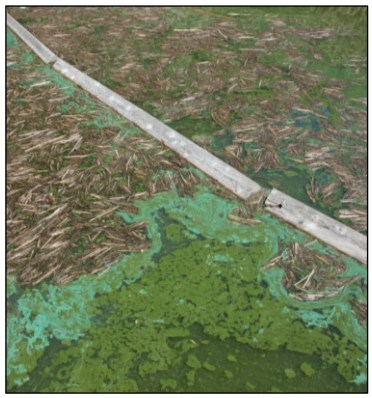
Toxigenic Cyanobacterial Trends in the Middle Klamath River, 2005-2016

Laurel Genzoli and Jacob Kann


Prepared by

Aquatic Ecosystem Sciences LLC

for

Karuk Department of Natural Resources

Toxic Cyanobacteria Trends in the Middle Klamath River, 2005-2016

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EXECUTIVE SUMMARY

The Klamath River experiences harmful algal blooms (HABs) dominated by the cyanobacteria *Microcystis aeruginosa*. Although dense *Microcystis* blooms and associated microcystin toxins originate in the lacustrine waters of the Copco and Iron Gate impoundments, cyanobacterial cells and toxin are transported downstream as far as the Klamath River Estuary, with *Microcystis* cell density and microcystin toxin levels in the middle and lower Klamath River consistently exceeding World Health Organization and California public health thresholds (e.g., SWRCB 2010; CCHAB 2016).

As a result of the public health risks associated with high concentrations of *Microcystis* and associated microcystin toxin in the Klamath River system, a public health sampling program for HABs was implemented to inform public health postings for river and reservoir safety. The Klamath River HAB monitoring program consists of grab samples taken approximately weekly once the toxin or potentially toxigenic cyanobacteria have been measured in the river, generally from June through October, and these samples supplement algae and toxin samples taken as part of a monthly monitoring program. Samples analyzed in this study were collected longitudinally beginning in the source reservoirs and extending downstream on the Klamath River from below Iron Gate Dam to Orleans, CA.

Twelve years of monitoring for planktonic cyanobacteria and associated microcystin toxin has provided a rich source of data in which to analyze spatial and temporal trends, the effectiveness of current monitoring techniques, relationships between cell densities and toxins, and the history of public health exceedances. We compiled data from public health and baseline sampling programs collected by Pacific Corp and the Karuk Department of Natural Resources from above Copco Reservoir to Orleans, CA. We assessed variability in cell and toxin data between duplicate samples and sample types. In addition, we assessed spatial and temporal trends in cyanobacteria and associated toxin, as well as the relationships between absolute cell densities and microcystin toxin. Finally, we calculated annual public health exceedances of cells and toxins, allowing for seasonal and inner-annual comparisons of public health exceedances.

Microcystis and associated microcystin toxins were present seasonally at levels above public health thresholds in the Klamath River below Iron Gate Dam during every year of this study (2005-2016). *Microcystis* was elevated in the Klamath River from July to November, and public health thresholds were most often exceeded in August, September, and October. Although there were consistent exceedances of *Microcystis* and microcystin toxin at sites from below Iron Gate Dam to Orleans, there was a general downstream decline in concentrations of toxigenic algae and toxins. Mean concentration of *Microcystis* at Orleans was 15% of the concentration at the Klamath River Below Iron Gate, although the number of samples that exceeded level II public health thresholds (5000 cells/mL) at Orleans declined by 50% from the number of exceedances below Iron Gate.

Increased concentrations of *Microcystis* generally resulted in increased concentrations of the cyano-toxin, microcystin. When *Microcystis* cell densities were low, microcystin toxin was always low in the river, but when *Microcystis* cell densities were high, microcystin levels ranged from low to high. Variation in the relationship between microcystin toxin and cell densities (toxin-to-cell ratios or cell quota) occurred both among years and within seasons.

Notwithstanding genetic shifts, which are the likely cause of the cell quota variability, cell counts of toxigenic cyanobacteria continue to remain a strong line of evidence in indicating possible public health risk.

Results from analyses of the variation in cell densities and toxins due to sampling and lab procedures, as well as the natural variation in the river, were intended to inform future sampling efforts and public health notifications. These analyses showed that microcystin toxin samples were more accurate than cell counts, although multiple types of samples are helpful in confirming the presence of bloom conditions that result in a public health risk. Cell density and toxin concentrations in public health shoreline grab samples tended to co-vary with the open-channel baseline samples. However, shoreline samples were higher in general, and some shoreline samples showed much higher concentrations reflecting increased *Microcystis* in edge areas where entrapped cells concentrate.

Microcystis and associated toxins at times showed high variation within a single day, with cell densities varying from as much as 50,000 to 100,000 cells/mL, and microcystin from less than 1 to over 20 µg/L in a 24-h period. These changes were generally progressive throughout the day (versus spiking up and down) and daily maximum cyanobacterial periods at Klamath River below Iron Gate Dam and at Seiad Valley were most common during night and early morning hours, although this pattern did not always occur and occurred with variable strength. Large within-day changes in cyanobacteria in the river below Iron Gate Dam are likely due to daily changes in algae buoyancy in the reservoir, which is then reflected in vertical distribution of cells relative to the Iron Gate intake prior to transport down river. Further study of the daily patterns in cyanobacteria density in relation to reservoir dynamics and use of real-time phycocyanin monitors will help to better understand the observed short-term temporal shift in *Microcystis* levels. Further, public health samples should be collected during the morning hours, generally prior to 11:00 am, after which values tend to decline. Posting notices should acknowledge the possible short-term variation in cell and toxin concentrations.

Twelve years of sampling for toxigenic *Microcystis* in the Klamath River revealed general trends and deviations from expected patterns. Identifying the hotspots for algal exceedances in both space and time will inform future monitoring and public health warnings. Understanding the relationship between cell densities and toxin concentrations, as well as the variation in these relationships lays a platform to ask questions about the driving forces behind toxin levels. It is important to note that patterns highlighted in this report reflect general patterns observed between 2005 and 2016, and given the observed variability in spatial-temporal dynamics, as well as the potential for environmental controls to alter the expression of toxic vs. non-toxic *Microcystis* strains, it is important that future public health decisions be based on continued monitoring.

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* The electronic appendix is embedded as an attachment in the Portable Document Format (PDF) version of this report. To access the attachments, open the report in Adobe Acrobat and then click the paper clip icon to open the Attachments panel.

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1 INTRODUCTION

1.1 DESCRIPTION OF STUDY AREA

The Klamath River is one of the major salmon spawning and rearing rivers of the Western United States. Its uppermost tributaries originate in Southern Oregon and drain into Upper Klamath Lake, the Link River and Lake Ewauna, where the Klamath River begins. From this point, the river flows through a series of irrigation and hydroelectric impoundments, including Keno, J.C. Boyle, Copco (No. 1 and No. 2), and Iron Gate Reservoirs. Below Iron Gate Dam, the river flows 190 miles to the Pacific Ocean, mostly through a confined, bedrock canyon. The climate is Mediterranean, with cool, wet winters featuring rainfall at lower elevations and snow at higher elevations, and hot, dry summers that are moderated in downstream reaches by a cooling maritime influence. High winter and spring discharges, exceeding 100,000 ft^3/s every one to two years, are derived from heavy rain and snowmelt floods from tributaries below Iron Gate Dam. Summer and early autumn flows are low and these flows are primarily from Iron Gate Dam, with additional flows coming from the regulated Trinity River. Although sampling for cyanobacteria is conducted throughout the Klamath River Watershed, the geographical focus area of this report was from Copco Reservoir to Orleans, CA (River miles 203 to 59).

1.2 BACKGROUND

1.2.1 TOXIGENIC CYANOBACTERIA IN THE KLAMATH RIVER

Cyanobacterial harmful algal blooms (cyanoHABs) and cyanotoxins are a worldwide problem. Through exposure routes of recreational contact including ingestion or inhalation (e.g., Backer et al. 2010), contaminated drinking water (Jetoo et al. 2015; Backer et al. 2015),food crops (Miller and Russel 2017; Lee et al. 2017), and consumption of fish or shellfish (e.g., Ibelings and Chorus 2007; Hardy et al. 2015; Preece et al. 2015), cyanobacteria have been implicated in numerous human and animal illnesses (e.g., Loftin et al. 2016). CyanoHABs in the middle Klamath River are well documented, with the Klamath River from Copco No. 1 Reservoir (RM 203) to Iron Gate Dam (RM 190) listed as impaired for cyanobacterial toxicity (USEPA 2008). In addition, targets for *Microcystis* cell density and associated hepatotoxin concentration have been developed by the California North Coast Regional Water Board (NCRWQCB 2010) and were approved by the US Environmental Protection Agency pursuant to Clean Water Act (CWA) Section 303(d)(2).

The primary species responsible for the Klamath River toxic blooms, *Microcystis aeruginosa*, consistently produces cell densities and microcystin toxin[1] levels that exceed public health guideline levels both in Copco and Iron Gate reservoirs (e.g., Jacoby and Kann 2007, Kann and Corum 2009, Raymond 2010), and high cell and toxin densities are transported to the Klamath River downstream of the reservoirs (e.g., Otten et al. 2015; Kann and Bowman 2012, Kann and Corum 2009; Fetcho 2011). Studies have also shown that bioaccumulation of microcystin has occurred in a variety of Klamath River fish species and freshwater mussels even

[1] Microcystin is a potent hepatotoxin capable of causing death or severe liver damage (e.g., OEHHA 2012).

when ambient microcystin concentrations were near or below detection in the lower river (Kann et al. 2010, Kann et al. 2013).

Although dense *Microcystis* blooms primarily originate in the lacustrine waters of the Copco and Iron Gate impoundments, cyanobacterial cells and associated toxins originating in the impoundments are transported downstream as far as the Klamath River estuary (Otten et al. 2015). *Microcystis* cell density and microcystin toxin levels in these downstream reaches have consistently exceeded World Health Organization, USEPA, and California public health thresholds (SWRCB 2010; CCHAB 2016; USEPA 2016).

1.2.2 KLAMATH RIVER PUBLIC HEALTH THRESHOLDS

Klamath River Public heath thresholds for microcystin toxin and *Microcystis* cell density evaluated in this report are from the Karuk Tribe and are based on action levels described in OEHHA (2012) and expanded upon by Kann (2014). These tribal public health thresholds consist of a Level I Health Advisory Warning at 0.8 µg/L microcystin and 1000 cells/mL *Microcystis*, and a Level II Health Advisory Warning at 4.0 µg/L microcystin and 5000 cells/mL *Microcystis.* The state of California provides public health posting levels consisting of a cautionary posting at 0.8 µg/L microcystin and 4000 cells/mL *Microcystis,* and Tier I Warning and Tier II Danger postings at 6.0 µg/L and 20 µg/L microcystin, respectively (CCHAB 2016). In addition, the Environmental Protection Agency recently developed draft recreational ambient water quality criterion of 4 µg/L for microcystin (USEPA 2016), which corresponds to the Karuk Tribe Level II warning.

1.2.3 PUBLIC HEALTH MONITORING FOR KLAMATH RIVER HABs

As a result of the public health risks associated with high concentrations of *Microcystis* and associated microcystin toxin in the Klamath River, a variety of entities, including the Bureau of Reclamation, PacifiCorp, and the Karuk and Yurok Tribes participate in a public health sampling program for cyanoHABs to inform public health postings for river and reservoir safety[2]. The program began in 2005 after initial discovery of the toxic blooms in 2004 (Kann 2005). Initially, the focus was on collecting surface water samples in Copco and Iron Gate reservoirs at public access locations, and adding microcystin toxin analysis to the baseline sampling in the Klamath River below Iron Gate Dam. Weekly public health monitoring was implemented in 2009 in the Klamath River from below Iron Gate Dam to the estuary during late summer and autumn, when microcystin is passed from Iron Gate Reservoir into the Klamath River. The river cyanoHAB monitoring program consists of grab samples taken approximately weekly once toxin or potentially toxigenic cyanobacteria have been measured in the river.

[2] The public heath monitoring is facilitated by the Klamath Blue-green Algae Workgroup and is composed of monitoring organizations, health officials and interested parties working to inform the public regarding Klamath Basin blue-green algae blooms and the potential health risks. http://www.kbmp.net/collaboration/klamath-blue-green-algae-workgroup

1.3 STUDY GOALS

Toxin concentrations and cell density data from public health and baseline sampling efforts have been used to inform public health notifications for over a decade, but have not been analyzed as one complete data set[3] to assess long-term trends in the Klamath River. The goal of this study was to assess the spatial and temporal patterns of toxigenic cyanobacteria, with an emphasis on *Microcystis aeruginosa* in the middle Klamath River from the time of initial monitoring in 2005 through 2016. Our main goals were to: 1) assess the variability in sampling methods by analyzing duplicate sample data of cell counts and toxins and by comparing the public health and baseline data collection methods, 2) describe the longitudinal trends from Iron Gate Reservoir to Orleans, 3) describe the temporal trends on scales ranging from within-day to inter-annual, and 4) to assess the variability in the relationship between cell density and toxin concentrations by exploring these relationships through space and time. To inform future monitoring and event planning and activities involving water contact, we evaluated trends within the context of public health thresholds.

2 METHODS

2.1 DATA COLLECTION

2.1.1 PUBLIC HEALTH GRAB SAMPLE DATA COLLECTION

Pacific Corp and the Karuk Tribe collected grab samples to monitor cyanobacteria in Copco and Iron Gate Reservoirs and along the mid-Klamath River from below Iron Gate Dam to Orleans as part of baseline and public health monitoring programs (Figure 1, Table 1). Pacific Corp and others sampled additional sites above Copco Reservoir. The Yurok Tribe sampled sites below Orleans, but those data were not included in this analysis.

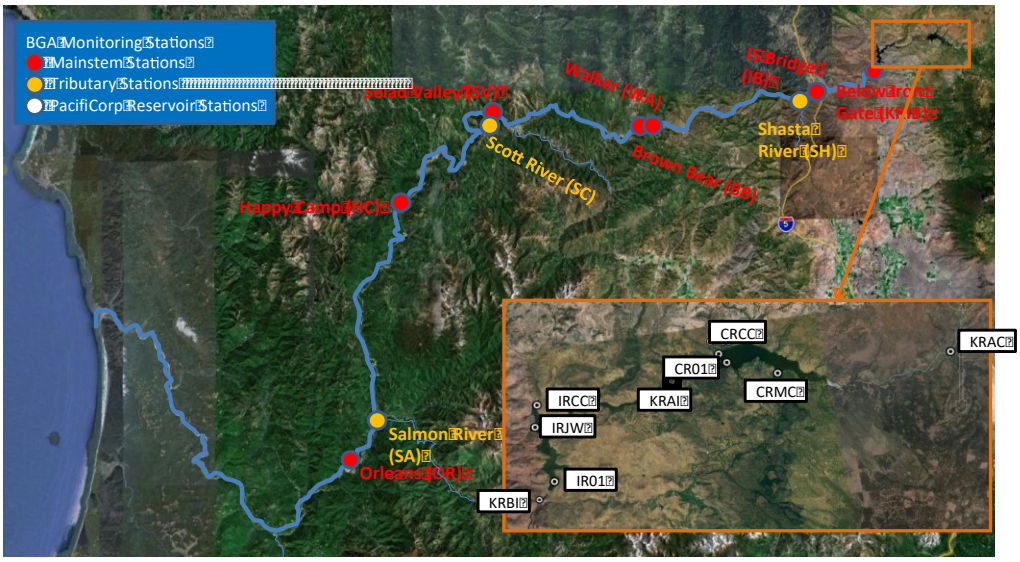

Figure 1. Cyanobacteria monitoring stations on the middle Klamath River

[3] Previous analyses compared inter-annual data collected between 2005-2010 (Kann and Bowman 2012)

Table 1. Location information for 18 cyanobacteria monitoring sites along the mid-Klamath River.

Site Code	North Latitude	West Longitude	River Mile	Site Description	Sampling Program
Copco Reservoir					
CRMC	41°58.441	122°17.869	201.5	Mallard Cove Boat Ramp	Public Health
CRCC	41°59.035	122°19.802	200	Copco Cove Boat Ramp	Public Health
CR01	41°58.932	122°19.694	198.6	Copco Res. Near Dam	Both
Iron Gate Reservoir					
IRCC	41°58.368	122°26.114	192.8	Camp Creek Boat Ramp	Public Health
IRJW	41°57.721	122°26.425	192.8	Jay Williams Boat Ramp	Public Health
IR01	41°56.330	122°25.930	190.2	Iron Gate Res. Near Dam	Both
River between Reservoirs					
KRAC	41°58.345	122°12.101	206.4	Klamath Above Copco	Baseline
KRAI	41°58.380	122°21.900	196.4	Klamath Above Iron Gate	Baseline
Klamath River below Iron Gate Dam (mainstem sites)					
KRBI	41°55.865	122°26.532	189.7	Below Iron Gate Dam	Both
IB	41°51.417	122°34.233	176	I-5 Bridge	Public Health
WA	41°50.252	122°51.811	157	Walker Bridge	Baseline
BB	41°49.399	122°57.650	150	Brown Bear River Access	Public Health
SV	41°50.561	123°13.132	128.5	Seiad Valley at Sluice Box	Both
HC	41°43.780	123°25.775	108.4	Happy Camp	Both
OR	41°18.336	123°31.895	59.1	Orleans	Both
Tributary sites					
SA	41°22.617	123°28.633	0	Mouth of Salmon River	Baseline
SC	41°46.100	123°01.567	0	Mouth of Scott River	Baseline
SH	41°49.390	122°35.700	0	Mouth of Shasta River	Baseline

Limited baseline sampling was initiated in 2005 in Copco and Iron Gate reservoirs and in the Klamath River below Iron Gate Dam. Beginning in 2009 baseline[4] water quality monitoring consisting of grab samples for algal species enumeration and microcystin toxin analysis were generally collected every two weeks from May through October, with monthly collection from November through April in some years (Figure 2, Figure 3). Baseline samples in the river were collected from the upper one-foot of the thalweg (open-channel, denoted OC) using a 14 L churn splitter[5].

[4] The Karuk Tribe, Yurok Tribe and Pacific Corp conducted regular water quality monitoring via grab samples and data sondes on the Klamath River from below Iron Gate Dam to the estuary. Grab samples are analyzed for a suite chemical, biological, and physical water quality parameters. The baseline-monitoring program predates the public health programs.

[5] These open-water samples were collected at wadable depths towards the center of the channel in well-mixed areas of noticeably moving water.

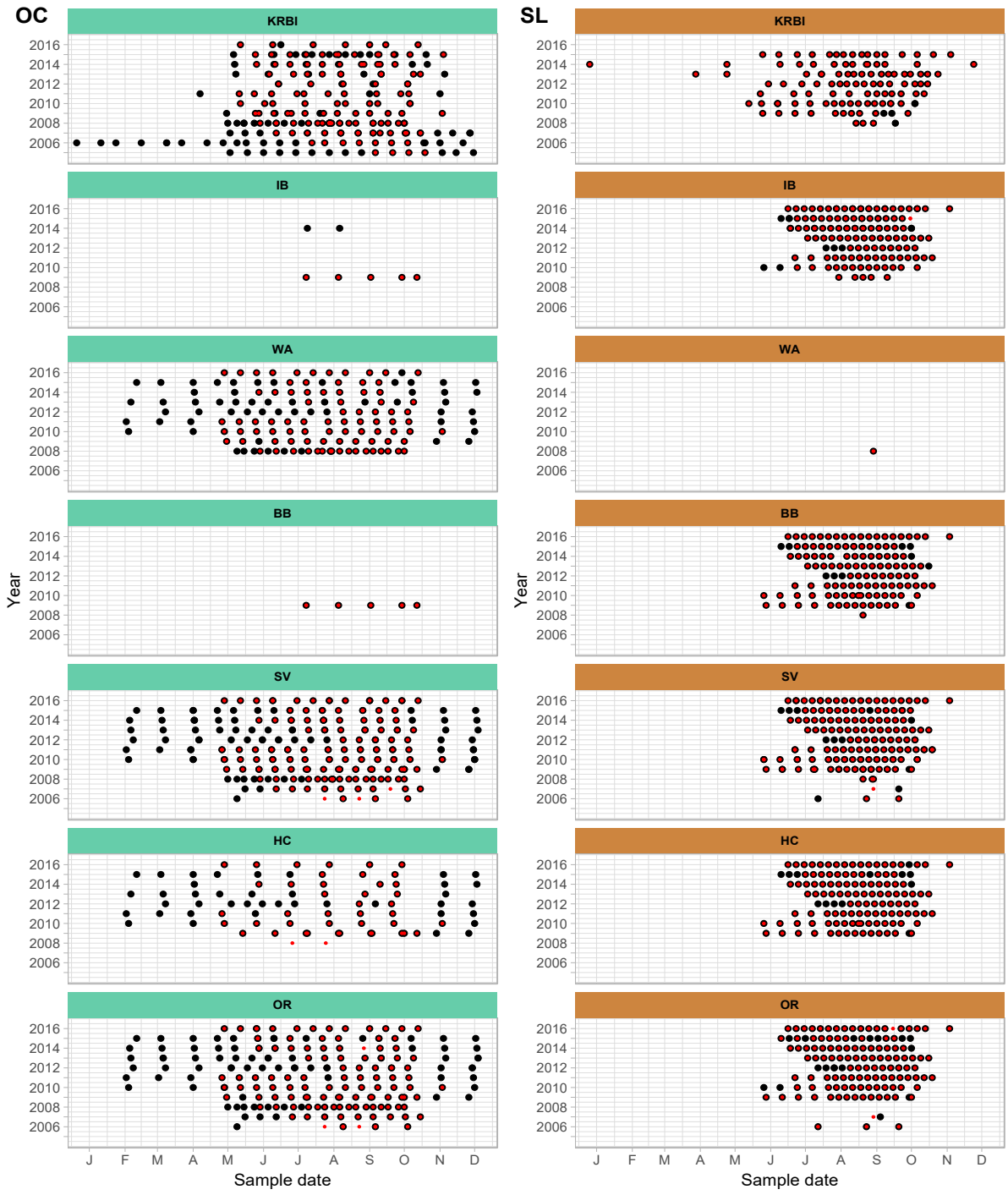

Figure 2. Sample frequency by site on the Klamath River below Iron Gate Dam. Open channel samples (OC, baseline) are displayed with green headings on the left column, and shoreline grabs (SL, public health) are displayed with brown headings on the right side of the figure. Black dots represent samples where algal species were identified and enumerated, red dots are toxin samples, and red dots with black borders (majority of points) represent both parameters.

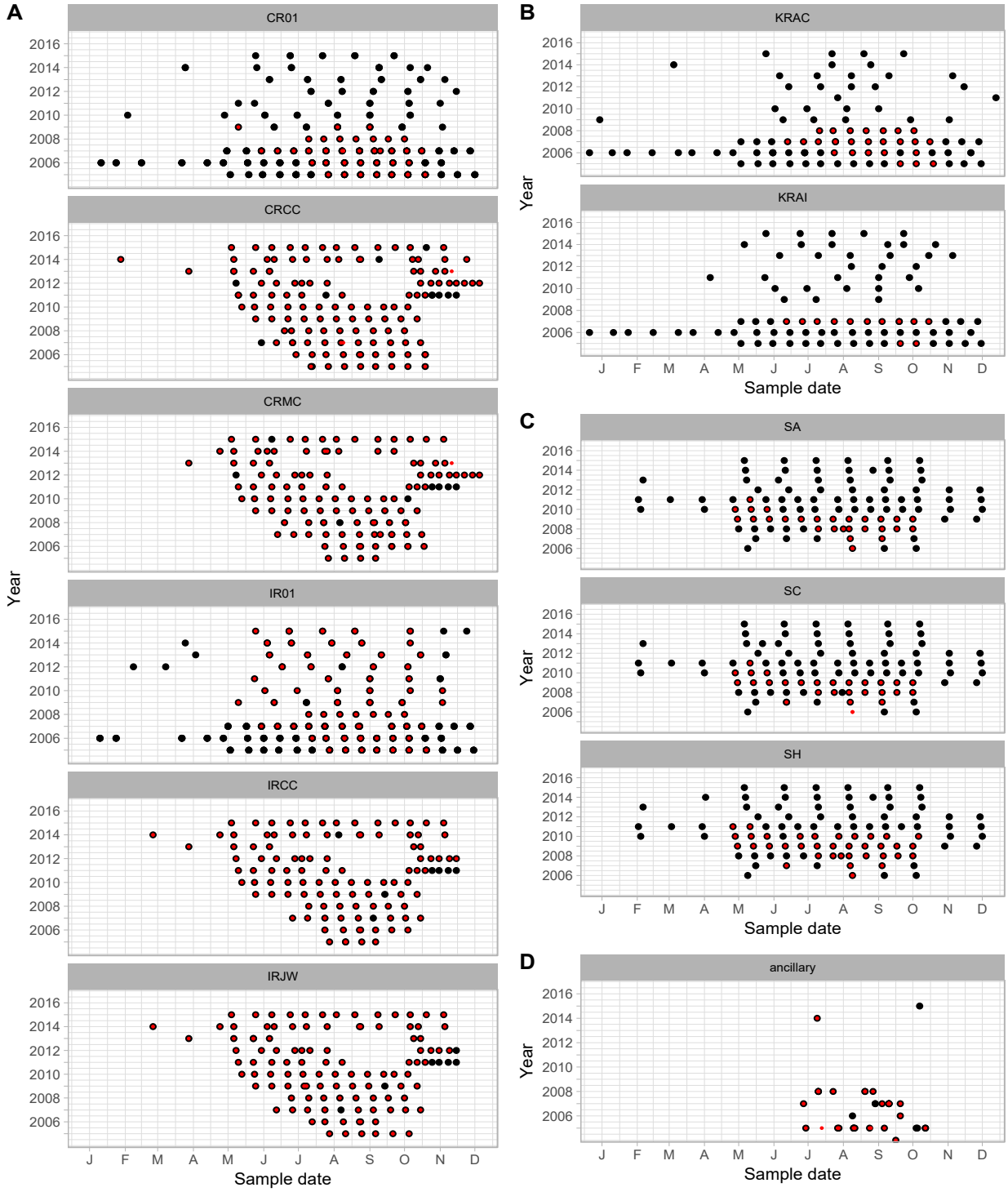

Figure 3. Sample frequency by site in Iron Gate and Copco reservoirs (panel A), river sections within the reservoir reach (panel B), mouths of major tributaries below Iron Gate Dam (panel C), and miscellaneous sites (panel D). All samples are shoreline grab sample from public health monitoring. Black dots represent samples where algal species were identified and enumerated, red dots are toxin samples, and red dots with black borders (majority of points) represent both parameters.

For reservoir open water sites, baseline samples were collected by boat at a depth of 0.5 meters using a Van Dorn bottle and depth integrated samples (0-8 meters) were collected using a hose sampler (Klamath blue green algae working group, 2009). At Walker Bridge, where sampling was conducted from the bridge, a Van Dorn bottle was used to collect the water sample. Baseline samples collected using Van Dorn bottles were put into a churn splitter prior to decanting into sample bottles for analysis of microcystin toxin and cyanobacterial cell density. The public health monitoring program targets surface algal material taken from shoreline or river-edge areas (denoted SL), where human and animal contact is common. These samples were collected weekly once toxin or toxin producing species were identified or suspected in water body being sampled (generally from August through October, Figure 2 and Figure 3). The protocol requires the person collecting the sample to seek out the area of "Reasonable Maximum Exposure" within the public health access point. Surveyors conducted an initial visual survey of the public access area to identify a location likely having a greater presence of cyanobacteria. Samples were collected from the top 10 cm of the water column at the identified location using a wide-mouth jar, and sample bottles were filled from the jar after gently inverting the jar to maintain a well-mixed sample (Figure 4). This protocol was used when sampling from the river or reservoirs.

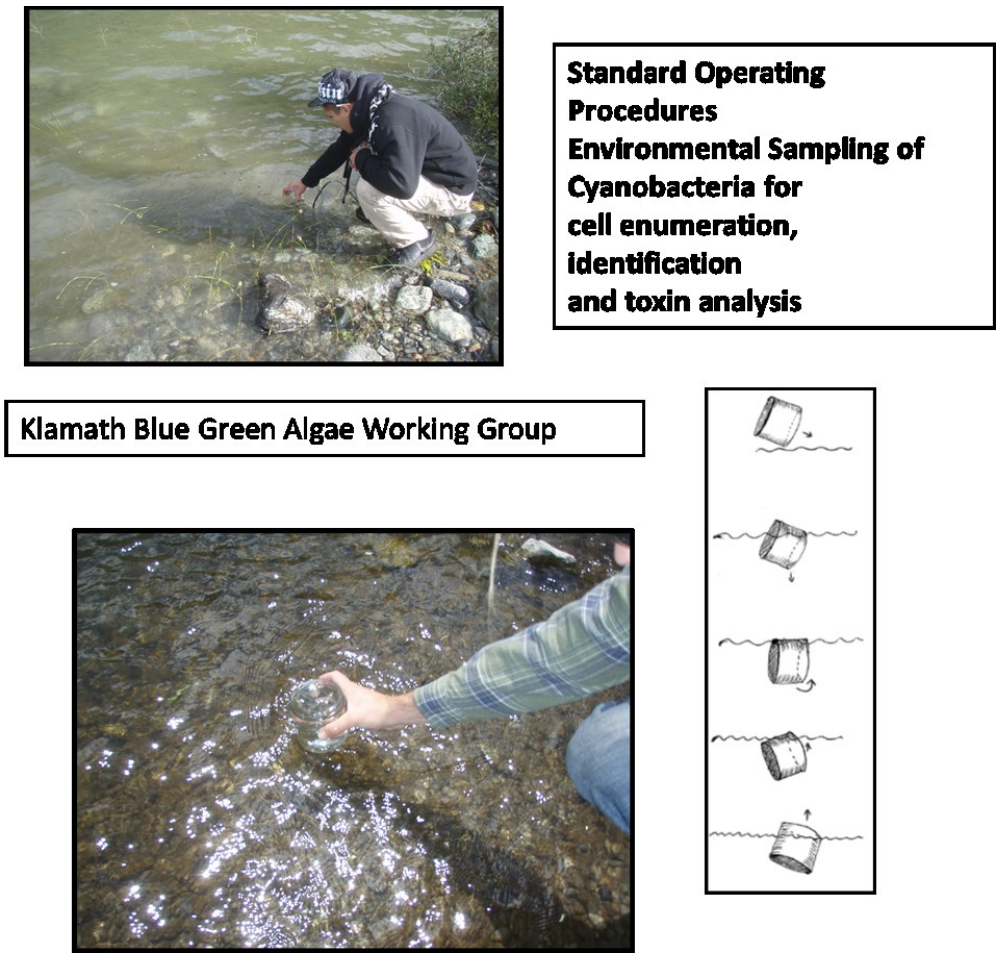

Figure 4. Klamath Blue Green Algae Working Group public health grab sample collection methodology.

Both baseline and public health sampling programs utilized the standard operating procedure (SOP) developed by the Klamath Blue-Green Algae Working Group[6], and samples were collected for algal cell enumeration and microcystin toxin analysis. Samples for microscopic determination of species composition and cell density were preserved in Lugol's Iodine and analyzed by Aquatic Analysts in Friday Harbor, WA. Samples for microcystin toxin were collected in glass vials, frozen, and subsequently placed in a cooler with gel-ice and shipped overnight air to the USEPA Region 9 Laboratory in Richmond, CA for analysis of microcystin toxin using ELISA[7] methodology. All samples were placed in a cooler with ice to keep cool and protect from light between sample collection and shipping.

A set of "blind duplicate" quality assurance samples were collected for approximately 10% of the samples for cell density and microcystin toxin. Quality assurance (QA) sampling was performed by splitting samples in the field using a churn splitter, or by partitioning the sample from the public health grab samples. One of the pair of split samples was designated as the "blind" and sent with its associated split for analysis of both cell density and microcystin toxin.

2.1.2 ASSESMENT OF WITHIN DAY VARIATION IN CYANOBACTERIA

In order to assess diel trends in cell density and microcystin toxin in 2013 an ISCO automated sampler was installed in the Klamath River at the USGS gauging station below Iron Gate Dam. Three automated trials were conducted; at the public health posting on July 30th - 31st, at the assumed algal peak on September 17th - 18th,and at the de-posting of public health warnings on October 26th - 27th. Each sample event consisted of seven samples taken over a 24-hour period starting at noon and taken every four hours. Three replicate samples were taken at each sampling event over the 24-hour period for a total of 21 samples.

In 2014 six ISCO sampling events occurred at four stations: KRBI and KAT were sampled two times, and SV and OR were each sampled one time. Three replicate samples were taken at each sampling event for toxin analysis and a single sample was analyzed for cyanobacterial cell enumeration. All samples were collected from the ISCO sampler at the end of the 24-hour sampling period and cell counts and toxin concentrations were prepared and analyzed as described above for the public health sampling program.

[6] http://www.kbmp.net/images/stories/pdf/KHSA/Cyanobacteria_Sampling_SOP.pdf
[7] Enzyme-Linked ImmunoSorbent Assay using EnviroLogix QuantiPlate Kit designed for quantitative laboratory detection of microcystin toxin in surface water samples (quantitation limit is 0.18 µg/L)

Table 2. Dates and sites of 24-hour cyanobacteria sampling events with the number of samples and replicates taken during each sampling effort.

Year	Start Date	Site	Trial	Samples per 24 hour period	Replicates per sample: Microcystin	Replicates per sample: cell counts
2013	July 30	KRBI	1	7	3	3
2013	September 17	KRBI	2	7	3	3
2013	October 26	KRBI	3	7	3	3
2014	July 30	KRBI	4	7	3	1
2014	August 5	KAT	5	7	3	1
2014	September 2	KRBI	6	7	3	1
2014	September 4	SV	7	7	3	1
2014	September 6	OR	8	7	3	1
2014	September 9	KAT	9	7	3	1

2.1.3 ASSESMENT OF WITHIN DAY VARIATION IN CYANOBACTERIA: SONDE-BGA DATA

Continuous water quality data sondes equipped with phycocyanin probes (sonde-BGA) were deployed by the Karuk Tribe, Yurok Tribe, and Pacific Corp at seven mainstem stations from just below Iron Gate Dam to Turwar (as described in Genzoli and Kann 2016). These probes measure florescence of the phycocyanin pigment and provide results as relative florescent units or a qualitative estimate of cyanobacteria cell density based on the phycocyanin fluorescence. The sondes recorded phycocyanin pigment readings continuously over the monitoring period (generally May through October) at 30-minute intervals. We used the hourly mean of the sonde-BGA estimates, and only included data that passed the quality assurance analysis conducted by Genzoli and Kann (2016). This high-frequency data collection allowed for the investigation of within-day variation in riverine cyanobacteria conditions based on phycocyanin pigment concentrations.

2.2 ANALYSIS

Since the long-term database, which covered 12 years of sampling, had occasional inconsistencies in site names, we standardized site names and categorized site type within the cyanobacteria database. Seven regularly monitored long-term sites were identified along the mainstem of the Klamath River below Iron Gate Dam. Of these, we standardized site names (i.e. “IG” and “KRBI” were standardized to “KRBI”) and combined sites that had river mile designations less than one mile apart, such as Walker Bridge (WA), which was listed as 157.5 in earlier years, and as river mile 157 in more recent sampling years. Similarly, we standardized site codes in the reservoir public health sites when the site descriptions were associated with different four-letter codes.

Sampling locations were categorized as either mainstem, tributary, reservoir, or river between reservoir for this analysis. We labeled sites of river mile 190 or less as “mainstem” sites, with the exception of sites with river mile of 0, which were all sites taken at the mouth of the

major tributaries (Shasta, Scott, Salmon); these sites were characterized as "tributary". Sites with river mile above 190 were characterized as "reservoir" if taken from Copco or Iron Gate Reservoirs, and "river between reservoir" for the two sites that were in river sections between or above the reservoirs (KRAC, KRAI). Finally, sites that were sampled infrequently (fewer than 20 samples per site) were designated as "ancillary".

To assess seasonal species composition of Klamath River cyanobacteria we calculated the weekly mean cell densities by species or genus using data from all Klamath River mainstem sites and all years analyzed in this study. The weekly means of both absolute and percent composition of cyanobacterial cell density were then plotted over the sampling season.

We analyzed sample accuracy by comparing paired samples. We paired samples indicated as duplicates in the database, as well as any samples taken under the same sampling program on the same day and site. We assumed two samples taken on the same day and site were duplicates, and confirmed that samples were taken at the same time when the sample time was available for those samples. For both *Microcystis* and microcystin, we plotted the duplicate sample against the original sample with a 1 to 1 line to assess scatter in the relationship. Additionally, we calculated the relative percent difference between each pair of original and duplicate samples as:

$$\frac{|\,O - D\,|}{\left(\frac{O + D}{2}\right)} \times 100$$

Where O is the original sample and D is the duplicate. Similarly, we assessed the variation between sampling programs (public health vs. baseline monitoring) by pairing the open channel and shoreline grabs collected at the same site and same day for *Microcystis* cell density and microcystin toxin and calculated the difference between open-channel samples and shoreline samples.

We assessed longitudinal trends in *Microcystis* and microcystin concentrations from Iron Gate Dam to Orleans by calculating the percent of cells and toxins that were transferred from the Iron Gate Reservoir baseline station to the river below the dams. We assessed downstream declines in *Microcystis* using boxplots, quantile regressions by river mile, and locally weighted regression (LOESS). To assess changes in Klamath River cyanobacteria through time, we used LOESS smoothers and box plots to assess variation in seasonal trends among years.

Further, to assess spatial and temporal patterns in the context of public health exceedances, we calculated the percent of samples that exceeded public health thresholds by month and year, and included those thresholds on most graphs. Graphical displays were used to assess the relationships between *Microcystis* cell density and toxins, and the variation in those relationships among sites, years, and seasons. To evaluate microcystin cell quotas we calculated the ratio of toxin per 100,000 cells for all samples with paired cell density and toxin parameters. In addition, to determine the cell density predicted to occur at the upper end of the data distribution when microcystin exceeded public health thresholds, we plotted the 0.9 quantile of the relationship between cell density and toxin and compared them to established public health thresholds.

To further investigate the relationship between microcystin toxin and *Microcystis* cell density, we utilized a nonparametric cross-tabulation approach previously used for establishing water quality thresholds (e.g, Heiskary and Walker 1988, Walker and Havens 1995; Kann and Smith 1999). Following methods described in Kann and Smith (1999), paired grab sample microcystin concentration and *Microcystis* cell density were ordered by ascending cell density, divided into data intervals chosen to maximize interval evenness, and the median cell density for each interval was computed. The frequency of grab sample microcystin observations within each interval that exceeded chosen toxin thresholds (beginning at 1 µg/L and continuing in 1 µg/L increments to 8 µg/L) was then computed as an exceedance frequency (expressed here as a percentage of the total observations within each interval) and plotted against the median *Microcystis* cell density for each interval. In order to describe the functional relationship and to facilitate determination of specific probabilities that grab sample toxin concentrations would exceed public health posting standards at any given *Microcystis* cell density, we constructed correlative contours following Lodwick and Whittle (1970) using the linear interpolation function in SYSTAT version 13.1 (Systat 2009).

Finally, to assess the degree of water quality exceedances in the context of current standards, we calculated the percent of samples that exceeded the Karuk Tribal water quality standards for microcystin toxin and *Microcystis* cell density in the Klamath River below Iron Gate Dam during bloom periods. We calculated exceedances for both the level I and level II thresholds (described above) by month and by year, as well as for all months and years combined for the months of July, August, September, and October.

3 RESULTS/DISCUSSION

3.1 SPATIAL PATTERNS IN CYANOBACTERIA

3.1.1 SPECIES COMPOSITION

The planktonic cyanobacterial community in the Klamath River below Iron Gate Dam was dominated by *Microcystis aeruginosa* from mid-July through mid-November, which is also the time that cyanobacteria concentrations were regularly elevated above 1000 cells/mL (Figure 5). *Anabaena* and *Aphanizomenon* were dominant before and after the period of *Microcystis* dominance, but these periods generally showed low weekly mean cell densities (Figure 5). Other species from the genera *Gloeotrichia*, *Oscillatoria*, and *Pseudanabaena* were occasionally present, but at low cell densities and percent dominance, with weekly means using samples from all river sites showing generally low numbers and few detections of these species.

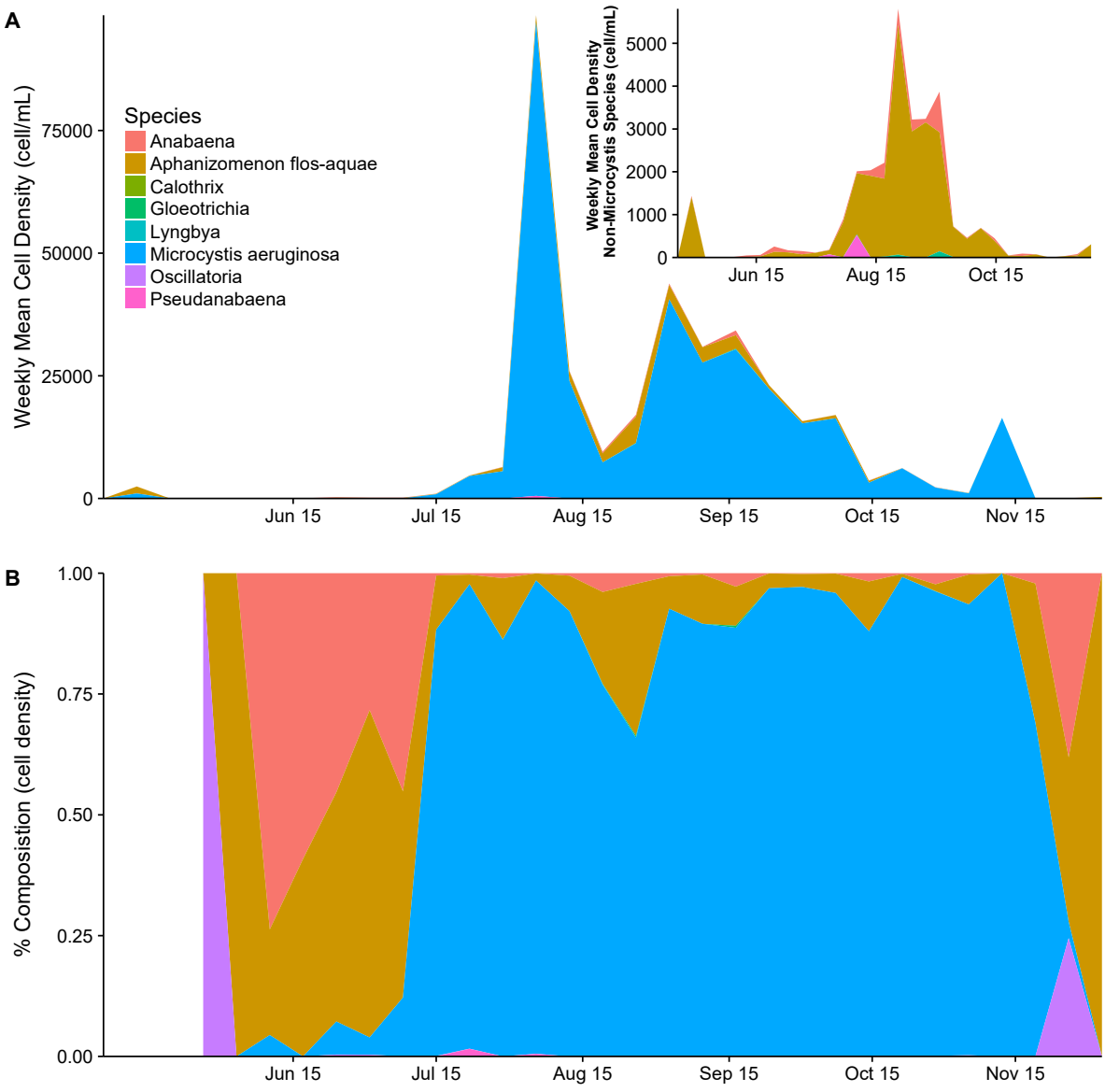

Figure 5. Weekly mean cyanobacterial cell density by species with plot-inset of non-*Microcystis* species to show weekly mean cell density of species other than *Microcystis* (panel A), and weekly mean % composition of cell density by species (panel B). Data from all mainstem river sites was used.

Because cyanobacteria species, including *Gloeotrichia*, *Oscillatoria*, and *Anabaena* that can produce microcystin as well as other cyanotoxins, were found only at low densities in the Klamath River over the last decade, the following analyses focuses only on *Microcystis* and associated microcystin toxin in the Klamath River and its source reservoirs. Future monitoring should continue to identify these species, and protective measures taken if levels increase.

3.1.2 DUPLICATE SAMPLES

Although variability was evident, duplicate samples of cyanobacterial cell density and microcystin toxin generally reflected the cell density and toxin concentration of the paired

sample (Figure 6). Cell density samples showed higher variability than toxin samples, with medians of the relative percent difference between cell density and toxin concentration of 40% vs. 20% (Figure 7). The smaller relative percent difference between the toxin concentrations and their paired duplicates suggest that that toxin concentration provides a more accurate measurement of the potential health effects of a *Microcystis* dominated cyanoHAB than cell counts. Nonetheless, cell counts or another secondary measurement of cyanoHAB risk is helpful in confirming the presence and severity of the bloom effects.

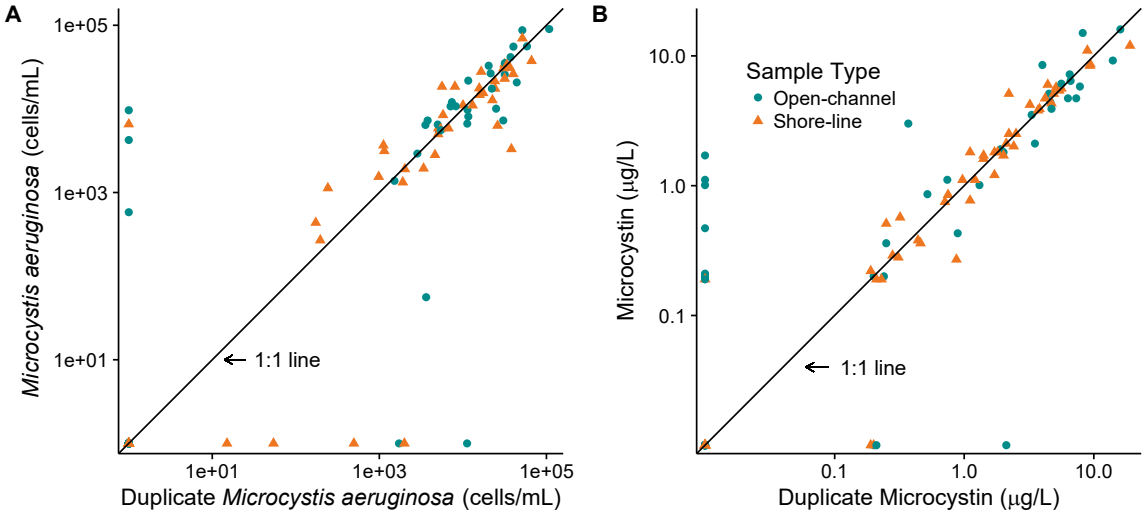

Figure 6. *Microcystis aeruginosa* cell density (panel A) and microcystin toxin concentration (panel B) as predicted by paired duplicate samples from Klamath River sites below Iron Gate Dam. Line is 1:1 line, green circles represent open-channel samples and brown triangles represent Shore-line grab samples.

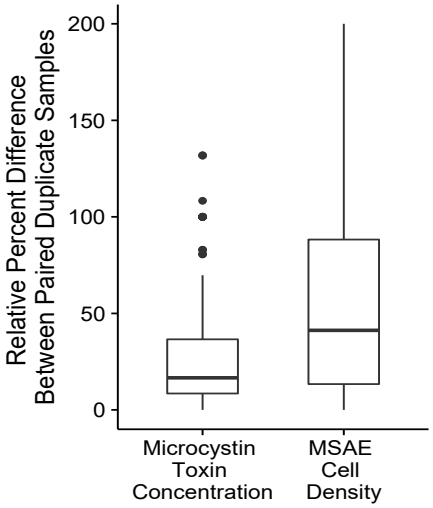

Figure 7. Boxplots of the relative percent difference between paired duplicates of Klamath River mainstem samples for both cell density and microcystin toxin.

Cell density and toxin samples and their paired duplicates, although generally reflective of one another, occasionally spanned public health thresholds (Figure 8). Of 130 duplicates, paired samples of *Microcystis* cell density spanned public health thresholds in 12 instances; five

pairs spanned the level I threshold, four pairs spanned the level II threshold, and three pairs of samples had a sample below the level I and above the level II threshold. For microcystin toxin, 13 samples spanned thresholds, with three spanning the level II threshold, and 10 spanning the level I threshold. No toxin samples spanned both level I and II thresholds. With approximately 10% of duplicate samples for both cell counts and toxins spanning across one or both public health thresholds, having data from a second analyte (i.e., both toxin and cell density) can support public health posting decisions. In addition, cell density measurements are helpful for understanding long-term ecological dynamics and determining microcystin cell quota.

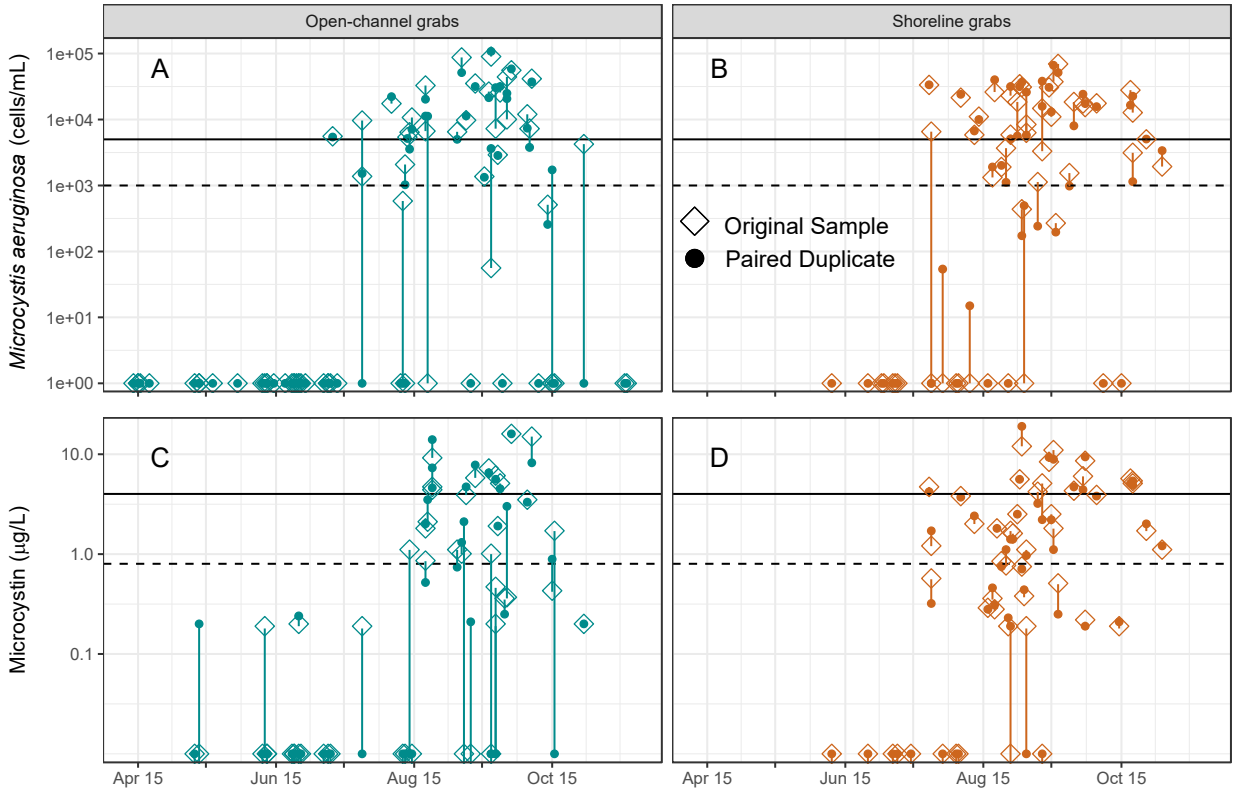

Figure 8. Original sample (open diamond) with the paired duplicate sample (solid circle) connected by a vertical line for *Microcystis* (panels A and B) and Microcystin toxin (panels C and D). Panels A and C show open-channel samples and panels B and D show shoreline samples. Solid black lines and dashed black lines are the level II and level I public health thresholds, respectively.

3.1.3 RELATIONSHIP BETWEEN SHORELINE AND OPEN-CHANNEL SAMPLES

Shoreline and open-channel grabs often reflected similar cell densities, but at times shoreline samples were much higher than open-channel samples (Figure 9). For shoreline cell counts of *Microcystis*, 72% of samples were higher than the paired open-channel sample, and 30% of those samples were more than 2x higher than the open-channel samples. For microcystin toxin, 55% of samples were higher than the paired open-channel samples and 15% of the shoreline samples were more than twice as high as the open channel samples. The shoreline samples reflect the build-up of cells and toxins, which can get trapped in the channel margins and build up in high concentrations relative to the main channel (Kann 2012).

Most of the shoreline samples that had substantially higher concentrations of toxins and higher cell densities than the corresponding open-channel grabs occurred in 2008 and 2009. It is unknown if these higher relative concentrations of shoreline samples in 2008 and 2009 were due to different river dynamics, or to sampling methods. For example, specific flows could cause water along parts of the shoreline to be entrapped, and if these flows corresponded to times of higher *Microcystis* cell concentrations, we would expect to see relatively higher cell and toxin concentrations along the shoreline than in the thalweg. Alternatively, sampling methods may have shifted slightly over the years with changes in sampling crews. Although the open-channel thalweg samples should be similar when taken from anywhere in the well-mixed main channel, we would not expect samples taken along the shoreline to be heterogeneous because water is entrapped in some places at higher rates than others.

The protocol for collecting the shoreline public health samples states that the person collecting water should search for an area of reasonable maximum exposure within the public health access point to take the grab sample from. Selecting the point of reasonable maximum exposure is inherently subjective, and would depend on the distance a field technician traveled and which micro-site the technician ultimately chose for sampling. Comparing samples from along the shoreline at different micro-locations could inform where samples should be taken to reflect the highest likely cyanobacteria and toxin concentrations.

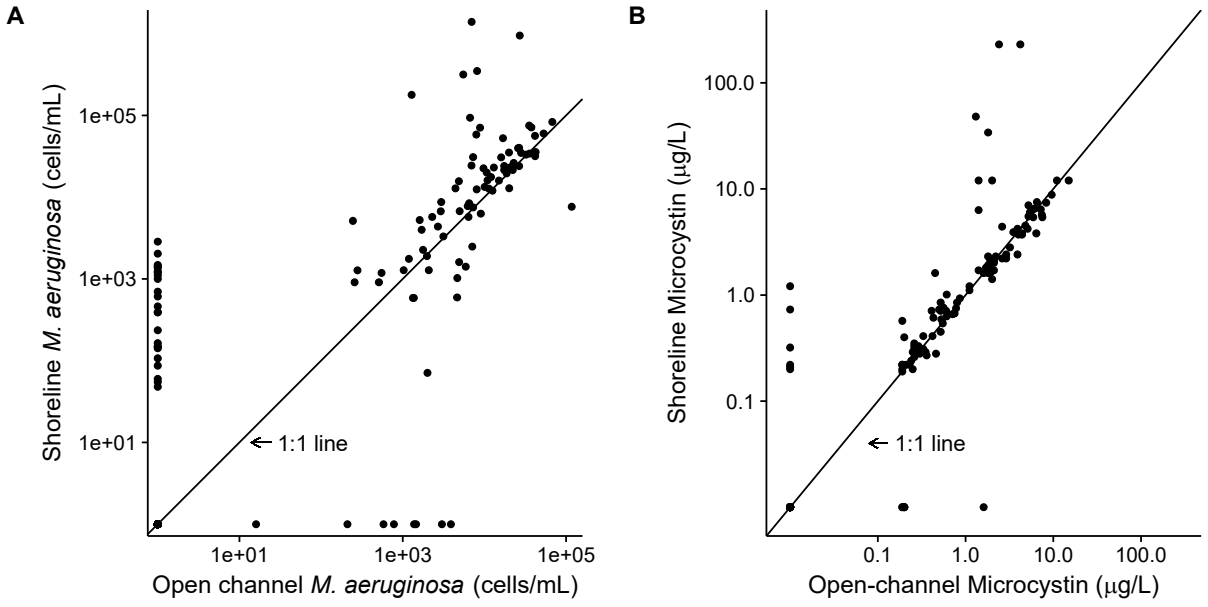

Figure 9. Shoreline *Microcystis* cell density (panel A) and shoreline microcystin toxin concentration (panel B) as predicted by open channel samples taken on the same day and from the same site from Klamath River sites below Iron Gate Dam. Points above the 1:1 line indicate that shoreline samples were higher and points below the 1:1 line indicate that open channel samples were higher.

3.1.4 RELATIONSHIP BETWEEN RESERVOIR AND RIVER SAMPLES

Microcystis aeruginosa and associated microcystin toxin concentrations were substantially higher in the reservoirs than the flowing portions of the Klamath River. For example, surface grab samples collected in the reservoirs from July through October to assess public health risk had significantly higher concentrations of *Microcystis* and microcystin than in the Klamath River below Iron Gate Dam, and cell counts showed only six detections (out of 54 samples, half of which occurred in 2015) of *Microcystis* above Copco Reservoir during these months (Figure 10). Median concentrations of *Microcystis* and microcystin were an order of magnitude higher in Copco Reservoir than in Iron Gate Reservoir, and median concentrations in the river below Iron Gate Dam were yet an order of magnitude lower than in Iron Gate Reservoir. The flowing river site located immediately above Iron Gate Reservoir had higher median cell densities higher than the river below Iron Gate Reservoir (which included sites spanning 130 miles of river), but much lower than Copco Reservoir, likely due to subsurface reservoir releases and breaking up of cellular colonies in the hydropower facilities.

The patterns shown in the 11 years of *Microcystis* data from above Copco Reservoir to the Klamath River below Iron Gate Reservoir supports the understanding that Copco and Iron Gate reservoirs are the major source of *Microcystis* to the Klamath River below the dams. For example, QPCR assays showed that *Microcystis* was a minor constituent of the phytoplankton community above Copco and Iron Gate Reservoirs, and that the highly prolific populations within the reservoirs originated primarily internally (Otten et al. 2015; Otten and Dreher 2017). Although concentrations of *Microcystis* can occasionally spike in Upper Klamath Lake, they are generally 2 to 3 orders of magnitude lower than in Copco reservoir (Eldridge et al. 2013; Kann 2017; KHSA 2017). These lower concentrations in Upper Klamath Lake, combined with apparent lack of cell survival during transport downstream, results in rare detections of *Microcystis* and associated microcystin toxin above Copco Reservoir (Figure 10). In contrast, *Microcystis* grows prolifically in the lacustrine waters of Copco and Iron Gate reservoirs, where the lake-like environment, paired with plentiful nutrients, supports high densities of cyanobacteria, with *Microcystis* dominating by mid-summer. The cyanobacteria begin growing along shoreline margins in calm and warm water but eventually populates the entire reservoir allowing release through the reservoir outtake and into the Klamath River below Iron Gate Dam.

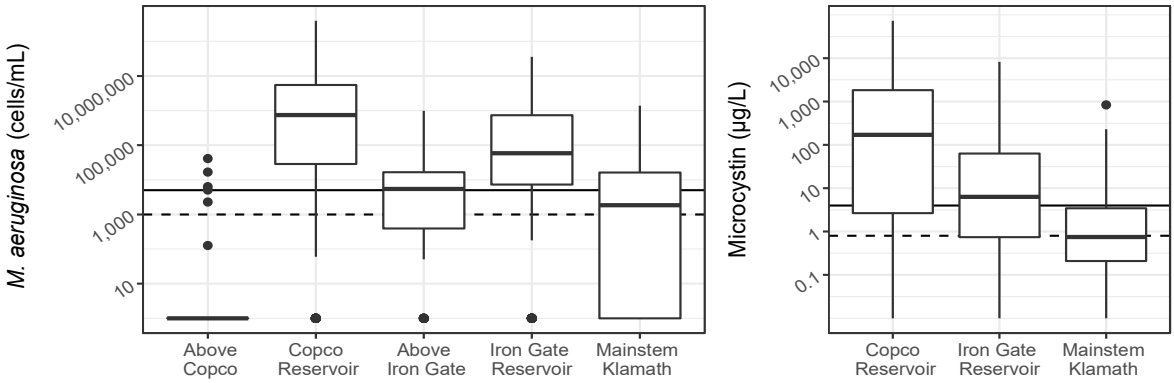

Figure 10. Box plots of *Microcystis* and Microcystin toxin from public health samples during July – October from 2005 to 2015 for the mid-Klamath. Mainstem Klamath is all sites from below Iron Gate to Orleans and "Above Copco" and "Above Iron Gate" are flowing river sites above the respective reservoirs; samples here were a mix of public health and base-line due to limited samples after 2008.

Levels of *Microcystis* and microcystin toxin in the Klamath River below Iron Gate Dam reflected concentrations in the reservoir at the Iron Gate log boom, but did not follow a one to one relationship. *Microcystis* cells and toxins were lower in the river than the reservoir when comparing samples taken on the same day (Figure 11). The few occasions where samples were higher in the river below the reservoir can likely be attributed to variability in the samples that were apparent even when duplicates taken in the same time and place were compared (Figure 6, Figure 8), or due to daily variation in concentrations of cells and toxins (see section 3.2.2). Integrated samples taken throughout the top eight meters of the reservoir related to river samples below the reservoir with less variability than samples taken near the reservoir surface (0.5 m). This is expected because releases from Iron Gate Dam are integrated releases from a depth of approximately 10 m. Surface samples, on the other hand, are expected to be more concentrated due to buoyancy dynamics and the tendency of *Microcystis* cells to accumulate in upper well-lit layers during the day to maximize photosynthesis.

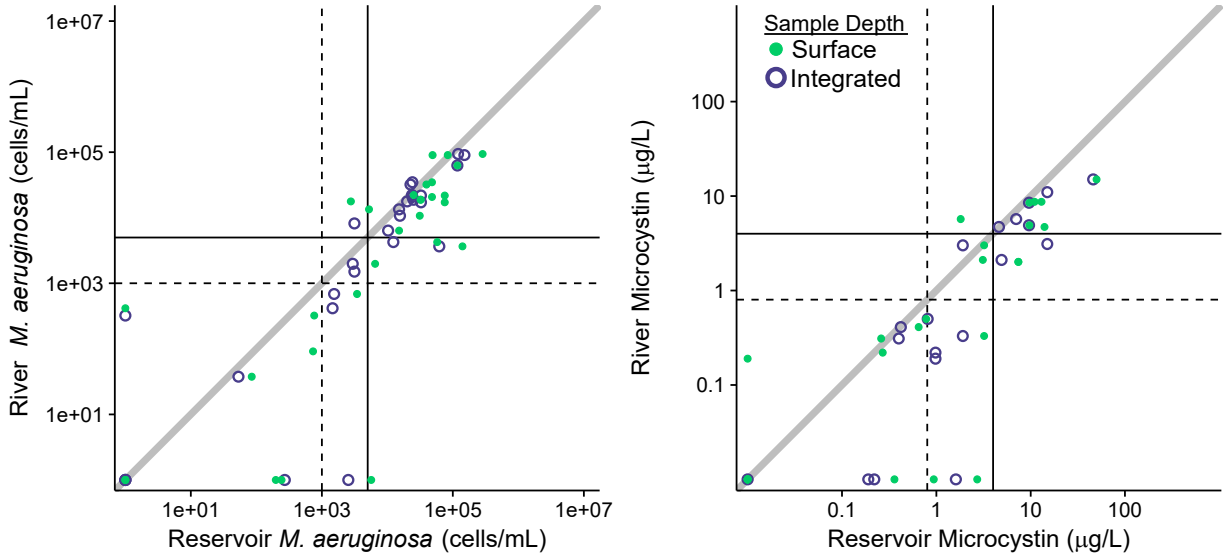

Figure 11. Klamath River *Microcystis* cell density (left panel) and microcystin toxin concentration (right panel) below Iron Gate Dam versus cell density and toxin concentration in Iron Gate Reservoir at the log boom baseline monitoring site. Solid green circles are from reservoir surface samples (0.5 m) and open indigo circles represent reservoir-integrated samples (combined 0-8 m). Grey line is the 1 to 1 line. Solid and dashed black lines are the level II and level I public health thresholds, respectively.

Graham et al. (2012) found that downstream of a reservoir with a hypolimnetic release, microcystin toxins were generally less than 10% of that measured in the reservoir, whereas on the Klamath, paired reservoir and river toxin concentrations averaged approximately 70% of the concentration measured in the reservoir on the same day (63% for integrated reservoir samples and 76% for 0.5 m grab samples based on 19 and 21 sample pairs, respectively). Transfer of cells was slightly higher with 77% and 88% of cells transferred to the river, based on integrated and 0.5 m grab samples, respectively. This indicated that cell density and toxin concentration in samples below the reservoir more closely approximated the 0.5 m depth in the reservoir than they did the integrated sample. This was not expected given the reservoir withdrawal depth of ~10 m and the 0-8 m depth of the integrated sample. Upon further examination we determined

that integrated samples were on average 137% of cell densities and 114% of toxin concentrations of the 0.5 m samples. Higher algal and toxin concentrations may be due to the influence of dense cells and toxin on the very surface of the water which is not captured in the 0.5 m grabs, but would be captured in the integrated samples. A transfer rate of cells and toxins from Iron Gate Reservoir to the Klamath River (e.g., river samples are ~70% of reservoir samples) is helpful for general understanding, but with high variability in the differences between individual samples above and below the reservoir, river toxin and cell estimates should not be based on reservoir concentrations. It should be noted that the ~30% estimated decrease below Iron Gate is based on monthly baseline samples, and that public health samples taken at the water surface show substantially lower concentrations below Iron Gate when compared to public health samples taken in Iron Gate Reservoir (Figure 10).

3.1.5 LONGITUDINAL PATTERNS BELOW IRON GATE DAM

Microcystis cell density and microcystin toxin decreased longitudinally from below Iron Gate Reservoir to Orleans. The median and upper quartiles of the cell density and toxin concentrations generally decreased from below Iron Gate Dam, although highest individual values of cell density and toxins occurred at Brown Bear and Seiad Valley sites, approximately 40 and 60 miles downriver from Iron Gate (Figure 12). These few high samples occurred in the shoreline samples, where entrapment of cells and toxins along the shoreline can occur. Data from July to October for all years indicated that median cell densities of *Microcystis* at KRBI and IB were consistently above the level II public health threshold. Median cell densities were above the level I threshold at WA, BB, and SV, and medians were below both thresholds at HC and OR. Upper quartile cell density values were above the level II public health threshold at all sites from July to October (only shoreline grabs at HC and OR), indicating that in these months, more than 25% of samples exceeded 5000 cells/mL at all sites. Similar to cell densities, toxin concentrations decreased downstream, with median toxin levels above the level I public health threshold from KRBI to SV, and below the threshold from SV to OR. The upper quartiles of toxin concentration crossed the level I public health threshold at all sites, but only crossed the level II threshold at KRBI, IB, and BB.

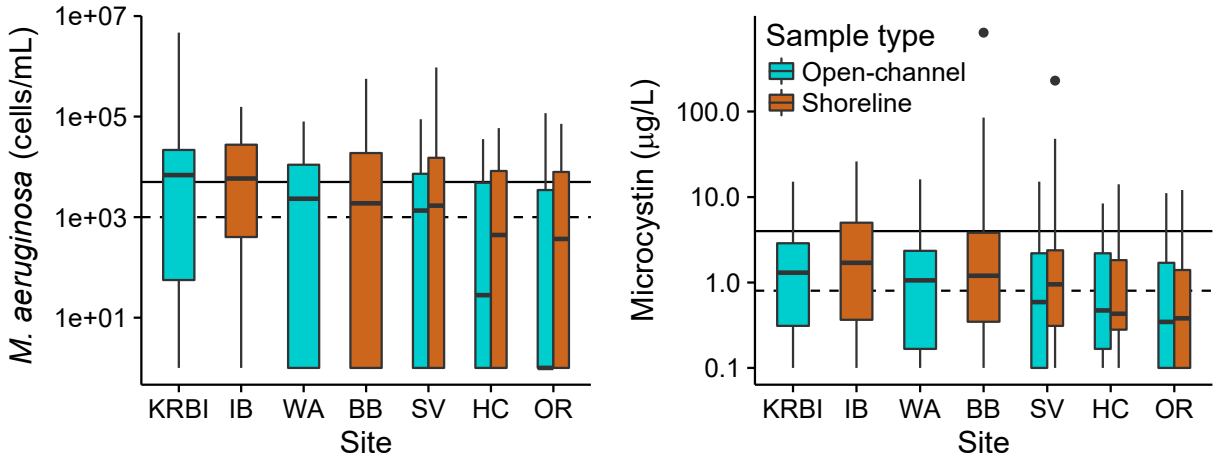

Figure 12. Boxplots of *Microcystis* and Microcystin concentrations on the Klamath River from below Iron Gate Dam to Orleans from July through October. Blue boxes are open channel and brown boxes are shoreline samples. Solid and dashed black lines are level II and I public health thresholds, respectively.

The longitudinal decrease in cell density and toxin concentration could be described by a linear relationship in the 0.5 and 0.9 quantiles of the data, by river mile. Shoreline samples had similar slopes and slightly higher intercepts for the 0.9 quantile of the cell density data and for the 0.5 and 0.9 quantiles of the toxin data than corresponding open-channel samples (Figure 13). The slope was steeper for the median of the *Microcystis* cell density open channel samples than the shoreline samples, indicating that there was less dissipation of algal cells along shore margins in a downstream direction than in the main channel. This longitudinal decrease in cell density and toxin concentration is expected due to the natural death and breakdown of cells and associated toxins without replacement in the river. High water velocity and constant mixing in the Klamath River from below Iron Gate Dam to above the estuary creates an environment that is not supportive of planktonic algae reproduction (Reynolds 1984, Otten et al. 2015).

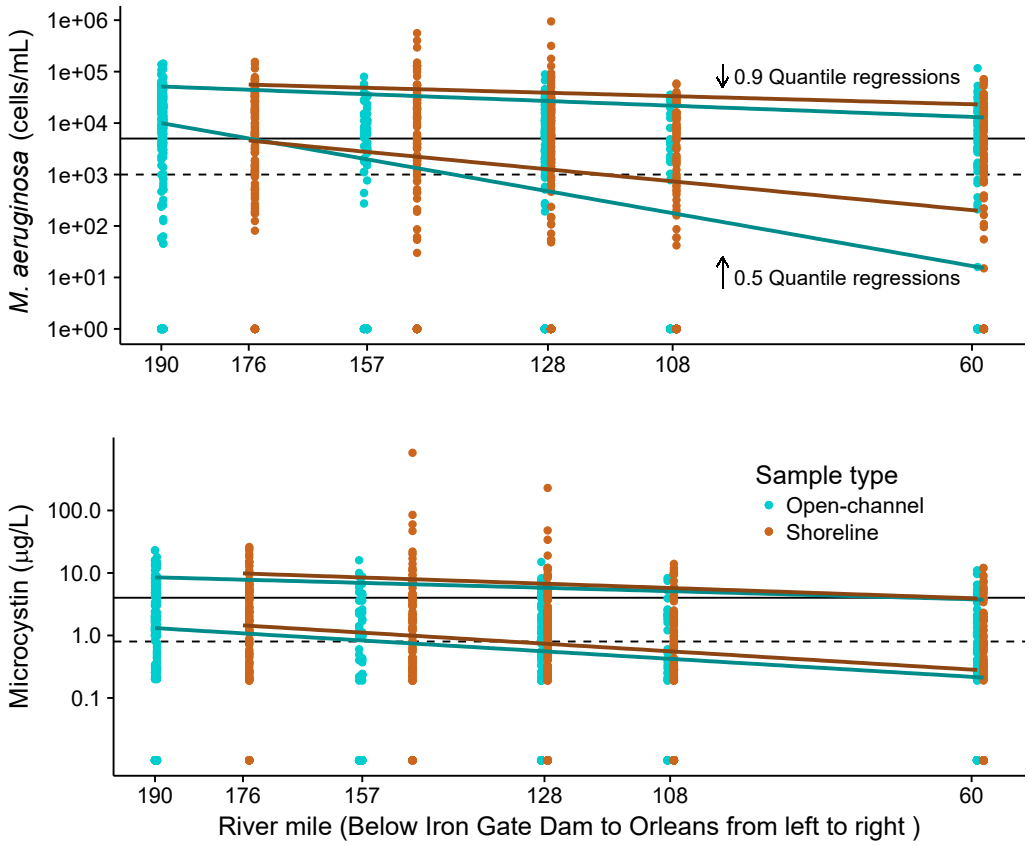

Figure 13. *Microcystis* cell density (top panel) and microcystin concentration (bottom panel) by river mile on the Klamath River from below Iron Gate Dam to Orleans (listed from left to right) from July–October. Blue points are open channel samples and brown points are shoreline samples; upper corresponding regression lines are on the 0.9 quantiles of the data and lower regression lines are on the 0.5 quantiles. Solid and dashed black lines are level II and level I public health thresholds, respectively.

Longitudinal decreases in the mean of *Microcystis* cell density and microcystin toxin occurred during each month from July through October, but there was variability among months (Figure 14), indicating longitudinal and seasonal variability in cyanobacteria in the Klamath River. The July LOESS was lowest at all sites and the September LOESS was generally highest. The LOESS of the toxins generally reflected that of the *Microcystis* cell density LOESS for any given month.

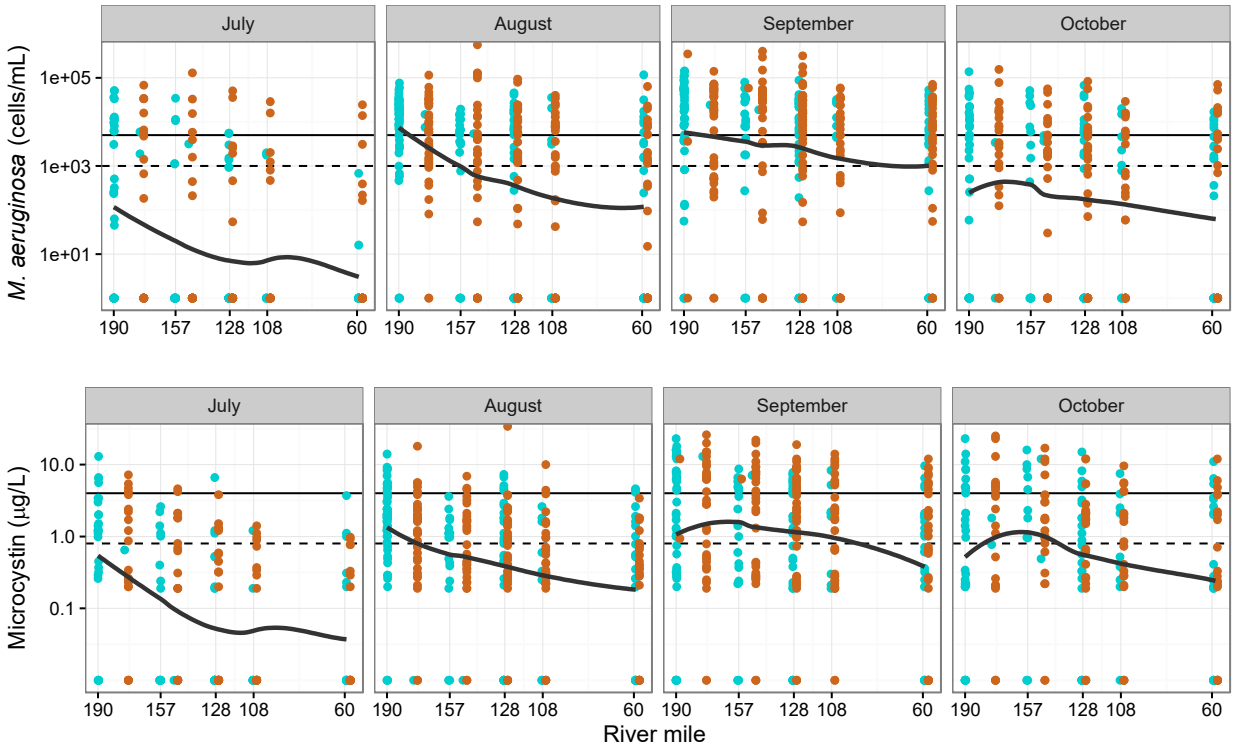

Figure 14. *Microcystis* cell density (top panels) and microcystin concentration (bottom panels) by river mile on the Klamath River from below Iron Gate Dam to Orleans by month. Blue points represent open channel samples and brown points represent shoreline samples. Thick black lines are LOESS smoothers, horizontal solid and dashed black lines are level II and level I public health thresholds, respectively.

3.2 TEMORAL PATTERNS IN CYANOBACTERIA

3.2.1 SEASONAL AND INTERANNUAL VARIATION IN *MICROCYSTIS* AND MICROCYSTIN

There was notable seasonal and interannual variation in the density of *Microcystis* and concentrations of mycrocystin toxin in the Klamath River below Iron Gate Dam. Seasonal variation occurred in every year of the study, although the timing and magnitude of the bloom conditions varied. Using the LOESS smoother to represent the data trend relative to public health thresholds through time, *Microcystis* cell density from all Klamath River sites from below Iron Gate Dam to Orleans crossed the level II public health threshold in most years, although in 2015 and 2016 the LOESS peaked at or below the level I public thresholds (Figure 15). For the years that the LOESS crossed the level II public health threshold, the date that the mean of the data crossed that threshold ranged from August 1st in 2014 to September 15th in 2011. Dates of bloom decrease, as indicated by the seasonal LOESS, also varied, ranging from early September to after the monitoring season ended in late October. Although these seasonal LOESS patterns show patterns in *Microcystis* cell density that allows comparison among years, since the LOESS lines represent the central tendency they are not intended to represent among-year public health risk.

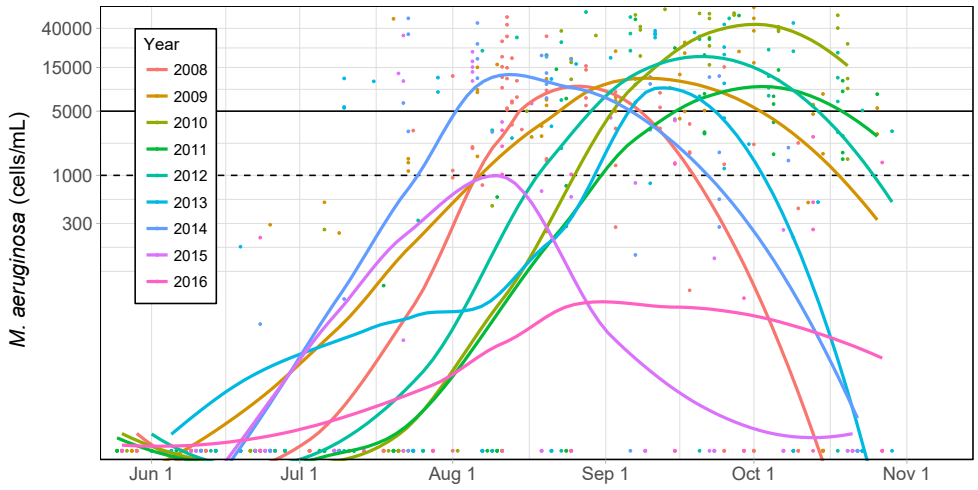

Figure 15. Microcystin cell density by date with seasonal LOESS smoothers for each year. Y-axis is truncated to see detail in the LOESS smoothers, and thus excludes data points above 56,000 cells/mL. Solid black lines and dashed black lines are the level II and level I public health thresholds, respectively.

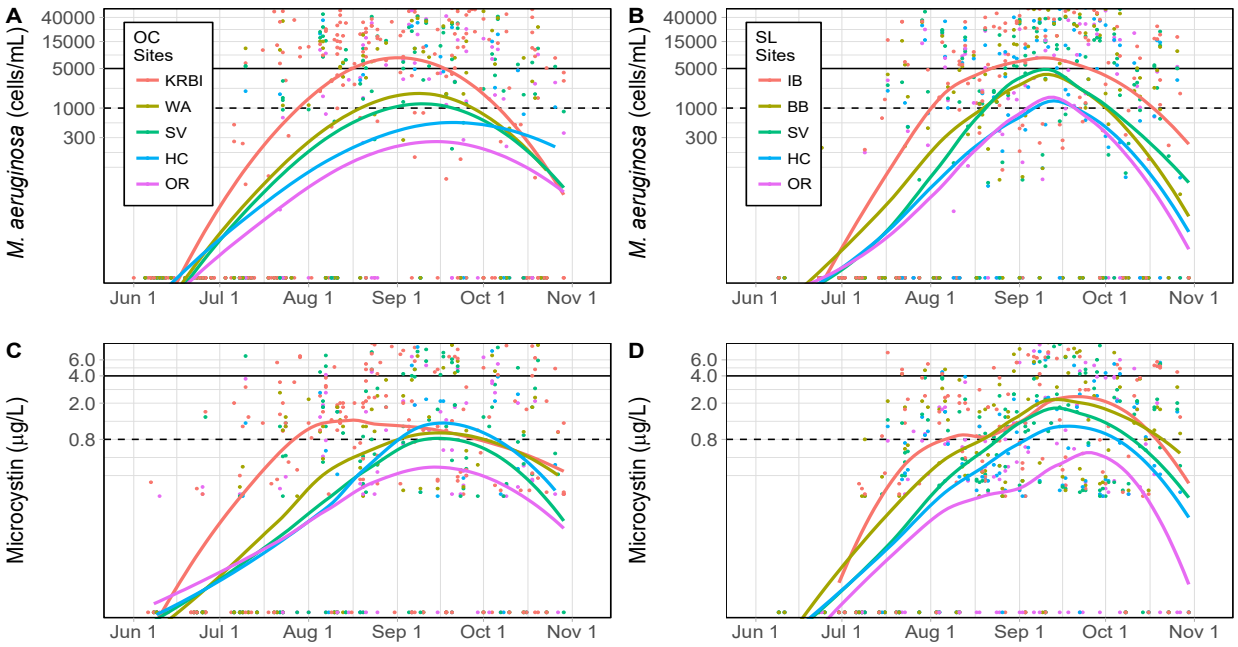

Figure 16. Microcystin cell density (panels A and B) and microcystin toxin (panels C and D) with seasonal LOESS smoothers for each site with all years of data. Panels A and C show Open-channel samples and Panels B and D show shoreline samples. Y-axis is truncated to see detail in the LOESS smoothers, and thus excludes high data points. Solid black lines and dashed black lines are the level II and level I public health thresholds, respectively.

There was similar seasonal variation in microcystin concentrations as in *Microcystis* cell density, but sampling for toxin data was not consistent enough among years to compare interannual trends (Figure 16, Figure 2). The seasonal pattern was reflected similarly at all sites, and displayed the longitudinal decrease shown in Figure 12, Figure 13, and Figure 14. The similar seasonal pattern among sites, in both cell density and toxins, is expected not only in bloom magnitude as shown above, but also in seasonal timing (Figure 16), because the algae originate in the upstream reservoirs and are transported downstream past these sites within a matter of days (Otten et al. 2015).

Boxplots are a useful tool for examining temporal trends throughout the data distribution, with trends in the upper quartile (75^{th} percentile) of the data especially relevant to understanding public health risks. In the open channel grabs, which were collected bi-weekly from May through October (with monthly samples collected in some years in all months), the upper quantile of the river data from all sites never crossed the level I public health threshold in May, June, or July, while about half of the years had upper quartiles of cell and toxin data at or above at least the level I threshold in August. By September, all years except 2015 and 2016 had cell density upper quartiles above the level II public health threshold (Figure 17). Boxplots of shoreline grabs from the public health samples, which were consistently collected in August, September, and October, show a similar patter to the open-channel grab samples, but shoreline grabs from August in particular show higher levels of cell density and toxins than the corresponding open-channel grabs (Figure 18).

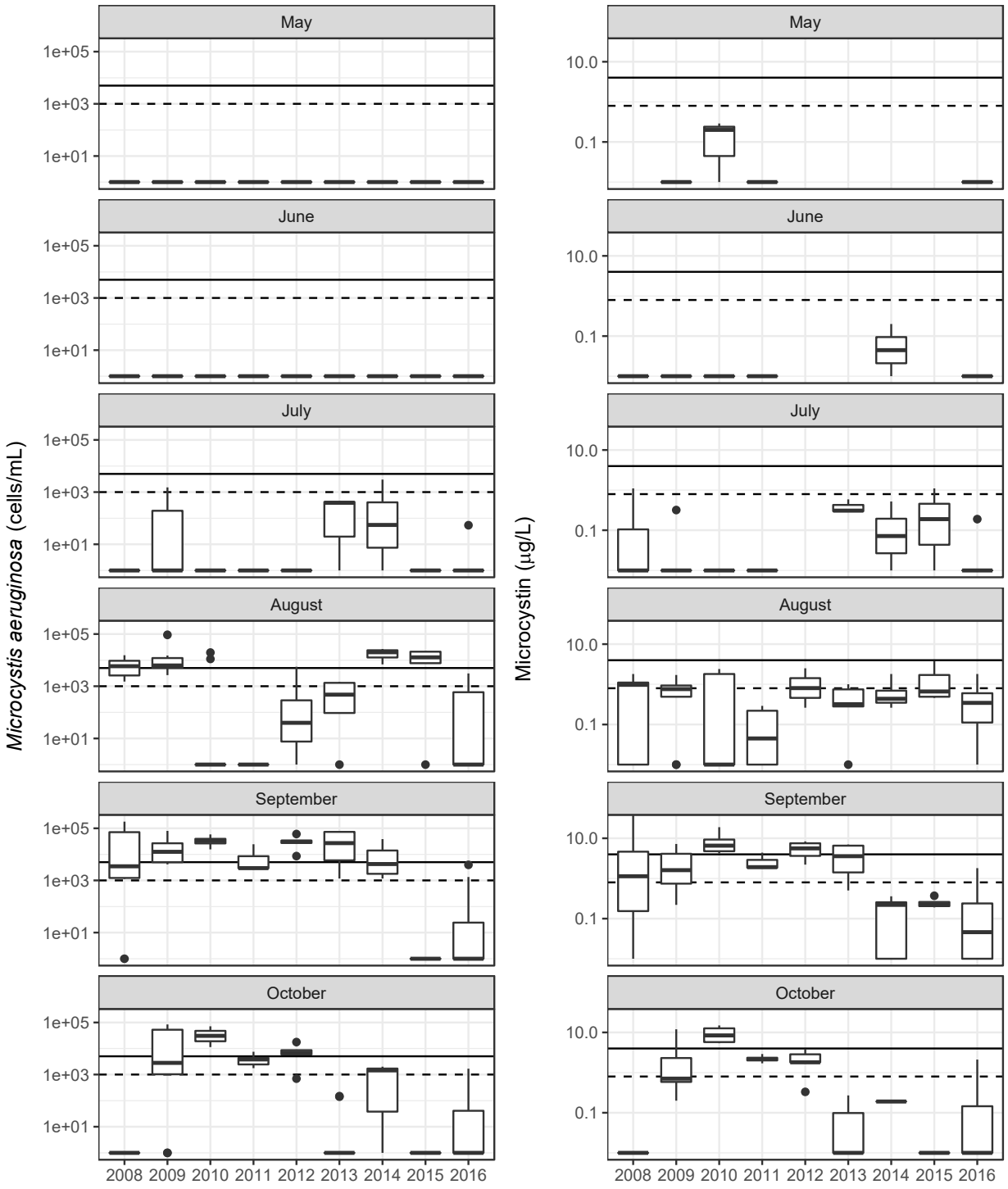

Figure 17. Boxplots of microcystin cell density (left panels) and microcystin concentration (right panels) by year for Klamath River sites below Iron Gate Dam. Solid and dashed black lines are the level II and level I public health thresholds, respectively. Samples are open-channel grabs from baseline monitoring.

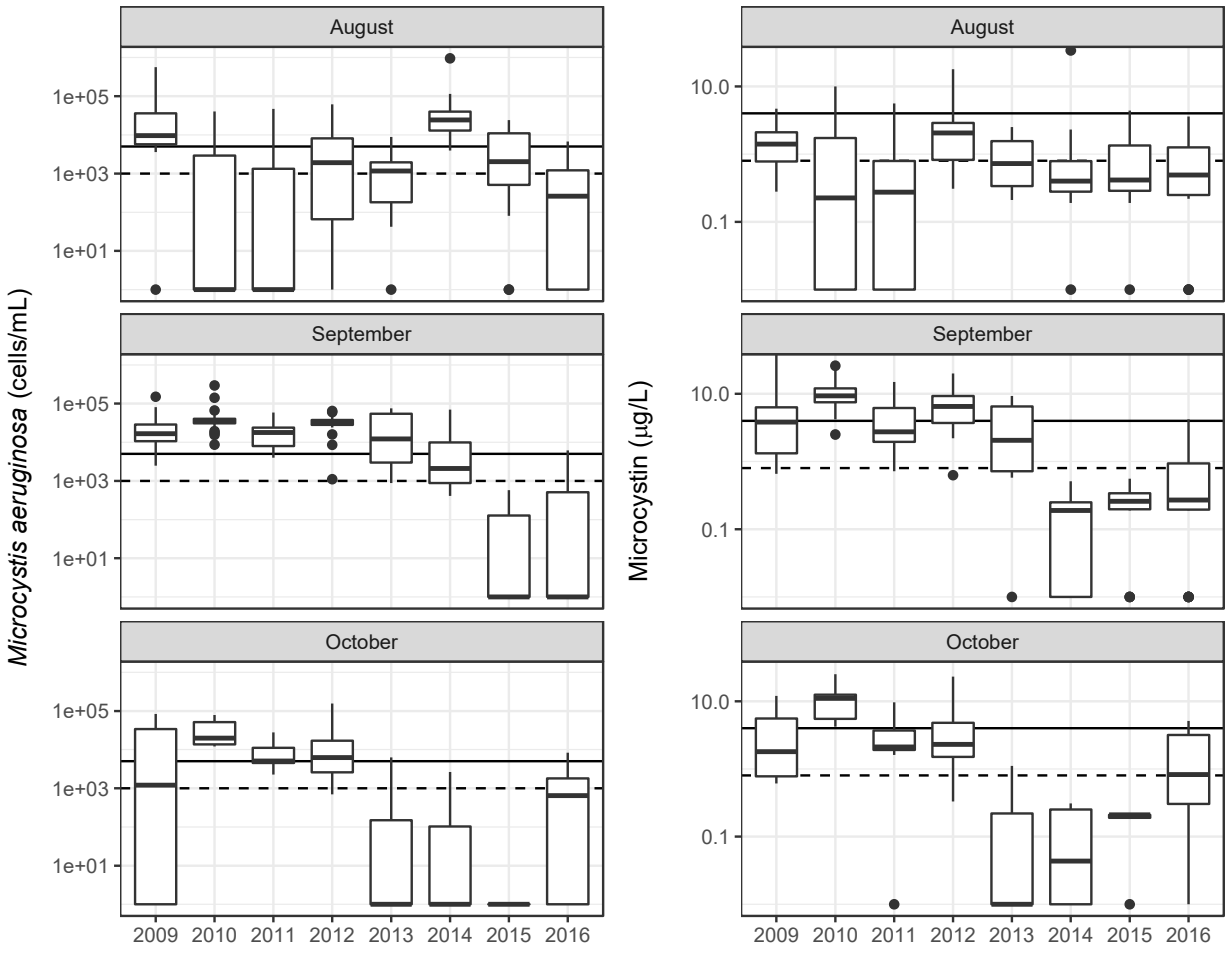

Figure 18. Boxplots of *Microcystis* cell density (left panels) and microcystin concentration (right panels) by year for Klamath River sites below Iron Gate Dam. Solid and dashed black lines are the level II and level I public health thresholds, respectively. Samples are shoreline grabs from public health monitoring.

3.2.2 WITHIN-DAY VARIATION IN CYANOBACTERIA CELL DENSITY, MICROCYSTIN TOXIN CONCENTRATION, AND HIGH-FREQUENCY OPTICAL MEASUREMENTS FROM SONDES

Variation in cyanobacterial cell density and toxin concentrations were common within a 24-hour period on the Klamath River below Iron Gate Dam. Within-day variation was evident during bloom periods at all sites sampled, from below Iron Gate Dam (KRBI) to the Klamath at Turwar (KAT). ISCO samples analyzed for microcystin toxin taken at KRBI on July 30 and 31 in 2013 show a range in the mean of the three replicate samples from less than 0.8 (the Tribal level I public health advisory) to more than 20 µg/L of microcystin (five-times the Tribal level II public health advisory). A second sampling event in September of 2013 at KRBI showed within-day variation in microcystin from ~7 to ~16 µg/L (Figure 19). In 2014, the one sampling event with microcystin levels > 4 µg/L showed relatively consistent toxin concentrations over the 24-hour period (Figure 20), and during periods of low microcystin levels, variation in toxin was low.

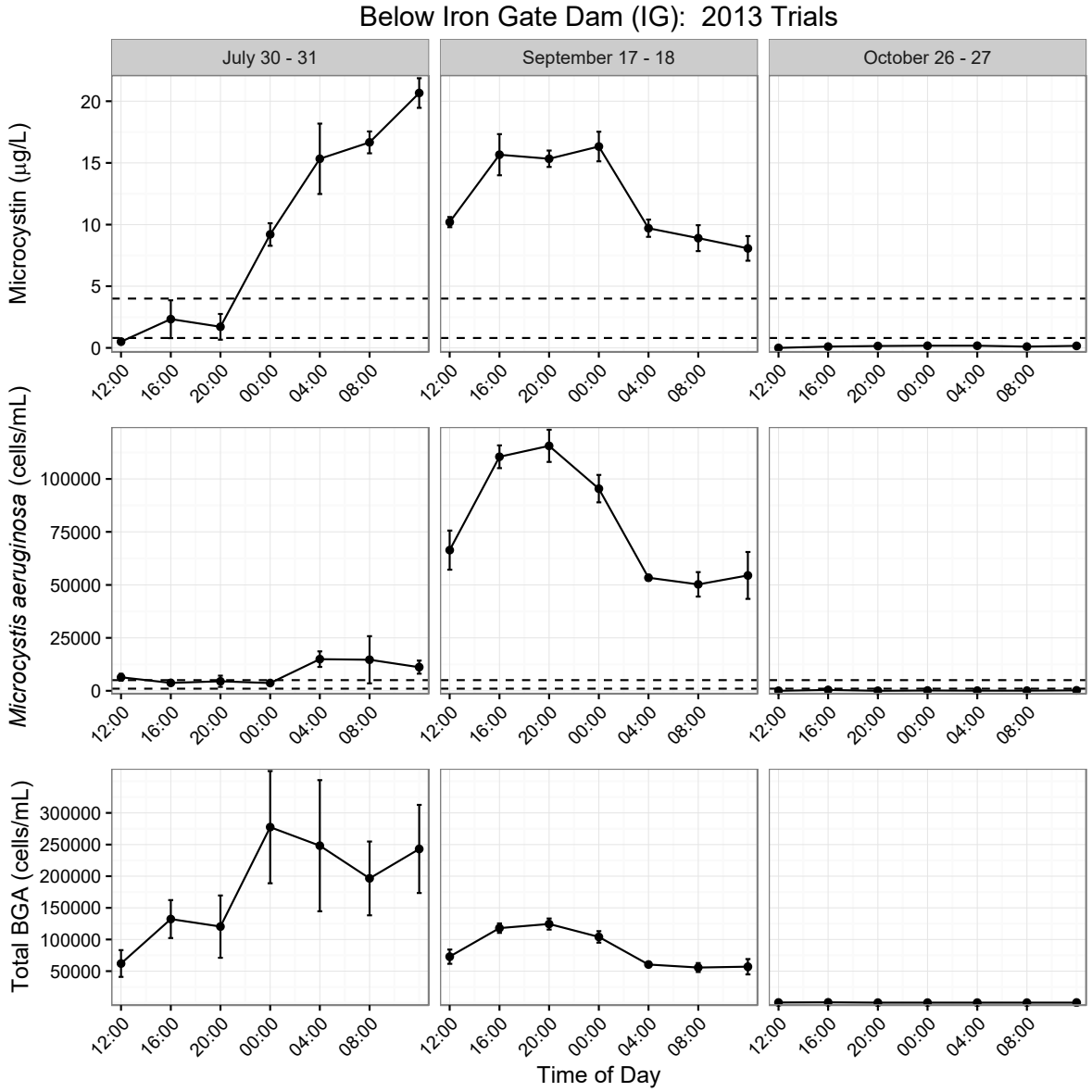

Figure 19. Microcystin toxin, *Microcystis aeruginosa* cell density, and total cyanobacterial cell density by time of day during three diel-sampling efforts on the Klamath River below Iron Gate Dam in 2013. Black points are the mean of three replicates and error bars show +/- one standard error of the mean. Grey background indicates night-time hours.

Variation in toxin concentrations was reflective of variation in cell counts during the 2013 sampling. The variation in toxin followed the variation in total cyanobacterial cell counts rather than only the *Microcystis* cell counts (Figure 19), possibly driven by high levels of *Gloeotrichia* in the July 2013 samples, which can also produce microcystin. In October when total cyanobacterial cell density and *Microcystis* cell densities were low, toxins were also low (< 1 µg/L microcystin). Triplicate samples collected in 2013 further confirmed that daily variation in cells and toxins was not a product of sampling or lab variability (Table 3).

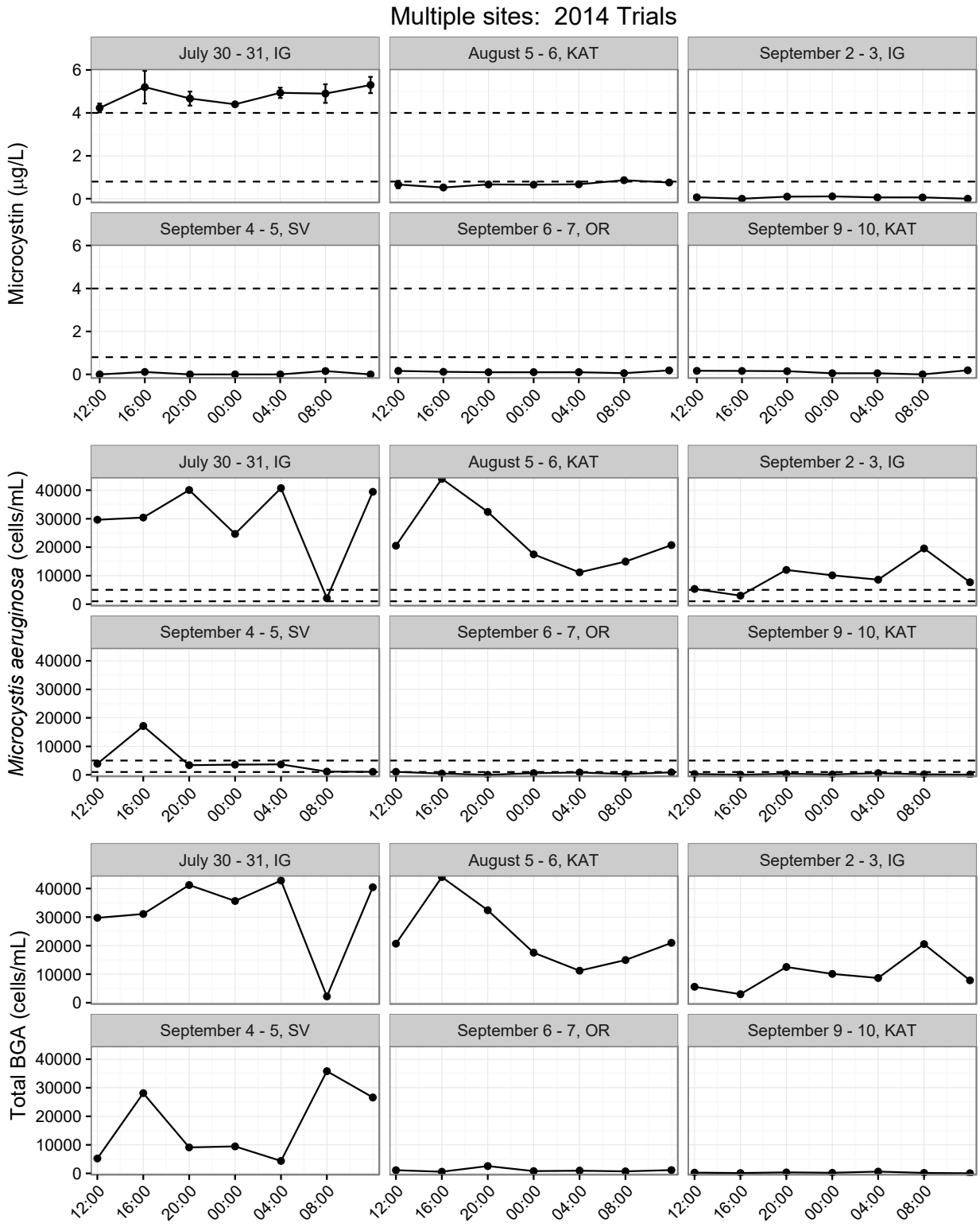

Figure 20. Microcystin toxin, *Microcystis aeruginosa* cell density, and total cyanobacterial cell density by time of day during six diel-sampling efforts on the Klamath River below Iron Gate Dam, at Seiad Valley, at Orleans, and at Turwar in 2014. For microcystin, black points are the mean of three replicates and error bars show +/- one standard error of the mean. Cell densities are based on single samples. Grey background indicates night-time hours.

Table 3. Duplicates of microcystin, *Microcystis* (MSAE), and total cyanobacteria (Total BGA) taken during ISCO sampling by date, site, ISCO position, and sampling event.

Date	ISCO Site	ISCO Event	Microcystin (µg/L)	Microcystin Duplicate (µg/L)	MSAE (cells/mL)	MSAE Duplicate (cells/mL)	Total BGA (cells/mL)	Total BGA Duplicate (cells/mL)
2013-07-30	KRBI_1B	1	7.4	0.48	4085	4672	110375	41444
2013-07-30	KRBI_3C	1	5.4	3.8	5164	9734	76544	67931
2013-07-31	KRBI_7A	1	17	23	17410	16677	311024	380484
2013-09-18	KRBI_1A	2	12	9.6	82683	80971	94174	91247
2013-09-18	KRBI_7C	2	6.9	8.4	45100	67685	46350	72143
2013-10-27	KRBI_2B	3	0	0.15	34	1021	313	1367
2013-10-27	KRBI_5A	3	0	0.16	93	0	93	0
2014-07-30	KRBI_1C	6	5.4	3.9	NA	NA	NA	NA
2014-07-31	KRBI_4C	6	4.4	4.4	NA	NA	NA	NA

In 2014, cell counts were variable within the 24-hour sampling period and were not consistently reflected by toxin concentrations. *Microcystis* cell counts were above the Tribal level II public health advisory in four of the six sampling events, but toxin concentrations were only above the level II advisory during the July sampling event at KRBI (see below discussion on microcystin cell quota for further detail on 2014 dynamics). Variation in *Microcystis* cell density during that July sampling event ranged from ~40,000 to ~2000 cells/mL, despite relatively constant toxin levels ranging from ~4-5 µg/L microcystin.

Samples taken from within a 24-hour period were often much higher than the public health or baseline samples taken on near-by dates (Figure 21). Some diel samples collected below Iron Gate Dam in 2013 and 2014 from a 24-hour period were much higher than any standard samples collected during the monitoring season, by as much as three-fold. When baseline and public health grab samples were near 0, the diel samples generally reflected these no-detect values (Figure 21, Figure 22).

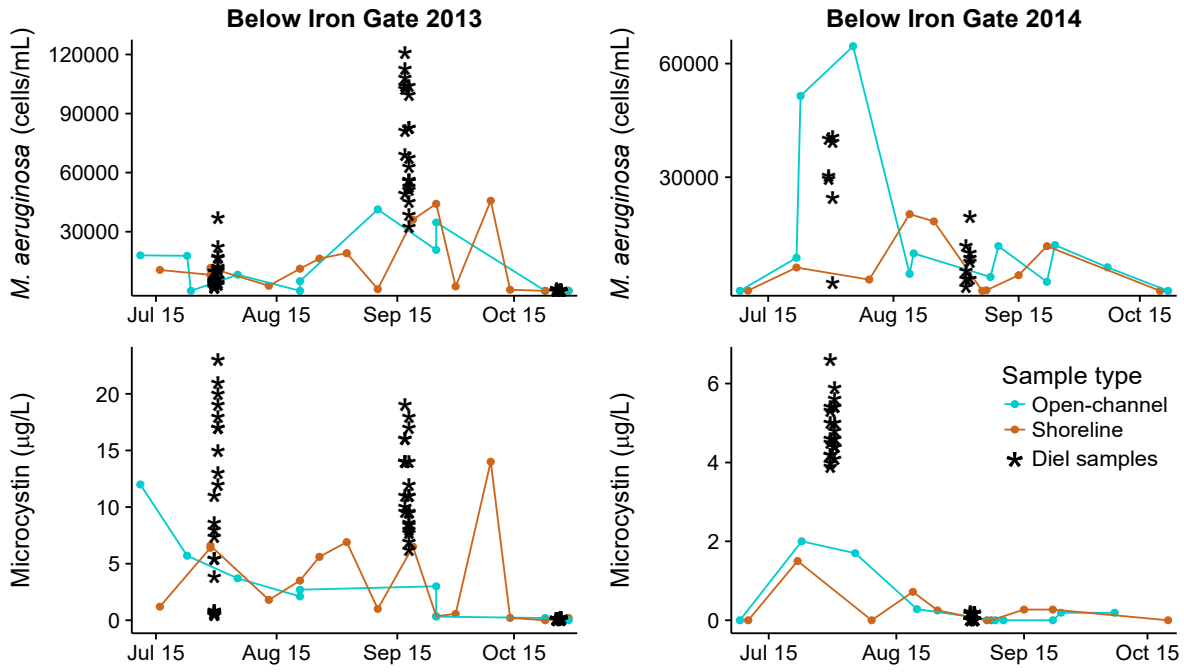

Figure 21. *Microcystis aeruginosa* cell density and microcystin toxin concentration from weekly and bi-weekly grab samples from the open channel (baseline samples, blue) and the shoreline (public health samples, brown) at Klamath Below Iron Gate in 2013 and 2014. Black asterisks show the cell counts and toxin concentration from the diel ISCO samples.

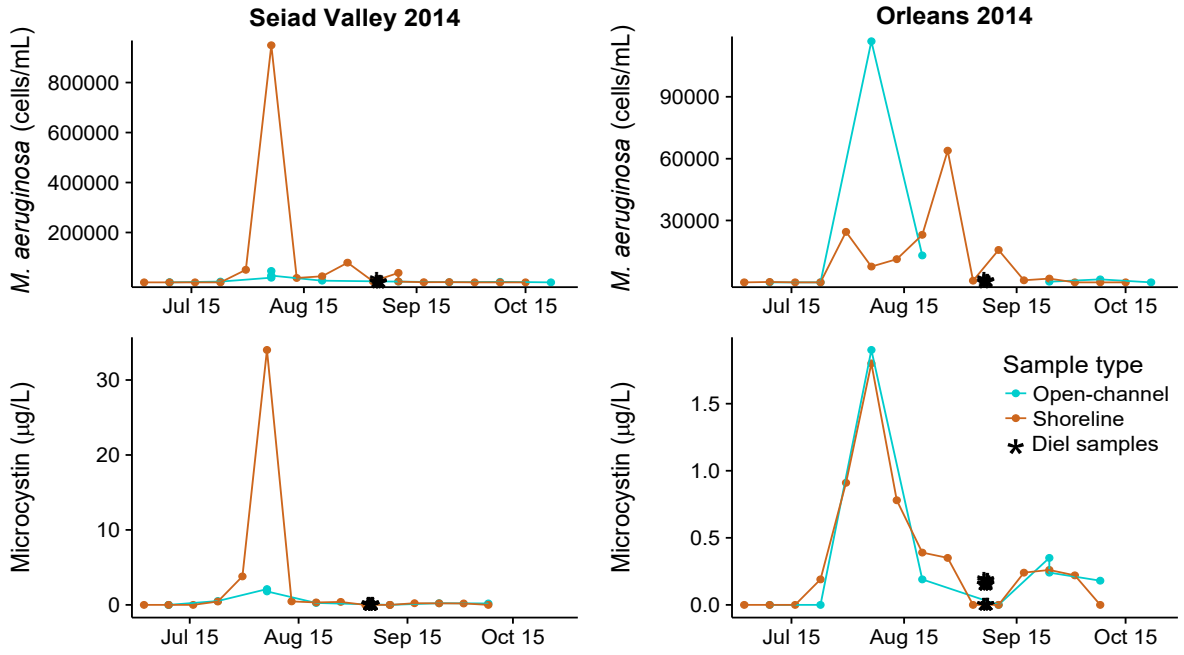

Figure 22. *Microcystis aeruginosa* cell density and microcystin toxin concentration from weekly and bi-weekly grab samples from the open channel (baseline samples, blue) and the shoreline (public health samples, brown) for Seiad Valley and Orleans from 2014. Black asterisks show the cell counts and toxin concentration from the diel ISCO samples.

Variation in estimated blue-green algae densities based on high-frequency measurements of phycocyanin pigment from data sondes (sonde-BGA) further suggest that diel variation in cyanobacteria is common in the Klamath River. The median difference in daily sonde-BGA data in August was approximately 5800 and 2200 at KRBI and SV, respectively. Course scale examination (i.e., examining an entire summer season of hourly data on one plot) of the sonde-BGA data revealed that within-day variation is present (Figure 23), and further examination of these short-scale time periods revealed somewhat regular daily patterns of variation (Figure 24, Figure 25). Further, the daily fluctuation at KRBI was mirrored at SV from September 29 to October 7 of 2011 with a lag of ~1.75 days (42 hours; Figure 24). These dates showed a daily low that transitioned to a daily high approximately12 hours later, and although this pattern was evident at both KRBI and SV, the minimum and maximum values, as well as the magnitude shifted between the two sites (Figure 24, Figure 25).

Examination of three-weeks of phycocyanin data from August of 2014 showed similar diel patterns at SV to those observed in 2011, although noise due to hourly fluctuation in the data made diel fluctuations less obvious at KRBI during this period (Figure 25). To reduce noise in the data from this three-week period in August of 2014, we grouped the hourly sonde values during each week (of the three-week period) into four-hour categories, and then used boxplots to examine the median and ranges of values of each four-hour period. At KRBI, phycocyanin values were higher from mid-night to late morning, and then generally decreased in the afternoon and evening. This pattern was more apparent during the first and third weeks of the analysis, with the week from August 13 to 18 showing a weaker diurnal pattern. At SV, the diurnal pattern in the sonde data was more distinct, where the weekly high medians occurred from 4-7:00 am for all three weeks and the weekly low medians occurred in the 4-7:00 pm hours (Figure 25).

Daily maximum cyanobacterial concentrations in the Klamath River at KRBI and SV were most common during the night and early morning. Although the timing and magnitude of daily maximum sonde-BGA levels were highly variable at times (and at times no apparent diel variation existed), eight years of daily data revealed diurnal patterns. In order to identify the most common periods in the daily high cyanobacterial concentration, we identified the hour of the day that the maximum sonde-BGA value occurred at KRBI and SV for July – October, using data from 2007 to 2014. Daily maximums occurred most often during the evening hours, through the night, and into mid-morning (Figure 26), while daily minimum values most often occurred from late-morning to mid-evening. This overall pattern indicates that an optimal public health sampling program for the Klamath River should target sample collection during early- to mid-morning hours.

Algal dynamics in the reservoir likely influence daily fluctuations in cell densities and toxin concentrations downstream. Factors such as light and nutrient supply control diurnal buoyancy dynamics in planktonic cyanobacteria. *Microcystis* colonies often move upward from the early morning until noon and sink from the surface in the afternoon and night (e.g., Cui et al. 2016). A limited study in Copco Reservoir showed a diurnal pattern of *Microcystis* concentration accumulating in the upper water column during the afternoon and early evening, and an increase in concentration at deeper depths in the early morning that may facilitate intake entrainment. (unpublished data; Moisander 2008). Such patterns could explain the observed nighttime increase in *Microcystis* cell density below Iron Gate Dam as cells sink downward at night making them increasingly susceptible to entrainment in the Iron Gate intake.

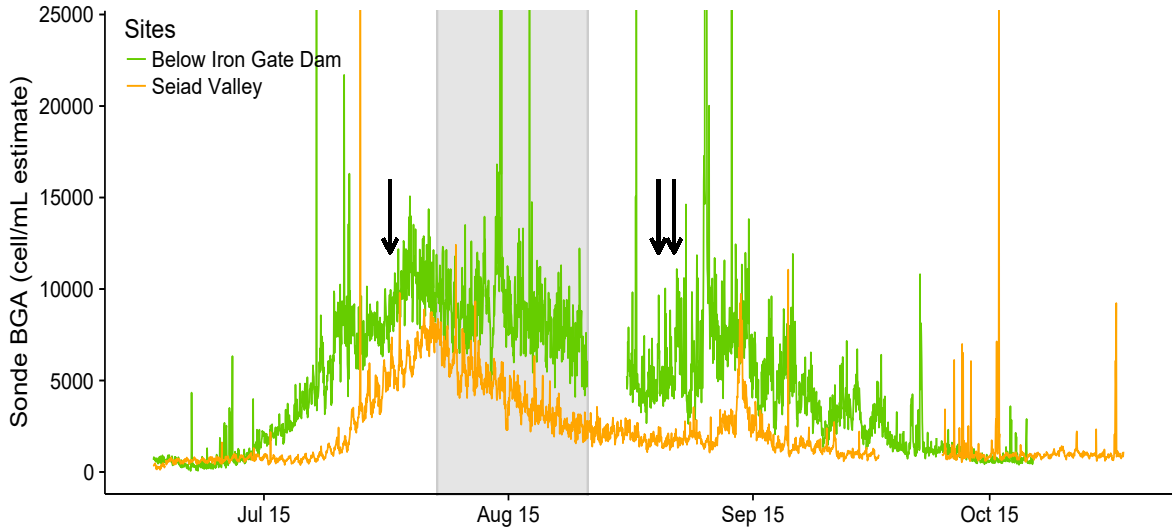

Figure 23. Hourly phycocyanin (sonde-BGA) as an estimate of cells/mL in the Klamath River below Iron Gate Dam (KRBI, green lines) and at Seiad Valley (SV, orange lines) for June through October, 2014. Black arrows indicate dates of diel ISCO sampling at either KRBI or SV, and grey background highlights the three weeks in mid-August when diel variation in sonde-BGA data was analyzed.

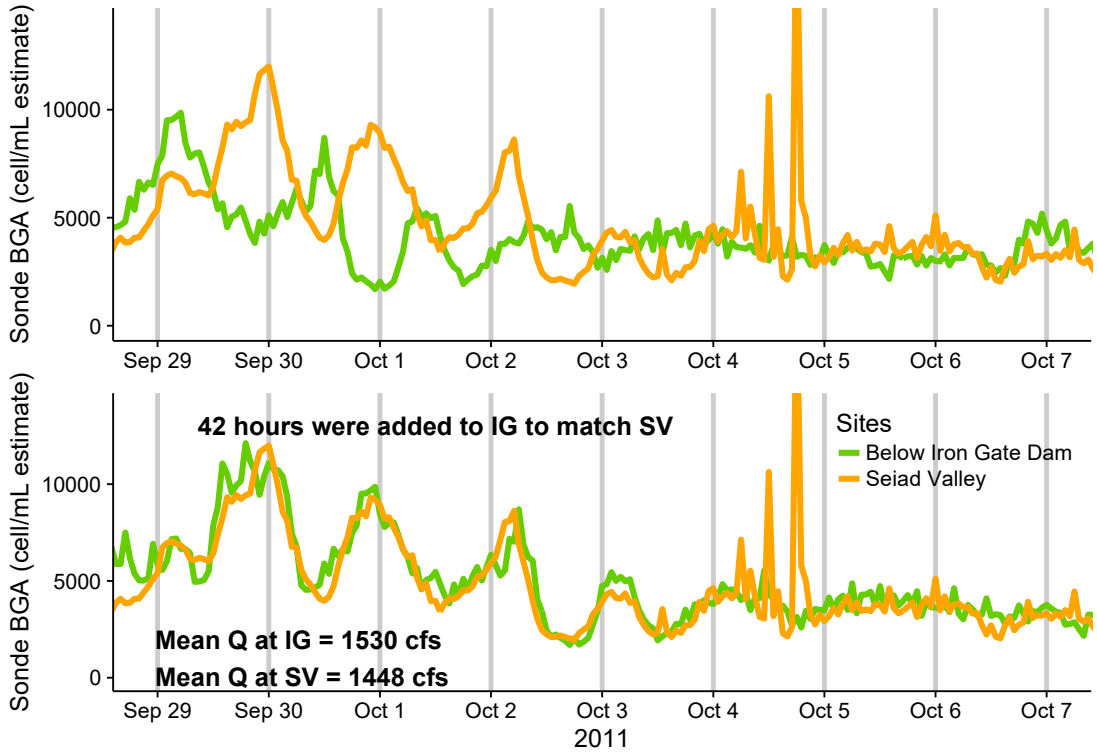

Figure 24. Hourly phycocyanin (sonde-BGA) as an estimate of cells/mL in the Klamath River below Iron Gate Dam (KRBI, green lines) and at Seiad Valley (SV, orange lines) for eight days in 2011 when SV closely mirrored KRBI. Adding 42 hours to the sample time at KRBI caused the data to line up, indicating that algal pulse arrived downstream ~1.75 days after passing KRBI.

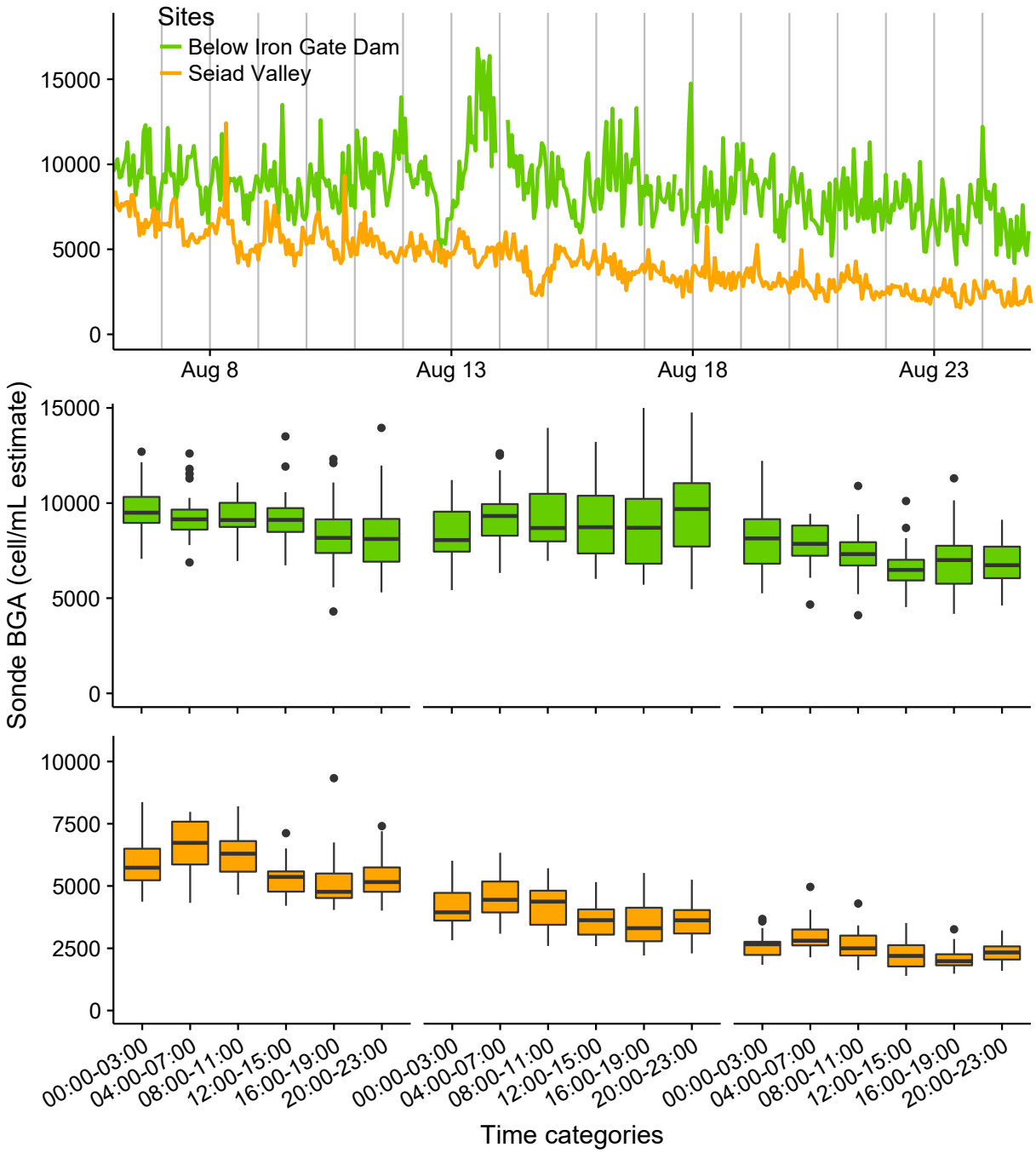

Figure 25. Hourly phycocyanin (sonde-BGA) as an estimate of cells/mL in the Klamath River below Iron Gate Dam (KRBI, green lines) and at Seiad Valley (SV, orange lines) with grey vertical lines showing mid-night each day. Box plots show hourly Sonde data binned in four-hour categories for one week per panel. KRBI is top 3 panels (green boxes) and SV is bottom 3 panels (orange boxes); blue background indicates night and white is day-time. Dates for boxplots correspond to dates in top panel directly above each boxplot panel.

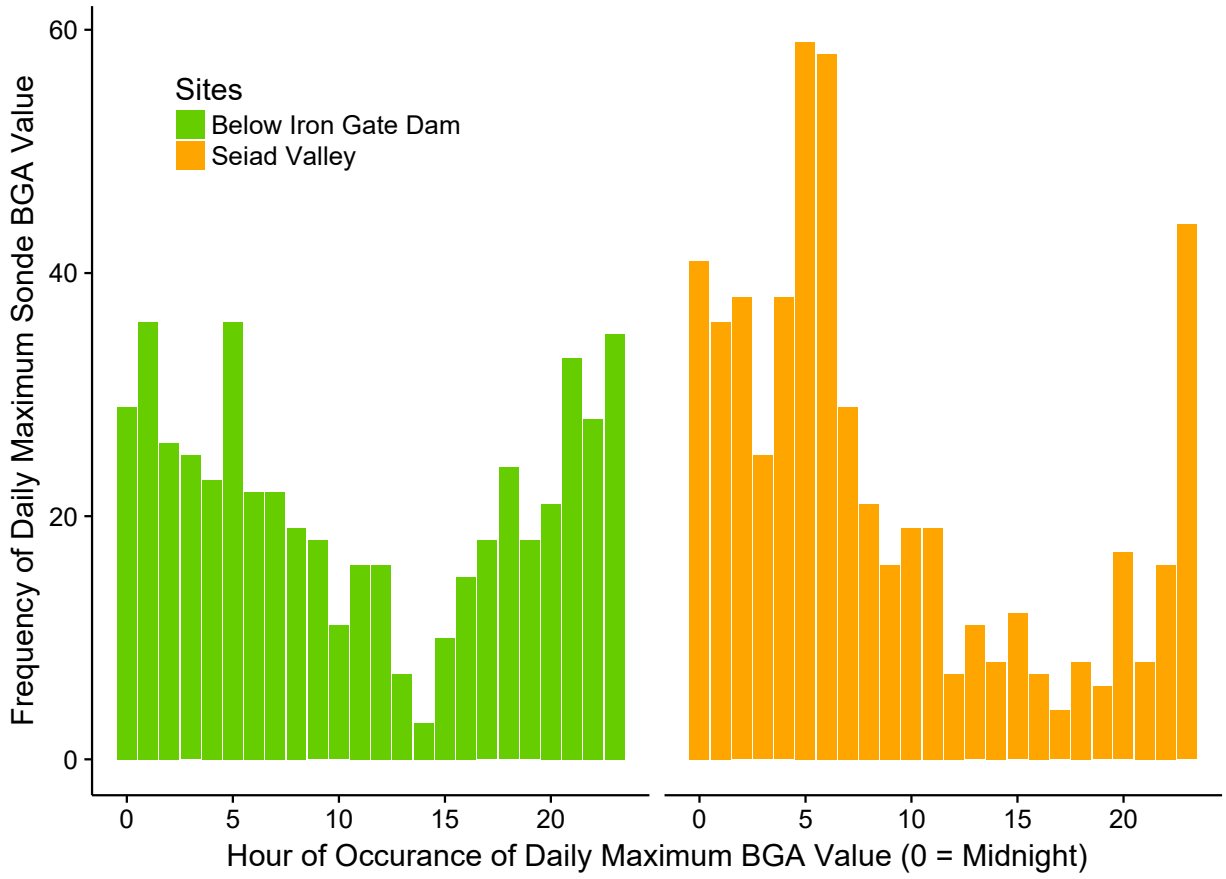

Figure 26. Frequency histograms showing the count of the daily maximum sonde-BGA values that occurred each hour of the day from July – October during 2007 – 2014 at Klamath River below Iron Gate (KRBI) and at Seiad Valley (SV).

Although the riverine diurnal patterns observed in the high-frequency sonde-BGA data are likely related to buoyancy patterns in the reservoirs, these patterns were not always consistent. Variation in nutrients and light due to cloud cover or turbidity (and even self shading from algal cells) can lead to varying vertical patterns in cell density from day to day (e.g., Oliver et al. 2012). Additionally, windy conditions in the reservoir may cause the cells to mix, changing their buoyancy dynamics and patterns of transference downstream. Other variation in cell and toxin concentrations may be due to the patchy nature of algal cell colonies, which can be clumped, and therefore result in variable densities of cells and toxins. A rapid increase in algal growth may also have the potential to dominate algal density dynamics over the influence of diurnal pattern. The cyanobacterial cells leaving the reservoir propagate downstream, thus reflecting the daily changes in the flowing river, which is well-mixed and not likely to experience buoyancy dynamics.

Within day variation in microcystin toxin and cyanobacterial cell density has implications for public health monitoring. Four of the nine diel ISCO sampling events showed some *Microcystis* samples below the Tribal level II advisory while other samples in the same 24-hour period exceeded the 5000 cell/mL threshold by as much as eight-fold. Depending on when samples are taken, dramatically different results occurred, which would lead to different posting

decisions, and thus Tribal members and the general public's choice to engage in water contact activities. Agencies should avoid sampling during the likely daily low periods and use conservative public health thresholds that are still protective in light of the daily variability. Further studies of reservoir algal dynamics may help managers to predict when regular diel variation would be expected, while more in-depth riverine studies would help predict how the algal pulses travel downstream, and if riverine dynamics have the potential to influence the diel variation.

3.3 RELATIONSHIP BETWEEN CYANOBACTERIAL CELL COUNTS AND TOXIN CONCENTRATIONS

3.3.1 INTERANNUAL AND LONGITURDINAL PATTERNS

Microcystin toxin increased with increasing cell density in the Klamath River, although the relationship between toxin concentration and cell density was variable. The relationships between cell density and toxin concentration were similar among sites on the Klamath River below Iron Gate Dam, and among reservoir and river sites. Seasonal variability was evident in the ratio of microcystin toxin to *Microcystis* cell density, but the largest source of variation was among years, where both toxin-to-cell ratios (toxin cell quota), as well as the seasonal pattern of these ratios, were highly variable.

Increased *Microcystis* cell density generally resulted in increased microcystin toxin, although the resulting toxin levels were highly variable, and at times toxin concentrations were very low, even with high *Microcystis* cell densities (Figure 27). This pattern resulted in a relationship best described using quantile regression on the upper (0.9) quantile of the data, where an upper limit to toxin production is likely based on a physiological maximum that *Microcystis* cells can produce due to the genetic strains and environmental conditions present over the last decade (Bozarth et al. 2010, Otten et al. 2015; Otten and Dreher 2017). Although factors such as light intensity can modulate toxic gene expression, environmental factors controlling expression of toxin producing genes are poorly understood (Makower et al., 2014). It is important to note that novel genetic strains or environmental conditions could potentially increase this upper limit in future years. Noting the patterns in cell density and toxin concentrations at the upper end of the distribution is important because these high levels pose the largest risk to public health.

Although low *Microcystis* cell density always resulted in low toxin concentrations (rarely >4 µg/L), higher cell densities resulted in a range of toxin concentrations. Cells of microcystin producing species[8] need to be present, or recently present, for toxin production. Therefore, high toxin and low cell counts would only be expected if extra-cellular toxin was present (possibly washed downstream after cells had settled out of the water column or with cell lysis), or, if other microcystin producing species were present that were not considered in this analysis. These factors may also help to explain times when moderate to high *Microcystis* cell density was paired with microcystin concentrations higher than predicted by the 0.9 quantile regression, as occurred a few times in the Klamath River system during the study period (Figure 27). Conversely, low

[8] For the Klamath River system these include *Microcystis*, *Gloeotrichia*, *Planktothrix*, and *Anabaena*.

levels of microcystin often occurred with high *Microcystis* cell density. Toxin levels below and at the detection limit of 0.18 µg/L regularly occurred in reservoir and river sites at cell densities up to approximately 100,000 cells/mL. In the river, few samples of *Microcystis* were above 100,000 cells/mL, so it is unknown if very low toxin concentrations would continue to be present with higher cell densities. At reservoir sites, where higher cell densities were common, toxin concentrations were nearly always elevated at cell densities above 100,000 cells/mL, although the variation in toxin concentration above this cell density was on the scale of four orders of magnitude (i.e., could range from 1 to 1000 µg/L).

Seasonal variation was present in the microcystin cell quota, but interannual differences were stronger drivers of variation. Although highly variable, cell quota tended to be lower in August and September in reservoir and river sites over the 2008 to 2016 period (Figure 28). This weak seasonal pattern was present in the Klamath River below Iron Gate in many years, but not all, and in 2015 and 2016 the reverse occurred, with September displaying the highest seasonal toxin to cell ratios (Figure 29, Figure 30). We did not attempt to assess the seasonal cell quota patterns among years in the reservoirs because mid-summer reservoir public health data was not collected from 2011 to 2013.

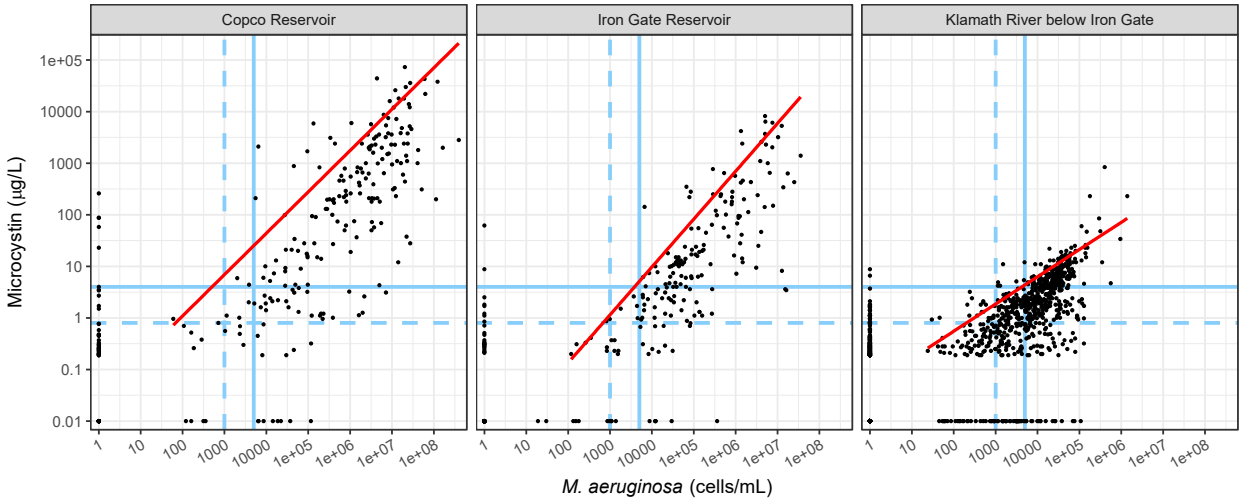

Figure 27. Microcystin toxin (µg/L) by *Microcystis aeruginosa* (cell/mL) for Copco Reservoir, Iron Gate Reservoir, and the Klamath River below Iron Gate using data from all available public health monitoring stations as well as baseline stations in the river for all years. Solid and dashed blue lines are the level II and level I public health thresholds, respectively. Red lines are the 0.9 quantile regression.

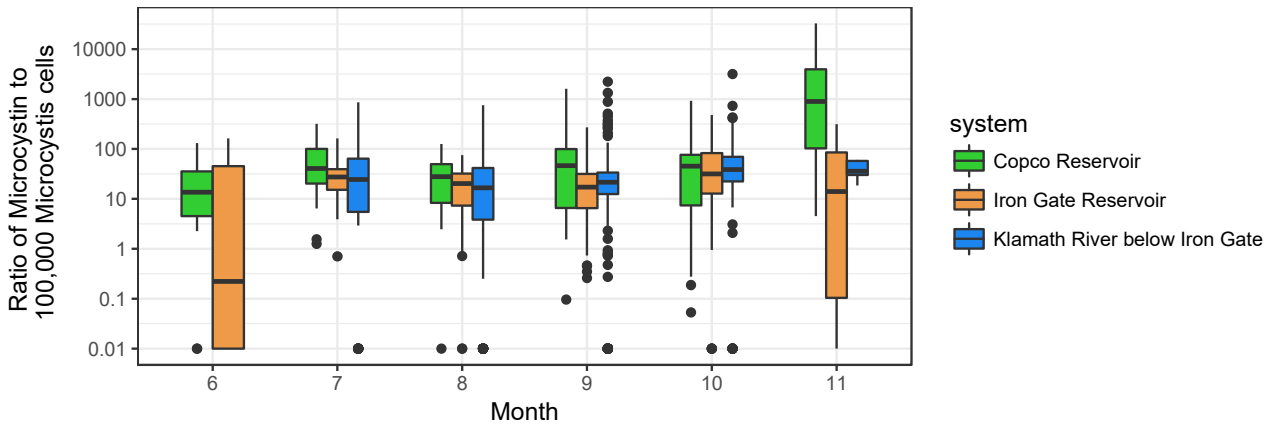

Figure 28. Monthly boxplots of the ratio of Microcystin (µg/L) to 100,000 cells/mL of *Microcystis* for Copco Reservoir, Iron Gate Reservoir, and the Klamath River below Iron Gate using data from all years and sites.

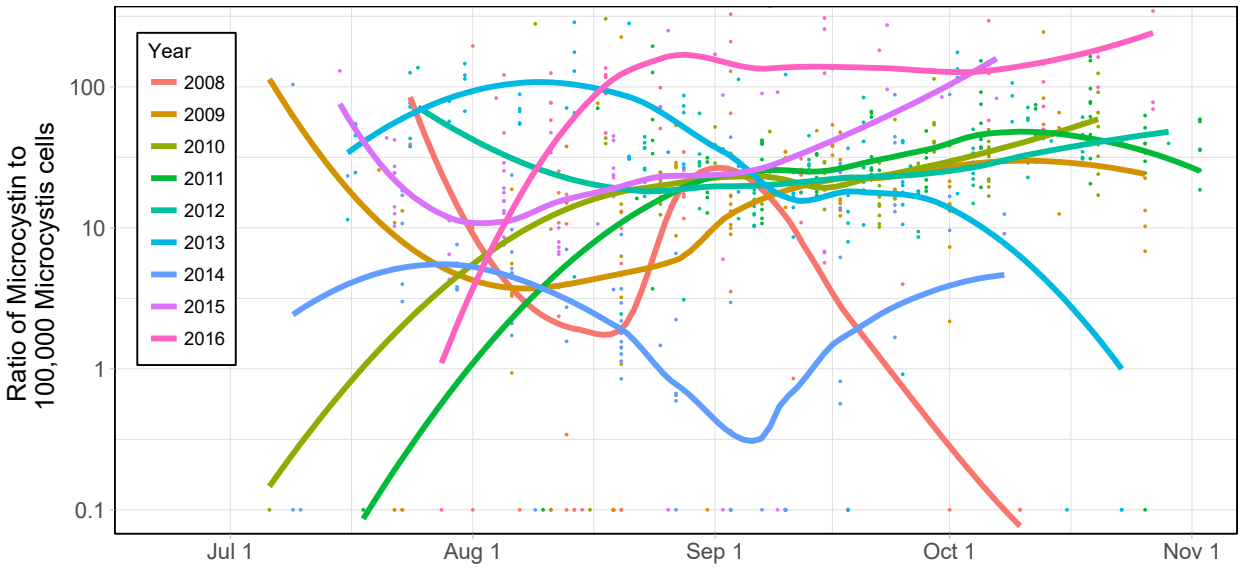

Figure 29. Seasonal LOESS of the ratio of Microcystin (µg/L) to 100,000 cells/mL of *Microcystis* by year in the Klamath River below Iron Gate Dam.

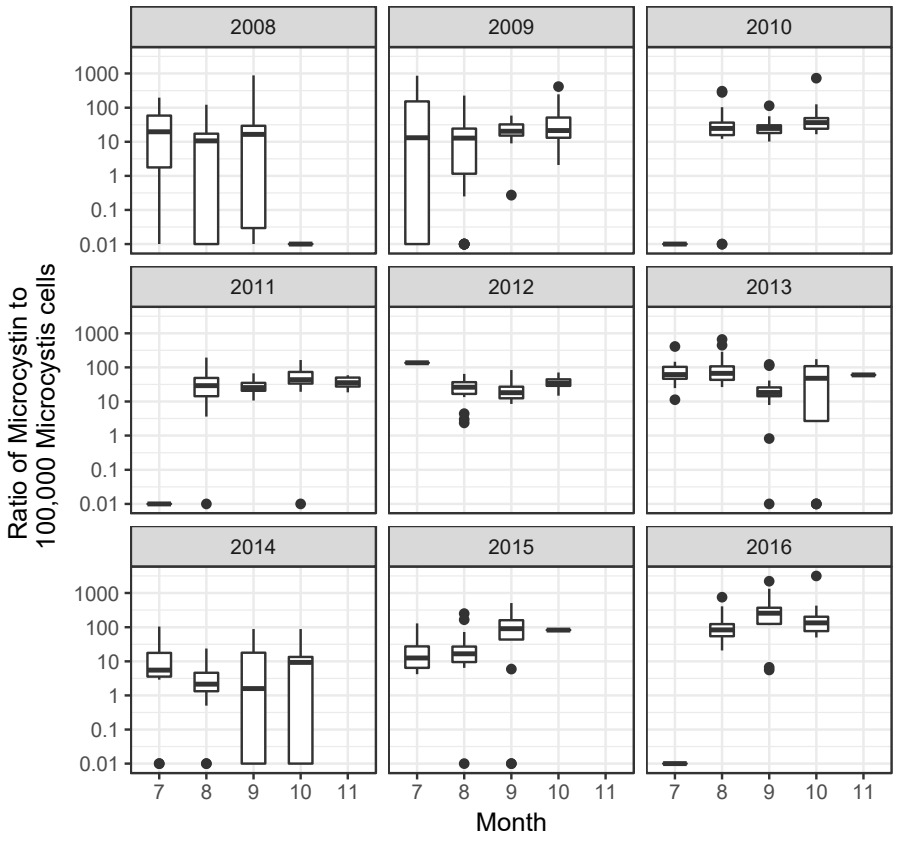

Figure 30. Monthly boxplots of the ratio of Microcystin (µg/L) to 100,000 cells/mL of *Microcystis* for the Klamath River below Iron Gate using data sites and all sample types.

The overall microcystin cell quota was variable among years, with median quotas lowest in 2014, followed by 2007 and 2008. Subsequent to the 2014 low, cell quota reached the highest values within the 10-year period in 2016 (Figure 31). For 2007, cell quotas were intermediate (Figure 31), which was likely driven by the genetic shift that occurred between July and September when toxigenic potential (mcyB copy number) was lower during the September peak than it was for July (Bozarth et al. 2010). QPCR results from Otten and Dreher (2017) tend to corroborate observations from these microcystin cell quota data, showing that Copco and Iron Gate reservoirs undergo periods of higher and lower toxigenicity. For example, 2014 had a strong reduction in toxigenicity relative to several years prior (Otten and Dreher 2017).

In addition to the overall interannual cell quota variation (e.g., Figure 31), there was also interannual variation in the shape of the relationship between cell density and toxin levels. For example, in 2012 and 2013, the relationship between *Microcystis* and microcystin toxin was relatively linear, with few occurrences of high cell densities and corresponding low toxin levels (Figure 32). In addition, more recent years tended to show greater scatter in the relationship between cell density and toxin concentrations, with cell densities being a poorer predictor of toxin concentrations at moderate to high cell densities (Figure 32). This may be due to multiple genetic strains present in these more recent years, which lead to variable toxin production, or environmental stresses causing cells to produce fewer toxins (e.g., Bozarth et al 2010; Otten and Dreher 2017).

The highest levels of toxins and cells counts were variable among years, as indicated by variation in the slope and intercept of the upper 0.9 quantile. During many years (2009, 2011, 2012, 2013, 2016), the 0.9 quantile crossed the level II public health threshold at very near the intersection of the 5000 cell/mL and 4 µg/L levels, indicating that 10% of samples were predicted to exceed 4 µg/L when cell density was 5000 cells/mL. In 2010, this intersection point was elevated to ~6.8 µg/L, indicating more toxin per cell in the upper quantile of the data, while in 2014 and 2015 the intersection point was lower (1 and 2.4 µg/L, respectively), indicating less toxin per cell in the upper quantile in these years (Figure 32).

Copco and Iron Gate reservoirs generally showed higher microcystin cell quotas than river sites. For example, the slope of the 0.9 quantile regression was steepest at Copco, and both reservoirs had steeper slopes of the 0.9 quantile regression line compared to the river sites (Figure 27). Median microcystin cell quota was highest in Copco Reservoir during all months, while cell quotas were more similar between Iron Gate Reservoir and river stations (Figure 28). The similarity between toxin quotas in Iron Gate and the Klamath River below Iron Gate Dam are expected based on genetic research showing that underlying genetic heterogeneity, which can play a major role in determination of toxin levels, was lower between Iron Gate *Microcystis* and those below the dam, than it was between Copco Reservoir and Iron Gate Reservoirs (Otten et al. 2015). For example, Otten et al (2015) via phycological genetic and toxin analyses identified distinction between the population dynamics within Copco and Iron Gate reservoir, but not between those of Iron Gate Reservoir and the river downstream (Otten et al. 2015).

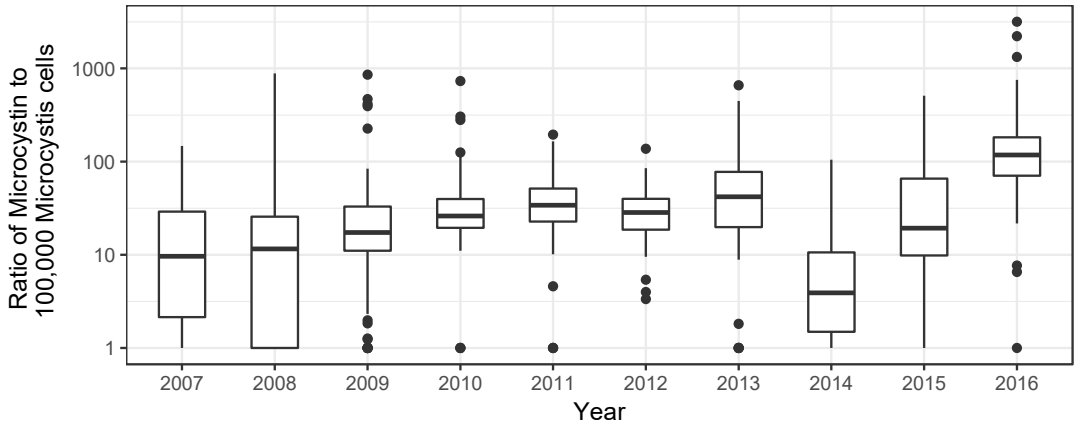

Figure 31. Annual boxplots of the ratio of Microcystin (µg/L) to 100,000 cells/mL of *Microcystis* for the Klamath River below Iron Gate using data from all river sites and both sample types (OC and SL) from July through October.

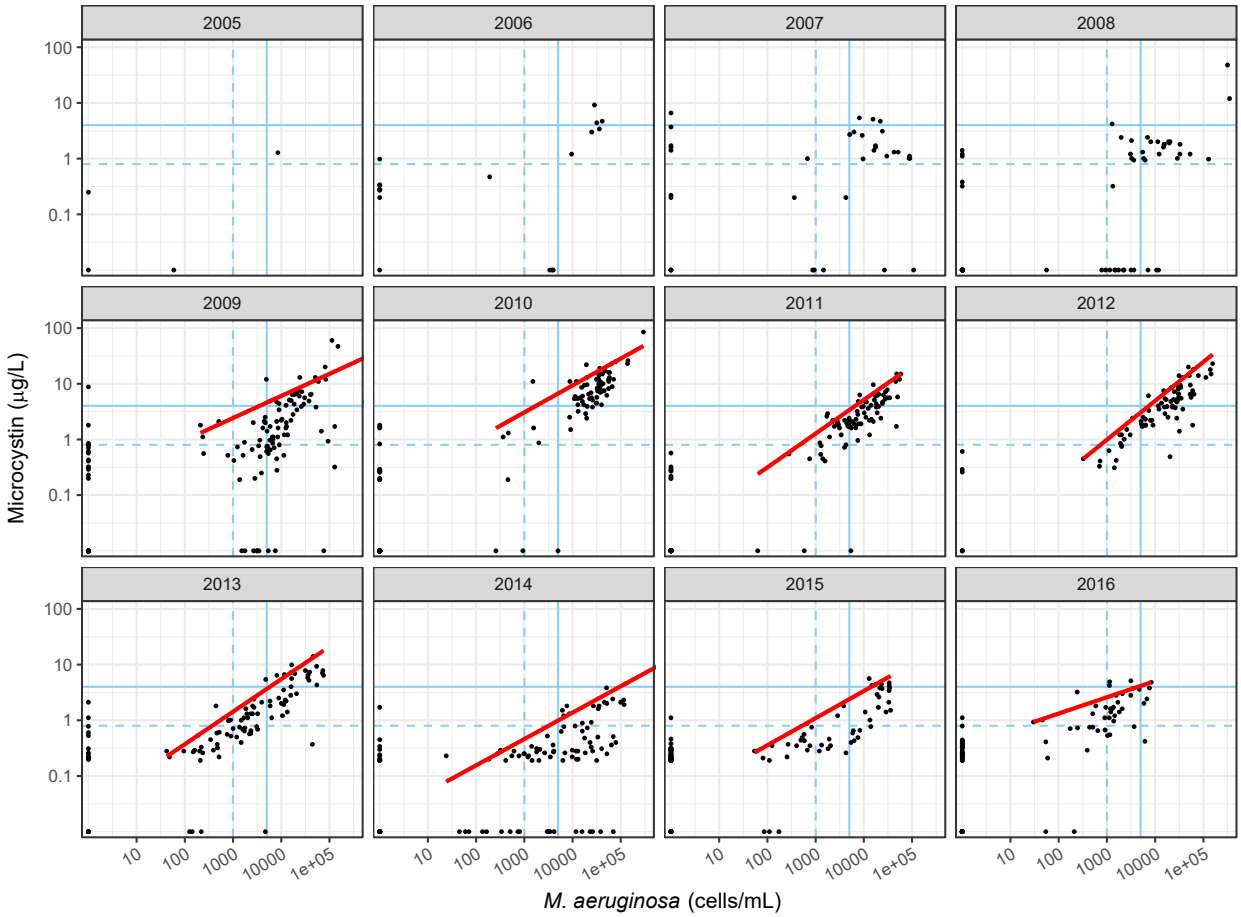

Figure 32. Microcystin toxin (µg/L) by *Microcystis* aeruginosa (cell/mL) for the Klamath River below Iron Gate using data from all available public health monitoring stations as well as baseline stations. Solid and dashed blue lines are the level II and level I public health thresholds, respectively. Red lines are the 0.9 quantile regression.

Although the relationship between cell density and toxin concentration was similar among Klamath River sites below Iron Gate Dam, there appeared to be a slight longitudinal decrease in the upper end of the distribution (Figure 33). This pattern was further confirmed by the 0.9 quantile regression predicting microcystin values above 4 µg/L at the most upriver sites (KRBI, IB, BB), near 4 µg/L at SV, and values less than 4 µg/L at the downriver sites of HC and OR when *Microcystis* cell density was 5000 cells/mL (Figure 34). Median toxin-to-cell ratios were similar longitudinally, with apparent longitudinal trends likely being attributed, in part, to sample type, rather than position along the river (open-channel vs. shoreline grabs, Figure 33). The longitudinal decrease in the upper quantile of the data may be explained by less optimal growing environment in the river causing the cells to not maintain maximum toxin production.

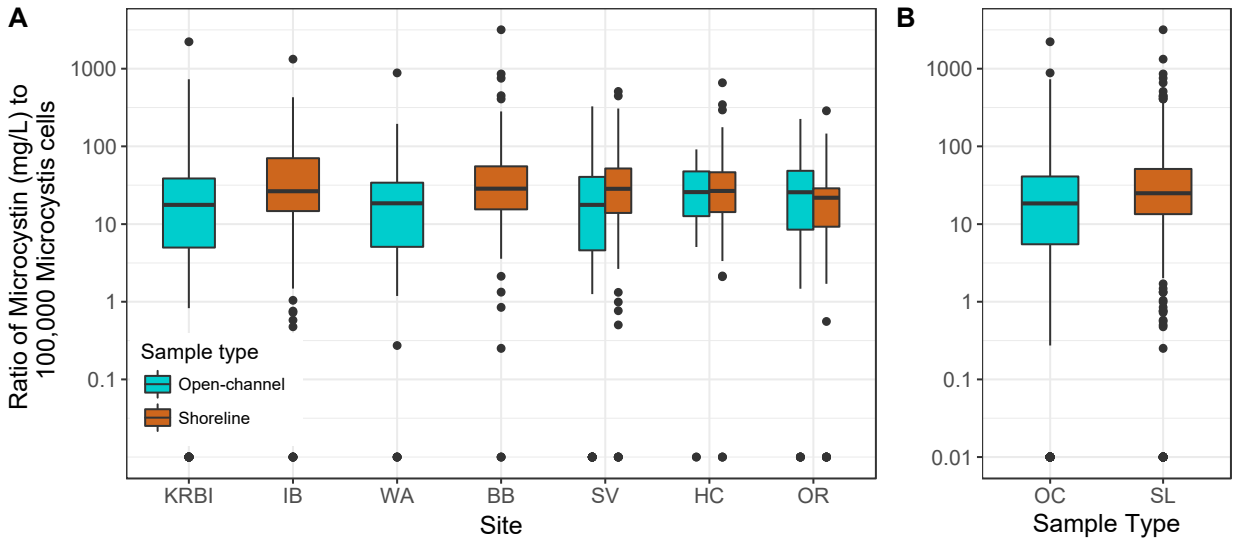

Figure 33. Boxplots by site (panel A) and sample type (panel B) of the ratio of Microcystin (µg/L) to 100,000 cells/mL of *Microcystis* for the Klamath River below Iron Gate using data from July through October for all years.

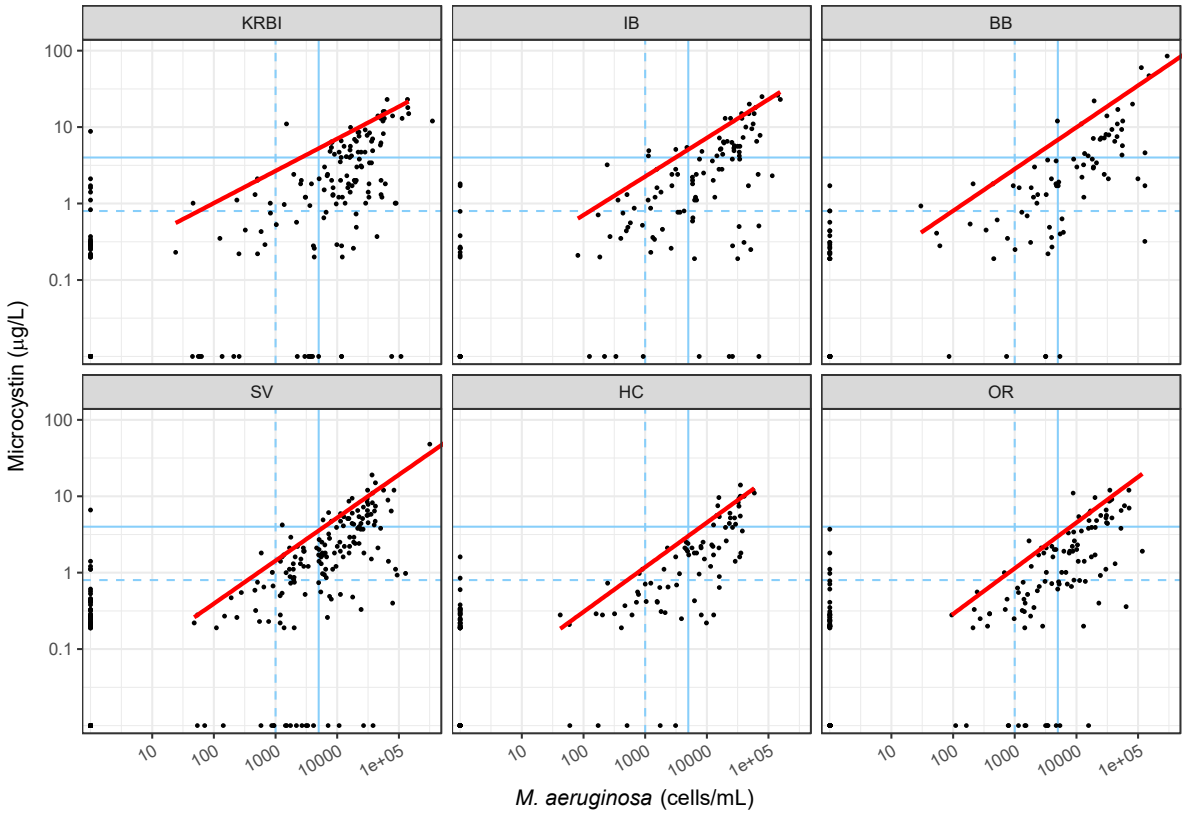

Figure 34. Microcystin toxin (µg/L) by *Microcystis* aeruginosa (cell/mL) for sites along the Klamath River below Iron Gate using data from all available public health monitoring stations and baseline stations for all years. Solid and dashed blue lines are the level II and level I public health thresholds, respectively. Red lines are the 0.9 quantile regression.

3.3.2 ESTIMATING PROBABILITIES OF EXCEEDING PUBLIC HEALTH THRESHOLDS FOR MICROCYSTIN BASED ON *MICROSYSTIS* CELL DENSITY

The above 0.9 quantile regression for the Klamath River below Iron Gate Reservoir can be used to predict the *Microcystis* cell densities at which 10% of the microcystin toxin samples exceeded the Tribal public health advisories[9] (Figure 27). For example, the 0.9 quantile regression of microcystin toxin data as predicted by cell density exceeded the Tribal level I public health advisory of 0.8 µg/L microcystin at a level of ~300 cells/mL, and exceeded the Tribal level II public health advisory of 4 µg/L at a level of ~5000 cells/mL (Figure 27). In other words, when the *Microcystis* cell density was 5000 cells/mL, 10% of the toxin samples were predicted to be above 4 µg/L. Quantile regression provides a means to evaluate predictive relationships with parts of the response variable distribution even when there may be a weak or no predictive relationship between the mean of the response variable and the predictive variable (in this case *Microcystis* cell density) or when the underlying assumptions of linear regression such as homogeneity of variance may not be met (Cade and Noon 2003). This method was previously employed to determine phycocyanin levels[10] that were protective of public health thresholds for microcystin toxin and *Microcystis* cell density on the Klamath River (Genzoli and Kann 2016).

As described in Genzoli and Kann (2016), aside from quantile regression, other non-parametric probability methods that compute the percent exceedances of a particular level of a response variable within a given range of a predictive variable (e.g., Kann and Smith 1999) can also help determine public health thresholds. Such nonparametric cross-tabulation models have been used successfully for establishing thresholds for algal bloom frequencies, phosphorus criteria, and pH (Heiskary and Walker 1988, Havens 1994, Walker and Havens 1995; Kann and Smith 1999), and require no assumptions about the shape or functional form of the underlying relationships (Kann and Smith 1999). Additionally, the probability of exceedance analyses allow for flexibility in user interpretation by including the full range in probabilities (0-100%) and a range of public health thresholds.

In this case the probability of exceedance analysis was combined with a contouring algorithm to produce a tool allowing the *Microcystis* cell density that corresponds to any chosen probability (frequency) of exceedance value of various public health thresholds for microcystin toxin concentration to be determined (Figure 35). For instance, if one is interested in the cell density at which the Tribal level II public health threshold for microcystin (4 µg/L) was exceeded 10% or 20% of the time, the analysis shows that this occurred at 5000 and 8000 cells/mL, respectively (Figure 35). Alternately stated, the level II public health advisory was exceeded 10% of the time at a *Microcystis* cell density level of 5000 cells/mL and 20% of the time at 8000 cells/mL. The 10% value of 5000 cells/mL from the probability analysis corroborates the 5000 cells/mL as computed from the quantile regression above. Another approach to determining protective values can be determined from the point above which probabilities increase rapidly (Genzoli and Kann 2016); for the probability of exceedance for the level I threshold this occurred at 1000 cells/mL *Microcystis*, and at 5000 cells/mL for the level II

[9] Regression analysis of the upper conditional quantile (in this case the 0.90 quantile) is more protective of public health than traditional regression on the conditional mean, and quantile regression does not require data to be normally distributed (Yu et al. 2003, Munir et al. 2011).

[10] Continuously collected at various Klamath River stations using YSI Sondes (see Genzoli and Kann 2016).

threshold. The current analysis utilizing a 12-year dataset arrives at similar values as derived previously when Karuk Tribe public health thresholds were developed using a 8-year database (Kann 2014).

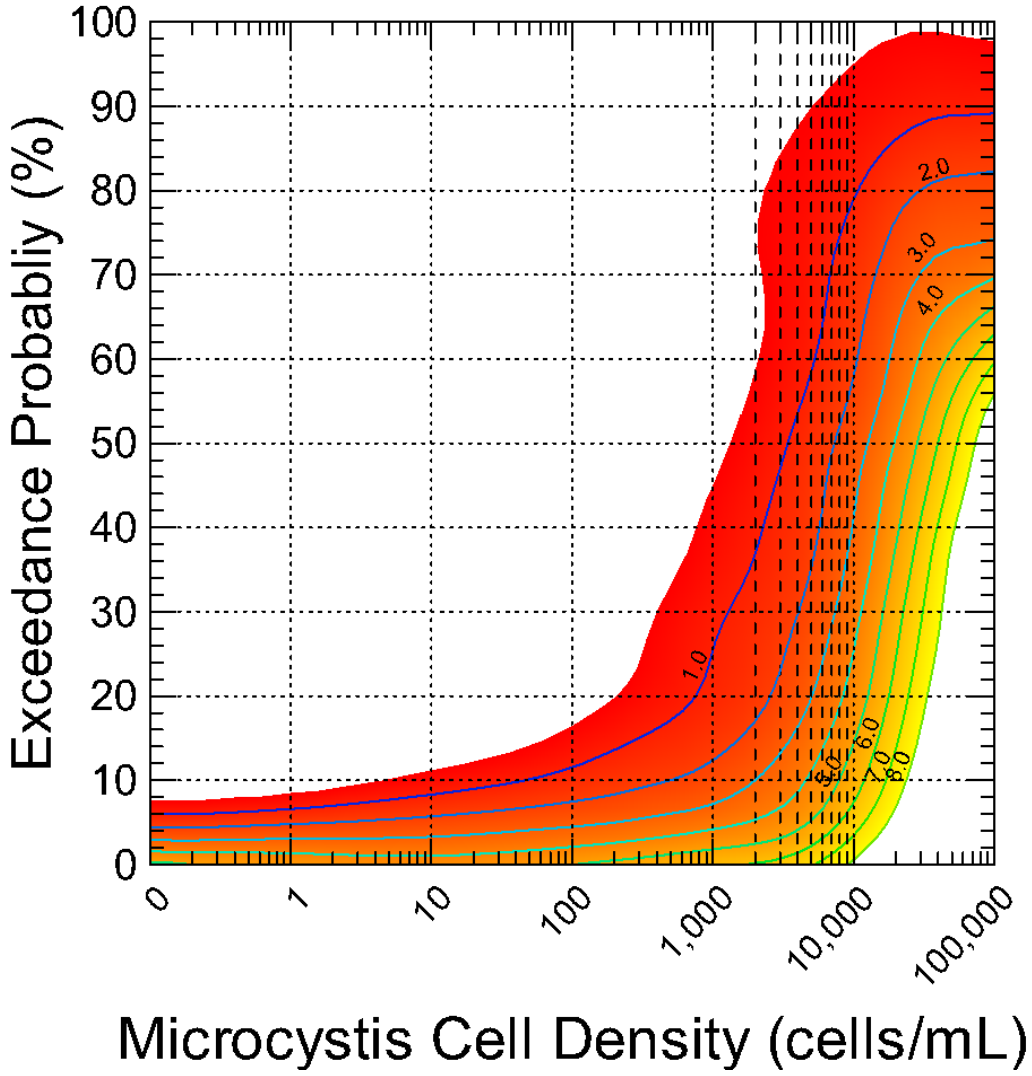

Figure 35. Probability of exceeding critical Klamath River microcystin levels at varying *Microcystis* cell densities. For any chosen cell density level (x-axis) the corresponding exceedance probability for a given microcystin toxin level is shown on the y-axis. Microcystin toxin levels are shown as shaded colors and range from 1 µg/L (red) to 8 µg/L (yellow). Computed probabilities of exceedance were calculated based on data from all Klamath River monitoring sites from 2007 to 2016.

The protectiveness of these public health thresholds and those in Kann (2014) is also confirmed by the relationship between *Microcystis* cell density and microcystin toxin concentration (Figure 27) showing that there are relatively few exceedances of the 0.8 µg/L and 4.0 µg/L public health warning levels at *Microcystis* cell densities at or below 1000 cells/ml and 5,000 cells/ml, respectively. The upper left quadrants defined by the intersection of 0.8 µg/L and 1000 cells/ml and 4.0 µg/L and 5,000 cells/ml in Figure 27 represent false negatives, which from a public health perspective should be avoided.

3.4 HISTORIC EXCEEDANCES OF PUBLIC HEALTH THRESHOLDS

The Klamath River below Iron Gate Dam regularly experienced public health exceedances during the last 12 years in which monitoring was performed for toxigenic *Microcystis aeruginosa* and the associated microcystin toxin. During July through October when *Microcystis* was most commonly present in the river, 56% of cell density samples exceeded the level I threshold and 40% of samples exceeded the level II public health threshold (

Table 4). Similarly, 48% and 20% of toxin samples exceeded the level I and II thresholds for microcystin toxin, respectively (Table 5). These percentages are based on over 1000 samples taken from both the open channel and along shoreline margins at seven river sites below Iron Gate Dam. September showed the highest percentage of exceedances for both parameters and both threshold levels, while July had the fewest exceedances during these summer months. During four years, more than half of July - October samples were above the level II public health threshold for *Microcystis* cell density (2007, 2009, 2010, and 2012), although only 2010 and 2012 had toxin samples exceeding the level II toxin threshold at similarly high percentages (

Table 4, Table 5).

Table 4. Percent of samples exceeding public health thresholds for *Microcystis* aeruginosa on the Klamath River using data from below Iron Gate Dam to Orleans by month. Means for all years ("Mean Years"), July – October ("4-mo Mean"), and total samples ("n(Jul-Oct)") are presented. Data is from combine baseline and public health monitoring samples.

Percent of samples exceeding public health thresholds: *Microcystis aeruginosa*											
	Level I threshold (1000 cells/mL)					***Level II threshold (5000 cells/mL)***					
Year	**Jul**	**Aug**	**Sep**	**Oct**	**4-mo Mean**	**Jul**	**Aug**	**Sep**	**Oct**	**4-mo Mean**	**n (Jul -Oct)**
2005	0%	50%	100%	0%	38%	0%	50%	100%	0%	38%	8
2006	25%	100%	67%	0%	45%	25%	100%	17%	0%	30%	20
2007	29%	100%	100%	33%	65%	29%	100%	100%	17%	59%	37
2008	15%	94%	71%	11%	61%	5%	76%	36%	0%	40%	90
2009	32%	93%	100%	67%	77%	11%	75%	88%	33%	56%	142
2010	0%	38%	100%	100%	64%	0%	31%	100%	96%	61%	128
2011	0%	33%	100%	100%	63%	0%	20%	84%	58%	44%	136
2012	0%	69%	97%	90%	76%	0%	43%	95%	71%	61%	124
2013	62%	50%	95%	5%	52%	34%	15%	68%	5%	30%	139
2014	34%	100%	68%	20%	58%	27%	89%	30%	4%	40%	140
2015	33%	68%	7%	0%	30%	31%	51%	0%	0%	23%	150
2016	0%	41%	19%	33%	24%	0%	5%	3%	7%	4%	128
Mean Years	23%	66%	74%	49%	56%	16%	47%	58%	31%	40%	1242

Table 5. Percent of samples exceeding public health thresholds for microcystin toxin on the Klamath River using data from below Iron Gate Dam to Orleans by month. Means for all years ("Mean Years"), July – October ("4-mo Mean"), and total samples ("n(Jul-Oct)") are presented. Data is from combine baseline and public health monitoring samples.

Percent of samples exceeding public health thresholds: *Microcystin toxin*											
	Level I threshold (0.8 µg/L)					***Level II threshold (4 µg/L)***					
Year	**Jul**	**Aug**	**Sep**	**Oct**	**4-mo Mean**	**Jul**	**Aug**	**Sep**	**Oct**	**4-mo Mean**	**n (Jul -Oct)**
2005	NA	NA	100%	0%	33%	NA	NA	0%	0%	0%	3
2006	100%	100%	18%	0%	46%	0%	89%	0%	0%	29%	28
2007	83%	100%	83%	25%	68%	17%	43%	0%	0%	11%	37
2008	62%	68%	52%	0%	54%	0%	3%	22%	0%	9%	79
2009	18%	50%	87%	59%	56%	4%	11%	51%	28%	25%	134
2010	0%	51%	100%	100%	67%	0%	13%	98%	91%	53%	126
2011	0%	26%	97%	97%	59%	0%	5%	44%	31%	22%	133
2012	0%	70%	95%	90%	85%	0%	20%	74%	32%	46%	92
2013	65%	53%	71%	5%	46%	26%	3%	35%	3%	16%	128
2014	26%	32%	0%	0%	17%	3%	3%	0%	0%	2%	126
2015	48%	31%	0%	0%	22%	15%	9%	0%	0%	6%	110
2016	0%	33%	28%	44%	26%	0%	0%	3%	11%	3%	125
Mean Years	27%	47%	60%	50%	48%	6%	10%	36%	22%	20%	1121

4 CONCLUSIONS

Microcystis and associated microcystin toxins were present seasonally in the Klamath River Below Iron Gate Dam during every year of this study (2005-2016) at levels above public health thresholds. *Microcystis* cell density was elevated in the Klamath River from July to November, and public health thresholds were most often exceeded in August, September, and October, when 65% and 48% of river samples exceeded the level I and level II public health thresholds, respectively. Klamath River *Microcystis* levels were highly seasonal, with distinct bloom periods generally lasting two to three months. Years with earlier bloom onset generally had earlier declines. In addition to distinct seasonal patterns in *Microcystis* in the Klamath River, the magnitude and duration of the blooms varied among years. More than 50% of July – October samples in 2007, 2009, 2010, and 2012 exceeded the level II public health threshold of 5000 cells/ml in the river, and the 0.9 quantile of *Microcystis* concentrations in those years exceeded level II public health threshold by more than eight times. Meanwhile 2015 and 2016 had lower bloom magnitudes, with 23% and 4% of samples exceeding the level II public health threshold in those years. Similarly, 48% and 20% of toxin samples exceeded the level I (0.8 µg/L) and level II (4 µg/L) thresholds for microcystin toxin, respectively. Exceedances of the level II threshold were lower (<6%) during 2015-2016 than it was for most previous years.

Although there were consistent exceedances of *Microcystis* and microcystin toxin at sites from below Iron Gate Dam to Orleans, there was a general downstream decline in toxigenic algae. Mean concentration of *Microcystis* at Orleans was 15% of the concentration at the Klamath River Below Iron Gate, although the number of samples that exceeded level II public health thresholds at Orleans only declined by 50% from the number of exceedances below Iron Gate. Due to initially high *Microcystis* concentrations below Iron Gate that were many times above the level II threshold, the large decline in cell concentrations in the 130 river miles from Iron Gate Dam to Orleans did not translate to an equally large decline in exceedances

Public health stations in the reservoirs showed substantially higher concentrations of *Microcystis* and microcystin toxin than down-river sites, but the Iron Gate base-line sampling station generally reflected only slightly higher concentrations than the river below Iron Gate Dam. *Microcystis* cell density in the Klamath River below Iron Gate Dam was roughly 70% of the concentration sampled above the Iron Gate Reservoir outflow at the log boom. However, public health samples taken at the water surface in the river show substantially lower concentrations (an order of magnitude) below Iron Gate when compared to public health samples taken in Iron Gate Reservoir.

Understanding the variation in samples of cell densities and toxins due to sampling and lab procedures, as well as the natural variation in the river, will help inform future sampling efforts and public health notifications. Microcystin toxin samples were more accurate than cell counts, although multiple types of samples are helpful in confirming the presence of bloom conditions that result in a public health risk. The median of the relative percent difference of the paired duplicates was approximately 20% for toxin samples versus 40% for cell counts, indicating more accuracy in toxin samples than cell counts. Sources of variation between duplicate samples can occur due to patchy distribution of algal cells and associated toxins within the churn-splitter, or counting errors and extrapolation during laboratory microscopy analyses.

Public health shoreline grab samples generally reflected the baseline samples from the open-channel, although shoreline samples were generally higher, and some shoreline samples reflected much higher concentrations of toxigenic algae than open channel grabs. Although shoreline samples generally reflected the open channel grabs, they differed by more than the difference expected based only on lab methods (approximately 97% median relative percent difference compared to 40% with paired duplicates). Shoreline samples are expected to have higher concentrations of *Microcystis* than open channel grabs because samples were collected from edge areas where entrapped cells concentrate.

Microcystis and associated toxins varied within a single day. Cell densities varied from as much as 50,000 to 100,000 cells/mL, while microcystin varied from less than 1 to over 20 µg/L. These changes were generally progressive throughout the day (versus spiking up and down) and the timing of high and low samples were often consistent. Examination of high-frequency phycocyanin data (sonde-BGA) suggested that higher concentrations of cyanobacteria are most common at KRBI and SV in the night and early morning, with lower concentrations in the late morning and early afternoon. Large within day changes in cyanobacteria in the river below Iron Gate Dam are likely due to daily changes in algae buoyancy in the reservoir, which is then transported down river. Although variable, the overall observed diel pattern indicates that an optimal public health sampling program for the Klamath River should target sample collection during early- to mid-morning hours.

Increased concentrations of *Microcystis* generally resulted in increased concentrations of the cyano-toxin, microcystin. When *Microcystis* cell densities were low, microcystin toxin was always low in the river, but when *Microcystis* cell densities were high, microcystin could be low or high. Variation in the relationship between microcystin toxin and cell densities (toxin-to-cell ratios or cell quota) occurred both among years and within seasons. Previous genetic research (e.g., Otten et al. 2015) suggests that different genetic strains of *Microcystis* are likely the main cause of variation in cell to toxin ratios. The protectiveness of previous public health cell density thresholds was confirmed by this study, where during the 2005-2016 period there were relatively few exceedances of the 0.8 µg/L and 4.0 µg/L public health warning levels at *Microcystis* cell densities at or below 1000 cells/ml and 5,000 cells/ml, respectively. Cell counts of toxigenic cyanobacteria remain a strong line of evidence in indicating possible public health risk, but at times can over estimate toxin concentrations, so whenever possible direct measurements of microcystin is important.

Twelve years of sampling for toxigenic *Microcystis* in the Klamath River revealed general trends and deviations from expected patterns. Identifying the hotspots for algal exceedances in both space and time will inform future monitoring and public health warnings. Understanding the relationship between cell densities and toxin concentrations, as well as the variation in these relationships lays a platform to ask questions about the driving forces behind toxin levels. It is important to note that patterns highlighted in this report reflect general patterns observed between 2005 and 2016, and given the observed variability in spatial-temporal dynamics, as well as the potential for environmental controls to alter the expression of toxic vs. non-toxic *Microcystis* strains, it is important that future public health decisions be based on continuing monitoring efforts.

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Disclaimer

Due to the patchy nature of blue-green algal blooms it is possible for higher Microcystis aeruginosa densities (and therefore higher microcystin toxin concentrations) to have been present in locations not covered in these surveys, particularly along shorelines or protected coves and backwaters during calm conditions of little to no wind. Recreational users should always avoid contact with water whenever noticeable surface concentrations of algae are evident. Moreover, because pets or other domestic animals are the most likely to ingest contaminated water, these animals should not be allowed access to areas of either noticeable surface concentrations of algae or when an obvious green to blue-green appearance is evident.

APPENDIX A: TRIBAL AND CALIFORNIA POSTING GUIDELINES FOR PUBLIC HEALTH ADVISORIES

Karuk Tribe Public Health Guidelines for Cyanobacterial Toxins and Cells for the Klamath River and Tributaries.

From: Karuk Tribe Water Quality Control Plan. February 2014.

Table 4 Cyanobacterial toxin and cell density criteria.

Parameter	Designated Uses	Standard	Rationale for Standard
Microcystis aeruginosa cell density	Drinking water (MUN)	**Below detection**	The Minnesota (2012a, 2012b) Heinze-based BMDL short-term non-cancer "Health Based Value" of 0.04 µg/L essentially does not allow for the detection of any cells.
	Contact: Cultural (CUL-1)) Recreational ((REC-1)	**<1,000 cells/mL:** Initial media outreach and general informational signage. Begin routine monitoring.	Cell density corresponding to OEHHA "Action Level"
		<5,000 cells/mL: *Additional Media outreach and specific public health postings that warning against water contact due to levels that are 5x the OEHHA "action level"*	Cell density corresponding to 5x OEHHA "Action Level"
		<10,000 cells/mL: Repeat Media outreach and specific public health postings warning against water contact due to levels that are 10x the OEHHA "action level"	Cell density corresponding to 10x OEHHA "Action Level"
Total microcystin toxin concentration[1]	Drinking water (MUN)	**<0.04 µg/L total microcystins**[2]	Minnesota (2012a, 2012b) Heinze-based BMDL short-term non-cancer "Health Based Value" of 0.04 µg/L.
	Contact: Cultural (CUL-1) Recreational (REC-1)	**<0.8 mg/L total microcystin**: Initial media outreach and general informational signage. Begin routine monitoring.	OEHHA "Action Level"
		<4.0 mg/L total microcystin: *Additional Media outreach and specific public health postings that warn against water contact due to levels that are 5x the OEHHA "action level"*	5x OEHHA "Action Level"
		<8.0 mg/L total microcystin: Repeat Media outreach and specific public health postings warning against water contact due to levels that are 10x the OEHHA "action level"	10x OEHHA "Action Level"
Total potentially toxigenic blue-green algal species[3]	Contact: Cultural (CUL-1) Recreational (REC-1)	**<100,000 cells/mL or cyanobacterial scums**	WHO/SWRCB guidelines
Anatoxin-a	Contact: Cultural (CUL-1) Recreational (REC-1)	**<90 µg/L**	OEHHA (2012)
Cyanotoxins in Fish/Shellfish	Shellfish Harvest (SHELL), Fish Consumption, FC)	**<10 ng/g microcystins, <5000 ng/g anatoxin, <4 ng/g cylindrospermopsin** (wet weight)	OEHHA (2012)

[1] While there are numerous congeners of microcystin (e.g., microcystin-LA, RR, and YR) the most extensive toxicological information is available for the microcystin-LR congener. However, the literature indicates that most of these congeners appear to have similar toxicological effects (OEHHA 2012). Therefore, the toxicity criteria apply to the total of all microcystin congeners (if measured separately the concentration of the various congeners is summed), or if ELISA methodology is used then the reported value is already assumed to represent the total.

[2] Value based on the older WHO studies, and although OEHHA (2012) did not evaluate drinking water "action levels", the Minnesota Department of Health (2012) utilized the same Heinze-based BMDL of 0.0064 mg/kg/day that OEHHA used to arrive at a short-term non-cancer "Health Based Value" of 0.04 µg/L.

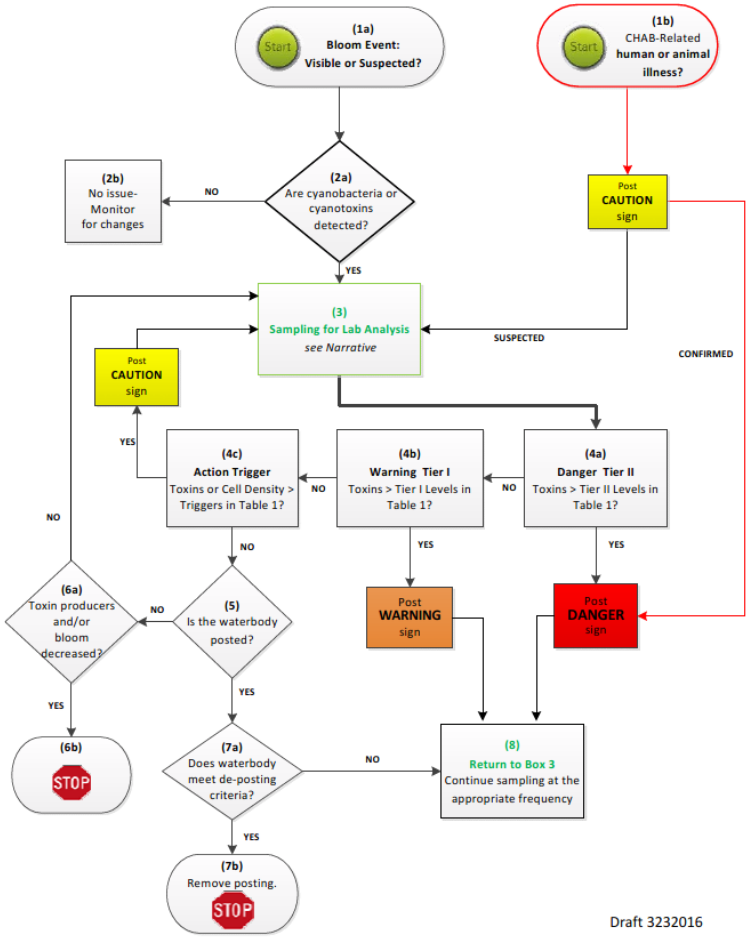
[3] Includes: *Anabaena, Microcystis, Planktothrix, Gloeotrichia* and *Oscillatoria*

California Water Quality Monitoring Council CCHAB Guidelines

http://www.mywaterquality.ca.gov/monitoring_council/cyanohab_network/index.html

http://www.mywaterquality.ca.gov/monitoring_council/cyanohab_network/docs/tree_narrative.pdf

Figure 1. Decision Tree for Posting and De-posting Health Advisories for CyanoHABs
Proposed changes to consider for Voluntary CHAB Guidance



http://www.mywaterquality.ca.gov/monitoring_council/cyanohab_network/docs/triggers.pdf

Table 1. CyanoHAB Trigger Levels for Human Health

	Caution Action Trigger	Warning TIER I	Danger TIER II
Primary Triggers [a]			
Total Microcystins [b]	**0.8** µg/L	**6** µg/L	**20** µg/L
Anatoxin-a	**Detection** [c]	**20** µg/L	**90** µg/L
Cylindrospermopsin	**1** µg/L	**4** µg/L	**17** µg/L
Secondary Triggers			
Cell Density *(Toxin Producers)*	**4,000** cells/mL	--	--
Site Specific Indicators of Cyanobacteria	Blooms, scums, mats, ect.	--	--

[a] The primary triggers are met when ANY toxin exceeds criteria.
[b] Microcystins refers to the sum of all measured microcystin variants. (See Box 3)
[c] Must use an analytical method that detects ≤ 1µg/L Anatoxin-a.