Phototrophic Bacteria in an Archean Analog System

Introduction: Life is thought to have first evolved in the ferruginous (anoxic, iron rich) environments of Earth's oceans in the Archean era (4.0-2.5 mya). Since then, all geochemical cycles, including the iron cycle, have been directly tied with microbial metabolisms. **By determining which microbes make contributions to iron cycling in analogous environments in the present, inferences can be drawn to better understand iron cycling in the Archean era. Additional information about Archean iron cycling can be extrapolated from biosignatures in the rock record.** The Earth's oceans spent a long period of time in the ferruginous conditions of the Archean. Potentially, other worlds both in our solar system and beyond could contain similar habitats. The more that is known about the Earth's biosphere and biogeochemical cycles, the better the predictions that can be made of similar cycles on exoplanets. One of the environments considered an Archean ocean analog is Brownie Lake, located in Minnesota, US.[2] The lake consists of an oxic surface layer that transitions to an anoxic layer beginning at depths of ~3 m.[2] Just above the sediment, the dissolved Fe(II) concentration is high, >1 μM.[2] The conditions of the anoxic zone, combined with the site's accessibility, make it an excellent analog for studying microorganisms similar to those found in the Archean oceans.

Early microbial life forms in the Archean era likely included anoxygenic phototrophs, anoxic microbes that utilize light as an energy source for their metabolisms. Among them would have been photoferrotrophs (iron oxidizing phototrophs) which likely thrived due to the abundance of dissolved Fe(II).[5] The production of oxygen by the later evolving oxygenic phototrophs (cyanobacteria) contributed to the Great Oxidation Event (GOE)[5] at the end of the Archean eon. Oxygen produced from this process would have also oxidized iron in the environment. It is presently under debate which of the two microbial metabolisms had a greater contribution to the formation of banded iron formations (BIFs), iron rich rock layers found in the geological record beginning in the Archean.[5] While the Archean ocean was certainly ideal for photoferrotrophy, the conditions were less favorable for oxygenic phototrophs as the high iron concentrations led to increased intracellular buildup of reactive oxygen species.[4] Still, there is evidence for oxygenic photosynthesis in Archean oceans in the form of "whiffs" of oxygen found in the geologic record before the GOE.[1] This implies that early oxygenic phototrophs could have been present and contributed to BIF formation. It is possible that oxygenic phototroph populations rose and fell in cycles.

Hypothesis: Throughout seasonal changes, Brownie Lake undergoes geochemical and temperature changes corresponding to the motion of the oxycline through the water column.[2] I hypothesize that the community composition of microbial populations at various depths in Brownie Lake will change following the seasonal motion of the oxycline. For instance, when the oxycline moves upwards in the lake, oxygenic photosynthesizers at that depth will decrease due to the conditions becoming more iron rich and less oxic. Photoferrotrophs will be examined for potential biosignatures, such as carbon and iron isotopes, for detecting these microbes in Archean era rocks. Answering the questions of who is in these environments and what they are doing are pertinent to the study of how the Earth and life evolved together.

Objectives: Metagenomic and cultivation approaches will be used to continue characterization of microorganisms in **Brownie Lake**, to uncover the identity, abundance, and metabolic capabilities of different groups at multiple depths of the lake. **[Aim 1]** This study will use metagenomics to address how the microbial community and metabolic capabilities present in the anoxic zone change over a period of time. In particular, how closely does the location of microbial populations follow the seasonal changing location of anoxic waters? **[Aim 2]** Due to the high iron concentrations of the Archean oceans, it is likely that photoferrotrophs were important primary producers. Previous work in Brownie Lake has led to the isolation of a photoferrotroph belonging to the bacterial phylum Chlorobi.[3] In addition, photoferrotrophic isolates will be cultivated. Characterization of the isolates will identify potential biosignatures for these groups in Archean rocks as well as connect genotypes revealed from metagenomics to observed phenotypes.

Methods: [Aim 1] Samples will be taken seasonally from various depths in the lake. 16S rRNA gene sequencing will be conducted to get an overview of community composition. Metagenomes will be generated from samples found to have cyanobacteria or photoferrotrophic signatures (bacteriochlorophyll genes, iron oxidation genes, etc). This approach will generate an understanding of community composition, by determining what genes are present and what taxa they are associated with. Every month, samples will be taken from different depths in the lake. Patterns demonstrating which species are associated with which depths and geochemical parameters (dissolved oxygen concentration, Fe(II) concentration, pH, etc) will be noted. **[Aim 2]** Water from depths containing genomes indicative of photoferrophy will be combined with anoxic freshwater media and incubated with light at wavelengths at an intensity too low to support oxygenic photosynthesis.[3] Isolation will be done via dilution. In the event that new isolates cannot be obtained after multiple attempts, photoferrotrophic isolates will be acquired from a collaborating lab to be tested for biosignatures specific to photoferrotrophy. The isolates will be tested to determine what biosignatures they exhibit, with the goal being to identify new biosignatures specific to photoferrotrophic metabolisms, such as a specific iron isotope signature produced as a result of their metabolism.

Intellectual Merit: Discovering the roles played by different taxa in biogeochemical cycles is a fundamental question to the study of basic science. Presently there are a multitude of uncharacterized microorganisms in the environment that may be contributing to the iron cycle. This research will be important in identifying the key players in the iron cycle, connecting genotypes revealed through metagenomics with phenotypes revealed by studying isolates. Studying Brownie Lake allows us to see how cyanobacteria and photoferrotrophs in the present respond to seasonal environmental changes, allowing for potential insight as to **how Archean cyanobacteria and photoferrotrophs populations evolved**. In addition, much remains to be discovered about the physiology of photoferrotrophs, as few have been cultured in the lab. This study will **reveal potential biosignatures** that could be used to search for evidence of these organisms in Archaean sediments, or even on other worlds.

Broader Impacts: Research will be shared yearly with the general public through the **Science in Nature seminar program** at Itasca State Park. The park advertises the talks, which draw groups of up to 100 people each month. To reach more of the public, the research will be visually presented in comic format that will be published online in the **Community of Scholars Program (COSP) magazine** yearly. This comic, along with demonstration props such as a soil core and plates of bacteria, will be part of an interactive presentation at a farmer's market through **Market Science,** a program at the University of Minnesota that allows for enriching interactions between scientists and visitors to farmer's markets. In 2019, over 9,000 people visited Market Science stalls created by over 200 volunteer scientists. In addition, undergraduate researchers will be actively solicited from the **North Star STEM Alliance** (an organization that serves Minnesotan minority students in STEM) to receive mentoring and research experience in isolating and characterizing organisms.

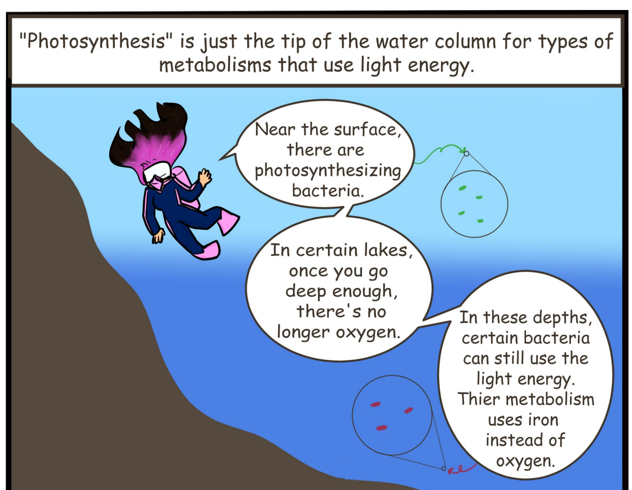

Figure 1. Example of a frame that would appear in the comic.

Citations: [1]Anbar et al. (2007). *Science.* [2]Lambrecht et al. (2019). *Geobiology.* [3]Lambrecht,N. (2019). *Iowa State University, PhD dissertation*. [4]Swanner et al.(2015).*Nature Geoscience.* [5]Thompson et al.(2019). *Science Advances*.