US EPA BENTHIC HABS DISCUSSION GROUP WEBINAR

March 26, 2024, 9:00am – 10:30am Pacific Standard Time

Webinar registration: https://zoom.us/webinar/register/WN_g-sc9lq0STKiGdf38UFl1Q




GUEST SPEAKERS:

BRANNON WALSH, EPA

CHRISTOPHER NIETCH, EPA & ROCHELLE LABIOSA, EPA

ROSALINA STANCHEVA CHRISTOVA, GEORGE MASON U.; SYDNEY BROWN, GEORGE MASON U.; ABEER SOHRAB, UNIVERSITY OF UTAH

I. AGENDA

I **Welcome, Agenda Overview, Introductions, and Announcements**
Margaret Spoo-Chupka, Eric Zimdars, and Keith Bouma-Gregson

II **Presentation: Overview of USEPA National HAB Program**
Guest Speaker – Brannon Walsh

III **Presentation: USEPA Regions Research Assessing Field Sampling and Analytical Procedures for Characterizing Risk Posed by Harmful Benthic Cyanobacteria in Streams and Rivers**
Guest Speaker – Christopher Nietch & Rochelle Labiosa

IV **Speakers: Effect of Culture Conditions on Growth and Toxin Production of *Microcoleus* Species (Cyanobacteria) Isolate from Streams in California**
Guest Speaker – Rosalina Stancheva Christova, Sydney Brown, & Abeer Sohrab

I. INTRODUCTIONS

Name	Affiliation	Contact Information
Margaret Spoo-Chupka	Metropolitan Water District of Southern CA	Phone: 909-392-5127 Email: MSpoo-Chupka@mwdh20.com
Keith Bouma-Gregson	U.S. Geological Survey	Phone: 510-230-3691 Email: kbouma-gregson@usgs.gov
Eric Zimdars	U.S. Army Corps of Engineers	Phone: 206-764-3506 Email: Eric.S.Zimdars@usace.army.mil
Janice Alers-Garcia	U.S. EPA, Washington, DC	Phone: 202-566-0756 Email: Alers-Garcia.Janice@epa.gov

I. ANNOUNCEMENTS

- Upcoming Meetings
  - 13th International Conference on Toxic Cyanobacteria
    - https://ictc13.gr/
    - Chania, Crete
    - May 4-8, 2025
  - 12th U.S. Symposium on Harmful Algae
    - https://neiwpcc.org/events/ushab12/
    - Portland, ME
    - October 27th-November 1st ; Call for Abstracts Open, Closes May 8th
  - U.S. EPA Harmful Algal Blooms, Hypoxia, and Nutrients Research Webinar Series
    - https://www.epa.gov/water-research/harmful-algal-blooms-hypoxia-and-nutrients-research-webinar-series
  - National Association of Lake Managers/California Association of Lake Managers
    - https://www.nalms.org/nalms2024/
    - South Lake Tahoe, CA/NV
    - November 5-8; Call for Abstracts Open

ITEM II: GUEST PRESENTATION

OVERVIEW OF USEPA NATIONAL HAB PROGRAM

BRANNON WALSH, U.S. EPA

ITEM III: GUEST PRESENTATION

U.S. EPA Regions Research Assessing Field Sampling and Analytical Procedures for Characterizing Risk Posed by Harmful Benthic Cyanobacteria in Streams and Rivers

CHRISTOPHER T. NIETCH, RESEARCH ECOLOGIST U.S.EPA/ORD

&

ROCHELLE LABIOSA, PHYSICAL SCIENTIST U.S. EPA REGION 10

ITEM IV: GUEST PRESENTATION

Effect of Culture Conditions on Growth and Toxin Production of *Microcoleus* Species (Cyanobacteria) Isolate from Streams in California

ROSALINA STANCHEVA CHRISTOVA, GEORGE MASON UNIVERSITY; SYDNEY BROWN, GEORGE MASON UNIVERSITY; ABEER SOHRAB, UNIVERSITY OF UTAH

THANKS FOR ATTENDING TODAY'S MEETING!

United States Environmental Protection Agency

USEPA National HAB Program Overview

Brannon Walsh, USEPA

HABHRCA IWG Liaison

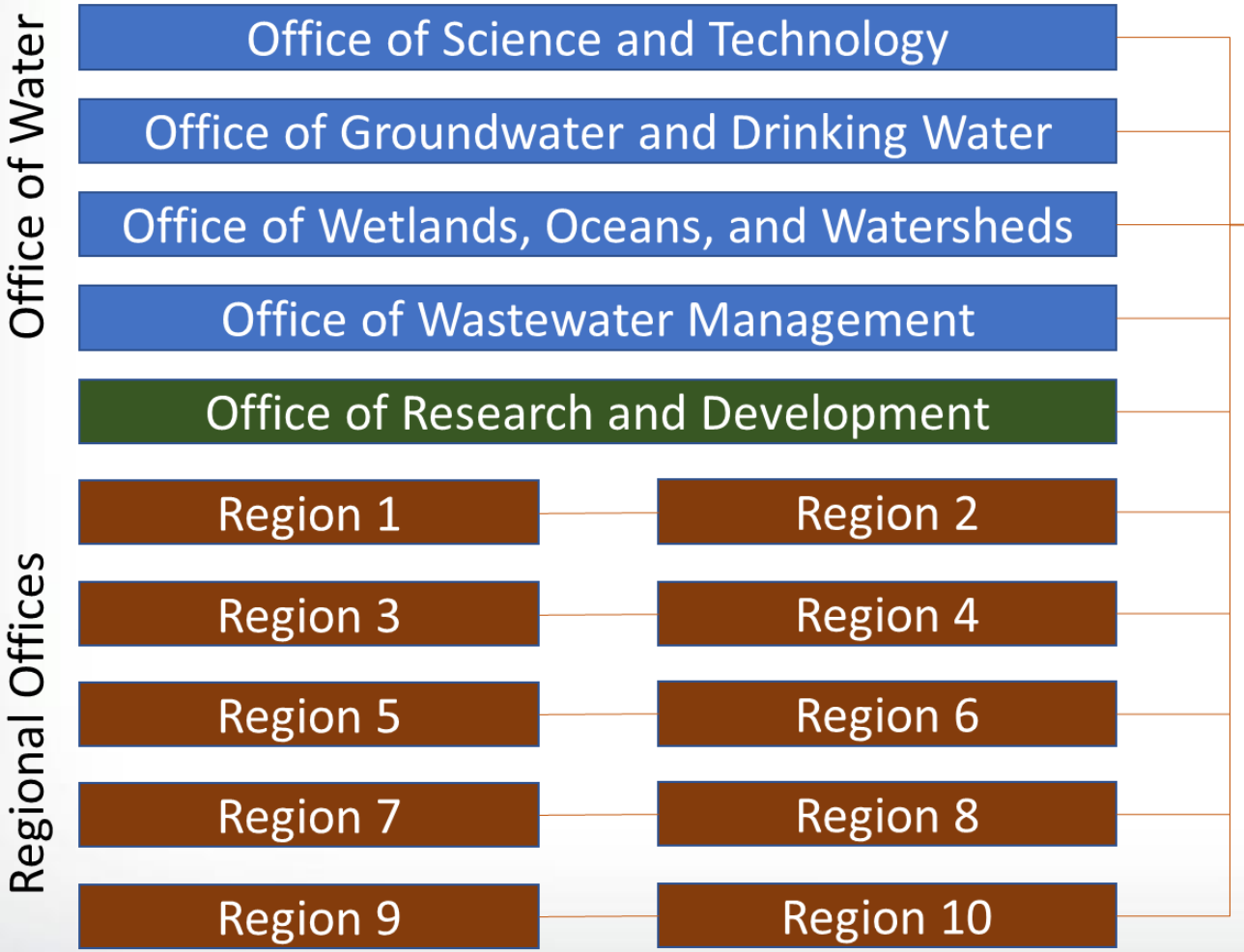
Presentation for

Benthic HABs Discussion Group

March 26, 2024

USEPA National HAB Program

USEPA National HAB Program – Improving Intra-agency HAB Coordination



USEPA National HAB Program

Lead: Michael Paul

"Serves as the center for communication, coordination, and collaboration among EPA programs and regional offices involved in HAB-related work."

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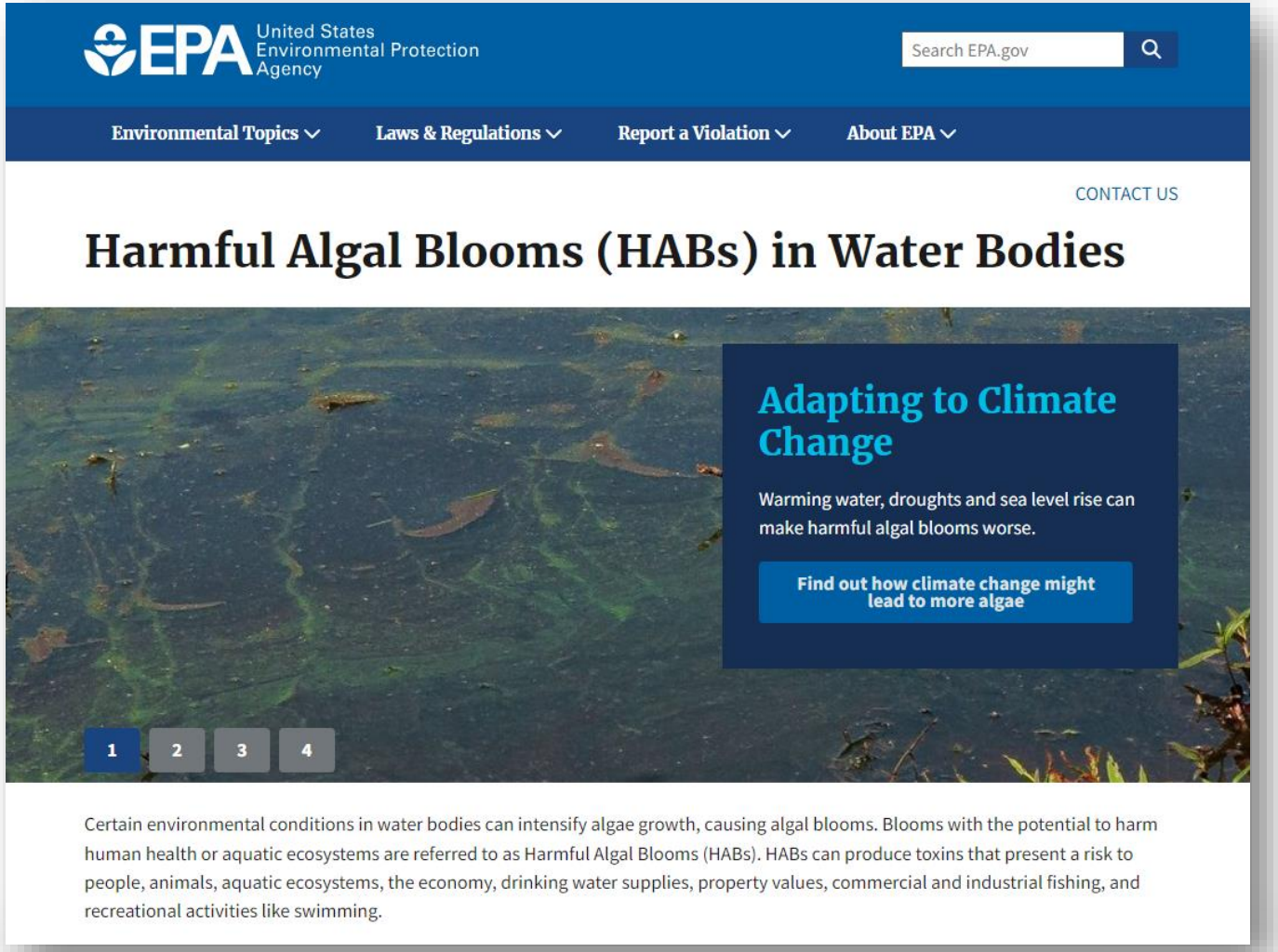
USEPA National HAB Program

- Prevent
- Monitor
- Forecast
- Control
- Response



New Website!
Hot off the Press!!

https://www.epa.gov/habs

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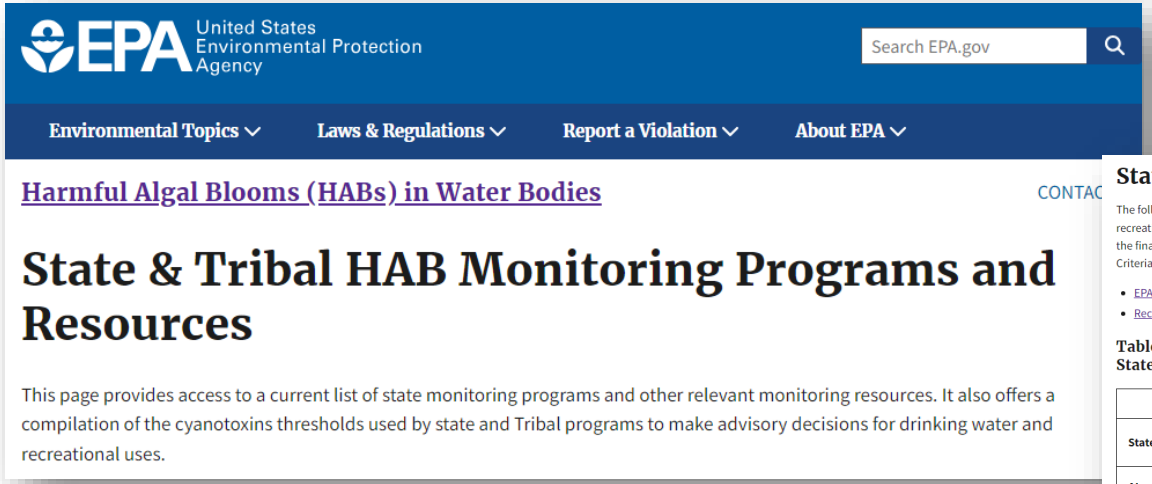
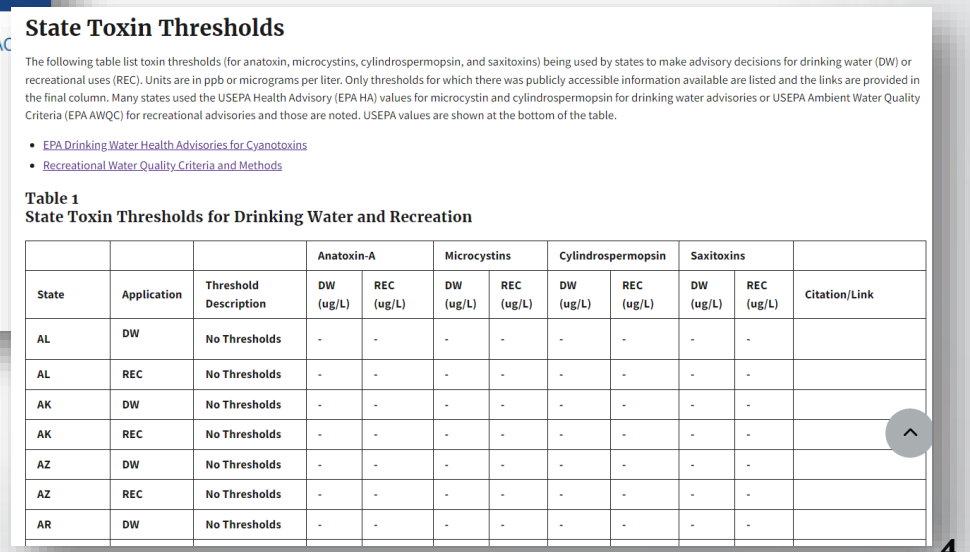
Cyanotoxin	Drinking Water Health Advisory (10-day)	
	Bottle-fed infants and pre-school children	School-age children and adults
Cylindrospermopsin	0.7 µg/L	3.0 µg/L
Microcystins	0.3 µg/L	1.6 µg/L

Table. Recommended magnitude for cyanotoxins.

Microcystins	Cylindrospermopsin
8 µg/L	15 µg/L





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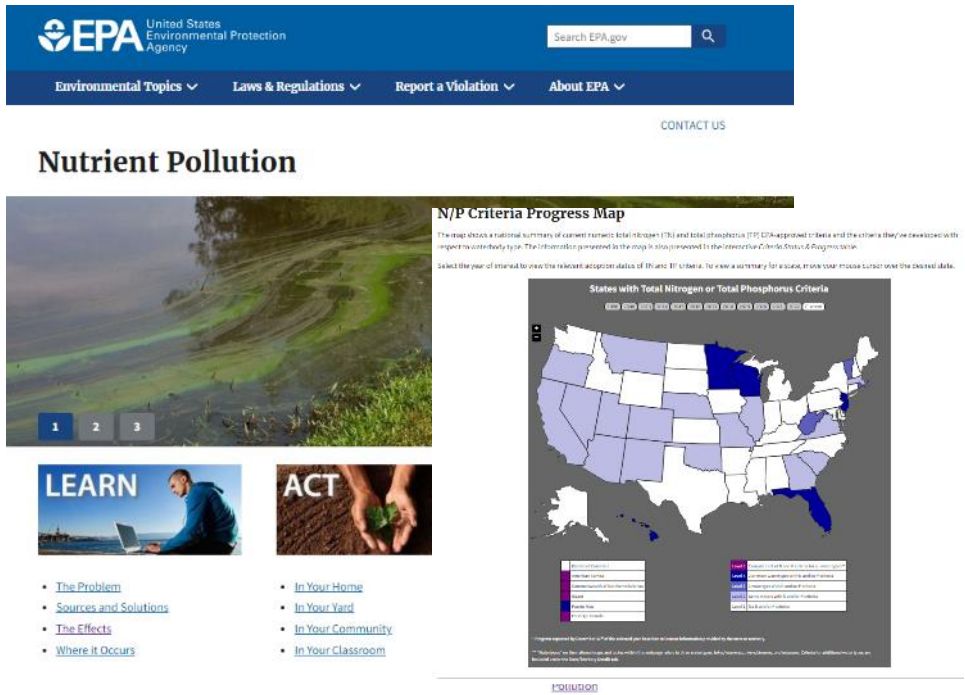

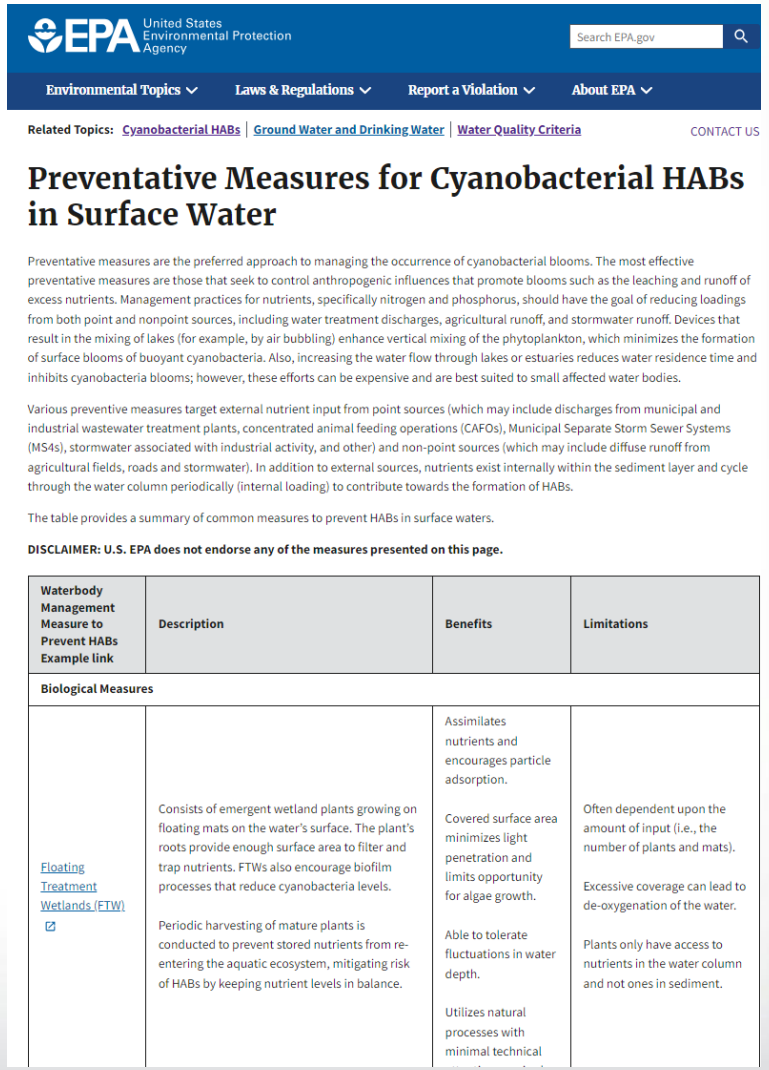

Preventative Measures for Cyanobacterial HABs in Surface Water

Preventative measures are the preferred approach to managing the occurrence of cyanobacterial blooms. The most effective preventative measures are those that seek to control anthropogenic influences that promote blooms such as the leaching and runoff of excess nutrients. Management practices for nutrients, specifically nitrogen and phosphorus, should have the goal of reducing loadings from both point and nonpoint sources, including water treatment discharges, agricultural runoff, and stormwater runoff. Devices that result in the mixing of lakes (for example, by air bubbling) enhance vertical mixing of the phytoplankton, which minimizes the formation of surface blooms of buoyant cyanobacteria. Also, increasing the water flow through lakes or estuaries reduces water residence time and inhibits cyanobacteria blooms; however, these efforts can be expensive and are best suited to small affected water bodies.

Various preventive measures target external nutrient input from point sources (which may include discharges from municipal and industrial wastewater treatment plants, concentrated animal feeding operations (CAFOs), Municipal Separate Storm Sewer Systems (MS4s), stormwater associated with industrial activity, and other) and non-point sources (which may include diffuse runoff from agricultural fields, roads and stormwater). In addition to external sources, nutrients exist internally within the sediment layer and cycle through the water column periodically (internal loading) to contribute towards the formation of HABs.

The table provides a summary of common measures to prevent HABs in surface waters.

DISCLAIMER: U.S. EPA does not endorse any of the measures presented on this page.

Waterbody Management Measure to Prevent HABs Example link	Description	Benefits	Limitations
Biological Measures			
Floating Treatment Wetlands (FTW)	Consists of emergent wetland plants growing on floating mats on the water's surface. The plant's roots provide enough surface area to filter and trap nutrients. FTWs also encourage biofilm processes that reduce cyanobacteria levels. Periodic harvesting of mature plants is conducted to prevent stored nutrients from re-entering the aquatic ecosystem, mitigating risk of HABs by keeping nutrient levels in balance.	Assimilates nutrients and encourages particle adsorption. Covered surface area minimizes light penetration and limits opportunity for algae growth. Able to tolerate fluctuations in water depth. Utilizes natural processes with minimal technical	Often dependent upon the amount of input (i.e., the number of plants and mats). Excessive coverage can lead to de-oxygenation of the water. Plants only have access to nutrients in the water column and not ones in sediment.

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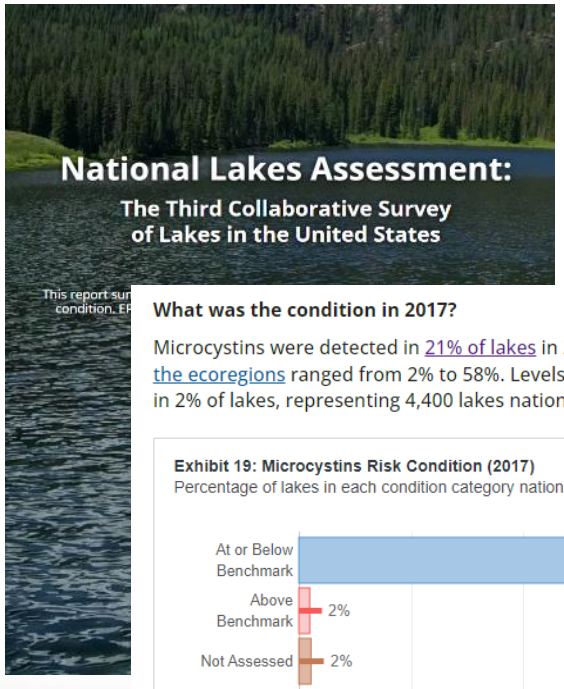
Cyanobacteria Assessment Network Application (CyAN app)

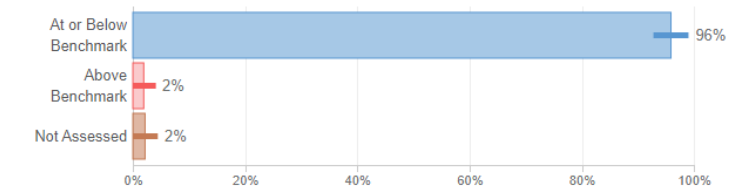
Make faster decisions related to cyanobacterial algal blooms



What was the condition in 2017?

Microcystins were detected in 21% of lakes in 2017. The detection of microcystins in the ecoregions ranged from 2% to 58%. Levels exceeded EPA's recreational criterion in 2% of lakes, representing 4,400 lakes nationally, as shown below.

Exhibit 19: Microcystins Risk Condition (2017)
Percentage of lakes in each condition category nationally



For more details, download the data for this chart, or visit the NLA dashboards for ecoregional data on microcystins risk.

BECOME A BLOOMWATCH BETA TESTER

WHAT IS BLOOMWATCH? | HOW DOES IT WORK? | WHERE ARE THE BLOOMS?

Cyanotoxins and the Safe Drinking Water Act: Drinking Water Protection Act, Contaminant Candidate List and the Unregulated Contaminant Monitoring Rule

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Emerging HAB Topics

Benthic HABs Discussion Group

Mission Statement:

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

Calendar of Webinars:

- Benthic HABs Discussion October 17, 2023

Related Info

Past Benthic Webinars

Contact Information:

Benthic HAB Workgroup Facilitators – Contact us to join the workgroup or to be a presenter!

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Eric Zimdars	U.S. Army Corps of Engineers	Phone: 206-764-3506 Email: Eric.S.Zimdars@usace.army.mil
Margaret Spoo-Chupka	Metropolitan Water District of Southern CA	Phone: 909-392-5127 Email: MSpoo-Chupka@mwdh2o.com
Keith Bouma-Gregson	U.S. Geological Survey	Phone: 510-230-3691 Email: kbouma-gregson@usgs.gov
Janice Alers-Garcia	US EPA, Washington, DC	Phone: 202-566-0756 Email: Alers-Garcia.Janice@epa.gov

*** Disclaimer:** The information presented in the Benthic HABs Discussion Group Webinars does not constitute an official endorsement by the U.S. government.

Developing Standardized Methods for Sampling, Analyzing and Assessing Benthic Harmful Algal Blooms

United States Environmental Protection Agency

epa.gov/research

Innovative Science for a Sustainable Future

Background

Benthic harmful cyanobacterial blooms (HCBs) and their toxins pose a significant environmental threat to domestic animals, wildlife, and humans, and have impacted drinking water treatment operations in recent years. Specifically, dog and cattle deaths have

have recently experienced benthic HCBs. Researchers are looking to characterize locations on wadeable streams and wadeable areas of larger rivers where high exposure risks have the potential to occur, such as places where children and pets (i.e., dogs) play in water, wade, or have the potential

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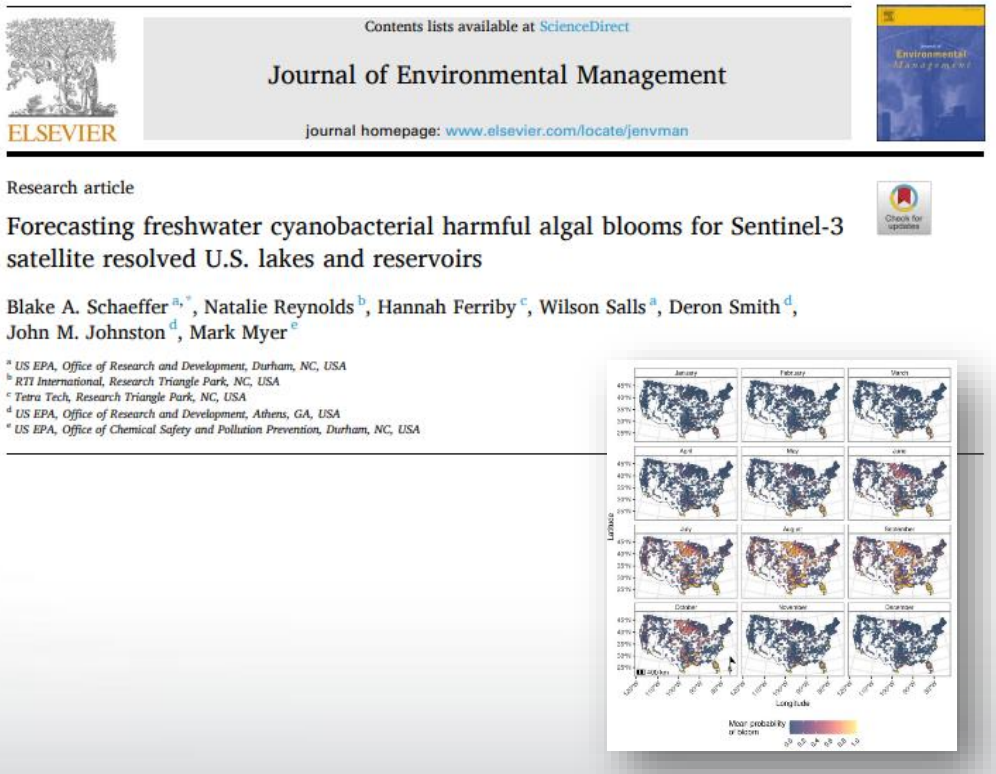
Make faster decisions related to cyanobacterial algal blooms



Contents lists available at ScienceDirect

Journal of Environmental Management

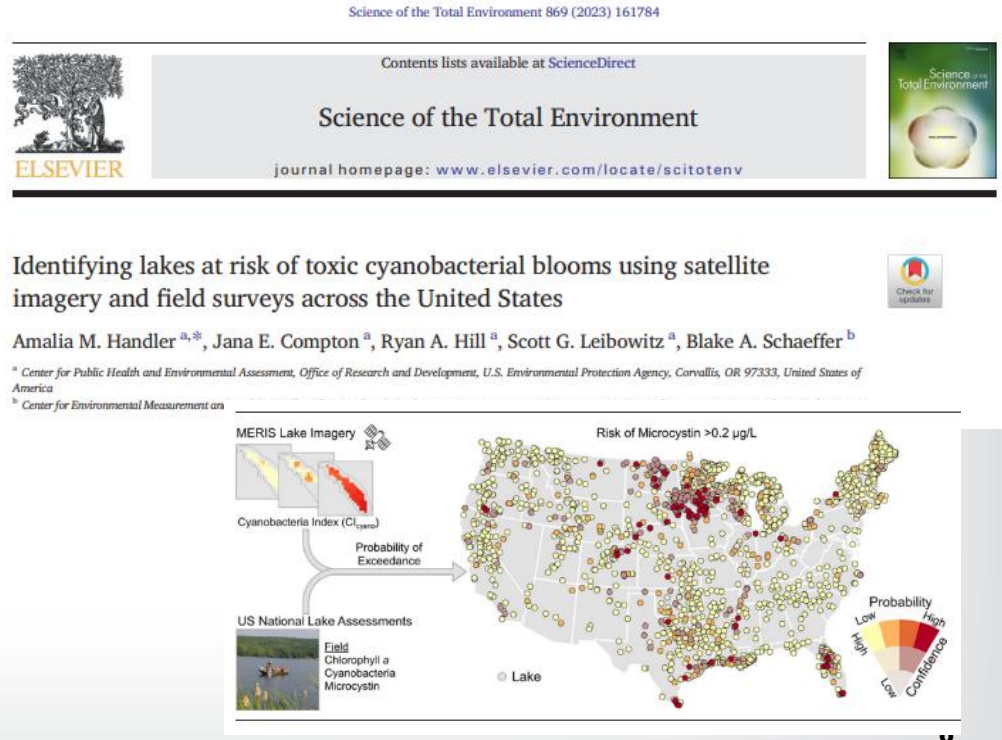
journal homepage: www.elsevier.com/locate/jenvman

Research article

Forecasting freshwater cyanobacterial harmful algal blooms for Sentinel-3 satellite resolved U.S. lakes and reservoirs

Blake A. Schaeffer [a,*], Natalie Reynolds [b], Hannah Ferriby [c], Wilson Salls [a], Deron Smith [d], John M. Johnston [d], Mark Myer [e]

[a] US EPA, Office of Research and Development, Durham, NC, USA
[b] RTI International, Research Triangle Park, NC, USA
[c] Tetra Tech, Research Triangle Park, NC, USA
[d] US EPA, Office of Research and Development, Athens, GA, USA
[e] US EPA, Office of Chemical Safety and Pollution Prevention, Durham, NC, USA



Science of the Total Environment 869 (2023) 161784

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Identifying lakes at risk of toxic cyanobacterial blooms using satellite imagery and field surveys across the United States

Amalia M. Handler [a,*], Jana E. Compton [a], Ryan A. Hill [a], Scott G. Leibowitz [a], Blake A. Schaeffer [b]

[a] Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Corvallis, OR 97333, United States of America
[b] Center for Environmental Measurement an

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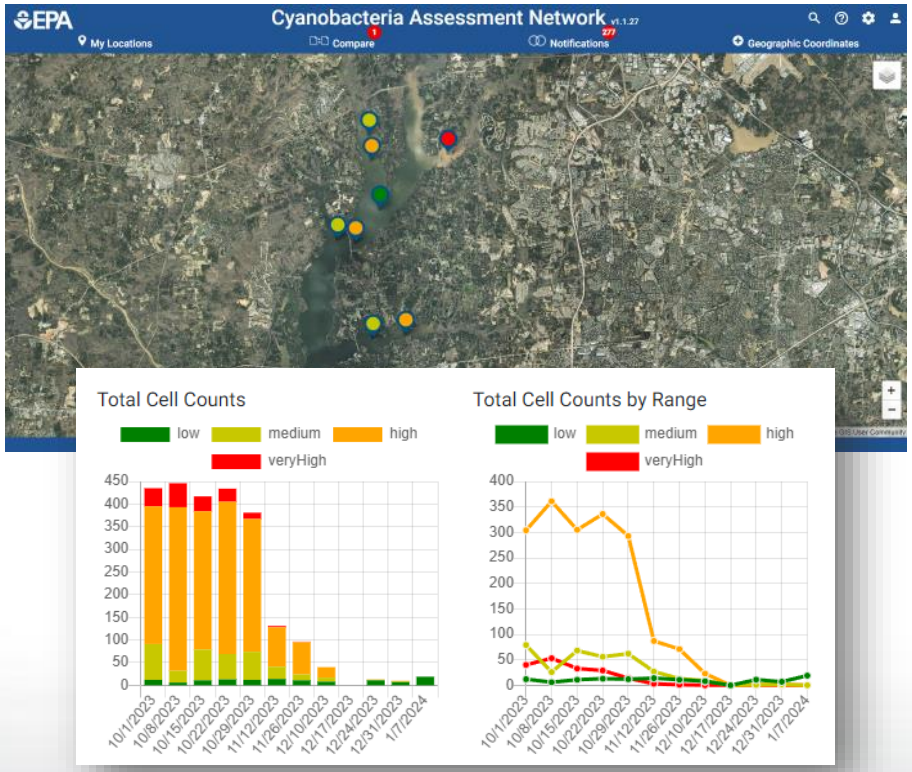
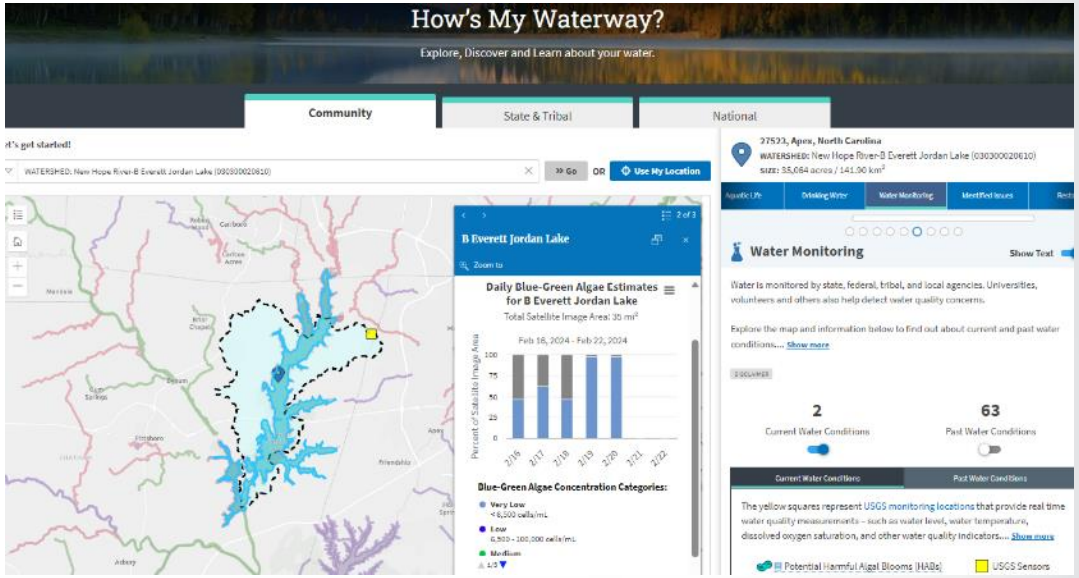
Operationalizing CyAN Forecasts





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Summary of Cyanotoxins Treatment in Drinking Water

Conventional water treatment (consisting of coagulation, sedimentation, filtration and chlorination) can generally remove cyanobacterial cells and low levels of toxins. However, water systems may face challenges providing drinking water during a severe bloom event, when there are high levels of cyanobacteria and cyanotoxins in drinking water sources.

Once cyanobacteria and/or their cyanotoxins are detected in the surface water supplying the water system, the treatment system operators can act to remove or inactivate them in a number of ways. Some treatment options are effective for some cyanotoxins, but not for others. Effective management strategies depend on understanding the growth patterns and species of cyanobacteria that dominates the bloom, the properties of the cyanotoxins (i.e., intracellular or extracellular), and appropriate treatment processes. For example, oxidation of microcystin depends on the chlorine dose, pH and the temperature of the water. Applying the wrong treatment process at a specific state in treatment could damage cells and result in the release rather than removal of cyanotoxins.

The table below summarizes the effectiveness of different types of water treatment to remove intact cyanobacteria cells and treatment processes that are effective in removing extracellular dissolved toxins of several of the most important cyanobacteria. Drinking water operators are encouraged to monitor the treated water to confirm the removal of cyanotoxins.

A Summary of Cyanotoxin Treatment Processes and Their Relative Effectiveness

Treatment Process	Relative Effectiveness
Intracellular Cyanotoxins Removal (Intact Cells)	
Pre-treatment oxidation	Oxidation often stresses or lyses cyanobacteria cells releasing the cyanotoxin to the water. If oxidation is required to meet other treatment objectives, consider using lower doses of an oxidant less likely to lyse cells. If oxidation at higher doses must be used, sufficiently high doses should be used to not only lyse cells but also destroy total toxins present (see extracellular cyanotoxin removal).
Coagulation/ Sedimentation/ Filtration	Effective for the removal of intracellular toxins (cyanobacteria cells). Ensure that captured cells accumulated in sludge are removed frequently to release toxins. Ensure that sludge supernatant is not

Control Measures for Cyanobacterial HABs in Surface Water

Measures can be employed once blooms have already occurred to control the phytoplankton blooming rate and to remove blooms. The table provides a summary of the common physical and chemical measures for cyanobacterial blooms in surface waters and their respective effectiveness and limitations.

To learn more about ways to manage cyanobacterial blooms visit: Report: Solutions for managing cyanobacterial blooms: A scientific summary for policy makers (PDF) or the ITRC's Strategies for Preventing and Managing Benthic Harmful Cyanobacterial Blooms Management Criteria Tool.

DISCLAIMER: *U.S. EPA does not endorse any of the measures presented on this page.*

A Summary of Waterbody Management Measures for Cyanobacterial Blooms

Waterbody Management Measure	Description	Effectiveness	Limitations
Physical Controls			
Aeration	Aerators operate by pumping air through a diffuser near the bottom of the waterbody, resulting in the formation of plumes that rise to the surface and create vertical circulation cells as they propagate outwards from the aerator. This mixing of the water column disrupts the behavior of cyanobacteria to migrate vertically in addition to limiting the accessibility of nutrients.	Successfully implemented in small ponds and waterbodies. May also provide more	Generally more efficient in deeper water columns. Also highly
Hydrologic manipulations	Manipulation of inflow/outflow of water in the system to disrupt stratification and control cyanobacterial growth.		
	Mechanical mixers are usually surface-		

Cyanotoxin Management Tools for Public Water Systems

The following resources can help public water systems plan for and manage cyanotoxins in their drinking water. Key resources provide information on treating, monitoring and communicating the risks of cyanotoxins in drinking water.

Preparing a cyanotoxin management plan

- Recommendations for Public Water Systems to Manage Cyanotoxins in Drinking Water
- Cyanotoxin Management Plan Template and Example Plans

Treating cyanotoxins

- Water Treatment Optimization for Cyanotoxins Document
- Comprehensive Performance Evaluation Protocol to Address Harmful Algal Blooms and Associated Cyanotoxins
- Summary of Cyanotoxins Treatment in Drinking Water
- Cyanobacteria and Cyanotoxins: Information for Drinking Water Systems Fact Sheet

Freshwater Cyanotoxins				
Techniques	**Anatoxins**	**Cylindrospermopsins**	**Microcystins**	**Saxitoxins**
Biological Assays				
Mouse	Yes	Yes	Yes	
Protein Phosphatase Inhibition Assays (PPIA)	No	No	Yes	
Neurochemical	Yes	No	No	
Enzyme-Linked Immunosorbent Assays (ELISA)	Yes	Yes	Yes	Yes
Chromatographic Methods ***Gas Chromatography***				
Gas Chromatography with Flame Ionization Detection (GC/FID)	Yes	No	No	No
Gas Chromatography with Mass Spectrometry (GC/MS)	Yes	No	No	No
				Yes
				Yes
				Yes
				Yes
Liquid Chromatography Single Quadrupole Mass Spectrometry (LC/MS)	Yes	Yes	Yes	Yes

Determination of Cyanotoxins in Drinking and Ambient Freshwaters

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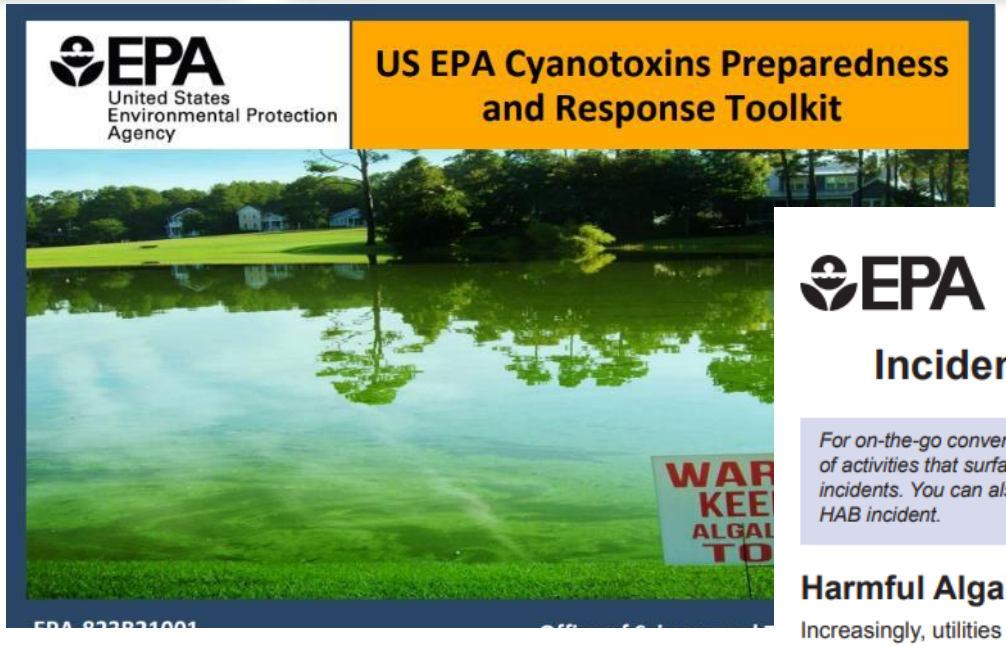
- **Response**



Monitoring and Responding to Cyanobacteria and Cyanotoxins in Recreational Waters

This information is intended for recreational waterbody managers, which may include public health officials, lake managers, or other state, local or tribal officials, involved in monitoring water quality and protecting the health of people and animals that use waterbodies within their jurisdiction.

DISCLAIMER: *This information does not impose legally binding requirements on EPA, states, tribes, or the public, nor does it confer legal rights. It does not constitute a regulation, nor does it change or substitute for any Clean Water Act provision or EPA regulation. Any mention of trade names, products, or services does not convey and should not be interpreted as conveying official EPA approval, endorsement, or recommendation for use.*

On this page:

- Visual signs of a Cyanobacterial Bloom
- Developing an Emergency Response Plan for Cyanotoxins

Related Information

- Communicating about Cyanobacterial Blooms in Recreational Waters
- Nutrient Pollution Policy and Data
- Recreational Water Quality Criteria or Swimming Advisories for Cyanotoxins
- Final Technical Support Document: Implementing 2019 Recommended Recreational Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin

Incident Action Checklist – Harmful Algal Blooms

For on-the-go convenience, the actions in this checklist are divided up into three "rip & run" sections and are examples of activities that surface water utilities can take to: prepare for, respond to and recover from harmful algal bloom (HAB) incidents. You can also populate the "My Contacts" sections with critical information that your utility may need during the HAB incident.

Harmful Algal B

Increasingly, utilities fac… elivery of safe drinking water to their cu… n lead to prolonged

PRESS RELEASE

RECREATIONAL WATER CLOSURE ISSUED

FOR IMMEDIATE RELEASE

Media Contact: [insert name, title, telephone and fax number, and e-mail of spokesperson]

WHY IS THERE A CLOSURE?

- [Cyanotoxin *or* cyanobacteria name], a toxin produced by cyanobacteria (formerly known as blue-green algae) was detected in the water at levels that could cause harm at [location] on [date].
- Samples collected on [dates] show [cyanotoxins *or* cyanobacteria name] in [location] at [levels and/or ranges], which are above the state-designated recreational water health advisory levels.

WHAT SHOULD I DO?

- Do not swim, wade or come in contact with the water, scum, foam or algae at [location].
- Seek medical attention if you or family members are experiencing illness after swimming or playing in water. Recreational waters containing [cyanotoxin *or* cyanobacteria name] at levels exceeding the state's guidelines for issuing a Health Advisory can put you at risk of various adverse health effects including upset stomach, vomiting and diarrhea. Exposure to concentrations of cyanotoxins higher than the state's guideline values could potentially result in more serious illnesses, including liver or kidney damage.
- Animals may be vulnerable to adverse health effects of [cyanotoxin *or* cyanobacteria name] at the detected levels indicated above. Contact a veterinarian if animals show signs of illness.
- If you, your family members or your animals have experienced adverse [cyanotoxin- *or* cyanobacteria-related] health effects, please contact [State or local Health Department] to report the illness.

USEPA National HAB Program

Outreach and Other Resources

- Prevent
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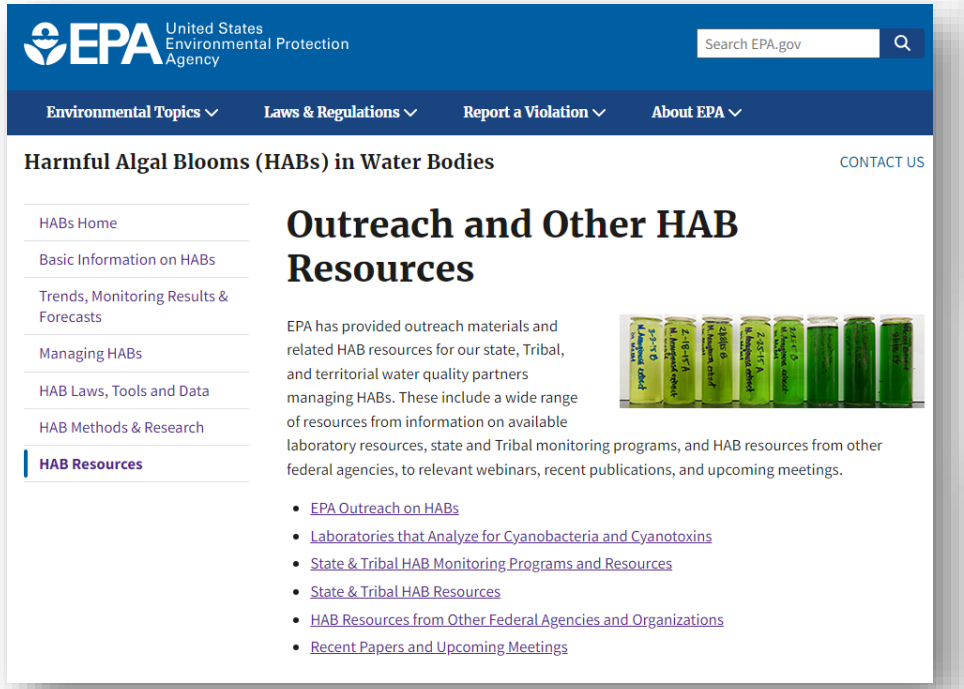

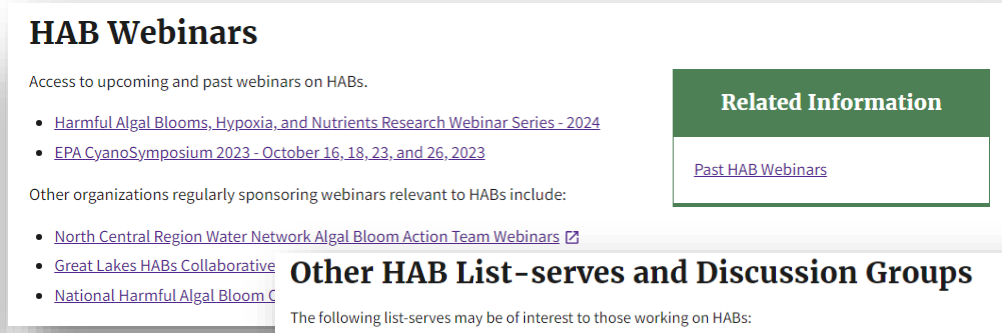

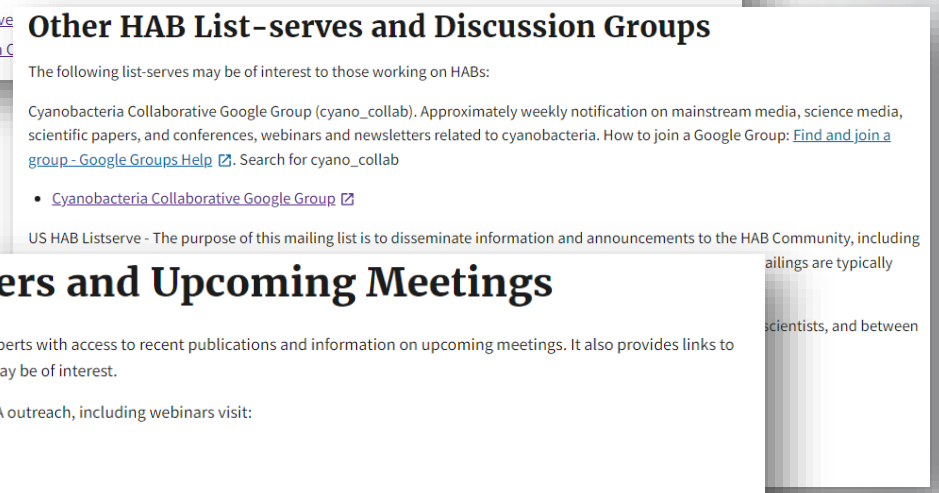

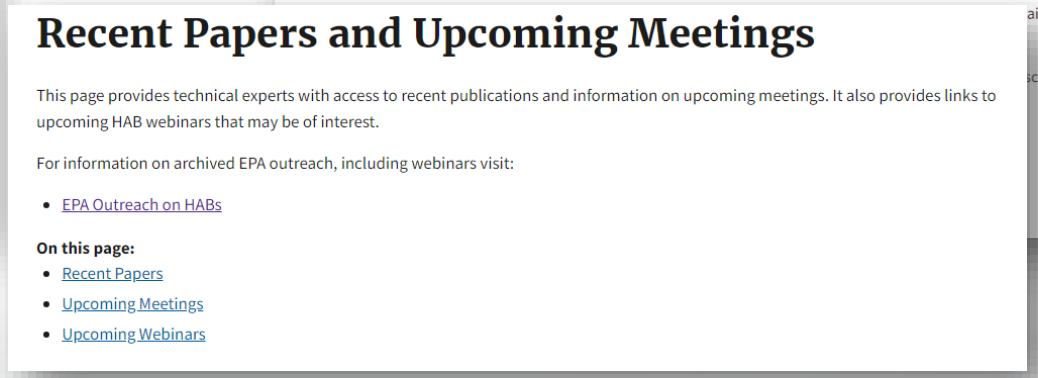

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USEPA National HAB Program

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https://www.epa.gov/habs

12th U.S. Symposium on Harmful Algae

October 27-November 1, 2024

Holiday Inn Portland by the Bay in Portland, Maine.

https://neiwpcc.org/events/ushab12/

Search: US HAB Meeting 2024

Image from Lower Calf Creek, UT, Training Site– 15Aug2023

USEPA Regions Research

Assessing field sampling and analytical procedures for characterizing risk posed by harmful benthic cyanobacteria in streams and rivers

Chris Nietch[1] and Rochelle Labiosa[2]

[1]Ecologist, USEPA, ORD, Cincinnati, OH; [2]Physical Scientist, USEPA, R10, Seattle, WA

The Issue

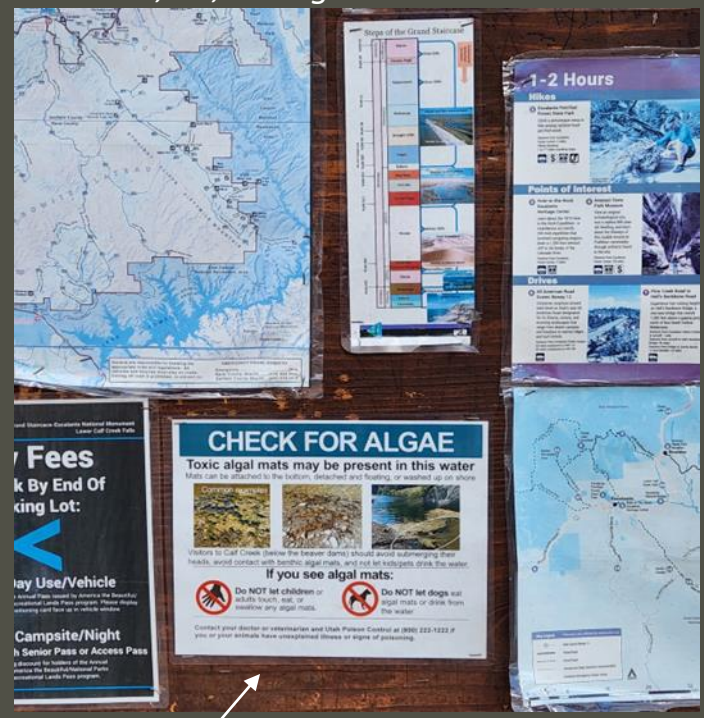
- Benthic harmful cyanobacteria blooms (HCBs) pose a significant threat to domestic animals, wildlife, and humans
- State, tribal, and local agencies need greater understanding of the risk posed by benthic HCBs
- The Interstate Technology and Regulatory Council (ITRC) developed guidance, but did not provide specific recommendations for characterizing risk quickly and effectively
- Results will inform sampling protocols and analyses that will help partners develop plans that use common approaches and inform decisions about when to post and remove alerts

Benthic HCB in the Eel River, CA

underwater view of toxin producing Microcoleus mat

Periphyton is the biofilm of streams and rivers, a mixture of algae, cyanobacteria, heterotrophic microbes, and detritus that is attached to submerged surfaces in most aquatic ecosystems.

Kiosk at Lower Calf Creek Falls Trailhead, Escalante, UT, 17Aug2023



Benthic HCB Signage used by CA and UT-DEQ

ROAR _ Core Research Team

Tina Laidlaw (R8), Chris Nietch (ORD), Jim Lazorchak (ORD) – **oversight**

Laura Webb (R7), Tina Laidlaw (R8), Rochelle Labiosa (R10), Avery Tatters and Chris Nietch (ORD) – **field procedures and ecological measures**

Heath Mash and Toby Sanan (ORD: LCMSMS), Marcie Tidd (R8: ATX/STX ELISA), Meghan Dunn (R10: Nutrients), Hillary Snook (R1: MYC/CYL), and Laura Webb (R7: AFDM/CHLA/Phyco) – **analytical chemistry**

Jingrang Lu (ORD: QPCR) and Erik Pilgrim (ORD: DNA Metabarcoding) – **molecular biology**

Chris Nietch, Nate Smucker, Avery Tatters (ORD) – **ecological interpretations**

External partners (E.g., Dana Michels (R9); Rich Fadness, Michael Thomas, Carly Nilson, and Marisa VanDyke (CA Water Boards)), Hannah Bonner, UTDEQ, Robyn Henderek, NPS, WA USGS, and many others – **expert advice and sampling effort**

Benthic HCB ROAR -Strategy for 2023

Goals

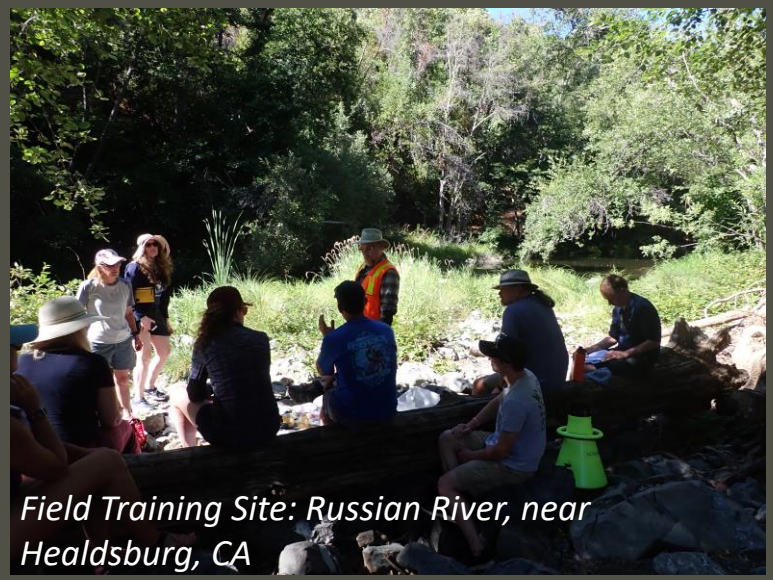
- Each of 7 field teams (representing EPA Regions 3, 5, 7, 8, 9, & 10) sampled one site of interest; 2 events (4 visits total)
- Test different in-stream sampling methodologies
- Conduct "experiments" to quantify effectiveness of proposed methods
- Test-run sample transfer, processing, and analyses logistics and methods
- Develop and test data acquisition and management strategies

Project Scheduling

- Study design, write QAPP, and write SOP, February - June
- Field crew training, July – August
  - Two field training events provided: Santa Rosa, CA and Escalante, UT
- Field sampling, September - October
- Field measurements managed by the end of January 2024
- Lab analyses completed by end of February 2024
- March - April 2024, analyze and interpret data
- May - June 2024, study design, etc. for 2024 benthic HCB season

Lessons learned and results from 2023 are informing the sampling design for 2024, including sampling more sites but doing fewer procedures at each

Images from Rich Fadness, CA Water Board, Russian River

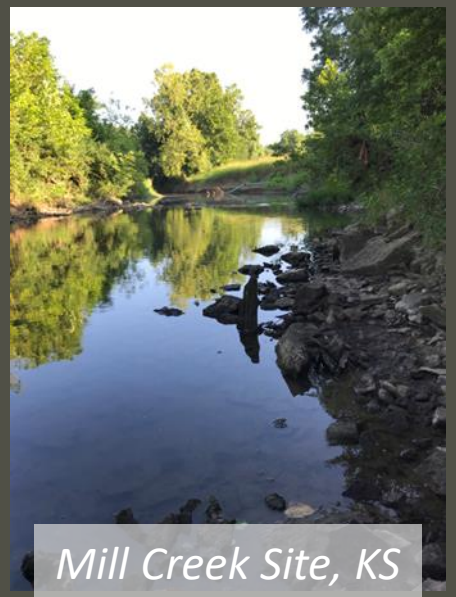


Field Training Site: Russian River, near Healdsburg, CA

2023 Benthic HCB Study Sites

Field Team	Sample ID*	State	City	2023 sampling locations	Lat, Long
Region 3	**R3NFSR**	**VA**	**Strasburg**	North Fork Shenandoah River (Strasburg Town Park)*	38.972861, -78.351111
ORD (Region 5)	**R5SMR**	**OH**	**St. Marys**	St. Mary's River at St. Marys	40.535342, -84.378224
Region 7	**R7IC**	**KS**	**Kansas City**	Mill Cree near Shawnee Station	39.017313, -94.815727
Region 8	**R8RS1**	**UT**	**Escalante**	Lower Calf Creek near Escalante	37.795997, -111.413100
Region 9	**R9AR**	**CA**	**Sacramento**	American River @ Oregon Bar	38.863448, -121.058674
Region 9	**R9SFER_01**	**CA**	**Phillipsville**	South Fork Eel River @ Phillipsville	40.199195, -123.775900
Region 10	**ROAR_R10ColLG**	**WA**	**Richland**	Columbia River, Leslie Groves Park	46.312003, -119.261317

St. Marys River Site, OH



Mill Creek Site, KS

North Fork Shenandoah Site, VA

Two main objectives of Benthic HCB ROAR sampling procedures design in 2023

Transect Methods

Characterize the relative extent of potential toxin-producing benthic cyanobacteria at the reach scale and within the context of reach ecology, including the whole periphyton community

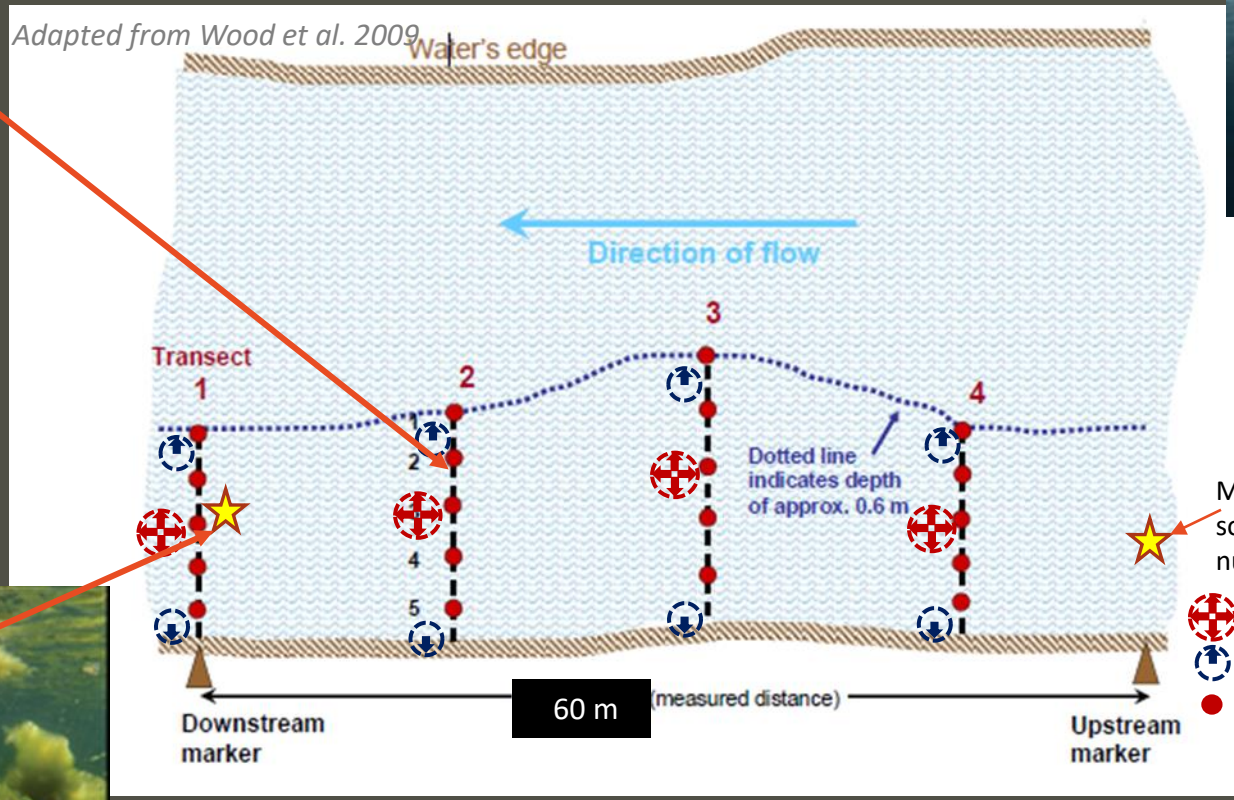
Disturbance Methods

Assess the potential risk of toxin exposure through direct contact that might occur during human recreation or use of the reach by domestic pets, livestock, and wildlife, or through drinking water whose source was contaminated with cyanotoxin(s)

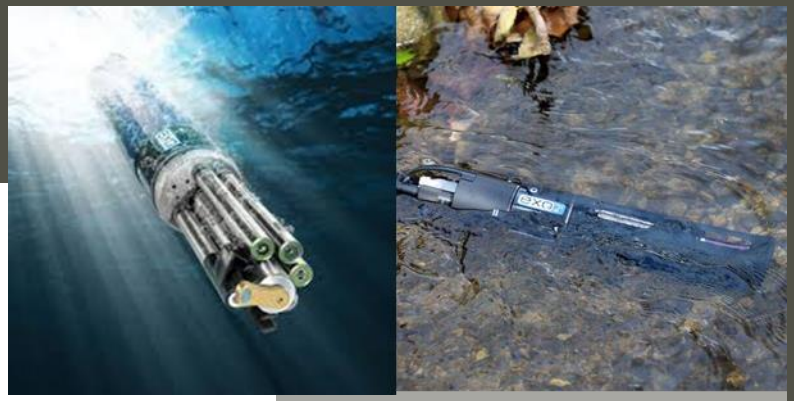
Transect method set-up: channel sections >10m

Sampling locations for:

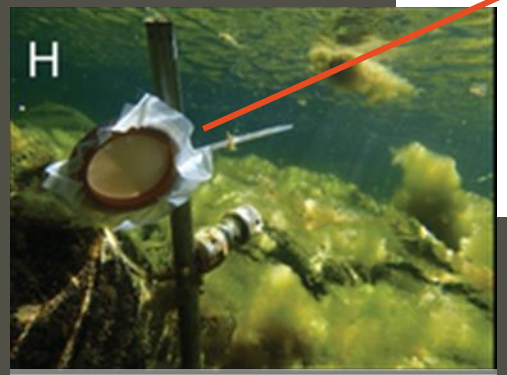
- Substrate type
- %Cover - Bathyscope visual assessment
- Periphyton Composite -Sample substrate(s) from 8 of the bathyscope locations; 2 in each transect.
- Disturbance Sample – 1 location with max cyanobacteria cover



Zig-zag set-up scheme used for stream reaches under 10m width



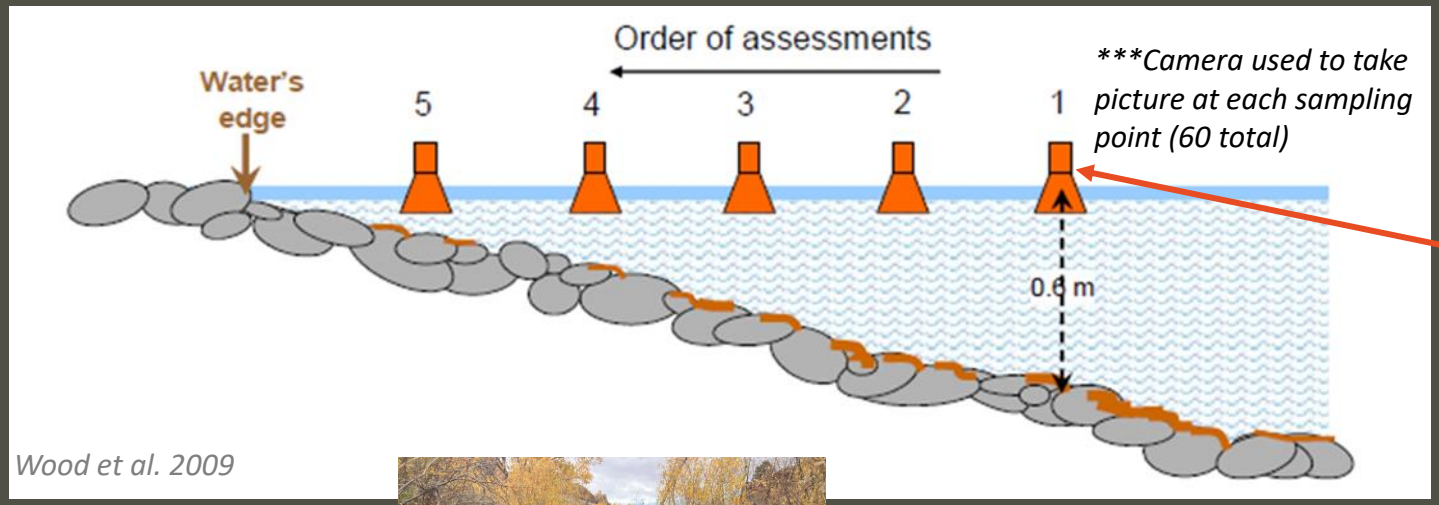
Multiparameter data sonde – YSI EXO-type shown, or similar



SPATT sampler in the Eel River, CA. Figure 2 in Bouma-Gregson et al 2018

Convex densiometer for estimating canopy cover

Transect Sampling Procedure: Cross section of transect and bathyscope viewing for %Cover

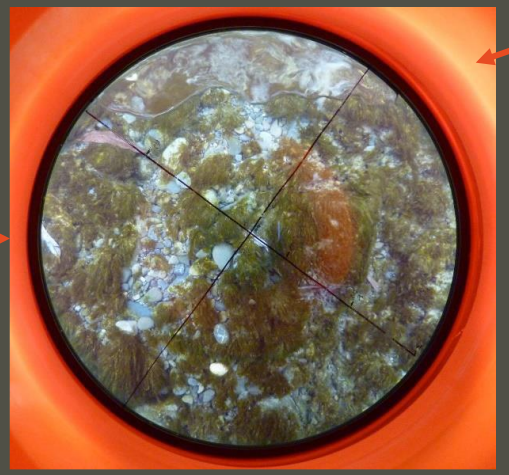


Wood et al. 2009

***Camera used to take picture at each sampling point (60 total)

Bathyscope

bathyscope use, Lower Calf Creek, UT



Example, bathyscope view occupied by 60% green filaments (NIWA 2021)

St. Mary's River Site, OH. Note strandings and floating chunks of benthic mats

Disturbance Sampling Procedure: Considered and tested different approaches

The goal: Obtain a 'standardized' sample that characterizes the exposure potential (worse-case scenario)

Location sampled: Target area with highest cyanobacterial presence

One approach: UDEQ-NPS (2021) – "stomp and catch" calls for stepping in an approximate 1 m^2 area 5 s while scooping water from the disturbed area using a 2.5-gallon bucket and attempting to capture any dislodged mats in the bucket ITRC (2022b).

Concern: Toxin quantities not normalized to a known area or quantity of mat material to compare across sites or establish future guideline

Test disturbance sampling with Surber or Hess sampler

1. Bulk Biomass Sample
2. Water Parcel Sample

HESS Sampler

Confluence to American River, CASouth Crew – 26Sep2023

Lower Calf Creek, Study Site, Escalante, UT

Questions and experiments to inform effectiveness of in-reach sampling procedures

1. %Cover validation experiment

- Does the transect set-up and sampling protocol adequately characterize the extent of benthic cyanobacteria in the section of concern?

2. Benthic HCB vs. NRSA periphyton composite sample acquisition experiment

- A NRSA reach set-up is overlayed and extended from the Benthic HCB reach to determine how the periphyton composite measures compare.

3. Disturbance sampling techniques adjustment and comparison experiment

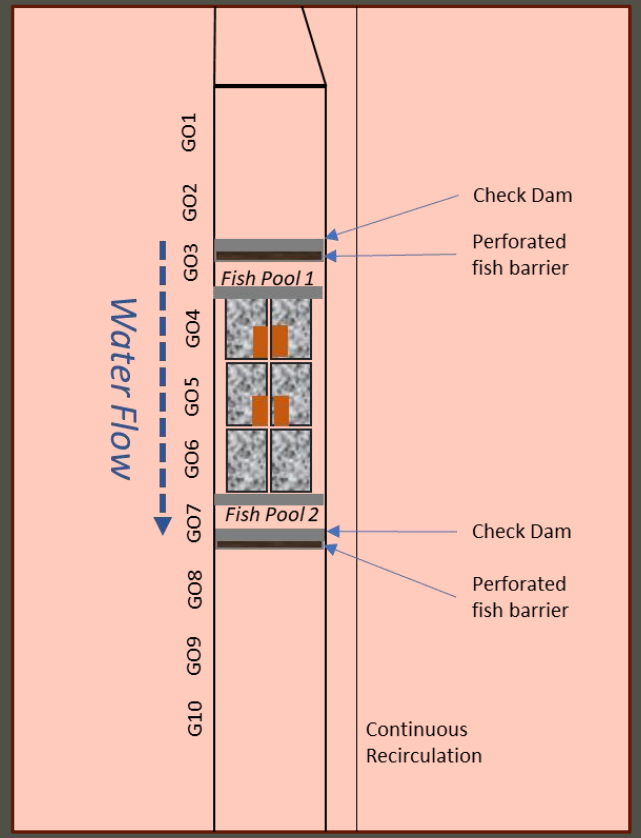
- Does adjusting the disturbance sampling technique so that measures of toxins in the bulk biomass disturbed as well as in a parcel of the disturbed water column prove relevant to the risk characterization?
- Conduct a “stomp-and-catch” disturbance sampling approach to the Hess/Surber sampling technique to determine if a standardizable approach is practical and more informative.

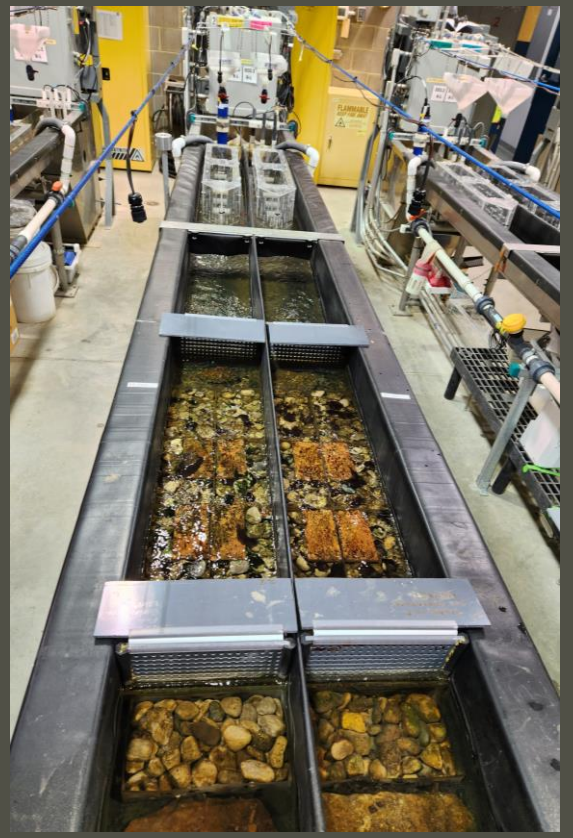
4. *Benthic HCB reach scale spatial variability experiments*

- How does spatial variability effect the risk characterization?
- Periphyton composite samples are processed separately, and additional disturbance samplings are scheduled.

5. Longer-term multiparameter sonde deployment (diel dissolved oxygen, pH, and temp)

- Teams with access to a multi-parameter sonde and that can perform a longer-term deployment (i.e., at least 7 d or over the entire period of SPATT deployment).





Experimental Stream Facility – 2023 Study – Benthic Cyanobacteria Mesocosm study

- *Controlled cyanobacteria dominance of periphyton for three strains of cyanobacteria*
- *Measurements to assess effects on stream insects and a native fish (central stoneroller)*

2023 Analytical Plan

	Periphyton I&E Microscopy by contractor (GLEC) FlowCam by Heath Mash	**Cyano microscopy** by Avery Tatters	**Metabarcoding** (using 16s and cyano-specific primers) by Erik Pilgrim	**qPCR** by Jingrang Lu	**Cyanotoxins - ELISA** anatoxin and saxitoxin by Marcie Tidd (R8) ; microcystin and cylindrospermopsin by Hilary Snook (R1)	**Toxins - LC/MS/MS** Toby Sanan focuses on periphyton[1] Heath Mash focuses on water and SPATTs	**Chlorophyll, Phycocyanin, Dry Wt/AFDM** by Laura Webb (R7)	**Nutrients, Chlorophyll, and Phycocyanin** -TKN, TP, NO2-3, DisP, & Anions by R10 Laboratory -Chl and Phyco by Laura Webb (R7)
Periphyton Transect Sampling Method *Obj: Estimate of relative abundance of cyanobacteria at the reach scale that can be compared to other reach-scale measures*								
Disturbance Sample **(test different sampling approaches)** *Obj: Evaluate exposure to benthic mats. Target worst case scenario identified when using viewing bucket. Normalized by area.*								
Surface Water Grab Samples (+ Additional water sample also collected during SPATT retrieval)								
SPATTs								

[1]Toby's analysis: LC-MS/MS for anatoxins (+ homo, dihydro), cylindrospermopsins (couple of congeners) + microcystins (typical suite of 14-15), using labeled toxins for isotope dilution

Samples Scheduled for 2023

Karen Odkins, California Department of Fish and Wildlife, Statewide HAB Coordinator, CaSouth Crew Member preparing for field sampling

Analysis	Matrix	Total # of Samples
TP/TKN and NO3+NO2	SW	28
Dissolved P	SW	35
Anions	SW	28
Toxins-LC/MS/MS	SPATTs	28
Toxins-LC/MS/MS	SW and Periphyton	245
ELISA_ATX-STX	SW and Periphyton	217
ELISA_MC-CYL	SW and Periphyton	217
Cyanobacteria Microscopy	Periphyton	189
FlowCam	SW and Periphyton	217
Periphyton AlgaeI&E	Periphyton	28
QPCR	SW and Periphyton	224
Metabarcoding	Periphyton	147
Dry Weight and AFDM	Periphyton	147
Chl-a	SW and Periphyton	224
Phycocyanin	SW and Periphyton	224
Total		***2198***

98% of samples scheduled were collected

✓ *Data delivered*

South Fork Eel River, near Phillipsville, CA: 17Oct2024

CANorth Crew: Rich Fadness/Mike Thomas, CA Water Board

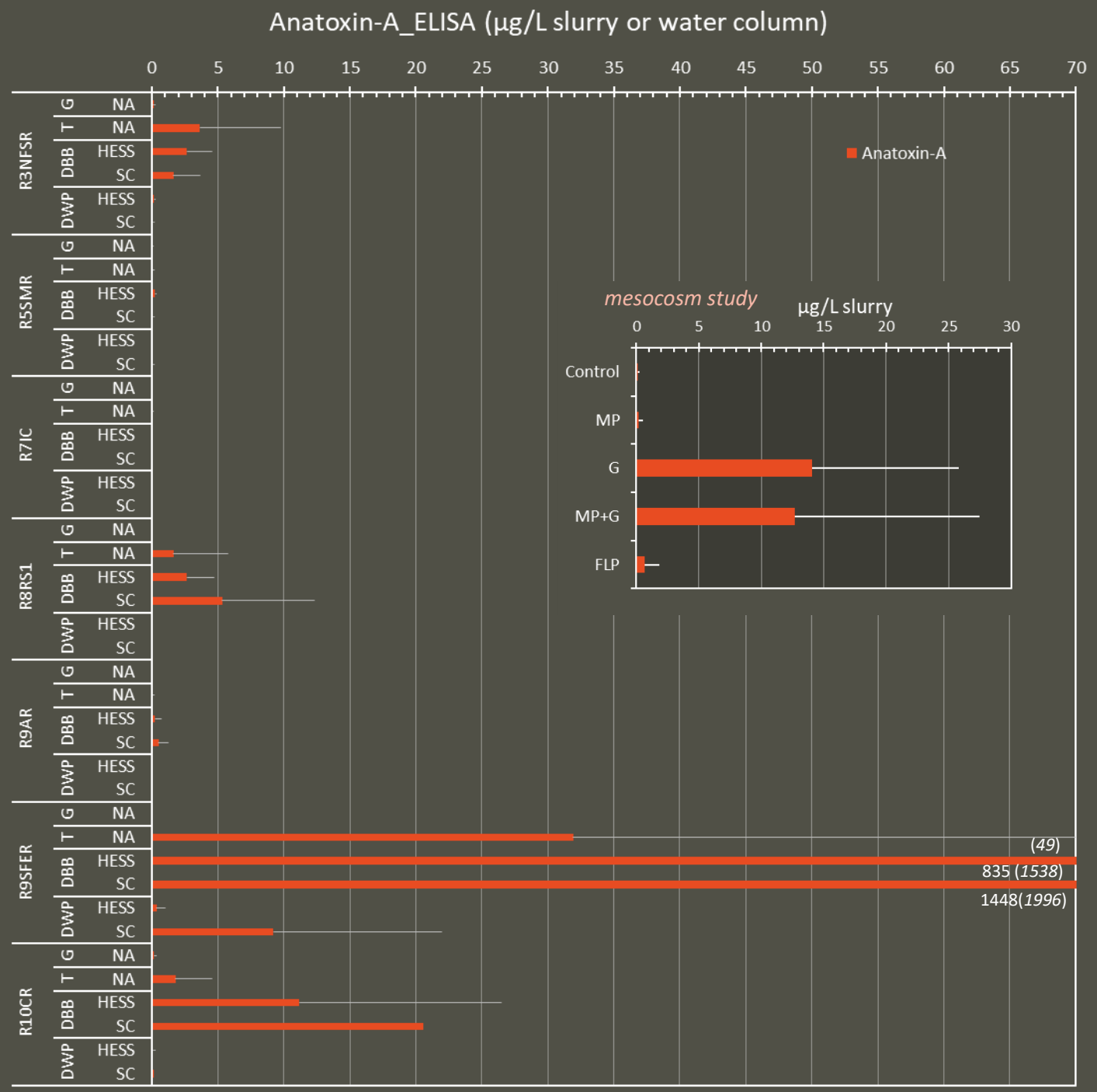
T1.1-SP2: NoAlgae(25%), Cyano(75%)

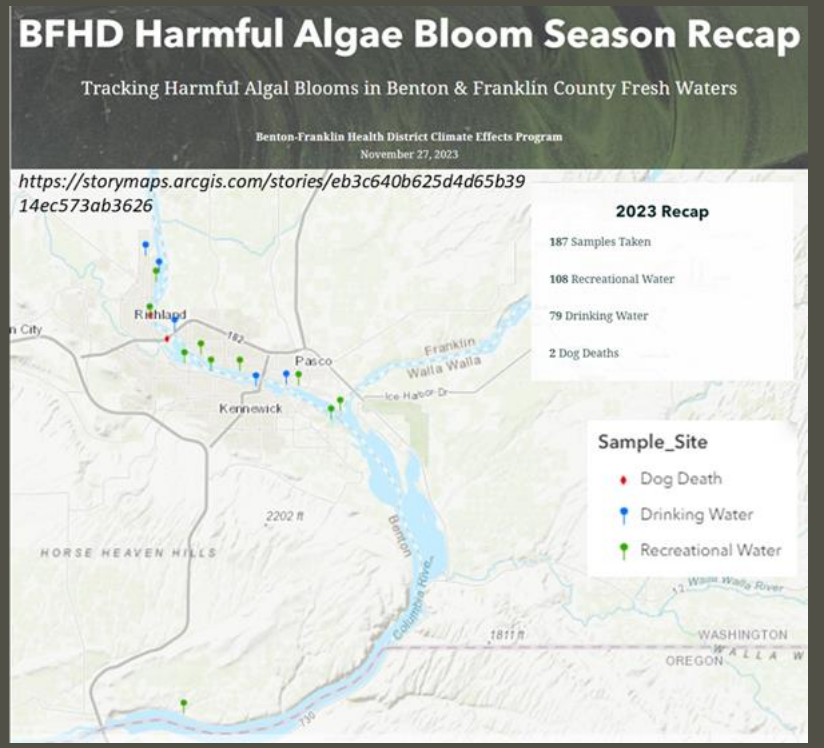
T2.1-SP2: NoAlgae(70%), Cyano(10%), GreenFil(20%)

Preliminary Anatoxin A results

Field Site Sample Types
G= Surface water grab
T= Periphyton transect (composite)
DBB= Disturbed benthic biomass
DWP= Disturbed water parcel
HESS= Sampled with Hess sampler
SC = Sampled with stomp and catch

Mesocosm Study Treatments (i.e., cultured strains of cyanobacteria isolates)
Control = No cyano strain addition
MP = *Microcoleus/Phormidium* strain
G = *Geitlerinema* strain
MP+G = Both strains added
FLP = Florida *Phormidium* strain





Benthic HCB ROAR in the news - 2023

Primary methods/guidelines reviewed for 2023 sampling procedures

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- Wood, S. A., L. T. Kelly, K. Bouma-Gregson, J.-F. Humbert, H. D. Laughing house IV, J. Lazorchak, T. G. McAllister, A. McQueen, K. Pokrzywinski, J. Puddick, C. Quiblier, L. A. Reitz, K. G. Ryan, Y. Vadeboncoeur, A. Zastepa, and T. W. Davis. 2020. Toxic benthic freshwater cyanobacterial proliferations: Challenges and solutions for enhancing knowledge and improving monitoring and mitigation. Freshwater Biology 65:1824-1842.

Effect of culture conditions on growth and toxin production of *Microcoleus* species (Cyanobacteria) isolated from streams in California

Rosalina Stancheva[1], Abeer Sohrab[2], Sydney M. Brown[1]

[1]Department of Environmental Science and Policy, George Mason University, Fairfax, VA

[2]Department of Civil and Environmental Engineering, University of Utah, Salt Lake City, UT

E-mail: *rchris13@gmu.edu* (Corresponding author R. Stancheva Christova)

US EPA Benthic HABs Discussion Group | March 26, 2024

Road Map

Research Project: URoL:EN: Understanding the rule of life facilitating the proliferation of toxic cyanobacterial benthic mats in flowing freshwaters

Research team:

Ramesh Goel (PI) and Harry Sundar (Co-PI) – University of Utah, Salt Lake City, UT
Joanna Blaszczak (Co-PI) and Robert Shriver (Co-PI) – University of Nevada, Reno, NV
Rosalina Stancheva Christova (Co-PI) – George Mason University, Fairfax, VA

- Part 1: *Microcoleus* monoclonal strains from streams in the Western US
  - Dr. Rosalina Stancheva
- Part 2: Molecular taxonomy of *Microcoleus* from streams in California
  - Abeer Sohrab (Ph.D. student at the University of Utah)
- Part 3: Establishing baseline *Microcoleus* life histories
  - Sydney Brown (Ph.D. student at George Mason University)

Introduction

- *Microcoleus* (Oscillatoriales) is a benthic mat-forming cyanobacterium
- Some species produce neurotoxins
  - Anatoxin-a (ATX)
  - Dihydroanatoxin-a (dhATX)
  - Homoanatoxin-a
  - Dihydrohomoanatoxin-a
- Common in streams
- Dog kills

Microcoleus from Zion National Park, 2023

The Mysterious Dog-Killing Bacteria Plaguing a Popular National Park 2022

END TIMES

A few weeks ago, Zion National Park was forced to warn visitors against the Narrows due to a toxic bacteria spreading through the park's waterways.

Daniel Modlin | Published Nov. 25, 2022 4:48AM EST

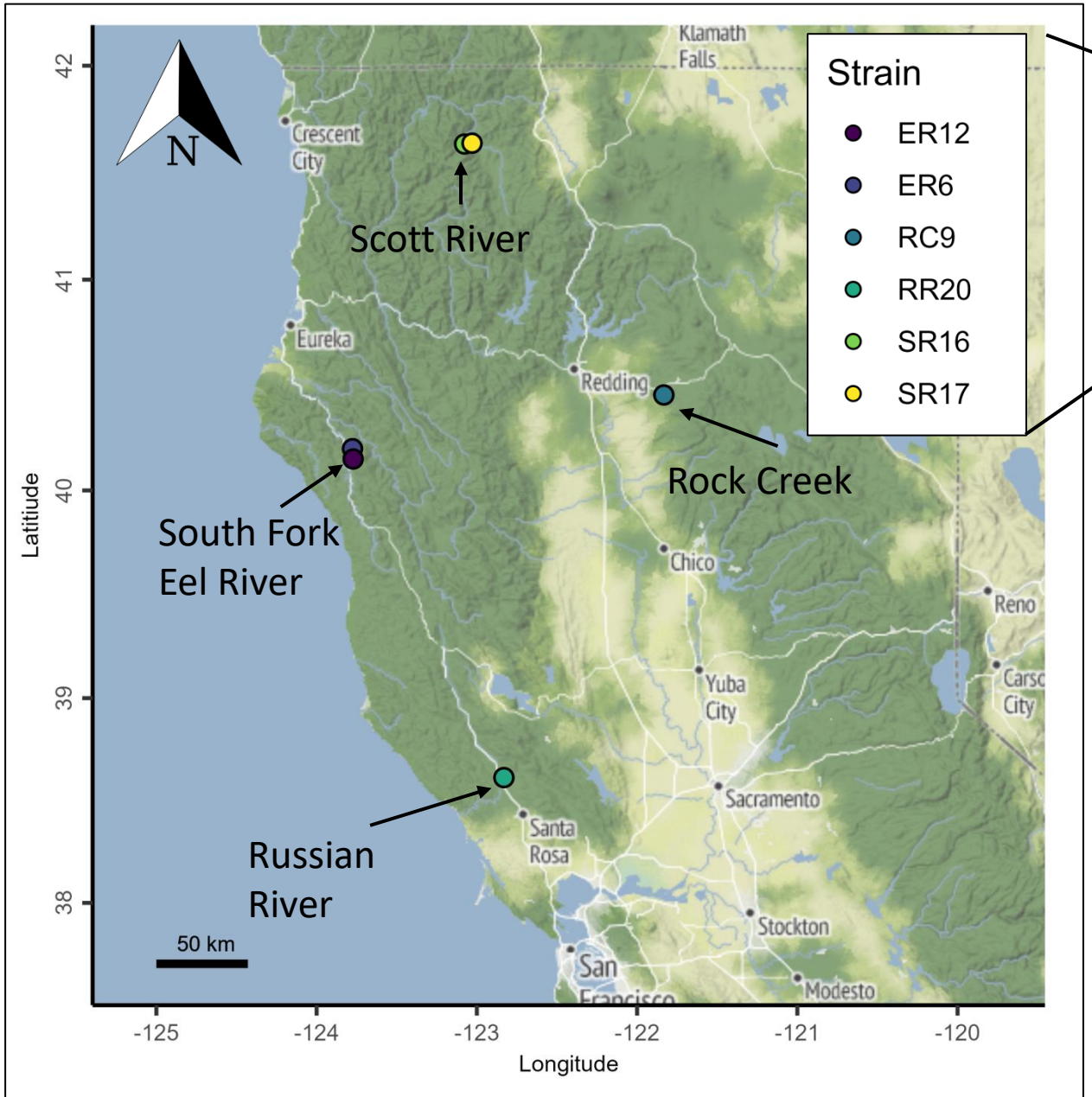
https://www.thedailybeast.com

Dog dies on Russian River, tests positive for toxic algae 2015

The test results are preliminary. Sonoma County public health officials are deciding what to do next, including whether to urge people and their pets to avoid the Russian River.

https://www.pressdemocrat.com

Study Area: Northern California



Sampling sites, dates and strains

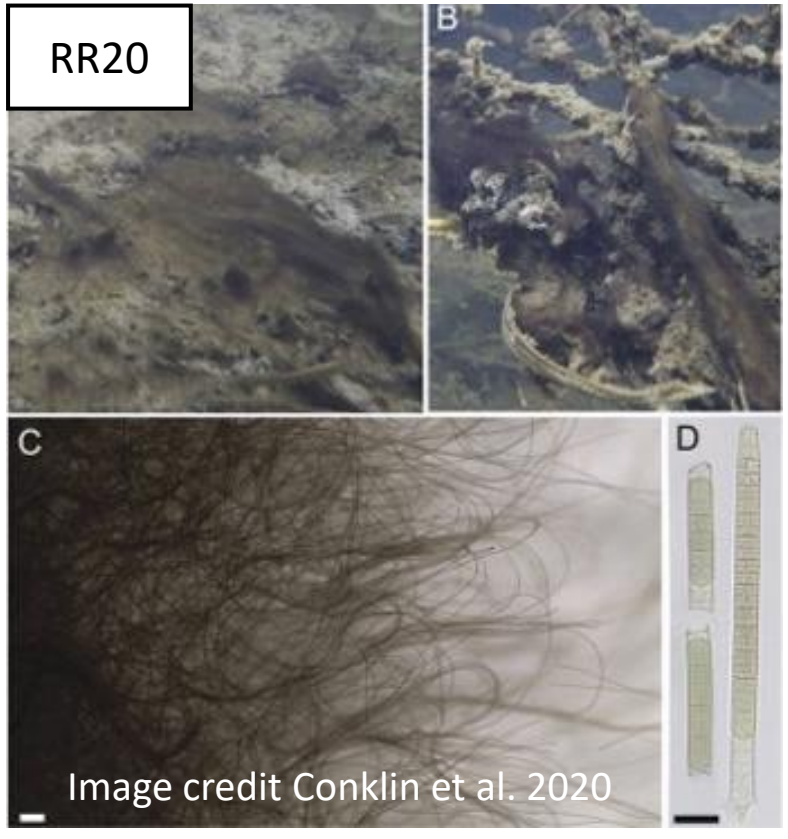
- Russian River (10.1.15) – **RR20 (PTRS1)**: dhATX
  - *M. anatoxicus* Stancheva & Conklin (PTRS1)
- Rock Creek (10.7.20) – **RC9**: dhATX
- South Fork Eel River (8.23.22)
  - **ER6, ER12**: non-toxic
- Klamath River Watershed (9.20.22)
  - Indian Scotty (**SR16**)
  - Scott River Jones Beach (**SR17**)
  - ATX+dhATX

Monoclonal *Microcoleus* Strains

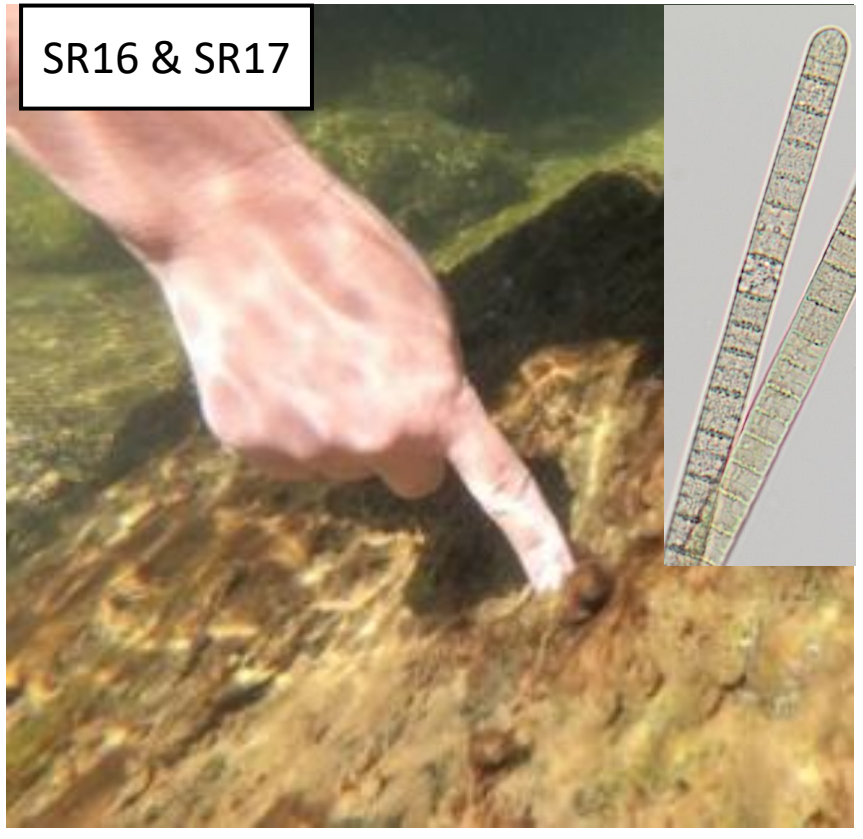
Stream	pH	Conductivity (µS/cm)	Ortho-P (mg/L)	Nitrate (mg/L)	Toxins	N_2-fixers (algae)	Strains
Eel River	8.6-8.9	236.8-248.4	0.0038-0.0042	0.015-0.023	None	Present	ER6 & ER12
Rock Creek	7.1-8.4	80.7-96.5	0.0051-0.046	0.05-0.06	dhATX	Absent	RC9
Russian River	7.96-8.07	209-238	0.031-0.071	0.023-0.13	dhATX & ATX*	Present	RR20 (PTRS1)
BG11	7.2	2200	5.1	245	NA	NA	NA

ER6 & ER12

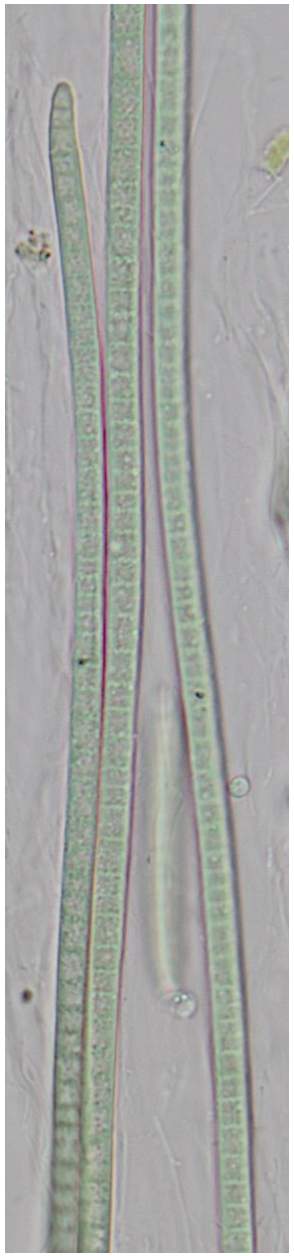
RR20



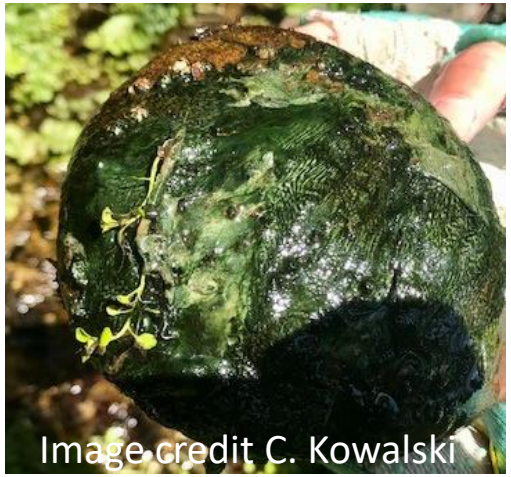
SR16 & SR17



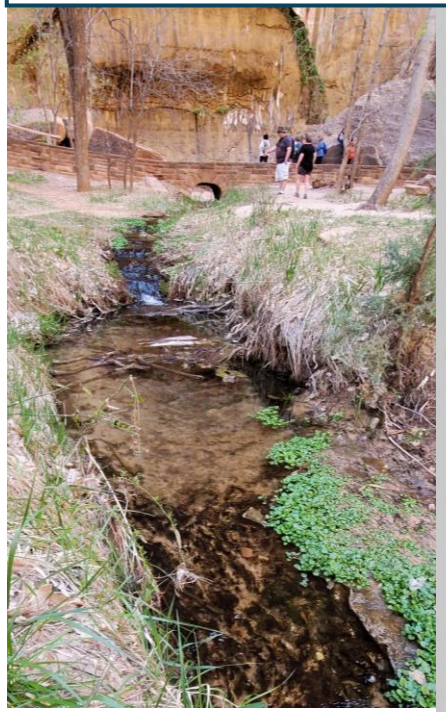
Study Area: Southern California and Utah

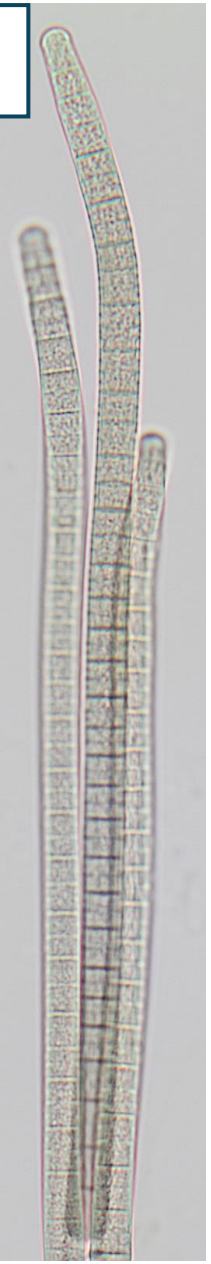


SC15



Zion National Park



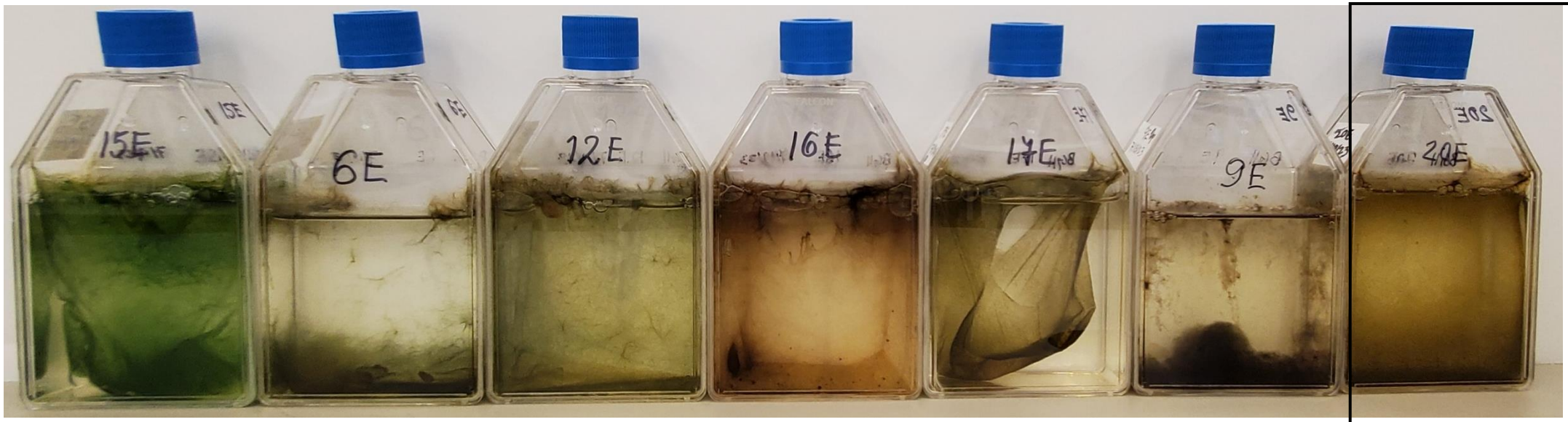


Sampling sites, dates and strains

- Pine Creek, southern CA (6.14.21)
  - Strain **SC15 -** non toxic
- North Fork Virgin River tributary crossing Riverside walk (4.27.23)
- Zion National Park, Utah
- ATX and dhATX

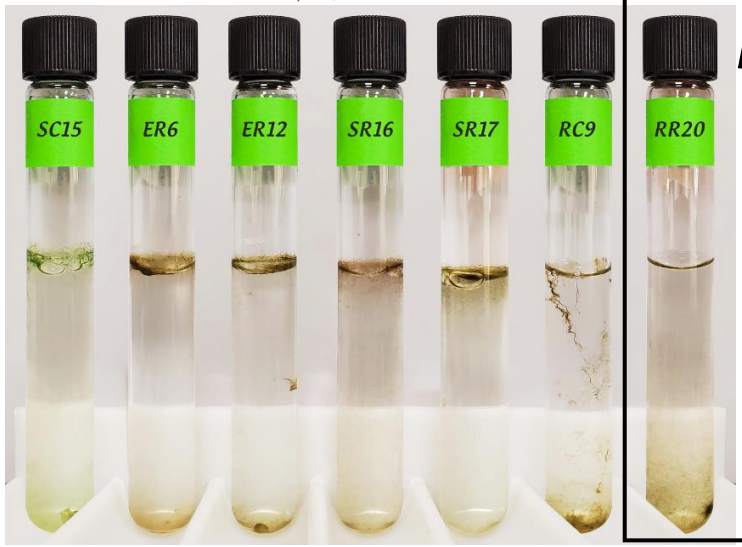
Monoclonal *Microcoleus* Strains

- *Microcoleus* strains grown in 200 mL BG11 for 6 months (January 2024)



- *Microcoleus* strains grown in 20 mL BG11 for 40 days (April 2023)

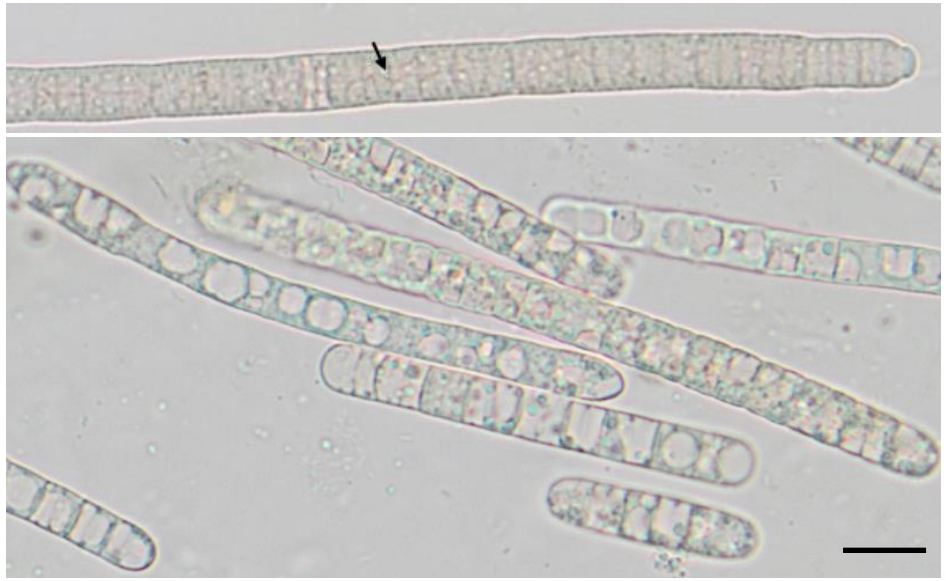
- Differences in growth pattern, mat color and characteristics



M. anatoxicus type strain PTRS1

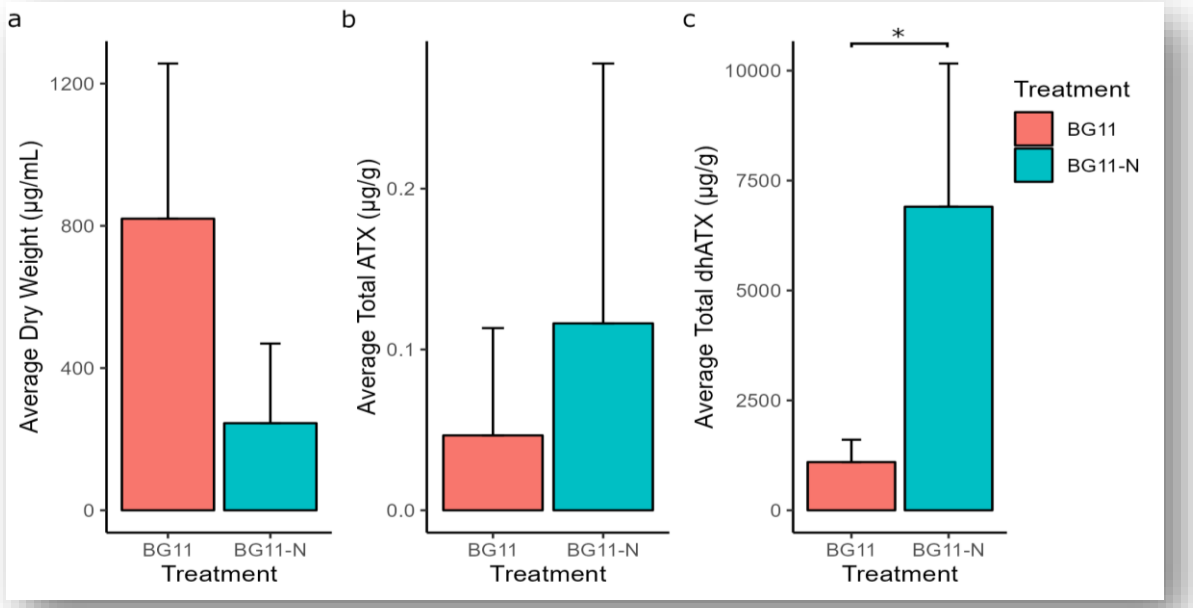
M. anatoxicus PTRS2 Nitrogen Depletion Tolerance

- **Nutrient depletion experiment**
  - PTRS2, June 2019
  - BG11 and BG11-N for 40 days
  - Dry weight, ATX and dhATX at day 40
- **Growth conditions**
  - 125 ml Pyrex® glass Erlenmeyer flasks
  - 21°C, light irradiance 100 µmoles·m^{-2}·s^{-1}
  - 12:12 hr light/dark cycle
- **Results**
  - Reduced cell growth
  - Increased production of ATX and dhATX
  - Cells were keratomized, without storage granules
  - Heath et al. 2014 reported similar response of *M. autumnalis* to N reduction

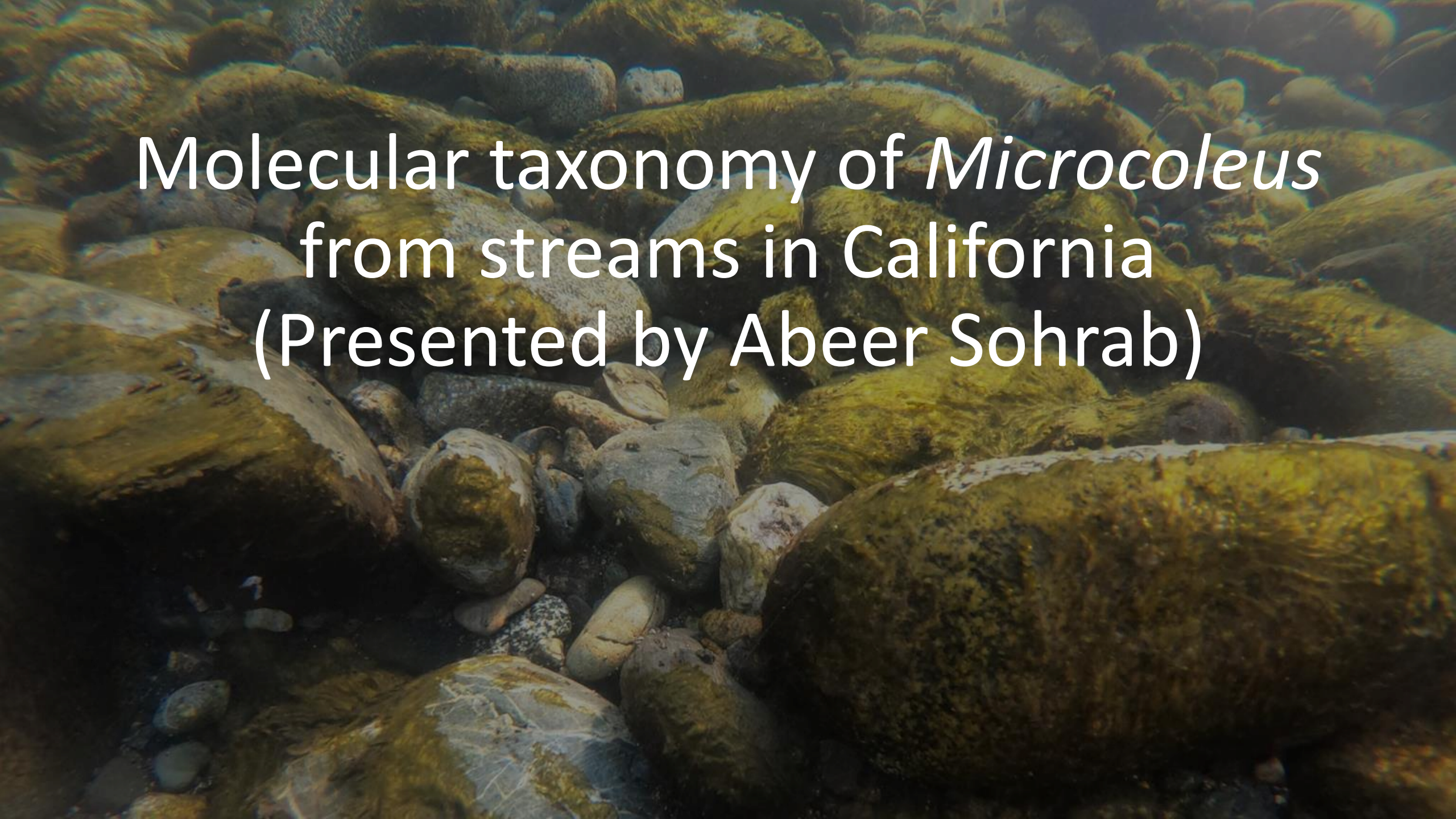


BG11 control

BG11-N



Bars represent average value ± 95% confidence interval



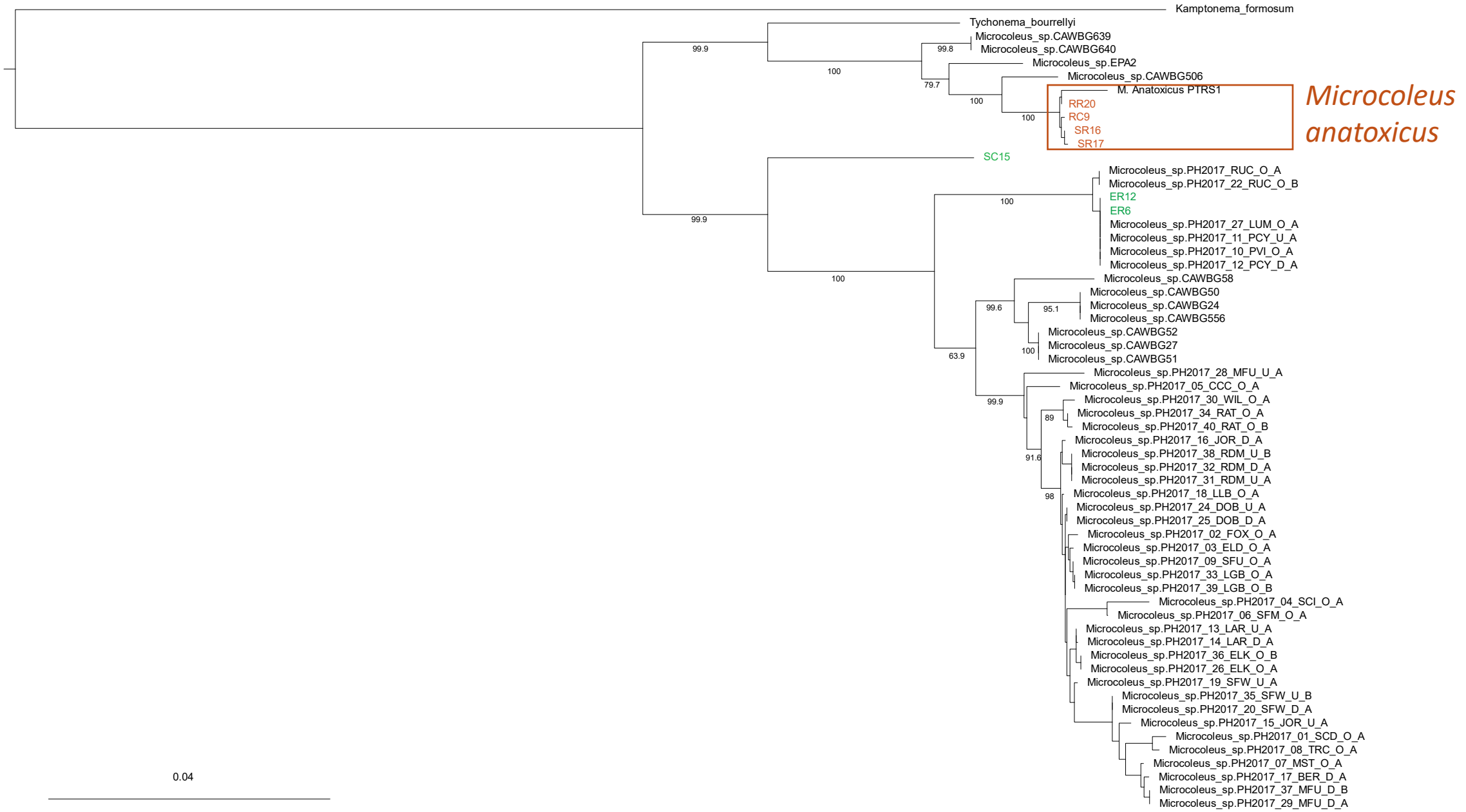
Molecular taxonomy of *Microcoleus* from streams in California (Presented by Abeer Sohrab)

Molecular Methods

- DNA was extracted from 7 *Microcoleus* strains described above by the QIAGEN All Prep DNA/RNA Kit
- Single *Microcoleus* MAGs of completeness > 95% and contamination < 5% were successfully formed from each sample
- **Phylogeny Tree**: Maximum-likelihood trees with branch supports were constructed based on **concatenated alignments of 120 single-copy core marker genes** obtained from GTDB-Tk v0.2.1
- Trees were built using the ultrafast bootstrap approximation in IQ-TREE v1.6.9. and visualized using FigTree Version - v1.4.4
- The tree was rooted at midpoint with bootstrapping values greater that 50% shown

GENOMES	COMPLETENESS	CONTAMINATION
R6	97.96	0.23
R9	95.22	0.66
R12	97.85	0.23
R15	98.18	0.00
R16	96.64	0.22
R17	95.48	0.22
R20	96.69	0.00

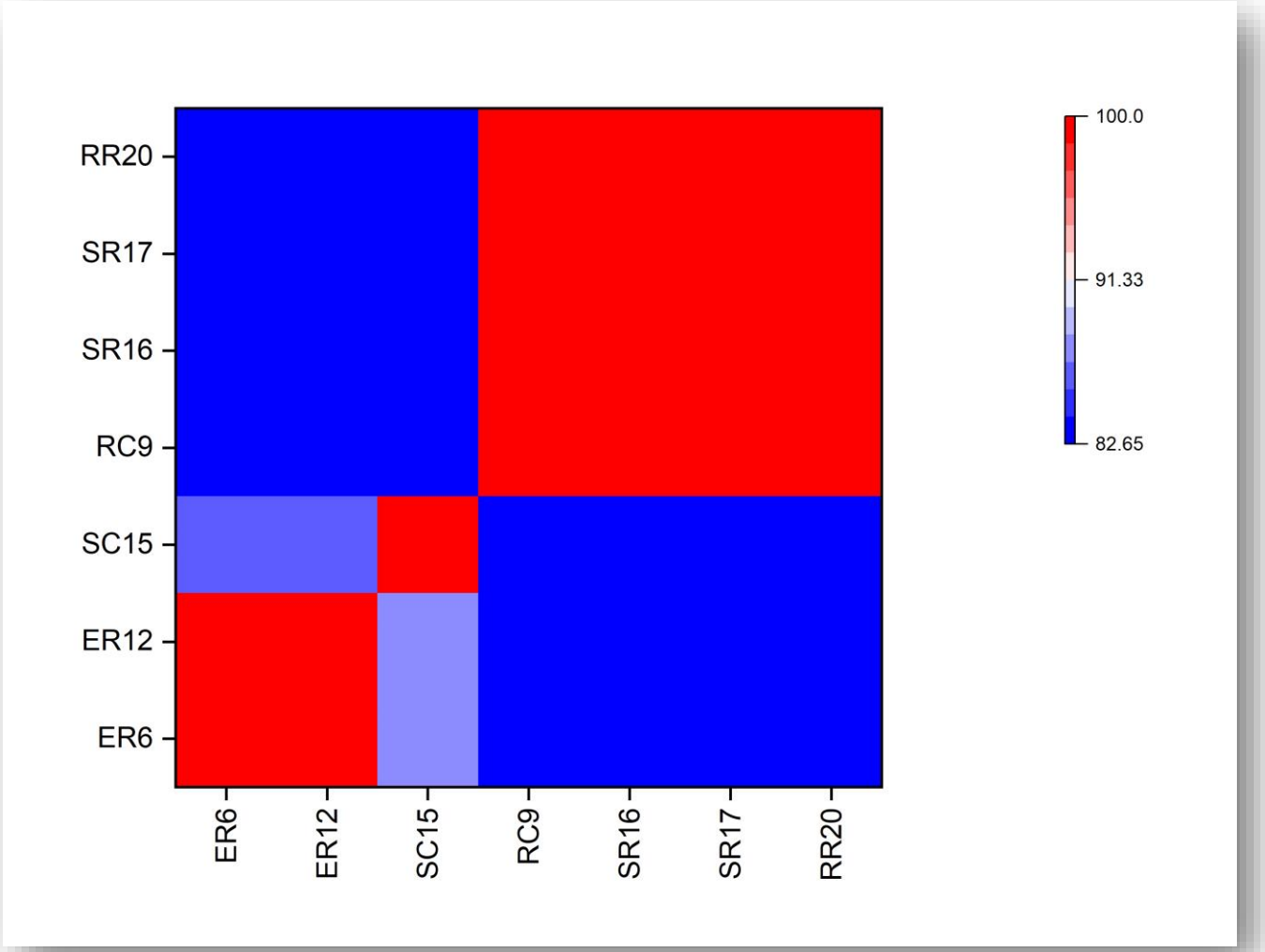
Microcoleus Phylogeny



Microcoleus Genome Comparison

Comparison of genomes based on average nucleotide identity (ANI)

- Non-toxic strains ER12 and ER6 are classified into the same cluster of non-toxic species as described by Tee et al. (2021)
- Toxic strains RC9, SR16, SR17, and RR20 (*M. anatoxicus* type strain PTRS1) group together in the cluster associated with toxic *Microcoleus* species
- Non-toxic strain SC15 from southern California is distinctive, forming its own cluster, as indicated in the heat map



Heat map showing ANI similarity for all 7 *Microcoleus* genomes.

Establishing baseline *Microcoleus* life histories (Presented by Sydney Brown)

Research collaborators/coauthors: Jordan Zabrecky[2] , Joanna Blaszczak[2], R. Christian Jones[1], Abeer Sohrab[3], Gregory L. Boyer[4], Bofan Wei[4], Laurel Genzoli[5], Kalina M. Manoylov[6], T. Reid Nelson[1], Robert Shriver[2], Ramesh Goel[3], Rosalina Stancheva[1]

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[4]Department of Chemistry, State University of New York College of Environmental Science and Forestry, Syracuse, NY

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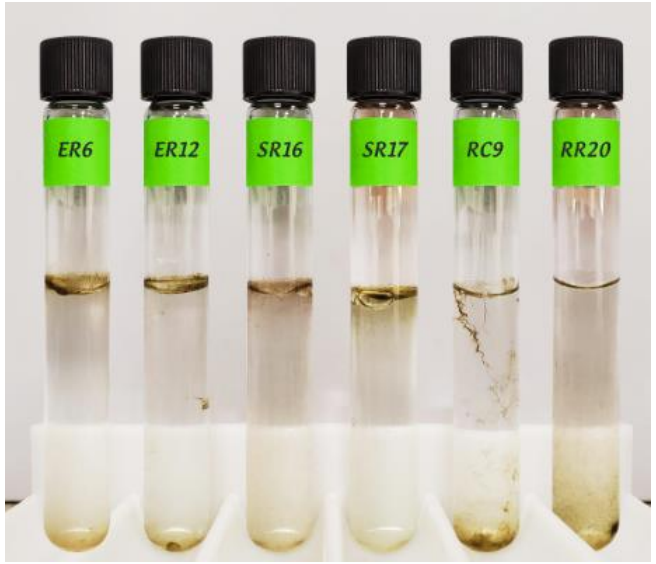
[7]Department of Biological Sciences, California State University San Marcos, San Marcos, CA

Experiment Hypothesis

Study goals

- Characterize growth rates and toxin production of *Microcoleus* strains with varying toxicity



Hypotheses

1. Non-toxic strains will grow better than toxic strains under laboratory conditions (previously demonstrated by Heath et al. 2016)
2. Peak toxin-production will have negative effect on cell growth (analyzed by specific growth rate)

Experiment Setup

- Unialgal monoclonal non-axenic strains maintained in BG 11
- Cultures inoculated with 1 mL stock solution with filaments and incubated for 49 days starting
  - 20 mL liquid BG11 in 30 mL tubes (UTEX; Austin, TX)
  - Temperature: 21°C
  - Light irradiance: 100 µmoles* m^{-2} s^{-1}
  - 12:12 hr light/dark cycle

Biomass Analyses

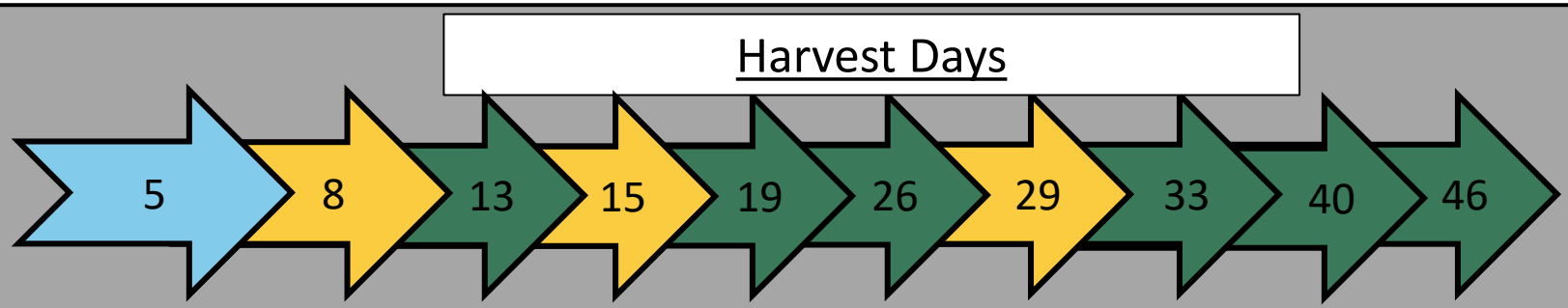
Chlorophyll *a*
72 samples (12 per strain)
Grown in duplicates

Cell density & biovolume
165 samples (~28 per strain)
Grown in triplicates

Dry weight
56 samples (14 per toxic strain)
Grown in duplicates

ATX & dhATX Analyses

56 samples (14 per toxic strain)
Grown in duplicates



Cell density/biovolume & toxins

Cell density/biovolume, chl *a*, & toxins

Cell density/biovolume only

Experiment Methods

Biomass Analyses

Cell density & Biovolume

- Cell density calculated per unit of volume using modified APHA 1992 methods
- Cell density converted to biovolume (better correlation with other biomass measures)
  (Hillebrand et al. 1999)

Chlorophyll *a*

- Samples filtered, frozen, and measured fluorometrically (Parsons et al. 1984, Wetzel et al. 1991)

Dry Weight

- Samples filtered, freeze dried, and weighed

ATX and dhATX Analyses

- LCMS/MS

Intracellular: Analyzed toxins in cells on filter

Extracellular: Analyzed toxins in filtrate

Statistical Analyses

Growth curves: Calculated via R: A language and environment for statistical computing

(R Core Team 2024)

- During exponential phase (up to day 29), slopes (growth rates) were calculated using GLM gamma family with log link
  - Confirmed best fit by AIC
- Tukey corrected pair-wise comparisons run on contrasts
  - 'emtrends' package (Lenth 2024)

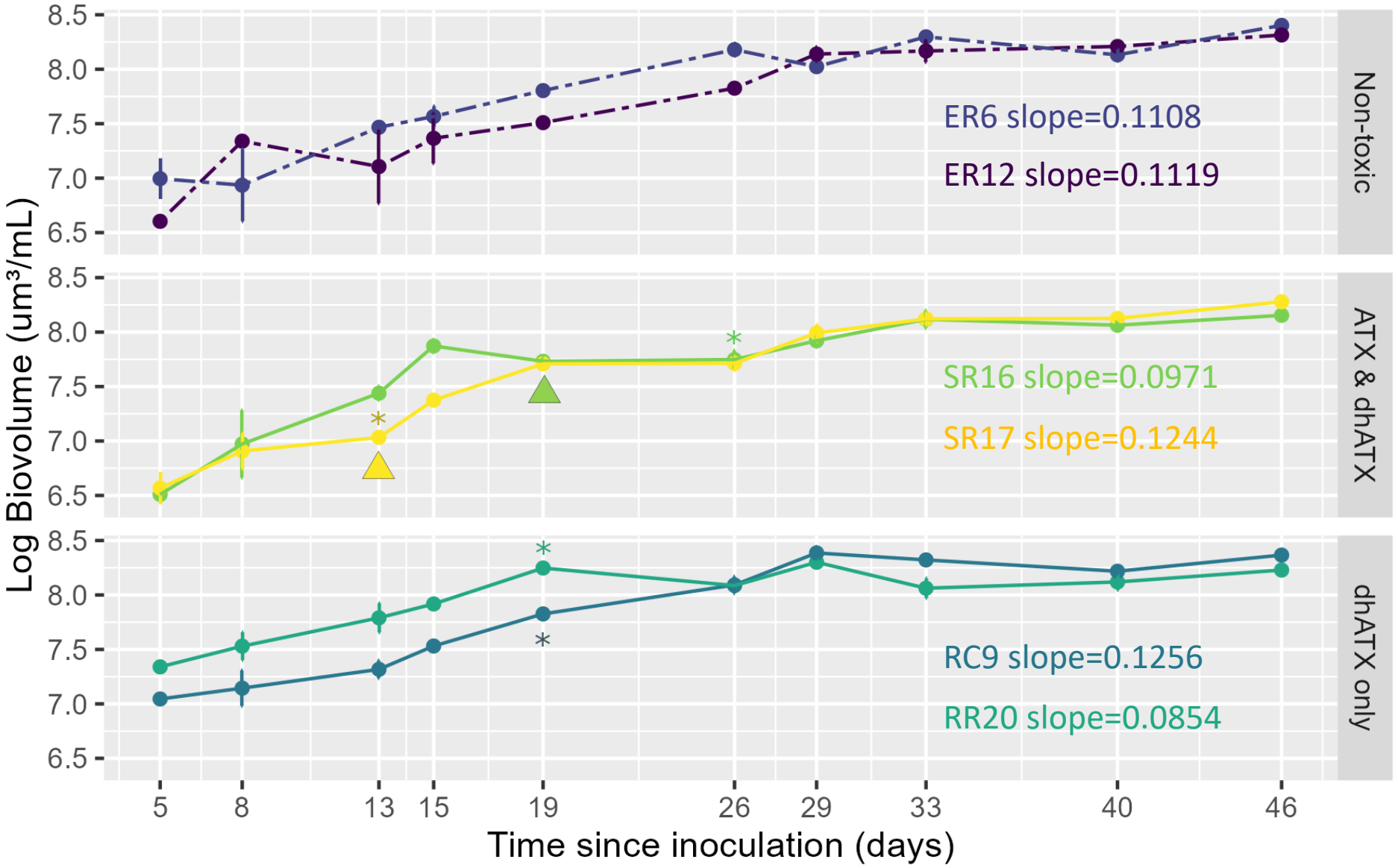
Statistical Analyses

Specific Growth Rate

$$\mu = \frac{ln(X_n) - ln(X_{n-1})}{T_n - T_{n-1}}$$

Growth Curves - Biovolume

- During the exponential phase, there is no difference in growth rates across all strains



t-ratio ranging from
-2.04 to 2.10
p>0.05 for all

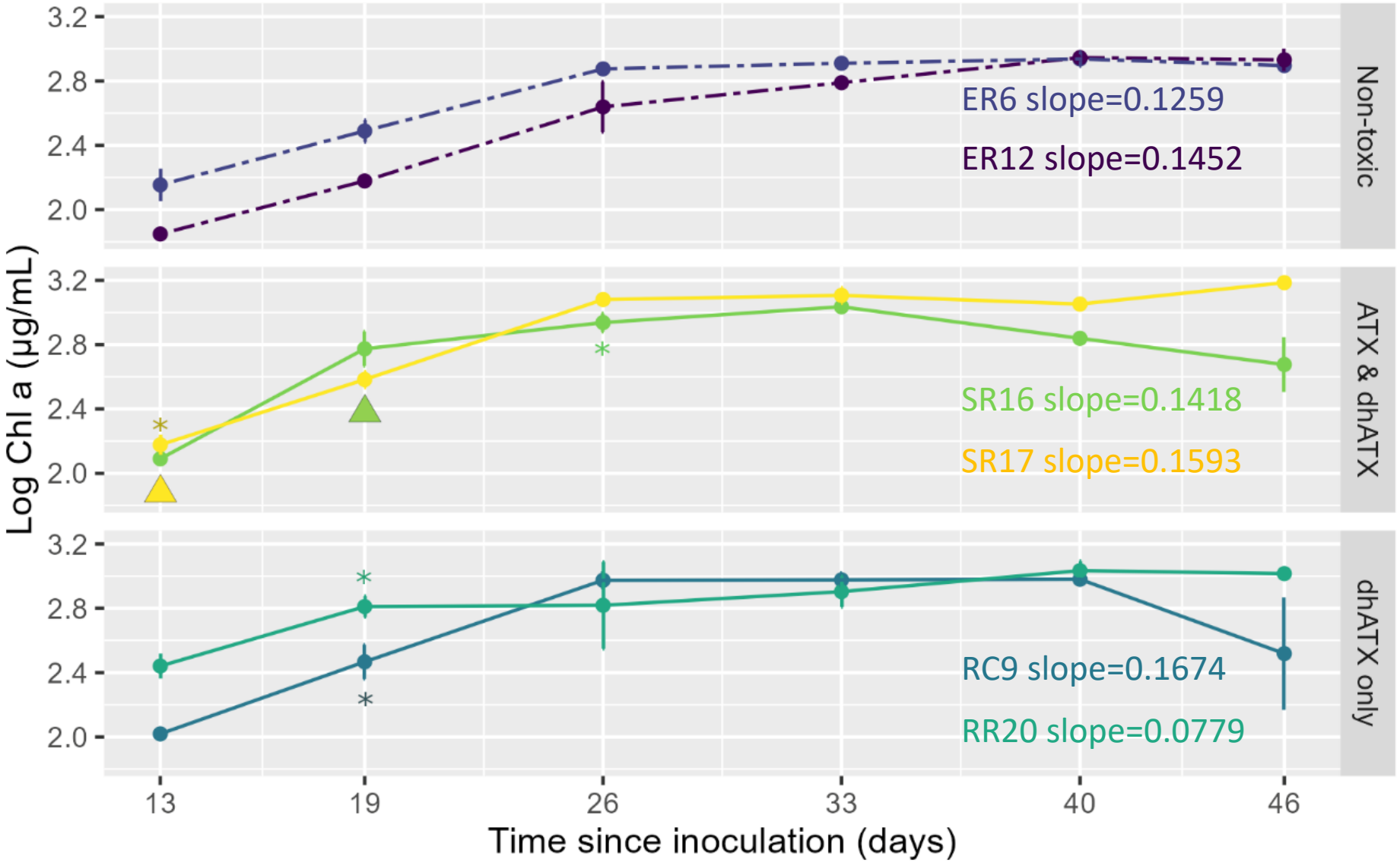
▲ peak ATX production

* peak dhATX production

Each point represents average value ± 95% confidence interval

Growth Curves – Chlorophyll *a*

- During the exponential phase, there is no difference in growth rates across all strains



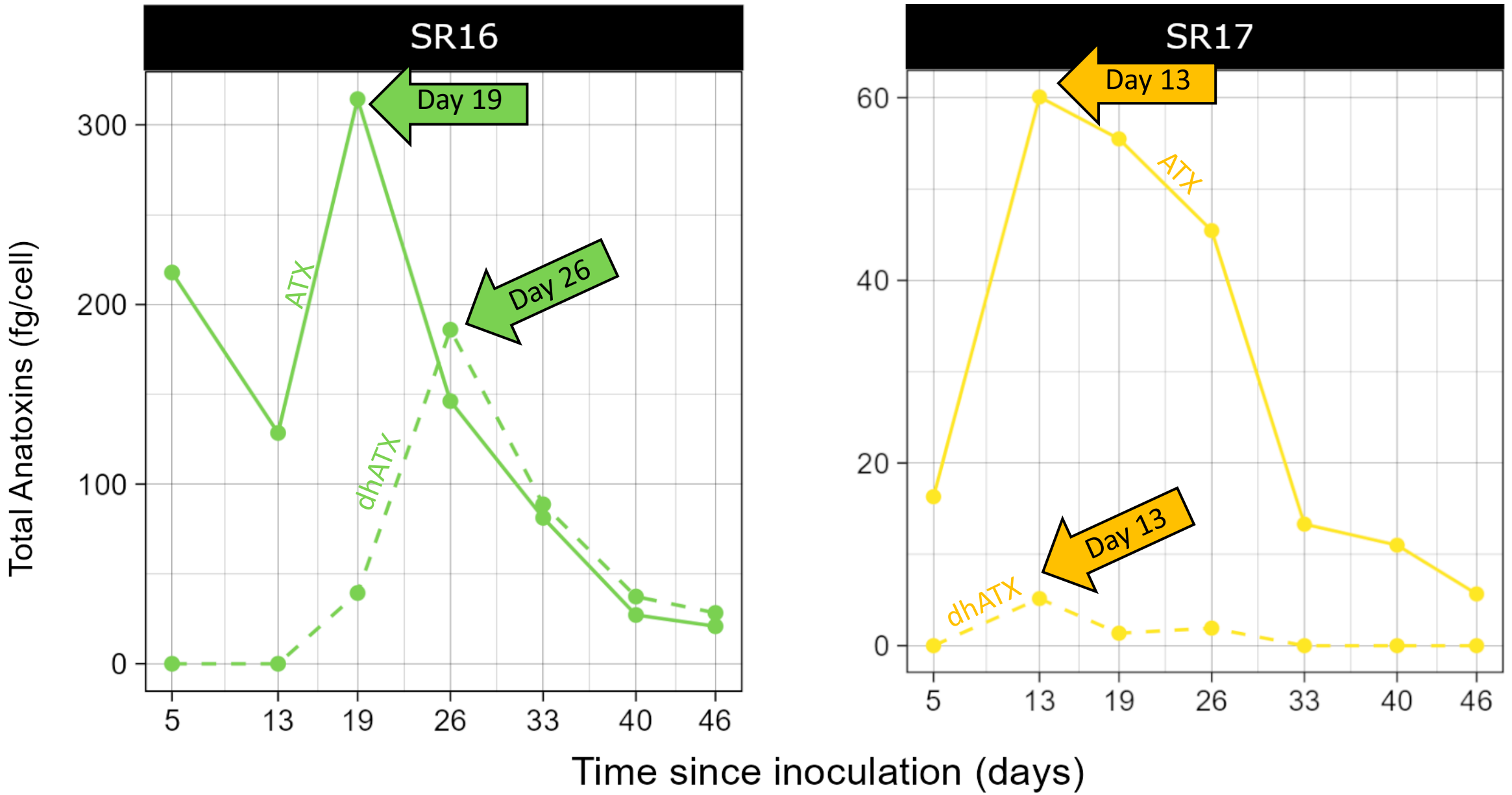
t-ratio ranging from -2.245 to 2.467
p>0.05 for all

▲ peak ATX production

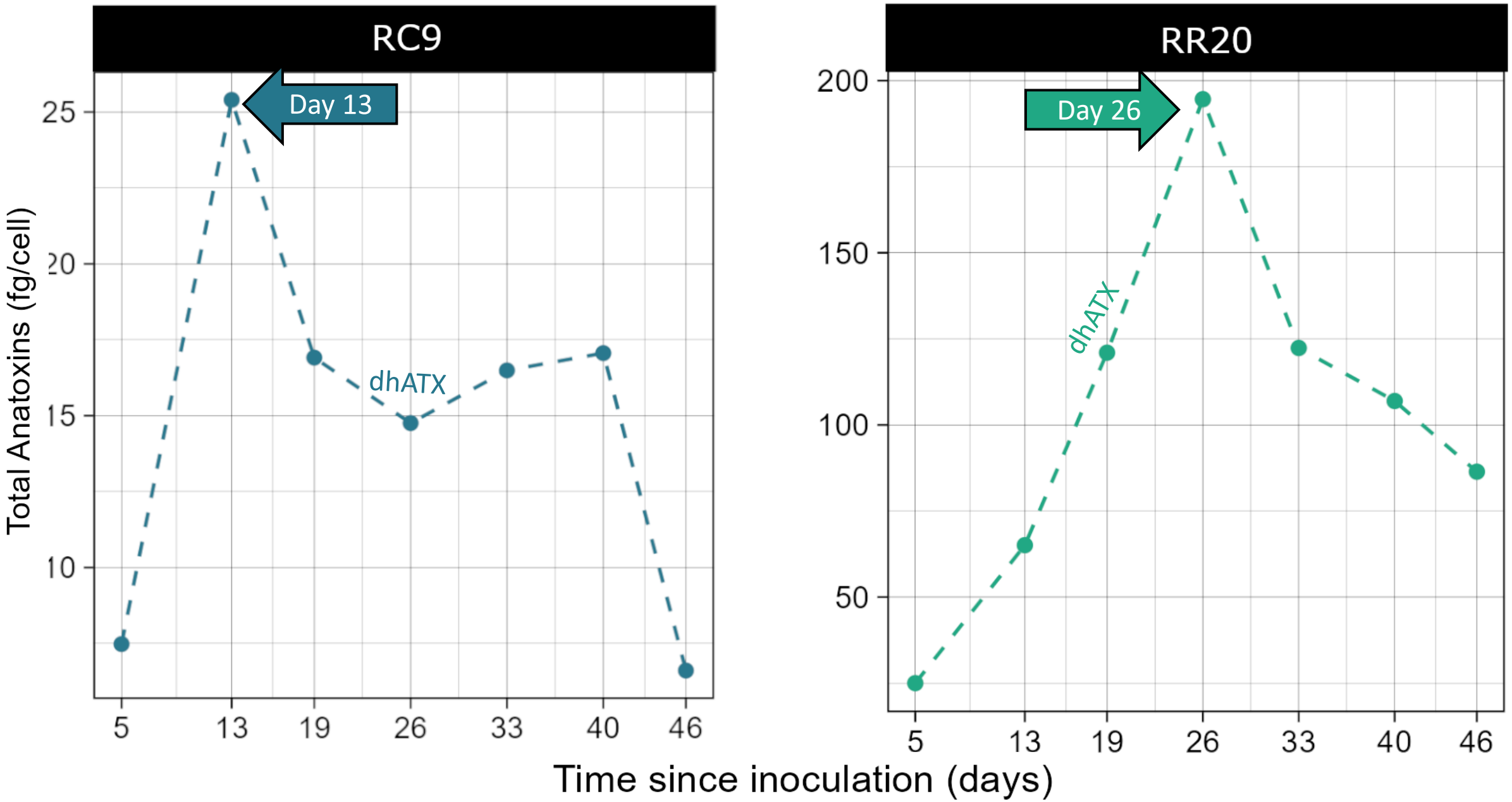
* peak dhATX production

Each point represents average value ± 95% confidence interval

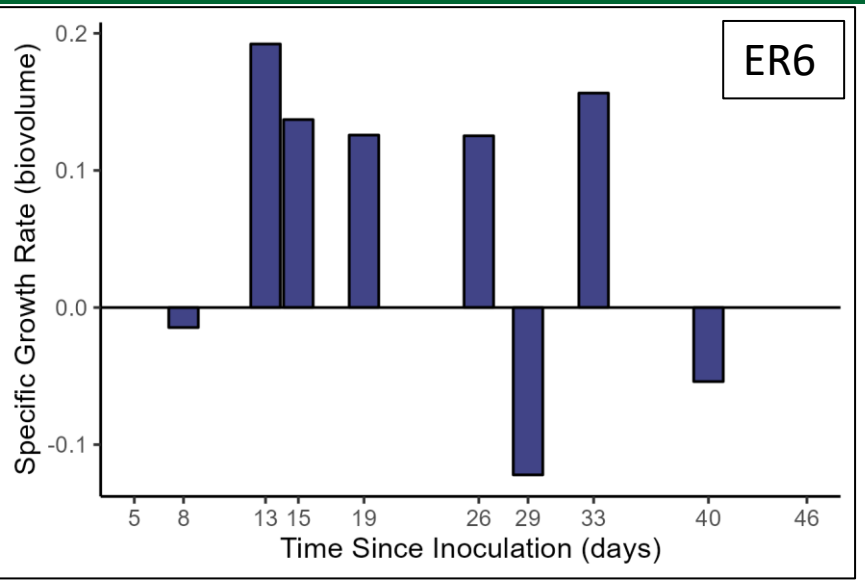
ATX and dhATX Production

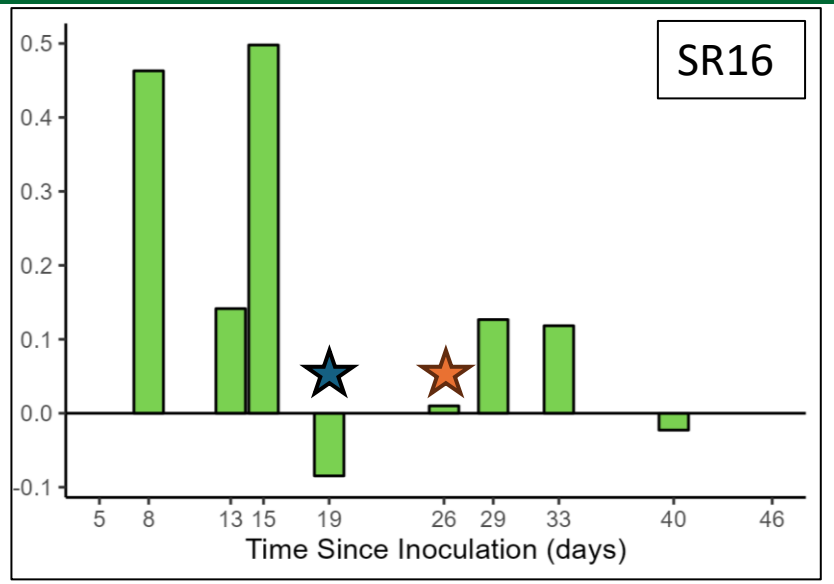


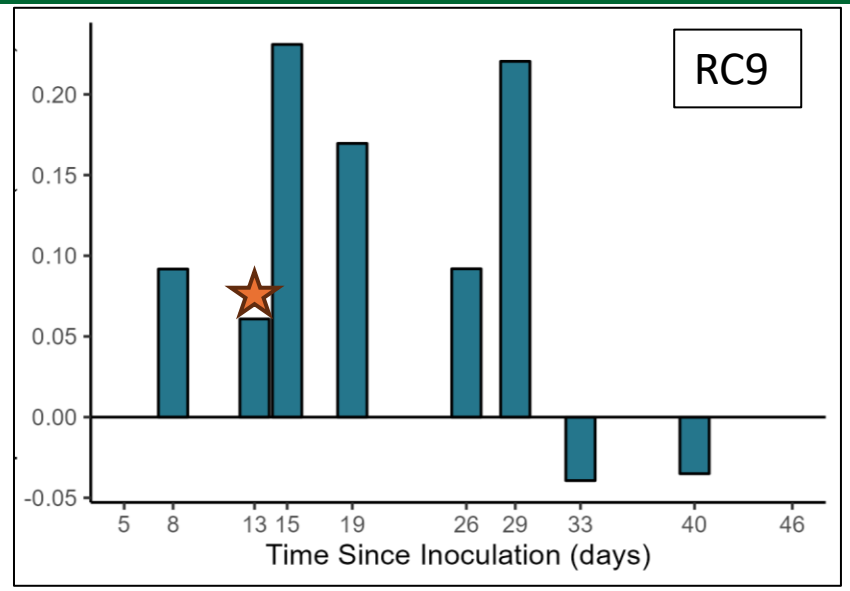
dhATX Production



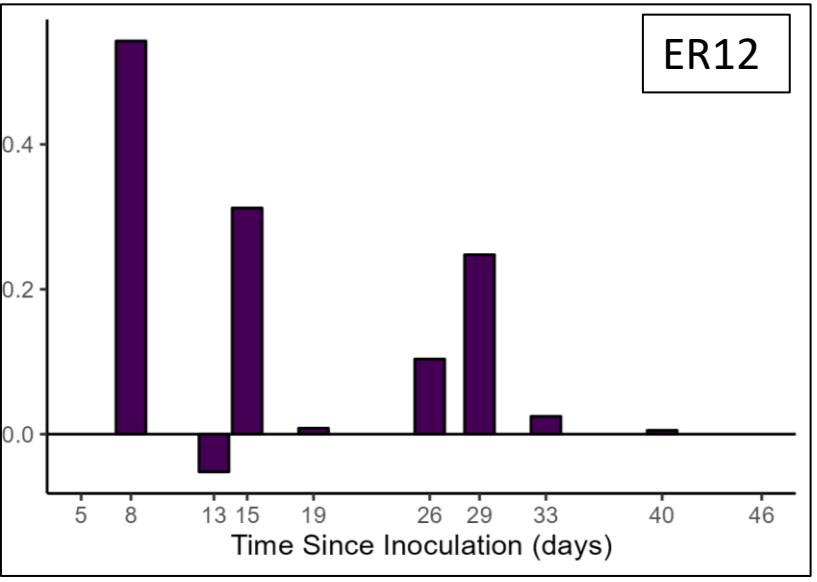
Specific Growth Rates

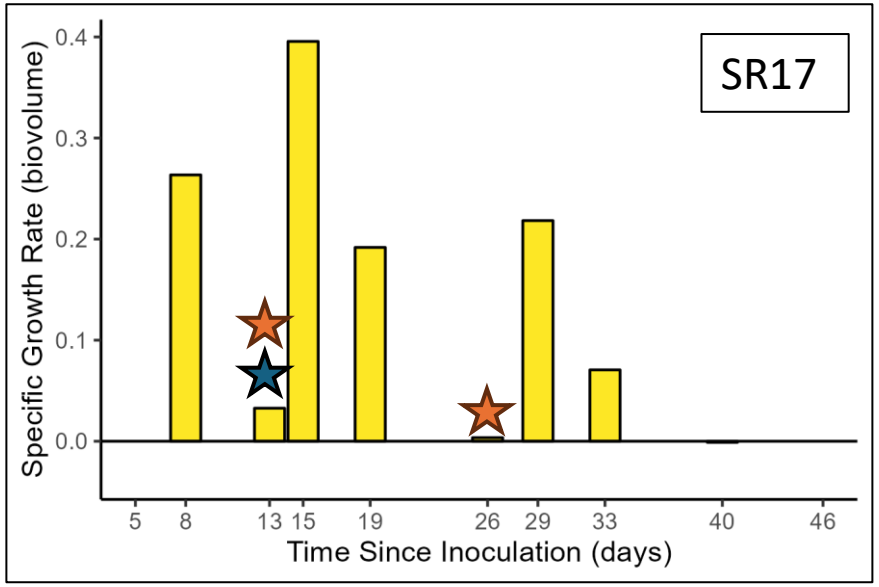


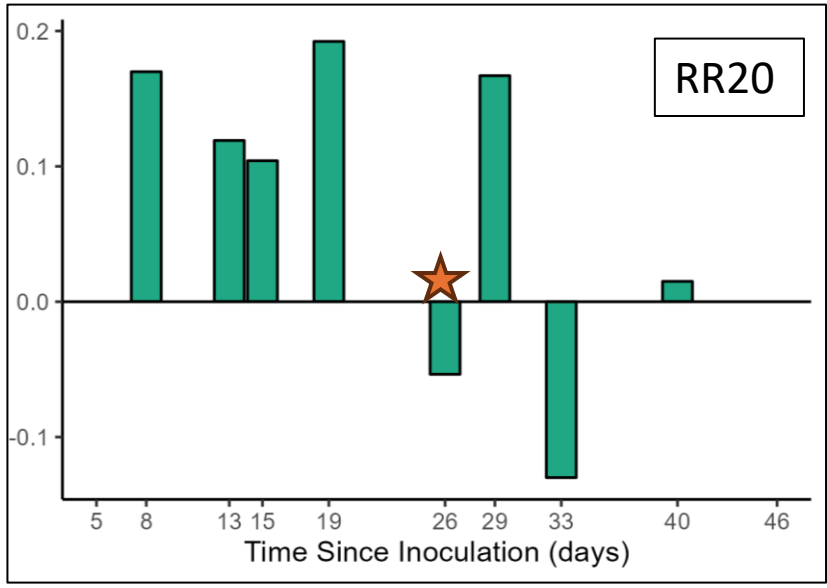




Legend: peak dhATX peak ATX

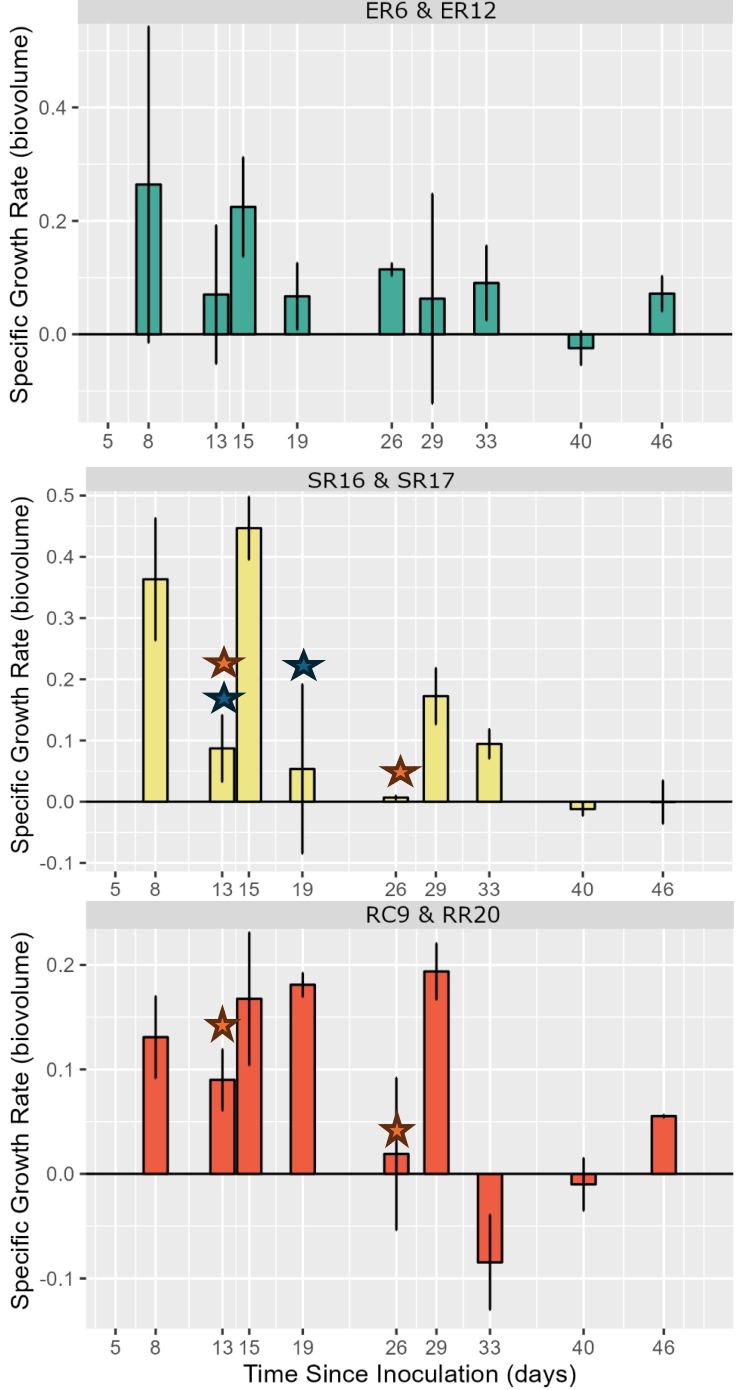






Specific Growth Rates

- Non-toxic strains had a more or less steady decrease in specific growth rate over time
- Peak ATX & dhATX production corresponds with reduced specific growth rates

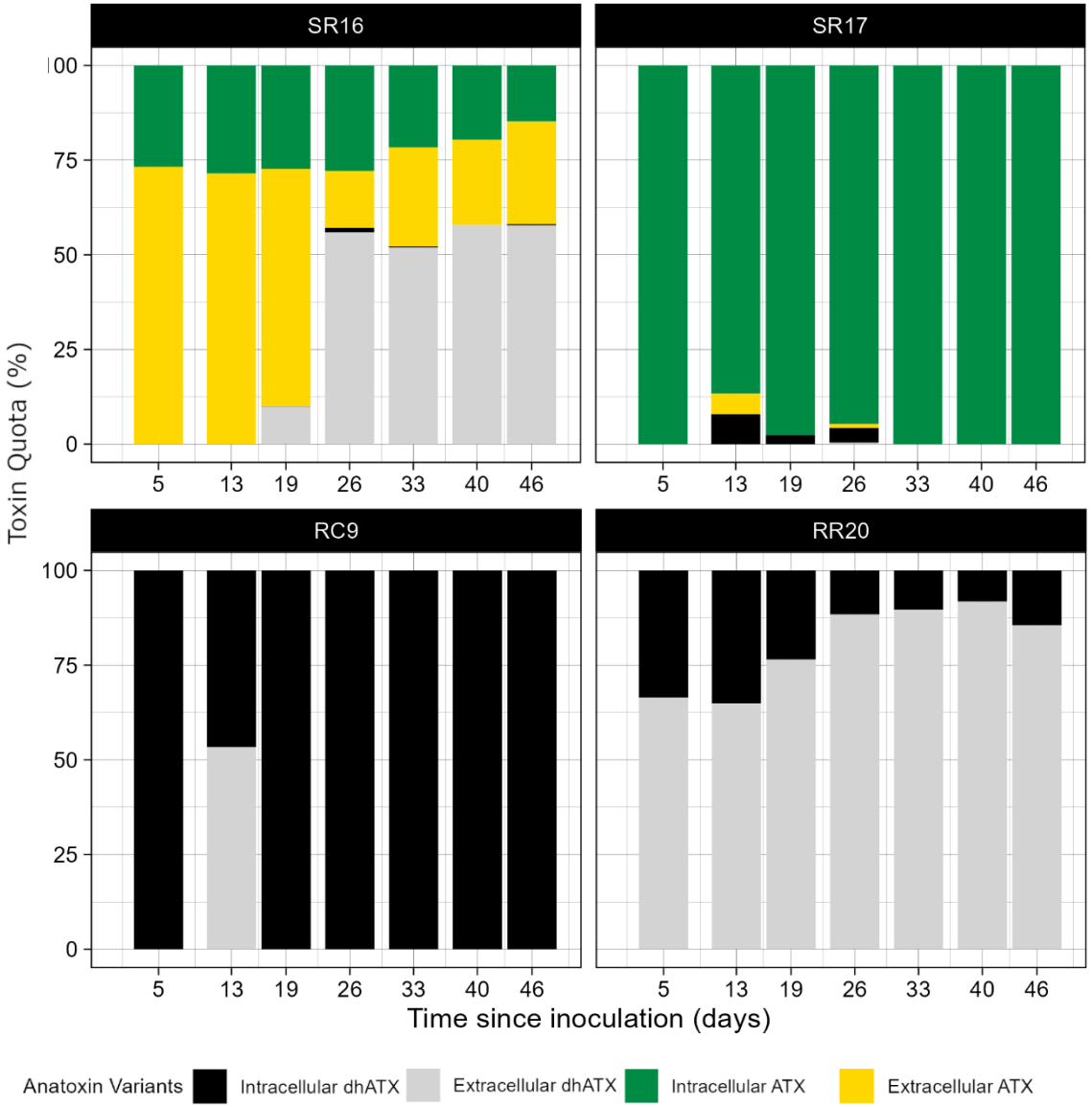


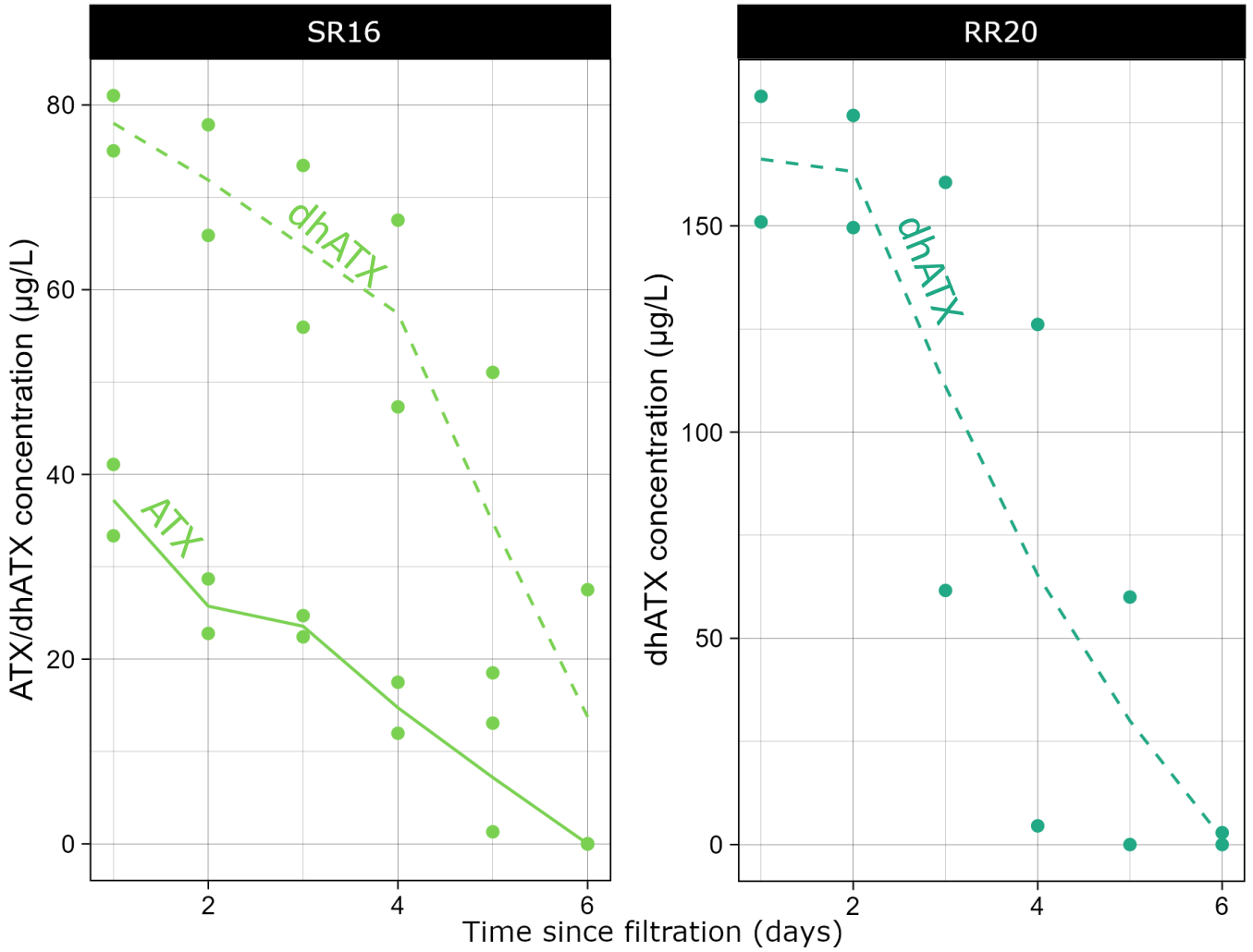
Legend: ★ peak dhATX ★ peak ATX

Bars represent average value ± 95% confidence interval

Extracellular vs. Intracellular Toxins

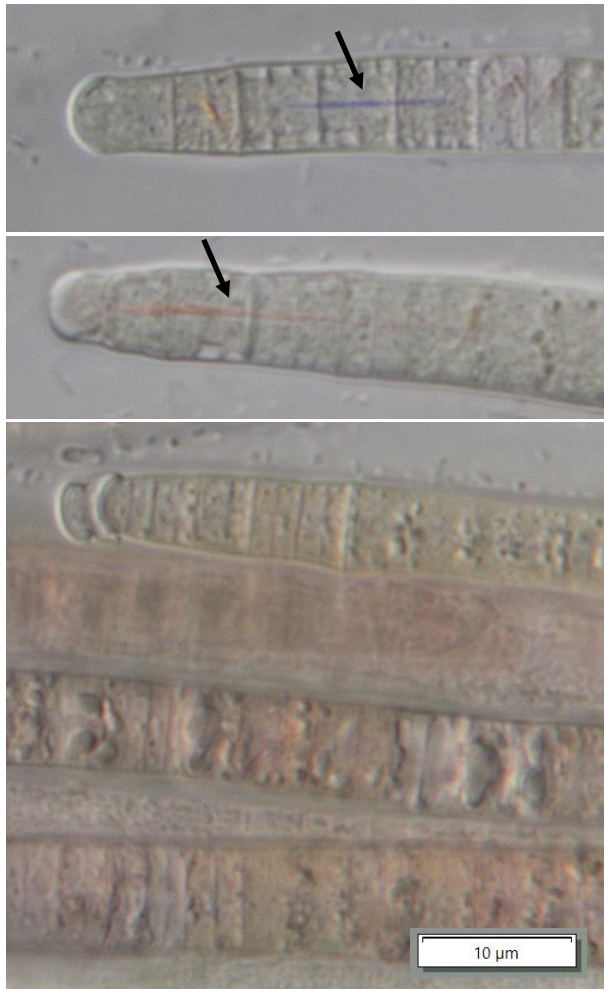
- Extracellular toxins prevail in strains SR16 & RR20 but not in SR17 & RC9



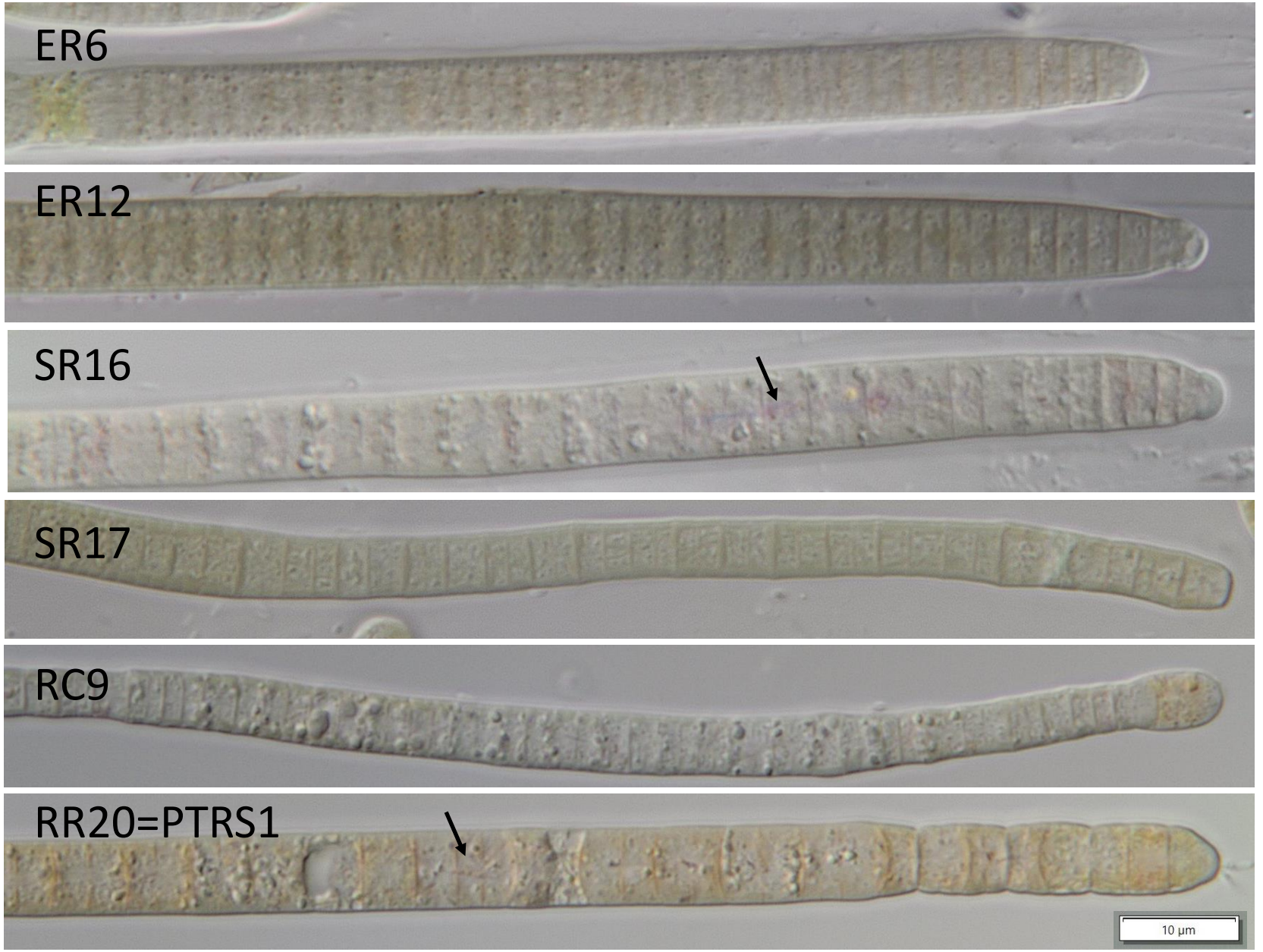


- ATX & dhATX degrade in 6 days

Filament Morphology



Damaged cell walls
of the most toxic strains
SR16 and RR20
40 days in culture



Take home Messages

Study outcomes

- Toxic and non-toxic strains represent two different species, the toxic is *M. anatoxicus*
- Toxic and non-toxic strains showed similar overall growth curves and slopes
  - All strains reached stationary phase around day 30, when mats were formed
- ATX and dhATX production peaked at day 13 for SR17, but later for SR16 (days 19 and 26)
- SR17 maximum toxin concentrations are five times lower than SR16
- dhATX production peaked at day 13 (RC9) and day 26 (RR20)
- RC9 maximum toxin concentrations are eight times lower than RR20
- The later maximum in toxin production corresponds with higher total toxin concentration and dominance of extracellular portion

Hypotheses revisited

1. Non-toxic strains will grow better than toxic strains under laboratory conditions (previously demonstrated by Heath et al. 2016)

   Rejected: Overall growth rates are comparable between toxic and non-toxic species under high nutrient conditions in the current culture condition

2. Peak toxin-production will have negative effect on cell growth (analyzed by specific growth rate)

   Supported: Both ATX and dhATX peak production corresponds with reduced cell growth

Future Research

Current experiment considerations which need further explorations

- BG11 is a high nutrient medium
- Batch cultures – we do not know how the culture chemistry change affects growth and toxin production
- Size of containers effects timing of reaching stationary phase
- Measure nutrient change in our culture conditions
  - Loss of N in the medium may trigger toxin production
- Filament morphology, cytology characterization and staining

Acknowledgements

- Dr. Goel lab
- Dr. Blaszczak lab
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