

Application of genetic tools for improved cyanobacterial bloom monitoring in the Klamath River system: Implications for public health monitoring



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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. 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). There are challenges with both cell counts and additional laboratory work that tests the limits of available funding.

The application of molecular diagnostic tools to cyanobacteria monitoring affords several potential benefits over traditional approaches that may ultimately improve Klamath water quality monitoring by providing: faster results, lower cost, higher sample throughput, greater accuracy at low cell concentrations, and results that are not subjective. The two new tools evaluated in this study are Quantitative Polymerase Chain Reaction (QPCR) analysis and the use of CyanArrays. The goal of this study was to demonstrate 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.

Introduction

Cyanobacterial blooms have been a recurring summer feature in Upper Klamath Lake for decades, *Aphanizomenon* akinetes have 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 the lower reservoirs during the summer and fall based on Klamath Basin Monitoring Program (KBMP) data extending back to 2001. 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 these 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 throughout the Klamath River system, including *Aphanizomenon*, *Gloeotrichia*, *Planktothrix*, *Oscillatoria* and *Anabaena* (also called *Dolichospermum*); however, to date only *Microcystis* has been directly linked to toxin-production. Anatoxin-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 besides microcystin. As such, the presence of anatoxin-a strongly suggests that there is at least one other cyanotoxin-producing cyanobacterium within the Klamath River system.

The purpose of this study was to screen for the presence of cyanotoxin genes involved in the biosynthesis of anatoxin-a (ANTX), cylindrospermopsin (CYN), microcystin (MC) and saxitoxin (STX) using a gene amplification assay (CyanArray). The second objective of this study was to assess the utility of using real-

time QPCR for quantifying total and toxigenic (microcystin-producing) *Microcystis*, and to assess how each of the molecular methods compares with microscope cell counting and toxin measurements.

Hypotheses

H₁ - Molecular assays targeting the genes responsible for toxin biosynthesis are reliable surrogates for estimating the presence (via CyanArray) or concentration (via QPCR) of cyanotoxins.

H₂ - Molecular assays are more effective predictors of toxicity than microscopic cell counts because of their higher sensitivity and specificity (i.e., ability to discern toxic from nontoxic cells).

Methods

Study Sites

Samples were collected from 22 locations spanning from Upper Klamath Lake to the lower estuary (Turwar). Samples collected for this project were subsample of normal sampling that occurred 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). There are 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 located 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 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.

Regardless of the program under which they were collected, samples sent to the USEPA for toxin analysis were placed in an amber glass bottles. 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 were 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. Details on the overall water quality monitoring program are available in the 2016 study plan (PacifiCorp 2016).

Toxin Measurements

Enzyme linked immunosorbent assays (ELISA) were used to quantify total microcystin concentrations (intracellular + extracellular fraction) from 123 of the collected water samples. The reporting limit for the microcystin ELISA assay was 0.10 microgram per liter ($\mu\text{g/L}$). Analyses were completed at the EPA Region 9 laboratory following standard methods. In a few instances, based on molecular results or cell density estimates, some samples were further evaluated for the presence of indicated toxin by either ELISA (Bend Genetics) or by liquid chromatography/mass spectrometry (Green Water Laboratory). Specifically, LC-MS/MS was used to analyze three samples for anatoxin-a and ELISA was used to analyze one sample for cylindrospermopsin and saxitoxin.

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 $\pm 20\%$; only those algae believed to be alive (intact membrane/chloroplast) were counted. The detection limit is considered to be 1 cell per milliliter (cell/mL).

Molecular Assays

Upon receipt of samples by Bend Genetics, the sample temperatures were measured by infrared thermometer, then the 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 centigrade ($^{\circ}\text{C}$) or extracted immediately using a Mo Bio Powerlyzer Power Soil DNA extraction kit. QPCR was used to quantify total *Microcystis* by targeting the photopigment gene c-phycoyanin (*cpcB*) and microcystin-producing *Microcystis* was quantified by targeting the microcystin synthase E gene (*mcyE*) as previously described (Otten et al., 2015). The results provided a quantitative estimate of 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 both assays target single copy genes, each gene is equivalent to one *Microcystis* cell. The extracted DNA was also screened for the presence of cyanotoxin genes involved in the biosynthesis of anatoxin-a, cylindrospermopsin, microcystin and saxitoxin, using CyanArrays specifically designed for each toxin. These assays were applied to 123 of the 126 samples collected. In the presence of an appropriate concentration of target genes the reaction mixture changed from a pale yellow color to reddish-purple (Figure 1) and samples with higher starting DNA concentrations typically generate stronger shifts in color. CyanArray assay results provided qualitative assessments of the likely presence or absence of toxin genes in each sample. For environmental samples, the limit of detection for the assay was determined to be $\sim 2,000$ gene copies/mL based on side-by-side comparisons with QPCR data and by spiking known concentrations of control DNA into environmental DNA extracts.

Statistical Methods

Inter-method comparisons were made using log-log regression. In instances where one paired value was above the reporting limit and the other below, values below were considered to be half the reporting limit for statistical purposes. In instances where both paired values were below the reporting limits, these “double negative” data were removed from the analysis.

Results and Discussion

Cell Counts

Potentially toxigenic cyanobacteria (PTOX), a designation which includes the genera:

Anabaena/Dolichospermum, *Aphanizomenon*, *Gloeotrichia*, *Microcystis*, *Oscillatoria*, and *Planktothrix* were observed microscopically in 76/125 samples. *Microcystis* sp. was observed in 48 samples, *Dolichospermum* sp. (formerly *Anabaena*) in 21 samples, *Aphanizomenon* sp. in 49 samples, *Planktothrix/Oscillatoria* in 3 samples and *Gloeotrichia* sp. in 4 samples. *Planktothrix* and *Oscillatoria* were never abundant, peaking at concentrations of 81 and 23 cells/mL, respectively. Likewise, *Dolichospermum* concentrations were never significantly elevated, with concentrations peaking at 19,191 cells/mL. *Dolichospermum* was generally rare throughout the system with the exception of Copco and Iron Gate reservoirs, where it exceeded 10,000 cells/mL on three occasions. *Gloeotrichia* sp. was the next most abundant cyanobacterium, reaching as high as 689,765 cells/mL and exceeding 10,000 cells/mL on two other occasions. Similar to *Dolichospermum*, elevated concentrations of *Gloeotrichia* only occurred within Copco and Iron Gate reservoirs. By far the most dominant cyanobacteria throughout the system were *Aphanizomenon flos-aquae* and *Microcystis*. *Aphanizomenon* concentrations exceeded 10,000 cells/mL in 30 out of the 49 samples it was observed in (min=48, max=43,439,500 cells/mL). The Upper Klamath Lake sites contained the greatest concentrations of *Aphanizomenon*, followed by Copco Reservoir and Link Dam. The highest concentration of *Aphanizomenon* observed below Iron Gate Reservoir was 5,727 cells/mL at the I-5 Bridge site. *Microcystis* concentrations exceeded 10,000 cells/mL in 24 out of the 48 samples that it was observed in (min=27, max=144,972,763 cells/mL). Copco Reservoir contained the highest concentrations of *Microcystis*, followed by Iron Gate Reservoir and Upper Klamath Lake (specifically Howard’s Bay and Moore Park). The highest concentration of *Microcystis* occurring below Iron Gate Reservoir was 6,792 cells/mL at site KRBI.

QPCR and CyanArray

The QPCR assays quantified total *Microcystis* sp. (*cpcB*) and toxigenic *Microcystis* sp. (*mcyE*) by targeting single copy genes. Of the 126 samples analyzed, 104 samples (82.5%) contained *Microcystis* sp. concentrations above the limit of quantification (LOQ) of 100 gene copies/mL and toxigenic *Microcystis* sp. was quantified in 95 samples (75.4%). The CyanArray assays detected microcystin genes in 70 of 123 samples (56.9%), anatoxin-a genes in 24 samples (19.5%), cylindrospermopsin genes in 3 samples (2.4%) and saxitoxin genes in 1 sample (0.8%). The detection limit for these assays was ~2,000 gene copies/mL.

Cyanotoxins

Microcystin was detected in 88 of 123 samples (71.5%) by ELISA. The average concentration across all positive samples was 284 µg/L and concentrations ranged from 0.10 to 11,000 µg/L. Of the 123 samples analyzed for microcystin, 26 exceeded the Draft statewide guidelines on cyanobacteria in recreational waters (CCHAB Network 2016) warning level of 6 µg/L and 46 exceeded the caution level of 0.8 µg/L. Of the samples analyzed in this study, microcystin concentrations were consistently elevated in the Upper Klamath Lake sites and in Copco and Iron Gate Reservoir (Figure 2).

Three samples were analyzed for anatoxin-a by LC-MS/MS and all were below the reporting limit of 0.1 µg/L. Two of these samples tested positive for anatoxin-a genes via CyanArray, suggesting that the genetic results were false positives or that the limit of detection of the CyanArray was less than that for LC-MS/MS. Because 24 samples (19.5%) were positive for anatoxin-a using the CyanArrays, an anatoxin-a follow-up study is in ongoing. This effort will utilize QPCR to quantify anatoxin-a genes from all 126 samples collected and these data will be compared with the CyanArray results in order to determine the efficacy of the anatoxin-a assay. DNA sequencing will be conducted on samples verified to contain anatoxin-a genes, these data will be used to identify the specific anatoxin-a producer(s). The results of this investigation will be presented in a separate report.

Comparison of Methods

Cell counts and microcystins

All prevalent PTOX cyanobacteria (i.e., *Anabaena*, *Aphanizomenon* and *Microcystis*) exhibited a positive relationship with microcystin concentration (Figure 3). The strongest relationship (Adj. $R^2=0.83$) was between *Microcystis* cells and microcystin. Combining these data into total estimates of PTOX abundance, including those samples where toxin was measured but no cyanobacteria were observed, lowers the correlation coefficient slightly to Adj. $R^2=0.62$.

There were 20 samples with quantifiable microcystin concentrations (min=0.10, max=0.40 µg/L) when no cyanobacteria were observed microscopically. In the Klamath River system there is presently only evidence for microcystin production by the genus *Microcystis*. Microcystin production has never been observed in *Aphanizomenon flos-aquae* anywhere in the world. More regionally, the genomes of naturally occurring and cultured *Aphanizomenon flos-aquae* strains have been isolated from Upper Klamath Lake and Iron Gate Reservoir and sequenced by shotgun metagenomics and found to lack cyanotoxin genes (Driscoll, 2016). Of the other PTOX genera, *Planktothrix* is the most common producer of microcystins in North American waters and we are unaware of any reports of microcystin-producing *Dolichospermum* in North America (it has only been reported in Northern Europe and Australia). Throughout the Klamath system in 2016, *Planktothrix* was only observed twice and its cell concentration peaked at 81 cells/mL. Because it was only present twice at very low levels, it cannot explain the discrepancy between the cell counts and toxin measurements and is therefore not presented in Figure 3. *Dolichospermum* at times was abundant when microcystins were elevated, most notably in Copco Cove on 8-Jun-16 when the microcystin concentration was 3.7 µg/L and *Dolichospermum* was present at 503 cells/mL and no other cyanobacteria were observed. However, the QPCR results indicated that toxigenic *Microcystis* was

actually present in that sample at a concentration of 12,269 *mcyE* cell equivalents/mL, providing evidence that *Microcystis* was present but missed by the cell counting. An even more striking example of this comes from a sample collected from Howard's Bay Park in Upper Klamath Lake on 28-Jun-16 when the microcystin concentration was 130 µg/L but only *Aphanizomenon* was observed (43.4 million cells/mL). QPCR estimates of total *Microcystis* indicated its presence at about 97,000 cell equivalents/mL, of which about 28% were had the toxin-producing *mcyE* gene.

In this context, the identification of *Microcystis* in only 48 samples when 88 samples contained measurable concentrations of microcystin suggests that either the cell counts consistently missed potentially toxigenic *Microcystis* or that there are other microcystin-producing genera in the system. Because they were not detected by microscopy, any other microcystin-producing cyanobacteria would likely need to be benthic mat forming species. Under the assumption that *Microcystis* sp. is the sole producer of microcystin in the system, then the microscopy false negative detection rate of *Microcystis* could be as high as 49% (43 non-detects out of 88 samples). One complication in this assessment is the possibility that *Microcystis* cells may lyse and release toxin into the water column and this toxin may persist for a period of days to weeks (Schmidt et al., 2014). The inclusion of QPCR into the study design enabled us to assess the likelihood that microcystins were present extracellularly. If this were true, then samples with microcystins, but no *Microcystis*, should also test negative for *Microcystis* genes, since the turnover rate of extracellular DNA in lakes is estimated to only require about 12 hours (Paul et al., 1989). This topic is discussed in detail further below.

The cell count data suggests that when other taxa are numerically dominant, it is not uncommon for the *Microcystis* present in the samples to be underrepresented or completely missed. By comparing QPCR estimates of total *Microcystis* (*cpcB*-possessing) with microscope cell counts of *Microcystis* we get an idea of how large this error rate could be (Figure 4). *Microcystis* was detected by QPCR in 56 more samples ($n=104$) than by microscopy ($n=48$); for samples in which *Microcystis* was not observed microscopically, QPCR estimates of *Microcystis* abundance ranged from 148 to 7 million cell equivalents/mL. In contrast, there was only one sample where *Microcystis* was detected by cell counting in which QPCR failed to identify *Microcystis*, and that sample contained 81 cells/mL, which is below the reporting limit for the QPCR assay.

QPCR (*mcyE*) for estimating microcystin concentrations

QPCR estimates of microcystin genes were related to microcystin concentrations using log-log regression (Figure 5). Toxin gene abundance was found to strongly and positively correlate with microcystin concentration (Adj. $R^2=0.67$). The limit of detection for QPCR is about 100 gene copies and by comparing QPCR estimates of *mcyE* genes to microcystin concentrations has been estimated that 100 *mcyE* possessing cells/mL should correspond to about 0.04 µg/L of microcystin (Otten et al., 2015). This is less than the microcystin reporting limit of 0.10 µg/L. In order to produce 0.10 µg/L of microcystin, it is estimated that approximately 360 (± 75) toxigenic cells/mL would be required. There were 11 samples that were below the reporting limit for microcystin, but above the reporting limit for *mcyE* genes. Of these, three samples had gene concentrations below 360 *mcyE* cell equivalents/mL and the remaining eight

exceeded this threshold. All but one of the exceedances consisted of gene concentrations ranging from 756 to 5,793 *mcyE* cell equivalents/mL. The one outlier (KRBI on 8/10/16) contained 153,464 *mcyE* cell equivalents/mL and 6,792 *Microcystis* cells/mL via microscopy, but no quantifiable microcystin in the ELISA analysis. It is unclear why such a large discrepancy between toxin gene abundance and toxin concentration would exist. One plausible explanation is that the *Microcystis* population in that sample was no longer actively growing, but instead was in stationary phase which has been shown to result in a significant decrease in *mcyE* gene expression, and therefore toxin production (Kaebernick et al., 2000). Regardless of the explanation, if we consider these 8 samples to be false positives, then the false positive rate could be as high as 8.8% (8 out of 91 samples) for the QPCR assay. Note that three samples with quantifiable *mcyE* could not be compared because microcystin analysis was not completed for them.

False negatives were defined as those samples which contained a measurable amount of microcystin but in which the *mcyE* genes concentrations were below the reporting limit. There were 7 out of 88 samples that contained between 0.10 - 0.21 µg/L microcystin and less than 100 *mcyE* cell equivalents/mL. It is worth noting, however, that six of these samples contained 0.10 - 0.12 µg/L, which is essentially at the ELISA limit of detection (0.1 µg/L) and that the largest outlier (0.21 µg/L) had an estimated 76 *mcyE* cell equivalents/mL, which is near the reporting limit for the QPCR assay. Therefore, the false negative rate could be as high as 8% or as low as 1.1% if the ELISA results near the detection limit are actually false positives. Most importantly, no QPCR false negatives exceeded the caution level of 0.6 µg/L microcystin.

CyanArray for detecting microcystin genes

The efficacy of the CyanArray microcystin assay was assessed by comparing the CyanArray results (presence or absence of *mcyE* toxin genes) relative to measured microcystin concentrations or to QPCR estimates of *mcyE* gene abundance. The limit of detection for the CyanArray assay is approximately 2,000 gene copies/mL. In general, as *mcyE* concentrations increased, the probability of positive CyanArray results also increased (Figure 6). For samples containing no detectable *mcyE* genes, 19 out of 22 were negative by CyanArray, a false positive rate of 13.6%. Of the 81 samples that exceeded 2,000 *mcyE*/mL, 16 of them were negative by CyanArray, a false negative rate of 20%.

We also assessed the utility of using CyanArray for estimating cyanotoxin risks based on the relationship between *mcyE* concentration and microcystin concentration as described in Otten et al. (2015). Based on that relationship, it was estimated that 2,000 *mcy* genes should correspond to a microcystin concentration of 0.36 µg/L (± 0.08). For the 35 samples with microcystin concentrations below the reporting limit (0.10 µg/L), 32 of the CyanArray assays were negative and 3 were positive, a false positive rate of 8.6% (Figure 6A). Of the 85 samples with microcystin concentrations above the reporting limit, 22 of them were negative by CyanArray, a false negative rate of 26%. However, 17 of the 22 false negatives occurred at microcystin concentrations of 0.44 µg/L or less, which is approximately what the limit of detection is expected to be for the CyanArray assay. In this context, there were 5 samples that were falsely negative out of the 51 that exceeded 0.44 µg/L microcystin for a more accurate false negative rate of 9.8%. The microcystin concentrations in these five false negatives ranged from 3.7 to 130 µg/L. The paired QPCR data from these five samples indicated the presence of elevated *mcyE* genes, ranging from 12,269 to 3.6

million *mcyE* cell equivalents/mL. Thus, the evidence indicates that these five CyanArray samples clearly failed, although the reason why is uncertain. Reaction failure could be caused by the presence of inhibitory substances such as certain salts, metals, or humics. However, sample processing controls were run to assess for this contingency, and in all cases where false positives occurred the controls showed no sign of inhibition. Similarly, the positive QPCR results suggest that reaction contamination is unlikely because QPCR is also sensitive to these same types of reaction inhibitors.

Summary

H₁ - Molecular assays targeting the genes responsible for toxin biosynthesis are reliable surrogates for estimating the presence (via CyanArray) or concentration (via QPCR) of cyanotoxins.

Conclusion - QPCR assays targeting microcystin genes proved to be a reliable predictor (Adj. R²=0.67) of cyanotoxin concentrations spanning over five orders of magnitude. False positive and false negative rates were around 8% for the *mcyE* QPCR assay. Notably, no QPCR false negatives occurred when microcystin concentrations approached or exceeded the California caution level (0.6 µg/L). The CyanArray assays showed a positive relationship with *mcyE* concentration and had a false negative rate of about 10% in relation to microcystin concentrations above 0.44 µg/L and a false positive rate of 8.6%. The CyanArray assays were unable to accurately predict the presence of low microcystin concentrations (in the range of 0.10 - 0.40 µg/L). Most importantly, there were five negative CyanArray sample results that turned out to contain microcystin concentrations ranging from 3.7 to 130 µg/L. These data suggest that for public health purposes, QPCR offers a superior assessment of microcystin risk. The CyanArray may be useful as an additional component of a monitoring program, for example to screen for microcystin genes when elevated *Microcystis* cell concentrations are observed; however, public health decisions should not be based on the CyanArray results alone.

H₂ - Molecular assays are more effective predictors of toxicity than microscopic cell counts because of their higher sensitivity and specificity.

Conclusion - Microcystin was quantified in 88 out of 123 samples, additionally there were 43 samples with quantifiable microcystins in which *Microcystis* was not observed by microscope cell counting. QPCR identified *Microcystis* in 104 out of 126 samples, 95 of which also had *mcyE* gene concentrations above the reporting limit. The QPCR results identified *mcyE* genes in 35 of the 43 samples that contained microcystins but had no *Microcystis* observed microscopically. Therefore, the false negative rate for the cell counts is estimated to be approximately 40% (35 out of 88 samples). In addition to this high false negative rate, several of these samples contained elevated microcystin concentrations, with values ranging from 0.10 to 130 µg/L. Because of this high false negative rate and the potential for high toxin samples to be missed by microscopy, QPCR was found to be the most reliable method for assessing cyanotoxin risks based on cell abundances where the cell abundances are either direct cell counts or genetic cell equivalents. Under this framework, QPCR results indicating the presence of toxin genes could be followed-up by directly testing for the relevant toxin(s).

Proposed Future Directions

The large discrepancy between QPCR assessments of *Microcystis* and the microscopy results could only be attributed to the presence of other numerically dominant co-occurring cyanobacteria in a few of the samples in question. Several of the samples with microcystin and *mcyE* genes where *Microcystis* was not observed by microscopy contained little to no cyanobacteria. Based on this, we hypothesize that *Microcystis* cells may at times exhibit a unicellular morphology (i.e., non-colonial form) that is generally indistinguishable from other picoplanktonic phytoplankton. It is the colonial morphology that primarily distinguishes *Microcystis* from other coccoidal, non-colony forming algae such as *Synechocystis* sp. or *Chroococcus* sp. It is not unusual for *Microcystis* colonies to disaggregate into a unicellular morphology upon placement into culture conditions (Zhang et al., 2007; Sun et al., 2016). To our knowledge no study has ever assessed what percentage of *Microcystis* cells occur as colonies versus individual cells under natural conditions, although one study did use size fractionation to estimate the percentage of the *Microcystis* population comprised of colonies less than 50 μm , 50-100 μm and greater than 100 μm (Kurmayer et al., 2003). In that study, the authors observed that the largest size class comprised the smallest fraction of the total cell number. In cell counting, there is a natural tendency to preferentially count larger celled organisms or colonies. Because of this unless a specific objective of the study is to characterize the picoplankton, then they tend to be significantly underestimated (Paerl, 1978). In the Klamath River public health samples, only potentially toxigenic cyanobacteria were enumerated, not unicellular cyanobacteria that we hypothesize may at times include a substantial proportion of the total *Microcystis* population. This question of colonial versus unicellular contributions to *Microcystis* total abundance could be easily addressed using the molecular tools assessed in this study. For example, samples could be size fractionated by first filtering reservoir water through a larger pore size filter (greater than 10 μm) which would retain *Microcystis* colonies while still passing individual cells through the filter; the average diameter of a *Microcystis* cell is about 5 μm . The flow through (filtrate) could then be refiltered onto the normal 1.2 μm pore size filters that we use for routine monitoring. Both could be quantified by QPCR and the proportion of the population present in a unicellular form versus a colonial form could be assessed.

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Tables and 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	

Figure 1. Photograph of CyanArray colorimetric detection of toxin genes, higher concentrations of toxin genes illicit a stronger color change.

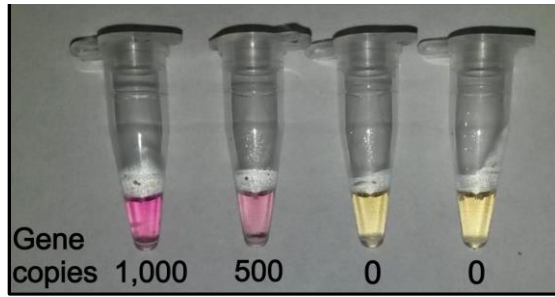


Figure 2. Heat map displaying LOG₁₀ transformed microcystin concentrations observed throughout the Klamath River system in 2016. The sites are ordered from Upper Klamath Lake to the lower estuary (Turwar). See Table 1 for site codes.

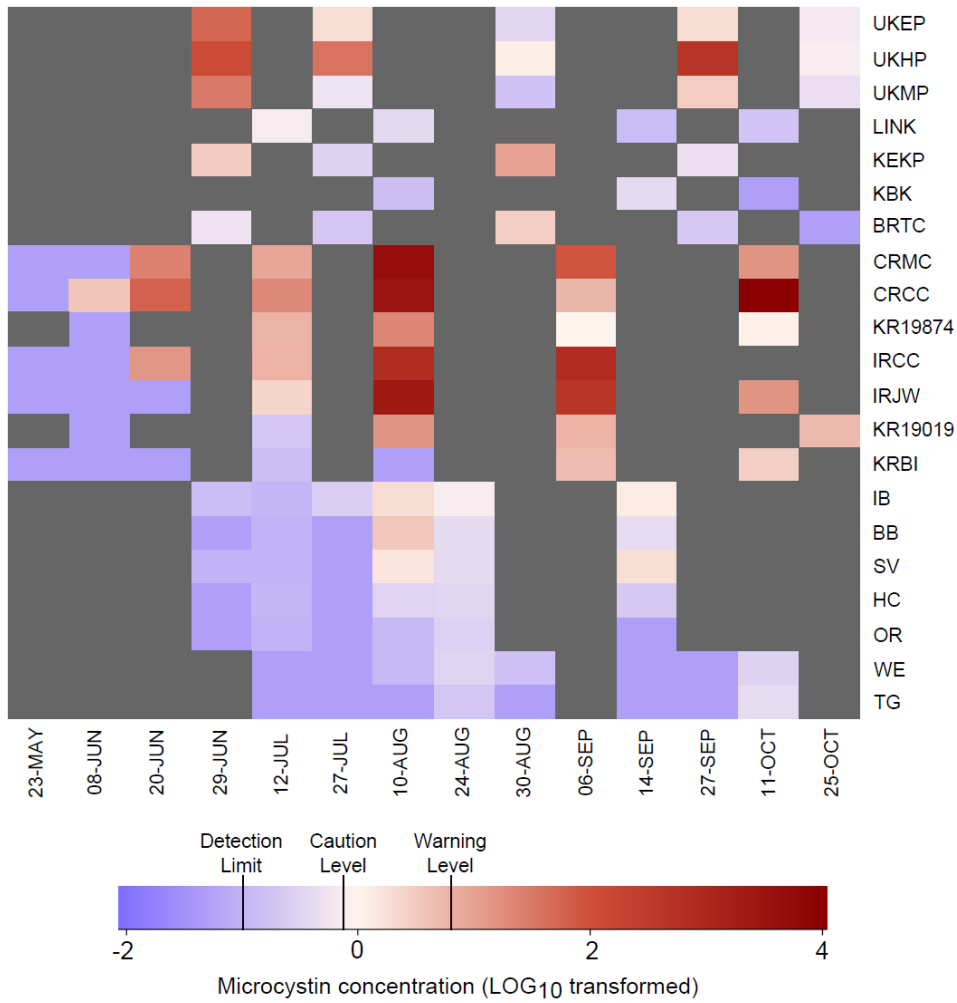


Figure 3. Relationship between cell counts of *Microcystis* (A), *Aphanizomenon* (B), *Anabaena* (C), and (D) potentially toxigenic cyanobacteria (PTOX) and microcystin concentration

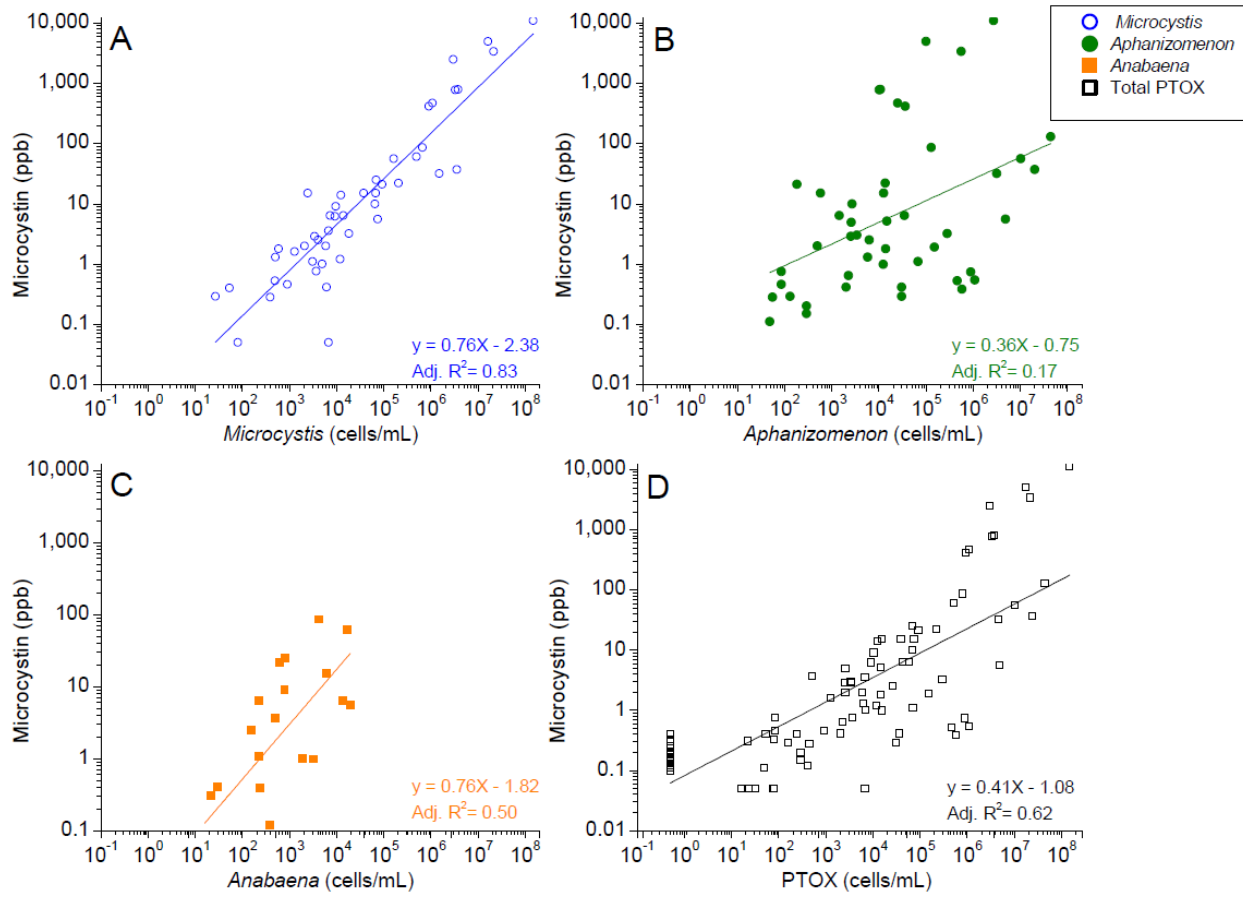


Figure 4. Comparison of microscope estimates of *Microcystis* abundance with QPCR estimates of total *Microcystis* (*cpcB*-possessing).

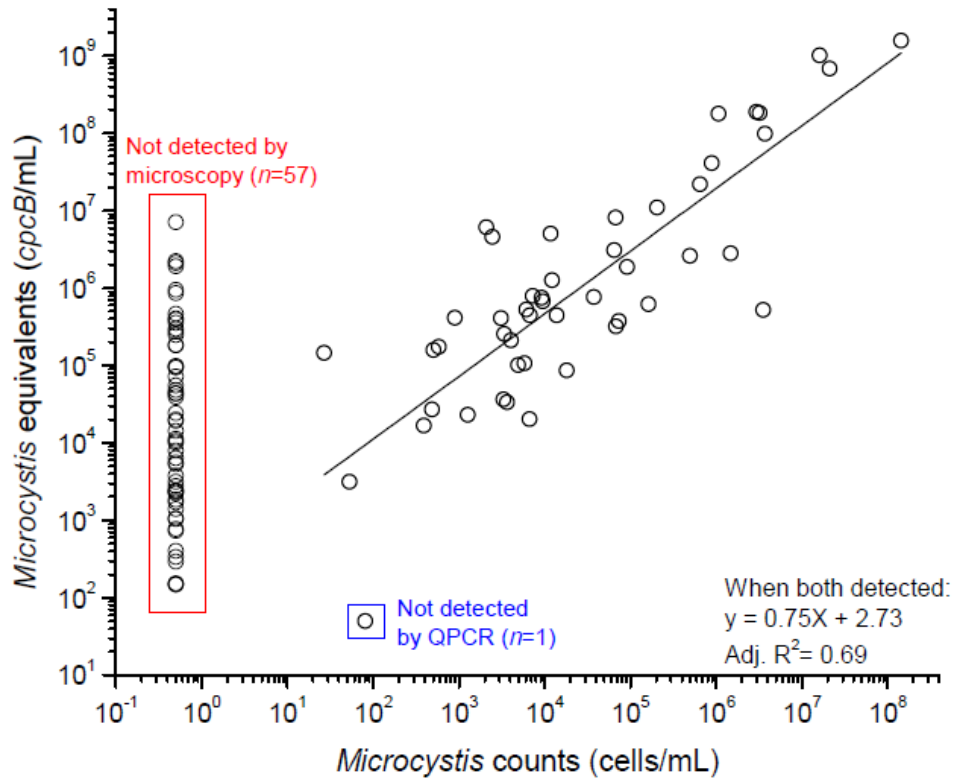


Figure 5. Relationship between microcystin-producing *Microcystis* (*mcyE*) and microcystin concentration. Concentrations below the reporting limits were set at half the RL (0.05 µg/L for microcystin and 50 *mcyE* cell equivalents/mL for QPCR).

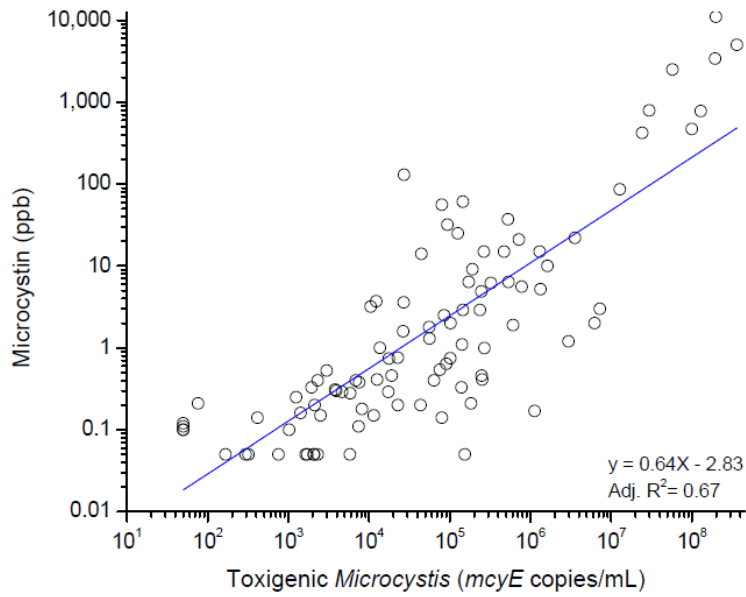
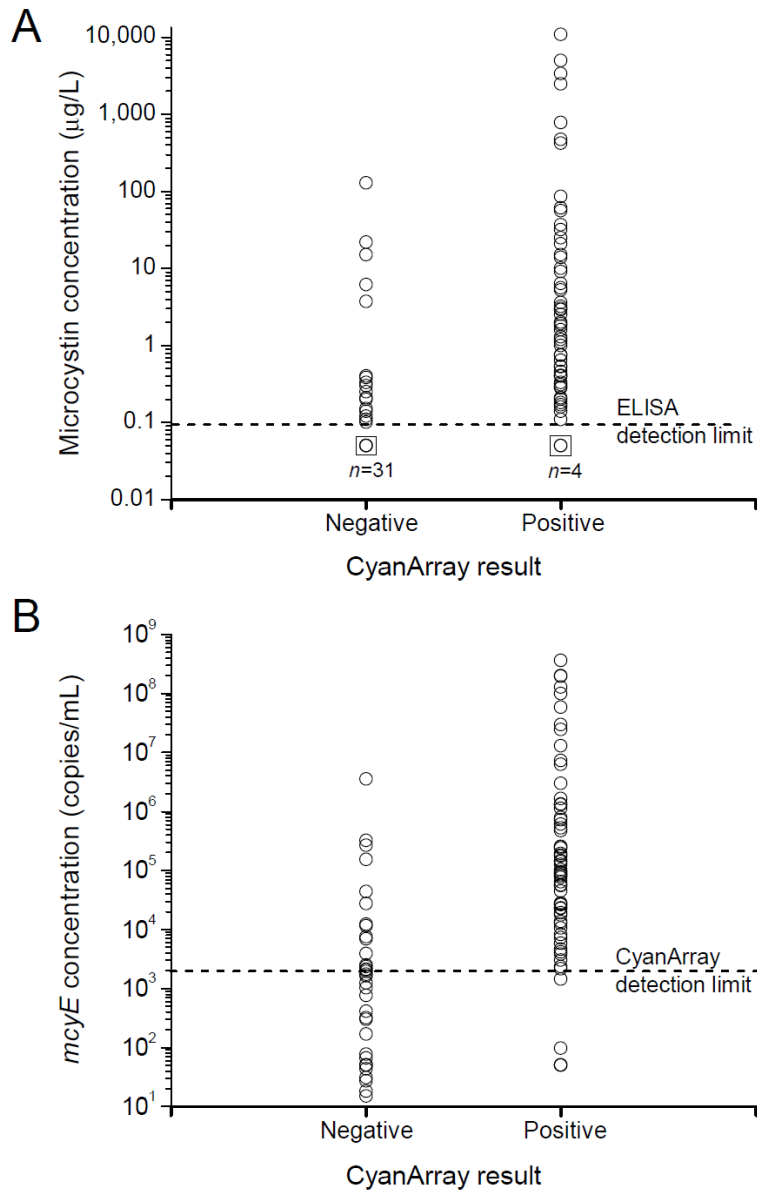


Figure 6. Comparisons of microcystin concentration (A) or *mcyE* gene concentration (B) relative to CyanArray microcystin assay results



APPENDICES

Appendix 1. Potentially toxic cyanobacterial (PTOX) cell concentrations (cells/mL) determined by microscopic counting

Sample ID	Sample Date	Site ID	Location	Total Density (cells/mL)	MIC	DOL	APHA	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			

Where: MIC = *Microcystis*, DOL = *Dolichospermum*, APHA = *Aphanizomenon*, PLANK = *Planktothrix*, OSC = *Oscillatoria*, GLO = *Gloeotrichia*

Appendix 2. Microcystin concentrations, QPCR results (total *Microcystis* = *cpcB* gene copies/mL, toxigenic *Microcystis* = *mcyE* gene copies/mL) and CyanArray (Cray) toxin gene results (anatoxin-a, cylindrospermopsin, microcystin and saxitoxin).

Sample ID	Sample Date	Location	Microcystin (µg/L)	QPCR - <i>cpcB</i> (copies/mL)	QPCR - <i>mcyE</i> (copies/mL)	Cray ANTX	Cray CYN	Cray MC	Cray STX
KR16800	5/23/16	CRMC	ND	1,059	296	NEG	NEG	NEG	NEG
KR16801	5/23/16	CRCC	ND	6,336	321	NEG	NEG	NEG	NEG
KR16802	5/23/16	IRCC	ND	72	18	NEG	NEG	NEG	NEG
KR16803	5/23/16	IRJW	ND	27	ND	NEG	NEG	NEG	NEG
KR16804	5/23/16	KRBI	ND	18	ND	NEG	NEG	NEG	NEG
KR16090	6/8/16	KRBI	ND	153	167	NEG	NEG	NEG	NEG
KR16091	6/8/16	Iron Gate Log Boom	ND	18	ND	NEG	NEG	NEG	NEG
KR16096	6/8/16	Copco Log Boom	ND	1,704	1,646	NEG	NEG	NEG	NEG
KR16805	6/8/16	CRMC	ND	89	31	NEG	NEG	NEG	NEG
KR16806	6/8/16	CRCC	3.70	2,208,267	12,269	NEG	NEG	NEG	NEG
KR16807	6/8/16	IRCC	ND	1,036	43	POS	NEG	NEG	NEG
KR16808	6/8/16	IRJW	ND	ND	ND	NEG	NEG	NEG	NEG
KR16809	6/8/16	KRBI	ND	294	66	NEG	NEG	NEG	NEG
KR16105	6/20/16	KRBI	ND	11,237	756	NEG	NEG	NEG	NEG
KR16810	6/20/16	CRMC	25.00	320,704	125,898	NEG	NEG	POS	NEG
KR16811	6/20/16	CRCC	61.00	2,594,238	145,536	NEG	NEG	POS	NEG
KR16812	6/20/16	IRCC	14.00	1,258,047	44,736	NEG	NEG	POS	NEG
KR16813	6/20/16	IRJW	1.00	100,869	13,676	NEG	NEG	POS	NEG
KR16814	6/20/16	KRBI	ND	14,233	2,091	NEG	NEG	NEG	NEG
2016KHSA-35	6/21/16	Link Dam	Not run	36,449	7,191	POS	NEG	POS	NEG
2016KHSA-38	6/21/16	Keno Dam	Not run	54,994	4,117	POS	NEG	POS	NEG
BRTC16002	6/28/16	Boyle - Topsy Camp	0.53	27,134	2,997	NEG	NEG	POS	NEG
KEKP16002	6/28/16	Keno Park	3.20	85,926	10,515	NEG	NEG	POS	NEG
UKEP16002	6/28/16	Eagle Ridge	56.00	616,277	80,059	NEG	NEG	POS	NEG
UKHP16002	6/28/16	Howard's Bay	130.00	96,748	27,227	NEG	NEG	NEG	NEG

Sample ID	Sample Date	Location	Microcystin (µg/L)	QPCR - <i>cpcB</i> (copies/mL)	QPCR - <i>mcyE</i> (copies/mL)	Cray ANTX	Cray CYN	Cray MC	Cray STX
UKMP16002	6/28/16	Moore Park	32.00	2,801,424	93,574	POS	NEG	POS	NEG
BB062916-SG	6/29/16	Brown Bear	ND	10	ND	NEG	NEG	NEG	NEG
HC062916-SG	6/29/16	Happy Camp	ND	ND	ND	NEG	NEG	NEG	NEG
IB062916-SG	6/29/16	I-5 Bridge	0.14	1,397	414	NEG	NEG	NEG	NEG
OR062916-SG	6/29/16	Orleans	ND	ND	ND	POS	NEG	NEG	NEG
SV062916-SG	6/29/16	Seiad Valley	0.10	36	18	NEG	NEG	NEG	NEG
2016KHSA-40	7/12/16	Link Dam	0.74	71,936	17,610	NEG	NEG	POS	NEG
2016KHSA-44	7/12/16	Keno Dam	Not run	461,768	27,701	POS	NEG	POS	NEG
KR16114	7/12/16	Iron Gate Log Boom	0.20	93,600	43,614	NEG	NEG	NEG	NEG
KR16119	7/12/16	Copco Log Boom	6.20	747,374	323,488	NEG	NEG	NEG	NEG
KR16815	7/12/16	CRMC	9.10	673,577	191,596	NEG	NEG	POS	NEG
KR16816	7/12/16	CRCC	21.00	1,876,816	720,067	NEG	NEG	POS	NEG
KR16817	7/12/16	IRCC	6.40	444,234	172,338	NEG	NEG	POS	NEG
KR16818	7/12/16	IRJW	2.50	210,018	84,965	NEG	NEG	POS	NEG
KR16819	7/12/16	KRBI	0.15	24,295	11,502	NEG	NEG	NEG	NEG
TG071216-SG	7/12/16	Turwar	ND	ND	ND	NEG	NEG	NEG	NEG
BB071316-SG	7/13/16	Brown Bear	0.10	2,754	1,027	POS	NEG	NEG	NEG
HC071316-SG	7/13/16	Happy Camp	0.11	2,441	ND	NEG	NEG	POS	NEG
IB071316-SG	7/13/16	I-5 Bridge	0.11	20,110	7,448	NEG	NEG	NEG	NEG
OR071316-SG	7/13/16	Orleans	0.10	ND	ND	NEG	NEG	NEG	NEG
SV071316-SG	7/13/16	Seiad Valley	0.10	728	ND	POS	NEG	NEG	NEG
WE071316-SG	7/13/16	Weitchpec	ND	ND	ND	NEG	NEG	NEG	NEG
BRTC16004	7/26/16	Boyle - Topsy Camp	0.20	43,129	22,742	NEG	NEG	POS	NEG
KEKP16004	7/26/16	Keno Park	0.29	7,922	4,649	NEG	NEG	POS	NEG
TG072616-SG	7/26/16	Turwar	ND	ND	ND	NEG	NEG	NEG	NEG
UKEP16004	7/26/16	Eagle Ridge	1.90	949,251	609,724	NEG	NEG	POS	NEG
UKHP16004	7/26/16	Howard's Bay	37.00	518,867	528,589	NEG	NEG	POS	NEG
UKMP16004	7/26/16	Moore Park	0.54	43,515	75,487	NEG	NEG	POS	NEG

Sample ID	Sample Date	Location	Microcystin (µg/L)	QPCR - <i>cpcB</i> (copies/mL)	QPCR - <i>mcyE</i> (copies/mL)	Cray ANTX	Cray CYN	Cray MC	Cray STX
BB072716-SG	7/27/16	Brown Bear	ND	335	96	NEG	NEG	POS	NEG
HC072716-SG	7/27/16	Happy Camp	ND	18	ND	NEG	NEG	POS	NEG
IB072716-SG	7/27/16	I-5 Bridge	0.25	2,406	1,247	NEG	NEG	NEG	NEG
OR072716-SG	7/27/16	Orleans	ND	ND	ND	NEG	NEG	NEG	NEG
SV072716-SG	7/27/16	Seiad Valley	ND	ND	ND	NEG	NEG	NEG	NEG
WE072716-SG	7/27/16	Weitchpec	ND	ND	15	NEG	NEG	NEG	NEG
2016KHSA-51	8/9/16	Link Dam	0.38	148	7,540	NEG	NEG	NEG	NEG
2016KHSA-55	8/9/16	Klamath below Keno	0.15	5,599	2,501	NEG	NEG	NEG	NEG
TG080916-SG	8/9/16	Turwar	ND	ND	ND	POS	NEG	NEG	NEG
BB081016-SG	8/10/16	Brown Bear	3.60	20,195	27,036	NEG	NEG	POS	NEG
HC081016-SG	8/10/16	Happy Camp	0.30	3,782	3,927	NEG	NEG	NEG	NEG
IB081016-SG	8/10/16	I-5 Bridge	2.00	106,525	102,281	NEG	NEG	POS	NEG
KR16137	8/10/16	Iron Gate Log Boom	15.00	768,055	264,543	NEG	NEG	NEG	NEG
KR16825	8/10/16	CRMC	5,000.00	1,010,749,563	361,196,489	NEG	POS	POS	POS
KR16827	8/10/16	IRCC	790.00	98,824,670	29,591,878	NEG	NEG	POS	NEG
KR16828	8/10/16	IRJW	2,500.00	188,593,211	57,837,348	NEG	NEG	POS	NEG
KR16829	8/10/16	KRBI	ND	445,106	153,464	NEG	NEG	NEG	NEG
OR081016-SG	8/10/16	Orleans	0.12	ND	ND	NEG	NEG	NEG	NEG
SV081016-SG	8/10/16	Seiad Valley	1.60	23,028	26,465	NEG	NEG	POS	NEG
WE081016-SG	8/10/16	Weitchpec	0.12	ND	ND	NEG	NEG	NEG	NEG
KR16142	8/11/16	Copco Log Boom	22.00	10,998,062	3,558,107	NEG	NEG	NEG	NEG
KR16826	8/11/16	CRCC	3,400.00	679,757,552	194,612,685	POS	POS	POS	NEG
TG082316-SG	8/23/16	Turwar	0.20	2,341	2,129	POS	NEG	POS	NEG
BB082416-SG	8/24/16	Brown Bear	0.40	3,131	2,314	NEG	NEG	POS	NEG
HC082416-SG	8/24/16	Happy Camp	0.33	2,246	1,942	POS	NEG	NEG	NEG
IB082416-SG	8/24/16	I-5 Bridge	0.76	33,600	22,883	NEG	NEG	POS	NEG
OR082416-SG	8/24/16	Orleans	0.28	16,792	5,834	POS	NEG	POS	NEG
SV082416-SG	8/24/16	Seiad Valley	0.40	10,052	6,878	POS	NEG	NEG	NEG

Sample ID	Sample Date	Location	Microcystin (µg/L)	QPCR - <i>cpcB</i> (copies/mL)	QPCR - <i>mcyE</i> (copies/mL)	Cray ANTX	Cray CYN	Cray MC	Cray STX
WE082416-SG	8/24/16	Weitchpec	0.31	5,295	3,808	POS	NEG	POS	NEG
BRTC16006	8/30/16	Boyle - Topsy Camp	2.90	256,633	145,768	POS	NEG	POS	NEG
KEKP16006	8/30/16	Keno Park	10.00	3,082,722	1,635,340	NEG	NEG	POS	NEG
TG083016-SG	8/30/16	Turwar	ND	3,132	2,078	POS	NEG	NEG	NEG
UKEP16006	8/30/16	Eagle Ridge	0.33	247,745	139,466	POS	NEG	POS	NEG
UKHP16006	8/30/16	Howard's Bay	1.20	5,060,743	2,958,188	NEG	NEG	POS	NEG
UKMP16006	8/30/16	Moore Park	0.17	1,912,652	1,125,739	NEG	NEG	POS	NEG
WE083116-SG	8/30/16	Weitchpec	0.16	1,852	1,420	NEG	NEG	POS	NEG
KR16160	9/6/16	Iron Gate Log Boom	6.40	789,307	537,035	Not Run	Not Run	Not Run	Not Run
KR16835	9/6/16	CRMC	86.00	21,816,130	12,817,609	NEG	NEG	POS	NEG
KR16836	9/6/16	CRCC	5.60	373,974	780,823	POS	NEG	POS	NEG
KR16837	9/6/16	IRCC	780.00	182,740,012	128,921,907	NEG	NEG	POS	NEG
KR16838	9/6/16	IRJW	420.00	41,037,218	24,236,096	NEG	NEG	POS	NEG
KR16165	9/7/16	Copco Log Boom	0.99	357,231	268,705	Not Run	Not Run	Not Run	Not Run
KR16839	9/7/16	KRBI	4.90	405,934	246,340	Not Run	Not Run	Not Run	Not Run
2016KHSA-62	9/13/16	Link Dam	0.14	271,389	80,181	NEG	NEG	POS	NEG
2016KHSA-66	9/13/16	Klamath below Keno	0.40	297,360	63,976	POS	NEG	POS	NEG
TG091316-SG	9/13/16	Turwar	ND	403	ND	NEG	NEG	NEG	NEG
BB091416-SG	9/14/16	Brown Bear	0.41	525,905	252,780	NEG	NEG	POS	NEG
HC091416-SG	9/14/16	Happy Camp	0.21	1,062	76	POS	NEG	NEG	NEG
IB091416-SG	9/14/16	I-5 Bridge	1.30	158,763	56,286	NEG	NEG	POS	NEG
OR091416-SG	9/14/16	Orleans	ND	19,155	1,719	NEG	NEG	NEG	NEG
SV091416-SG	9/14/16	Seiad Valley	1.80	174,980	55,642	NEG	NEG	POS	NEG
WE091416-SG	9/14/16	Weitchpec	ND	770	27	NEG	NEG	NEG	NEG
BRTC16008	9/27/16	Boyle - Topsy Camp	0.21	183,299	183,090	NEG	NEG	POS	NEG
KEKP16008	9/27/16	Keno Park	0.46	412,174	248,646	NEG	NEG	POS	NEG
TG092716-SG	9/27/16	Turwar	ND	150	ND	NEG	NEG	NEG	NEG
UKEP16008	9/27/16	Eagle Ridge	2.00	6,100,445	6,237,038	POS	NEG	POS	NEG

Sample ID	Sample Date	Location	Microcystin (µg/L)	QPCR - <i>cpcB</i> (copies/mL)	QPCR - <i>mcyE</i> (copies/mL)	Cray ANTX	Cray CYN	Cray MC	Cray STX
UKHP16008	9/27/16	Howard's Bay	470.00	177,872,199	99,907,945	NEG	NEG	POS	NEG
UKMP16008	9/27/16	Moore Park	3.00	7,084,999	7,240,034	NEG	NEG	POS	NEG
WE092816-SG	9/28/16	Weitchpec	ND	ND	ND	NEG	POS	POS	NEG
2016KHSA-73	10/11/16	Link Dam	0.18	39,109	8,224	NEG	NEG	POS	NEG
2016KHSA-77	10/11/16	Klamath below Keno	ND	48,078	5,793	NEG	NEG	POS	NEG
KR16188	10/11/16	Copco Log Boom	1.10	410,007	141,786	NEG	NEG	POS	NEG
KR16845	10/11/16	CRMC	15.00	8,146,848	472,802	NEG	NEG	POS	NEG
KR16846	10/11/16	CRCC	11,000.00	1,566,148,491	198,632,925	NEG	NEG	POS	NEG
KR16848	10/11/16	IRJW	15.00	4,556,398	1,306,309	NEG	NEG	POS	NEG
KR16849	10/11/16	KRBI	2.90	862,454	237,762	NEG	NEG	POS	NEG
TG101116-SG	10/11/16	Turwar	0.41	181,901	12,612	NEG	NEG	POS	NEG
WE101216-SG	10/12/16	Weitchpec	0.29	145,942	17,375	POS	NEG	POS	NEG
BRTC16010	10/25/16	Boyle - Topsy Camp	ND	10,633	2,315	NEG	NEG	NEG	NEG
KR16183	10/25/16	Iron Gate Log Boom	5.20	2,106,740	1,339,576	NEG	NEG	POS	NEG
UKEP16010	10/25/16	Eagle Ridge	0.64	401,789	90,668	NEG	NEG	POS	NEG
UKHP16010	10/25/16	Howard's Bay	0.75	393,062	101,870	NEG	NEG	POS	NEG
UKMP16010	10/25/16	Moore Park	0.46	98,378	19,122	POS	NEG	POS	NEG