

---

TENMILE LAKES TOXIC ALGAL SAMPLING PROGRAM: 2012 DATA  
SUMMARY REPORT

---



Prepared By:

**Jacob Kann, Ph.D.**

**Lisa Bowater**

AQUATIC ECOSYSTEM SCIENCES, LLC

295 East Main St., Suite 7

Ashland, OR 97520

Prepared For:

**Tenmile Lakes Basin Partnership**

P.O. Box L

Lakeside OR 97520

March 2013

# TABLE OF CONTENTS

List of Tables .....	3
List of Figures.....	3
Background.....	4
Methods .....	4
Results .....	6
<i>2012 Cell Density and Toxin Trends-Standard Stations</i> .....	6
<i>2012 Cell Density and Toxin Trends-Ancillary Stations</i> .....	10
<i>Quality Assurance Related to Species Identification, Cell Density, and Algal Toxins</i> .....	13
Summary.....	19
Literature Cited.....	20
Appendix I: Lake Superior State University Environmental Analysis Laboratory Phytoplankton Reports .....	23
Appendix II: Aquatic Analysts Phytoplankton Reports.....	40
Appendix III: PhycoTech Laboratories Phytoplankton Report; N16: 9/4/12 .....	43
Appendix IV: Multi Lab Phytoplankton Comparison .....	44
Appendix V: Lake Superior State University Environmental Analysis Laboratory Algal Toxin Results .....	46
Appendix VI: GreenWater Laboratories Algal Toxin Results.....	55
Appendix VII: Oregon State University Dreher Lab Results .....	58
Appendix VIII: Oregon DHS and Tenmile Lakes Harmful Algal Bloom Information .....	62

**LIST OF TABLES**

Table 1. Algal Cell Density for Potentially Toxigenic Species in Tenmile Lakes, 2012 (see below description for public health color coding). Blue shaded stations refer to ancillary stations located in the vicinity of visual blooms. ....9

Table 2. Cell density and toxin results for Ancillary Stations, Tenmile Lakes, 2009-2012. ....11

Table 3. Inter-lab cell density analysis for various Tenmile Lake stations, 2012. ....13

Table 4. Inter-lab comparison (blue-green algae only) for South Tenmile Lake station Davis Dock, September 17, 2012. All species and parameters are contained in Appendix IV. ....15

Table 5. Toxin duplicate results for Ancillary Stations, Tenmile Lakes, 2012. ....16

**LIST OF FIGURES**

Figure 1. Location of standard and ancillary toxic algal sampling stations in Tenmile Lakes, 2012. ....4

Figure 2. Tenmile Lakes Monitoring Coordinator Richard Litts collecting plankton net samples July 9, 2012. ....5

Figure 3. Cell Density of total Anabaena, Microcystis aeruginosa and Coelosphaerium naegelianum in Tenmile Lakes at standard sampling stations, 2012. The pink shaded symbols in the lower graph show the two values of Microcystis, the rest of the values are Coelosphaerium. ....8

Figure 4. Bloom conditions in Coleman Arm, South Tenmile Lake, September 17, 2012. ....10

Figure 5. Cell Density of total Anabaena, Microcystis aeruginosa and Coelosphaerium naegelianum in Tenmile Lakes at all sampling stations, 2012. ....12

Figure 6. Micrograph of a Coelosphaerium/Woronichinia colony from Davis Dock in South Tenmile Lake on August 20, 2012. ....14

Figure 7. Cell density of Microcystis aeruginosa, Coelosphaerium naegelianum and total Anabaena at standard open-water stations in Tenmile Lakes, 2002-2012; time-series of individual data points (a), and box plots grouped by station (b). ....18

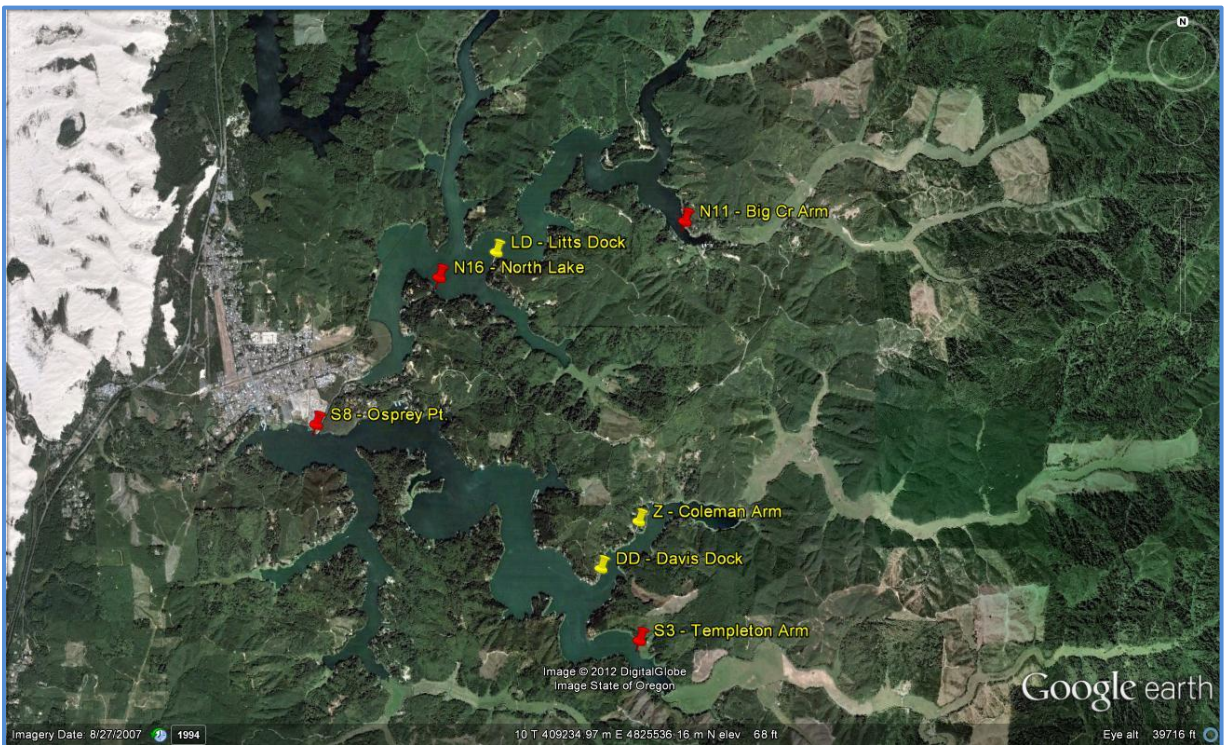
Figure 8. Cell density of Microcystis aeruginosa, Coelosphaerium naegelianum and total Anabaena in Tenmile Lakes during September, 2002-2012; boxes represent the distribution of both North and South Tenmile Lakes combined. ....18

## BACKGROUND

Long-term public health monitoring for potentially toxigenic cyanobacteria (blue-green algae) in Tenmile Lakes has occurred since 2002 (Kann 2008; Kann 2012; Jacoby and Kann 2007). Continued monitoring of Tenmile Lakes occurred in 2012 to assess the dynamics of potentially toxic blue-green algal species, including *Microcystis aeruginosa*, *Gloeotrichia echinulata*, and various *Anabaena* species. *Microcystis* and *Gloeotrichia* can produce hepatotoxins (known as microcystins), and *Anabaena* produces both neurotoxins (anatoxin-a) and microcystins. Both toxins are capable of harmful effects to animals and humans (Chorus and Bartram 1999). A toxic bloom of *M. aeruginosa* was first documented in Tenmile Lakes in September of 1997, prompting the Oregon Department of Health to issue a health advisory recommending that the lakes not be used for drinking water (numerous private homes around the lakes utilize treated lake water for potable purposes) and that contact recreation be avoided (Kann and Gilroy 1997). The goal of the 2012 sampling, performed by the Tenmile Lakes Basin Partnership, was to determine cell density and toxin levels of these potentially toxigenic species at a limited number of sampling stations. Cell density and toxin levels of potentially toxigenic species were then compared to drinking water guidance levels for lakes and reservoirs (e.g., Yoo et al. 1995; Chorus and Bartram 1999), as well as State of Oregon recreational guideline values (Oregon DHS 2011; Stone and Bress 2007).

## METHODS

Four long-term standard sampling stations (2 in each lake) were monitored to cover a major arm and open-water location in each lake (Fig. 1; red symbols). Stations S8 and N16 are centrally located and S3 and N11 are located near the terminus of Templeton Arm and Big Creek Arm, respectively. These stations were sampled 9 times beginning May 30<sup>th</sup> and ending October 1<sup>st</sup>, 2012.



**Figure 1. Location of standard and ancillary toxic algal sampling stations in Tenmile Lakes, 2012.**



At these standard stations, samples were collected mid-day (to approximate conditions that may pose a maximum public health risk) and integrated over the upper 1/3 of the water column at the open-water stations (S8 and N16), and over the entire water column at the shallow stations (S3 and N11). At each of the established sampling locations a vertical tow ranging between 1 to 2.5 meters of the water column (depending on location) was made using a 64- $\mu\text{m}$  plankton net (Figure 2).



**Figure 2. Tenmile Lakes Monitoring Coordinator Richard Litts collecting plankton net samples July 9, 2012.**

In addition, grab-samples were obtained from a series of ancillary stations (Figure 1; yellow symbols) that were sampled to assess blooms that were visibly noticeable in areas not overlapping the standard long-term stations. The protocol calls for surface grab samples to be collected at the long-term stations when visible surface accumulations are present. In this fashion presence of cyanobacterial cells that may migrate within the water column are captured with the integrated samples, while maximum concentrations relative to public health exposure are captured with the surface grab samples.

For both standard (where filtered contents of 3 replicate hauls were composited in a bucket) and ancillary stations, contents were placed in a 250 ml opaque sample bottle containing 1% Lugol's preservative and shipped to Ben Southwell of Lake Superior State University Environmental Analysis Laboratory (LSSU-EAL)<sup>1</sup>, who performed a microscopic analysis for algal cell density (cells  $\text{ml}^{-1}$ ). The combined density of *Microcystis aeruginosa* (MSAE) and *Gloeotrichia echinulata* (GTEC) is computed in table 1 because both MSAE and GTEC are potential

---

<sup>1</sup> LSSU-EAL is a contract lab with Oregon Health Authority (OHA) and because prices were substantially lower than previous labs for both algal counts and toxin the TLBP sent samples to LSSU during 2012. The lab change necessitated that various cross-laboratory comparisons be performed to evaluate trends relative to previous years. These comparisons are discussed below.

microcystin producers (Carey et al. 2007). On several occasions duplicate samples were preserved and shipped to Aquatic Analysts, INC and PhycoTech Laboratories for QA purposes.

For toxin analyses at standard stations the contents of 3 replicate hauls taken with a tube sampler were composited in a bucket and approximately 600 mls were placed in a 1 liter bottle with no preservative. These samples, along with 600 mls of the ancillary station grab-samples were shipped overnight air with blue-ice to LSSU-EAL for HPLC/PDA<sup>2</sup> analysis of microcystin, anatoxin-a and cylindrospermopsin.<sup>3</sup> For QA purposes limited duplicate toxin samples were sent to CyanoLab (division of GreenWater Labs in Palatka, FL) for the enzyme linked immunosorbent assay (ELISA) of microcystin toxin and cylindrospermopsin, and to Oregon State University (OSU) for cylindrospermopsin using LC-MS/MS with LOQ=0.005 µg/L (5 ng/L) and LOD=0.001 µg/L (1 ng/L). Dr. Theo Dreyer's lab at OSU also provided a genetic analysis of the same OSU sample analyzed for cylindrospermopsin.

## **RESULTS**

### **2012 Cell Density and Toxin Trends-Standard Stations**

Results from the first sample trip (May 30<sup>th</sup>) showed that all samples were well below drinking water and recreational public health guidelines for cell density<sup>4</sup> and algal toxin levels for both *Anabaena* and *Microcystis aeruginosa*<sup>5</sup> in North and South Tenmile Lakes. On June 18<sup>th</sup> *Anabaena* cell density increased at the standard South Lake station S3 and the North Lake stations N11 and N16, exceeding the WHO Alert Level 1 drinking water guideline (Yoo et al. 1995; also known as the increased vigilance level for drinking water systems) of 500 cells ml<sup>-1</sup> for potentially toxigenic species (Table 1; Figure 3). Given variable toxin production the actual algal toxin levels are generally more informative with respect to public health risk, and as expected based on the low cell densities, algal toxin results for samples taken on June 18<sup>th</sup> show that all samples tested for microcystin were below the OHA and WHO drinking water microcystin guideline level of 1 µg/L. Microcystin was present only at station N11 (0.3 µg/L) (Table 1), and anatoxin-a and cylindrospermopsin levels were below the detection limit of 0.2 µg/L.

On the following sample date of July 9<sup>th</sup>, results showed that all samples were well below cell density and algal toxin public health alert limits for both *Anabaena* and *Microcystis aeruginosa* in North and South Tenmile Lakes (Table 1; Figure 3). *Microcystis aeruginosa* was not present at any of the standard sampling stations. *Anabaena* cell density decreased at the standard South

---

<sup>2</sup> High Performance Liquid Chromatography/Photo Diode Array

<sup>3</sup> In previous years health advisories and media outreach were initiated based upon cell density and not toxin concentration; however in 2012 the option of basing public health advisories on toxin levels was initiated by the OHA (2012).

<sup>4</sup> Results described in this section are as reported by LSSU\_EAL; as shown in the below QA section, cell density levels were substantially lower than previous years due to laboratory method bias and are not comparable to public health comparisons made in previous years. Thus, cell density results in this section are intended to show general seasonal trends but not absolute levels. Fortunately toxin analyses were performed for each sample such that public health guidelines could still be adequately evaluated.

<sup>5</sup> A change to 2012 reporting is the inclusion of *Coelosphaerium naegelianum* (alternatively identified as *Woronichinia naegeliana*) in Table1. Although generally not considered to be a toxigenic species, *Coelosphaerium* has similar morphology to *Microcystis* and there is some indication that the previous laboratory may have included *Coelosphaerium* in its counts of *Microcystis* (see further elaboration below). *Coelosphaerium* did not exceed 500 cells/ml at any of the standard stations during 2012.

Lake station S3 and the North Lake stations N11 and N16, with levels falling below the public health alert limits. Station S8 showed an increase in *Anabaena* but levels remained low (Table 1, Figure 3). Microcystin was detected at stations N16 (0.42 µg/L) and S8 (0.30 µg/L). Anatoxin-a and cylindrospermopsin levels were below the detection limit of 0.2 µg/L.

The next three sample dates of July 23<sup>rd</sup>, August 6<sup>th</sup> and August 20<sup>th</sup> resulted in low levels of *Anabaena* cell density at all of the standard North and South Lake stations. All levels were below cell density public health alert limits for *Anabaena*. *Microcystis* was not present during any of these three sampling dates. No toxins were detected on July 23<sup>rd</sup> at any standard stations. August 6<sup>th</sup> had low levels of microcystin at stations S3 (0.38 µg/L) and S8 (0.20 µg/L). Anatoxin-a was detected at stations S8 (0.58 µg/L) and N16 (0.27 µg/L) on August 6<sup>th</sup>. Microcystin and anatoxin-a samples from August 20<sup>th</sup> were all below detection limits at the standard stations (Table 1); however, cylindrospermopsin was detected at N16 (2.7 µg/L) exceeding the OHA provisional acute drinking water toxicity value of 1 µg/L for cylindrospermopsin (in finished water-- at which time OHA recommends that public water systems issue a “Do Not Drink” advisory). However, the cylindrospermopsin level was below the recreational guideline of 6 µg/L (OHA 2012). It was not clear which algal species was responsible for the cylindrospermopsin production; although low levels of *Coelosphaerium*, *Anabaena*, and *Aphanizomenon flos-aquae* were present, no *Cylindrospermopsis* was detected. In addition, other QA samples were not able to confirm the presence of cylindrospermopsin in Tenmile Lakes (see below).

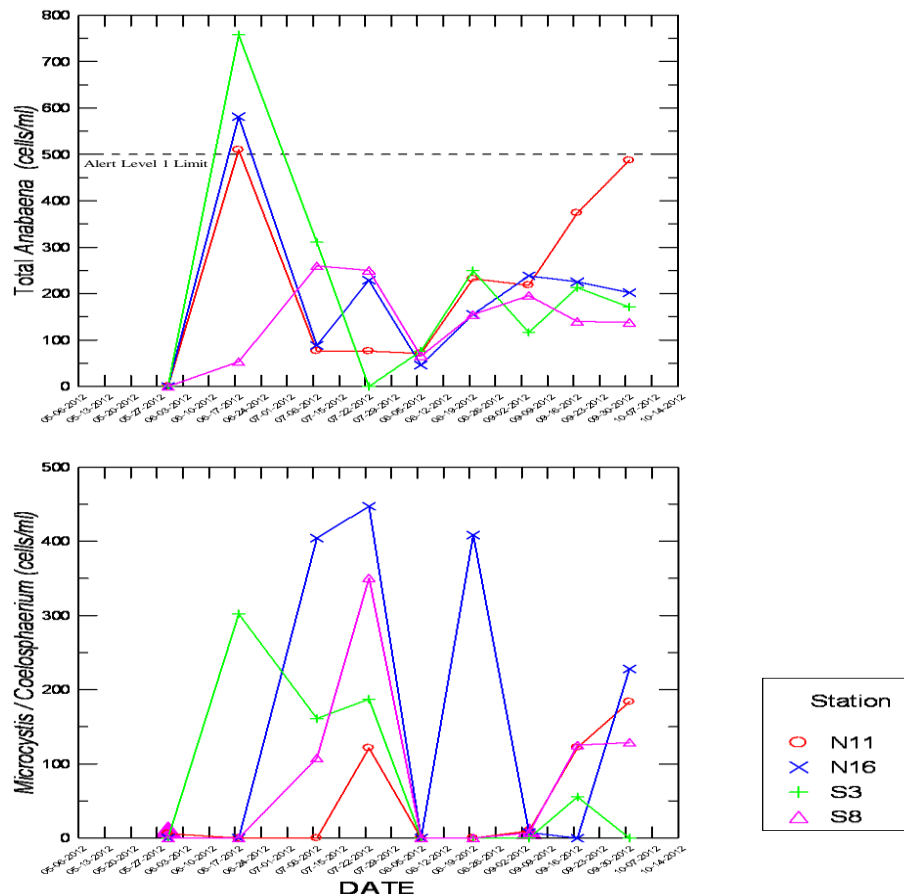
Overall May through July biovolume for standard stations was dominated by the diatom *Fragilaria crotonesis* (Appendix I). Biovolume throughout August was dominated by the dinoflagellate *Ceratium hirundinella* and the cyanobacterium (blue-green) *Aphanizomenon flos-aquae* (APFA; Appendix I). Although APFA has been shown to produce neurotoxins in other areas of the world, doubt exists whether the reported species are in fact the same APFA found in this region, and evidence indicates that toxin-producing species differ from those typically occurring in Oregon or other areas of North America (Li et al. 2000). Various Chrysophytes, Cryptophytes, Dinoflagellates, Blue-greens, Greens and Diatoms comprised the remainder of the biovolume at all stations (Appendix I).

Levels of *Anabaena* and *Microcystis* remained low throughout September at the standard stations. On September 4<sup>th</sup> samples were well below public health alert limits for both *Anabaena* and *Microcystis aeruginosa* in North and South Tenmile Lakes (Table 1). *Microcystis aeruginosa* was detected at a very low level at station S8 (9 cells/ml) and was not detected at any of the other standard sampling stations throughout the remaining sampling season. Microcystin was only detected in the North Lake at stations N11 (0.30 µg/L) and N16 (0.52 µg/L). Anatoxin-a and cylindrospermopsin were not present at any stations on September 4<sup>th</sup>.

On September 17<sup>th</sup> *Anabaena* was present at low levels at all of the standard sampling stations (less than 400 cells/ml) (Table 1, Figure 3). Microcystin was present again in the North Lake at stations N11 (0.20 µg/L) and N16 (0.30 µg/L). Anatoxin-a and cylindrospermopsin were not present at any stations. Biovolume throughout September continued to be dominated by the dinoflagellate *Ceratium hirundinella* and the cyanobacterium (blue-green) *Aphanizomenon flos-aquae* along with the diatom *Melosira granulata*.

Overall, blue-green algae (cyanobacteria) levels remained low into October. Samples from October 1<sup>st</sup> showed low levels of total *Anabaena* at all open water stations. MSAE was not present at any stations. Microcystin was only detected in the South Lake at stations S3 (0.40 µg/L) and S8 (0.20 µg/L). Anatoxin-a and cylindrospermopsin continued to be below detection limits (Table 1). In October the predominant species were *Ceratium hirundinella* followed by the diatom *Melosira granulata* (Appendix I).

Cell densities at the standard stations in North Tenmile Lake only exceeded the WHO alert level 1 guideline twice during 2012 (N11 and N16 on 6/18) and the standard stations in South Tenmile Lake only exceeded the WHO alert level 1 guideline once during 2012 (S3 on 6/18). Cell densities of potentially toxigenic species at the standard open-water stations in both North and South Lakes remained well below OHA Recreational Guideline levels during 2012. However, see footnote 3 above regarding low cell densities during 2012. Microcystin remained below the OHA and WHO drinking water microcystin guideline level of 1 µg/L throughout 2012. Cylindrospermopsin exceeded the OHA provisional acute finished drinking water toxicity value of 1 µg/L only once in 2012 (N11 on 8/20).



**Figure 3. Cell Density of total *Anabaena*, *Microcystis aeruginosa* and *Coelosphaerium naegelianum* in Tenmile Lakes at standard sampling stations, 2012. The pink shaded symbols in the lower graph show the two values of *Microcystis*, the rest of the values are *Coelosphaerium*.**



**Table 1. Algal Cell Density for Potentially Toxicogenic Species in Tenmile Lakes, 2012 (see below description for public health color coding). Blue shaded stations refer to ancillary stations located in the vicinity of visual blooms.**

Station	Date	<i>Microcystis</i> + <i>Gloeotrichia</i> (cells/ml)	Total <i>Anabaena</i> (cells/ml)	<i>Coelosphaerium</i> <i>naegelianum</i> (cells/ml)	Microcystin (µg/L)	Anatoxin-a (µg/L)	Cylindrospermopsin (µg/L)
S3	5/30/2012	0	1	0			
S8	5/30/2012	11	0	0			
N11	5/30/2012	0	0	6			
N16	5/30/2012	0	0	0			
S3	6/18/2012	0	757	302	0.00	0.00	0.00
S8	6/18/2012	0	53	0	0.00	0.00	0.00
N11	6/18/2012	0	510	0	0.30	0.00	0.00
N16	6/18/2012	0	581	0	0.00	0.00	0.00
S3	7/9/2012	0	311	161	0.00	0.00	0.00
S8	7/9/2012	0	260	108	0.30	0.00	0.00
N11	7/9/2012	0	77	0	0.00	0.00	0.00
N16	7/9/2012	0	89	404	0.42	0.00	0.00
S3	7/23/2012	0	0	187	0.00	0.00	0.00
S8	7/23/2012	0	250	351	0.00	0.00	0.00
N11	7/23/2012	0	76	122	0.00	0.00	0.00
N16	7/23/2012	0	228	447	0.00	0.00	0.00
S3	8/6/2012	0	76	0	0.38	0.00	0.00
S8	8/6/2012	0	65	0	0.20	0.58	0.00
N11	8/6/2012	0	71	0	0.00	0.00	0.00
N16	8/6/2012	0	46	0	0.00	0.27	0.00
S3	8/20/2012	0	249	0	0.00	0.00	0.00
S8	8/20/2012	0	155	0	0.00	0.00	0.00
N11	8/20/2012	0	232	0	0.00	0.00	0.00
N16	8/20/2012	0	155	409	0.00	0.00	2.70
Davis	8/20/2012	0	69,258	0	0.67	0.00	3.20
S3	9/4/2012	0	116	0	0.00	0.00	0.00
S8	9/4/2012	9	196	7	0.00	0.00	0.00
N11	9/4/2012	0	218	9	0.30	0.00	0.00
N16	9/4/2012	0	238	8	0.52	0.00	0.00
Davis	9/4/2012	9,521	104,261	390,703	1.45	0.00	0.52
Litts Dock	9/4/2012	0	23,379	50,276	0.21	0.00	0.21
S3	9/17/2012	0	213	56	0.00	0.00	0.00
S8	9/17/2012	0	140	125	0.00	0.00	0.00
N11	9/17/2012	0	374	122	0.20	0.00	0.00
N16	9/17/2012	0	226	0	0.30	0.00	0.00
Davis	9/17/2012	6,657	44,887	72,938	0.00	0.00	0.00
S3	10/1/2012	0	171	0	0.40	0.00	0.00
S8	10/1/2012	0	138	129	0.20	0.00	0.00
N11	10/1/2012	0	487	184	0.00	0.00	0.00
N16	10/1/2012	0	202	228	0.00	0.00	0.00
South Lake Z	10/1/2012	0	28,361	234,453	0.30	0.00	0.00

\*Exceeds World Health Organization Alert Level 1 increased vigilance guideline level of 500 cells/ml for potentially toxicogenic species in drinking water systems.

\*\*Exceeds World Health Organization Alert Level 2 public health posting guideline level of 2000 cells/ml for potentially toxicogenic species in drinking water systems.

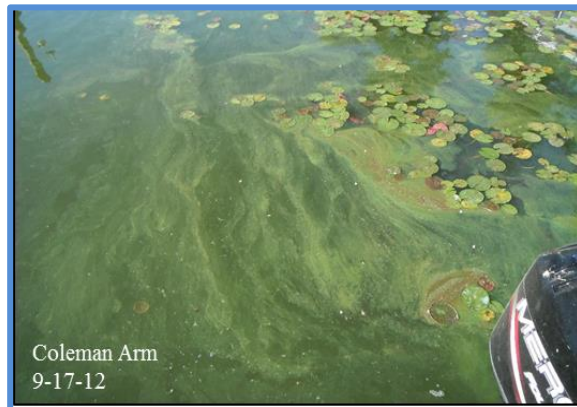
\*\*\*Exceeds State of Oregon Recreational Guideline Levels of 40,000 cells/ml for *Microcystis* or 100,000 cells/ml for *Anabaena*.

\*\*\*\* Exceeds the Oregon Department of Human Services and WHO drinking water microcystin guideline level of 1 µg/l or the Oregon Public Health Department provisional acute drinking water toxicity value of Cylindrospermopsin 1 µg/l.

Blue color indicates toxin levels above the method detection limit.

### ***2012 Cell Density and Toxin Trends-Ancillary Stations***

As noted above (see methods), several ancillary (non-standard) stations were sampled to account for patchy concentrations of cyanobacteria (Figure 4) occurring in areas that did not overlap the standard stations (i.e., S3, S8, N11, and N16). The first such event was noted during the August 20<sup>th</sup> sampling and an additional sample was collected from South Tenmile Lake at station Davis Dock (see Figure 1 above for location). Results from this station showed a concentration of *Anabaena planctonica* (69,258 cells/ml) exceeding the WHO Alert Level 2 drinking water guideline of 2,000 cells/ml (at which time OHA and local health services typically issue a public alert for drinking water lakes and reservoirs) (Table 2; Figure 5). However, no anatoxin-a (the toxin typically associated with *Anabaena*) was detected. Curiously, as noted above for N16 on this date, cylindrospermopsin was detected at a value of 3.2 µg/L (Table 2) despite the lack of *Cylindrospermopsis* detection. However, the presence of cylindrospermopsin was not confirmed in a duplicate sample analyzed at Greenwater Laboratory (Table 4).



**Figure 4. Bloom conditions in Coleman Arm, South Tenmile Lake, September 17, 2012.**

A subsequent sample taken at the Davis Dock station on September 4<sup>th</sup> showed *Microcystis* levels exceeding the WHO Alert Level 2 guideline (9,521 cells/ml) and an increase in *Anabaena* (104,261 cells/ml) which exceeded the State of Oregon Recreational Guideline Level (Table 2). Although *Microcystis* did not exceed the recreational guideline limit of 40,000 cells/ml at Davis Dock, consistent with the elevated counts, levels of microcystin (1.45 µg/L) did exceed the OHA and WHO drinking water guideline of 1 µg/L. Previous reporting indicates that *Microcystis* cell densities in that range typically exceed the drinking water guideline (Kann 2012). A duplicate sample taken from Davis Dock was analyzed at Greenwater Laboratory and also exceeded the OHA and WHO drinking water guideline for microcystin (3.9 µg/L Table 3).

An additional ancillary sample was collected on September 4<sup>th</sup> in the North Lake at station LD (Litts Dock); results showed an *Anabaena* concentration of 23,379 cells/ml exceeding the WHO Alert Level 2 limit, no detected anatoxin-a, and despite no detection of *Microcystis*, microcystin and cylindrospermopsin were both detected at low levels of 0.21 µg/L (Table 2). However, a

duplicate sample for this station (Litts Dock) was analyzed by Greenwater Laboratory and resulted in non-detects for both microcystin and cylindrospermopsin (Table 3).

On September 17<sup>th</sup> the Davis Dock station exceeded the WHO Alert Level 2 for both *Microcystis* (6,657 cells/ml) and *Anabaena* (44,887 cells/ml) (Table 2). However none of the three toxins were detected in this sample. On October 1<sup>st</sup> station South Lake Z was sampled resulting in no detection of *Microcystis* and a level of *Anabaena* (28,361) exceeding the Who Alert Level 2 guideline (Table 2). In this case, despite the *Anabaena* detection and lack of *Microcystis* detection, microcystin was the only toxin detected (0.30 µg/L) on October 1<sup>st</sup> (Table 2).

**Table 2. Cell density and toxin results for Ancillary Stations, Tenmile Lakes, 2009-2012.**

Station	Location	Date	Microcystis aeruginosa (cells/ml)	Total Anabaena (cells/ml)	Coelosphaerium naegelianum (cells/ml)	Microcystin (µg/L)	Anatoxin-a (µg/L)	Cylindrospermopsin (µg/L)	Exceedance of microcystin TDI of 0.04 µg/kg/day for a 20kg (44lb) child ingesting 100 mls <sup>1</sup> (x greater than TDI)
X	North Lake	9/8/2009	300,940	605,160		20.0	ND		2.5
South Lake Z	South Lake	9/15/2009	2,158,388	2,137		2365.0			295.6
South Lake Z	South Lake	9/21/2009	1,008,139	17,932		910.0			113.8
Z1	South Lake	10/5/2009	3,197,474	182,681		1410.0	0.6		176.3
Z1	South Lake	10/20/2009	4,664,468	853,143		1265.0	2.0		158.1
Carlson 1	North Lake	9/21/2010	3,051,153	1,232,887		460.0	ND		57.5
Carlson 1	North Lake	9/27/2010	5,939,379	314,811		149.0	ND		18.6
County Boat Ramp	South Lake	9/27/2010	1,518,783	2,301,942		705.0			88.1
X	North Lake	11/16/2010	4,446,479	669,090		645.0			80.6
N16	North Lake	11/29/2010	4,636	904		1.2			0.2
X	North Lake	11/29/2010	1,326,471	433,491		11.0			1.4
South Lake Z	South Lake	8/22/2011	96,643	939,046		0.7	ND		0.1
South Lake Intake	South Lake	8/24/2011				0.2			0.0
South Lake Tap	South Lake	8/24/2011				ND			
South Lake Z	South Lake	9/6/2011	0	15,292,913		3.00	ND		0.4
South Lake Z	South Lake	9/20/2011	452,443	882,516					
Davis	South Lake	9/20/2011	671,256	2,382,958		1.36			
Davis Dock 1	South Lake	9/30/2011				0.95			0.1
Davis Dock 2	South Lake	9/30/2011				1.58			0.2
Davis Tap	South Lake	9/30/2011				0.09			0.0
Coleman Upper	South Lake	9/30/2011				1.03			
Upper Carlson	North Lake	10/3/2011				0.78			0.1
South Lake Z	South Lake	10/3/2011	12,249	1,757		0.67			0.1
Davis	South Lake	10/3/2011	7,466	5,515		0.89			0.1
South Lake Z	South Lake	11/1/2011				NT	ND		
Davis	South Lake	8/20/2012	0	69,258	0	0.67	0.00	3.20	0.1
Davis	South Lake	9/4/2012	9,521	104,261	390,703	1.45	0.00	0.52	0.2
Litts Dock	North Lake	9/4/2012	0	23,379	50,276	0.21	0.00	0.21	0.0
Davis	South Lake	9/17/2012	6,657	44,887	72,938	0.00	0.00	0.00	0.0
South Lake Z	South Lake	10/1/2012	0	28,361	234,453	0.30	0.00	0.00	0.0

\*Exceeds World Health Organization Alert Level 1 increased vigilance guideline level of 500 cells/ml for potentially toxigenic species in drinking water systems.

\*\*Exceeds World Health Organization Alert Level 2 public health posting guideline level of 2000 cells/ml for potentially toxigenic species in drinking water systems.

\*\*\*Exceeds State of Oregon Recreational Guideline Levels of 40,000 cells/ml for *Microcystis* or 100,000 cells/ml for *Anabaena*.

\*\*\* Exceeds the Oregon Department of Human Services and WHO drinking water microcystin guideline level of 1 µg/l or the Oregon Public Health Department provisional acute drinking water toxicity value of Cylindrospermopsin 1 µg/l.

ND= Non Detect

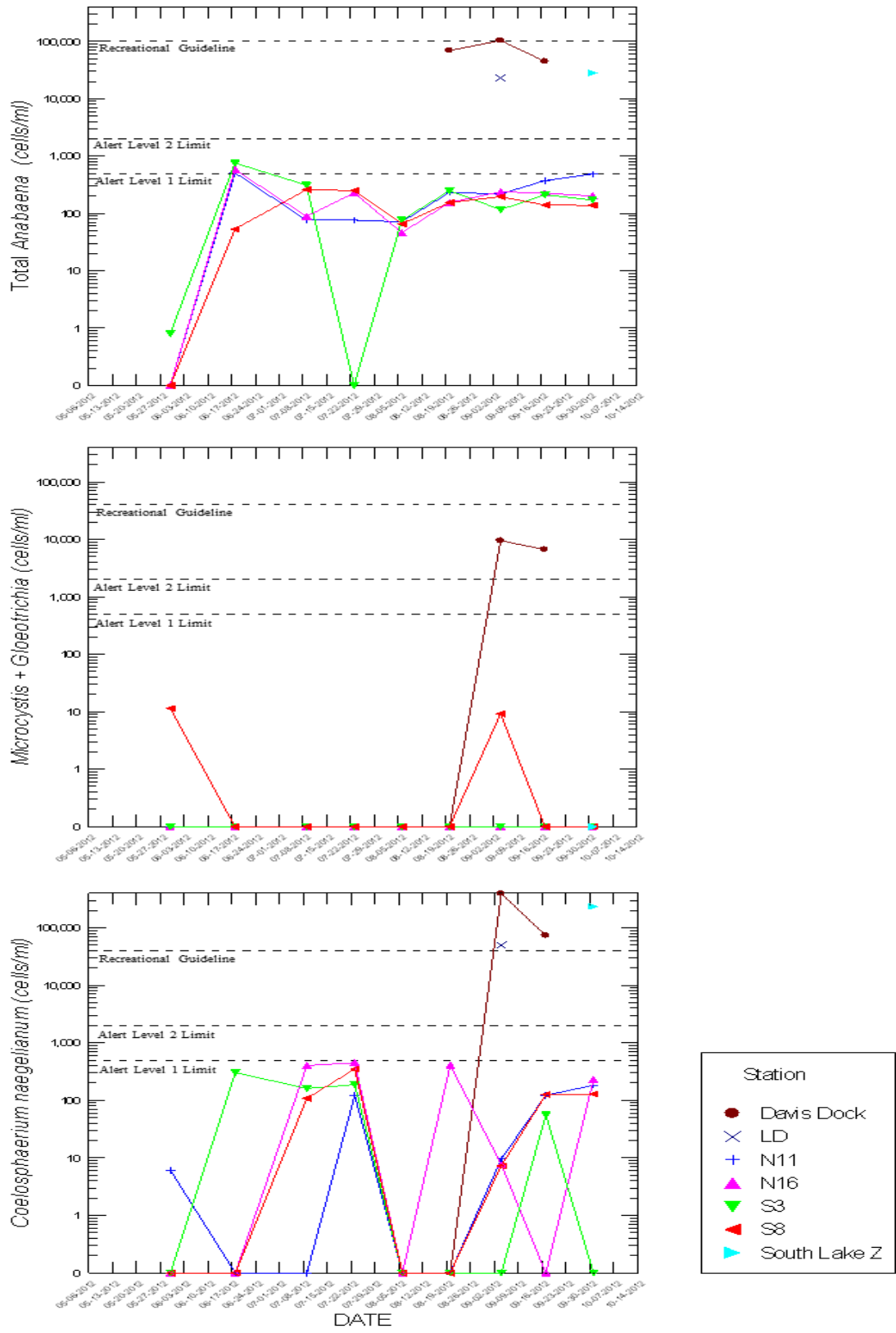


Figure 5. Cell Density of total *Anabaena*, *Microcystis aeruginosa* and *Coelosphaerium naegelianum* in Tenmile Lakes at all sampling stations, 2012.

**Quality Assurance Related to Species Identification, Cell Density, and Algal Toxins**

As noted above in the methods and results sections, in 2012 there were several issues related to presence of *Coelosphaerium*, cell density values, and detection of the toxin cylindrospermopsin. In June of 2012 the blue-green alga *Coelosphaerium naegelianum*<sup>6</sup> was detected by LSSU-EAL, and because 1) this species had not been detected previously by Aquatic Analysts (the lab providing phytoplankton data between 2002 and 2011), 2) it has morphological similarity with *Microcystis aeruginosa* (Figure 6), and 3) *Microcystis* was not detected until September of 2012 when it was historically detected earlier, duplicate lake samples were analyzed by additional labs (Table 3).

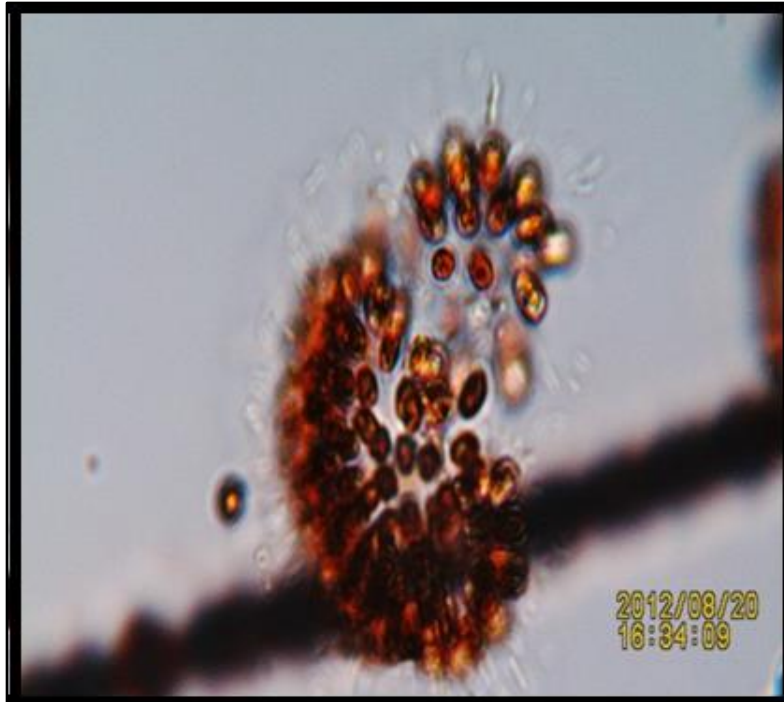
Both split samples from September 4<sup>th</sup> indicate that what Aquatic Analysts (AA) identifies as *Microcystis*, both LSSU and PhycoTech identify as *Coelosphaerium/Woronichinia*. For example, for station N16 both LSSU (8 cells/ml) and PhycoTech (287 cells/ml) reported *Coelosphaerium* or *Woronichinia*, while Aquatic Analysts reported only *Microcystis* (248 cells/ml). Given the similarity in cell density between PhycoTech and Aquatic Analysts it appears they are enumerating the same species, and even though the cell density is much lower for LSSU (see below for explanation), they are confirming of the *Coelosphaerium/Woronichinia* identification (Table 3).

**Table 3. Inter-lab cell density analysis for various Tenmile Lake stations, 2012.**

Species	Cells/ml		
	LSSU	AA	PhycoTech
5/30/2012 S8 Dup			
Aphanizomenon flos-aquae	11	87	NA
Microcystis aeruginosa	11	0	NA
9/4/2012 N16 Dup			
Anabaena planctonica	238	1,085	288
Aphanizomenon flos-aquae	963	5,993	6,708
Aphanizomenon issatschenkoi	0	0	83
Aphanocapsa delicatissima	0	0	131
Pseudanabaena limnetica	0	0	42
Synechocystis	0	0	3
Coelosphaerium naegelianum	8	0	0
Woronichinia naegeliana	0	0	287
Microcystis	0	248	0
9/4/2012 DD Dup			
Anabaena flos-aquae	0	2,967	NA
Anabaena planctonica	104,261	579,476	NA
Aphanizomenon flos-aquae	370,053	1,203,458	NA
Coelosphaerium naegelianum	390,703	0	NA
Microcystis aeruginosa	9,521	1,113,258	NA

<sup>6</sup>*Coelosphaerium naegelianum* is currently regarded as a synonym of *Woronichinia naegeliana*.





**Figure 6. Micrograph of a *Coelosphaerium*/*Woronichinia* colony from Davis Dock in South Tenmile Lake on August 20, 2012 (Photo credit: Richard Litts).**

The DD sample also shows that while LSSU reported both *Microcystis* and *Coelosphaerium*, Aquatic Analysts only reported *Microcystis*, indicating a lack of distinction between the two species for AA (Table 3). In the case of DD, the relatively low microcystin concentration (1.45 µg/L; Table 2) also indicates that *Coelosphaerium* was high relative to *Microcystis* because the former is generally considered to be non-toxic, and a value of >1 million cells/ml of *Microcystis* as reported by AA would typically be associated with a much higher concentration of microcystin (though not always).

Finally, a second set of laboratory splits from DD on 9/17/12 also shows that for the three labs that found either *Microcystis* or *Coelosphaerium* (lab C did not detect either species) all identified *Coelosphaerium*/*Woronichinia* (Table 4). Since microcystin was not detected in this sample (Table 2) it is likely that the predominant species was indeed *Coelosphaerium*-*Woronichinia*. It is unclear whether *Coelosphaerium*/*Woronichinia* is a relatively recent occurrence in Tenmile Lakes, or if it had been previously identified as *Microcystis*. However, as shown in Table 2, the very high microcystin concentrations in previous years are consistent with the levels of *Microcystis* present, so clearly *Microcystis* has been dominant in previous years.

Aside from the species identification issue, it also became apparent that LSSU\_EAL cell density estimations were substantially lower than previous years for all of the blue-green species (e.g., *Aphanizomenon*, *Anabaena*, etc.). For example, both AA and PhycoTech reported similar values for *Aphanizomenon* and *Coelosphaerium*/*Woronichinia* that were about 6x and 31x higher respectively, than LSSU\_EAL (N16; (Table 3). Likewise, for DD, values for AA for the major

species were 3x to 5x higher than those of LSSU-EAL (Table 3). For DD on 9/17, cell density values were also substantially lower than all 3 of the other labs for species commonly identified among the labs (Table 4)

**Table 4. Inter-lab comparison (blue-green algae only) for South Tenmile Lake station Davis Dock, September 17, 2012. All species and parameters are contained in Appendix IV (other differences in biovolume and species identification were also apparent but are not discussed here).**

Sample: Davis Dock 9-17-12	LSSU	Lab A	Lab B	Lab C
Species	Cells/ml	Cells/ml	Cells/ml	Cells/ml
<i>Anabaena planctonica</i>	44,887	1,015,846	275,000	330,950
<i>Anabaena spiroides (crassa)</i>			7,500	
<i>Aphanizomenon flos-aquae</i>	256,028	5,006,463	1,280,000	1,949,602
<i>Aphanizomenon sp.</i>			500,000	
<i>Coelosphaerium naegelianum*</i>	72,938	14,099,542	1100**	
<i>Gloeotrichia echinulata</i>			750***	
<i>Microcystis aeruginosa</i>	6,657	263,289	9,000	
<i>Microcystis wesenbergii (tenta)</i>			14,000	
Oscillatoriales: <i>Pseudanabaenaceae</i>			72,000	
Oscillatoriales: <i>Pseudanabaenaceae</i>			300***	

\*Lab A and Lab B alternate name: *Woronichinia*

\*\*value is colonies/ml not cells/ml

\*\*\*Value is in filaments/ml not cells/ml

A review of the LSSU-EAL protocol for extrapolating from algal units/ml to algal cells /ml indicated a non-standard protocol that is not consistent with standard methods (e.g., APHA, USGS, or EPA, among others). Aside from using the average of the maximum and minimum (where the minimum is always a value of one) number of cells in an algal unit as opposed to a true average as performed by other laboratories, LSSU also used a non-standard definition for an “algal unit”. Standard methods specify that the colony is the “unit” to be counted-- these are usually termed "natural units" or "algal units"<sup>7</sup>. The reason for this is that it is assumed that what

<sup>7</sup> From EPA (2010): "For the purposes of determining the numbers of organisms to count, colonies and filaments are considered one organism."

From Australia (1999): "Because of the statistics used to calculate the precision levels of algal counts (see section 4.1.10, ‘Statistics of counting’), algal units must be enumerated. ‘Algal units’ may be single cells, filaments or colonies depending on the species’ usual life form."

From WHO (1999): "Counting cyanobacteria involves defining the units to be counted. The majority of planktonic cyanobacteria are present as filamentous or colonial forms consisting of a large number of cells which are often difficult to distinguish. The accuracy of quantitative determination depends on the number of counted objects (e.g. cells or colonies); the relative error is approximately indirectly proportional to the square root of the number of objects counted. The number of colonies, not the number of cells, is decisive for accurate enumeration."

is microscopically enumerated has settled or is filtered in a random and independent fashion. The assumption of random distribution of independent settled items (in this case algal units) is essential for accurate determination of cell density when colonial species are involved. For example as noted by WHO (1999): “The number of colonies, not the number of cells, is decisive for accurate enumeration.”

In contrast to the standard definition of an algal unit, the LSSU\_EAL protocol defines an algal unit, not as an entire filament or colony, but as a square on a Whipple grid: “An algal unit is described as a section of a filament or colony through the use of a calibrated Whipple grid” (Enumeration of Contaminant Candidate List Cyanobacteria in Source Water Samples; Methods document provided by LSSU\_EAL). Such squares containing a portion of a colony would not constitute randomly settled and independent units and thus violates the assumptions necessary to produce statistically valid cell density counts. Unfortunately, data could not be post-corrected because algal colonies or filaments (i.e., the natural counting units) were not reported. Thus, while the 2012 data from LSSU-EAL provide the general seasonal trend they are not adequate for evaluating the density of potentially toxigenic species with respect to public health thresholds. Fortunately, algal toxin testing was performed on all samples allowing public health thresholds to be evaluated for Tenmile Lakes during 2012.

Finally, several cross-laboratory comparisons were performed to evaluate 2012 alga toxin data. As noted above, for the first time in Tenmile Lakes the algal toxin cylindrospermopsin was detected by LSSU\_EAL despite the lack of detection of *Cylindrospermopsis raciborskii*, the most common species associated with cylindrospermopsin toxin production<sup>8</sup>. Although, as shown in Table 3, *Aphanizomenon issatschenkoi* which has been associated with cylindrospermopsin production (Wood et al. 2007), was detected by PhycoTech in very low numbers at N16 on September 4th. However, the presence of cylindrospermopsin in duplicate samples sent to an alternative laboratory was not confirmed (Table 5; Appendix VI). Since the discrepant results could have been due to methodological differences (LSSU uses HPLC-PDA while GreenWater uses the enzyme linked immunosorbent assay or ELISA), an additional set was analyzed by LSSU using ELISA. These results, although lower than the HPLC-PDA results still confirmed the presence of cylindrospermopsin (Appendix V). In addition, for 2 of the 3 duplicate samples GreenWater did not detect microcystin, even when LSSU found levels of 0.67 and 0.21 µg/L (Table 5).

**Table 5. Toxin duplicate results for Ancillary Stations, Tenmile Lakes, 2012.**

Station	Lab	Date	Microcystin (µg/L)	Anatoxin-a (µg/L)	Cylindrospermopsin (µg/L)
Davis Dock	LSSU	8/20/2012	0.67	0.00	3.20
Davis Dock Dup	Greenwater	8/20/2012	0.00		0.00
Davis Dock	LSSU	9/4/2012	1.45	0.00	0.52
Davis Dock Dup	Greenwater	9/4/2012	3.90		0.00
Litts Dock	LSSU	9/4/2012	0.21	0.00	0.21
Litts Dock Dup	Greenwater	9/4/2012	0.00		0.00

\* Exceeds the Oregon Department of Human Services and WHO drinking water microcystin guideline level of 1 µg/l or the Oregon Public Health Department provisional acute drinking water toxicity value of Cylindrospermopsin 1 µg/l.

<sup>8</sup> Cylindrospermopsin is a potent liver and kidney toxin typically produced by *Cylindrospermopsis raciborskii*, but also has been associated with several *Anabaena* and *Aphanizomenon* species.

To further understand these differences in toxin detection, two samples that tested positive for cylindrospermopsin were sent to the laboratories of Drs. Theo Dreyer and Claudia Maier at Oregon State University for both genetic and cylindrospermopsin analysis<sup>9</sup>. The genetic analysis (Appendix VII) for the DD sample taken on 4 Sept 2012 provided evidence that *Cylindrospermopsis* is present (although it was not identified in any of the samples), but that uncertainty existed about the presence of cylindrospermopsin biosynthetic genes (toxin producing genes). This analysis provided evidence that *Anabaena* and *Microcystis* were also present in the sample, as were anatoxin-a and microcystin biosynthetic genes (Appendix VII).

However, the OSU LC-MS/MS MC testing found no detections for cylindrospermopsin in either of the two samples (N16 8/20/12 and DD 9/4/12; Claudia Maier personal communication) that tested positive for cylindrospermopsin by LSSU. Interestingly, there were no detections for anatoxin-a, MC-LA, -LR, -YR, and -RR<sup>10</sup>, despite microcystin being detected by both LSSU and Greenwater (Table 5 and Appendix V; both MC-LR [0.89 µg/L] and MC-LA [0.56 µg/L]).

In summary:

1. for DD on 9/4 both OSU (LC-MS/MS) and Greenwater (ELISA) showed no cylindrospermopsin detected while LSSU showed cylindrospermopsin detected using both HPLC/PDA (0.52 µg/L) and ELISA (0.34 µg/L).
2. for DD on 9/4 both LSSU (HPLC/PDA) and Greenwater (ELISA) showed microcystin detected but OSU (LC-MS/MS) did not detect microcystin.
3. for N16 on 8/20 LSSU showed detection of cylindrospermopsin (2.7 µg/L) while OSU did not.
4. for N16 on 8/20 both LSSU and OSU showed no microcystin detected.

At this point it is unclear whether cylindrospermopsin is present in Tenmile Lakes, one lab found it, two labs did not, and the genetic analysis is equivocal on the presence of cylindrospermopsin biosynthetic genes. The genetic analysis also indicated the presence of *Cylindrospermopsis*; however, none of the three labs detected this species in phytoplankton analyses. Clearly, further work is necessary to confirm the presence of cylindrospermopsin toxin in Tenmile Lakes.

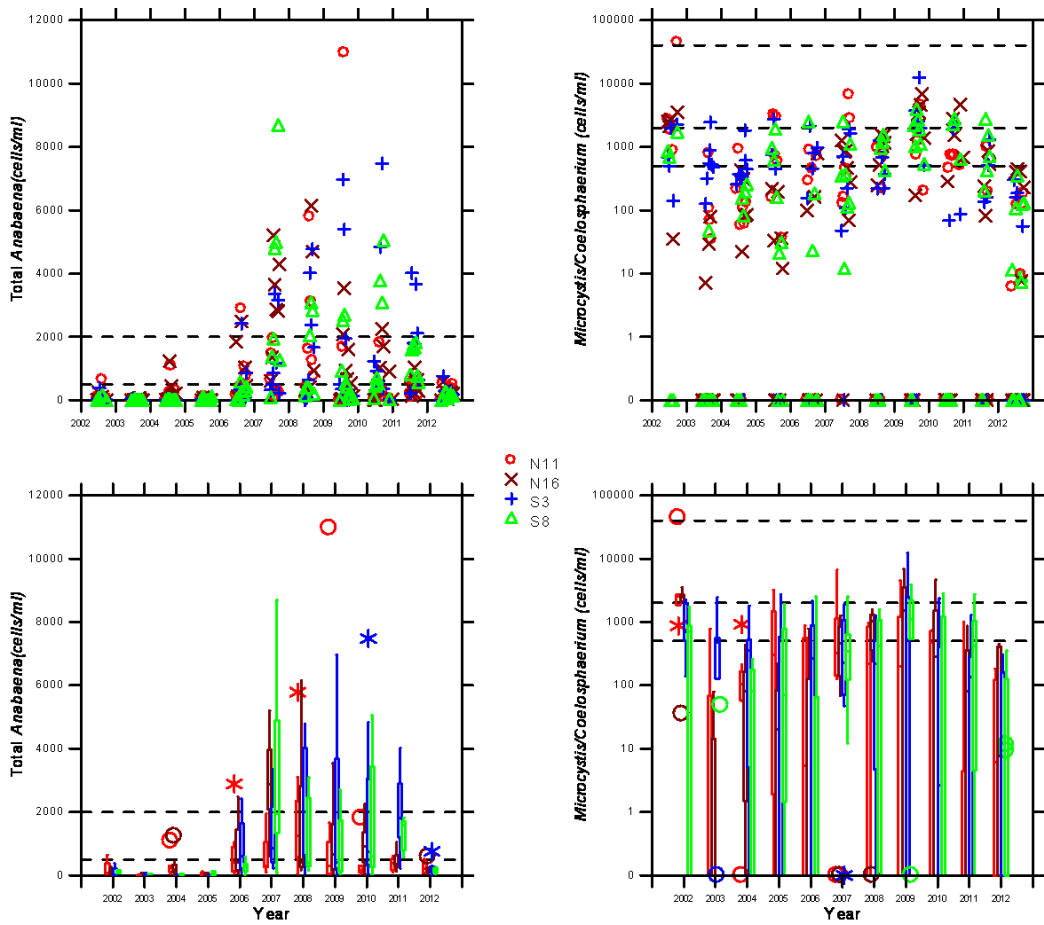
### **2002-2012 Comparison**

Previous reports included an inter-annual comparison of cyanobacterial cell densities (see Kann 2012 for a summary of 2002-2011 data); however, given the lower cell densities reported by LSSU in 2012 these across-year comparisons (Figure 7 and Figure 8) are not valid for 2012.

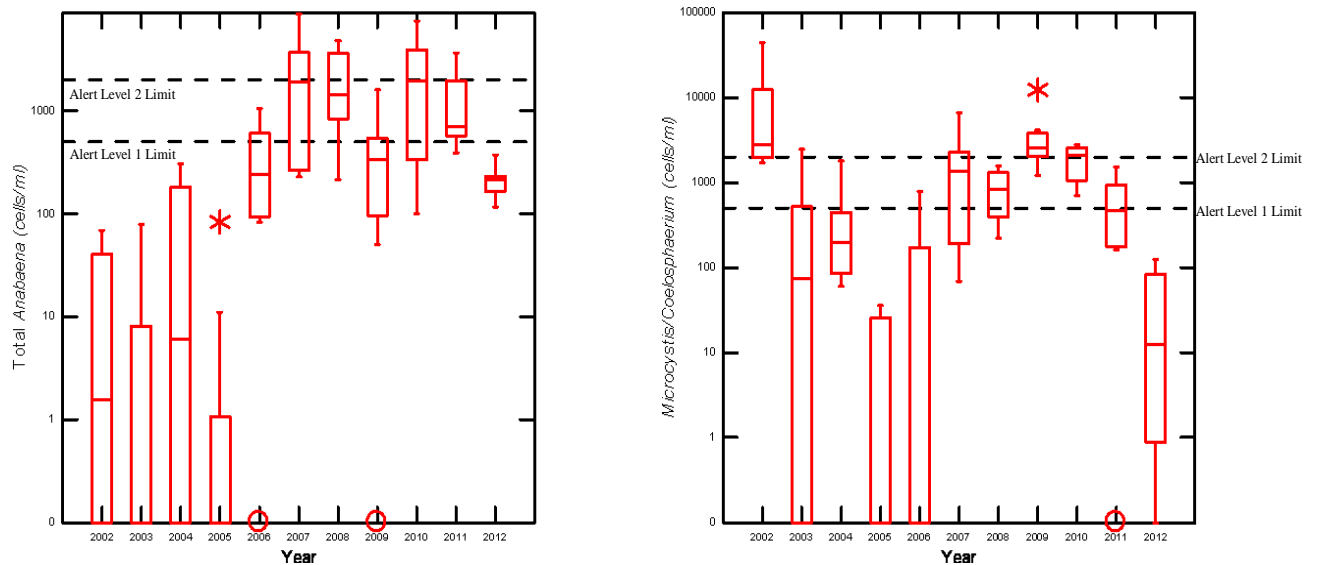
---

<sup>9</sup> Claudia Maier, Dept. of Chemistry (claudia.maier@oregonstate.edu). Theo Dreher, Dept. of Microbiology (theo.dreher@oregonstate.edu)

<sup>10</sup> OSU LC-MS/MS MC panel measures only of 4 microcystin (MC) congeners: MC-LR, RR, LA, and YR.



**Figure 7.** Cell density of *Microcystis aeruginosa*, *Coelosphaerium naegelianum* and total *Anabaena* at standard open-water stations in Tenmile Lakes, 2002-2012; time-series of individual data points (a), and box plots grouped by station (b).



**Figure 8.** Cell density of *Microcystis aeruginosa*, *Coelosphaerium naegelianum* and total *Anabaena* in Tenmile Lakes during September, 2002-2012; boxes represent the distribution of both North and South Tenmile Lakes combined.



## **SUMMARY**

2012 marks the first year for which public health monitoring was based on direct algal toxin measurements (as opposed to cell density of potentially toxigenic cyanobacteria) in Tenmile Lakes. Cell density values provided by LSSU used non-standard laboratory methods that appear to cause reported cell densities to be biased low relative to alternative laboratories. Thus, 2012 cell density results could not be compared to previous years. However, because toxin values were available for all samples this did not affect the ability to compare to public health thresholds.

Initial exceedances of public health guideline values for drinking water began in late August at N16 and Davis Dock. However, these were for cylindrospermopsin, and as described above, the presence of this toxin is uncertain at this time, and further work would be required to confirm its presence. There was only one occurrence of microcystin exceeding the drinking water guideline level in 2012, with a value of 1.45 µg/L detected at Davis Dock on 9/4/2012. This is in contrast to previous years when both *Microcystis* and microcystin greatly exceeded both drinking water and recreational public health guidelines (e.g., see Table 2 above)

Although localized blooms and blue-green algal scums were noted that clearly had the potential to exceed recreational guidelines, toxin concentrations remained relatively low. One possibility is that *Coelosphaerium/Woronichinia* may have replaced *Microcystis* as the dominant species during 2012. The presence of *Coelosphaerium* may also explain why levels of microcystin in 2011 were lower than expected based on the microcystin-cell density relationship for previous years (Kann 2012). Other explanations may include either environmental factors, genetic factors, or both leading to variable ratios of toxin produced per unit algal density (Kann 2012; Bozarth et al.). The previous laboratory appeared to not have distinguish between *Microcystis* and *Coelosphaerium/Woronichinia*.

Finally, because reported levels indicate the general trend but cannot guarantee that levels of potentially toxigenic species or their toxins at a particular location do not exceed guideline values, and the fact that cyanobacterial cells have been previously reported in home-owner drinking water treatment systems (see Kann 2007), drinking water protection efforts should always be in place. Patchy distribution of blue-green algae and accumulation in localized areas commonly occurs, and although levels of all potentially toxigenic cyanobacteria were well below the recreational guidelines of 40,000 cells/ml for *Microcystis* or 100,000 cells/ml for *Anabaena* at the standard sampling stations, they were greatly exceeded in adjacent areas.

Thus, as previously noted in Tenmile Lakes monitoring memoranda: “...those utilizing the lake for drinking water should always follow Oregon Health Division recommendations for purification (attached). In addition, recreational users should always avoid contact with water whenever noticeable surface concentrations of algae are evident or when the lake has an obvious green to blue-green appearance.”

## Disclaimer

Due to the patchy nature of blue-green algal blooms it is possible for higher *Microcystis* and *Anabaena* densities (and therefore higher microcystin or anatoxin concentrations) to be present in areas not sampled in this survey, particularly along shorelines or during calm conditions of little to no wind. Given the lakes' demonstrated history of toxic *Microcystis* and *Anabaena* blooms, and the fact that all areas of the lake cannot be tested at all times, those utilizing the lake for drinking water should always follow Oregon Health Division recommendations for purification (see Appendix III). In addition, recreational users should always avoid contact with water whenever noticeable surface concentrations of algae are evident or when the lake has an obvious green to blue-green appearance. Moreover, because pets or other domestic animals are the most likely to ingest contaminated water, these animals should not be allowed access to the lakeshore whenever either noticeable surface concentrations of algae or an obvious green to blue-green appearance is evident.

---

## LITERATURE CITED

- Hötzel, G. and R. Croome (Australia). 1999. A Phytoplankton Methods Manual for Australian Freshwaters. Land and Water Resources Research and Development Corporation. LWRRDC Occasional Paper 22/99. <http://npsi.gov.au/files/products/national-river-health-program/pr990300/pr990300.pdf>
- Bozarth, C.S. A. D. Schwartz, J. W. Shepardson, F. S. Colwell, and Theo W. Dreher. 2010. Population Turnover in a *Microcystis* Bloom Results in Predominantly Nontoxigenic Variants Late in the Season. *Applied and Environmental Microbiology* 76: 5207–5213.
- Carey, C.C., and J.F. Haney, and K.L. Cottingham. 2007. First report of microcystin-LR in the cyanobacterium *Gloeotrichia echinulata*. *Environmental toxicology* 22:337-339.
- Chorus, I. and J. Bartram (WHO). 1999. Toxic Cyanobacteria in Water; A Guide to their Public Health Consequences, Monitoring and Management. World Health Organization Report. E & F Spon, London and New York. 416 p.
- EPA. 2010. Standard Operating Procedure for Phytoplankton Analysis. LG401. Revision 05, February 2010. [http://www.epa.gov/greatlakes/monitoring/sop/chapter\\_4/lg401.pdf](http://www.epa.gov/greatlakes/monitoring/sop/chapter_4/lg401.pdf)
- Jacoby, J.M., and J. Kann. 2007. The occurrence and response to toxic cyanobacteria in the Pacific Northwest, North America. *Lake and Reserv. Manage.* 23:123-143.
- Kann, J. 2008. Tenmile Lakes toxic algal sampling program: 2008 data summary report. Prepared by Aquatic Ecosystem Sciences LLC for Tenmile Lakes Basin Partnership, P.O. Box L, Lakeside OR 97520. Dec. 2008.
- Kann, J. 2010. Tenmile Lakes toxic algal sampling program: 2009 data summary report. Prepared by Aquatic Ecosystem Sciences LLC for Tenmile Lakes Basin Partnership, P.O. Box L, Lakeside OR 97520. April 2010.
- Kann, J. 2011. Tenmile Lakes toxic algal sampling program: 2010 data summary report. Prepared by Aquatic Ecosystem Sciences LLC for Tenmile Lakes Basin Partnership, P.O. Box L, Lakeside OR 97520. May 2011.
- Kann, J. 2012. Tenmile Lakes toxic algal sampling program: 2011 data summary report. Prepared by Aquatic Ecosystem Sciences LLC for Tenmile Lakes Basin Partnership, P.O. Box L, Lakeside OR 97520. February 2012.

- Kann, J., and D. Gilroy. 1998. Tenmile Lakes toxic *Microcystis* bloom, September-November 1997. Oregon Health Division Technical Report. Environmental Services and Consultation Center for Environment and Health Systems, OHD, 800 NE Oregon St., Ste.608, Portland, OR 97232.
- Kann, J. and C.Bowman. 2012. Middle Klamath river Toxic Cyanobacteria Trends, 2011 . Technical Memorandum. Prepared by Aquatic Ecosystem Sciences, LLC, Ashland, Oregon and the Karuk Tribe Department of Natural Resources for the Karuk Tribe Department of Natural Resources, Orleans, California.
- Li, R., W.W. Carmichael, Y. Liu, and M.M. Watanabe. 2000. Taxonomic re-evaluation of *Aphanizomenon flos-aquae* NH-5 based on morphology and 16S rRNA gene sequences. *Hydrobiologia*. 438:99-105.
- Oregon DHS. 2011. Public Health Advisory Guidance for Toxigenic Cyanobacteria in Recreational Waters. <http://www.oregon.gov/DHS/ph/hab/index.shtml>
- Oregon Health Authority (OHA). 2012. Oregon Harmful Algae Bloom Surveillance (HABS) Program: Public Health Advisory Guidelines for Harmful Algae Blooms in Freshwater Bodies. Public Health Division Office of Environmental Public Health Research & Education Section [https://public.health.oregon.gov/HealthyEnvironments/Recreation/HarmfulAlgaeBlooms/Pages/resources\\_for\\_samplers.aspx](https://public.health.oregon.gov/HealthyEnvironments/Recreation/HarmfulAlgaeBlooms/Pages/resources_for_samplers.aspx)
- Stone, D. and W. Bress. 2007. Addressing public health risks for cyanobacteria in recreational freshwaters: the Oregon and Vermont Framework. *Integr. Environ. Assess. Manage.* 3:137–143.
- Yoo, S.R., W.W. Carmichael, R.C. Hoehn, and S.E. Hrudy. Cyanobacterial (blue-green algal) toxins: a resource guide. AWWA Research Foundation and American Water Works Association. Denver, CO. 229 p. (ISBN 0-89867-824-2)
- Wood, S. A., J. P. Rasmussen, P. T. Holland, R. Campbell, and A. L. M. Crowe (2007). First Report of the cyanotoxin Anatoxin-A from *Aphanizomenon issatschenkoi* (cyanobacteria). *Journal of Phycology* 43 (2): 356–365.

**Appendix I: Lake Superior State University Environmental Analysis  
Laboratory Phytoplankton Reports**

**Appendix II: Aquatic Analysts Phytoplankton Reports**

**Appendix III: PhycoTech Laboratories Phytoplankton Report**

**Appendix IV: Multi Lab Phytoplankton Comparison**

**Appendix V: Lake Superior State University Environmental Analysis  
Laboratory Algal Toxin Results**

**Appendix VI: GreenWater Laboratories Algal Toxin Results**

**Appendix VII: Oregon State University Dreher Lab Results**

**Appendix VIII: Oregon DHS and Tenmile Lakes Harmful Algal Bloom  
Information**

**APPENDIX I: LAKE SUPERIOR STATE UNIVERSITY ENVIRONMENTAL ANALYSIS  
LABORATORY PHYTOPLANKTON REPORTS**

Note that the adjusted cells/ml column reflects actual cell densities corrected for volume of water filtered.



Report to: Richard Litts  
Tenmile Lakes Watershed  
Lakeside, Oregon  
541-759-2414

N11						
Species	Cells/mL	Adjusted Cells/mL	Density Percent	Biovolume	Biovolume Percent	Phylum
Fragilaria mesolepta	1129.7	4.7	9.9%	1186.7	14.7%	Diatom
Melosira ambigua	1002.9	4.1	8.8%	2433.3	30.2%	Diatom
Fragilaria crotonesis	288.2	1.2	2.5%	997.2	12.4%	Diatom
Synedra radians	269.0	1.1	2.4%	398.9	5.0%	Diatom
Asterionella formosa	165.2	0.7	1.5%	149.7	1.9%	Diatom
Nitzschia acicularis	19.2	0.1	0.2%	22.2	0.3%	Diatom
Sphaerocystis Schroeteri	3024.8	12.5	26.6%	436.1	5.4%	Green
Dinobryon divergens	80.7	0.3	0.7%	39.9	0.5%	Dinoflagella
Coelosphaerum naegelianum	1452.4	6.0	12.8%	17.9	0.2%	Blue-green
Aphanizomenon flos-aquae	1139.1	4.7	10.0%	295.6	3.7%	Blue-green
Uroglenopsis americana	2807.5	11.6	24.7%	2077.0	25.8%	Ochrophyta
Totals	11378.6	46.9	100.0%	8054.5	100.0%	

S3						
Genus	Cells/mL	Adjusted Cells/mL	Density Percent	Biovolume	Biovolume Percent	Phylum
Asterionella formosa	2587.4	12.9	42.3%	2829.3	31.7%	Diatom
Fragilaria crotonesis	1105.7	5.5	18.1%	4616.4	51.7%	Diatom
Fragilaria mesolepta	248.5	1.2	4.1%	315.0	3.5%	Diatom
Melosira ambigua	114.1	0.6	1.9%	334.0	3.7%	Diatom
Sphaerocystis Schroeteri	800.2	4.0	13.1%	139.2	1.6%	Green
Dinobryon divergens	653.3	3.2	10.7%	422.1	4.7%	Dinoflagella
Aphanizomenon flos-aquae	475.5	2.4	7.8%	148.9	1.7%	Blue-green
Anabaena plantonica	135.3	0.7	2.2%	123.1	1.4%	Blue-green
Totals	6120	30.4	100.0%	8928.0	100.0%	
		0.0				



N16						
Genus	Cells/mL	Adjusted Cells/mL	Density Percent	Biovolume	Biovolume Percent	Phylum
Melosira ambigua	3474.3	14.9	14.6%	8783.1	32.3%	Diatom
Fragilaria crotonesis	3460.6	14.9	14.5%	12476.6	45.9%	Diatom
Asterionella formosa	2252	9.7	9.5%	2126.5	7.8%	Diatom
Fragilaria mesolepta	600.2	2.6	2.5%	656.9	2.4%	Diatom
Melosira angustissima	439.6	1.9	1.8%	471.7	1.7%	Diatom
Cyclotella stelligera	33.8	0.1	0.1%	8.0	0.0%	Diatom
Sphaerocystis Schroeteri	8578	36.8	36.0%	1288.6	4.7%	Green
Dinobryon divergens	139.5	0.6	0.6%	71.8	0.3%	Dinoglagella
Aphanizomenon flos-aquae	4850.1	20.8	20.4%	1311.5	4.8%	Blue-green
Totals	23828	102.3	100.0%	27194.7	100.0%	

S8						
Genus	Cells/mL	Adjusted Cells/mL	Density Percent	Biovolume	Biovolume Percent	Phylum
Asterionella formosa	6373.8	27.7	36.7%	6087.7	46.6%	Diatom
Melosira ambigua	1230	5.3	7.1%	3145.2	24.1%	Diatom
Melosira angustissima	1159.8	5.0	6.7%	1258.8	9.6%	Diatom
Cyclotella stelligera	160.6	0.7	0.9%	38.3	0.3%	Diatom
Staurastrum sp.	342.4	1.5	2.0%	356.8	2.7%	Green
Sphaerocystis Schroeteri	2617.2	11.4	15.1%	397.7	3.0%	Green
Athrodesmus sp.	76.1	0.3	0.4%	198.2	1.5%	Green
Microcystis aeruginosa	2637.4	11.4	15.2%	749.3	5.7%	Blue-green
Aphanizomenon flos-aquae	2588.8	11.2	14.9%	708.1	5.4%	Blue-green
Mallomonas sp.	118.3	0.5	0.7%	18.0	0.1%	Ochrophyta
Cocconeis placentula	59.2	0.3	0.3%	118.2	0.9%	Ochrophyta
Totals	17363.6	75.4	100.0%	13076.2	100.0%	

650 W. Easterday Ave., Sault Ste. Marie, MI 49783  
 Telephone:906-635-2076 Fax:906-635-2266  
 www.lssu.edu

**Environmental Analysis Laboratory**

Report to: Richard Littz, Tenmile Lakes Watershed, Lakeside, Oregon

S8 (6/18/2012)						
Genus	Cells/mL	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Dinobryon divergens	187676.7	1094.8	50.94%	131380.2	35.72%	Dinoflagella
Asterionella formosa	75910.9	442.8	20.61%	97423.9	26.49%	Diatom
Aphanizomenon flos-aquae	39942.0	233.0	10.84%	14679.4	3.99%	Blue-green
Sphaerocystis schroeteri	20684.3	120.7	5.61%	4223.2	1.15%	Green
Fragilaria crotonesis	16737.6	97.6	4.54%	82018.3	22.30%	Diatom
Anabaena planctonica	9129.6	53.3	2.48%	9746.3	2.65%	Blue-green
Fragilaria mesolepta	8749.2	51.0	2.37%	13015.1	3.54%	Diatom
Staurastrum sp.	7481.2	43.6	2.03%	10474.2	2.85%	Green
Melosira granulata	951.0	5.5	0.26%	1386.9	0.38%	Diatom
Cocconeis placentula	634.0	3.7	0.17%	1701.3	0.46%	Diatom
Gomphonema subclavatum	507.2	3.0	0.14%	1775.3	0.48%	Diatom
Totals:	368403.7	2149.1	100.00%	367824.3	100.00%	

N11 (6/18/2012)						
Genus	Cells/mL	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Dinobryon divergens	91470.8	534.7	34.78%	64167.5	27.63%	Dinoflagella
Anabaena sp.	87183.6	509.7	33.15%	34657.3	14.92%	Blue-green
Asterionella formosa	35706.2	208.7	13.58%	45921.7	19.77%	Diatom
Dinobryon bavaricum	14393.5	84.1	5.47%	10097.2	4.35%	Dinoflagella
Fragilaria crotonesis	11103.6	64.9	4.22%	54524.7	23.48%	Diatom
Melosira granulata	9715.6	56.8	3.69%	14199.1	6.11%	Diatom
Aphanizomenon flos-aquae	8224.9	48.1	3.13%	3029.2	1.30%	Blue-green
Staurastrum sp.	3289.9	19.2	1.25%	4615.8	1.99%	Green
Sphaerocystis schroeteri	1233.7	7.2	0.47%	252.4	0.11%	Green
Cosmarium sp.	642.6	3.8	0.24%	788.8	0.34%	Green
Totals:	262964.4	1537.3	100.00%	232253.8	100.00%	

## Environmental Analysis Laboratory

N16 (6/18/2012)						
Genus	Cells/mL	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Dinobryon divergens	154632.6	709.4	31.83%	85130.6	20.97%	Dinoflagella
Anabaena sp.	126673.2	581.1	26.07%	39518.2	9.73%	Blue-green
Aphanizomenon flos-aquae	66791.9	306.4	13.75%	19304.9	4.75%	Blue-green
Asterionella formosa	64300.3	295.0	13.24%	64899.1	15.98%	Diatom
Fragilaria crotonesis	47550.0	218.1	9.79%	183245.4	45.13%	Diatom
Sphaerocystis schroeteri	15976.8	73.3	3.29%	2565.4	0.63%	Green
Melosira granulata	9890.4	45.4	2.04%	11343.8	2.79%	Diatom
Totals:	485815.2	2228.8	100.00%	406007.4	100.00%	

S3 (6/18/2012)						
Genus	Cells/mL	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Anabaena sp.	115863.5	757.3	39.34%	51498.3	18.73%	Blue-green
Dinobryon divergens	53850.4	352.0	18.28%	42238.3	15.36%	Dinoflagella
Coelosphaerum naegelianum	46218.6	302.1	15.69%	906.3	0.33%	Green
Asterionella formosa	33575.1	219.5	11.40%	48281.0	17.56%	Diatom
Fragilaria crotonesis	19875.9	129.9	6.75%	109129.7	39.68%	Diatom
Staurastrum sp.	7845.8	51.3	2.66%	12307.9	4.48%	Green
Sphaerocystis schroeteri	6181.5	40.4	2.10%	1414.2	0.51%	Green
Oocystis elliptica	4755.0	31.1	1.61%	2331.0	0.85%	Green
Cosmarium sp.	4456.1	29.1	1.51%	6116.6	2.22%	Green
Aphanizomenon flos-aquae	1902.0	12.4	0.65%	783.2	0.28%	Blue-green
Total:	294523.8	1925.1	100.00%	275006.6	100.00%	



**LAKE SUPERIOR**  
STATE UNIVERSITY

**Environmental Analysis Laboratory**

Report to: Richard Litts, Tenmile Lakes Watershed, Lakeside, Oregon

<b>S8 (7/9/2012)</b>						
Genus	Cells/mL	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Ceratium hirundinella	34996.8	176.1	9.35%	1725770.3	85.21%	Dinoflagellate
Fragilaria mesolepta	98034.5	493.3	26.20%	125790.5	6.21%	Diatom
<b>Anabaena planctonica</b>	<b>51607.6</b>	<b>259.7</b>	<b>13.79%</b>	<b>47521.9</b>	<b>2.35%</b>	<b>Blue-green</b>
Dinobryon divergens	64192.5	323.0	17.16%	38760.9	1.91%	Dinoflagellate
Melosira ambigua	5848.7	29.4	1.56%	17334.0	0.86%	Diatom
Melosira granulata	12743.4	64.1	3.41%	16030.8	0.79%	Diatom
Synedra radians	8368.8	42.1	2.24%	15159.8	0.75%	Diatom
<b>Aphanizomenon flos-aquae</b>	<b>47708.5</b>	<b>240.1</b>	<b>12.75%</b>	<b>15123.9</b>	<b>0.75%</b>	<b>Blue-green</b>
Mallomonas sp.	4279.5	21.5	1.14%	8182.9	0.40%	Chrysophyta
Asterionella formosa	5832.8	29.3	1.56%	6457.0	0.32%	Diatom
Staurastrum sp.	3296.8	16.6	0.88%	3981.4	0.20%	Green
Coelosphaerum naegelianum	21429.2	107.8	5.73%	3234.9	0.16%	Green
Rhodomonas minuta	15444.2	77.7	4.13%	1554.3	0.08%	Cryptophyta
Cosmarium depressum	380.4	1.9	0.10%	402.0	0.02%	Green
Totals:	374163.7	1882.7	100.00%	2025304.4	100.00%	

<b>N11 (7/9/2012)</b>						
Genus	Cells/mL	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Fragilaria crotonesis	33708.2	178.3	39.34%	149809.9	77.42%	Diatom
<b>Anabaena planctonica</b>	<b>14455.2</b>	<b>76.5</b>	<b>16.87%</b>	<b>13995.9</b>	<b>7.23%</b>	<b>Blue-green</b>
Melosira granulata	8730.2	46.2	10.19%	11547.5	5.97%	Diatom
Dinobryon bavaricum	10651.2	56.4	12.43%	6762.5	3.49%	Dinoflagellate
Dinobryon divergens	6847.2	36.2	7.99%	4347.3	2.25%	Dinoflagellate
Asterionella formosa	3651.8	19.3	4.26%	4250.7	2.20%	Diatom
<b>Aphanizomenon flos-aquae</b>	<b>6433.5</b>	<b>34.0</b>	<b>7.51%</b>	<b>2144.4</b>	<b>1.11%</b>	<b>Blue-green</b>
Cosmarium depressum	456.5	2.4	0.53%	507.2	0.26%	Green
Sphaerocystis Schroeteri	741.8	3.9	0.87%	137.4	0.07%	Green
Totals:	85675.6	453.3	100.00%	193502.8	100.00%	

N16 (7/9/2012)						
Genus	Cells/mL	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Fragilaria crotonesis	101744.3	554.6	27.97%	465886.5	76.38%	Diatom
Dinobryon divergens	89267.2	486.6	24.54%	58393.4	9.57%	Dinoflagellate
Melosira granulata	17118.0	93.3	4.71%	23328.3	3.82%	Diatom
Aphanizomenon flos-aquae	54270.4	295.8	14.92%	18637.8	3.06%	Blue-green
Anabaena planctonica	16230.4	88.5	4.46%	16190.9	2.65%	Blue-green
Coelosphaerum naegelianum	74178.0	404.4	20.39%	12130.7	1.99%	Green
Oocystis elliptica	4216.1	23.0	1.16%	7078.7	1.16%	Green
Asterionella formosa	4691.6	25.6	1.29%	5626.4	0.92%	Diatom
Staurastrum sp.	2028.8	11.1	0.56%	2654.2	0.44%	Green
Totals:	363744.8	1982.8	100.00%	609927.1	100.00%	

S3 (7/9/2012)						
Genus	Cells/mL	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Fragilaria crotonesis	115033.0	706.5	41.19%	593470.8	81.57%	Diatom
Anabaena planctonica	40431.1	248.3	14.48%	45442.7	6.25%	Blue-green
Dinobryon divergens	43773.2	268.8	15.68%	32261.7	4.43%	Dinoflagellate
Asterionella formosa	18218.4	111.9	6.52%	24616.8	3.38%	Diatom
Melosira granulata	8640.5	53.1	3.09%	13267.1	1.82%	Diatom
Staurastrum sp.	4945.2	30.4	1.77%	7289.4	1.00%	Green
Coelosphaerum naegelianum	26247.6	161.2	9.40%	4836.2	0.66%	Green
Anabaena lemmermannii	10162.1	62.4	3.64%	4369.0	0.60%	Blue-green
Sphaerocystis Schroeteri	7608.0	46.7	2.72%	1635.4	0.22%	Green
Dictyosphaerium ehrenbergianum	4184.4	25.7	1.50%	385.5	0.05%	Green
Total:	279243.5	1715.1	100.00%	727574.7	100.00%	

Toxin Producing Algae

650 W. Easterday Ave., Sault Ste. Marie, MI 49783  
 Telephone: 906-635-2076 . Fax: 906-635-2266  
[www.lssu.edu](http://www.lssu.edu)



**LAKE SUPERIOR**  
STATE UNIVERSITY

**Environmental Analysis Laboratory**

Report to: Richard Litts, Tenmile Lakes Watershed, Lakeside, Oregon

<b>S8 (7/23/2012)</b>					
Genus	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Asterionella formosa	140.7	9.10%	30951.3	3.50%	Diatom
Aphanizomenon flos-aquae	55.2	3.57%	3475.8	0.39%	Blue-green
Melosira granulata	139.8	9.04%	34942.1	3.96%	Diatom
Fragilaria mesolepta	457.1	29.56%	70856.2	8.02%	Diatom
Anabaena planctonica	250.1	16.17%	45770.4	5.18%	Blue-green
Cosmarium depressum	3.1	0.20%	643.7	0.07%	Green
Ceratium hirundinella	69.9	4.52%	684864.5	77.52%	Dinoflagellate
Coelosphaerum naegelianum	350.6	22.67%	10519.4	1.19%	Green
Sphaerocystis Schroeteri	11.0	0.71%	386.2	0.04%	Green
Dictyosphaerium ehrenbergianum	69.0	4.46%	1034.5	0.12%	Green
Totals:	1546.5	100.00%	883444.1	100.00%	

<b>S3 (7/23/2012)</b>					
Genus	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Melosira granulata	977.2	48.95%	244294.2	29.46%	Diatom
Aphanizomenon flos-aquae	336.7	16.86%	21209.6	2.56%	Blue-green
Rhodomonas minuta	29.3	1.47%	587.0	0.07%	Cryptophyta
Ceratium hirundinella	46.0	2.31%	451181.8	54.41%	Dinoflagellate
Asterionella formosa	19.0	0.95%	4178.0	0.50%	Diatom
Fragilaria mesolepta	400.8	20.08%	102210.8	12.33%	Diatom
Coelosphaerum naegelianum	187.3	9.38%	5619.6	0.68%	Green
Total:	1996.4	100.00%	829281.0	100.00%	

Potential toxin producer

<b>N16 (7/23/2012)</b>					
Genus	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
<b>Aphanizomenon flos-aquae</b>	240.9	15.54%	15175.2	1.80%	Blue-green
Melosira granulata	192.7	12.43%	48175.3	5.71%	Diatom
Dinobryon divergens	207.0	13.35%	24837.1	2.94%	Cryptophyta
Ceratium hirundinella	69.0	4.45%	676120.1	80.16%	Dinoflagellate
Asterionella formosa	72.0	4.64%	15832.4	1.88%	Diatom
Coelosphaerum naegelianum	447.3	28.85%	13417.7	1.59%	Green
Staurastrum sp.	5.9	0.38%	1427.4	0.17%	Green
<b>Anabaena planctonica</b>	90.4	5.83%	16543.8	1.96%	Blue-green
Fragilaria mesolepta	87.4	5.64%	22294.5	2.64%	Diatom
<b>Anabaena lemmermannii</b>	138.0	8.90%	9658.9	1.15%	Blue-green
Totals:	1550.5	100.00%	843482.4	100.00%	

<b>N11 (7/23/2012)</b>					
Genus	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
<b>Aphanizomenon flos-aquae</b>	62.2	13.06%	3915.5	5.78%	Blue-green
Melosira granulata	69.4	14.58%	17340.1	25.59%	Diatom
Dinobryon bavaricum	11.9	2.51%	1432.0	2.11%	Cryptophyta
Gyrosigma sp.	1.7	0.35%	828.7	1.22%	Diatom
Navicula sp.	0.5	0.10%	288.4	0.43%	Diatom
Dinobryon divergens	16.0	3.35%	1914.3	2.82%	Diatom
Asterionella formosa	17.7	3.73%	3901.4	5.76%	Diatom
Fragilaria mesolepta	76.8	16.14%	19579.7	28.89%	Diatom
Coelosphaerum naegelianum	121.5	25.54%	3645.4	5.38%	Green
Sphaerocystis schroeteri	5.0	1.05%	174.0	0.26%	Green
<b>Anabaena planctonica</b>	27.8	5.85%	5095.4	7.52%	Blue-green
Melosira ambigua	9.7	2.04%	5710.7	8.43%	Diatom
Dictyosphaerium ehrenbergianu	5.4	1.13%	80.8	0.12%	Green
Staurastrum sp.	2.0	0.42%	477.3	0.70%	Green
<b>Anabaena lemmermannii</b>	48.3	10.15%	3381.8	4.99%	Blue-green
Totals:	475.8	100.00%	67765.5	100.00%	





**LAKE SUPERIOR**  
STATE UNIVERSITY

**Environmental Analysis Laboratory**

Report to: Richard Litts, Tenmile Lakes Watershed, Lakeside, Oregon

<b>S8 (8/06/2012)</b>					
Genus	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Aphanizomenon flos-aquae	767.1	81.02%	48329.9	32.64%	Blue-green
Sphaerocystis schroeteri	66.0	6.97%	2308.7	1.56%	Green
Anabaena planctonica	32.7	3.45%	5985.7	4.04%	Blue-green
Anabaena lemmermannii	32.7	3.45%	2289.6	1.55%	Blue-green
Melosira granulata	27.4	2.90%	6853.3	4.63%	Diatom
Dinobryon divergens	8.4	0.89%	1009.3	0.68%	Cryptophyta
Ceratium hirundinella	8.3	0.87%	80900.1	54.64%	Dinoflagellate
Rhodomonas	2.9	0.30%	57.1	0.04%	Cryptophyta
Staurastrum sp.	1.4	0.15%	336.4	0.23%	Green
Totals:	946.9	100.00%	148070.2	100.00%	

<b>S3 (8/06/2012)</b>					
Genus	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Aphanizomenon flos-aquae	232.5	58.19%	14645.0	24.20%	Blue-green
Anabaena planctonica	65.6	16.42%	12004.3	19.84%	Blue-green
Melosira granulata	54.1	13.54%	13523.8	22.35%	Diatom
Sphaerocystis schroeteri	15.2	3.81%	532.2	0.88%	Green
Asterionella formosa	11.5	2.87%	2521.4	4.17%	Diatom
Anabaena lemmermannii	10.7	2.67%	746.2	1.23%	Blue-green
Fragilaria mesolepta	6.5	1.62%	1647.4	2.72%	Diatom
Ceratium hirundinella	1.5	0.37%	14610.3	24.15%	Dinoflagellate
Dinobryon divergens	1.5	0.37%	178.9	0.30%	Cryptophyta
Cosmarium sp.	0.4	0.11%	93.9	0.16%	Green
Rhodomonas sp.	0.1	0.04%	3.0	0.00%	Cryptophyta
Total:	399.5	100.00%	60506.3	100.00%	

Potential toxin producer

<b>N16 (8/06/2012)</b>					
Genus	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Aphanizomenon flos-aquae	560.0	57.68%	35281.3	24.91%	Blue-green
Melosira granulata	142.1	14.64%	35531.1	25.08%	Diatom
Sphaerocystis Schroeteri	122.6	12.63%	4292.6	3.03%	Green
Fragilaria mesolepta	78.5	8.08%	20006.5	14.12%	Diatom
Anabaena planctonica	46.0	4.74%	8416.5	5.94%	Blue-green
Dinobryon divergens	11.9	1.23%	1428.5	1.01%	Cryptophyta
Asterionella formosa	6.1	0.63%	1349.1	0.95%	Diatom
Ceratium hirundinella	3.6	0.37%	35350.7	24.96%	Dinoflagellate
Totals:	970.9	100.00%	141656.4	100.00%	

<b>N11 (8/06/2012)</b>					
Genus	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Aphanizomenon flos-aquae	177.3	46.49%	11167.1	12.43%	Blue-green
Anabaena planctonica	71.0	18.62%	12989.8	14.46%	Blue-green
Melosira ambigua	55.4	14.53%	32635.4	36.32%	Diatom
Sphaerocystis Schroeteri	37.5	9.84%	1313.2	1.46%	Green
Melosira granulata	33.4	8.77%	8361.1	9.31%	Diatom
Scenedesmus sp.	2.4	0.62%	118.6	0.13%	Green
Ceratium hirundinella	2.3	0.61%	22828.8	25.41%	Dinoflagellate
Asterionella formosa	2.0	0.52%	439.3	0.49%	Diatom
Totals:	381.3	100.00%	89853.3	100.00%	

Richard Litts, Tenmile Lakes Watershed, Lakeside, Oregon

<b>N11 (8/20/2012)</b>					
Genus	Adjusted Cells/m	Density Percen	Biovolume (uL/m)	Biovolume percen	Group
Aphanizomenon flos-aquae	454.0	86.67%	28600.6	17.52%	Blue-green
Anabaena planctonica	232.0	44.30%	42462.7	26.01%	Blue-green
Melosira granulata	123.2	23.52%	30799.8	18.86%	Diatom
Spirogyra ternata	58.6	11.19%	58619.8	35.90%	Green
Sphaerocystis schroeterii	43.0	8.21%	1504.6	0.92%	Green
Asterionella formosa	5.9	1.12%	1289.6	0.79%	Diatom
Totals:	523.8	100.00%	163277.1	100.00%	
					Toxin Producing Algae
<b>N16 (8/20/2012)</b>					
Genus	Adjusted Cells/m	Density Percen	Biovolume (uL/m)	Biovolume percen	Group
Aphanizomenon flos-aquae	668.5	46.71%	42117.5	7.03%	Blue-green
Coelospharium naegelianur	408.5	28.54%	12254.2	2.05%	Green
Anabaena planctonica	98.3	6.87%	17988.1	3.00%	Blue-green
Fragilaria mesolepta	91.9	6.42%	23422.2	3.91%	Diatom
Anabaena lemmermannii	56.8	3.97%	3975.5	0.66%	Blue-green
Ceratium hirundinella	49.5	3.46%	485216.6	81.00%	Dinoflagellate
Melosira granulata	44.2	3.09%	11043.1	1.84%	Diatom
Asterionella formosa	12.9	0.90%	2829.9	0.47%	Diatom
Cosmarium	0.7	0.05%	152.9	0.03%	Green
Totals:	1431.2	100.00%	599000.1	100.00%	

S3 (8/20/2012)					
Genus	Adjusted Cells/m	Density Percen	Biovolume (uL/m)	Biovolume percen	Group
Aphanizomenon flos-aquae	1277.5	53.22%	80481.7	8.83%	Blue-green
Melosira granulata	412.4	17.18%	103111.5	11.31%	Diatom
Navicula cuciclia	366.2	15.26%	54932.0	6.03%	Green
Anabaena planctonica	249.2	10.38%	45598.3	5.00%	Blue-green
Ceratium hirundinella	63.3	2.64%	620007.1	68.03%	Dinoflagellate
Asterionella formosa	23.1	0.96%	5085.6	0.56%	Diatom
Fragilaria mesolepta	8.7	0.36%	2210.5	0.24%	Diatom
Total:	2400.4	100.00%	911426.7	100.00%	
					Toxin Producing Algae

S8 (8/20/2012)					
Genus	Adjusted Cells/m	Density Percen	Biovolume (uL/m)	Biovolume percen	Group
Aphanizomenon flos-aquae	921.0	58.83%	58025.1	24.34%	Blue-green
Melosira granulata	242.3	15.48%	60574.7	25.41%	Diatom
Sphaerocystis shroeterii	205.6	13.13%	7194.5	3.02%	Green
Anabaena planctonica	154.9	9.90%	28349.0	11.89%	Blue-green
Rhodomonas minuta	18.8	1.20%	375.1	0.16%	Cryptophyte
Ceratium hirundinella	8.4	0.54%	82719.2	34.70%	Dinoflagellate
Dinobryon divergens	8.3	0.53%	993.0	0.42%	Dinoflagellate
Selenastrum westii	4.7	0.30%	94.3	0.04%	Green
Nitzschia	1.5	0.10%	74.5	0.03%	Diatom
Totals:	1565.5	100.00%	238399.4	100.00%	

Davis (8/20/2012)					
Genus	Adjusted Cells/m	Density Percen	Biovolume (uL/m)	Biovolume percen	Group
Anabaena planctonica	64439.8	59.62%	11792476.1	79.56%	Blue-green
Aphanizomenon flos-aquae	23255.1	21.52%	1465072.6	9.88%	Blue-green
Sphaerocystis schroeterii	13846.6	12.81%	484629.6	3.27%	Green
Anabaena spiroides	4818.4	4.46%	645665.6	4.36%	Blue-green
Melosira granulata	1420.2	1.31%	355040.0	2.40%	Diatom
Oocystis lucustris	253.6	0.23%	78108.8	0.53%	Green
Ankistrodesmus	50.7	0.05%	1268.0	0.01%	Green
Total:	108084.3	100.00%	14822260.6	100.00%	
					Toxin Producing Algae



**LAKE SUPERIOR**  
STATE UNIVERSITY  
Environmental Analysis Laboratory

Report to: Richard Litts, Tenmile Lakes Watershed, Lakeside, Oregon

N11 (9/4/2012)						
Species	Cell/mL	Adjusted Cell/mL	Percentage	Biovolume	Biovolume Percent	Group
<b>Aphanizomenon flos-aquae</b>	<b>118716.5</b>	<b>1393.85</b>	<b>71.18%</b>	<b>87812.64</b>	<b>37%</b>	<b>Blue-Green</b>
Fragilaria mesolepta	5777.325	67.83	3.46%	17297.06	7%	Diatom
Melosira granulata	13789.5	161.90	8.27%	40475.66	17%	Diatom
Aphanocapsa elachista	1648.4	19.35	0.99%	580.62	0%	Blue-Green
Coelosphaerium naegelianum	808.35	9.49	0.48%	284.73	0%	Green
<b>Anabaena planctonica</b>	<b>18570.92</b>	<b>218.04</b>	<b>11.13%</b>	<b>39901.56</b>	<b>17%</b>	<b>Blue-Green</b>
Dinobryon bavaricum	760.8	8.93	0.46%	1071.91	0%	Chrysoophyta
Frustulia sp.	824.2	9.68	0.49%	2903.08	1%	Diatom
Asterionella formosa	1585	18.61	0.95%	4094.09	2%	Diatom
Spirogyra ternata	2092.2	24.56	1.25%	24564.54	10%	Green
Dinobryon divergens	380.4	4.47	0.23%	535.95	0%	Chrysoophyta
Synedra radians	1331.4	15.63	0.80%	5627.51	2%	Diatom
Melosira ambigua	380.4	4.47	0.23%	2630.64	1%	Diatom
Staurastrum sp.	63.4	0.74	0.04%	178.65	0%	Green
Ceratium hirundinella	63.4	0.74	0.04%	7294.92	3%	Dinoflagellate
Sum:	166792.2	1958.31	100.00%	235253.56	100%	

N16 (9/4/2012)						
Species	Cells/mL	Adjusted Cells/mL	Percentage	Biovolume	Biovolume Percent	Group
<b>Aphanizomenon flos-aquae</b>	<b>121379.3</b>	<b>962.55</b>	<b>70.07%</b>	<b>60640.75</b>	<b>31%</b>	<b>Blue-green</b>
<b>Anabaena planctonica</b>	<b>30026.94</b>	<b>238.12</b>	<b>17.33%</b>	<b>43575.42</b>	<b>22%</b>	<b>Blue-green</b>
Melosira granulata	9414.9	74.66	5.44%	18665.31	10%	Diatom
Asterionella formosa	4992.75	39.59	2.88%	8710.48	4%	Diatom
Coelosphaerium naegelianum	951	7.54	0.55%	226.25	0%	Green
Ankistrodesmus	285.3	2.26	0.16%	56.56	0%	Green
Fragilaria mesolepta	2678.65	21.24	1.55%	5416.71	3%	Diatom
Dinobryon divergens	2789.6	22.12	1.61%	2654.62	1%	Chrysoophyta
Ceratium hirundinella	697.4	5.53	0.40%	54198.52	28%	Dinoflagellate
Sum:	173215.8	1373.62	100.00%	194144.62	100%	

S3 (9/4/2012)						
Species	Cells/mL	Adjusted Cells/mL	Percentage	Biovolume	Biovolume Percent	Group
<b>Aphanizomenon flos-aquae</b>	<b>240850.3</b>	<b>1339.65</b>	<b>49.08%</b>	<b>84398.09</b>	<b>11%</b>	<b>Blue-green</b>
Melosira granulata	159216.4	885.59	32.44%	221397.56	28%	Diatom
Asterionella formosa	30127.68	167.58	6.14%	36866.62	5%	Diatom
<b>Anabaena planctonica</b>	<b>20922</b>	<b>116.37</b>	<b>4.26%</b>	<b>21296.06</b>	<b>3%</b>	<b>Blue-green</b>
Ceratium hirundinella	7100.8	39.50	1.45%	387060.03	49%	Dinoflagellate
Fragilaria mesolepta	29509.18	164.14	6.01%	41854.51	5%	Diatom
Dinobryon divergens	3022.067	16.81	0.62%	2017.11	0%	Chrysoophyta
Sum:	490748.4	2729.63	100.00%	794889.98	100%	

S8 (9/4/2012)						
Species	Cells/mL	Adjusted Cells/mL	Percentage	Biovolume	Biovolume Percent	Group
Melosira granulata	34711.5	238.88	15.08%	59719.48	24%	Diatom
<b>Aphanizomenon flos-aquae</b>	<b>118925.7</b>	<b>818.42</b>	<b>51.68%</b>	<b>51560.72</b>	<b>20%</b>	<b>Blue-Green</b>
Microcystis aeruginosa	1331.4	9.16	0.58%	320.69	0%	Blue-Green
Staurastrum sp.	317	2.18	0.14%	523.57	0%	Green
<b>Anabaena planctonica</b>	<b>28434.9</b>	<b>195.68</b>	<b>12.36%</b>	<b>35810.09</b>	<b>14%</b>	<b>Blue-Green</b>
Synedra nana	1141.2	7.85	0.50%	3926.76	2%	Diatom
Ceratium hirundinella	1141.2	7.85	0.50%	76964.50	30%	Dinoflagellate
Asterionella formosa	4739.15	32.61	2.06%	7175.06	3%	Diatom
Coelosphaerium naegelianum	1046.1	7.20	0.45%	215.97	0%	Blue-green
Dinobryon bavaricum	475.5	3.27	0.21%	392.68	0%	Chrysoophyta
Dinobryon divergens	760.8	5.24	0.33%	628.28	0%	Chrysoophyta
Sphaerocystis shroeterii	32238.9	221.86	14.01%	7765.17	3%	Green
Fragilaria mesolepta	4850.1	33.38	2.11%	8511.25	3%	Diatom
Sum:	230113.5	1583.60	100.00%	253514.22	100%	

DD (9/4/2012)						
Species	Cells/mL	Adjusted Cells/mL	Percentage	Biovolume	Biovolume Percent	Group
Aphanizomenon flos-aquae	370053.1	370053.12	42.07%	23313346.56	34%	Blue-Green
Anabaena planctonica	104261.3	104261.30	11.85%	19079817.90	28%	Blue-Green
Coelosphaerium naegelianum	390702.5	390702.50	44.42%	11721075.00	17%	Blue-Green
Melosira granulata	1902	1902.00	0.22%	475500.00	1%	Diatom
Asterionella formosa	1854.45	1854.45	0.21%	407979.00	1%	Diatom
Microcystis aeruginosa	9520.567	9520.57	1.08%	333219.83	0%	Blue-Green
Ceratium hirundinella	1331.4	1331.40	0.15%	13047720.00	19%	Dinoflagellate
Sum:	879625.3	879625.34	100.00%	68378658.29	100%	

LD (9/4/2012)						
Species	Cells/mL	Adjusted Cells/mL	Percentage	Biovolume	Biovolume Percent	Group
Aphanizomenon flos-aquae	130699.1	130699.10	63.92%	8234043.30	59%	Blue-Green
Anabaena planctonica	23378.75	23378.75	11.43%	4278311.25	30%	Blue-Green
Navicula	126.8	126.80	0.06%	19020.00	0%	Diatom
Coelosphaerium naegelianum	50276.2	50276.20	24.59%	1508286.00	11%	Blue-Green
Sum:	204480.9	204480.85	100.00%	14039660.55	100%	

Potential toxin producer

S8 (9/17/2012)					
Genus	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Melosira granulata	1362.6	68.24%	340640.7	43.80%	Diatom
Aphanizomenon flos-aquae	177.5	8.89%	11181.2	1.44%	Blue-green
Anabaena planctonica	140.1	7.02%	25641.1	3.30%	Blue-green
Fragilaria mesolepta	138.1	6.91%	35207.8	4.53%	Diatom
Coelosphaerium naegelianum	125.3	6.27%	3758.6	0.48%	Green
Ceratium hirundinella	36.5	1.83%	357481.9	45.97%	Dinoflagellate
Asterionella formosa	11.3	0.56%	2475.0	0.32%	Diatom
Staurastrum	5.5	0.27%	1309.1	0.17%	Green
Totals:	1996.7	100.00%	777695.5	100.00%	

N11 (9/17/2012)					
Genus	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Aphanizomenon flos-aquae	1639.6	71.12%	103295.3	34.96%	Blue-green
Anabaena planctonica	373.7	16.21%	68388.0	23.15%	Blue-green
Coelosphaerium naegelianum	122.0	5.29%	3660.1	1.24%	Green
Melosira granulata	91.8	3.98%	22955.4	7.77%	Diatom
Melosira ambigua	49.7	2.16%	29294.9	9.92%	Diatom
Sphaerocystis Schroeterii	12.8	0.55%	446.4	0.15%	Green
Ceratium hirundinella	6.8	0.30%	66655.6	22.56%	Dinoflagellate
Ankistrodesmus	6.0	0.26%	148.8	0.05%	Green
Scenedesmus	2.1	0.09%	106.3	0.04%	Green
Navicula	0.9	0.04%	493.1	0.17%	Diatom
Totals:	2305.4	100.00%	295443.8	100.00%	

N16 (9/17/2012)					
Genus	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Aphanizomenon flos-aquae	2245.9	88.33%	141491.7	70.55%	Blue-green
Anabaena planctonica	225.5	8.87%	41270.0	20.58%	Blue-green
Melosira granulata	71.2	2.80%	17804.1	8.88%	Diatom
Totals:	2542.6	100.00%	200565.8	100.00%	

S3 (9/17/2012)					
Genus	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Aphanizomenon flos-aquae	2868.0	60.26%	180683.3	11.02%	Blue-green
Melosira granulata	1462.9	30.74%	365726.5	22.30%	Diatom
Anabaena planctonica	212.7	4.47%	38925.8	2.37%	Blue-green
Ceratium hirundinella	106.0	2.23%	1038875.2	63.35%	Dinoflagellate
Coelosphaerium naegelianum	55.5	1.17%	1665.3	0.10%	Green
Fragilaria mesolepta	54.6	1.15%	13925.6	0.85%	Diatom
Total:	4759.7	100.00%	1639801.6	100.00%	

Davis (9/17/2012)					
Genus	Adjusted Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Aphanizomenon flos-aquae	256028.2	64.32%	16129777.9	52.02%	Blue-green
Coelosphaerium naegelianum	72937.7	18.32%	2188132.1	7.06%	Green
Anabaena planctonica	44887.2	11.28%	8214357.6	26.49%	Blue-green
Melosira granulata	12933.6	3.25%	3233400.0	10.43%	Diatom
Microcystis aeruginosa	6657.0	1.67%	419391.0	1.35%	Blue-green
Fragilaria Mesolepta	2567.7	0.65%	654763.5	2.11%	Diatom
Sphaerocystis Schroeterii	1014.4	0.25%	35504.0	0.11%	Green
Scenedesmus	570.6	0.14%	28530.0	0.09%	Green
Asterionella formosa	475.5	0.12%	104610.0	0.34%	Diatom
Total:	398072.0	100.00%	31008466.1	100.00%	
					Toxin Producing Algae





**LAKE SUPERIOR**  
STATE UNIVERSITY  
Environmental Analysis Laboratory

Report to: Richard Litts, Tenmile Lakes Watershed, Lakeside, Oregon

<b>S8 (10/1/2012)</b>					
Genus	Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Melosira granulata	1897.5	81.76%	474367.0	62.25%	Diatom
Anabaena planctonica	138.3	5.96%	25317.1	3.32%	Blue-green
Aphanizomenon flos-aquae	38.7	1.67%	2437.4	0.32%	Blue-green
Euglena sp.	8.7	0.37%	2162.8	0.28%	Euglenoid
Coelosphaerium naegelianum	128.8	5.55%	3864.1	0.51%	Blue-green
Ntischia sp.	1.4	0.06%	216.3	0.03%	Diatom
Fragilaria mesolepta	42.3	1.82%	10785.0	1.42%	Diatom
Cryptomonas sp.	3.4	0.14%	100.9	0.01%	Cryptomonad
Asterionella formosa	27.2	1.17%	5991.7	0.79%	Diatom
Ceratium hirundinella	24.0	1.04%	235501.4	30.91%	Dinoflagellate
Dinobryon divergens	10.6	0.46%	1268.8	0.17%	Chryophyte
Totals:	2320.9	100.00%	762012.6	100.00%	

<b>N16 (10/1/2012)</b>					
Genus	Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Aphanizomenon flos-aquae	946.2	62.58%	59608.7	19.82%	Blue-green
Melosira granulata	112.3	7.43%	28078.4	9.34%	Diatom
Anabaena planctonica	201.5	13.32%	36865.7	12.26%	Blue-green
Coelosphaerium naegelianum	228.2	15.09%	6845.8	2.28%	Blue-green
Nitschia sp.	1.8	0.12%	267.4	0.09%	Diatom
Ceratium hirundinella	17.2	1.14%	168886.7	56.16%	Dinoflagellate
Sphaerocystis Schroeterii	4.8	0.31%	166.4	0.06%	Green
Totals:	1511.9	100.00%	300719.0	100.00%	

<b>N11 (10/1/2012)</b>					
Genus	Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Aphanizomenon flos-aquae	747.4	39.34%	47084.4	15.22%	Blue-green
Anabaena planctonica	487.4	25.65%	89185.1	28.83%	Blue-green
Melosira granulata	130.3	6.86%	32566.6	10.53%	Diatom
Asterionella formosa	135.0	7.11%	29707.6	9.60%	Diatom
Dinobryon divergenes	204.3	10.76%	24520.8	7.93%	Chrysophyte
Coelosphaerium naegelianum	183.7	9.67%	5512.1	1.78%	Blue-green
Navicula sp.	2.0	0.11%	306.5	0.10%	Diatom
Ceratium hirundinella	8.2	0.43%	80101.1	25.90%	Dinoflagellate
Cosmarium sp.	1.5	0.08%	321.8	0.10%	Diatom
Totals:	1899.8	100.00%	309306.0	100.00%	

<b>S3 (10/1/2012)</b>					
Genus	Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Aphanizomenon flos-aquae	1212.2	47.12%	76371.4	4.47%	Blue-green
Melosira granulata	946.6	36.79%	236654.7	13.86%	Diatom
Ceratium hirundinella	134.7	5.23%	1319584.1	77.27%	Green
Asterionella formosa	64.3	2.50%	14155.1	0.83%	Diatom
Anabaena planctonica	170.5	6.63%	31194.7	1.83%	Blue-green
Aphanocapsa elachista	5.7	0.22%	57.3	0.00%	Blue-green
Gomphosphaeria aponina	30.1	1.17%	7520.4	0.44%	Blue-green
Melosira ambigua	8.6	0.33%	22251.9	1.30%	Diatom
Total:	2572.7	100.00%	1707789.7	100.00%	

<b>Z (10/1/2012)</b>					
Genus	Cells/mL	Density Percent	Biovolume (uL/mL)	Biovolume percent	Group
Aphanizomenon flos-aquae	80408.1	22.46%	5065710.7	24.27%	Blue-green
Anabaena planctonica	28360.9	7.92%	5190050.8	24.87%	Blue-green
Coelosphaerium naegelianum	234453.2	65.50%	7033596.0	33.70%	Blue-green
Melosira granulata	11919.2	3.33%	2979800.0	14.28%	Diatom
Asterionella formosa	2694.5	0.75%	592790.0	2.84%	Diatom
Cryptomonas sp.	126.8	0.04%	6340.0	0.03%	Cryptomonad
Total:	357962.7	100.00%	20868287.5	100.00%	



Phytoplankton Sample Analysis					
<b>Sample:</b>		Tenmile Lake			
<b>Sample Site:</b>		DD			
<b>Sample Depth:</b>					
<b>Sample Date:</b>		4-Sep-12			
<b>Total Density (#/mL):</b>		116,014			
<b>Total Biovolume (um<sup>3</sup>/mL):</b>		195,116,545			
<b>Trophic State Index:</b>		87.9			
<b>Species</b>	<b>Density #/mL</b>	<b>Density Percent</b>	<b>Biovolume um<sup>3</sup>/mL</b>	<b>Biovolume Percent</b>	<b>Group</b>
1 Aphanizomenon flos-aquae	75,216	64.8	75,817,847	38.9	bluegreen
2 Anabaena planctonica	32,193	27.7	106,044,045	54.3	bluegreen
3 Microcystis aeruginosa	4,154	3.6	8,906,063	4.6	bluegreen
4 Melosira distans alpigena	1,038	0.9	472,512	0.2	diatom
5 Melosira granulata	742	0.6	1,550,313	0.8	diatom
6 Fragilaria crotonensis	742	0.6	872,329	0.4	diatom
7 Melosira ambigua	445	0.4	262,144	0.1	diatom
8 Melosira granulata angustissima	445	0.4	411,686	0.2	diatom
9 Asterionella formosa	297	0.3	97,914	0.1	diatom
10 Cyclotella stelligera	148	0.1	8,160	0.0	diatom
11 Anabaena flos-aquae	148	0.1	198,796	0.1	bluegreen
12 Achnanthes lanceolata	148	0.1	26,704	0.0	diatom
13 Botryococcus braunii	148	0.1	427,263	0.2	green
14 Synedra rumpens	148	0.1	20,770	0.0	diatom
Aphanizomenon flos-aquae cells/mL =		1,203,458			
Microcystis aeruginosa cells/mL =		1,113,258			
Anabaena planctonica cells/mL =		579,476			
Anabaena flos-aquae cells/mL =		2,967			
Aphanizomenon flos-aquae heterocysts/mL =		25,962			
Anabaena planctonica heterocysts/ml =		14,094			
Anabaena flos-aquae heterocysts/mL =		148			
Anabaena planctonica akinetes/mL =		148			
Note: whole water grab sample					
<b>Aquatic Analysts</b>			<b>Sample ID:</b> PT02		



**APPENDIX III: PHYCOTECH LABORATORIES PHYTOPLANKTON REPORT; N16: 9/4/12**

sample_date	division	genus	species	morph	coloniality	coloniality_state	average_cells_per_natural_unit	concentration_natural_unit_per_ml	Plankton_net_corrected_concentration_natural_unit_per_ml	algal_cell_concentration_cells_per_ml	Plankton_net_corrected_algal_cell_concentration_cells_per_ml	relative_algal_cell_concentration_percent	nitrogen_concentration_percent	taxa_count	alternate_taxa_name
9/4/2012	Bacillariophyta	Acanthoceras	zachariasii	.	Cell-Nonmotile	Free	1	212	2	212	2	0.02%	0.28%	1	
9/4/2012	Bacillariophyta	Asterionella	formosa	.	Colonial-Nonmotile	Attached	2.5	1482	12	3705	29	0.37%	1.94%	7	
9/4/2012	Bacillariophyta	Aulacoseira	granulata	straight	Filament	Attached	5.246	3388	27	17772	141	1.79%	4.44%	16	Melosira granulata
9/4/2012	Bacillariophyta	Aulacoseira	granulata	curled	Filament	Attached	7.5	212	2	1588	13	0.16%	0.28%	1	Melosira granulata
9/4/2012	Bacillariophyta	Cocconeis	placentula	.	Cell-Nonmotile	Free	1	212	2	212	2	0.02%	0.28%	1	
9/4/2012	Bacillariophyta	Fragilaria	crotonensis	.	Lateral-Filament	Attached	17.8	423	3	7538	60	0.76%	0.56%	2	
9/4/2012	Bacillariophyta	Nitzschia	subacicularis	.	Cell-Motile	Free	1	212	2	212	2	0.02%	0.28%	1	
9/4/2012	Bacillariophyta	Synedra	delicatissima	.	Cell-Nonmotile	Free	1	847	7	847	7	0.09%	1.11%	4	
9/4/2012	Chlorophyta	.	.	2-9.9 um s	Cell-Nonmotile	Free	1	212	2	212	2	0.02%	0.28%	1	
9/4/2012	Chlorophyta	Chlamydomonas	.	.	Cell-Motile	Free	1	3388	27	3388	27	0.34%	4.44%	16	
9/4/2012	Chlorophyta	Dictyosphaerium	pulchellum	.	Colonial-Nonmotile	Attached	4	212	2	847	7	0.09%	0.28%	1	
9/4/2012	Chlorophyta	Monomastix	minuta	.	Cell-Motile	Free	1	212	2	212	2	0.02%	0.28%	1	
9/4/2012	Chlorophyta	Monoraphidium	arcuatum	monoraphi	Cell-Nonmotile	Free	1	212	2	212	2	0.02%	0.28%	1	Ankistrodesmus falcatus
9/4/2012	Chlorophyta	Pediastrum	duplex	.	Colonial-Nonmotile	Attached	1	212	2	212	2	0.02%	0.28%	1	
9/4/2012	Chlorophyta	Selenastrum	gracile	.	Cell-Nonmotile	Free	4	212	2	847	7	0.09%	0.28%	1	
9/4/2012	Chrysophyta	Dinobryon	bavaricum	.	Colonial-Motile	Free	1	423	3	423	3	0.04%	0.56%	2	
9/4/2012	Chrysophyta	Dinobryon	bavaricum	.	Colonial-Motile	Attached	3	212	2	635	5	0.06%	0.28%	1	
9/4/2012	Chrysophyta	Mallomonas	.	.	Cell-Motile	Free	1	635	5	635	5	0.06%	0.83%	3	
9/4/2012	Cryptophyta	Cryptomonas	erosa	.	Cell-Motile	Free	1	423	3	423	3	0.04%	0.56%	2	
9/4/2012	Cryptophyta	Rhodomonas	minuta	.	Cell-Motile	Free	1	212	2	212	2	0.02%	0.28%	1	Rhodomonas lacustris
9/4/2012	Cyanophyta	Anabaena	planctonica	.	Filament	Attached	57.2222	635	5	36347	288	3.66%	0.83%	3	
9/4/2012	Cyanophyta	Aphanizomenon	flos-aquae	.	Multi-Filament	Attached	28.1333	30066	238	845850	6708	85.22%	39.44%	142	
9/4/2012	Cyanophyta	Aphanizomenon	issatschenkoi	.	Multi-Filament	Attached	24.8	423	3	10502	83	1.06%	0.56%	2	
9/4/2012	Cyanophyta	Aphanocapsa	delicatissima	.	Colonial-Nonmotile	Attached	26	635	5	16515	131	1.66%	0.83%	3	
9/4/2012	Cyanophyta	Pseudanabaena	limnetica	.	Filament	Attached	25	212	2	5293	42	0.53%	0.28%	1	Pseudanabaena limnetica
9/4/2012	Cyanophyta	Synechocystis	.	>1 um sp	Cell-Nonmotile	Free	1	423	3	423	3	0.04%	0.56%	2	
9/4/2012	Cyanophyta	Woronichinia	naegeliana	.	Colonial-Nonmotile	Free	1	28584	227	28584	227	2.88%	37.50%	135	Coelosphaerium naegeliana
9/4/2012	Cyanophyta	Woronichinia	naegeliana	.	Colonial-Nonmotile	Attached	12	635	5	7622	60	0.77%	0.83%	3	Woronichinia naegeliana
9/4/2012	Euglenophyta	Trachelomonas	volvocina	.	Cell-Motile	Free	1	423	3	423	3	0.04%	0.56%	2	
9/4/2012	Pyrrhophyta	Ceratium	hirundinella	.	Cell-Motile	Free	1	423	3	423	3	0.04%	0.56%	2	
9/4/2012	Pyrrhophyta	Peridinium	umbonatum	.	Cell-Motile	Free	1	212	2	212	2	0.02%	0.28%	1	
<b>TOTALS</b>							<b>235.2015</b>	<b>76223</b>	<b>604</b>	<b>992538</b>	<b>7871</b>	<b>100.00%</b>	<b>100.00%</b>		

***APPENDIX IV: MULTI LAB PHYTOPLANKTON COMPARISON***



Sample: Davis Dock 9-17-12

Species	LSSU				Lab A					Lab B					Lab C					
	Cells/ml	Density (%)	Biovolume (uL/mL)	Biovolume (%)	Density (units/ml)	Biovolume (mic3/unit)	Biovolume (total/ml)	Cells/ml	Biovolume (%)	Cells/ml	um3/cell	Biovolume (um3/ml)	Biovolume (%)	Colonies/ml	Filaments/ml	Density (#/ml)	Density (%)	Biovolume (um3/ml)	Biovolume (%)	Cells/ml
<i>Achnanthes lanceolata</i>																406	0.3	73,004	0.0	
<i>Achnanthes minutissima</i>																406	0.3	20,279	0.0	
<i>Anabaena platonica</i>	44,887	11.28	8,214,358	25.49	19,226	27,687	532,303,110	1,015,846	20.64	275,000	680	187,091,667	18.98			20,684	14	60,563,784	30	330,950
<i>Anabaena spiroides (crassa)</i>										7,500	697	5,224,175	0.53							
<i>Anabaena TL A</i>					3,272	483	1,580,601		0.10											
<i>Ankistrodesmus falcatius</i>										100	91	9,119	0.00							
<i>Aphanizomenon flos-aquae</i>	256,028	64.32	16,129,778	52.02	68,313	7,329	500,646,322	5,006,463	19.41	1,280,000	132	168,876,736	17.14			102,611	70	122,824,902	61	1,949,602
<i>Aphanizomenon sp.</i>										500,000	68	34,006,200	3.45							
<i>Aphanocapsa sp. (tenta)</i>										2,500	48	119,222	0.01							
<i>Asterionella formosa</i>	476	0.12	104,610	0.34	818	460	376,334		0.01		40	482	19,292	0.00		7,300	5	1,927,295	1	
<i>Botryococcus sp.</i>											234,453	28,134,400	2.85	120						
<i>Botryococcus sp.</i>											1,025,733	11,283,067	1.14	11						
<i>Caloneis ventricosa</i>															406	0	99,366	0		
<i>Ceratium hirundinella</i>										25	60,000	1,500,000	0.15							
<i>Ceratium hirundinella(cyst)</i>										5	30,000	150,000	0.02							
<i>Chlamydomonas sp.</i>															811	1	263,624	0		
<i>chrysophyte (unicell)</i>										33	5,572	183,891	0.02							
<i>Chronulina TL A</i>					2,337	2,145	5,013,886		0.19											
<i>Cocconeis placentula</i>					409	643	263,025		0.01											
<i>Cocconeis sp.</i>										10	2,198	21,980	0.00							
<i>Coelosphaerium naegelianum*</i>	72,938	18.32	2,188,132	7.06	818	1,668,412	1,364,955,664	14,099,542	52.93		471,000	518,100,000	52.57	1,100						
<i>Coelastrum sp.</i>											11,488	126,370	0.01	11						
colonial nannoplankton										100	1,436	143,603	0.01							
<i>Cymbella sp.</i>										5	64,621	323,106	0.03							
<i>Dictyosphaerium sp.</i>										400	87	34,828	0.00							
<i>Dimorphococcus sp.</i>										44	158	6,966	0.00							
<i>Euastrum sp.</i>										1	125,600	125,600	0.01							
filamentous chrysophyte										650	678	440,856	0.04							
filamentous cyanophyte										600	8	4,906	0.00							
<i>Fragilaria crotonensis</i>					409	8,938	3,656,163		0.14	400	600	240,000	0.02							
<i>Fragilaria Mesolepta</i>	2,568	0.65	654,764	2.11																
<i>Gloeotrichia echinulata</i>											9,420	7,065,000	0.72		750					
<i>Gomphonema sp.</i>										11	2,800	30,800	0.00							
<i>Kirchneriella sp.</i>										500	33	16,747	0.00							
<i>Limnithrix TL A</i>					3,272	2,059	6,738,009	177,316	0.26											
<i>Melosira ambigua</i>															1,217	1	2,364,952	1		
<i>Melosira distans v. alpigena</i>					818	2,062	1,686,957	30,992	0.07						811	1	638,781	0		
<i>Melosira granulata v. angust.</i>					3,682	1,319	4,855,931		0.19						4,867	3	3,163,489	2		
<i>Melosira granulata</i>	12,934	3.25	3,233,400	10.43	1,636	1,571	2,570,523		0.10						3,650	2	8,833,435	4		
<i>Melosira varians</i>										50	8,792	439,600	0.04							
<i>Melosira/Aulacoseira spp. complex</i>										15,000	687	10,303,125	1.05							
<i>Melosira/Aulacoseira spp. complex</i>										600	735	440,856	0.04							
<i>Microcystis aeruginosa</i>	6,657	1.67	419,391	1.35	818	181,830	148,758,154	263,289	5.77	9,000	87	783,626	0.08							
<i>Microcystis wesenbergii (tenta)</i>										14,000	107	1,504,743	0.15							
nannoplankton (unicells)										300	4,187	1,256,000	0.13							
nannoplankton (unicells)										600	904	542,592	0.06							
<i>Navicula sp.</i>										11	3,517	38,685	0.00							
<i>Nitzschia acicularis</i>					2,337	101	236,085		0.01											
<i>Oocystis sp.</i>										1,200	226	271,296	0.03							
Oscillatoriales: Pseudanabaenaceae										72,000	51	3,693,017	0.37							
Oscillatoriales: Pseudanabaenaceae											594	178,250	0.02	300						
<i>Pediastrum duplex</i>											6,154	6,154	0.00	1						
<i>Pseudanabaena TL A</i>					409	299	122,308	5,824	0.00											
<i>Rhodomonas minuta</i>															406	0	8,112	0		
<i>Scenedesmus arcuatus/bijuga asmbly</i>											670	29,474	0.00	44						
<i>Scenedesmus**</i>	571	0.14	28,530	0.09							256	5,642	0.00	22						
<i>Scenedesmus quadricauda</i>															406	0	105,450	0		
<i>Sphaerocystis schroeterii</i>	1,014	0.25	35,504	0.11																
<i>Synedra radians</i>															406	0	146,007	0		
<i>Synedra rumpens</i>					2,337	400	934,990		0.04						2,028	1	283,903	0		
<i>Synedra spp.</i>										1,200	181	217,602	0.02							
<i>Synedra spp.</i>										300	440	131,880	0.01							
Thalassiosirales										600	205	123,101	0.01							
<i>Trachelomonas hispida</i>					409	3,040	1,243,537		0.05											
<i>Trachelomonas sp. (sph)</i>										300	2,571	771,341	0.08							
<i>Trachelomonas volvocina</i>					409	3,054	1,249,264		0.05											
undet pennate diatom										20	2,051	41,029	0.00							
undetermined unicell										44	33,493	1,473,707	0.15							
Unk. Biflag cell PWB15A					2,337	34	79,474		0.00											
Unk. Non-thec. Dinoflag					2,337	628	1,467,935		0.06											

\* Lab A and Lab B alternate name: *Microcystis*  
 \*\* Lab B alternate name: *Scenedesmus sp.*

2012 Tenmil Aquatic Ecosystem Sciences LLC

**APPENDIX V: LAKE SUPERIOR STATE UNIVERSITY ENVIRONMENTAL ANALYSIS  
LABORATORY ALGAL TOXIN RESULTS**



Date: June 22<sup>th</sup>, 2012

To: Richard Litts, Monitoring Coordinator  
Tenmile Lakes Basin Partnership

From: Mr. Benjamin Southwell ph: (906) 635-2076 bsouthwell@lssu.edu  
Environmental Laboratory Manager and Chemist, LSSU

Subject: Data Report for Cyanotoxin Samples Supplied 6/18/2012

Listed below are the analysis results for the supplied samples. Detection limits for each toxin listed are 0.2 ug/L. (Microcystin = MYC)

Sample Name	Collection Date	Anatoxin-a (ug/L)	Cyclindrospermopsin (ug/L)	MYC-RR (ug/L)	MYC-YR (ug/L)	MYC-LR (ug/L)	MYC-LF (ug/L)	MYC-LW (ug/L)
N11	6/18/2012	<0.2	<0.2	<0.2	0.3	<0.2	<0.2	<0.2
N16	6/18/2012	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
S3	6/18/2012	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
S8	6/18/2012	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2

650 W. Easterday Ave., Sault Ste. Marie, MI 49783  
Telephone: 906-635-2076 Fax: 906-635-2266  
www.lssu.edu



Date: July 11<sup>th</sup>, 2012

To: Richard Litts, Monitoring Coordinator  
Tenmile Lakes Basin Partnership

From: Mr. Benjamin Southwell ph: (906) 635-2076 bsouthwell@lssu.edu  
Environmental Laboratory Manager and Chemist, LSSU

Subject: Data Report for Cyanotoxin Samples Supplied 7/10/2012

Listed below are the analysis results for the supplied samples. Detection limits for each toxin listed are 0.2 ug/L. (Microcystin = MYC)

Sample Name	Collection Date	Anatoxin-a (ug/L)	Cyclindrospermopsin (ug/L)	MYC-RR (ug/L)	MYC-YR (ug/L)	MYC-LR (ug/L)	MYC-LA (ug/L)	MYC-LF (ug/L)	MYC-LW (ug/L)
N11	7/9/2012	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
N16	7/9/2012	<0.2	<0.2	<0.2	<0.2	0.42	<0.2	<0.2	<0.2
S3	7/9/2012	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
S8	7/9/2012	<0.2	<0.2	<0.2	<0.2	<0.2	0.3	<0.2	<0.2

650 W. Easterday Ave., Sault Ste. Marie, MI 49783  
Telephone: 906-635-2076 \* Fax: 906-635-2266  
www.lssu.edu



Date: July 26<sup>th</sup>, 2012

To: Richard Litts, Monitoring Coordinator  
Tenmile Lakes Basin Partnership

From: Mr. Benjamin Southwell ph: (906) 635-2076 bsouthwell@lssu.edu  
Environmental Laboratory Manager and Chemist, LSSU

Subject: Data Report for Cyanotoxin Samples Supplied 7/24/2012

Listed below are the analysis results for the supplied samples. Detection limits for each toxin listed are 0.2 ug/L. (Microcystin = MYC)

Sample Name	Collection Date	Anatoxin-a (ug/L)	Cyclindrospermopsin (ug/L)	MYC-RR (ug/L)	MYC-YR (ug/L)	MYC-LR (ug/L)	MYC-LA (ug/L)	MYC-LF (ug/L)	MYC-LW (ug/L)
N11	7/23/2012	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
N16	7/23/2012	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
S3	7/23/2012	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
S8	7/23/2012	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2

650 W. Easterday Ave., Sault Ste. Marie, MI 49783  
 Telephone: 906-635-2076 \* Fax: 906-635-2266  
[www.lssu.edu](http://www.lssu.edu)



Date: August 10<sup>th</sup>, 2012

To: Richard Litts, Monitoring Coordinator  
Tenmile Lakes Basin Partnership

From: Mr. Benjamin Southwell ph: (906) 635-2076 bsouthwell@lssu.edu  
Environmental Laboratory Manager and Chemist, LSSU

Subject: Data Report for Cyanotoxin Samples Supplied 8/7/2012

Listed below are the analysis results for the supplied samples. Detection limits for each toxin listed are 0.2 ug/L. (Microcystin = MYC)

Sample Name	Collection Date	Anatoxin-a (ug/L)	Cyclindrospermopsin (ug/L)	MYC-RR (ug/L)	MYC-YR (ug/L)	MYC-LR (ug/L)	MYC-LA (ug/L)	MYC-LF (ug/L)	MYC-LW (ug/L)
N11	8/6/2012	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
N16	8/6/2012	0.27	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
S3	8/6/2012	<0.2	<0.2	<0.2	<0.2	0.38	<0.2	<0.2	<0.2
S8	8/6/2012	0.58	<0.2	<0.2	<0.2	<0.2	<0.2	0.2	<0.2

650 W. Easterday Ave., Sault Ste. Marie, MI 49783  
Telephone: 906-635-2076 \* Fax: 906-635-2266  
www.lssu.edu



Date: August 24<sup>th</sup>, 2012

To: Richard Litts, Monitoring Coordinator  
Tenmile Lakes Basin Partnership

From: Mr. Benjamin Southwell ph: (906) 635-2076 bsouthwell@lssu.edu  
Environmental Laboratory Manager and Chemist, LSSU

Subject: Data Report for Cyanotoxin Samples Supplied 8/20/2012

Listed below are the analysis results for the supplied samples. Detection limits for each toxin listed are 0.2 ug/L. (Microcystin = MYC)

Sample Name	Collection Date	Anatoxin-a (ug/L)	Cyclindrospermopsin (ug/L)	MYC-RR (ug/L)	MYC-YR (ug/L)	MYC-LR (ug/L)	MYC-LA (ug/L)	MYC-LF (ug/L)	MYC-LW (ug/L)
N11	8/20/2012	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
N16	8/20/2012	<0.20	2.70	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
S3	8/20/2012	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
S8	8/20/2012	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
DD	8/20/2012	<0.20	3.20	<0.20	<0.20	0.46	<0.20	<0.20	0.21

650 W. Easterday Ave., Sault Ste. Marie, MI 49783  
Telephone: 906-635-2076 Fax: 906-635-2266  
www.lssu.edu



Date: September 7<sup>th</sup>, 2012

To: Richard Litts, Monitoring Coordinator  
Tenmile Lakes Basin Partnership

From: Mr. Benjamin Southwell ph: (906) 635-2076 bsouthwell@lssu.edu  
Environmental Laboratory Manager and Chemist, LSSU

Subject: Data Report for Cyanotoxin Samples Supplied 9/5/2012

Listed below are the analysis results for the supplied samples. Detection limits for each toxin listed are 0.2 ug/L. (Microcystin = MYC)

Sample Name	Collection Date	Anatoxin-a (ug/L)	Cyclindrospermopsin (ug/L)	MYC-RR (ug/L)	MYC-YR (ug/L)	MYC-LR (ug/L)	MYC-LA (ug/L)	MYC-LF (ug/L)	MYC-LW (ug/L)
N11	9/4/2012	<0.20	<0.20	<0.20	<0.20	<0.20	0.30	<0.20	<0.20
N16	9/4/2012	<0.20	<0.20	<0.20	<0.20	0.25	<0.20	<0.20	0.27
S3	9/4/2012	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
S8	9/4/2012	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
DD	9/4/2012	<0.20	0.52	<0.20	<0.20	0.89	0.56	<0.20	<0.20
LD	9/4/2012	<0.20	0.21	<0.20	<0.20	0.21	<0.20	<0.20	<0.20

650 W. Easterday Ave., Sault Ste. Marie, MI 49783  
Telephone: 906-635-2076 \* Fax: 906-635-2266  
www.lssu.edu





Date: September 21<sup>st</sup>, 2012

To: Richard Litts, Monitoring Coordinator  
Tenmile Lakes Basin Partnership

From: Mr. Benjamin Southwell ph: (906) 635-2076 bsouthwell@lssu.edu  
Environmental Laboratory Manager and Chemist, LSSU

Subject: Data Report for Cyanotoxin Samples Supplied 9/18/2012

Listed below are the analysis results for the supplied samples. Detection limits for each toxin listed are 0.2 ug/L. (Microcystin = MYC)

Sample Name	Collection Date	Anatoxin-a (ug/L)	Cylindrospermopsin (ug/L)	MYC-RR (ug/L)	MYC-YR (ug/L)	MYC-LR (ug/L)	MYC-LA (ug/L)	MYC-LF (ug/L)	MYC-LW (ug/L)
N11	9/17/2012	<0.20	<0.20	<0.20	<0.20	<0.20	0.20	<0.20	<0.20
N16	9/17/2012	<0.20	<0.20	<0.20	<0.20	<0.20	0.30	<0.20	<0.20
53	9/17/2012	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
58	9/17/2012	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
DD	9/17/2012	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20

650 W. Easterday Ave., Sault Ste. Marie, MI 49783  
Telephone: 906-635-2076 Fax: 906-635-2266  
www.lssu.edu



Date: October 5th, 2012

To: Richard Litts, Monitoring Coordinator  
Tenmile Lakes Basin Partnership

From: Mr. Benjamin Southwell ph: (906) 635-2076 bsouthwell@lssu.edu  
Environmental Laboratory Manager and Chemist, LSSU

Subject: Data Report for Cyanotoxin Samples Supplied 10/2/2012

Listed below are the analysis results for the supplied samples. Detection limits for each toxin listed are 0.2 ug/L. (Microcystin = MYC)

Sample Name	Collection Date	Anatoxin-a (ug/L)	Cyclindrospermopsin (ug/L)	MYC-RR (ug/L)	MYC-YR (ug/L)	MYC-LR (ug/L)	MYC-LA (ug/L)	MYC-LF (ug/L)	MYC-LW (ug/L)
N11	10/1/2012	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
N16	10/1/2012	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
S3	10/1/2012	<0.20	<0.20	<0.20	<0.20	0.40	<0.20	<0.20	<0.20
S8	10/1/2012	<0.20	<0.20	<0.20	<0.20	0.20	<0.20	<0.20	<0.20
Z	10/1/2012	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	0.30

650 W. Easterday Ave., Sault Ste. Marie, MI 49783  
Telephone: 906-635-2076 Fax: 906-635-2266  
www.lssu.edu



Date: October 1<sup>st</sup>, 2012

To: Richard Litts, Monitoring Coordinator  
Tennmile Lakes Basin Partnership

From: Mr. Benjamin Southwell ph: (906) 635-2076 bsouthwell@lssu.edu  
Environmental Laboratory Manager and Chemist, LSSU

Subject: Data Report for Cyanotoxin Samples

Listed below are the ELISA analysis results for the supplied samples.  
Detection limits for each toxin listed are 0.05 ug/L.

Sample Name	Collection Date	Cyclindrospermopsin (ug/L)
DD	8/20/2012	0.93
DD	9/4/2012	0.34
LD	9/4/2012	0.12

NOTE: These analyzes were performed on samples that were stored for several weeks.  
This data is used solely to confirm the results (HPLC-PDA) reported previously.

650 W. Easterday Ave., Sault Ste. Marie, MI 49783  
Telephone: 906-635-2076 Fax: 906-635-2266  
www.lssu.edu

## Appendix VI: GreenWater Laboratories Algal Toxin Results

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

Contact: [markaubel@greenwaterlab.com](mailto:markaubel@greenwaterlab.com)  
[amandafoss@greenwaterlab.com](mailto:amandafoss@greenwaterlab.com)



**Tenmile Lakes Basin Partnership**


**CYLINDROSPERMOPSIN RESULTS**

Tested on: 9/14/2012  
 Method: Enzyme-Linked ImmunoSorbent Assay (ELISA)  
 Analyte: Cylindrospermopsin  
 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)
DD 8/20/12	1x	none	ND	1	80	112	ND	ND
	1x	none	ND	1	80	112	ND	
DD 9/4/12	1x	none	ND	1	80	-	ND	ND
	1x	none	ND	1	80	-	ND	
L 9/4/12	1x	none	ND	1	80	-	ND	ND
	1x	none	ND	1	80	-	ND	

ND = Not detected above LOD/LOQ  
 LOD/LOQ = 0.1 µg/L  
 LFB = 1.0 µg/L CYN  
 LFM = 1.0 µg/L CYN

Submitted by:

  
 Mark T. Aubel, Ph.D.  
 9/14/2012

Date:

Submitted to: Richard Litts  
 TLBP  
 PO Box L  
 Lakeside, OR 97449  
[tlbp@oresys.com](mailto:tlbp@oresys.com)

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

Contact: [markaubel@greenwaterlab.com](mailto:markaubel@greenwaterlab.com)  
[amandafoss@greenwaterlab.com](mailto:amandafoss@greenwaterlab.com)



Tenmile Lakes Basin Partnership

MICROCYSTIN RESULTS

Tested on: 9/13/2012  
 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)
DD	1x	none	4.26	1	92	-	4.26	3.9
9/4/12	1x	none	3.60	1	92	-	3.60	

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:

9/14/2012

Submitted to:

Richard Littis  
 TLBP  
 PO Box L  
 Lakeside, OR 97449  
[tlbp@presys.com](mailto:tlbp@presys.com)

**Cylindrospermopsin and Microcystin Analysis Report**

Project: TLBP (Tenmile Lake)

Sample Identification

Sample Collection Date

DD	8/20/12
DD	9/4/12
L	9/4/12

**Toxin** – Cylindrospermopsin (CYN), microcystin (MC)

**Sample Prep** – The samples were ultra-sonicated to lyse cells and release toxins. A duplicate DD (8/20/12) sample was spiked (Lab Fortified Matrix, LFM) at 1.0 µg/L CYN, which provided quantitative capability and additional qualitative confirmation.

**Analytical Methodology** – A microcystins enzyme linked immunosorbent assay (ELISA) was utilized for the quantitative and sensitive congener-independent detection of MCs. The current assay is sensitive to down to an LOD/LOQ of 0.15 µg/L for total MCs. The average recovery of a laboratory fortified blank (LFB) spiked with 1 µg/L MCLR was 92%.

A cylindrospermopsin enzyme linked immunosorbent assay (ELISA) was also utilized for the quantitative detection of CYN. The current assay is sensitive down to a LOD/LOQ of 0.1 µg/L for CYN. A lab fortified blank (LFB) spiked with 1.0 µg/L CYN was recovered at 80%.

**Summary of ANTX-A/MC Results**

<u>Sample</u>	<u>CYN level</u> (µg/L)	<u>MC level</u> (µg/L)
DD (8/20/12)	ND	—
DD (9/4/12)	ND	3.9
L (9/4/12)	ND	—

ND = Not detected above the LOD  
LOD/LOQ = 0.1 µg/L (CYN), 0.15 µg/L (MC)

Submitted by: 

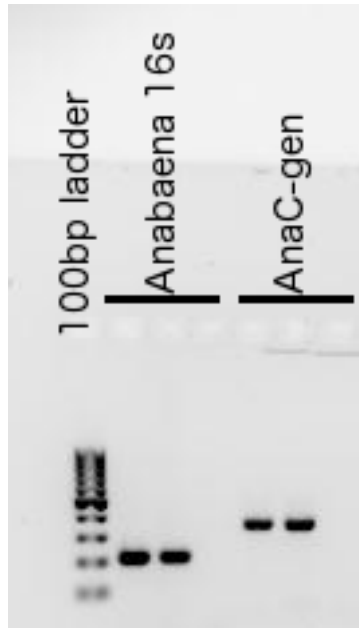
Mark T. Aubel, Ph.D.

Date: 9/14/12

## **APPENDIX VII: OREGON STATE UNIVERSITY DREHER LAB RESULTS**

### **Ten Mile Lake PCR Analysis; 14 November, 2012 Connie Bozarth, Dreher Lab, Oregon State University**

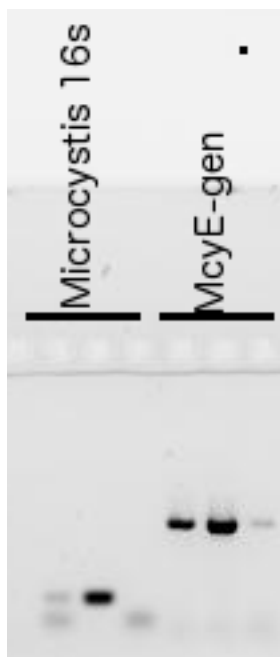
Sample was collected 9-04-12 and received in Corvallis (frozen) 10-04-12 (sent by Jake Kann). The sample was thawed, and 5ml was filtered through a Whatman GF/C filter. The filter was stored at -80 prior to DNA extraction using a MO-BIO Power Plant extraction kit with bead beating. Each PCR reaction contained 10ng genomic DNA. Negative results (with Ten Mile template) were spiked with appropriate positive control templates to check for inhibition of the polymerase reaction from the Ten Mile sample. In no case was sample inhibition a problem. For each set of detection primers, three templates were included: (first lane) Ten Mile lake sample, (second lane) positive control and (third lane) a no template, negative control.



Gel 1:

**Anabaena 16S** primers will detect all *Anabaena sp* 16S. These results show that *Anabaena sp* are present in the sample.

**AnaC-gen** will detect the AnaC gene of the anatoxin-a producing cassette in *Anabaena sp.*, *Aphanizomenon sp.* and *Oscillatoria sp.*, but does not distinguish between these three genera. These results show that anatoxin-a producers are present in the sample, but does not determine which genus is the producer.

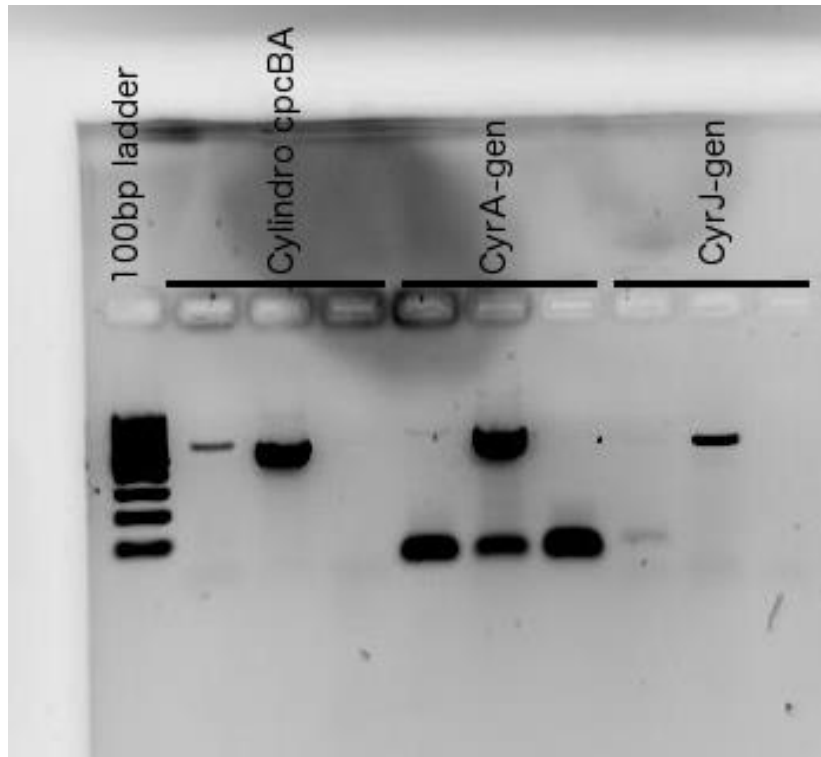


Gel 2:

**Microcystis 16S** primers will detect all *Microcystis sp* 16S in the sample. These results show that *Microcystis sp* is present in the sample.

**McyE-gen** primers will detect the *mcyE* gene of the microcystin-producing cassette of *Microcystis sp.*, *Anabaena sp.*, and *Oscillatoria sp.* These primers also detect the nodularin biosynthetase gene cluster of *Nodularia sp.* There is a small detection in the negative control, but the signals in both the positive control and the Ten Mile sample are well above the negative control. These results show that microcystin producers are present in the sample but does not determine which genus is the producer





Gel 3:

**Cylindro cpcBA** primers will detect the *cpcBA* intergenic region in *Cylindrospermopsis sp.* These results show that *Cylindrospermopsis sp.* is present in the sample. This PCR product was sequenced and is 99.5% identical to *C. raciborskii* (Florida strain F).

**CyrA-gen** and **CyrJ-gen** primers will detect *cyrA* and *cyrJ* genes in the cylindrospermopsin cassette of *Cylindrospermopsis sp.*, *Aphanizomenon sp.*, *Raphidiopsis sp.* and *Umezakia sp.* Although cylindrospermopsin production has been reported in *Anabaena sp.*, there isn't enough information in the data base to determine whether these primer pairs will detect cylindrospermopsin in *Anabaena sp.* These results are inconclusive as to the presence of cylindrospermopsin producers in the sample. In the gel above, very faint signals were detected for *cyrA* and *cyrJ*, but these results were not repeatable in a conclusive way. There was not enough of the amplicon present to try for a sequence confirmation.

The sample had been frozen and thawed prior to DNA isolation and few intact cells were present (microscopic examination). Therefore, the material captured on the filter (from which DNA was extracted) was probably much reduced. Samples have been submitted for LC-MS/MS analysis; results are pending.

**Conclusion:** There's evidence that *Cylindrospermopsis* is present, but not certain about the presence of cylindrospermopsin biosynthetic genes, and toxin analyses are pending. *Anabaena* and *Microcystis* are also present, as are anatoxin-a and microcystin biosynthetic genes.

From: Dreher, Theo <theo.dreher@oregonstate.edu>  
Sent: Wednesday, December 12, 2012 5:14 PM  
To: Jacobkann@aol.com  
Cc: tlbp@presys.com; Maier, Claudia  
Subject: Re: algal toxin testing Ten Mile Lake, suspected cylindrospermopsin

Jake,

Yes, we did get the results, with no detections for cylindrospermopsin, in addition to no detections for anatoxin-a, MC-LA, -LR, -YR, and -RR in either of your two samples, N16 (8/20/12) and DD (9/4/12).

You said you had a positive result for one sample from one lab/technique but not another. Mind sharing which lab reported the positive result?

Theo

Theo W. Dreher,  
Pernot Professor and Chair  
Department of Microbiology  
Oregon State University  
Corvallis, OR 97331  
Tel. 541-737-1795  
E-mail theo.dreher@oregonstate.edu

From: "Jacobkann@aol.com" <Jacobkann@aol.com>  
Date: Wednesday, December 12, 2012 4:52 PM  
To: Theo Dreher <theo.dreher@oregonstate.edu>  
Cc: "tlbp@presys.com" <tlbp@presys.com>  
Subject: Re: algal toxin testing Ten Mile Lake, suspected cylindrospermopsin

Hi Theo-- any results yet for cylindrospermopsin on the Tenmile sample? Thanks.

Jake

---

Jacob Kann, Ph.D.  
Aquatic Ecosystem Sciences, LLC  
295 East Main St., Suite 7  
Ashland, OR 97520  
voice: 541-482-1575  
fax: 541-552-1024  
email: jacobkann@aol.com  
www.aquatic-ecosciences.com

## ***APPENDIX VIII: OREGON DHS AND TENMILE LAKES HARMFUL ALGAL BLOOM INFORMATION***

**Oregon Health Division**  
**Drinking water treatment guidance**  
**August 31, 2001**

### **DHS Contact Information:**

Harmful Algae Program Coordinator: Jennifer Ketterman at 877-290-6767

If she is not available call the main line for the Office of Environmental

Public Health at: (971) 673 – 0440 or

Toll Free: (877) 290 – 6767.

1. Treatment systems should consist of sand filtration followed by chlorination, followed by activated charcoal filtration. It is essential that sand filtration be done before disinfection to remove as many algal cells as possible without killing or rupturing them.
2. Chlorination systems should be capable of maintaining at least 1 ppm of chlorine residual for at least 20 minutes contact time before the water enters the activated charcoal system.
3. The final step in the process should be effective activated charcoal treatment to remove toxin remaining after the sand filtration and disinfection processes.
4. All treatment equipment used should meet NSF standard 53, and should be adequately sized to treat the maximum amount of water that you use. Treatment equipment needs regular monitoring and servicing to assure that it functions properly.
5. Ideally all water entering your home should be treated as recommended. It is possible to treat only water used in the kitchen, but this increases chances that animals or pets would inadvertently drink untreated water.

As more monitoring is done and toxin levels are measured this advisory may be altered. The advisory is to remain in effect until specifically changed or lifted by county and state health officials.

## FACT SHEET

### Toxic *Microcystis* Blooms in Tenmile Lakes

(information modified from Oregon Health Division Document: Hazards from *Microcystis aeruginosa* in Fresh Water – <http://www.ohd.hr.state.or.us/esc/docs/mafact.htm>)

#### ➤ What is a toxic bloom of *Microcystis aeruginosa*?

*Microcystis aeruginosa* is a species of blue-green algae that grows naturally in many surface waters. In most bodies of fresh water and most weather conditions it does not pose a hazard to wildlife or human beings. However, under certain conditions (such as when the water is warm with abundant nutrients) *Microcystis aeruginosa* can grow more rapidly than normal. The result can be excessive numbers of large colonies that form floating masses on the water surface or that are dispersed within the water column. These occurrences are called "algal blooms". *Microcystis aeruginosa* can produce natural toxins (called microcystins) that are very potent, and these toxins are higher in concentration during bloom conditions. The microcystin toxins are produced and contained inside the *Microcystis* cells, and are released to the water when the cells die and disintegrate. Also, since the cells are very small, they can be ingested along with the water. Toxin levels in a water body tend to be higher near shorelines and at the surface of the water where animal and human contact is most likely.

#### ➤ What are the primary toxic effects of these blooms?

The primary toxic effect of microcystins is on the liver. At very high doses, death of liver cells and destruction of blood vessels in the liver can result in serious injury and possibly death. Though less is known about the long-term effects of microcystin toxins, animal studies have shown these toxins can cause chronic liver damage and may promote the formation of liver tumors. These effects are more likely to occur if exposure is frequent over a long period of time.

The levels of toxin necessary to produce immediate or acute illness in humans and animals are much higher than levels that may cause chronic liver injury. Drinking water standards are usually based on chronic effects. Currently, there is no drinking water standard in the U.S. for microcystins. Canada, Australia, and Great Britain have developed a guideline level of 1 microgram toxin per liter of water, or 1 part per billion (1 ppb). During algal blooms, toxin levels can greatly exceed 1 ppb.

➤ **How is it determined when the water becomes safe once a bloom is reported?**

Changes in weather or in other conditions in a water body influence the growth of blue-green algae. Generally, cooler weather, rainfall, and reduced sunshine will lead to reductions in algal growth and toxin levels. Algal blooms generally peak and die off rapidly and toxin levels in the water decline over days or weeks. Only blue-green algae experts can distinguish visually between different kinds of algal growth, and are able to determine when blooms have disappeared. Testing of the water is the only way to be certain that toxin levels are no longer dangerous.

➤ **When does the Oregon Health Division Issue Warnings?**

**Drinking Water** -- When measured or estimated toxin levels reach 1 ug/l the Department of Human Services, Office of Public Health Systems issues public advisories or warnings. These will include warnings regarding the use of water for drinking or food preparation unless the water has been treated following specific guidelines for destroying and removing toxins. Animals should be kept away from water during periods when microcystin toxin levels exceed 1 ug/l, because drinking the water can cause serious or even fatal illness.

**Contact Recreation** -- If levels are high enough to pose hazards for swimming, water-skiing or other direct skin contact activities, the advisories will warn against water contact. Generally skin hazards occur where the water has a green or blue-green color or where there are visible clumps or mats of algae present in the water. When measured toxin levels reach 5 ug/L or cell counts reach 15,000 cells/ml, contact recreation is considered unsafe.

➤ **Can testing ensure that all areas of the lake are safe?**

No, due to the patchy nature of blue-green algal blooms it is possible for higher *Microcystis* densities (and therefore higher microcystin toxin concentrations) to be present in areas not sampled in a given survey, particularly along shorelines or during calm conditions of little to no wind. Therefore, when a lake has a demonstrated history of algal toxicity or the presence of known toxin producing algal species, those utilizing the lake for drinking water should always follow Oregon Health Division recommendations for purification. In addition, recreational users should always avoid contact with water whenever noticeable surface concentrations of algae are evident or when the lake has an obvious green to blue-green appearance.

➤ **Are domestic animals at risk during blooms?**

Yes, pets or other domestic animals are the most likely to ingest contaminated water, these animals should not be allowed access to the lakeshore whenever either noticeable surface concentrations of algae or an obvious green to blue-green appearance is evident.

➤ **Is it safe to eat fish and other aquatic life?**

Clams, mussels, snails and other shellfish should not be eaten during microcystin advisory periods, but it is believed that fish can be safely eaten if they are cleaned and all internal organs discarded. Internal organs of such fish may be toxic even to animals.

➤ **How much does testing cost?**

Samples must be shipped to qualified laboratories for analysis. A microscopic determination to quantify the number of *Microcystis* colonies and cells costs \$90 per sample. A specialized test to analyze for the microcystin toxin concentration costs \$100 per sample (overnight shipping costs not included), and for anatoxins the cost is \$250/sample .

NOTE: Additional information can be obtained online from the Oregon Harmful Algae Bloom Surveillance Program: <http://www.oregon.gov/DHS/ph/hab/>