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Addendum 2 to Quality Assurance Project Plan

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

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

November 2020

Approved by:

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

Note: The numbered headings in this document correspond to the headings used in the original QAPP. Only relevant sections are included. Therefore, some numbered headings are missing, and the text begins at 3.0.

3.0 Background

3.1 Introduction and Problem Statement

During the summer and fall of 2019, the Washington State Department of Ecology (Ecology) deployed a multi-parameter water quality data logger (sonde) in Spanaway Lake, Pierce County (Wong and Hobbs, 2020). The main goal of the study was to establish a relationship between sonde measurements and cyanobacteria harmful algae bloom (cyanoHABs) events that could be used as a predictive tool. A fluorometric probe on the sonde allowed for the continuous measurement of phycocyanin, the main pigment in cyanobacteria. Microscopic analysis showed that the dominant cyanobacteria during the cyanoHAB blooms were *Microcystis* sp., *Dolichospermum* sp., and *Woronichinia* sp. The main cyanotoxin produced during the blooms was microcystin.

In lakes that experience recurrent cyanoHABs, a common question is whether the presence of cyanotoxins is a relatively recent phenomenon (~ last 20-30 years). The analysis of lake sediment cores has been used to decipher the historical prevalence of cyanobacteria through time (about the last 100 years) (Zastepa et al., 2017). In the summer of 2018, Hobbs and Wong (2019) recovered a sediment core from Anderson Lake, Jefferson County. Using algal pigment remains they were able to show that cyanobacteria have been a dominant feature in the lake phytoplankton over the last 300 years. The management implications of this work are that any future strategies to reduce cyanoHABs need to acknowledge the naturally high concentrations of nutrients in the lake.

Building on the previous study of Spanaway Lake in 2019 (Wong and Hobbs, 2020), this project will assess the history of cyanobacteria in Spanaway Lake using a lake sediment core. The study will use the same algal proxies as the Anderson Lake study (Hobbs and Wong, 2019) and also analyze the sediments directly for the cyanotoxin, microcystin. In addition, sediment will be analyzed for the presence of cyanotoxin-producing genes.

3.2 Study area and surroundings

Spanaway Lake is a natural kettle lake located in Pierce County, WA (Figure 1). The lake is approximately 1 km^2 in area with a maximum depth of around 8.5 m. The main surface water inlet originates from a wetland at the south end of the lake. The main outlet is Spanaway Creek at the north end of the lake. The hydrology is dominated by groundwater inputs to the lake (Pierce County 2017). Development around the lake is largely residential, with more than 170 single family homes and 160 multi-family residences surrounding the lake (Pierce County 2017). A public park with a boat launch and swimming beach occupies the northeastern end of the lake. Popular recreational activities include boating, swimming, and fishing.

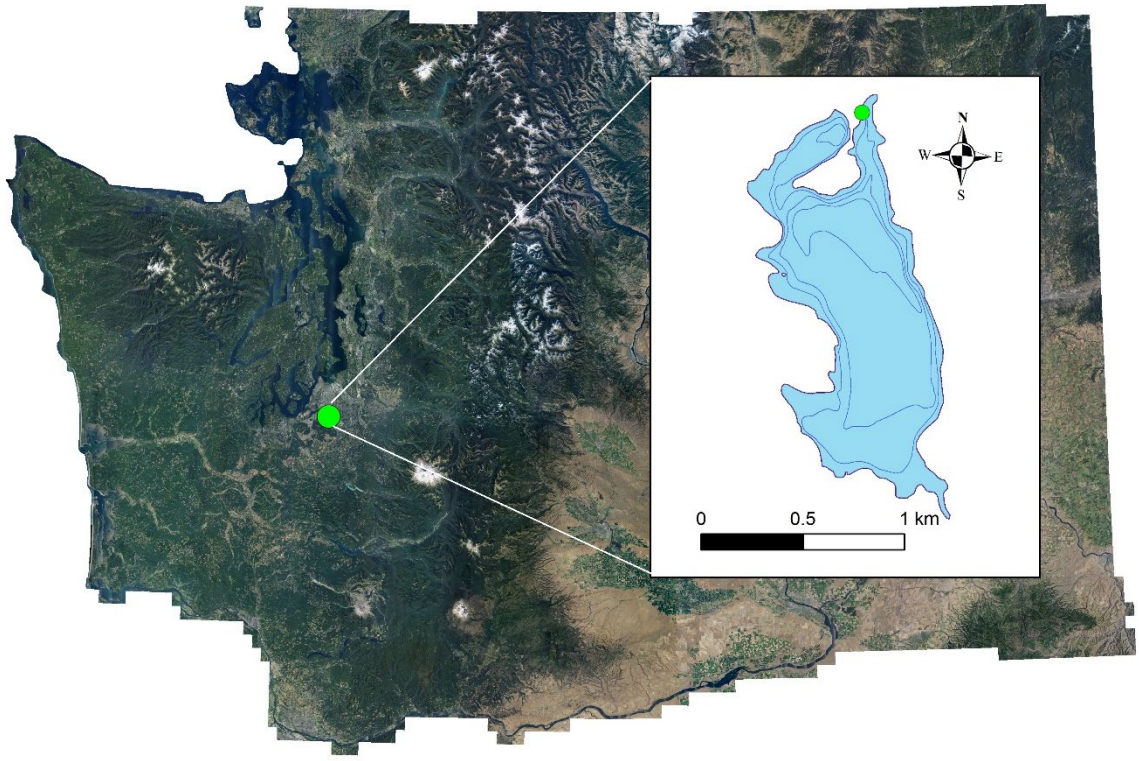

Figure 1: Site location map. Inset map shows location of sampling site (green dot) for the 2019 study (Wong and Hobbs, 2020).

3.2.3 Summary of previous studies and existing data

Spanaway Lake has experienced blooms of toxin-producing cyanobacteria (Figure 2). Nutrient inputs to the lake and internal nutrient cycling, particularly phosphorus, are likely the main drivers of the blooms (Pierce County 2017). Microcystin levels exceeding WA Department of Health's guideline of 6 µg/L have led to Pierce County's issuance of caution advisories and lake closures every year over the past decade. Between 2006 and 2016, the number of days per year that the lake was under health advisory due to toxic blooms averaged 179 days, ranging from 64 to 318 days (Pierce County 2016). From 2007 to present, microcystin concentrations in samples collected from the lake that exceeded the health guideline ranged from 6.4 to 6,279 µg/L. Cyanobacteria blooms are typically most productive in mid to late summer.

Figure 2: Photo of cyanobacteria bloom on Spanaway Lake, September 12, 2019.

A 2018 study of Spanaway Lake shoreline sediments found that microcystin can infiltrate into shoreline sediments and porewaters from the surface water during a cyanoHAB bloom (Preece et al., 2020). Furthermore, microcystins can adhere and persist in the shoreline sediments following subsidence of the bloom in the surface waters (Figure 3). Based on human health risk modeling, it does not appear that the accumulation of microcystin in lakeshore sediments and porewater pose a human health concern.

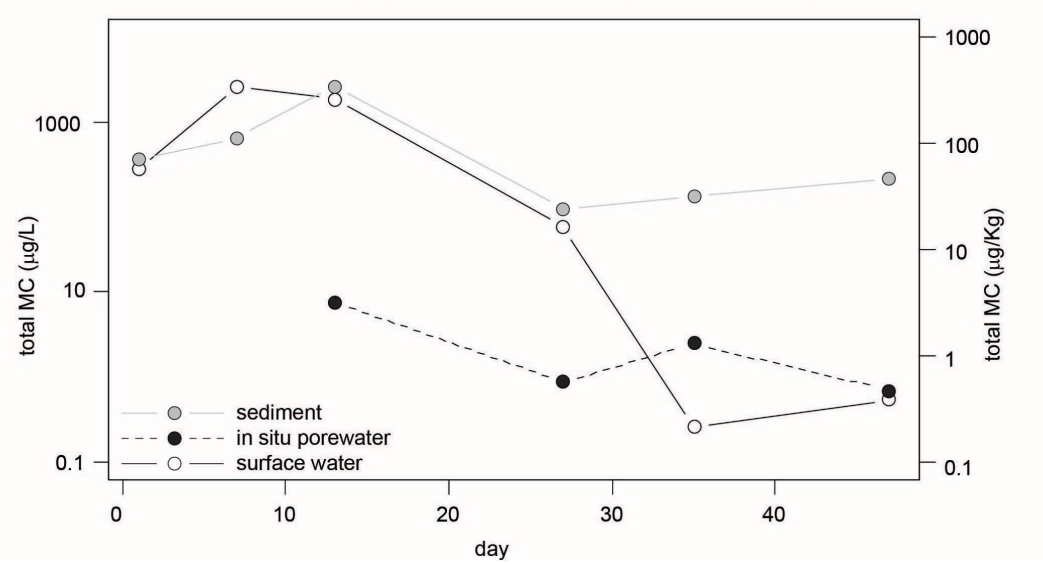

Figure 3: Microcystin at the shoreline of Spanaway Lake.

Microcystin concentrations in sediments (µg/Kg), porewater (µg/L) and surface waters (µg/L) (as measured by ELISA using log scale) over 47 days at Spanaway Lake north beach.

During the Wong and Hobbs (2020) study, USEPA and Ecology collected and analyzed additional samples for DNA (Lu, 2019; Lu et al., 2020), to complement Ecology's continuous cyanobacteria pigment measurements. This was an exploratory analysis for the genes found to produce microcystin (*unpublished data*). Results from this pilot work suggested a complex relationship between microcystin concentrations in the water and the presence of microcystin genes (McyA and McyE). There appears to be a strong power (log-log) relationship at low to moderate microcystin concentrations (0.15 – 120 µg/L), however at very high microcystin concentrations (>2000 µg/L) the microcystin gene copies are at the lowest abundance. Further data analysis with the USEPA ORD lab is necessary to understand these observations.

4.0 Project Description

4.1 Project goals

The goal of this project is to establish the historical prevalence of cyanobacteria in Spanaway Lake, Pierce County using a dated sediment core.

4.2 Project objectives

The objective of this study is to assess the trends in concentrations of algal pigments, microcystin variants and microcystin genes in sediment subsamples representing the last ~150 years.

4.4 Tasks required

Specific tasks under this project include the following:

- Coordinate sampling with the Toxic Studies Unit – PBT Monitoring group for shared sampling goals.
- Collect a sediment core from the deepest point in Spanaway Lake and subsample immediately following recovery.
- Apportion the sediment subsamples for various analyses.
- Freeze or freeze dry all sediment samples depending on analysis.
- Submit samples to the laboratories.
- Establish an age-depth relationship for the sediment core.
- Review and assess data quality and laboratory results.
- Write a report documenting the historical prevalence of cyanobacteria in Spanaway Lake.

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	Provides internal review of the QAPP and approves the final QAPP.
William Hobbs, PhD TSU, SCS Phone: 360-407-7512	Project Manager	Writes the QAPP. Oversees field sampling and transportation of samples to the laboratory. Conducts QA review of data, analyzes and interprets data, and enters data into EIM. Manages receives analytical results from all labs (see Section 9.4). Writes the draft report and final report.
Callie Mathieu and Jakub Bednarek TSU, SCS Phone: 360-407-6965 (Mathieu)	Project Scientists	Helps collect samples and records field information. Assists with collection permits.
James Medlen TSU, SCS Phone: 360-407-6194	Unit Supervisor for the Project Manager	Provides internal review of the QAPP, approves the budget, and approves the final QAPP.
Alan Rue Manchester Environmental Laboratory Phone: 360-871-8801	Director	Reviews and approves the final QAPP. Analyzes water samples for supplemental nutrient parameters.
Francis Sweeney King County Environmental Lab Phone: 206-477-7117	Director, Aquatic Toxicology	Reviews draft QAPP, coordinates with Project Manager. Analyzes sediment samples for microcystins.
Rochelle Labiosa US Environmental Protection Agency Phone: 206-553-1172	Region 10 Project Manager - Innovation Grant	Reviews draft QAPP, coordinates with Project Manager for the analysis of sediments for microcystin genes.
Arati Kaza Phone: 360-407-6964	Ecology Quality Assurance Officer	Reviews the draft QAPP and approves the final QAPP. May comment on the final report.

EAP: Environmental Assessment Program; EIM: Environmental Information Management database;
QAPP: Quality Assurance Project Plan; SCS: Statewide Coordination Section; TSU: Toxic Studies Unit

5.4 Proposed project schedule

The proposed project schedule assumes no further delays due to compliance with Ecology's Safe Start Plan with respect to the COVID pandemic; see section *7.5 Possible challenges and contingencies*.

Table 2. Proposed schedule for completing field and laboratory work, data entry into the Environmental Information Management (EIM) database, and reports.

Field and laboratory work	Due date	Lead staff
Field work completed	November 2020	William Hobbs
Laboratory analyses completed	April 2021	
Environmental Information Management (EIM) database		
EIM Study ID	WHOB008	
Product	Due date	Lead staff
EIM data loaded	April 2021	TBD
EIM data entry review	May 2021	William Hobbs
EIM complete	June 2021	TBD
Final report		
Author lead / support staff	William Hobbs	
Schedule		
Draft due to supervisor	June 2021	
Draft due to client/peer reviewer	July 2021	
Final (all reviews done) due to pub coordinator	August 2021	
Final report due on web	September 2021	

5.5 Budget and funding

The detailed budget for the laboratory expenses is outlined in Table 3. All laboratory contracts are handled by the project manager.

Table 3. Detailed project budget and funding.

	Number of samples	Number of QA samples	Cost per sample ($)	In-house cost per sample ($)	Contract ($)	Subtotal ($)
C:N & isotopes	20	20	15	–	600	600
loss-on-ignition	25	–	50	1,250	–	1,250
pigments	17	2	105	–	1,995	1,995
microcystin variants	17	2	235	–	4,465	4,465
radioisotopes	15	–	150	–	2,250	2,250
			Total	$1,250	$9,310	$10,560

7.0 Study Design

7.2 Field data collection

7.2.1 Sampling locations and frequency

The sediment core will be collected from the deepest area of Spanaway Lake (Figure 4). The Toxics Studies Unit of Ecology's Environmental Assessment Program (EAP) has a well-established program focused on the use of sediment cores to inform the long-term trends of persistent, bioaccumulative, and toxic (PBT) chemical deposition to Washington lakes. The coring of Spanaway Lake for this project is a shared goal with the PBT program. The same approaches and methods will be followed as described in the QAPP for the sediment core program (Mathieu, 2016).

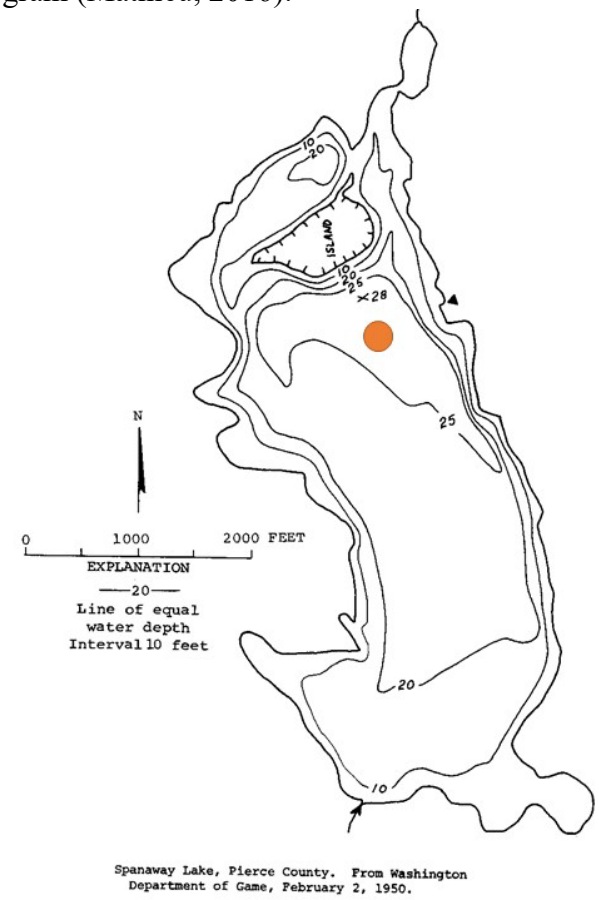

Figure 4: Bathymetric map of Spanaway Lake showing the proposed core location (orange dot).

The sediment core will be subsampled immediately following recovery aboard the boat. All samples will be frozen upon return from the field. Samples will be shipped to the lab frozen or freeze dried, depending on the analytical method requirements.

7.5 Possible challenges and contingencies

All field work and lab work must comply with Ecology's Safe Start Plan (Ecology, 2020) in response to the COVID-19 pandemic. Field work conducted under this plan adheres to Ecology's COVID protocols and has been reviewed and approved by EAP program management for implementation. Field activities must meet a "trifecta" where the employee's official residence, duty station and work site are all in the same phase of re-opening under the Governor's Safe Start guidelines.

It is possible that delays to the project occur due to the COVID pandemic, which impacts the *Logistical Problems*, *Practical Constraints* and *Schedule Limitations* of this QAPP.

8.0 Field Procedures

8.1 Invasive species evaluation

Field personnel for this project are required to be familiar with and follow the procedures described in SOP EAP070, *Minimizing the Spread of Invasive Species* (Parsons et al., 2018). Our study area is not considered to be of high concern for invasive species. Sampling events will be day trips, with sufficient time in between to allow for decontamination by drying (48 hours).

8.8 Other activities

All field activities will comply with the COVID-19 guidelines for conducting field work. Field work will be carried out by five or fewer people. The field work requires the following precautions:

- All employees will undergo a health screen by their supervisor prior to entry into the field.
- Travel to the field site in separate vehicles
- Face coverings on the boat during sampling
- Processing of the samples at Ecology Headquarters and the EAP Operations Center will require advance planning and adherence to building re-entry guidelines.

9.0 Laboratory Procedures

9.1 Lab and field procedures table

In addition to the procedures described in the original QAPP, we will submit samples to the US EPA Office of Research and Development in Cincinnati, OH for qPCR analysis (Table 4). The qPCR assays are used to amplify genes from general microcystin producers (mcyAcy1F/R) and toxic *Microcystis* (mcyEmc4F/R).

Table 4. Laboratory procedures for the qPCR assay.

Laboratory	Analyte	Sample matrix	Samples	Expected range	Method detection limit (DNA recovery)	Reporting (DNA base pairs library match)	Analytical (instrumental) method
EPA ORD, Cincinnati OH	qPCR	Sediment	20	100 to 250 base pairs	0.5 ng/mL	97% identity match	Agilent 2100 Bioanalyzer

EPA ORD = US Environmental Protection Agency, Office of Research and Development

9.3 Special method requirements

This follow-up study will use the non-standard methods established by the USEPA ORD lab for qPCR (Lu et al., 2020). Water samples were successfully analyzed as a pilot study by the ORD lab during the 2019 sample collections for the Wong and Hobbs (2020) study, but the results were not published because the samples were exploratory. The Qiagen DNA extraction kits for soils that will be used for the sample preparation of the sediments are off-the-shelf, tried extraction kits for researchers working with soils and sediments. Samples will be frozen in 2ml microfuge tubes, shipped frozen and thawed prior to extraction. Extraction will take place in the sample microfuge tube so not to contaminate the sample.

Existing waivers from the original studies for the analysis of pigments and microcystin variants will apply to this follow-up study.

10.0 Quality Control Procedures

10.1 Table of field and laboratory quality control

Table 5. Quality control samples, types, and frequency.

Parameter	Field Replicates	Check Standards[a]	Method Blanks	Analytical Duplicates	Matrix Spikes[b]
qPCR	NA	Each sample	1/batch	Each sample	Each sample

a Check standards consist of comparison with neat DNA.
b Matrix spike consists of the TaqMan Exogenous Internal Positive Control Reagents (a VIC-labeled probe) (Life Technologies).

15.0 References

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