SWP Cyanotoxin Monitoring - 2015

Last update: 7/16

Sample analysis conducted by Greenwater Laboratories, Palatka, Florida (see Methods workshhet for description)

Southern Field Division

*Date	Pyramid Lake PY001 (0.4m)				Pyramid Lake PY001 (20 m)				Pyramid Lake PY003 (1m)				Pyramid Lake PY005 (1m)				Castaic Lake CA002 (0.4m)				Silverwood Lake SI002 (0.4 m)				Lake Perris PE002 (0.4m)			
	MC	CYN	STX	ANT	MC	CYN	STX	ANT	MC	CYN	STX	ANT	MC	CYN	STX	ANT	MC	CYN	STX	ANT	MC	CYN	STX	ANT	MC	CYN	STX	ANT
04/20/15																	0.17	ND	ND	ND								
04/21/15	npt	npt	npt	npt																								
04/28/15																									ND	0.19	ND	ND
04/29/15																					ND	ND	ND	ND				
5/18-19/2015	5.74	npt	npt	npt													ND	ND	ND	ND	ND	ND	ND	ND	npt	0.16	npt	npt
05/26/15																												
6/8-9/2015	**79.50**	ND	ND	ND													0.24	ND	ND	ND	ND	npt	npt	npt	ND	0.16	npt	npt
06/22/15																	ND	npt	npt	npt								
06/23/15	**81.50**	npt	npt	npt																					npt	0.12	npt	npt
06/24/15																					ND	npt	npt	npt				
06/30/15	**10.30**	npt	npt	npt	**0.32**	npt	npt	npt	**0.23**	npt	npt	npt																
07/06/15	**3.68**	npt	npt	npt	**0.85**	npt	npt	npt	**1.39**	npt	npt	npt	**4.53**	npt	npt	npt	npt	npt	npt	npt								
07/07/15																									npt	0.10	npt	npt
07/08/15																					0.14	ND	ND	ND				
07/13/15	**3.20**	npt	npt	npt	**1.70**	npt	npt	npt	**2.80**	npt	npt	npt	**0.21**	npt	npt	npt												
07/20/15																	npt	npt	npt	npt					npt	ND	npt	npt
07/21/15	**7.32**	npt	npt	npt	**1.54**	npt	npt	npt					**4.73**	npt	npt	npt					ND	npt	npt	npt				
08/03/15																	npt	npt	npt	npt								
08/04/15	**10.55**	npt	npt	npt	**0.60**	npt	npt	npt	**3.71**	npt	npt	npt	**17.80**	npt	npt	npt												
08/17/15																									ND	0.36	ND	ND
08/18/15	**6.08**	npt	npt	ND	**2.16**	npt	npt	npt	**1.63**	npt	npt	npt	**7.20**	npt	npt	npt	npt	ND	ND	ND	**0.53**	npt	npt	npt				
08/19/15																												
9/14-15/2015	4.00	npt	npt	npt	2.21	npt	npt	npt	5.20	npt	npt	npt	3.10	npt	npt	npt	npt	npt	npt	npt					npt	0.29	npt	npt
09/17/15																					0.64	npt	npt	npt				
09/22/15																												
9/28-29/2015	1.04	npt	npt	npt	1.12	npt	npt	npt	0.76	npt	npt	npt	0.86	npt	npt	npt	0.20	npt	npt	npt	3.71	npt	npt	npt	npt	0.22	npt	npt
10/12-13/2015	0.31	npt	npt	npt	0.26	npt	npt	npt	0.32	npt	npt	npt	0.35	npt	npt	npt	ND	npt	npt	npt	0.39	npt	npt	npt	npt	0.19	npt	npt
10/21/15																												
10/26/15																	0.15	npt	npt	npt					npt	0.30	npt	npt
10/27/15	0.26	npt	npt	npt	1.05	npt	npt	npt	0.26	npt	npt	npt	0.30	npt	npt	npt					1.80	npt	npt	npt				
11/17/15																					2.28	npt	npt	npt				

Units = ug/L

Toxins – Microcystins (MC), cylindrospermopsin (CYN), saxitoxin (STX), anatoxin-A (ANT)

ND = Not detected above the LOD;

"npt" = No potential toxin producers observed by microscopy and ELISA analysis not conducted

LOD = 0.05 µg/L (ANT & STX), 0.1 µg/L CYN, 0.15 µg/L MC

****** Indicates LC/MS conducted to determine concentration of microcystin variants (see MC-variant worksheet)

PTOX species, detectable toxin levels and variant concentration determined by LC/MS and external standard.

Toxins – Anatoxin-a (ANT) cylindrospermopsin (CYN), microcystin (MC), saxitoxin (STX)

Date	Station (drop list)	Site depth (m)	Toxin (Drop list)	Conc. (ug/L)	PTOX Species (Drop list)	(Drop list)	(Drop list)		Congener concentration determined by LC/MS
4/20/2015	Castaic	CA02-1m	MC	0.17	*Dolichospermum*				
4/28/2015	Perris	PE02-1m	CYN	0.19	*Dolichospermum*	*Aphanizomenon*			
5/18/2015	Perris	PE02-1m	CYN	0.16	*Aphanizomenon*				
5/19/2015	Pyramid	PY03-1m	MC	5.74	*Microcystis*				MC-LA = 2 ug/L
6/8/2015	Pyramid	PY03-1m	MC	79.50	*Microcystis aeruginosa*	*Worenchinia naegelianum*	*Dolichospermum*	*Gloeotrichia*	MC-LA = 74 ug/L; MC-LR = < 1 ug/L
6/8/2015	Perris	PE02-1m	CYN	0.15	*Worenchinia naegelianum*				
6/9/2015	Castaic	CA02-1m	MC	0.24	*Microcystis*				
6/23/2015	Pyramid	PY01-1m	MC	81.50	*Microcystis aeruginosa*	*Worenchinia naegelianum*	*Gloeotrichia*		MC-LA = 50 ug/L
6/23/2015	Perris	PE02-1m	CYN	0.12	Aphanizomenon				
6/30/2015	Pyramid	PY01-1m	MC	10.30	Microcystis	Gloeotrichia			MC-LA = 19 ug/L
6/30/2015	Pyramid	PY01-20m	MC	0.32	Microcystis				
6/30/2015	Pyramid	PY03-1m	MC	0.23	Microcystis	Aphanizomenon			
7/6/2015	Clifton Court FB		MC	0.37	Microcystis	Aphanizomenon			
7/6/2015	Pyramid	PY01-1m	MC	3.68	Microcystis				
7/6/2015	Pyramid	PY01-20m	MC	0.85	Microcystis				
7/6/2015	Pyramid	PY03-1m	MC	1.39	Microcystis	Aphanizomenon			
7/6/2015	Pyramid	PY05-1m	MC	4.53	Microcystis	Aphanizomenon	*Gloeotrichia*		MC-LA = 1.8 ug/L
7/6/2015	Pyramid	Yellowbar	MC	3.80	Microcystis	Gloeotrichia			
7/6/2015	Pyramid	Vaquero	MC	3.10	Microcystis	Aphanizomenon			
7/7/2015	Perris	PE02-1m	CYN	0.10	Dolichospermum				
7/8/2015	Silverwood		MC	0.14	Worenchinia naegelianum				
7/13/2015	Pyramid	PY01-1m	MC	3.20	Microcystis				
7/13/2015	Pyramid	PY01-20m	MC	1.70	Microcystis				
7/13/2015	Pyramid	PY03-1m	MC	2.80	Microcystis	Aphanizomenon			
7/13/2015	Pyramid	PY05-1m	MC	0.21	Microcystis				
7/15/2015	NBA Barker SI PP		MC	0.73	Planktothrix	Cuspidothrix	*Dolichospermum*		
7/15/2015	NBA Barker SI PP		ANTX-A	.05-0.1	Planktothrix	Cuspidothrix	*Dolichospermum*		
7/20/2015	Check 13		MC	0.39	Microcystis				
7/21/2015	Pyramid	PY01-1m	MC	7.32	Microcystis				
7/21/2015	Pyramid	PY01-20m	MC	1.54	Microcystis				
7/21/2015	Pyramid	PY05-1m	MC	4.73	Microcystis	Gloeotrichia			MC-LA (1.5-4.5 ppb) and MC-LR (1-3 ppb)
8/4/2015	Pyramid	PY01-1m	MC	10.55	Microcystis				
8/4/2015	Pyramid	PY01-20m	MC	0.60	Microcystis				
8/4/2015	Pyramid	PY03-1m	MC	3.71	Microcystis				
8/4/2015	Pyramid	PY05-1m	MC	17.80	Microcystis				MC-LA (7 ug/L); MC-LR (1 ug/l)
8/10/2015	Clifton Court FB		MC	0.17	Microcystis	Dolichospermum			
8/10/2015	SLR Gianelli		MC	1.58	Microcystis	Dolichospermum	*Worenchinia naege*	Aphanizomenon	
8/10/2015	Check 13		MC	1.66	Microcystis	Dolichospermum	*Aphanizomenon*		
8/17/2015	Perris	PE02-1m	CYN	0.36	Dolichospermum	Chrysosporum ovalisporum			
8/18/2015	Pyramid	PY01-1m	MC	6.08	Microcystis	Planktothrix	*Dolichospermum*		
8/18/2015	Pyramid	PY01-20m	MC	2.16	Microcystis				
8/18/2015	Pyramid	PY05-1m	MC	7.20	Microcystis	Limnoraphis birgei			MC-LA, MC-LR
8/18/2015	Pyramid	PY03-1m	MC	1.63	Microcystis				
8/19/2015	NBA Barker SI PP		MC	0.21	Dolichospermum	Aphanizomenon			
8/24/2015	Clifton Court FB		MC	0.26	Microcystis	Planktothrix	*Dolichospermum*		
8/24/2015	Check 13		MC	4.75	Dolichospermum	Microcystis			
8/24/2015	SLR Gianelli		MC	1.65	Microcystis	Dolichospermum	Aphanizomenon		
8/31/2015	Banks		MC	0.24	Microcystis	Aphanizomenon			
8/31/2015	Clifton Court FB		MC	0.47	Microcystis	Planktothrix	Aphanizomenon	*Dolichospermum*	
9/14/2015	Check 13		**MC**	1.09	Planktothrix	Dolichospermum	Microcystis	*Aphanizomenon*	
9/14/2015	SLR Gianelli		MC	1.27	Microcystis	Dolichospermum	Planktothrix	*Aphanizomenon*	
9/14/2015	Clifton Court FB		MC	0.29	Aphanizomenon	Planktothrix	Planktothrix	*Aphanizomenon*	
9/14/2015	Perris		CYN	0.29	Aphanizomenon				
9/15/2015	Pyramid	PY01-1m	MC	4.00	Microcystis	Aphanizomenon	Limnoraphis birgei		
9/15/2015	Pyramid	PY01-20m	MC	2.21	Microcystis	Aphanizomenon	Limnoraphis birgei		
9/15/2015	Pyramid	PY05-1m	MC	3.10	Microcystis	Aphanizomenon			
9/15/2015	Pyramid	PY03-1m	MC	5.20	Microcystis	Aphanizomenon	Dolichospermum	*Planktothrix*	
9/16/2015	NBA Barker SI PP		**MC**	0.50	Cuspidothrix	Dolichospermum	*Aphanizomenon*		
9/17/2015	Silverwood		**MC**	0.64	Microcystis				
9/21/2015	Clifton Court FB		MC	0.20	Microcystis	Aphanizomenon			
9/28/2015	Castaic	CA02-1m	MC	0.20	Pseudanabaena				
9/29/2015	Pyramid	PY01-1m	MC	1.04	Microcystis	Aphanizomenon			
9/29/2015	Pyramid	PY01-20m	MC	1.12	Microcystis				
9/29/2015	Pyramid	PY05-1m	MC	0.86	Microcystis	Aphanizomenon			
9/29/2015	Pyramid	PY03-1m	MC	0.76	Microcystis	Aphanizomenon			
9/28/2015	Perris	PE02-1m	CYN	0.22	Aphanizomenon				
9/29/2015	Silverwood	SI02-1m	MC	3.71	Woronichinia naegeliana	Microcystis	*Limnoraphis birgei*		
9/28/2015	SLR Pacheco PP		MC	1.63	Microcystis				
9/28/2015	Check 13		MC	0.41	Microcystis	Dolichospermum	*Planktothrix*	Aphanizomenon	
9/28/2015	Check 13		STX	0.05	Microcystis	Dolichospermum	*Planktothrix*	Aphanizomenon	
9/28/2015	SLR Gianelli		MC	0.57	Microcystis				
10/13/2015	Pyramid	PY01-1m	MC	0.31	Microcystis				
10/13/2015	Pyramid	PY01-20m	MC	0.26	Microcystis				
10/13/2015	Pyramid	PY05-1m	MC	0.35	Microcystis				
10/13/2015	Pyramid	PY03-1m	MC	0.32	Microcystis				
10/13/2015	Silverwood	SI02-1m	MC	0.39	Woronichinia naegeliana	Microcystis	*Limnoraphis birgei*		
10/12/2015	Perris	PE02-1m	CYN	0.19	Anabaenopsis	Aphanizomenon			
10/14/2015	SLR Pacheco PP		MC	1.65	Microcystis	Planktothrix			
10/14/2015	Check 13		MC	0.86	Microcystis	Dolichospermum	*Planktothrix*	Aphanizomenon	
10/14/2015	SLR Gianelli		MC	0.70	Microcystis				
10/26/2015	Perris		CYN	0.30	Aphanizomenon				
10/27/2015	Silverwood		MC	1.80	Woronichinia naegeliana	Microcystis	Limnoraphis birgei		
10/26/2015	Castaic	CA02-1m	MC	0.15	Aphanizomenon	Pseudanabaena			
10/27/2015	Pyramid	PY01-1m	MC	0.26	Microcystis				
10/27/2015	Pyramid	PY01-20m	MC	1.05	Microcystis				
10/27/2015	Pyramid	PY05-1m	MC	0.30	Microcystis				
10/27/2015	Pyramid	PY03-1m	MC	0.26	Microcystis				
10/26/2015	SLR Pacheco PP		MC	1.84	Microcystis	Planktothrix			
10/26/2015	Check 13		MC	0.95	Microcystis	Planktothrix	Dolichospermum	Aphanizomenon	
10/26/2015	SLR Gianelli		MC	1.00	Microcystis	Woronichinia naegeliana	Planktothrix	Dolichospermum	
11/9/2015	SLR Pacheco PP		MC	0.41	Planktothrix	Microcystis			
11/9/2015	Check 13		MC	0.54	Microcystis	Planktothrix	Dolichospermum	Aphanizomenon	
11/9/2015	SLR Gianelli		MC	0.16	Microcystis	Planktothrix	Dolichospermum	Aphanizomenon	
11/17/2015	Silverwood		MC	2.28	Microcystis	Woronichinia naegeliana	Aphanizomenon		
12/7/2015	SLR Pacheco PP		MC	0.22	Microcystis				

Methods- Greenwater Laboratories, Palatka, FL

1. PTOX Screening Method- Microscopic

One mL from of the sample was preserved with Lugol's iodine solution and allowed to settle. Entire samples were scanned at 100X for the presence of potentially toxigenic (PTOX) cyanobacteria using a Nikon Eclipse TE100 Inverted Microscope equipped with phase contrast optics. Higher magnification was used as necessary for identification.

2. **Analytical Methodology –ELISA**

Cylindrospermopsin (CYN), microcystin (MC), saxitoxin (STX)
Sample Prep – The samples were ultra-sonicated to lyse all cells and release toxins. Duplicate samples (Lab Fortified Matrix, LFM) were spiked at 1.0 µg/L CYN, 1.0 µg/L MCLR and 0.2 µg/L STX, which provided quantitative capability and additional qualitative confirmation.

MC's
A microcystins enzyme linked immunosorbent assay (ELISA) was utilized for the quantitative and sensitive congener-independent detection of MCs. The current assay is sensitive down to a LOD/LOQ of 0.15 µg/L for total MCs. The average recovery of a laboratory fortified blank (LFB) spiked with 1 µg/L MCLR was 87%.

CYN
A cylindrospermopsin enzyme linked immunosorbent assay (ELISA) was also utilized for the quantitative detection of CYN. The current assay is sensitive down to a LOD/LOQ of 0.1 µg/L for CYN. A lab fortified blank (LFB) spiked with 1.0 µg/L CYN was recovered at 87%.

PARALYTIC SHELLFISH TOXINS / SAXITOXIN
PST's /SXT
A saxitoxin enzyme linked immunosorbent assay (ELISA) was utilized for the quantitative detection of saxitoxin. The current assay is sensitive down to a LOD/LOQ of 0.02 µg/L bsaxitoxin. The LFB (0.2 µg/L STX spike) recovery was 86%.

3. **Analytical Methodology –Microcystin Congeners**
Sample Prep – The sample was ultra-sonicated to lyse cells and release toxins. Solid phase extraction (SPE - Strata X) was utilized to pre-concentrate the sample (100x) for LC/MS/MS confirmatory analysis and identification of microcystin congeners/variants.

Analytical Methodology – LC/MS (scan from 200-1500 *m/z*) was used to screen for the most common microcystin variants. LC/MS/MS was utilized for confirmation of seven microcystin variants; MC-RR, MC-YR, MC-LR, MC-dmLR, MC-LA, MC-LW, and MC-LF. The following transitions were monitored: MC-RR (519.5→452.7 & 1038.5→1020.5 *m/z*), MC-YR (1045.5→1027.6 *m/z*), MC-LR (995.5→553.3 & 599.5 *m/z*), dmMC-LR (981.5→852.5 *m/z*), MC-LA (910.5 & 932.5 →904.4 & 419.1 *m/z*), MC-LW (1025.5 & 1047.6→640.2 & 1019.5 *m/z*), and MC-LF (986.5 & 1008.5 → 419.1 & 980.5 *m/z*). Approximation of microcystin concentrations was achieved using an external standard. The method detection limits (MDLs) ranged from 0.05– 0.1 µg/L for MCs and were based on instrument sensitivity and concentration of extract.
Toxins – Anatoxin-a (ANTX-A)

Sample Prep – The sample was ultra-sonicated to lyse cells and release toxins. Solid phase extraction (SPE - Strata X) was utilized to pre-concentrate the sample (100x) for ANTX-A analysis. A duplicate sample was spiked (lab fortified matrices, LFM) at 0.1 µg/L ANTX-A, which provided quantitative capability and additional qualitative confirmation.

Analytical Methodology – Liquid chromatography/ mass spectrometry/ mass spectrometry (LC- MS/MS) was utilized for the determination of ANTX-A. The $[M+H]^+$ ion for ANTX-A (*m/z* 166) was fragmented and the major product ions (*m/z* 149, 131, 107, and 91) provided both specificity and sensitivity. The current methodology established a limit of detection (LOD) of 0.05 µg/L and a limit of quantification (LOQ) of 0.1 µg/L for ANTX-A.

A microcystins enzyme linked immunosorbent assay (ELISA) was utilized for the quantitative and sensitive congener-independent detection of MCs. The current assay is sensitive down to a LOD/LOQ of 0.15 µg/L for total MCs. The average recovery of the lab fortified blanks (LFB) spiked with 1 µg/L MCLR was 110% with an LFM of 106%.