TECHNICAL MEMORANDUM

Summary of 2006 Toxic *Microcystis aeruginosa* and Microcystin Trends in Copco and Iron Gate Reservoirs, CA

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INTRODUCTION

Copco and Iron Gate Reservoirs (the lowermost projects of PacifiCorp's Klamath Hydropower Project-- KHP) experienced extensive blooms of toxigenic *Microcystis aeruginosa* (MSAE) in 2004 and 2005 (Kann and Corum 2006; Jacoby and Kann 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 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 not detectable in the Klamath River directly above the reservoirs, detectable levels of both parameters were found directly below the reservoirs in 2005.

A similar toxic algal monitoring program was undertaken by the Karuk Tribe during July-November, 2006. The following technical memorandum summarizes 2006 toxigenic MSAE trends in Copco and Iron Gate Reservoirs and in the Klamath River directly above and below the reservoir complex.

METHODS

Station Location

During the 2006 sampling season, MSAE cell density, cell biovolume, and microcystin toxin samples were collected from a variety of shoreline and open-water sites, including standard open-water locations (Table 1 and Figure 1; Stations IR01, IR03, and CR01) and shoreline stations specifically sampled to assess the extent of toxic MSAE in the vicinity of public recreational access points (Figure 1). KRAC is a Klamath River station above Copco Reservoir, while KRBI, SV (Seiad Valley), and OR (Orleans) are Klamath River stations below Iron Gate Reservoir (Figure 1).

Sample Collection and Lab Analysis

Shoreline and open-water samples taken at the surface consisted of grab samples of surface algal material, and both open-water samples taken at 1 m and samples collected at river stations KRAC and KRAI were taken with a Van-Dorn water collection bottle (KRAI data have not yet been received from the laboratory). 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). Phytoplankton laboratory reports are contained in Electronic Appendix E1.

Table 1. Phytoplankton/microcystin sampling locations in Copco and Iron Gate Reservoirs and Klamath River stations, 2006.

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
IR03	N41 57.876 W122 25.389	Iron Gate Res. Upper ½	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
KRBI	N41 55.865 W122 26.532	Klamath River Below Iron Gate Reservoir	River
SV	N41 50.561 W 123 13.132	Seiad Valley	River
OR	N 41 18.336 W 123 31.895	Orleans	River

Figure 1. Location of Copco and Iron Gate Reservoir and Klamath River toxic cyanobacteria sampling stations, 2006.

Samples for microcystin toxin collected between July and the end of September were placed in a cooler with gel-ice and shipped overnight air to Wright State University (WSU) in Dayton, OH (CyanoHab Services Lab of Dr. Wayne Carmichael). Due to closure of the WSU lab in October, samples collected in October and November were frozen at Karuk Tribal facilities and were shipped in February of 2007 to the EPA Region 9 Laboratory in Richmond, CA. Both WSU and EPA samples were analyzed for 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.

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. 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 an 18kg child ingesting 100 mls of reservoir water. 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.

RESULTS/DISCUSSION

Quality Assurance Samples

2006 QA samples generally showed very good agreement between split samples for both cell density and microcystin (Figures 2a), with the majority of cell density and toxin splits falling within ±25% of the relative percent difference (RPD; defined as $(((x_1-x_2)/(x_1+x_2/2))x100)$. Of toxin samples falling outside of 25% RPD, the absolute values of the paired samples were low (e.g., 1.6/2.4 and 2.7/4.4μg/L; Appendix II), with differences of 0.8 and 1.7 μg/L, respectively. Of cell or colony count samples falling outside of 25% RPD, the highest values of 200% occurred when colony density was less than 400/ml in one sample and no colonies were detected in the split sample (Appendix II). Other paired samples for cell and colony density that fell outside 25% RPD do indicate variability stemming from sample splitting (i.e., use of a churn splitter) and or subsampling during laboratory microscopic analysis. However, an analysis of paired samples with respect to public health threshold values showed that there were no instances

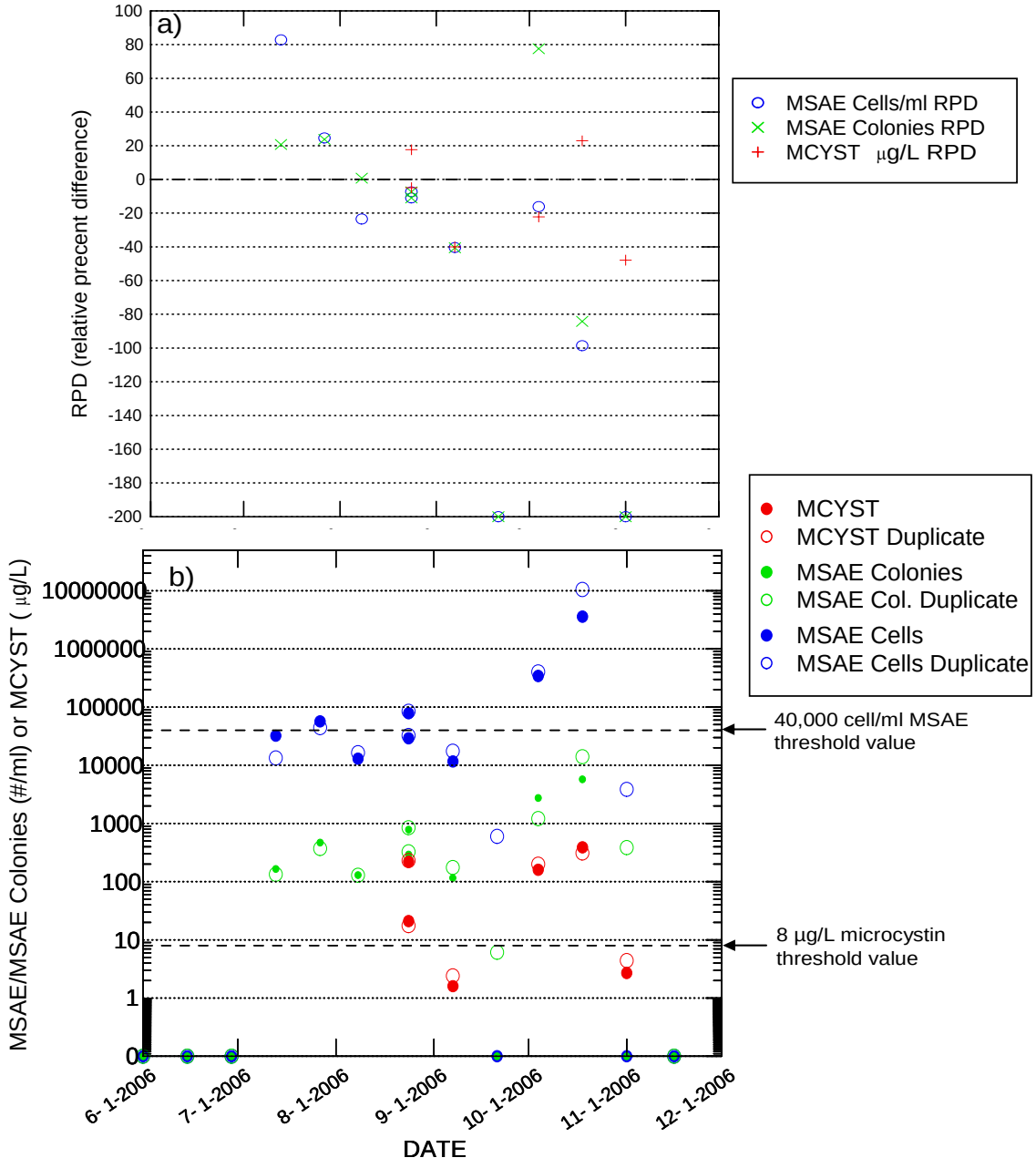


Figure 2. Analysis of field duplicate samples for MSAE cell density and microcystin concentration (MCYST); relative percent difference (RPD) (a), and paired sample values (b).

when management relative to these values would have differed based on duplicate variability (Figure 2b). Thus, utilized phytoplankton and toxin methodology had adequate sensitivity relative to public health threshold values.

2006 Temporal Trends

Similar to the timing in 2005, the first visual detection of a cyanobacterial bloom in the reservoir system in 2006 was noted during regular biweekly sampling on July 12th and 13th at station CRCC in Copco Reservoir. Subsequent analyses showed >11 million MSAE cells per ml and 2286 µg/L of microcystin at station CRCC (Table 2). These high cell concentration and toxin levels exceeded the SWRCB/OEHHA thresholds by hundreds of times, and the associated high microcystin toxin concentration of 2286 µg/L was greater than 315 times the tolerable daily intake (Table 2). Data from July 13th illustrate that despite low cell density (2492 cells/ml) at the open water station CR01 (Table 2), localized heavy blooms have the potential to occur due to MSAE buoyancy and the concentrating effect of wind. MSAE levels in Iron Gate were relatively low (13,777 cells/ml at IR01) and MSAE was not detected either upstream of the reservoirs at KRAC or downstream of Iron Gate Dam at station KRBI (Table 2).

By the next sample period of July 26-27, data clearly showed that blooms of MSAE and associated microcystin toxin had increased substantially in intensity and extent since the July 13th sample period (Table 2; Figures 2 and 3). All reservoir stations on July 27th exceeded the SWRCB/OEHHA public health threshold (Figure 3a). In fact, the maximum MSAE cell count of over 393 million cells/ml at CRCC exceeded the 40,000 cells/ml threshold by 9835 times (Table 2). This is the highest cell density recorded for the reservoirs to date. The open-water stations CR01 and IR01 also exceeded the SWRCB/OEHHA level by 409 and 163 times, respectively. Toxin concentrations were also well above the threshold level (Figure 3b), with maximum toxin concentrations higher than maxima measured in 2005. Both shoreline and open-water stations exceeded the SWRCB/OEHHA guideline of 8 µg/L (of microcystin), with levels exceeding the threshold by as much as 352 times at station CRCC on 7/27 where a value of 2813 µg/L (2.8 mg/L) was reported. A 40 lb child accidentally ingesting 100 mls at this station would exceed the TDI by over 388 times.

Although no MSAE was detected at KRAC above Copco Reservoir on 7-26, 35,985 cells/ml were detected at KRBI below Iron Gate Reservoir; MSAE was not detected further downstream at either Seiad Valley (SV) or Orleans (OR) on 7-26 (Table 2; Figures 3a and 4a). Although microcystin at KRBI (3.4 µg/L) was elevated relative to KRAC, it was lower than the threshold guideline of 8 µg/L (Figures 3b and 4b). Despite the lack of MSAE detected at Orleans (OR), microcystin was detected at a low level of 0.97 µg/L.

Overall MSAE cell density on Aug 8th was lower than July 27th; however, all reservoir stations continued to exceed the MPAHEL of 100,000 cells/ml (Figure 3a). Again, MSAE was not detected at KRAC above Copco Reservoir, but 24,929 cells/ml were detected at KRBI below Iron Gate Reservoir (Figure 3a), and due to an omission no cell density samples (see below for toxin results) were collected at either Seiad Valley (SV) or Orleans (OR). All shoreline and open-water stations exceeded the threshold of 8 µg/L of microcystin. Although the maximum MSAE cell count of over 393 million cells/ml at CRCC on Jul 27th declined to a maximum of ~26.5 million cells/ml on Aug 8th, microcystin toxin level increased over 4x to 12,176 µg/L (or 12.18 mg/L; Table 2 and Figure 3b).

Table 2. ***Microcystis aeruginosa*** **cell density, microcystin toxin concentration, and risk exceedance for toxigenic cyanobacteria in Copco and Iron Gate Reservoirs, 2006.**

DATE	STATION NAME	DEPTH	*Microcystis aeruginosa* (cells/ml)	*Anabaena sp.* (cells/ml)[1]	Microcystin Total (µg/L)[2]	Exceedance of SWRCB/OEHHA[3] risk level of 40,000 cells/ml *Microcystis or Planktothrix* (x greater than 4[5] cells/ml)	Exceedance of SWRCB/OEHHA[3] risk level of 8 µg/L microcystin (x greater than 8 µg/L)	Exceedance of TDI[4] of 0.04 µg/kg/day for a 40 lb (18kg) child ingesting 100 mls (x greater than TDI)
7/12/2006	KRAC	0	0	0	ns	0		
7/13/2006	KRBI	0	0	0	ns	0		
7/13/2006	CR01	1	2,492	0	ns	0		
7/13/2006	CRCC	0	11,783,212	6,086	2286.00	295	286	315.7
7/13/2006	IR01	1	13,377	0	ns	0.33		
7/26/2006	KRAC	0	0	0	ns	0		
7/27/2006	CR01	0	16,340,580	0	1003.00	409	125	138.5
7/27/2006	CRCC	0	393,395,000	0	2813.00	9835	352	388.5
7/27/2006	IR01	0	6,504,808	0	650.00	163	81	89.8
7/27/2006	IRJW	0	25,043,386	32,214	430.00	626	54	59.4
7/27/2006	KRBI	0	35,985	0	3.40	1	0	0.5
7/26/2006	SV	0	0	0	ns	0		
7/26/2006	OR	0	0	0	0.97	0.0	0.1	0.1
8/7/2006	KRAC	0	0	0	2.00	0	0	0.3
8/8/2006	CR01	0	2,371,806	0	507.00	59	63	70.0
8/8/2006	CRCC	0	26,487,302	0	12176.00	662	1522	1681.8
8/7/2006	CRMC	0	23,575,000	0	3779.00	589	472	522.0
8/8/2006	IR01	0	1,170,405	0	87.00	29	11	12.0
8/7/2006	IRJW	0	13,717,917	0	341.00	343	43	47.1
8/7/2006	IRCC	0	1,999,113	0	113.00	50	14	15.6
8/8/2006	IR03	0	379,668	0	ns	9		
8/7/2006	KRBI	0	24,929	0	3.00	1	0	0.4
8/7/2006	SV	0	ns	0	6.70		1	0.9
8/7/2006	OR	0	ns	0	4.10		0.5	0.6
8/23/2006	KRAC	0	0	0	0.45	0	0	0.1
8/24/2006	CR01	0	92,250	0	17.60	2	2	2.4
8/23/2006	CRCC	0	16,093,579	0	3839.00	402	480	530.2
8/23/2006	CRMC	0	8,388,600	0	1543.00	210	193	213.1
8/24/2006	IR01	0	434,893	0	231.00	11	29	31.9
8/23/2006	IRJW	0	121,401	0	15.90	3	2	2.2
8/23/2006	IRCC	0	7,492,880	0	2032.00	187	254	280.7
8/23/2006	KRBI	0	28,423	0	9.20	1	1	1.3
8/23/2006	SV	0	41,299	0	7.30	1	1	1.0
8/23/2006	OR	0	31,801	0	4.60	0.8	0.6	0.6
9/6/2006	KRAC	0	0	0	0.00	0	0	0.0
9/7/2006	CR01	0	95,838	0	1.40	2	0	0.2
9/7/2006	CRCC	0	3,728,535	0	206.10	93	26	28.5
9/7/2006	CRMC	0	1,028,844	0	19.00	26	2	2.6
9/7/2006	IR01	0	17,524	0	2.40	0	0	0.3
9/7/2006	IR03	0	45,585		11.90	1	1	1.6
9/7/2006	IRJW	0	11,897	0	0.68	0	0	0.1
9/7/2006	IRCC	0	29,530	0	10.10	1	1	1.4
9/6/2006	KRBI	0	3,735	0	0.69	0	0	0.1
9/6/2006	SV	0	9,555	0	0.57	0	0	0.1
9/6/2006	OR	0	3,356	0	0.39	0.1	0.0	0.1
9/20/2006	KRAC	0	0	0	0.00	0	0	0.0
9/21/2006	CR01	0	22,259	0	0.00	1	0	0.0
9/20/2006	CRCC	0	2,628,528	0		66	0	0.0

DATE	STATION NAME	DEPTH	*Microcystis aeruginosa* (cells/ml)	*Anabaena sp.* (cells/ml)[1]	Microcystin Total (µg/L)[2]	Exceedance of SWRCB/OEHHA[3] risk level of 40,000 cells/ml *Microcystis or Planktothrix* (x greater than 4[5] cells/ml)	Exceedance of SWRCB/OEHHA[3] risk level of 8 µg/L microcystin (x greater than 8 µg/L)	Exceedance of TDI[4] of 0.04 µg/kg/day for a 40 lb (18kg) child ingesting 100 mls (x greater than TDI)
9/20/2006	CRMC	0	3,312,031	0	209.00	83	26	28.9
9/21/2006	IR01	0	12,177	0	0.00	0	0	0.0
9/20/2006	IRJW	0	8,786	0	0.00	0	0	0.0
9/20/2006	IRCC	0	8,301	0	0.19	0	0	0.0
9/20/2006	KRBI	0	3,982	0	0.00	0	0	0.0
9/20/2006	SV	0	190	0	0.00	0	0	0.0
9/20/2006	OR	0	0	0	0.00	0.0	0.0	0.0
10/4/2006	KRAC	0	0	0	ns	0		
10/5/2006	CR01	0	756	0	0.45	0	0	0.1
10/4/2006	CRCC	0	2,774,943	0	630.00	69	79	87.0
10/4/2006	CRMC	0	403,718	0	200.00	10	25	27.6
10/5/2006	IR01	0	0	0	0.27	0	0	0.0
10/5/2006	IR03	0	604	0	ns	0		
10/4/2006	IRCC	0	346	0	0.40	0	0	0.1
10/4/2006	KRBI	0	0	0	0.17	0	0	0.0
10/4/2006	SV	0	0	0	0.26	0	0	0.0
10/4/2006	OR	0	0	0	0.27	0.0	0.0	0.0
10/18/2006	KRAC	0	0	0	0.14	0	0	0.0
10/19/2006	CR01	0	313	0	0.07	0	0	0.0
10/18/2006	CRCC	0	51,250,000	0	310.00	1281	39	42.8
10/18/2006	CRMC	0	10,570,313	0	310.00	264	39	42.8
10/19/2006	IR01	0	10,208	0	0.24	0	0	0.0
10/19/2006	IR03	0	0	0	0.13	0	0	0.0
10/18/2006	IRCC	0	14,455	0	0.21	0	0	0.0
10/18/2006	KRBI	0	47	0	0.33	0	0	0.0
10/18/2006	SV	0	0	0	0.32	0	0	0.0
10/18/2006	OR	0	0	0	0.27	0.0	0.0	0.0
11/1/2006	KRAC	0	0	0	ns	0		
11/2/2006	CR01	0	313	0	ns	0		
11/1/2006	CRCC	0	3,855	0	4.40	0	1	0.6
11/1/2006	CRMC	0	27,234,581	0	28.00	681	4	3.9
11/1/2006	KRBI	0	0	0	ns	0.0		

[1]*Anabaena flos-aquae* (ABFA) is another potentially toxigenic cyanobacteria that can produce the neurotoxin, anatoxin-a. Because cell counts were lower than the MPAHEL of 100,000 cells/ml, ABFA is not discussed further in this report.

[2]ns=not sampled; July-September samples analyzed by Wright State University, Dayton, OH; October-November samples analyzed by USEPA Region 9 Laboratory, Richmond, CA.

[3]From: 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 (DRAFT June 2007)

[4]Tolerable Daily Intake-- World Health Organization (1998).

CRCC 7-27-06

CRCC 7-27-06

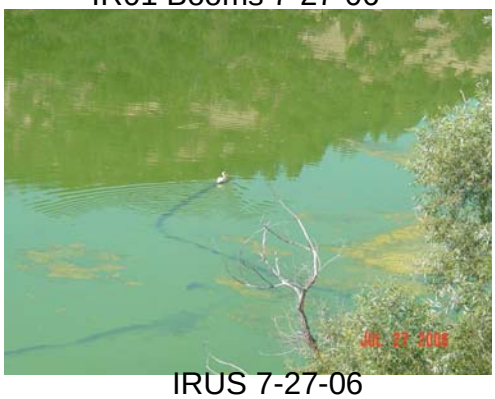
CR01 7-27-06

KRAI downstream 07-27-06

KRAI 7-27-06

IR01 Booms 7-27-06

IR017-27-06



IRUS 7-27-06

Figure 2. *Microcystis aeruginosa* blooms in Copco and Iron Gate Reservoirs; July 26-27, 2006.

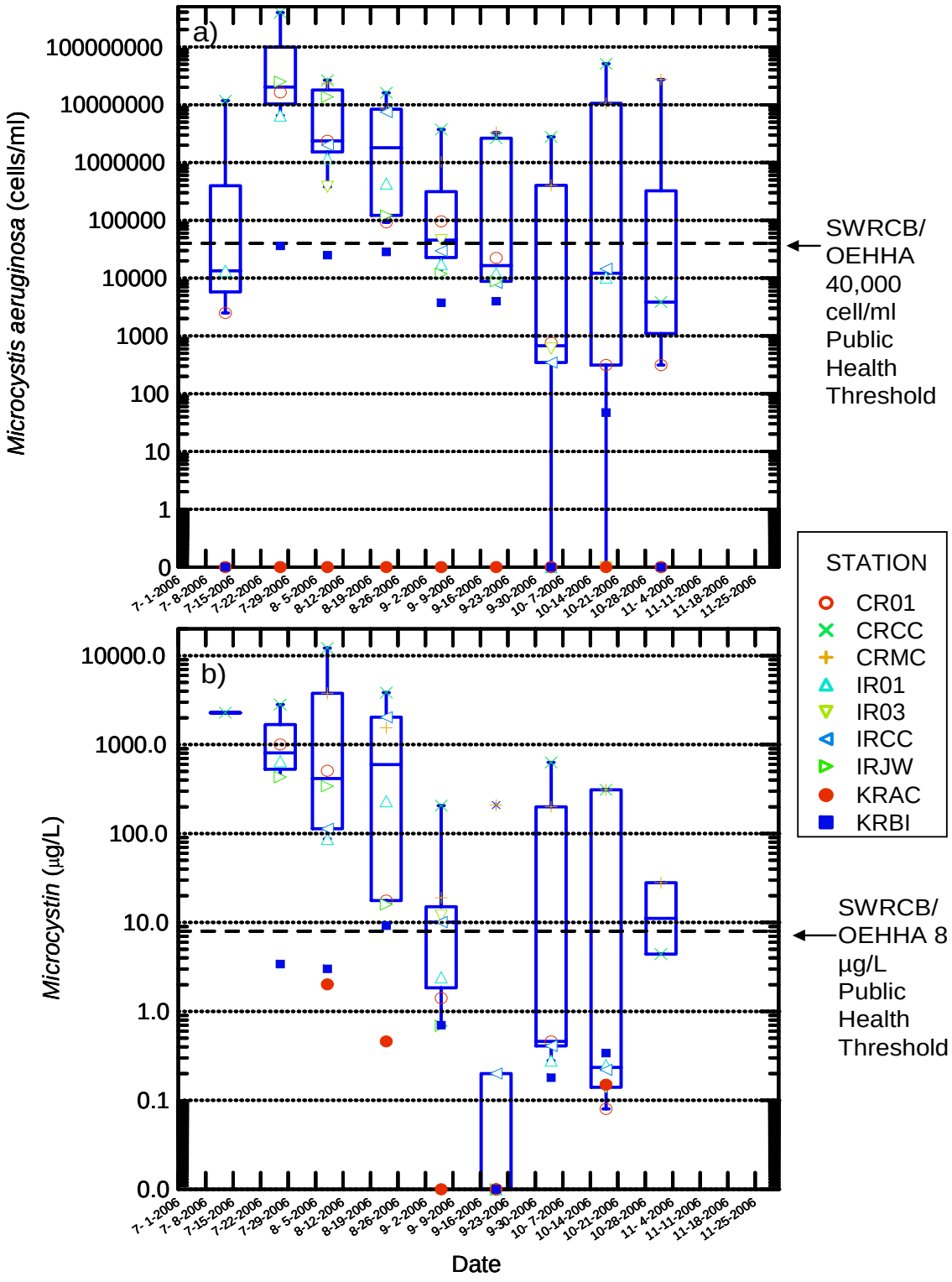


Figure 3. Time-series of MSAE cell density (a) and microcystin toxin concentration (b) for Copco and Iron Gate Reservoir stations, 2006. The blue box is for the reservoir stations only; the river stations KRAC and KRBI are shown independently.

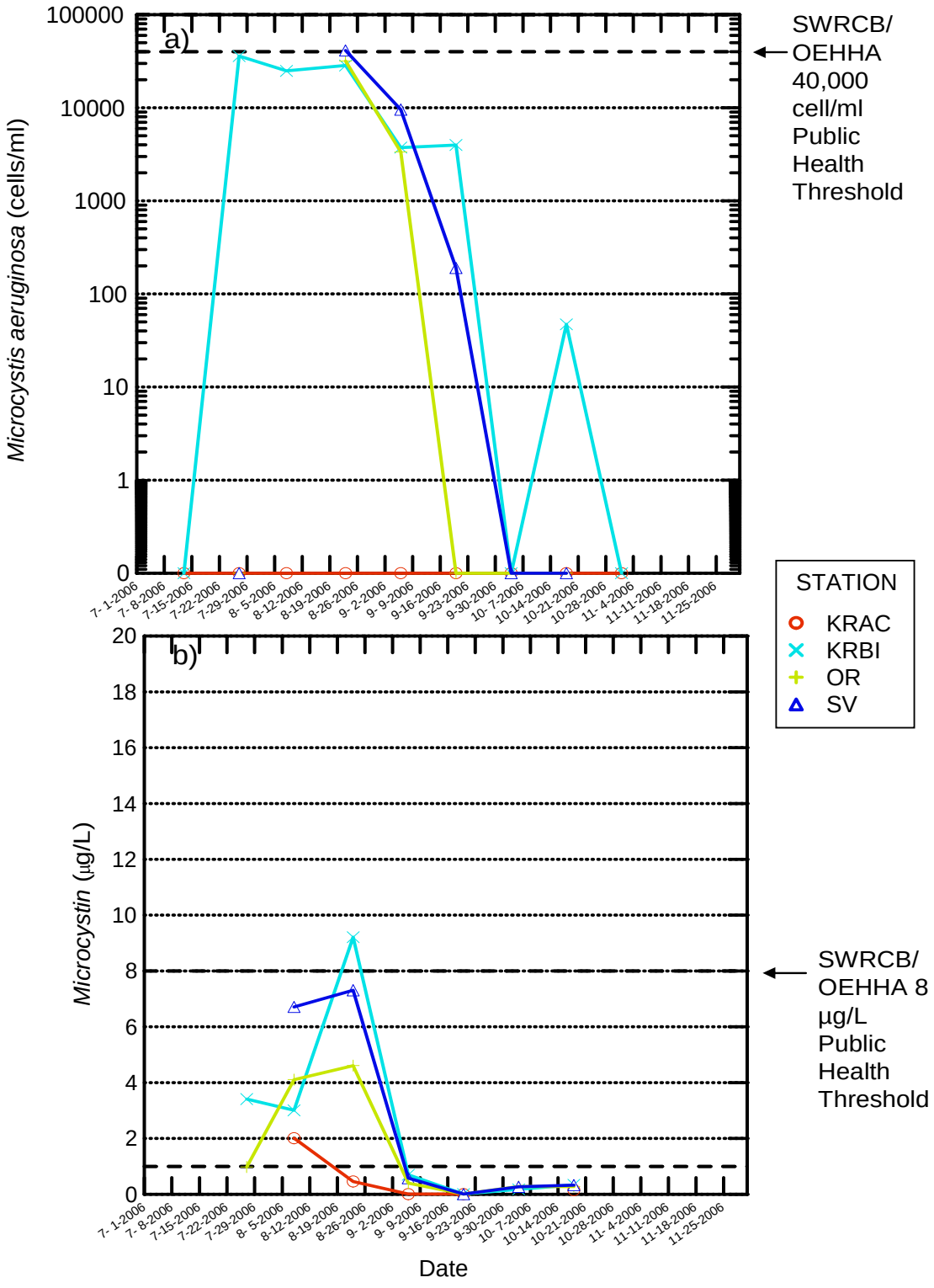


Figure 4. Time-series of MSAE cell density (a) and microcystin toxin concentration (b) for Klamath River Stations KRAC, KRBI, SV, and OR, 2006

Variable toxin production per unit cell count was also demonstrated during the 2005 sampling season; where although there was a general trend of increasing toxin with increasing cell density in 2005, there was also variability in the toxin to unit cell density ratio (Kann and Corum 2006 and see discussion below). The microcystin levels of 3779 µg/L at CRMC and 12,176 µg/L at CRCC are the highest yet recorded in these reservoirs and are among the highest recorded in the world.

Despite the non-detect for MSAE cells at Klamath River Station KRAC (above Copco Reservoir) on 8/7, microcystin toxin was measured at a level of 2.0 µg/L (Table 2; Figure 4b). During this same period (Aug 7th) cell densities of ~21,000 to 24,000 cells per ml as well as low microcystin levels (<3.0 µg/L)were measured by USBR upstream from KRAC in the reach between Link River and J.C. Boyle (Fetcho 2007). This trend indicates downstream transport of low levels of MSAE cells and toxin from these areas. However, at station KRBI downstream from Iron Gate, microcystin increased by 50% (relative to KRAC) to 3.0 µg/L on 8/7. Microcystin concentrations at Seiad Valley (SV) and Orleans (OR) were further elevated to 6.7 and 4.1 µg/L, respectively (samples for cell density were not available for these stations on 8/7). Given that blooms can recur in slower moving or backwater areas of the river, these results indicate the potential for both higher toxin concentrations and for toxin accumulation in fish tissue.

Although overall MSAE cell density continued to decline on August 24th, all reservoir stations continued to exceed public health threshold values for both cell density and microcystin (Figure 3a,b). Several stations (CRCC, CRMC, and IRCC) had >1500 µg/L (1.5mg/L) of microcystin (Table 2 and Figure 3). The microcystin levels of 3839 µg/L at CRCC and 2031 µg/L at IRCC are still among the highest recorded in these reservoirs. Similar to the previous sample period, MSAE was not detected at KRAC above Copco Reservoir, but 28,423 cells/ml were detected at KRBI below Iron Gate Reservoir, 41,299 cells/ml at Seiad Valley (SV) and 31,801 cells/ml at Orleans (OR). The Seiad Valley station exceeded the Posting Level threshold of 40,000 cells/ml (Figure 4a). Again, although MSAE cells were not detected at Klamath River Station KRAC (above Copco Reservoir), microcystin toxin was measured at a low level of 0.45 µg/L (Table 2). At station KRBI downstream from Iron Gate, microcystin increased by 20 times (relative to KRAC) to 9.2 µg/L and exceeded the Posting Level of 8 µg/L (Figure 4b). Concentrations at Seiad Valley (SV) and Orleans (OR) were 7.3 and 4.6 µg/L, respectively.

Levels continued to decline in both reservoirs during September, and by September 20th only CRCC and CRMC in Copco Reservoir exceeded threshold levels for public health (Figure 3). Although counts slightly rebounded in October at Iron Gate stations, an overall leveling off was observed through the Nov 1-2nd sample period; where, with the exception of Copco Stations CRCC and CRMC, all other reservoir stations were below both the World Health Organization MPAHEL of 100,000 cells/ml and the public health threshold posting Level of 40,000 cells/ml level (Figure 3). During this same period MSAE was again not detected at KRAC above Copco, and declined to levels that were not detectable at river stations KRBI below Iron Gate Reservoir, Seiad Valley (SV), and Orleans (OR) (Table 2; Figure 4).

Microcystin levels in the reservoirs also declined though the September period, and with the exception of CRMC most stations were lower than 1µg/L by September 20-21 (Table 2; Figure 3b). Likewise, microcystin at all river stations dropped below 1µg/L as well (Figure 4).

2006 Spatial Trends

Lower MSAE and toxin levels below Iron Gate despite relatively high levels at IR01 reflect water withdrawal (4-6.4m below surface) below the depths were MSAE tends to be more concentrated (Kann and Asarian 2007). The Iron Gate open-water station IR01 tended to have higher MSAE than the open-water Copco station CR01 in 2006. However, the Copco shoreline stations CRCC and CRMC consistently showed both higher MSAE and microcystin than all other stations (Figure 5a,b).

As noted above, all in-reservoir stations as well as downstream stations showed higher levels of both MSAE and microcystin than did the upstream station KRAC (Figure 5a,b; stations ordered upstream (left) to downstream (right)). Although results from samples collected by USBR in the reaches below Upper Klamath Lake (upstream from KRAC) showed minimal MSAE cells and microcystin toxin in those reaches (Fetcho 2007), levels were extremely low compared to those in Copco and Iron Gate Reservoirs. Moreover, despite these low upstream levels, no MSAE cells were detected at KRAC during the entire season (Figure 5a). On two occasions in 2006 low microcystin levels were detected at KRAC; however, toxin levels then increased substantially both in the reservoirs and downstream. Microcystin exceeded the 8 µg/L level at KRBI on one occasion in 2006 (Figure 5,b). A maximum level of 7.1 µg/L microcystin was also measured downstream at Weitchpec (Fetcho 2007).

2006 Cell Density-Microcystin Relationship

Similar to 2005, toxin concentration followed the same general seasonal trajectory as MSAE cell concentration (Figure 3a,b). Although the relationship between MSAE cell density and microcystin toxin is variable (as noted above), a scatter plot fitted with a distance weighted least squares smoother (DWLS) shows a general increasing trend of toxin concentration with cell density (Figure 6). These data also indicate microcystin was detected (although values sere generally less than 1 µg/L) on several instances when MSAE cells were not detected (Figure 6)

Similar to, although not as extreme as 2005 (Kann and Corum 2006), later season (October-November) variability in microcystin production per unit cell density (red circles; Figure 6) was also evident in 2006. However, unlike 2005, the ratio of toxin per unit MSAE cell was not substantially lower in October than it was for July-September (Figure 7).

The relationship of MSAE cell density vs. microcystin relative to public health thresholds indicates that the majority of 8 µg/L microcystin exceedances occurred at MSAE levels greater than 40,000 cells/ml (shaded upper right quadrant; Figure 6). Although there were a few instances when 8 µg/L was exceeded at ~ 38,000 cells/ml (upper left quadrant: Figure 7), the SWRCB/OEHHA public health posting level of 40,000 cells/ml MSAE is adequate to indicate when recreational health thresholds are exceeded.

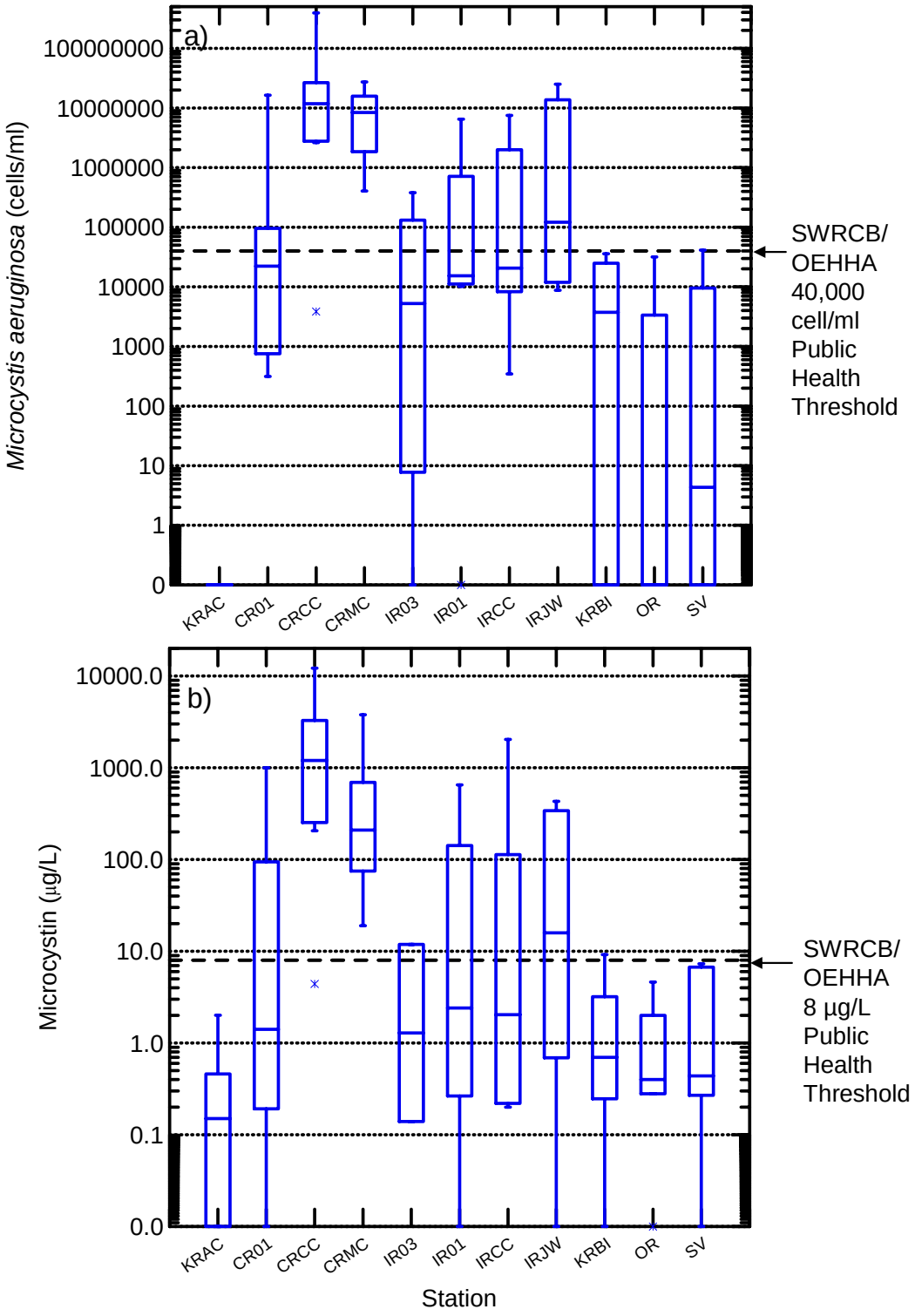


Figure 5. MSAE cell density (a) and microcystin toxin concentration (b) for Klamath River Stations ordered from upstream (left) to downstream (right), 2006.

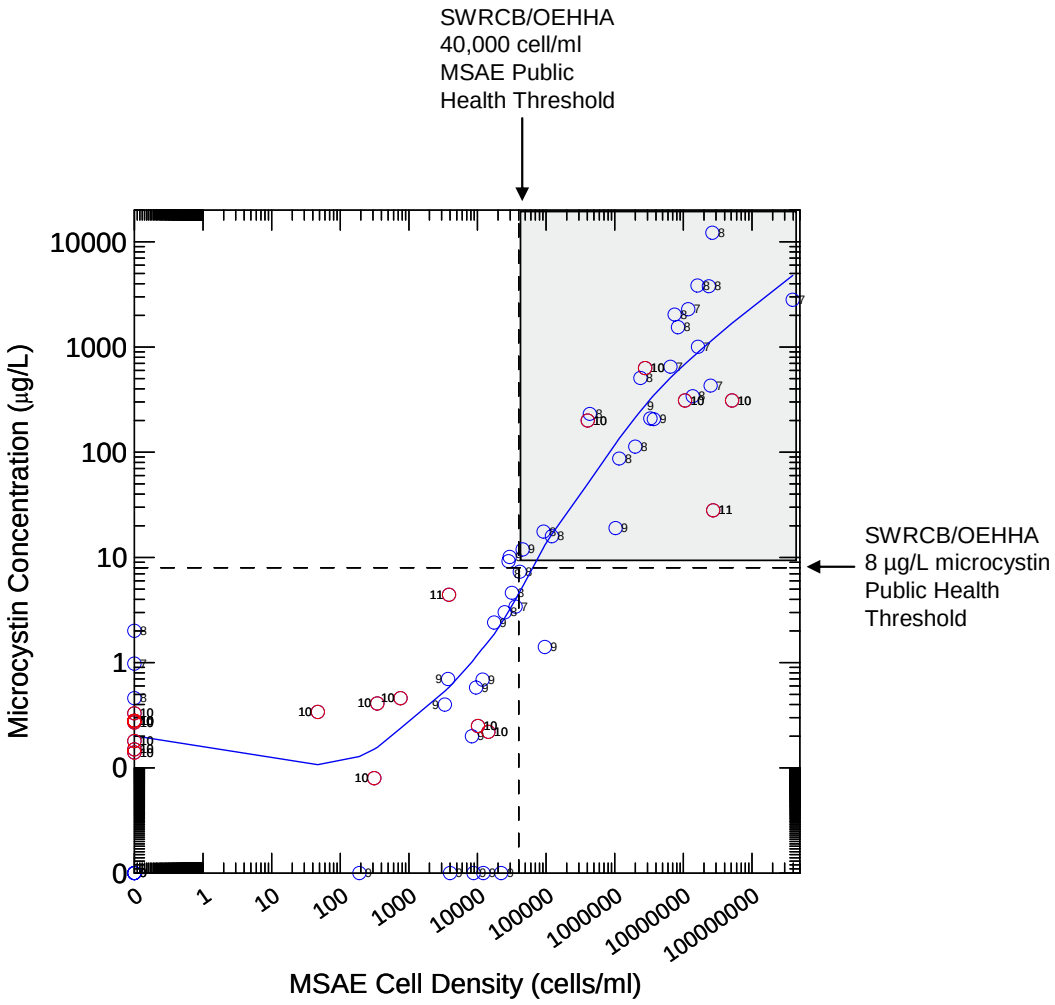


Figure 6. Relationship between MSAE cell density and microcystin toxin concentration (symbol labels are sample dates and October-November dates are shown in red); shown with distance weighted least squares (DWLS) smoother applied to all data, Copco and Iron Gate Reservoirs, 2005.

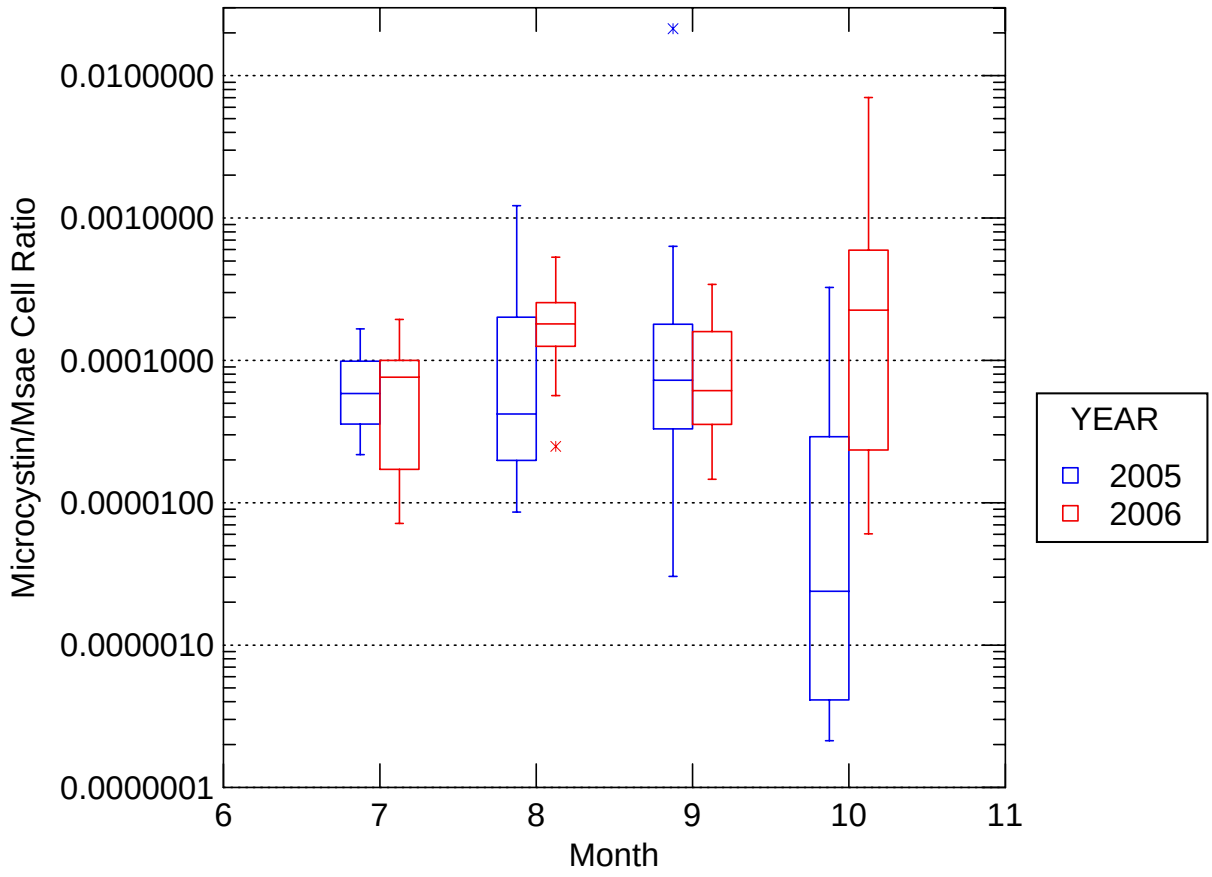


Figure 7. Box plot of the ratio of microcystin toxin per unit MSAE cell density for Copco and Iron Gate Reservoirs, 2005 and 2006. Lower hinge of the box is the lower quartile (25th percentile), upper hinge is the upper quartile (75th percentile), and the whiskers are values 1.5x the upper or lower quartile.

Comparison with 2005 Trends

The general seasonal trend in 2006 was similar to 2005, and public health threshold values were exceeded by hundreds of times in both years (Figure 8a,b). However, both MSAE and microcystin tended to be higher in July and August of 2006 than in 2005, with the reverse true for September (Figure 8a,b). Although MSAE was lower in October of 2006 than in 2005, microcystin tended to be higher in 2006, further illustrating the between-year differences in late season toxin production noted above. The trends above Copco (KRAC) and below Irongate were similar in 2005 and 2006, with no MSAE detected at KRAC in either year, and elevated values shown at KRBI (Figure 8).

The July and September ratio of microcystin to MSAE cell density was similar between 2005 and 2006; however, 2006 showed a higher ratio in August and October of 2006 than occurred for those months in 2005 (Figure 7).

Summary

Similar to 2005, the 2006 sampling program demonstrated widespread and high abundance of toxigenic MSAE blooms in Copco and Iron Gate reservoirs from July-October. MSAE cell density and toxin concentrations in 2006 exceeded public health thresholds by 10 to over 1000 times during these months. Likewise, a 40 pound child accidentally ingesting 100 milliliters of reservoir water would have exceeded the WHO tolerable daily intake level by 10 to over 1600 times during dense bloom periods.

MSAE Blooms and microcystin concentrations peaked in late-July through early-August, and although values continued to exceed public health thresholds through the fall period, an overall declining trend was observed. In general, the Iron Gate decline preceded the Copco decline by several weeks. Also similar to 2005, cell density data indicated that MSAE cells were not detectable in the Klamath River directly above the reservoirs in 2006. During the period of intense blooms in both Copco and Iron Gate reservoirs, the station above Copco (station KRAC) showed non-detects for MSAE. Conversely, the stations below Iron Gate (stations KRBI, SV, and OR), although lower in concentration than the reservoirs, followed a similar seasonal trajectory as the reservoir stations. Low concentrations of microcystin at KRAC on two occasions indicate that microcystin can be transported from upstream areas despite the lack of MSAE detection at KRAC. However, stations below Iron Gate were further elevated (by as much as 20 times on 8/23) compared to the river above Copco Reservoir.

Sample stations were intended to be representative of surface conditions at both shoreline and open-water locations. Although for the overall reservoir study samples were collected at multiple depths (e.g., 1m, 5m, 10m, 25m; Kann and Asarian 2007), the surface samples analyzed herein were specifically collected to assess recreational contact with surface water. Analyses of samples from multiple depths did show a decrease in MSAE as depth decreased; however, MSAE was still detectable and occasionally prevalent even at deeper depths (Kann and Asarian 2007). In addition, satellite imagery clearly shows the widespread nature of cyanobacterial blooms when they occur, and that the locations of sampling stations for this study are not unique with respect to overall spatial distribution of blooms (Figure 9).

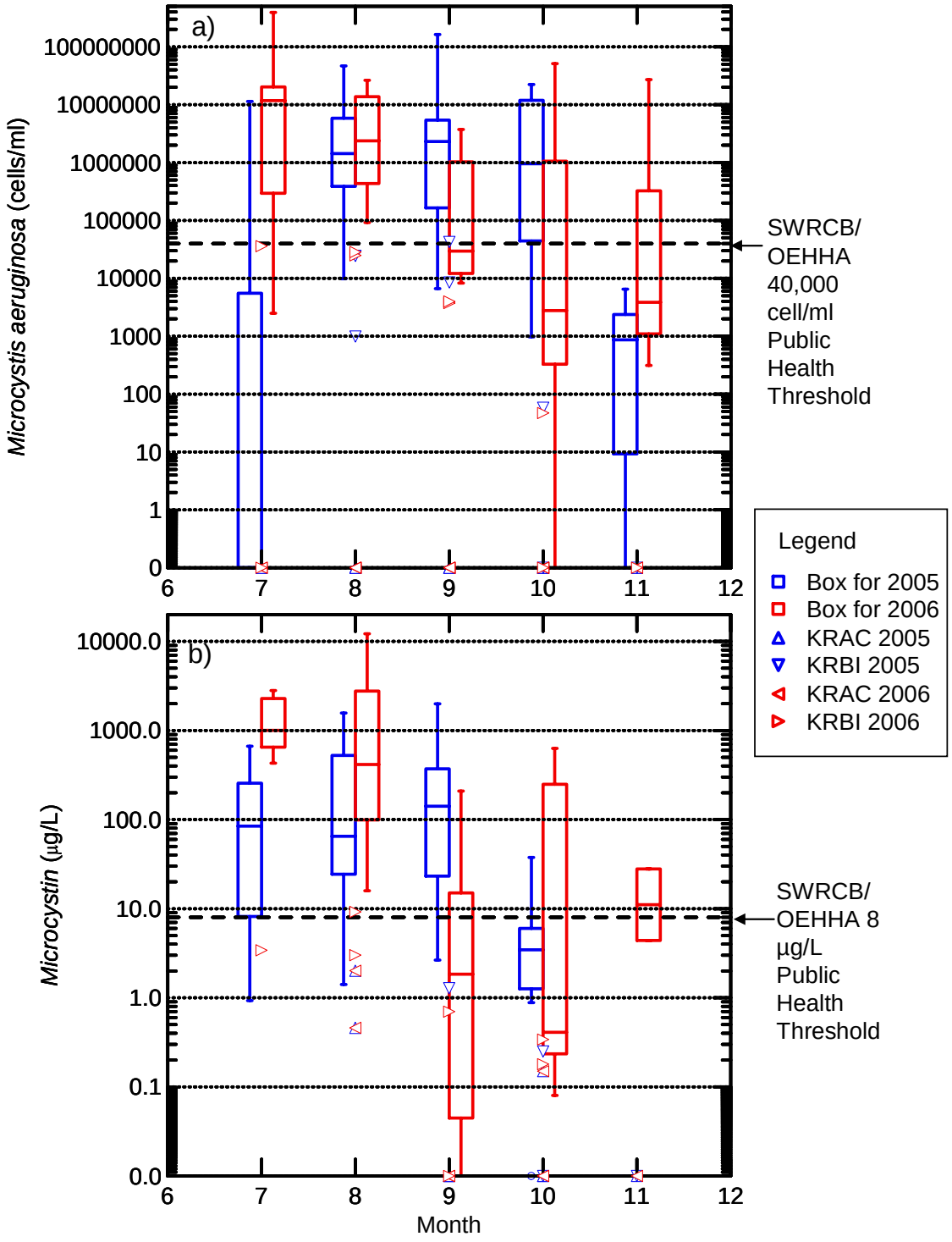


Figure 8. MSAE cell density (a) and microcystin toxin concentration (b) compared between 2005 and 2006.

Figure 9. Satellite imagery of Copco and Iron Gate Reservoirs, CA. Image downloaded from Google Earth.

Similar to 2005, the relationship between MSAE cell density and microcystin toxin in 2006 was variable (although less so than 2005 on a whole-season basis), but showed a strong increasing trend of toxin concentration with cell density. Copco and Iron Gate Reservoir data from 2006 indicate that the SWRCB/OEHHA public health posting levels of 40,000 cells/ml for MSAE and 8 μg/L for microcystin are well supported by the relationship between MSAE cell density and microcystin toxin concentration.

Given these threshold values, as well as the Australian guideline of 50,000 cells/ml MSAE (at which point a water body is considered to be unsuitable for primary contact recreation; NHMRC 2005) and the WHO guideline considering a cyanobacterial scum in a bathing area to be cause for a high probability of adverse health effects (at which point they recommend "immediate action to control scum contact"; WHO 2003), MSAE bloom conditions in Copco and Iron Gate Reservoirs in 2006 represented a clear public health risk with respect to water contact recreation.

Maximum MSAE cell density and microcystin concentrations measured in 2006 were higher than those in 2005, and were among the highest reported in the literature (e.g., Chorus and Bartram 1999). The maximum microcystin value of 12,176 μg/L exceeded the 8 μg/L threshold level by 1522 times. Monitoring data in 2006 show that the 2005 conditions were not anomalous and that toxigenic blooms are likely to be a recurring phenomenon.

High MSAE cell density (10 to1000's of times higher than guideline levels), the presence of scums in shoreline and open-water areas, and high microcystin toxin concentrations in Copco and Iron Gate Reservoirs, necessitates prevention of primary contact recreation in, and either indirect or direct ingestion of, contaminated water in the Klamath River system.

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

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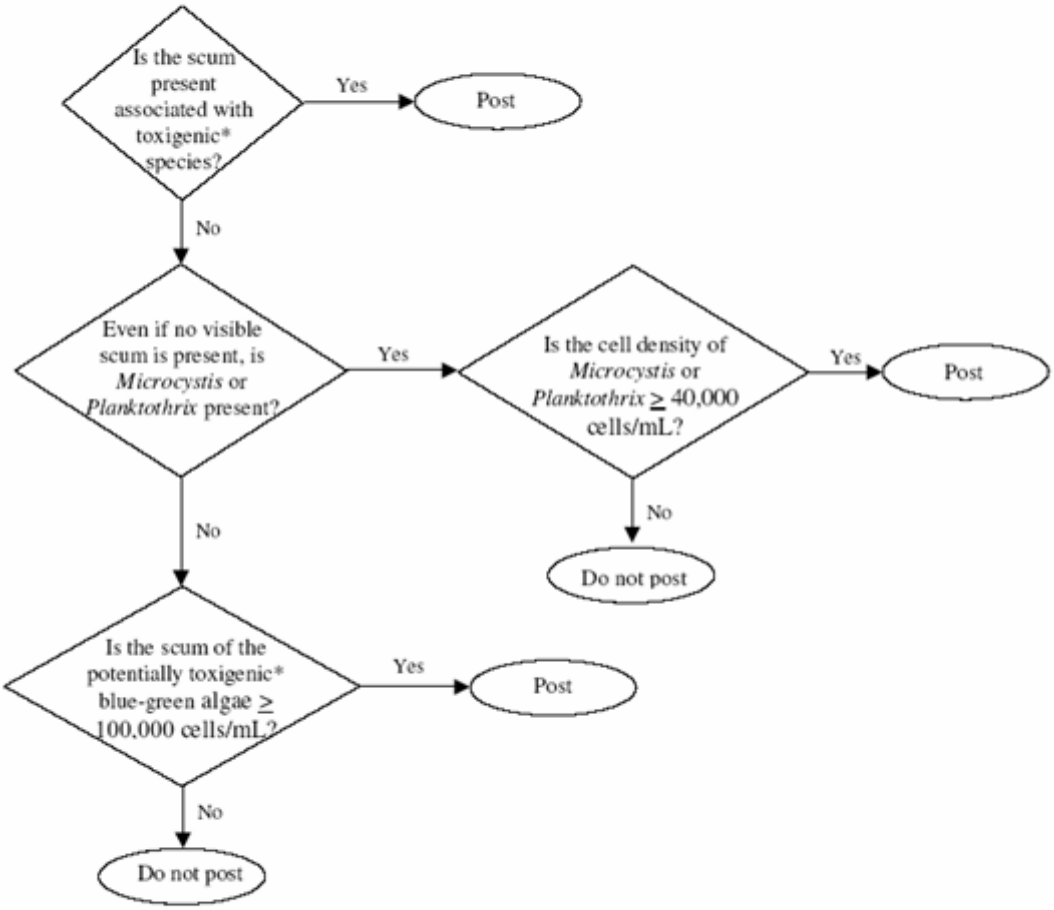
Appendix I

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 (DRAFT June 2007) http://www.waterboards.ca.gov/bluegreenalgae/index.html

Posting Decisions:

- If visible scum is present: Post warning signs and distribute informational brochures.
- When sampling with microbial identification is available, the following decision chart is recommended:



*Potentially toxic blue-green algae that have been detected in California include those of the genera *Anabaena, Microcystis, Aphanizomenon, and Gloeotrichia.* Additional blue-green algae that are known to be potentially toxic may be added to this list.

Appendix II

Quality Assurance Data for Split Samples Collected in the Field.

				Microcystis Colonies (#/ml)				Microcystis Cells (#/ml)				Microcystin (µg/L)			
Date	Sample Station	Duplicate Station Code	Depth	Sample	Blind Duplicate	Sample-Duplicate Difference	RPD	Sample	Blind Duplicate	Sample-Duplicate Difference	RPD	Sample	Blind Duplicate	Sample-Duplicate Difference	RPD
6/1/2006	CR01	CR03	1	0	0	0		0	0	0					
6/15/2006	IR01	IR05	1	0	0	0		0	0	0					
6/29/2006	CR01	CR03	1	0	0	0		0	0	0					
7/13/2006	IR01	IR05	1	165	134	31	21	32,258	13,377	18,881	83				
7/27/2006	IR01	IR05	1	471	371	100	24	57,014	44,543	12,471	25				
8/8/2006	CR01	CR03	5	130	129	1	1	12,978	16,437	-3,459	-24				
8/24/2006	CR01	CR03	0						92,250			21	17.6	3.4	18
8/24/2006	CR01	CR03	1	292	326	-34	-11	29,182	32,572	-3,390	-11				
8/24/2006	IR01	IR05	0						434,893			220	231	-11	-5
8/24/2006	IR01	IR05	1	786	846	-60	-7	78,583	84,563	-5,980	-7				
9/7/2006	IR01	IR05	0	116	175	-59	-41	11,639	17,524	-5,885	-40	1.6	2.4	-0.8	-40
9/21/2006	IR01	IR05	1	0	6	-6	-200	0	601	-601	-200				
10/4/2006	CRMC	CR03	0	2,746	1,212	1,534	78	343,447	403,718	-60,271	-16	160	200	-40	-22
10/18/2006	CRMC	CR03	0	5,745	14,094	-8,349	-84	3,590,764	10,570,313	-6,979,549	-99	390	310	80	23
11/1/2006	CRCC	CR03	0	0	385	-385	-200	0	3,855	-3,855	-200	2.7	4.4	-1.7	-48
11/16/2006	IR01	IR05	1	0	0	0		0	0	0					