# Blue-Green Algae Toxins in Washington Lakes: Screening Fish Tissues for Microcystins and Anatoxin-a

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Cover photo: Blue-green algae bloom in Steilacoom Lake, 10/20/09 (Don Russell)

# Blue-Green Algae Toxins in Washington Lakes: Screening Fish Tissues for Microcystins and Anatoxin-a

by
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Waterbody Number(s):

| | |
|---|---|
| Ketchum Lake | WA-03-9110 |
| Waughop Lake | WA-12-9090 |
| Anderson Lake | WA-17-9010 |
| Steilacoom Lake | WA-12-9080 |
| American Lake | WA-12-9010 |
| Leland Lake | WA-17-9050 |

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# Abstract

Blue-green algae blooms in lakes can pose a human health concern. Although most blooms are not toxic, some blue-greens produce toxins that affect the liver and nervous system of animals, including humans. The toxins of particular concern are microcystins and anatoxin-a. The primary exposure pathways are through drinking water and recreation. In addition, consumption of fish containing blue-green toxins represents a poorly studied, but potentially, important exposure route for humans.

The Washington State Department of Ecology conducted a screening survey to assess the presence of these toxins in muscle and liver tissue from game fish in six Western Washington lakes that had blue-green blooms in 2008. Microcystins were detected in all 33 samples analyzed, with higher concentrations in liver than muscle. Anatoxin-a was analyzed in a subset of 8 samples but was not detected.

The microcystin analysis suffered from low recovery in spiked tissue samples (38-49%) and poor precision. Follow-up study is recommended to obtain higher quality microcystin data that can be used to better assess the human health risk.

Anatoxin-a spike recoveries were extremely low (4-13%), which was anticipated. Anatoxin may be too unstable to accumulate or is simply not taken up by fish.

# Acknowledgements

The authors would like to thank the following people for their contribution to this study:

- Don Russell, American Lake property owner, originally proposed this study and provided much useful information on the subject of toxic blue-green algae in local lakes.
- Robert Arnold, Ketchum Lake property owner, provided fish samples from the lake.
- Adam Couto, Richard Eltrich, and Dan Collins, Washington Department of Fish and Wildlife, provided fish samples from Anderson, Leland, American, Steilacoom, and Waughop Lakes.
- Dr. John Berry, Florida International University, extracted the project samples and analyzed for microcystins. Dr. Berry generously provided this service at no charge.
- Dr. Gabriela Hannach, King County Environmental Laboratory, analyzed anatoxin-a.
- Dr. Joan Hardy, Washington State Department of Health, advised on study design and provided useful comments on the project report.
- Washington State Department of Ecology staff:
    - Kathy Hamel, Water Quality Program, provided information on the study lakes, advised on the study, and reviewed the project report.
    - Dale Norton, Environmental Assessment Program, reviewed the project report.
    - Joan LeTourneau, Cindy Cook, and Gayla Lord, Environmental Assessment Program, edited and formatted the final report.

# Introduction

Blue-green algae blooms in lakes can pose a human health concern. Although most blooms are not toxic, some blue-greens produce nerve or liver toxins. People have become ill after swimming or water skiing in lakes with toxic blue-greens. Rarely, people experience symptoms such as stomach pain, vomiting, diarrhea, and skin rashes. Pets and wildlife have died after exposure to toxic blue-greens in Washington lakes, but worldwide there are no confirmed deaths of humans from recreational exposure to algal toxins.

Consumption of fish containing blue-green toxins represents a poorly studied but potentially important exposure route for humans. A growing body of literature documents detection of these compounds in fish tissues. The toxins of particular concern are microcystins and anatoxin-a.

Toxic algae blooms have been documented at an increasing rate in Washington lakes over the past 25 years. In light of the known uptake of blue-green toxins by fish and the potential for adverse human health effects, the Washington State Department of Ecology (Ecology) initiated a screening study to test for the presence of microcystins and anatoxin-a in fish samples. The samples were collected from six Western Washington lakes that experienced blue-green algae blooms in 2008.

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# Background on Blue-Greens[1]

Cyanobacteria, commonly known as blue-green algae, are bacteria that contain photosynthetic pigments similar to those found in algae and plants. Their ability to fix nitrogen directly from the atmosphere gives them a competitive advantage over other algae. Many blue-greens have gas vacuoles that keep them near the surface where there is more light for photosynthesis. Colonies may clump together, forming a surface scum which causes water quality problems in lakes.

A bloom can consist of one or a mixture of two or more types of blue-greens. The genera *Microcystis, Anabaena,* and *Aphanizomenon* account for the vast majority of blue-green blooms in Washington lakes and can produce microcystins or anatoxin-a. About 70+ variants of microcystins are known. The most common forms are microcystin-LR and microcystin-RR. (L and R stand for the amino acid groups leucine and arginine.)

Microcystins primarily affect the liver. Anatoxin-a affects nerve synapses. Microcystins are relatively stable and can remain in the water for days or weeks after a bloom has disappeared. Anatoxin-a is a much less stable compound.

Some *Microcystis* species produce microcystins. *Anabaena* species produce several kinds of toxins that include microcystins, anatoxin-a, anatoxin-a(s), and saxitoxins. Anatoxin-a(s) has a different structure and mode of action than anatoxin-a and is thought to be relatively uncommon. Saxitoxins cause paralytic shellfish poisoning in marine waters. It is only occasionally detected in freshwater. *Aphanizomenon flos-aquae* is also a known producer of anatoxin-a and saxitoxin.

A bloom of blue-green algae can potentially be found somewhere in Washington nearly any month of the year. Most blooms occur during the summer. However, toxic blooms can also arise in the winter. American Lake in Pierce County has a history of toxic *Anabaena* episodes during the winter at low water temperatures (7-8$^{o}$C). Blue-green algae blooms typically occur when plant nutrients such as phosphorus and nitrogen are in plentiful supply. However, factors needed for a bloom are complex. No individual environmental cause or particular set of conditions clearly controls their formation. Even blooms caused by known toxin producers may not produce toxins or may produce toxins at undetectable levels.

Blue-greens cannot maintain an abnormally high population for long and will rapidly die and disappear after 1-2 weeks. If conditions remain favorable, another bloom can replace the previous one, making it appear as one continuous bloom lasting for up to several months.

Toxic blue-greens are an emerging public health issue (Stone and Bress, 2007). The primary exposure pathways of concern have been drinking water and recreational exposure. Consumption of fish containing blue-green toxins represents a poorly studied, but potentially

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[1] Some of this information comes from the Washington State Department of Health, Office of Environmental Health, Safety, and Toxicology cyanobacteria web page. www.doh.wa.gov/ehp/algae/whatarecyanobacteria.htm

important, exposure route for humans (Ibelings and Chorus, 2007; Stone and Bress, 2007; Wilson et al., 2008). Microcystins are heat stable and do not break down during cooking (Harada et al., 1996). These compounds are suspected liver carcinogens, which could prove significant to humans following continuous, low-level exposure. Due to its instability, anatoxin-a is more difficult to analyze than microcystins.

Blue-green toxins can be extremely toxic to mammals. Table 1 compares their lethal dose with other more well-known poisons. These data are from animal experiments.

Table 1. Comparison of Blue-Green Algae Toxins with Other Known Poisons.
*(from Hamel, 2009.)*

| Blue-Green Toxin | LD-50* (ug/Kg) | | Other Poisons | LD-50 (ug/Kg) |
|---|---|---|---|---|
| Saxitoxin | 9 | | Ricin | 0.02 |
| Anatoxin-a (s) | 20 | | Cobra venom | 20 |
| Microcystin LR | 50 | | Curare | 500 |
| Anatoxin-a | 50 | | Strychnine | 2,000 |

*Lethal dose to 50% of test population.

Freshwater and brackish-water fish have been known to accumulate microcystins in their tissues, including muscle, liver, and other organs (Kotak et al., 1996; Magalhães et al., 2001; Sipiä et al., 2001; Xie et al., 2005; Ibelings et al., 2005; Gkelis et al., 2006; Wood et al., 2006; Wilson et al., 2008; Kann, 2008). Concentrations are usually highest in the liver. Less is known about uptake of other blue-green toxins, although their bioaccumulation potential in fish is generally considered to be low.

Laboratory experiments have shown rapid loss of microcystins once fish are removed from exposure. Half-lives on the order of 1-10 days have been reported for microcystins in muscle and liver tissue (Adamovsky et al., 2007). In other words, most of the microcystin would be expected to be eliminated from fish tissue soon after concentrations drop to low levels in the surrounding water.

# Project Description

Microcystins and anatoxin-a were analyzed in the muscle and liver of game fish collected in association with blue-green algae blooms in six Western Washington lakes during the summer and fall of 2008. Fish samples were obtained from Ketchum, Anderson, Leland, American, Steilacoom, and Waughop Lakes (Figure 1).

The objective was to obtain screening-level data that could be used in a preliminary assessment of the potential for human health concerns from fish consumption. The trigger for sampling was elevated levels of microcystins or anatoxin-a in algae samples collected by local health departments. The project was conducted by Ecology's Environmental Assessment Program (EA Program) at the request of the Ecology Water Quality Program (WQ Program).

Local health departments and the WQ Program monitored bloom conditions in local lakes to determine when and where fish samples should be collected. The EA Program was notified of significant blooms and contacted the Washington Department of Fish and Wildlife (WDFW) to coordinate a fish collection. The Ketchum Lake fish were provided by Robert Arnold, a property owner on the lake.

The tissue samples were analyzed by Florida International University, North Miami (microcystins) and the King County Environmental Laboratory, Seattle (anatoxin-a). Thirty-three microcystin samples and eight anatoxin samples were analyzed in all. Fewer samples were analyzed for anatoxin because of the lower likelihood of its detection.

The data were provided to the Washington State Department of Health for their use in assessing the human health concern. The present report is limited to a description of the 2008 screening study and presentation of the fish tissue data.

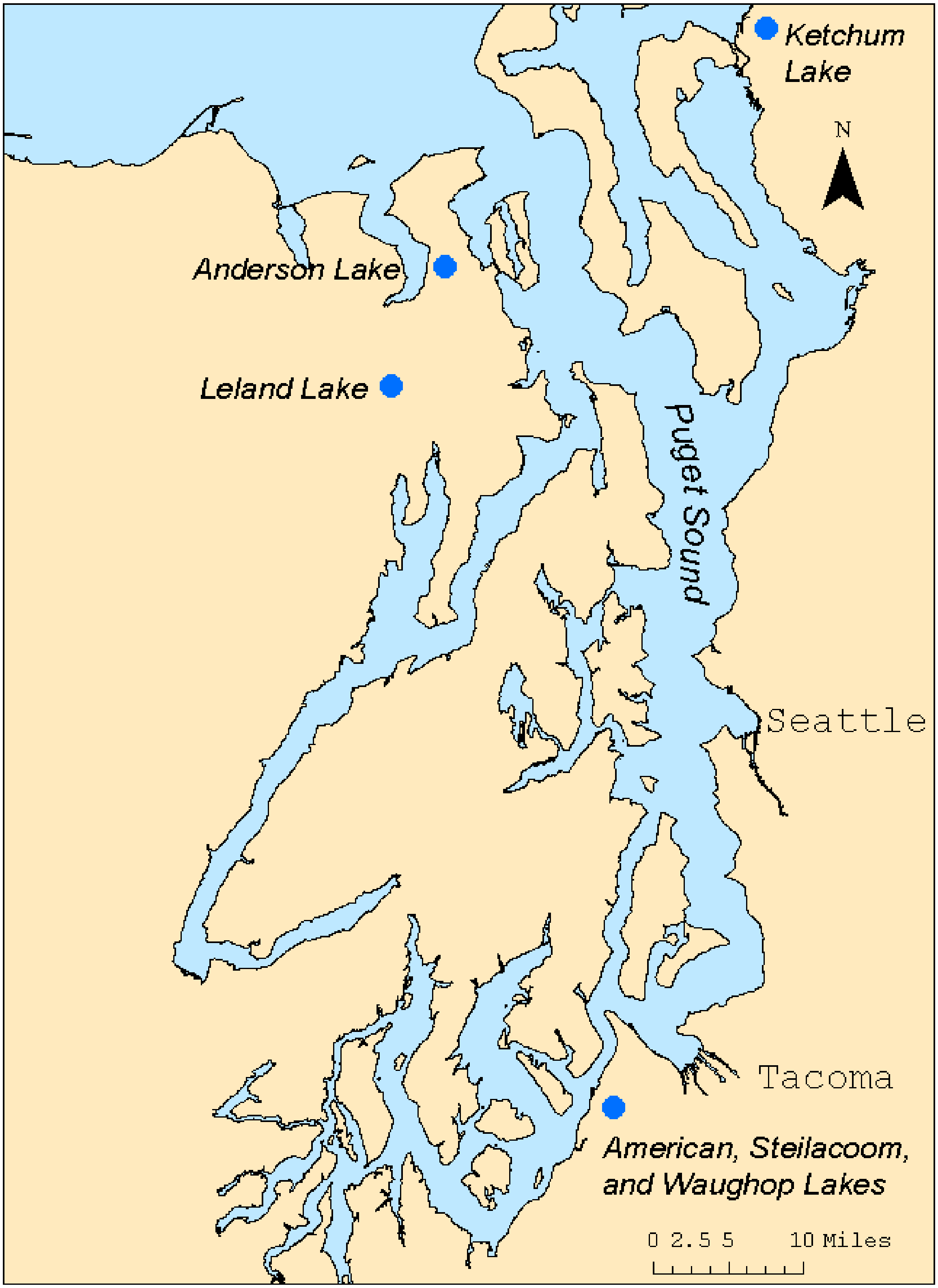



Figure 1. Lakes Where Fish Were Collected to Screen for Microcystins and Anatoxin-a.

# Samples Analyzed

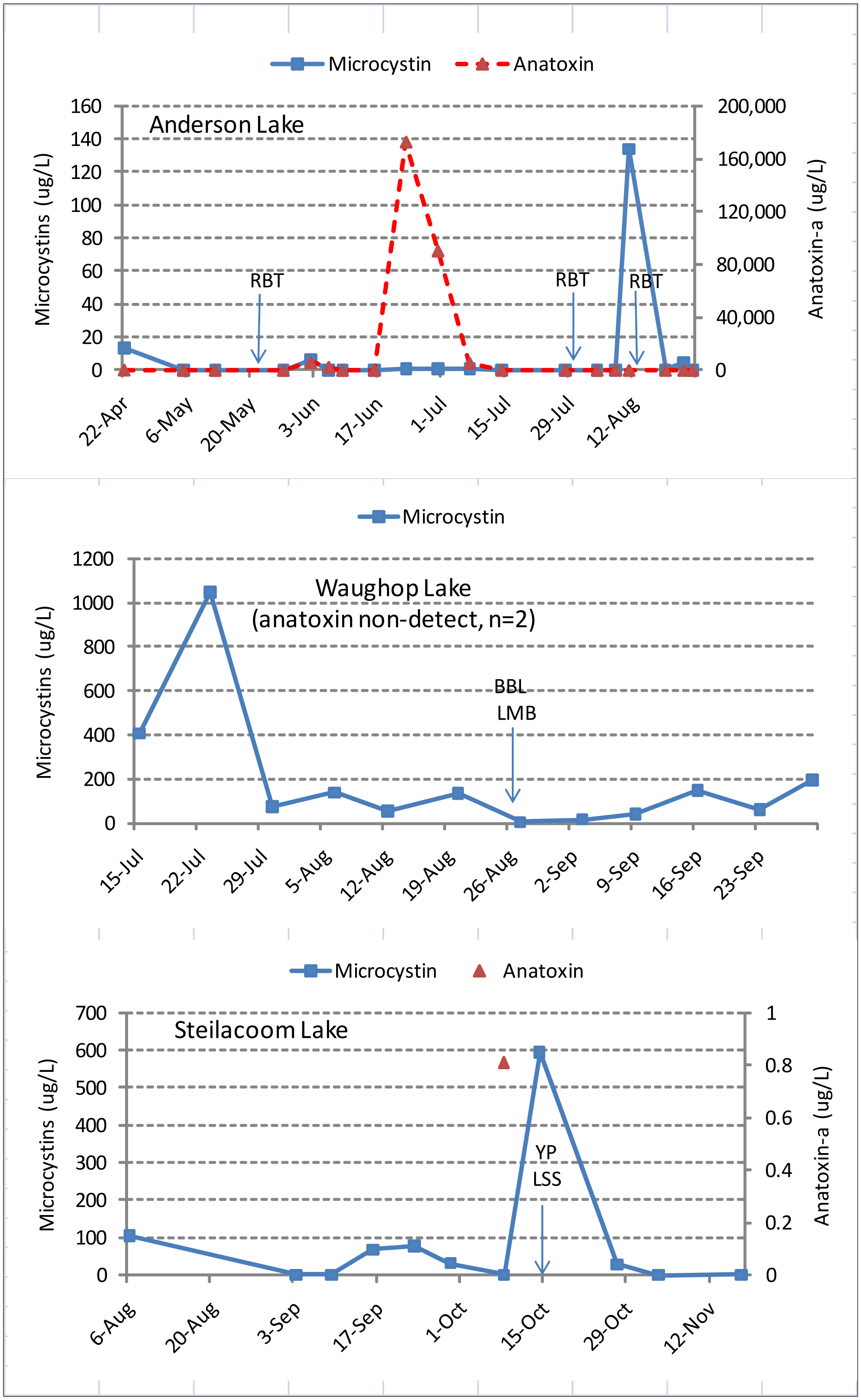
Locations, fish species, and sample collection dates for this project are shown in Table 2.

Table 2. Fish Samples Obtained for Microcystin and Anatoxin Analysis.

| Lake | County | Species | Date | Collector |
|---|---|---|---|---|
| Anderson Lake | Jefferson | Rainbow Trout | 22-May-08 | Dan Collins WDFW |
| | | | 31-Jul-08 | Adam Couto WDFW |
| | | | 13-Aug-08 | |
| American Lake | Pierce | Kokanee | 30-May-08<br>11-Sep-08 | Richard Eltrich WDFW |
| Ketchum Lake | Snohomish | Rainbow Trout | 26-Aug-08<br>2-8 Sept 08 | Robert Arnold |
| | | Yellow Perch | 28-29 Aug 08 | |
| Waughop Lake | Pierce | Brown Bullhead<br>Largemouth Bass | 27-Aug-08 | Adam Couto WDFW |
| Leland Lake | Jefferson | Largemouth Bass<br>Yellow Perch<br>Rainbow Trout | 15-Oct-08 | Adam Couto WDFW |
| Steilacoom Lake | Pierce | Yellow Perch<br>Largescale Sucker | 15-Oct-08 | Adam Couto WDFW |

Appendix A has data on microcystin and anatoxin concentrations in algae samples collected from these lakes during 2008 as part of Ecology's Freshwater Algae Control Program (www.ecy.wa.gov/programs/wq/plants/algae/index.html). Because these samples were from the surface scum that formed during blooms, the concentrations do not necessarily reflect the microcystin or anatoxin levels fish are exposed to through the water column. Anatoxin was not analyzed for all six lakes.

The timing of the fish collections relative to the algal blooms is illustrated in Figure 2. The work schedule of the WDFW biologists who collected fish for this study dictated when fish could be collected from Anderson, Waughop, Steilacoom, Leland, and American Lakes. The Ketchum Lake fish were also samples of opportunity collected by a lake resident. As a result, although an effort was made to collect fish during or soon after a bloom, the sampling was not timed to coincide with algae blooms in a consistent way.


Microcystin
Anatoxin
Anderson Lake
Microcystins (ug/L)
Anatoxin-a (ug/L)
160
140
120
100
80
60
40
20
0
200,000
160,000
120,000
80,000
40,000
0
RBT
RBT
RBT
22-Apr
6-May
20-May
3-Jun
17-Jun
1-Jul
15-Jul
29-Jul
12-Aug
Microcystin
Waughop Lake
(anatoxin non-detect, n=2)
Microcystins (ug/L)
1200
1000
800
600
400
200
0
BBL
LMB
15-Jul
22-Jul
29-Jul
5-Aug
12-Aug
19-Aug
26-Aug
2-Sep
9-Sep
16-Sep
23-Sep
Microcystin
Anatoxin
Steilacoom Lake
Microcystins (ug/L)
Anatoxin-a (ug/L)
700
600
500
400
300
200
100
0
1
0.8
0.6
0.4
0.2
0
YP
LSS
6-Aug
20-Aug
3-Sep
17-Sep
1-Oct
15-Oct
29-Oct
12-Nov

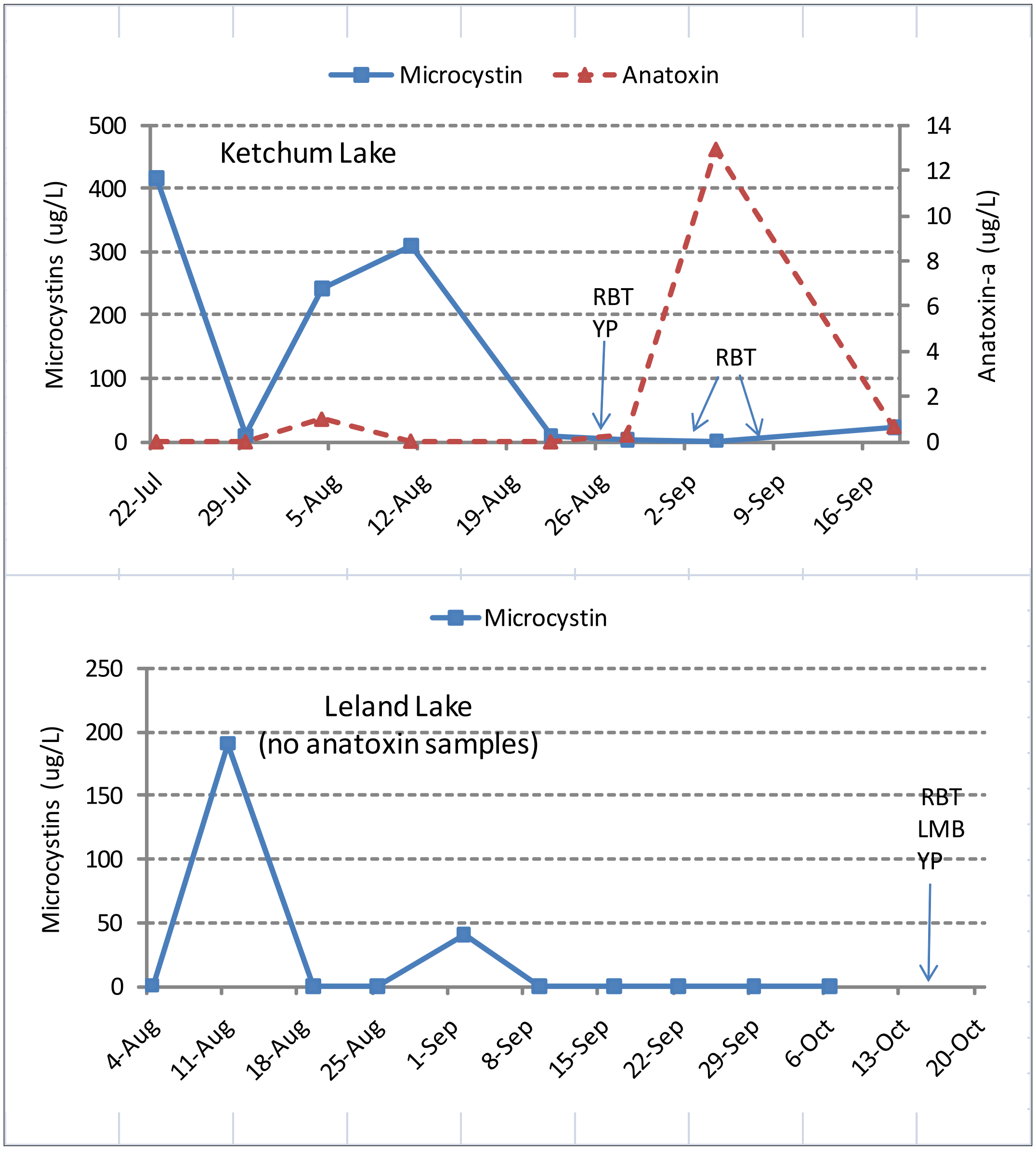



Figure 2. Blue-Green Algae Blooms in Screening Study Lakes, Showing When Fish Samples Were Collected in 2008.

*RBT = rainbow trout, BBL = brown bullhead, LMB = largemouth bass, YP = yellow perch, LSS = Largescale Sucker.*

The water quality guidelines used in Washington to assess the risk from recreational exposure to blue-green toxins are 6 ug/L (parts per billion) for microcystins and 1 ug/L for anatoxin-a (Hardy, 2008).

Of the 53 lakes Ecology tested for microcystins in 2008 (Hamel, 2009), 40 lakes had detectable concentrations > 0.05 µg/L (75%), 18 lakes had levels over the state recreational guidance of 6 µg/L (34%), and 14 lakes had levels higher than 50 µg/L (26%). Waughop, Steilacoom, Ketchum, and Leland Lakes were among the 14 with the highest concentrations. The microcystin concentration of 1,050 ug/L in Waughop Lake in July was the third highest recorded that year. The highest concentration in 2008 was 4,620 µg/L from Ohop Lake in Pierce County.

Of the 24 lakes tested for anatoxin-a in 2008 (Hamel, 2009), 18 lakes had detectable levels of anatoxin-a (75%) and eight lakes had levels over the state recreational guidance of 1 µg/L (29%). Anderson, Steilacoom, Ketchum, and Leland Lakes were among those with the highest concentrations. Of the six lakes sampled for the present study, the anatoxin-producing *Anabaena* bloom in Anderson Lake was by far the most severe. The anatoxin-a concentration of 172,640 µg/L detected in Anderson Lake in July is one of the highest concentrations reported worldwide (Hamel, 2009). The anatoxin bloom in Leland Lake (22 ug/L) occurred in June; a fish collection could not be arranged at that time.

As a result of the *Anabaena* bloom, Anderson Lake was closed to recreation by state parks. Local health departments posted warning signs and notified the residents of Steilacoom, Waughop, Ketchum, and Leland Lakes about the blooms.

Despite a history of toxic *Anabaena* blooms, none were observed in American Lake during 2008. It has been theorized that subsurface *Anabaena* blooms occur in American Lake in the relatively narrow and nutrient-rich layer between the warmer surface water and colder deep water layers, known as the metalimnion. American Lake sustains a popular fishery for kokanee, a land-locked sockeye salmon. Kokanee are zooplankton feeders and may consume *Daphnia*, a microscopic crustacean, that concentrate in the metalimnion to feed on *Anabaena*. (Don Russell, personal communication, 7/27/08 email). Subsurface maxima for blue-green algae and their toxins have been reported in other lakes (Lindholrn and Meriluoto, 1991; Albay et al., 2003).

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# Tissue Preparation

The fish were collected by gill net or hook and line. Fish selected for samples were killed by a blow to the head, put in plastic bags, and placed on ice as soon as possible. The fish were transported to Ecology headquarters on ice or frozen if transport was delayed. At headquarters, the fish were measured for length and weight, individually wrapped in aluminum foil, put in plastic bags, and frozen pending preparation of tissue samples.

Tissue samples were prepared following the EA Program standard operating procedure (SOP) (Sandvik, 2006). The fish were thawed enough to remove the foil wrapper, scaled, and rinsed under tap water, followed by a deionized water rinse.

The fish were either analyzed individually or as composites of tissues from two to five fish. The entire fillet from one or both sides of each fish was removed with stainless steel knives and homogenized in a Kitchen-Aid blender to uniform color and consistency. The fillets were analyzed skin-on, except bullheads were skin-off. The muscle samples were placed in glass jars with Teflon lid liners, cleaned to EPA (1990) QA/QC specifications. After filleting, the body cavity was opened, and the liver removed and placed in plastic vials.

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. Cleaning of knives, cutting boards, and the blender 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 shipment with chain-of-custody record to Dr. John Berry, Florida International University (FIU). The liver samples were homogenized at FIU.

Appendix B has a list of the samples prepared for microcystin and anatoxin analysis. The lengths, weights, and sex of the fish used in the samples are shown in Appendix C.

# Laboratory Analysis

## Microcystins

Samples for microcystin analysis were extracted and analyzed at FIU. Microcystins were analyzed by enzyme-linked immunosorbent assay (ELISA). In these types of tests, the sample competes with an enzyme solution for the binding sites on an antibody specific for microcystins. The reaction is a measure of the amount of microcystins in the sample.

Portions (~1-5 g) of tissue were homogenized and sequentially extracted in 20 mL of 75% methanol (in water) for 24 hours, followed by 20 mL of 75% methanol + 0.05% acetic acid. Extracts were pooled, and aliquots (150 µL) of the pooled extracts were taken to dryness in vacuo and subsequently re-taken in the same volume (150 µL) of phosphate buffered saline (PBS). Duplicate aliquots (50 µL each) were analyzed for microcystins by ELISA kits obtained from Abraxis, Inc., Warminster PA, as per the manufacturer's instructions.

Each pooled extract was tested in replicate (analyzed twice). Duplicate extractions were prepared and analyzed separately for four of the samples (7, 8, 38 and 39). In addition, two fish tissue samples were spiked with microcystin-LR (50 ng) as a matrix spike and matrix spike duplicate. The matrix spikes were extracted and analyzed the same as field samples. Given the limited amount of liver tissue, matrix spikes were only prepared for muscle tissue. In addition, a sample of MilliQ water was spiked with microcystin-LR (50 ng) and analyzed as a spiked blank.

## Anatoxin-a

FIU conducted a separate extraction for anatoxin-a. The tissue samples were extracted three times in acidic methanol (1% HCl 1 M), as per James et al. (1997). The extracts were then shipped to the King County Environmental Laboratory where they were analyzed by Dr. Gabriela Hannach.

The tissue extracts were analyzed following SOP #457vD (2009) and James et al. (1998). The dried extracts were resuspended in borate buffer. Anatoxin-a was converted into a fluorescent derivative using 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F), and the fluorescent compound was then separated and detected by isocratic HPLC. Liquid chromatography was performed with an Agilent 1200 series system using a Zorbax $C_{18}$ column, 45% acetonitrile-water as the mobile phase and fluorometric detection at 470 nm (excitation) and 530 nm (emission). Calibration used anatoxin-a analytical standards purchased from Biomol and was compared with standards from A.G. Scientific for inter-lab consistency.

# Data Quality

## Spike Recoveries

Spike recoveries provide an indication of loss of target compounds during analysis or bias due to interferences from components in the sample matrix. The recoveries achieved for microcystin spikes in fish tissue (matrix spikes) were rather low, approximately 38% (Appendix D). Likewise, recovery from the spiked blank was only 49%. These spikes were prepared with purified microcystin-LR, rather than a certified reference standard. Therefore, there is some uncertainty as to the true concentration in the spiked samples.

As previously noted, anatoxin-a is an unstable compound. There was some recovery of anatoxin-a in matrix spikes, but it was extremely low at 13% in muscle and 4% in liver. Somewhat improved recoveries of 20-38% were achieved in spikes of laboratory water.

## Split Samples

Separate aliquots of selected fish muscle and liver samples were analyzed to assess the precision of the data generated for this project (Table 3). The precision of the microcystin data was generally poor, particularly for liver tissue. Anatoxin was not detected in the duplicates.

Table 3. Precision on Duplicate Samples Analyzed for Microcystins and Anatoxin-a.
*(ug/Kg wet weight; parts per billion).*

| Sample No. (0902058-) | Species | Tissue | Dup. #1 | Dup. #2 | RPD |
|---|---|---|---|---|---|
| **Microcystins** | | | | | |
| 7 | Brown Bullhead | muscle | 1.4 | 0.8 | 51% |
| 8 | | liver | 38 | 9.1 | 123% |
| 38 | Rainbow Trout | muscle | 1.1 | 4.5 | 120% |
| 39 | | liver | 90 | 4.8 | 180% |
| **Anatoxin-a** | | | | | |
| 26 | Rainbow Trout | muscle | <1.7 | <2.0 | not detected |
| 27 | | liver | <14 | <38 | not detected |

RPD = relative percent difference (range of duplicates as percent of mean).

The reason for within-sample variability in the microcystin analysis is unknown. The analyst suggested it could be a product of the ELISA kit itself, plate reader calibration, or inhomogeneity of the tissue samples.

Better precision was achieved in the replicate measurements conducted on each sample extract (Appendix D). The relative percent difference (RPD) between microcystin replicates averaged 36% and 23%, respectively, for muscle and liver. There was good agreement (13% RPD) between a matrix spike and matrix spike duplicate for microcystin in muscle tissue (Appendix D).

In similar Ecology studies analyzing other types of organic compounds, the residues measured in duplicate fish muscle tissue samples homogenized using the same protocols as in the present study have typically agreed within 20% or better (e.g., Seiders and Deligeannis, 2009; Johnson et al., 2007). For muscle tissue at least, this suggests but does not rule out sample inhomogeneity as being the cause of the current problem.

For the samples in Table 3, the average of duplicate results or the lower detection limit (anatoxin) was used in the remainder of this report.

# Results

## Microcystins

Results of the microcystin analysis are summarized in Table 4. Concentrations are reported in units of ug/Kg wet (fresh) weight, which is equivalent to parts per billion. N is the number of samples. The mean and median are the average and middle values, respectively, in the data set. The 90$^{th}$ percentile is the concentrations exceeded by 10% of the samples. The data for individual samples follow in Table 5.

Table 4. Summary of Results for Microcystins in Fish Tissue.
*(estimated concentration in ug/Kg wet weight; parts per billion.)*

| | Muscle | Liver |
|---|---|---|
| N = | 20 | 11 |
| Mean | 15 | 66 |
| Median | 14 | 64 |
| Minimum | 0.9 | 7.2 |
| Maximum | 53 | 169 |
| 90th Percentile | 33 | 96 |

Table 5. Individual Sample Results for Microcystins.
*(estimated concentration in ug/Kg wet weight; parts per billion.)*

| Sample No. (0902058-) | Lake | Date | Species | Tissue | Microcystin |
|---|---|---|---|---|---|
| 3 | American | 11-Sep-08 | Kokanee | muscle | 53 |
| 5 | | " | " | liver | 75 |
| 7 | Waughop | 27-Aug-08 | Brown Bullhead | muscle | 1.1 |
| 8 | | " | | liver | 23 |
| 9 | | " | | muscle | 1.0 |
| 10 | | " | | muscle | 10 |
| 11 | | " | Largemouth Bass | muscle | 17 |
| 12 | Leland | 15-Oct-08 | Largemouth Bass-small | muscle | 13 |
| 22 | | | Largemouth Bass-large | muscle | 28 |
| 13 | | | Yellow Perch | muscle | 25 |
| 14 | | | Rainbow Trout | muscle | 1.5 |
| 15 | | | | liver | 75 |
| 16 | Steilacoom | 15-Oct-08 | Yellow Perch | muscle | 21 |
| 17 | | | | muscle | 11 |
| 18 | | | | muscle | 32 |
| 19 | | | | liver | 48 |
| 20 | | | Largescale Sucker | muscle | 22 |
| 23 | Anderson | 22-May-08 | Rainbow Trout | muscle | 15 |
| 24 | | | | liver | 64 |
| 29 | | 13-Aug-08 | Rainbow Trout -small | muscle | 1.5 |
| 30 | | | | liver | 96 |
| 31 | | | | whole | 49 |
| 32 | | | Rainbow Trout -large | muscle | 0.9 |
| 33 | | | | liver | 40 |
| 34 | Ketchum | 26-Aug-08 | Rainbow Trout | muscle | 2.2 |
| 35 | | | | liver | 78 |
| 40 | | | | carcass* | 70 |
| 38 | | 2-8 Sept-08 | Rainbow Trout -small | muscle | 2.8 |
| 39 | | | | liver | 48 |
| 41 | | | Rainbow Trout -large | muscle | 14 |
| 42 | | | | liver | 7.2 |
| 36 | | 28-29 Aug-08 | Yellow Perch | muscle | 38 |
| 37 | | | | liver | 169 |

*Whole fish less muscle and liver.

Microcystin concentrations were almost always higher in liver than muscle. Microcystins were detected in all samples at concentrations ranging from 0.9 – 53 ug/Kg in muscle to 7.2 – 169 ug/Kg in liver. Mean concentrations were 15 ug/Kg and 66 ug/Kg in muscle and liver, respectively (Figure 3). Concentrations in a whole fish sample and a carcass sample (fillet and liver removed) were intermediate between those measured in muscle and liver (Table 4).

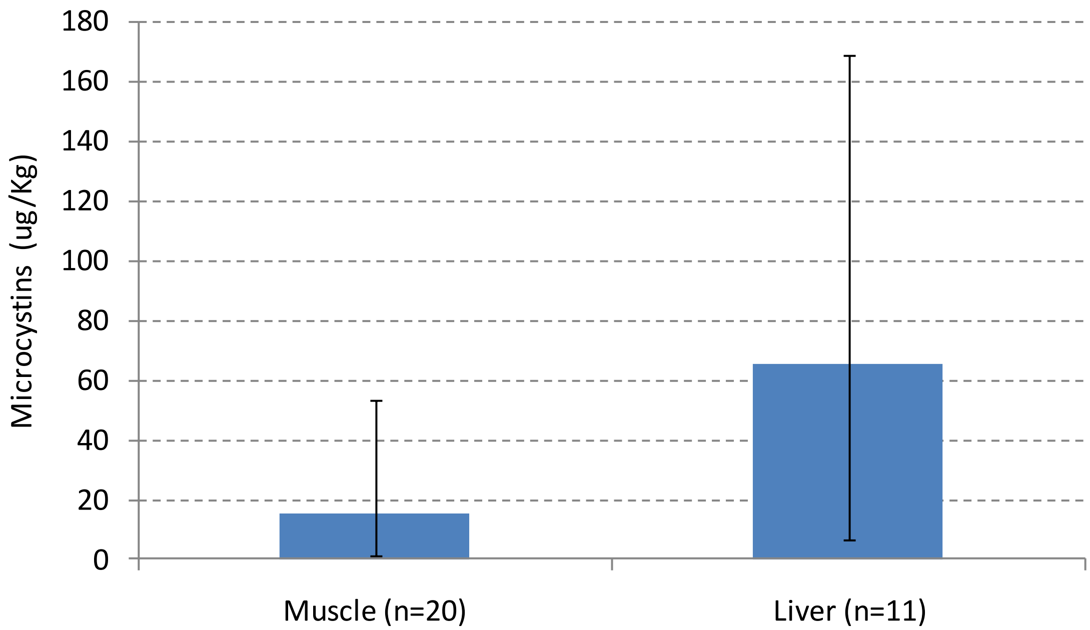
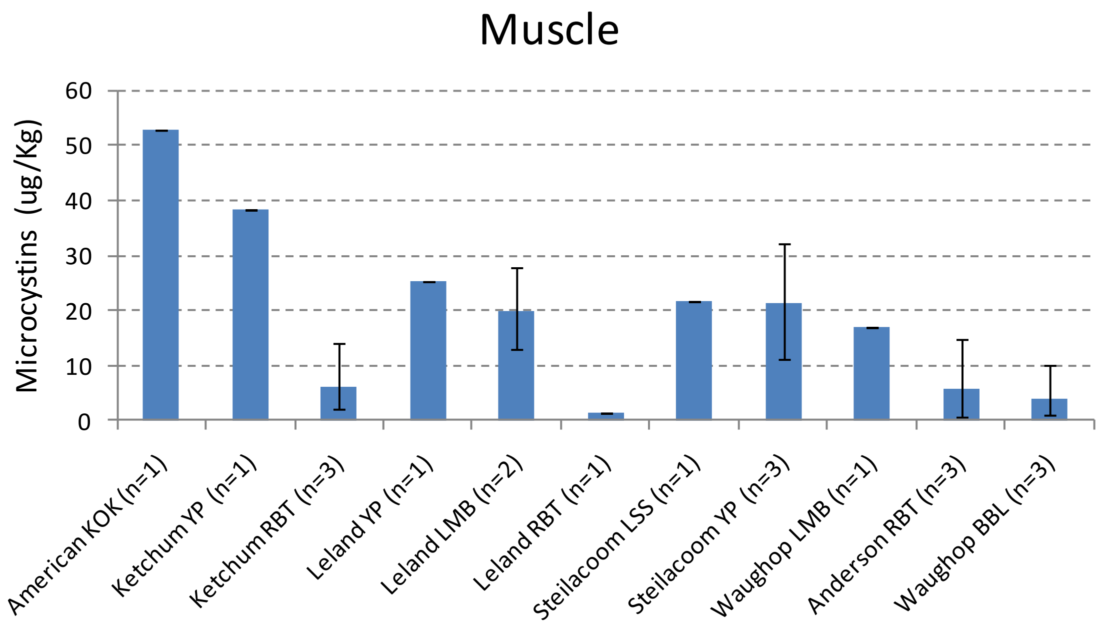



Figure 3. Mean and Range of Estimated Concentrations of Microcystins in Fish Muscle and Liver Samples.

The microcystin data are plotted by lake and species in Figure 4. Overall, the results suggest that fish from American, Ketchum, and Leland Lakes had the higher microcystin concentrations. Many factors, however, potentially affect these results, including but not limited to the species analyzed, fish size, and the severity and timing of algae blooms in relation to when the fish were collected. The relatively high microcystin concentration in muscle tissue from American Lake kokanee may be an indicator they were feeding on subsurface *Anabaena* blooms, as postulated earlier in this report.



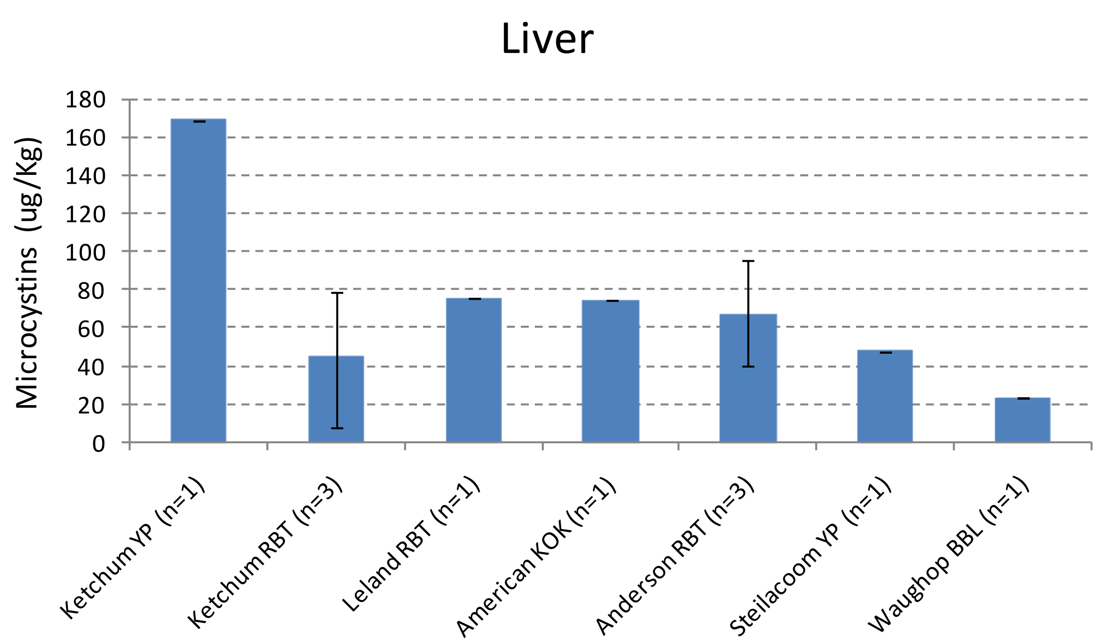




Figure 4. Estimated Concentrations and Range of Microcystins by Lake and Fish Species.

*(KOK = kokanee, YP = yellow perch, RBT = rainbow trout, LMB = largemouth bass, LSS = Largescale Sucker, BBL = brown bullhead.)*

## Anatoxin-a

Anatoxin-a was analyzed in a subset of the fish tissue samples. Samples were selected from American Lake due to its history of *Anabaena* blooms and from Anderson Lake due to the highly toxic anatoxin bloom that occurred during the 2008 study period (see Figure 2).

As noted previously, anatoxin-a is a relatively unstable compound and was poorly recovered from spiked samples. Anatoxin-a was not detected in any of the eight fish tissue extracts analyzed (Table 6). Detection limits ranged from 1.5 – 2.6 ug/Kg in muscle and whole fish to 6.4 – 14 ug/Kg in liver.

Table 6. Results for Anatoxin-a.
*(estimated concentration in ug/Kg wet weight; parts per billion).*

| Sample No. (0902058-) | Lake | Date | Species | Tissue | Anatoxin-a |
|---|---|---|---|---|---|
| 1 | American | 30-May-08 | Kokanee | muscle | <1.5 |
| 2 | | | | liver | <6.4 |
| 3 | | 11-Sep-08 | | muscle | <2.6 |
| 4 | | | | liver | <8.2 |
| 6 | | | | whole | <2.1 |
| 26 | Anderson | 31-Jul-08 | Rainbow Trout | muscle | <1.7 |
| 27 | | | | liver | <14 |
| 28 | | | | whole | <2.1 |

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# Comparison with Other Studies

Table 7 compares the microcystin results from the screening study to data reported in similar investigations in other parts of the U.S. These studies have employed both immunoassay and more rigorous liquid chromatography/mass spectrometry (LC/MS) techniques to analyze microcystins. Similar data were not located on anatoxin, which apparently is rarely analyzed in fish tissue.

The microcystin concentrations estimated in the present study generally fall in the mid-range of those reported in fish from other lakes and reservoirs subject to blue-green algae blooms. A high order of variability in the data appears to be a feature common to several of these studies. Some of this variability can be attributed to the samples being collected over a period of months (e.g., Wilson et al., 2008). Field and/or analytical variability are likely important factors in others (e.g., Kann, 2008).

Table 7. Microcystin Concentrations Compared to Results from Similar Investigations in Other U.S. Waterbodies.

| Waterbody | Species | Tissue | N = | Microcystins (ug/Kg, wet) | | Method | Reference |
|---|---|---|---|---|---|---|---|
| | | | | Median | Range | | |
| Klamath River: Iron Gate Reservoir | Yellow Perch | Muscle | 19 | 2.7 | ND - 229 | LC/MS | Kann (2008) |
| Copco Reservoir | | Muscle | 19 | 141 | ND - 422 | | |
| | | Liver | 6 | 124 | ND - 473 | | |
| Lake Erie | Yellow Perch | Muscle | 68 | ~0.2 | 0.1 - 0.8 | immunoassay | Wilson et al. (2008)* |
| | | Liver | 68 | ~40 | 3 - 240 | | |
| | Four species | Muscle | 31 | NR | 7.3-10.4 | immunoassay | Schuster et al. (2006) |
| Fremont Lake (Nebraska) | Channel Catfish White Crappie Largemouth Bass | Muscle | 6 | 240 | ND - 320 | immunoassay | Carmichael (2006a,b) |
| | | Liver | 6 | 200 | ND - 270 | | |
| | | Muscle | 4 | 200 | 130 - 250 | LC/MS | |
| | | Liver | 4 | 90 | 70 - 110 | | |
| Six Washington lakes | Six species | Muscle | 20 | 14 | 0.9 - 53 | immunoassay | present 2008 study |
| | | Liver | 11 | 64 | 7.2 - 169 | | |

*Dry weight data divided by 5 to convert to wet weight-based concentrations; medians estimated from Figure 3.
ND = not detected.
LC/MS = liquid chromatography/mass spectrometry.
NR = not reported.

The Carmichael (2006a,b) data show lower microcystin levels in fish samples analyzed by LC/MS than by ELISA. Most studies, however, have concluded that ELISA tends to underestimate microcystin concentration in fish tissue and other types of environmental samples (e.g., Rapala et al., 2002; Babica et al., 2006, Bruno et al., 2006).

According to Dr. John Berry of FIU (7/17/2009 email):

"It is fairly well established that solvent extraction followed by ELISA underestimates total microcystin, specifically because much of the toxin (on the order of 75%) is covalently bound to protein phosphatases. This same problem could be expected to give a high degree of variability in the measurement. Indeed, it is probably safe to assume that whatever levels of microcystin we measure with ELISA are probably a significant underestimation. That said, this would not explain the variability between two aliquots of the same extract."

In this regard it should be noted that it is the unbound fraction that is considered to be more bioavailable and therefore the greater human health concern (Wilson et al., 2008; Smith and Boyer, 2009).

# Conclusions

Results of this 2008 screening study show that microcystins are accumulated in the muscle and liver of Washington freshwater fish exposed to blue-green algae blooms. Concentrations were almost always higher in liver than in muscle. These findings are consistent with similar studies done elsewhere. The low recovery of quality control samples spiked with microcystin suggests the concentrations found may be underestimates.

Anatoxin-a was not detected in muscle or in liver. The inability to detect anatoxin-a even in fish exposed to a highly significant anatoxin-producing bloom (Anderson Lake) is noteworthy. Anatoxin-a may be too unstable to accumulate or is simply not taken up by fish.

# Recommendations

Given confirmation that microcystins accumulate in fish exposed to blue-green algae blooms in Washington lakes, further investigation appears warranted. Eight recommendations follow:

1. Additional fish sampling should be conducted at regularly timed intervals during and following microcystin blooms to better determine the extent and duration of elevated concentrations. Different feeding types should be monitored if possible to identify the species with the greatest potential for bioaccumulation.

2. Water column samples should be analyzed in conjunction with the fish samples to more accurately chart the course and magnitude of the blooms fish are exposed to. Ideally, this would include an assessment of the fraction of microcystin in dissolved and particulate form. The sampling design should take the effects of water column stratification into account.

3. Follow-up studies will likely rely on ELISA due to its low cost. However, at least a subset of samples should be analyzed by LC/MS or similar more rigorous methods as a check on the accuracy of the ELISA data. These methods can also provide information on the types and relative amounts of microcystins present (LR, RR, YR, etc.).

4. Steps should be taken to ensure the accuracy of ELISA data. Potential improvements identified in the present study include use of a certified microcystin standard, checks on plate reader calibration, and verifying sample homogeneity.

5. LC/MS analysis would benefit from use of a non-target microcystin variant spiked into all samples as a means of gauging recovery of target compounds. If not available, Smith and Boyer (2009) have suggested using thiol-LR as a microcystin surrogate. (Use of surrogates is not appropriate for ELISA.)

6. In view of the stability of microcystins, sediments and beach material from lakes with a history of blue-green blooms should be screened for these compounds.

7. Follow-up studies should consider screening for other blue-green toxins with potential for bioaccumulation. For example, very low levels of saxitoxin (<1 ug/Kg) appeared to be present in the Waughop Lake fish samples from the present study (Dr. John Berry, 10/24/09 email). Saxitoxin was also recently detected in algae samples from Waughop Lake (Hamel, 2009).

8. WDOH should review any new data collected on blue-green toxins in fish from Washington lakes.

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# Appendices

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# Appendix A. Monitoring Data on Microcystins and Anatoxin-a Concentrations in Algae Samples from Lakes Screened for Blue-Green Toxins in Fish Tissue (ug/L, parts per billion)

| Date (2008) | Microcystins | Anatoxin-a |
|---|---|---|
| **Anderson Lake** | | |
| 22-Apr | 14 | 300 |
| 5-May | <0.05 | not analyzed |
| 12-May | <0.05 | 0.85 |
| 27-May | <0.05 | no data |
| 2-Jun | 6.2 | 6,000 |
| 6-Jun | not analyzed | 2,353 |
| 9-Jun | <0.05 | not analyzed |
| 16-Jun | <0.05 | 66 |
| 23-Jun | 0.58 | 172,640 |
| 30-Jun | 0.47 | 90,256 |
| 7-Jul | 0.65 | 5,280 |
| 14-Jul | 0.19 | 2.0 |
| 28-Jul | 0.09 | 3.9 |
| 4-Aug | 0.08 | 0.08 |
| 8-Aug | not analyzed | 201 |
| 11-Aug | 134 | not analyzed |
| 19-Aug | 0.45 | 1.8 |
| 23-Aug | 4.1 | not analyzed |
| 25-Aug | not analyzed | 0.53 |
| **Waughop Lake** | | |
| 22-May | not analyzed | ND |
| 15-Jul | 406 | ND |
| 23-Jul | 1,050 | not analyzed |
| 30-Jul | 74 | not analyzed |
| 6-Aug | 138 | not analyzed |
| 12-Aug | 56 | not analyzed |
| 20-Aug | 136 | not analyzed |
| 27-Aug | 4.7 | not analyzed |
| 3-Sep | 17 | not analyzed |
| 9-Sep | 42 | not analyzed |
| 16-Sep | 148 | not analyzed |
| 23-Sep | 60 | not analyzed |
| 29-Sep | 195 | not analyzed |

| Date (2008) | Microcystins | Anatoxin-a |
|---|---|---|
| **Ketchum Lake** | | |
| 22-Jul | 416 | not analyzed |
| 29-Jul | 10 | not analyzed |
| 4-Aug | 242 | <1.0 |
| 11-Aug | 309 | <0.03 |
| 22-Aug | 8.6 | not analyzed |
| 28-Aug | 3.0 | 0.30 |
| 4-Sep | 1.5 | 13 |
| 18-Sep | 23 | <0.67 |
| **Steilacoom Lake** | | |
| 6-Aug | 104 | not analyzed |
| 3-Sep | 0.36 | not analyzed |
| 9-Sep | 0.72 | not analyzed |
| 16-Sep | 68 | not analyzed |
| 23-Sep | 76 | not analyzed |
| 29-Sep | 31 | not analyzed |
| 8-Oct | 0.15 | 0.81 |
| 14-Oct | 594 | not analyzed |
| 27-Oct | 28 | not analyzed |
| 3-Nov | 0.08 | not analyzed |
| 17-Nov | 0.16 | not analyzed |
| **Leland Lake** | | |
| 4-Aug | 0.68 | not analyzed |
| 11-Aug | 191 | not analyzed |
| 19-Aug | 0.25 | not analyzed |
| 25-Aug | 0.51 | not analyzed |
| 2-Sep | 41 | not analyzed |
| 9-Sep | 0.36 | not analyzed |
| 16-Sep | 0.20 | not analyzed |
| 22-Sep | 0.19 | not analyzed |
| 29-Sep | 0.11 | not analyzed |
| 6-Oct | 0.08 | not analyzed |
| **American Lake** | | |
| No visible blooms in 2008; no algae samples collected. | | |

ND = not detected.

Data Source: https://fortress.wa.gov/ecy/toxicalgae/InternetDefault.aspx.

## Appendix B.  Fish Samples Analyzed for Microcystins and Anatoxin-a

| Lake | Species | Collection Date | Tissue | Sample No. (0902058-) | Number of Individuals | Sample Wt. (grams) | Analysis | | Note |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Microcystin | Anatoxin | |
| American Lake | Kokanee | 30-May-08 | muscle | -1 | 4 | 75 | | 1 | |
| | | | liver | -2 | 4 | 17 | | 1 | |
| | Kokanee | 11-Sep-08 | muscle | -3 | 5 | 75-86 | 1 | 1 | 2 jars |
| | | | liver | -4 | 5 | 19 | | 1 | |
| | | | | -5 | 5 | 17 | 1 | | |
| | | | whole | -6 | 5 | 116 | | 1 | without liver |
| Waughop Lake | Brown Bullhead | 27-Aug-08 | muscle | -7* | 4 | 37 | 1 | | |
| | | | liver | -8* | 4 | 6 | 1 | | |
| | | | muscle | -9 | 4 | 38 | 1 | | |
| | | | muscle | -10 | 4 | 58 | 1 | | |
| | Largemouth Bass | | muscle | -11 | 1 | 28 | 1 | | |
| Leland Lake | Largemouth Bass | 15-Oct-08 | muscle | -12 | 3 | 43 | 1 | | small fish |
| | | | muscle | -22 | 2 | 22 | 1 | | large fish |
| | Yellow Perch | | muscle | -13 | 5 | 48 | 1 | | |
| | Rainbow Trout | | muscle | -14 | 3 | 70 | 1 | | |
| | | | liver | -15 | 3 | 10 | 1 | | |
| Steilacoom Lake | Yellow Perch | 15-Oct-08 | muscle | -16 | 1 | 25 | 1 | | |
| | | | muscle | -17 | 1 | 33 | 1 | | |
| | | | muscle | -18 | 1 | 30 | 1 | | |
| | | | liver | -19 | 3 | 8 | 1 | | |
| | Largescale Sucker | | muscle | -20 | 1 | 20 | 1 | | |

| Lake | Species | Collection Date | Tissue | Sample No. (0902058-) | Number of Individuals | Sample Wt. (grams) | Analysis | | Note |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Microcystin | Anatoxin | |
| Anderson Lake | Rainbow Trout | 27-May-08 | muscle | -23 | 5 | 80 | 1 | 1 | 2 jars |
| | | | liver | -24 | 5 | 26 | 1 | 1 | 1 vial |
| | | 31-Jul-08 | muscle | -25 | 1 | 63 | | 1 | large fish |
| | | | muscle | -26* | 5 | 22 | | 1 | small fish |
| | | | liver | -27* | 5 | 4 | | 1 | |
| | Rainbow Trout | 31-Jul-08 | whole | -28 | 5 | 102 | | 1 | |
| | | 13-Aug-08 | muscle | -29 | 5 | 35 | 1 | | small fish |
| | | | liver | -30 | 5 | 6 | 1 | | small fish |
| | | | whole | -31 | 5 | 111 | 1 | | small fish |
| | | | muscle | -32 | 1 | 65 | 1 | | large fish |
| | | | liver | -33 | 1 | 8 | 1 | | large fish |
| Ketchum Lake | Rainbow Trout | 26-Aug-08 | muscle | -34 | 1 | 26 | 1 | | |
| | | | liver | -35 | 1 | 2 | 1 | | |
| | | | remainder | -40 | 1 | 83 | 1 | | |
| | Yellow Perch | 28-29 Aug 08 | muscle | -36 | 3 | 23 | 1 | | |
| | | | liver | -37 | 3 | 3 | 1 | | |
| | Rainbow Trout | 2-8 Sept-08 | muscle | -38* | 3 | 78 | 1 | | small fish |
| | | | liver | -39* | 3 | 5 | 1 | | small fish |
| | | 4-Sep-08 | muscle | -41 | 1 | 93 | 1 | | large fish |
| | | | liver | -42 | 1 | 10 | 1 | | large fish |
| | | | | | | Total Samples | 33 | 11 | |
| | | | | | | Lab Splits | 4 | 2 | |
| | | | | | | Total Analyses | 37 | 13 | |

*extract and analyze duplicate subsamples.

# Appendix C. Biological Data on Fish Samples Analyzed for Microcystins and Anatoxin-a

| Lake | Species | Collection Date | Sample No. (0902058-) | Total Length (mm) | Weight (grams) | Sex |
|---|---|---|---|---|---|---|
| American Lake | Kokanee | 30-May-08 | -1 and -2 | 345 | 420 | M |
| | | | | 333 | 364 | M |
| | | | | 326 | 349 | F |
| | | | | 288 | 246 | M |
| | | 11-Sep-08 | -3 and -4 | 302 | 250 | F |
| | | | | 355 | 445 | F |
| | | | | 330 | 364 | M |
| | | | | 348 | 458 | M |
| | | | | 350 | 431 | M |
| | | | -5 and -6 | 340 | 373 | M |
| | | | | 354 | 435 | M |
| | | | | 335 | 373 | F |
| | | | | 309 | 304 | M |
| | | | | 326 | 336 | M |
| Waughop Lake | Brown Bullhead | 27-Aug-08 | -7 and -8 | 220 | 138 | ind* |
| | | | | 214 | 115 | ind |
| | | | | 218 | 128 | ind |
| | | | | 220 | 135 | ind |
| | | | -9 | 230 | 140 | ind |
| | | | | 225 | 137 | ind |
| | | | | 216 | 115 | ind |
| | | | | 211 | 109 | ind |
| | | | -10 | 221 | 140 | ind |
| | | | | 226 | 136 | ind |
| | | | | 230 | 151 | ind |
| | | | | 228 | 137 | ind |
| | Largemouth Bass | 27-Aug-08 | -11 | 221 | 115 | M |
| Leland Lake | Largemouth Bass | 15-Oct-08 | -12 | 231 | 143 | F |
| | | | | 232 | 162 | M |
| | | | | 226 | 144 | M |
| | | | -22 | 340 | 749 | M |
| | | | | 400 | 1,141 | F |
| | Yellow Perch | | -13 | 202 | 105 | F |
| | | | | 187 | 87 | F |

| Lake | Species | Collection Date | Sample No. (0902058-) | Total Length (mm) | Weight (grams) | Sex |
|---|---|---|---|---|---|---|
| Leland Lake | Yellow Perch | 15-Oct-08 | | 209 | 113 | M |
| | | | | 185 | 77 | M |
| | | | | 200 | 110 | M |
| | Rainbow Trout | | -14 and -15 | 217 | 369 | M |
| | | | | 3242 | 356 | F |
| | | | | 295 | 295 | ind |
| Steilacoom Lake | Yellow Perch | 15-Oct-08 | -16 | 208 | 126 | M |
| | | | -17 | 185 | 105 | M |
| | | | -18 | 187 | 93 | M |
| | | | -19 | composite of -16, -17, and -18 | | |
| | Largescale Sucker | 15-Oct-08 | -20 | 210 | 98 | ind |
| Anderson Lake | Rainbow Trout | 27-May-08 | -23 and -24 | 330 | 415 | F |
| | | | | 300 | 228 | M |
| | | | | 305 | 354 | F |
| | | | | 343 | 453 | ind |
| | | | | 325 | 400 | M |
| | | 31-Jul-08 | -25 | 352 | 501 | M |
| | | | -26 and -27 | 184 | 75 | ind |
| | | | | 166 | 59 | ind |
| | | | | 182 | 76 | ind |
| | | | | 172 | 66 | ind |
| | | | | 180 | 71 | ind |
| | | | -28 | 160 | 46 | ind |
| | | | | 164 | 55 | ind |
| | | | | 164 | 57 | ind |
| | | | | 160 | 54 | ind |
| | | | | 160 | 51 | ind |
| | | 13-Aug-08 | -29 and -30 | 162 | 59 | ind |
| | | | | 174 | 70 | ind |
| | | | | 170 | 66 | ind |
| | | | | 178 | 75 | ind |
| | | | | 175 | 76 | ind |
| | | | -31 | 160 | 53 | ind |
| | | | | 158 | 50 | ind |
| | | | | 163 | 54 | ind |
| | | | | 158 | 56 | ind |
| | | | | 155 | 53 | ind |
| | | | -32 and -33 | 350 | 500 | M |

| Lake | Species | Collection Date | Sample No. (0902058-) | Total Length (mm) | Weight (grams) | Sex |
|---|---|---|---|---|---|---|
| Ketchum Lake | Rainbow Trout | 26-Aug-08 | -34, -35, and -40 | 271 | 205 | F |
| | Yellow Perch | 28-29 Aug-08 | -36 and -37 | 170 | 65 | M |
| | | | | 149 | 46 | M |
| | | | | 160 | 61 | M |
| | Rainbow Trout | 2-8 Sept-08 | -38 and -39 | 235 | 162 | F |
| | | | | 290 | 258 | F |
| | | | | 288 | 237 | F |
| | | 4-Sep-08 | -41 and -42 | 450 | 1,040 | F |

*ind = indeterminate.

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# Appendix D. Microcystin Results from Dr. John Berry, Florida International University

| Sample | Tissue | | Replicate 1 | | Replicate 2 | | Average (ng/g) | RPD (%) |
|---|---|---|---|---|---|---|---|---|
| | | TissMass (g) | [MC] (ng/mL) | ng/g | [MC] (ng/mL) | ng/g | | |
| 3 | Muscle | 3.58 | 3.9 | 43.7 | 5.5 | 62.0 | 52.9 | 34.7 |
| 5 | Liver | 2.75 | 5.1 | 73.7 | 5.2 | 75.5 | 74.6 | 2.4 |
| 7A | Muscle | 4.85 | 0.2 | 1.6 | 0.1 | 1.2 | 1.4 | 27.6 |
| 7B | Muscle | 4.09 | 0.1 | 0.9 | 0.1 | 0.7 | 0.8 | 27.6 |
| 8A | Liver | 2.40 | 2.3 | 38.1 | 2.3 | 37.6 | 37.9 | 1.2 |
| 8B | Liver | 4.00 | 0.9 | 8.7 | 0.9 | 9.5 | 9.1 | 8.3 |
| 9 | Muscle | 5.75 | 0.1 | 0.8 | 0.2 | 1.2 | 1.0 | 41.0 |
| 10 | Muscle | 5.46 | 1.2 | 8.9 | 1.6 | 12.1 | 10.5 | 30.6 |
| 11 | Muscle | 4.05 | 1.9 | 19.1 | 1.5 | 14.8 | 16.9 | 25.8 |
| 12 | Muscle | 4.21 | 1.6 | 15.5 | 1.1 | 10.8 | 13.1 | 35.3 |
| 13 | Muscle | 3.65 | 2.4 | 26.4 | 2.2 | 24.1 | 25.3 | 9.1 |
| 14 | Muscle | 3.69 | 0.1 | 1.5 | 0.1 | 1.6 | 1.5 | 5.9 |
| 15 | Liver | 2.52 | 4.2 | 66.0 | 5.3 | 84.6 | 75.3 | 24.6 |
| 16 | Muscle | 4.28 | 2.8 | 25.9 | 1.8 | 16.5 | 21.2 | 44.0 |
| 17 | Muscle | 4.73 | 1.6 | 13.3 | 1.0 | 8.7 | 11.0 | 41.1 |
| 18 | Muscle | 4.09 | 3.3 | 32.3 | 3.3 | 31.9 | 32.1 | 1.2 |
| 19 | Liver | 3.45 | 3.4 | 39.2 | 4.8 | 56.1 | 47.7 | 35.3 |
| 20 | Muscle | 4.90 | 2.8 | 23.2 | 2.5 | 20.0 | 21.6 | 14.5 |
| 22 | Muscle | 4.31 | 2.9 | 26.9 | 3.1 | 28.3 | 27.6 | 4.8 |
| 23 | Muscle | 3.52 | 0.3 | 3.8 | 2.3 | 25.6 | 14.7 | 148.1 |
| 24 | Liver | 3.81 | 5.7 | 59.6 | 6.6 | 69.3 | 64.5 | 15.1 |
| 29 | Muscle | 4.72 | 0.1 | 1.1 | 0.2 | 2.0 | 1.5 | 60.5 |
| 30 | Liver | 2.41 | 6.7 | 111.3 | 4.9 | 81.1 | 96.2 | 31.4 |
| 31 | Whole | 4.56 | 5.2 | 45.6 | 5.9 | 51.7 | 48.6 | 12.7 |

| Sample | Tissue | | Replicate 1 | | Replicate 2 | | Average (ng/g) | RPD (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | TissMass (g) | [MC] (ng/mL) | ng/g | [MC] (ng/mL) | ng/g | | | | |
| 32 | Muscle | 3.79 | 0.1 | 1.1 | 0.1 | 0.7 | 0.9 | 44.8 | | |
| 33 | Liver | 3.97 | 4.1 | 40.8 | 3.8 | 38.2 | 39.5 | 6.6 | | |
| 34 | Muscle | 3.45 | 0.2 | 2.7 | 0.1 | 1.7 | 2.2 | 44.8 | | |
| 35 | Liver | 1.52 | 3.3 | 88.1 | 2.6 | 67.9 | 78.0 | 25.8 | | |
| 36 | Muscle | 4.67 | 4.4 | 38.0 | 4.5 | 38.4 | 38.2 | 1.2 | | |
| 37 | Liver | 1.30 | 5.4 | 166.2 | 5.6 | 171.3 | 168.8 | 3.0 | | |
| 38A | Muscle | 5.23 | 0.2 | 1.6 | 0.1 | 0.7 | 1.1 | 79.6 | | |
| 38B | Muscle | 3.98 | 0.5 | 5.5 | 0.4 | 3.6 | 4.5 | 42.9 | | |
| 39A | Liver | 2.33 | 5.0 | 85.6 | 5.5 | 95.4 | 90.5 | 10.9 | | |
| 39B | Liver | 4.16 | 0.7 | 7.1 | 0.2 | 2.4 | 4.8 | 100.2 | | |
| 40 | Carcass | 3.34 | 6.2 | 73.6 | 5.6 | 67.2 | 70.4 | 9.1 | | |
| 41 | Muscle | 5.07 | 1.5 | 11.5 | 2.1 | 16.2 | 13.9 | 34.1 | | |
| 42 | Liver | 3.65 | 0.6 | 6.1 | 0.8 | 8.3 | 7.2 | 31.2 | | |
| QC Samples: | Tissue | TissMass (g) | Replicate 1 | | Replicate 2 | | Average (ng/g) | Average | RPD (%) | % Recovery |
| | | | [MC] (ng/mL) | ng/g (or ng) | [MC] (ng/mL) | ng/g (or ng) | | | | |
| Unspiked Tissue | Muscle | 2.16 | 4.2 | 77.9 | 5.0 | 93.1 | 85.5 | 86.2 | 1.6 | |
| | Muscle | 1.89 | 4.0 | 83.9 | 4.2 | 89.9 | 86.9 | | | |
| Matrix Spike | Muscle | 1.98 | 4.5 | 91.1 | 5.5 | 111.1 | 101.1 | 94.8 | 13.3 | 37.7 |
| Matrix Spike Duplicate | Muscle | 2.42 | 5.8 | 95.5 | 4.9 | 81.5 | 88.5 | | | |
| Spike Blank | None | NA | 0.6 | 25.6 | 0.6 | 23.2 | 24.4 | | | 48.8 |

MC = microcystins.

# Appendix E. Acronyms and Units of Measurement

## Acronyms

| | |
|---|---|
| Ecology | Washington State Department of Ecology |
| EIM | Environmental Information Management database |
| ELISA | Enzyme-linked immunosorbent assay |
| FIU | Florida International University |
| LC/MS | Liquid chromatography/mass spectrometry |
| QA/QC | Quality assurance and quality control |
| RPD | Relative percent difference |
| SOP | Standard operating procedures |
| WDFW | Washington Department of Fish and Wildlife |

## Units of Measurement

| | |
|---|---|
| g | gram, a unit of mass |
| Kg | kilograms (1,000 grams) |
| mL | milliliters |
| mm | millimeters |
| N | number of samples |
| ng | nanogram (one billionth of a gram) |
| ug/Kg | micrograms per kilogram (parts per billion) |
| µg/L | micrograms per liter (parts per billion) |