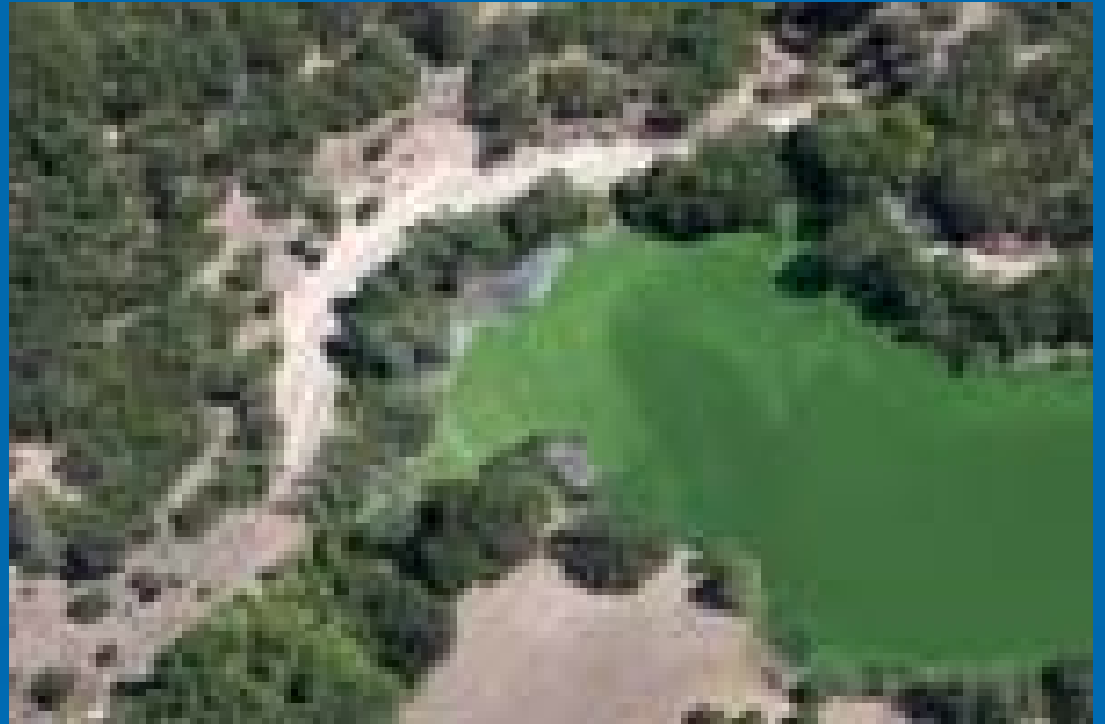
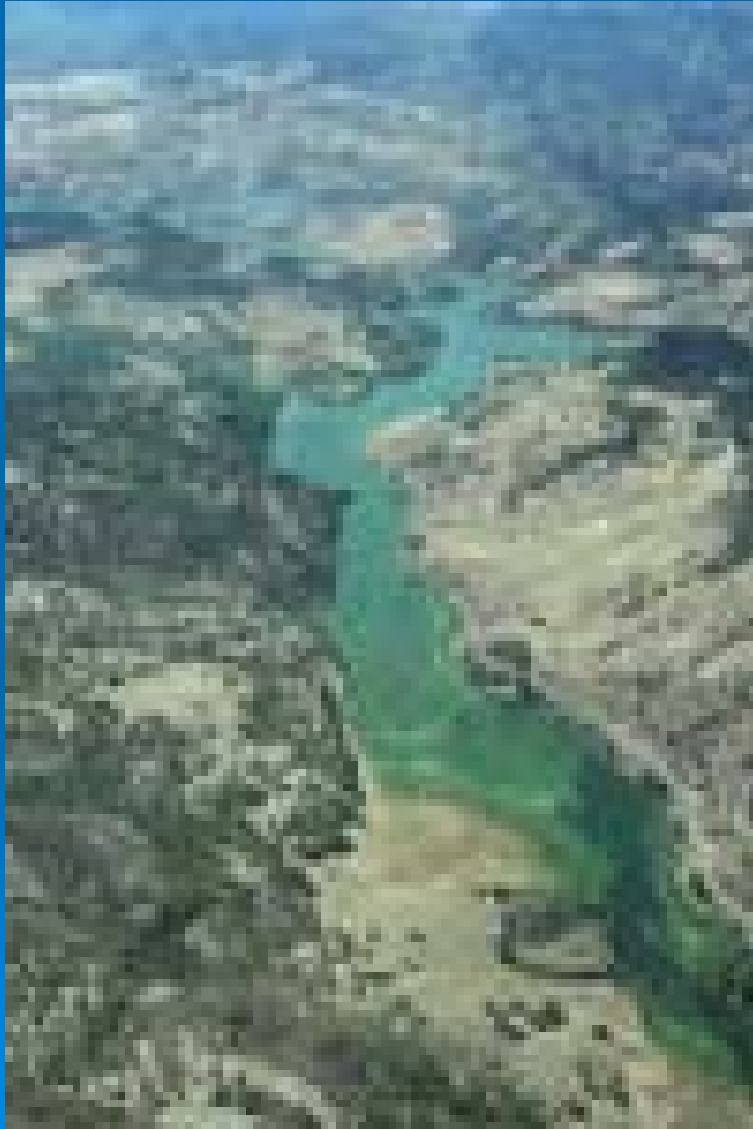


Microcystin Toxins in California Klamath River Lake Success Pinto Lake and Pajaro River 2007

Dave Crane, Abdou Mekebri and Gloria Blondina
CDFG Water Pollution Control Laboratory (WPCL)
2005 Nimbus Road
Rancho Cordova, CA 95670

Melissa A. Miller, DVM, PhD
Marine Wildlife Veterinary Care and Research Center
Department of Fish and Game and
University of California, Davis
1451 Shaffer Rd.
Santa Cruz, CA 95060

Iron Gate and Copco Reservoirs

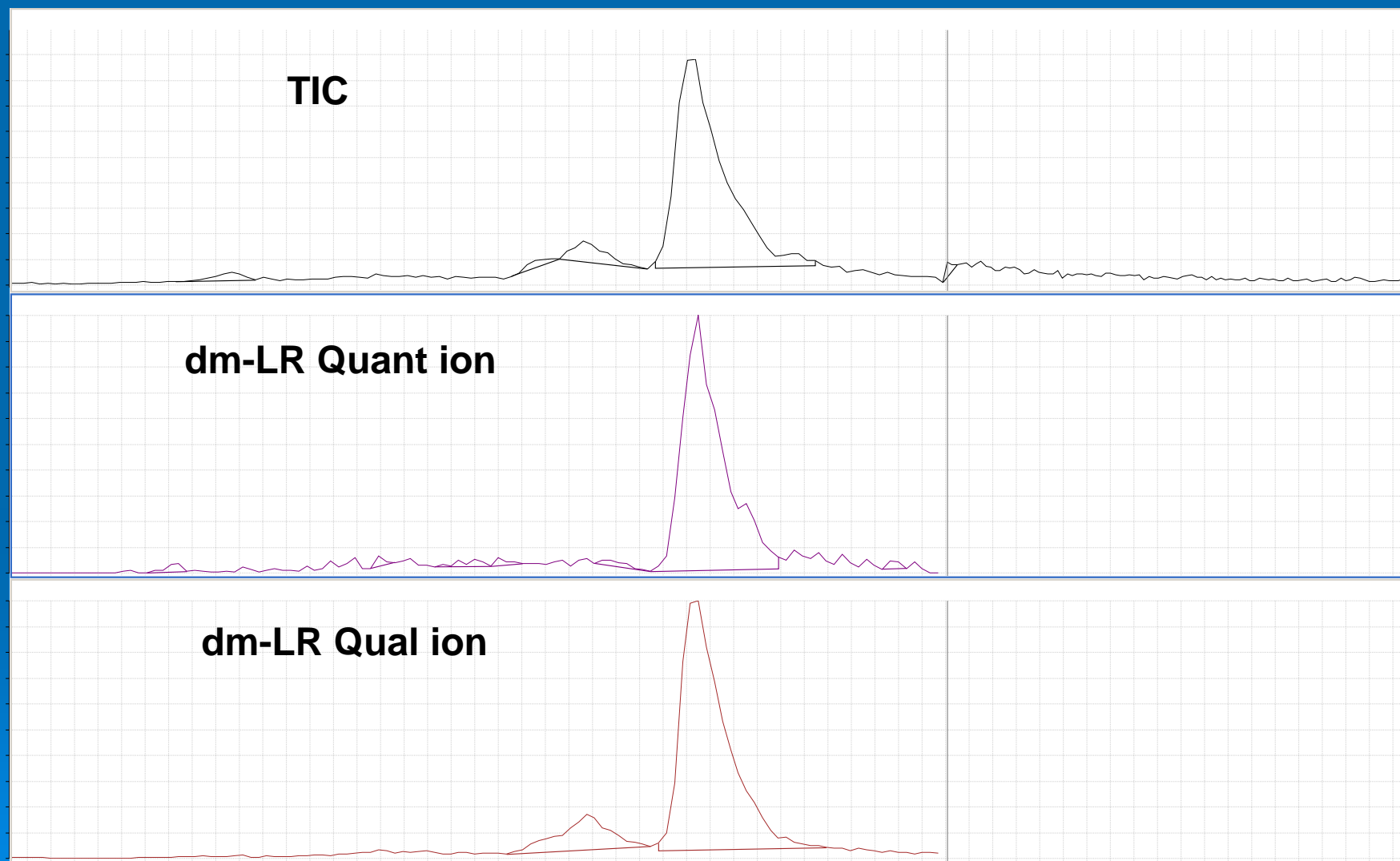


Summary of Microcystin Tissue Results (ppb)

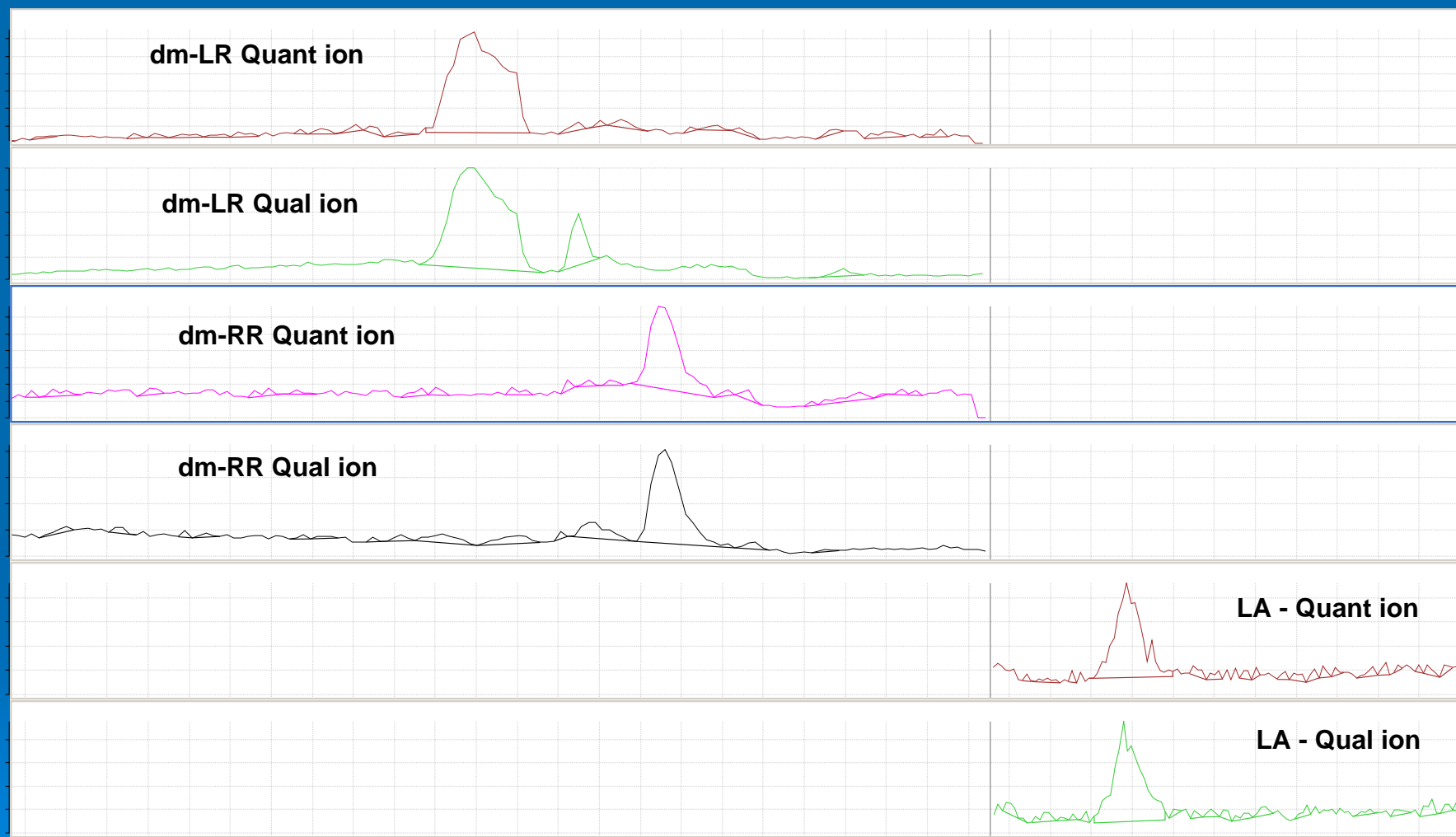
Microcystin Analytes	Irongate Reservoir		Copco Reservoir			Klamath River
	Yellow Perch		Yellow Perch		Mussel	Mussel
	Fillet	Liver	Fillet	Liver		
MCY-RR		ND-15.7				ND-136
MCY-dm-RR*		ND-42.2		25.0-61.6		
MCY-LR						58.1-396
MCY-dm-LR*	ND-227		58.4-422	138-426		5.10-36.6
MCY-YR	ND-4.23		ND-3.16			
MCY-LA		ND-12.7		7.88-14.7	32.3-57.0	138-2,200
MCY-LW						
MCY-LF						ND-14.5

* Demethyl analog quantified as parent compound.

Copco Reservoir – Yellow Perch Fish Tissue

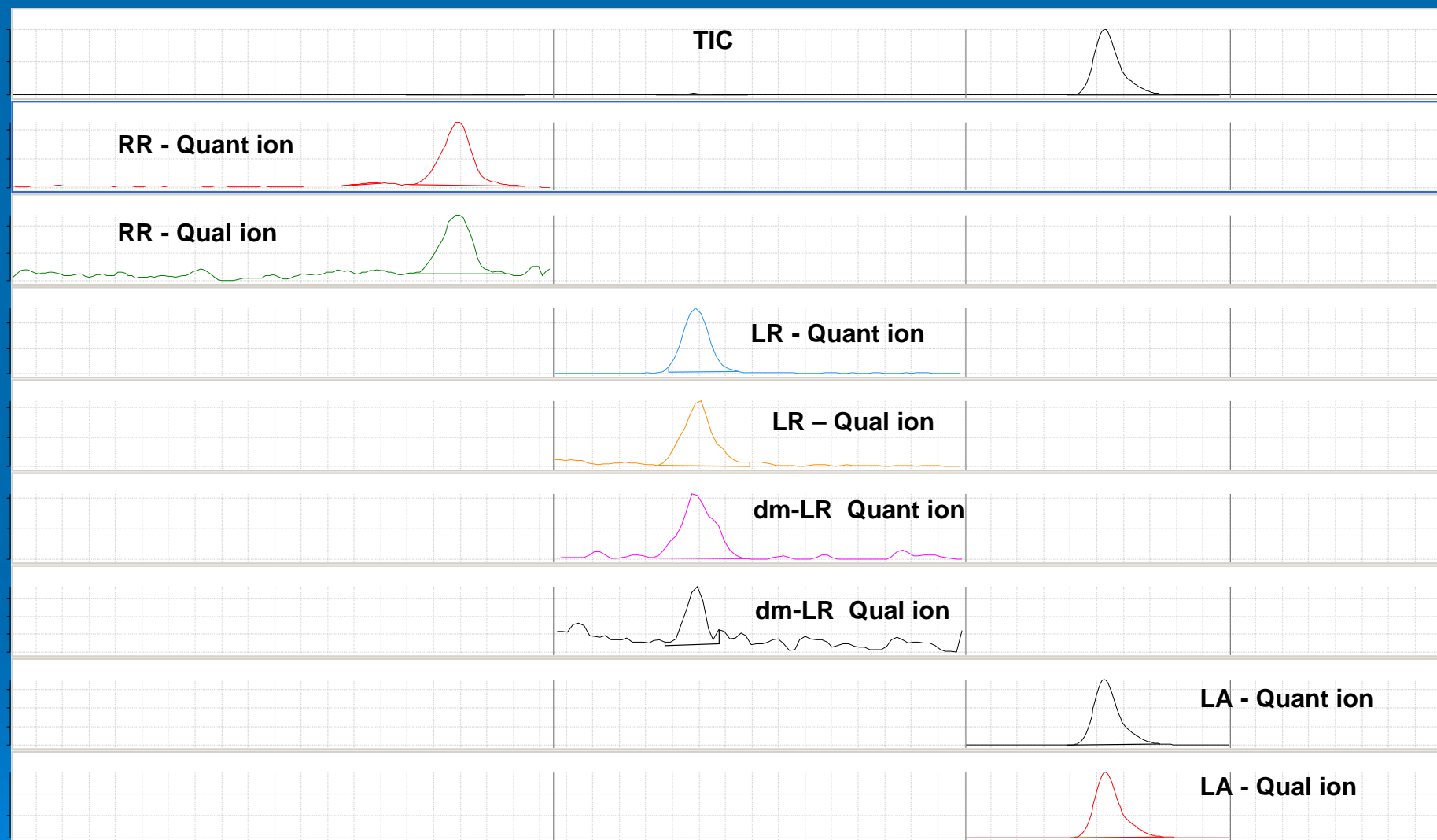


Copco Reservoir - Yellow Perch Liver Tissue



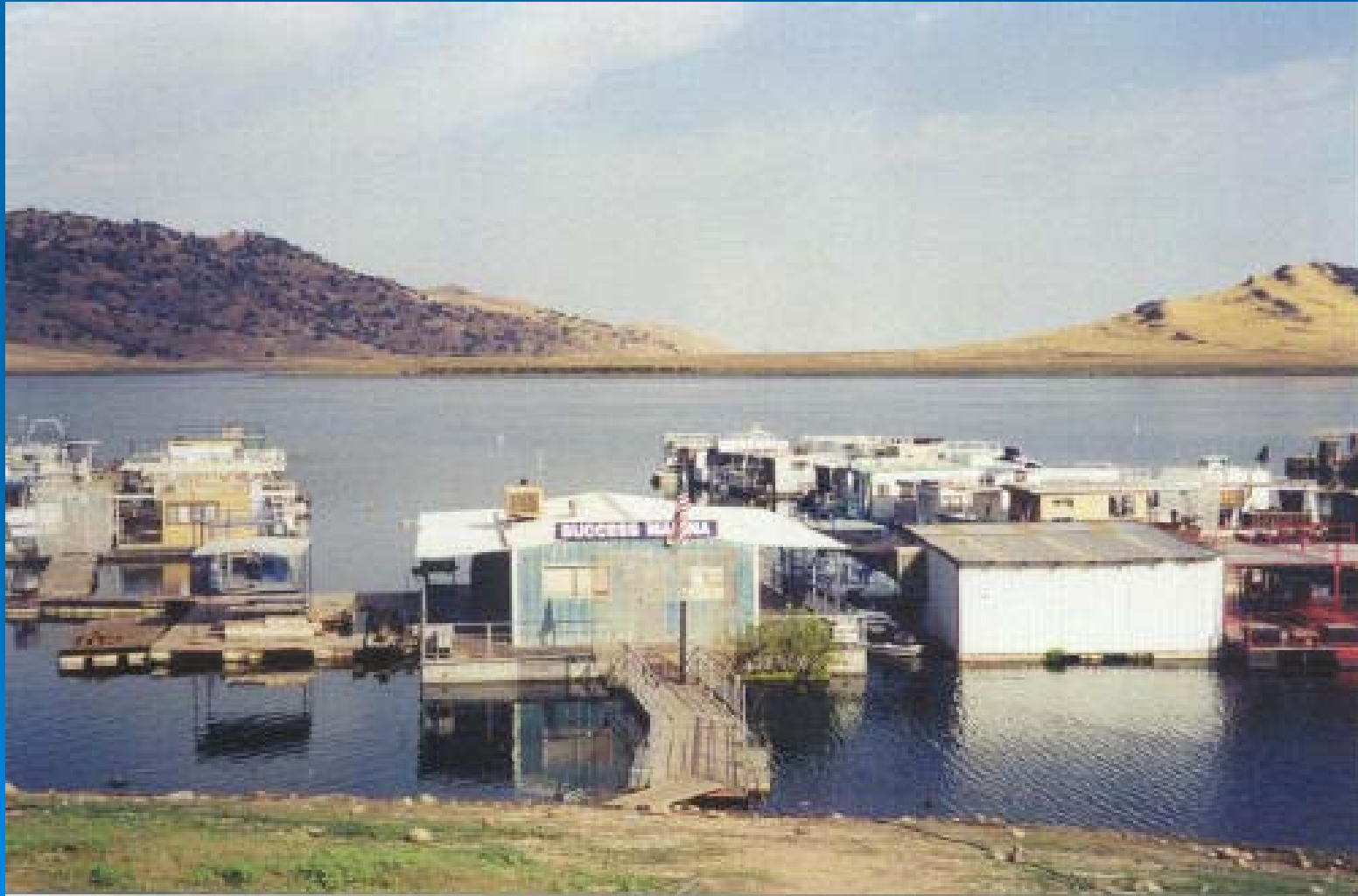
Analysis by LC-MS/MS: Abdou Mekebri & Gloria Blondina

Klamath River near I-5 (Mussel)



Analysis by LC-MS/MS: Abdou Mekebri & Gloria Blondina

Lake Success



Lake Success

Elevation: 692 ft

Capacity: 82,300 acre ft

County: Tulare

River Basin: Tule R.

Hydrologic Area: Tulare L.

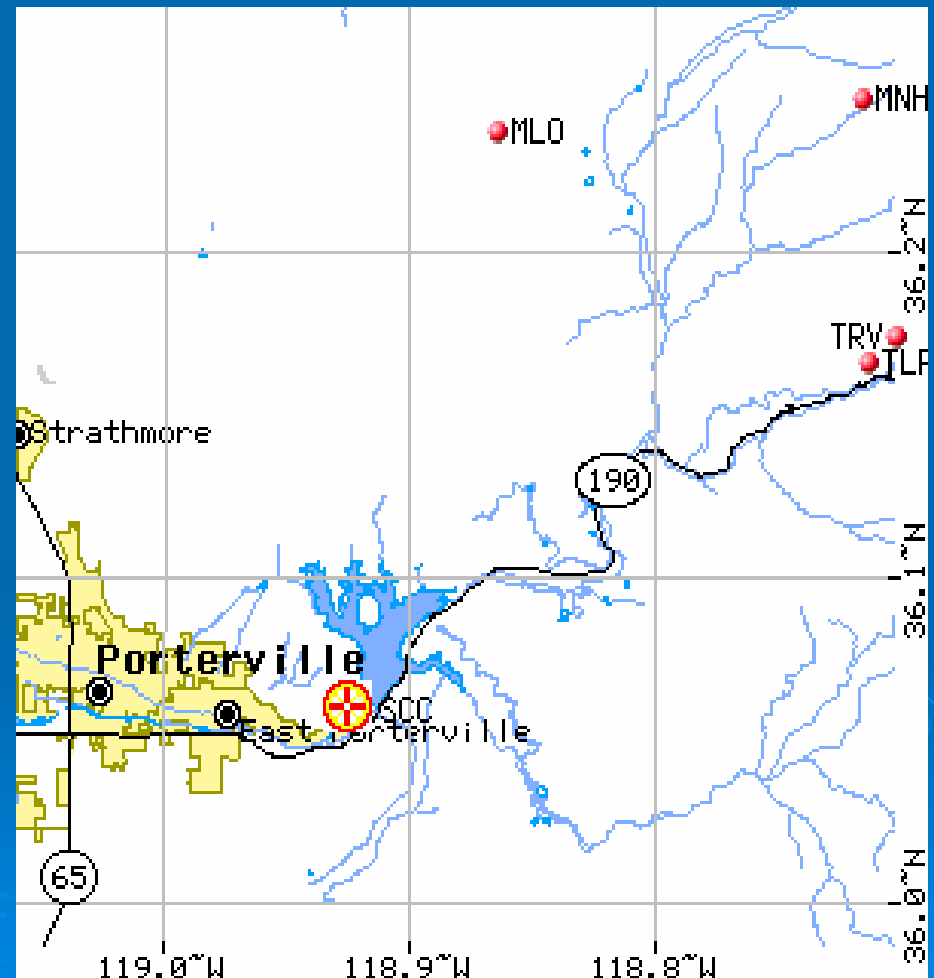
Latitude: 36.0610°N

Longitude: 118.9220°W

Average Storage:

July-36,633 acre ft

August-20,621 acre ft



- The US Army Corps of Engineers reported a major fish die-off at Lake Success in the middle of July 2007.
- 10 western grebes were also observed dead around the lake.
- An algal bloom was observed the second week of July and dead fish, primarily small bass and crappie, first appeared the week of July 15th.
- The fish die-off peaked between July 25 and August 4, which included crappie, bass, catfish, and carp.

- Approximately 5,000 fish died. Dead grebes were observed August 3.
- One dead black crappie was submitted to the Department of Fish and Game's (DFG) Fish Health Laboratory.
- *Flavobacterium columnare* (Columnaris disease) was observed on the gills.
- A mixed liver/gut tissue analyzed by the DFG Water Pollution Control Laboratory revealed this fish also had detectable microcystin concentrations (400 ppb wet wt).

- It is unclear if the bacterial infection or the toxin was the direct cause of death for the crappie that was sent to the laboratory.
- Logically both would have contributed to the fish death. Currently, very little information is available in the literature about microcystin thresholds or toxicity in fish.

Investigation report by: Andy Gordous, Ph.D.

CDFG

1234 E. Shaw Avenue
Fresno, CA 93710

Note: This is the fourth incident Andy has been involved with this past 10 years that includes an algal bloom with a large fish die-off and dead or sick western or Clark's grebes.

Common variables of these incidents included:

- a large water body reduced to a smaller area due to draw down or drought for three of the four incidences
- very high summer air temperatures during July, August and September
- alkaline conditions
- algal bloom
- large numbers of dead fish and sick or dead western/Clark's grebes. Other waterbirds such as cormorants, white pelicans and waterfowl also have been observed using these areas at the same time of a bloom, but appeared not to be as sensitive as the grebes

Tissue Algal Toxin Concentrations from One Dead Black Crappie Collected at Lake Success, Tulare County, California.

	Fish Liver & Stomach Content	Fish Liver & Stomach Content Duplicate	Mean
	ng/g (ppb) Wet wt.	ng/g (ppb) Wet wt.	ng/g (ppb) Wet wt.
Microcystin-RR	<1.0	<1.0	
Microcystin-Demethyl-RR	<1.0	<1.0	
Microcystin-LR	172	225	198
Microcystin-Demethyl-LR	<1.0	<1.0	
Microcystin-YR	51.7	60.1	55.9
Microcystin-LA	79.0	96.8	87.9
Microcystin-LW	46.4	68.5	57.5
Microcystin-LF	<1.0	<1.0	
Domoic Acid	<5.0	<5.0	
Okadaic Acid	<2.0	<2.0	
Total Microcystins	349	450	399

Source: David Crane, California Department of Fish and Game, Office of Spill Prevention and Response, Water Pollution Control Laboratory, 2005 Nimbus Road, Rancho Cordova, California 95670

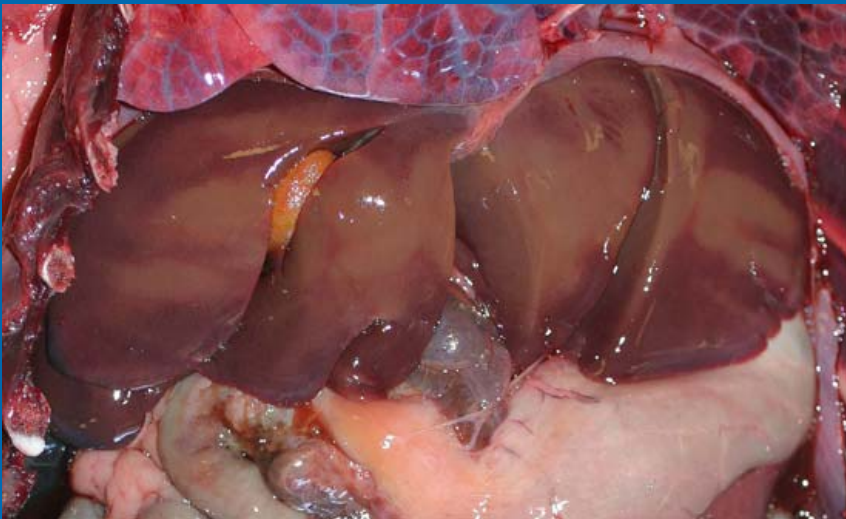
Putative microcystin toxicosis in southern sea otters



Melissa A. Miller, Abdu Mekebri, Dave Crane, Raphael Kudela, Gregg Langlois, Sharon Toy-Choutka, Dane Hardin, Clare Dominik, Eva Berberich, Michael Murray and David Jessup



Normal liver




Diseased liver

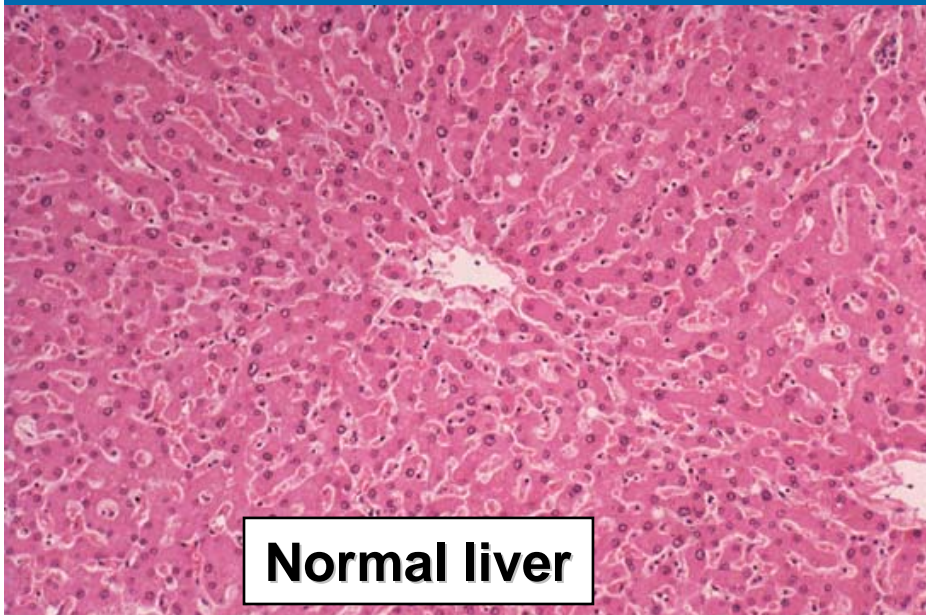
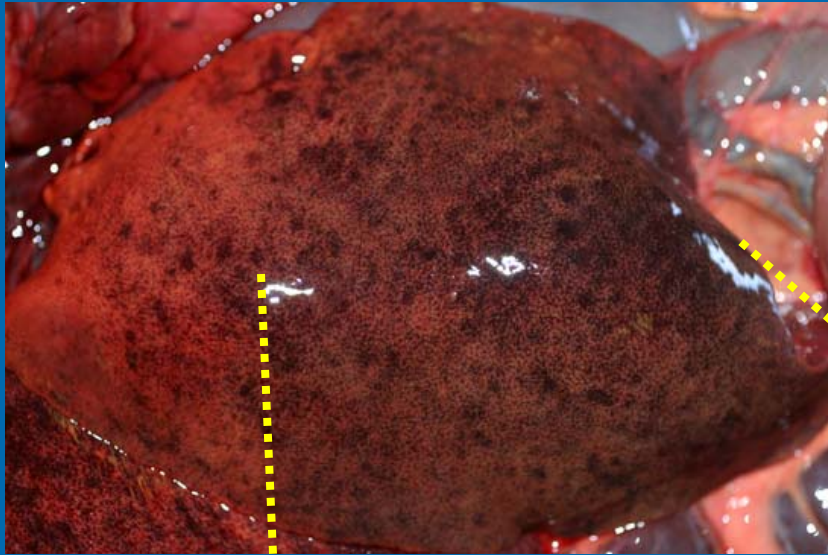


Test results

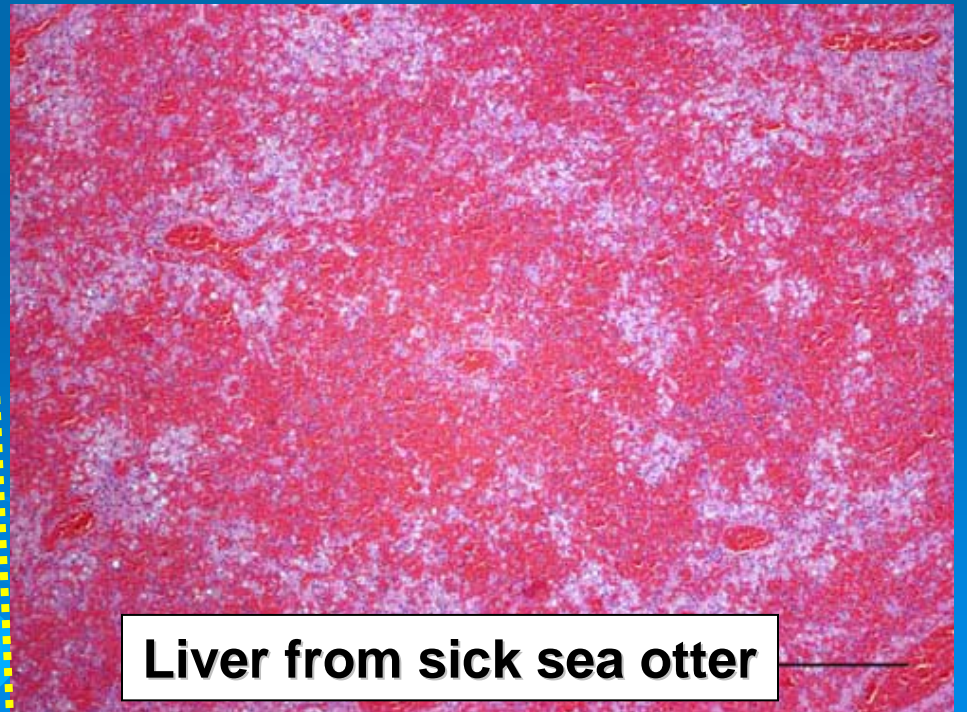
➤ Bacterial culture	Negative
➤ Testing for <i>Leptospira</i>	Negative
➤ Protozoa	Negative

Microscopic lesions in liver
supportive of hepatic toxin





Normal liver



Liver from sick sea otter

Possible environmental sources

➤ Freshwater:

- warm
- stagnant
- nutrient-enriched



➤ Marine?:

- Marine cyanobacterium
- Scattered reports + coral
- Salmon in net pens
- NO MARINE MAMMALS?



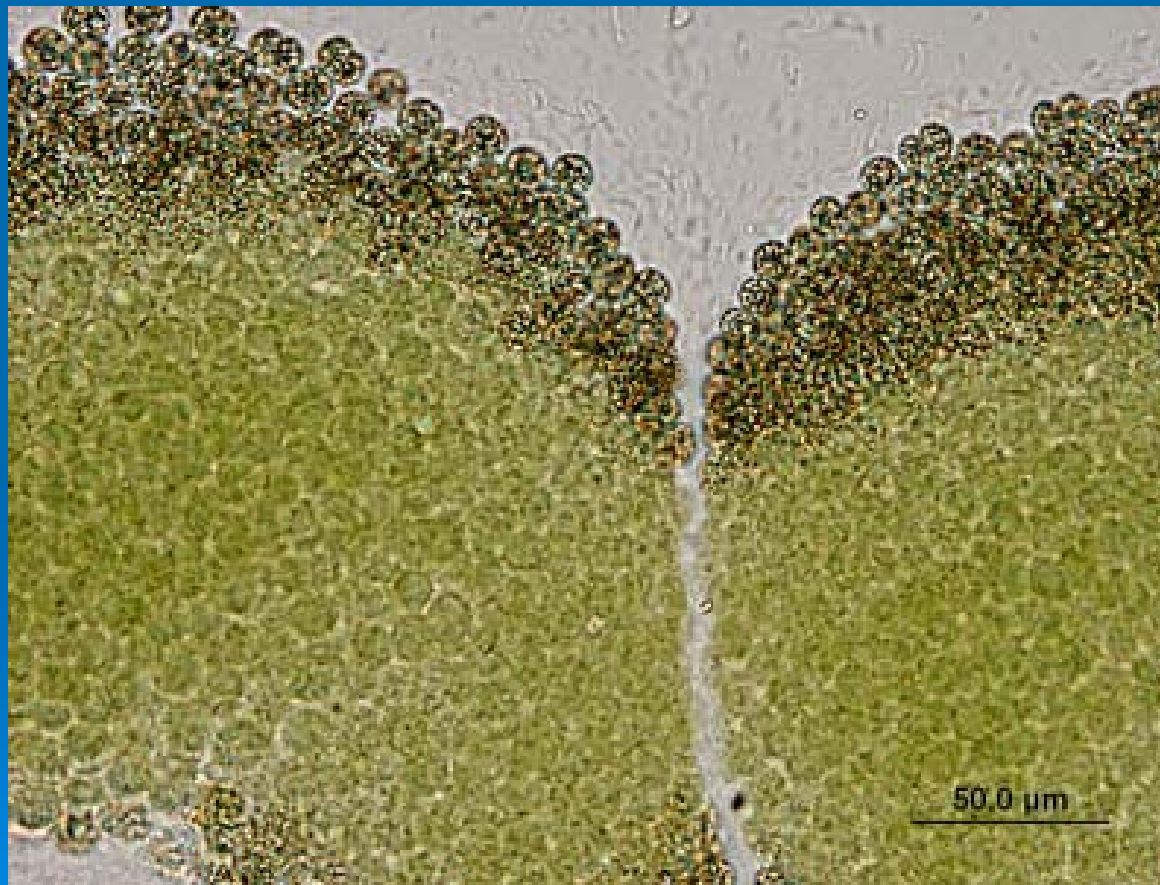
Next steps....

- Local area testing: All local watersheds→Pajaro
- Networking with local experts
 - DHS
 - CDFG-WPCL
 - State Water Board
 - Regional Water Board
 - City of Watsonville
 - CSUMB
 - UCSC

Pinto Lake and Pajaro River



Microcystis spp detected!!



Summary of Microcystin Toxins in Water/Surface Scum (ppb)

	Pinto Lake 1' deep water end of boat ramp	Pinto Lake surface scum beginning of ramp	Corralitos Creek at Coolidge Ave deep water (~2')	Corralitos Creek at Coolidge Ave rock scrape
Microcystin Analytes	water	water	water	water
MCY-RR		33.3		
MCY-dm-RR*				
MCY-LR	33.3	760,000	2.06	0.811
MCY-dm-LR*	1.67	33,000		
MCY-YR		44.4		
MCY-LA	61.1	2,100,000	6.58	1.90
MCY-LW				
MCY-LF		7.29		

* Demethyl analog quantified as parent compound.

Summary of Microcystin Toxins in Water (ppb)

	Pajaro River at Thurwachter Bridge deep in channel	Pajaro River at Thurwachter Bridge surface near edge	Kelly Lake Watsonville	Kelly Lake Watsonville
Microcystin Analytes	water	water	water	water
MCY-RR				
MCY-dm-RR*				
MCY-LR			5.08	0.594
MCY-dm-LR*				
MCY-YR				
MCY-LA	1.10	0.789		
MCY-LW				
MCY-LF				

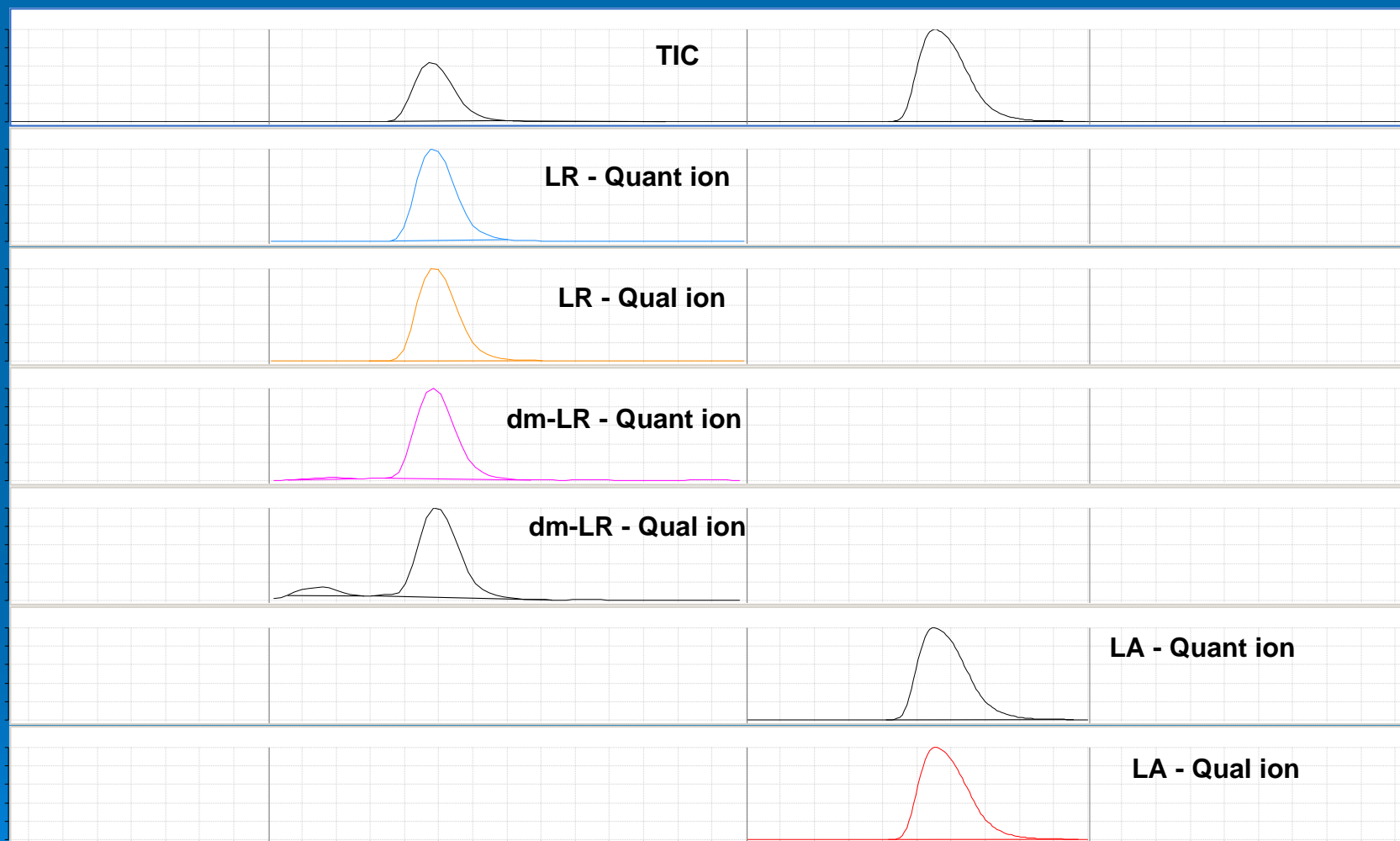
* Demethyl analog quantified as parent compound.

Summary of Microcystin Sea Otter Liver Results (ppb)

Sea Otter Liver MCY Results				
Microcystin Analytes	L-445-07-1	L-445-07-2	L-445-07-3	L-445-07-4
MCY-RR	ND	61.6	104	38.4
MCY-dm-RR*				
MCY-LR	ND		348	
MCY-dm-LR*				
MCY-YR				
MCY-LA				
MCY-LW				
MCY-LF				

* Demethyl analog quantified as parent compound.
L-445-07-1 – Blind Control Sample

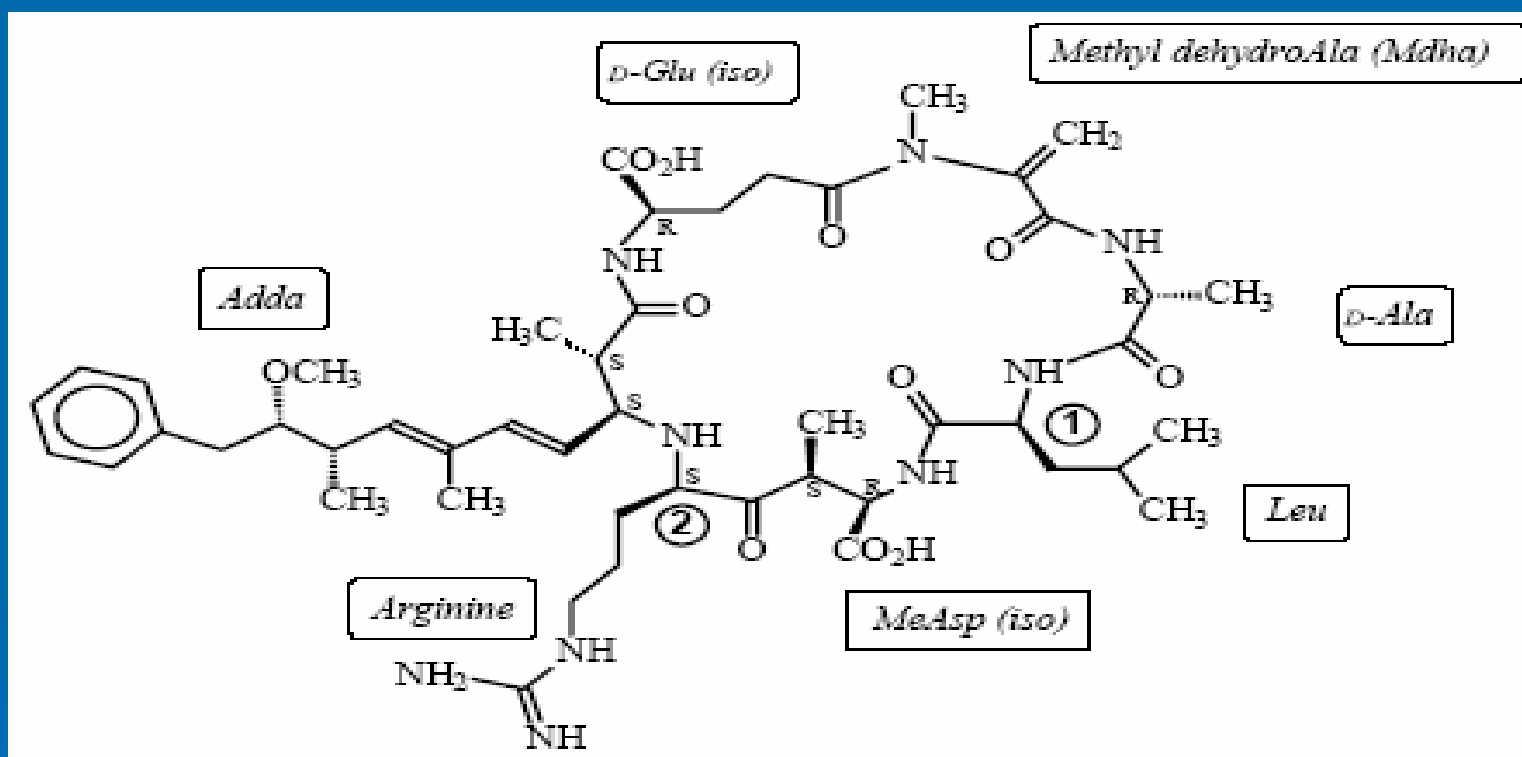
Pinto Lake - Water



Analysis by LC-MS/MS, Abdou Mekebri & Gloria Blondina

$\times 10^2$ + MRM (995.70001 \rightarrow 135.19922) WorklistData08.d

Chemical Structure of Microcystins (cyanobacterial hepatotoxins)

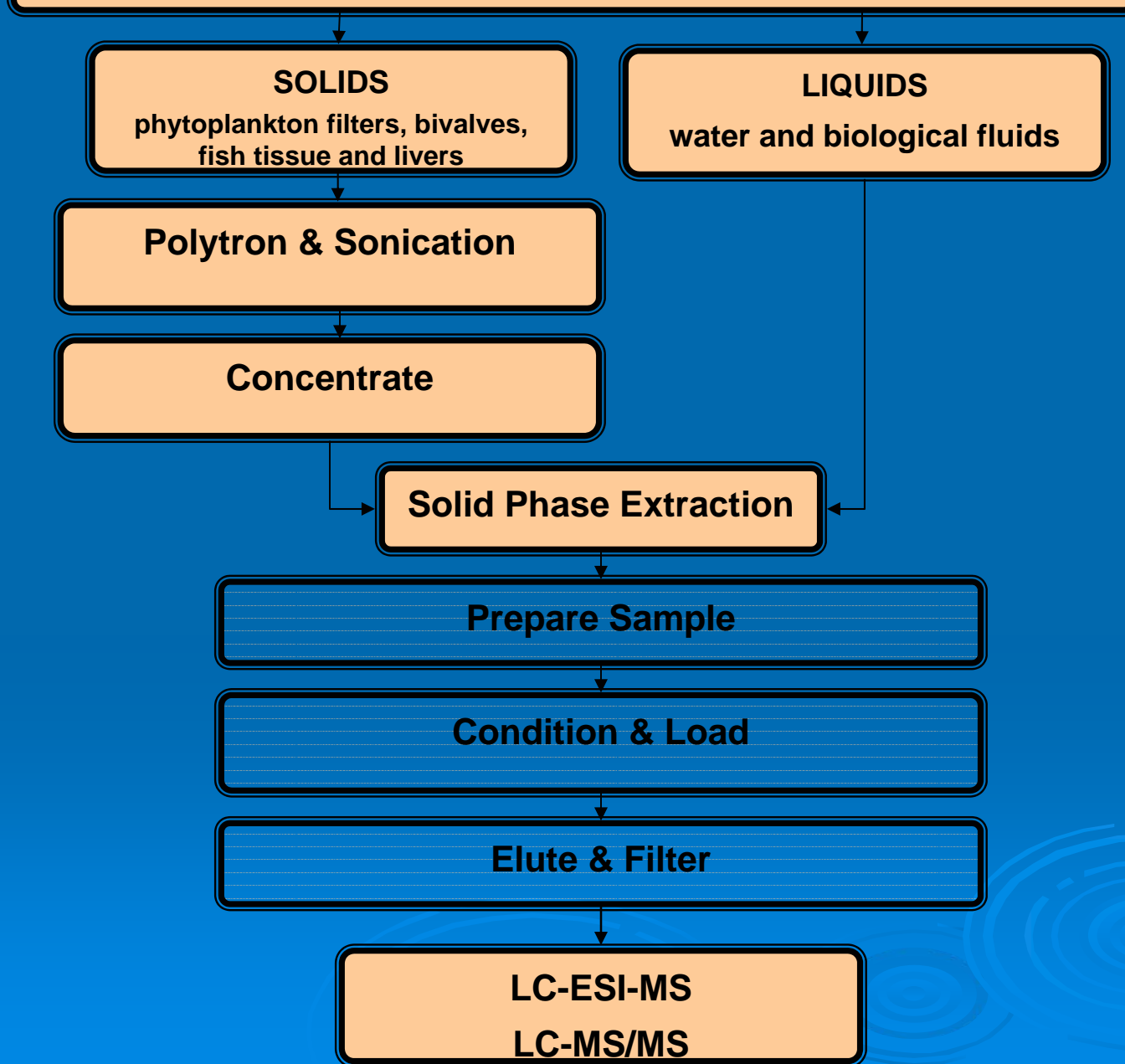


The generic structure of a microcystin. Variations occur primarily at positions 1 and 2. For example, Microcystin-LR contains the amino acids leucine (L) and arginine (R) at positions 1 and 2 respectively. Microcystin-RR has arginine at both positions. (Harada, 1996).

Analytical Method Development and Validation of Microcystins in Bivalves



Methods for Toxin Analysis



LC/MS/MS PARAMETERS

Chromatograph conditions:

- Column: Waters Atlantis dC18, 3 μ m, 2.1 x 100 mm
- Mobile phase A: water with 0.1 % formic acid
- Mobile phase B: acetonitrile with 0.1 % formic acid
- Pump parameters: gradient

Time (min)	A (%)	B (%)
0	95	5
2	95	5
10	50	50
13	25	75
18	25	75
19	95	5
20	95	5

- Flow rate: 0.250 ml/min
- Run time: 20 minutes
- Column temperature: 38°C
- Injection volume: 20 μ L

LC/MS/MS PARAMETERS

MS Conditions: API-ES in positive ion mode

- Drying gas flow: 12 L/min
- Drying gas temperature: 350°C
- Nebulizer gas pressure: 40 psig
- Capillary voltage: 4000

MS Parameters:

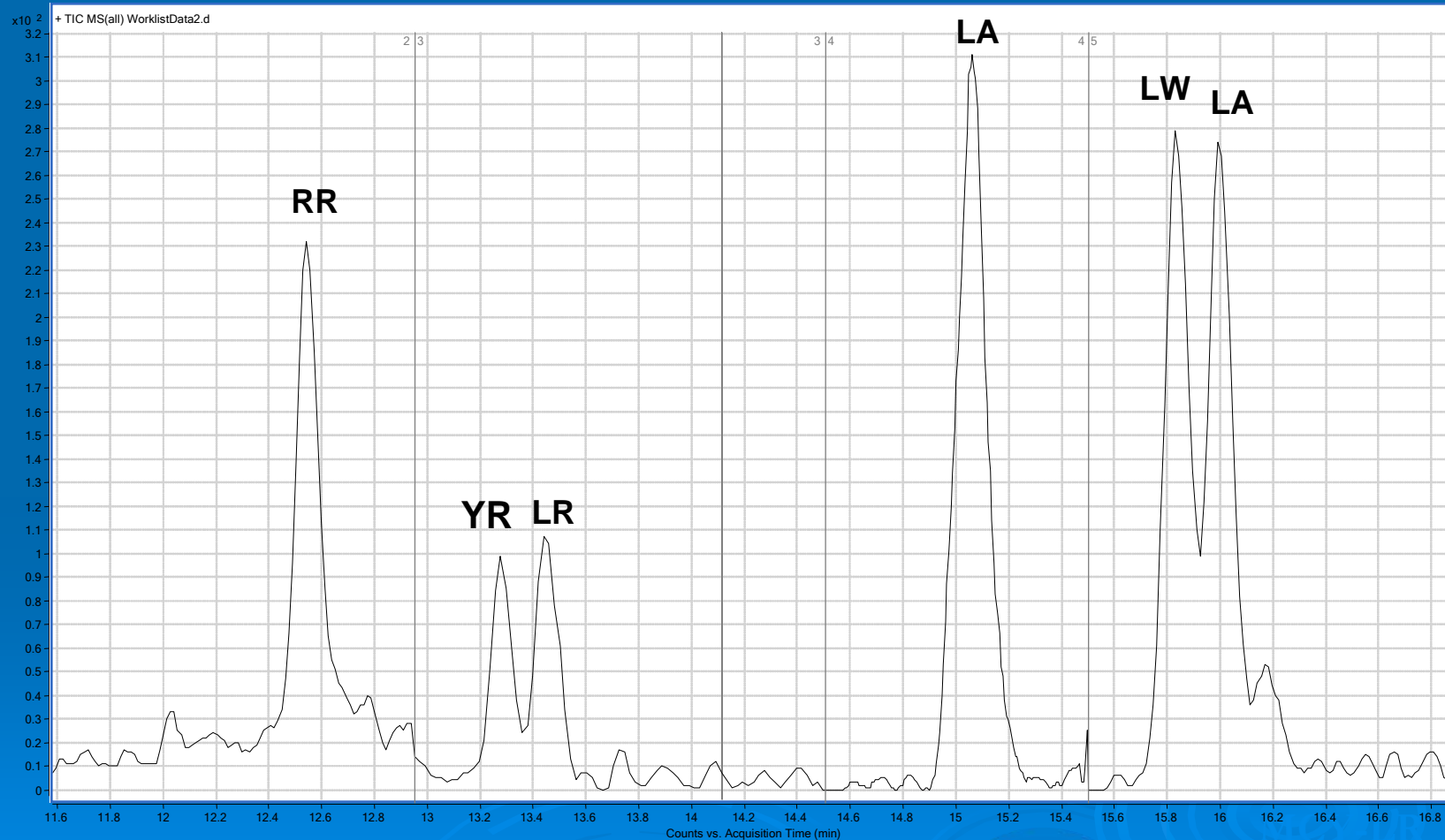
Time Segment #	Time (min)	Target Compound	Scan Type	Precursor Ion	Product Ions	Fragment (V)	Collision Energy (V)
1	0	n/a	MS2 Scan (To Waste)				
2	5.0	MCY-RR	MRM	520	213, 135.2	110	50
		MCY-Demethyl-RR	MRM	512.8	213, 135.2	110	50
3	12.95	MCY-LR	MRM	995.7	213, 135.2	110	70
		MCY-Demethyl-LR	MRM	981.7	213, 135.2	110	70
		MCY-YR	MRM	1045.6	213, 135.2	110	70
4	14.5	MCY-LA	MRM	910.6	213, 135.2	110	70
5	15.5	MCY-LW	MRM	1026.6	213, 135.2	110	70
		MCY-LF	MRM	987.6	213, 135.2	110	70

Microcystin Method Validation

Microcystin Congeners	Sacramento-American River Water			Fortified Filters 1 µm retention size			Fish Tissue (Rainbow Trout)			Fish Livers (Rainbow Trout)		
	Spike Level = 5 µg/L			Spike Level = 0.2 µg/g			Spike Level = 0.2 µg/g			Spike Level = 0.2 µg/g		
	W1	W2	W3	F1	F2	F3	T1	T2	T3	L1	L2	L3
MCY-RR	102	99.6	102	73.8	79.2	76.0	102	103	97.8	81.6	77.0	83.8
MCY-LR	104	103	111	87.2	89.6	86.8	90.1	97.5	89.7	78.4	84.4	82.4
MCY-YR	114	115	125	103	111	110	83.8	80.8	83.0	89.0	83.2	87.0
MCY-LA	92.0	89.9	101	82.0	77.0	84.0	96.0	98.0	97.4	88.6	76.4	84.8
MCY-LF	85.6	74.0	84.8	92.0	84.8	80.8	89.0	80.0	82.0	79.8	68.8	85.4
MCY-LW	66.7	72.3	81.9	38.8	44.8	52.7	86.2	87.6	83.5	78.4	84.4	82.4
Nodularin	100	101	113	94.7	89.7	92.0	106	107	100	110	114	99.4

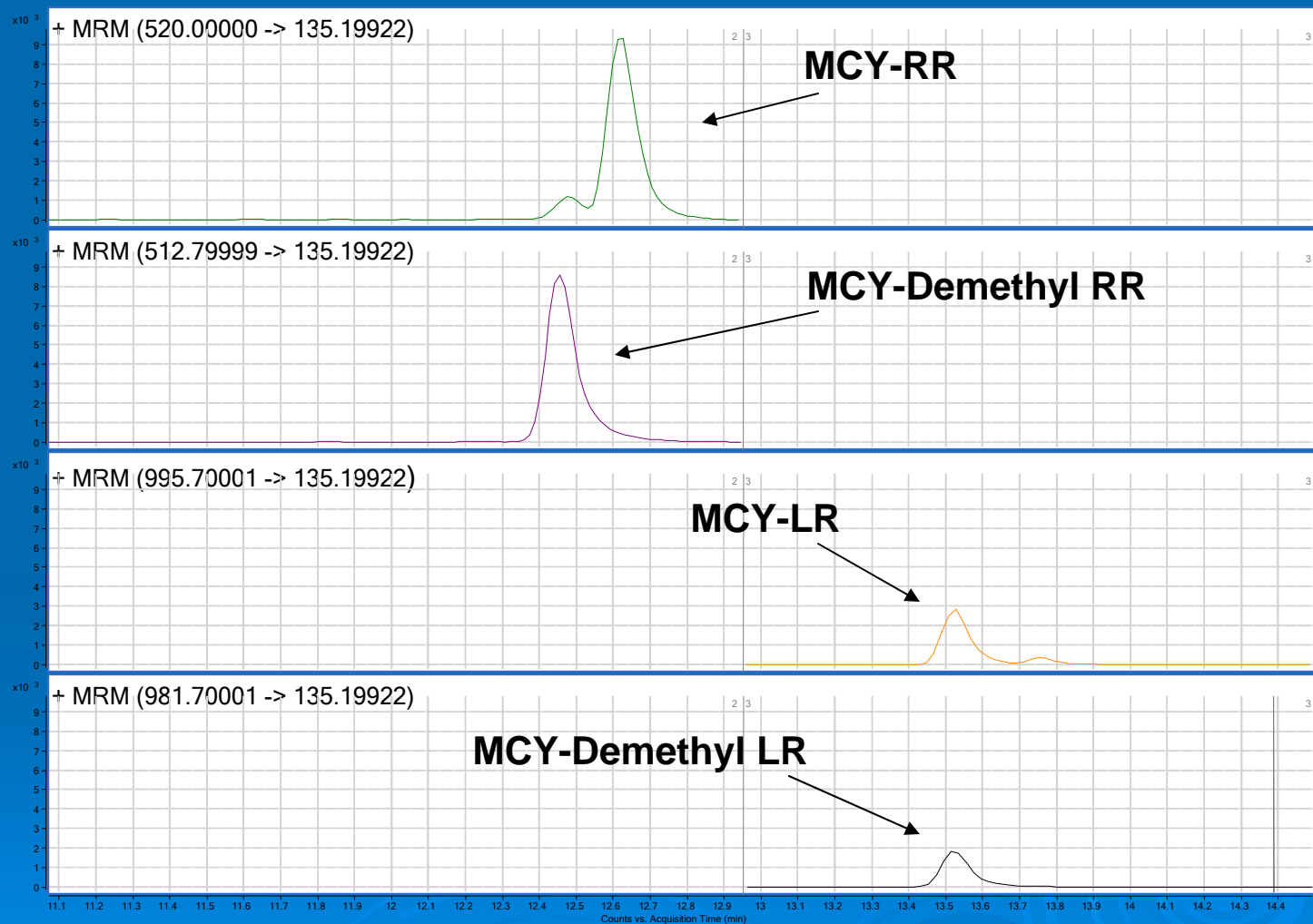
LC/MS/MS

Microcystin Standard – 1 $\mu\text{g/L}$



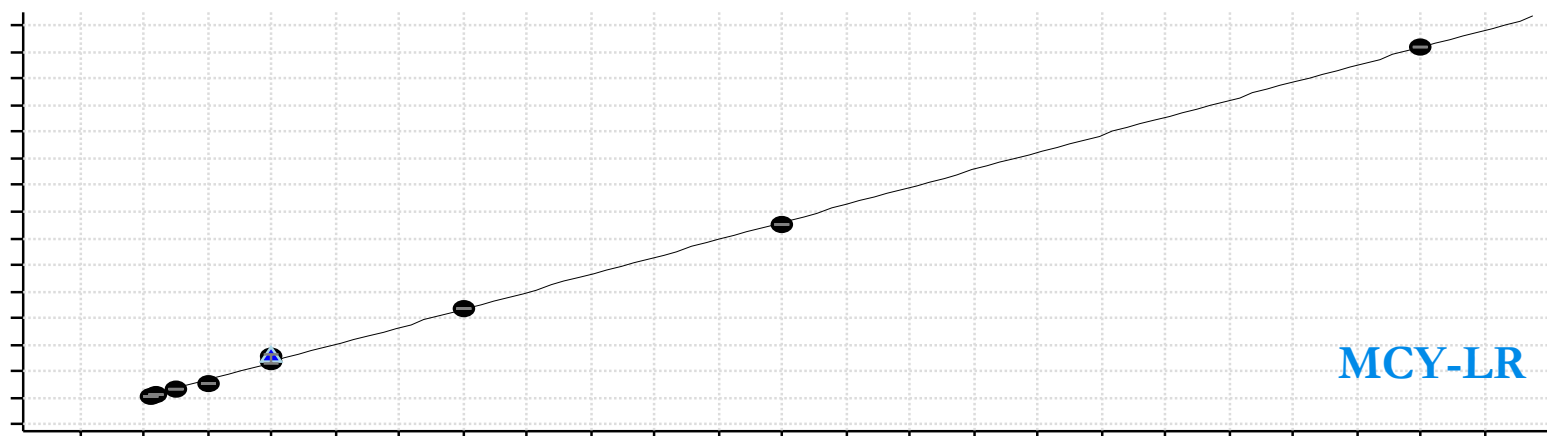
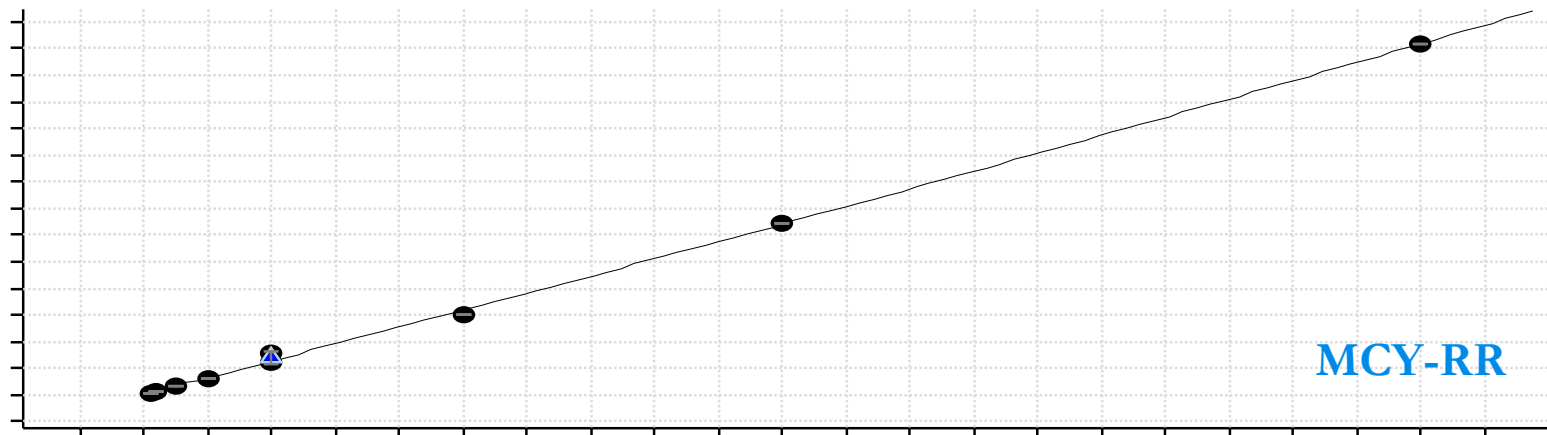
LC/MS/MS

Extracted chromatogram



LC/MS/MS

8 Point Calibration Curves
1.00-200 ppb ($\mu\text{g/L}$) range



espon

$$R^2 = 0.99907041$$

, 9 Poi

545.54

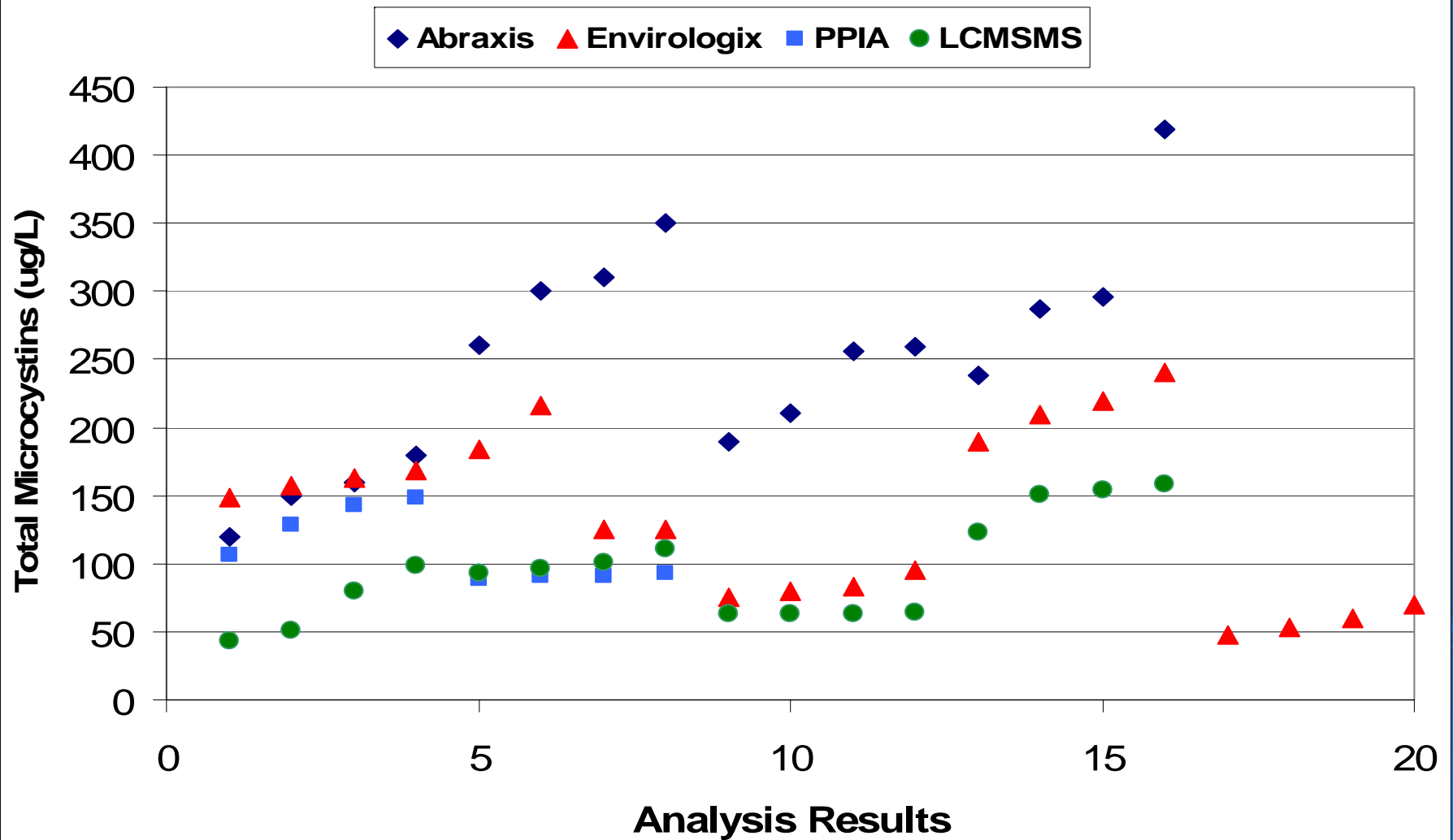
Florida Department of Environmental Protection Round Robin - 2007

Participation in nation-wide round robin exercise with eleven agencies/laboratories for the analysis of microcystins by ELISA, PPIA and LC/MS/MS

Ten water samples:

- 3 Replicates FDEP standard
- 3 Replicates University of Texas culture
- 4 Replicates Lake Munson sample

FDEP Round Robin - Lake Munson Culture Results



FDEP Round Robin - University of Texas *Microcystis* Culture Results

