SAMPLING AND ANALYSIS PLAN – PHASE IV FOR TOXIC CYANOBACTERIA IN LAKE WASHINGTON, LAKE SAMMAMISH, AND LAKE UNION

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1 Introduction

Toxic cyanobacteria have been increasingly detected in western Washington lakes since the first documented toxic episode in American Lake in 1989 (Jacoby et al. 1994, Jacoby and Kann 2007). State health officials are concerned that the rate of occurrence appears to be increasing over time leading to the possibility of increased human and animal exposure to cyanotoxins. Toxic cyanobacteria blooms are emerging as a national and international issue (Ecology 2007).

Mass accumulations or "blooms" of cyanobacteria in freshwater ecosystems are primarily caused by nutrient, particularly phosphorus (P), enrichment. Cyanobacteria blooms can cause surface scums, decreased water column transparency, dissolved oxygen depletion and unpalatable drinking water due to taste and odors. Some cyanobacteria also produce toxic compounds ("cyanotoxins") that have caused livestock, wildlife and pet fatalities worldwide (reviewed by Carmichael 1994; Chorus 2001). Although many cyanobacteria blooms are not toxic, a bloom that is not toxic one day may become toxic during the same growing season.

Table 1. General features of cyanotoxins. (Modified from Chorus and Bartram 1999).

Toxin Group	Primary Target organ in mammals	Cyanobacterial genera
Microcystins	Liver	*Microcystis, Anabaena, Planktothrix (Oscillatoria), Nostoc, Hapalosiphon, Anabaenopsis*
Nodularian	Liver	*Nodularia*
Anatoxin-a	Nerve Synapse	*Anabaena, Planktothrix (Oscillatoria), Aphanizomenon*
Anatoxin-a (S)	Nerve Synapse	*Anabaena*
Aplysiatoxins	Skin	*Lyngbya, Schizothrix, Planktothrix (Oscillatoria)*
Cylindrospermopsins	Liver	*Cylindrospermopsis, Aphanizomenon*
Lyngbyatoxin-a	Skin, G.I. Tract	*Lyngbya*
Saxitoxins	Nerve Axons	*Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis*
(LPS)	Potential irritant; affects any exposed tissue	ALL

1. Many structural variants may be known for each toxin group.
2. Not produced by all species of a particular genus
3. Whole cells of toxic species elicit widespread tissue damage to kidney and lymphoid tissue.

Cyanotoxins include a broad, diverse range of chemicals and mechanisms of toxicity (Carmichael 1994; Sivonen and Jones 1999). Major classes of cyanotoxins include the cyclic peptides, which are primarily hepatotoxins (microcystins and nodularins); alkaloids and an organophosphate, which are strong neurotoxins (anatoxin-a, anatoxin-a (S), and saxitoxins); a cyclic guanide alkaloid, which inhibits protein synthesis (cylindrospermopsin); lipopolysaccharides, which have pyrogenic properties; and dermatoxic alkaloids (aplysiatoxins and lyngbyatoxins) (Chorus 2001). Toxin groups and the cyanobacteria genera known to produce them are listed in Table 1. This Phase IV SAP will focus on the two toxins; microcystin and anatoxin-a.

Microcystins are the most commonly tested and detected cyanotoxins in Washington and have been found at water concentrations from <1 to 4,810 µg/L (Jacoby and Kann 2007). These hepatotoxins are of concern due to their prevalence and potential to harm animals and humans. Hepatotoxins damage liver tissues, and at high doses can cause liver failure and death (Carmichael 1994). Hepatotoxins with seven amino acids are called microcystins (produced by species of *Microcystis, Planktothrix,* and *Anabaena*) and those with five amino acids are nodularins (produced by *Nodularin spumigena*). The mechanism of toxicity involves the inhibition of the specific protein phosphatase enzymes possessed by all eukaryotic cells. In addition, microcystins are suspected tumor-promoters and teratogens (Falconer 1998). These toxins have been associated with elevated rates of primary liver cancer in people drinking waters with high densities of cyanobacteria (Yu 1989).

While microcystins appear to be more common than neurotoxins, neurotoxins are notoriously potent and rapid acting poisons that have caused severe animal poisonings in North America, Europe and Australia (WHO 2003, Botana 2007). The neurotoxin anatoxin-a is an alkaloid with high toxicity. It acts as a post-synaptic, depolarizing, neuromuscular blocking agent. Depending upon the size of the animal and amount of the toxin present, illness or death may occur within a minutes to a few hours after exposure. Signs of anatoxin-a poisoning are staggering, paralysis, muscle twitching, gasping, and convulsions – all potentially leading to death. Anatoxin-a can be produced by some species of cyanobacteria including *Anabaena*, *Aphanizomenon, Cylindrospermum*, *Planktothrix* (*Oscillatoria), and Microcystis* spp.. Determination of anatoxin-a in natural waters has been challenging due to its typically low concentration and/or rapid degradation to nontoxic degradation products (Botana 2007).

1.1 Project Background

The toxic bloom of *Microcystis aeruginosa* in Lake Sammamish during 1997 and subsequent detection of microcystins by Johnston and Jacoby (Johnston and Jacoby 2003) in 1999 prompted King County to develop a sampling and analysis plan for the measurement of microcystins in the major King County lakes in 2002. King County began routinely collecting samples for the analysis of microcystin, during summer and fall of 2002 and has continued to date with modifications documented in the Phase II SAP in 2005, Addendum to Phase II SAP in 2006, and the Phase III SAP in 2007. This Phase IV of the Cyanotoxicity SAP is based upon results of the County's 2003-2007 monitoring efforts (as discussed below) with modifications to increase efficiency and to better meet project objectives.

The assessment of cyanotoxins throughout Washington State has primarily focused on microcystins due to their widespread occurrence and potential for chronic toxicity. However, blooms that test negative for microcystins may have other cyanotoxins present. For example, a 2007 bloom in Anderson Lake in Jefferson County tested negative for microcystins but was highly toxic with anatoxin-a (Ecology 2007). The increasing detection of anatoxin-a in western Washington lakes (*e.g.*, American Lake, Pierce County, December 1989 and 2007; Kitsap Lake, Kitsap County, October 2001; and Jefferson County 2006) in association with animal deaths has prompted King County to expand cyanotoxicity testing to include this neurotoxin. In 2007 the King County Environmental Laboratory (KCEL) purchased the highly sensitive equipment necessary to test for anatoxin-a. The method adapted at KCEL uses fluorimetric derivatization with HPLC (High Pressure Liquid Chromatography). Regular testing for anatoxin-a will begin with the method development in 2008. The inclusion of anatoxin-a as part of the cyanotoxicity monitoring program will allow the County to further define the potential threat of this neurotoxin in lakes Washington, Sammamish, and Union.

1.1.1 Summary of Lake Sammamish 1997 & 1999 Cyanotoxin Study

Cyanobacterial activity and environmental conditions that may promote toxic cyanobacteria were investigated in Lake Sammamish during summer and fall 1999 (Johnston and Jacoby 2003). Microcystins were detected using enzyme linked immunosorbent assay (ELISA) during late August and early September 1999 despite low cyanobacterial abundance. In contrast to other studies in which concentrations varied substantially throughout a lake (Carmichael & Gorham 1981; Jacoby et.al. 1994) similar microcystin concentrations were detected throughout Lake Sammamish at all depths, ranging between 0.19 to 3.8 $\mu g\ L^{-1}$, with the exception of the boat launch where a surface concentration reached 43 $\mu g\ L^{-1}$.

During the toxic episodes in 1997 and 1999, *Microcystis* was associated with a stable water column, increased surface total phosphorus concentrations (> 10 $\mu g\ L^{-1}$), surface temperatures greater than 22°C, high total nitrogen to phosphorus ratios (> 30) and increased water column transparency (up to ~5.5 m). Microcystin:chlorophyll-*a* (chl-*a*) ratios varied from 0.4 to 6.4, with higher ratios in the hypolimnion during the toxic episode in 1999. Migration of *Microcystis* and *Anabaena* occurred in both the deep and shallow portions of the lake. Migration rates were more than 2 times higher at the shallow station, and the migrating cyanobacteria were dominated by *Microcystis* (89 to 99% of the total biovolume) at both stations. External loading of nutrients due to the large storm event that preceded the 1997 toxic episode may have provided the nutrients needed to fuel that bloom. Despite the lack of rain and subsequent external runoff, toxic *Microcystis* occurred in 1999. The migration of *Microcystis* from the nutrient-rich sediments may have contributed to the toxic population detected in 1999.

1.1.2 King County Microcystin Monitoring Results 2002-2007

King County had two parallel microcystin monitoring efforts in 2002. The first County monitoring effort included samples collected at all 25 routinely monitored Major Lake stations beginning in spring 2002. These samples were processed using a 0.45 μm syringe-filter and analysis with the ELISA method. No microcystins were detected using these protocols.

The second effort, as described in the 2002 SAP, was focused on determination of environmental conditions that may trigger toxin production and the evaluation of microcystin extraction methods. This effort involved the monitoring of six stations in lakes Union, Washington, and Sammamish. Samples from this effort were either refrigerated or treated with one of two extraction processes (freezing and sonication) prior to analysis by protein phosphatase inhibition assay (PPIA) and ELISA. Cell lysing is assumed to be representative of a recreational exposure in which a swimmer ingests ambient water and cells as a combined dose. Based on the results of initial effort, it was determined that the best method for cell lysing was a combination of freezing and sonication.

In 2005, the program was modified to include sites in the Swimming Beach Monitoring Program. Coordination of both the Major Lakes Monitoring and the Swimming Beach Monitoring programs provided weekly sample collection throughout most of the algae productive growing season and to better track cyantoxins in areas of high recreational use.

The results of the microcystin monitoring efforts are summarized below, in Table 2, and illustrated in Figures 1a-f.

Toxins

- Microcystins were detected in all three lakes in 2003 – 2006. Lake Sammamish had only one detectable sample in 2006.
- Concentrations in all but two of the 806 samples were < 1.0 µg/L, the threshold for drinking water recommended by the World Health Organization (WHO 1988).
- The highest microcystin concentrations were measured on May 30, 2006 during a bloom in the Matthews Beach area of Lake Washington.
- swimming area = 1.15 µg/L (PPIA) and 1.35 µg/L (ELISA) microcystin LR equivalents
- cyanobacteria scum = 52.6 µg/L (PPIA) and 47.1 g/L (ELISA) microcystin LR equivalents
- In general, the PPIA test found more detectable levels of microcystin than the ELISA test in 2003, 2004, and 2005. This was reversed in 2006 in Lake Washington and Lake Union. Since the two methods measure different forms of microcystin, this may indicate that the form of the toxin varies from year to year.

Phytoplankton

- *Anabaena flos-aquae* and *Anacystis sp*. were the dominant cyanobacteria present in Lake Washington and Lake Union samples with detectable microcystin concentrations.
- *Anabaena flos-aquae, Anacystis sp*., and *Aphanizomenon flos-aquae* were the most consistently detected cyanobacteria in Lake Sammamish samples with detectable microcystin concentrations.

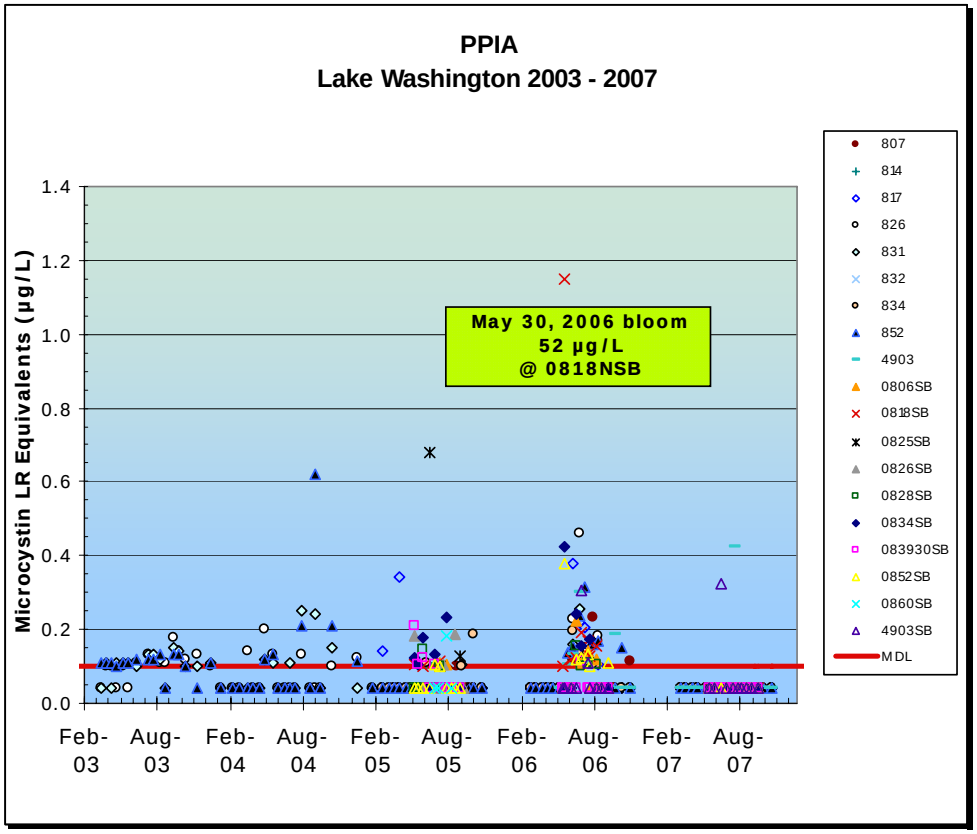
- The highest cell counts occurred at Matthews Beach in Lake Washington during the May 30, 2006 bloom. Cell counts of *Anabaena flos-aquae* measured 348,440 cells/ml in the scum and 60,500 cells/ml in the swimming beach area.

- Concentrations of microcystins showed no significant correlation with total phosphorus (1 m), temperature (1 m), or chlorophyll *a*. This may indicate that conditions favoring cyanobacterial growth are uncoupled from the production and release of toxins.

Table 2. Summary of King County Toxic Cyanobacteria Monitoring Results through October 2007.

	Samples Collected	Date of Maximum Value	Station	Major Species	Cells/ml dominant cyanobacteria	Maximum Total Microcystin[1] (µg/L)	Analysis Method[2]
Sammamish							
Sept 1997				*Microcystin aeruginosa*		478-588 µg/g total microcystins	
1999 Aug - Sept				*Microcystis aeruginosa*		1-43	ELISA
2003 Apr - Dec	n = 30	Aug 5/Nov 3	0612	*Gomphosphaeria aponina* *unknown colonial cyanobact.* *Chroococcus sp.,* *Anacystis sp.* *Aphanizomenon* *Anabaena circinalis/flos-aquae*	7,040 / 352 5,280 1,760 1,980 380 / 200 120/140	0.13	PPIA
2004 Jan - Nov	n = 36	Jun 21	0612	*Chroococcaceae* *Anacystis sp.* *Aphanizomenon flos-aquae* *Anabaena sp*	3,600 800 330 20	0.17	PPIA
2005 Mar - Oct	n = 84	May 17	0614	*Aphanizomenon flos-aquae* *Anabaena sp*	680 150	0.155	PPIA
2006 Mar - Nov	n = 89	--	--	--	--	< MDL	
Union							
2003 Apr - Dec	n = 15	Oct 7/Nov 3	A522	*unknown colonial cyanobacteria* *Anacystis sp.* *Oscillatoriaceae* *Anabaena sp.*	1,560 / 1,400 200 308 20 / 560	0.14	PPIA

1. Total microcystin LR equivalents (extra- and intracellular). Sample preparation includes cell lysing by freezing and sonication prior to analysis.
2. Both PPIA and ELISA analysis done on all samples. Method that resulted in maximum total microcystin value for the year is listed here.


PPIA
Lake Washington 2003 - 2007
Microcystin LR Equivalents (µg/L)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
May 30, 2006 bloom
52 µg/L
@ 0818NSB
Feb-03
Aug-03
Feb-04
Aug-04
Feb-05
Aug-05
Feb-06
Aug-06
Feb-07
Aug-07
807
814
817
826
831
832
834
852
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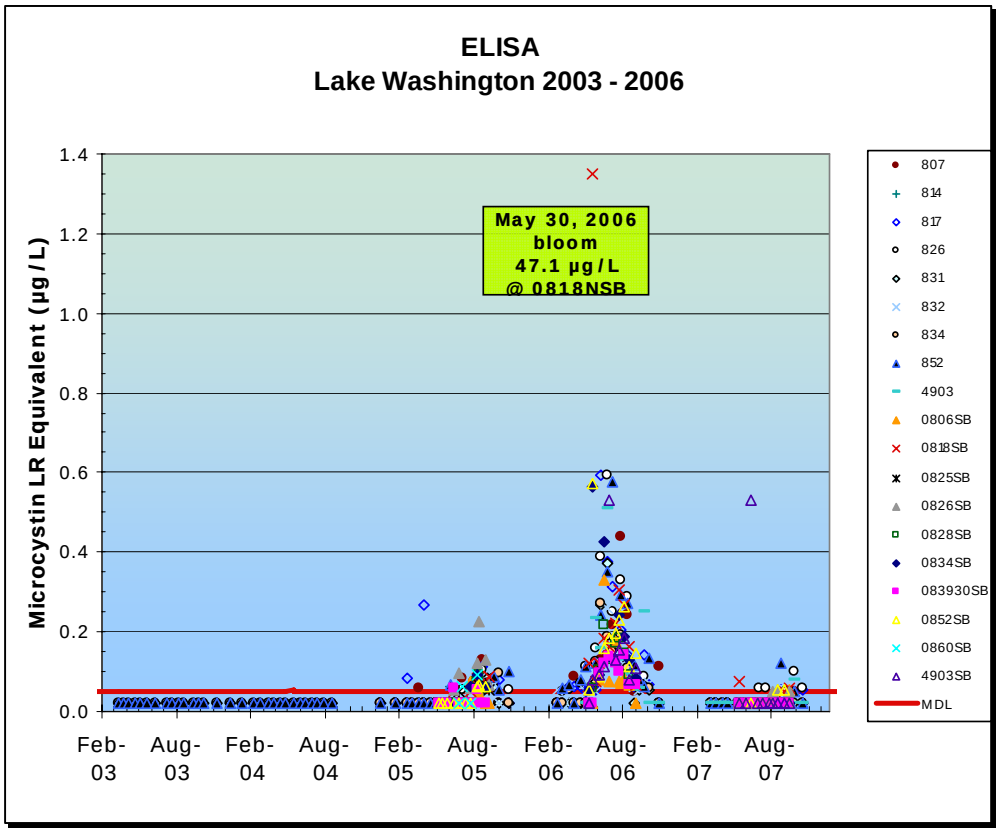
0852SB
0860SB
4903SB
MDL


ELISA
Lake Washington 2003 - 2006
Microcystin LR Equivalent (µg/L)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
May 30, 2006
bloom
47.1 µg/L
@ 0818NSB
Feb-03
Aug-03
Feb-04
Aug-04
Feb-05
Aug-05
Feb-06
Aug-06
Feb-07
Aug-07
807
814
817
826
831
832
834
852
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4903SB
MDL

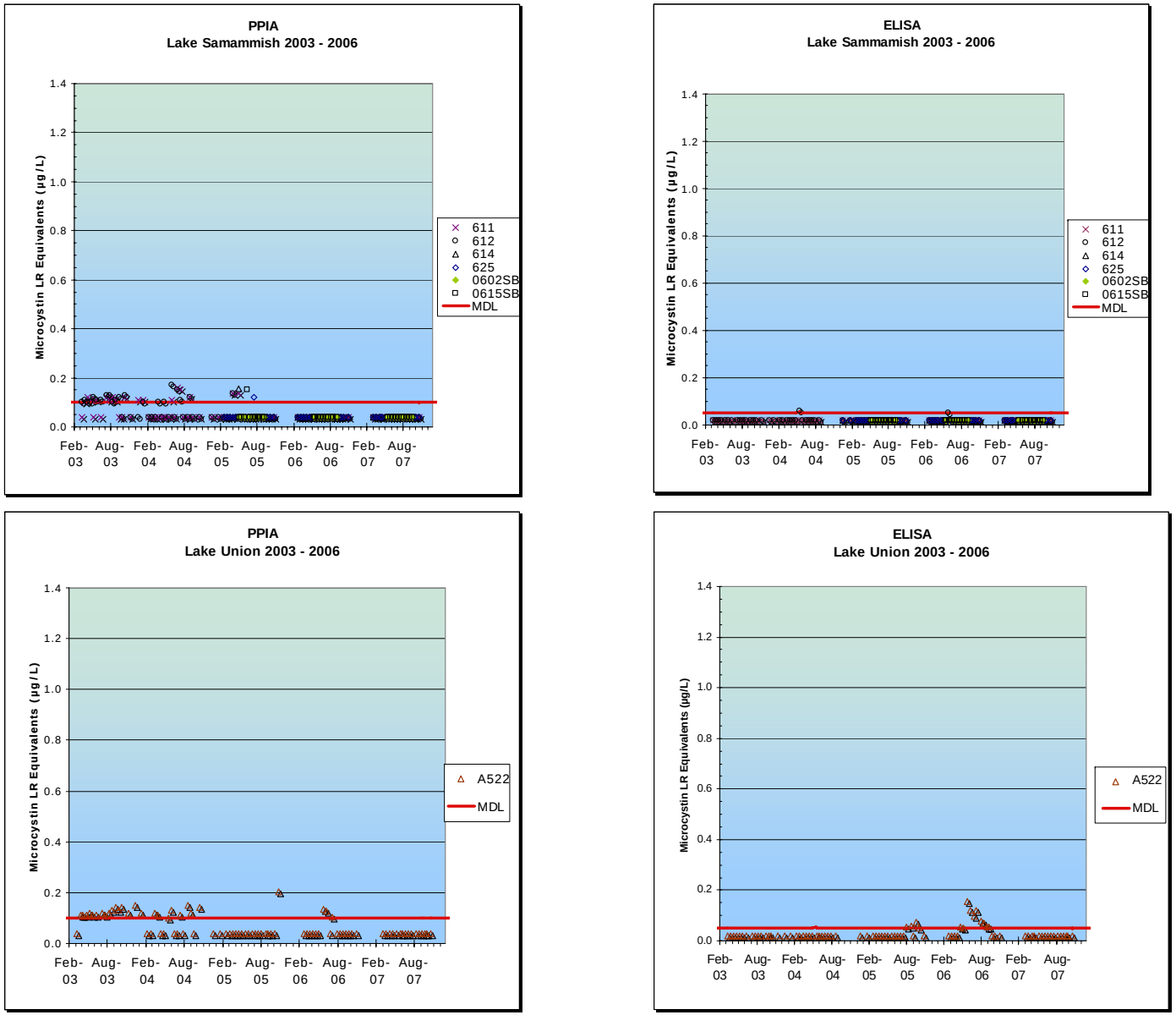

Figure 1a to 1f. Results of the 2003 – 2007 Cyanobacteria Toxicity testing in samples collected from lakes Washington, Sammamish, and Union using both PPIA and ELISA.

1.2 Regulatory Status of Cyanotoxin Criteria and Guidelines

In 2005, the Washington State Legislature established funding for a Freshwater Algae Control Program (RCW 43.21A.667) through the Department of Ecology (Ecology) to assist local governments in the management of freshwater algae problems. As part of this program Ecology partnered with the Washington Department of Health (WaDOH) to develop recreational guidelines values for cyanotoxins. WaDOH has recommended a three-tiered approach using the recreational guidance values of 6.0 µg/L microcystins and 1 µg/L anatoxin-a for managing Washington Lakes (WaDOH, May 2008). More information about this three-tiered management approach can be found at the Washington State Department of Health webpage: http://www.doh.wa.gov/ehp/algae/guidelines.htm.

1.3 Study Area Description

Sampling locations are listed in Table 3. Stations have been modified from previous versions of this SAP to include all swimming beaches that are currently being sampled through the Swimming Beach Monitoring Program (Figure 2). One deep water station on each of the three major lakes will be sampled through the Major Lakes Routine Sampling Program (A522, Lake Union; 0612, Lake Sammamish; 0852, Lake Washington). These deep water stations were part of previous cyanotoxin monitoring efforts as shown on Figure 3. If "blooms" are observed at other locations on the lakes, additional grab samples may be collected and analyzed. If "bloom" samples are collected at other than established stations, coordinates will also be collected.

Table 3. Stations and Sampling Parameters at Each Location.

Swimming Beach Monitoring Program				Major Lakes Monitoring Program			
Station	Locator	Parameter[1]	Annual # samples/ parameter	Station	Locator	Parameter[1]	Annual # samples/ parameter
130th Place	0805SB	M/H	25/25	Lk Union	A522	M/Q	16/20
Juanita	0806SB	M/H/A	25/25/13	Lk Samm	0612	M/Q	16/20
Seward Pk.	0813SB	M/H	25/25	Lk Wa	0852	M/Q	16/20
Matthews	0818SB	M/H/A	25/25/13				
Mt Baker	0820SB	M/H	25/25				
Magnuson	0826SB	M/H	25/25				
Magnuson Off Leash	0826OLA	M/H	25/25				
G. Coulon	0828SB	M/H/A	25/25/13				
Meydenbauer	0834SB	M/H/A	25/25/13				
Newcastle	83930SB	M/H	25/25				
Madison Park	0852SB	M/H	25/25				
Madrona	SD007SB	M/H	25/25				
L. Burbank	SD017SB	M/H	25/25				
Green Lk	A734SB	M/H/A	25/25/13				
Pritchard	4903SB	M/H	25/25				
Idylwood	0602SB	M/H	25/25				
Lk Samm Park	0615SB	M/H	25/25				

*PARAMETER CODES:
M = microcystin analysis
H = collect quantitative phytoplankton samples and hold for analysis pending cyanotoxin values.
Q = quantitative phytoplankton analysis routinely done
A = anatoxin-a analysis

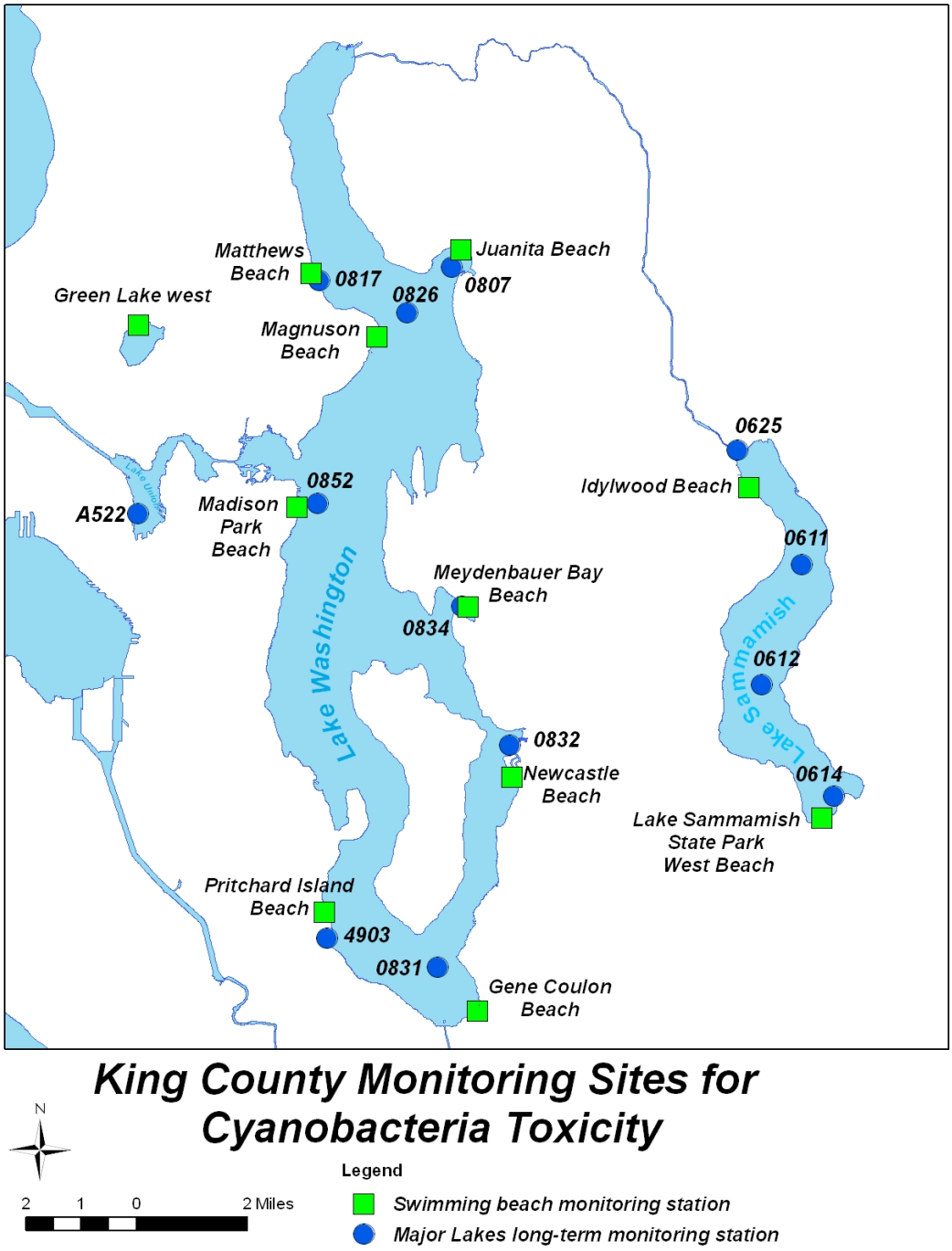

Figure 2. 2006 – 2007 Major Lakes Cyanobacteria Toxicity Monitoring Program Sampling Locations.

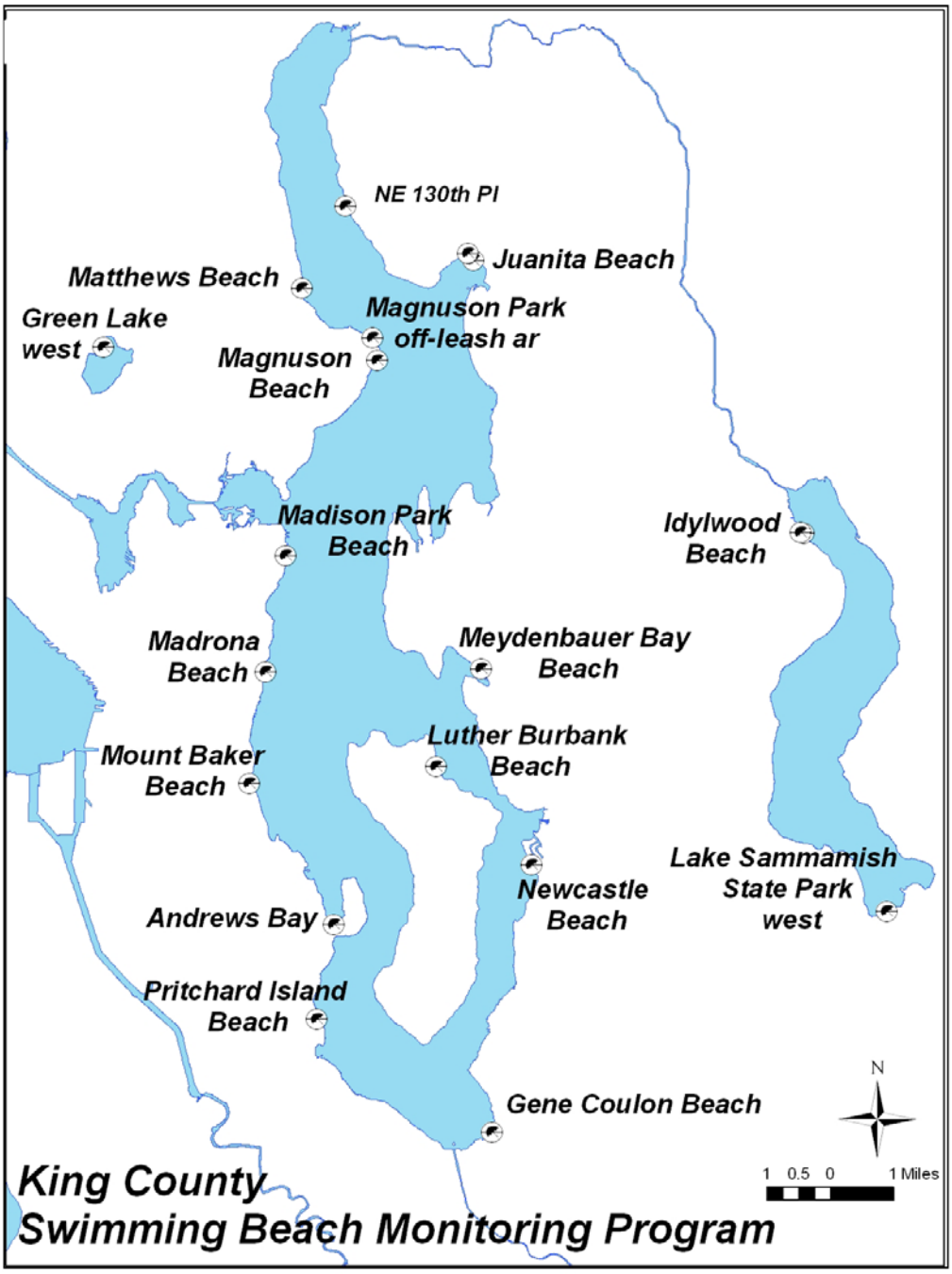

Figure 3. 2008 Swimming Beach Monitoring Program Sampling Locations.

1.4 Project Objectives

The objectives of the Phase IV Cyanobacteria Toxicity Program in Lake Washington, Lake Sammamish and Lake Union Study are to:

- Evaluate the presence of microcystins, a hepatoxin, and anatoxin-a, a neurotoxin, to protect human health
- Estimate concentrations and geographic extent of the toxicity, should it be present
- Establish relationships between microcystin and cyanobacteria species/abundance.
- Evaluate environmental conditions leading to toxin production in cyanobacteria.

The study will provide decision-makers with information and recommendations regarding recreational water use during cyanobacterial blooms and will lead to improved management of King County lakes for the protection of human health.

2 Study Design

2.1 Approach

This Phase IV survey is primarily designed to evaluate the presence/absence of the cyanobacterial toxins microcystins and anatoxin-a, and secondarily to estimate concentrations and geographic extent of the toxicity, should it be present. The monitoring efforts described in this Phase IV SAP began in 2008.

Sample collection will utilize the combined efforts of the Routine Major Lakes Sampling Program and the Swimming Beach Monitoring Program. Table 3 lists the sampling sites included in this cyanotoxicity monitoring effort. Table A (attached) lists all Major Lake sampling sites and swimming beach sites and illustrates how microcystin and quantitative phytoplankton sample collection is coordinated with these programs. The Major Lakes Routine Monitoring Program collects samples once per month from November through February, and twice per month from March through October. Swimming Beach Monitoring occurs weekly from mid-May through mid-September. Because high cyanotoxin concentrations have often been detected in Washington State in early autumn, additional sampling at the swimming beach sites will take place in October by WLRD staff. Table 4 lists the sampling schedule for these combined efforts. In addition to swimming beaches, one sample will be collected from a mid-lake station in all three water bodies in King County's Major Lakes Program (Sammamish, Washington and Union) to estimate open water distribution of any toxicity measured.

Quantitative phytoplankton samples will be collected at each site listed in Table 3. Phytoplankton samples collected as part of the Major Lakes Routine Monitoring will be analyzed regularly. Phytoplankton samples collected as part of the Swimming Beach Monitoring Program will be held for analysis pending measured concentrations of cyanotoxins.

The following three types of sampling scenarios are included in this study:

1. Routine Major Lakes Sampling. Three deep water sampling sites were selected at Routine Major Lake monitoring locations in order to estimate geographical extent of toxicity, if detected, to relate cell volumes of cyanobacteria to other environmental parameters measured as part of the routine monitoring efforts, and to continue to provide quantitative phytoplankton data for lake modeling efforts.

   An aliquot of the sample collected as part of the routine sampling effort will be used for this study. Sample collection in the Routine Major Lakes program has been modified to incorporate use of an integrated composite sample at the deep water sites. Section 2.3.1 describes the sample collection methods.

   Microcystin will be measured by PPIA and ELISA using freezing and sonication as the extraction methods (described in the Phase II SAP). Chl-*a*/pheo-*a* (pheophytin *a*) analysis will be conducted on these Major Lake samples as part of this Routine Major Lakes Monitoring effort. See Major Lakes Monitoring Program SAP for further discussion.

   NOTE that quantitative phytoplankton enumeration and identification will be performed for the three samples collected from these Major Lake stations as part of this Phase IV Cyanobacteria Study effort (Table 3 and Table A). Data from the quantitative phytoplankton analyses is stored in LIMS and will be stored in a database being established by WLRD's Science Section.

2. Swimming Beach Monitoring. The major component of this sampling scenario will be conducted by the laboratory's Environmental Services Section (ESS) staff as part of the Swimming Beaches Monitoring Program. The seventeen swimming beaches that are part of the seasonal monitoring for fecal coliform bacteria are included in this Phase IV of the Toxic Cyanobacteria Study (Table 3).

   Microcystin will be measured by PPIA and ELISA using the freezing and sonication extraction method (described in the Phase II SAP). In addition, five of the swimming beaches (0806SB, 0818SB, 0828SB, 0834SB, A734WSB) will be sampled for anatoxin-a analysis in 2008 as KCEL works on anatoxin-a method development. KCEL is adapting a highly sensitive method for anatoxin-a which uses fluorimetric derivatization with HPLC. The expectation is that in subsequent years all samples collected as part of the cyanotoxicity program will have both microcystin and anatoxin-a analysis.

   Sufficient sample volume will be collected for microcystin testing, quantitative phytoplankton identification and enumeration, and anatoxin-a where indicated. Quantitative phytoplankton identification and enumeration samples collected from swimming beaches will be archived and analyzed if determined necessary by high cyanotoxin concentrations. If toxins are present, quantitative phytoplankton identification and enumeration will be determined using the same methodology as for the Routine Major Lakes sampling effort. Sample collection will be a surface dip.

In addition, ESS will routinely visually inspect the waters at other swimming beach stations for cyanobacteria blooms while conducting the Swimming Beaches program. Up to 10 samples may be collected per bloom event, followed by Project Manager evaluation, and subsequent decisions regarding appropriate next steps.

3. Bloom Sampling. Focused sampling efforts will be made to collect blooms or accumulations of cyanobacteria if they are present within the visual distance of routine lakes sampling sites or as part of the Small Lakes Monitoring Program. A bloom will be defined by a visually observable accumulation of phytoplankton in the water column or as a surface accumulation. Coordinates will be obtained for these grab samples and a LIMS locator created. New locator names will be consistent with the naming convention system established for the Major Lakes and/or Small Lakes Programs. Up to 10 samples may be collected during a bloom event, at which time the Toxic Cyanobacteria Study Project Manager will evaluate such data as is available and discuss with the laboratory available options for proceeding with the bloom investigation.

   Sufficient volume will be collected for toxicity testing, as well as phytoplankton quantitative enumeration and identification. Microcystin will be measured by ELISA and PPIA on these discrete samples using extraction methods described in the Phase II SAP. Anatoxin-a will be measured by HPLC. If toxins are present, quantitative phytoplankton identification and enumeration may be determined.

Table 4. Summary of Cyanotoxin and Phytoplankton Weekly Sample Collection Schedule by Major Lakes Monitoring and Swimming Beach Programs.

		Major Lakes Monitoring		Swimming Beach Monitoring		
		# Microcystins per week	# Phytos ANALYZED	# Microcystins per week	# Phytos COLLECTED	# Anatoxin per week
JAN	1 sample	0	4			
FEB	1 sample	0	4			
MAR	2nd week	4	4			
	4th week	3	3			
APR	2nd week	4	4			
	4th week	3	3			
MAY	1st week					
	2nd week	4	4	18	17	
	3rd week			17	17	
	4th week	3	3	17	17	
JUN	1st week			17	17	
	2nd week	4	4	18	17	
	3rd week			17	17	
	4th week	3	3	17	17	
JUL	1st week			17	17	
	2nd week	4	4	18	17	
	3rd week			17	17	
	4th week	3	3	17	17	
	5th week			17	17	
AUG	1st week			17	17	5
	2nd week	4	4	18	17	5
	3rd week			17	17	5
	4th week	3	3	17	17	5
SEP	1st week			17	17	5
	2nd week	4	4	18	17	5
	3rd week			17	17	5
	4th week	3	3	17	17	5
	5th week			17	17	5
OCT	1st week			17	17	5
	2nd week	4	4	18	17	5
	3rd week			17	17	5
	4th week	3	3	17	17	5
NOV	1 sample		4			
DEC	1 sample		4			
Total	56	72	431	425	65	

2.2 Timeline

This Phase IV SAP will be implemented from March 2008 and continue through December 2010. It is expected that anatoxin-a will become regular part of cyanotoxin monitoring for all stations in the program in 2009. In 2010, the program will be reevaluated and modifications made as necessary. Previous sampling programs will be discontinued.

2.3 Sampling Procedures

Protocols for the sampling and analysis of microcystins do not currently exist. However, a working group of the International Organization for Standardization is currently developing such protocols (Chorus, personal communication, April 24, 2002). The following sampling procedures are based on methods of Carmichael (2001), Chorus (2001), Johnston and Jacoby (2002).

Table 5. Sample Container & Preservation Requirements

Parameter	Matrix	Container	Preservation	Hold time
Quantitative Phytoplankton	Liquid	•1x 250- ml Plastic Amber Wide Mouth (AWM)	•Lugol's solution pre-preserved •Store at room temperature in the dark	•365 days
Microcystins ELISA (MLR-ELISA)	Liquid	250- ml Glass, AWM	4°C	24 to 48 hours then freeze
Microcystins PPIA (MLR-PPIA)	Liquid	250- ml Glass, AWM (same bottle as collected for MLR-ELISA)	4°C	24 to 48 hours then freeze
Anatoxin-a HPLC	Liquid	2x 1– L Plastic Amber bottle (2 per station)	4°C	1 day for filtration 28 days for analysis

Notes:
AWM – Amber wide mouth bottle

2.3.1 Water sample collection and storage procedure to test for toxins:

Samples will be collected using the site-specific collection method identified above in Section 2.1 (e.g., integrated composite or surface grab).

Integrated Composite technique: Vertically integrated composite samples are collected using a weighted length of ¾-inch tygon tubing let down vertically through the water column as done for the Routine Major Lakes sampling program. This tube is marked so that when fully extended, the distance from the mark at the water surface to the end of the tube is 10 m. The tube is plugged at the submerged end by a check valve and retrieved. The tube contains a vertically integrated sample of the lake from surface to 10 meters. The sample is decanted into a stainless steel bowl and homogenized before sub-sampling for microcystin, chl-*a*, pheo-*a* and phytoplankton enumeration. If more than one tube is collected, combine the water in the steel bowl prior to filling sample containers. Aliquots for microcystin analysis will be poured into a 250 mL glass AWM bottle, leaving some headspace for freezing. The sample bottle should not be pre-rinsed with sample.

Swimming Beach surface grabs: For surface grabs, fill the 250 mL glass, AWM bottle by dipping the bottle mouth-down into the water. With a sweeping arch, collect water from approximately (my arm is not 2 meter long – let's say about 1 ½ feet below the surface, leaving some headspace to allow for freezing.

Other sampling protocol notes:

- Label the bottles if not pre-labeled.
- Place the sample bottles in a cooler with ice packs (no preservative required).
- Sub-samples will be removed from the 250 mL glass bottle and frozen within 24 to 48 hours of arrival at the KCEL. Bottles and vials should be slanted to prevent breakage during freezing. Samples must be stored frozen for a minimum of 12 hours to insure complete freezing of the sample.

2.3.2 Water sample collection and storage procedure for quantitative identification of cyanobacteria.

Quantitative cyanobacteria identification and enumeration will be conducted at the three Major Lake stations as part of this Phase IV SAP for Toxic Cyanobacteria (Table 3 and Table A). Quantitative phytoplankton identification and enumeration will be conducted by KCEL.

In addition, samples for quantitative identification and enumeration will be collected and preserved at the designated Swimming Beach sites in the event that high microcystin and/or anatoxin-a concentrations warrant further investigation. A 250 mL aliquot will be collected and placed in properly labeled opaque bottles (typically 250 mL plastic amber wide mouth) and preserved with a sufficient amount of concentrated Lugol's solution to turn the sample light red; typically 1.75ml Lugol's is the preferred amount for 250 mL bottle. Care should be taken that samples are covered tightly and stored in the dark until analyzed.

In the event that algal blooms are sampled (as per #3 in section 2.1), samples will be collected and preserved as described above.

3 Laboratory Analysis

ELISA and PPIA assays are suitable for rapid and sensitive detection of microcystins. These methods are useful for preliminary toxin screening for both cyanobacterial samples and extra cellular microcystins in the water (Chu *et* al. 1990; Chorus 2001). ELISA is based on the structure of the microcystin molecule and requires antibodies against microcystins whereas PPIA is based on the toxic effects of microcystins. The PPIA method is preferred for waters that may contain toxic forms of microcystins and nodularins.

ELISA and PPIA are suitable as indicating tests for the analysis of extracellular microcystins at concentrations at or above 0.05 µg/ L. ELISA is the most sensitive and simple method, but has the potential for false positive reactions (Chorus 2001). PPIA provides preliminary information on the toxicity of microcystins in comparison to the microcystin content measured by ELISA. The KCEL has developed Standard Operating Procedures (SOPs) for the measurement of microcystins using ELISA (SOP 440v2) and microcystins and nodularins using PPIA (SOP 443v1) in water.

3.1 Microcystin Toxin Structure and Cross-Reactivity Analysis Summary

Microcystins are a group of cyclic heptapeptide hepatotoxins produced by species of the common bloom-forming genera of cyanobacteria including *Microcystis*, *Anabaena, Nostoc* and *Oscillatoria*. These toxins contain two variable L-amino acids, three D-amino acids and two unusual amino acids. There are now over 50 different microcystins which have been structurally characterized and which differ primarily in the two L-amino acids and methylation or demethylation of the two unusual amino acids. These microcystins all contain the Adda amino acid, which is essential for expression of their biological activity. Nodularins are monocyclic pentapeptide liver toxins produced by the cyanobacterium *Nodularia*. Nodularins contain Adda but lack one of the L- and D-amino acids found in microcystins. Both microcystins and nodularin have been found to be potent inhibitors of protein phosphatase (PP) isozyme types 1 and 2A. The inhibitory action of the toxins on PP1 is considered a basis for their toxicity and forms the basis for the PP1 inhibition assay. Currently several methods have been developed to detect and quantify cyanotoxins. However, there is no single method that provides adequate monitoring for all cyanotoxins. Many of the microcystins and nodularins in environmental samples will be detected by a combination of the ELISA and PPIA methods.

3.1.1 Sample Preparation for Microcystin Toxin Assay

To measure total microcystin concentrations (extra- and intracellular) in the water samples, sample preparation will include a cell lysing step prior to analysis.

The objective of the cell lysing is to generate a sample in which all microcystins (extra- and intracellular) have been converted into a free form that can be measured by ELISA

and PPIA, thus providing a close approximation of the total concentration in the ambient sample (extra- and intracellular). The resulting concentration should be representative of a recreational exposure in which a swimmer ingests ambient water and cells as a combined dose. If samples were analyzed without lysing, results would be reported as *Extracellular Microcystins.* Since all samples collected for this study will be analyzed following lysing, results will be reported as *Total Microcystins*. Note ELISA measures only free microcystin, not the amount chemically bound to the cell or molecular components such as protein phosphatase enzymes.

Based on previous cyanobacteria toxin work done, laboratory staff recommended combining extraction methods. Therefore, each sample will be prepared for analysis by the following lysing process:

- 10-ml aliquots will be frozen for a minimum of 12 hours and then
- Thawed at room temperature and then immediately sonicated (ultrasonic disruption) using the Vibra Cell Sonicator.
- Samples will be filtered through a 0.45 μm filter prior to analysis.

NOTE: Green pigments and associated substances in 0.45 μm filtrate can mask the presence of microcystins. Additional filtration to 5000 nominal molecular weight limit (NMWL) will be performed when the filtrate appears colored to remove pigments and associated substances that may interfere with the assay. Since the ELISA requires 50 μL per replicate, a scaled up version of the ultra filtration system, perhaps including centrifugation, may be most efficient (see attachment for further discussion). The method detection limit (MDL) is 0.05 μg/L as microcystin-LR equivalents. MDL for the PPIA is 0.1 μg/L as microcystin-LR equivalents.

Holding times for microcystin analysis in frozen samples have not been established to date. Other studies have shown that microcystins do not readily degrade in frozen samples (Chorus, personal communication, April 24, 2002). Deep-freezing samples that have been freeze-dried will ensure sample preservation; however, even wet-frozen samples demonstrate no substantial loss in microcystin concentration over months or years. Storage of dried samples at air temperature should be avoided because absorbed moisture from the air may activate the bacteria (Chorus, personal communication, April 24, 2002). Based on KCEL SOP(s) 440v2 and 443v1, a conservative holding time for frozen samples of 7 days will be employed. Holding times for the filtrate at 4 ºC are being determined.

3.1.2 Microcystins– ELISA

The ELISA test kit uses polyclonal antibodies that bind either microcystins or a microcystin-enzyme conjugate. Microcystins in the sample compete with the microcystin-enzyme conjugate for a limited number of antibody binding sites. Since the same number of antibody binding sites are available on every test well, and each test well receives the same number of microcystin-enzyme conjugate molecules, a sample that contains a low concentration of microcystins allows the antibody to bind many microcystin-enzyme conjugate molecules. The result is a dark blue solution. Conversely,

a high concentration of microcystins allows fewer microcystin-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution. The plate kit does not differentiate between microcystin-LR and other microcystin variants but detects their presence to differing degrees. At 50% inhibition the concentrations are: MC-LR 0.31 μg/L, MC-RR 0.32 μg/L, MC-YR 0.38 μg/L and NODLN 0.47 μg/L.

3.1.3 Microcystins –PPIA

The enzyme protein phosphatase is inhibited in a concentration-dependent manner by microcystins. Subsequent exposure of the enzyme to a substrate that forms a colored product reveals the degree of enzyme inhibition. Comparison of sample results with those of known standards quantifies the level of microcystins in the sample.

3.2 Anatoxin-a Toxin Structure and Cross-Reactivity Analysis Summary

The neurotoxin anatoxin-a is an alkaloid with high toxicity (LD_{50} i.p. mouse 200 μg/kg). It acts as a post-synaptic, depolarizing, neuromuscular blocking agent. There is evidence that anatoxin-a can be produced by some species of *Anabaena*, *Aphanizomenon, Cylindrospermum* and *Oscillatoria*.

The determination of anatoxin-a in natural waters has been challenging due to its typically low concentration. The highly sensitive method adapted at KCEL uses fluorimetric derivatization with HPLC. Anatoxin-a is converted into a fluorescent derivative using 4-fluoro-7-nitro-2,1,3-benzoxadiazole and the fluorescent compound is then separated and detected by HPLC. Further increase in sensitivity is accomplished by concentrating the algal cells on a filter. This method will thus only measure intracellular toxin. Since anatoxin-a is easily degraded once outside the cell, we can assume that the filtration step will retain most or all of the active toxin present in the sample.

3.2.1 Sample Preparation for Anatoxin-a Toxin Assay

Anatoxin-a degrades readily, especially in sunlight and at high pH. At KCEL the bottles are immediately transferred to a 4 °C chamber. The samples are filtered onto glass fiber filters (45 mm Whatman 934-AH) within 24 hours of arrival and the filters stored in cryovials at -20 °C (2 filters per station). The filtrate is discarded.

KCEL is in the process of developing a SOP for the measurement of anatoxin-a in water samples using isocratic HPLC with fluorescence detection. The method will be under development during the 2008 season. The reference method is James et al. (1998).

<u>Extraction</u>: Anatoxin-a is extracted from cells by treating the filter with 100% methanol (freezing and sonication). The extract may or may not be dried prior to derivatization. Alternatively, a whole water sample is extracted, cleaned and concentrated using a weak cation exchange solid phase extraction column. The resulting extract is evaporated under nitrogen and stored at -20 °C.

<u>Derivatization:</u> Dried samples or standards are derivatized with 4-Fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) following the method of James et al. (1998). The reaction is

terminated at 60 minutes, when samples are centrifuged or filtered and transferred to autosampler vials.

Chromatography: HPLC analysis is performed with an Agilent 1200 series system using a Zorbax C_{18} column, 55% acetonitrile-water as the mobile phase and fluorimetric detection at 470 nm (excitation) and 530 nm (emission). Run time is 45 min per injection. The system can be programmed to run a complete batch overnight (e.g., blank, 5 standards, 2 spikes, 5-8 samples).

Anatoxin-a analytical standards are purchased commercially, diluted and run along with samples to create a 5-point calibration curve (25 – 225 µg/L). Methyl pipecolinate (1.25 mg/L) is added to all samples and standards and used as an internal or reference standard. Dihydroanatoxin and epoxyanatoxin (non-toxic anatoxin-a degradation products) can be synthesized in house and added to the standards. Natural samples spiked with anatoxin-a will also be run routinely.

Although an MDL has not been determined it is expected that, based on 1 L samples and adequate detection at 25 µg/L (lowest standard), the MDL would lie in the range 0.025 – 0.050 µg/L (i.e., a 500 – 1000 fold sample concentration). Strong matrix effects could, however, significantly affect the level of detection for certain samples. The WHO recommended threshold for anatoxin-a in drinking water is 3 µg/L (J. Hardy, personal communication).

3.3 Analytical Procedures

Samples will be analyzed using the procedures and detection limits listed in the table below.

Table 6. Laboratory Analysis Summary

Parameter	Reference	Method Detection Limit	Reporting Detection Limit
Microcystins by ELISA	KCEL SOP 440v2	0.05 µg/L	0.05 µg/L
Microcystins and Nodularins by PPIA	KCEL SOP 443v1	0.1 µg/L	0.1 µg/L
Anatoxin-a by HPLC	(see section 3.2.1 above).	TBD	TBD

4 Data Quality Objectives

The procedures and practices described in this studyspecific SAP are designed to generate data of sufficient quality to support decision making as discussed above and in the Major Lakes Monitoring Program SAP; King County, 2005. Critical elements of laboratory data quality objectives are discussed in this section. Procedures to attain these data quality objectives are discussed throughout this document. In particular, Section 7.0, Quality Control Procedures, addresses many of the procedures necessary to obtain data that meet these data quality objectives.

4.1.1 Laboratory Precision

Laboratory precision will be assessed using laboratory duplicate QC samples. When both sample results are at or exceed the MDL the RPD (relative percent difference) should be less than 25 %. An RPD cannot be determined unless both values are at or above the MDL since no values are reported if <MDL. Note that the MDL and the Reporting Detection Limit (RDL) are the same for both the ELISA and PPIA.

If one value is >MDL and the other <MDL, a RPD is still calculated using zero for the less <MDL value.

4.1.2 Field Precision

Information regarding the precision of sampling procedures will be obtained by collecting field replicates. The data user should take the information obtained by collecting field replicates into account when making decisions based on data generated under this SAP.

4.1.3 Bias

Bias is an indicator of the accuracy of analytical data. For this project, laboratory control samples or blank spikes, whichever are available, will be used to assess bias. Results should be within 20% of the true value or within the criteria provided with the purchase of the control sample.

Bias will also be assessed by the evaluation of field blank and method blank data. Analytical results for method blanks should be less than the MDL.

The use of matrix spike recovery data will provide additional information regarding method performance on actual samples. The laboratory will use professional judgment regarding assessment of data quality and any subsequent action taken as a result of matrix spike recoveries.

4.1.4 Representativeness

This survey is primarily designed to evaluate the presence/absence of cyanobacterial toxicity, and secondarily to estimate concentrations and geographic extent of the toxin distribution, should it be present. Representative samples will be obtained through the following practices:

- The use of generally accepted sampling procedures will allow for the collection of representative samples.
- Subsampling within the KCEL will be conducted according to lab standard operating procedures. These procedures are designed to obtain representative subsamples.

Note that additional practices to be used to obtain representative data are described in the site specific SAP; Major Lakes Monitoring Program SAP, King County, released in 2005.

4.1.5 Comparability

Data comparability will be obtained through the use of standard sampling procedures and analytical methods. Additionally, adherence to the procedures and QC approach contained in this SAP will provide for comparable data throughout the duration of this project. Before making changes to sample collection, storage or analysis procedures, each must be evaluated to verify that comparability will not be compromised.

4.1.6 Completeness

Completeness will be evaluated by the following criteria:

- The number of usable data points compared to the projected data points as detailed in this SAP.
- Compliance with the data quality criteria as presented in this section.
- Compliance with specified holding times.

The goal for the above criteria is to obtain 100% data completeness. However, where data are not complete, decisions regarding re-sampling and/or re-analysis will be made by a collaborative process involving both data users and data generators. These decisions will take into account the project data quality objectives as presented above.

5 Data Reduction, Review, and Reporting

Data reduction, review and reporting will be performed under the KCEL's standard operating procedures. Laboratory data will be provided to data recipients within 30 days of sample receipt or within 30 days of the decision to analyze archived samples. Data reports will include sufficient information to conduct the data assessment. Field measurements will also undergo standard review and reporting procedures. Data will be reported in the standard laboratory-reporting format. This includes an analytical result, MDL and RDL, if available.

Protocols will be worked out with the KCEL for the rapid turn around of selected samples in the event of a bloom episode that could have potential public health implications. Preliminary project data, required in the event of a bloom episode that

could have potential public health implications, will be reported using KCEL Preliminary Data Reporting Form followed by final data as soon as practical.

Final project data will be presented to the project and program managers in a format that may include the following:

- KCEL Comprehensive Reports consisting of spreadsheets of analytical and field parameters;
- Case narratives for ELISA and PPIA results prepared by the Aquatic Toxicology Unit;
- Unit narratives of chemistry and microbiology data including supporting QC documentation (provided by the KCEL) in the event of analytical or data anomalies.
- Cyanobacteria identification and biovolume determinations conducted by KCEL, as KCEL SOP 544v0.

6 Project Organization

Project team members and their responsibilities are summarized below. All team members are staff of the King County Department of Natural Resources and Parks, Water and Land Resources Division.

Table 7. Project Team Members

Name/Telephone	Title	Affiliation	Responsibility
Katherine Bourbonais (206) 684-2382	Laboratory Project Manager	Environmental Laboratory	Coordination of analytical activities, lab QA/QC and data reporting.
David Robinson (206) 684-2329	Environmental Scientist	Environmental Laboratory	Coordination of sampling activities, field QA/QC, and field analyses for the Major Lakes Program
Judy Ochs (206) 684-2347	Environmental Scientist	Environmental Laboratory	Coordination of sampling activities, field QA/QC, and field analysis for the Swimming Beach Program
Debra Bouchard (206) 263-6343	Water Quality Planner	Water & Land Resources	Project manager for the Toxic Cyanobacteria

			Study, coordination between various groups at the lab
Colin Elliott (206) 684-2343	Quality Assurance Officer	Environmental Laboratory	Overall laboratory QA/QC.
Gabriela Hannach (206) 684-2301	Aquatic Toxicologist	Environmental Laboratory	Coordination of toxicity analysis; Anatoxin-a method development
Jim Buckley (206) 684-2314	Aquatic Toxicologist	Environmental Laboratory	PPIA, Anatoxin-a method development

7 Quality Control Procedures

7.1 Field Quality Control Procedures

Over the course of this project, field QC samples will be collected at the frequency listed below. It is recommended that a set of field QC samples be collected during the first sampling effort to provide an initial indication of field sampling precision and bias.

Table 8. Field Quality Control Samples

Type of Quality Control Sample	Description	Frequency
Field Replicate	A second sample generated from the same sampling location as the initial sample, but from a second sampler deployment. Used as an indicator of field sampling precision.	Over the course of the project, 1 per sampling event, done at a random site. If more than 20 sites are done in a day, 2 field replicates should be collected.

7.1.1 QC Practices for Field Measurements

Sampling for this Toxic Cyanobacteria Study is conducted concurrently with the Routine/Ambient Major Lakes Monitoring program and the Swimming Beach Monitoring Program. Therefore QA practices are covered under those SAPs/SOPs.

7.2 Laboratory Quality Control Procedures

The KCEL is accredited by the Washington State Department of Ecology. As a requirement of this accreditation, the lab is audited by the Washington State Department

of Ecology. Additionally, the KCEL participates regularly in US EPA inter-laboratory performance evaluation studies.

7.2.1 Frequency of quality control samples

All samples will be analyzed at the KCEL. The frequency of quality control samples to be performed for this project is shown in the following table. QC samples shown below may not be available for all lab analyses.

Table 9. Laboratory Quality Control Samples (microcystins and anatoxins only)

Type of Quality Control Sample	Description	Frequency
Method Blank	An aliquot of clean reference matrix carried through the analytical process and used as an indicator of contamination.	1 per sample batch. Maximum sample batch size equals 20 samples.
Laboratory Control Sample	Solution of known analyte concentration, processed through the entire analytical procedure and used as an indicator of method accuracy and precision.	1 per sample batch, as available. Maximum sample batch size equals 20 samples.
Spike Blank	Known concentration of target analyte(s) introduced to clean reference matrix, processed through the entire analytical procedure and used as an indicator of method performance.	Used if a laboratory control sample is not available. 1 per sample batch. Maximum sample batch size equals 20 samples.
Matrix Spike	Known concentration of target analyte(s) introduced to a separate portion of sample, processed through the entire analytical procedure and used as an indicator of method performance.	1 per sample batch. Maximum sample batch size equals 20 samples.

In addition to the QC samples specified above, the following QC samples will be performed on samples from this project at the frequency listed below:

Table 10. Additional Laboratory Quality Control Samples (all parameters)

Type of Quality Control Sample	Description	Frequency
Lab Duplicate	A second aliquot of a sample, processed concurrently and identically with the initial sample, used as an indicator of method precision.	One per batch of samples for microcystins and anatoxin. Once per quarter for quantitative phytoplankton testing.

KCEL laboratory QC samples for microcystins analysis and associated control limits are summarized below. These QC samples will be analyzed at a frequency of one per analytical batch 20 or fewer samples.

Table 11. Laboratory QC Requirements

Parameter	Method Blank	Duplicate RPD	Negative Control	CS % Recovery
Microcystins	<MDL	25%	<0.1 ppb	NA
Anatoxin-a	TBD	TBD	TBD	TBD

Notes:
CS- Check Standard (positive control equivalent to Laboratory Control Sample)
MDL – Method Detection Limit
NA – Not Applicable
RPD – Relative Percent Difference
TBD – Anatoxin QC limits To Be Determined during method development

7.3 Corrective Action

KCEL standard operating practice is to detect and correct analytical difficulties during sample analysis. Should the lab have difficulty in meeting the data quality objectives outlined in this SAP, the lab will work with the data user to develop and implement corrective action.

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9 Attachment –THE INFLUENCE OF PIGMENTS ON RESULTS IN THE PROTEIN PHOSPHATASE INHIBITION ASSAY

Lab Analysis conducted by Jim Buckley

020123 JB

In the ELISA for microcystins, it is not uncommon to observe anomalous %B values of greater than 100% for environmental samples. The same is true for the PPIA. When the samples from the *Aphanizomenon* bloom in Green Lake were analyzed by PPIA, samples filtered to 0.45 um retained a green color and yielded %B values much greater than 100%. This provided an opportunity to test for pigment removal with an ultrafiltration system consisting of a filter of 5000 nmwl and centrifugation at 15,000 g.

The following table shows results of three days of tests of several treatments of Green Lake SE (GL SE) and Aquatheater (GL AT) samples designed to identify the influence of pigments, probably mostly chlorophylls, on the results of PPIA. Values in bold print are from samples that were green in color following filtration to 0.45um.

Terms:
0.45 = filtration to 0.45 um with glass fiber prefilter and 0.45 um Millipore Millex filter
5000 = filtration to 5000 nominal molecular weight limit (nmwl) Millipore Ultrafree centrifuge unit
control = assay mixture including sample but without PP enzyme
blank = negative control without PP enzyme
centrifuge = 10 min. at 15,000g

Sample /Treatment	GL SE 1		GL SE 2		GL AT 1		GL AT 2	
	%B	ug/L	%B	ug/L	%B	ug/L	%B	ug/L
12-29-01								
0.45 + 5000	90	0.095	92	0.087	90	0.093	86	0.111
control	2		-15		3		4	
blank = 0.069								
1-8-02								
0.45	**373**	**0.000**	**442**	**0.000**	95	0.082	92	0.092
0.45 + 5000	94	0.085	95	0.081	91	0.094	91	0.095
control	-0.1		-1.0		-1.0		-4	
blank = 0.107								
1-10-02								
0.45	**391**		**489**					
control	**285**		**376**					
0.45 + centrifuge	**355**		**421**					
control	**257**		**332**					
blank = 0.075								

12-29-01
The samples were filtered to 0.45 (green) and then to 5000 (clear) and assayed for microcystins. Results showed microcystins present at 0.111 ug/L in the GL AT 2 sample. All other samples gave results < MDL of 0.1 μg/L. Control %B values were ≤ 4, indicating little or no apparent endogenous PP activity.

1-8-02
Samples from GL SE 1 and 2 filtered to 0.45 um only gave very high %B values (**373** and **442**) due to the OD from the green pigments and perhaps from other substances also. In comparison, samples GL AT 1 and 2, also filtered to 0.45 um, were clear and gave typical %B values (95 and 92). When the 0.45-filtered

GL SE 1 and 2 samples were further treated by filtration to 5000 nmwl thereby removing the green color and putative other substances, the %B values were reduced from **373** to 94 and **442** to 95. This indicates that the 5000 nmwl filtration step is effective in removal of pigments and other substances that can confound results of the PPIA. Filtration of GL AT 1 and 2 to 5000 nmwl only slightly changed the % B values (95 to 91 and 92 to 91) and resulting levels of microcystins. Control values for these samples were all very low (-0.1 to -1.0).

1-10-02
Samples were green following filtration to 0.45 um only. Subtraction of the control (without PP) from the assay (with PP) yields values (mean = 102) that are close to the negative control (with PP) indicating little or no endogenous PP activity in these samples.

GL SE 1: **391** – **285** = 106 A % B value of ≈ 100 indicates a sample reading ≈ negative control

355 – **257** = 98

GL SE 2: **489** – **376** = 113

421 – **332** = 89

Centrifugation alone yielded a small white button for both samples. However, the high %B values were only reduced by 9 to 14 %, indicating that only a small amount of the high OD is due to particulates.

GL SE 1: **391** – **355** = 36 or 9% of %B is removable by centrifugation

GL SE 2: **489** – **421** = 68 or 14% of %B is removable by centrifugation

Conclusions

1. Green pigments and associated substances in 0.45 um filtrate give artificially high %B values that can mask the presence of microcystins.

2. Filtration to 0.45 um and then to 5000 nmwl removed these pigments and associated substances that, in this case, gave high %B values that could mask otherwise detectable levels of microcystins.

3. It is useful to run Controls to check for apparent endogenous protein phosphatase activity in samples. In the present samples, there was no apparent protein phosphatase activity.

4. Centrifugation only of samples previously filtered to 0.45 um showed that 9 to 14 % of the high %B values was due to particulates amenable to removable by filtration.

5. For the Green Lake SE samples, the yield from the ultrafiltration system was 120 uL each which was adequate for the PPIA which requires 20 uL per replicate. To be useful for the ELISA which requires 100 uL per replicate, a scaled up version of the ultrafiltration system, perhaps including centrifuge, would be most efficient.

Table A. 2008 Sampling Schedule for Cyanobacteria Toxicity and Quantitative Phytoplankton in both the Routine Major Lakes Program and Swimming Beach Programs.

Major Lakes and Toxic Cyanobacteria Phytoplankton Sampling Schedule

IC = Integrated composite sample ("the tube")
S = Discrete surface sample
c = chlorophyll

Q = Quantitative phytoplankton analysis.
M = microcystin

NOTE: ALL sites have chlorophyll samples

		0826	0852 [a]	0831	0807	4903	0834	0817	0832	625	0611	0612	0614	A522
Sampling Method =>		IC	IC	IC	S	S	S	S	S	S	IC	IC	S	IC
JAN	1 sample	c	Qc	c	c	c	c	c	c	c	c	Qc	c	Qc
FEB	1 sample	c	Qc	c	c	c	c	c	c	c	c	Qc	c	Qc
MAR	2nd week	c	QcM	c	c	c	c	c	c	c	c	QcM	c	QcM
	4th week	c	QcM	c	c	c	c	c	c	c	c	QcM	c	QcM
APR	2nd week	c	QcM	c	c	c	c	c	c	c	c	QcM	c	QcM
	4th week	c	QcM	c	c	c	c	c	c	c	c	QcM	c	QcM
MAY	1st week													
	2nd week	c	QcM	c	c	c	c	c	c	c	c	QcM	c	QcM
	3rd week													
	4th week	c	QcM	c	c	c	c	c	c	c	c	QcM	c	QcM
JUN	1st week													
	2nd week	c	QcM	c	c	c	c	c	c	c	c	QcM	c	QcM
	3rd week													
	4th week	c	QcM	c	c	c	c	c	c	c	c	QcM	c	QcM
JUL	1st week													
	2nd week	c	QcM	c	c	c	c	c	c	c	c	QcM	c	QcM
	3rd week													
	4th week	c	QcM	c	c	c	c	c	c	c	c	QcM	c	QcM
	5th week													
AUG	1st week													
	2nd week	c	QcM	c	c	c	c	c	c	c	c	QcM	c	QcM
	3rd week													
	4th week	c	QcM	c	c	c	c	c	c	c	c	QcM	c	QcM
SEP	1st week													
	2nd week	c	QcM	c	c	c	c	c	c	c	c	QcM	c	QcM
	3rd week													
	4th week	c	QcM	c	c	c	c	c	c	c	c	QcM	c	QcM
	5th week													
OCT	1st week													
	2nd week	c	QcM	c	c	c	c	c	c	c	c	QcM	c	QcM
	3rd week													
	4th week	c	QcM	c	c	c	c	c	c	c	c	QcM	c	QcM
NOV	1 sample	c	Qc	c	c	c	c	c	c	c	c	Qc	c	Qc
DEC	1 sample	c	Qc	c	c	c	c	c	c	c	c	Qc	c	Qc

a. One field replicate sample collected at 0852 for microcystin, chlorophyll, **and quantitative phytoplankton analysis** once a month.

Swimming Beach Toxic Cyanobacteria Phytoplankton Sampling Schedule

**** samples will be collected on alternate weeks from Major Lakes sampling**

S = Discrete surface sample
H = Collect quantitative phytoplankton samples and hold pending microcystin values.
a = anatoxin

M = microcystin

NOTE: **phytoplankton analysis will be done only if necessary due to high microcystin concentrations**
Quantitative phytoplankton samples will be analyzed by King County staff.

			0805ASB	806SB*	0813SB	0818SB	0820SB	826SB	828SB	826OLA	834SB	83930SB	852SB	A734WSB	SD007SB	SD017SB	4903SB	602SB	615SB
Sampling Method =>			S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
JAN	1 sample																		
FEB	1 sample																		
MAR	2nd week																		
	4th week																		
APR	2nd week																		
	4th week																		
MAY	1st week																		
	2nd week	13-May	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
	3rd week	20-May	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
	4th week	27-May	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
JUN	1st week	3-Jun	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
	2nd week	10-Jun	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
	3rd week	17-Jun	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
	4th week	24-Jun	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
JUL	1st week	1-Jul	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
	2nd week	8-Jul	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
	3rd week	15-Jul	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
	4th week	22-Jul	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
	5th week	29-Jul	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH	MH
AUG	1st week	5-Aug	MH	MHa	MH	MHa	MH	MH	MHa	MH	MHa	MH	MH	MHa	MH	MH	MH	MH	MH
	2nd week	12-Aug	MH	MHa	MH	MHa	MH	MH	MHa	MH	MHa	MH	MH	MHa	MH	MH	MH	MH	MH
	3rd week	19-Aug	MH	MHa	MH	MHa	MH	MH	MHa	MH	MHa	MH	MH	MHa	MH	MH	MH	MH	MH
	4th week	26-Aug	MH	MHa	MH	MHa	MH	MH	MHa	MH	MHa	MH	MH	MHa	MH	MH	MH	MH	MH
SEP	1st week	2-Sep	MH	MHa	MH	MHa	MH	MH	MHa	MH	MHa	MH	MH	MHa	MH	MH	MH	MH	MH
	2nd week	9-Sep	MH	MHa	MH	MHa	MH	MH	MHa	MH	MHa	MH	MH	MHa	MH	MH	MH	MH	MH
	3rd week	16-Sep	MH	MHa	MH	MHa	MH	MH	MHa	MH	MHa	MH	MH	MHa	MH	MH	MH	MH	MH
	4th week	23-Sep	MH	MHa	MH	MHa	MH	MH	MHa	MH	MHa	MH	MH	MHa	MH	MH	MH	MH	MH
	5th week	30-Sep	MH	MHa	MH	MHa	MH	MH	MHa	MH	MHa	MH	MH	MHa	MH	MH	MH	MH	MH
OCT **	1st week	7-Oct	MH	MHa	MH	MHa	MH	MH	MHa	MH	MHa	MH	MH	MHa	MH	MH	MH	MH	MH
	2nd week	14-Oct	MH	MHa	MH	MHa	MH	MH	MHa	MH	MHa	MH	MH	MHa	MH	MH	MH	MH	MH
	3rd week	21-Oct	MH	MHa	MH	MHa	MH	MH	MHa	MH	MHa	MH	MH	MHa	MH	MH	MH	MH	MH
	4th week	28-Oct	MH	MHa	MH	MHa	MH	MH	MHa	MH	MHa	MH	MH	MHa	MH	MH	MH	MH	MH
NOV	1 sample																		
DEC	1 sample																		

* One field replicate sample collected at 0806SB for microcystin analysis once a month.
** Samples collected beyond swim beach program (Bob Brenner)

Major Lakes and Swimming Beach
Toxic Cyanobacteria & Phytoplankton Sampling

Total Samples Collected per Sampling Week

		Major Lakes		Swim Beach		
		# Microcystins	# Phytos ANALYZED	# Microcystins	# Phytos COLLECTED	# Anatoxin
		per sampling week	per sampling week	per sampling week	per sampling week	per sampling week
JAN	1 sample	0	**4**			
FEB	1 sample	0	**4**			
MAR	2nd week	**4**	**4**			
	4th week	3	3			
APR	2nd week	**4**	**4**			
	4th week	3	3			
MAY	1st week					
	2nd week	**4**	**4**	**18**	17	
	3rd week			17	17	
	4th week	3	3	17	17	
JUN	1st week			17	17	
	2nd week	**4**	**4**	**18**	17	
	3rd week			17	17	
	4th week	3	3	17	17	
JUL	1st week			17	17	
	2nd week	**4**	**4**	**18**	17	
	3rd week			17	17	
	4th week	3	3	17	17	
	5th week			17	17	
AUG	1st week			17	17	5
	2nd week	**4**	**4**	**18**	17	5
	3rd week			17	17	5
	4th week	3	3	17	17	5
SEP	1st week			17	17	5
	2nd week	**4**	**4**	**18**	17	5
	3rd week			17	17	5
	4th week	3	3	17	17	5
	5th week			17	17	5
OCT	1st week			17	17	5
	2nd week	**4**	**4**	**18**	17	5
	3rd week			17	17	5
	4th week	3	3	17	17	5
NOV	1 sample		**4**			
DEC	1 sample		**4**			
nnual Total		56	72	431	425	65