Probing the Pigments and Physiology of Modern Cyanobacterial Mats that are Analogs of Life on Early Earth

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Abstract

Recently discovered submerged sinkholes in Lake Huron are low-oxygen, high-sulfur extreme environments for microbial life. These habitats mimic conditions on Earth's early shallow seas and could aid our search for extraterrestrial life. My thesis work will probe the pigments and physiology of these modern day mat ecosystems under *in situ* and in laboratory conditions in order to understand how they adapt to variable light quality and intensity. Cyanobacteria-dominated microbial mats will be grown in varying light intensities and examined for pigment composition via HPLC - providing insight about their light adaptive capabilities. *In situ* observations will reveal whether variable pigment composition and ratios reflect functional diversity, provide a glimpse into Earth's past and support future conservation efforts of these novel Great Lakes ecosystems.

Introduction

Three billion years ago our planet was inhabited exclusively by microbes. The ocean and atmosphere contained not oxygen, but sulfur, H_2 and primarily CO_2 (Lunine, 2006). Under these conditions, cyanobacteria-dominated microbial mats thrived in the shallow proterozoic seas (Falkowski et al. 2008; Johnston et al. 2009). Modern analogs of these ancient photosynthetic bacteria have been found in low-oxygen, high-sulfur submerged sinkholes of Lake Huron that are relatively unexplored (Ruberg et al. 2008; Biddanda et al. 2012, figure 1) Photosynthesis evolved 3.5 Bya eventually leading to the Great Oxygen Event (GOE), and is responsible for the oxygenated environment today (Falkowski et al. 2008; Blankenship et al.

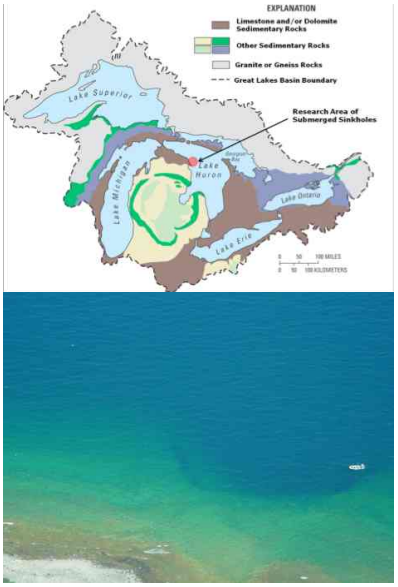

Figure 1. Top: Surface geology of the Great Lakes Basin highlighting karst limestone aquifers where sinkholes are likely to occur and our study site, Bottom: Aerial view of MIS

Figure 2. Top: ROV image of cyanobacterial mats at MIS, Bottom: 100X microscopic image of cyanobacterial filaments from sinkhole floor.

2007). However, early photosynthesis was quite different from the process seen in modern plants. Anoxygenic photosynthesis (non-oxygen evolving) occurred first, wherein instead of water, elements like sulfur and iron were utilized as electron donors and acceptors (Falkowski et al. 2008; Blankenship et al. 2007). The cyanobacterial mats present in Middle Island Sinkhole (MIS, figure 2) can conduct oxygenic as well as anoxygenic photosynthesis - an ability which makes them a model of photosynthetic evolution on early Earth and can give insight into what Earth was like billions of years ago (Biddanda et al. 2009/2012; Nold et al. 2010; Voorhies et al. 2012).

Phormidium and *Oscillatoria* are the two dominant genera in MIS (Biddanda et al. 2012; Nold et al. 2010, figure 3) where they thrive at depths of 75ft. where only 10% of surface irradiance is available (Ruberg et al. 2008) -- making photoacclimation a key life strategy (Bryant and Cohen-Bazire 1981). Interestingly, the mat surface is characterized by a strikingly low species diversity (Voorhies et al. 2012).

My research will gain insight into how a system with such low species diversity can possess such a versatile physiology. *Major questions I wish to address are how these mats use photosynthetic pigments to survive in the modern period, what pigments they contain, and to what extent, if at all, they use their pigments to adapt to changing light quality.* My research aims to study photosynthetic life in this easily accessible novel ecosystem representative of deep time that can serve as a model for life in Earth's extreme environments.

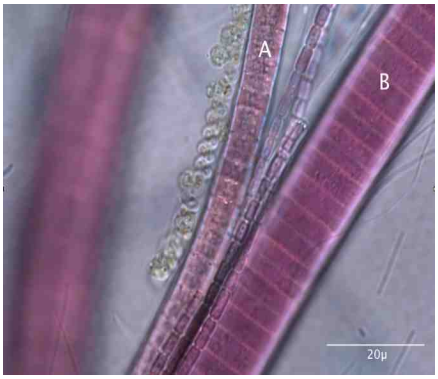

Figure 3. 1000X image of common MIS mat filament types. A - Phormidium-like filament, B - Oscillatoria-like filament. Photograph taken by Michael Snider.

Materials and Methods Overview

Study Site

Field work will be conducted from May 2012 to September 2012 at Middle Island Sinkhole which is located in Lake Huron off the coast of Alpena, MI (N 45.19843°N, W 083.32721°W). Work will be conducted through an ongoing partnership with Thunder Bay National Marine Sanctuary (figure 4 top).

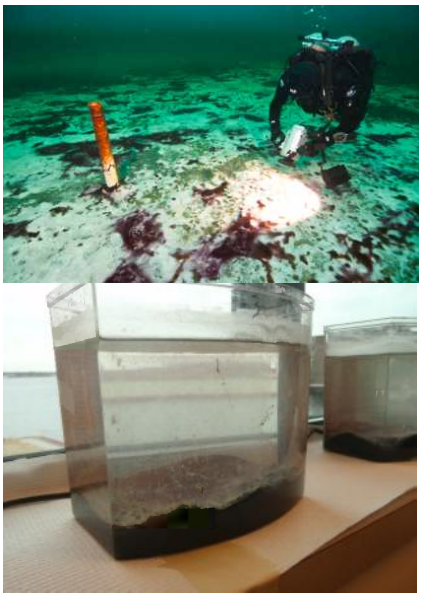

Figure 4. Top: Thunder Bay National Marine Sanctuary Divers filming video at Middle Island Sinkhole, Bottom: Culture tank where microbial mats are grown and analyzed in the laboratory.

Pigment Composition

Mat samples will be collected in opaque core tubes from MIS via dive support. Purple and green mats, as well as white "streamer" samples will be collected. Samples will be kept on ice for transportation back to the laboratory. Subsamples of the mats will be isolated, filtered and frozen for pigment extractions. Frozen filters will be extracted and analyzed for pigment composition using reverse phase HPLC (Heukelem and Thomas 2001; Steinman et al. 1997), and spectrophotometric analysis (Lawrenz et al. 2011).

The remaining samples will be cultured in aquaria tanks (figure 4 bottom) with experimentally altered light and nutrient conditions. Culture tanks will be filled with synthetic media (617mg/L magnesium sulfate, 280mg/L calcium; based off of previous groundwater analysis) or BG-11 (cyanobacteria growth media), and placed across a gradient of light intensity from 0 to 200 μmol photons m^{-2} s^{-1}. All tanks will be placed in a 10°C incubator with 16:8hr light:dark cycle. Cyanobacteria biomass will be sampled and processed for pigment analysis (as above) for 8 weeks. We hypothesize that different mat types (purple, green, white) have different pigment profiles and pigment ratios reflecting the functional diversity present in these cyanobacterial groups. Data will be analyzed for significant changes in the pigment profile across light and nutrient groups, and if possible over time, based on pigment concentrations

determined from HPLC and spectrophotometry using multiple linear regression analysis in *R stats* software.

Pigment Function

Collected samples will be analyzed immediately upon retrieval with a Phyto-PAM and Phyto-ED attachment by plucking the dense surface cell growth, homogenizing by vortexing, then transferring to the Phyto-ED quartz cuvette and analyzing for quantum efficiency and constructing rapid light curves (RLC). Care will be taken to not expose samples to light before analysis. The phyto-PAM calculates quantum efficiency (Q_E, dimensionless) by finding maximum and minimum fluorescence values ($Q_E = F_M - F_O / F_M$). Photosynthetic capacity data is generated by performing RLCs, which finds Q_E over a broad range of light intensities. This parameter quantitatively estimates physiological capacity of cells.

The experimentally grown mats will be periodically (every 48hrs) analyzed for pigment function in the same way as above. Analysis will always be conducted at the same time to minimize changes in previous light history. This process will illustrate the process of short and long term photoacclimation through pigment function over time, which may correlate to changes in the pigment profile. We hypothesize that mats grown in lower light treatments will show increased photosynthetic efficiency and capacity, and that different mat types will exhibit distinct PAM characteristics, and changes in PAM values will reflect changes in the pigment composition and ratio. Quantum efficiency and light capacity data will be analyzed with multiple linear regression analysis with *R stats* for significant differences ($\alpha=0.05$) between mat types, treatment groups, and effects of time.

Dissemination of Information

I plan to publish my research in a peer reviewed journal like *Applied and Environmental Microbiology*, as well as present my findings at a *American Society for Microbiology* poster or oral presentation. I will be presenting results at the University of Michigan in the Fall for the annual Michigan Space Grant Conference.

Timeline

	Fall '11	Winter '12	Spring '12	Summer '12	Fall '12	Winter '13	Spring '13
Literature Review	X	X					
Experimental Design	X	X	X				
Sample Collection			X	X			
Sample Processing				X	X		
Statistical Analysis				X	X		
Manuscript Writing					X	X	
Conferences and Seminars					X	X	X
Education and Outreach					X	X	X
Thesis Defense							X

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Budget

Culture Supplies			
BG-11 Media (4)	Cyanobacteria growth media, 500mL	Fisher Scientific	$218.40
Calcium (3)	Nutrient for cultures, 100g	Fisher Scientific	$94.38
Magnesium Sulfate	Nutrient for cultures, 500g	Fisher Scientific	$60.44
Neutral Density Filters	For altering light intensity in cultures	Fisher Scientific	$94.25
Small Plastic Fishtanks (6)	Tanks for growing mat cultures	Petco	$95.82
Phyto-PAM supplies			
Culture tubes	Translucent polypropylene snap tubes	Fisher Scientific	$258.08
Filter discs	Filter out organics to zero Phyto-PAM readings, 47mm, 0.2µm	Fisher Scientific	$119.79
Transfer pippetes	Homogenization and transferring of cells to cuvette (500)	Fisher Scientific	$49.89
		TOTAL	**$991.05**