



# Cyanobacteria Monitoring Program

Rhode Island  
2011 Report  
RIDEM REQ. NO. 1180565/1194117

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ESS Project No. R298-010

Revised January 30, 2013





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*Prepared For:*

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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 2011.

## 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 2011, 15 cyanobacteria samples were collected from 13 water bodies, distributed across the state as far north and west as Glocester, as far south as Charlestown, and as far east as Pawtucket (Table A and Figure 1). The water bodies visited ranged from a very small, urban, manmade pond (Slater Memorial Park Pond) to a relatively large coastal pond (Trustom Pond). Of the 13 water bodies sampled, 11 were sampled as part of the annual screening program and 2 were sampled in response to reports of algae blooms from the public.

The water bodies selected for screening level monitoring in the 2011 monitoring year were chosen based on anecdotal historical presence of algal blooms and chlorophyll *a* data collected by University of Rhode Island Watershed Watch (URI-WW) during the growing seasons between 2000 and 2009. Of the water bodies reviewed by RIDEM, the ten with the highest mean and maximum chlorophyll *a* and total phosphorus concentrations were selected as screening level monitoring locations in 2011. Additionally, Trustom Pond was selected for screening based on anecdotal evidence of previous cyanobacteria blooms. Water bodies were selected for response level monitoring as prompted by specific public or agency requests to investigate suspected algae blooms.

**Table A. Water Bodies Sampled by the Cyanobacteria Monitoring Program in 2011**

Sampling Program	Water Body	Location	Long	Lat	WBID	Acres
Screening Level	Barber Pond	South Kingstown	-71.56448	41.4992	RI0008039L-14	28.16
	Breakheart Pond	Exeter/West Greenwich	-71.70335	41.59605	RI0008040L-15	43.79
	Fenner Pond	Cranston	-71.42036	41.76986	RI0006017L-08	19.47
	Lake Washington	Glocester	-71.75853	41.91716	RI0005047L-04	40.89
	Mashapaug Pond	Providence	-71.43553	41.79313	RI0006017L-06	76.75
	Roger Williams Park Ponds	Providence	-71.41414	41.77731	RI0006017L-05	113.95
	Slater Memorial Park Pond	Pawtucket	-71.34652	41.8712	none	4.60
	Spectacle Pond	Cranston	-71.44225	41.79402	RI0006017L-07	38.81
	Trustom Pond	Charlestown	-71.58356	41.37669	RI0010043E-08	179.20
	Valley Falls Pond	Central Falls	-71.39055	41.89884	RI0001003L-02	37.97
	Warwick Pond	Warwick	-71.41531	41.72472	RI0007024L-02	84.72
Response Level	Slack Reservoir	Smithfield	-71.55258	41.86584	RI0002007L-03	133.61
	Spring Lake Reservoir #2 (Lower J.L. Curran Reservoir)	Cranston	-71.54574	41.74314	RI0006016L-02	18.08

ESS collected each of the screening level samples in mid to late 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 access sampling location, at a minimum.

Each screening level cyanobacteria sample was sent to GreenWater Laboratory (the lab) for identification/enumeration. Microcystin samples were analyzed by the lab if the cell count for a given sample was greater than 50,000 cells per milliliter (mL), the microcystin analysis threshold established in the project-specific QAPP. Samples with cell counts lower than the threshold were not analyzed for microcystins.

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. Each response level monitoring sample was first screened by RIDEM staff to determine if a substantial number of potentially toxigenic cyanobacteria were present within the sample. Samples with substantial numbers of these cells were sent to the lab for identification/enumeration. Microcystins were subsequently analyzed by the lab if the cell count for a given sample exceeded 50,000 cells/mL.

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

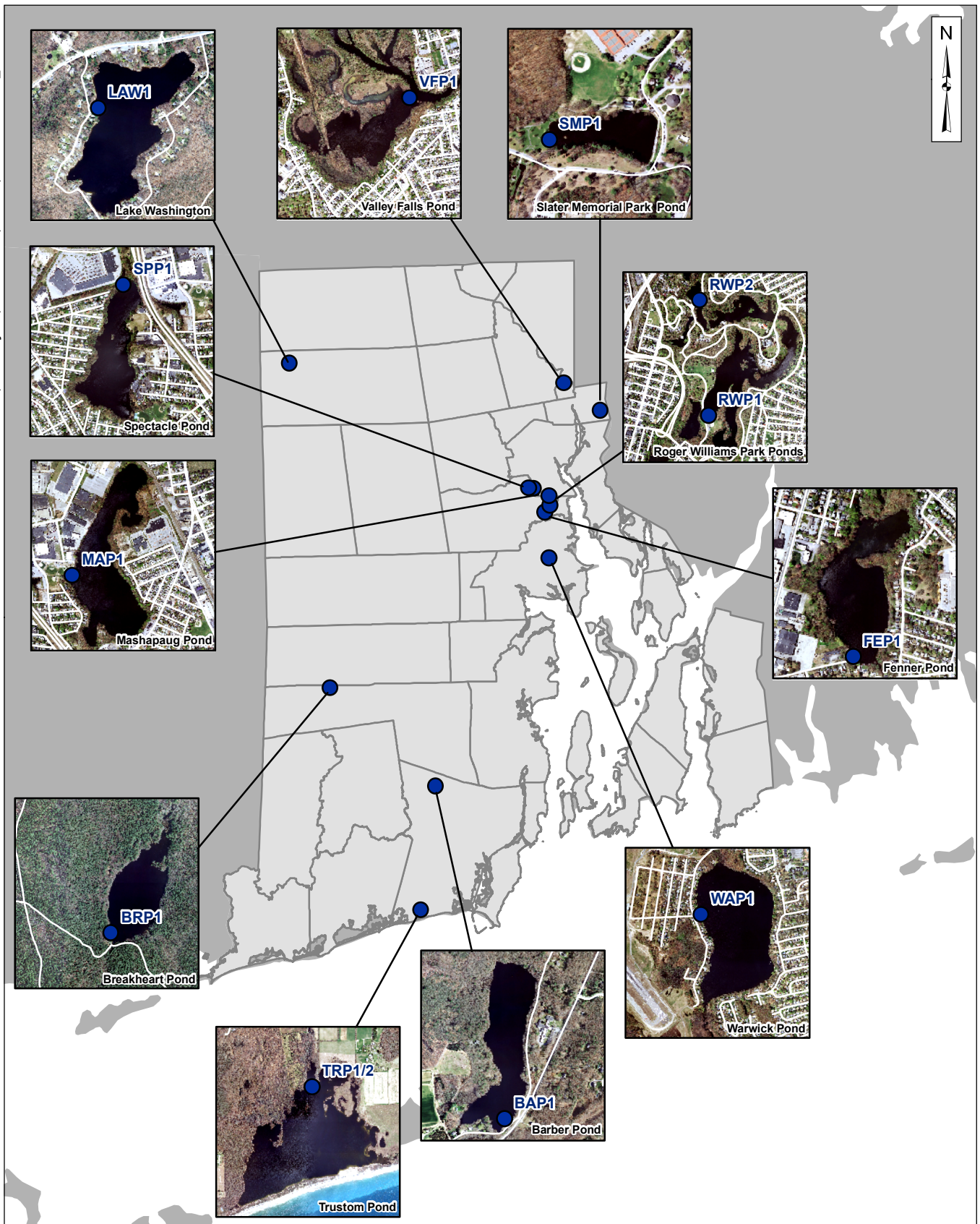
### **3.0 RESULTS**

#### **3.1 Cyanobacteria**

Cell densities ranged from fewer than 2,500 cells/ml to more than 15 million cells/mL (Table B). Overall, cell density exceeded 50,000 cells/mL in 10 samples from 8 water bodies.

Potentially toxigenic cyanobacteria species were identified in 11 samples from 9 water bodies (Table B). The potentially toxigenic cyanobacteria species that most frequently dominated samples were *Woronichinia naegeliana* and *Anabaena spp.* *Microcystis ichthyoblabe* and *Planktothrix suspensa* were dominant or co-dominant species with *Woronichinia naegeliana* at the two stations sampled in Roger Williams Park. Complete cyanobacteria identification and enumeration results may be found in Appendix A.

Measured microcystin levels from the 10 samples selected for microcystin analysis ranged from 0.3 µg/L to 82 µg/L and suggest that a relationship with cell density may potentially be described by a power function (Table B, Figure 2). Additional data from other blooms in Rhode Island would be needed to further evaluate the strength and significance of this relationship. Complete microcystin laboratory results are presented in Appendix B.



### RIDEM CYANOBACTERIA MONITORING Rhode Island








Scale: 1" = 50,000'  
0 10 Miles








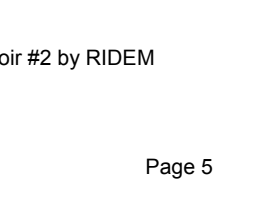
Source: 1) RIGIS, Political Boundaries, 2001  
2) RIGIS, Orthos, 2011  
3) ESS, GPS Locations, 2011

### 2011 Cyanobacteria Screening Level Monitoring Sites

Figure  
1

**Table B. Summary of 2011 Cyanobacteria Sampling Program Results**

Water Body	Station ID	Date	Cell Density (cells/mL)	Exceeded Cell Count Threshold?	Microcystin Level (µg/L)	Dominant Species	Photograph*
Mashapaug Pond	MAP1	9/21/2011	15,205,667	Yes	15.3	<ul style="list-style-type: none"> <li>• <i>Woronichinia naegeliana</i> (PTOX)</li> <li>• <i>Anabaena planctonica</i> (PTOX)</li> </ul>	
Spring Lake Reservoir #2 (Lower J.L. Curran Reservoir)	SPR1	9/23/2011	13,556,869	Yes	35	<ul style="list-style-type: none"> <li>• <i>Anabaena sp.</i> (PTOX)</li> </ul>	
Slater Memorial Park Pond	SMP1	9/21/2011	5,545,811	Yes	0.6	<ul style="list-style-type: none"> <li>• <i>Woronichinia naegeliana</i> (PTOX)</li> </ul>	
Slack Reservoir	SLR1	9/26/2011	3,284,778	Yes	82	<ul style="list-style-type: none"> <li>• <i>Woronichinia naegeliana</i> (PTOX)</li> </ul>	
Roger Williams Park Ponds	RWP2	9/21/2011	618,884	Yes	2.3	<ul style="list-style-type: none"> <li>• <i>Woronichinia naegeliana</i> (PTOX)</li> <li>• <i>Microcystis ichthyoblabe</i> (PTOX)</li> </ul>	
Spectacle Pond	SPP1	9/21/2011	333,038	Yes	6.1	<ul style="list-style-type: none"> <li>• <i>Woronichinia naegeliana</i> (PTOX)</li> </ul>	
Warwick Pond	WAP1	9/14/2011	126,727	Yes	0.3	<ul style="list-style-type: none"> <li>• <i>Cyanogrannis ferruginea</i></li> </ul>	

Water Body	Station ID	Date	Cell Density (cells/mL)	Exceeded Cell Count Threshold?	Microcystin Level (µg/L)	Dominant Species	Photograph*
Roger Williams Park Ponds	RWP1	9/21/2011	85,067	Yes	0.5	<ul style="list-style-type: none"> <li><i>Planktothrix suspensa</i> (PTOX)</li> </ul>	
Trustom Pond	TRP2 (field duplicate for TRP1)	9/21/2011	54,965	Yes	5	<ul style="list-style-type: none"> <li><i>Woronichinia naegeliana</i> (PTOX)</li> </ul>	
Trustom Pond	TRP1	9/21/2011	52,623	Yes	3	<ul style="list-style-type: none"> <li><i>Woronichinia naegeliana</i> (PTOX)</li> </ul>	
Valley Falls Pond	VFP1	9/14/2011	9,101	No	NS	<ul style="list-style-type: none"> <li><i>Planktolyngbya limnetica</i></li> <li><i>Aphanothece</i> sp.</li> </ul>	
Barber Pond	BAP1	9/14/2011	5,584	No	NS	<ul style="list-style-type: none"> <li><i>Anabaena planctonica</i> (PTOX)</li> </ul>	
Lake Washington	LAW1	9/14/2011	4,818	No	NS	<ul style="list-style-type: none"> <li><i>Woronichinia naegeliana</i> (PTOX)</li> </ul>	
Fenner Pond	FEP1	9/21/2011	3,824	No	NS	<ul style="list-style-type: none"> <li>Small cyanophyte cell pairs</li> </ul>	
Breakheart Pond	BRP1	9/14/2011	2,456	No	NS	<ul style="list-style-type: none"> <li><i>Aphanocapsa planctonica</i></li> </ul>	

NS = not sampled

\*All photos by ESS, except Slack Reservoir and Spring Lake Reservoir #2 by RIDEM

PTOX = potentially toxicogenic species



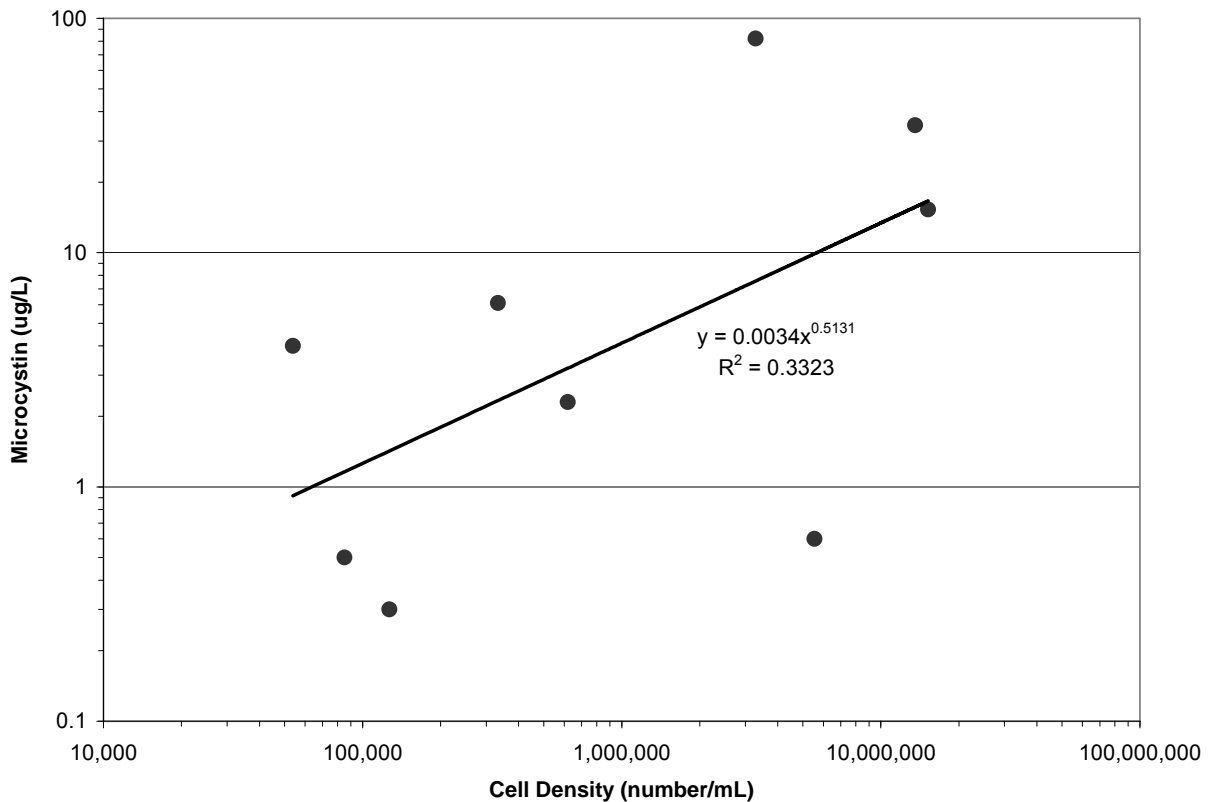


Figure 2. Cyanobacteria Cell Density and Microcystin Levels in Samples Exceeding 50,000 Cells/mL

### 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. For instance, a trend of increasing water temperature and dissolved oxygen values from morning to afternoon was observed during the monitoring efforts conducted on September 21. 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 most locations, except for Breakheart Pond (4.9 mg/L at BRP1) and the Roger Williams Park Ponds (3.4 at RWP1) (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 Trustom Pond (920.1  $\mu\text{S}/\text{cm}$ ) (Table C). However, Trustom Pond is a coastal pond that may receive some water quality influences from marine waters, typically during overflow or natural breaching associated with large storms. It is possible that the observed specific conductance and salinity levels at Trustom Pond were associated with the aftermath of Tropical Storm Irene (which made landfall on August 31). Outside of Trustom Pond, the highest specific conductance was recorded at Mashapaug Pond (351.6  $\mu\text{S}/\text{cm}$ ), which is located in a highly urbanized watershed.

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 1.00 meter (i.e., pond

bottom). Additional Secchi depth measurements were collected at ponds where a boat allowed access to deeper water and provide a better indication of actual water clarity at the time of sampling. Water clarity was most impaired at Slater Memorial Park Pond, where the pond-wide bloom reduced Secchi depth to less than 0.25 meters (Table C). Water clarity was also less than 1.00 meter in Fenner Pond, Mashapaug Pond, and at Station RWP2 in the Roger Williams Park Ponds. Since a cyanobacteria bloom was not evident at Fenner Pond, the reduced water clarity observed was likely due to staining (water color was a dark brown) and/or possibly a bloom sustained by another phytoplankton taxonomic group (e.g., green algae).

**Table C. Water Quality Observed during Cyanobacteria Screening**

Waterbody	Station ID	Date	Time	Air Temp (°C)	Water Temp (°C)	DO (mg/L)	DO (%)	Spec. Cond (µS/cm)	Salinity (ppt)	Secchi Depth (m)	Secchi Depth at Deep Hole (m)**
Warwick Pond	WAP1	9/14/2011	0840	21.8	23.1	9.5	111.5	237.6	0.1	1.00*	1.13
Barber Pond	BAP1	9/14/2011	1021	23.0	22.8	7.9	91.5	77.3	0.0	1.00*	1.13
Breakheart Pond	BRP1	9/14/2011	1206	NA	22.5	4.9	56.4	79.8	0.0	1.00*	NA
Lake Washington	LAW1	9/14/2011	1130	26.5	23.8	8.4	99.5	178.0	0.1	1.00*	1.75
Valley Falls Pond	VFP1	9/14/2011	1515	28.2	22.7	8.1	93.4	297.5	0.1	1.00*	Same
Fenner Pond	FEP1	9/21/2011	0745	16.6	18.7	6.5	69.2	314.7	0.2	0.70	Same
Roger Williams Park Ponds	RWP1	9/21/2011	0830	17.8	19.1	3.4	34.8	335.1	0.2	1.00*	NA
Roger Williams Park Ponds	RWP2	9/21/2011	0850	18.2	18.8	5.2	55.9	323.9	0.2	0.75	NA
Spectacle Pond	SPP1	9/21/2011	0950	NA	20.5	6.6	73.3	285.9	0.1	1.00*	1.20
Mashapaug Pond	MAP1	9/21/2011	1100	NA	22.3	6.4	74.3	351.6	0.2	0.25	0.80
Slater Memorial Park Pond	SMP1	9/21/2011	1220	NA	22.4	11.5	131.6	88.0	0.0	<0.25	NA
Trustom Pond	TRP1/2	9/21/2011	1447	26.7	20.7	12.5	139.4	920.1	0.5	1.00*	NA

\*On bottom

\*\*Only reported where boat was used for sampling and Secchi depth was greater than at sampling location.

### **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 due to equipment malfunction or lack of availability.

Cyanobacteria QA/QC requirements were met for all screening level monitoring samples. Among the response level samples, the sample from Spring Lake Reservoir #2 was held for four days. This is outside the holding time for enumeration outlined in the project-specific QAPP but is within the holding time for microcystin analysis. However, Jane Sawyers, the RIDEM algal taxonomist examined the Lower J.L. Curran Reservoir sample on the day of collection and had already determined that cell counts were excessive, thus triggering microcystin analysis by the lab. Therefore, it is reasonable to conclude that

exceedance of hold time for enumeration/identification analysis did not have a substantive impact on the decision to have the lab analyze the sample for microcystins or the results of the microcystin analysis.

All internal lab 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 Trustom Pond (TRP1/TRP2). Cell density and microcystin levels for the field duplicate were within QC limits.

#### **4.0 DISCUSSION AND CONCLUSIONS**

ESS visited 11 water bodies statewide and collected 13 cyanobacteria samples as part of the 2011 screening level monitoring program. An additional two samples were collected by RIDEM in response to active blooms.

Cyanobacteria densities exceeded the 50,000 cells/mL threshold established in the project-specific QAPP in 10 samples from 8 water bodies, initiating analysis of microcystins. Among these samples, microcystin levels ranged from 0.3 µg/L to 82 µg/L. Microcystin guidelines for recreational contact generally range from 14 µg/L (Massachusetts Department of Public Health, undated) to 20 µg/L (World Health Organization, 2003). The Rhode Island health advisory guidelines trigger an advisory when the cyanobacteria cell count exceeds 70,000 cells/mL or microcystin-LR level of lysed cells is 14 µg/L or higher. Both of the Rhode Island health advisory guideline levels were exceeded in the two response level samples from Lower J.L. Curran Reservoir and Slack Reservoir. Additionally, microcystin levels in Mashapaug Pond exceeded the Rhode Island health advisory guideline of 14 µg/L.

Microcystin levels may be associated with cell density, although exceedance of Rhode Island recreational contact guidelines for microcystins only occurred in samples where cell density was greater than 3 million cells/mL. The highest microcystin levels measured were associated with blooms dominated by *Woronichinia naegeliana* or *Anabaena* spp. Data collected from future monitoring events could potentially be pooled with the 2011 data to better determine whether elevated microcystin levels can be predicted based on cell density and dominant species.

Investigating the factors contributing to the observed cyanobacteria blooms 2011 is beyond the scope of this study. However, phosphorus total maximum daily loads (TMDL) have been prepared for several of the ponds sampled during the 2011 cyanobacteria monitoring program, including Mashapaug Pond, Spectacle Pond, the Roger Williams Park Ponds, Warwick Pond, and Barber Pond. Of these, only Warwick Pond and Barber Pond were not observed to host cyanobacteria blooms during screening level sampling in 2011. Cyanobacteria are able to fix nitrogen from the atmosphere, allowing them to take advantage of excess phosphorus in the water column and multiply rapidly under favorable environmental conditions.

Four ponds exceeded the 50,000 cells/mL project-specific QAPP threshold during 2011 sampling but have not been officially identified as impaired waters or incorporated into a TMDL. These include Trustom Pond, Slater Memorial Park Pond, Lower J.L. Curran Reservoir, and Slack Reservoir. All of these except Trustom Pond also exceeded Rhode Island health advisory guidelines.

Trustom Pond cyanobacteria sampling under the 2011 monitoring program suggests a low-end bloom (~50,000 cells/mL) may have been occurring at the time, although microcystin levels were below Rhode Island recreational contact guidelines. This pond was previously characterized as a eutrophic water body with evidence of cyanobacteria blooms in September and October of 2010 (Faubl, 2011). Data collected during these blooms indicated that *Anabaena* was the dominant taxon and microcystin levels were less than 1.0 µg/L. Watershed sources (e.g., agricultural runoff) and waterfowl were identified as the major sources of phosphorus to the pond, with waterfowl potentially contributing as much or more phosphorus to the pond as watershed loading.

Cyanobacteria sampling at Slater Memorial Park Pond under the 2011 monitoring program confirmed that a dense bloom was occurring with a density of more than 5.5 million cells/mL (although microcystin levels were well below Rhode Island recreational contact guidelines). This is a small urban pond and field observations during sampling suggest that the immediate pond surroundings receive heavy use from visitors and resident waterfowl. Observed activities such as waterfowl feeding likely contribute nutrients to the pond and further investigation and quantification of local and watershed sources is recommended.

Lower J.L. Curran Reservoir and Slack Reservoir were also identified as water bodies with active cyanobacteria blooms through response level sampling in 2011. Microcystin levels in both water bodies exceeded Rhode Island recreational contact guidelines. No readily available water quality data were located for Lower J.L. Curran Reservoir. According to URI Watershed Watch data, Slack Reservoir phosphorus levels have not been excessive in recent years, typically averaging less than 20 µg/L. However, average chlorophyll-*a* levels have occasionally spiked above 10 µg/L at Slack Reservoir, suggesting that the water body has a history of algae blooms.

In sum, the 2011 cyanobacteria monitoring program successfully detected several active blooms and also documented that bloom intensity, species composition, and microcystin levels can be quite variable even across a single water body. Follow-up studies to document pollutant sources (particularly phosphorus) and identify appropriate in-lake management actions and watershed BMPs for addressing these sources and resulting blooms may be warranted, especially in water bodies where blooms corresponded with elevated microcystin levels or where TMDLs have not yet been developed.

## **5.0 REFERENCES**

- ESS Group, Inc. (ESS). 2011. Quality Assurance Project Plan. Rhode Island Department of Environmental Management Cyanobacteria Monitoring Program. Revised October 13, 2011.
- Faubl, S. 2011. A Management-oriented Water Quality Investigation of Trustom Pond National Wildlife Refuge (Major Paper). University of Rhode Island.
- Massachusetts Department of Public Health. Date unspecified. MDPH Guidelines for Cyanobacteria in Freshwater Recreational Water Bodies in Massachusetts.
- World Health Organization. 2003. Guidelines for Safe Recreational Water Environments, Volume 1: Coastal and Fresh Waters.

## Appendix A

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### Cyanobacteria Identification and Enumeration Lab Reports



## ESS Group Cyanobacteria ID and Enumeration Report

Prepared: October 17, 2011

Prepared By: GreenWater Laboratories

Samples: 5 (collected on 9/14/11)

1. BAP1
2. BRP1
3. LAW1
4. VFP1
5. WAP1

### Sample 1: BAP1

Total cyanobacteria cell numbers in the BAP1 sample collected on 9/14/11 were 5,584 cells/mL. The dominant cyanophyte species in the sample was *Anabaena planctonica* (3,176 cells/mL; Fig. 1).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 4,198 cells/mL (75.2% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Anabaena planctonica* (3,176 cells/mL), *Woronichinia naegeliana* (871 cells/mL), *Aphanizomenon ovalisporum* (62 cells/mL), *Microcystis aeruginosa* (53 cells/mL), *Cylindrospermopsis raciborskii* (32 cells/mL) and *Planktothrix* sp. (4 cells/mL).

### Sample 2: BRP1

Total cyanobacteria cell numbers in the BRP1 sample collected on 9/14/11 were 2,456 cells/mL. The most abundant cyanophyte species in the sample was *Aphanocapsa planctonica* (726 cells/mL).

No potentially toxigenic cyanobacteria (PTOX Cyano) were observed in the BRP1 sample.

### Sample 3: LAW1

Total cyanobacteria cell numbers in the LAW1 sample collected on 9/14/11 were 4,818 cells/mL. The most abundant cyanophyte species in the sample was *Woronichinia naegeliana* (3,901 cells/mL).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 4,199 cells/mL (87.2% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Woronichinia naegeliana* (3,901 cells/mL), *Microcystis* cf. *smithii* (166 cells/mL) and *Anabaena/Aphanizomenon* sp. (132 cells/mL).

Sample 4: VFP1

Total cyanobacteria cell numbers in the VFP1 sample collected on 9/14/11 were 9,101 cells/mL. The dominant cyanophyte species in the sample were *Planktolyngbya limnetica* (3,484 cells/mL; Fig. 2) and *Aphanothece* sp. (3,157 cells/mL; Fig. 3).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 391 cells/mL (4.3% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Anabaena/Aphanizomenon* sp. (209 cells/mL), *Woronichinia naegeliana* (72 cells/mL), *Anabaena* sp. (54 cells/mL), *Planktothrix suspensa* (41 cells/mL), *Aphanizomenon* sp. (6 cells/mL), *Aphanizomenon issatschenkoi* (6 cells/mL) and *Cylindrospermopsis raciborskii* (3 cells/mL).

Sample 5: WAP1

Total cyanobacteria cell numbers in the WAP1 sample collected on 9/14/11 were 126,727 cells/mL. The dominant cyanophyte species in the sample was *Cyanogranis ferruginea* (98,805 cells/mL; Fig. 4).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 6,737 cells/mL (5.3% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Anabaena planctonica* (2,382 cells/mL), *Aphanizomenon ovalisporum* (1,588 cells/mL), *Cylindrospermopsis raciborskii* (1,361 cells/mL; Fig. 5), *Aphanizomenon* cf. *gracile* (771 cells/mL), *Microcystis* cf. *smithii* (288 cells/mL), *Woronichinia naegeliana* (272 cells/mL) and *Microcystis wesenbergii* (75 cells/mL).



Fig. 1 *Anabaena planctonica* 400X (scale bar = 10 $\mu$ m)

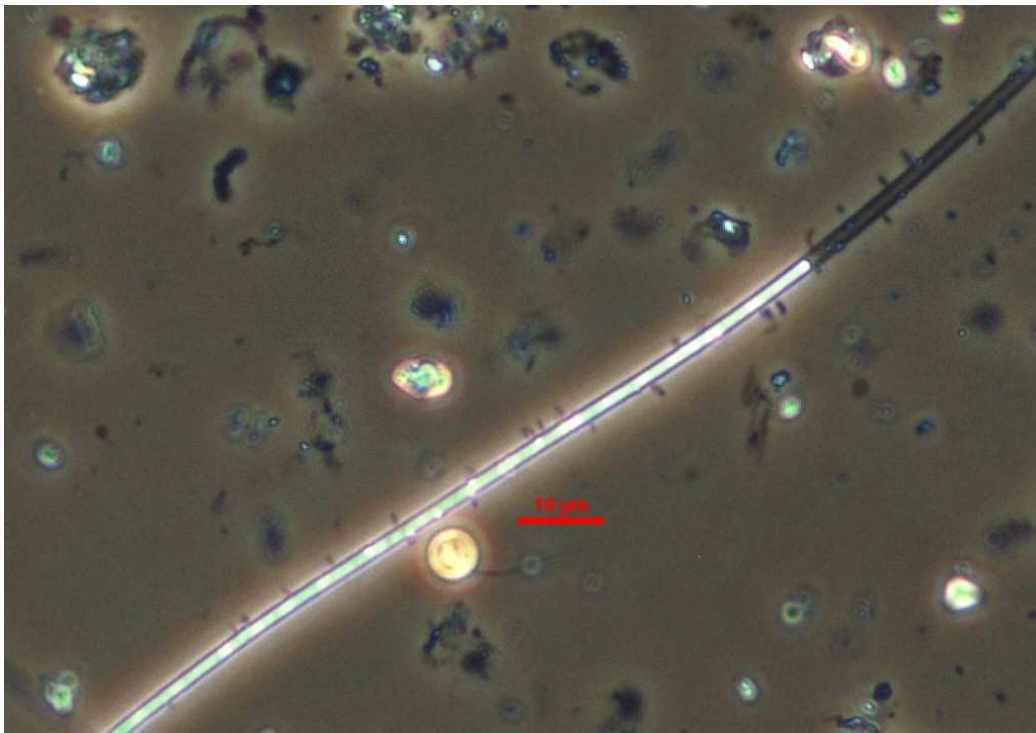


Fig. 2 *Planktolyngbya limnetica* 400X (scale bar = 10µm)

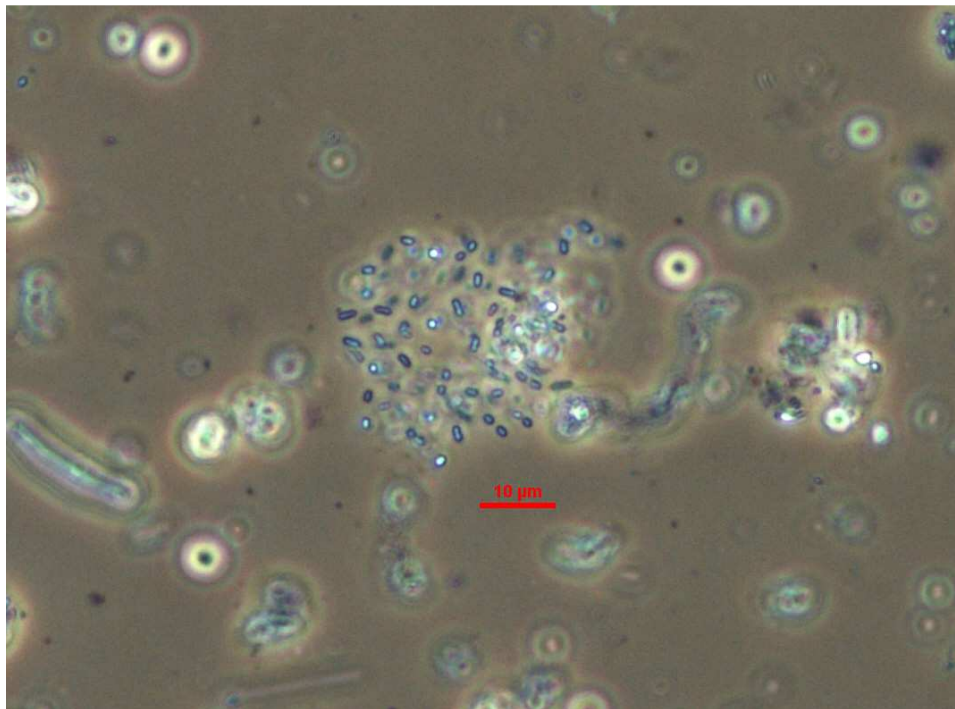


Fig. 3 *Aphanothece* sp. 400X (scale bar = 10µm)



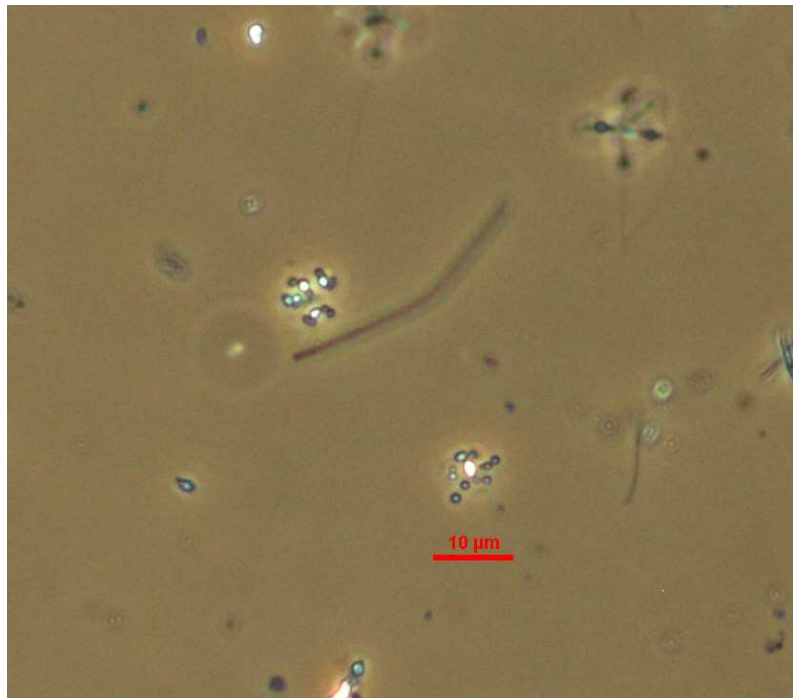


Fig. 4 *Cyanogranis ferruginea* 400X (scale bar = 10µm)

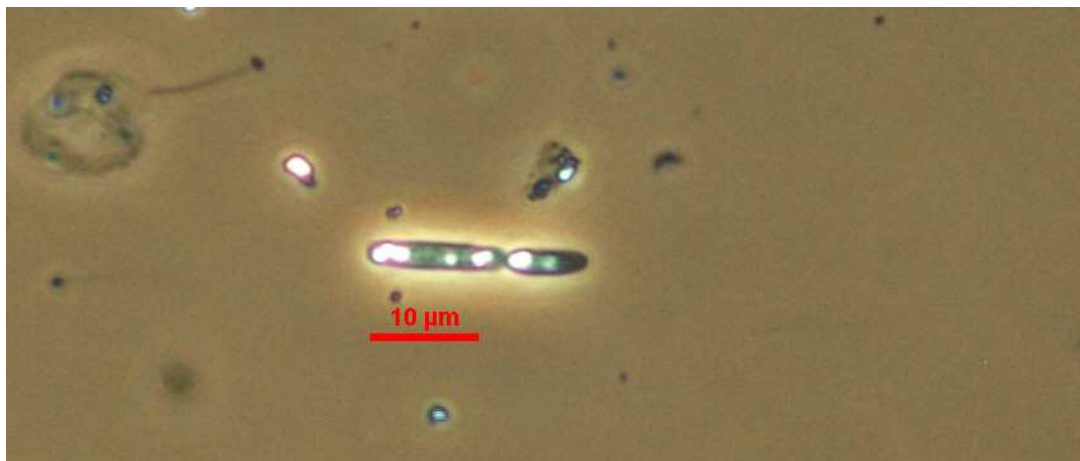


Fig. 5 *Cylindrospermopsis raciborskii* 400X (scale bar = 10µm)

## ESS Group Cyanobacteria ID and Enumeration Report

Prepared: October 17, 2011

Prepared By: GreenWater Laboratories

Samples: 8 (collected on 9/21/11)

1. MAP1
2. RWP1
3. RWP2
4. SMP1
5. SPP1
6. TRP1
7. TRP2
8. FEP1

### Sample 1: MAP1

Total cyanobacteria cell numbers in the MAP1 sample collected on 9/21/11 were 15,205,667 cells/mL. The dominant species in the sample was *Woronichinia naegeliana* (10,896,625 cells/mL; Fig. 1). Many loose cells and cell pairs of *W. naegeliana* were present.

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 15,205,667 cells/mL (100% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Woronichinia naegeliana* (10,896,625 cells/mL), *Anabaena planctonica* (2,953,248 cells/mL; Fig. 2), *Microcystis botrys* (469,526 cells/mL; Fig. 3), *Microcystis ichthyoblabe* (258,579 cells/mL), *Anabaena/Aphanizomenon* sp. (222,288 cells/mL), *Aphanizomenon* cf. *flos-aquae* (206,410 cells/mL), *Microcystis wesenbergii* (179,191 cells/mL) and *Microcystis aeruginosa* (19,800 cells/mL).

### Sample 2: RWP1

Total cyanobacteria cell numbers in the RWP1 sample collected on 9/21/11 were 85,067 cells/mL. The most abundant species in the sample was *Planktothrix suspensa* (40,647 cells/mL; Fig. 4).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 49,685 cells/mL (58.4% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Planktothrix suspensa* (40,647 cells/mL), *Woronichinia naegeliana* (4,112 cells/mL), *Microcystis wesenbergii* (2,087 cells/mL), *Microcystis ichthyoblabe* (1,270 cells/mL), *Anabaena planctonica* (953 cells/mL), *Microcystis* sp. (362 cells/mL), *Aphanizomenon issatschenkoi* (138 cells/mL), *Aphanizomenon* cf. *flos-aquae* (60 cells/mL) and *Anabaena/Aphanizomenon* sp. (56 cells/mL).

Sample 3: RWP2

Total cyanobacteria cell numbers in the RWP2 sample collected on 9/21/11 were 618,884 cells/mL. The most abundant species in the sample were *Woronichinia naegeliana* (254,270 cells/mL) and *Microcystis ichthyoblabe* (204,142 cells/mL; Fig. 5).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 593,071 cells/mL (95.8% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Woronichinia naegeliana* (254,270 cells/mL), *Microcystis ichthyoblabe* (204,142 cells/mL), *Anabaena planctonica* (58,521 cells/mL), *Planktothrix suspensa* (32,209 cells/mL), *Microcystis* cf. *smithii* (21,889 cells/mL), *Anabaena/Aphanizomenon* sp. (8,166 cells/mL), *Aphanizomenon* cf. *flos-aquae* (5,897 cells/mL), *Microcystis* spp. unicells and cell pairs (4,310 cells/mL), *Microcystis wesenbergii* (2,193 cells/mL) and *Aphanizomenon issatschenkoi* (1,474 cells/mL).

Sample 4: SMP1

Total cyanobacteria cell numbers in the SMP1 sample collected on 9/21/11 were 5,545,811 cells/mL. The dominant species in the sample was *Woronichinia naegeliana* (5,494,811 cells/mL). Many loose cells and cell pairs of *W. naegeliana* were present.

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 5,545,811 cells/mL (100% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Woronichinia naegeliana* (10,896,625 cells/mL) and *Anabaena* sp. (51,000 cells/mL; Fig. 6).

Sample 5: SPP1

Total cyanobacteria cell numbers in the SPP1 sample collected on 9/21/11 were 333,038 cells/mL. The dominant species in the sample was *Woronichinia naegeliana* (203,461 cells/mL). Many loose cells and cell pairs of *W. naegeliana* were present.

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 330,697 cells/mL (99.3% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Woronichinia naegeliana* (203,461 cells/mL), *Anabaena planctonica* (57,462 cells/mL), *Microcystis ichthyoblabe* (31,453 cells/mL), *Aphanizomenon* cf. *flos-aquae* (15,122 cells/mL), *Microcystis* spp. unicells and cell pairs (14,819 cells/mL), *Microcystis wesenbergii* (3,432 cells/mL), *Microcystis* cf. *smithii* (2,948 cells/mL) and *Microcystis botrys* (2,000 cells/mL).

Sample 6: TRP1

Total cyanobacteria cell numbers in the TRP1 sample collected on 9/21/11 were 52,623 cells/mL. The dominant species in the sample was *Woronichinia naegeliana* (34,477 cells/mL). Many loose cells and cell pairs of *W. naegeliana* were present.

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 40,513 cells/mL (77.0% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Woronichinia naegeliana* (34,477 cells/mL), *Microcystis aeruginosa* (3,744 cells/mL), *Microcystis* cf. *smithii* (715 cells/mL), *Microcystis wesenbergii* (544 cells/mL), *Snowella*

*lacustris* (470 cells/mL), *Microcystis* spp. (347 cells/mL) and *Aphanizomenon* sp. (216 cells/mL).

#### Sample 7: TRP2

Total cyanobacteria cell numbers in the TRP2 sample collected on 9/21/11 were 54,965 cells/mL. The dominant species in the sample were *Woronichinia naegeliana* (24,860 cells/mL) and *Microcystis aeruginosa* (16,740 cells/mL; Fig. 7).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 42,378 cells/mL (77.1% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Woronichinia naegeliana* (24,860 cells/mL), *Microcystis aeruginosa* (16,740 cells/mL), *Microcystis* sp. (499 cells/mL), *Anabaena* sp. (96 cells/mL), *Aphanizomenon issatschenkoi* (95 cells/mL), *Aphanizomenon* sp. (60 cells/mL), *Microcystis wesenbergii* (18 cells/mL) and *Anabaena* sp. (10 cells/mL).

#### Sample 8: FEP1

Total cyanobacteria cell numbers in the FEP1 sample collected on 9/21/11 were 3,824 cells/mL. The dominant species in the sample were pairs of small (<5µm) cyanophyte cells.

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 770 cells/mL (20.1% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Woronichinia naegeliana* (588 cells/mL) and *Microcystis* sp. (182 cells/mL).



Fig. 1 *Woronichinia naegeliana* 200X (scale bar = 50μm)



Fig. 2 *Anabaena planctonica* 400X (scale bar = 50μm)

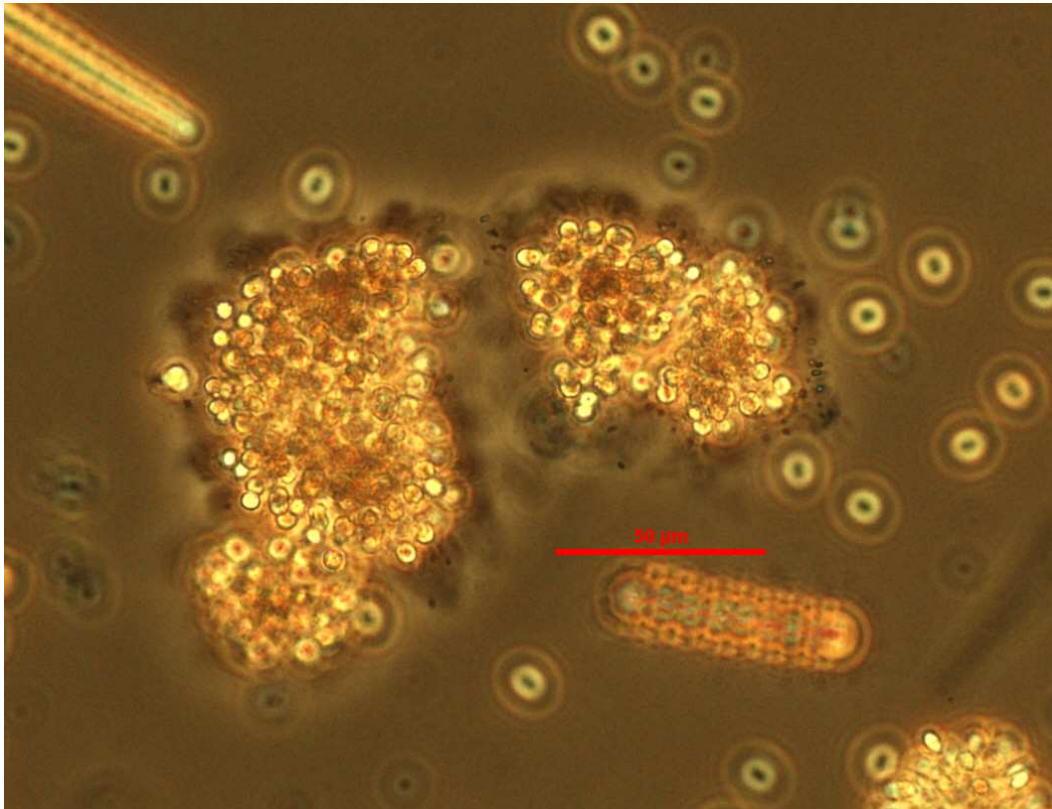


Fig. 3 *Microcystis botrys* 200X (scale bar = 50µm)



Fig. 4 *Planktothrix suspensa* 400X (scale bar = 10µm)

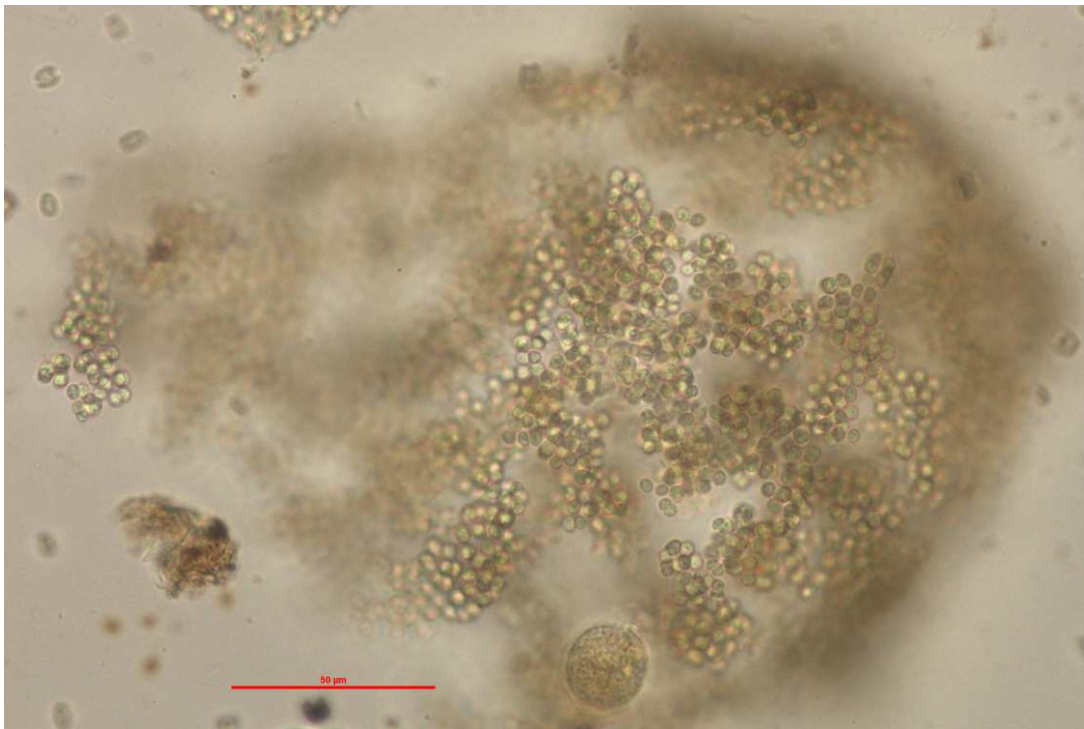


Fig. 5 *Microcystis ichthyoblabe* 400X (scale bar = 50 $\mu$ m)



Fig. 6 *Anabaena* sp. 400X (scale bar = 10 $\mu$ m)

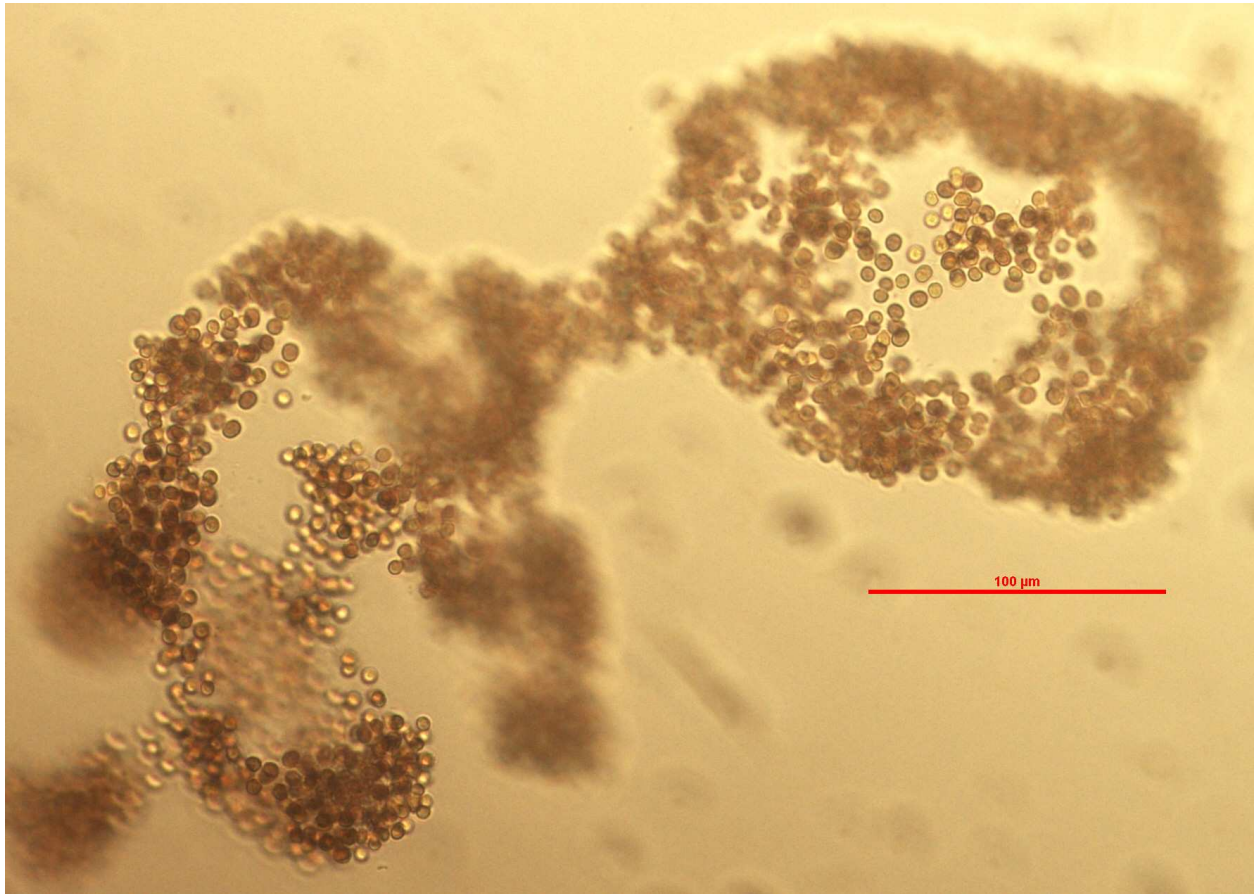


Fig. 7 *Microcystis aeruginosa* 200X (scale bar = 100 $\mu$ m)



## ESS Group Cyanobacteria ID and Enumeration Report

Prepared: September 29, 2011

Prepared By: GreenWater Laboratories

Samples: 2

1. Spring Lake Reservoir (collected on 9/23/11)
2. Slacks Reservoir (collected on 9/26/11)

### Sample 1: Spring Lake Reservoir

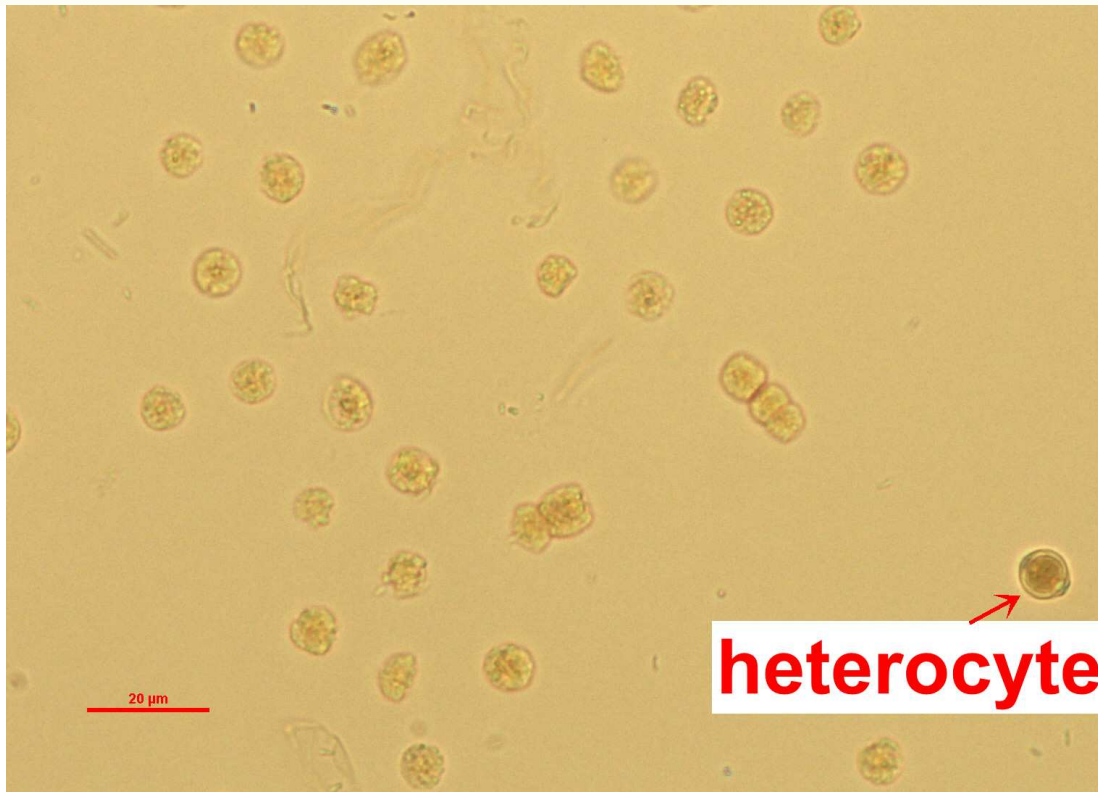
Total cyanobacteria cell numbers in the Spring Lake Reservoir sample collected on 9/23/11 were 13,556,869 cells/mL. The dominant species in the sample was an *Anabaena* species (12,411,809 cells/mL). The *Anabaena* consisted mostly of loose unicells indicating the recent crash of a bloom (Fig. 1). Some loose akinetes were observed (Fig. 2) which most resembled those of *Anabaena planctonica*.

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 13,436,652 cells/mL (99.1% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Anabaena* sp. (12,411,809 cells/mL), *Woronichinia naegeliana* (966,270 cells/mL; Fig. 3) and *Microcystis botrys* (Fig. 4).

### Sample 2: Slacks Reservoir

Total cyanobacteria cell numbers in the Slacks Reservoir sample collected on 9/26/11 were 3,284,778 cells/mL. The most abundant species in the sample was *Woronichinia naegeliana* (2,022,666 cells/mL; Fig. 5).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 3,110,515 cells/mL (94.7% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Woronichinia naegeliana* (2,022,666 cells/mL), *Anabaena lemmermannii* (358,382 cells/mL; Fig. 6), *Microcystis aeruginosa* (267,350 cells/mL; Fig. 7), *Microcystis botrys* (217,751 cells/mL; Fig. 8), *Microcystis* sp. (134,583 cells/mL; Fig. 9), *Microcystis wesenbergii* (76,818 cells/mL; Fig. 10), *Anabaena planctonica* (20,868 cells/mL; Fig. 11) and *Anabaena* sp. (12,097 cells/mL).



**heterocyte**

Fig. 1 *Anabaena* sp. loose unicells 400X (scale bar = 20µm)



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



Fig. 3 *Woronichinia naegeliana* 400X (scale bar = 20µm)

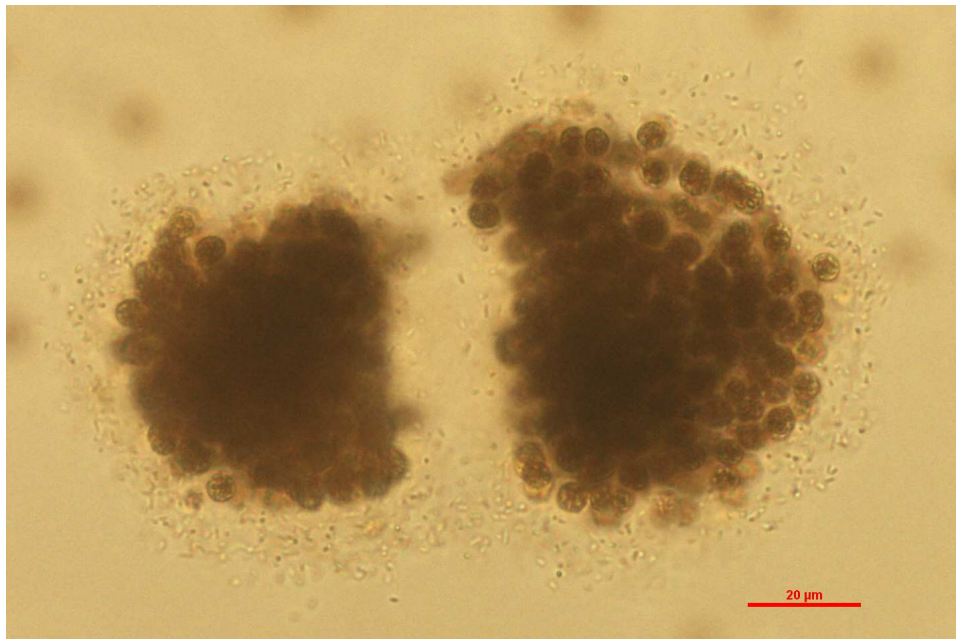


Fig. 4 *Microcystis botrys* 400X (scale bar = 20µm)



Fig. 5 *Woronichinia naegeliana* 400X (scale bar = 20μm)



Fig. 6 *Anabaena lemmermannii* 400X (scale bar = 10μm)

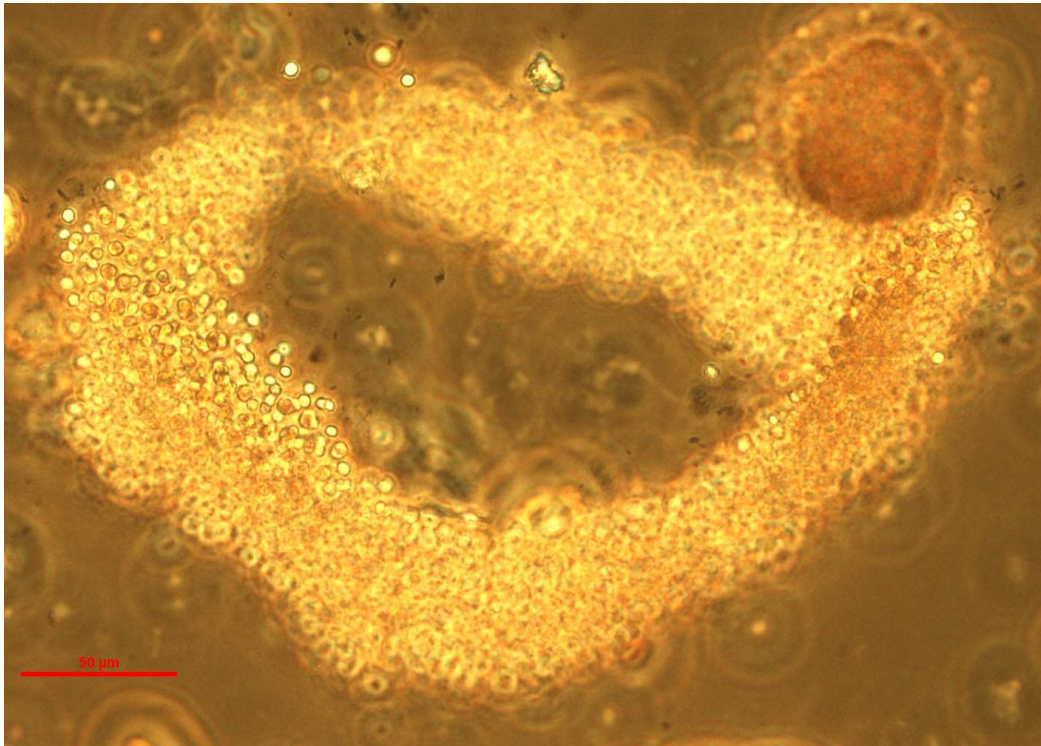


Fig. 7 *Microcystis aeruginosa* 200X (scale bar = 50 $\mu$ m)

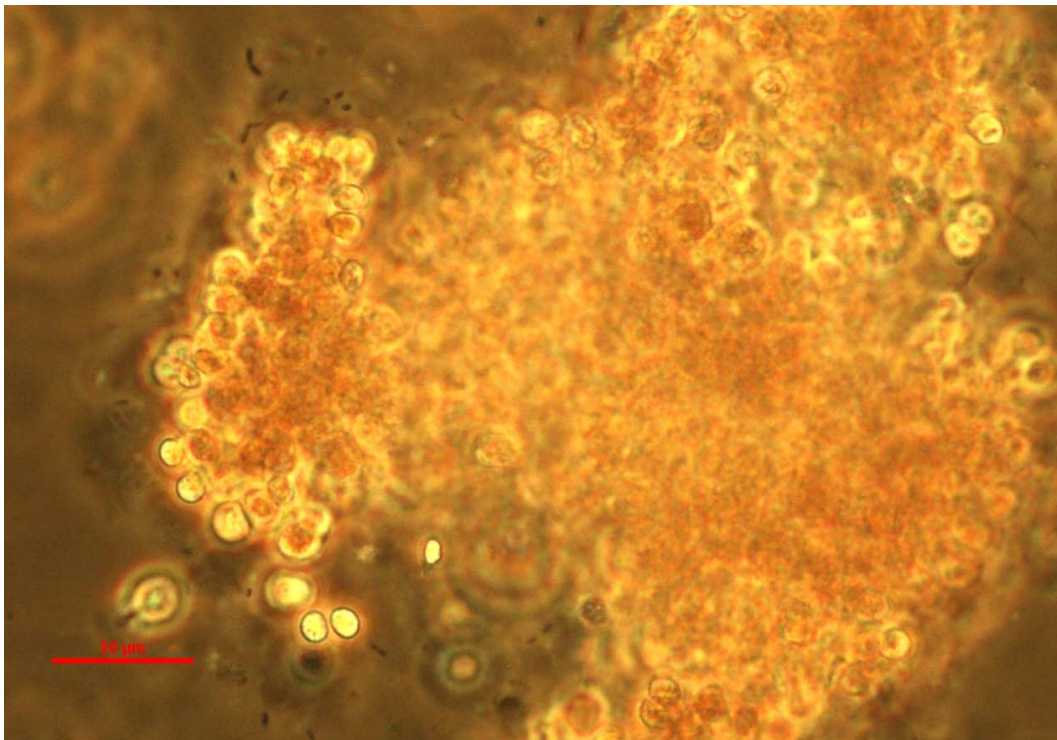


Fig. 8 *Microcystis botrys* 400X (scale bar = 20 $\mu$ m)

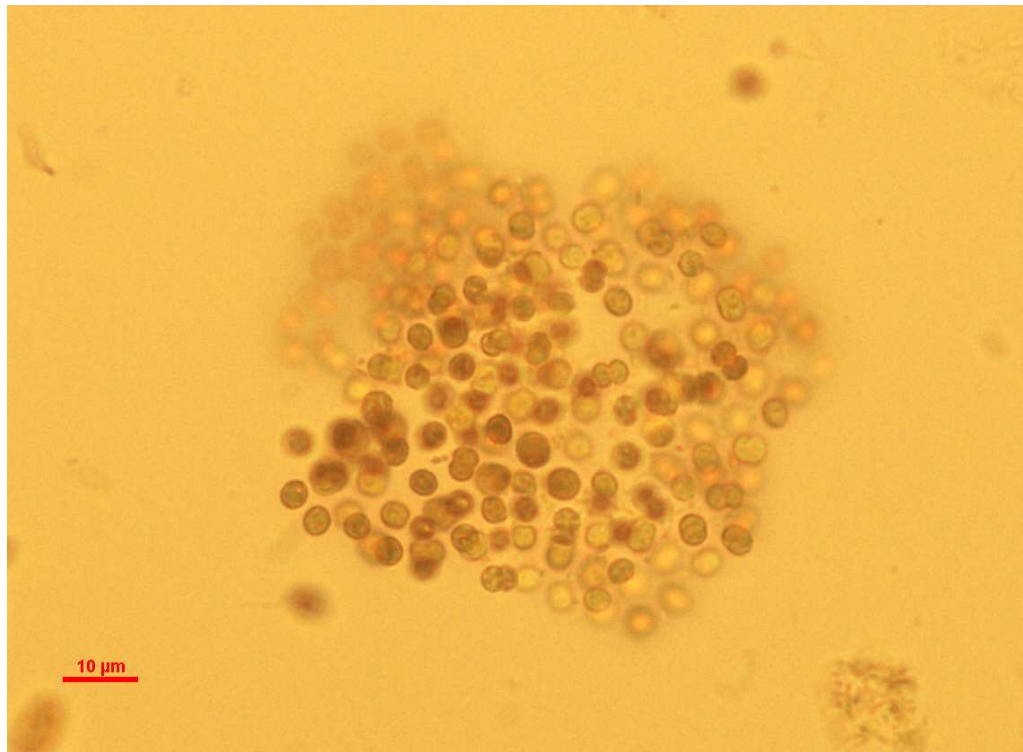


Fig. 9 *Microcystis* sp. 400X (scale bar = 10 $\mu$ m)

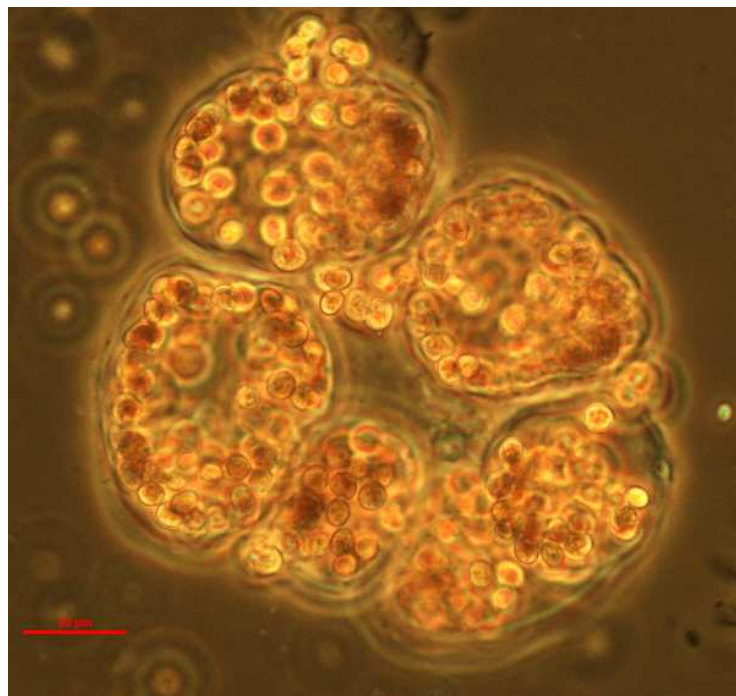


Fig. 10 *Microcystis wesenbergii* 400X (scale bar = 20 $\mu$ m)



Fig. 11 *Anabaena planctonica* 400X (scale bar = 10μm)

## Appendix B

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### Microcystin Lab Reports





**Microcystin Analysis Report****Project: ESS Group**  
(Warwick Pond-WAP1)Sample Identification

Warwick Pond (WAP1)

Sample Collection Date

110914

**Toxin** – Microcystin (MC)

**Sample Prep** – The sample was ultrasonicated to lyse cells and release toxins. A duplicate sample was spiked (lab fortified matrix, LFM) with 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 94%.

**Summary of Results**

<u>Sample</u>	<u>MC levels</u> (µg/L)
Warwick Pond	≈ 0.3

ND = not detected above the LOD/LOQ

LOD/LOQ = 0.15 µg/L

Submitted by:



Mark T. Aubel, Ph.D.

Date:

10/26/11

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**ESS Group****MICROCYSTIN RESULTS**

**Tested on:** 10/25/2011  
**Method:** Enzyme-Linked ImmunoSorbent Assay (ELISA)  
**Analyte:** Microcystins  
**Analyzed by:** 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)
Warwick Pond (WAP1)	1x	none	0.29	1	94	102	0.29	0.3
9/14/11	1x	none	0.25	1	94	102	0.25	

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.  
 10/25/2011

Date:

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**Microcystin Analysis Report**  
**Project: ESS Group**Sample IdentificationSample Collection Date

RWP1	110921
RWP2	110921
SPP1	110921
SMP1	110921
MAP1	110921

**Toxin** – Microcystin (MC)

**Sample Prep** – The samples were ultrasonicated to lyse cells and release toxins. Several sample dilutions (1:10) was necessary to accommodate calibrated. A duplicate RWP1 sample was spiked (lab fortified matrix, LFM) with 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 106%.

**Summary of Results**

<u>Sample</u>	<u>MC levels</u> (µg/L)
RWP1	≈ 0.5
RWP2	≈ 2.3
SPP1	≈ 6.1
SMP1	≈ 0.6
MAP1	≈ 15.3

LOD/LOQ = 0.15 µg/L

Submitted by:



Mark T. Aubel, Ph.D.

Date:

9/23/11

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ESS Group									
MICROCYSTIN RESULTS									
<b>Tested on:</b>		9/22/2011 & 9/23/2011							
<b>Method:</b>		Enzyme-Linked ImmunoSorbent Assay (ELISA)							
<b>Analyte:</b>		Microcystins							
<b>Analyzed by:</b>		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)	
RWP1 9/21/11	1x	none	0.52	1	106	131	0.52	0.5	
	1x	none	0.56	1	106	131	0.56		
RWP2 9/21/11	1x	1:10	0.19	10	106	-	1.90	2.3	
	1x	1:10	0.27	10	106	-	2.70		
SPP1 9/21/11	1x	1:10	0.57	10	106	-	5.70	6.1	
	1x	1:10	0.65	10	106	-	6.50		
SMP1 9/21/11	1x	none	0.58	1	106	-	0.58	0.6	
	1x	none	0.62	1	106	-	0.62		
MAP1 9/21/11	1x	1:10	1.63	10	106	-	16.30	15.3	
	1x	1:10	1.42	10	106	-	14.20		

LOD/LOQ = 0.15 µg/L  
 LFB = 1.0 µg/L MCLR  
 LFM = 1.0 µg/L MCLR

Submitted by:

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**ESS Group****MICROCYSTIN RESULTS**

**Tested on:** 10/6/11 & 10/7/11  
**Method:** Enzyme-Linked ImmunoSorbent Assay (ELISA)  
**Analyte:** Microcystins  
**Analyzed by:** 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)
Trustem Pond 1 9/21/11	1x	1:2	1.47	2	96	-	2.9	3
	1x	1:2	1.47	2	96	-	2.9	
Trustem Pond 2 9/21/11	1x	none	4.40	1	105	-	4.4	5
	1x	none	4.82	1	105	-	4.8	

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:

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**Microcystin Analysis Report**  
**Project: ESS Group**Sample IdentificationSample Collection DateTrustem Pond 1  
Trustem Pond 2110921  
110921**Toxin** – Microcystin (MC)

**Sample Prep** – The samples were ultrasonicated to lyse cells and release toxins. The Trustem Pond 1 sample was diluted (1:2) to accommodate the calibrated linear range.

**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 recoveries of laboratory fortified blanks (LFB) spiked with 1 µg/L MCLR was 105% (10/6/11) and 96% (10/7/11).

**Summary of Results**

<u>Sample</u>	<u>MC levels</u> (µg/L)
Trustem Pond 1	≈ 3
Trustem Pond 2	≈ 5

LOD/LOQ = 0.15 µg/L

Submitted by:



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10/7/11

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**ESS Group****MICROCYSTIN RESULTS**

**Tested on:** 9/28/11 & 9/29/11  
**Method:** Enzyme-Linked ImmunoSorbent Assay (ELISA)  
**Analyte:** Microcystins  
**Analyzed by:** 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)
Slacks Reservoir 9/26/11	1x	1:100	0.70	100	100	-	70.0	82
	1x	1:100	0.93	100	100	-	93.0	
Spring Lake Reservoir #2 9/23/11	1x	1:50	0.64	50	96	-	32.0	35
	1x	1:50	0.74	50	96	-	37.0	

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:

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**Microcystin Analysis Report**  
**Project: ESS Group**Sample IdentificationSample Collection Date

Slacks Reservoir

110926

Spring Lake Reservoir #2

110923

**Toxin** – Microcystin (MC)

**Sample Prep** – The samples were ultrasonicated to lyse cells and release toxins. Both the Slacks (1:100) and Spring Lake (1:50) samples required dilution to accommodate the calibrated linear range.

**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 recoveries of laboratory fortified blanks (LFB) spiked with 1 µg/L MCLR was 100% (9/28/11) and 96% (9/29/11).

**Summary of Results**

<u>Sample</u>	<u>MC levels</u> (µg/L)
Slacks Reservoir	≈ 82
Spring Lake Reservoir #2	≈ 35

LOD/LOQ = 0.15 µg/L

Submitted by:



Mark T. Aubel, Ph.D.

Date:

9/29/11