TECHNICAL MEMORANDUM

Toxigenic *Microcystis aeruginosa* bloom dynamics and cell density/chlorophyll *a* relationships with microcystin toxin in the Klamath River, 2005-2008.

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May 2009

INTRODUCTION

As outlined previously (Kann 2007; Kann and Corum 2007; Jacoby and Kann 2007; Kann and Corum 2006) Copco and Iron Gate Reservoirs (the lowermost projects of PacifiCorp's Klamath Hydropower Project-- KHP) experienced extensive blooms of toxigenic *Microcystis aeruginosa* (MSAE) from 2004-2007. These blooms were associated with high levels of microcystin, a potent hepatotoxin capable of causing chronic liver damage and acting as a tumor promoter (Carmichael 1995; Chorus et al. 1999; Chorus 2001).-

The results of the 2005-2007 sampling program demonstrated widespread and high abundance of toxigenic MSAE blooms in Copco and Iron Gate reservoirs from July-October, exceeding World Health Organization Moderate Probability of Adverse Health Effect Levels (WHO MPHAEL) for both cell density and toxin by 10 to over 1000 times Although both cell density and toxin data indicated that MSAE cells and microcystin were either not detectable or detected at very low levels in the Klamath River directly above the reservoirs, levels of both parameters increased directly below the reservoirs in all years. In addition, bioaccumulation studies undertaken in 2007 showed accumulation of microcystin toxin in muscle and/or liver tissues of yellow perch, hatchery salmon, and freshwater mussels (Mekebri et al. 2009; Kann 2008; Kanz 2008). Microcystin levels in yellow perch filets from Copco and Iron Gate Reservoirs and freshwater mussels from the Klamath River below the reservoirs exceeded public health threshold values for safe consumption (Kann 2008; OEHHA 2008)

Similar to previous years, a toxic algal monitoring program was undertaken by the Karuk Tribe during July-October, 2008. The following technical memorandum summarizes 2008 toxigenic MSAE trends in Copco and Iron Gate Reservoirs and in the Klamath River directly above and below the reservoir complex. In addition, as a follow-up to previous analysis relating microcystin concentration to MSAE cell density (Kann and Corum 2006; 2007), both microcystin concentration and MSAE density was related to chlorophyll *a*, a commonly reported indicator of algal biomass.

METHODS

Station Location

During the 2008 sampling season, MSAE cell density, cell biovolume, and microcystin toxin samples were collected from a variety of reservoir shoreline and open-water sites, including standard open-water locations (Table 1 and Figure 1; Stations IR01 and CR01) and shoreline stations specifically sampled to assess the extent of toxic MSAE in the vicinity of public recreational access points (Figure 1). River stations consisted of standard stations KRAC (located above Copco Reservoir), and KRBI, WA (added in 2008), SV (Seiad Valley), and OR (Orleans) located below Iron Gate Reservoir (Figure 1). Control stations near the mouths of the Shasta River (SH), Scott River (SC), and the Salmon River (SA) were also sampled (Figure 1).

In addition, based on past sampling and observations by USFWS Arcata Office in 2003 and 2005, as well as observations during 2007 of river-edge accumulations of MSAE, several new ancillary stations were sampled in 2008 specifically to determine MSAE and microcystin levels in near-shore river habitat below Iron Gate Reservoir (Table 1; Figure 1).

Table 1. Phytoplankton/microcystin sampling locations in Copco and Iron Gate Reservoirs and Klamath River stations, 2008.

STATION NAME	STATION LAT/LON	Station Description	Shoreline (SL) or Open Water (OW)
CR01	N41 58.932 W122 19.694	Copco Res. Near Dam	OW
CRCC	N41 59.035 W122 19.802	Copco Res. Copco Cove Boat Ramp/Recreation Area	SL
CRMC	N41 58.441 W122 17.869	Copco Res. Mallard Cove Boat Ramp/Recreation Area	SL
IR01	N41 56.330 W122 25.930	Iron Gate Res. Near Dam	OW
IRCC	N41 58.368 W122 26.114	Iron Gate Res. Camp Creek Boat Ramp/Recreation Area	SL
IRJW	N41 57.721 W122 26.425	Iron Gate Res. Jay Williams Boat Ramp/Recreation Area	SL
KRAC	N41 58.345 W122 12.101	Klamath River Above Copco Reservoir	River-OW
KRBI	N41 55.865 W122 26.532	Klamath River Below Iron Gate Reservoir	River-OW
WA	N41 50.252 W122 51.811	Located downstream of the town of Klamath River. Samples were collected off of Walker Bridge.	River-OW
SV	N41 50.561 W 123 13.132	Seiad Valley at Sluice Box River Access	River-OW
OR	N41 18.336 W 123 31.895	Orleans just north of Orleans Bridge	River-OW
BRBE	N41 49.399 W122 57.650	Brown Bear River Access just east of Horse Creek	River-SL
KRRP	N41 48.972 W123 07.591	Rocky Point River Access located just west of Hamburg	River-SL
SVMN	N41 50.209 W123 10.484	New 49ers mining claim just east of Seiad Valley. Large backwater.	River-SL
SVFD	N41 50.068 W123 11.780	Seiad Valley Fish Disease Site Located just outside of Seiad Valley	River-SL
BVFI	N 41 52.025 W122 48.585	Beaver Creek Fish Disease Site Located just upriver of Seiad Valley	River-SL

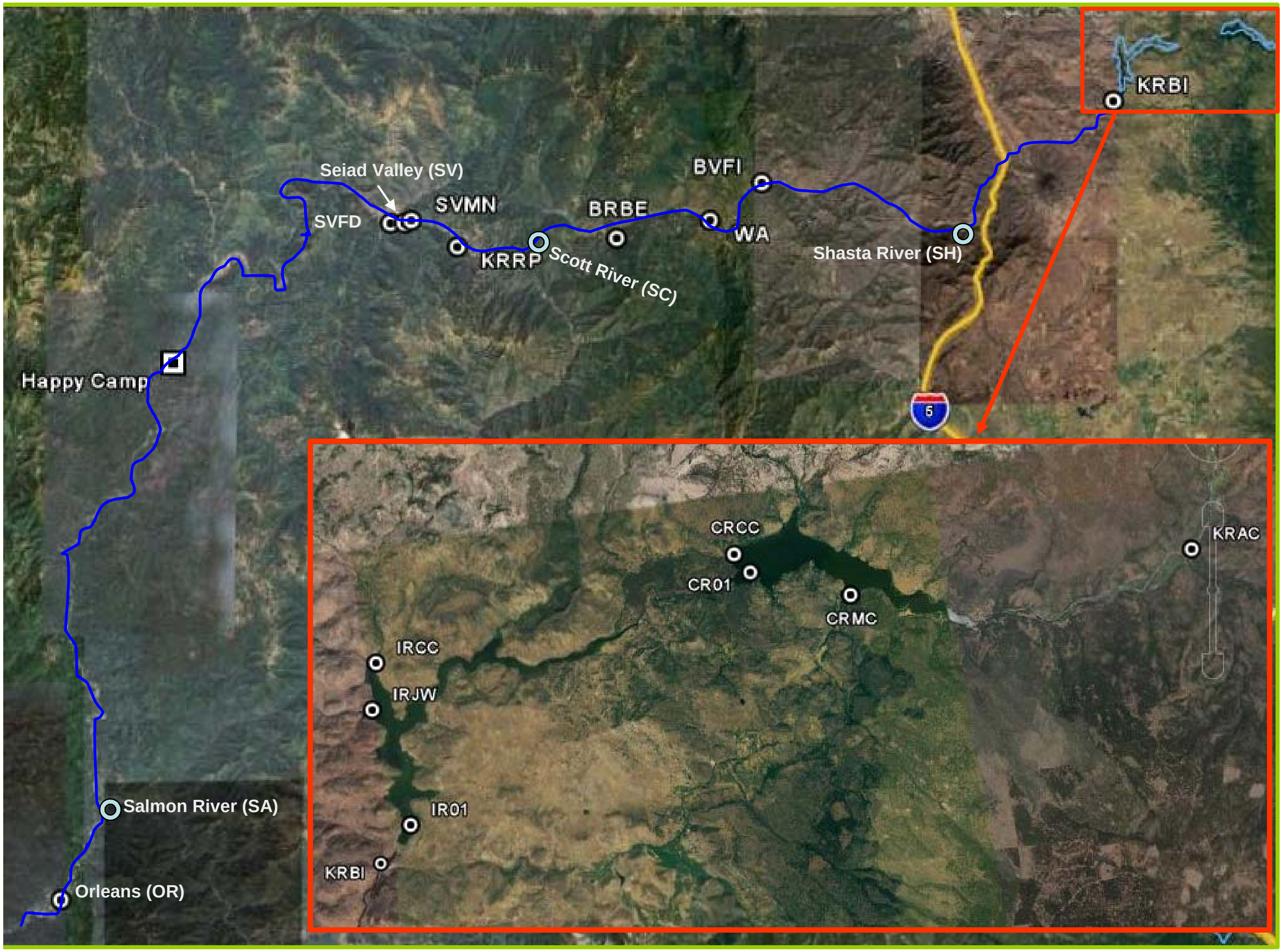

Figure 1. Location of Copco and Iron Gate Reservoir and Klamath River toxic cyanobacteria sampling stations, 2008.

Sample Collection and Lab Analysis

Reservoir shoreline and open-water samples taken at the surface consisted of grab samples of surface algal material (denoted SG in the depth column of Appendix I below), and river open-water (in mixed areas of noticeable velocity) samples were taken with a Van-Dorn water collection bottle at KRAC and WA and then composited in a churn splitter. At the remaining standard river stations (denoted OC in the depth column of Appendix I) samples were collected with a churn by wading towards the center of the channel (in mixed areas of noticeable velocity), submersing and filling the churn prior to distributing to appropriate sample bottles.

Samples collected at ancillary river stations consisted of a grab sample collected near the river edge in areas of low velocity. Coinciding with other diel nutrient studies, samples were also occasionally collected at multiple times in a 24 hour period. Samples for microscopic determination of phytoplankton density and biovolume were preserved in Lugol's Iodine and sent to Aquatic Analysts in White Salmon, WA where enumeration and biovolume measurements are determined according to APHA Standard Methods (1992).

Because previous work showed higher variability among split samples when MSAE levels were in the lower range (e.g. 0-30,000 cells/ml), samples from the Klamath River stations were enumerated at an increased counting resolution that included cells counts of potentially toxigenic blue-green algae in an area 4x that of the usual 100 algal unit count performed by the laboratory. This provided an effective algal unit count of 400 for potentially toxigenic species. Phytoplankton laboratory reports are contained in Electronic Appendix I.

Samples for microcystin toxin were collected in glass vials, which were frozen at Karuk Tribal facilities, 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 methodology (microcystin laboratory reports and methodology are contained in Electronic Appendix E2—also see Fetcho (2007) for a comprehensive description of laboratory methods and detection limits).

A minimum of one set of "blind duplicate" quality assurance samples per trip were collected for cell density and microcystin toxin. Quality assurance (QA) sampling was performed by splitting samples in the field using a churn splitter. One of the pair of split samples was disguised and sent with its associated split for analysis of both cell density and microcystin toxin. Due to a laboratory mix-up, the blind duplicate was analyzed at normal 100 count while the original was processed at the increased resolution described above (4x).

Comparison to Public Health Threshold Values

Cell density and toxin concentration were compared to California State Water Resources Control Board (SWRCB) and Office of Environmental Health and Hazard Assessment (OEHHA) public health guideline levels that are similar to those used by the state of Oregon (Stone and Bress (2007). These levels are 40,000 cells/ml of MSAE and 8 μg/L of microcystin and are also consistent with recent Australian analysis of health risk threshold values (NHMRC 2005).

The SWRCB/OEHHA levels are specific for MSAE and microcystin, whereas previously used World Health Organization (WHO) threshold values for Moderate Probability of Adverse Health

Effects (MPAHEL as published in documents for the WHO and EPA: e.g., Falconer el al. 1999 and Chorus and Cavalieri 2000) are general levels for a variety of toxigenic cyanobacteria. These WHO guidelines indicated 4 μg/L of microcystin constituted a low probability of adverse health effects and 20 μg/L constituted a moderate probability of adverse health effects.

Microcystin concentration was also compared to the tolerable daily intake level (TDI: 0.04 µg microcystin per kg of body weight/day as described in WHO 1998) computed for a 20kg child ingesting 100 mls of reservoir water. The TDI as computed here for a 20kg child is equivalent to the exceedance of the 8µg/L public health guideline value described by SWRCB/OEHHA (www.waterboards.ca.gov/water_issues/programs/bluegreen_algae/docs/bga_volguidance.pdf). The WHO (Falconer et al. 1999) also lists cyanobacterial scums in swimming areas as having a high probability of adverse health effects (i.e., the potential to cause acute poisoning) and recommends immediate action to prevent contact with scums.

As noted above, public health threshold values were also evaluated by relating microcystin concentration to MSAE cell density as well as chlorophyll *a*. Chlorophyll *a* (CHL) data were collected as part of routine water quality monitoring carried out by the Karuk Tribe, with analyses performed at Aquatic Research, INC in Seattle, WA (see Kann and Asarian 2007 for analytical and collection details). Paired CHL, MSAE, and microcystin data for those dates and stations where data were collected simultaneously were then used in subsequent empirical analyses.

RESULTS/DISCUSSION

Quality Assurance Samples

Analysis of 2008 MSAE duplicate samples was somewhat complicated by differing counting resolution on the original sample vs. the duplicate, whereby when the counting resolution (4X or non-4X) between the paired sample and duplicate differs the comparison becomes one of both counting resolution and precision. These qualifications notwithstanding, samples generally showed good agreement between split samples for MSAE cell density (Table 2).

The 4X counts were higher than non-4X counts in all but one instance (8/10/2008) and there was only one date where the 4X sample exceeded the 40,000 cells/ml MSAE threshold when the non-4X did not. Microcystin analyses showed less variability than MSAE cell counts with very good agreement between paired microcystin samples and duplicates (Table 2). With respect to public health threshold values there were no instances when management based on the 8 µg/L microcystin threshold would have differed, and only one instance when management based on the 40,000 cells/ml MSAE threshold would have differed (8/11/2008: 53,102 cells/ml vs. 11,345 cells/ml). However, the higher value was likely due to increased counting resolution rather than decreased precision between the split samples. Field blanks for MSAE density were all non detects (ND), while one of the microcystin field blanks (10/14/2008) showed a very low level of 0.1 µg/L microcystin. Overall, the utilized phytoplankton and toxin methodology had adequate sensitivity relative to public health threshold values.

Table 2. Field duplicate samples for MSAE cell density and microcystin concentration.

Lab ID	Date Collected	Sample ID[1]	Type	Microcystin µg/L	*M. aeruginosa* cells/mL
LT11	5/28/2008	KRBI052808-OC	sample		ND
LT12	5/28/2008	KRBI052808-OC	duplicate		ND
LT13	5/28/2008	KRBL052808-OC	blank		ND
0809053-01/LT36	6/25/2008	KRBI062508-OC	sample	ND	ND
0809053-06/LT37	6/25/2008	KRBL062508-OC	duplicate	ND	ND
0809053-07/LT38	6/25/2008	KRID062508-OC	blank	ND	ND
LT84	7/23/2008	KRID072308-OC (not 4X)	sample		ND
LT82	7/23/2008	KRBI072308-OC (4X)	duplicate		3073
LT83	7/23/2008	KRBL072308-OC	blank		ND
LT97	8/5/2008	CRMC080508-SG	duplicate	6.3	15187
LT96	8/5/2008	CRJD080508-SG	blank	7.3	564
LX21	8/10/2008	KRBI081008-0900 (4X)	sample		9013
0809011-18/LX30	8/10/2008	KRID081008-0900 (not 4X)	duplicate	1.80	15252
0809011-16/LX26	8/11/2008	KRBI081108-1030 (4X)	sample	1.20	53102
0809011-14/LX31	8/11/2008	KRID081108-1030 (not 4X)	duplicate	2.00	11345
0809054-07/LX38	8/19/2008	KRBI081908-OC (4X)	sample	1.80	32805
0809053-08/LX40	8/19/2008	KRID081908-OC (not 4X)	duplicate	ND	20350
0809053-20/LX98	9/17/2008	KRBI091708-OC (4X)	sample	ND	55
0809053-17/MB02	9/17/2008	KRID091708-OC (not 4X)	duplicate	ND	3632
0809053-11/MB01	9/17/2008	KRBL091708-OC	blank	ND	ND
0809050-06/LX76	9/18/2008	CRMC091808-SG	sample	99.00	27612
0809050-03/LX77	9/18/2008	CRMD091808-SG	duplicate	88.00	ND
0811040-07/MB34	10/14/2008	KRBI101408-OC (4X)	sample	0.16	ND
0811039-07/MB40	10/14/2008	KRID101408-OC (4x)	duplicate	0.11	ND
0811039-12/MB27	10/14/2008	KRBL101408-OC	blank	0.10	ND
0811039-03/MB25	10/15/2008	CRMC101508-SG	sample	23.00	ND
0811039-01/MB24	10/15/2008	CRMD101508-SG	duplicate	13.00	17019

[1]Note: when the counting resolution (4X or non-4X) between split samples differs the comparison becomes one of both counting resolution and than precision.

2008 Temporal/Spatial Trends

Similar to the timing of other years, the first visual detection of a cyanobacterial bloom in the reservoir system in 2008 was noted during the first week of July (Appendix I). Although station CRCC in Copco Reservoir had a microcystin concentration of 58 µg/L on 7/2 (exceeding the public health TDI by 7.3x), no MSAE was detected. However, *Anabaena* and *Planktothrix* were detected at low levels but it is not known if these species were responsible for the microcystin production. MSAE then exceeded the 40,000 cell/ml public health threshold on 7/8 at CRCC with a microcystin concentration of 3.8 µg/L (Figure 2). During the first full biweekly sampling on 7/23 none of the standard sampling stations (CR01, CRMC, CRCC, IR01, IRCC, and IRJW:

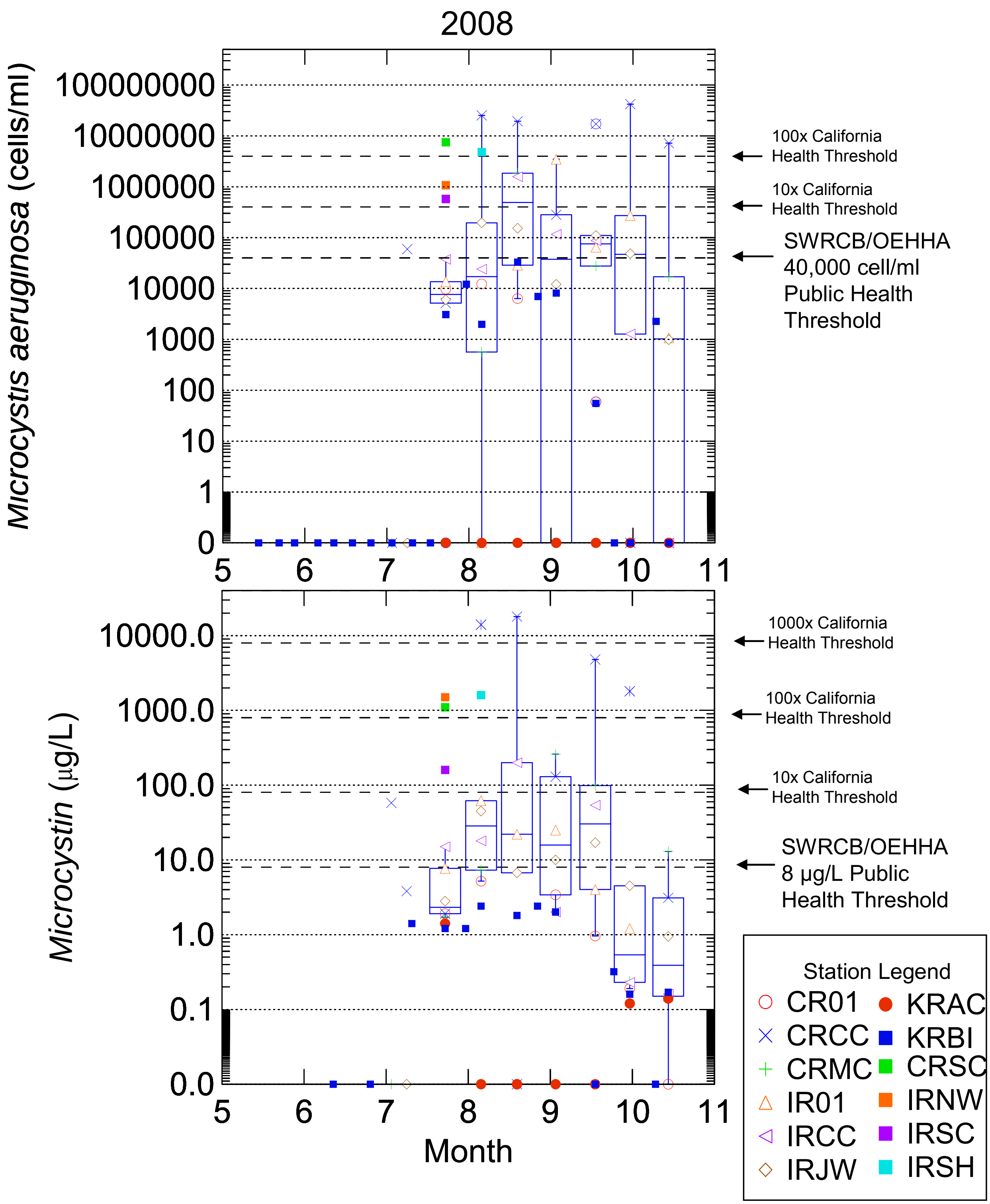

Figure 2. Time-series of MSAE cell density (a) and microcystin toxin concentration (b) for Copco and Iron Gate Reservoir stations, 2008. The box plot (blue box) is for standard reservoir stations CR01. CRMS, CRCC, IR01, IRCC, IRJW only; the river stations KRAC and KRBI, and additional reservoir stations CRSC, IRNW, IRSC, and IRSH are shown independently.

note that the box-plot boxes are for data from these stations only; other stations are plotted independently) exceeded 40,000 cells/ml, and only IRCC exceeded 8 µg/L (Figure 2). However, other stations specifically sampled to assess areas that appeared more concentrated showed much higher levels, exceeding public health guideline values by 10 to over 100 times (Figure 2; stations CRSC, IRNW, and IRSC). Such patchy distributions underscore the need to supplement standard sampling stations, especially prior to initial public health postings.

With the exception of the open-water station CR01, which remained low through the season, overall MSAE and microcystin levels continued to increase in early and mid August, before undergoing a slight decline in early September (Figure 2). During this period the relative decline in MSAE was greater than that of microcystin, especially for Copco Reservoir, where microcystin at CRMC and CRCC remained over 100 µg/L (Appendix I; Figure 2). An evaluation of the ratio of toxin produced per unit MSAE indicates that unlike September 2007 (Kann 2007 Tech Memo shown below in Appendix II) when a sharp decline in the ratio of toxin produced per unit MSAE occurred, the decline in September 2008 was less pronounced (Figure 3), with an increased ratio occurring in Copco Reservoir relative to Iron Gate Reservoir (Figure 4). Of the four years, the ratio of toxin produced per unit MSAE in 2008 ranked among the highest for both July and August (Figure 3).

Although some lingering high values of both MSAE and microcystin continued at CRCC, all other stations were below the 8 µg/L threshold for microcystin by late September (Figure 2). Similar to previous years MSAE was not detected upstream of the reservoirs at KRAC but was detected downstream of Iron Gate Dam at station KRBI (Appendix I; Figure 2).

A comparison of station distributions for 2008 shows a trend of low detections for microcystin at KRAC, increasing but low MSAE and microcystin at CR01, and moderate to high levels of both parameters at the remaining reservoir stations (Figure 5). Despite showing lower overall MSAE at CRMC, microcystin at CRMC was similar to other reservoir stations in 2008 (Figure 5). The trend at CRMC is due in part to a non-detect for MSAE on 9/3/08 that was accompanied by a relatively high microcystin value of 260 µg/L (Appendix I). Likewise, a low MSAE value of 27,612 cells/ml at CRMC on 9/18 was associated with a microcystin concentration of 99 µg/L (Appendix I). To the extent that these values indicate that non-cell bound microcystin was higher overall in Copco Reservoir, such a trend would also contribute to the increased toxin to cell ratio described above.

Continuing downstream to KRBI and below to Orleans (OR), levels of both MSAE and toxin were elevated relative to KRAC; however, none of these river samples taken in the mixed portion of the channel exceeded the threshold guideline values of 40,000 cells/ml MSAE or 8 µg/L microcystin (Figure 5). This is in direct contrast to surface samples taken in areas of low velocity in Klamath River edge habitat (Figure 6; see methods above for station description). These data show that for river-edge samples MSAE cell density and microcystin concentration always exceeded (often by over 10x) the 40,000 cells/ml MSAE and 8 µg/L microcystin public health guideline values, even when open-water samples did not (Figure 6). The edge-water concentrations typically observed are illustrated in Figure 7. From a public health perspective these data illustrate that low MSAE or toxin values in open-water (collected in mixed areas of

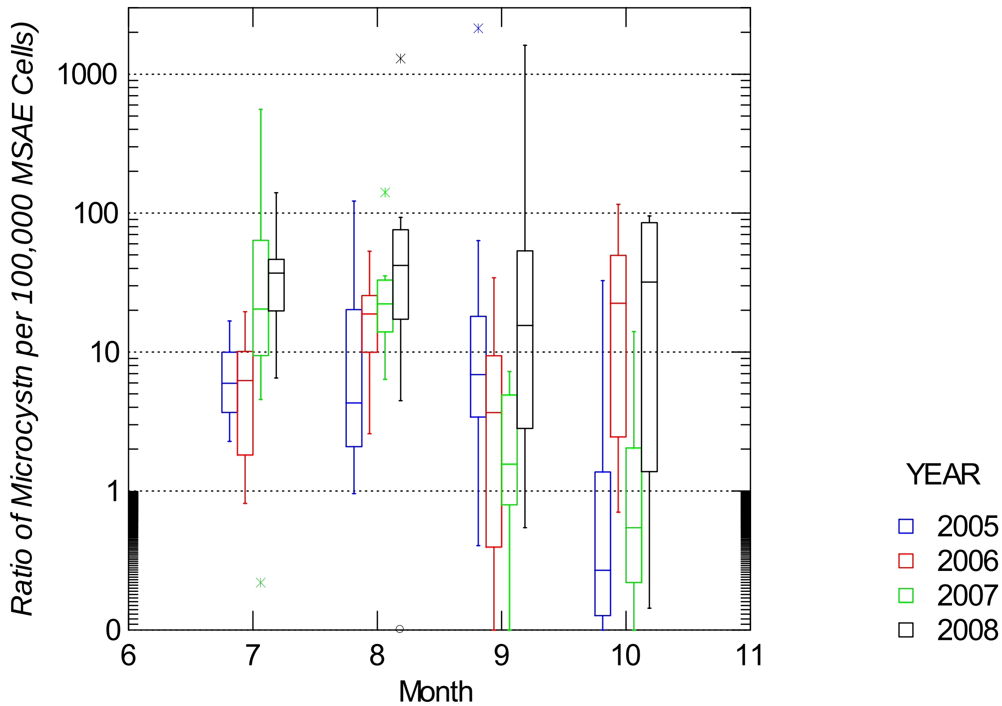

Figure 3. Box plot of the ratio of microcystin concentration per 100,000 MSAE cells in Copco and Iron Gate Reservoirs, 2005-2008.

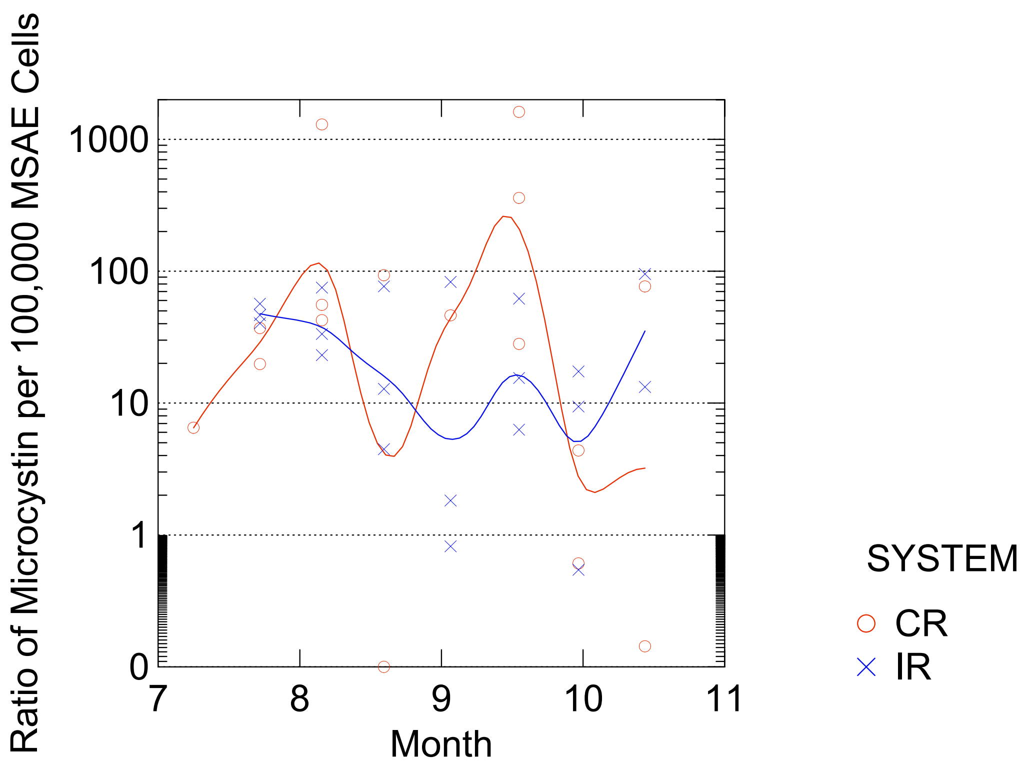

Figure 4. Ratio of microcystin concentration per 100,000 MSAE cells compared between Copco and Iron Gate Reservoirs for 2008. Trend line is the distance weighted least squares smoother; red Copco, blue Iron Gate.

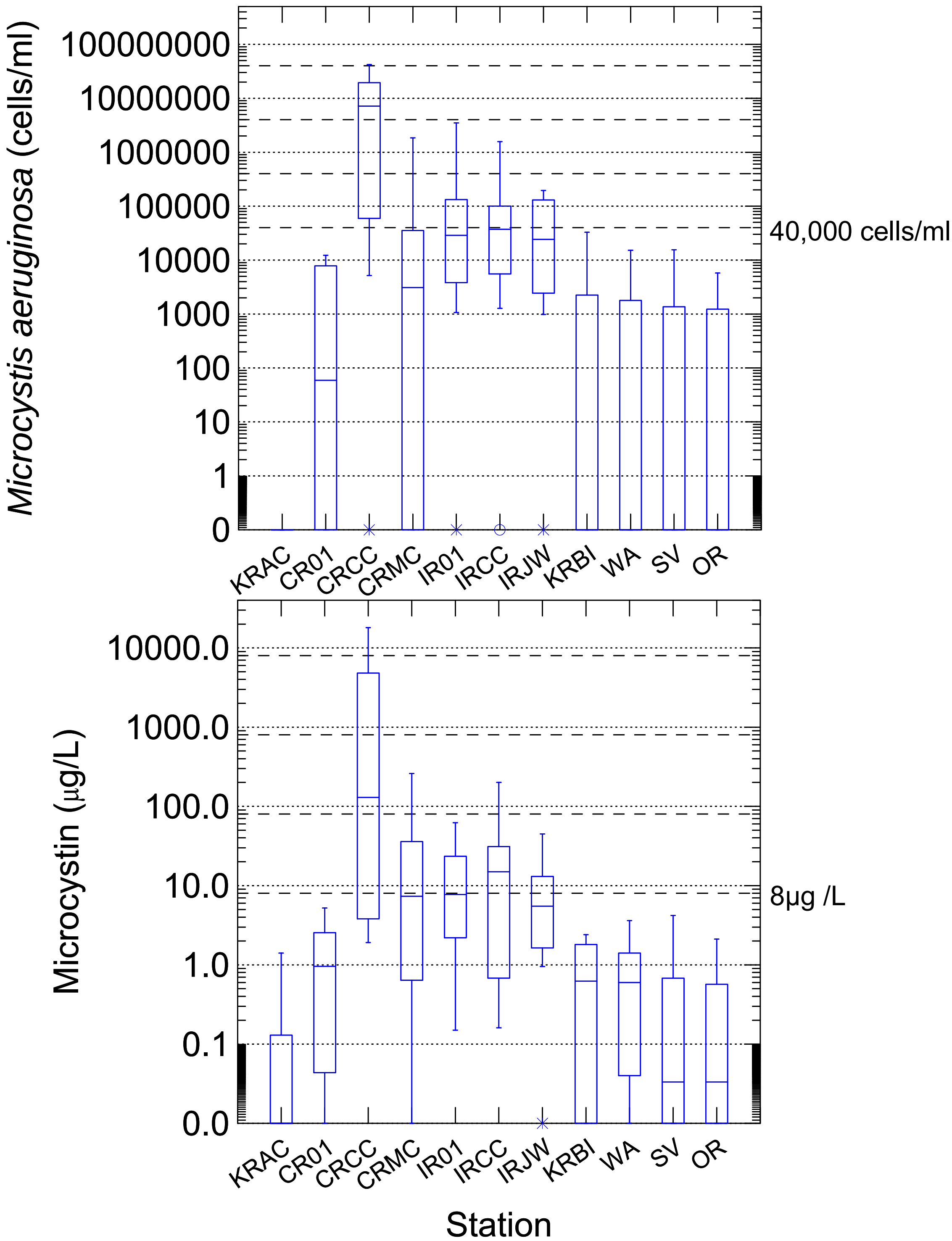

Figure 5. Station comparison of MSAE cell density (a) and microcystin toxin concentration (b) for Klaamth River and Reservoir locations, 2008. Stations ordered longitudinally left (upstream) to right (downstream).

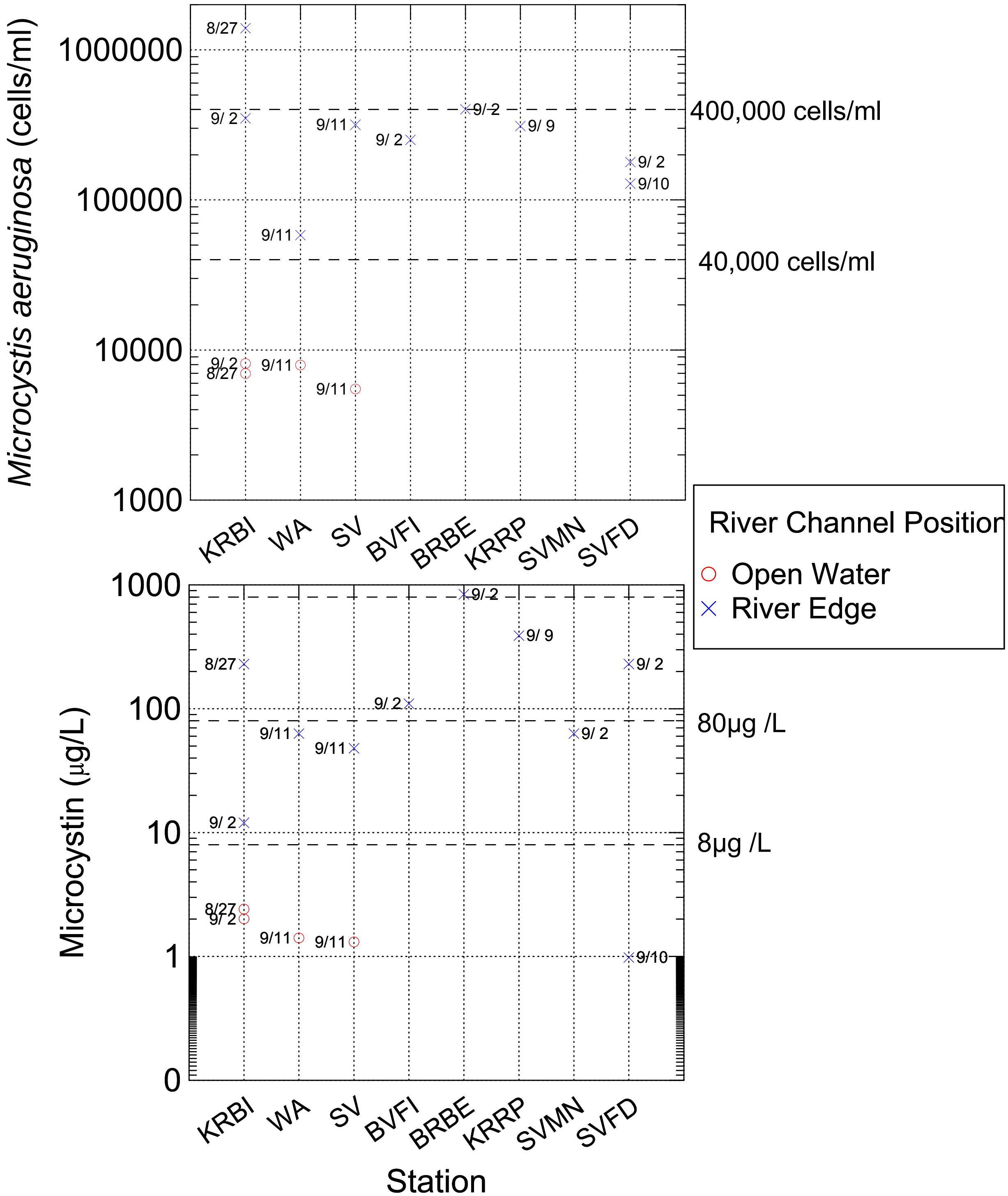

Figure 6. MSAE cell density (a) and microcystin toxin concentration (b) for Klamath River stations sampled in areas of low velocity at the river's edge, 2008.

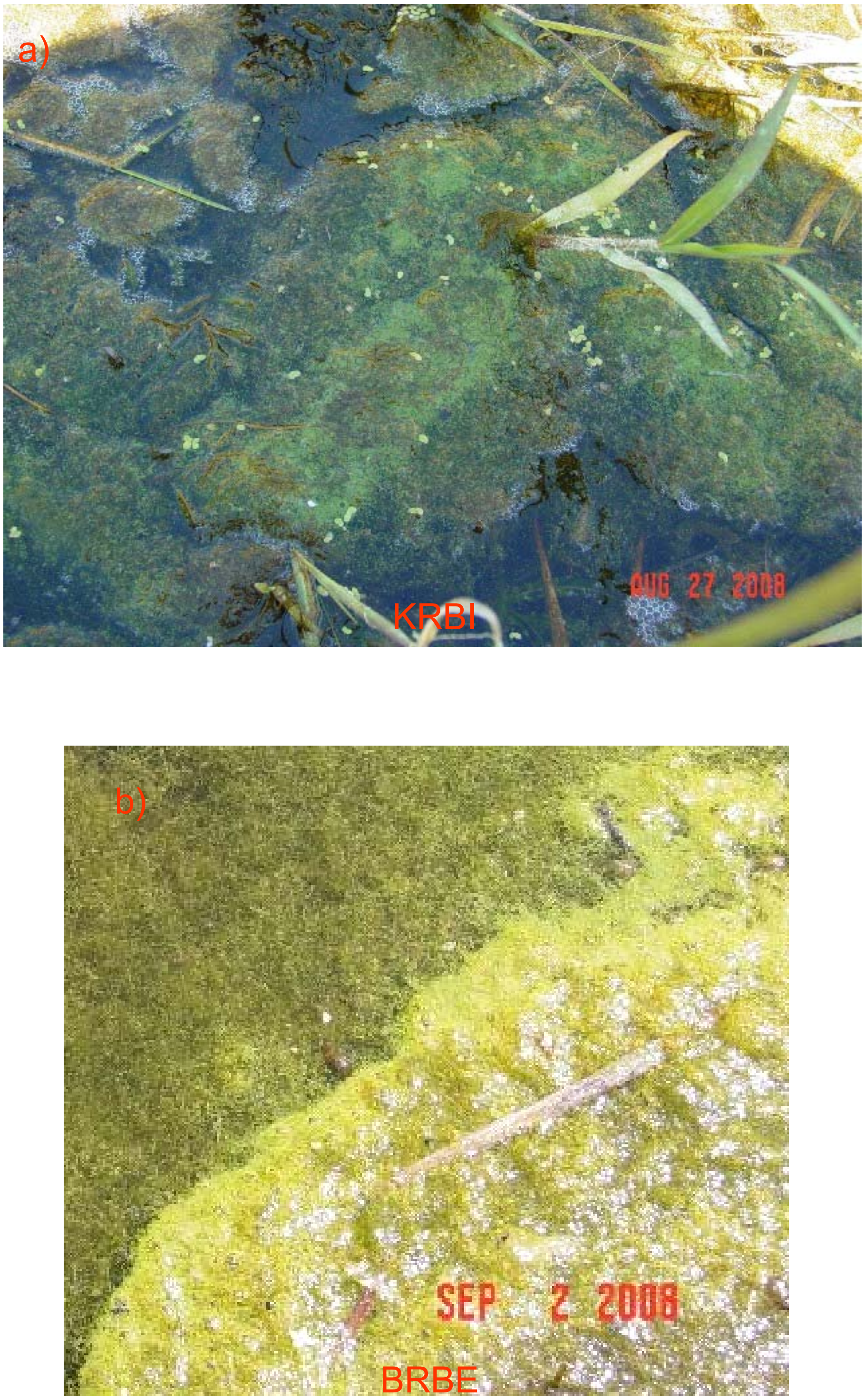

Figure 7. Example of algal accumulations at edge locations along the Klamath River; a) KRBI on 8-27-08, and b) Brown Bear River Access (BRBE) on 9-2-08.

higher velocity) Klamath River samples often translates to values exceeding public health thresholds in river-edge areas. The collection of diel MSAE and toxin samples provided a snapshot to further evaluate short term temporal variability relative to public health thresholds (Figure 8). These data show that for the brief period evaluated that MSAE density and microcystin toxin concentration generally showed low variability in the diel samples; however, there were two instances when MSAE density exceeded the public health threshold, even when other diel samples did not (Figure 8). This illustrates the possibility of false negatives inherent in standard biweekly sampling programs, especially when values are close to public health thresholds.

With the exception of one instance of low MSAE cell density (656 cells/ml) and microcystin concentration (1.4 µg/L) detected at the mouth of the Salmon River (SA) on 7/23, neither MSAE nor microcystin was detected in any other tributary samples during 2008 (Appendix I). The cause of and or source of the low levels at the mouth of the Salmon River is unknown, but given the low level and infrequent detection, is not of general concern at this time.

Inter-annual Comparisons

A comparison of 2008 reservoir data for both Copco and Iron Gate (standard stations CR01, CRMC, CRCC, IR01, IRCC, IRJW only to facilitate inter-annual trends) with 2005-2007 data shows both the median 2008 MSAE density and overall distribution to be lower than the previous three years (Figure 9a). Further breakdown shows that this trend is not only true for the reservoirs as a whole, but for Copco and Iron Gate individually as well (Figure 9b). For example, the upper quartile values (top line of the box or 75th percentile in Figure 9) for both reservoirs were notably lower in 2008 than other years, with the difference even more apparent for Iron Gate Reservoir (Figure 9b). As noted above, the skewing of the lower quartile downward for Copco Reservoir is caused by several values of zero MSAE even when microcystin was detected at relatively high levels.

Despite an upper quartile microcystin value that was lower for 2008 than previous years, the overall distribution of both reservoirs combined (Figure 10a), was not consistently lower than previous years as was MSAE density (Figure 9a). Likewise, although distribution of microcystin toxin concentration tended to be lower in Iron Gate Reservoir than in Copco Reservoir in all years, the overall distribution in either reservoir for 2008 was not consistently lower than previous years (Figure 10b).

The observed inter-annual trend of lower 2008 MSAE density without a similar trend observed for microcystin is consistent with higher toxin to cell ratio noted for 2008 as well as several values of zero MSAE even when microcystin was detected at relatively high levels. Numerous environmental factors can influence both microcystin cell quota (e.g., temperature and/or genetic shifts in MSAE strain composition) as well as inter-annual bloom dynamics (Moisander et al 2009a and 2009b), and although beyond the scope of this technical memo, a brief look at two factors (temperature and wind), known to influence year-to-year cyanobacterial bloom dynamics shows that a partial explanation of lower MSAE density in 2008 may be due to somewhat lower air temperatures and higher wind speeds (used here as potential indicators of water column warming and mixing) in July and August of 2008 (Figure 11).

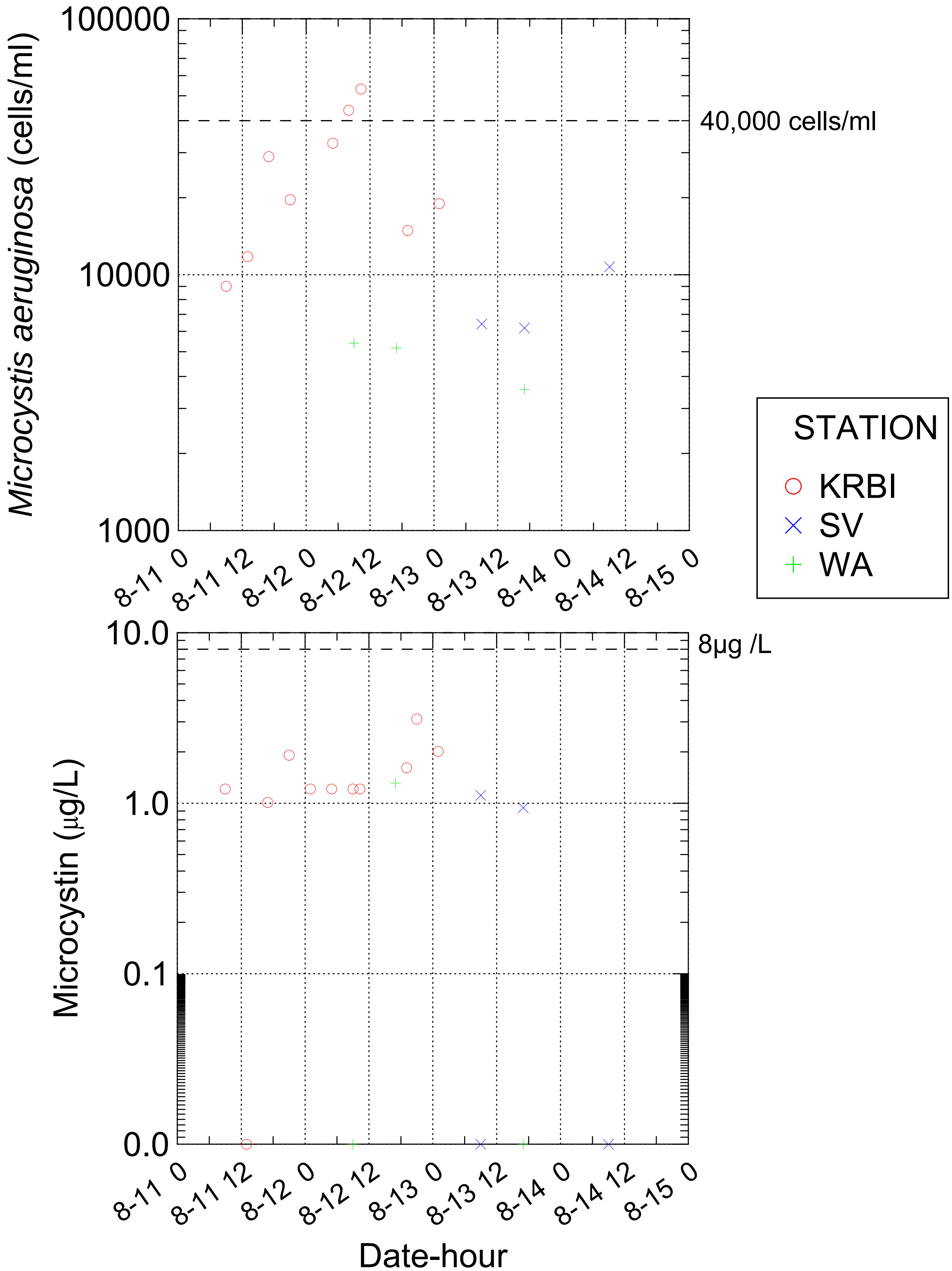

Figure 8. Diel trends in MSAE cell density (a) and microcystin toxin concentration (b) for Klamath River stations, August 11-14, 2008.

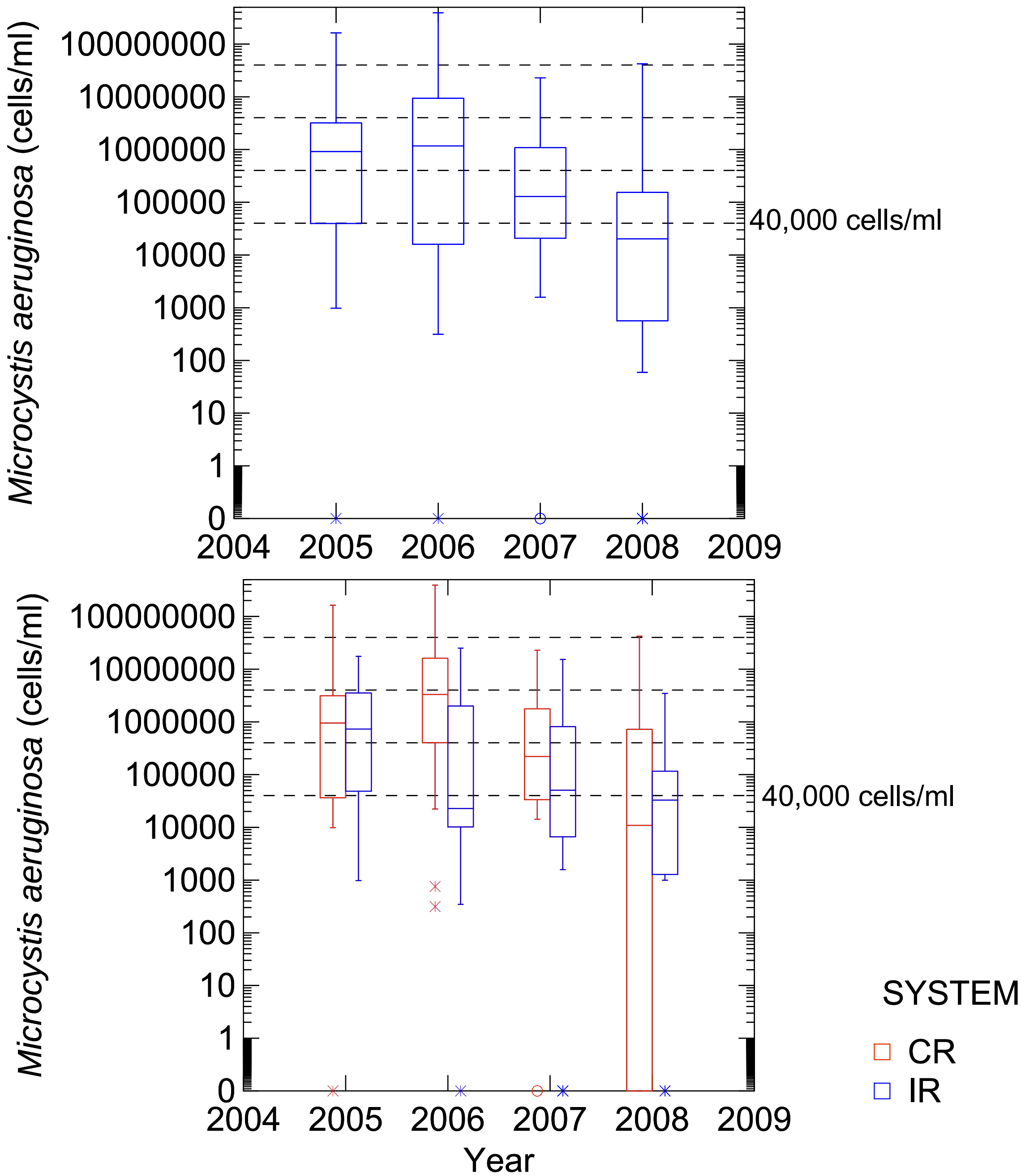

Figure 9. Inter-annual trends in MSAE cell density for standard reservoir stations during July-October for both reservoirs combined (a) and Copco and Iron Gate Reservoirs individually (b), 2005-2008.

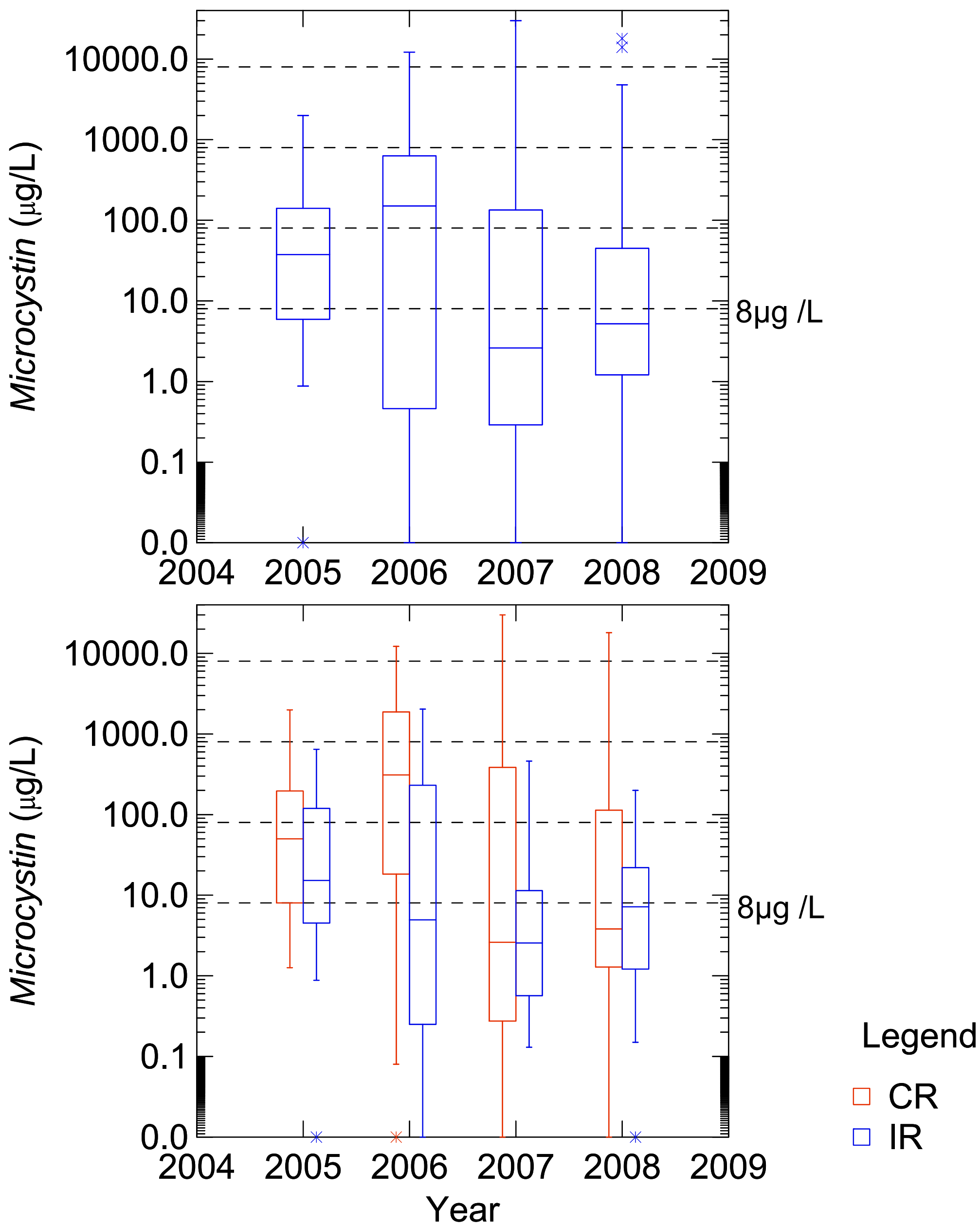

Figure 10. Inter-annual trends in microcystin concentration for standard reservoir stations during July-October for both reservoirs combined (a) and Copco and Iron Gate Reservoirs individually (b), 2005-2008.

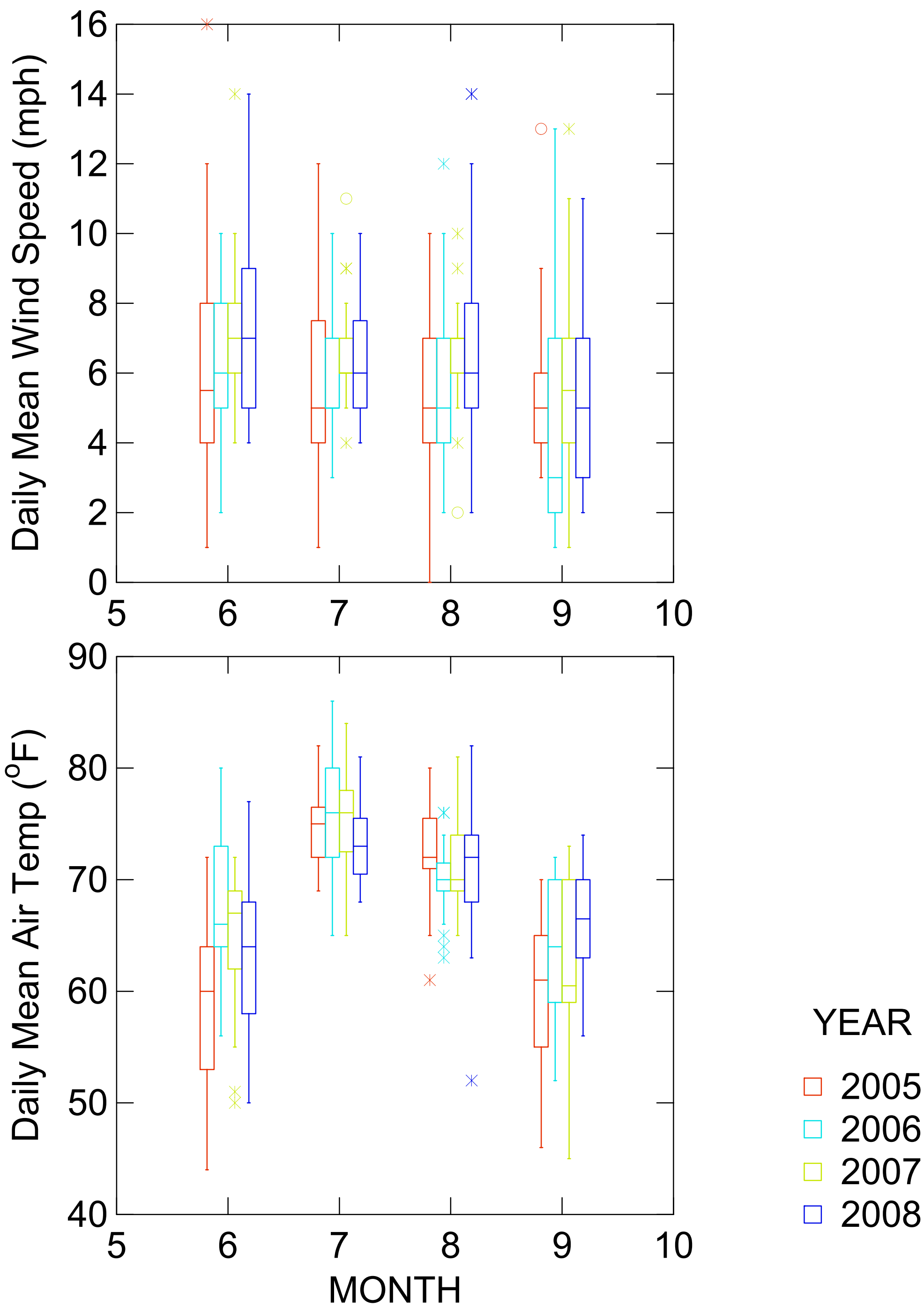

Figure 11. Indication of regional mean daily air temperature and wind speed. 2005-2008. Data obtained for meteorological station KSIY located in the town of Montague, Siskiyou County, California: downloaded from: http://www.wunderground.com/history/airport/KSIY/2009/4/23/CustomHistory.html

In addition, field crews noted particularly windy and choppy conditions during many of the sampling events in 2008, particularly at CR01. Such mixed conditions would also explain the consistently lower MSAE and microcystin at CR01 relative to other stations (Figure 5).

MSAE Cell Density-Microcystin Concentration Relationships

Although the relationship between MSAE cell density and microcystin toxin is variable (particularly when low or zero levels of MSAE are associated with high microcystin levels or with changing microcystin to cell ratios noted above), similar to plots produced for past years (Kann and Corum 2006; 2007) a scatter plot of 2008 data fitted with a distance weighted least squares smoother (DWLS) shows a general increasing trend of toxin concentration with cell density (Figure 12). As noted above, data points along the y-axis depict instances of microcystin detection when MSAE cells were not detected.

The 2008 relationship of MSAE cell density vs. microcystin relative to public health thresholds continues to indicate that the majority of 8 µg/L microcystin exceedances occurred at MSAE levels greater than 40,000 cells/ml (upper right quadrant; Figure 12). As noted above in Figure 6, the Klamath River stations exceeding the threshold values were those collected near the river-edge (green circles in upper right quadrant; Figure 12). However, there were also several exceedances of 8 µg/L for the reservoir stations when MSAE cell density was less than 40,000 cells/ml (upper left quadrant: Figure 12). This is consistent with expectations based upon variable toxin production and presence of non-cell bound toxin as described above. These relationships continue to indicate that generally (but not always) the SWRCB/OEHHA guideline value for MSAE density is protective of the 8 µg/L microcystin moderate probability of adverse health effect threshold.

Further analysis of the relationship among MSAE cell density, microcystin concentration, and chlorophyll a (CHL) was performed using the World Health Organization low probability of adverse health effect guideline values of 20,000 cells/ml MSAE and 4 µg/L microcystin. Whereas the SWRCB/OEHHA guidelines are considered to protect against a moderate probability of adverse effects, this lower threshold is utilized by the CA North Coast Regional Water Quality Control Board (NCRWQCB) for Total Maximum Daily Load calculations that are expected to further reduce the probability that adverse levels could pose a public health issue.

The Klamath River TMDL CHL target of 10 µg/L was established based on the framework described in "*Technical Approach To Develop Nutrient Numeric Endpoints for California*" (CA NNE) (Tetra Tech 2006) and is the guidance recommended by the California State Water Resources Control Board Planning Standards & Implementation Unit. Although the 10 µg/L CHL level in the CA NNE is the boundary condition for impairment, since the Klamath is a naturally productive and somewhat eutrophic system, the target value of 10 µg/L CHL was deemed appropriate (Tetra Tech 2008). Moreover, previous work by Walker (1985) and Downing et al. (2001) demonstrates that the probability of nuisance algal blooms and a shift to blue-green algal biomass increases rapidly beyond 10 µg/L CHL. Thus, within the Klamath basin the margin of error is small for reducing the probability of nuisance algal blooms dominated by blue-green algae. The below analyses provides site-specific confirmation for the selection of the CA NNE CHL target of 10 µg/L.

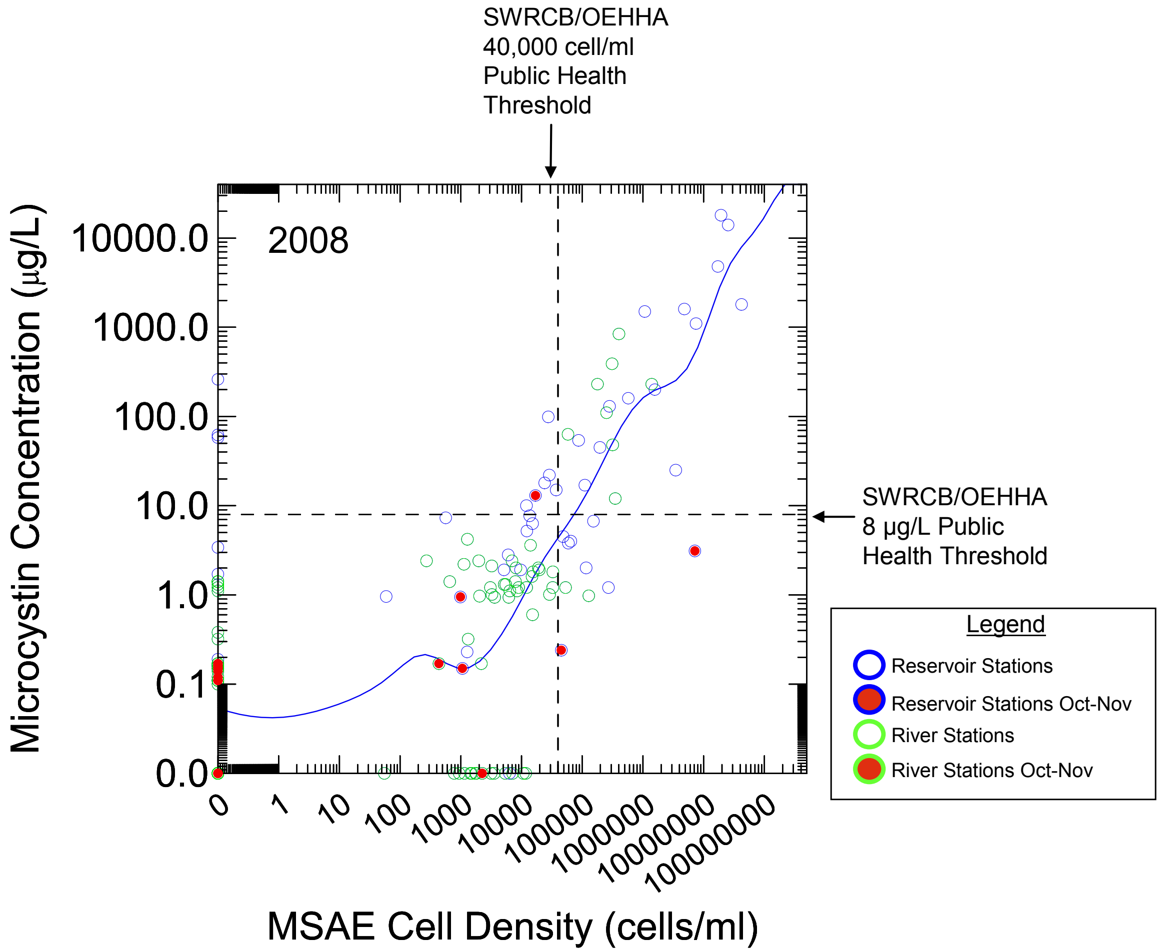

Figure 12. Relationship between MSAE cell density and microcystin toxin concentration; shown with distance weighted least squares (DWLS) smoother applied to all data, 2008.

Scatter plots for the 2005-2007 (this period was utilized because paired CHL data were available) relationships between MSAE cell density and microcystin concentration, CHL and MSAE cell density, and CHL and microcystin concentration are shown in Figure 13. Although there tended to be a greater number of microcystin exceedances at low MSAE cell density in 2008, the 2005-2007 relationship shows relatively few exceedances of the 4 µg/L microcystin threshold when MSAE density was less than 20,000 cells/ml (Figure 13a). Also note the Oct-Nov decrease in toxin production for those years (solid red circles; Figure 13a) that was not apparent in 2008.

The relationships between CHL and either MSAE cell density or microcystin (Figure 13b,c) showed greater variability than the MSAE vs. microcystin relationship (Figure 13a). However, given that CHL is a measure of all algae present and not only MSAE, such increased variability is not unexpected. Moreover, CHL indicates active photosynthesis and would not account for algal cells that may be in an older physiological state, yet such cells would still be enumerated microscopically. This is the likely cause of the relatively low CHL values associated with high (>1 million cells/ml) MSAE in Oct-Nov (solid red circles: Figure 13b). Nonetheless, the relationships still indicated linear trends between CHL and both MSAE cell density and microcystin toxin. Thus, high CHL tended to be associated with increased bloom density of MSAE as well as increased microcystin concentration. These relationships also indicate that there were few exceedances of the WHO low probability of adverse health effects levels of 20,000 cells/ml MSAE or 4 μg/L microcystin at the NCRWQCB 10 μg/L CHL level (Figure 13a,b).

Given variability in the various relationships shown in Figure 13, an alternative nonparametric cross-tabulation probability approach outlined by Kann and Smith (1999) was utilized to assess public health exceedance probabilities. Methodology for this approach is described in Kann and Smith (1999) and involves computation of exceedance probabilities for a chosen threshold value (in this case the focus was on either 20,000 cells/ml MSAE or 4 μg/L microcystin) within ordered groups of an independent variable (in this case either MSAE cell density or chlorophyll *a*). Here, the probability (frequency) of exceeding MSAE cell density or microcystin concentration was then plotted against the median value for MSAE cell density or CHL for each ordered group.

Probability models computed for all stations Jun-Sep, reservoir-only Jun-Sep data, all stations Jun-Aug, and reservoir-only Jun-Aug show an increasing trend in the probability of exceeding the various WHO microcystin levels as MSAE cell density increases (Figure 14a,b,c,d). For example, at an MSAE cell density level of 20,000 cells/ml that there was a ~35% and 50% probability of exceeding 4 μg/L microcystin for the all station Jun-Sep and Jun-Aug models, respectively (Figure 14a,b). Exceedance probabilities for the reservoir-only models (Figure 14c,d) were very similar to those using all reservoir and river stations (Figure 14a,b). Because reservoir microcystin toxin to MSAE cell density ratio as well as absolute values are typically higher earlier in the season (e.g., July and August), the Jun-Aug models show a greater frequency of exceedance, thus providing a more conservative indication of risk with respect to public health. Likewise, the SWRCB/OEHHA public health guidance level of 8 μg/L had 15% and 20% exceedance probabilities at an MSAE cell density of 20,000 cells/ml, and ~25% and ~40% at an MSAE cell density of 40,000 cells/ml for the Jun-Sep and Jun-Aug models. Although cell density and toxin are clearly highly related, the inherent variability in the cell density-toxin relationship (e.g., low cell density can be associated with high toxin when a greater proportion of toxin is in an aqueous form) translates to public health exceedances for microcystin at MSAE cell densities lower than designated thresholds.

As expected based on scatter plot relationships (Figure 13b,c), probability models using CHL as the independent variable also show an increasing trend in the probability of exceeding the various WHO microcystin and MSAE cell density levels as CHL increases (Figure 15a,b; note that lower sample size for CHL data precluded model development for the Jun-Aug only period). These plots show that there was ~30% and ~25% probability of exceeding 20,000 cells/ml MSAE and 4 µg/L microcystin at a CHL level of 10 µg/L, and that exceedance probabilities for the SWRCB/OEHHA public health guidance levels of 20,000 cells/ml and 8 μg/L were both ~15% (Figure 15a,b).

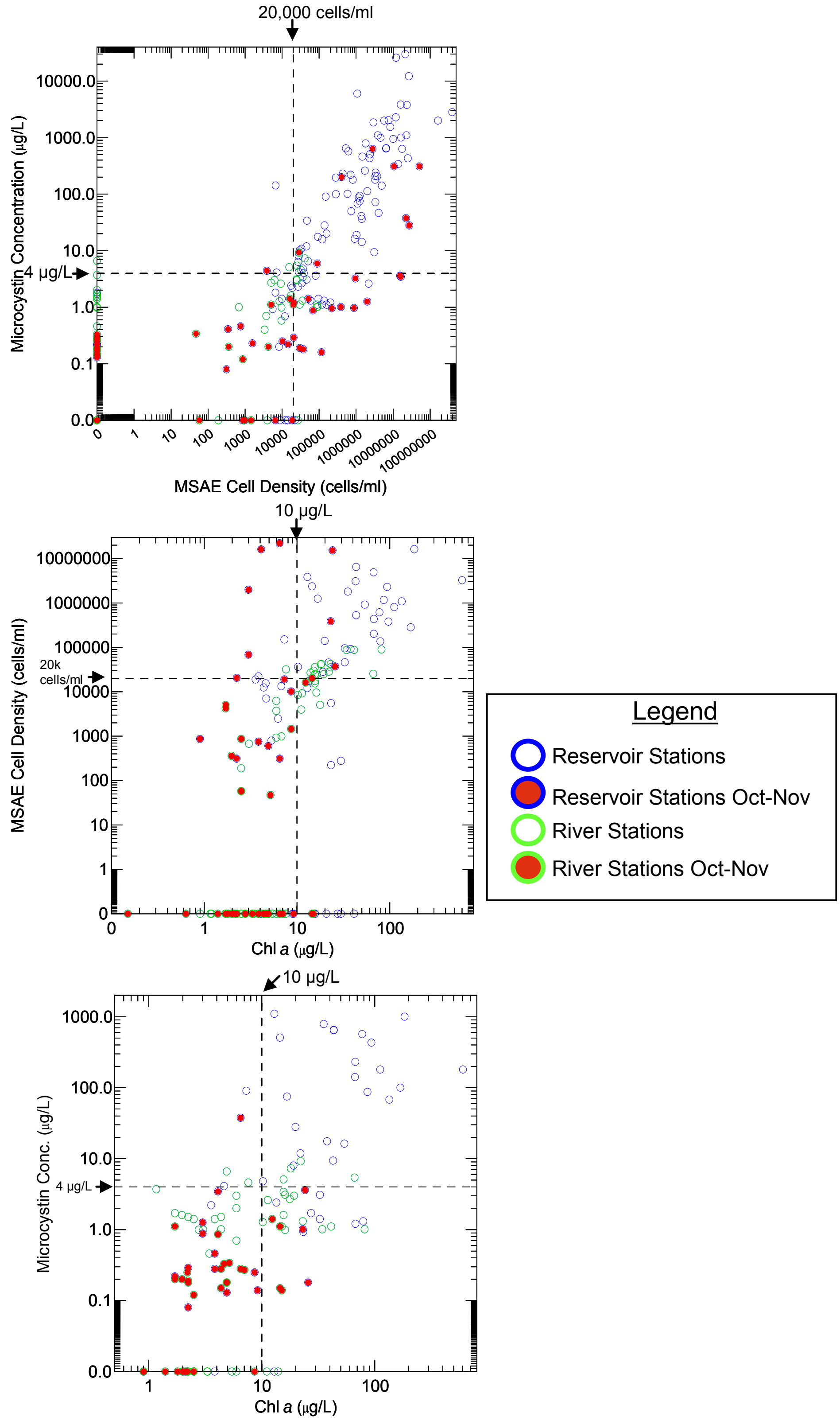

Figure 13. Relationship between MSAE cell density and microcystin toxin concentration (a); between chlorophyll a concentration and MSAE cell density (b); and between chlorophyll a concentration and microcystin toxin concentration (c), 2005-2007.

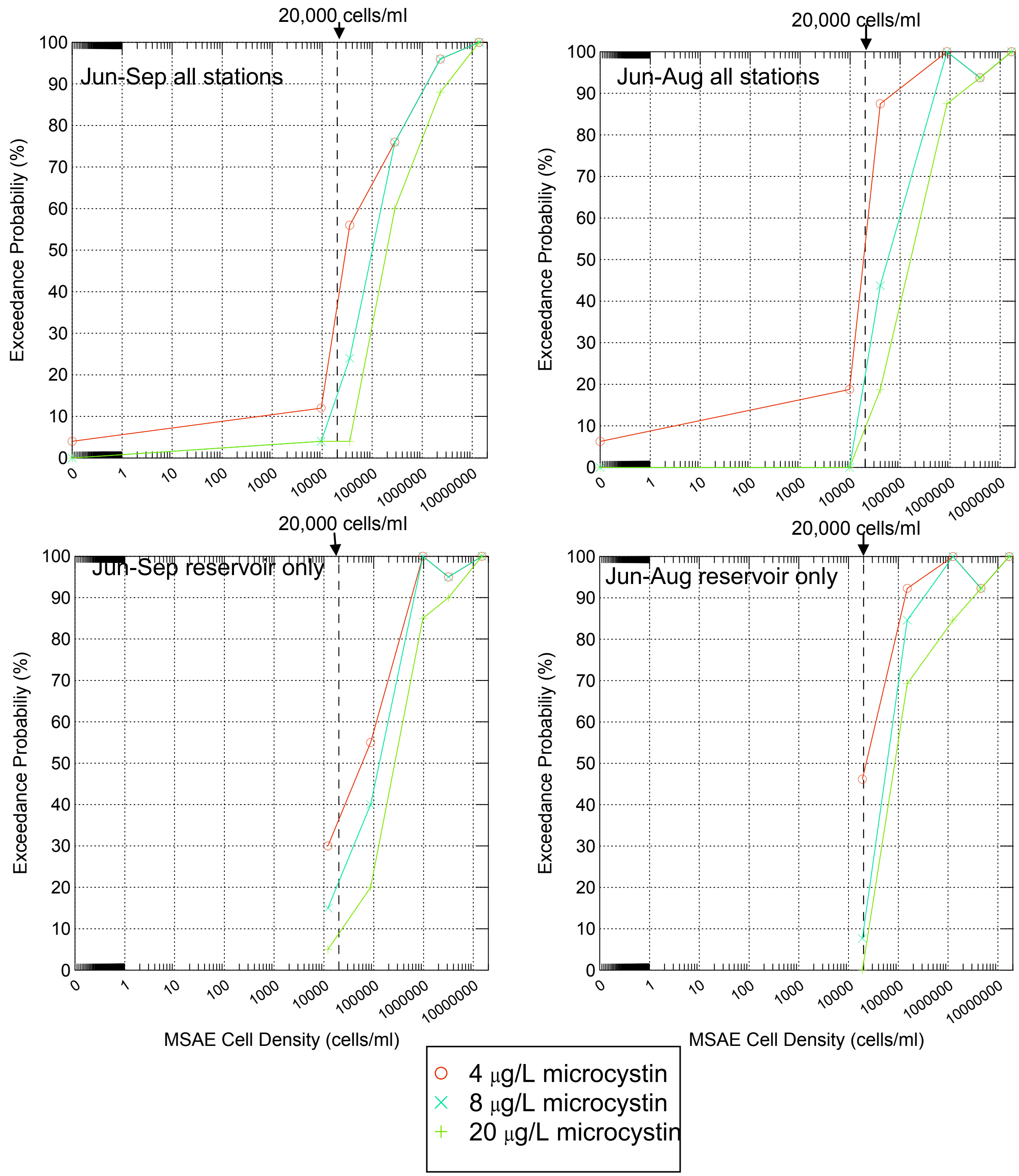

Figure 14. Probability of exceeding various WHO public health microcystin toxin levels at varying MSAE cell density for all stations Jun-Sep (a), all stations Jun-Aug (b), reservoir-only Jun-Sep (c), and reservoir-only Jun-Aug (d) in Copco and Iron Gate Reservoirs and the Klamath River, 2005-2007. Exceedance probability is computed using nonparametric cross-tabulation method described in Kann and Smith (1999).

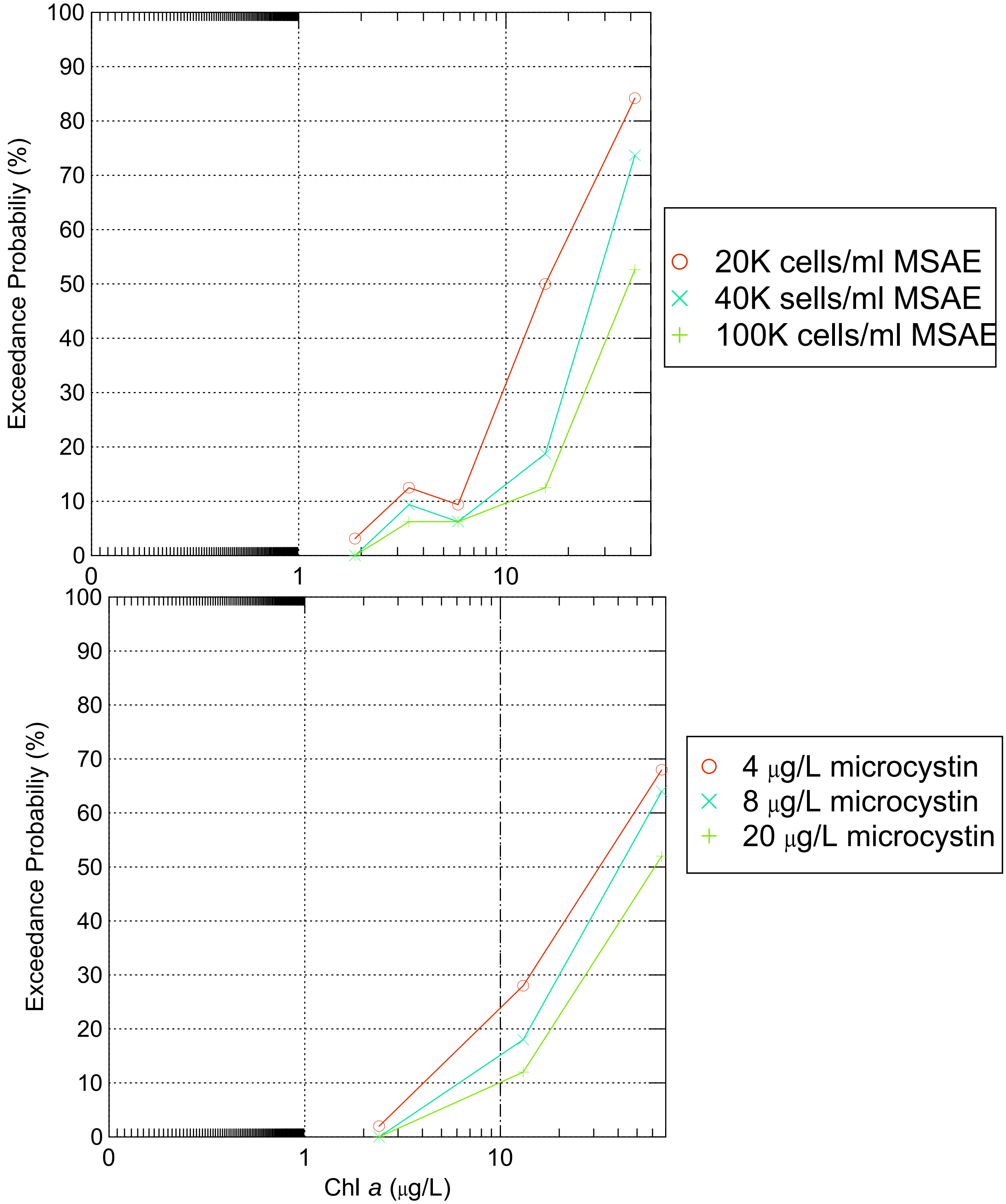

Figure 15. Probability of exceeding various WHO public health MSAE cell density levels at varying CHL concentration (a), and probability of exceeding various WHO public health microcystin toxin levels at varying CHL concentration (b) in Copco and Iron Gate Reservoirs and the Klamath River, 2005-2007. Exceedance probability is computed using nonparametric cross-tabulation method described in Kann and Smith (1999).

By demonstrating increasing trends in response variables with either increasing CHL or MSAE cell density, the above relationships provide a robust basis for evaluation of public health guidance values for toxic cyanobacteria in the Klamath River system. As noted above, the probability models show exceedances of guideline levels below either the NCRWQCB/TMDL or SWRCB/OEHHA thresholds. However, plotted probabilities represent an interval around the median of the independent variable and thus include values above and below any chosen value.

An analysis of exceedances directly above or below a given threshold (in other words the trend is not being evaluated over the entire range) shows that when CHL was less than 10 µg/L that the exceedance frequencies of the public health thresholds for MSAE density or microcystin concentration were less than 10% (Table 3).

Likewise, when MSAE cell density was less than 20,000 cells/ml, maximum exceedance frequencies were 14.3% and 7,1% for 4 µg/L and 8 µg/L microcystin.. Frequency of exceedance for 8 µg/L microcystin when MSAE cell density was below 40,000 cells per ml was 16.7% (Jun-Sep) and 7.7% Jun-Aug (Table 3). The higher frequency for the computation period that includes September is likely due to a tendency towards increased aqueous vs. cell-bound toxin during the fall months.

SUMMARY

Although somewhat lower than 2005-2007, the 2008 sampling program demonstrated widespread and high abundance of toxigenic MSAE blooms in Copco and Iron Gate reservoirs from July-September, continuing to exceeded public health thresholds by numerous times during these months. During the first full biweekly sampling on 7/23 none of the standard sampling stations (CR01, CRMC, CRCC, IR01, IRCC, and IRJW) exceeded 40,000 cells/ml, and only IRCC exceeded 8 µg/L. However, other stations specifically sampled to assess areas that appeared more concentrated showed much higher levels, exceeding public health guideline values by 10 to over 100 times. Such patchy distributions underscore the need to supplement standard sampling stations, especially prior to initial public health postings.

With the exception of the open-water station CR01, which remained low through the season, overall MSAE and microcystin levels continued to increase in early and mid August, before undergoing a slight decline in early September During this period the relative decline in MSAE was greater than that for microcystin, especially for Copco Reservoir. Of the four years of study (2005-2008), the ratio of toxin produced per unit MSAE in 2008 ranked among the highest for both July and August, with an increased ratio occurring in Copco Reservoir relative to Iron Gate Reservoir in September. In addition, although median and overall distribution of MSAE cell density in both reservoirs in 2008 was lower than the previous three years, the overall distribution of microcystin concentration was not consistently lower than previous years in either Copco or Iron Gate Reservoir.

Table 3. Percent exceedance for MSAE cell densities and microcystin toxin concentrations at threshold chlorophyll a of 10 µg/L, and percent exceedance for microcystin toxin concentrations at threshold MSAE cell density of 20,000 cells/ml; Klamath River, California 2005-2007.

	MSAE cell density percent exceedance for Chl<10 µg/L			MSAE cell density percent exceedance for Chl≥10 µg/L		
	20,000 cells/ml	40,000 cells/ml	100,000 cells/ml	20,000 cells/ml	40,000 cells/ml	100,000 cells/ml
all stations all months	8.2	5.2	4.1	69.6	49.3	34.8
reservoirs only; Jun-Aug	7.1	7.1	7.1	66.7	59.3	55.6
	Microcystin conc. percent exceedance for Chl<10 µg/L			Microcystin conc. percent exceedance for Chl≥10 µg/L		
	4 µg/L	8 µg/L	20 µg/L	4 µg/L	8 µg/L	20 µg/L
all stations all months	7.4	2.9	2.9	47.4	40.4	29.8
reservoirs only; Jun-Aug	insufficient sample size			89.5	84.2	68.4
	Microcystin conc. percent exceedance for MSAE<20,000 cells/ml			Microcystin conc. percent exceedance for MSAE≥20,000 cells/ml		
	4 µg/L	8 µg/L	20 µg/L	4 µg/L	8 µg/L	20 µg/L
all stations; Jun-Sep	7.6	1.3	1.3	78.5	70.1	58.9
all stations; Jun-Aug	10.4	0.0	0.0	88.6	75.7	65.7
reservoirs only; Jun-Sep	14.3	7.1	7.1	86.7	81.1	70.0
reservoirs only; Jun-Aug	14.3	0.0	0.0	94.9	86.4	78.0
	Microcystin conc. percent exceedance for MSAE<40,000 cells/ml			Microcystin conc. percent exceedance for MSAE≥40,000 cells/ml		
reservoirs only; Jun-Sep	37.5	16.7	4.2	88.8	87.5	78.8
reservoirs only; Jun-Aug	46.2	7.7	0.0	96.2	94.3	86.8

Similar to previous years MSAE was not detected and microcystin was detected at low levels upstream of the reservoirs at KRAC. Downstream of the reservoir complex at station KRBI and below to Orleans (OR), levels of both MSAE and toxin were elevated relative to KRAC; however, none of these river samples taken in the mixed portion of the channel exceeded the threshold guideline values of 40,000 cells/ml MSAE or 8 µg/L microcystin in 2008. This was in direct contrast to surface samples taken in areas of low velocity in Klamath River edge habitat that showed MSAE cell density and microcystin concentration exceeded (often by over 10x) the 40,000 cells/ml MSAE and 8 µg/L microcystin public health guideline values, even when open-water samples did not. From a public health perspective these data illustrate that low MSAE or toxin values in open-water higher velocity Klamath River samples often translates to values exceeding public health thresholds in river-edge areas. In addition, diel MSAE and toxin samples indicated that MSAE density and microcystin toxin concentration generally showed low variability on a diel basis; however, there were two instances when MSAE density exceeded the public health threshold, even when other diel samples did not. The increased spatial and temporal sampling resolution for the Klamath River stations illustrates the possibility of false negatives (with respect to public health thresholds) inherent in the standard biweekly open-water sampling program carried out over the past 4 years.

Similar to previous years, 2008 data showed a general increasing trend of toxin concentration with cell density, and continued to indicate that the majority of 8 µg/L microcystin exceedances occurred at MSAE levels greater than 40,000 cells/ml indicating that the SWRCB/OEHHA guideline value for MSAE density is protective of the 8 µg/L public health level. Further analyses utilizing WHO low probability of adverse health effect guideline values of 20,000 cells/ml MSAE and 4 µg/L microcystin (as presented in the Klamath River TMDL) as the dependant variables and either MSAE cell density or CHL as the independent variables indicated that there were few exceedances of the WHO low probability of adverse health effects level of 4 µg/L microcystin at 20,000 cells/ml MSAE or at the NCRWQCB TMDL 10 µg/L CHL level. Moreover, high CHL tended to be associated with not only increased microcystin concentration but also with increased bloom density of MSAE.

Nonparametric models confirmed an increasing trend in the probability of exceeding the various WHO microcystin levels as both MSAE cell density and CHL increased. For example, at an MSAE cell density level of 20,000 cells/ml that there was a ~35% and 50% probability of exceeding 4 µg/L microcystin for the all station Jun-Sep and Jun-Aug models, respectively. Higher early-season absolute values of microcystin as well as higher toxin to MSAE cell density ratios thus provide a more conservative indication of risk with respect to public health. Probability models using CHL as the independent variable also showed an increasing trend in the probability of exceeding the various WHO microcystin and MSAE cell density levels as CHL increased, with a ~30% and ~25% probability of exceeding 20,000 cells/ml MSAE and 4 µg/L microcystin at a CHL level of 10 µg/L. Further analysis when CHL was below the given 10 µg/L threshold showed that of exceedances of public health thresholds for MSAE density or microcystin concentration were less than 10%, providing site-specific confirmation for the selection of the CA NNE CHL target of 10 µg/L.

Disclaimer

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

Acknowledgements

Funding for this project for algae speciation has been provided in part through a contract with the State Water Resources Control Board (State Water Board). The contents of this document do not necessarily reflect the views and policies of the State Water Board, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. Funding for this project was also provided by EPA Region IX and the Klamath Blue Green Algae Workgroup.

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Appendix I.

***Microcystis aeruginosa* cell density, microcystin toxin concentration, and risk exceedance for toxigenic cyanobacteria in Copco and Iron Gate Reservoirs, 2008. Blank cells indicate no sample taken; zero values indicate below detection (microcystin) or not detected (algae).**

DATE	STATION NAME	Station Description	DEPTH	*Microcystis aeruginosa* (cells/ml)	*Anabaena sp.* (cells/ml)	Microcystin Total (µg/L)	Exceedance of SWRCB[1] risk level of 40,000 cells/ml *Microcystis or Planktothrix* (x greater than 4[5] cells/ml)	Exceedance of microcystin TDI of 0.04 µg/kg/day for a 20kg (44lb) child ingesting 100 mls[2] (x greater than TDI)
5/14/2008	KRBI	Below Iron Gate	0C[3]	0	0		0.0	
5/14/2008	OR	Orleans	0C	0	0		0.0	
5/14/2008	SA	Mouth of Salmon River	0C	0	0		0.0	
5/14/2008	SC	Mouth of Scott River	0C	0	0		0.0	
5/14/2008	SH	Mouth of Shasta River	0C	0	0		0.0	
5/14/2008	SV	Seaid Valley	0C	0	0		0.0	
5/22/2008	KRBI	Below Iron Gate	0C	0	0		0.0	
5/22/2008	OR	Orleans	0C	0	0		0.0	
5/22/2008	SV	Seaid Valley	0C	0	0		0.0	
5/22/2008	WA	Walker Bridge	0C	0	0		0.0	
5/28/2008	KRBI	Below Iron Gate	0C	0	0		0.0	
5/28/2008	OR	Orleans	0C	0	0		0.0	
5/28/2008	SA	Mouth of Salmon River	0C	0	0		0.0	
5/28/2008	SC	Mouth of Scott River	0C	0	0		0.0	
5/28/2008	SH	Mouth of Shasta River	0C	0	0		0.0	
5/28/2008	SV	Seaid Valley	0C	0	0		0.0	
5/28/2008	WA	Walker Bridge	0C	0	0		0.0	
6/5/2008	KRBI	Below Iron Gate	0C	0	0		0.0	
6/5/2008	OR	Orleans	0C	0	0		0.0	
6/5/2008	SV	Seaid Valley	0C	0	0		0.0	
6/5/2008	WA	Walker Bridge	0C	0	0		0.0	

DATE	STATION NAME	Station Description	DEPTH	*Microcystis aeruginosa* (cells/ml)	*Anabaena sp.* (cells/ml)	Microcystin Total (µg/L)	Exceedance of SWRCB[1] risk level of 40,000 cells/ml *Microcystis or Planktothrix* (x greater than 4[5] cells/ml)	Exceedance of microcystin TDI of 0.04 µg/kg/day for a 20kg (44lb) child ingesting 100 mls[2] (x greater than TDI)
6/11/2008	KRBI	Below Iron Gate	0C	0	0	0	0.0	0.0
6/11/2008	OR	Orleans	0C	0	0	0	0.0	0.0
6/11/2008	SA	Mouth of Salmon River	0C	0	0		0.0	
6/11/2008	SC	Mouth of Scott River	0C	0	0		0.0	
6/11/2008	SH	Mouth of Shasta River	0C	0	0	0	0.0	0.0
6/11/2008	SV	Seaid Valley	0C	0	0	0	0.0	0.0
6/11/2008	WA	Walker Bridge	0C	0	0	0	0.0	0.0
6/18/2008	KRBI	Below Iron Gate	0C	0	0		0.0	
6/18/2008	OR	Orleans	0C	0	0		0.0	
6/18/2008	SV	Seaid Valley	0C	0	0		0.0	
6/18/2008	WA	Walker Bridge	0C	0	0		0.0	
6/25/2008	KRBI	Below Iron Gate	0C	0	0	0	0.0	0.0
6/25/2008	OR	Orleans	0C	0	0	0	0.0	0.0
6/25/2008	SA	Mouth of Salmon River	0C	0	0		0.0	
6/25/2008	SC	Mouth of Scott River	0C	0	0		0.0	
6/25/2008	SH	Mouth of Shasta River	0C	0	0		0.0	
6/25/2008	SV	Seaid Valley	0C	0	0	0	0.0	0.0
6/25/2008	WA	Walker Bridge	0C	0	0	0	0.0	0.0
7/2/2008	CRCC	Copco Reservoir Copco Cove	SG[4]	0	48,304	58	0.0	7.3
7/2/2008	CRMC	Copco Reservoir Mallard Cove	SG	0	0	0	0.0	0.0
7/2/2008	KRBI	Below Iron Gate	0C	0	0		0.0	
7/2/2008	OR	Orleans	0C	0	0		0.0	
7/2/2008	SV	Seaid Valley	0C	0	0		0.0	
7/2/2008	WA	Walker Bridge	0C	0	0		0.0	
7/8/2008	CRCC	Copco Reservoir Copco Cove	SG	59,342	0	3.8	1.5	0.5

DATE	STATION NAME	Station Description	DEPTH	*Microcystis aeruginosa* (cells/ml)	*Anabaena sp.* (cells/ml)	Microcystin Total (µg/L)	Exceedance of SWRCB[1] risk level of 40,000 cells/ml *Microcystis or Planktothrix* (x greater than 4^5 cells/ml)	Exceedance of microcystin TDI of 0.04 µg/kg/day for a 20kg (44lb) child ingesting 100 mls[2] (x greater than TDI)
7/8/2008	IRJW	Iron Gate Jay Williams Boat Dock	SG	0	0	0	0.0	0.0
7/10/2008	KRBI	Below Iron Gate	0C	0	0	1.4	0.0	0.2
7/10/2008	OR	Orleans	0C	0	0	0	0.0	0.0
7/10/2008	SA	Mouth of Salmon River	0C	0	0		0.0	
7/10/2008	SC	Mouth of Scott River	0C	0	0		0.0	
7/10/2008	SH	Mouth of Shasta River	0C	0	0		0.0	
7/10/2008	SV	Seaid Valley	0C	0	0	0	0.0	0.0
7/10/2008	WA	Walker Bridge	0C	0	0	0	0.0	0.0
7/17/2008	KRBI	Below Iron Gate	0C	0	0		0.0	
7/17/2008	OR	Orleans	0C	0	0		0.0	
7/17/2008	SV	Seaid Valley	0C	0	0		0.0	
7/17/2008	WA	Walker Bridge	0C	0	69		0.0	
7/23/2008	CR01	Copco Open Water near Dam	0C	9,664	0	1.9	0.2	0.2
7/23/2008	CRCC	Copco Reservoir Copco Cove	SG	5,154	0	1.9	0.1	0.2
7/23/2008	CRMC	Copco Reservoir Mallard Cove	SG	0	0	1.7	0.0	0.2
7/23/2008	CRSC	Copco Open Water Scum	SG	7,501,327	0	1100	187.5	137.5
7/23/2008	IR01	Iron Gate Open Water near Dam	SG	13,615	19	7.7	0.3	1.0
7/23/2008	IRCC	Iron Gate Camp Creek Rec. Area	SG	37,338	708	15	0.9	1.9
7/23/2008	IRJW	Iron Gate Jay Williams Boat Dock	SG	6,040	369	2.8	0.2	0.4
7/23/2008	IRNW	Iron Gate Narrows	SG	1,072,938	0	1500	26.8	187.5
7/23/2008	IRSC	Iron Gate Open Water Scum	SG	574,804	0	160	14.4	20.0
7/23/2008	KRBI	Below Iron Gate	0C	3,073	0	1.2	0.1	0.2
7/23/2008	OR	Orleans	0C	0	0	1.1	0.0	0.1

DATE	STATION NAME	Station Description	DEPTH	*Microcystis aeruginosa* (cells/ml)	*Anabaena sp.* (cells/ml)	Microcystin Total (µg/L)	Exceedance of SWRCB[1] risk level of 40,000 cells/ml *Microcystis or Planktothrix* (x greater than 4[5] cells/ml)	Exceedance of microcystin TDI of 0.04 µg/kg/day for a 20kg (44lb) child ingesting 100 mls[2] (x greater than TDI)
7/23/2008	SA	Mouth of Salmon River	0C	656	0	1.4	0.0	0.2
7/23/2008	SC	Mouth of Scott River	0C	0	0	0	0.0	0.0
7/23/2008	SH	Mouth of Shasta River	0C	0	0	1.3	0.0	0.2
7/23/2008	SV	Seaid Valley	0C	0	0	1.2	0.0	0.2
7/23/2008	WA	Walker Bridge	0C	0	0	1.4	0.0	0.2
7/24/2008	KRAC	Above Copco	0C	0	0	1.4	0.0	0.2
7/31/2008	KRBI	Below Iron Gate	0C	12,103	52	1.2	0.3	0.2
7/31/2008	OR	Orleans	0C	0	0	0	0.0	0.0
7/31/2008	SV	Seaid Valley	0C	942	0	0	0.0	0.0
7/31/2008	WA	Walker Bridge	0C	1,128	0	2.2	0.0	0.3
8/4/2008	KRBI	Below Iron Gate	0C	1,974	206	2.4	0.0	0.3
8/4/2008	WA	Walker Bridge	0C	2,040	0	0.96	0.1	0.1
8/5/2008	CR01	Copco Open Water near Dam	SG	12,279	7,532	5.2	0.3	0.7
8/5/2008	CRCC	Copco Reservoir Copco Cove	SG	25,292,371	0	14000	632.3	1750.0
8/5/2008	CRMC	Copco Reservoir Mallard Cove	SG	564	952	7.3	0.0	0.9
8/5/2008	IR01	Iron Gate Open Water near Dam	SG	0	0	62	0.0	7.8
8/5/2008	IRCC	Iron Gate Camp Creek Rec. Area	SG	24,003	158	18	0.6	2.3
8/5/2008	IRJW	Iron Gate Jay Williams Boat Dock	SG	195,506	13,584	45	4.9	5.6
8/5/2008	IRSH	Iron Gate Spring Hill Boat Ramp	SG	4,804,077	0	1600	120.1	200.0
8/5/2008	KRAC	Above Copco	0C	0	0	0	0.0	0.0
8/5/2008	SH	Mouth of Shasta River	0C	0	94	0	0.0	0.0
8/6/2008	OR	Orleans	0C	783	0	0	0.0	0.0
8/6/2008	SA	Mouth of Salmon River	0C	0	0	0	0.0	0.0
8/6/2008	SC	Mouth of Scott River	0C	0	0	0	0.0	0.0

DATE	STATION NAME	Station Description	DEPTH	*Microcystis aeruginosa* (cells/ml)	*Anabaena sp.* (cells/ml)	Microcystin Total (µg/L)	Exceedance of SWRCB[1] risk level of 40,000 cells/ml *Microcystis or Planktothrix* (x greater than 4^5 cells/ml)	Exceedance of microcystin TDI of 0.04 µg/kg/day for a 20kg (44lb) child ingesting 100 mls[2] (x greater than TDI)
8/6/2008	SV	Seaid Valley	0C	0	0	0	0.0	0.0
8/10/2008	KRBI-0900	Below Iron Gate 0900 hrs	0C	9,013	0	1.2	0.2	0.2
8/10/2008	KRBI-1300	Below Iron Gate 1300 hrs	0C	11,753	4	0	0.3	0.0
8/10/2008	KRBI-1700	Below Iron Gate 1700 hrs	0C	28,903	33	1	0.7	0.1
8/10/2008	KRBI-2100	Below Iron Gate 2100 hrs	0C	19,614	108	1.9	0.5	0.2
8/11/2008	KRBI-0100	Below Iron Gate 0100 hrs	0C		0	1.2		0.2
8/11/2008	KRBI-0500	Below Iron Gate 0500 hrs	0C	32,629	27	1.2	0.8	0.2
8/11/2008	KRBI-0800	Below Iron Gate 0500 hrs		43,861	23		1.1	
8/11/2008	KRBI-0900	Below Iron Gate 0900 hrs			0	1.2		0.2
8/11/2008	KRBI-1030	Below Iron Gate 1030 hrs	0C	53,102	32	1.2	1.3	0.2
8/11/2008	KRBI-1910	Below Iron Gate 1910 hrs	0C	14,874	55	1.6	0.4	0.2
8/11/2008	KRBI-2100	Below Iron Gate 2100 hrs	0C		0	3.1		0.4
8/11/2008	WA-0900	Walker Bridge 0900 hrs	0C	5,397	0	0	0.1	0.0
8/11/2008	WA-1700	Walker Bridge 1700 hrs	0C	5,171	0	1.3	0.1	0.2
8/12/2008	KRBI-0100	Below Iron Gate 0100 hrs	0C	18,943	54	2	0.5	0.3
8/12/2008	SH	Mouth of Shasta River	0C	0	0	0	0.0	0.0
8/12/2008	SV-0900	Seiad Valley 0900 hrs	0C		0	0		0.0
8/12/2008	SV-1700	Seiad Valley 1700 hrs	0C	6,187	0	0.93	0.2	0.1
8/12/2008	WA-0900	Walker Bridge 0900 hrs	0C	6,417	0	1.1	0.2	0.1
8/12/2008	WA-1700	Walker Bridge 1700 hrs	0C	3,561	0	0	0.1	0.0
8/13/2008	SC	Mouth of Scott River	0C	0	0		0.0	
8/13/2008	SV	Seaid Valley	0C	7,119	0	0	0.2	0.0
8/13/2008	SV-0900	Seiad Valley 0900 hrs	0C	10,731	0	0	0.3	0.0
8/14/2008	OR-0835	Orleans 0835 hrs	0C	3,160	0	0	0.1	0.0
8/14/2008	SA	Mouth of Salmon River	0C	0	0	0	0.0	0.0

DATE	STATION NAME	Station Description	DEPTH	*Microcystis aeruginosa* (cells/ml)	*Anabaena sp.* (cells/ml)	Microcystin Total (µg/L)	Exceedance of SWRCB[1] risk level of 40,000 cells/ml *Microcystis or Planktothrix* (x greater than 4[5] cells/ml)	Exceedance of microcystin TDI of 0.04 µg/kg/day for a 20kg (44lb) child ingesting 100 mls[2] (x greater than TDI)
8/19/2008	CR01	Copco Open Water near Dam	SG	6,352	0	0	0.2	0.0
8/19/2008	CRCC	Copco Reservoir Copco Cove	SG	19,363,426	0	18000	484.1	2250.0
8/19/2008	CRMC	Copco Reservoir Mallard Cove	SG	1,845,631	0		46.1	
8/19/2008	IR01	Iron Gate Open Water near Dam	SG	28,740	0	22	0.7	2.8
8/19/2008	IRCC	Iron Gate Camp Creek Rec. Area	SG	1,575,134	0	200	39.4	25.0
8/19/2008	IRJW	Iron Gate Jay Williams Boat Dock	SG	153,424	6,932	6.7	3.8	0.8
8/19/2008	KRBI	Below Iron Gate	0C	32,805	0	1.8	0.8	0.2
8/19/2008	OR	Orleans	0C	1,514	0	0	0.0	0.0
8/19/2008	SA	Mouth of Salmon River	0C	0	0	0	0.0	0.0
8/19/2008	SC	Mouth of Scott River	0C	0	0	0	0.0	0.0
8/19/2008	SH	Mouth of Shasta River	0C	0	0	0	0.0	0.0
8/19/2008	SV	Seaid Valley	0C	1,450	44	0	0.0	0.0
8/19/2008	WA	Walker Bridge	0C	8,448	0	1.1	0.2	0.1
8/20/2008	KRAC	Above Copco	0C	0	0	0	0.0	0.0
8/21/2008	OR	Orleans	0C			1		0.1
8/27/2008	KRBI	Below Iron Gate	0C	6,968	0	2.4	0.2	0.3
8/27/2008	KRBI	Below Iron Gate	SG	1,394,139	6,461	230	34.9	28.8
8/27/2008	OR	Orleans	0C	5,773	0		0.1	
8/27/2008	SV	Seaid Valley	0C	15,575	0	1.8	0.4	0.2
8/27/2008	WA	Walker Bridge	0C	14,125	0	3.6	0.4	0.5
9/2/2008	BRBE	River edge at Brown Bear River Access	SG	401,672	0	840	10.0	105.0
9/2/2008	BVFI	Beaver Creek Fish Disease Site	SG	251,305	0	110	6.3	13.8
9/2/2008	CR01	Copco Open Water near Dam	SG	0	0	3.4	0.0	0.4

DATE	STATION NAME	Station Description	DEPTH	*Microcystis aeruginosa* (cells/ml)	*Anabaena sp.* (cells/ml)	Microcystin Total (µg/L)	Exceedance of SWRCB[1] risk level of 40,000 cells/ml *Microcystis or Planktothrix* (x greater than 4^5 cells/ml)	Exceedance of microcystin TDI of 0.04 µg/kg/day for a 20kg (44lb) child ingesting 100 mls[2] (x greater than TDI)
9/2/2008	CRCC	Copco Reservoir Copco Cove	0C	281,875	0	130	7.0	16.3
9/2/2008	IR01	Iron Gate Open Water near Dam	SG	3,475,206	0	25	86.9	3.1
9/2/2008	IRCC	Iron Gate Camp Creek Rec. Area	SG	116,130	0	2	2.9	0.3
9/2/2008	IRJW	Iron Gate Jay Williams Boat Dock	SG	12,080	4,953	10	0.3	1.3
9/2/2008	KRBI	Below Iron Gate	0C	8,116	0	2	0.2	0.3
9/2/2008	KRBI	Below Iron Gate	SG	349,672	1,474	12	8.7	1.5
9/2/2008	OR	Orleans	0C	3,237	0	2.1	0.1	0.3
9/2/2008	SA	Mouth of Salmon River	0C	0	0	0	0.0	0.0
9/2/2008	SC	Mouth of Scott River	0C	0	0	0	0.0	0.0
9/2/2008	SV	Seaid Valley	0C	1,284	0	4.2	0.0	0.5
9/2/2008	SVFD	Siead Valley Fish Disease Site	SG	178,547	0	230	4.5	28.8
9/2/2008	SVMN	Mining claim river access upstream of Seiad (town)	SG		0	63		7.9
9/2/2008	WA	Walker Bridge	0C	272	0	2.4	0.0	0.3
9/3/2008	CRMC	Copco Reservoir Mallard Cove	SG	0	0	260	0.0	32.5
9/3/2008	KRAC	Above Copco	0C	0	0	0	0.0	0.0
9/3/2008	SH	Mouth of Shasta River	0C	0	0	0	0.0	0.0
9/9/2008	KRRP	River edge at Rocky Point	SG	310,689	0	390	7.8	48.8
9/10/2008	SVFD	Siead Valley Fish Disease Site	SG	128,332	0	0.97	3.2	0.1
9/11/2008	KRBI	Below Iron Gate	SG	3,606	0	0.93	0.1	0.1
9/11/2008	OR	Orleans	0C	3,251	0	1	0.1	0.1
9/11/2008	SV	Seaid Valley	OC	5,503	0	1,3	0.1	0.2
9/11/2008	SV	Seaid Valley	SG	316,828	0	48	7.9	6.0
9/11/2008	WA	Walker Bridge	0C	7,938	0	1.4	0.2	0.2

DATE	STATION NAME	Station Description	DEPTH	*Microcystis aeruginosa* (cells/ml)	*Anabaena sp.* (cells/ml)	Microcystin Total (µg/L)	Exceedance of SWRCB[1] risk level of 40,000 cells/ml *Microcystis or Planktothrix* (x greater than 4[5] cells/ml)	Exceedance of microcystin TDI of 0.04 µg/kg/day for a 20kg (44lb) child ingesting 100 mls[2] (x greater than TDI)
9/11/2008	WA	Walker Bridge	SG	58,336	0	63	1.5	7.9
9/17/2008	CR01	Copco Open Water near Dam	0C	59	0	0.95	0.0	0.1
9/17/2008	CRCC	Copco Reservoir Copco Cove	SG	17,149,136	0	4800	428.7	600.0
9/17/2008	IR01	Iron Gate Open Water near Dam	0C	64,772	0	4	1.6	0.5
9/17/2008	IRCC	Iron Gate Camp Creek Rec. Area	0C	87,403	0	54	2.2	6.8
9/17/2008	IRJW	Iron Gate Jay Williams Boat Dock	SG	110,381	0	17	2.8	2.1
9/17/2008	KRBI	Below Iron Gate	0C	55	0	0	0.0	0.0
9/17/2008	OR	Orleans	0C	1,147	0	0	0.0	0.0
9/17/2008	SA	Mouth of Salmon River	0C	0	0	0	0.0	0.0
9/17/2008	SC	Mouth of Scott River	0C	0	0	0	0.0	0.0
9/17/2008	SV	Seaid Valley	0C	1,746	0	0	0.0	0.0
9/17/2008	WA	Walker Bridge	0C	1,792	0	0	0.0	0.0
9/18/2008	CRMC	Copco Reservoir Mallard Cove	0C	27,612	0	99	0.7	12.4
9/18/2008	KRAC	Above Copco	0C	0	0	0	0.0	0.0
9/18/2008	SH	Mouth of Shasta River	0C	0	0	0	0.0	0.0
9/24/2008	KRBI	Below Iron Gate	0C	0	0	0.31	0.0	0.0
9/24/2008	OR	Orleans	0C	1,321	0	0.31	0.0	0.0
9/24/2008	SV	Seaid Valley	0C	0	0	0.37	0.0	0.0
9/24/2008	WA	Walker Bridge	0C	15,178	0	0.59	0.4	0.1
9/30/2008	CR01	Copco Open Water near Dam	SG	0	0	0.18	0.0	0.0
9/30/2008	CRCC	Copco Reservoir Copco Cove	SG	42,168,500	0	1800	1054.2	225.0
9/30/2008	IR01	Iron Gate Open Water near Dam	SG	270,600	0	1.2	6.8	0.2
9/30/2008	IRCC	Iron Gate Camp Creek Rec. Area	SG	1,274	0	0.22	0.0	0.0

DATE	STATION NAME	Station Description	DEPTH	*Microcystis aeruginosa* (cells/ml)	*Anabaena sp.* (cells/ml)	Microcystin Total (µg/L)	Exceedance of SWRCB[1] risk level of 40,000 cells/ml *Microcystis or Planktothrix* (x greater than 4^5 cells/ml)	Exceedance of microcystin TDI of 0.04 µg/kg/day for a 20kg (44lb) child ingesting 100 mls[2] (x greater than TDI)
9/30/2008	IRJW	Iron Gate Jay Williams Boat Dock	SG	48,382	0	4.5	1.2	0.6
9/30/2008	KRBI	Below Iron Gate	0C	0	0	0.15	0.0	0.0
9/30/2008	OR	Orleans	0C	0	0	0.1	0.0	0.0
9/30/2008	SA	Mouth of Salmon River	0C	0	0	0	0.0	0.0
9/30/2008	SC	Mouth of Scott River	0C	0	0	0	0.0	0.0
9/30/2008	SV	Seaid Valley	0C	2,177	0	0.16	0.1	0.0
9/30/2008	WA	Walker Bridge	0C	0	0	0.16	0.0	0.0
10/1/2008	CRMC	Copco Reservoir Mallard Cove	SG	45,289	0	0.23	1.1	0.0
10/1/2008	KRAC	Above Copco	0C	0	0	0.11	0.0	0.0
10/1/2008	SH	Mouth of Shasta River	0C	0	0	0.1	0.0	0.0
10/9/2008	KRBI	Below Iron Gate	0C	2,255	0	0	0.1	0.0
10/9/2008	OR	Orleans	0C	0	0	0.14	0.0	0.0
10/9/2008	SV	Seaid Valley	0C	0	0	0.14	0.0	0.0
10/9/2008	WA	Walker Bridge	0C	436	0	0.16	0.0	0.0
10/14/2008	CR01	Copco Open Water near Dam	SG	0	0	0	0.0	0.0
10/14/2008	CRCC	Copco Reservoir Copco Cove	SG	7,175,000	0	3.1	179.4	0.4
10/14/2008	IR01	Iron Gate Open Water near Dam	SG	1,065	0	0.14	0.0	0.0
10/14/2008	IRCC	Iron Gate Camp Creek Rec. Area	SG	0	0	0.15	0.0	0.0
10/14/2008	IRJW	Iron Gate Jay Williams Boat Dock	SG	988	0	0.94	0.0	0.1
10/14/2008	KRBI	Below Iron Gate	0C	0	0	0.16	0.0	0.0
10/14/2008	OR	Orleans	0C	0	0	0.16	0.0	0.0
10/14/2008	SA	Mouth of Salmon River	0C	0	0	0	0.0	0.0
10/14/2008	SC	Mouth of Scott River	0C	0	0	0	0.0	0.0

DATE	STATION NAME	Station Description	DEPTH	*Microcystis aeruginosa* (cells/ml)	*Anabaena sp.* (cells/ml)	Microcystin Total (µg/L)	Exceedance of SWRCB[1] risk level of 40,000 cells/ml *Microcystis or Planktothrix* (x greater than 4[5] cells/ml)	Exceedance of microcystin TDI of 0.04 µg/kg/day for a 20kg (44lb) child ingesting 100 mls[2] (x greater than TDI)
10/14/2008	SH	Mouth of Shasta River	0C	0	0	0	0.0	0.0
10/14/2008	SV	Seaid Valley	0C	0	0	0.1	0.0	0.0
10/14/2008	WA	Walker Bridge	0C	0	0	0.15	0.0	0.0
10/15/2008	CRMC	Copco Reservoir Mallard Cove	0C	17,019	0	13	0.4	1.6
10/15/2008	KRAC	Above Copco	0C	0	0	0.13	0.0	0.0

[1]From: Blue Green Algae Work Group of the State Water Resources Control Board and Office of Environmental Health and Hazard Assessment: *Cyanobacteria in California Recreational Water Bodies Providing Voluntary Guidance about Harmful Algal Blooms, Their Monitoring, and Public Notification* (Sep 2008: www.waterboards.ca.gov/water_issues/programs/bluegreen_algae/docs/bga_volguidance.pdf)
[2]The TDI or tolerable daily intake (e.g., WHO 1999: www.who.int/water_sanitation_health/resourcesquality/toxicyanbact/en/) as computed here for a 20kg child is equivalent to the exceedance of the 8µg/L microcystin value as shown in Appendix 6 of document in footnote 1 above.
[3]0C denotes sampling near mid-channel mixed region
[4]SG denotes surface grab-sampling near shoreline region of low mixing

Appendix II

AQUATIC ECOSYSTEM SCIENCES LLC

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November 1, 2007 Toxic Cyanobacteria Memo Prepared by Aquatic Ecosystem Sciences for the Karuk Tribe of California.

November 1, 2007

RE: Change in Microcystin to Cell Density Ratio

Recent microcystin toxin data (performed using ELISA) from the EPA Richmond Lab indicates a change in toxin production beginning in September of 2007. Despite elevated MSAE density in September and early October (Figure 1a) toxin concentrations were substantially lower during this same period (Figure 1b). A plot of the ratio of microcystin per 100,000 cells of MSAE shows a dramatic drop in September of 2007 (Figure 2). With the exception of one sample date in late-October of 2005 and one in September of 2006, the ratio upper quartile values beginning in September of 2007 are the lowest of the 2005-2007 period (Figure 2). Further illustrating lower toxin production per unit MSAE are data from 9-24-07 which show that despite MSAE density in the hundreds of thousands of cells per ml that microcystin was often near the quantification level of 1.8 µg/L (Table 1). This is pictorially illustrated in Figures 3 and 4, where obviously dense MSAE concentrations were associated with very low microcystin production.

While there is a good probability the decline in toxin production is real, and may be due to environmental conditions (e.g., temperature), because we have not seen such a sustained dramatic decline in the past, I recommend we confirm the ELISA results by running some of the remaining sample material (presumably being held by the EPA lab) at another lab. It would be good to use LC-MS and the U.C. Davis lab is probably the best lab for this purpose. I recommend sending, at a minimum, the two samples from 9-24-07 that have high MSAE density and low microcystin (e.g., Copco mid-point and URA downriver of plunge line) along with the actual General Biology microcystin standard used by EPA. This type of cross-laboratory QA would be very informative and provide greater confidence in the data.

Please let me know what you think or if you have any questions. Thank you.

Sincerely,

Jacob Kann, Ph.D.
Aquatic Ecologist

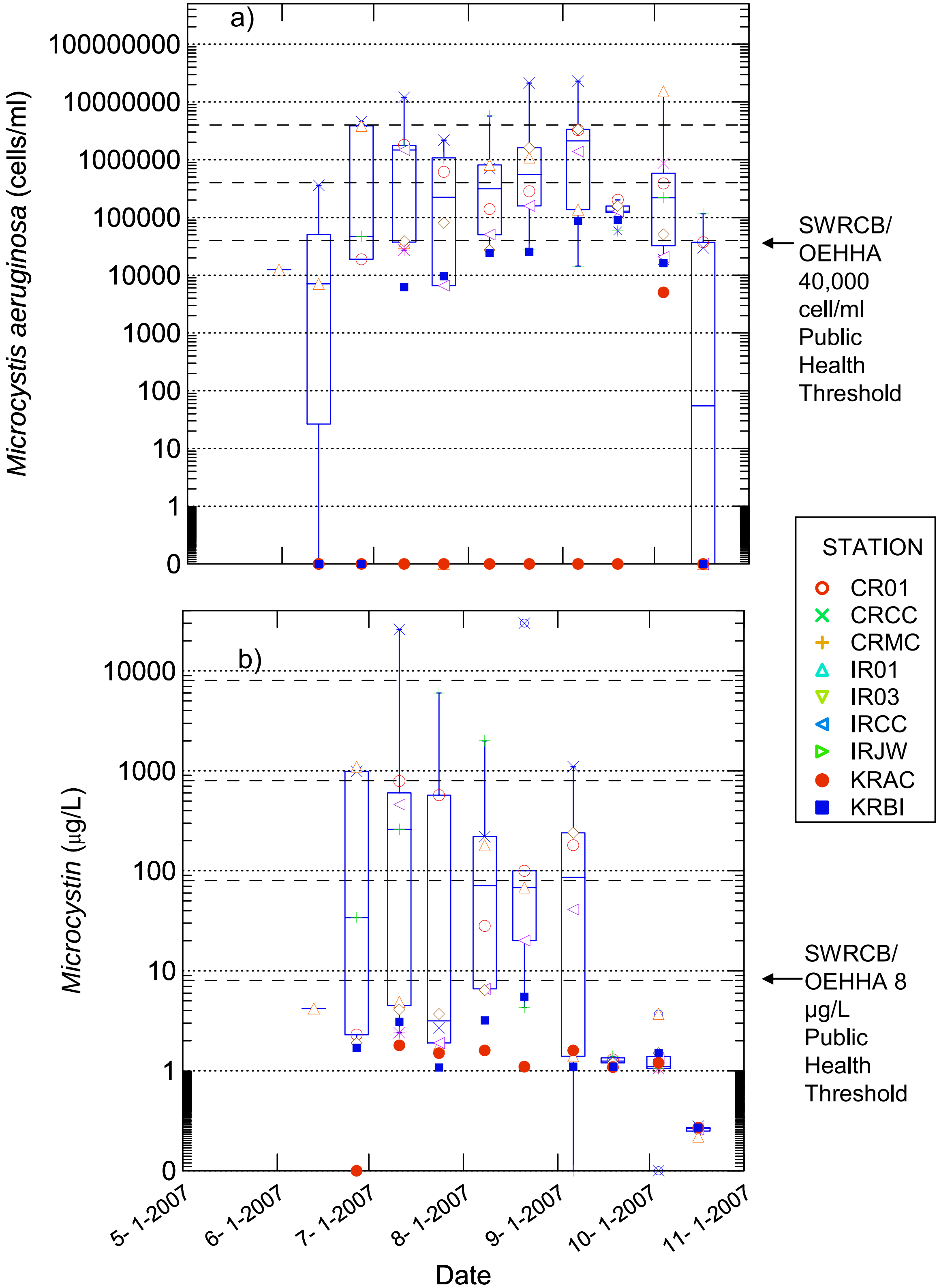

Figure 1. Time-series of MSAE cell density (a), and microcystin concentration (b) for Copco and Iron Gate Reservoir stations, 2007. The blue box is for the reservoir stations only; the river stations KRAC and KRBI are shown independently. Lab results are preliminary and subject to change pending final seasonal QA.

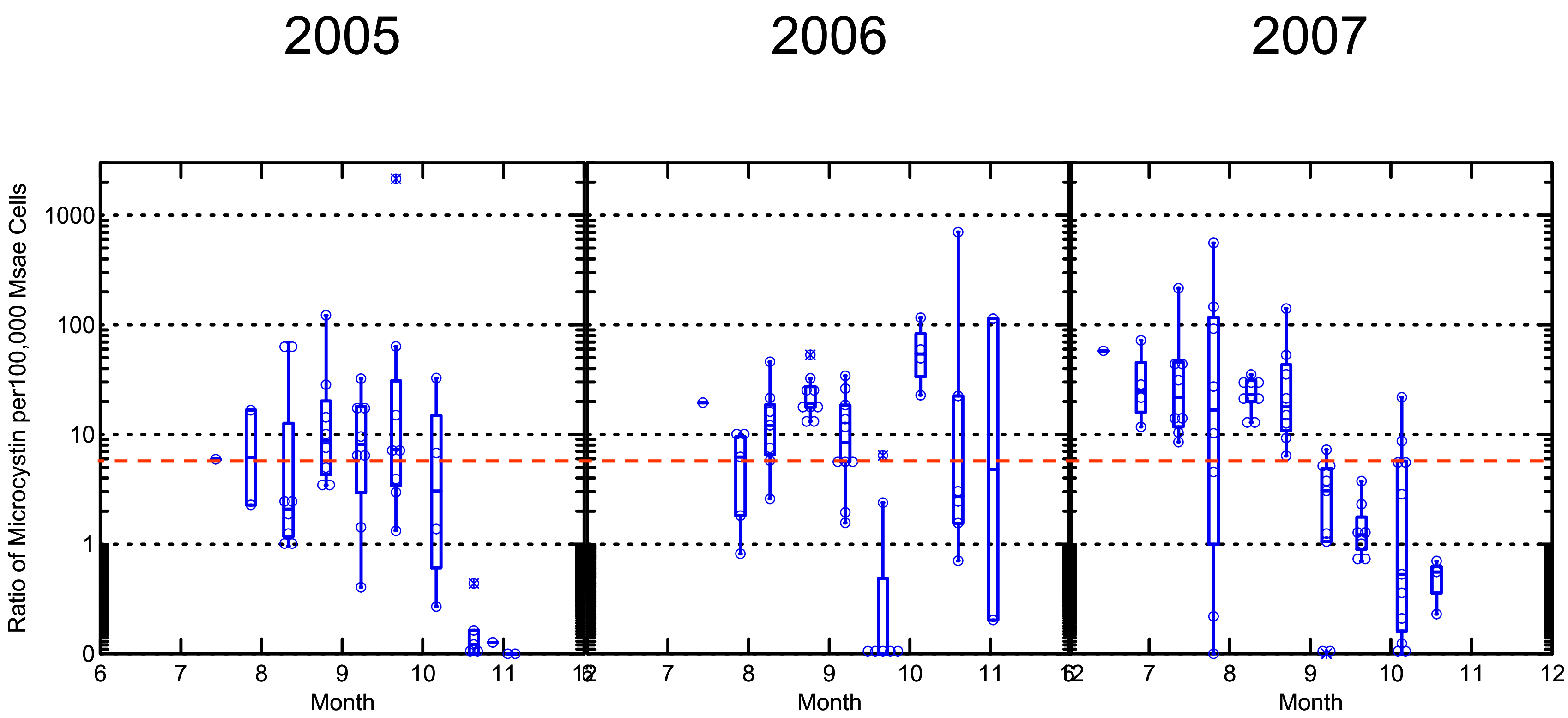

Figure 2. Ratio of microcystin per 100,000 MSAE cells, 2005-2007. (red dashed line show September-October 2007 upper quartile maximum). note: **Lab results are preliminary and subject to change pending final seasonal QA.**

Table 1. Cell density and microcystin concentration in Copco Reservoir, 9-24-07 (note: Lab results are preliminary and subject to change pending final seasonal QA).

DATE	STATION NAME	DEPTH	*Microcystis aeruginosa* (cells/ml)	Microcystin Total (µg/L)	Exceedance of SWRCB[1] risk level of 40,000 cells/ml *Microcystis or Planktothrix* (x greater than 4[5] cells/ml)	Exceedance of SWRCB[1] risk level of 8 µg/L microcystin (x greater than 8 µg/L)	Exceedance of TDI of 0.04 µg/kg/day for a 40 lb (18kg) child ingesting 100 mls (x greater than TDI)	Toxin:Cell Density Ratio (toxin per 100,000 cells)
9/24/2007	near CR01	0	33,896	1.60	0.8	0	0	4.720321
9/24/2007	Mallard Cove Boat Ramp	0	1,145,883	18.00	28.6	2	2	1.570841
9/24/2007	Mallard Cove Inlet	0	5,104,467	180.00	127.6	23	25	3.526323
9/24/2007	Copco Reservoir Mid-Point	0	343,367	1.80	8.6	0	0	0.524220
9/24/2007	URA (down-river of plunge line)	0	770,842	1.30	19.3	0	0	0.168647
9/24/2007	URA-dup (down-river of plunge line)	0		1.50		0	0	
9/24/2007	UR (near bridge up-river of plunge line)	0	0	1.40	0.0	0	0	
9/24/2007	KRBI (algal strands from rock)	0	684,956	1.60	17.1	0	0	0.233592
9/24/2007	KRBI (water column)	0	17,410	1.70	0.4	0	0	9.764503
9/24/2007	KRBI-dup	0		1.70		0	0	
9/24/2007	Ager Bridge Fishing Access	0	23,446	1.70	0.6	0	0	7.250704

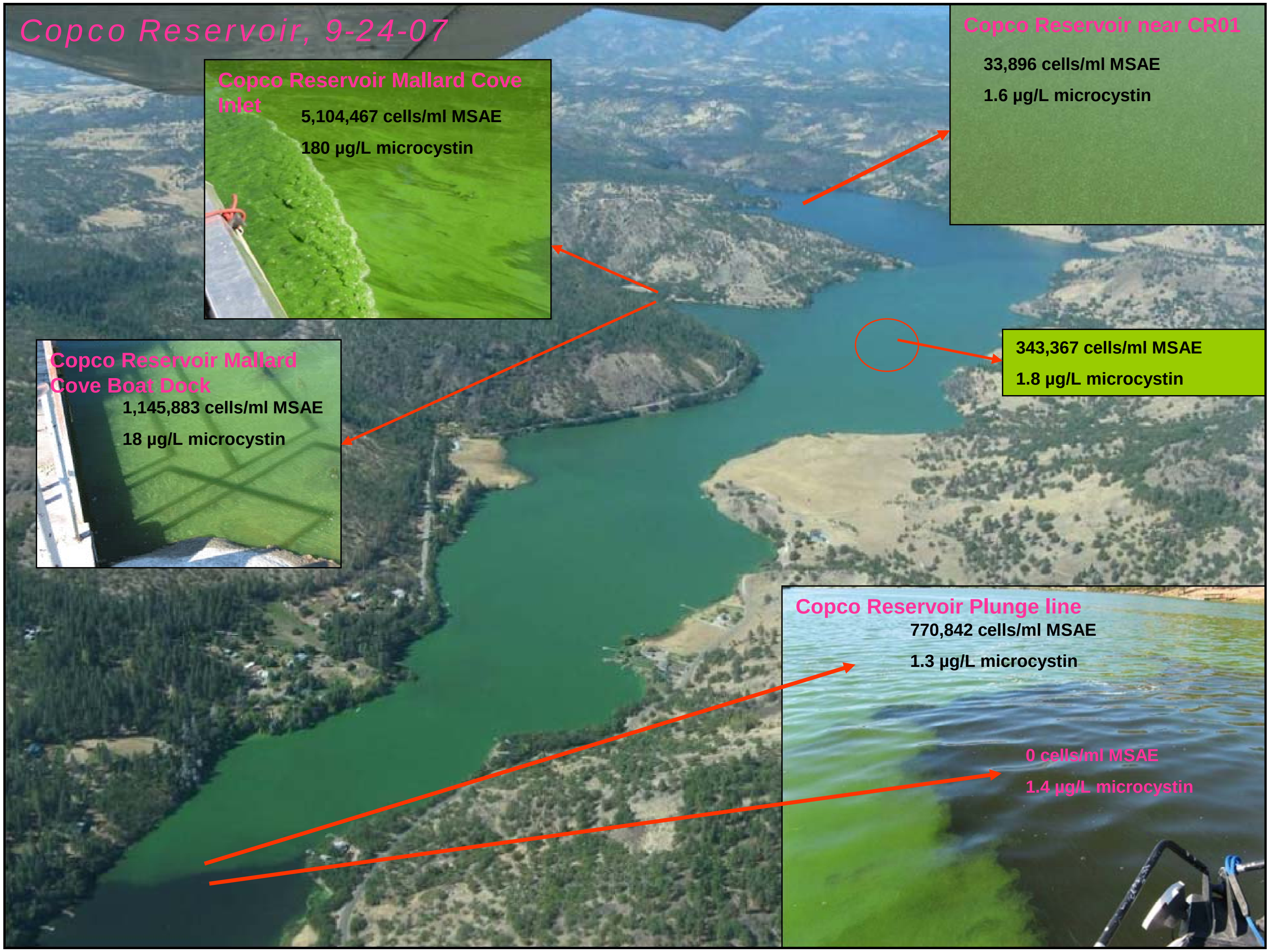

Figure 3. MSAE cell density and microcystin concentration in Copco Reservoir on 9-24-07. Note: Lab results are preliminary and subject to change pending final seasonal QA.

Figure 4. MSAE cell density and microcystin concentration associated with attached algae below Iron Gate Dam (KRBI) on 9-24-07. Note: Lab results are preliminary and subject to change pending final seasonal QA.