Application of genetic tools for improved cyanobacterial bloom monitoring in the Klamath River system: The molecular identification of anatoxin-a producers

Prepared by: Tim Otten, PhD, MPH
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Executive Summary

Evaluation of new tools for analysis of algae species and toxins in water samples from the Klamath River were conducted in 2016. Overall, 126 samples were collected at 22 locations throughout the Klamath River basin. Samples were analyzed using microscopy, enzyme linked immunosorbent assay (ELISA), quantitative polymerase chain reaction (QPCR), CyanArrays, and liquid chromatography/mass spectrometry (LC-MS/MS). Comparison of results from these different methods indicated that the false positive (20%) and false negative (57%) results for the CyanArrays limited their usefulness in anatoxin-a monitoring. QPCR conversely appeared to be a more superior tool for identifying anatoxin-a genes in the samples. Microscopy documented few anatoxin-a producers, and none in some samples with relatively high levels of the toxin. Additionally, the abundance of *Dolichospermum* (a potential anatoxin-a producer) was not related to toxin levels indicating that the source of the anatoxin-a was likely a different species. This discrepancy led to an investigation into possible anatoxin-a producers using metagenomic approaches. Amplification and sequencing of the anatoxin-a genes in the samples indicated that the DNA sequences were nearly identical and were highly similar to those derived from *Oscillatoria* sp. or *Phormidium* sp. This analysis also indicated that the producer of anatoxin-a was not likely to be from the order Nostocales which includes *Dolichospermum* and *Aphanizomenon,* two relatively common genera in the Klamath River. Further resolution was pursued by using shotgun metagenomic sequencing, a method that sequences and assemblies all DNA in a sample, to identify the anatoxin-a producer. The average coverage depth for the anatoxin-a genes was substantially less than the coverage depth for the overall samples indicating that the anatoxin-a producer was less abundant than the average bacterium or algal cell in the samples. The anatoxin-a- genes recovered from this process were compared to known anatoxin-a sequences in genetic databases. This analysis showed that the assembled gene sequences were closely related to that from *Oscillatoria*, however, the similarity was below what was expected from *Oscillatoria*, indicating that another species was likely present. Unfortunately, there are no complete anatoxin-a gene clusters for *Phormidium* available for comparison. Further analysis extracted the 16S ribosomal (rRNA) gene, which is a common marker used in bacterial community analysis. Comparison of the recovered 16S rRNA gene with known gene sequences indicated that the Klamath sequence was most closely related to *Phormidium* or *Tychonema*; both are benthic mat-forming species. The fact that the shotgun sequencing was only able to generate fragments of the genome and the general absence of any *Oscillatoria*-like cyanobacteria in the microscopy analysis collectively suggested that the anatoxin-a producer was a benthic species that was underrepresented in the water column grab samples.

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Background

Under Interim Measure 15, PacifiCorp provides a fixed amount of funding per year for comprehensive Klamath water quality monitoring. Incremental increases in labor, supplies, shipping, and laboratory costs are beginning to limit the amount of work that can be completed under the program with the available funds. Analysis conducted for the public health program currently includes identification and counts of blue green algae cells and laboratory analysis for toxins other than microcystin (e.g., anatoxin-a, cylindrospermopsin, saxitoxin).

The application of molecular diagnostic tools for cyanobacteria monitoring affords several potential benefits over traditional approaches that may ultimately improve Klamath water quality monitoring efforts by providing: faster results, lower cost, higher sample throughput, greater accuracy at low cell concentrations, and results that are not subjective. Two of the genetic tools evaluated in this study were real-time quantitative polymerase chain reaction (QPCR) analysis and the use of CyanArrays for visual detection of cyanotoxin genes. The goal of this study was to assess the effectiveness, accuracy, and reliability of these molecular diagnostic tools for monitoring cyanobacterial toxins in the Klamath River. If effective and accurate, it may be possible to change the existing analysis protocols, reduce the need for laboratory testing to just those samples where toxins are present, and speed the return of data useful to informing public health decision makers. As will be discussed, the first effort resulted in more frequent observations of anatoxin-a in samples than in previous years. This led to the second major objective of this study which was to identify the specific cyanobacteria responsible for anatoxin-a production in the Klamath River system. In order to accomplish this task, a combination of traditional and next generation (e.g., shotgun metagenomics) DNA sequencing methods were employed.

Introduction

Cyanobacterial blooms have been a recurring summer feature in Upper Klamath Lake for decades, *Aphanizomenon* akinetes (the dormant 'resting' phase of cyanobacteria) have been identified from sediment cores dating as far back as the late 1800s (Eilers et al., 2004). In modern times, cyanobacterial blooms have consistently been observed in Copco and Iron Gate reservoirs during the summer and fall since 2001 (Raymond, 2003).

The ability to synthesize cyanotoxins is not a fixed genetic trait; while certain genera of cyanobacteria contain representatives able to produce toxins, the presence of toxin genes varies from strain to strain. In the Klamath River system, recent studies have established that at least one strain of *Microcystis* sp. is capable of producing the hepatotoxin microcystin (Bozarth et al., 2010). Other potential cyanotoxin producers are at times observed, including *Aphanizomenon*, *Gloeotrichia*, *Planktothrix*, *Oscillatoria*, and *Anabaena* (also called *Dolichospermum*); however, to date only *Microcystis* has been directly linked to toxin-production in the Klamath River system. Anatoxin-a, a neurotoxin, has been detected on several occasions in recent years, often in the

lower reaches of the river including Weitchpec and Orleans (Yurok Tribe pers. comm.). *Microcystis* populations from around the world have never been found to produce any cyanotoxin except microcystin. As such, the presence of anatoxin-a strongly suggests that there is at least one other cyanotoxin-producing cyanobacterium in the Klamath River system.

Anatoxin-a, and its derivatives homoanatoxin-a and dihydroanatoxin-a, have been found to occur in various genera of cyanobacteria, including: *Anabaena*/*Dolichospermum*, *Aphanizomenon*, *Cylindrospermopsis*, *Oscillatoria*, *Phormidium*, and *Raphidiopsis*. For several of these genera, the anatoxin-a gene clusters have been sequenced, revealing both similarities and differences between them (Figure 1). The gene cluster is comprised of approximately nine genes that span about 30,000 base pairs of DNA.

The purpose of this study was to screen for the presence of genes involved in the biosynthesis of anatoxin-a using a gene amplification assay (CyanArray). The second objective of this study was to assess the utility of using real-time QPCR for quantifying toxigenic (anatoxin-a -producing) cyanobacteria. The third objective of the study was to use DNA sequencing to identify which cyanobacteria are capable of anatoxin-a production in the Klamath River system.

Hypotheses

H_1 - Molecular assays targeting the genes responsible for toxin biosynthesis are reliable surrogates for estimating the presence (via CyanArray) or abundance (via QPCR) of anatoxin-a producing cyanobacteria.

H_2 – DNA sequencing (shotgun metagenomics) applied to samples containing elevated anatoxin-a genes will enable identification of the producer(s).

Methods

Study Sites

Samples were collected from 22 locations spanning an area of the Klamath River from Upper Klamath Lake to the lower estuary (Turwar). Samples collected for this project were subsamples from normal sampling efforts that occur at monthly or bi-weekly intervals (Table 1). Several agencies were involved in the sampling effort, including: U.S. Bureau of Reclamation, Oregon Department of Environmental Quality, PacifiCorp, Karuk Tribe, and Yurok Tribe.

Field Methods

A total of 126 discrete samples were collected during the study period from a subset of the sites in the Baseline and Public Health programs (Table 1). These are the two monitoring programs that take place

concurrently on the Klamath River every year and all phytoplankton samples were collected in accordance with the standard operating procedures developed for these programs (KBGAWG, 2009). The Baseline program focuses on water chemistry, algae species, and microcystin at 24 sites from Link River dam to the estuary. Sampling at these sites occurs in the open flowing water at 0.5 m depth. The second effort is the Public Health program. The focus of Public Health sampling is to provide information that helps evaluate risk to the public from cyanobacteria and associated toxins. Public Health sampling occurs in accordance with a protocol developed by the Klamath Blue Green Algae Working Group (2009) and is focused on areas that are publically accessible (e.g., boat launches, campgrounds, etc.). At each of these sample points, the person collecting the sample evaluates the area and collects a sample from the upper 10 centimeters at a site where it visually appears cyanobacteria are the most concentrated. It is important to note that monitoring efforts in the Klamath River do not presently include routine anatoxin-a testing.

Samples for species identification were preserved in 1% Lugol's solution and samples for genetic analysis were placed in sterile 50 mL polypropylene tubes. Except for the species identification samples, all other samples where packed on ice in coolers and shipped as soon as possible. Because they were preserved, the speciation samples did not require ice for shipping. Details on how sample sites were selected, samples collected, splits, and associated record keeping are available in the 2009 Standard Operating Procedure (Klamath Blue Green Algae Working Group, 2009). Details on the overall water quality monitoring program are available in the 2016 study plan (PacifiCorp, 2016).

Toxin Measurements

Anatoxin-a analysis was only conducted on a small set of samples based on molecular results or cell density estimates. Each of these samples were evaluated for the presence of anatoxin-a by enzyme linked immunosorbent assay (ELISA; Bend Genetics) or by liquid chromatography/mass spectrometry (LC-MS/MS; Green Water Laboratory). Specifically, LC-MS/MS was used to analyze three samples for anatoxin-a and ELISA for one sample. Additionally, the Yurok Tribe conducted anatoxin-a analysis on two of the sample splits using LC-MS/MS.

Phytoplankton Enumeration

Cyanobacterial identification and enumeration was conducted on 125 out of the 126 samples by Aquatic Analysts, Inc. Permanent microscope slides were prepared by concentrating the sample by filtration onto 0.45 micron (µm) membrane filters and counting cells across a measured transect of the slide using a Zeiss phase contrast microscope (1000X magnification), congruent with standard method SM10200F for phytoplankton counting techniques. For each sample, a minimum of 100 algal units was counted, resulting in an accuracy of ±20%; only those algae believed to be alive (having intact membrane/chloroplast) were counted. The detection limit is considered to be 1 cell per milliliter (cell/mL).

Molecular Assays

Upon receipt by Bend Genetics, samples were vacuum concentrated onto 1.2 µm pore size by 25 millimeter (mm) diameter glass fiber filters (Whatman GF/C). The filters were stored at -20 degrees Celsius (°C) or extracted immediately using a Mo Bio Powerlyzer Power Soil DNA extraction kit. QPCR was used to quantify total anatoxin-a producing cyanobacteria by targeting one of the genes responsible for anatoxin-a biosynthesis (*anaC*) using *anaC*-gen primers with SYBR Green I stain as previously described by Rantala-Ylinen et al (2011). The results provided a quantitative estimate of toxin gene concentrations by relating the amplified fluorescence from environmental samples to a standard curve made from serially diluted synthetic gene constructs (gBlocks; IDTDNA) of known concentrations. All 126 samples that were collected were analyzed in duplicate with an iQ5 multi-color real-time PCR detection system (Bio-Rad). The limit of detection for the QPCR assays was 100 gene copies/mL. Since the assay targeted single copy genes, each gene is equivalent to one anatoxin-a producing cell.

The extracted DNA was also visually screened for the presence of anatoxin-a genes using the CyanArray method for 123 of the 126 samples collected. In the presence of an appropriate concentration of target genes the reaction mixture changes from a pale yellow color to reddish-purple (Figure 2) and samples with higher starting DNA concentrations typically generate stronger shifts in color. CyanArrayassay results provided qualitative assessments of the likely presence or absence anatoxin-a genes in each sample. For environmental samples, the limit of detection for the assay was determined to be about 2,000 gene copies/mL based on side-by-side comparisons with QPCR data and by spiking known concentrations of control DNA into environmental DNA extracts.

Bioinformatics

Samples that tested positive for anatoxin-a genes by both CyanArray and QPCR method were PCR amplified using the *anaC*-gen primers. PCR products were visualized under ultraviolet light following gel electrophoresis and DNA from positive samples was purified using a Monarch PCR Clean-up kit (New England Biolabs). The purified DNA was bi-directionally Sanger sequenced using an ABI 3730 Capillary Electrophoresis genetic analyzer (Applied Biosystems) with a BigDye Terminator v3.1 cycle sequencing kit (ThermoFisher). The DNA sequences were manually trimmed based on their chromatographs and aligned with reference sequences from GenBank corresponding to anatoxin-a producing *Anabaena*, *Aphanizomenon*, *Cuspidothrix*, *Oscillatoria*, and *Phormidium* using Geneious v9 software.

Samples with anatoxin-a genes confirmed by Sanger sequencing were shotgun sequenced at the Center for Genome Research and Biocomputing (CGRB) at Oregon State University. One nanogram of DNA per sample was prepared using a Nextera XT library prep kit, each DNA library was pooled at equimolar

concentrations with about 25 nanograms (ng) of prepared DNA per sample and sequenced using an Illumina HiSeq 3000 instrument with 151 base pair (bp), paired-end reads and about 450 bp insert sizes. All sequencing reads were quality screened using Trimmomatic (Bolger et al., 2014), only those with quality (phred) scores over 30 were retained. The sequences were interleaved using the program khmer and only those with mate pairs and a minimum length of 50 bp were further retained. All sequencing reads were pooled and randomly sub-sampled to 375 million reads and assembled using IDBA-UD (Peng et al., 2012); an assembler specifically designed to handle highly uneven read depths characteristic of metagenome datasets. Assembled genome fragments (contigs) were binned to the lowest assignable taxonomic level using PhylopythiaS+ (Gregor et al., 2014) and mmgenome (Albertsen et al., 2013); these programs utilize a combination of approaches for assigning contigs to organisms that include: basic local alignment search tool (BLAST), tetranucleotide frequency, %GC content, kmer length, read coverage depth, and essential gene content. The sequencing reads from each individual sample were mapped to the concatenated multi-sample assembly using Burrows-Wheeler Aligner (BWA) (Li et al. 2009) in order to determine the read coverage (average number of sequencing reads per nucleotide) of each contig. All contigs were screened for genes involved in anatoxin-a biosynthesis using a combination of local alignment searches (both nucleotide and amino acid) and hidden Markov models implemented through Prokka (Seemann, 2014). Genome bins containing anatoxin-a genes were extracted and screened for phylogenetic markers (e.g., 16S rRNA genes). A cyanobacterial 16S rRNA gene sequence was extracted from the anatoxin-a bin and its phylogeny was inferred relative to other cyanobacterial 16S rRNA reference genes obtained from the NCBI GenBank database using a Bayesian framework carried out in MrBayes v.3.1.2 with a general time reversible substitution model with gamma distributed variation.

Results

Of the 126 samples collected during the summer, 24 samples were flagged as potentially containing anatoxin-a producing cyanobacteria based on the results of the anatoxin-a gene visual detection assay (CyanArray; Appendix I). QPCR was also used to quantify *anaC* genes from all 126 samples, of which 41 contained *anaC* genes above the limit of quantification (100 copies/mL; Appendix I). Fourteen of these samples contained over 2,000 *anaC* gene copies/mL, which was the detection limit for the CyanArray assay. Out of those 14 samples, the CyanArray assays were negative for eight of them, indicating a false negative rate of 57%. Conversely, the false positive rate of the CyanArray assay, defined as those samples exhibiting a positive result when the QPCR results were negative, was estimated to be 20% (16/79 samples). For comparison, the false negative and false positive rates previously observed for the microcystin CyanArray assay, were 8% and 8.6%, respectively (Otten, 2017). Based on these data, it appears that QPCR is a superior DNA based tool for detecting and quantifying anatoxin-a genes in the Klamath River system.

Even though QPCR indicated the presence of anatoxin-a genes in 32% of samples collected throughout the Klamath River in 2016, *Aphanizomenon flos-aquae* was generally the only potential anatoxin-a producer present at elevated cell concentrations based on microscopy results (Appendix II). However, in the Klamath River it is believed that *A. flos-aquae* does not produce anatoxin-a; this inference is based on the genome of an *A. flos-aquae* strain isolated from Upper Klamath Lake that was shown to lack cyanotoxin genes (Driscoll et al., 2017). Since *Microcystis* sp. has never been found to produce anatoxin-a, the only other potential anatoxin-a producers observed by microscopy were *Dolichospermum*, which was found in 21/126 samples (17%) and *Planktothrix*/*Oscillatoria*, which was observed in three samples (2.4%). There were only six samples where *anaC* genes were observed by QPCR and *Dolichospermum* was observed by microscopy; however, there was no relationship in these samples between *anaC* and *Dolichospermum* abundance (Figure 3). These data suggested that the source of the anatoxin-a genes was not *Dolichospermum*.

Based on the QPCR results, all samples that exhibited elevated *anaC* gene concentrations (over 1,000 copies/mL) were PCR amplified and prepared for DNA sequencing of the *anaC* gene. A total of 10 sets of PCR amplicons were advanced for Sanger sequencing that represented seven discrete river grab samples. Nine out of the 10 samples produced high quality sequencing reads, these were aligned along with representative sequences from a variety of anatoxin-a producing cyanobacteria genera (Figure 4). This analysis indicated that the Klamath River samples, spanning multiple collection dates across three locations [JC Boyle Reservoir (BRTC), Orleans (OR), and Weitchpec (WE)], all contained identical or nearly identical *anaC* sequences. This suggested that the producer could be the same organism in each location, although the small amount of sequence information (about 360 bp DNA) that the Sanger sequences provided was inadequate to definitively determine if it was the same organism in each sample. The alignment of the Sanger-generated sequences indicated that all of the Klamath sequences were highly similar to *anaC* sequences derived from *Oscillatoria* sp. or *Phormidium* sp. Both of these species are filamentous non-nitrogen fixing cyanobacteria within the cyanobacterial order Oscillatoriales. Importantly, this analysis demonstrated that the producer was not likely to be a nitrogen fixer from the order Nostocales, which includes common Klamath River cyanobacteria such as *Dolichospermum* (or *Anabaena*) and *Aphanizomenon.*

Shotgun metagenomic sequencing was used in an effort to definitively identify the producer(s) of anatoxin-a from three samples (JC Boyle Reservoir – BRTC16006; Weitchpec – WE082416; and Orleans – OR091416) where *anaC* sequences were already confirmed. Unlike PCR-directed methods that only amplify a portion of a gene (e.g., *anaC*), shotgun metagenomics is the process of sequencing all DNA present in a sample. This is a non-targeted process, whereby portions of DNA from all bacteria, archaea, or eukaryota present in the sample are sequenced to varying degrees based on the number of sequencing reads generated per run, the

abundance of taxa and their relative diversity in a sample. As such, organisms present at higher concentrations tend to be more deeply sequenced (higher representation of the dataset) than rarer taxa. Overall, there were 874,679 contigs assembled from about 280 million sequencing reads. The combined assembly spanned over 780 megabase pairs (Mbp) of DNA sequence; for comparison, the average cyanobacterial genome is about 5.0 Mbp. The average contig coverage depth from each sample, a proxy for average organismal abundance, was 13.1X, 6.1X, and 4.3X for the Weitchpec, Orleans, and JC Boyle samples, respectively. Because sequencing effort was comparable across these three samples (about 85 million reads/sample), the difference in coverage depths is largely a result of differences in species diversity at these sites, with the JC Boyle sample containing the highest microbial diversity (and therefore lowest average contig coverage depth). All contigs from the three datasets were combined and screened for the presence of anatoxin-a genes, of which 20 contigs were identified, these had an average coverage depth of only 3.6X. Based on these coverage depths, it can be inferred that the anatoxin-a producer was less abundant than the average bacterium or algal cell in the three water column samples. Generally, coverage depths of 8X or greater are typically needed in order to generate draft genomes from aquatic metagenomic datasets (Otten et al., 2016).

The 20 anatoxin-a gene fragments recovered from the combined metagenomes spanned about 18,000 base pairs of the anatoxin-a gene cluster. The gene sequences were compared with all sequences in the NCBI GenBank database in order to find their closest relative using the basic local alignment search tool (BLAST). The closest organismal match and their percent nucleotide similarity were recorded for each sequence (Table 2). The results indicated that the majority of the sequences were most closely related to anatoxin-a genes from *Oscillatoria* strains, however, the average nucleotide similarity was only about 92% similar. For comparison, the anatoxin-a gene cluster from two strains of *Oscillatoria* sp. (PCC 6506 & PCC 6407) was previously sequenced and found to be 100% identical (Méjean et al., 2014). As such, these results expand upon the *anaC* Sanger sequences and indicate that, while the Klamath anatoxin-a genes are most closely related to *Oscillatoria* in the database, it is likely that they are not actually derived from *Oscillatoria* or else the percent similarity would be considerably higher. Unfortunately, there is not a complete anatoxin-a gene cluster from *Phormidium* that has been previously published or deposited into NCBI GenBank that can be used for a better comparison. Therefore, Klamath anatoxin-a gene sequences appear to currently represent novel genetic information. In the future, these sequences can be reanalyzed when there are more anatoxin-a gene sequences publicly available in order to gain further resolution into their host.

The relatively low sequencing coverage of the anatoxin-a containing genome made assembly problematic, however, by using the anatoxin-a genes as a marker we were able to extract a number of other co-occurring sequences using two dimensional binning (Figure 5). Within this extraction bin there were a number

of cyanobacterial sequences related to *Oscillatoria*; most useful of which was a contig containing a cyanobacterial 16S ribosomal (rRNA) gene. The 16S rRNA gene is a common phylogenetic marker used in studies of bacterial community diversity and phylogeny. The recovered 16S rRNA gene was aligned with other cyanobacterial genes from public databases and visualized as a phylogenetic tree (Figure 6). The result indicated that the Klamath sequence was most closely related to *Phormidium* sp. or *Tychonema* sp. because it grouped together in the same clade (branch) of the tree. Notably, both *Phormidium* and *Tychonema* genera are part of the family Oscillatoriales and are benthic mat-forming cyanobacteria.

Because the genetic data indicated that a member of the Oscillatoriales family is responsible for anatoxin-a biosynthesis in the Klamath River system, then they should have also been detected by cell counting when *anaC* genes or anatoxin-a were measured. However, neither *Oscillatoria* nor any other related genus such as *Phormidium* or *Tychonema* were ever observed in any sites where *anaC* genes or anatoxin-a were observed. Of the two samples that were collected in which anatoxin-a was detected, *anaC* genes were not detected in either sample even though the anatoxin-a concentrations were 4.4 µg/L and 4.6 µg/L (TG091316-SG and WE091416-SG, respectively). Since none of these samples contained any *Oscillatoria*, nor were *anaC* genes observed, these results suggest that the producer is likely of benthic or attached (epiphytic) origin and the toxins were being released extracellularly into the water column.

Discussion

For several years it has been recognized that there is at least one strain of anatoxin-a producing cyanobacteria endemic to the Lower Klamath River where positive anatoxin-a results have been occasionally observed; however, the identity of the producer remained unresolved. By way of DNA sequencing, we now know that *Phormidium*, *Tychonema*, or some other closely related cyanobacterium likely exist within parts of the Klamath River watershed and that their population is comprised of members capable of biosynthesizing anatoxin-a and/or its analogues. The sequencing depth employed on the three samples that were shotgun sequenced should have been more than ample to generate draft genomes of these toxigenic strains. However, only fragments of the genome could be extracted from the mixed microbial assemblage present in the samples. This fact, along with the general absence of any *Oscillatoria*-like cyanobacteria reported from microscopy analysis of samples throughout the 2016 monitoring season, suggests that the causative agent(s) are of benthic origin and were therefore underrepresented in the water column grab samples analyzed in this study.

Anatoxin-a producing benthic cyanobacteria have been reported to occur in both the Russian and Eel rivers in Northern California and in the Umpqua River in Southern Oregon (Bouma-Gregson et al., 2017). An extensive survey of California wadeable stream algae documented *Phormidium* in greater than one-third of

systems investigated, making it the fourth most commonly encountered benthic cyanobacteria genera in California (Fetscher et al., 2015). Conversely, *Tychonema* sp. was not reported in any of the surveys; however, there are many similar morphological features shared between *Tychonema* and *Phormidium* which could have led to grouping of these two genera (Figures 7 and 8). Interestingly, less than 1 percent of samples analyzed during a previous study of benthic algae in the lower Klamath River were reported to contain cyanobacteria and no cyanobacteria from the family Oscillatoriales were observed (Yurok Tribe et al., 2011). The lack of congruence between this 2011 study and the present one suggests one of several potential possibilities: 1) benthic cyanobacteria are relatively recent colonizers to the Klamath River system; 2) their abundance was previously reduced by scouring that occurred during higher flow (pre-drought) years; 3) their preferred habitat was not sampled in the aforementioned Yurok Tribe study; or 4) that the bulk of their biomass occurs in the tributaries feeding into the main stem of the Klamath River that were not sampled in either study.

One way to better establish the identity of the anatoxin-a producer would be to conduct lateral transects of the river during the summer in order to better understand the habitat used by potential anatoxin-a producers. In the Eel River, benthic cyanobacterial mats have been found to become more extensive during low flow periods of the summer and in areas where water tends to stagnate (Power et al., 2015). However, when flows become too low it has been shown that *Phormidium* mats tend to slough off and float away due to the accumulation of oxygen bubbles generated during photosynthesis (McAllister, 2015). Additional insights into their preferred habitat structure may also be drawn by comparing similar systems that are known to harbor benthic anatoxin-a producing cyanobacteria. For example, in New Zealand *Phormidium* mats tend to proliferate in moderately flowing waters with little shading, low dissolved soluble reactive phosphorus (SRP < 0.01 mg/L), slightly elevated dissolved inorganic nitrogen (DIN > 0.1 mg/L) concentrations and in areas where stable substrates such as bedrook, boulder, and cobble are abundant (McAllister et al., 2016).

An important feature of these mats that should be recognized before beginning any targeted monitoring for these taxa is that *Phormidium* mats have been shown to be comprised of a mixture of toxic and nontoxic strains and their relative abundances are highly variable across time and space (Wood et al., 2012). Therefore, single grab samples may poorly represent the public health risk associated with what can become at times spatially extensive mats.

The most likely source of anatoxin-a genes found in this study of the Klamath River is from benthic sources. It's expected that at times these mats and/or their cells may slough off into the water column, be transported downstream, and occasionally are collected in a grab sample. The relatively low abundance of genes and toxins observed during this study likely reflect that water column grab samples are not best suited for assessing risks from benthic algae. Attention should be given to these benthic mats because they comprise a

public health risk. Anatoxin-a is not the only cyanotoxin risk that may be associated with benthic mats, as they have been found to also produce cylindrospermopsin, microcystin, and saxitoxin.

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Literature Cited

Albertsen M, Hugenholtz P, Skarshewski A, Tyson GW, Nielson KL, Nielsen PH. (2013). Genome sequences of rare, uncultured bacteria obtained by differential coverage binning of multiple metagenomes. Nature Biotechnology 31:533-538.

Bolger AM, Lohse M, Usadel B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114-2120.

Bozarth, C.S., Schwartz, A.D., Shepardson, J.W., Colwell, F.S., Dreher, T.W. (2010). Population Turnover in a *Microcystis* Bloom Results in Predominantly Nontoxigenic Variants Late in the Season. Applied Environmental Microbiology 76(15):5207-5213.

Bouma-Gregson, K., Power, M.E., Bormans, M. (2017). Rise and fall of toxic benthic freshwater cyanobacteria in the Eel River: Buoyancy and dispersal. Harmful Algae 66:79-87.

Driscoll CB, Otten TG, Brown NM, Dreher TD. (2017). Toward long-read metagenomics: complete assembly of three novel genomes from bacteria dependent on a diazotrophic cyanobacterium in a freshwater lake co-culture. Standards in Genomic Sciences 12:9.

Eilers, J.M., Kann, J., Cornett, J., Moser, K., St. Amand, A. (2004). Paleolimnological Evidence of Change in a Shallow, Hypereutrophic Lake: Upper Klamath Lake, Oregon, USA. Hydrobiologia 520:7-18.

Fetscher, A.E., Howard, M.D.A., Stancheva, R., Kudela, R.M., Stein, E.D., Sutula, M.A., et al. (2015). Wadeable streams as widespread sources of benthic cyanotoxins in California, USA. Harmful Algae 49:105-116.

Gregor I, Dröge J, Schirmer M, Quince C, McHardy AC. (2014). PhyloPythiaS+: A self-training method for the rapid reconstruction of low-ranking taxonomic bins from metagenomes. arXiv 1406.7123

Kaminski, A., Bober, B., Lechowski, Z., Bialczyk, J. (2013). Determination of anatoxin-a stability under certain abiotic factors. Harmful Algae 28:83-87.

Klamath Blue Green Algae Working Group. (2009). Standard Operating Procedures: Environmental sampling of cyanobacteria for cell enumeration, identification, and toxin analysis, version 6. Available online at: http://www.kbmp.net/images/stories/pdf/KHSA/Cyanobacteria_Sampling_SOP.pdf.

Li, H., Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**:1754-1760.

McAllister, T.G., Wood, S.A., Hawes, I. (2016). The rise of toxic benthic *Phormidium* proliferations: A review of their taxonomy, distribution, toxin content and factors regulating prevalence and increased severity. Harmful Algae 55:282-294.

Méjean, A., Paci, G., Gautier, V., Ploux, O. (2014). Biosynthesis of anatoxin-a and analogues (anatoxins) in cyanobacteria. Toxicon 91:15-22.

Otten, T.G., Crosswell, J.R., Mackey, S., Dreher, T.W. (2015). Application of molecular tools for microbial source tracking and public health risk assessment of a *Microcystis* bloom traversing 300km of the Klamath River. Harmful Algae 46:71-81.

Otten, T.G., Graham, J.L., Harris, T.D., Dreher, T.W. (2016). Elucidation of taste-and-odor producing bacteria and toxigenic cyanobacteria in a Midwestern drinking water supply reservoir by shotgun metagenomic analysis. Applied Environmental Microbiology 82(17):5410-5420.

Otten, T.G. (2017). Application of genetic tools for improved cyanobacterial bloom monitoring in the Klamath River system: Implications for public health monitoring. Technical report prepared for PacifiCorp. April 12, 2017.

Peng, Y., Leung, H.C., Yiu, S.M., Chin, F.Y. (2012). IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. Bioinformatics 28:1420-1428.

Power, M.E., Bouma-Gregson, K., Higgins, P., Carlson, S.M. (2015). The Thirsty Eel: Summer and Winter Flow Thresholds that Tilt the Eel River of Northwestern California from Salmon-Supporting to Cyanobacterially Degraded States. Copeia 1:200-211.

Rantala-Ylinen, A, Kana S, Wang H, Rouhiainen L, Wahlsten M, et al. 2011. Anatoxin-a Synthetase Gene Cluster of the Cyanobacterium *Anabaena* sp. Strain 37 and Molecular Methods to Detect Potential Producers. Applied Environmental Microbiology 77(20):7271-7278.

Raymond, R. (2003). Memorandum to Todd Olson, PacifiCorp, regarding Klamath Algae (*Microcystis aeruginosa*).

Seemann, T. (2014). Prokka: rapid prokaryotic genome annotation. Bioinformatics 30(14):2068-2069.

Shams, S., Capelli, C., Cerasino, L., Ballot, A., Dietrich, D.R., Sivonen, K., Salmaso, N. (2015). Anatoxin-a producing *Tychonema* (Cyanobacteria) in European waterbodies. Water Research 69:68-79.

Wood, S., Smith, F.M., Heath, M.W., Palfroy, T., Gaw, S., et al. (2012). Within-mat variability in anatoxin-a and homoanatoxin-a production among benthic *Phormidium* (cyanobacteria) strains. Toxins 4(10):900-912.

Yurok Tribe, Karuk Tribe, and Watercourse Engineering, Inc. 2011. KHSA: Klamath River Periphyton Pilot Study Summary Report.

TABLES & FIGURES

Table 1. Study sampling sites and frequency (May - October, 2016)

Site ID	Location	# Samples	Frequency
UKEP	Upper Klamath Lake at Eagle Ridge County Park	5	Monthly
UKHP	Upper Klamath Lake at Howard's Bay Park	5	Monthly
UKMP	Upper Klamath Lake at Moore Park	5	Monthly
LINK	Link Dam	5	Monthly
KENO	Keno Dam	2	Monthly
KEKP	Keno Reservoir at Keno Park	4	Monthly
KBK	Klamath River Below Keno Reservoir	3	Monthly
BRTC	J.C. Boyle Reservoir at Topsy Campground	5	Monthly
CRMC	Copco Reservoir at Mallard Cove	7	Monthly
CRCC	Copco Reservoir at Copco Cove	7	Monthly
KR19874	Copco Buoy Line	5	Monthly
IRCC	Iron Gate Reservoir at Camp Creek	6	Monthly
IRJW	Iron Gate Reservoir at Williams Boat Ramp	7	Monthly
KR19019	Iron Gate Log Boom	5	Monthly
KR18973	Klamath River below Iron Gate (KRBI)	9	Monthly (2 in June)
IB	Klamath River at I-5 Rest Area	6	Monthly (2 in July)
BB	Klamath River at Brown Bear River Access	6	Monthly (2 in July)
SV	Klamath River at Seiad Valley	6	Monthly (2 in July)
HC	Klamath River at Happy Camp	6	Monthly (2 in July)
OR	Klamath River at Orleans	6	Monthly (2 in July)
WE	Klamath River at Weitchpec	8	Monthly (2 in July)
TG	Klamath River at Turwar	8	Monthly (2 in July)
	Total Samples	**126**	

Table 2. Assembled contigs that encode for anatoxin-a biosynthesis genes, their closest match in the NCBI GenBank database and their position relative to the anatoxin-a gene cluster in *Oscillatoria* sp. (PCC6506) shown in Figure 1.

Contig #	Gene(s)	Length	Closest match	% Identity	Osc coords
1	anaBCD	3266	*Oscillatoria*	91	12233 - 15504
3	hyp - anaI	1905	*Oscillatoria*	90	5985 - 7920
4	anaE	1455	*Oscillatoria*	94	16536 - 17991
6	anaF	1337	*Oscillatoria*	91	22168 - 23510
8	anaE	1056	*Oscillatoria*	94	18069 - 19124
9	anaJ	1036	*Oscillatoria*	87	9572 - 10608
13	anaE	894	*Oscillatoria*	96	15575 - 16468
16	anaF	876	*Anabaena*	94	23716 - 24591
23	anaE	758	*Oscillatoria*	92	19626 - 20383
25	anaF	716	*Anabaena*	96	26713 - 27428
34	anaF	630	*Cuspidothrix*	93	23836 - 24465
53	anaG	549	*Anabaena*	96	28211 - 28759
54	anaF	548	*Anabaena*	91	24573 - 25120
60	anaE	533	*Oscillatoria*	95	20723 - 21255
68	anaEF	512	*Anabaena*	95	21630 - 22144
72	anaF	505	*Anabaena*	94	25240 - 25744
131	anaE	412	*Oscillatoria*	95	19270 - 19681
159	anaF	389	*Nostocales*	92	26151 - 26539
165	anaA	381	*Oscillatoria*	85	10802 - 11185
187	anaG	359	*Anabaena*	95	27820 - 28179

Figure 1. Diagram of the anatoxin-a gene clusters sequenced from four different strains of cyanobacteria (from Méjean et al., 2014).

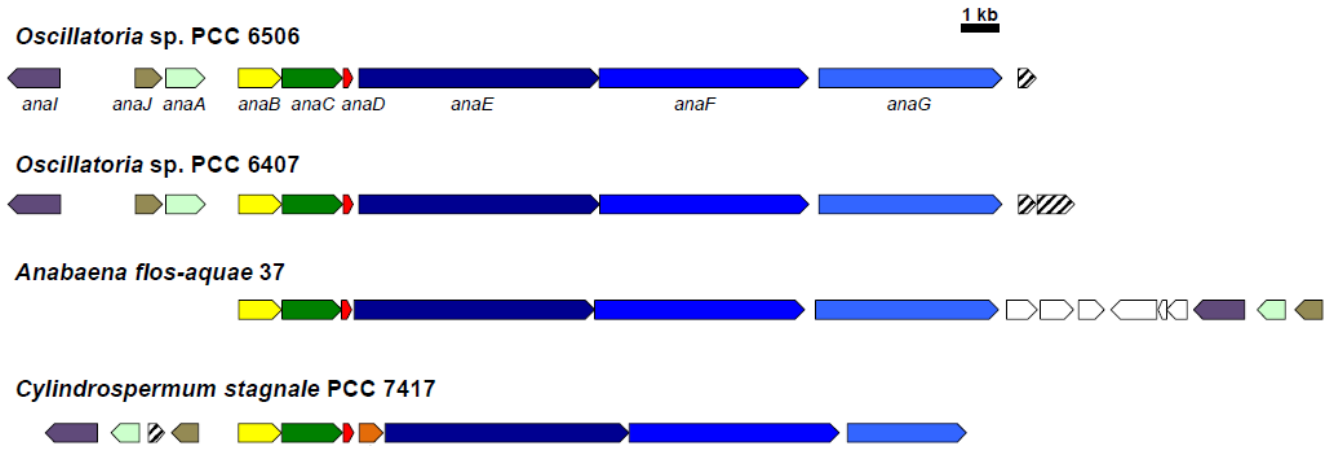


Figure 2. Photograph of CyanArray colorimetric detection of toxin genes; higher toxin gene concentrations illicit a stronger color change.

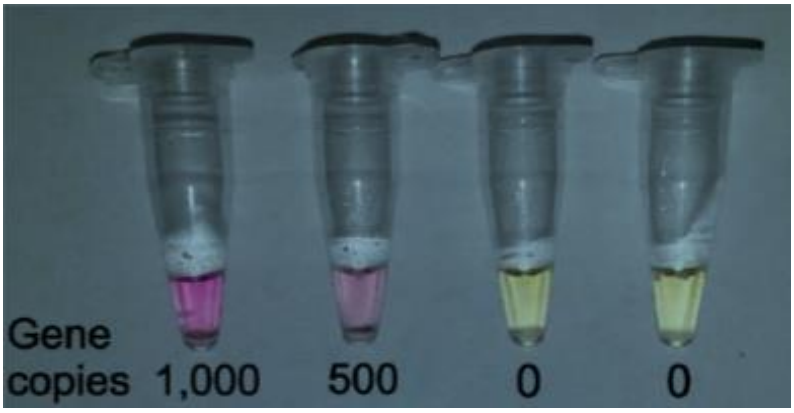


Figure 3. Comparison of *anaC* gene abundance relative to *Dolichospermum* sp. cell abundance (*n*=6).

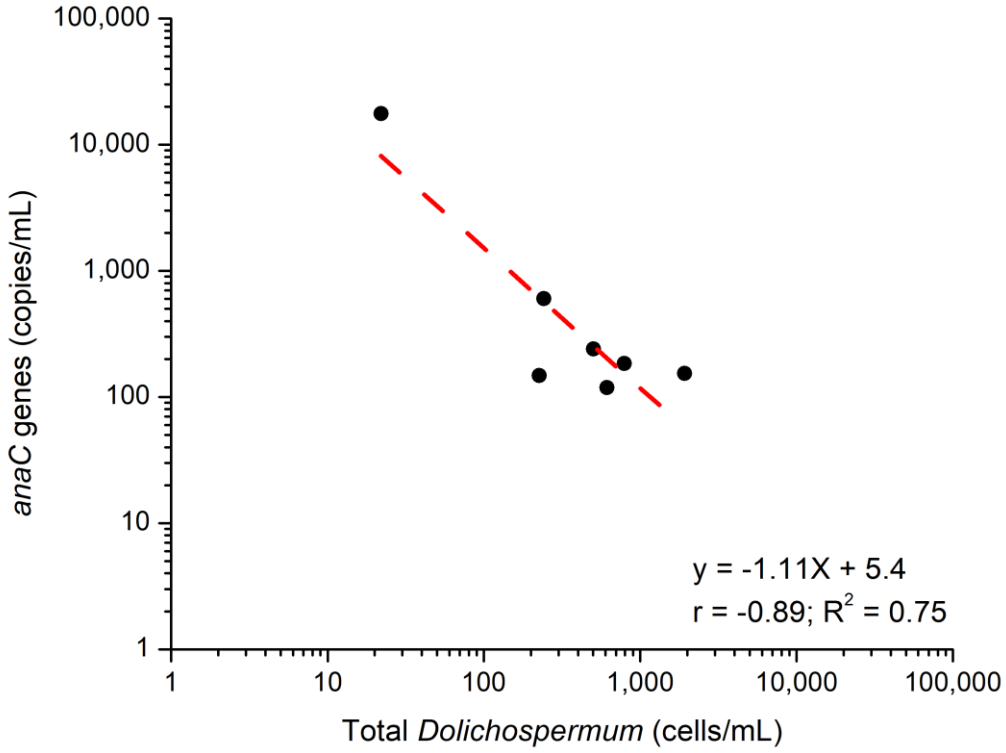


Figure 4. Nucleotide alignments of *anaC* amplicons from the Klamath River (Rows 1-9) and from specific genera obtained from NCBI GenBank (Rows 10-15). Colored nucleotides in rows 12-15 indicate dissimilarity with anatoxin-a gene sequences collected from Klamath River samples that eliminate those genera as likely anatoxin-a producers in the Klamath River. The v1 and v2 designation in the identity column indicates if the sequence was derived from a single PCR run or if it needed to be reamplified (v2) in order to generate enough DNA for sequencing purposes. Site IDs: WE = Weitchpec, OR = Orleans, and BRTC = JC Boyle Reservoir.

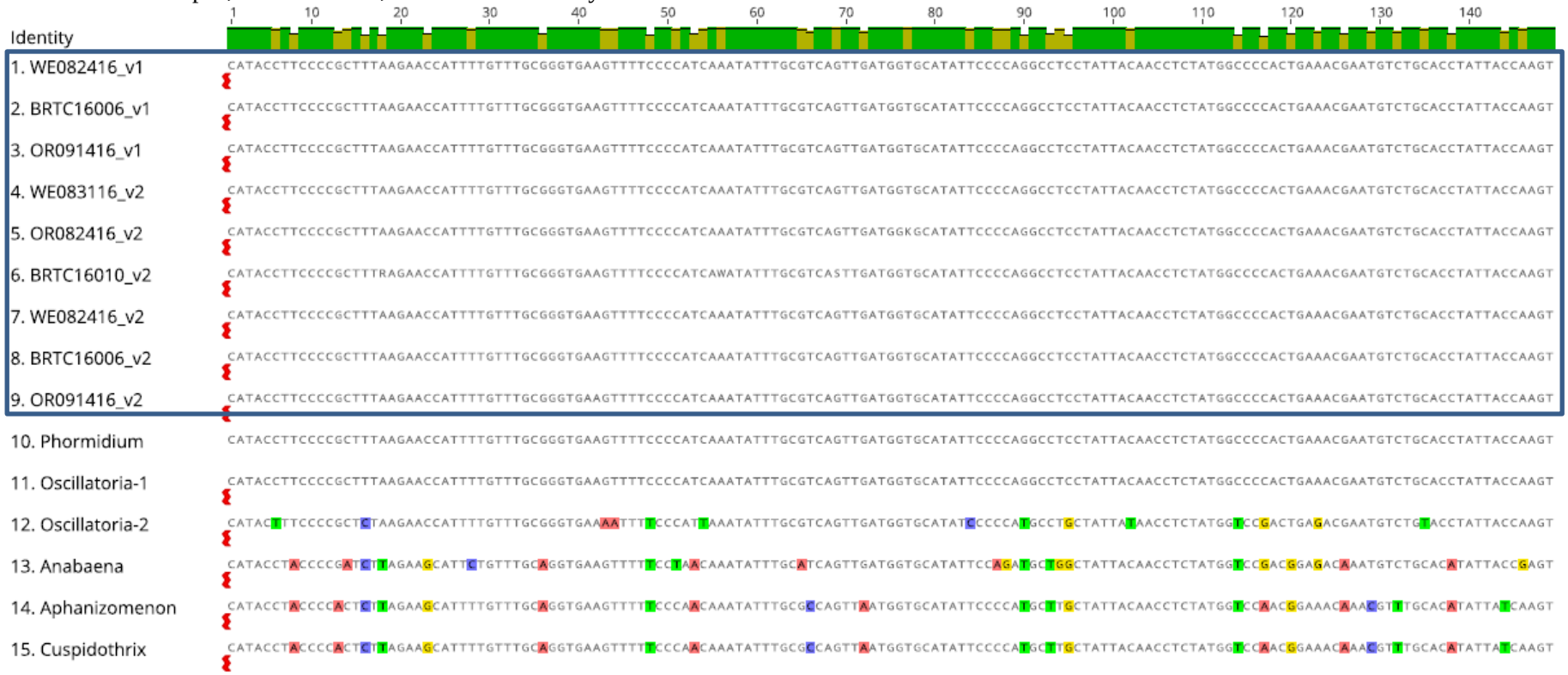


Figure 5. Example of how genes which co-occur in their relative abundance (similar contig coverage) with anatoxin-a genes (highlighted red circles) are extracted using two-dimensional differential binning. Each colored circle comprises a genome fragment (contig), larger circles denote longer contigs and axes denote contig coverage depth.

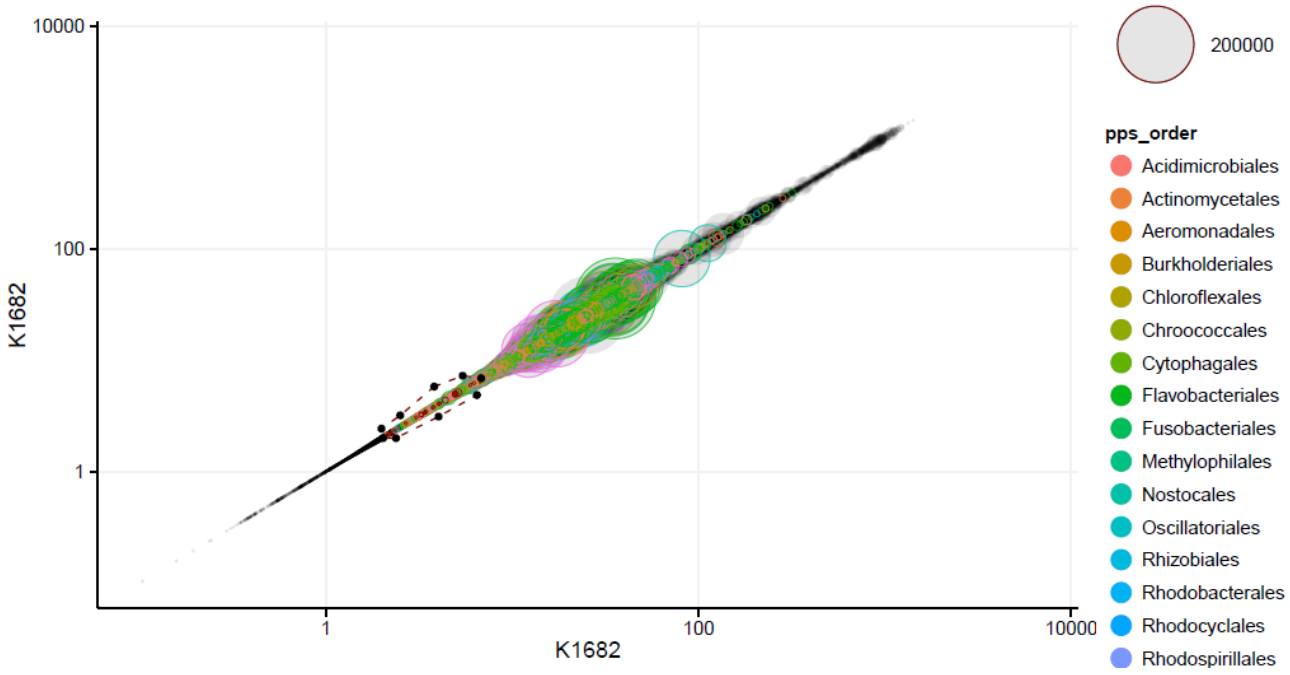


Figure 6. Phylogenetic inference of cyanobacterial 16S rRNA gene sequences places the Klamath River 16S rRNA shotgun sequence (red box) as a close relative to *Phormidium* sp. and *Tychonema* sp.

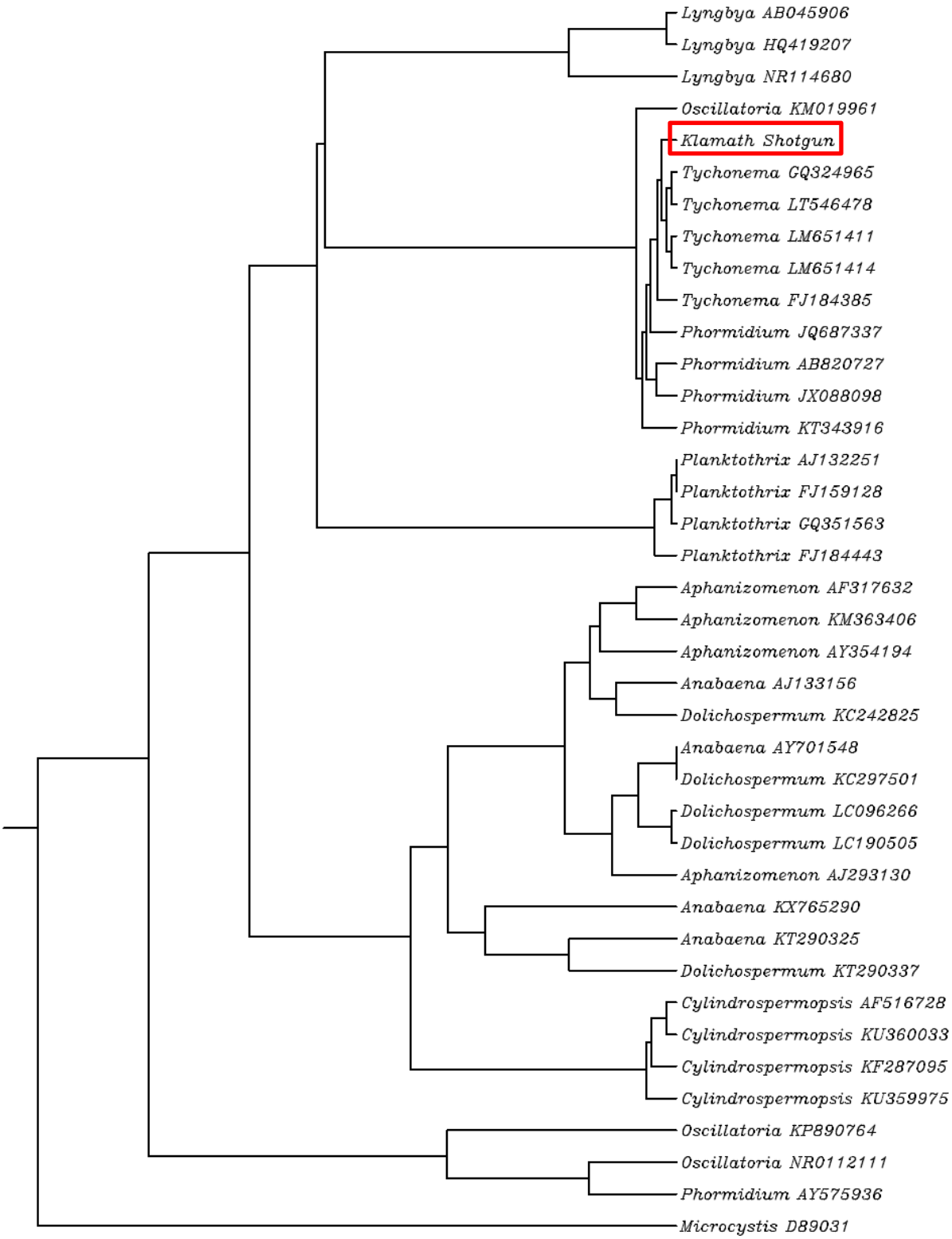


Figure 7. Photographs of benthic mats of *Phormidium* in rivers and photomicrographs of *Phormidium* sp. and *Tychonema* sp. filaments. (A) microscope image of typical *Phormidium* sp. filaments; (B) Dense accumulation of Phormidium sp. on a rock, left side peeled back to show fine sediment accumulation typically observed beneath mats; (C,D) benthic mats in river bottoms. Images from McAllister et al., 2016.

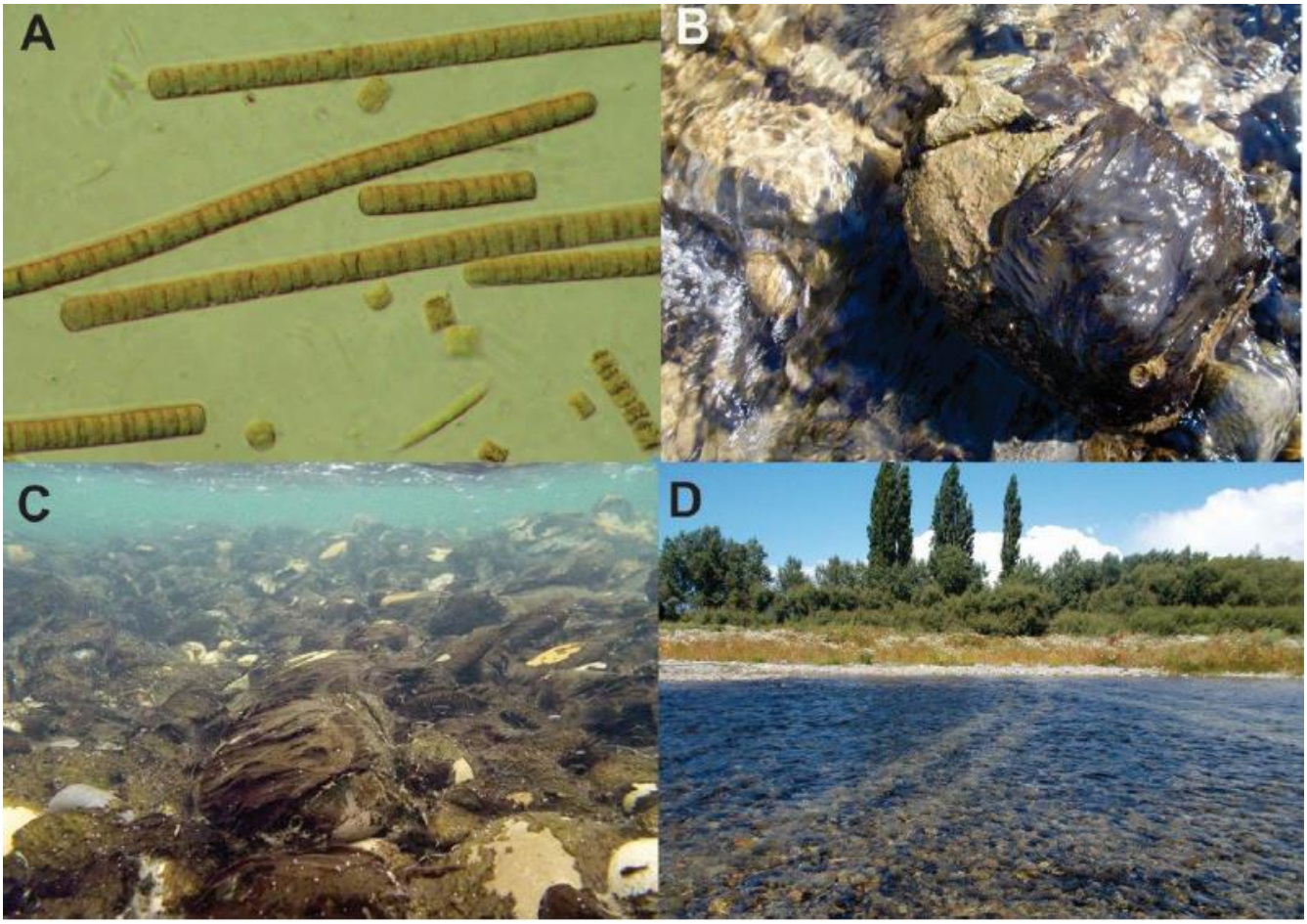
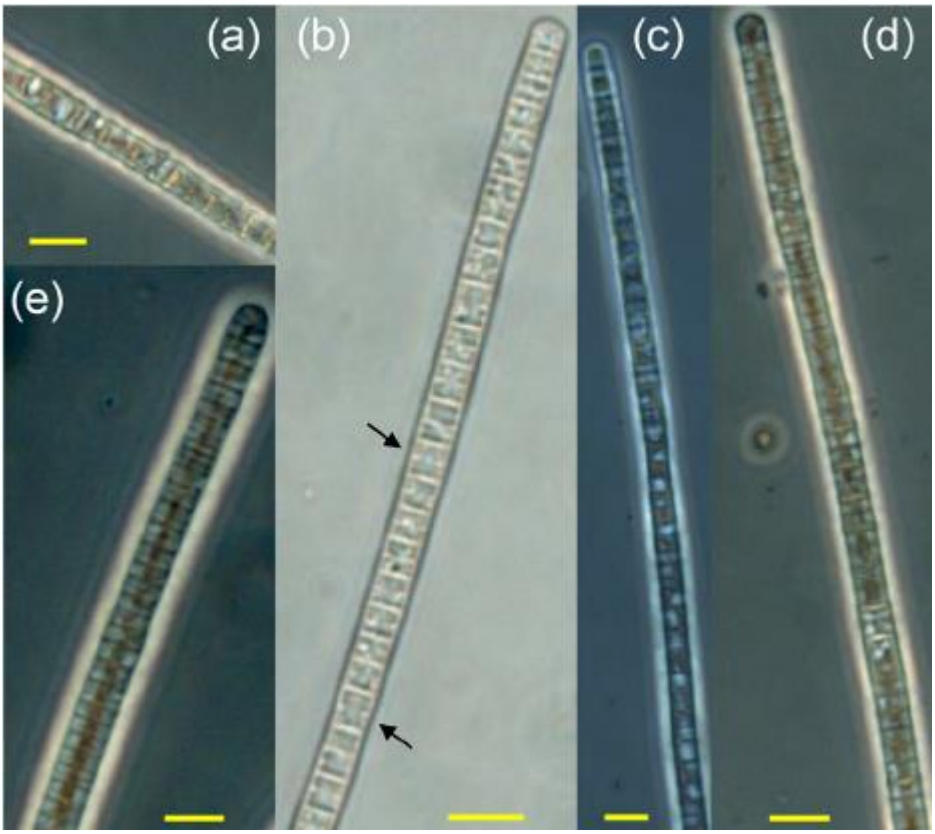


Figure 8. Photomicrographs of anatoxin-a producing *Tychonema* isolates collected from Lake Garda, Italy. Images from Shams et al., 2015.



Appendix I. Anatoxin-a concentrations and DNA results for 2016 Klamath River special study

Sample ID	ANTX (µg/L)	CyanArray	QPCR (*anaC*/mL)	95% CI	Comment
KR16800		Neg	177	202	
KR16801		Neg	112	130	
KR16802		Neg	ND		U
KR16803		Neg	ND		U
KR16804		Neg	ND		U
KR16805		Neg	ND		U
KR16806		Neg	240	132	
KR16807		**Pos**	ND		U
KR16808		Neg	1,054	674	
KR16809		Neg	ND		U
KR16090		Neg	ND		U
KR16091		Neg	ND		U
KR16096		Neg	ND		U
2016KHSA-35		**Pos**	3,172		
2016KHSA-38		**Pos**	ND		U
KR16810		Neg	ND		U
KR16811		Neg	ND		U
KR16812		Neg	ND		U
KR16183		Neg	154	48	
KR16814		Neg	ND		U
KR16105		Neg	ND		U
BRTC16002		Neg	205	111	
KEPC16002		Neg	ND		U
UKMP16002	0.26	**Pos**	58,982	1,806	
UKHP16002		Neg	306	28	
UKEP16002		Neg	365	48	
SV062916		Neg	ND		U
OR062916-SG		**Pos**	2,077		
BB062916		Neg	ND		U
HC062916		Neg	ND		U
IB062916		Neg	ND		U
2016KHSA-40		Neg	164	29	
2016KHSA-44		**Pos**	ND		U
IB071316		Neg	ND		U
SV071316		**Pos**	ND		U
BB071316		**Pos**	ND		U
HC071316		Neg	ND		U
OR071316		Neg	ND		U
TG071216		Neg	100	27	C1,J
WE071316		Neg	96	50	C1,J
KR16815		Neg	184	94	
KR16816		Neg	100	0	C1,J
KR16817		Neg	ND		U
KR16818		Neg	ND		U
KR16819		Neg	ND		U
KR16114		Neg	ND		U
KR16119		Neg	ND		U

Sample ID	ANTX (µg/L)	CyanArray	QPCR (*anaC*/mL)	95% CI	Comment
BRTC16004		Neg	ND		U
KEKP16004		Neg	ND		U
UKMP16004		Neg	115	8	
UKHP16004		Neg	ND		U
UKEP16004		Neg	102	13	
IB072716-SG		Neg	ND		U
HC072716-SG		Neg	ND		U
BB072716-SG		Neg	ND		U
SV072716-SG		Neg	ND		U
OR072716-SG		Neg	ND		U
TG072616-SG		Neg	ND		U
WE072716-SG		Neg	ND		U
2016KHSA-51		Neg	134	15	
2016KHSA-55		Neg	ND		U
TG080916		**Pos**	ND		U
WE081016-SG		Neg	361	37	
OR081016-SG		Neg	ND		U
HC081016-SG		Neg	ND		U
SV081016-SG		Neg	ND		U
BB081016-SG		Neg	ND		U
IB081016-SG		Neg	ND		U
KR16825		Neg	4,164	1,758	
KR16826	<0.05	**Pos**	ND		U
KR16827		Neg	5,582	1,807	
KR16828		Neg	17,364	1,152	
KR16829		Neg	ND		U
KR16137		Neg	184	23	
KR16142		Neg	119	63	
OR082416		**Pos**	3,747	2,291	
HC082416-SG		**Pos**	ND		U
SV082416-SG		**Pos**	603	11	
BB082416-SG		Neg	ND		U
IB082416-SG		Neg	515	279	
TG082316		**Pos**	ND		U
WE082316		**Pos**	17,611	2,516	
BRTC16006		**Pos**	8,229	3,521	
KEKP16006		Neg	795	344	
UKMP16006		Neg	738	261	
UKHP16006		Neg	ND		U
UKEP16006		**Pos**	ND		U
TG083016		**Pos**	ND		U
WE083116		Neg	5,163	1,432	
KR16835		Neg	ND		U
KR16836	<0.05	**Pos**	ND		U
KR16837		Neg	18,732	943	
KR16838		Neg	6,641	880	
KR16839		Not Run	ND		U
KR16160		Not Run	ND		U

Sample ID	ANTX (µg/L)	CyanArray	QPCR (*anaC*/mL)	95% CI	Comment
KR16165		Not Run	ND		U
2016KHSA-62		Neg	ND		U
2016KHSA-66		**Pos**	804	22	
OR091416-SG		Neg	31,582	2,456	
HC091416-SG		**Pos**	ND		U
SV091416-SG		Neg	ND		U
BB091416-SG		Neg	ND		U
IB091416-SG		Neg	546	115	
TG091316-SG	4.6	Neg	ND		U
WE091416-SG	4.7	Neg	ND		U
BRTC16008		Neg	1,046	686	
KEKP16008		Neg	1,154	95	
UKMP16008		Neg	920	333	
UKHP16008		Neg	ND		U
UKEP16008		**Pos**	ND		U
TG092716-SG		Neg	ND		U
WE092816-SG		Neg	ND		U
2016KHSA-73		Neg	ND		U
2016KHSA-77		Neg	917	34	
TG101116-SG		Neg	ND		U
WE101216-SG		**Pos**	ND		U
KR16845		Neg	ND		U
KR16846		Neg	ND		U
KR16848		Neg	ND		U
KR16849		Neg	ND		U
KR16188		Neg	148	33	
BRTC16010		Neg	2,100	393	
UKMP16010		**Pos**	ND		U
UKHP16010		Neg	ND		U
UKEP16010		Neg	ND		U
KR16183		Neg	ND		U

Appendix II. Potentially toxic cyanobacterial (PTOX) cell concentrations (cells/mL) determined by microscopic counting. MIC = *Microcystis*, DOL = *Dolichospermum*, AFA = *Aphanizomenon flos-aquae*, PLANK = *Planktotrhix*, OSC = *Oscillatoria*, GLO = *Gloeotrichia*

Sample ID	Sample Date	Site ID	Location	Total Density (cells/mL)	MIC	DOL	AFA	PLANK	OSC	GLO
KR16800	5/23/16	CRMC	CRMC	0						
KR16801	5/23/16	CRCC	CRCC	0						
KR16802	5/23/16	IRCC	IRCC	0						
KR16803	5/23/16	IRJW	IRJW	0						
KR16804	5/23/16	KRBI	KRBI	0						
KR16090	6/8/16	KR18973	KRBI	0						
KR16091	6/8/16	KR19019	Iron Gate Log Boom	0						
KR16096	6/8/16	KR19874	Copco Log Boom	0						
KR16805	6/8/16	CRMC	CRMC	0						
KR16806	6/8/16	CRCC	CRCC	503		503				
KR16807	6/8/16	IRCC	IRCC	0						
KR16808	6/8/16	IRJW	IRJW	0						
KR16809	6/8/16	KRBI	KRBI	0						
KR16105	6/20/16	KR18973	KRBI	77		77				
KR16810	6/20/16	CRMC	CRMC	69,214	68,407	807				
KR16811	6/20/16	CRCC	CRCC	513,458	496,424	17,034				
KR16812	6/20/16	IRCC	IRCC	12,272	12,272					
KR16813	6/20/16	IRJW	IRJW	6,860	4,940	1,920				
KR16814	6/20/16	KRBI	KRBI	16		16				
2016KHSA-35	6/21/16	KR25440	Link Dam	686,244	3,341		682,903			
2016KHSA-38	6/21/16	KR2460	Keno Dam	No Data						
BRTC16002	6/28/16	BRTC	Boyle - Topsy Camp	456,882	490		456,392			
KEKP16002	6/28/16	KEKP	Keno Park	297,115	18,090		279,025			
UKEP16002	6/28/16	UKEP	Eagle Ridge	10,267,054	163,293		10,103,761			

Sample ID	Date	Site ID	Location	Total Density (cells/mL)	MIC	DOL	APHA	PLANK	OSC	GLO
UKHP16002	6/28/16	UKHP	Howard's Bay	43,439,500			43,439,500			
UKMP16002	6/28/16	UKMP	Moore Park	4,642,481	1,485,481		3,157,000			
BB062916-SG	6/29/16	BB	Brown Bear	0						
HC062916-SG	6/29/16	HC	Happy Camp	0						
IB062916-SG	6/29/16	IB	I-5 Bridge	0						
OR062916-SG	6/29/16	OR	Orleans	0						
SV062916-SG	6/29/16	SV	Seiad Valley	0						
2016KHSA-40	7/12/16	KR25440	Link Dam	876,954			876,954			
2016KHSA-44	7/12/16	KR2460	Keno Dam	33,162			33,162			
KR16114	7/12/16	KR19019	Iron Gate Log Boom	293			293			
KR16119	7/12/16	KR19874	Copco Log Boom	9,279	9,279					
KR16815	7/12/16	CRMC	CRMC	10,394	9,601	793				
KR16816	7/12/16	CRCC	CRCC	92,066	91,883		183			
KR16817	7/12/16	IRCC	IRCC	58,341	13,917	13,981	1,450			28,993
KR16818	7/12/16	IRJW	IRJW	26,477	4,050	153	6,259			16,015
KR16819	7/12/16	KRBI	KRBI	0						
TG071216-SG	7/12/16	TG	Turwar	0						
BB071316-SG	7/13/16	BB	Brown Bear	0						
HC071316-SG	7/13/16	HC	Happy Camp	48			48			
IB071316-SG	7/13/16	IB	I-5 Bridge	0						
OR071316-SG	7/13/16	OR	Orleans	0						
SV071316-SG	7/13/16	SV	Seiad Valley	0						
WE071316-SG	7/13/16	WE	Weitchpec	23					23	
BRTC16004	7/26/16	BRTC	Boyle - Topsy Camp	0						
KEKP16004	7/26/16	KEKP	Keno Park	30,067			30,067			
TG072616-SG	7/26/16	TG	Turwar	0						
UKEP16004	7/26/16	UKEP	Eagle Ridge	150,457			150,457			
UKHP16004	7/26/16	UKHP	Howard's Bay	23,722,950	3,545,070		20,177,880			

Sample ID	Sample Date	Site ID	Location	Total Density (cells/mL)	MIC	DOL	APHA	PLANK	OSC	GLO
UKMP16004	7/26/16	UKMP	Moore Park	1,082,400			1,082,400			
BB072716-SG	7/27/16	BB	Brown Bear	0						
HC072716-SG	7/27/16	HC	Happy Camp	0						
IB072716-SG	7/27/16	IB	I-5 Bridge	0						
OR072716-SG	7/27/16	OR	Orleans	0						
SV072716-SG	7/27/16	SV	Seiad Valley	0						
WE072716-SG	7/27/16	WE	Weitchpec	0						
2016KHSA-51	8/9/16	KR25440	Link Dam	576,336			576,336			
2016KHSA-55	8/9/16	KBK	Klamath below Keno	294			294			
TG080916-SG	8/9/16	TG	Turwar	81	81					
BB081016-SG	8/10/16	BB	Brown Bear	6,729	6,729					
HC081016-SG	8/10/16	HC	Happy Camp	0						
IB081016-SG	8/10/16	IB	I-5 Bridge	5,859	5,859					
KR16137	8/10/16	KR19019	Iron Gate Log Boom	39,019	37,639		572			808
KR16825	8/10/16	CRMC	CRMC	17,025,250	16,236,000		99,485			689,765
KR16827	8/10/16	IRCC	IRCC	3,731,488	3,720,750		10,738			
KR16828	8/10/16	IRJW	IRJW	2,956,887	2,956,887					
KR16829	8/10/16	KRBI	KRBI	6,792	6,792					
OR081016-SG	8/10/16	OR	Orleans	403		381		22		
SV081016-SG	8/10/16	SV	Seiad Valley	1,278	1,278					
WE081016-SG	8/10/16	WE	Weitchpec	0						
KR16142	8/11/16	KR19874	Copco Log Boom	220,346	206,202	614	13,530			
KR16826	8/11/16	CRCC	CRCC	21,718,238	21,169,275		548,963			
TG082316-SG	8/23/16	TG	Turwar	0						
BB082416-SG	8/24/16	BB	Brown Bear	53	53					
HC082416-SG	8/24/16	HC	Happy Camp	81				81		
IB082416-SG	8/24/16	IB	I-5 Bridge	3,671	3,671					
OR082416-SG	8/24/16	OR	Orleans	446	390		56			

Sample ID	Sample Date	Site ID	Location	Total Density (cells/mL)	MIC	DOL	APHA	PLANK	OSC	GLO
SV082416-SG	8/24/16	SV	Seiad Valley	242		242				
WE082416-SG	8/24/16	WE	Weitchpec	22		22				
BRTC16006	8/30/16	BRTC	Boyle - Topsy Camp	3,391	3,391					
KEKP16006	8/30/16	KEKP	Keno Park	67,553	64,845		2,708			
TG083016-SG	8/30/16	TG	Turwar	33		33				
UKEP16006	8/30/16	UKEP	Eagle Ridge	0						
UKHP16006	8/30/16	UKHP	Howard's Bay	11,776	11,776					
UKMP16006	8/30/16	UKMP	Moore Park	0	0					
WE083116-SG	8/30/16	WE	Weitchpec	0						
KR16160	9/6/16	KR19019	Iron Gate Log Boom	42,082	7,281	224	34,577			
KR16835	9/6/16	CRMC	CRMC	785,439	654,054	4,228	127,157			
KR16836	9/6/16	CRCC	CRCC	4,903,745	73,887	19,191	4,810,667			
KR16837	9/6/16	IRCC	IRCC	3,250,623	3,240,556		10,067			
KR16838	9/6/16	IRJW	IRJW	931,315	895,235		36,080			
KR16165	9/7/16	KR18974	Copco Log Boom	15,680		3,245	12,435			
KR16839	9/7/16	KRBI	KRBI	2,582			2,582			
2016KHSA-62	9/13/16	KR25440	Link Dam	0						
2016KHSA-66	9/13/16	KBK	Klamath below Keno	0						
TG091316-SG	9/13/16	TG	Turwar	0						
BB091416-SG	9/14/16	BB	Brown Bear	36,241	6,144	30	30,067			
HC091416-SG	9/14/16	HC	Happy Camp	0	0					
IB091416-SG	9/14/16	IB	I-5 Bridge	6,234	507		5,727			
OR091416-SG	9/14/16	OR	Orleans	0	0					
SV091416-SG	9/14/16	SV	Seiad Valley	14,447	586		13,861			
WE091416-SG	9/14/16	WE	Weitchpec	0						
BRTC16008	9/27/16	BRTC	Boyle - Topsy Camp	0						
KEKP16008	9/27/16	KEKP	Keno Park	904	904					
TG092716-SG	9/27/16	TG	Turwar	0						

Sample ID	Sample Date	Site ID	Location	Total Density (cells/mL)	MIC	DOL	APHA	PLANK	OSC	GLO
UKEP16008	9/27/16	UKEP	Eagle Ridge	2,582	2,092		490			
UKHP16008	9/27/16	UKHP	Howard's Bay	1,102,674	1,077,630		25,044			
UKMP16008	9/27/16	UKMP	Moore Park	3,398			3,398			
WE092816-SG	9/28/16	WE	Weitchpec	0						
2016KHSA-73	10/11/16	KR25440	Link Dam	0						
2016KHSA-77	10/11/16	KBK	Klamath below Keno	0						
KR16188	10/11/16	KR19874	Copco Log Boom	70,413	3,101	226	67,086			
KR16845	10/11/16	CRMC	CRMC	73,818	67,650	6,168				
KR16846	10/11/16	CRCC	CRCC	147,678,763	144,972,763		2,706,000			
KR16848	10/11/16	IRJW	IRJW	15,145	2,461		12,684			
KR16849	10/11/16	KRBI	KRBI	2,563			2,563			
TG101116-SG	10/11/16	TG	Turwar	2,020			2,020			
WE101216-SG	10/12/16	WE	Weitchpec	158	27		131			
BRTC16010	10/25/16	BRTC	Boyle - Topsy Camp	0						
KR16183	10/25/16	KR19019	Iron Gate Log Boom	14,734			14,734			
UKEP16010	10/25/16	UKEP	Eagle Ridge	2,255			2,255			
UKHP16010	10/25/16	UKHP	Howard's Bay	85			85			
UKMP16010	10/25/16	UKMP	Moore Park	85			85			