

**Characterization of Algal Blooms Affecting Wyoming Irrigation Infrastructure:
Microbiological Groundwork for Effective Management**
Final Report

Principal Investigators:

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Abstract:

Eutrophication, resulting from increased nutrient input into a water body, is one of the most pervasive water quality problems in the United States, affecting lakes, estuaries, streams, and wetlands. Eutrophication is often driven by human activities such as agriculture, where fertilizer run-off and soil erosion are major sources of the nutrient load. The effects of eutrophication include algal/cyanobacteria blooms, leading to hypoxia of the water column and subsequent decline in submerged vegetation, and fish kills. Locally, management of algal blooms represents a significant cost to maintaining the irrigation infrastructure in Wyoming. The effectiveness and environmental impact of these algae treatment strategies are not well understood. It is very difficult to estimate or monitor the total amount of algacides released into the environment, and the full range of species affected remains unknown. Development of more effective algae treatment strategies is hampered by a *knowledge gap*: we have not identified the key algal and bacterial species and processes involved in establishing, maintaining, and degrading algal blooms in Wyoming lakes. *We are working to address this knowledge gap and thus provide a sound microbiological foundation for long-term development of more targeted, effective algae treatment strategies.* In order to achieve this objective, we are (1) Characterizing algae/cyanobacteria species responsible for blooms, (2) Characterizing the role of bloom-associated bacteria, and (3) Developing model systems to test bacterial/algal interactions. Our long-term goal is to anticipate the type and severity of the bloom and propose predictive management strategies (as opposed to the reactive treatment protocols currently employed).

Progress:

Objectives:

- (1) Characterize algae/cyanobacteria species responsible for blooms,
- (2) Characterize the role of bloom-associated bacteria, and
- (3) Develop model systems to test bacterial/algal interactions.

Methodology:

A. Field Work. We have worked at two sites: Labonte Lake in Laramie (impacted urban lake), and Rock Lake (impacted agricultural lake). Sample types included the lake sediment, water column, and any macroscopically observed algal bloom material. Samples were subdivided for water quality analysis, microscopy, and DNA extraction.

B. Laboratory Work. Water quality analyses included total nitrogen, total phosphorus, dissolved oxygen, and dissolved organic carbon (DOC). Phase-contrast light microscopy was

used to monitor the development of blooms. Profiling of the microbial community was performed by ribosomal RNA gene 454 FLX pyrosequencing. Sequencing was performed by Research and Testing Laboratories LLC (Lubbock, TX). We are performing separate analyses of bacterial, archaeal, and algal populations. Small-subunit (16S) rRNA genes were analyzed for Bacteria and Archaea, and large-subunit (23S) rRNA genes for algae. This very large amount of sequencing was achieved by multiplexing the 454 runs with the use of bar-coded PCR primers. We were able to simultaneously sequence 16S/23S rRNA genes from all samples on a PicoTiter plate, yielding approximately 5,000 sequence reads per sample. Low-quality sequences were removed and primer sequences trimmed and de-coded using in-house Perl scripts. DOTUR was used to assign sequences to OTUs (Operational Taxonomic Units) at 96% identity, then one sequence representing each of the resulting OTUs was selected for inclusion in a multiple sequence alignment from which a phylogenetic tree was constructed (RaxML). This tree was used to cluster the samples with UniFrac. BLAT, the BLAST-like alignment tool, was used to compare sequences against sequence databases obtained from one of the publicly available rRNA sequence resources. Matches with weak homologies were filtered out, and then each read assigned to a specific taxonomic group, resulting in a weighted phylogeny.

Algal microcosms were established in Year 2 to study specific interactions between algae and bacteria under controlled conditions. Four microcosm types were set up: three in which we simulated eutrophication events by addition of different amounts of extra nitrogen and phosphorus, and a control untreated microcosm. We ran the microcosms in triplicate for 4 weeks and sampled weekly, yielding samples on which pyrosequencing has been performed. We also determined biomass dry weight and total chlorophyll, as indicators of overall growth, and phototroph growth, respectively. Analysis of these data is still underway. Comparison of the lake and microcosm data will allow us to determine whether the algal-bacterial interactions observed in our microcosms reflect the natural relationships occurring in the lake.

Principal Findings:

We collected samples over the course of 2009 bloom development and collapse in Labonte Lake. This involved monthly sampling in May and October, with bimonthly sampling during the intervening 4 months, resulting in a total of 10 time points. We also collected peak bloom samples from Rock Lake. Analysis of water quality (Table 1) in LaBonte Lake indicated that dissolved oxygen increased until peak algal bloom (mid July), and then decreased during bloom decay. Total organic carbon displayed an inverse relationship to dissolved oxygen, while total nitrogen and phosphorus exhibited smaller fluctuations. Water quality data for samples taken at Rock Lake at peak bloom (mid July to early August) fairly closely resembled LaBonte Lake data from the same time period (Table 1).

We have generated 141,155 bacterial 16S rDNA sequences, and 133,371 algae 23S rDNA plastid sequences. Archaeal 16S rDNA sequencing has also been performed, but we experienced problems with non-specificity of the archaeal primers. This limited the usefulness of the data obtained. Analysis of algal sequences is still underway, and the results described in the remainder of this section refer only to bacterial sequences. Changes in bacterial populations were compared between sediment, water, and algal mat samples from LaBonte Lake. Different time-points for these three sample types shared broad similarities in phylum-level composition, but each sample type displayed different shifts in composition over time (Figure 1). For example, the most variably abundant phylum in the water appeared to be the Actinobacteria, while this

phylum remained relatively low in abundance in the sediment, where greater shifts in Chloroflexi were observed. At deeper levels of taxonomic classification, there was more variation in composition over time within one type of sample and across sample types. This was most evident at the level of species-equivalents, where few operational taxonomic units (OTUs) were found consistently throughout the sampling regime (Table 2). The OTUs that persisted throughout the sampling never accounted for more than 2% of the total sequences, and were represented by only a single sequence when all sequence libraries were compared. These data suggest that LaBonte Lake contains temporally and spatially diverse microbial communities, with a ‘core’ bacterial population often present in low abundance. Peak bloom bacterial populations in this lake were also compared with those recovered from Rock Lake. Taxonomic analysis (Figure 2) and similarity clustering (Figure 3) indicated that communities from the same sample type (sediment, water, or algal mat) in different lakes were more alike than communities from different sample types within the same lake, indicating strong ecotype differentiations in the freshwater systems under study.

Significance:

The microbial community sequencing performed in our project has resulted in the most exhaustive description of the bacterial community in a eutrophic lake performed to date. It will provide an excellent foundation for selection of bacterial species and functions most relevant to bacteria-algal interactions, for further study. Lastly, it will serve as a reference point for future comparison of microbial communities associated with algal blooms in other lakes. Such comparative analysis will be important to future determination of the most effective management strategies that can be applied in lakes and reservoirs where algal blooms adversely affect irrigation.

Student support:

Undergraduate researcher Sage McCann was supported and trained by this project during the summer of 2009. He was also included as a co-author on an abstract presented at the 2010 International Symposium on Microbial Ecology (see below). Sage (graduated Spring 2009, Molecular Biology) was previously an INBRE Transition Scholar, i.e. a Wyoming community college student supported by NIH INBRE funds to participate in research after transfer to UW. Therefore WRP support for Sage allowed further research training for a community college transfer student. Sage is currently pursuing graduate studies in Pharmacy at UW. An additional student (Kristie Capson, Molecular Biology) was supported in Summer 2010. Postdoctoral fellow Blaire Steven also received training for the duration of the project.

Publications (student and postdoc names underlined):

1. Steven, B., S. E. Dowd, K. H. Schulmeyer, and N. L. Ward. Diversity and abundance of planctomycete populations associated with an algal bloom in a eutrophic lake. In press at Aquatic Microbial Ecology.
*** Note: the work described in this paper was performed prior to obtaining WRP funding, but the results informed the conduct of our project and therefore the paper is included here.
2. Steven, B. and N. L. Ward. Pyrosequencing-based characterization of bacterial, archaeal, and algal population dynamics in a freshwater algal bloom. In preparation for The ISME Journal (Nature Publishing Group).

Presentations (student names underlined):

1. **Steven, B., and N. Ward.** Deep sequencing of ribosomal RNA genes during an algal bloom in a eutrophic lake: a primer for metagenomic sequencing. DOE Joint Genome Institute 5th Annual User Meeting: Genomics of Energy & Environment. Walnut Creek, CA. March 24-26, 2010.
2. **Steven, B., S. McCann, K. H. Schulmeyer, and N. L. Ward.** Characterization of the microbial diversity associated with algal blooms in a eutrophic freshwater lake. 13th International Symposium on Microbial Ecology. Seattle, WA. August 22-27, 2010.

Funding:

We submitted a proposal ('Metatranscriptomic analysis of bacterial-algal interactions: an ecological foundation for enhancing algal biofuel and geoengineering initiatives') that was selected for funding support in 2010 by the US Department of Energy's Community Sequencing Program (CSP), **PI: Naomi Ward**. This is not a traditional award mechanism in which funds are distributed to the University, but rather a peer-reviewed program that allows researchers to compete for access to the high-throughput sequencing resources of the DOE's Joint Genome Institute. We leveraged the support provided by USGS/WRP to obtain this funding, which will help us to establish a program that uses both bioinformatic and experimental approaches to characterize algal-bacterial interactions.

Table 1 Physical and chemical parameters of study sites

	Sample Date	Water Temp. (°C)	pH	Dissolved Oxygen mg l⁻¹	Total Nitrogen mg l⁻¹	Total Phosphorus mg l⁻¹	Total Organic Carbon mg l⁻¹	Algal bloom status
<i>Labonte Lake</i>	May 12	15.6	8.9	3.7	2.8	0.3	14	Pre-bloom
	June 2	12.7	8.7	2.8	1.5	0.2	13	Algal bloom
	June 16	17.0	9.0	4.8	1.2	0.2	18	
	July 16	24.9	9.3	11.2	1.3	0.2	10	
	July 28	21.1	9.2	8.3	1.8	0.2	14	
	June 28	24.4	9.7	10.4	1.8	0.2	14	
	August 3	21.8	8.7	4.4	1.6	0.4	13	
	August 17	15.9	7.9	2.2	1.4	0.4	13	
	August 31	18.4	7.4	5.0	4.0	0.5	20	
	September 14	12.8	7.2	1.2	2.5	0.6	18	
	September 28	11.8	8.4	4.3	1.9	0.4	23	Post-bloom
<i>Rock Lake</i>	July 9	20.6	8.5	8.2	1.1	0.1	10	Algal bloom
	July 21	31.9	8.4	8.4	3.3	0.1	15	
	August 4	16.3	8.0	4.3	3.5	0.1	12	

Table 2 Operational Taxonomic Units (OTUs) shared between lakes and across sampling time points

Sample	Comparison	Shared OTUs
LaBonte Lake	Rock Lake	693
LaBonte Lake algal mat	Rock Lake algal mat	182
LaBonte Lake water	Rock Lake water	187
LaBonte Lake sediment	Rock Lake sediment	255
LaBonte algal mat (all time points)	-	7
LaBonte water (all time points)	-	0
LaBonte sediment (all time points)	-	0
Rock Lake algal mat (all time points)	-	40
Rock Lake water (all time points)	-	28
Rock lake sediment (all time points)	-	18

Figure 1. Taxonomic diversity of 16S rRNA gene sequences in LaBonte Lake over the development of an algal bloom. A). Phylum level taxonomic assignments. B). Family level taxonomic assignments. Only relative abundances of the dominant groups are plotted, so the bars do not sum to 100%.

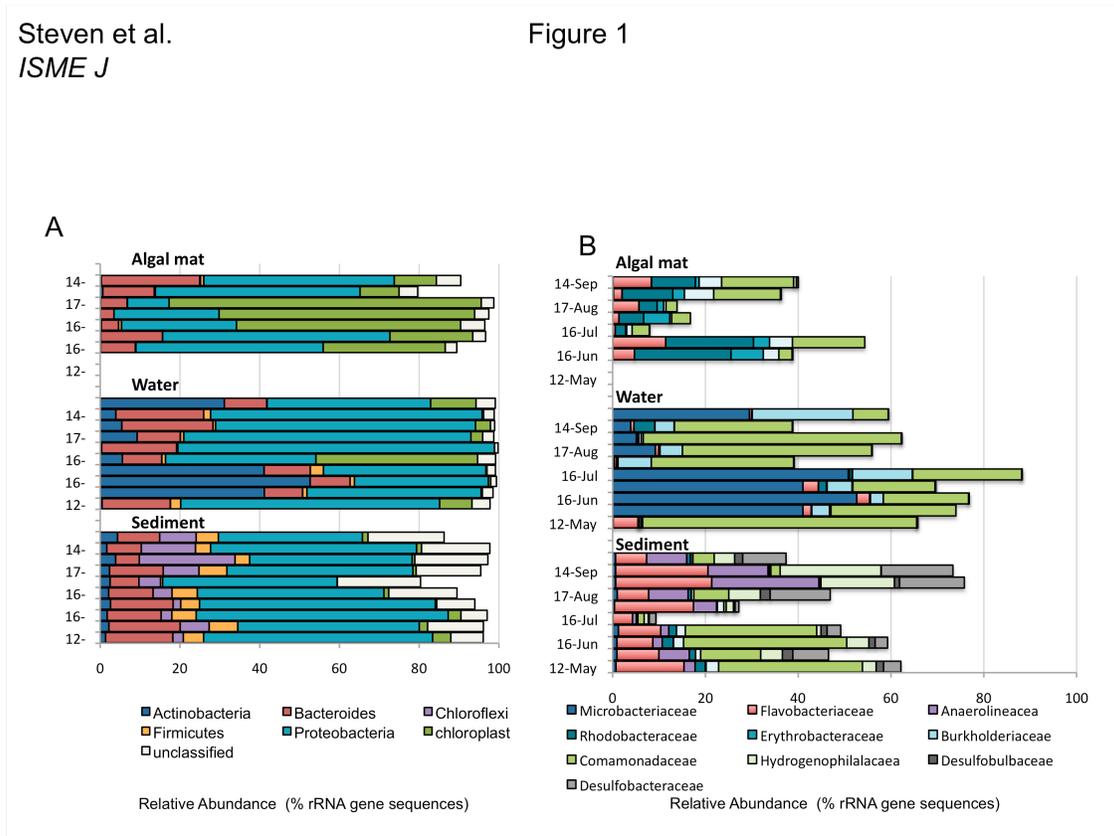


Figure 2. Comparison of LaBonte Lake and Rock Lake microbial communities. Each chart represents a lake environment where the outside ring represents LaBonte Lake and the inside ring represents Rock Lake. Relative abundances (percent of sequence libraries) of the dominant groups are indicated either inside the ring or with the phylum label.

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Figure 2

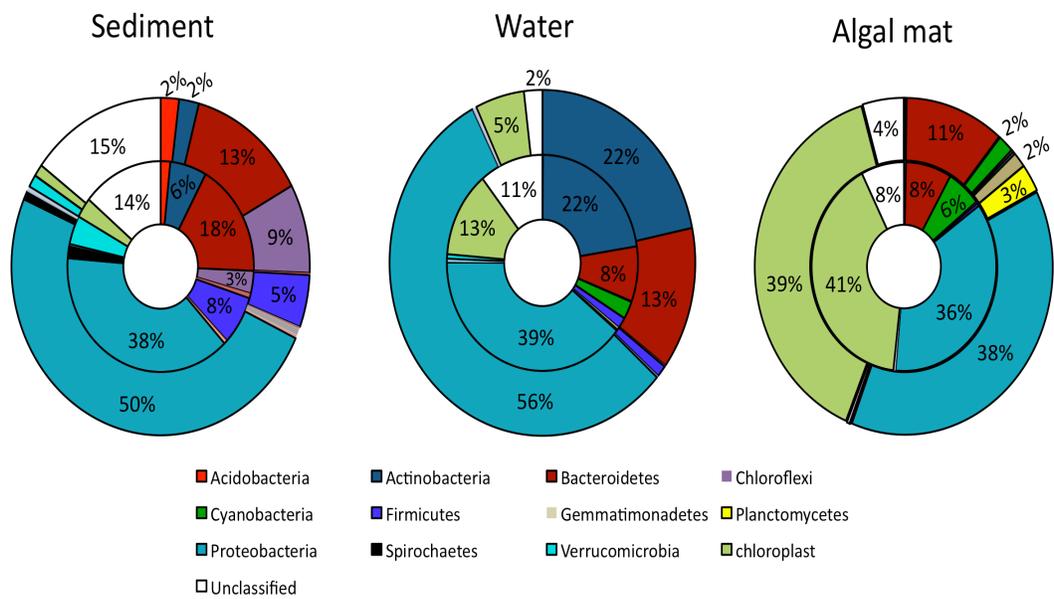


Figure 3. Clustering of sequence libraries. Similarity in community structure was determined using the abundance based similarity index and plotted as a dendrogram. Branch lengths are proportional to the fraction of individuals that belong to shared OTUs.

