in the mat (Riding, 2000). Of course, there can be many other metabolic routes within this scheme, as well as microbial interactions that enforce or disrupt the expected distribution (Visscher and Stolz, 2005). An alternative model of microbialite dynamics involves most non-photosynthetic metabolisms in close proximity to the photosynthetic (cyanobacteria) metabolisms, with both methanogenesis and sulfate reduction sometimes occurring at the surface

of the mat instead of the predicted anoxic zones (Paerl and Pickney, 1996). These unexpected configurations create a heterogeneous environment with resource pockets instead of a simple layered arrangement (Visscher and Stolz, 2005).

On the other hand, dissolved solutes may have a huge impact on the microbial metabolisms present or possible within a system. For instance, the Great Salt Lake's brine solution does not allow certain microbes to grow even if nutrients (nitrate or iron) are available. Only those microbes that are both halophilic and have the metabolic capabilities can thrive. Four main "functional metabolic units" within microbialites have been described: photosynthesis (including nitrogen-fixing cyanobacteria), aerobic respiration, sulfide oxidation, and sulfate reduction (Dupraz et al., 2009; Ionescu et al., 2015; Visscher and Stolz, 2005). Specific microbial taxa and their associated metabolic processes in microbial mats are addressed elsewhere and is not expected to differ greatly for microbialite systems (Buhring et al., 2009; Visscher and Stolz, 2005).

Figure 1.4. A reduction-oxidation scheme showing energy yield for five different electron acceptors for respiration. Microbes that can utilize a high-energy electron acceptor will outcompete other microbes at a given location in the substrate.

1.5 *Lipidomics*

Lipidomics is the study of cellular lipid composition (Killops and Killops, 2013). The central dogma of molecular biology is that DNA is translated into RNA, which is then transcribed into proteins. These proteins can be structural or functional and lead to the regulation and expression of the four major macromolecules in cells: carbohydrates, proteins, nucleic acids, and lipids. Often, researchers will rely on DNA to determine the genetic 'potential' of the cell. However, just because a particular gene is present does not mean that the cell will express it. Instead, researchers can use RNA or metabolomics to learn about the cellular expression of a given gene which will be influenced by the cell's environmental conditions, cell-cycle stage, and activity of metabolic pathways (though RNA does not always indicate protein expression either). Likewise, lipids can be used to characterize cellular physiology due to the abundance or variety of lipids present. Lipid analysis is unique in metabolomics because lipids are hydrophobic, unlike charged DNA or proteins. This can both constrain and expand the possibilities for research questions and methodologies. For microbial macro-structures in particular, lipids have been used to identify the bacterial source and the environmental conditions of growth when paired with stable isotope analyses (Zarzycki and Potka, 2015; Shields, 2017).

Common lipid biomarkers in natural environments include sterols, fatty acids, and alcohols (Arts and Wainmann, 1999). Sterols are produced by eukaryotic organisms (green algae and diatoms in freshwater systems) and are cellular membrane components. The sterol type, abundance, and location within a membrane dictates the rigidity or fluidity of the membrane. Sterols are produced in different amounts by different organisms and can generally be used as a biomarker for eukaryotes (Rampen et al., 2010). For instances, green algae have been shown to produce high abundances of C_{29} sterols relative to C_{27} or C_{28} (Kodner et al., 2008). Fatty acids

are long carbon chains with a carboxylic acid (hydrophilic) head group that make up the bulk of cellular membranes. Fatty acids produced by microalgae and bacteria typically range from 12 to 26 carbons long and can be saturated (no double bonds) or unsaturated (one to multiple double bonds). Chain length and degree of saturation also impact membrane fluidity (Arts and Wainmann, 1999). Odd carbon number fatty acids (C_{15} and C_{17}) are typically associated with bacteria as bacteria use a different substrate to synthesize these fatty acids (Allen et al., 2010). Longer chain even-numbered fatty acids (greater than C_{22}) tend to be biomarkers of higher plants or diatoms and algae (Allen et al., 2010). Both fatty acids and sterols can be thought of as non-specific biomarkers (Zimmerman and Canuel, 2001).

1.6 Hopanoid biomarkers

Hopanoids are a type of lipid ubiquitously produced by microorganisms and are important indicators of life that are well-preserved in the rock record (Newman et al., 2016; Zarzycki and Portka, 2015). While they appear to be structurally unstable with 5 linked carbon rings with various modifications (methylations and large oxygenated functional groups) (Figure 1.5), hopanoids are actually very stable even under the high heat and pressure conditions that materials undergo during diagenesis and fossilization (Pearson and Rusch, 2009). As such, hopanoids have been found in rocks up to 50 Ma old (Talbot et al., 2007; Rohmer, 1979). Because of the prevalence of these stable compounds they may be the most abundant organic compounds on Earth (Zarzycki and Portka, 2015) and there is much interest in utilizing hopanoid composition in tracking the evolution of life on Earth (Newman et al., 2016).
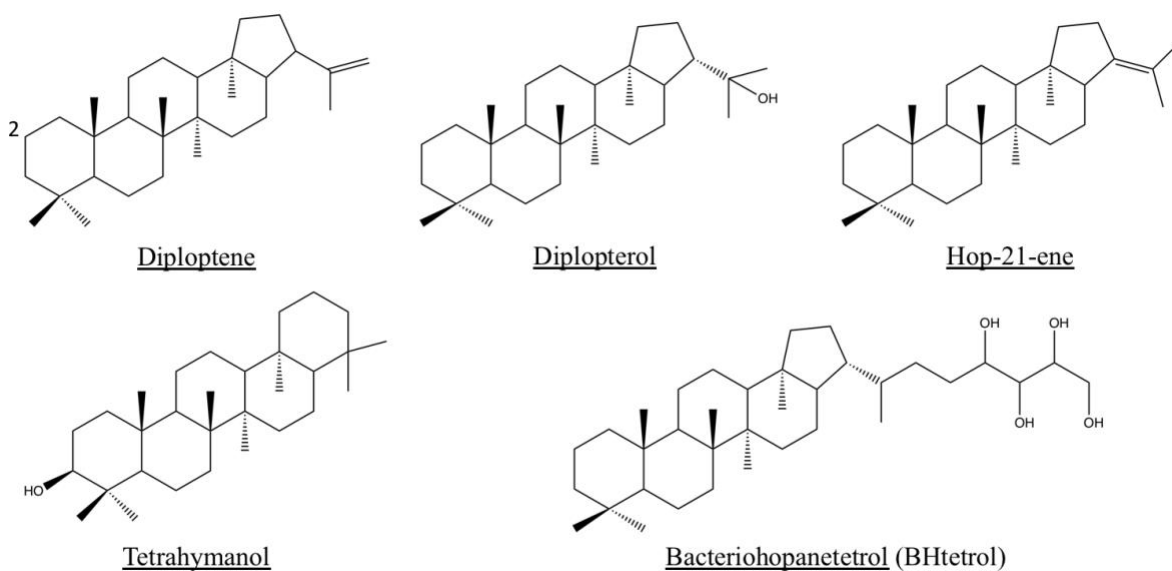
Figure 1.5. Key hopanoid structures including hydrocarbons (diploptene and hop-21-ene) and the functionalized hopanoids (diplopterol, tetrahymanol, and BHtetrol). The carbon 2 position indicated on diploptene is commonly methylated in all of these structures for certain bacteria, known as 2-methyl hopanoids.

Hopanoids are synthesized in a single cyclization step by a squalene-hopene cyclase (*shc*) or oxidosqualene cyclase (OSC) in bacteria (Siedenburg and Jendrossek, 2011). Recent work by Welander et al. (2010) showed that 2-methyl hopanoids are methylated by S-adenosyl methionine transferase (SAM methylase), encoded by the *hpnP* gene in cyanobacteria and alphaproteobacteria. Archaea and purple and green sulfur bacteria lack *shc* genes and do not produce hopanoids at all (Rezanka et al., 2010). Tetrahymanol is unique in that its biosynthesis goes to a gammacerane (5-six carbon rings) instead of four six-carbon rings and one five-carbon ring (Bravo et al., 2001). General information about pertinent hopanoids is provided in Table 1.2 including known sources, functionality, carbon numbers, and molecular weight.

Table 1.2 Relevant hopanoid biomarkers and their known sources

Hopanoid	Molecular Formula	Molecular Weight (g/mol)	Functionality	Modern Sources	References
Diploptene	$C_{30}H_{50}$	410.72	1 double bond	higher plants, mosses, lichen, fungi, ferns, cyanobacteria, gram-negative bacteria, *Geobacter*	Rezanka et al., 2010; Hartner et al., 2005
Hop-21-ene	$C_{30}H_{50}$	410.72	1 double bond	Co-occurs with diploptene	Rohmer et al., 1984
Diplopterol	$C_{30}H_{52}O$	428.75	1 hydroxyl	gram-negative bacteria, ciliates, and most diploptene-producing organisms	Bravo et al., 2001; Babiak et al., 1975; Rohmer et al., 1984
Tetrahymanol	$C_{30}H_{52}O$	428.75	1 hydroxyl	freshwater and marine ciliates, anaerobic free-living protists, anaerobic rumen fungus, alphaproteobacteria, *Methylomicrobium*, likely *Desulfovibrio*	Welander et al., 2010
Bacterio-hopanetetrol (BHtetrol)	$C_{35}H_{62}O_4$	546.86	4 hydroxyls	cyanobacteria, strict anaerobic bacteria, anammox bacteria, thermophiles, sulfur reducers, *Frankia* (nitrogen fixer), acetic acid bacteria, methanotrophs, purple non-sulfur bacteria, gram-negative bacterium *Z. mobilis*	Rohmer et al., 1984; Rezanka et al., 2010
2-methyl bacterio-hopanepolyol	$C_{36}H_{65}O_4$	561.86	Variable but includes methylated C2	cyanobacteria, *Methylobacterium*, *Bradyrhizobium*, *Beijerinckia*, acetic acid bacteria, alphaproteobacteria	Rezanka et al., 2010; Talbot et al., 2008; Welander et al., 2010

The functional role of hopanoids in living microbes is not fully understood (Talbot et al. 2008). Table 1.3 summarizes potential functions of hopanoids in cellular biology. The sterol-surrogate function is the most likely and relies on the similarities between hopanoids and sterols in structure and cellular location (Talbot et al. 2008). Sterols in eukaryotes are membrane constituents that make the membrane more rigid or more fluid depending on ambient temperatures and sterol abundance. Hopanoids are thought to serve the same purpose in prokaryotes (Ourisson et al., 1987; Talbot et al. 2008). These membrane-modifying molecules can also influence membrane permeability as well as the other functions (Table 1.3) to varying degrees.

Although the sterol-surrogate hypothesis is widely accepted and often assumed, scientists have prioritized the idea that hopanoids help a cell tolerate environmental stressors. Such stressors include changes in pH, temperature, or environmental stress specifically related to a community-style lifestyle as is found for microbial mats, stromatolites, microbialites, or biofilms (Saenz et al., 2012). 2-methyl hopanoids were found to be enriched in outer microbial membranes, which are directly interacting with external environmental factors (Kulkarni et al., 2013). Another line of evidence is the presence of abundant 2-mehtyl hopanoids in plant-microbe symbioses. A hopanoid-mutant, lacking a gene to produce 2-methyl hopanoids, could not survive under low oxygen and low pH (Kulkarni, 2015).

Cyanobacteria and alphaproteobacteria are thought to be the primary hopanoid producers in environmental samples (Talbot et al., 2008; Zarzycki and Portka, 2015). This is a complicating variable for determining hopanoid function because many different microbes potentially produce hopanoids: cyanobacteria, alphaproteobacteria (purple non-sulfur bacteria and methylotrophic bacteria), gram-negative chemoautotrophs, gram-negative chemoheterotrophs, gram-positive

chemoheterotrophs, actinomycetes, planctomycetes, and sulfate-reducing bacteria (Allen et al., 2010; Burns et al., 2011; Papineau et al., 2005). For this reason, the hopanoid profile may be useful for identifying groups of organisms or a particular environmental niche, rather than individual taxa. This community approach is useful for both modern and ancient microbial systems (Ricci et al., 2014) because in the environment, many organisms work together to build a microbialite. Studying individual microbes and their hopanoid productivity is only a piece of the story.

Table 1.3 Summary of potential functions of membrane hopanoids in microbes

Potential Function	References
Sterol surrogate (regulates permeability or fluidity)	Newman et al., 2016; Rohmer et al., 1979; Saenz et al., 2012 & 2015; Sessions et al., 2013; Zarzycki & Portka, 2015
Lipid ordering or sub-compartmentalization	Saenz et al., 2012
Tolerance to ethanol or other antimicrobial toxins	Saenz et al., 2012; Rezanka et al., 2010
Preventing water loss or cell dissociation	Saenz et al., 2012
Barrier to O_2 diffusion (nitrogen fixing bacteria)	Saenz et al., 2012; Bravo et al., 2001;
Tolerance to changes in pH	Saenz et al., 2012
Microbe-microbe interactions	Ricci et al., 2014
Tolerance to changes in temperature	Zarzycki & Portka, 2015
Involvement in plant-microbe interactions	Newman et al., 2016; Ricci et al., 2014
Tolerance to other environmental stressors	Garcia Costas et al., 2011; Kulkarni et al., 2013; Newman et al., 2016; Saenz et al., 2012; Zarzycki & Portka, 2015

Hopanoid production initially was linked to oxygenic photosynthetic microorganisms, so they were interpreted as markers in the rock record for the oxygenation of the atmosphere (Brocks et al., 1999). However, this has been disproven (French et al., 2015) as many anaerobic organisms can also produce hopanoids (Welander et al., 2010). There are weak associations between nitrogen fixation and hopanoid concentrations in cells, but a direct relationship between hopanoids and nitrogen fixation has not been demonstrated (Newman et al., 2016; Saenz et al., 2012). In one study, Berry et al. (1991) showed that bacteriohopanetetrol (BHT) constituted 60% of all lipids in one type of nitrogen fixing bacteria in legumes.

There have been many efforts to determine the modern distribution of hopanoid-producing organisms. In a two-year sampling campaign of the global ocean metagenome, researchers found that only 4% of cells had genes for squalene-hopene cyclases (shc), the key biosynthetic enzyme for hopanoid production (Pearson and Rusch, 2009). Talbot et al. (2008) studied 58 cyanobacteria and determined that 49 produce hopanoids; this work confirmed a previous analysis by Saenz et al. (2012) that found 26 distinct hopanoids, in 56 different cyanobacteria, each one containing 1-8 different hopanoid molecules.

Linking hopanoids to their cyanobacterial source with high specificity allows for the use of hopanoid composition as a 'fingerprint' of microbial communities, especially those dominated by a cyanobacterial primary-producer. While promising, there is opposition to this approach since many types of organisms produce hopanoids, complicating the interpretation of both modern and ancient hopanoid compositions (Arp et al., 1999). Furthermore, researchers have shown that hopanoid abundance varies considerably over time and is dynamic within the cell (Zarzycki and Portka, 2015), complicating past and current research on these molecules. However, with more research, hopanoids will be valuable for interpreting microbial evolution on

Earth from systems with fossilized macro-structural evidence of the microbial origins of life (microbialites) but where typical molecular biology techniques are impossible (Talbot et al., 2008; Burns et al., 2011; Sessions et al., 2013).

1.7 Gas-chromatography for lipidomics

Lipids are distinctive within metabolomics due to their partial or complete hydrophobicity (Sessions et al., 2013). Hopanoids, sterols, and fatty acids are all cellular-membrane components with amphiphilic (hydrophobic and hydrophilic) characteristics. Depending on the analyte of interest, multiple steps are required to separate more neutral (sterol) components from the more hydrophilic (fatty acid) components. Other lipids, like hopanoids, require extensive extraction procedures (vigorous shaking) to solubilize the molecules and separate them from the rest of the cellular debris (Rohmer et al., 1984). There are also special considerations for the choice of internal standard and solvents for isolation of hopanoids and subsequent analysis by gas chromatography.

Gas chromatography (GC)-mass spectrometry (MS) is a standard analytical instrument for molecule identification and quantification (Figure 1.6). A liquid with the analyte is injected onto the gas-chromatography column. The liquid is vaporized and the molecules travel through the column at a rate that depends on their affinity for the stationary phase of the column. When studying lipids, one might choose a non-polar column which will separate non-polar molecules according to their affinity for non-polar stationary phase versus the carrier gas (helium). Molecules elute at a certain time from the initial injection and can be compared on different instruments using a relative retention time. After the GC, the molecules (now separated) are ionized using an electron beam. Molecular bonds break apart in a predictable manner and ions are deflected off their trajectory according to their mass to charge ratio (*m/z*). The mass-

spectrometer detector counts the number of ions at a particular *m/z* in a given time interval, which is then displayed as the unique MS fingerprint for a given GC peak. This method can be used to identify compounds and quantify their abundance in a given sample using peak height or peak area relative to a known standard concentration.

Optimization of the GC-MS instrumentation for hopanoid analysis was completed by Sessions et al. (2013). This methodology was developed for the lab-cultured microbe *Rhodopseudomonas palustris*. The GC-MS column and temperature program were carefully selected to optimize hopanoid recovery using high temperature columns without needing to remove the hopanoid functional groups prior to analysis. Identification was expedited by using single ion monitoring (SIM) for *m/z* 191 for most hopanoids and SIM *m/z* 205 for 2-methyl hopanoid homologs (Figure 1.7) (Fischer et al., 2005).
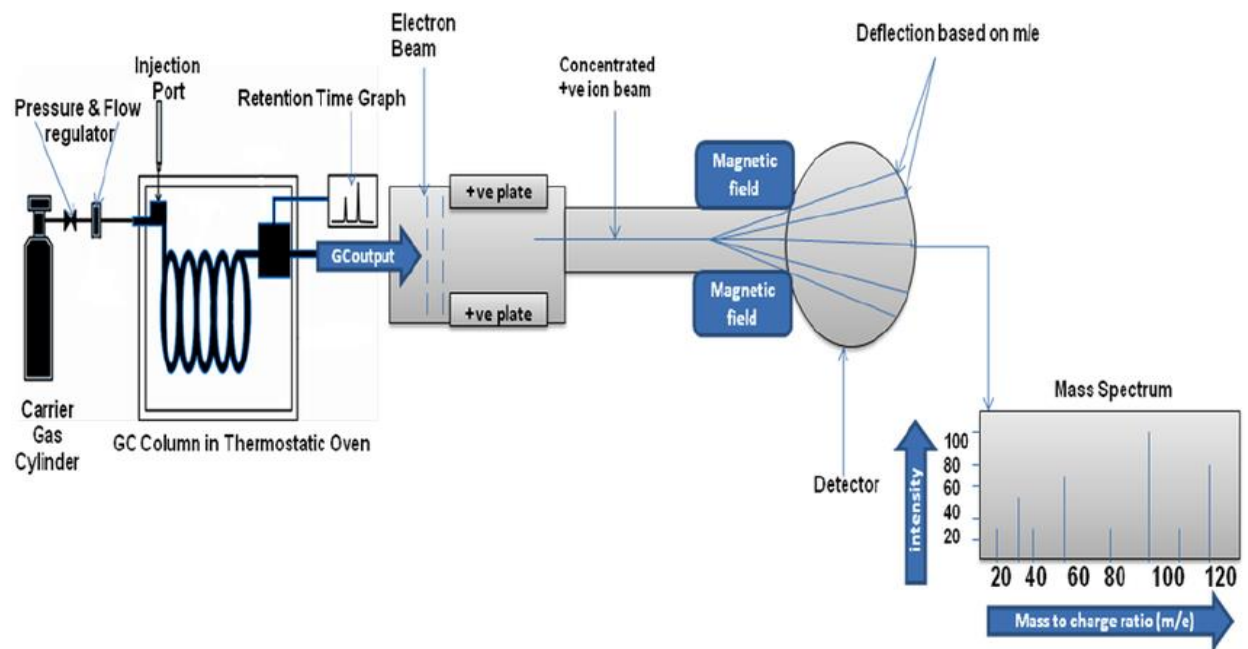
Figure 1.6. Gas chromatography-mass spectrometry schematic (from Kumar et al., 2015).
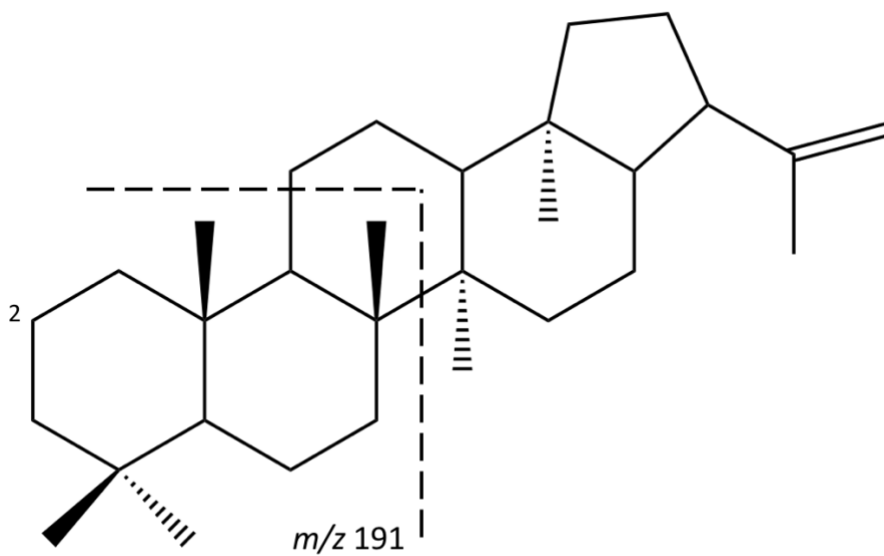
Figure 1.7. Major ionization fragment (*m/z* 191) for hopanoids using diploptene as an example.

Methylation at position 2 results in an ion fragment of *m/z* 205.

1.8 Importance of learning about modern microbial carbonates

Lipid biomarkers, including hopanoids, are just one of many potential techniques (e.g. genomics, metabolomics, isotopes, mineralogy) for learning about microbial ecology, each with its own strengths and weaknesses. When combined together they provide the richest possible interpretation of complex systems. Hopanoids have been described as a promising field of research (Newman et al., 2016); it is imperative that work be done to learn about the current distribution of hopanoids in the environment, what organisms produce them and why, and whether they are useful tools for interpreting the rock record. Modern microbialites are a powerful study subject due to their prevalence in the rock record, their modern global distribution, and their prevalence in a wide range of environmental conditions. The carbonate framework that gives microbialites so much value also results in potentially challenging analytical methodologies. Despite this drawback, standardized lipidomics procedures are possible and necessary for comprehensive microbialite studies.

Chapter 2 describes the lipid and hopanoid biomarker composition of microbialites from Green Lakes State Park. Chapter 3 uses the same methodology for microbialites at the Great Salt Lake. The Appendices include results from hopanoid analyses for several other environmental systems including Sulfur Springs in NY, coral animal and zooxanthellae from FL, and cultured microbial samples from the Green Lakes microbialites. None of these additional projects yielded hopanoid biomarkers. This shows how hopanoid production is a feature of microbialites, not necessarily found in all microbial systems. Conclusions are drawn regarding hopanoid and lipid composition of the microbial communities in carbonates from each lake and applications for future work.

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Chapter 2: Hopanoid and lipid biomarker composition of freshwater microbialites in Fayetteville Green Lake, New York

Abstract

The microbialites at Fayetteville Green Lake are a rare example of actively accreting carbonate structures in a freshwater meromictic lake due to biologic activity. Prior work suggested the presence of hopanoid biomarkers in these microbial structures. Hopanoids are cellular membrane constituents that are thought to be indicative of microbial communities occurring in highly osmotic, oxygen-limited conditions. Tightly growing microbes in these communities require adaptations, like membrane-rigidifying hopanoids, to cope with stress. Hopanoids have been found both in modern actively growing carbonate structures (stromatolites) and in ancient, fossilized carbonate structures, making them extremely useful for differentiating carbonates of biologic origin from those that are not. In this work, gas chromatography-mass spectrometry is used for lipid analysis. Abundance and composition of fatty acids, sterols, and hopanoids is used to assess community composition through three core depths into the microbialite surface. Eight hopanoids (diploptene, hop-21-ene, diplopterol, tetrahymanol, bacteriohopanetetrol, and their 2-methyl forms) were found in the Fayetteville Green Lake microbialites with significant composition shifts with depth in the water column and with core depth into the microbialite surface. Hopanoid composition changes with core depth are indicative of carbonate mineralization-dissolution dynamics due to heterotrophic activity in these microbialites. This work expands prior knowledge of the diversity of hopanoids in environmental samples and allows for comparison among other freshwater microbialites.

Key Words

hopanoid, lipid biomarker, Fayetteville Green Lake, microbial community, freshwater reef

1 Introduction

1.1 Ancient and modern microbial carbonates

Recent melting of snowfields in Greenland revealed the oldest known life on early Earth, 3.7 billion years old (Nutman et al., 2016). Similar to other ancient biogenic structures, life was preserved as stromatolites, microbial communities whose metabolism results in the trapping and binding of minerals in a layered structure that is then recorded in the rock record (Nutman et al., 2016; van Kranendonk et al., 2008). Determining that layered rock features have a biological origin is not trivial; researchers rely on morphology, stable isotopes, elemental composition, and chemical biomarkers to link these macrostructures to the microscopic cells that produced them (Newman et al., 2016). This is possible by comparing the ancient microbial-structures in Greenland (Nutman et al., 2016) or Australia (Allwood et al., 2006) to modern actively growing microbial communities like stromatolites at Shark Bay, Australia (Foster and Green, 2011); or by comparison with the freshwater equivalent, microbialites, in Cuatro Ciénegas, Mexico (Nitti et al., 2012); Pavilion Lake, Canada (Brady et al., 2014); or Fayetteville Green Lake, USA (Thompson et al., 1990; Patterson, 2014). These actively growing structures offer insight into the growth, function, and dynamics of microbial carbonates including the mineral-accretion process, the spatial distribution of microbial metabolism, the temporal or seasonal variability in their growth, and their ecological function in a variety of environments (Newman et al., 2016). Once these characteristics are well understood, it may be possible to further understand the evolution of life on early Earth including the role of microbial metabolism and impacts of changing environmental growth conditions through time.

1.2 Hopanoid and lipid biomarkers

One of the most useful tools for studying microbialite structures is via biomarker analysis (Zarzycki and Portka, 2015). A biomarker is a chemical compound derived from a specific living organism that can be used to link the molecule to its source (Farrimond et al., 1999). Biomarker analysis allows for the identification and quantification of an organism even after it is no longer present or living (Newman et al., 2016). Lipid biomarker analyses can include phospholipid fatty acid profiling, a fast and relatively inexpensive method for characterizing taxonomy (Pages et al., 2015). For instance, odd chain fatty acids (iso- and anteiso-C15:O and C17:O) are produced specifically by heterotrophic bacteria (Allen et al., 2010) while even-chain di- or poly-unsaturated fatty acids (C16:2, C18:3, C18:4, and C18:2ω6) are common biomarkers for autotrophs like diatoms and cyanobacteria (Brady et al., 2014). These different molecules can be quantified and then compared as the autotrophic to heterotrophic lipid ratio to describe community composition (Zimmerman and Canuel, 2001). Sterols are also useful biomarkers for detecting eukaryotic organisms. Higher plants, zooplankton, diatoms, phytoplankton, and cyanobacteria produce C27-C29 sterols (Zimmerman and Canuel, 2001).

Bacteria do not produce sterols but instead produce hopanoids, which serve a similar role in cellular membrane structure; however, the exact cellular role of hopanoids is not yet known (Saenz et al., 2015; Wu et al., 2015). Hopanoid functions may include: regulation of membrane permeability or fluidity (Rohmer et al., 1979), lipid ordering and sub-compartmentalization, ethanol and antimicrobial toxin tolerance, water loss prevention, blocking oxygen diffusion in nitrogen fixing bacteria, pH change tolerance, microbe-microbe interactions, temperature change tolerance, involvement in plant-microbe interactions, and tolerance to environmental stressors (Garcia Costas et al., 2012; Ricci et al., 2014; Saenz et al., 2012; Zarzycki and Portka, 2015).

Hopanoids are valuable as a biomarker because they have been observed in ancient microbialite structures (Brocks et al., 1999, 2005; Hefter et al., 1993). While genetic analyses and microscopy are extremely useful for characterizing living or recently living microbial communities, both genetic material and distinctive cellular structures can be lost through remineralization of carbonates or degradation (Newman et al., 2016). Hopanoids are therefore extremely useful since they survive the action of heterotrophic degradative enzymes as well as the high heat and pressure conditions of fossilization (Brocks et al., 1999). Not only are these molecules present in ancient microbialites, they are also predicted to be distinctive to particular microbial groups (Talbot et al., 2007). Our current knowledge of the role of hopanoids and their distribution within organisms is limited, however as they are well preserved in the fossil record these compounds may be a source of information about paleoclimates, paleoenvironmental conditions, and the composition of ancient microbial systems. Our first steps in understand their importance in ancient systems, requires a greater understanding of the role of hopanoid biomarkers in living systems; it will be important to discern their current distribution in microbial carbonates, their variability with different environmental conditions, and their specificity to living taxa (Talbot et al., 2008; Newman et al., 2016).

1.3 Study Site: Fayetteville Green Lake, NY

Fayetteville Green Lake (FGL) is a ~50 m deep, meromictic, hardwater lake, carved by glacial waterfall pour-offs about 14,000 years ago (Hilfinger and Mullins, 1997; Thompson et al., 1990). Along the shores of this wind-protected lake, microbialites grow as shelves outward from the steep dolomite rock shore (Syracuse Formation) to depths of 10 m where the rock turns to crumbly shale (Vernon Shale Formation) (Thompson et al., 1990). The FGL microbialites are known as "freshwater reefs" for their similarity to saltwater reefs that support entire ecosystems

by providing substrate for other organisms' growth, habitat structure, and the base of the food web for higher organisms (Hughes et al., 2003). In comparison to coral-based saltwater reefs with an animal-dinoflagellate symbiosis, the FGL primary producers instead are cyanobacteria, diatoms, and green algae (Thompson et al., 1990; Wilhelm and Hewson, 2012). Aquatic mosses and sponges grow on the carbonate substrate while zooplankton, invertebrates, mollusks, and fish graze. Microbialites also form on all woody debris that falls into the lake from the surrounding forest. So, not only do microbialites thrive at different locations around the lake under variable growth conditions, but they also use different growth substrates, shelf and wood-types, which may have an impact on the microbial community structure.

Investigations of the FGL microbialite community diversity are limited. Thompson et al. (1990) identified two dominant cyanobacteria, *Oscillatoria* (spp.) and *Synechococcus* (spp.) at the surface of the microbialites. Carbonate accretion and macro structural growth were attributed to *Synechococcus* in the work of Thompson et al. (1990), the same organism thought to be responsible for the annual whiting events in FGL. Wilhelm and Hewson (2012) expanded this work on cyanobacteria; richness and diversity of cyanobacteria increased with depth in the water column, as measured by automated rRNA intergenic spacer analysis. Deeper assemblages (1 m) were more stable than shallower (0.5 m) microbial communities. Differences were attributed to environmental conditions including temperature, light intensity and quality, and habitat stability (Wilhelm and Hewson, 2012).

Subsequent work by Patterson (2014) identified two additional filamentous cyanobacterial groups, *Leptolyngbya* and *Arthrospira*. Work on seasonal accretion cycles in FGL showed both *Oscillatoria* and *Synechococcus* were responsible for accretion at the surface of the microbialite with diatoms and green algae also present (Patterson, 2014). While initial carbonate

precipitation can be attributed to cyanobacteria, subsequent dissolution and remineralization

processes are likely due to the action of heterotrophic organisms (Figure 2.1) (Gallagher et al.,

2010; Nitti et al., 2012) that are expected to become more abundant further from the

phototrophic surface where light and oxygen become deplete toward the interior (Dupraz et al.,

2014).
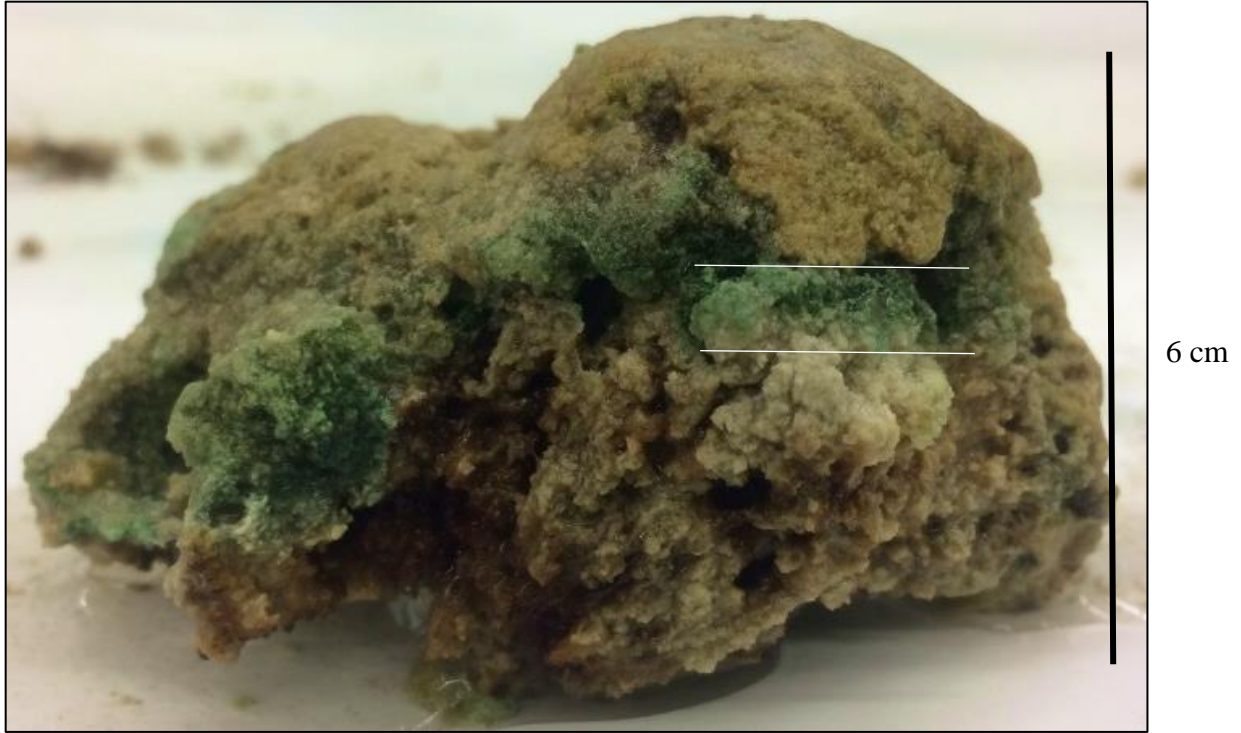
Figure 2.1 Microbialite fragment from Fayetteville Green Lake, NY. Soft, yellow upper layer (0-1 cm) with a hard, dark green layer underneath (1-3 cm). White to dark-red, hard layer (3-6 cm) at the base.

1.4 Objective

The objective of this work is to provide the first hopanoid composition analysis of a freshwater-microbialite, contributing to our understanding of the global distribution of modern hopanoids in carbonate microbialites. It is our aim to determine variability in molecular composition (lipids and hopanoids) of microbialites: 1) at different sites, 2) at different depths in the water column, 3) on different substrates, and 4) with core depth. We hypothesize that FGL microbialites contain lipid and hopanoid biomarkers that vary in composition at different depths in the lake, with core depth into the matrix, and on different substrates due to environmental differences (e.g. light, temperature, nutrients). Community composition changes, as described by community-level biomarker analysis, are predicted to be an indicator of precipitation-dissolution processes of the carbonate matrix.

2 Methods

2.1 Sample collection

Microbialite samples were collected from Fayetteville Green Lake, NY at three sites along the east (site 1) and west (sites 2 and 3) shores using a hammer and chisel in 2014 (NYS Parks Permit # 2014-GRL-005) and 2017 (NYS Parks Permit # 2017-GRL-006) by snorkeling (Figure 2.2). Sites were selected based on greatest abundance of microbialites and through consultation with Green Lake State Park management. Ten samples were taken at each site and 3 depths (1 m, 2 m, and 3 m). Ten wood samples were also collected at each site at 1 or 2-meter depths in 2017. All surficial algae and mosses were washed off microbialite samples before being placed in labelled Ziploc bags, transported on ice, and stored frozen at -20°C until further processing. Core depth portions (0-1 cm, 1-3 cm, and 3-6 cm) of shelf and wood-substrate samples were separated using a chisel and razor in the laboratory. All samples were freeze-dried

at -60°C for at least 36 hours. Samples were then ground using a mortar and pestle and stored

frozen at -20°C.

Light attenuation was measured once at three sites in FGL around noon in August 2017

using a LICOR light intensity instrument (LI-190R) with a SPQA 5446 round bulb and a Q

102582 light sensor. Data was recorded from a handheld LI-1500 data logger.
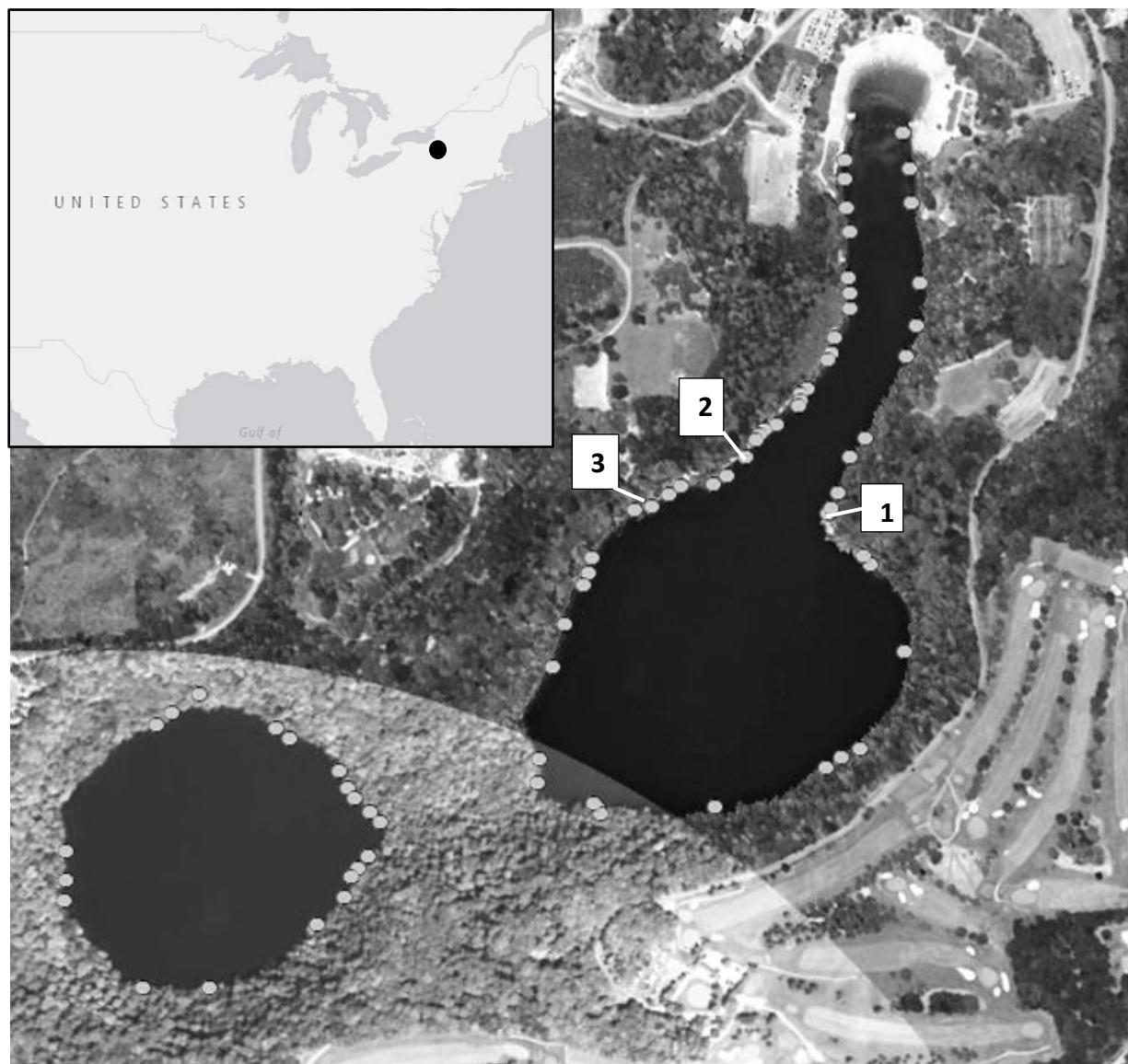
Figure 2.2 Microbialite locations (circles) at Green Lakes State Park including Fayetteville

Green Lake (right) and Round Lake (left). Sampling sites are labelled 1 (43.05163 N, 75.96387

W), 2 (43.05225 N, 75.96756 W), and 3 (43.05309 N, 75.96574 W) on the East and West shores

of Green Lake. There is a well-developed swimming beach on the North shore and multi-use

trails around both lakes. Inset shows location of Green Lakes State Park in NY, USA.

2.2 Fayetteville Green Lake microbialite cultures

Z8 media (Appendix B) was selected for culturing the cyanobacteria of the FGL microbialite based on its success in supporting the growth of general freshwater cyanobacteria (Staub, 1961; Kotai, 1972; NIVA, 1976). Autoclaved media was inoculated using a sterile probe stabbed into the active portion of the microbialite (green layer). Cultures were incubated for 25 days at 15°C on 12-hour day-night cycles with daily stirring. A Zeiss Telaval 31 light microscope was used to visualize cultures and photographs were taken with a Nikon D5000 digital camera with and without Lugol's Iodine Stain to observe the diversity of the microbialite community. Cyanobacteria, green algae, and diatoms were identified with algae and cyanobacteria guides (Whitford and Schumacher, 1984; Ettl et al., 2000).

2.3 Organic carbon and carbonate content

Total organic carbon and calcium carbonate content were determined for all microbialite samples following standard loss on ignition (LOI) procedures (Dean, 1994). Ground, freeze-dried microbialite material (1.0 g) was dried at 60°C for 36 hours. Clean, dry ceramic crucibles were used to heat samples at 550°C for four hours (organic carbon content) and subsequently to 1000°C for two hours (carbonate content). Organic carbon and carbonate content were calculated as percent of the initial mass of the dry microbialite.

2.4 Hopanoid Analysis

2.4.1 Extraction and derivatization

Optimized hopanoid extraction methods developed by Sessions et al. (2013) were followed; 5-10 g of freeze-dried microbialite sample were extracted by sonicating with 10 mL of 1:2:0.9 dichloromethane (DCM):methanol:water for 20 minutes in 50 mL Teflon centrifuge tubes. The mixture was centrifuged at 2000 rpm for 10 minutes. The organic phase was collected

with a glass pipette and filtered through pre-combusted cotton (500°C; 1 hr). Two additional extractions of the microbialite were added to the initial extract with 10 mL of 1:1 DCM:water. The combined total organic phase was then dried under N_2 gas, transferred with DCM to storage vials, completely dried under N_2 gas, and stored at -20°C.

The samples were derivatized for GC-MS by reacting the dried extract with 100 µL of 1:1 acetic anhydride:pyridine for 20 minutes at 70°C. Next, 50-100 µL of 70 µg/L cholestane (Sigma) in pyridine was added to each sample as the internal standard. The samples were immediately transferred to GC vials, gently evaporated under N_2 gas to a known volume and injected onto the GC-MS. Blanks were extracted for every set of five microbialite samples.

2.4.2 Gas chromatography-mass spectrometric analysis

Hopanoids were identified and quantified by gas chromatography-mass spectrometry (GC-MS) using a Perkin-Alma Clarus 580 GC and Clarus SQ85 quadrupole MS and data were analyzed using TurboMass software. The derivatized samples were analyzed on a DB-5 (J&W Scientific) column (30 m, ID 0.25 mm, film thickness 0.25 µm). A 1 µL sample was injected on the column using an autosampler with the injector in splitless mode at 300°C. The oven program was 100°C (2.0 min hold) to 250°C at 15°C/min and then to 320°C (30 min hold) at 15°C/min. The helium carrier gas was set to a constant flow rate of 10.0 ml/min. The mass spectrometer was operated in full scan mode over 50-620 EI+ (MS transfer line at 250°C and ion source at 200°C).

Hopanoids were identified following Sessions et al. (2013) using diagnostic ion peaks, relative retention times, and original hopanoid spectra (Summons and Jahnke, 1992). The total ion chromatogram (TIC) and single ion monitoring (SIM) at *m/z* 191 and 205 were manually integrated and concentrations were determined based on the cholestane internal standard.

Because cholestane does not have a major ion peak at *m/z* 191, a conversion factor was determined for each sample by comparing the TIC area to the SIM *m/z* 191 area for the diploptene peak (~22.7 min). This conversion factor was used to calculate the relative area of each hopanoid peak. Concentrations (µg/g C_{org}) were determined based on mass of organic carbon in each sample (LOI). Fifteen of thirty-three samples from 2014 lacked sufficient material for LOI analysis, thus the average percent organic carbon (0.4% $\pm$ 0.1) was used to calculate concentration (µg/g C_{org}) for these samples.

2.5 Lipid Analysis

2.5.1 Extraction and derivatization

Three microbialite samples at each of three sites were extracted for both fatty acid content and neutral sterol lipids. Only 1 m deep samples were analyzed based on prior investigation showing no difference in lipid content with depth. Lipid extractions followed standard protocols (Teece et al., 2011). Lipids were extracted from ~2 g of dried and ground microbialite by adding 2 mL of 1:1 DCM:methanol and vortex mixing for 5 minutes. Clean glass pipettes were used to transfer the total lipid extract (TLE) to a new vial and the extraction was repeated twice more. The combined TLE was evaporated to dryness under N_2 gas (50°C). Fatty acids and neutral sterols were then separated by alkaline hydrolysis. Two mL of 5% KOH in methanol were added to the dry TLE and heated for one hour (70°C). Samples were allowed to cool, 2 mL of water were added, then 5 mL of 9:1 hexane:ether were added, mixed by inversion, and allowed to separate. The neutral sterol fraction was transferred to a separate vial and the extraction was performed twice more before drying under N_2 gas (50°C). The aqueous layer was acidified to pH 2 with the addition of 1 mL of 6N HCl. The resulting fatty acids were then

extracted with 5 mL of 9:1 hexane:ether three times. The combined fatty acids were dried under N_2 gas (50°C) and all vials were stored at -20°C for up to two weeks.

Fatty acids were then methylated; 5 μL of toluene were added to the vial with 0.5 mL of methanolic HCl (Supelco). The solution was quickly vortexed then heated for one hour (60°C). The lid-seal was broken, and samples were allowed to cool for 3 minutes, then 1 mL of milliQ water was added. The fatty acid methyl esters were extracted into a new vial with 2 mL of hexane three times and dried under N_2 gas (50°C).

Both neutral sterols and fatty acid methyl esters were silylated for gas chromatography-mass spectrometry. Five μL of toluene were added to each vial, vortexed, then evaporated under N_2 gas (50°C). Five μL of DCM and 10 μL of N,O-Bis(trimethylsilyl)trifluoro-acetamide (BSTFA) (Supelco) were added, vortexed, and heated for 30 minutes (60°C). Samples cooled for 1 minute and dried under N_2 gas. Six μL of DCM were added, vortexed, and evaporated under N_2 gas (50°C). Finally, using 0.8 mL of DCM, samples were transferred to GC vials and injected on the GC-MS. In addition, one cod liver oil standard solution was methylated, silylated, and run on the GC-MS for lipid identification with C_{23} standard (100 μL of 200 mg/L). Blanks were extracted with every set of five microbialite samples.

2.5.2 Gas chromatography-mass spectrometric analysis

Fatty acid methyl esters and neutral sterols were separately analyzed and identified using a Perkin-Alma Clarus 580 GC and Clarus SQ85 quadrupole MS and data were analyzed using TurboMass software. The derivatized samples were analyzed on a DB-5 (J&W Scientific) column (30 m, ID 0.25 mm, film thickness 0.25 µm). A 1 µL sample was injected on the column using an autosampler with the injector in splitless mode at 280°C. The oven program was 60°C (1.0 min hold) to 140°C at 15°C/min and then to 300°C (15.0 min hold) at 4°C/min. The helium

carrier gas was set to a constant flow rate of 10.0 ml/min. The mass spectrometer was operated in full scan mode over 50-620 EI+ (MS transfer line at 250°C and ion source at 200°C).

Fatty acid methyl esters (FAMEs) and neutral sterols were identified using diagnostic molecular ions and relative retention times based on the cod liver oil standard. The TIC was manually integrated for each peak of interest. Both FAME and neutral sterol content were calculated as proportion of the total FAME or neutral sterols in a given sample.

2.6 Statistical analysis

All statistical analyses were completed in Statistical Analysis System (SAS University Edition software, copyright 2014). T-tests were used to test for significant differences in hopanoid concentration by year and substrate. One-way analysis of variance (ANOVA) with post-hoc Tukey HSD were used to test for significant differences in hopanoid and lipid concentrations by site, water depth, and core depth. To analyze biomarker composition, each was calculated as proportion of the total then transformed prior to statistical testing with the logit transformation [$\hat{y}=\ln (y/(1-y))$] to normalize the data (Warton and Hui, 2011). Four default tests were used to test for normality including Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling. Tests were considered significant at $\alpha=0.05$ for all analyses.

3 Results

3.1 Fayetteville Green Lake microbialite cultures

Culture media became green with microbial growth after 20 days of incubation. Subsequent observation with light microscopy revealed a mixed population of diatoms, green algae, and cyanobacteria. Green algae included *Oedogonium* (spp.), *Scenedesmus* (spp.), *Ankistrodesmus* (spp.), and *Cosmarium* (spp.) Cyanobacteria included *Synechococcus* (spp.), *Oscillatoria* (spp.), *Calothrix* (spp.), *Chroococcus* (spp.), and *Planktothrix* (spp.) (Figure 2.3).
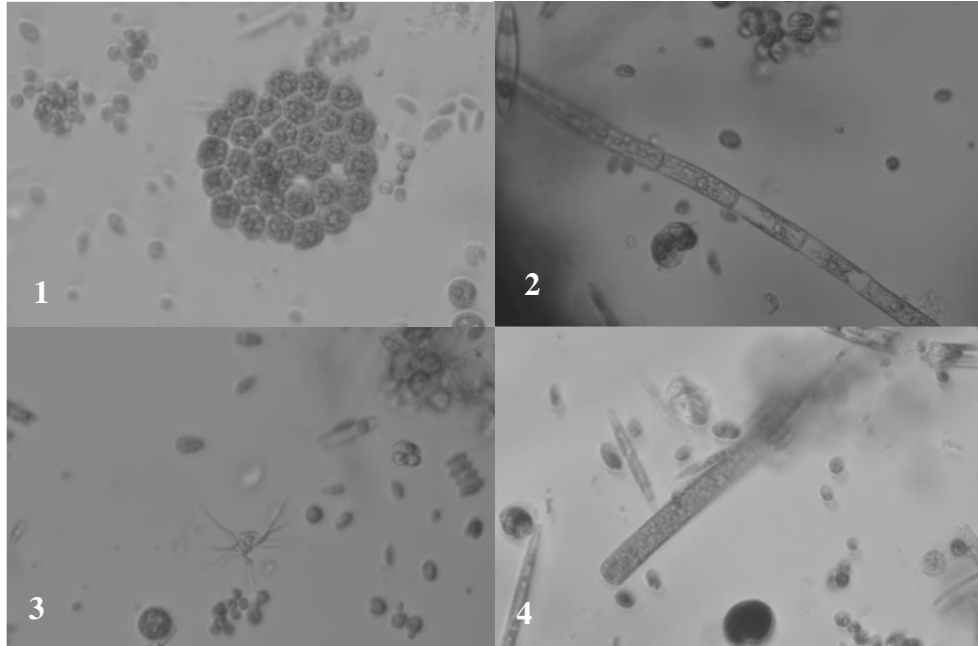
Figure 2.3 Cultured cyanobacteria, diatoms, and algae from Fayetteville Green Lake. 1) Image includes *Synechococcus* (spp.), and diatoms. 2) Image includes *Oedogonium* (spp.) and *Choococcus* (spp.). 3) Image includes *Scenedesmus* (spp.), *Ankistromdesmus* (spp.). 4) Image includes *Oscillatoria* (spp.), diatoms, and *Synechococcus* (spp.).

3.2 Organic carbon and carbonate content

There were no statistical differences in organic carbon (0.4% $\pm$ 0.2) or carbonate content (5.7% $\pm$ 0.3) by site, depth, substrate, or year. There were, however, differences by core depth with all core depths significantly different for both organic carbon ($F_{(3,53)}$=25.66, p<0.0001) and carbonate content ($F_{(3,53)}$=5.13, p=0.00034). The surface of the microbialite (0-1 cm) contained more organic carbon (0.6% $\pm$ 0.1) and less carbonate (5.5% $\pm$ 0.3) than progressively deeper layers (C_{org}=0.3% $\pm$ 0.1 and $C_{carbonate}$= 5.9% $\pm$ 0.4 at 3-6 cm core depth).

3.3 Hopanoid identification and quantification

Eight hopanoids were present in FGL microbialites (Table 2.1). Diploptene was the most abundant and present in every sample. The diploptene peak overlapped with the hop-21-ene peak around 22.67 minutes (Figure 2.4). Bacteriohopanetetrol (BHtetrol), tetrahymanol, and their 2-methyl forms were also present. A highly abundant 2-methyl hopanoid eluted just 0.2 min in front of the diploptene peak. Sessions et al. (2013) identify three potential compounds at this location with similar diagnostic ions, all of which were in this one peak. The GC-MS method was not able to separate these compounds completely, and it was not possible to confirm the identity of this 2-methyl hopanoid. The term methyl-diplop[†] was adopted to account for the potential presence of 2-methylhop-17(21)ene, 2-methylhop-22-(29)-ene, 2-methylhop-21-ene as one wide peak. Appendix A includes all mass spectra for these compounds.

Table 2.1 Hopanoids present in Fayetteville Green Lakes microbialites. Common name, elution time relative to diploptene, diagnostic ions, and key SIM masses are listed for each compound. Base peak for the diagnostic ions is bold. (Modified from Sessions et al., 2013).

Hopanoid	Common Name	Structure	Relative Retention Time	Diagnostic Ions (m/z)	SIM (m/z)
	cholestane internal standard		0.8	372, 357, 218, **217**, 95	
2-methylhop-17(21)-ene	methyl-diplop[†]	I	0.99	424, 380, 355, 313,245,**205**, **189**, 161,121, 95	205
2-methylhop-22-(29)-ene		II			
2-methylhop-21-ene		III			
hop-22(29)-ene	diploptene	IV	1.00	410, 299, **191**, 189, 95	191
hop-21-ene		V	1.00	410, 341, **191**, 189, 121	191
hopan-22-ol	diplopterol	VI	1.05	428, 395, **191**, 189, 149, 95	191
2-methyltetrahymanol		VII	1.24	484, 424, 249, **205**, 189, 83	205
tetrahymanol	tetrahymanol	VIII	1.25	470, 410, 249, **191**, 189, 69	191
2-methylbacteriohopanetetrol		IX	1.62	493, 383, **205**, 95	205
bacteriohopanetetrol	BHtetrol	X	1.65	493, 369, **191**, 95	191
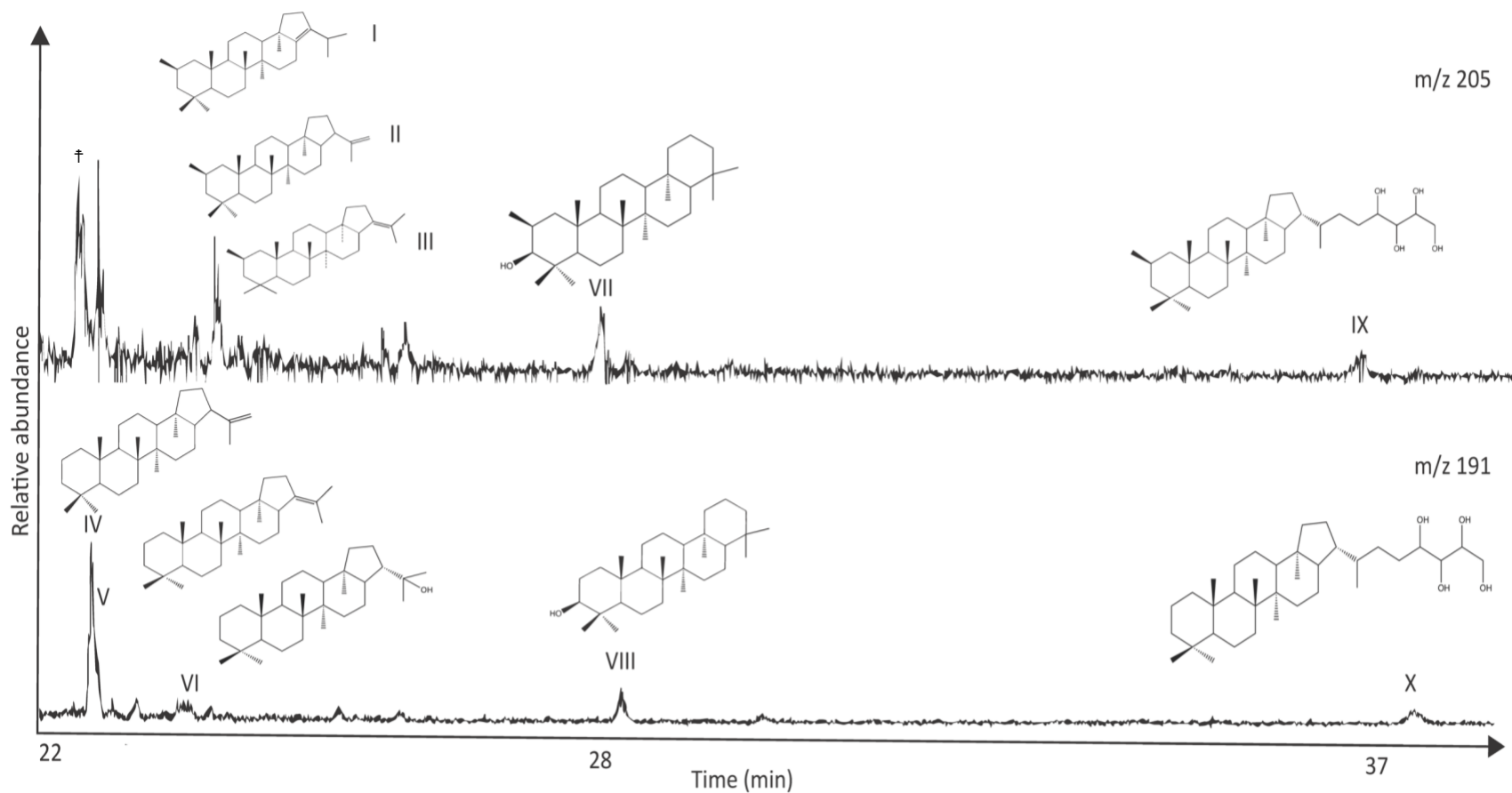
Figure 2.4 Gas chromatography trace for m/z 191 (hopanoids) and m/z 205 (2-methyl hopanoids) (sample J30-1-3 from site 2, 2 m deep). Molecular labels correspond to Table 1. The methyl-diplop peak (†) represents a mixture of compounds I-III. All hydroxyl groups were acetylated prior to GC run.

3.4 Hopanoid concentration

For individual hopanoids, concentrations ranged from 4.8 ± 2.0 µg/g C_{org} to 82.7 ± 52.1 µg/g C_{org}. The mean sum of all hopanoids was between ~190-230 $\pm$ 58 µg/g C_{org}. There were no differences in the individual hopanoid concentrations at the three sites (1, 2, 3) or three depths (1 m, 2 m, 3 m). For core depth, there were differences in hopanoid concentration for diploptene $(F_{(2,36)}=8.92, p=0.0007)$ and hop-21-ene $(F_{(2,34)}=6.85, p=0.0032)$ that resulted in large differences in the total hopanoid concentration at the surface $(205 \pm 21$ µg/g $C_{org})$ compared to deeper layers: 1-3 cm, $(130 \pm 12$ µg/g $C_{org})$ and 3-6 cm $(112 \pm 9$ µg/g $C_{org})$. Individual hopanoid concentrations are listed in Table 2.2 for each site, depth, core depth, and substrate.

Table 2.2 Hopanoid concentrations in Fayetteville Green Lake microbialites. Sample number is in parentheses after each mean and standard deviation for eight hopanoids at 3 sites, 3 depths, 3 core depths, and 2 substrate types. BDL indicates "below detection limit" which was 9.1 µg/g C_{org}.

[Hopanpoid] (µg/g C_{org})	Site			Depth (m)		
	1	2	3	1	2	3
methyl-diplop	31.6 ± 9.3 (4)	35.8 ± 21.7 (3)	42.6 ± 14.2 (5)	38.0 ± 8.8 (6)	41.9 ± 21.4 (4)	25.7 ± 12.7 (2)
diploptene	80.2 ± 46.7 (10)	72.1 ± 42.2 (9)	74.9 ± 54.3 (8)	86.5 ± 42.3 (15)	49.7 ± 39.4 (6)	75.7 ± 56.9 (6)
hop-21-ene	18.2 ± 18.4 (10)	16.3 ± 7.8 (8)	21.0 ± 17.3 (8)	21.3 ± 16.1 (14)	10.2 ± 7.5 (6)	20.2 ± 17.1 (6)
diplopterol	12.8 ± 8.9 (6)	9.4 ± 5.8 (6)	13.0 ± 7.3 (7)	14.2 ± 8.0 (8)	BDL	12.4 ± 6.6 (6)
2-methyl tetrahymanol	27.1 ± 18.6 (8)	25. 5 ± 17.3 (7)	24.7 ± 11.9 (7)	22.5 ± 13.7 (11)	28.7 ± 20.6 (5)	29.5 ± 15.8 (6)
tetrahymanol	13.4 ± 10.7 (10)	9.3 ± 3.9 (8)	16.1 ± 9.6 (8)	11.8 ± 7.7 (14)	11.1 ± 6.7 (6)	17.5 ± 12.8 (6)
2-methyl BHtetrol	9.5 ± 4.4 (5)	23.2 ± 10.1 (6)	13.5 ± 5.6 (6)	12.5 ± 5.5 (6)	19.8 ± 13.0 (5)	15.6 ± 8.1 (6)
BHtetrol	16.6 ± 17.5 (10)	17.7 ± 10.5 (7)	20.4 ± 16.9 (8)	11.6 ± 5.8 (13)	21.1 ± 17.8 (6)	29.3 ± 20.7 (6)

[Hopanpoid] (µg/g C_{org})	Core Depth (cm)			Substrate Type	
	0-1	1-3	3-6	Shelf	Wood
methyl-diplop	44.9 ± 27.5 (9)	39.2 ± 24.6 (12)	29.2 ± 15.8 (12)	37.5 ± 4.6 (11)	34.7 (1)
diploptene	75.0 ± 55.6 (13)	30.0 ± 19.9 (13)	21.2 ± 12.7 (13)	70.8 ± 9.3 (22)	98.5 ± 24.2 (5)
hop-21-ene	16.4 ± 13.4 (12)	BDL	BDL	17.8 ± 2.8 (21)	21.5 ± 9.3 (5)
diplopterol	BDL	BDL	BDL	11.8 ± 1.7 (18)	11.2 (1)
2-methyl tetrahymanol	26.8 ± 19.6 (6)	19.4 ± 15.9 (11)	18.6 ± 12.4 (10)	25.8 ± 3.4 (19)	26.2 ± 13.1 (3)
tetrahymanol	9.2 ± 9.1 (11)	BDL	BDL	12.8 ± 2.9 (21)	13.8 ± 4.3 (5)
2-methyl BHtetrol	11.6 ± 6.2 (5)	11.6 ± 9.1 (8)	19.5 ± 16.0 (9)	15.7 ± 9.0 (17)	-
BHtetrol	12.5 ± 10.5 (10)	9.2 ± 9.3 (11)	BDL	19.7 ± 3.6 (20)	11.8 ± 3.0 (5)

3.5 Hopanoid composition

There were no significant differences in relative amounts of individual hopanoids across the two years of the study and across the three sites. Hopanoid hydrocarbons account for 61% and functionalized hopanoids were 38% of the total. 2-methylhopanoids made up 28% of all hopanoids. Diploptene alone was responsible for 35% of all hopanoids present (Figure 2.5).
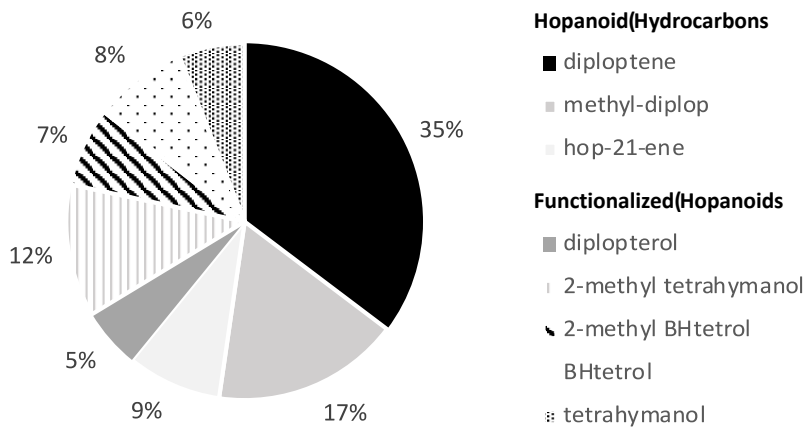
Figure 2.5 Hopanoid composition of the average FGL microbialite including data from 2 years, 2 substrates, 3 sites, and 3 depths using samples through the entire microbialite (0-6 cm). This is the overall hopanoid profile of FGL microbialites.

3.5.1 Hopanoid composition by water depth and light attenuation

Two hopanoids were significantly more abundant at 1 m depth than deeper in the water column: diploptene ($F_{(2,24)}=4.65$, $p=0.02$) and hop-21-ene ($F_{(2,23)}=3.90$, $p=0.03$). The abundance of diploptene decreased from 48% at the surface to 30% (2 m) and 32% (3 m). Hop-21-ene decreased from 12% at the surface to 7% (2 m) and 8% (3 m). In contrast, BHtetrol was the only hopanoid that became more abundant with depth; increasing from 7% at the surface to 12% at 2 m and 13% at 3 m ($F_{(2,22)}=4.31$, $p=0.03$) (Figure 2.6).

There was a change in light intensity in the water column measured at three locations in Green Lake at noon in August 2017. On this single sampling day, microbialites received 80% of surface irradiance growing at 1 m depth, 60% at 2 m, and 50% at 3 m (Figure 2.6 inset).
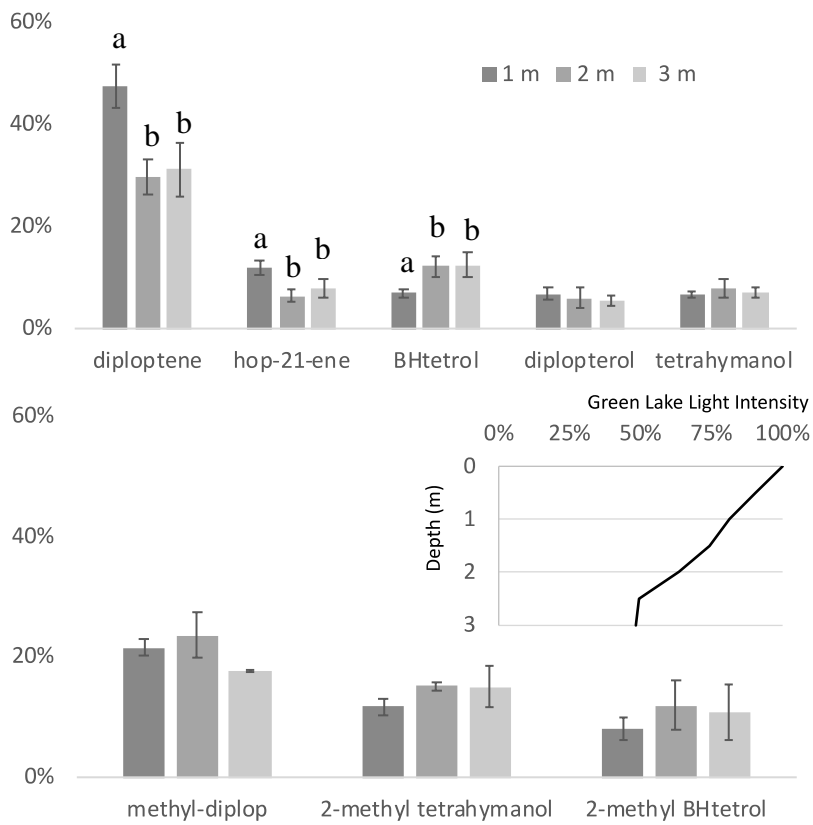
Figure 2.6 Hopanoid composition (percent of total hopanoids) with depth in the water column in FGL microbialites. Statistically significant differences in hopanoid composition are indicated with bars labelled 'a' different from 'b' for an individual hopanoid. Inset graph shows average light attenuation with depth at noon in August 2017.

3.5.2 Hopanoid composition of shelf and wood substrates

      Diploptene accounted for a greater proportion (61%) of hopanoids in microbialites growing on wood substrates than in shelf substrates (35%) (T(25)=3.74, p=0.01). The difference in proportion for the two substrates is related to the absence of 2-methyl BHtetrol in wood-substrate microbialite samples (Figure 2.7).
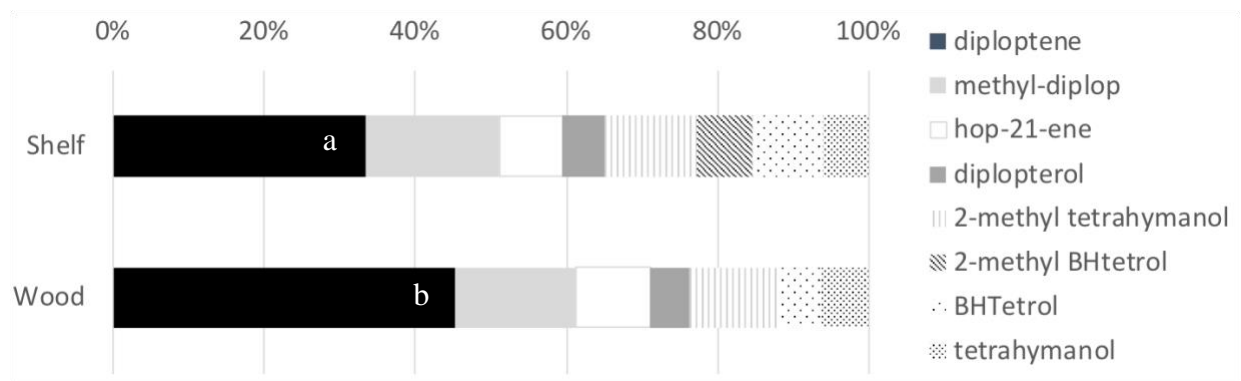
Figure 2.7 Hopanoid composition for shelf (0-6 cm) (n=20) and wood (0-6 cm) (n=5) substrates of FGL microbialites. Greater abundance of diploptene in the wood compared to shelf samples is indicated by labels 'a' and 'b'.

3.5.3 Hopanoid composition from three core depths

The hopanoid composition shifts from the surface to the interior of the microbialite material (Figure 2.8). For shelf-type microbialites, diploptene is proportionally greater at the surface (45%) than in the 1-3 cm portion (27%) or the 3-6 cm portion (22%) ($F_{(2,23)}=5.99$, p=0.008). Similarly, hop-21-ene is proportionally greater at the surface (11%) than in the 1-3 cm (7%) or 3-6 cm (6%) portions ($F_{(2,23)}=4.13$, p=0.0294). In contrast, wood-substrate microbialites exhibit no differences in hopanoid composition with core depth. Table 2.3 provides hopanoid composition details for sites, depths, core depths, and substrates.
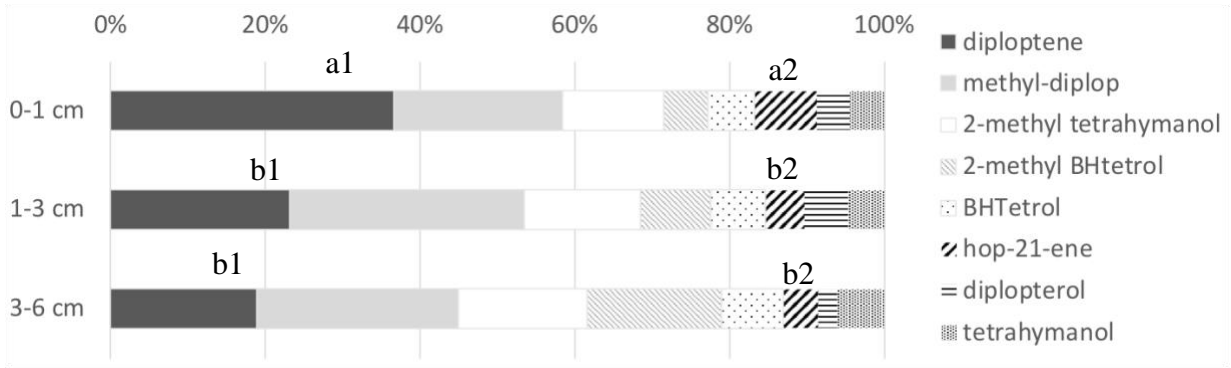
Figure 2.8 Hopanoid composition changes with core depth into the FGL microbialite matrix for shelf-substrate. Significant differences in proportion are indicated for diploptene (a1) and hop-21-ene (a2) with 'a' different from 'b' for an individual hopanoid.

Table 2.3 Hopanoid relative abundance (percent of total hopanoids) in Fayetteville Green Lake microbialites. Sample number is in parentheses after each mean and standard deviation for eight hopanoids at 3 sites, 3 depths, 3 core depths, and 2 substrate types.

Percent of Total Hopanoids	Site			Depth (m)		
	1	2	3	1	2	3
methyl-diplop	20.4 ± 3.2 (4)	19.1 ± 2.3 (3)	24.2 ± 6.7 (5)	21.6 ± 3.4 (6)	23.7 ± 7.7 (4)	17.8 ± 0.1 (2)
diploptene	47.7 ± 18.0 (10)	37.3 ± 17.7 (9)	33.4 ± 9.1 (8)	47.6 ± 16.7 (15)	29.7 ± 8.2 (6)	31.4 ± 13.0 (6)
hop-21-ene	9.10 ± 4.9 (10)	10.5 ± 5.6 (8)	10.0 ± 7.0 (8)	12.0 ± 5.8 (14)	6.5 ± 3.2 (6)	8.1 ± 4.6 (6)
diplopterol	6.4 ± 4.1 (6)	5.2 ± 2.4 (6)	7.1 ± 3.2 (7)	6.9 ± 3.6 (8)	6.1 ± 4.3 (5)	5.6 ± 2.4 (6)
2-methyl tetrahymanol	14.5 ± 6.9 (8)	14.6 ± 5.7 (7)	11.2 ± 3.4 (7)	11.8 ± 4.8 (11)	15.2 ± 1.7 (5)	15.1 ± 8.3 (6)
tetrahymanol	7.3 ± 2.7 (10)	5.8 ± 1.5 (8)	8.1 ± 3.9 (8)	6.7 ± 2.0 (14)	7.9 ± 4.7 (6)	7.1 ± 2.9 (6)
2-methyl BHtetrol	6.1 ± 3.6 (5)	16.6 ± 11.9 (6)	7.5 ± 2.8 (6)	8.1 ± 4.6 (6)	12.1 ± 9.5 (5)	10.9 ± 11.6 (6)
BHtetrol	9.3 ± 5.6 (10)	10.1 ± 4.4 (7)	9.5 ± 4.7 (8)	7.0 ± 2.6 (13)	12.3 ± 5.1 (6)	12.6 ± 5.6 (6)

Percent of Total Hopanoids	Core Depth (cm)			Substrate Type	
	0-1	1-3	3-6	Shelf	Wood
methyl-diplop	29.5 ± 7.2 (9)	35.2 ± 11.4 (12)	31.6 ± 11.7 (12)	21.4 ± 5.2 (11)	23.9 (1)
diploptene	46.4 ± 20.5 (13)	29.1 ± 13.1 (13)	29.8 ± 22.6 (13)	35.3 ± 13.2 (22)	60.7 ± 13.6 (5)
hop-21-ene	10.3 ± 4.8 (12)	6.7 ± 3.3 (13)	5.5 ± 2.6 (12)	9.5 ± 5.3 (21)	11.3 ± 3.4 (5)
diplopterol	7.2 ± 2.4 (8)	4.5 ± 2.3 (5)	4.7 ± 3.5 (2)	6.4 ± 3.2 (18)	3.3 (1)
2-methyl tetrahymanol	10.6 ± 4.5 (6)	14.7 ± 5.4 (11)	15.5 ± 7.1 (10)	13.7 ± 5.8 (19)	12.3 ± 4.6 (3)
tetrahymanol	6.5 ± 6.1 (11)	5.6 ± 2.0 (12)	7.0 ± 3.1 (12)	6.9 ± 3.1 (31)	7.7 ± 1.8 (5)
2-methyl BHtetrol	7.1 ± 4.3 (5)	9.4 ± 6.0 (8)	15.6 ± 7.4 (9)	10.2 ± 8.6 (17)	
BHtetrol	7.9 ± 5.2 (10)	7.7 ± 5.6 (11)	8.8 ± 4.7 (9)	10.1 ± 5.2 (20)	7.5 ± 2.1 (5)

3.6 Lipid identification and relative abundance

Fatty acids ranged from C14 to C26 (Table 2.4). Saturated fatty acids made up ~40% of all fatty acids, while mono-saturated fatty acids accounted for 39% and polyunsaturated fatty acids accounted for 19%. The most abundant fatty acid was C16:0 (palmitic acid) making up 29% of the total fatty acids. The next most abundant were algal fatty acids C16:1ω9 and C18:1ω9 at ~15% and 14%, respectively. The heterotrophic derived odd carbon chain length (C15 and C17) fatty acids made up only 4% of the total and each was less than 1%.

A range of sterols were present in the 0-6 cm microbialite samples including $C_{27}\Delta^{5,22}$, $C_{27}\Delta^5$ (cholesterol), C_{27}-stanol (cholestanol), 24-MeC$_{28}\Delta^{5,22}$, 24-MeC$_{28}\Delta^5$, 24-EtC$_{29}\Delta^{5,22}$, 24-EtC$_{29}\Delta^5$ (Table 2.4). The most abundant sterol in Green Lakes microbialites was 24-EtC$_{29}\Delta^{5,22}$ (36%) and 24-EtC$_{29}\Delta^5$ (32%) followed by $C_{27}\Delta^5$ (16%).

Table 2.4 Average lipid composition of FGL microbialites. 1) Fatty acid composition (% of the total fatty acids) including saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids of nine 0-6 cm portion FGL microbialite samples from three sites combined. 2) Heterotrophic lipid biomarker relative abundance separated from autotrophic biomarkers. 3) Seven sterols from the same nine FGL microbialite samples (percent of total sterols).

Autotrophic Fatty Acids	3
Relative Abundance (%) 4	
Saturated Fatty Acids (SFA)	
14:0	3.0 ± 1.0 5
16:0	28.7 ± 8.7 6
18:0	4.5 ± 4.0
20:0	0.9 ± 1.4
22:0	1.0 ± 0.8
24:0	0.9 ± 0.7
26:0	1.3 ± 0.7
SFA Sum	40 ± 10
Monosaturated Fatty Acids (MUFA)	
16:1w9	14.9 ± 3.4
16:1w7	3.6 ± 2.1
18:1w9	14.0 ± 2.4
18:1w7	6.2 ± 1.5
MUFA Sum	39 ± 5
Polyunsaturated Fatty Acids (PUFA)	
16:2	1.7 ± 0.5
18:2w6	8.6 ± 3.8

Autotrophic PUFA Continued	
18:3	1.2 ± 0.7
18:4	0.7 ± 0.2
20:4w6	1.9 ± 0.8
20:5w3	3.1 ± 1.7
22:6w3	1.6 ± 1.0
PUFA Sum	19 ± 4
Heterotrophic Fatty Acids	
Relative Abundance (%)	
Saturated Fatty Acids (SFA)	
iso-15:0	0.9 ± 0.3
anteiso-15:0	0.7 ± 0.3
cyclo-17:0	0.8 ± 0.4
iso-17:0	0.9 ± 0.9
anteiso-17:0	0.4 ± 0.1
17:0	0.5 ± 0.3
Sum	4 ± 1

Sterol	
Relative Abundance (%)	
$C_{27}\Delta^{5,22}$	1.8 ± 1.0
$C_{27}\Delta^{5}$ cholesterol	16.0 ± 4.5
C_{27} cholestanol	3.4 ± 1.6
$24\text{-MeC}_{28}\Delta^{5,22}$	5.2 ± 1.4
$24\text{-MeC}_{28}\Delta^{5}$	5.4 ± 2.7
$24\text{-EtC}_{29}\Delta^{5,22}$	36.2 ± 7.5
$24\text{-EtC}_{29}\Delta^{5}$	32.4 ± 5.3

3.7 Lipid composition at 3 sites and 3 core depths

There was only one statistically significant difference in the fatty acid or sterol composition at different sites in Green Lake. C16:0 was significantly less abundant at site 1 (19%) than at site 3 (36%) ($F_{(2,6)}$=8.19, p=0.0193).

There were statistically significant differences in the heterotrophic bacteria-derived fatty acid composition with core depth; three key biomarkers were more abundant in the second deepest layer (1-3 cm) than at the surface (0-1 cm). Anteiso-C15:0 was more abundant in the 1-3 cm portion (0.9 %) than at the surface (0.6 %) ($F_{(2,3)}$=13.00, p=0.0333). Cyclo-C17:0 was more abundant in the 1-3 cm portion (2%) than at the surface (1%) ($F_{(2,3)}$=17.74, p=0.0218). And anteiso-C17:0 was more abundant in the 1-3 cm portion (2%) than at the surface (1%) ($F_{(2,3)}$=17.17, p=0.0228).

When heterotrophic (C15 and C17) fatty acid proportions were summed separately from all other fatty acids, heterotrophic-fatty acids were more abundant in deeper core depths (7-8%) than at the surface of the microbialite (4%). Among the heterotrophic fatty acids, cyclo-C17:0 and iso-C17:0 are primarily responsible for these differences with core depth. At the same time a shift in the autotrophic proportions was observed (Figure 2.9). Autotrophic-associated saturated fatty acid biomarkers increased from 30% at the surface of the microbialite to 65% in the 1-3 cm portion and 74% in the 3-6 cm portion. Mono-unsaturated fatty acids similarly shifted from 47% (0-1 cm) to 22% (1-3 cm) and 15% (3-6 cm). Poly-unsaturated fatty acids shift from 20% (0-1 cm) to 7% (1-3 cm) to 5% (3-6 cm).
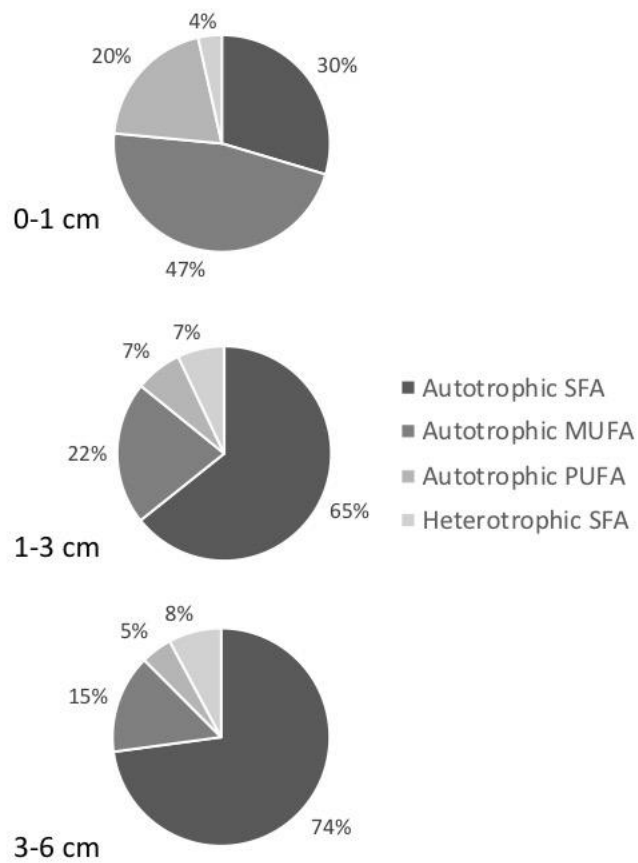
Figure 2.9 Autotrophic and heterotrophic fatty acid composition in Green Lakes microbialites at the surface (0-1 cm) to deeper layers (1-3 cm and 3-6 cm). Heterotrophic fatty acid biomarkers include iso-C15:0, anteiso-C15:0, cyclo-C17:0, C17:0, iso-C17:0, and anteiso-C17:0. All others are considered autotrophic biomarkers.

3.8 Community composition indices

3.8.1 2-methylhopanoid index

For each core depth, the concentration of each hopanoid and its 2-methyl hopanoid were used to calculate the 2-methylhopanoid index (2-MeHI), a ratio of the 2-methyl concentration to the sum of the 2-methyl and its desmethyl hopane. This index has been used to study ancient oceanic anoxic events as well as ancient carbonate depositional environments where 2-MeHI values range from 0-0.3 (Knoll et al., 2007). Index values for FGL range from 0.3 at the surface to 0.8 in the 1-3 cm portion of the microbialite. The 2-MeHI for diploptene was calculated using a sum of the diploptene, hop-21-ene, and diplopterol peaks to account for degradation trends commonly observed in the rock record as well as the complexity of the methyl-diplop peak that included at least three different hopanoid isomers. The diploptene and BHtetrol indices increase toward the deeper layers of the microbialite. Tetrahymanol does not exhibit this trend (Table 2.5).

3.8.2 Homohopane index

The extent of degradation of hopanoids can be calculated with the homohopane index [$C_{35}/(\Sigma C_{31-35})$]. A higher index within the core of the microbialite (0.2 for 3-6 cm portion and 0.16 for the 1-3 cm portion) indicates less degradation, under the assumption that longer side chains are only preserved under anoxic conditions (Peters and Moldowan, 1991) similar to the 2-MeHI discussed above. The higher index at the core of the microbialite (0.2) is associated with anoxic conditions compared to a lower index which was observed at the surface 0-1 cm layer (0.1) (Rashby et al., 2007, Newman et al., 2016) (Table 2.5).

3.8.3 Heterotrophic to autotrophic ratio with lipid biomarkers

The heterotrophic and autotrophic community biomarkers were compared for shelf-substrate and wood-substrate microbialites for the three core depths. The heterotrophic to autotrophic ratio was calculated as [Σ(*iso-* and *anteiso-*C15:0 and *iso-* and *anteiso-*C17:0)/Σ(C16:2, C18:3, C18:4, C18:2ω6, C16:1ω7, C18:1ω9, C18:1ω7, and C16:0)] (Zimmerman and Canuel, 2001). An increase in the heterotrophic to autotrophic ratio was observed with core depth from 0.04 (0-1 cm) to 0.1 (1-3 cm and 3-6 cm) for shelf microbialites (Table 2.5).

Table 2.5 Three community composition indices by core depth in FGL microbialites (shelf-substrates only). 1) The 2-methyl hopanoid index values are listed for diploptene, tetrahymanol, and BHtetrol. A larger ratio indicates more anoxic conditions. 2) Homohopane index with a higher value corresponding to anoxic conditions. 3) Heterotrophic to autotrophic ratio calculated with lipid biomarkers showing lower relative abundance of heterotrophs at the surface of the FGL microbialite.

2-Methyl Hopanoid Index			Homohopane Index	H/A Ratio
	Diploptene (C30)	Tetrahymanol (C30)	BHtetrol (C35)		
0-1 cm	0.3	0.7	0.5	0.1	0.04
1-3 cm	0.5	0.8	0.6	0.16	0.1
3-6 cm	0.5	0.7	0.7	0.2	0.1

4 Discussion

4.1 Community composition of FGL microbialites

Fayetteville Green Lakes (FGL) is the first, to our knowledge, active freshwater microbialite system to be analyzed for hopanoid production. Diploptene, tetrahymanol, and bacteriohopanetetrol as well as their 2-methyl forms were present in all samples. Trends in hopanoid abundance provide insight into FGL microbial community composition beyond what can be understood from culturing or traditional lipid analyses. Culturing of FGL microbialites in media designed to promote cyanobacterial growth also promoted green algae and diatom growth; chemoorganoheterotrophs could not be identified with this method. Lipid analysis provided a general overview of the heterotrophic and autotrophic communities; the heterotrophic to autotrophic ratio shifted from 0.04 at the surface of the microbialite to 0.1 in deeper layers. Fatty acid and sterol analysis also corroborated culturing methods, showing markers for photoautotrophic algae, cyanobacteria, and diatoms at the surface (0-1 cm).

The highly active microbialite surface was dominated by diploptene as well as autotrophic biomarkers of fatty acids (16:1ω7, 16:1ω9, 18:1ω7, 18:1ω9) and eukaryotic (algal) sterols (C$_{29}$). Diploptene and hop-21-ene are proportionally high within this first 0-1 cm layer and are markers for autotrophic non-eukaryotic organisms within a closely packed community. The only known eukaryotic source of diploptene are terrestrial ferns, which are not expected to contribute to diploptene concentrations in FGL microbialites (Ageta et al., 1964; Rohmer et al., 1984; Prahl et al., 1992). Therefore, cyanobacteria and other bacteria are the most likely sources of diploptene and hop-21-ene at the surface of microbialites.

Moving deeper into the microbialite matrix, oxygen is expected to quickly deplete, and light is no longer available. At 1-3 cm, there is a change in microbialite coloration, carbonate

density and hardness, organic carbon content, as well as changes in the hopanoid and fatty acid composition. The decrease in percent PUFAs suggests fewer photosynthetic organisms with core depth and preferential degradation of PUFAs over SFAs, as expected based on lipid degradation studies from other environments (Harvey et al., 1986; Sun and Wakeham, 1994; Sun et al., 1997; Teece et al., 1998). Community change is also indicated by the increased relative abundance of bacteriohopanetetrol and decreasing diploptene biomarkers accompanied by an increase in the homohopane index, which is associated with anaerobic conditions (Peters and Moldowan, 1991). Sulfate is likely an available electron acceptor in metabolism for FGL microbialites due to the high concentration of both carbonate and sulfate from the groundwater that comes into the lake at ~12 meters carrying ions from surrounding gypsum rock deposits (Thompson et al., 1990). No lipid biomarkers specific to sulfate-reducing organisms (i.e., 10-Me16:0) were identified from the samples collected. These have been helpful in other microbial carbonates for identifying sulfate-reducers (Brady et al., 2010; Riding, 2000; Dupraz et al., 2009).

Deeper layers of the microbialite matrix (3-6 cm) appear red to black with large, re-mineralized carbonate pockets (Patterson, 2014). These hardened and dense carbonate patches intermixed with open pockets are evidence of the activity of chemo heterotrophic organisms that cause dissolution and re-mineralization of the carbonate (alkalinity engine) as well as iron-sulfide deposits, common to anoxic microbial sediment (Dupraz et al., 2004, 2009). The presence of sulfate-reducing organisms at these depths have been shown in other systems to be extremely important in determining the carbonate-macrostructure due to metabolic regulation of carbonate dissolution and precipitation (Brady et al., 2014; Dupraz et al., 2004).

Previously, cyanobacteria have been the primary focus of FGL microbialite studies (Thompson et al., 1990; Patterson, 2014; Wilhelm and Hewson, 2012). They are thought to be

the primary drivers of accretion due to exo-polysaccharide production as well as the base of these food-webs. Cyanobacteria are localized to the outer surfaces of the microbialite where they have a competitive advantage for resources (Patterson, 2014). Both filamentous and coccoid cyanobacteria secrete an exo-polysaccharide (EPS) matrix at the surface of the microbialite which results in carbonate accretion and carbon-substrates (food) for the microbialite food-web (Wilhelm and Hewson, 2012). Because of this crucial role in building the microbialite macro-structure, *Synechococcus* (spp.) and *Arthrospira* (spp.) had been the primary focus of FGL studies (Patterson, 2014; Thompson et al., 1990; Wilhelm and Hewson, 2012). However, these organisms are not known to produce hopanoids (Rohmer et al., 1984). Therefore, the abundance of diploptene and hop-21-ene can be attributed to other cyanobacteria, *Oscillatoria* (spp.), or bacteria presently unidentified or unconfirmed due to few prior investigations.

While the fatty acid composition changes dramatically over a short distance (0-6 cm) through the matrix, hopanoid composition shifts only slightly. There are two potential explanations for these trends: 1) the microbial community is changing from the surface to the deeper layers of the microbialite and 2) the biomarkers are preserved in the structure from active-growth which occurs only at the surface (0-1 cm). The results of this study indicate that while there is an abundant and thriving surface community, there is also a rich heterotrophic community deeper in the microbialite that controls the breakdown of organic molecules and alteration of the carbonate deposit. The inner heterotrophic community actually is quite important and ultimately determines the preserved biomarker composition and carbonate structure over the long-term.

While cyanobacteria have been linked to hopanoid production in laboratory cultures, not all FGL hopanoids can be attributed to them. Tetrahymanol was also an important biomarker of

the FGL microbialites and was found in constant proportion (~6%) in most samples. Tetrahymanol has recently been linked to the alphaproteobacteria, aerobic methanotrophic gammaproteobacteria, and sulfate-reducing deltaproteobacteria through genetic analysis (Banta et al., 2015). These proteobacterial groups are metabolically versatile (Newman et al., 2016), which may explain why tetrahymanol does not change dramatically from the surface to the core of the microbialite or with depth in the water column, as the diversity of organisms that produce it may occur throughout. An alternative explanation is that tetrahymanol is produced at the surface and then is not degraded by heterotrophs at lower layers. Tetrahymanol and gammacerane (tetrahymanol is the metabolic precursor) are common biomarkers in anoxic marine sediments (Ten Haven et al., 1989) up to 2.67-2.46 billion years old (Waldbauer et al., 2009).

Similarly, the 2-methylhopanoids were found in constant proportion throughout the FGL microbialite matrix. Despite the changing diploptene proportion with core depth, its methylated form was no more abundant at the surface than at 3-6 cm. Recent genetic analyses have shown that 2-methyl hopanoids cannot be confidently linked to cyanobacteria. Proteobacteria and acidobacteria contain the hpnP gene for methylating hopanoids at the C2 position (Welander et al., 2010). Given these two lines of evidence, it is most likely that the hopanoids found in FGL microbialites are produced by microbes from the surface (autotrophs with diploptene) down to at least 6 cm into the microbialite (heterotrophs with 2-methyl hopanoids, BHtetrol, and tetrahymanol). Roughly dividing FGL microbialites into three depths may not fully capture microbial transitions. A more fine-tuned approach at the millimeter scale may be necessary to fully distinguish hopanoid biomarker differences.

Bacteriohopanetretrol was the only hopanoid to become more abundant with increasing matrix core depth. BHtetrol has been studied in paleo systems (Zarzycki and Portka, 2015), modern marine cyanobacteria (Saenz et al., 2012) and lab cultured proteobacteria (Sessions et al., 2013). BHtetrol has not been observed in freshwater microbialite systems previously. It is unlikely to be a cyanobacterial biomarker since its abundance increased with core depth. Non-cyanobacterial sources tend to be associated with nitrogen metabolism. For instance, in marine sediments, BHtetrol was linked to anaerobic ammonium oxidizing bacteria (Rush et al., 2014) and more specifically studied in a globally-significant nitrogen-fixing cyanobacterium (Saenz et al., 2012). Freshwater microbialites from Cuatro Cienegas had a significant abundance of *Nitrospira* (nitrite oxidizer) in deeper layers (Nitti et al., 2012).

4.2 Lipid biomarkers in microbialites from two freshwater lakes (Pavilion Lake and Green Lake)

There is a striking similarity in lipid biomarker composition in microbialites at Fayetteville Green Lake, New York and Pavilion Lake, British Colombia (a 65 m deep oligotrophic freshwater lake ~12,000 years old) despite large differences in morphology and depth of growth (Brady et al., 2010). Fatty acid abundances were similar for microbialites in the two lakes for long (>C20:0) even-carbon chain fatty acids (~2-3%), for odd-carbon chain fatty acids (~3%), for C16:1 and C18:1 fatty acids (>35%), and poly-unsaturated fatty acids (<20%). The short-chain fatty acids were proportionally greater in FGL microbialites (37%) than in Pavilion Lake microbialites (20-25%). For heterotrophic markers, C15:0 and C17:0 contributed 9-10% in Pavilion Lake microbialites and only 4% in FGL microbialites. While cyclo-C17:0 was present in microbialites in both lakes, Pavilion Lake microbialites also contained the 10-Me-C16:0 biomarker for sulfate-reducers (Brady et al., 2014), while FGL microbialites did not.

Similar to FGL, Pavilion lake microbialites contain *Synechococcus* (spp.) and *Oscillatoria* (spp.) cyanobacteria. Brady et al. (2014) also attribute lipid biomarkers to diatoms in the microbialites. Green algae and eukaryotes are also important contributors to the fatty acid and sterol composition of FGL microbialites and were not a focus of the Brady et al. (2014) study.

This similarity in lipid biomarkers in microbialites at Green Lake and Pavilion Lake as well as the minimal shifts in lipid composition with water column depth in both lakes is surprising considering the substantial differences in environmental conditions (light intensity, temperature, pressure) that occur with depth in both lakes. In FGL, microbialites occur from the surface down to 10 meters. In Pavilion lake, microbialites occur from 7 to 24 m depths. Across these distances, there is significant decrease in light intensity (shown in Figure 6 and reported in Lim et al., 2004). Surface-level microbial communities may experience more significant temperature changes than those at depth. Similar or stable microbial communities in these different conditions suggests that microbialites are likely to be more ubiquitous than previously thought, potentially present in many other lakes in a consistent assemblage. This has been discussed in prior microbialites work as the "global microbialite microbiome" (Foster and Green, 2011; Paerl et al., 2000; White III et al., 2015). In this case, FGL microbialite genetic biomarkers may be comparable to Pavilion Lake's, and we could expect to find hopanoid biomarkers in many other systems. Any differences in hopanoid composition at each site (despite similar genetic composition) may indicate hopanoid function, which is currently not well known.

4.3 Freshwater microbialites and their marine counterparts

A similar hopanoid composition analysis has been completed previously in stromatolites at Shark Bay, Bahamas (Pages et al., 2015). While eight hopanoids were present in all layers of

the Green Lakes microbialites, Shark Bay stromatolites contained only three hopanoids in four distinct layers (no 2-methyl hopanoids). Diploptene was present in the first three layers of Shark Bay stromatolites (1-4%). In the fourth layer, the hopanoid abundance increased to 23% (C27 and C30 hopenes and C31 hopane) (Pages et al., 2015). This community shift was attributed to anaerobic sulfur reducing bacteria in layer 4 using fatty acid markers that were also used in this study for chemotrophic bacteria (iso- and anteiso- C15:0 and C17:0). In Shark Bay stromatolites hopanoids were associated with the microbialite sulfur cycle. In FGL microbialites, the connection between hopanoids and sulfur metabolism was not so clear, further evidence is warranted.

5 Conclusion

Hopanoid composition of microbialites at FGL changed with depth in the water column (1-3 m) and with core depth into the matrix of the microbialite (0-6 cm). Wood-substrates for microbialite development exhibit a similar hopanoid composition as shelf-substrates, but with an even greater proportion of diploptene. The FGL microbialites have a distinct hopanoid fingerprint comprised of >30% diploptene, <10% each of BHtetrol and tetrahymanol, and ~10% each of 2-methyl hopanoids.

FGL microbialite hopanoids support the hypothesis that hopanoids are indicators of a particular niche of closely-packed, oxygen-limited, high osmolarity conditions where sessile microbial communities grow (Newman et al., 2016). Variable microbial metabolisms FGL hopanoids exhibit shifts by core depth with diploptene and hop-21-ene associated with the photoautotrophic surface layers and BHtetrol associated with non-oxygenic metabolisms in deeper layers. Tetrahymanol and 2-methyl hopanoids did not change in abundance throughout

the matrix, indicating either that the organisms that produce them are able to grow throughout the 0-6 cm profile or that these molecules are preserved.

There is a similarity in the lipid composition of FGL and Pavilion Lake microbialites despite large differences in morphology. This supports the idea that individual community members may be extremely diverse (up to 600 'species' in Bahamian stromatolites (Baumgartner et al., 2006)), but that the metabolic niches are conserved, and spatially arranged as has also been observed in stromatolites and microbial mats (Dupraz et al., 2009; Wilhelm and Hewson, 2012) as well as other freshwater microbialites (Nitti et al., 2012). There may be a hopanoid composition similar to FGL in the Pavilion Lake microbialites and other freshwater microbialite communities. A key feature of these systems is that they exhibit stable community composition horizontally (by site) despite great variability in macro-structure and environmental conditions, but variable community composition over the short vertical scale (Nitti et al., 2012).

Hopanoid analysis allowed for a better investigation of the microbial community of FGL microbialites than culturing or lipid analysis alone. This study provides the first hopanoid analysis of freshwater microbialites and adds Central New York's hardwater meromictic lake to the list of environmental locations for hopanoid-producing communities. Hopanoid biomarker changes with core depth indicate that there is active microbial growth, mainly heterotrophic, below the surface (1-3 cm, 3-6 cm) which could account for the dissolution-remineralization processes in Fayetteville Green Lake microbialite structures (Brady et al., 2010; Dupraz et al., 2009; Patterson, 2014; Nitti et al., 2012).

Acknowledgements

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Appendix A

Hopanoid biomarkers in FGL microbialites.

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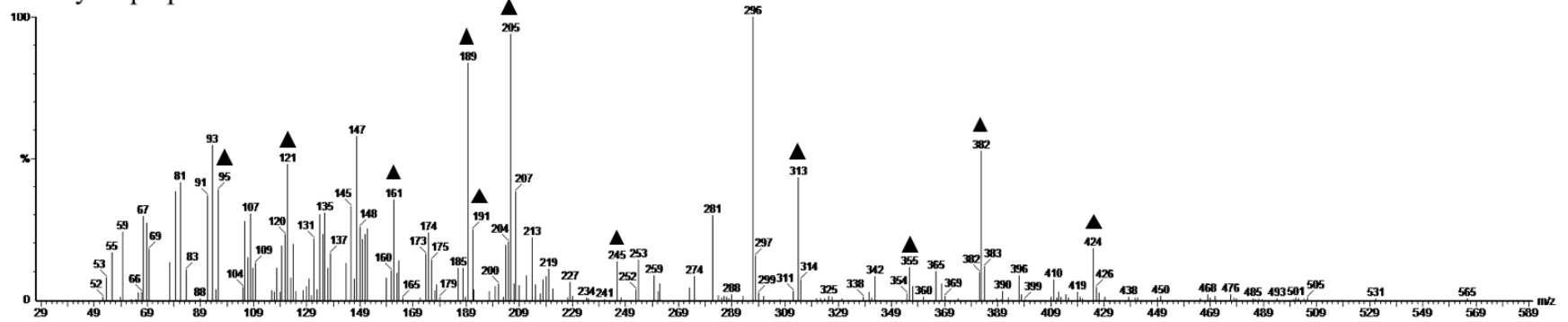
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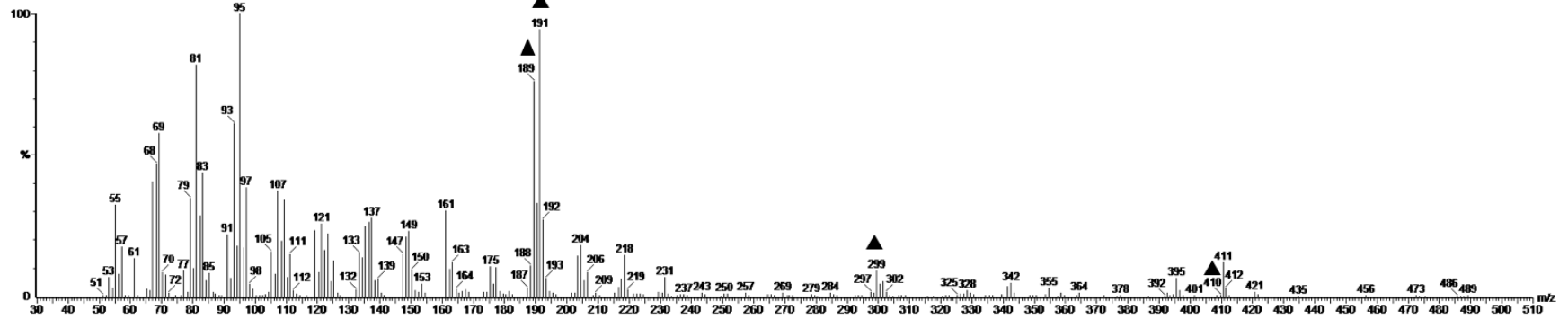
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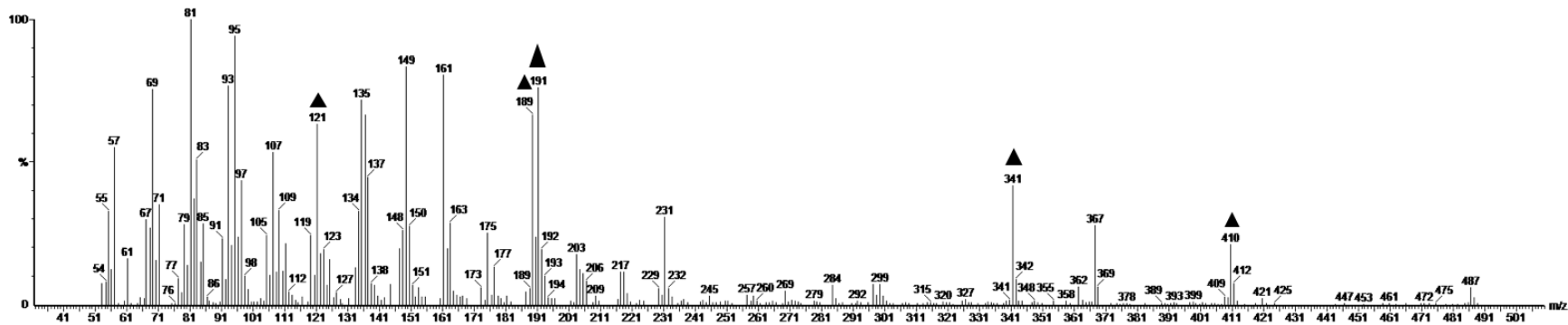
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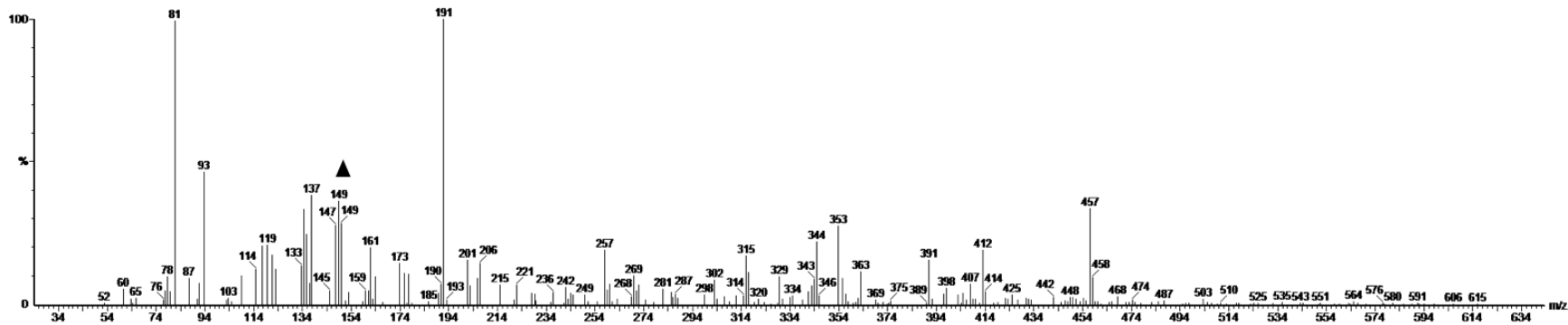
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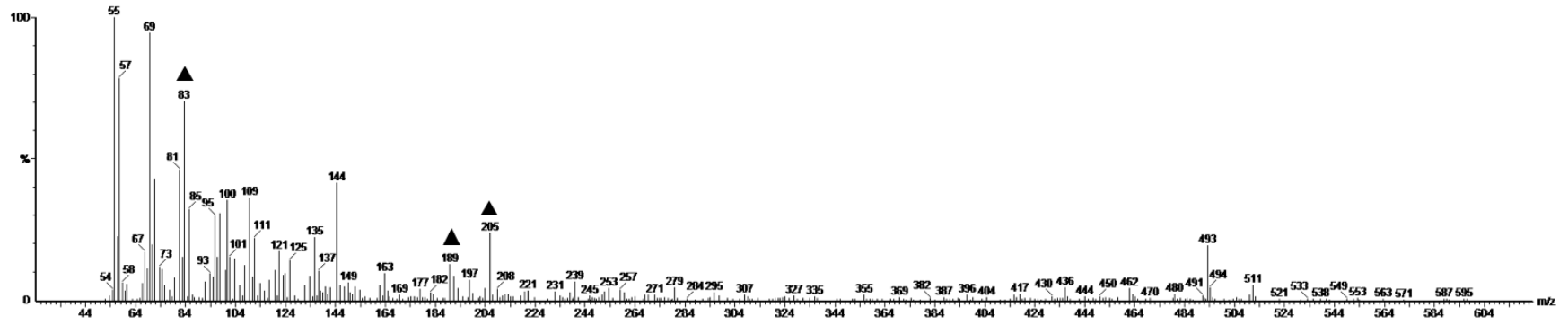
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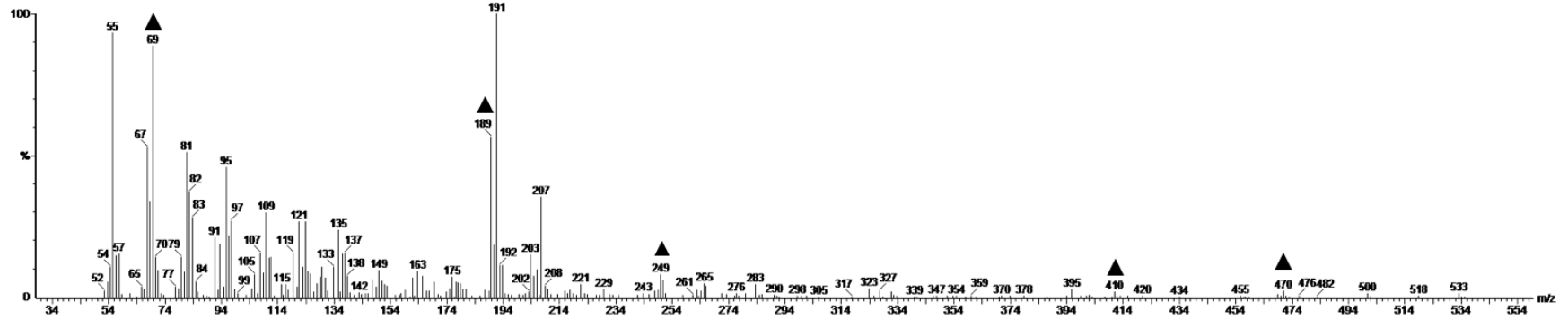
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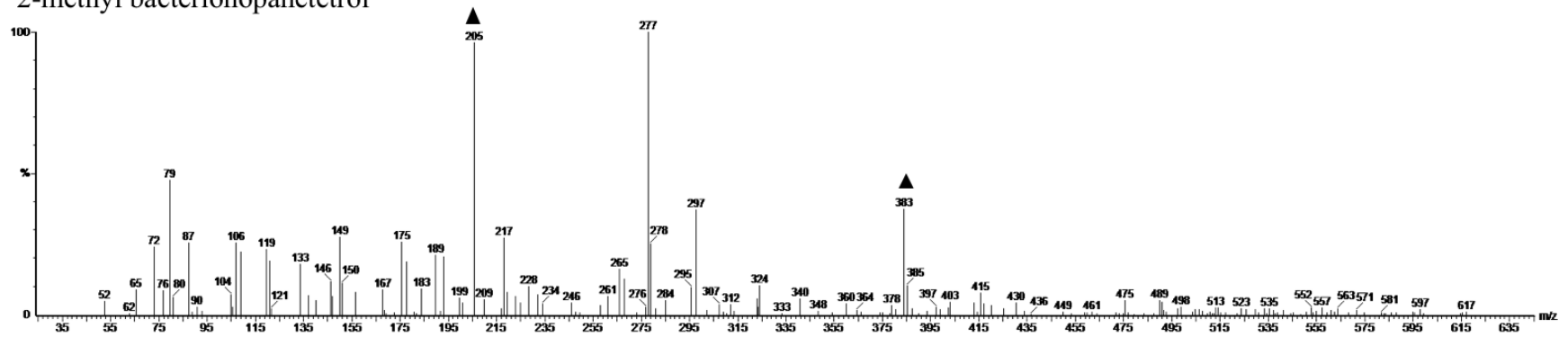
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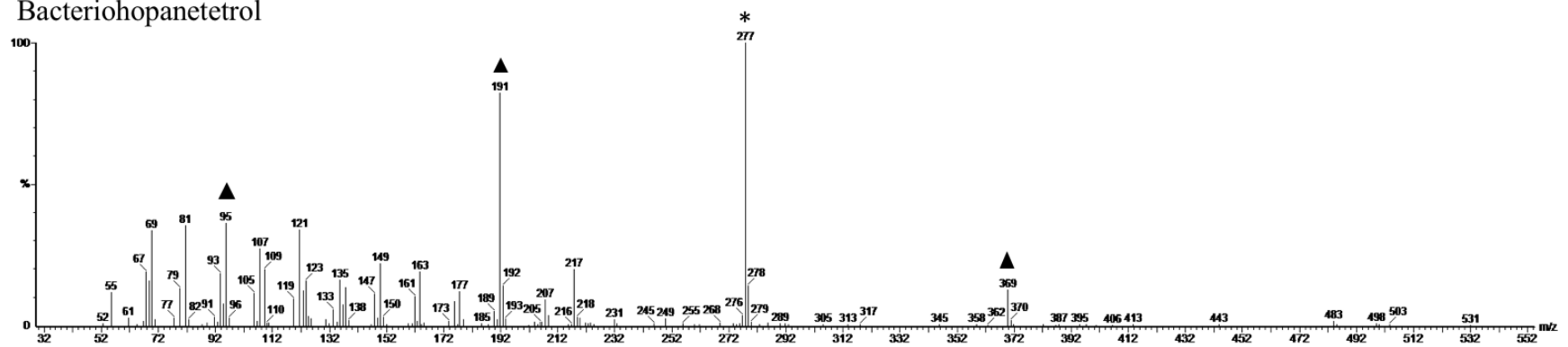
Appendix B

Z8 culture was prepared following exactly the standard procedure (Staub, 1961; Kotai, 1972; NIVA, 1976), using proportions from 4 solutions diluted to 1 L with DI water, autoclaved, and pH adjusted to 6-7. 1) Ten mL of solution 1 (46.7 g $NaNO_3$, 5.9 g $Ca(NO_3)_2 \cdot 4H_2O$, 2.5 g $MgSO_4 \cdot 7H_2O$ diluted to 1 L DI water). 2) Ten mL of solution 2 (3.1 g K_2PO_4 and 2.1 g Na_2CO_3 diluted to 1 L of DI water). 3) Ten mL of solution 3 (2.80 g $FeCl_3 \cdot 6H_2O$ dissolved in 100 mL of 0.1 N HCl EDTA solution (10 mL) and 3.9 g EDTA-Na_2 in 100 mL of 0.1 N NaOH (9.5 mL) and then diluted to 1 L DI water). 4) 1 mL of solution 4 which was a combination of metals in trace concentration and diluted to 1 L of DI water. Solution 4 contained 1 mL each of the following: 0.330 g/100 mL $Na_2WO_4 \cdot 2H_2O$, 0.880 g/100 mL $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 1.20 g/100 mL KBr, 0.83 g/100 mL KJ, 2.87 g/100 mL $ZnSO_4 \cdot 7H_2O$, 1.55 g/100 mL $Cd(CNO_3)_2 \cdot 4H_2O$, 1.46 g/100 mL $Co(NO_3)_2 \cdot 6H_2O$, 1.25 g/100 mL $CuSO_4 \cdot 5H_2O$, 1.98 g/100 mL $NiSO_4(NH_4)_2SO_4 \cdot 6H_2O$, 0.410 g/100 mL $Cr(NO_3)_3 \cdot 9H_2O$, 4.74 g/100 mL $KAl(SO_4)_2 \cdot 12H_2O$; and 10 mL of 0.089 g/100 mL V_2O_5 and 100 mL of a solution containing 3.10 g H_3BO_3 and 1.60 g of $MnSO_4 \cdot H_2O$ diluted to 1000 mL DI.

Chapter 3: Lipid biomarkers in Great Salt Lake microbialites (Utah, USA)

Abstract

The Great Salt Lake food web starts with a thriving microbial community built on carbonate mounds (microbialites) which provide substrate and nutrients for brine shrimp, fly larvae, and thousands of migrating birds. These microbialite structures are a complex community of microorganisms whose net metabolism results in the precipitation of carbonate to form massive bottom mounds around the lake shore. These structures host the majority of the lake's biotic diversity with Eukarya, Bacteria, and Archaean components all tightly packed into a centimeter-deep space. This study used lipid biomarkers to learn about the microbial dynamics of Great Salt Lake microbialites. Diploptene, tetrahymanol, and bacteriohopanetetrol were found in all microbialite locations and were preserved to at least 3 cm depth in the carbonate substrate. Lipid biomarker analysis suggests that Great Salt Lake microbialites share a microbial composition with other freshwater and marine microbialites from around the world. Understanding the internal microbial interactions of the Great Salt Lake microbialite structures reveals the biotic complexity of this halophilic environment and allows for comparison with modern and ancient examples of carbonate-producing microbial communities in extreme environments.

1 Introduction

The Great Salt Lake (GSL) appears to be a lifeless, barren environment, too salty (6-28% in the southern arm (Stephens, 1990)) to support an intricate food web and for many years was viewed as biologically deficient (Stephens, 1974). There is a limited fishery (Rainwater killifish, *Lucania parva*) (Arnow and Stephens, 1990), and the only aquatic invertebrates include brine shrimp (*Artemia franciscana*) and brine flies (*Ephydra gracilis* and *Ephydra hians*) (Wurtsbaugh and Gliwicz, 2001; Wurtsbaugh et al., 2011). Despite an apparent lack of biotic productivity, brine shrimp grow to high density in the water column and brine flies thickly coat the mud beaches along the shore (Wurtsbaugh and Gliwicz, 2001; Wurtsbaugh et al., 2011). Brine shrimp are harvested from the Great Salt Lake and sold as feed in aquaculture (Wurtsbaugh and Gliwicz, 2001). These organisms are key food sources for migrating water birds, the highest trophic level for this aquatic ecosystem (Roberts and Conover, 2014; Wurtsbaugh et al., 2011).

A highly productive invertebrate population in Great Salt Lake relies on primary producers in the water column (Wurtsbaugh and Gliwicz, 2001). The larval life-stage of both shrimp and flies also relies on a hard substrate for attachment (Baskin, 2014; Roberts and Conover, 2014). The lake-bottom is primarily composed of mud and oolitic sand, except along lake shores where carbonate mounds actively grow due to the metabolic activity of a complex, halophilic microbial community (Roberts and Conover, 2014; Wurtsbaugh et al., 2011). These microbial structures are called microbialites, a type of microbialite similar to those in other saline environments like Highborne Cay, Bahamas (Bowlin et al., 2012) or Shark Bay, Australia (Nitti et al., 2012). In these microbial communities, metabolisms are typically spatially and/or temporally arranged such that a large number of taxonomic groups co-exist within a small space competing for limited nutrients (Brady et al., 2014; Mobberley et al., 2015). Metabolic types

may include photoautotrophs (limited by light and oxygen) as well as microbes linked to nitrogen, sulfur, and iron cycles (Patterson, 2014). The microbialites in Great Salt Lake are currently known to host the cyanobacteria (photoautotrophic), *Aphanothece packardii*, (Chagas et al., 2016; Stephens, 1974) as well as sulfate reducers, like *Desulfohalobium utahensis* (Brandt et al., 2001; Kjeldsen et al., 2007; Pace et al., 2016; Schneider et al., 2013) and methanogens (Paterek and Smith, 1985).

These microbialites are the foundation of the Great Salt Lake food web. Genetic analyses of this community completely alter the perception of the Great Salt Lake as biologically-deficient (Lindsay et al., 2016). The lake supports a wide range of microorganisms (Eukarya, Bacteria, Archaea) whose net metabolic activity causes the precipitation of carbonate-mounds along the bottom of the lake (Dupraz and Visscher, 2005; Lindsay et al., 2016). These microbial mounds provide food and substrate for the brine shrimp and flies (Roberts and Conover, 2014) and may also be important in the bioaccumulation of methyl-mercury from the water column, through the invertebrate populations (152-659 ng Hg g^{-1} dry weight), and then to water birds (8000 ng Hg g^{-1} dry weight) (Wurtsbaugh et al. 2011).

The GSL microbialites are reminiscent of other actively accreting microbialites or stromatolites that occur globally and serve as modern analogues to ancient carbonate-producing microbial structures that have been preserved in the rock record through 80% of Earth history (Allen et al., 2010; Cerqueda-Garcia et al., 2016; Dupraz and Visscher, 2005; Mobberley et al., 2015; Pace et al., 2016). These ancient microbes are the oldest known life forms on Earth and are therefore valuable for learning about the evolution of life (Burns et al., 2011; Talbot et al., 2008). For example, Great Salt Lake microbialites may be compared to the ancient Green River formation microbialites with both systems hosting a high density of invertebrates (Chidsey et al.,

2015). Modern and ancient microbial carbonates have a wide variety of forms. While GSL microbialites appear to be a carpet of carbonate along the lake bed, the ones in Fayetteville Green Lake, NY are thick shelves (Chapter 2), the ones at Pavilion Lake are bottom-mounds (Brady et al., 2010), and the marine microbialites at Shark Bay have a variety of forms including flat sheets and pinnacles depending on the microbial community (Suosaari et al., 2015).

Great Salt Lake microbialites also serve as a rare saline microbial-carbonate (non-marine) thought to be the type of environment where early life could have started (Chagas et al., 2016). Another modern hypersaline lake with microbialites has been studied at Eleuthera Island, Bahamas (Dupraz et al., 2004). In fossilized structures, researchers generally cannot use molecular techniques that are common for modern structures. DNA, proteins, and carbohydrates quickly degrade (Burns et al., 2011; Nitti et al., 2012; Talbot et al., 2008), and morphology is often difficult to decipher for determining what is biogenic and what is not (Burns et al., 2011; Chagas et al., 2016). Instead, lipid biomarkers are preserved intact in these ancient structures that can connect knowledge of modern biology to ancient systems (Allen et al., 2010; Zarzycki and Portka, 2015).

Lipid biomarkers are molecules that can be linked to the specific organisms that produced them (Allen et al., 2010; Jungblut et al., 2009). For instance, diatoms tend to produce polyunsaturated fatty acids (PUFAs) with a long carbon-chain length, so that when C20:4ω6 or C20:5ω3 are present, diatoms are most likely present (de Cavalho and Caramujo, 2014); however, the specificity is not perfect. For instance, *Bacteroidetes*, eukaryotes, and other marine microbes also produce these PUFAs (Allen et al., 2010); however, identifying a number of organism-specific lipid biomarkers can help. Fatty acids (partially-hydrophobic) and sterols (hydrophobic) are cell-membrane constituents; their hydrophobicity alters their interaction with

decomposers (Harvey et al., 1986; Krasowska and Sigler, 2014). Hopanoid biomarkers are one highly persistent lipid biomarker from environmental settings which are also stable under high pressure and heat conditions (Sessions et al., 2013).

Hopanoids are similar in structure and function (membrane constituents) to eukaryotic sterols (cholesterol) (Figure 3.1) but are associated with prokaryotic sources like cyanobacteria and proteobacteria (Talbot et al., 2003; Welander et al., 2010). They contain five carbon rings with functional group variety off the fifth ring (R-groups) (Kannenberg and Poralla, 1999). Some hopanoids (bacteriohopanetetrol or C35) have hydrophobic and hydrophilic properties with oxygen atoms on the R-group (Talbot et al., 2003). These unique functional groups are expected to have different roles in regulating membrane fluidity/rigidity as well as being potential markers for specific metabolisms (Saenz et al., 2012) or a specific environmental niche (Ricci et al., 2014; Saenz et al., 2012).
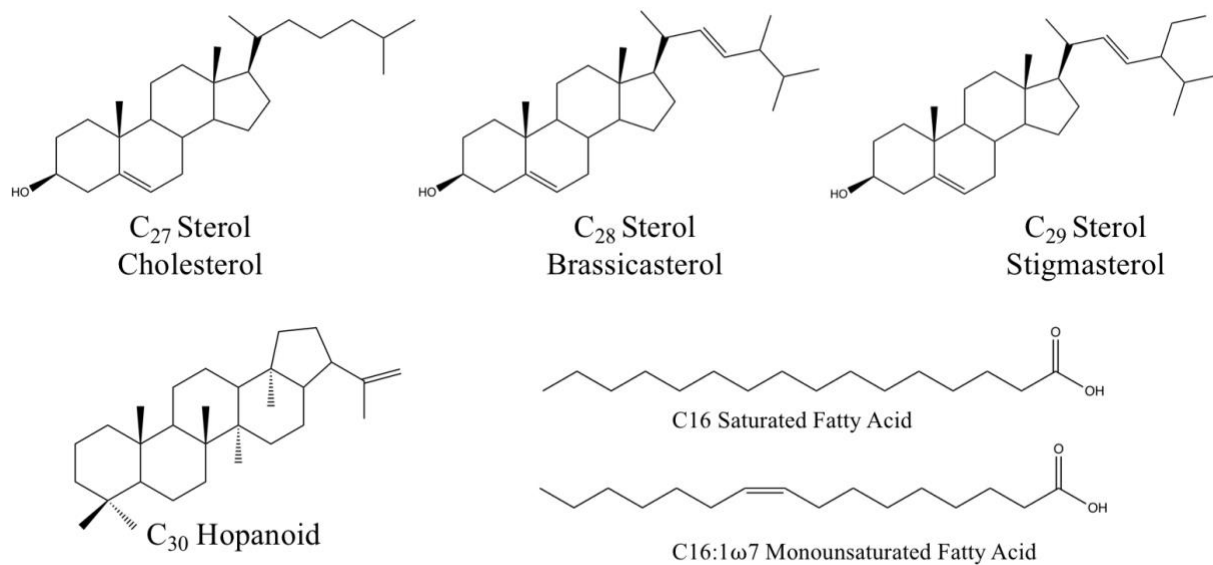
Figure 3.1 Structural comparison of lipid biomarkers including three sterols (cholesterol,

brassicasterol, stigmasterol), hopanoid (diploptene), and saturated and monounsaturated palmitic

acid (C16 fatty acid).

While cyanobacteria are known sources of hopanoids, recent genetic analysis has shown squalene-hopene cyclase (shc) genes for hopanoid biosynthesis are much broader taxonomically (Ricci et al., 2014). Tetrahymanol in particular is strongly associated with proteobacteria, microorganisms that are metabolically flexible, but not tied to oxygenic photosynthesis (as was once thought to be a requirement for hopanoid biosynthesis) (Banta et al., 2015; Saenz et al., 2015). Additionally, the known sources of 2-methyl hopanoids (methylation at carbon 2 on the first ring) have expanded to include both cyanobacteria and proteobacteria due to genetic analysis of hopanoid enzymes (Welander et al., 2010).

The use of hopanoid biomarkers in paleo systems is currently limited by lack of understanding about the distribution of hopanoid producing organisms and their cellular role (Talbot et al., 2008). For that reason, this work aims to use standardized methods (Sessions et al., 2013) to extract hopanoid biomarkers from the Great Salt Lake microbialites, while linking these compounds to lipid biomarkers more generally to contribute to both modern microbial ecology (Martin-Creuzburg et al. 2008) and ancient microbial community structure. The main objectives are 1) to determine if microbial communities in Great Salt Lake microbialites are consistent across different sites and 2) to determine the relationship between Great Salt Lake microbialites and other extant microbialites (freshwater and marine) based on microbial lipid biomarkers.

2 Methods

2.1 Sample collection

In July 2017 samples were collected from five separate microbialites from each of three sites in the southern arm of Great Salt Lake, UT (Figure 3.2) under a Right of Entry Permit from the State of Utah (#41000610). Sites were accessed after consultation with State Park managers by walking and wading (Antelope Island site) or with a 15' aluminum drift boat with outboard

motor (Fremont Island and GSL Marina sites). Microbialites were barely visible from the boat and accessed directly by wading in the 3-4' deep water. Samples were separated from larger microbialites using a hammer and chisel and stored on ice in Zip-Lock bags (Figure 3.3). Samples were immediately rinsed with deionized water and then stored frozen under dry ice and shipped to SUNY-ESF for storage (-20°C). Underlying grey carbonate deposits were removed from the active dark-colored microbial mat (between 1-5 cm thick) using a razor and chisel. Large feathers were removed from the active microbial mat portions (brine fly casings were left in the matrix), then samples were freeze-dried for at least 36 hours (-60°C), ground with a mortar and pestle, and stored for analysis (-20°C).

A subset of Fremont Island samples was separated into three core depths 0-1 cm, 1-2 cm, and 2-3 cm using a chisel and razor (n=2 at each depth). These were also freeze-dried (36 hours, -60°C), ground, and stored for analysis (-20°C).
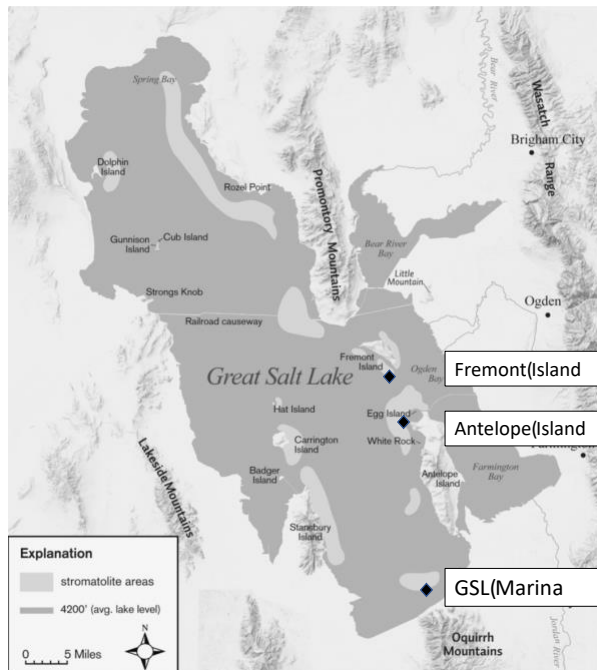
Figure 3.2 Map of sampling locations in the Great Salt Lake, UT (Modified from Utah Geological Survey, 2012). Light coloration shows microbialite locations against grey lake background. Dark diamonds are sampling sites including Fremont Island (41.13046 N, 112.31272 W), Antelope Island (41.04006 N, 112.2782 W), and Great Salt Lake (GSL) Marina (40.73744 N, 112.21815 W).

thin green mat

thicker black mat

brine fly larvae

Figure 3.3 Microbialite from Great Salt Lake (Fremont Island site) sampled in July 2017. Left side of the sample contains a thin light green microbial mat that is different from the right side of the image with a thicker dark green-black microbial mat where brine fly larvae casings are attached. Total depth of the microbial layer is ~1 cm before the substrate becomes grey carbonate.

2.2 Organic carbon and carbonate content

Microbialite material (1.0 g) was completely dried (60°C, 36 hours) then stored in clean, dry ceramic crucibles and heated to 550°C for four hours in a muffle furnace. Mass difference was calculated as the organic carbon content. Subsequent heating to 1000°C for two hours determined the carbonate content following standard methods (Dean, 1994).

2.3 Hopanoid Analysis

2.3.1 Extraction and derivatization

Procedures for hopanoid extraction and derivatization were followed without modification from Chapter 2, which were modified from methods for lab-cultured microbes, optimized by Sessions et al. (2013). Ground microbialite samples (6 g) were extracted by sonicating with 10 mL of 1:2:0.9 dichloromethane (DCM):methanol:water (20 min) in 50 mL Teflon centrifuge tubes. Samples were centrifuged (2000 rpm, 10 min) and the organic phase was collected by filtering through a glass pipette packed with pre-combusted glass wool (500°C, 1 hr). Two additional extracts were added to the initial extract using 10 mL of 1:1 DCM:water. The combined total organic phase was gently dried under N_2 gas and stored at -20°C in 2 mL storage vials.

The total organic extract was derivatized for gas-chromatography mass spectrometry using 100 µL of 1:1 acetic anhydride:pyridine (70°C, 20 min). The samples were immediately transferred to GC vials with inserts after adding 100 µL of cholestane (Sigma) internal standard in pyridine (70 µg/L). Samples were immediately injected onto the GC-MS and extraction blanks were measured with every set of five microbialite samples.

2.3.2 Gas chromatography-mass spectrometric analysis

Hopanoids were analyzed by gas chromatography-mass spectrometry (GC-MS) using a

Perkin-Alma Clarus 580 GC and Clarus SQ85 quadrupole MS and TurboMass software. Derivatized samples (1 µL) were injected (splitless mode, 300°C) on a DB-5 (J&W Scientific) column (30 m, ID 0.25 mm, film thickness 0.25 µm). The oven program was 100°C (2.0 min hold) to 250°C at 15°C/min and then to 320°C (30 min hold) at 15°C/min. The helium carrier gas was set to a constant flow rate of 10.0 ml/min. The mass spectrometer was operated in full scan mode over 50-620 EI+ (MS transfer line at 250°C and ion source at 200°C).

Diagnostic ion peaks, relative retention times, and original hopanoid spectra (Summons and Jahnke, 1992) were used to identify hopanoids in GSL samples (Sessions et al., 2013). Total ion chromatogram (TIC) peaks were manually integrated as were hopanoid peaks with single ion monitoring (SIM) at m/z 191, to determine relative peak areas based on the cholestane internal standard. Because cholestane does not have a major ion peak at m/z 191, a conversion factor was determined for each sample by comparing the TIC area to the SIM m/z 191 area for the diploptene peak (~22.7 min). This conversion factor was used to calculate the relative area of each hopanoid peak. Concentrations (µg/g C_{org}) were determined based on mass of organic carbon in each sample (LOI). Hopanoid proportions were determined as a fraction of total hopanoids in a given sample.

2.4 Lipid Analysis

2.4.1 Extraction and derivatization

Fatty acids and sterols were extracted from five samples at each site. Dry, ground microbialites (2 g) were vortex mixed (5 min) with 1:1 DCM:methanol (2 mL). The total lipid extract (TLE) was transferred to a new vial using a clean glass pipette and the extraction was repeated twice more. The combined TLE was dried completely under N_2 gas (50°C). Fatty acids and neutral sterols were then separated by alkaline hydrolysis using 2 mL of KOH (5%) in

methanol (1 hr, 70°C). Samples were cooled, and 2 mL of water was added. Then 9:1 hexane:ether (5 mL) was added, samples were inverted to mix and allowed to separate. The neutral sterol fraction was transferred with a clean glass pipette to a separate vial and the extraction was repeated twice, then dried under N_2 gas (50°C). The aqueous layer was acidified to pH 2 with 6N HCl (1 mL). The resulting fatty acids were then extracted with 9:1 hexane:ether (5 mL) three times. All samples included a $C_{23:0}$ (Sigma) (100 μL of 200 mg/L in pyridine) and cholestane (Sigma) (100 μL of 200 mg/L in hexane) internal standards. The combined fatty acids were dried under N_2 gas (50°C) and all vials were stored frozen at -20°C for up to two weeks.

Fatty acids were methylated by adding 5 μL of toluene then 0.5 mL of methanolic HCl (Supelco), vortex mixed, and heated (1 hr., 60°C). The cap seal was broken, samples were cooled (3 min), then milliQ water (1 mL) was added. The fatty acid methyl esters were extracted into a new vial with hexane (2 mL) three times and dried under N_2 gas (50°C).

Both neutral sterols and fatty acid methyl esters were silylated for gas chromatography-mass spectrometry by first adding toluene (5 μL), vortex mixing, and evaporating under N_2 gas (50°C). Next, DCM (5 μL) and N,O-Bis(trimethylsilyl)trifluoro-acetamide (BSTFA) (Supelco) (10 μL) were added, vortex mixed, and heated (30 min, 60°C). Samples were cooled (1 min) and dried under N_2 gas (50°C). Samples were transferred to GC vials with DCM (0.8 mL) and injected on the GC-MS immediately. Cod liver oil was also extracted using the above methods. Blanks were extracted with every set of 5 microbialite samples.

2.4.2 Gas chromatography-mass spectrometric analysis

Fatty acid methyl esters and sterols were separately analyzed and identified using a Perkin-Alma Clarus 580 GC and Clarus SQ85 quadrupole MS with TurboMass software. The derivatized samples (1 μL) were injected (splitless mode, 280°C) on a DB-5 (J&W Scientific)

column (30 m, ID 0.25 mm, film thickness 0.25 µm). Oven program was 60℃ (1.0 min hold) to 140℃ at 15℃/min and then to 300℃ (15.0 min hold) at 4℃/min. The helium carrier gas was set to a constant flow rate of 10.0 ml/min. The mass spectrometer was operated in full scan mode over 50-620 EI+ (MS transfer line at 250℃ and ion source at 200℃).

Diagnostic molecular ions and relative retention times allowed for the identification of fatty acids methyl esters (FAMEs) and comparison with known FAMEs in the cod liver oil standard. The TIC was manually integrated for each peak of interest to determine concentration, taking into account the mass of organic carbon in each sample. Both FAME and neutral sterol content were calculated as proportion of the total FAME or neutral sterols in a given sample.

2.5 Statistical analysis

All statistical analyses were completed in Statistical Analysis System (SAS University Edition software, copyright 2014). One-way analysis of variance (ANOVA) with post-hoc Tukey HSD were used to test for significant differences in hopanoid and lipid concentrations by site and by core depth at one site (Fremont Island). To analyze biomarker composition, each proportion was transformed prior to statistical testing with the logit transformation [$\hat{y}=\ln(y/(1-y))$] to normalize the data (Warton and Hui, 2011). Tests for normality included four default tests from SAS: Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling. Tests were considered significant at $\alpha=0.05$ for all analyses.

3 Results

3.1 Organic carbon and carbonate content

The organic carbon and carbonate content of microbialite samples was similar at the three sites (Fremont Island, Antelope Island, and GSL Marina) and for the three core depths in the samples from Fremont Island. The average organic carbon content for GSL microbialites was

1.5% ± 0.9. The average carbonate content for GSL microbialites was 4.3% ± 0.7. Three of the GSL Marina samples contained 3-5% organic carbon, which was much greater than the average organic carbon content based on Grubb's Test for Outliers. Organic carbon content was therefore used as an adjustment on the hopanoid and lipid quantification so that concentration was not influenced by the total amount of organic matter present in any given sample.

3.2 Hopanoid identification and quantification

Three hopanoids found in the GSL microbialites matched those identified by Sessions et al. (2013): diploptene, tetrahymanol, and bacteriohopanetetrol (BHtetrol). Two unidentified peaks eluted before the diploptene peak (22.7 min). These peaks had diagnostic ions closely resembling diploptene and not the other potential hopanoids that are listed in Sessions et al. (2013) (i.e., hop-21-ene or hop-17(21)-ene). These peaks are therefore suspected to be structural isomers of diploptene (Appendix A). In prior work, there has been some debate as to whether the isomers of diploptene are naturally present or an artifact of the analytical process (Garcia Costas et al., 2011; Sessions et al., 2013). To account for this uncertainty in further data analysis, the diploptene isomers were summed.

Similarly, two potential isomers of tetrahymanol were found in many of the GSL microbialite samples (Appendix A), so they were summed. Elution times and diagnostic ions for all three hopanoid biomarkers and their isomers is shown in Table 3.1. All composition analyses (using proportions and not concentrations) use each of the six hopanoids separately, not sums of isomers to remain comparable to hopanoid studies of other systems (i.e. Green Lakes microbialites in NY).

Table 3.1. Six hopanoid biomarkers were found in GSL microbialites including diploptene and two isomers, tetrahymanol and one isomer, and BHtetrol. Retention times are listed relative to diploptene (22.7 min). Diagnostic ions are listed with the base peak bolded. Relative abundances (percent of the total hopanoid) are shown for each site in the Great Salt Lake (n=5 microbialites at each site). Limit of detection was 0.05%.

	GC-MS		Relative Abundance (%)		
	Relative retention time	Diagnostic Ions	Fremont Island	GSL Marina	Antelope Island
Diploptene	1.00	410, 299, **191**, 189, 95	BDL	28 ± 23	8.5 ± 5
Diploptene$_a$	0.93	410, 299, **191**, 189, 95	49 ± 17	35 ± 17	38 ± 13
Diploptene$_b$	0.93	410, **191**, 95	36 ± 25	17 ± 4	18 ± 9
Tetrahymanol	1.26	470, 410, 249, **191**, 189, 69	9 ± 3	15 ± 8	20 ± 2
Tetrahymanol$_a$	1.27	341, 327, **191**, 69	5 ± 1	5 ± 1	10 ± 5
BHtetrol	1.65	493, 369, **191**, 95	4 ± 2	5 ± 2	14 ± 2

3.3 Hopanoid concentration

The total hopanoid concentration (as measured with the SIM *m/z* 191 peaks) in GSL microbialites was ~70 ± 30 µg/g C_{org} (n=15). There was no significant difference in total hopanoid concentration at the three sites (n=5): Fremont Island (60 ± 40 µg/g C_{org}), Antelope Island (90 ± 60 µg/g C_{org}), and GSL Marina (30 ± 10 µg/g C_{org}). For individual hopanoids, BHtetrol was consistently the least concentrated (8 ± 9 µg/g C_{org}) while diploptene was most concentrated (40 ± 20 µg/g C_{org}). Tetrahymanol concentration was 20 ± 15 µg/g C_{org}.

3.4 Hopanoid composition

3.4.1 Hopanoid composition by site

The relative abundances of tetrahymanol and BHtetrol were different between the three sites with Antelope Island microbialites having significantly more tetrahymanol (20%) compared to Fremont Island microbialites (9%) and GSL Marina microbialites (15%) ($F_{(2,9)}$=5.56, p=0.0268) (Figure 3.4). Similarly, BHtetrol at Antelope Island microbialites (14%) was significantly more abundant than at Fremont Island microbialites (4%) and GSL Marina microbialites (5%) ($F_{(2,7)}$=20.4, p=0.0012).

Despite great differences in texture, coloration, and density of material for the Fremont Island core depth samples (n=2 at each depth), there were no significant differences in hopanoid composition in the core depth portions (Figure 3.5).
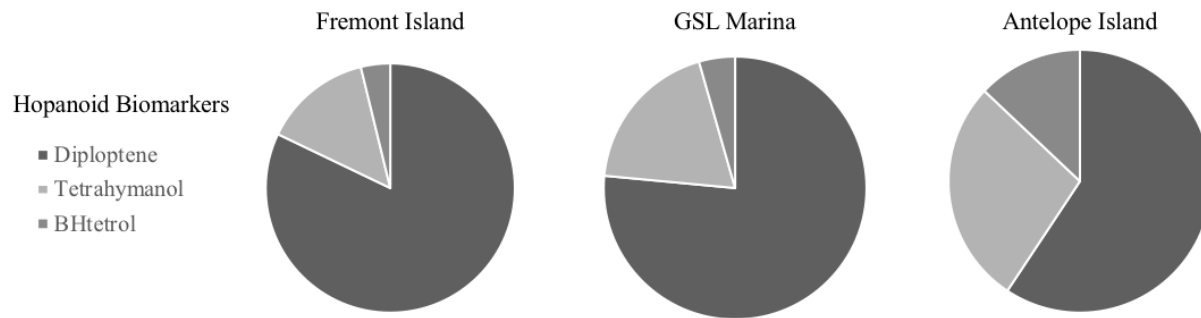
Figure 3.4 Hopanoid biomarker comparison for the three Great Salt Lake sites. Antelope Island has a slightly different composition with greater abundances of tetrahymanol and BHtetrol than the Fremont Island and GSL Marina microbialites.
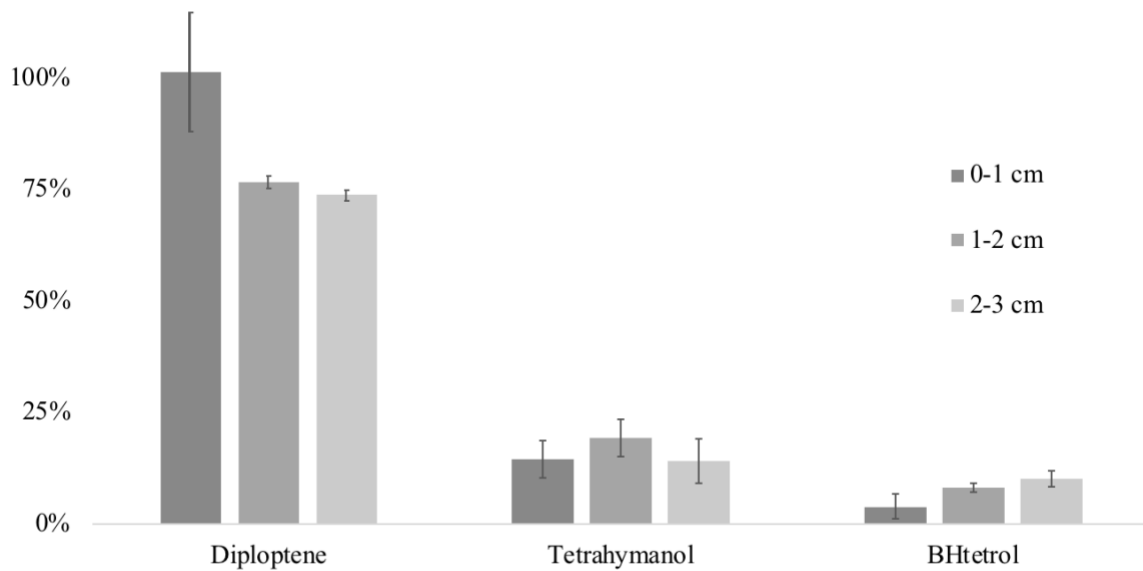
Figure 3.5 Hopanoid relative abundance through three core depths of Fremont Island samples. There were no significant differences in hopanoid composition from the surface (0-1 cm) to the interior of the microbialite matrix (1-3 cm). Error bars indicate standard error.

3.5 Lipid identification and relative abundance

Even-length chain saturated fatty acids (SFAs) C14:0-C26:0 were found in all GSL microbialites with C16:0 the most abundant SFA (23%). Monounsaturated C16 and C18 accounted for 57% of the total fatty acids with C18:1ω9 the most abundant of all fatty acids (29%). Polyunsaturated fatty acids (PUFAs) including C16:2, C18:3, C18:2ω6, C18:2ω7, and C20:4ω6, C20:5ω3 made up only 4% of fatty acids in the microbialites. Heterotrophic lipid markers (12% of the total) included unsaturated C15 and C21, *iso-* and *anteiso*-C15, *iso-* and *anteiso*-C17 as well as *cyclo*-C17 and C19:1 (Table 3.2).

Microbialites contained six sterols, even chain-length C14-C26 alcohols, and C15-ol, C17-ol, and C19-ol (Table 3.3). Sterols included cholesterol (43% of total sterols), cholestanol (10%), 24-MeC$_{28}\Delta^{5,22}$ (2%), 24-MeC$_{28}\Delta^{5}$ (6%), 24-MeC$_{29}\Delta^{5,22}$ (4%), 24-MeC$_{29}\Delta^{5}$ (40%). C16-ol was the most abundant alcohol (45%), followed by C22-ol (15%) and C19-0l (14%) (Table 3.3).

There were no differences in the total concentration of each lipid fraction across the three sites: fatty acids (120 ± 60 mg/g C$_{org}$), alcohols (380 ± 260 µg/g C$_{org}$), and sterols (610 ± 290 µg/g C$_{org}$).

Table 3.2 Autotrophic and heterotrophic fatty acid composition of Great Salt Lake microbialites. Sample number in parentheses.

	Fatty Acid Relative Abundance (%)		
	Fremont Island	GSL Marina	Antelope Island
	Autotrophic Saturated Fatty Acids		
C14:0	0.4 ± 0.2 (5)	0.3 ± 0.2 (5)	0.4 ± 0.2 (7)
C16:0	25.5 ± 3.4 (5)	23.9 ± 3.4 (5)	21.1 ± 5.7 (7)
C18:0	3.0 ± 1.0 (5)	3.1 ± 1.6 (5)	2.2 ± 1.0 (7)
C20:0	0.1 ± 0.0 (5)	0.1 ± 0.1 (5)	0.1 ± 0.0 (6)
C22:0	0.1 ± 0.0 (5)	0.1 ± 0.1 (4)	0.1 ± 0.0 (4)
C24:0	0.1 ± 0.1 (4)	0.1 ± 0.1 (4)	0.1 ± 0.0 (6)
C26:0	0.1 ± 0.0 (5)	0.1 ± 0.0 (5)	0.1 ± 0.0 (6)
Sum	29.3 ± 3.5	27.6 ± 3.8	24 ± 5.7
	Autotrophic Monounsaturated Fatty Acids		
C16:1w9	5.2 ± 2.8 (5)	10.1 ± 9.0 (5)	6.1 ± 1.7 (7)
C16:1w7	15.3 ± 10.3 (5)	7.1 ± 1.2 (6)	9.0 ± 4.8 (7)
C18:1w9	25.0 ± 8.7 (5)	28.7 ± 28.6 (5)	32.8 ± 29.0 (7)
Sum	45.5 ± 13.8	45.9 ± 30.0	47.9 ± 29.4
	Autotrophic Polyunsaturated Fatty Acids		
C16:2	1.6 ± 0.6 (5)	0.9 ± 0.6 (5)	0.7 ± 0.4 (7)
C18:3	0.1 ± 0.1 (2)	-	0.0 ± 0.0 (4)
C18:2w6	1.9 ± 0.9 (5)	1.9 ± 0.9 (5)	1.3 ± 0.8 (7)
C20:4w6	0.5 ± 0.2 (4)	0.1 ± 0.1 (5)	0.2 ± 0.2 (6)
C20:5w3	1.6 ± 1.0 (4)	0.2 ± 0.1 (5)	0.4 ± 0.3 (6)
Sum	5.7 ± 1.5	3.1 ± 1.1	2.7 ± 0.9
	Heterotrophic Saturated Fatty Acids		
iso-C15:0	0.7 ± 0.3 (5)	1.7 ± 0.9 (5)	1.4 ± 0.8 (7)
anteiso-C15:0	0.4 ± 0.2 (5)	0.8 ± 0.5 (5)	0.8 ± 0.5 (7)
C15:0	0.2 ± 0.0 (5)	0.2 ± 0.1 (5)	0.2 ± 0.1 (7)
cyclo-C17:0	0.8 ± 0.3 (5)	1.0 ± 0.6 (5)	1.1 ± 0.6 (7)
iso-C17:0	0.4 ± 0.1 (5)	0.5 ± 0.3 (5)	0.4 ± 0.2 (7)
anteiso-C17:0	0.9 ± 0.4 (5)	1.0 ± 0.4 (5)	0.9 ± 0.4 (7)
C21:0	0.5 ± 0.2 (5)	0.6 ± 0.3 (5)	0.7 ± 0.5 (7)
Sum	3.8 ± 0.7	5.9 ± 0.1	5.4 ± 0.1
	Heterotrophic Monounsaturated Fatty Acids		
C19:1	6.7 ± 2.0 (5)	7.4 ± 4.2 (4)	7.5 ± 2.7 (7)
C18:1w7	8.9 ± 1.2 (5)	9.1 ± 0.5 (5)	11.6 ± 2.2 (7)
Sum	15.6 ± 2.3	16.5 ± 4.2	19.1 ± 3.5

Table 3.3 Neutral lipid composition of Great Salt Lake microbialites including sterols and alcohols.

	Sterol Relative Abundance (%)		
	Fremont Island	GSL Marina	Antelope Island
$C_{27}\Delta^5$ cholesterol	34.1 ± 20.3 (8)	52.5 ± 28.7 (6)	40.9 ± 8.8 (10)
C_{27} cholestanol	6.8 ± 2.6 (5)	12.7 (1)	8.9 ± 2.9 (5)
$24\text{-MeC}_{28}\Delta^{5,22}$	3.1 ± 2.4 (5)	0.9 ± 0.5 (5)	2.3 ± 0.5 (5)
$24\text{-MeC}_{28}\Delta^5$	7.1 ± 3.6 (5)	4.6 ± 4.9 (5)	5.7 ± 1.1 (5)
$24\text{-EtC}_{29}\Delta^{5,22}$	2.2 ± 0.9 (4)	7.3 ± 6.2 (4)	3.6 ± 0.8 (4)
$24\text{-EtC}_{29}\Delta^5$	44.6 ± 18.1 (3)	38.5 ± 20.3 (3)	35.7 ± 6.4 (3)

	Alcohol Relative Abundance (%)		
	Fremont Island	GSL Marina	Antelope Island
C14-ol	2.4 ± 1.2 (5)	4.7 ± 2.3 (5)	2.4 ± 2.0 (7)
C15-ol	1.7 ± 1.0 (5)	1.8 ± 0.7 (5)	2.4 ± 1.3 (7)
C16-ol	39.3 ± 23.6 (5)	52.5 ± 21.2 (5)	41.8 ± 23.2 (7)
C17-ol	3.9 ± 1.4 (5)	2.2 ± 0.8 (5)	5.9 ± 7.6 (7)
C18-ol	8.5 ± 2.0 (4)	4.5 ± 1.4 (5)	5.4 ± 1.6 (7)
C19-ol	13.0 ± 4.1 (4)	9.9 ± 2.6 (5)	19.2 ± 10.4 (5)
C20-ol	5.1 (1)	1.7 ± 0.3 (2)	1.2 ± 0.4 (4)
C22-ol	19.8 ± 6.7 (4)	10.0 ± 12.9 (5)	13.6 ± 17.2 (7)
C24-ol	14.0 ± 9.4 (5)	11.1 ± 11.2 (5)	9.6 ± 4.0 (7)
C26-ol	7.6 ± 2.2 (3)	3.3 ± 3.3 (4)	5.5 ± 1.9 (6)

3.6 Lipid composition by site

Fatty acid content was not consistent across the three sites in the Great Salt Lake microbialites (Figure 3.6). Two MUFAs ($C16:1\omega9$ and $C18:1\omega7$) and three PUFAs ($C16:2$, $C20:4\omega6$, and $C20:5\omega3$) differed in proportion by site. The diatom biomarker $C20:4\omega6$ was significantly more abundant at Fremont Island (0.5%) than at GSL Marina (0.1%) ($F_{(2,12)}=4.790$, p=0.03). The diatom biomarker $C20:5\omega3$ was more abundant at Fremont Island (1.6%) than at both of the other sites (0.2-0.4%) ($F_{(2,12)}=7.840$, p=0.007). The biomarker $C16:2$ was also more abundant at Fremont Island (1.6%) than Antelope Island (0.7%) ($F_{(2,14)}=3.770$, 0.049). For the MUFAs, $C16:1\omega9$, another photoautotrophic biomarker was significantly more abundant at GSL Marina (10%) than at the other two sites (5-6%) ($F_{(2,14)}=4.5$, p=0.031). Finally, $C18:1\omega7$, which can be a photoautotrophic maker or heterotrophic marker was significantly more abundant at Antelope Island (11%) than at the other two sites (Fremont Island=9% and GSL Marina=9%) ($F_{(2,14)}=5.640$, p=0.016). The only significant difference in alcohol composition was for C18-ol with higher abundance at Fremont Island (9%) than the other two sites (5%) ($F_{(2,16)}=4.5$, p=0.0326). For all other lipids, there were no significant differences in proportion at each of the three sites (sterol composition shown in Figure 3.7). The majority of lipids were present in every sample; however, $C18:3$ was not measured in any samples from GSL Marina.
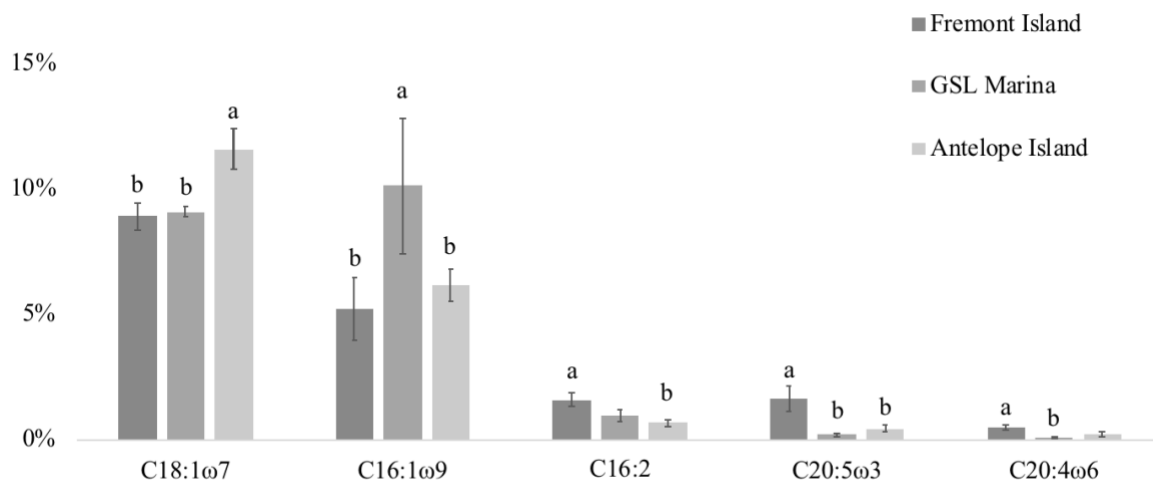
Figure 3.6 Variability in MUFA and PUFA proportions in Great Salt Lake microbialite samples from Fremont Island, GSL Marina, Antelope Island. Columns labelled 'a' are significantly different from those labelled 'b'.
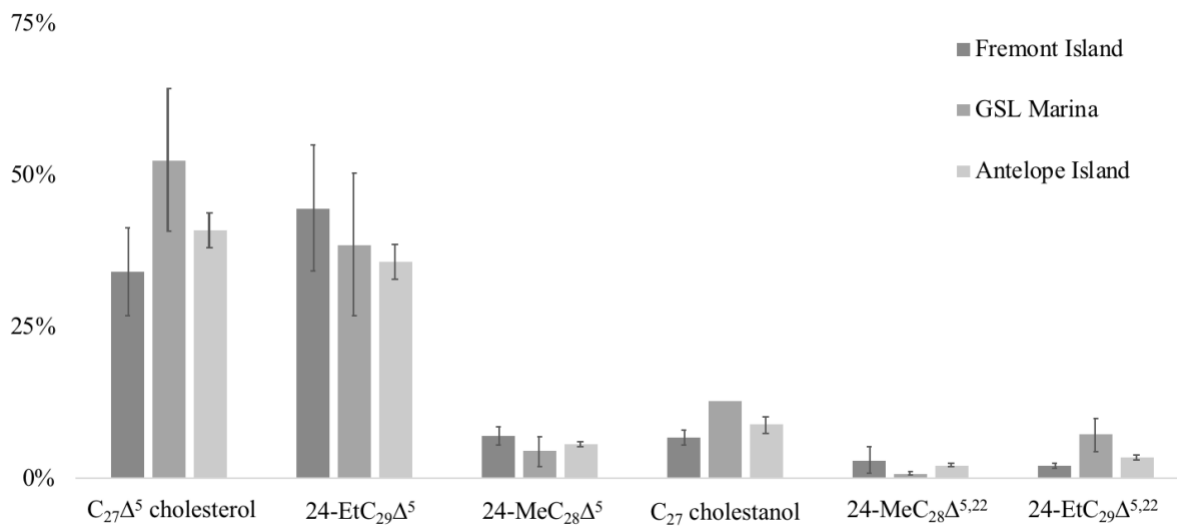
Figure 3.7 Sterol composition of Great Salt Lake microbialites. No significant differences by

site. Error bars show standard error.

4 Discussion

4.1 Hopanoid and lipid biomarkers for community composition

Three previously identified hopanoids (diploptene, tetrahymanol, and BHtetrol) were found in Great Salt Lake microbialites (Sessions et al., 2013); hopanoid biomarker evidence for life was preserved in GSL microbialites to at least 3 cm in the substrate. Two additional diploptene isomers as well as an additional tetrahymanol isomer were observed in the GSL microbialite hopanoid chromatograms (Appendix A). Despite a visibly diminished active microbial community deeper in the carbonate deposits (1-3 cm), the hopanoid biomarkers were found in consistent proportion from the surface to the interior, indicating little to no degradation over time. In GSL microbialites, hopanoids (diploptene, tetrahymanol, and BHtetrol) are preserved, unlike the microbialites of Fayetteville Green Lake, NY (Chapter 2).

Hopanoid biomarkers were different at Antelope Island than the other two GSL sites (Fremont Island and GSL Marina). BHtetrol was previously shown to be a potential biomarker for a heterotrophic (non-photosynthetic) microbial community (Chapter 2). Tetrahymanol is also thought to have non-photosynthetic sources. Both of these hopanoids were found in greater proportion at Antelope Island than the other two sites. Additionally, MUFA and PUFA biomarkers showed differences by site, generally supporting the idea of a heterotrophic-rich community at Antelope Island site. Table 3.4 summarizes the evidence for each site.

The lipid biomarker C18:1ω7 was significantly more abundant at Antelope Island microbialites than the other sites. This biomarker accounts for ~10% of all autotrophic lipid biomarkers in both Great Salt Lake and 10-18% of markers in Hamelin Pool microbialites (Allen et al., 2010). C18:1ω7 was characteristic of gram-negative marine bacteria (Oliver and Colwell, 1973) in previous work. There are a wide variety of potential C18:1ω7-producing gram-negative

bacteria in GSL, which was also the most abundant microbial group by genetic analysis (Lindsay et al., 2016).

Diatom MUFA and PUFA biomarkers (Allen et al., 2010; Buhring et al., 2009) were significantly more abundant at Fremont Island (C16:2, C20:4w6, C20:5w3) and GSL Marina (C16:1w9). Sterols were a dominant fraction of GSL microbialites, indicating relative importance of eukaryotic community members with major contributions from cholesterol (~40%) and the C_{29} cyanobacterial markers (~45%) (Abdo et al., 2010) with minor contributions from the C_{28} diatom markers (~6%) (Allen et al., 2010). There were no differences in sterol biomarkers at the three sites.

Overall, these markers indicate phototrophic-rich communities at Fremont Island and GSL Marina compared to Antelope Island. It is hard to say based on lipid biomarkers which phototroph Antelope Island may be lacking compared to the other sites. Markers for diatoms (sterols) and cyanobacteria or algae (C17-ol and multiple MUFAs) were present all sites. The key difference is only the enrichment of C18:1ω7 (gram-negative bacteria) in Antelope Island microbialites. An autotroph-deplete community at Antelope Island could explain textural differences between it and the other sites; crumbly well-developed mats (thicker layers of exo-polymeric substances) were observed at sites enriched in diatom and cyanobacterial lipids.

Table 3.4 Site differences summary including statistically significant findings in bold.

	Fremont Island	GSL Marina	Antelope Island
Observations	mm thick active microbial mat (black-green). Solidly attached to underlying carbonate Macro-structures were large, flat bottom-mounds with oolitic sand in between	Very different texture. Large, friable, black-green crumbles, effortlessly detached from underlying carbonate deposit Gas bubbles escaping microbialite structure Macro-structure thicker, contiguous carpet	cm thick active microbial mat (black-green). Solidly attached to underlying carbonate Macro-structures were similar to Fremont Island
Hopanoid Biomarkers	85% diploptene 9% tetrahymanol 4% BHtetrol	79% diploptene 15% tetrahymanol 5% BHtetrol	64% diploptene **20% tetrahymanol** **15% BHtetrol**
Fatty Acid Biomarkers	More abundant **C16:2** **C20:5w3** **C20:4w6**	More abundant **C16:1w9**	More abundant **C18:1w7**
Sterol Biomarkers		No significant differences	
Alcohol Biomarkers	More abundant **C18-ol**		
External Evidence			Genetic analyses 2 miles west of this site showed evidence of a community dominated by Bacteria (90%) (proteobacteria, bacteroidetes, cyanobacteria) with Eukarya (7%) and Archaea (2%) as minor components

4.2 Microbialite community variability

Microbialites are common in the rock record, but much rarer today (Riding, 2000) with structures actively growing in a wide variety of environments (Riding, 2000; White III et al., 2015). Microbialite researchers are curious if there is a "shared global microbialite microbiome", a community of microbial components that assemble in a regular way in each of these environments to produce these carbonate structures (White III et al., 2015). It is thought that the common microbial community is vertically structured, each metabolic unit preferring a precise placement in relation to the others to maximize growth and survival (Riding, 2000). Pace et al. (2016) showed Great Salt Lake microbialites have a more heterogeneous configuration, with overlapping metabolic units, which contribute uniquely to different carbonate dissolution-remineralization processes within the substrate. This is similar to the Fayetteville Green Lakes (Chapter 2) accretion story (Patterson, 2014).

Similarities in the accretion stories of the Great Salt Lake microbialites and Fayetteville Green Lake microbialites seems to support the notion of a global microbialite microbiome. Do the lipid biomarker characteristics paint the same picture? Are Great Salt Lake microbialites more similar to freshwater (FGL, Pavilion Lake, Cuatro Cienegas) or marine microbialites (Shark Bay, Hamelin Pool)?

Diploptene, tetrahymanol, and BHtetrol were the major hopanoid molecules of FGL and GSL in a roughly 5:1:1 ratio for both systems. These are the same hopanoid markers that have been found in marine (Allen et al., 2010; Buhring et al., 2009) and freshwater microbialites (Nitti et al., 2012). The presence of 2-methyl hopanoids is not necessarily based on water chemistry; microbialites at both Fayetteville Green Lake (freshwater) and Hamelin Pool (marine) produce these markers (Allen et al., 2010). Hopanoid biomarkers mark transitions in biogeochemical

conditions within microbialites (Chapter 2). This was also found by Blumenberg et al. (2013) in their work with 2-methyl hopanoids. The lack of hopanoid change with core depth in Great Salt Lake microbialites may be related to the thinness of the active layer (as compared to FGL's 6 cm deep active layer, Chapter 2). Anoxic conditions may be measurable with a hopanoid index (2-methyl hopanoid index (Knoll et al., 2007) or homohopane index (Peters and Moldowan, 1991) for GSL microbialites if thinner sections had been used for analysis. Or perhaps the microbial community arrangement at GSL does not fit the typical vertical oxic-anoxic interphase and instead possesses pockets of oxic or anoxic conditions, a heterogeneous micro-landscape (Pace et al., 2016). Micro-environments would encourage hopanoid production without the expected layering.

The presence of hopanoids in FGL and GSL microbialites as well as other locations around the globe suggests hopanoids are key markers. The debate remains whether they are markers for specific organisms (Brocks et al., 1999), stressful growth conditions (Saenz et al., 2012), a biogeochemical condition (Blumenberg et al., 2013), or a combination. Diploptene, hop-21-ene, diplopterol, and bacteriohopanetetrol are common in microbialites (Allen et al., 2010; Blumenberg et al., 2013; Buhring et al., 2009; Nitti et al., 2012); tetrahymanol has been found in Hamelin Pool (marine) microbialites (Alllen et al., 2010).

Lipid biomarkers from marine and freshwater microbialites tell a similar story. Microbialite fatty acid composition in both marine and freshwater includes 25-50% SFAs, 20-60% MUFAs, 4-20% PUFAs, and 10-30% heterotrophic biomarkers. SFAs are typically 14 and 28 carbons long, MUFAs include C:16 and C:18, PUFAs include a combination of C16:2, C18:2, C18:3, C18:4, C20:4, C20:5. Only Great Salt Lake and Cuatro Cienegas contained C19:1 of unknown importance (Nitti et al., 2012). Great Salt Lake microbialites contain a combination of

marine and freshwater characteristics, and thus cannot be grouped with one or the other. This is

quite surprising considering the dramatic difference in environmental conditions at these

different lakes. The microbialite community assemblage, whether halophilic or not, contributes a

"shared global lipid profile" across multiple years of study (Brady et al., 2010; Chapter 2) and

variable morphologies (Brady et al., 2010; Allen et al., 2010) or substrate types (Chapter 2).

5 Conclusion

Great Salt Lake microbialites contain three major hopanoid biomarkers: diploptene, tetrahymanol, and BHtetrol which are preserved in this system down to at least 3 cm into the microbialite substrate. Hopanoids as well as lipid biomarkers were quite different in Antelope Island microbialites compared to the other two sites, indicating an autotroph-deplete microbial community at Antelope Island. While autotrophic markers were relatively low, the eukaryotic and heterotrophic biomarkers were no different at this site, indicating that there is still an active community there.

Great Salt Lake microbialite biomarkers add to the growing evidence for a shared global microbialite microbiome. Despite differences in environment, accretion styles, dominant cyanobacterial types, and macro-structure, Great Salt Lake microbialite biomarkers are comparable to both freshwater and marine microbialites around the world.

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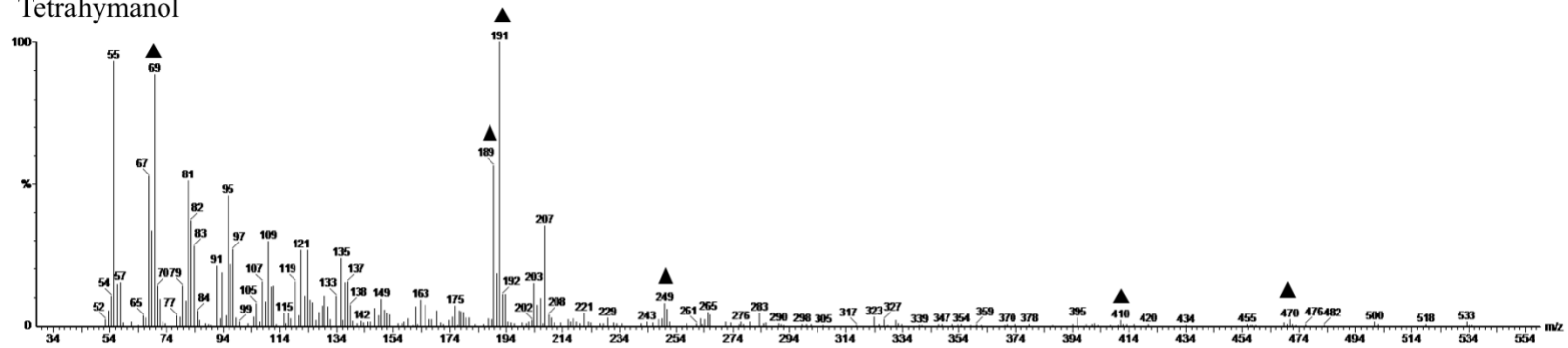
Gas-chromatogram of hopanoids in Utah microbialites. Each hopanoid is labelled and asterisks indicate non-hopanoid peaks. Mass

spectra of each hopanoid follow with diagnostic ions marked with black triangles and asterisks indicating co-eluting peaks.
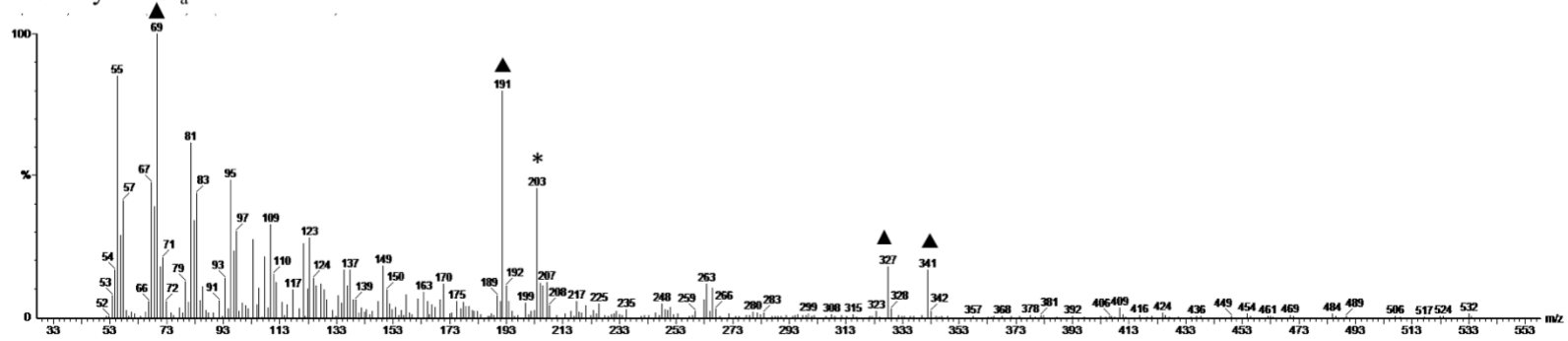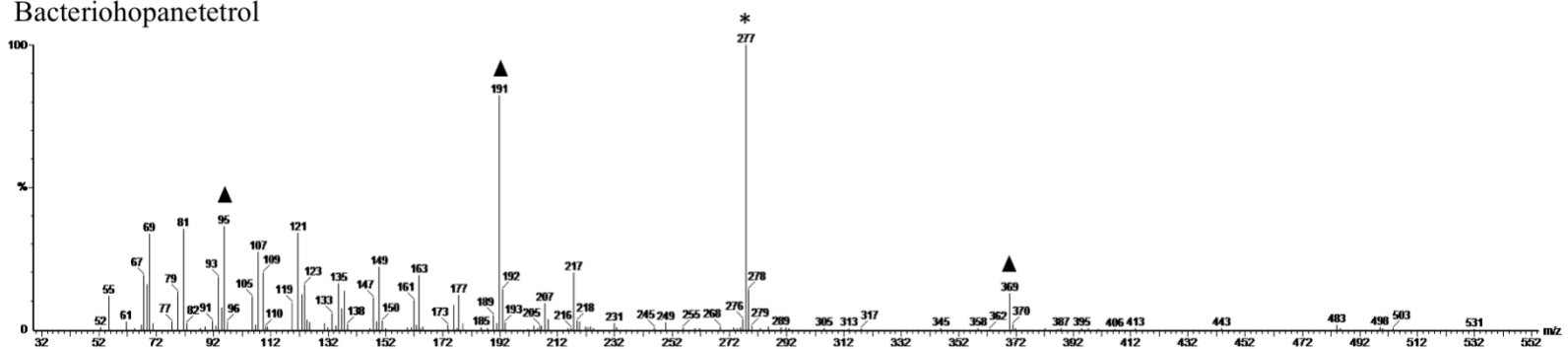
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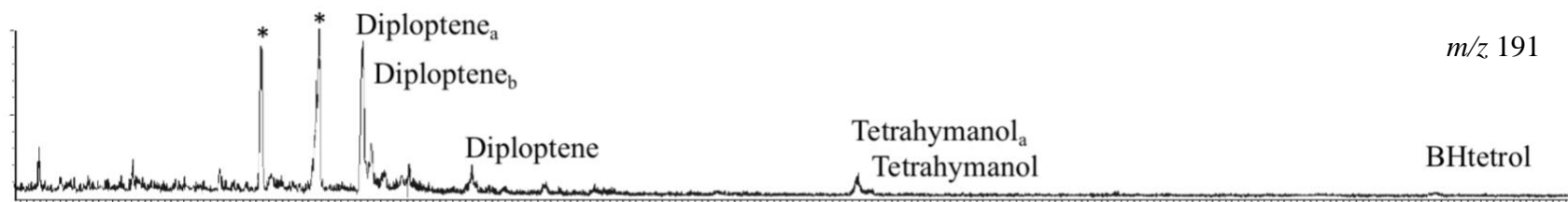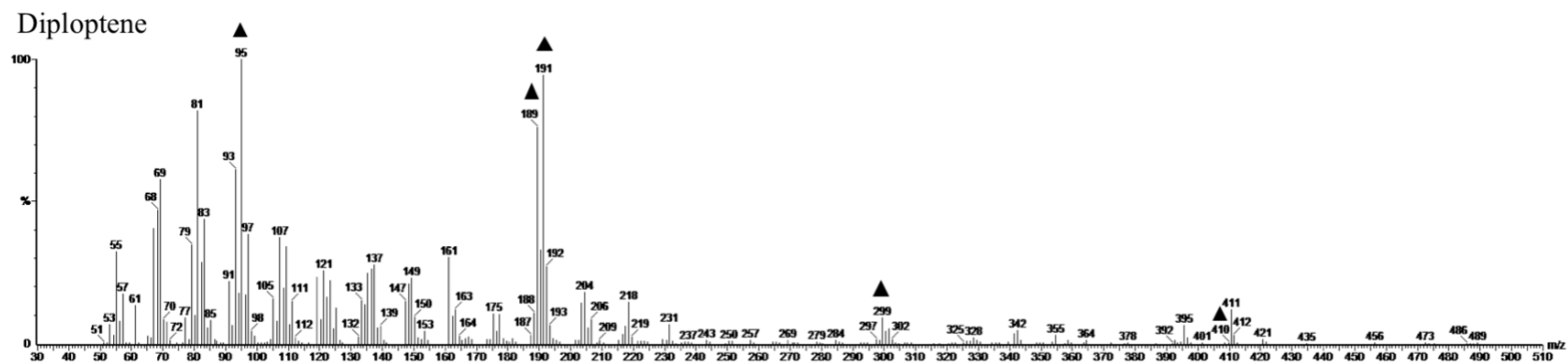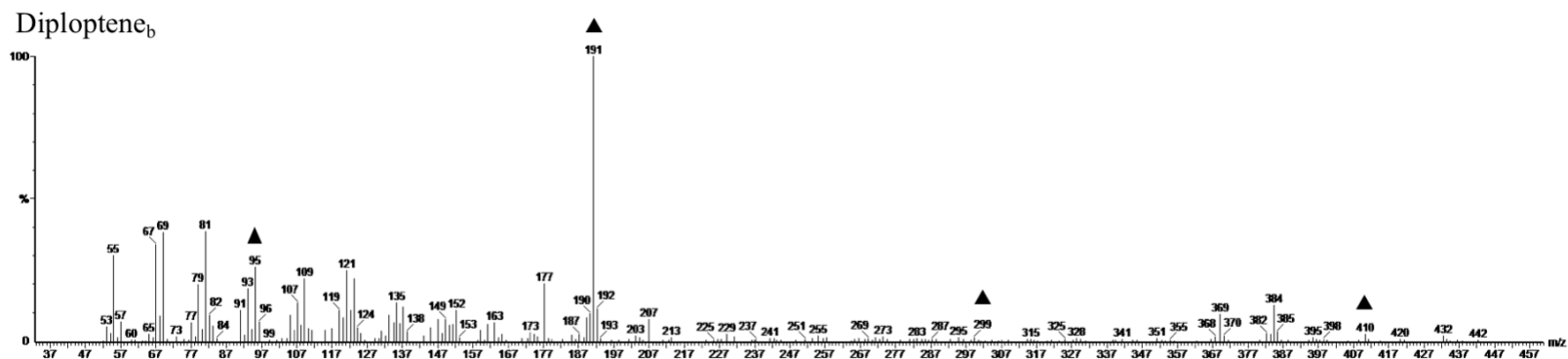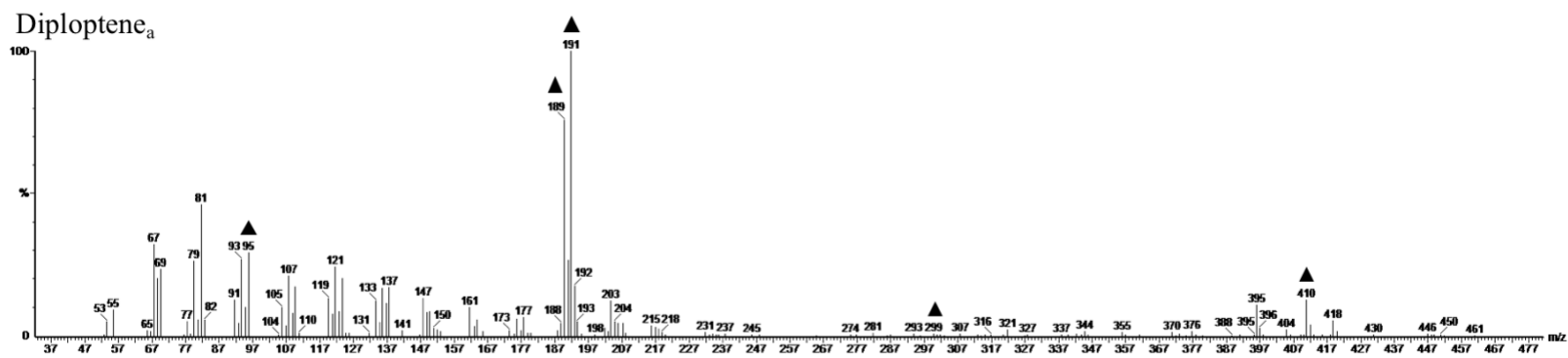
Tetrahymanol

Tetrahymanol$_a$

Bacteriohopanetetrol

Chapter 4: Conclusion

Value of research and implications for future work

This was the first detailed study of hopanoids in freshwater microbialites (Chapter 2) that successfully showed that hopanoid biomarker composition is as complex as it is in marine microbialites (Allen et al., 2010; Buhring et al., 2009). Previous work indicated the presence of a limited number of hopanoids in freshwater microbialites through the non-specific lipid extraction (Cuatro Cienegas) (Nitti et al., 2012); however, using the hopanoid-specific extraction protocol of Sessions et al. (2013) provided more detail of the biomarker composition. This work may encourage other microbial community researchers to employ hopanoid markers in their work.

This work also expands the current breadth of knowledge of microbialites with significant additions to the microbialite story at Fayetteville Green Lake, beyond accretion, and at Great Salt Lake in terms of spatial variability in structure development. This work points out and challenges a couple of key assumptions about lipid and hopanoid biomarkers that should be addressed with future work. Cyanobacteria are not the only hopanoid-producing community members, and the cyanobacterial source should never be assumed, even for the 2-methyl forms (Allen et al., 2010; Newman et al., 2016). There are important differences in hopanoid composition with core depth that correspond to the successional maturity of the microbial community. At FGL, hopanoid biomarkers may indicate biogeochemical changes from the surface to interior of the microbialite. The similarity of hopanoid composition across many sites (Chapter 2 and 3) and by water column depth (Chapter 2) corroborate prior investigations of microbialite community stability using lipid biomarkers (Brady et al., 2014). Microbialite assemblage is relatively steady through time and space if they are at the same developmental

stage. For instance, at GSL, hopanoid and lipid biomarkers were able to discern differences in developmental stage (thick EPS build up vs. thin mat) (Chapter 3).

There are remaining microbialite questions that this work and prior investigations examine. For instance, there is still little understanding of the main sources and reasons for the production of hopanoids. Microbial cultures of the FGL microbialite that were subsequently analyzed for hopanoid content, did not contain hopanoids (Appendix A). The change in growth conditions under laboratory conditions relative to in situ growth was enough to prevent hopanoid production or the growth of the primary hopanoid-producing organisms. There is much work to be done to link these two puzzle pieces together. On a related note, stable isotopes would be an appropriate tool for future researchers to use to trace the flow of carbon (Grey, 2016) through microbialites. This may provide a more detailed perspective of how microbialites actually grow as well as what factors may limit their growth (light, oxygen, carbon sources for heterotrophic metabolism, sulfur species). This would add to the current genomics-type analyses to identify the microbial community.

Another question that remains is, are hopanoid fingerprints for each microbialite unique? How well does that fingerprint hold up over time and with diagenesis? FGL microbialites would be a good candidate for this type of study, having well-preserved and relatively undisturbed microbialite structures that are easily accessible. It is also not known what organisms produce the tetrahymanol marker that is present in FGL, GSL, Hamelin Pool (Allen et al., 2010), and likely other microbialites around the world. Genetic work with tetrahymanol should help with this problem (Welander, 2010).

There are also unanswered questions about lipid biomarkers in microbialites. What is the source of the C_{29} sterol found in very high abundance in FGL (Chapter 2) and Hamelin Pool

(Allen et al., 2010)? Allen et al. (2010) attributed it to a bivalve and its associated zooxanthellae. FGL does have an invasive zebra mussel, but this seems an unlikely source for such a widely abundant sterol biomarker; zebra mussels were not widely distributed in microbialite samples nor abundant. Perhaps there are algal sources in both systems that would be a more reasonable source. It is also not known what organisms produce the C19:1 biomarker (Allen et al., 2010; Chapter 3).

Overall, this work emphasizes the value of microbialites at Fayetteville Green Lake at Green Lakes State Park and the Great Salt Lake. Both lakes currently have a permitting process to protect microbialites. FGL currently prohibits lake-wide swimming and motorized watercrafts to mitigate visitor impacts on microbialite structures. GSL does not have these protections. In both lakes microbialites are ubiquitous, though that does not mean that this resource should be misused. In order to preserve microbialites for future research and visitor enjoyment, it is important to maintain and strengthen protections where necessary so that structures may be maintained and thrive long into the future for both public enjoyment and scientific inquiry. This work provides impetus for future scientific investigations of microbialites in both FGL and GSL in the context of global microbialites.

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Appendices

Appendix A: Hopanoids from microbialite cultures (Fayetteville Green Lake, NY)

Green lake microbialites were cultured in the laboratory as reported in Chapter 2. Following microscopy and microorganism identification, samples were centrifuged (2000 rpm, 10 min) and the media was decanted. The microbial biomass was freeze-dried (-60°C, 72 hours) then extracted for hopanoids using the standard hopanoid protocol (Chapter 2 and 3). All six cultures were extracted without being isolated to individual colonies and run on the GC-MS for hopanoid content with a cholestane internal standard and a method blank. None of the six culture samples had hopanoid peaks.

It is likely that Z8 media selected for certain organisms (those identified in Chapter 2) and not other organisms, which may have either produced hopanoids themselves, or contributed to environmental conditions that encouraged hopanoid production. Recent hopanoid research favors the notion that hopanoid-membrane constituents are related to stress tolerance, allowing cells to survive pH change, temperature change, or crowded living conditions (Garcia Costas et al., 2012; Kulkarni et al., 2013; Saenz et al., 2012; Zarzycki and Portka, 2015). Culture conditions are quite different from the growth conditions of Green Lake. It is reasonable to say that microorganisms were freed from hopanoid-producing conditions and thus, hopanoids were not measured in the resulting biomass.

A second hypothesis, and potentially more useful to hopanoid research, is that Z8 media favored *Synechococcus* and many types of green algae. Prior work has shown that *Synechococcus* does not produce hopanoids (Rohmer et al., 1984). Perhaps this is additional evidence that the Green Lake microbialite proteobacteria are primarily responsible for the hopanoids in Green Lake microbialites and were not culturable in the culture conditions.

Appendix B: Hopanoids from chemolithic microbial mats (Sulfur Springs, NY)

About 2.2 miles south of Chittenango, NY there is a sulfur spring with sulfate-rich groundwater flowing to the surface and into Chittenango Creek, NY. At the mouth of this spring and downstream toward the river, a thick mat of white sulfur bacteria grows on leaf, wood, and rock substrates. This white microbial mat is more the consistency of algae or lichen as it forms flowing filaments in the water (Figure A.1). These sulfate reducers can be smelled from the road as hydrogen sulfide gas is produced through their metabolism.

These sulfur bacterial mats were sampled in April 2017 at two sites into clean and acid-washed Nalgene bottles: leaf material at the opening of the spring and closer to the river west of the road. The white microbial mat was washed off all leaf and wood debris with DI water in the laboratory. This white material was freeze dried (-60°C, 72 hours) then extracted using the standard hopanoid extraction protocol (Chapter 2 and 3) with a cholestane internal standard and a blank. These samples were run on the GC-MS and neither sample contained hopanoid peaks.

Figure A.1. Sulfur Spring as it flows out of the ground (2.2 miles south of Chittenango, NY).

White filaments are sulfate oxidizing bacteria (consuming hydrogen sulfide).

Appendix C: Hopanoids from coral (Florida Keys, USA)

Four coral species were collected from the Florida Keys in 2010: *Montastraea cavernosa*, *Sidasterea siderians*, *Porites astreoides*, *Montastraea faveolata*. The coral system is similar to the microbialites that are the focus of this work. Just like a coral-reef, the microbialites in Fayetteville Green Lake, NY and Great Salt Lake, UT provide habitat or substrate that other organisms rely on for survival. Carbonate-producing microbial communities and carbonate-producing corals are also the base of their ecosystems, providing high density of primary producers whose metabolism feeds higher trophic level organisms.

The purpose of this work was to determine whether the animal or zooxanthellae portions of corals produce hopanoids. Each coral was separated into its constituents: animal and zooxanthellae then freeze dried (-60°C, 72 hours) and stored frozen (-20°C) as described in Teece et al. (2011). The standard hopanoid protocol (Chapters 2 and 3) was used to extract hopanoids from both the animal and zooxanthellae portions of the coral. Samples were run on the GC-MS and no hopanoid peaks were found for the animal or zooxanthellae of any of the four coral species.

Appendix D: Mapping Fayetteville Green Lakes microbialites

There are several very large microbialite shelves in Fayetteville Green Lake that are visible from shore along the east and west shorelines. Researchers and managers question whether these are the only microbial structures, the extent to which microbialites grow on other substrates in the lake, and whether they also grow deeper in the water column (invisible from shore). To better understand microbialite distribution (including smaller structures), Fayetteville Green Lake microbialites were surveyed in summer 2017 by snorkeling the perimeter of both Green and Round Lakes (NYS Parks Permit # 2017-GRL-006).

Microbialites were observed as shelf structures primarily on the east and west shores of Green Lake and the west to southern shore of Round Lake. Green Lake microbialite shelves grow close to the surface down to about 12 m (the bottom was not visible) (Thompson et al. 1990). The microbialites in Round Lake tend to grow as shelves deeper in the water column than those at Green Lake. In both lakes, microbialites grow on wood debris (large trees) that falls into the lake. These microbialites encircle the entire trunk of the tree, growing outward from the trunk in all directions despite limited access to incoming light (upward only).

This work provided the first exhaustive search of microbialites in Green Lake. Park Managers now know that management of this unique park resource is not limited to the shelf at Dead Man's Point. The current regulations against swimming or using motorized watercraft are appropriate for protecting the other microbialites. In addition, Park Managers know for the first time that there are significant microbialite structures in Round Lake, a National Historic Landmark with higher protection status than Green Lake.

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Saenz, J.P., Waterbury, J.B., Eglington, T.I., Summons, R.E., 2012. Hopanoids in marine cyanobacteria: probing their phylogenetic distribution and biological role. Geobiology 10, 311-319.

Teece, M.A., Estes, B., Gelsleichter, E., Lirman, D., 2011. Heterotrophic and autotrophic assimilation of fatty acids by two scleractinian corals, *Montastraea faveolata* and *Porites astreoides*. Limnology and Oceanography 56(4), 1285-1296.

Thompson, J.B., Ferris, F.G., Smith, D.A., 1990. Geomicrobiology and sedimentology of the mixolimnion and chemocline in Fayetteville Green Lake, New York. SEPM Society for Sedimentary Geology 5(1), 52-75.

Zarzycki, P.K., Portka, J.K., 2015. Recent advances in hopanoids analysis: Quantification protocols overview, main research targets and selected problems of complex data exploration. Journal of Steroid Biochemistry and Molecular Biology 153, 3-26.

Vita: Sierra D. Jech

307.250.4043, sjech11@gmail.com

EDUCATION

M.S.	Environmental Chemistry. SUNY-ESF, Syracuse, NY. May 2018
B.S.	Chemistry (ACS Approved). University of Wyoming, Laramie, WY. 2016
B.S.	Earth Systems Science (Biology). University of Wyoming, Laramie, WY. 2016
Minor	Queer Studies. University of Wyoming, Laramie, WY. 2016

EMPLOYMENT

Fall 2016-present	Teaching Assistant Gen Chemistry Labs, SUNY-ESF
Summer 2016/5	Herpetology Field Technician, Wyoming Natural Diversity Database
2012-2015	Field and Lab Work for several UWYO labs (see Research Experience)
2007-2011	Night Manager, Cashier, Cook, Server, Peter's Café and Bakery
2006-2007	Housekeeping, Green Gables Inn

RESEARCH EXPERIENCE

M.S. Graduate Research
Fall 2016-present *SUNY-ESF,* Dr. Mark Teece; Syracuse, New York
- Sample extraction and derivatization for GC-MS analysis of lipids and hopanoids in Green Lakes, NY microbialites and Great Salt Lake, UT microbialites
- Preparation of peer-reviewed manuscript including data analysis in SAS and R
- Surveying and mapping Green Lakes microbialites, creating an educational video about Green Lakes for the Environmental Education Center, training and work at Central NY State Parks as part of Friends of Recreation, Conservation, and Environmental Stewardship (FORCES)
- Field work at the Great Salt Lake, UT sampling living microbialites summer 2017

Wyoming Natural Diversity Database
Summer 2015-2016 *University of Wyoming*, Dr. Wendy Estes-Zumpf; Laramie, Wyoming
- Herpetology field technician (2015) and field leader (2016)
- Field surveys throughout Southern Wyoming
- Surveys for the Wyoming Toad reintroduction site monitoring program
- Data quality checking and database management

Microbiology Capstone
Fall 2016 *University of Wyoming*, Rachel Watson and John Willford; Laramie, Wyoming
- Wrote a project proposal, carried out research, designed a presentation poster, and presented to community partners
- Worked with the ACRES Student Farm to determine optimal blue oyster mushroom cultivation conditions as a group project for Microbiology Capstone course

Footprint of an International Sporting Event

2015-present *University of Wyoming*, Rachel Watson and Dr. Christi Boggs; Laramie, Wyoming

- Wrote collaborative Special Projects Grant through the HAUB School of the Environment and Natural Resources
- Investigated the Footprint of an International Sporting Event at the World University Games in Strbske Pleso, Slovakia, 2015
- Individual project: Quantification of ski wax (fluorocarbons and hydrocarbons) in snow samples using GC/MS
- World University Games in Almaty, Kazakhstan, 2017 conducted research on Perception of Fluorocarbon Bans in the Ski community by interviewing wax technicians and coaches about health and environmental risk
- Presented work in 2016 at the Shepard Symposium on Social Justice and at the 2017 conference for the Action Research Network of the Americas (ARNA) with funding from the SUNY-ESF Graduate Student Association
- Preparation of peer-reviewed publication with Rachel Watson to be completed winter 2018

Benkman Laboratory

Summer 2014 *University of Wyoming*, Dr. Craig Benkman; Laramie, Wyoming

- Collected soils in Yellowstone National Park for Lodgepole Pine evolution study related to seed predation by squirrels
- Measured soil bulk density, soil texture, soil depth for peer-reviewed publication (citation below)
- Field crew leader (3 weeks)

Murphy Laboratory

Summer 2013 *University of Wyoming*, Dr. Melanie Murphy and Charlotte Gabrielsen; Laramie, WY

- Received UW EPSCoR Grant for individual project
- Focused on relationships between land-use, and amphibian presence/absence in the pothole wetland from Montana to Minnesota
- Presented at Undergraduate Research Days at University of Wyoming
- Field technician for Charlotte Gabrielsen's project on water availability in the Plains and Prairie Pothole Region

Pendall Laboratory

Summer 2012 *University of Wyoming*, Dr. Elise Pendall; Laramie, WY

- Field experience and data entry for the Prairie Heating and CO_2 Exchange (PHACE) project
- Laboratory experience for several projects including bark beetle and nutrient cycling, soil respiration under snowpack, PHACE, and metabolomics
- Root and soil picking, soil and plant grinding, acidification of soils, Phospholipid Lipid Fatty Acid extractions, and soil incubations
- Research Experience for Undergraduates (REU) funding
- Individual research on respiration rates at PHACE sites

PUBLICATIONS

Benkman, C. W., Jech, S., Talluto, M.V. 2016. From the ground up: biotic and abiotic features that set the course from genes to ecosystems. Ecology and Evolution. 1-7.

Watson, R.M., Boggs, C.N., with contributions from Bochanski, K. J., Solvang, S., Jech, S., Wiswell, S., Sulser, A.E., Noren, B. 2016. Climate, competition and curriculum collide: Designing an integrated action-learning course with international competition and environmental sustainability curriculum. Journal of Action Research Network of the Americas. 4th Annual ARNA Conference Proceedings.

PRESENTATIONS in Graduate Program

2017	Gliding into Action: Integrating skiing and climate change research at the Action Research Network of the Americas (ARNA) Conference. Cartagena, Colombia
2017	Microbialite biomarkers and the growth of unusual freshwater microbialites in a Central New York lake. Poster session for Spotlight in Graduate Research. Syracuse, NY
2017	State University of New York Faculty Senate Graduate Research Conference. Microbialite biomarkers and the growth of unusual freshwater microbialites in a Central New York lake. Saratoga Springs, NY

GRANTS, SCHOLARSHIPS, and AWARDS in Graduate Program

2017	Sussman Foundation Fellowship. Internship at Green Lakes State Park conducting thesis work and science outreach with the Park
2017	SUNY-ESF Chemistry Outstanding Teaching Assistant Award
2017	Pathfinder Fellowship from the Consortium of Universities for the Advancement of Hydrologic Science, Inc. for work on Great Salt Lake microbialites
2017	Travel Grant from SUNY-ESF Graduate Student Association for travel to Cartagena, Colombia to present Gliding into Action: Integrating skiing and climate change research

COMMUNITY SERVICE AND VOLUNTEER WORK in Graduate Program

Fall 2017	Organized 10 SUNY-ESF students for the Post-Landfill Action Network's Students for Zero Waste Conference with support from ESF Sustainability Office
2017	Volunteered with Friends of Recreation, Conservation and Environmental Stewardship in the NY State Parks doing manual invasive species removal