

# US EPA Benthic HABs Discussion Group

[webpage](#)

You are invited to a Zoom webinar.

When: Mar 26, 2024 09:00 AM Pacific Time (US and Canada)

Topic:

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## AGENDA

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- 1) Introduction and announcements  
-upcoming meetings, recent papers, other news, etc.
- 2) Brannon Walsh, USEPA Environmental Scientist

### OVERVIEW OF USEPA NATIONAL HAB PROGRAM

- 3) Christopher T. Nietch, Ph.D., Research Ecologist USEPA/ORD & Rochelle Labiosa, Ph.D., Physical scientist USEPA Region 10  
USEPA REGIONS RESEARCH ASSESSING FIELD SAMPLING AND ANALYTICAL PROCEDURES FOR CHARACTERIZING RISK POSED BY HARMFUL BENTHIC CYANOBACTERIA IN STREAMS AND RIVERS  
Abstract: U.S. states, tribes, and local agencies need consistent approaches to characterize the relative risk posed by harmful benthic cyanobacteria proliferations and to inform mitigation practices. USEPA is addressing this need with multi-year, multi-site field research and controlled experiments. In 2023, research focused on evaluating in-stream sampling methods that quantify spatial extent at the reach scale and assess the toxin exposure risk from disturbing benthic mats. A stream mesocosm study designed to control the relative dominance of specific strains of benthic cyanobacteria was conducted in parallel with the field effort. Field measurements were scheduled among seven field crews, making multiple visits to seven pilot test sites across six states (CA/KS/OH/UT/VA/WA). Laboratory analyses are ongoing and include general water quality variables, pigments, microscope counts, DNA metabarcoding of periphyton samples, qPCR analyses for toxin biosynthesis genes, and cyanotoxin-specific analyses. All periphyton samples were collected such that measures are normalized to stream bed area and periphyton biomass. The same analyses were run on samples from the mesocosm study, but in addition, effects on macroinvertebrates and fish were tested. Preliminary results provide rationale for streamlining field sampling techniques so that more sites can be surveyed in 2024 and insight into the variation in benthic cyanobacteria growth forms, community structures, toxins, and their potential effects on stream biota to help guide recommendations for risk assessment in the future.

- 4) Dr. Rosalina Stancheva Christova, Ph.D., George Mason University Assistant Professor & Sydney Brown, Ph.D. Student, George Mason University

EFFECT OF CULTURE CONDITIONS ON GROWTH AND TOXIN PRODUCTION OF *MICROCOLEUS* SPECIES (CYANOBACTERIA) ISOLATE FROM STREAMS IN CALIFORNIA

*Microcoleus* is a mat-forming benthic cyanobacterium recently recognized as an environmental problem in streams nationwide due to production of anatoxin-a (ATX), a neurotoxin implicated in dog deaths globally. However, environmental conditions stimulating its growth and toxin-production are little known. We cultured four toxin-producing unialgal, non-axenic strains determined by metagenomic analysis to be 99% ANI similar to *Microcoleus anatoxicus* Stancheva & Conklin isolated from the Russian River in 2015. This species initially produced ATX and higher concentrations of dihydroanatoxin-a (dhATX), but over time ATX production has become undetectable. We compared the growth and toxin-production of *M. anatoxicus* type strain PTRS1 and new strain RC9 from Rock Creek, both producing detectable dhATX only during the experiment, to strains SR16 and SR17, isolated from the Klamath River watershed which produced higher levels of ATX than dhATX. We also used two non-toxic *Microcoleus* strains ER6 and ER12 isolated from mats in the Eel River. All six strains were grown in batch monocultures for 46 days in liquid BG11 medium and reached stationary phase developing surface mats by day 30, with exception for RC9. The toxin production reached its maximum at day 13 for SR17 and at days 19 and 26 for the rest of the strains. All strains displayed storage granules along the cross-cell walls, which decreased rapidly at day 15 in toxic strains only during the peak of the toxin production. Experiments with increased salinity concentrations showed maximum growth of *M. anatoxicus* in oligohaline waters with salinity of 4.6 ppt. Moderate salinity stress (up to 7.8 ppt) did not affect the growth and dhATX production significantly. In contrast, higher salinity above 9.3 ppt had a detrimental effect on cell growth and significantly suppressed dhATX production. *M. anatoxicus* grown for 40 days in nitrogen-deplete BG11 medium formed mats with significantly elevated dhATX, and slightly increased ATX concentrations. Morphological observations of storage granules, thylakoids and extracellular polysaccharide sheath were used to help understand trade-offs and energetic expense of toxin-production.

**Benthic HAB Workgroup Facilitators**

See [website](#) for content and recordings of previous meetings. Contact us if you would like to be added to our list serve and receive communications.

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