Exploring the Use of Fluorometric Sensors to Monitor Harmful Algal Blooms in Lakes

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Abstract

During the summer and fall of 2019, the Washington State Department of Ecology (Ecology) deployed a multi-parameter data logger (sonde) in Spanaway Lake, Pierce County. 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.

The sonde was equipped with a fluorescence probe to measure both chlorophyll-*a* and phycocyanin, the latter being the main pigment in cyanobacteria. Eleven grab samples were collected during the deployment to compare laboratory-measured data to the sonde data. Cyanotoxins (microcystin and anatoxin-a) were also measured in the grab samples.

The dominant cyanobacteria during the cyanoHAB blooms were *Microcystis* sp., *Dolichospermum* sp., and *Woronichinia* sp. A statistically significant relationship was found between phycocyanin measured in the field and the lab. Furthermore, a statistically significant relationship was observed between lab-measured phycocyanin and microcystin concentrations. A predictive relationship between field-measured phycocyanin and microcystin concentrations appears possible based on this pilot dataset.

We recommend continuing to develop the use of in situ fluorescence as a monitoring tool for cyanoHAB detection and possible early warning of toxins in lake water.

Background

Cyanobacteria Blooms

Cyanobacteria are photosynthetic bacteria common to aquatic ecosystems. In lakes, the occurrence of cyanobacterial blooms may be attributed to many factors including temperature, turbulence and mixing, sunlight, nutrient levels, lake morphology, and lake ecology (Bellinger and Sigee 2010).

Some species of cyanobacteria are also capable of producing toxins (cyanotoxins) that are harmful to humans and other animals. Exposure to toxins occur by ingestion, inhalation, and direct contact with skin. Acute health effects include damage to the liver and nervous system, with symptoms such as vomiting and diarrhea, allergic reactions, fever, headaches, muscle and joint pain, mouth ulcers, and blisters (EPA 2014). Commonly detected cyanotoxins in the U.S. include microcystins, cylindrospermopsin, and anatoxins. These are produced by genera that include *Microcystis*, *Dolichospermum* (formerly *Anabaena*), *Planktothrix*, *Aphanizomenon*, and *Cylindrospermopsis*, among others.

When harmful (toxin-producing) algae blooms occur, they impact the lake's recreational value, ecological health, and viability as a drinking water resource. They pose health threats to humans, pets, livestock, and wildlife. Public warnings or closures may be needed to help protect the lake users and their pets.

Algal Fluorescence Sensors

Monitoring of cyanobacteria harmful algal blooms (cyanoHABs) traditionally consists of collecting water quality samples that may include nutrients, chlorophyll-*a*, algal species counts and abundance, cyanotoxins, as well as measurements of water temperature, dissolved oxygen, pH, and conductivity.

In vivo fluorescence is a potentially useful and practical technology for monitoring cyanoHABs in real-time. It works by measuring the direct fluorescence of pigments in living algal cells in the water, and then determining algal production in terms of relative fluorescence units (RFUs). Benefits of the sensor technology include collection of real-time, unattended measurements of algal production at high temporal resolution (up to every minute).

Applications to lake management include integrating sensor data with public health posting guidelines. Risk levels for exposure to harmful cyanotoxin levels can be developed, as well as real-time public access to these data and information (Genzoli and Kann 2016). Limitations include uncertainties in the relationships between phycocyanin fluorescence sensor measurements and laboratory-measured pigment and cyanotoxin concentrations (Genzoli and Kann 2016).

Goal and Objectives

The goal of this 2019 study was to assess the use of chlorophyll-*a* and phycocyanin fluorescence sensors as tools for continuously monitoring cyanoHABs in lakes. It is part of a larger body of work examining cyanotoxins in lakes within the Puget Sound region (Hobbs 2018).

The objectives of the study were to:
(1) Deploy a multi-parameter sonde equipped with a fluorescence total algae sensor in Spanaway Lake, Pierce County during one full algae bloom season;
(2) Collect surface water grab samples for laboratory analyses of chlorophyll-*a*,

phycocyanin, microcystin, and anatoxin-a; (3) Collect phytoplankton samples for identification of cyanobacteria and other algae; and (4) Determine seasonal trends and correlations between algal pigment concentrations, algal fluorescence measurements, and cyanotoxin concentrations.

Methods

Study Location

Spanaway Lake is a natural kettle lake located in Pierce County, WA (Figure 1). It is approximately 1 km^2 in area with a maximum depth of around 8.5 m (Smith et al. 1998). The main surface water inlet is Spanaway Creek originating from a wetland at the south end of the lake. The main outlet is Spanaway Creek on the north end of the lake. Groundwater appears to be the largest source of inflow 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.

In its recent history, Spanaway Lake has experienced blooms of toxin-producing cyanobacteria (Figure 2). The blooms are likely fueled by excessive nutrients in the lake, particularly total phosphorus (Pierce County 2017). Microcystin levels

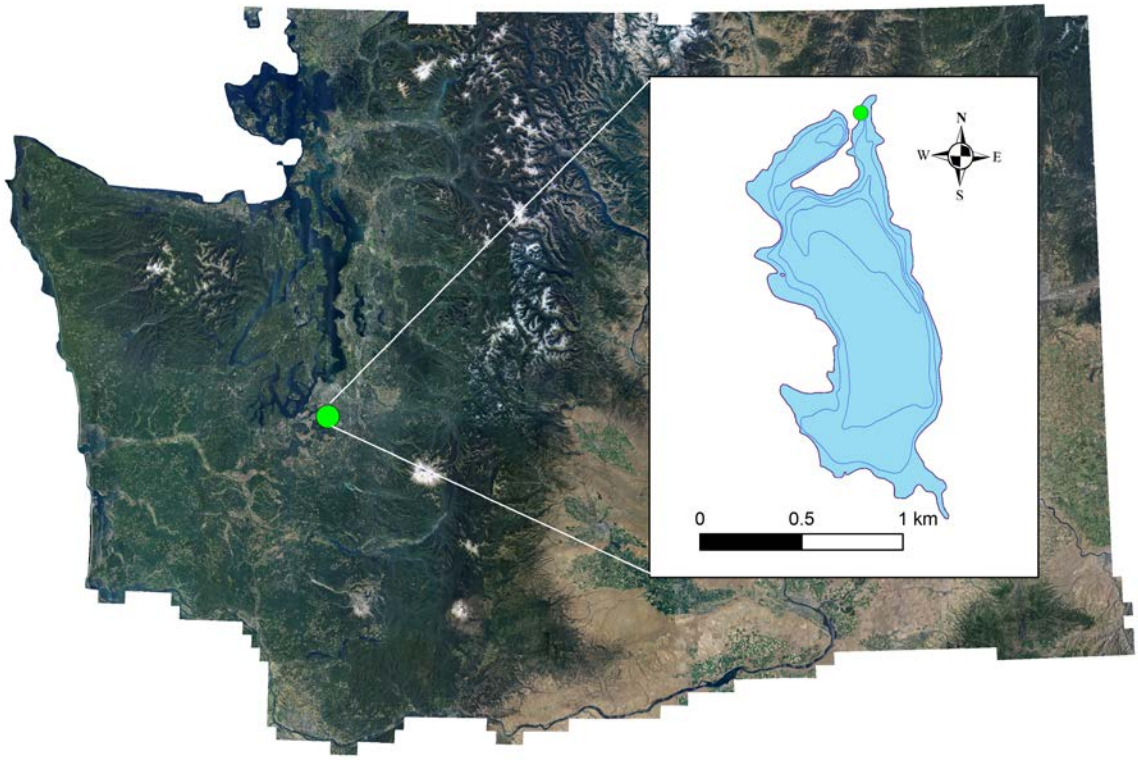

Figure 1. Map location of Spanaway Lake. Inset map shows location of sampling site (green dot) in Spanaway Lake.

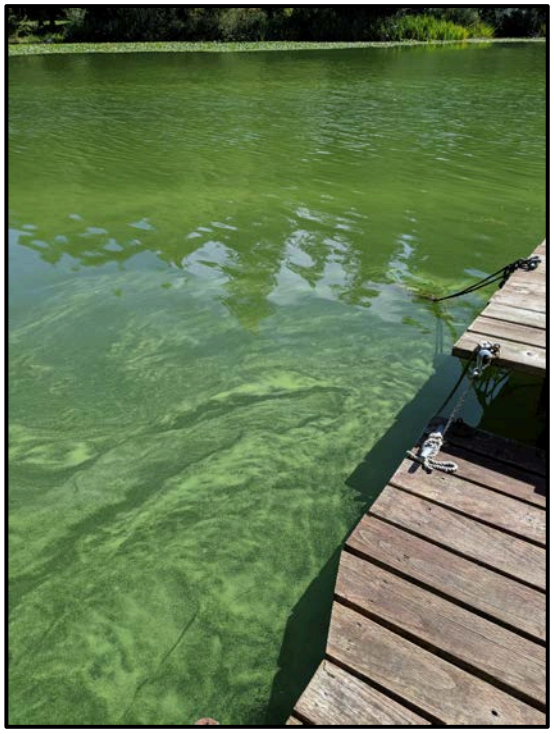

Figure 2. Cyanobacterial bloom on Spanaway Lake, Sep. 12, 2019.

exceeding WA Department of Health's guideline of 6 µg/L has 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.

Field Methods

Our sampling site was located at the northern end of the lake, close to public beaches and where cyanobacteria are known to accumulate during a bloom. From June 19–December 3, 2019, a calibrated multi-parameter sonde (YSI EXO 3) was installed on a private dock, with permission from the homeowner. The sonde was secured to the dock so that the sensors were approximately 0.2 m below the water surface.

The sonde was programmed to collect continuous hourly measurements of chlorophyll-*a* and phycocyanin fluorescence, water temperature, dissolved oxygen, pH, and conductivity.

Measurements of chlorophyll-*a* (the green pigment produced by all algae and cyanobacteria) provide estimates of total algal production. Measurements of phycocyanin (an accessory pigment produced by cyanobacteria) provide estimates of cyanobacteria production. Phycocyanin and chlorophyll-*a* produce different fluorescence signatures (light absorption and emission wavelengths). Because of this, the two pigments can be distinguished by using sensors that emit different peak absorption wavelengths corresponding to the two different pigments (~470 nm for chlorophyll-*a* and ~590 nm for phycocyanin).

During each site visit, we also collected surface water grab samples for chlorophyll-*a*, phycocyanin, microcystin, and anatoxin-*a*. Samples were collected at ~0.2 m water depth. We recorded the YSI field measurements concurrent with each surface water grab sample collection. Phytoplankton samples for taxonomic identification were collected using a 20 µm phytoplankton net and preserved with 10% formalin.

All samples were stored in a cooler on ice at <4°C and shipped overnight to the respective laboratories for further processing. Further details of field methods are provided in this study's Quality Assurance Project Plan (Wong and Hobbs 2019).

Laboratory Methods

Chlorophyll-*a* samples were analyzed by the Institute for Watershed Studies (IWS), Bellingham, WA, using SM10200H. Phycocyanin samples were also analyzed by IWS using methods adapted from EPA (2017) and Kasinak et al. (2015).

A laboratory split for each chlorophyll-*a* sample was analyzed by Manchester Environmental Laboratory, Port Orchard, WA, using SM10200H.

Microcystin samples were analyzed by King County Environmental Laboratory (KCEL) using Abraxis ADDA enzyme-linked immunosorbent assay (ELISA) test kits. Anatoxin-a was also analyzed by KCEL using an Agilent high performance liquid chromatograph coupled to a triple quadrupole mass spectrometer with an electrospray ionization source (Oehrle et al. 2010).

Phytoplankton taxa in each sample were identified at Ecology headquarters using a light microscope at 400x and regional taxonomic keys (Wehr and Sheath 2003, Matthews 2016,).

Quality Assurance

Field duplicates for each laboratory analyte were collected and analyzed at 10% of the total number of samples collected. Field blank samples were also collected for chlorophyll-*a* and phycocyanin at 10% of the total number of samples.

Regular bi-weekly maintenance and calibration checks were performed on the YSI sonde. This involved cleaning biofouling from the sonde, inspecting and backing up data, and re-calibrating the sonde's sensors if calibration checks did not meet quality assurance criteria.

Quality assurance results for YSI measurements are provided in Appendix A. Overall, the chlorophyll-*a*, pH, conductivity, and dissolved oxygen sensors performed well, with 95% of data accepted either without or with qualification. A lower acceptance rate was observed with the phycocyanin data, with 75% of data accepted without or with qualification.

During Aug 14-28, Sep 12-30, and Oct 28-Nov 14, battery issues resulted in data gaps in which the sonde did not log any data.

The relative percent difference (RPD) for field duplicates was: microcystin–36%; chlorophyll-*a*–15%; and phycocyanin–2%. All laboratory quality assurance samples met the method quality objectives as outlined in the Quality Assurance Project Plan (Hobbs 2018).

Results & Discussion

Seasonal Trends: Continuous Field Measurements

Seasonal trends in water quality parameters show water temperatures, dissolved oxygen concentrations, and pH increasing in the early summer and peaking in August to early September (Figure 3). This trend is indicative of heightened photosynthetic activity in warmer waters, characteristic of an algal bloom. As the bloom declines, there is a drop in pH and dissolved oxygen in mid-September.

The highest chlorophyll-*a* and phycocyanin fluorescence measurements were observed between late August and October. Maximum concentrations of chlorophyll-*a* (659 µg/L) and microcystin (2,108 µg/L) from our grab samples

occurred on September 30, corresponding to a spike in phycocyanin and chlorophyll-*a* fluorescence measurements (Figure 3, Table 1).

Phytoplankton Taxa

The two most common phytoplankton taxa observed in our samples during the bloom period were *Microcystis* sp. and *Dolichospermum* sp. (Appendices B), two genera capable of producing microcystin. *Dolichospermum* is known to also produce anatoxin-a; however, concentrations of anatoxin-a were not observed above the detection limit in our samples. *Woronichinia* sp. was frequently present in our samples as well. *Woronichinia* is commonly associated with blooms of *Microcystis* and *Dolichospermum*. Although its toxicity has been less studied, it has been reported to produce toxins (Bober and Bialczyk 2017).

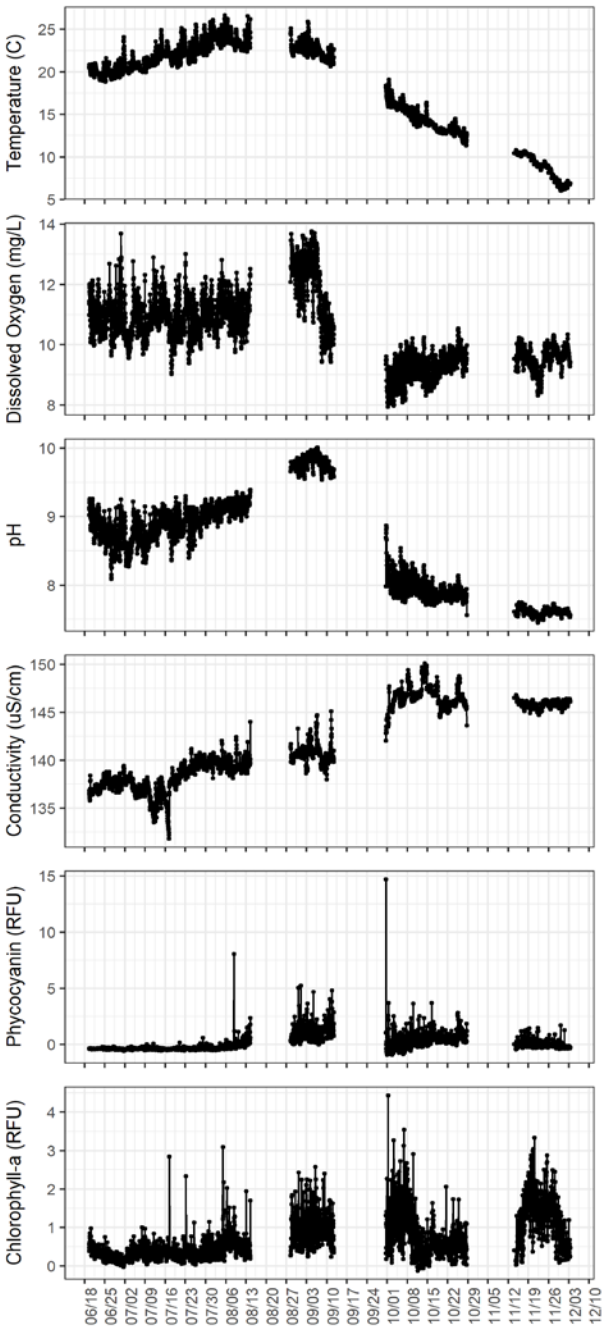

Figure 3. Seasonal trends in water quality parameters collected hourly from Spanaway Lake.

Table 1. Summary statistics for surface water grab samples and hourly YSI sonde field measurements.

	Method	N	Median	Minimum	Maximum
Chlorophyll-*a* (µg/L; IWS)	Grab	11	8.0	<1.0	415
Chlorophyll-*a* (µg/L; MEL)	Grab	12	15.3	0.5	659
Phycocyanin (µg/L)	Grab	11	34.6	<5.0	3936
Microcystin (µg/L)	Grab	11	3.0	<0.15	2,108
Anatoxin-a (µg/L)	Grab	11	<0.01	<0.01	<0.01
Temperature (°C)	Sonde	2,837	20.4	6.0	26.6
Chlorophyll-a (RFU)	Sonde	2,837	0.49	-0.12	4.4
Phycocyanin (RFU)	Sonde	2,837	-0.17	-0.91	14.7
Dissolved Oxygen (mg/L)	Sonde	2,837	10.4	7.9	13.8
pH	Sonde	2,837	8.7	7.5	10.0
Conductivity (µS/cm)	Sonde	2,836	140	132	150

Algal Pigments & Cyanotoxins

One of our objectives was to determine if field measurements of chlorophyll-*a* and phycocyanin fluorescence were correlated with laboratory-measured concentrations of chlorophyll-*a*, phycocyanin, and microcystin in grab samples. We used linear regression to model the relationships between phycocyanin fluorescence and laboratory phycocyanin, chlorophyll-*a*, and microcystin (Figure 4). The data were log-transformed to achieve a normal or near-normal distribution.

Phycocyanin fluorescence was strongly correlated with laboratory phycocyanin (r^2=0.78), chlorophyll-*a* (r^2=0.89), and microcystin concentrations (r^2=0.85; Figure 4). No correlations were found between chlorophyll-*a* fluorescence and any of the other algal variables (Appendix C). Overall, the results suggest that the phycocyanin fluorescence sensor may be a promising tool for monitoring trends in phycocyanin (cyanobacteria biomass) and microcystin in Spanaway Lake.

Management Implications

Using the strong relationships we observed between the field and lab measurements for cyanoHABs and toxins in Spanaway Lake, it seems possible to predict when exceedances of public health thresholds might occur. For example, based on our model of phycocyanin fluorescence and microcystin, we can predict that future microcystin samples will exceed the 6 µg/L threshold 95% of the time when phycocyanin fluorescence measurements are above approximately 3 RFU (Figure 5). Between approximately 0.2 and 3 RFU, microcystin concentrations can be expected to exceed 6 µg/L more than 50% of the time. Below 0.2 RFU, microcystin samples can be expected to be lower than 6 µg/L more than 50% of time.

In order to improve predictive capabilities, a better model fit using additional data would be helpful.

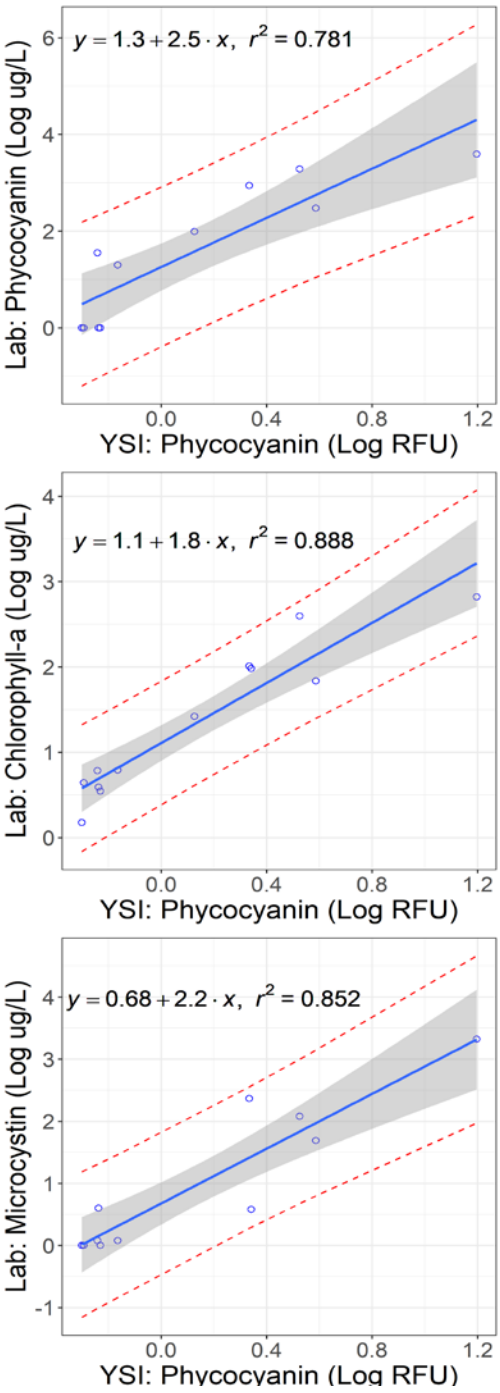

Figure 5. Linear regression between field-measured phycocyanin and lab-measured phycocyanin (top), lab-measured chlorophyll-*a* (middle), and lab-measured-microcystin (bottom).

The 95% confidence (gray shade) and prediction (red dashed line) intervals are also shown. Note data are log transformed.

Despite the strong relationships found for Spanaway Lake, caution should be exercised in applying the same relationship to another lake. The methods and tools developed in this study should be viewed as site-specific. Furthermore, we should also acknowledge the spatial variability inherent in cyanoHAB detection and monitoring; the approach taken in this study is suited to monitoring a public swim beach, not the entire lake.

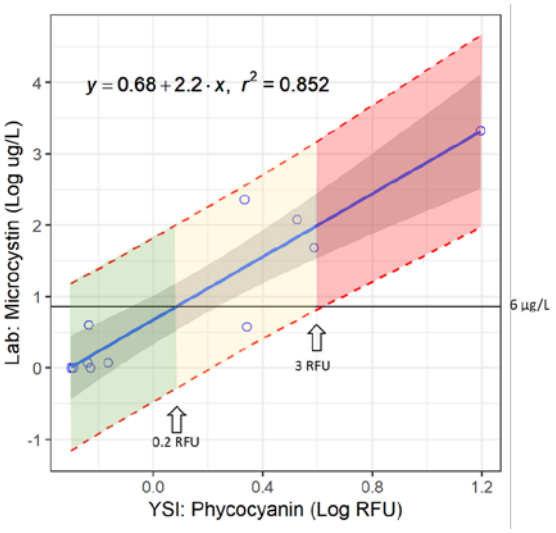

Figure 4. Predictive relationship between phycocyanin RFU (field) and microcystin (lab).

Gray shaded area is the 95% confidence interval for the regression. Red dashed lines are the 95% confidence limits for the predictive relationship. Green, yellow, and red shading corresponds to sections of the predictive relationship where the chances of exceeding the recreational microcystin threshold (6 µg/L) are <50%, >50%, and >95% respectively.

Conclusions

During the summer and fall of 2019, cyanoHABs in Spanaway Lake persisted from mid-August through mid-October. We deployed a YSI sonde in the lake to continuously measure pigments (phycocyanin and chlorophyll-*a*), dissolved oxygen, temperature, pH and conductivity. Eleven grab samples were used to establish relationships between field and laboratory measured parameters. The following conclusions can be made from this study:

- We found a statistically significant relationship between phycocyanin measured in the field (RFU) and the lab (µg/L).
- We found a statistically significant relationship between lab-measured phycocyanin and microcystin concentrations (µg/L).
- The dominant cyanobacteria during the cyanoHAB blooms were *Microcystis* sp., *Dolichospermum* sp., and *Woronichinia* sp.
- A predictive relationship between field-measured phycocyanin and laboratory-measured microcystin concentrations in the water appears possible from this pilot dataset.

Recommendations

The following recommendations can be made from this 2019 study:

- Suggest not using YSI's Total Algae Sensor for cyanoHAB monitoring in this lake because we did not find the chlorophyll RFU data to be reliable as an estimate of chlorophyll-*a* concentrations in the water.
- Replicate this study for an additional season of continuous monitoring in Spanaway Lake.
- Develop the capacity of real-time monitoring using satellite transmission of the sonde data.
- Communicate further and plan with the Pierce County Health Department on the utility and capacity of the findings as well as future technology development.

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Appendix A. YSI Measurement Quality Objectives

Lists of weeks and percentage of total hours that parameters fit the Measurement Quality Objective (MQO) categories of *Accept*, *Qualify*, or *Reject* Data.

Parameter	Units	MQO	Accept	Qualify	Reject	% Accepted	% Qualified	% Rejected
Chlorophyll-*a*	RFU	Accept: ≤ ±1.0 Qualify: > ±1.0, ≤ ±2.0 Reject: > ±2.0	6/19/19-8/1/19 8/14/19-9/30/19 10/10/19-10/28/19 11/15/19-12/3/19	8/2/19-8/13/19 10/29/19-11/14/19	10/1/19-10/9/19	77.3	17.4	5.4
Phycocyanin	RFU	Accept: ≤ ±1.0 Qualify: > ±1.0, ≤ ±2.0 Reject: > ±2.0	6/19/19-7/1/19 8/2/19-8/28/19 10/29/19-12/3/19	7/2/19-7/17/19 8/29/19-9/12/19 10/10/19-10/28/19	7/18/19-8/1/19 9/13/19-10/9/19	44.9	29.9	25.1
pH	std. units	Accept: ≤ ±0.2 Qualify: > ±0.2, ≤ ±0.8 Reject: > ±0.8	6/19/19-7/17/19 8/29/19-12/3/19	7/18/19-8/28/19	-	74.9	25.1	0
Conductivity	uS/cm	Accept: ≤ ±5 RPD[1] Qualify: > ±5 RPD, ≤ ±15 RPD Reject: > ±15 RPD	6/19/19-12/3/19	-	-	100	0	0
Dissolved Oxygen	mg/L	Accept: ≤ ±0.3 Qualify: > ±0.3, ≤ ±0.8 Reject: > ±0.8	6/19/19-12/3/19	-	-	100	0	0

[1] Relative Percent Difference

Appendix B. List of Phytoplankton Taxa

List of phytoplankton taxa observed in 12 samples collected from our sampling site on the north shore of Spanaway Lake. Dominant taxa in samples are marked "D" with red-colored cells. Subdominant taxa in samples are marked "S" with yellow-colored cells. Taxa that were present, but not commonly encountered, are marked "P" with blue-colored cells.

Taxa	19-Jun	1-Jul	17-Jul	1-Aug	14-Aug	28-Aug	12-Sep	30-Sep	9-Oct	28-Oct	14-Nov	3-Dec
Asterionella sp.	D	D	P	D	P				P	D	P	P
Ceratium sp.	P		P	P		P				P		
Cymbella sp.		P							P			
Dinobryon sp.	P		P	P	P							
Dolichospermum sp.	S	S	S	S	D	D	D	D	D	D	S	S
Eudorina sp.	P					P						
Fragilaria sp.	P	D	D	D		P	S	P	S	P	P	S
Microcystis sp.		S		S	D	D	D	D	D	S	P	D
Pandorina sp.		P										
Scenedesmus sp.		P										
Staurastrum sp.										P	D	
Tabellaria sp.		P										
Volvox sp.					P							
Woronichinia sp.		S	P	P	D	P	S	D	S	D	S	D
Undetermined Colony 1								P				
Undetermined Colony 2				P								
Undetermined Diatom 1 (Tiny)	P											
Undetermined Diatom 2 (Filament)		P										
Undetermined Diatom 3 (Filament)							P					
Undetermined Diatom 4 (Filament)		P										
Undetermined Diatom 5 (Coccoid)									P			
Undetermined Filament				P								

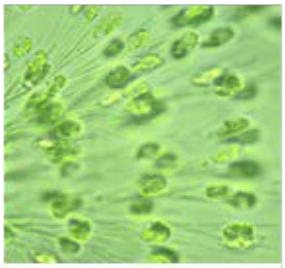
Appendix B cont'd. Phytoplankton Images

Images of selected phytoplankton taxa observed in samples collected from June through December 2019 at our sampling site on the north shore of Spanaway Lake. All images are captured at 400x under light microscopy unless noted.

Asterionella sp.

Ceratium sp.

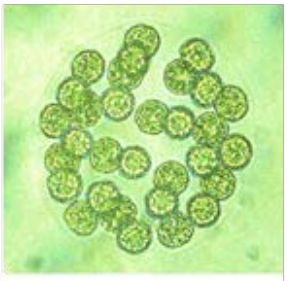
Cymbella sp.



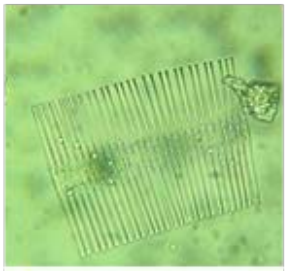
Dinobryon sp.

Dolichospermum sp. (100x)

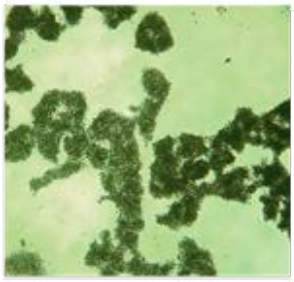
Dolichospermum sp.



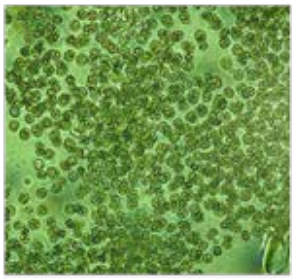
Eudorina sp.



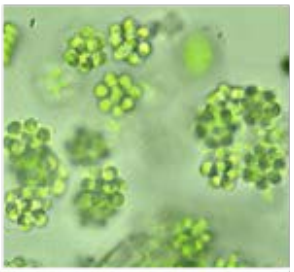
Fragilaria sp.



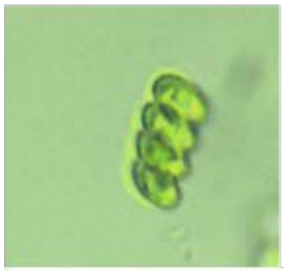
Microcystis sp. (100x)



Microcystis sp.

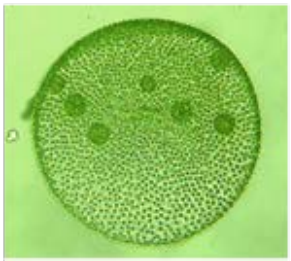


Pandorina sp.



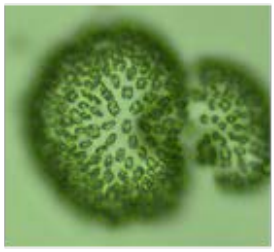
Scenedesmus sp.

Staurastrum sp.



Volvox sp. (100x)

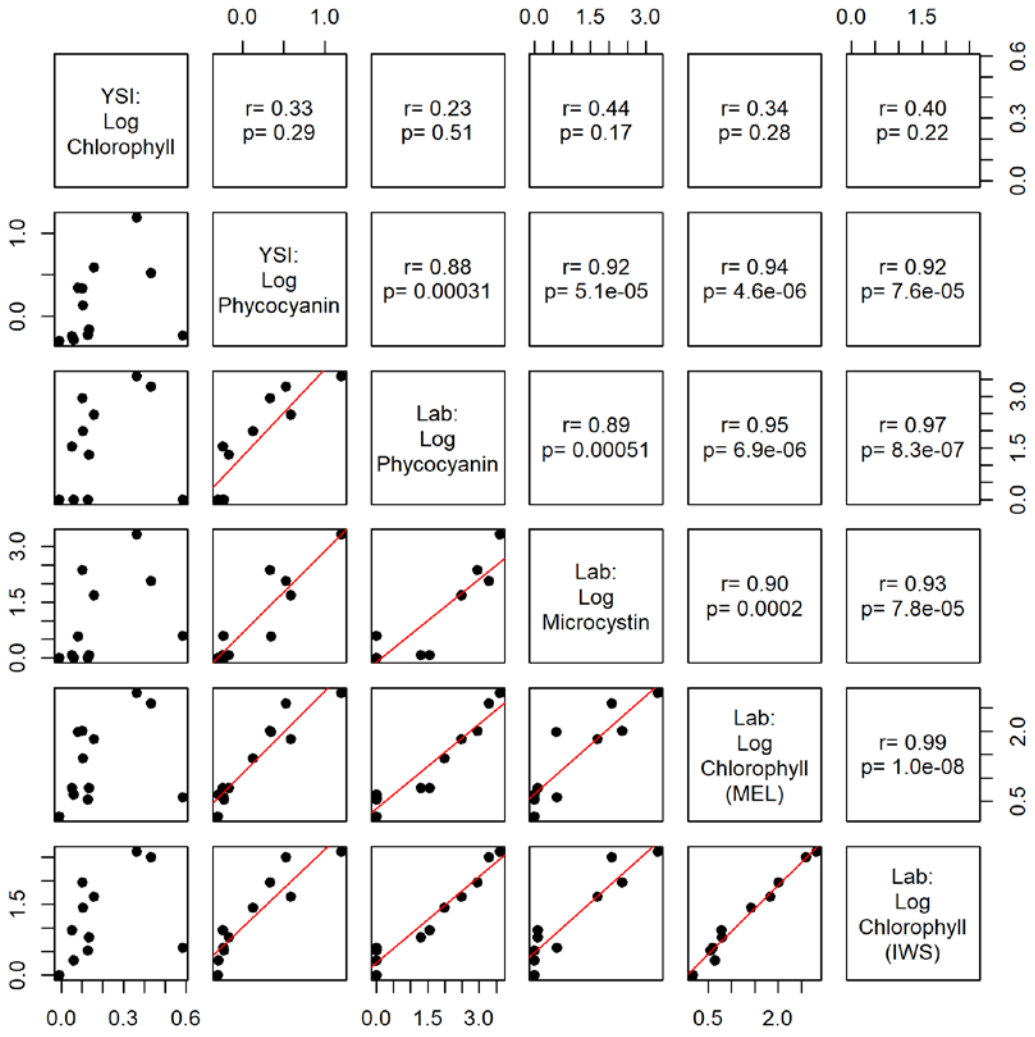
Woronichinia sp.



Woronichinia sp.

Appendix C. Correlations of Field and Lab Measurements

A Pearson correlation matrix of the field and laboratory measured parameters.
Red regression lines are included for those linear relationships that are statistically significant ($p<0.05$). All data are log transformed.



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