Chapter 22: Field methods in the study of toxic cyanobacterial blooms: results and insights from Lake Erie Research

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Abstract

Sound field methodologies are an essential prerequisite in the development of a basic understanding of toxic cyanobacteria blooms. Sample collection, on–site processing, storage and transportation, and subsequent analysis and documentation are all critically dependent on a sound field program that allows the researcher to construct, with minimal uncertainty, linkages between bloom events and cyanotoxin production with the ecology of the studied system. Since 1999, we have collected samples in Lake Erie as part of the MELEE (Microbial Ecology of the Lake Erie Ecosystem) and MERHAB–LGL (Monitoring Event Responses for Harmful Algal Blooms in the Lower Great Lakes) research programs to develop appropriate tools and refine methods necessary to characterize the ecology of the reoccurring cyanobacterial blooms in the systems. Satellite imagery, large ship expeditions, classical and novel molecular tools have been combined to provide insight into both the cyanobacteria responsible for these events as well as into some of the environmental cues that may facilitate the formation of toxic blooms. This information, as well new directions in cyano–specific monitoring will be presented to highlight needs for field program monitoring and/or researching toxic freshwater cyanobacteria.

Introduction

Responses to toxic cyanobacterial events in freshwater systems require, as a first step, the identification of the event in question. Advanced field methods, designed to identify potential events and provide subsequent confirmation, are a critical need in cyanobacterial harmful algal bloom (CHAB) management. This need begets the development of sound approaches and tools for field applications, which will provide a systematic, tier–based response to events (i.e., a series of response ranging from casual observation to event validation and management action). This paper reviews the presently available technologies for field use to identify, confirm and characterize CHABs.

Given the global scale of CHAB events, an appropriate tier–based structure is required that allows for primary identification of a potential event, localized confirmation of this event, and subsequent characterization of the diversity and activity of the community members associated with the event. Combined with an identification of essential future need items, the goal of this paper is to provide the reader with insight into the current state of the available monitoring systems.

Early identification systems: The application of satellites and sentinel warning systems.

Although CHAB events occur on regional scales of meters to kilometers, a broad–based approach to monitoring freshwater systems for potential CHABs is ideal. While not necessarily applicable for small bodies of water (such as rivers and ponds), large bodies of water (e.g., the Laurentian Great Lakes) can be effectively monitored using available satellite imagery. Application of satellite imagery to monitoring large scale oceanic events is well established, with proxies for plankton biomass, sea surface temperature, sea surface height, etc. (Field et al. 1998). Application of these tools as sentinels for potential CHAB events in freshwater environments is enticing, as large scale imagery can be used to provide basic insight into phytoplankton distribution on time–scales associated with satellite flyover. As an example, current imagery for Lake Erie (which has been plagued by reoccurring blooms of toxic *Microcystis aeruginosa* since 1995, (Brittain et al. 2000)) includes true color satellite data (Fig. 1a) as well as inferred products such as phycocyanin (Vincent et al. 2004). While potentially limited by cloud albedo (Fig. 1b, August 23, 2005), this imagery nonetheless provides a simple, global approach to bloom monitoring.

One limitation of these imagery products is that they are currently based solely on the presence of inferred pigments. While blooms of toxic cyanobacteria do lead to significant concentrations of water column chlorophyll a (Rinta–Kanto et al. 2005), other non–toxic phytoplankton can similarly bloom and result in "false alarms" (Fig. 1c, May 3, 2005, see Color Plate 8). As such, any potential events identified by these tools will require more specific ground based confirmation.

Sentinel systems, incorporated into existing or newly deployed buoys, *in situ* monitoring devices, etc. also provide valuable insight into water column conditions that can be used as a first–alert to CHAB events. Predicated upon similar analytical methods as the satellites, currently available early monitoring systems have examined chlorophyll and phycocyanin as proxies for algal biomass. An expanded distribution of these sensors, combined with networking to a single source hub, is an ideal first step in the development of a global monitoring system for North American freshwater environments. The ongoing development of the IOOS (Integrated Ocean Observing System, see http://ocean.us/) and linkage to region nodes such as the proposed GLOS (Great Lakes Observing System, see http://www.glc.org/glos/) should provide stakeholders and end users with the potential to rapidly assess water conditions. Expansion of these sensors to mobile platforms (e.g., Coast Guard vessels, regional car and passenger ferries, public and privately managed docks, etc.) can be expected to further enhance coverage. These integrated networks represent the ideal ground based system for the incorporation of emerging CHAB sensors (discussed later). Moreover, many of the available sentinels can be deployed in small water bodies (e.g., ponds, rivers, reservoirs) that are typically below the resolution of satellites. As with any remote data collection method though, these systems require field sampling and confirmation prior to any action to deal with the bloom event.

Response to potential CHAB events, sample collection and processing.

The collection of a potentially toxigenic water sample that will be used to make management decisions of public access and use of a water resource requires appropriate and timely handling. In studies involving toxic samples particular attention needs to be paid to safety. While no prescription for preventative measures is given here (due to the diversity of sample and toxin types covered in this general overview) it is strongly recommended that newer personnel seek guidance from senior scientists and err on the side of caution concerning exposure risk.

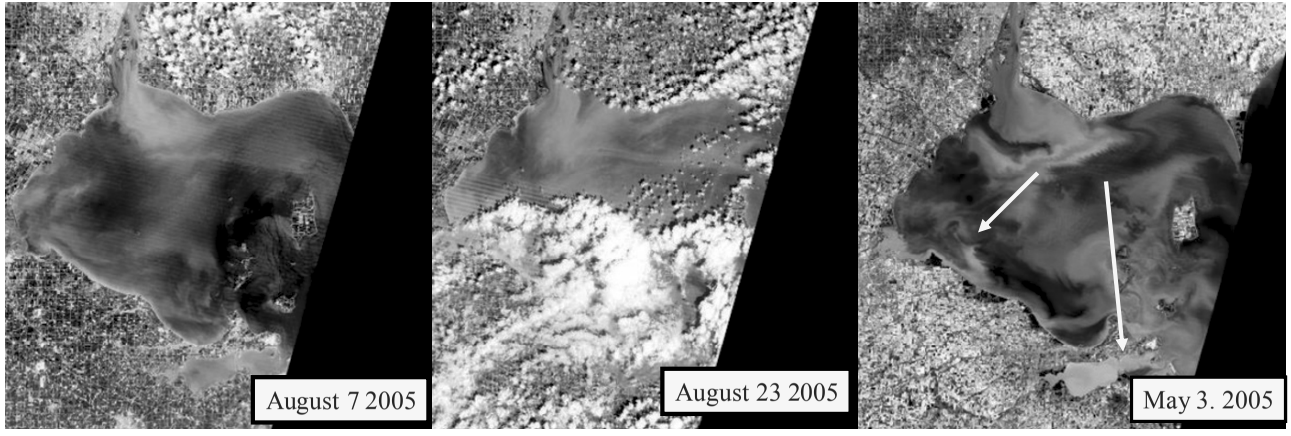

Fig. 1. LandSat 7 image of the western basin of Lake Erie for August 7, 2005. Greenish coloration of the water column in Sandusky Bay and at the mouth of the Maumee River suggests the onset of seasonal cyanobacterial blooms. (See Color Plate 8).

In field studies, the collection of water samples is commonly dependent upon the sampling site (riverine, pond, large lake) and end use of the sample (e.g., measurement of toxin molecular markers, measurement of toxin concentrations, processing for experiment or culture conditions). For samples to be processed for cellular genetics or toxin content, water collection onto appropriate filters (e.g., 0.2–µm polycarbonate filters) needs to be completed in a manner that ensures trace levels of contamination between samples does not occur. For the analysis of molecular markers (by PCR, microarray, etc.) sample storage is a critical issue as nucleic acids decay. If samples are processed in the field this issue is moot, but if they are to be transported to a laboratory then considerations of buffering and refrigeration, appropriate for the planned analyses must be considered. Several companies now provide stabilizing solutions for RNA and DNA that can be used to reduce degradation during transport. Preservation of samples for analytical measures of toxin concentration are being dealt with in companion papers (Lawton this volume, Meriluoto this volume).

Toxic bloom confirmation – a first level response to a potential threat.

As stated above, sentinel systems to autonomously monitor aquatic bodies for CHAB events require confirmation of any potential positive results. As a first step, a rapid, easy to use and cost–effective diagnostic can be employed to quickly ascertain whether an event is occurring. These tools are now commonplace and can take many forms: strip tests, reactive tube assays, etc. They are used in widely from the detection of pathogenic bacteria (like *E. coli* O157:H7) to home pregnancy tests. Compact and easy to use, these assays typically lack the sensitivity of more refined laboratory techniques, but provide a simply binary (yes/no) output and require little training to employ or interpret. Currently the commercial market consists of slightly more complicated tools (e.g., multiwell ELISA type assays), but it is anticipated that significant demand will drive the development of simpler tools in the near future.

Bloom confirmation and event characterization

Once a bloom occurrence has been identified, it is important for several reasons for full characterization to be carried out. Management of blooms and toxic events as well as public awareness campaigns regarding risks associated with exposure are dependent upon information concerning the responsible CHAB species, the toxin produced, and environmental conditions associated with the event. Collection and archiving of information regarding events remains one of the best scientific tools that may, in the future, allow for a better understanding of factors associated with bloom initiation.

Obviously, characterization of toxins produced during these events is a critical factor. Techniques to characterize and quantify toxins vary almost as widely as the toxins themselves. Moreover, ranges in sensitivity, cross–reactivity, and equipment/technical expertise are also broad. While an important consideration for the analysis of field samples, it should be noted that most of these current tools (like those of the molecular biologist, below) require transport of the sample to a research laboratory as equipment is often too cumbersome for field use. For more details on approaches to measuring toxins, see papers by Lawton and Meriluoto (this issue).

If available, microscopy provides important morphological information that can be used to provide a preliminary characterization of the CHAB associated community. The fact that species of potentially toxic cyanobacteria can be rapidly distinguished from others by morphology remains one of the best tools of the cyanobacteriologist (Chorus and Bartram 1999). However, while this gross morphology allows for distinction to be made between different taxa (a dying art in the scientific world !), morphology cannot be used to distinguish between toxic *vs* non–toxic cells (Ouellette and Wilhelm 2003). Indeed, one consideration in the development of new techniques for the analysis of toxic blooms is that they must be able to make these distinctions: as such the introduction of molecular tools to the CHAB research community holds great promise.

Given the limitations of microscopy, researchers have globally turned toward molecular tools to identify cyanobacteria as well as characterize their ability to produce toxins. Phylogenetically, cyanobacteria have been characterized by several researchers based on the sequence of their 16S rDNA (e.g., (Urbach et al. 1992; Nelissen et al. 1992; Nubel et al. 1997)). This allows researchers to place cyanobacteria into a general phylogeny (*vis a vis* (Woese 2000)). As shown in Fig. 2, this information can be useful as cells will cluster, for the most part, based on their taxonomy. Indeed, one strength of the molecular approach is how it often can corroborate ex-

isting taxonomies based on classical (i.e., morphological) approaches (Castenholz 1992; Komárek 2003). As such, sequences from taxonomically unidentified isolates or natural populations can be quickly characterized and a determination as to their potential phylogeny provided.

A set of problems however arise when using the standard "universal marker" (16S rDNA) for phylogeny:

1. The 16S rDNA marker in itself does not allow the user to determine the toxigenic potential of the organism in question, as phylogeny based on this marker has no relation to the potential of cells to produce any of the diverse cyanotoxins.
2. Identification of any organism is based on the quality of the information in the databases (i.e., GenBank, EMBL, the Ribosomal Database Project, *etc*.). In some cases the information in the databases may be misleading, out of date or even incorrect.
3. Application of such a general tool to natural systems is often confounded by an overabundance of non–target organisms. For example, in studies conducted during *Microcystis* blooms in the western basin of Lake Erie, PCR amplification of cyanobacterial 16S rDNA have yielded only *Synechococcus*–like sequences, even though *Microcystis* were abundant during the sample periods (Ouellette et al. 2005).

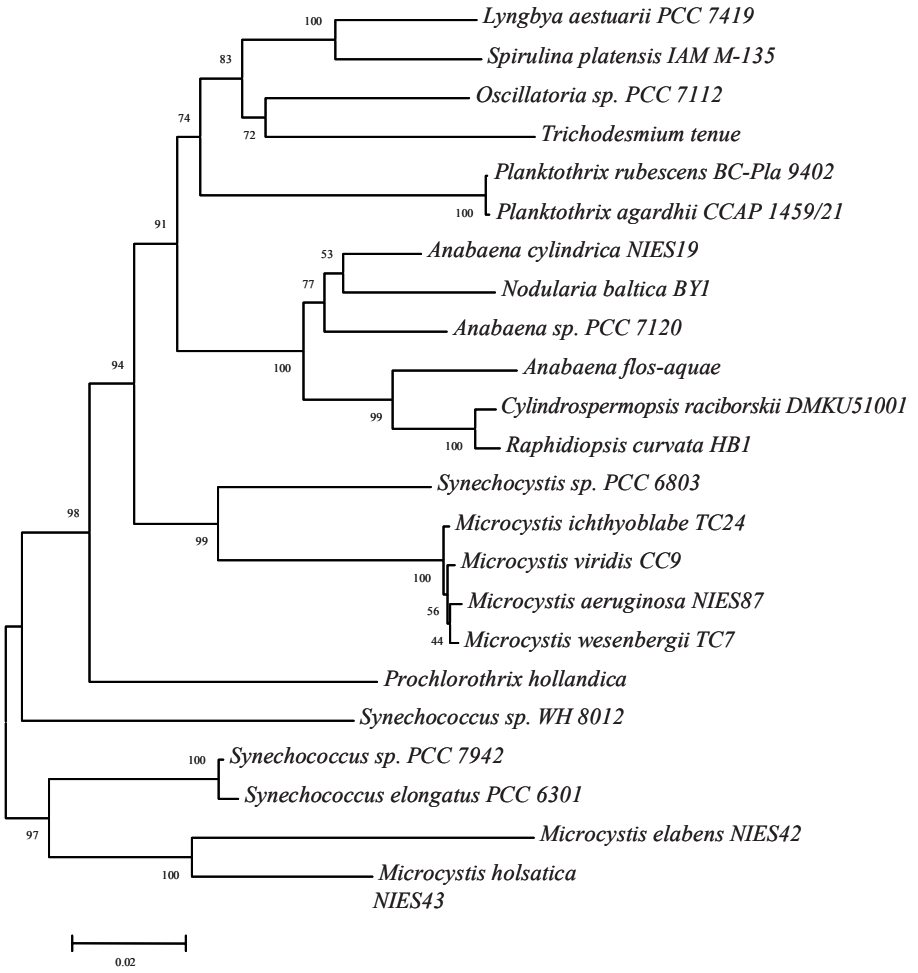

Fig. 2. Phylogenetic analysis of 16s rDNA sequencings of common cyanobacteria. Sequences were acquired from the ribosomal database project, aligned and edited in BioEdit (Hall TA 1999) and dendogram created using the neighbor-joining approach with Mega3 Kumar et al. 1994).

As such, while generic makers of microbial phylogeny are useful with organisms in culture, more specific molecular makers are desired for studies with mixed communities. Functional genes, such as the *nifH* (nitrogen fixation pathway), and conserved regions such as the intergenic *cpcBA–IGS* (between *cpcB* and *cpcA*, which encode for phyobilisome subunits) have been shown to be effective at discriminating specific groups of potentially toxigenic cyanobacteria (Dyble et al. 2002). More specifically, a

number of studies have genetic elements directly associated with the production of specific toxins (e.g., (Nonneman and Zimba 2002; Kaebernick et al. 2000; Neilan et al. 2003)). Identification of these genes is of interest as they can be used in populations, alone or with other makers, to not only determine which type of cells may be present, but also whether or not the specific cell line is potentially toxigenic (Ouellette and Wilhelm 2003). While holding great promise for the future, the specificity of these genetic markers also delimits one of their flaws: the specificity of PCR primers for certain toxin genes means that related by different genetic elements are easily missed by these approaches. Moreover, it means that the development of probes is limited to only those strains that we have genetic information for – as such the identification of the > 70 variants of microcystin is a current significant issue.

Once developed though, the movement of these tools from binary (presence/absence) data collection to quantitative data collection is now relatively easy. Quantitative PCR (qPCR, sometimes also known as real–time PCR) is an approach that allows an investigator to estimate the abundance of copies of a target gene of choice in an original sample. Several research groups have now developed and published information using a variety of genetic elements as targets, and have been able to quantify *Anabaena* and *Microcystis* in natural samples (Vaitomaa et al. 2003; Rinta–Kanto et al. 2005; Kurmayer and Kutzenberger 2003). This breakthrough has allowed researchers to not only accurately quantify toxigenic cyanobacteria in natural environments, but has allowed them to do it effectively amongst a background of other cyanobacterial populations.

Future directions for field studies in toxic cyanobacterial blooms

Rapid advancement in monitoring approaches, a better understanding of the causes and phylogenetic diversity, and the development of a national reporting system for CHABs should all be priorities for federal agencies, scientists and system managers. A great deal of excitement and anticipation surround large scale monitoring plans (such as the GLOS described above). However, these approaches need to be tempered with insight into the development of tools (e.g., bioreporters, cytotoxicity monitors, etc.) that can be incorporated into a systems of deployable sentinels that can monitor smaller, regional areas at risk. Expanded distribution of sensors to other platforms (e.g., commercial ferries, government vessels and coastal docks, etc.) will in part offset “gaps” in the system.

Along with enhanced vigilance, the continued insight into the genetic mechanisms of toxin production and the organisms that are capable of these biochemistries, coupled with the development of advanced autonomous tools to characterize communities based on molecular markers, will allow for the determination of both the presence (DNA) and activity (protein or product) of genetic systems capable of producing toxin production. Linkage of these systems to remotely deployable biosensors (e.g., bioluminescent bacterial bioreporter systems, (Layton et al. 1998; Mioni et al. 2003) that can be incorporated into real time microsensors (Simpson et al. 1999) should allow for accurate characterization of cell abundance, toxin concentrations and toxin activity. When coupled to deployable sentinels, and supported by other technologies (e.g., fluorescence based sensing of pigments, etc.) these tools will provide a powerful first alert to CHABs. Moreover, the continued advancement of our understanding of the environmental conditions that lead to CHAB events will provide new avenues to target sensors.

Perhaps a final consideration that needs to be made is that there currently remains no reporting system within North America (an important point as many blooms occur in waters that cross national boundaries) where information can be archived. Modern computer software and nearly universal internet access amongst researchers and managers provide the necessary framework for the development of a national repository for this information. Information on system chemistry, microbial diversity, toxins and physiochemical parameters should all be collected that could, through data mining approaches, provide insight into the causes of CHABs and assist in future management decisions.

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