Mobile toolkit for rapid in-field screening of freshwater Harmful Algal Blooms

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Caption: GIS based, publically available map of HABs locations and microscope photographs on 0.5 mm gridded backgrounds

Abstract

This project aimed to develop inexpensive and rapid field screening tools for microcystin producing cyanobacterial Harmful Algal Blooms (HABs). During the 2020 HABs season, we trained 10 volunteers in Cayuga and Canandaigua Lakes how to collect and share images of suspected HABs using inexpensive WiFi microscopes. Using ImageJ opensource image analysis software we generated correlations between image colony abundance and the levels of microcystin (MC) (R squared of 0.48) and chlorophyll A, a general measure of algal density (R squared = 0.64). Additionally, our team performed qPCR for MC synthase genes on DNA extracted from a subset of HABs and background non-bloom samples. Using both benchtop and handheld devices for qPCR we found excellent sensitivity of the method for screening for high toxin HABs (87.5-96%). Other key findings are that: multiple strains of Microcystis (MC-producing genus) are present in the Finger Lakes and that one, specific to Canandaigua Lake, has a very high MC/chloral ratio; blooms arose quickly from non-bloom samples and non-bloom samples immediately preceding the event had detectable levels of the MC toxin gene. The work strengthened relationships among the community partners and trained community members on tools for HABs monitoring. In addition to one publication submitted to Water Research journal, and two others in preparation our team also disseminated the results via webinars, a newsletter in the Cayuga Lake Watershed Network newsletter, and the creation of a publicly available GIS based map that shows microscope images associated with the different locations sampled.
Three Summary Points of Interest

• Inexpensive Digital WiFi microscope kits deployed by 10 volunteers and our team were able to distinguish bloom from nonbloom samples and distinguish between MC-producing and non-MC-producing cyanobacterial species
• A qPCR workflow on a handheld field qPCR device performed well in distinguishing toxic from nontoxic water samples
• Canandaigua Lake has high MC/chloral ratios than Cayuga Lake in both non-bloom and bloom samples and both microscope images and 16S rRNA based community analysis support that this lake has a unique and highly toxic species/strain of Microcystis

Keywords

HABs, volunteer science, microscopy, Finger Lakes, qPCR, Microcystin

Policy implication statement; products of interest and/or upcoming events

This project has implications for policy in that it can inform beach managers on when to take a “clearance” sample after the HAB passes (in order to open the beach again).

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Introduction
The Finger Lakes (FLs) region of NY has seen a steep increase in occurrences of cyanobacterial harmful algal blooms (cHABs) over the last decade. Since 2016, local volunteer groups have been established in a grassroots effort to monitor blooms across the FLs. One of the FLs, Cayuga Lake (CY), had the most reported cHABs of all NY water bodies in 2020 and 2021 (NYHABs website: https://www.dec.ny.gov/chemical/83332.html). The Cayuga Lake HABs Monitoring Program, initiated by the Community Science Institute (CSI), the Cayuga Lake Watershed Network (CLWN) and Discover Cayuga Lake (DCL) in 2018 and implemented annually from June to October on CY, recruited and trained “HABs Harrier” volunteers to collect and document suspicious blooms and deliver samples to CSI’s state-certified lab for measurement of chlorophyll A and microcystin (MC) as well as microscopic identification of cyanobacteria taxa using compound microscopes. Volunteers patrolled slightly >50% of the 96 miles of CY shoreline in 2020. Patterns from three years of data collected by the Cayuga Lake HABs Monitoring Program (2018-2020) showed that cHABs were dominated by the genus Microcystis, and a strong correlation was observed between the concentrations of MC and chlorophyll A if Microcystis was present.

Results & Discussion
We collected more than one hundred samples from across the Finger Lakes with most coming from Cayuga Lake and Canandaigua Lake. Samples had MC levels ranging from below detection (<0.3 ug/L) to >1500 ug/L. Other samples were collected from Keuka, Seneca and Honeoye Lakes. We will report on results related to DNA analysis followed by the inexpensive image analysis.

DNA analysis:
Major findings regarding DNA analysis by 16S profiling showed that Microcystis is indeed the main MC producer in the Finger Lakes and that the other major bloom producer, Dolichospermum, can make dense blooms but with no to low MC level. The analysis also highlighted some common genera (e.g. Pseudoanabaena) that colonize with Microcystis and may provide nitrogen to the community via nitrogen fixation. The community analysis work guided our qPCR primer selection to ensure that the assay would capture all relevant genera of MC producers in Cayuga and Canandaigua Lakes. The qPCR assay for the mcyA gene (a key gene in the synthesis of MC toxin) showed good performance for screening if the sample was a high MC sample or not (Figure 1; Table 1). More details are provided in the attached manuscript which has been submitted for review for publication.

Figure 1: Log-log plot of microcystin level in 2020 Finger Lakes samples versus qPCR gene copy abundances using a portable , battery powered qPCR device.
Table 1: Sensitivity and specificity of qPCR methods for detecting high toxin HABs.

<table>
<thead>
<tr>
<th>Method</th>
<th>n</th>
<th>Test</th>
<th>δt</th>
<th>p-value</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bench-top</td>
<td>28</td>
<td>Non-inferiority</td>
<td>0.05</td>
<td>0.014</td>
<td>75.9%</td>
<td>75.9%</td>
</tr>
<tr>
<td>Handheld</td>
<td>29</td>
<td>Non-inferiority</td>
<td>0.15</td>
<td>0.211</td>
<td>96.0%</td>
<td>60.0%</td>
</tr>
<tr>
<td>Handheld vs Bench-top</td>
<td>23</td>
<td>Equivalence</td>
<td>0.15</td>
<td>0.313</td>
<td>96.3%</td>
<td>75.9%</td>
</tr>
</tbody>
</table>

*δt: equivalence limit

**Results if two borderline toxic samples (MC = 3.31 and 3.85 µg/L) were considered as toxic samples

The image-based coding program showed promising results as a preliminary screening tool to determine whether a bloom can be categorized as toxic. The program’s outputted bloom density was a more accurate predictor of chlA levels than it was for MC levels (See Figure 2 and 3).

Figure 2: Image Analysis workflow for the project.

Field Microscope Images Analysis Workflow

1. Very high data
   - Image data
   - Colony Area (colony density in plot)
   - RGB (color) info
2. Neural Networks
   - Algorithms for predicting toxic bloom sample from images

Table 2: Results of correlations using ImageJ software to identify colonies.

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However, the coding program requires more calibration before it can be used reliably. While the program correctly identified ROIs for images with high chlA and MC levels with larger, clearly defined cyanobacteria colonies, it struggled to identify ROIs for images with low chlA and MC levels with minimal colonies. In images with few, ill-defined colonies, the program created “ghost colonies”. “Ghost colonies” cause an overestimation of the samples’ bloom density, which in turn causes the program to inaccurately classify a non-toxic bloom as toxic. This limited the applicability of the program to chlA and MC levels that are greater than the NYSDEC threshold for safe recreation. Future work to improve the program includes creating new iterations that distinguish cyanobacteria colonies from non-harmful objects, like pollen and green algae. By recognizing the lack of colonies before fitting ROIs to the image, it would prevent the creation of “ghost colonies” and widen the application of the program to blooms with smaller chlA and MC levels.

We also created a GIS based map for public access (Figure 4):

Figure 4: Publicly available map on ArcGIS from 2020 pilot test of the scope kits on Canandaigua and Cayuga Lakes. Locations of samples are colored by how high the microcystin toxin was (red being the most highly toxic). Each dot is clickable and opens up information and associated microscope images (shown here is a photo with the 500 micron grid background).

https://www.arcgis.com/home/webmap/viewer.html?webmap=97e6f1dbd69475s676619470d1ad577&extent=-77.7602,42.3464,-76.3663,42.9615.

Understanding the microbiology of HABs in Cayuga Lake and other Finger Lakes narrows down the types of organisms laboratories should consider when determining whether a bloom is toxic. This knowledge will help train future HAB Harrier Microscopy volunteers in the type of organisms to look for when sampling potential HABs. In addition, knowing the dominant cyanobacteria species will help inform which shapes the coding program should identify in its ROIs. For example: Dolichospermum colonies tended to appear as thin and curly at a magnification of 500
microns; Pseudanabaena colonies tended to appear as straight, segmented cells; and Microcystis appeared as globular, rounded groups of cells. In future updates of the coding program, the shape and border of ROIs can be refined to identify different cyanobacteria. This would improve bloom density estimates and further strengthen the relationship between outputted bloom density and chlA. Interestingly Canandaigua Lake compared to Cayuga Lake, had much higher MC-to-chlorophyllA levels and also had distinct shapes of Microcystis colonies as well as unique strains in 16S libraries. All of these data are suggesting distinct communities which result in different toxin expression levels.

Figure 5: Comparison of 2020 samples from Cayuga and Canandaigua Lakes, showing the generally higher MC level in both non-bloom and HABs samples. Additionally, representative images from under the inexpensive microscopes are shown on the right.

With global increases of HABs in freshwater bodies, it is critically important to have effective identification and timely public health responses. Combining community-sourced data with traditional scientific methods such as culturing, microscopy and coding programs can facilitate substantial improvements to monitoring and information sharing programs and lessen the risks to human health, ecosystems and the tourism economy.

Policy Implications
This project has implications for policy in that it can inform beach managers on when to take a “clearance” sample after the HAB passes (in order to open the beach again).

Additionally, the tool could aid in mitigation efforts. For example technology is being explored by the Army Corps that use air flotation/skimmers to remove HABs colonies from the water body. Simply on site imaging can help prioritize areas for removal as well as to document removal effectiveness – by examining how many colonies remain.

Methods
The work partnered with community members and local nonprofits to gather and analyze suspicious HABs and also non-bloom background samples. ChlorophyllA, microcystin (MC) and compound microscopy were performed by CSI and FLI partners. The Cornell partners developed an inexpensive microscope field kit and trained 10 volunteers on its use. We collected hundreds of images and used ImageJ opensource software to effectively detect HABs colonies in digital images and compared the results to the levels of chlorophyll A and MC (as well as which genera/species were identified using more expensive compound microscopy). Additionally, for some samples, DNA was extracted and analyzed for qPCR levels of the mcyA (MC toxin synthase gene) and 16 S rRNA community analysis. The qPCR assay was tested on two devices: one benchtop and one field-portable. Gene concentrations of mcyA were also compared to the levels of chloral and MC.

Outreach Comments
This work relied heavily on outreach to volunteer HABS monitors. Because the Covid19 pandemic prevented almost all in person training in summer/fall 2020, our team developed online training materials and also help weekly zoom sessions with HABs microscope volunteers. Additionally, our team held outdoor outreach events related to HABs in 2020 in coordination with the Sciencenter museum.

Student Training
The project supported one PhD student and also partly supported two undergraduates: one at Cornell and one at Hobart and William Smith Colleges/The Finger Lakes Institute.

Notable Awards & Achievements
See Publications/Presentations
Also follow on funding was received by Engaged Cornell to support further development and dissemination of the microscope toolkit.

Publications/Presentations
Please list publications, presentations, book chapters, reports, conference proceedings, etc. resulting from this project.

Publication 1 in review at Water Research (co-authored with community partners, Finger Lakes Institute and
Community Science Institute). Title: “Harmful Algal Blooms in Cayuga Lake, NY: Development of Mobile qPCR Assay for Onsite Screening”

Publication 2 in preparation; Title: “Investigating the Applicability of an Image-Based Bloom Toxicity Screening Tool for Harmful Algal Blooms”

Conference proceedings at the General meeting of the American Society for Microbiology, June 2022, Washington DC (PhD student Nan Wang was chosen for a talk)

Newsletter article published with the Cayuga Lake Watershed Network’s Network News: LINK.

Presentations:
Talk at the Cayuga Lake Watershed Network Fall Conference. Title “Rapid tools to screen samples for elevated microcystin”

Talk at the HABs Upstate Symposium, Fall 2021; Title:” $40 digital microscope kit for rapid onsite cyanobacteria screening”
Presentation in Fall 2021 at the Tompkins County Water Quality Research Council meeting

Sciencenter presentations (introduction to cyanobacteria and algae using the inexpensive microscopes).

Additional final reports related to water resource research are available at http://wri.cals.cornell.edu/news/research-reports

References
https://doi.org/10.1016/j.hal.2015.12.007

Chaffin et al., 2018.
https://doi.org/10.1016/j.hal.2018.02.001

Wang et al, 2022 (in Review Water Research, March 2022)

Websites:
Team’s GIS map of images by location of sample (also includes links to ArtStor long term image storage/open access). Publicly available map on ArcGIS from 2020 pilot test of the scope kits on Canandaigua and Cayuga Lakes
https://www.arcgis.com/home/webmap/viewer.html?webmap=97e6fb1dbd694755a67619470d1ad577&extent=-77.7602,42.3464,-76.3663,42.9615.

Appendices (if needed)
1. Manuscript in review for publication
2. pdf of presentations given to the community about the inexpensive microscopy project. Basis of manuscript in preparation

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