

**AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2018 – 2020**

TITLE: *Volatile organic compound production during summer algal blooms in Upper Klamath Lake, Oregon*

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EXECUTIVE SUMMARY: Annual harmful algal blooms (HABs) threaten aquatic environments by producing a wide array of chemical compounds, including toxins and other harmful metabolites, that degrade habitat and increase disease susceptibility in fish and other aquatic animals. Many of these chemical compounds cause either directly or indirectly, a range of severe to deadly health problems in aquatic animals and humans. Annual economic losses in the United States due to HABs are conservatively estimated at \$2-4 billion, with additional hidden losses associated with consumer anxiety and aborted recreation opportunities (Anderson et al. 2000, Watson et al. 2015). The frequency and severity of HABs are increasing due to climate change and nutrient loading from agriculture and other human-related activities (Hallegraeff 1993, Anderson et al. 2008). The interactions between harmful chemical metabolites, the environment, and fish health are strongly correlated but not well understood (Watson et al. 2015). Furthermore, management of fisheries is severely challenged by the unpredictability of HAB development, toxin production, and bloom demise, and a profound lack of knowledge about the full range of chemicals produced by HABs. This research project used high-resolution analytical technology to identify volatile organic compounds (VOCs) that signal HAB trajectories and that impact fish health, development, or edibility. Improved prediction of HABs and better understanding of the chemical ecology causing negative impacts on aquatic health will give waterway managers the critical information needed to determine appropriate and timely management strategies. VOC profiles from samples collected at our study site, Upper Klamath Lake, OR clustered strongly by sampling date. Furthermore, specific VOCs were unique to sample date and partitioned into groups based on their seasonal dynamics, with some VOCs peaking in intensity with microcystin toxin concentrations. Our preliminary analysis provides good evidence that VOCs can be used as early-warning indicators of HAB trajectories.

OBJECTIVES: The overarching goal of this project was to characterize VOCs production in Upper Klamath Lake, OR (UKL) to understand their identity, biological sources, and environmental variability. To achieve our goal we:

1. Measured the diversity of VOCs in UKL across the course of algal blooms in 2018 and 2019.
2. Measured environmental factors, including community composition and toxin concentrations, across the course of algal blooms in 2018 and 2019.

3. Analyze VOC and environmental data to understand the relationships between VOC production and toxin production

With the data now in-hand from the objectives completed above, we are now in the process of using more complex data analysis tools to achieve the final objectives to:

4. Elucidate ecosystem factors that govern VOC production.
5. Explore the feasibility of using VOCs as early warning indicators of toxic HABs.

PROCEDURES: Funding for this work supported five trips to UKL during 2018 and seven trips to UKL during 2019 (Table 1). These trips focused on sampling from three sites: North Agency Lake (east side), Eagle Point and Howard Bay (both on the west side of UKL). This project benefited from linked funding of the Dreher ARF proposal because it expanded sampling to nearby canals that serve as irrigation sources. Sampling was conducted from the spring through fall seasons to capture samples dominated by different cyanobacteria and a range of toxin concentrations (Fig 1). On-site abiotic properties were measured and samples were processed for VOC composition, chlorophyll, carbon and nitrogen concentrations, microscopic identification, and toxin analysis. Subsampling for culture isolates has supported a new collection of HAB-forming cyanobacteria in the lab that will be further analyzed for VOC production (Table 1). Samples were also collected for sequence analyses. All samples (64 total) have been extracted, prepped and are now being sequenced using Illumina paired-end MiSeq at the Center for Genome Research and Biocomputing.

Data analysis: We are using clustering and network analyses to determine ecological factors important in regulating VOC profiles. We anticipate these factors to include biogeochemical parameters such as community composition, inorganic nutrients, toxin production, temperature, and pH.

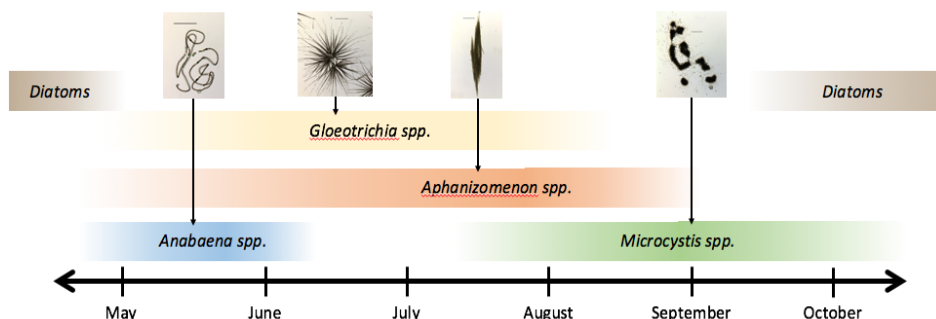
Table 1: Summary of samples collected at and around UKL. Each UKL and canal sample (white) includes community composition by 16S sequencing, VOC profiles by PTR-ToF/MS, and environmental parameters (cell counts, incident light, conductivity, chlorophyll, carbon and nitrogen). For each unialgal isolate, (grey), VOC profiles are being obtained during active growth and following senescence. All samples and isolates will be sequenced for their metagenomes.

Year	Samples collected				Unialgal isolates			
	UKL sites	time points	Canals sites	time points	<i>Gloeotrichia</i>	<i>Microcystis</i>	<i>Aphanizomenon</i>	<i>Anabaena</i>
2018	3	5	4	5	2	0	4	3
2019	3	7	2	7	2	3	6	3
Total	3	12	4	12	4	3	10	6

SIGNIFICANT ACCOMPLISHMENTS TO DATE:

We hypothesized that the VOCs measured in UKL would demonstrate seasonal dynamics coincident with shifts in the algal communities leading to HAB and toxin production. VOC signatures from samples collected in UKL from spring to late fall, 2018, clustered strongly by sampling date, when the microbial community was dominated by different cyanobacterial species at different growth stages (Figs 1 and 2). In both years UKL was dominated by diatoms in May but

Fig 1. General progression of dominant cyanobacteria during the annual bloom cycle at UKL. Toxin concentrations typically peak in August but can be present in high concentrations from July through October.



the algal community shifted to the cyanobacteria *Gloeotrichia* in July and then to *Aphanizomenon* in August through October, although there were differences in the timing of the succession dynamics, and *Microcystis* did not accumulate appreciably in 2018. Two of the sites, Howards Bay and Eagle Point showed more similar VOC patterns than North Agency (2019). These data and other environmental conditions suggested that the cyanobacterial bloom progresses north to south, allowing us to use the north-south gradient as a higher resolution time dimension for our investigation of toxic HAB trajectories.

Specific VOC masses (M+1) were unique to sample date and partitioned into groups based on their seasonal dynamics, with some VOCs peaking in intensity with microcystin concentrations (Fig 4). These results provide strong evidence that VOC signatures detected by automated sensors could provide early warning of HAB trajectories. Broad patterns of seasonality were observed across all VOCs (Fig 4). VOCs in May samples were largely negatively correlated when compared to VOCs in samples collected later in the year, especially August. The pattern shifts through the year, suggesting a clear annual cycle of VOC production.

Our isolates cultured from sub-samples at UKL are being sampled for VOC profiles during active growth and immediately following senescence to test the idea that VOCs produced at different stages of growth would also be detected in UKL when the species representing the isolates are at similar stages of growth *in situ*. VOC profiles from one of our *Gloeotrichia* isolates showed a strong growth phase-dependence, and some noxious and taste/odor VOCs were produced at higher levels during exponential phase in both *Gloeotrichia* and *Aphanizomenon* (Fig 3). VOCs that are common to cultured isolates and field samples will give strong evidence for the algal species involved. Thus, these unialgal isolates will provide supporting evidence for the key microorganisms and their physiologies responsible for VOC production dynamics observed during the UKL bloom cycle. These cultures will serve as important resources for testing hypotheses generated from our data analyses about other environmental drivers of VOCs.

An interesting side-project has developed through our isolation of *Aphanizomenon* collected from UKL. This cyanobacterium exhibits flexible morphology, but the causes are unknown. In fact, most labs struggle to maintain *Aphanizomenon* in culture, and Lindsay has

maintained some isolates for over 1.5 y. We have observed the different morphologies in our cultures (Fig 5), and are now conducting a systematic analysis of environmental conditions that trigger different structures to form.

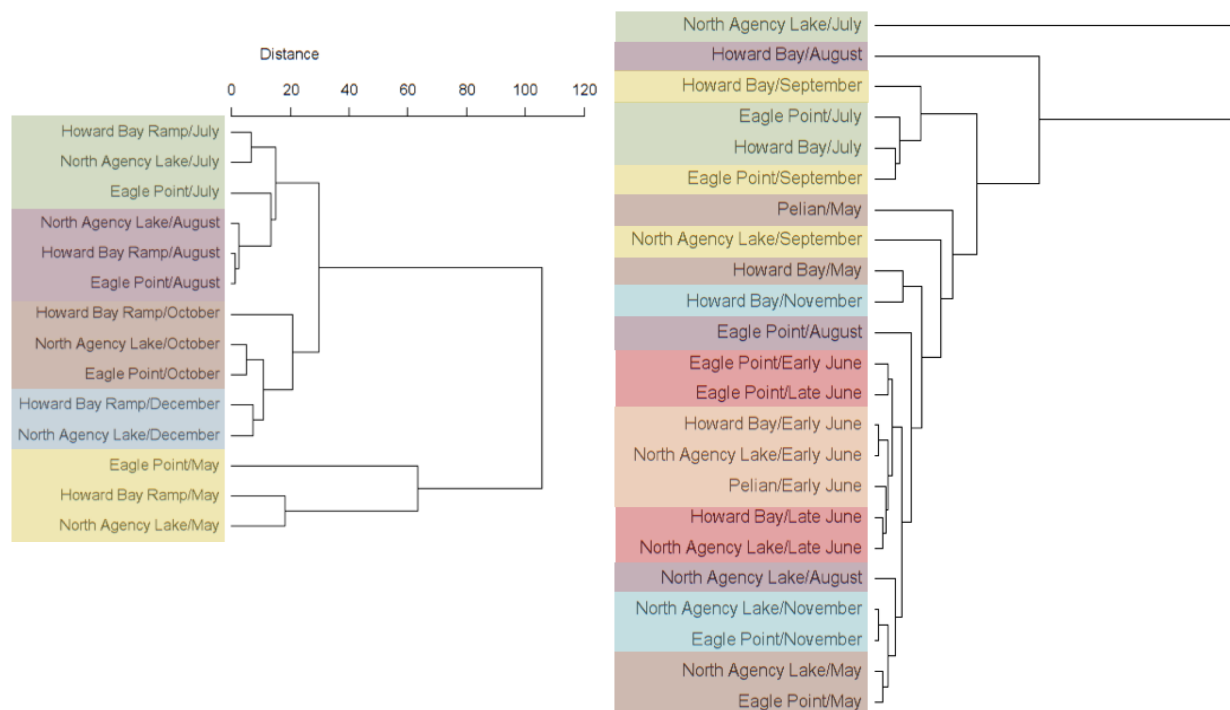


Fig 2. Cluster dendrograms of VOC profiles from samples collected at UKL in May (yellow), July (green), August (pink), October (brown), and December (blue) in 2018 (**Left**) and in 2019 (**Right**). In 2019, July samples along with some August and September samples clustered most distantly from June, November, and May samples. Samples from these latter sampling dates were dominated by diatoms and clustered at the bottom of the dendrogram, distinct from July, August, and September samples that correspond with the HAB and period of toxicity. Samples that do not cluster together but that were collected on the same date, but at different sites, likely reflect the physiological status of the dominant cyanobacteria. For example, August samples, which are distributed throughout the dendrogram reflect the progression of the *Aphanizomenon* bloom and its toxicity: Howard Bay (most toxic)>Eagle Point>North Agency (post-bloom, least toxic).

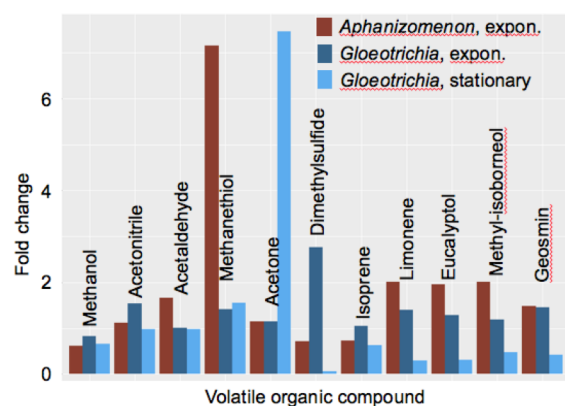


Fig 3. VOC profiles shift depending on species and physiology.

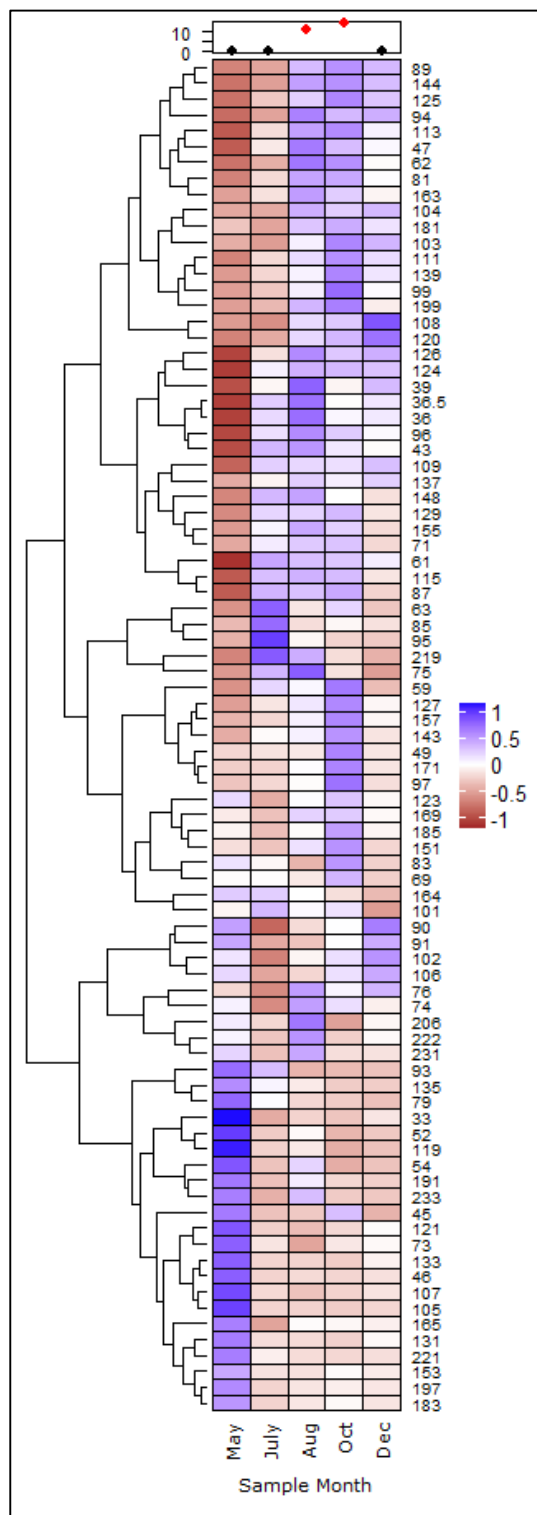


Fig 4. Dendrogram clustering of UKL 2018 samples by VOC composition (Y axis). Samples were grouped by calendar month (X axis). Each row corresponds to VOCs with mass numbers (+1) binned to the 0.5 mass unit shown on the right. Color intensity is the strength of the correlation between mass signal and month of year. The data show strong correlations exist between the volatilome and HAB trajectory (microcystin concentration in ppb shown at top of the figure and red points are toxin levels above the EPA recreational threshold).



Fig 5. *Aphanizomenon* forming “fluffs” (top) and “flakes” (bottom), morphological expressions of their fascicles. We hypothesize that morphological differences are triggered by light availability.



ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

- Additional funding was received from the Department of Microbiology in the form of a Graduate Teaching Assistantship (Winter, 2019).
- Tartar award, Dept. of Microbiology: \$7,000 (2019)
- Tartar award, Dept. of Microbiology: \$7,000 (2020)
- SciRIS-II, College of Science: \$10,000 (2020)

FUTURE FUNDING POSSIBILITIES:

- Pending: Rural Data Science for the Public Good, USDA NIFA & the Bill and Melinda Gates Foundation: \$ 10,000 (2020)
- Pending: Collaboration with CGRB, Brett Tyler - CC*Team Oregon Big Data Research and Education Team, NSF Campus Cyberinfrastructure (2021)

SUMMARY STATEMENT:

We are grateful to the Agricultural Research Foundation for funding this pilot project. We are now able to leverage the preliminary data and high-dimension data set to gain new support for our idea that VOCs can be used as early warning indicators of toxic HAB events. This project benefits from a two-year data-set of environmental monitoring at UKL (2018-2019). Samples were collected from three locations in UKL and in nearby canals used for irrigation of cropland (Table 1). Samples captured periods of the annual bloom cycle at UKL that were dominated by different algal species to capture a broad range of biological and chemical characteristics occurring across the annual HAB events (Fig 1).

Our high-dimension data set includes: community composition by 16S sequencing, VOC profiles, and environmental parameters (cell counts, incident light, conductivity, chlorophyll, carbon and nitrogen). These discrete samples are supplemented by continuous USGS buoy monitoring of temperature, wind direction, dissolved oxygen, and pH.

The next step is to use cutting-edge statistical tools for high-dimensional data to model the associations between microbiome composition, the volatilome and the environment. We have begun working with Duo Jiang (Assistant Professor, Statistics) to apply dimension reduction methods such as sparse principal component analysis (Zou et al. 2012), canonical association analysis (Witten and Tibshirani, 2009), and regularized regression techniques including lasso and group lasso (Yuan and Lin, 2005) to identify VOC and microbiome signatures that coincide with shifts in the UKL microbial communities. Furthermore, we will assess the potential for integrating multiple types of information including microbiome, volatilome, and environmental data in addition to time-of-year to boost the power of predicting toxin and HAB trajectories.