DEPARTMENT OF ECOLOGY
State of Washington

Addendum 3 to Quality Assurance Project Plan

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

September 2021

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Addendum 3 to
Quality Assurance Project Plan

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

by William Hobbs

September 2021

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EAP: Environmental Assessment Program

Table of Contents

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, 2020a). 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. The study identified microcystin as the main cyanotoxin produced during the blooms.

Ecology's previous work established the utility of using the fluorometric probe to infer cyanobacteria production and associated microcystin production over time at a single sample location in the lake. However, the earlier work did not investigate the associations among water column nutrients, cyanobacteria communities, and microcystin production. The current project will assess water column nutrient concentrations, cyanobacteria communities, and microcystin-producing genes from May to November 2021 in Spanaway Lake. We will also measure the concentration and composition of microcystin in the water. Overall, this project contributes to our further understanding and development of techniques and indicators to rapidly identify cyanoHABs.

Ecology's earlier work on cyanoHABs has also included investigations into the historical prevalence of cyanobacteria at Anderson Lake, Jefferson County using a dated sediment core (Hobbs et al., 2021). At the request of Washington State Parks and Recreation, the current project will also investigate the historical presence of cyanobacteria in Pass Lake within Deception Pass State Park. The study will use the same algal proxies as previous work and also analyze an age-dated sediment core for the presence of cyanotoxin-producing genes, as per the original QAPP (Hobbs, 2018).

3.2 Study area and surroundings

Details on Spanaway Lake have been covered in previous Quality Assurance Project Plans and reports (Wong and Hobbs, 2020a; Hobbs, 2020).

Pass Lake is located on Fidalgo Island in Skagit County, within the Deception Pass State Park. It is a small kettle lake (~95 acres) with a maximum depth of about 20-25 feet (Figure 1). There is one engineered outlet stream on the southwest shoreline which drains to Bowman Bay in the Puget Sound (Figure 2). The outlet flow is controlled by lake level reaching a culvert. It is not clear exactly when the culvert was installed, but likely in the 1930s when many construction projects took place in Deception Pass State Park. A small perennial inlet stream is present on the northeast shoreline of the lake. There has been no major hydrologic study of the lake, but it is likely a seepage or spring lake where groundwater inputs dominate the hydrology. The lake watershed is mainly forested parkland with a park residence on the north shore (Figure 3).

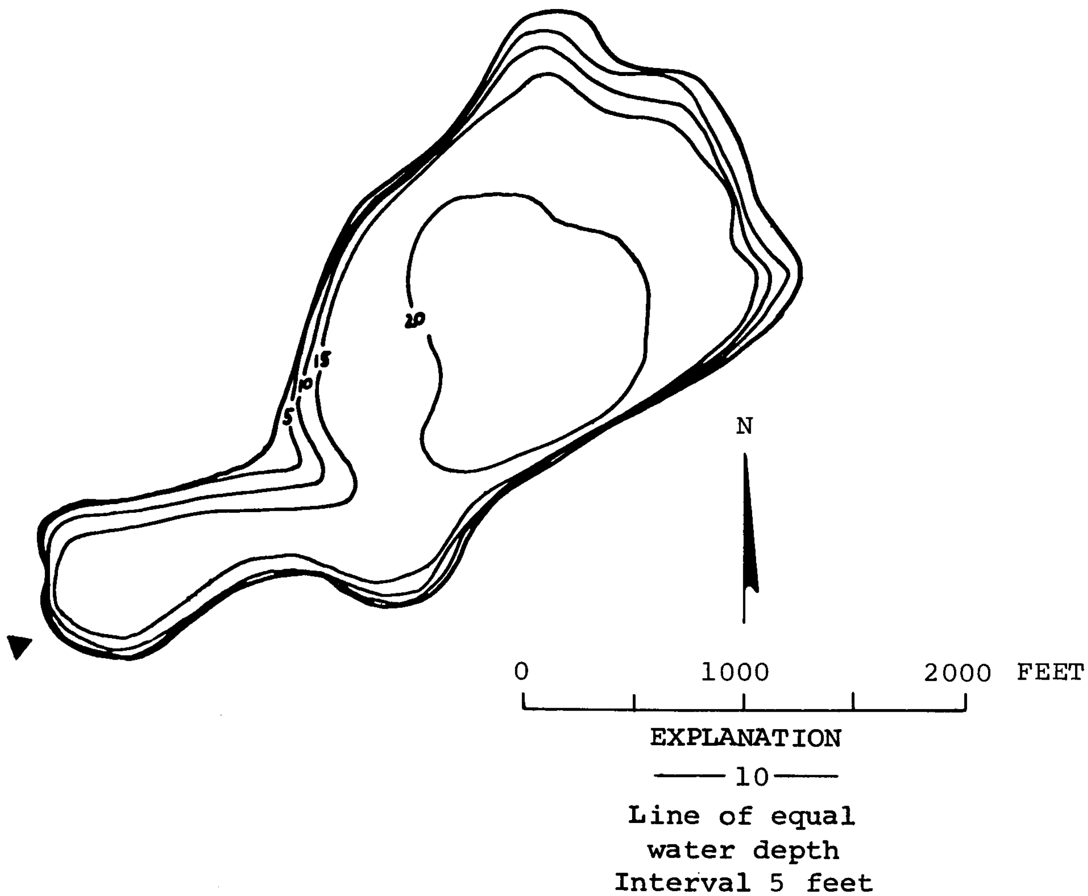

Figure 1. Bathymetric map of Pass Lake, Deception Pass State Park.

Since the early 1900s, the lake has been managed as a trout fishery by the Washington Department of Fish and Wildlife (formerly the Department of Game). There have been many fish introductions and two major fish eradications using rotenone (1946 and 1959) (Personal communications: Justin Spinelli, Washington Department of Fish and Wildlife, and Julie Morse, Washington State Parks and Recreation).

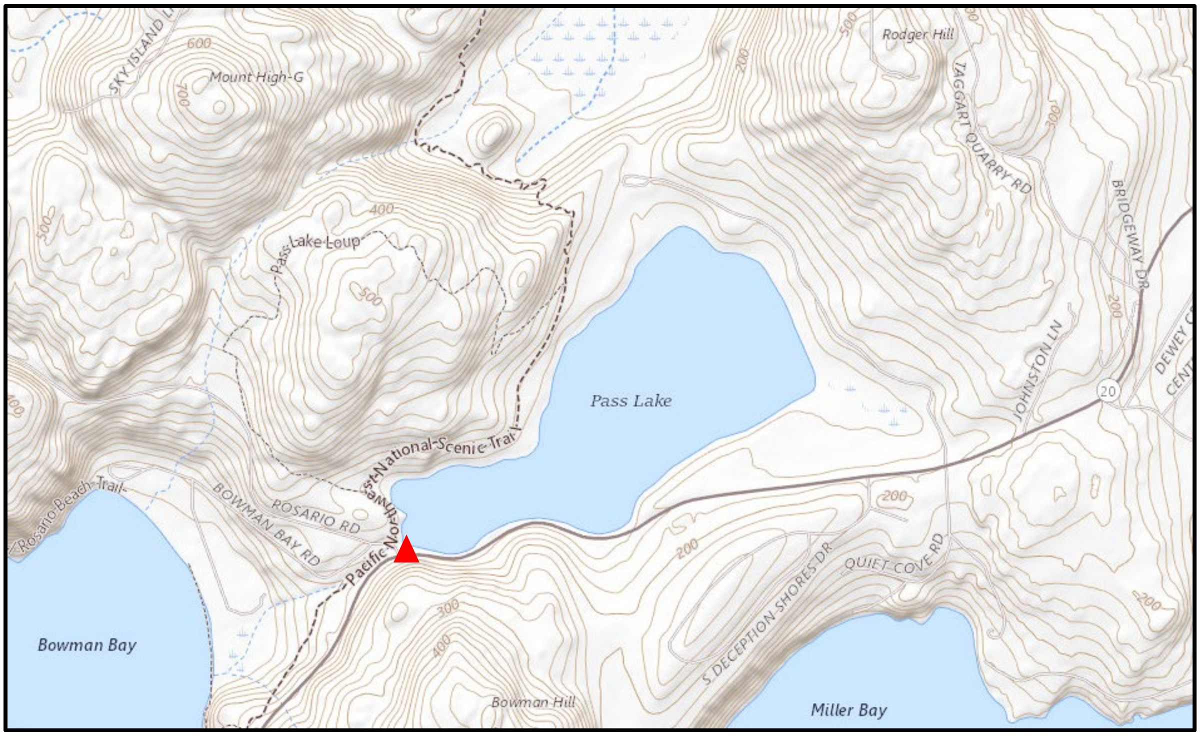

Figure 2. Topographic map of the Pass Lake watershed.
Red triangle is the location of the outlet.

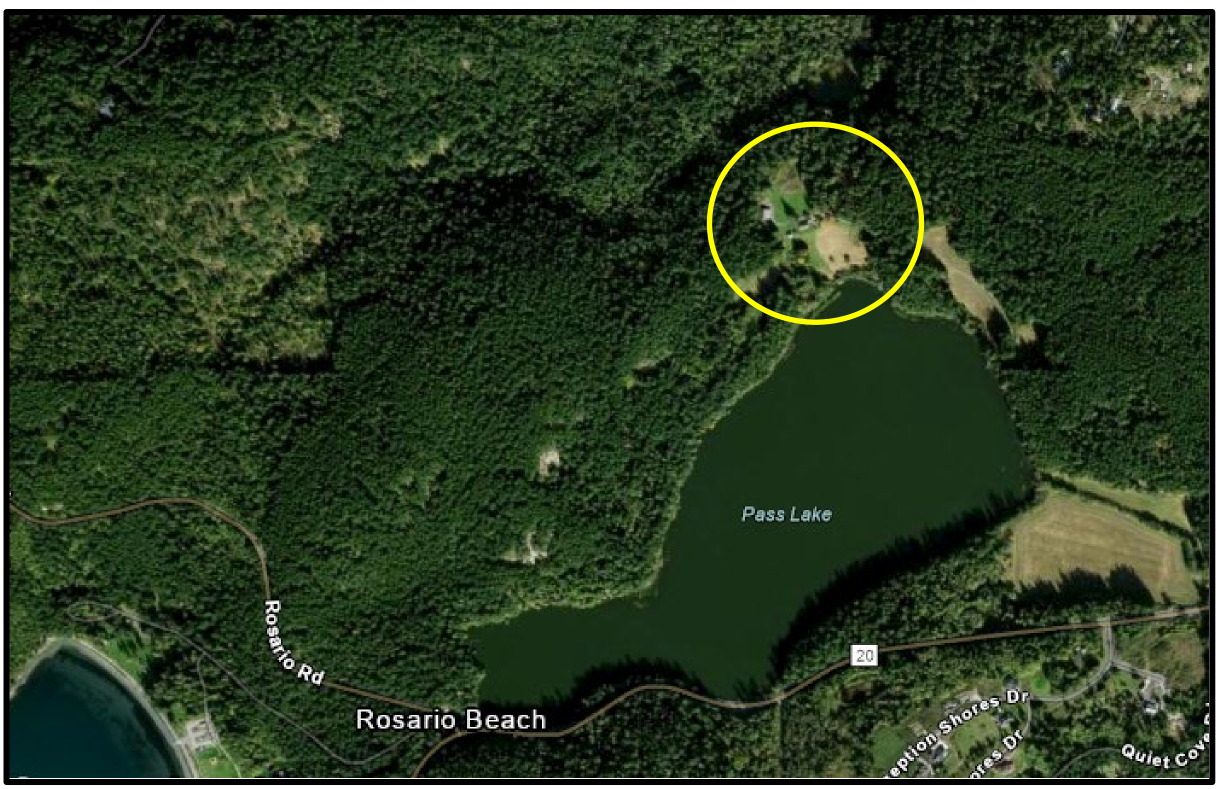

Figure 3. Arial photograph of Pass Lake.
The park residence is highlighted by the yellow circle.

3.2.2 Parameters of interest and potential sources

The majority of the parameters have been described in the previous QAPP and addenda. There are several additional water quality parameters that will be collected at Spanaway Lake to characterize water chemistry and nutrient concentrations (Table 1). Monthly samples will be collected for major anions (bromide, chloride, fluoride, nitrite, nitrate and sulfate) and major cations (calcium, magnesium, sodium and potassium). In addition, to previously sampled nutrient parameters (see Hobbs, 2018; total phosphorus, total nitrogen, ammonia, and nitrite-nitrate), we will sample orthophosphate.

Table 1. List of the water quality parameters of interest at Spanaway Lake.

Parameter	Sample frequency	Depth
Microcystin variants	monthly	Integrated surface water sample
Total Microcystin - ELISA	weekly	Integrated surface water sample
Chlorophyll *a*	weekly	Integrated surface water sample and profile
Phycocyanin	weekly	Integrated surface water sample and profile
Total phosphorus (TP)	weekly	Integrated surface water sample and bottom waters
Orthophosphate (PO_4)	weekly	Integrated surface water sample and bottom waters
Nitrite-Nitrate (NO_2–NO_3)	weekly	Integrated surface water sample and bottom waters
Ammonia (NH_3)	weekly	Integrated surface water sample and bottom waters
Total persulfate nitrogen (TPN)	weekly	Integrated surface water sample
Major cations and metals	monthly	Integrated surface water sample and bottom waters
Major anions	monthly	Integrated surface water sample and bottom waters

Major anions = bromide, chloride, fluoride and sulfate;
Major cations and metals = calcium, potassium, magnesium, sodium, iron and aluminum.

3.2.3 Summary of previous studies and existing data

Previous results from Spanaway Lake are covered in the QAPP addendum by Hobbs (2020).

The water quality of Pass Lake has been monitored by the Institute for Watershed Studies at Western Washington University (WWU) since 2006. Shoreline grab samples have been taken once or twice a year during the spring and summer (Table 2). We will not be monitoring nutrients in Pass Lake for this study. The purpose of Table 2 is to show that Pass Lake appears to have similar nutrient concentrations from year to year.

Table 2. Water quality monitoring results of Pass Lake (Institute for Watershed Studies, WWU).

Sample date	Dissolved oxygen (mg/L)	Temp (° C)	pH	SpC (µS/cm)	Chl a (µg/L)	Alk (mg/L)	Turbidity (NTU)	NH_3 (µg/L)	TN (µg/L)	NO_3 (µg/L)	TP (µg/L)	SRP (µg/L)
8/22/2006	8.5	19.9	8.8	296	NA	81.2	6.53	<10	862.5	<10	29.6	<3
3/27/2007	10.6	9.5	7.6	278	9	73.3	2.51	69.7	674.3	<10	13.2	<3
8/14/2007	9.7	20.1	8.4	291	41.2	79.6	6.68	<10	793.2	<10	29.9	4.6
5/27/2008	9.6	17	8	280	1.7	73	0.87	<10	NA	<10	NA	<3
8/27/2008	8.8	20	8.6	288	9.9	76.3	4.1	20.7	655.7	<10	30.5	5.6
5/14/2009	9.9	16.2	8.1	276	1.4	70.1	1.01	15.7	576	<10	7.8	<3
8/24/2009	9.7	20.3	8.5	294	11.2	77.4	3.6	<10	737	<10	31.7	6.6
3/25/2010	9.7	10.5	8.1	281	NA	75.4	2.13	15.9	668	<10	11.5	<3
7/7/2010	8.5	19.2	8.1	284	6.5	75	2.96	<10	705	<10	17.1	5.5
7/20/2011	7.6	19.5	8.1	279	5.9	73.6	2	<10	527	<10	8.6	6.9
7/24/2012	9.1	19.2	8.1	278	6.2	72.4	2.07	<10	580	<10	15	5.1
7/31/2013	9.1	20.3	8.3	284	16.6	76.3	2.52	<10	682.3	<10	19.6	<3
9/3/2014	8.2	19.7	8.4	59.8	19.7	79.4	5.38	<10	579	<10	20.1	<3
7/13/2015	8.1	21	7.8	282	14.1	76.5	3.2	10.9	667.1	<10	27.5	<3
7/6/2016	8.1	19.5	7.7	269	0.1	73.9	2.9	<10	719.9	<10	22	<3
7/10/2017	NA	NA	7.7	274	5.3	64.8	0.9	15.1	600.7	<10	24.7	3.3
7/23/2018	9.4	22.1	8.7	271.1	13.5	69.5	3.46	<10	680	<10	26.7	<3
8/19/2019	10	20.7	8.7	282.7	33.2	76	9.15	<10	834	<10	74	6.6
8/31/2020	9	20	8.8	277.9	73.5	75.4	32.1	15.3	2076	<10	231.2	11.9

Temp = temperature; SpC = specific conductance; Chl a = chlorophyll a; Alk = alkalinity; NH_3 = ammonia;
TN = total nitrogen; NO_3 = nitrate; TP = total phosphorus; SRP = soluble reactive phosphorus;
Data available at https://www.wwu.edu/iws/

Since 2012, Pass Lake has been experiencing noticeable blooms of cyanobacteria that have been sampled and analyzed for cyanotoxins under Ecology's Freshwater Algae Program. Data are available at https://www.nwtoxicalgae.org/. Both microcystin and anatoxin-a are prevalent in Pass Lake and generally concentrations are highest around September and October (Figures 4 and 5). It is likely this time of the year when the lake is undergoing mixing as the surface water temperatures decrease.

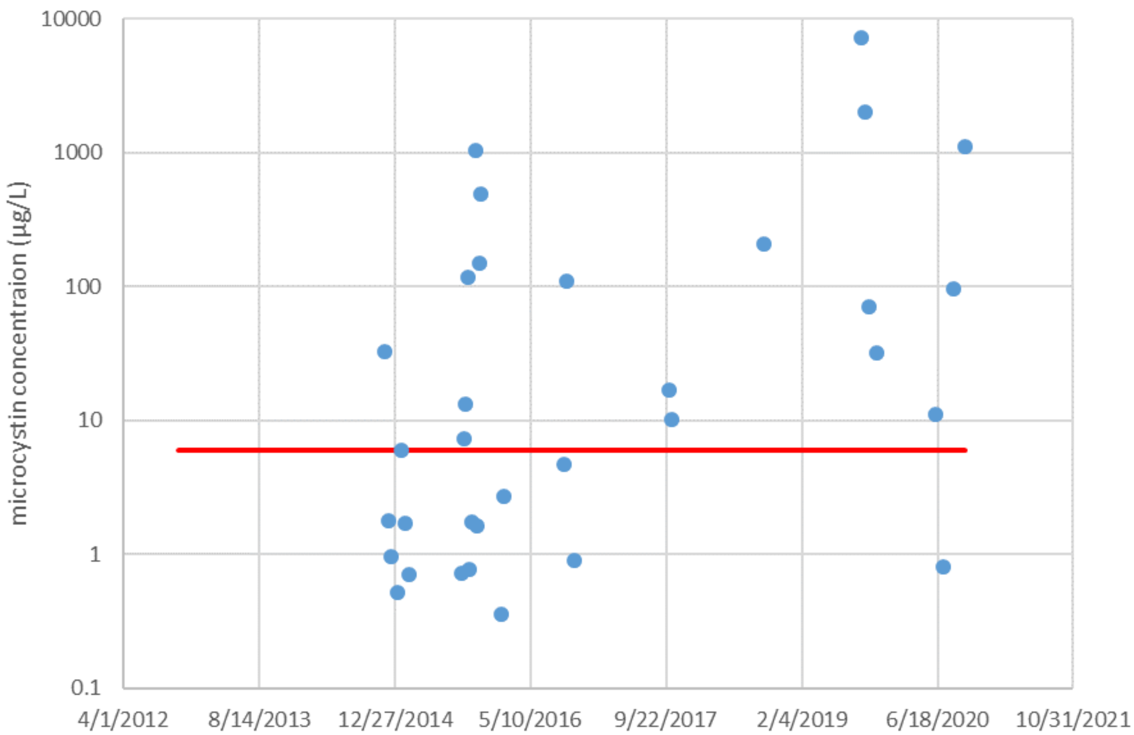

Figure 4. Microcystin concentrations at Pass Lake since 2012.

Red line is the Washington State Department of Health Recreational guideline (6 µg/L).

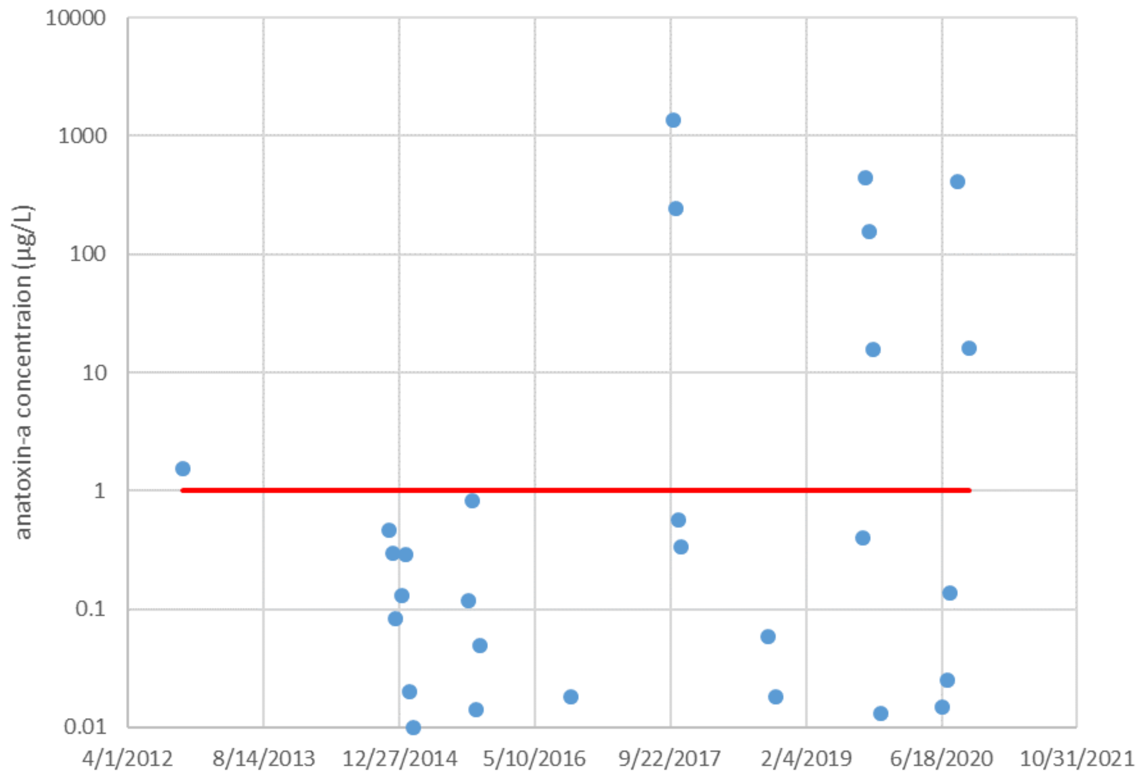

Figure 5. Anatoxin-a concentrations at Pass Lake since 2012.

Red line is the Washington State Department of Health Recreational guideline (1 µg/L).

4.0 Project Description

4.1 Project goals

The goals of this project are to establish:

- Associations among nutrients, cyanobacteria communities, and cyanotoxin production in Spanaway Lake during the summer of 2021.
- The historical prevalence of cyanobacteria in Pass Lake, in Deception Pass State Park, using a dated sediment core.

4.2 Project objectives

The objectives of this study are to:

- Collect weekly samples from a central sample location on Spanaway Lake to assess nutrient concentrations in the surface and bottom waters and corresponding cyanobacteria communities and microcystin production.
- Assess the historic prevalence of cyanobacterial pigments and cyanotoxin-producing genes in the sediments of Pass Lake, Deception Pass State Park.

4.4 Tasks required

Specific tasks under this project include the following:

- Write a QAPP addendum for the project.
- Measure weekly water column profiles of Spanaway Lake at the deepest point in the lake using a multiprobe sonde. Parameters include dissolved oxygen, conductivity, pH, temperature, fluorometric chlorophyll a (in reflectance units; RFU) and phycocyanin (RFU).
- Collect weekly samples from Spanaway Lake at the central deepest point in the lake, from the surface and deep waters.
- Collect phytoplankton samples and net tows for identification of algal groups from Spanaway Lake.
- Collect a sediment core from Pass Lake at the deepest location.
- Subsample the core at Ecology for dating, geochemical analysis, and sedimentary algal pigments.
- Construct an age–depth model for the sediment core.
- Review and assess data quality and laboratory results.
- Write separate short reports for the monitoring of Spanaway Lake and the sediment core from Pass Lake.

5.0 Organization and Schedule

5.1 Key individuals and their responsibilities

Table 3. 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, approves the budget, 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.
James Medlen TSU, SCS Phone: 360-407-6194	Unit Supervisor for the Project Manager	Provides internal review of the QAPP, and approves the final QAPP.
Alan Rue Manchester Environmental Laboratory Phone: 360-871-8801	Director	Reviews and approves the final QAPP. Oversees analysis of 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 EPA 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;
EPA: US Environmental Protection Agency.

5.2 Special training and certifications

A research permit with Washington State Parks and Recreation will be required to collect a core from Pass Lake. Ecology will apply for the research permit in the summer of 2021, and coring will take place in the fall of 2021.

5.4 Proposed project schedule

The proposed project schedule (Tables 4-6) assumes no further delays due to compliance with Ecology's response plan to the COVID pandemic.

Table 4. Proposed schedule for completing field and laboratory work.

Field and laboratory work	Due date	Lead staff
Field work completed	November 2021	William Hobbs
Laboratory analyses completed	April 2022	MEL and contract labs

Table 5. Proposed schedule for data entry into the Environmental Information Management (EIM) database. EIM Study ID WHOB008.

Product	Due date	Lead staff
EIM data loaded	June 2022	TBD
EIM data entry review	July 2022	William Hobbs
EIM complete	August 2022	TBD

Table 6. Proposed schedule for project reporting.

Tasks	Due Date	Lead staff
Draft due to supervisor	September 2022	William Hobbs
Draft due to client/peer reviewer	October 2022	William Hobbs
Final (all reviews done) due to pub team	November 2022	William Hobbs
Final report due on web	December 2022	William Hobbs

5.5 Budget and funding

The detailed budget for the laboratory expenses is outlined in Tables 7 and 8. All laboratory contracts are handled by the project manager and not through MEL. For the Spanaway Lake water samples (Table 8), some in-house lab costs will be billed in FY21 (~$3,200); all remaining lab costs will be billed in FY22.

Table 7. Detailed project budget for water quality monitoring at Spanaway Lake

	Epi	Hypo/ Chl max	Samples	QA	Per sample cost	In-house ($)	Contract ($)	Lab
Total phosphorus	26	26	52	6	20	1160	-	MEL
Orthophosphate	26	26	52	6	20	1160	-	MEL
Total persulfate nitrogen	26	-	26	6	20	640	-	MEL
Ammonia/ NO_3-NO_2	26	26	52	6	30	1740	-	MEL
Chlorophyll a	26		26	6	50	1600	-	MEL
Major anions	5	5	10	3	65	845	-	MEL
Major cations and metals	5	5	10	3	100	1300	-	MEL
POC-PN with stable isotopes	26	-	26	6	15	-	480	UCSC
Phycocyanin	14	-	14	3	50	-	850	WWU
MC Elisa	26	-	26	6	65	-	2080	KCEL
MC variants	5	-	5	-	175	-	875	KCEL
MC genes	26	26	52	-	0	-	0	EPA-ORD
					Total	**8,445**	**4,285**	**$12,730**

Major anions = bromide, chloride, fluoride, and sulfate.
Major cations and metals = calcium, potassium, magnesium, sodium, iron, and aluminum.
Epi = epilimnion (surface waters); Hypo/Chl max = hypolimnion (bottom waters) or chlorophyll a maximum;
NO_3-NO_2 = nitrate-nitrite as N; POC-PN = particulate organic carbon and particulate nitrogen;
MC = microcystin; MEL = Manchester Environmental Lab; UCSC = University of California- Santa Cruz;
WWU = Western Washington University; KCEL = King County Environmental Lab;
EPA-ORD = US Environmental Protection Agency – Office of Research and Development.

Table 8. Detailed project budget for the Pass Lake sediment core.

	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	20	2	105	–	2,310	2,310
Radioisotopes (alpha)	16	–	150	–	2,400	2,400
Radioisotopes (gamma)	10	–	150	–	1,500	1,500
			Total	**$1,250**	**$6,810**	**$8,060**

C:N = Carbon : Nitrogen (molar)

6.0 Quality Objectives

6.2 Measurement quality objectives

The measurement quality objectives (MQOs) for the analytical data in this study are detailed in Table 9. For completeness all the water quality parameters are included as well as being detailed in the previous QAPP and associated addenda (Hobbs, 2018; Wong and Hobbs, 2020b). All sediment parameter MQOs follow the QAPP by Hobbs (2020).

6.2.1 Targets for precision, bias, and sensitivity

Table 9: Measurement quality objectives for water quality parameters.

Parameter	Verification standards (LCS, CRM, CCV) (% recovery limits)	Spiked blank (% recovery limits)	Duplicate samples (RPD[b])	Matrix spikes (% recovery limits)	Matrix spike duplicates (RPD[b])	Lowest concentrations of interest
Microcystin variants	CCV low: 50–150 CCV mid: 70–130 CCV high: 70–130	70–130	40	70–130	40	0.2 µg/L
Microcystin - ELISA	PC 70 – 130	NA	60 – 140	0 – 45	50 – 150	0.15 µg/L
Chlorophyll a	CCV 90–110	NA	20	NA	NA	0.004 mg/L
Phycocyanin	NA	<Reporting Limit	NA	20	NA	8 µg/L
TP	CCV 90–110	80–120	20	75–125	20	0.0024 mg/L
NO_2–NO_3	CCV 90–110	80–120	20	75–125	20	0.01 mg/L
NH3	CCV 90–110	80–120	20	75–125	20	0.01 mg/L
Total persulfate N	CCV 90–110	80–120	20	75–125	20	0.025 mg/L
Orthophosphate	CCV 90–110	80–120	20	75–125	20	0.003 mg/L
Sodium	LCS 85-115%	70-130%	<30%	75-125%	<30%	0.025 mg/L
Magnesium	LCS 85-115%	70-130%	<30%	75-125%	<30%	0.025 mg/L
Potassium	LCS 85-115%	70-130%	<30%	75-125%	<30%	0.25 mg/L
Calcium	LCS 85-115%	70-130%	<30%	75-125%	<30%	0.025 mg/L
Iron	LCS 85-115%	70-130%	<30%	75-125%	<30%	0.025 mg/L
Aluminum	LCS 85-115%	70-130%	<30%	75-125%	<30%	0.025 mg/L
Sulfate	LCS 90-110%	70-130%	<30%	75-125%	<30%	0.30 mg/L
Chloride	LCS 90-110%	70-130%	<30%	75-125%	<30%	0.10 mg/L
Bromide	LCS 90-110%	70-130%	<30%	75-125%	<30%	0.025 mg/L
Fluoride	LCS 90-110%	70-130%	<30%	75-125%	<30%	0.10 mg/L

[a] LCS = laboratory control sample; CRM = certified reference materials;
CCV = continuing calibration verification standard.

[b] Relative Percent Difference

7.0 Study Design

7.2 Field data collection

7.2.1 Sampling locations and frequency

The sample location on Spanaway Lake remains the same as detailed in the previous QAPP (Hobbs, 2020).

The sample location on Pass Lake will target the deepest location (Figure 1). The sediment core will be collected using a percussion-type corer as per the original QAPP (Hobbs, 2018). The sediment core will be subsampled following transport back to the Ecology, Headquarters lab, in Lacey, Washington. All samples will be frozen following subsampling. Samples will be shipped to the lab frozen or freeze dried, depending on the analytical method requirements.

7.5 Possible challenges and contingencies

Current and future COVID policies and protocols applicable to all field work will be followed.

There are no foreseeable issues of access to Spanaway Lake or Pass Lake.

Possible challenges with the sediment core collection on Pass Lake is recovering enough sediment (length of core) to achieve the necessary radioisotope threshold for dating. As a contingency we have included the necessary budget to accommodate additional sample analysis.

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).

8.2 Measurement and sampling procedures

Representative water quality samples of the surface waters of Spanaway Lake will be collected using an integrated sampler. The upper 1m of the epilimnion of the lake water will be homogenized and distributed into sample containers. This approach follows standard limnological protocols as described in EPA's guidance for the National Lakes Assessment (USEPA, 2017a). Water samples representative of the bottom waters or hypolimnion, will be collected with Kemmerer bottle to capture a discrete sample at depth.

8.3 Containers, preservation methods, holding times

All necessary containers, preservatives and holding times for all water quality samples are listed in Table 10. Sediment containers have been described in the previous QAPP and associated addenda (Hobbs, 2018; Hobbs 2020).

Table 10. Sample containers, preservation, and holding times for water quality samples.

Parameter	Minimum quantity required	Container	Preservative	Holding time
MC variants	100 ml	125 ml amber glass bottle	cool at 4°C or freeze	48 hrs (1 month frozen)
MC - ELISA	100 ml	125 ml amber glass bottle	cool at 4°C or freeze	48 hrs (1 month frozen)
Chlorophyll *a*	0.25–1 L, filtered	field filter in glass tube	acetone	24 hrs to filtration; 28 days after filtration
Phycocyanin	400 mL	500 mL amber polyethylene bottle	cool to 4°C, overnight shipping	60 days after frozen
TP	60 ml	125 ml clear Nalgene	1:1 HCl	28 days
PO_4	60 ml	125 ml amber Nalgene	cool at 4°C	48 hrs field filtered
NO_2–NO_3	60 ml	125 ml clear Nalgene	1:1 H_2SO_4	28 days
NH_3	60 ml	125 ml clear Nalgene	1:1 H_2SO_4	28 days
Total persulfate N	60 ml	125 ml clear Nalgene	1:1 H_2SO_4	28 days
Major cations and metals	100 ml	500 mL HDPE bottle; field filtered	1:1 HNO_3, cool to ≤6°C	6 months
Major anions	60 ml	500 mL HDPE	cool to ≤6 °C	28 days

Major anions = bromide, chloride, fluoride and sulfate.
Major cations and metals = calcium, potassium, magnesium, sodium, iron and aluminum.
NO_3-NO_2 = nitrate-nitrite as N.

9.0 Laboratory Procedures

9.1 Lab and field procedures table

Table 11. Measurement methods (laboratory).

Lab	Analyte	Sample matrix	Samples	Expected range of results	Method detection limit	Reporting limit	Sample prep method	Analytical (instrumental) method
KCEL	Microcystin variants	Water	5	<MDL to 100 µg/L	0.04 µg/L	0.2 µg/L	KCEL SOP 469 (Mekebri et al 2009)	KCEL SOP 473 (Mekebri et al. 2009)
KCEL	Microcystin - ELISA	Water	26	Limit – 4000 µg/L	0.15 µg/L	0.15 µg/L	KCEL SOP #465	ELISA-Abraxis ADDA (KCEL SOP #465)
MEL	Chlorophyll a	Water	26	1 µg/L to 100 µg/L	NA	0.004 to 0.05 mg/L	SM10200-H1	SM10200-H3
IWS-WWU	phycocyanin	Water	14	<Reporting Limit – 20 µg/L	8 µg/L	8 µg/L	USEPA (2017b)	EPA (2017); Kasinak et al. (2015)
MEL	TP	Water	52	<MRL to 1 mg/L	0.005 mg/L	0.0024 mg/L	SM4500-P B5	SM4500-P H
MEL	orthophosphate	Water	52	<MRL to 1 mg/L	0.005 mg/L	0.0024 mg/L	SM4500 PG	SM4500-P G
MEL	NO_2-NO_3	Water	52	<MRL to 1 mg/L	0.005 mg/L	0.01 mg/L	SM4500NO3I	SM4500-NO3 I
MEL	NH_3	Water	52	<MRL to 1 mg/L	0.006 mg/L	0.01 mg/L	SM4500NH3	SM4500-NH3 H
MEL	Total persulfate N	Water	26	<MRL to 2 mg/L	0.013 mg/L	0.025 mg/L	SM4500-N B	SM4500-N B
MEL	Major cations and metals	water	10	0.025–500 µg/L	0.025 µg/L	0.025 µg/L	EPA 200.7	EPA 200.7
MEL	Major anions	water	10	0.025–500 µg/L	0.025–0.3 µg/L	0.025–0.3 µg/L	NA	EPA 300.0
EPA	mRNA	Water and Sediment core	75	100 to 250 base pairs	0.5 ng/mL	97% identity match base pairs library	(sediment) Qiagen – Rneasy Powersoil total RNA kit	Agilent 2100 Bioanalyzer
MEL	LOI	Sediment core	25	1 – 80%	1%	1%	ASTM D7348-13	LOI (Heiri et al., 2001)
SMM	^{210}Pb radioisotopes	Sediment core	16	< 0.45 - 30 pCi/g	NA	0.45 pCi/g	Eakins and Morrison, 1978	Alpha Spectroscopy (Eakins and Morrison, 1978)
Dr. Rolf Vinebrooke	algal pigments	Sediment core	22	0.1 to 2000 nmole pigment	NA	0.1 nmole	Leavitt and Hodgson, 2001	HPLC (Mantoura and Llewellyn, 1983)
UC-Santa Cruz	TOC:N and isotopes	Sediment core and particulates	66	0.1 - 2.0 (%N); 1.0 - 15 (%C)	NA	0.10%	lyophilization	ⱡ stable isotopes of N and C

KCEL: King County Environmental Lab; MEL: Manchester Environmental Lab; SMM: Science Museum of Minnesota; IWS-WWU: Institute for Watershed Studies – Western Washington University; LOI: loss-on-ignition;
ⱡ Costech Elemental Analyzer, Conflo III, MAT253

9.4 Laboratories accredited for methods

All analyses for nutrients will be carried out at MEL. Other parameters are being analyzed by contract labs using non-accredited methods. The following contract labs will be used during this project:

- Water MC variants and ELISA –King County Environmental Lab (not accredited; waiver required).
- Water and sediment mRNA – US Environmental Protection Agency, Office of Research and Development, Cincinnati, OH (not accredited, waiver required).
- Sediment and particulate TOC-TN and isotopes – University of Santa Cruz, Isotope Lab (not accredited; waiver required).
- Sediment core algal pigments – Dr. Rolf Vinebrooke, University of Alberta (not accredited; waiver required).
- Sediment core radioisotopes – Science Museum of Minnesota (not accredited; waiver required).

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