# Cyanobacteria Monitoring Program
# 2012 Report

Rhode Island
RIDEM REQ. NO. 1180565/1194117

**PREPARED FOR:**
Rhode Island Department of Environmental Management
Office of Water Resources
Promenade Street
Providence, Rhode Island 02915

**PREPARED BY:**
ESS Group, Inc.
401 Wampanoag Trail, Suite 400
East Providence, Rhode Island 02915

ESS Project No. R298-011

Revised January 30, 2013

**CYANOBACTERIA MONITORING PROGRAM**
**2012 REPORT**
**Rhode Island**
**RIDEM REQ# 1180565/1194117**

*Prepared For:*

**Rhode Island Department of Environmental Management**
Office of Water Resources
235 Promenade Street
Providence, Rhode Island 02908

*Prepared By:*

**ESS Group, Inc.**
401 Wampanoag Trail, Suite 400
East Providence, Rhode Island 02915

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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 2012.

## 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 2012, 19 cyanobacteria samples were collected from 14 water bodies, distributed across the state from Lincoln and Pawtucket to Newport and Charlestown (Table A). Of the 14 water bodies sampled, 11 were sampled as part of the annual screening program, 2 were sampled in response to reports of algae blooms from the public, and 1 was sampled as part of both the screening and response sampling programs.

The water bodies selected for screening level monitoring in the 2012 monitoring year included several that were previously sampled in 2010 or 2011, 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 2012 include Pasquisett Pond, Scott Pond, and Blackamore Pond. 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 2012**

| Sampling Program | Water Body | Location | Long | Lat | WBID | Acres |
|---|---|---|---|---|---|---|
| Screening Level | Barber Pond | South Kingstown | -71.56448 | 41.4992 | RI0008039L-14 | 28.16 |
| | Pasquisett Pond | Charlestown | -71.633 | 41.42423 | RI0008039L-06 | 76.61 |
| | Almy Pond | Newport | -71.3107 | 41.45849 | RI0010047L-01 | 49.85 |
| | Scott Pond | Lincoln | -71.4087 | 41.89542 | RI0001003L-01 | 42.13 |
| | Blackamore Pond | Cranston | -71.44439 | 41.775731 | RI0006018L-06 | 20.44 |
| | Turner Reservoir | East Providence | -71.3432 | 41.8419 | RI0004009L-01B | 85.10 |
| | 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 |
| | Warwick Pond | Warwick | -71.41531 | 41.72472 | RI0007024L-02 | 84.72 |
| | Spring Lake Reservoir #2 (Lower J.L. Curran Reservoir) | Cranston | -71.54574 | 41.74314 | RI0006016L-02 | 18.08 |

| Sampling Program | Water Body | Location | Long | Lat | WBID | Acres |
|---|---|---|---|---|---|---|
| Response Level | Mashapaug Pond | Providence | -71.43553 | 41.79313 | RI0006017L-06 | 76.75 |
| | Melville Pond | Portsmouth | -71.27175 | 41.58399 | RI0007029L-01 | 13.60 |
| Screening and Response Level | Slack Reservoir | Smithfield | -71.55258 | 41.86584 | RI0002007L-03 | 133.61 |

ESS collected each of the screening level samples in mid-August. 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. As in previous years, microcystin samples were automatically 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. In 2012, RIDEM requested that all samples be analyzed for microcystin, regardless of cell count.

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.

## <u>3.0 RESULTS</u>

### <u>3.1 Cyanobacteria</u>

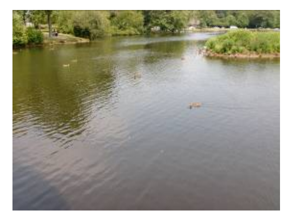
Cell densities in 2012 ranged from 94 cells/mL to more than 5 million cells/mL (Table B). Cell density exceeded 50,000 cells/mL (the microcystin analysis threshold established in the project-specific QAPP) in 12 samples from 10 water bodies.

Potentially toxigenic cyanobacteria species were identified in 15 samples from 11 water bodies (Table B). *Microcystis* spp., *Anabaena* spp., *Aphanizomenon* spp., *Woronichinia naegeliana*, and *Plantothrix suspensa* were the primary dominant or co-dominant species in these samples. However, *Sphaerospermopsis (Anabaena) aphanizomenoides* was also found as a co-dominant in one sample from Almy Pond. Complete cyanobacteria identification and enumeration results may be found in Appendix A.

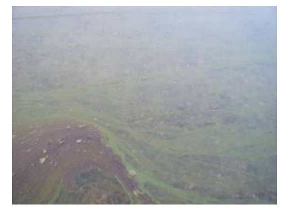
Measured microcystin levels in 2012 ranged from not detected at 0.15 µg/L to 48 µg/L (Table B). The highest microcystin levels were found in samples with high cell densities. However, not all samples with high cell densities demonstrated correspondingly high microcystin concentrations. The higher microcystin levels were measured in samples dominated by certain taxa, including *Microcystis* spp., *Anabaena* spp., *Woronichinia naegeliana*, and *Plantothrix suspensa*.

Complete microcystin laboratory results are presented in Appendix B.

## Table B. Summary of 2012 Cyanobacteria Sampling Program Results

| Water Body | Station ID | Date | Cell Density (cells/mL) | Microcystin Level (µg/L) | Dominant Species | 2012 Photograph | 2011 Photograph* |
|---|---|---|---|---|---|---|---|
| Almy Pond | ALP1 | 8/16/2012 | 5,009,668 | 1.5 | • *Aphanocapsa planctonica (PTOX)*<br>• *Aphanizomenon gracile (PTOX)*<br>• *Sphaerospermopsis aphanizomenoides (PTOX)* |  | No photo available |
| Mashapaug Pond | MAP1/ MASH P | 8/8/2012 | 311,293 | 7.0 | • *Microcystis botrys (PTOX)*<br>• *M. ichthyoblabe (PTOX)*<br>• *M. wesenbergii (PTOX)* | No photo available |  |
| J.L. Curran Reservoir (Spring Lake Reservoir #2) | SPR1/UCR1 | 8/16/2012 | 202,213 | 0.2 | • *Aphanizomenon cf. ovalisporum (PTOX)* |  |  |
| Slater Memorial Park Pond | SMP1 | 8/17/2012 | 4,783 | 0.27 | • *Unidentified cyanophyte unicells*<br>• *Microcystis spp.(PTOX)* |  |  |
| | SMP2 (field duplicate station) | 8/17/2012 | 1,530 | 0.29 | • *Unidentified cyanophyte unicells* | | |

## Table B. Summary of 2012 Cyanobacteria Sampling Program Results

| Water Body | Station ID | Date | Cell Density (cells/mL) | Microcystin Level (µg/L) | Dominant Species | 2012 Photograph | 2011 Photograph* |
|---|---|---|---|---|---|---|---|
| Slack Reservoir | SLR1 | 8/17/2012 | 13,649 | 0.16 | • *Woronichinia naegeliana (PTOX)* |  | |
| | SLR1/SLK2 | 9/24/2012 | 902,080 | 48 | • *Woronichinia naegeliana (PTOX)*<br>• *Anabaena cicinalis (PTOX)* |  |  |
| | SLK3 | 9/24/2012 | 50,376 | 3.7 | • *Woronichinia naegeliana (PTOX)*<br>• *Aphanocapsa incerta* | No photo available | |
| Roger Williams Park Ponds | RWP1 | 8/16/2012 | 315,581 | *0.15*** | • *Aphanizomenon* cf. *flos-aquae (PTOX)*<br>• *Cuspidothrix issatscenkoi (PTOX)* |  |  |
| | RWP2 | 8/16/2012 | 252,864 | *0.15*** | • *Cuspidothrix issatscenkoi (PTOX)* |  |  |

## Table B. Summary of 2012 Cyanobacteria Sampling Program Results

| Water Body | Station ID | Date | Cell Density (cells/mL) | Microcystin Level (µg/L) | Dominant Species | 2012 Photograph | 2011 Photograph* |
|---|---|---|---|---|---|---|---|
| Spectacle Pond | SPP1 | 8/16/2012 | 32,417 | *0.15*** | • *Anabaena planctonica (PTOX)* |  |  |
| Warwick Pond | WAP1 | 8/17/2012 | 5,230 | *0.15*** | • *Cyanogrannis ferruginea* |  |  |
| Scott Pond | SCP1 | 8/17/2012 | 455,079 | 0.67 | • *Pseudanabaena sp.*<br>• *Aphanizomenon spp. (PTOX)* |  | No photo available |
| Barber Pond | BAP1 | 8/16/2012 | 2,241,352 | 4.8 | • *Planktothrix suspensa (PTOX)* |  |  |
| | BAP2 | 8/16/2012 | 3,657 | 0.4 | • *Planktothrix suspensa (PTOX)*<br>• *Woronichinia naegeliana (PTOX)* | | |

## Table B. Summary of 2012 Cyanobacteria Sampling Program Results

| Water Body | Station ID | Date | Cell Density (cells/mL) | Microcystin Level (µg/L) | Dominant Species | 2012 Photograph | 2011 Photograph* |
|---|---|---|---|---|---|---|---|
| Blackamore Pond | BMP1 | 8/16/2012 | 387,060 | *0.15*** | • *Aphanizomenon spp.(PTOX)*<br>• *Cyanogranis ferruginea* |  | No photo available |
| Pasquiset Pond | PAP1 | 8/17/2012 | 560,111 | 5.3 | • *Microcystis* cf. *aeruginosa (PTOX)* |  | No photo available |
| Melville Pond | MEP1 | 7/23/2012 | 183,422 | *0.15*** | • *Anabaena planctonica (PTOX)*<br>• *Woronichinia naegeliana (PTOX)* | No photo available | No photo available |
| Turner Reservoir | TUR1 | 8/17/12 | 94 | *0.15*** | • *Unidentified cyanophyte unicells* |  | No photo available |

NS = not sampled; PTOX = potentially toxigenic species
*All photos by ESS, except Slack Reservoir photo at station SLK2, taken by RIDEM
** Reported value is the quantitation limit. Microcystins were not detected at this level.

## 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 6.1 mg/L at J.L. Curran Reservoir to 13.1 mg/L at Warwick Pond (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 Turner Reservoir (404.6 µS/cm) (Table C). Turner Reservoir is an impoundment on the highly urbanized Ten Mile River. The lowest specific conductance (74.9 µS/cm) was measured at Slater Memorial Park Pond.

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 to 1.50 meter (i.e., pond bottom), depending on the pond bottom substrate and slope. Additional Secchi depth measurements were collected at selected ponds, particularly where water clarity appeared to vary significantly over the pond area. Water clarity was lowest at Almy Pond, where the pond-wide bloom reduced Secchi depth to approximately 0.25 meters (Table C). Water clarity was also less than 1.00 meter in Blackamore Pond, the Roger Williams Park Ponds, Pasquiset Pond, and Scott Pond.

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

| Waterbody | Station ID | Date | Time | Water Temp (°C) | DO (mg/L) | DO (%) | Spec. Cond (µS/cm) | Salinity (ppt) | Secchi Depth (m) |
|---|---|---|---|---|---|---|---|---|---|
| Almy Pond | ALP1 | 8/16/2012 | 0945 | 25.3 | 6.9 | 82.5 | 274.5 | 0.1 | 0.25 |
| Barber Pond | BAP1 | 8/16/2012 | 1100 | 26.4 | 6.5 | 79.8 | 76.3 | 0.0 | 1.00* |
| Barber Pond | BAP2 | 8/16/2012 | 1115 | 26.8 | 6.4 | 81.3 | 76.8 | 0.0 | 3.00* |
| J.L. Curran Reservoir | SPR1**/ UCR1 | 8/16/2012 | 1230 | 28.6 | 6.1 | 78.9 | 140.4 | 0.1 | 1.00* |
| Spectacle Pond | SPP1 | 8/16/2012 | 1345 | 28.8 | 7.4 | 92.6 | 284.0 | 0.2 | 1.00* |
| Blackamore Pond | BMP1 | 8/16/2012 | 1430 | 29.2 | 9.3 | 119.7 | 358.7 | 0.3 | 0.9 |
| Roger Williams Park Ponds | RWP1 | 8/16/2012 | 1450 | 29.4 | 10.8 | 143.7 | 346.8 | 0.3 | 1.00* |
| Roger Williams Park Ponds | RWP2 | 8/16/2012 | 1505 | 29.2 | 7.0. | 93.2 | 265.4 | 0.2 | 0.75 |
| Pasquiset Pond | PAP1 | 8/17/2012 | 0900 | 26.6 | 6.8 | 84.9 | 102.6 | 0.1 | 0.75 |
| Warwick Pond | WAP1 | 8/17/2012 | 1050 | 27.4 | 13.1 | 163.6 | 234.1 | 0.2 | 1.25* |
| Slack Reservoir | SLR1 | 8/17/2012 | 1200 | 27.7 | 6.6 | 83.0 | 174.5 | 0.1 | 1.50* |
| Scott Pond | SCP1 | 8/17/2012 | 1300 | 28.4 | 11.3 | 144.2 | 381.3 | 0.3 | 0.9 |
| Slater Memorial Park Pond | SMP1 | 8/17/2012 | 1400 | 27.2 | 7.7 | 97.5 | 74.9 | 0.0 | 0.75* |
| Turner Reservoir | TUR1 | 8/17/2012 | 1423 | 28.8 | 12.6 | 170.9 | 404.6 | 0.3 | 1.0* |

*On bottom
**2011 ID Code

## 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 Slater Memorial Park Pond (SMP1/SMP2). 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 12 water bodies statewide and collected 15 cyanobacteria samples as part of the 2012 screening level monitoring program. An additional four samples were collected by RIDEM in response to active blooms, including two at Slack Reservoir, one at Mashapaug Pond, and one at Melville Pond. In the following discussion, these data are compared to and pooled with observations from 2011 to examine potential relationships.

### A Brief Comparison of Results from 2011 and 2012

As in 2011, the cyanobacteria monitoring program successfully detected and documented the intensity of multiple active blooms across the state. Cyanobacteria densities in 2012 exceeded the 50,000 cells/mL threshold established in the project-specific QAPP in 12 samples from 10 water bodies. Measured microcystin levels exceeded the World Health Organization (2003) drinking water guideline of 1 µg/L in 6 samples from 5 water bodies and the recreational contact guideline of 20 µg/L in a single sample collected at Slack Reservoir on September 24. Rhode Island health advisory guidelines for cell count (70,000 cells/mL) were exceeded in 11 samples from 10 water bodies although guidelines for microcystin (14 µg/L) were only exceeded in a single sample collected at Slack Reservoir on September 24. The September 24, 2012 Slack Reservoir sample coincided with a cell density of approximately 900,000 cells/mL. This represents just the third instance since the monitoring program began where microcystin concentrations exceeded the WHO recreational contact guidelines and fourth where they exceeded the Rhode Island health advisory guidelines.

In 2011, cell counts exceeded Rhode Island health advisory guidelines in microcystin levels in 8 samples from 7 water bodies. However, the Rhode Island health advisory guidelines for microcystin were only observed in two samples, both of which contained cell densities greater than 3 million cells/mL (Slack Reservoir and J.L. Curran Reservoir [Spring Lake Reservoir #2]).

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).

### Examining the Pooled 2011 and 2012 Data

The pooled 2011 and 2012 data indicate a positive relationship between cell density and microcystin concentration (Figure 1). However, the variance in microcystin concentration clearly increases with increasing cell density.

When dominance by microcystin-producing taxa is factored into the relationship, it is apparent that blooms dominated by these taxa tend to produce microcystin in higher concentrations than blooms dominated by other cyanobacteria taxa (Figure 1). The two exceptions in the dataset include an early-season *Anabaena planctonica* sample from Melville Pond and the 2011 *Woronichinia naegeliana* bloom at Slater Memorial Park Pond. The reason for the lower-than-expected microcystin concentrations observed in these two blooms is uncertain.

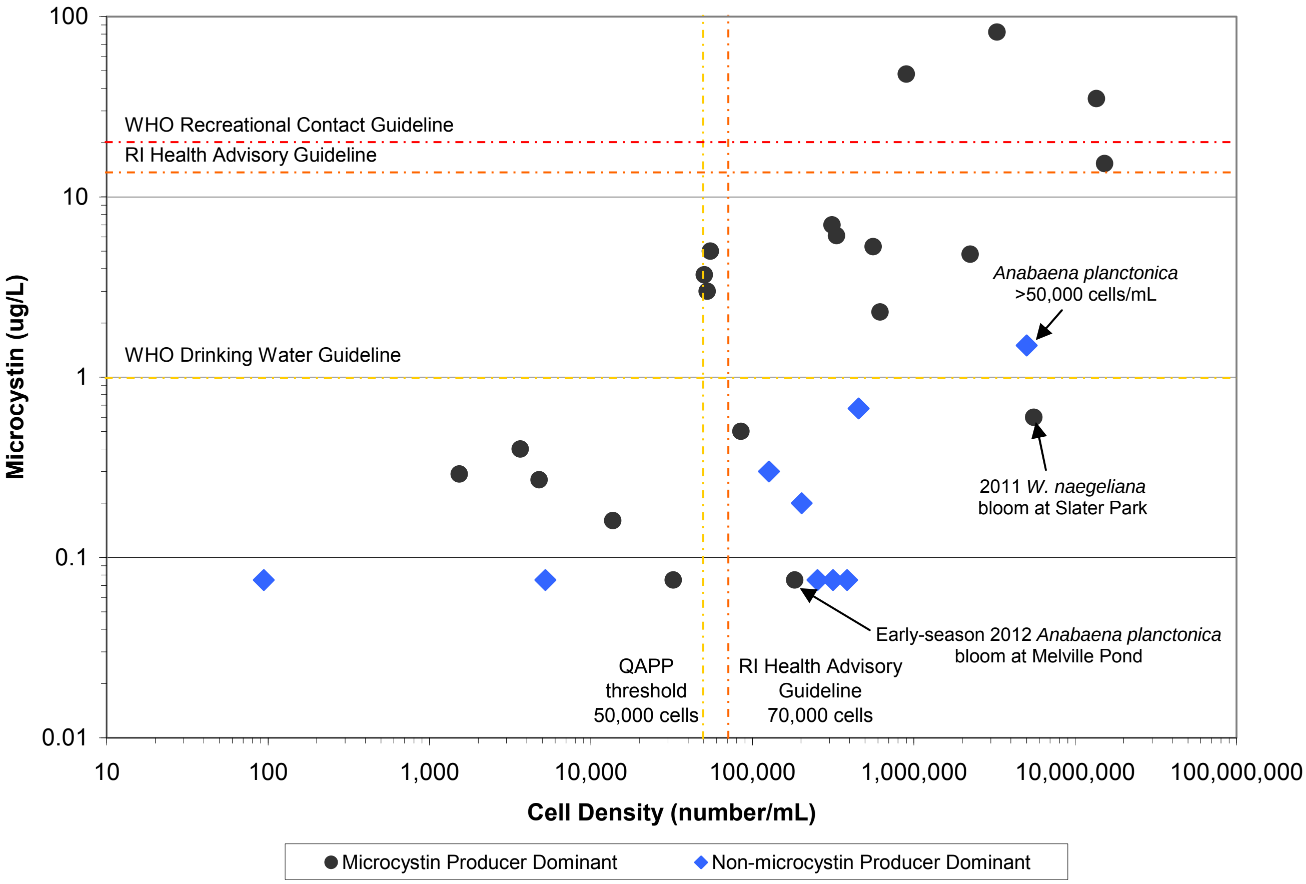



**Figure 1. Cyanobacteria Cell Density and Microcystin Levels (Pooled 2011 and 2012 Data)**

Among samples with cell densities above the Rhode Island health advisory guideline only one sample not dominated by microcystin-producing taxa exceeded the WHO drinking water guidelines and none exceeded the Rhode Island health advisory criteria for microcystin (Figure 1). The single bloom exceeding WHO drinking water guidelines was dominated by *Aphanocapsa planctonica* (not a suspected microcystin producer) but *Anabaena planctonica* (a potential microcystin producer) was also present at a density exceeding 50,000 cells/mL. This suggests that focusing future cell density counts on potential microcystin-producing taxa (rather than enumerating all cyanobacteria or all potentially toxigenic taxa) may be sufficient for identifying blooms likely to be producing elevated concentrations of microcystin.

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. RIDEM's decision to analyze microcystin in all samples (even those with cell densities below 50,000 cells/mL) collected in 2012 provided a glimpse of the range of microcystin concentrations that might be expected at the lower end of cyanobacteria cell densities. Cyanobacteria cell densities below 50,000 cells/mL have not yet produced microcystin concentrations above WHO drinking water guidelines and cell densities below 70,000 cells/mL have not yet resulted in concentrations above Rhode Island health advisory guidelines. However, with additional data, particularly data collected over the duration of a bloom (i.e., inception to senescence), it may be possible to establish the lower cell density threshold required to generate excessive microcystins with greater confidence.

**Other Observations**

The 2012 cyanobacteria monitoring program served as a reminder that blooms and production of associated toxins are dynamic with respect to time and space. Due to the early blooms observed at Barber Pond and Slack Reservoir, these water bodies were more extensively sampled and provide additional information on temporal and spatial variability.

Barber Pond was sampled by ESS during an active cyanobacteria bloom. This bloom was visibly characterized by a bright bluish surface scum at the public access location. However, cell density was more diffuse in offshore waters, where small clumps of cyanobacteria were observed in suspension below the water surface. Both cell density and microcystin concentration were much higher in the sample collected from surface scum at the public access location.

Slack Reservoir was sampled by ESS at a time when no direct evidence of a cyanobacteria bloom was observed despite the presence of several recently killed yellow and white perch washed up on the beach. The sample results from that day confirmed the unremarkable cyanobacteria cell density and microcystin concentration at the time. When Slack Reservoir was resampled by RIDEM just over a month later, a bloom was visible at the surface and both cyanobacteria cell density and microcystin concentration were substantially higher than before. However, the difference in both cell density and microcystin concentration at Slack Reservoir varied by an order of magnitude between the two locations sampled on the same date.

One variable that did not differ appreciably over space or time within each of these ponds was the dominant cyanobacteria species. *Woronichinia naegeliana* was the dominant species at Slack Reservoir in the 2011 sample and each sample collected over the span of more than one month in 2012. *Planktothrix suspensa* was the dominant species in each of the two samples from Barber Pond.

Investigating the factors contributing to the observed cyanobacteria blooms remains beyond the scope of this study. Phosphorus certainly plays a role in the development of cyanobacteria blooms and total maximum daily loads (TMDL) for phosphorus have been prepared for several of the ponds sampled in 2012, including Almy Pond, Mashapaug Pond, Spectacle Pond, the Roger Williams Park Ponds, Warwick Pond, and Barber Pond. Of these, Warwick Pond was the only pond not observed to host a cyanobacteria bloom during screening level sampling in 2012.

Phosphorus may not be the only nutrient responsible for creating conditions suitable for production of microcystin. While some cyanobacteria taxa (including microcystin producers like *Anabaena* spp.) are able to fix nitrogen from the atmosphere, allowing them to take advantage of excess phosphorus in the water column, taxa such as *Microcystis* spp. and *Woronichinia naegeliana* are not and may be more sensitive to available nitrogen. Additionally, there is growing evidence that nitrogen enrichment may actually enhance microcystin production by these species (Downing et al 2005, Van de Waal et al. 2010).

These observations underscore the importance of continuing to pursue watershed and lake management strategies that promote broad spectrum pollutant reductions, where possible.

## 5.0 REFERENCES

Chen, H., J.M. Burke, T. Mosindy, P.M. Fedorak, and E.E. Prepas. 2009. Cyanobacteria and microcystin-LR in a complex lake system representing a range in trophic status: Lake of the Woods, Ontario, Canada. Journal of Plankton Research, 31(9): 993-1008.

Downing, T.G., C. Meyer, M.M. Gehringer, and M. van de Venter. 2005. Microystin content of *Microcystis aeruginosa* is modulated by nitrogen uptake rate relative to growth rate or carbon fixation rate. Environmental Toxicology, 20(3): 257-262.

ESS Group, Inc. (ESS). 2011. Quality Assurance Project Plan. Rhode Island Department of Environmental Management Cyanobacteria Monitoring Program. Revised October 13, 2011.

ESS Group, Inc. (ESS). 2012. Rhode Island Cyanobacteria Monitoring Program 2011 Report.

Massachusetts Department of Public Health. Date unspecified. MDPH Guidelines for Cyanobacteria in Freshwater Recreational Water Bodies in Massachusetts.

Van de Waal, D.B., G. Ferreruela, L. Tonk, E. Van Donk, J. Huisman, P.M. Visser, and H.C.P. Matthijs. 2010. Pulsed nitrogen supply induces dynamic changes in the amino acid composition and microcystin production of the harmful cyanobacterium *Planktothrix agardhii*. FEMS Microbiology Ecology, 74(2): 430-438.

Willame, R., T. Jurczak, J. Iffly, T. Kull, J. Meriluoto, and L. Hoffmann. 2005. Distribution of hepatotoxic cyanobacterial blooms in Belgium and Luxembourg. Hydrobiologia, 551: 99-117.

World Health Organization. 2003. Guidelines for Safe Recreational Water Environments, Volume 1: Coastal and Fresh Waters.

# Appendix A

## Cyanobacteria Identification and Enumeration Lab Reports

# ESS Group PTOX Cyanobacteria ID and Enumeration Report

Prepared: July 27, 2012
Prepared By: GreenWater Laboratories

Samples: 1
1. Melville Pond (collected on 7/23/12)

<u>Sample 1: Melville Pond</u>
Total potentially toxigenic (PTOX) cyanobacteria cell numbers in the Melville Pond sample collected on 7/23/12 were 183,422 cells/mL. Potentially toxigenic species observed in the sample included *Anabaena planctonica* (98,959 cells/mL; Fig. 1), *Woronichinia naegeliana* (83,951 cells/mL; Fig. 2) and *Microcystis wesenbergii* (512 cells/mL; Fig. 3). Many loose cells and cell pairs of *W. naegeliana* were present.


Fig. 1 *Anabaena planctonica* 400X (scale bar = 10µm)


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


Fig. 3 *Microcysits wesenbergii* 400X (scale bar = 10µm)

# ESS Group PTOX Cyanobacteria ID and Enumeration Report

Prepared: August 13, 2012
Prepared By: GreenWater Laboratories

Samples: 1
1. Mashapaug Pond (collected on 8/08/12)

Sample 1: Mashapaug Pond
Total potentially toxigenic (PTOX) cyanobacteria cell numbers in the Mashapaug Pond sample collected on 8/8/12 were 311,293 cells/mL. Potentially toxigenic species observed in the sample included *Microcystis botrys* (123,149 cells/mL; Fig. 1), *Microcystis ichthyoblabe* (86,864 cells/mL; Fig. 2), *Microcystis wesenbergii* (47,516 cells/mL; Fig. 3) *Anabaena planctonica* (25,918 cells/mL; Fig. 4), *Microcystis* sp. (15,080 cells/mL; Fig. 5), *Aphanizomenon* cf. *flos-aquae* (6,597 cells/mL), *Woronichinia naegeliana* (3,616 cells/mL) and *Anabaena crassa* (2,553 cells/mL).


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

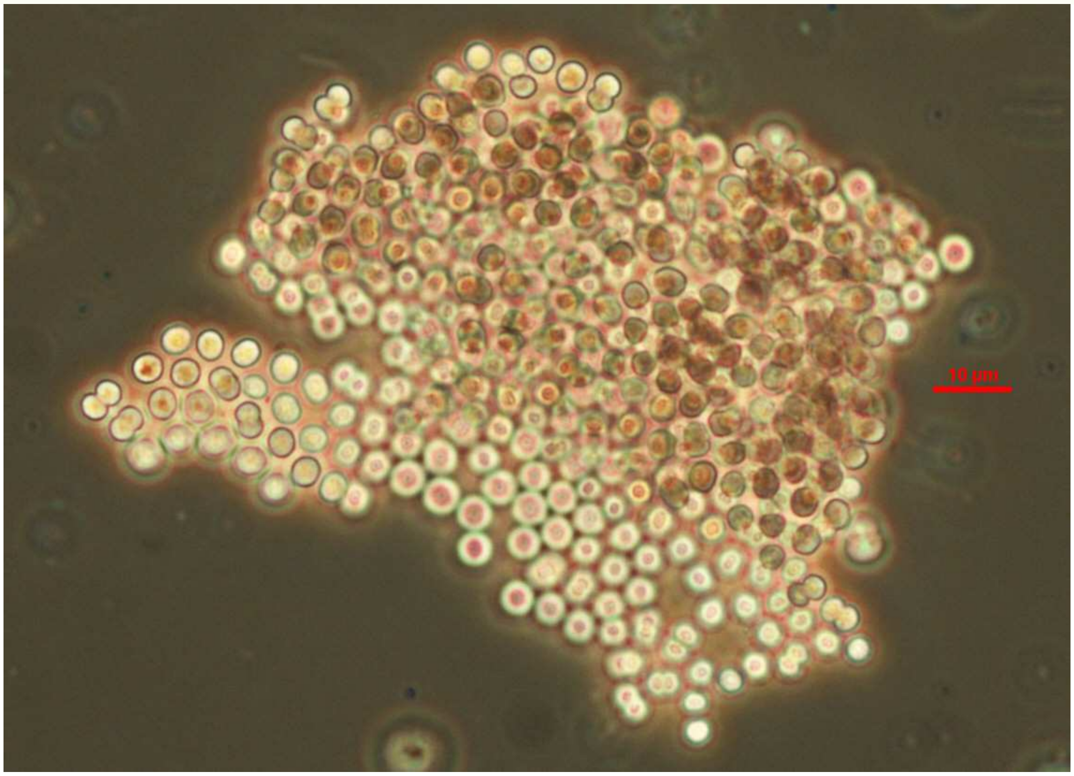


Fig. 2 *Microcystis ichthyoblabe* 400X (scale bar = 10µm)

Fig. 3 *Microcystis wesenbergii* 400X (scale bar = 20µm)


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

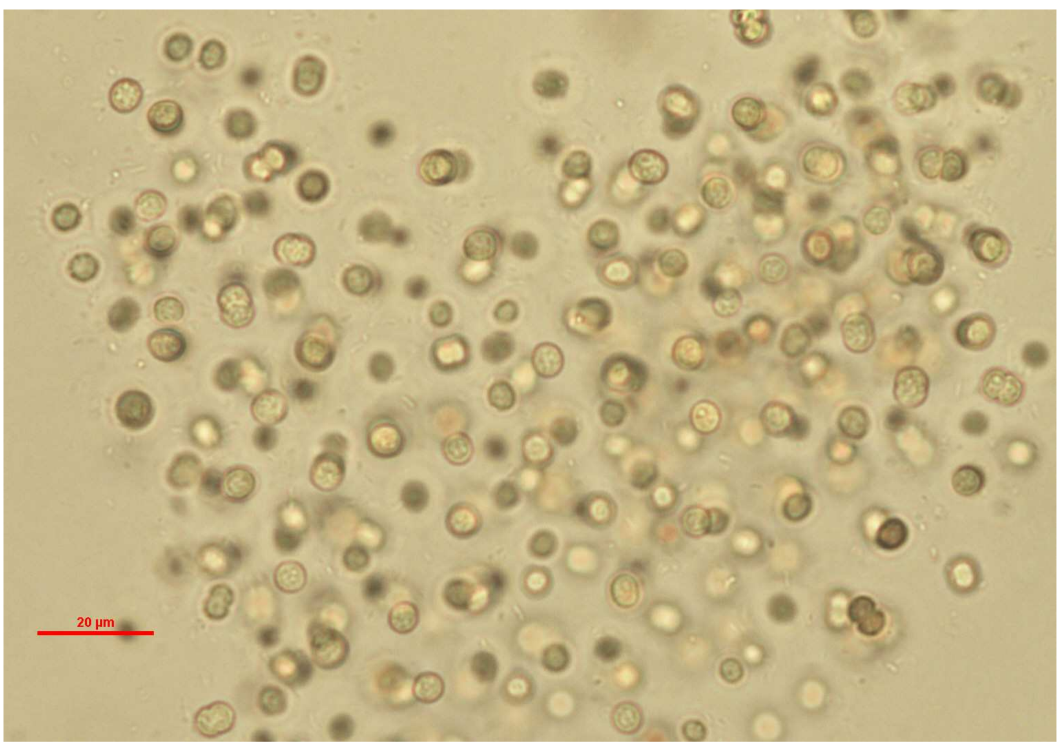



Fig. 5 *Microcystis* sp. 400X (scale bar = 20µm)

# ESS Group Cyanobacteria ID and Enumeration Report

Prepared: August 22, 2012
Prepared By: GreenWater Laboratories

Samples: 1 (collected on 8/16/12)
1. Almy Pond

Sample 1: Almy Pond
Total cyanobacteria cell numbers in the Almy Pond sample collected on 8/16/12 were 5,009,668 cells/mL. The dominant species in the sample was *Aphanocapsa planctonica* (3,387,396 cells/mL; Fig. 1). Many loose unicells and cell pairs of *A. planctonica* were present (Fig. 2).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 498,346 cells/mL (9.9% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Aphanizomenon gracile/Sphaerospermopsis aphanizomenoides* (408,404 cells/mL; Figs. 3-4), *Anabaena* sp. (84,822 cells/mL; Fig. 5), *Anabaena* sp. (3,120 cells/mL), *Snowella lacustris* (940 cells/mL), *Planktothrix agardhii* (660 cells/mL) and *Microcystis wesenbergii* (400 cells/mL). Filaments of *Aphanizomenon gracile* and *Sphaerospermopsis aphanizomenoides* (formerly *Aphanizomenon aphanizomenoides*) lacking akinetes could not be reliably distinguished and so these two species were counted together.

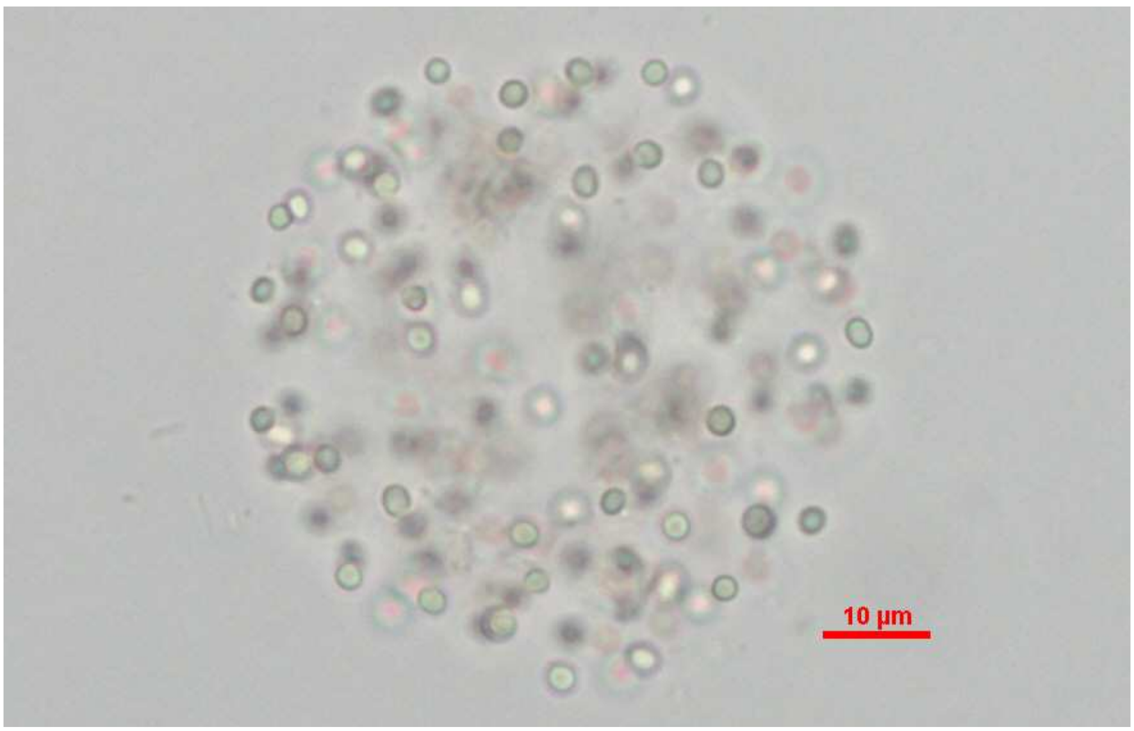



Fig. 1 *Aphanocapsa planctonica* 400X (scale bar = 10µm)

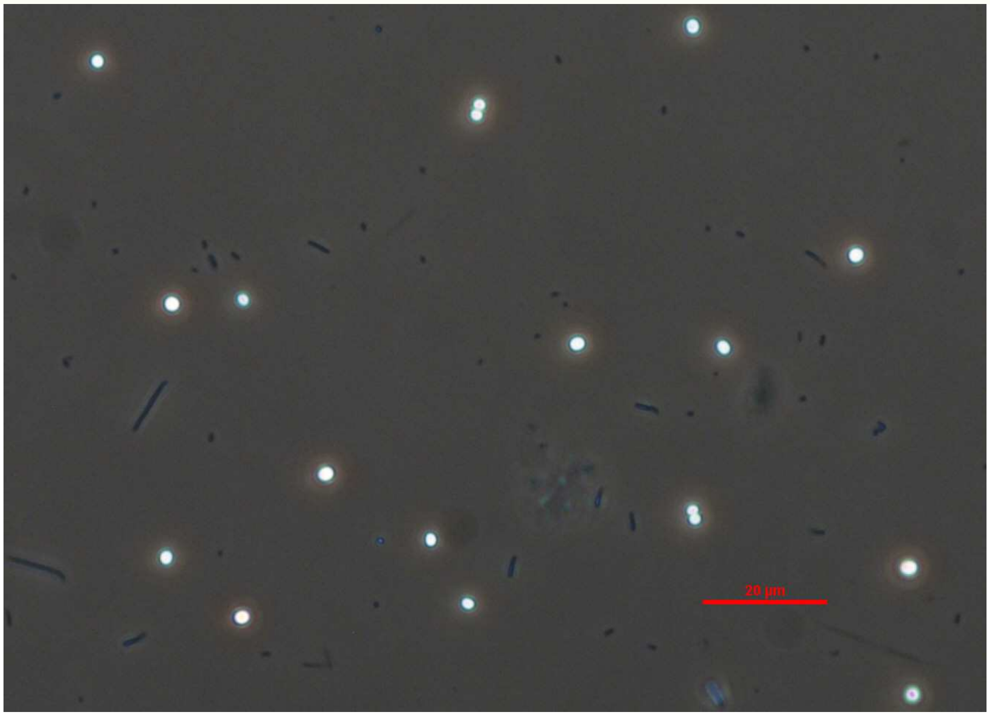



Fig. 2 *Aphanocapsa planctonica* unicells and cell pairs 400X (scale bar = 20µm)

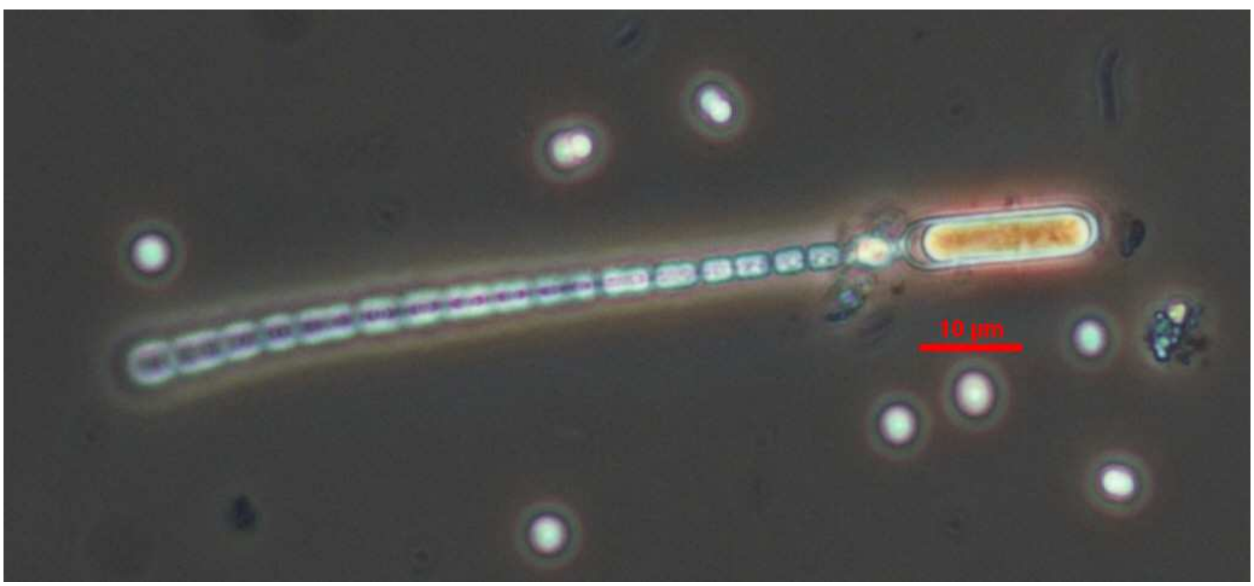



Fig. 3 *Aphanizomenon gracile* 400X (scale bar = 10µm)


Fig. 4 *Sphaerospermopsis aphanizomenoides* 400X (scale bar = 10µm)


Fig. 5 *Anabaena* sp. 400X (scale bar = 20µm)

# ESS Group Cyanobacteria ID and Enumeration Report

Prepared: August 27, 2012
Prepared By: GreenWater Laboratories

Samples: 2 (collected on 8/16/12)

1. BAP1
2. BAP2

Sample 1: BAP1

Total cyanobacteria cell numbers in the BAP1 sample collected on 8/16/12 were 2,241,352 cells/mL. The dominant species in the sample was *Planktothrix suspensa* (2,186,531 cells/mL; Figs. 1-3). *Planktothrix suspensa* may just be a form of *Planktothrix agardhii* with more elongated aerotopes and less attenuated trichome ends.

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 2,224,073 cells/mL (99.2% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Planktothrix suspensa* (2,186,531 cells/mL), *Woronichinia naegeliana* (27,762 cells/mL), *Microcystis botrys* (4,880 cells/mL), *Anabaena* cf. *lemmermannii* (2,945 cells/mL), *Microcystis* sp. (1,850 cells/mL), *Aphanizomenon* sp. (60 cells/mL) and *Anabaena* sp. (45 cells/mL).

Sample 2: BAP2

Total cyanobacteria cell numbers in the BAP2 sample collected on 8/16/12 were 3,657 cells/mL. The dominant species in the sample were *Planktothrix suspensa* (1,524 cells/mL) and *Woronichinia naegeliana* (1,115 cells/mL; Fig. 4).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 3,201 cells/mL (87.5% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Planktothrix suspensa* (1,524 cells/mL), *Woronichinia naegeliana* (1,115 cells/mL), *Microcystis* sp. (466 cells/mL), *Microcystis botrys* (95 cells/mL) and *Anabaena* cf. *lemmermannii* (1 cell/mL).


205 Zeagler Drive, Suite 302 • Palatka, FL 32177
info@greenwaterlab.com • www.greenwaterlab.com
386.328.0882,voice • 386.328.9646,fax
cyanomail@cyanolab.com • www.cyanolab.com

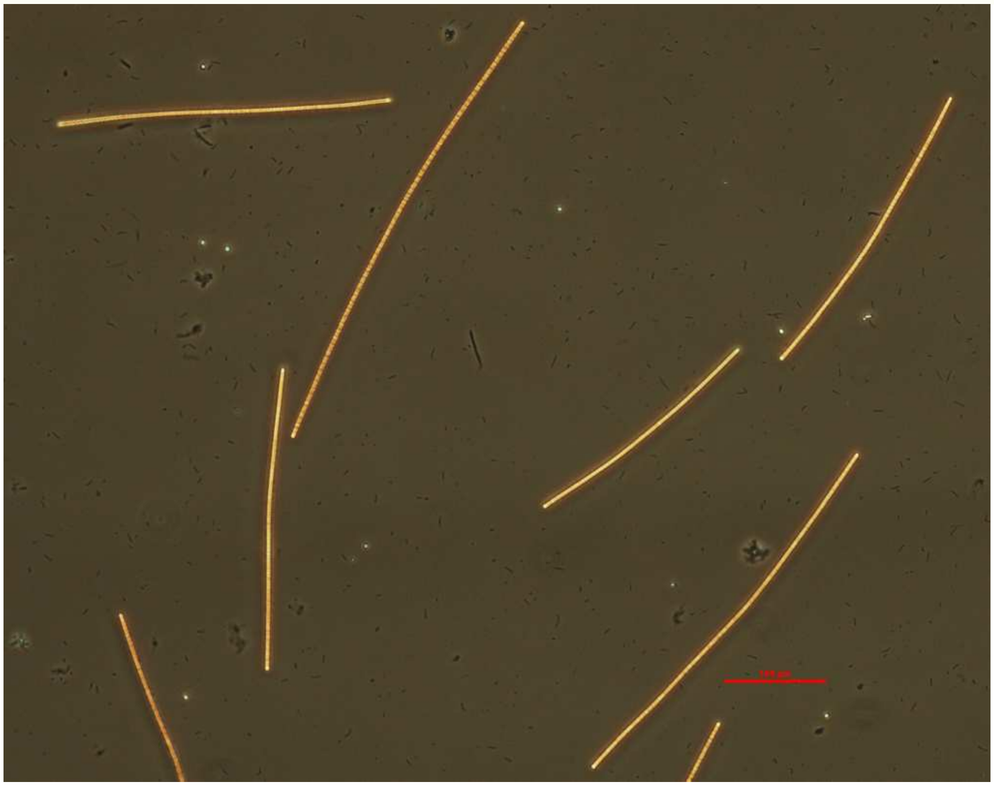


Fig. 1 *Planktothrix suspensa* 100X (scale bar = 100µm)


Fig. 2 *Planktothrix suspensa* 400X (scale bar = 20µm)

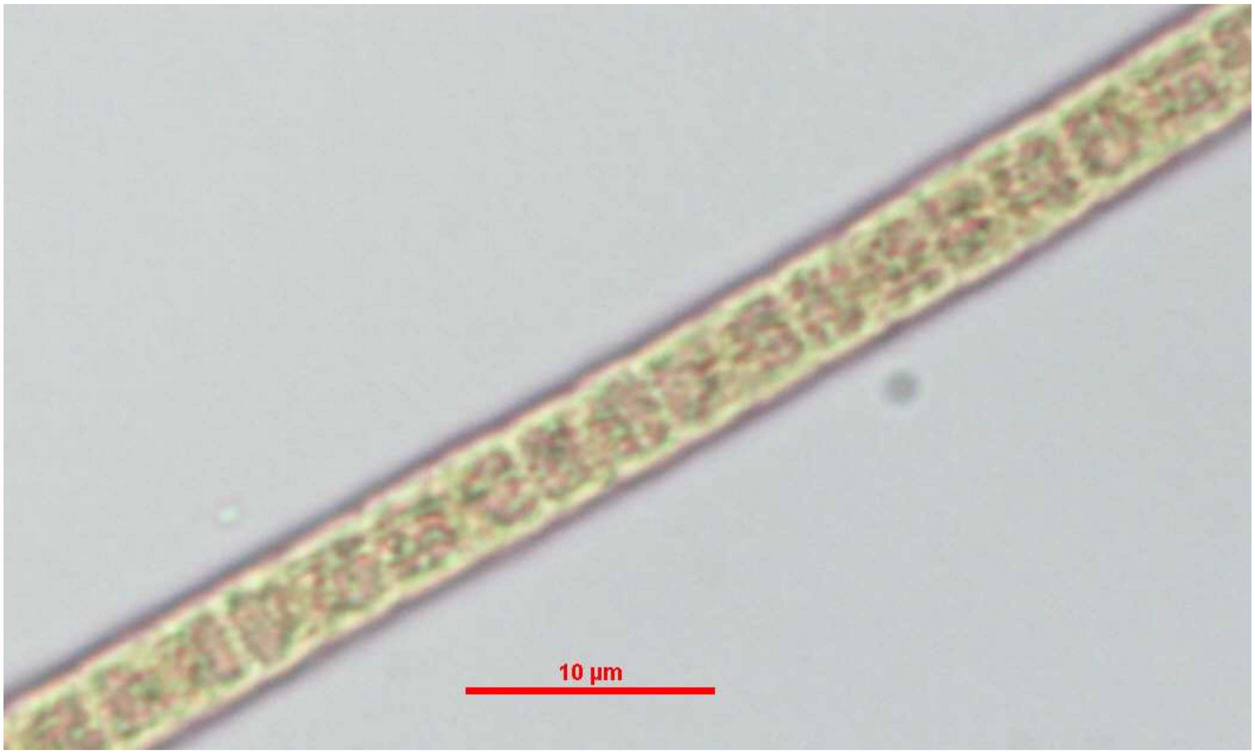



Fig. 3 *Planktothrix suspensa* 1000X (scale bar = 10µm)


Fig. 4 *Woronichinia naegeliana* 400X (scale bar = 10µm) Arrow pointed at mucilaginous stalks

# ESS Group Cyanobacteria ID and Enumeration Report

Prepared: September 3, 2012
Prepared By: GreenWater Laboratories

Samples: 3 (collected on 8/16/12)

1. RWP1
2. RWP2
3. UCR1

Sample 1: RWP1

Total cyanobacteria cell numbers in the RWP1 sample collected on 8/16/12 were 315,581 cells/mL. The dominant species in the sample were *Aphanizomenon* cf. *flos-aquae* (144,512 cells/mL; Fig. 1) and *Cuspidothrix issatschenkoi* (113,096 cells/mL; Fig. 2).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 313,981 cells/mL (99.5% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Aphanizomenon* cf. *flos-aquae* (144,512 cells/mL), *Cuspidothrix issatschenkoi* (113,096 cells/mL), *Cylindrospermopsis raciborskii* (52,778 cells/mL), *Anabaena smithii* (2,199 cells/mL), *Microcystis* unicells and cell pairs (942 cells/mL), *Microcystis wesenbergii* (306 cells/mL), *Microcystis* sp. (90 cells/mL) and *Microcystis ichthyoblabe* (58 cells/mL). The *Cylindrospermopsis raciborskii* morphotype in the sample (Fig. 3) in the past would have been identified as a species of *Raphidiopsis*, however, recent molecular evidence indicates that most (if not all) *Raphidiopsis* are actually forms of *Cylindrospermopsis* lacking terminal heterocytes and having acute end cells.

Sample 2: RWP2

Total cyanobacteria cell numbers in the RWP2 sample collected on 8/16/12 were 252,864 cells/mL. The dominant species in the sample was *Cuspidothrix issatschenkoi* (195,976 cells/mL).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 249,144 cells/mL (98.5% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Cuspidothrix issatschenkoi* (195,976 cells/mL), *Cylindrospermopsis raciborskii* (27,763 cells/mL), *Aphanizomenon* cf. *flos-aquae* (16,331 cells/mL), *Microcystis wesenbergii* (4,263 cells/mL), *Anabaena smithii* (1,361 cells/mL), *Microcystis* unicells and cell pairs (1,180 cells/mL), *Microcystis* sp. (998 cells/mL), *Microcystis ichthyoblabe* (819 cells/mL) and *Woronichinia naegeliana* (453 cells/mL).

Sample 3: UCR1

Total cyanobacteria cell numbers in the UCR1 sample collected on 8/16/12 were 202,213 cells/mL. The dominant species in the sample was a species of *Aphanizomenon* most closely matching *Aphanizomenon ovalisporum* (140,742 cells/mL; Figs. 4-6). The akinete shape and size fits with *A. ovalisporum*, however, the length and swollen ends of some vegetative cells differs from most descriptions of this species.

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 196,007 cells/mL (96.9% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Aphanizomenon* cf. *ovalisporum* (140,742 cells/mL), *Anabaena planctonica* (22,619 cells/mL), *Aphanizomenon* cf. *flos-aquae* (22,619 cells/mL), *Anabaena*/*Aphanizomenon* sp. (6,911 cells/mL), *Woronichinia naegeliana* (2,670 cells/mL), *Microcystis* sp. (314 cells/mL), *Microcystis* sp. (114 cells/mL) and *Microcystis* sp. (18 cells/mL).


Fig. 1 *Aphanizomenon* cf. *flos-aquae* 400X (scale bar = 10µm)

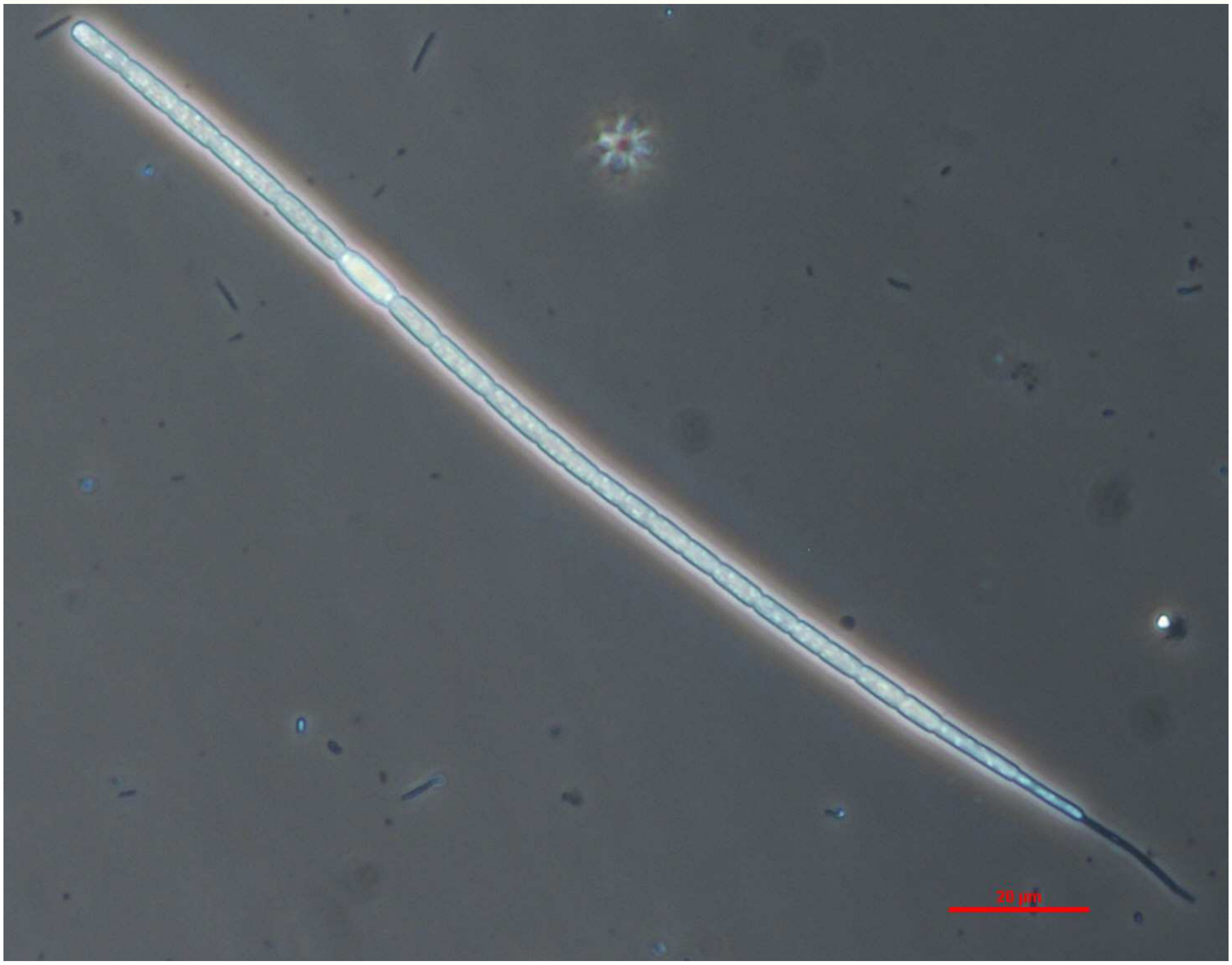



Fig. 2 *Cuspidothrix issatschenkoi* 400X (scale bar = 20µm)

Fig. 3 *Cylindrospermopsis raciborskii* 400X (scale bar = 20µm)

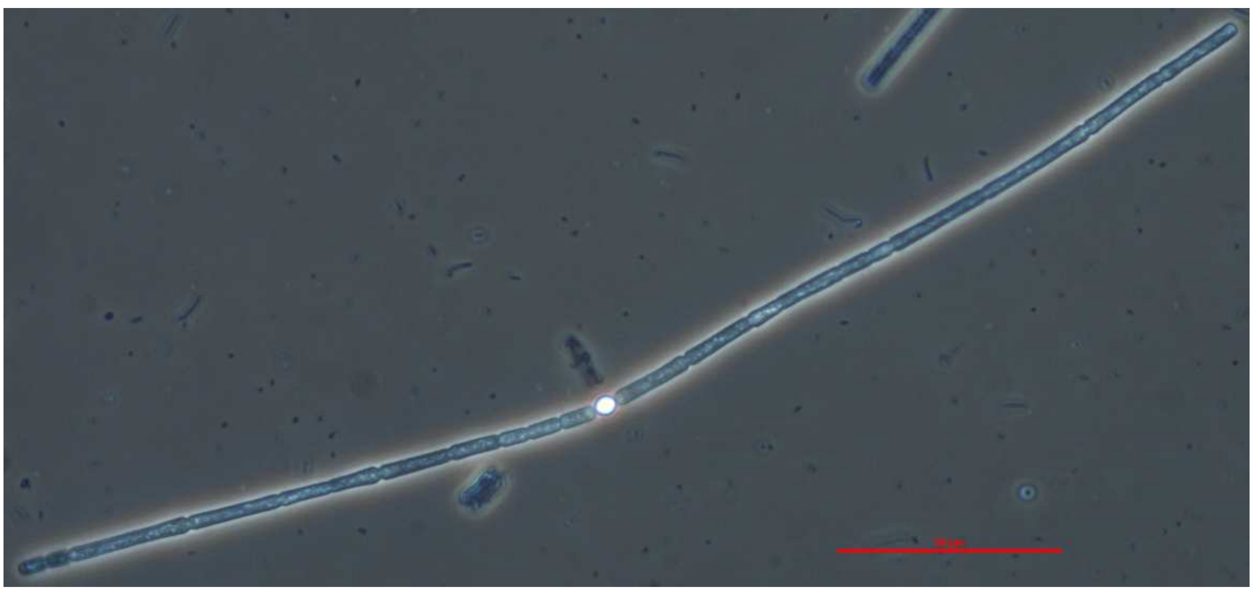


Fig. 4 *Aphanizomenon* cf. *ovalisporum* 400X (scale bar = 50µm)


Fig. 5 *Aphanizomenon* cf. *ovalisporum* 400X (scale bar = 20µm)


Fig. 6 *Aphanizomenon* cf. *ovalisporum* 400X (scale bar = 20µm)

# ESS Group Cyanobacteria ID and Enumeration Report

Prepared: September 4, 2012
Prepared By: GreenWater Laboratories

Samples: 1 (collected on 8/16/12)
1. SPP1

Sample 1: SPP1
Total cyanobacteria cell numbers in the SPP1 sample collected on 8/16/12 were 32,417 cells/mL. The dominant species in the sample was *Anabaena planctonica* (21,488 cells/mL; Fig. 1).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 30,783 cells/mL (95.0% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Anabaena planctonica* (21,488 cells/mL), *Aphanizomenon* sp. (4,273 cells/mL; Fig. 2), *Woronichinia naegeliana* (2,432 cells/mL), *Aphanizomenon* cf. *flos-aquae* (1,696 cells/mL), *Microcystis* sp. (471 cells/mL), *Microcystis* sp. (157 cells/mL), *Microcystis* spp. unicells and cell pairs (157 cells/mL), *Microcystis wesenbergii* (92 cells/mL) and *Anabaena* cf. *crassa* (17 cells/mL).


Fig. 1 *Anabaena planctonica* 400X (scale bar = 10µm)

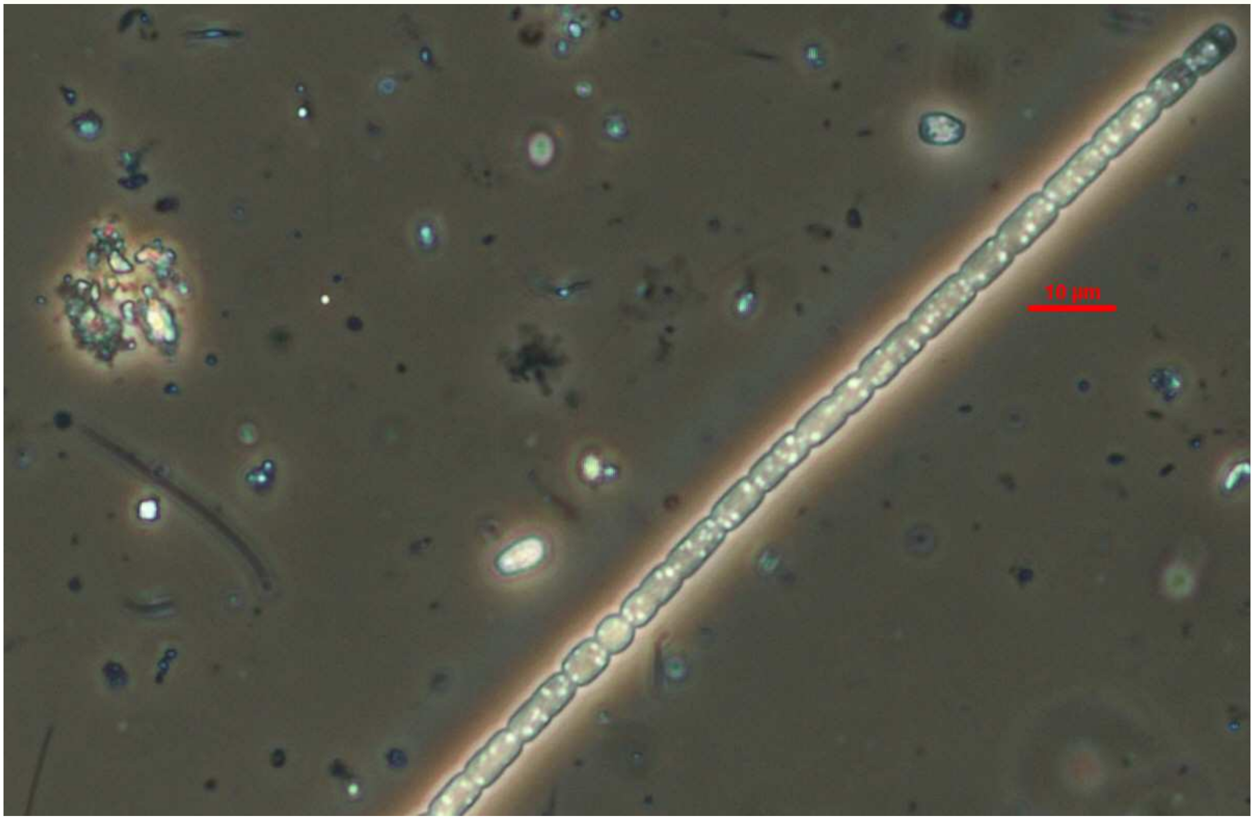



Fig. 2 *Aphanizomenon* sp. 400X (scale bar = 10µm)

# ESS Group Cyanobacteria ID and Enumeration Report

Prepared: September 13, 2012
Prepared By: GreenWater Laboratories

Samples: 6

1. BMP1 (collected on 8/16/12)
2. SCP1 (collected on 8/17/12)
3. SMP1 (collected on 8/17/12)
4. SMP2 (collected on 8/17/12)
5. TUR1 (collected on 8/17/12)
6. WAP1 (collected on 8/17/12)

Sample 1: BMP1

Total cyanobacteria cell numbers in the BMP1 sample collected on 8/16/12 were 387,060 cells/mL. The dominant species in the sample were *Aphanizomenon* cf. *klebahnii* (244,100 cells/mL; Fig. 1) and *Aphanizomenon* sp. (82,466 cells/mL; Fig. 2).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 352,540 cells/mL (91.1% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Aphanizomenon* cf. *klebahnii* (244,100 cells/mL), *Aphanizomenon* sp. (82,466 cells/mL), *Woronichinia naegeliana* (12,526 cells/mL), *Anabaena planctonica* (8,953 cells/mL), *Microcystis* sp. (4,477 cells/mL) and *Microcystis wesenbergii* (18 cells/mL).

Sample 2: SCP1

Total cyanobacteria cell numbers in the SCP1 sample collected on 8/17/12 were 455,079 cells/mL. The dominant species in the sample were filamentous species including *Pseudanabaena* sp. (104,614 cells/mL; Fig. 3), *Aphanizomenon* sp. (96,132 cells/mL; Fig. 4) and *Aphanizomenon gracile* (84,822 cells/mL; Fig. 5).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 304,657 cells/mL (66.9% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Aphanizomenon* sp. (96,132 cells/mL), *Aphanizomenon gracile* (84,822 cells/mL), *Planktothrix* sp. (55,606 cells/mL; Fig. 6), *Anabaena planctonica* (54,035 cells/mL; Fig. 7), *Cuspidothrix issatschenkoi* (5,184 cells/mL), *Microcystis* sp. (3,770 cells/mL), *Aphanizomenon* sp. (3,456 cells/mL), *Aphanizomenon* cf. *flos-aquae* (589 cells/mL), *Cylindrospermopsis raciborskii* (471 cells/mL), *Microcystis* sp. (340 cells/mL) and *Microcystis wesenbergii* (252 cells/mL).

Sample 3: SMP1

Total cyanobacteria cell numbers in the SMP1 sample collected on 8/17/12 were 4,783 cells/mL. The dominant species in the sample were small (5um) unicells (1,853 cells/mL) and *Microcystis* spp. (1,332 cells/mL).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 1,559 cells/mL (32.6% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Microcystis* unicells and cell pairs (1,131 cells/mL), *Anabaena* cf. *circinalis* (165 cells/mL), *Microcystis wesenbergii* (101 cells/mL), *Microcystis botrys* (58 cells/mL), *Microcystis* sp. (42 cells/mL), *Woronichinia naegeliana* (32 cells/mL) and *Aphanizomenon* cf. *flos-aquae* (30 cells/mL).

Sample 4: SMP2

Total cyanobacteria cell numbers in the SMP2 sample collected on 8/17/12 were 1,530 cells/mL. The dominant species in the sample were small (5µm) unicells (1,131 cells/mL).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 264 cells/mL (17.3% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Microcystis* unicells and cell pairs (142 cells/mL), *Microcystis wesenbergii* (45 cells/mL), *Microcystis* sp. (37 cells/mL), *Anabaena* cf. *circinalis* (24 cells/mL) and *Woronichinia naegeliana* (16 cells/mL).

Sample 5: TUR1

Total cyanobacteria cell numbers in the TUR1 sample collected on 8/17/12 were 94 cells/mL. The only cyanophyte species in the sample were small (5µm) unicells (94 cells/mL).

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

Sample 6: WAP1

Total cyanobacteria cell numbers in the WAP1 sample collected on 8/17/12 were 5,230 cells/mL. The dominant species in the sample was the colonial species *Cyanogranis ferruginea* (4,653 cells/mL; Fig. 8).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 27 cells/mL (0.5% of total cyanobacteria cell numbers). *Microcystis wesenbergii* (27 cells/mL) was the only PTOX Cyano species observed in the sample.

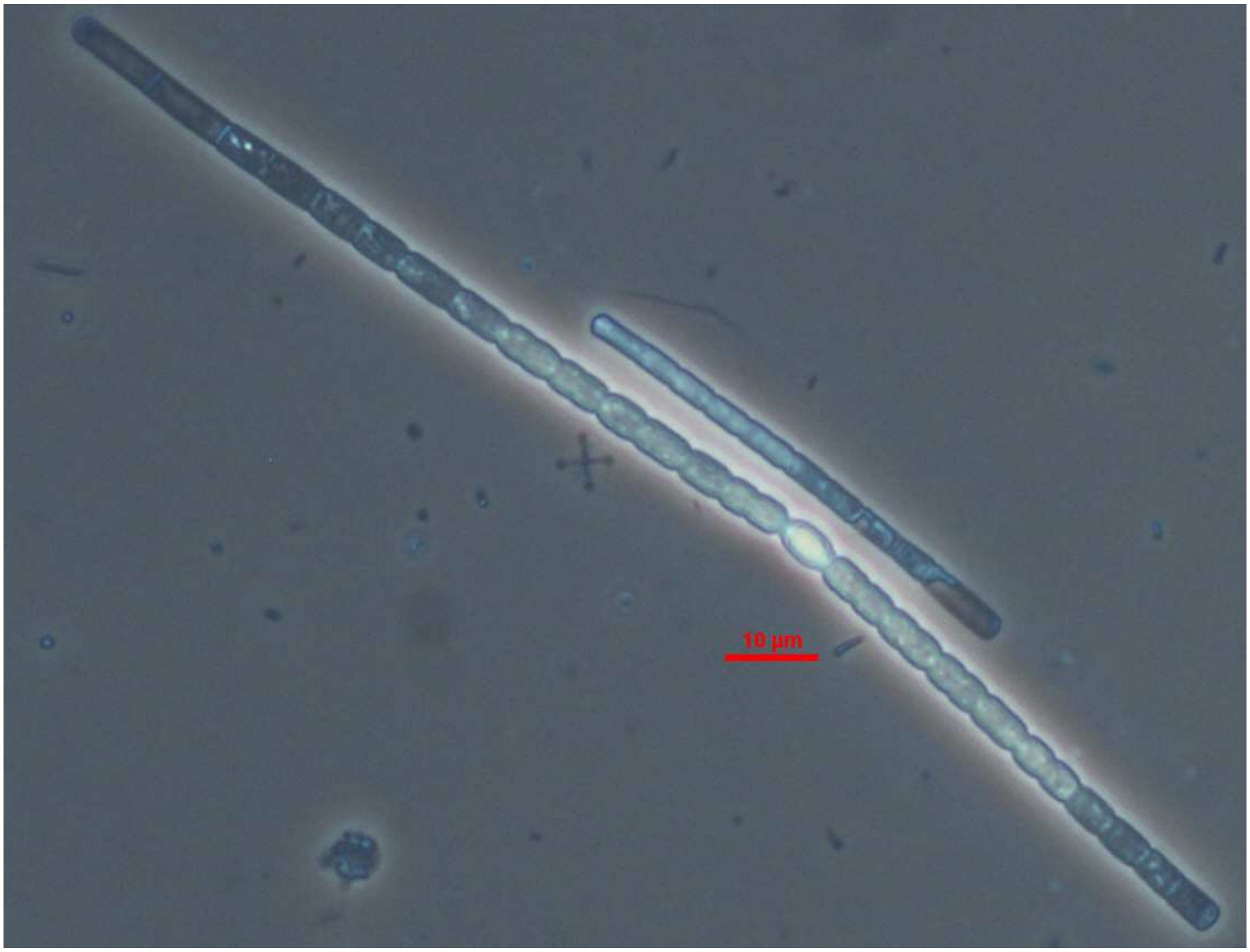



Fig. 1 *Aphanizomenon* cf. *klebahnii* BMP1 400X (scale bar = 10µm)

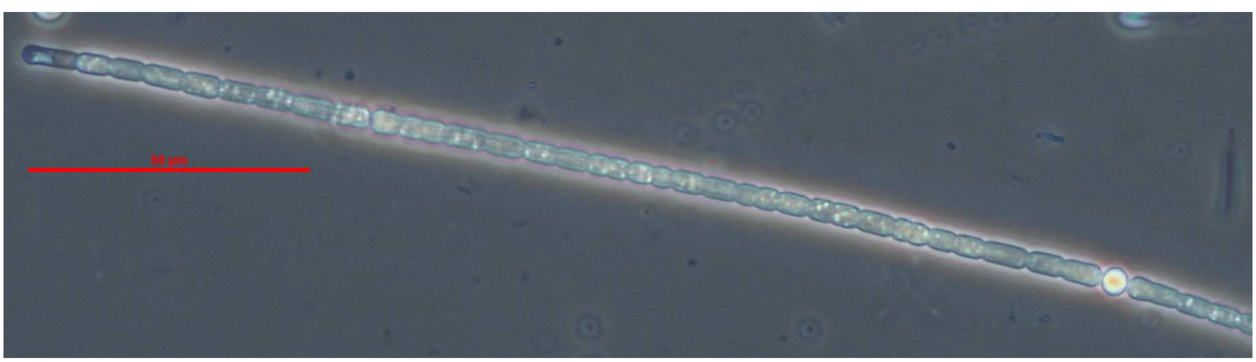


Fig. 2 *Aphanizomenon* sp. BMP1 400X (scale bar = 50µm)

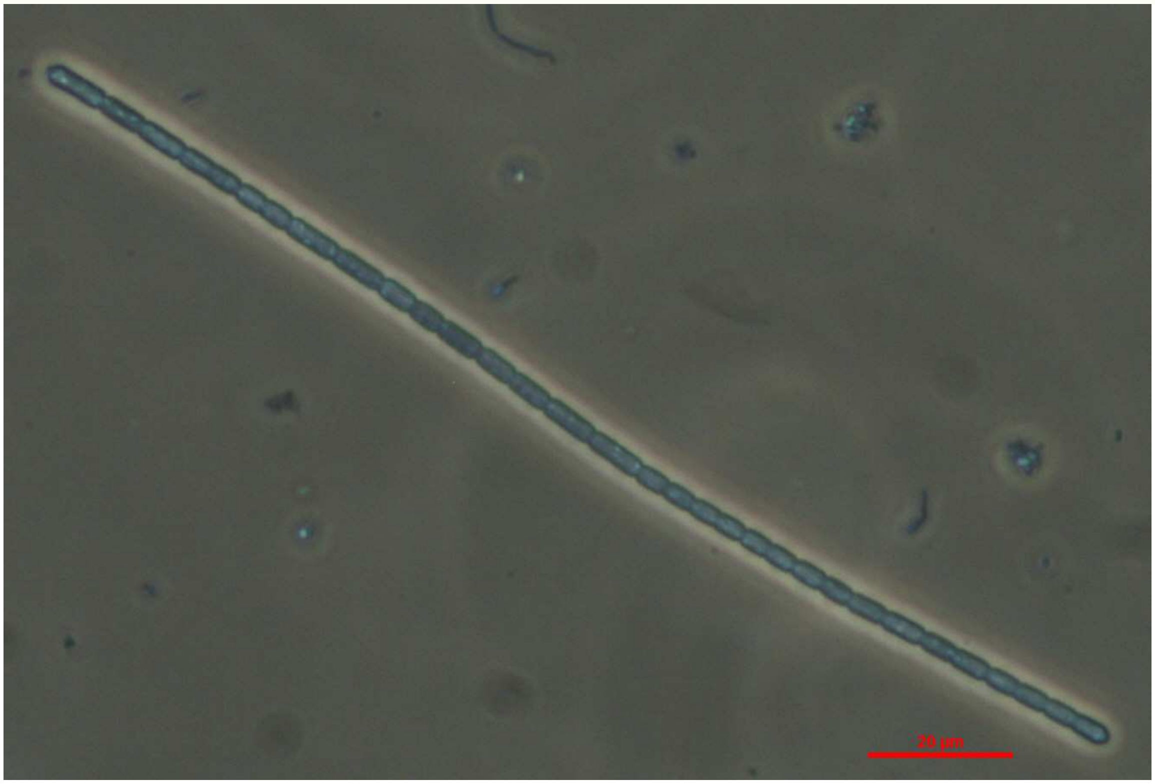



Fig. 3 *Pseudanabaena* sp. SCP1 400X (scale bar = 20µm)

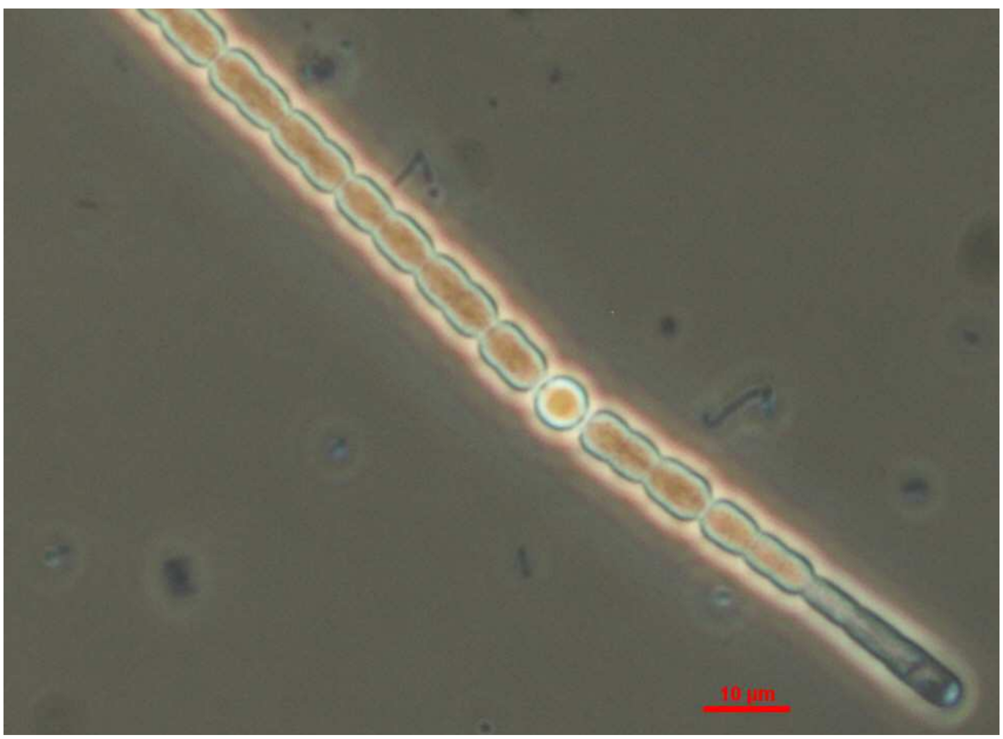



Fig. 4 *Aphanizomenon* sp. SCP1 400X (scale bar = 10µm)

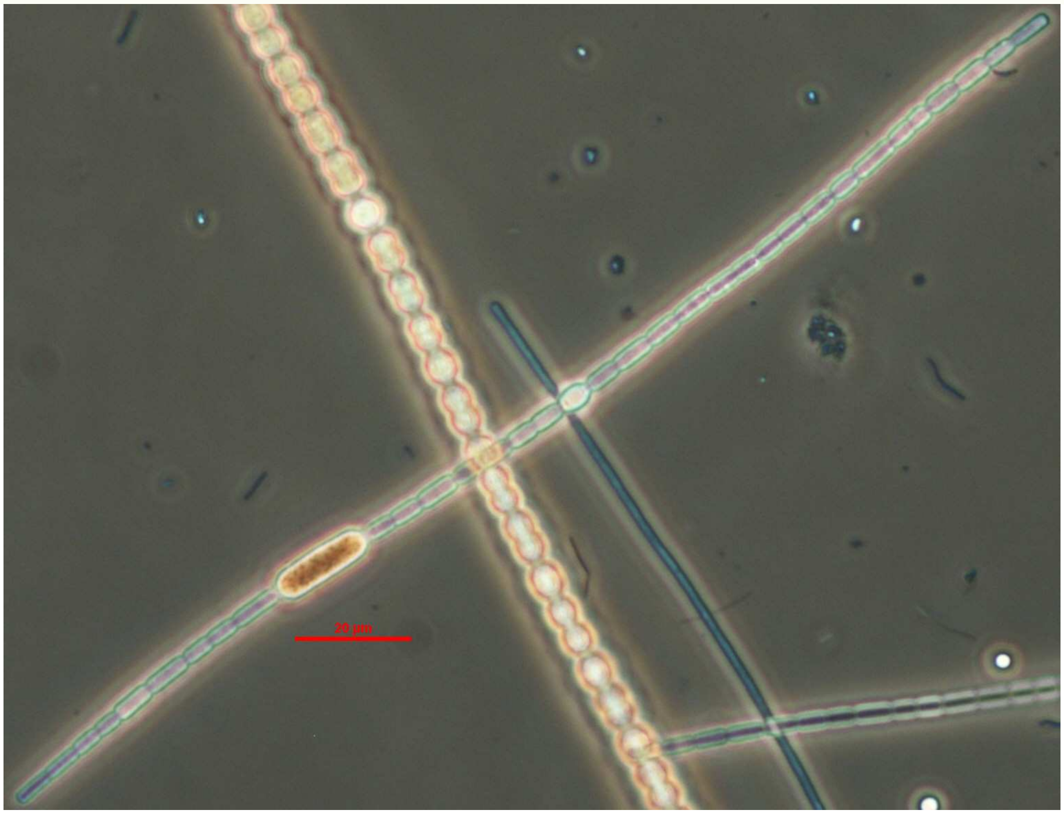



Fig. 5 *Aphanizomenon gracile* SCP1 400X (scale bar = 20µm)

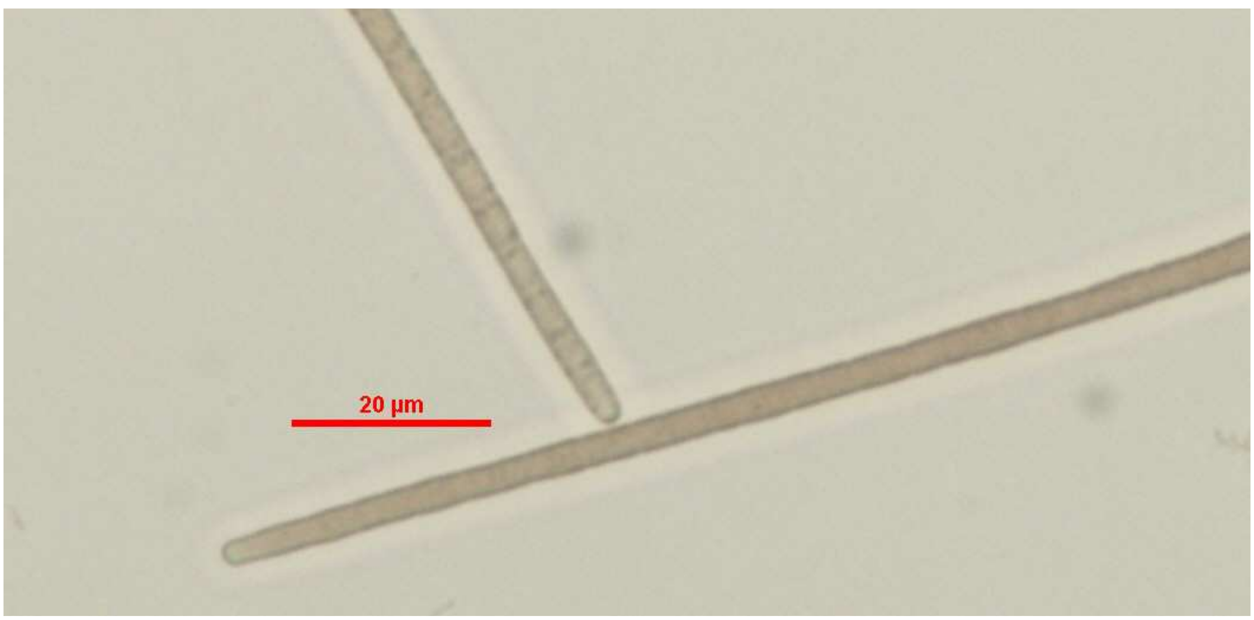



Fig. 6 *Planktothrix* sp. SCP1 400X (scale bar = 20µm)


Fig. 7 *Anabaena planctonica* SCP1 400X (scale bar = 50µm)

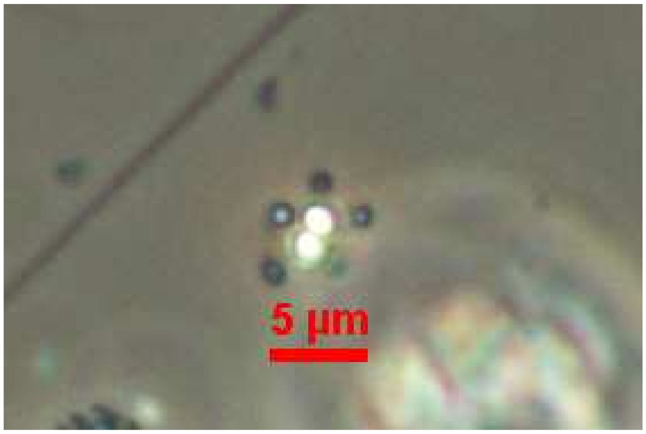



Fig. 8 *Cyanogranis ferruginea* WAP1 400X (scale bar = 5µm)

# ESS Group Cyanobacteria ID and Enumeration Report

Prepared: September 4, 2012
Prepared By: GreenWater Laboratories

Samples: 1 (collected on 8/17/12)

1. PAP1

Sample 1: PAP1

Total cyanobacteria cell numbers in the PAP1 sample collected on 8/17/12 were 560,111 cells/mL. The dominant species in the sample was *Microcystis* cf. *aeruginosa* (555,428 cells/mL; Fig. 1). The size of the cells and the nature of the colonial mucilage (extending past the outer cells of the colony) fit the description of *M. aeruginosa*, however, no large colonies exhibiting the characteristic clathration (holes) in the colony were observed.

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 556,966 cells/mL (99.4% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Microcystis* cf. *aeruginosa* (555,428 cells/mL), *Anabaena* sp. (1,178 cells/mL), *Microcystis botrys* (240 cells/mL) and *Anabaena* sp. (120 cells/mL).


Fig. 1 *Microcystis* cf. *aeruginosa* 400X (scale bar = 10µm)

# ESS Group Cyanobacteria ID and Enumeration Report

Prepared: September 3, 2012
Prepared By: GreenWater Laboratories

Samples: 1 (collected on 8/17/12)
1. SLR1

Sample 1: SLR1
Total cyanobacteria cell numbers in the SLR1 sample collected on 8/17/12 were 13,649 cells/mL. The dominant species in the sample was *Woronichinia naegeliana* (9,048 cells/mL; Fig. 1). The majority of the *W. naegeliana* was in the form of unicells and cell pairs.

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 11,270 cells/mL (82.6% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Woronichinia naegeliana* (9,048 cells/mL), *Microcystis wesenbergii* (1,241 cells/mL), *Microcystis* unicells and cell pairs (314 cells/mL), *Microcystis botrys* (245 cells/mL), *Anabaena* sp. (181 cells/mL), *Anabaena* spp. (115 cells/mL), *Microcystis* sp. (102 cells/mL), *Microcystis* sp. (13 cells/mL), *Anabaena* cf. *lemmermannii* (9 cells/mL) and *Anabaena* sp. (2 cells/mL).

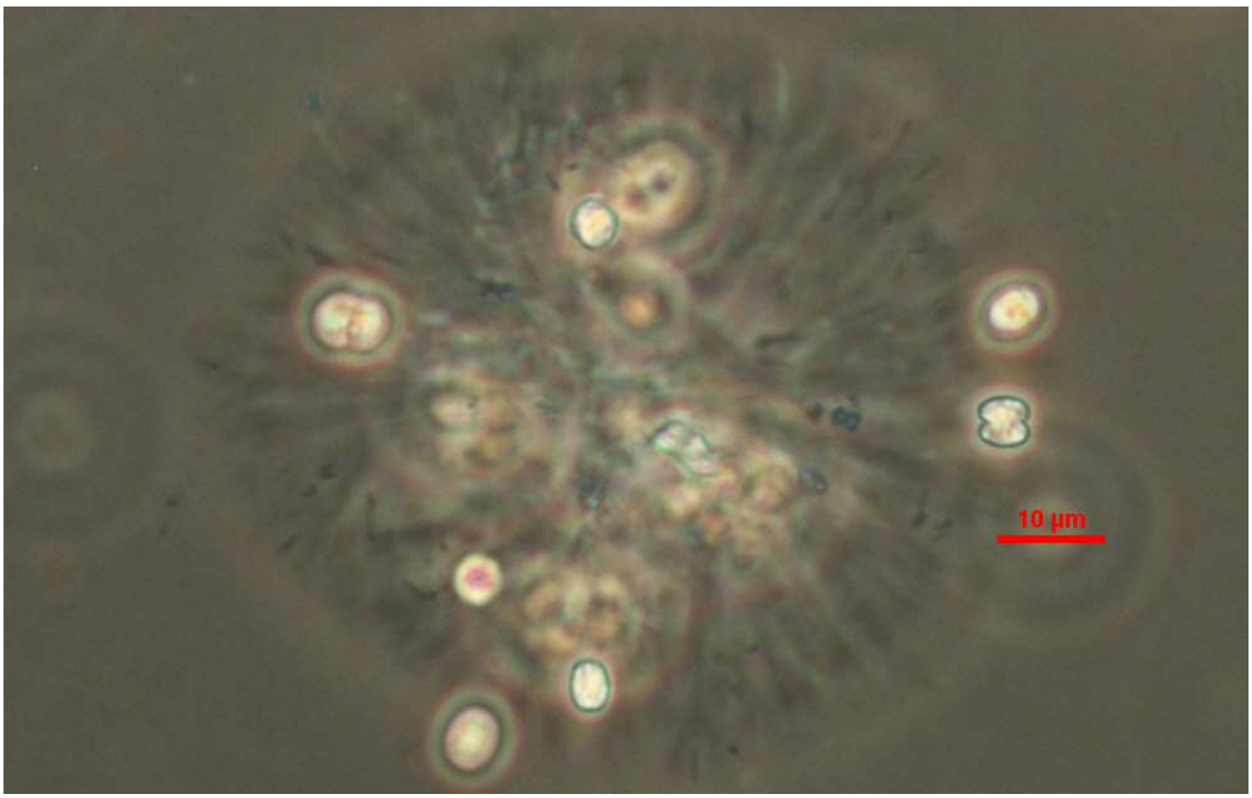



Fig. 1 *Woronichinia naegeliana* 400X (scale bar = 10µm)

# ESS Group Cyanobacteria ID and Enumeration Report

Prepared: September 28, 2012
Prepared By: GreenWater Laboratories

Samples: 2 (collected on 9/24/12)
1. SLK2
2. SLK3

Sample 1: SLK2
Total cyanobacteria cell numbers in the SLK2 sample collected on 9/24/12 were 902,080 cells/mL. The dominant species in the sample were *Woronichinia naegeliana* (395,052 cells/mL; Fig. 1), *Anabaena circinalis* (197,919 cells/mL; Fig. 2), *Microcystis aeruginosa* (131,003 cells/mL; Fig. 3) and *Microcystis ichthyoblabe* (104,929 cells/mL; Fig. 4).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 878,206 cells/mL (97.4% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Woronichinia naegeliana* (395,052 cells/mL), *Anabaena circinalis* (197,919 cells/mL), *Microcystis aeruginosa* (131,003 cells/mL), *Microcystis ichthyoblabe* (104,929 cells/mL), *Microcystis wesenbergii* (23,248 cells/mL), *Microcystis botrys* (20,499 cells/mL), *Anabaena lemmermannii* (4,005 cells/mL) and *Anabaena planctonica* (1,550 cells/mL).

Sample 2: SLK3
Total cyanobacteria cell numbers in the SLK3 sample collected on 9/24/12 were 50,376 cells/mL. The dominant species in the sample was *Woronichinia naegeliana* (22,436 cells/mL).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 35,706 cells/mL (70.9% of total cyanobacteria cell numbers). Potentially toxigenic species observed in the sample included *Woronichinia naegeliana* (22,436 cells/mL), *Anabaena circinalis* (8,796 cells/mL), *Microcystis wesenbergii* (1,774 cells/mL), *Microcystis aeruginosa* (1,263 cells/mL), *Microcystis ichthyoblabe* (1,113 cells/mL), *Microcystis botrys* (223 cells/mL), *Anabaena planctonica* (67 cells/mL), *Anabaena/Aphanizomenon* sp. (19 cells/mL) and *Microcystis* sp. (15 cells/mL).


205 Zeagler Drive, Suite 302 • Palatka, FL 32177
info@greenwaterlab.com • www.greenwaterlab.com
386.328.0882,voice • 386.328.9646,fax
cyanomail@cyanolab.com • www.cyanolab.com


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


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

Fig. 3 *Microcystis aeruginosa* 200X (scale bar = 100µm)

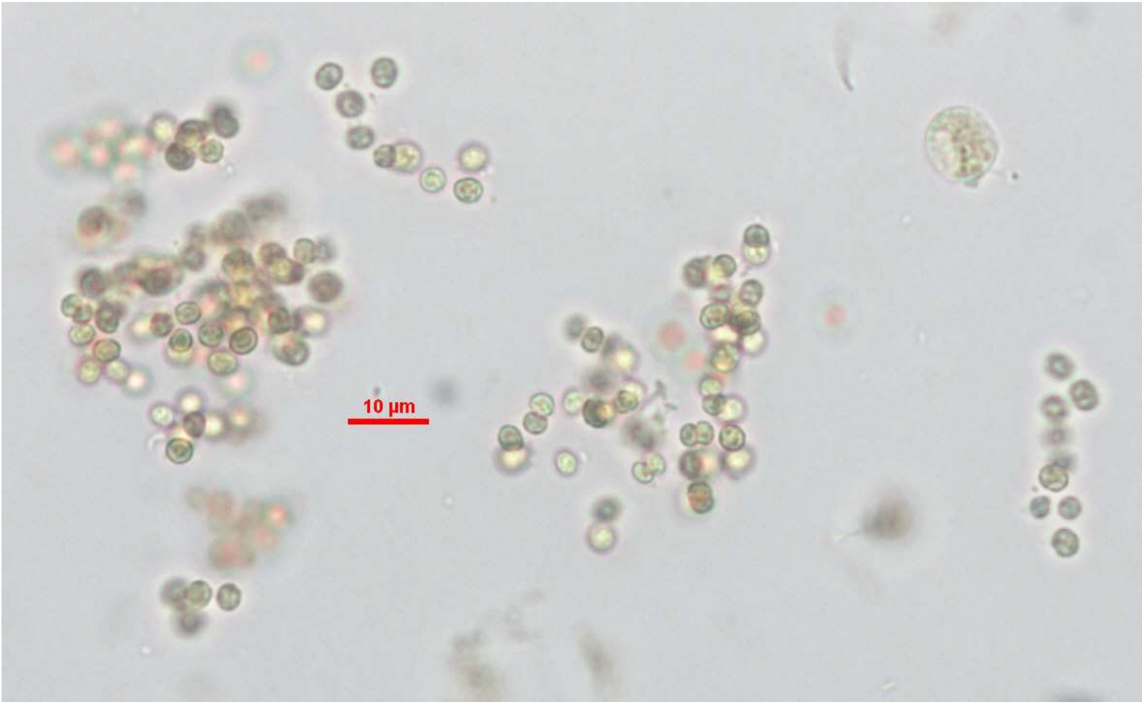



Fig. 4 *Microcystis ichthyoblabe* 400X (scale bar = 10µm)

| Sample | Sampling Date | Genus | Species | Algal Group | Counting Unit | Cells/Unit | Species Units/mL | Species Cells/mL | PTOX Cyano Total Units/mL | PTOX Cyano Total Cells/mL |
|---|---|---|---|---|---|---|---|---|---|---|
| Melville Pond | 7/23/2012 | Anabaena | planctonica | Cyanobacteria | filament | 35 | 2,827 | 98,959 | 45,198 | 183,422 |
| Melville Pond | 7/23/2012 | Woronichinia | naegeliana (unicell) | Cyanobacteria | cell | 1 | 30,473 | 30,473 | | |
| Melville Pond | 7/23/2012 | Woronichinia | naegeliana (colony) | Cyanobacteria | colony | 277 | 108 | 29,916 | | |
| Melville Pond | 7/23/2012 | Woronichinia | naegeliana (cell pair) | Cyanobacteria | colony | 2 | 11,781 | 23,562 | | |
| Melville Pond | 7/23/2012 | Microcystis | wesenbergii | Cyanobacteria | colony | 64 | 8 | 512 | | |

| Sample | Sampling Date | Genus | Species | Algal Group | Counting Unit | Cells/Unit | Species Units/mL | Species Cells/mL | PTOX Cyano Total Units/mL | PTOX Cyano Total Cells/mL |
|---|---|---|---|---|---|---|---|---|---|---|
| Mashapaug Pond | 8/08/2012 | Microcystis | botrys | Cyanobacteria | colony | 392 | 314 | 123,149 | 24,076 | 311,293 |
| Mashapaug Pond | 8/08/2012 | Microcystis | ichthyoblabe (colony) | Cyanobacteria | colony | 20 | 3,456 | 69,114 | | |
| Mashapaug Pond | 8/08/2012 | Microcystis | wesenbergii | Cyanobacteria | colony | 121 | 393 | 47,516 | | |
| Mashapaug Pond | 8/08/2012 | Anabaena | planctonica | Cyanobacteria | filament | 55 | 471 | 25,918 | | |
| Mashapaug Pond | 8/08/2012 | Microcystis | sp. (colony) | Cyanobacteria | colony | 77 | 157 | 12,095 | | |
| Mashapaug Pond | 8/08/2012 | Microcystis | ichthyoblabe (unicell) | Cyanobacteria | cell | 1 | 10,524 | 10,524 | | |
| Mashapaug Pond | 8/08/2012 | Microcystis | ichthyoblabe (cell pair) | Cyanobacteria | colony | 2 | 3,613 | 7,226 | | |
| Mashapaug Pond | 8/08/2012 | Aphanizomenon | cf. flos-aquae | Cyanobacteria | filament | 28 | 236 | 6,597 | | |
| Mashapaug Pond | 8/08/2012 | Anabaena | crassa | Cyanobacteria | filament | 65 | 39 | 2,553 | | |
| Mashapaug Pond | 8/08/2012 | Woronichinia | naegeliana (unicell) | Cyanobacteria | cell | 1 | 2,199 | 2,199 | | |
| Mashapaug Pond | 8/08/2012 | Microcystis | sp. (cell pair) | Cyanobacteria | colony | 2 | 942 | 1,885 | | |
| Mashapaug Pond | 8/08/2012 | Woronichinia | naegeliana (cell pair) | Cyanobacteria | colony | 2 | 628 | 1,257 | | |
| Mashapaug Pond | 8/08/2012 | Microcystis | sp. (unicell) | Cyanobacteria | cell | 1 | 1,100 | 1,100 | | |
| Mashapaug Pond | 8/08/2012 | Woronichinia | naegeliana (colony) | Cyanobacteria | colony | 40 | 4 | 160 | | |

| Sample | Sampling Date | Genus | Species | Algal Group | Counting Unit | Cells/Unit | Species Units/mL | Species Cells/mL | Cyano Total Units/mL | Cyano Total Cells/mL |
|---|---|---|---|---|---|---|---|---|---|---|
| Almy Pond | 8/16/2012 | Aphanocapsa | planctonica (unicell) | Cyanobacteria | cell | 1 | 2,877,676 | 2,877,676 | 3,147,606 | 5,009,668 |
| Almy Pond | 8/16/2012 | Geitlerinema/Jaaginema | sp. | Cyanobacteria | filament | 19 | 37,699 | 716,278 | | |
| Almy Pond | 8/16/2012 | Aphanizomenon/Sphaerospermopsis | gracile/aphanizomenoides | Cyanobacteria | filament | 13 | 31,416 | 408,404 | | |
| Almy Pond | 8/16/2012 | Aphanocapsa | planctonica (cell pair) | Cyanobacteria | colony | 2 | 175,928 | 351,856 | | |
| Almy Pond | 8/16/2012 | Planktolyngbya | limnetica | Cyanobacteria | filament | 41 | 6,283 | 257,609 | | |
| Almy Pond | 8/16/2012 | Aphanocapsa | planctonica (colony) | Cyanobacteria | colony | 67 | 2,356 | 157,864 | | |
| Almy Pond | 8/16/2012 | Anabaena | sp. | Cyanobacteria | filament | 27 | 3,142 | 84,822 | | |
| Almy Pond | 8/16/2012 | Coelosphaerium | kuetzingianum | Cyanobacteria | colony | 34 | 2,356 | 80,110 | | |
| Almy Pond | 8/16/2012 | Aphanocapsa | conferta | Cyanobacteria | filament | 48 | 785 | 37,699 | | |
| Almy Pond | 8/16/2012 | Pseudanabaena | mucicola | Cyanobacteria | filament | 3 | 6,283 | 18,849 | | |
| Almy Pond | 8/16/2012 | Aphanocapsa | sp. | Cyanobacteria | colony | 41 | 140 | 5,740 | | |
| Almy Pond | 8/16/2012 | Coelomoron/Coelosphaerium | sp. | Cyanobacteria | colony | 45 | 100 | 4,500 | | |
| Almy Pond | 8/16/2012 | cyanophyte unicell | sp. | Cyanobacteria | cell | 1 | 3,142 | 3,142 | | |
| Almy Pond | 8/16/2012 | Anabaena | sp. | Cyanobacteria | filament | 13 | 240 | 3,120 | | |
| Almy Pond | 8/16/2012 | Snowella | lacustris | Cyanobacteria | colony | 47 | 20 | 940 | | |
| Almy Pond | 8/16/2012 | Planktothrix | agardhii | Cyanobacteria | filament | 33 | 20 | 660 | | |
| Almy Pond | 8/16/2012 | Microcystis | wesenbergii | Cyanobacteria | colony | 20 | 20 | 400 | | |

| Sample | Sampling Date | Genus | Species | Algal Group | Counting Unit | Cells/Unit | Species Units/mL | Species Cells/mL | Cyano Total Units/mL | Cyano Total Cells/mL |
|---|---|---|---|---|---|---|---|---|---|---|
| BAP1 | 8/16/2012 | Planktothrix | suspensa | Cyanobacteria | filament | 96 | 22,776 | 2,186,531 | 48,618 | 2,241,352 |
| BAP1 | 8/16/2012 | Woronichinia | naegeliana (unicell) | Cyanobacteria | cell | 1 | 15,708 | 15,708 | | |
| BAP1 | 8/16/2012 | cyanophyte colony | sp. | Cyanobacteria | colony | 15 | 785 | 11,781 | | |
| BAP1 | 8/16/2012 | Woronichinia | naegeliana (cell pair) | Cyanobacteria | colony | 2 | 3,927 | 7,854 | | |
| BAP1 | 8/16/2012 | Microcystis | botrys | Cyanobacteria | colony | 122 | 40 | 4,880 | | |
| BAP1 | 8/16/2012 | Woronichinia | naegeliana (colony) | Cyanobacteria | colony | 70 | 60 | 4,200 | | |
| BAP1 | 8/16/2012 | cyanophyte unicell, oval/rod 2.5-5um | spp. | Cyanobacteria | cell | 1 | 3,927 | 3,927 | | |
| BAP1 | 8/16/2012 | Anabaena | cf. lemmermannii | Cyanobacteria | filament | 5 | 589 | 2,945 | | |
| BAP1 | 8/16/2012 | Microcystis | sp. | Cyanobacteria | colony | 370 | 5 | 1,850 | | |
| BAP1 | 8/16/2012 | cyanophyte cell pair | spp. | Cyanobacteria | colony | 2 | 785 | 1,571 | | |
| BAP1 | 8/16/2012 | Aphanizomenon | sp. | Cyanobacteria | filament | 6 | 10 | 60 | | |
| BAP1 | 8/16/2012 | Anabaena | sp. | Cyanobacteria | filament | 9 | 5 | 45 | | |
| BAP2 | 8/16/2012 | Planktothrix | suspensa | Cyanobacteria | filament | 97 | 16 | 1,524 | 914 | 3,657 |
| BAP2 | 8/16/2012 | Woronichinia | naegeliana (unicell) | Cyanobacteria | cell | 1 | 534 | 534 | | |
| BAP2 | 8/16/2012 | Microcystis | sp. | Cyanobacteria | colony | 168 | 2 | 403 | | |
| BAP2 | 8/16/2012 | Woronichinia | naegeliana (cell pair) | Cyanobacteria | colony | 2 | 157 | 314 | | |
| BAP2 | 8/16/2012 | Woronichinia | naegeliana (colony) | Cyanobacteria | colony | 34 | 8 | 267 | | |
| BAP2 | 8/16/2012 | cyanophyte colony | sp. | Cyanobacteria | colony | 7 | 31 | 220 | | |
| BAP2 | 8/16/2012 | cyanophyte cell pair | spp. | Cyanobacteria | colony | 2 | 63 | 126 | | |
| BAP2 | 8/16/2012 | Microcystis | botrys | Cyanobacteria | colony | 159 | 1 | 95 | | |
| BAP2 | 8/16/2012 | cyanophyte colony | sp. | Cyanobacteria | colony | 10 | 8 | 79 | | |
| BAP2 | 8/16/2012 | Microcystis | sp. | Cyanobacteria | cell | 1 | 63 | 63 | | |
| BAP2 | 8/16/2012 | cyanophyte unicell, oval/rod 2.5-5um | spp. | Cyanobacteria | cell | 1 | 31 | 31 | | |
| BAP2 | 8/16/2012 | Anabaena | cf. lemmermannii | Cyanobacteria | filament | 4 | 0.2 | 1 | | |

| Sample | Sampling Date | Genus | Species | Algal Group | Counting Unit | Cells/Unit | Species Units/mL | Species Cells/mL | Cyano Total Units/mL | Cyano Total Cells/mL |
|---|---|---|---|---|---|---|---|---|---|---|
| RWP1 | 8/16/2012 | Aphanizomenon | cf. flos-aquae | Cyanobacteria | filament | 23 | 6,283 | 144,512 | 12,063 | 315,581 |
| RWP1 | 8/16/2012 | Cuspidothrix | issatschenkoi | Cyanobacteria | filament | 36 | 3,142 | 113,096 | | |
| RWP1 | 8/16/2012 | Cylindrospermopsis | raciborskii | Cyanobacteria | filament | 42 | 1,257 | 52,778 | | |
| RWP1 | 8/16/2012 | Anabaena | smithii | Cyanobacteria | filament | 28 | 79 | 2,199 | | |
| RWP1 | 8/16/2012 | Microcystis | spp. (unicell) | Cyanobacteria | cell | 1 | 628 | 628 | | |
| RWP1 | 8/16/2012 | cyanophyte filament | sp. | Cyanobacteria | filament | 4 | 157 | 628 | | |
| RWP1 | 8/16/2012 | Aphanocapsa | planctonica | Cyanobacteria | colony | 16 | 39 | 628 | | |
| RWP1 | 8/16/2012 | cyanophyte unicell, sphere 2.5-5um | spp. | Cyanobacteria | cell | 1 | 314 | 314 | | |
| RWP1 | 8/16/2012 | Microcystis | spp. (cell pair) | Cyanobacteria | colony | 2 | 157 | 314 | | |
| RWP1 | 8/16/2012 | Microcystis | wesenbergii | Cyanobacteria | colony | 102 | 3 | 306 | | |
| RWP1 | 8/16/2012 | Microcystis | sp. | Cyanobacteria | colony | 45 | 2 | 90 | | |
| RWP1 | 8/16/2012 | Microcystis | ichthyoblabe | Cyanobacteria | colony | 58 | 1 | 58 | | |
| RWP1 | 8/16/2012 | Snowella | litoralis | Cyanobacteria | colony | 28 | 1 | 28 | | |
| RWP2 | 8/16/2012 | Cuspidothrix | issatschenkoi | Cyanobacteria | filament | 20 | 9,799 | 195,976 | 16,084 | 252,864 |
| RWP2 | 8/16/2012 | Cylindrospermopsis | raciborskii | Cyanobacteria | filament | 18 | 1,542 | 27,763 | | |
| RWP2 | 8/16/2012 | Aphanizomenon | cf. flos-aquae | Cyanobacteria | filament | 18 | 907 | 16,331 | | |
| RWP2 | 8/16/2012 | Microcystis | wesenbergii | Cyanobacteria | colony | 114 | 37 | 4,263 | | |
| RWP2 | 8/16/2012 | cyanophyte unicell, sphere 2.5-5um | spp. | Cyanobacteria | cell | 1 | 1,361 | 1,361 | | |
| RWP2 | 8/16/2012 | Anabaena | smithii | Cyanobacteria | filament | 30 | 45 | 1,361 | | |
| RWP2 | 8/16/2012 | Aphanocapsa | sp. | Cyanobacteria | colony | 7 | 181 | 1,270 | | |
| RWP2 | 8/16/2012 | Microcystis | sp. | Cyanobacteria | colony | 44 | 23 | 998 | | |
| RWP2 | 8/16/2012 | Microcystis | ichthyoblabe | Cyanobacteria | colony | 81 | 10 | 819 | | |
| RWP2 | 8/16/2012 | Microcystis | spp. (unicell) | Cyanobacteria | cell | 1 | 817 | 817 | | |
| RWP2 | 8/16/2012 | cyanophyte unicell, oval/rod 2.5-5um | spp. | Cyanobacteria | cell | 1 | 635 | 635 | | |
| RWP2 | 8/16/2012 | Microcystis | spp. (cell pair) | Cyanobacteria | colony | 2 | 181 | 363 | | |
| RWP2 | 8/16/2012 | cyanophyte filament | sp. | Cyanobacteria | filament | 3 | 91 | 272 | | |
| RWP2 | 8/16/2012 | Woronichinia | naegeliana (unicell) | Cyanobacteria | cell | 1 | 272 | 272 | | |
| RWP2 | 8/16/2012 | Woronichinia | naegeliana (cell pair) | Cyanobacteria | colony | 2 | 91 | 181 | | |
| RWP2 | 8/16/2012 | cyanophyte cell pair | spp. | Cyanobacteria | colony | 2 | 91 | 181 | | |
| UCR1 | 8/16/2012 | Aphanizomenon | cf. ovalisporum | Cyanobacteria | filament | 32 | 4,398 | 140,742 | 11,392 | 202,213 |
| UCR1 | 8/16/2012 | Anabaena | planctonica | Cyanobacteria | filament | 24 | 942 | 22,619 | | |
| UCR1 | 8/16/2012 | Aphanizomenon | cf. flos-aquae | Cyanobacteria | filament | 18 | 1,257 | 22,619 | | |
| UCR1 | 8/16/2012 | Anabaena/Aphanizomenon | sp. | Cyanobacteria | filament | 44 | 157 | 6,911 | | |
| UCR1 | 8/16/2012 | Snowella | litoralis | Cyanobacteria | colony | 49 | 79 | 3,848 | | |
| UCR1 | 8/16/2012 | Woronichinia | naegeliana (unicell) | Cyanobacteria | cell | 1 | 1,728 | 1,728 | | |
| UCR1 | 8/16/2012 | cyanophyte unicell, sphere 2.5-5um | spp. | Cyanobacteria | cell | 1 | 1,571 | 1,571 | | |
| UCR1 | 8/16/2012 | Woronichinia | naegeliana (cell pair) | Cyanobacteria | colony | 2 | 471 | 942 | | |
| UCR1 | 8/16/2012 | cyanophyte cell pair | spp. | Cyanobacteria | colony | 2 | 314 | 628 | | |
| UCR1 | 8/16/2012 | Microcystis | sp. | Cyanobacteria | cell | 1 | 314 | 314 | | |
| UCR1 | 8/16/2012 | cyanophyte unicell, oval/rod 2.5-5um | spp. | Cyanobacteria | cell | 1 | 157 | 157 | | |
| UCR1 | 8/16/2012 | Microcystis | sp. | Cyanobacteria | colony | 38 | 3 | 114 | | |
| UCR1 | 8/16/2012 | Microcystis | sp. | Cyanobacteria | colony | 18 | 1 | 18 | | |

| Sample | Sampling Date | Genus | Species | Algal Group | Counting Unit | Cells/Unit | Species Units/mL | Species Cells/mL | Cyano Total Units/mL | Cyano Total Cells/mL |
|---|---|---|---|---|---|---|---|---|---|---|
| SPP1 | 8/16/2012 | Anabaena | planctonica | Cyanobacteria | filament | 38 | 565 | 21,488 | 3,255 | 32,417 |
| SPP1 | 8/16/2012 | Aphanizomenon | sp. | Cyanobacteria | filament | 34 | 126 | 4,273 | | |
| SPP1 | 8/16/2012 | Aphanizomenon | cf. flos-aquae | Cyanobacteria | filament | 18 | 94 | 1,696 | | |
| SPP1 | 8/16/2012 | Woronichinia | naegeliana (unicell) | Cyanobacteria | cell | 1 | 1,225 | 1,225 | | |
| SPP1 | 8/16/2012 | Woronichinia | naegeliana (cell pair) | Cyanobacteria | colony | 2 | 597 | 1,194 | | |
| SPP1 | 8/16/2012 | Aphanocapsa | sp. | Cyanobacteria | colony | 10 | 94 | 942 | | |
| SPP1 | 8/16/2012 | Microcystis | sp. | Cyanobacteria | colony | 30 | 16 | 471 | | |
| SPP1 | 8/16/2012 | Cyanogranis | ferruginea | Cyanobacteria | colony | 3 | 94 | 283 | | |
| SPP1 | 8/16/2012 | cyanophyte unicell | spp. | Cyanobacteria | cell | 1 | 188 | 188 | | |
| SPP1 | 8/16/2012 | Microcystis | sp. | Cyanobacteria | colony | 262 | 1 | 157 | | |
| SPP1 | 8/16/2012 | Microcystis | spp. (cell pair) | Cyanobacteria | colony | 2 | 63 | 126 | | |
| SPP1 | 8/16/2012 | cyanophyte cell pair | spp. | Cyanobacteria | colony | 2 | 63 | 126 | | |
| SPP1 | 8/16/2012 | cyanophyte unicell, oval/rod 2.5-5um | spp. | Cyanobacteria | cell | 1 | 94 | 94 | | |
| SPP1 | 8/16/2012 | Microcystis | wesenbergii | Cyanobacteria | colony | 46 | 2 | 92 | | |
| SPP1 | 8/16/2012 | Microcystis | spp. (unicell) | Cyanobacteria | cell | 1 | 31 | 31 | | |
| SPP1 | 8/16/2012 | Anabaena | cf. crassa | Cyanobacteria | filament | 84 | 0.2 | 17 | | |
| SPP1 | 8/16/2012 | Woronichinia | naegeliana (colony) | Cyanobacteria | colony | 64 | 0.2 | 13 | | |

| Sample | Sampling Date | Genus | Species | Algal Group | Counting Unit | Cells/Unit | Species Units/mL | Species Cells/mL | Cyano Total Units/mL | Cyano Total Cells/mL |
|---|---|---|---|---|---|---|---|---|---|---|
| BMP1 | 8/16/2012 | Aphanizomenon | cf. klebahnii | Cyanobacteria | filament | 21 | 11,624 | 244,100 | 31,736 | 387,060 |
| BMP1 | 8/16/2012 | Aphanizomenon | sp. | Cyanobacteria | filament | 15 | 5,498 | 82,466 | | |
| BMP1 | 8/16/2012 | Cyanogranis | ferruginea | Cyanobacteria | colony | 10 | 2,670 | 26,703 | | |
| BMP1 | 8/16/2012 | Anabaena | planctonica | Cyanobacteria | filament | 38 | 236 | 8,953 | | |
| BMP1 | 8/16/2012 | Woronichinia | naegeliana (unicell) | Cyanobacteria | cell | 1 | 6,911 | 6,911 | | |
| BMP1 | 8/16/2012 | Microcystis | sp. | Cyanobacteria | colony | 114 | 39 | 4,477 | | |
| BMP1 | 8/16/2012 | Woronichinia | naegeliana (cell pair) | Cyanobacteria | colony | 2 | 2,199 | 4,398 | | |
| BMP1 | 8/16/2012 | cyanophyte colony | sp. | Cyanobacteria | colony | 18 | 157 | 2,827 | | |
| BMP1 | 8/16/2012 | Aphanocapsa | sp. | Cyanobacteria | colony | 15 | 157 | 2,356 | | |
| BMP1 | 8/16/2012 | cyanophyte unicell | sp. | Cyanobacteria | cell | 1 | 1,257 | 1,257 | | |
| BMP1 | 8/16/2012 | Woronichinia | naegeliana (colony) | Cyanobacteria | colony | 31 | 39 | 1,217 | | |
| BMP1 | 8/16/2012 | cyanophyte unicell, oval/rod 2.5-5um | spp. | Cyanobacteria | cell | 1 | 785 | 785 | | |
| BMP1 | 8/16/2012 | cyanophyte cell pair | sp. | Cyanobacteria | colony | 2 | 157 | 314 | | |
| BMP1 | 8/16/2012 | Aphanocapsa | conferta | Cyanobacteria | colony | 90 | 2 | 180 | | |
| BMP1 | 8/16/2012 | cyanophyte colony | sp. | Cyanobacteria | colony | 50 | 1 | 50 | | |
| BMP1 | 8/16/2012 | Coelosphaerium | sp. | Cyanobacteria | colony | 46 | 1 | 46 | | |
| BMP1 | 8/16/2012 | Microcystis | wesenbergii | Cyanobacteria | colony | 9 | 2 | 18 | | |
| SCP1 | 8/17/2012 | Pseudanabaena | sp. | Cyanobacteria | filament | 37 | 2,827 | 104,614 | 14,783 | 455,079 |
| SCP1 | 8/17/2012 | Aphanizomenon | sp. | Cyanobacteria | filament | 34 | 2,827 | 96,132 | | |
| SCP1 | 8/17/2012 | Aphanizomenon | gracile | Cyanobacteria | filament | 27 | 3,142 | 84,822 | | |
| SCP1 | 8/17/2012 | Planktothrix | sp. | Cyanobacteria | filament | 177 | 314 | 55,606 | | |
| SCP1 | 8/17/2012 | Anabaena | planctonica | Cyanobacteria | filament | 43 | 1,257 | 54,035 | | |
| SCP1 | 8/17/2012 | cyanophyte filament | sp. | Cyanobacteria | filament | 27 | 1,571 | 42,411 | | |
| SCP1 | 8/17/2012 | Cuspidothrix | issatschenkoi | Cyanobacteria | filament | 44 | 118 | 5,184 | | |
| SCP1 | 8/17/2012 | Microcystis | sp. | Cyanobacteria | colony | 32 | 118 | 3,770 | | |
| SCP1 | 8/17/2012 | Aphanizomenon | sp. | Cyanobacteria | filament | 11 | 314 | 3,456 | | |
| SCP1 | 8/17/2012 | cyanophyte unicell, sphere 2.5-5um | spp. | Cyanobacteria | cell | 1 | 1,571 | 1,571 | | |
| SCP1 | 8/17/2012 | cyanophyte filament | sp. | Cyanobacteria | filament | 120 | 7 | 840 | | |
| SCP1 | 8/17/2012 | cyanophyte cell pair | spp. | Cyanobacteria | colony | 2 | 314 | 628 | | |
| SCP1 | 8/17/2012 | Aphanizomenon | cf. flos-aquae | Cyanobacteria | filament | 15 | 39 | 589 | | |
| SCP1 | 8/17/2012 | Cylindrospermopsis | raciborskii | Cyanobacteria | filament | 12 | 39 | 471 | | |
| SCP1 | 8/17/2012 | Microcystis | sp. | Cyanobacteria | colony | 170 | 2 | 340 | | |
| SCP1 | 8/17/2012 | cyanophyte unicell, oval/rod 2.5-5um | spp. | Cyanobacteria | cell | 1 | 314 | 314 | | |
| SCP1 | 8/17/2012 | Microcystis | wesenbergii | Cyanobacteria | colony | 36 | 7 | 252 | | |
| SCP1 | 8/17/2012 | Snowella | litoralis | Cyanobacteria | colony | 22 | 2 | 44 | | |
| SMP1 | 8/17/2012 | cyanophyte unicell, oval/rod 2.5-5um | spp. | Cyanobacteria | cell | 1 | 1,005 | 1,005 | 3,046 | 4,783 |
| SMP1 | 8/17/2012 | cyanophyte unicell, sphere 2.5-5um | spp. | Cyanobacteria | cell | 1 | 848 | 848 | | |
| SMP1 | 8/17/2012 | Microcystis | spp. (unicell) | Cyanobacteria | cell | 1 | 817 | 817 | | |
| SMP1 | 8/17/2012 | Aphanocapsa | sp. | Cyanobacteria | colony | 6 | 94 | 565 | | |
| SMP1 | 8/17/2012 | cyanophyte tetrad | spp. | Cyanobacteria | colony | 4 | 94 | 377 | | |
| SMP1 | 8/17/2012 | Microcystis | spp. (cell pair) | Cyanobacteria | colony | 2 | 157 | 314 | | |
| SMP1 | 8/17/2012 | Geitlerinema | splendidum | Cyanobacteria | filament | 26 | 8 | 204 | | |
| SMP1 | 8/17/2012 | Anabaena | cf. circinalis | Cyanobacteria | filament | 55 | 3 | 165 | | |
| SMP1 | 8/17/2012 | Aphanocapsa | sp. | Cyanobacteria | colony | 20 | 8 | 157 | | |
| SMP1 | 8/17/2012 | Microcystis | wesenbergii | Cyanobacteria | colony | 504 | 0.2 | 101 | | |
| SMP1 | 8/17/2012 | Microcystis | botrys | Cyanobacteria | colony | 145 | 0.4 | 58 | | |
| SMP1 | 8/17/2012 | Pseudanabaena | sp. | Cyanobacteria | filament | 6 | 8 | 47 | | |
| SMP1 | 8/17/2012 | Microcystis | sp. | Cyanobacteria | colony | 53 | 1 | 42 | | |
| SMP1 | 8/17/2012 | Woronichinia | naegeliana (colony) | Cyanobacteria | colony | 79 | 0.4 | 32 | | |
| SMP1 | 8/17/2012 | Aphanizomenon | cf. flos-aquae | Cyanobacteria | filament | 19 | 2 | 30 | | |
| SMP1 | 8/17/2012 | Merismopedia | glauca | Cyanobacteria | colony | 84 | 0.2 | 17 | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SMP1 | 8/17/2012 | cyanophyte filament | sp. | Cyanobacteria | filament | 13 | 0.2 | 3 | | |
| SMP2 | 8/17/2012 | cyanophyte unicell, sphere 2.5-5um | spp. | Cyanobacteria | cell | 1 | 754 | 754 | 1,307 | 1,530 |
| SMP2 | 8/17/2012 | cyanophyte unicell, oval/rod 2.5-5um | spp. | Cyanobacteria | cell | 1 | 377 | 377 | | |
| SMP2 | 8/17/2012 | Microcystis | spp. (unicell) | Cyanobacteria | cell | 1 | 126 | 126 | | |
| SMP2 | 8/17/2012 | cyanophyte tetrad | spp. | Cyanobacteria | colony | 4 | 31 | 126 | | |
| SMP2 | 8/17/2012 | Microcystis | wesenbergii | Cyanobacteria | colony | 75 | 1 | 45 | | |
| SMP2 | 8/17/2012 | Microcystis | sp. | Cyanobacteria | colony | 37 | 1 | 37 | | |
| SMP2 | 8/17/2012 | Anabaena | cf. circinalis | Cyanobacteria | filament | 30 | 1 | 24 | | |
| SMP2 | 8/17/2012 | Microcystis | spp. (cell pair) | Cyanobacteria | colony | 2 | 8 | 16 | | |
| SMP2 | 8/17/2012 | Woronichinia | naegeliana (cell pair) | Cyanobacteria | colony | 2 | 8 | 16 | | |
| SMP2 | 8/17/2012 | cyanophyte filament | sp. | Cyanobacteria | filament | 13 | 1 | 8 | | |
| SMP2 | 8/17/2012 | Pseudanabaena | sp. | Cyanobacteria | filament | 10 | 0.2 | 2 | | |
| TUR1 | 8/17/2012 | cyanophyte unicell, oval/rod 2.5-5um | spp. | Cyanobacteria | cell | 1 | 63 | 63 | 94 | 94 |
| TUR1 | 8/17/2012 | cyanophyte unicell, sphere 2.5-5um | spp. | Cyanobacteria | cell | 1 | 31 | 31 | | |
| WAP1 | 8/17/2012 | Cyanogranis | ferruginea | Cyanobacteria | colony | 3 | 1,551 | 4,653 | 2,102 | 5,230 |
| WAP1 | 8/17/2012 | Dactylococcopsis | irregularis | Cyanobacteria | cell | 1 | 373 | 373 | | |
| WAP1 | 8/17/2012 | cyanophyte unicell, oval/rod 2.5-5um | spp. | Cyanobacteria | cell | 1 | 157 | 157 | | |
| WAP1 | 8/17/2012 | Microcystis | wesenbergii | Cyanobacteria | colony | 31 | 1 | 27 | | |
| WAP1 | 8/17/2012 | cyanophyte unicell, sphere 2.5-5um | spp. | Cyanobacteria | cell | 1 | 20 | 20 | | |

| Sample | Sampling Date | Genus | Species | Algal Group | Counting Unit | Cells/Unit | Species Units/mL | Species Cells/mL | Cyano Total Units/mL | Cyano Total Cells/mL |
|---|---|---|---|---|---|---|---|---|---|---|
| PAP1 | 8/17/2012 | Microcystis | cf. aeruginosa (colony) | Cyanobacteria | colony | 19 | 23,562 | 447,673 | 105,190 | 560,111 |
| PAP1 | 8/17/2012 | Microcystis | cf. aeruginosa (unicell) | Cyanobacteria | cell | 1 | 54,977 | 54,977 | | |
| PAP1 | 8/17/2012 | Microcystis | cf. aeruginosa (cell pair) | Cyanobacteria | colony | 2 | 26,389 | 52,778 | | |
| PAP1 | 8/17/2012 | Merismopedia | punctata | Cyanobacteria | colony | 262 | 12 | 3,144 | | |
| PAP1 | 8/17/2012 | Anabaena | sp. | Cyanobacteria | filament | 5 | 236 | 1,178 | | |
| PAP1 | 8/17/2012 | Microcystis | botrys | Cyanobacteria | colony | 120 | 2 | 240 | | |
| PAP1 | 8/17/2012 | Anabaena | sp. | Cyanobacteria | filament | 10 | 12 | 120 | | |

| Sample | Sampling Date | Genus | Species | Algal Group | Counting Unit | Cells/Unit | Species Units/mL | Species Cells/mL | Cyano Total Units/mL | Cyano Total Cells/mL |
|---|---|---|---|---|---|---|---|---|---|---|
| SLR1 | 8/17/2012 | Woronichinia | naegeliana (unicell) | Cyanobacteria | cell | 1 | 5,278 | 5,278 | 7,476 | 13,649 |
| SLR1 | 8/17/2012 | Woronichinia | naegeliana (cell pair) | Cyanobacteria | colony | 2 | 1,696 | 3,393 | | |
| SLR1 | 8/17/2012 | Aphanocapsa | sp. | Cyanobacteria | colony | 22 | 63 | 1,382 | | |
| SLR1 | 8/17/2012 | Microcystis | wesenbergii | Cyanobacteria | colony | 79 | 16 | 1,241 | | |
| SLR1 | 8/17/2012 | Pseudanabaena | sp. | Cyanobacteria | filament | 9 | 94 | 848 | | |
| SLR1 | 8/17/2012 | Woronichinia | naegeliana (colony) | Cyanobacteria | colony | 16 | 24 | 377 | | |
| SLR1 | 8/17/2012 | Microcystis | botrys | Cyanobacteria | colony | 153 | 2 | 245 | | |
| SLR1 | 8/17/2012 | Microcystis | spp. (unicell) | Cyanobacteria | cell | 1 | 188 | 188 | | |
| SLR1 | 8/17/2012 | Anabaena | sp. | Cyanobacteria | filament | 23 | 8 | 181 | | |
| SLR1 | 8/17/2012 | cyanophyte tetrad | spp. | Cyanobacteria | colony | 4 | 31 | 126 | | |
| SLR1 | 8/17/2012 | Microcystis | spp. (cell pair) | Cyanobacteria | colony | 2 | 63 | 126 | | |
| SLR1 | 8/17/2012 | Anabaena | sp. | Cyanobacteria | filament | 36 | 3 | 115 | | |
| SLR1 | 8/17/2012 | Microcystis | sp. | Cyanobacteria | colony | 13 | 8 | 102 | | |
| SLR1 | 8/17/2012 | Snowella | litoralis | Cyanobacteria | colony | 100 | 0.2 | 20 | | |
| SLR1 | 8/17/2012 | Microcystis | sp. | Cyanobacteria | colony | 16 | 1 | 13 | | |
| SLR1 | 8/17/2012 | Anabaena | cf. lemmermannii | Cyanobacteria | filament | 9 | 1 | 9 | | |
| SLR1 | 8/17/2012 | cyanophyte filament | sp. | Cyanobacteria | filament | 15 | 0.2 | 3 | | |
| SLR1 | 8/17/2012 | Anabaena | sp. | Cyanobacteria | filament | 12 | 0.2 | 2 | | |

| Sample | Sampling Date | Genus | Species | Algal Group | Counting Unit | Cells/Unit | Species Units/mL | Species Cells/mL | Cyano Total Units/mL | Cyano Total Cells/mL |
|---|---|---|---|---|---|---|---|---|---|---|
| SLK2 | 9/24/2012 | Woronichinia | naegeliana (colony) | Cyanobacteria | colony | 318 | 1,021 | 324,681 | 84,401 | 902,080 |
| SLK2 | 9/24/2012 | Anabaena | circinalis | Cyanobacteria | filament | 21 | 9,425 | 197,919 | | |
| SLK2 | 9/24/2012 | Microcystis | aeruginosa | Cyanobacteria | colony | 556 | 236 | 131,003 | | |
| SLK2 | 9/24/2012 | Microcystis | ichthyoblabe (colony) | Cyanobacteria | colony | 41 | 2,199 | 90,163 | | |
| SLK2 | 9/24/2012 | Woronichinia | naegeliana (unicell) | Cyanobacteria | cell | 1 | 43,982 | 43,982 | | |
| SLK2 | 9/24/2012 | Woronichinia | naegeliana (cell pair) | Cyanobacteria | colony | 2 | 13,195 | 26,389 | | |
| SLK2 | 9/24/2012 | Microcystis | wesenbergii | Cyanobacteria | colony | 148 | 157 | 23,248 | | |
| SLK2 | 9/24/2012 | Microcystis | botrys | Cyanobacteria | colony | 87 | 236 | 20,499 | | |
| SLK2 | 9/24/2012 | Aphanocapsa | sp. | Cyanobacteria | colony | 13 | 942 | 12,252 | | |
| SLK2 | 9/24/2012 | Aphanocapsa | sp. | Cyanobacteria | colony | 30 | 314 | 9,425 | | |
| SLK2 | 9/24/2012 | Microcystis | ichthyoblabe (unicell) | Cyanobacteria | cell | 1 | 9,111 | 9,111 | | |
| SLK2 | 9/24/2012 | Microcystis | ichthyoblabe (cell pair) | Cyanobacteria | colony | 2 | 2,827 | 5,655 | | |
| SLK2 | 9/24/2012 | Anabaena | lemmermannii | Cyanobacteria | filament | 51 | 79 | 4,005 | | |
| SLK2 | 9/24/2012 | Anabaena | planctonica | Cyanobacteria | filament | 31 | 50 | 1,550 | | |
| SLK2 | 9/24/2012 | cyanophyte tetrad | spp. | Cyanobacteria | colony | 4 | 314 | 1,257 | | |
| SLK2 | 9/24/2012 | Pseudanabaena | mucicola | Cyanobacteria | filament | 3 | 314 | 942 | | |
| SLK3 | 9/24/2012 | Woronichinia | naegeliana (unicell) | Cyanobacteria | cell | 1 | 13,299 | 13,299 | 16,204 | 50,376 |
| SLK3 | 9/24/2012 | Aphanocapsa | incerta | Cyanobacteria | colony | 460 | 26 | 12,043 | | |
| SLK3 | 9/24/2012 | Anabaena | circinalis | Cyanobacteria | filament | 24 | 367 | 8,796 | | |
| SLK3 | 9/24/2012 | Woronichinia | naegeliana (colony) | Cyanobacteria | colony | 189 | 26 | 4,948 | | |
| SLK3 | 9/24/2012 | Woronichinia | naegeliana (cell pair) | Cyanobacteria | colony | 2 | 2,094 | 4,189 | | |
| SLK3 | 9/24/2012 | Microcystis | wesenbergii | Cyanobacteria | colony | 313 | 6 | 1,774 | | |
| SLK3 | 9/24/2012 | Microcystis | aeruginosa | Cyanobacteria | colony | 947 | 1 | 1,263 | | |
| SLK3 | 9/24/2012 | Microcystis | ichthyoblabe (colony) | Cyanobacteria | colony | 85 | 13 | 1,113 | | |
| SLK3 | 9/24/2012 | Aphanocapsa | sp. | Cyanobacteria | colony | 18 | 52 | 942 | | |
| SLK3 | 9/24/2012 | Aphanothece | sp. | Cyanobacteria | colony | 13 | 52 | 681 | | |
| SLK3 | 9/24/2012 | cyanophyte filament | sp. | Cyanobacteria | filament | 8 | 52 | 419 | | |
| SLK3 | 9/24/2012 | cyanophyte tetrad | spp. | Cyanobacteria | colony | 4 | 105 | 419 | | |
| SLK3 | 9/24/2012 | Microcystis | botrys | Cyanobacteria | colony | 167 | 1 | 223 | | |
| SLK3 | 9/24/2012 | cyanophyte cell pair | spp. | Cyanobacteria | colony | 2 | 52 | 105 | | |
| SLK3 | 9/24/2012 | Anabaena | planctonica | Cyanobacteria | filament | 25 | 3 | 67 | | |
| SLK3 | 9/24/2012 | cyanophyte unicell, sphere 2.5-5um | spp. | Cyanobacteria | cell | 1 | 52 | 52 | | |
| SLK3 | 9/24/2012 | Anabaena/Aphanizomenon | sp. | Cyanobacteria | filament | 56 | 0.3 | 19 | | |
| SLK3 | 9/24/2012 | Microcystis | sp. | Cyanobacteria | colony | 46 | 0.3 | 15 | | |
| SLK3 | 9/24/2012 | Pseudanabaena | sp. | Cyanobacteria | filament | 31 | 0.3 | 10 | | |

# Appendix B

## Microcystin Lab ReportsTitle

# Microcystin Analysis Report
**Project: ESS Group**
(Melville Pond)

| Sample Identification | Sample Collection Date |
|---|---|
| Melville Pond | 7/23/12 |

**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 recoveries for a laboratory fortified blank (LFB) spiked with 1 µg/L MCLR and the LFM were 94% and 113%, respectively.

**Summary of Results**

| Sample | MC levels (µg/L) |
|---|---|
| Melville Pond | ND |

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

Submitted by: Mark T. Aubel, Ph.D.
Date: 7/25/12

**GreenWater Laboratories**
205 Zeagler Drive
Suite 302
Palatka FL 32177
Ph (386) 328-0882
Fax (386) 328-0882

Contact: markaubel@greenwaterlab.com
amandafoss@greenwaterlab.com

**ESS Group**

**MICROCYSTIN RESULTS**

**Tested on:** 7/25/2012
**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) |
|---|---|---|---|---|---|---|---|---|
| Melville Pond | 1x | none | ND | 1 | 94 | 113 | ND | ND |
| 7/23/12 | 1x | none | ND | 1 | 94 | 113 | 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: Mark T. Aubel, Ph.D.
Date: 7/25/2012

Submitted to: Matt Ladewig
ESS Group
401 Wampanoag Trail
Suite 400
East Providence RI 02915
(401) 330-1204
mladewig@essgroup.com

# Microcystin Analysis Report
**Project: ESS Group**
(Mashapaug Pond)

| Sample Identification | Sample Collection Date |
|---|---|
| MASH P (Mashapaug Pond) | 8/8/12 |

**Toxin –** Microcystin (MC)

**Sample Prep –** The sample was ultrasonicated to lyse cells and release toxins. A sample dilution (1:2) was necessary to acquire an absorbance value within range of the standard curve.

**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 93%.

**Summary of Results**

| Sample | MC levels (µg/L) |
|---|---|
| MASH P (Mashapaug Pond) | 7.0 |

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

Submitted by: Mark T. Aubel, Ph.D.
Date: 8/10/12

**GreenWater Laboratories**
205 Zeagler Drive
Suite 302
Palatka FL 32177
Ph (386) 328-0882
Fax (386) 328-0882

Contact: markaubel@greenwaterlab.com
amandafoss@greenwaterlab.com

**ESS Group**

## MICROCYSTIN RESULTS

**Tested on:** **8/10/2012**
**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) |
|---|---|---|---|---|---|---|---|---|
| Mash P (Mashapaug Pond) | 1x | 1:2 | 3.73 | 2 | 93 | - | 7.5 | 7.0 |
| 8/8/12 | 1x | 1:2 | 3.23 | 2 | 93 | - | 6.5 | |

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: 8/10/2012

Submitted to: Matt Ladewig
ESS Group
401 Wampanoag Trail
Suite 400
East Providence RI 02915
(401) 330-1204
mladewig@essgroup.com

# Microcystin Analysis Report
**Project: ESS Group**
(Almy Pond)

| Sample Identification | Sample Collection Date |
|---|---|
| Almy Pond (ALP1) | 8/16/12 |

**Toxin –** Microcystin (MC)

**Sample Prep –** The sample was ultrasonicated to lyse cells and release toxins.

**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**

| Sample | MC levels (µg/L) |
|---|---|
| Almy Pond (ALP1) | 1.5 |

LOD/LOQ = 0.15 µg/L

Submitted by: ______________
Mark T. Aubel, Ph.D.
Date: 8/23/12

**GreenWater Laboratories**
205 Zeagler Drive
Suite 302
Palatka FL 32177
Ph (386) 328-0882
Fax (386) 328-0882

Contact: markaubel@greenwaterlab.com
amandafoss@greenwaterlab.com

**ESS Group**

## MICROCYSTIN RESULTS

**Tested on:** 8/23/2012
**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) |
|---|---|---|---|---|---|---|---|---|
| ALP1 Almy Pond | 1x | none | 1.52 | 1 | 94 | - | 1.5 | 1.5 |
| 8/16/12 | 1x | none | 1.55 | 1 | 94 | - | 1.6 | |

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: 8/23/2012

Submitted to: Matt Ladewig
ESS Group
401 Wampanoag Trail
Suite 400
East Providence RI 02915
(401) 330-1204
mladewig@essgroup.com

# Microcystin Analysis Report

**Project: ESS Group**
(Barber Pond)

| Sample Identification | Sample Collection Date |
|---|---|
| BAP1 (Barber Pond- Launch) | 8/16/12 |
| BAP2 (Barber Pond- Center) | 8/16/12 |

**Toxin –** Microcystin (MC)

**Sample Prep –** The samples were ultrasonicated to lyse cells and release toxins. A duplicate BAP2 sample was spiked (lab fortified matrix, LFM) 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 94%.

## Summary of Results

| Sample | MC levels (µg/L) |
|---|---|
| BAP1 (Barber Pond- Launch) | 4.8 |
| BAP2 (Barber Pond- Center) | 0.4 |

LOD/LOQ = 0.15 µg/L

Submitted by: ______________
Mark T. Aubel, Ph.D.

Date: 8/17/12

**GreenWater Laboratories**
205 Zeagler Drive
Suite 302
Palatka FL 32177
Ph (386) 328-0882
Fax (386) 328-0882

Contact: markaubel@greenwaterlab.com
amandafoss@greenwaterlab.com

**ESS Group**

## MICROCYSTIN RESULTS

**Tested on:** 8/17/2012
**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) |
|---|---|---|---|---|---|---|---|---|
| BAP1 Barber Pond (Launch) | 1x | none | 5.00 | 1 | 94 | - | 5.0 | 4.8 |
| 8/16/12 | 1x | none | 4.53 | 1 | 94 | - | 4.5 | |
| BAP2 Barber Pond (Center) | 1x | none | 0.37 | 1 | 94 | 118 | 0.4 | 0.4 |
| 8/16/12 | 1x | none | 0.36 | 1 | 94 | 118 | 0.4 | |

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: 8/17/2012

Submitted to: Matt Ladewig
ESS Group
401 Wampanoag Trail
Suite 400
East Providence RI 02915
(401) 330-1204
mladewig@essgroup.com

# Microcystin Analysis Report

**Project: ESS Group**

| Sample Identification | Sample Collection Date |
|---|---|
| UCR1 (Upper Curran Reservoir) | 8/16/12 |
| SPP1 (Spectacle Pond) | 8/16/12 |
| BMP1 (Blackamore Pond) | 8/16/12 |
| RWP1 (Roger Williams Park Pond) | 8/16/12 |
| RWP2 (Roger Williams Park Pond) | 8/16/12 |
| WAP1 (Warwick Pond) | 8/17/12 |
| SLR1 (Slack's Reservoir) | 8/17/12 |
| SCP1 (Scott Pond) | 8/17/12 |
| SMP1 (Slater Memorial Park Pond) | 8/17/12 |
| SMP2 (Slater Memorial Park Pond) | 8/17/12 |
| TUR1 (Turner Reservoir) | 8/17/12 |

**Toxin –** Microcystin (MC)

**Sample Prep –** The samples were ultra-sonicated to lyse all cells and release toxins. Duplicate samples of BMP1, SLR1, SCP1 and SMP1 were spiked (lab fortified matrix, LFM) 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 recoveries for laboratory fortified blanks (LFB) spiked with 1 µg/L MCLR ranged from 83 - 94%.


205 Zeagler Drive, Suite 302 • Palatka, FL 32177
info@greenwaterlab.com • www.greenwaterlab.com
386.328.0882,voice • 386.328.9646,fax
cyanomail@cyanolab.com • www.cyanolab.com

**Summary of Results**

| Sample | MC levels (µg/L) |
|---|---|
| UCR1 (Upper Curran Reservoir) | 0.20 |
| SPP1 (Spectacle Pond) | ND |
| BMP1 (Blackamore Pond) | ND |
| RWP1 (Roger Williams Park Pond) | ND |
| RWP2 (Roger Williams Park Pond) | ND |
| WAP1 (Warwick Pond) | ND |
| SLR1 (Slack's Reservoir) | 0.16 |
| SCP1 (Scott Pond) | 0.67 |
| SMP1 (Slater Memorial Park Pond) | 0.27 |
| SMP2 (Slater Memorial Park Pond) | 0.29 |
| TUR1 (Turner Reservoir) | ND |

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

Submitted by: Mark T. Aubel, Ph.D.
Date: 9/6/12

**GreenWater Laboratories**
205 Zeagler Drive
Suite 302
Palatka FL 32177
Ph (386) 328-0882
Fax (386) 328-0882

Contact: markaubel@greenwaterlab.com
amandafoss@greenwaterlab.com

**ESS Group**

**MICROCYSTIN RESULTS**

**Tested on:** 8/17/2012, 8/28/12 & 9/5/12
**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) |
|---|---|---|---|---|---|---|---|---|
| UCR1 Upper Curran Reservoir | 1x | none | 0.19 | 1 | 94 | - | 0.19 | 0.20 |
| 8/16/12 | 1x | none | 0.20 | 1 | 94 | - | 0.20 | |
| SPP1 Spectacle Pond | 1x | none | 0.06 | 1 | 94 | - | ND | ND |
| 8/16/12 | 1x | none | 0.07 | 1 | 94 | - | ND | |
| BMP1 Blackamore Pond | 1x | none | 0.10 | 1 | 94 | 123 | ND | ND |
| 8/16/12 | 1x | none | 0.11 | 1 | 94 | 123 | ND | |
| RWP1 Roger Willams Park Pond | 1x | none | 0.05 | 1 | 94 | - | ND | ND |
| 8/16/12 | 1x | none | 0.03 | 1 | 94 | - | ND | |
| RWP2 Roger Willams Park Pond | 1x | none | 0.11 | 1 | 94 | - | ND | ND |
| 8/16/12 | 1x | none | 0.16 | 1 | 94 | - | ND | |
| WAP1 Warwick Pond | 1x | none | 0.07 | 1 | 84 | - | ND | ND |
| 8/17/12 | 1x | none | 0.07 | 1 | 84 | - | ND | |
| SLR1 Slack's Reservoir | 1x | none | 0.13 | 1 | 83 | 104 | 0.13 | 0.16 |
| 8/17/12 | 1x | none | 0.19 | 1 | 83 | 104 | 0.19 | |
| SCP1 Scott Pond | 1x | none | 0.64 | 1 | 84 | 116 | 0.64 | 0.67 |
| 8/17/12 | 1x | none | 0.69 | 1 | 84 | 116 | 0.69 | |
| SMP1 Slater Memorial Park Pond | 1x | none | 0.26 | 1 | 84 | 79 | 0.26 | 0.27 |
| 8/17/12 | 1x | none | 0.27 | 1 | 84 | 79 | 0.27 | |
| SMP2 Slater Memorial Park Pond | 1x | none | 0.26 | 1 | 84 | - | 0.26 | 0.29 |
| 8/17/12 | 1x | none | 0.31 | 1 | 84 | - | 0.31 | |
| TUR1 Turner Reservoir | 1x | none | 0.00 | 1 | 84 | - | ND | ND |
| 8/17/12 | 1x | none | 0.00 | 1 | 84 | - | 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: 9/5/2012

Submitted to: Matt Ladewig
ESS Group
401 Wampanoag Trail
Suite 400
East Providence RI 02915
(401) 330-1204
mladewig@essgroup.com

GreenWater laboratories
aquatic analysis ... research ... consulting

# Microcystin Analysis Report

**Project: ESS Group**

(Pasquiset Pond)

| <u>Sample Identification</u> | <u>Sample Collection Date</u> |
|---|---|
| PAP1 (Pasquiset Pond) | 8/17/12 |

**Toxin –** Microcystin (MC)

**Sample Prep –** The sample was ultra-sonicated to lyse all cells and release toxins. A sample dilution (1:10) was necessary to accommodate the calibrated range for ELISA analysis.

**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 83%.

**Summary of Results**

| <u>Sample</u> | <u>MC levels</u> (µg/L) |
|---|---|
| PAP1 (Pasquiset Pond) | 5.3 |

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

Submitted by: Mark T. Aubel, Ph.D.
Date: 8/28/12

Cyano LAB
205 Zeagler Drive, Suite 302 • Palatka, FL 32177
info@greenwaterlab.com • www.greenwaterlab.com
386.328.0882,voice • 386.328.9646,fax
cyanomail@cyanolab.com • www.cyanolab.com

**GreenWater Laboratories**
205 Zeagler Drive
Suite 302
Palatka FL 32177
Ph (386) 328-0882
Fax (386) 328-0882

Contact: markaubel@greenwaterlab.com
amandafoss@greenwaterlab.com

**ESS Group**

**MICROCYSTIN RESULTS**

**Tested on:** **8/28/2012**
**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) |
|---|---|---|---|---|---|---|---|---|
| PAP1 Pasquiset Pond | 1x | 1:10 | 0.50 | 10 | 83 | - | 5.0 | 5.3 |
| 8/17/12 | 1x | 1:10 | 0.55 | 10 | 83 | - | 5.5 | |

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: 8/28/2012

Submitted to: Matt Ladewig
ESS Group
401 Wampanoag Trail
Suite 400
East Providence RI 02915
(401) 330-1204
mladewig@essgroup.com

# Microcystin Analysis Report
**Project: ESS Group**
(Slack Reservoir)

| Sample Identification | Sample Collection Date |
|---|---|
| SLK2 | 9/24/12 |
| SLK3 | 9/24/12 |

**Toxin –** Microcystin (MC)

**Sample Prep –** The samples were ultrasonicated to lyse cells and release toxins. A dilution (1:10) of sample SLK2 was necessary to accommodate the calibrated range for ELISA analysis.

**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 81%.

## Summary of Results

| Sample | MC levels (µg/L) |
|---|---|
| SLK2 | 48 |
| SLK3 | 3.7 |

LOD/LOQ = 0.15 µg/L

Submitted by: Mark T. Aubel, Ph.D.

Date: 9/26/12

**GreenWater Laboratories**
205 Zeagler Drive
Suite 302
Palatka FL 32177
Ph (386) 328-0882
Fax (386) 328-0882

Contact: markaubel@greenwaterlab.com
amandafoss@greenwaterlab.com

**ESS Group**

**MICROCYSTIN RESULTS**

**Tested on:** 9/26/2012
**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) |
|---|---|---|---|---|---|---|---|---|
| SLK#2 | 1x | 1:10 | 4.90 | 10 | 81 | - | 49.0 | 48 |
| 9/24/12 | 1x | 1:10 | 4.70 | 10 | 81 | - | 47.0 | |
| SLK#3 | 1x | none | 3.60 | 1 | 81 | - | 3.60 | 3.7 |
| 9/24/12 | 1x | none | 3.83 | 1 | 81 | - | 3.83 | |

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: 9/26/2012

Submitted to: Matt Ladewig
ESS Group
401 Wampanoag Trail
Suite 400
East Providence RI 02915
(401) 330-1204
mladewig@essgroup.com