BENTHIC HABS WORKGROUP WEBINAR

OCTOBER 30, 2019 - 12:30 PM to 2:00 PM Pacific Daylight Time

Web Meeting Address: https://usace.webex.com/meet/jade.l.young

Meeting Number: 968 579 710

Phone Number: 1-888-363-4735

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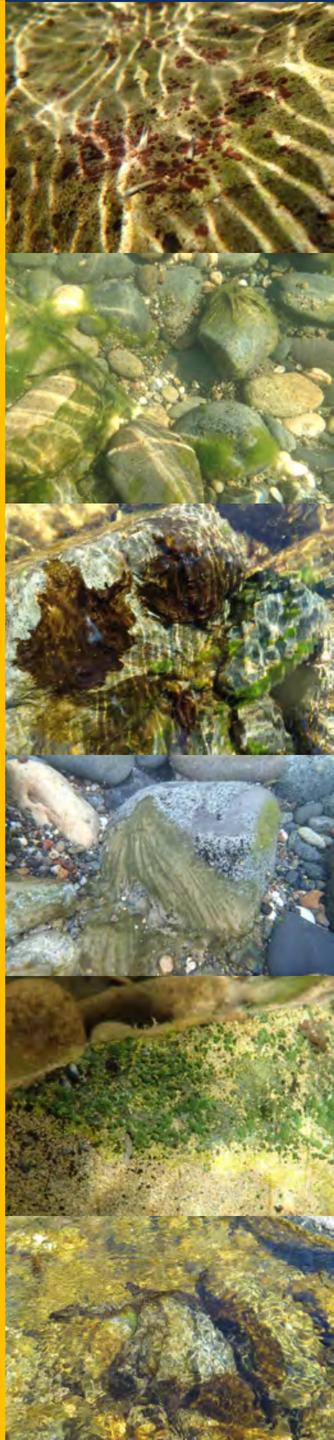
GUEST SPEAKER: KEITH BOUMA-GREGSON

PRESENTATION: CRYPTIC CYANOTOXIN PRODUCERS IN BENTHIC MATS

ITEM I

Welcome, Introductions & Agenda Overview

Margaret Spoo-Chupka



WELCOME & INTRODUCTIONS

Where to Find Us:

https://www.epa.gov/cyanohabs/epa-newsletter-and-collaboration-and-outreach-habs#benthic

An official website of the United States government.

EPA United States Environmental Protection Agency

Environmental Topics | Laws & Regulations | About EPA | Search EPA.gov

Related Topics: Cyanobacterial HABs | Ground Water and Drinking Water | Water Quality Criteria

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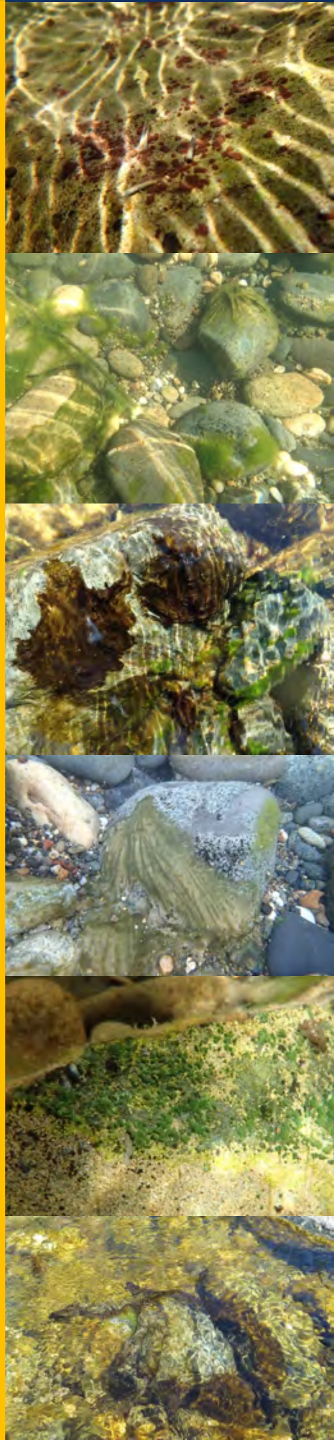
EPA Newsletter and Collaboration and Outreach on HABs

On this page:

- EPA Freshwater HABs Monthly Newsletters
- HABs Webinars
- Benthic HABs Discussion Group
- Inland HABs Discussion Group



AGENDA OVERVIEW

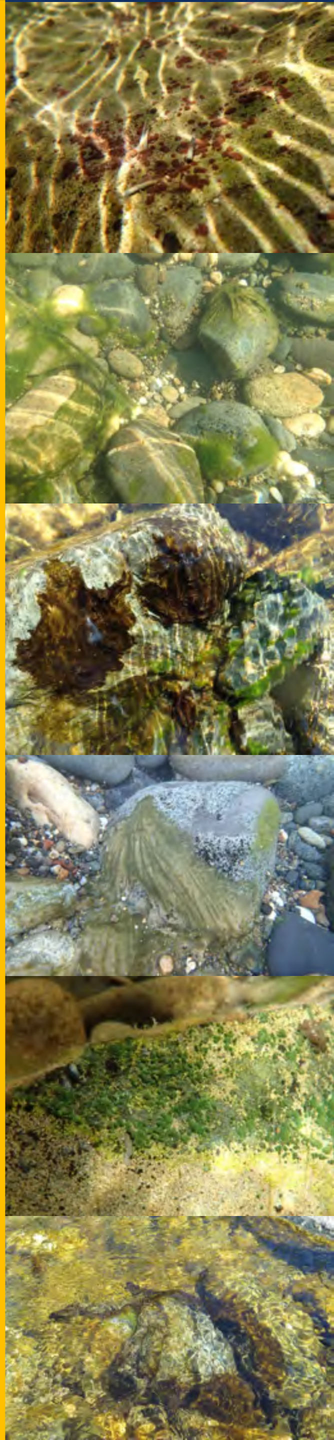
I **Welcome, Introductions & Agenda Overview (10 MIN)**
Margaret Spoo-Chupka

II **Benthic HABs Workgroup 2019 Member Survey Summary (30 MIN)**
Jade Young

III **Presentation: Cryptic cyanotoxin producers in benthic mats (30 MIN)**
Guest Speaker – Keith Bouma-Gregson

IV **Open Discussion, Benthic HABs article, Journal Publications & Upcoming Meetings (10 MIN)**
Christine Joab & Benthic HAB members

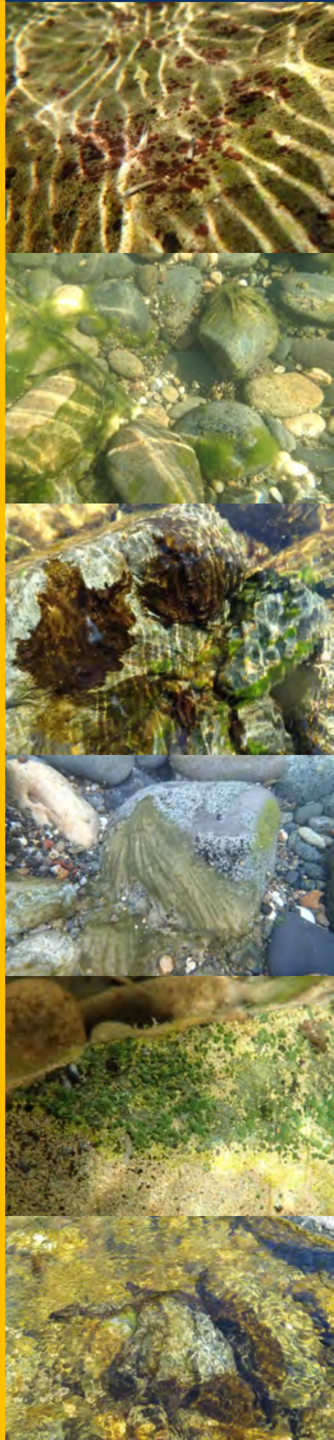
V **2020 Schedule, Wrap Up & Next Steps (10 MIN)**
Facilitators & Benthic HAB members



ITEM II

Benthic HABs Workgroup
2019 Member Survey Summary

Jade Young



Benthic HABs Discussion Group 2019 Member Survey Results

Presented for the Benthic HABs Discussion Group on 30 OCT 2019 by co-facilitator Jade Young
US Army Corps of Engineers, Louisville District
Jade.L.Young@usace.army.mil
502-315-7439

MISSION STATEMENT

"The mission of this international collaborative is to accelerate mutual understanding of benthic HABs in rivers and lake systems, by sharing data and monitoring protocols, experiences and lessons learned."

Facilitators

Christine Joab

Environmental Scientist

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Quality Control Board, California

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Jade Young

Limnologist

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Louisville District, Kentucky

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Jade.L.Young@usace.army.mil

Summary of previous affiliations and topics

UC Berkeley

Cawthron Institute, New Zealand

Metropolitan Water District of Southern CA

North Coast Regional WQ Control Board, CA

Southern CA Coastal Water Research Program

CA State University, San Marcos

University of CA, Davis

Northern KY University

US Army Corps of Engineers

- Temporal and spatial distribution
- SPATT samplers
- Toxic species
- Challenges
- Ecology & toxins
- Monitoring and management
- Research
- Monitoring tools
- Laboratory methods
- Taxonomic information
- Management techniques
- Results
- Downstream dispersal
- Toxin synergy
- Culturing methods
- Toxin measurement methods
- Invertebrate toxicity

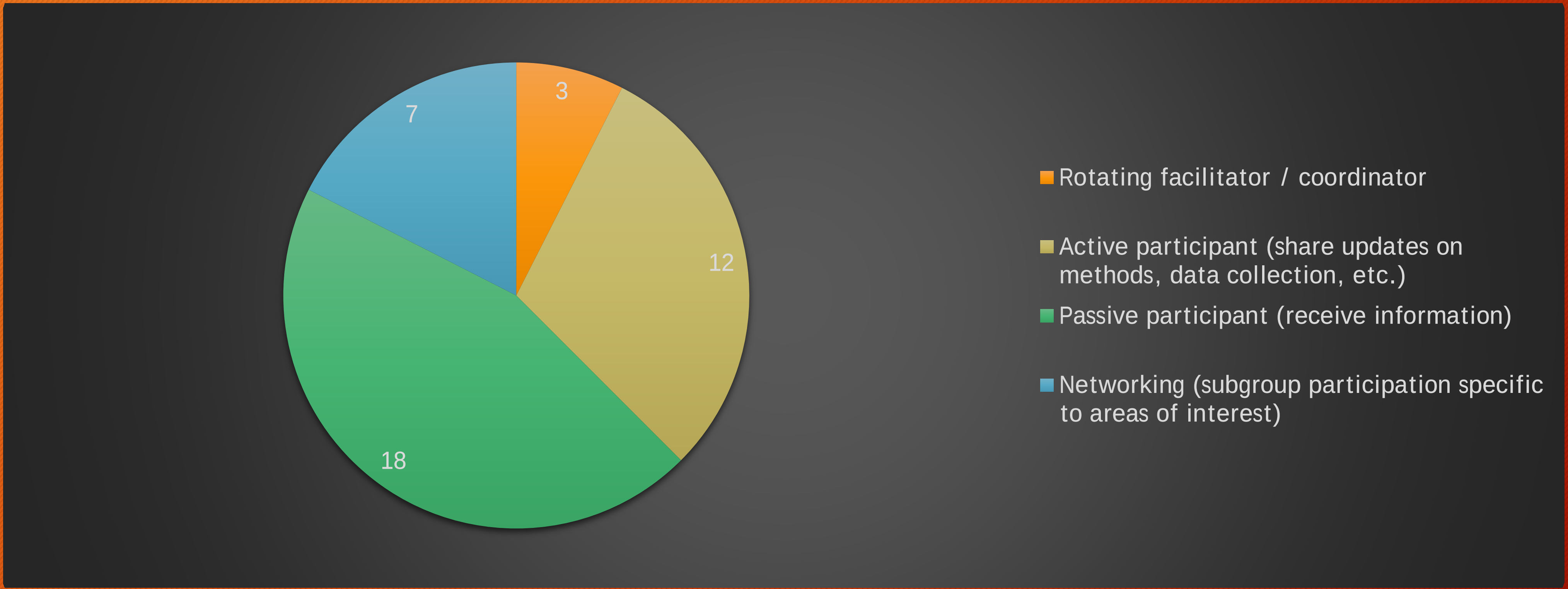
2019 Membership Re-survey

Summary of Responses

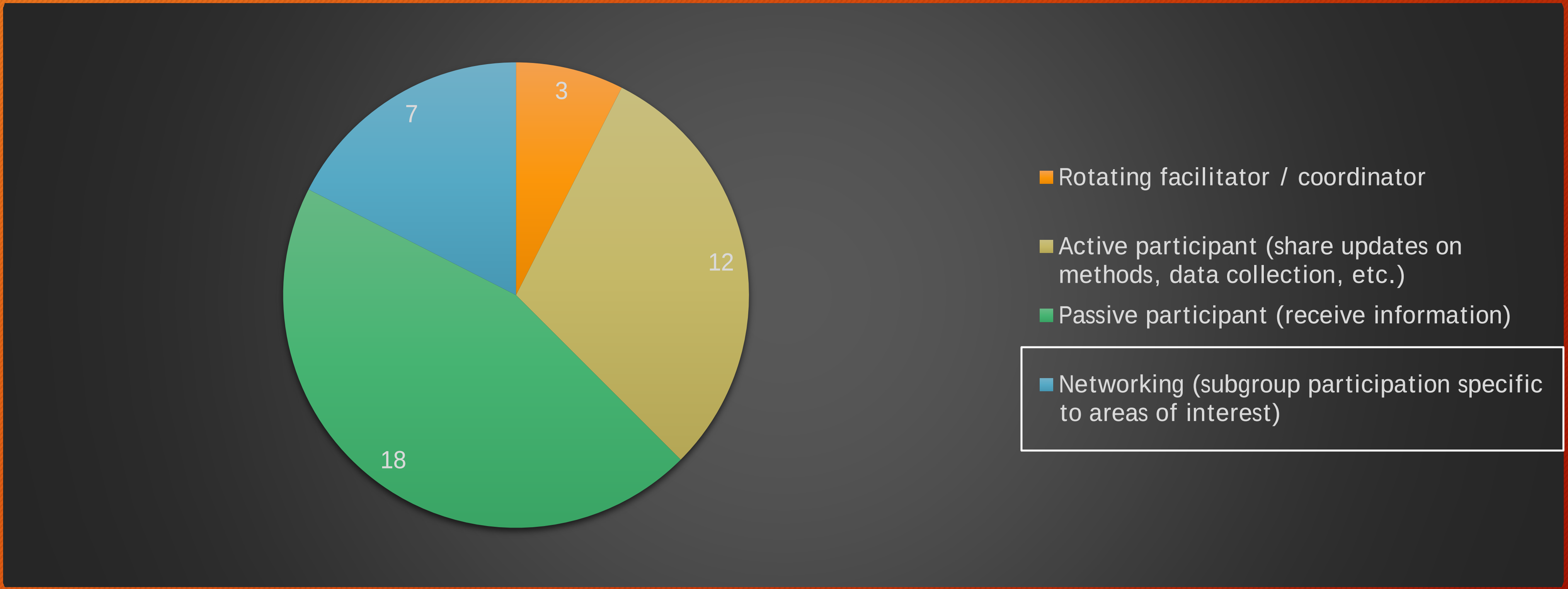
- 40 responses collected
- 17 new members
- New agencies represented

Anabaena sp. mat in the Russian River by Rich Fadness.

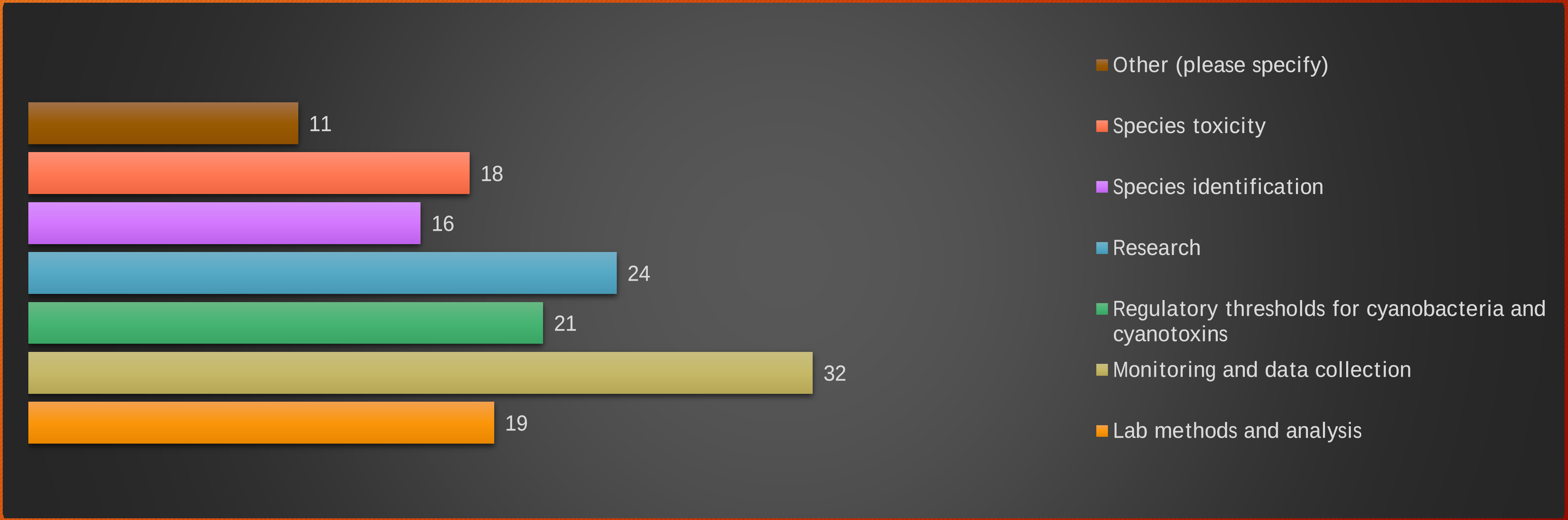
As a member of the Benthic HABs Discussion Group, which role is of most interest to you?



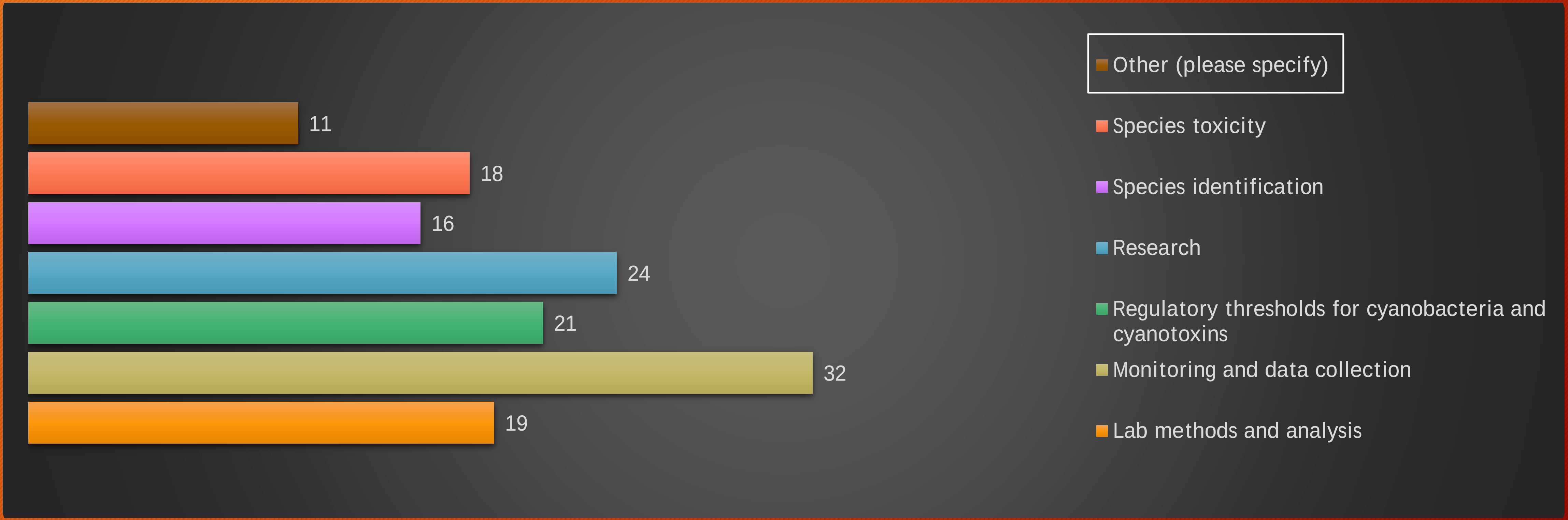
As a member of the Benthic HABs Discussion Group, which role is of most interest to you?



Do you have an area of interest or expertise?



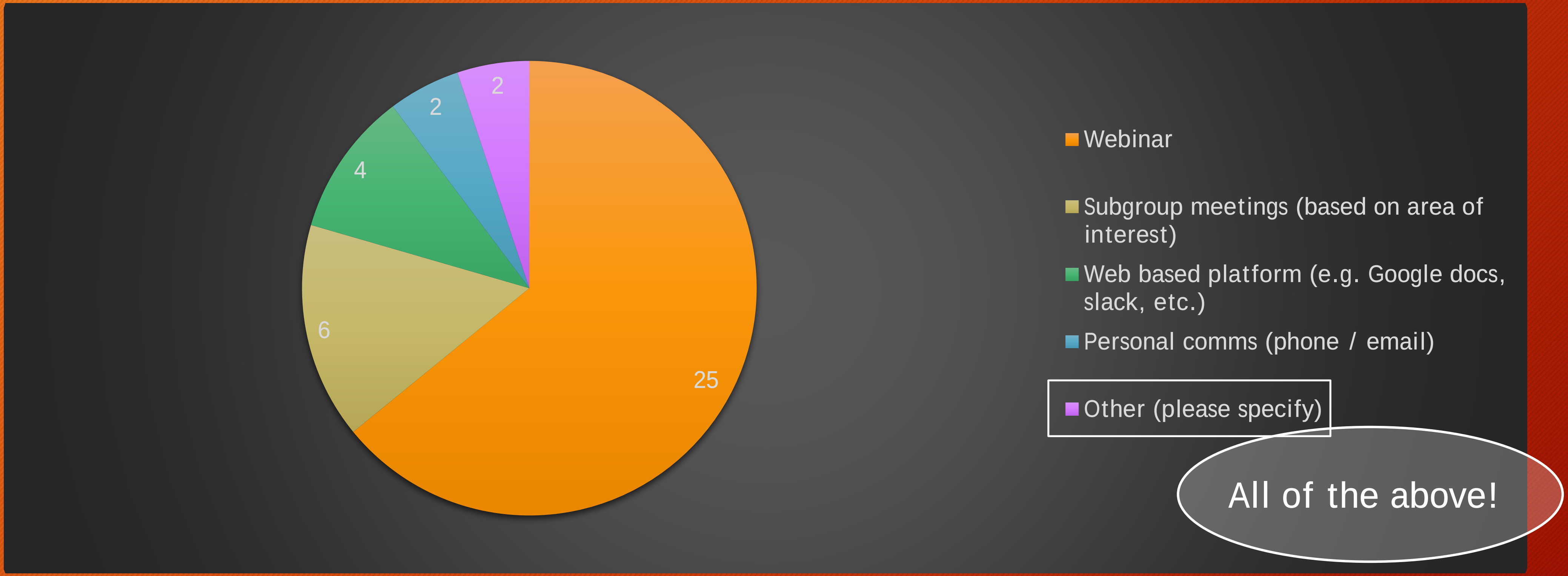
Do you have an area of interest or expertise?



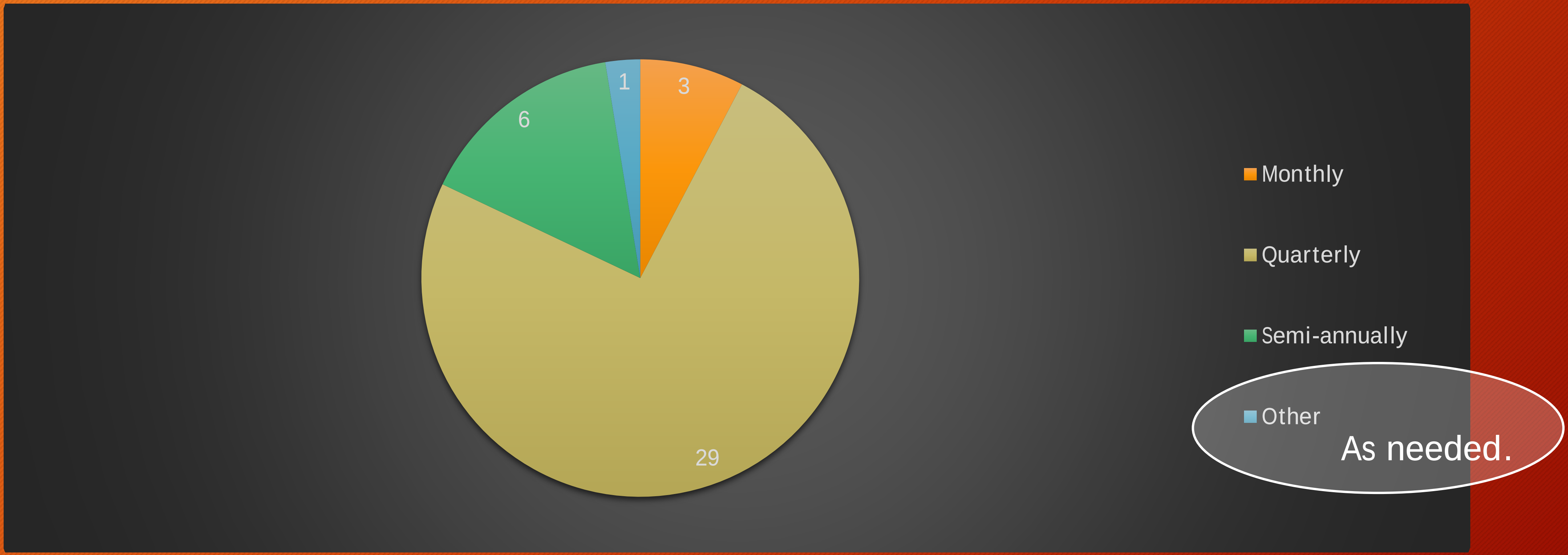
Other areas of interest / expertise of members

- Water treatment and impacts to treatment
- Any information that would assist reservoir projects
- Water quality modeling
- Biomass and fluorometry of phycocyanin
- Policy
- Mitigation
- Drinking water treatment for cyanotoxins
- Environmental drivers

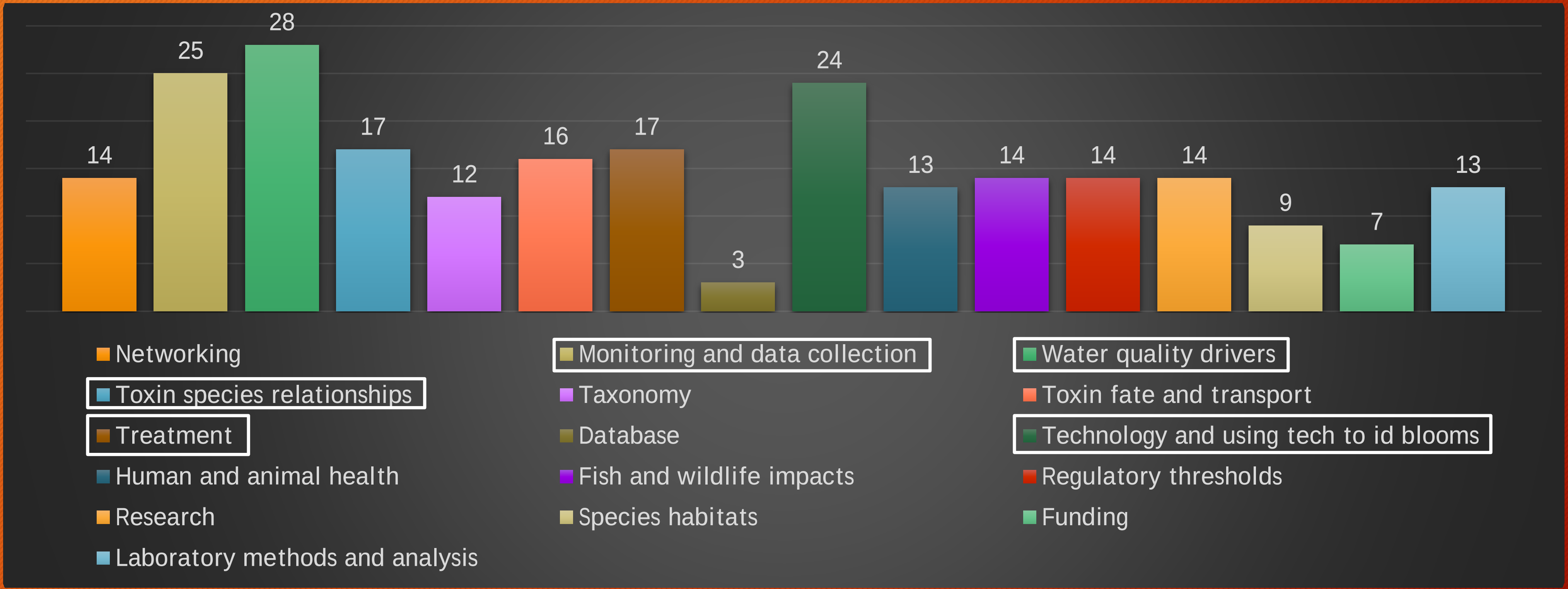
Information sharing preferences



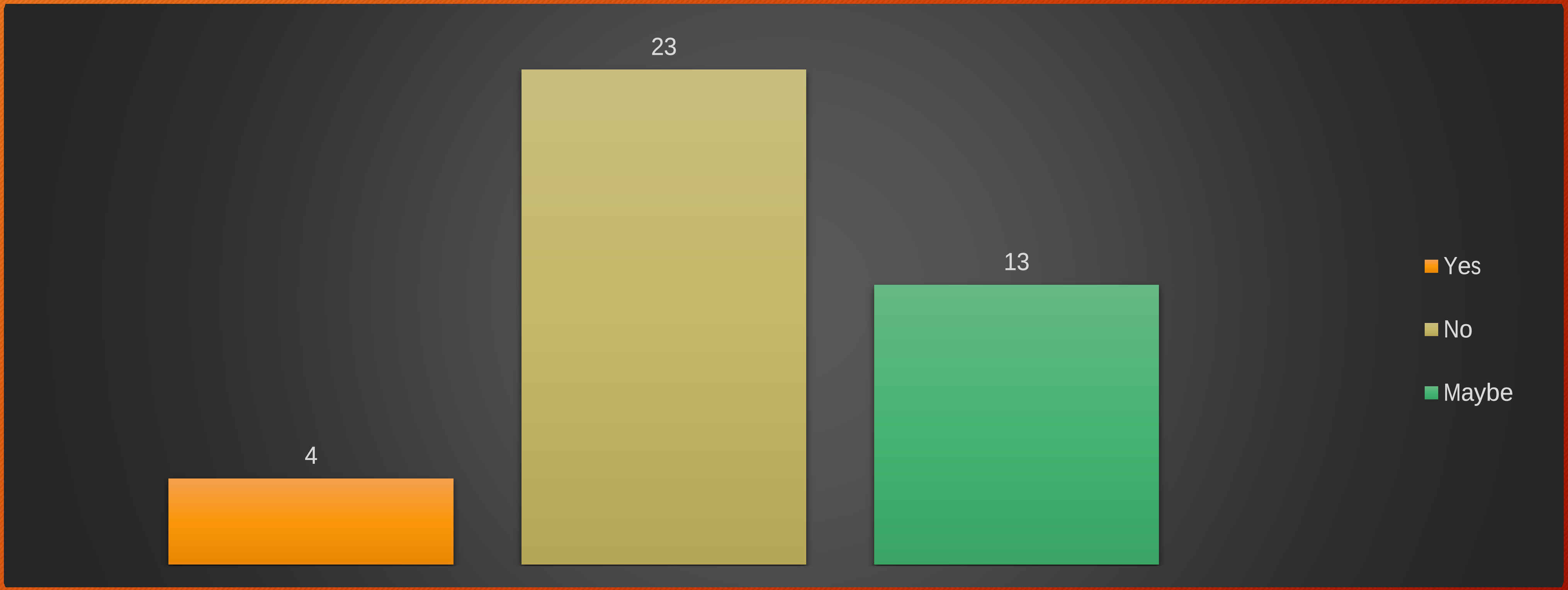
Preferred workgroup meeting frequency



What are your top 5 categories of interest that you would like to see in this workgroup?



Would you be interested in presenting?



Other info shared:

- I am interested in inspiring research to find what environmental factors trigger the biochemical production of toxins and to clearly identify the biochemical production pathway. If we know what triggers gene expression for toxin production we can focus on the true cause of toxin production and control.
- Thanks for running this group - it's an excellent initiative. I hope it can continue!
- Interested in the use of phycocyanin as a proxy for HABs
- I have learned a lot from this group.
- I am mainly interested to hear about data gaps and research gaps. I manage a research program so understanding the needs is key for me to make sure my program is relevant.

Future directions

This is a member driven group.

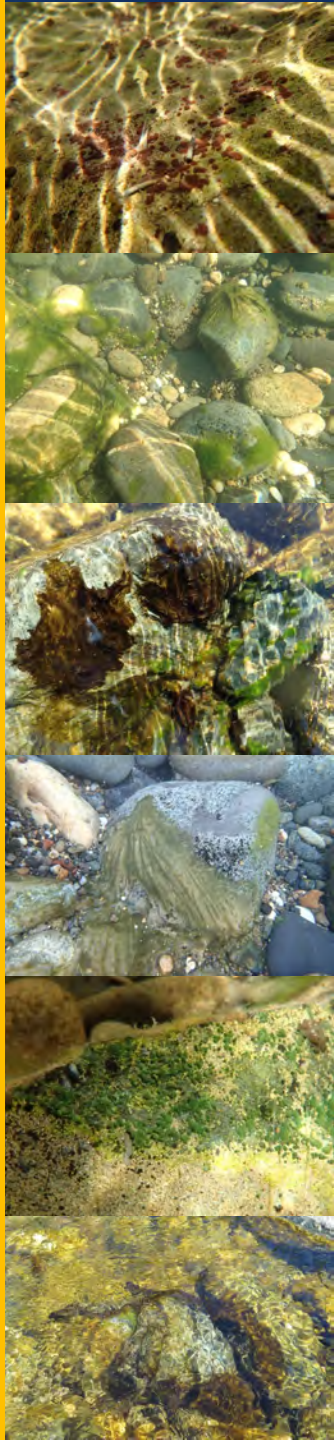
We may be in contact with you about survey responses.

We'll be using the survey results to direct our efforts but you can send feedback any time.

ITEM III

Guest Presentation:
Cryptic cyanotoxin producers in benthic mats

Keith Bouma-Gregson

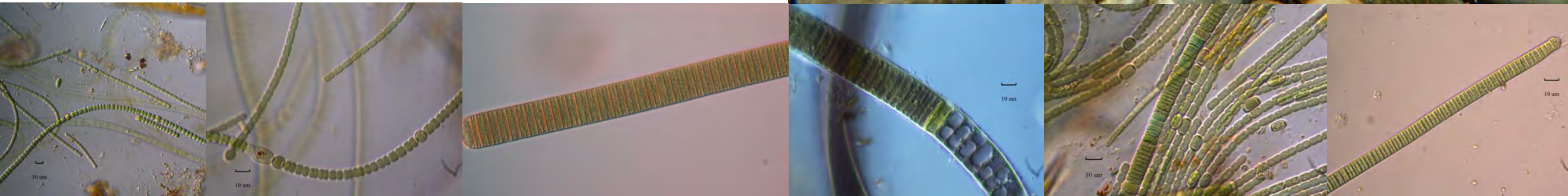


Cryptic cyanotoxin producers in benthic mats

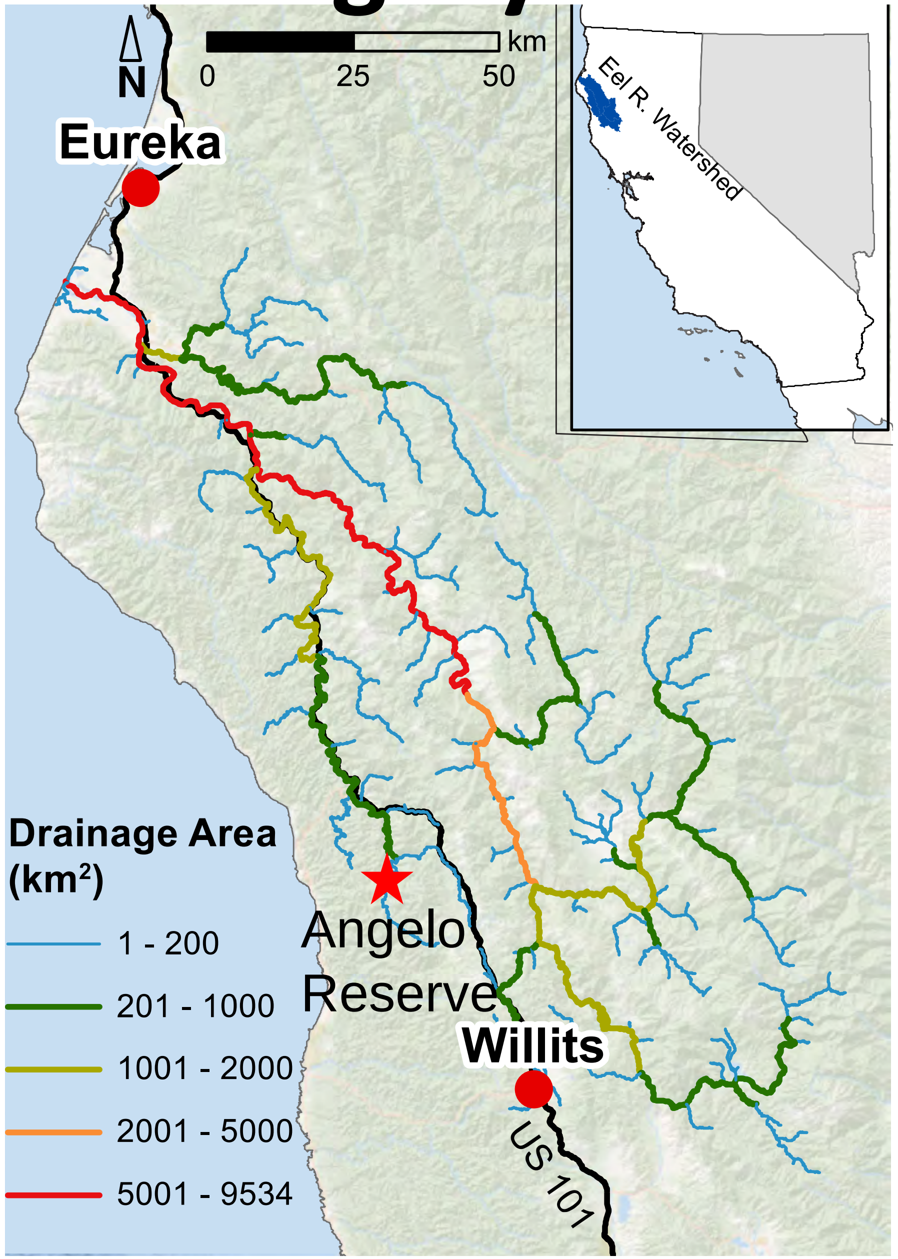
Keith Bouma-Gregson, Ph.D.
California State Water
Resources Control Board

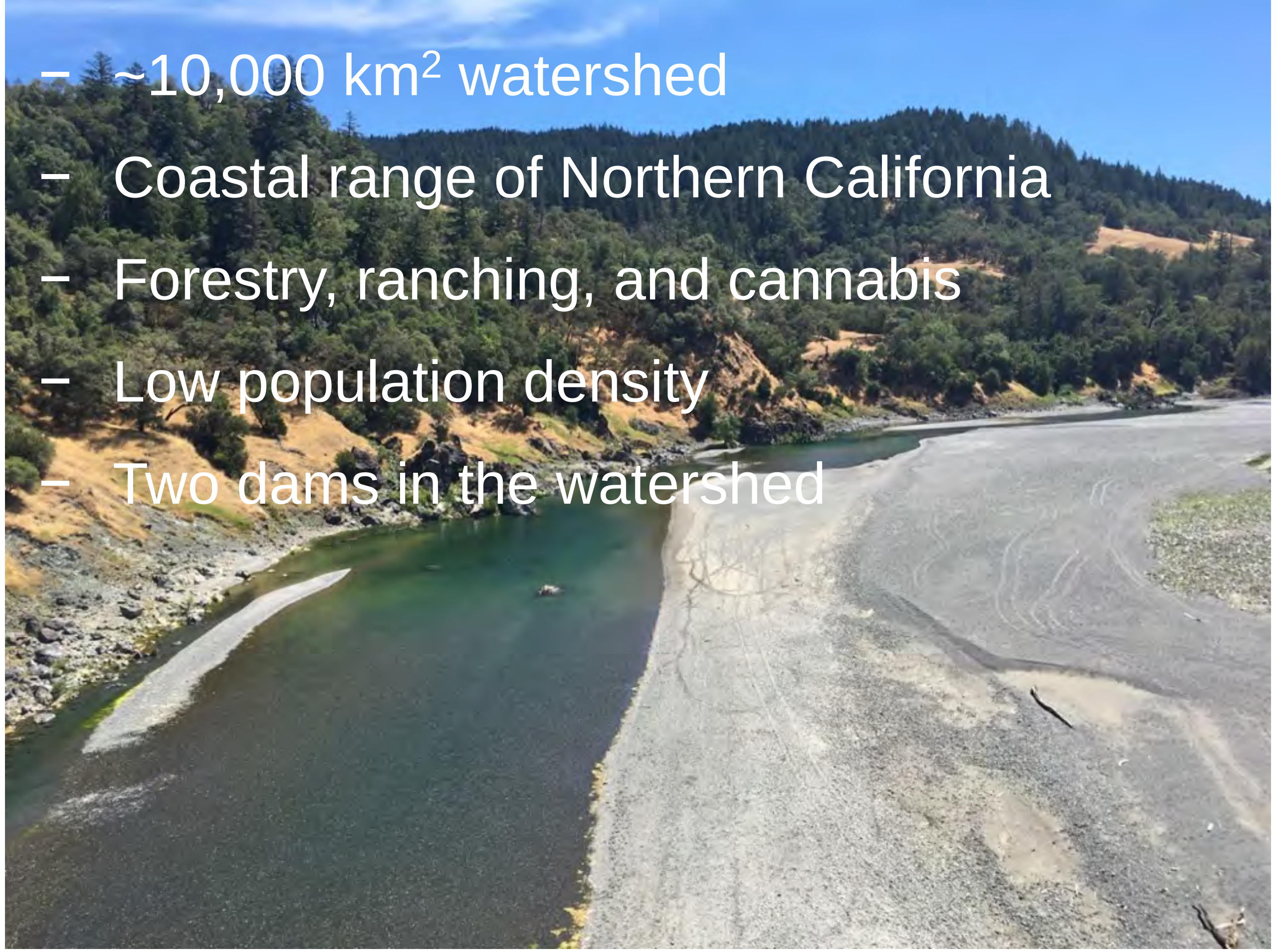
Laura Kelly, Ph.D.
Cawthron Institute, New Zealand

Benthic HABs Workgroup
Oct. 30, 2019



The mighty Eel River





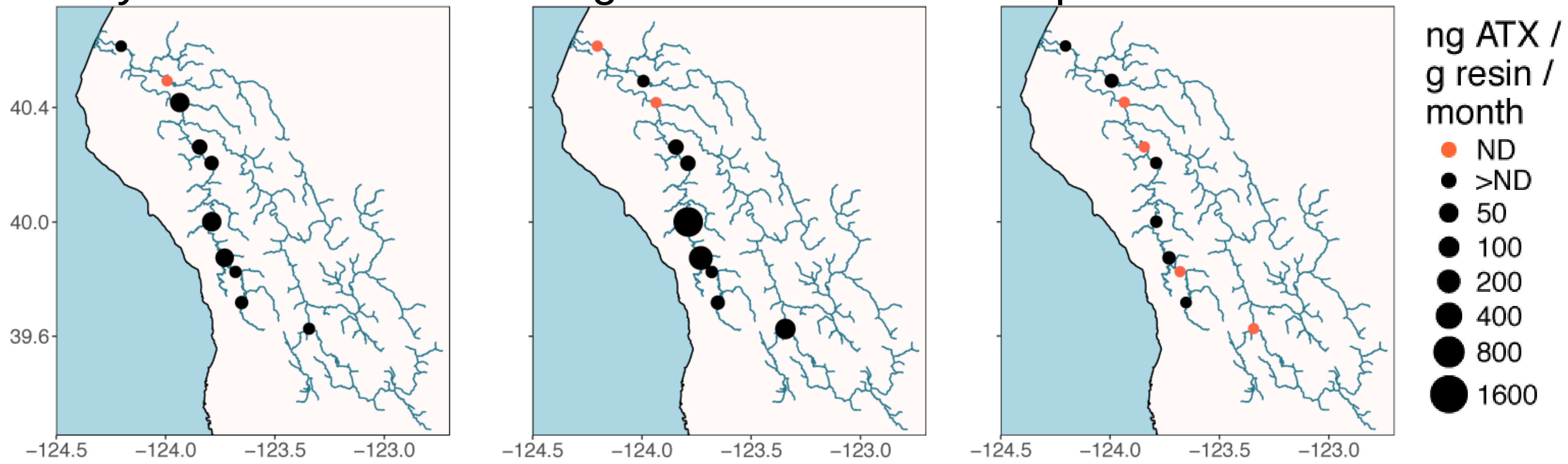
- ~10,000 km² watershed
- Coastal range of Northern California
- Forestry, ranching, and cannabis
- Low population density
- Two dams in the watershed

The mighty Eel River

- Monitoring sites 2013-2015
- Collected mat samples
- Measured cyanotoxin concentrations
- Environmental parameters

Anatoxin-a present throughout watershed

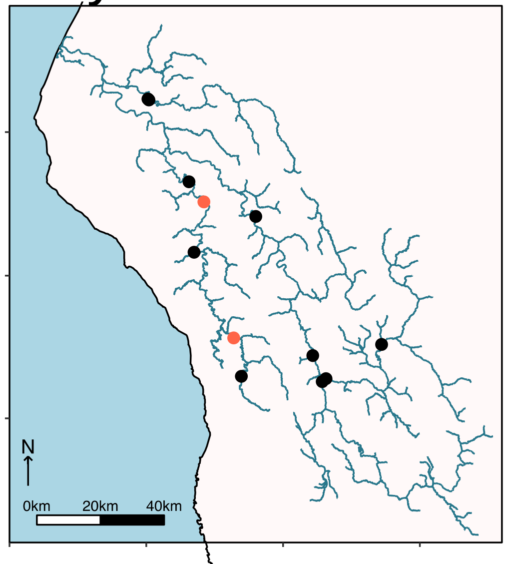
2014 July August September



SPATT samplers

2013-2015

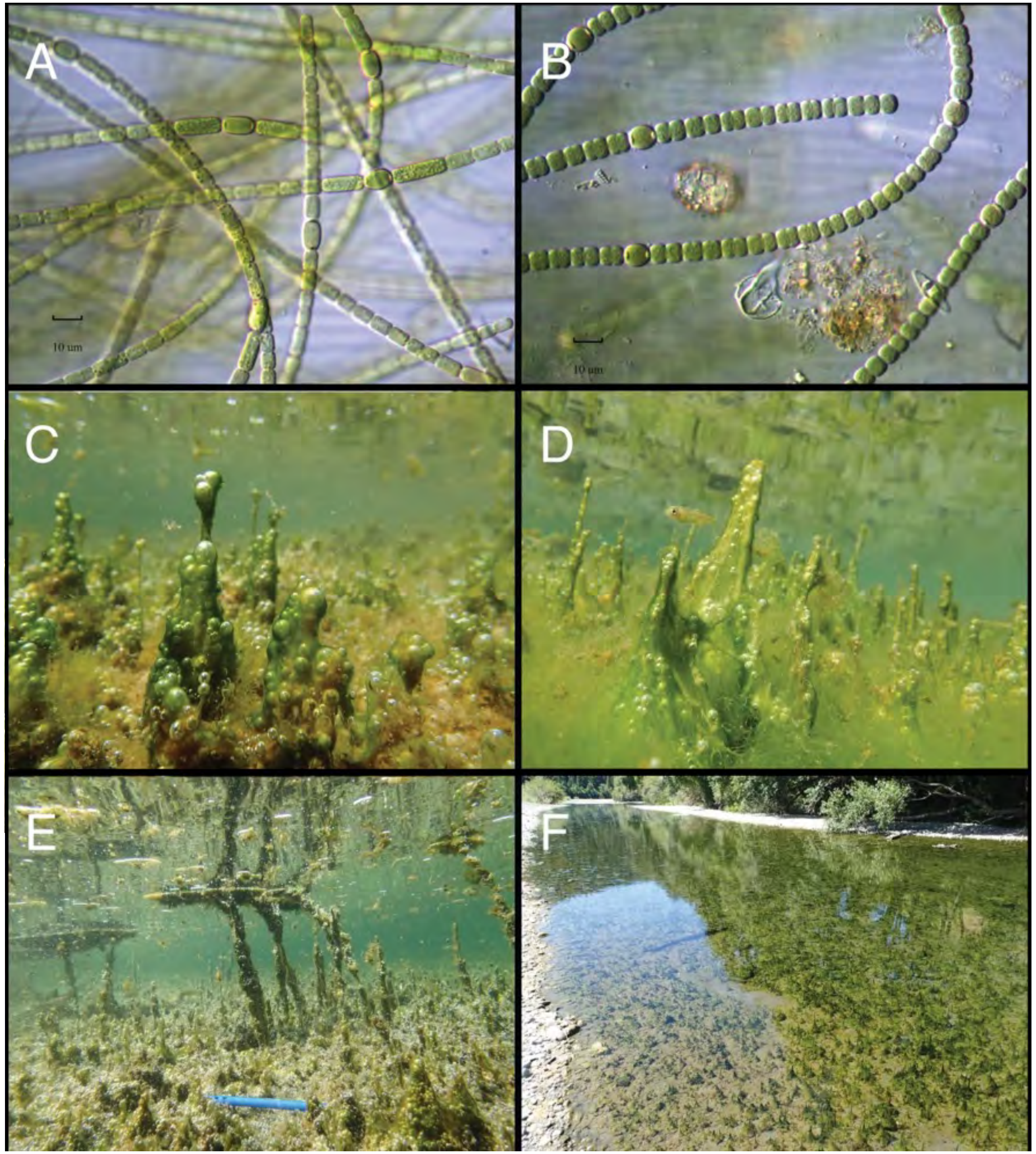
2015 July



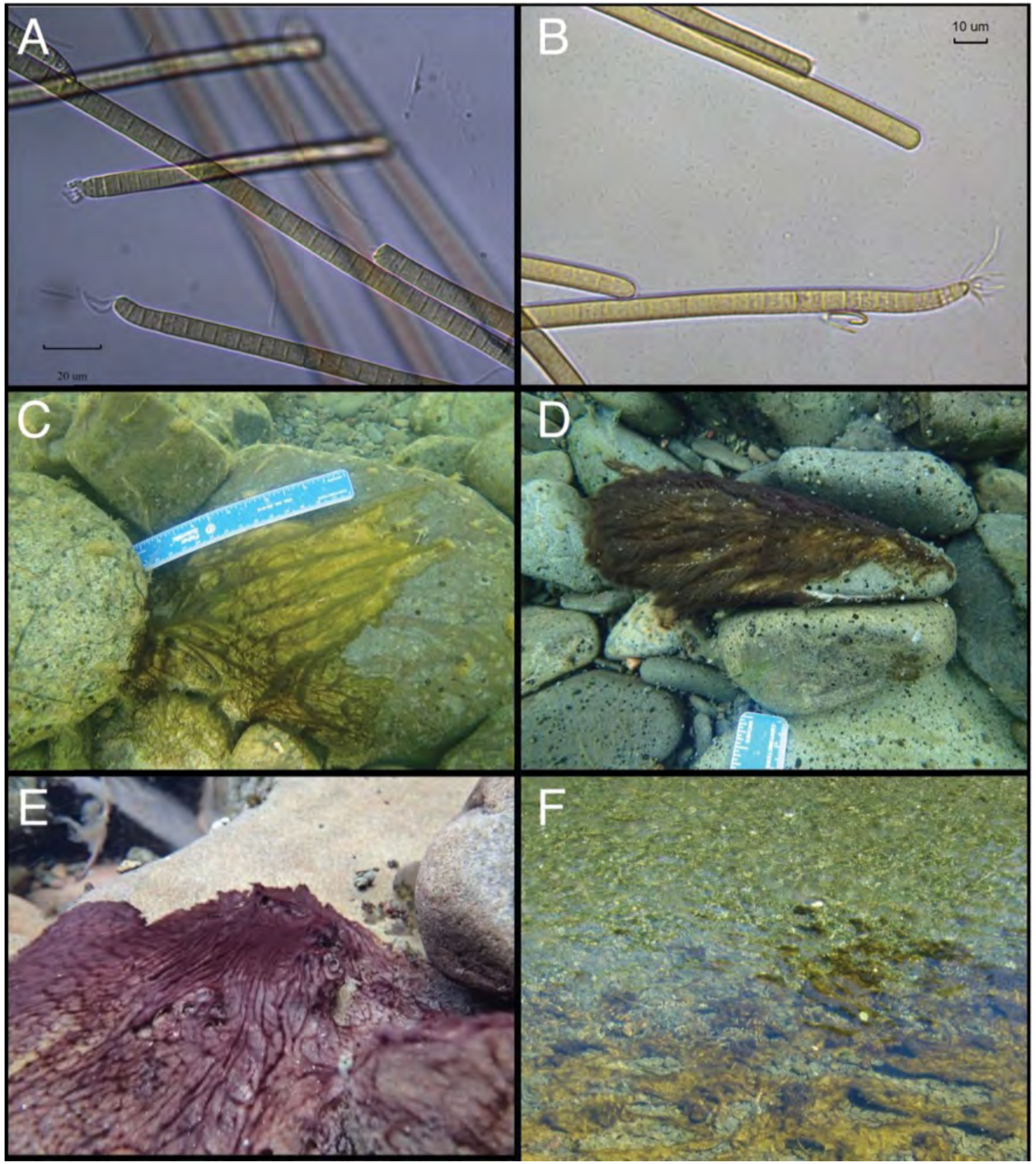
Bouma-Gregson et al., 2018 *PLoS ONE*

Anatoxin-a higher than microcystin

Anabaena spp. (Nostocales)



Microcoleus spp. (Oscillatoriales)



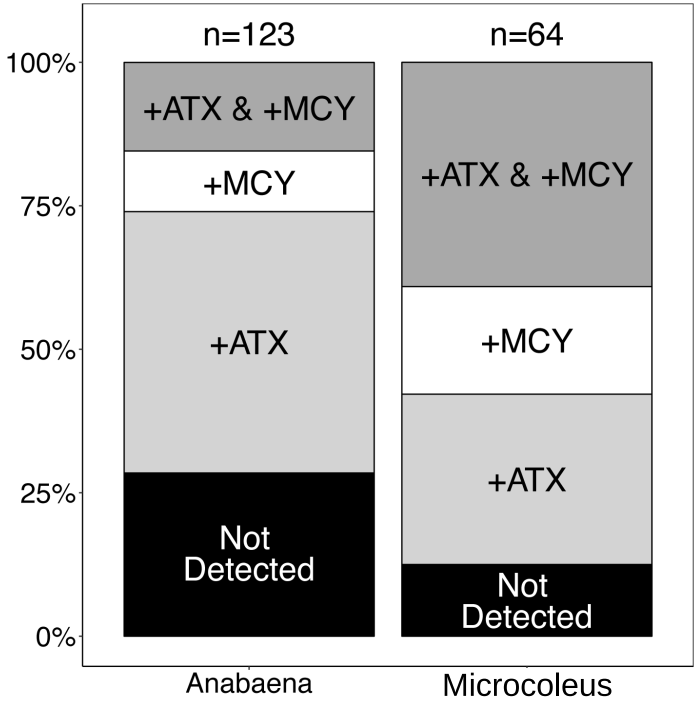
Bouma-Gregson et al., 2018 *PLoS ONE*

Both *Anabaena* and *Microcoleus*-dominated mats contain cyanotoxins

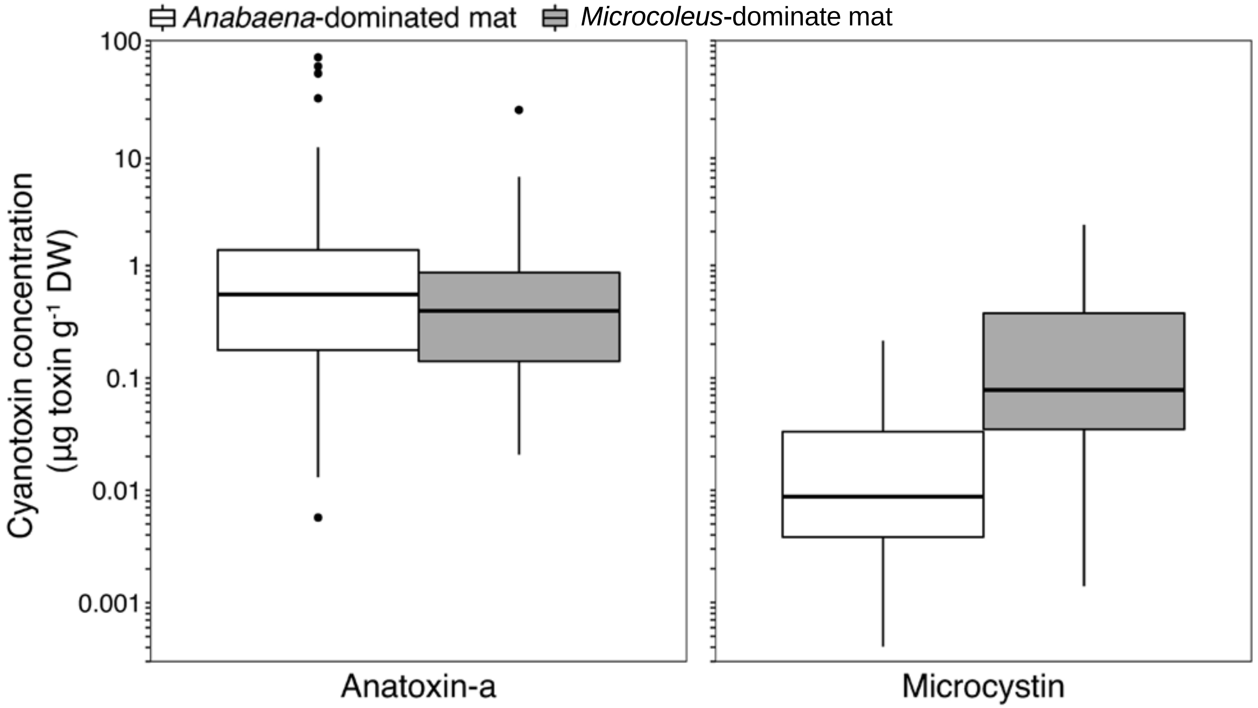
Cyanotoxins frequently detected in mats

Average anatoxin-a concentrations similar, but maximum concentrations in *Anabaena*-dominated mats

Cyanotoxin detections

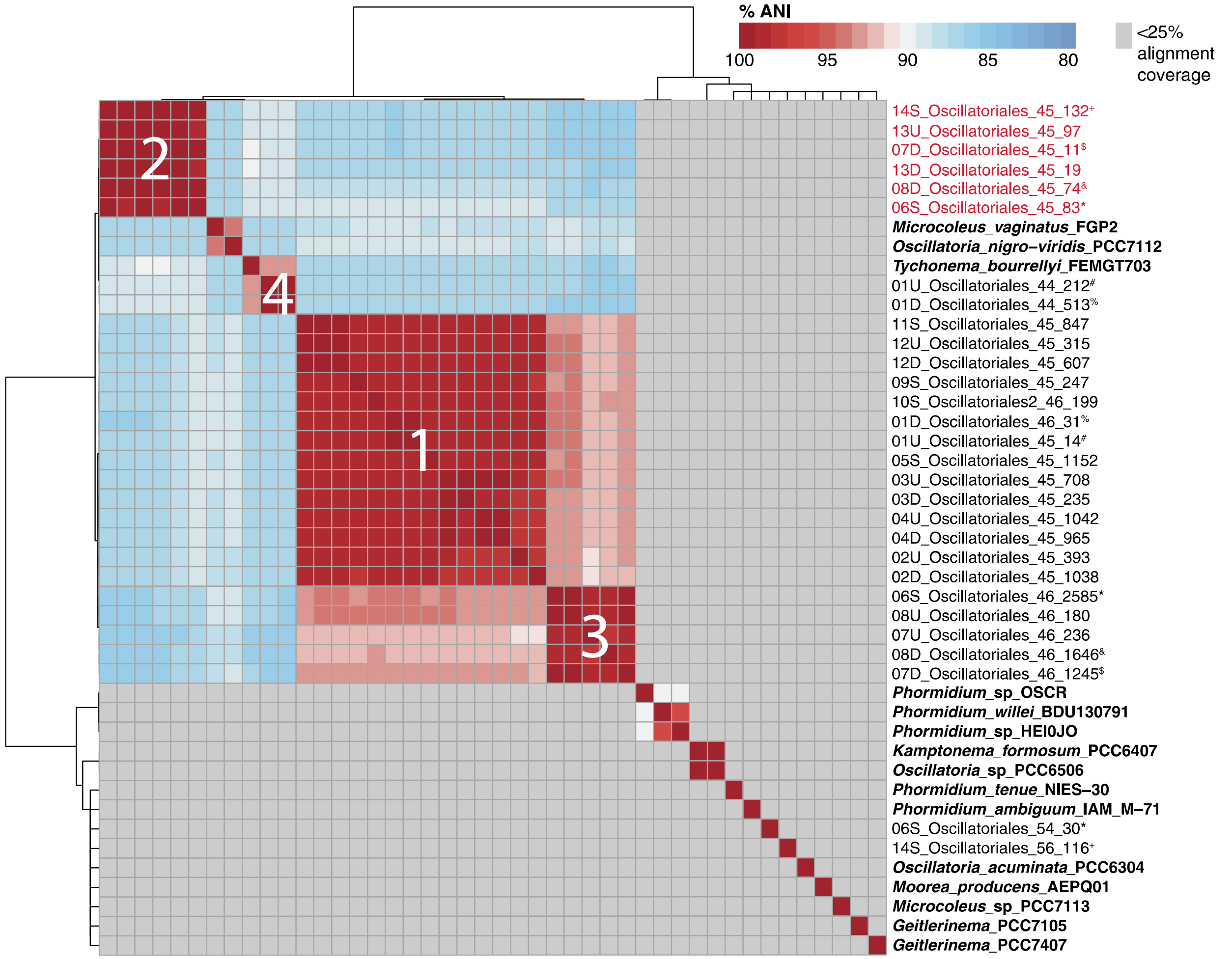


Cyanotoxin concentrations

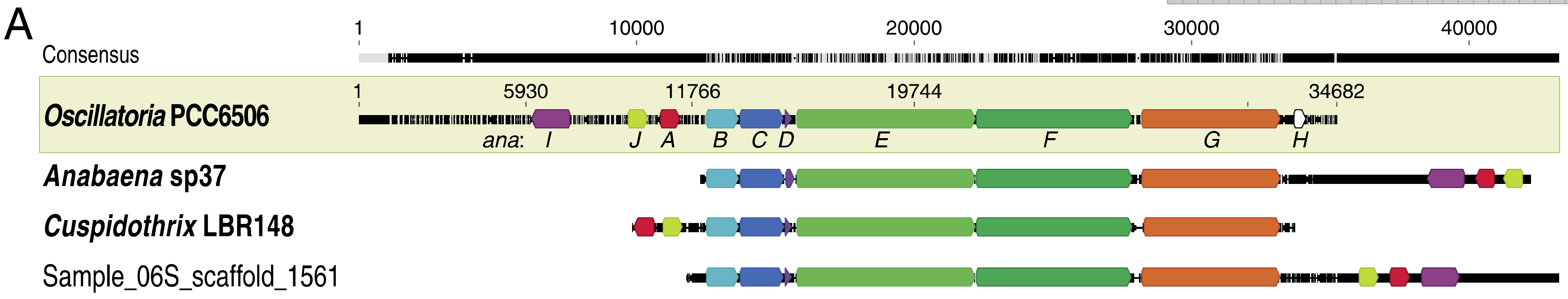


Microcoleus biodiversity

- Potentially novel *Microcoleus* species and strains
- Anatoxin-a biosynthesis genes recovered only in Species 2



Anatoxin biosynthesis gene cluster



Bouma-Gregson et al. 2019, *ISME*

Microcoleus anatoxin production

Isolate cultures tested for anatoxin-a, microcystin, nodularin, and cylindrospermopsin

No detections for microcystin, saxitoxin, cylindrospermopsin

7 isolate *Microcoleus* cultures with no anatoxin-a detects

Cultures producing anatoxin-a

Genus	River	# of strains
Microcoleus	Eel	2
Microcoleus	Russian	3
Microcoleus	Garcia	2

Image: R. Stancheva

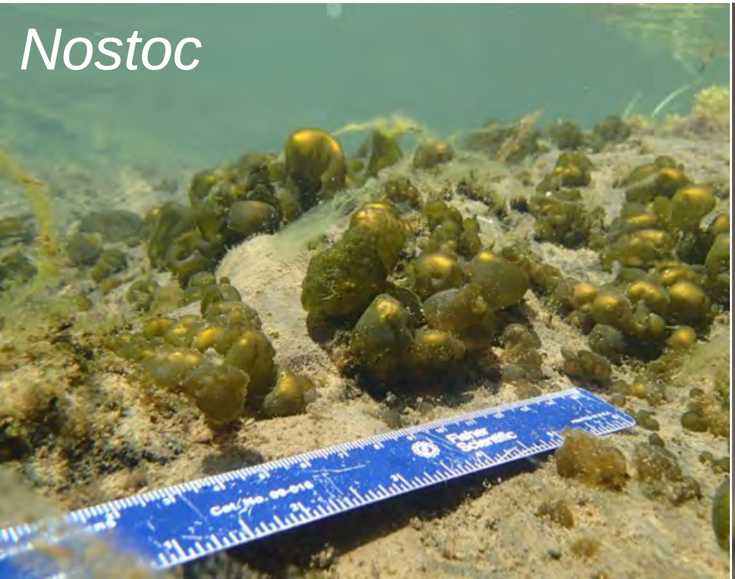

What else is producing cyanotoxins?

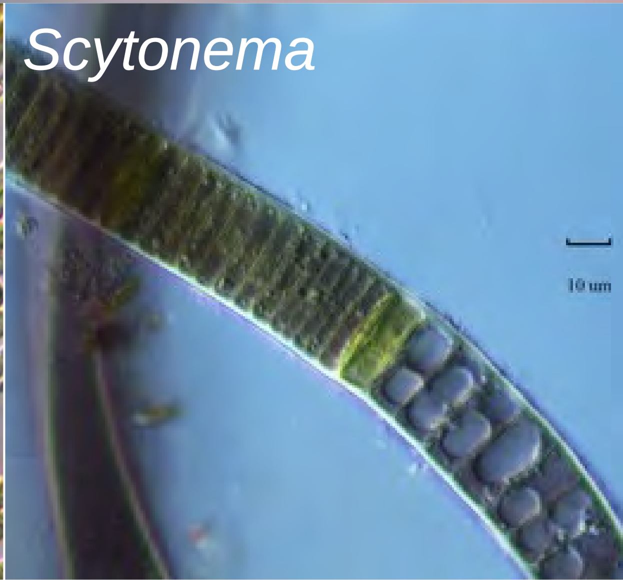
Other cyanotoxins in the Eel River watershed

Nodularin: frequently detected from SPATT

Cylindrospermopsin: infrequent on SPATT and in mats

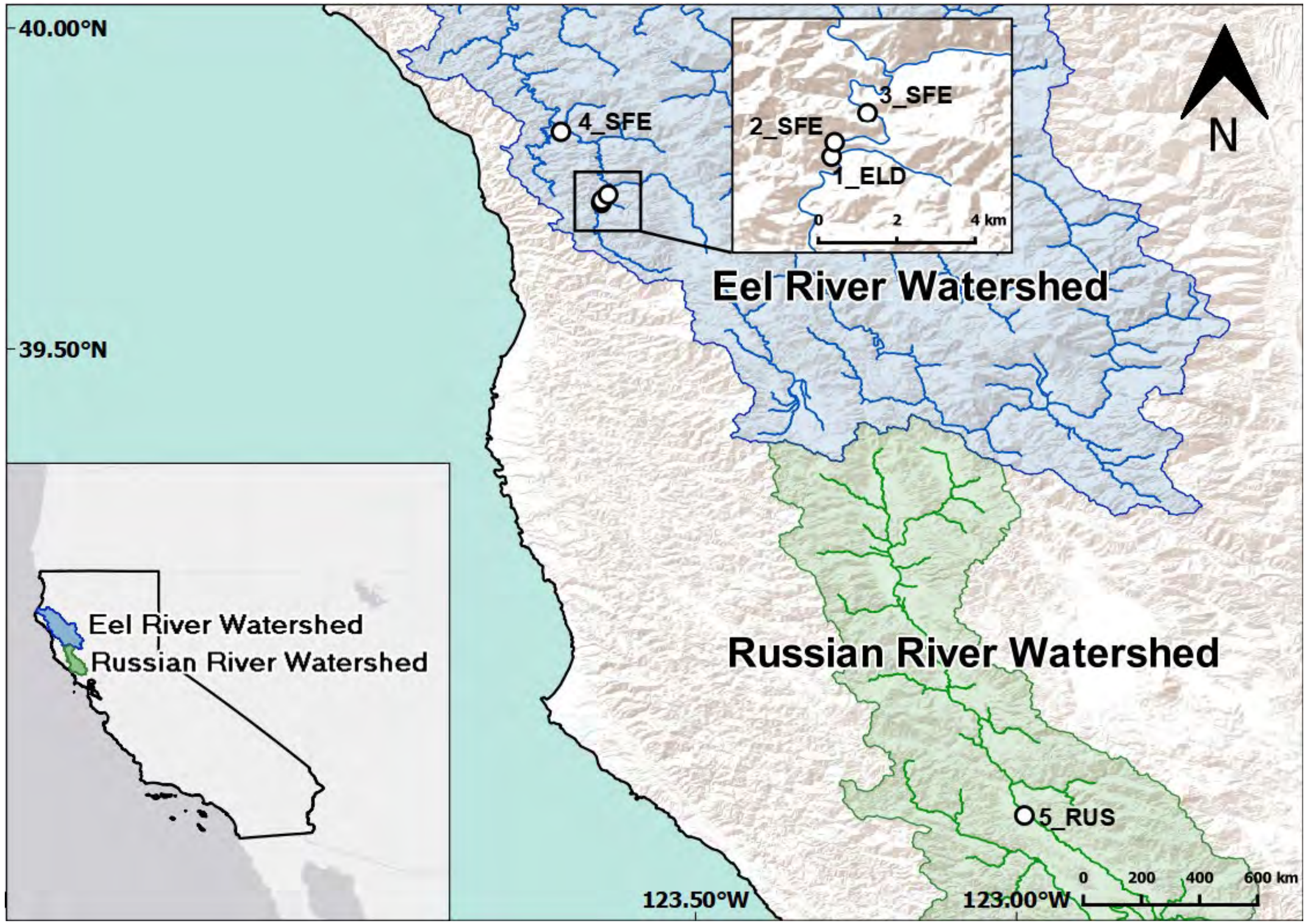
Saxitoxin: infrequently detected in cyano. mats





Summer 2018 sampling

1. Variation in anatoxin concentrations
2. Investigate cyanotoxin producers



NZ Catalyst Grant

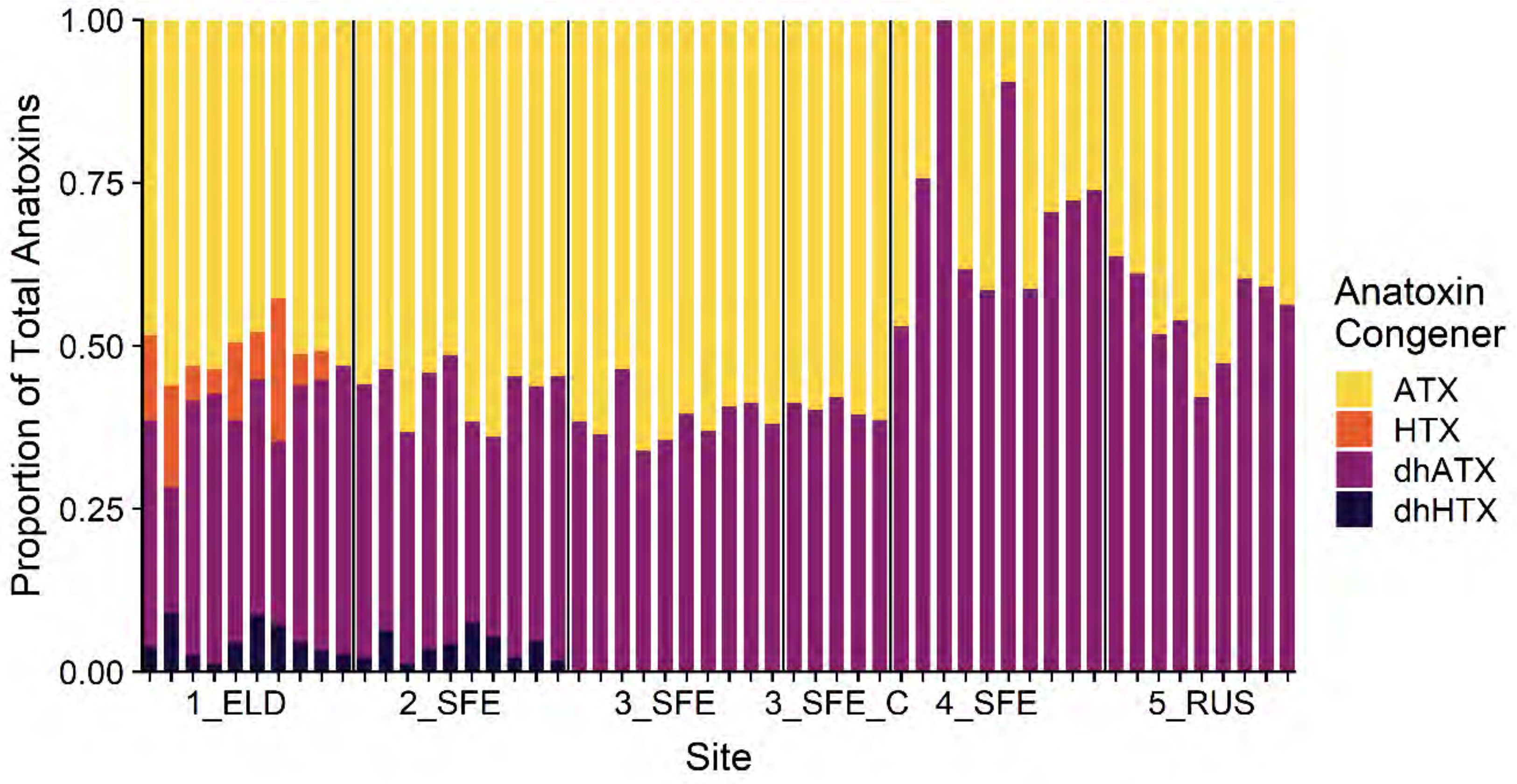
Kelly et al., in review

Sampled:
- *Microcoleus*
- *Anabaena*
- *Cladophora*

Multiple anatoxin congeners detected

Both anatoxin-a and dihydro-anatoxin-a present in *Microcoleus* mats

Need to understand variants to prevent underestimates of cyanotoxin concentrations



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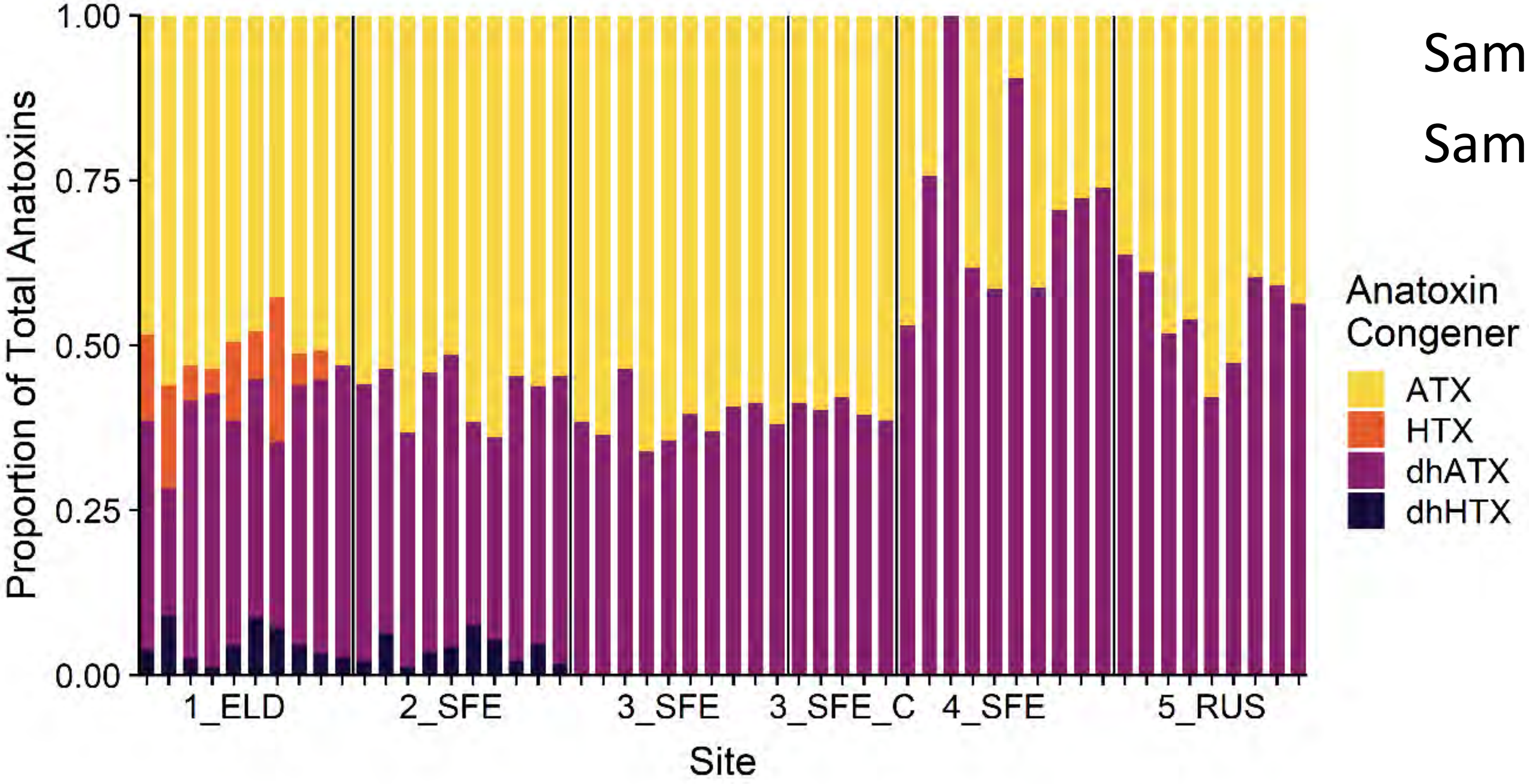

Sample	Anatoxin-a (ug/L)	Dihydro-anatoxin-a (ug/L)
Sample 1	0.66	331.2
Sample 2	0.38	363.4
Sample 3	0.47	483.3

Anderson et al. 2018, *Env. Tox. and Chem.*

Anatoxin variation driven by cyano. genotypes

$$ATX\ Quota = \frac{toxins}{qPCR} = \frac{\frac{\mu g\ toxin}{DW}}{\frac{gene\ copies}{DW}} = \frac{\mu g\ toxin}{\cancel{DW}} \times \frac{\cancel{DW}}{gene\ copies} = \frac{pg\ toxin}{cell}$$

Wood and Puddick, 2017, Marine Drugs
Kelly et al. 2018, Toxins

1 anaC gene copy per cell

Anatoxin variation driven by cyano. genotypes

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Wood and Puddick, 2017, Marine Drugs
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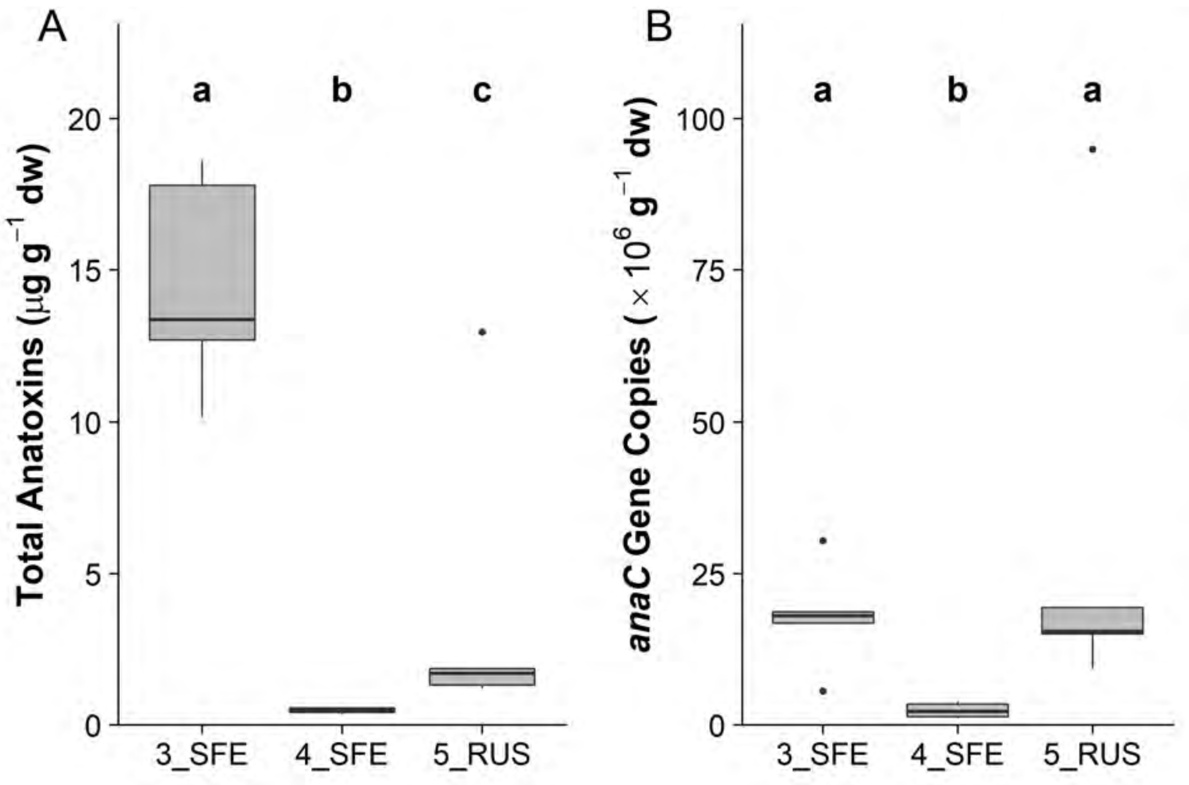
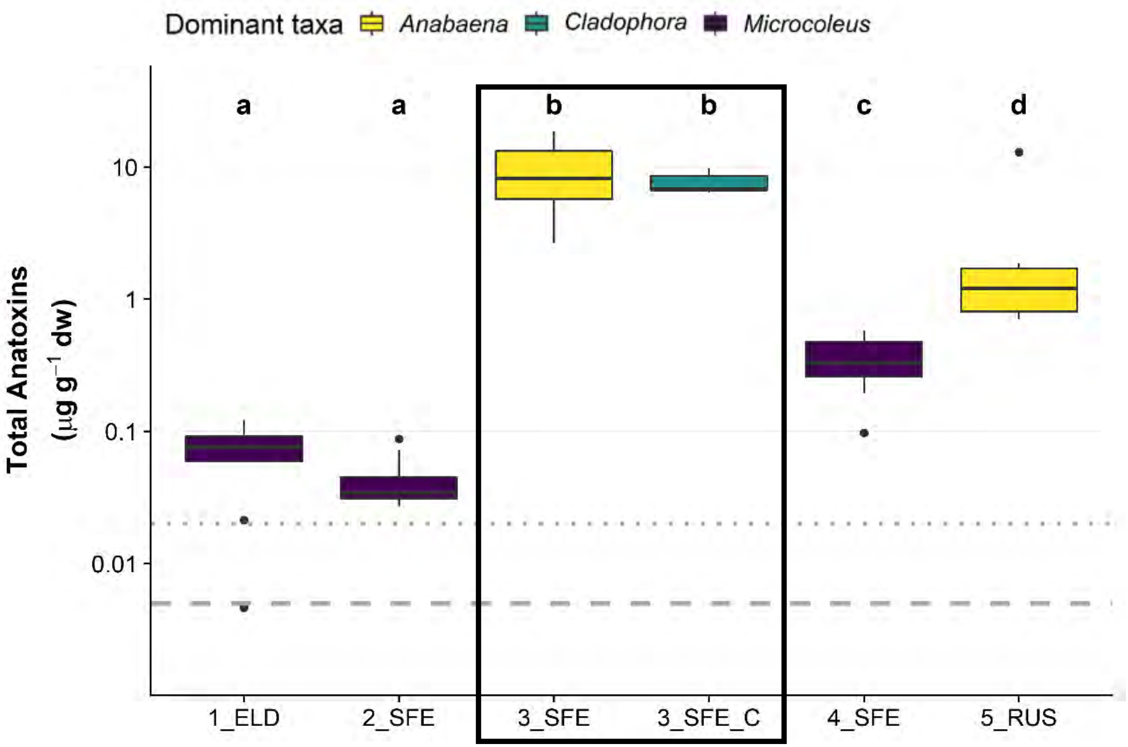


Cryptic anatoxin production



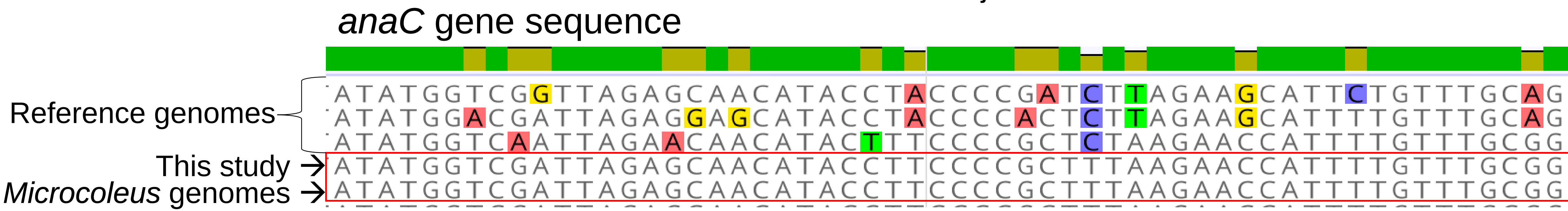
Anabaena & *Cladophora* assemblage contained anatoxins

Cryptic anatoxin production

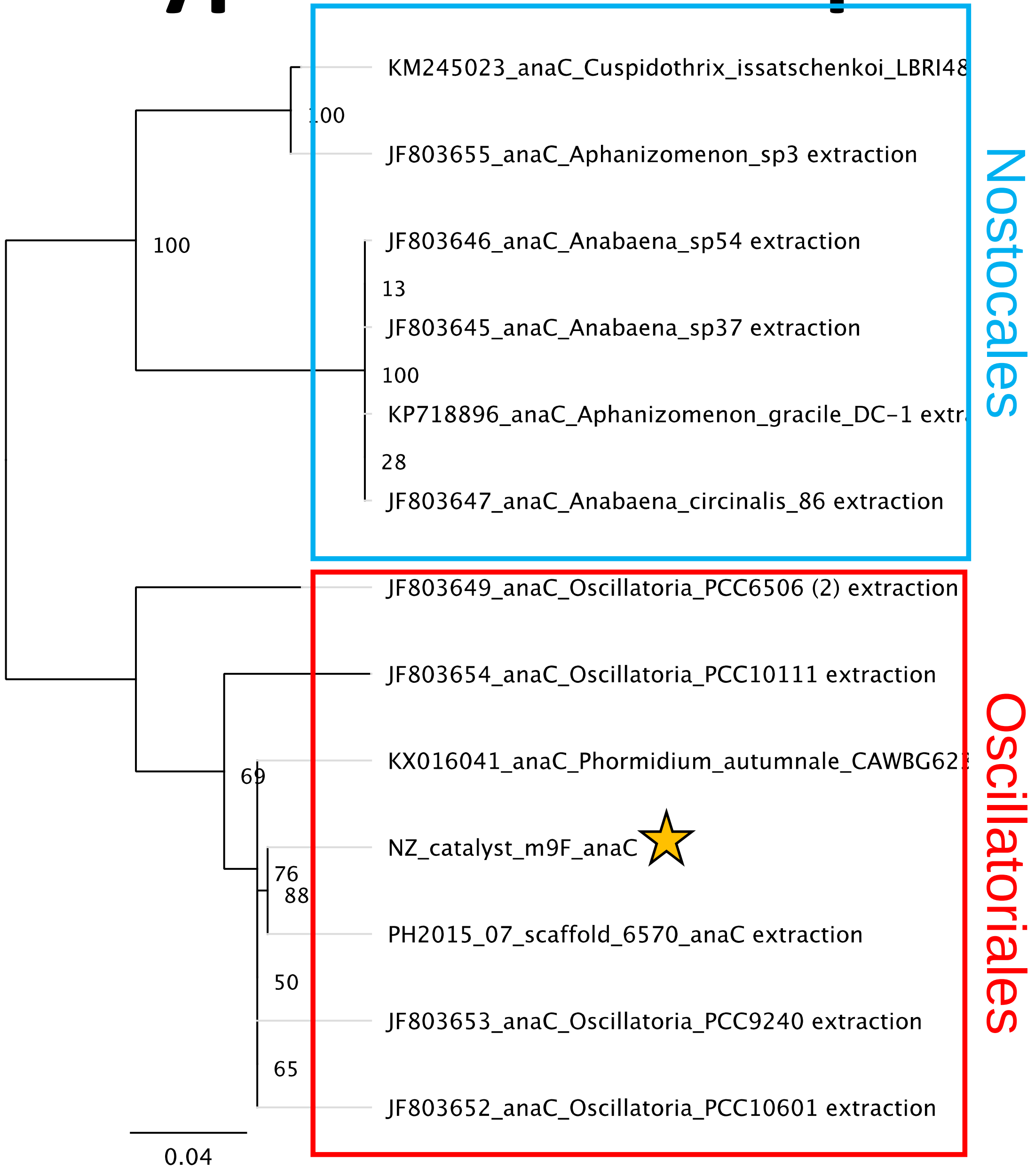
Sequenced 33 *anaC* genes (282 bp)
All sequences were 100% identical

Also 100% identical with anaC from Microcoleus draft genomes

Mejean et al. 2014



Cryptic anatoxin production



- 15 sequences 282 bp long (4 identical)
- Aligned with MUSCLE to build RAxML tree (in Geneious)
- Nostocales and Oscillatoriales sequences form separate clades (80-90% nucleotide identity between clades)

- Two isolate cultures (Russian and Garcia rivers) no ATX detections
- Two draft *Anabaena* genomes, no ATX genes

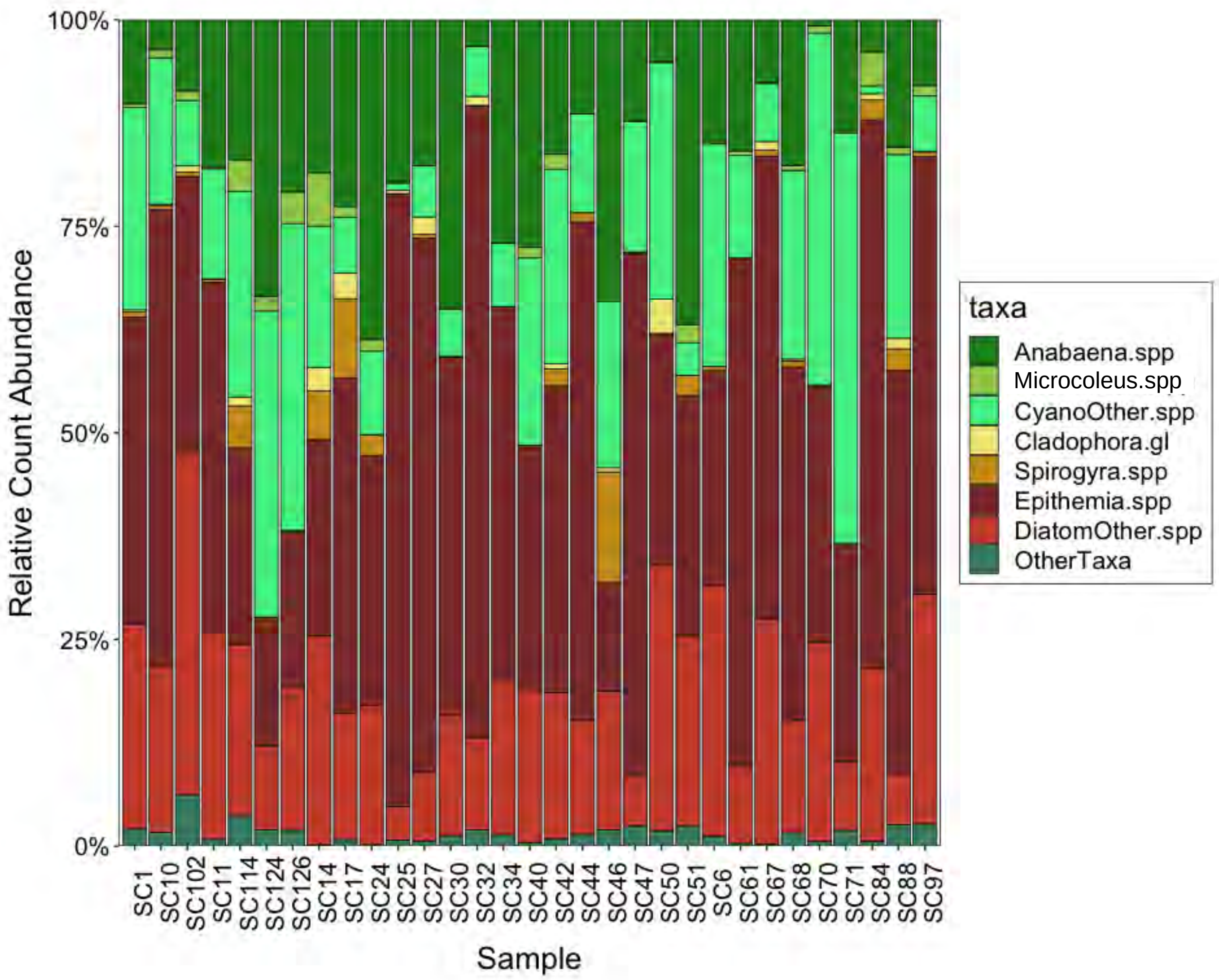
Cryptic anatoxin production

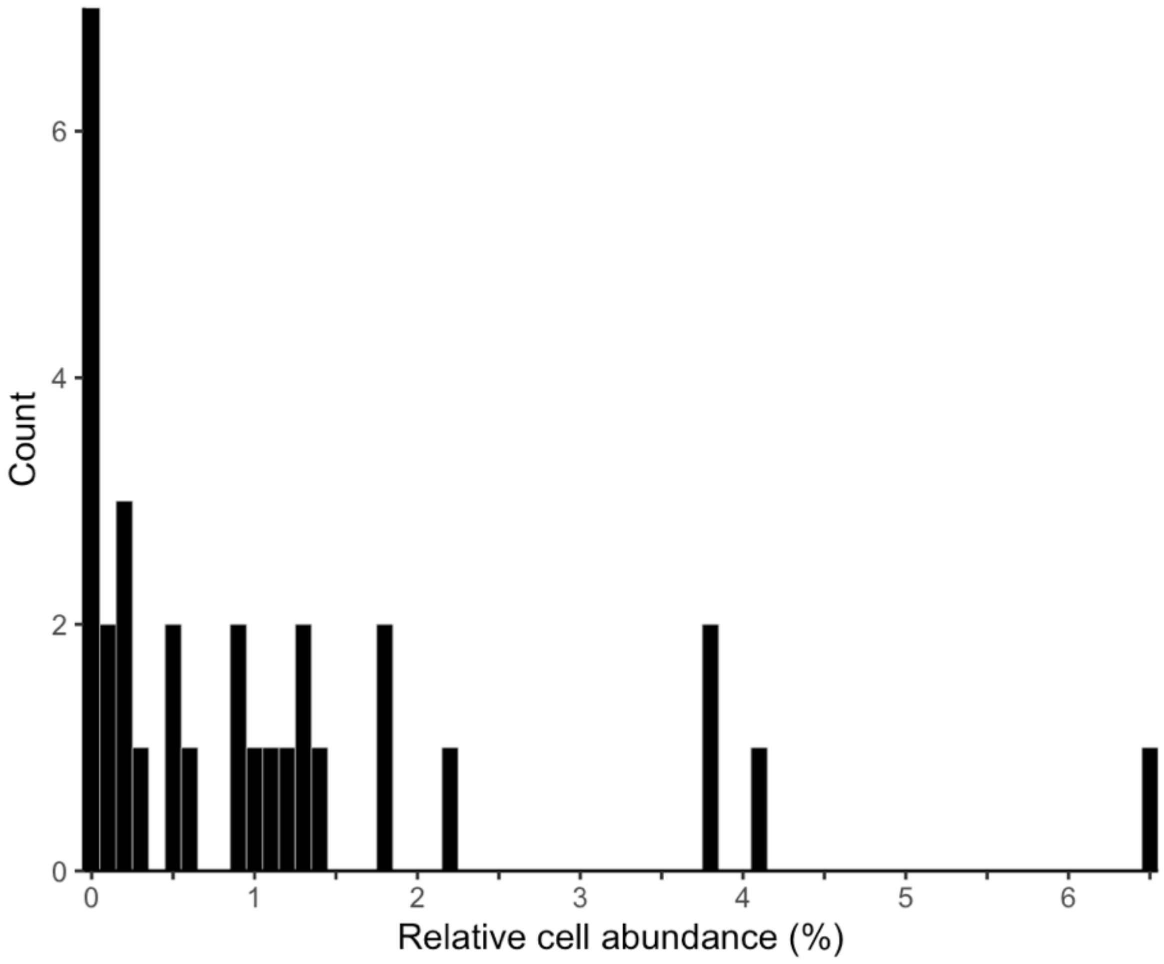
Oscillatoriales inhabit
Anabaena-dominated mats

Microcoleus common, but rare, in *Anabaena* mats

31 *Anabaena* mat samples collected in 2014

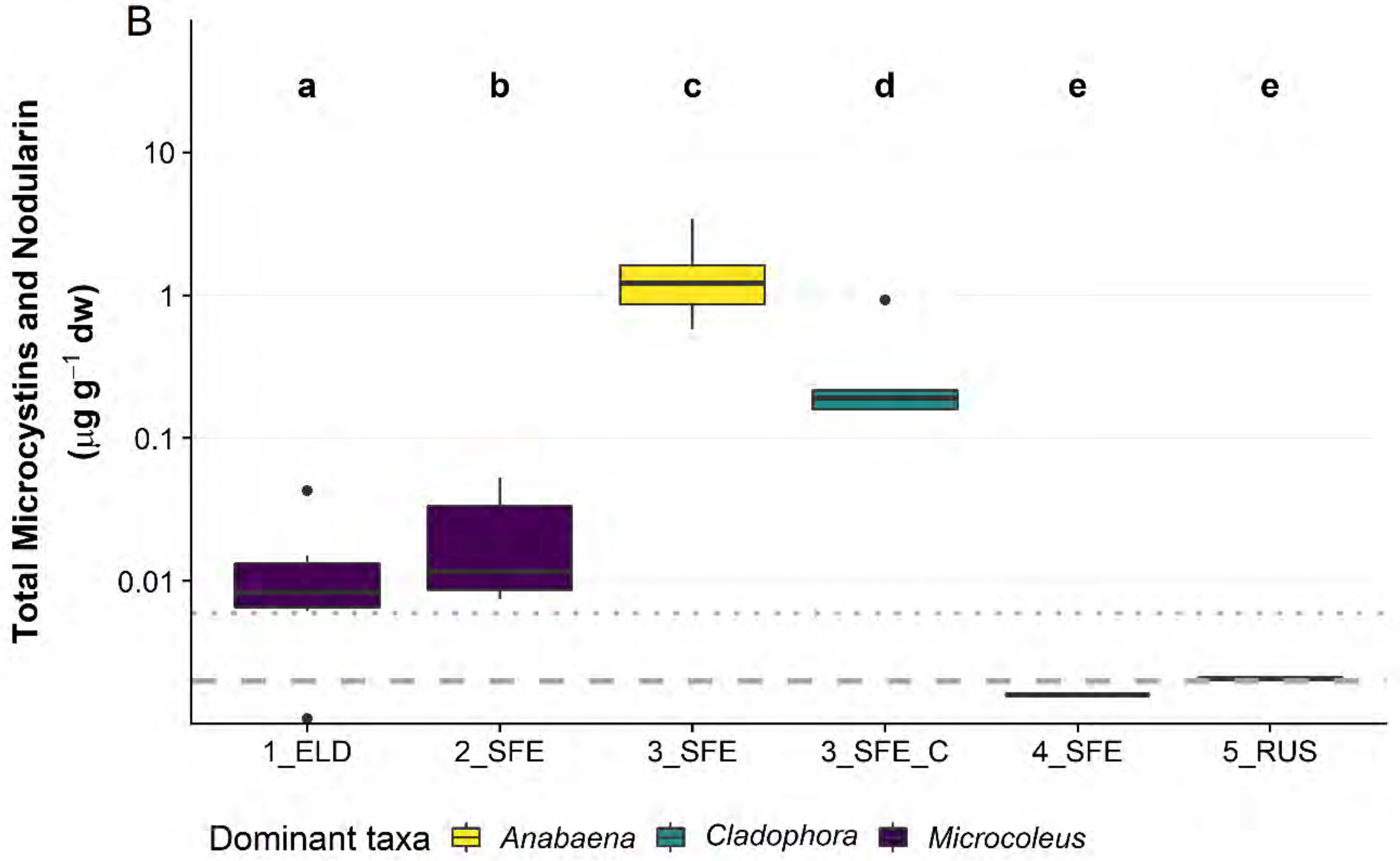
Microcoleus observed	*Microcoleus* absent
24 (77%)	7 (23%)





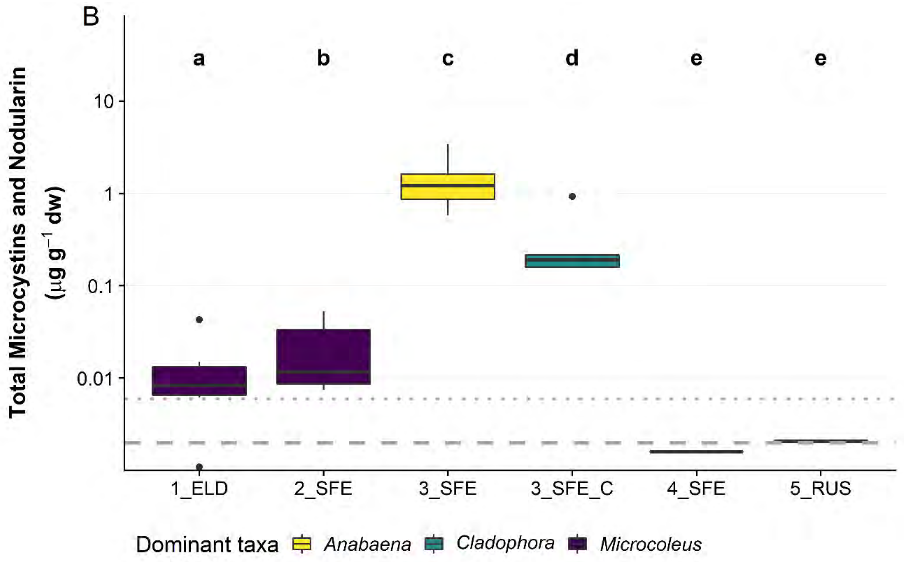
Cryptic nodularin production

Anabaena and *Cladophora* mats have higher MCY/NOD concentrations than *Microcoleus*

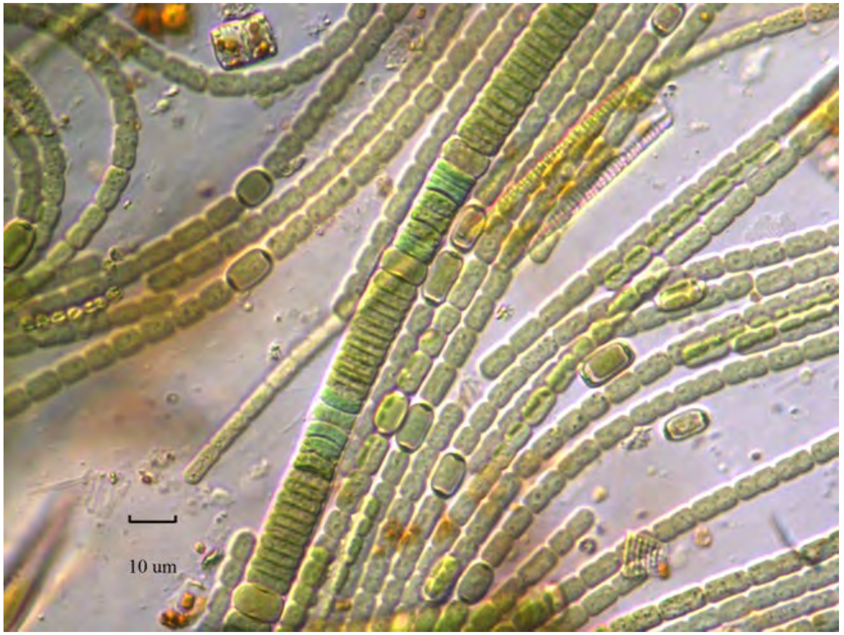


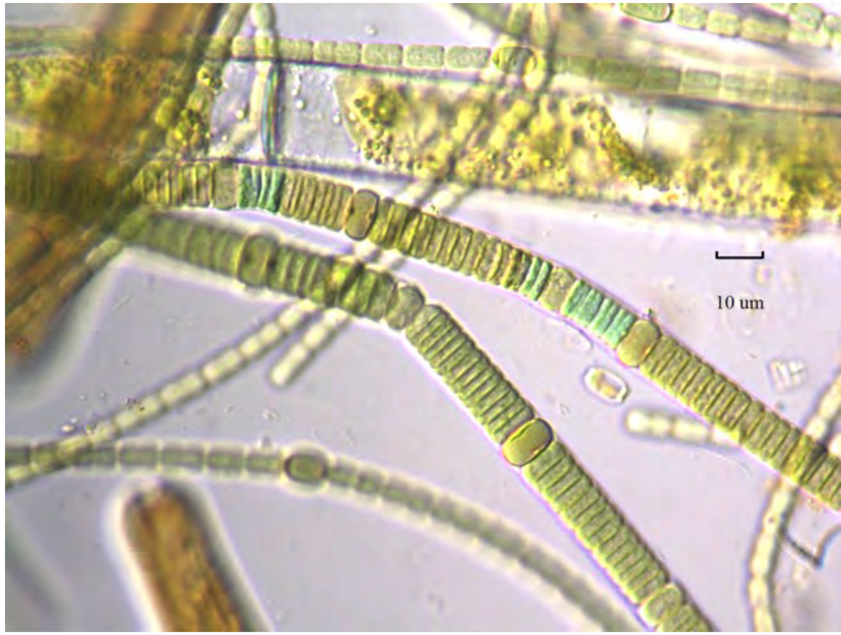
Cryptic nodularin production

- Nodularin *ndaF* gene detected in *Anabaena and Cladophora* mats
- 99.7% nucleotide identity to *Nodularia spumigena*



Microscope images from 2013



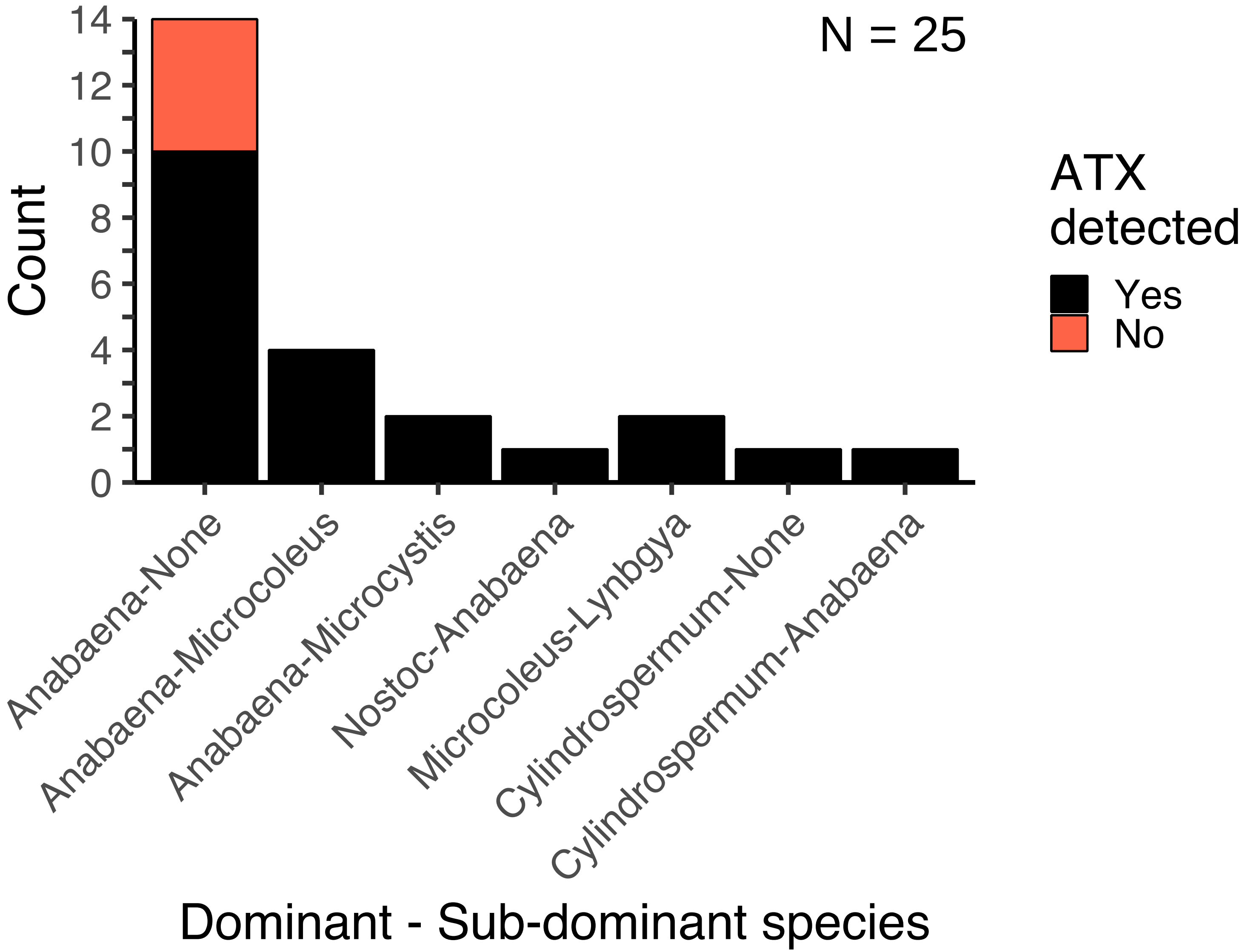


Anabaena collections 2019

- 25 mat samples collected in 2019
- 7 rivers in Northern California

Analyses:

- 16S cyano-assemblage composition
- PCR and sequencing of anaC gene



Conclusions

- Multiple cyanotoxins produced within wadeable streams
- Cryptic diversity and strain heterogeneity within cyanobacterial species
- Toxin quota (toxins per cell) has less variation than toxins per mat mass
- Dominant biomass may not be the dominant cyanotoxin producer
    - *Anabaena, Cladophora, Nodularia*
- Nodularia diversity and nodularin production needs more study

Management implications

- Doesn’t matter who is producing toxins, but whether or not mat contains toxins
    - Messaging, ”Don’t eat algae” or “Don’t eat *Microcoleus*”
- More work on anatoxin toxicity and variant production

Acknowledgements

CAWTHRON INSTITUTE

SWAMP

the ROYAL SOCIETY of NEW ZEALAND
TE APĀRANGI

CATALYST FUND
SEEDING

NSF

CALIFORNIA WATER BOARDS
North Coast - R1

California State University
SAN MARCOS

Natural Reserve System
UNIVERSITY OF CALIFORNIA

EPA
United States Environmental Protection Agency

Questions?

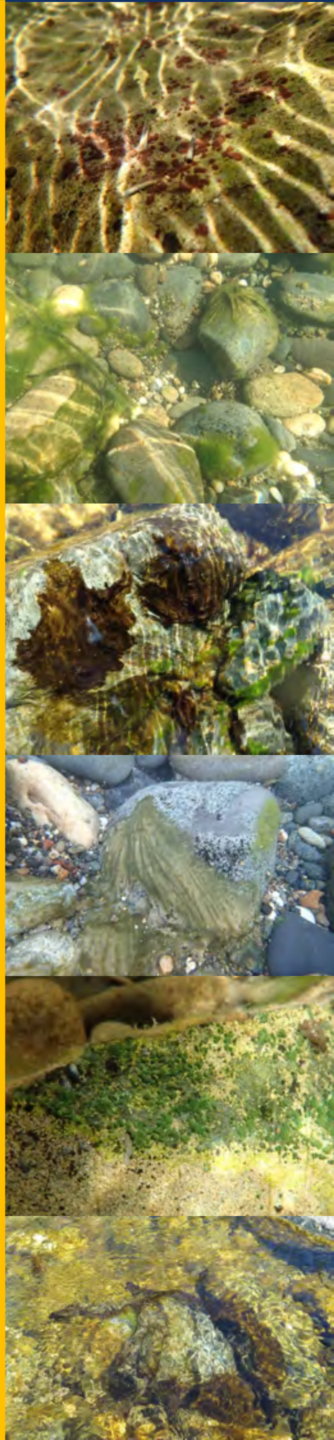
Keith Bouma-Gregson | California State Water Resources Control Board

keith.bouma-gregson@waterboards.ca.gov | 916-322-830

ITEM IV

Open Discussion, Publications & Upcoming Meetings

Christine Joab



BENTHIC HABS WORKGROUP - ARTICLE

A Deeper Look at HABs by Margaret Spoo-Chupka, Jade Young, and Rich Fadness
North American Lake Management Society
LakeLine Magazine, Summer 2019 (Vol 39. No. 2) https://www.nalms.org/lakeline-magazine/

A Deeper Look at HABs

Margaret Spoo-Chupka, Jade Young, and Rich Fadness

Cyanobacteria that have the potential to cause HABs can be found in a diverse array of aquatic systems, from the highly visible planktonic blooms to less conspicuous benthic mats. The potential risks and detrimental effects of planktonic blooms have been well studied in recent years. Most alert-level framework monitoring programs and guidelines are based on planktonic cyanobacteria and their associated toxins (EPA).

To date, benthic cyanobacteria that grow on substrates in aquatic ecosystems have been overlooked in risk assessments. Toxin production in benthic cyanobacteria mats has been documented worldwide and linked to dog and livestock deaths in various countries. Despite the potential detrimental effects they present, benthic populations have been largely overlooked because they are less visible and therefore, more difficult to detect. This article aims to bring awareness to those who manage aquatic systems to look deeper, beyond the water's surface, when evaluating and responding to HABs in their systems.

Benthic communities in lakes and rivers

The term "periphyton" refers to the complex communities of phototrophs attached to submerged surfaces in aquatic ecosystems, which can include benthic cyanobacteria. Environmental controls that can influence the periphytic communities and the benthic cyanobacteria within include physical disturbances, light, temperature, nutrients, and grazing. The influence each environmental factor has on the communities can vary and depends on the habitat.

Benthic cyanobacteria have been found to inhabit all ecological niches within the riverine system, from slow-moving backwater locations to swift water riffle and cascade habitats. In some instances, a river reach can contain several different habitats containing dozens of cyanobacteria species with the potential to release several cyanotoxins at the same time. Periods of stable flow, temperature, and light availability provide an environment conducive to the proliferation of periphytic communities that may lead to benthic HABs.

Benthic cyanobacteria in lakes are commonly found in the periphyton of shallow near-shore waters or littoral zone where light penetrates to sediments. They frequently form mat communities that exhibit complex ecological interactions among the diverse assemblage of organisms. The spatial distribution of cyanobacteria mats in lakes is largely dependent on light availability, which in turn is affected by lake size, morphometry, and water clarity. The taxonomic composition of these mats is also influenced by light availability; for example some potentially harmful genera of cyanobacteria, like *Phormidium* spp., can be found under low-light conditions due to the presence of phycobilins, photosynthetic pigments that can capture longer wavelengths of light.

Planktonic blooms, common in eutrophic lakes, can reduce water clarity and limit light penetration to the benthos. Water clarity in oligotrophic lakes is high compared to eutrophic lakes and favors deeper growth of potentially toxigenic cyanobacteria mats. Lake managers should not assume that oligotrophic lakes with seemingly high water quality cannot be a source of HABs.

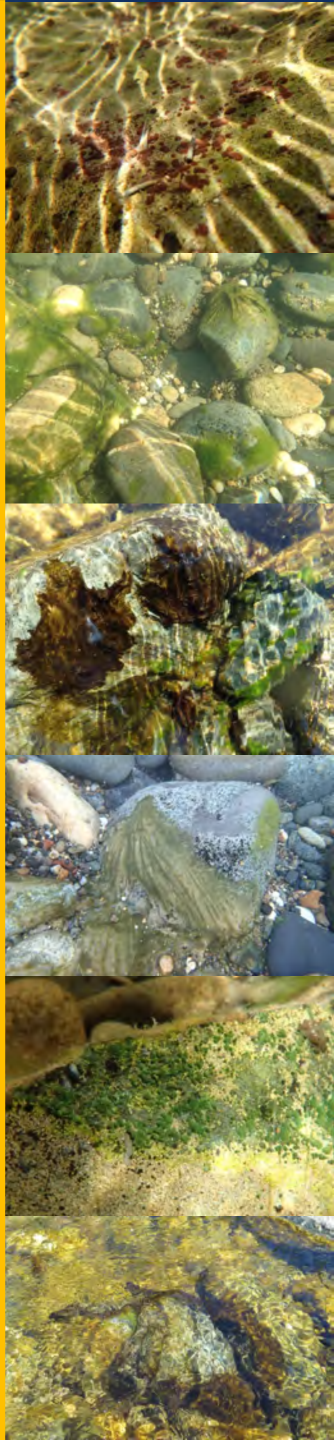
Toxins within

Benthic cyanobacteria are capable of producing several cyanotoxins such as hepatotoxins, neurotoxins, and dermatoxins. These toxins are known to contribute to human and animal illness and, in the worst case scenario, death. Reports of benthic HABs contributing to animal poisonings have increased in recent years. In Northern California's rivers, several dog deaths have been attributed to benthic cyanotoxin poisonings since 2000. Due to the inconspicuous nature of benthic cyanobacteria, there has been a lack of research into the health risks associated with benthic cyanobacteria. The ability to quantify the health risks requires new research and the development of new tools for risk assessment.

Despite these challenges, there are countries (e.g., Scotland, New Zealand, Cuba) that are responding to these needs. Periphytic communities are complex, composed of numerous organisms and substrates. They are also less accessible than the planktonic community and, therefore, more difficult to observe and sample.

Only two countries, Cuba and New Zealand, have introduced guidelines for monitoring benthic cyanobacteria. In both cases, the action triggers are based upon percent coverage of the benthos by potentially toxigenic cyanobacteria species. This type of guidance requires the determination of the toxigenic potential of cyanobacteria assemblages, which can be difficult, requiring time-consuming microscopy or DNA analysis and potentially cost-prohibitive toxin analysis.

Benthic cyanobacteria have been shown to produce toxins that are harmful to humans, animals, and aquatic life. It is important that water managers work together with regulators to develop protocols and establish water quality criteria that protects the public, animals



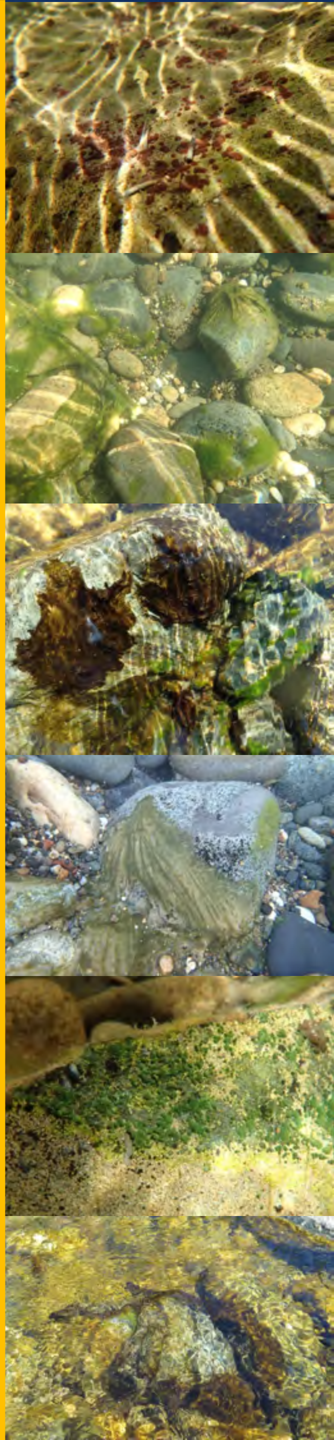
(FAIRLY RECENT) PUBLICATIONS ON BENTHIC HABS

- **Spatial and Temporal Variation in Paralytic Shellfish Toxin Production by Benthic *Microseira (Lyngbya) wollei* in a Freshwater New York Lake**
  Zacharias J. Smith, Robbie Martin, Bofan Wei, Steven Wilhelm, Gregory Boyer
  Toxins 2019, 11(1), 44; https://www.mdpi.com/2072-6651/11/1/44

- **Spatial and Temporal Variability in the Development and Potential Toxicity of *Phormidium* Biofilms in the Tarn River, France**
  Isidora Echenique-Subiabre, Maxime Tenon, Jean-Francois Humbert, Catherine Quiblier
  Toxins 2018, 10(10), 418;
  https://www.mdpi.com/2072-6651/10/10/418/htm

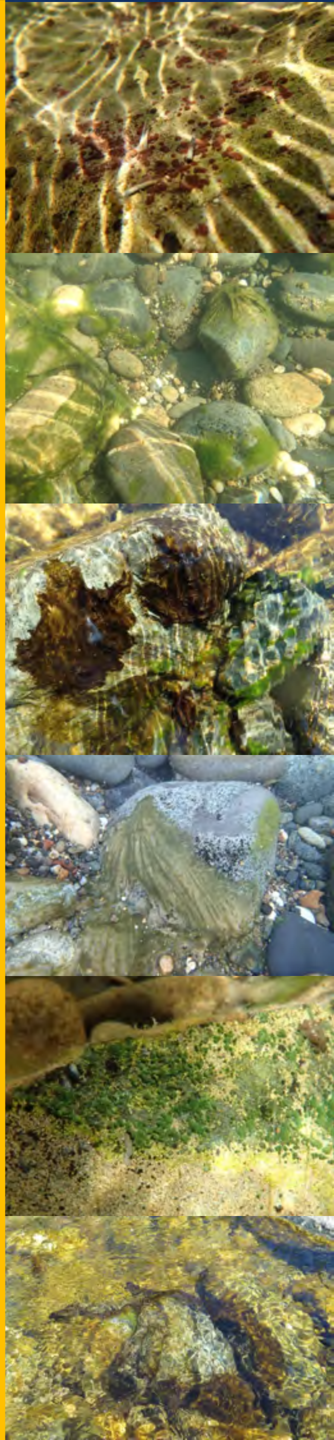
- **Impacts of microbial assemblage and environmental conditions on the distribution of anatoxin-a producing cyanobacteria within a river network**
  Keith Bouma-Gregson, Matthew Olm, Alexander Probst, Karthik Anantharaman
  The ISME Journal (2019) https://doi.org/10.1038/s41396-019-0374-3

- **Others to share?**



UPCOMING HAB MEETINGS

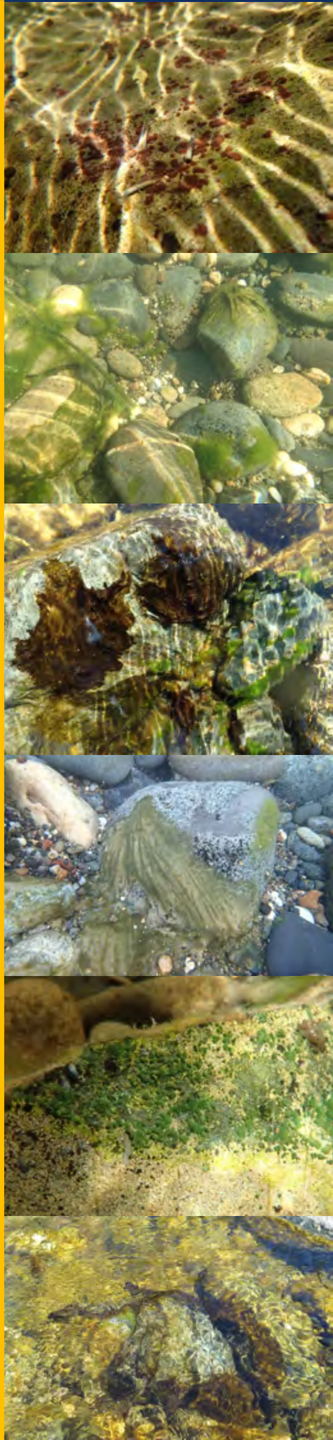
- **10th US HAB Symposium – Nov 3-8, 2019 – Orange Beach, Alabama**
  - http://ushabs.com/
- **NALMS Meeting – Watershed Moments – Nov. 11-15, 2019 – Burlington, Vermont**
  - https://www.nalms.org/nalms2019/ Several HAB Sessions
- **Water Quality Technology Conference – Nov. 3-7, Dallas, Texas**
  - https://www.awwa.org/Events-Education/Water-Quality-Technology Several HAB Sessions
- **Any others to share?**



ITEM V

2020 Schedule, Wrap Up & Next Steps

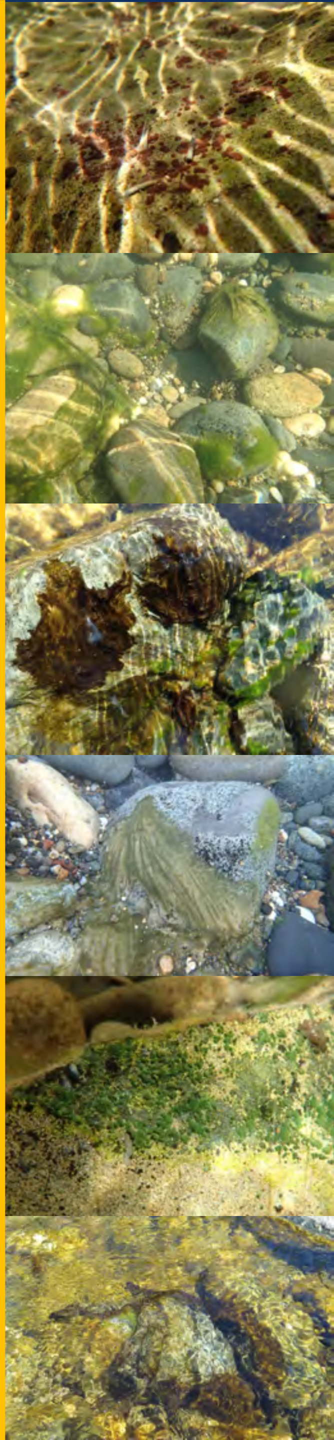
Facilitators & Benthic HAB members



PROPOSED 2020 SCHEDULE

JANUARY 2020	JULY 2020
Tentative speaker **Zach Smith - benthic HABs and a paralytic shellfish toxin in a freshwater New York lake**	**Speakers TBD**
MAY 2020	**OCTOBER 2020**
Speakers TBD	**Speakers TBD**

We'd love to hear from you on your research or monitoring program!



WRAP UP & NEXT STEPS

- Presentation material posted to Benthic HABs Workgroup webpage
  https://www.epa.gov/cyanohabs/epa-newsletter-and-collaboration-and-outreach-habs#benthic
- Send additional questions on presentation to:
  Keith.Bouma-Gregson@waterboards.ca.gov
- If you would like to present, contact the Benthic HAB facilitators.
- If you'd like to be added to the distribution list, contact the Benthic HAB facilitators.
- Our next Workgroup webinar is being scheduled for January 2020.
- Benthic HAB Facilitators:
  Christine Joab Christine.Joab@waterboards.ca.gov
  Margaret Spoo-Chupka Mspoo-Chupka@mwdh2o.com
  Jade Young Jade.L.Young@usace.army.mil
  Dr. Lesley D'Anglada Danglada.Lesley@epa.gov