Identification of Toxic Benthic Cyanobacteria in Three Northern California Rivers

Rich Fadness
North Coast Regional Water Quality Control Board

Monitoring for the Protection of Public Health

(EXPANDED)

Identification of Toxic Benthic Cyanobacteria in Three Northern California Rivers

SWAMP
Surface Water Ambient Monitoring Program

SCCWRP
Established 1969

California State University
SAN MARCOS

Sheath
California Primary Algae Laboratory

CALIFORNIA
DEPARTMENT OF FISH & WILDLIFE

Bend Genetics

Marine Pollution Studies Laboratory
Granite Canyon

Kudela Lab
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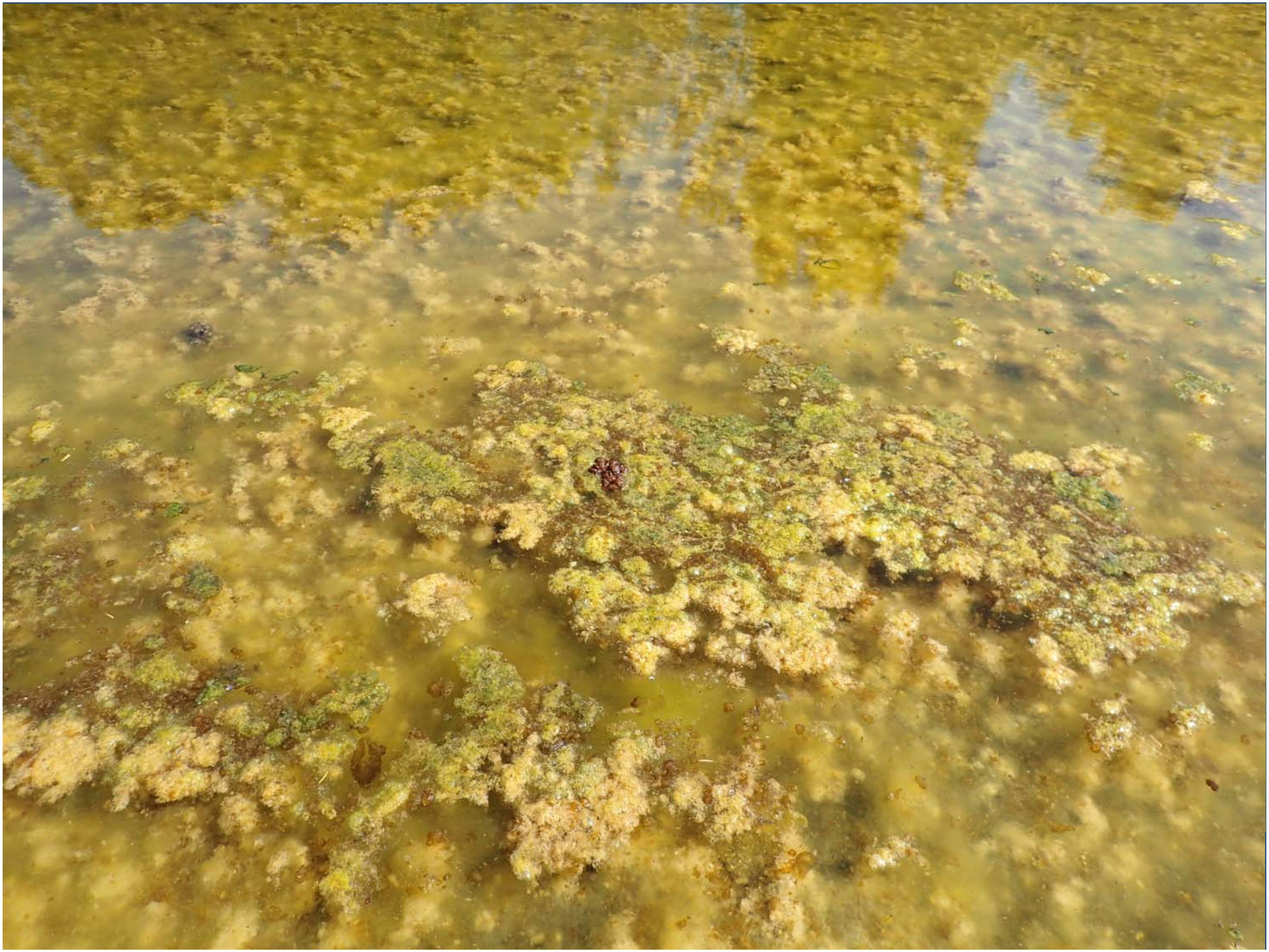
Identification of Toxic Benthic Cyanobacteria in Three Northern California Rivers

- *2015 Discussion and 2016 Sample Design Development*
  Rich Fadness
- *2016 Monitoring Results*
  Meredith Howard
- *Monoculturing Cyanobacteria Species*
  Rosalina Stancheva
- *Toxicity Testing of 2015 Monocultured Cyanobacteria*
  Brian Anderson
- *2018 Design Changes, Thoughts, and Next Steps*
  Rich Fadness
- *Questions - Discussion*

Identification of Toxic Benthic Cyanobacteria in Three Northern California Rivers

An investigation into the presence of benthic cyanobacteria in Northern California was initiated in response to a citizen complaint related to multiple dog illnesses after swimming in the Russian River. Further examination of the dogs suggested that a cyanotoxic response was NOT responsible for their symptoms, however our field investigation had begun.



Initial Water Column and Integrated Algal Mat Toxin Testing *(RESULTS)*

		114RR3173	114RR3119	114RR2940	114RR2036	114RR1898	114RR1515	114RR1325	114RR0898	OEHHA 2012 Criteria (ug/L)
		UPSTREAM to DOWNSTREAM →								
		Water Column Grab Sample								
8/10/2015	Microcystin / Nodularin	-	-	-	-	-	<0.10	-	ND	Advisory - 0.8
8/17/2015		-	-	-	-	-	-	-	-	
8/24/2015		ND	ND	ND	ND	ND	-	ND	ND	
8/31/2015		ND	ND	ND	ND	ND	-	ND	ND	
8/10/2015	Anatoxin	-	-	-	-	-	-	-	-	Advisory - 90
8/17/2015		-	-	-	-	-	ND	-	ND	
8/24/2015		-	-	-	-	-	-	-	-	
8/31/2015		-	-	-	-	-	-	-	-	
8/17/2015	Cylindrospermopsin	-	-	-	-	-	-	-	-	Advisory - 4

		114RR3173	114RR3119	114RR2940	114RR2036	114RR1898	114RR1515	114RR1325	114RR0898	2012 Criteria
		UPSTREAM to DOWNSTREAM →								
		Integrated Streambank Algal Mat Sample								
8/10/2015	Microcystin / Nodularin	-	-	-	-	-	<0.10	-	0.47	NONE
8/17/2015		-	-	1.1	<0.10	2.92	0.12	-	12.75	
8/24/2015		0.13	4.726	0.369	0.101	0.103	-	<0.10	0.178	
8/31/2015		<0.10	0.11	ND	ND	0.1	-	<0.10	0.5	
8/10/2015	Anatoxin	-	-	-	-	-	-	-	-	NONE
8/17/2015		-	-	-	-	-	0.62	-	ND	
8/24/2015		-	-	-	-	-	-	-	-	
8/31/2015		-	-	-	-	-	-	-	-	
8/17/2015	Cylindrospermopsin	-	-	ND	ND	ND	0.06	-	0.09	NONE

Initial Cyanobacteria Species Identification

Algal Species	Potential Toxicity	Possible Toxicants
Aphanothece elabens	Yes	Microcystins
Calothrix breviarticulata	Yes	Microcystins
Cylindrospermum stagnale	Yes	Microcystins
Geitlerinema amphibium	Yes	Microcystins
Geitlerinema splendidum	Yes	Microcystins
Nodularia spumigena	Yes	Nodularin, Microcystins
Oscillatoria limosa	Yes	Microcystins
Oscillatoria tenuis	Yes	Microcystins, Anatoxin, Cylindrospermopsin
Trichormus cf rotundosporus	Yes	Microcystins
Calothrix fusca	Possible	Microcystins
Gloeotrichia sp	Possible	Microcystins
Nostoc paludosum	Possible	Microcystins
Oscillatoria princeps	Possible	Microcystins
Phormidium cf tergestinum	Possible	Anatoxin
Phormidium subfuscum	Possible	Anatoxin
Pseudanabaena mucicola	Possible	Microcystins
Anabaena sp	Unknown	Microcystins, Cylindrospermopsin
Anabaena sp 2	Unknown	Microcystins, Anatoxin, Cylindrospermopsin
Aphanothece sp	Unknown	Microcystins, Cylindrospermopsin
Cylindrospermum sp	Unknown	Microcystins, Anatoxin, Cylindrospermopsin
Dolichospermum sp 2	Unknown	Microcystins, Anatoxin
Geitlerinema sp	Unknown	Microcystins, Anatoxin


SOAR

Dog dies on Russian River, tests positive for toxic algae

THE PRESS DEMOCRAT | September 3, 2015

…on Aug. 29, a golden retriever visiting the region with its owners died during a paddle down the Russian River, very shortly after getting in the water.

The dog suffered seizures more common to Anatoxin-a, the neurotoxin later determined to be the cause of the dog's death.

The incident occurred on the eve of the busy Labor Day holiday weekend, which brings thousands of people to the Russian River.

…the small amounts of Anatoxin-a detected in the river over the last weeks of summer never exceeded the first trigger level on the state guidance, requiring signs cautioning visitors to stay away from algae scums and keep their pets and livestock away…

Post-Dog Death Anatoxin Concentrations

(surface water ELISA analysis ug/L)

	9/3/2015	9/7/2015	9/14/2015	9/21/2015	9/28/2015	10/5/2015
Cloverdale River Park		ND	ND	ND	ND	ND
Del Rio Woods		14.4	ND	41.6	ND	ND
Camp Rose		ND	ND	19.2	ND	ND
Healdsburg Memorial Beach		ND	ND	ND	ND	ND
200' downstream of put-in	16.6					
Middle Corona	17.9					
50' downstream of rope swing	12.7					
150' downstream of rope swing	ND					
Mid-Martinelli	18.5					
Steelhead Beach		ND	ND	48.9	ND	ND
ForestvilleAccess Beach		ND	ND	ND	ND	ND
Sunset Beach		ND	ND	ND	ND	ND
Johnson's Beach		ND	ND	25.9	ND	ND
Monte Rio Beach		ND	ND	ND	ND	ND
Patterson Point		ND	ND	ND	ND	ND

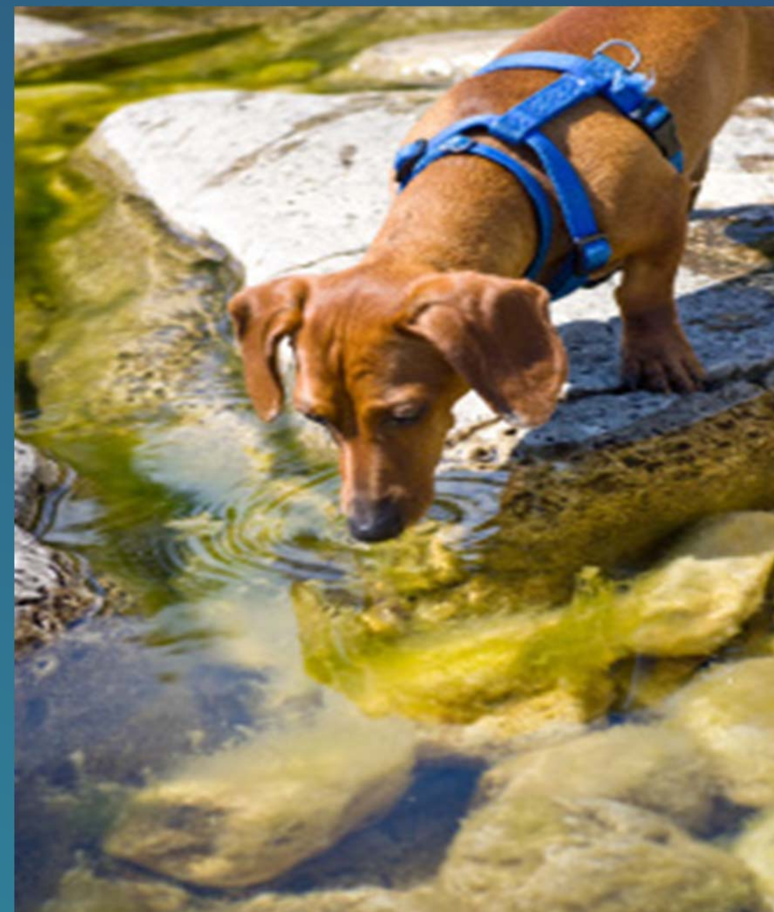
2012 Action Criteria:

Advisory – 90 ug/L

2016 Action Criteria:

Caution - Detect
Warning - 20 ug/L
Danger - 90 ug/L


fineart
america



Mendocino County issues algae alert for Eel River after dog's death

THE PRESS DEMOCRAT | September 25, 2015

…It was the second confirmed canine fatality in two months linked to toxic algae and swimming in a North Coast stream. The first case involved a dog that died during an outing on the Russian River.

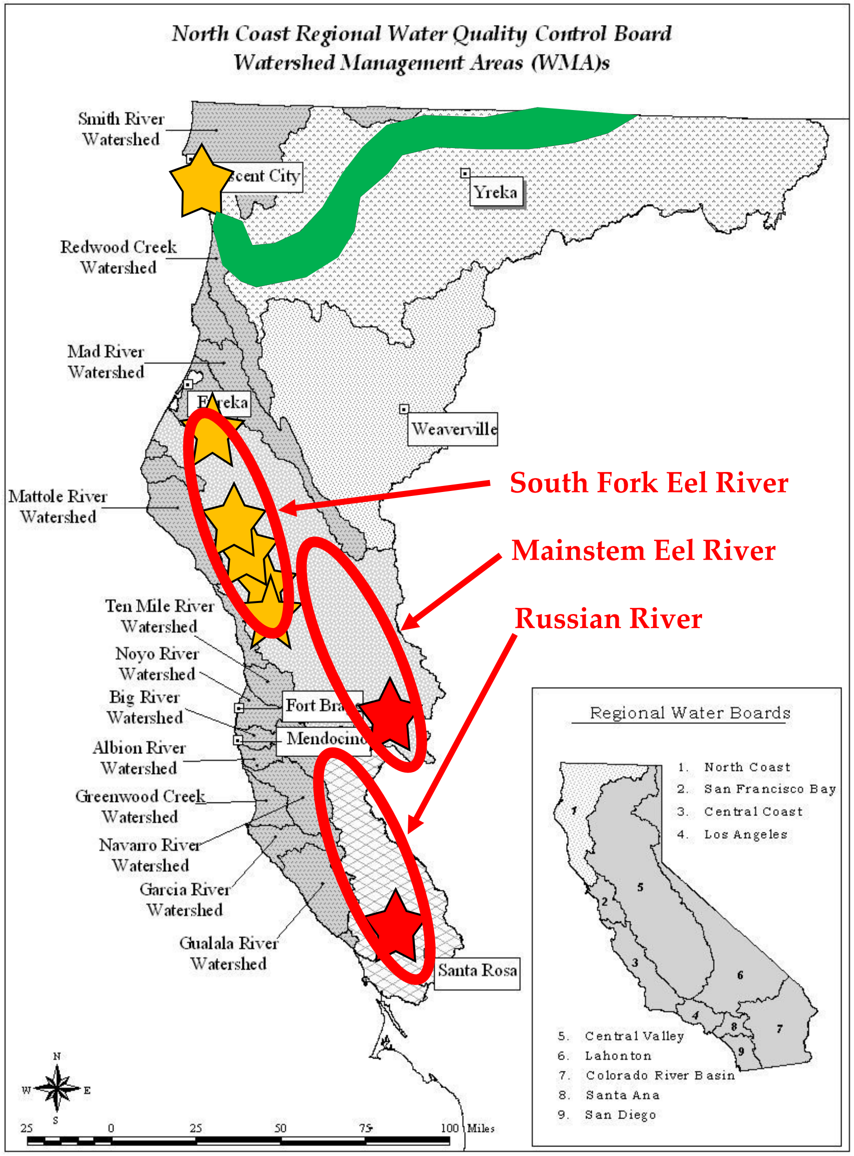
In the latest case, the stricken dog had been swimming in the Eel River at a Potter Valley-area campground before becoming ill…

…Mendocino County officials … are advising people to avoid any water body in which they can see the telltale signs of algae growth, including algal scum and mats that can be seen floating in the water or collecting along the shore…

2015 Public Health Monitoring in Review

- Heavy economic toll leads to review, research and coordination between multiple agencies.
- Ad-hoc effort from 2015 left us with unanswered questions:
  - What does benthic cyanobacteria look like?
  - What are the various genus and species present?
  - Where is it?
  - What toxins does it produce?
  - How toxic is it?
  - When is it toxic?
  - How do we monitor for it?

North Coast
Regional Water Quality Control Board



Water Facts
Approximately 20,000 square miles in size (larger than 9 other states)
340 miles of coastline
27,000 acres of lakes
Approximately 28,000 stream miles (~45,000 km)
Receives more than 35% of state's total annual rainfall
Average annual rainfall varies from 20" to more than 120"

Solid **P**hase **A**dsorption **T**oxin **T**racking (SPATT) Deployments

Water Column and Reach Integrated Algal Mat Collection

(Toxin Testing)

Reach Integrated Algal Mat Collection

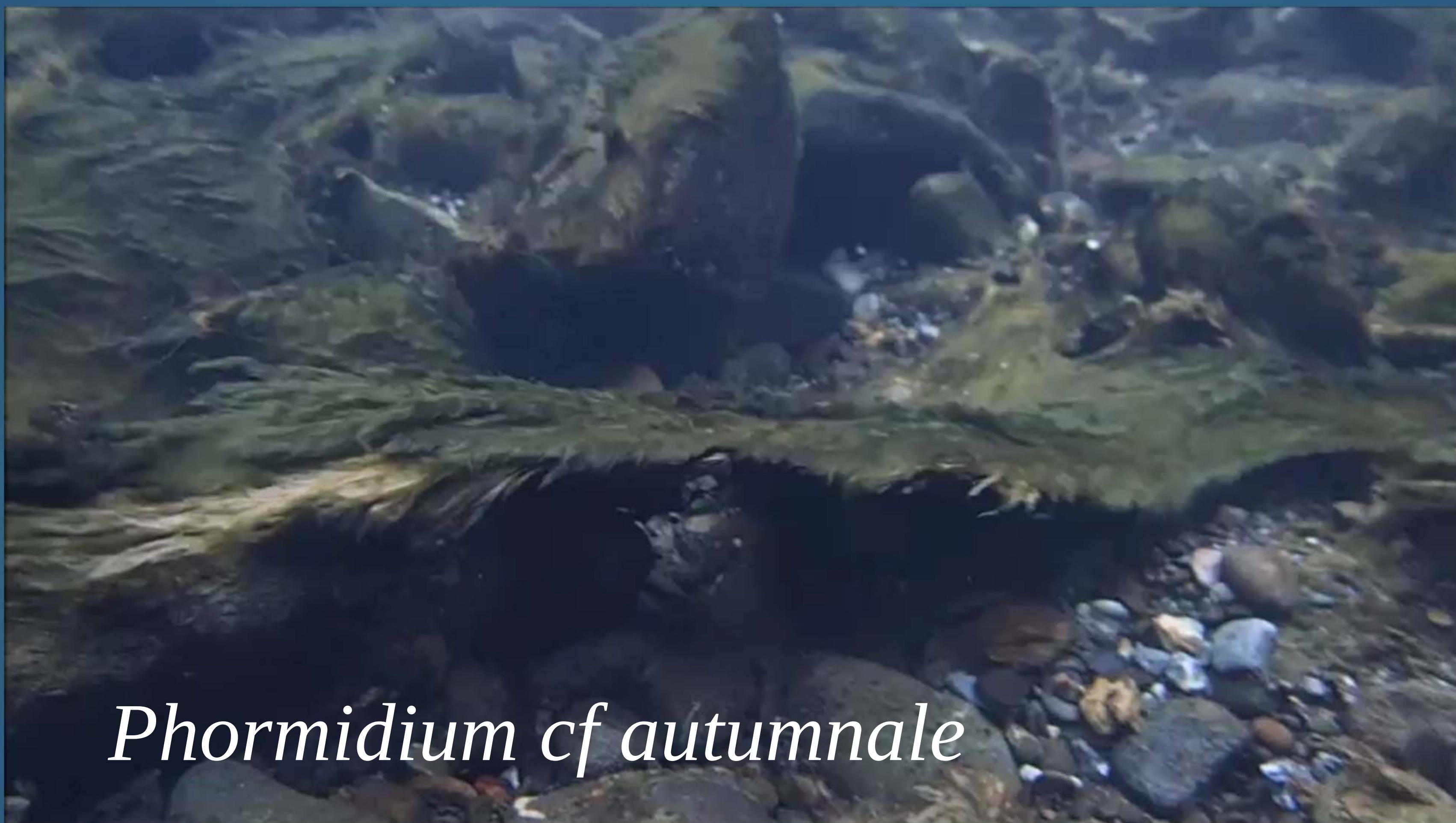
(Toxin Testing)

Reach Integrated Algal Mat (ELISA testing in ug/L)					
Station Code	Date	ATX	CYL	MCY	SXT
114RR2655	8/15/2016	**2204**		**0.1**	
114RR2655	8/30/2016	**2054**		**0.1**	ND
113GAR042	8/16/2016	**1446**	**0.1**	**0.4**	**0.3**
114RR5407	8/15/2016	**1002**		**0.6**	
114RR5407	8/30/2016	**619**		**0.1**	ND
114RR4234	8/30/2016	**391**		**0.1**	ND
114RR4234	8/2/2016	**365**	ND	ND	ND
114RR4234	8/15/2016	**246**		**0.6**	
111ER6381	7/12/2016	**224**		ND	
111SF2423	8/1/2016	**90.9**	ND	ND	ND
113GAR030	8/17/2016	**66.7**	**0.1**	**50.5**	**0.0**
114RR5407	8/2/2016	**53.3**	ND	**0.6**	ND
111ER6381	9/1/2016	**35.8**		**0.2**	ND
111SF2423	8/17/2016	**31.5**		**0.8**	
111ER8102	8/15/2016	**18.5**		**0.5**	
111ER8102	9/6/2016	**13.6**		ND	ND
111SF2423	9/1/2016	**11.9**		**0.1**	ND
114RR4234	7/13/2016	**7.1**		ND	
111SF6856	8/1/2016	**6.7**	ND	ND	ND

Dominant (Single-Species) Mat Toxin Testing

Dominant (Single-Species) Mat Toxin Testing

Dominant (Single-Species) Mat Toxin Testing



Dominant (Single-Species) Mat Toxin Testing

Dominant (Single-Species) Mat
Toxin Testing

Dominant (Single-Species) Mat Toxin Testing *(AFDM Normalization)*

Reach Integrated Algal Mat (ELISA testing in ug/L)					
Station Code	Date	ATX	CYL	MCY	SXT
114RR2655	8/15/2016	2204		0.1	
114RR2655	8/30/2016	2054		0.1	ND
113GAR042	8/16/2016	1446	0.1	0.4	0.3
114RR5407	8/15/2016	1002		0.6	
114RR5407	8/30/2016	619		0.1	ND
114RR4234	8/30/2016	391		0.1	ND
114RR4234	8/2/2016	365	ND	ND	ND
114RR4234	8/15/2016	246		0.6	
111ER6381	7/12/2016	224		ND	
111SF2423	8/1/2016	90.9	ND	ND	ND
113GAR030	8/17/2016	66.7	0.1	50.5	0.0
114RR5407	8/2/2016	53.3	ND	0.6	ND
111ER6381	9/1/2016	35.8		0.2	ND
111SF2423	8/17/2016	31.5		0.8	
111ER8102	8/15/2016	18.5		0.5	
111ER8102	9/6/2016	13.6		ND	ND
111SF2423	9/1/2016	11.9		0.1	ND
114RR4234	7/13/2016	7.1		ND	
111SF6856	8/1/2016	6.7	ND	ND	ND

Dominant (Single-Species) Mat (ELISA testing in ug/L)						
Station Code	Mat #	Date	ATX	CYL	MCY	SXT
114RR2655	2	9/12/16	**>15750**	ND	0.45	0.05
114RR2655	4	9/29/2016	**8143**		**0.7**	
114RR2655	1	9/12/2016	**8115**	ND	**0.2**	ND
114RR5407	1	9/12/2016	**3396**	ND	**0.4**	ND
114RR5407	2	9/12/2016	**2631**	ND	**0.3**	**0.0**
114RR5407	1	10/3/2016	**1217**		**0.4**	
114RR2655	2	9/29/2016	**787**		**0.3**	
114RR2655	1	9/29/2016	**126**		ND	
114RR7396	3	9/30/2016	**110**		**2.3**	
114RR5407	2	10/3/2016	**94.5**		**0.9**	
114RR2655	3	9/29/2016	**48.2**		**0.2**	
111ER8102	5	9/15/2016	**45.3**	ND	**0.5**	**0.1**
111ER6381	1	9/14/2016	**38.0**	ND	**0.9**	ND
114RR4234	1	9/30/2016	**26.4**		**4.7**	
114RR7396	1	9/30/2016	**21.0**		**0.3**	
111ER8102	3	9/15/2016	**15.6**	ND	**0.3**	**0.1**
111SF2423	2	10/1/2016	**14.3**		**2.2**	ND
111ER8102	1	9/15/2016	**12.5**	ND	**0.9**	**0.1**
111ER8102	6	9/15/2016	**11.5**	ND	**0.2**	**0.1**

Dominant (Single-Species) Mat Toxin Testing *(AFDM Normalization)*

Waterbody	Date Collected	Dominant Species	Anatoxin, Total (ug/L)	AFDM (mg/L)	Anatoxin (mg/kg dry wt)
Russian River	9/12/2016	Phormidium	>15750	9030	**>1744**
Russian River	9/12/2016	Phormidium	2631	1940	**1356.0**
Russian River	9/12/2016	Phormidium	8115	6670	**1217.0**
Russian River	9/12/2016	Phormidium	3396	7290	**466.0**
Eel River	9/15/2016	Not Identified	45.3	800	**57.0**
Eel River	9/15/2016	Cylindrospermum	15.6	1850	**8.4**
Eel River	9/14/2016	Oscillatoria	38.0	4950	**7.7**
Eel River	9/15/2016	Geitlerinema	11.5	2370	**4.8**
Russian River	9/12/2016	Nostoc (?)	4.84	2140	**2.3**
Eel River	9/15/2016	Geitlerinema	0.68	600	**1.1**
Eel River	9/15/2016	Phormidium	12.5	12400	**1.0**
Eel River	9/15/2016	Cylindrospermum	7.20	8690	**0.8**
South Fork Eel River	9/14/2016	Not Identified	2.87	4120	**0.7**
Russian River	9/12/2016	Anabaena	1.70	3980	**0.4**
South Fork Eel River	9/14/2016	Phormidium	2.80	10200	**0.3**
South Fork Eel River	9/14/2016	Not Identified	0.36	1410	**0.3**
South Fork Eel River	9/14/2016	Scytonema	2.48	10300	**0.2**
Eel River	9/14/2016	Not Identified	0.67	8350	**0.1**
Russian River	9/12/2016	Phormidium	0.49	9930	**0.1**

Summary of the 2016 Field Survey in the Russian and Eel Rivers Conducted by the North Coast Regional Water Quality Control Board

Meredith Howard

SCCWRP

Southern California Coastal Water Research Project

Russian and Eel Rivers Cyanotoxin Field Study

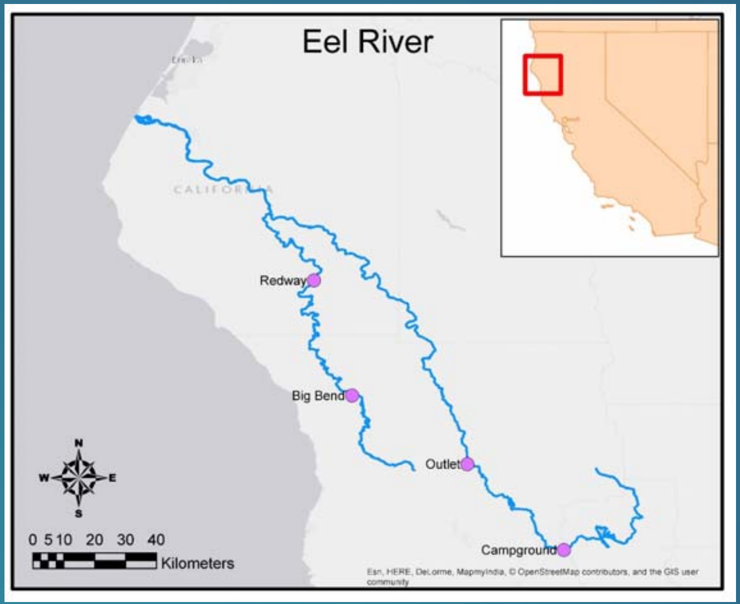
Monitoring Sites:

- July – Oct 2016, sampling 2X per month

Cyanotoxins measured from 3 sample types:

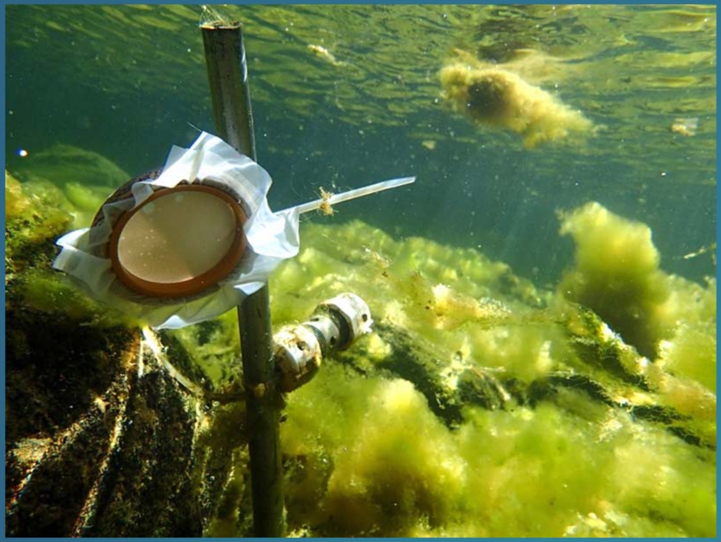
- Whole water (particulate and dissolved)
- SPATT passive samplers (dissolved)
- Cyanobacterial mats (particulate)

Cyanotoxins measured using LC-MS or ELISA:
(microcystins, anatoxin, nodularin, saxitoxin, cylindrospermopsin)



Solid Phase Adsorption Toxin Tracking

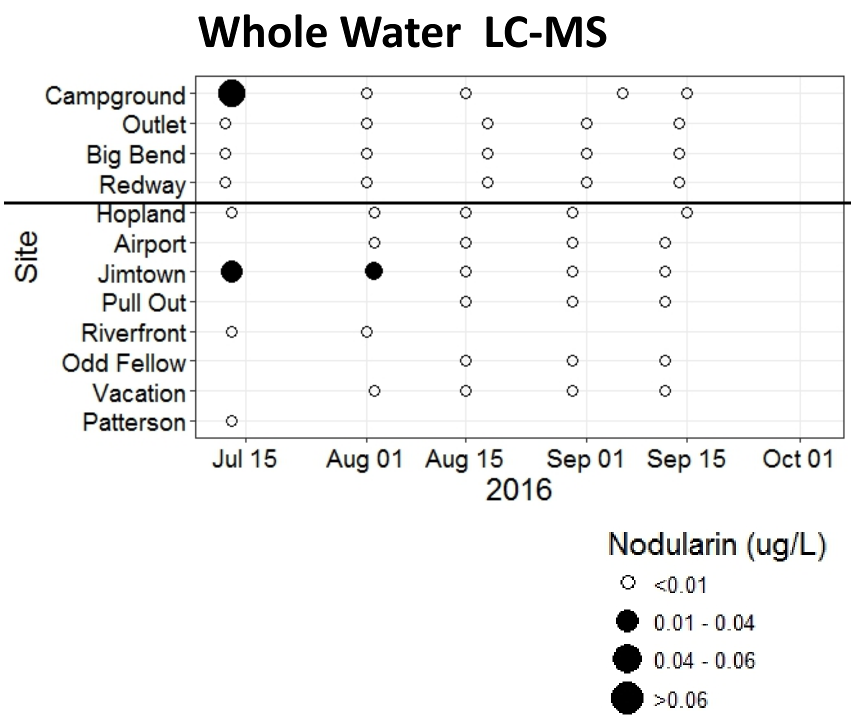
- Passive Sampler that is time-integrative
- Continuous toxin detection to capture ephemeral events
- Applicable in all waterbody types and for many different toxins
- Low cost, simple and easy to deploy/recover
- Not applicable to health advisory thresholds (ng/g)
- Only measures dissolved toxins not total toxins

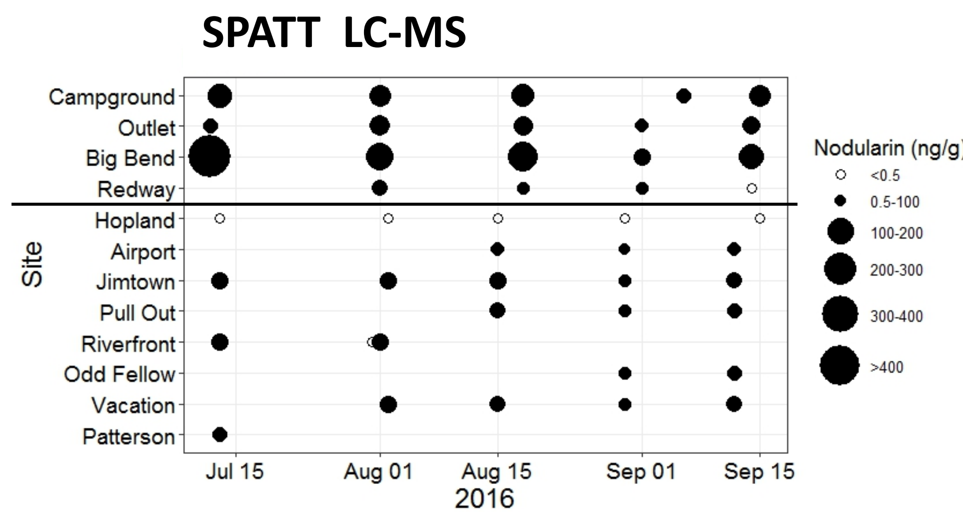


Picture: Keith Bouma-Gregson

Each Sample Type Contributes a Different Piece of the Toxin Story

Nodularin



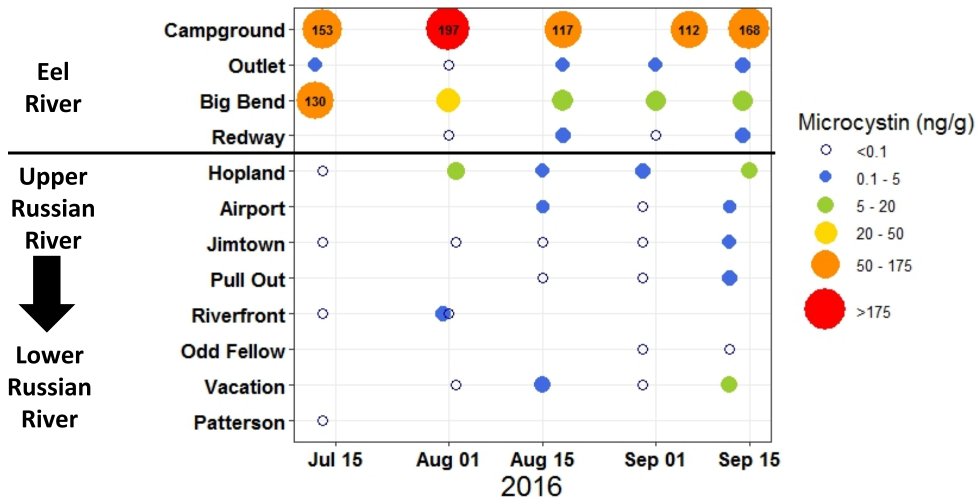


Sample Types (LC-MS)	Nodularin
SPATT (ng g^{-1})	bd - 450
Water (μg L^{-1})	bd - 0.06
Cyanobacterial Mats (μg L^{-1})	Not Tested

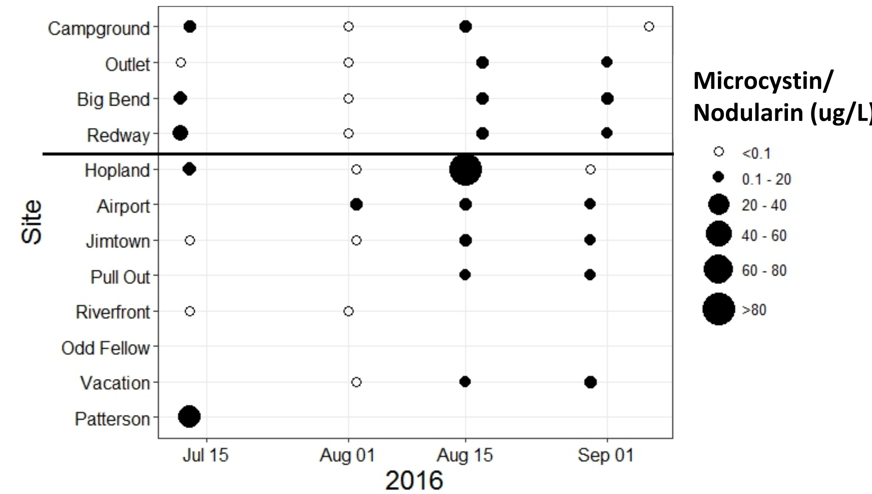
bd – Below Detection Limit

Microcystins

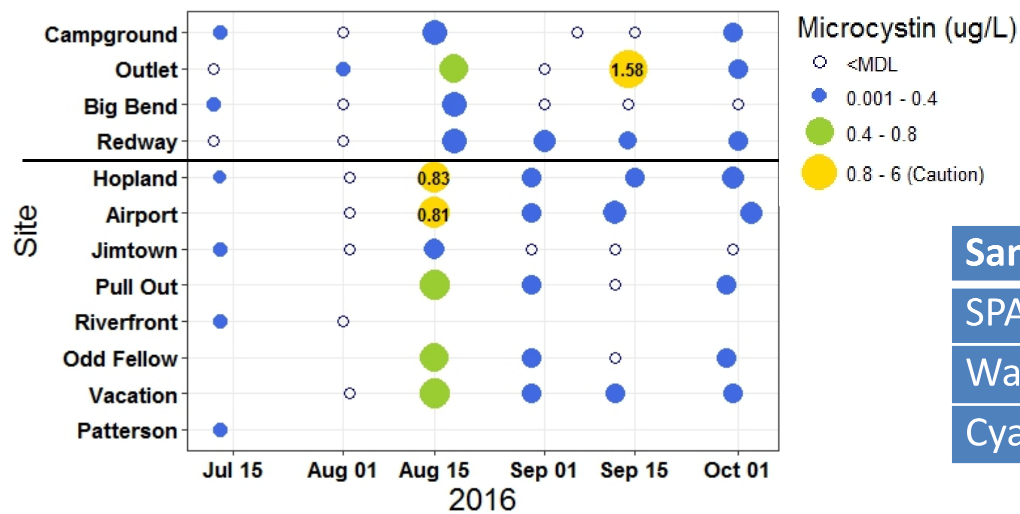
SPATT LC-MS



Cyanobacteria Mats - ELISA



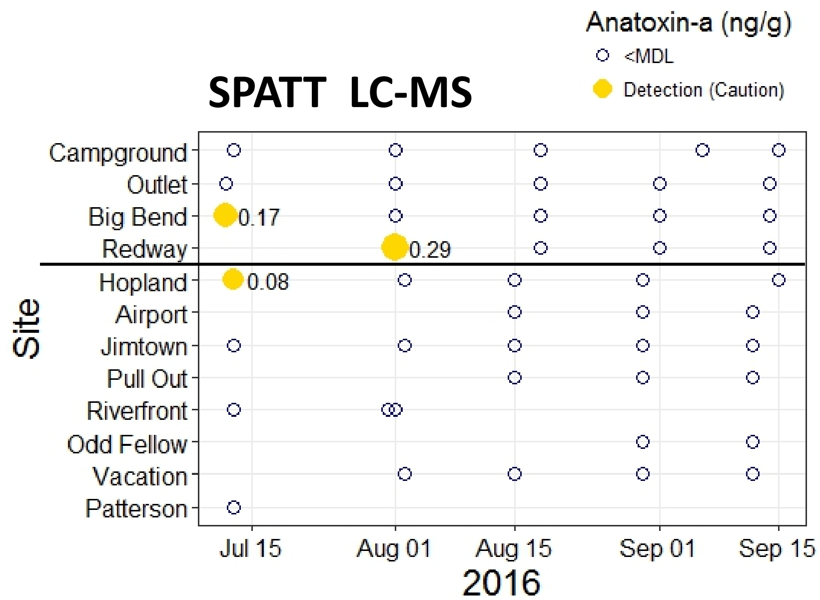
Whole Water LC-MS

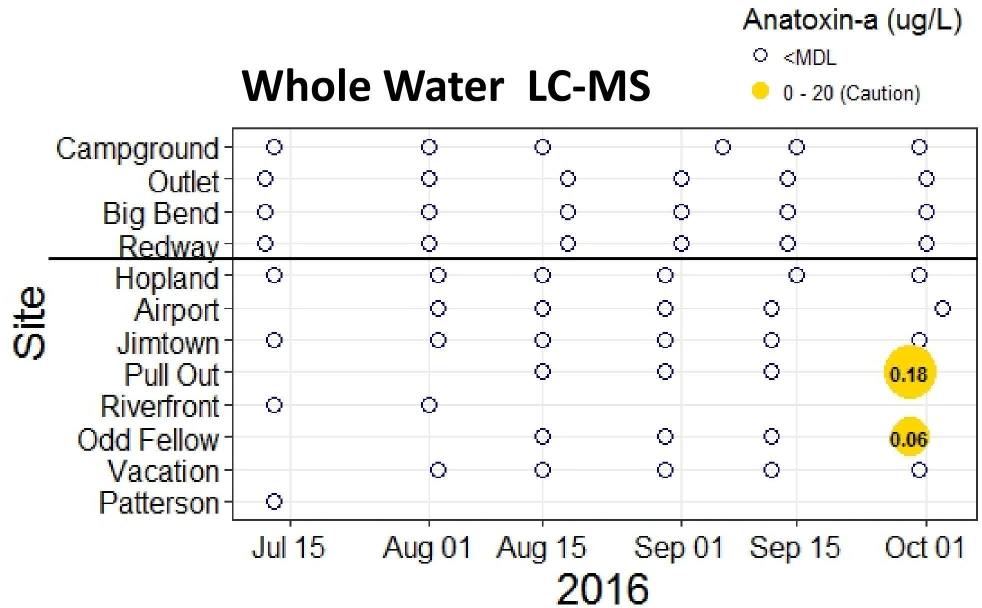


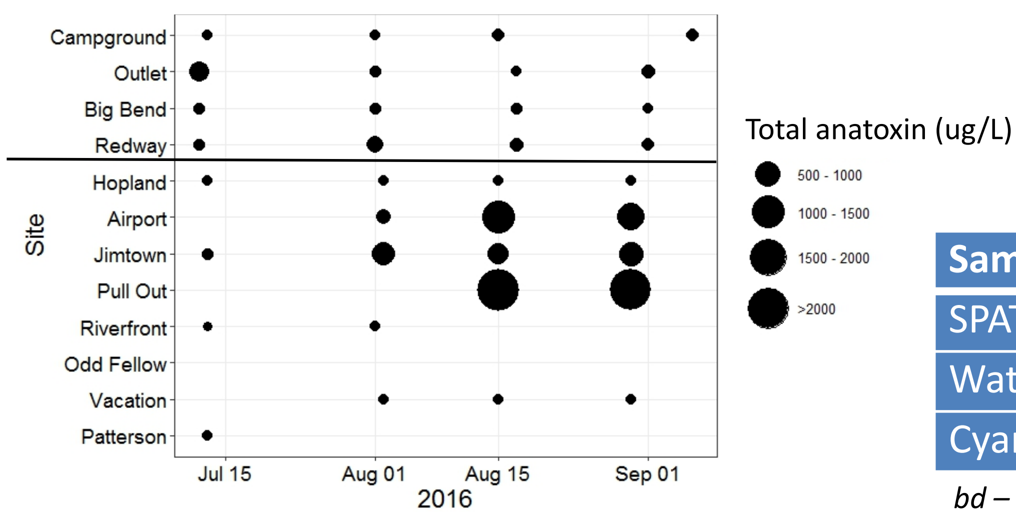
Sample Types	
SPATT (ng g^{-1})	bd - 197
Water (µg L^{-1})	bd – 1.5
Cyanobacterial Mats (µg L^{-1})	bd - 86

bd – Below Detection Limit

Analysis Method Contributes to the Toxin Story

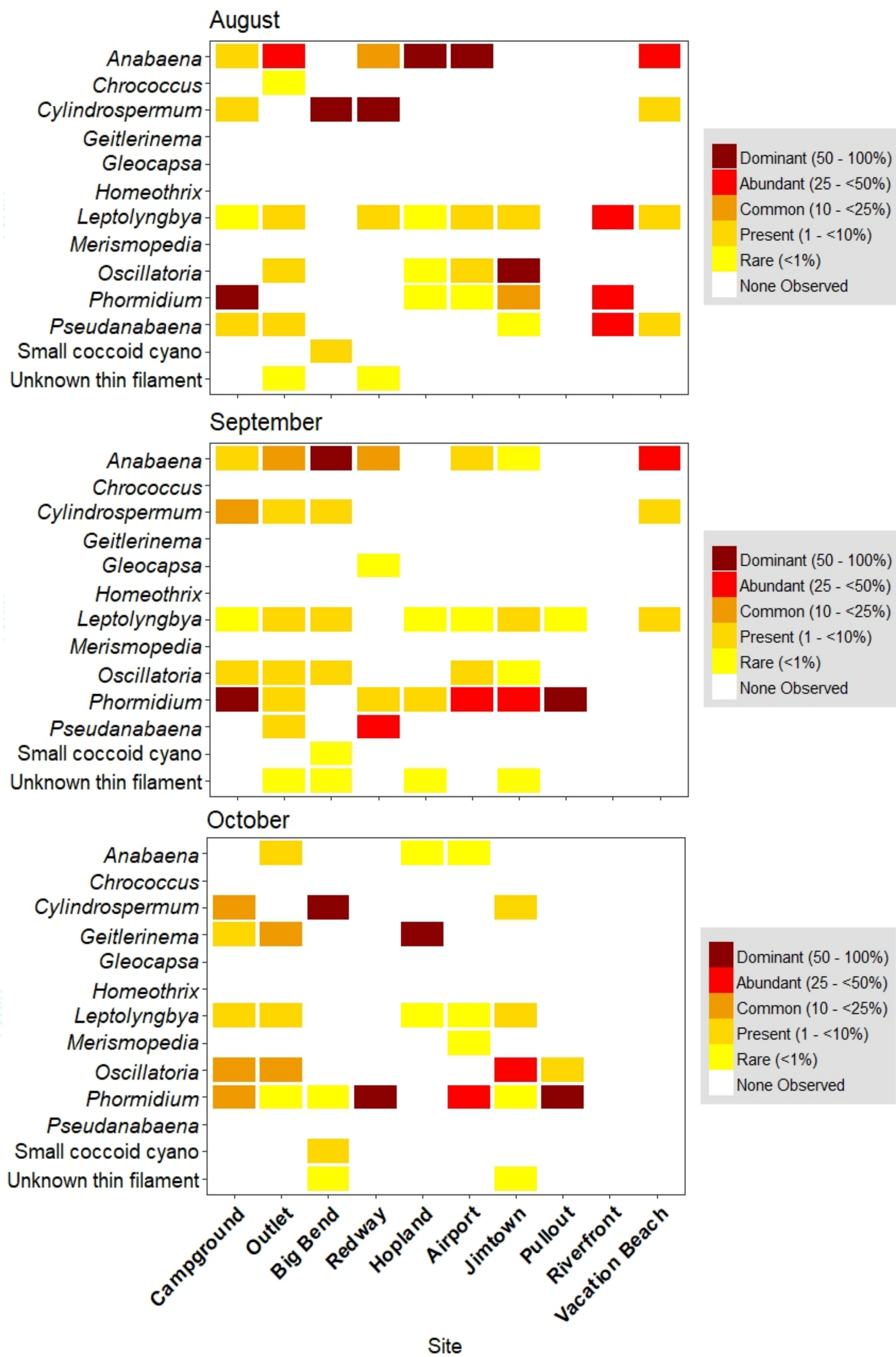






Sample Types	
SPATT (ng g^{-1})	bd – 0.17
Water (µg L^{-1})	bd
Cyanobacterial Mats (µg L^{-1})	0.4 - >15,750

bd – Below Detection Limit

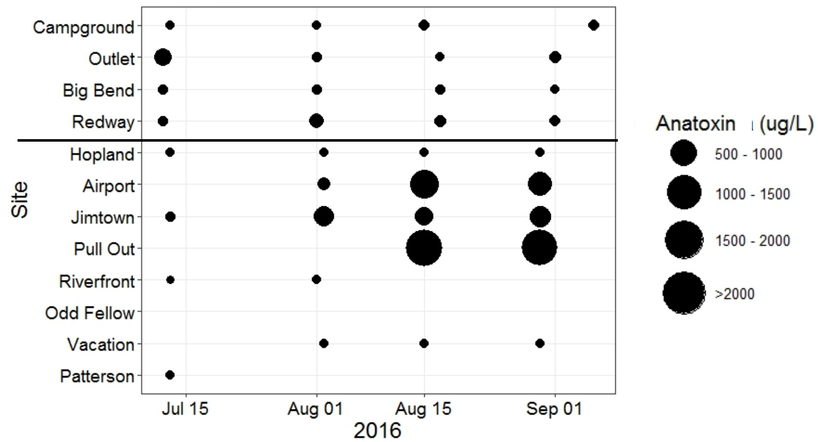


Benthic Cyanobacteria Mat Samples Indicated Cyanobacterial Dominance of Potential Toxin-Producing Cyanobacteria

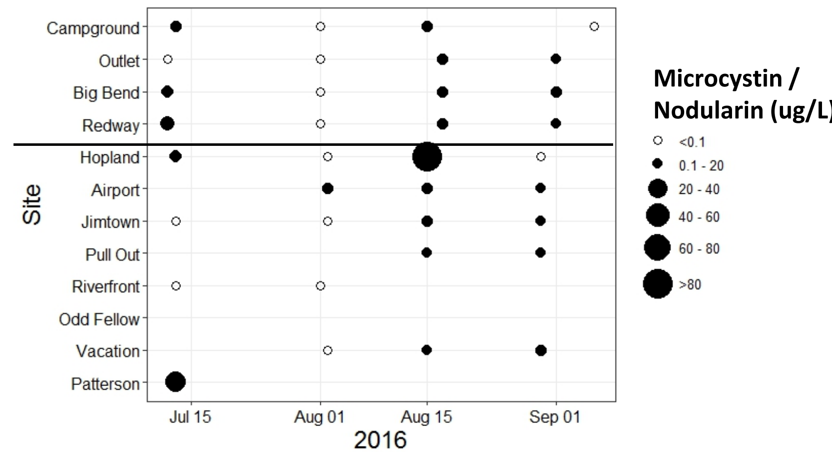
Relative Abundance (semi-quantitative)
Reach-integrated sample collection

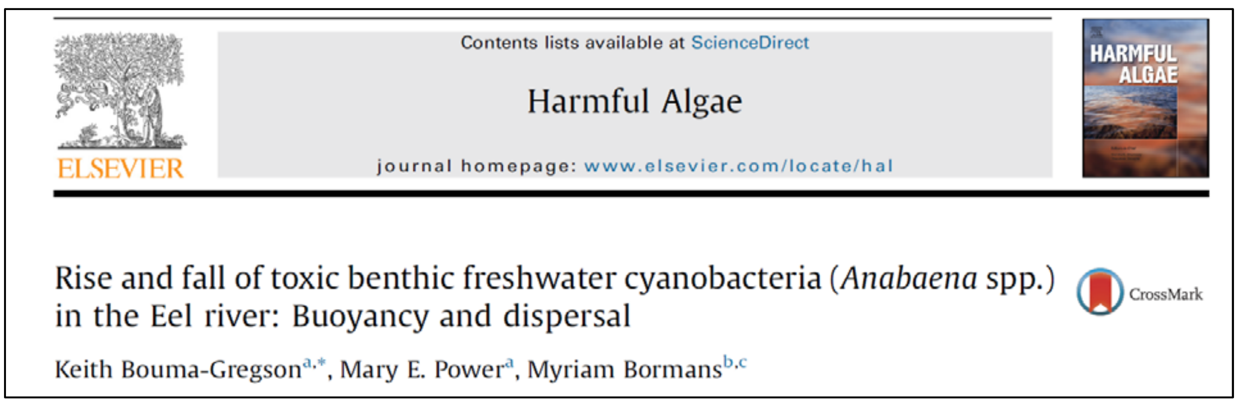
Buoyancy of Cyanobacterial Mats Increases Downstream Dispersal

Anatoxin



Microcystin / Nodularin

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HARMFUL ALGAE

Rise and fall of toxic benthic freshwater cyanobacteria (*Anabaena* spp.) in the Eel river: Buoyancy and dispersal

CrossMark

Keith Bouma-Gregson[a,*], Mary E. Power[a], Myriam Bormans[b,c]

- **Release of floating clumps from mats that are able to maintain buoyancy**
- **Buoyancy mechanism increases downstream dispersal distances**

Benthic River Blooms Can Have Impacts Downstream

toxins
MDPI

Article

Microcystin Prevalence throughout Lentic Waterbodies in Coastal Southern California

Meredith D. A. Howard [1,*], Carey Nagoda [2], Raphael M. Kudela [3], Kendra Hayashi [3], Avery Tatters [4], David A. Caron [4], Lilian Busse [5], Jeff Brown [1], Martha Sutula [1] and Eric D. Stein [1]

Contents lists available at ScienceDirect
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Evidence of freshwater algal toxins in marine shellfish: Implications for human and aquatic health

Corinne M. Gibble[a,*], Melissa B. Peacock[a,b,c], Raphael M. Kudela[a]
[a] Ocean Sciences Department, 1156 High Street, University of California, Santa Cruz, CA 95064, USA
[b] San Francisco Estuary Institute, 4911 Central Avenue, Richmond Ca 94804, USA
[c] Native Environmental Science, Northwest Indian College, 2522 Kwina Rd, Bellingham, Wa, 98226, USA

ELSEVIER
Harmful Algae
Volume 73, March 2018, Pages 138-147

Blurred lines: Multiple freshwater and marine algal toxins at the land-sea interface of San Francisco Bay, California

Melissa B. Peacock [a, b, c], Corinne M. Gibble [b, d], David B. Senn [d], James E. Cloern [e], Raphael M. Kudela [b]

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toxins
MDPI

Article

Multiple Stressors at the Land-Sea Interface: Cyanotoxins at the Land-Sea Interface in the Southern California Bight

Avery O. Tatters [1,*], Meredith D.A. Howard [2], Carey Nagoda [3], Lilian Busse [4], Alyssa G. Gellene [1] and David A. Caron [1]

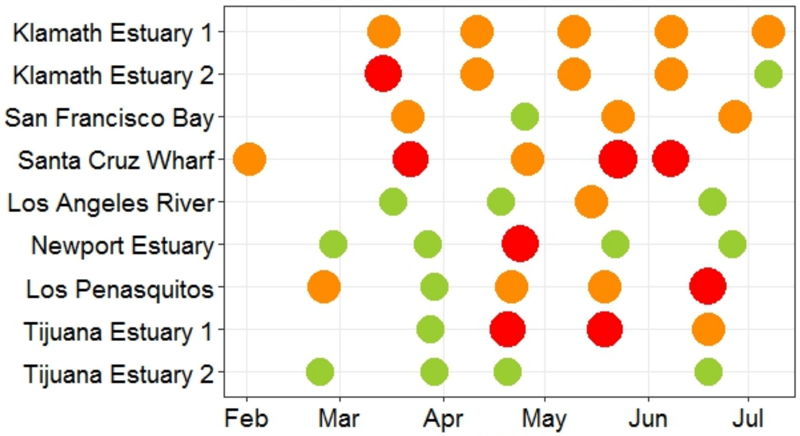
OPEN ACCESS Freely available online
PLoS one

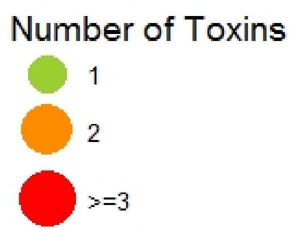
Evidence for a Novel Marine Harmful Algal Bloom: Cyanotoxin (Microcystin) Transfer from Land to Sea Otters

Melissa A. Miller[1,2,*], Raphael M. Kudela[2], Abdu Mekebri[3], Dave Crane[3], Stori C. Oates[1], M. Timothy Tinker[4], Michelle Staedler[5], Woutrina A. Miller[6], Sharon Toy-Choutka[1], Clare Dominik[7], Dane Hardin[7], Gregg Langlois[8], Michael Murray[5], Kim Ward[9], David A. Jessup[1]

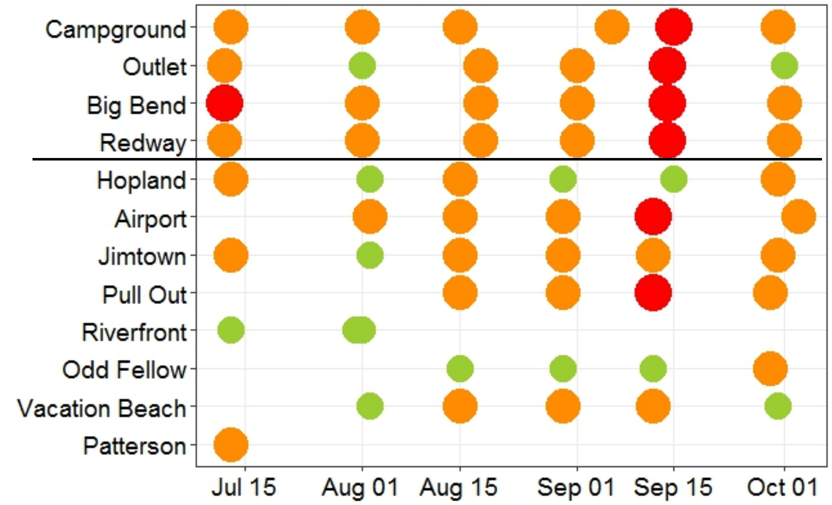
Synergistic Stressors :
Simultaneous Detection of Multiple Toxins

MERHAB Ocean and Estuarine Sites 2017

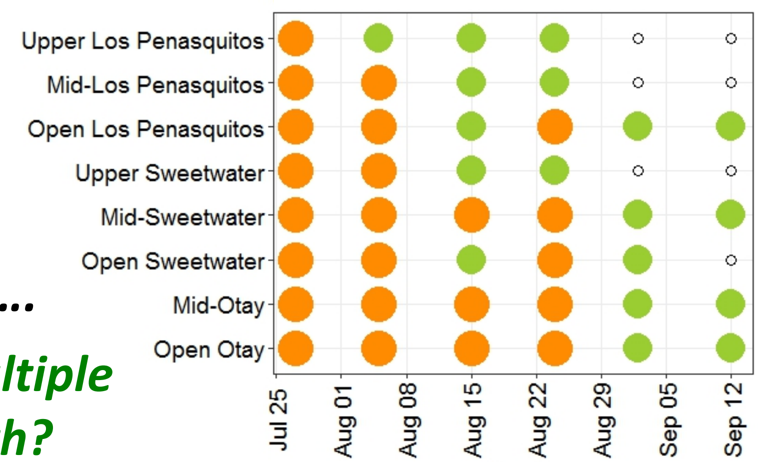




Russian and Eel Rivers - 2016



San Diego Sites 2016



Recreational and drinking water health thresholds are based on single toxin exposure….

What are the consequences of exposure to multiple toxins for human, wildlife and ecological health?

Isolation and culturing of toxigenic cyanobacteria from Russian River, Garcia River and Eel River: 2015 and 2017

Rosalina Stancheva
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California Primary Algae Lab for SWAMP
California State University San Marcos

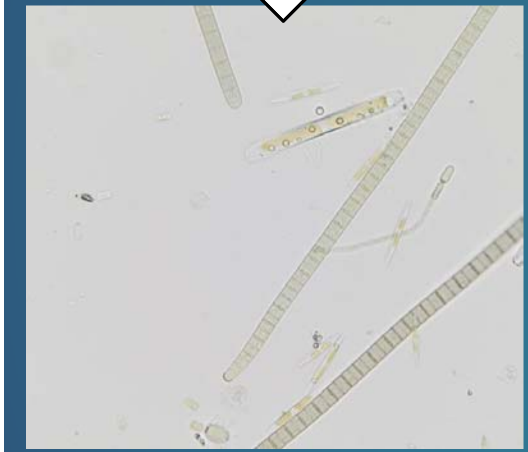
Culturing Methods


Russian River to be closely monitored this summer to guard against harmful algae blooms

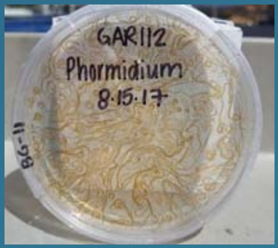
(2 of 7) Rich Fadness, the coordinator of the Surface Water Ambient Monitoring Program (SWAMP) with the North Coast Regional Water Quality Board, takes a sample of algae to see if it contains any blue-green algae, at Monte Rio Beach in Monte Rio, on Thursday, June 23, 2016. (BETH SCHLANKER/ The Press Democrat)

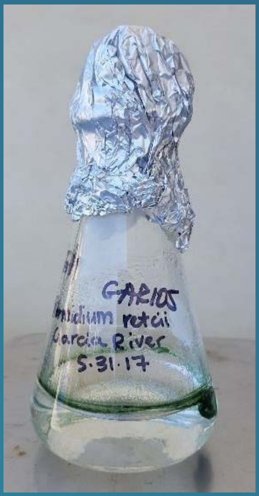
MARY CALLAHAN
THE PRESS DEMOCRAT | June 23, 2016

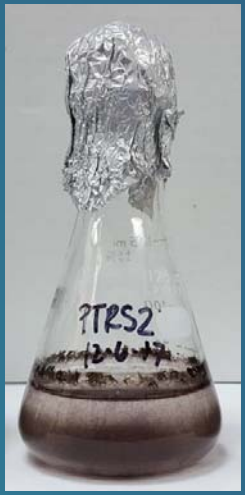
- Blue Green 11 Medium (BG11), liquid and solid 1% or 1.5% agar
- Glassware, limited use of disposable plastic containers
- 20^{o}C, 12h:12h light/dark cycle, 40 µmol photons m-2 s-1
- Initial cyanobacteria isolation on solid medium: 3-6 transfers every 10 days
- Monoculture transfer to a liquid medium
- Continuing growth with regular transfer in new medium every 2 months
- Regular microscope check for culture purity and documentation (Olympus BX41 microscope with Olympus SC30 digital camera)
- Monocultures are NOT axenic
- Cyanobacteria continuously produce toxins in culture

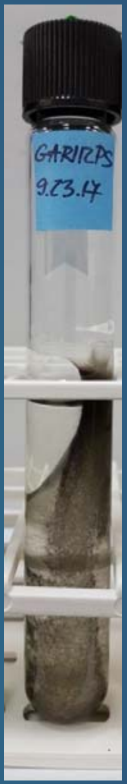


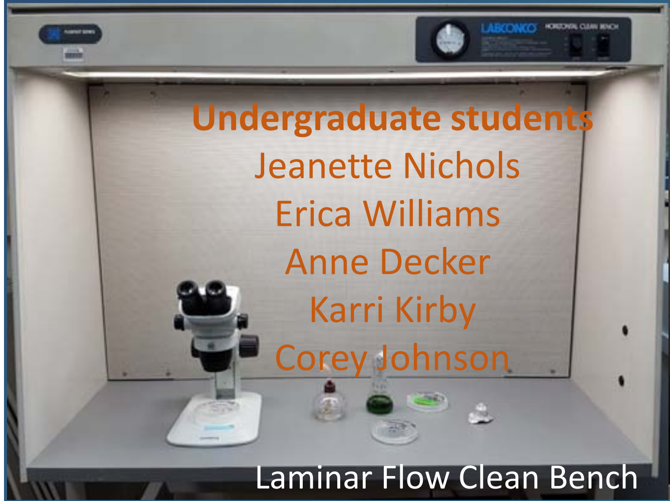
Single-species
cyanobacterial mat does not exist!









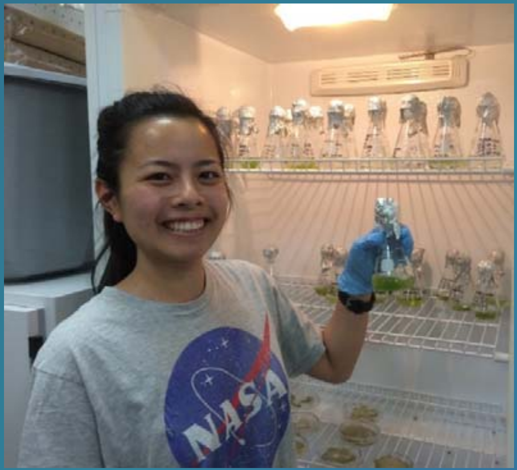


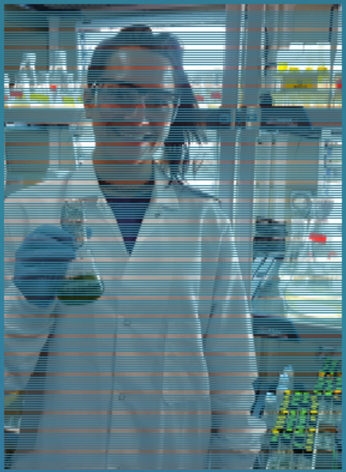
Undergraduate students
Jeanette Nichols
Erica Williams
Anne Decker
Karri Kirby
Corey Johnson

Laminar Flow Clean Bench

Biosafety Cabinet

Walk-in Culturing Room





Support from Professors Betsy Read and Robert Sheath and taxonomists Cricket Fuller and Kim Conklin!

Toxin Measurement Methods

ELISA:

Anatoxin (ATX), Cylindrospermopsin (CYL), Microcystin/Nodularin (MCY/NOD), Saxitoxin (STX)

- *Dan Orr*
- *Tim Otten*

LC-MS/MS:

Anatoxin-a, Homoanatoxin-a, Dihydroanatoxin-a, Dihydrohomoanatoxin-a, Cylindrospermopsin

- *Susie Wood*

Molecular Methods

Complete genome sequencing of anatoxin-producing strains

- *Tim Otten*
- *Betsy Read*
- *Keith Bouma-Gregson*

RESULTS:

12 ATX-producing cyanobacteria strains established

ATX-a (µg/L)	dhATX (µg/L)	Method	Date	Strain	Genus	Sample site	River	Sample date
maybe	maybe	LC-MS/MS	3/15/17	AOJN1**	*Anabaena oscillarioides*	114RR3119	Russian	10/1/15
0.66	331	LC-MS/MS	3/15/17	PTRS1**	*Phormidium*	114RR3119	Russian	10/1/15
0.38	363	LC-MS/MS	3/15/17	PTRS2**	*Phormidium*	114RR3119	Russian	10/1/15
0.47	483	LC-MS/MS	3/15/17	PSRS3H**	*Phormidium*	114RR5652	Russian	10/1/15
Total Anatoxin (µg/L)		**Method**	**Date**	**Strain**	**Genus**	**Sample site**	**River**	**Sample date**
0.14		ELISA	11/13/17	RRAPS	*Phormidium*	114RR5407	Russian	8/3/17
0.10		ELISA	11/13/17	RRAAF	*Anabaena+Geitlerinema*	114RR5407	Russian	8/3/17
>125		ELISA	11/13/17	GAR112PS*	*Phormidium*	113GAR112	Garcia	5/31/17
3.36		ELISA	11/13/17	GAR046PS	*Phormidium*	113GAR046	Garcia	8/1/17
2.86		ELISA	11/13/17	GAR105PS*	*Phormidium*	113GAR105	Garcia	5/30/17
0.10		ELISA	11/13/17	GAR112AA	*Anabaena+Amoeba*	113GAR112	Garcia	5/31/17
0.65		ELISA	11/13/17	RR2PA	*Phormidium*	111ER8102	Eel	8/17/17
2.60		ELISA	11/13/17	RR3PS*	*Phormidium*	111ER8102	Eel	8/17/17

All strains ELISA tested negative for microcystin/nodularin and cylindrospermopsin (*Orr, Otten*)

****Complete genome sequencing data (*Tim Otten*)**

ONLY Anatoxin pathways found in three Phormidium strains

*** Complete genome sequencing data (*Keith Bouma-Gregson*)**

Phormidium strains from Russian River (2015 and 2017)

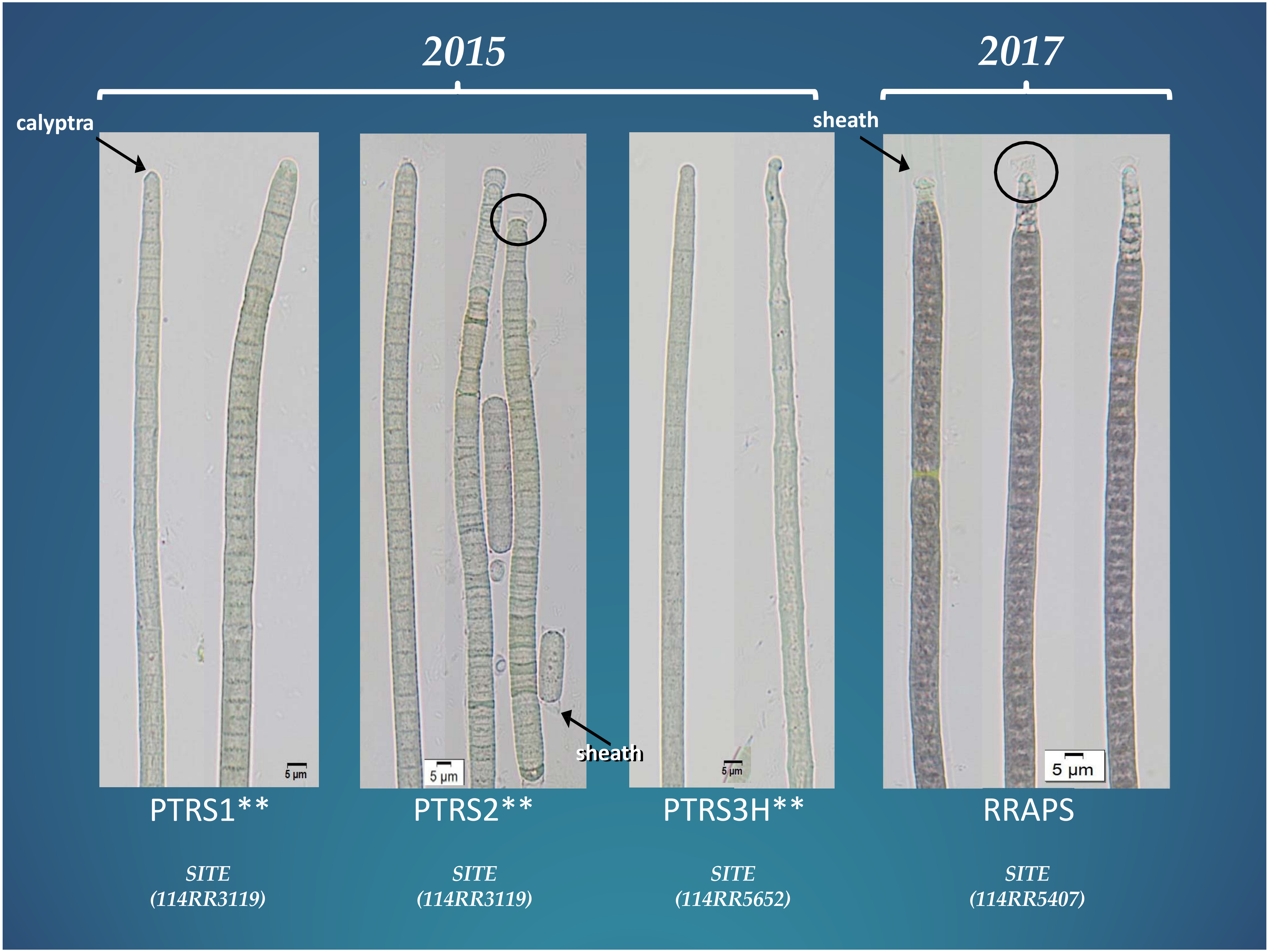
2015

PTRS1 PTRS2 PTRS3H

PTRS1 PTRS2 PTRS3H RRAPS

2017

RRAPS



2015
2017
calyptra
sheath
sheath
5 µm
5 µm
5 µm
5 µm
PTRS1**
PTRS2**
PTRS3H**
RRAPS
SITE
(114RR3119)
SITE
(114RR3119)
SITE
(114RR5652)
SITE
(114RR5407)


Anabaena strains from Russian River
heterocyte
akinete
10 µm
Anabaena and *Geitlerinema*
5 µm
10 µm
AOJN1 (*SITE 114RR3119*)
2015
RRAAF (*SITE 114RR5407*)
2017

Anabaena and *Phormidium* strains Garcia River (2017)

GAR112AA

SITE
(*113GAR112*)

GAR112PS*

SITE
(*113GAR112*)

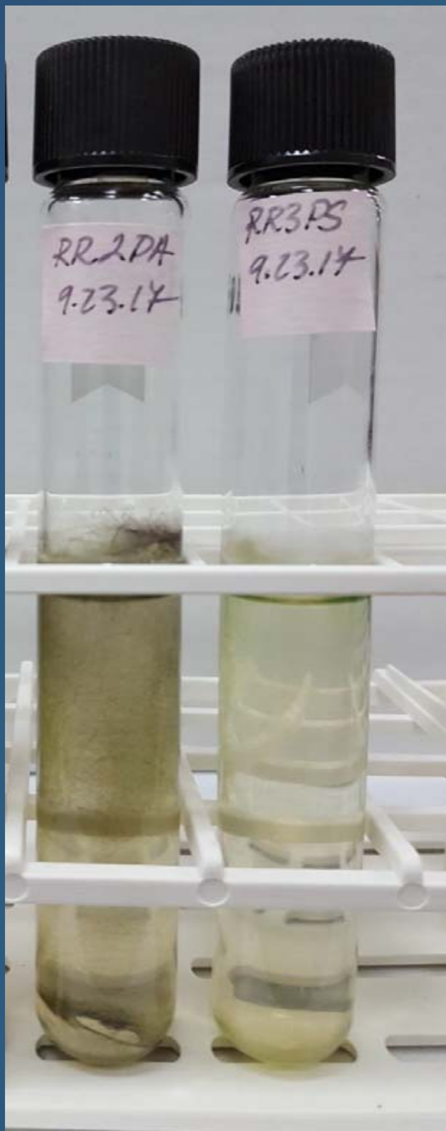
GAR046PS

SITE
(*113GAR046*)

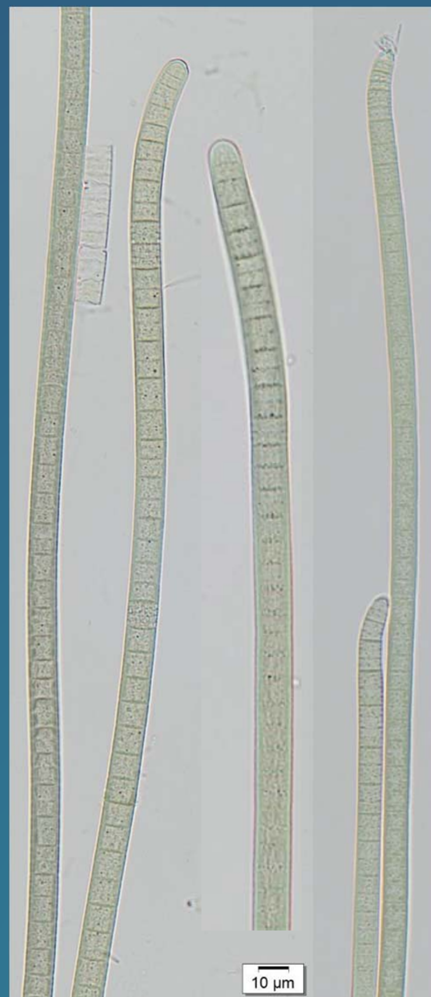
GAR105PS*

SITE
(*113GAR105*)

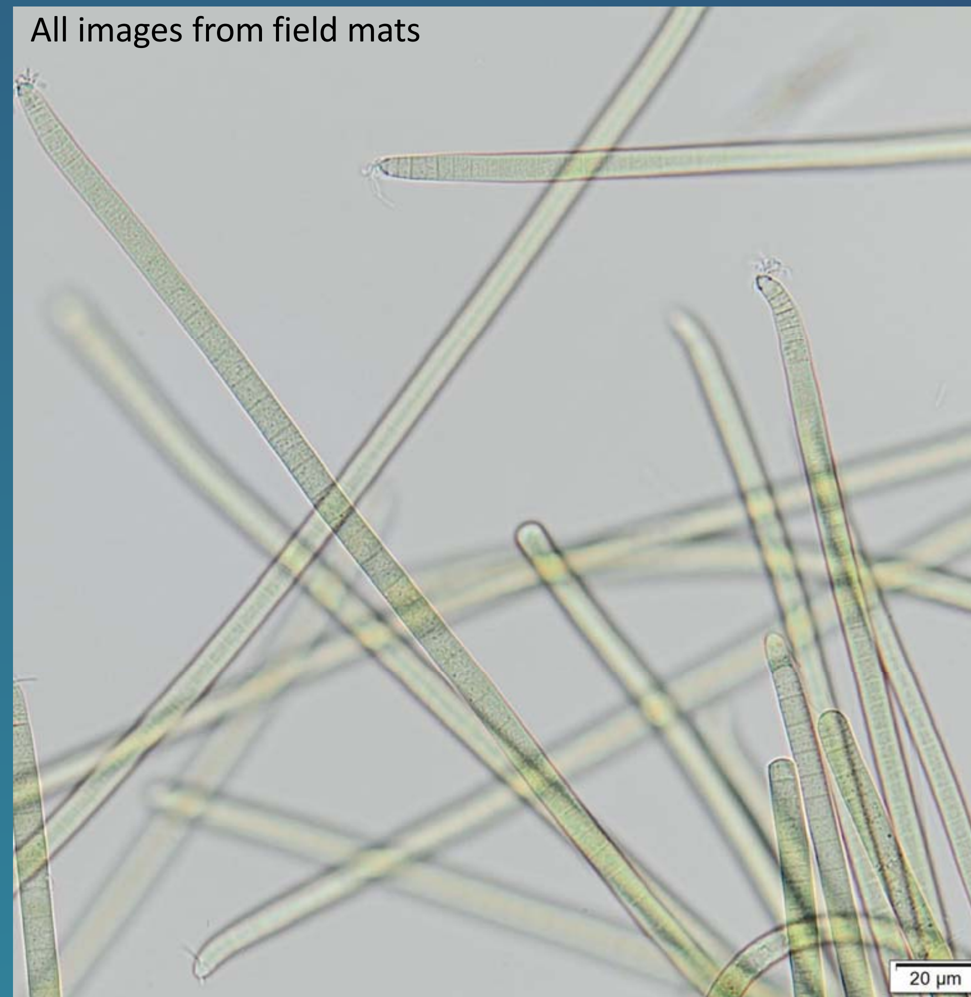
Phormidium strains from Eel River (2017)



SITE
(111ER8102)



RR3PS*



RR2PA

RESULTS:

7 non-toxigenic (?) cyanobacteria strains established

Strain	Genus	Sample site	River	Sample date
GAR105PR*	*Phormidium*	113GAR105	Garcia	5/30/17
GAR061CM	*Cylindrospermum*	113GAR061	Garcia	8/2/17
GAR112PP	*Pseudanabaena*	113GAR112	Garcia	5/31/17
Eel07Phor*	*Phormidium*	Fox Creek	Eel	7/19/17
Eel06Phor*	*Phormidium*	Mud Creek	Eel	7/20/17
RR5PA	*Phormidium*	111ER8102	Eel	8/17/17
GAJN1**	*Geitlerinema*	114RR3119	Russian	10/1/15

ELISA testing:

Negative for ATX, MCY and STX

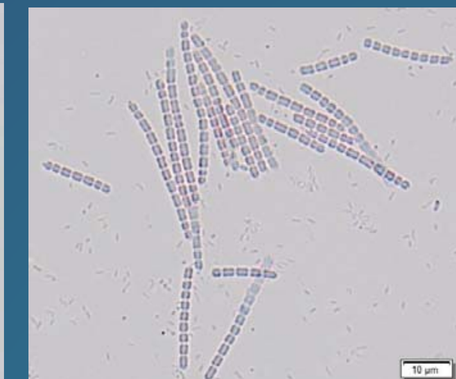
(CYL detected in GAJN1 only)

Non-toxigenic cyanobacteria strains morphology

2015

2017

GAJN1**

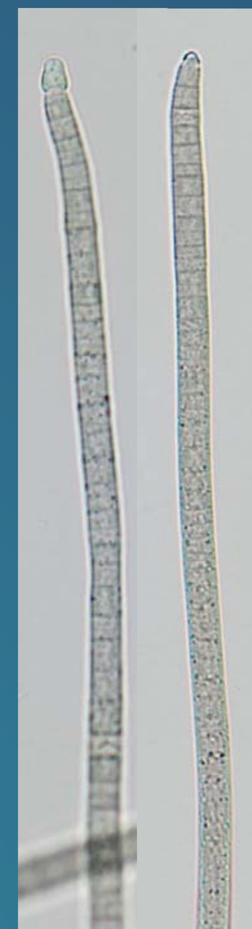


GAR112PP

GAR061CM

GAR105PR*

EEL06*



EEL07*

RR5PA

Extracts from benthic anatoxin-producing *Phormidium* are toxic to three macroinvertebrate taxa at environmentally relevant concentrations

Brian Anderson

University of California, Davis
Granite Canyon Marine Pollution Studies Lab

Algal toxins and environmental monitoring

- Impacts to water supply, wild and domestic animals, recreational use, and human health
- Increasing impacts expected, linked to climate change (Roelke et al. 2012)
- Freshwater cyanotoxins:
  - Microcystin (hepatotoxin) - SPoT monitors sediments
    - Trend monitoring
    - Bioaccumulation in invertebrates – trophic transfer
  - Anatoxin (neurotoxin) - water
    - Dog deaths
    - Invertebrate impacts

Anatoxin from Russian River algae: Invertebrate Toxicity

- Samples from benthic cyanobacterial mats collected at two sites on Russian River: *(October 2015)*
- California State University, San Marcos Species identification and culturing: *(Rosalina Stancheva)*
- Strain isolation and culture using BG11 medium
- Three *Phormidium* strains isolated for this study

First steps:

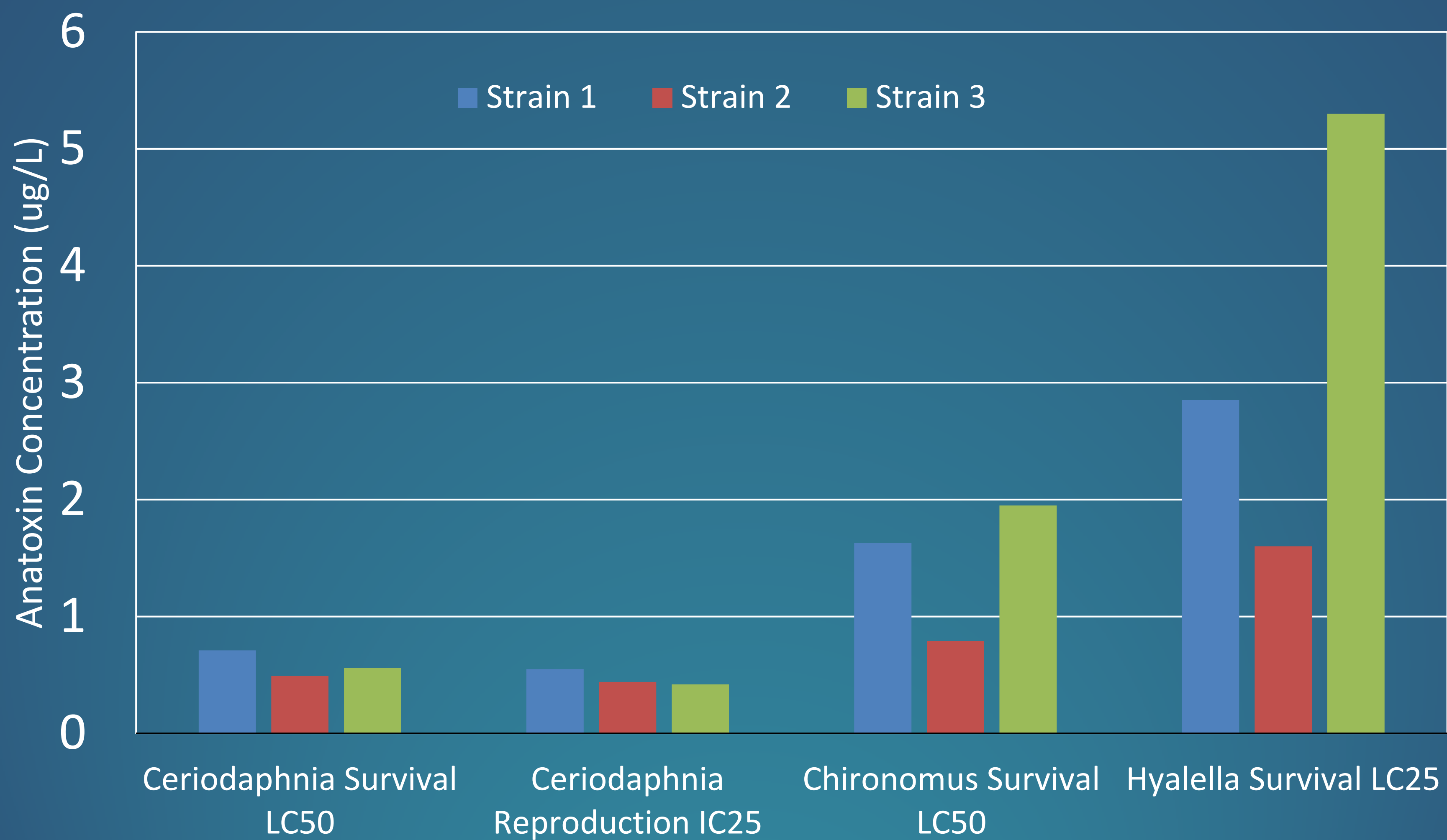
- BG 11 media pre-testing
- *Ceriodaphinia dubia* pre-testing

Anatoxin Toxicity Tests (water)

- Water samples from *Phormidium* cultures analyzed for anatoxin by ELISA and anatoxin-a by LCMSMS
- *Ceriodaphnia dubia*: 7-day chronic (survival and reproduction)
- *Chironomus dilutus*: 4-day acute
- *Hyalella azteca*: 4-day acute
- Dilution series based on BG11 tolerance and sensitivity to anatoxin (C. dubia).



Anatoxin Toxicity Threshold Levels (ELISA)



Toxic to all three species

Anatoxin Variant Analysis by LC/MS

Concentrations of anatoxin variants and cylindropersmopsin measured by liquid chromatography-mass spectrometry in cell material extracted from the three *Phormidium* strains used for toxicity tests.

Phormidium strain	Anatoxin-a	Homoanatoxin-a	Dihydro-anatoxin-a	Dihydro-homoanatoxin-a	Cylindrospermopsin
	µg/g of dry culture				
Strain 1	0.66	ND	331.2	ND	ND
Strain 2	0.38	ND	363.4	ND	ND
Strain 3	0.47	ND	483.3	ND	ND

ND = not detected.

Anatoxin Toxicity Results

- Water from 3 cultures highly toxic to three invertebrate species commonly used in surface water monitoring
- Toxicity to Hyalella and Chironomus under-estimated using 4d exposures
- LC-MS/MS analysis showed anatoxin variant is dihydroanatoxin-a

Potential for Impacts on Stream Macroinvertebrates

- Relationship to instream concentrations?
  - Fetscher et al. (2015) reported that one third of the streams from a large dataset in California contained one or more cyanotoxins with benthic origin
  - Previously reported SMC results showed unexplained *C. dubia* mortality at headwater and reference sites
  - Russian River anatoxin concentrations:
    - SPATT samplers and water column samples showed non-detects for anatoxin-a (LCMS analysis)

Russian River Mat Extracts

Concentrations of anatoxin measured in cyanobacterial mat samples collected at two stations on the Russian River in September, 2016. Toxin analyses were conducted with ELISA.

Russian River Site	Dominant species	Anatoxin (µg/L)	Ash Free Dry Mass (mg/L)	Anatoxin (µg/g dry wt.)
114RR2655	Phormidium A	8,115	6,670	1216
114RR2655	Phormidium B	>15,750	9,030	>1,744
114RR5407	Phormidium B	3,396	72,90	466
114RR5407	Phormidium C	2,631	1,940	1,356

Implications for Surface Water Monitoring

1) Presence of cyanotoxins could account for toxicity to invertebrates not attributed to traditional suite of chemical analytes

2) This may affect interpretation of surface water toxicity and bioassessment monitoring

3) ELISA provides good screening data for cyanotoxins, but LCMSMS provides quantification of specific variants or homologues

4) Next Steps?

Publication in review

In-stream concentrations of algal toxins: Is there a potential for impacts

Relative sensitivity of other native invertebrates

2015-2017

Benthic Cyanobacteria

Project Collaborators

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