BUREAU OF WATER

South Carolina Department of Health and Environmental Control

2018 South Carolina Cyanotoxin Distribution Project

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Technical Report No. 022-2020

S.C. Department of Health and Environmental Control

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Prepared By:

Emily Bores and Lindsey Lachenmyer

SCDHEC – Bureau of Water, Aquatic Science Programs

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Executive Summary

There is growing concern in the United States about harmful algal blooms (HABs), which occur due to rapid algal growth. HABs can cause harm to humans and their localized environment. One of the most common types of HABs, especially in freshwater, is due to cyanobacteria, a microscopic organism. HABs of cyanobacteria can produce toxins in high enough concentrations to impact human health and freshwater or marine life. Cyanobacteria toxins (cyanotoxins) can also produce taste and odor issues in drinking water, which increases the need for improved drinking-water treatment. In order to protect public health and the environment, the Department developed and commenced a HABs Monitoring Program, particularly cyanobacteria toxins, that began in 2018. This assessment report covers the cyanotoxin work completed that year.

The main purpose and general questions posed to be addressed by this assessment were:

- Establish baseline data for cyanotoxin distribution in State reservoirs and influent and selected streams
- Did any monthly-monitoring or event-driven sampling exceed any recommended U.S. Environmental Protection Agency (USEPA) criteria?
- Were there notable potential correlative relationships between cyanotoxin concentrations and other physicochemical water quality parameters measured?

From August to November 2018, samples were collected from 62 monthly-monitored sites across several South Carolina reservoirs and selected influent stream for two (2) cyanotoxins: microcystins and cylindrospermopsin. The monthly-monitored sites were coordinated with other sampling conducted by SCDHEC regional field staff which allowed data comparison to other parameters collected contemporaneously. Also, water samples from seven (7) event-driven samples were analyzed for microcystins and cylindrospermopsin in the August to November 2018 sampling period.

The toxin results for the monthly-monitoring and event-driven sampling were less than 1 microgram per liter (ug/L) for both microcystins and cylindrospermopsin. These concentrations were well less than the USEPA's recommended recreational action levels of 8 ug/L for microcystins and 15 ug/L for cylindrospermopsin. Almost half of the monthly samples collected had detectable concentrations of microcystins, whereas only one (1) monthly sample had a detectable level of cylindrospermopsin. Lake Whelchel in Cherokee County had the highest concentrations of microcystins in comparison to the other waterbodies. Lake Murray had the only monthly-monitoring sample with a detectable level of cylindrospermopsin. A correlation analysis was conducted for the monthly-monitoring microcystin data for Lake Murray, which had the largest sample size with detectable levels. No relationships could be concluded when comparing microcystins concentration to some of the other parameters (dissolved oxygen, pH, temperature, total phosphorous, nitrogen: phosphorus ratio, and chlorophyll A) collected in Lake Murray. However, this was a small sample size and a larger dataset would likely prove beneficial for future correlation analyses.

Overall, this assessment will aid in establishing a baseline for cyanobacteria toxin levels in waterbodies across South Carolina and provide insight into enhancement of the HABs Monitoring Program. The future goals of the HABs program include continuing to develop the Statewide sampling strategy and to collect samples during the full algal growing season of May through October (typical).

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 lead to harmful effects on freshwater or marine life and humans. There is an increased need for monitoring cyanotoxin concentrations in waterbodies and water treatment plants due to HABs that have impacted drinking water (Jetto, Grover, & Krantxberg, 2015). The U.S. Environmental Protection Agency (USEPA) has formulated health advisory criteria for two (2) cyanotoxins (microcystins and cylindrospermopsin) (U.S. Environmental Protection Agency, 2019). and recreational advisory criteria (U.S. Environmental Protection Agency, 2015b,c). Exposure to microcystins can lead to liver, reproductive, developmental, kidney, and gastrointestinal effects (U.S. Environmental Protection Agency, 2019). Exposure to 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) and its predecessor has had a robust surface water monitoring network since the 1950s; however, cyanotoxins have not been included in the suite of analytes normally tested due to a lack of instrument capability to achieve the necessary detection limits to yield a dataset that can be interpreted. Analytical methods have improved greatly, and it is now possible to detect cyanotoxins at low concentrations. As a result, SCDHEC created the HABs Monitoring Program in 2018 in order to monitor cyanotoxins in South Carolina. The ability to measure cyanotoxins in South Carolina's waters will establish a baseline for their spatial distribution and allow for an improved assessment of environmental conditions associated with cyanotoxins. The characterization of South Carolina's waterways is the first step in the process for effective environmental management and understanding where and under what conditions threats may occur.

Purpose of Assessment

The purpose of this assessment was to examine cyanotoxins in the surface water reservoirs of South Carolina, and some of their influent streams and rivers, and to evaluate the potential hazards to drinking water facilities. The cyanotoxin concentrations were also intended to be used in accordance with USEPA guidance to determine risk for recreational and aquatic life uses for waterbodies of the State. The data was used to identify potential water bodies of concern and for future assessment of their potential algal production. However, a decision for action would occur when the cyanotoxin concentrations were greater than the recommended USEPA guidelines (Tables 1 and 2). Thus, the goal of such action would be to prevent potential or further risk to the water body, water facilities, and/or recreational 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 (ug/L)	School age children and adults (ug/L)
Microcystins	0.3	1.6
Cylindrospermopsin	0.7	3.0

a. U.S. Environmental Protection Agency, 2015b,c
b. ug/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 (ug/L)[a,b]	Cylindrospermopsins Concentration (ug/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. ug/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 earlier this year (2020).

Methods

SCDHEC Bureau of Water (BOW), Aquatic Science Programs (ASP), collected cyanotoxin samples from August 2018 to November 2018. The 2018 sampling season was extended by one (1) month into November to account for the delayed start of the season as well as a loss of samples due to Hurricane Florence in September.

During the 2018 HAB sampling season, there were two (2) different types of sampling conducted in the State: monthly-monitoring at various waterbodies and event-driven sampling response due to complaints,

such as visually observed algal blooms and fish kills. A total of 27 freshwater bodies were sampled during the monthly-monitoring; seven (7) freshwater samples were collected due to an event-driven response.

Monthly-Monitoring

The 62 sites (Table 3 and Figure 1) that were sampled monthly from August 2018 to November 2018 were based on the Ambient Water Quality Monitoring Program sites in 2018, see State of South Carolina Monitoring Strategy for 2018 (SCDHEC 2017). The SCDHEC Ambient Water Quality Monitoring Program collected samples from 244 Base Sites that were sampled monthly year-round and tested for various parameters including temperature, chlorophyll, nutrients, metals, etc. Therefore, coordination with the SCDHEC Ambient Water Quality Monitoring Program sample collection allowed a wider array of water bodies sampled for cyanotoxins, and it provided an opportunity to compare cyanotoxin results to other water quality parameters.

There were 221 total samples at the end of the sampling season that were tested for two (2) cyanotoxins: microcystin and cylindrospermopsin. The sample collection, field analysis, handling, preservation, and Chain of Custody (COC) was completed according to the SCDHEC Determination of Total Microcystins and Cylindrospermopsin in Ambient Water Standard Operating Procedure (SOP) (Appendix 1) and the 2018 HAB Quality Assurance Project Plan (Appendix 2). Samples were frozen at –20^{o}C at the SCDHEC ASP lab for a holding time not to exceed two (2) weeks. The field manager oversaw the transportation of the samples and the COCs to the SCDHEC ASP lab.

Samples were analyzed for microcystin and cylindrospermopsin using Enzyme Linked Immunosorbent Assay (ELISA). The analysis was based on USEPA method 546 (U.S. Environmental Protection Agency, 2015a) with guidance from the supply provider, Abraxis. The primary instrumentation required for analysis, the necessary equipment, and the ELISA methodology is located in the SCDHEC Determination of Total Microcystins and Cylindrospermopsin in Ambient Water SOP, Appendix 1.

Table 3: Sampling site locations.

Site	Regional Lab	Description	Latitude	Longitude
B-327	Greenville	Monticello Lake	34.329669	-81.302637
B-339	Greenville	Lake Bowen	35.112851	-82.045531
B-345	Midlands	Parr Reservoir	34.262086	-81.33538
CL-019	Greenville	Lake Jocassee	34.959888	-82.923614
Cl-041	Greenville	Clarks Hill Reservoir	33.669994	-82.207614
Cl-069	Aiken	Langley Pond	33.522261	-81.843207
Cl-089	Midlands	Lake Wateree	34.336849	-80.704999
CW-016F	Lancaster	Fishing Creek Reservoir	34.677783	-80.877187
CW-033	Midlands	Cedar Creek Reservoir	34.542652	-80.877738
CW-057	Lancaster	Fishing Creek Reservoir	34.605283	-80.891043
CW-174	Midlands	Cedar Creek Reservoir	34.558159	-80.891665
CW-197	Midlands	Lake Wylie	35.137560	-81.059423
CW-201	Midlands	Lake Wylie	35.028120	-81.047666
CW-207	Midlands	Lake Wateree	34.402490	-80.788392
CW-230	Midlands	Lake Wylie	35.022540	-81.008718

Site	Regional Lab	Description	Latitude	Longitude
CW-231	Midlands	Lake Wateree	34.536496	-80.874886
PD-327	Florence	Lake Robinson	34.467522	-80.169800
RL-06435	Midlands	Lake Whelchel	35.109882	-81.637977
RL-18079	Midlands	Lake Murray	34.084683	-81.312869
RL-18081	Greenville	Lake Keowee	34.836635	-82.89471
RL-18083	Florence	Lake Wateree	34.436043	-80.856582
RL-18085	Greenville	Lake Jocassee	35.033045	-82.923425
RL-18087	Midlands	Lake Robinson	34.481743	-80.169963
RL-18089	Greenville	Lake Bowen	35.103173	-82.023042
RL-18092	Greenville	Lake Richard B. Russell	34.117097	-82.617832
RL-18096	Midlands	Lake Murray	34.01548	-81.353929
RL-18099	Midlands	Lake Murray	34.096573	-81.479843
RL-18100	Aiken	Strom Thurman Reservoir	33.956206	-82.397233
RL-18136	Greenville	Broadway Lake	34.458843	-82.594253
RL-18137	Greenville	Lake Blalock	35.089201	-81.88065
RL-18138	Greenville	Lake Rabon	34.516053	-82.131542
R:-18139	Greenville	Lake Cooley	35.00175	-82.104137
RL-18141	Greenville	Lake Tugaloo	34.737998	-83.347911
RL-18142	Greenville	Lake J. Robinson	35.002929	-82.308294
RL-18143	Greenville	Lake Yonah	34.689975	-83.340806
RL-18144	Greenville	Lake Cunningham	34.977418	-82.256092
RL-18146	Midlands	Cedar Creek Reservoir	34.539757	-80.890175
RL-18151	Greenville	Lake Keowee	34.841024	-82.903165
S-022	Greenville	Lake Greenwood	34.327828	-82.084925
S-024	Greenville	Lake Greenwood	34.307961	-82.110082
S-131	Greenville	Lake Greenwood	34.279142	-82.058652
S-211	Midlands	Lake Murray	34.098439	-81.476470
S-213	Midlands	Lake Murray	34.125146	-81.433674
S-222	Midlands	Lake Murray	34.080157	-81.562536
S-308	Midlands	Lake Greenwood	34.346724	-82.108837
S-309	Midlands	Lake Murray	34.131457	-81.604810
S-310	Midlands	Lake Murray	34.115117	-81.599895
S-311	Greenville	Boyd Mill Pond	34.454740	-82.201920
SV-098	Greenville	Lake Russell	34.070411	-82.642967
SV-200	Greenville	Lake Hartwell	34.611708	-83.226227
SV-236	Greenville	Lake Hartwell	34.595426	-82.907767
SV-268	Greenville	Lake Hartwell	34.597196	-82.821775
SV-331	Greenville	Lake Secession	34.331881	-82.575844
SV-335	Greenville	Lake Jocassee	35.032026	-82.915140
SV-336	Greenville	Lake Jocassee	34.995929	-82.979349
SV-338	Greenville	Lake Keowee	34.826901	-82.897685
SV-339	Greenville	Lake Hartwell	34.511242	-82.809785
SV-340	Greenville	Lake Hartwell	34.403249	-82.839061
SV-357	Greenville	Lake Russell	34.192024	-82.630926
SV-361	Greenville	Lake Keowee	34.733950	-82.918264

Site	Regional Lab	Description	Latitude	Longitude
SV-363	Greenville	Lake Hartwell	34.480026	-82.945395
SV-372	Greenville	Stephens Creek Reservoir	33.592784	-82.123327

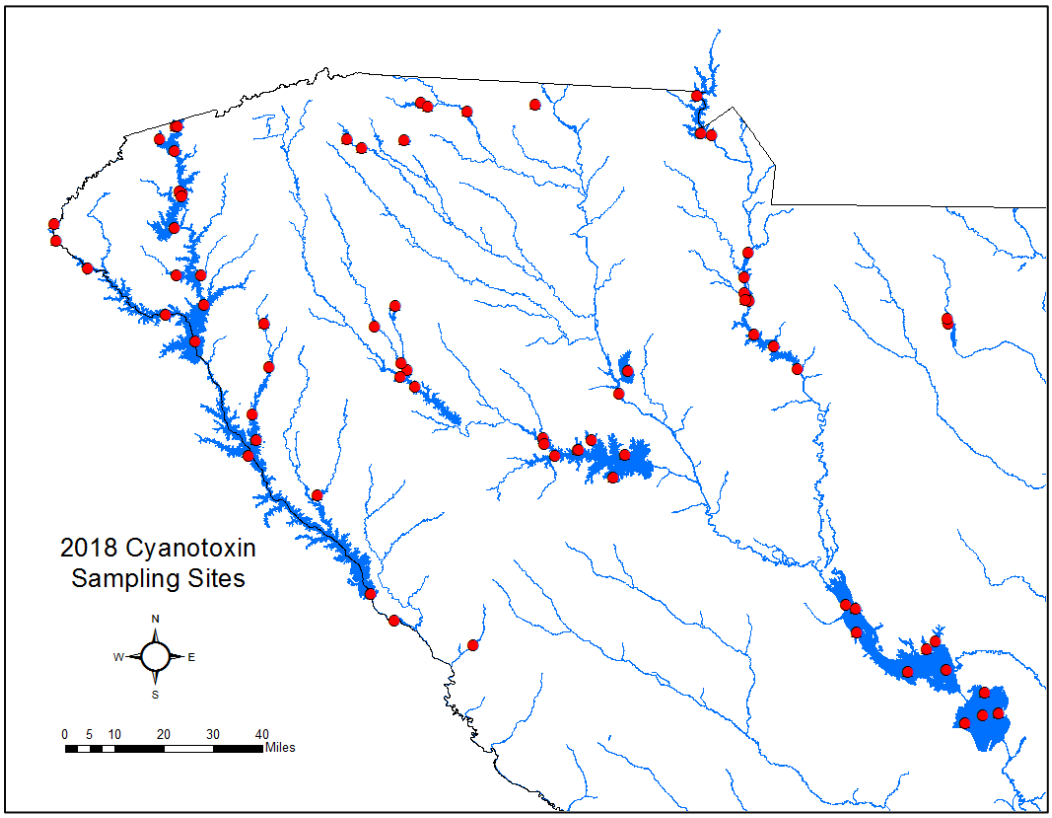

Figure 1: Sampling site locations.

Event-Driven Samples

There were seven (7) samples collected in response to complaints reporting algal blooms, fish kills, and taste and odor issues received during the HABs 2018 sampling season. Grab samples and phytoplankton tow nets were taken in the respective areas after a complaint was received. At the lab, the samples were observed under the microscope for algal identification and then analyzed for microcystins and/or cylindrospermopsin if the species identified was a potential toxin producing species.

The sample collection, handling, and preservation was completed according to the SCDHEC Determination of Total Microcystins and Cylindrospermopsin in Ambient Water SOP (Appendix 1) and the 2018 HAB Quality Assurance Project Plan (Appendix 2). Samples were frozen at –20°C at the SCDHEC ASP lab for a holding time not to exceed two (2) weeks. The field manager oversaw the transportation of the samples and the COCs to the SCDHEC ASP lab.

Samples identified with cyanobacteria were analyzed for the toxins microcystin and cylindrospermopsin using Enzyme Linked Immunosorbent Assay (ELISA). The analysis was based on USEPA method 546 (U.S. Environmental Protection Agency, 2015a) with guidance from the supply provider, Abraxis. The primary instrumentation required for analysis, the necessary equipment, and the ELISA methodology is located in

the SCDHEC Determination of Total Microcystins and Cylindrospermopsin in Ambient Water SOP, Appendix 1.

Quality Assurance/ Quality Control

SCDHEC ASP performed quality assurance and quality control on all samples. All 221 samples analyzed for microcystins in 2018 met the quality control requirements and were considered valid results. Seven (7) of the results for cylindrospermopsin did not meet the requirements for quality control and were discarded. Thus, 214 samples were analyzed for cylindrospermopsin.

Statistical Analyses

Statistical analyses were performed comparing microcystin concentrations to other parameters also collected [dissolved oxygen, pH, temperature, total phosphorus, nitrogen to phosphorous ratio (N:P ratio), and chlorophyll A]. Only quantifiable data, toxin concentration values within the method detection limit, was used for analyses. Only one (1) cylindrospermopsin sample had a quantifiable amount; therefore, cylindrospermopsin concentrations were not analyzed.

There were 27 lakes sampled from the 62 sites selected for the monthly-monitoring in 2018. 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 quantifiable samples per month over the course of four months; thus, equating to a minimum of twelve samples total. There was only one lake that met the sample size criteria: Lake Murray. Pearson correlation coefficients in Microsoft Excel were calculated for the Lake Murray dataset 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 (ug/L). 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.

Results

Monthly-Monitoring

From August 2018 through November 2018, a total of 221 samples were collected for microcystins and cylindrospermopsin. Some stations were not sampled in September due to hazardous weather conditions from Hurricane Florence.

Microcystins

Of the 221 samples analyzed for microcystins, 46% had quantifiable amounts of microcystin present (≥ 0.100 ug/L); all were less than 1 ug/L. These quantifiable results were well less than the USEPA recreational action level of 8 ug/L. The maximum concentration observed from the sites was 0.371 ug/L at station RL-18079 on Lake Whelchel in August. Lake HB Robinson, Lake Yonah, and Tugaloo Lake had all samples less than the detection limit (<0.100 ug/L).

Seventeen (17) of the 27 lakes sampled had more than one (1) sample with quantifiable amounts of microcystin (Figure 2). Lake Whelchel had the highest average quantifiable concentration ($\bar{x}$=0.339 ug/L, SE=0.026) and Lake Jocassee had the lowest quantifiable average microcystin concentration ($\bar{x}$=0.112

ug/L, SE=0.009). Refer to Appendix 3 to see the microcystin concentrations of individual sites analyzed each month, organized based on lake location.

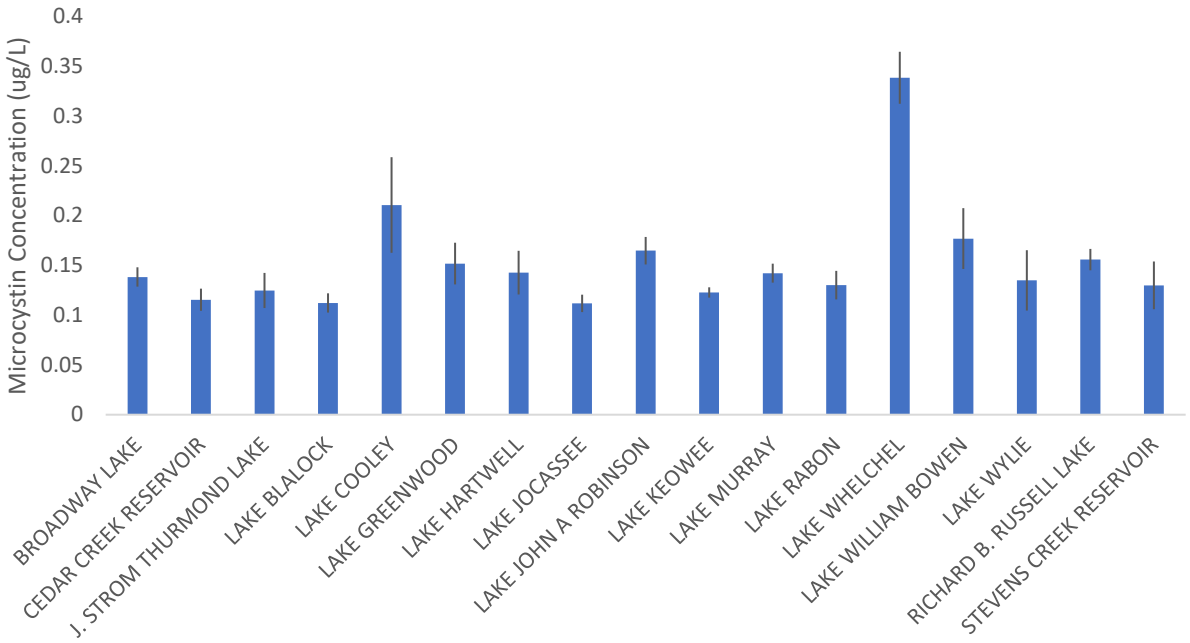

Figure 2: Average quantifiable microcystin concentration (ug/L) per lake in 2018. There were 17 lakes that had more than one sample with quantifiable concentrations. The error bars represent +/- one (1) standard error.

There were no strong correlations in Lake Murray between microcystin concentration versus dissolved oxygen, pH, temperature, total phosphorous, N:P ratio, or chlorophyll A (Table 4). The highest positive correlation value was 0.19 when comparing microcystin concentration versus dissolved oxygen. The lowest negative correlation value was -0.35 for microcystin concentration versus N:P ratio.

Table 4: Pearson correlation coefficient results comparing microcystin concentration in Lake Murray to dissolved oxygen (mg/L), pH, temperature (°C), total phosphorous (mg/L), N:P ratio, and chlorophyll A (ug/L). Strong relationships could not be determined between microcystin concentration and any of the previously mentioned parameters for any of the lakes.

Water Quality Parameter	Microcystin Concentration Correlation
Dissolved Oxygen	0.19
pH	0.17
Temperature	0.01
Total Phosphorous	-0.14
N:P	-0.35
Chlorophyll A	-0.01

Cylindrospermopsin

Only one (1) station of the 214 samples analyzed for cylindrospermopsin had a quantifiable concentration (≥0.040 ug/L). This site was S-213 on Lake Murray with a concentration of 0.050 ug/L, analyzed in October.

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:

- 46% of the 221 samples analyzed for microcystins were quantifiable (≥ 0.100 ug/L).
- All of the quantifiable microcystin samples were less than the EPA-recommended recreational action level of 8 ug/L.
- There were no correlations between microcystin concentration and dissolved oxygen, pH, temperature, total phosphorous, N:P ratio, and chlorophyll A in Lake Murray.
- Only one (1) station of the 214 samples analyzed for cylindrospermopsin was quantifiable (≥0.040 ug/L).
- The lone (1) cylindrospermopsin sample that was quantifiable was less than the USEPA recommended recreational action level of 15 ug/L.

Event-Driven Samples

Throughout the 2018 season, the SCDHEC BOW ASP section received complaints on seven (7) potential HABs throughout the State. Four (4) of the seven (7) samples had quantifiable levels of cyanotoxins (Table 5). The greatest concentration of microcystins (0.250 ug/L) and cylindrospermopsin (0.060 ug/L) was at Broad Creek Landing in response to a reported fish kill. All measured cyanotoxin concentrations were below recommended USEPA action levels.

Table 5: Description and cyanotoxin (microcystins and cylindrospermopsin) results from 2018 algal bloom complaints.

Sample Location	Sample Description	Microcystin (ug/L)[a]	Cylindrospermopsin (ug/L) [a]
Lake Wateree	Brown red algal sample on Lake Wateree	0.127	BDL
Goose Creek Reservoir	Random Sample Grab (no bloom present)	BDL	BDL
Fish Kill- Old State Road	Fish kill in a private pond in Cameron, SC	BDL	BDL
Elms of Charleston	Bloom at a housing complex in Charleston	BDL	BDL
Walton Pond	Fish Kill in Chapin	BDL	0.040
Wateree Cove	Cove by Lugoff-Elgin water intake	0.128	BDL
Broad Creek Landing	Fish kill	0.250	0.060

a. ug/L = micrograms per liter (parts per billion)
b. BDL = Below Detection Limits

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:

- Three (3) of the seven (7) HAB complaint samples were quantifiable for microcystins (≥0.100 ug/L).
- Two (2) of the seven (7) HAB complaint samples were quantifiable for cylindrospermopsin (≥0.040 ug/L).
- All microcystins and cylindrospermopsin results were less than the EPA-recommended action levels.

Discussion and Conclusions

Overall, the 2018 monthly-monitoring cyanotoxin results for microcystins and cylindrospermopsin showed toxin concentrations far less than USEPA recreational action standards throughout the lakes sampled in South Carolina. Almost half of the samples tested showed a quantifiable concentration of microcystin, whereas only one (1) site (S-213 in Lake Murray) had a quantifiable concentration of cylindrospermopsin. A similar study examining microcystin in 187 Florida lakes also had a majority of samples without quantifiable concentrations; it reported that only 29% of the samples had detectable concentrations of microcystins (≥0.100 ug/L) (Bigham, Hoyer, & Canfield Jr., 2009). All quantifiable concentrations for microcystins and cylindrospermopsin in this study were less than 1 ug/L, which was well less than the USEPA's recommended recreational action value of 8 ug/L and 15 ug/L respectively. Therefore, there was no immediate concern to the recreation activities on the sampled lakes. However, it is important to note that the samples that were analyzed in this 2018 sampling season from the monthly-monitoring sites were from open water sites, not actual algal blooms. Cyanotoxin concentrations were expected to be lower due to the reduced presence of algae and likely low cyanotoxin production. Furthermore, the samples were usually collected in the middle of lake channels, whereas algal blooms are normally found in shallower water, such as coves, where wind and wave action move algae to those areas. The results from this monthly-monitoring data and from the study in Florida (Bigham, Hoyer, & Canfield Jr., 2009) show that there are low detectable levels of cyanotoxins, particularly microcystins, that occur on freshwater lakes without a bloom present.

The event-driven samples that were analyzed from algal blooms also showed low or undetectable values of microcystins and cylindrospermopsin. Out of these events, Broad Creek Landing reported the highest concentrations of microcystins and cylindrospermopsin. All microcystin and cylindrospermopsin concentrations for event-driven samples were less than 1 ug/L, well less than the USEPA-recommended recreation action values. These low concentrations suggested that the dominant species in those specific algal blooms were not producing significant amounts of either cyanotoxin during that time. This being the HAB Programs first year, we anticipate encountering and sampling more event-driven samples with potentially higher concentrations in the future as the program is further established.

The correlation results comparing microcystins to dissolved oxygen, pH, temperature, total phosphorus, N:P ratio, and chlorophyll A for Lake Murray showed that there were no relationships between the microcystin concentration and any of the above parameters. This dataset was small and did not include data from any algal blooms; consequently, the absence of meaningful correlation results was anticipated.

Correlation between microcystins concentration and chlorophyll A was unlikely because chlorophyll A includes all general algal production, not only cyanobacteria. Sampling for chlorophyll A in water systems is important to monitor algal growth and the ensuing standing stock of phytoplankton, but it is not a strong indicator for cyanobacteria presence. It would be beneficial in future analyses to analyze phycocyanin, a pigment more indicative of cyanobacteria presence, and compare it to cyanotoxin concentrations. The presence of phycocyanin in a system does not indicate that a bloom is producing toxins, rather it shows there is potential toxin producing cyanobacteria in the system.

One (1) of the main goals of the HAB Program is to establish cyanotoxin spatial distribution data in South Carolina waterbodies. These 2018 results have (a) contributed to starting a cyanotoxin concentration baseline for South Carolina waterbodies and (b) provided insight towards cyanotoxin presence/absence expectations. The data in Figure 2 and Appendix 3 can assist in depicting which South Carolina lakes contained quantifiable 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 may be an important lake to monitor toxins and HABs in future algal blooming seasons because it produced the greatest monitored microcystin concentrations in 2018 and serves as the primary drinking source for the town of Gaffney. Even though no correlations between microcystin concentration and other parameters were discerned in this assessment, a larger data set may provide better insight into relationships between cyanotoxin concentrations and other collected parameters. The microcystin and cylindrospermopsin concentration data collected in 2018 were informative, but the 2018 sampling season had data missing from a significant portion of the algal growing season: May through July. Therefore, it is imperative for future sampling plans to collect data through the entire algal growing season.

Overall Summary:

- The monthly sampling and event-driven sampling resulted in no immediate concern for recreation activities on the sampled lakes due to the low concentrations of microcystins and cylindrospermopsin.
- There were no correlations between microcystins concentration and other parameters measured in Lake Murray. Future analyses would benefit from a larger data set that also included samples from algal blooms.
- The data gathered in 2018 helps establish cyanotoxin spatial distribution data, and this data will contribute to future sampling plans and provide insight into lakes that should be monitored more often.
- Future sampling should incorporate the entire algal growing season from May through October.

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

Determination of Total Microcystins and Cylindrospermopsin in Ambient Water

Bureau of Water- Aquatic Science Programs

June 1, 2020

_____	_____
Author	Date
_____	_____
Manager, Aquatic Science Programs	Date
_____	_____
Assistant Bureau Chief- BOW	Date

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 10ºC or higher than 30ºC). 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 10ºC during the first 48 hours after collection. A temperature of greater than 10ºC 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 (4-8ºC). The solutions must be allowed to reach room temperature (20-25 ºC) 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 35ºC 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 ≥70% and ≤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. $r^2 \geq 0.98$
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 Project
Prepared by Emily Bores
South Carolina Department of Health and Environmental Control
2600 Bull St.
Columbia, SC 29201
August 2018

Project Manager: Emily Bores
Department of Health and Environmental Control, Columbia, SC

Lead Organization: Department of Health and Environmental Control, Columbia SC

Project Location: South Carolina

Project Manager: Emily Bores Date: 08/29/18
Emily Bores

SC DHEC BOW: Date: 2018-08-29
Bryan Rabon, Aquatic Science Program, Manager

SCDHEC QAM: Date: 8/29/18
Nydia Burdick, QAM Designee

EPA Region 4 QA Officer: DAD signed for Date: 10/4/18
Liza Montalvao, US EPA, Region 4

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
Nydia Burdick	QAM Designee	Environmental Laboratory Certification Office	803-896-0862	burdicnf@dhec.sc.gov
Carmen Woodward	Field Manager	SC DHEC- Greenville Office	864-241-1090	woodwacg@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.

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

Nydia Burdick- Will review and approve the QAPP

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.

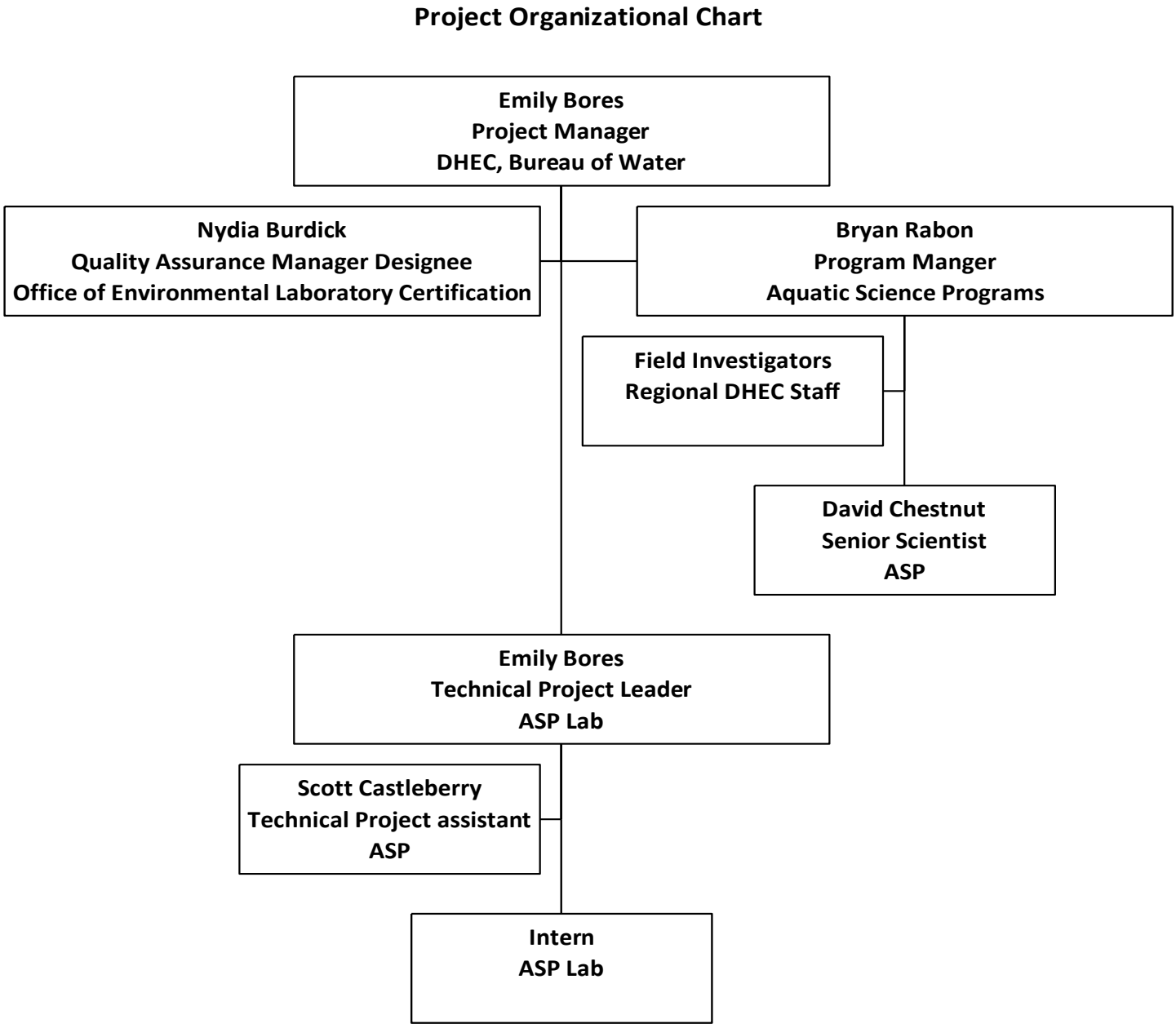


Figure 1 Project Organization Chart

A5. Problem Definition/Background

The goal of this project is to characterize the occurrence of cyanotoxins in surface waters from reservoirs in South Carolina and use the results from the analysis to include potential risks to drinking water facilities and 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 for detection of these toxins in surface water have made clear the need and opportunity to better characterize the extent and levels of these 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 has 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 with drinking water intakes, particularly those that have reported taste and order issues in the recent past. Some event driven testing will be conducted and may include large rivers in addition to lakes. The event driven testing will target algal blooms that may be observed or reported during the 2018 growing season. 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 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. Table 2 for the EPA draft Recreational Criteria or Swimming Advisory Recommendations for Microcystins and Cylindrospermopsin.

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	4	Not to be exceeded	One day	8	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. Approximately 219 water samples will be collected by regional staff members monthly via grab sample at approximately 73 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 the course of normal monthly ambient monitoring of select reservoirs and lakes during the months of August through October 2018. Refer to the State of South Carolina Monitoring Strategy for Calendar Year 2018, Technical Report No, 0802-17. Due to the holding time for cyanotoxins, samples must be frozen 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 with additional guidance and expertise from Abraxis personnel. 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	3/23/18	04/01/18
QAPP Approval	SCDHEC	05/01/18	07/30/18
Sampling Begins	SCDHEC	07/30/18	10/31/18
Lab Reports	SCDHEC	08/01/18	11/30/18
Data Validation	SCDHEC	10/31/18	11/31/18
Final Report Due	SCDHEC	10/31/18	11/31/18

Table 4. Site Locations

Station	Regional Lab	Description	Latitude	Longitude
B-327	Greenville	Monticello Lake- Lower Impoundment between large islands	34.32966927326	-81.30263710763
B-339	Greenville	Lake Bowen 0.3 MI W of SC 9	35.11285121982	-82.0455309651
B-345	Richland	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-042	Santee Cooper	Lake Marion Forebay; Spillway Marker 44- SC-022	33.45076263603	-80.18610938467
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

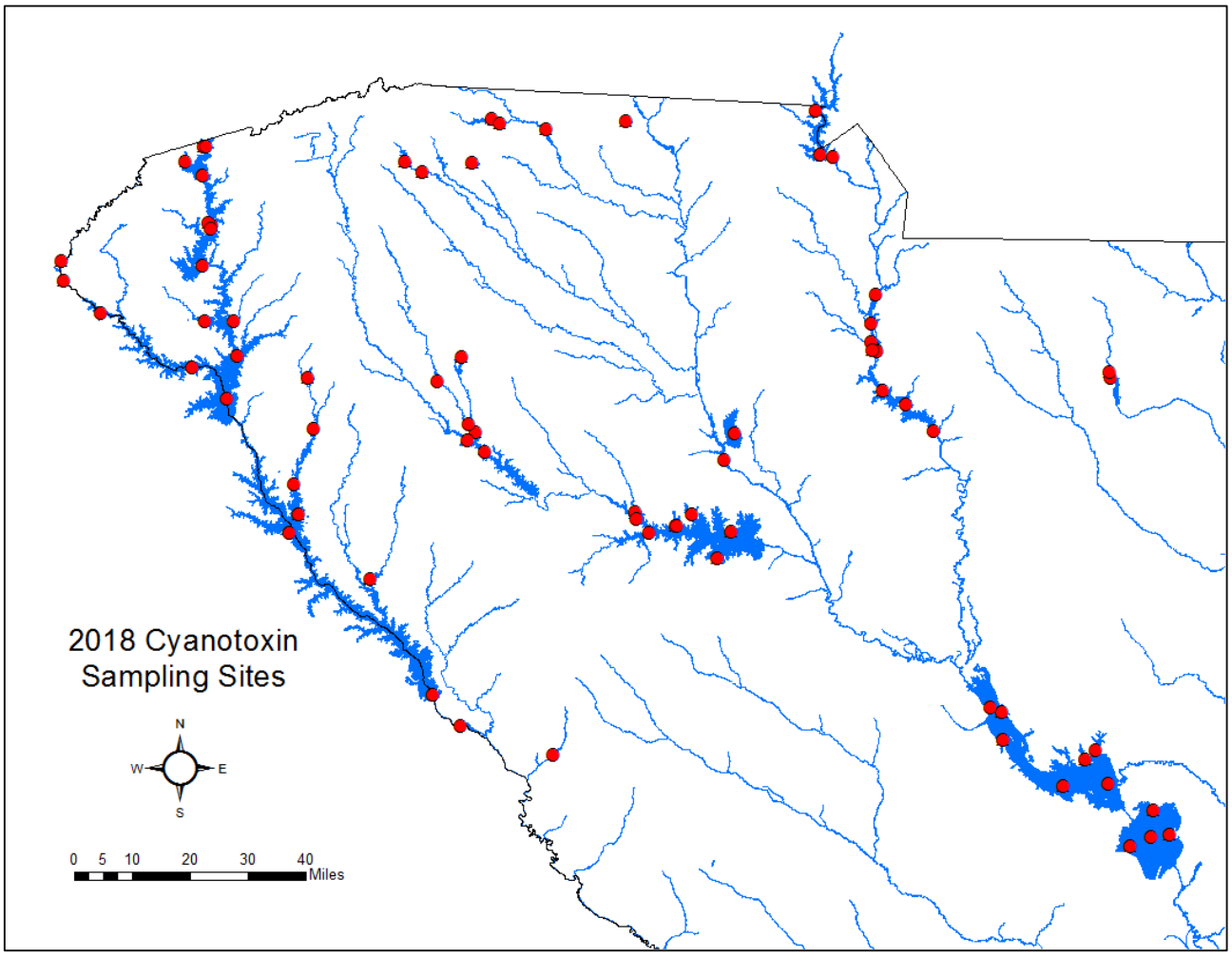
Station	Regional Lab	Description	Latitude	Longitude
CW-197	Midlands	Lake Wylie above Mill Creek arm at end of S-46-557	35.13756014086	-81.05942285366
CW-201	Midlands	Lake Wylie North Lakewoods S/D at Ebenezer access	35.02811990158	-81.0476664737
CW-207	Midlands	Lake Wateree at end of S-20-291	34.40248974794	-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-06435	Midlands	Lake Whelchel 3 MI NE of Gaffney	35.1098815563	-81.6379766407
RL-18078	Santee Cooper	Lake Moultrie near Bonneau Beach near South Facing docks off	33.321624	-80.002816
RL-18079	Midlands	Lake Murray approx 270 YDS SSW of the house at the end of point	34.084683	-81.312869
RL-18081	Greenville	Lake Keowee 0.7 MI NNE of SV-338 approx 50 YDS SW of tip of island	34.836635	-82.89471
RL-18083	Florence	Lake Wateree approx. 0.25 miles NE of Lake Wateree State Park Boat ramp	34.436043	-80.856582
RL-18085	Greenville	Lake Jocassee 50 YDS SW of Western Tip of Cove at point between horsepasture river and toxaway river	35.033045	'82.923425
RL-18087	Midlands	Lake Robinson cove near upstream end of Lake near end of road S-13-7391	34.481743	-80.169963
RL-18089	Greenville	Lake Bowen near shoreline directly opposite north woodfin ridge drive	35.103173	-82.023042
RL-18092	Greenville	Lake Richard B. Russell approx. 0.35 mi SSW of Latimer ramp behind island	34.117097	-82.617832

Station	Regional Lab	Description	Latitude	Longitude
RL-18094	Santee Cooper	Lake Marion Potato Creek arm Santee National Wildlife Refuge	33.511004	-80.251902
RL-18095	Santee Cooper	Lake Moultrie approx 2.8 mi SSE from Cross	33.29322	-80.120892
RL-18096	Midlands	Lake Murray in Beaver Dam Creek Cove near end of Pine Point Drive	34.01548	-81.353929
RL-18098	Santee Cooper	Lake Moultrie along NE area of lake S of Crooked Bay behind island	33.381417	-80.051544
RL-18099	Midlands	Lake Murray Buffalo Creek Arm directly across from S-211 near end of Bethel Church Rd	34.096573	-81.479843
RL-18100	Aiken (?)	Strom Thurman Reservoir on Long Cane Creek arm approx. 0.5 miles SW of SC-28 bridge	33.956206	-82.397233
RL-18136	Greenville	Broadway Lake opposite small cove nearshore along lakeside drive	34.458843	-82.594253
RL-18137	Greenville	Lake Blalock approx 0.25 miles SSW past Buck Creek Road approx off end of Bishop Drive	35.089201	-81.88065
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-18141	Greenville	Lake Tugaloo Approx on State line approx across from bull sluice rd	34.737998	-83.347911
RL-18142	Greenville	Lake J. Robinson near Shore opposite the end of Harbor Master Lane	35.002929	-82.308294
RL-18143	Greenville	Lake Yonah near west bank approx 2.3 mi downstream of Tugaloo dam	34.689975	-83.340806
RL-18144	Greenville	Lake Cunningham approx directly off end of Lake Cunningham Circle	34.977418	-82.256092

Station	Regional Lab	Description	Latitude	Longitude
RL-18146	Midlands	Cedar Creek Reservoir Debutary Creek arm in cove approx. 90 yds. N of debutary boat ramp	34.539757	-80.890175
RL-18151	Greenville	Lake Keowee approx 0.15mi SSW of end of point north drive	34.841024	-82.903165
S-022	Greenville	Reedy Fork of Lake Greenwood at S-30-29	34.32782770413	-82.08492453465
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
SC-010	Santee Cooper	Upper Lake Marion at Channel Marker 150	33.56127465182	-80.49869691038
SC-016	Santee Cooper	Lake Marion @ Channel Marker 69; use Santee Cooper SC-016	33.445997	-80.321198
SC-039	Santee Cooper	Upper Lake Marion 2.0KM below Rimini Railroad Trestle	33.63210598531	-80.5025856819
ST-034	Santee Cooper	Lake Marion at railroad Trestle at Lone Star SC-008	33.64353438324	-80.53527662631
ST-036	Santee Cooper	Lake Marion; Wyboo Creek arm directly S of Clubhouse bridge SC-023A	33.5342596349	-80.22129822653
ST-037	Santee Cooper	Lake Moultrie at channel marker 17-SC-030	33.31465436253	-80.05694242055

Station	Regional Lab	Description	Latitude	Longitude
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
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 3 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 use the results from the analysis to include potential risks to drinking water facilities and 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. 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 5 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- 73 sites located in lakes throughout South Carolina will be sampled once a month for 3 months in 2018. 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 > 4.0 ug/L in recreational waters, Bryan Rabon will be notified and additional samples for toxin and phytoplankton analysis may be collected. If microcystin values are < 1.6 ug/L in any of the drinking reservoirs or < 4.0 ug/L in recreation waters, no immediate action will be taken, and the lakes will continue to be routinely monitored.

5. 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.

6. Specify limits on decision error- Although this is an investigative type study, limiting error are important. 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.

7. Optimize the design for obtaining the data- It is believed that 73 sites sampled once a month for a 3-month period, producing approximately 219 samples, will be enough to help characterize the occurrence of cyanotoxins in the reservoirs of SC. The sufficient quality of samples and their analysis for harmful toxins could also help identify 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. 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 off 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 off 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 2018 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 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 designee for approval. Once the 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 sent out. If there are only minor changes to a few pages, these pages will be sent out with directions of which pages to pull out from the QAPP and which to insert. A signature page will be sent out with the updated QAPP and/or QAPP portions so the recipient must sign 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 08/01/18 and end on 10/31/18. Samples will be collected once a month during the algal growing season (August-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 off the current 2018 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 73 sites will each be sampled once a month for 3 months, equating to about 219 total samples being tested for cyanotoxins (microcystins and cylindrospermopsin). 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, 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 and with a GPS unit to verify the latitude and longitude. 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 most likely, field staff will return within a week to resample the site. It is also possible that 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.
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 1.0 L 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 to not 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. 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 2018* 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 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. It is important to document if the sample integrity or data quality has been adversely affected.

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 evolves or results.

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.

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% ≤15% allowed for 1 pair 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

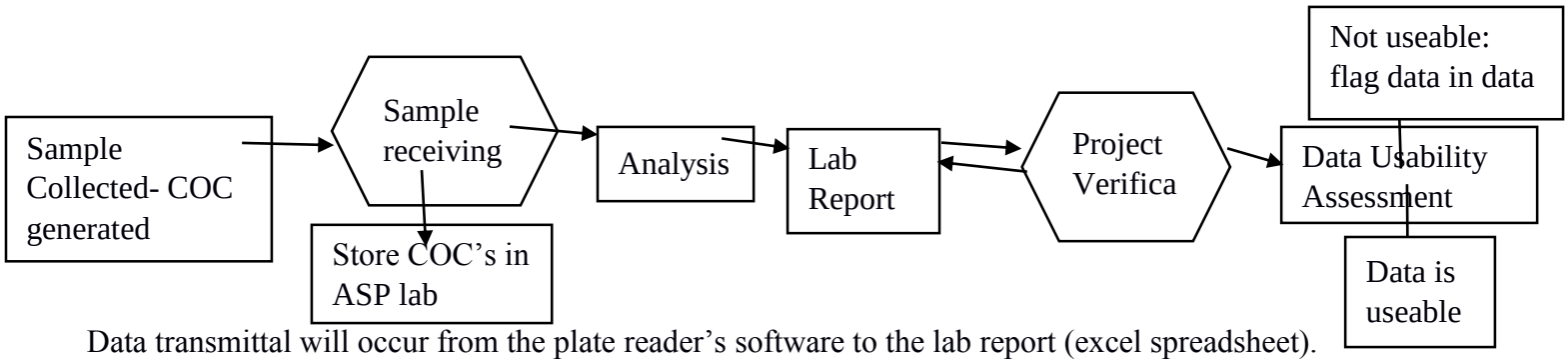
				manager), ASP lab
Syringe Filters	All	Glass microfiber filter, 30mm with 1.2 µm pore size	Office prep area-room temp	Emily Bores (Project manager), ASP lab
PET Storage Bottles	All	Has to be PET material, at least 1L volume	Office prep area-room temp	Emily Bores (Project manager), ASP lab
PTFE Discs	US 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 (ie 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 this project may be used as reference and/or guidance for 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 (ie 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. Field Sampling Technical System Audits (TSA) will be performed at the start of field sampling activities for ambient monitoring. Refer to Section 2.7.6 of the State of South Carolina Monitoring Strategy for the Quality Assurance Assessment conducted for ambient monitoring. The QAM is responsible for responding to and resolving all quality assurance problems and needs. The QAM will initiate corrective action to adverse conditions that compromise quality in the field or laboratory. A thorough review of the complete data review process, including a review of the sample analysis verification, sampling and analysis validation, and data usability steps, to ensure that the process

conforms to the procedure specified in the QAPP. 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 and the client.

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 a training and capacity building project 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.

Literature Cited

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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.

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 Bottle Lot #	31633							
Enterococci (Q-tray) P1 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 2018 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.