Cyanobacteria Monitoring Program 2013 Report

Rhode Island
RIDEM REQ. NO. 1180565/1194117

PREPARED FOR:
Rhode Island Department of Environmental Management
Office of Water Resources
235 Promenade Street
Providence, Rhode Island 02908

PREPARED BY:
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ESS Project No. R298-012

Revised March 12, 2014

CYANOBACTERIA MONITORING PROGRAM
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1.0 INTRODUCTION

ESS Group, Inc. (ESS) was contracted by the Rhode Island Department of Environmental Management (RIDEM) to conduct cyanobacteria monitoring in surface waters of the state of Rhode Island. Cyanobacteria (also known as blue-green algae) are a photosynthetic group of organisms naturally found in surface waters as phytoplankton, floating colonies, or attached to substrate. Under certain conditions, cyanobacteria may grow at high densities (blooms) and release toxins into the water degrading taste and odor and potentially raising public health risks, particularly for contact recreation. The Rhode Island cyanobacteria monitoring program was developed to screen for, respond to, and characterize blooms in the state's fresh waters. This annual report provides a summary of the cyanobacteria monitoring program methodology and results for 2013.

2.0 METHODS

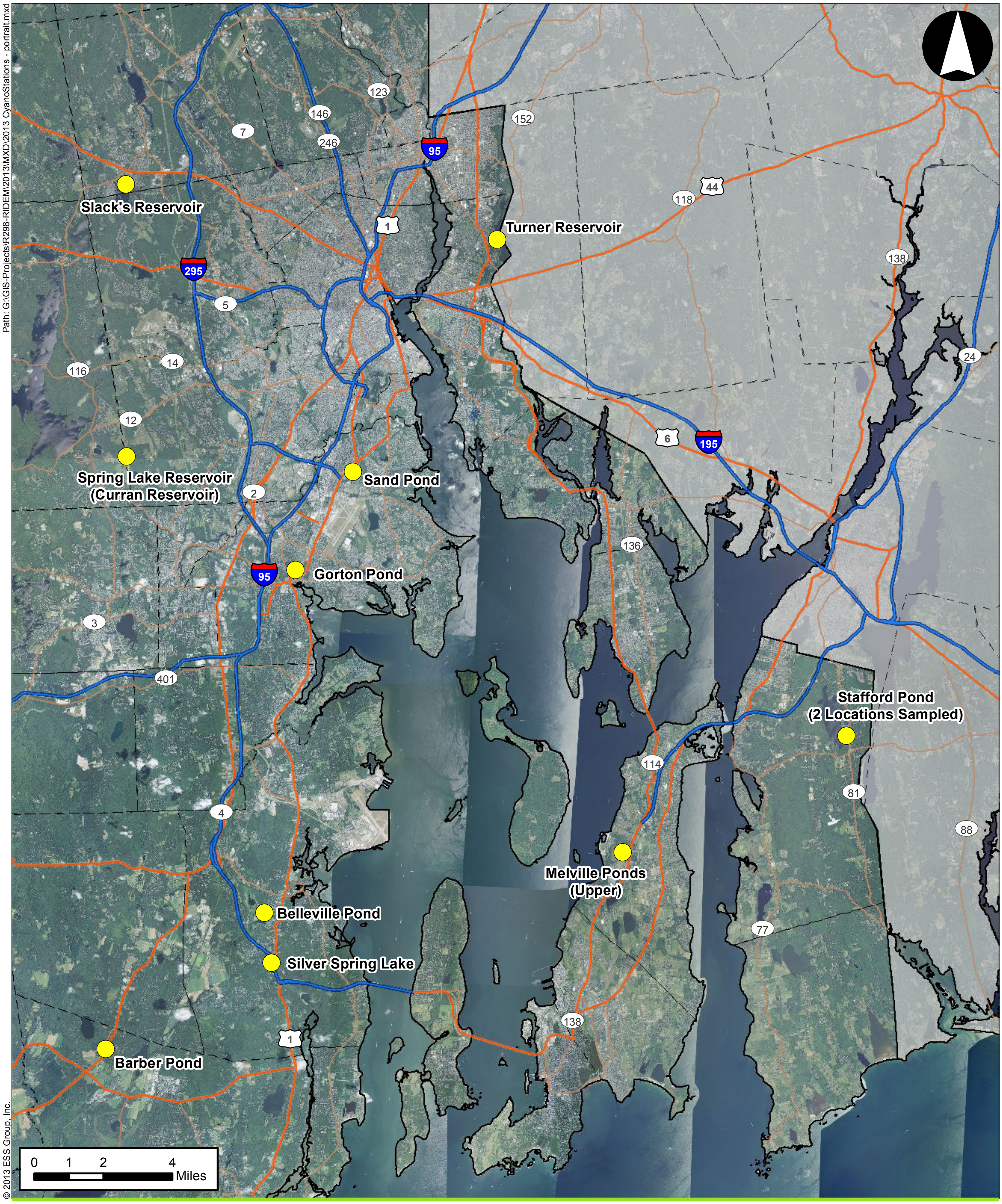
A summary of the monitoring program methodology is presented in this section. For a full description of methodology used by this program, please refer to the project-specific Quality Assurance Project Plan (QAPP)(ESS, 2011).

Two types of sampling were completed as part of the cyanobacteria monitoring program: screening level and response level monitoring. Water quality parameters measured by this program for each type of sampling included both *in situ* parameters (Secchi depth, temperature, dissolved oxygen, and specific conductance) and laboratory-based analysis (enumeration and microcystins). In 2013, 14 cyanobacteria samples were collected from 12 water bodies, distributed across the state from Smithfield to Portsmouth (Table A, Figure 1). Of the 12 water bodies sampled, 10 were sampled as part of the annual screening program and 2 were sampled in response to reports of algae blooms from the public.

Screening Level Sampling

The water bodies selected for screening level monitoring in the 2013 monitoring year included several that were previously sampled at some point during the 2010 to 2012 period, as well as some new ponds with anecdotal evidence of algal blooms, excessive phosphorus, and/or high levels of chlorophyll *a*. The new screening level ponds in 2013 include Stafford Pond, Sand Pond, Gorton Pond, Belleville Pond, and Silver Spring Lake. Water bodies were selected for response level monitoring as prompted by specific public or agency requests to investigate suspected algae blooms.

Mallards dabble along shoreline of Melville Pond during *Woronichinia naegeliana* bloom in September 2013.



ess group
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RIDEM Biomonitoring
Rhode Island

1 inch = 4 miles

Source: 1) NAIP, Ortho Imagery, 2012
2) RIDEM, Sample Locations, 2013
3) ESRI, Major Highways, 2010

Legend

Cyanobacteria Monitoring Station

Cyanobacteria Monitoring Stations
2013

Figure 1

Table A. Water Bodies Sampled by the Cyanobacteria Monitoring Program in 2013

Sampling Program	Water Body	Location	Long	Lat	WBID	Acres
Screening Level	Spring Lake Reservoir #2 (Curran Reservoir)	Cranston	-71.5512	41.7508	RI0006016L-02	46.23
	Slack's Reservoir	Smithfield, Johnston	-71.551689	41.86521667	RI0002007L-03	133.61
	Turner Reservoir	East Providence	-71.3432	41.8419	RI0004009L-01B	85.1
	Melville Ponds (Upper)	Portsmouth	-71.273522	41.584381	RI0007029L-01	13.59
	Silver Spring Lake	North Kingstown	-71.469939	41.538203	RI0010044L-02	18.75
	Barber Pond	North Kingstown	-71.33575	41.30893	RI0008039L-14	28.16
	Sand Pond (North of Airport)	Warwick	-71.424306	41.744411	RI0006017L-09	12.21
	Stafford Pond	Tiverton	-71.148244	41.632994	RI0007037L-01	480.13
	Gorton Pond	Warwick	-71.456414	41.703136	RI0007025L-01	58.30
	Belleville Pond	North Kingstown	-71.473939	41.559161	RI0007027L-02	130.27
Response Level	Mashapaug Pond	Providence	-71.43553	41.79313	RI0006017L-06	76.75
	Roger Williams Park Ponds	Providence	-71.41414	41.77731	RI0006017L-05	113.95

ESS collected each of the screening level samples in mid-September. Screening level samples were collected from the surface (elbow deep and shallower) in at least one location at each water body, typically at the public access point. If no official public access point was present, samples were collected from the most readily accessible location. Where algae blooms were only observed away from the public access, ESS collected a second sample from the bloom. *In situ* water quality parameters were measured at the sampling location.

Each screening level cyanobacteria sample was sent to GreenWater Laboratory for identification/ enumeration. Additionally, microcystin levels were analyzed by the lab for each sample, regardless of cell count.

Response Level Sampling

RIDEM staff collected response level cyanobacteria samples using similar methods to those used for screening level sample collection. However, response level sampling focused only on collection of samples from active blooms. Response level monitoring samples were first screened by RIDEM staff to determine if a substantial number of cyanobacteria were present within the sample. Samples with substantial numbers of these cells were sent to the lab for detailed identification/enumeration and microcystin analysis.

All samples sent to the lab were shipped via overnight delivery and were accompanied by a completed chain-of-custody.

3.0 RESULTS

3.1 Cyanobacteria

Cyanobacteria cell densities in 2013 ranged from 457 cells/mL to more than 2 million cells/mL (Table B). Cell density exceeded 50,000 cells/mL (the microcystin analysis threshold established in the project-specific QAPP) in 5 samples from 4 water bodies.

Potentially toxigenic cyanobacteria species were identified in 13 samples from 11 water bodies (Table B). *Woronichinia naegeliana, Microcystis* spp., *Aphanizomenon* spp., *Anabaena* spp., *Chrysosporum* sp.(Family: Nostocaceae), and *Cuspidothrix issatschenkoi* .(Family: Nostocaceae) were the primary dominant or co-dominant species in these samples. Complete cyanobacteria identification and enumeration results may be found in Appendix A.

Measured microcystin levels in 2013 ranged from not detected at 0.15 µg/L to 11.5 µg/L (Table B). The highest microcystin levels were generally found in samples with high cell densities. However, as in previous years, not all samples with high cell densities demonstrated correspondingly high microcystin concentrations. The higher microcystin levels were measured in samples dominated by certain taxa, including *Microcystis* spp., *Anabaena* spp. and *Woronichinia naegeliana.*

Complete microcystin laboratory results are presented in Appendix B.

3.2 Water Quality

Some water quality parameters (particularly temperature and dissolved oxygen) tend to be sensitive to diurnal trends and should be interpreted cautiously when comparing instantaneous water quality across multiple water bodies. Therefore, the analysis of water quality results will focus on summarizing the data and identifying potentially extreme values.

Instantaneous dissolved oxygen measurements were above state standards for fresh waters (5.0 mg/L) at each location, ranging from 5.3 mg/L at Belleville Pond to 9.4 mg/L at Turner Reservoir (Table C). In some cases, dissolved oxygen levels were supersaturated (i.e., greater than 100%), a condition that may result from high levels of primary productivity in the surveyed lakes and ponds.

Specific conductance was highest at Sand Pond (365.5 µS/cm) in Warwick (Table C). The lowest specific conductance (81.5 µS/cm) was measured at Barber Pond.

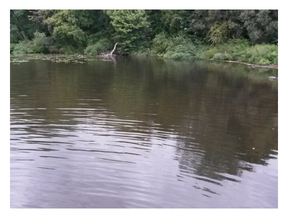
Table B. Summary of 2013 Cyanobacteria Sampling Program Results

Water Body	Station ID	Date	Cell Density (cells/mL)	Microcystin Level (µg/L)	Dominant Species	2013 Photograph†	2012 Photograph†	2011 Photograph†
Mashapaug Pond	MP/MAP1/ MASH P	7/24/13	252,361	1.0	• *Microcystis ichthyoblabe (PTOX)* • *Anabaena planctonica (PTOX)*	No photo available	No photo available	
J.L. Curran Reservoir (Spring Lake Reservoir #2)	SPR1/UCR1	9/17/13	251,556	0.2	• *Chrysosporum ovalisporum (PTOX)* • *Aphanizomenon cf. flos-aquae (PTOX)*			
Slack Reservoir	SLR1/SLK2	9/17/13	37,631	8.6	• *Woronichinia naegeliana (PTOX)*			
Roger Williams Park Ponds (Polo/Willow Ponds)	PL	7/24/13	2,328,543	11.5	• *Microcystis wesenbergii (PTOX)*	No photo available	No photo available	No photo available
Barber Pond	BAP1	9/17/13	14,721	0.77	• *Microcystis aeruginosa (PTOX)*			

Table B. Summary of 2013 Cyanobacteria Sampling Program Results

Water Body	Station ID	Date	Cell Density (cells/mL)	Microcystin Level (µg/L)	Dominant Species	2013 Photograph†	2012 Photograph†	2011 Photograph†
Gorton Pond	GRP1	9/17/13	22,675	0.22	• *Cyanogranis ferruginea*		No photo available	No photo available
Sand Pond	SDP1	9/17/13	1,580	*0.15**	• *Pseudanabaena sp.* • Unicellular cyanophytes		No photo available	No photo available
Silver Spring Lake	SSL1	9/17/13	1,478	*0.15**	• Cyanophyte cell pairs		No photo available	No photo available
Belleville Pond	BLP1	9/17/13	457	*0.15**	• *Pseudanabaena sp.*		No photo available	No photo available

Table B. Summary of 2013 Cyanobacteria Sampling Program Results

Water Body	Station ID	Date	Cell Density (cells/mL)	Microcystin Level (µg/L)	Dominant Species	2013 Photograph†	2012 Photograph†	2011 Photograph†
Stafford Pond	STP1	9/16/13	8,071	*0.15**	• *Aphanizomenon sp.* (PTOX)		No photo available	No photo available
	STP2	9/16/13	6,189	*0.15**	• *Chrysosporum/ Sphaerospermopsis sp.(PTOX)*		No photo available	No photo available
Melville Pond	MLP1/MEP1	9/16/13	86,189**	0.20**	• *Woronichinia naegeliana (PTOX)*		No photo available	No photo available
Turner Reservoir	TUR1	9/16/13	1,559	*0.15**	• *Cuspidothrix issatschenkoi (PTOX)*			No photo available

NS = not sampled; PTOX = potentially toxigenic species
* Reported value is the quantitation limit. Microcystins were not detected at this level.
**Average of two duplicate samples collected for QA/QC purposes
†All photos by ESS, except Slack Reservoir photograph from 2011, which was taken by RIDEM

Because cyanobacteria samples were primarily collected by wading into the water at shoreline access points, water clarity (as measured by Secchi depth) was limited to approximately 1.0 meters (i.e., pond bottom), depending on the pond bottom substrate and slope. Additional Secchi depth measurements were collected Stafford Pond, where the sample at station STP2 was collected from a central area of the pond. Water clarity was not observed to be less than 1.0 meter at any location and ranged as high as 2.2 meters at Stafford Pond.

Table C. Water Quality Observed during 2013 Cyanobacteria Screening

Waterbody	Station ID	Date	Time	Water Temp (°C)	DO (mg/L)	DO (%)	Spec. Cond (µS/cm)	Salinity (ppt)	Secchi Depth (m)
Stafford Pond	STP1	9/16/2013	14:30	20.8	8.0	90.0	125.7	0.1	1.0*
Stafford Pond	STP2	9/16/2013	14:00	21.4	7.4	83.3	126.0	0.1	2.2
Melville Pond	MLP1/MEP1**	9/16/2013	15:00	21.4	8.9	100.2	291.4	0.3	1.0*
Melville Pond	MLP2/MEP1**	9/16/2013	15:10	21.5	8.8	101.0	291.1	0.3	1.0*
Turner Reservoir	TUR1	9/16/2013	14:00	22.1	9.4	107.1	270.6	0.2	1.0*
Slack's Reservoir	SLR1	9/17/2013	8:40	16.7	5.9	62.1	193.7	0.2	1.0*
J.L. Curran Reservoir	CUR1/SPR1/UCR1**	9/17/2013	9:30	19.9	8.2	89.3	123.8	0.1	1.0*
Sand Pond	SDP1	9/17/2013	10:00	20.1	6.6	73.0	365.5	0.3	1.0*
Gorton Pond	GRP1	9/17/2013	10:20	19.4	9.0	97.0	292.1	0.3	1.0*
Belleville Pond	BLP1	9/17/2013	10:45	17.4	5.3	51.1	141.8	0.1	1.0*
Silver Spring Lake	SSL1	9/17/2013	11:15	17.9	7.5	80.4	201.5	0.2	1.0*
Barber Pond	BAP1	9/17/2013	12:15	20.3	9.0	94.0	81.5	0.1	1.0*

*On bottom
**ID code reference for previous years

3.3 Quality Assurance/Quality Control

All water quality QA/QC requirements were met during screening level monitoring by ESS. Water quality data was not collected by RIDEM staff during response level monitoring at all stations due to equipment malfunction or lack of availability.

Cyanobacteria sampling QA/QC requirements were met for all screening and response level monitoring samples and all internal lab QA/QC requirements were met for each sample. Additionally, one field duplicate was collected in accordance with the rate specified by the project-specific QAPP. The duplicate sample was collected from the same location in Melville Pond (MLP1/MLP2). Cell density and microcystin levels for the field duplicate were within the relative percent difference limits set in the QAPP.

4.0 DISCUSSION AND CONCLUSIONS

ESS visited 10 water bodies statewide and collected 12 cyanobacteria samples as part of the 2013 screening level monitoring program. An additional two samples were collected by RIDEM in response to active blooms, including one at Mashapaug Pond and one at the Polo Lake/Willow Lake portion of the Roger Williams Park Ponds. These data are briefly examined in the context of the larger 2011 to 2013 dataset below.

A Brief Comparison of Results from 2011 to 2013

As in 2011 and 2012, the cyanobacteria monitoring program successfully detected and documented the intensity of multiple active blooms across the state. However, fewer exceedances of Rhode Island or World Health Organization recreational guidelines were documented in 2013 than in either of the previous years.

Cyanobacteria densities in 2013 exceeded the 50,000 cells/mL threshold established in the project-specific QAPP in five samples from four water bodies. Measured microcystin levels did not exceed the World Health Organization (2003) recreational contact guideline of 20 µg/L in any samples. Rhode Island health advisory guidelines for cell count (70,000 cells/mL) were exceeded in five samples from four water bodies although guidelines for microcystin (14 µg/L) were not exceeded in any samples.

In 2011, the highest microcystin levels measured were associated with blooms dominated by *Woronichinia naegeliana* or *Anabaena* spp. The highest microcystin levels in 2012 were associated with dominance by these taxa but also included blooms dominated by *Microcystis* spp. and *Planktothrix suspensa*. *Anabaena* spp. and *Microcystis* spp. blooms have long been recognized as potential generators of microcystins. However, both *Woronichinia naegeliana* and *Planktothrix* spp. also have a documented association with elevated microcystin levels in European and North American lakes and ponds (e.g., Willame et al 2005, Chen et al. 2009). In 2013, the highest microcystin levels were associated with blooms dominated by *Woronichinia naegeliana* or *Microcystis* spp.

Examining the Pooled 2011 to 2013 Data

The pooled 2011 to 2013 data reinforce the positive relationship between cell density and microcystin concentration observed previously (e.g., ESS 2013) (Figure 1). However, as previously noted, the variance in microcystin concentration does tend to rise with increasing cell density.

The majority of the 2013 data fall within the range of values previously documented for microcystin concentration and cell density. However, one point (associated with a *Woronichinia naegeliana* bloom at Slack Reservoir) was characterized by anomalously high microcystin levels for the observed cell density. Notably, the lab observed a large number of empty mucilaginous stalks systems in the Slack Reservoir sample, indicating that *W. naegeliana* densities may have been much higher prior to sampling. This would seem to provide a potential mechanism for release of toxins, resulting in higher than expected microcystin levels.

Although the Rhode Island health advisory guidelines are currently set at a cyanobacteria density of 70,000 cells/mL, the lower threshold at which excessive levels of microcystins are actually produced in Rhode Island cyanobacteria blooms remains difficult to define. Among the complicating factors in examining this issue is the fact that, during a bloom cycle, microcystin concentrations may remain elevated even as cell density declines (as apparently occurred at Slack Reservoir in 2013).

Investigating the factors contributing to the observed cyanobacteria blooms remains beyond the scope of this study. However, as observed in previous annual reports, in the absence of direct cause-and-effect relationships for blooms in Rhode Island, it is wise to pursue balanced watershed and lake management strategies that result in broad spectrum pollutant loading reductions.

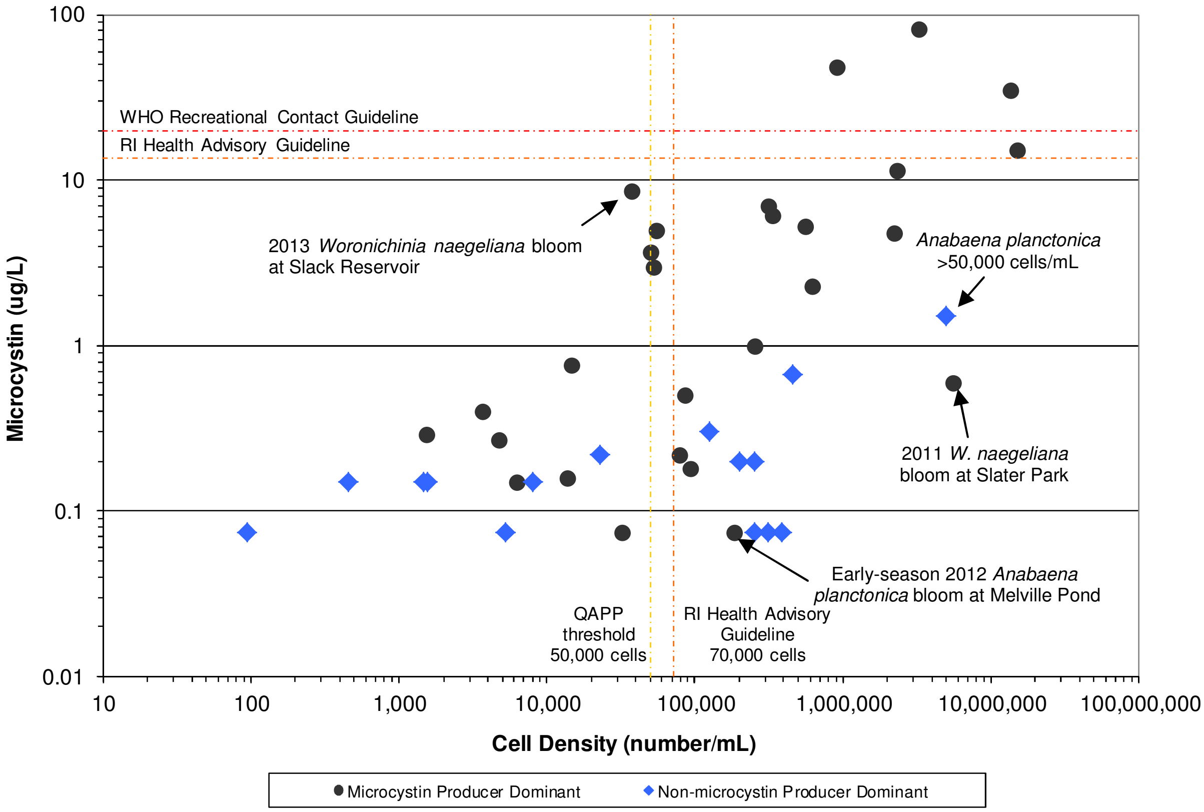


Figure 2. Cyanobacteria Cell Density and Microcystin Levels (Pooled 2011 to 2013 Data)

Interpreting patterns in cyanobacteria bloom species composition, environmental triggers and production of toxins is complicated by the fact that cyanobacteria taxonomy is rapidly evolving. In particular, the application of new molecular techniques to cyanobacteria taxonomy has recently resulted in some significant revisions (Komárek 2014). These revisions challenge much of the conventional wisdom about toxigenic and non-toxigenic species and may provide new insights into the observed variability in toxin production within genera or even species. They also serve as a reminder that taxonomic identification of cyanobacteria, while useful, yet has room to mature for use in policy-making.

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Appendix A

Cyanobacteria Identification and Enumeration Lab Reports

ESS Group Cyanobacteria ID and Enumeration Report

Prepared: October 18, 2013
Prepared By: GreenWater Laboratories

Samples: 8

1. STP1 (collected on 9/16/13)
2. STP2 (collected on 9/16/13)
3. TUR1 (collected on 9/16/13)
4. SAN1 (collected on 9/17/13)
5. GOR1 (collected on 9/17/13)
6. BEL1 (collected on 9/17/13)
7. SSL1 (collected on 9/17/13)
8. BAR1 (collected on 9/17/13)

Sample 1: STP1

Total cyanobacteria cell numbers in the STP1 sample collected on 9/16/13 were 8,071 cells/mL. Total potentially toxigenic (PTOX) cyanobacteria cell numbers were 6,660 cells/mL (82.5% of total cyanobacteria numbers). Potentially toxigenic species observed in the sample included *Aphanizomenon* sp. (3,958 cells/mL; Fig. 1), *Aphanizomenon* cf. *flos-aquae* (1,571 cells/mL; Fig. 2) and *Chrysosporum*/*Sphaerospermopsis* sp. (1,131 cells/mL; Fig. 3).

Sample 2: STP2

Total cyanobacteria cell numbers in the STP2 sample collected on 9/16/13 were 6,189 cells/mL. Total potentially toxigenic (PTOX) cyanobacteria cell numbers were 4,084 cells/mL (66.0% of total cyanobacteria numbers). Potentially toxigenic species observed in the sample included *Chrysosporum*/*Sphaerospermopsis* sp. (2,262 cells/mL), *Aphanizomenon* cf. *flos-aquae* (1,257 cells/mL) and *Aphanizomenon* sp. (565 cells/mL).

Sample 3: TUR1

Total cyanobacteria cell numbers in the TUR1 sample collected on 9/16/13 were 1,559 cells/mL. Total potentially toxigenic (PTOX) cyanobacteria cell numbers were 1,114 cells/mL (71.5% of total cyanobacteria numbers). Potentially toxigenic species observed in the sample included *Cuspidothrix issatschenkoi* (534 cells/mL; Fig. 4), *Microcystis* cf. *ichthyoblabe* (174 cells/mL; Fig. 5), *Anabaena circinalis* (141 cells/mL; Fig. 6), *Woronichinia naegeliana* (110 cells/mL), *Microcystis* spp. cell pairs (63 cells/mL), *Microcystis wesenbergii* (33 cells/mL), *Anabaena* cf. *crassa* (31 cells/mL), *Microcystis* sp. (16 cells/mL) and *Anabaena* sp. (12 cells/mL).

Sample 4: SAN1

Total cyanobacteria cell numbers in the SAN1 sample collected on 9/17/13 were 1,580 cells/mL. Total potentially toxigenic (PTOX) cyanobacteria cell numbers were 10 cells/mL (0.6% of total cyanobacteria numbers). Potentially toxigenic species observed in the sample included *Woronichinia naegeliana* (8 cells/mL) and *Chrysosporum*/*Sphaerospermopsis* sp. (2 cells/mL).

Sample 5: GOR1
Total cyanobacteria cell numbers in the GOR1 sample collected on 9/17/13 were 22,675 cells/mL. Total potentially toxigenic (PTOX) cyanobacteria cell numbers were 71 cells/mL (0.3% of total cyanobacteria numbers). Potentially toxigenic species observed in the sample included *Woronichinia naegeliana* (62 cells/mL), *Microcystis wesenbergii* (6 cells/mL) and *Anabaena*/*Aphanizomenon* sp. (3 cells/mL).

Sample 6: BEL1
Total cyanobacteria cell numbers in the BEL1 sample collected on 9/17/13 were 457 cells/mL. No potentially toxigenic (PTOX) cyanobacteria were observed in the sample.

Sample 7: SSL1
Total cyanobacteria cell numbers in the SSL1 sample collected on 9/17/13 were 1,478 cells/mL. Total potentially toxigenic (PTOX) cyanobacteria cell numbers were 3 cells/mL (0.2% of total cyanobacteria numbers). Potentially toxigenic species observed in the sample included *Aphanizomenon* sp. (3 cells/mL).

Sample 8: BAR1
Total cyanobacteria cell numbers in the BAR1 sample collected on 9/17/13 were 14,721 cells/mL. Total potentially toxigenic (PTOX) cyanobacteria cell numbers were 11,418 cells/mL (77.6% of total cyanobacteria numbers). Potentially toxigenic species observed in the sample included *Microcystis aeruginosa* (9,283 cells/mL; Fig. 7), *Snowella lacustris* (1,665 cells/mL; Fig. 8), *Planktothrix* sp. (432 cells/mL), *Woronichinia naegeliana* (34 cells/mL), *Microcystis* sp. (3 cells/mL) and *Anabaena* sp. (1 cell/mL).

Fig. 1 *Aphanizomenon* sp. STP1 400X (scale bar = 20µm)

Fig. 2 *Aphanizomenon* cf. *flos-aquae* STP1 400X (scale bar = 20µm)

Fig. 3 *Chrysosporum/Sphaerospermopsis* sp. STP1 400X (scale bar = 20µm)

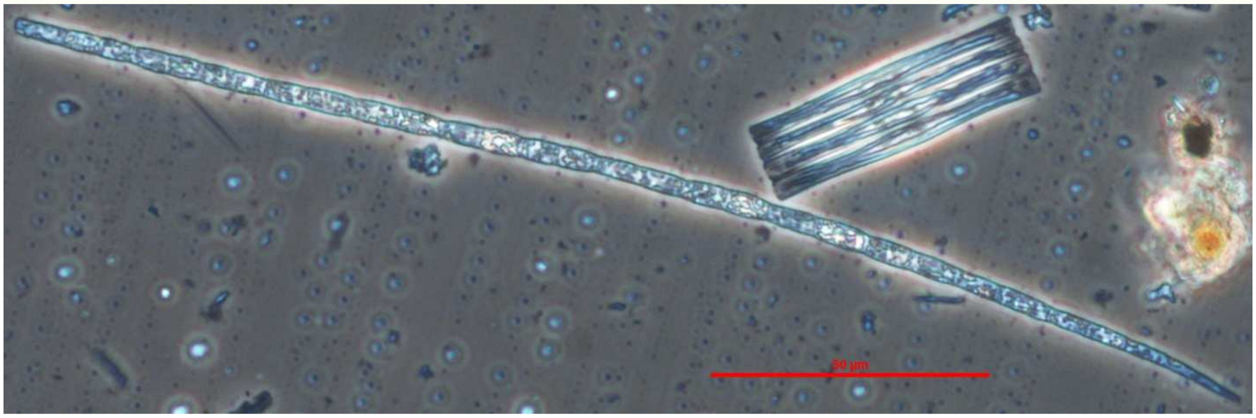

Fig. 4 *Cuspidothrix issatschenkoi* TUR1 400X (scale bar = 50µm)

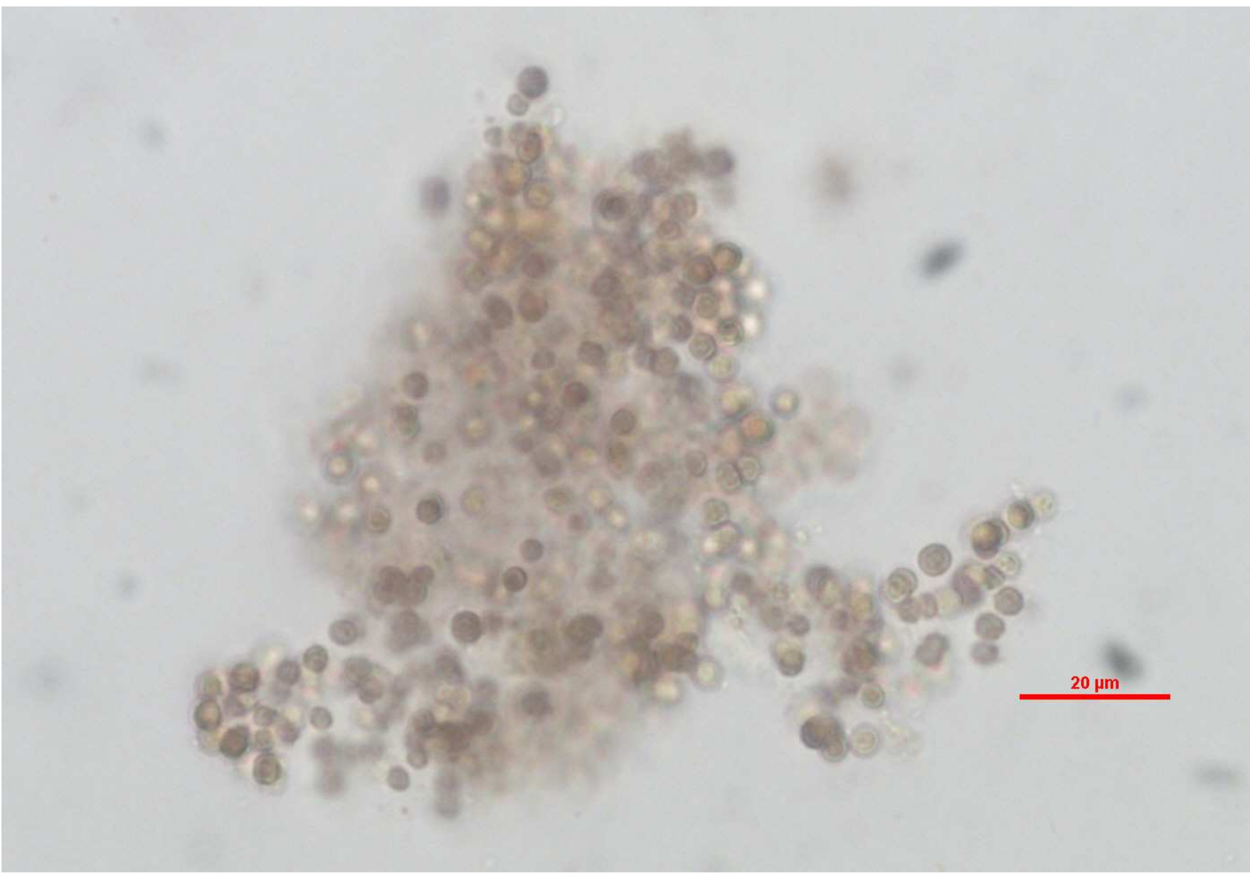

Fig. 5 *Microcystis* cf. *ichthyoblabe* TUR1 400X (scale bar = 20µm)

Fig. 6 *Anabaena circinalis* TUR1 200X (scale bar = 50µm)

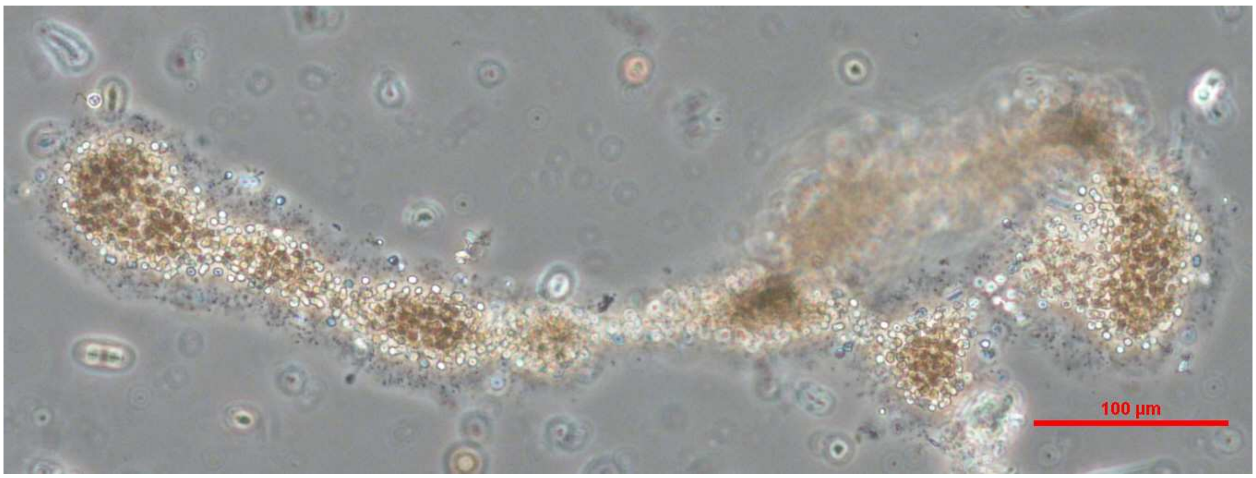

Fig. 7 *Microcystis aeruginosa* BAR1 100X (scale bar = 100µm)

Fig. 8 *Snowella lacustris* BAR1 400X (scale bar = 10µm)

ESS Group Cyanobacteria ID and Enumeration Report

Prepared: July 27, 2013
Prepared By: GreenWater Laboratories

Samples: 2 (collected on 7/24/13)

1. Mashapaug Pond
2. Polo-Willow Ponds

Sample 1: Mashapaug Pond

Total cyanobacteria cell numbers in the Mashapaug Pond sample collected on 7/24/13 were 252,361. Total potentially toxigenic (PTOX) cyanobacteria cell numbers were 252,313 cells/mL (99.98% of total cyanobacteria numbers). Potentially toxigenic species observed in the sample included *Microcystis ichthyoblabe* (86,707 cells/mL; Fig. 1), *Anabaena planctonica* (80,110 cells/mL; Fig. 2), *Microcystis wesenbergii* (45,239 cells/mL; Fig. 3), *Microcystis* spp. unicells and cell pairs (10,682 cells/mL), *Anabaena crassa* (10,681 cells/mL; Fig. 4), *Microcystis aeruginosa* (8,370 cells/mL), *Woronichinia naegeliana* (4,162 cells/mL), *Microcystis* sp. (2,906 cells/mL), *Aphanizomenon* cf. *flos-aquae* (2,356 cells/mL) and *Microcystis botrys* (1,100 cells/mL).

Sample 2: Polo-Willow Ponds

Total cyanobacteria cell numbers in the Polo-Willow Ponds sample collected on 7/24/13 were 2,328,543. Total potentially toxigenic (PTOX) cyanobacteria cell numbers were 2,322,064 cells/mL (99.7% of total cyanobacteria numbers). Potentially toxigenic species observed in the sample included *Microcystis wesenbergii* (1,308,463 cells/mL; Fig. 5), *Microcystis ichthyoblabe* (325,152 cells/mL; Fig. 6), *Microcystis* sp. (227,764 cells/mL; Fig. 7), *Anabaena planctonica* (169,645 cells/mL; Fig. 8), *Microcystis aeruginosa* (150,206 cells/mL; Fig. 9), *Microcystis* spp. unicells and cell pairs (105,243 cells/mL), *Anabaena crassa* (10,603 cells/mL; Fig. 10), *Aphanizomenon* cf. *flos-aquae* (7,854 cells/mL), *Cuspidothrix issatschenkoi* (6,676 cells/mL), *Woronichinia naegeliana* (4,712 cells/mL), *Cylindrospermopsis raciborskii* (4,516 cells/mL), *Microcystis viridis* (730 cells/mL) and *Microcystis botrys* (500 cells/mL).

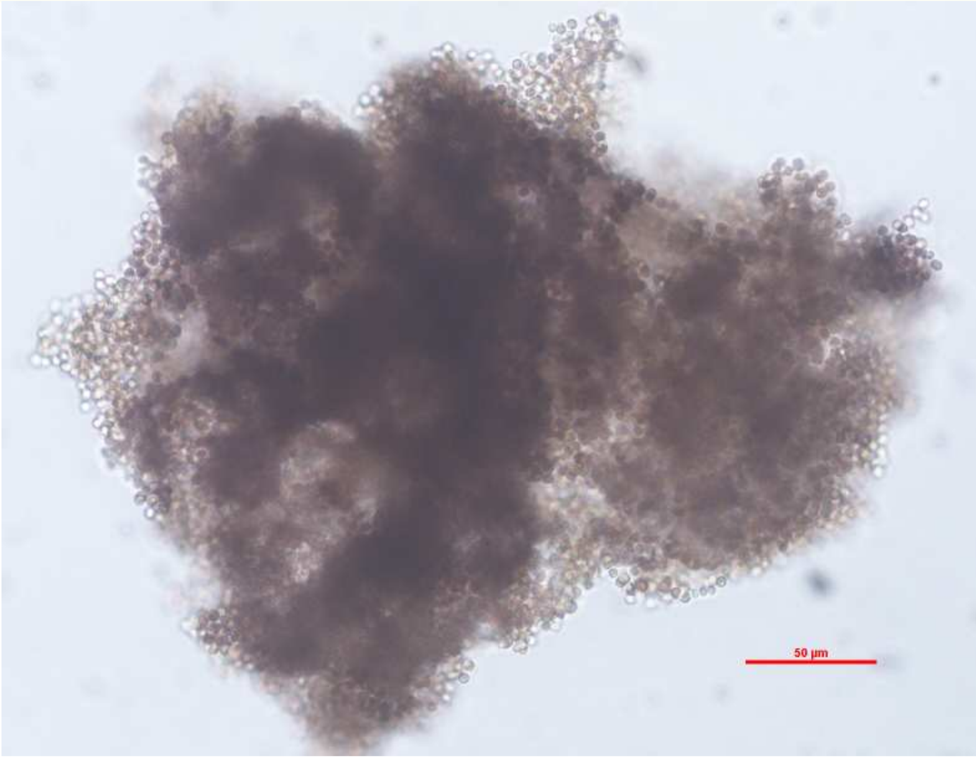

Fig. 1 *Microcystis ichthyoblabe* MP 200X (scale bar = 50µm)

Fig. 2 *Anabaena planctonica* MP 400X (scale bar = 20µm)

Fig. 3 *Microcystis wesenbergii* MP 200X (scale bar = 20µm)

Fig. 4 *Anabaena crassa* MP 400X (scale bar = 50µm)

Fig. 5 *Microcystis wesenbergii* PL 200X (scale bar = 100µm)

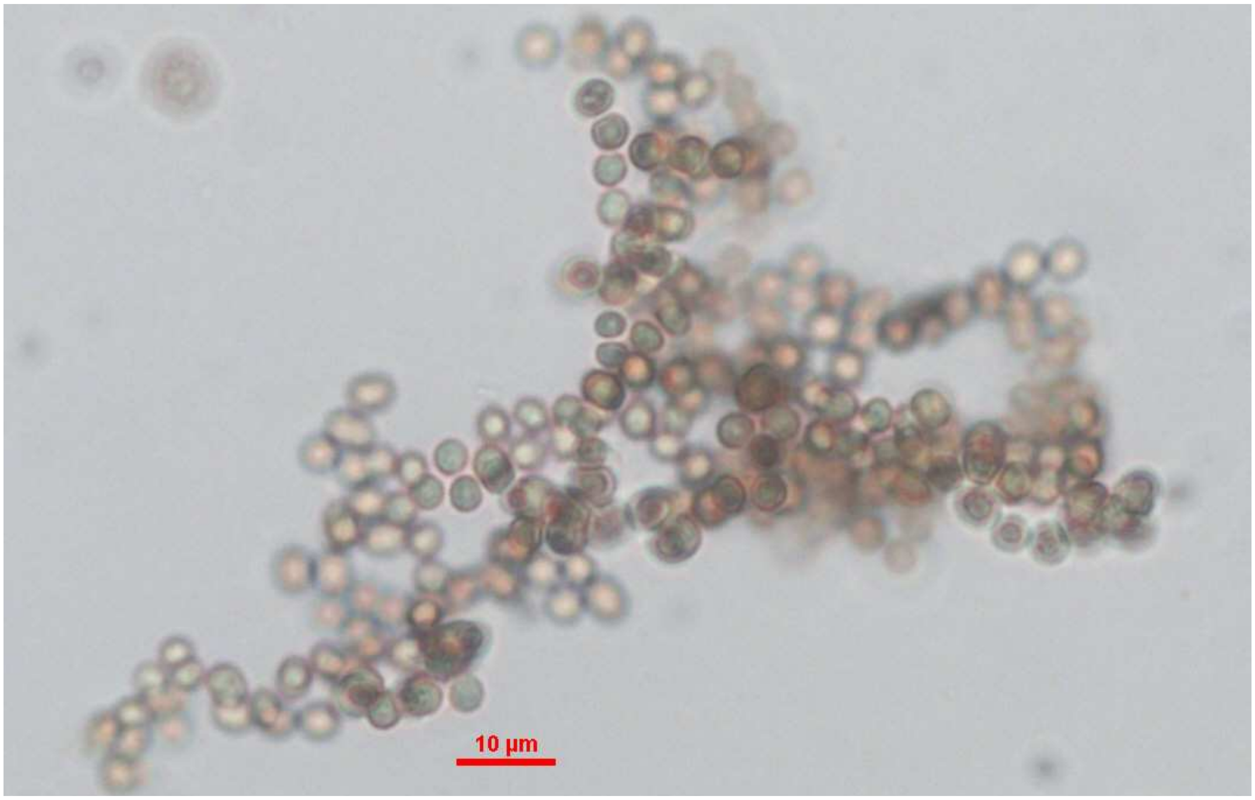

Fig. 6 *Microcystis ichthyoblabe* PL 400X (scale bar = 10µm)

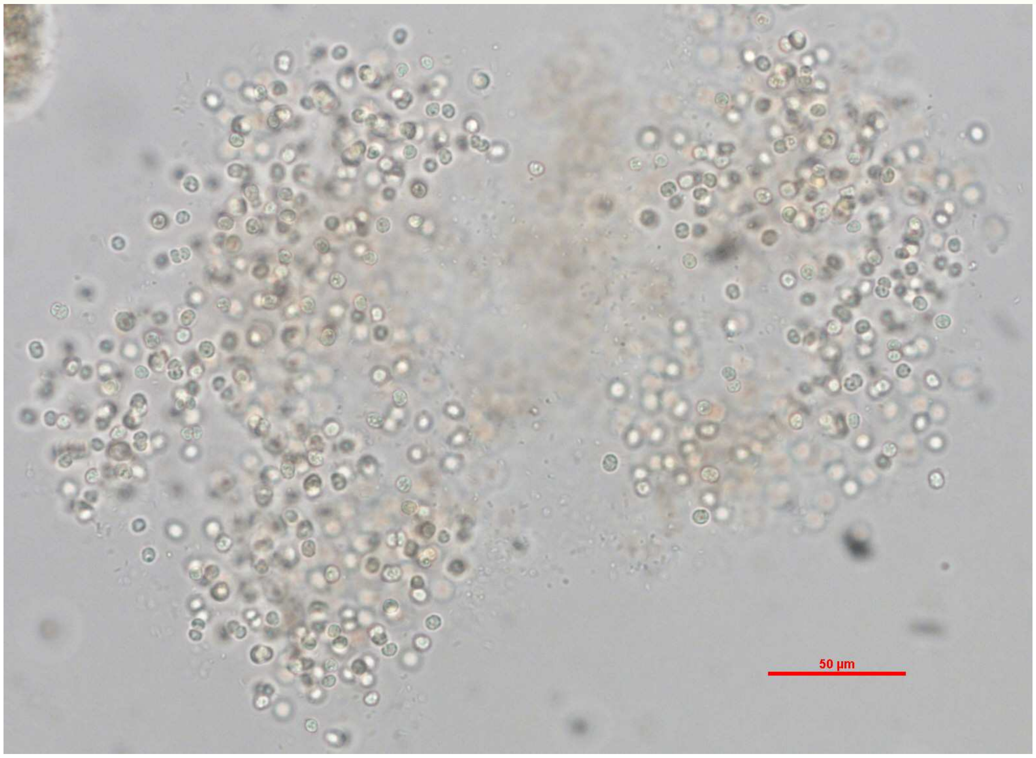

Fig. 7 *Microcystis* sp. PL 200X (scale bar = 50µm)

Fig. 8 *Anabaena planctonica* PL 400X (scale bar = 20µm)

Fig. 9 *Microcystis aeruginosa* PL 200X (scale bar = 50µm)

Fig. 10 *Anabaena crassa* PL 400X (scale bar = 20µm)

ESS Group Cyanobacteria ID and Enumeration Report

Prepared: September 20, 2013
Prepared By: GreenWater Laboratories

Samples: 2 (collected on 9/16/13)
1. MLP1
2. MLP2

Sample 1: MLP1
Total cyanobacteria cell numbers in the MLP1 sample collected on 9/16/13 were 163,328 cells/mL. Total potentially toxigenic (PTOX) cyanobacteria cell numbers were 163,303 cells/mL (99.98% of total cyanobacteria numbers). Potentially toxigenic species observed in the sample included *Woronichinia naegeliana* (121,003 cells/mL; Fig. 1), *Anabaena planctonica* (32,986 cells/mL; Fig. 2), *Microcystis* cf. *ichthyoblabe* (4,241 cells/mL; Fig. 3), *Aphanizomenon* cf. *flos-aquae* (4,005 cells/mL; Fig. 4), *Microcystis* spp. unicells (1,047 cells/mL) and *Anabaena* sp. (20 cells/mL). The majority of *W. naegeliana* was in the form of unicells and cell pairs.

Sample 2: MLP2
Total cyanobacteria cell numbers in the MLP2 sample collected on 9/16/13 were 130,990 cells/mL. Total potentially toxigenic (PTOX) cyanobacteria cell numbers were 129,934 cells/mL (99.2% of total cyanobacteria numbers). Potentially toxigenic species observed in the sample included *Woronichinia naegeliana* (96,643 cells/mL), *Anabaena planctonica* (29,452 cells/mL), *Aphanizomenon* cf. *flos-aquae* (2,199 cells/mL), *Microcystis* cf. *ichthyoblabe* (1,025 cells/mL), *Microcystis* spp. unicells (524 cells/mL), *Anabaena* sp. (54 cells/mL) and *Microcystis botrys* (38 cells/mL). The majority of *W. naegeliana* was in the form of unicells and cell pairs.

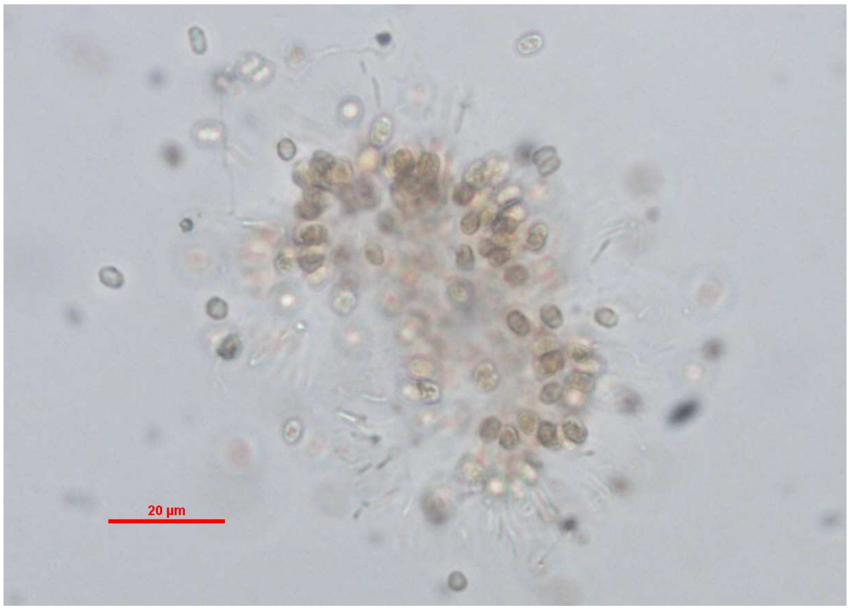

Fig. 1 *Woronichinia naegeliana* 400X (scale bar = 20µm)

Fig. 2 *Anabaena planctonica* 400X (scale bar = 10µm)

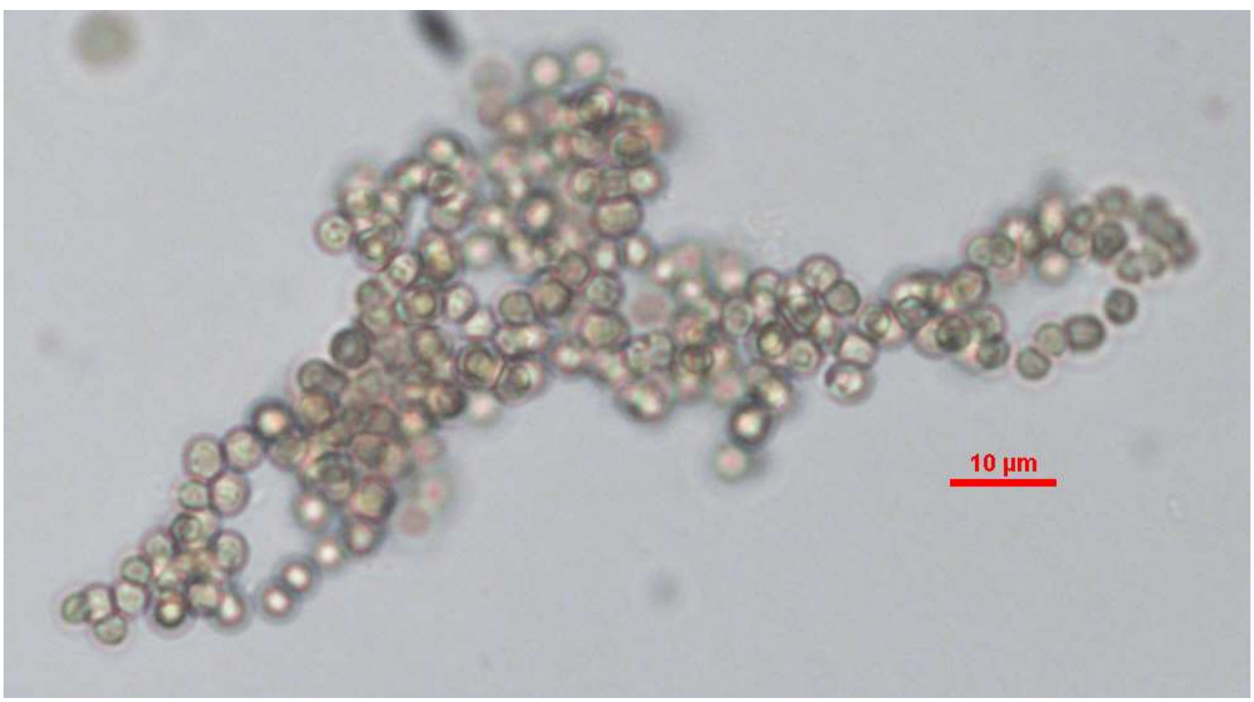

Fig. 3 *Microcystis* cf. *ichthyoblabe* MP 400X (scale bar = 10µm)

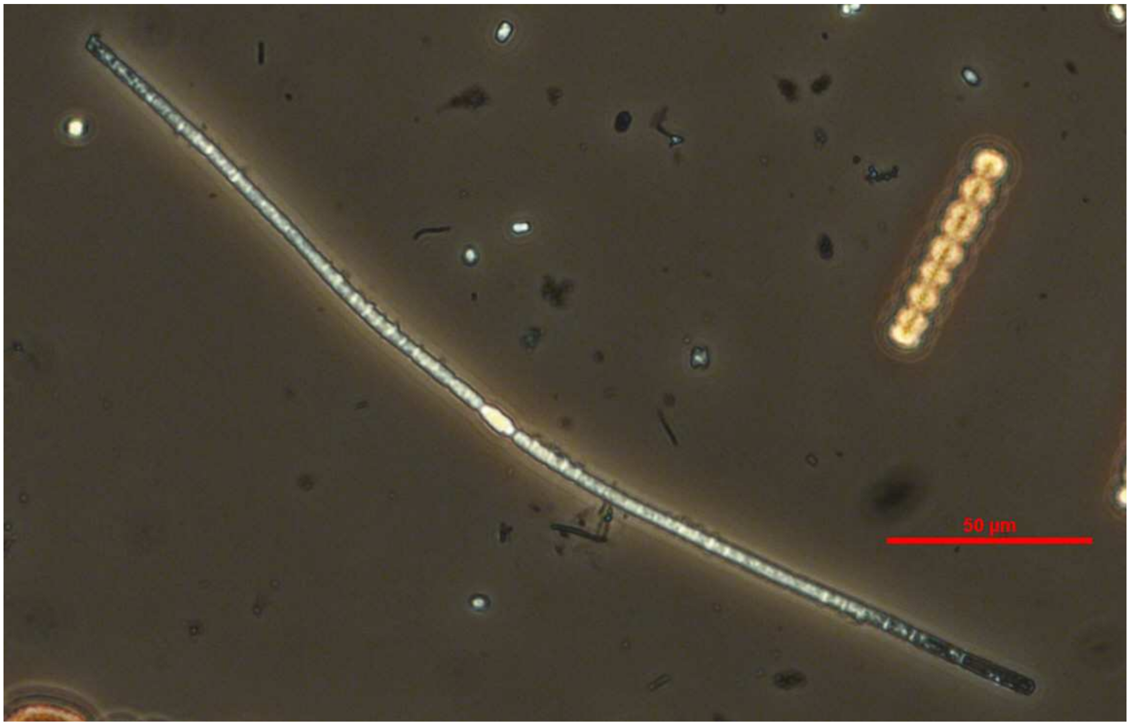

Fig. 4 *Aphanizomenon* cf. *flos-aquae* 200X (scale bar = 50µm)

ESS Group Cyanobacteria ID and Enumeration Report

Prepared: September 26, 2013
Prepared By: GreenWater Laboratories

Samples: 2 (collected on 9/17/13)
1. SLA1
2. CUR1

Sample 1: SLA1
Total cyanobacteria cell numbers in the SLA1 sample collected on 9/17/13 were 37,631 cells/mL. Total potentially toxigenic (PTOX) cyanobacteria cell numbers were 37,003 cells/mL (98.3% of total cyanobacteria numbers). Potentially toxigenic species observed in the sample included *Woronichinia naegeliana* (34,479 cells/mL; Fig. 1), *Microcystis* sp. (1,340 cells/mL), *Microcystis wesenbergii* (864 cells/mL) and *Microcystis* sp. (320 cells/mL). The presence of large numbers of dead *Woronichinia naegeliana* cells (Fig. 2) and empty mucilaginous stalk systems (Fig. 3) indicates the density of this species was significantly higher prior to time of collection. The *Microcystis* species observed also did not appear to be in a healthy condition.

Sample 2: CUR1
Total cyanobacteria cell numbers in the CUR1 sample collected on 9/17/13 were 251,556 cells/mL. Total potentially toxigenic (PTOX) cyanobacteria cell numbers were 250,849 cells/mL (99.7% of total cyanobacteria numbers). Potentially toxigenic species observed in the sample included *Chrysosporum ovalisporum* (formerly *Aphanizomenon ovalisporum*; 123,699 cells/mL; Fig. 4), *Aphanizomenon* cf. *flos-aquae* (101,787 cells/mL; Fig. 5), *Planktothrix isothrix* (11,153 cells/mL; Fig. 6), *Anabaena planctonica* (11,074 cells/mL; Fig. 7), *Woronichinia naegeliana* (1,885 cells/mL), *Microcystis* sp. unicells and cell pairs (707 cells/mL), *Microcystis* sp. (460 cells/mL), *Snowella lacustris* (60 cells/mL) and *Radiocystis* sp. (25 cells/mL).

Fig. 1 *Woronichinia naegeliana* SLA1 400X (scale bar = 20µm)

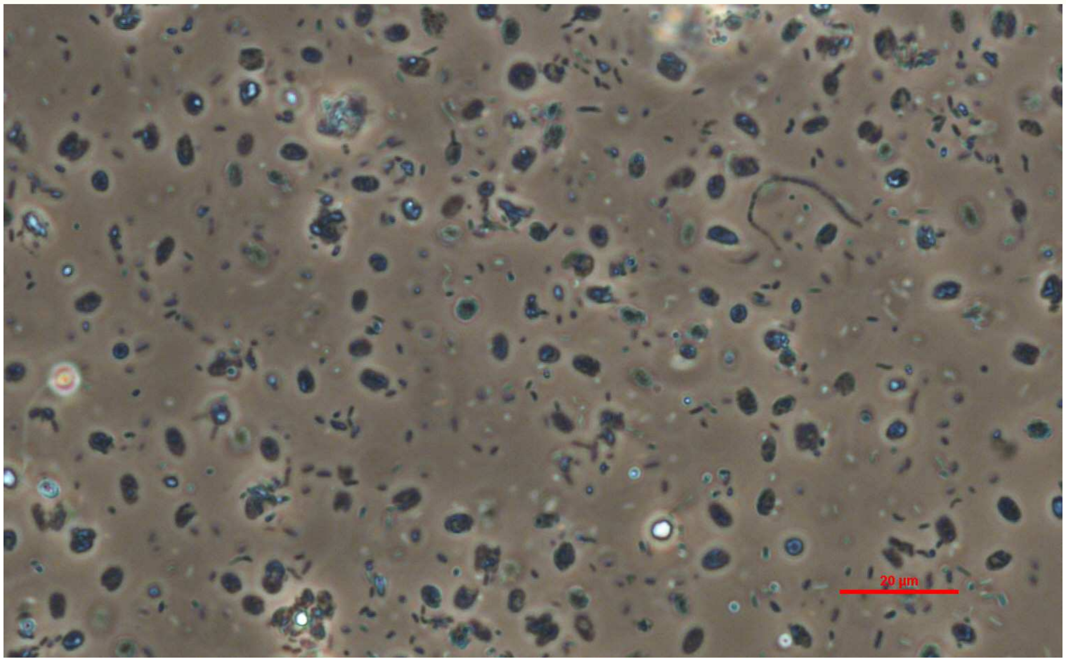

Fig. 2 *Woronichinia naegeliana* SLA1 dead cells 400X (scale bar = 20µm)

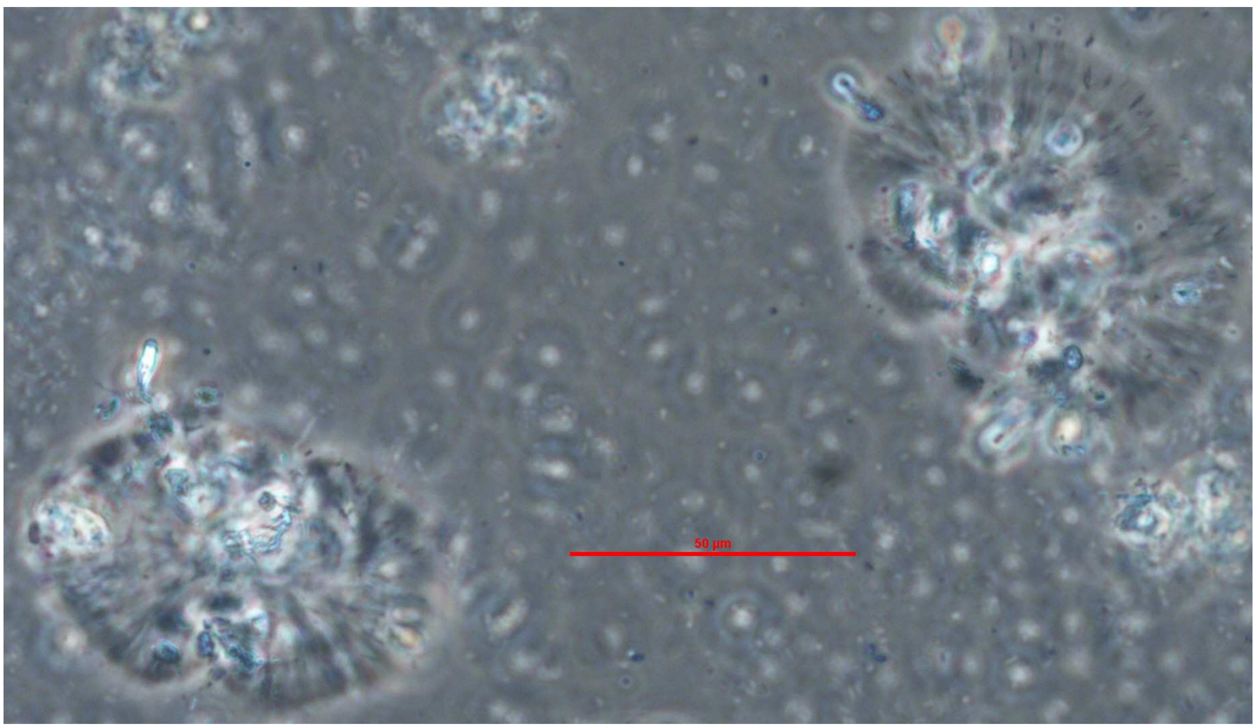

Fig. 3 *Woronichinia naegeliana* empty mucilage SLA1 400X (scale bar = 50µm)

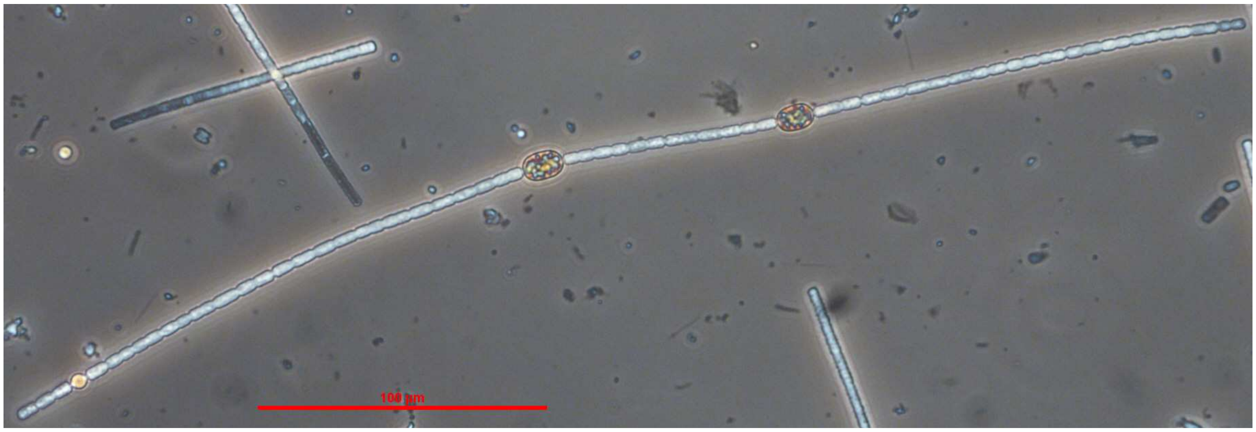

Fig. 4 *Chrysosporum ovalisporum* CUR1 200X (scale bar = 100µm)

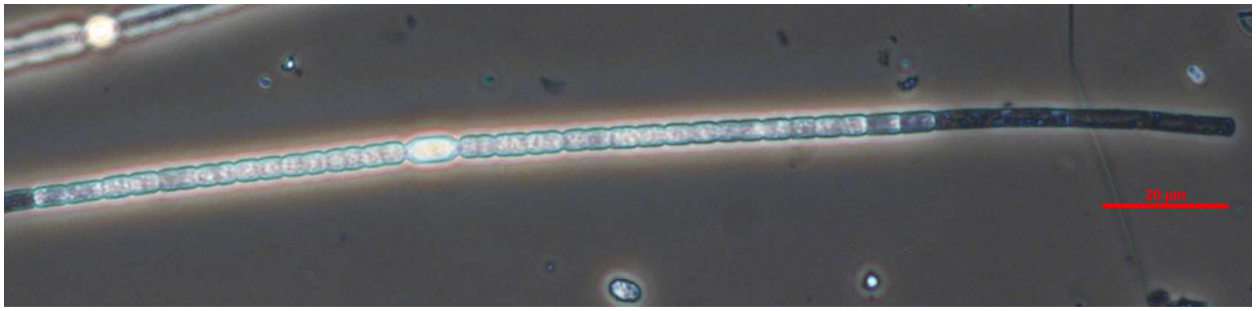

Fig. 5 *Aphanizomenon* cf. *flos-aquae* CUR1 400X (scale bar = 20µm)

Fig. 6 *Planktothrix isothrix* CUR1 400X (scale bar = 10µm)

Fig. 7 *Anabaena planctonica* CUR1 400X (scale bar = 20µm)

Sample	Date	Genus	Species	Algal Group	Unit	Cells/Unit	Species Units/mL	Species Cells/mL	Cyano Total Units/mL	Cyano Total Cells/mL	PTOX Cyano Total Units/mL	PTOX Cyano Total Cells/mL
STP1	9/16/2013	Aphanizomenon	sp.	Cyanobacteria	filament	18	220	3,958	479	8,071	408	6,660
STP1	9/16/2013	Aphanizomenon	cf. flos-aquae	Cyanobacteria	filament	10	157	1,571				
STP1	9/16/2013	Pseudanabaena	sp.	Cyanobacteria	filament	38	31	1,194				
STP1	9/16/2013	Chrysosporum/Sphaerospermopsis	sp.	Cyanobacteria	filament	36	31	1,131				
STP1	9/16/2013	Pseudanabaena	sp.	Cyanobacteria	filament	17	8	134				
STP1	9/16/2013	cyanophyte cell pair	spp.	Cyanobacteria	colony	2	31	63				
STP1	9/16/2013	Aphanocapsa	sp.	Cyanobacteria	colony	104	0.2	21				
STP2	9/16/2013	Chrysosporum/Sphaerospermopsis	sp.	Cyanobacteria	filament	36	63	2,262	353	6,189	220	4,084
STP2	9/16/2013	Aphanizomenon	cf. flos-aquae	Cyanobacteria	filament	10	126	1,257				
STP2	9/16/2013	Pseudanabaena	sp.	Cyanobacteria	filament	38	31	1,194				
STP2	9/16/2013	Aphanocapsa	sp.	Cyanobacteria	colony	104	8	817				
STP2	9/16/2013	Aphanizomenon	sp.	Cyanobacteria	filament	18	31	565				
STP2	9/16/2013	cyanophyte unicell, oval/rod 2.5-5um	spp.	Cyanobacteria	cell	1	63	63				
STP2	9/16/2013	Synechococcus	sp.	Cyanobacteria	cell	1	31	31				
TUR1	9/16/2013	Cuspidothrix	issatschenkoi	Cyanobacteria	filament	34	16	534	263	1,559	138	1,114
TUR1	9/16/2013	Coelosphaerium	sp.	Cyanobacteria	colony	780	0.4	312				
TUR1	9/16/2013	Microcystis	cf. ichthyoblabe	Cyanobacteria	colony	218	1	174				
TUR1	9/16/2013	Anabaena	circinalis	Cyanobacteria	filament	176	1	141				
TUR1	9/16/2013	cyanophyte unicell, sphere 2.5-5um	spp.	Cyanobacteria	cell	1	126	126				
TUR1	9/16/2013	Woronichinia	naegeliana (unicell)	Cyanobacteria	cell	1	63	63				
TUR1	9/16/2013	Microcystis	spp. (cell pair)	Cyanobacteria	colony	2	31	63				
TUR1	9/16/2013	Woronichinia	naegeliana (cell pair)	Cyanobacteria	colony	2	24	47				
TUR1	9/16/2013	Microcystis	wesenbergii	Cyanobacteria	colony	165	0.2	33				
TUR1	9/16/2013	Anabaena	cf. crassa	Cyanobacteria	filament	77	0.4	31				
TUR1	9/16/2013	Microcystis	sp.	Cyanobacteria	colony	80	0.2	16				
TUR1	9/16/2013	Anabaena	sp.	Cyanobacteria	filament	20	1	12				
TUR1	9/16/2013	Geitlerinema/Jaaginema	sp.	Cyanobacteria	filament	23	0.2	5				
TUR1	9/16/2013	Pseudanabaena	sp.	Cyanobacteria	filament	15	0.2	3				
SAN1	9/17/2013	Pseudanabaena	sp.	Cyanobacteria	filament	12	63	754	825	1,580	8	10
SAN1	9/17/2013	cyanophyte unicell, oval/rod 2.5-5um	spp.	Cyanobacteria	cell	1	660	660				
SAN1	9/17/2013	cyanophyte cell pair	spp.	Cyanobacteria	colony	2	63	126				
SAN1	9/17/2013	cyanophyte unicell, sphere 2.5-5um	spp.	Cyanobacteria	cell	1	31	31				
SAN1	9/17/2013	Woronichinia	naegeliana (unicell)	Cyanobacteria	cell	1	8	8				
SAN1	9/17/2013	Chrysosporum/Sphaerospermopsis	sp.	Cyanobacteria	filament	8	0.2	2				
GOR1	9/17/2013	Cyanogranis	ferruginea	Cyanobacteria	colony	8	2,513	20,106	2,730	22,675	47	71
GOR1	9/17/2013	Planktolyngbya	f. limnetica	Cyanobacteria	filament	50	31	1,571				
GOR1	9/17/2013	Aphanocapsa	sp.	Cyanobacteria	colony	15	16	236				
GOR1	9/17/2013	cyanophyte filament	sp.	Cyanobacteria	filament	52	4	204				
GOR1	9/17/2013	Aphanocapsa	sp.	Cyanobacteria	colony	8	16	126				
GOR1	9/17/2013	Pseudanabaena	sp.	Cyanobacteria	filament	28	4	110				
GOR1	9/17/2013	Komvophoron/Pseudanabaena	sp.	Cyanobacteria	filament	6	16	94				
GOR1	9/17/2013	cyanophyte unicell, oval/rod 2.5-5um	spp.	Cyanobacteria	cell	1	63	63				
GOR1	9/17/2013	Geitlerinema	splendidum	Cyanobacteria	filament	73	1	51				
GOR1	9/17/2013	Woronichinia	naegeliana (unicell)	Cyanobacteria	cell	1	31	31				
GOR1	9/17/2013	Woronichinia	naegeliana (cell pair)	Cyanobacteria	colony	2	16	31				
GOR1	9/17/2013	Pseudanabaena	sp.	Cyanobacteria	filament	7	4	27				
GOR1	9/17/2013	cyanophyte unicell, sphere 2.5-5um	spp.	Cyanobacteria	cell	1	16	16				
GOR1	9/17/2013	Microcystis	wesenbergii	Cyanobacteria	colony	60	0.1	6				
GOR1	9/17/2013	Anabaena/Aphanizomenon	sp.	Cyanobacteria	filament	26	0.1	3				
BEL1	9/17/2013	Pseudanabaena	sp.	Cyanobacteria	filament	10	31	314	99	457	0	0
BEL1	9/17/2013	cyanophyte unicell, oval/rod 2.5-5um	spp.	Cyanobacteria	cell	1	47	47				

BEL1	9/17/2013	cyanophyte filament	sp.	Cyanobacteria	filament	10	4	39				
BEL1	9/17/2013	cyanophyte cell pair	spp.	Cyanobacteria	colony	2	16	31				
BEL1	9/17/2013	cyanophyte filament	sp.	Cyanobacteria	filament	54	0.3	16				
BEL1	9/17/2013	Geitlerinema	splendidum	Cyanobacteria	filament	45	0.2	9				
SSL1	9/17/2013	cyanophyte cell pair	spp.	Cyanobacteria	colony	2	503	1,005	686	1,478	0.2	3
SSL1	9/17/2013	Pseudanabaena	sp.	Cyanobacteria	filament	10	24	236				
SSL1	9/17/2013	cyanophyte unicell, oval/rod 2.5-5um	spp.	Cyanobacteria	cell	1	126	126				
SSL1	9/17/2013	Pseudanabaena	sp.	Cyanobacteria	filament	20	2	32				
SSL1	9/17/2013	cyanophyte unicell, sphere 2.5-5um	spp.	Cyanobacteria	cell	1	31	31				
SSL1	9/17/2013	Aphanocapsa	sp.	Cyanobacteria	colony	74	0.2	15				
SSL1	9/17/2013	Geitlerinema/Jaaginema	sp.	Cyanobacteria	filament	28	0.4	11				
SSL1	9/17/2013	Snowella	litoralis	Cyanobacteria	colony	51	0.2	10				
SSL1	9/17/2013	Pseudanabaena	sp.	Cyanobacteria	filament	21	0.4	8				
SSL1	9/17/2013	Aphanizomenon	sp.	Cyanobacteria	filament	17	0.2	3				
BAR1	9/17/2013	Microcystis	aeruginosa	Cyanobacteria	colony	394	24	9,283	845	14,721	59	11,418
BAR1	9/17/2013	Snowella	lacustris	Cyanobacteria	colony	53	31	1,665				
BAR1	9/17/2013	cyanophyte filament	sp.	Cyanobacteria	filament	11	94	1,037				
BAR1	9/17/2013	Aphanocapsa	sp.	Cyanobacteria	colony	18	31	565				
BAR1	9/17/2013	Pseudanabaena	sp.	Cyanobacteria	filament	14	31	440				
BAR1	9/17/2013	Planktothrix	sp.	Cyanobacteria	filament	135	3	432				
BAR1	9/17/2013	Geitlerinema/Jaaginema	sp.	Cyanobacteria	filament	6	63	377				
BAR1	9/17/2013	cyanophyte unicell, oval/rod 2.5-5um	spp.	Cyanobacteria	cell	1	346	346				
BAR1	9/17/2013	Pseudanabaena	mucicola	Cyanobacteria	filament	4	63	251				
BAR1	9/17/2013	cyanophyte cell pair	spp.	Cyanobacteria	colony	2	94	188				
BAR1	9/17/2013	cyanophyte unicell, sphere 2.5-5um	spp.	Cyanobacteria	cell	1	63	63				
BAR1	9/17/2013	Woronichinia	naegeliana (colony)	Cyanobacteria	colony	170	0.2	34				
BAR1	9/17/2013	cyanophyte filament	sp.	Cyanobacteria	filament	137	0.2	27				
BAR1	9/17/2013	Komvophoron	sp.	Cyanobacteria	filament	39	0.2	8				
BAR1	9/17/2013	Microcystis	sp.	Cyanobacteria	colony	17	0.2	3				
BAR1	9/17/2013	Anabaena	sp.	Cyanobacteria	filament	3	0.2	1				

Sample	Date	Genus	Species	Algal Group	Unit	Cells/Unit	Species Units/mL	Species Cells/mL	Cyano Total Units/mL	Cyano Total Cells/mL	PTOX Cyano Total Units/mL	PTOX Cyano Total Cells/mL
Mashapaug Pond	7/24/2013	Microcystis	ichthyoblabe	Cyanobacteria	colony	92	942	86,707	16,022	252,361	16,021	252,313
Mashapaug Pond	7/24/2013	Anabaena	planctonica	Cyanobacteria	filament	30	2,670	80,110				
Mashapaug Pond	7/24/2013	Microcystis	wesenbergii	Cyanobacteria	colony	72	628	45,239				
Mashapaug Pond	7/24/2013	Anabaena	crassa	Cyanobacteria	filament	34	314	10,681				
Mashapaug Pond	7/24/2013	Microcystis	aeruginosa	Cyanobacteria	colony	310	27	8,370				
Mashapaug Pond	7/24/2013	Microcystis	spp. (cell pair)	Cyanobacteria	colony	2	2,827	5,655				
Mashapaug Pond	7/24/2013	Microcystis	spp. (unicell)	Cyanobacteria	cell	1	5,027	5,027				
Mashapaug Pond	7/24/2013	Microcystis	sp.	Cyanobacteria	colony	74	39	2,906				
Mashapaug Pond	7/24/2013	Woronichinia	naegeliana (unicell)	Cyanobacteria	cell	1	2,827	2,827				
Mashapaug Pond	7/24/2013	Aphanizomenon	cf. flos-aquae	Cyanobacteria	filament	30	79	2,356				
Mashapaug Pond	7/24/2013	Woronichinia	naegeliana (cell pair)	Cyanobacteria	colony	2	628	1,257				
Mashapaug Pond	7/24/2013	Microcystis	botrys	Cyanobacteria	colony	110	10	1,100				
Mashapaug Pond	7/24/2013	Woronichinia	naegeliana (colony)	Cyanobacteria	colony	78	1	78				
Mashapaug Pond	7/24/2013	Chroococcus	limneticus	Cyanobacteria	colony	48	1	48				
Polo-Willow Ponds	7/24/2013	Microcystis	wesenbergii	Cyanobacteria	colony	119	10,995	1,308,463	118,211	2,328,543	117,622	2,322,064
Polo-Willow Ponds	7/24/2013	Microcystis	ichthyoblabe	Cyanobacteria	colony	23	14,137	325,152				
Polo-Willow Ponds	7/24/2013	Microcystis	sp.	Cyanobacteria	colony	580	393	227,764				
Polo-Willow Ponds	7/24/2013	Anabaena	planctonica	Cyanobacteria	filament	36	4,712	169,645				
Polo-Willow Ponds	7/24/2013	Microcystis	aeruginosa	Cyanobacteria	colony	255	589	150,206				
Polo-Willow Ponds	7/24/2013	Microcystis	spp. (unicell)	Cyanobacteria	cell	1	59,690	59,690				
Polo-Willow Ponds	7/24/2013	Microcystis	spp. (cell pair)	Cyanobacteria	colony	2	22,776	45,553				
Polo-Willow Ponds	7/24/2013	Anabaena	crassa	Cyanobacteria	filament	54	196	10,603				
Polo-Willow Ponds	7/24/2013	Aphanizomenon	cf. flos-aquae	Cyanobacteria	filament	20	393	7,854				
Polo-Willow Ponds	7/24/2013	Cuspidothrix	issatschenkoi	Cyanobacteria	filament	34	196	6,676				
Polo-Willow Ponds	7/24/2013	Cyanogranis	libera	Cyanobacteria	colony	11	589	6,479				
Polo-Willow Ponds	7/24/2013	Cylindrospermopsis	raciborskii (straight)	Cyanobacteria	filament	23	196	4,516				
Polo-Willow Ponds	7/24/2013	Woronichinia	naegeliana (unicell)	Cyanobacteria	cell	1	2,356	2,356				
Polo-Willow Ponds	7/24/2013	Woronichinia	naegeliana (cell pair)	Cyanobacteria	colony	2	785	1,571				
Polo-Willow Ponds	7/24/2013	Woronichinia	naegeliana (colony)	Cyanobacteria	colony	4	196	785				
Polo-Willow Ponds	7/24/2013	Microcystis	viridis	Cyanobacteria	colony	146	5	730				
Polo-Willow Ponds	7/24/2013	Microcystis	botrys	Cyanobacteria	colony	100	5	500				

Sample	Date	Genus	Species	Algal Group	Unit	Cells/Unit	Species Units/mL	Species Cells/mL	Cyano Total Units/mL	Cyano Total Cells/mL	PTOX Cyano Total Units/mL	PTOX Cyano Total Cells/mL
MLP1	9/16/2013	Woronichinia	naegeliana (unicell)	Cyanobacteria	cell	1	72,256	72,256	93,084	163,328	93,083	163,303
MLP1	9/16/2013	Woronichinia	naegeliana (cell pair)	Cyanobacteria	colony	2	17,540	35,081				
MLP1	9/16/2013	Anabaena	planctonica	Cyanobacteria	filament	20	1,649	32,986				
MLP1	9/16/2013	Woronichinia	naegeliana (colony)	Cyanobacteria	colony	58	236	13,666				
MLP1	9/16/2013	Microcystis	cf. ichthyoblabe	Cyanobacteria	colony	36	118	4,241				
MLP1	9/16/2013	Aphanizomenon	cf. flos-aquae	Cyanobacteria	filament	17	236	4,005				
MLP1	9/16/2013	Microcystis	spp. (unicell)	Cyanobacteria	cell	1	1,047	1,047				
MLP1	9/16/2013	Pseudanabaena	sp.	Cyanobacteria	filament	25	1	25				
MLP1	9/16/2013	Anabaena	sp.	Cyanobacteria	filament	20	1	20				
MLP2	9/16/2013	Woronichinia	naegeliana (unicell)	Cyanobacteria	cell	1	60,999	60,999	79,293	130,990	79,253	129,934
MLP2	9/16/2013	Woronichinia	naegeliana (cell pair)	Cyanobacteria	colony	2	16,231	32,463				
MLP2	9/16/2013	Anabaena	planctonica	Cyanobacteria	filament	25	1,178	29,452				
MLP2	9/16/2013	Woronichinia	naegeliana (colony)	Cyanobacteria	colony	27	118	3,181				
MLP2	9/16/2013	Aphanizomenon	cf. flos-aquae	Cyanobacteria	filament	14	157	2,199				
MLP2	9/16/2013	Microcystis	cf. ichthyoblabe	Cyanobacteria	colony	25	41	1,025				
MLP2	9/16/2013	Aphanocapsa	sp.	Cyanobacteria	colony	26	39	1,021				
MLP2	9/16/2013	Microcystis	spp. (unicell)	Cyanobacteria	cell	1	524	524				
MLP2	9/16/2013	Anabaena	sp.	Cyanobacteria	filament	18	3	54				
MLP2	9/16/2013	Microcystis	botrys	Cyanobacteria	colony	19	2	38				
MLP2	9/16/2013	Pseudanabaena	sp.	Cyanobacteria	filament	35	1	35				

Sample	Date	Genus	Species	Algal Group	Unit	Cells/Unit	Species Units/mL	Species Cells/mL	Cyano Total Units/mL	Cyano Total Cells/mL	PTOX Cyano Total Units/mL	PTOX Cyano Total Cells/mL
SLA1	9/17/2013	Woronichinia	naegeliana (colony)	Cyanobacteria	colony	73	471	34,400	759	37,631	563	37,003
SLA1	9/17/2013	Microcystis	sp.	Cyanobacteria	colony	335	4	1,340				
SLA1	9/17/2013	Microcystis	wesenbergii	Cyanobacteria	colony	108	8	864				
SLA1	9/17/2013	Pseudanabaena	sp.	Cyanobacteria	filament	12	39	471				
SLA1	9/17/2013	Microcystis	sp.	Cyanobacteria	colony	320	1	320				
SLA1	9/17/2013	Synechococcus	sp.	Cyanobacteria	cell	1	157	157				
SLA1	9/17/2013	Woronichinia	naegeliana (unicell)	Cyanobacteria	cell	1	79	79				
CUR1	9/17/2013	Chrysosporum	ovalisporum	Cyanobacteria	filament	45	2,749	123,699	9,978	251,556	9,349	250,849
CUR1	9/17/2013	Aphanizomenon	cf. flos-aquae	Cyanobacteria	filament	24	4,241	101,787				
CUR1	9/17/2013	Planktothrix	isothrix	Cyanobacteria	filament	71	157	11,153				
CUR1	9/17/2013	Anabaena	planctonica	Cyanobacteria	filament	47	236	11,074				
CUR1	9/17/2013	Woronichinia	naegeliana (unicell)	Cyanobacteria	cell	1	1,100	1,100				
CUR1	9/17/2013	Woronichinia	naegeliana (cell pair)	Cyanobacteria	colony	2	393	785				
CUR1	9/17/2013	Microcystis	sp. (cell pair)	Cyanobacteria	colony	2	236	471				
CUR1	9/17/2013	Microcystis	sp.	Cyanobacteria	colony	230	2	460				
CUR1	9/17/2013	cyanophyte unicell, oval/rod 2.5-5um	spp.	Cyanobacteria	cell	1	393	393				
CUR1	9/17/2013	Microcystis	sp. (unicell)	Cyanobacteria	cell	1	236	236				
CUR1	9/17/2013	cyanophyte cell pair	spp.	Cyanobacteria	colony	2	79	157				
CUR1	9/17/2013	cyanophyte unicell, sphere 2.5-5um	spp.	Cyanobacteria	cell	1	157	157				
CUR1	9/17/2013	Snowella	lacustris	Cyanobacteria	colony	60	1	60				
CUR1	9/17/2013	Radiocystis	sp.	Cyanobacteria	colony	50	1	25				

Appendix B

Microcystin Lab Reports

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Contact: markaubel@greenwaterlab.com
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ESS Group, Inc.

MICROCYSTIN RESULTS

Tested on: 7/26/2013
Method: Enzyme-Linked ImmunoSorbent Assay (ELISA)
Analyte: Microcystins
Analyzed by: Alicia Gard-Kaminkow

Sample ID/ Date Collected	Initial Conc. Factor	Dilution Ratio	Assay Value, ug/L	Final Dilution Factor	Avg. LFB Recovery(%)	Avg. LFM Recovery(%)	Final Concentration (ug/L)	Average (ug/L)
MP	1x	none	0.94	1	96	109	0.94	1.0
7/24/13	1x	none	0.99	1	96	109	0.99	
PL	1x	1:10	1.15	10	96	—	11.48	11.5
7/24/13	1x	1:10	1.16	10	96	—	11.55	

ND = Not detected above LOD/LOQ
LOD/LOQ = 0.15 µg/L
LFB = 1.0 µg/L MCLR
LFM = 1.0 µg/L MCLR

Submitted by: Mark T. Aubel, Ph.D.
Date: 7/26/2013

Submitted to: Matt Ladewig
ESS Group, Inc.
401 Wampanoag Trail
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Tel. (401) 434-5560
mladewig@essgroup.com

Microcystin Analysis Report

Project: ESS Group

(Mashapaug and Polo-Willow Ponds)

Sample Identification	Sample Collection Date
MP (Mashapaug Pond)	7/24/13
PL (Polo-Willow Pond)	7/24/13

Toxin – Microcystin (MC)

Sample Prep – The samples were ultrasonicated to lyse cells and release toxins. A sample dilution (1:10) was necessary for the Polo-Willow sample to acquire an absorbance value within range of the standard curve. A duplicate MP sample (Lab Fortified Matrix, LFM) was spiked at 1.0 µg/L MCLR, which provided quantitative capability and additional qualitative confirmation.

Analytical Methodology – A microcystins (MC) enzyme linked immunosorbent assay (ELISA) from Abraxis LLC was utilized for the quantitative and sensitive congener-independent detection of MCs. The ELISA kit is sensitive down to a limit of detection/quantification (LOD/LOQ) of 0.15 µg/L. The average recovery for a laboratory fortified blank (LFB) spiked with 1 µg/L MCLR was 96%.

Summary of Results

Sample	MC levels (µg/L)
MP (Mashapaug Pond)	1.0
PL (Polo-Willow Pond)	11.5

ND = not detected above the LOD/LOQ
LOD/LOQ = 0.15 µg/L

Submitted by: [signature]
Mark T. Aubel, Ph.D.
Date: 7/26/13

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ESS Group

MICROCYSTIN RESULTS

Tested on: 9/18/13 & 9/19/13
Method: Enzyme-Linked ImmunoSorbent Assay (ELISA)
Analyte: Microcystins
Analyzed by: Alicia Carter & Amanda Foss

Sample ID/ Date Collected	Initial Conc. Factor	Dilution Ratio	Assay Value, ug/L	Final Dilution Factor	Avg. LFB Recovery(%)	Avg. LFM Recovery(%)	Final Concentration (ug/L)	Average (ug/L)
Slack's Reservoir - SLA1	1x	1:10	0.94	10	106	–	9.4	**8.6**
9/17/2013	1x	1:10	0.78	10	106	–	7.8	
Curran Reservoir - CUR1	1x	none	0.20	1	106	–	0.20	**0.20**
9/17/2013	1x	none	0.20	1	106	–	0.20	
Sand Pond - SAN1	1x	none	0.03	1	105	118	ND	**ND**
9/17/2013	1x	none	0.05	1	105	118	ND	
Gorton Pond - GOR1	1x	none	0.25	1	105	–	0.25	**0.22**
9/17/2013	1x	none	0.18	1	105	–	0.18	
Bellville Pond - BEL1	1x	none	0.06	1	105	–	ND	**ND**
9/17/2013	1x	none	0.04	1	105	–	ND	
Silver Spring Lake - SSL1	1x	none	0.03	1	105	98	ND	**ND**
9/17/2013	1x	none	0.03	1	105	98	ND	
Barber Pond - BAR1	1x	none	0.79	1	105	–	0.79	**0.77**
9/17/2013	1x	none	0.75	1	105	–	0.75	

ND = Not detected above LOD/LOQ
LOD/LOQ = 0.15 µg/L
LFB = 1.0 µg/L MCLR
LFM = 1.0 µg/L MCLR

Submitted by: Amanda Foss, M.S.
Date: 9/19/2013

Submitted to:
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Microcystin Data Report

Project: ESS Group

Sample Identification	Sample Collection Date
Slack's Reservoir - SLA1	9/17/13
Curran Reservoir - CUR1	9/17/13
Sand Pond - SAN1	9/17/13
Gorton Pond - GOR1	9/17/13
Bellville Pond - BEL1	9/17/13
Silver Spring Lake - SSL1	9/17/13
Barber Pond - BAR1	9/17/13

Toxins –microcystins (MC)

Sample Prep
The samples were ultra-sonicated to lyse cells and release toxins. The samples were analyzed using ELISA without pre-concentration. The Slack's Reservoir sample (SLA1) required dilution in order to fall within range of the standard curve. Duplicate samples of SAN1 and SSL1 were spiked (lab fortified matrix, LFM) at 1.0 µg/L MCLR, which provided quantitative capability and additional qualitative confirmation.

Analytical Methodology
A microcystins enzyme linked immunosorbent assay (ELISA) was utilized for the quantitative and sensitive congener-independent detection of MCs. The current assay is sensitive down to a LOD/LOQ of 0.15 µg/L for total MCs. The average recovery of the lab fortified blanks (LFB) spiked with 1 µg/L MCLR was 105%.

Summary of Results

Sample	MC Levels (µg/L)
Slack's Reservoir - SLA1	**8.6**
Curran Reservoir - CUR1	**0.2**
Sand Pond - SAN1	**ND**
Gorton Pond - GOR1	**0.2**
Bellville Pond - BEL1	**ND**
Silver Spring Lake - SSL1	**ND**
Barber Pond - BAR1	**0.8**

LOD/LOQ = 0.15 µg/L

Submitted by: [signature]
Mark T. Aubel, Ph.D.
Date: 9/19/13

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ESS Group

MICROCYSTIN RESULTS

Tested on: 9/18/13 & 9/19/13
Method: Enzyme-Linked ImmunoSorbent Assay (ELISA)
Analyte: Microcystins
Analyzed by: Alicia Carter & Amanda Foss

Sample ID/ Date Collected	Initial Conc. Factor	Dilution Ratio	Assay Value, ug/L	Final Dilution Factor	Avg. LFB Recovery(%)	Avg. LFM Recovery(%)	Final Concentration (ug/L)	Average (ug/L)
Stafford Pond - STP2	1x	none	0.10	1	105	–	ND	**ND**
9/16/2013	1x	none	0.14	1	105	–	ND	
Stafford Pond - STP1	1x	none	0.08	1	105	135	ND	**ND**
9/16/2013	1x	none	0.08	1	105	135	ND	
Melville Pond - MLP1	1x	none	0.20	1	105	–	0.20	**0.18**
9/16/2013	1x	none	0.16	1	105	–	0.16	
Melville Pond - MLP2	1x	none	0.20	1	106	–	0.20	**0.22**
9/16/2013	1x	none	0.23	1	106	–	0.23	
Turner Reservoir - TVR1	1x	none	0.06	1	105	–	ND	**ND**
9/16/2013	1x	none	0.04	1	105	–	ND	

ND = Not detected above LOD/LOQ
LOD/LOQ = 0.15 µg/L
LFB = 1.0 µg/L MCLR
LFM = 1.0 µg/L MCLR

Submitted by: Amanda Foss
Amanda Foss, M.S.
Date: 9/19/2013

Submitted to: Matt Ladewig
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GreenWater laboratories — aquatic analysis ... research ... consultation

Microcystin Data Report

Project: ESS Group

Sample Identification	Sample Collection Date
Stafford Pond – STP2	9/16/13
Stafford Pond – STP1	9/16/13
Melville Pond – MLP1	9/16/13
Melville Pond – MLP2	9/16/13
Turner Reservoir – TVR1	9/16/13

Toxins –microcystins (MC)

Sample Prep

The samples were ultra-sonicated to lyse cells and release toxins. The samples were analyzed using ELISA without pre-concentration. A duplicate STP1 sample was spiked (Lab Fortified Matrix, LFM) at 1.0 µg/L MCLR, which provided quantitative capability and additional qualitative

Analytical Methodology

A microcystins enzyme linked immunosorbent assay (ELISA) was utilized for the quantitative and sensitive congener-independent detection of MCs. The current assay is sensitive down to a LOD/LOQ of 0.15 µg/L for total MCs. The average recovery of the lab fortified blanks (LFB) spiked with 1 µg/L MCLR was 105%.

Summary of Results

Sample	MC Levels (µg/L)
Stafford Pond – STP2	**ND**
Stafford Pond – STP1	**ND**
Melville Pond – MLP1	**0.2**
Melville Pond – MLP2	**0.2**
Turner Reservoir – TVR1	**ND**

LOD/LOQ = 0.15 µg/L

Submitted by: [signature]
Mark T. Aubel, Ph.D.

Date: 9/19/13

CyanoLAB