# The Historical Prevalence of Cyanobacteria in Pass Lake, Skagit County, Washington

**Environmental Assessment Program**
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## Abstract

Pass Lake in Skagit County (Washington) experiences annual harmful cyanobacterial blooms that produce high concentrations of the toxins, anatoxin-a and microcystin. The goal of this project was to understand whether cyanobacteria have been a dominant part of the lake phytoplankton historically.

A 70cm sediment core was collected and an age-depth model established a chronology of sediment accumulation back to roughly 1600 AD (~400 years). Sediment accumulation rates showed a period of increased accrual starting in the 1930s (and ending around 1975) — when the lake shoreline was initially developed by the Civilian Conservation Corps for Deception Pass State Park. Algal pigments representative of cyanobacteria were measured in the historical sediment deposits (~400 years old) along with the microcystin-producing gene marker McyE. Data from this study suggests that cyanobacteria were historically present in Pass Lake, however degradation of some of the pigments and gene markers prevented establishing a detailed long-term history for the lake.

Opportunistic water samples were collected and analyzed by qPCR[2] for genetic indicators of toxin production and nitrogen fixation. The samples showed an abundance of toxin-producing genes for microcystin and anatoxin-a; this coincided with very high measured concentrations of these toxins during routine shoreline sampling around the same time.

[1] Washington State Department of Ecology, Olympia, WA

[2] Quantitative Polymerase Chain Reaction

# Publication Information

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- HUC numbers: 12: 171100190101 – Fidalgo Island-Frontal Similk Bay

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# Background

Cyanobacteria (blue-green algae) are common in many inland waters worldwide. This diverse group of photosynthetic bacteria are generally classified as benthic (residing on sediments or rocks) or planktonic (free-floating in the water column). The most widely recognized are the planktonic species. Some species can produce toxins (collectively called cyanotoxins) harmful to humans and wildlife.

Previous monitoring[3] of Pass Lake since 2007 has documented harmful concentrations of the cyanotoxins, anatoxin-a (ATX) and microcystin (MC) in shoreline accumulations (Figure 1). The timing of the blooms is generally in late-summer to early fall. Concentrations of ATX have been high enough in the past (2020) to cause a large mortality event of brown bats, porcupine, and swallows (personal communications Julie Morse, WA State Parks). Typically, the lake has experienced closures in the late-summer due to concerns over exposure to cyanotoxins.

Anatoxins are neurotoxins (affecting nerve function) and are the likely toxin that cause death in dogs (Puschner et al. 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).

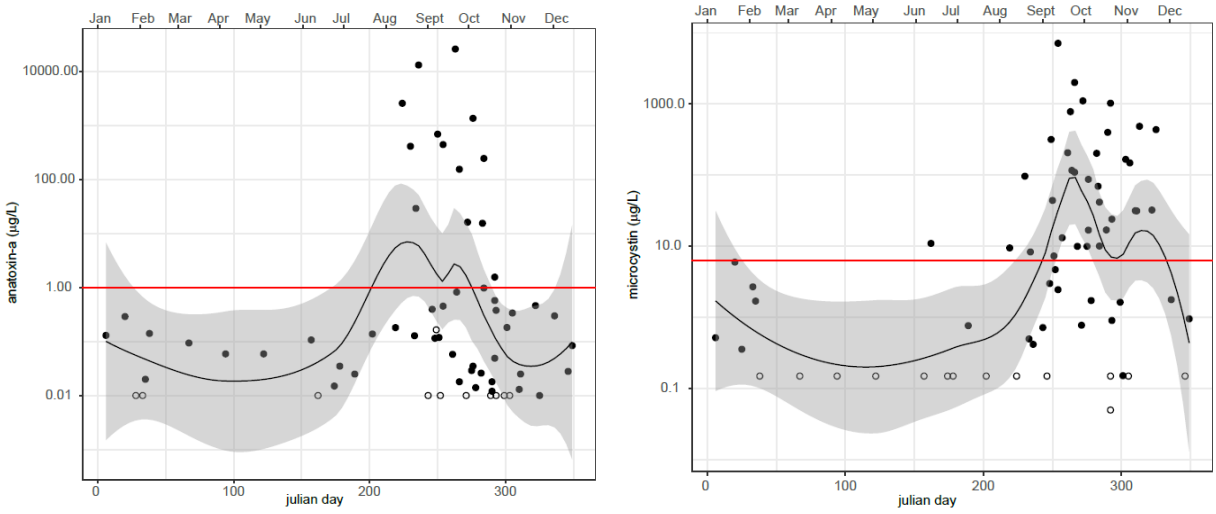


**Figure 1. Anatoxin-a and microcystin levels in samples collected from Pass Lake, 2012 – 2022.**

Black dots are measured concentrations and white dots are below the analytical detection limit.
Red horizontal lines are the Washington State Department of Health (DOH) recreational guidelines (Hardy, 2008).

[3] https://www.nwtoxicalgae.org/Data.aspx?SiteID=174

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 can provide a proxy of cyanobacteria growth over time (Pal et al. 2015). Specific pigments are attributed to certain groups of algae (Leavitt and Hodgson 2001) and therefore changes over time represent how algal communities have changed. The genes that produce the toxins have also been successfully measured in lake sediment studies (Pal et al. 2015; Hobbs et al. 2021) and can indicate the presence and changes in these toxins over time. The goal of this study was to analyze a sediment core from Pass Lake and ask the question: Have cyanobacteria been prevalent in the lake over the last ~150 years? If so, is there any indication that toxins were produced during this period?

# Methods

## Study Site

Pass Lake is located on Fidalgo Island in Skagit County, within the Deception Pass State Park (Figure 2). It is a small kettle lake (~95 acres) with a maximum depth of approximately 20 – 25 feet (Figure 3). There is one engineered outlet stream on the southwest shoreline which drains to Bowman Bay in the Puget Sound (Figure 3). 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 with the Civilian Conservation Corps. 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 2. Washington State map with the location of Pass Lake.**


**Figure 3. (left) Pass Lake watershed, (right) lake bathymetry and lake sediment core location (red dot).**

Bathymetry contours are in feet.

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 numerous fish introductions, a fish eradication in 1919 using dynamite and two 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).

The lake's submerged plant community was assessed in 2018 by the Washington State Department of Ecology[4]. The invasive plant, Eurasian water-milfoil (*Myriophyllum spicatum*) was confirmed genetically and growing in dense stands throughout the lake; Reed canary grass (*Phalaris arundincea*), another invasive species (sp.), was also present in the lake. Pondweed (*Potamogeton* sp.), muskwort (*Chara* sp.), pond lily (*Nuphar polysepala*), waterweed (*Elodea canadensis*) and several species (spp.) of sedges (*Carex* spp.) were present within the native plant community.

There has been no assessment of the phytoplankton community composition in Pass Lake. Occasionally, qualitative observations of phytoplankton are made on the cyanotoxin samples submitted to King County Environmental Lab. *Dolichospermum* spp. have been routinely observed, along with *Microcystis* sp. and *Aphanizomenon* sp.

The Institute for Watershed Studies at Western Washington University has collected annual shoreline grab samples at Pass Lake[5] for nutrients and conventional water quality parameters since 2006. Sampling usually occurs in July or August. Generally, total phosphorus concentrations have ranged from 7.8 µg/L to 231 µg/L with a median of 23.4 µg/L (mean 35.6 µg/L); total nitrogen concentrations have ranged from 527 µg/L to 2076 µg/L with a median of 680 µg/L (mean 756 µg/L).

[4] https://apps.ecology.wa.gov/lakes/?CustomMap=y&BBox=-13656664,6174722,-13647118,6179500&Opacity=0.9&Basemap=bmHybrid&Layers=0&ReachCode=17110019019082

[5] https://iws.wwu.edu/?page=lakeslist

## Field Methods

A 70-cm long sediment core was recovered from the deepest point in the lake (approx. 25 feet) using a percussion-type sediment corer on September 30, 2021 (Reasoner 1986; Glew et al. 2001). The lake was closed for recreational activities at the time of sampling due to high toxin concentrations. The sediment core was sectioned on the shore of the lake at 1-cm intervals, and samples were frozen within 24 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 US EPA's Office of Research and Development, Cincinnati, OH 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.

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).

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).

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 hours 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.

Analysis of the sediment intervals for toxin-producing genes using qPCR was carried out by US EPA's Office of Research and Development. 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 – 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 30 s, 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.

# Results and Discussion

## Sediment Dating

A reliable age-depth model using the constant rate of supply model (Appleby and Oldfield 1978) 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 approximately 35 cm. Typically, the background $^{210}$Pb activity is reached at a sediment age of ~150 years and thereafter ages must be extrapolated.

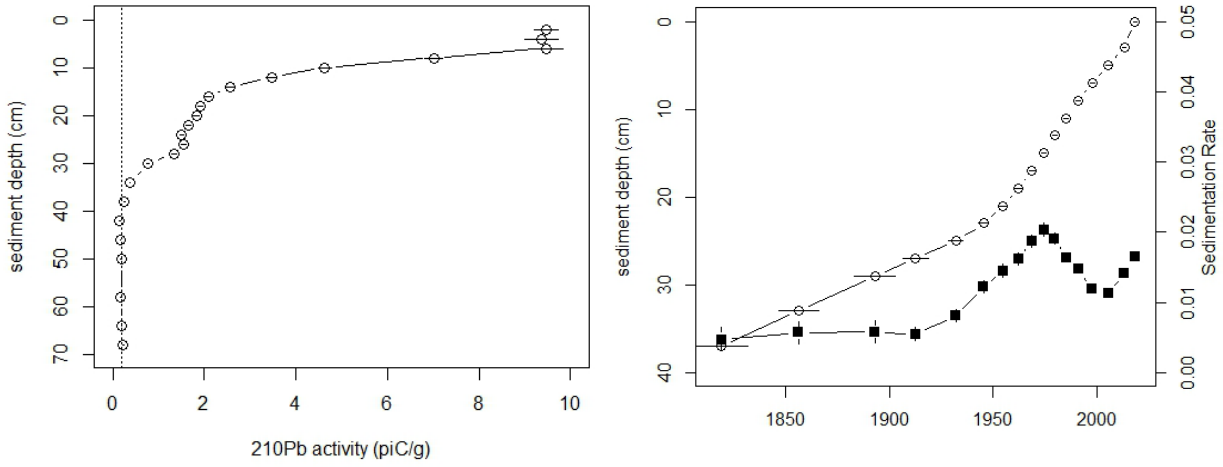


**Figure 4. Sediment radioisotopes and estimated ages.**

**Left:** 210Pb activity with sediment depth; dashed vertical line represents the supported 210Pb.

**Right:** estimated sediment ages (o) and error with sediment depth; sediment accumulation rate on the secondary y-axis (■).

To estimate the age of the sediment below the supported activity, we extrapolated the mass accumulation rate of the sediment and calculated ages based on the measured dry bulk density of the sediment (Binford 1990). The approximate age of the bottom of the sediment core (70cm) was ~1600 AD or roughly 400 years old. The ages inferred from this extrapolation method should be viewed as rough estimates.

## Sediment Accumulation and Composition

The rate at which sediment has accumulated at the core location has changed slightly over time (Figure 4). Sediment accumulation remained relatively constant from ~1820 – 1930; from 1930 – 1975, there is an increase in the accumulation rate; and post-1975, the rate of accumulation decreases until 2000. Post-2000 there appears to be a slight increase in the accumulation rate. It is possible that the increase in sediment accumulation rate from 1930 – 1975 coincides with increased development and filling of the shoreline and increased sediment inputs from the watershed (Figure 5). In the mid-1800s the linear rate of sedimentation is around 0.19 cm/yr, increasing to 0.33 cm/yr by 1975.

**Figure 5. Historic photographs of shoreline development.**

**Left:** 1934; **Right:** same location,1935

The sediment at the bottom of Pass Lake is composed primarily of organic matter with a fraction of mineral material (~30%) (Figure 6). The composition of the sediment has been relatively constant over the last ~400 years or so.

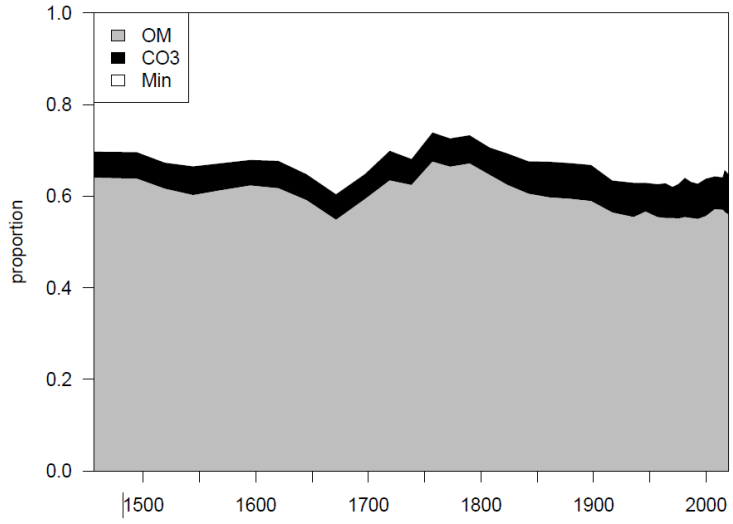


**Figure 6. Sediment composition over time.**

OM = organic matter; CO3 = carbonate content; Min = mineral matter

## Fossil Algal Pigments and qPCR of Cyanobacteria

The sediments of Pass Lake contain several algal pigments (Figure 7). Chlorophylls and carotenoids from different algal groups were identifiable. There were several pigments associated with cyanobacteria throughout the sediment core (Figure 8). 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 which 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 echinenone, from filamentous, colonial and picocyanobacteria (Schlüter et al. 2016; Schlüter et al. 2018).

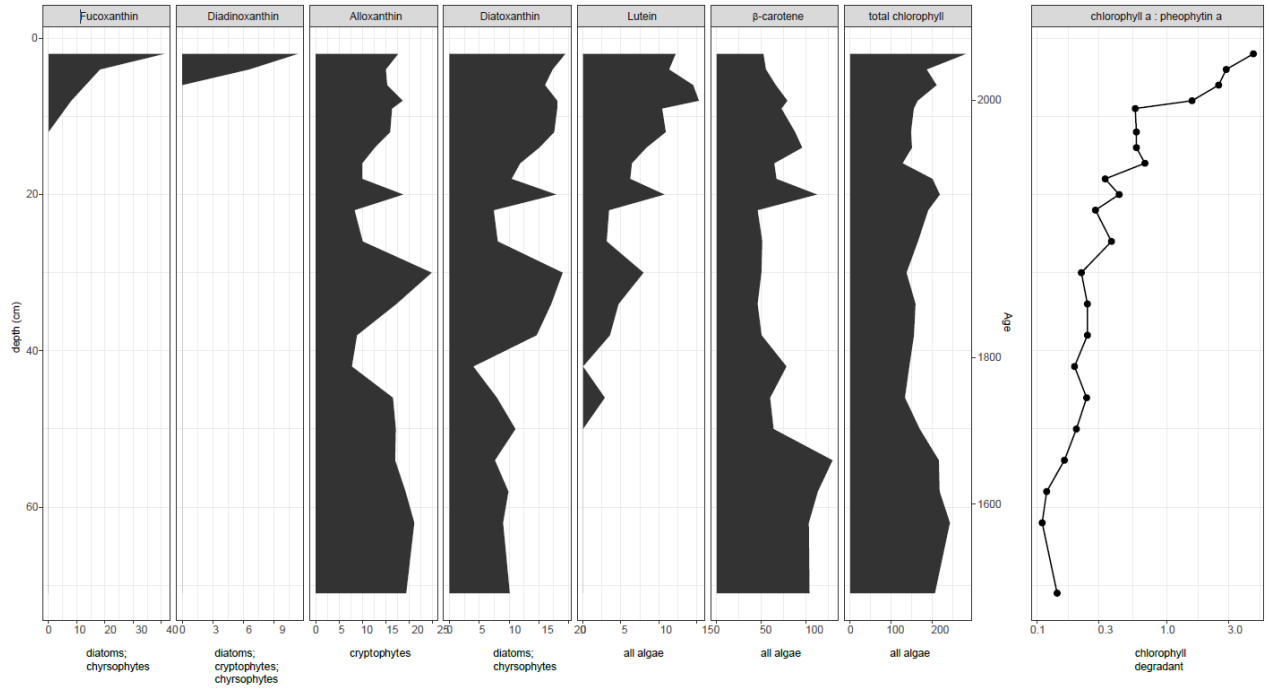


**Figure 7. Algal pigment concentrations (µg/g OM) over time in Pass Lake.**

OM = organic matter.

Other pigments found in the sediment record included: fucoxanthin and diatoxanthin (diatoms and chrysophytes); alloxanthin (cryptophytes); general pigments of lutein and beta-carotene 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* (chl:pheo) 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. It is worth noting that primary chlorophylls are particularly sensitive to degradation and some pigments in the sediments are more stable over time (e.g., alloxanthin, zeaxanthin, canthaxanthin, echinenone and diatoxanthin; Leavitt

and Hodgson 2001). In the Pass Lake core the chl:pheo ratio declined throughout the core, suggesting that the post-depositional environment for some pigments was not particularly stable and the records should be interpreted accordingly.

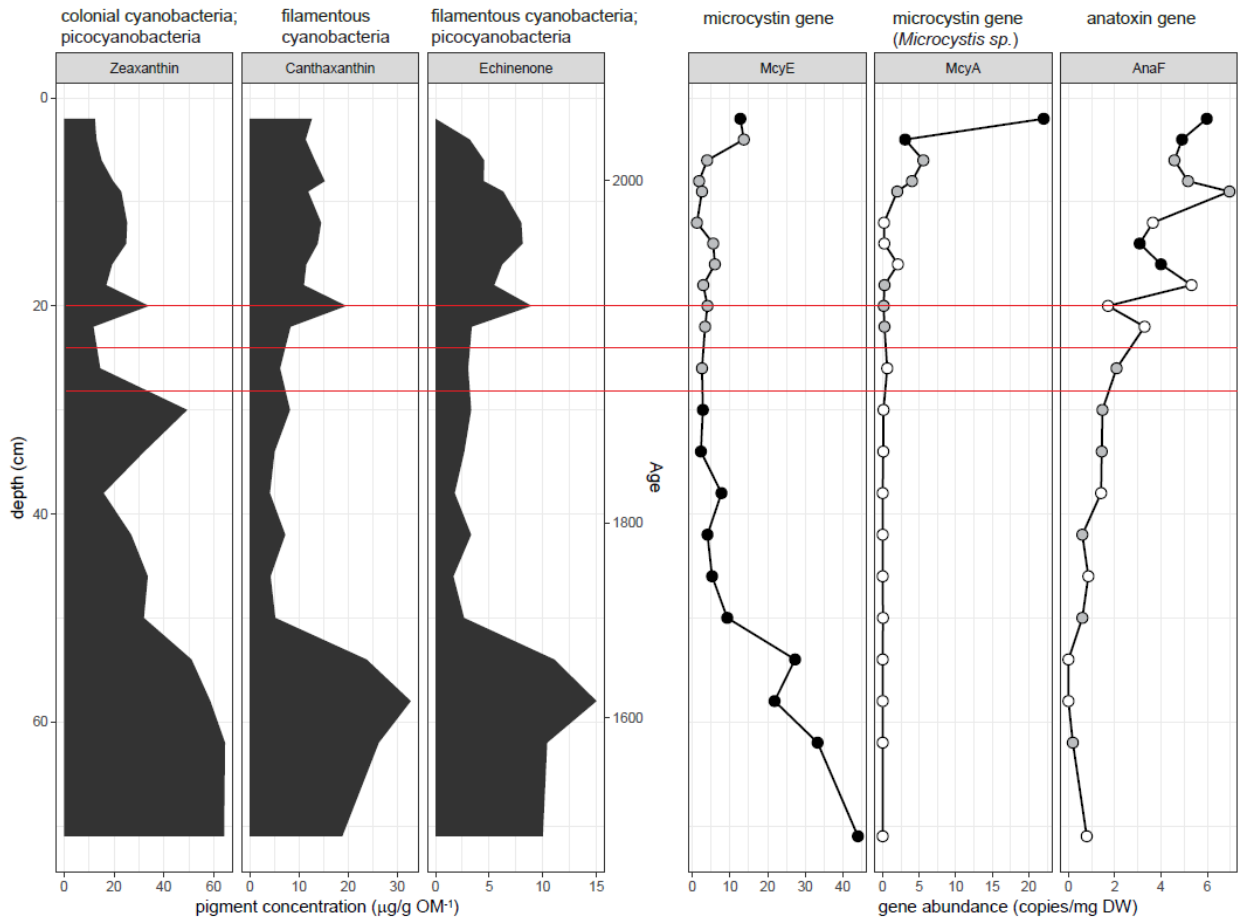


**Figure 8. Cyanobacteria pigment concentrations and microcystin qPCR in sediments.**

OM = organic matter, DW = dry weight; red horizontal lines represent fish eradication events on the lake in 1919 (dynamite), 1946 (rotenone) and 1959 (rotenone). Gene abundance data points are filled to describe the data reliability: white = non-detect or U-flag, grey = estimate or J-flag, and black = positive identification

The abundance of algal pigments over time does suggest that common algal groups have been present in the lake for the last ~ 400 years or so (i.e., down to the bottom of the sediment core). Interestingly the carotenoids indicative of cyanobacteria, which are more chemically stable, appear to have had a higher abundance historically (Figure 8). The sediment record suggests a decrease in abundance during the 17$^{th}$ century.

Coincident with the historically higher abundance of cyanobacteria pigments is a higher concentration of the McyE gene, present in cyanobacteria for microcystin production. Gene markers for anatoxin production (AnaF) were present in the core from present to the mid-1970s; for some of the older samples (~1700) we were able to get positive amplification of the AnaF genes, but detection was too low to reliably quantify. AnaC genes were only present in the upper sediments and were at low concentrations (below the detection limit).

There have been three major fish management impacts to the lake — namely the dynamiting of the lake in 1919 and rotenone treatments in 1946 and 1959 (Figure 8). There are two observations worth noting, (1) a short-term increase in algal and cyanobacteria abundance coinciding with the 1959 rotenone treatment, and (2) a general increase in anatoxin genes following the period of fish eradication (1919 – 1959) (Figure 8). It's likely that changes in the algal ecology of the lake are also related to climatic factors and nutrient cycling.

General 16S rRNA gene-based markers for cyanobacteria were measured throughout the core but appeared to be impacted by preservation issues. There is a positive relationship between 16S gene abundance and the indicator of labile pigment preservation chl:pheo ($r^2 = 0.96$; $p<0.001$) (Figure A-1). It therefore appears that the 16S rRNA markers are sensitive to degradation in the lake sediments. Other DNA markers do not seem to reflect the indicator of labile pigment preservation, suggesting more reliable preservation. Furthermore, there was recoverable DNA throughout the core and the quality was adequate, suggesting that target genes could be analyzed if present.

At the time of the sediment sampling, there was a dense cyanobacteria bloom on Pass Lake and an opportunistic sample of the water was collected from an accumulation near the shore and the center of the lake at the core site. The water was sampled and analyzed for qPCR as per methods described in Hobbs (2023). Both samples showed an abundance of toxin-producing genes for microsystin and anatoxin-a (Table A-3), both for total DNA and for the reverse transcription (RT) of the gene indicating that it's active. As expected, due to the bloom, the gene concentrations were much higher at the shoreline accumulation. Anatoxin-a concentrations at the shoreline prior to the sediment coring in September 2021 were 692 µg/L (9/7) and 26,100 ug/L (9/20)[6]. Microcystin concentrations in the same shoreline accumulation were 44.2 µg/L (9/7) and 782 µg/L (9/20).

Indicator genes for nitrogen fixation and general cyanobacteria genes were also in high abundance in the water sample. There was no indication of the high-affinity phosphate transporter gene (phosphate stress), saxitoxin gene or cylindrospermopsin gene.

## Conclusions

The successful dating of a sediment core from Pass Lake describes the increase in sediment accumulation beginning in the 1930s when the lake shoreline was developed for park access. Sediment accumulation continues to increase until the mid-1970s when it decreases slightly. Through this time the sediment composition remains relatively consistent.

Overall, there is an indication that cyanobacteria were present in the lake ~400 years ago and producing microcystin (McyE gene). It appears that anatoxin-a production may have been occurring historically and post-1975 there is an increase in the presence of the anatoxin gene, AnaF.

The impacts of short-term fish eradication efforts in 1919,1946, and 1959 are not consistently evident in algal or cyanobacteria changes in the sediment record. It does appear that poor preservation of some of the pigment and qPCR markers occurred that limits the ability to provide a detailed long-term history of the lake beyond the known management history.

[6] https://www.nwtoxicalgae.org/Data.aspx?SiteID=174

## Recommendations

Contemporary samples for toxin and cyanobacteria qPCR successfully detailed several rRNA and DNA targets. This genetic tool is worth considering in future monitoring of this lake.

Despite the annual cyanobacteria blooms that take place on Pass Lake, very little detailed understanding exists of the phytoplankton and cyanobacteria succession over the year, and possible abiotic drivers (e.g., nutrients, water column mixing) of the cyanobacteria communities. Further detailed sampling is worth pursuing.

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# Appendix A.

**Table A-1: Timeline of fish and lake management actions.**
**Compiled by Julie Morse, WA State Parks.**

| Date | Note | Document type |
|---|---|---|
| 1936 | One outlet to salt, no surface inlet, fed by two underground springs, max ~50 feet<br>• Excellent trout until 1918/19<br>• 1919 lake dynamited | Correspondence (Skagit Sportsmen's Association, Anacortes Chapter) |
| 1944 | Washington Department of Game (WDG) restricts fishing to fly only, January 9, 1941 | News Article ("The Inside on the Outdoors" by Enos Bradner) |
| 1946 | Rotenone treatment to eradicate Yellow Perch — compete with trout and potential complicate operation of Bowman's Bay facility | In Washington Department of Fish and Wildlife (WDFW) database and referenced in Correspondence (WA Department of Game, 1946) |
| 1947 | Screen for Bowman's Bay facility inlet not properly in place | Correspondence (WDG) |
| 1947 | • Estimate lake productivity 190–229 pounds of Cutthroat Trout per acre<br>• Reference to Rotenone concentration, "One part of 5% rotenone to two million parts of water used, and there has been, no indication of an incomplete kill." | Report of Sampling Results (WDG) |
| 1952 | Request to open for all fishing gear | Correspondence (authors illegible) |
| 1959 | • Rotenone treatment to eradicate Yellow Perch — compete with trout<br>• 400,000 adult and 1 million juvenile YP eradicated<br>• one adult catfish (no other detected) | Pre- and post-treatment Info (WDG) |
| 1963 | Game Commission Reinstate fly fishing only for 1964 season | Correspondence (WDG, Chief Fishery Division) |
| 1975 | • Reference to current harvest regulations — daily limit: 5, minimum size: 6 inches<br>• split season spring-opener to June 30, mid-September to mid-November | Fishery Management Report (WDG) |
| 1976 | Reduced stocking densities to increase size of average fish (in reference to proposals of increasing minimum size to 12 inches or eliminating harvest all together) | Multiple correspondences (WDG) |
| 1977 | Reference to new harvest regulations — daily limit: 3, minimum size: 12 inches | Study Proposal (Fidalgo Fly Fishermen) |
| 1979 | Reducing numbers of stocked Rainbow | March Weekly Activity Summary |
| 1988 | • Reference to "shiner population". Maybe Fathead Minnow?<br>• Atlantic Salmon introduced (starting 1986) as predator to control "undesirable species"<br>• harvest regulations are one 18-inch trout per day | Correspondence (WDG) |

| Date | Note | Document type |
|---|---|---|
| 1989 | • Brown Trout mistakenly stocked in Pass<br>• open year-round, one trout over 18” per day<br>• Atlantic Salmon not effective at controlling shiners<br>• Local staff intended to propose the lake for a rotenone treatment, but intend to wait to see if Browns are successful in suppressing “shiners” | Correspondence (WDG) |
| 1996 | “bluegill” which are likely Green Sunfish were introduced around 1992 | Bio Notes Comments on Voluntary Catch Cards |
| 1997 | Proposal to adopt Catch-and-Release only in 1998 | WDFW |
| 2014 | Fidalgo Fly Fishers approached Spinelli with a request to adjust stocking practices to improve condition and average length of trout | — |
| 2014 | Fidalgo Fly Fishers began coordinating annual volunteer angling survey to track catch rates and sizes of fish recruiting to the fishery from March-April<br>Average angler catch rate, length, and weight | — |
| 2015 | WDFW initiated annual gill netting to monitor population size structure and condition of trout<br>Average net catch rate, length, weight, and condition | — |
| 2018 | Yellow Perch detected in WDFW annual gill netting<br>Estimated age of those fish is between age 1 and 2 using westside average length-at-age. | — |

**Table A-2: 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 | 16S all cyanobacteria | 56 | Nubel et al., 1997 and Urbach et al., 1992 |
| Micr 209F | ATGTGCCGCGAGGTGAAACCTAAT | Microcystis 16S genus | 64 | Neilan 1997 |
| Micr 409R | TTACAATCCAAAGACCTTCCTCCC | Microcystis 16S genus | 64 | Neilan 1997 |
| HepF | TTTGGGGTTAACTTTTTTGGGCATAGTC | All Microcystin producers | 54 | Jungblut and Neilan 2006 |
| HepR | AATTCTTGAGGCTGTAAATCGGGTTT | All Microcystin producers | 54 | Jungblut and Neilan 2006 |
| mcyA-MSF | ATCCAGCAGTTGAGCAAGC | Microcystin/ Microcystis specific | 60 | Tillet et al., 2001 and Furukawa et al., 2006 |
| mcyA-MS2R | GCCGATGTTTGGCTGTAAAT | Microcystin/ Microcystis specific | 60 | Tillet et al., 2001 and Furukawa et al., 2006 |
| mcyE Mic F3 | GTTATGTTTGCCGGCTCCTA | Toxic Microcystis | 60 | This study |
| mcyE Mic R3 | GTGCCTAGACTTAAGGGTTGAG | Toxic Microcystis | 60 | This study |
| AnaCF1 | GGTCCTGGGTTGATGACAGG | General anatoxin | 60 | Chen et al., 2017 |
| AnaCR1 | CGGTAGCCCCGACTCTTAATC | General anatoxin | 60 | Chen et al., 2017 |

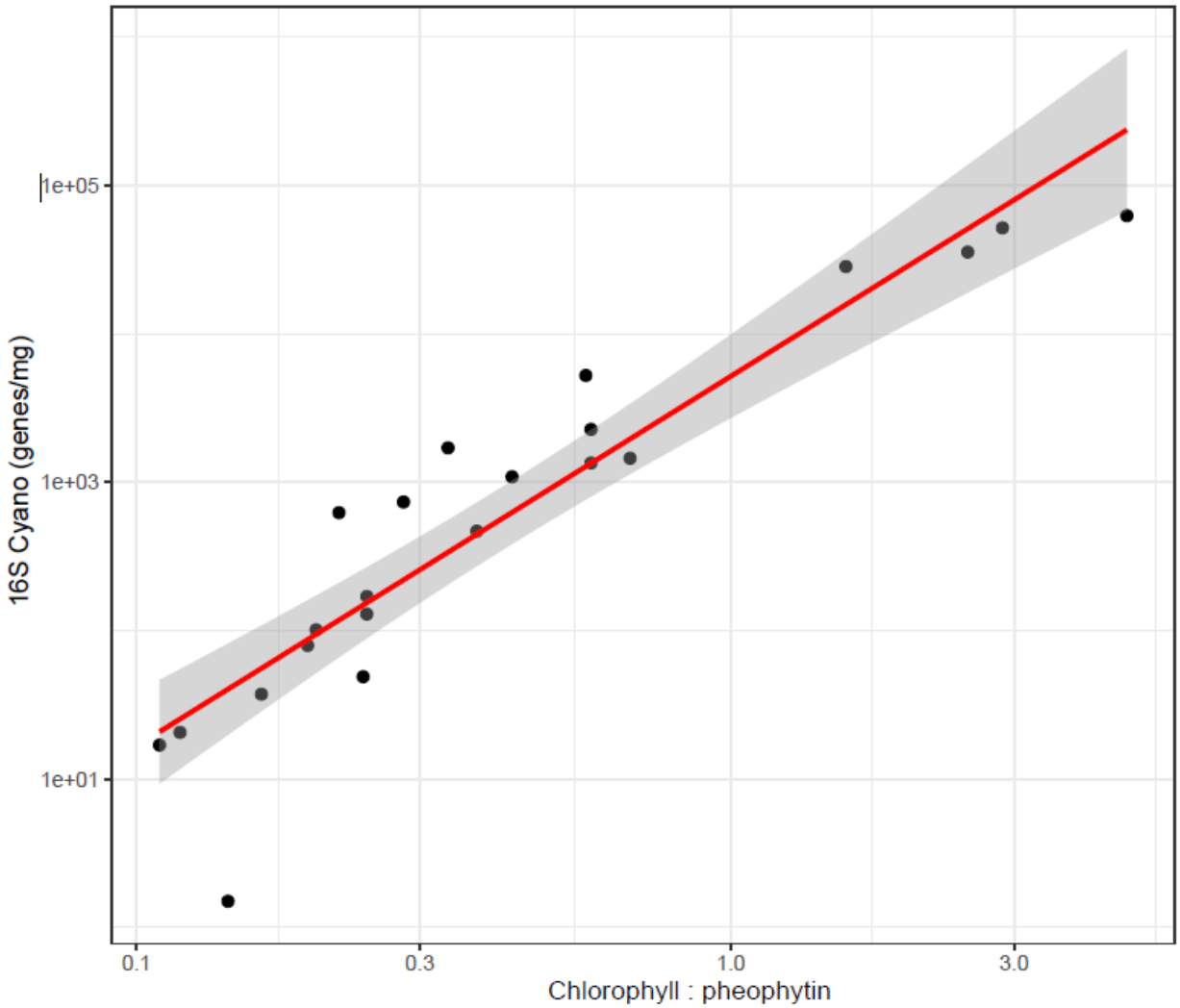


**Figure A-1: linear regression between qPCR 16S for cyanobacteria and indictor of pigment preservation (chlorophyll: pheophytin).**

**Table A-3: qPCR results (gene copies/ml) for water samples collected on September 30, 2021.**

| Target | Primer | DNA | Pass lake-center | Pass lake-shore (boat launch) |
|---|---|---|---|---|
| **16S all cyano** | 16S 108F/377R | 16SCyano | 456619 | 522876 |
| **16S all cyano** | 16S 108F/377R | 16SCyano-RT | 992708608 | 7685466240 |
| **Microcystis 16S genus** | 209F/409R | 16S-Mic | 4866 | 61764 |
| **Microcystis 16S genus** | 209F/409R | 16SMic-RT | 54164168 | 3783328000 |
| **all microcystin producers** | Hep F/R | McyEcyano | 2305 | 29023 |
| **all microcystin producers** | Hep F/R | McyEcyao-RT | 13 | 672 |
| **microcystin/Microcystis specific** | MSF/MS2R | McyA-Mic | 1665 | 18999 |
| **microcystin/Microcystis specific** | MSF/MS2R | McyAMic-RT | 48 | 987 |
| **toxic Microcystis** | McyEmic F3R3 | McyEmic | 1287 | 14271 |
| **toxic Microcystis** | McyEmic F3R3 | McyEmic-RT | 100 | 8014 |
| **general anatoxin** | AnaC F1/R1 | AnaC | 1507 | 4865 |
| **general anatoxin** | AnaC F1/R1 | AnaC-RT | 5512 | 59387 |
| **general saxotoxin** | Sxt F1/R1 | Sxt | 0 | 0 |
| **general saxotoxin** | Sxt F1/R1 | Sxt-RT | 0 | 0 |
| **general cylindro** | Cyr F7/R7 | Cylindro | 0 | 0 |
| **general cylindro** | Cyr F7/R7 | Cylindro-RT | 0 | 0 |
| **nif in Anabaena** | NifAnaF6/R6 | Nif-Ana | 3310 | 10983 |
| **nif in Anabaena** | NifAnaF6/R6 | NifAna-RT | 88355 | 1240494 |
| **nif in Nostoc** | NifNos F3/R3 | Nif-Nos | 3232 | 19290 |
| **nif in Nostoc** | NifNos F3/R3 | NifNos-RT | 5618 | 368649 |
| **pstS in Anabaena (mainly A. flos-Aquae)** | Psts F3/R3 | Psts | below detection | below detection |
| **pstS in Anabaena (mainly A. flos-Aquae)** | Psts F3/R3 | Psts-RT | 0 | 0 |

## References for Appendix A

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