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South Carolina Department of Health and Environmental Control

2020 South Carolina Cyanotoxin Distribution Project

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Technical Report No. 004-2022

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Executive Summary

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

In 2018, the South Carolina Department of Health and Environmental Control (SCDHEC) initiated the HABs Monitoring Program to investigate the effects that cyanotoxins have on human health and the environment within the State. This assessment report covers the cyanotoxin work completed in 2020. In 2020, SCDHEC aimed to:

- Continue establishing baseline data for cyanotoxin distribution in State reservoirs,
- Expand cyanotoxin monitoring network to include estuaries, rivers, and influent streams,
- Monitor drinking water intakes with a history of HABs and/or taste and odor issues,
- Issue recreational advisories for waterbodies that exceed SCDHEC's state standards, and
- Identify potential correlative relationships between cyanotoxin concentrations and other physicochemical water quality parameters.

In 2020, samples were collected and analyzed for microcystins from 92 monthly-monitored sites across South Carolina reservoirs, estuaries, and influent streams. Microcystin samples were collected from May 1 to October 31. The monthly-monitored sites were coordinated with routine sampling conducted by SCDHEC regional field staff, which allowed data comparison to other parameters collected contemporaneously. In addition to monthly monitoring of lake and estuarine sites, samples were collected from an additional five (5) lakes at six (6) drinking water intakes with past algal issues, including taste and odor complaints. Twelve (12) samples from eleven (11) water bodies in response to the occurrence of possible HAB conditions (event-driven samples) were also collected from April through October.

In general, monthly-monitoring concentrations were less than 1 microgram per liter (µg/L) for microcystins, except for one (1) sample collected from Lake Hartwell (1.15 µg/L). Concentrations greater than the analytical detection level (≥ 0.100 µg/L for ADDA ELISA method or ≥ 0.016 µg/L for SAES ELISA method) were observed in 74% of samples analyzed for microcystins. Toxin concentrations in all monthly-monitoring samples were less than SCDHEC's recreational standard of 8 µg/L for microcystins.

Microcystins were also detected at all six (6) drinking water intakes. The drinking water intakes at Lake Rabon and Lake Whelchel had at least one (1) sample that exceeded USEPA 10-day drinking water health advisory value of 0.3 µg/L for microcystins; however, the treatment processes at the drinking water facilities were able to reduce microcystin concentrations to less than the detection limit.

One event-driven sample at Bear Creek, a flood control stormwater pond in Lancaster County, exceeded the SCDHEC state recreational standard value of 8 µg/L for microcystins. SCDHEC worked with Lancaster County Stormwater Management to distribute this information and advised closure of the adjacent park area.

There were two (2) recreational advisories issued in 2020 at Lake Edgar Brown and Lake Whelchel for toxin concentrations greater than SCDHEC's state recreational standard. Advisories were removed once the microcystin concentrations were below 8 ug/L and the bloom had dissipated.

Correlation analyses were conducted for monthly-monitoring microcystin concentration data for Lake Greenwood, Lake Hartwell, Lake Murray, and Lake Wateree. No strong relationships were determined for microcystin concentrations and water quality parameters including dissolved oxygen, pH, temperature, total phosphorous, nitrogen: phosphorus ratio, and chlorophyll *a* for any of the lakes.

This assessment builds on the 2018 and 2019 pilot year studies and broadens the baseline understanding of cyanotoxin distributions across the State. In 2020, the HABs Monitoring Program expanded by testing estuaries and influent streams, monitor drinking water lake sources, and issuing recreational advisories. Future goals of the HABs Monitoring Program include evaluating additional toxins, such as anatoxin and saxitoxin, which will further enhance the State's growing understanding of cyanotoxin distributions.

Introduction and Background

Harmful algal blooms (HABs) are an increasing concern in U.S. waters. These blooms occur when algae grow excessively in response to elevated nutrient concentrations, typically from non-point source runoff due to a variety of land-uses. In high enough densities, blue-green algae, or cyanobacteria, can impact aquatic life and human health by degrading water quality and producing cyanotoxins. There is growing recognition of the need for increased monitoring of cyanotoxin concentrations in waterbodies and in the water treatment process (Jetto, Grover, & Krantxberg, 2015). The U.S. Environmental Protection Agency (USEPA) has provided health advisory criteria (U.S. Environmental Protection Agency, 2019) and recreational advisory criteria (U.S. Environmental Protection Agency, 2015b,c) for two (2) cyanotoxins (microcystins and cylindrospermopsin). Exposure to high levels of microcystins can lead to liver, reproductive, developmental, kidney, and gastrointestinal effects (U.S. Environmental Protection Agency, 2019). Exposure to high levels of cylindrospermopsin can affect the liver, kidneys, and have potential effects to red blood cells (U.S. Environmental Protection Agency, 2019).

The South Carolina Department of Health and Environmental Control (SCDHEC) has maintained a robust surface water monitoring network since the 1950s. With the advancement of cyanotoxin analytical methods, SCDHEC established the HABs Monitoring Program in 2018 to monitor cyanotoxins statewide. A primary objective of the HABs Monitoring Program is to establish a baseline and context for interpretation of cyanotoxin concentrations in South Carolina's waters, which was accomplished with the adoption of the USEPA's recreational advisory criteria (Table 1) in SCDHEC's State standards in 2020.

Table 1: USEPA and SCDHEC 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 other recreational water activities.

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

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

Purpose of Assessment

The purpose of the 2020 assessment was to examine cyanotoxin distributions in South Carolina reservoirs, estuaries, rivers, and influent streams, and to determine potential risks for recreational and aquatic life uses for waterbodies of the State. Cyanotoxin concentrations were also compared to USEPA drinking

water health advisories (Table 2) to identify potential hazards to drinking water facilities. The data were used to identify reservoirs of potential concern and will guide future assessment activities. In 2020, monitoring activities primarily focused on analyzing microcystin toxins based on results from the previous two (2) years. Since no cylindrospermopsin producing cyanobacteria species were identified in 2020, this cyanotoxin was not measured during the field program.

Table 2: USEPA 10-day health advisory values for microcystins and cylindrospermopsin in drinking water.

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

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

Note: The recommended USEPA criteria for recreational waters protection shown in Table 1 were adopted as enforceable State water quality standards in 2020.

Methods

SCDHEC Bureau of Water (BOW) Aquatic Science Programs (ASP) collected cyanotoxin samples from April 2020 to December 2020 for microcystins. Three (3) types of sampling were conducted as part of the 2020 study: monthly-monitoring at waterbodies throughout the State, sampling at drinking water intakes with a history of algal issues (drinking water lake source monitoring), and sampling in response to complaints (event-driven). The event-driven sampling included visually observed algal blooms and a fish kill in response to citizen and stakeholder complaints. A total of 18 freshwater bodies and 38 estuaries and influent streams were regularly sampled during the monthly-monitoring component, six (6) drinking water lake intakes, and twelve (12) samples were collected at eleven (11) different water bodies due to event-driven responses. In 2020, the USEPA criteria for recreational water quality and swimming advisories for microcystins and cylindrospermopsin were adopted as State water quality standards.

Monthly-Monitoring

Ninety-two (92) sites were sampled monthly from May 2020 to October 2020 (Table 3 and Figure 1). These sites were selected from the 2020 list of Ambient Water Quality Monitoring Program sites (SCDHEC, 2020b). The 2020 Ambient Water Quality Monitoring Program collected monthly samples from a total of 244 Base Sites for water quality parameters including temperature, chlorophyll *a*, nutrients, metals, etc. providing an opportunity to compare cyanotoxin results to other water quality parameters.

A total of 523 samples were analyzed for microcystins. Sample collection, field analysis, handling, preservation, and Chain of Custody (COC) followed SCDHEC Determination of Total Microcystins and Cylindrospermopsin in Ambient Water Standard Operating Procedure (SOP) (Appendix 1) and the 2020 HAB Quality Assurance Project Plan (Appendix 2). The field manager oversaw the transportation of the

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

Samples were analyzed for microcystins using the Enzyme Linked Immunosorbent Assay (ELISA) technique described in SCDHEC Determination of Total Microcystins and Cylindrospermopsin in Ambient Water SOP (Appendix 1). The analysis is based on USEPA method 546 (U.S. Environmental Protection Agency, 2015a) with guidance from the assay provider, Abraxis. Microcytsins/Nodularins ADDA ELISA and SAES ELISA plates were used for this analysis, with detection limits of 0.100 ug/L and 0.016 ug/L, respectively.

Table 3: Sampling site locations for monthly-monitoring.

Site	Regional Lab	Description	Latitude	Longitude
B-327	Midlands	Monticello Lake	34.3297	-81.3026
B-339	Greenville	Lake Bowen	35.1128	-82.0455
B-345	Midlands	Parr Reservoir	34.2621	-81.3354
CL-019	Greenville	Lake Jocassee	34.9599	-82.9236
Cl-041	Greenville	J. Strom Thurmond	33.6699	-82.2076
Cl-089	Midlands	Lake Wateree	34.3368	-80.7049
CSTL-102	Charleston	Ashley River	32.9584	-80.2010
CSTL-107	Beaufort	Coosawhatchie River	32.5883	-80.9238
CW-016F	Lancaster	Fishing Creek Reservoir	34.6777	-80.8772
CW-033	Midlands	Cedar Creek Reservoir	34.5426	-80.8777
CW-057	Lancaster	Fishing Creek Reservoir	34.6053	-80.8910
CW-174	Midlands	Cedar Creek Reservoir	34.5581	-80.8917
CW-197	Midlands	Lake Wylie	35.1376	-81.0594
CW-201	Midlands	Lake Wylie	35.0281	-81.0477
CW-207	Midlands	Lake Wateree	34.4025	-80.7884
CW-207B	ASP	Lake Wateree	34.4039	-80.7827
CW-208	ASP	Lake Wateree	34.4219	-80.8674
CW-230	Midlands	Lake Wylie	35.0225	-81.0087
CW-231	Midlands	Lake Wateree	34.5365	-80.8749
LCR-02	ASP	Lake Wateree	34.4858	-80.8998
LCR-03	ASP	Lake Wateree	34.4254	-80.8439
LCR-04	ASP	Fishing Creek Reservoir	34.6204	-80.8862
MD-001	Beaufort	Beaufort River	32.4456	-80.6632
MD-004	Beaufort	Beaufort River	32.3653	-80.6779
MD-043	Charleston	Cooper River	32.9629	-79.9212
MD-045	Charleston	Cooper River	32.8453	-79.9335
MD-049	Charleston	Ashley River	32.8758	-80.0815
MD-052	Charleston	Ashley River	32.7966	-79.9719
MD-069	Charleston	Intracoastal Waterway	32.7728	-79.8422
MD-077	Florence	Sampit River	33.3574	-79.2940
MD-115	Charleston	Wando River	32.9228	-79.9273
MD-116	Beaufort	Broad River	32.3848	-80.7838
MD-117	Beaufort	Chechessee	32.3741	-80.8361
MD-118	Beaufort	New River	32.2360	-81.0129
MD-120	Beaufort	Dawho River	32.6366	-80.3418

Site	Regional Lab	Description	Latitude	Longitude
MD-129	Beaufort	Great Swamp	32.4061	-81.0187
MD-130	Charleston	Folly River	32.6596	-79.9433
MD-142	Florence	Waccamaw River	33.4083	-79.2171
MD-173	Beaufort	May River	32.2104	-80.8423
MD-174	Beaufort	Broad Creek	32.1804	-80.7740
MD-176	Beaufort	Colleton River	32.3323	-80.8774
MD-202	Charleston	Stono River	32.7857	-80.1075
MD-206	Charleston	Stono River	32.6744	-80.0046
MD-209	Charleston	Bohicket Creek	32.6223	-80.1643
MD-248	Charleston	Cooper River	32.8905	-79.9627
MD-252	Beaufort	Combahee River	32.5643	-80.5570
MD-253	Beaufort	Ashepoo River	32.5330	-80.4484
MD-256	Beaufort	Unnamed Creek	32.3399	-80.5078
MD-257	Beaufort	Ramshorn Creek	32.1288	-80.8890
MD-258	Beaufort	Ramshorn Creek	32.1110	-80.8986
MD-259	Beaufort	Wright River	32.0943	-80.9489
MD-260	Beaufort	S. Edisto River	32.5673	-80.3901
MD-261	Charleston	Yonges Island Creek	32.6947	-80.2229
MD-262	Charleston	N. Edisto River	32.6059	-80.2293
MD-264	Charleston	Wando River	32.8584	-79.8959
MD-266	Charleston	Casino Creek	33.0751	-79.3941
MD-267	Charleston	Five Fathom Creek	33.0366	-79.4769
MD-269	Charleston	Sewee Bay	32.9367	-79.6550
MD-271	Charleston	Hamlin Sound	32.8269	-79.7746
MD-273	Charleston	Kiawah River	32.6080	-80.1274
MD-275	Florence	Pee Dee River	33.4222	-79.2246
MD-277	Florence	Parsonnage Creek	33.5529	-79.0339
MD-278	Florence	Winyah Bay	33.2735	-79.0340
MD-281	Beaufort	Parrot Creek	32.4953	-80.5553
MD-282	Beaufort	Morgan River	32.4438	-80.6069
PD-325	Florence	Black River	33.4138	-79.2504
PD-327	Lancaster	Lake Robinson	34.4675	-80.1698
S-022	Greenville	Lake Greenwood	34.3278	-82.0849
S-024	Greenville	Lake Greenwood	34.3079	-82.1101
S-131	Greenville	Lake Greenwood	34.2791	-82.0587
S-211	Midlands	Lake Murray	34.0984	-81.4765
S-213	Midlands	Lake Murray	34.1251	-81.4337
S-222	Midlands	Lake Murray	34.0802	-81.5625
S-308	Midlands	Lake Greenwood	34.3467	-82.1088
S-309	Midlands	Lake Murray	34.1315	-81.6048
S-310	Midlands	Lake Murray	34.1151	-81.5999
S-311	Greenville	Boyd Mill Pond	34.4547	-82.2019
SV-098	Greenville	Lake Russell	34.0704	-82.6429
SV-200	Greenville	Lake Hartwell	34.6117	-83.2262
SV-236	Greenville	Lake Hartwell	34.5954	-82.9078

Site	Regional Lab	Description	Latitude	Longitude
SV-268	Greenville	Lake Hartwell	34.5972	-82.8218
SV-331	Greenville	Lake Secession	34.3319	-82.5758
SV-335	Greenville	Lake Jocassee	35.0320	-82.9151
SV-336	Greenville	Lake Jocassee	34.9959	-82.9793
SV-338	Greenville	Lake Keowee	34.8269	-82.8977
SV-339	Greenville	Lake Hartwell	34.5112	-82.8098
SV-340	Greenville	Lake Hartwell	34.4032	-82.8391
SV-357	Greenville	Lake Russell	34.1920	-82.6309
SV-361	Greenville	Lake Keowee	34.7339	-82.9183
SV-363	Greenville	Lake Hartwell	34.4800	-82.9454
SV-372	Greenville	Stephens Creek Reservoir	33.5928	-82.1233
SV-374	Greenville	Lake Hartwell	34.5721	-82.8299

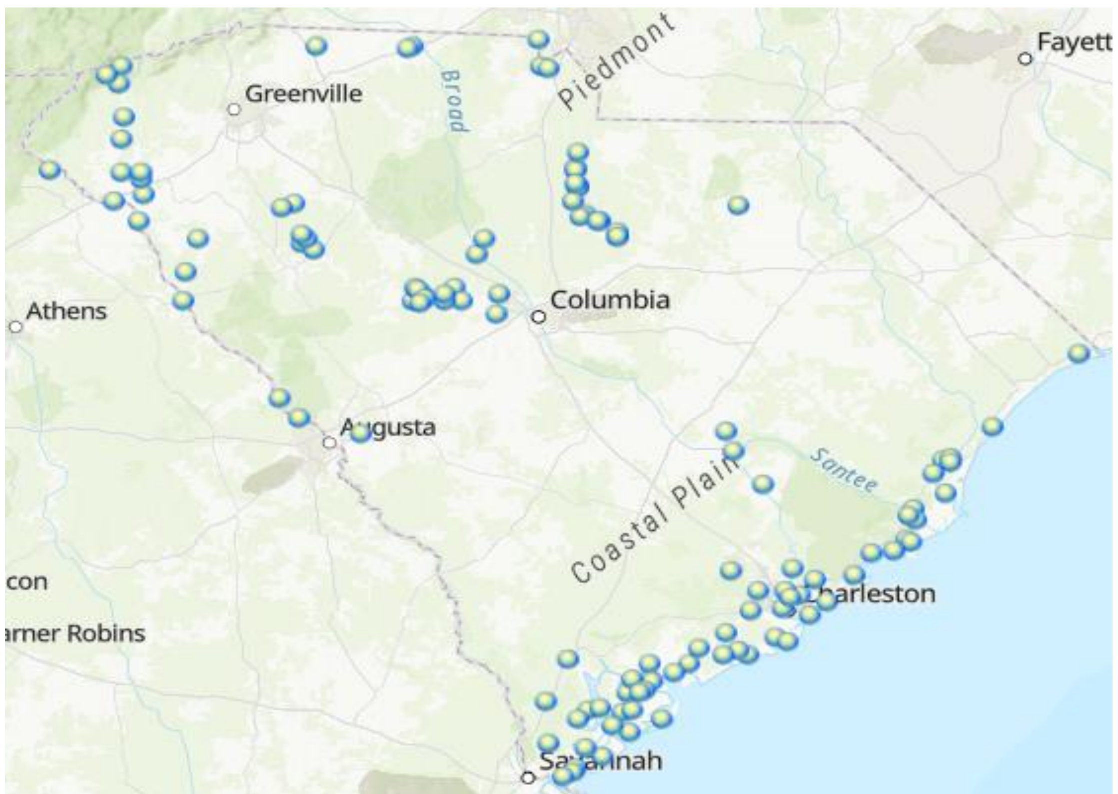

Figure 1: 2020 monthly-monitoring sampling site locations.

Drinking Water Lake Source Monitoring

Five (5) lakes were sampled monthly from May through December 2020 in proximity to intakes for six (6) different drinking water facilities (Table 4). The lakes and drinking water intake sampling sites were selected based on previous algal issues and taste and odor complaints. A total of 41 samples were collected from the drinking water lakes and analyzed for microcystins and chlorophyll *a*. Most samples were collected near the drinking water facility intakes; however, additional samples were collected at other parts of the lakes if algal blooms were occurring.

Drinking water sample collection, field analysis, handling, preservation, and laboratory analysis followed the same procedures as described above in the Monthly-Monitoring section.

Table 4: Sampling site locations for five (5) lakes that were monitored at their respective drinking water source intakes.

Lake	Drinking Water Facility	Latitude	Longitude
Lake Bowen	Spartanburg Water System	35.1113	-81.9728
Lake Greenwood	Greenwood CPW	34.2604	-82.0294
Lake Murray	City of Columbia	34.0215	-81.2326
	City of West Columbia	34.0978	-81.2313
Lake Rabon	Laurens CPW	34.4785	-82.1398
Lake Whelchel	Gaffney BPW	35.1079	-81.6222

Event-Driven Samples

Twelve (12) samples were collected in response to complaints reporting algal blooms, fish kills, and/or taste and odor issues during the 2020 sampling season. Sample locations are described in Table 6 below. Toxin samples and/or phytoplankton tow nets were collected after a complaint was received. Samples were observed under the microscope for algal identification at the SCDHEC ASP laboratory and analyzed for microcystins and/or cylindrospermopsin if the species identified was a potential toxin producing species.

Sample collection, field analysis, handling, and preservation followed the same procedures as described above in the Monthly-Monitoring section. Samples identified with cyanobacteria were analyzed via the same procedures as described above in the Monthly-Monitoring section.

Advisories

In 2020, recreational advisories were issued when one (1) sample exceeded SCDHEC's state standards for microcystins and/or cylindrospermopsin toxins. If a recreational advisory is issued on a waterbody with a drinking water intake, drinking water providers were contacted and recommended to have the finished drinking water tested for toxins. Recreational advisories remained in place until a cyanotoxin result below the recreational state standard was returned and the bloom had dissipated. The public was notified about recreational advisories that were issued or lifted via press releases and postings on the SCDHEC HABs webpage: https://scdhec.gov/environment/your-water-coast/harmful-algal-blooms.

Quality Assurance/ Quality Control

In total, 499 of the 523 samples analyzed for microcystins in 2020 passed quality control requirements. Quality Control Requirements can be found in section 10.5 of SCDHEC's Determination of Total Microcystins and Cylindrospermopsin in Ambient Water SOP (Appendix 1). SCDHEC also participated in the Abraxis Cyanotoxins Proficiency Testing Program for recreational water as a check on the accuracy of ASP's routine sample analysis. Performance was evaluated by calculating a z-score metric based on the analysis results of four (4) surface water standards fortified with purified Microcystin-LR, Microcystin-RR, Microcystin-YR, and/or nodularins (toxins produced by *Nodularia sp.*, a cyanobacterium). The z-score metric is as follows:

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

Where:

z= the z score (Standard score)

x= the reported value of analyte

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

σ= the estimate of variation (proficiency standard deviation)

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

Results Obtained	Rating
$z \leq 2$	Satisfactory
$2 < z < 3$	Questionable
$z \geq 3$	Unsatisfactory

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

Sample Number	Result (µg/L) [a]	Z-Score	Evaluation
1	5.68	0.416	Satisfactory
2	1.18	0.721	Satisfactory
3	9.05	1.291	Satisfactory
4	<0.015	N/A [b]	Satisfactory

a. µg/L = micrograms per liter (parts per billion)

b. Z-score is not calculated when the sample is a blank (no microcystins present)

Statistical Analyses

Pearson correlation coefficients were calculated to determine if there were linear relationships between concentrations of microcystins and pH, dissolved oxygen (mg/L), temperature (°C), total phosphorous (mg/L), N:P ratio, and chlorophyll *a* (µg/L) in water bodies that met sample size requirements. Only detectable data (toxin concentration values greater than or equal to the method detection limit) were

used for analyses. Microcystin concentration data were considered detectable when result(s) were ≥ 0.016 ug/L for SAES ELISA plates and ≥0.100 ug/L for ADDA ELISA plates.

Fifty-six water bodies across the State were sampled as part of the 2020 monthly-monitoring program. Due to different hydrologic characteristics among the water bodies, lakes were analyzed individually. Water bodies with a minimum sample size of three (3) detectable samples per month over the course of six (6) months were analyzed: Lake Greenwood, Lake Hartwell, Lake Murray, and Lake Wateree.

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

Results

Monthly-Monitoring

From May 2020 through October 2020, a total of 523 samples were collected for microcystins. Of the 499 samples meeting QA/QC guidelines for microcystins, 74% had concentrations greater than or equal to the method detection limit. The maximum microcystin concentration was 1.15 ug/L at station SV-339 on Lake Hartwell in May 2020. All other microcystin concentrations were less than 1 µg/L and all microcystin concentrations were less than the SCDHEC recreational action level of 8 µg/L.

A total of 38 estuarine and influent streams were sampled at 46 different sites during the 2020 monitoring season. Thirty-three (33) of the 38 estuarine and influent streams had more than one (1) sample with detectable amounts of microcystins (Figure 2). Wright River had the highest average microcystin concentration (mean ($\bar{x}$)=0.343 µg/L, standard error (SE)=0.239); Waccamaw River had the lowest average microcystin concentration ($\bar{x}$=0.021 µg/L, SE=0.002).

All 18 freshwater lakes had more than one (1) sample with detectable amounts of microcystins (Figure 3). J. Strom Thurmond Lake had the highest average microcystin concentration ($\bar{x}$=0.210 µg/L, SE=0.014); Lake Jocassee had the lowest average microcystin concentration ($\bar{x}$=0.037 µg/L, SE=0.013). Refer to Appendix 3 to see the microcystin concentrations of individual sites analyzed each month, organized based on lake location.

Microcystins did not strongly correlate with dissolved oxygen, pH, temperature, total phosphorous, N:P ratio, or chlorophyll *a* in Lake Greenwood, Lake Hartwell, Lake Murray, and Lake Wateree with coefficients ranging from -0.37 to 0.45 (Table 5).

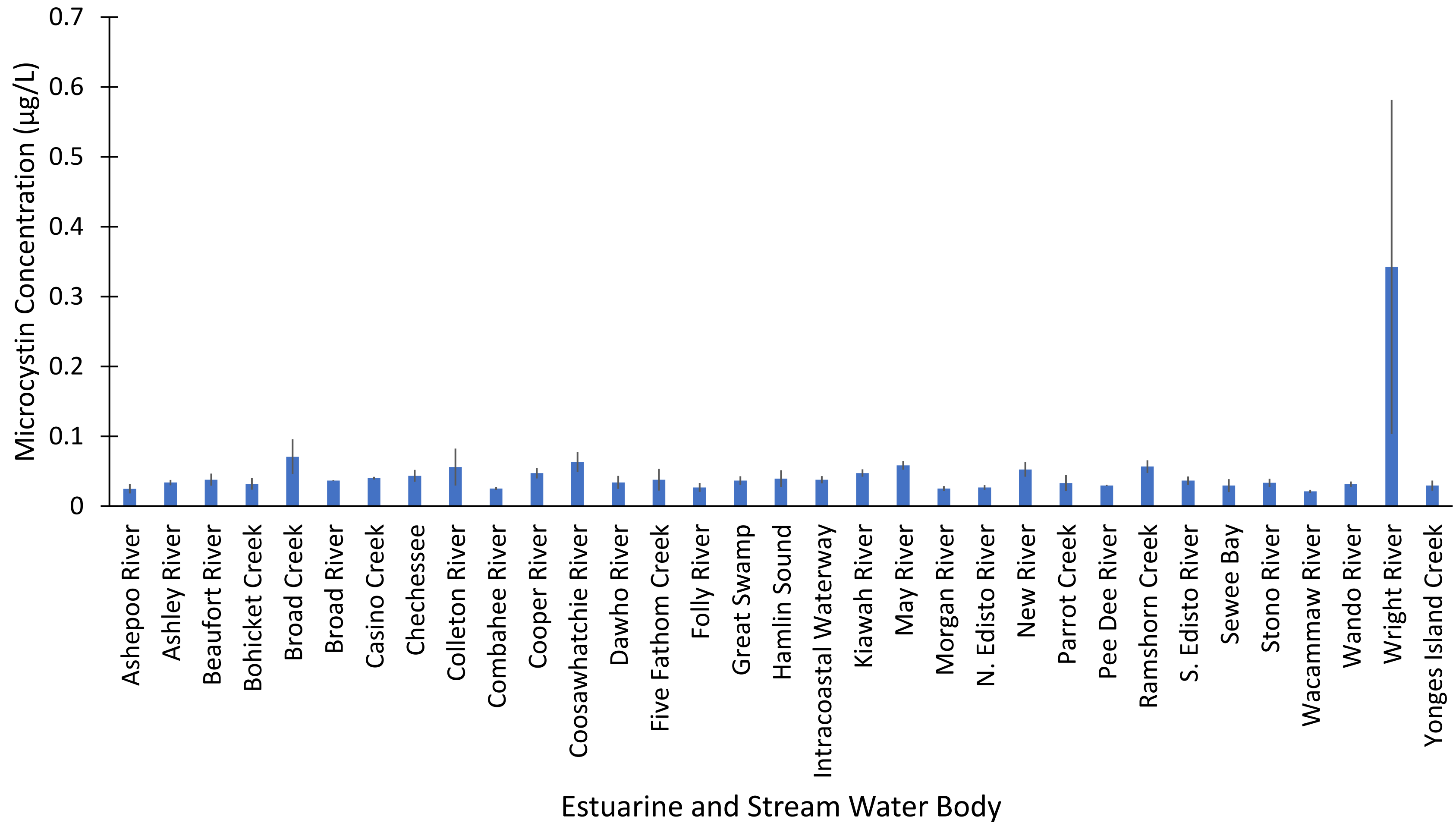

Figure 2: Average detectable microcystin concentrations (µg/L) estuarine and influent streams sampled in 2020. There were 33 estuaries and influent streams that had more than one (1) sample with quantifiable concentrations. The error bars represent +/- one (1) standard error.

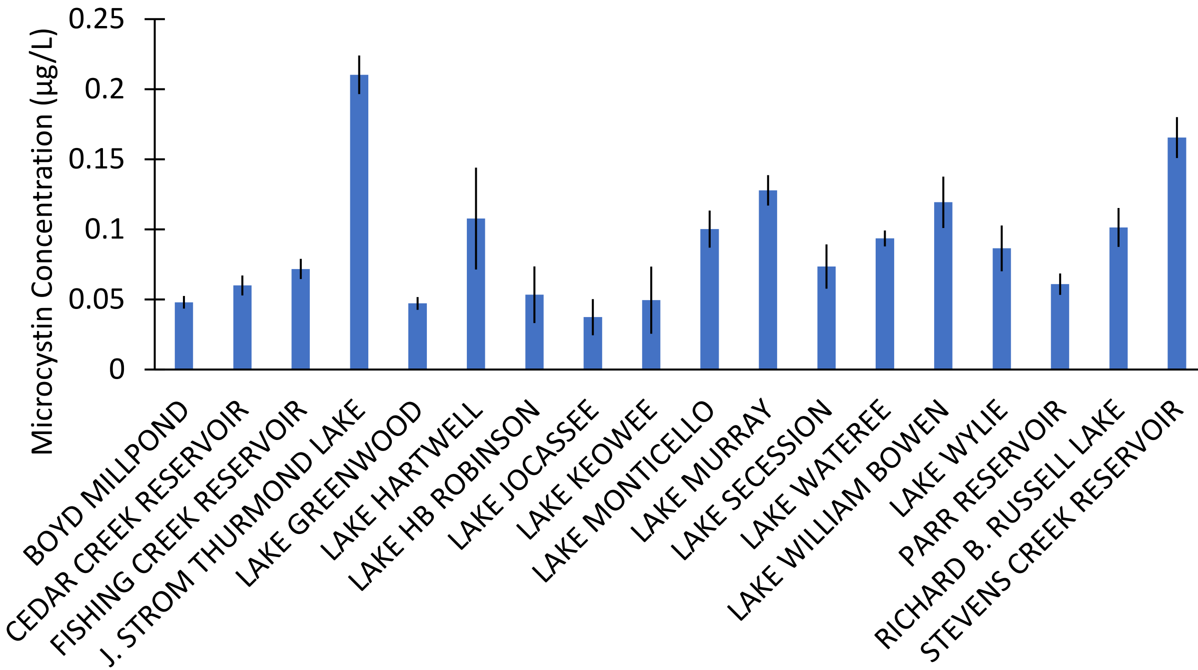

Figure 3: Average detectable microcystin concentrations (µg/L) per freshwater lake in 2020. There were 18 lakes that had more than one (1) sample with quantifiable concentrations. The error bars represent +/- one (1) standard error.

Table 5: Pearson correlation coefficient results comparing microcystin concentrations (µg/L) in Lake Greenwood, Lake Hartwell, Lake Murray, and Lake Wateree to dissolved oxygen (mg/L), pH, temperature (°C), total phosphorous (mg/L), N:P ratio, and chlorophyll *a* (µg/L).

Water Body	**Microcystin Concentrations Correlation for Respective Water Quality Parameters**					
	Dissolved Oxygen	pH	Temperature	Total Phosphorous	N:P	Chlorophyll *a*
Lake Greenwood	-0.07	-0.13	-0.02	-0.37	0.45	0.23
Lake Hartwell	0.30	0.01	-0.35	-0.05	0.19	-0.05
Lake Murray	-0.12	0.18	0.29	-0.21	0.10	-0.21
Lake Wateree	-0.02	0.08	0.01	-0.10	-0.06	0.16

<u>Summary of Monthly-Monitoring Findings</u>

- 74% of the 499 samples analyzed for microcystins were detectable (≥ 0.100 µg/L for ADDA ELISA or ≥ 0.016 µg/L for SAES ELISA method).

- All microcystin samples were less than the USEPA recommended recreational action level of 8 µg/L.
- There were no strong correlations between microcystin concentrations and dissolved oxygen, pH, temperature, total phosphorous, N:P ratio, and chlorophyll *a* in Lake Greenwood, Lake Hartwell, Lake Murray, or Lake Wateree.

Drinking Water Lake Source Monitoring

From May through December 2020, 42 samples were collected for microcystins at five (5) different lakes for six (6) different drinking water facilities. Thirty (30) of the 42 samples were collected at the drinking water facilities intakes. Samples collected near the Gaffney BPW drinking water intake at Lake Whelchel had the highest average microcystin concentration ($\bar{x}$=2.428 µg/L, SE=2.043); the Greenwood CPW drinking water intake samples at Lake Greenwood had the lowest average microcystin concentration ($\bar{x}$=0.052 µg/L, SE=0.008). Lake Bowen (Spartanburg Water System), Lake Greenwood (Greenwood CPW), and Lake Murray (City of Columbia and City of West Columbia) samples were below the USEPA 10-day drinking water health advisory values of 0.3 µg/L for bottle fed infants and pre-school aged children and 1.6 µg/L for school age children and adults (Figure 4). One (1) sample near the Lake Rabon drinking water intake had a microcystin concentration above 0.3 µg/L (microcystin concentration was 0.398 µg/L); however, the treatment process at Laurens CPW was able to remove microcystins and the toxin was not present in final drinking water. All samples collected near the Lake Whelchel (Gaffney BPW) drinking water intake had a microcystin concentration above 0.3 µg/L, and one sample was above 1.6 µg/L (microcystin concentration was 10.600 µg/L). The treatment process at Gaffney BPW was also able to remove the toxin, and it was not detected in finished drinking water.

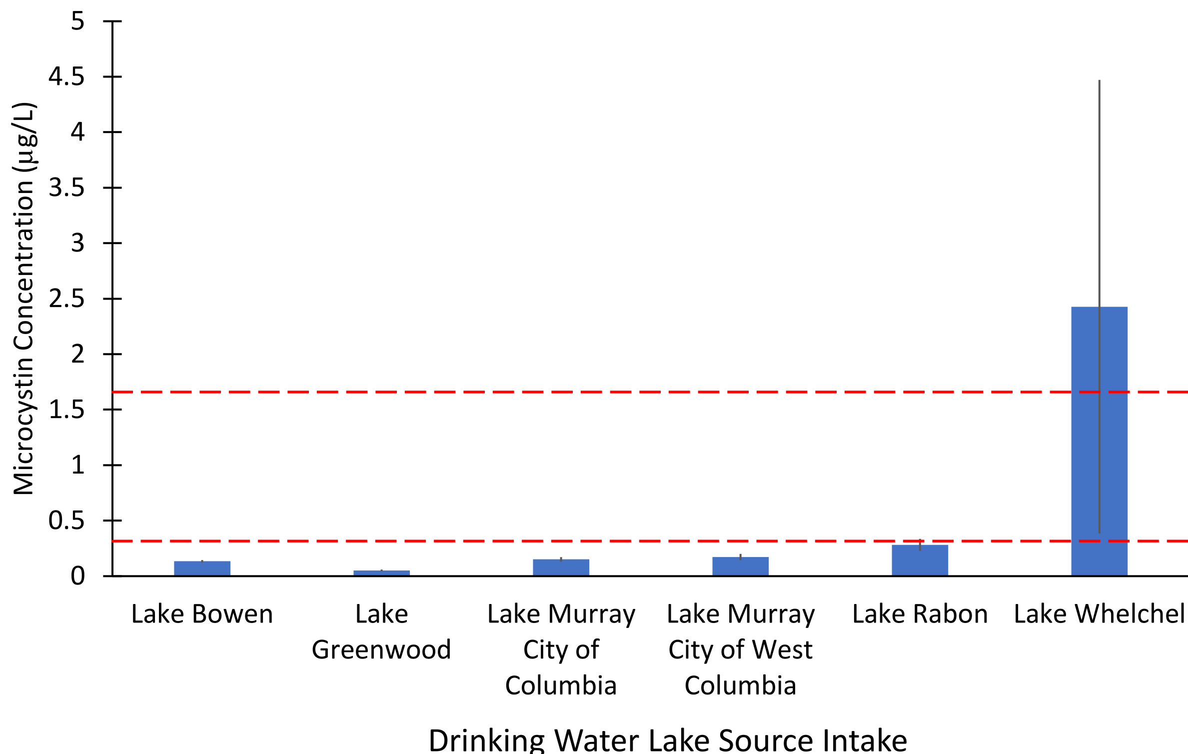

Figure 4: Average detectable microcystin concentrations (µg/L) per drinking water source intake in 2020. There were five (5) lakes sampled for six (6) different drinking water facilities. The red lines indicate the USEPA drinking water 10-day health advisory values of 0.3 for bottle fed infants and pre-school children and 1.6 µg/L for school age children and adults. The highest average microcystin concentration occurred at Lake Whelchel. All samples were below 0.5 µg/L at the Lake Whelchel drinking water intake, except for one sample with a concentration of 10.600 µg/L. The error bars represent +/- one (1) standard error.

Eleven (11) additional drinking water lake samples were collected at algal blooms that occurred on Lake Rabon and Lake Whelchel. One (1) bloom that occurred on Lake Rabon near the public boat landing at Lake Rabon Park had elevated microcystin concentration of 6.08 µg/L but was below the SCDHEC state recreational standard of 8 µg/L. Lake Whelchel had a persistent HAB from the end of August through November 2020. A total of ten (10) samples were collected at additional areas within the lake, including at the boat landing and in open water near the center of the lake. Five (5) samples collected had microcystin concentrations at 20 µg/L or higher during the bloom. SCDHEC issued a recreational advisory for the duration of the bloom. See the Advisory section below for specific details.

Summary of Drinking Water Lake Source Sample Findings

- Microcystins were detected in samples collected near all six (6) drinking water intakes in 2020 (≥ 0.100 µg/L for ADDA ELISA or ≥ 0.016 µg/L for SAES ELISA method).
- Lake Bowen (Spartanburg Water System), Lake Greenwood (Greenwood CPW), and Lake Murray (City of Columbia and City of West Columbia) samples were below the USEPA 10-day drinking water health advisory values of 0.3 µg/L for bottle fed infants and pre-school aged children and 1.6 µg/L for school age children and adults.

- One sample at the Lake Rabon drinking water intake had a microcystin concentration above 0.3 µg/L (microcystin concentration was 0.398 µg/L); however, the treatment process at Laurens CPW was able to remove microcystins and the toxin was not present in final drinking water.
- All samples collected at the Lake Whelchel (Gaffney BPW) drinking water intake had microcystin concentrations above 0.3 µg/L, with one sample above 1.6 µg/L. The treatment process at Gaffney BPW was also able to remove microcystins, and the toxin was not detected in the finished drinking water.

Event-Driven Samples

Throughout the 2020 season, the SCDHEC BOW ASP section received complaints on twelve (12) potential HABs. Of the twelve (12) complaint blooms, nine (9) were identified to be cyanobacteria blooms with the potential to produce microcystins. All nine (9) cyanobacteria samples had detectable levels of microcystins (Table 6). The highest concentration of microcystins (>20 µg/L) was at Bear Creek, a stormwater pond in Lancaster.

Table 6: Description and microcystin concentration (µg/L) results from 2020 algal bloom complaints with the associated date of the HAB. Microscopic images of cyanobacteria for four (4) of the designated blooms can be found in Appendix 4.

Sample Location	Sample Description	Collection Date	Microcystins (µg/L) [a]
Abbeville Drinking Water reservoir	*Lyngbya sp.*	04/16/2020	0.144
James Island- Hollis Lake	*Microcystis sp* and *Dolichospermum sp.* bloom	04/24/2020	2.0
Heritage Lakes	*Dolichospermum sp.* bloom	05/19/2020	1.9
Lake Hartwell	Filamentous mats of nontoxic *Oedogonium* and *Spirogyra*	06/10/2020	N/A[b]
Blythewood	*Planktothrix sp.* bloom in private pond	06/11/2020	0.276
Mount Pleasant	*Aphanizomenon sp.* bloom in drainage canal[c]	06/18/2020	N/A[b]
Bear Creek, Lancaster	*Microcystis sp.* Bloom[c]	06/22/2020	> 20
Bear Creek, Lancaster	*Microcystis sp.* and *Dolichospermum sp.* bloom	06/25/2020	3.0
Lancaster Reservoir, Lancaster	*Microcystis sp.* and *Dolichospermum sp.* bloom[c]	06/29/2020	5.75
Lake Edgar Brown	Undetermined brown algae bloom (dinoflagellate bloom) with small fish kill	07/13/2020	1.4
Lake Wateree	*Phormidium sp.* Bloom[c]	07/23/2020	1
Hilton Head Island	*Chrysoporum ovalisporum*	08/21/2020	N/A[b]

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

Summary of Event-Driven Sample Findings

- All nine (9) of the HAB complaint samples detected microcystins (≥ 0.100 µg/L for ADDA ELISA or ≥ 0.016 µg/L for SAES ELISA method).
- One (1) of the HAB complaint samples was greater than the SCDHEC state recreational action value of 8 µg/L for microcystins. This sample was at Bear Creek in Lancaster and had a microcystin concentration of >20 ug/L.

Advisories

The recommended USEPA recreational water quality and swimming advisory criteria for microcystins and cylindrospermopsin (Table 1) were adopted as enforceable State water quality standards in 2020. Two (2) recreational advisories were issued in 2020 for microcystin concentrations higher than SCDHEC's state standard of 8 µg/L (Table 7). The advisories were lifted once microcystin concentrations were below 8 ug/L and the bloom had dissipated.

The first advisory was issued at Lake Edgar Brown in mid-August following a sample with a microcystin concentration of 9.50 µg/L. The advisory was lifted in October when a second consecutive sample had a microcystin concentration below 8 µg/L. The second advisory was issued at Lake Whelchel from late August until the beginning of December in 2020. The highest microcystin concentration at >40 µg/L was observed at the boat landing in August. The advisory was lifted when the microcystin concentration decreased to 1.85 µg/L on December 4.

Table 7: Two (2) recreational HAB advisories were issued in 2020 due to microcystin concentrations µg/L) greater than SCDHEC's state standard of 8 µg/L. Samples were routinely collected at the water body until the advisory was lifted. The initial and ending microcystin concentrations were when the advisory was issued and lifted, respectively.

Water Body	Advisory Issued	Advisory Lifted	Initial Microcystin Concentrations (µg/L)[a]	Ending Microcystin Concentrations (µg/L)[a]
Lake Edgar Brown	8/19/2020	10/23/2020	9.50	0.893
Lake Whelchel	8/27/2020	12/04/2020	>40	1.850

a. µg/L = micrograms per liter (parts per billion)

Discussion and Conclusions

A primary goal of the HAB Monitoring Program is to establish cyanotoxin spatial distribution data in South Carolina waterbodies. These 2020 results have (a) contributed to a cyanotoxin concentration baseline for South Carolina waterbodies and (b) provided insight towards cyanotoxin presence/absence expectations. In 2018 and 2019, monthly samples were analyzed for both cylindrospermopsin and microcystins. However, the data from both of those years demonstrated that cylindrospermopsin was not present in most samples and if detected was present at very low concentrations (SCDHEC, 2020a; SCDHEC, 2021). As a result, the HAB Monitoring Program focused on expanding monitoring microcystins in South Carolina water bodies during the 2020 season and did not regularly analyze samples for cylindrospermopsin. Total number of samples for microcystins increased by 49% from 2019 to 2020 and microcystins were detected in 74% of the samples that passed QA/QC. SCDHEC expanded the HABs Monitoring Program in 2020 by sampling estuaries and influent streams, monitoring six (6) drinking water intakes at five (5) lakes, and issuing recreational water advisories when cyanotoxin levels were above State standards.

Overall, the results from the 2020 monthly-monitoring for microcystins in lakes showed toxin concentrations less than 2 µg/L, below SCDHEC's recreational standards. Estuaries were monitored for cyanotoxins for the first time in 2020. While all microcystin concentrations for estuaries were below 1 µg/L, these data are important milestones in establishing baseline toxin levels along the coast. The prevalence of algal toxins and toxin producing species in brackish and saltwater environments is not well understood. Salinity tolerances for algal species which produce microcystins are still being evaluated; however, some toxin producing species are not significantly impacted by salinity (Preece, Hardy, Moore, & Bryan, 2017). The low cyanotoxin concentrations observed as part of the monthly-monitoring data suggest that generally recreational activities in South Carolina are not an immediate concern. Maintaining and expanding monthly-monitoring in the future field seasons will help in identifying localized elevated cyanotoxin concentrations in additional environments. A limitation of the monthly-monitoring sampling sites is that they are fixed open-water locations. Cyanobacteria blooms often occur in shallow coves or along shorelines.

The event-driven sampling is a more targeted component of the HAB Program, which provides insight into potential cyanotoxin producing HABs in nearshore environments. Microcystin concentrations in event-driven samples ranged from 0.144 µg/L to >20 µg/L. The HAB at Bear Creek, a flood control pond in Lancaster County, was the only event-driven sample that had a microcystin concentration exceeding the SCDHEC state recreational standard of 8 ug/L. SCDHEC BOW ASP worked closely with Lancaster County Stormwater management to notify the homeowners on Bear Creek, and the public park was temporarily closed until the bloom dissipated. A recreational advisory could not be issued at the time since Bear Creek was not an official SCDHEC regulated waterbody of the state. Two (2) recreational advisories were issued in 2020 for Lake Edgar Brown and Lake Whelchel. The HABs at these water bodies were identified by SCDHEC staff while conducting routine sampling. The longest advisory occurred at Lake Whelchel, which lasted for approximately three (3) months.

SCDHEC's HAB Monitoring Program collaborated with six (6) drinking water facilities in 2020 to monitor drinking water intakes at five (5) lakes: Lake Bowen, Lake Greenwood, Lake Murray, Lake Rabon, and Lake Whelchel. Microcystins were detected at all drinking water intakes, but Lake Rabon and Lake Whelchel were the only drinking water intakes that had at least one sample greater than the USEPA 10-day drinking water health advisory value of 0.3 µg/L for bottle fed infants and pre-school aged children. The treatment process at Laurens CPW (Lake Rabon) and Gaffney BPW (Lake Whelchel) was able to remove microcystins and the toxin was not present in the finished drinking water. As HABs continue to expand and increase in frequency and duration, monitoring drinking water intakes and collaborating with drinking water facilities will continue to be a vital component of the HAB Monitoring Program.

No strong relationships were observed in the monthly-monitoring correlation results comparing microcystin concentrations to dissolved oxygen, pH, temperature, total phosphorus, N:P ratio, and chlorophyll *a* for Lake Greenwood, Lake Hartwell, Lake Murray, and Lake Wateree. The lack of a clear relationship among these monitoring variables suggests that the periodic occurrence of toxin producing cyanobacteria species is more complex than a single variable correlation in the same time and space (Davis, Berry, Boyer, & Gobler, 2009; Paerl & Otten, 2012; Wiltsie, Schnetzer, Green, Vander Borgh, & Fensin, 2018) or is related to environmental variables not routinely measured as part of the ambient monitoring program. Further, these lake-by-lake datasets are small and likely not robust enough for meaningful correlation. More data over the next several years will build on the past three (3) years of data and may provide a clearer understanding of patterns in cyanotoxin production.

In conclusion, the monthly-monitoring cyanotoxin results were generally lower than the SCDHEC state recreational standards, suggesting recreational activities in South Carolina were not an immediate concern. Estuaries were included for the first time in the 2020 cyanotoxin monthly-monitoring. While initial microcystin concentrations were low, continuing to monitor the estuarine environment in future years will improve and expand SCDHEC's understanding of harmful cyanobacteria presence along the coast. Two (2) recreational advisories were issued in August for Lake Edgar Brown and Lake Whelchel, which lasted approximately two (2) and three (3) months respectively. There was one (1) event-driven sampling event at Bear Creek in Lancaster County where microcystin concentrations exceeded SCDHEC recreational state standards. In this case, SCDHEC worked with Lancaster County to disseminate the information for user education and protection. SCDHEC also worked with drinking water facilities to monitor six (6) different drinking water intakes at five (5) lakes for microcystins. Microcystins were present at each drinking water intake, but the drinking water treatment successfully removed the toxin. Even though no strong correlations between microcystin concentrations and other environmental parameters were discerned in this assessment, a larger dataset over several years may provide better insight into relationships among these variables. The HAB Monitoring Program continues to work on educating South Carolina residents on HABs. In 2020 an informational rack card was created to provide an additional educational resource to users of private and public water bodies (see Appendix 5). Future goals of the HABs Monitoring Program include expanding the statewide cyanotoxin study to include other toxins, such as anatoxin and saxitoxin.

Overall Summary:

- 2020 completed the third year of the HAB Monitoring Program. The data gathered in 2018, 2019, and 2020 will be used to inform future sampling plans and provide insights into lakes that the agency may consider monitoring more frequently.
- The monthly-monitoring sampling suggest no immediate concern for recreation activities due to the low concentrations of microcystins in open water settings.
- Estuarine water bodies were included in the monthly-monitoring sampling for the first time in 2020.
- There was one (1) event-driven sample at Bear Creek, a privately owned pond, that exceeded the SCDHEC state standard of 8 µg/L. SCDHEC worked with Lancaster County on ways to distribute appropriate information and advised closure of the public park area.
- SCDHEC adopted USEPA recreational guidelines for cyanotoxins in 2020, which allows the Department to issue advisories for water bodies of the state when cyanotoxins are greater than above guidelines. Two (2) recreational advisories were issued in August for Lake Edgar Brown and Lake Whelchel, which lasted approximately two (2) and three (3) months respectively.
- There were no strong correlations between microcystin concentrations and other parameters measured in Lake Greenwood, Lake Hartwell, Lake Murray, and Lake Wateree. Future analyses would benefit from a larger data set that also includes samples from algal blooms and examines a combination of factors.
- An informational rack card was created at the end of the 2020 season to provide an educational HAB resource to private and public water bodies. See Appendix 5 for the HAB rack card.

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

Revision 1

Determination of Total Microcystins and Cylindrospermopsin in Ambient Water

Bureau of Water- Aquatic Science Programs

June 1, 2020

Author	06/02/2020 Date
Manager, Aquatic Science Programs	2020-06-01 Date
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1. SCOPE AND APPLICATION

1.1 Method Description

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

2. METHOD SUMMARY

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

2.1 Microcystins

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

2.2 Cylindrospermopsin

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

3. DEFINITIONS

3.1 Analysis Batch

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

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

3.2 Well Replicates

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

3.3 Use of Well Replicate Absorbance Values

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

3.4 Use of Well Replicate Concentrations

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

3.5 Calibration Standards

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

3.6 Calibration Curve

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

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

3.7 Four-parameter Logistic Equation

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

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

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

3.8 Quality Control Sample (QCS)

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

4. HEALTH AND SAFETY WARNINGS

4.1 Microcystins

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

4.2 Cylindrospermopsin

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

4.3 Cylindrospermopsin Seawater Sample Reagent

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

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

5. INTERFERENCES

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

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

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

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

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

6. SAMPLE HANDLING, PRESERVATION, AND STORAGE

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

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

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

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

7. INSTRUMENTATION AND EQUIPMENT

7.1 Adda ELISA Test Kits- 96-well Microtiter Plates

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

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

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

7.3 Disposable plastic tips for pipettes

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

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

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

7.4 Vials for freezing samples

7.4.1 Vials, Glass, Clear, 4 mL with caps

7.4.2 Vials, Glass, Clear, 40mL with caps

7.5 Syringes and Filters for Lysing

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

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

7.6 500 mL PETG containers with PTFE septa lined lids

7.7 Parafilm for plate covering

8. REAGENTS, STANDARDS, AND CONSUMABLE MATERIALS

8.1 Analysis Kit

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

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

9. INSTRUMENT CALIBRATION PROCEDURES

9.1 Micropipetters

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

9.2 Calibration Procedure

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

9.3 Calibration Acceptance Criteria

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

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

10. Procedures

10.1 Sample Lysing Procedure by Freeze-Thaw

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

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

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

10.2 Seawater Sample Preparation

10.2.1 Microcystins

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

10.2.2 Cylindrospermopsin

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

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

10.3 Test Preparation

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

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

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

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

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

10.4 Assay Procedures

10.4.1 Microcystins

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

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

10.4.2 Cylindrospermopsin

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

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

10.5 Running an Assay

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

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

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

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

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

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

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

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

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

10. 5 QUALITY CONTROL

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

10.5.1 Initial Demonstration of Capability (IDC)

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

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

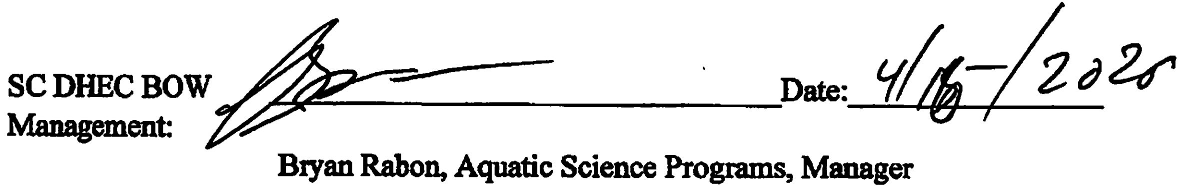
SC Cyanotoxin Distribution Study
Prepared by Emily Bores
February 5, 2020

Project Manager: Emily Bores, Aquatic Science Programs

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

Project Manager: ____________________ Date: 04/20/2020
Emily Bores, Aquatic Science Programs

SC DHEC BOW Management:



Bryan Rabon, Aquatic Science Programs, Manager

SC DHEC BEHS: ____________________ Date: 04/15/2020
Elizabeth Basil, Assistant Bureau Chief

SCDHEC QAM: ____________________ Date: 4/16/2020
David Graves, QAM

EPA Region 4 QA Officer: ELIZABETH SMITH (Digitally signed by ELIZABETH SMITH Date: 2020.05.04 08:51:53 -04'00') Date: ____________
Elizabeth Smith, US EPA, Region 4

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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
Taylor Shearer	ASP personnel	SC DHEC	803-898-1538	shearetv@dhec.sc.gov
David Graves	QAM	Environmental Affairs	803-898-4272	gravesda@dhec.sc.gov
Alexander Grubbs	Field Personnel	SC DHEC- Greenville Office	864-372-3263	grubbsaw@dhec.sc.gov
Chad E. Johnson	Field Manager	SC DHEC- Lancaster Office	803-285-7461	johnsoce@dhec.sc.gov
Caitlin Smith	Field Personnel	SC DHEC- Midlands Office	803-896-0620	Smithc5@dhec.sc.gov
Stephanie Jacobs	Lab Manager	SC DHEC- Aiken Office	803-642-1637	jacobssa@dhec.sc.gov
Allyson Muller	Field Manager	SC DHEC- Charleston Office	843-953-0150	mulleram@dhec.sc.gov
Sarah Brower	Field Manager	SC DHEC- Beaufort Office	843-846-1030	browersr@dhec.sc.gov
Dave Chestnut	Project Validation	SCDHEC	803-898-4066	chestnde@dhec.sc.gov

A4 Project/Task Organization

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

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

David Graves- 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.

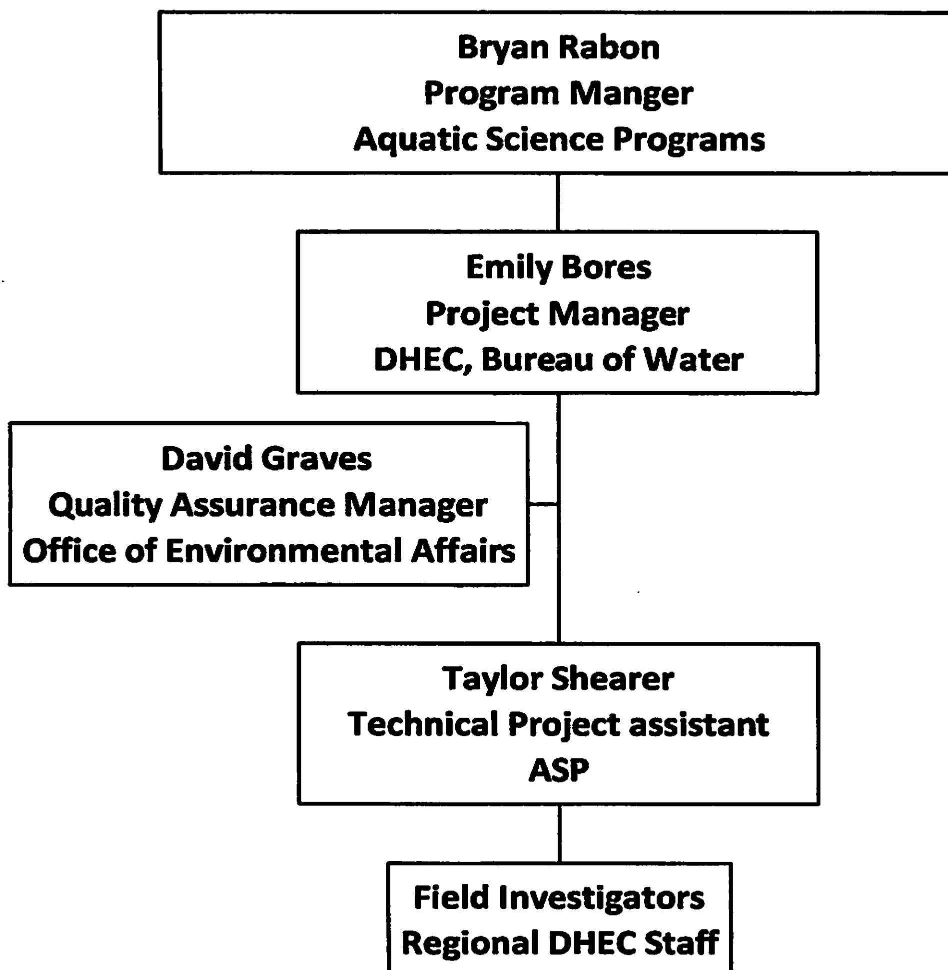


Figure 1 Project Organization Chart

A5. Problem Definition/Background

The goal of this project is to continue to characterize the occurrence of cyanotoxins in surface waters from reservoirs in South Carolina. The results will be used with data from 2018 and 2019 to continue to assess potential risks to drinking water facilities, as well as recreational and aquatic life uses for waterbodies of the state. Recent events associated with toxic algal blooms in Toledo (Jetoo et al. 2015), EPAs (2015) release of health advisories for cyanotoxins in drinking water and improved analytical methods have made clear the need to better characterize the presence of cyanotoxins in the state's reservoirs. Despite the increased knowledge of eutrophication and harmful algal blooms (HABs) in SC's coastal waters, HABs of inland freshwaters remains less clear. Although SCDHEC and its predecessors have had a robust monitoring network of surface water since the 1950s, cyanotoxins have not been included in the suit of analytes normally tested. While certain measures of eutrophication such as chlorophyll a, nitrogen, phosphorus, and water clarity may show correlation with cyanotoxins, these measures alone do not provide a full picture of environmental conditions associated with toxins. With improved analytical methods it is now possible to detect cyanotoxins at lower levels, which can provide the baseline for their occurrence in SC. The characterization of waterways is the first step in the process for effective environmental management and knowing where and under what conditions threats may occur is a critical first step to mitigate harm to human and environmental health.

We propose, therefore, to conduct a statewide survey of cyanotoxins in the lakes and estuaries of South Carolina. The survey will focus on lakes being sampled from the 2020 base ambient sites as well as samples collected from the 2020 base estuarine sites. In addition to the base lake and estuarine sites, samples from 5 lakes will be collected near their drinking water intakes on a monthly basis. These 5 lakes were chosen based on past algal issues, including taste and odor complaints. Some event driven testing will be conducted and may include large rivers in addition to lakes and estuaries. Combined with other water quality variables and geospatial data, a better understanding of cyanotoxins in freshwaters and estuaries will be achieved. With EPAs (2015) recent release of health advisories thresholds in drinking water for microcystins, this cyanotoxin will be targeted. While this project is focused on toxin analysis for recreational waters only, if there are 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. See Table 2 for the EPA draft Recreational Criteria or Swimming Advisory Recommendations for Microcystins. The event driven testing will target algal blooms that may be observed or reported during the 2020 growing season.

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

Application of Recommended Values	Microcystins		
	Magnitude (ug/L)	Frequency	Duration

Swimming Advisory	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)

A6. Project/Task Description

As stated previously, the purpose of this proposed project is to better understand the occurrence of cyanotoxins in the lakes of South Carolina, in continuation of the sampling efforts from the 2018 and 2019 sampling season. A total of approximately 650 samples will be collected by regional staff. Monthly grab samples will be collected at approximately 105 stations and will be shipped via overnight courier to the Aquatic Science Programs'(ASP) cyanotoxin lab in Columbia. These samples will be taken during normal monthly ambient monitoring of select reservoirs and estuaries during the months of May through October 2020. Refer to the State of South Carolina Monitoring Strategy for Calendar Year 2020, Technical Report No, 1008-19. An additional 5 lakes will be sampled monthly as close as practical to their drinking water intakes due to previous issues with taste and odor. Due to the holding time for cyanotoxins, all samples will be frozen in 40mL vials within 24 hours at -20 C or lower (holding time at -20 is 2 weeks). The transport of samples to the ASP cyanotoxin lab should occur within 24 hours from the regions. At the lab, samples will be tested for total microcystins 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. The ELISA plates being used will be SAES- Streptavidin Amplified Enhanced Sensitivity, to allow for analysis of both fresh and saltwater samples. Training and additional guidance was also provided from the provider, Abraxis. Additionally, samples may be collected due to event driven algal blooms and/or waters with taste and odor problems. Phytoplankton taxonomic analysis may also be conducted on samples when applicable. Table 3 provides the project activities and their anticipated date of initiation and completion. Table 4 provides the locations of the sampling locations for the drinking water facilities. Table 5 provides the SC DHEC station codes and site descriptions. Sites for this project were chosen from the current list of 2020 sites as well as their proximity to a public water source. 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	02/01/2020	03/02/2020

QAPP Approval	SCDHEC	02/05/2020	04/30/2020
Sampling Begins	SCDHEC	05/04/2020	10/31/2020
Lab Reports	SCDHEC	05/30/2020	11/10/2020
Data Validation	SCDHEC	06/01/2020	11/30/2020
Final Report Due	SCDHEC	12/01/2020	12/31/2020

Table 4. Drinking Water Site Locations

Lake Name	Lab	Drinking water Facility	Latitude	Longitude
Lake Greenwood	ASP	Greenwood CPW	34.2603526	-82.0293682
Lake Rabon	ASP	Laurens CPW	34.478479	-82.139813
Lake Murray	ASP	City of West Columbia and City of Columbia	34.097777 34.021534	-81.231318 -81.232625
Lake Bowen	ASP	Spartanburg Water System	35.111289	-81.972796
Lake Whelchel	ASP	Gaffney BPW	35.107865	-81.622177

Table 5. Site Locations

Station	Regional Lab	Description	Latitude	Longitude
B-327	Central Midlands	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	Central Midlands	Parr Reservoir in Forebay near dam	34.26208554189	-81.33538487819
CL-019	Greenville	Lake Jocassee in Forebay equidistant from dam and shorelines	34.95988763468	-82.92361397724
CL-041	Greenville	Clarks Hill Reservoir in Forebay near dam	33.66999442019	-82.20761435616
CL-069	Aiken	Langley Pond in Forebay near dam	33.5222610417	-81.8432066618
CL-089	Midlands	Lake Wateree in Forebay equidistant from dam and shorelines	34.33684850575	-80.70499959935
CW-016F	Lancaster	Fishing Creek Reservoir 2 mi. below Cane Creek	34.67778314931	-80.87718655105

Station	Regional Lab	Description	Latitude	Longitude
CW-033	Midlands	Cedar Creek Reservoir 100 m N of dam	34.5426516318	-80.87773762794
CW-057	Lancaster	Fishing Creek Reservoir 75 ft. above dam near Great Falls	34.60528283986	-80.89104250062
CW-174	Midlands	Cedar Creek Reservoir at Unimp. Road AB JCT with Rocky Creek	34.55815953884	-80.8916653521
CW-197	Midlands	Lake Wylie above Mill Creek arm at end of S-46-557	35.13756014086	-81.05942285366
CW-201	Midlands	Lake Wylie North Lakewoods S/D at Ebenezer access	35.02811990158	-81.0476664737
CW-207	Midlands	Lake Wateree at end of S-20-291	34.40248974794	-80.78839167726
CW-207B	Midlands	Mid Lake Wateree	34.4039	-80.7827
CW-208	Midlands	Lake Wateree at S-20-101 11 miles ENE Winnsboro	34.4219226293	-80.86743212474
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
LCR-02	Midlands	Lake Wateree upstream of Wateree Creek Arm	34.4817	-80.9001
LCR-03	Midlands	Lake Wateree off Dutchman Creek arm	34.4254	-80.8439
LCR-04	Midlands	Fishing Creek Reservoir Midlake off Bear Creek Arm	34.6204	-80.8862
MD-001	Beaufort	Beaufort River above Beaufort at Channel Marker 231	32.44563386949	-80.66322125342
MD-004	Beaufort	Beaufort River at junction with Battery Creek near marker 42	32.36529134346	-80.67789557314
MD-043	Beaufort	Cooper River at channel marker 72 near USN Ammo Depot	32.96290012385	-7992123591461
MD-045	Beaufort	Cooper River above mouth of shipyard creek at channel buoy 49	32.84532743	-79.93346899899
MD-049	Charleston	Ashley River at Magnolia Gardens	32.8758482832	-80.08147390945
MD-052	Charleston	Ashley River at Salrr Bridge	32.79655336431	-79.97193838412
MD-069	Charleston	Intracoastal waterway at SC 703 E Mt Pleasant	32.77282850304	-79.84221069087

Station	Regional Lab	Description	Latitude	Longitude
MD-077	Myrtle Beach??	Sampit River at US 17	33.35741249322	-79.29399372267
MD-115	Charleston	Wando River at SC 41	32.92280921729	-79.82734089263
MD-116	Beaufort	Broad River at SC 170 7.5 miles SW of Beaufort	32.38477775245	-80.78380589943
MD-117	Beaufort	Chechessee River at SC 170 10.5 miles SW of Beaufort	32.37405584367	-80.8360677473
MD-118	Beaufort	New River at SC 170 9 miles west of Bluffton	32.23596776794	-81.0128595401
MD-120	Charleston	Dawho River at SC 174 9 Mi N of Edisto Beach SP	32.63658170895	-80.34185351325
MD-129	Beaufort	Great Swamp at U.S. 17	32.4060985216	-81.01871610562
MD-130	Charleston	Folly River at SC 171	32.65961753556	-79.94334175075
MD-142	Myrtle Beach	Wacammaw River downstream of Butler Island at marker 86	33.40826895929	-79.21707619442
MD-173	Beaufort	May River 1.8 miles SE of Bluffton out from end of S-07-461	32.21038205018	-80.84226621333
MD-174	Beaufort	Broad Creek opposite end of S-07-80	32.18041955466	-80.7740148536
MD-176	Beaufort	Colleton River at Colleton Neck at junction with Chechessee river	32.33230257137	-80.87736135055
MD-202	Charleston	Stono River at S-10-20 2 miles upstream of Clemson Exp Station	32.78572668309	-80.10748582768
MD-206	Charleston	Stono River at Abbapoola Creek	32.67443347229	-80.00458969335
MD-209	Charleston	Bohicket Creek at Fickling Creek	32.62230612481	-80.16433818575
MD-248	Charleston	Cooper River at Mark Clark Bridge 1-526	32.8905488956	-79.96269694133
MD-252	Beaufort	Combahee River off Fields Point Landing off end of S-15-161	32.56425104067	-80.55697391071
MD-253	Beaufort	Ashepoo River at Public Oyster ground 14-19	32.53296809641	-80.44844018266
MD-256	Beaufort	Unnamed Creek between harbor river and stony river 16-21	32.33994756891	-80.50781796438
MD-257	Beaufort	Ramshorn Creek at Cooper River 19-03	32.1288301718	-80.88987349545
MD-258	Beaufort	Ramshorn Creek at New River 19-07	32.11096107166	-80.89857916165
MD-259	Beaufort	Wright River 1.5 miles upstream from Fields Cut 19-20	32.09431706968	-80.94887684008

Station	Regional Lab	Description	Latitude	Longitude
MD-260	Charleston	S. Edisto River at Northern Confluence with Alligator Creek 13-20	32.56726404851	-80.3900702786
MD-261	Charleston	Yonges Island Creek; Marker #90 12-03	32.69473169577	-80.22295893957
MD-262	Charleston	N Edisto River at Leadenwah Creek 12-08	32.60590740978	-80.22932922218
MD-264	Charleston	Wando River at I-526 Mark Clark Expressway -09B-15	32.85842094488	-79.89590184826
MD-266	Charleston	Casino Creek at Closure Line - 06B-16	33.07509375369	-79.39409759572
MD-267	Charleston	Five Fathom Creek at Bull River -07-06A	33.0366115845	-79.47689624498
MD-269	Charleston	Sewee Bay at Moores Landing - 08-09	32.9367375924	-79.65497814354
MD-271	Charleston	Hamlin Sound -08-02	32.8268721303	-79.77456462671
MD-273	Charleston	Kiawah River on the Flats -11-21	32.60800793194	-80.12742971635
MD-275	Myrtle Beach	Pee Dee River at White House Plantation	33.42224881377	-79.22459332197
MD-277	Myrtle Beach	Parsonnage Creek at Inlet Port Basin -04-17	33.55291880646	-79.03395826617
MD-278	Myrtle Beach	Winyah Bay Main Channel; Buoy 19A Range E -05-20	33.2735051089	-79.24263237088
MD-281	Beaufort	Parrot Creek and Coosaw River Marker #1 Shellfish 14-10	32.4953580259	-80.555346251
MD-282	Beaufort	Morgan River at Confluence with Warsaw Flatsshellfish 16A-35	32.4438202642	-80.6069005252
PD-325	Myrtle Beach	Black River at S-22-489 4 miles NE Georgetown	33.41380456204	-79.25037552266
PD-327	Florence	Lake Robinson at S-13-346 5 MI E Mcbee by boat ramp	34.46752201266	-80.1698000394
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

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

Station	Regional Lab	Description	Latitude	Longitude
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
SV-374	Greenville	Lake Hartwell- Eighteen Mile Creek arm approx. 227 yards SW of 18 mile Creek Boat Landing	34.5721409	-82.8299353

Figure 2 Sampling Locations

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

The overall data quality objective is to collect water samples for identification of potentially toxigenic algal species and cyanotoxin analysis via ELISA methodology. Samples will be collected once per month for 6 months from each site to assess distribution during the algal growing season. Objectives for accuracy, precision, representativeness, comparability, and completeness are summarized below. Specific data quality indicators are provided in Table 6.

DQOs

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

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

2. Identify inputs to the study - Specific Microcystin concentrations in water samples via ELISA assay and possible identification of phytoplankton taxonomy.

3. Define the Study Boundaries- 105 sites located in lakes and estuaries throughout South Carolina will be sampled once a month for 6 months in 2020. See table 4 and 5 for locations of sampling sites.

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

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

6. Optimize the design for obtaining the data- It is believed that 105 sites sampled once a month for a 6-month period, producing approximately 650 samples, will continue to provide an adequate baseline characterization of the occurrence of cyanotoxins in the reservoirs and estuaries of SC. The quality of samples and their analysis for harmful toxins will continue to be important in identifying more potential sites to be added to the sampling list the following year due to potential risks associated with high cyanotoxin concentrations in certain reservoirs, as well as specific areas that are "hot spots" for cyanotoxin blooms.

Table 6. 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. $r^2 \geq 0.98$	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:

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

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

Bias

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

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

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

Accuracy for the project will be based on the average of the well replicates analyzed for the known standards in the test kit. Thus, accuracy for this project will be assessed by the percent recovery of the analyzed value of a microcystin 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 2020 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 7).

A9 Documentation and Records

QAPP Formulation and Distribution
Emily Bores is responsible for writing, maintaining and distributing the QAPP. The approved QAPP will be distributed electronically. If the QAPP needs to be revised during the study period, the person in charge of the QAPP will do so and submit to the QAM, or designee, for approval.

Once the revised QAPP is approved, the updated QAPP is sent to those individuals on the distribution list. If there are major changes to the QAPP, then the entire document will be distributed. If there are only minor changes to a few pages, these pages will be distributed with directions of which pages to pull from the QAPP and which to insert. A delivery receipt request will be sent with the updated QAPP and/or QAPP portions, so the recipient must reply indicating that they have received the updates and are using them.

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

Other records generated by this project:
The information in Table 7 is an itemized list of the records generated by the project and how they are stored.

Table 7. 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

Routine sampling will begin 05/04/20 and end on 10/31/20. Samples will be collected once a month during the algal growing season (May-October). See Table 3 in section A6 for the list of proposed sampling activities for this project.

Description of Sample Design Strategy and Sample Sites

The sampling locations were chosen by SC DHEC based on the current 2020 lake and estuary site sampling schedule. If affected by cyanotoxins, these sites could affect human health due to their use for recreational activities, drinking water, and possible harvesting areas in estuaries. The sample locations for this project are provided in Table 4 and 5 of section A6. The 105 sites will each be sampled once a month for 6 months, equating to about 650 total samples being tested for Microcystins. 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, with the exception of the 5 drinking water facility sites. The drinking water sites will be identified with the name of the lake and intake where the sample was taken at. These sites are listed in Table 4 and Table 5 of section A6. All the sites will be accessed by boat via public boat landings or public docks. If a private dock is used for an algal bloom complaint, consent from the landowner must be obtained before the sample can be taken. In the field, the site locations will be located via the description provided in Table 4 and 5. The samples collected will be grab samples and collected from the surface 0.3m below the water surface. Samples will be identified with the site name and the sampling date.

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

B2 Sampling Methods Requirement

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

1. The sample will be collected at the site location using a boat or dock to reach the area.
2. The COC is filled out just prior to sample collection (see appendix).
4. A 500mL Polyethylene Terephthalate Glycol bottle will be used and the samples will be collected via grab sample 0.3m below the surface. A minimum of 300 mL of sample must be collected.
5. Once the bottle is filled, the sample lid will immediately be replaced. No preservative is needed for the samples that are solely being analyzed for toxins.
6. Samples are not to be composited, split, or filtered in the field.
7. The sample information is written on the bottle and logbook. This includes
   a. Site name
   b. Date and Time of collection
8. The time the sample was collected is written on the COC and logbook.
9. Samples will be placed in ice in coolers immediately. Coolers will be shipped via State courier overnight to the ASP lab in Columbia where the samples will be placed in the freezer (-20 C). The temperature blank in the cooler must be ≤ 10°C 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 2020 for the Ambient Surface Water Quality Monitoring Program.
12. If any problems occur during sampling, the Field manager is responsible for any corrective action that needs to be taken.

B3 Sample Handling and Custody Requirements

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

Sample Identification

Each sample will be identified using the SC DHEC station number labeled on the sample container, with the exception of the 5 drinking water intakes. These samples will be identified with the lake and intake name. These codes are provided in Table 4 and 5 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 toxin Microcystin 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 8. The primary instrumentation required for analysis is listed in Table 8 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 8 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 8. Analytical Method and Performance Criteria

Analyte	Matrix	SOP	Rev # and Date	Method Ref	Instrument	Test Sensitivity
Total Microcystins	**Water**	**8/28/18**	**Rev 1 03/2020**	**EPA 546, Ohio EPA DES 701.0 Version 2.2, Abraxis product inserts**	**Abraxis 8-channel microplate reader; Model 4303**	**0.016 ppb (µg/L) for SAES plate and 0.100 ppb (ug/L) for ADDA**

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 logbooks, or on project paperwork including the case narrative.

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

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

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

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

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

B5 Quality Control Requirements

An initial demonstration of capability (IDC) must be successfully performed prior to analyzing field samples. Refer to the attached SOP for IDC requirements. The QC requirements in Table 9 are considered the minimum acceptable QC protocol. EPA Region 4 confirmed that the QC measures described below are satisfactory for ambient water sampling.

Table 9. 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 $r^2 \geq 0.98$	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 10. 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 11. 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	Contact technical support for direction	

	the device needs to be recalibrated.		

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 12. 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'	Analyst	Section 5.3.2.4 in Abraxis Model 4303 Manual

			as action to take when result is out of range, and to set the valid time, in days/hours.		
Controls	Set the amount of time in days, hours, or both, that the controls should remain valid	Set up the out of range and the 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	Once a control reaches the end of the valid time period, the calibration tab will indicate "expired"	Analyst	Section 5.3.2.6
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

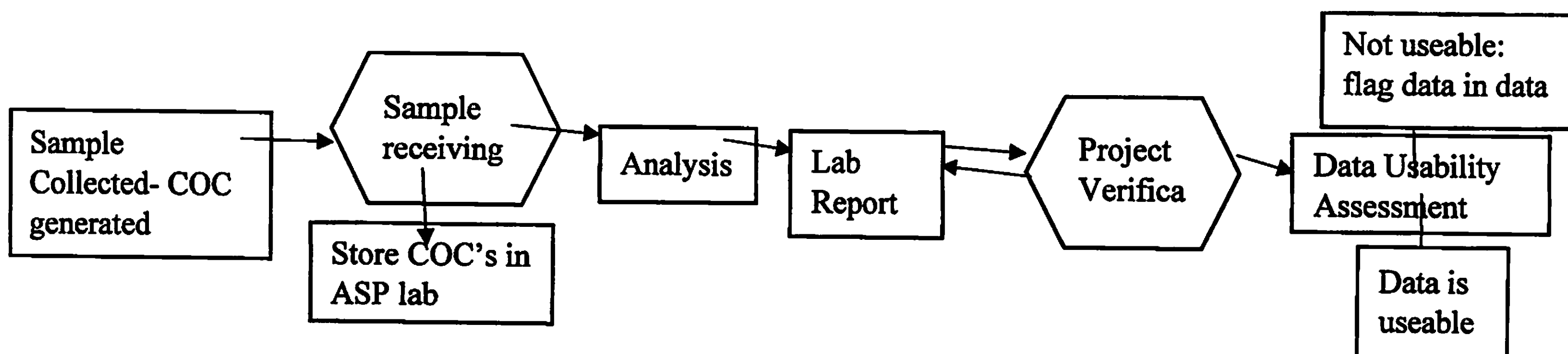
Latex Gloves	All	No holes	1 box of appropriate size in lab	Emily Bores (Project manager), ASP lab
4mL and 40mL vials	All	Borosilicate glass with PTFE-lined caps. Glass not broken.	Office prep area-room temp	Emily Bores (Project manager), ASP lab
Luer Slip Syringe	All	3mL with Luer-Lock connection	Office prep area-room temp	Emily Bores (Project manager), ASP lab
Syringe Filters	All	Glass microfiber filter, 25mm with 0.45 µm pore size	Office prep area-room temp	Emily Bores (Project manager), ASP lab
PETG Storage Bottles	All	Has to be PETG material, at least 500 mL volume	Office prep area-room temp	Emily Bores (Project manager), ASP lab
PTFE Discs	US Plastics	Discs must be PTFE, 38mm disc for 500mL 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 ADDA SAES ELISA plates	Abraxis	Kits must be complete (i.e. include all standards) and not broken. Must be within expiration dates	Refrigerator at 4-8 C	Emily Bores (Project manager), ASP lab
Pipette tips	All	Must have volume of at least 50µL and up to 300µL	Office prep area-room temp	Emily Bores (Project manager), ASP lab

Precision Dispenser (PD) Tips	All	Volume of 1mL	Office prep area-room temp	Emily Bores (Project manager), ASP lab

B9 Data Acquisition Requirements for Non-Direct Measurements
Since there is little known about the occurrence of cyanotoxins in the lakes of SC and this is an investigative study in order to better understand the possible distribution, there are no intended sources of previously collected data (not applicable) and other information that will be used in this project. The data collected in the 2018 and 2019 project is used as reference for the 2020 lakes. Data collected from the estuaries in 2020 has no reference. Data from both years may be used as reference and/or guidance for any future projects.

B10 Data Management

Figure 3. Data Management Scheme



Data transmittal will occur from the plate reader's software to the lab report (excel spreadsheet). The software will allow for the data to be downloaded electronically on the computer via excel file. The analysts are responsible for the data transmittal and the project manager is responsible for reviewing each transmittal. David Chestnut is responsible for the data quality during the process. He will review the data in the generated lab report to make sure that the results were accurately recorded and check for any errors. If any errors are found in the lab report, the project manager is responsible for correcting that error. The data from the COC (i.e. field parameters such as temperature, pH, etc.) and the data generated from the analysis will be recorded electronically via excel spreadsheet. Data can be retrieved through this spreadsheet on the computer. The hard copies of the COC will be archived in the ASP lab for at least 10 years. The excel spreadsheet of the data will be maintained for 10+ years. If possible (permitting space requirements), do not dispose of the COC or lab reports even after the 10-year deadline.
The microplate reader and Abraxis reader software are the hardware and software items that will need to be routinely tested and upgraded. Refer to Table 12. This software and hardware are proprietary and are acceptable for this project. For ELISA Instrument Maintenance, Operation, and

Preventative Maintenance. If updates are required for the test menu, contact the dealer at Abraxis. Also refer to the User manual (Abraxis Reader Operator's Manual Doc. 4303 Rev. D) for more assistance.

Section C Assessment and Oversight

C1 Assessment and Response Actions

Since this is a short-term research project, few assessments will be conducted. The Project Manager is responsible for responding to and resolving all quality assurance problems and needs. The Project Manager will initiate corrective action to adverse conditions that compromise quality in the field or laboratory. A thorough periodic review of the complete data review process, including a review of the sample analysis verification, sampling and analysis validation, and data usability steps, will be conducted to ensure that the process conforms to the procedure specified in the QAPP. Elizabeth basil is responsible for field QA/QC and the project manager is responsible for the Lab QA/QC. Any evaluation or progress reports requested by USEPA Region 4 will be addressed directly to Region 4.

C2 Reports to Management

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

Section D Data Validation and Usability

D1 Data Review, Verification and Validation

Table 13. Data Criteria

Item	Data Standards		If this criterion is not met, is the sample rejected or flagged?
Sample Temperature	Sample temperature blank is below 10°C		Flagged (may be rejected at analyst's discretion)
Analysis Time	Two weeks from time of sampling if in a -20C freezer.		Flagged

Hold Time	Samples arrives at the lab within 24 hours after collection		Flagged (may be rejected at analyst's discretion)
ELISA calibration	See Table 5		Analysis Batch invalid
Well Replicates	See Table 5		Samples invalid
Quality Control Sample (QCS)	See Table 5		Flagged (may be rejected at analyst's discretion). Reanalysis if possible

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

A The analyte was analyzed in replicate. Reported value is an average of the replicates
P Sample improperly preserved and/or collected
R The presence of absence of the analyte cannot be determined from the data due to sever quality control problems. The data are rejected and considered unusable.
U The analyte was not detected at or above the reporting limit

D2 Validation and Verification Methods

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

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

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

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

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

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

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

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

David Chestnut will be responsible for validating all components of the project data/information. See Table 14 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.

Table 14. 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 lakes and estuaries to determine the future direction of a State HABs surveillance program. As this is primarily an investigative study one of the important outcomes is the evaluation of the performance of all aspects of this project and recommendations for future improvements. Any limitations on data due to issues found during verification and validation will be included in the final report.

E. Revision History

Date	Revision	Change	Section
Feb 2019	1.0	Added Revision History	E
Feb 2019	1.0	Updated Background Information for 2019	A5
Feb 2019	1.0	Updated Draft Swimming Advisory Numbers	Table 2
Feb 2019	1.0	Updated number of sites and sampling period for 2019	A6, A7, B1
Feb 2019	1.0	Updated Project Actions for 2019	Table 3
Feb 2019	1.0	Updated site descriptions for 2019	Table 4
Feb 2019	1.0	2019 sampling locations map	Figure 2
Feb 2020	2.0	Updated Distribution List	A3- Table 1
Feb 2020	2.0	Updated Project Organization Chart	A4- Figure 1
Feb 2020	2.0	Updated Background Information for 2020	A5
Feb 2020	2.0	Removed Cylindrospermopsin Information from Table 2	A5
Feb 2020	2.0	Updated Number of Sample and Sites for 2020	A6
Feb 2020	2.0	Added new table (Table 4) of drinking water sampling locations	A6
Feb 2020	2.0	Updated project activities for 2020	A6- Table 4
Feb 2020	2.0	Updated Site Locations	A6- Table 5
Feb 2020	2.0	Updated map of 2020 site locations	A6- Figure 2
Feb 2020	2.0	Updated DQOS	A7
Feb 2020	2.0	Updated Schedule of Sampling and Design Strategy	B1
Feb 2020	2.0	Updated Step 4 to 500mL PETG	B2
Feb 2020	2.0	Removed analytical method for Cylindrospermopsin	B4- Table 8
Feb 2020	2.0	Updated SOP number for Microcystins ADDA SAES	B4- Table 8
Feb 2020	2.0	Updated supplies to 500mL PETG from 1L	B8
Feb 2020	2.0	Microcystins ADDA plate changed to Microcystins ADDA SAES plate	B8
Feb 2020	2.0	Updated sampling for 2020	B9

Literature Cited

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

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

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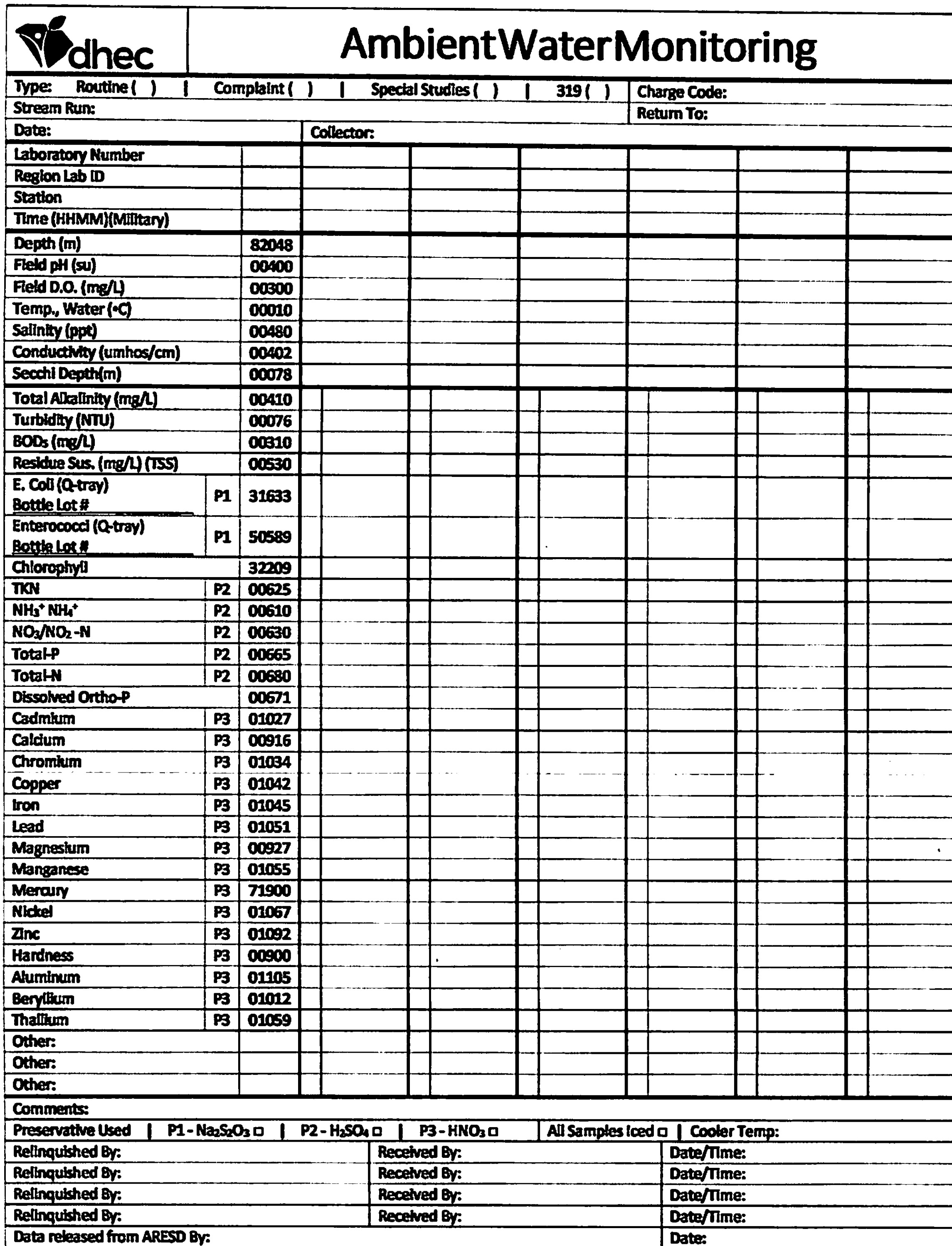
EPA. 2016. Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin: Draft. U.S. Environmental Protection Agency, Office of Water. EPA 822-P-16-002.

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

Appendix



dhec

AmbientWaterMonitoring

Type: Routine ( ) | Complaint ( ) | Special Studies ( ) | 319 ( ) | Charge Code:

Stream Run: | Return To:

Date: | Collector:

Field	Bottle	Code						
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) Bottle Lot #	P1	31633						
Enterococci (Q-tray) Bottle Lot #	P1	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 2020 microcystin analyses, which are organized by water body, sites within those water bodies, and the analytical results for each of the sites based on the sampling month.

Water Body	Site	Microcystin Concentration (µg/L) [a]						
		Apr	May	Jun	Jul	Aug	Sep	Oct
Ashepoo River	MD-253	- [b]	0.019	BDL [c]	0.0175	-	0.0385	BDL
Ashley River	MD-049	-	BDL	-	-	BDL	0.04	0.0575
	CSTL-102	-	-	-	-	0.0385	0.0285	0.0395
	MD-052	-	-	0.0255	0.0165	0.028	0.03	BDL
Beaufort River	MD-001	-	-	BDL	BDL	0.0205	-	0.0385
	MD-004	-	-	BDL	0.0315	BDL	-	0.0615
Black River	PD-325	-	BDL	BDL	-	-	-	-
Bohicket Creek	MD-209	-	-	BDL	0.0465	0.019	0.047	0.016
Boyd Mill Pond	S-311	-	BDL	BDL	-	BDL	0.0525	0.0435
Broad Creek	MD-174	-	BDL	BDL	0.1195	0.0555	0.0375	-
Broad River	MD-116	-	-	BDL	0.0375	0.037	0.036	BDL
Casino Creek	MD-266	-	-	0.04	0.035	-	0.0445	0.041
Cedar Creek Reservoir	CW-033	-	0.0515	BDL	0.068	-	0.0645	0.062
	CW-174	-	BDL	0.022	0.0735	-	0.0785	-
Chechessee	MD-117	-	-	BDL	BDL	0.035	-	0.052
Colleton River	MD-176	-	-	BDL	0.0825	BDL	BDL	0.0295
Combahee River	MD-252	-	BDL	BDL	BDL	-	0.023	0.0275
Cooper River	MD-043	-	-	BDL	0.048	0.0855	0.041	0.043
	MD-045	-	-	0.028	0.0505	0.038	0.045	0.0165
	MD-248	-	-	0.018	0.0405	0.126	0.055	0.0275
Coosawhatchie River	CSTL-107	-	-	BDL	0.099	0.032	0.0505	0.072
Dawho River	MD-120	-	0.036	BDL	0.059	BDL	0.016	0.0255
Fishing Creek Reservoir	LCR-04	BDL	BDL	0.0285	0.11	0.093	0.108	0.0595
	CW-016F	-	-	0.065	0.076	0.126	0.0865	-
	CW-057	-	-	0.0195	0.043	0.0845	0.0935	0.0565
Five Fathom Creek	MD-267	-	-	BDL	0.069	0.019	-	0.026
Folly River	MD-130	-	BDL	0.0175	0.027	0.045	0.018	-
Great Swamp	MD-129	-	BDL	BDL	BDL	0.0315	0.0295	0.049
Hamlin Sound	MD-271	-	BDL	BDL	0.016	0.0475	0.055	-
Intracoastal Waterway	MD-069	-	BDL	BDL	0.0295	0.0475	0.0365	BDL
J. Strom Thurmond	CL-041	-	0.1805	-	0.2255	0.25	0.2175	0.178
Kiawah River	MD-273	-	BDL	BDL	0.0375	0.0495	0.0555	-
Lake Bowen	B-339	-	BDL	0.09	0.151	0.1245	0.0665	0.1645

Lake Greenwood	S-022	-	BDL	0.0575	0.0745	0.034	0.082	0.058
	S-024	-	BDL	BDL	-	0.038	0.0515	0.0295
	S-131	-	-	0.021	0.059	0.05	0.0375	0.028
	S-308	-	BDL	0.016	0.033	0.0415	0.0775	0.0605
Lake Hartwell	SV-200	-	BDL	BDL	0.0405	BDL	0.0205	BDL
	SV-236	-	BDL	0.089	0.0665	0.0685	0.0785	0.07
	SV-268	-	0.1175	-	0.0335	0.0485	-	BDL
	SV-339	-	1.1535	0.0385	0.0675	0.057	0.111	0.0375
	SV-340	-	BDL	0.076	-	0.044	-	0.0835
	SV-363	-	BDL	0.1255	0.079	0.089	0.097	0.0745
	SV-374	-	BDL	0.0765	0.0855	BDL	0.081	0.064
Lake Jocassee	CL-019	-	BDL	0.0285	-	BDL	0.024	BDL
	SV-335	-	BDL	0.0185	-	BDL	BDL	0.0165
	SV-336	-	0.111	BDL	-	BDL	0.0165	0.0465
Lake Keowee	SV-338	-	BDL	0.016	BDL	0.031	BDL	0.019
	SV-361	-	BDL	BDL	0.144	BDL	0.0375	BDL
Lake Murray	S-211	-	0.1085	0.112	0.193	0.126	0.056	0.149
	S-213	-	0.136	0.0985	0.183	0.185	0.175	0.1705
	S-222	-	0.231	0.2715	-	0.123	0.1155	0.069
	S-309	-	0.108	BDL	0.11	0.0835	0.0585	0.038
	S-310	-	BDL	0.165	0.155	0.0965	0.076	0.059
Lake Robinson	PD-327	-	0.108	-	0.027	0.0595	0.019	-
Lake Russell	SV-098	-	0.037	-	BDL	0.1155	0.096	0.071
	SV-357	-	0.142	0.175	-	0.088	0.0695	0.1185
Lake Secession	SV-331	-	0.017	0.081	0.1095	0.0665	0.0935	-
Lake Wateree	CL-089	-	BDL	0.106	0.067	0.1505	0.1145	0.0905
	CW-207	-	BDL	0.05	0.071	0.124	0.0835	0.07
	CW-207B	0.119	0.152	0.0555	0.0865	0.134	0.101	0.064
	CW-208	0.101	BDL	0.1595	0.067	0.253	0.141	0.145
	CW-231	0.113	0.157	0.0275	0.075	0.15	0.112	0.084
	LCR-02	BDL	BDL	0.0255	0.064	0.097	0.098	0.082
	LCR-03	BDL	0.127	0.1305	0.0955	0.1355	0.1135	0.0755
Lake Wylie	CW-197	-	0.0225	0.0275	BDL	0.062	-	0.140
	CW-201	-	0.022	0.1545	BDL	0.129	-	0.1565
	CW-230	-	0.018	0.082	BDL	0.1485	-	0.075
May River	MD-173	-	BDL	BDL	0.053	0.515	0.071	-
Monticello Lake	B-327	-	0.1315	0.056	-	0.107	0.0875	0.119
Morgan River	MD-282	-	BDL	0.026	0.019	BDL	0.031	BDL
N. Edisto River	MD-262	-	-	BDL	0.0335	0.023	0.024	-
New River	MD-118	-	BDL	0.0475	0.083	BDL	0.044	0.036
Parr Reservoir	B-345	-	0.0765	0.0355	0.0415	0.074	0.0785	0.0595
Parrot Creek	MD-281	-	BDL	BDL	0.022	BDL	0.0445	BDL

Parsonnage Creek	MD-277	-	-	0.0405	-	-	-	-
Pee Dee River	MD-275	-	0.029	0.0305	-	-	-	-
Ramshorn Creek	MD-257	-	BDL	BDL	0.0385	0.057	0.056	-
	MD-258	-	BDL	BDL	0.028	0.07	0.0905	-
S. Edisto River	MD-260	-	0.0305	BDL	BDL	BDL	BDL	0.0425
Sampit River	MD-077	-	0.03	BDL	-	-	-	-
Sewee Bay	MD-269	-	0.0165	BDL	0.021	0.0235	0.057	BDL
Stephens Creek Reservoir	SV-372	-	0.190	-	-	-	0.1395	0.167
Stono River	MD-202	-	BDL	-	-	0.0235	0.044	0.0195
	MD-206	-	BDL	BDL	0.035	0.0555	0.023	-
Unnamed Creek	MD-256	-	-	BDL	-	0.0215	-	-
Waccamaw River	MD-142	-	0.0235	0.019	-	-	-	-
Wando River	MD-115	-	-	-	0.029	BDL	0.028	0.05
	MD-264	-	-	BDL	-	0.0295	0.024	0.029
Winyah Bay	MD-278	-	BDL	BDL	-	-	-	-
Wright River	MD-259	-	BDL	BDL	0.0205	0.8095	0.198	-
Yonges Island Creek	MD-261	-	-	BDL	0.042	0.017	0.0295	BDL

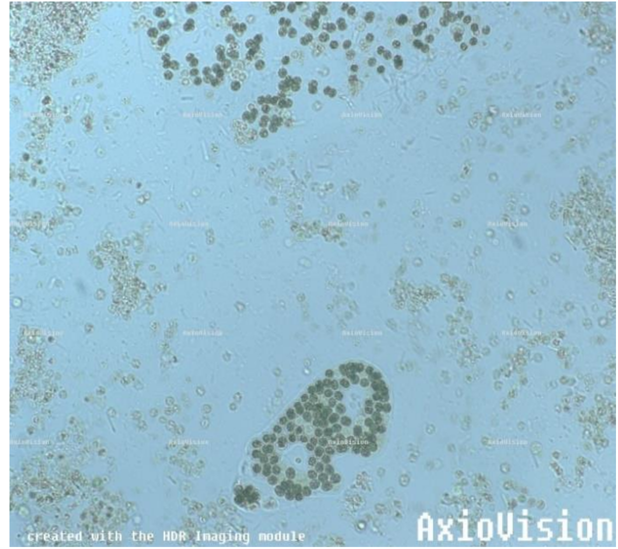
a. µg/L = micrograms per liter (parts per billion)

b. No data available

c. BDL= below detection limit

Appendix 4: Microscopic images of cyanobacteria from the 2020 HAB complaint sites.

Aphanizomenon sp bloom in drainage canal, Mount Pleasant- 06/18/20



Microcystis sp. bloom at Bear Creek, Lancaster- 06/22/20

Dolichospermum sp. bloom on Lancaster Reservoir, Lancaster- 06/29/20

Phormidium sp. Bloom on Lake Wateree- 07/23/20

Appendix 5: Informational HAB rack card

What are harmful algal blooms (HABS)?

Harmful algal blooms are when microscopic organisms, called phytoplankton, overgrow under the right conditions in water bodies. Not all algal blooms are harmful, but some blooms can produce toxins that affect humans, animals, and the environment.

What do HABS look like?

Algal blooms can be associated with thick scums, mats, or layers on the surface of the water. Algal blooms can also be blue, green, blue-green, brown, and/or red in color. They can also be associated with a musty smell. However, not all algal blooms can be seen.

What should I do if I see an algal bloom?

You cannot tell from just looking at an algal bloom if it's toxic or not. If the water is discolored or looks abnormal, it's best to avoid swimming, boating, fishing, etc. in the area. Remember:

"WHEN IN DOUBT, STAY OUT"