TECHNICAL MEMORANDUM

Tenmile Lakes Toxic Cyanobacteria Monitoring: 2009 Supplemental Toxin Results

Prepared for: **Tenmile Lakes Basin Partnership**

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Although results for the 2009 sampling season were previously summarized (e.g., see Kann 2010), additional funding was available to process several archived toxin samples that were frozen at the time of sample collection in 2009. Similar to several toxin samples previously analyzed in 2009, the additional frozen samples were shipped overnight air to CyanoLab (division of GreenWater Labs in Palatka, FL) for the enzyme linked immunosorbent assay (ELISA) of microcystin toxin (see lab results in Appendix I below). Results for these stations (Figure 1) were combined with previous cyanotoxin results (which also included the LC/MS analysis to determine anatoxin-a and saxitoxin on two dates in October), and are shown below in Table 1.

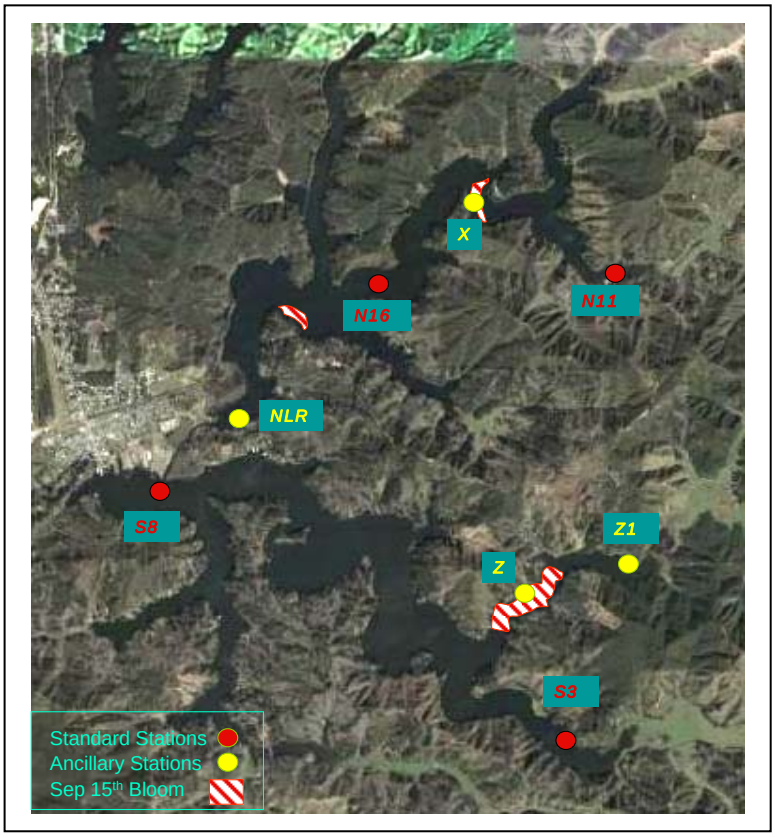

Figure 1. Location of standard and ancillary toxic algal sampling stations in Tenmile Lakes, 2009.

Table 1. Cell density and algal toxin results for select 2009 samples; Tenmile Lakes, Oregon.

Station	Date	*Microcystis aeruginosa* (cells/ml)	*Gloeotrichia echinulata* (cells/ml)	*Microcystis* + *Gloeotrichia* (cells/ml)	*Anabaena flos-aquae* (cells/ml)	*Anabaena planktonica* (cells/ml)	*Anabaena circinalis* (cells/ml)	*Anabaena sp.* (cells/ml)	Total *Anabaena* (cells/ml)	*Aphanizomenon flos-aquae* (cells/ml)	Microcystin (µg/L)	Anatoxin-a (µg/L)	Saxitoxin (µg/L)	Exceedance of microcystin TDI of 0.04 µg/kg/day for a 20kg (44lb) child ingesting 100 mls[1] (x greater than TDI)
X	9/8/2009	300,940	0	300,940	0	605,160	0	0	605,160	560,880	20.0	nd	nd	2.5
Z	9/15/2009	2,158,388	0	2,158,388	0	2,137	0	0	2,137	145,667	2365.0	nt	nt	295.6
Z	9/21/2009	1,008,139	0	1,008,139	0	17,932	0	0	17,932	64,571	910.0	nt	nt	113.8
Z1	10/5/2009	3,197,474	0	3,197,474	39,783	86,400	56,498	0	182,681	197,085	1410.0	0.6	nt	176.3
Z1	10/20/2009	4,664,468	0	4,664,468	770,835	82,308	0	0	853,143	886,215	1265.0	2.0	nt	158.1

nd=non detect
nt=not tested
[1]The TDI or tolerable daily intake (e.g., WHO 1999: http://www.who.int/water_sanitation_health/resourcesquality/toxicyanbact/en/) as computed here for a 20kg child is equivalent to the exceedance of the 8 µg/L microcystin guideline value as recommended by the State of Oregon.

***Exceeds World Health Organization Alert Level 1 increased vigilance guideline level of 500 cells ml^{-1} for potentially toxigenic species in drinking water systems.**
****Exceeds World Health Organization Alert Level 2 public health posting guideline level of 2000 cells ml^{-1} 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* or 8 µg/L of microcystin.**

As noted in 2009 biweekly toxic algal memos, patchy concentrations of blue-green algae were occurring in Tenmile Lakes beginning around September 8th and continuing through October. At that time ancillary samples were collected in areas of accumulated blue-green algal material (e.g., see Figure 2 depicting conditions on September 15th). These results showed that the blue-green blooms were comprised of high concentrations of *Anabaena planctonica, Anabaena flos-aquae*, *Aphanizomenon flos-aquae*, and *Microcystis aeruginosa,* and that cell density levels greatly exceeded both drinking water and recreational guideline levels for public health (Table 1; State of Oregon posting guidelines for recreational water bodies are 40,000 cells/ml for *Microcystis* and 100,000 cells/ml for *Anabaena*).

Figure 2. September 15th bloom conditions at varying locations Tenmile Lake (see map above).

Algal toxin results showed that the September 8th sample at station "X" had 20 µg/L of microcystin, exceeding the 8 µg/L State of Oregon recreational posting guideline by 2.5x, and confirming that *Microcystis* levels exceeding 40,000 cells/ml can be associated with microcystin toxin levels that constitute a high probability of adverse health effects for recreational users of the lake (microcystin is a potent liver toxin associated with both chronic and acute health effects). Note that 2,000 cells/ml is the advisory level for using lake-water for potable purposes.

The September 8th results also supported statements in earlier memos that *Anabaena planktonica* has not been generally found to be associated with the algal neurotoxin anatoxin-a (despite a cell density of >600,000 cells/ml, anatoxin-a was not detected). Likewise, although *Aphanizomenon flos-aquae* has been associated with toxin production in other regions, the blooms typically experienced in Oregon have not been associated with toxin production; at a cell density of >560,000 cells/ml neither anatoxin nor saxitoxin was detected in the Tenmile sample.

A sample collected one week later at station "Z" continued to show prevalent blue-green algae, including a very high density of *Microcystis* (2,158,388 cells/ml) that was associated with a microcystin toxin level of 2365 µg/L. ***This toxin level exceeded the Oregon public health guideline level for recreation by ~296 times (Table 1), and is among the highest levels recorded in the region.*** Such levels have been associated with bioaccumulation of toxin in both fish and freshwater mussels in other systems (e.g., the Klamath River in northern California; Kann 2008 and 2010). A subsequent sample at station "Z" on September 21st continued to show both high *Microcystis* cell density (>1 million cells/ml) and microcystin concentration that exceeded the recreational public health guideline by >113 times (Table 1).

Microcystin concentration also persisted at high levels at station "Z1" on both October 5th (1410 µg/L or >176 times the public health guideline), and October 20th (1265 µg/L or 158 times the public health guideline). In addition, on those dates *Anabaena flos-aquae* was detected along with low levels of the neurotoxin anatoxin-a. However, despite a large increase in *Anabaena flos-aquae* on October 20th (770,835 cells/ml), anatoxin-a only increased slightly to 2 µg/L (Table 1).

These cyanotoxin results clearly show that during the late-summer through fall period of 2009 that Tenmile Lakes experienced toxic cyanobacterial blooms that greatly exceeded not only the drinking water guidelines for the hepatotoxin microcystin, but also exceeded recreational guidelines for public health by hundreds of times. Depending ion the efficacy of treatment systems, such microcystin concentrations in the vicinity of homeowner drinking water intakes could pose a serious public health threat.

Due to the patchy nature of blue-green algal blooms it is possible for higher Microcystis aeruginosa and Anabaena flos-aquae densities (and therefore higher microcystin toxin and 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 blooms, and the fact that all areas of the lake cannot be tested at all times, those utilizing the lake for drinking water should <u>always</u> follow Oregon Health Division recommendations for purification (attached). In addition, recreational users should <u>always</u> 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.

References for Alert Levels

Kann, J. 2009. Microcystin Bioaccumulation in Klamath River Fish and Freshwater Mussel Tissue: Preliminary 2007 Results. Technical Memorandum Prepared for the Karuk Tribe Department of Natural Resources. April 2009.

Kann, J. 2010. Microcystin Bioaccumulation in Klamath River Freshwater Mussel Tissue: 2009 Results. Technical Memorandum Prepared for the Karuk Tribe Department of Natural Resources. July 2010.

Kann, J. 2010. Tenmile Lakes Toxic Algal Sampling Program: 2009 Data Summary Report. Tenmile Lakes Basin Partnership, Lakeside OR 97520

Falconer et al. 1999. Safe levels and safe practices. Pages 155-177 *in*: I. Chorus and J. Bartram, editors. *Toxic Cyanobacteria in water: a guide to their public health consequences*. World Health Organization Report. E & FN Spon, London and New York.

Stone, D., and W. Bress. 2007. Addressing Public Health Risks for Cyanobacteria in Recreational Freshwaters: The Oregon and Vermont Framework. Integrated Environmental Assesment and Managment; 3(1): 137 - 143 (2007). http://www.oregon.gov/DHS/ph/hab/docs/Stone_cyano_rec.pdf

Yoo, S.R., W.W. Carmichael, R.C. Hoehn, and S.E. Hrudy. 1995. 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)

Appendix I: GreenWater Labs Algal Toxin Results

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

Anatoxin-a, Microcystin and Saxitoxin Analysis Report
Project: TLBP
(Tenmile – North Lake)

Sample Identification	Sample Collection Date
Tenmile - North Lake	9/8/09

Toxin – Anatoxin-a (ANTX-A), microcystin (MC), saxitoxin (STX)

Sample Prep – The sample was ultra-sonicated to lyse cells and release toxins. Solid phase extraction (SPE) was also utilized for ANTX-A prep and preconcentration (100x). Duplicate samples were spiked with 0.1 µg/L of ANTX-A and 0.5 µg/L STX, which provided quantitative capability and additional qualitative confirmation.

Analytical Methodology – Liquid chromatography/ mass spectrometry/ mass spectrometry (LC/MS/MS) was utilized for the determination of ANTX-A. The $[M+H]^+$ ion for ANTX-A (*m/z* 166) was fragmented and the major product ions (*m/z* 149, 131, 107, and 91) provided both specificity and sensitivity. The current methodology established a detection limit of 0.05 µg/L and a quantification limit of 0.1 µg/L for ANTX-A.

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 a detection/quantification limit of 0.15 µg/L for total MCs.

A saxitoxin enzyme linked immunosorbent assay (ELISA) was utilized for the quantitative detection of saxitoxin. The current assay is sensitive down to a detection/quantification limit of 0.1 µg/L saxitoxin.

Summary of ANTX-A/MC/STX Results

Sample	ANTX-A level (µg/L)	MC level (µg/L)	STX level (µg/L)
Tenmile – North Lake	ND	≈ 20	ND

ND = Not detected above the detection limit
Detection Limit = 0.05 µg/L (ANTX-A), 0.15 µg/L (MC), 0.1 µg/L (STX)
Limit of Quantification = 0.1 µg/L (ANTX-A & STX), 0.15 µg/L (MC)

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SAXITOXIN RESULTS

Tested on:	9/16/2009
Method:	Enzyme-Linked ImmunoSorbent Assay (ELISA)
Analyte:	Saxitoxins
Analyzed by:	Amanda Foss

Sample ID/ Date Collected	Initial Conc. Factor	Dilution Ratio	Assay Value, ug/L	Final Dilution Factor	Avg. Std. Recovery(%)	Avg. Spike Recovery(%)	Final Concentration (ug/L)	Average (ug/L)
Tenmile North Lake	1x	none	ND	1	99	96	ND	ND
9/8/2009	1x	none	ND	1	99	96	ND	

ND = Not detected above Quantification limit
Quantification limit = 0.10 µg/L
Standard = 0.5 µg/L STX
Sample spike = 0.5 µg/L STX

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MICROCYSTIN RESULTS

Tested on:	9/16/2009
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. Std. Recovery(%)	Final Concentration (ug/L)	Average (ug/L)
Tenmile North Lake	1x	1:10	1.97	10	100	19.7	20
9/8/2009	1x	1:10	2.01	10	100	20.1	
		1:100	0.20	100	100	20.0	
		1:100	0.20	100	100	20.0	

ND = Not detected above Quantification limit
Quantification limit = 0.15 µg/L
Standard = 1 µg/L MCLR
Sample spike = 1 µg/L MCLR

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

Anatoxin-a and Microcystin Analysis Report

Project: TLBP (Tenmile Lake)

Sample Identification	Sample Collection Date
Tenmile Z	090915
Tenmile Z	090921
Tenmile Z1	091005
Tenmile Z1	091020

Toxin – Anatoxin-a (ANTX-A), microcystin (MC)

Sample Prep – The sample was ultra-sonicated to lyse cells and release toxins. Solid phase extraction (SPE) was also utilized for ANTX-A prep and preconcentration (100x). A duplicate sample of Z1 (10/20/09) was spiked with 0.1 µg/L of ANTX-A (Lab Fortified Matrix, LFM), which provided quantitative capability and additional qualitative confirmation.

Analytical Methodology – Liquid chromatography/ mass spectrometry/ mass spectrometry (LC/MS/MS) was utilized for the determination of ANTX-A. The $[M+H]^+$ ion for ANTX-A (*m/z* 166) was fragmented and the major product ions (*m/z* 149, 131, 107, and 91) provided both specificity and sensitivity. The current methodology established a limit of detection (LOD) of 0.05 µg/L and a limit of quantification (LOQ) of 0.1 µg/L for ANTX-A.

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. Significant dilutions (i.e. 1000x) were necessary prior to analysis.

Summary of ANTX-A/MC Results

Sample	ANTX-A level (µg/L)	MC level (µg/L)
Tenmile Z (090915)	–	≈ 2365
Tenmile Z (090921)	–	≈ 910
Tenmile Z1 (091005)	≈ 0.6	≈ 1410
Tenmile Z1 (091020)	≈ 2.0	≈ 1265

ND = Not detected above the LOD
LOD = 0.05 µg/L (ANTX-A), 0.15 µg/L (MC)
LOQ = 0.1 µg/L (ANTX-A), 0.15 µg/L (MC)

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Tenmile Lake Basin Partnership

MICROCYSTIN RESULTS

Tested on: 6/24/2010
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(%)	Final Concentration (ug/L)	Average (ug/L)
Tenmile Z	1x	1:1000	2.35	1000	100	2350.0	2365
9/15/2009	1x	1:1000	2.38	1000	100	2380.0	
Tenmile Z	1x	1:1000	0.92	1000	100	920.0	910
9/21/2009	1x	1:1000	0.90	1000	100	900.0	
Tenmile Z1	1x	1:1000	1.30	1000	100	1300.0	1410
10/5/2009	1x	1:1000	1.52	1000	100	1520.0	
Tenmile Z1	1x	1:1000	1.18	1000	100	1180.0	1265
10/20/2009	1x	1:1000	1.35	1000	100	1350.0	

ND = Not detected above LOD/LOQ
LOD/LOQ = 0.15 µg/L
LFB (Lab Fortified Blank = 1 µg/L MCLR

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