BUREAU OF WATER

South Carolina Department of Health and Environmental Control

# 2019 South Carolina Cyanotoxin Distribution Project

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Technical Report No. 001-2021

S.C. Department of Health and Environmental Control

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SCDHEC – Bureau of Water, Aquatic Science Programs

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# Executive Summary

Harmful algal blooms (HABs) are an emerging concern in the United States. In freshwater environments, HABs are generally caused by excessive growth of cyanobacteria, or blue-green algae. Cyanobacteria blooms can degrade water quality through increased water column turbidity that reduces light availability for ecologically important vegetation. Die-offs of these blooms reduce oxygen levels that can lead to fish kills. Some cyanobacteria species produce toxins (cyanotoxins) harmful to humans, livestock, and wildlife. In high enough concentrations, cyanotoxins can also cause nuisance taste and odor issues in drinking water and increase the cost of water treatment.

In 2018, the South Carolina Department of Health and Environmental Control (SCDHEC) initiated the HABs Monitoring Program to investigate the effects that cyanotoxins have on human health and the environment within the State. This assessment report covers the cyanotoxin work completed in 2019. In 2019, SCDHEC aimed to:

- Continue establishing baseline data for cyanotoxin distribution in State reservoirs following 2018,
- Detect monthly-monitoring or event-driven (sampling in response to complaints) cyanotoxin exceedances of any recommended U.S. Environmental Protection Agency (USEPA) criteria, and
- Identify potential correlative relationships between cyanotoxin concentrations and other physicochemical water quality parameters.

In 2019, samples were collected from 72 monthly-monitored sites across several South Carolina reservoirs and influent streams for two (2) cyanotoxins: microcystins and cylindrospermopsin. Microcystin samples were collected from May to October, while cylindrospermopsin samples were collected from May to July. The monthly-monitored sites were coordinated with routine sampling conducted by SCDHEC regional field staff, which allowed data comparison to other parameters collected contemporaneously. In addition, 13 event-driven samples were collected from April 2019 to October 2019.

In general, monthly-monitoring concentrations were less than 1 microgram per liter (µg/L) for both microcystins and cylindrospermopsin. Concentrations greater than the detection levels were observed in 69% of samples analyzed for microcystins and in 28% of samples analyzed for cylindrospermopsin. Toxin concentrations were less than the USEPA recommended recreational action levels of 8 µg/L for microcystins and 15 µg/L for cylindrospermopsin. One event-driven sample at Anne Springs Close Greenway, a privately owned pond, exceeded the USEPA microcystins action value of 8 µg/L. SCDHEC worked with park owners to distribute this information and advised closure of the area.

Correlation analyses were conducted for monthly-monitoring microcystins data for Cedar Creek Reservoir, Lake Hartwell, Lake Murray, and Lake Wateree. No strong relationships were determined for microcystins concentration and water quality parameters including dissolved oxygen, pH, temperature, total phosphorous, nitrogen: phosphorus ratio, and chlorophyll *a* for any of the above lakes. A weak correlation was observed in Lake Hartwell for microcystins concentration and dissolved oxygen. However, this weak correlation was based on limited data.

This assessment builds on the 2018 pilot year study and expands the baseline understanding of cyanotoxin distributions across the State. Future goals of the HABs Monitoring Program include development of a statewide cyanotoxin sampling strategy and adoption of USEPA recreation action levels into State standards.

## Introduction and Background

An increasing concern in U.S. waters are harmful algal blooms (HABs), which occur when algae colonies grow excessively and produce toxins. Increased algal growth and population density are usually caused by an increase in nutrients in a water body, typically from nonpoint source runoff from a variety of land-uses. Cyanobacteria, or blue-green algae, are often found in these nutrient-rich waters and can release toxins (known as cyanotoxins) into their aquatic environment. Cyanotoxins in high enough concentrations, or through bioaccumulation, can impact aquatic life and human health. There is growing recognition of the need for increased monitoring of cyanotoxin concentrations in waterbodies and water treatment plants (Jetto, Grover, & Krantxberg, 2015). The U.S. Environmental Protection Agency (USEPA) has formulated health advisory criteria (U.S. Environmental Protection Agency, 2019) and recreational advisory criteria (U.S. Environmental Protection Agency, 2015b,c) for two (2) cyanotoxins (microcystins and cylindrospermopsin). Exposure to high levels of microcystins can lead to liver, reproductive, developmental, kidney, and gastrointestinal effects (U.S. Environmental Protection Agency, 2019). Exposure to high levels of cylindrospermopsin can affect the liver, kidneys, and have potential effects to red blood cells (U.S. Environmental Protection Agency, 2019).

The South Carolina Department of Health and Environmental Control (SCDHEC) has maintained a robust surface water monitoring network since the 1950s. However, cyanotoxins have not been routinely monitored due to analytical limitations. Analytical methods for cyanotoxins have improved greatly, and in 2018 SCDHEC established the HABs Monitoring Program to monitor cyanotoxins statewide. A primary objective of the HABs Monitoring Program is to establish a baseline for cyanotoxins in South Carolina's waters. This baseline will provide valuable insight into the spatial and seasonal distribution of cyanotoxins which will allow for an improved assessment of environmental threats and management options associated with these toxins.

## Purpose of Assessment

The purpose of this assessment was to examine cyanotoxin distributions in South Carolina reservoirs and influent streams and rivers, and to identify potential hazards to drinking water facilities. Cyanotoxin concentrations were also compared to USEPA health advisories (Table 1 and 2) to determine potential risks for recreational and aquatic life uses for waterbodies of the State. The data were used to identify potential reservoirs of concern and will guide future assessment activities.

**Table 1**: USEPA 10-day health advisory values for microcystins and cylindrospermopsin in drinking water.

| Cyanotoxin | USEPA 10-day Drinking Water Health Advisory [a, b] | |
|---|---|---|
| | Bottle Fed Infants and pre-school children (µg/L) | School age children and adults (µg/L) |
| Microcystins | 0.3 | 1.6 |
| Cylindrospermopsin | 0.7 | 3.0 |

a. U.S. Environmental Protection Agency, 2015b, c
b. µg/L = micrograms per liter (parts per billion)

**Table 2**: USEPA recreational water quality and swimming advisory criteria for microcystins and cylindrospermopsin. Recreational water activities, such as rowing, fishing, boating, etc., have a lower chance of water ingestion than swimming; thus, swimming has a shorter duration and frequency criteria than recreational water activities.

| Use | USEPA Criteria | | Duration | Frequency |
|---|---|---|---|---|
| | Microcystins Concentration (μg/L) [a, b] | Cylindrospermopsin Concentration (μg/L) [a, b] | | |
| Recreational Water Quality | 8 | 15 | One in 10-day assessment period across a recreational season | Not more than three excursions in a recreational season in more than one year |
| Swimming | 8 | 15 | One day | Not to be exceeded |

a. U. S. Environmental Protection Agency, 2019
b. μg/L = micrograms per liter (parts per billion)

Note: The recommended USEPA criteria for recreational waters protection shown in Table 2 were adopted as enforceable State water quality standards in 2020.

# Methods

SCDHEC Bureau of Water (BOW) Aquatic Science Programs (ASP) collected cyanotoxin samples from May 2019 to October 2019 for microcystins. Cylindrospermopsin samples were collected from May 2019 to July 2019. Two (2) types of sampling were conducted as part of the 2019 study: monthly-monitoring at various waterbodies and sampling in response to complaints (event-driven), such as visually observed algal blooms and fish kills. A total of 25 freshwater bodies were regularly sampled during the monthly-monitoring component and 13 samples were collected due to event-driven responses.

## Monthly-Monitoring

Seventy-two (72) sites were sampled monthly from May 2019 to October 2019 (Table 3 and Figure 1). These sites were selected from the 2019 list of Ambient Water Quality Monitoring Program sites. (SCDHEC, 2019). The 2019 Ambient Water Quality Monitoring Program collected monthly samples from a total of 244 Base Sites for water quality parameters including temperature, chlorophyll *a*, nutrients, metals, etc. Therefore, utilizing sites from the Ambient Water Quality Monitoring Program provided an opportunity to compare cyanotoxin results to other water quality parameters.

A total of 551 samples were analyzed for microcystins or cylindrospermopsin. Sample collection, field analysis, handling, preservation, and Chain of Custody (COC) followed SCDHEC Determination of Total Microcystins and Cylindrospermopsin in Ambient Water Standard Operating Procedure (SOP) (Appendix 1) and the 2019 HAB Quality Assurance Project Plan (Appendix 2). The field manager oversaw the

transportation of the samples and the COCs to the SCDHEC ASP laboratory. Samples were frozen at –20°C for a holding time not to exceed two (2) weeks.

Samples were analyzed for microcystins and cylindrospermopsin using the Enzyme Linked Immunosorbent Assay (ELISA) technique described in SCDHEC Determination of Total Microcystins and Cylindrospermopsin in Ambient Water SOP, Appendix 1. The analysis is based on USEPA method 546 (U.S. Environmental Protection Agency, 2015a) with guidance from the assay provider, Abraxis.

**Table 3**: Sampling site locations.

| Site | Regional Lab | Description | Latitude | Longitude |
|---|---|---|---|---|
| B-327 | Greenville | Monticello Lake | 34.3297 | -81.3026 |
| B-339 | Greenville | Lake Bowen | 35.1128 | -82.0455 |
| B-345 | Midlands | Parr Reservoir | 34.2621 | -81.3354 |
| CL-019 | Greenville | Lake Jocassee | 34.9599 | -82.9236 |
| Cl-041 | Greenville | J. Strom Thurmond | 33.6699 | -82.2076 |
| Cl-089 | Midlands | Lake Wateree | 34.3368 | -80.7049 |
| CW-016F | Lancaster | Fishing Creek Reservoir | 34.6777 | -80.8772 |
| CW-033 | Midlands | Cedar Creek Reservoir | 34.5426 | -80.8777 |
| CW-057 | Lancaster | Fishing Creek Reservoir | 34.6053 | -80.8910 |
| CW-174 | Midlands | Cedar Creek Reservoir | 34.5581 | -80.8917 |
| CW-197 | Midlands | Lake Wylie | 35.1376 | -81.0594 |
| CW-201 | Midlands | Lake Wylie | 35.0281 | -81.0477 |
| CW-207 | Midlands | Lake Wateree | 34.4025 | -80.7884 |
| CW-207B | ASP | Lake Wateree | 34.4039 | -80.7827 |
| CW-208 | ASP | Lake Wateree | 34.4219 | -80.8674 |
| CW-230 | Midlands | Lake Wylie | 35.0225 | -81.0087 |
| CW-231 | Midlands | Lake Wateree | 34.5365 | -80.8749 |
| LCR-01 | ASP | Fishing Creek Reservoir | 34.6591 | -80.8855 |
| LCR-02 | ASP | Lake Wateree | 34.4858 | -80.8998 |
| LCR-03 | ASP | Lake Wateree | 34.4254 | -80.8439 |
| LCR-04 | ASP | Fishing Creek Reservoir | 34.6204 | -80.8862 |
| LCR-05 | ASP | Fishing Creek Reservoir | 34.6274 | -80.8817 |
| PD-327 | Florence | Lake Robinson | 34.4675 | -80.1698 |
| RL-13081 | Midlands | Parr Reservoir | 34.2684 | -81.3376 |
| RL-19149 | Lancaster | Cedar Creek | 34.5578 | -80.8694 |
| RL-19150 | Lancaster | Lake Whelchel | 35.1079 | -81.6297 |
| RL-19154 | Midlands | Lake Murray | 34.0695 | -81.6186 |
| RL-19155 | Greenville | Lake Jocassee | 35.0391 | -82.9334 |
| RL-19158 | Greenville | Lake Murray | 34.4817 | -80.0084 |
| RL-19159 | Greenville | Lake Keowee | 34.8181 | -82.8876 |
| RL-19165 | Greenville | Lake Secession | 34.2704 | -82.6046 |
| RL-19166 | Midlands | Lake Wateree | 34.4365 | -80.8869 |
| RL-19167 | Greenville | Lake Keowee | 34.7181 | -82.9670 |
| RL-19170 | Midlands | Lake Monticello | 34.3076 | -81.2935 |
| R-19174 | Midlands | Lake Murray | 34.0921 | -81.3441 |
| RL-19177 | Greenville | Lake Russell | 34.0967 | -82.6331 |

| Site | Regional Lab | Description | Latitude | Longitude |
|---|---|---|---|---|
| RL-19178 | Lancaster | Lake Wylie | 35.1116 | -81.0867 |
| RL-19179 | Greenville | Lake Hartwell | 34.5170 | -82.8089 |
| RL-19251 | Midlands | Lake Yonah | 34.6919 | -83.3418 |
| RL-19253 | Greenville | Lake Blalock | 35.0988 | -81.8981 |
| RL-19254 | Lancaster | Cedar Creek Reservoir | 34.5604 | -80.8708 |
| RL-19255 | Greenville | Lake Tugaloo | 34.7295 | -83.3528 |
| RL-19256 | Lancaster | Lake Eureka | 34.6391 | -79.8953 |
| RL-19257 | Greenville | Lake Blalock | 35.0954 | -81.8808 |
| RL-19258 | Lancaster | Great Falls Reservoir | 34.5863 | -80.8928 |
| RL-19259 | Charleston | Goose Creek Reservoir | 32.9722 | -80.0362 |
| S-022 | Greenville | Lake Greenwood | 34.3278 | -82.0849 |
| S-024 | Greenville | Lake Greenwood | 34.3079 | -82.1101 |
| S-131 | Greenville | Lake Greenwood | 34.2791 | -82.0587 |
| S-211 | Midlands | Lake Murray | 34.0984 | -81.4765 |
| S-213 | Midlands | Lake Murray | 34.1251 | -81.4337 |
| S-222 | Midlands | Lake Murray | 34.0802 | -81.5625 |
| S-308 | Midlands | Lake Greenwood | 34.3467 | -82.1088 |
| S-309 | Midlands | Lake Murray | 34.1315 | -81.6048 |
| S-310 | Midlands | Lake Murray | 34.1151 | -81.5999 |
| S-311 | Greenville | Boyd Mill Pond | 34.4547 | -82.2019 |
| SV-098 | Greenville | Lake Russell | 34.0704 | -82.6429 |
| SV-200 | Greenville | Lake Hartwell | 34.6117 | -83.2262 |
| SV-236 | Greenville | Lake Hartwell | 34.5954 | -82.9078 |
| SV-268 | Greenville | Lake Hartwell | 34.5972 | -82.8218 |
| SV-331 | Greenville | Lake Secession | 34.3319 | -82.5758 |
| SV-335 | Greenville | Lake Jocassee | 35.0320 | -82.9151 |
| SV-336 | Greenville | Lake Jocassee | 34.9959 | -82.9793 |
| SV-338 | Greenville | Lake Keowee | 34.8269 | -82.8977 |
| SV-339 | Greenville | Lake Hartwell | 34.5112 | -82.8098 |
| SV-340 | Greenville | Lake Hartwell | 34.4032 | -82.8391 |
| SV-357 | Greenville | Lake Russell | 34.1920 | -82.6309 |
| SV-361 | Greenville | Lake Keowee | 34.7339 | -82.9183 |
| SV-363 | Greenville | Lake Hartwell | 34.4800 | -82.9454 |
| SV-372 | Greenville | Stephens Creek Reservoir | 33.5928 | -82.1233 |
| SV-374 | Greenville | Lake Hartwell | 34.5721 | -82.8299 |

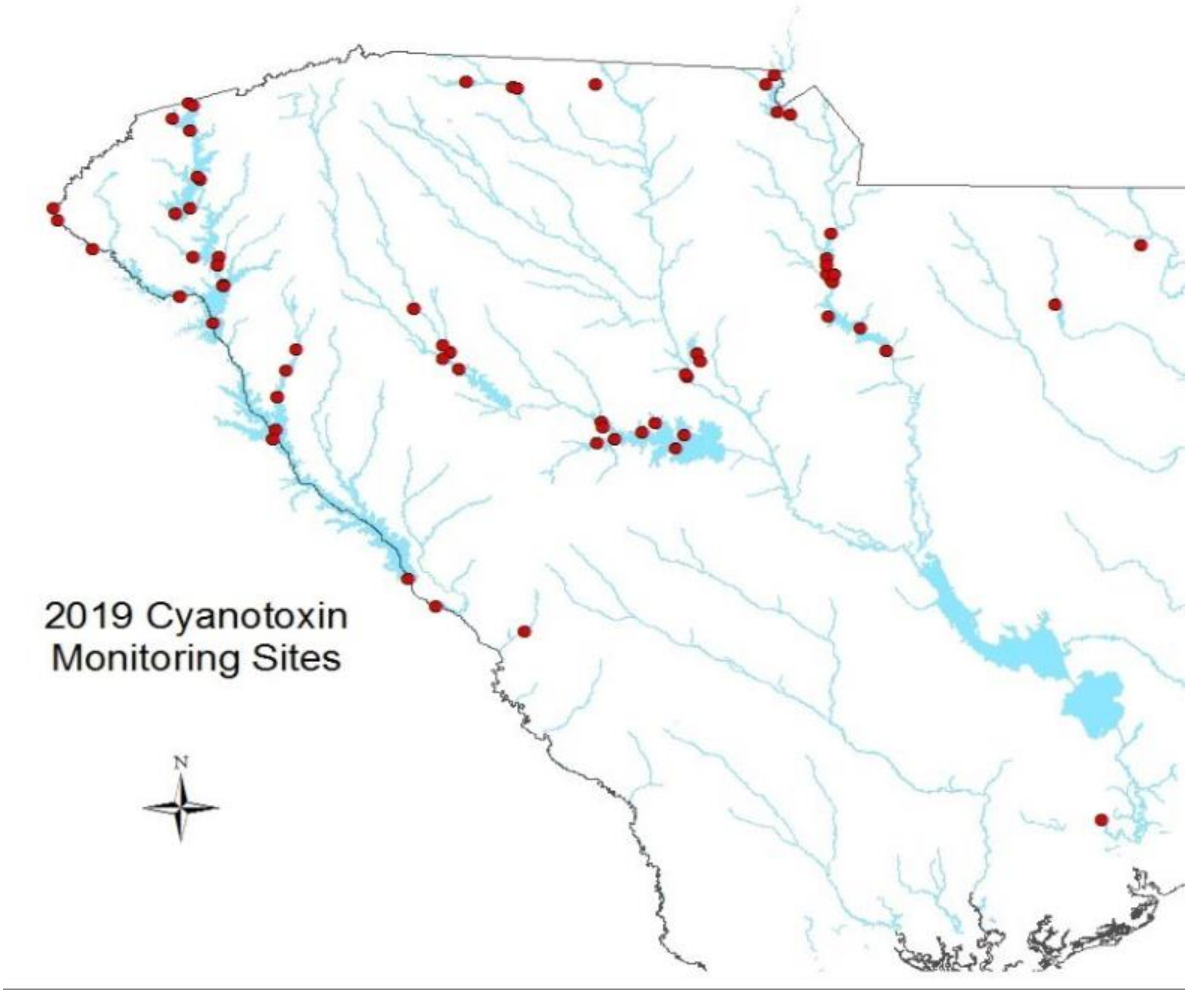


**Figure 1**: Monthly-Monitoring sampling site locations.

## Event-Driven Samples

Thirteen (13) samples were collected in response to complaints reporting algal blooms, fish kills, and/or taste and odor issues during the HABs 2019 sampling season. Grab samples and phytoplankton tow nets were collected after a complaint was received. Samples were observed under the microscope for algal identification at the SCDHEC ASP laboratory and analyzed for microcystins and/or cylindrospermopsin if the species identified was a potential toxin producing species.

Sample collection, field analysis, handling, preservation, and Chain of Custody (COC) followed SCDHEC Determination of Total Microcystins and Cylindrospermopsin in Ambient Water Standard Operating Procedure (SOP) (Appendix 1) and the 2019 HAB Quality Assurance Project Plan (Appendix 2). The field manager oversaw the transportation of the samples and the COCs to the SCDHEC ASP laboratory. Samples were frozen at –20$^{o}$C for a holding time not to exceed two (2) weeks.

Samples identified with cyanobacteria were analyzed for microcystins and cylindrospermopsin using the Enzyme Linked Immunosorbent Assay (ELISA) technique described in SCDHEC Determination of Total Microcystins and Cylindrospermopsin in Ambient Water SOP, Appendix 1. The analysis is based on USEPA method 546 (U.S. Environmental Protection Agency, 2015a) with guidance from the assay provider, Abraxis.

## Quality Assurance/ Quality Control

347 of the 373 samples analyzed for microcystins and 169 of the 178 samples analyzed for cylindrospermopsin in 2019 passed the quality control requirements.

SCDHEC also participated in the Abraxis Cyanotoxins Proficiency Testing Program for recreational water as a check on the accuracy of our routine sample analysis. Performance was evaluated by calculating a z-score metric based on the analysis results of four (4) surface water standards fortified with purified Microcystin-LR, Microcystin-RR, Microcystin- YR, and/or nodularins (toxins produced by *Nodularia spumigena*, a cyanobacterium). The z-score metric is as follows:

$$z = \frac{(x - X)}{\sigma}$$

Where:

$z$= the z score (Standard score)

$x$= the reported value of analyte

$X$= the assigned value, the best estimate of the *true* concentration

$\sigma$= the estimate of variation (proficiency standard deviation)

The following interpretations for z-scores in proficiency testing schemes are recommended:

| Results Obtained | Rating |
|---|---|
| z ≤ 2 | Satisfactory |
| 2 < z < 3 | Questionable |
| z ≥ 3 | Unsatisfactory |

The results for SCDHEC's proficiency testing for each of the four (4) samples are listed in the table below.

| Sample Number | Result (µg/L) [a] | Z-Score | Evaluation |
|---|---|---|---|
| 1 | 9.99 | 0.053 | Satisfactory |
| 2 | 1.80 | 0.768 | Satisfactory |
| 3 | 8.93 | 0.079 | Satisfactory |
| 4 | 0.20 | N/A[b] | Questionable |

a. µg/L = micrograms per liter (parts per billion)
b. Z-score is not calculated when the sample is a blank (no microcystins present)

## Statistical Analyses

Pearson correlation coefficients were calculated to determine if there were linear relationships between microcystin concentrations versus pH, dissolved oxygen (mg/L), temperature (°C), total phosphorous (mg/L), N:P ratio, and chlorophyll *a* (µg/L) in lakes that meet sample size requirements (see below). Only detectable data (toxin concentration values greater than or equal to the method detection limit) were used for analyses. Cylindrospermopsin was not sampled during the entire growing season (May through October); therefore, cylindrospermopsin concentrations were not analyzed. Microcystin data were considered detectable when result(s) were ≥ 0.100 ug/L.

There were 25 lakes sampled from the 72 sites selected for monthly monitoring in 2019. These lakes spanned across the State of South Carolina and had various waters feeding into and out of the lakes. Thus, it was determined to analyze lakes individually rather than combining samples across water bodies due to diversity in water dynamics between lakes. The lake analysis selection was based off a minimum sample size of three detectable samples per month over the course of six months: thus, equating to a minimum of eighteen samples total. There were four water bodies that met the sample size criteria for microcystins: Cedar Creek Reservoir, Lake Hartwell, Lake Murray, and Lake Wateree.

Pearson correlation matrix output values range from -1 to 1, where values closer to -1 indicate a strong inverse relationship and values closer to 1 indicate a strong positive relationship. Matrix values that are closer to zero indicates no linear relationship. All data analyses were made using Microsoft Excel.

# Results

## Monthly-Monitoring

From May 2019 through October 2019, a total of 373 samples were collected for microcystins and from May 2019 through July 2019, a total of 178 samples were collected for cylindrospermopsin.

### Microcystins

Of the 347 samples meeting QA/QC guidelines for microcystins, 69% were greater than or equal to the method detection limit of ≥ 0.100 µg/L. All microcystins concentrations were less than both 1 µg/L and the USEPA recreational action level of 8 µg/L. The maximum concentration observed was 0.745 µg/L at station RL-19150 on Lake Whelchel in October.

All 25 lakes had at least one sample with detectable amounts of microcystins. Twenty-four of the 25 lakes had more than one (1) sample with detectable amounts of microcystins (Figure 2). Lake Whelchel had the highest average microcystins concentration ($\bar{x}$=0.422 µg/L, SE=0.110); Great Falls Reservoir had the lowest average microcystins concentration ($\bar{x}$=0.123 µg/L, SE=0.010). Refer to Appendix 3 to see the microcystin concentrations of individual sites analyzed each month, organized based on lake location.

Microcystins did not strongly correlate with dissolved oxygen, pH, temperature, total phosphorous, N:P ratio, or chlorophyll *a* in Cedar Creek Reservoir, Lake Hartwell, Lake Murray, and Lake Wateree with coefficients ranging from -0.44 to 0.46. (Table 4). A weak correlation was observed for microcystins and dissolved oxygen in Lake Hartwell (0.63).

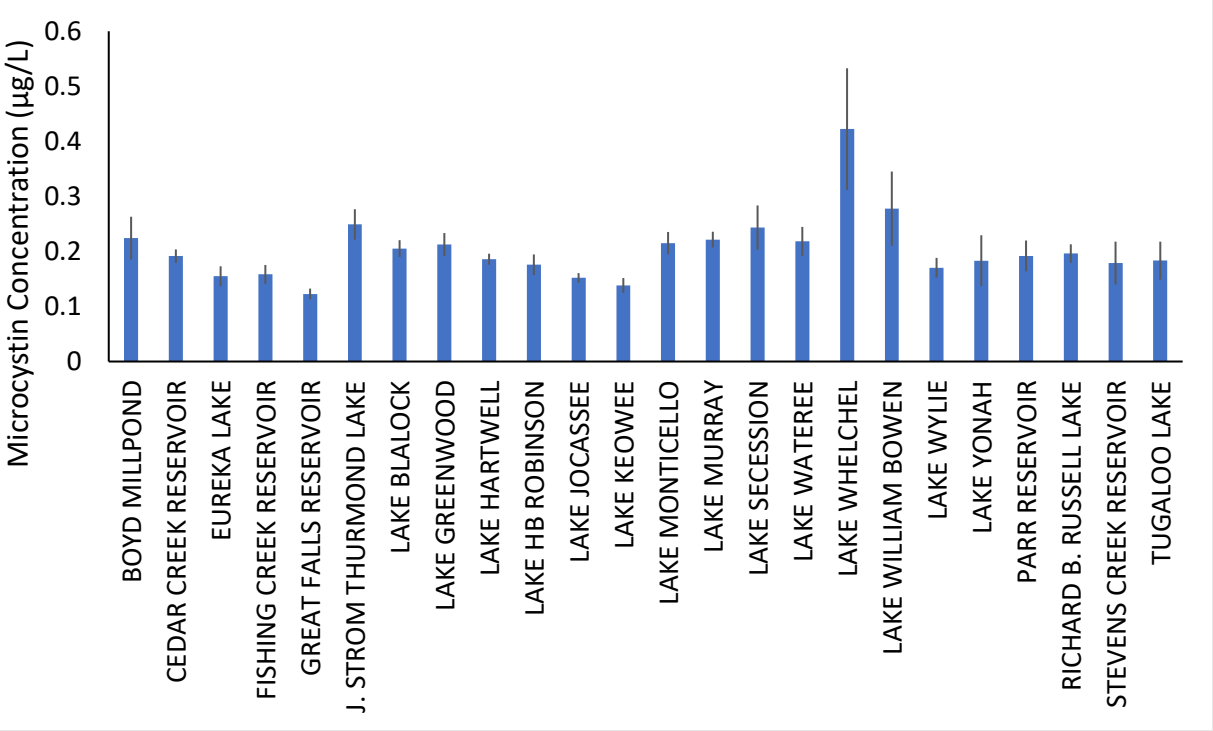


**Figure 2**: Average detectable microcystin concentration (µg/L) per lake in 2019. There were 24 lakes that had more than one sample with quantifiable concentrations. The error bars represent +/- one (1) standard error.

**Table 4**: Pearson correlation coefficient results comparing microcystin concentration (µg/L) in Cedar Creek Reservoir, Lake Hartwell, Lake Murray, and Lake Wateree to dissolved oxygen (mg/L), pH, temperature (°C), total phosphorous (mg/L), N:P ratio, and chlorophyll *a* (µg/L).

| **Water Body** | **Microcystin Concentration Correlation for Respective Water Quality Parameters** | | | | | |
|---|---|---|---|---|---|---|
| | Dissolved Oxygen | pH | Temperature | Total Phosphorous | N:P | Chlorophyll *a* |
| Cedar Creek Reservoir | -0.25 | 0.10 | 0.46 | -0.24 | 0.19 | -0.21 |
| Lake Hartwell | 0.63 | 0.17 | 0.20 | -0.40 | -0.44 | 0.38 |
| Lake Murray | -0.17 | 0.00 | -0.15 | 0.46 | -0.04 | 0.24 |
| Lake Wateree | 0.15 | 0.20 | -0.01 | -0.27 | -0.13 | 0.17 |

## Cylindrospermopsin

Of the 169 samples meeting QA/QC guidelines for cylindrospermopsin, 28% of them were greater than the method detection limit of ≥0.040 µg/L. All cylindrospermopsin concentrations were less than both 1 µg/L and the USEPA recreational action level of 15 µg/L. The maximum concentration observed was 0.107 µg/L at station CW-057 in Fishing Creek Reservoir in July.

Twenty-two (22) of the 25 lakes sampled had at least one (1) sample with detectable amounts of cylindrospermopsin. Nine (9) water bodies had more than one (1) sample with detectable amounts of

cylindrospermopsin (Figure 3). Lake Keowee had the highest average cylindrospermospin concentration ($\bar{x}$=0.078 µg/L, SE=0.007); Lake Wateree had the lowest average cylindrospermopsin concentration ($\bar{x}$=0.027 µg/L, SE=0.006).

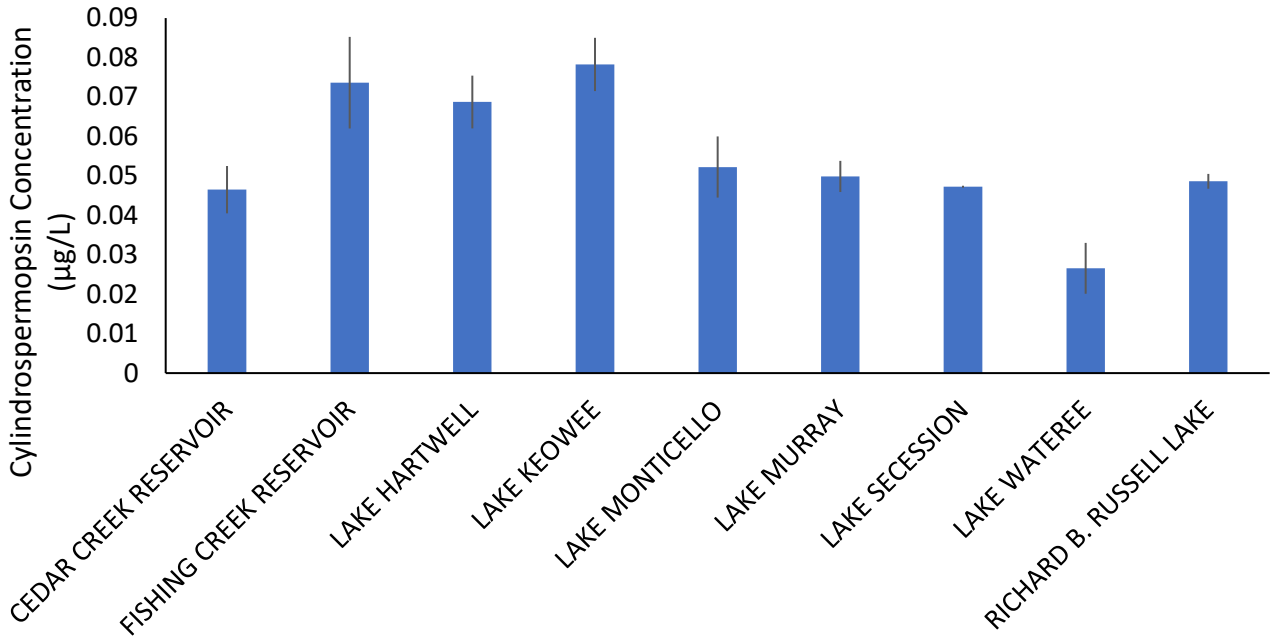


**Figure 3**: Average detectable cylindrospermopsin concentration (µg/L) per lake in 2019. There were nine (9) lakes that had more than one sample with detectable concentrations. The error bars represent +/- one (1) standard error.

Summary of Monthly-Monitoring Findings

Within the limiting context of the chemical parametric coverages selected; the number of samples collected; and, the time period of sample collection, the cyanotoxin data demonstrated:

- 69% of the 347 samples analyzed for microcystins were detectable (≥ 0.100 µg/L).
- All microcystins samples were less than the USEPA recommended recreational action level of 8 µg/L.
- There were no strong correlations between microcystin concentration and dissolved oxygen, pH, temperature, total phosphorous, N:P ratio, and chlorophyll *a* in Cedar Creek Reservoir, Lake Hartwell, Lake Murray, or Lake Wateree. There was a weak correlation (0.63) in Lake Hartwell between microcystin concentration and dissolved oxygen.
- 28% of the 169 samples analyzed for cylindrospermopsin were detectable (≥0.040 µg/L).
- All cylindrospermopsin samples were less than the USEPA recommended recreational action level of 15 µg/L.

## Event-Driven Samples

Throughout the 2019 season, the SCDHEC BOW ASP section received complaints on 13 potential HABs throughout the State. Nine (9) of the thirteen (13) samples had detectable levels of cyanotoxins (Table 5). The greatest concentration of microcystins (>40 µg/L) was at Anne Springs Close Greenway in response to a *Microcystis sp.* bloom in their dog park pond, which was greater than the USEPA recommended recreational value of 8 µg/L. Anne Springs Close Greenway also had another pond, their Dairy Pond, with

microcystins concentrations greater than the USEPA recommended recreational values (>8 µg/L); however, this pond was inaccessible to the public.

**Table 5**: Description and cyanotoxin (microcystins and cylindrospermopsin) results from 2019 algal bloom complaints with the associated date of the HAB. Microscopic images of cyanobacteria for four (4) of the designated blooms can be found in Appendix 4.

| Sample Location | Sample Description | Collection Date | Microcystin (µg/L) [a] | Cylindrospermopsin (µg/L) [a] |
|---|---|---|---|---|
| Goose Creek Reservoir | *Dolichospermum sp.* bloom[d] | 04/16/2019 | 0.167 | BDL |
| Lake Wateree | *Lyngbya wollei* bloom by Lugoff-Elgin drinking water intake | 06/7/2019 | 4.45 | BDL |
| Lake Rabon | Taste and odor issues during a *Dolichospermum sp.* bloom[d] | 08/2019 | 0.256 | N/A |
| Lake Hartwell | Citizen complaint of green algae at northern portion of the lake | 08/23/2019 | 0.154 | N/A |
| Coopers | Fish kill in Chapin | 8/16/2019 | BDL | N/A |
| Berry Shoals Pond | Citizen complaint- no algal bloom was present at time of sampling | 9/18/2019 | BDL | N/A |
| Anne Springs Close Greenway (Dog Park Pond) | *Microcystis sp.* bloom[d] | 09/19/19 | > 40 | N/A |
| Anne Springs Close Greenway (Haigler Pond) | *Microcystis sp.* bloom | 9/23/2019 | 0.455 | N/A |
| Anne Springs Close Greenway (Dairy Pond) | *Microcystis sp.* bloom | 9/23/2019 | > 8 | N/A |
| West Columbia drinking water intake | Lake Murray taste and odor issue | 09/2019 | BDL | N/A |
| Columbia drinking water intake | Lake Murray taste and odor issue | 09/2019 | BDL | N/A |
| Lake Wateree | *Trichormus sp.* bloom[d] | 09/23/2019 | 0.720 | N/A |
| Lake Greenwood | Desmid (green algae) bloom complaint | 10/04/2019 | 0.781 | N/A |

a. µg/L = micrograms per liter (parts per billion)
b. BDL = Below Detection Limits
c. N/A= Not Applicable
d. Microscope image of the associated cyanobacteria can be found in Appendix 4

Summary of Event-Driven Sample Findings

Within the limiting context of the chemical parametric coverages selected; the number of samples collected; and, the time period of sample collection, the cyanotoxin data demonstrated:

- Nine (9) of the thirteen (13) HAB complaint samples detected microcystins (≥0.100 µg/L).
- The two (2) HAB complaint samples analyzed for cylindrospermopsin were less than detection limits (≥0.040 µg/L).
- Two (2) of the HAB complaint samples were greater than the USEPA recreational guideline of 8 µg/L for microcystins. Both samples were at Anne Springs Close Greenway: one at their dog park (>40 µg/L), and the other at their Dairy Pond (>8 µg/L), a pond not accessible to the public.

## Discussion and Conclusions

One (1) of the main goals of the HAB Monitoring Program is to establish cyanotoxin spatial distribution data in South Carolina waterbodies. These 2019 results have (a) contributed to starting a cyanotoxin concentration baseline for South Carolina waterbodies and (b) provided insight towards cyanotoxin presence/absence expectations. Data from the first two years of the HABs Monitoring Program has shown higher cyanotoxin concentrations on average in 2019 than reported in 2018 (SCDHEC, 2020), which may be due to sampling the entire growing season in 2019. The data in Figure 2 and Appendix 3 can assist in depicting which South Carolina lakes contained detectable amounts of microcystins. The cyanotoxin data can also be referenced when examining drinking water intake areas that could be impacted by future HABs. For instance, Lake Whelchel is an important lake to monitor toxins and HABs in future algal blooming seasons because it has produced the greatest monitored microcystin concentrations in 2018 and 2019 and serves as the primary drinking source for the town of Gaffney.

Overall, the 2019 monthly monitoring results for microcystins and cylindrospermopsin showed toxin concentrations less than 1 µg/L, which was well less than the USEPA recreational action standards throughout South Carolina lakes. These low toxin levels, if maintained going forward, suggest that recreational activities in South Carolina are not an immediate concern. The monthly-monitoring sampling sites are fixed open-water locations, whereas cyanobacteria blooms tend to occur in shallow coves or along shorelines. As such, this program component may not capture localized elevated cyanotoxin concentrations in the nearshore environment.

The event-driven sampling is a more targeted component of the HAB Program, which provides insight into potential cyanotoxin HABs in nearshore environments. The 2019 event-driven samples analyzed from algal blooms showed higher concentrations of microcystins than from the event-driven samples in 2018. Anne Springs Close Greenway, a privately owned recreational park, contained the only events with microcystin concentrations greater than the USEPA recommended recreational action value of 8 µg/L. An advisory was not issued at the time since the ponds were privately owned and SCDHEC had not yet adopted the USEPA recreational guidelines for cyanotoxins in the 2019 State standards; however, SCDHEC BOW ASP worked with Anne Springs Close Greenway Park owners during this *Microcystis sp*. bloom on ways to distribute this information. It was advised that the areas of concern should close and/or signs posted around the park to notify the public. SCDHEC BOW ASP also suggested posting the information on social media pages for Anne Springs Close Greenway. SCDHEC did adopt the USEPA recreational guidelines for cyanotoxins in 2020 during the HAB Monitoring Program's third year. Adopting these standards in

2020 allowed SCDHEC the capability to issue advisories on waterbodies of the State and to expand HAB educational guidance and assistance in South Carolina.

The monthly-monitoring correlation results comparing microcystins to dissolved oxygen, pH, temperature, total phosphorus, N:P ratio, and chlorophyll *a* for Cedar Creek Reservoir, Lake Hartwell, Lake Murray, and Lake Wateree produced no strong relationships between the microcystin concentration and any of the above parameters. The presence of microcystins may be present due to the periodic occurrence of toxin producing cyanobacteria that are not captured by obvious changes in the associated physical and chemical data. Several studies have shown that a combination of environmental factors may influence cyanotoxin production (Davis, Berry, Boyer, & Gobler, 2009) (Paerl & Otten, 2012) (Wiltsie, Schnetzer, Green, Vander Borgh, & Fensin, 2018). Thus, it would be beneficial in future analyses to examine the combination of several factors in relation to cyanotoxin concentration rather than linear correlations. There was a weak positive correlation in Lake Hartwell between microcystin concentration and dissolved oxygen. However, the other three lakes did not show any similar trends, and a North Carolina study did not see a correlation between microcystin concentration and dissolved oxygen (Wiltsie, Schnetzer, Green, Vander Borgh, & Fensin, 2018). The samples in Lake Hartwell were typically collected around midday when dissolved oxygen can often fluctuate due to temperature and photosynthetic activity. The microcystin values in Lake Hartwell were low and within a narrow range, so it would be particularly beneficial to look at dissolved oxygen if a future cyanobacteria HAB occurs there. Overall, the dataset for each lake was small and did not include data from any algal blooms; consequently, the absence of meaningful correlation results was anticipated. More data over the next several years will build on the 2018 and 2019 dataset and will provide a clearer understanding of any patterns or relationships occurring with cyanotoxin production. Future data analysis would benefit from looking at the data over several years to examine any potential seasonal patterns and the combination of physicochemical parameters on cyanotoxin production.

In conclusion, the monthly-monitoring results were less than the USEPA recreational action standards, suggesting recreational activities in South Carolina were not an immediate concern. There were two (2) event-driven sampling events within the same park where concentrations were measured that were greater than USEPA recreational action standards. In those cases, SCDHEC worked with the park owners to disseminate the information for user education and protection. Even though no strong correlations between microcystin concentration and other parameters were discerned in this assessment, a larger dataset over several years may provide better insight into relationships between cyanotoxin concentrations and other water quality parameters. Because the microcystins and cylindrospermopsin concentration data collected in 2019 were informative, the HAB Monitoring Program will continue to use these data to develop the Program's goals.

Overall Summary:

- This was the second year of the HAB Monitoring Program. The data gathered in 2018 and 2019 helps establish cyanotoxin spatial distribution data; these data will be used to inform future sampling plans and provide insight into lakes that should be monitored more often.
- This was the first year that sampling incorporated the entire algal growing season from May through October.
- The monthly-monitored sampling resulted in no immediate concern for recreation activities on the sampled lakes due to the low concentrations of microcystins and cylindrospermopsin.

- There were two (2) event-driven samples at Anne Springs Close Greenway, a privately owned pond, that exceeded the USEPA action value of 8 µg/L. SCDHEC worked with the park owners on ways to distribute this information and advised closure of the area. SCDHEC adopted USEPA recreational guidelines for cyanotoxins in 2020, which allows the Department to issue advisories for water bodies of the state when cyanotoxins are greater than above guidelines.
- There were no strong correlations between microcystins concentration and other parameters measured in Cedar Creek Reservoir, Lake Hartwell, Lake Murray, and Lake Wateree. There was a weak positive correlation in Lake Hartwell comparing microcystin concentration and dissolved oxygen. However, this trend was not observed in any of the other lakes. Future analyses would benefit from a larger data set that also includes samples from algal blooms and examines a combination of factors.

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**Appendix 1:** Standard Operating Procedure for Determination of Total Microcystins and Cylindrospermopsin in Ambient Water

S.C. Department of Health and Environmental Control

# Determination of Total Microcystins and Cylindrospermopsin in Ambient Water

## Bureau of Water- Aquatic Science Programs

**June 1, 2020**

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# 1. SCOPE AND APPLICATION

## 1.1 Method Description

These methods are used for the determination of algal toxins in ambient water, including (extracellular and intracellular) microcystins and cylindrospermopsin via enzyme-linked immunosorbent assay (ELISA). The detection limit for the Microcystin ADDA assay is 0.10 ppb (µg/L) and the detection limit for the Microcystins ADDA SAES assay is 0.016 ppb (µg/L). The detection limit for the Cylindrospermopsin assay is 0.040 ppb (µg/L). The detection limit for using the seawater sample treatment solution for Cylindrospermopsin is 0.015ppb (ug/L).

# 2. METHOD SUMMARY

The method is an immunoassay for the quantitative and sensitive cogener-independent detection of Microcystins and Nodularins and Cylindrospermopsin in ambient water samples. The testing is completed in a 96-well microtiter plate.

## 2.1 Microcystins

The test is an indirect competitive ELISA for the cogener-independent detection of Microcystins and Nodularins. It is based on the recognition of Microcystins, Nodularins, and their cogeners by specific antibodies. Microcystins, nodularins, and their cogeners when present in a sample and a Microcystins-protein analogue immobilized on the plate compete for binding sites of antibodies in solution. The plate is then washed and a second antibody-HRP label is added. After a second washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of Microcystins present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

## 2.2 Cylindrospermopsin

The test is a direct competitive ELISA for the detection of Cylindospermopsin. It is based on the recognition of Cylindrospermopsin by specific antibodies. Cylindrospermopsin, when present in a sample, and a Cylindrospermopsin-HRP analogue compete for the binding sites of rabbit anti-Cylindrospermopsin antibodies in solution. The anti-Cylindospermopsin antibodies are then bound by a second antibody (goat anti-rabbit) immobilized on the wells of the microtiter plate. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of Cylindrospermopsin present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

## 3. DEFINITIONS

### 3.1 Analysis Batch

Standards, samples, and quality control elements are assayed on a single 96-well plate using identical lots of reagents and wells. Each plate by definition is an Analysis Batch, regardless of the number of wells included. Quality control samples must be analyzed in each Analysis Batch at the frequencies prescribed. Each Analysis Batch includes the following elements:

- Calibration Standards
- Quality Controls
- Field samples (ambient water)

### 3.2 Well Replicates

Within the Analysis Batch, this method requires each calibration standard, field sample, and QC sample to be assayed in two wells. These two wells are called well replicates. Two values are associated with each well replicate: an absorbance measured by the plate reader, and a concentration calculated from this absorbance.

### 3.3 Use of Well Replicate Absorbance Values

For each set of well replicates, the percent coefficient of variation (%CV) is calculated from the two absorbance values. The %CV of the absorbance values for calibration standards must meet QC criteria. The %CV of the absorbance values for all field and QC samples must meet the limits. Refer to Table 2 for QC criteria.

### 3.4 Use of Well Replicate Concentrations

For each set of well replicates, the mean is calculated from the two concentration values. The mean concentration must be used for reporting field sample results. The mean must be used in all method calculation and for evaluating results against QC limits.

### 3.5 Calibration Standards

Solutions of Microcystin and Cylindrospermopsin toxins provided in the ELISA kit or prepared in the laboratory that are appropriate for the measurement range of the ELISA kit.

### 3.6 Calibration Curve

The calibration points are modelled using a four-parameter logistic function, relating concentration (x-axis) to the measured absorbance in the wells (y-axis). Note the inverse relationship between concentration and response. The zero calibration standard gives the highest absorbance and the highest calibration standard gives the lowest absorbance. Note also that the slope, or sensitivity, of

the ELISA response is greatest in the middle of the curve and tends toward zero slope at extreme low and high concentrations.

### 3.7 Four-parameter Logistic Equation

$$y = \frac{(a-d)}{1+(\frac{x}{c})^b} + d$$

y= absorbance
x= concentration
a= absorbance at the bottom plateau
b= slope related term at the inflection point
c= concentration at the inflection point= $EC_{50}$
d= absorbance at the top plateau

The coefficients, a, b, c, and d, are calculated by the data reduction software using regression analysis.

### 3.8 Quality Control Sample (QCS)

A solution containing microcystin toxins or cylindrospermopsin toxins at a known concentration that is obtained from a source different from the source of calibration standards. The purpose of the QCS is to verify the accuracy of the primary calibrations standards.

## 4. HEALTH AND SAFETY WARNINGS

### 4.1 Microcystins

The standard solution in the test kit contain small amounts of Microcystins. The substrate solution contains tetramethylbenzidine (TMB) and the stop solution contains diluted sulfuric acid. Avoid contact of the TMB and stopping solution with skin and mucous membranes. If these reagents come in contact with skin, wash with water.

### 4.2 Cylindrospermopsin

The standard solutions in the test kit contain small amounts of Cylindrospermopsin. The substrate solution contains tetramethylbenzidine (TMB) and the stop solution contains diluted sulfuric acid. Avoid contact of the TMB and stopping solution with skin and mucous membranes. If these reagents come in contact with skin, wash with water.

### 4.3 Cylindrospermopsin Seawater Sample Reagent

Irritant to skin and mucous membranes. May cause eye irritation in susceptible persons. The chemical, physical, and toxicological properties of this reagent have not been thoroughly investigated.

**4.4** Each laboratory is responsible for maintaining an awareness of OSHA regulations regarding safe handling of any chemicals used in this method. A reference file of Safety Data Sheets should be made available to all personnel involved in the analysis. Handle samples and standards using appropriate personal protective equipment.

## 5. INTERFERENCES

**5.1** Numerous organic and inorganic compounds commonly found in water samples have been tested and found not to interfere with this test. However, due to high variability of compounds that may be found in water samples, test interferences caused by matrix effects cannot be completely excluded.

**5.2** Samples containing methanol must be diluted to a concentration <1% methanol to avoid matrix effects.

**5.3** Mistakes in handling the test can cause errors. Possible sources for such errors include: inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, and extreme temperatures during the test performance (lower than 10°C or higher than 30°C). The assay procedure should be performed away from direct sunlight.

**5.4** To avoid cross contamination between samples, do not reuse plastic syringes for filtering. Thoroughly clean glass containers if they are reused. Do not reuse septa from bottle containing ambient water samples.

**5.5** As with any analytical technique, positive results requiring regulatory action should be confirmed by an alternative method.

## 6. SAMPLE HANDLING, PRESERVATION, AND STORAGE

**6.1** Collect samples in 500 mL polyethylene terephthalate glycol (PETG) containers with Polytetrafluoroethylene (PTFE) lined septa lids. Use of other types of plastic collection and/or storage containers may result in adsorptive loss of Microcystins, producing inaccurate (falsely low) results. Ambient water samples do not need to be treated after collection. Freeze samples upon arrival at the laboratory. Samples can be stored in the freezer for up to 2 weeks. When freezing, allow adequate volume for expansion and place the sample container on its side to prevent breakage.

**6.2** Place samples on ice immediately. The temperature blank in the cooler must not exceed 10°C during the first 48 hours after collection. A temperature of greater than 10°C is acceptable if transit time is short and the samples do not have sufficient time to chill. In this case, examine the ice packs in the cooler. If they remain frozen, the samples are valid. Based on holding time (see section 6.1), refrigerate or freeze samples upon arrival to the laboratory.

**6.3** Samples may be filter and assayed any time after lysing if within 14 days of collection. If not assayed immediately, store lysed samples by freezing in glass

vials with PTFE-faced septa, for example, 1 mL of lysed and filtered sample held in a 4mL vial.

## 7. INSTRUMENTATION AND EQUIPMENT

**7.1** Adda ELISA Test Kits- 96-well Microtiter Plates

- **7.1.1** Microcystins/Nodularins- Abraxis PN 520011
- **7.1.2** Microcystins-ADDA SAES- Abraxs PN 520011SAES
- **7.1.3** Cylindrospermopsin- Abraxis PN 522011
- **7.1.4** Standards
  1. Microcystins ADDA: (6): 0, 0.15, 0.40, 1.0, 2.0. 5.0 ppb, 1mL each
  2. Microcystins ADDA SAES: (6): 0, 0.05, 0.15, 0.4, 1.5, 5.0 ppb, 1mL each
  3. Cylindrospermopsin: (7): 0, 0.05, 0.10, 0.25, 0.50, 1.0, 2.0 ppb, 1mL each
- **7.1.5** Control:
  1. Microcystins: 0.75 ± 0.185 ppb, 1 mL
  2. Cylindrospermopsin: 0.75 ± 0.15ppb, 1 mL
- **7.1.6** Sample Diluent, 25 mL, for use as a Laboratory Reagent Blank and for dilution of samples above the range of the standard curve
- **7.1.7** Antibody Solution
  1. Microcystins ADDA: 6mL
  2. Microcystins ADDA SAES, 6mL
  3. Cylindrospermopsin: rabbit anti-Cylindrospermopsin, 6 mL
- **7.1.8** Conjugate Solution
  1. Microcystins ADDA: Anti-Sheep-HRP conjugate solution, 12 mL
  2. Microcystins-ADDA SAES Conjugate Solution, 12mL
  3. Cylindrospermopsin: Cylindrospermopsin-HRP conjugate solution (vortex before use), 6 mL
- **7.1.9** Wash Buffer (5X) Concentrate, 100 mL, must be diluted prior to use
- **7.1.10** Substrate (Color) Solution (TMB), 12 mL
- **7.1.11** Stop Solution
  1. 6 mL for Microcystins
  2. 12mL for Cylindrospermopsin
- **7.1.12** Cylindrospermopsin Seawater Sample Treatment Solution, 45 test

**7.2** Cyanotoxin Manual Assay System- Abraxis PN 475010S. Includes:

- **7.2.1** Microplate Reader, Model 4303
- **7.2.2** Pipette, transfer, 10-100 µL, adjustable
- **7.2.3** Pipette, repeating, manual
- **7.2.4** Pipette, multichannel, 8-tip, adjustable
- **7.2.5** Basin, reagent, for multichannel, 50/bag
- **7.2.6** Rack for 4mL vials, 48-postion (4x12)

**7.3** Disposable plastic tips for pipettes

**7.3.1** Cartridges, Repeater, 1mL, bx/100- PN 70468

**7.3.2** Tips, Pipette, 10-200µL, 96/bx- PN 300002

**7.3.3** Tips, Pipette, 30-300µL, 96/bx- PN 300004

**7.4** Vials for freezing samples

**7.4.1** Vials, Glass, Clear, 4 mL with caps

**7.4.2** Vials, Glass, Clear, 40mL with caps

**7.5** Syringes and Filters for Lysing

**7.5.1** All plastic Luer-Lok syringes, 3mL, from Thermofisher Scientific

**7.5.2** Glass Fiber Syringe Filters, 25mm, 1.2µm,

**7.6** 500 mL PETG containers with PTFE septa lined lids

**7.7** Parafilm for plate covering

## 8. REAGENTS, STANDARDS, AND CONSUMABLE MATERIALS

### 8.1 Analysis Kit

Store kits according to manufacturer's instructions. Standards and reagents may be used until the manufacturer's expiration date.

**8.1.1** Both the Microcystin and Cylindrospermopsin kits should be stored in the refrigerator (4-8°C). The solutions must be allowed to reach room temperature (20-25 °C) before use. Consult state, local, and federal regulations for proper disposal of all reagents.

## 9. INSTRUMENT CALIBRATION PROCEDURES

### 9.1 Micropipetters

Micropipetters must be verified each year for accuracy. Verification of accuracy is done by pipetting DI water and then weighing to determine if it is accurate. This check must be done for 50µL, 100 µL, and 250 µL.

### 9.2 Calibration Procedure

A calibration is required with each Analysis Batch. Use the concentrations stated in the kit instructions. Do not add additional calibration levels or eliminate any levels. Use the calibration standards provided in the original kit. Each calibration standard must be added to at least two wells.

### 9.3 Calibration Acceptance Criteria

The calibration curve is validated by evaluating the %CV of the absorbance values for the well replicates representing each calibration level, and the correlation coefficient of the four-parameter logistic curve. Calculate the %CV for each of the paired absorbance values, including the "zero" standard. The %CV for each pair must be less than, or equal to, 10%. However, one pair is allowed to exceed 10% providing the %CV is less than, or equal to, 15%. The square of the correlation coefficient ($r^2$) of the four-parameter curve must be greater than, or equal to, 0.98.

If the calibration fails the %CV limits or $r^2$ is less than 0.98, then the entire Analysis Batch is invalid. Assay the samples in a subsequent Analysis Batch. Freeze the filtered samples if this Analysis Batch cannot be completed on the same day as the original attempt. Each sample must be within the 14-day holding time for the repeat assay.

## 10. Procedures

### 10.1 Sample Lysing Procedure by Freeze-Thaw

**10.1.1** Mix samples thoroughly and immediately transfer 5 to 10 mL of each field sample into a 40 mL vial to begin three freeze-thaw cycles. If the sample was previously frozen, only two freeze-thaw cycles are needed (once it has thawed, it has undergone the first freeze/thaw cycle). Smaller vials may be used, but reduce the sample volume to less than 25% of vial capacity.

**10.1.2** Once sample is completely frozen, remove from freezer and thaw. To speed up the process, vials may be immersed in a 35°C in a water bath until completely thawed. Ensure samples are completely frozen and completely thawed during each cycle.

**10.1.3** Filter 1 to 2 mL of each lysed sample into a 4mL vial using a glass-fiber syringe filter. Samples are ready for immediate analysis.

### 10.2 Seawater Sample Preparation

**10.2.1** Microcystins

1. No matrix effects have been observed with seawater salinities (salinity up to 38 parts per thousand) using the ADDA SAES ELISA plate

**10.2.2** Cylindrospermopsin

1. Weigh 0.1 g of Cylindrospermopsin Seawater Sample Treatment reagent into a clean, appropriately labeled 4mL glass vial
2. Add 1mL of brackish water or seawater sample to the vial
3. Vortex for 1 minute. Allow the sample to settle for 10 minutes
4. Pipette the supernatant into an appropriately labeled microcentrifuge tube. Centrifuge for 5 minutes at 13,000 rpm. The sample will separate into 3 laters: a solid, white precipitate (bottom layer), a clear liquid (center layer), and a very thin white film (on top of the liquid layer).
5. Pipette the clear liquid (center layer) into a clean, appropriately labeled 4mL glass vial. Avoid pipetting the very thin white film

6. Dilute the supernatant 1: 3 with DI H2O (I.e. 333 uL supernatnat and 667 ul DI H2O). The sample can then be analyzed using the Abraxis Cylindrospermopsin ELISA Kit.

**10.3 Test Preparation**

**10.3.1** Verify kit standards and reagents are used prior to the expiration date. Allow the reagents and samples to reach ambient temperature before analysis. The assay procedure must be performed away from direct sunlight.

**10.3.2** Remove the number of microtiter plate strips required from the resealable pouch. The remaining strips are stored in the pouch with the desiccant (tightly sealed)

**10.3.3** The standards, control, sample diluent, antibody enzyme conjugate, substrate, and stop solutions are ready to use and do not require any further dilutions

**10.3.4** Dilute the wash buffer (5X) concentrate at a ratio of 1:5 with deionized or distilled water. If using the entire bottle (100mL), add to 400mL of deionized or distilled water and mix thoroughly.

**10.3.5** The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously. See Table 1.

**10.4 Assay Procedures**

**10.4.1 Microcystins**

1. Add 50µL of the standard solutions, control, or samples into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. Add 50µL of the antibody solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for 90 minutes at room temperature.
3. Remove the covering, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. Wash the strips three times using the diluted wash buffer. Please use at least a volume of 250 µL of 1X wash buffer for each well and each washing step. Blot the inverted plate after each wash step on a stack of paper towels. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.

4. Add 100 µL of the enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strip for 30 minutes at room temperature.
5. Remove the covering, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. Wash the strip three times using the diluted wash buffer. Please use at least a volume of 250 µL of 1X wash buffer for each well and each washing step. Blot the inverted plate after each wash step on a stack of paper towels. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.
6. Add 100 µL of substrate (color) solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for 20-30 minutes at room temperature. Protect the strips from sunlight.
7. Add 50 µL of stop solution to the wells in the same sequence as for the substrate (color) solution using a multi-channel pipette or a stepping pipette.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

**10.4.2 Cylindrospermopsin**

1. Add 50 µL of the standards, control (QCS), LRB, or samples into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. Add 50 µL of the enzyme conjugate solution to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette.
3. Add 50 µL of the antibody solution to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for 45 minutes at room temperature.

4. Remove the covering, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. Wash the strips four times using the diluted wash buffer. Please use at least a volume of 250 µL of 1X wash buffer for each well and each washing step. Blot the inverted plate after each wash step on a stack of paper towels. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.
5. Add 100 µL of substrate (color) solution to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette. Cover the wells in the same sequence as for the substrate (color) solution using a multi-channel, stepping or electronic repeating pipette.
6. Add 100 µL of stop solution to the wells in the same sequence as for the substrate (color) solution using a multi-channel, stepping, or electronic repeating pipette.
7. Read the absorbance at 450nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

**10.5 Running an Assay**

**10.5.1** Place the plate instrument with well A-1 at the rear right corner so that row 1 is going into the reader first. As you press the first row back and down you will feel slight tension on the plate stretching the carrier so that the front fits in. The plate requires a snug fit.

**10.5.2** When using a strip tray, make sure wells are pushed down into tray so that they will not cause the plate to jam or entry. Use care that well tabs do not extend over other wells. Do not place the tabbed ends of strips in row 1; they should be in row 12. Be sure to place the strips in the order in which Blanks, Calibrators and Samples are to be read.

**10.5.3** For best results, do not fill wells completely; 200-250 µL depending on well total volume is the maximum fill recommended when the mixing feature is used.

**10.5.4** Plate Layout is the default window for Abraxis Reader and displays when the program is started. There are several options: Load Plate, Save Plate, Reset, Re-Assign, Read Plate or Remove. Once samples have been assigned, press the Read Plate button to run. Results are displayed as delta Abs for fixed time read, and delta Abs/min for non-fixed time kinetic. Refer to the "AReader Abraxis Model 4303 Operators Manual" for more information on running an assay.

**10.5.5** Sample analyses resulting in a higher concentration than the highest standard in the calibration curve must be diluted within the calibration range and reanalyzed to obtain accurate results. Samples may not be diluted in the well plate. If a sample is diluted, the final values must be

calculated by multiplying the result by the proper dilution factor. Report calculated values.

**10.5.6** Save and print a copy of the calibration curve and sample results as part of the laboratory's record maintenance protocol.

**10.5.7** Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the standards.

**10.4.7.1** Samples with lower absorbances than a standard will have concentrations of Microcystins or Cylindrospermopsin greater than the standard. Samples which have higher absorbances than a standard will have concentrations of Microcystins or Cylindrospermopsin less than that standard.

## 10. 5 QUALITY CONTROL

QC requirements include the IDC, and QC elements associated with each Analysis Batch. This section describes each QC parameter, its required frequency, and the performance criteria that must be met in order to satisfy EPA data quality objectives. These QC requirements are considered the minimum acceptable QC protocol. Laboratories are encouraged to institute additional QC practices to meet their specific needs.

### 10.5.1 Initial Demonstration of Capability (IDC)

The IDC must be successfully performed prior to analyzing field samples. A plate with all calibration standards, controls, and LRB, plus 10 field samples, must be ran in duplicate wells for the IDC. The IDC must be performed by each analyst, when a new analyst begins work or whenever a change in analytical performance.

When conducting the IDC, the analyst must meet the calibration requirements specified in section 9 for the standards. The %CV for each pair must be less than, or equal to, 10%. However, one pair is allowed to exceed 10% providing the %CV is less than, or equal to, 15%. All samples must have a %CV of less than 15%. If the analyst fails to meet the %CV limits or $r^2 = 0.98$ for the given standards, then their batch is invalid and they must perform the analysis in a subsequent Analysis Batch. The mean recovery of the QCS must also have a percent recovery $\geq$70% and $\leq$130% of the true value. If the analyst fails to meet the percent recovery during the IDC, then the analysis batch is invalid and must be performed again in a subsequent Analysis Batch.

### 10.5.2. Criterion for Replicate Wells

All field and QC samples are added to at least two wells. The %CV of the absorbance values measured for the well replicates must be less than, or equal to, 15%. Calculate the %CV as follows:

$$\%CV = \frac{Standard\ Deviation\ of\ Absorbances}{Mean\ Absorbance} x100\%$$

If the %CV exceeds 15% for a field sample or QC sample, then that sample is invalid. Note that the well replicates of calibration standards must meet a different set of criteria for %CV.

### 10.5.3 Quality Control Standard (QCS)

A secondary source QCS must be analyzed with each batch of samples to verify the concentration of the calibration curve. If a QCS is already included in the kit, it may be used if it has a different lot number than the calibration standards and was prepared from a separate primary stock. Acceptance limits must be within ±25% of true value. QCS values exceeding the acceptance limits require action and reanalysis of sample(s) with results greater than the concentration of an acceptable Low-CV in the same analytical batch. If reanalysis is not possible, all sample concentration results greater than an acceptable Low-CV analyzed in the same batch must be appropriately qualified and noted in the final report.

## 11 DATA REDUCTION, VALIDATION, AND REPORTING

### 11.1 Quantitation

A four-parameter logistic curve fit must be used. Other curve-fitting models are not permitted. Calculate the sample concentration for each well using the multipoint calibration. For each field and QC sample, average the two concentration values from each well. Use this mean to report sample results and to evaluate QC results against acceptance limits. Final results should be rounded to two significant figures.

### 11.2Exceeding the Calibration Range

If a result exceeds the range of the calibration curve, dilute the sample with reagent water. Analyze the diluted sample in a subsequent Analysis Batch. Incorporate the dilution factor into the final concentration calculations. Report the dilution factor with the sample result.

## 12 WASTE MANAGEMENT

The EPA requires that laboratory waste management practices be consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. In addition, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions.

## 13 REFEREENCES

EPA Method 546, "*Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by Adda Enzyme-Linked Immunosorbent Assay*; EPA 815-B-16-011; Office of Water: Cincinnati, OH, August 2016.

## 14 REVISION HISTORY

| Revision | Date | Summary | Section |
|---|---|---|---|
| 1 | 03/05/20 | Added limit detection for Microcystins ADDA-SAES and for use of Cylindrospermopsin seawater sample treatment | 1.1 |
| 1 | 03/05/2020 | Added safety information about the Cylindrospermopsin seawater sample treatment | 4.3 |
| 1 | 03/05/20 | Added limitations with methanol | 5.2 |
| 1 | 03/05/20 | Changed 1 L PETG container to 500mL | 6.1 |
| 1 | 03/05/20 | Added Microcystins ADDA-SAES test kit supplies | 7.1 |
| 1 | 03/05/20 | Added Cylindrospermopsin seawater sample treatment to supplies | 7.1.12 |
| 1 | 03/05/20 | Changed 1 L PETG container to 500mL | 7.6 |

## 15 Tables, Figures, and Method Performance Data

**Table 1. Working Scheme of microtiter plate**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | **Std 0** | **Std 4** | **Sample 2** | | | | | | | | | |
| **B** | **Std 0** | **Std 4** | **Sample 2** | | | | | | | | | |
| **C** | **Std 1** | **Std 5** | **Sample 3** | | | | | | | | | |
| **D** | **Std 1** | **Std 5** | **Sample 3** | | | | | | | | | |
| **E** | **Std 2** | **Control** | **Etc.** | | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **F** | **Std 2** | **Control** | **Etc.** | | | | | | | | | |
| **G** | **Std 3** | **Sample 1** | | | | | | | | | | |
| **H** | **Std 3** | **Sample 1** | | | | | | | | | | |

** Note: The working scheme of the Cylindrospermopsin plate contains an additional standard. Thus well G2 and H2 will be used for Standard 6 and the samples will start in the wells in column 3.

**Table 2. Analysis Batch QC Requirements**

| Method Reference | Requirement | Specification and Frequency | Acceptance Criteria |
|---|---|---|---|
| 9 | ELISA Calibration- with provided standards | Use kit-recommended levels and concentrations. Two well replicates per standard. | %CV of absorbance ≤10%; ≤15% allowed for 1 pair.<br><br>$r^2 \geq 0.98$ |
| 3.2 | Well Replicates | Assay field and QC samples in two wells | Sample invalid if %CV of absorbance values > 15% |
| 3.11 | Quality Control Sample (QCS) | Assay 1 QCS for each new lot of calibration standards. Prepare the QCS near the $EC_{50}$ with MC-LR from a source independent of the calibration standards. | Percent recovery ≥70% and ≤130% of the true value. |

**Appendix 2**: SC Cyanotoxin Distribution Quality Assurance Project Plan

## Section A. Project Management

**A1 Title Page**

SC Cyanotoxin Distribution Study
Prepared by Emily Bores
April 29, 2019

Project Manager: Emily Bores, Aquatic Science Programs

Lead Organization: South Carolina Department of Health and Environmental Control
2600 Bull St.
Columbia, SC 29201

Project Manager: Emily Bores Date: 05/07/19
Emily Bores, Aquatic Science Programs

SC DHEC BOW Management: Date: 5/7/19
Bryan Rabon, Aquatic Science Programs, Manager

SC DHEC BEHS: Date: 5/8/19
Elizabeth Basil, EA BEHS Assistant Bureau Chief

SCDHEC QAM: David Graves Date: 5-7-19
David Graves, QAM

SCDHEC BEHS: Nydia Burdick Date: 5-7-19
Nydia Burdick, QA liason

EPA Region 4 QA Officer: Date: 5/17/19
Elizabeth Smith, US EPA, Region 4
for Liza Montalvo

## A2 Table of Contents

## A3 Distribution List

**Table 1 Distribution List**

| Name | Title | Organization | Phone | Email |
|---|---|---|---|---|
| Emily Bores | Project Manager and Lab Contact | SC DHEC | 803-898-4837 | boreseb@dhec.gov.sc |
| Bryan Rabon | ASP Manager | SC DHEC | 803-898-4402 | raboneb@dhec.sc.gov |
| David Graves | QAP | Environmental Laboratory Certification Office | 803-898-4272 | gravesda@dhec.sc.gov |
| Nydia Burdick | QA Liaison | Environmental Laboratory Certification Office | 803-896-0862 | burdicnf@dhec.sc.gov |
| Alexander Grubbs | Field Personnel | SC DHEC- Greenville Office | 864-372-3263 | grubbsaw@dhec.sc.gov |
| Chad E. Johnson | Field Manager | SC DHEC- Lancaster Office | 803-285-7461 | johnsoce@dhec.sc.gov |
| Matt Miller | Field Manager | SC DHEC- Midlands Office | 803-896-0620 | millermw@dhec.sc.gov |
| Stephanie Jacobs | Lab Manager | SC DHEC- Aiken Office | 803-642-1637 | jacobssa@dhec.sc.gov |
| Allyson Muller | Field Manager | SC DHEC- Charleston Office | 843-953-0150 | mulleram@dhec.sc.gov |
| Sarah Brower | Field Manager | SC DHEC- Beaufort Office | 843-846-1030 | browersr@dhec.sc.gov |
| Dave Chestnut | Project Validation | SCDHEC | 803-898-4066 | chestnde@dhec.sc.gov |

## A4 Project/Task Organization

**Emily Bores**- is the Project Manager and is responsible for developing and maintaining the QAPP. She is also the technical project leader for the ASP cyanotoxin lab. She will analyze incoming samples as well as train and supervise additional staff members in analysis.

**Taylor Shearer**- ASP staff member who will assist in the analysis and identification of cyanotoxin samples.

**Scott Castleberry-** ASP staff member who will assist in the analysis and identification of cyanotoxin samples.

**David Graves**- Will review and approve the QAPP

**Nydia Burdick**- QAPP liaison

**Bryan Rabon**- Will provide guidance and expertise from SC DHEC.

**David Chestnut-** Validator of the samples and data.

**Field Investigators**- regional staff members who will collect cyanotoxin monthly samples from SC reservoirs.

**Intern-** Summer intern for the Aquatic Science Programs who will be trained to assist in the analysis of cyanotoxin samples.

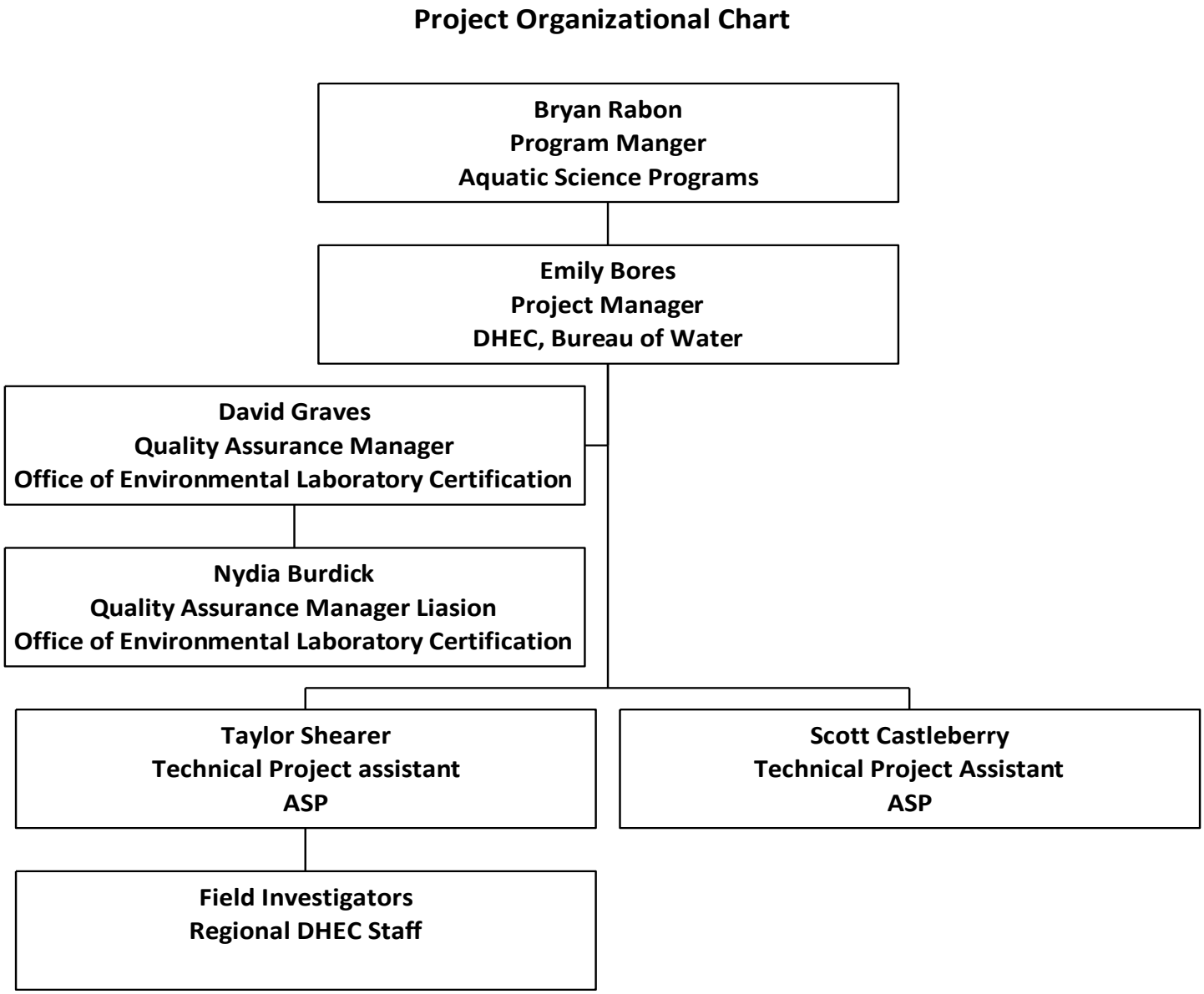



**Figure 1 Project Organization Chart**

## A5. Problem Definition/Background

The goal of this project is to characterize the occurrence of cyanotoxins in surface waters from reservoirs in South Carolina. The results will be used to assess potential risks to drinking water facilities, as well as recreational and aquatic life uses for waterbodies of the state. Recent events associated with toxic algal blooms in Toledo (Jetoo et al. 2015), EPAs (2015) release of health advisories for cyanotoxins in drinking water and improved analytical methods have made clear the need to better characterize the presence of cyanotoxins in the state's reservoirs. Despite the increased knowledge of eutrophication and harmful algal blooms (HABs) in SC's coastal waters, HABs of inland freshwaters remains less clear. Although SCDHEC and its predecessors have had

a robust monitoring network of surface water since the 1950s, cyanotoxins have not been included in the suit of analytes normally tested. While certain measures of eutrophication such as chlorophyll a, nitrogen, phosphorus, and water clarity may show correlation with cyanotoxins, these measures alone do not provide a full picture of environmental conditions associated with toxins. With improved analytical methods it is now possible to detect cyanotoxins at lower levels, which can provide the baseline for their occurrence in SC. The characterization of waterways is the first step in the process for effective environmental management and knowing where and under what conditions threats may occur is a critical first step to mitigate harm to human and environmental health.

We propose, therefore, to conduct a statewide survey of cyanotoxins in the lakes of South Carolina. The survey will focus on lakes being sampled from the 2019 ambient sites as well as some additional sites picked from the 2018 sampling locations. Some event driven testing will be conducted and may include large rivers in addition to lakes. Combined with other water quality variables and geospatial data, a better understanding of cyanotoxins in freshwaters will be achieved. With EPAs (2015) recent release of health advisories thresholds in drinking water for microcystin and cylindrospermopsin, these two cyanotoxins will be targeted. While this project is focused on toxin analysis for recreational waters only, if there is a high concentrations of toxins in the lake there may be a potential for toxins to get into the drinking water. For reference, EPA's 10-day Health Advisory values for school age children and adults is 1.6 ug/L for microcystins and 3 ug/L cylindrospermopsin. See Table 2 for the EPA draft Recreational Criteria or Swimming Advisory Recommendations for Microcystins and Cylindrospermopsin. The event driven testing will target algal blooms that may be observed or reported during the 2019 growing season.

**Table 2. Draft Recreational Criteria or Swimming Advisory Recommendations for Microcystins and Cylindrospermopsin**

| Application of Recommended Values | Microcystins | | | Cylindrospermopsin | | |
|---|---|---|---|---|---|---|
| | Magnitude (ug/L) | Frequency | Duration | Magnitude (ug/L) | Frequency | Duration |
| Swimming Advisory | 8 | Not to be exceeded | One day | 15 | Not to be exceeded | One day |
| Recreational Water Quality Criteria | | No more than 10 percent of days | Recreational season (up to one calendar year) | | No more than 10 percent of days | Recreational season (up to one calendar year) |

## A6. Project/Task Description

As stated previously, the purpose of this proposed project is to better understand the occurrence of cyanotoxins in the lakes of South Carolina, in continuation of the sampling efforts from the 2018 sampling season. A total of approximately 460 water samples will be collected by regional staff. Monthly grab samples will be collected at approximately 76 sites in SC and will be shipped via overnight courier to the Aquatic Science Programs'(ASP) cyanotoxin lab in Columbia. These samples will be taken during normal monthly ambient monitoring of select reservoirs and lakes during the months of May through October 2019. Refer to the State of South Carolina Monitoring Strategy for Calendar Year 2019, Technical Report No, 0802-17. Due to the holding time for cyanotoxins, all samples will be frozen in 40mL vials within 24 hours at -20 C or lower (holding time at -20 is 2 weeks). The transport of samples to the ASP cyanotoxin lab should occur within 24 hours from the regions. At the lab, samples will be tested for total microcystins and cylindrospermopsin by Enzyme Linked Immunosorbent Assays (ELISA) methodology via a microplate reader and associated software. Samples will be analyzed based on the ELISA methodology in EPA method 546. Training and additional guidance was also provided from the provider, Abraxis. Additionally, samples may be collected due to event driven algal blooms and/or waters with taste and odor problems. Phytoplankton taxonomic analysis may also be conducted on samples when applicable. Table 3 provides the project activities and their anticipated date of initiation and completion. Table 4 provides the SC DHEC station codes and site descriptions. Sites for this project were chosen from the current list of 2018 sites as well as their proximity to a public water source. Figure 2 is a map of SC with all the locations for the sampling sites identified. Sampling events may be delayed in the cases of serious droughts or rain events.

**Table 3. Project Activities**

| Activity | Organization | Anticipated Start Date(s) | Anticipated Date(s)of Completion |
|---|---|---|---|
| Site Determination | SCDHEC | 1/03/19 | 01/30/19 |
| QAPP Approval | SCDHEC | 02/01/19 | 05/2019 |
| Sampling Begins | SCDHEC | 05/2019 | 10/31/19 |
| Lab Reports | SCDHEC | 06/01/19 | 11/10/18 |
| Data Validation | SCDHEC | 06/01/19 | 11/31/19 |
| Final Report Due | SCDHEC | 10/31/19 | 11/31/19 |

**Table 4. Site Locations**

| Station | Regional Lab | Description | Latitude | Longitude |
|---|---|---|---|---|
| B-327 | Central Midlands | Monticello Lake- Lower Impoundment between large islands | 34.32966927326 | -81.30263710763 |

| Station | Regional Lab | Description | Latitude | Longitude |
|---|---|---|---|---|
| B-339 | Greenville | Lake Bowen 0.3 MI W of SC 9 | 35.11285121982 | -82.0455309651 |
| B-345 | Central Midlands | Parr Reservoir in Forebay near dam | 34.26208554189 | -81.33538487819 |
| CL-019 | Greenville | Lake Jocassee in Forebay equidistant from dam and shorelines | 34.95988763468 | -82.92361397724 |
| CL-041 | Greenville | Clarks Hill Reservoir in Forebay near dam | 33.66999442019 | -82.20761435616 |
| CL-069 | Aiken | Langley Pond in Forebay near dam | 33.5222610417 | -81.8432066618 |
| CL-089 | Midlands | Lake Wateree in Forebay equidistant from dam and shorelines | 34.33684850575 | -80.70499959935 |
| CW-016F | Lancaster | Fishing Creek Reservoir 2 mi. below Cane Creek | 34.67778314931 | -80.87718655105 |
| CW-033 | Midlands | Cedar Creek Reservoir 100 m N of dam | 34.5426516318 | -80.87773762794 |
| CW-057 | Lancaster | Fishing Creek Reservoir 75 ft. above dam near Great Falls | 34.60528283986 | -80.89104250062 |
| CW-174 | Midlands | Cedar Creek Reservoir at Unimp. Road AB JCT with Rocky Creek | 34.55815953884 | -80.8916653521 |
| CW-197 | Midlands | Lake Wylie above Mill Creek arm at end of S-46-557 | 35.13756014086 | -81.05942285366 |
| CW-201 | Midlands | Lake Wylie North Lakewoods S/D at Ebenezer access | 35.02811990158 | -81.0476664737 |
| CW-207 | Midlands | Lake Wateree at end of S-20-291 | 34.40248974794 | -80.78839167726 |
| CW-230 | Midlands | Lake Wylie at Dam; under powerlines | 35.02254041376 | -81.00871832877 |
| CW-231 | Midlands | Lake Wateree headwaters approx. 50 yds. downstream confluence Cedar Creek | 34.5364955341 | -80.87488591149 |
| PD-327 | Florence | Lake Robinson at S-13-346 5 MI E Mcbee by boat ramp | 34.46752201266 | -80.1698000394 |
| RL-13081 | Central Midlands | Parr Reservoir approx. 0.5 MI E MCBEE by boat | 34.2684205 | -81.3375885 |

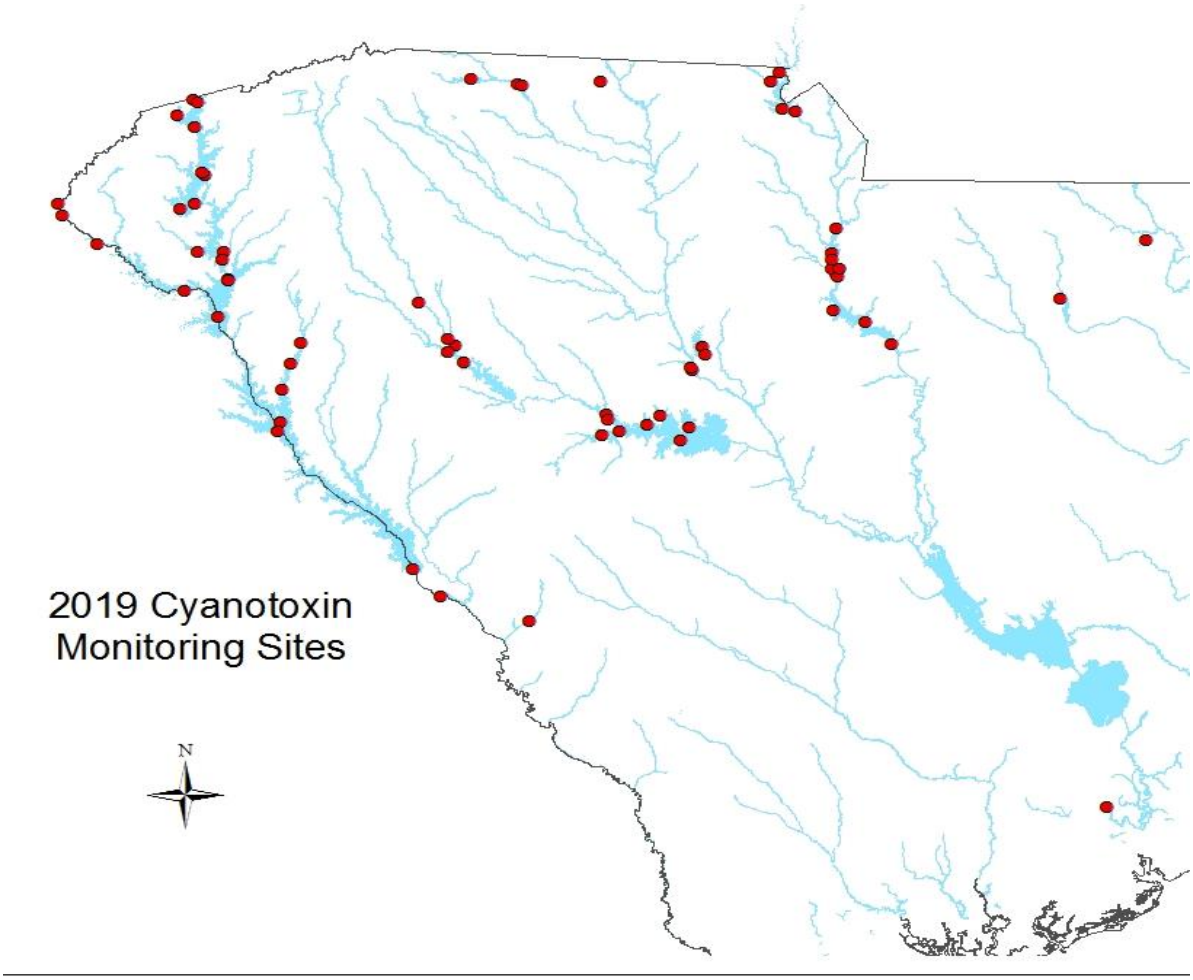
| Station | Regional Lab | Description | Latitude | Longitude |
|---|---|---|---|---|
| RL-19149 | Catawba | Cedar Creek reservoir ~100 M NW of end of powerline easement on eastern side of lake | 34.557795 | -80.869363 |
| RL-19150 | Catawba | Lake Whelchel- 670 m ENE of Boat landing on western side of lake | 35.107915 | -81.629668 |
| RL-19154 | L. Savannah | Lake Murray Big Creek Arm Across Lake from Shinner Ln | 34.069528 | -81.61858 |
| RL-19155 | Greenville | Lake Jocassee confluence of Horsepasture Creek and Bearcamp | 35.039171 | -82.933357 |
| RL-19156 | | Lake Moultrie- 0.80MI NNW of Augustus M flood boat landing | 33.322915 | -80.008371 |
| RL-19158 | Midlands | Lake Robinson cove near upstream end of Lake near end of road S-13-7391 | 34.481743 | -80.169963 |
| RL-19159 | Greenville | Lake Keowee at the end of Kelly Creek Arm- nearshore- approx. 0.3 miles ESE of Cedar Bluff Court Cul-de-sac | 34.818138 | -82.88761 |
| RL-19164 | Central Midlands | Parr Reservoir approx. 0.5 MI NNW of B-345 | 33.476220 | -80.307580 |
| RL-19165 | Greenville | Lake Secession midway between 3rd Avenue Point and Turtle Point | 34.270358 | -82.604573 |
| RL-19166 | Central Midlands | Lake Wateree- near end of Taylor Creek arm | 34.436545 | -80.886864 |
| RL-19167 | Greenville | Lake Keowee- in cove in the V of Arrowhead trail and cliffwick | 34.718135 | -82.96703 |
| RL-19168 | | | 33.32374 | -80.1010536 |
| RL-19170 | Central Midlands | Lake Monticello- in cove located half way between ends of lighted lane and fireside drive | 34.307568 | -81.293468 |
| RL-19174 | Central Midlands | Lake Murray- 133 meters NNE of cove off of Putnam Dr | 34.092189 | -81.344055 |
| RL-19176 | Pee Dee | Lake Marion- 1.25 MI WSW of bridge of SC-260 over church branch | 33.519757 | -80.2129 |
| RL-19177 | Greenville | Lake Russell- 0.8 MI SSW of the Swimming Beach and the end of | 34.096751 | -82.633133 |

| Station | Regional Lab | Description | Latitude | Longitude |
|---|---|---|---|---|
| | | Day use Rd- Calhoun Falls State Recreational Area | | |
| RL-19178 | Catawba | Crowders Creek Arm- 125 Yd ENE of Bridge | 35.111649 | -81.086748 |
| RL-19179 | Greenville | Lake Hartwell- 0.5 MI SSW of Boat landing at portman marina- nearshore at point on west side of uninhabited island | 34.517005 | -82.808994 |
| RL-19251 | Greenville | Lake Yonah- 0.7 Mi North of dam | 34.691933 | -83.341828 |
| RL-19253 | Greenville | Lake Blalock- in cove in between dancing Brooke LN and Dancing Water Dr- 135M SE of Cul-De-Sac at the end of dancing Brooke Ln | 35.098794 | -81.898066 |
| RL-19254 | Catawba | Cedar Creek Reservoir- 15M East of Bowden Island Shoreline | 34.560391 | -80.870757 |
| RL-19255 | Greenville | Lake Tugaloo- Center of Lake 1 MI North of Dam | 34.72946 | -83.352801 |
| RL-19256 | Catawba | Eureka Lake- 0.3 MI East of Swimming Beach on lake | 34.639144 | -79.895267 |
| RL-19257 | Greenville | Lake Blalock- 75M NW of the end of Davis Trading Post RD | 35.095426 | -81.880795 |
| RL-19258 | Catawba | Great Falls Reservoir- Western side of lake 0.7 Mi NNW of Dam | 34.586287 | -80.892762 |
| RL-19259 | Trident | Goose Creek Reservoir- 0.25 MI WSW from center of Goose Creek Primary School | 32.972221 | -80.036219 |
| RL-18136 | Greenville | Broadway Lake opposite small cove nearshore along lakeside drive | 34.458843 | -82.594253 |
| RL-18138 | Greenville | Lake Rabon North Rabon arm near headwaters near east bank | 34.516053 | -82.131542 |
| RL-18139 | Greenville | Lake Cooley Jordan Creek arm off end of Andre Drive | 35.00175 | -82.104137 |
| RL-18142 | Greenville | Lake J. Robinson near Shore opposite the end of Harbor Master Lane | 35.002929 | -82.308294 |
| S-022 | Greenville | Reedy Fork of Lake Greenwood at S-30-29 | 34.32782770413 | -82.08492453465 |

| Station | Regional Lab | Description | Latitude | Longitude |
|---|---|---|---|---|
| S-024 | Greenville | Lake Greenwood; Headwaters; US S-30-33 | 34.30796139287 | -82.11008169299 |
| S-131 | Greenville | Lake Greenwood at US 221 7.6mi NNW 96 | 34.2791422726 | -82.05865234935 |
| S-211 | Midlands | Hollands Landing Lake Murray off S-36-26 at end of S-36-3 | 34.09843911162 | -81.47647071452 |
| S-213 | Midlands | Lake Murray at S-36-15 | 34.12514632317 | -81.43367351171 |
| S-222 | Midlands | Lake Murray; Little Saluda arm at SC 391 | 34.08015740659 | -81.56253556103 |
| S-308 | Richland (Laurens) | Lake Greenwood; Reedy River arm; 150 yards US Rabon Creek | 34.34672448649 | -82.10883717482 |
| S-309 | Richland (Newberry) | Lake Murray; Bush River arm; 4.6 km US SC 391 | 34.13145718979 | -81.60480965259 |
| S-310 | Richland (Newberry) | Lake Murray; Saluda River arm; US Bush River; 3.8 KM US SC 391 | 34.11511713204 | -81.59989492506 |
| S-311 | Greenville | Boyd Mill Pond 0.6km W of dam | 34.45474035788 | -82.20191995164 |
| SV-098 | Greenville | Lake Russell at SC 72 3.1 mi SW of Calhoun Falls | 34.07041123611 | -82.64296730781 |
| SV-200 | Greenville | Tugaloo River arm of Lake Hartwell at US 123 | 34.61170811855 | -83.2262275002 |
| SV-236 | Greenville | Lake Hartwell at S-37-184 6.5mi SSE of Seneca | 34.59542649222 | -82.9077665746 |
| SV-268 | Greenville | Lake Hartwell- Eighteen Mile Creek arm at S-04-1098 | 34.59719859963 | -82.82177535664 |
| SV-331 | Greenville (Anderson) | Lake Secession; 1 ¼ MI below SC Route 28 | 34.33188084214 | -82.57584405972 |
| SV-335 | Greenville | Lake Jocassee at Toxaway; Horse Pasture; and Laurel Fork Confluence | 35.03202556123 | -82.91514019701 |
| SV-336 | Greenville | Lake Jocassee at Confluence of Thompson and Whitewater Rivers | 34.99592876746 | -82.97934904167 |
| SV-338 | Greenville | Lake Keowee above SC Route 130 and dam | 34.82690126626 | -82.89768505093 |

| Station | Regional Lab | Description | Latitude | Longitude |
|---|---|---|---|---|
| SV-339 | Greenville | Lake Hartwell; Seneca River arm at USACE buoy between S-14 and S-15 | 34.51124259177 | -82.80978476766 |
| SV-340 | Greenville | Lake Hartwell; main body at USACE WQ buoy between markers 11 and 12 | 34.40324891528 | -82.83906135828 |
| SV-357 | Greenville | Lake Russell; Rocky river arm between markers 48 and 49; DS Felkel | 34.19202426554 | -82.63092646246 |
| SV-361 | Greenville | Lake Keowee in forebay of Little River dam | 34.73395040312 | -82.91826415278 |
| SV-363 | Greenville | Lake Hartwell off Glenn Ford Landing US Beaverdam Creek cove | 34.48002595316 | -82.94539509097 |
| SV-372 | Greenville | Stephens Creek Reservoir/ Savannah River at SC 28; Walk in from GA side | 33.5927839022 | -82.1233268586 |

**Figure 2 Sampling Locations**




## A7 Data Quality Objectives (DQOs) and Data Quality Indicators (DQIs)

The overall data quality objective is to collect water samples for identification of potentially toxigenic algal species and cyanotoxin analysis via ELISA methodology. Samples will be collected once per month for 6 months from each site to assess distribution during the algal

growing season. Objectives for accuracy, precision, representativeness, comparability, and completeness are summarized below. Specific data quality indicators are provided in Table 5.

**DQOs**

State the problem- To better understand the occurrence of cyanotoxins in the lakes of South Carolina and the potential risks to drinking water facilities, as well as recreational and aquatic life uses for waterbodies of the state.

1. Identify the decision- This study is an investigative study, so it is possible that there may not be any decisions or actions made from the data obtained. We are studying the distribution of toxins in SC to determine what (if any) water bodies are potential spots for high algal toxin production. We are using these results to not develop a routine monitoring program but to know what potential water bodies of concern are and assess their potential algal production in the future. However, if a situation arises where the cyanotoxin levels in a specific reservoir is above the suggested EPA draft standards (see Table 2), a decision for further action may be called for to prevent any potential or further risk to the water body and its water facilities and/or recreational activities. See number 4 for what decisions should be made in these case by case situations.

2. Identify inputs to the study - Specific Cyanotoxin (i.e. Microcystin and cylindrospermopsin) concentrations in water samples via ELISA assay and possible identification of phytoplankton taxonomy.

3. Define the Study Boundaries- 76 sites located in lakes throughout South Carolina will be sampled once a month for 6 months in 2019. Additional samples may be taken from lakes sampled in 2018 that are not being sampled in 2019. See table 4 and figure 2 for locations of sampling sites.

4. Analytical approach/Decision rule – If microcystin values are < 1.6 ug/L in any of the drinking reservoirs or < 8.0 ug/L in recreation waters, no immediate action will be taken, and the lakes will continue to be routinely monitored. If microcystin values are > 1.6 ug/L in any of the drinking reservoirs or > 8.0 ug/L in recreational waters, Bryan Rabon will be notified and additional samples for toxin and phytoplankton analysis may be collected If sample analysis through this project reveals extreme concentrations of cyanobacteria in recreation waters, the DHEC South Carolina Harmful Algal Bloom response guidance document should be referred to.

5. Specify limits on decision error- Accuracy will be assured by using known standards of microcystin and cylindrospermopsin concentration for each plate that is analyzed. Precision of the samples is determined by using at least 2 well replicates for each sample analyzed on each plate. Samples being collected are to determine if there is a presence or absence of

toxins in the lakes. Since these samples are being collected from routine lake sampling sites, representativeness will be obtained by the other in situ and water samples collected from the same location. Comparability will not be used due to the unique nature of this study and the lack of historical data, but the data may be used for comparability in future studies. In order to achieve comparability for future studies, the same sampling and analytical methods should be used. Completeness of this study is important and thus the goal of this project is to have at least 90% completion. If completion is not met, the project manager will review the incompleteness of the project and if necessary, may require additional sampling after October.

6. Optimize the design for obtaining the data- It is believed that 76 sites sampled once a month for a 6-month period, producing approximately 460 samples, will provide an adequate baseline characterization of the occurrence of cyanotoxins in the reservoirs of SC. All ambient sites will be sampled again in 2019 due to the presence of microcystins in numerous samples analyzed in 2018. The quality of samples and their analysis for harmful toxins will continue to be important in identifying more potential sites to be added to the sampling list the following year due to potential risks associated with high cyanotoxin concentrations in certain reservoirs, as well as specific areas that are "hot spots" for cyanotoxin blooms.

**Table 5. Data Quality Indicators**

| QA Sample Type | Frequency | Acceptance Limit | Corrective Action |
|---|---|---|---|
| ELISA Calibration | Two well replicates per standard | %CV of absorbance ≤10%; ≤15% allowed for 1 pair.<br><br>$r^2$≥0.98 | If the calibration fails the %CV limits or $r^2$is less than 0.98, then the entire Analysis batch is invalid. Assay the samples in a subsequent Analysis Batch. |
| Well Replicates | Assay field and QC samples in at least two wells | Sample invalid if %CV of absorbance values >15% | Sample is invalid and must be noted in results. |
| Quality Control Sample (QCS) | Assay 1 QCS for each new lot of calibration standards. | Percent recovery ≥70% and ≤130% of the true value | QCS exceeding the acceptance limits require reanalysis of samples with results greater than the concentration of an LCRC in the same analytical batch. If reanalysis is not possible, |

| | | | |
|---|---|---|---|
| | | | all sample concentration results greater than an acceptable LCRC analyzed in the same batch must be appropriately qualified and noted in the final report. |

**Precision**

Precision is a measure of agreement among replicate measurements of the same property, under prescribed similar conditions. Precision is expressed in terms of the relative percent difference (RPD) between measurements and is computed as follows:

$$\text{RPD}= \frac{(A-B)}{\frac{(A+B)}{2}} \times 100$$

Precision for this project will be based on the well replicates for the samples in order to assure that the results are valid.

**Bias**

Bias is the systematic occurrence of persistent distortion of a measurement process that causes errors in one direction. Bias assessments for environmental measurements are made using personnel, equipment, and spiking materials or reference materials as independent as possible from those used in the calibration of the measurement system. Bias will be addressed by using standards outside the lab for the calibration of the measurement system as well as using the same equipment and materials to grab all representative samples for the project.

**Accuracy**

Accuracy is a measure of the closeness of an individual measurement or the average of a number of measurements to the true value. Accuracy is determined by analyzing a reference material of known pollutant concentration or by reanalyzing a sample to which a material of known concentration or amount of pollutant has been added. Accuracy is usually expressed as percent recovery. Accuracy is calculated as follows:

$$\%\ \text{Recovery}= \frac{[Analyzedvalue]}{[Truevalue]} \times 100$$

Accuracy for the project will be based on the average of the well replicates analyzed for the known standards in the test kit. Thus, accuracy for this project will be assessed by the percent recovery of the analyzed value of a microcystin or cylindrospermopsin standard over the true value of that standard.

**Comparability**

Comparability is the qualitative term that expresses the confidence that two data sets can contribute to a common analysis and interpolation. In a laboratory analysis, term comparability focuses on method type comparison, holding times, stability issues, and aspects of overall analytical quantitation. EPA approved sampling and analytical methods will be used so that the data is comparable to other studies using these EPA methods. Since this study is based on determining the presence/absence of toxins in SC reservoirs, there is no data set that we will be comparing ours too. However, we will be basing some of our methods for analysis off of EPA Method 546 and the directions that come with the Abraxis test kits.

**Representativeness**

Representativeness is a measure of the degree to which data accurately and precisely represent a characteristic of a population parameter at a sampling point or for a process condition or environmental condition. Representativeness is a qualitative term that should be evaluated to determine whether in situ and other measurements are made and physical samples collected in such a manner that the resulting data appropriately reflect the media and phenomenon measured or studied. Representativeness is established via adherence to specified site criteria, and under implementation of sample collection and analytical SOPs. Representativeness for this project will be ensured by having samples collected for toxins at all the routine lake sampling sites for the 2019 summer. This will ensure proper sample collection by regional staff members as well as provides other environmental conditions of the sampling site, such as pH, temperature, chlorophyll, etc.

**Completeness**

Completeness is a measure of the amount of valid data obtained from a measurement system, expressed as a percentage of the number of valid measurements that should have been collected (i.e., measurements that were planned to be collected.) The degree to which lack of completeness affects the outcome of the study is a function of many variables ranging from deficiencies in the number of field samples acquired to failure to analyze as many replications as deemed necessary by the QAPP and DQOs. Completeness for this study is 90%.

**Method Sensitivity**

Sensitivity is the capability of a method or instrument to discriminate between measurement responses representing different levels of a variable of interest. Sensitivity is determined from the value of the standard deviation at the concentration level of interest. It represents the minimum difference in concentration that can be distinguished between two samples with a high degree of

confidence. Sensitivity for this project is based off the Abraxis plate reader. The plate reader has an optical measurement range of 0.00 to 4.0 absorbance units. With this range and the standards provided with the kit, a curve with the controls and calibrators will be created and stored. Concentrations of the samples and controls are calculated using the stored standard curve. Refer to the Abraxis User manual for more information on the method sensitivity of the plate reader.

**A8 Training and Certification**

Regional DHEC staff members are certified for the collection of water quality samples and will be briefed on the additional collection method for cyanotoxins via QAPP. The ASP staff will be certified and trained for cyanotoxin analysis via the kit provider, Abraxis. Initial Demonstration of Capability (IDC) must be performed before the staff member can analyze samples or when a new analyst begins work. A continuing demonstration of capability (CDC) is performed annually by each analyst or whenever a change in analytical performance caused by either a change in instrument hardware or operating conditions would dictate the MDL must be recalculated **(**Refer to SOP Section 10). The project manager is responsible for assuring that all analysts satisfy the IDC's and CDC's. Documentation for IDC's and CDCs are maintained by the laboratory and stored in a binder at the ASP lab (see Table 5).

**A9 Documentation and Records**

**QAPP Formulation and Distribution**
Emily Bores is responsible for writing, maintaining and distributing the QAPP. The approved QAPP will be distributed electronically. If the QAPP needs to be revised during the study period, the person in charge of the QAPP will do so and submit to the QAM, or designee, for approval. Once the revised QAPP is approved, the updated QAPP is sent to those individuals on the distribution list. If there are major changes to the QAPP, then the entire document will be distributed. If there are only minor changes to a few pages, these pages will be distributed with directions of which pages to pull from the QAPP and which to insert. A delivery receipt request will be sent with the updated QAPP and/or QAPP portions, so the recipient must reply indicating that they have received the updates and are using them.

**Data Report package:**
Data will be reported in electronic Excel spreadsheet and electronic PDFs of resulting curves from the analysis. The values will be reported in parts per billion (ppb) or micrograms per liter (ug/L), which are equivalent. Another data report may be included in the report package containing taxonomic analysis of phytoplankton. Table 6 delineates the items that will be in the Excel spreadsheet with numerical data. The project manager is responsible for updating and reviewing the excel sheet.

**Other records generated by this project**:

The information in Table 6 is an itemized list of the records generated by the project and how they are stored.

**Table 6. Project Records and Archives**

| Item | Produced by: | Hardcopy/Electronic | Storage Location/Time | Archival | Disposal (Time) |
|---|---|---|---|---|---|
| Chain of Custody | Field personnel | Hardcopy | Filled out in field and shipped with samples. | Stored at ASP | 10 years |
| Corrective Action Reports | Program Manager | Electronic | Reported in excel sheet with data results | ASP-cyanotoxin folder | 10 years |
| Sample Prep Form | Laboratory personnel | Hard Copy | Stored in folder | ASP | |
| Training Logs, including IDCs and CDCs | Laboratory personnel | Excel | Initial Demonstration of Performance records for each analyst | ASP-cyanotoxin folder | 10 years |
| Data Report | Laboratory personnel | Both | Stored in folder on computer with a hard copy print off for the cyanotoxin folder | ASP Lab | 10 years |
| QC Narrative | Laboratory personnel | Both | Stored in folder on computer with a hard copy print off for the cyanotoxin folder | ASP Lab | 10 years |

# Section B Measurement/Data Acquisition

## B1 Sampling Process Design (Experimental Design)

### Schedule of Project Sampling Activities

Sampling will begin 05/01/19 and end on 10/31/19. Samples will be collected once a month during the algal growing season (May-October). See Table 3 in section A6 for the list of proposed sampling activities for this project.

### Description of Sample Design Strategy and Sample Sites

The sampling locations were chosen by SC DHEC based on the current 2019 lake site sampling schedule. If affected by cyanotoxins, these sites could affect human health due to their use for recreational activities and drinking water. The sample locations for this project are provided in Table 4 and Figure 2 of section A6. The 76 sites will each be sampled once a month for 6 months, equating to about 460 total samples being tested for Microcystins. Samples from 2018 showed overall, no sites with quantifiable amounts of Cylindrospermopsin. Since we missed the early part of the sampling season in 2018 (May-July) we will plan to analyze samples for Cylindrospermopsin from May 2019 - July 2019. If we see no quantifiable results during that time period for Cylindrospermopsin, sampling will be discontinued. Samples from regional staff will be overnighted via State courier to the cyanotoxin lab in the Aquatic Science Programs once collected.

The sites being sampled for this project are established DHEC sites and will thus be identified by their DHEC numbers. These sites are listed in Table 4 of section A6. All the sites will be accessed by boat via public boat landings or public docks. If a private dock is used for an algal bloom complaint, consent from the landowner must be obtained before the sample can be taken. In the field, the site locations will be located via the description provided in Table 4. The samples collected will be grab samples and collected from the surface 0.3m below the water surface. Samples will be identified with the site name and the sampling date.

The weather will be the main source of variability for this project. Sampling dates and times may have to be rescheduled due to weather events such as thunderstorms, hurricanes, droughts, etc. as they may affect field sampling locations and activities. If the sites become inaccessible, sampling will not occur, and field staff will return within a week to resample the site. If the site becomes permanently inaccessible, another site may be substituted for sampling on the same waterbody.

## B2 Sampling Methods Requirement

### Sample Collection SOP:

A single water sample for cyanotoxins and/or phytoplankton analysis will be collected once a month at each site.
All sample collection, field analysis, handling, preservation, and Chain of Custody (COC) will be done as follows:

1. The sample will be collected at the site location using a boat or dock to reach the area.
2. The COC is filled out just prior to sample collection (see appendix).
4. A 1L Polyethylene Terephthalate bottle will be used and the samples will be collected via grab sample 0.3m below the surface. A minimum of 500 mL of sample must be collected.
5. Once the bottle is filled, the sample lid will immediately be replaced. No preservative is needed for the samples that are solely being analyzed for toxins.
6. Samples are not to be composited, split, or filtered in the field.
7. The sample information is written on the bottle and logbook. This includes
    a. Site name
    b. Date and Time of collection
8. The time the sample was collected is written on the COC and logbook.
9. Samples will be placed in ice in coolers immediately. Coolers will be shipped via State courier overnight to the ASP lab in Columbia where the samples will be placed in the freezer (-20 C). The temperature blank in the cooler must be ≤ 10ºC upon arrival of the samples in the lab.
10. Since the samples are collected via grab samples directly into the sterilized container, there is no additional sampling equipment that needs to be cleaned or decontaminated.
11. There is no additional in situ or continuous monitoring for this project beyond what is specified in the State of South Carolina Monitoring Strategy for CY 2019 for the Ambient Surface Water Quality Monitoring Program.
12. If any problems occur during sampling, the Field manager is responsible for any corrective action that needs to be taken.

**B3 Sample Handling and Custody Requirements**

Samples for toxin analysis should be shipped via State courier overnight to the ASP lab in Columbia (within 24 hours of sampling). At the lab, samples will be transferred into a 40mL vial and frozen in a -20 C freezer. If samples are frozen at -20 C the holding time is 2 weeks. The field managers will be responsible to oversee the transportation of the samples and the chain of custody sheet to the ASP lab. Once the COC is signed, and the samples are relinquished to the laboratory, then the cooler is opened, and the temperature blank is read. This temperature is documented on the COC. Besides the COC and the bottle, each sample grab time will be logged in the Field Investigators Field Log book. The Field Log book is kept with the field manager when not in the field. The project manager will be responsible for keeping in contact with the field managers and making sure the transportation of samples occurs efficiently and on time. The COC is provided at the end of the QAPP.

**Sample Identification**
Each sample will be identified using the SC DHEC station number labeled on the sample container. These codes are provided in Table 4 of section A6. At the lab, sample custody forms are compared to sample container labels to ensure all samples are accounted for.

**Sample Labeling**
The date, time, and location of the site will be labeled directly on the lid of the sampling container by field personnel using a sharpie. The bottle is labeled directly before or after the sample is collected.

**B4 Analytical Methods**

Samples will be analyzed for the toxins Microcystins and Cylindrospermopsin using Enzyme Linked Immunosorbent Assay (ELISA). The analysis is based off EPA method 546 with technical guidance from the supply provider, Abraxis. The analytical SOP for the ELISA is referenced in Table 7. The primary instrumentation required for analysis is listed in Table 7 and all other necessary equipment is listed in the individual SOP that is attached as an appendix. The method performance criteria are found in Table 7 and in the individual SOP that is attached as an appendix. The turnaround time for this analysis is 2 weeks. Since this project is for the analysis of ambient water only, the analytical methods being used have been approved by the EPA. Chris D. Decker, the Regional Water Quality Monitoring Coordinator for US EPA Region 4, stated

“Since your project involves collecting ambient water rather than drinking water, we do not have any reservations with the QC measures described below. In addition, your plan to follow the advice of the test manufacturer and NOAA when analyzing ambient water is technically sound.”

**Table 7. Analytical Method and Performance Criteria**

| Analyte | Matrix | SOP | Rev # and Date | Method Ref | Instrument | Test Sensitivity |
|---|---|---|---|---|---|---|
| Total Microcystins | Water | 8/28/18 | Rev 1 06/2018 | EPA 546, Ohio EPA DES 701.0 Version 2.2, Abraxis product inserts | Abraxis 8-channel microplate reader; Model 4303 | 0.10 ppb (µg/L) |
| Cylindrospermopsin | Water | 8/28/18 | Rev 1 06/2018 | EPA 546, Ohio EPA DES 701.0 Version 2.2, Abraxis product inserts | Abraxis 8-channel microplate reader; Model 4303 | 0.040 ppb (µg/L) |

**Sample Disposal at the Laboratory**
Samples are scheduled for disposal at the ASP based on their holding times; after 2 weeks from the date they were frozen and after the sample has already been successfully analyzed. Analysts must verify with the project manager before disposing of any samples. Water samples are disposed on site in the lab's sanitary sewer (the sink). No disposal form is needed for the project file.

**Corrective Action Procedures**
Each individual engaged in analytical laboratory activities should be alert to problems, deviations from approved procedures, out-of-control events, or other issues that may require corrective action. The appropriate response is determined by the event. The responsibility for resolution of deviations and reporting them lies with the project manager. Briefly, deviations are classified as simple, minor, and major occurrences:

**Simple Deviation:** A simple deviation is a deviation from project control limits. The situation is documented either in log books, or on project paperwork including the case narrative.

**Corrective Action**- Document the situation and look for opportunity to correct the situation.

**Minor Deviation**- A minor deviation is defined as method or protocol deviation that does not appear to adversely impact the quality of the data. A minor deviation may evolve into a major deviation if an impact on data quality occurs.

**Corrective Action**- Determination of a minor deviation will be initiated by the project manager. The corrective action will be established to assure the highest quality of data is produced and that all limits are met. It is possible for a minor deviation to result in a major deviation depending upon all circumstances.

**Major Deviation**- A major deviation is defined as an occurrence or method or protocol deviation with an impact on project data quality or a negative effect on the outcome of a test or analysis.

**Corrective Action**- Formal documentation. Data will be invalidated and analysis must be repeated, if possible.

**B5 Quality Control Requirements**

An initial demonstration of capability (IDC) must be successfully performed prior to analyzing field samples. Refer to the attached SOP for IDC requirements. The QC requirements in Table 8 are considered the minimum acceptable QC protocol. EPA Region 4 confirmed that the QC measures described below are satisfactory for ambient water sampling.

**Table 8. Analytical QC Samples**

| Requirement | Specification and Frequency | Acceptance Criteria | Corrective Action |
|---|---|---|---|
| ELISA Calibration | Use kit-recommended levels and concentrations. Two well replicates per standard | %CV of absorbance ≤10%<br>≤15% allowed for 1 pair<br><br>$r^2$≥0.98 | If the calibration fails the %CV limits or $r^2$ is less than 0.98, then the Analysis Batch is invalid. Assay the samples in a subsequent Analysis Batch. |
| Well replicates | Assay field and QC samples in two wells | Sample invalid if %CV of absorbance values >15% | If the %CV exceeds 15% for a field sample of QC sample, then that sample is invalid. |
| Quality Control Sample (QCS) | Assay 1 QCS for each new lot of calibration standards. Prepare the QCS near the EC50 with MC-LR from a source independent of calibration standards | Percent recovery ≥70% and ≤130% of the true value | QCS values exceeding the acceptance limits require |

**Table from EPA Method 546**

## B6 Instrument/Equipment Testing, Inspection Maintenance

**Table 9. Maintenance for Field Equipment**

| Instrument | Type of Maintenance | Frequency | Parts needed/Location | Person responsible |
|---|---|---|---|---|
| Hand held GPS | Batteries changed | As needed-minimally once per year | AA batteries/ storage cabinet/shelves in field office | Operator |
| Boat | Maintain boat for reliable working conditions | Quarterly and as needed | As needed dependent on repair | operator |

**Table 10. ELISA Instrument Maintenance, Operation, and Preventative Maintenance**

| Maintenance | Activity | Performed by | Corrective Action |
|---|---|---|---|
| Lamp Replacement | Adjustment and/or replacement of lamp anytime the "Lamp Output Low" message is generated. | Analyst | If the signal drops below 1 volt, the message will be triggered, and the lamp will need to be replaced. |
| Voltage Meter | Select Voltage Meter from the maintenance option on the toolbar in Abraxis reader | Analyst | Acceptable voltage readings are within in the "greater than 2.0" and "less than 10.0" range |
| Firmware Update | Allows the user to update to a new firmware version. | Analyst with help from technical support | Enables user to browse a list of files. Technical support will advise which file to select. |
| Calibration Lock/Unlock | Emergency use only be authorized personnel in case the device needs to be recalibrated. | Contact technical support for direction | |

Note- there are no user-serviceable parts inside the instrument. Refer servicing to qualified service personnel. Use only factory-authorized parts. Failure to do so may void the warranty.

Refer to Section 6 of the A Reader Abraxis Model 4303 Operators Manual for any issues with troubleshooting.

## B7 Instrument Calibration and Frequency

Calibration records for equipment will be kept on Excel file as well as hard copy in the ASP Lab.

**Table 11. Instrument Calibration and Frequency for ELISA reader**

| Calibration Procedure | Frequency of Calibration | Acceptance Criteria | Corrective Action (CA) | Person Responsible for CA | SOP Reference |
|---|---|---|---|---|---|
| Standard Properties | Every time an analysis is conducted | Enter the concentration for each standard used | | Analyst | 5.3.2.2 in Abraxis Model 4303 Operators manual |
| Curve Valid Time | Set the amount of time in days, hours, or both, that the standard curve should remain valid. | If no entry is made for Day(s) or Hour(s), expiration will be set at the default of (7) days | Once a calibration curve reaches the end of the valid time period, the Calibration Tab will indicate "expired". Set the amount of time. | Analyst | 5.3.2.3 in Abraxis Model 4303 Operators manual |
| Blank Properties | When 'use blank' is selected, the properties button is enabled. | Whatever valid time period the analyst assigns to the blank | Click on properties to enter an absorbance range value and gain access to options of 'issue warning' or 'invalidate tests' as action to take when result is out of range, and to set the valid time, in days/hours. | Analyst | Section 5.3.2.4 in Abraxis Model 4303 Manual |
| Controls | Set the amount of time in days, | Set up the out of range and the Valid | Once a control reaches the end of the valid time | Analyst | Section 5.3.2.6 |

| | | | | | |
|---|---|---|---|---|---|
| | hours, or both, that the controls should remain valid | Time the Control (s). If no entry is made for Day(s) or Hour(s) expiration will be set at the default of (7) days | period, the calibration tab will indicate "expired" | | |
| QC Criteria | Whenever a new parameter for controls need to be entered | Acceptable ranges for controls are entered in QC criteria. | To enter parameters for your controls, select the QC criteria button to clock on the control desired and then on the operators and values you require. | Analyst | Section 5.3.2.7 |

**B8 Inspection/Acceptance Requirements for Supplies and Consumables**

| Item | Vendor | Acceptance Criteria | Handling/Storage Conditions | Person responsible for inspection and tracking |
|---|---|---|---|---|
| Latex Gloves | All | No holes | 1 box of appropriate size in lab | Emily Bores (Project manager), ASP lab |
| 4mL and 40mL vials | All | Borosilicate glass with PTFE-lined caps. Glass not broken. | Office prep area-room temp | Emily Bores (Project manager), ASP lab |
| Luer Slip Syringe | All | 3mL with Luer-Lock connection | Office prep area-room temp | Emily Bores (Project |

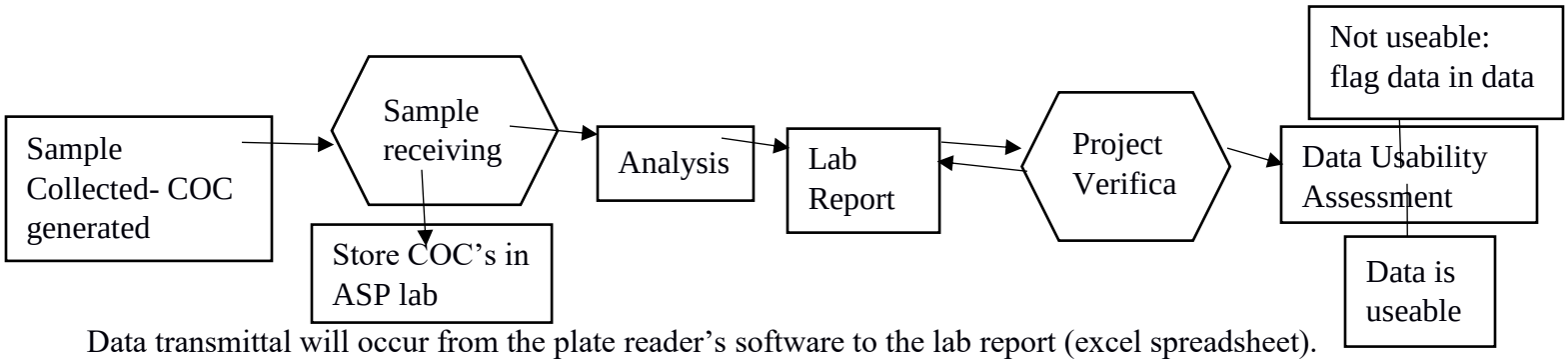
| | | | | |
|---|---|---|---|---|
| | | | | manager), ASP lab |
| Syringe Filters | All | Glass microfiber filter, 30mm with 1.2 µm pore size | Office prep area-room temp | Emily Bores (Project manager), ASP lab |
| PET Storage Bottles | All | Has to be PET material, at least 1L volume | Office prep area-room temp | Emily Bores (Project manager), ASP lab |
| PTFE Discs | US Plastics | Discs must be PTFE, 38mm disc for 1L bottle | Office prep area-room temp | Emily Bores (Project manager), ASP lab |
| Parafilm | All | | Office prep area-room temp | Emily Bores (Project manager), ASP lab |
| Microcystins and Cylindrospermopsin ELISA plates | Abraxis | Kits must be complete (i.e. include all standards) and not broken. Must be within expiration dates | Refrigerator at 4-8 C | Emily Bores (Project manager), ASP lab |
| Pipette tips | All | Must have volume of at least 50µL and up to 300µL | Office prep area-room temp | Emily Bores (Project manager), ASP lab |
| Precision Dispenser (PD) Tips | All | Volume of 1mL | Office prep area-room temp | Emily Bores (Project manager), ASP lab |

**B9 Data Acquisition Requirements for Non-Direct Measurements**

Since there is little known about the occurrence of cyanotoxins in the lakes of SC and this is an investigative study in order to better understand the possible distribution, there are no intended sources of previously collected data (not applicable) and other information that will be used in this project. The data collected in the 2018 project is used as reference for the 2019 project. Data from both years may be used as reference and/or guidance for any future projects.

B10 Data Management

**Figure 3. Data Management Scheme**




Data transmittal will occur from the plate reader's software to the lab report (excel spreadsheet). The software will allow for the data to be downloaded electronically on the computer via excel file. The analysts are responsible for the data transmittal and the project manager is responsible for reviewing each transmittal. David Chestnut is responsible for the data quality during the process. He will review the data in the generated lab report to make sure that the results were accurately recorded and check for any errors. If any errors are found in the lab report, the project manager is responsible for correcting that error. The data from the COC (i.e. field parameters such as temperature, pH, etc.) and the data generated from the analysis will be recorded electronically via excel spreadsheet. Data can be retrieved through this spreadsheet on the computer. The hard copies of the COC will be archived in the ASP lab for at least 10 years. The excel spreadsheet of the data will be maintained for 10+ years. If possible (permitting space requirements), do not dispose of the COC or lab reports even after the 10-year deadline.

The microplate reader and Abraxis reader software are the hardware and software items that will need to be routinely tested and upgraded. Refer to Table 11. This software and hardware are proprietary and are acceptable for this project. For ELISA Instrument Maintenance, Operation, and Preventative Maintenance. If updates are required for the test menu, contact the dealer at Abraxis. Also refer to the User manual (Abraxis Reader Operator's Manual Doc. 4303 Rev. D) for more assistance.

# Section C Assessment and Oversight

## C1 Assessment and Response Actions

Since this is a short-term research project, few assessments will be conducted. The Project Manager is responsible for responding to and resolving all quality assurance problems and needs. The Project Manager will initiate corrective action to adverse conditions that compromise quality in the field or laboratory. A thorough periodic review of the complete data review process, including a review of the sample analysis verification, sampling and analysis validation, and data usability steps, will be conducted to ensure that the process conforms to the procedure specified in the QAPP. Elizabeth basil is responsible for field QA/QC and the project manager is responsible

for the Lab QA/QC. Any evaluation or progress reports requested by USEPA Region 4 will be addressed directly to Region 4.

**C2 Reports to Management**

A final QA management report including the summary of the project, QA/QC, training, conformance and nonconformance of project activities, etc. will be submitted as a final report to the EPA once the sampling and analysis is completed. The report will also include status of the project, schedule delays, results of data review activities in terms of amount of usable data generated, required corrective actions and effectiveness of the implemented corrective actions, data usability assessments in terms of DQIs, and limitations on the use of the data generated. The project manager will write this report and submit it the Bureau of Water's Division of Water Quality Management, Assessment and Protection, for final review and reporting of all monitoring results to the EPA.

## Section D Data Validation and Usability

**D1 Data Review, Verification and Validation**

**Table 12. Data Criteria**

| Item | Data Standards | | If this criterion is not met, is the sample rejected or flagged? |
|---|---|---|---|
| Sample Temperature | Sample temperature blank is below 10ºC | | Flagged (may be rejected at analyst's discretion) |
| Analysis Time | Two weeks from time of sampling if in a -20C freezer. | | Flagged |
| Hold Time | Samples arrives at the lab within 24 hours after collection | | Flagged (may be rejected at analyst's discretion) |
| ELISA calibration | See Table 5 | | Analysis Batch invalid |
| Well Replicates | See Table 5 | | Samples invalid |
| Quality Control Sample (QCS) | See Table 5 | | Flagged (may be rejected at analyst's discretion). Reanalysis if possible |

When reporting data, the following example data flags will be used where appropriate:

**A** The analyte was analyzed in replicate. Reported value is an average of the replicates

**P** Sample improperly preserved and/or collected

**R** The presence of absence of the analyte cannot be determined from the data due to sever quality control problems. The data are rejected and considered unusable.

**U** The analyte was not detected at or above the reporting limit

**D2 Validation and Verification Methods**

**Data Validations**

Prior to their release from the laboratory data will be validated. Validation is defined as the process through which data are accepted or rejected and consists of proofing, verifying editing, and technical reviewing activities. Data validation will occur at multiple levels as data are collected and processed. These levels include:

Individuals recording data during field or laboratory operations are responsible for verifying their work at the end of the day to ensure that the data are complete and accurate.

Analysts and instrument users are responsible for monitoring the instrument operation to ensure that the instrument has been properly calibrated.

Laboratory analysts and project Managers are responsible for verifying analytical and supporting documentation to assess sample holding times and conditions, equipment calibration, and sample integrity. As an additional measure of acceptability, the results of QC samples are compared to the project DQOs of section A7.

Technical staff is responsible for reviewing the data for scientific reasonableness.

All manual entries into databases and spreadsheets are verified, either through proofing or by double entry/comparison programs and all calculations performed by hand are checked for accuracy.

Complete data packages including sample and analysis plan, hard copies of instrument outputs, and summary data sheets are provided to the laboratory technical leader or designee for review. Analytical data packages are reviewed against a checklist. Data are reviewed to ensure that the data are accurate, traceable, defensible, and complete, as compared to the planning documents and/or project requirements. Concerns that can be corrected will be corrected before the data are released. Deviations are required to be summarized and provided to the client.

Data that do not meet the established criteria for acceptance may be flagged, not reported, or reported with an explanation of the limitations, at the discretion of the Project Manager.

David Chestnut will be responsible for validating all components of the project data/information. See Table 13 for items that are used for validation. Following internal data validation and the correction of any errors discovered, the data will be forwarded to the project manager. The project manager reviews the field data and ensures that for every sample sent to the laboratory, a result was received. This check will ensure that the sample data is complete. The project manager will determine completeness was achieved. Completeness is expressed as a percentage of the number of valid measurements that should have been collected (see section A7).

If issues arise from the validation and verification, the project manager is responsible for conveying these results to data users. The goal of this project is to reach 90% completeness and if this is not achieved, then the Project Manager may contact the data users as well as the Field Sampling Staff and Laboratory that the project will be extended to increase the amount of valid data. Once the data has been determined to have met project quality objectives, it will then be logged into the database, STORET.

**Table 13. QA Items Validated**

| QA Item | Comments/Purpose |
|---|---|
| Chain-of-custody for each sample | Must include sampling location and include the handling of the sample from collection to final disposal. Preservation information and condition of the sample upon receipt to the lab must also be included. This allows the Validator to assess if sample treatment was according to the QAPP and allow the Validator to look for anomalies such as time travel (example: when the sample arrives at the lab before it has been collected) |
| Methods and SOPs (sampling and analysis) | Must be checked against what was originally dictated in the QAPP. If deviations exist, the validator would assess the impact. |
| Detection Limit information for each method and analysis | The Validator would determine if the detection limit requirement was met by the lab. If not, the Validator would assess the impact of this on the study. |
| List of Qualifier Flags from the lab and an explanation for each | Depending on the flag, the Validator will assess the impact of these flags. The list of these flags will be reported and kept in the binder with the results from each analysis. |
| Sample chronology (time of receipt, extraction and analysis) | Will allow the Validator to determine that the sample was within hold time when analyzed and to note anomalies. |
| Calibration Data associated with each sample analysis | The Validator will determine if the standards and controls ran with the samples in an analysis batch pass the calibration requirements. |
| Documentation of Laboratory Method/ SOP Deviations | The lab may report this, and the verifier will include it in the report, or the verifier may well note this as part of the verification process and report it. The Validator will assess the impact of this on the study. |
| Reporting Forms with actual results | These are checked for transcription errors by the Validator. |

**D3 Reconciliation and User Requirements**

The primary data user is the South Carolina Department of Health and Environmental Control. The intended use of this project is to investigate the occurrence of potentially toxigenic algae in South Carolina reservoirs to determine the future direction of a State HABs surveillance program. As this is primarily an investigative study one of the important outcomes is the evaluation of the performance of all aspects of this project and recommendations for future improvements. Any limitations on data due to issues found during verification and validation will be included in the final report.

## E. Revision History

| Date | Revision | Change | Section |
|---|---|---|---|
| Feb 2019 | 1.0 | Added Revision History | E |
| Feb 2019 | 1.0 | Updated Background Information for 2019 | A5 |
| Feb 2019 | 1.0 | Updated Draft Swimming Advisory Numbers | Table 2 |
| Feb 2019 | 1.0 | Updated number of sites and sampling period for 2019 | A6, A7, B1 |
| Feb 2019 | 1.0 | Updated Project Actions for 2019 | Table 3 |
| Feb 2019 | 1.0 | Updated site descriptions for 2019 | Table 4 |
| Feb 2019 | 1.0 | 2019 sampling locations map | Figure 2 |

## Literature Cited

DHEC 2015. South Carolina Harmful Algal Bloom Response Guidance. South Carolina Department of Health and Environmental Control. Bureau of Water, Aquatic Biology Section. Columbia SC.

EPA. 2015. Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by Adda Enzyme-Linked Immunosorbent Assay. U.S. Environmental Protection Agency, Office of Ground Water and Drinking Water. EPA 815-B-16-011.

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EPA. 2016. Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin: Draft. U.S. Environmental Protection Agency, Office of Water. EPA 822-P-16-002.

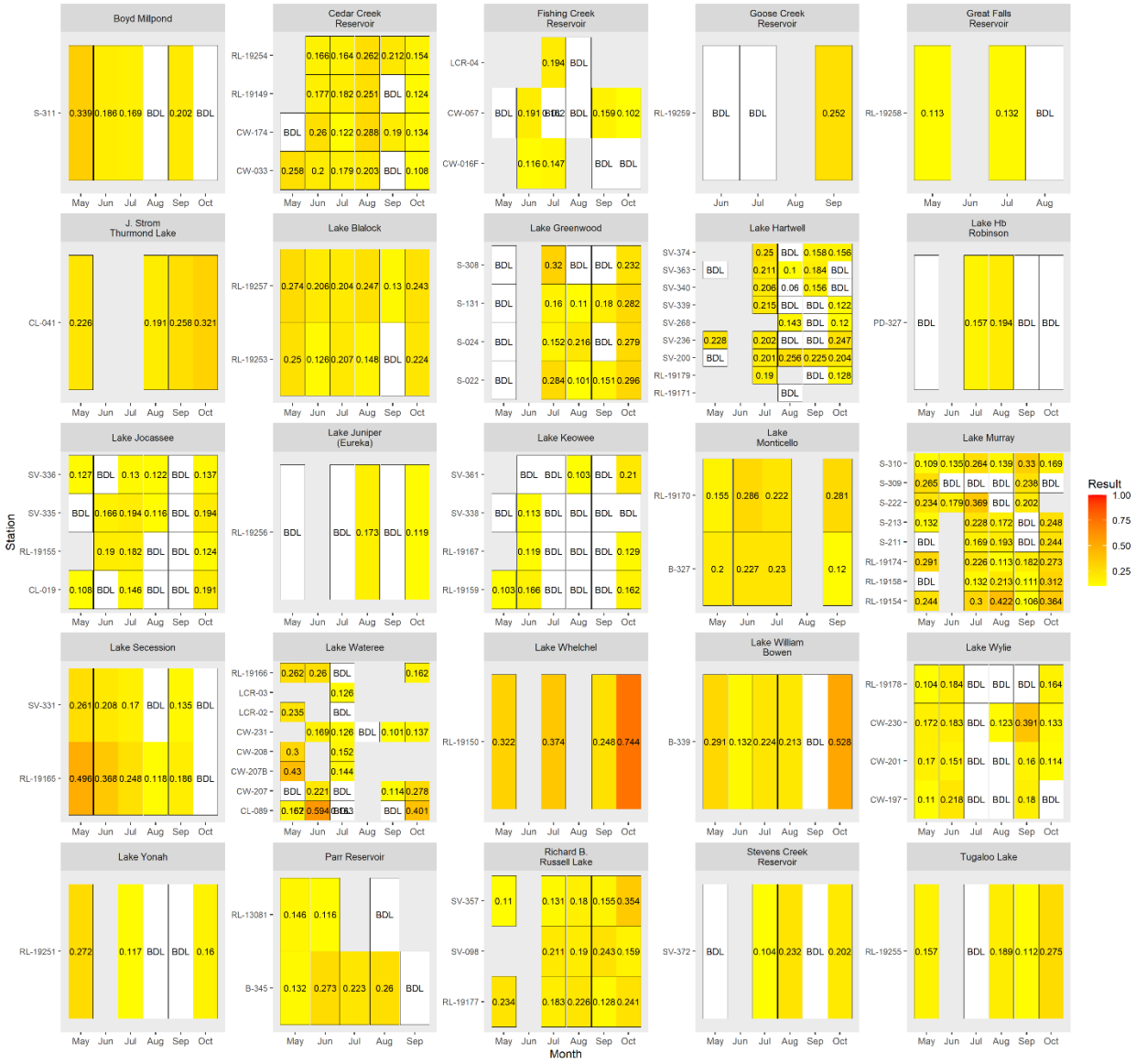
Jetoo, S. Grover, V. and Krantzberg, G. 2015. The Toledo drinking water advisory: Suggested application of the water safety planning approach. *Sustainability* (7): 9787-9808.

## Appendix

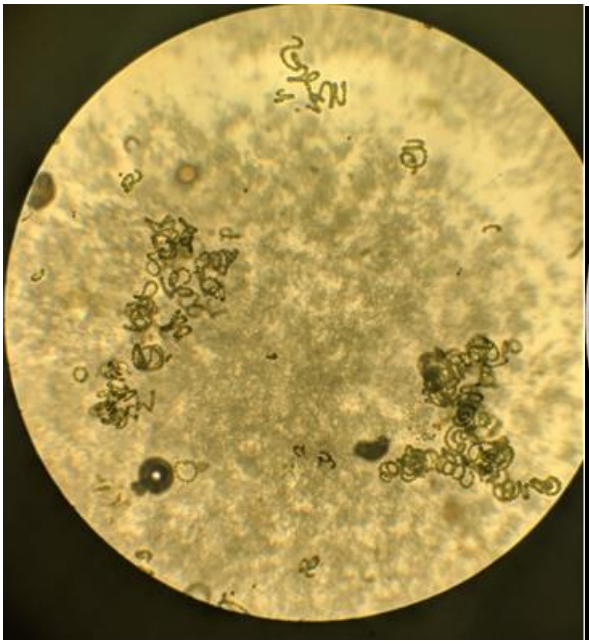
| dhec | Ambient Water Monitoring | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Type: Routine ( ) \| Complaint ( ) \| Special Studies ( ) \| 319 ( ) | | | | | Charge Code: | | | |
| Stream Run: | | | | | Return To: | | | |
| Date: | | Collector: | | | | | | |
| Laboratory Number | | | | | | | | |
| Region Lab ID | | | | | | | | |
| Station | | | | | | | | |
| Time (HHMM)(Military) | | | | | | | | |
| Depth (m) | 82048 | | | | | | | |
| Field pH (su) | 00400 | | | | | | | |
| Field D.O. (mg/L) | 00300 | | | | | | | |
| Temp., Water (◦C) | 00010 | | | | | | | |
| Salinity (ppt) | 00480 | | | | | | | |
| Conductivity (umhos/cm) | 00402 | | | | | | | |
| Secchi Depth(m) | 00078 | | | | | | | |
| Total Alkalinity (mg/L) | 00410 | | | | | | | |
| Turbidity (NTU) | 00076 | | | | | | | |
| $BOD_5$ (mg/L) | 00310 | | | | | | | |
| Residue Sus. (mg/L) (TSS) | 00530 | | | | | | | |
| E. Coli (Q-tray) P1<br>Bottle Lot # | 31633 | | | | | | | |
| Enterococci (Q-tray) P1<br>Bottle Lot # | 50589 | | | | | | | |
| Chlorophyll | 32209 | | | | | | | |
| TKN P2 | 00625 | | | | | | | |
| $NH_3^+$ $NH_4^+$ P2 | 00610 | | | | | | | |
| $NO_3/NO_2$ -N P2 | 00630 | | | | | | | |
| Total-P P2 | 00665 | | | | | | | |
| Total-N P2 | 00680 | | | | | | | |
| Dissolved Ortho-P | 00671 | | | | | | | |
| Cadmium P3 | 01027 | | | | | | | |
| Calcium P3 | 00916 | | | | | | | |
| Chromium P3 | 01034 | | | | | | | |
| Copper P3 | 01042 | | | | | | | |
| Iron P3 | 01045 | | | | | | | |
| Lead P3 | 01051 | | | | | | | |
| Magnesium P3 | 00927 | | | | | | | |
| Manganese P3 | 01055 | | | | | | | |
| Mercury P3 | 71900 | | | | | | | |
| Nickel P3 | 01067 | | | | | | | |
| Zinc P3 | 01092 | | | | | | | |
| Hardness P3 | 00900 | | | | | | | |
| Aluminum P3 | 01105 | | | | | | | |
| Beryllium P3 | 01012 | | | | | | | |
| Thallium P3 | 01059 | | | | | | | |
| Other: | | | | | | | | |
| Other: | | | | | | | | |
| Other: | | | | | | | | |
| Comments: | | | | | | | | |
| Preservative Used \| P1 - $Na_2S_2O_3$ □ \| P2 - $H_2SO_4$ □ \| P3 - $HNO_3$ □ | | | | | All Samples Iced □ \| Cooler Temp: | | | |
| Relinquished By: | | | Received By: | | | Date/Time: | | |
| Relinquished By: | | | Received By: | | | Date/Time: | | |
| Relinquished By: | | | Received By: | | | Date/Time: | | |
| Relinquished By: | | | Received By: | | | Date/Time: | | |
| Data released from ARESD By: | | | | | | Date: | | |

DHEC 3271 (01/2018) White - **Central Office**; Canary - **Lab**; Pink - **District**

**Appendix 3**: Results of 2019 microcystin analyses, which are organized by Lakes, sites within those lakes, and the analytical results for each of the sites based on the sampling month. Results that are below the detection limit (BDL) are white. The results that are yellow can be compared to the scale for the right concentration comparison.

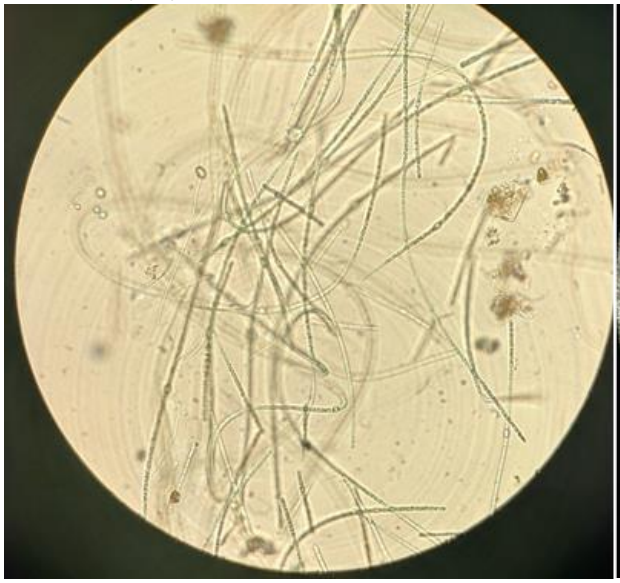


**Appendix 4:** Microscopic images of cyanobacteria from the 2019 HAB complaint sites.

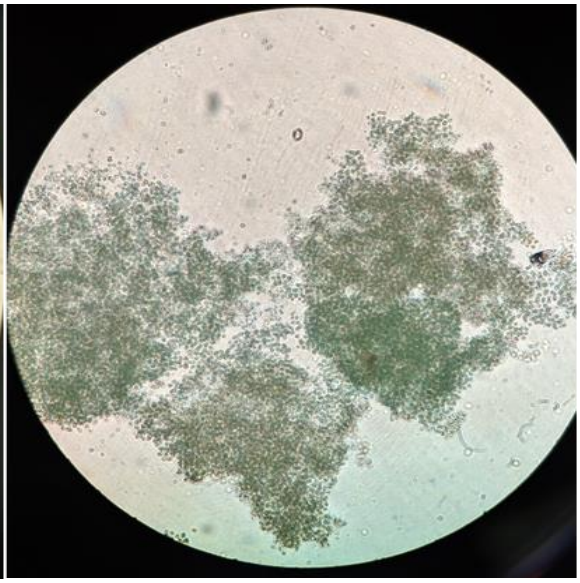


*Dolichospermum sp.* bloom on Goose Creek Reservoir- 04/16/19

*Trichormus sp.* on Lake Wateree- 09/23/19



*Dolichospermum sp.* on Lake Rabon 08/2019



*Microcystis sp.* bloom on Anne Springs Close Greenway 09/19/2019