The Historical Prevalence of Cyanobacteria in Spanaway Lake, Pierce County, Washington

Environmental Assessment Program
Publication 22-03-009
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Abstract

Spanaway Lake in Pierce County (Washington) experiences annual harmful cyanobacterial blooms that produce high concentrations of the toxin, microcystin. The goal of this project was to understand whether (1) cyanobacteria have been a dominant part of the lake phytoplankton historically (~150 years ago) and (2) whether microcystin was produced historically.

The project entailed:

- Collecting a lake sediment core.
- Establishing sediment ages using radioisotopes.
- Analyzing the sediment intervals for fossil algal pigments, microcystin concentrations, and microcystin-producing genes.

pigment analysis of the sediments recorded a diverse cyanobacteria community in the lake, extending back to about 1850. This finding suggests that the physical and chemical (i.e. nutrients), characteristics of the lake supported cyanobacteria prior to urbanization. Measured pigments in modern sediments represented both colonial cyanobacteria (zeaxanthin and myxoxanthophyll) and filamentous cyanobacteria (canthaxanthin).

Microcystin compounds (MCs) were measured throughout the sediment core, with the MC-LA variant making up over 97-100% of the total MC. Copies of cyanobacterial 16S rRNA gene were measured throughout the sediment core. Microcystin-producing genes (mcyE) linked to several cyanobacterial genera (i.e., *Anabaena*, *Microcystis* and *Planktothrix*) were detected back to 1850 with a period of no detection from about 1900-1950. Microcystin-producing genes specific to *Microcystis* (mcyA) were detected in the sediment record after 1985. Overall, Spanaway Lake has a long history of cyanobacteria production; however, changes in the pigment abundance and microcystin-producing genes suggest that since 1985, the lake has had a greater dominance of cyanobacteria with higher levels of toxin production.

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Background

Cyanobacteria (blue-green algae) are common in many inland waters worldwide. This diverse group of photosynthetic bacteria has a variety of life histories and habitat niches; however, the most widely recognized are the planktonic (open water) species. Some species can produce toxins (collectively called cyanotoxins) harmful to humans and wildlife.

Spanaway Lake in Pierce County (Washington) has a long history of cyanobacterial blooms dominated by *Microcystis sp.*, *Dolichospermum sp.*, and *Woronichinia sp*. (Wong and Hobbs, 2019; Pierce County, 2016). Previous monitoring[5] of the blooms since 2007 has documented harmful concentrations of the cyanotoxin, microcystin in shoreline accumulations (Figure 1).

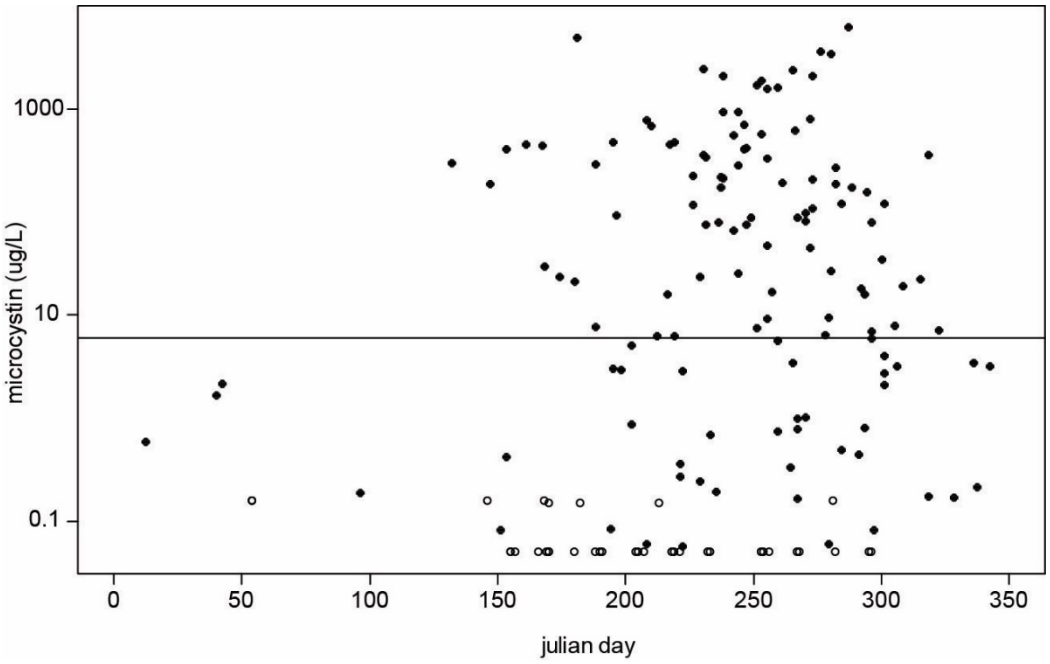

Figure 1. Microcystin in shoreline samples collected from Spanaway Lake, 2007-2020.

Black dots are measured concentrations and white dots are below the analytical detection limit.
Horizontal line is the Washington State Department of Health (DOH) recreational guideline (6 µg/L; Hardy, 2008).

Microcystins (MCs) are hepatotoxins (affecting the liver) consisting of over 200 variants or congeners, although seven congeners commonly comprise the bulk of the total MC in lake water (Catherine et al., 2017).

Lake sediment cores are an effective way of describing long-term ecological changes and the historical context of lakes over the last ~150 years or more. Analyzing lake sediment cores for the pigments in the algal cells that are deposited on the lake bottom is a proxy of cyanobacteria over time (Pal et al., 2015). Specific pigments are

[5] https://www.nwtoxicalgae.org/Data.aspx?SiteID=75

attributed to certain groups of algae (Leavitt and Hodgson, 2001) and therefore changes over time represent how algal communities have changed. Microcystins have been successfully measured in lake sediment cores spanning the last 150 years (Efting et al., 2011; Zastepa et al., 2017). Furthermore, cyanobacterial toxin genes have also been successfully measured in lake sediment studies (Pal et al, 2015; Hobbs et al, 2021). The goal of this study was to analyze a sediment core from Spanaway Lake and ask the question: Have cyanobacteria been prevalent in the lake over the last ~150 years? If so, is there any indication that microcystin was historically present in this lake?

Methods

Study Site

Spanaway Lake is an urban lake located in Pierce County (Washington) (Figure 2). The lake has a long history of shoreline development and recreation. Much of the immediate lake shoreline is residential with a park and golf course to the northeast of the lake (Figure 3). The surrounding watershed is mostly residential with about 30% undeveloped land situated on the Joint Base Lewis-McChord (JBLM) to the southwest of the lake. As early as the 1890s a railway was built to the park to make it a recreation destination. In 1967, the golf course was completed and opened. It was not until the late 1990s that the golf course clubhouse and other park facilities were connected to a sanitary sewer system; the same is also true of the Coffee Creek Condominiums adjacent to the main surface water inlet for the lake. All residences on the lake shore and many within the watershed rely on on-site septic systems (OSS). It is estimated that there are more than 4000 OSS in the watershed (Pierce County, 2016).

Figure 2. Washington State map with the location of Spanaway Lake.

The lake is 110 ha (272 acres) in area and has a maximum and mean depth of 8.5 m (28 ft) and 4.9 m (16 ft), respectively (Figure 3). Spanaway Lake is a kettle lake and the hydrology is heavily influenced by groundwater inputs, possibly up to around 65% of the inflow (Pierce County, 2016). Surface water enters the lake at the southern end from Coffee Creek which originates in a wetland complex on JBLM land. The primary surface outlet, Spanaway Creek, is at the north end of the lake, although it is also possible that a portion of the lake water leaves via groundwater in the north end of the lake (Pierce County, 2016).

The lake's submerged plant community was assessed in 1996, 2002, 2008, 2009 and 2017 by the Washington State Department of Ecology[6]. Pondweeds (*Potamogeton* spp.), muskwort and water-lilies have dominated the communities since 1996. The invasive plants, Yellow flag *Iris* and Reed canary grass have been present since

[6] https://apps.ecology.wa.gov/coastalatlas/tools/LakeDetail.aspx?ReachCode=17110019001229

1996; the invasive Curly leaf pondweed seems to have increased in density over time. There have been numerous applications of both glyphosate and diquat dibromide to reduce the density of pondweeds and water-lilies.

Spanaway Lake supports populations of largemouth and smallmouth bass, yellow perch and rock bass. Rainbow trout are stocked by the Washington Department of Fish and Wildlife. Other fish found in the lake include common carp, pumpkinseed, sculpin, brown bullhead and cutthroat trout (Caromile and Jackson, 2002).

There has been one year of quantitative study of phytoplankton community composition and succession in Spanaway Lake, from Oct 2014-Oct 2015 (Pierce County, 2016). The study found that for most of the year cyanobacterial species were the most dominant phytoplankton (~90% of the community), with some of the highest counts during the winter months. The spring (April-May) was dominated by diatoms and green algae and then cyanobacteria became dominant again by June. Cyanobacteria were denser in the bottom waters from June through September, until fall mixing occurred and cyanobacteria became higher in the upper waters or mixed throughout the water column.

Figure 3. (left) Spanaway Lake, (middle) lake bathymetry and lake sediment core location and (right) cross-section of the sediment core.

Bathymetry contours are in feet.

A 40-cm sediment core was retrieved.

Field Methods

A 40-cm long sediment core was recovered from the deepest point in the lake using a gravity box corer (11 x 11 x 50cm) with a sterilized acrylic liner (Glew et al., 2001). The sediment core was sectioned on the boat at 1-cm intervals, and samples were frozen within 12 hours. Samples intended for DNA analysis were collected from the center of each sediment interval using sterile stainless steel scooptulas. DNA samples were shipped frozen to the lab and stored at -80°C until analysis. Following the removal of the sample used for DNA analyses the remaining sediment interval was transferred to sterile tubes after the edges of the sediment in contact with the core liner were trimmed and discarded. All samples were freeze-dried and stored in the freezer at -20°C to avoid photodegradation of the algal pigments (Leavitt and Hodgson, 2001).

Laboratory Methods

Laboratory quality objectives and procedures were outlined in the Quality Assurance Project Plan (QAPP; Hobbs, 2020). Measurement quality objectives defined in the QAPP were satisfied, any exceptions have been detailed in this section.

Sediment composition was determined using loss-on-ignition (LOI), which describes the relative percent of organic matter, carbonate, and mineral content (Heiri et al., 2001). The content and stable isotope ratios of carbon (C) and nitrogen (N) were measured at the Stable Isotope Laboratory – University of California Santa Cruz using a CE Instruments NC2500 elemental analyzer coupled to a Thermo Scientific DELTAplus XP isotope ratio mass spectrometer via a Thermo-Scientific Conflo III.

Sediment subsamples were dated using the activity and modelled decay of radioisotopes (^{210}Po-^{210}Pb) (Appleby, 2001). Samples (0.2 - 0.5 g DW) were analyzed by the St. Croix Watershed Research Station at the Science Museum of Minnesota using alpha spectroscopy (Eakins and Morrison, 1978). The constant rate of supply model was then used to establish an age-depth model (Appleby and Oldfield, 1978). An independent radioisotopic age marker was established using ^{137}Cs measured by gamma spectroscopy using ~3g DW of sediment. Measurements of the background ^{214}Pb by gamma spectroscopy also provided confirmation of supported ^{210}Pb for the age-depth model.

Total metals (copper, aluminum, phosphorus, iron and manganese) were measured using EPA method 6020B by ICP-MS. (USEPA, 2014). Sediment samples were digested with concentrated nitric acid as per EPA method 3050B (USEPA, 1996). The instrument was calibrated with a NIST traceable standard and all calibration checks were within acceptance limits. All laboratory quality control (QC) samples met the project acceptance limits, except for the matrix spike sample recovery for iron which was outside acceptance limits due to the high natural concentration of iron in the sediments. The results for iron were subsequently reported as estimates.

Sediments were analyzed for algal pigment concentrations by Dr. Rolf Vinebrooke, University of Alberta, using reverse-phase high pressure liquid chromatography (HPLC, Leavitt and Hodgson, 2001). All samples were first freeze-dried, and then homogenized using a mortar and pestle prior to extraction of

the pigments. The extraction solution consisted of acetone, methanol, and water (80:15:5 v/v), which was added to a pre-weighed amount of sediment from each sample contained within a borosilicate vial for 24 h in darkness at 10 °C. Pigments were separated using a Agilent (Agilent Technologies Canada Inc., Mississauga, Ont.) model 1100 system with a Varian Microsorb 100 C-18 column (10-cm long, 5-µm particle size) and detected by an inline HP Series 1100 diode array detector (435-nm detection wavelength) and a fluorescence detector (435-nm excitation wavelength, 667-nm detection wavelength). Pigment concentrations were normalized to the organic matter concentration of the sediment and chlorophyll a and derivatives were summed and reported as a *total* chlorophyll concentration.

Microcystin compounds (MCs) were analyzed by King County Environmental Laboratory (KCEL), as described in Preece et al. (2021). Briefly, two analytical methods (ELISA and LC-MS/MS) were used to measure MCs in the sediment. Sediment samples were freeze-dried and homogenized. A 2 g portion was weighed into a glass vial with 10 ml of extraction solvent (0.1 M EDTA, 0.1M $Na_4P_2O_7$) and the pH was adjusted to between 3 and 3.4 using trifluoroacetic acid (Chen et al., 2006). The sediment slurry was vortexed, and spikes were added to QC samples for spiked blank, matrix spike, and matrix spike duplicate. Samples were sonicated and centrifuged and the supernatant was filtered prior to solid phase extraction on Waters Oasis HLB 3 cc extraction columns.

For the LC MS/MS, analysis seven microcystin congeners and total toxin nodularin were measured. The toxin, nodularin was included as part of KCEL's analytical suite. No evidence of laboratory contamination was noted in the blank samples. The batch MS/MSD recoveries exceeded the QC limits for the variants nodularin and MC-YR; the high MS/MSD recoveries were not a concern to data quality. All continuing calibration checks met the QC limits for these variants. Continuing calibration checks had average recoveries of: 105% (MC-RR), 98% (MC-LR), 101% (MC-YR), 104% (MC-LA), 108% (MC-LY), 112% (MC-LW), 101% (MC-LF), and 110% (Nodularin). The detection limit for the LC-MS/MS method was 0.05 µg/g DW.

MC-ADDA ELISA kits were obtained from Abraxis LLC. (Warminster, PA, USA). This ELISA targets the ADDA moiety common to all MC variants and nodularin. ELISA analyses were conducted following instructions described by the manufacturer. Samples were analyzed in duplicate and diluted per ELISA kit instructions to contain <5% methanol. No evidence of false positives was noted when compared to the LC-MS/MS data. The detection limit for the ELISA method was 0.75 µg/g DW.

Analysis of the sediment intervals for toxin-producing genes using qPCR was carried out by US EPA's Office of Research and Development, Cincinnati, OH. DNA extractions were performed using the Dneasy PowerLyzer Power Soil Kit (Qiagen, Hilden, Germany). Samples were centrifuged at 2000 rpm for 3 minutes and excess water was removed from the sediment samples. Sediment from each sample was aseptically placed in a bead tube provided in the extraction kit and weighed. Duplicates were taken from each sample and the amount of sediment extracted ranged from 220 to 310 mg. The manufacturer's standard operating procedure was followed for

DNA extraction using the vortex adapter for homogenization and DNA was eluted with 100 µL of elution buffer. DNA extracts were stored at -20 C until qPCR analysis could be performed.

We conducted qPCRs using an Applied Biosystems™ QuantStudio™ 6 Flex Real-Time PCR System (Applied Biosystems, Foster City, California, USA). Each reaction mixture (final volume 20 µL) contained 10 µL of 2× qPCR SYBR® Green Master Mix (Life Technologies, Carlsbad, CA), 0.25 µM primers (each; Integrated DNA Technologies, Inc., Coralville, IA), and 2 µL of template DNA. The thermal cycling included 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C extension for 30s, or the annealing temperatures (Table A-1).

Each qPCR was run with a 7-point standard curve ranging from $5x10^6$ to $5x10^0$ copies/µL. The template for the standard curve was a linearized plasmid with the PCR target cloned into an Invitrogen™ pCR™4-TOPO™ Vector (Thermo Fisher Scientific Inc., Waltham, MA). The qPCR primers and targets can be found in Table A-1. Original DNA template and a 10-fold dilution of the DNA were run in the assays to detect inhibition in the samples. Inhibition was defined by having less than 3.0 Ct values between the undiluted and diluted DNA aliquots used as templates. If inhibition was detected the quantity for the diluted DNA template was used to estimate the gene copies in the sample; if no inhibition was detected the value for the undiluted DNA was reported.

Statistical Methods

To detect statistically relevant changes in the trends of pigments over time, nonparametric Mann-Kendall tests of the individual pigment trends were used (Taranu et al. 2015). The Mann-Kendall test is a rank correlation analysis and the level of significance is assessed using the trend coefficient (tau) and p-value. In addition, a principal component analysis was conducted on the relative pigment concentrations to explore changes in pigments over time. All data were centered and standardized. The first two axes were significant as compared to a random broken-stick model and explained a cumulative total of 61% of the variability in the dataset. All analyses are completed in R (R Core Team, 2020) with the vegan package (Oksanen et al., 2019).

Results and Discussion

Sediment Dating

A reliable age-depth model was established for the sediment core based on conformable, exponential decay of unsupported or excess ^{210}Pb activity (Figure 4). The model is dependent on measuring the supported (background) ^{210}Pb activity, which was reached at a depth of 40 cm. The background ^{210}Pb activity was also independently confirmed by measuring ^{214}Pb activity; these two radioisotopes should be in equilibrium. In addition, ^{137}Cs activity was independently measured to establish the 1963 peak of atmospheric bomb testing (Appleby, 2001). As shown in Figure 4 the modeled 1963 date and the measured ^{137}Cs peak overlap.

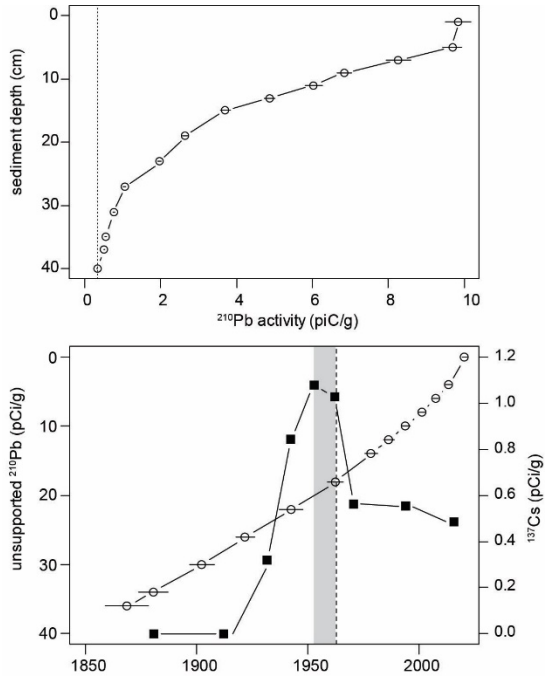

Figure 4. Sediment radioisotopes and estimated ages.

Top: ^{210}Pb activity with sediment depth; dashed vertical line represents the supported ^{210}Pb (confirmed with ^{214}Pb).
Bottom: estimated sediment ages (o) and error with sediment depth; measured 137Cs activity (■) confirming the modeled 1963 date (vertical dashed line).

Sediment Accumulation and Composition

The rate at which sediment has accumulated at the core location has changed slightly over time (Figure 5). Sediment accumulation remained relatively constant from ~1850-1940; from 1940-1975, there is an increase in the accumulation rate; and post-1975, the rate of accumulation is again relatively constant. It is possible that the increase in sediment accumulation rate from 1940-1975 coincides with increased development of the shoreline and increased sediment inputs from the watershed. Since 1900 there has been a linear rate of sedimentation of around 0.26 cm/yr.

The sediment at the bottom of Spanaway Lake is composed primarily of mineral material with a large fraction of organic matter (~40%) (Figure 5). The composition of the sediment has been relatively constant over the last ~150 years.

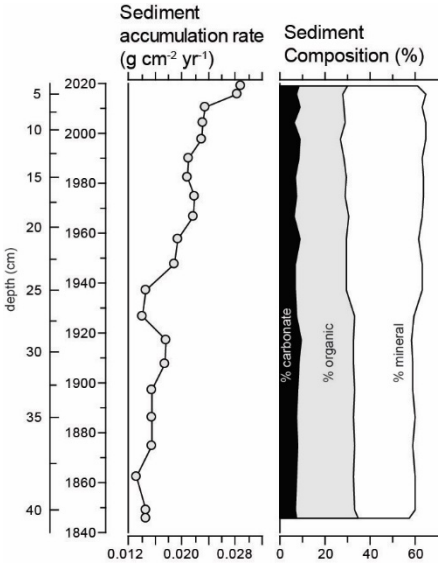

Figure 5. Sediment dry mass accumulation rate and composition over time.

Metal Concentrations

Sediment core trends of the metals copper (Cu), aluminum (Al), phosphorus (P), iron (Fe), and manganese (Mn) were analyzed to (1) look at possible inputs of Cu from historic in-lake copper sulfate treatments to reduce algae growth, and (2) attain baseline data on the concentrations of Fe, Mn, and Al. The latter being of interest in case any future in-lake treatments are planned using alum ($Al_2(SO_4)_3$) or zerovalent Fe filings.

The time trend of both Cu and Al mirror each other, showing a steady increase in concentration beginning in the early 1900s (Figure 6). Concentrations of Cu plateau at around 1985 and then begin to decrease to concentrations similar to the

1930s. The trend of Cu and Al in Spanaway likely reflects inputs (atmospheric and via surface waters from the watershed) from the operation of the Tacoma Asarco copper smelter which was operable from 1920-1985. Gawel et al. (2014) documented arsenic and lead inputs to regional lakes, including Spanaway, from the Asarco smelter. It does not appear that historic copper sulfate treatments were decipherable using the sediment record.

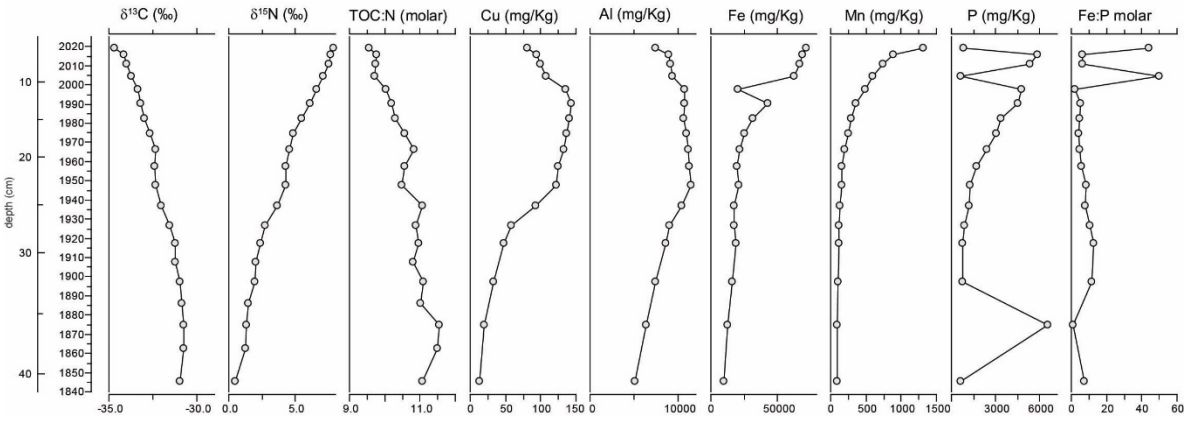

Figure 6. Sediment geochemistry of the Spanaway Lake core.

Both Fe and Mn are redox sensitive metals, and as such they can become mobile in the sediments under anoxic (absence of oxygen) conditions (Engstrom and Wright, 1984). In the deeper parts of lakes, only the upper few millimeters of lake sediment that is contacting the water contains oxygen; below that, oxygen is consumed by microbial activity. As oxygen is consumed Fe and Mn are no longer stable and therefore reduced forms appear to migrate upwards as new sediment falls to the bottom of the lake. The sediment core trends for these metals in Spanaway Lake are fairly typical (Engstrom and Wright, 1984), with an increasing trend towards the surface as the metals accumulate near the sediment-water interface.

Iron and Mn are also the two main metals that bind labile and dissolved bioavailable forms of phosphorus in the sediments. Phosphorus is often the most important nutrient supporting algal growth in lakes. The analysis of metals and P in Spanaway Lake sediments for this study is not sufficient to understand the internal loading of P from the sediments when Fe and Mn bonds break under anoxic conditions. There are two sediment intervals worth noting in the record when the molar ratio of Fe:P is high (>15; Jensen et al., 1992) and we might expect there to be retention of P in the sediments: the surface sample (~2019) and 6-7cm depth (~2005). However, the high Fe:P ratio is due to low concentrations of P in these sediment intervals which may be due to movement within the sediments. Lastly, the highest measured P in the sediment was a historic sample at around 1875; the cause of this is not clear.

Fossil Algal Pigments

The sediments of Spanaway Lake have several well-preserved algal pigments (Figure 7). Chlorophylls and carotenoids from different algal groups were identifiable. Most importantly, there were several pigments associated with cyanobacteria throughout the sediment core (i.e. since ~1850). The most abundant

cyanobacteria pigment was the carotenoid zeaxanthin which has been associated with colonial cyanobacteria, like *Microcystis* spp., and picocyanobacteria (e.g. *Synechococcus* spp.) (Paerl et al. 1983; Krajewska et al., 2019; Bianchi et al., 2000; Romero- Viana et al., 2010). In addition, the carotenoid canthaxanthin was identified throughout the sediment record and has been associated with filamentous heterocystous (Nostocales) cyanobacteria (e.g. *Anabaena* and *Dolichospermum* spp.) (Jeffrey and Vesk, 1997; Tse et al., 2015; Krajewska et al., 2019). Lastly, low concentrations of the carotenoid myxoxanthophyll, from both filamentous and colonial cyanobacteria (Schlüter et al., 2016; Srivastava et al., 2017; Schlüter et al., 2018), were measured during 1950-1975 and ~1900. Other pigments found in the sediment record included: fucoxanthin and diatoxanthin (diatoms and chrysophytes); alloxanthin (cryptophytes) and primary chlorophylls and degradates (general algal production).

Any study relying on the measurement of sediment parameters from cores should consider possible alterations following deposition that may impact the interpretation of temporal trends. It is common when interpreting trends of sedimentary pigments to use the ratio of chlorophyll a: pheophytin *a* as a rough estimate of post-depositional diagenesis (Leavitt and Hodgson, 2001), where pheophytin *a* is the primary degradation product and a shift to more of it through the core would suggest greater diagenesis. In the Spanaway Lake core the ratio was very stable beyond the surface sample (0.33 ± 0.09; mean ± SD) suggesting a reliable record of past algal production.

There have been some changes in the algal pigments in Spanaway Lake since ~1850 (Figure 7). It appears that the abundance of diatoms (diatoxanthin) has significantly decreased over time (tau = -0.73; p = 0.000089). It also appears that the cyanobacteria community has changed over time based on (1) the presence of myxoxanthophyll at ~1900 and during ~1950-1975, and (2) a significant decrease in zeaxanthin abundance occurring pre-1950 (tau = -0.63; p = 0.0007). Zeaxanthin may be associated with picocyanobacteria, which can produce toxins but this is likely rare and has not been well-studied (Śliwińska-Wilczewska et al. 2018); zeaxanthin is also associated with *Microcystis* spp. (Smit et al., 1983; Krajewska et al., 2019), which are present in the modern cyanobacteria community and do produce toxins.

A period of notable change across all pigments is evident around ~1980 when there is a decrease in pigment abundance (i.e. algae production) (Figure 7). This decrease in pigment concentration is not due to changes in the sediment accumulation (i.e. dilution) or composition. Using a multivariate analysis to look at the relative changes in pigment concentration through time (Figure 8) there is a transition occurring from ~1975-1985. Altogether, we identified three distinct periods since 1850:

1. 1850-1975: total algae production is high (total chlorophyll a and lutein), there are periods of cyanobacteria producing myxoxanthophylls and green algae producing chlorophyll b; diatom and picocyanobacteria abundance are high; filamentous cyanobacteria are abundant
2. 1975-1985: a period of transition; algae production is the lowest for the sediment core.
3. ~1985-present: cyanobacteria abundance is high, relative to other types of algae; total algae production is roughly the same as historic levels; diatom abundance is low.

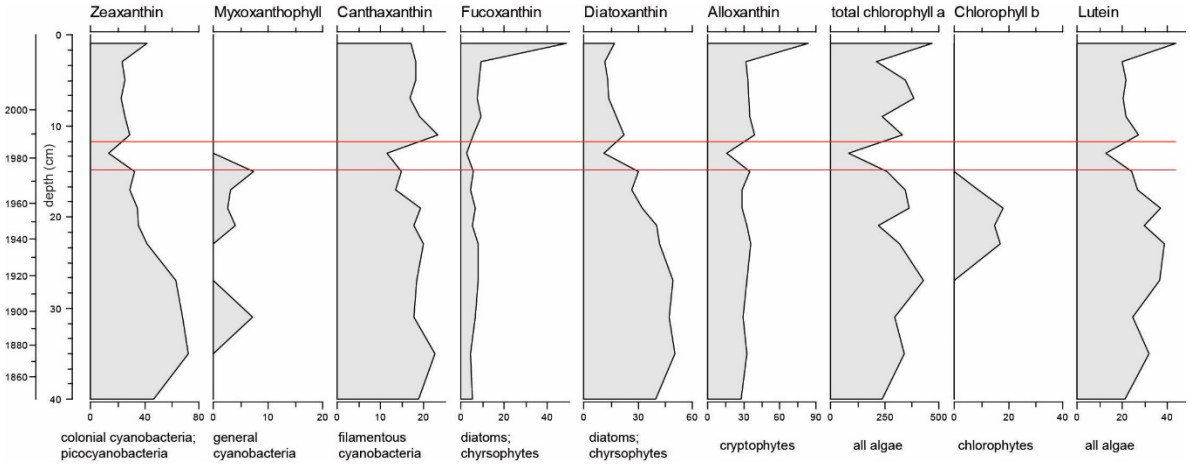

Figure 7. Algal pigment concentrations (µg/g OM) over time in Spanaway Lake.

Horizontal red lines represent a period of transition (1975-1985) as discussed in the text. OM = organic matter.

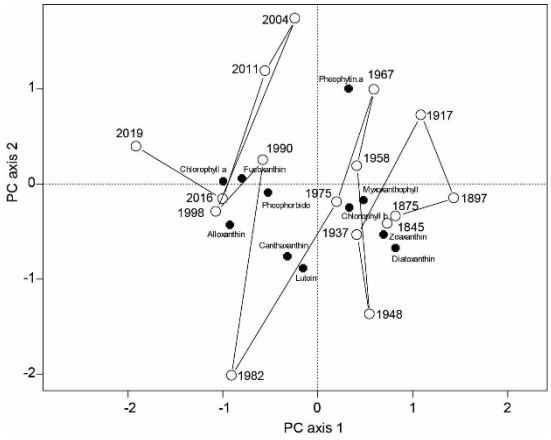

Figure 8: Principal Component Analysis of relative pigment concentrations.

Both PC axis 1 and 2 are significant when compared to a random broken-stick model. PC1 explains 35% of the variance, PC2 explains 26%.

Sediment Microcystin and qPCR of Cyanobacteria

The microcystin congener MC-LA, as measured by the LC MS/MS method, was detected throughout the sediment core. Of the seven MC congeners measured by LC MS/MS, MC-LA is the dominant congener in Spanaway Lake with minor amounts of MC-LR (Table A-2). Preece et al. (2021) showed a similar MC congener composition among other environmental media in Spanaway Lake (i.e. water and shoreline sediments). Total microcystin, as measured by the ELISA method, was not detectable below a depth of 18cm (~1960) in the sediment core (Figure 9). The ELISA method has a higher method detection limit, so the lack of detection below 18cm is not unexpected.

We used a 16S rRNA gene-based assay to detect total cyanobacteria. The results indicate that cyanobacterial populations were present in each sample throughout the core (Figure 9). *Microcystis*-specific 16S rRNA genes were not detected throughout the core. Gaps in detection occurred pre-1920, and at around 1950 and 1965. The presence of microcystin-producing genes (mcyE) linked to cyanobacteria genera such as *Anabaena*, *Microcystis* and *Planktothrix* (Jungblut and Neilan, 2006; Lu et al., 2020) were detected as early as 1850 with a period of no detection from ~1900-1950. Lastly,

the microcystin-producing genes specific to the genus *Microcystis* (mcyA) were found in the sediment record after 1985. No genes indicative of anatoxin-a production (anaC) were detected in the sediment core. Annual public health sampling of shoreline waters for cyanotoxins (2007 to present) corroborates the lack of measurable anatoxin-a produced by cyanobacteria in Spanaway Lake. All qPCR QC passed the laboratory measurement quality objectives, which means that a sufficient amount was extracted and the purity was adequate.

Detectable concentrations of MC-LA in the Spanaway Lake sediment since ~1850 suggests that microcystin producers have been in the lake since this time. There is general agreement between the presence of cyanobacteria pigments and the presence of cyanobacterial 16S rRNA gene; however, there is not a linear relationship or direct correlation between these two measurements. The presence of the mcyE genes decreases in the early 1900s, which is when the decrease in zeaxanthin begins; this transition may represent a historical reduction in microcystin producers. At ~ 1940, myxoxanthophyll increased and 16S rRNA gene copies for cyanobacteria and mcyE gene increased back to pre-1900 concentrations.

For both the pigment and the qPCR data, the post-1985 period of lake history is characterized by a dominance of cyanobacteria production and the presence of microcystin-producing genes. Post-1985 concentrations of MC-LA in the sediments are the highest measured and the *Microcystis* specific gene mcyA increases dramatically in abundance.

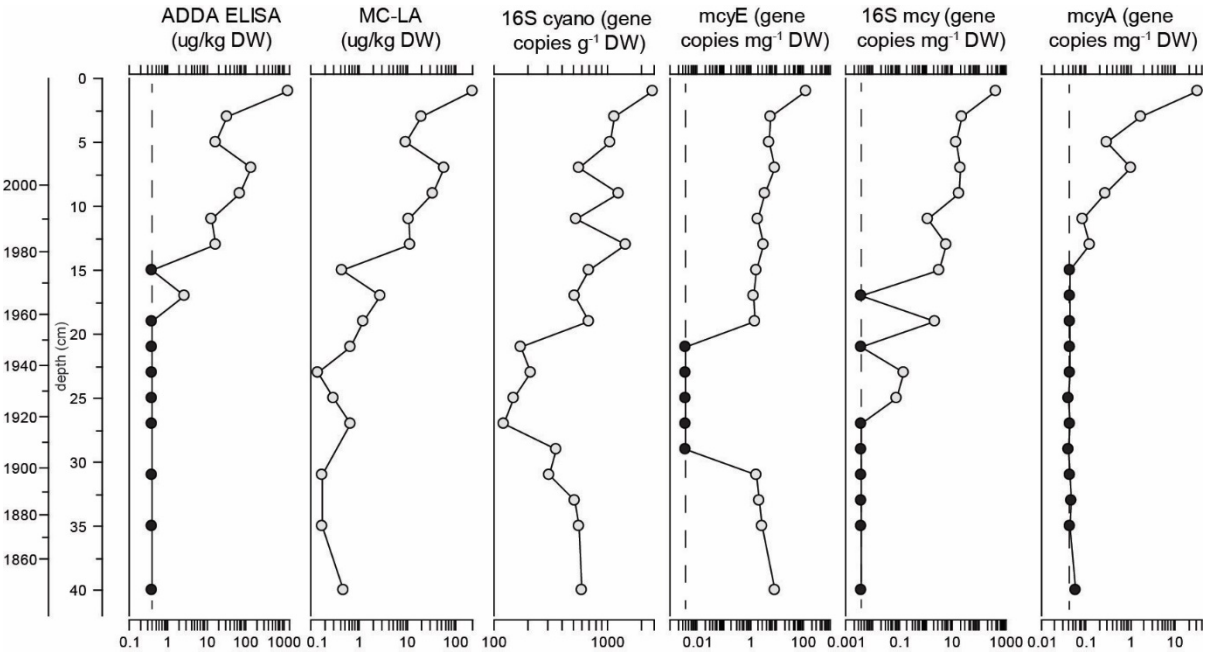

Figure 9. Microcystin and microcystin-producing genes (mcy) over time.

Vertical dashed lines represent the level of method detection; black dots are concentrations <MDL.

Conclusions

The sediment record from Spanaway Lake provides historical context to the prevalence and composition of cyanobacterial communities in the lake over the last ~150 years. Conclusions from this work include:

- The sediment accumulation rate to the bottom of Spanaway Lake increased slightly during ~1940-1975.
- Copper concentrations in the sediment record do not reflect historic copper sulfate herbicide treatments to limit algae growth; rather the Cu trend reflects the regional influence of smelting activities from the Asarco smelter in Tacoma.
- Iron concentrations in the sediment are naturally high and exhibit a trend with depth that suggests a mobility in the sediments with changes in oxygen content and redox.
- The physical, and likely chemical, setting of the lake naturally supports a diverse cyanobacteria community.
- Direct measurements of microcystin-LA and microcystin-producing genes (mcyE) in sediments that are ~150 years old confirms that toxins were produced historically.
- It is likely that the post-1985 period of lake history had a dominance of cyanobacteria with higher levels of toxin production. Post-1985, *Microcystis* spp. (mcyA) are contributing to the total microcystin along with other toxin producers (mcyE).

Recommendations

Based on the findings that Spanaway Lake had a long history of microcystin-producing cyanobacteria blooms, the following recommendation can be made:

- Any management plan for the control or mitigation of cyanobacteria blooms should acknowledge the historical prevalence of cyanobacteria in the lake and historically nutrient-rich waters. For instance, the efficacy or duration of short-term treatments of the water column or sediments may be limited in a naturally nutrient-rich lake.
- A better understanding of the cyanobacteria community (taxa) and the seasonality of N and P cycling, would provide important information on the drivers of toxin production.

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Appendix

Table A-1: qPCR primers and targets.

Primer	Sequence	Target	Annealing Temp	Source
Cyan108F	ACGGGTGAGTAACRCGTRA	16S all cyanobacteria	56	Nubel et al, 1997 and Urbach et al, 1992
Cyan 377R	CCATGGCGGAAAATTTCCC			
Micr 209F	ATGTGCCGCGAGGTGAAACCTAAT	Microcystis 16S genus	64	Neilan 1997
Micr 409R	TTACAATCCAAAGACCTTCCTCCC			
HepF	TTTGGGGTTAACTTTTTTGGGCATAGTC	All Microcystin producers	54	Jungblut and Neilan 2006
HepR	AATTCTTGAGGCTGTAAATCGGGTTT			
mcyA-MSF	ATCCAGCAGTTGAGCAAGC	Microcystin/ Microcystis specific	60	Tellit et al, 2001 and Furukawa et al, 2006
mcyA-MS2R	GCCGATGTTTGGCTGTAAAT			
mcyE Mic F3	GTTATGTTTGCCGGCTCCTA	Toxic Microcystis	60	This study
mcyE Mic R3	GTGCCTAGACTTAAGGGTTGAG			
AnaCF1	GGTCCTGGGTTGATGACAGG	General anatoxin	60	Chen et al, 2017
AnaCR1	CGGTAGCCCCGACTCTTAATC			

Table A-2: Sediment microcystin concentrations using both LC MS/MS and ELISA assay.

Upper depth	Lower depth	Estimated age	Total MC by LCMS (ug/kg DW)	Total MC by ELISA (ug/kg DW)	MC-LA (ug/kg DW)	MC-LF (ug/kg DW)	MC-LR (ug/kg DW)	MC-LW (ug/kg DW)	MC-LY (ug/kg DW)	MC-RR (ug/kg DW)	MC-YR (ug/kg DW)	Nodu-larin (ug/kg DW)
0	1	2019.0	223.33	1307.73	218.00	0.463	4.24	<MDL	0.37	<MDL	0.017 J	<MDL
2	3	2015.6	19.33	33.58	19.20	<MDL	0.15	<MDL	<MDL	<MDL	<MDL	<MDL
4	5	2010.6	9.56	17.05	9.43	<MDL	0.13	<MDL	<MDL	<MDL	<MDL	<MDL
6	7	2004.3	57.16	145.06	56.50	0.073	0.55	<MDL	<MDL	<MDL	<MDL	<MDL
8	9	1997.7	34.41	72.77	34.00	<MDL	0.46	<MDL	<MDL	<MDL	<MDL	<MDL
10	11	1990.2	10.85	13.94	10.80	<MDL	0.067	<MDL	<MDL	<MDL	0.017 J	<MDL
12	13	1982.3	11.56	17.16	11.50	<MDL	0.042 J	<MDL	<MDL	<MDL	<MDL	<MDL
14	15	1974.6	0.46	<MDL	0.45	<MDL	0.01 J	<MDL	<MDL	<MDL	<MDL	<MDL
16	17	1966.5	2.81	2.66	2.81	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
18	19	1957.6	*1.35	<MDL	1.25	<MDL	0.049 J	<MDL	<MDL	<MDL	0.044 J	<MDL
20	21	1947.8	0.67	<MDL	0.65	<MDL	<MDL	<MDL	<MDL	<MDL	0.02 J	<MDL
22	23	1937.0	0.17	<MDL	0.14	<MDL	0.024 J	<MDL	<MDL	<MDL	<MDL	<MDL
24	25	1926.7	0.30	<MDL	0.30	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
26	27	1917.2	0.68	<MDL	0.66	<MDL	0.021 J	<MDL	<MDL	<MDL	<MDL	<MDL
30	31	1897.1	0.17	<MDL	0.17	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
34	35	1874.9	0.19	<MDL	0.17	<MDL	0.022 J	<MDL	<MDL	<MDL	<MDL	<MDL
39	40	1845.5	0.48	<MDL	0.48	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL

*Laboratory duplicate analysis of this sample was 1.26 ug/kg DW (relative percent difference of 6%).
"J" qualifier represents and estimated concentration below the reporting limit.
MDL = method detection limit (0.05 ug/kg DW for the LC MS/MS method; 0.75 ug/kg DW for the ELISA method).

Publication Information

This report is available on the Department of Ecology's website at:
https://apps.ecology.wa.gov /publications/SummaryPages/2203009.html.

Data for this project are available in Ecology's EIM Database. Study ID: WHOB008

The Activity Tracker Code for this study is: 21-008.

Suggested Citation:
W. Hobbs , E. Frame , F. Sweeney, I. Struewing, N. Sienkiewicz, J. Lu , E. Villegas and R. Labiosa. 2022. The Historical Prevalence of Cyanobacteria in Spanaway Lake, Pierce County, Washington. Publication 22-03-009. Washington State Department of Ecology, Olympia. https://apps.ecology.wa.gov /publications/SummaryPages/2203009.html.

Water Resource Inventory Area (WRIA) and 12-digit Hydrologic Unit Code (HUC) numbers for the study area:

- WRIAs: 12 – Chambers - Clover watershed
- HUC numbers: 171100190302 – Clover Creek; HUC8: 17110019 – Puget Sound

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