**Quality Assurance Program Plan (QAPP)**
**For the**

# CYANOBACTERIA MONITORING COLLABORATIVE PROGRAM

By the

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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)*.

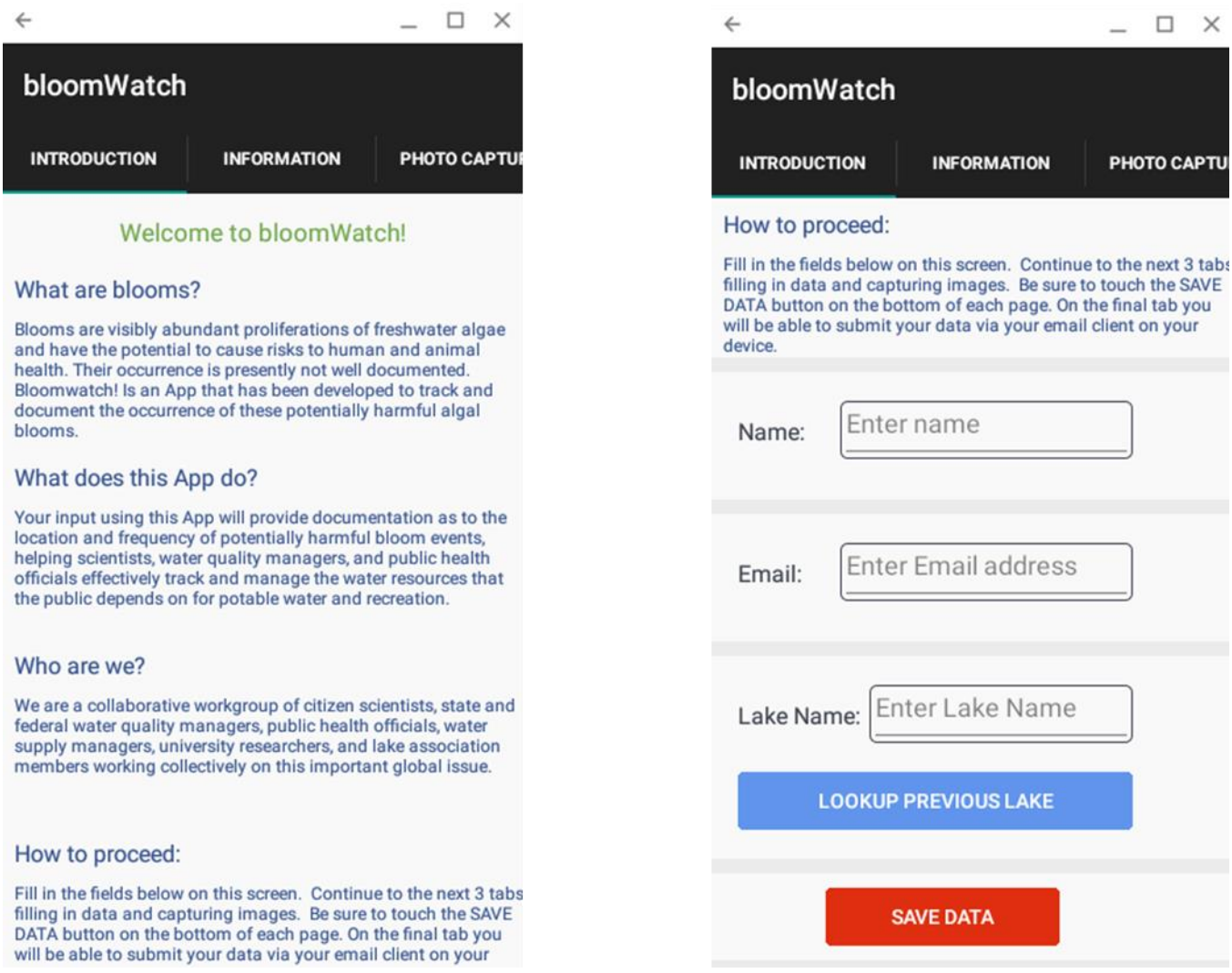



***Figure 1****: Introductory and general information page for bloomWatch. Page/screen 1 of 4.*

**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.

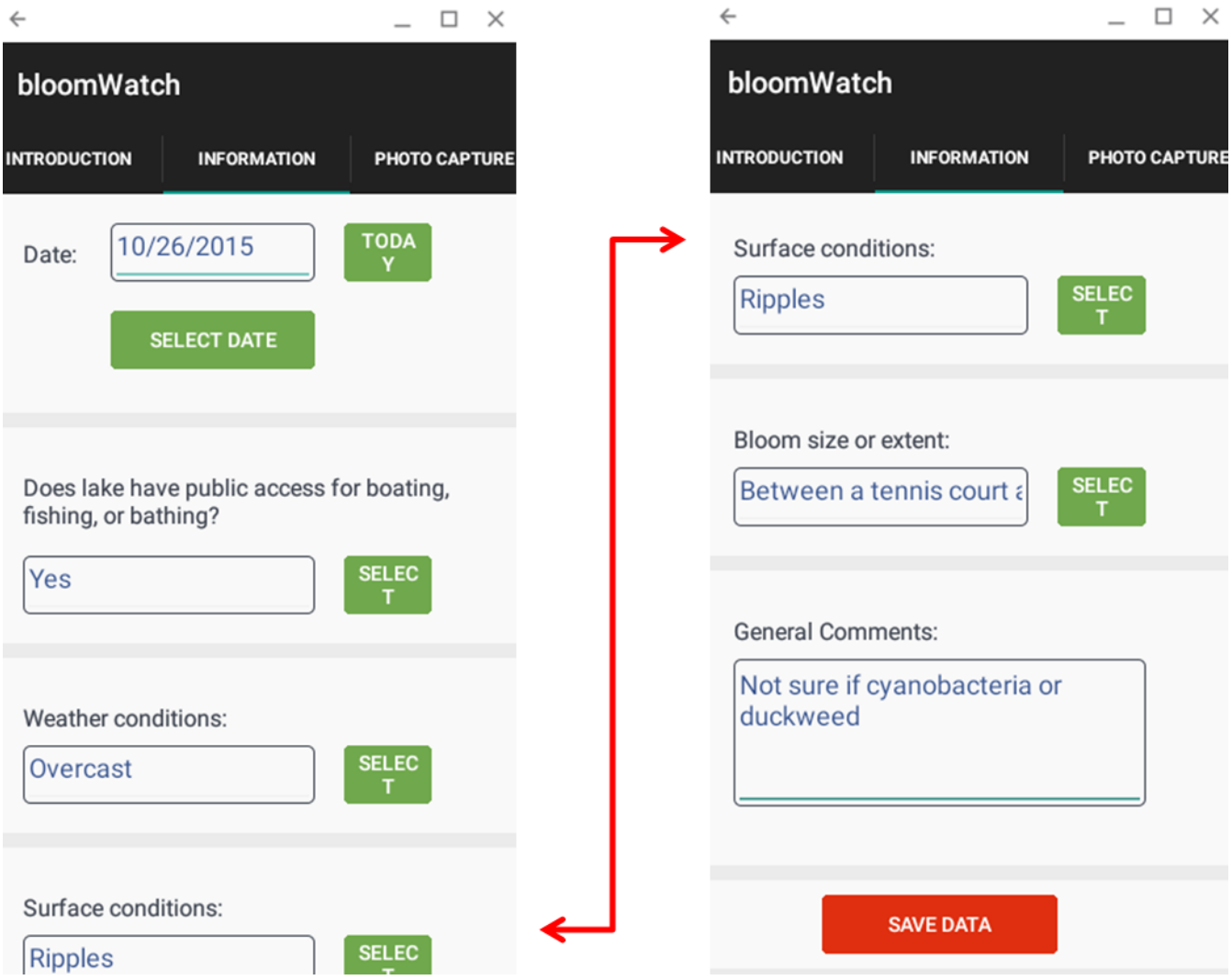



***Figure 2:*** *Lake conditions and bloom size screen. Page/screen 2 of 4.*

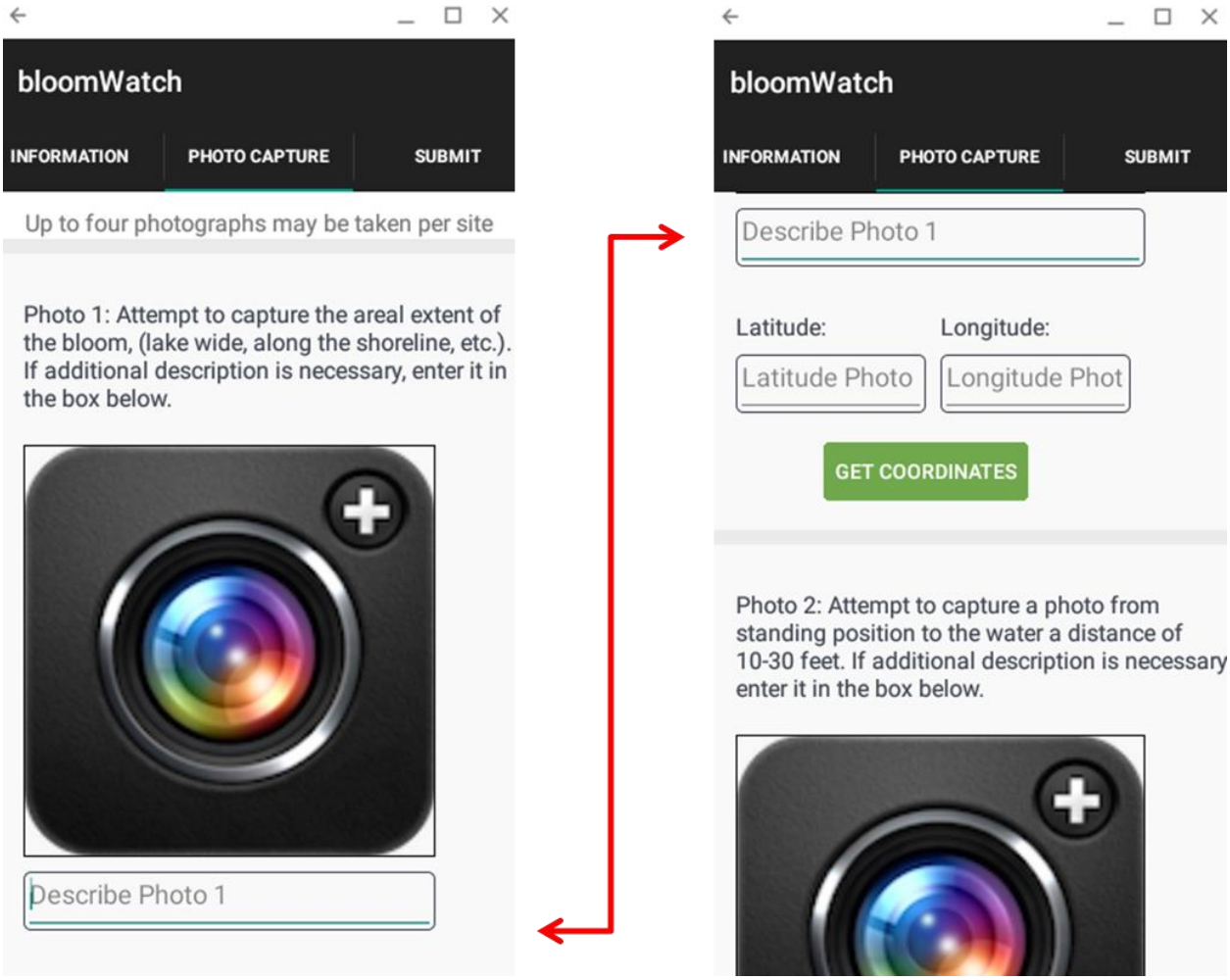



***Figure 3:*** *Image capture screen. Page/screen 3 of 4*

**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*

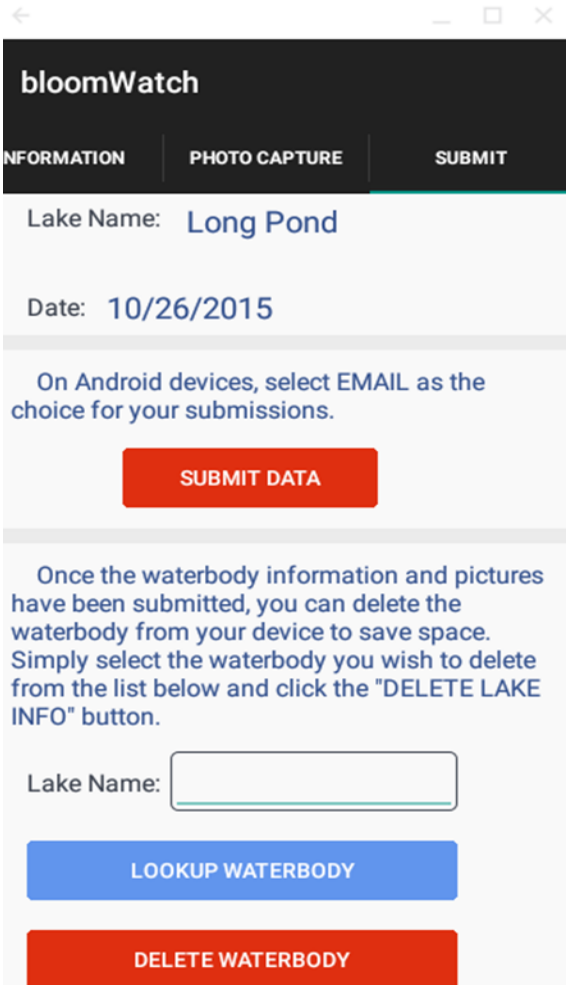



***Figure 7**: BloomWatch data submittal page. Screen 4 of 4.*

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

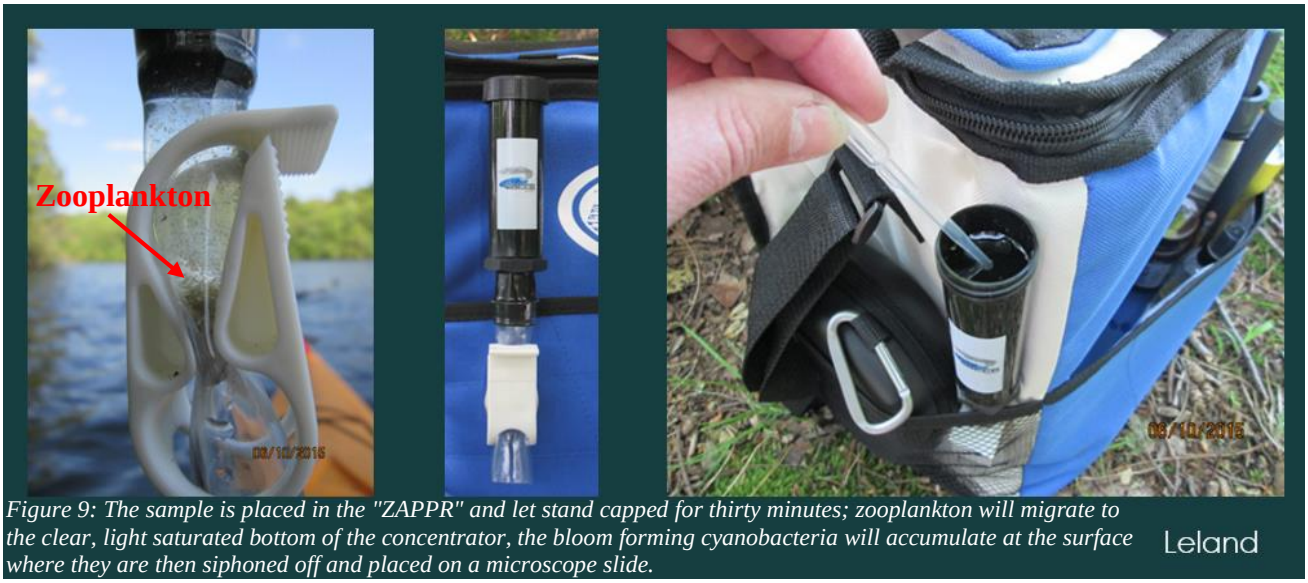



*Figure 9: The sample is placed in the "ZAPPR" and let stand capped for thirty minutes; zooplankton will migrate to the clear, light saturated bottom of the concentrator, the bloom forming cyanobacteria will accumulate at the surface where they are then siphoned off and placed on a microscope slide.*

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.

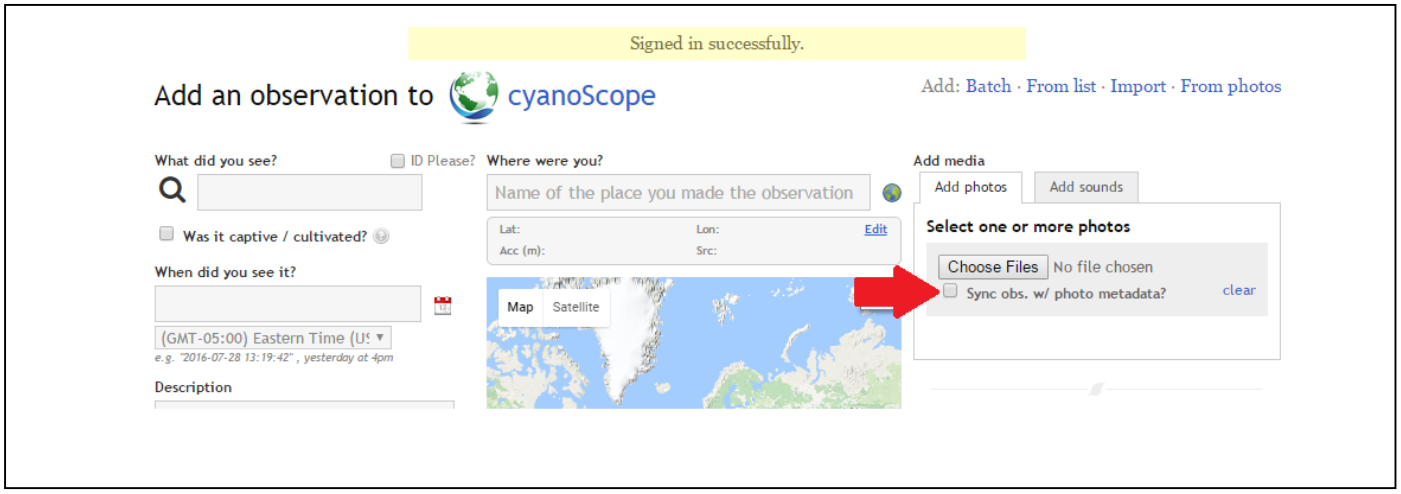
To submit a cyanobacteria observation to the cyanoScope project, navigate to http://www.inaturalist.org/projects/cyanoscope and click on the "Add Observations" button. The following instructions will guide you through adding a single observation. *A single observation is a microscope photo of a single 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.

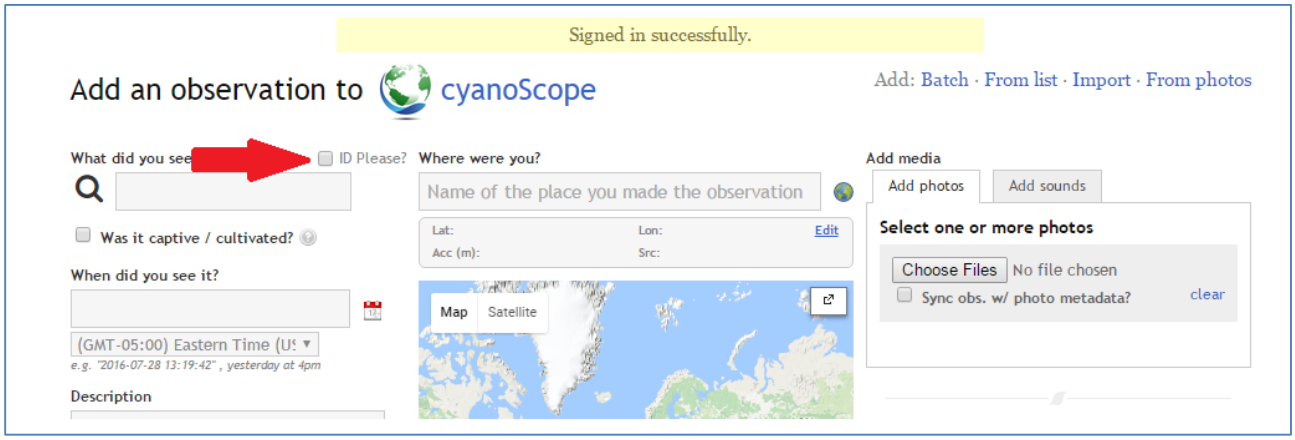
Adding location information to observations is critical for this project. This can be accomplished using several methods:

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.

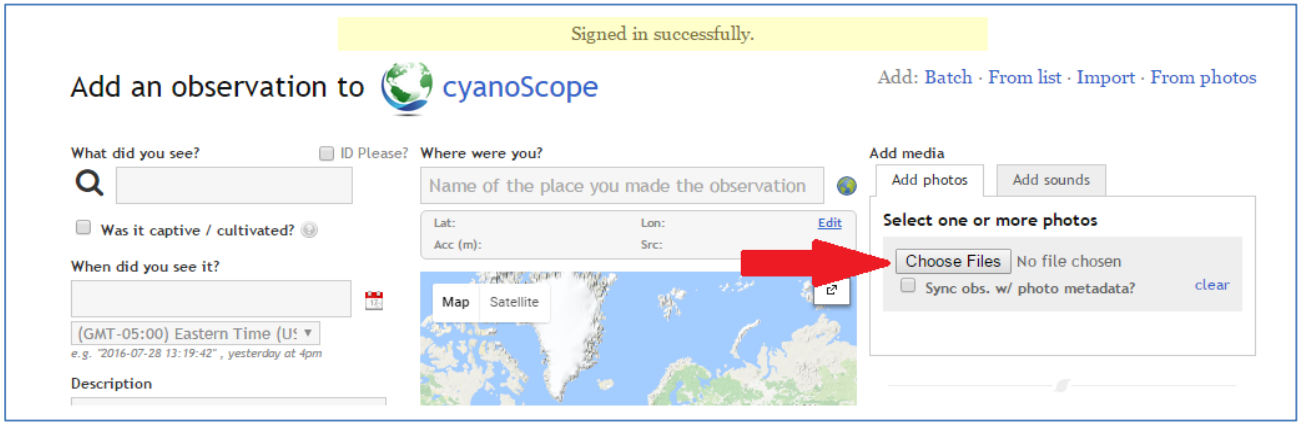



2. **Use the map interface to either navigate with a cursor or type in location name:** If you type in the name of your sampling location, be sure it is the correct location. Lake and ponds names are generally very common (i.e. there are several waterbodies named Silver Lake in New Hampshire).
3. **Type 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

# APPENDIX B

## Recreational Action Levels for Health Advisories

# APPENDIX C

## HAB Regulations and Outreach Matrix

# APPENDIX D

## Fluorometer Calibration and User Manual

# APPENDIX E

## Secondary Standards Log Sheet

# APPENDIX F

## Sample Bottle Labels Sheet

# APPENDIX G

## CMC Program Database Structure and Format