Evaluation of phycocyanin probes as a monitoring tool for toxigenic cyanobacteria in the Klamath River below Iron Gate Dam

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Klamath Tribal Water Quality Consortium
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EXECUTIVE SUMMARY

The Klamath River experiences harmful algal blooms dominated by the cyanobacterium *Microcystis aeruginosa*. Although dense *Microcystis* blooms and associated 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, leading to public health concerns for the entire middle and lower Klamath River. *Microcysti*s cell density and microcystin toxin levels in these reaches have consistently exceeded World Health Organization and California public health thresholds during summer and fall (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 monitoring program for harmful algal blooms was implemented to inform public health postings for river and reservoir safety. Limitations of the sampling program, including sample processing times and costs, have led to augmenting the monitoring program with optical probes that provide instantaneous estimates of cyanobacterial conditions in the river by measuring phycocyanin, a pigment unique to cyanobacteria. This technology provides real-time monitoring that has the potential to inform public health warnings for the river. However, due to uncertainties in the relationships between phycocyanin and cell density or toxin concentrations, direct relationships between laboratory cell counts or toxin concentrations and phycocyanin readings are needed to better understand and use real-time data from phycocyanin probes for public health management on the Klamath River.

In this study, we investigated the relationship between estimates of cyanobacteria (used interchangeably with the term blue-green algae or BGA) concentrations from the real-time phycocyanin probes and grab samples, which were analyzed for BGA cell density, species composition, and toxin concentrations. A variety of techniques were used to examine these relationships, including spearman rank correlation, locally weighted polynomial regression, quantile regression, and probability analysis. We presented our findings in the context of current public health posting thresholds, and we suggested ways to use the phycocyanin data to communicate BGA health risks to the public.

BGA conditions in the river were well represented by real-time data from the phycocyanin probes. Although the magnitude often differed between sonde estimates of BGA concentration and grab sample cell concentration, this was primarily an artifact of the original relative fluorescent units (RFU) converted to an estimated cell density based on within-probe conversion by the manufacturer (YSI). For example, daily mean cell density (estimated from RFUs) from the phycocyanin probes consistently underestimated grab BGA cell concentrations at readings above ~3500 cells/mL, while lower probe readings, although primarily underestimating grab cell density, were associated with cell counts both lower and higher than associated probe readings. Overall, sonde BGA reported as cell density was significantly related to both grab sample cell density and microcystin toxin (Spearman's rho=0.76 and 0.64; $p<0.001$). In addition, quantile regression demonstrated that the 0.9 quantiles of both grab sample cell density and toxin were positively related to the sonde estimates of cell density.

Setting the phycocyanin probes to record as RFUs instead of cell density will facilitate the development of relationships between phycocyanin probe readings and sampled cell density and toxin levels that are easier to interpret. Reporting phycocyanin as cell density leads to

confusion between units of measured cell density from grab samples and the estimated cell density units from the phycocyanin probes, especially when considering units in the context of public health thresholds. Setting sondes to report both RFUs and cells/mL will help transition into using RFUs, which most new phycocyanin probes report in exclusively.

Notwithstanding the issue of converting from RFUs to cell density, we determined that the real-time phycocyanin data can be used to determine changes in riverine BGA conditions and to inform public health decisions. We provided a graphical tool where daily mean sonde BGA values can be compared to sonde BGA thresholds that correspond to any chosen exceedance probability for a particular public health posting level, and provide examples of how this exceedance level can translate into templates to display the phycocyanin data. As an example, we determined that sonde BGA values of 500 and 2000 cells/mL corresponded to approximately a 10% probability that the Tribal level I and II public health warning thresholds for microcystin concentration would be exceeded (the 10% probability level was 3000 cells/mL for the California CyanoHAB Tier I warning Level of 6 µg/L microcystin). Phycocyanin levels corresponding to the 10% exceedance probability were chosen in part because 10% was near the inflection point, where the probability of exceedance began to rapidly increase at higher sonde BGA levels. Using the tribal sonde exceedance level of 2000 cells/ml, we found that public health exceedances were most common closer to Iron Gate Dam, but that phycocyanin levels were above 2000 cells/mL during all of September at all sites during some years.

We examined spatial and temporal trends of BGA blooms in the Klamath River over eight years of phycocyanin monitoring. There was a general longitudinal decrease in sonde BGA concentrations from below Iron Gate Dam to Turwar. Maximum bloom magnitudes were lowest below the confluence of the Trinity River, where sonde BGA estimates at Turwar were about a third of those immediately below Iron Gate Dam. Seasonal variation in sonde BGA was more pronounced than the variation among years, although among year differences were present in both the timing and magnitude of peak BGA conditions. Seasonal changes in riverine BGA densities occurred in all eight years of this study, with bloom conditions present from mid-July to November.

Day to day variation in BGA was high during the bloom, making real-time phycocyanin probes a useful tool to indicate riverine BGA conditions, especially between grab sample events. Phycocyanin readings changed more than 5000 cell/mL from one day to the next during bloom periods, and increased and decreased more than 10,000 cells/mL within one week when the bloom was starting and ending. Many of these changes in sonde BGA occurred between grab samples, making the use of real-time phycocyanin probes highly complementary to traditional grab sample monitoring.

The phycocyanin probes provide data in real-time that can be used to inform public health decisions about cyanoHABs in the Klamath River. Analyses between the real-time phycocyanin data and grab sample density and microcystin toxin showed that the probes sufficiently reflected cyanobacterial conditions in the Klamath River such that phycocyanin data can be used as additional evidence signifying bloom conditions that warrant notification to river users. Possible applications for use of the real-time phycocyanin data include increasing grab sample frequency based on changes in phycocyanin, integrating phycocyanin levels into public health posting guidelines, and creating tools for the public to access the real-time data displayed against risk levels from this analysis based on the current public health warning levels.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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.

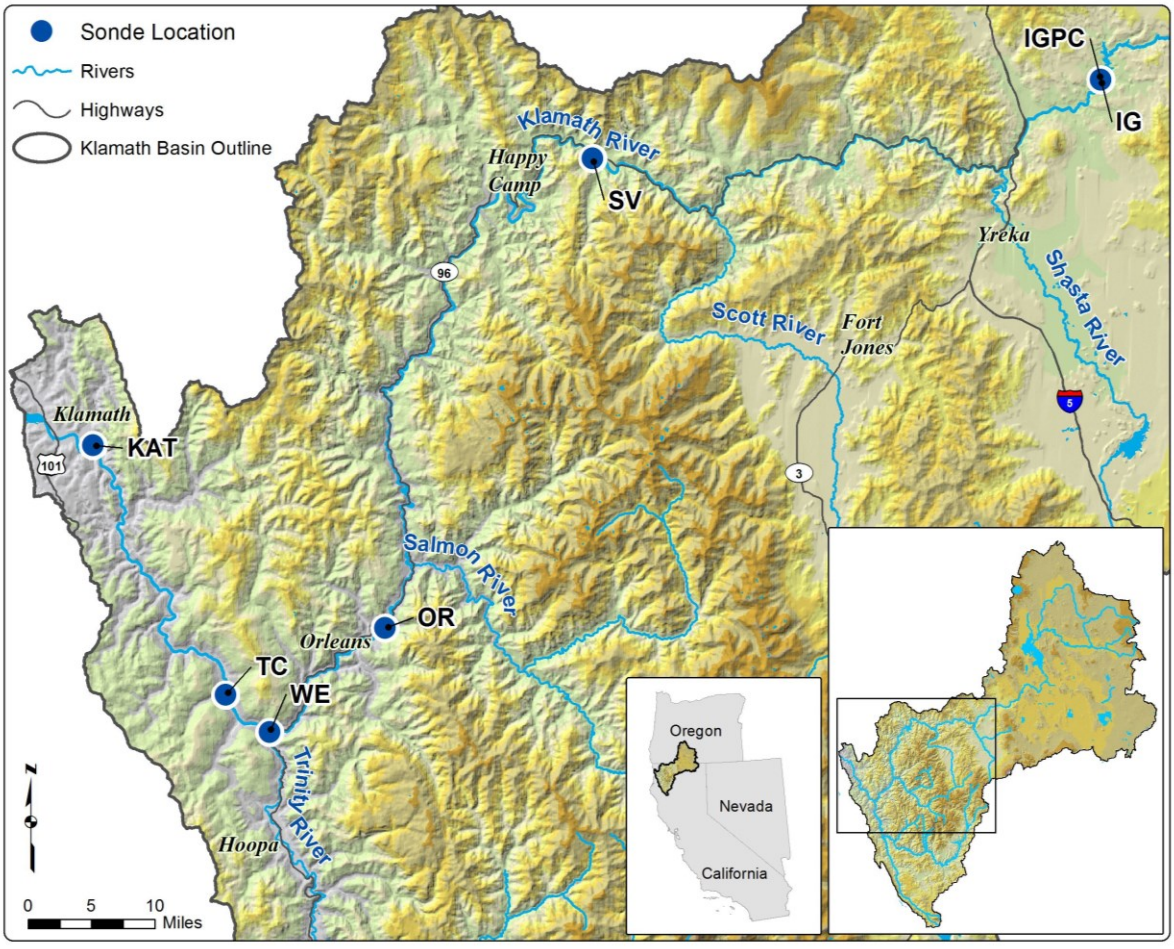

Figure 1. Location of continuous water quality monitoring stations (YSI data sondes fitted with phycocyanin probes) on the mainstem of the Klamath River below Iron Gate Dam.

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 contact including ingestion or inhalation while recreating, drinking contaminated water, and consumption of fish or shellfish, cyanoHABs 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 Weitchpec (RM 43) listed as impaired for cyanobacterial toxicity during summer and fall months (U.S. EPA 2008, U.S. EPA 2015). Due to consistent annual blooms posing public health threats, 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 guidelines both in Copco and Iron Gate Reservoirs (e.g., Jacoby and Kann 2007, Kann and Corum 2009, Raymond 2010) and downstream of the reservoirs in the Klamath River (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 (Fetcho 2006, Kann 2008, Kann et al. 2010, Kann et al. 2013, Mekebri et al. 2009, Hardy et al. 2015). Moreover, microcystin has been shown to bioaccumulate in Klamath River freshwater mussels even when ambient microcystin concentrations were near or below detection in the lower river (Kann et al. 2010).

Although dense *Microcystis* blooms primarily occur in the lacustrine waters of the Copco and Iron Gate impoundments where they originate, cyanobacterial cells and associated toxins from the impoundments are transported downstream as far as the Klamath River estuary (Otten et al. 2015). Summer and autumn *Microcystis* cell density and microcystin toxin levels in these downstream reaches often exceed World Health Organization and California public health thresholds (e.g., SWRCB 2010; CCHAB 2016).

1.2.2 PUBLIC HEALTH MONITORING FOR KLAMATH RIVER CYANOHABs

As a result of the public health risks associated with high concentrations of *Microcystis* and associated microcystin toxin in the Klamath River system, 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 Klamath River cyanoHAB monitoring program currently implemented by the Karuk and Yurok Tribes consists of grab samples taken approximately weekly once toxin or potentially toxigenic cyanobacteria have been measured in the river. Limitations of the sampling program

[1] Microcystin is a potent hepatotoxin capable of causing death or severe liver damage (e.g., OEHHA 2012).
[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 the potential health risks. http://www.kbmp.net/collaboration/klamath-blue-green-algae-workgroup

include intervals of a week or more between samples when bloom conditions may rapidly change[3], a one-to-two week processing time by laboratories for microcystin toxin and cyanobacterial cell density, and the significant cost of collecting, processing and shipping samples. These limitations are especially critical to the Klamath River Tribes given their cultural connection to the Klamath River, including ceremonial, subsistence, and recreational use. Thus, the need to ensure safety of tribal members using the Klamath River led the tribes to augment traditional grab samples with continuous monitoring technology. This technology provides real-time monitoring that has the potential to inform public health warnings for the river.

1.2.3 CONTINUOUS MONITORING OF CYANOHABs

Real-time monitoring of phycocyanin, a pigment unique to cyanobacteria, is an emerging tool used to indicate the presence and relative abundance of these species in freshwater. Phycocyanin probes are typically fitted to continuous water quality monitoring probes (data sondes) deployed for extended periods of time and capable of recording data at the sub-hourly interval. These probes measure florescence of the phycocyanin pigment and provide results as relative florescent units (RFU). An estimate of cyanobacteria cell density is also calculated by some probes, converting phycocyanin fluorescence to qualitative estimates of cell density. BGA[4] cell density estimates from phycocyanin probes have generally been found to correlate well with microscopically determined cell density from grab samples in a variety of ecosystems and laboratory settings (Brient et al. 2008, Bastien et al. 2011, McQuaid et al. 2011). Although these studies have often found strong linear relationships between BGA estimates from phycocyanin probes and cell counts from grab samples, researchers and the probe manufacturer have expressed the importance of a post-calibration step in order to obtain quantitative estimates of BGA cell density from phycocyanin probes (YSI 2012, Bastein et al. 2011). This post calibration step is needed for quantitative estimates because pigment concentration in BGA cells varies due to species, strain, cell size, and environmental factors (YSI 2012). Additionally, high levels of turbidity and chlorophyll *a* can interfere with phycocyanin probe readings (McQuaid et al. 2010, Bowling et al. 2013).

Using phycocyanin probes for early or real-time detection of BGA does not allow for post calibration with laboratory cell counts due to sampling intervals and processing time as discussed above. Therefore, we compared raw data from the probes to the laboratory cell counts and toxin concentrations to establish relationships between sonde BGA and traditional grab sample cell counts and toxin concentrations, and to suggest ways in which these probe estimates can be used to inform public health management of BGA blooms in the Klamath River.

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[3] For example, previous diel sampling occurring over several days has shown that microcystin and *Microcystis* cell density were variable with respect to public health threshold exceedance on a shorter than weekly time interval (Kann 2014).

[4] The manufacturer (YSI) of the phycocyanin probe analyzed in this study use the term "Phycocyanin Blue-Green Algae Sensor (abbreviated BGA-PC), and when converting RFU to cells/mL they report as BGA cells/mL: https://www.ysi.com/File%20Library/Documents/Specification%20Sheets/E35-6131-6132-Blue-Green-Algae-Sensors.pdf
Thus in this report we use the term sonde BGA or BGA probe to denote the phycocyanin probe and sonde BGA cells/mL to denote estimated cyanobacterial cell density. Grab BGA is then utilized to distinguish from sonde BGA and denotes cyanobacterial cell density as determined microscopically from river grab samples (see below methods).

1.3 STUDY GOALS

The primary study goal was to assess the reliability of phycocyanin probes to monitor cyanobacteria (particularly toxigenic *Microcystis aeruginosa*) to inform public health warnings, and to use the continuous data from the phycocyanin probes to explore BGA dynamics through space and time in the Klamath River. We analyzed the phycocyanin data from seven sites on the Klamath River from below Iron Gate Dam to the mouth of the Klamath River from 2007 to 2014. Our specific objectives were to 1) assess the reliability of BGA probe data by performing a QA/QC analysis on all BGA probe data by comparing BGA data from probes to cell counts from grab samples and by comparing phycocyanin data from adjacent sites, 2) determine the relationship between BGA probe readings and BGA grab values for cell density and toxin, 3) provide a method to relate BGA probe readings to Klamath River Tribes and State of California public health guidelines, and 4) explore temporal and spatial patterns of BGA in the Klamath River below Iron Gate Dam using the multi-year BGA probe data, including bloom timing and magnitude, as well as spatial dynamics of cyanobacteria in the Klamath River.

2 METHODS

2.1 DATA COLLECTION

Real-time phycocyanin and grab sample data were collected at monitoring sites along the mainstem of the Klamath River (Figure 1) from just below Iron Gate Dam (river mile 189.5) to Turwar (river mile 5.8; just upstream of the Klamath Estuary). PacifiCorp, the Karuk Tribe, and the Yurok Tribe collected the data utilized in this report (Table 1).

Table 1. Phycocyanin monitoring sites on the Klamath River.

Site Code	Site Description	River Mile	Latitude	Longitude	Elevation (feet ASL)	Data Collection Entity
IGPC	Below Iron Gate Dam	189.5	41.931674	-122.439825	2172	Pacific Corp
IG	Below Iron Gate Dam	189.1	41.931083	-122.442200	2169	Karuk
SV	Klamath at Seiad Valley	128.6	41.842683	-123.218867	1355	Karuk
OR	Klamath at Orleans	59.1	41.305600	-123.531583	358	Karuk
WE	Klamath at Weitchpec	43.5	41.186183	-123.700556	194	Yurok
TC	Klamath above Tully Creek	38.5	41.222960	-123.770478	183	Yurok
KAT	Klamath at Turwar	5.8	41.511071	-123.979477	22	Yurok

2.1.1 SONDE DATA COLLECTION

As described in Asarian and Kann (2013), continuous water quality data sondes equipped with phycocyanin probes were deployed by the Karuk Tribe, Yurok Tribe, and Pacific Corp at

seven mainstem stations from just below Iron Gate Dam to Turwar (Figure 1, Table 1). The duration of the monitoring seasons varied by year and site, but generally occurred from May through October (Figure 2). Pacific Corp operated one sonde below Iron Gate Dam, the Karuk Tribe operated three sondes from below Iron Gate Dam to Orleans, and the Yurok Tribe operated three sondes from Weitchpec to Turwar. The Yurok Tribe used YSI 6600 EDS sondes and the Karuk Tribe used YSI 6600 V2 sondes for the duration of this study. Pacific Corp deployed a YSI 6600 series sonde (model unknown) from 2008 until 2013, and upgraded to a YSI EXO sonde in 2014. All 6600 series sondes were equipped with YSI-6131 phycocyanin blue-green algae sensors.

Sonde calibration and maintenance was conducted similarly by all three data collection entities. Sondes were generally recalibrated and cleaned every two weeks. Phycocyanin sensors were calibrated via a one-point calibration process using de-ionized water as the zero point. Sondes operated by the Yurok Tribe Environmental Program were also checked for sensor drift using rhodamine dye. No post calibration of data was carried out by any of the data collection entities[5]. Sondes were generally set to log data every 30 minutes, with the exception of the sonde operated by Pacific Corp from 2012 to 2014, for which data was logged every 15 minutes and an hourly mean was reported.

As noted above, sondes operated by the Karuk and Yurok Tribes recorded BGA cells/mL, which is based on a within-probe conversion of phycocyanin fluorescence (YSI 2012). The sonde operated by Pacific Corp reported both RFUs and cells/mL from 2008 through 2013, and then reported only RFU in 2014. As noted by YSI (2012), the output of the sensor is automatically processed via the sonde firmware to provide readings in either relative fluorescent units (RFU) or cells/mL of phycocyanin-containing BGA. Both RFU and cells/mL are relative measures of real-time phycocyanin fluorescence and the conversion to cells/mL is based on a semi-quantitative comparison to fluorescence readings form a Turner Designs fluorometer[6]. Although YSI indicates the conversion from RFU to cells/mL is based on using a standard multiplier of 2800[7], our analysis of PacifiCorp data from 2008 to 2013 when RFU and cells/mL were both simultaneously reported showed a multiplier of 2133 (Figure 3). Thus, in order to compare the RFU-only data to the rest of the data set, we converted RFU to the cells/mL estimate using the multiplier of 2133.

[5] YSI suggests post calibrating sonde cell density data by calculating a correction ratio between measured cell density and YSI sonde readings.

[6] According to YSI (2012): "The range estimate for the YSI 6131 sensor is based on the fact that its reading in an empirical sample of PC-containing algae is about 40% less than that of the industry standard fluorometer from Turner Designs which is configured for PC-BGA. In the estimation experiment, a Turner Cyclops sensor was fixed on its middle range and then its voltage reading in a PC-BGA culture (*Microcystis aeruginosa*) was determined. The YSI 6131 sensor was placed in the same culture and its sensitivity found to be about 40% less in terms of the percent of full scale deflection relative to the Turner sensor on its middle range. Since Turner Designs has designated the middle range of its sensor as 0-200,000 cells/mL, the YSI sensor is estimated to have a range of about 40% more or 280,000 cells/mL. Naturally, this range is only an estimation for both the YSI and Turner sensors because of the general limitations of in vivo fluorescence measurements described above."

[7] Personal communication with YSI technical services indicated the conversion constant from RFU to cells/mL is 2800 and is based on the ratio between the 0-100 range for RFU and the 0-280,000 for cells/mL (see footnote 6 for derivation of the cells/mL range).

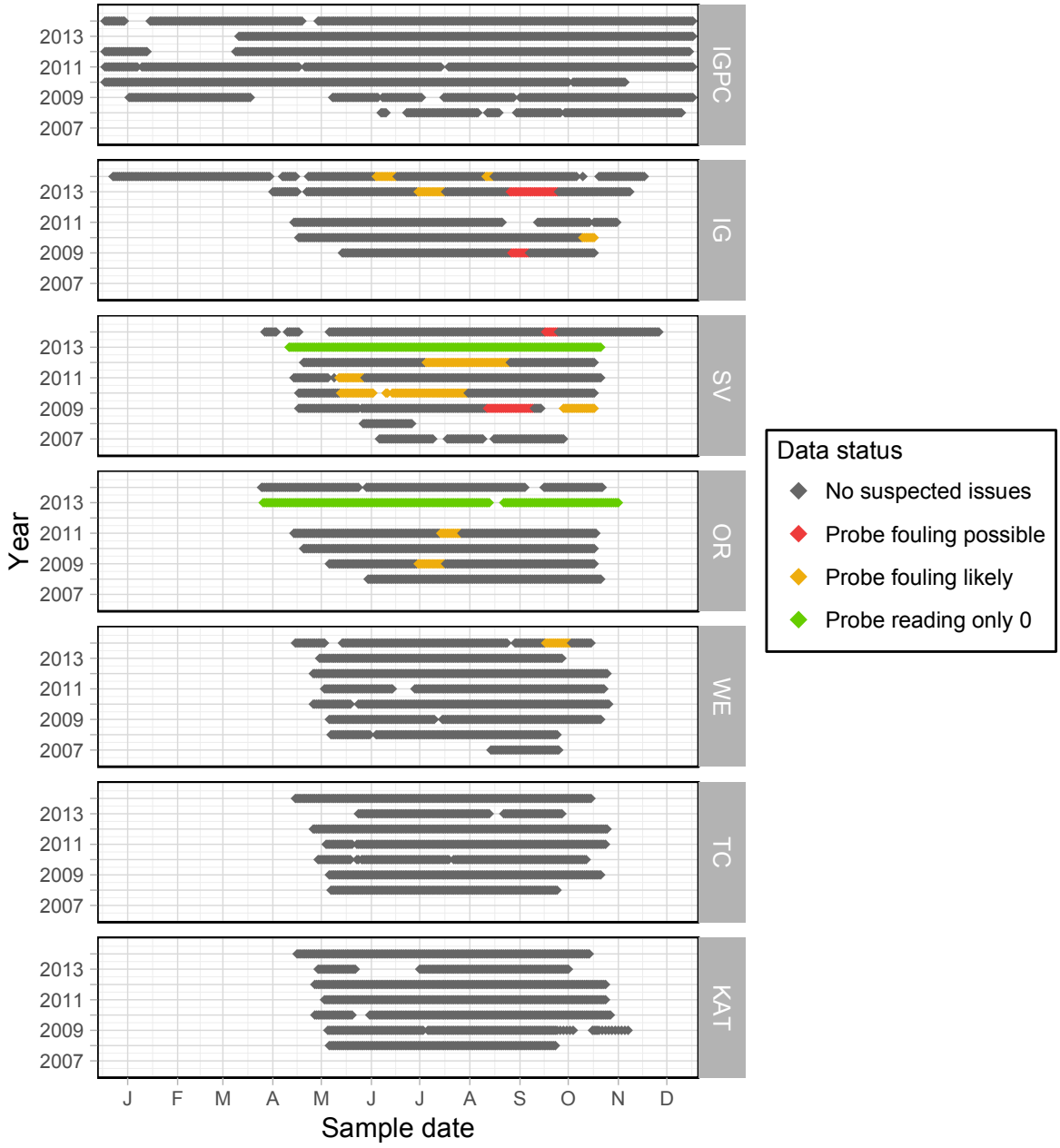


Figure 2. Timing of sonde BGA measurements by year and day at the seven long-term sonde sites on the Klamath River below Iron Gate Dam from 2007 to 2014. Red, yellow, and green points are days with quality assurance concerns. Month abbreviations on the x-axis are at the 15th of each month.

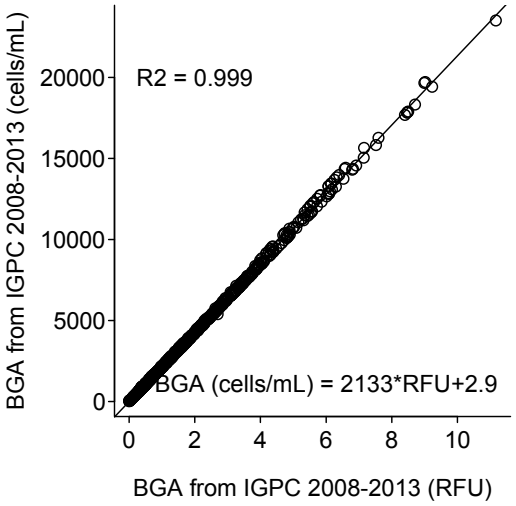


Figure 3. Daily mean sonde BGA (cells/mL) from the Iron Gate sonde operated by Pacifi Corp from 2008 to 2013 as predicted by the daily mean of sonde BGA (RFUs) reported the same day.

2.1.2 PUBLIC HEALTH GRAB SAMPLE DATA COLLECTION

As noted above, PacifiCorp and the Karuk and Yurok Tribes participate in a cyanoHab public health monitoring program to inform public health postings for river and reservoir safety. The public heath monitoring is facilitated by the Klamath Blue-green Algae Workgroup and consists of grab samples taken approximately biweekly (PacifiCorp) to weekly (Karuk and Yurok Tribes) for Klamath River stations. Samples of algal material taken from shoreline or river-edge areas (denoted SL) utilized the standard operating procedure (SOP) developed by the Klamath Blue-Green Algae Working Group[8], and river open-water samples (denoted OC) were collected from the upper one foot of the water column using a 14 L churn splitter[9]. Samples for microscopic determination of phytoplankton density and biovolume 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[10] methodology.

2.2 QUALITY CONTROL OF SONDE DATA

We identified and flagged suspect phycocyanin data. According to the YSI manual, optical probes, including phycocyanin probes, are highly prone to biological fouling, which can result in data spikes and offsets. We plotted the raw phycocyanin data for each site and each year, overlaid by the mean of daily phycocyanin and total BGA cell density from the grab samples (Appendix A). In cases where large spikes in the raw data were smoothed out by daily means, we retained the data (QA code = 0 denoting data passed QA criteria) and relied on the daily mean to smooth these short-term spikes in the raw data.

[8] http://www.kbmp.net/collaboration/klamath-hydroelectric-settlement-agreement-monitoring
[9] These open-water samples were collected at wadable depths towards the center of the channel in well-mixed areas of noticeably moving water.
[10] Enzyme-Linked ImmunoSorbent Assay using EnviroLogix QuantiPlate Kit designed for quantitative laboratory detection of microcystin toxin in shoreline water samples (quantitation limit is 0.18 µg/L)

When spikes in daily means of estimated BGA cell density were substantially higher (> ~10,000 cell/mL) than the grab samples collected near the time of the spike, we further investigated these spikes by comparing the daily means of BGA estimates at the sites in question to the daily means of BGA estimates at near-by sites. We plotted the daily mean of the BGA estimates during spikes with the daily means of BGA estimates at the two closest sites. In cases where we suspected probe malfunction at the near-by sites, we used the next closest sites for comparison. In addition we overlaid grab sample data from all sites and plotted sonde calibration dates to determine whether there were obvious discontinuities associated with calibration.

We flagged data as likely fouled (QA code = 1 denoting data did not pass QA criteria) when spikes were not reflected at near-by sites and were substantially higher than concurrent grab samples from all river sites. When spikes in daily mean sonde data were higher than concurrent grab samples, but some similar patterns occured at one or more nearby sites, we flagged data as fouling possible (QA code = 2). For SV and OR in 2013, where sonde data reported zeros with no variation over long periods of time even when adjacent stations and grab samples showed positive fluctuations (see Appendix B), we flagged the data as flat-lined (QA code = 3). When flagging data for suspected sonde malfunction, we flagged only the obvious spike when a single large spike was present. When start and end dates of possible sonde malfunction was difficult to determine, we flagged data from the entire calibration period (Appendix B). In many cases, suspected probe fouling occurred in the middle of a calibration period and ended when the sonde was re-calibrated, which would be expected when probe malfunction was due to biological fouling.

2.3 ANALYSIS

2.3.1 SELECTION OF SONDE AND GRAB SAMPLE METRICS

In order to establish the most representative statistic to characterize BGA patterns in the Klamath River we compared cell counts from open channel and shoreline grab samples (grab BGA) taken near sonde sites to BGA cell density estimates from phycocyanin probes (sonde BGA) on hourly, daily, and weekly scales. From the 30-minute interval probe readings we calculated hourly means, daily means, daily trim means (10% trim), minimums, maximums, quartile ranges, medians, and moving averages (4, 8, and 14-day). We assessed the degree of correlation between the BGA grab samples and the sonde BGA summary statistics using the nonparametric Spearman's Rank Correlation Coefficient. Conservatively, we only included data with a QA code of 0 (no suspected probe malfunction) when calculating Spearman correlation coefficients. Additionally, we plotted the mean of the daily sonde BGA with both open channel grab (OC) and shoreline grab (SL) samples to visually assess the differences in relationship between the OC and SL samples for both grab cell counts and toxin concentrations.

2.3.2 RELATIONSHIP BETWEEN SONDE BGA AND GRAB BGA

To assess the degree to which phycocyanin probes reflected cell counts and toxin concentrations from grab samples we related sonde BGA cell density estimates from phycocyanin sensors to grab samples taken near the sonde locations. Spearman's rank correlation was used to compare BGA cell density and toxin concentrations from both the open channel and the shoreline grab samples to the daily mean of the sonde BGA cell density. Additionally, we

evaluated plots of grab BGA vs. sonde BGA and grab toxin concentration vs. sonde BGA to visually assess the general relationship between the grab and sonde data. For the grab cell count data vs. sonde BGA we assessed data relative to a one-to-one line to determine whether sondes were under or overestimating grab sample data.

To determine species-specific relationships we compared sonde BGA to grab sample cell density of the three most commonly observed BGA (cyanobacteria) groups in the lower Klamath River. We plotted the cell density from grab samples for each species by the proportion of the sonde BGA estimate represented by each species (based on the grab sample cell density proportion), and calculated the proportion of each species by dividing cell density for each species by total cell density. Additionally, we compared sonde BGA estimates to grab sample cell biovolume. We estimated BGA biovolume by multiplying grab BGA cell density estimates for each species by the mean cell biovolume for each species based on data from Upper Klamath Lake (48 μm^3 for MSAE, 78 μm^3 for ANAB, and 120 μm^3 for APFA; Kann et al. 2015). We did not attempt to calculate biovolume for species listed as "other" because this category was a mix of species of different sizes. We then plotted total grab BGA cell biovolume by sonde BGA cell density estimates, and species-specific biovolume by the proportion of the sonde BGA estimate represented by each species.

2.3.3 RELATING SONDE DATA TO PUBLIC HEALTH POSTING THRESHOLDS

2.3.3.1 BLOOM TIMING

We estimated the timing of the BGA bloom on the Klamath River to establish the range of dates (bloom season) in which bloom conditions have occurred on the Klamath River from 2007 to 2014 using sonde BGA data, grab sample BGA cell counts, and grab sample toxin concentrations from both open channel and shoreline grab samples. In defining the bloom timing, we used the 0.9 quantile of the data, which has more relevance to public health than the statistical mean because it bases the timing of the bloom on the upper quantile of the sample values, leading to establishment of bloom timing that is more conservative relative to public health protection (Yu et al. 2003). For each BGA data type (i.e., sonde BGA, grab BGA, and grab toxin concentration) we quantified bloom timing by first computing the nonparametric locally weighted polynomial regression (LOESS) through the 0.9 quantile of BGA data by day of year, and then determining the time of year when the 0.9 quantile LOESS regression line crossed the Yurok Tribe Level II public health posting standards[11].

[11] Klamath River Public heath thresholds for microcystin toxin and *Microcystis* cell density used in primary report analyses are from the Yurok and Karuk Tribes and are based on action levels described in OEHHA (2012) and expanded upon by Kann (2015). 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.* In addition, the state of California also 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 4.0 µg/ and 20 µg/L microcystin, respectively. Graphical analyses depicting sonde BGA cell density relative to the California thresholds are contained in Appendix F. Both Tribal and California public health thresholds are described in more detail in Appendix C.

2.3.3.2 QUANTILE REGRESSION

Using quantile regression (Cade and Noon 2003) we related sonde BGA estimates to the 0.9 quantile of the grab sample cell density data and the 0.9 quantile of the grab sample toxin data, and interpreted the resulting relationships in the context of established public health standards (Appendix C). For the grab sample cell density data, the relationship was described linearly and for the grab toxin data the relationship was best modeled using a second order polynomial due to non-linearity of the upper quantile as predicted by the sonde BGA data. We identified where the 0.9 quantile of the sonde BGA data crossed the 1000 and 5000 cells/mL threshold for the cell density data, and the 0.8 and 4 µg/L threshold for the toxin data, to establish the sonde BGA level in which 10% of corresponding grab samples exceeded public health posting standards.

2.3.3.3 PROBABILITY OF EXCEEDANCE

To further investigate the relationship between phycocyanin probe readings and the grab sample data in the context of the public health posting standards, 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 daily mean sonde BGA were ordered by ascending sonde BGA, divided into data intervals chosen to maximize interval evenness, and the median sonde BGA cell density for each interval was computed. The frequency of grab sample microcystin observations within each interval that exceeded chosen thresholds (beginning at 0.8 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 sonde BGA cell density for each interval. A similar analysis was conducted for the relationship between grab sample cell density and sonde BGA estimates. Finally, in order to describe the functional relationship and to facilitate determination of specific probabilities that grab sample cell counts or toxin concentrations would exceed public health posting standards at any given sonde BGA estimate, we constructed correlative contours following Lodwick and Whittle (1970) using the linear interpolation function in SYSTAT version 13.1 (Systat 2009).

2.3.4 SPATIAL AND TEMPORAL TRENDS IN SONDE BGA

Longitudinal and temporal patterns in sonde BGA cell density estimates for the eight-year study period were analyzed. We used box plots and LOESS (locally weighted scatterplot smoothing) curves through the daily mean of the sonde BGA data to document and compare BGA magnitudes and bloom timing within and among years. To assess the variation in sonde BGA between grab sample intervals when changing BGA concentrations were not captured by grab samples, we calculated daily and weekly changes in mean daily sonde BGA. We compared sonde BGA estimates among sites to investigate longitudinal patterns in riverine BGA.

3 RESULTS/DISCUSSION

3.1 SELECTION OF DATA USED IN ANALYSIS

3.1.1 IDENTIFICATION OF PROBE ERRORS AND ASSIGNMENT OF QA CODES

We identified phycocyanin probe errors at four sites and six years (Figure 2). Spikes in sonde BGA data that were not associated with increased grab BGA cell concentrations or spikes at adjacent sites were most common at SV and IG (Appendix B). Fewer spikes occurred at OR and WE, and we identified no data spikes at IGPC, TC or KAT. In addition to spikes in the data likely associated with biological fouling, phycocyanin probe data at SV and OR in 2013 flat-lined at zero for most of the monitoring season. Some data editing was performed prior to data acquisition for this report at IGPC, WE, TC, and KAT during some years (M. Hanington, personal communication, 2 Februray 2016; D. Ebert personal communication 1 June 2016), possibly explaining the lower number of probe errors at these sites.

We were able to perform quality assurance measures on the phycocyanin data with a relatively high degree of confidence on the Klamath River. For example, confirming the initial flagging of sonde BGA data as described above (based on spikes not associated with spikes at adjacent sites or concomitant increases in grab BGA and flat-lining), the suspect data points were generally outliers when plotted against grab sample cell density and toxin data (Figure 4). Low grab BGA (cells density and toxin concentrations) with corresponding high sonde BGA probe readings were likely due to biological fouling, while high grab sample cell counts and toxin concentrations with corresponding low BGA probe readings were due to the phycocyanin probes from SV and OR in 2013 that were flat-lined near zero during the monitoring season. When data spikes that were not characteristic of the rest of the data set occurred, we compared the data in question to concurrent data from nearby probes, which should reflect similar changes in BGA. In a few cases (QA Code =2), there were increases in sonde BGA data that were uncharacteristic of the larger data set, and nearby sites also had uncharacteristic increases in BGA sonde data that were somewhat reflective of the data in question (eg. Seiad Valley in 2009, see appendix B). In these cases, possible sources of interference including high levels of chlorophyll, turbidity and dissolved organic material may explain some of the variability in the data (Bowling et al. 2013, McQuaid et al. 2010), or lower levels of biofouling.

We expected the seven probes located along the river to experience riverine BGA conditions due to planktonic algae being sourced from the reservoirs above the study sites. Although the timing and magnitude of the changes in phycocyanin were offset due to water travel time, dilution, and algal cell break-down and settling, we still observed similar patterns in phycocyanin among sites. These similar patterns helped us to confirm that comparing sonde BGA from adjacent sites was a good way to identify suspect data, especially when assessing the quality of data in real-time when grab sample cell count do not exist or results are not available.

The general process for the quality assurance of phycocyanin data used in this report could be applied to other water bodies, but in ecosystems such as lakes or very low velocity rivers where bloom dynamics may be more variable among sites, it would be prudent to deploy two nearby phycocyanin sensors to allow for identification of sensor malfunction. In addition to relying on multiple sensors, it is important to understand the basic dynamics of bloom timing and

magnitude in the ecosystem being monitored so that researchers and natural resource managers can quickly identify non-characteristic sonde BGA data. This is especially true when using the real-time data to inform public health posting (see section 3.3) to avoid false public health advisories.

3.1.2 COMPARISON OF SONDE BGA DATA TO GRAB SAMPLE TYPE

Public health grab samples analyzed for total BGA cell density collected from river edges (shoreline grabs; SL) vs. the well-mixed open channel (OC) related similarly to BGA estimates from the sondes. Grab data were positively related to sonde data for both grab types. Shoreline grab samples had a slightly higher correlation coefficient with sonde daily means when including no-detects (Spearman's rank correlation coefficient = 0.78) than open channel grabs (Spearman's rank correlation coefficient = 0.76). The pattern was opposite when excluding no-detects, in which the Spearman's rank correlation coefficient was 0.76 for OC grabs and 0.72 for SL grabs (Table 2). Visual examination of the relationship between grab data and sonde data re-enforced that the relationship between sonde data and grab sample types were similar (Figure 4). The SL samples had a higher upper range for both total cell counts and microcystin (90^{th} quantile = 50,700 cells/L and 7.6 µg/L respectively) compared to the OC samples (90^{th} quantile = 21,900 cells/L and 4.2 µg/L respectively).

Table 2. Spearman's rank correlation coefficients for summary statistics of sonde BGA data to open channel and shoreline grab samples of BGA cell counts with no detects removed from all data sets. All correlation coefficients were statistically significant (*p-value* < 0.05).

Data metric	**Spearman's rank correlation coefficient**	
	Open channel grabs	Shoreline grabs
Maximum daily BGA	0.69	0.67
75th quantile of daily BGA	0.77	0.71
Median of daily BGA	0.76	0.73
25th quantile of daily BGA	0.74	0.73
Minimum of daily BGA	0.73	0.69
14-day moving average of BGA	0.71	0.67
8-day moving average of BGA	0.73	0.68
4-day moving average of BGA	0.75	0.70
Mean daily BGA	0.76	0.72
Mean daily BGA (10% trim)	0.76	0.71
Hourly mean BGA	0.75	0.73

Although we expected the ranked correlation to be higher between sonde BGA and OC grab data than between sonde BGA and SL grab data, both OC and SL sample types had relatively similar ranked correlation coefficients and relationships with the sonde BGA data. Thus, we selected the OC grabs as the primary data source to use in comparing sonde data to grab sample data in this analysis because they were specifically collected from well mixed,

flowing parts of the river in closer proximity to the locations of the BGA probes and in locations more representative of the river condition where the probes were located.

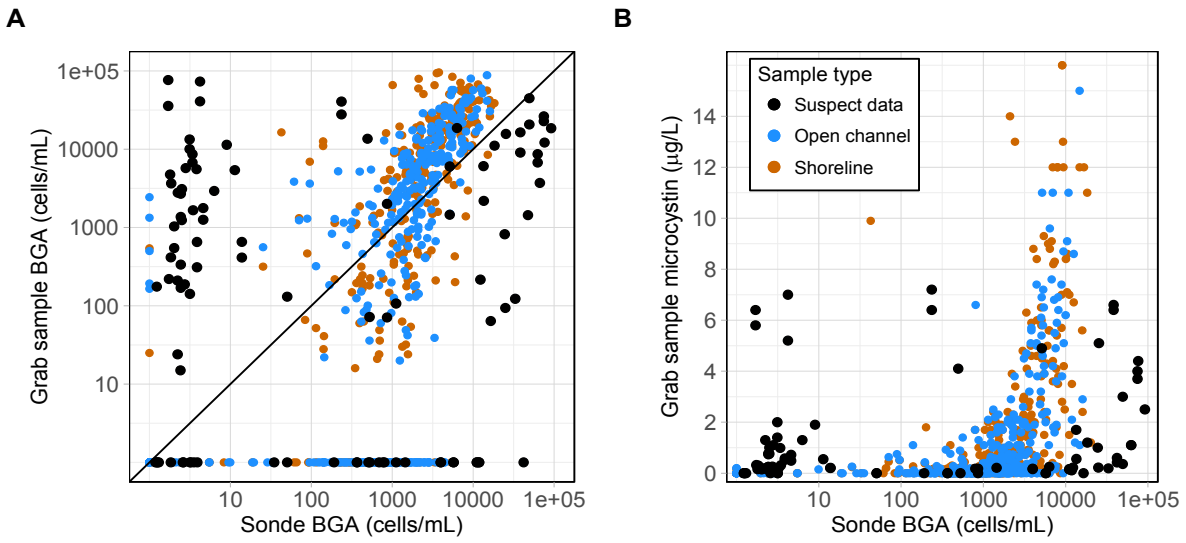


Figure 4. Grab sample BGA (cells/mL, panel A) and grab sample microcystin (µg/L, panel B) by sonde BGA for all sites and years. Brown points are shoreline grab samples, blue points are open channel grab samples, and black points are grab samples of either type taken on days when sonde data was flagged as suspect.

3.1.3 SELECTION OF SONDE BGA DATA METRIC

Assessment of the data metrics used to summarize the 30-minute interval probe readings (hourly means, daily means, daily 10% trim means, minimums, maximums, quartile ranges, medians, and moving averages [4, 8, and 14-day]) indicated that the relationship with grab BGA data was somewhat insensitive to choice of data metric. The relationship between the sonde BGA metric and the OC grab sample BGA cell density yielded similar Spearman's correlation coefficients, ranging from 0.69 for the daily maximum sonde BGA to 0.77 for the 75th quantile of daily sonde BGA readings (Table 2).

Of the 11 summary statistics considered to represent the sonde BGA data in this analysis, all summary statistics were similarly related to the grab sample BGA data. Although the 75th quantile of the daily sonde BGA data had a slightly higher r-squared value and correlation coefficient, we considered the daily mean to be a more intuitive value to use to represent the data set, and the difference in performance against the grab data was not large enough to warrant using the 75th quantile throughout this report (Table 2).

3.2 RELATIONSHIP BETWEEN MEAN DAILY SONDE BGA AND GRAB SAMPLE DATA

3.2.1 COMPARISON OF DAILY MEAN SONDE BGA TO GRAB CELL DENSITY

BGA cell densities from grab samples were positively related to BGA estimates from sonde readings. At cell densities < 3500 cells/mL, sonde BGA both overestimated (points below the 1:1 line) and underestimated (points above the 1:1 line) grab BGA cell densities, whereas at higher cell densities, grab sample cell densities commonly underestimated by the sonde BGA (Figure 5, panel A). This underestimation clearly indicates that the manufacturer conversion used to program the probe to provide readings in cells/mL as opposed to reporting in RFUs (as noted in the methods and acknowledged by the manufacturer) is not an accurate record of BGA cell density for the Klamath River.

Species composition explained some of the variability in the relationship between sonde BGA data and grab BGA data at low cell densities. At sonde BGA cell density estimates below 2000 cells/mL, species other than *Microcystis aeruginosa* (MSAE) were often dominant (Figure 5, panel B). Sonde BGA most strongly underestimated grab BGA cell densities in samples where *Aphanizomenon flos-aquae* (APFA), *Anabaena sp.* (ANAB), or species listed as "other" in the database were dominant. Because phycocyanin probes measure pigments associated with BGA cells, probes can overestimate cell density for larger algal species and underestimate cell density for smaller celled species (YSI 2012; Bastien et al. 2011)[12]. Individual cells of ANAB and APFA are larger (~78 and 120 μm^3 respectively) relative to MSAE (~48μm^3), possibly explaining the overestimation of cyanobacteria cell density when species other than MSAE were dominant. Additionally, the presence of larger colonial forms of cyanobacteria of any species can result in underestimation of sonde BGA cell density because the probe was designed to detect microscopic, free-floating cells rather than larger colonial forms[13] (YSI 2012).

Although grab BGA biovolume was also positively related to sonde BGA cell density, the Spearman's rank correlation coefficient (Spearman's rho = 0.73) did not improve as would be expected based on previous studies (Zamyadi et al. 2012). Converting BGA cell density to biovolume based on mean cell sizes from Upper Klamath Lake eliminated some of the scatter at the lower end of BGA cell densities. However, because *Microcystis* is the dominant species in the Klamath River (86% of individual cells in the dataset), the biovolume conversion mostly affects periods when non-*Microcystis* species were prevalent, which may explain the improvement at the lower end of the grab BGA vs. sonde BGA relationship (Figure 5; panel C). Although sonde BGA readings have often been found to more closely relate to grab sample biovolume, which standardizes for cell size (Zamyadi et al. 2012, Kong et al. 2014),

[12] The BGA probe is measuring relative florescence and not actual cell density and assuming the pigment is proportional to cell size (i.e., a larger cell would have more pigment than a smaller one) a similar RFU may be recorded with a greater number of cells of a smaller species such as *Microcystis* as it would with a fewer number of cells of a larger species such as *Aphanizomeno*n.

[13] As noted by YSI (2012): "when present in high concentrations, colonies of BGA can often be seen with the naked eye and may resemble fine grass cutting or take the form of small irregular clumps or pinhead-sized spheres. When BGA colonize into these forms, the sensitivity of the YSI sensor in terms of the fluorescence per cell of BGA is reduced because it has been designed to detect microscopic, free-floating cells and not large, macroscopic floating particles. Thus, the sensor is likely to underestimate the total amount of BGA present in the water when clumps are present."

phycocyanin probes measure a pigment of BGA, and do not directly measure either cell density or biovolume. Therefore, phycocyanin levels can be variable among species due to cell size, as well as the amount of pigment in different species, and even within species due to algal cell condition and variability among populations (Macário et al. 2015). Additionally, in this study biovolume was estimated based on standard cell sizes and rather than measured for each sample, reducing the accuracy of the biovolume estimates.

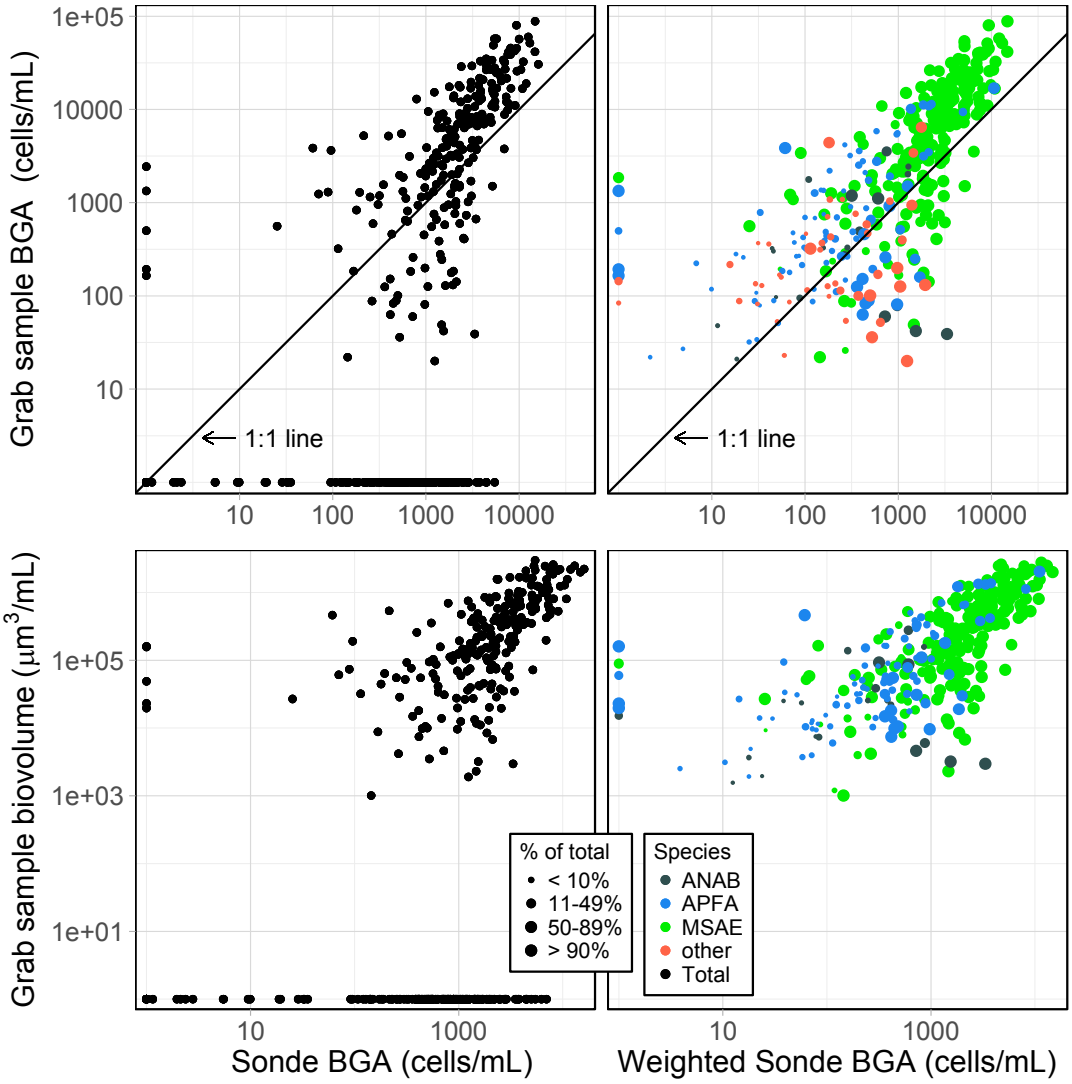


Figure 5. Total grab BGA cell density (panel A) and species-specific grab cell density (panel B) vs. estimated daily mean sonde BGA cell density (panel A) and the weighted estimated daily mean sonde cell density (panel B). Grab sample biovolume (panel C) and species-specific grab sample biovolume (panel D) vs. the daily mean sonde BGA cell density (panel C) and the weighted estimated daily mean sonde BGA cell density (panel D). Colors differentiate species type (ANAB = *Anabaena sp.*, APFA = *Aphanizomenon flos-aquae*, MSAE = *Microcystis aeruginosa*) and point size indicates the percent of each grab sample represented by each species (panels B,D).

Underestimation of grab BGA cell density by sonde BGA occurred at all stations but both underestimation and overestimation were somewhat more prevalent at down-river than up-river sites (Figure 6). Although overestimation of grab BGA data by sondes (points below the 1:1 line, Figure 5, Figure 6) often occurred when species other than MSAE were dominant, there were also occasions of overestimation of grab BGA cell density by sonde BGA when MSAE was dominant. Because phycocyanin probes rely on optical measurements, interference from turbidity and non-blue-green algal species can cause overestimates of phycocyanin relative to cell density (Bowling et al. 2013, McQuaid et al. 2010). Overestimations were most common at sites lower on the river, where higher turbidity levels may be more common. Additionally, the phycocyanin probes detect the florescence of extra-cellular materials (Bastien et al. 2011) as well as intra-cellular pigment, and BGA cells originating in the reservoir likely deteriorate as they travel downstream, increasing the probability of measuring phycocyanin pigment from extra-cellular material even when cell counts at downstream sites may be low.

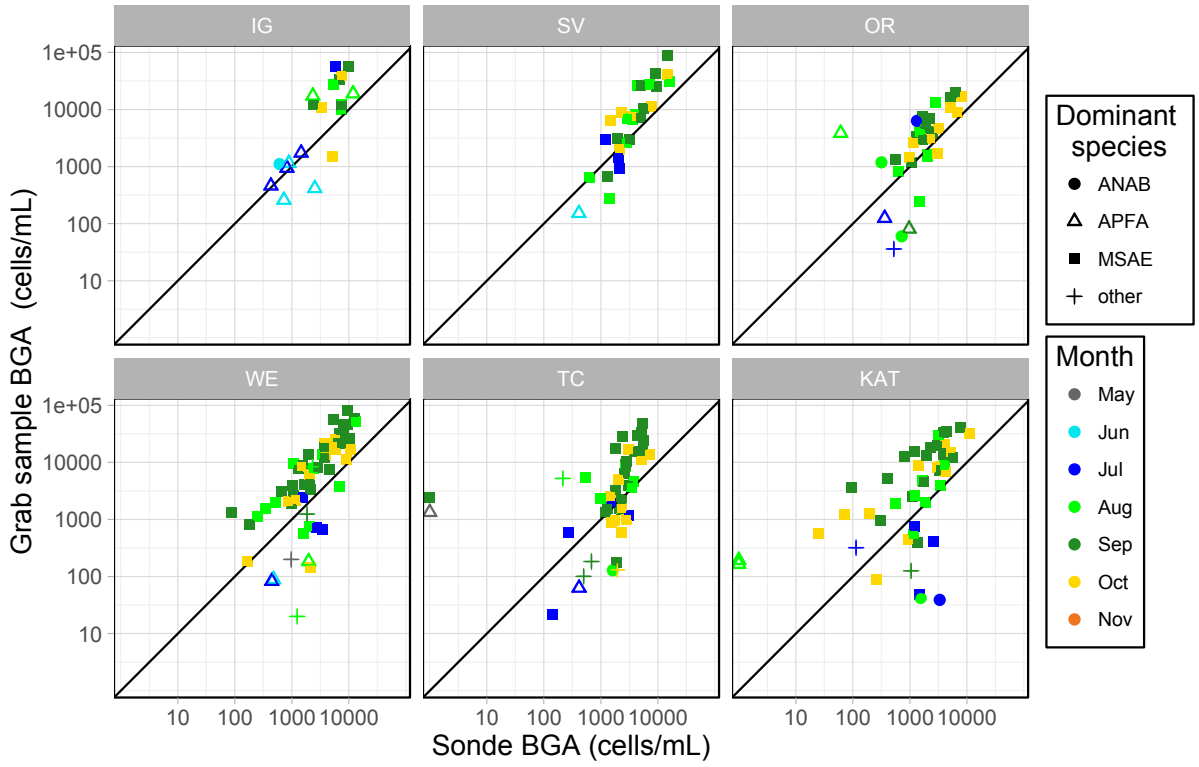


Figure 6. Grab sample BGA cell density from open channel grabs by sonde BGA at each site. Point shapes indicate dominant species in the grab sample and colors indicate month of sample. Black line is 1:1 line.

3.2.2 COMPARISON OF SONDE BGA TO GRAB SAMPLE MICROCYSTIN

Sonde BGA readings related non-linearly to grab sample microcystin toxin concentrations (Spearman's rho=0.64) in the Klamath River below Iron Gate Dam (Figure 4; panel B). At sonde readings < 1000 cells/mL, microcystin concentrations were generally below 2 µg/L at all sites. At sonde readings > 1000 cells/mL, microcystin concentrations could be high or low (0.05 quantile = 0 µg/L and 0.95 quantile = 7.4 µg/L). This pattern indicates that at higher sonde readings, additional factors beyond the density of BGA cells (as indicated by the sonde)

controlled toxin levels. This is an expected pattern given that expression of toxin producing variants of *Microcystis* is variable within a season and inter-annually in the Klamath River system (e.g., Bozarth et al. 2010). Otten et al (2015) also showed varying percentage of the number of toxic *Microcystis* cell equivalents (mcyE/mL) in Klamath River populations. Moreover, the sonde BGA is detecting other non-toxic cyanobacteria such as *Aphanizomenon*, and microcystin concentrations above 4 µg/L generally occurred only when *Microcystis* was the dominant species (Figure 7), and high microcystin concentrations occurred later in the summer at all sites (Figure 7).

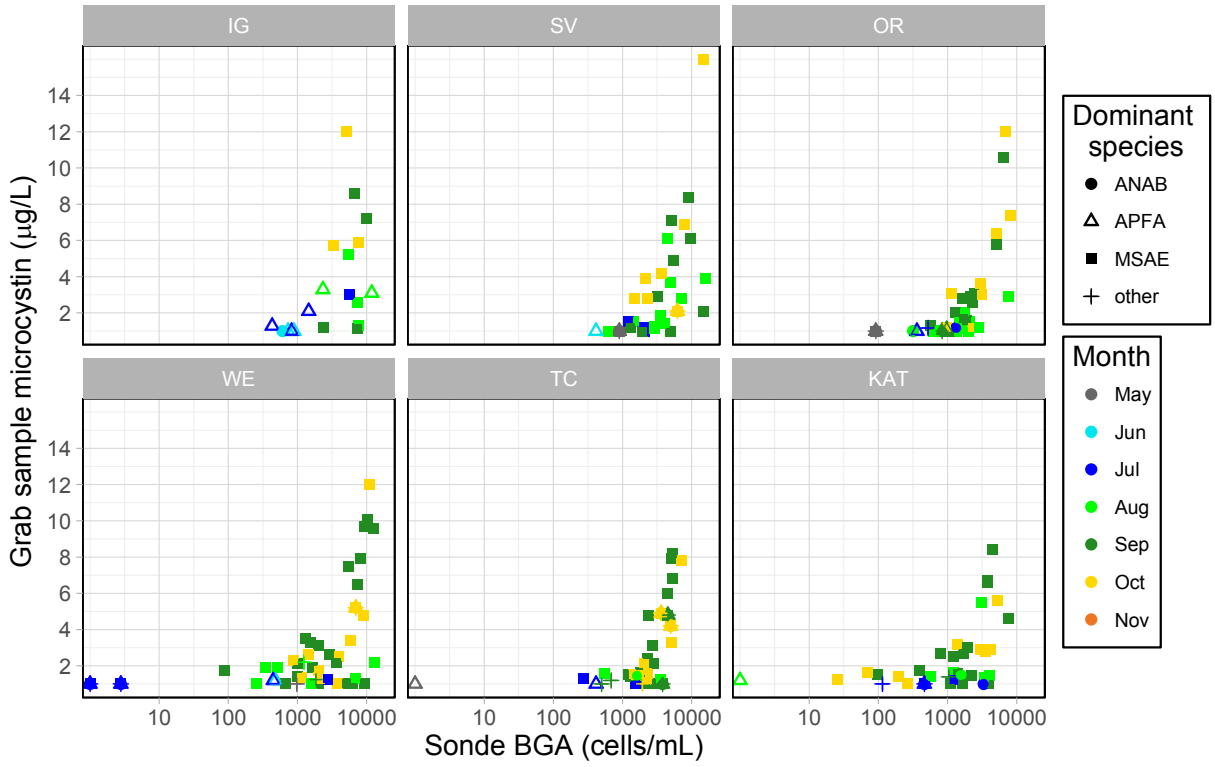


Figure 7. Grab sample microcystin from open channel grabs by sonde BGA at each site. Point shapes indicate dominant species in the grab sample and colors indicate month of sample.

3.2.1 TIMING AND MAGNITUDE OF BGA BLOOMS

Although sonde BGA reflected qualitative changes in bloom timing and magnitude, as expected based on the overall underestimation of grab sample cell density when sonde BGA is reported in cells/mL, the magnitude of sonde BGA density was lower than grab sample cell density over the course of the season (Figure 8). For example, sonde BGA seasonally increased and peaked concomitant with OC and SL grab sample BGA data, but due the underestimation of grab sample magnitude, the point at which the 0.9 quantile LOESS exceeded Tribal level II public health limit of 5000 cells/mL occurred three weeks later than indicated by grab sample data (Figure 8). The LOESS curves of both OC grab samples and sonde BGA data remained above 5000 cells/mL until mid-October. The LOESS of the shoreline grab samples declined below 5000 cells/mL slightly later, possibly due to build-up on the river edge only occurring after higher BGA concentrations were reached in the well-mixed water column.

These differences in estimates of bloom timing (based on crossing of the public health thresholds) and magnitude between the grab sample data and the sonde data do not reflect the accuracy of the phycocyanin probes, but rather are an artifact of the manufacturer conversion allowing RFUs to be reported as an estimate of cell density (cells/mL). Future analyses would be simplified by having the probes record RFUs and not estimated cell density. In this fashion, RFUs could be directly related to grab samples and public health thresholds without the need to consider whether the sonde estimated cell density either under- or overestimates grab sample cell density.

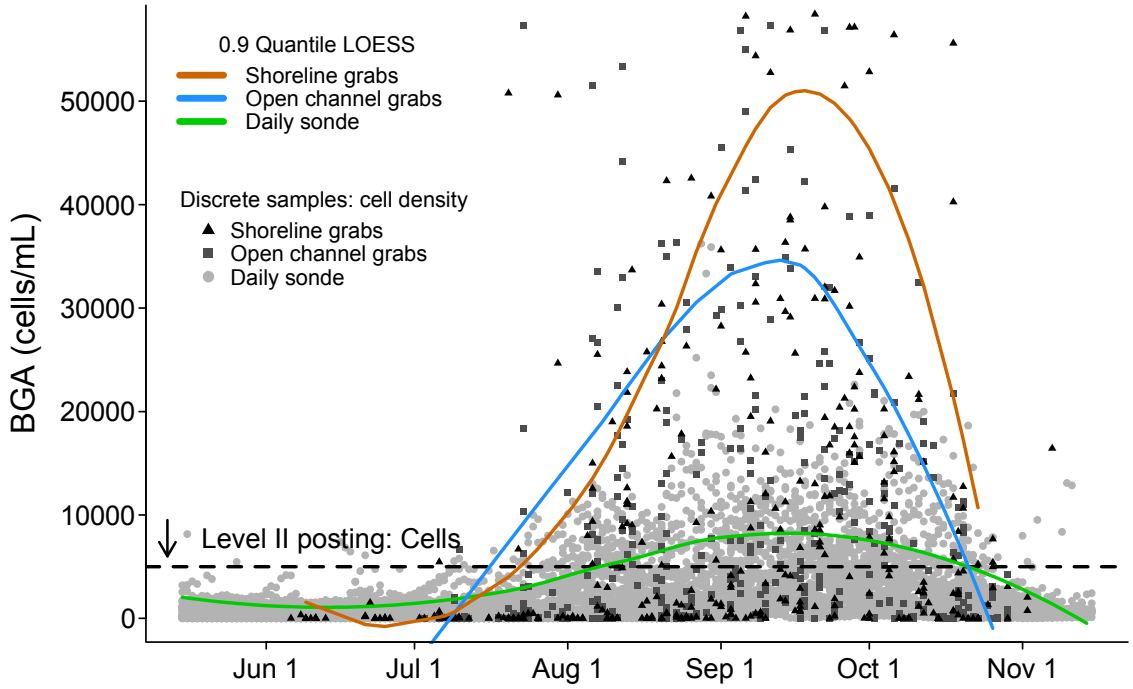


Figure 8. BGA cell density by date from shoreline grabs (SL), open channel grabs (OC), and daily mean sonde BGA (as reported from YSI phycocyanin probes. Lines show the 0.9 quantile LOESS fit for each data type and dashed lines show the Tribal level II public health advisory for cell density.

3.2.2 ESTIMATING PROBABILITY OF EXCEEDING PUBLIC HEALTH POSTING GUIDELINES BASED ON SONDE BGA DATA

Through the use of quantile regression, we calculated sonde BGA levels that predicted when 10% of the grab sample cell counts exceeded the Tribal public health advisories. Regression analysis of the upper conditional 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). The 0.9 quantile regression of grab sample BGA cell density as predicted by sonde BGA values exceeded the level I public health advisory of 1000 cells/mL at a sonde BGA level of <500 cells/mL (near sonde detection limit), and the level II public health advisory of 5000 cells/mL was exceeded at a sonde BGA level of ~1200 cells/mL (Figure 9). In other words, when the phycocyanin probes read ~1200 cells/mL, 10% of the grab sample cell counts were above 5000 cells/mL. The 0.9 quantile regression of the grab sample toxin data as predicted by the sonde BGA values exceeded the Tribal level I public

health advisory of 0.8 µg/L microcystin at a sonde BGA level of 1000 cells/mL, and exceeded the Tribal level II public health advisory of 4 µg/L at a sonde BGA level of ~3000 cells/mL. As expected based on the above relationships showing that the sonde BGA data generally under predicted grab BGA data at higher BGA concentrations, these results demonstrate that the upper limits of grab sample cell density and toxin concentrations (0.9 quantile) are predicted to exceed Tribal public health advisories at correspondingly lower sonde BGA readings.

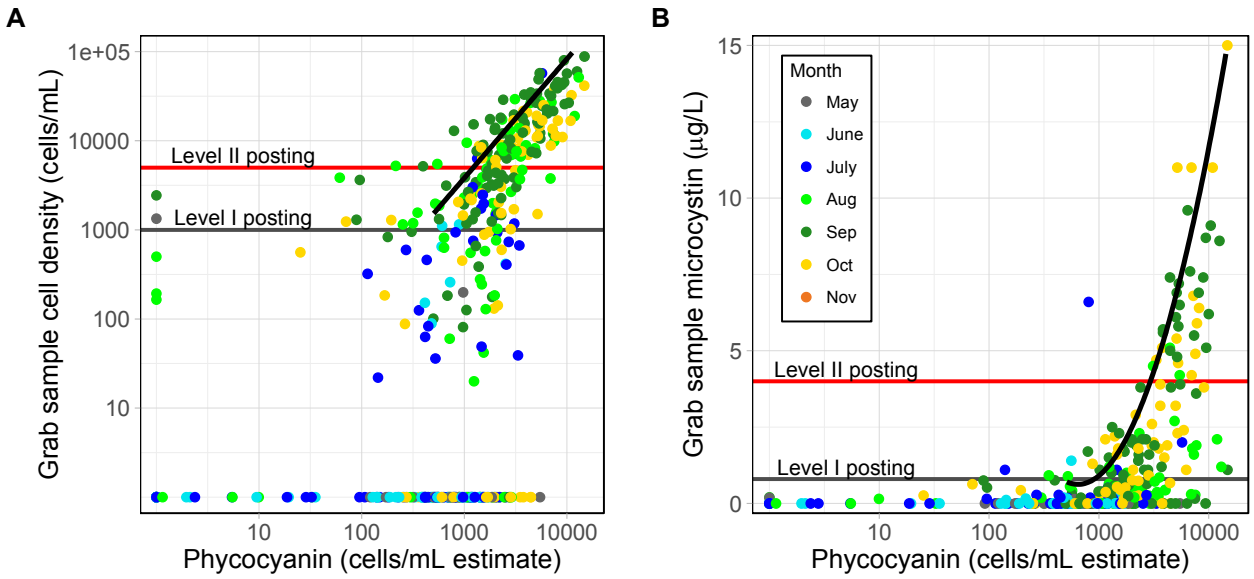


Figure 9. BGA cell density (panel A) and microcystin concentration (panel B) from grab samples by sonde BGA. Colors indicate month during which sample was collected. Black line is the 0.9 quantile regression, and horizontal lines indicate level I and II Tribal health posting threshold.

Quantile regressions provide a means to provide 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 sonde BGA) or when the underlying assumptions of linear regression such as homogeneity of variance may not be met (Cade and Noon 2003). Moreover, regressions using upper quantile values then help to determine thresholds more protective of public heath even when variability or non-linearity occurs in the underlying relationships (e.g. Figure 9).

High variability in the data and violation of parametric assumptions associated with linear regression[14] between sonde BGA (the predictive variable) vs. grab BGA or grab toxin (response variables) hampered establishment of simple predictive models. 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 for when the BGA probes indicate that either BGA cell density or microcystin toxin exceeds various public health warning and or posting levels. Such nonparametric cross-tabulation models have been used successfully for

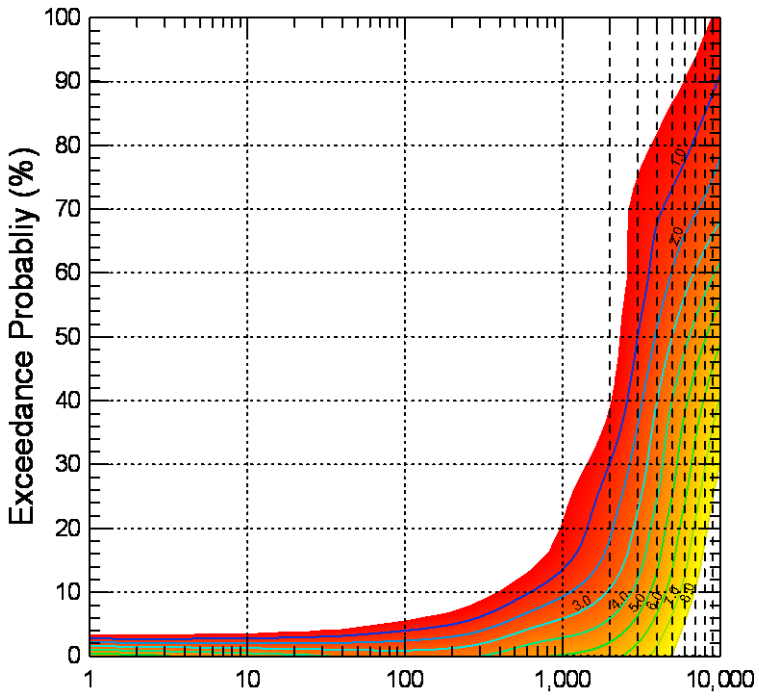
[14] For example even with transformation, error residuals violated assumptions of normality for the linear model between sonde BGA and grab BGA

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.

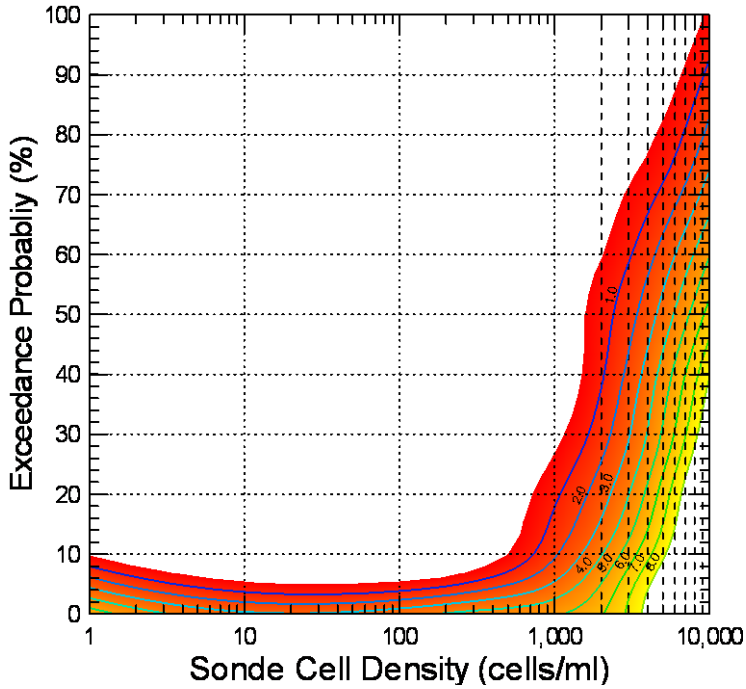
We combined the probability of exceedance analysis with a contouring algorithm to produce a series of graphs allowing the sonde BGA level that corresponds to any chosen probability (frequency) of exceedance value of the various public health thresholds for BGA toxin concentration (Figure 10) or BGA cell density (Appendix D) to be determined. Such analyses provide flexibility for public health managers to determine an acceptable level of risk. For example, for a conservative approach with respect to public health, one can choose a 10% exceedance probability or the point above which probabilities increase sharply[15], alternatively one can take a less conservative approach and choose higher exceedance probabilities. For instance, if one is interested in the corresponding BGA sonde cell density at which the Tribal level II public health advisory (microcystin level of 4 µg/L) 10% or 20% probability was exceeded, the analysis shows that this occurred at ~2500 and 3500 cells/mL, respectively (Figure 10; panel A). Alternately stated, the level II public health advisory was exceeded 10% of the time at a BGA sonde cell density level of ~2500 cells/mL and 20% of the time at 3500 cells/mL. The 10% value of 2500 cells/mL is similar to the 3000 cells/mL as computed from the quantile regression above. The point above which probabilities increase rapidly occurred at 2000 cells/mL and was slightly lower than the 10% exceedance level of 2500 cells/mL (Figure 10; panel A). Due to the sharp increase in slope above 2000 cells/mL, the exceedance probability of 4 µg/L microcystin jumps to ~50% at a BGA sonde value of 5000 cells/mL. In addition, the point above which probabilities increased rapidly occurred at 2500 cells/mL for the 6 µg/L microcystin level corresponding to the California CyanoHAB Warning Tier I advisory.

Although near the lower range of stated detection limits for the phycocyanin probe (220 cells/mL, YSI 2012; 1500 cells/mL Bastein et al. 2012), the analysis indicated that the probability of exceeding the Tribal level I public health advisory of 0.8 µg/L microcystin rapidly increased above a sonde BGA density of ~500 cells/mL (the 0.8 µg/L curve is the left-most edge from the 1 µg/L curve in Figure 10). As expected based on higher toxin values in near-shore areas where cells accumulate along the river margin (Kann and Corum 2009), the corresponding sonde BGA cell density was 2000 cells/mL for the 10% probability of exceeding the Tribal level II public health advisory (Figure 10; panel B), a value equal to the sonde BGA cell density when levels increased rapidly in the open channel analysis (Figure 10; panel A).

[15]As was done for determining exceedance of critical *Microcystis aeruginosa* cell density levels for the Klamath River TMDL chlorophyll-a target of 10 ug/L; NCRWQCB 2010). Often the point of rapid increase is the curve inflection point but can vary depending upon cure shape.



Panel A: Open channel grab samples



Panel B: Shoreline grab samples

Figure 10. Probability of exceeding critical Klamath River microcystin levels at varying sonde BGA cell densities. For any chosen sonde BGA 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 2014 during May 15 through November 15. Panel A was created using toxin concentrations from open channel grab samples and panel B used shoreline grab samples.

Similar exceedance analyses for observed cell density as the response variable showed that exceedance of grab BGA cell densities was positively related to sonde BGA cell density, and as shown above, sonde BGA generally underestimated grab BGA (Appendix D). For example the 10% probability of exceeding the Tribal level II public health advisory of 5000 cells/mL for open channel samples occurred at a sonde BGA cell density of ~1000 cells/mL, and the expanded scale (Appendix D; bottom figure for open channel samples) analysis showed that there was a 50% exceedance of grab BGA cell density of 20,000 cells/mL at a sonde BGA density of 6000 cells/mL. The point of rapid increase occurred at a sonde BGA cell density of ~500 cells/mL. Although this is near the detection limit for the probes, it does indicate that for the Klamath River system one can expect a rapid increase in the probability of exceeding various grab BGA cell density thresholds at relatively low sonde BGA values.

As with toxin values, the relationship with shoreline grab samples showed higher exceedance probabilities at a given sonde BGA density than did the open channel analysis (Appendix D). At 1000 cells/mL sonde BGA, the probability of exceeding the Tribal level II public health advisory increased to 20%. Given probe detection limit issues at values closer to the inflection point, it appears that sonde BGA values of 1000 cells/mL provide a conservative indication of the level above which is associated with increased risk of exceeding the Tribal level II public health advisory. For example, at 2000 cells/mL sonde BGA the probability of exceeding 5000 cells/mL increases to ~20% for open channel grabs and ~40% for shoreline grabs, whereas at 1000 cells/mL sonde BGA the probabilities were 10% and ~18%, respectively.

3.3 APPLICATION OF REAL-TIME PHYCOCYANIN MONITORING TO PUBLIC HEALTH MANGEMENT

The phycocyanin probes provide data in real-time that can be used to inform public health decisions about cyanoHABs in the Klamath River. The phycocyanin probes sufficiently reflected the cyanobacteria conditions in the Klamath River to be used as additional evidence signifying bloom conditions that warrant notification to river users. Possible applications for use of the real-time phycocyanin data include increasing grab sample frequency based on changes in phycocyanin, integrating phycocyanin levels into public health posting guidelines, and making the real-time data available to the public in the context of risk levels via on-line resources or daily printouts.

To set phycocyanin levels that signify possible health risks, selection of an appropriate risk level (chosen exceedance value or curve inflection point) should first be made. As an example, we selected sonde BGA levels of 500 cells/mL and 2000 cells/mL, corresponding to the levels above which the probability of exceeding the Tribal Level I and Level II public health advisories for microcystin rapidly increased in OC samples, and corresponding to a ~10% probability of exceeding public health guidelines using SG microcystin samples.

Using these chosen sonde levels to indicate “sonde exceedances”, we compared the continuous probe data to the number of sonde exceedances that occurred both seasonally and inter-annually over the past eight years of monitoring, focusing our analysis on the Tribal level II public health advisory threshold (Figure 11). Most sonde exceedances occurred August through

October[16], and during some months 100% of daily mean sonde BGA values exceed this threshold (Figure 11). As expected based on the longitudinal decline in BGA cells and toxin (e.g., Otten et al. 2015; Kann and Asarian 2006), the number of sonde exceedances declined downstream from Iron Gate Reservoir. However, even at the most downstream stations, sonde exceedances occurred 80-100% of days in some months (e.g., see September and October of 2010 at TC or August of 2014 at WE, TC and KAT; Figure 11).

Using the sonde BGA levels of 500 cells/mL and 2000 cells/mL, corresponding to the levels above which the probability of exceeding the Tribal Level I and Level II public health advisories rapidly increased, it is apparent that the bi-weekly to weekly grab sampling intervals (grey vertical lines in Figure 12), although mostly protective of public health[17], can often miss peaks of BGA that likely warrant public health warnings[18]. For example, within some 2014 grab sampling intervals such as those near September 15th for SV, OR and TC, the BGA phycocyanin probe exceeded the 2000 cell/mL sonde BGA level corresponding to the Tribal level II public health advisory, even when the beginning and ending of the intervals showed values below the that level (Figure 12). Thus, the use of the continuous BGA probes for detecting periods when public health exceedances are likely occurring is highly complementary to the weekly grab sampling protocol, and can be used to supplement such programs to minimize public health risk.

Rapid changes in phycocyanin levels, which indicate changes to riverine BGA conditions, could be used to inform public health officials and the public either in real-time or at a minimum with a significantly shorter time lag than grab sample data. Because grab samples are collected every one to two weeks, and require an additional four to seven days minimum for processing of cell density[19], grab sample results can easily have a two-week delay from the time algal conditions change to receiving sample results. A major strength of the phycocyanin data is that it is available in real-time, and can thus be used to indicate river conditions prior to the receipt of grab sample results. Examination of the phycocyanin data from multiple sites will help confirm that the patterns and general magnitudes are not likely due to probe malfunction. These data can then be used to implement interim public health warnings, or as others have done, graphical displays similar to that shown in Figure 12 could be produced with the real time data (e.g., Loisa et al. 2015). The public could access these data figures via a website or postings at river access points.

[16] This is similar to grab sample cell counts which also regularly exceeded Tribal level II public health advisories (5000 cells/mL) in August as well as September and October, but again is in contrast to the Tribal level II public health advisory of 4 µg/L microcystin, which was more regularly exceeded in September and October (e.g. see Figure 9b). As noted above, this reflects the seasonality in the occurrences of higher microcystin values, which lagged those of *Microcystis* cell density.

[17] Especially since once a posting occurs, de-posting requires that toxin results are below the guideline level of 0.8 µg/L for two consecutive sampling events that are at least one week apart (see Yurok guidelines Appendix C).

[18] An example year of 2014 is shown in Figure 12 using the Yurok guidelines and all years are shown in Appendix E for Yurok health advisory levels and in Appendix F for California Water Quality Monitoring Council CyanoHAB Network (CCHAB) public health guidelines.

[19] Grab samples for microcystin toxin generally take two weeks before results are available for public health managers.

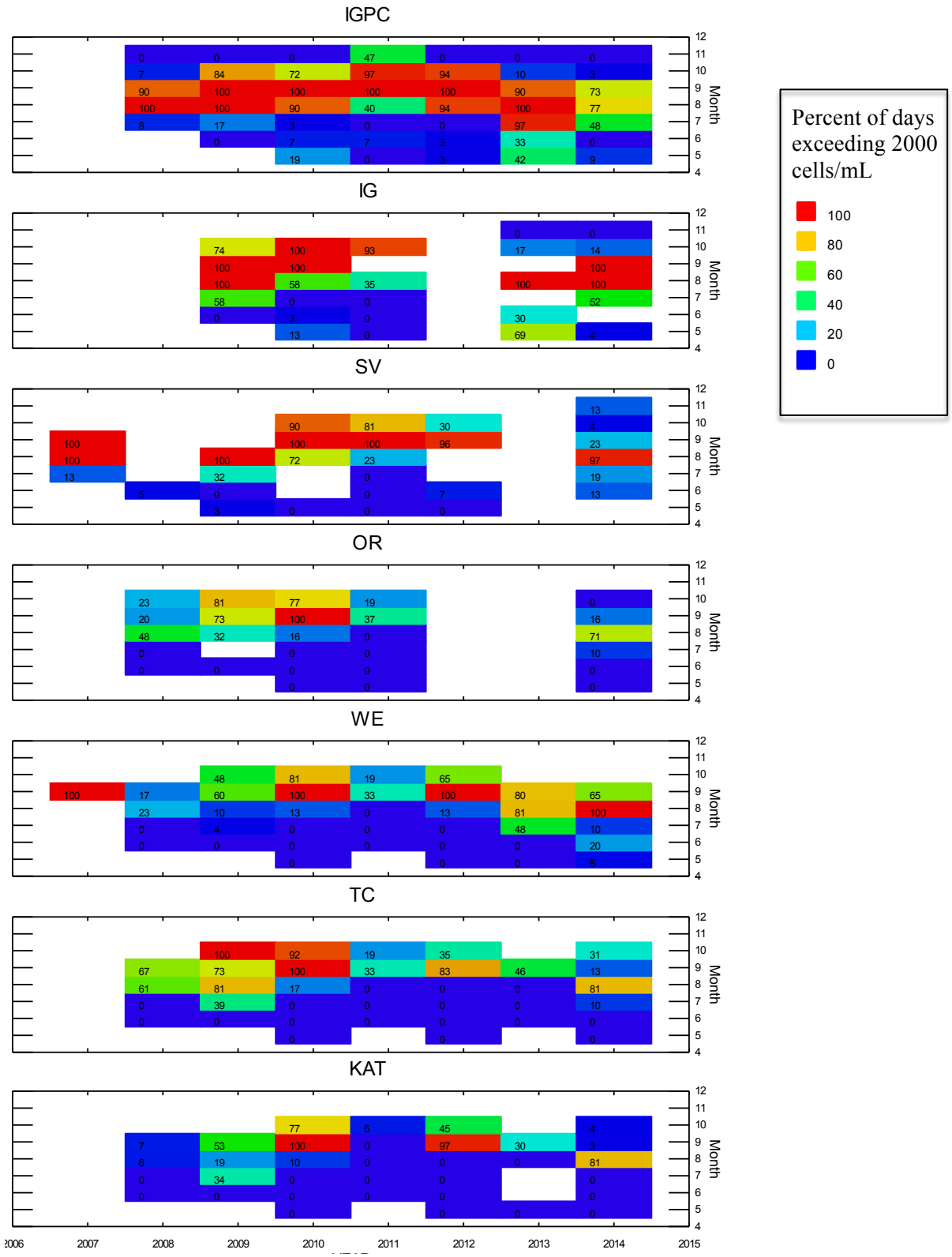


Figure 11. The percent of days at each of seven Klamath River sites that exceeded 2000 cells/mL (sonde estimate) for each month (y axis) and year (x axis) are indicated on each tile and are categorized by color.

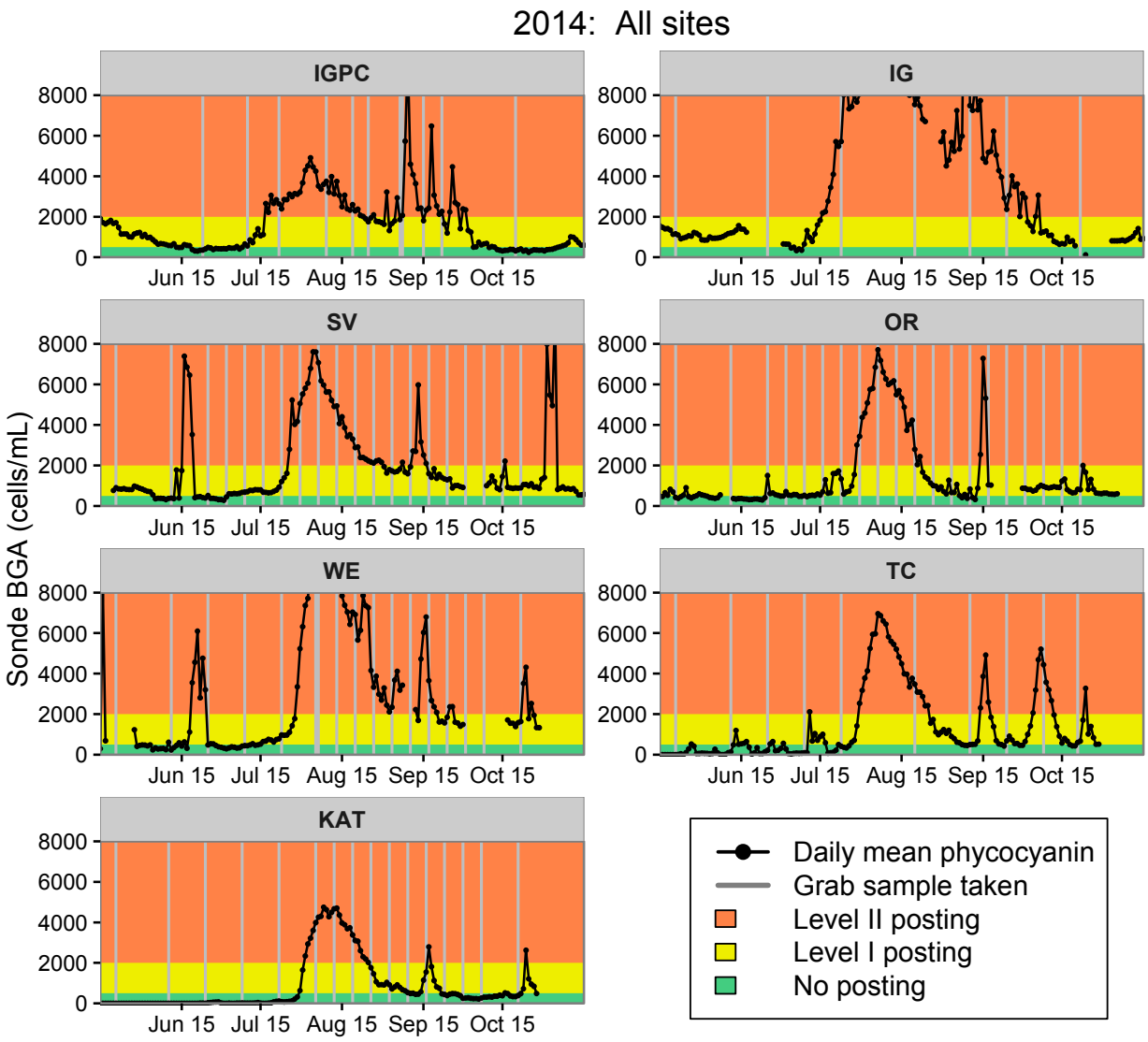


Figure 12. Mean daily sonde BGA at Klamath River sites during 2014 as reported from YSI phycocyanin probes. Background colors indicate possible risk levels as determined from probability of exceedance analyses. Green < 500 cells/mL; Yellow > 500 cells/mL and < 2000 cells/mL; Orange > 2000 cells/mL. Grey vertical lines indicate when grab samples were collected.

Although false advisories are possible using the phycocyanin data, increases in phycocyanin that occur in the interval between grab sampling events or increases that began prior to grab sample results were received, suggest that a delay in posting or missing postings altogether is also a risk when relying on grab sample data alone. The Seiad Valley site in 2014 provides an example of how using the phycocyanin data would have provided a more timely warning of the cyanoHAB threat in the Klamath River. Phycocyanin increased to levels above

2000 cells/mL (the BGA probe estimate corresponding to the point at which the probability of exceeding the Level II warning limit of 4 µg/L rapidly increases) on July 26th, and continued to increase to > 6000 cells/mL into early August (Figure 12). Although these levels would have warranted public health advisories based on exceedance of the Level II limit on July 26th, grab sample results at Seiad Valley from July 30th were the first samples to show toxin concentrations and cell counts above the Level I health advisory, and these sample results were not received until August 5, 2014, 10 days after the phycocyanin data suggested a rapid increase in riverine BGA. In this event, the increase in the bloom magnitude was large enough (from 0 cells/mL on 23 July to 50,590 cell/mL on 30 July) to bring with it visible algal scums that led to public health postings on 31 July that were based on the presence of algal scums. However, most postings are generally based on the results of grab sample data and are thus subject to even longer delays.

Choosing sonde exceedance levels to base postings or other actions on will require balancing the risk of missing or delaying posting of potentially dangerous bloom conditions (false negatives) with not over-posting when bloom condition are not present (false positives). If one wanted to minimize false positives, a BGA sonde value of 3500 cells/mL provides an upper limit above which false positives are minimal[20]. However, at a sonde value of 3500 cells/mL the probability of exceedance of the Tribal Level II warnings increased to 25% for microcystin toxin (4 µg/L level; Figure 10B), and 70% for cell density (5000 cell/mL level; Appendix D). Moreover, the number of false negatives nearly doubles from 18 at 2000 cells/ml to 35 at 3500 cells/mL.

Implementing the phycocyanin probe data into the decision making framework and using the data as an additional tool to inform monitoring and convey public health risk to river users can improve public safety on the Klamath River. Furthermore, presenting data in a user friendly and accessible framework has the potential to allow for informed decision-making by river users. As noted above, sonde BGA reported as cell density (cells/mL) is estimated by the sonde manufacturer from relative florescent units (RFUs), but as an estimate does not represent a 1:1 correlation with cell density as directly measured using microscopic analysis. Thus, to avoid the issue of needing to compare and relate sonde BGA readings presented as estimated cells/mL to grab sample cell density directly measured in cells/mL, we recommend that sonde BGA readings be recorded as RFUs, and that the probability of exceedance and other relationships be determined using RFUs and not sonde estimated cells/mL.

[20]Of the five false positives above 3500 cells/ml, three had detectable levels of microcystin, two of which were over the Tribal level II public health advisory, suggesting that even though cells were not detected in these samples, the probes detected the presence of a cyanoHAB, leaving only two true false positives above the sonde level of 3500 cells/mL.

3.4 SPATIAL AND TEMPORAL TRENDS IN SONDE BGA

3.4.1 ANNUAL AND SEASONAL VARIATION IN SONDE BGA

Elevated levels of cyanobacteria occurred in all years of this study in the Klamath River, although variation in the timing and magnitude of bloom conditions was apparent among years (Figure 13, Figure 14). The highest bloom magnitude as indicated from the LOESS regression of the mean daily sonde BGA data occurred in 2007, while the lowest magnitude occurred in 2008 (Figure 14). However, fewer sites were operational during these early years of monitoring, which may have influenced these patterns. For example, in 2007 only Seiad Valley and Weitchpec were operational, whereas in 2008, Iron Gate and Seiad were not in operation. The years 2010 and 2014 had the next highest bloom magnitudes (mean sonde BGA = 3460 and 2040 cell/mL estimate respectively). Lower magnitude bloom years included 2011 and 2013 (mean sonde BGA = 1440 and 2660 cell/mL estimate respectively). Box plots, which show medians and interquartile range of daily mean sonde data from May 15 to November 15, show similar cyanobacterial trends, with 2007 showing highest median and upper-quartile values, followed by 2010 with a relatively high upper-quartile (but not higher medians), and 2008 and 2011 were relatively low BGA years, as documented by the phycocyanin probes. The remainder of the years had intermediate BGA densities estimates during the six-month monitoring season, although interpretation of high vs. low BGA years is dependent on both the maximum bloom magnitude and bloom length, and therefore, a simple seasonal mean is not indicative of public health risk without understanding the length and magnitude of bloom periods (Figure 13).

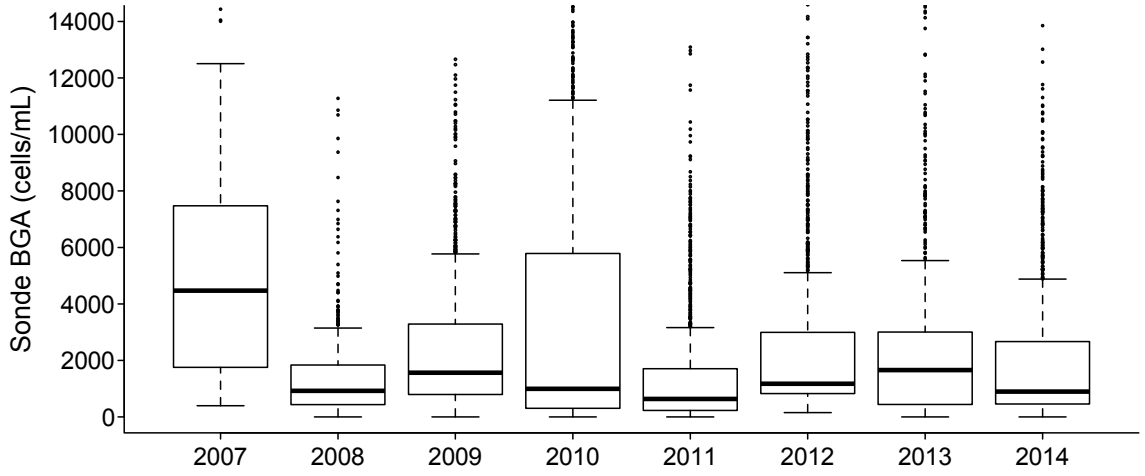


Figure 13. Box plots of daily mean sonde BGA at all sites by year.

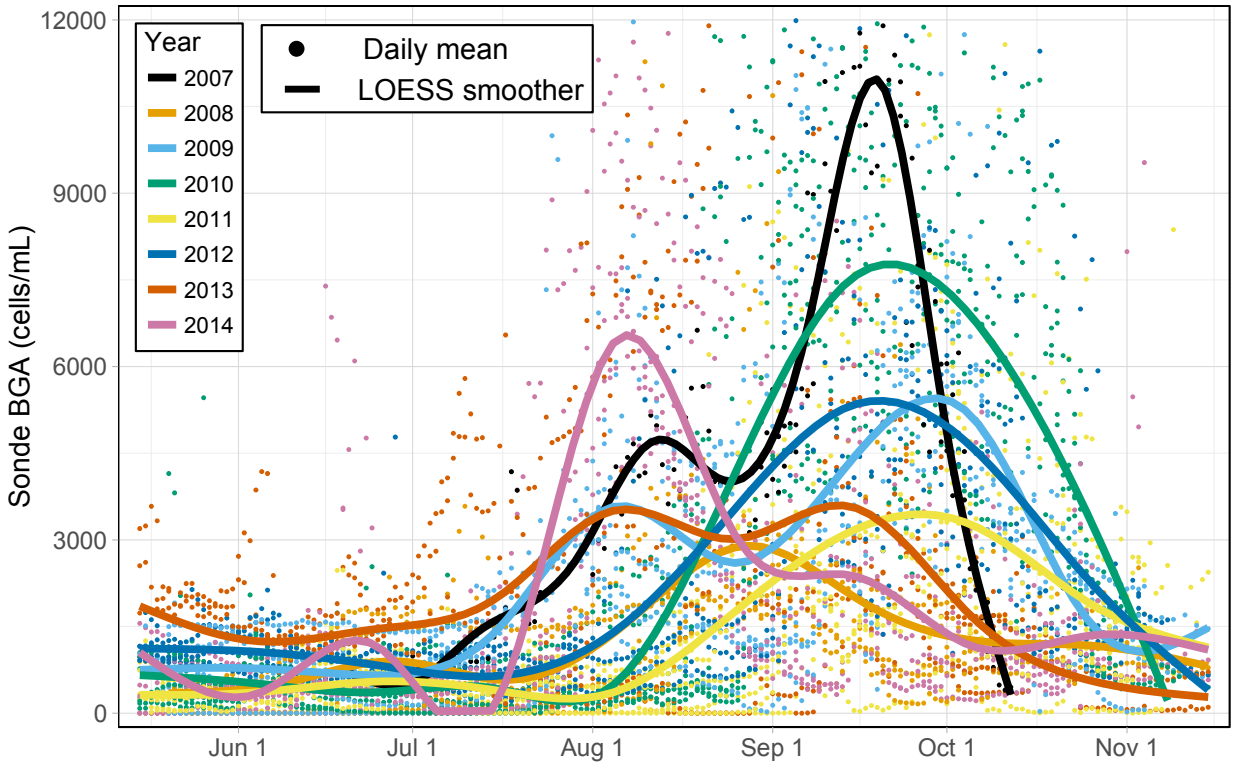


Figure 14. Daily means of sonde BGA for all sites and years by date on the Klamath River. LOESS curves show seasonal trends in mean BGA by year.

The season in which cyanobacterial bloom conditions were present in the Klamath River ranged from mid-July through October, with the time that the bloom started, ended, and the total length of the bloom varying among years (Figure 14). In 2014, the bloom peaked in early August, which is the earliest bloom peak during the study period. More commonly, the bloom peaked in mid- to late-September. Sonde BGA levels decreased following the bloom peak, with some years returning to pre-bloom BGA conditions in early November. In 2007, 2008, 2013, and 2014, BGA conditions returned to pre-bloom levels approximately three weeks earlier. Variability in the start and end times of the bloom led to variation in bloom duration. Because most planktonic cyanobacteria in the Lower Klamath River originate in the upstream reservoirs (Otten et al. 2015), the timing and magnitude of the bloom in any given year is related to reservoir conditions. Variability in tributary inputs will also influence BGA cell densities via dilution.

3.4.2 DAY-TO-DAY VARIATION IN BGA

At times sonde BGA cell density values showed large daily fluctuations, indicating changes in BGA density between grab sampling intervals (Figure 12). The largest daily fluctuations in sonde BGA cell density occurred during peak bloom periods, with daily increases and decreases of >5000 cells/mL common. In addition, increases and decreases in sonde BGA values of >10,000 cell/mL sometimes occurred over the course of one week during the onset of the bloom and at the end of the bloom. Daily fluctuations in sonde BGA were generally higher at upriver sites (standard deviation for IG, IGPC, and SV = 3860, 3560, 4500 cells/mL

respectively), with decreasing daily fluctuations at lower river sites (standard deviation for OR, WE, TC, and KAT = 2470, 3420, 1580, 1930 respectively). Day to day fluctuations in BGA often resulted in BGA densities that were above level II public health posting guidelines, and these increases often occurred in the interval between grab samples. Thus, as noted above, real-time BGA monitoring in the interval between grab sampling events could be used to inform river managers and the greater public to changing riverine BGA conditions that are common on the day-to-day scale on the Klamath River.

3.4.3 LONGITUDINAL TRENDS IN BGA

There was a distinct longitudinal trend of decreasing sonde BGA from upriver to downriver on the Klamath below Iron Gate Reservoir. Highest cell density estimates occurred just below the dam, and decreased downstream. One exception to this pattern occurred at Orleans, where upper-quartile and median cell density estimates were lower than at Weitchpec, despite Weitchpec being approximately 15 miles downstream from Orleans (Figure 15).

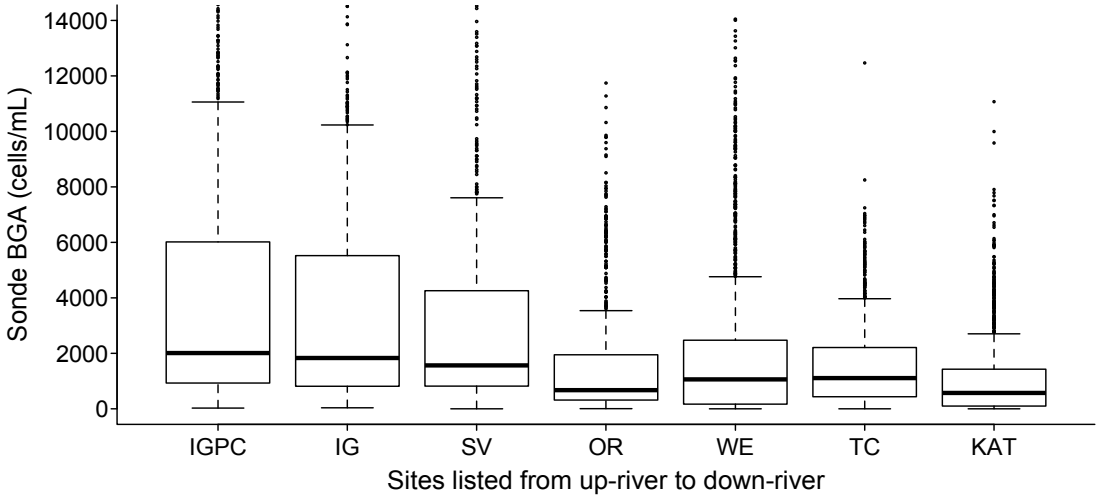


Figure 15. Box plots of daily mean sonde BGA from 2007 to 2014 by site.

The longitudinal decrease in sonde BGA from upriver to downriver was most pronounced between Iron Gate and Seiad Valley and Seiad Valley and Orleans (Figure 16), where sites are separated by ~60 river miles. Despite Weitchpec and Tully Creek being only five miles apart, there was a notable decrease in BGA estimates between these two sites when algal densities were high (Figure 16), likely due to dilution by the Trinity River, which enters just below the Weitchpec monitoring station and dilutes the BGA concentration. Since endemic populations of *Microcystis* were not found in flowing regions of the Klamath River (Otten et al. 2015), and flowing waters typically do not provide good habitat for planktonic cyanobacteria (Reynolds 1984), the decreases in BGA cell density from upriver to downriver are most likely due to dilution from tributaries, settling and trapping of cyanobacterial cells in low velocity edge areas, and the physical forces of the flowing river that likely break down cells and associated algal pigments.

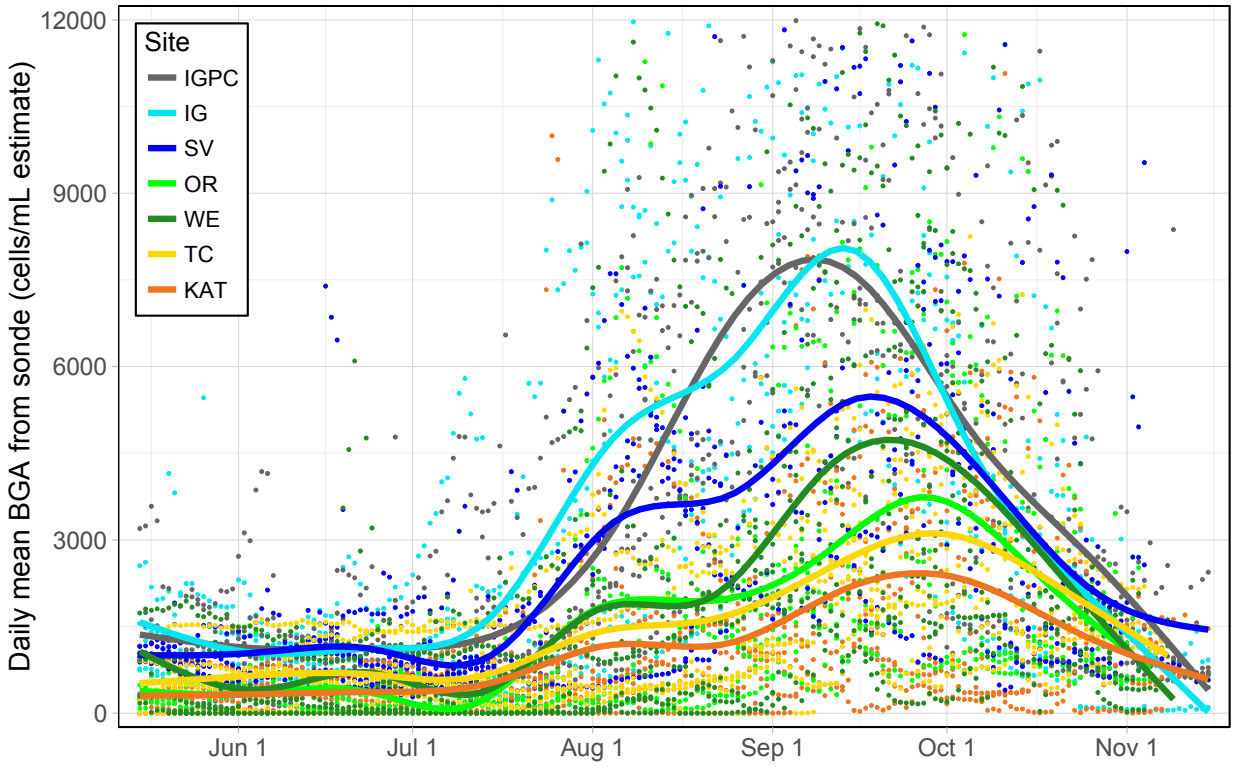


Figure 16. Daily means of sonde BGA by site on the Klamath River with LOESS curves by site.

The longitudinal patterns in sonde BGA estimates were generally similar among years, with some exceptions (Figure 17). The two Iron Gate sondes and the Seiad sonde reported higher BGA concentrations than other sites, except in 2012 and 2014. In 2012, missing data from Seiad likely led to lower mean estimates of BGA cell density at that site, whereas in 2014, the Iron Gate sonde operated by Pacific Corp reported the lowest BGA estimates from any site, despite the Karuk Iron Gate site reporting the second highest BGA concentration that year. The IGPC sonde was updated to a YSI EXO model in 2014, and although YSI states that the BGA output from the EXO sonde should be comparable to the 6600 series sondes (personal communication, YSI technical support), the low values reported in 2014 warrant caution in comparing output from the EXO sonde to data from other sondes.

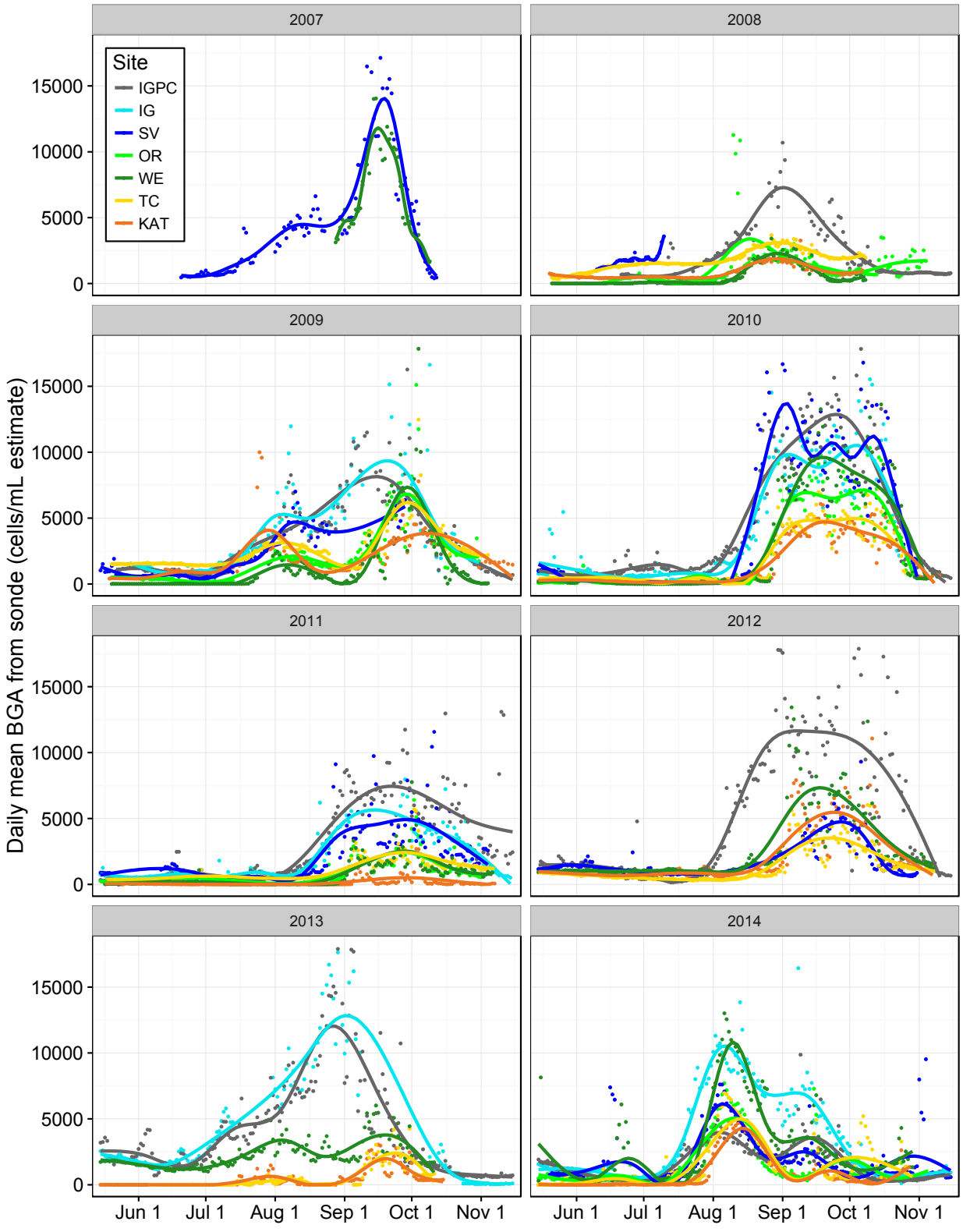


Figure 17. Daily means of sonde BGA on the Klamath River with LOESS curves by site and year.

4 CONCLUSIONS

The analysis provided herein develops system-specific relationships between real-time fluorescence of cyanobacterial pigment and cell density and microcystin toxin from grab samples collected longitudinally in the Klamath River. Data from phycocyanin probes generally reflected the cyanobacterial conditions in the Klamath River. Furthermore, the real-time data can be used to inform public health management of toxic algal blooms as a complementary source of information to the grab sample cell density, toxin concentration, and visual observations currently being used. Although real-time phycocyanin monitoring is becoming common in many water bodies, creating models to predict exceedance thresholds based on real-time data and to implement these models is rare (Francy et al. 2016), but the extensive data collection efforts and cyanobacterial bloom events on the Klamath River have allowed for the examination of these relationships, with relevant application to public health management.

Phycocyanin concentrations from real-time sensors represented the blue-green algae conditions in the Klamath River below Iron Gate Dam. Although the magnitude often differed between sonde estimates of BGA concentration and grab sample cell concentration, the fluctuations in BGA cell counts and microcystin toxin were well represented by the probe data. After removing phycocyanin data that were likely due to probe issues such as biofouling, grab sample cell concentrations increased with increasing phycocyanin (recorded as cell density based on within-probe conversion from RFUs to cells/mL). The increase was non-linear, with probe cell density data consistently underestimating BGA cell concentrations at readings above ~3500 cells/mL, while lower probe readings, although primarily underestimating grab cell density, were associated with cell counts both lower and higher than associated probe readings. Lower grab sample cell counts relative to BGA probe cell density (overestimates), occurred more often when species other than *Microcystis* were dominant and often occurred at lower probe readings that approached probe detection limits. Microcystin also increased non-linearly with increasing phycocyanin measurements. For example, when phycocyanin levels were low (< 1000 cell/mL estimate), microcystin was always low (< 2 µg/L). When phycocyanin levels were high, microcystin could be low or high, but the frequency of higher microcystin values was substantially greater than lower ones above sonde BGA values of 2000 cell/mL. For example, at sonde BGA values of 10,000 cells/mL, the frequency of concentrations above 4 µg/L (Tribal level II public health advisory) was >60%.

Setting the phycocyanin probes to record as RFUs instead of cell density will facilitate the development of relationships between phycocyanin probe readings and sampled cell density and toxin levels that are easier to interpret. Reporting phycocyanin as cell density leads to confusion between units of measured cell density from grab samples and the estimated cell density units from the phycocyanin probes, especially when considering units in the context of public health thresholds. Setting sondes to report both RFUs and cells/mL will help transition into using RFUs, which most new phycoyanin probes report in exclusively.

Real-time phycocyanin data can be used to inform public health management of toxic cyanobacterial blooms on the Klamath River. Despite non-linearities and apparent under/overestimation issues in the relationships between sonde BGA and grab sample data, we were able to quantify sonde BGA levels associated with public health warning levels for both cell density and toxin. We provided a graphical tool where daily mean sonde BGA values can be compared to sonde BGA thresholds that correspond to any chosen exceedance probability for a

particular public health posting level, and provided examples of how this exceedance level can translate into templates to display the phycocyanin data. As an example, we selected sonde BGA values of 500 and 2000 cells/mL, which corresponded to approximately a 10% probability that the Tribal level I and II public health warning thresholds for microcystin concentration would be exceeded. Phycocyanin levels corresponding to the 10% exceedance probability were chosen in part because 10% was near the inflection point, where the probability of exceedance began to rapidly increase at higher sonde BGA levels. Using these example "sonde exceedance levels", we found that sonde exceedances were most common closer to Iron Gate Dam, but that phycocyanin levels were above 2000 cells/mL during all of September at all sites during some years.

Quantification using these methods allows for the real-time phycocyanin data to be used as an early warning system to signal changes in riverine BGA conditions relative to public health warning levels. Implications for using the real time data range from notifying sampling entities to collect non-scheduled grab samples, to issuing public health warnings, to creating tools for the public to access the real-time data displayed against risk levels from this analysis based on the current public health warning levels.

Distinct longitudinal and temporal patterns emerged in the multi-year, high frequency phycocyanin data set, with implications to public health awareness and management. There was a longitudinal decrease in BGA concentrations from below Iron Gate Dam to Turwar, and a corresponding decrease in "sonde exceedances". Maximum bloom magnitudes were lowest below the confluence of the Trinity River, where sonde BGA estimates at Turwar were about a third of those below Iron Gate Dam. Seasonal variation in phycocyanin was more pronounced than the variation among years. Bloom conditions were present from mid-July to November, and most commonly peaked in September. Day to day variation in BGA was high during the bloom, making the use of real-time phycocyanin probes a useful tool to indicate riverine BGA conditions, especially during the interval between grab sample events. Phycocyanin readings showed daily fluctuations of more than 5000 cell/mL during bloom periods, and increased and decreased more than 10,000 cells/mL within one week when the bloom was starting or ending. Many of these changes in phycocyanin concentrations occurred during intervals between grab samples or before results from grab samples were received, making the probe data useful as a real-time indicator of changing BGA conditions.

Phycocyanin probes indicate bloom conditions in real time, and can be used as a tool for managers to implement early public health warnings of potentially toxic bloom conditions when laboratory results from grab samples are not available or pending. This analysis was a first step toward implementing the sonde BGA data into the cyanoHAB public health awareness framework. From here, decisions will need to be made about how to implement the sonde BGA data, while learning how to work with and interpret the phycocyanin data in real time. Although this analysis could be performed in other aquatic ecosystems or with different BGA sensors, the outcomes of this report are specific to the Klamath River below Iron Gate Dam, and the relationships in other water bodies or with upgraded sensors will need to be investigated, as relationships may differ.

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6 ACKNOWLEDGMENTS

This report was prepared for the Klamath Tribal Water Quality Consortium using funds awarded to the Consortium by the U.S. EPA Region 9 and administered by the Karuk Tribe. The Yurok Tribe, Karuk Tribe, and Pacific Corp contributed data to this report. We also acknowledge the diligent field crews from the Yurok and Karuk Tribes who collected field data and maintained the water quality data sondes. Constructive comments from Andy Lincoff of the U.S. EPA, Katherine Carter and Steven Butkus from the Northcoast Water Quality Control Board, and Randy Turner from the Klamath Basin Monitoring Partnership improved this report.

AAAPPENDIX A: RAW DATA FROM SONDES AND GRAB SAMPLES

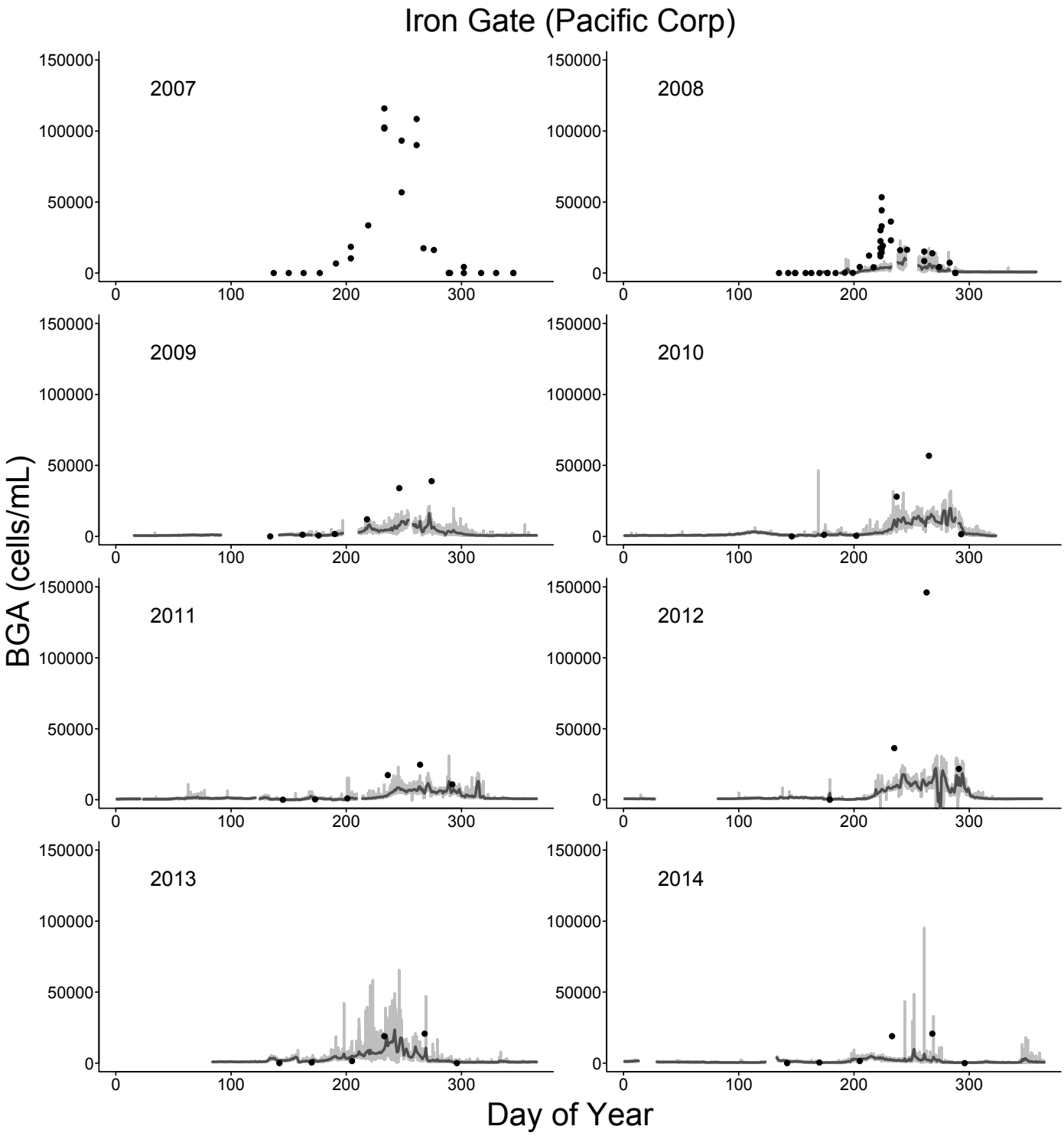


Figure A1. Raw data (light grey lines) and daily means (dark grey line) of blue-green algae (bga) cell density estimates from the phycocyanin probe located below Iron Gate dam, operated by Pacific Corp. Black points indicate bga cell density from grab samples taken near the sonde location.

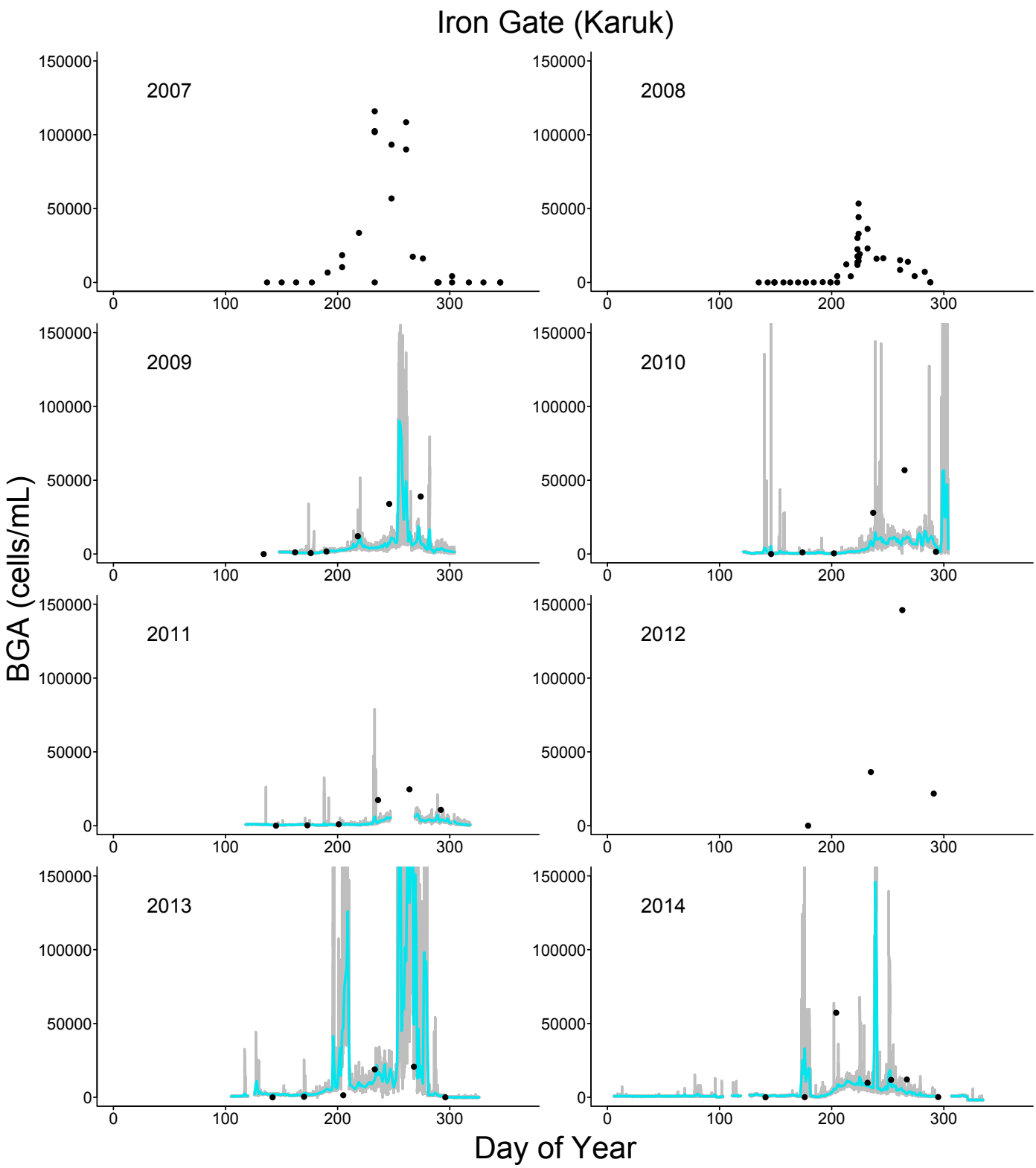


Figure A 2. Raw data (light grey lines) and daily means (blue line) of blue-green algae (bga) cell density estimates from the phycocyanin probe located below Iron Gate dam, operated by the Karuk Tribe. Black points indicate bga cell density from grab samples taken near the sonde location.

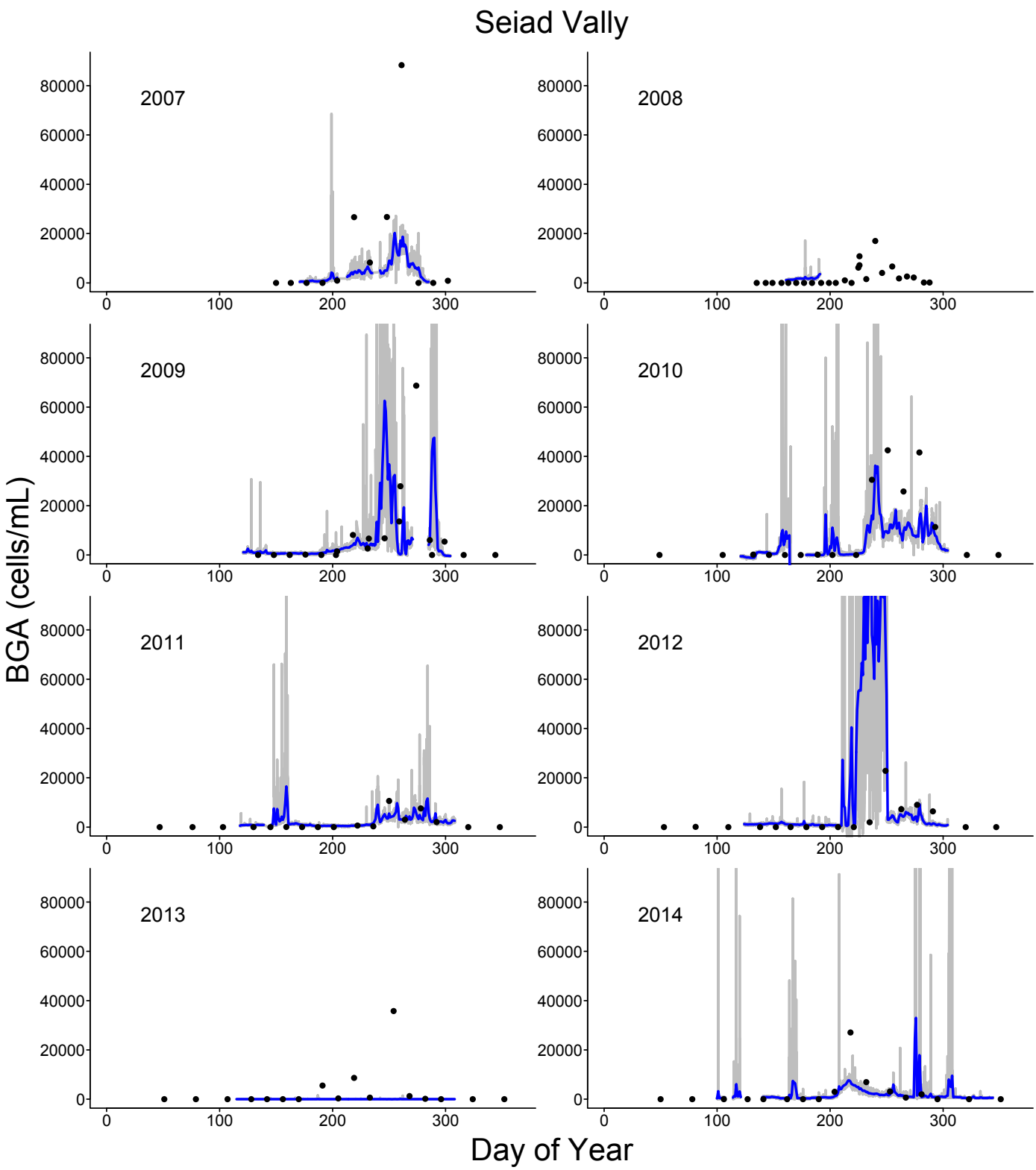


Figure A 3. Raw data (light grey lines) and daily means (blue line) of blue-green algae (bga) cell density estimates from the phycocyanin probe located near Siead Valley. Black points indicate bga cell density from grab samples taken near the sonde location.

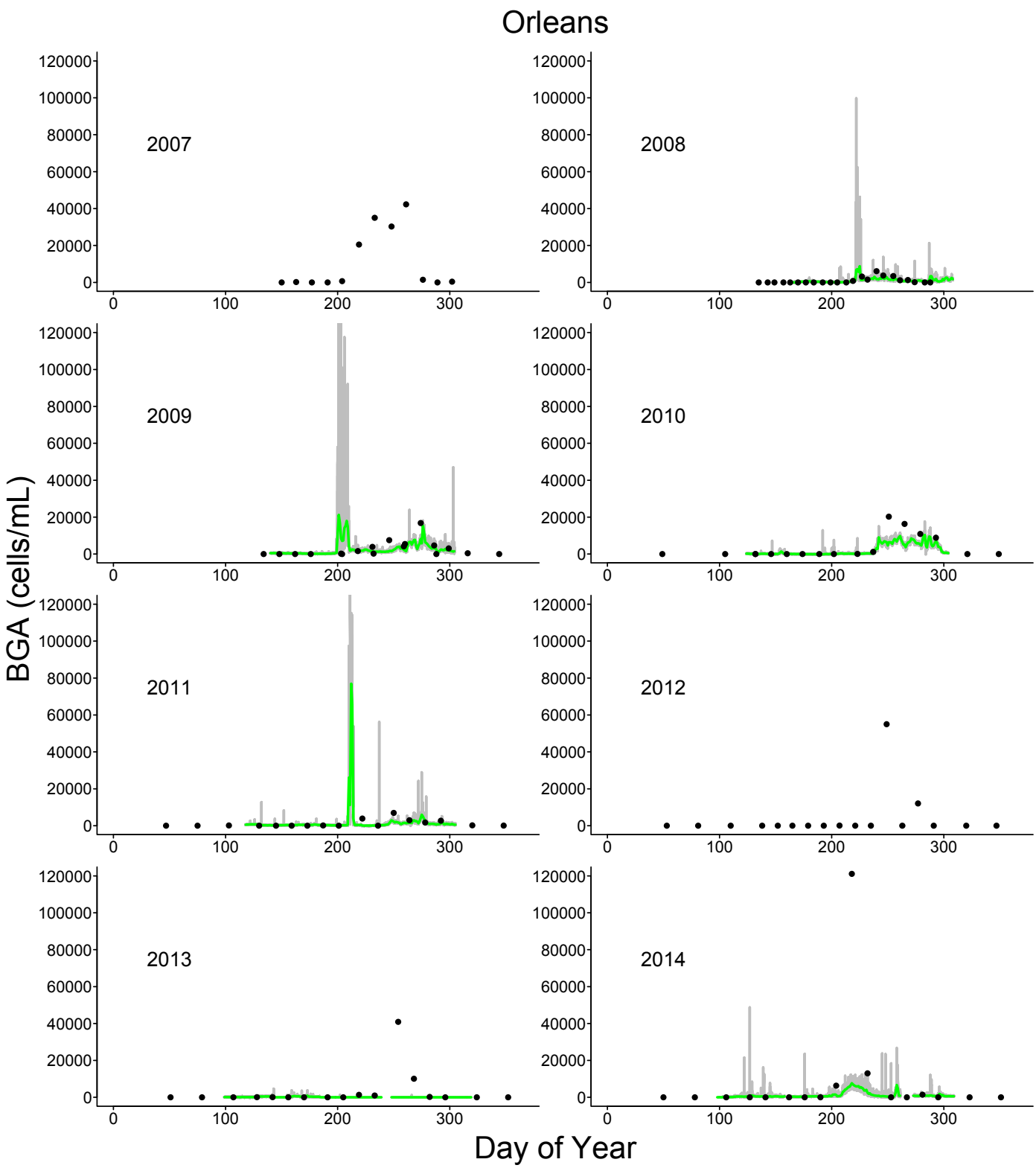


Figure A4. Raw data (light grey lines) and daily means (green line) of blue-green algae (bga) cell density estimates from the phycocyanin probe located near Orleans. Black points indicate bga cell density from grab samples taken near the sonde location.

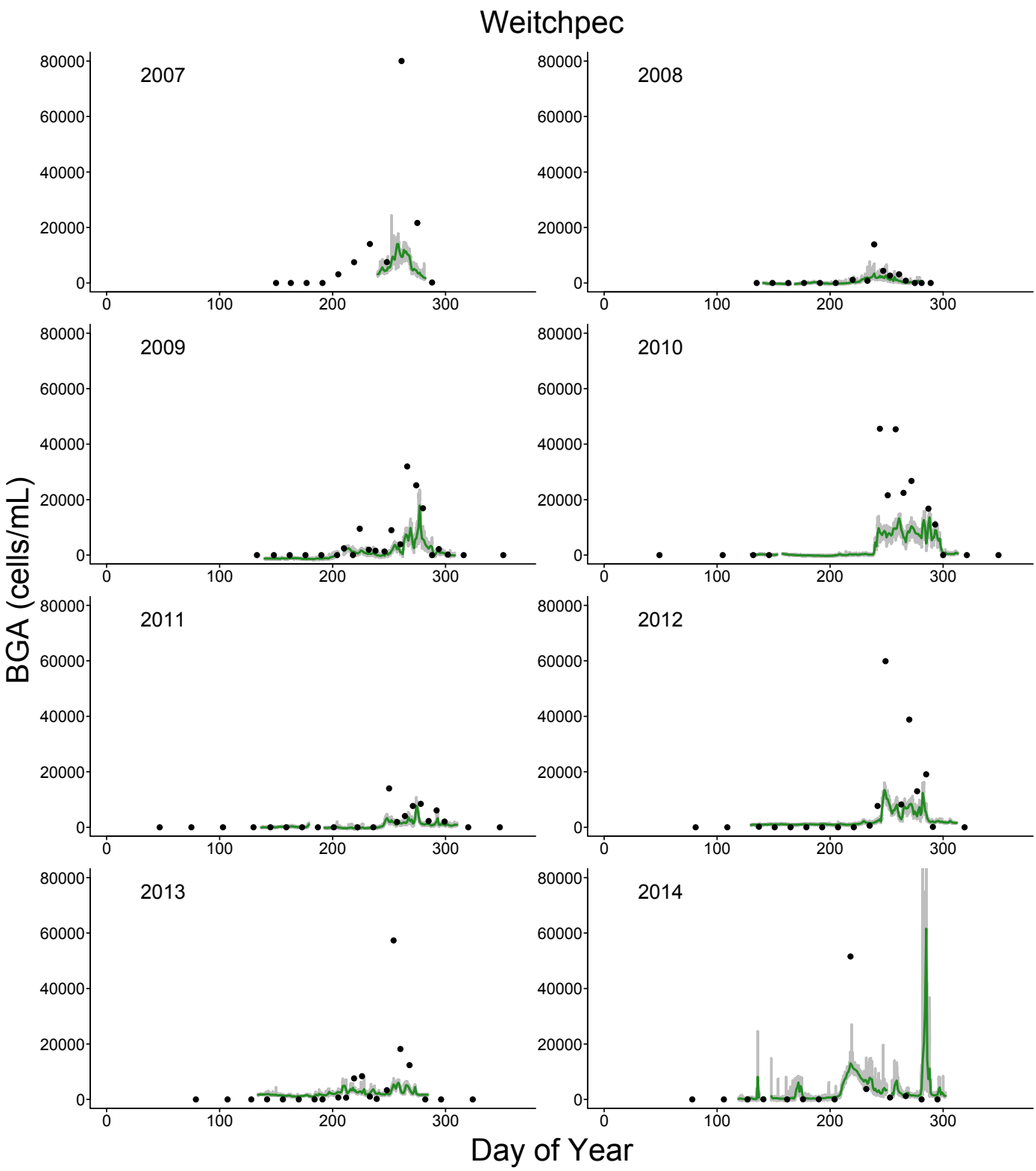


Figure A5. Raw data (light grey lines) and daily means (green line) of blue-green algae (bga) cell density estimates from the phycocyanin probe located near Weitchpec. Black points indicate bga cell density from grab samples taken near the sonde location.

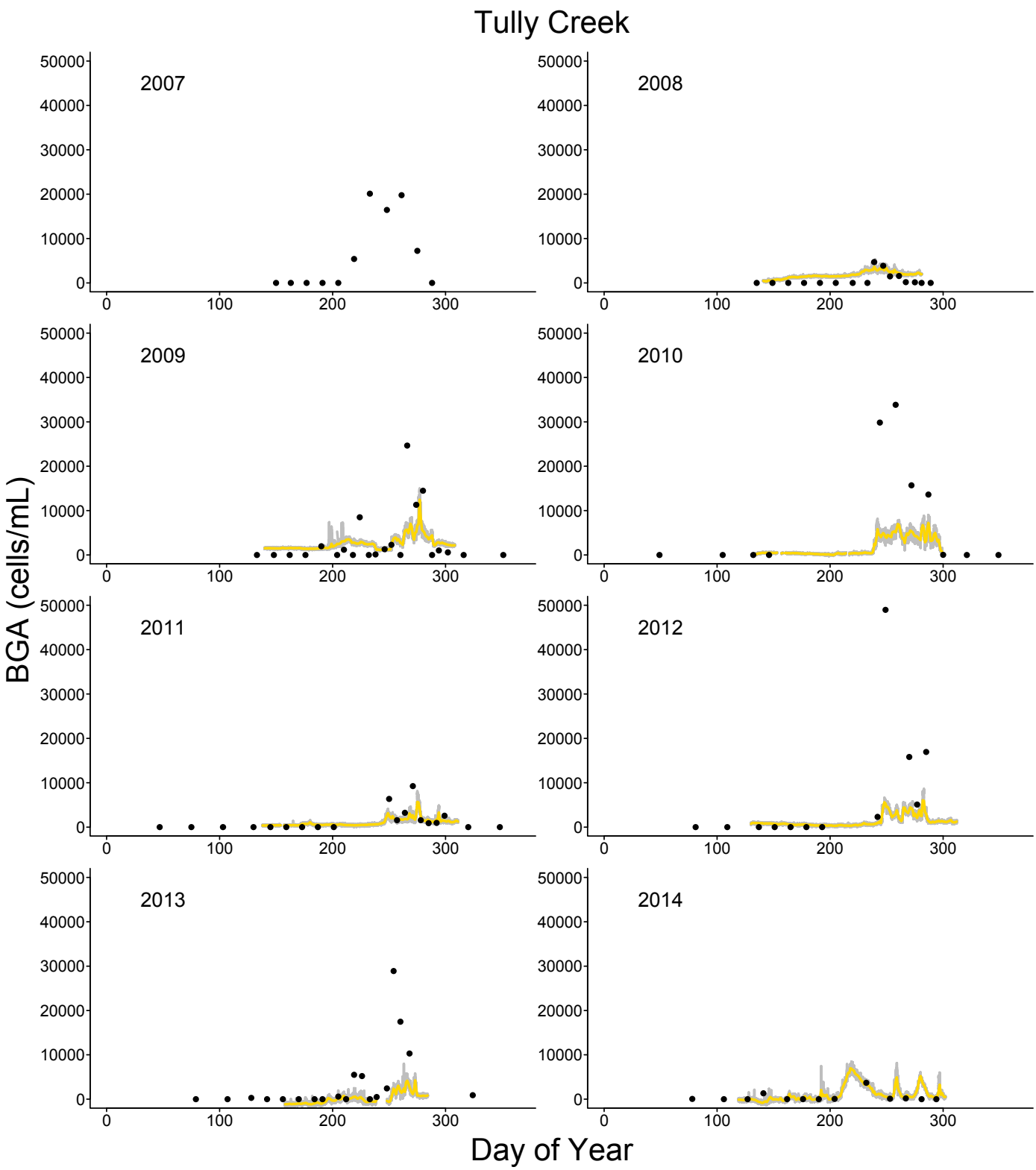


Figure A6. Raw data (light grey lines) and daily means (yellow line) of blue-green algae (bga) cell density estimates from the phycocyanin probe located below Tully Creek. Black points indicate bga cell density from grab samples taken near the sonde location.

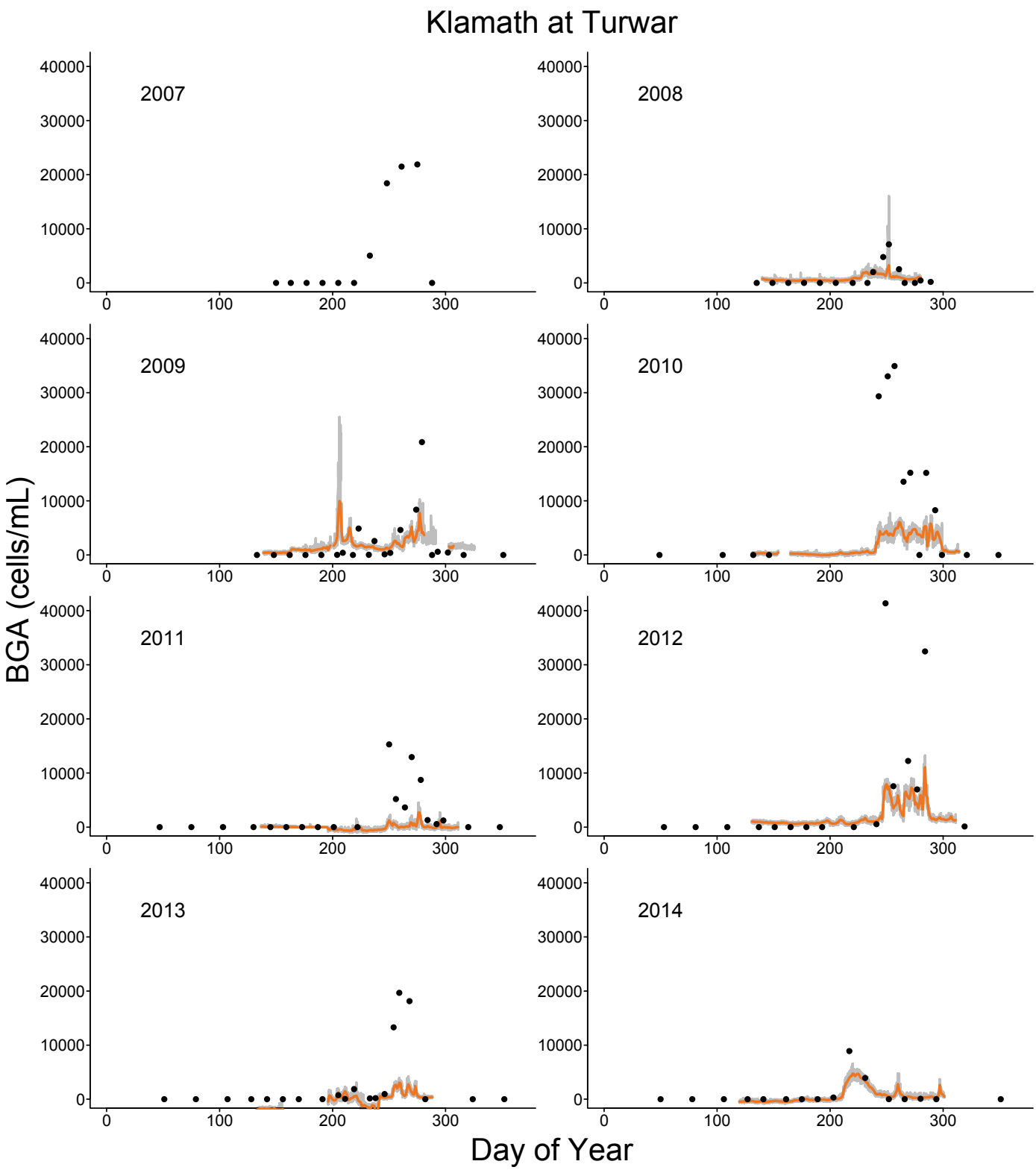


Figure A7. Raw data (light grey lines) and daily means (orange line) of blue-green algae (bga) cell density estimates from the phycocyanin probe located below near Turwar. Black points indicate bga cell density from grab samples taken near the sonde location.

APPENDIX B: SONDE DATA SPIKES COMPARED TO GRAB SAMPLES AND NEAR-BY SITES

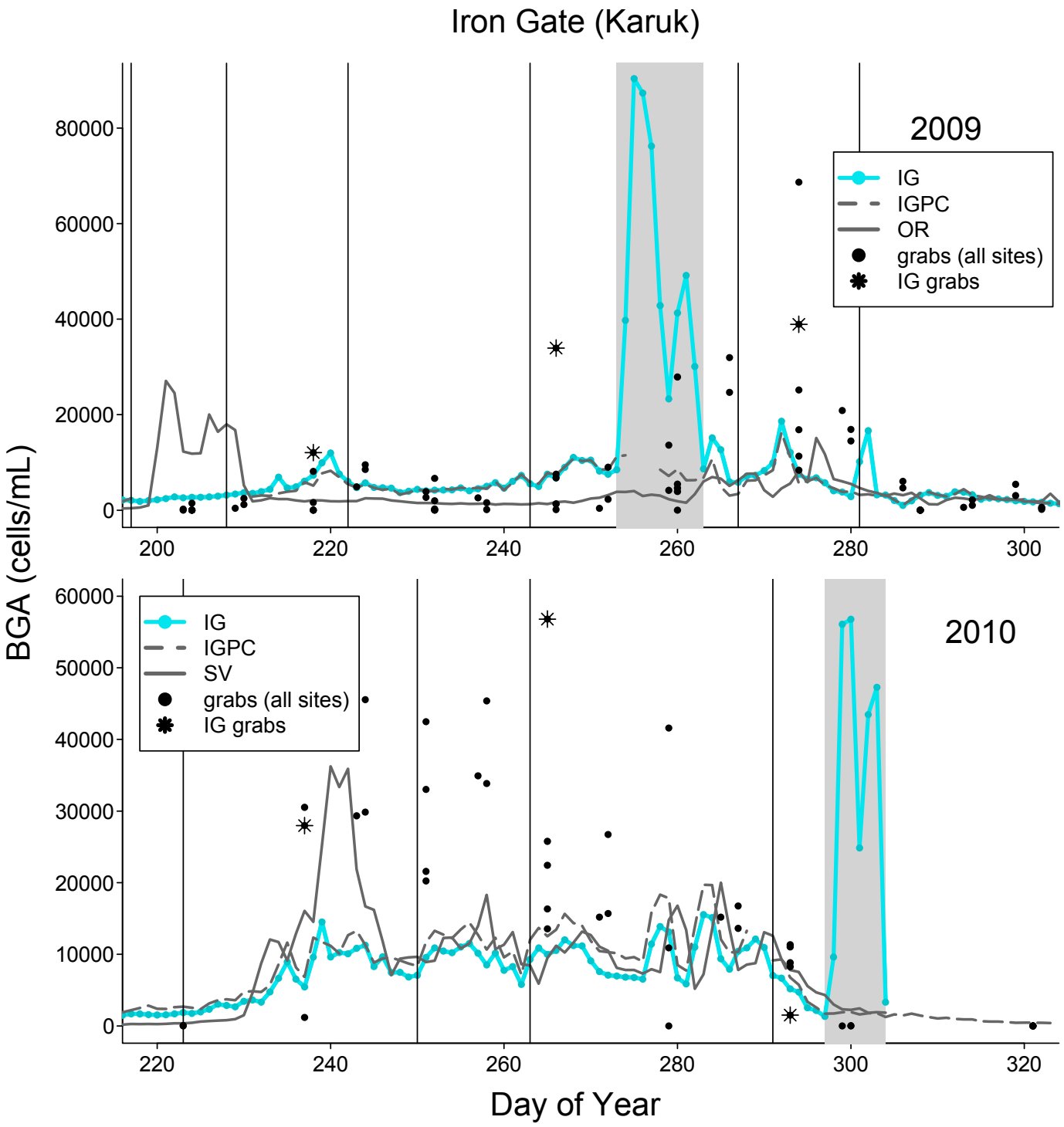


Figure B1. Daily means of phycocyanin (as a cell/mL estimate) at the Iron Gate sonde (IG, blue line), the Iron Gate sonde operated by Pacific Corp (IGPC, dashed grey line), and the Seiad Valley (2010) or Orleans (2009) sonde (SV/OR, solid grey line) in 2009 (top panel) and 2010 (bottom panel). Vertical lines show calibration dates for the IG sonde and black points are grab samples of total BGA from all river sites, with stars indicated grab samples from IG. Graph areas with grey backgrounds are calibration periods when probe fouling was likely, identified by high daily means of phycocyanin not reflected by near-by sites or grab samples during the same time-period.

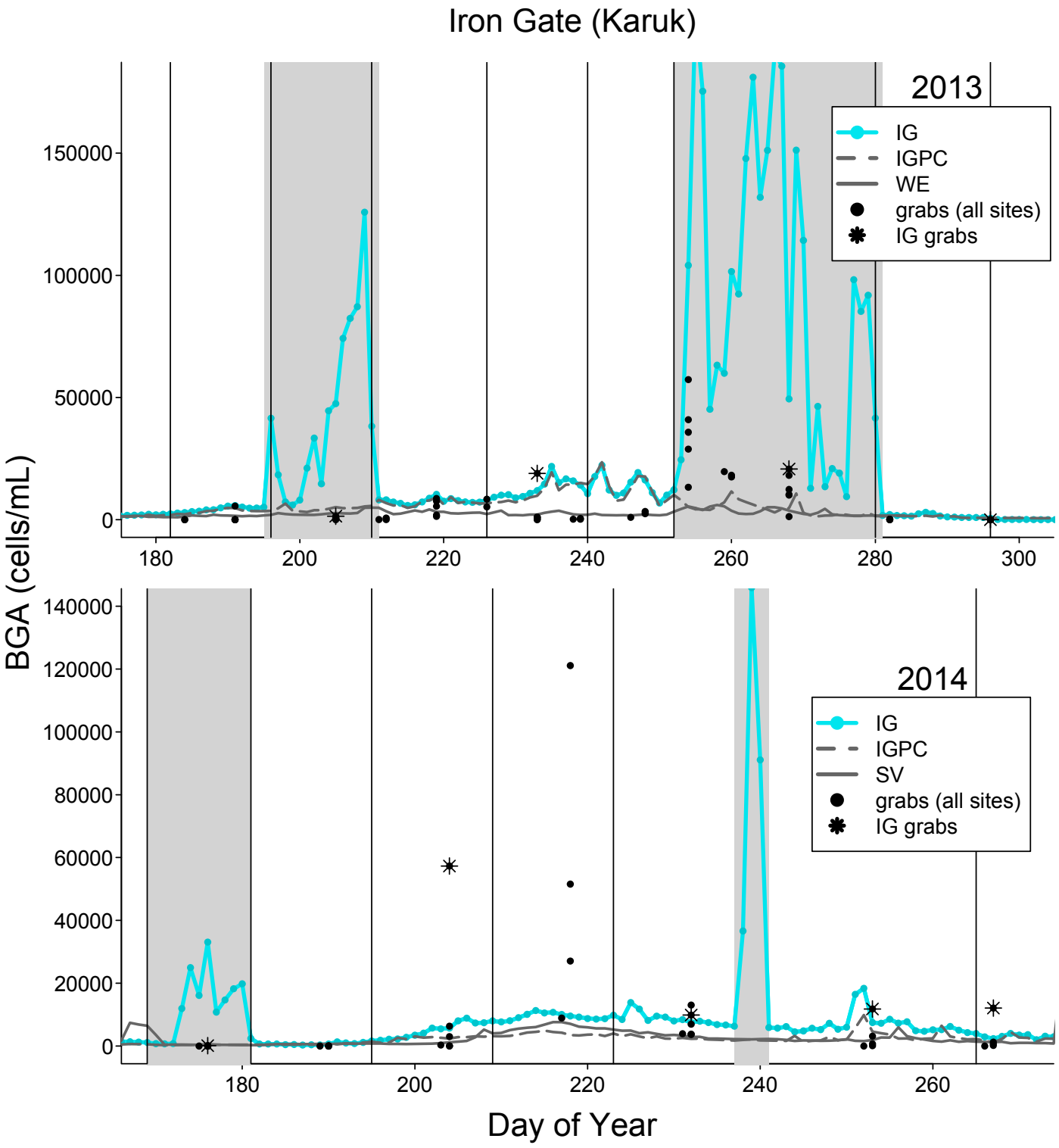


Figure B2. Daily means of phycocyanin (as a cell/mL estimate) at the Iron Gate sonde (IG, blue line), the Iron Gate sonde operated by Pacific Corp (IGPC, dashed grey line), and the Seiad Valley (2014) or Weitchpec (2013) sonde (SV/WE, solid grey line) in 2013 (top panel) and 2014 (bottom panel). Vertical lines show calibration dates for the IG sonde and black points are grab samples of total BGA from all river sites, with stars indicated grab samples from IG. Graph areas with grey backgrounds are calibration periods when probe fouling was likely, identified by high daily means of phycocyanin not reflected by near-by sites or grab samples during the same time-period.

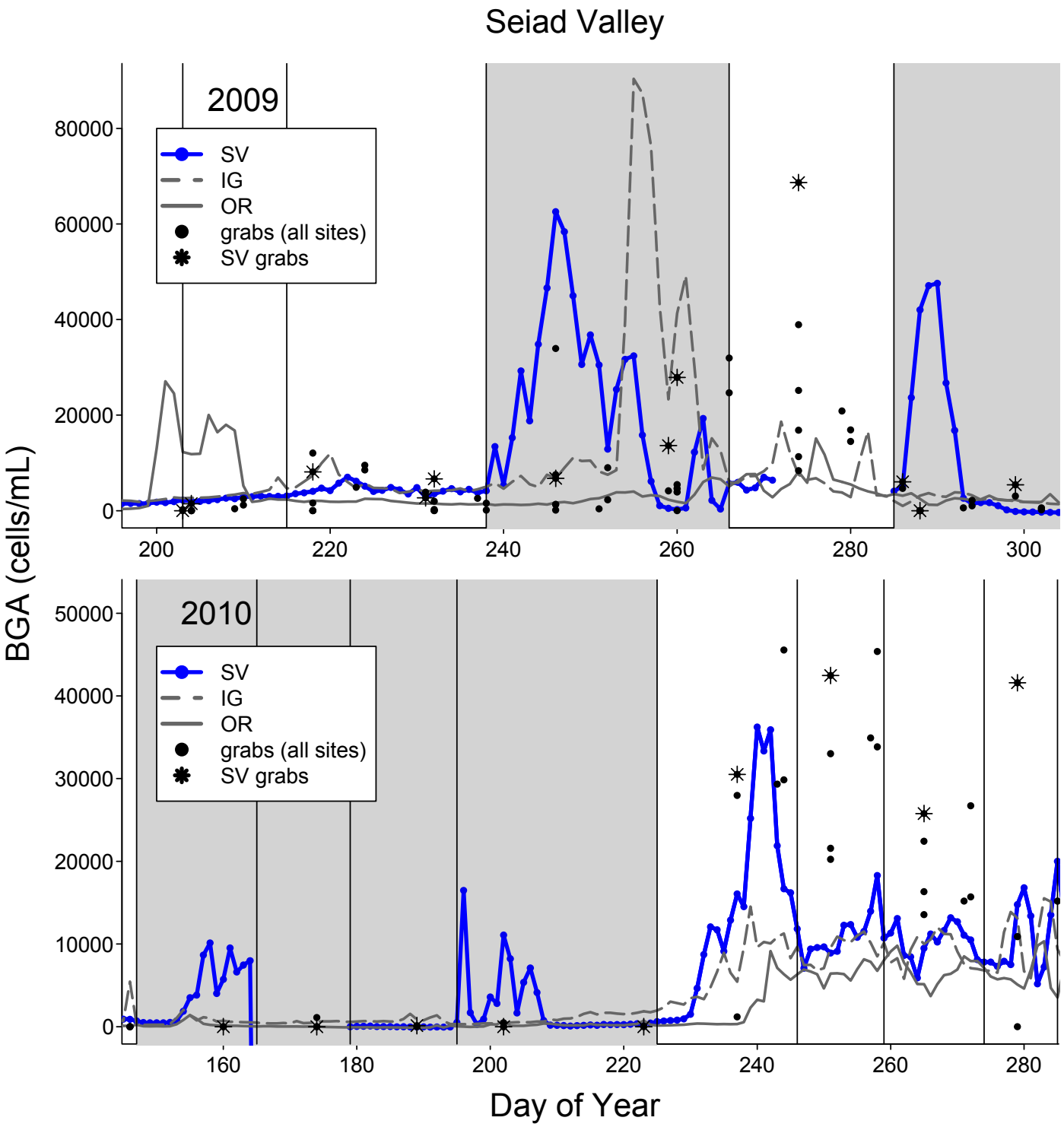


Figure B3. Daily means phycocyanin (as a cell/mL estimate) at the Seiad Valley sonde (SV, dark blue line), the Iron Gate sonde (IG, dashed grey line), and the Orleans sonde (OR, solid grey line). Vertical lines show calibration dates for the SV sonde and black points are grab samples of total BGA from all river sites, with stars indicated grab samples from SV. Graph area with the grey background indicates calibration periods when probe fouling was likely, identified by high daily means of phycocyanin not reflected by near-by sites or grab samples during the same time-period.

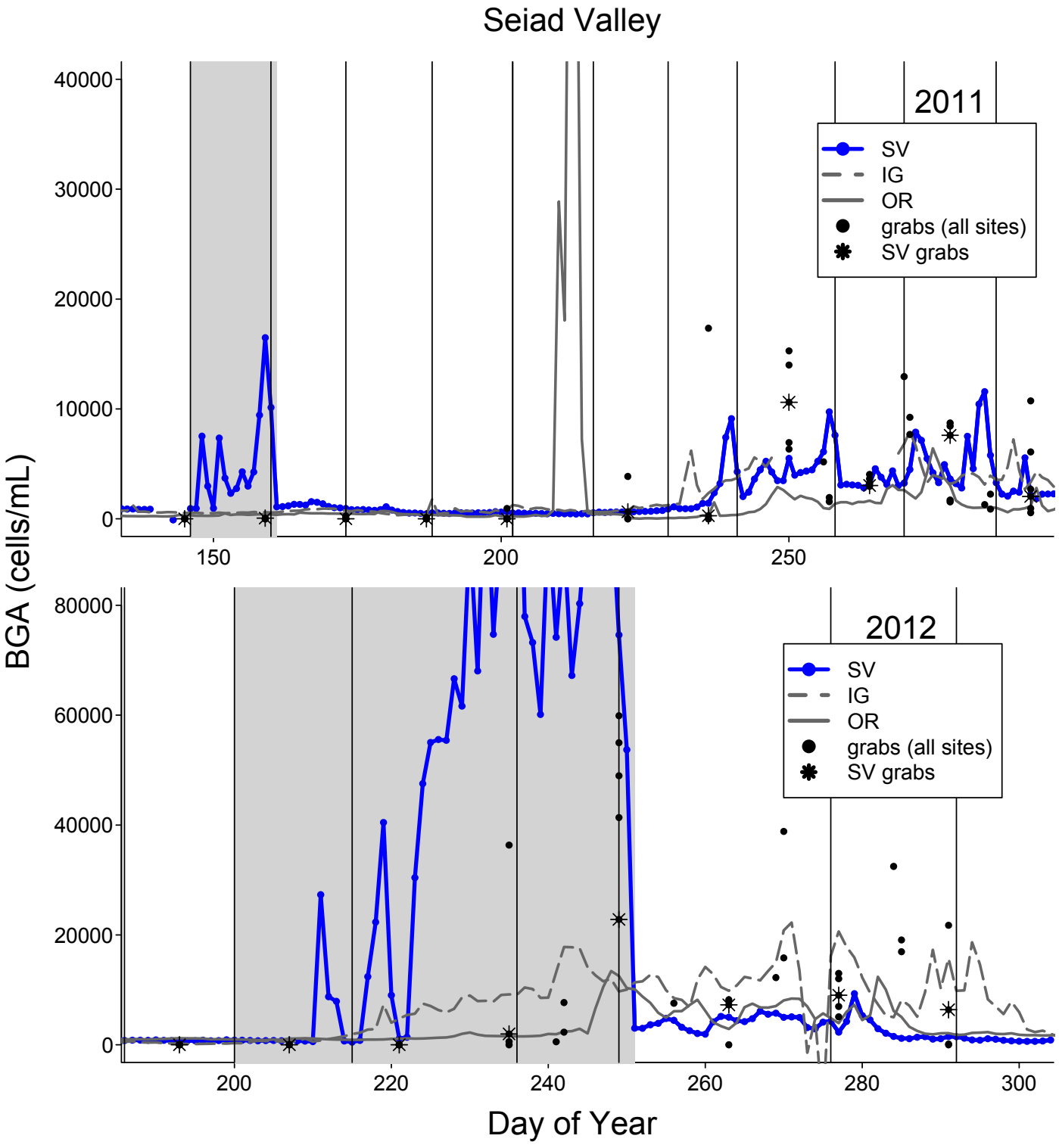


Figure B4. Daily means of phycocyanin (as a cell/mL estimate) at the Seiad Valley sonde (SV, dark blue line), the Iron Gate sonde (IG, dashed grey line), and the Orleans sonde (OR, solid grey line). Vertical lines show calibration dates for the SV sonde and black points are grab samples of total BGA from all river sites, with stars indicated grab samples from SV. Graph area with the grey background indicates calibration periods when probe fouling was likely, identified by high daily means of phycocyanin not reflected by near-by sites or grab samples during the same time-period.

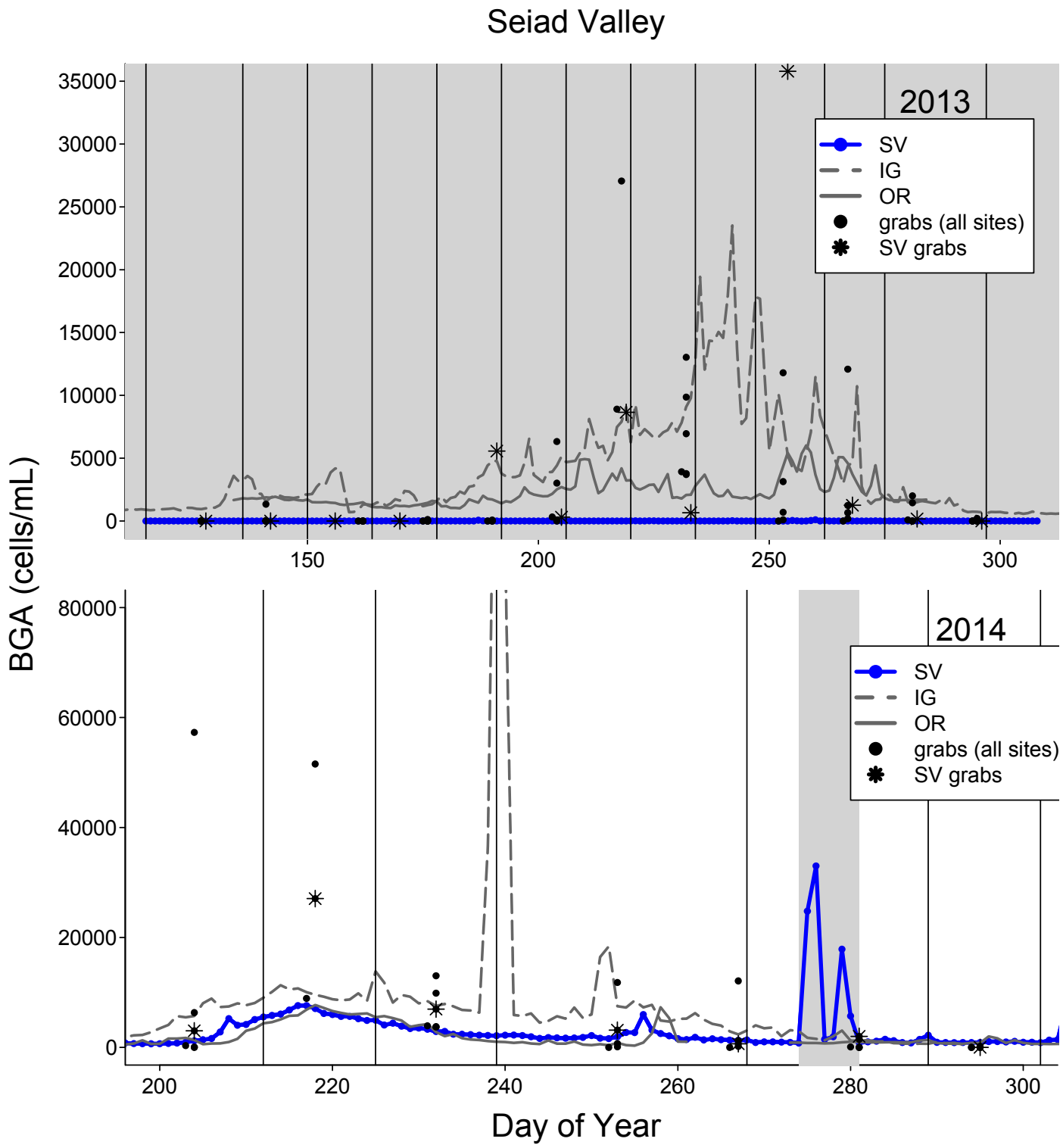


Figure B5. Daily means of phycocyanin (as a cell/mL estimate) at the Seiad Valley sonde (SV, dark blue line), the Iron Gate sonde (IG, dashed grey line), and the Orleans sonde (OR, solid grey line). Vertical lines show calibration dates for the SV sonde and black points are grab samples of total BGA from all river sites, with stars indicated grab samples from SV. Graph area with the grey background indicates calibration periods when probe fouling was likely, identified by high daily means of phycocyanin not reflected by near-by sites or grab samples during the same time-period.

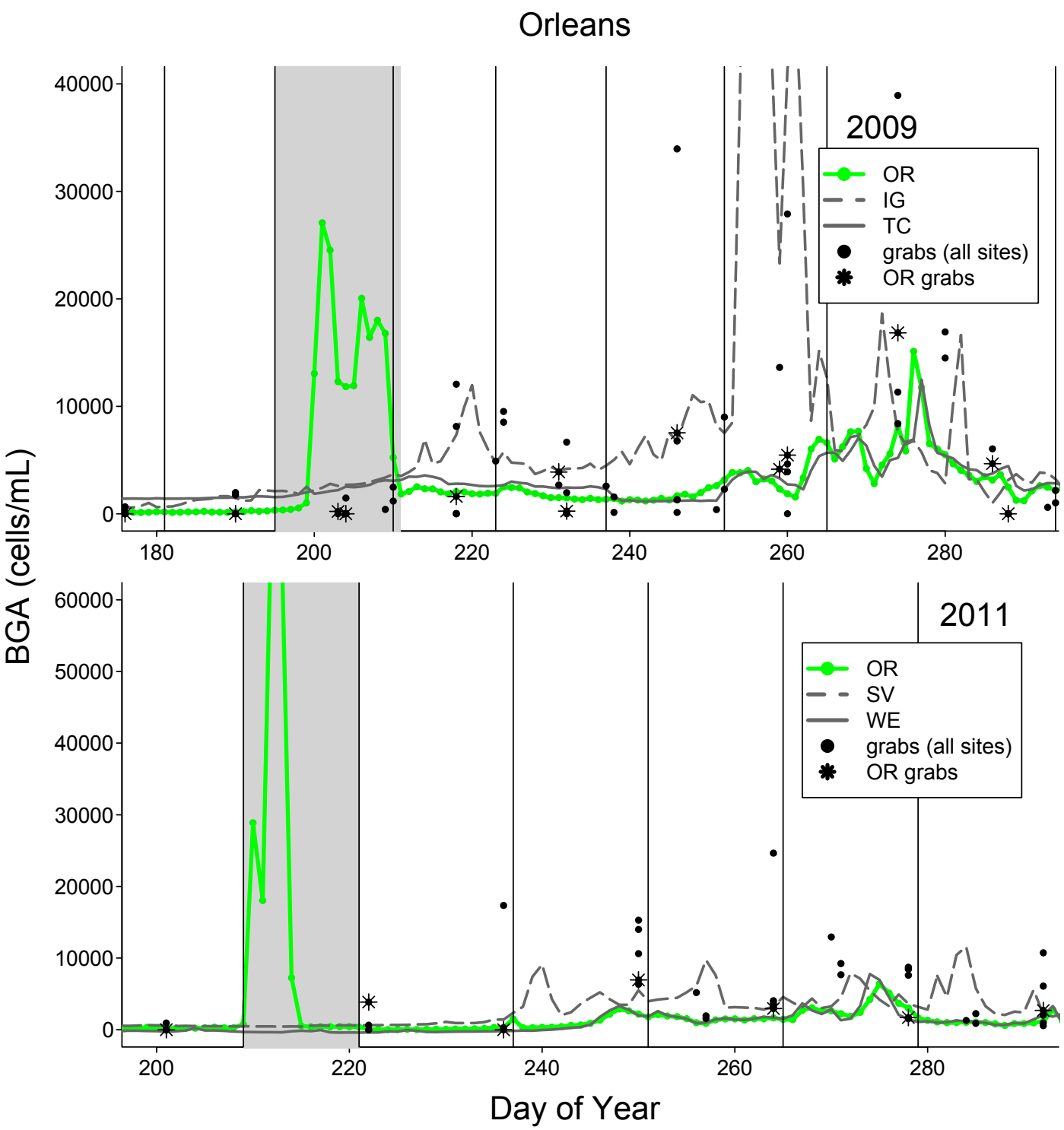


Figure B6. Daily means of phycocyanin (as a cell/mL estimate) at the Orleans sonde (OR, green line), the Iron Gate or Seiad Valley sonde(IG or SV, dashed grey line), and the Tully Creek or Weitchpec sonde (TC or WE, solid grey line). Vertical lines show calibration dates for the OR sonde and black points are grab samples of total BGA from all river sites, with stars indicated grab samples from OR. Graph area with the grey background indicates calibration periods when probe fouling was likely, identified by high daily means of phycocyanin not reflected by near-by sites or grab samples during the same time-period.

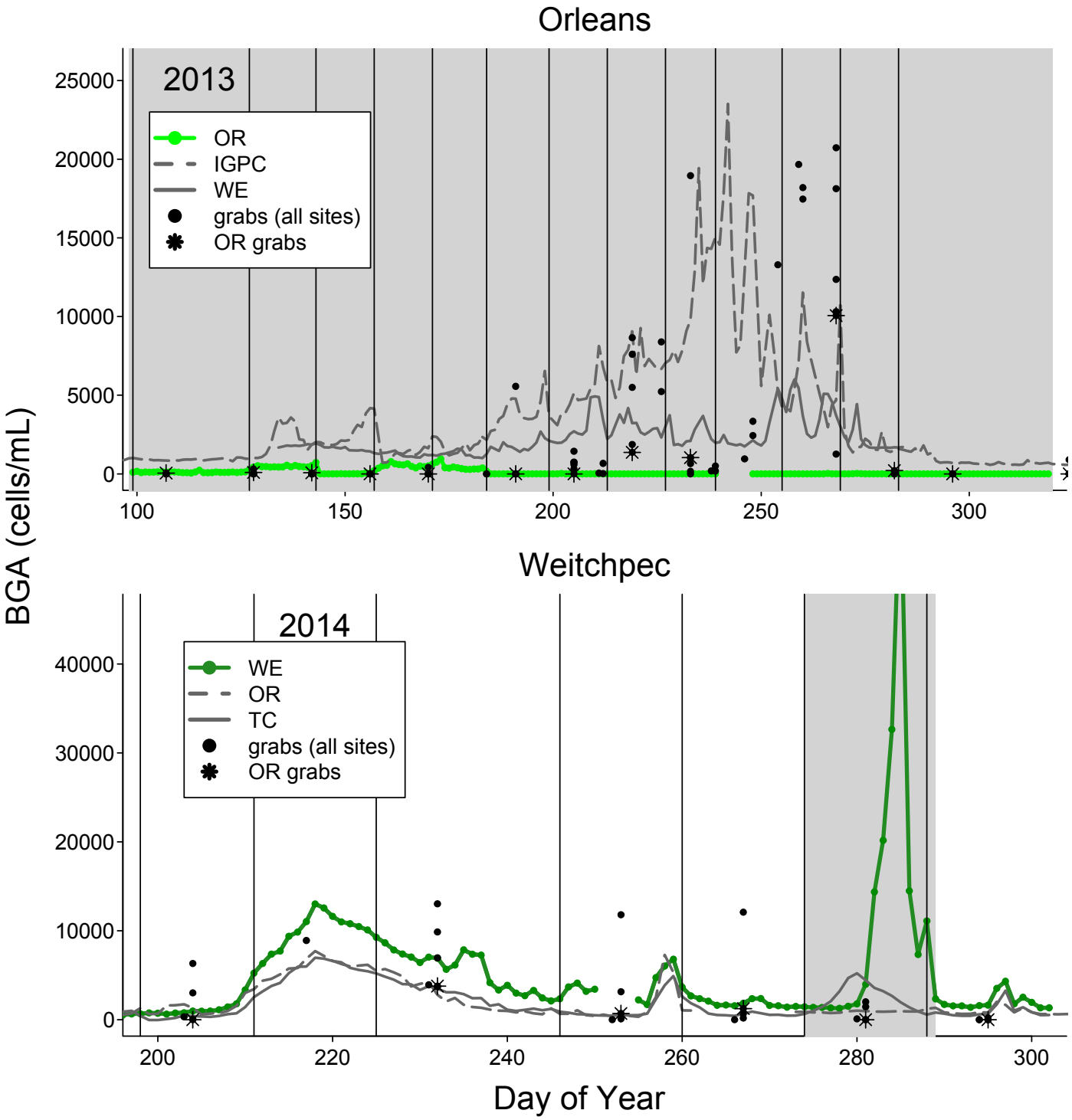


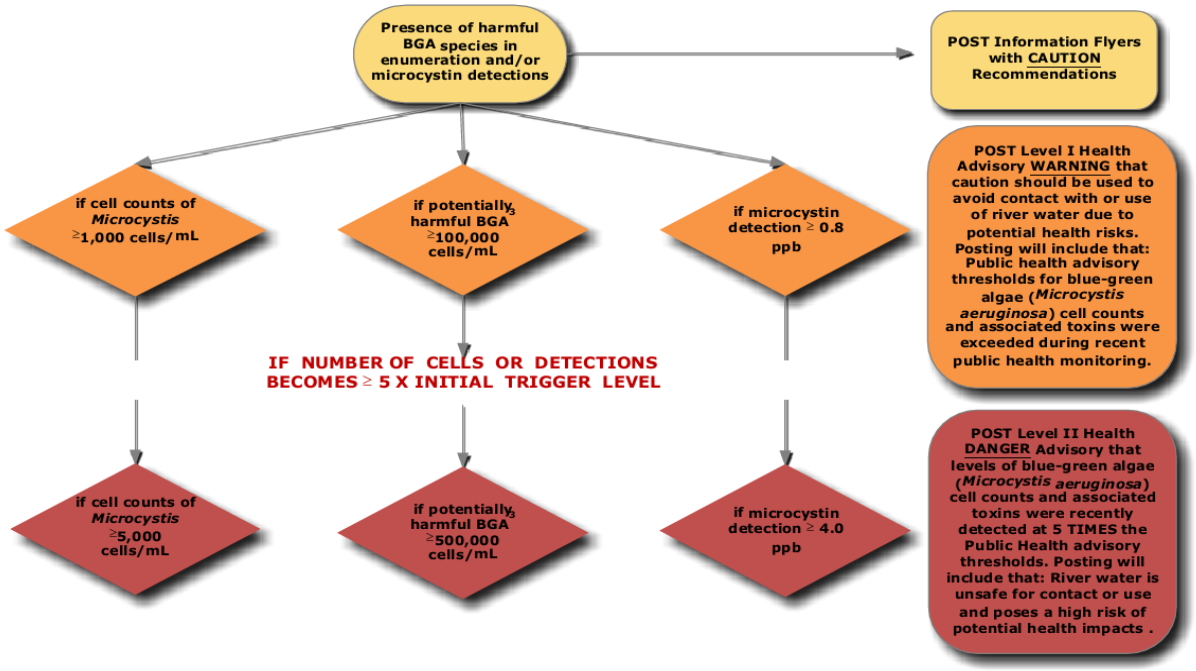
Figure B7. Daily means of phycocyanin (as a cell/mL estimate) at the Orleans sonde (OR, green line, top panel) and the Weitchpec sonde (WE, dark green line, bottom panel), the Iron Gate or Orleans sonde (IG or OR, dashed grey line), and the Weitchpec or Tully Creek sonde (WE or TC, solid grey line). Vertical lines show calibration dates for the OR and WE sondes and black points are grab samples of total BGA from all river sites, with stars indicated grab samples from OR and TC. Graph area with the grey background indicates calibration periods when probe fouling was likely, identified by high daily means of phycocyanin not reflected by near-by sites or grab samples during the same time-period.

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

Yurok Tribe Environmental Program (YTEP) Memorandum

2016 *Posting* Guidelines for Public Health Advisories

To ensure that people have the knowledge necessary to make informed decisions regarding the potential risks to their health and are not exposed to concentrations of microcystin in Klamath River water that could cause adverse health effects, YTEP will be posting Public Health Advisory signs within the exterior boundaries of the Yurok Reservation based on the decision tree below. A WARNING flyer will be posted using the recommended level[1] of 0.8 µg/L as the maximum dose a child swimmer could be exposed to with little to no risk of harm. Additionally, while we agree with statements that cell counts are not a good indication of toxin levels, they do provide an early warning of the likelihood of toxin presence and as such will contribute to the safeguarding of the Public Health. Therefore, YTEP has incorporated algal cell counts[2] into the decision tree along with microcystin levels.



2016 *De-Posting* Guidelines

The removal and de-posting of Public health advisories and flyers will be based on **TOXIN ANALYSIS**.
After toxin results are below the guideline level of 0.8 µg/L for two consecutive sampling events that are at least one week apart, advisories will be lifted and flyers removed.

- If the dominant species of blue-green algae is known to produce microcystin and anatoxin-a, it is recommended that BOTH toxins be tested prior to lifting an advisory.)
- In some situations there may be reason, such as reported illness and/or persistence of the toxin, to prolong the advisory beyond the recommended waiting period.

[1]CalEPA, OEHHA, SWRCB. 2012. Toxicological Summary & Suggested Action Levels to Reduce Potential Adverse Health Effects of Six Cyanotoxins.
[2]Kann, J. January 17, 2014. Technical Memorandum: Evaluation of Cyanobacteria and Cyanobacterial toxins with reference to Selection of Water Quality Criteria for the Karuk Tribe of California.
[3]Potentially toxic blue-green algae that have been detected in California include those of the genera *Anabaena, Microcystis , Aphanizomenon, Planklothrix / Oscillitoria*, and *Gloeotrichia.* This list may be added to as additional blue-green algae that have toxic potential become known.

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.

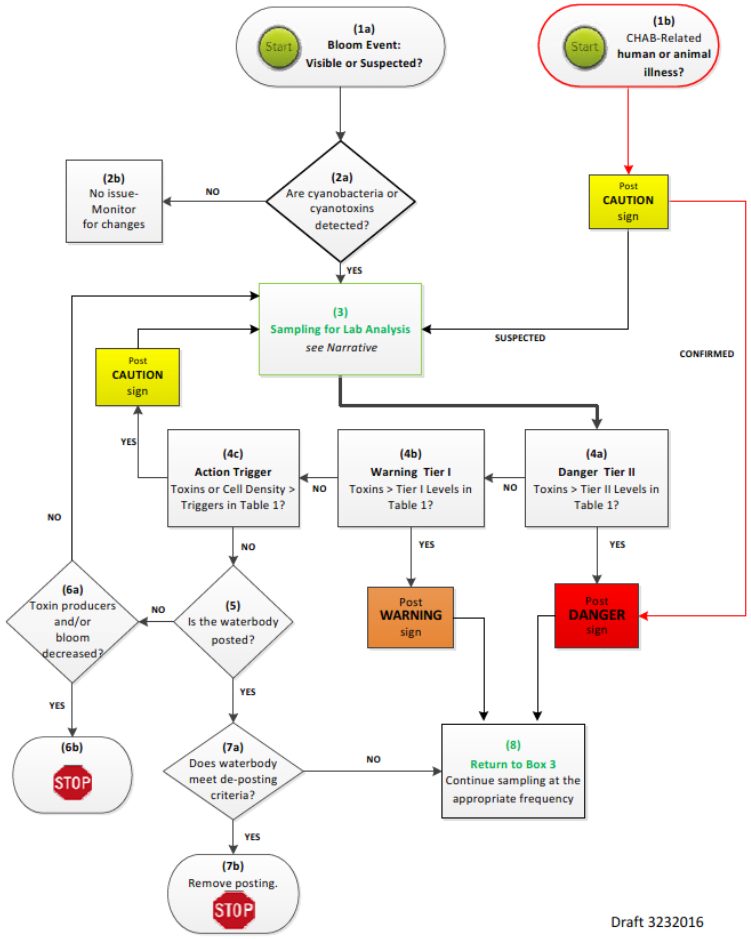
[3] Includes: *Anabaena, Microcystis, Planktothrix, Gloeotrichia* and *Oscillatoria*

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



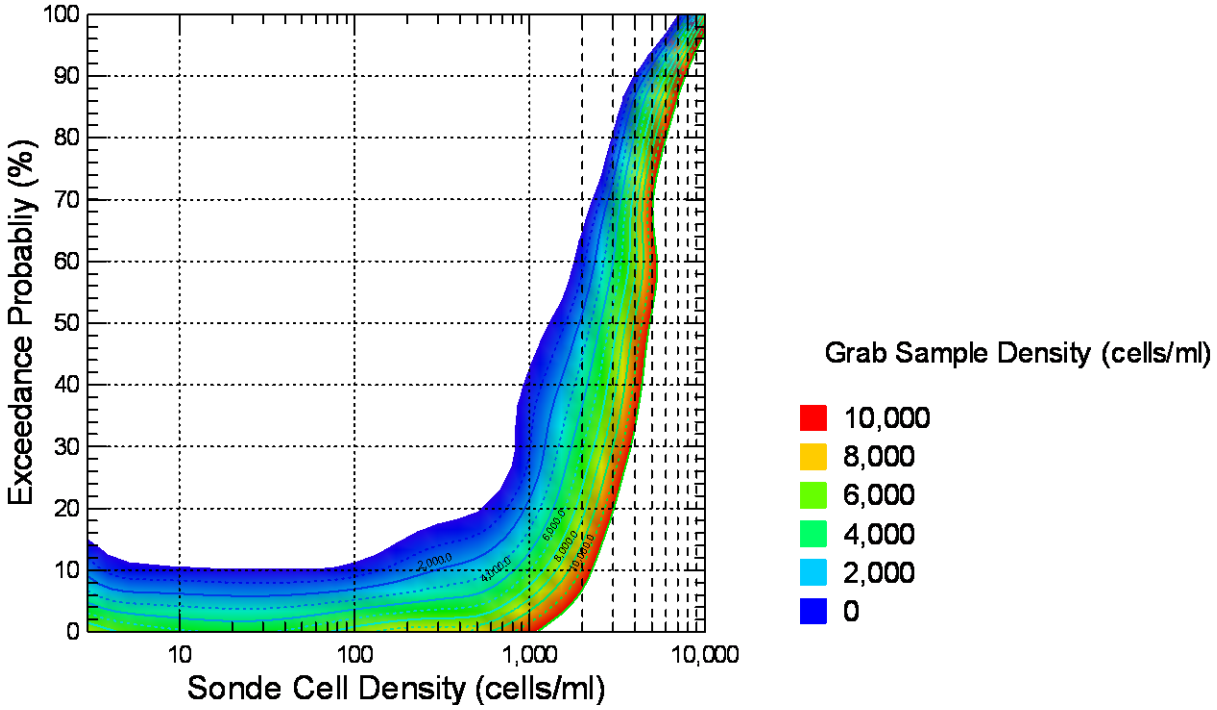
http://www.mywaterquality.ca.gov/monitoring_council/cyanohab_network/docs/triggers.pdf

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.

APPENDIX D: PROBABILITY OF EXCEEDANCE PLOTS FOR GRAB SAMPLE CELL COUNTS



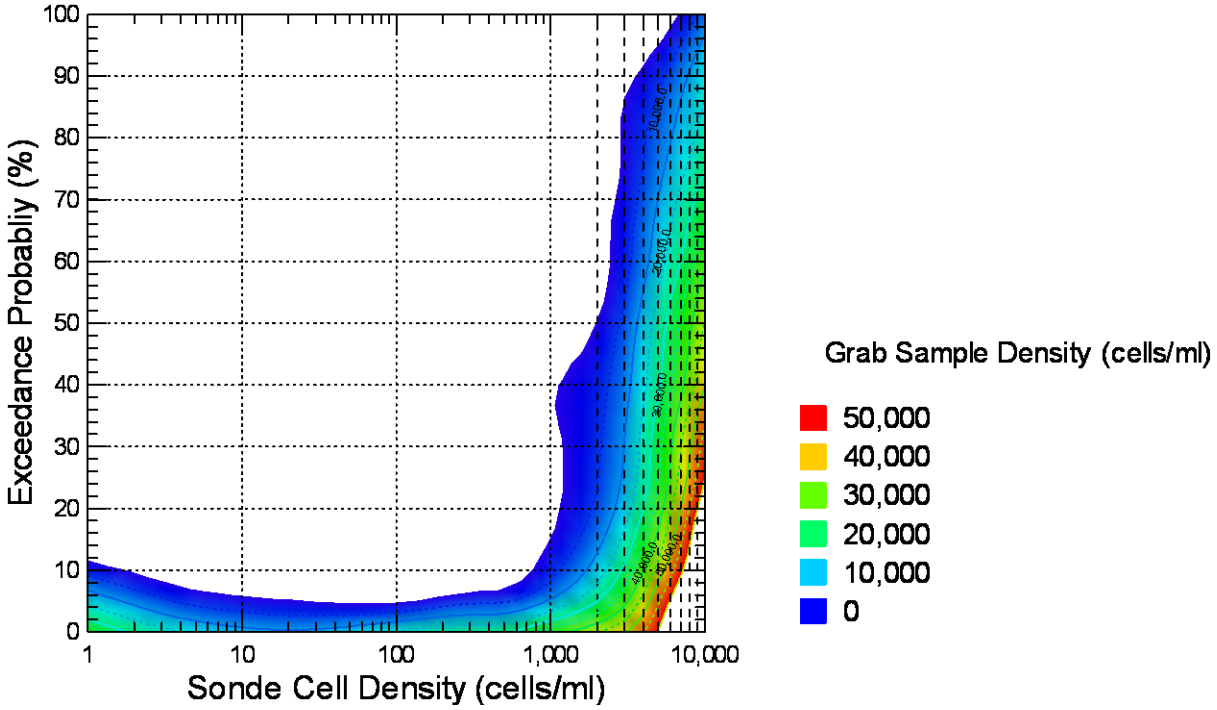


Figure D 1. Phycocyanin concentration (expressed as sonde cell density) predicts the probability (y-axis) that blue-green algae (BGA) cell counts will exceed a given cell density category. Probabilities of exceedance were calculated based on open channel grab samples from all Klamath River monitoring sites from 2007 to 2014 during May 15 through November 15. Panel A categorizes data into more narrow categories, giving more detail at lower grab sample densities, while panel B has wider categories for use with higher grab sample cell densities.

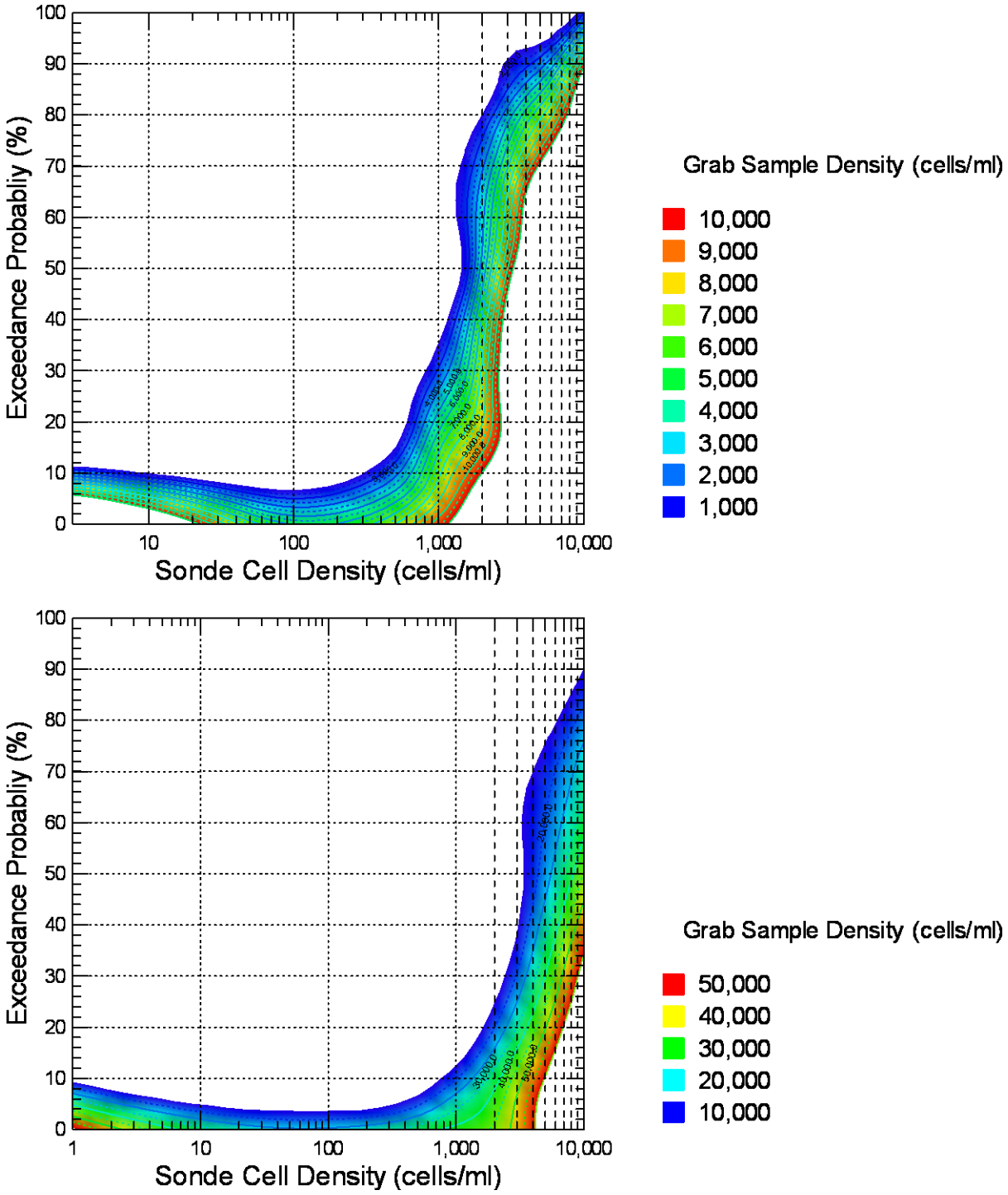


Figure D 2. Phycocyanin concentration (expressed as sonde cell density) predicts the probability (y-axis) that blue-green algae (BGA) cell counts will exceed a given cell density category. Probabilities of exceedance were calculated based on shoreline grab samples from all Klamath River monitoring sites from 2007 to 2014 during May 15 through November 15. Panel A categorizes data into more narrow categories, giving more detail at lower grab sample densities, while panel B has wider categories for use with higher grab sample cell densities.

APPENDIX E: HISTORIC PHYCOCYANIN DATA IN THE CONTEXT OF YUROK TRIBAL HEALTH ADVISORY WARNING LEVELS

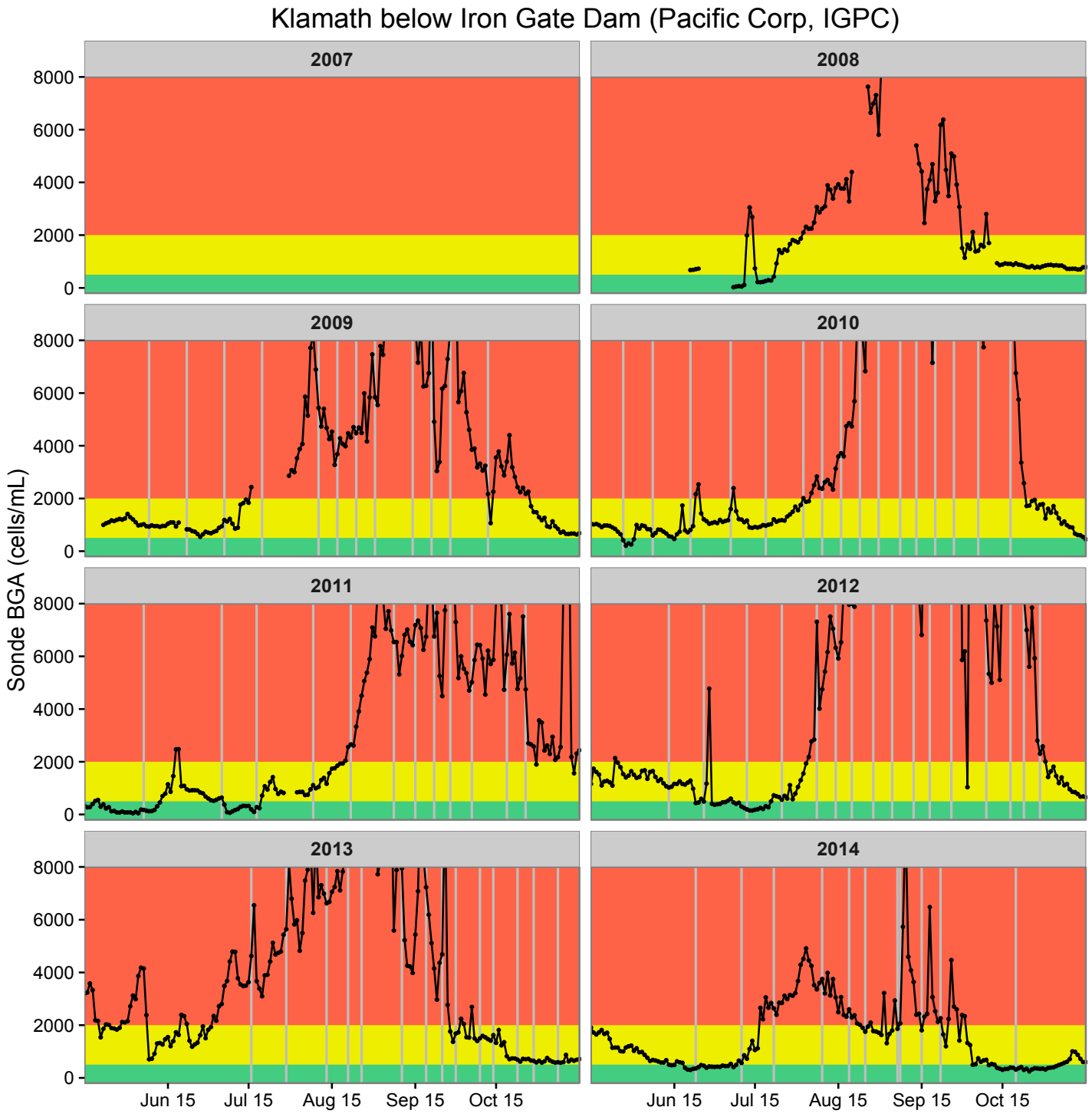


Figure E 1. Daily mean phycocyanin time series (black line with points) with shaded background colors corresponding to sonde BGA levels above which the probability of exceeding public health thresholds rapidly increases, as determined from probability of exceedance analysis for microcystin and sonde BGA. Green < 500 cells/mL; Yellow > 500 cells/mL and < 2000 cells/mL and corresponds to 0.8 µg/L (Level I Health Warning); Orange > 2000 cells/mL and corresponds to 4.0 µg/L (Level II Health Danger). Verticle grey bars represent dates of grab samples.

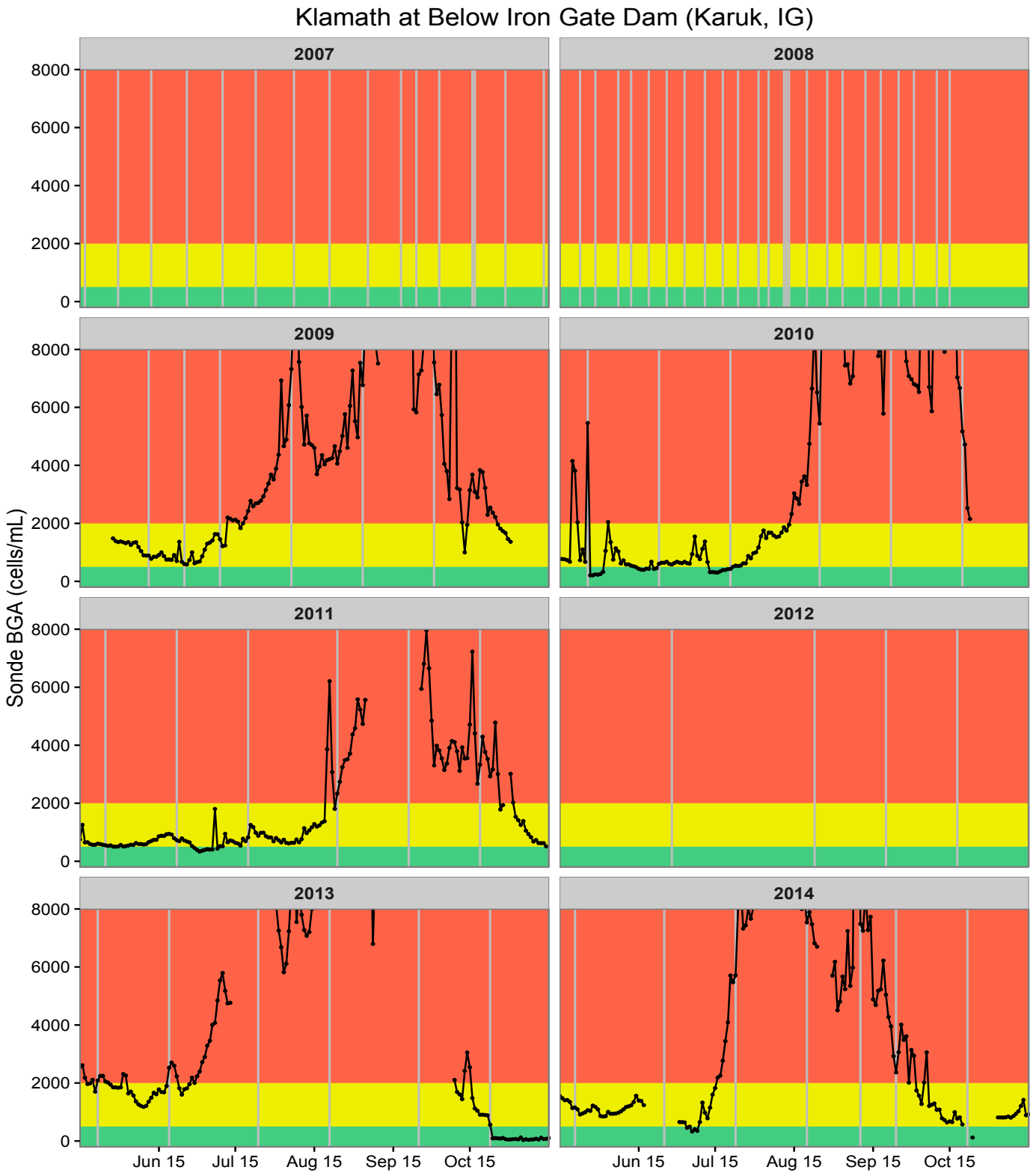


Figure E 2. Daily mean phycocyanin time series (black line with points) with shaded background colors corresponding to sonde BGA levels above which the probability of exceeding public health thresholds rapidly increases, as determined from probability of exceedance analysis for microcystin and sonde BGA. Green < 500 cells/mL; Yellow > 500 cells/mL and < 2000 cells/mL and corresponds to 0.8 µg/L (Level I Health Warning); Orange > 2000 cells/mL and corresponds to 4.0 µg/L (Level II Health Danger). Verticle grey bars represent dates of grab samples.

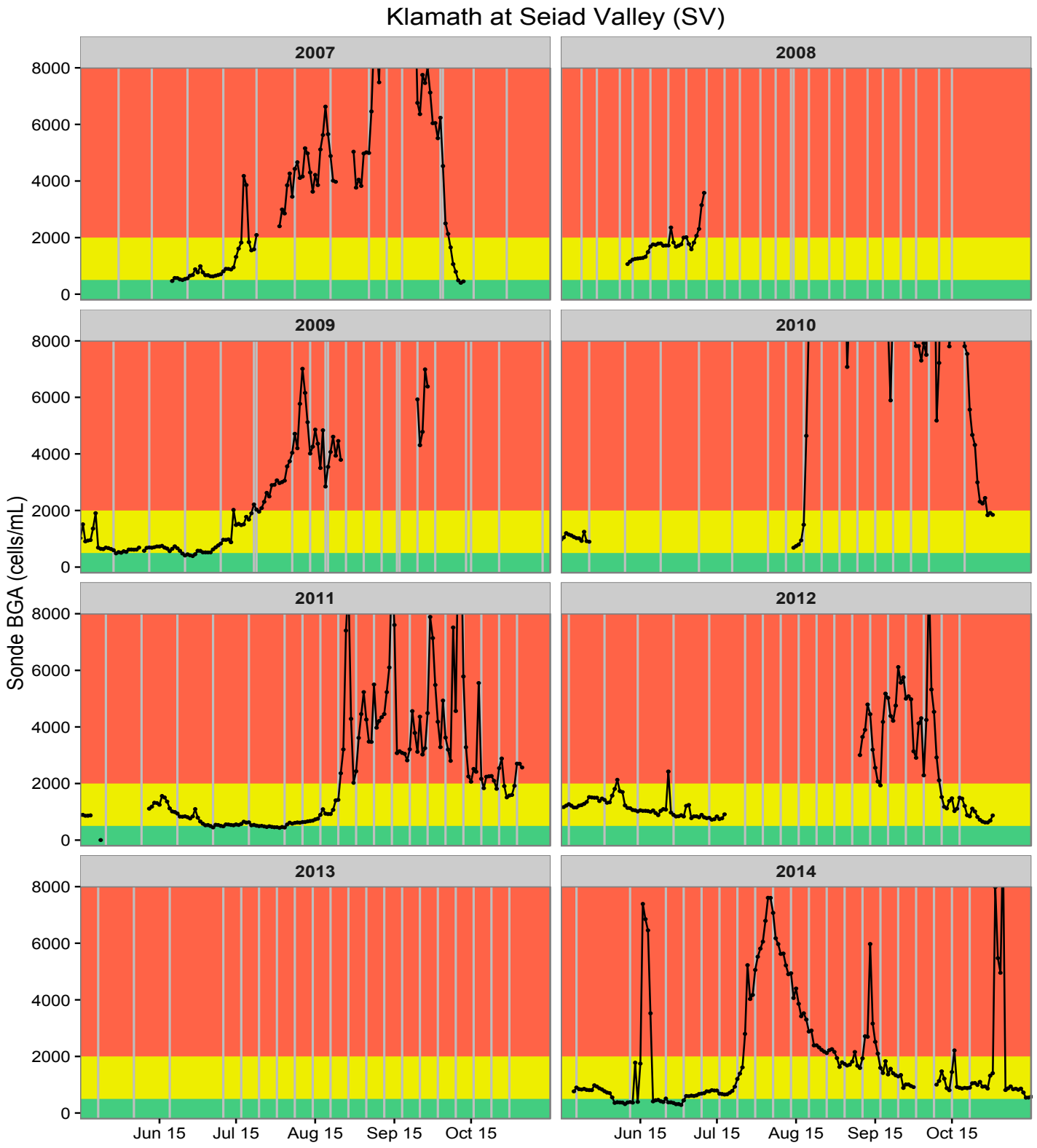


Figure E 3. Daily mean phycocyanin time series (black line with points) with shaded background colors corresponding to sonde BGA levels above which the probability of exceeding public health thresholds rapidly increases, as determined from probability of exceedance analysis for microcystin and sonde BGA. Green < 500 cells/mL; Yellow > 500 cells/mL and < 2000 cells/mL and corresponds to 0.8 µg/L (Level I Health Warning); Orange > 2000 cells/mL and corresponds to 4.0 µg/L (Level II Health Danger). Verticle grey bars represent dates of grab samples.

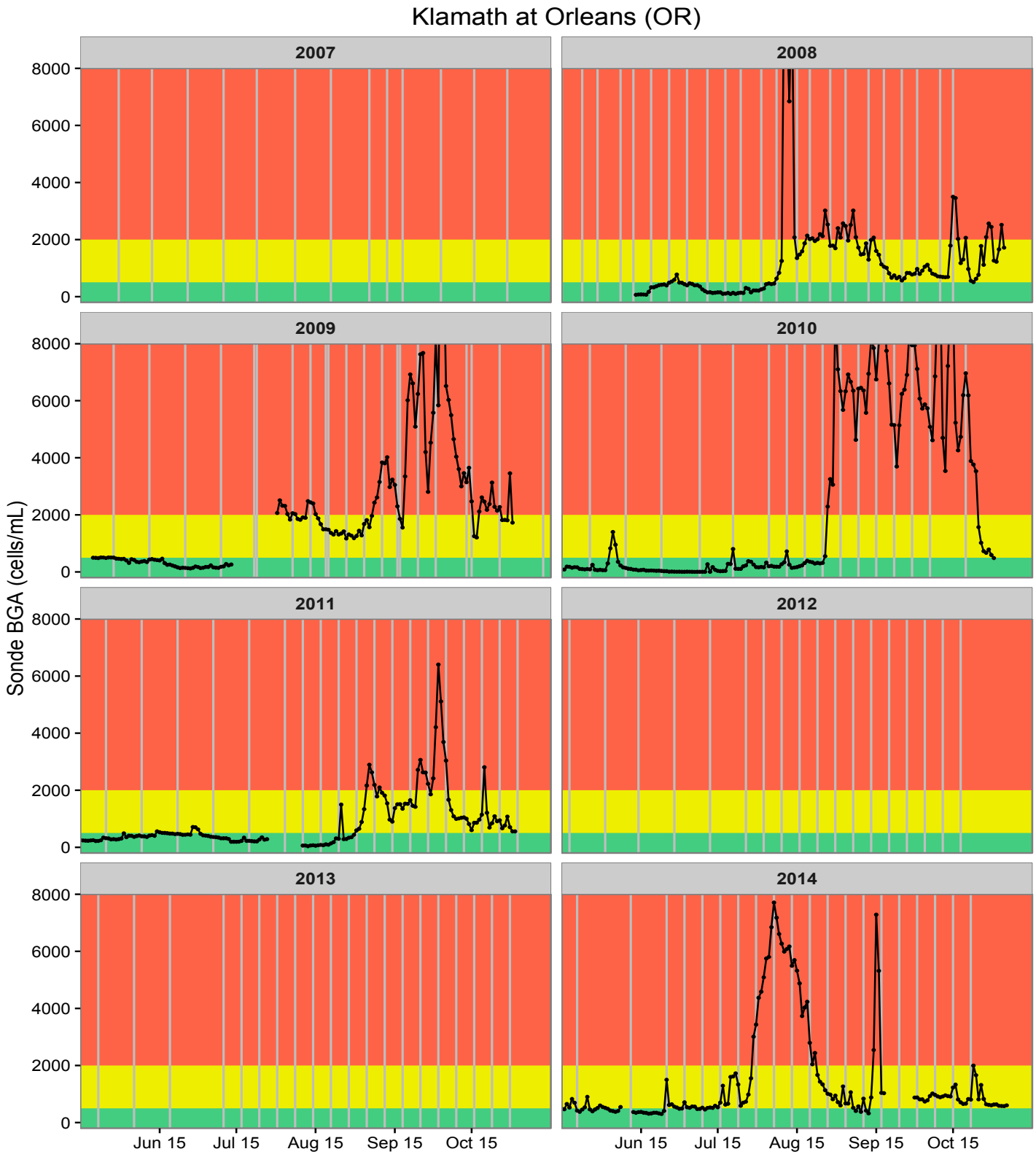


Figure E 4. Daily mean phycocyanin time series (black line with points) with shaded background colors corresponding to sonde BGA levels above which the probability of exceeding public health thresholds rapidly increases, as determined from probability of exceedance analysis for microcystin and sonde BGA. Green < 500 cells/mL; Yellow > 500 cells/mL and < 2000 cells/mL and corresponds to 0.8 µg/L (Level I Health Warning); Orange > 2000 cells/mL and corresponds to 4.0 µg/L (Level II Health Danger). Verticle grey bars represent dates of grab samples.

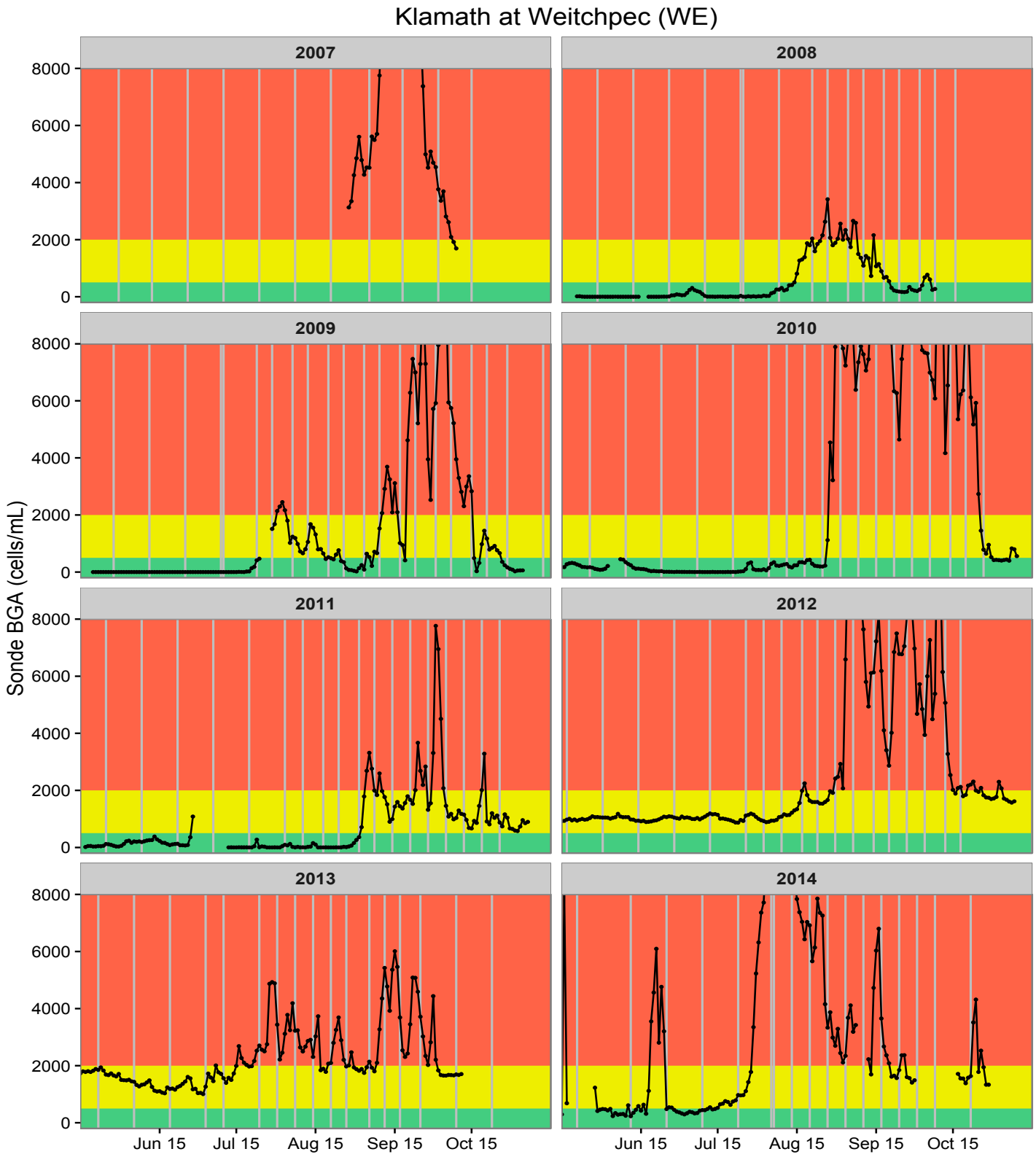


Figure E 5. Daily mean phycocyanin time series (black line with points) with shaded background colors corresponding to sonde BGA levels above which the probability of exceeding public health thresholds rapidly increases, as determined from probability of exceedance analysis for microcystin and sonde BGA. Green < 500 cells/mL; Yellow > 500 cells/mL and < 2000 cells/mL and corresponds to 0.8 µg/L (Level I Health Warning); Orange > 2000 cells/mL and corresponds to 4.0 µg/L (Level II Health Danger). Verticle grey bars represent dates of grab samples.

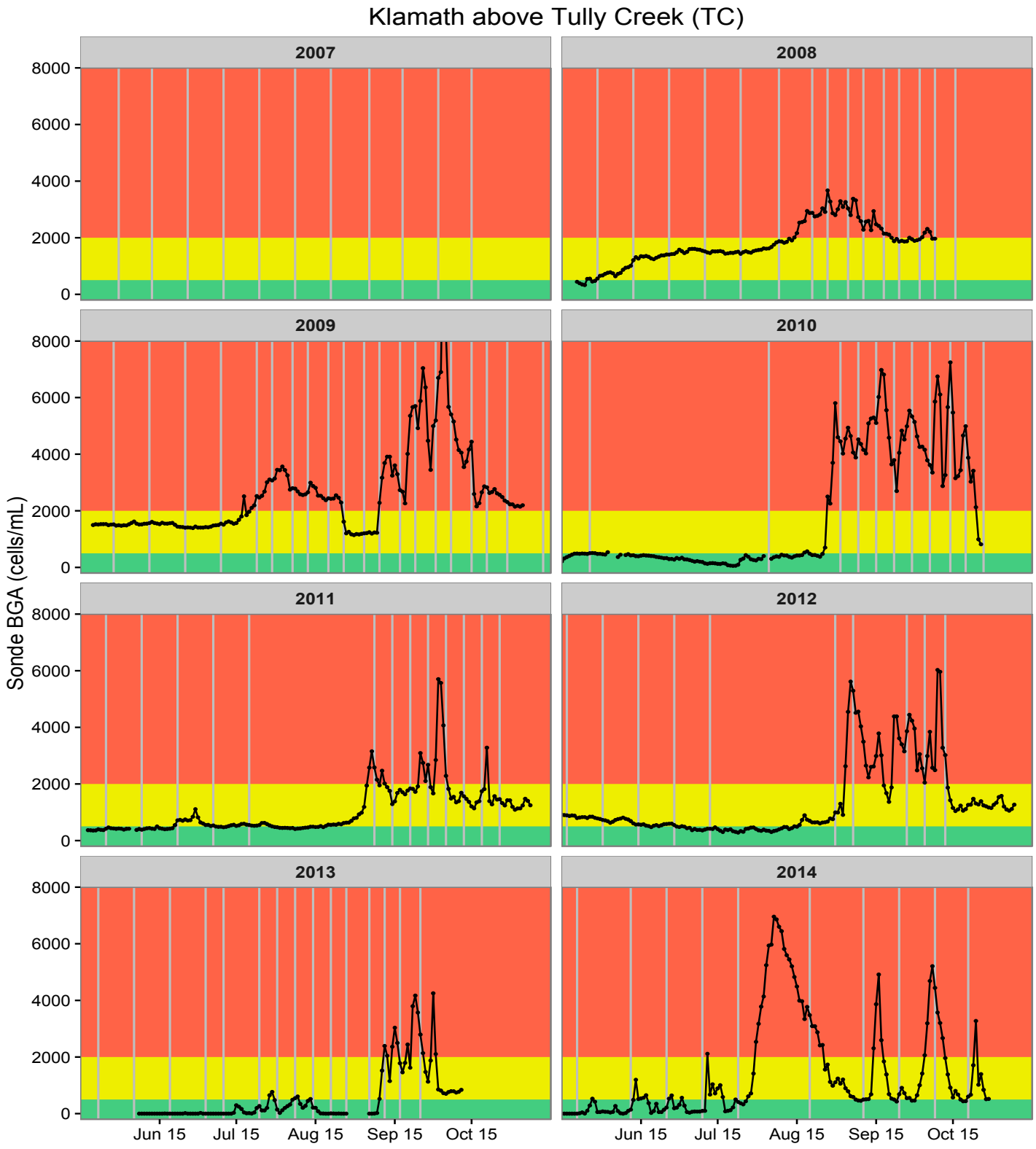


Figure E 6. Daily mean phycocyanin time series (black line with points) with shaded background colors corresponding to sonde BGA levels above which the probability of exceeding public health thresholds rapidly increases, as determined from probability of exceedance analysis for microcystin and sonde BGA. Green < 500 cells/mL; Yellow > 500 cells/mL and < 2000 cells/mL and corresponds to 0.8 µg/L (Level I Health Warning); Orange > 2000 cells/mL and corresponds to 4.0 µg/L(Level II Health Danger). Verticle grey bars represent dates of grab samples.

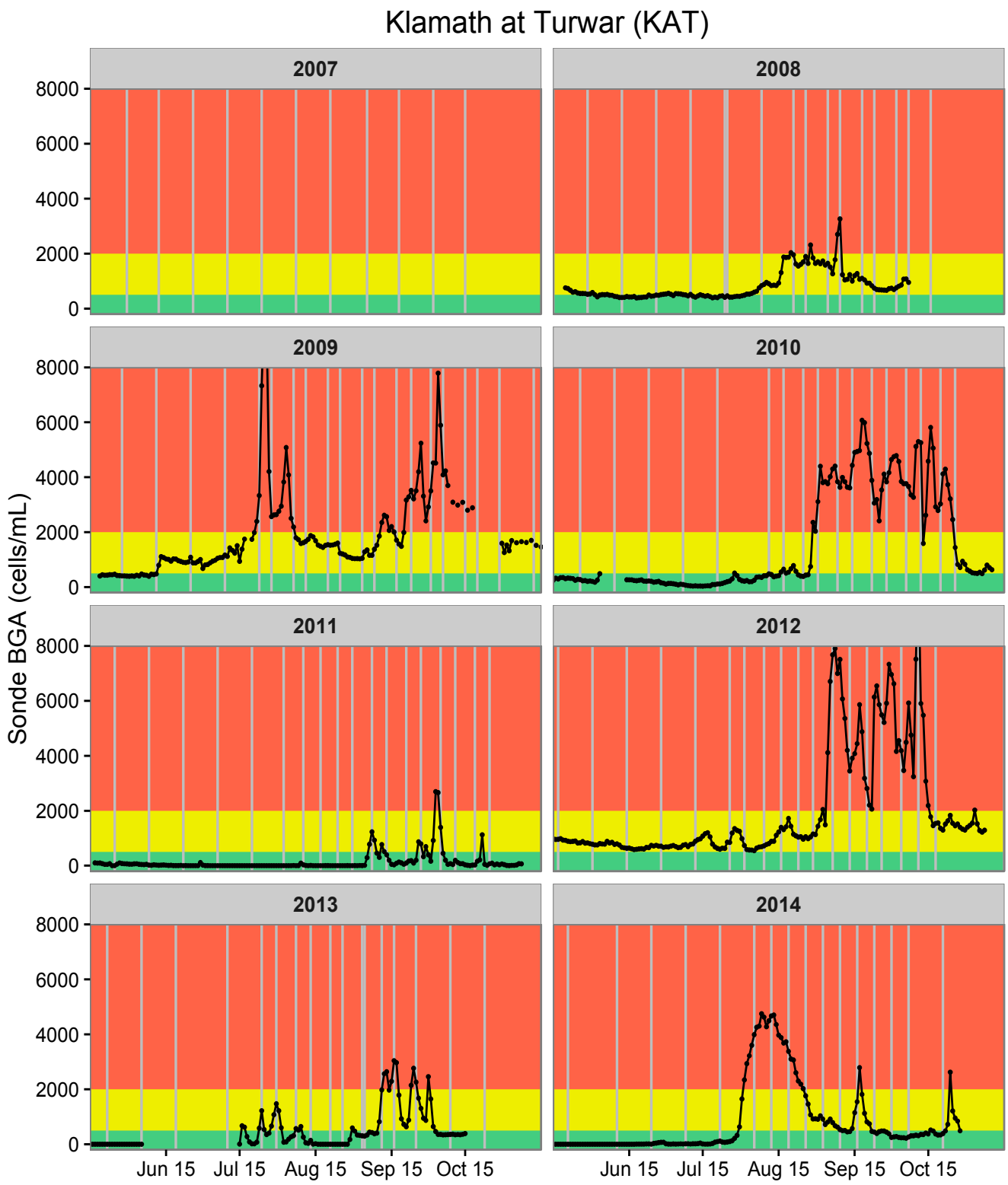


Figure E 7. Daily mean phycocyanin time series (black line with points) with shaded background colors corresponding to sonde BGA levels above which the probability of exceeding public health thresholds rapidly increases, as determined from probability of exceedance analysis for microcystin and sonde BGA. Green < 500 cells/mL; Yellow > 500 cells/mL and < 2000 cells/mL and corresponds to 0.8 µg/L (Level I Health Warning); Orange > 2000 cells/mL and corresponds to 4.0 µg (Level II Health Danger). Verticle grey bars represent dates of grab samples.

APPENDIX F: HISTORIC PHYCOCYANIN DATA IN THE CONTEXT OF CALIFORNIA WATER QUALITY MONITORING COUNCIL CCHAB GUIDELINES

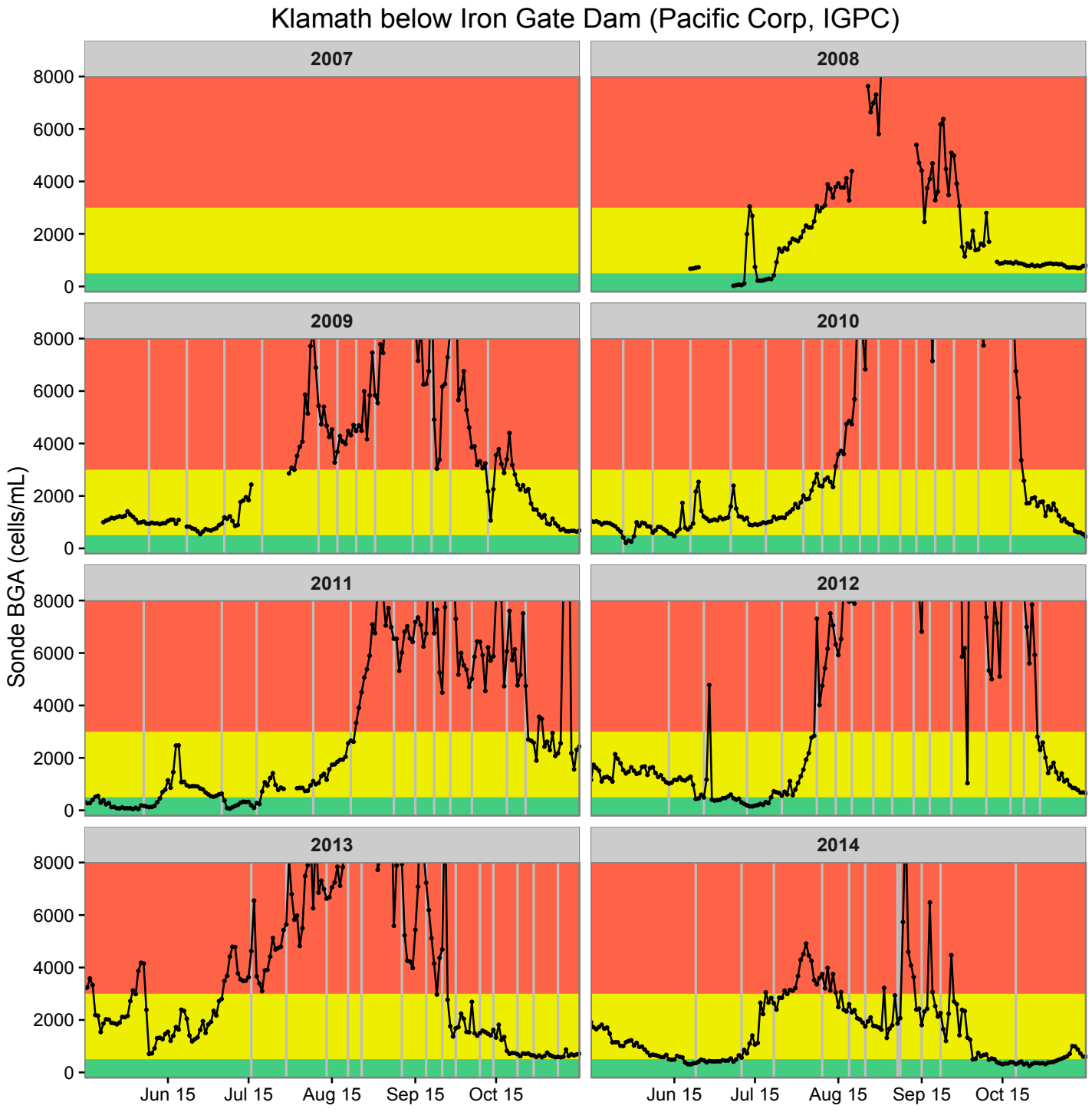


Figure F 1. Daily mean phycocyanin time series (black line with points) with shaded background colors corresponding to sonde BGA levels above which the probability of exceeding public health thresholds rapidly increases, as determined from probability of exceedance analysis for microcystin and sonde BGA. Green < 500 cells/mL; Yellow > 500 cells/mL and < 3000 cells/mL and corresponds to 0.8 µg/L (California CCHAB Caution Action Trigger); Orange > 3000 cells/mL and corresponds to 6.0 µg (Warning TIER I Action Trigger). Verticle grey bars represent dates of grab samples.

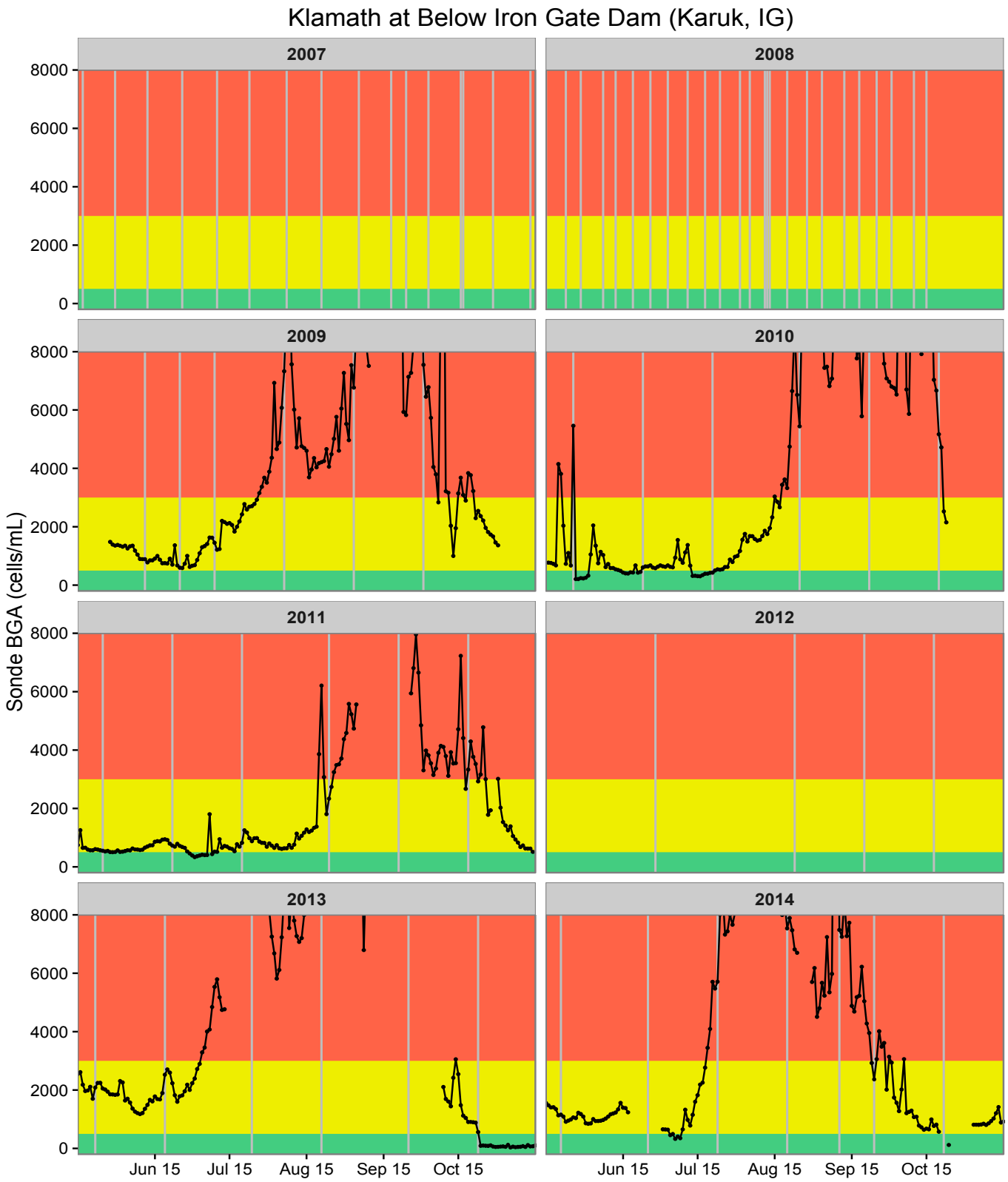
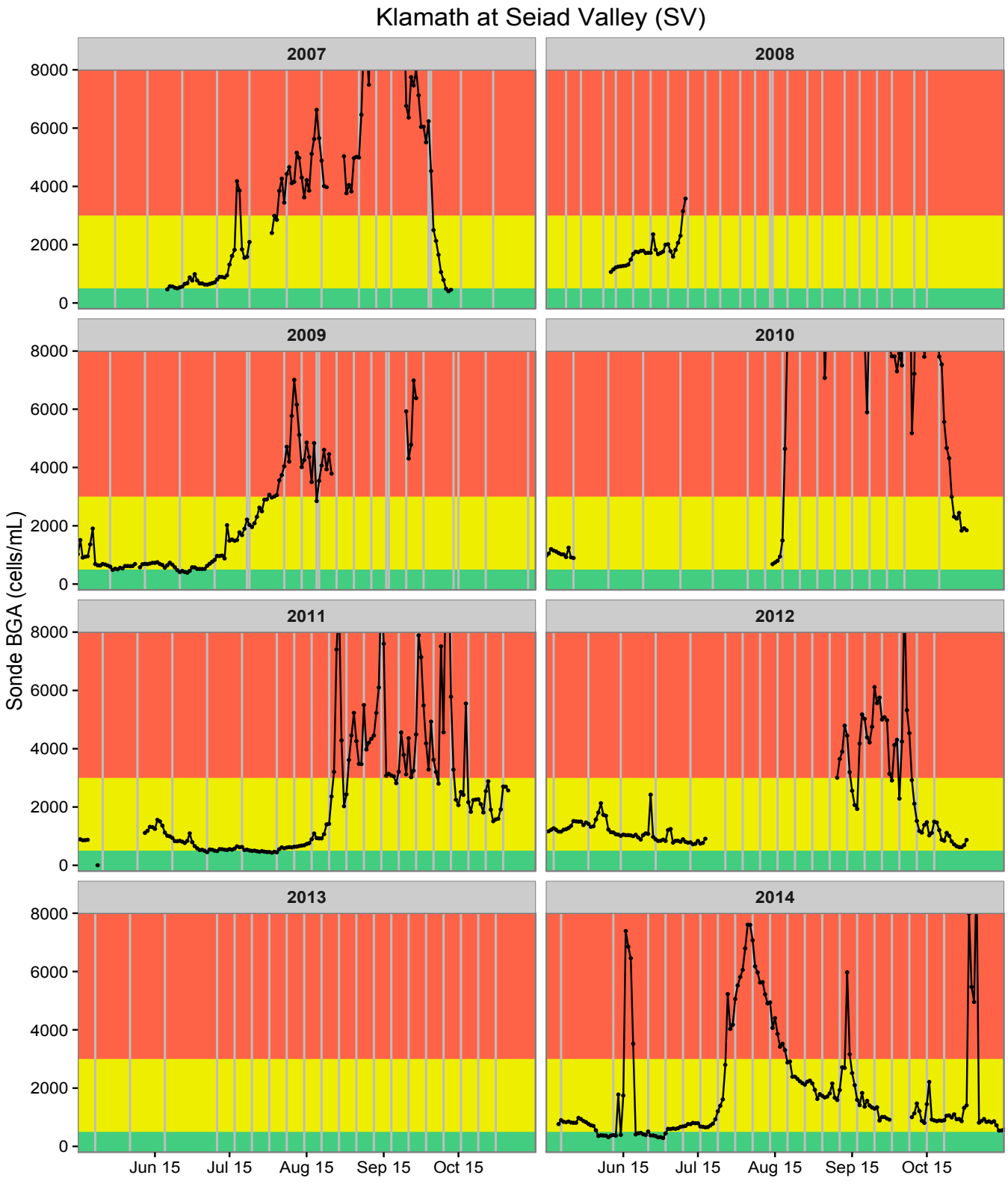


Figure F 2. Daily mean phycocyanin time series (black line with points) with shaded background colors corresponding to sonde BGA levels above which the probability of exceeding public health thresholds rapidly increases, as determined from probability of exceedance analysis for microcystin and sonde BGA. Green < 500 cells/mL; Yellow > 500 cells/mL and < 3000 cells/mL and corresponds to 0.8 µg/L (California CCHAB Caution Action Trigger); Orange > 3000 cells/mL and corresponds to 6.0 µg (Warning TIER I Action Trigger). Verticle grey bars represent dates of grab samples.



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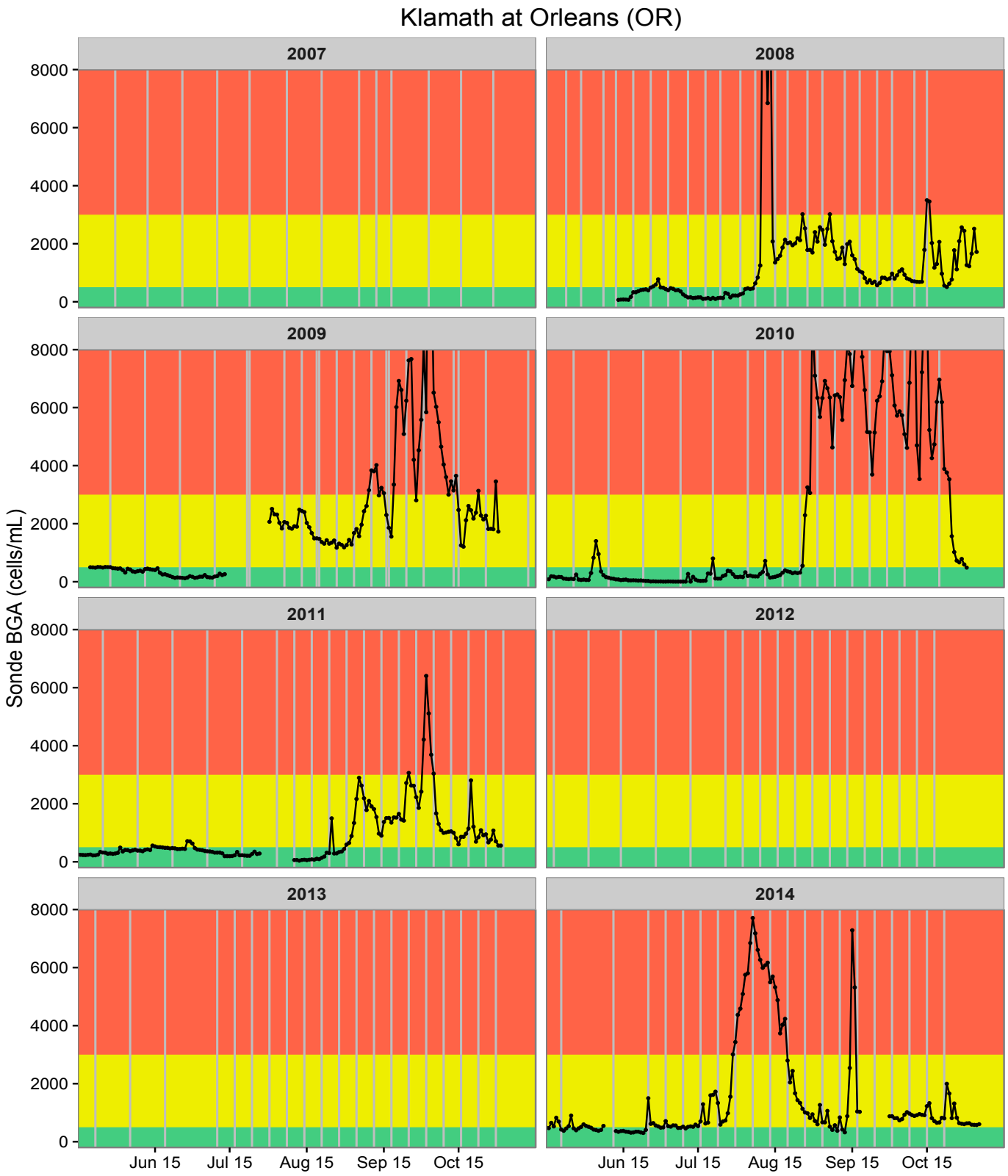
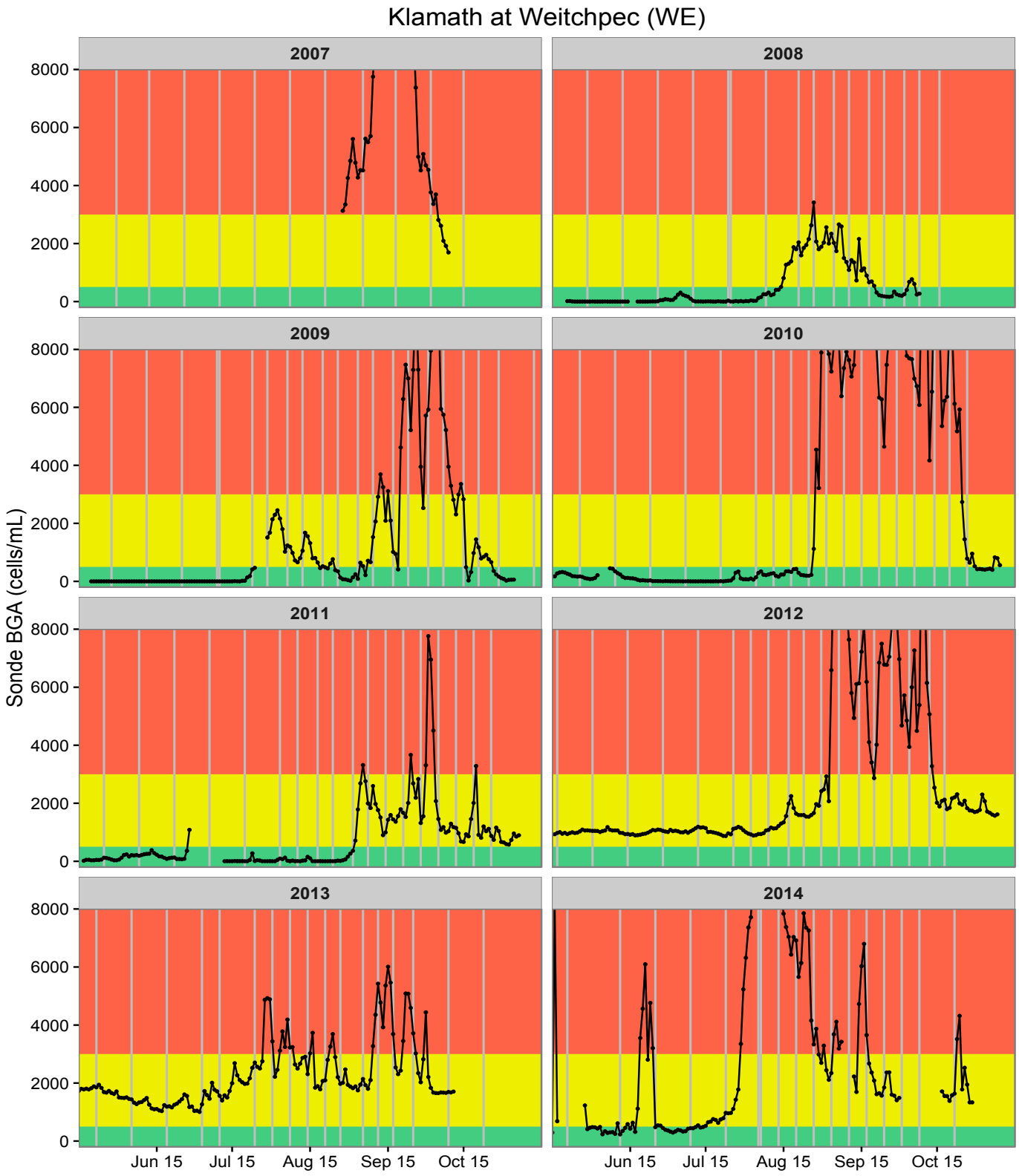


Figure F 4. Daily mean phycocyanin time series (black line with points) with shaded background colors corresponding to sonde BGA levels above which the probability of exceeding public health thresholds rapidly increases, as determined from probability of exceedance analysis for microcystin and sonde BGA. Green < 500 cells/mL; Yellow > 500 cells/mL and < 3000 cells/mL and corresponds to 0.8 µg/L (California CCHAB Caution Action Trigger); Orange > 3000 cells/mL and corresponds to 6.0 µg (Warning TIER I Action Trigger). Verticle grey bars represent dates of grab samples.



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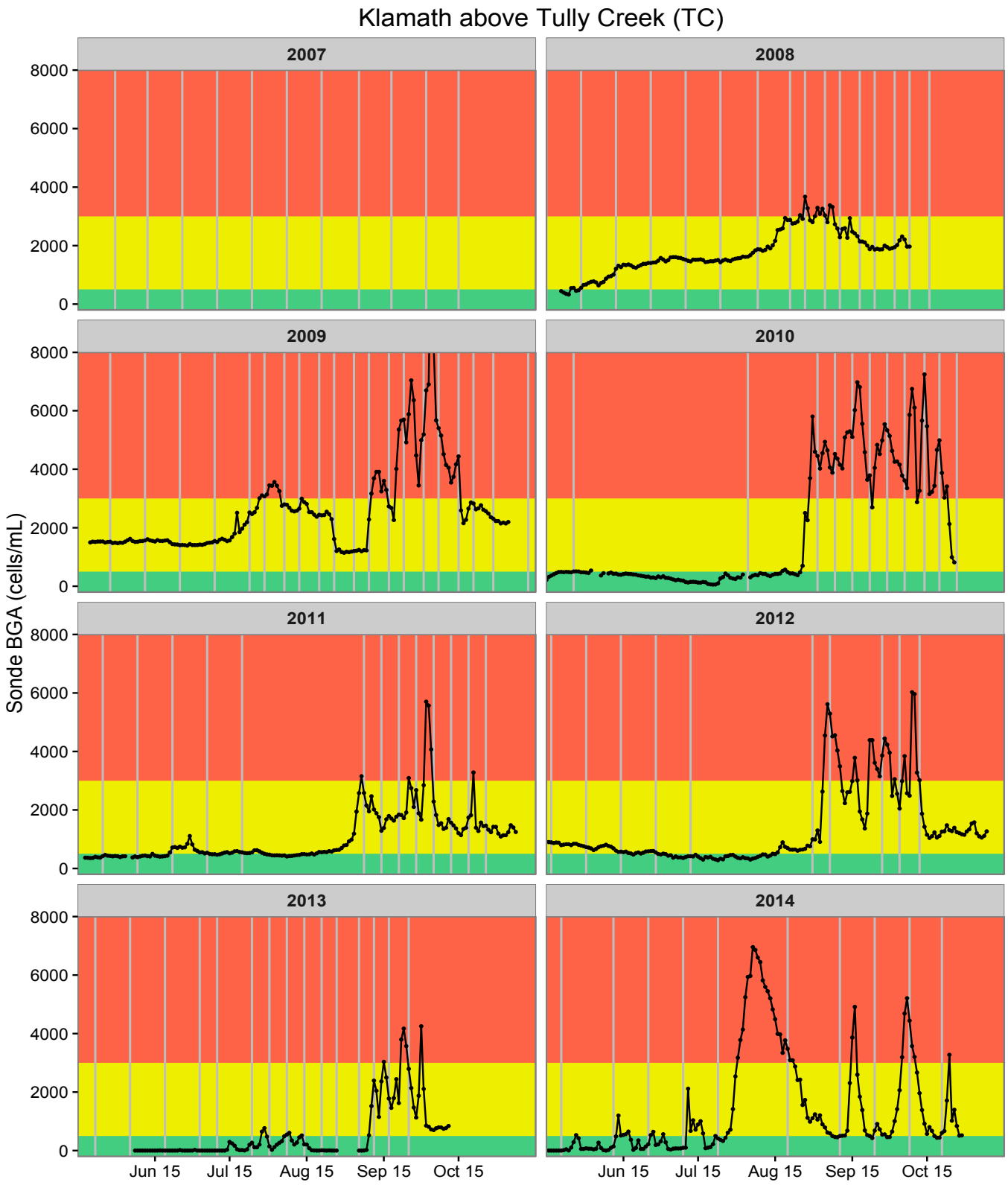

Figure F 6. Daily mean phycocyanin time series (black line with points) with shaded background colors corresponding to sonde BGA levels above which the probability of exceeding public health thresholds rapidly increases, as determined from probability of exceedance analysis for microcystin and sonde BGA. Green < 500 cells/mL; Yellow > 500 cells/mL and < 3000 cells/mL and corresponds to 0.8 µg/L (California CCHAB Caution Action Trigger); Orange > 3000 cells/mL and corresponds to 6.0 µg (Warning TIER I Action Trigger). Verticle grey bars represent dates of grab samples.

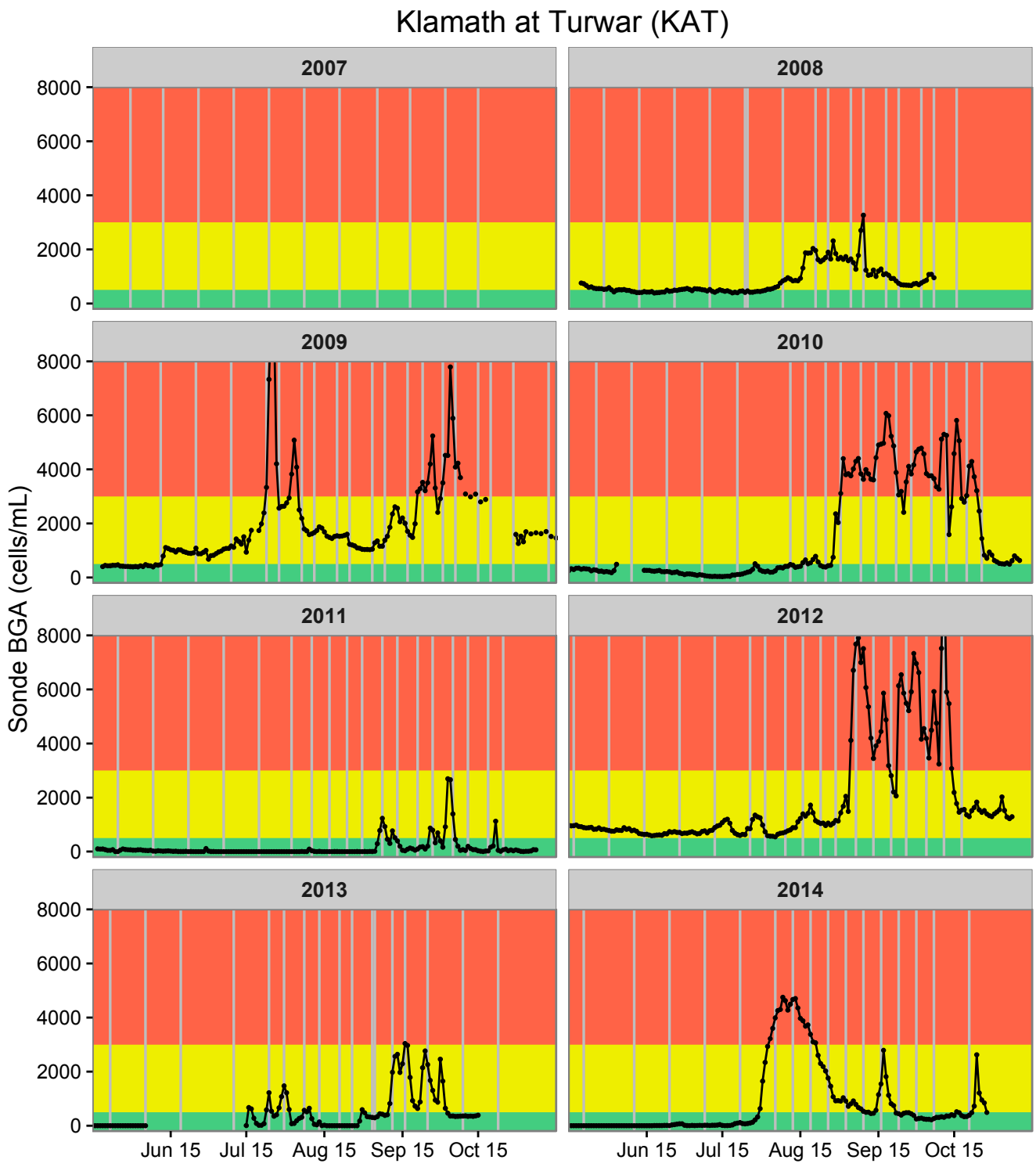

Figure F 7. Daily mean phycocyanin time series (black line with points) with shaded background colors corresponding to sonde BGA levels above which the probability of exceeding public health thresholds rapidly increases, as determined from probability of exceedance analysis for microcystin and sonde BGA. Green < 500 cells/mL; Yellow > 500 cells/mL and < 3000 cells/mL and corresponds to 0.8 µg/L (California CCHAB Caution Action Trigger); Orange > 3000 cells/mL and corresponds to 6.0 µg (Warning TIER I Action Trigger). Verticle grey bars represent dates of grab samples.