Microcystins and Other Blue-Green Algae Toxins Analyzed in Fish and Sediments from Washington Lakes

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Cover photo: Blue-green algae bloom in Kapowsin Lake, Pierce County, 11/4/2010.
(Photo courtesy of Don Russell, Tacoma, WA.)

Microcystins and Other Blue-Green Algae Toxins Analyzed in Fish and Sediment from Washington Lakes

by

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Waterbody Number(s):

Anderson Lake	WA-17-9010
Bay Lake	WA-15-9010
Black Lake	WA-23-9010
Cassidy Lake	WA-07-9150
Ketchum Lake	WA-03-9110
Lake Spokane	WA-53-9020
Pattison Lake	WA-13-9130
Spanaway Lake	WA-12-9070
Waughop Lake	WA-12-9090

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Table of Contents

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List of Figures

Page

List of Tables

Page

Abstract

Microcystins and other blue-green algae toxins were analyzed in fish and sediment samples collected from nine Washington lakes with recurrent toxic algae blooms. The samples were analyzed by enzyme-linked immunosorbent assay (ELISA) and liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS). Until recently, exposure pathways of concern for these compounds have focused on drinking water, swallowing water while swimming, and dermal contact through recreational activities. Exposure to blue-green toxins through fish consumption is a relatively new area of investigation.

Microcystins or microcystin-like compounds were found to be elevated in fish liver relative to muscle and other tissues. Microcystin-LA (MC-LA) was the only variant specifically detected in fish among the nine microcystins analyzed by LC/MS/MS. The detections – 2.5 to 12 ug/Kg (parts per billion) – were in four fish liver samples. Fish fillets showed no accumulation of the microcystins targeted in this analysis.

MC-LA and microcystin-LR (MC-LR) residues were found in the lake sediment samples. These compounds may exhibit long-term persistence. Deep water sediments consistently had higher concentrations than shallow water sediments. The maximum concentrations recorded were 52 ug/Kg for MC-LA and 42 ug/Kg for MC-LR.

The other blue-green toxins analyzed – anatoxin-a, saxitoxin, domoic acid, and okadaic acid – were not detected in fish or sediment.

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Background

In 2008, the Washington State Department of Ecology (Ecology) tested for the presence of microcystins and anatoxin-a in fish from six western Washington lakes that were experiencing blue-green algae (cyanobacteria) blooms (Johnson, 2010a). These compounds are highly toxic to animals, including humans, and are an emerging public health issue.

Microcystins particularly affect the liver. Anatoxin-a is a neurotoxin. Until recently, exposure pathways of concern for these compounds have focused on drinking water, swallowing water while swimming, and dermal contact through recreational activities. Exposure to blue-green toxins through fish consumption is a relatively new area of investigation. Information on the biology and toxicity of blue-greens can be found on the Washington State Department of Health (WDOH) cyanobacteria website (www.doh.wa.gov/ehp/algae/whatarecyanobacteria.htm) and Ecology's Freshwater Algae Control Program website (www.ecy.wa.gov/programs/wq/plants/algae/index.html).

For Ecology's 2008 study, microcystins were analyzed by an enzyme-linked immunosorbent assay method (ELISA). Significant concentrations appeared to be present in fish from most of the lakes surveyed. The accuracy of these data, however, was difficult to assess. Anatoxin-a, analyzed by high pressure liquid chromatography (HPLC), was not detected. It may be too unstable to accumulate or is simply not taken up by fish.

A third blue-green compound, saxitoxin, was tentatively identified by ELISA in fish from the one lake it was tested for, a lake known to produce saxitoxin. Saxitoxin is a neurotoxin primarily associated with paralytic shellfish poisoning (PSP or "red tide") in marine waters.

The 2008 report recommended further sampling and analysis to obtain accurate and verifiable data on microcystins and saxitoxin in fish from Washington lakes. In view of the stability of microcystin compounds, it was also recommended that lake sediments be screened for their presence. Results of these efforts, conducted in 2010 and 2011, are reported here.

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Project Description

Ecology's 2010-2011 investigations employed liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) in an effort to detect and quantify specific microcystin variants in fish and sediment. It was hoped that the LC/MS/MS results could be further used to evaluate the sensitivity and correspondence of ELISA as applied to microcystins in fish. If confidence can be established in ELISA, the method holds promise as a relatively quick and economical screening tool for assessing health risks to fish consumers. Although not a focus of this study, the LC/MS/MS method also provides data on anatoxin-a, as well as the shellfish toxins domoic acid and okadaic acid.

During the summer and fall of 2010, fish fillet samples were obtained from eight Washington lakes in association with significant blue-green algae blooms and analyzed for microcystins by ELISA and LC/MS/MS. Saxitoxin was analyzed in fish from two lakes using a separate ELISA method. In March the following year, sediment samples were collected from six lakes with a documented history of blue-green blooms dating back to 1999. These samples were analyzed by LC/MS/MS only.

Results from 2010 showed a need to analyze fish samples known to be positive for important microcystin variants to better compare the two methods. Therefore, a second set of samples was obtained in 2011 that included four archived fish muscle homogenates provided by the California Department of Fish & Game (CDFG). These samples had previously been analyzed by LC/MS/MS (2007) and reported to contain elevated levels of the desmethyl-LR microcystin variant. Fish livers were also analyzed in 2011.

Objectives of the 2010 and 2011 investigations were to:

1. Obtain accurate, verifiable data on microcystins in fish fillets and liver.
2. Evaluate the usefulness of the ELISA method for microcystins in fish tissue.
3. Investigate the accumulation of microcystins in lake sediments.
4. Test for the presence of saxitoxin in fish from a known saxitoxin producing lake.
5. Provide data to help WDOH determine if the presence of microcystins in fish tissue or sediments represents an exposure concern for the public.

Project samples were analyzed by the Water Pollution Control Laboratory of CDFG (LC/MS/MS) and the King County Environmental Laboratory (KCEL; ELISA). CDFG had recently validated an LC/MS/MS method for microcystins in water and tissue. KCEL uses ELISA and other methods to do algae identification and toxicity testing for Ecology's Freshwater Algae Control Program.

The Ecology Environmental Assessment Program (EAP) conducted the 2010 and 2011 studies with the assistance of the Ecology Water Quality Program, Program Development Services Section, Technical Services Unit (PDS-TSU) and the WDOH Office of Environmental Health, Safety, and Toxicology and Office of Shellfish and Water Protection. PDS-TSU operates the Freshwater Algae Control Program and had requested the microcystin fish tissue investigation.

WDOH developed state recreational guidance values for microcystins, anatoxin-a, and saxitoxin (Hardy, 2008, 2011). WDOH's guidance values and lake protocol help local health departments decide whether to post a lake for blue-green toxins and advise on how to communicate that information to the public.

This study followed a Quality Assurance Project Plan (Johnson, 2010b) developed in accordance with the Ecology guidance in Lombard and Kirchmer (2004).

Samples Analyzed

Lakes Sampled

Fish and sediment samples were collected from the lakes listed below (Table 1). For logistical reasons, field work focused mainly on western Washington lakes. One reservoir in eastern Washington, Lake Spokane (Spokane River), was also sampled. Locations of the lakes are shown in Figures 1 and 2.

Table 1. Lakes Where Fish and Sediment Samples were Collected in 2010-2011 and Analyzed for Blue-Green Toxins.

Lake	County	Surface Area (acres)	Maximum Depth (feet)	Latitude	Longitude	Fish and Sediment Samples	Fish Samples Only	Sediment Samples Only
Waughop	Pierce	230	22	47 10 09	122 33 41	x		
Spanaway		22	28	47 07 11	122 26 45			x
Bay		129	11	47 14 24	122 45 22			x
Ketchum	Snohomish	20	30	48 16 48	122 20 27	x		
Cassidy		125	20	48 02 51	122 05 28	x		
Pattison	Thurston	257	20	46 59 54	122 46 15		x	
Black		576	40	47 38 39	121 43 25		x	
Anderson	Jefferson	59	29	48 00 54	122 48 04	x		
Spokane	Spokane	5,020	~75	47 53 41	117 41 12		x	

Figure 1. Location of Fish and Sediment Samples Collected from Western Washington Lakes in 2010-2011.

Figure 2. Location of Fish Samples Collected from Lake Spokane in Eastern Washington in 2010.

Fish Collection

It is not possible to predict when or where blue-green blooms will occur. Although blooms can be observed somewhere in Washington almost any time of year, most occur in July through October (Hamel, 2009). The trigger for fish sampling in the present study was detection of high levels of microcystins in algae samples collected through Ecology's Freshwater Algae Control Program. PDS-TSU alerted EAP to blooms that seemed significant enough to warrant fish sampling. Appendix A shows the microcystin levels reported prior to or soon after the fish collections in 2010 and 2011.

Fish appear to take up microcystins quickly (Tencala and Dietrich, 1997: Magalhaes et al., 2001; Dyble et al., 2011). Depuration (loss) is also reported to be fairly rapid once fish are removed from exposure (Adamovsky et al., 2007; Dyble et al., 2011). Therefore, an attempt was made to collect fish soon after high toxin levels were reported. Field work for other projects and logistical difficulties sometimes delayed mobilization for fish sampling.

Up to three fish species were collected from each lake. Popular sport fish such as rainbow trout, bass, and perch, as well as a bottom-feeding species such as suckers and bullheads were targeted. Composite samples were analyzed to provide a cost-effective estimate of toxin concentrations. Where possible, each sample consisted of pooled tissues from five individual fish. In some instances, only a few fish were encountered during collection efforts and sample size was lower than desired.

The fish samples analyzed in 2010 are listed in Table 2. Because human health concern was the initial impetus for this study, all of the 2010 samples were fillets. The Washington Department of Fish & Wildlife (WDFW) Arlington Fish Hatchery (listed first in Table 2), is the source of the rainbow trout sampled in Ketchum Lake. The sample analyzed was collected at the hatchery approximately one week before Ketchum Lake was stocked.

Two types of ELISA kits were initially used for the 2010 samples: Envirologix™ (www.envirologix.com) and Abraxis anti-ADDA (www.abraxiskits.com). The primary difference is that the Abraxis kit detects a part of the microcystin molecule common to all microcystins whereas Envirologix detects a part of the molecule that is not common to all microcystins. Over 80 microcystin variants have been reported in environmental samples (Zurawell et al., 2005). Envirologix primarily, although not exclusively, measures the MC-LR form. At present, MC-LR is the primary public health concern during algae blooms. Because a wider range of microcystins are targeted, ADDA ELISA is the more frequent choice for fish tissue work cited in the literature.

Table 2. Fish Fillet Samples Collected in 2010 and Analyzed for Microcystins and Saxitoxin.

Lake/Location	Species	Collection Date	Microcystins			Saxitoxin
			Envirologix ELISA	ADDA ELISA	LC/MS/MS	ELISA
Arlington Fish Hatchery	Rainbow trout	19-Apr	x	x		
Waughop	Rainbow trout	27-May	x		x	x
Ketchum	Pumpkinseed	19-Jul	x		x	
	Yellow perch		x		x	
	Brown bullhead				x	
	Yellow perch	12-Aug	x		x	
	Pumpkinseed				x	
	Rainbow trout	13-Aug			x	
		24-Aug	x		x	x
Cassidy	Largemouth bass	26-Aug	x		x	
	Pumpkinseed		x	x	x	
	Yellow perch		x	x	x	
Anderson	Rainbow trout	27-Aug	x	x		
		28-Aug	x	x		
Pattison	Largemouth bass	8-Sep	x	x	x	
	Brown bullhead				x	
	Rock bass				x	
Cassidy	Largemouth bass	21-Sep			x	
	Yellow perch		x	x	x	
	Pumpkinseed				x	
Black	Largemouth bass	18-Oct			x	
	Cutthroat		x		x	
	Brown bullhead		x		x	
Spokane	Largescale sucker	26-Oct	x			
	Lake whitefish		x			
	Northern pikeminnow		x			

Table 3 shows the fish samples analyzed for 2011. Both fillets and liver were analyzed in an effort to provide the laboratories with material that had a range of microcystin concentrations. ELISA testing was limited to the ADDA kit. All samples were submitted blind to the laboratories.

The California yellow perch samples listed in Table 3 were from a study of microcystin bioaccumulation in Klamath River (Oregon) reservoirs (Kann, 2008). CDFG provided these known positive microcystin samples to Ecology where they were re-homogenized, split into separate aliquots, and sent to KCEL and CDFG.

The 2011 samples also included a cutthroat trout fillet from the Upper St. Joe River in northern Idaho, collected for a separate Ecology study (Johnson et al., 2011). This part of the St. Joe flows through relatively undeveloped National Forest land. It was assumed the cutthroat sample would not contain detectable amounts of microcystins.

Table 3. Fish Fillet and Liver Samples Analyzed for Microcystins in 2011.

Site/ Lake	Species	Collection Date	ADDA ELISA	LC/MS/MS
Western Washington Samples				
Ketchum Lake	Rainbow trout	2-Jul-11	x*	x
Waughop Lake	Rainbow trout	3-Aug-11	x	x
	Largemouth bass	3-Aug-11	x	x
		14-Aug-11	x	x
Cassidy Lake	Largemouth bass	23-Sep-11	x	x
	Yellow perch		x*	x
California Samples (MC positive)				
Copco Reservoir	Yellow perch #23	7-Sep-07	x	x
	Yellow perch #29		x	x
Iron Gate Reservoir	Yellow perch #17	6-Sep-07	x	x
	Yellow perch #18		x	x
Idaho Sample (background)				
Upper St. Joe River	Cutthroat trout	28-Sep-10	x*	x*

*Liver not analyzed due to insufficient sample.

The fish samples obtained for the 2010-2011 studies are listed individually in Appendix B, along with dates of capture, lengths, and weights.

Sediment Sampling

Microcystins are highly resistant to degradation and have the potential to persist in lakes long after a bloom has past. Babica et al. (2006) report detection of microcystins in freshwater sediments and cite several other studies with similar findings. Overwintering of cyanobacteria cells in freshwater sediments has been documented (Ihle et al., 2005).

Monitoring data from Ecology's Freshwater Algae Control Program suggest that microcystins dissipate fairly quickly from the water column, but the extent to which this is due to sedimentation or other processes is unknown. If deposition is an important pathway, then the sediments could act as a reservoir for uptake by fish or toxic effects to benthic organisms. Recreational exposure is also a potential concern along beaches. In view of these considerations, lake sediments were screened for the presence of microcystins.

PDS-TSU provided a list of lakes with a documented history of significant microcystin concentrations in algae samples collected during blooms. The data were reviewed to select six lakes that exhibited histories ranging from a single modest bloom in 2008 to multiple significant blooms going back to 1999 (Appendix C).

Two composite sediment samples were collected from each lake: one from the deepest part and one from a shallower near-shore area (Table 4). Babica et al. (2006) observed higher microcystin concentrations in deepwater sediments and concluded that the cold, dark conditions were better for overwintering of cyanobacterial cells. Each sediment sample consisted of the top 2cm surface layer so as to reflect recent depositional history.

Gravel or dense aquatic plant growths were encountered in the nearshore areas of Waughop and Spanaway Lakes. The shallow water samples for these sites were therefore collected at depths only slightly less than the corresponding deep water sample.

The sediments were collected during late winter (March). This was done in view of winter-spring maximums reported in studies of microcystins in lake sediments (Babica et al., 2006; Ihle et al., 2005) and to avoid collecting in the midst of active blooms that could contaminate the samples during collection.

Table 4. Sediment Samples Collected in 2011 and Analyzed for Microcystins by LC/MS/MS.

Lake	Collection Date	Depth of Water (feet)
Ketchum	29-Mar	21
		5
Anderson	25-Mar	24
		10
Cassidy	29-Mar	22
		7
Bay	23-Mar	12
		5
Waughop	23-Mar	15
		13
Spanaway	23-Mar	28
		21

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Sampling Methods

Fish

Fish were collected by electroshocking, gill net, or hook and line, following Environmental Assessment Program (EAP) standard operating procedures (SOP; Sandvik, 2006a). Where size limits applied, only legal size fish were taken for analysis. For species with no size limits, only those large enough to reasonably be retained for consumption were taken, to the extent possible.

Fish selected for analysis were killed by a blow to the head. The fish were put in new plastic bags, and placed on ice soon after collection. The fish were transported to Ecology headquarters on ice or frozen if transport was delayed by more than two days.

At Ecology headquarters, each fish was given a unique identifying number and its length and weight recorded. The fish were individually wrapped in aluminum foil, put in plastic bags, and kept frozen pending preparation of tissue samples.

Tissue samples were prepared following the EAP SOP (Sandvik, 2006b). Techniques to minimize potential for sample contamination were used. People preparing the samples wore non-talc nitrile gloves and worked on heavy duty aluminum foil or a polyethylene cutting board. The gloves and foil were changed between samples; the cutting board was cleaned between samples as described below.

The fish were thawed enough to remove the foil wrapper and rinsed with tap water, then deionized water to remove any adhering debris. For 2010, the fish were scaled, except for bullheads where the skin was discarded because it is not eaten. For 2011, all fish fillet samples were skin off in an effort to improve sample homogeneity. For both years, the entire fillet from one or both sides was removed with stainless steel knives and homogenized in a KitchenAid blender. Fish liver samples were homogenized with an IKA-Ultra-Turrax dispersion tool.

All tissues were homogenized to uniform color and consistency. Fillet homogenates were placed in 2 or 4 oz. glass jars that had been cleaned to EPA (1990) QA/QC specifications. The smaller liver and CDFG homogenates were put in small plastic vials. Each homogenate was split into two aliquots, one for CDFG and one for KCEL. A few of the liver samples were too small to split and were analyzed by CDFG only.

Cleaning of resecting instruments, cutting boards, and blender parts was done by washing in tap water with Liquinox detergent, followed by sequential rinses with tap water, de-ionized water, and pesticide-grade acetone. The items were then air dried on aluminum foil in a fume hood before use.

The tissue samples were refrozen for overnight shipment with chain-of-custody record to the analyzing laboratories. The samples were maintained at or near freezing during shipment.

Sediment

Sediment sampling followed the EAP SOP (Blakley, 2008). The samples were collected using a 0.02 m^2 stainless steel Ponar grab. A grab was considered acceptable if not over-filled with sediment, overlying water was not excessively turbid, the sediment surface was relatively flat, and the desired depth penetration had been achieved.

All samples were composites of the top 2 cm layer, consistent with routine practice for sediment studies where recent depositional history is of interest (Ecology, 2008). After siphoning off overlying water, the surface layer from each of three grabs per sampling site was removed with a stainless steel scoop, placed in a stainless steel bowl, and homogenized by stirring. Material touching the side walls of the grab was not taken.

Subsamples of the homogenized sediment were put into 4 oz. glass jars cleaned to EPA (1990) QA/QC specifications and placed on ice immediately upon collection. The samples were returned to Ecology HQ and held frozen until transport with chain-of-custody record to CDFG.

Stainless steel implements used to collect and manipulate the sediments were cleaned by washing with Liquinox detergent, followed by sequential rinses with tap water, deionized water, and pesticide-grade acetone. The equipment was then air dried and wrapped in aluminum foil. Cleaning of the Ponar between sampling sites within each lake consisted of thorough brushing with lake water.

Analysis Methods

Table 5 summarizes the methods used to analyze blue-green algae toxins in this study.

Table 5. Analysis Methods.

Matrix / Analytes	Extraction	Analytical Method	Reference	Laboratory
Fish Tissue				
Microcystins (9), anatoxin-a, domoic acid, okadaic acid	methanol	LC/MS/MS	Mekebri et al. (2009)	CDFG
Microcystins		ELISA	Envirologix* Abraxis† / Wilson et al. (2008)	KCEL
Saxitoxin		ELISA	Bioo Scientific**	KCEL
Sediment				
Microcystins (9), anatoxin-a, domoic acid, okadaic acid	methanol	LC/MS/MS	Mekebri et al. (2009)	CDFG

*KCEL SOP 440V.2: Microcystin Analysis of Waters and Envirologix ® Microcystin High Sensitivity Plat Kit Manual, Version 1-13-03, Envirologix Inc., Portland, ME.

†Anti-ADDA Kit #520011, Abraxis, Warminster, PA

** MaxSignal® Saxitoxin Test Kit #1034-01, Bioo Scientific Corp., Austin, TX.

CDFG: California Dept. of Fish & Game, Water Pollution Control Laboratory

KCEL: King County Environmental Laboratory

CDFG recently validated its LC/MS/MS method for analyzing microcystins in water and tissue (Mekebri et al., 2009). Nine microcystin variants and several other algae toxins are quantified (Table 6). The L, R, Y, A, F, and W designations stand for the microcystin variable amino acids leucine, arginine, tyrosine, alanine, phenylalanine, and tryptophan, respectively. Microcystin-LR, -RR, -YR, and -LA are the most important toxins of human health concern associated with blue-green blooms.

Table 6. Microcystins and Other Toxins Analyzed by the California Department of Fish & Game LC/MS/MS Method.

MC-LR
MC-Desmethyl-LR
MC-RR
MC-Desmethyl-RR
MC-YR
MC-LA
MC-LW
MC-LF
MC-LY
Anatoxin A
Domoic acid
Okadaic acid

The strength of the ELISA response varies with the type of microcystin variant encountered. Table 7 illustrates the relative response (cross-reactivity) of the two ELISA tests for the microcystins analyzed by LC/MS/MS in this study. An ELISA's cross-reactivity pattern coupled with the analytes present will impact whether a measured value under- or over-reports actual concentrations. With more than 80 microcystins, most cross-reactivities are unknown (Loftin et al., 2010).

Table 7. Cross-Reactivity of Envirologix and Abraxis ADDA ELISA for Microcystins.
(from Loftin et al., 2010, except MC-DMLR from Fischer et al., 2001)

Assay	Percent Cross Reactivity							
	LR	LA	LF	RR	DMLR	LW	LY	YR
Envirologix	100	62	?	54	?	?	?	35
Abraxis-ADDA	100	125	108	91	157	114	?	81

To further illustrate with a modified example from Loftin et al. (2010):

ELISA Response = Σ (Cross-Reactivity x Actual Variant Concentration)
Example: Theoretical Concentration = 1 ppb MC-LR + 1 ppb MC-LA = 2 ppb
Abraxis-ADDA ≈ 2.25 ppb
Envirologix ≈ 1.62 ppb

The LC/MS/MS method use in this project has not yet been accredited by Ecology www.ecy.wa.gov/programs/eap/labs/lab-accreditation.html. A waiver for its use was obtained from the Ecology QA Officer.

Data Quality

Quality control samples analyzed for this project included method blanks, spiked blanks, matrix spikes, matrix spike duplicate, surrogate spikes, and laboratory duplicates, as appropriate. Results were within the QC limits established by the analyzing laboratories, except as described below.

The LC/MS/MS and saxitoxin data from this study have been entered into Ecology's Environmental Information Management System (EIM; www.ecy.wa.gov/eim).

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Results and Discussion

Microcystins in Fish Tissues

Ecology's 2010 Samples

ELISA

KCEL initially analyzed the 2010 fish fillet samples by Envirologix ELISA which responds primarily to the MC-LR variant. Recoveries of MC-LR matrix spikes[1] were inconsistent and sometimes low in these samples (21-34%). A subset of seven samples was then analyzed by variant independent ADDA ELISA where more acceptable recoveries were achieved (65% on average). The results are compared with Envirologix ELISA (Table 8).

Table 8. ELISA Results for Microcystins in Selected Fish Fillet Samples from 2010 (ug/Kg, wet weight; parts per billion).

Site/Lake	Species	Date	Abraxis ADDA	Envirologix
Arlington hatchery	Rainbow trout	19-Apr	7.0	1.0
Cassidy	Pumpkinseed	26-Aug	8.7	0.31
	Yellow perch	26-Aug	7.7	0.76
		21-Sep	8.0	0.47
Pattison	Largemouth bass	8-Sep	8.2	0.25
Anderson	Rainbow trout	27-Aug	6.3	2.2
	Rainbow trout (dup.)		11	2.4
	Rainbow trout	28-Aug	9.6	0.38

Microcystins appeared to be present in all samples. Concentrations measured with ADDA ELISA – 6.3 to 11 ug/Kg – were higher than Envirologix ELISA by about an order of magnitude. There was no apparent correlation between the two tests. Because of different cross-reactivity patterns (see Table 7), close agreement between Envirologix and ADDA ELISA would only be expected when analyzing purified MC-LR. Results of a duplicate analysis conducted on one of the Anderson Lake samples differed by almost a factor of two for ADDA ELISA, indicating that the precision of these data may be poor (Table 8).

The narrow range of results achieved with ADDA ELISA suggests it was responding primarily to other constituents in the tissues than microcystins. Although significant microcystin blooms had been reported in both Cassidy and Pattison Lakes prior to the fish collections, similar or higher concentrations were obtained for fish samples from the WDFW Arlington hatchery and

[1] The term "matrix spikes" refers to known amounts of target chemicals spiked into samples prior to extraction and analysis. Recovery of matrix spikes provides an estimate of positive or negative bias in the data.

Anderson Lake. Water for this hatchery comes from a series of natural springs, an unlikely source of microcystins. The Anderson Lake samples were dead rainbow trout obtained during a WDFW fish kill investigation. Although Anderson has a history of significant blue-green activity, there had been no evidence of a bloom prior to the kill.

Results potentially more indicative of microcystin exposure were obtained using ADDA ELISA to analyze various tissues from yellow perch collected at Ketchum Lake following blue-green blooms in August (Table 9). Microcystin levels in liver and gut were much higher than either muscle or skin. The pattern liver > gut > muscle/skin generally fits expectations of how bioaccumulative chemicals would be taken up by fish following ingestion.

Table 9. ADDA ELISA Results for Microcystins in Yellow Perch Collected from Ketchum Lake in 2010 (ug/Kg, wet weight; parts per billion).

Collection Date	Size (weight / length)	Tissue	Microcystins
24-Aug	103 g / 200 mm	Muscle	5.1
		Skin	7.6
		Gut + contents	87
		Liver	109
26-Aug	112 g / 205 mm	Muscle	2.9
		Gut + contents	68
		Liver	56

LC/MS/MS

Twenty of the fish fillet samples from 2010 were analyzed by LC/MS/MS for nine specific microcystin variants. None of these microcystins was detected at or above 0.5 ug/Kg. This suggests that the response seen in the ADDA ELISA test was due to other microcystins or microcystin-like compounds. The three other blue-green toxins screened with the LC/MS/MS method – anatoxin-a, domoic acid, and okadaic acid – were also not detected. Detection and reporting limits[2] for this analysis are shown in Table 10.

[2] Detection limit is the lowest concentration that can be detected by an instrument with correction for the effects of sample matrix and method-specific parameters such as sample preparation. Reporting limit is the lowest concentration that can be reliably achieved within specified limits of precision and accuracy during routine laboratory operating conditions.

Table 10. Detection and Reporting Limits for 2010 Fish Samples Analyzed by LC/MS/MS.

Compound	Detection Limit	Reporting Limit
MC-RR	0.50	1.0
MC-Desmethyl-RR	0.50	1.0
MC-LR	0.50	1.0
MC-Desmethyl-LR	0.50	1.0
MC-YR	0.50	1.0
MC-LA	0.50	1.0
MC-LW	0.50	1.0
MC-LF	0.50	1.0
MC-LY	0.50	1.0
Anatoxin A	5.0	10
Domoic acid	2.0	5.0
Okadaic acid	1.0	2.0

Ecology's 2011 Samples

Four types of fish samples were analyzed by ADDA ELISA and LC/MS/MS in 2011:

- Fillet and liver samples from fish collected in western Washington lakes with blue-green blooms.
- Archived fillet samples obtained through CDFG which were known to test positive for specific microcystins.
- A fish fillet sample from a pristine Idaho river assumed to be free of microcystins.

Results are summarized in Table 11. Three samples shown as being analyzed in duplicate agreed within 13% to 56% for ELISA and 2% for LC/MS/MS.

Based on ELISA, microcystin levels appeared to be distinctly different among the Washington and California samples (Figure 3). Concentrations ranged from 2.0 to 5.9 ug/Kg in Washington fillets, 5.5 to 35 ug/Kg in California fillets, and 35 to 132 ug/Kg in Washington livers. The Idaho cutthroat fillet, initially assumed to represent background, gave an ELISA response of 12 ug/Kg, roughly midway between the Washington and California fillet samples.

MC-LA was detected in the LC/MS/MS analysis of these samples, but only in fish livers (Table 11). Detected concentrations ranged from 2.5 to 14 ug/Kg. Detection and reporting limits for the other microcystin variants, anatoxin-a, domoic acid, and okadaic acid were the same as achieved the previous year (see Table 10).

Table 11. ADDA ELISA and LC/MS/MS Results for Microcystins in Fish Tissue Samples Analyzed for 2011 (ug/Kg, wet weight; parts per billion).

Lake	Species	Date	ADDA ELISA	LC/MS/MS (MC-LA)
Fillet				
Washington Samples				
Ketchum Lake	Rainbow trout	2-Jul-11	3.5	<1.0
Waughop Lake	Rainbow trout	3-Aug-11	5.9	<1.0
	Largemouth bass	3-Aug-11	2.0	<1.0
		14-Aug-11	2.4	<1.0
Cassidy Lake	Largemouth bass	23-Sep-11	2.9	<1.0
	Yellow perch		3.2	<1.0
California Samples				
Copco Reservoir	Yellow perch #23	7-Sep-07	23	<1.0
	Yellow perch #29		9.8	<1.0
	Yellow perch #29 (dup.)		5.5	<1.0
Iron Gate Reservoir	Yellow perch #17	6-Sep-07	22	<1.0
	Yellow perch #17 (dup.)		18	<1.0
	Yellow perch #18		35	<1.0
Idaho Sample				
Upper St. Joe River	Cutthroat trout	28-Sep-10	12	<1.0
Liver				
Washington Samples				
Ketchum Lake	Rainbow trout	2-Jul-11	na	<1.0
Waughop Lake	Rainbow trout	3-Aug-11	132	<1.0
	Largemouth bass	3-Aug-11	90	12
		14-Aug-11	64	9.2
	Largemouth bass (dup.)		56	9.4
Cassidy Lake	Largemouth bass	23-Sep-11	35	2.5
	Yellow perch		na	14

na: not analyzed

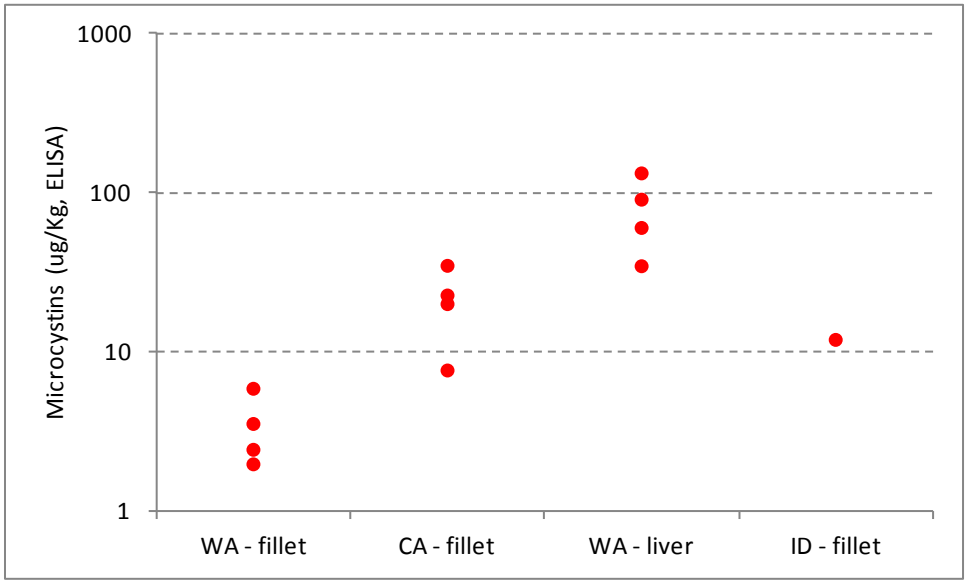

Figure 3. Microcystin Levels in Fish Tissue Samples Analyzed by ADDA ELISA in 2011 (log scale; duplicates averaged).

There was evidence of a correlation between the ELISA response and MC-LA concentrations in largemouth bass liver (Figure 4). Again, the much higher levels seen by ELISA suggest the presence of other microcystins or microcystin-like compounds not analyzed by LC/MS/MS. For unknown reasons, the only other fish liver sample analyzed by both methods (rainbow trout) had widely disparate results.

CDFG had originally identified significant concentrations of desmethyl-LR (80 – 422 ug/Kg) in the California perch fillets when these samples were first analyzed back in 2007. In view of the lack of detections when re-analyzed, CDFG reviewed their LC/MS/MS output from 2007. A standard had not been available for desmethyl-LR at that time, and it had been quantified based on MC-LR, the parent compound. A standard has since been obtained, allowing CDFG to determine that desmethyl-LR had been misidentified and the peak was due to an unknown microcystin.

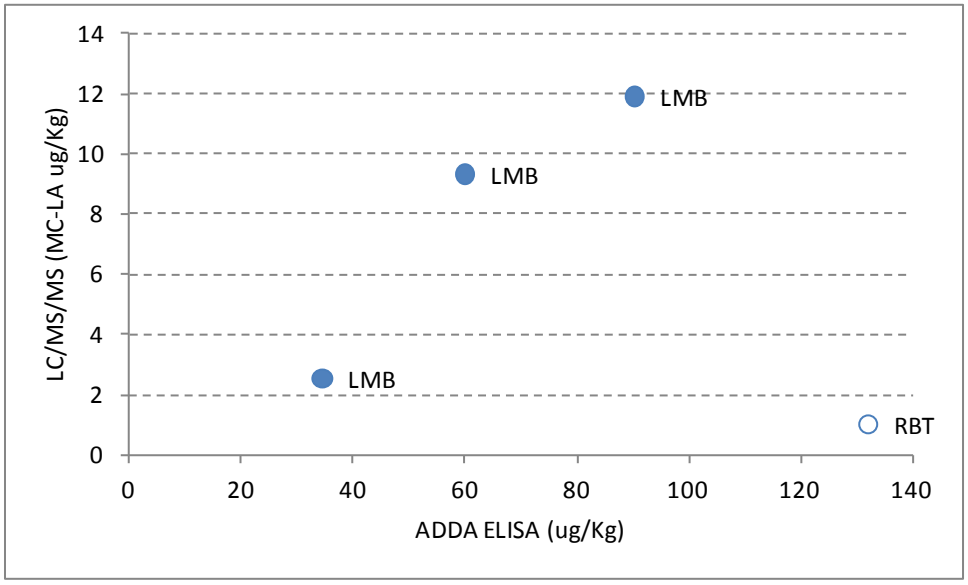

Figure 4. Comparison of ADDA ELISA and LC/MS/MS Results on Fish Liver Samples from Waughop and Cassidy Lakes, 2011.

LMB: largemouth bass, RBT: rainbow trout; duplicates averaged; LC/MS/MS results are for MC-LA.

Other Fish Tissue Studies

A number of studies have now evaluated microcystin accumulation by fish. Most of these efforts have employed HPLC, ADDA ELISA, LC/MS, or, more recently, LC/MS/MS. HPLC is subject to false positives (Ohio EPA, 2010) and LC/MS has been shown to overestimate microcystin concentrations (Kohoutek et al., 2010; Berry et al., 2011).

The findings on Ecology's 2008 and 2010-11 samples were compared with other recent investigations where ADDA ELISA has been used to analyze freshwater fish potentially affected by blue-green algae blooms (Table 12). All of these studies from other areas of the world have found similar levels in fish muscle, with average concentrations confined to the relatively narrow range of 5.0 to 14 ug/Kg. Higher levels are consistently measured in liver, averaging between 50 and 200 ug/Kg.

ELISA does not differentiate among microcystins present in a sample and a positive response does not necessarily indicate the presence of variants currently viewed as a human health concern during blue-green algae blooms. ADDA ELISA is also known to respond to an ADDA-like molecule in fish that is unrelated to microcystins (Boyer, 2012; Caulfield, 2012). As a result, microcystins are overestimated using this test because results are never zero on a fish tissue sample regardless of its source. LC/MS/MS is now regarded as the method of choice for analyzing microcystins in environmental samples due to improved specificity and sensitivity (Lawton and Edwards, 2005; Mekebri et al., 2009; Kohoutek et al., 2011).

Table 12. Summary of Results from Recent Investigations where Fish Tissue was Analyzed for Microcystins by ADDA ELISA (mean concentrations).

Waterbody	Species	Tissue	N =	Microcystins (ug/Kg, wet)	Reference
Western Wash. lakes (5)	4 species	Muscle	14	5.6	present study
		Liver	6	50	
Western Wash. lakes (6)	6 species	Muscle	20	14	Johnson (2010a)
		Liver	11	64	
Lago de Patzcuaro (Mexico)	Carp	Muscle	?	5.0	Berry et al. (2011)
		Liver	?	94	
Lake Albufera (Spain)	Mullet	Muscle	103	5.0	Romo et al. (2012)
		Liver	103	200	
Greek lakes (13)	Carp	Muscle	130	7.1	Papadimitriou et al. (2010)
		Liver	130	124	
Lake Ontario and Lake Erie	17 species	Muscle	57	7.8	Poste et al. (2011)

Gies-Asteggiante et al. (2011) recently compared ADDA ELISA with LC/MS/MS for analyzing microcystins spiked into fish tissue samples. They concluded that ELISA was able to correctly assess the presence or absence of MC-LR, -LA, -YR, and -RR when concentrations were greater than 10 ug/Kg, but that it had shortcomings for quantitative purposes. Gies-Asteggiante et al. suggested ADDA ELISA could be used as an initial screening tool for fish followed by confirmation of presumed positive samples by LC/MS/MS or, conversely, as confirmation for LC/MS/MS.

Kohoutek et al. (2010) used LC/MS/MS to conduct an extensive study of microcystins in 148 edible fish tissue samples from five European reservoirs with dense cyanobacterial blooms. All samples were below the limits of detection (1.2 ug/Kg for -RR and 5.4 ug/Kg for -YR and -LR). Other recent LC/MS/MS studies that have found little or no accumulation of important microcystin variants in fish muscle include CH2M Hill (2009), DeBlois et al. (2009), Prendergast and Foster (2010), Bruno et al. (2012), and Ohio EPA (2012). These finding are in line with LC/MS/MS results from the present study where target microcystins were not detected (<1.0 ug/Kg) in approximately 30 fish fillet samples collected in association with blue-green blooms in Washington lakes.

Both ELISA and LC/MS/MS underestimate the total microcystin content in fish tissues because of their inability to extract enzyme-bound toxin (Berry et al., 2011). Studies cited in Berry et al. suggest the total concentration could be an order of magnitude higher. While it has been assumed that bound microcystins are relatively benign, there is recent evidence they may become bioavailable in the digestive system of fish consumers (Smith et al., 2010). The extent of underestimation and its relevance to human health remain to be determined in future studies.

Microcystins in Sediments

Ecology's 2011 Samples

Sediment samples were collected in March 2011 from six western Washington lakes with a history of blue-green algae blooms. The lakes included Waughop, Ketchum, Cassidy, and Andersen, all of which were sample for fish in 2010/11. Sediment samples were also collected from Bay and Spanaway Lakes, previously unsampled. The known bloom history of these lakes (Appendix C) ranged from a single, relatively modest bloom in 2008 (Bay Lake) to chronic, highly toxic blooms dating back to at least 1999 (Waughop Lake).

Two composite sediment samples were collected at each lake, one in deep water and one in shallower water. Gravel or dense aquatic plant growths in Waughop and Spanaway made it necessary to collect the "shallow" sample in somewhat deeper water than originally intended.

The sediment samples were analyzed by LC/MS/MS (Table 13). Of the nine microcystins analyzed, only MC-LR and MC-LA were detected. Both compounds were detected in all lakes except for Spanaway where only MC-LA was found. Concentrations ranged from 2.9 to 52 ug/Kg, with the highest levels in Ketchum and Waughop Lakes. Duplicate analyses conducted on two of the sediment samples agreed within 24% or better, providing an estimate of the variability associated with these data.

Table 13. Microcystins Detected in Sediment Samples Collected in 2011 and Analyzed by LC/MS/MS (ug/Kg, dry weight; parts per billion).

Lake	Date	Depth (feet)	MC-LR	MC-LA
Ketchum	29-Mar	21	**37**	< 0.5
		21 (dup.)	**47**	< 0.5
		5	< 0.5	< 0.5
Anderson	25-Mar	24	**30**	**11**
		10	**2.9**	< 0.5
Cassidy	29-Mar	22	**15**	**31**
		7	< 0.5	**28**
Bay	23-Mar	12	< 0.5	**52**
		5	**3.0**	**4.6**
Waughop	23-Mar	15	**10**	**18**
		13	**6.0**	**15**
Spanaway	23-Mar	28	< 0.5	**11**
		28 (dup.)	< 0.5	**11**
		21	< 0.5	< 0.5

Sediment samples from deeper parts of the lakes had consistently higher levels of the sum of MC-LR and MC-LA than those taken in shallower water (Figure 5). The difference between deep vs. shallow water was particularly marked for Ketchum, Anderson, Cassidy (-LR only), and Bay Lakes. This finding is consistent with Babica et al. (2006) and may be due to the colder temperatures and lower light levels at depth. Organic content of the samples could also play a role but was not analyzed.

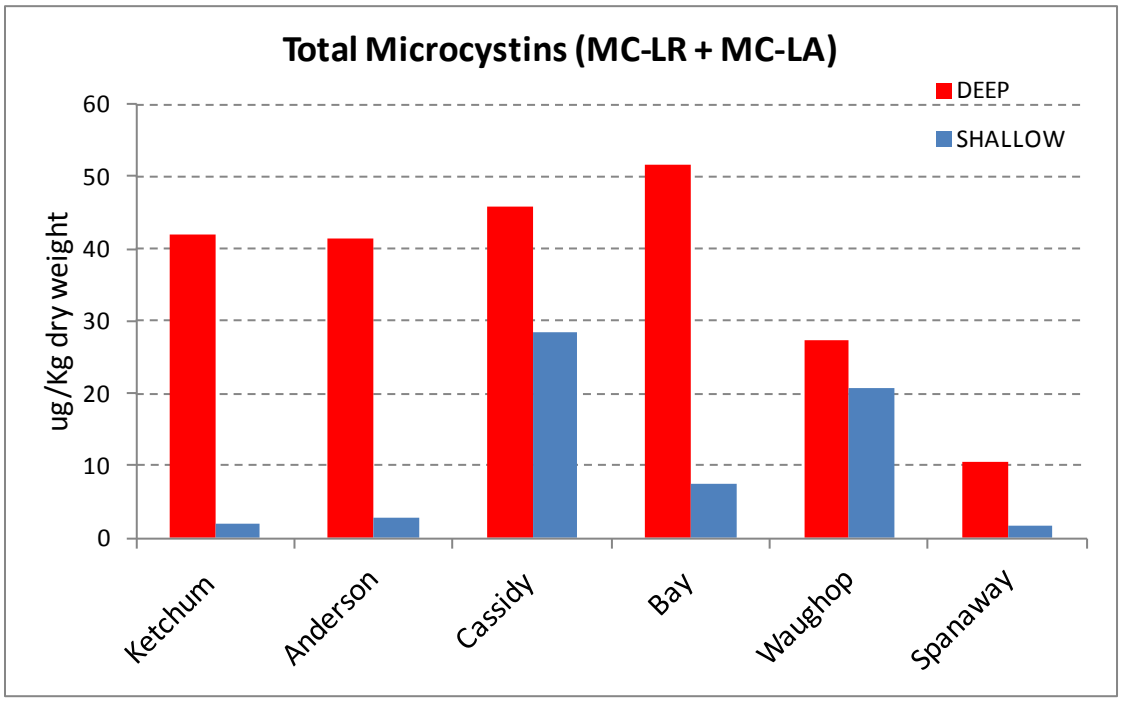

Figure 5. Microcystin Levels in Lake Sediment Samples Collected in 2011: Deep vs. Shallow Water.

MC-LA was present in much higher concentrations than MC-LR in all lakes except Ketchum and Anderson (Figure 6). Fish liver samples were analyzed from three of these lakes: Ketchum, Cassidy, and Waughop (see Table 11). Liver concentrations were consistent with sediment concentrations, to the extent that where MC-LA was detected in fish livers (Cassidy and Waughop), it also appeared to predominate in sediment. Conversely, where MC-LA was not detected in livers (Ketchum) it was also not detected in the sediments.

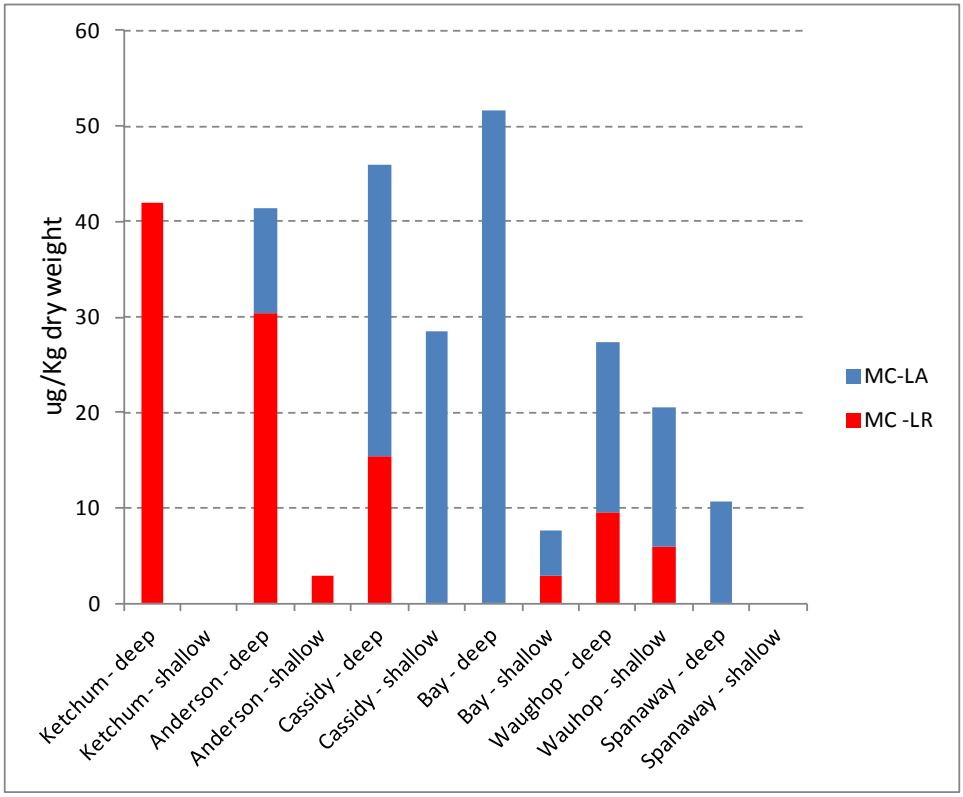

Figure 6. Microcystin Levels in Lake Sediment Samples Collected in 2011: MC-LR vs. MC-LA.

Microcystin levels in the sediments showed no obvious relationship with the history of blue-green algae blooms (Appendix C). For example, the highest MC-LA concentration was found in Bay Lake which had only a single known modest bloom in 2008. Although Waughop had experienced the most frequent and severe blooms among lakes sampled (and for western Washington in general), MC-LR concentrations were similar to or lower than less severely affected lakes.

Two factors could be contributing to the apparent lack of correlation between sediment levels and bloom history of these lakes. First, Ecology's monitoring data for microcystin-producing blue-green algae are obtained using Envirologix ELISA which is more sensitive to - LR than - LA (see Table 8). Second, because the algae samples for the monitoring program are generally collected from surface scums that accumulate along the shoreline, the concentrations reported may not be representative of the lake as a whole. At present, the presence, seasonal variation, and geographical distribution of microcystin variants are not known for western Washington lakes.

Detection and reporting limits for the other blue-green toxins analyzed but not detected in the sediment samples are shown (Table 14). Anatoxin-a has been a public health concern in some of these lakes, Anderson in particular (Hamel, 2009). It was not detected in any of the sediment samples at or above 2.5 ug/Kg.

Table 14. Detection and Reporting Limits for Microcystins and Other Toxins Not Found in Sediment Samples.

Compound	Detection Limit (ug/Kg)	Reporting Limit (ug/Kg)
MC-RR	0.25	0.50
MC-Desmethyl-RR	0.25	0.50
MC-Desmethyl-LR	0.25	0.50
MC-YR	0.25	0.50
MC-LW	0.25	0.50
MC-LF	0.25	0.50
MC-LY	0.25	0.50
Anatoxin A	2.5	5.0
Domoic acid	1.0	2.5
Okadaic acid	0.50	1.0

Other Sediment Studies

A number of investigations have demonstrated that sedimentation is an important fate process for microcystin-producing blue-green algae (Preston et al., 1980; Ihle et al., 2005; Verspagen et al., 2005; Babica et al., 2006; Welker et al., 2007; Mohamed et al., 2007: Chena et al., 2008; Wormer et al., 2011). However, only one study was located, Efting et al. (2011), that used LC/MS/MS to quantify specific microcystin compounds in lake bottom sediments.

Efting et al. analyzed MC-LR, -LA, -RR, and -LW in sediment cores from three Nebraska lakes. MC-LR was detected in all three lakes at concentrations ranging from 1 to 230 ug/Kg, with higher concentrations closer to the surface. MC-LR was found in core sections estimated to have been laid down as early as 1832. A dramatic increase in concentrations was observed in one lake during the 1980s, assumed to be human- induced.

MC-LA detections in the cores were “negligible” (Efting, 2012), which contrasts with findings from the present study. MC-RR and -LW were not found.

Saxitoxin Analysis of Fish Tissue

Waughop Lake in Tacoma, Pierce County, is the only lake in Washington producing saxitoxin at levels of potential human health concern. Saxitoxin was first reported in algae samples by Hamel (2009). Five other Washington lakes have produced saxitoxin levels just above the detection limit during the past four years. Saxitoxin was tentatively identified at low levels (< 1 ug/Kg) in fish samples collected from Waughop in 2008 and analyzed by ELISA (Johnson, 2010a).

Saxitoxin was analyzed in selected fish fillet samples collected from Waughop and Ketchum Lakes in 2010. An ELISA kit that specifically recognizes saxitoxin, as well as other PSP toxins to a lesser degree, was used. No saxitoxin was detected at or above approximately 1.0 to 2.0 ug/Kg (Table 15).

Table 15. Results of Saxitoxin Analysis in Selected Fish Fillet Samples from 2010 (ug/Kg, wet weight; parts per billion).

Lake	Species	Date	Saxitoxin
Waughop	Rainbow trout	27-May	< 1.2
			< 1.2 (dup)
			< 2.0
Ketchum	Rainbow trout	24-Aug	< 1.2

Summary and Conclusions

Microcystins and four other blue-green algae toxins were analyzed in fish and sediment samples collected from nine Washington lakes subject to algae blooms. Two ELISA methods were used to screen fish tissues for microcystin content. An LC/MS/MS method was employed to analyze fish and sediment for nine specific microcystins, anatoxin-a, domoic acid, and okadaic acid. Saxitoxin was tested in a few fish samples using a separate ELISA method.

Microcystins particularly affect the liver. Anatoxin-a is a neurotoxin. Saxitoxin is a neurotoxin primarily associated with paralytic shellfish poisoning in marine waters. Domoic acid and okadaic acid are also shellfish toxins, included in this study by virtue of being on the analyte list for LC/MS/MS.

Envirologix ELISA responds primarily to the MC-LR variant and therefore may underestimate total microcystin content in fish tissues. The ADDA ELISA test showed microcystins or microcystin-like compounds were elevated in fish liver relative to muscle and other tissues.

MC-LA was the only variant specifically detected in fish among the nine microcystins analyzed by LC/MS/MS. The detections – 2.5 to 12 ug/Kg (parts per billion) – were in four fish liver samples. Fish fillets showed no accumulation of the microcystins targeted in the analysis. This finding is consistent with similar, recent studies done elsewhere. The LC/MS/MS limit of detection for microcystins in livers and fillets was 1.0 ug/Kg.

MC-LA and MC-LR residues were found in lake sediment samples. These compounds may exhibit long-term persistence. Deep water sediments consistently had higher concentrations than shallow water sediments, possibly due to the colder temperatures and lower light levels at depth. MC-LA predominated over -LR in four of the six lakes sampled. The maximum concentrations recorded were 52 ug/Kg for -LA and 42 ug/Kg for -LR.

ELISA does not differentiate among microcystins present in a sample and a positive response does not necessarily indicate the presence of variants currently viewed as a human health concern. ADDA ELISA appears to be a useful tool for screening fish tissue samples but only when concentrations are elevated. In the present study, it was able to differentiate between low, moderate, and higher levels of microcystins or microcystin-like compounds in fish tissue. There was some limited evidence of a correspondence between ADDA ELISA and MC-LA concentrations in fish liver.

Anatoxin-a, domoic acid, and okadaic acid were not detected. LC/MS/MS detection limits for fish tissue were 5.0, 2.0 and 1.0 ug/Kg, respectively. Detection limits in sediment were 2.5, 1.0, and 0.5 ug/Kg.

Saxitoxin was tested in fish fillet samples from two lakes, one of which – Waughop – is known to be a saxitoxin producer. Saxitoxin was not detected at or above approximately 1.0 to 2.0 ug/Kg.

Recommendations

- LC/MS/MS is the recommended method for future investigations of microcystins in fish and sediment. ADDA ELISA may be useful for ranking these types of samples for microcystin content but only when elevated concentrations are present.
- Additional investigation is warranted into the potential for significant microcystin bioaccumulation concentrations occurring in fish organs, whole fish, or in marine shellfish potentially affected by lake discharges.
- Microcystin uptake by fish muscle appears to result in relatively low tissue concentrations. Confidence in applying this conclusion to other Washington lakes could be improved with a better understanding of levels and types of microcystin variants in the water, sediments, and food items that fish are exposed to prior to sampling and analysis.
- Research is needed to determine which microcystins or microcystin-like compounds in fish tissue are giving a positive response in the ADDA ELISA test and the extent to which they are toxic.
- The potential for microcystins in lake sediments exerting toxic effects on fish or invertebrates should be assessed.
- If future fish tissue studies use saxitoxin ELISA, a subset of samples should be analyzed by more traditional chemistry such as HPLC for better specificity.

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Appendices

Appendix A. Microcystin Levels Reported in Lakes Prior to Fish Collections in 2010 and 2011

Tables A-1 and A-2 highlight monitoring data from Ecology's Freshwater Algae Control Program that were the trigger for fish sampling in the present study. (https://fortress.wa.gov/ecy/toxicalgae/InternetDefault.aspx)

A water quality guideline of 6 ug/L (parts per billion) is used in Washington to assess the risk from recreational exposure to microcystins from blue-green algae exposure (Hardy, 2008). The local health authority determines what actions, if any, are taken to protect human health from algal toxins. Actions range from no action, recreational advisories, recreational closures, to closure of the entire water body for all activities. Ecology posts the actions taken by the local health authority on its on-line database.

Washington lakes had a relatively low level of algal bloom activity in 2010, partly due to unseasonably cool weather. As a result, the microcystin levels associated with fish sampling were relatively low compared to previous years.

Table A-1. Microcystin Levels that Triggered Fish Sampling in 2010.

Lake	Microcystin Levels in Algae Samples Prior To or Soon After Fish Sampling (highest concentrations reported)				Date of Fish Samples (2010)	Rationale for Fish Sampling
	Date (2010)	Conc. (ug/L)	Date (2010)	Conc. (ug/L)		
Waughop	26-Apr	1,330	6-May	422	27-May	significant recent bloom
Ketchum	7-Jul	25	- -	- -	19-Jul	recreational guideline exceeded
	4-Aug	8.4	- -	- -	12-Aug	recreational guideline exceeded
					24-Aug	recreational guideline exceeded
Cassidy	30-Aug	16	13-Sep	732	26-Aug	recreational guideline exceeded
Anderson	24-Aug	<0.05	- -	- -	27-28-Aug	WDFW request, fish kill investigation
Pattison	30-Aug	15- 54	14-Sep	8 - 37	8-Sep	recreational guideline exceeded
Cassidy	13-Sep	732	- -	- -	21-Sep	significant recent bloom
Black	27-Sep	669	12-Oct	167	18-Oct	significant recent bloom
Lower Long	23-Sep-09	18,700	- -	- -	26-Oct	extreme bloom 2009

Recreational guideline: 6 ug/L
- -: no samples or < 6 ug/L

The bloom alerts that trigger fish sampling in 2011 are shown in Table A-2. 2011 also proved to be a period of relatively low blue-green algae activity. In spite of this, a sample set was obtained that represented a fairly wide range of microcystin levels in lakes. This included a highly significant bloom in Waughop Lake during July and August.

Table A-2. Microcystin Levels that Triggered Fish Sampling in 2011.

Lake	Microcystin Levels in Algae Samples Prior to or Soon after Fish Sampling (highest concentrations reported)				Date of Fish Samples (2011)	Rationale for Fish Sampling
	Date (2011)	Conc. (ug/L)	Date (2011)	Conc. (ug/L)		
Ketchum	June	10 - 45	5-Jul	50	1-2 July	recreational guideline exceeded
Waughop	26-Jul	3,610	4-Aug	7,082	3-Aug	significant ongoing bloom
Waughop	4-Aug	7,082	17-Aug	3 - 25	14-Aug	significant recent bloom
Cassidy	6-Sep	432	15-Sep	312	23-Sep	significant ongoing bloom

Recreational guideline: 6 ug/L

Appendix B. Length and Weight Data for Fish Samples

Table B-1. Fish Analyzed for Microcystins and Other Blue-Green Toxins for 2010.

Sample No.	Lake/ Site	Species	Date (2010)	Total Length (mm)	Weight (gm)
1	McGovern Creek Hatchery	Rainbow Trout	19-Apr	163	43
				205	100
				210	106
				166	49
				187	67
2	Waughop	Rainbow Trout	27-May	353	555
3				325	468
4	Ketchum	Pumpkinseed	19-Jul	125	41
				118	31
				117	34
				116	32
				115	30
				115	31
				113	29
				112	27
5	Ketchum	Yellow Perch	19-Jul	251	196
				240	180
				225	151
				221	122
				209	115
6	Ketchum	Yellow Perch	12-Aug	270	268
				210	134
				202	104
				196	99
				192	85
7	Ketchum	Pumpkinseed	12-Aug	125	40
				119	37
				113	30
				108	31
				108	29
				106	26
				105	35
				103	24
				103	26

Sample No.	Lake/ Site	Species	Date (2010)	Total Length (mm)	Weight (gm)
8	Ketchum	Rainbow Trout	24-Aug	272	172
				240	109
				227	110
9	Cassidy	Largemouth Bass	26-Aug	427	1426
				386	899
				377	883
				368	642
				365	732
11	Cassidy	Pumpkinseed	26-Aug	170	131
				165	121
				160	103
				158	113
				158	104
12	Cassidy	Yellow Perch	26-Aug	217	127
				186	79
				171	68
				164	61
				161	51
13	Anderson	Rainbow Trout	27-Aug	135-166	28-54
14	Anderson	Rainbow Trout	28-Aug	480	1136
				466	1064
				443	881
15	Pattison	Largemouth Bass	8-Sep	201	117
				185	85
				179	72
				178	86
				176	75
16	Pattison	Brown Bullhead	8-Sep	158	62
				160	56
17	Pattison	Rock Bass	8-Sep	221	206
				220	196
				205	152
18	Cassidy	Largemouth Bass	21-Sep	325	492
				315	399
				301	356
				290	313
				286	321

Sample No.	Lake/ Site	Species	Date (2010)	Total Length (mm)	Weight (gm)
19	Cassidy	Yellow Perch	21-Sep	198	88
				180	73
				180	64
				176	63
				175	63
20	Cassidy	Pumpkinseed	21-Sep	134	49
				132	54
				143	63
				151	75
				164	93
21	Black	Largemouth Bass	18-Oct	220	148
				262	273
				248	213
				236	185
				256	285
22	Black	Cutthroat Trout	18-Oct	290	253
				400	550
				282	238
				282	238
				327	359
23	Black	Brown Bullhead	18-Oct	238	199
				281	229
				270	259
24	Black	Largescale Sucker	18-Oct	not recorded	
27	Ketchum	Rainbow Trout	13-Aug	221	94
			21-Aug	210	85
28	Ketchum	Brown Bullhead	19-Jul	211	104
				152	50
29	Pooled Waughop rainbow trout samples #2 and #3				
25	Lower Long	Largescale Sucker	26-Oct	514	1564
				515	1488
				509	1642
				503	1558
				465	1173
				446	934
				459	999
				494	1285
				440	988

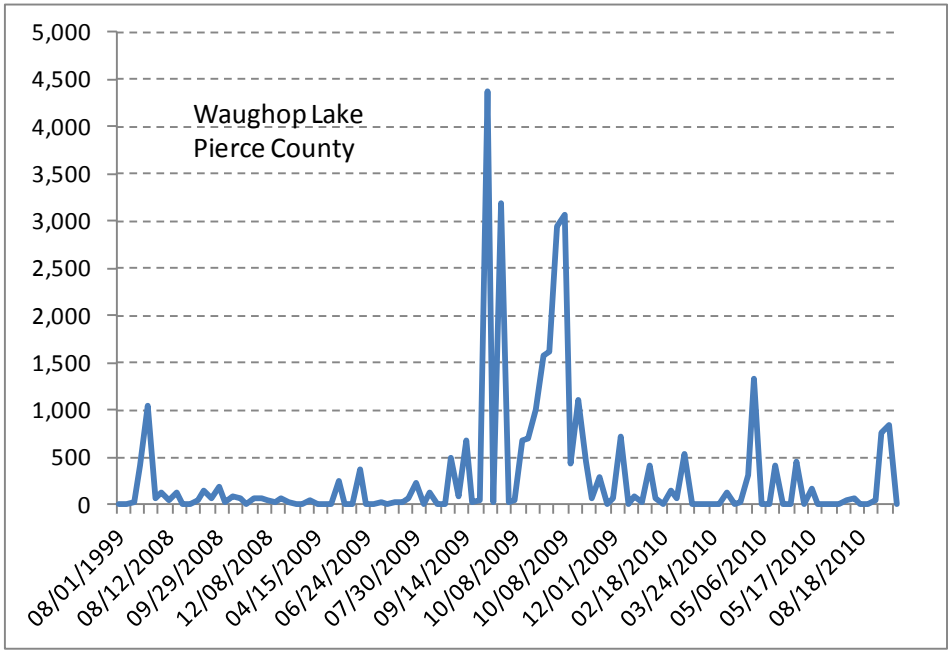
Sample No.	Lake/ Site	Species	Date (2010)	Total Length (mm)	Weight (gm)
26	Lower Long	Lake Whitefish	26-Oct	259	173
				273	185
				375	490
30	Lower Long	Northern Pikeminnow	26-Oct	403	575
				436	695
				392	512
				400	585
				395	498

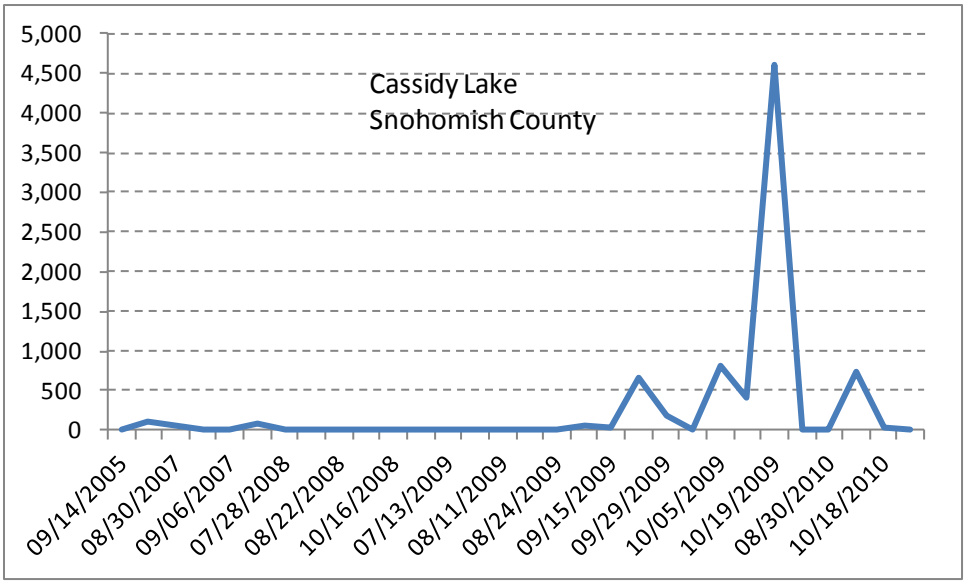
Table B-2. Fish Analyzed for Microcystins and Other Blue-Green Toxins for 2011.

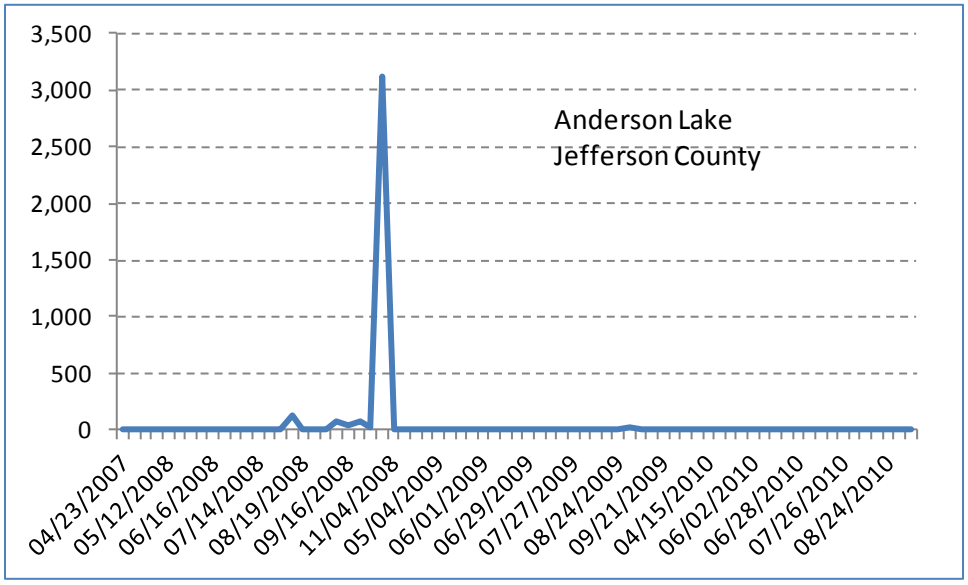
Lake/ Location	Date	Species	Length	Weight	Sample No.	
					Fillet	Liver
Ketchum	1-2 July-11	Rainbow Trout	238	144	11	12
			262	184		
			270	198		
			247	156		
			245	149		
Waughop	3-Aug-11	Rainbow Trout	577	1,787	1	6
		Largemouth Bass	375	972	2	7
			383	1,211		
Waughop	14-Aug-11	Largemouth Bass	298	476	3	8
			300	531		
Cassidy	23-Sep-11	Largemouth Bass	240	162	4	9
			251	216		
			246	220		
			333	597		
			295	307		
Cassidy	23-Sep-11	Yellow Perch	205	117	5	10
			212	114		
			201	99		
			197	91		
			190	84		
COPCO Reservoir (lower)	2007	Yellow Perch	- -	- -	13	- -
					14	
Iron Gate Reservoir (upper)	2007	Yellow Perch	- -	- -	15	- -
					16	
Upper St. Joe River	28-Sep-10	Cutthroat Trout	220	106	17	- -
			204	68		

Appendix C. History of Microcystin Blooms (Algae Samples) in Lakes Where Sediment Samples Were Collected in March 2011

(microcystin concentrations in ug/L; parts per billion)



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11/04/2008
05/04/2009
06/01/2009
06/29/2009
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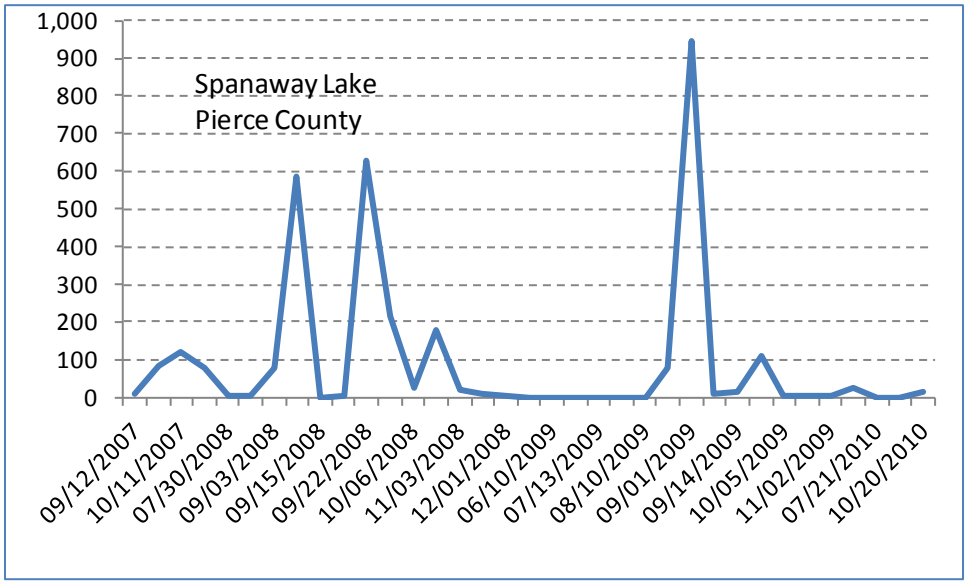
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800
700
600
500
400
300
200
100
0
Spanaway Lake
Pierce County
09/12/2007
10/11/2007
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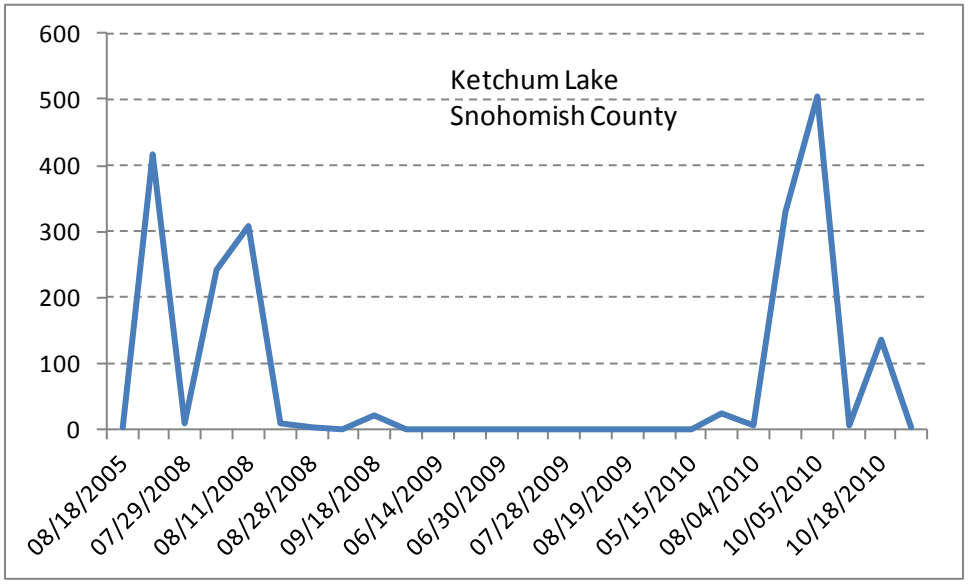
09/03/2008
09/15/2008
09/22/2008
10/06/2008
11/03/2008
12/01/2008
06/10/2009
07/13/2009
08/10/2009
09/01/2009
09/14/2009
10/05/2009
11/02/2009
07/21/2010
10/20/2010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200
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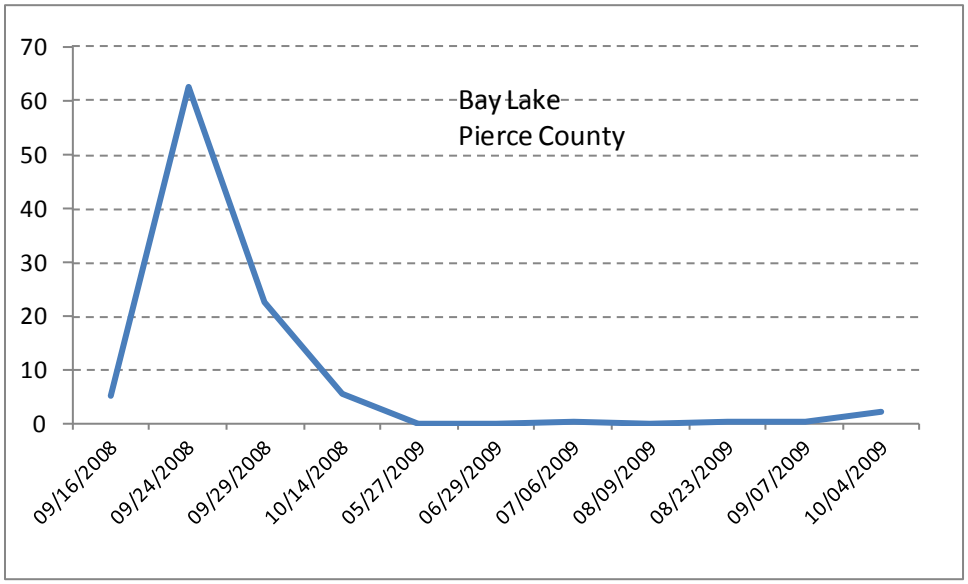
10/05/2010
10/18/2010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60
50
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30
20
10
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09/16/2008
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09/29/2008
10/14/2008
05/27/2009
06/29/2009
07/06/2009
08/09/2009
08/23/2009
09/07/2009
10/04/2009

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Appendix C. Glossary, Acronyms, and Abbreviations

Glossary

Anatoxin-a: A neurotoxin produced by members of the blue-green algae genera *Anabaena*, *Planktothrix*, and *Aphanizomenon*.

Cyanobacteria: Blue-green algae.

Domoic acid: A marine toxin produced by the diatom (algae) *Pseudo-nitzschia* that causes Amnesic Shellfish Poisoning.

Microcystins: A liver toxin produced by the blue-green algae species *Microcystis*, *Anabaena*, and others.

Okadaic acid: A marine toxin produced by the dinoflagellate (algae) *Dinophysis*.

Saxitoxin: A neurotoxin produced by members of the blue-green algae genera *Anabaena*, *Aphanizomenon*, *Lyngbya*, and *Cylindrospermopsis* and causing Diarrhetic Shellfish Poisoning.

Acronyms and Abbreviations

ADDA	3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid
CDFG	California Department of Fish & Game
EAP	Environmental Assessment Program
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management system
ELISA	enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
HPLC	high pressure liquid chromatography
KCEL	King County Environmental Laboratory
LC/MS	liquid chromatography/mass spectrometry
LC/MS/MS	liquid chromatography/mass spectrometry/mass spectrometry
MC	microcystin
PDS-TSU	Program Development Services Section, Technical Services Unit
PSP	paralytic shellfish poisoning
QA/QC	quality assurance/quality control
RPD	relative percent difference
SOP	standard operating procedures
WDFW	Washington Department of Fish & Wildlife
WDOH	Washington Department of Health

g	gram
mm	millimeter
ug/Kg	micrograms per kilogram (parts per billion)
ug/L	micrograms per liter (parts per billion)