SWP Cyanotoxin Monitoring - 2016

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

Southern Field Division																														
	Pyramid Lake PY001 (1m)				Pyramid Lake PY001 (20m)				Pyramid Lake Emigrant Landing Swim Beach				Pyramid Lake Vaquero Swim Beach (1m)				Castaic Lake CA002 (1m)				Silverwood Lake SI002 (1m)				Silverwood Lake Cleghorn (1m)				Lake Perris PE002 (1m)			
*Date	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	MC	CYN	STX	ANT
04/25/16																	ND	ND	ND	ND									npt	npt	npt	npt
04/26/16	ND	npt	npt	npt																	npt	npt	npt	npt								
05/23/16	npt	npt	npt	npt													ND	ND	ND	ND									ND	npt	npt	npt
05/24/16																					npt	npt	npt	npt								
06/06/16																	0.5	ND	ND	ND									ND	0.16	ND	ND
06/07/16	0.77	npt	npt	npt																	ND	ND	ND	ND								
06/20/16																													ND	0.13	ND	ND
06/21/16																					0.22	ND	ND	ND								
06/27/16																	0.2	npt	npt	npt												
06/28/16	4.98	ND	ND	ND																												
07/06/16	5.2	npt	npt	npt									26	npt	npt	npt																
07/11/16																	ND	ND	ND	ND									ND	ND	ND	ND
07/12/16	18.6	npt	npt	npt									14	npt	npt	npt					0.28	npt	npt	npt								
07/19/16	6.6	npt	npt	npt	6	npt	npt	npt	5.3	npt	npt	ND	6.3	npt	npt	npt	npt	npt	npt	npt												
07/21/16									3.6	npt	npt	npt	3.3	npt	npt	npt																
07/25/16	11.5	npt	npt	npt	1.34	npt	npt	npt	3.88	npt	npt	npt	5	npt	npt	npt					1.09	npt	npt	npt	17.2	ND	ND	ND				
07/27/16																													npt	ND	npt	npt
08/02/16	3.75	npt	npt	npt	0.57	npt	npt	npt	0.87	npt	npt	npt	3.4	npt	npt	npt																
08/04/16																					0.55	npt	npt	npt	381	npt	npt	npt				
08/08/16																	npt	npt	npt	npt												
08/09/16	3.25	npt	npt	npt	0.43	npt	npt	npt	2.36	npt	npt	npt	3.23	npt	npt	npt													ND	ND	ND	ND
08/10/16																					ND	npt	npt	npt	0.36	npt	npt	npt				
08/16/16	4.75	npt	npt	npt	0.34	npt	npt	npt	1.29	npt	npt	npt	3.48	npt	npt	npt					ND	npt	npt	npt	0.64	npt	npt	npt				
08/22/16																																
08/23/16	not sampled				not sampled				0.41	npt	npt	npt	3.93	npt	npt	npt					npt	npt	npt	npt	0.15	npt	npt	npt	ND	0.11	ND	ND
08/25/16																					ND	npt	npt	npt	ND	npt	npt	npt				
08/29/16	0.25	npt	npt	npt	ND	npt	npt	npt	ND	npt	npt	npt	0.3	npt	npt	npt	ND	npt	npt	npt												
09/06/16																					ND	npt	npt	npt	ND	npt	npt	npt				
09/08/16	3.00	npt	npt	npt	2.93	npt	npt	npt	2.10	npt	npt	npt	1.7	npt	npt	npt																
09/12/16																	ND	npt	npt	npt	npt	npt	npt	npt	ND	npt	npt	npt				
09/14/16	0.76	npt	npt	npt	0.70	npt	npt	npt	0.83	npt	npt	npt	0.96	npt	npt	npt																
09/20/16	0.20	npt	npt	npt	0.26	npt	npt	npt	0.49	npt	npt	npt	0.45	npt	npt	npt																
09/26/16	ND	npt	npt	npt	ND	npt	npt	npt	0.20	npt	npt	npt	ND	npt	npt	npt	npt	npt	npt	npt									npt	ND	npt	npt
09/27/16																					ND	ND	ND	ND								
10/05/16	ND	npt	npt	npt	ND	npt	npt	npt	ND	npt	npt	npt	ND	npt	npt	npt																
10/10/16																													ND	npt	npt	npt
10/11/16	npt	npt	npt	npt	npt	npt	npt	npt	ND	npt	npt	npt	ND	npt	npt	npt	npt	npt	npt	npt	npt	npt	npt	npt	ND	npt	npt	npt				
10/24/16																	npt	npt	npt	npt	npt	npt	npt	npt	ND	npt	npt	npt				
10/26/16																													npt	npt	npt	npt
10/27/16	npt	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
5/31/2016	Lake Del Valle	1	MC	0.16	*Microcystis*	Woronichinia naegeliana	*Aphanizomenon*	Aphanothece		
6/6/2016	Castaic	1	MC	0.45	*Dolichospermum*	*Aphanocapsa*	*Microcystis*	Aphanizomenon		
6/7/2016	Pyramid	1	MC	0.77	*Microcystis*					
6/6/2016	Perris	1	CYN	0.16	*Aphanocapsa*	*Dolichospermum*				
6/20/2016	Perris	1	CYN	0.13	*Dolichospermum*					
6/21/2016	Silverwood	1	MC	0.22	*Dolichospermum*	Limnoraphis robusta				
6/20/2016	Clifton Court FB	1	MC	0.22	*Dolichospermum*	Microcystis	*Aphanizomenon*			
6/28/2016	Castaic	1	MC	0.23	*Microcystis*	*Dolichospermum*				
6/28/2016	Pyramid	1	MC	4.98	Microcystis	Dolichospermum	*Woronichinia naegeliana*	Aphanizomenon		MC-RR (0.2 ppb), MC-LR (0.4 ppb), MC-LA (0.8 ppb)
7/6/2016	Pyramid	1	MC	5.20	Microcystis	Woronichinia naegeliana	*Dolichospermum*			MC-RR (0.1 ppb), MC-YR (0.6 ppb), HtYR (0.2 ppb), MC-LR (0.6 ppb), [Dha7]MC-LR (0.1 ppb), MC-LA (1.2 ppb), MC-LF (0.1 ppb)
7/6/2016	Pyramid-Vaquero Swim Beach	1	MC	26.00	Microcystis	Woronichinia naegeliana	*Dolichospermum*	Aphanizomenon		MC-RR (0.1 ppb), MC-YR (0.4 ppb), HtYR (1.7 ppb), MC-LR (2.3 ppb), [DAsp3]MC-LR (0.10 ppb), [Dha7]MC-LR (0.1 ppb), MC-HilR (0.1 ppb), MC-LA (4.0 ppb)
7/12/2016	Pyramid	1	MC	18.60	Dolichospermum	Microcystis	*Woronichinia naegeliana*	Gloeotrichia	Aphanizomenon	
7/12/2016	Pyramid-Vaquero Swim Beach	1	MC	13.70	Microcystis	Woronichinia naegeliana	*Dolichospermum*	Aphanizomenon		
7/11/2016	Banks	1	MC	1.26	Aphanizomenon	Dolichospermum				
7/11/2016	Clifton Court FB	1	MC	1.02	Dolichospermum	Microcystis	*Aphanizomenon*			
7/11/2016	Dyer Res	1	MC	0.26	Aphanizomenon	Planktothrix	*Microcystis*	Dolichospermum	Pseudanabaena	
7/12/2016	Silverwood	1	MC	0.28	Dolichospermum	Microcystis				
7/11/2016	Check 13	1	MC	1.02	Aphanizomenon	Oscillatoria/Phormidium	*Dolichospermum*			
7/11/2016	SLR Gianelli	1	MC	1.15	Microcystis					
7/19/2016	Pyramid-Emigrant Landing Swim Beach	1	MC	5.30	Microcystis	Planktothrix	*Aphanizomenon*	Dolichospermum	Woronichinia naegeliana	
7/19/2016	Pyramid	1	MC	6.60	Microcystis	Aphanizomenon	*Woronichinia naegeliana*	Dolichospermum		
7/19/2016	Pyramid	20	MC	6.00	Microcystis	Woronichinia naegeliana	*Aphanizomenon*	Dolichospermum		
7/19/2016	Pyramid-Vaquero Swim Beach	1	MC	6.30	Microcystis	Woronichinia naegeliana	*Dolichospermum*	Aphanizomenon		
7/21/2016	Pyramid-Emigrant Landing Swim Beach	1	MC	3.60						
7/21/2016	Pyramid-Vaquero Swim Beach	1	MC	3.30						
7/25/2016	Pyramid	1	MC	11.50	Microcystis	Aphanizomenon	*Dolichospermum*			
7/25/2016	Pyramid	20	MC	1.34	Microcystis	Planktothrix				
7/25/2016	Pyramid-Vaquero Swim Beach	1	MC	5.00	Microcystis	Aphanizomenon	*Woronichinia naegeliana*			
7/25/2016	Pyramid-Emigrant Landing Swim Beach	1	MC	3.88	Microcystis	Planktothrix	*Aphanizomenon*	Dolichospermum	Pseudanabaena	
7/25/2016	Silverwood	1	MC	1.09	Dolichospermum	Microcystis	*Aphanocapsa*			
7/25/2016	Silverwood-Cleghorn	1	MC	17.20	Microcystis	Dolichospermum	*Planktothrix*			
7/25/2016	Banks	1	MC	0.23	Aphanizomenon	Dolichospermum				
7/25/2016	Clifton Court FB	1	MC	0.31	Microcystis	Aphanizomenon				
7/25/2016	Check 13	1	MC	0.81	Aphanizomenon	Dolichospermum	*Microcystis*	Woronichinia naegeliana		
7/25/2016	SLR Pacheco PP	1	MC	2.80	Dolichospermum	Aphanizomenon	*Microcystis*	Pseudanabaena		
7/25/2016	SLR Gianelli	1	MC	1.60	Microcystis	Dolichospermum				
7/25/2016	O'Neill Forebay North Beach	1	MC	0.25	Microcystis	Dolichospermum	*Aphanizomenon*			
8/2/2016	Pyramid	1	MC	3.75						
8/2/2016	Pyramid	20	MC	0.57						
8/2/2016	Pyramid-Vaquero Swim Beach	1	MC	3.40						
8/2/2016	Pyramid-Emigrant Landing Swim Beach	1	MC	0.87						
8/4/2016	Silverwood	1	MC	0.55						
8/4/2016	Silverwood-Cleghorn	1	MC	381.00						
8/8/2016	Check 13	1	MC	0.15	Aphanizomenon	Microcystis				
8/8/2016	SLR Pacheco PP	1	MC	3.04	Dolichospermum	Aphanizomenon	*Microcystis*			
8/8/2016	SLR Gianelli	1	MC	0.20	Aphanizomenon	Microcystis	*Dolichospermum*			
8/8/2016	SLR Basalt Boat Launch	1	MC	3.08	Microcystis	Aphanizomenon	Dolichospermum	Woronichinia naegeliana		

8/9/2016	Pyramid	1	MC	3.25	Microcystis	Aphanizomenon	*Woronichinia naegeliana*			
8/9/2016	Pyramid	20	MC	0.43	Microcystis					
8/9/2016	Pyramid-Vaquero Swim Beach	1	MC	3.23						
8/9/2016	Pyramid-Emigrant Landing Swim Beach	1	MC	2.36						
8/22/2016	Check 13	1	MC	0.16	Microcystis	Aphanizomenon				
8/22/2016	SLR Pacheco PP	1	MC	2.85	Dolichospermum	Aphanizomenon	Microcystis			
8/22/2016	SLR Gianelli	1	MC	0.20	Aphanizomenon	Microcystis	Dolichospermum			
8/22/2016	O'Neill Forebay North Beach	1	MC	0.46	Microcystis	Dolichospermum				
8/22/2016	SLR Basalt Boat Launch	1	MC	2.18	Microcystis	Aphanizomenon	Dolichospermum			
8/22/2016	Clifton Court FB	1	MC	0.33	Microcystis	Aphanizomenon	Dolichospermum	*Pseudanabaena*	*Phormidium*	
8/22/2016	Banks	1	MC	0.33	Microcystis	Aphanizomenon				
8/23/2016	Silverwood-Cleghorn	1	MC	0.15						Note: no PTOX species observed in sample. Toxin analysis recommended due to previous MC detections.
8/23/2016	Perris	1	CYN	0.11	Aphanizomenon	Planktothrix	*Dolichospermum*			
9/12/2016	Check 13	1	MC	0.27	Aphanizomenon	Dolichospermum				
9/12/2016	SLR Pacheco PP	1	MC	1.53	Microcystis	Aphanizomenon	*Pseudanabaena mucicola*	Dolichospermum	Pseudanabaena	
9/12/2016	SLR Gianelli	1	MC	0.24	Microcystis	Aphanizomenon	*Pseudanabaena*			
9/12/2016	Banks	1	MC	0.31	Microcystis	Aphanizomenon	*Dolichospermum*	Pseudanabaena	Pseudanabaena mucicola	
9/12/2016	Clifton Court FB	1	MC	0.16	Microcystis	Pseudanabaena mucicola	*Pseudanabaena*	Aphanizomenon		
9/19/2016	Check 13	1	MC	0.16						
9/19/2016	SLR Pacheco PP	1	MC	2.06						
9/19/2016	O'Neill Forebay North Beach	1	MC	0.15						
9/19/2016	SLR Basalt Boat Launch	1	MC	6.83						
10/24/2016	SLR Pacheco PP	1	MC	0.54	Microcystis	Aphanizomenon				
10/24/2016	SLR Basalt Boat Launch	1	MC	3.75	Microcystis	Aphanizomenon	*Dolichospermum*	Woronichinia naegeliana		
11/7/2016	SLR Pacheco PP	1	MC	0.45	Microcystis	Aphanizomenon				
11/7/2016	SLR Basalt Boat Launch	1	MC	3.08	Microcystis	Aphanizomenon	*Woronichinia naegeliana*	Dolichospermum		
11/14/2016	SLR Pacheco PP	1	MC	0.40	Microcystis	Dolichospermum	*Aphanizomenon*	Woronichinia naegeliana		
11/14/2016	SLR Basalt Boat Launch	1	MC	7.68	Microcystis	Aphanizomenon	*Dolichospermum*	Woronichinia naegeliana		
11/28/2016	SLR Pacheco PP	1	MC	0.19	Microcystis					
11/28/2016	SLR Basalt Boat Launch	1	MC	0.38	Microcystis	Aphanizomenon				

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 to 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%.