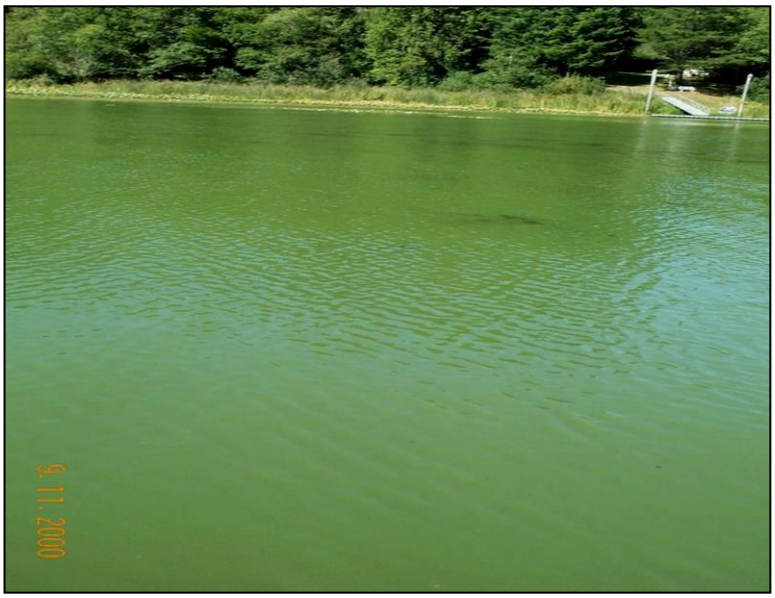
TENMILE LAKES TOXIC ALGAL SAMPLING PROGRAM: 2006 DATA SUMMARY REPORT



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BACKGROUND

Tenmile Lakes were sampled to assess the dynamics of the potentially toxic blue-green algal species, *Microcystis aeruginosa* and *Anabaena flos-aquae*. *Microcystis* produces hepatotoxins (known as microcystins), and *Anabaena* produces a neurotoxin (anatoxin-a). 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 Tenmmile 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 and that contact recreation be avoided (Kann and Gilroy 1997). The goal of 2006 sampling, performed by the Tenmile Lakes Basin Partnership, was to determine presence and numbers of these potentially toxic species at a limited number of sampling stations. Cell density of the two potentially toxigenic species was then compared to drinking water guidance levels (e.g., Yoo et al. 1995; Chorus and Bartram 1999).

METHODS

Four stations (2 in each lake) were sampled to cover a major arm and open-water location in each lake (Fig. 1). 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 8 times beginning June 26th and ending October 11th, 2006.

Year 2006 Tenmile Lakes Sample Site Locations

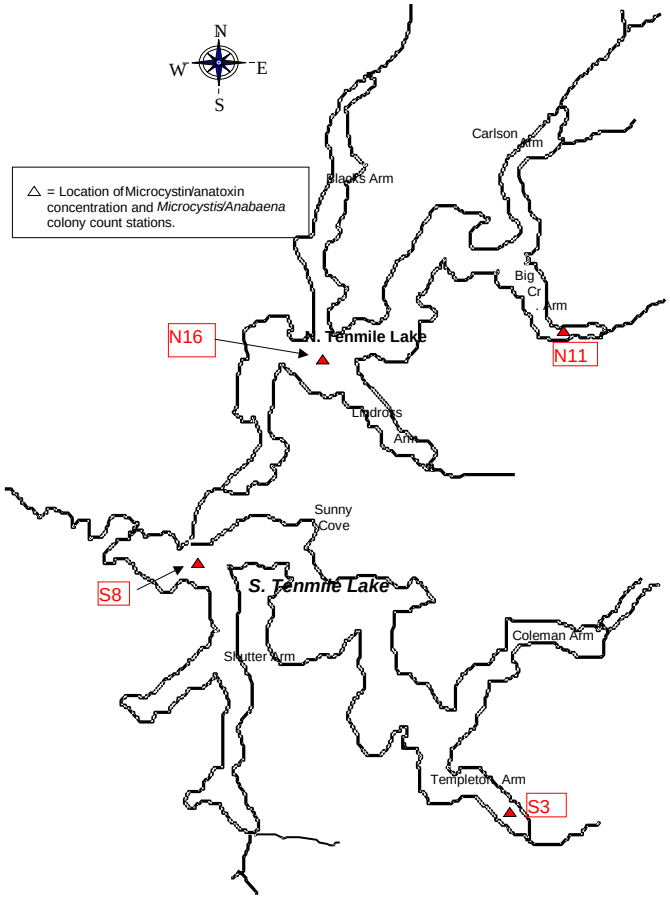

Figure 1. Location of standard toxic algal sampling stations in Tenmile Lakes, 2006.

Because the goal of the *M. aeruginosa* and *A. flos-aquae* sampling was to detect conditions that may pose human health hazards, samples were collected mid-day 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 sand 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-µm plankton net. In 2006 one additional sample from a concentrated patch of algae in Templeton Arm (Station X on attached lab sheets) was also collected on Oct 11th.

The filtered contents of 3 replicate hauls were composited in a bucket, and 2 portions of the filtered contents placed in sample bottles. The first portion was placed in a 250 ml opaque sample bottle containing 1% Lugol's preservative and shipped to plankton taxonomist Jim Sweet of Aquatic Analysts, INC., who performed a microscopic analysis for *Microcystis* and *Anabaena* density (cells ml^{-1}). The second portion was placed in a 1 liter bottle with no preservative and frozen at the TMLBP office. If counts received the following week from Aquatic Analysts, INC show that cell counts considerably exceed the Alert Level 2 threshold of 2000 cells ml^{-1}, the frozen samples are then shipped overnight air on ice to the laboratory of Dr. Wayne Carmichael at Wright State University (note that the WSU lab is no longer operational and 2007 samples will be shipped to Greenwater Labs in Palatka, FL). The enzyme linked immunosorbent assay (ELISA) is then used to determine microcystin toxins and LC/MS to determine anatoxin-a (Note: because health advisories and media outreach are initiated based upon cell density and not toxin concentration, toxin analysis is not prioritized when budgetary constraints exist or when cell counts are below 15,000 cells/ml).

Also in 2006, two samples of treated tap water and raw lake intake water were collected on 9/27 and 10/11 from a home located on Lindross Arm in North Tenmile Lake. In addition, a duplicate sampling tow for quality assurance was collected on five occasions (station D on attached lab sheets), and an additional sample on 9/27 was sub-sampled in triplicate to assess repeatability of cell density estimates.

RESULTS

2006 Trends

M. aeruginosa (MSAE) was present at values that were well below the Alert Level 1 guideline of 500 cells ml^{-1} (Yoo et al. 1995; also known as the increased vigilance level, Table 1; Figure 2) at stations S3 and N16 at the time of the first sample trip on June 26th. On this same date, station N16 had an *Anabaena flos-aquae* (ABFA) value (1849 cells/ml) that approached the Alert Level 2 Guideline of 2000 cells ml^{-1}(Yoo et al. 1995).

On July 11th *M. aeruginosa* exceeded the World Health Organization (WHO) Alert Level 2 guideline of 2000 cells ml^{-1} only at station S8 (2506 cells/ml; Figure 2; Table 1); both MSAE and ABFA levels were below 500 cells/ml at all other stations. MSAE cell density then declined at S8 but was elevated at S3 (2131 cells/ml) on July 24, with both MSAE and ABFA either relatively low or not detected at all other stations. For MSAE, levels at the four standard stations then remained below Alert Level 2 for the remainder of the 2006 season (Fig. 2; Table 1).

Table 1. Tenmile Lakes Algal Count and Toxin Results, 2006.

Station	*Lake*	*Date*	*Microcystis aeruginosa (cells/ml)*	*Anabaena flos-aquae (cells/ml)*	*Anabaena planktonica (cells/ml)*	*Anabaena circinalis (cells/ml)*	*Anabaena sp. (cells/ml)*	*Total Anabaena (cells/ml)*	*Microcystin (ug/L)*	*Anatoxin (ug/L)*
S3	S	26-Jun-06	154	0	0	0	0	0	NT*	NT
S8	S	26-Jun-06	0	26	0	0	0	26	NT	NT
N11	N	26-Jun-06	0	143	0	0	0	143	NT	NT
N16	N	26-Jun-06	99	1849	0	0	0	1849	NT	NT
S3	S	11-Jul-06	0	0	0	0	0	0	NT	NT
S8	S	11-Jul-06	2506	63	0	0	0	63	NT	NT
N11	N	11-Jul-06	288	0	0	0	0	0	NT	NT
N16	N	11-Jul-06	0	129	0	0	0	129	NT	NT
S3	S	24-Jul-06	2131	0	0	0	0	0	NT	NT
S8	S	24-Jul-06	0	0	0	0	0	0	NT	NT
N11	N	24-Jul-06	882	0	86	0	0	86	NT	NT
N16	N	24-Jul-06	0	0	49	0	0	49	NT	NT
S3	S	7-Aug-06	0	164	0	164	0	328	NT	NT
S8	S	7-Aug-06	0	326	189	70	0	585	NT	NT
N11	N	7-Aug-06	0	98	276	50	0	424	NT	NT
N16	N	7-Aug-06	0	0	196	0	0	196	NT	NT
S3	S	21-Aug-06	453	62	2317	41	0	2420	NT	NT
S8	S	21-Aug-06	23	0	278	0	0	278	NT	NT
N11	N	21-Aug-06	448	0	2885	0	0	2885	NT	NT
N16	N	21-Aug-06	0	184	2100	202	0	2486	NT	NT
S3	S	11-Sep-06	789	0	0	0	0	0	NT	NT
S8	S	11-Sep-06	184	90	92	0	0	182	NT	NT
N11	N	11-Sep-06	0	107	0	0	0	107	NT	NT
N16	N	11-Sep-06	163	280	70	0	0	350	NT	NT
S3	S	27-Sep-06	0	82	0	0	0	82	NT	NT
S8	S	27-Sep-06	0	105	209	0	0	314	NT	NT
N11	N	27-Sep-06	0	821	228	0	0	1049	NT	NT

Station	*Lake*	*Date*	*Microcystis aeruginosa (cells/ml)*	*Anabaena flos-aquae (cells/ml)*	*Anabaena planktonica (cells/ml)*	*Anabaena circinalis (cells/ml)*	*Anabaena sp. (cells/ml)*	*Total Anabaena (cells/ml)*	*Microcystin (ug/L)*	*Anatoxin (ug/L)*
N16	N	27-Sep-06	0	534	503	0	0	1037	NT	NT
S1 Lindross	N	27-Sep-06	0	326	41	0	0	367	NT	NT
S2 Lindross	N	27-Sep-06	735	392	221	0	0	613	NT	NT
S3	S	11-Oct-06	966	869	0	0	0	869	NT	NT
S8	S	11-Oct-06	0	211	189	27	0	427	NT	NT
N11	N	11-Oct-06	701	308	437	0	0	745	NT	NT
N16	N	11-Oct-06	780	193	266	0	0	459	NT	NT
X	S	11-Oct-06	0	2626	0	0	0	2626	NT	NT
L1 Lindross	N	11-Oct-06	0	580	232	0	0	812	NT	NT
L2 Lindross	N	11-Oct-06	0	756	571	33	0	1360	NT	NT

*NT=not tested because 2000 cell/ml Alert Level 2 threshold not considerably exceeded

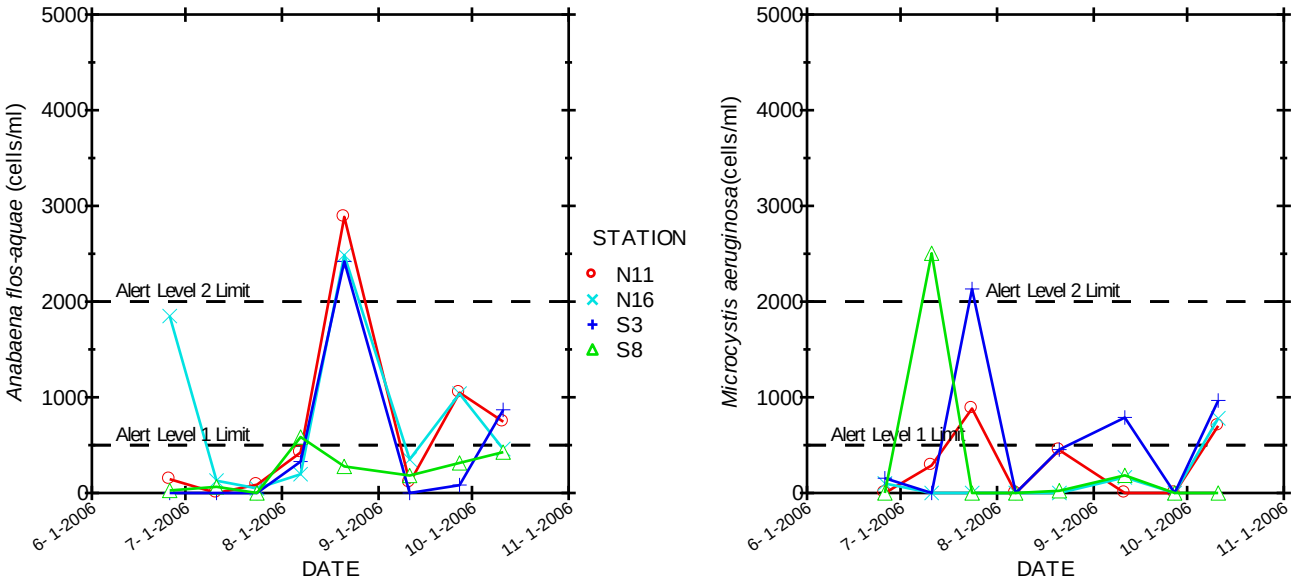

Figure 2. Cell Density of *Microcystis aeruginosa* and total *Anabaena sp.* in Tenmile Lakes, 2006.

Subsequent to the July 11th Alert Level 2 exceedances, Coos County Health Department and media outreach occurred. No Oregon Department of Human Services (OHS) news releases or lake postings were issued for *M. aeruginosa* in 2006, and due to relatively low density levels and budgetary constraints no microcystin analyses were performed.

ABFA at the 4 standard stations also remained below Alert Level 2 for the remainder of the season. However, on August 21st total *Anabaena* showed an increase above Alert Level 2 at all stations except S8, with the predominant *Anabaena* species being *A. planktonica* (Table 1). Total *Anabaena* declined on September 11th, but then increased again on September 27th and October 11th (although levels were below the Alert Level 2 threshold). The additional sample collected in Templeton Arm (Sample X in Table 1) from what appeared to be a more concentrated patch of algae than that observed at S3, did show a relatively higher concentration of ABFA (2626 cells/ml) than other stations. This indicates that although levels were still relatively low with respect to recreational guidelines (e.g., which would be 100,000 cells/ml for *Anabaena* and 40,000 cells/ml for MSAE; Stone and Bress 2007) that the potential for non-uniform distribution of algae exists. However, as shown below, the difference between counts at this station and the nearby station S3 are still within methodological counting resolution. No OHS news releases or lake postings were issued for *Anabaena* in 2006, and no anatoxin analyses were performed.

Sampling Resolution Issues

As noted in the methods, a duplicate plankton tow was collected on six occasions in 2006 to assess tow reproducibility and potential non-uniform cell density distribution at a station. These results show that aside from the September 27th duplicate that the difference between the original sample and the duplicate was low for both MSAE and total *Anabaena*, and that both samples were similar with respect to public health alert levels (Table 2).

Table 2. Difference in cell density between original sample tow and duplicate sample tow for MSAE and *Anabaena* in Tenmile Lakes, 2006.

Station	Date	Duplicate	*Microcystis aeruginosa* (cells/ml)	*Anabaena flos-aquae* (cells/ml)	*Anabaena planktonica* (cells/ml)	*Anabaena circinalis* (cells/ml)	*Anabaena sp.* (cells/ml)	Total *Anabaena* (cells/ml)	MSAE sample-duplicate difference	Total *Anabaena* sample-duplicate difference
N16	7/11		0	129	0	0	0	129		
N16-D	7/11	D	176	264	0	0	0	264	176	135
N16	7/24		0	0	49	0	0	49		
N16-D	7/24	D	0	0	196	0	0	196	0	147
N16	8/21		0	184	2100	202	0	2486		
N16-D	8/21	D	47	0	2945	71	0	3016	47	530
N16	9/11		163	280	70	0	0	350		
N16-D	9/11	D	654	82	0	0	0	82	491	268
N16	9/27		0	534	503	0	0	1037		
N16-D	9/27	D	3553	162	183	0	0	345	3553	692

The exception to the sample-duplicate agreement occurred on Sep 27th when MSAE was not detected at any of the usual 4 sampling stations (Table1; Fig. 2), yet the duplicate tow showed a level of 3553 MSAE cells/ml (Table 2). This level did exceed the WHO Alert Level 2 density of 2000 cells/ml (Alert Level 2 is the point at which advisories are issued for drinking water systems). These results underscore both the patchiness that algal densities exhibit in lake environments and the potential limitation of the resolution of laboratory determinations of algal density. To determine sensitivity of cell density estimates to laboratory microscopic analyses a repeat analysis by Aquatic Analysts on additional sub-samples of both the original and duplicate samples (N16 and N16-D) was performed for the September 27th samples. These results showed that while N16-D had higher density on the average than N16 (2433 vs. 828 cells/ml; indicating lake patchiness), that MSAE was also detected in the additional sub-sample analyses performed on N16 (Table 3).

Table 3. Repeated sub-sampling of the original (N16) and duplicate sample (N16-D) from September 27th, 2006.

Aquatic Analysts Repeated Sub-sampling for MSAE on Tenmile Sep 27 Duplicates		
Slide	**JU82**	**JU83**
Sample	**N16**	**N16-D**
	MSAE (cells/ml)	**MSAE (cells/ml)**
Original sub-sample	0	3553
2nd sub-sample	74	2116
3rd sub-sample	2502	1523
4th sub-sample	737	2538
Mean	828	2433
±95% Confidence Interval	1852	1361

The repeated sub-sampling analysis also showed that the 95% confidence intervals for these samples ranged between ±1361 to ±1852 cells per ml. Although this cell density resolution would be adequate relative to the recreational standard of 40,000 cells/ml MSAE, it is not adequate relative to the drinking-water Alert Level 2 level of 2000 cells/ml. Based on these results, future laboratory analyses for potentially toxigenic cyanobacteria in drinking-water lakes or reservoirs should be performed using increased sub-sampling resolution. For example, the number of total algal units counted could be increased from the current level of 100 algal units to 200 algal units. Although this method would incur additional costs, the above data indicate that for drinking-water systems (when low levels of a potentially toxigenic species need to be determined), such an increase in precision is necessary.

Sampling of Home Water Treatment System

Cell density data from a home water treatment system located on Lindross Arm in North Tenmile show that *Anabaena* cells were detected in samples collected directly from treated kitchen tap water (Table 4; Stations S1 and L1). On 9/27/06, 731 cells/ml of MSAE were found in the lake near the treatment system intake, and no MSAE cells were detected at the tap. However, of a total of 613 cells/ml of *Anabaena* at the intake, only ~40% were removed, with 367 cells/ml remaining at the tap. Likewise, on Oct 11th, of a total of 1360 cells/ml of *Anabaena* at the intake, only ~40% were removed, with 812 cells/ml remaining at the tap. Typical home treatment systems at Tenmile Lakes consist of sand filtration, chlorination, and activated carbon filtration. At this time it is unclear where in the treatment system the failure occurred. Although potentially toxigenic cyanobacteria levels were low in the lake in 2006, the potential for high tap water concentrations are possible should large in-lake blooms develop. This underscores the need for homeowners to ensure their treatment systems are operating effectively. However, in this case the water treatment system was newly installed and followed DHS 2001 guidelines (see Appendix I)for algal toxin treatment (J. Frederickson--TLBP; personal communication). Thus, it is imperative that additional testing be performed on treatment system efficacy and adjustments made to treatment protocol if warranted.

Table 4. Comparison of lake and treated tap water at Lindross Arm private residence, September 27th and October 11th, 2006.

Lab Station ID	Description	Date	*Microcystis aeruginosa (cells/ml)*	*Anabaena flos-aquae (cells/ml)*	*Anabaena planktonica (cells/ml)*	*Anabaena circinalis (cells/ml)*	*Anabaena sp. (cells/ml)*	*Total Anabaena (cells/ml)*
S1	Lindross Arm at Homeowner Tap	9/27/2006	0	326	41	0	0	367
S2	Lindross Arm in Lake at Homeowner Intake	9/27/2006	735	392	221	0	0	613
L1	Lindross Arm at Homeowner Tap	10/11/2006	0	580	232	0	0	812
L2	Lindross Arm in Lake at Homeowner Intake	10/11/2006	0	756	571	33	0	1360

2002-2006 Comparison

Density of MSAE in Tenmile Lakes in 2006 was somewhat lower than 2005, and slightly higher than both 2004 and 2003 (Fig. 3). By contrast, in 2002 density tended to be higher overall and there were several occasions when MSAE cell counts exceeded Alert Level 2, and one occasion of exceedance of Alert Level 3 (Fig. 3). However, ABFA cell density tended to be higher overall in 2006 than that for the 2002-2005 period (Fig. 3). There were two incidences when ABFA cell density exceeded Alert Level 2 in 2006.

Box plots with data grouped by station indicate the same trend of increased ABFA in 2006, with ABFA cell density tending to be somewhat higher in North Tenmile than South Tenmile (Fig 4). For MSAE, the stations located in Big Creek Arm (N11) and Templeton Arm (S3) tended to show higher MSAE cell density than open water stations (N16 and S8) in 2006 (Fig 4). Aside from 2002 when N16 showed high MSAE density, N11 and S3 tended to exhibit higher MSAE density than other stations.

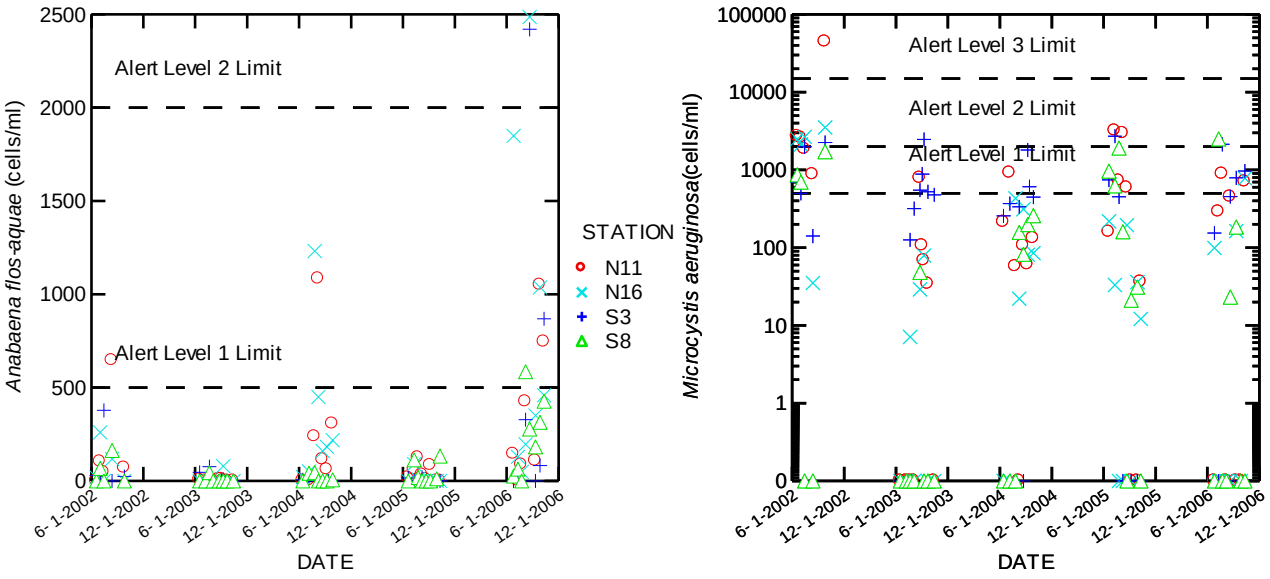

Figure 3. Density of *Microcystis aeruginosa* and *Anabaena flos-aquae* in Tenmile Lakes, 2002-2006.

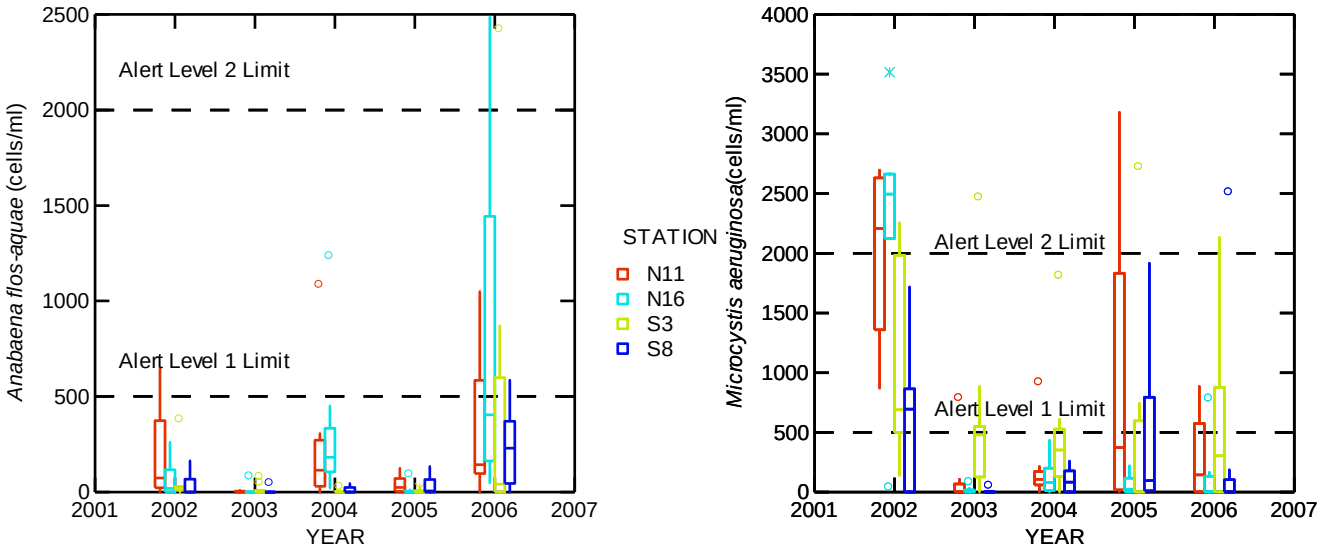

Figure 4. ***Microcystis aeruginosa*** **and** ***Anabaena flos-aquae*** **cell density grouped by station, Tenmile Lakes, 2002-2006 (2002 value of >44,000 cells/ml** ***M. aeruginosa*** **not shown to facilitate comparison among years).**

Summary

Although overall cell density values remained low in 2006 relative to recreational guidelines utilized by Oregon DHS (Stone and Bress 2007), there were 3 instances when either *Anabaena* or MSAE exceeded the Alert Level 2 of 2000 cells/ml. Duplicate samples as well as repeated sub-sampling (microscopic analysis) indicate that evaluation with respect to threshold levels should consider variation around cell density and lake patchiness. Particularly, lake home-owners who utilize treated lake water for household purposes should always ensure their treatment systems are operational and up to date. This is underscored by the above results for the home in Lindross Arm that showed low levels of *Anabaena* in treated tap water. Additional testing of treated tap water is essential for further evaluation of treatment system efficacy. Future laboratory analyses for potentially toxigenic cyanobacteria in drinking-water lakes or reservoirs should be performed using increased sub-sampling resolution. For example, the number of total algal units counted could be increased from the current level of 100 algal units to 200 algal units. Although this method would incur additional costs, the above data indicate that for drinking-water systems (when low levels of a potentially toxigenic species need to be determined), such an increase in precision is necessary.

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. 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.

A fact sheet about *Microcystis aeruginosa* and detailed recommendations for lake water treatment may be obtained from Ken Kauffman at 971-673-0435 or via E-mail at kenneth.w.kauffman@state.or.us or from the Coos County Health Department at (541) 756-2020. Information is also available on the worldwide web at http://www.oregon.gov/DHS/ph/envtox/mafact.shtml

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Literature Cited

Chorus, I. and J. Bartram. 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.

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.

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)

Electronic Appendix I: Aquatic Analysts, Inc Phytoplankton Reports (attached electronically)

Appendix I: Drinking water treatment guidance

Oregon Health Division **Contact Person: Ken Kauffman**
971-673-0435
August 31, 2001 **kenneth.w.kauffman@state.or.us**

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.