DEPARTMENT OF ECOLOGY
State of Washington

Addendum to Quality Assurance Project Plan

Prevalence and Persistence of Cyanotoxins in Lakes of the Puget Sound Basin

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Data for this project will be available on Ecology's Environmental Information Management (EIM) website at EIM Database. Search Study ID WHOB008.

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Addendum to Quality Assurance Project Plan

Prevalence and Persistence of Cyanotoxins in Lakes of the Puget Sound Basin

January 2020

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Signatures are not available on the Internet version.
EAP: Environmental Assessment Program

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3.0 Background

3.1 Introduction and problem statement

In summer 2018, the Toxics Studies Unit of the Washington State Department of Ecology (Ecology) conducted a field study to continuously monitor algal pigments in a local Puget Sound lake known to experience cyanobacterial blooms (Appendix A). The goal was to assess the feasibility of using continuous monitoring of algal pigments through in-situ fluorometry as a tool for gaging the onset and seasonal dynamics of cyanobacterial blooms.

The field study was part of a larger study examining the prevalence and persistence of cyanotoxins in the sediments and waters of Puget Sound lakes (Hobbs 2018). Cyanobacterial blooms may produce toxins that are harmful to the health of humans, pets, and wildlife. Real-time monitoring of algal pigments using fluorometry has potential to be a useful early-warning tool for lake managers.

This addendum describes continued field testing of fluorometric technology as a tool for monitoring cyanobacteria in lakes.

Sections of the original QAPP that do not require changes are not included in this addendum.

3.2 Study area and surroundings

3.2.2 Summary of previous studies and existing data

As part of the 2018 field study, a multiparameter monitoring instrument (YSI EXO3 sonde) was deployed in Black Lake, Olympia from June through October. Hourly measurements of fluorescence from chlorophyll *a* (a photosynthetic pigment in algae), fluorescence from phycocyanin (an accessory pigment in freshwater cyanobacteria), water temperature, dissolved oxygen, pH, and conductivity were recorded.

During each biweekly site visit, surface water grab samples for laboratory analyses of chlorophyll *a* were collected. The samples were collected concurrently with measurement readings from the YSI to compare grab sample results with instrument results.

A strong linear relationship was found between laboratory measurements of chlorophyll *a* and field measurements of phycocyanin (R^2=0.968, $p<0.01$; Figure 1). Curiously, no correlation was found between laboratory and field measurements of chlorophyll *a* (R^2=-0.281, p=0.28). In terms of seasonal trends, phycocyanin fluorescence began to gradually increase in August to a high in October, which corresponded with visible observations of lake surface water blooms.

Questions arising from this study include:

- Would similar correlative patterns between YSI measurements and chlorophyll *a* grab samples be observed in a different lake with different bloom dynamics?

- What types of algal species are present before, during, and after the surface water bloom?
- Can continuous monitoring of algal pigments and other water quality variables be used to predict the onset and levels of toxins associated with cyanobacterial blooms?

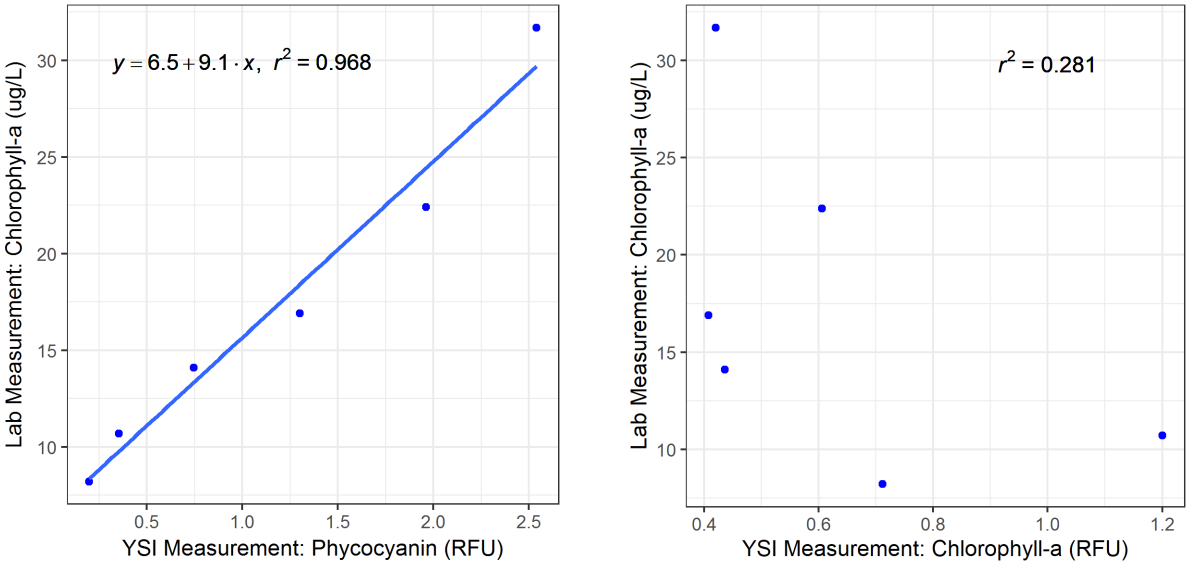

Figure 1. Left: XY graph showing relationship between phycocyanin fluorescence and laboratory-measured chlorophyll *a*. Right: XY graph showing lack of relationship between chlorophyll *a* fluorescence and laboratory-measured chlorophyll *a*. Data were collected from Black Lake, Olympia in summer 2018.

4.0 Project Description

4.1 Project goal

The project goal is to further assess the feasibility of using *in-situ* fluorometry of algal pigments as a tool for continuously monitoring toxic cyanobacterial blooms.

4.2 Project objectives

Project objectives are to:

- Deploy one multiparameter sonde equipped with a fluorometric algae sensor in a Puget Sound lake known to experience cyanobacterial blooms that produce toxins (microcystin or anatoxin-a).
- Collect 10 surface water grab samples for laboratory analyses of chlorophyll *a*, phycocyanin, and microcystin, and anatoxin-a concentrations.
- Collect phytoplankton samples for identification of cyanobacteria and other algae.
- Determine seasonal trends and correlations between water quality variables.

4.4 Tasks required

The tasks required include:

- Coordinate with analytical laboratories.
- Install sonde at secure location in the selected lake from early summer to late fall.
- Conduct biweekly site visits.
- Review and assess data quality for laboratory and continuous monitoring data.
- Enter algal pigment and cyanotoxin data into Ecology's EIM.
- Conduct data analysis and prepare final report.

5.0 Organization and Schedule

5.1 Key individuals and their responsibilities

Table 1. Organization of project staff and responsibilities.

Staff	Title	Responsibilities
Jessica Archer SCS, EAP Phone: 360-407-6698	EAP Client and Section Manager for the Project Manager	Clarifies scope of the project. Provides internal review of the QAPP addendum and approves the final addendum.
Siana Wong TSU, SCS Phone: 360-407-6432	Project Manager	Writes the QAPP addendum. Oversees field sampling and transportation of samples to the laboratory. Conducts QA review of data, analyzes and interprets data, and enters data into EIM. Writes the draft report and final report.
William Hobbs TSU, SCS Phone: 360-407-7512	Principal Investigator	Helps write QAPP addendum and final report, and serves as senior scientist for the project addendum.
Holly Young WCC, EAP Phone: 360-407-6022	Field Assistant	Helps collect samples and records field information.
James Medlen TSU, SCS Phone: 360-407-6194	Unit Supervisor for the Project Manager	Provides internal review of the QAPP addendum, approves the budget, and approves the final addendum.
Alan Rue Manchester Environmental Laboratory Phone: 360-871-8801	Director	Reviews and approves the final QAPP addendum.
Francis Sweeney King County Environmental Lab Phone: 206-477-7117	Director, Aquatic Toxicology	Reviews draft QAPP addendum, coordinates with Project Manager. Analyzes water samples for microcystin and anatoxin-a.
Robin Matthews Institute for Watershed Studies, WWU Phone: 360-650-3507	Director	Reviews draft QAPP addendum, coordinates with Project Manager. Analyzes water samples for chlorophyll *a* and phycocyanin.
Arati Kaza Phone: 360-407-6964	Quality Assurance Officer	Reviews and approves the draft QAPP addendum and the final addendum.

EAP=Environmental Assessment Program; EIM=Environmental Information Management database;
SCS= Statewide Coordination Section; TSU=Toxic Studies Unit; QAPP=Quality Assurance Project Plan;
WCC= Washington Conservation Corp; WWU=Western Washington University

5.4 Proposed project schedule

Table 2. Proposed schedule for field and laboratory work, EIM data entry, and final report.

Work type	Due date	Lead staff
Field and laboratory work		
Field work completed	December 2019	Siana Wong
Laboratory analyses completed	December 2019	n/a
Environmental Information System (EIM) database		
EIM data loaded [1]	April 2020	Siana Wong
EIM data entry review [2]	May 2020	To be determined
EIM complete [3]	June 2020	Siana Wong
Final report		
Draft due to supervisor	June 2020	S. Wong / W. Hobbs
Draft due to client/peer reviewer	July 2020	S. Wong / W. Hobbs
Final (all reviews done) due to publications coordinator	August 2020	S. Wong / W. Hobbs
Final report due on web	October 2020	n/a

5.5 Budget and funding

Table 3. Estimated budget for laboratory analyses.

	Number of Samples	Number of Field QC Samples	Total Number of Samples	Cost Per Sample	Contract Lab Subtotal
Chlorophyll *a*	10	4*	14	$30	$300
Chlorophyll *a* (laboratory split)	10	-	10	$50	$500
Phycocyanin	10	4	14	$20	$200
Microcystin	10	1	11	50	$550
Anatoxin-a	10	1	11	100	$1,100
				GRAND TOTAL:	**$2,650**

*IWS, the lab that will analyze chlorophyll *a* and phycocyanin does not charge for field QC samples.

6.0 Quality Objectives

6.2 Measurement quality objectives

Measurement quality objectives (MQOs) for all laboratory analytes and field measurements are listed in Tables 4 and 5, respectively.

Table 4. Measurement quality objectives for laboratory analytes.

Parameter	Verification Standards (% Recovery Limits)[a]	Field Blank	Spiked Blank (% Recovery Limits)	Duplicate Samples (RPD)	Matrix Spikes (% Recovery Limits)	Matrix Spike-Duplicates(RPD)	Lowest Concentrations of Interest (ug/L)
Chlorophyll *a*	-	<Reporting Limit	-	20	-	-	0.1 ug/L
Phycocyanin	-	<Reporting Limit	-	20	-	-	8.0 ug/L
Microcystin	PC 70 – 130	-	60 – 140	0 – 45	50 – 150	0 – 45	0.15 ug/L
Anatoxin-a	PC 70 – 130	-	50 – 150	0 – 45	50 – 150	0 – 45	0.01 ug/L

[a]Verification Standards include: LCS=Laboratory Control Sample; CRM=Certified Reference Materials; CCV=Continuing Calibration Verification standard; PC=Positive Control

Table 5. Measurement quality objectives for YSI sonde calibration checks.

Parameter	Units	Accept	Qualify	Reject
Chorophyll *a*	RFU[a]	< or = ± 1.0	> ± 1.0 and < or = ± 2.0	> ± 2.0
Phycocyanin	RFU	< or = ± 1.0	> ± 1.0 and < or = ± 2.0	> ± 2.0
pH	std. units	< or = ± 0.2	> ± 0.2 and < or = ± 0.8	> ± 0.8
Conductivity	uS/cm	< or = ± 5%	> ± 5% and < or = ± 15%	> ± 15%
Temperature	° C	< or = ± 0.2	> ± 0.2 and < or = ± 0.8	> ± 0.8
Dissolved Oxygen	% saturation	< or = ± 5%	> ± 5% and < or = ± 15%	> ± 15%
Dissolved Oxygen	mg/L	< or = ± 0.3	> ± 0.3 and < or = ± 0.8	> ± 0.8

[a]RFU=Relative Fluorescence Unit

7.0 Study Design

7.1 Study boundaries

The study will take place in Pierce County on Spanaway Lake. Spanaway Lake was selected because of its popularity as a water contact recreational area and because it is known to experience regular annual occurrences of toxic cyanobacterial blooms.

7.2 Field data collection

7.2.1 Sampling locations and frequency

A YSI sonde will be deployed at a secure, fixed location in Spanaway Lake on the north shore where surface blooms accumulate. The sonde will be set up to collect hourly water quality measurements from early summer to late fall. Surface water grab samples will be collected at the same location as the sonde every two weeks during scheduled site visits.

7.2.2 Field parameters and laboratory analytes to be measured

Field parameters to be measured by the YSI sonde are chlorophyll *a* fluorescence, phycocyanin fluorescence, water temperature, dissolved oxygen, pH, and conductivity.

Algae samples will be collected using a plankton net, and species will be identified using a light microscope at Ecology's Lacey headquarters.

Analytes to be collected as surface water grab samples and measured in the laboratory are chlorophyll *a*, phycocyanin, microcystin, and anatoxin-a.

7.4 Assumptions in relation to objectives and study area

Cyanobacterial blooms in Washington's freshwater lakes typically peak during the mid- to late-summer period. To ensure that a wide range of algal pigment concentrations are captured during monitoring, the field study period will be early summer through late fall. The study assumes that cyanotoxins will be present during cyanobacterial blooms in Spanaway Lake during this targeted monitoring period.

7.5 Possible challenges and contingencies

7.5.1 Logistical problems

The main logistical problem is to find a secure location along the shore of the lake to install the sonde. To remedy this, Ecology will work in agreement with private lake shoreline landowner(s) willing to grant lake access for installing the sonde and collecting water samples.

7.5.2 Practical constraints

There are no foreseeable practical constraints for this study.

7.5.3 Schedule limitations

Limitations include the QAPP addendum review timeline. To ensure that the field study does not miss the bloom cycle, sonde deployment would need to be initiated for sampling in June. An approval to begin work form would be necessary prior to any work being done.

8.0 Field Procedures

8.2 Measurement and sampling procedures

Procedures for collecting surface water grab samples and sonde measurements will follow guidelines in Ecology's SOPs:

- EAP015 – Manually Obtaining Surface Water Samples, Version 1.3 (Urmos-Berry, 2016).
- EAP033 – Hydrolab® DataSonde® and MiniSonde® Multiprobes, Version 2.1 (Anderson, 2016).

Specific field procedures are summarized below.

Continuous Monitoring

At a secure location on the north shore of Spanaway Lake, a calibrated multiparameter sonde (YSI EXO 3) will be installed, with the sensors deployed to within 0.3 m below the water surface. The sonde will be set up to collect hourly measurements. The following sonde maintenance tasks will be performed during each site visit:

1. Download data from the sonde as a backup. Upload to laptop computer.
2. Verify accuracy of data collection (e.g., no missing hours).
3. Perform a post-calibration check to determine if any drift has occurred.
4. Clean the sonde of any biofouling.
5. Re-calibrate sensors and replace batteries if necessary.
6. Collect and record measurements of current water quality conditions (concurrent with surface water grab sample collection).
7. Re-deploy sonde.

Surface Water Grab Samples

During each site visit, surface water grab samples will be collected for chlorophyll-*a*, phycocyanin, anatoxin-a, and microcystin. Chlorophyll *a* and phycocyanin samples will be collected first by triple-rinsing a clean 2-L amber wide-mouthed Nalgene "transfer" bottle with site water. The bottle will then be submerged to within 0.3 m below the water surface and filled with water adjacent to the sonde sensors. Water from the bottle will then be mixed, and transferred to one sample bottle for analyses of chlorophyll *a* and phycocyanin.

A laboratory split sample for chlorophyll *a* will also be collected during each visit. The sample will be collected by mixing, measuring, and field filtering remaining water from the transfer bottle. The resulting filter will be analyzed by Manchester Environmental Laboratory (MEL) in Port Orchard.

Microcystin and anatoxin-a samples will be collected by submerging one sample bottle to within 0.3 m below the water surface, gently scooping up water, and then capping. The sample bottle will be filled with minimal headspace.

Chlorophyll *a*, microcystin, and anatoxin-a samples will be stored in a cooler on ice to <4°C. All samples will be shipped on the same day overnight to the relevant laboratories.

YSI field measurements will also be recorded at the same time, location, and depth as grab sample collections.

Phytoplankton net tow

To supplement the algal pigment and cyanotoxin sample collection, samples for algae identification will be collected. A phytoplankton net with 20µm mesh will be drawn horizontally within 0.3 m below the water surface about 10 – 20 times to get a concentrated sample of algae. Algae samples will be preserved with 10% formalin at a concentration of 1 parts formalin to 9 parts water. Phytoplankton will be identified down to genus or species (if possible) under light microscopy using multiple taxonomic resources (John et al. 2002; Wehr and Sheath 2003; Matthews 2016) and online resources (AlgaeBase, PhycoKey, Diatoms of North America).

8.3 Containers, preservation methods, holding times

Table 6. Sample containers, preservation, and holding times.

Parameter	Matrix	Minimum Quantity Required	Container	Preservative	Holding Time
Chlorophyll *a*	Water	400 mL	500 mL amber polyethylene bottle	Cool to 4°C, Overnight Shipping	28 days after filtered and frozen
Chlorophyll *a* (laboratory split)	Water	250 – 1000 mL, filtered	Field filter in glass tube	Acetone	30 days after filtered and frozen
Phycocyanin	Water	400 mL	500 mL amber polyethylene bottle	Cool to 4°C, Overnight Shipping	60 days after frozen
Microcystin ELISA – Abraxis ADDA	Water	100 mL	250 mL amber glass bottle	Cool to 4°C, Overnight Shipping	48 hours to freeze, 7 days after frozen
Anatoxin-a	Water	100 mL	250 mL glass (amber, wide-mouth)	Cool to 4°C, Overnight Shipping	48 hours to freeze or acidify, 28 after frozen or acidified

8.5 Sample ID

Laboratory sample IDs will be assigned by the relevant laboratories: King County Environmental Laboratory (KCEL), Institute for Watershed Studies (IWS) at Western Washington University, and MEL. Field IDs will be assigned by the project manager.

9.0 Laboratory Procedures

9.1 Lab procedures table

Table 7. Measurement methods (laboratory).

Analyte	Sample Matrix	Samples	Expected Range of Results	Detection or Reporting Limit	Sample Prep Method	Analytical (Instrumental) Method	Laboratory
Chlorophyll *a*	Water	10	<Reporting Limit – 20 *u*g/L	0.1 *u*g/L	APHA (2012) #10200 H; IWS SOP 12	APHA (2012) #10200 H; IWS SOP 12	IWS[a]
Chlorophyll *a* (laboratory split)	Water	10	<Reporting Limit – 20 *u*g/L	0.1 *u*g/L	SM10200H1	SM10200H3	MEL[b]
Phycocyanin	Water	10	<Reporting Limit – 20 *u*g/L	8 *u*g/L	EPA (2017)	EPA (2017); Kasinak et al. (2015)	IWS
Microcystin	Water	10	<Reporting Limit – 4000 *u*g/L	0.15 *u*g/L	KCEL SOP #465	ELISA-abraxis ADDA (KCEL SOP #465)	KCEL[c]
Anatoxin-a	Water	10	<Reporting Limit – 100 *u*g/L	0.01 *u*g/L	KCEL SOP #466	LC/MS/MS (KCEL SOP #466, Oehrle et al. 2010)	KCEL

[a]IWS = Institute for Watershed Studies
[b]MEL = Manchester Environmental Laboratory
[c]KCEL = King County Environmental Laboratory

9.3 Special method requirements

This addendum includes non-standardized methods for analysis of phycocyanin. IWS has experience with and is currently set up to perform benchtop fluorometric analyses of phycocyanin. Because phycocyanin degrades quickly, samples submitted to IWS will be shipped overnight and stored frozen within 24 hours.

9.4 Laboratories accredited for methods

IWS will analyze phycocyanin and chlorophyll *a* samples pending approval of a completed laboratory accreditation waiver. IWS is an Ecology-accredited laboratory with many years of experience working with university research groups and government agencies. IWS is not currently accredited for phycocyanin and chlorophyll *a*. However, IWS has many years of experience with chlorophyll *a* analysis using EPA standard methods and is the only known regional laboratory set up to analyze phycocyanin.

For each sampling event, a laboratory split for chlorophyll *a* will be also collected and analyzed by MEL, which is accredited for chlorophyll *a*.

KCEL is an Ecology-accredited laboratory and is accredited for analyses of microcystins and anatoxin-a in potable and non-potable water.

10.0 Quality Control Procedures

10.1 Table of field and laboratory quality control

Table 8. Quality control samples, types, and frequency.

Parameter	Field		Laboratory			
	Blanks	Replicates	Check Standards	Method Blanks	Analytical Duplicates	Matrix Spike/Matrix Spike Duplicate
Chlorophyll *a*	2	2	1/batch	1/batch	1/batch	-
Phycocyanin	2	2	1/batch	1/batch	1/batch	-
Microcystin	-	1	1/batch[1]	1/batch	1/batch	1/batch
Anatoxin-a	-	1	1/batch[1]	1/batch	1/batch	1/batch

[1]Check standard performed as a positive control.

12.0 Audits and Reports

12.3 Frequency and distribution of reports

Results from this addendum will be published in the form of a final report.

12.4 Responsibility for reports

The project manager will be responsible for the final report.

14.0 Data Quality (Usability) Assessment

14.3 Data analysis and presentation methods

Continuous monitoring data will be plotted as seasonal trends. Summary statistics (mean, minimum, maximum) will also be calculated. Data collected from the sonde will be compared to data from the laboratory-analyzed grab samples. Exploratory analyses, such as scatterplot matrices and correlation tables, can be used to examine relationships among all water quality variables.

14.4 Sampling design evaluation

The sample design, including duration of monitoring, number and type of grab samples collected, and field collection procedures, is expected to be sufficient to draw conclusions and accomplish the goals and objectives of this addendum.

15.0 References

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16.0 Appendices

Appendix A.

Environmental Assessment Program Note: Lake Blue-Green Algae – Continuous Monitoring 2018 (separate attachment).