Quality Assurance Program Plan (QAPP)
For the
CYANOBACTERIA MONITORING COLLABORATIVE PROGRAM

By the

U.S. ENVIRONMENTAL PROTECTION AGENCY
ECOLOGY MONITORING TEAM
ECOSYSTEMS ASSESSMENT UNIT
OFFICE OF ENVIRONMENTAL MEASUREMENT & EVALUATION
NEW ENGLAND REGIONAL LABORATORY
11 TECHNOLOGY DRIVE
NORTH CHELMSFORD, MASSACHUSETTS 01863

Title and Approval Page:

Hilary Snook, Ecology Monitoring Team Project Lead — Date: 4/13/2017

Dr. Jeff Hollister, USEPA Atlantic Ecology Division — Date: 2017-04-04

Dr. Betty Kreakie, USEPA Atlantic Ecology Division — Date: 4-10-2017

Dr. James Haney, University of New Hampshire — Date: 4/18/17

Dr. Shane Bradt, University of New Hampshire Extension Office — Date: 4/20/17

Jasper Hobbs, New England Interstate Water Pollution Control Commission — Date: 4/18/17

Diane Switzer, EMT Team Leader — Date: 4/13/17

Katrina Kipp, Manager, Ecosystems Assessment Unit — Date: 4/10/17

Dave McDonald, Quality Assurance, Ecosystems Assessment Unit — Date: 4/13/17

Dr. Nora Conlon, EPA QA Chemist — Date: 4/25/2017

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1. Introduction

This Quality Assurance Project Plan has been written to fulfill the requirements based on QA/R-5, EPA Requirements for Quality Assurance Project Plans, as applicable, and to include those QAPP elements deemed to be pertinent to the successful implementation of this program. The (QAPP) was written in conjunction with the Ecosystem Monitoring Team Generic Quality Assurance Project Plan (QAPP), January 2017. In addition to fulfilling the QA/R-5 requirements and QAPP elements, the structure of this QAPP is designed to fulfill the purpose of being directly useable as a general program operations manual. The key QAPP elements can be found within this context.

1.1 QAPP Distribution and Organization

As a general rule, all individuals currently listed in the Cyanobacteria Monitoring Collaborative (CMC) program email group listserv (http://listserv.uri.edu/cgi-bin/wa?SUBED1=CYANO_COLLAB) will be able to receive electronic copies of the Quality Assurance Project Plan and any updates as they arise and as requested. Since the list is long, and does fluctuate based on participation interest and need, the distribution list will only be updated within this QAPP on an annual basis. The current distribution list can be found in ***Appendix A***, along with projected levels of member participation in the program. An up-to-date version of this QAPP will be posted on the Cyanos.org webpage and will be kept current for immediate reference.

All Cyanobacteria Monitoring Collaborative members participating in the program are responsible for following the procedures outlined in this QAPP and in any relevant SOPs.

2. Program/Task Organization

The regional Cyanobacteria Monitoring Collaborative (CMC) program is an ad hoc organization with a voluntary membership that consists of state water quality monitoring groups, citizen scientists, lake association members, large rivers groups, regional extension offices, non-governmental organizations (NGO's), Boards of Health, academic researchers, public water suppliers, federal agencies, and other interested groups. The collaborative is not constrained by geographic region or by organization affiliation. A CMC workgroup has been formed as a subset of the collaborative, and meets on approximately a quarterly basis to discuss and manage the program by consensus. The workgroup is moderated by EPA staff and is guided by the current state of the art research provided by the collaborative, USEPA headquarter guidance material, and national and global research on the topic. Roles and responsibilities are dependent on the interest and objective of the individual workgroup member or organization to meet his or her needs with the underlying premise that sampling, data, and respective quality assurance guidelines remain consistent throughout the workgroup as outlined in this QAPP. Open communication as means to exchange ideas throughout the workgroup is commonplace and strongly encouraged.

3. Background

Cyanobacteria are prokaryotic organisms that have characteristics more similar to bacteria than to algae, yet undergo photosynthetic processes much like their eukaryotic algal counterparts. They contain green (Chlorophyll) and blue-green (Phycocyanin) photosynthetic pigments which

absorb specific wavelengths of light from which they obtain their functional energy. Not only do these pigment molecules absorb specific wavelengths of light, but they also emit specific wavelengths, which subsequently, can be measured. Chlorophyll absorbs light at 440 nanometers, and re-emits light at 670 nanometers. Phycocyanin has a narrower band width, absorbing light at 620 nanometers and re-emitting at 650 nanometers. They occur in both freshwater and marine environments, and are considered fairly ubiquitous across most aquatic habitats. Certain species strains may contain secondary metabolites that are toxic and/or produce taste and odor issues in potable water. Many freshwater species have optimal growth rates in warm thermally stratified, nutrient rich waterbodies. However, they exist in almost all environments from clear nutrient poor lakes to desert sands, thermal hot springs, and under lake ice. Optimal growth conditions are also enhanced in waterbodies with low flushing rates/long residence times, and prevailing calm/overcast conditions. Many cyanobacteria species can outcompete algae by a unique ability to regulate their buoyancy and optimally position themselves in the water column. Several cyanobacteria genera also have a unique ability to harvest nitrogen from the atmosphere and convert it to biologically available ammonia, giving them yet another competitive advantage. Some of the most common nitrogen fixing cyanobacteria genera are *Anabaena, Aphanizomenon, Cylyindrospermopsis, Lyngbya, Nodularia, Oscillatoria, and Trichodesmium*.[2] Common non-nitrogen fixers include *Microcystis, Planktrothrix, Aphanocapsa, Raphidiopsis,* and *Woronochinia*. Most cyanobacteria thrive in warmer waters and can propagate by dividing three or more times daily, quickly building to heavily concentrated conditions. Increasing global temperatures along with more intense precipitation patterns that bring in more nutrients to surface water bodies from off the landscape all point to an increasing occurrence of harmful algal blooms.

Nutrient sources, such as agricultural runoff, waterfront lawn care practices, and poor wastewater treatment practices, have been linked to prolific growth rates of these bacteria, whereby they outcompete more commonly occurring algal species and form large surface scums or “blooms” within a waterbody. Concerns have recently emerged on the effects of the increasing intensity of precipitation patterns and their effects on runoff due to changing climate.

Blooms may not always be formed at the surface, the species *Planktothrix (Oscillatoria)* commonly blooms within the water body where a distinct vertical temperature transition occurs within the water column. In many waters, the major source of nutrients comes from within the waterbody itself, historically brought into the water from the surrounding landscape when public awareness about the short and long term effects of nutrient loads was nonexistent. Today these legacy effects provide rich and abundant nutrient pools from which these bacteria can take advantage and thrive. The resulting cyanobacteria surface scums are commonly referred to as Harmful Algal Blooms (HABs), or more appropriately, harmful cyanobacteria blooms.

Cyanobacteria associated HABs and the toxins they produce are becoming an increasing concern across the North American continent and globally. The frequency of occurrence is increasing and their toxicity over the years has been associated with numerous human and animal fatalities and sub-lethal health issues. This has direct implications to the use of recreational waterbodies for contact recreation, the susceptibility of public water supplies to HABs and their toxins, and the overall ecological degradation of aquatic resources. Most of these HAB incidents can been

directly associated with an overabundance of historical and present day nutrient influxes to the waterbody.

Harmful algal blooms due not necessarily have to be toxic in order to cause environmental and ecological harm. Dense surface blooms that lead to high accumulations of cyanobacteria or algal biomass can deplete dissolved oxygen levels critical for aquatic life, resulting in fish kills and die-offs of benthic organisms. Chronic bloom formations can lead to vast areas of hypoxia in freshwater and marine systems, such as in the Northern Gulf of Mexico and the Mississippi River delta. Although less common, non-cyanobacteria HABs may also produce toxins, such as the golden algae *Prymesium parvum*, responsible for annual fish kills in Texas and documented in at least nine other states as of 2008[4].

In the 1990s, the threats from these events became increasingly apparent, and in 1998 Congress authorized the Harmful Algal Bloom and Hypoxia Research and Control Act (HABHRCA). This was amended in 2004 with authorization of Public Law 108-456 (HABHRCA 2004) and again in 2014 with Public Law 113-124 (HABHRCA 2014), authorizing research funding and expanding on the National Oceanographic and Atmospheric Administration's (NOAA) mandate for understanding and addressing harmful algal blooms. The United States has seen a thirty-fold increase in hypoxic waterbodies since the 1960s, and impacts to over three hundred coastal systems[5].

The Safe Drinking Water Act requires the USEPA to publish a list of unregulated contaminants that are known or expected to occur in public water systems occurring at a frequency and at concentrations that would be of concern from a public health standpoint. This list is known as the Contaminant Candidate List, or CCL. Cyanobacteria and cyanotoxins have been on the CCL since 1998 through to the present time, and since 2015 the toxins anatoxin-a, cylindrospermopsin, and microcystin-LR are specified. The CCL represents priorities for the Unregulated Contaminant Monitoring Rule (UCMR) program, which collects occurrence data to evaluate contaminants that do not have an associated drinking water standard in place. These data are subsequently used to support any future regulatory determinations made by the agency.

In 2015, the highest number of HABs was recorded in the United States in both marine and freshwater environments. It is likely that some of this is due to increased awareness and monitoring, but in many cases the temporal and spatial extent of these occurrences has been unprecedented. In the Northern Pacific, much longer and larger than normal algal blooms occurred, extending from May until late in the year, from the Aleutian Islands of Alaska down through Southern California and Mexico. The resulting bloom generated an extraordinarily high abundance of the genus Pseudo-Nitzschia, producing the Domoic acid neurotoxin. The deaths of several whales, numerous gulls, forage fish, sea otters, and other marine life have been attributed to the bloom and its associated toxin, along with the closure of recreational Dungeness crab and razor clam fisheries in Washington and Oregon State, and sardine and anchovy fisheries in California. Domoic acid levels in some locations were 10-30 times higher than any previously recorded levels, and the 2015 bloom was unprecedented in its extent and duration[1].

As a direct result of heavy rains and nutrient runoff from agricultural operations, 2015 was also the largest freshwater algal bloom to date in Lake Erie, far surpassing the previous record

breaker occurring in 2011. The 2015 Lake Erie bloom covered more the 300 square miles, but stayed offshore, limiting impacts to recreational use and water supplies. A much smaller, but toxin containing bloom occurred nearshore in 2014 and shut down the Toledo public water supply, depriving close to one half million people of domestic water use for several days. The 2014 Toledo incident set Congress in motion to promulgate Public Law 114-45 in accordance with section 1459 of the Safe Drinking Water Act, as amended by the Drinking Water Protection Act. The P.L. 114-45 requires that a strategic plan for assessing and managing risks associated with algal toxins in public drinking water supplies be developed by the USEPA administrator, and in November of 2015 the EPA produced the *Algal Toxin Risk Assessment and Management Strategic Plan for Drinking Water.*[2] This document sets the stage and provides a road map for future EPA activities related to HAB's and drinking water such as algal toxins and their human health effects, development of health advisories, factors affecting HABs, analytical methods, monitoring, and treatment and source water protection options and practices.

Cyanobacterial toxins and taste-and-odor compounds are naturally produced by-products[6]. These by-products are produced depending on the "strain" of cyanobacteria present and not the species. A species grouping is established when 95% of their genome is identical, the remaining 5% making up the various "strains," which leads to innumerable gene coding for different attributes, such as toxicity. This implies that identification of cyanobacteria down to the species taxonomic level will not relinquish whether or not it is toxic, as a single species may have several strains within the same waterbody, some toxic and others not. The complexities of toxin production are not yet well understood, and much research is currently focused in this area.

The toxins produced by cyanobacteria fall into three broad categories: dermatoxins, hepatotoxins, and neurotoxins. Many of these toxins are extremely persistent, and are not eradicated or degraded by conventional means such as boil water orders or chlorination practices. In some cases these "purification" process can even be more detrimental, the process itself may cause cell rupture or death, releasing intracellular toxins previously retained with the cell. Human illnesses primarily associated with dermatoxins has been documented mostly from recreational exposures in the form of moderate to acute skin rashes, and eye and ear irritations. The exposure route is primarily through contact recreation in surface waters. Hepatotoxins are some of the most toxic, and directly affect the liver with the route of cyanobacteria exposure principally through inadvertent ingestion and inhalation via aquatic recreation, to direct ingestion via drinking water. In 1996, fifty-six human deaths in Caruaru Brazil were attributed to exposure from these toxins via dialysis treatment[7]. These toxins can be extremely acute, and have been known to have caused animal deaths in as little as twenty minutes from time of ingestion. Incidences of neurotoxicity has been less prevalent, but current research has alluded to connections between aerosolization of cyanobacteria β-N-methylamino-L-alanine (BMAA) and the prevalence of Amnio Lateral Schlerosis (ALS) clusters in near proximities to cyanobacteria seasonally dominated waterbodies.[8] The U.S. Geological Survey's 2008 Scientific Investigations Report 5038 summarizes some of the most common genera of cyanobacteria and the toxins associated with them (Table 1).

[All data included in this table are based on documented production in laboratory cultures; data based on circumstantial evidence, such as co-occurrence of genera and toxin or taste-and-odor compounds in environmental samples, were not included in this table. LYN, lyngbyatoxin-a; APL, aplysiatoxins; LPS, lipopolysaccharides; CYL, cylindrospermopsins; MC, microcystins; NOD, nodularins; ATX, anatoxins; BMAA, β-N-methylamino-L-alanine; NEO, neosaxitoxins; SAX, saxitoxins; GEOS, geosmin; MIB, 2-methylisoborneol]

Cyanobacterial Genera	Dermatoxins			Hepatotoxins			Neurotoxins				Tastes and odors	
	LYN	APL	LPS	CYL	MC	NOD	ATX	BMAA	NEO	SAX	GEOS	MIB
Colonial/filamentous												
Anabaena			X	X	X		X	X	X	X	X	
Anabaenopsis			X		X							
Aphanizomenon			X	X	X		X	X	X	X	X	
Aphanocapsa			X		X							
Cylindrospermopsis			X	X				X		X		
Microcystis			X		X			X				
Nodularia			X			X		X				
Oscillatoria (Planktothrix)	X	X	X		X		X	X		X	X	X
Pseudanabaena			X		X						X	X
Raphidiopsis			X	X			X					
Unicellular												
Synechococcus			X		X			X			X	X
Synechocystis			X		X			X				

Sources: Wu and others (1991), Wnorowski (1992), Blevins and others (1995), Carmichael (1997), Bláha and Maršálek (1999), Chorus and Bartram (1999), Domingos and others (1999), Saadoun and others (2001), Oudra and others (2002), Watson (2003), Huisman and others (2005), and Taylor and others (2005). A comprehensive list of known cyanobacterial toxin and taste-and-odor producers is not currently (2008) available in the literature. Combined, the references used to create this table may be used to create a fairly complete list of planktonic and benthic producers.

Table 1: *Common genera of planktonic cyanobacteria that contain toxin and taste-and-odor producing strains. Source: USGS 2008 Scientific Investigations Report 5038.*

Human and animal illnesses associated with cyanobacteria from recreational activities and drinking water span the full spectrum; headaches, nausea, muscular pain, fever, diarrhea, pneumonia, vomiting, flu symptoms, skin rashes, mouth ulcers, eye and ear irritations, throat infections, tumor promotion, increased incidence of cancer, and death.

The World Health Organization (WHO) developed guideline values for safe practices in managing recreational waterbodies, and the USEPA is currently in the process of developing numeric criteria for cyanobacteria levels in recreational waterbodies, anticipated to be released sometime in 2017. Data collected from various countries at the time of WHO guideline development showed that approximately 60% of all cyanobacteria samples had toxic variants, with microcystin being the dominant toxin. The WHO established a series of recreational guideline values based on increasing severity and type of exposure from skin irritation to the more serious health effects from ingestion and inhalation (Table 2). Many states currently use some variation of these guidelines to establish action levels for posting recreational waterbody health advisories. A current listing can be found in *Appendix B*. In the Northeastern United States, the New England Interstate Water Pollution Control Commission, along with the larger CMC working group, put together a Harmful Algal Bloom Regulations and Outreach Matrix to be used as a reference and guidance document, *Appendix C*.

WHO Recreational Exposure Guidelines			
Relative Probability of Acute Health Effects	**Cyanobacteria (cells/mL)**	**Microcystin-LR (µg/L)**	**Chlorophyll-a (µg/L)**
Low	< 20,000	<10	<10
Moderate	20,000-100,000	10-20	20-50
High	100,000-10,000,000	20-2,000	50-5,000
Very High	> 10,000,000	>2,000	>5,000

Table 2: *Current World Health Organization Recreational Exposure Guidelines*

In addition to recreational concerns, a large amount of attention has been focused on the increasing concern of health impacts from harmful cyanobacteria to our drinking water resources. The USEPA has published Health Advisories (HA) for two known cyanobacteria toxins, microcystin and cylindrospermopsin, for which it has been felt that enough scientific research and literature exists to warrant posting a health advisory. The HAs are published under the authority of the Safe Drinking Water Act (SDWA) and are used to help describe the duration of exposure at which no health effects are anticipated. These documents are developed to be used as technical guidelines for those state and public entities responsible for protecting public health and drinking water supply resources. The current HA guideline for these toxins over a ten day exposure period are listed in Table 3.

USEPA 10 Day Drinking Water Health Advisory		
Cyanotoxin	**Bottle-fed infants and pre-school children**	**School-age children and adults**
Microcystins	0.3 µg/L	1.6 µg/L
Cylindrospermopsin	0.7 µg/L	3 µg/L

Table 3: *Cyanobacteria concentration at which no adverse health effects are expected over a ten day exposure period.*

4.0 Problem Statement

Since 2013, state water quality, beach monitoring, and drinking water programs have become increasingly aware of the need to formally address the harmful algal bloom issue within their state boundaries. Public awareness and concern has resulted in more inquiries to these agencies with increasing pressure to address the issue, as the closure of bathing beaches and recreational waterbodies has increased in recent years.

Monitoring and studying cyanobacteria in a consistent manner that could be utilized to determine the relative risks to human and ecological health have been elusive at spatial scales larger than individual waterbodies or relatively small geographic areas. This limits the utility of data for determining regional “hotspots,” detrimental land use practices, impacts from changing climate patterns, geographic distributions of specific cyanobacteria genera and there toxin prevalence, documentation of known bloom occurrences and their distribution, and a host of other information that would be informative and useful for the management and prediction of HABs.

5.0 Program Description & Objective

This program is being developed in order to establish a cyanobacteria monitoring and bloom watch program. It is a continuous work in progress and is constantly evolving. It will provide the needed consistency in field sampling equipment and methods and generate data that compliments existing monitoring programs. The program will establish the consistency necessary to aggregate data for interpretations of bloom frequencies, cyanobacteria concentrations and species distributions, and associated toxicity. Although developed initially for the Northeastern United States, it can be applied anywhere and its widespread use is encouraged. The program will provide an educational component through trainings in algal identifications and field instrumentation use as well as field sampling, collection, and preservation protocols. The program architecture is designed to be used in a tiered manner, providing a baseline of information than can be added to in more detail and complexity as the level of resources and time allow, and based on the desires of the entities involved (Table 4). This approach embraces a broad spectrum of involvement, from the citizen scientist monitoring population to being able to expand to large public water suppliers, beach programs, and overseers of large recreational waterbodies and the like. The effort is designed to complement existing water quality monitoring programs that may currently reside at federal, state, and local levels, and to assist in establishing harmful algal bloom monitoring programs for any public water supplies that may wish to participate to further develop their programs. This approach provides the flexibility needed to integrate across various existing programs and associated budgets, yet provides enough uniformity that generated data can be aggregated across geopolitical and program boundaries.

The cyanobacteria monitoring collaborative program has three overlapping components or tiers: A bloom watch/tracking component, a cyanobacteria identification and documentation component, and a cyanobacteria monitoring component. Each expanding tier has a specific component objective associated with it. The bloomwatch tracking component was developed to enable lay people, citizen scientists and the like to be able to report on the presence of a bloom with the use of a smartphone App. This tier creates awareness while educating, and provides important information on where and when blooms are occurring. The second tier provides the opportunity to go beyond just documenting a bloom, by identifying what types of cyanobacteria may be present and if they are potential toxin producers. This information can be aggregated up with bloomwatch information, providing higher resolution on the prevalence and occurrence of cyanobacteria. The final Tier, or cyanomonitoring component, provides the opportunity to develop a monitoring program that will provide potential bloom forecasting and insights into the waterbody specific characteristics and behavior of cyanobacteria. All tier levels are designed to have a baseline level of effort with commensurate quality assurance and established methods.

5.1 BloomWatch (Tier 1)

The main objective of bloomWatch is to ***photographically document the spatial and temporal occurrence of a perceived bloom*** for further verification, while engaging and educating the lay person/citizen scientist on cyanobacteria and harmful algal blooms. Because of logistics and the variability of when and where HABs may occur, (blooms may only be visibly present for a few hours or less and at specific locations within a particular waterbody) it is imperative that efforts be made to engage the public's help. Local knowledge of where and when blooms are occurring is likely under reported, or not reported at all. When blooms are reported to a state water quality or health official, by the time officials can reach the location the bloom has often dissipated or

shifted from its prior location. Local citizens are usually the first to encounter a bloom condition, as they often occur in the early morning hours while individuals are out walking their dogs, getting in a morning run, or getting ready for the day's work. Images can be taken at any time and consist of three images per submittal, but must follow the prescribed format listed in this document.

	Potential Program Use	Purpose	Sample Location	Sample Frequency	Sample Type	Parameters
TIER I Bloom Watch	All State & Federal water programs, general public at large, Citizen Scientitsts, beach monitors, educational institutions, lay monitoring programs	Determine high probability that a cyanobacteria bloom is occurring (ie. vs pollen), utilize as an educational tool, document frequency of occurrence, possible hotspots, report occurrence to state	wherever a bloom appears to be occurring. This could be in open water and widespread, or a distinct scum line located at the shoreline. Any surface water body, anywhere	Whenever a suspected bloom occurs; anywhere, anyplace, anytime	Smartphone image & data submittal via the bloomWatch App (see Cyanos.org)	Waterbody name, weather conditions, water surface conditions, public access, smartphone images, locational Lat/Lon
TIER II CyanoScope	All State & Federal water programs, local and state boards of health, public surface drinking water suppliers, concerned citizens, Citizen Scientists, academia & educators	To track and document the locations and occurrence of potentially toxic cyanobacteria genera	Any surface water bodies	Anytime, anywhere, any frequency	Concentrated 53 Micron Plankton Net sample	Locational data (waterbody name, town, Lat/Lon), digital microscope image submittal
TIER III Cyanomonitoring	All State and Federal water monitoring programs, public drinking water supplies, lake & river associations, various stakeholders, researchers	track spatial and temporal distribution of cyanobacteria pigments, frequency of occurrence, long term trends, concentration levels, and potential toxicity	Minimum of one site per waterbody from the deep hole area or a specific shoreside location	Minimum baseline of one sample collected every other week from June 1 through September 30. Additional samples may be collected from other waterbodies, other sites, other depths, and other frequencies, as long as the minimum baseline is completed. Long term monitoring	Integrated tube sample from the surface to a depth of 3 meters, or a one meter integrated tube sample if collected from the shoreline	Chlorophyll a concentration, Phycocyanin concentration, possible toxin analysis

Table 4: *Matrix detail of the three Tiers/components of the Cyanobacteria Monitoring Collaborative program.*

5.1.a BloomWatch Tools: The only tools required for this program component is a smartphone and its accompanying bloomWatch App (http://cyanos.org/bloomwatch#Project-Overview). IOS phones must have version 7.1 or newer, and Androids must have version 4.0 or newer. By utilization of this App, it will be much easier to engage the public's help with a common tool that they already have. The App consists of four input screens; **Page/screen 1**) an introductory/welcome screen which explains the purpose of BloomWatch from which your name or affiliation name is entered, the relevant email address where images that were taken will be sent in addition to being captured in the crowdsourced database, and the name of the waterbody where the images were taken. Input screens have been designed so that previously entered information will pop up again on initial entry, providing consistent data input formatting. Input information is then saved prior to proceeding on to the next screen/page *(Figure 1)*.

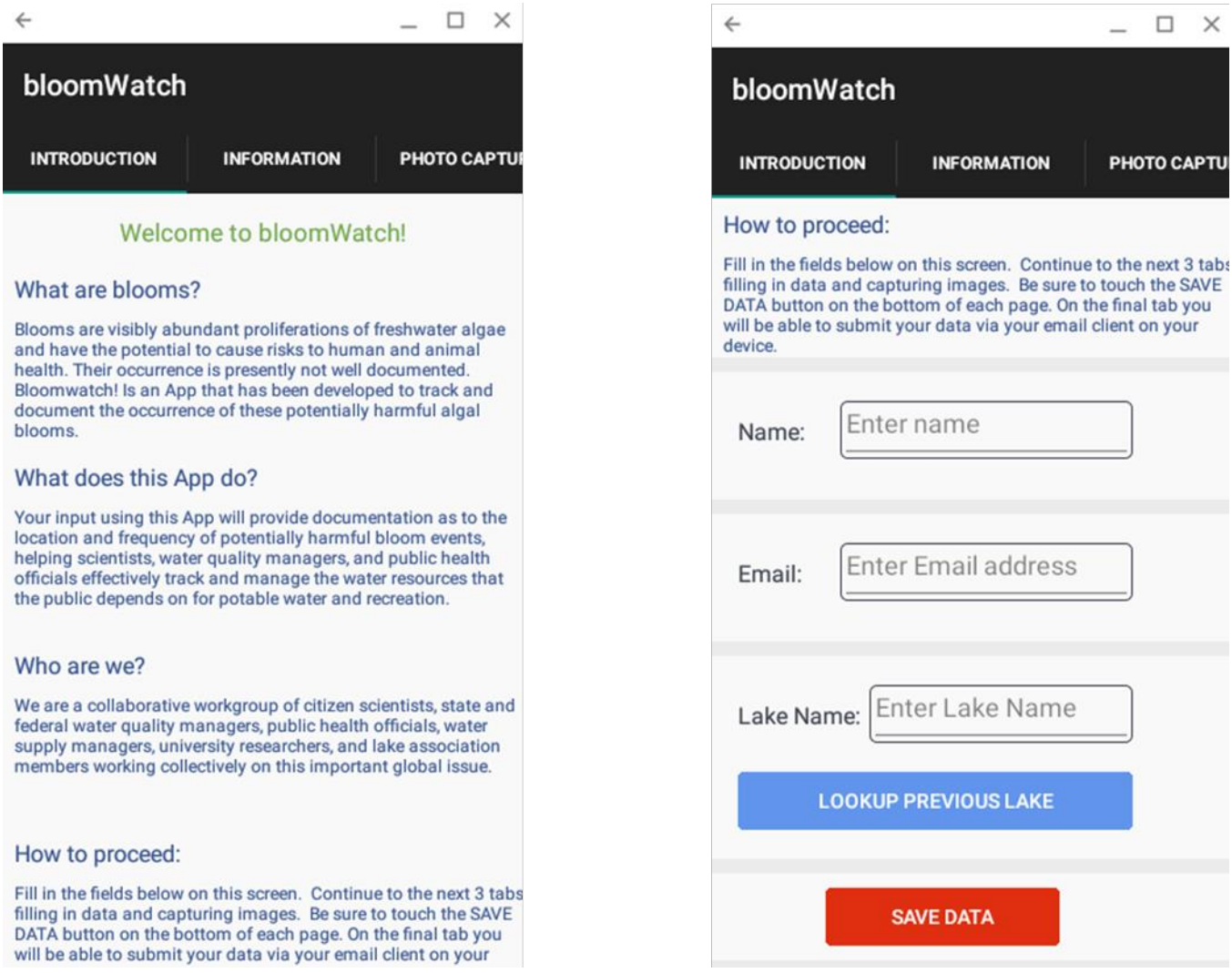


Figure 1*: Introductory and general information page for bloomWatch. Page/screen 1 of 4.*

Page/Screen 2) Screen 2, *(Figure 2),* captures general but important information on current weather and lake surface conditions that help provide a "weight of evidence" that a harmful cyanobacteria bloom has occurred. This information can be important indicators, as warm and calm overcast days are ideal conditions for bloom formation. The occurrence of breezy conditions may also be informative information as to a highly localized bloom in a certain location of the lake, positioned there by the prevailing wind. Bloom size is also important to help determine if the current bloom encompasses the entire lake, is concentrated on the surface or distributed throughout the water column, or is localized due to current lake and weather patterns. The app utilizes common items/places (i.e. tennis court) as a spatial frame of reference rather than measurable units (i.e. square meters), as it is easier and usually more accurate for people to visually quantify area in this manner.

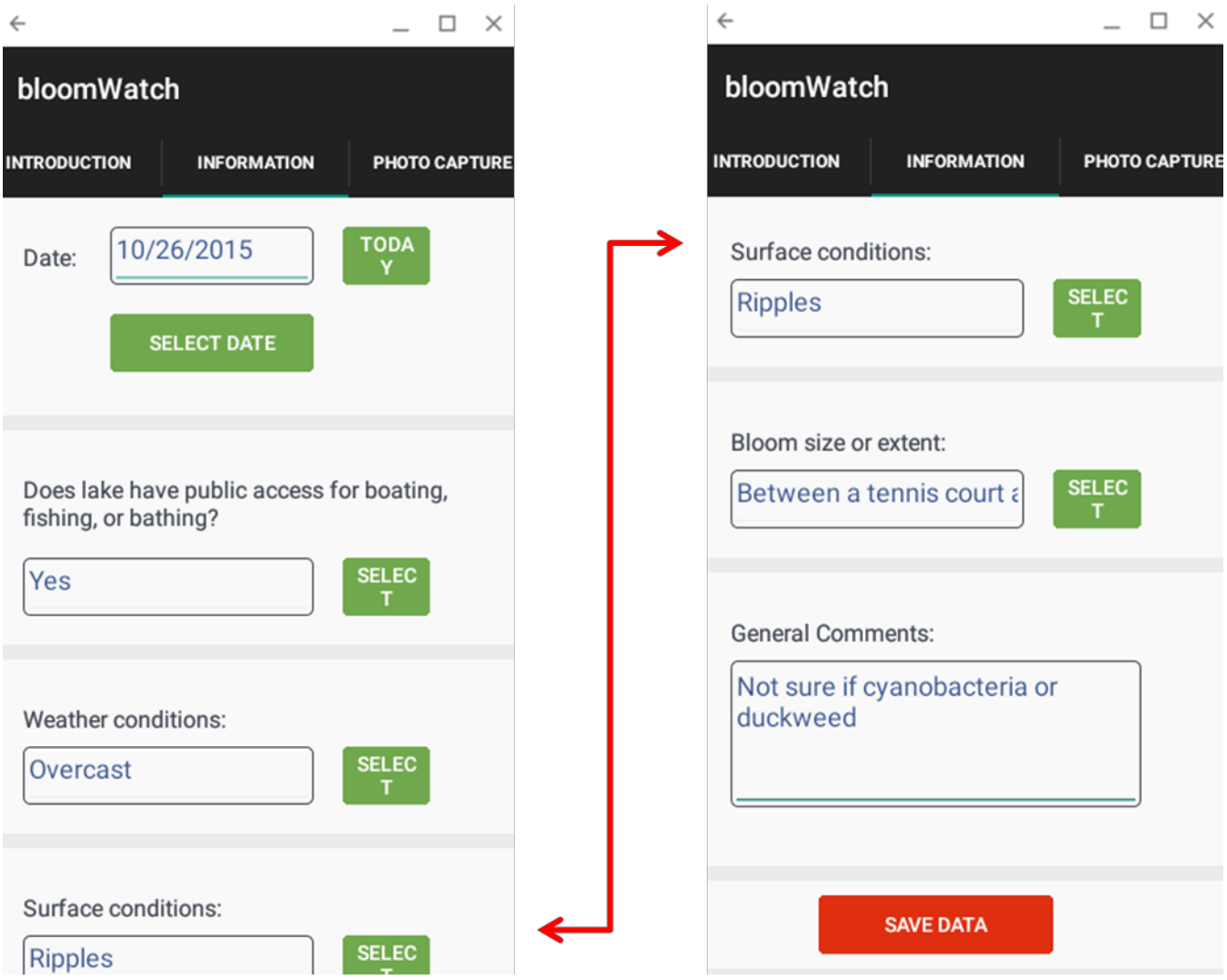


Figure 2: *Lake conditions and bloom size screen. Page/screen 2 of 4.*

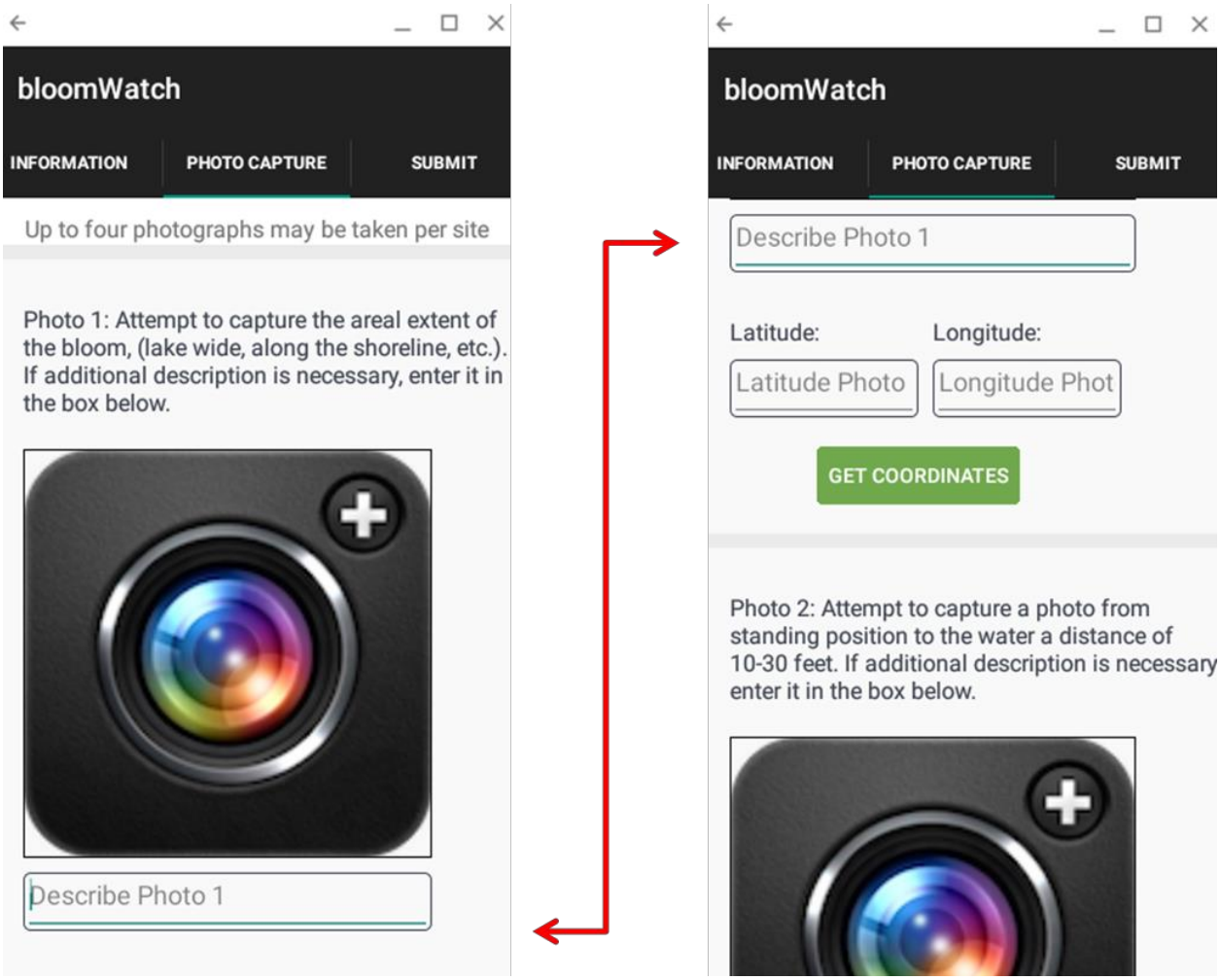


Figure 3: *Image capture screen. Page/screen 3 of 4*

Page/Screen 3) The photo screen page*, (Figure 3),* is designed for the capture and submittal of three separate and distinct images of a perceived bloom. The photos should all be taken from the same location, which is geo-located by turning your "location on" function in your smartphone or by selecting "get coordinates" directly from the app. The images need to be captured as follows: Image 1 is a large area photo that should show the extent of the bloom and should capture part of the shoreline in the picture and a large area of the lake and some skyline. This image helps verify the areal extent of the bloom while presenting an indication of current lake and weather conditions, ongoing lake activity at that point and time, and an indication of whether the bloom is waterbody wide or isolated to a discrete area. *Figure 4* depicts two good examples of first images. This first image is also used as the geo-referencing point that uses your smartphone internal GPS. Image 2 should capture an image from a standing position to the water surface at a distance of ten to thirty feet *(Figure 5).* This helps to document if the bloom is stacking up along the shoreline, has a surface scum or matt, is limited to a very narrow band along the shoreline or a small cluster, or appears to extend from the shoreline into the majority of the lake. The third image should be a close up photo of the bloom from three feet away or less, or

Figure 4: *Decent first images for the bloomWatch app showing some skyline, the shoreline, and an indication on the extent of the bloom.*

if possible, a picture of the bloom material in a clear glass container held out at arm's length. This image helps to verify if the bloom may be filamentous in nature, globular or in clumps, a thin film, accumulates at the surface, etc. all providing clues that help to determine the likelihood that it is a cyanobacteria bloom. The close-up or macro image may definitively determine not only the existence of a cyanobacteria bloom, but potentially what type of cyanobacteria *(Figure 6)*.

The final screen in the BloomWatch App is the data submittal screen *(Figure 7)*. This screen verifies that the waterbody name is correct and the date of when the images were taken. Once the images are submitted, they are sent to the crowdsourcing database CitSci.org where the BloomWatch images and accompanying data will be stored for public use. Your personal information, email address and name are protected and hidden from public view. The App is designed so that, if desired, upon pressing the submit data button, data and images will be automatically sent to key contact people. By pressing send, the data and images will not only be sent to the CitSci.org database, but also directly and immediately to these other pre-

Figure 5*: Good example of second image for bloomWatch App*

Figure 6: *Good example of third image for bloomWatch App submittal. Photo courtesy Des Moines Water Works*

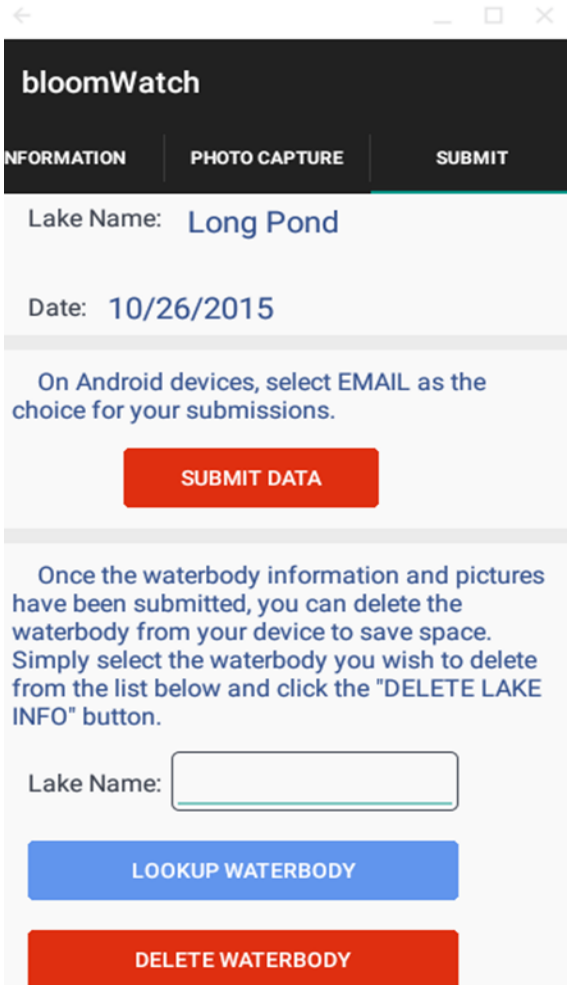


***Figure 7**: BloomWatch data submittal page. Screen 4 of 4.*

determined contacts. The App leads you through the data and image collection process step by step.

Note: *A few users have had some difficulty uploading images to the App, which, when downloaded, should automatically ask you for permission to access your location data for geo-referencing the images and to access the images on your phone. If these prompts do not appear and you receive an error message, go to the "applications manager" on your phone and manually allow permission for access. This should resolve the problem.*

5.1b A note about CitSci.org & bloomWatch

BloomWatch is a project that is established within CitSci.org, a crowdsourcing website that is designed to promote collaborative efforts between citizens and scientists to address local, regional, and global issues. The CitSci website was developed through Colorado State University's Natural Resources Ecology Lab, with initial funding from the National Science Foundation. The bloomWatch App gathers data collected by members of the Cyanobacteria Monitoring Collaborative, which is then automatically brought in to the bloomWatch project page at CitSci.org, where data can be analyzed, visualized, and shared among others with the same interests. This approach enhances environmental education at several levels, while advancing scientific understanding on critical environmental issues.

The bloomWatch App has the added flexibility of being able to be applied to discrete projects while simultaneously allowing submitted data to be aggregated up to the default comprehensive scale of the App. For any entity wishing to use bloomWatch as a part of their own project, all submissions will still go through the bloomWatch project page on CitSci.org. However, data on this page, which includes images, location, and water body name, can be constrained by a user or specifically selected for their own use. Submissions are still visible to the public and will be sent to any key state contacts, but "nested" project data can be parsed out. To simplify this process groups have a few options. The first is to have a code word that the group can insert into the "General Comments" section on the Information tab. The second option is to have each contributor for a certain project use the project name as part of their alias. For example, a project relating to alpine blooms could type AlpineBloom as one word in the general comments section, or have their members log-in as Alpine1, Alpine2, etc. This will allow the group to select out their individual project data from the larger dataset.

5.2 CyanoScope (Tier 2)

This "second tier" of the cyanomonitoring program is established in order ***to identify and determine the timing and spatial distribution of cyanobacteria genera*****,** assisting in the mapping and identification of potential toxin producing waterbodies as well as providing an educational component.

Figure 8: *Cyanobacteria monitoring kit & components*

Samples are collected on the lake, from the shoreline, or both utilizing a 50 micron plankton net, concentrated utilizing a specialized tool, and then observed and recorded utilizing a microscope and digital image capture software. Monitoring "kits" have been developed and put together to provide consistency and quality assurance while sampling *(Figure 8)*. Samples can be collected at any time, at any frequency, and at as many locations as desired, as the main goal is to determine the genera of cyanobacteria that may be residing in the waterbody.

5.2a Procedure for on the lake collection

Position your watercraft at your desired location for sample collection and record you position. This can be accomplished using several methods. If you have a GPS unit, you can simply record your latitude and longitude in a field notebook. Or, alternatively and less susceptible to transcription error, you can save a waypoint on your GPS unit. It is also possible to record your GPS location in the metadata of a photograph taken from your location with your smartphone. If these GPS-based methods are not available or forgotten, you can record your location later utilizing the mapping locator in the cyanoScope project in "add an observation" page. Sampling for cyanoScope can be done at any lake, pond, reservoir, or other water body to which you have access. Sampling can also be conducted at any frequency; even a one-time visit to a site is acceptable.

Once your location has been recorded, take out your plankton net and make sure the tube at the bottom of your net is pinched closed with the attached clip. Vertically lower your 53micron plankton net to your desired depth, ensuring that it does not come in contact with the bottom. The net should then be slowly retrieved at a rate of approximately one foot per second until your reach the surface. A slow retrieval is important because the mouth of the net can form a pressure wave that will actually displace organisms and plankton, preventing them from being captured in the net sample. Too slow of a retrieval and you won't be pushing the water through to capture your material. Once at the surface, vertically dip the lower two thirds of the net a couple times in the water to help wash material off the interior walls of the net and down into the lower plastic end of the net. You may also splash lake water against the outside of your net to help wash material down. Your "net sample" can now be transferred to the 500mL or larger opaque brown plastic bottle that came with your kit.

If you are collecting a sample from the shoreline, the plankton net may be tossed out away from the shoreline and then retrieved in a horizontal fashion back to shore. Care should be taken not to contact the bottom and fill the net with debris, yet retrieve at a slow enough rate not to push plankton away from the net opening. If there is a current bloom taking place along the shoreline, samples may also be collected utilizing a container. Processing the samples for slide mounting is the same for all approaches. **Note***: One should wear gloves as a safety precaution when handling any samples suspected of containing cyanobacteria.*

Once back on shore/office, gently mix your sample and transfer from the brown bottle into your ZAPPR up to the thread marks (make certain the tube on the bottom of your ZAPPR is closed first!). This should leave you approximately one quarter of an inch of air between the top of the cap and the surface of your sample. Place the cap snugly on the ZAPPR and place in an upright position, leaving it undisturbed for approximately thirty minutes. During this time, any bloom forming cyanobacteria, through buoyancy regulation and respiration, will move towards the surface of your sample in the darkened environment. Zooplankton, being attracted to the light, will migrate to the clear bottom portion of the ZAPPR resulting in a nicely separated sample *(Figure 9)*. After the thirty minute mark, GENTLY unscrew the cap of the ZAPPR. Any

Figure 9: The sample is placed in the "ZAPPR" and let stand capped for thirty minutes; zooplankton will migrate to the clear, light saturated bottom of the concentrator, the bloom forming cyanobacteria will accumulate at the surface where they are then siphoned off and placed on a microscope slide.

disturbance, even a slight tap, will cause the cyanobacteria to be redistributed in the water column away from the surface and you will have to wait several more minutes for them to float up to the surface again. Once the cap is off, use one of the small pipettes supplied with your kit to siphon off a small amount of sample from the surface of your sample. The cyanobacteria have a tendency to adhere to the sides of the tube, so utilize a sweeping motion with your pipette along the edge and at the surface to collect a good sample. Once collected, place a couple drops of sample on a clean glass slide, place a glass or plastic coverslip over the sample, and you are ready to view organisms under the microscope.[9]

5.2b Microscope Imaging Software

Any type of microscope software that allows taking of digital images can be used for the program along with a microscope of 40x magnification or greater. Digital software is included in

the cyanoScope kit however, and is quite simple to install and use. As long as the images are saved in an image format such as .jpg/.bmp/.tff, they will upload into the cyanoScope project.

5.2c Adding Observations to iNaturalist

CyanoScope is a project on iNaturalist.org. While cyanoScope is only designed to capture occurrence image data for cyanobacteria, iNaturalist allows users to submit observational data about all global biodiversity. Before you begin uploading cyanobacteria observations to cyanoScope, you must first have an iNaturalist account which you can set up within the website. Once set up, then you can join the cyanoScope project and start submitting your observations.

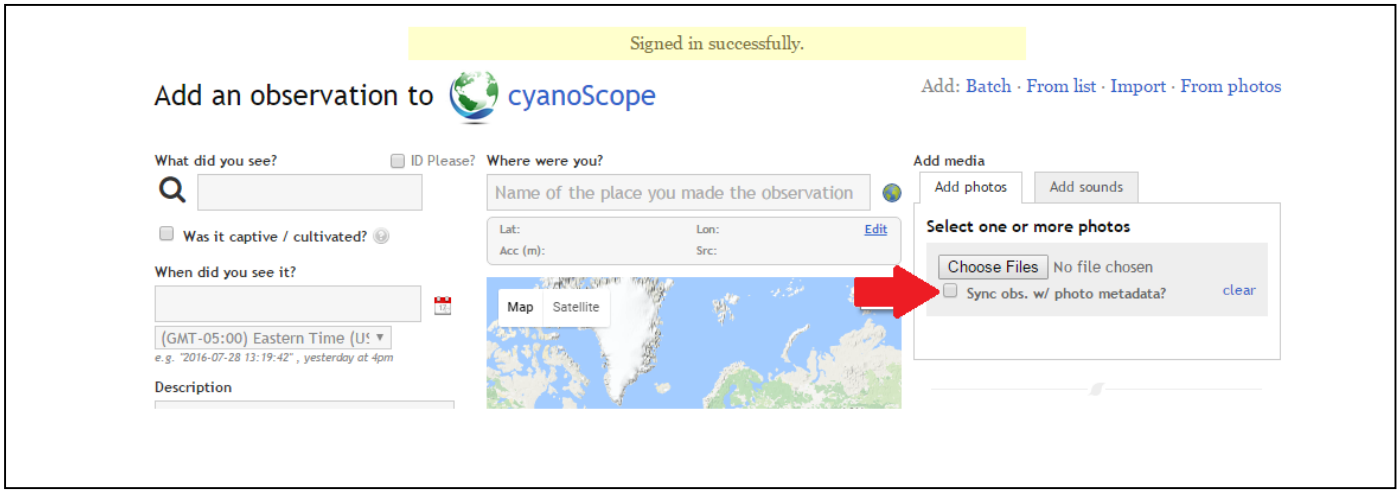
To submit a cyanobacteria observation to the cyanoScope project, navigate to http://www.inaturalist.org/projects/cyanoscope and click on the "Add Observations" button. The following instructions will guide you through adding a single observation. *A single observation is a microscope photo of a single genus from one net sample.* For example, photos of even the same genus from different sampling locations (even on the same water body) would be two different observations. Or conversely, photos of two different genera from the same net sample would be two observations. If you would like to submit multiple observations more efficiently, follow the "Batch" link at the top right of the cyanoScope observation page and follow the step by step procedure there.

Once you've joined the cyanoScope project on iNaturalist, you are able to submit observations. Simply click the "Add Observations" banner on the project's homepage

Note: Due to the difficulty of distinguishing between some cyanobacteria species, genus is the lowest taxonomic rank that will be used in the cyanoScope project.

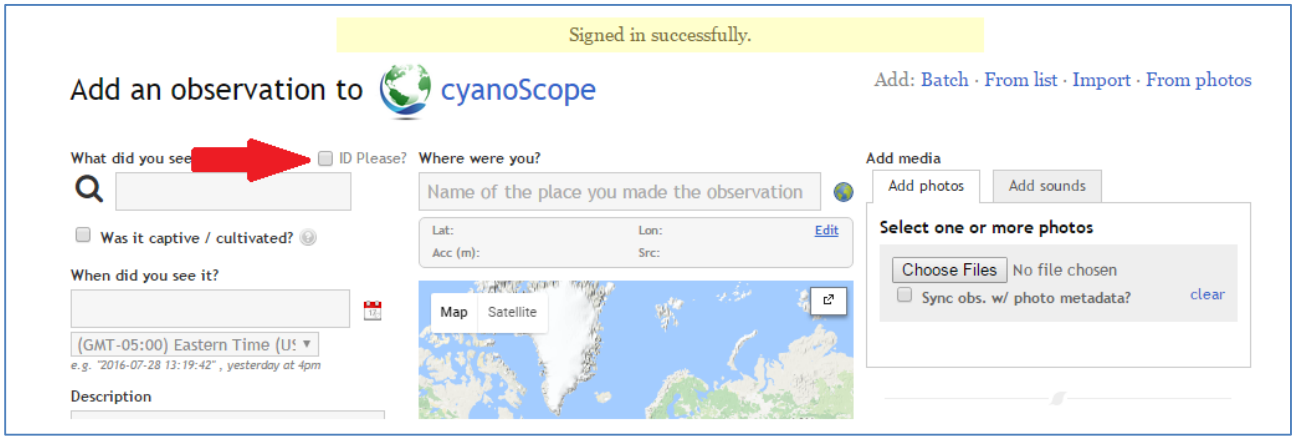
Adding location information to observations is critical for this project. This can be accomplished using several methods:

1. **Use photo metadata:** If you have a GPS-enabled smartphone, the location information will be captured in your photos' metadata. iNaturalist can directly read the location information if you check the box next to "Syn. obs. w/ photo metadata?" As a word of caution, ***be aware of where the photo was taken***. The location should be *where the sampling was conducted* and not where the microscope photo was taken. A photo of the sampling site can be used to capture the location information, but be sure to validate location information.

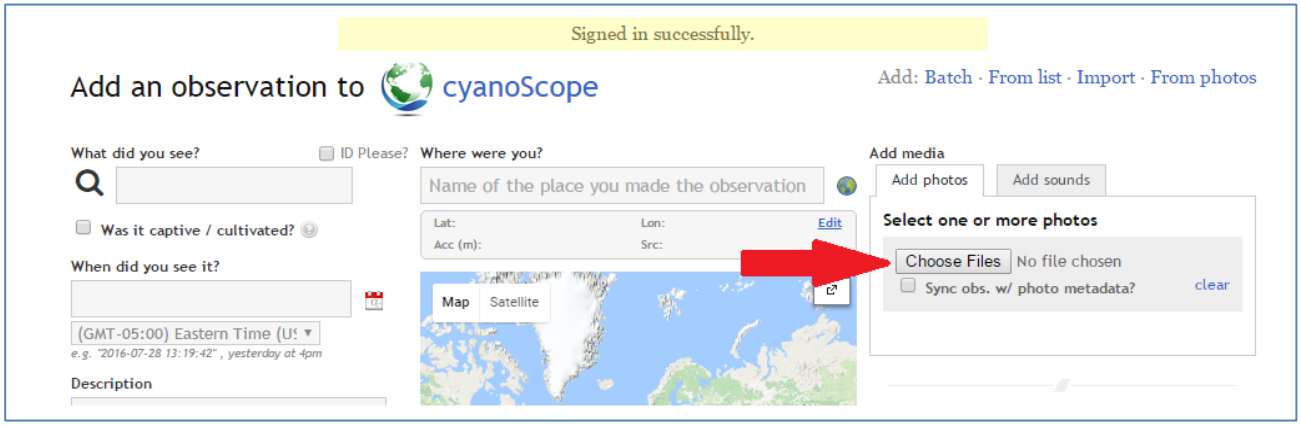


2. **Use the map interface to either navigate with a cursor or type in location name:** If you type in the name of your sampling location, be sure it is the correct location. Lake and ponds names are generally very common (i.e. there are several waterbodies named Silver Lake in New Hampshire).
3. **Type in GPS coordinates:** It is possible to directly type in your site's latitude and longitude. The map will navigate to the location, allowing you to double check that you have entered the correct coordinates. It is important to have at least four decimal places for position fixes to ensure reasonable location accuracy.

Once you have entered the spatial information, you will need to complete the remaining text boxes for the observation. Note that you do not need to identify the cyanobacteria in your observation. If you do not know the genera of observations, check the "ID Please?" box.



After all the information has been added, you need to upload your microscope photos. Click the "Choose Files" button in the "Add media" box and navigate to the photo location on your computer. You can upload multiple pictures for a single observation, if you feel that this might aid with identification.



Finally, click the "Save Observation" button at the bottom of the "Add Observation" page. After you submit an observation, several things will happen. Your observation will be shared with the entire cyanoScope community on iNaturalist. This will allow other users to propose an identification and to have conversations and ask questions about your observations. At this point the observation will be added to the cyanoScope project and will be included on the map. A preselected project curator/s will eventually verify an identification, the observation will be elevated to research grade and the ID will then be locked. Nonetheless, the conversations about the observation can continue.

5.3 CYANOMONITORING (Tier 3)

The principal objective of the cyanomonitoring component of the program is ***to track cyanobacteria development and dynamics within waterbodies and across waterbodies, assist in tracking trends due to climate changes and current and emerging land use practices, and assess waterbody/human health vulnerability to toxic cyanobacteria.*** This is the third tier of the program, which builds upon the two lower tiers and provides increasing resolution to the dynamic characteristics of cyanobacteria development in a waterbody.

5.3a Fluorometry

The cyanobacteria monitoring component is designed to focus on the relative concentrations of cyanobacteria found within the epilimnetic/photic zone of the lake through the use of fluorescence pigment measurements of chlorophyll and phycocyanin. Chlorophyll is a pigment found in all green plants and in cyanobacteria, and phycocyanin is a pigment found almost exclusively in cyanobacteria. These pigments primary function is to gather light energy from the sun, which through internal processes converts carbon dioxide and water into sugars, providing an energy source that can be used by the algae or cyanobacteria. These pigments absorb and emit light at specific wavelengths, elevating their energy states during the course of the reaction, and then quickly return to their original energy level. As the molecules return to their "normal" state, heat and photons of light are emitted. Fluorometers measure the intensity of the emitted light at specific wavelengths, which is directly proportional to its concentration. Due to these organisms self-sufficient ability to grow and develop, they are commonly referred to as primary producers. Please refer to *Appendix E* for details on fluorometer use and calibration. Instruments used for this program should have an established minimum detection limit (MDL) of 1-2ppb for phycocyanin, and 1ppb or less for chlorophyll. They should also provide a broad linear range from the 1-2ppb to 100,000ppb or greater for phycocyanin, and from 1ppb up to 2,500 ppb for chlorophyll. These levels provide adequate detection range values that will allow one to track the seasonal progression of phytoplankton and the development of harmful cyanobacteria blooms. Project action limits will vary, depending on the goals and objectives of individual users, as is the design and intent of the CMC. Calibration control limits must be within the bounds of the calibration standard to be considered acceptable (i.e. +/- 2ppb). This data will be posted on the cyanos.org webpage after QA vetting, and be presented utilizing various visualization tools as decided upon by the CMC working group in order to maximize the utility of the data.

5.3b Fluorometry Quality Assurance

Quality assurance is an essential part of any program and ensures that the data collected is not only accurate and precise, but will meet the needs of the end data users. The following lists the QA measures that are currently in place with the program to ensure that end user data is of the highest integrity.

- All fluorometry instruments are checked prior to each field day utilizing solid state secondary standards. These standards provide a quick and accurate check on the instruments primary calibration, and ensures that any drift in the instrument is identified quickly. Noticeable and continuous drift will require that the instrument be recalibrated immediately, or correction factors applied to the measured samples. A standardized secondary Standard log sheet has been crafted by the CMC for use with the program and can be found in *Appendix F*.
- Instruments will be calibrated on an annual basis before the start of the sampling season utilizing real pigment primary standards for phycocyanin and for chlorophyll within the dynamic range needed to track phytoplankton change and bloom progression.
- Any instrument group calibration will entail a serial dilution series to check the instruments MDL at the start of every season.
- Triplicate ***samples*** will be collected at least once per season, or for every 15 individual samples collected on a single waterbody at one designated site.

- Triplicate ***readings*** of a sample need to take place at least once for every 15 samples measured.

5.3c Ambient water sample collection for fluorometry

For standardization purposes, a three meter sample collection depth has been selected and is fairly representative as the depth to which sunlight penetrates the water surface enough to support primary production and hence the development of bloom forming cyanobacteria. Cyanomonitoring samples may be collected from open areas of the waterbody, or from the shoreline, depending upon the resources available to the sampler. At a minimum, **samples are to be collected every other week during the summer months from the beginning of June through September**, when algal blooms frequently occur and contact recreational use is at its highest. If desired, additional monitoring to the baseline sampling requirements can be added, but are left to the discretion and resources of the monitoring group depending on what their personal objectives might be. Increased sampling frequency, locations, and depths will only increase the resolution of the data and provide better insights to the dynamics and unique characteristics of the waterbody.

Open water sample collections will utilize an integrated tube sampler lowered into the water column from the surface to a depth of three meters. At a minimum, one sample every other week must be collected from the deep-hole area of the waterbody. If shore side samples are collected in lieu of or in addition to deep-hole samples, they need to be collected utilizing the integrated sampling tube for data consistency and quality assurance purposes. Samples should be consistently collected from the same locations throughout the sampling period, however, additional samples may be collected at other "non- index" locations at the discretion of the monitoring team and still be analyzed. For example, waterbodies with embayments or coves where blooms are known to occur or accumulate, drinking water supply intake locations, or important recreational areas such as beach/swimming areas are all good sample collection points.

6.0 Stepwise procedures for cyanomonitoring sample collection, preservation, and analysis

1. Proceed to your first fixed sampling location and record your index site GPS coordinates. If you are sampling on the lake, then your primary or first index station should be at the deep hole location of the lake. ***Location coordinates should be recorded in decimal degrees and contain at least four decimal places*** (i.e. Latitude 42.3645/Longitude 71.6634). This will provide a location accuracy of around 10 meters, which is acceptable.

2. Take out your integrated tube sampler and rinse the inside of the tube three times in the ambient water.

3. Lower the integrated tube (IT) sampler into the water column to the three meter depth mark or the one meter mark if sampling from the shoreline, place your thumb over the top of the tube opening to form a tight seal, and then pull the tube upwards from the bottom using the attached lanyard until the bottom opening is at the same height as the top of the tube. NOTE: *To the extent possible, samples should be collected at a given site as close to the same time of day as possible to provide consistency through your sampling efforts.*

4. Transfer the IT sample water into the 500mL brown plastic bottle, secure the lid, shake vigorously to rinse, and then pour out the sample. Take another three meter IT sample and dispense into the rinsed 500mL brown opaque plastic bottle and then cap tightly. Make sure to place this on ice in a darkened cooler until you can transfer to the smaller 125mL brown opaque sample bottles.

5. Prepare to transfer your IT sample from the 500ml bottle by first filling out the sample bottle label (see Appendix XX) and then attaching the label to your **125mL** sample bottle. *(Helpful hint: By filling out your labels first before placing on the container, they will be easier to fill out, and the ink will transfer better to the label than it will when on a damp and chilled sample bottle. Apply a strip of clear packing tape on to your bottle and over your label when completed).* Use a waterproof fine-point sharpie if possible for labeling. Information on the label should include contact name, waterbody name, state abbreviation, the station ID, sampling date in YYYY-MM-DD format, time in H:MM am/pm format, and sample type which the baseline is integrated tube (IT). The information and formatting on the label will then match the same format that is used for data entry in the program's database. An example of the database format can be found in Appendix G.

Waterbody Name _____
State _____
Station ID _____
Collector Name _____
Sampling Date __ __ __ __ - __ __ - __ __
Sampling Time ___ : __ __
Sample Type IT __ Other _____
Sample Depth 3m ___ 1m___ Other _____

***Figure 10**: Sample bottle label (see Appendix G)*

6. Shake gently, then transfer a portion of the 500mL sample into a pre-labeled 125mL brown plastic bottle, filling only up to the shoulder of the bottle to allow for expansion during freezing. For quality assurance purposes, a set of triplicate samples should be taken at **least once per season** if your sample volume is low, or **one triplicate set for every fifteen samples** collected. Make certain your bottles are tightly closed, then placed immediately in a plastic baggie on ice in a cooler until they can be frozen for future analysis. Samples should be frozen the same day. Samples can be kept frozen at -20°C for up to a year prior to analysis (Studies completed at the University of New Hampshire's Center for Freshwater Biology have shown no change in pigment concentrations after having been frozen for over two years).

NOTE**:** *Freezing samples provides consistency in analysis, normalization of samples across and within waterbodies, and provides a means where samples can be collected and preserved until a time is available to run analyses. This approach greatly expands the capability of collecting*

samples without worry of compromising sample integrity or dealing with logistical hurdles of getting samples to a laboratory within short time periods.

7. If the decision has been made to take ambient phycocyanin and chlorophyll fluorescence measurements before freezing the sample, then measurements can be taken at this time. Don't forget to blank your fluorometer and take temperature readings prior to taking measurements. If you plan to take ambient readings, but will not be able to do so right away, then place the samples on ice. Samples need to be processed and read under low light conditions at a temperature range between 20-24ºC, as photodegradation of these pigments can happen very rapidly and temperature can also affect the readings. If you did place your samples on ice, rewarm to this temperature range prior to reading. Gently mix the IT sample for 30 seconds prior to pipetting out the appropriate volume for your fluorometer cuvette. For details on fluorometer calibration and use, please see Appendix E.

8. Samples should be transferred as soon as possible to a freezer and frozen until analysis can be completed for phycocyanin, chlorophyll, and possible toxins.

9. Ensure that your sampling equipment is well rinsed at the new location if additional sites are to be sampled.

A choice can be made to collect samples from the shoreline rather than mid-lake if on-lake access is not available or if this approach falls more in line with collaborator program collection methods. Shore side sample collections are often how samples are acquired by local and state health departments and beach monitoring programs who are concerned about harmful algal blooms and their toxins in and around public swimming areas and beaches. It is also a common approach utilized when time and resources are limited and health officials and/or other monitoring entities need to visit many water bodies in a single day. The sampling frequency requirement is the same as for within lake sampling, minimum of one sample collected on an every other week basis from June through September. If access to water a meter or greater in depth cannot be reached from the shoreline or near shore area, then a shallower depth sample may be obtained, but the sample depth must be recorded and at least 50mL of sample volume must be collected (approximately ½ meter of integrated tube depth) in order to have enough sample for analysis.

Samples that are collected in the field at any of the above locations may be analyzed on site as stated above utilizing a hand held fluorometer for phycocyanin and chlorophyll. If possible, attempt to analyze at least one ambient sample on site per season to compare with itself after freezing. The fluorescence signal at least doubles when read after thawing a frozen sample, and this provides a good QA check. **Samples must however, be preserved by freezing** to be analyzed later, as is the required baseline protocol. If there is a need or desire to analyze samples within a short time period, then samples that are collected can be frozen solid for a minimum of four hours and then thawed to an ambient temperature range of 20-24ºC, well mixed, and then read for chlorophyll and phycocyanin pigment concentrations. Samples that are frozen can also be stored for extended periods beyond a year or more. Thawed samples can be refrozen for future toxin analysis, but may not be re-thawed to be used for subsequent pigment analysis, as these pigments will have degraded. It is extremely important that ***all samples be processed and***

read under low light conditions. No samples should be left open or exposed to light for any period. Frozen samples should be quickly thawed in a water bath at the upper optimal temperature range of 20-24°C and then read immediately after. The water bath can be as simple as a plastic dish tub filled with water at the appropriate temperature. Sample bottles should not be immersed any further than up to the shoulder of the bottle. Do not leave samples to slowly thaw out on lab countertops or left for any period of time as this can compromise the readings. Samples should be thawed out in a water bath to the optimal temperature range and then read immediately. ***The hold time should not exceed 20 minutes from the time the sample reaches temperature until the sample is read by the fluorometer***. Once read, they should be immediately placed back in the freezer if additional analysis for toxins is anticipated. All data needs to be entered into the standardized excel spreadsheet that can be downloaded off of the Cyanos.org webpage or by requesting a copy from one of the contacts from the Cyanos.org webpage. An example of the datasheet can be found in **Appendix G**.

A Note on Quality Assurance:

This program has been designed to meet multiple needs at multiple levels, from the individual lakeshore home owner interested specifically in their waterbody, to the drinking water supplier responsible for their community's water supply, to the government researchers looking for large data sets where they can determine large regional or national trends. Some intended uses may be purely educational while others can be of critical importance in managing a public resource for the benefit of a large population. In order to achieve these goals, whatever the scale, it is imperative that the baseline methods and procedures in this program are explicitly followed. Deviations from and lack of attention to detail on these baselines will compromise the data and limit its utility and benefit, not only to your individual goals, but also to the larger collaborative. We hope you will take the time and care necessary to make your program and the larger collaborative a continuing success!

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APPENDIX A

CMC Contact and Participation Matrix Chart

First Name	Last Name	Affiliation	Decision maker	QA manager	Field Sampler	Program developer	Program Trainer	Web Developer	flourometry analysis	Data Manager	Data User	Collaborative Member	bloomWatch	cyanoScope	Cyanomonitoring	Water Supplier	State/Tribal Government	local government	Federal Government	NGO	Lake/Watershed Association	University/Extension	Private Industry
Al	Collings	Lake Wickaboag Preservation										X									X		
Alex	Cahill	Lake Sunapee Protective Association										X											
Alex	Larochelle	Manchester Water Works			X				X	X	X	X	X	X	X	X							
Amy	Arsenault	Acton Wakefield Watershed Alliance			X						X	X	X	X	X						X		
Andrew	Madison	Granite State Rural Water Association	X		X						X	X				X							
Angela	Snell	Town of Shrewsbury									X	X						X					
Barbara	Kickham	Lake Quinsigamond Watershed Association	X		X	X					X	X									X		
Beth	Proko	Indian Lake Watershed Association	X		X	X					X	X	X	X							X		
Bill	Clougherty	Indian Lake Watershed Association	X									X									X		
Bob	Garnett	Milton Three Ponds										X									X		
Bryan	Dore	USEPA Boston										X							X				
Buck	Howe	Acton Wakefield Watershed Alliance										X	X								X		
Carl	Ullman	Owl lake Association										X	X								X		
Cheryl	Wood	Manchester Water Works	X			X						X				X							
Chet	Foster	FOWP										X	X								X		
Chris	Countie	Pennichuck Water Works																					
Chuck	Robbins	Lovell Lake Association/AWWA										X	X								X		
Dana	Padgett	Lovell Lake Association/AWWA										X	X								X		
Daniel	Leonard	Town of Meredith, NH	X			X					X	X				X							
Dave	Mankus	Great East lake Association/AWWA										X	X								X		
Dave	Miller	Manchester Water Works	X			X					X	X				X							
David	Bailey	Town of Sunapee, NH	X									X						X					
Delia	Kaye	Town of Concord, MA	X			X					X	X						X					
Don	Kretchmer	Lake Wentworth	X			X					X	X									X		
Ed	Pacheco	URI Watershed Watch			X							X	X		X								
Eileen	Pannetier	CEI Environmental	X			X					X	X											X
Eileen	Naughton	State Representative-District 21 (Warwick)	X									X					X						
Elaine	Papa	Buckeye Brook Coalition										X	X								X		
Elisabeth	Ccianciola	Charles River Watershed Association	X		X				X	X	X	X	X	X	X					X			
Evan	Philo	RI DOH Laboratories										X					X						
Gail	Colozzi and Bob Greene	Bear Camp Pond Association										X									X		
Gayle	Elsberry	Great East lake Association/AWWA										X	X								X		
Henry	Leibovitz	RI DOH Laboratories	X									X					X						
Hy	Rosen	Federation of Lake Garfield										X									X		
Ian	Rohrbacher	Rochester Water Department										X				X							
Jack	Sweeney	Lake Wickaboag Preservation										X	X								X		
Jaime	Rice	WDPH/CMRPHA	X								X	X						X					
Janice	Hunter	Great East lake Association/AWWA										X									X		
Janine	Gillum	Town of Wolfeboro										X				X		X					
Jasper	Hobbs	New England Interstate Water Pollution Control Commission				X		X		X		X	X							X			
Jeanne	Achille	Wilson Lake										X											
Jessica	Pearce	City of Portsmouth, NH										X						X					
Jim	Angers	City of Lebanon, NH	X		X	X					X	X				X							
Jim	McColl	City of Worcester										X						X					
Jim	Martel	Mascoma Lake Association										X	X								X		
John	Bergeron	Canaan Street lake Association	X		X						X	X									X		
John	Lynch	City of Lebanon, NH			X						X	X				X							
John	O'Neil	Manchester Water Works										X	X	X	X	X							
John	Ferrarone	Tatnuck Brook Association										X	X								X		
Julie	Hambrook	Mystic River Watershed Association			X						X	X	X	X							X		

Julio	Rodriguez	Federation of Lake Garfield										X									X		
Kaitlin	Carr	Acton Wakefield Watershed Alliance			X						X	X	X	X	X						X		
Kevin	Esposito	Town of Shrewsbury										X	X					X					
Koby	Ansalic	City of Worcester										X						X					
Kristin	Conte	Manchester Water Works			X	X			X	X	X	X	X	X	X	X							
Kristy	Sullivan	Lake Sunapee Protective Association										X	X								X		
Lee	Chase	Milton Three Ponds										X									X		
Lee	Bavis	Town of Meredith, NH										X						X					
Leo	Thuotte	Gorton Pond Watershed Watch										X									X		
Linda	Bacon	Maine DEP	X		X	X			X		X	X	X	X	X		X						
Marco	Phillipon	Concord, NH Water Department	X								X	X	X					X					
Marti	Ortiz	Lovell Lake Association/AWWA										X									X		
Marty	Goldstein	Federation of Lake Garfield										X									X		
Matt	Day	Pennichuck Water Works	X		X				X		X	X	X	X	X	X							
Michael	Mezzacapo	VLAP Canaan Street Lake			X						X	X									X		
Michael	Mendez	WDPH/CMRPHA	X								X	X						X					
Mike	Berberian	City of Worcester	X									X						X					
Mike	Kalinowski	Lake Quinsigamond Watershed Association										X	X								X		
Mike	Liberty	Liberty Concrete Cutting										X									X		
Nate	Fogg	Town of Wakefield	X								X	X						X					
Norman	Willard	White Pond Association									X	X									X		
Oscar	Ortiz	Lovell Lake Association/AWWA										X	X								X		
Pat	Myers	Concord, NH Water Department	X								X	X				X							
Pat	Theisen	Great East Lake Association/AWWA										X	X								X		
Pat	Tarpey	Lake Winnepesauki Association	X								X	X									X		
Pat and Ray	Boyd	Balch lake Association/AWWA										X	X								X		
Paul	Doucette	Lovell Lake Association/AWWA										X	X								X		
Paula	Inglee	Province Lake Association/AWWA										X	X								X		
Peter	Kulbacki	Town of Hanover, NH										X											
Philip	Leger	WPM/CMRPMA	X									X						X					
Rich	Masse	Lake Wentworth										X									X		
Richard and Pat	Edelstein	Lake Garfield Association										X	X								X		
Robin	Williams	Lake Buel Association										X									X		
Rosalind	Roth	Elephant Pond Association										X									X		
Rosemary	Stewart	Pine River Pond Association/AWWA										X									X		
Scott	Clang	Granite State Rural Water Association	X								X	X	X								X		
Scott	Almstrom	New Hampshire Lake Association										X									X		
Shirley and Noel	Cartwright	Province Lake Association/AWWA										X	X								X		
Stephanie	Thornton	Lovell Lake Association/AWWA										X									X		
Steve	Edelstein	Federation of Lake Garfield										X									X		
Sue and Frank	Burgess	Rock Pond Association										X									X		
Teriko	MacConnell	Lake Sunapee Protective Association	X		X							X	X	X	X						X		
Tim	Green	Rochester Water Department	X								X	X				X							
Wayne	Laroche	Lake Champlain International Advisory Council Member										X									X		
Wendy	German	Federation of Lake Garfield										X									X		
William	Longfellow	Passamaquoddy Tribe										X					X						
Yvonne	Buswell	Pine River Pond Association/AWWA										X									X		
Zoe	Hutcher	Federation of Lake Garfield										X									X		
Kristin	Divris	MA Dept. Environmental Protection			X	X			X		X	X		X	X		X						
Joanie	Beskenis	MA Dept. Environmental Protection			X	X			X		X			X	X		X						
Tracey	Lizotte	CT Dept. Energy & Environmental Protection			X				X	X	X	X		X	X		X						

APPENDIX B

Recreational Action Levels for Health Advisories

Interstate Harmful Algal Bloom Outreach Matrix

Prepared by NEIWPCC in cooperation with the States of Connecticut, Maine, Massachusetts, New Hampshire, New York, Rhode Island, and Vermont.
Last Updated July 2014. For more information, contact Dan Peckham, NEIWPCC.

		EPA / CDC / USGS	CT	MA	ME	NH	NY	RI	VT
Regulations / State Department Roles			Local Health Directors can open and close swimming facilities: **Statutory Authority:** **Connecticut General Statutes outlines enforcement authority under Chapter 98, Municipal Powers. Section 7-148:** - power to "control and operate" recreation places, public beaches and beach facilities - power to "regulate and prohibit swimming or bathing in the public or exposed places within the municipality" CT Public Health Code does not include a pertinent regulation specific for lakes and ponds, however; section 19-13-B34 may apply to impoundments.	**Statutory Authority: Massachusetts General Law 111, section 122:** Regulations relative to nuisances; examinations - power of local boards of health to take action against nuisances (including HABs) **Massachusetts General Law 11, section 5S: Public bathing waters**- describes roles and responsibilities of bathing beach operation **Bathing beach regulations: 105 CMR 445:** - Binding - The regulations allow for posting advisories at beaches for any potential health reason, including HABs. - Regulations moved through public process **Specific to Harmful Algae Blooms:** MDPH Guidelines for Cyanobacteria in Freshwater Recreational Water Bodies in Massachusetts Document	**DEP Rules (06-096 Chapter 581: 6B)** define algal blooms as "planktonic growth of algae which causes Secchi disk transparency to be less than 2.0 m." When DEP staff are asked about HABs, our standard answer pertains to general conditions (blooms, risk of contracting Girardia) rather than HABs (e.g., don't swallow water, some species irritate skin - shower after swimming, relate personal perspective that if it were my child and I couldn't see more than 6-7 feet into the water, I wouldn't want them swimming in the water because I might not be able to find them if they had an accident).	**DES:** No entities are required to test for cyanobacteria. However, if cyanobacteria is found, an advisory sign must be posted. Still researching the state statutes to determine if Health & Human Services has the authority to close a beach or lake. DES does NOT have that authority.	**DEC:** No specific regulations for HABs. A narrative standard for phosphorus and nitrogen references algae (Part 703.2) "none in amounts that will result in growths of algae, weeds and slimes that will impair the waters for their best uses." and taste-, color-, and odor-producing, toxic and other deleterious substances allows "None in amounts that will adversely affect the taste, color or odor thereof, or impair the waters for their best usages". The latter may be disaggregated to separate "toxins" and be interpreted through a numeric translator for citing HAB-related standards violations. DEC has integrated HABs sampling into their monitoring programs; encourages the public to submit reports; provides weekly updates on the public website based upon the information recieved; developed comprehensive web pages about blue-green algae & HABs and continues to improve the content; uses social media, DEC listserves and email to build awareness and direct the public to DEC & DOH information. **DOH/OPR:** developed response protocols for beach closures based on guidance, not regulations.	**Both salt and freshwater beach facilities** are required to conduct sampling to ensure safe swimming conditions as part of their recreational licenses. **The DEM's Office of Water Resources** screens lakes with reported blooms and lakes that have historically had high nutrient and/or chlorophyll a levels (factors that lead to cyanobacteria blooms) and responds to citizen complaints, as funding and manpower allow. **The agencies jointly** issue Health Advisories when any of the three guidelines (noted below), which indicate that a bloom exists, are met.	The Health Department offers Guidance Document Only: http://healthvermont.gov/enviro/bg_algae/documents/BGA_guide.pdf
Action Levels	**Advisories**		**Visual Rank Category 2 (or blue-green algae cells >20k/ml and < 100k):** Notify CT DPH, CT DEEP, Increase regular visual surveillance until conditions change.	**Further sampling** (toxin testing of lysed cells to ensure that guideline of **14 ppb (microcystin)** is not exceeded) if cell count exceeds 50,000 cells/mL. **Advisory:** Avoid contact with water if visible scum or mat, ≥14 µg/L microcystin-LR, or ≥70,000 cells/mL for cyanobacteria cell counts	No official guidance for closures/advisories, but **DEP Rules (06-096 Chapter 581: 6B)** define algal blooms as "planktonic growth of algae which causes Secchi disk transparency to be less than 2.0 m."	**Beach Advisories** issued when a bloom exists, with a cyanobacteria cell count exceeding 70,000 cells/mL OR >50% of the bloom is a cyanobacteria. A **Lake WARNING** is issued when a lake has no beach or the bloom covers area away from the beach. Action values the same as a beach advisory.	**DEC:** No official guidance for advisories for freshwater HABs. Guidance thresholds, based on literature review and analysis of other state's criteria, are used to categorize the alage bloom data received through DEC monitoring programs, volunteers, and the public. Specifically for the following DEC notification categories: **"Suspicious" blooms** = visual evidence consistent with BGA (spilled paint, pea soup, green streaks) **"Confirmed" blooms** = sample showing BG chlorophyll a > 30 ug/l or total chlorophyll a > 50 with dominance by BGA species; beach closure from DOH or OPR; chlorophyll > 20 with ancillary information **"Confirmed with high toxins"** = microcystis-LR > 20 ug/l or anatoxin-a levels > 4 ug/l; open water MC-LR > 10 and ancillary information (bloom increasing, unsampled denser shoreline blooms, etc.) **DOH and OPR:** advisory may be issued if DEC reports BGA near regulated beach but no evidence of bloom at beach, or if beach has reopened and some indication that bloom may return	Health Advisories issued when any of the following three guidelines, which indicate that a bloom exists, are met: - Evidence of a visible cyanobacteria scum or mat. - Cyanobacteria cell count exceeding 70,000 cells/mL. - Toxin (Microcystin-LR) level of lysed cells meeting or exceeding 14 ppb (ug/l).	VISUAL assessment: Post Beach at Category 2. Health alert- keep children and pets away from algae.
	Closures		**Visual Rank Category 3, or blue-green algae cells > 100k/ml:** POSTED BEACH CLOSURE: If public has beach access, alert water users that a blue-green algae bloom is present.	N/A - See Advisory.		N/A	**DEC:** Does not close freshwater waterbodies. Marine waters (immediately surrounding the sample location) are closed for shellfishing if mussels at a DEC monitoring sites test positive for Alexandrium. **DOH/OPR:** visual evidence of BGA bloom triggers beach closure; bloom cleared and MC-LR < 10 required to reopen beach. DEC regulated beaches would likely follow same protocol (blooms not reported at any DEC beaches since DOH protocol established)	N/A	**Visual:** Close Beach at Category 3 **Cell Count**: Close Beach <4000 potential producer cells/mL. **Toxins:** Close at >6 ug/L microcystin, >10ug/L anatoxin

Monitoring	**- What public messaging exists on how states are gathering data?** **- What monitoring can citizens take part in and What information are they asked for?**		No volunteering monitoring program actively managed by the state, but consultants and individual lake groups can conduct their own as desired. State swimming beaches are monitored by DEEP staff (weekly). Further efforts ongoing with EPA R1 (Monitoring and Analysis Focus Team).	- Section on "Recommendations for Monitoring or Advisory Posting" in: http://www.mass.gov/eohhs/docs/dph/environmental/exposure/protocol-cyanobacteria.pdf - Citizens are encouraged to report blooms (including a photo), but not involved with citizen monitoring	State program and volunteers in the Maine Volunteer Lake Monitioring Program monitor algal blooms using Secchi disk. Maine does not have any monitoring specific to HABs. We request that volunteers notify Maine DEP when algal blooms decrease Secchi transparencies below 2.0 meters.	Under Identification on DES Cyanobacteria webpage: "You may also collect a sample in a clean container (plastic or glass jar) and bring it to DES (link to directions to DES) to identify. Caution: If collecting a potentially toxic algal sample: Avoid contact with skin. Wear water-proof gloves when sampling and/or immediately wash all areas of the body where water contact has occurred." http://des.nh.gov/organization/commissioner/pip/factsheets/bb/documents/bb-13.pdf	DEC posts information about the programs and how the program that collect blue-green algae data: Citizens in lake communities that have a lake association may be able to participate in the Citizen Statewide Lake Assessment Program (CSLAP, http://www.dec.ny.gov/chemical/81576.html); information about what data is collected is available on DEC's website http://www.dec.ny.gov/chemical/81849.html - appx 120 lakes sampled 8x per summer for open water blooms and as needed when shoreline blooms observed. DEC monitoring conducted on other lakes; bloom samples collected when blooms observed Citizens not assoicated with a lake association may submit information about potential blooms using DEC's online form and limited sampling may be authorized under authority of DEC.	N/A	Lake Champlain Committee (LCC), Health and DEC monitor for blooms. Citizens may work with LCC to get trained as a volunteer monitor.
Reporting	**Protocol/Contacts**		**By Phone:** DPH: 860-509-7758 DEEP: 860-424-3020 **By Email:** algalbloomsCT@ct.gov, deep.algalblooms@ct.gov	**By Phone:** - MDPH - (617) 624-5757 MDPH responds to all received reports of blooms (reports come from state environmental and local health officials, parks staff, residents, and watershed associations). MDPH asks local health dept or individual reporting the bloom to email photos of the bloom before samplers are deployed.	**By Phone:** - DEP Lakes Staff: 207-287-3901 - Environmental and Occupational Health Program: 866-292-3474 in state. When a bloom is called in from a lake that has not supported chronic algal blooms in the past, DEP staff or VLMP staff will investigate extent and collect water samples for TP & Chl analysis.	**By email:** sonya.carlson@des.nh.gov or beaches@des.nh.gov **By Phone:** Cyano hotline: 603-419-0918	DEC: **Online:** http://www.dec.ny.gov/docs/water_pdf/algaereportform.pdf. **By Phone:** Contact regional DEC office or DEC HAB coordinators. DOH/OPR: blooms observed by beach managers reported by DOH/OPR HAB coordinators in Albany All data reported to any agency shared amongst all agency HAB coordinators	**By Phone:** Contact RIDEM at (401) 222-6800 (**By Email:** brian.zalewsky@dem.ri.gov or jane.sawyers@dem.ri.gov)	**By Phone:** 1-800-439-8550 **By Email:** AHS.VDHBlueGreenAlgae@state.vt.us
	Outreach/ Instructions		"If you believe that you have observed an algae bloom, follow the guidance listed above and contact your Local Public Health Agency (linked to Public Health Agencies webpage). You may also contact CT Department of Public Health (860-509-7758), or CT Department of Energy and Environmental Protection (860) 424-3020, or send an email to deep.algalblooms@ct.gov"	Reports requested via phone to MDPH. MDPH asks local health dept or individual reporting the bloom to email photos of the bloom before samplers are deployed.	"If you want to report a bloom, contact the DEP Lakes Staff at 207-287-3901. For information on health effects, contact the Environmental and Occupational Health Program (866-292-3474 in state). Out of State contact The World Health Organization (WHO)."	"You may use the tell-tale signs of cyanobacteria to initially identify its presence in the water, described in the Ecology section below. However, cyanobacteria may only be positively identified to the Genus level by microscopic identification. If you suspect a cyanobacteria bloom is occurring at your lake or pond, please call DES immediately at (603) 271-2457 or beaches@des.nh.gov and we will conduct a site visit. You may also collect a sample in a clean container (plastic or glass jar) and bring it to DES (link to directions to DES) to identify. Caution: If collecting a potentially toxic algal sample: Avoid contact with skin. Wear water-proof gloves when sampling and/or immediately wash all areas of the body where water contact has occurred."	"You are encouraged to include digital photographs as email attachments with the form (close-up, and landscape showing extent and location of bloom). If possible, please include an image from an online mapping application such as Google, Bing or Yahoo Maps, with a marker at the bloom location." (instructions included with on-line reporting form)	"To report a suspicious algae bloom, contact RIDEM at (401) 222-6800"	"To report a bloom to VDH, call 1-800-439-8550 or click the link on our website to send us an email."
Terminating an Advisory/Closure			**Health officials may justify lifting a blue-green algae bloom posting if observations meet either or both of the following two criteria:** - Visual assessment remains at the Category 1 condition for at least two successive and representative observational rounds one week apart - Cell count results of the water column indicate that blue-green algal cell abundance has markedly decreased over at least two successive and representative sampling rounds one week apart and is below 70,000 cells per ml. As the situation requires, health officials may consider additional confirmation through microcystin testing of the water column. As is stated for the above, the water column should be below the threshold for at least two successive and representative sampling rounds one week apart. CT DPH suggests a toxin threshold of 15 ug/l microcystin.	Advisories may be lifted after two successive and representative sampling rounds one week apart demonstrate cell counts or toxin levels below those at which an advisory would be posted. (Approach is similar to that of OR and Australia)	N/A	"When monitoring indicates that cyanobacteria are no longer present at levels that could harm humans or animals, the advisory or warning will be removed." The Beach Program will immediately resample all beaches upon issuing an advisory. The sign will be removed from the area when further samples indicate the concentration of the cyanobacteria species is below 50 percent of a sample.	**DEC** follows up with all reports received for updates to the status of bloom: **Resampled lakes-** once visual evidence and/or lab results indicate BGA bloom conditions have dissipated, waterbody removed from DEC nofification page **Unsampled lakes-** original or follow up- waterbodies that have not had an update for >= 4 weeks will be removed from the list on the DEC web page due to lack of information **All cases**- DEC still advises the public to be aware of blooms because blooms can come and go. **DOH/OPR-** beach reopened if bloom cleared and MC-LR < 10 (sampling not initiated until bloom has cleared)	Health advisories remain in effect for the remainder of the swimming season (first of November), unless follow-up sampling by a city, town, or third party indicate that the advisory can be lifted. Health Advisories may be lifted after two successive and representative sampling rounds, two weeks apart, demonstrate no evidence of an algal scum or mat and demonstrate cyanobacteria cell counts and toxin levels below threshold concentrations.	Advisory is lifted when water is visually clear and toxins are < 6 ug/L microcystin and 10 ug/L anatoxin

Advisories & Closures Outreach / Disclosure to Public			**Posting closure signs at swimming areas and advisory signs at other access points used for public recreation is the primary intervention. Some posting is up to local health director.** Further interventions include: - Notifying lake associations - Posting information for public access via the internet or local newspapers via a press release. Include information as to how the public can contact the CT DEEP for the most up-to-date information on the status of the blue-green algae bloom. - In some communities it may also be important to notify local Veterinarians and Physicians and keep them updated on the status of the blue-green algae bloom.	For any Advisory, signage should be posted at (all) water body entry points and should include the following: date of the posting, contact information for the posting authority, language (to be provided or reviewed by MDPH) advising against contact with the water, and a recommendation that pets accidentally entering the water be rinsed. **Current advisories are listed on MDPH website:** http://www.mass.gov/eohhs/gov/departments/dph/programs/environmental-health/exposure-topics/beaches-algae/algae-information.html	N/A	"DES will continue to monitor the water and will notify the appropriate parties regarding the results of initial and subsequent testing. **Public notification occurs through press releases and the DES website.**"	DEC posts waterbodies with bloom notifications on its website.The number of new waterbodies with blooms are announced in the Division of Water's weekly listserv email (Making Waves). DOH/OPR regulated beaches posted with signs (and some county DOH press releases) when beaches closed; signs removed or changed to advisory when beach reopened	Updated every year with new information on the year's blooms: http://www.health.ri.gov/publications/datareports/2013CyanobacteriaBloomsInRhodeIsland.pdf Beach closures are also posted on the state Beach Water Quality Information site: https://beaches.health.ri.gov/swim/ **For materials posted on the state website, see "Advisory Notices Materials" row**	Monitoring data are reported on the tracker: https://webmail.vdh.state.vt.us/vttracking/bluegreenalgae/d/ Conditions are reported on our website also http://healthvermont.gov/enviro/bg_algae/weekly_status.aspx
Advisory Notices Materials			See Section C of http://www.ct.gov/dph/lib/dph/environmental_health/pdf/guidance_to_lhd_for_blue-green_algaeblooms_in_rec_fresh_waters_june_2014.pdf	Signage posted at (all) water body entry points: http://village14.com/files/2012/08/Pages-from-CAUTION-sign_cyanobacteria-2012.pdf	N/A	http://des.nh.gov/organization/divisions/water/wmb/beaches/graphics/rec-exposure-caption.gif	http://www.health.ny.gov/publications/2849/images/sign2.jpg Similar signs available from DEC for posting at non-regulated sites (boat launches, common access points, etc.)	A letter is sent by HEALTH to town officials with signage to be posted at the point of access. DEM Fish and Wildlife will post if there is a state-owned boat ramp at the lake. http://www.southkingstownri.com/files/Health%20Advisory%20Barber%20Pond%20Cyanobacteria_ENG.pdf	See appendix E: http://healthvermont.gov/enviro/bg_algae/documents/BGA_guide.pdf
Drinking Water Advisories and Outreach				- Conducting drinking water outreach for cyanobacteria via presentations to public water suppliers. - New written outreach materials under review. - Another program working on drinking water actions levels for cyanobacteria (although all Mass. PWS have an Emergency Response Plan which details actions for any water supply emergency).	N/A	http://des.nh.gov/organization/commissioner/pip/factsheets/dwgb/documents/dwgb-4-15.pdf	No state protocol established. DEC does not provide information to the public about drinking water when waterbodies classified for drinking report blooms. DEC shares data with DOH, which may include information about drinking water in press releases.		Process for managing anatoxin and mirocystin detections in finished water samples for public water systems- this document exposires in January 2015. A **DO NOT DRINK** is ordered at anatoxin > 5 ug/L and microcystin at 10 ug/L. The document can be found at http://drinkingwater.vt.gov/wqmonitoring/pdf/practicealgaltoxindetections.pdf
Further Comments					Preliminary screening data indicate that toxic blooms are not a prevalent issue in Maine, but the state wants to be prepared for future bloom situations because changes in frequency and duration of precipitation events coupled with an extended growing season due to early ice-off and later ice-on, might change the types of blooms that occur in the state.				
Websites: HAB Landing Pages	**Health Agency**	**CDC:** http://www.cdc.gov/healthcommunication/toolstemplates/entertainmented/tips/algalblooms.html	http://www.ct.gov/dph/lib/dph/environmental_health/pdf/guidance_to_lhd_for_blue-green_algaeblooms_in_rec_fresh_waters_june_2014.pdf	http://www.mass.gov/eohhs/gov/departments/dph/programs/environmental-health/exposure-topics/beaches-algae/	N/A	N/A	http://www.health.ny.gov/environmental/water/drinking/bluegreenalgae.htm	http://www.health.ri.gov/healthrisks/poisoning/cyanobacteria/	http://healthvermont.gov/enviro/bg_algae/bgalgae.aspx
	Environmental Agency	**EPA:** http://www2.epa.gov/nutrient-policy-data/cyanobacterial-harmful-algal-blooms-cyanohabs_*** http://www2.epa.gov/nutrient-pollution/harmful-algal-blooms ***	http://www.ct.gov/deep/cwp/view.asp?A=2719&Q=510024	DW Only: http://www.mass.gov/eea/agencies/massdep/toxics/sources/cyanobacteria-in-drinking-water.html	http://www.maine.gov/dep/water/lakes/cynobacteria.htm	http://des.nh.gov/organization/divisions/water/wmb/beaches/cyano_bacteria.htm	http://www.dec.ny.gov/chemical/77145.html	http://www.dem.ri.gov/bart/habs.htm	http://www.anr.state.vt.us/dec/waterq/lakes/htm/lp_cyanobacteria.htm
Public Informational Documents		**CDC - Cyanos/Blooms:** http://www.cdc.gov/hab/cyanobacteria/pdfs/facts.pdf **CDC - Pets:** http://www.dem.ri.gov/programs/bnatres/agricult/pdf/algaepostr.pdf **CDC - Cyanobacteria:** http://www.cdc.gov/hab/cyanobacteria/pdfs/activities.pdf **USGS - Blooms:** http://pubs.usgs.gov/fs/2006/3147/pdf/FS2006_3147.pdf		**Flyer:** http://neiwpcc.org/neiwpcc_docs/AlgaeBlooms.pdf **Pets:** http://neiwpcc.org/neiwpcc_docs/protectpets.pdf		**Cyanos/Blooms:** http://des.nh.gov/organization/commissioner/pip/factsheets/wmb/documents/wmb-10.pdf	**BGA:** http://www.health.ny.gov/environmental/water/drinking/bluegreenalgae.pdf **Dogs:** http://www.seagrant.sunysb.edu/btide/pdfs/HABsBrochure0814.pdf	**Pets:** http://www.dem.ri.gov/programs/bnatres/agricult/pdf/algaepostr.pdf **Waterbody Management:** http://www.dem.ri.gov/programs/benviron/water/quality/pdf/algafact.pdf **Cyanos/Blooms:** http://www.uri.edu/ce/wq/ww/Publications/DEM_Cyanobacteria_%20fact%20sheet.pdf	**BGA/Blooms:** http://healthvermont.gov/enviro/bg_algae/documents/BGA_guide.pdf **Cyanos:** http://www.anr.state.vt.us/dec/waterq/lakes/docs/lp_cyanobacteria_basic_information.pdf **Reporting:** http://www.anr.state.vt.us/dec/waterq/lakes/docs/lp_cyanobacteria_If_you_suspect_a_bloom.pdf **Lake Champlain:** http://www.lakechamplaincommittee.org/fileadmin/files/Publications/2014_LCC_-_Flier_on_Distinguishing_Blue_Green_Algae_from_other_Lake_Phenomena.pdf
Tracker/Map Site			N/A	N/A	N/A	http://www2.des.state.nh.us/WaterShed_BeachMaps/WaterShed_BeachMaps.aspx	http://www.dec.ny.gov/chemical/83310.html	https://beaches.health.ri.gov/swim/	https://webmail.vdh.state.vt.us/vttracking/bluegreenalgae/d/

Contact			**Internal:** Stewart.chute@ct.gov, Charles.lee@ct.gov **Public:** algalbloomsCT@ct.gov, deep.algalblooms@ct.gov	**Internal:** Michael Celona, Algae Project Coordinator - Mike.Celona@state.ma.us **Public:** MDPH - (617) 624-5757	**Internal:** Linda Bacon - Linda.C.Bacon@maine.gov **Public:** DEP Lakes Staff - (207) 287-3901	**Internal:** sonya.carlson@des.nh.gov **Public:** beaches@des.nh.gov	**Internal:** karen.stainbrook@dec.ny.gov **Public:** dowinfo@dec.ny.gov; harmfulalgae@health.state.ny.us	**Internal:** Robert.Vanderslice@health.ri.gov, brian.zalewsky@dem.ri.gov, jane.sawyers@dem.ri.gov **Public:** Robert.Vanderslice@health.ri.gov, brian.zalewsky@dem.ri.gov, jane.sawyers@dem.ri.gov	**Internal:** **Public:** 1-800-439-8550 (in state), 1-802-863-7220 (out of state), AHS.VDHBlueGreenAlgae@state.vt.us

APPENDIX C

HAB Regulations and Outreach Matrix

Interstate Harmful Algal Bloom Regulations and Guidance Matrix

Prepared by NEIWPCC in cooperation with the States of Connecticut, Maine, Massachusetts, New Hampshire, New York, Rhode Island, and Vermont.
Last Updated July 2014. For more information, contact Dan Peckham, NEIWPCC.

	CT	MA	ME	NH	NY	RI	VT
Regulations	**Statutory Authority:** **Connecticut General Statutes outlines enforcement authority under Chapter 98, Municipal Powers. Section 7-148:** - power to "control and operate" recreation places, public beaches and beach facilities - power to "regulate and prohibit swimming or bathing in the public or exposed places within the municipality" **CT Public Health Code** does not include a pertinent regulation specific for lakes and ponds, however; section 19-13-B34 may apply to impoundments. Information below is from "Guidance to Local Health Departments For Blue–Green Algae Blooms in Recreational Freshwaters" (June 2014) - linked under Information Source(s): Web Access.	**Statutory Authority: Massachusetts General Law 111, section 122: Regulations relative to nuisances; examinations** - power of local boards of health to take action against nuisances (including HABs) **Massachusetts General Law 11, section 5S: Public bathing waters**- describes roles and responsibilities of bathing beach operation	**Title 38, Chapter 3, Article 4A: Section 465-A 1B** does not specifically mention HABs but does state: Class GPA (only water quality class for lakes) waters shall have a stable or decreasing trophic state, subject only to natural fluctuations and shall be free of culturally induced algal blooms which impair their use and enjoyment. DEP Rules **(06-096 Chapter 581: 6B)** define algal blooms as "planktonic growth of algae which causes Secchi disk transparency to be less than 2.0 m."	**DES:** No entities are required to test for cyanobacteria. However, if cyanobacteria is found, an advisory sign must be posted. Still researching the state statutes to determine if Health & Human Services has the authority to close a beach or lake. DES does NOT have that authority.	**DEC:** No specific regulations for HABs. A narrative standard for phosphorus and nitrogen references algae (Part 703.2) "none in amounts that will result in growths of algae, weeds and slimes that will impair the waters for their best uses." and taste-, color-, and odor-producing, toxic and other deleterious substances allows "None in amounts that will adversely affect the taste, color or odor thereof, or impair the waters for their best usages". The latter may be disaggregated to separate "toxins" and be interpreted through a numeric translator for citing HAB-related standards violations. **DOH:** https://www.health.ny.gov/regulations/nycrr/title_10/part_6/subpart_6-2.htm#s6215	None	Guidance Document Only: http://healthvermont.gov/enviro/bg_algae/documents/BGA_guide.pdf
Regulations Comments: - Type: Guidance, binding regulation, etc. - Purpose of the regulatory framework (e.g., alerting the public, assuring compliance) - Comments on how state reached the values, and how values were moved through the rulemaking process	- Guidance Document - Comprehensive document includes public alert and action value components, with less of a focus on watershed management/assuring compliance - VT processes and procedures were mirrored closely in the development of the CT approach	- **Bathing beach regulations: 105 CMR 445:** **-** Binding - The regulations allow for posting advisories at beaches for any potential health reason, including HABs. - Regulations moved through public process **Specific to Harmful Algae Blooms: MDPH Guidelines for Cyanobacteria in Freshwater Recreational Water Bodies in Massachusetts Document** - Goals: Respond to reports of blooms and collect samples as necessary and prevent exposures by recommending advisories as warranted - Values were proposed after conducting a literature review, and WHO and EPA recommendations were followed in developing a recreational water body concentration for microcystin (used TDI of 0.04 µg/kg of body weight per day, 35kg child, intake rate of 0.1L water per hour, and exposure duration of 1hr/day). Cell count guidelines considered available literature on associations between cell counts and toxin levels. Values were reviewed internally at DPH and by a small workgroup including MassDEP and Massachusetts Department of Conservation and Recreation.	State program and volunteers in the Maine Volunteer Lake Monitioring Program monitor algal blooms using Secchi disk. Maine does not have any monitoring specific to HABs; when DEP staff are asked about HABs, our standard answer pertains to general conditions (blooms, risk of contracting Girardia) rather than HABs (e.g., don't swallow water, some species irritate skin - shower after swimming, relate personal perspective that if it were my child and I couldn't see more than 6-7 feet into the water, I wouldn't want them swimming in the water because I might not be able to find them if they had an accident). Preliminary screening data indicate that toxic blooms are not a prevalent issue in Maine, but the state wants to be prepared for future bloom situations because changes in frequency and duration of precipitation events coupled with an extended growing season due to early ice-off and later ice-on, might change the types of blooms that occur in the state.	- Type: No requirements. Still researching the state statutes to determine if Health & Human Services has the authority to close a beach or lake. DES does NOT have that authority. - Purpose: Notify public if cyanobacteria are found	**DEC:** - No specific regulations for HABs, but WQS may be interpreted through a numeric translator for citing HAB-related standards violations. - DEC has integrated HABs sampling into their monitoring programs; encourages the public to submit reports; provides weekly updates on the public website based upon the information recieved; developed comprehensive web pages about blue-green algae & HABs and continues to improve the content; uses social media, DEC listserves and email to build awareness and direct the public to DEC & DOH information. - No official guidance for advisories for freshwater HABs. Guidance thresholds, based on literature review and analysis of other state's criteria, are used to categorize the alage bloom data received. **DOH/OPR:** - Regulations specify that certain parameters must be met for a water body to continue to be operated as a bathing beach. Regulations contain qualitative requirements for chemical, physical, and biological quality that are protective of public health; however, cyanobacteria and maximum concentrations of their associated compounds are not specified. Guidance developed response protocols for beach closures based on guidance, not regulations.	Type: Guidance, non-binding Purpose: Alerting the public Comments: Guidance has not undertaken the rule-making process. Guidance was developed based on MA studies/policy, which are based on WHO guidelines.	- Type: Guidance
Indicators Monitored	Visual scums Cell counts	Visual scums or mats Cell counts Toxin levels of lysed cells (Microcystic-LR)	Until 2014, Secchi Transparency only; we will use indicators recommended by Region 1 for the Regional project this summer.	Visual bloom Cell count Percent of the total cell count in an algae sample being identified as cyanobacteria.	Visual Evidence consistent with BGA (spilled paint, pea soup, green streaks) BG chlorophyll a, total chlorophyll a Microcystis-LR, Anatoxin-a Ancillary information (bloom increasing, unsampled denser shoreline blooms, etc.)	Visual scum/mat Cyanobacteria cell count Microcystin concentration	Visual Monitoring Cell Counts Species ID Toxins

Proactive Surveillance/ Sampling (DEP, DPH, Citizen) - Note inicator being sampled for each method	No volunteering monitoring program actively managed by the state, but consultants and individual lake groups can conduct their own as desired. State swimming beaches are monitored by DEEP staff (weekly). Further efforts ongoing with EPA R1 (Monitoring and Analysis Focus Team).	From 2009-2013, using CDC funding, MDPH conducted weekly monitoring at 5 locations with known history of blooms (MDPH) during the summer months. Tested for cyanobacteria count & ID, Microcystin, and water quality parameters (including pH, Turbidity, DO, Temp). That ceased after the CDC funding ended.	We request that volunteers notify Maine DEP when algal blooms decrease Secchi transparencies below 2.0 meters.	DES conducts routine sampling at freshwater beaches - the goal is to inspect each beach in the program three times between Memorial Day and Labor Day. Not all freshwater beaches sampled by the DES program are accessible to the general public.	DEC posts information about the programs and how the program that collect blue-green algae data: Citizens in lake communities that have a lake association may be able to participate in the Citizen Statewide Lake Assessment Program (CSLAP, http://www.dec.ny.gov/chemical/81576.html); information about what data is collected is available on DEC's website http://www.dec.ny.gov/chemical/81849.html.- appx 120 lakes sampled 8x per summer for open water blooms and as needed when shoreline blooms observed. DEC monitoring conducted on other lakes; bloom samples collected when blooms observed	Surveillance Program by Rhode Island Department of Environmental Management, as funding and manpower allows	16 VTDEC Long Term monitoring sites visited bi-weekly (cell counts, visual, toxins when bloom present) 70 Lake Champlain Committee volunteer sites (visual) 14 VDH monitoring sites (visual, cell count, toxins)
Sampling Methods - Note indicator being sampled for each method	**Sampling for cell counts, visual observations, (secchi depth)** **Sampling at the Shoreline** - Sampler should be using waders and long sleeved rubber gloves - Clearly mark sampling containers with required information (site #, date, time, etc.) - Wade to an approximate depth of three feet - Invert sample bottle(s) to collect a sample at approximately 18 inches below the surface - Decant water for required air space and/or pour into additional containers (if necessary), cap bottles - Visual observations – look to see if bottom is visible, if a scum on water's surface is present - Fill out chain of custody, including visual observations - Store samples in a cooler with ice until delivery to lab(s) **Sampling the Shoreline from a Dock, Wall, or Boat** - Sampler should be using long sleeved rubber gloves - Clearly mark sampling containers with required information (site #, date, time, etc.) - Choose a location that is approximately three feet deep (if possible) - Lean over to collect sample (if possible), or use a pole sampling device to collect sample - Invert sample bottle(s) to collect a sample at approximately 18 inches below the surface - Decant water for required air space and/or pour into additional containers (if necessary), cap bottles - Visual observations – look to see if bottom is visible, if a scum on water's surface is present - Use a Secchi disk with calibrated line to determine transparency and total depth - Fill out chain of custody, including visual observations - Store samples in a cooler with ice until delivery to lab(s) **Logistical Issues** 1. Long holding times may result in higher counts. [redacted]	- MDPH has a written sampling protocol. - Samples must be collected at 1 meter depth, six inches below the surface. - Hip waders and gloves are used. - Depth is fixed but lateral location is not. Sampling site is chosen based on bloom appearance and access to water to target worst case scenario for human exposure. - Samples must be placed on ice and analyzed for cell count and identification within 24 hours of collection. If a cell count sample exceeds 50,000 cells/mL, toxin testing of lysed cells should be done to ensure that guideline of 14 ppb is not exceeded. The lysing should consist of three freeze and thaw cycles.	State program and volunteers in the Maine Volunteer Lake Monitioring Program monitor algal blooms using Secchi disk. Maine does not have any monitoring specific to HABs.	The goal of the freshwater beach program is to inspect each beach in the program three times over between Memorial Day and Labor Day.	Citizen Statewide Lake Assessment Program (CSLAP, http://www.dec.ny.gov/chemical/81576.html); information about what data is collected is available on DEC's website http://www.dec.ny.gov/chemical/81849.html.- appx 120 lakes sampled 8x per summer for open water blooms and as needed when shoreline blooms observed. Full sampling procedures listed here: http://www.dec.ny.gov/docs/water_pdf/cslapsampro.pdf	At public access point (screening samples) or area of complaint (response samples), a single grab sample is collected for analysis of cyanobacteria cell count and microcystin concentration. If a bloom is noted in another area of the waterbody, a second grab sample can be collected from the bloom for cyanobacteria cell count and microcystin concentration	See QAPP for detail. Beach area whole water samples collected near surface at 1-2 feet in depth.
Indicator Action Limits/Levels (Advisories, Closures, Acute and Chronic)	**Visual Rank Category 2 (or blue-green algae cells >20k/ml and < 100k):** Notify CT DPH, CT DEEP, Increase regular visual surveillance until conditions change. **Visual Rank Category 3, or blue-green algae cells > 100k/ml:** POSTED BEACH CLOSURE: If public has beach access, alert water users that a blue-green algae bloom is present.	**Further sampling** (toxin testing of lysed cells to ensure that guideline of **14 ppb (microcystin)** is not exceeded) if cell count exceeds **50,000 cells/mL.** **Advisory**: Avoid contact with water if **visible scum or mat, ≥14 µg/L microcystin-LR, or ≥70,000 cells/mL** for cyanobacteria cell counts.	No official guidance for closures/advisories, but **DEP Rules (06-096 Chapter 581: 6B)** define algal blooms as "planktonic growth of algae which causes Secchi disk transparency to be less than 2.0 m."	**Beach Advisories** issued when a bloom exists, with a cyanobacteria cell count exceeding 70,000 cells/mL OR >50% of the bloom is a cyanobacteria. A **Lake WARNING** is issued when a lake has no beach or the bloom covers area away from the beach. Action values the same as a beach advisory. Beach closures not issued.	**"Suspicious" blooms** = visual evidence consistent with BGA (spilled paint, pea soup, green streaks) **"Confirmed" blooms** = sample showing BG chlorophyll a > 30 ug/l or total chlorophyll a > 50 with dominance by BGA species; beach closure from DOH or OPR; chlorophyll > 20 with ancillary information **"Confirmed with high toxins"** = microcystis-LR > 20 ug/l or anatoxin-a levels > 4 ug/l; open water MC-LR > 10 and ancillary information (bloom increasing, unsampled denser shoreline blooms, etc.) **DOH and OPR:** **Advisory:** bloom outside swimming area; **Closure:** bloom within swimming area, post advisory Advisory may be issued if DEC reports BGA near regulated beach but no evidence of bloom at beach, or if beach has reopened and some indication that bloom may return. Recommended:	If any of the following guidelines are met, then a recreational health advisory is issued: 1. A scum or mat of cyanobacteria. 2. Cyano cell count >70K cells/mL. 3. Microcystin concentration >14ug/L	Recommendation: **VISUAL** Post Beach at Category 2 Close Beach at Category 3 **Cell Count** Close Beach >4000 potential producer cells/mL **Toxins** Close >6 ug/L microcystin or >10ug/L anatoxin
Sources Referenced	**WHO** (Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management) **VTDOH** (Cyanobacteria (Blue-green Algae) Guidance for Vermont Communities.) **USEPA** (Exposure Factors Handbook) **Oberholster PJ, B. A.** (Microcystis aeruginosa: source of toxic microcystins in drinking water.) **Bress, D., & Stone, W.** (Addressing Public Health Risks for Cyanobacteria in Recreational Freshwaters: The Oregon and Vermont Framework.)	See pages 7-11 of the document linked below under Information Source(s): Web Access. **WHO, USEPA, VT, CA, OR, and Australia** were all key sources.	N/A	**WHO**	Guidance thresholds, based on literature review and analysis of other state's criteria, are used to categorize the alage bloom data received.	WHO. 1999. Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management. http://www.who.int/water_sanitation_health/resourcesquality/toxcyanbegin.pdf	Appendix G- Community Guidance Document - WHO, CDC, Scottish Executive Health Department, Providence of Quebec

Bloom Classification System	**Category 1:** Visible material is not likely cyanobacteria or water is generally clear. **Category 2:** Cyanobacteria present in low numbers. There are visible small accumulations but water is generally clear. **Category 3:** Cyanobacteria present in high numbers. Scums may or may not be present. Water is discolored throughout. Large areas affected. Color assists to rule out sediment and other algae.	**Advisory: Avoid contact with water** if visible scum or mat, ≥14 µg/L microcystin-LR, or ≥70,000 cells/mL for cyanobacteria cell counts.	Secchi transparency < 2.0 meters due to algal growth is defined as a nusiance algal bloom.	**Public Health Advisory:** Avoid contact with water	**"Suspicious" blooms** = visual evidence consistent with BGA (spilled paint, pea soup, green streaks) **"Confirmed" blooms** = sample showing BG chlorophyll a > 30 ug/l or total chlorophyll a > 50 with dominance by BGA species; beach closure from DOH or OPR; chlorophyll > 20 with ancillary information **"Confirmed with high toxins"** = microcystis-LR > 20 ug/l or anatoxin-a levels > 4 ug/l; open water MC-LR > 10 and ancillary information (bloom increasing, unsampled denser shoreline blooms, etc.) **DOH and OPR:** **Advisory** may be issued if DEC reports BGA near regulated beach but no evidence of bloom at beach, or if beach has reopened and some indication that bloom may return	None	**VISUAL** **Category 1:** Little or no Blue-Green algae present-clear water **Category 2:** Blue-Green algae present, but less than 'bloom levels' **Category 3:** Blue-Green algae bloom in progress
Labs Used	EcoAnalysts, Inc. Green Water Laboratories Northeast Laboratories, Inc. PhycoTech, Inc. State University of New York	Northeast Labs Greenwater Lab	We used State University of New York and University of New Hampshire for the screening study. We've used a semi-quantitative kit in our own lab since.		**CSLAP:** NYS Federation of Lake Associations, sent to SUNY - Syracuse, ESF **Non-CSLAP:** SUNY - Syracuse, ESF **DOH:** NYS DOH Office, Albany	GreenWater Laboratory, Palatka, FL	Vermont Public Health Lab
Reporting Protocol	**By Phone:** **DPH:** 860-509-7758 **DEEP:** 860-424-3020 **By Email:** algalbloomsCT@ct.gov, deep.algalblooms@ct.gov	Reports requested via phone to MDPH. MDPH asks local health dept or individual reporting the bloom to email photos of the bloom before samplers are deployed.	To report a bloom, contact the DEP Lakes Staff at 207-287-3901.	"If you suspect a cyanobacteria bloom is occurring at your lake or pond, please call DES immediately at 603-419-0918 (cyanobacteria hotline) or beaches@des.nh.gov… You may also collect a sample in a clean container (plastic or glass jar) and bring it to DES to identify."	**DEC:** **Online:** Citizens not assoicated with a lake association/CSLAP may submit information about potential blooms using DEC's online form (http://www.dec.ny.gov/docs/water_pdf/algaereportform.pdf) and limited sampling may be authorized under authority of DEC. **By Phone:** Contact regional DEC office or DEC HAB coordinators. **DOH/OPR:** blooms observed by beach managers reported by DOH/OPR HAB coordinators in Albany All data reported to any agency shared amongst all agency HAB coordinators	Email or call: Brian Zalewsky or Jane Sawyers at RIDEM	Call 800-439-8550 or 802-863-7220, or email AHS.VDHBlueGreenAlgae@state.vt.us.
Report Investigation	1) Visit the site of a reported bloom. 2) If justifiable (Category 2), notify State Agencies 3) Continue regular field observations. (See example field observation form in Appendix.) 4) If conditions deteriorate to Category 3, post the site and the area. 5) When visual conditions improve, take a water sample for microscopic analysis. 6) Wait approximately one week and sample again. 7) A: If justifiable, terminate the posting; B: Otherwise wait approximately one more week and sample again. 8) Repeat step 7 until termination or the end of the summer recreational season.	MDPH responds to all received reports of blooms (reports come from state environmental and local health officials, parks staff, residents, and watershed associations): - Asks local health dept or individual reporting the bloom to email photos of the bloom before samplers are deployed	When a bloom is called in from a lake that has not supported chronic algal blooms in the past, DEP staff or VLMP staff will investigate extent and collect water samples for TP & Chl analysis.	Upon receiving notice of a suspected cyanobacteria bloom, DES will conduct a site visit. The Beach Program will also immediately resample all beaches upon issuing an advisory.	**DEC:** Follows up with all reports received for updates to the status of bloom: once visual evidence and/or lab results indicate BGA bloom conditions have dissipated, waterbody removed from DEC nofification page **DOH/OPR:** Blooms observed by beach managers reported by DOH/OPR HAB coordinators in Albany. All data reported to any agency shared amongst all agency HAB coordinators	A picture is requested and reviewed by RIDEM for evidence of cyano bloom. A qualitative sample for citizen complaints is collected and reviewed by RIDEM (Jane Sawyers) for potential exceedance of the cyano cell count. If it may exceed, the sample is sent for quantitative cell count and microcystin analysis.	Site Visit by : Town Health Officer, LCC volunteer, VDH Staff, VTDEC Staff. Provide guidance document, signs and testing supplies if needed.
Intervention Protocol	Note: When issuing an advisory take note of all access points. Depending on the size of the bloom relative to the lake, and the location of the access point relative to the bloom, some access locations may not be impacted. **Visual Rank Category 1: [**Notifications: Not needed], [Further Monitoring: No change], [Public Posting: Not needed] **Visual Rank Category 2 (or blue-green algae cells >20k/ml and < 100k):** [Notifications: Notify CT DPH, CT DEEP], [Further Monitoring: Increase regular visual surveillance until conditions change.], [Public Posting: Not needed] **Visual Rank Category 3 (or blue-green algae cells > 100k/ml):** [Notifications: Update/inform CT DPH & CT DEEP and expand risk communication efforts. (See Risk Communication section.)], [Further Monitoring: Collect samples for analysis and/or increase frequency of visual assessment.], [Public Posting: POSTED BEACH CLOSURE: If public has beach access, alert water users that a blue-green algae bloom is present. POSTED ADVISORY: At other impacted access points.]	**Advisory:** Avoid contact with water if visible scum or mat, ≥14 µg/L microcystin-LR, or ≥70,000 cells/mL for cyanobacteria cell counts. **Once Avisory is posted:** Weekly testing until avisory can be lifted, but follow-up testing more frequently than weekly may be suggested based on weather conditions. Advisories may be lifted after two successive and representative sampling rounds one week apart demonstrate cell counts or toxin levels below those at which an advisory would be posted.	DEP staff are in contact with Department of Health and Human Services Center for Disease Control staff when specific questions/issues arise. The CDC has not engaged with DEP in moving to an Intervention Protocol due to lack of resources, although the need for such protocols has been discussed. Documented evidence of human or pet illness specifically due to ingestion of algal toxins would likely result in legislative appropriation of resources to develop advisory specifics.	At any beach, an **advisory is posted** if a potential toxin-producing cyanobacterial scum is present at the beach and cell dominance is greater than 50 percent of the sample total cell count. **Once an advisory is posted:** Follow up testing conducted until toxin-producing cyanobacterial scum is less than 50 percent of the total sample cell count.	DEC posts waterbodies with bloom notifications on its website.The number of new waterbodies with blooms are announced in the Division of Water's weekly listserv email (Making Waves). DOH/OPR regulated beaches posted with signs (and some county DOH press releases) when beaches closed; signs removed or changed to advisory when beach reopened	If the guidelines are exceed, then a letter is sent by RI Department of Health (HEALTH) to the town officials asking for signs to be posted at public access points and a press release is jointly issued by RIDEM/HEALTH.	Beach closure recommended at Category 3, Advisory sign at Category 2
Closure Protocol	**Visual Rank Category 3, or blue-green algae cells > 100k/ml:** POSTED BEACH CLOSURE: If public has beach access, alert water users that a blue-green algae bloom is present.	**Advisory:** Signage posted at (all) water body entry points.	None at this time (see above).	N/A	**DEC:** Does not close freshwater waterbodies. Marine waters (immediately surrounding the sample location) are closed for shellfishing if mussels at a DEC monitoring sites test positive for Alexandrium. **DOH/OPR:** visual evidence of BGA bloom triggers beach closure; bloom cleared and MC-LR < 10 required to reopen beach. DEC regulated beaches would likely follow same protocol (blooms not reported at any DEC beaches since DOH protocol established)	N/A	(Recommendations Only) Visual: Category 3 Toxins: Close >6 ug/L microcystin, >10ug/L anatoxin

Terminating an Advisory	**Health officials may justify lifting a blue-green algae bloom posting if observations meet either or both of the following two criteria:** - Visual assessment remains at the Category 1 condition for at least two successive and representative observational rounds one week apart - Cell count results of the water column indicate that blue-green algal cell abundance has markedly decreased over at least two successive and representative sampling rounds one week apart and is below 70,000 cells per ml. As the situation requires, health officials may consider additional confirmation through microcystin testing of the water column. As is stated for the above, the water column should be below the threshold for at least two successive and representative sampling rounds one week apart. CT DPH suggests a toxin threshold of 15 ug/l microcystin.	Advisories may be lifted after two successive and representative sampling rounds one week apart demonstrate cell counts or toxin levels below those at which an advisory would be posted. (Approach is similar to that of OR and Australia)	None at this time (see above).	The Beach Program will immediately resample all beaches upon issuing an advisory. The sign will be removed from the area when further samples indicate the concentration of the cyanobacteria species is below 50 percent of a sample.	**DEC** follows up with all reports received for updates to the status of bloom: **Resampled lakes-** once visual evidence and/or lab results indicate BGA bloom conditions have dissipated, waterbody removed from DEC nofification page **Unsampled lakes-** original or follow up- waterbodies that have not had an update for >= 4 weeks will be removed from the list on the DEC web page due to lack of information **All cases-** DEC still advises the public to be aware of blooms because blooms can come and go. **DOH/OPR-** beach reopened if bloom cleared and MC-LR < 10 (sampling not initiated until bloom has cleared)	A third party must follow up and sample the location with 2 samples at least 2 weeks apart meeting all of the above guidelines.	Visually clear and toxins less than 6 ug/L microcystin, 10ug/L anatoxin
Communications	**Posting closure signs at swimming areas and advisory signs at other access points used for public recreation is the primary intervention.** **Further interventions include:** - Notifying lake associations - Posting information for public access via the internet or local newspapers via a press release. Include information as to how the public can contact the CT DEEP for the most up-to-date information on the status of the blue-green algae bloom. - In some communities it may also be important to notify local Veterinarians and Physicians and keep them updated on the status of the blue-green algae bloom.	For any Advisory, signage should be posted at (all) water body entry points and should include the following: date of the posting, contact information for the posting authority, language (to be provided or reviewed by MDPH) advising against contact with the water, and a recommendation that pets accidentally entering the water be rinsed. Current advisories are listed on MDPH website.	None at this time (see above).	**Cyanobacteria Advisory Sign:** http://des.nh.gov/organization/divisions/water/wmb/beaches/graphics/rec-exposure-caption.gif **Beach Monitored and Safe Sign:** http://des.nh.gov/organization/divisions/water/wmb/beaches/graphics/monitored_sign.gif "DES will continue to monitor the water and will notify the appropriate parties regarding the results of initial and subsequent testing. Public notification occurs through press releases and the DES website."	**DEC** posts waterbodies with bloom notifications on its website.The number of new waterbodies with blooms are announced in the Division of Water's weekly listserv email (Making Waves). http://www.health.ny.gov/publications/2849/images/sign2.jpg Similar signs available from DEC for posting at non-regulated sites (boat launches, common access points, etc.) **DOH/OPR** regulated beaches posted with signs (and some county DOH press releases) when beaches closed; signs removed or changed to advisory when beach reopened	RIDEM and HEALTH have annual meetings and communicate regularly by email during active blooms.	Web Site CyanoTracker(Map) (https://webmail.vdh.state.vt.us/vttracking/bluegreenalgae/d/) Annual Media Release Social Media
Information Source(s): Web Access	http://www.ct.gov/dph/lib/dph/environmental_health/pdf/guidance_to_lhd_for_blue-green_algaeblooms_in_rec_fresh_waters_june_2014.pdf	http://www.mass.gov/eohhs/docs/dph/environmental/exposure/protocol-cyanobacteria.pdf	http://www.maine.gov/dep/water/lakes/cyanobacteria.htm	http://des.nh.gov/organization/divisions/water/wmb/beaches/cyano_bacteria.htm	http://www.dec.ny.gov/chemical/77145.html	http://www.dem.ri.gov/programs/benviron/water/quality/surfwq/pdfs/cyano11.pdf	http://healthvermont.gov/enviro/bg_algae/documents/BGA_guide.pdf
Contact	**Internal:** Stewart.chute@ct.gov, Charles.lee@ct.gov **Public:** algalbloomsCT@ct.gov	**Internal:** Michael Celona, Algae Project Coordinator - Mike.Celona@state.ma.us **Public:** MDPH - (617) 624-5757	**Internal:** Linda Bacon - Linda.C.Bacon@maine.gov **Public:** DEP Lakes Staff - (207) 287-3901	**Internal:** Sonya Carlson - sonya.carlson@des.nh.gov **Public:** (603) 271-2457 or beaches@des.nh.gov	**Internal:** karen.stainbrook@dec.ny.gov, scott.kishbaugh@dec.ny.gov **Public:** dowinfo@dec.ny.gov; harmfulalgae@health.state.ny.us	**Internal:** **Public:**	**Internal:** Sarah Vose, Andy Chevrefils 802-863-7220 AHS.VDHBlueGreenAlgae@state.vt.us **Public:** 802-863-7220 AHS.VDHBlueGreenAlgae@state.vt.us

APPENDIX D

Fluorometer Calibration and User Manual

FluoroQuik Fluorometer

User's Manual

Standard Operating procedure

Version 1.0

Edited for use with dual channel fluorometers and in conjunction with the Cyanobacteria Monitoring Collaborative (CMC)

Table of Contents:

1. FluoroQuik Fluorometer

1.1 Overview

This Standard Operating Procedure has been written to specifically address the operation of the AMISCIENCE two channel fluorometer designed and built by AmiScience and sold and distributed by Beagle BioProducts, as this is the principal fluorometer currently being used by the Cyanobacteria Monitoring Collaborative (CMC). Other fluorometers are acceptable for use, such as the Turner AquFluor unit. Key procedures that must be followed regardless of instrument type are noted throughout this document in order to ensure the highest level of data consistency and quality throughout the program. This SOP has been adapted from the original AmiScience user manual to specifically address the calibration, operation, and maintenance of the dual channel FluorQuik fluorometer and the protocols of the Cyanobacteria Monitoring Collaborative.

The FluoroQuik fluorometer utilized by the CMC is a portable instrument designed for multipurpose fluorescence measurements with two optical channels built into one unit. The instrument is simple to use, light in weight, and can be powered by either DC power adaptor or AA batteries.

1.2 Key Features

a. Using either 200-µL PCR tubes (model-A), 500-µL micro-centrifuge tubes (model-B), or 1-cm square cuvette (model-C).
b. LCD touch-screen display.
c. User-friendly software with “touch and test” operation.
d. USB interface for data management.
e. Larger than 5 logs of dynamic range (after proper calibration procedure).

1.3 Included Parts

a. The FluoroQuik fluorometer, carry case, sample tubes & pipettes.
b. 5VDC/2A power adapter.
c. Standard-USB-to-mini-USB cable.

d. Operation manual and USB driver/data management software disk.

2. System Operation

2.1 Principle of Operation

The FluoroQuik Fluorometer uses a single-wavelength light source to excite the sample which, as a result, emits a fluorescent signal of a specific wavelength detected by an internal photo sensor. The reading by the photo sensor, represented by RFU (Relative Fluorescence Unit) is used to calculate the nominal concentration of the sample when the fluorometer is properly calibrated.

As shown in Figure 1, during the calibration process, a "Blank" tube (zero concentration) and a "Standard" tube (known concentration) are separately measured by the fluorometer to obtain the RFU readings. The RFU readings and the concentration values are then used to generate a "linear calibration curve" which is stored in the non-volatile memory of the fluorometer. During the measurement operation, the sample's RFU is used to internally calculate the unknown concentration using interpolation or extrapolation based on the stored linear calibration curve.

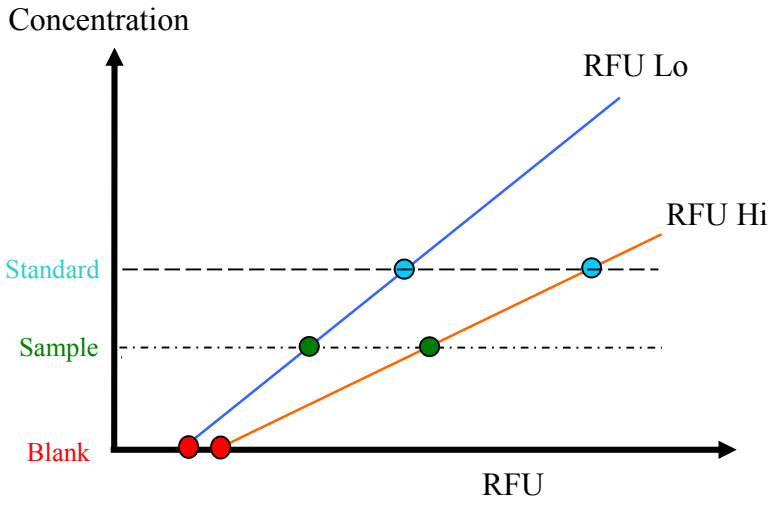

Figure 1

Note that in order to extend the measurable concentration range, two levels of excitation power are automatically used during the calibration and measurement steps, and two different RFU readings ("RFU Hi" and "RFU Lo") are obtained. When the sample's concentration is too high and the fluorescent signal saturates the photo sensor, the fluorometer automatically uses the

"RFU Lo" reading (and the associated linear calibration curve) to calculate the sample concentration, hence extending the upper measurement range. This does not change the output measurements.

In a dual-channel fluorometer, there are two independent excitation/emission wavelength pairs (Channel 1 and Channel 2) whose calibration curves are independently defined by the user, but each channel can define only one linear calibration curve (in other words, only one "Assay" for each channel.)

2.2 Power Up

The FluoroQuik Fluorometer can be powered by four AA batteries or the supplied power adapter (5VDC/2A). After connecting to power, switch the ON/OFF button on the upper-right of the unit to turn on the fluorometer. After a flash of the welcome screen, the screen automatically turns into the "**Main Menu**", as shown in Figure 2.

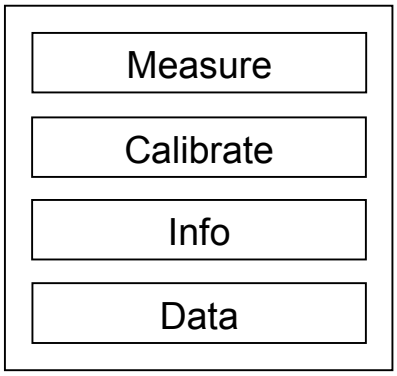

Figure 2. **Main Menu** screen

2.3 Calibration

a. In order to measure the concentration of unknown samples, a calibration procedure needs to be performed. If a calibration has already been completed, you can skip this calibration step and go to Sec. 2.4 to perform sample measurement. Once calibrated, the calibration curve is stored in a non-volatile memory and is not affected by powering the instrument on or off.

b. Touch "**Calibrate**" tab on the "**Main Menu**" screen. A confirmation screen asking "Create new calibration?" will show in order to prevent unintentional calibration steps. Touch

"**Return**" if you don't intend to perform the calibration, otherwise touch "**Continue**" to enter the channel selection screen. Select the Channel on which you want to calibrate your standard (1 Phycocyanin or 2 Chlorophyll).

NOTE: *For the Cyanobacteria Monitoring Collaborative, you will need to use phycocyanin and chlorophyll standards for calibrating your fluorometer. This helps reduce measurement error, improves on quality assurance, and provides standardization across the program.*

c. Now enter the "**Calibrate**" screen similar to Fig. 3. Put in the Blank tube in the sample chamber and close the cap (the blank tube does not need to be filled with de-ionized water). Make certain your tube is clean of fingerprints/debris and is oriented so that you can re-insert it if need be in the exact same position. Touch "**Blank**" to take the blank value.

d. After the Blank is read, the screen will look like the one shown in Fig. 4.

e. To set the nominal value of the Standard tube to calibrate the fluorometer, use the "<" and ">" arrow keys on the second row to move the underline to select the digit you want to change, and use the "+" or "–" keys to increase or decrease the value of the underlined digit. (A zero standard value is not allowed.)

f. Put the Standard tube in the chamber and touch the "**Measure**" tab to take the measurement. After a few seconds, "**Calibration Finished**" will show on the screen. Press "**Return**" to go back to "**Main Menu**".

g. If the Standard measured value is equal or less than the Blank, an error message "**Reading Too Low!**" will show. Prepare the right Blank or Standard and measure again.

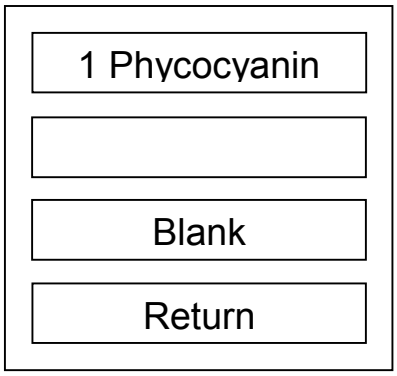

Figure 3. **Calibrate** screen

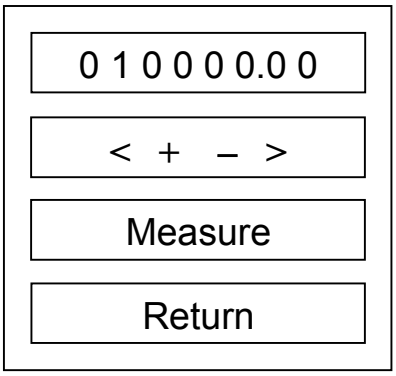

Figure 4. **Standard setting** screen

2.4 Measurement

a. Refer to Section 2.3 for calibration procedures if the fluorometer has not been calibrated.

b. Touch the "**Measure**" tab on the "**Main Menu**" screen to enter the Channel selection screen. In the screen you can select 1 Phycocyanin, or 2 Chlorophyll depending on which you want to measure your sample for. This selection will measure the sample and calculate the nominal concentration value using the linear calibration curve stored in the fluorometer.

c. Prepare sample tube (and blank tube if to be used), referring to Section 3.

d. Select the Channel to enter the "**Measure**" screen similar to Fig. 5.

e. If the fluorometer has been calibrated before, the Blank value is already stored in the fluorometer. But for low-concentration sample measurement, it is recommended that a new "Blank" is performed at this step to compensate for any minor inconsistencies in the walls of the sample tube. Insert the Blank tube into the testing chamber and secure the cap, and touch "**Blank**" to take the blank reading. The tube does not need to be filled with de-ionized water for a blank measurement.

f. Insert the sample tube and touch the "**Measure**" tab to start the sample measurement. The measurement result will be displayed on the "**Result**" screen in a few seconds, as shown in Figure 6. The timer clock will start from zero so you can do a follow-up measurement after a certain time. NOTE: *If doing repeat measurements of the same sample, wait a minimum of 10 seconds between measurements.*

g. If the reading is too high and saturates the photo-detector, an "**Over Limit**" message will display. In other cases where the concentration of material is too high, light cannot pass through the material and reach the measurement optics. This is commonly referred to as "quenching" and can be resolved by diluting your sample. This usually results in a lower reading (See section 3, Sample Preparation and Measurement Tips).

h. If you want to save the measurement data in the meter's on-board memory, you can touch the "**Save**" tab. The data will be saved in the memory of the specific assay/channel that you selected earlier, with the stored data sequential number displayed on the upper-right corner.

i. Touch "**Return**" to go back to the previous "**Measure**" screen. Touch "**Measure**" tab again will repeat the measurement. The timer clock will restart from zero.

j. If batteries are used as the power source, and the voltage has dropped too low and the accuracy of the measurement may be affected, a "**Battery Low**" warning message will show on the bottom of the screen during measurement. The batteries should be replaced as soon as possible.

NOTE: *Do not leave your batteries in the unit for any extended periods, as they can leak and damage the instrument. Always check the battery compartment for any corrosion or battery leakage prior to use.*

k. Touch "**Return**" tab will return to "**Measure**" screen, and touch "**Return**" again will go back to the "**Main Menu**".

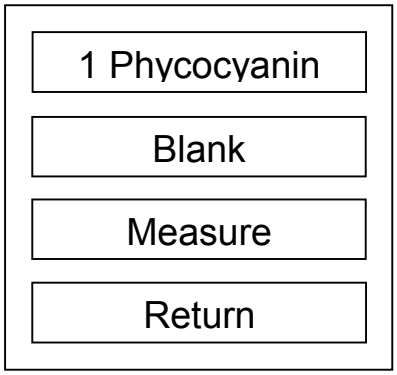

Figure 5. **Measure** screen

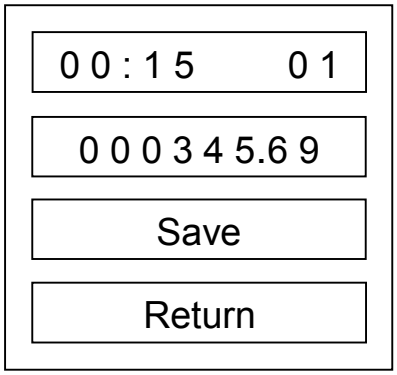

Figure 6. **Result** screen

2.5 Data Retrieval

a. Touch "**Data**" tab on the "**Main Menu**" screen will let you select the assay/channel in which you want to inspect the data. After selection the "**Data**" screen now shows similar to Fig. 7. The first row shows the saved data, and the second row shows the data sequential number. You can touch the left and right arrow key to change the data number to inspect other saved data.

b. If you want to erase the saved data of the assay/channel you are inspecting now, touch "**Erase All**" and confirm the action in the next screen. The data of the other assays/channel will not be affected by this erase.

c. Touch "**Return**" tab will return to "**Main Menu**" screen.

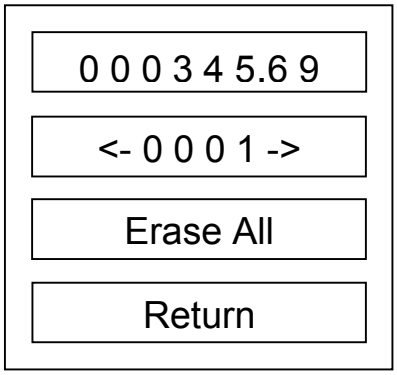

Fig. 7 "**Data**" screen

3. Sample Preparation and Measurement Tips

a. Prepare Standard and Sample solution within the concentration range that can be read by the fluorometer. You can use the "**RFU Hi**" or "**RFU Lo**" mode in the "**Measure**" function to measure the sample if you are not certain. It is also better that the Standard doesn't saturate the RFU Hi reading in order to maximize sensitivity.

> **Note***: If the ambient algal or cyanobacteria concentration is too high, the fluorometer may give an incorrect reading. This is caused when light passing from one side of the instrument and through the sample is blocked so much by the material in the sample that it cannot be appropriately measured by the detector on the receiving side of the instrument. In these cases, samples should be diluted until the proportion that the sample is diluted by reflects an equal reduction in concentration (i.e. diluting the sample by 50% shows a 50% reduction in concentration). This phenomenon is commonly referred to as "quenching."* A rule of thumb is if you cannot see through your sample in your fluorometer tube, then it is likely too concentrated (Fig. 8).

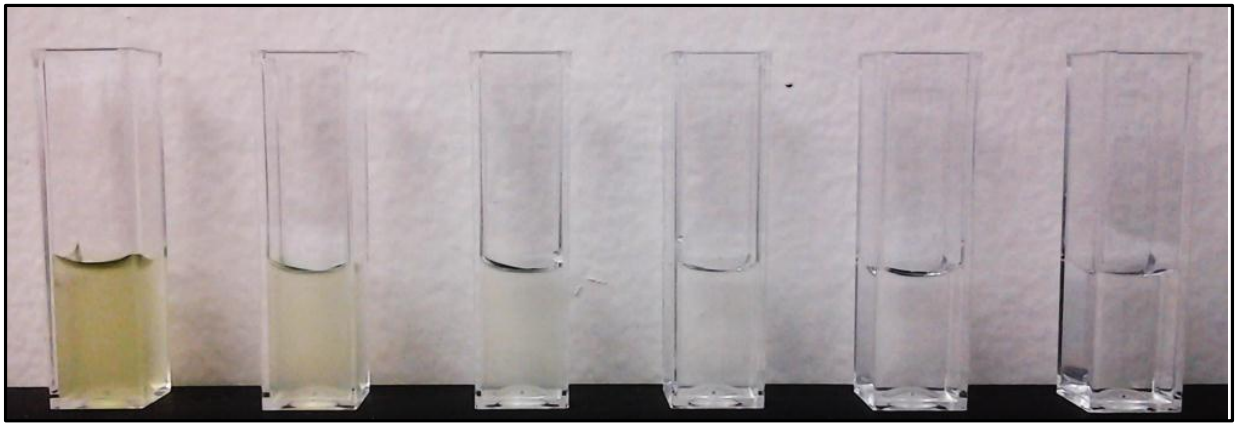

Fig. 8: Dilutions of a *Microcystis aeruginosa* sample. The left-most sample is too turbid to read accurately, but the fluorescence from all other samples can be accurately measured. The right-most sample, though it looks clear, still has a measureable amount of phycocyanin due to the cyanobacteria present, (Beagle BioProducts, April 2013).

b. Make sure the sample tube is clean internally before you put in the solution, and the outside of the tube is clean and dry. Any materials on the outside of the tube may cause measurement error.
c. If glass mini-tube, PCR tube, or micro-centrifuge tube is used, fill the tube with at least 200µL sample solution. For 1-cm cuvette, 1mL sample solution is needed.
d. Make sure no bubbles are in the sample solution.
e. Due to the poor tube-wall consistency of plastic tubes, if PCR tube or micro-centrifuge tube is used, align the cap-lip with the chamber mark so each time the measurement is consistent.
f. Allowing more than 10 seconds between each measurement can minimize the thermal build-up of the light source and maintain the measurement consistency.
g. Due to the possible variation of back-ground level produced by different sample tubes, for very low concentration measurements, you can use the same tube to perform the "Blank" reading, then remove the blank solution and fill with sample solution to perform the "Sample" reading. This technique can ensure the consistent back-ground level to achieve the optimal sensitivity.

4. Maintenance

a. Avoid over-filling the test tube and contaminate the outside wall of the tube. If the contamination is transferred to the inside wall of the test chamber, it may cause increased

signal level and hence reading error. If this happens, use a cotton swab with clean water or alcohol and gently clean the inside wall of the test chamber.

b. The touch screen can be periodically cleaned with alcohol or mild detergent.
c. If the meter will not be used for a while, remove the battery from the battery compartment before put into storage.
d. Always turn off the meter after use if the battery power is used, and remove batteries if the unit will not be in use for a week or longer.

APPENDIX E

Secondary Standards Log Sheet

Secondary Standard - Log Sheet

Fluorometer SN#:

Production Lot #:

Set #:

		Blank		Standard 1		Standard 2	
Concentration (ug/L)							
Channel		Phyco	Chloro	Phyco	Chloro	Phyco	Chloro
DATE	* Temp. °C						

*Temperature: These secondary standards are sensitive to temperature. Keep your digital thermometer and your solid state standards together and record your ambient temperature just before reading your standards. Keep these in a cool place out of the sun.

APPENDIX F

Sample Bottle Labels Sheet

Waterbody Name ___________________________
State _______
Station ID ________________________
Collector Name __________________
Sampling Date __ __ __ __ - __ __ - __ __
Sampling Time ___ : __ __
Sample Type IT __ Other ________
Sample Depth 3m ___ 1m___ Other ______________

Waterbody Name ___________________________
State _______
Station ID ________________________
Collector Name __________________
Sampling Date __ __ __ __ - __ __ - __ __
Sampling Time ___ : __ __
Sample Type IT __ Other ________
Sample Depth 3m ___ 1m___ Other ______________

Waterbody Name ___________________________
State _______
Station ID ________________________
Collector Name __________________
Sampling Date __ __ __ __ - __ __ - __ __
Sampling Time ___ : __ __
Sample Type IT __ Other ________
Sample Depth 3m ___ 1m___ Other ______________

Waterbody Name ___________________________
State _______
Station ID ________________________
Collector Name __________________
Sampling Date __ __ __ __ - __ __ - __ __
Sampling Time ___ : __ __
Sample Type IT __ Other ________
Sample Depth 3m ___ 1m___ Other ______________

Waterbody Name ___________________________
State _______
Station ID ________________________
Collector Name __________________
Sampling Date __ __ __ __ - __ __ - __ __
Sampling Time ___ : __ __
Sample Type IT __ Other ________
Sample Depth 3m ___ 1m___ Other ______________

Waterbody Name ___________________________
State _______
Station ID ________________________
Collector Name __________________
Sampling Date __ __ __ __ - __ __ - __ __
Sampling Time ___ : __ __
Sample Type IT __ Other ________
Sample Depth 3m ___ 1m___ Other ______________

Waterbody Name ___________________________
State _______
Station ID ________________________
Collector Name __________________
Sampling Date __ __ __ __ - __ __ - __ __
Sampling Time ___ : __ __
Sample Type IT __ Other ________
Sample Depth 3m ___ 1m___ Other ______________

Waterbody Name ___________________________
State _______
Station ID ________________________
Collector Name __________________
Sampling Date __ __ __ __ - __ __ - __ __
Sampling Time ___ : __ __
Sample Type IT __ Other ________
Sample Depth 3m ___ 1m___ Other ______________

Waterbody Name ___________________________
State _______
Station ID ________________________
Collector Name __________________
Sampling Date __ __ __ __ - __ __ - __ __
Sampling Time ___ : __ __
Sample Type IT __ Other ________
Sample Depth 3m ___ 1m___ Other ______________

Waterbody Name ___________________________
State _______
Station ID ________________________
Collector Name __________________
Sampling Date __ __ __ __ - __ __ - __ __
Sampling Time ___ : __ __
Sample Type IT __ Other ________
Sample Depth 3m ___ 1m___ Other ______________

*As of Oct1, 2016, this template will work with Avery label sheets #5911, 8371, 8471, and 8859

APPENDIX G

CMC Program Database Structure and Format

Field Name	Field Definition
org_id	Identifier for organization conducting the field and lab work. Drop down provides IDs used in past
contact_name	Primary contact for the organization. Drop down provides names used in past
email	Primary contacts email address. Drop down provides email addresses used in past
phone	Primary contacts phone number. Drop down provides values used in past
waterbody_id	Unique identifier for each individual waterbody. Drop down provides values used in past
waterbody_name	Name for waterbody. Drop down provides values used in past
state	State abbreviation. Drop down provides values used in past
town	Name of town. Drop down provides values used in past
station_id	Unique identifier for each individual station. May have multiple stations per waterbody. Drop down provides values used in past
station_description	Text description of the station. Drop down provides values used in past
station_type	Station type can either be offshore or nearshore. Drop down provides values used in past
station_longitude	Longitude of the station, must include at least 4 decimal points
station_latitude	Latitude of the station, must include at least 4 decimal points
station_location_source	Source for the location (e.g. GPS, google maps, etc.). Drop down provides values used in past
sample_id	Unique identifier for each sample at each station. May have multiple samples per station. Drop down provides values used in past
sample_date	Date sample was collected. Must be in YYYY-MM-DD format.
sample_time	Time sample was collected. Must be in H:MM AM/PM format.
sample_method	Method used to collect sample. Drop down provides values used in past
sample_depth_m	Depth at which sample was collected, in meters
water_temp_c	Temperature of water when sample was collected
analysis_id	Unique identifier for each cuvette (e.g. sub-sample) Possible to have multiple analyses per sample with each new analysis_id represented by a new cuvette.
analysis_date	Date analysis was conducted. Likely not the same as the sample date
dilution	Dilution used on sample. Should be a ratio. 2015 values messed up…
sample_temp_c	Temperature of water for analysis. Should be 20 C according to QAPP
chla_ugl	Concentration in ug/l of chlorophyll
phyco_ugl	Concentration in ug/l of phycocyanin
analysis_rep	Replicate of analysis. For each measurment (i.e. press of the button!) for a given cuvette increment the analysis_rep by one. For example, a single cuvette may be measured multiple times. Each of these would be a new analysis_rep.
fluorometer_type	Type of fluorometer used. Drop down provides values used in past
comments	Any comments about waterbody, station, sample, or analysis.
unique_id	A unique identifier that is a combination of waterbody ID, station ID, sample ID, analysis ID, and anlaysis rep. Should NOT be duplicated. Is generated automatically from other input IDs.