Welcome to the Benthic HABs Workgroup Webinar

March 30, 2021 08:30 AM to 10:00 AM Pacific Daylight Time

Web Meeting Registration:
https://zoom.us/webinar/register/WN_fVJ65TqSRk-sV8FjGKW_rw




US EPA Benthic HABs Discussion Group
https://www.epa.gov/cyanohabs/epa-newsletter-and-collaboration-and-outreach-habs#benthic

Facilitators
Keith Bouma-Gregson Keith.Bouma-Gregson@Waterboards.ca.gov
Margaret Spoo-Chupka Mspoo-Chupka@mwdh2o.com
Dr. Lesley D'Anglada Danglada.Lesley@epa.gov

AGENDA

I **Welcome, Introductions, Agenda Overview, Upcoming Events and Publications**
Keith Bouma-Gregson & Margaret Spoo-Chupka

IV **Announcements and Discussion**
Anthea Fredrickson, Lower Colorado River Authority, Austin, TX

III **Presentation: ITRC Benthic Harmful Cyanobacterial Bloom Team**
Dr. Beckye Stanton, California Office of Env. Health Hazard Assessment

IV **Presentation: Mass Occurrence of Anatoxin-a- and Dihydroanatoxin-a-Producing *Tychonema* sp. in Mesotrophic Reservoir Mandichosee (River Lech, Germany) as a Cause of Neurotoxicosis in Dogs**
Dr. Franziska Bauer, Dr. rer. nat.,
Limnological Research Station Iffeldorf, Germany, Aquatic Systems Biology Unit, Technical University of Munich, Hofmark

ITEM I: UPCOMING EVENTS

- Events:

10.5 US HAB Symposium – Emerging Voices and Blooming Careers – Virtual Meeting May 25-27. http://ushabs.com/

Society for Freshwater Science, May 23-27. https://sfsannualmeeting.org/

Interdisciplinary Freshwater Harmful Algal Bloom Workshop, June 7-8, 11:30am – 1:30 pm EST. https://www.ifhabworkshop.com/

ASLO Aquatic Sciences Meeting, June 22-27. https://www.aslo.org/2021-virtual-meeting/

75th Annual Phycological Society of America Meeting, July 13,15,20, and 22, 2021. https://www.psaalgae.org/psa-annual-meeting

ITEM I: RECENT PUBLICATIONS

- Publications:

Espinosa, C., Abril, M., Ponsá, S., Ricart, M., Vendrell-Puigmitjà, L., Ordeix, M., et al. (2021). Effects of the interaction between nutrient concentration and DIN:SRP ratio on geosmin production by freshwater biofilms. *Science of The Total Environment* 768, 144473. doi:10.1016/j.scitotenv.2020.144473.

Puddick, J., van Ginkel, R., Page, C. D., Murray, J. S., Greenhough, H. E., Bowater, J., et al. (2021). Acute toxicity of dihydroanatoxin-a from Microcoleus autumnalis in comparison to anatoxin-a. *Chemosphere* 263, 127937. doi:10.1016/j.chemosphere.2020.127937.

Thomson-Laing, G., Dyer, N., Whyte-Wilding, R. *et al.* In situ river experiments to explore variability in *Microcoleus autumnalis* mat expansion. (2021) *Hydrobiologia* **848,** 445–467. doi:10.1007/s10750-020-04453-1

Colas, S., Marie, B., Lance, E., Quiblier, C., Tricoire-Leignel, H., and Mattei, C. (2021). Anatoxin-a: Overview on a harmful cyanobacterial neurotoxin from the environmental scale to the molecular target. *Environmental Research* 193, 110590. doi:10.1016/j.envres.2020.110590.

ITRC Strategies for Preventing and Managing Harmful Cyanobacterial Blooms (HCBs). https://hcb-1.itrcweb.org/

ITEM II
ANNOUNCEMENTS AND DISCUSSION

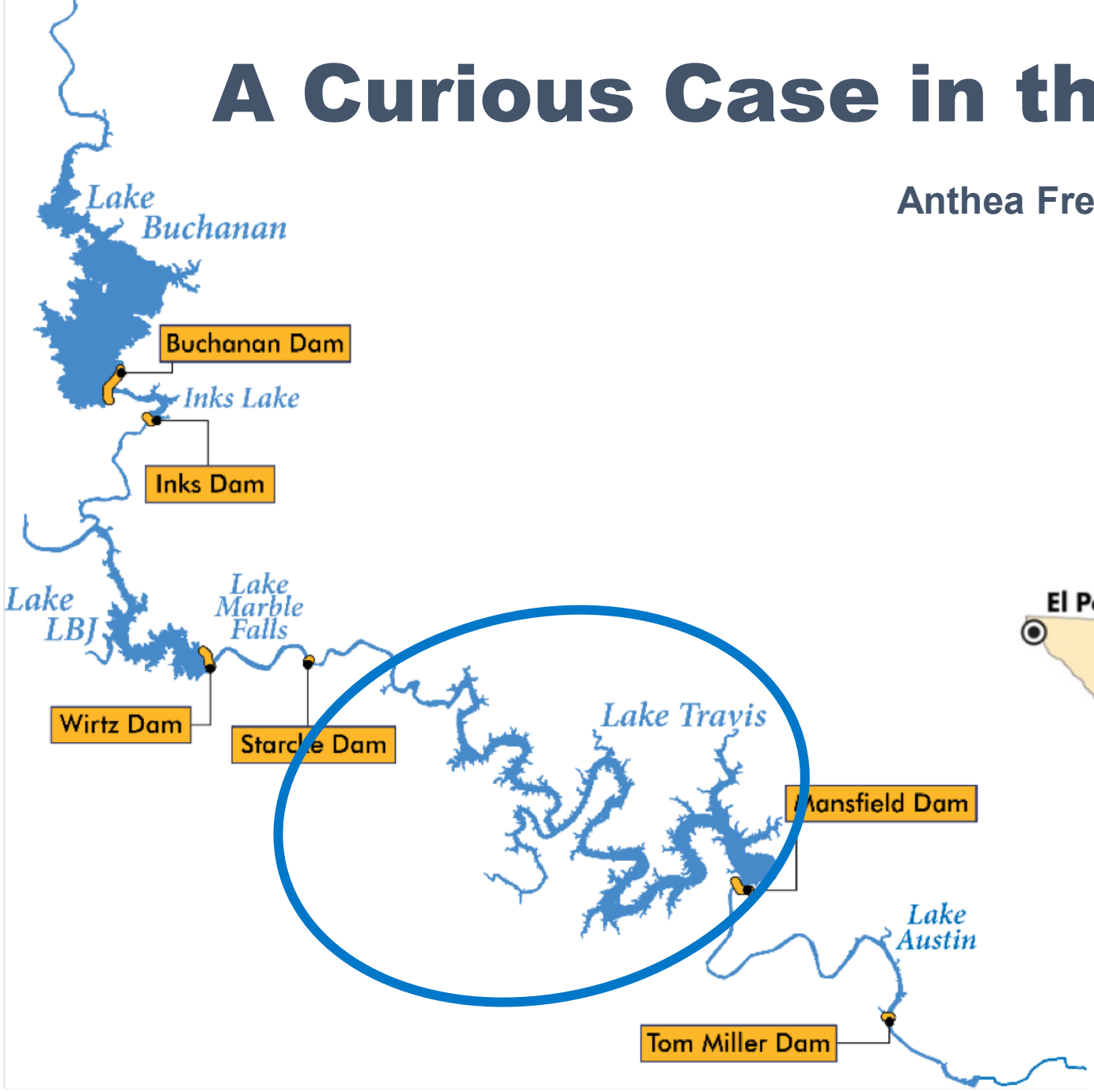
Anthea Fredrickson: *Lower Colorado River Authority, Water Quality Protection Colorado River (TX) update*

Other updates, announcements, or questions to pose to the group?

- Please raise your hand, and moderators will unmute you

A Curious Case in the Highland Lakes

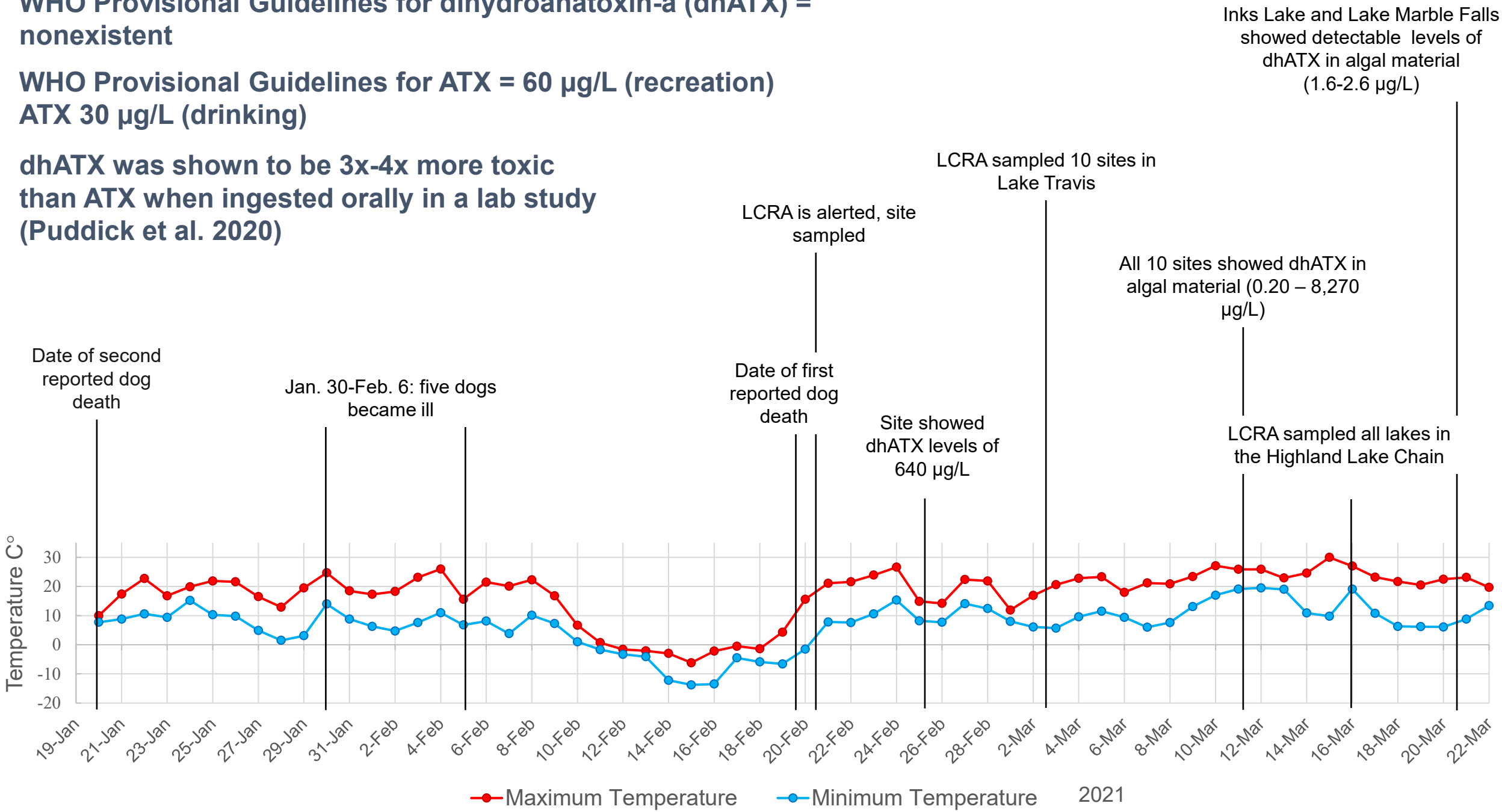
Anthea Fredrickson, LCRA Water Quality Coordinator
Anthea.Fredrickson@lcra.org



WHO Provisional Guidelines for dihydroanatoxin-a (dhATX) = nonexistent

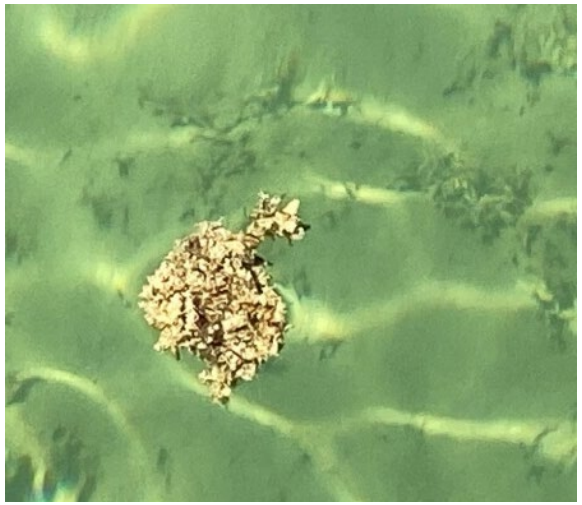
WHO Provisional Guidelines for ATX = 60 µg/L (recreation)
ATX 30 µg/L (drinking)

dhATX was shown to be 3x-4x more toxic than ATX when ingested orally in a lab study (Puddick et al. 2020)



Curiosities Continued

- Occurring in winter, both before and after record cold temperatures
- Toxins found in filamentous green algae (*Spirogyra, Cladophora*)
- *Phormidium/Microcoleus* was present in every sample that had detectable levels of dhATX
- Other species identified: *Oscillatoria, Anagnostidinema, Geitlerinema, Microcystis, Dolichospermum, Planktothrix*
- Another reservoir (Lake Belton) in an adjacent basin also had reported dog deaths in late February – similar benthic HAB there (dhATX)



ITEM III
ITRC BENTHIC HARMFUL CYANOBACTERIA BLOOM TEAM

Dr. Beckye Stanton, *California Office of Env. Health Hazard Assessment*

https://www.itrcweb.org/Team/Public?teamID=82

Advancing Environmental Solutions

ITRC Benthic Harmful Cyanobacteria Bloom (HCB) Team

BY:

BECKYE STANTON, CALIFORNIA OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT, ITRC BENTHIC HCB CO-TEAM LEADER

BEN HOLCOMB, UTAH DEPARTMENT OF ENVIRONMENTAL QUALITY, ITRC BENTHIC HCB CO-TEAM LEADER

ECOS

ERIS ENVIRONMENTAL RESEARCH INSTITUTE OF THE STATES

Benthic Harmful Cyanobacterial Blooms (HCBs)

January 2021 – December 2021

- Visit the Team Website
- HCB Team Fact Sheet

Team Leaders:

- Beckye Stanton

  rebecca.stanton@oehha.ca.gov

- Ben Holcomb

  bholcomb@utah.gov

Program Advisor

- Cherri Baysinger

  cbaysinger@socket.net

Interstate Technology and Regulatory Council

Strategies for Preventing and Managing Benthic Harmful Cyanobacterial Blooms

Fact Sheet

THE INTERSTATE TECHNOLOGY REGULATORY COUNCIL (ITRC) IS EXCITED TO START A TEAM IN JANUARY 2021 ON STRATEGIES FOR PREVENTING AND MANAGING HARMFUL CYANOBACTERIAL BLOOMS (BENTHIC)

The Interstate Technology Regulatory Council (ITRC) is a state-led coalition dedicated to reducing barriers to the use of innovative environmental technologies. ITRC represents over 1,000 individuals, across 50 states, working to produce guidance and training on innovative environmental solutions. Bringing together teams of state, federal, tribal, industry, academic, and stakeholder experts, ITRC broadens and deepens technical knowledge and reduces barriers to expedient regulatory approval. Since 1995, the collective success of this coalition has generated huge benefits to the environment, inspired new technical innovations, and saved hundreds of millions of dollars.

ITRC is a program of the Environmental Research Institute of the States, managed by the Environmental Council of the States. This partnership is based on a commitment to protect and improve human health and the environment across the country.

BENTHIC HARMFUL CYANOBACTERIAL BLOOMS (HCBs)

Freshwater inland lakes and reservoirs supply approximately 70% of our nation's drinking water and industry withdrawals. They serve as vibrant hubs for recreation, tourism, and local identity. Human activities can influence and alter their natural ecological equilibrium.

Harmful Cyanobacterial Blooms (HCBs) are complex ecological phenomenon that can occur where cyanobacteria proliferate and dominate aquatic ecosystems.

Much of what we know about HCBs is based on those planktonic forms that occur on the water surface or in the water column. Benthic HCBs grow along the bottom until pieces detach, float to the surface, or strand along the shoreline. As with planktonic HCBs, many benthic cyanobacteria produce toxins that can impact dermatologic, respiratory, hepatic, and neurologic systems. When these toxins are present in freshwater, they can threaten humans, wildlife, livestock, and pets.

HCB Strategies

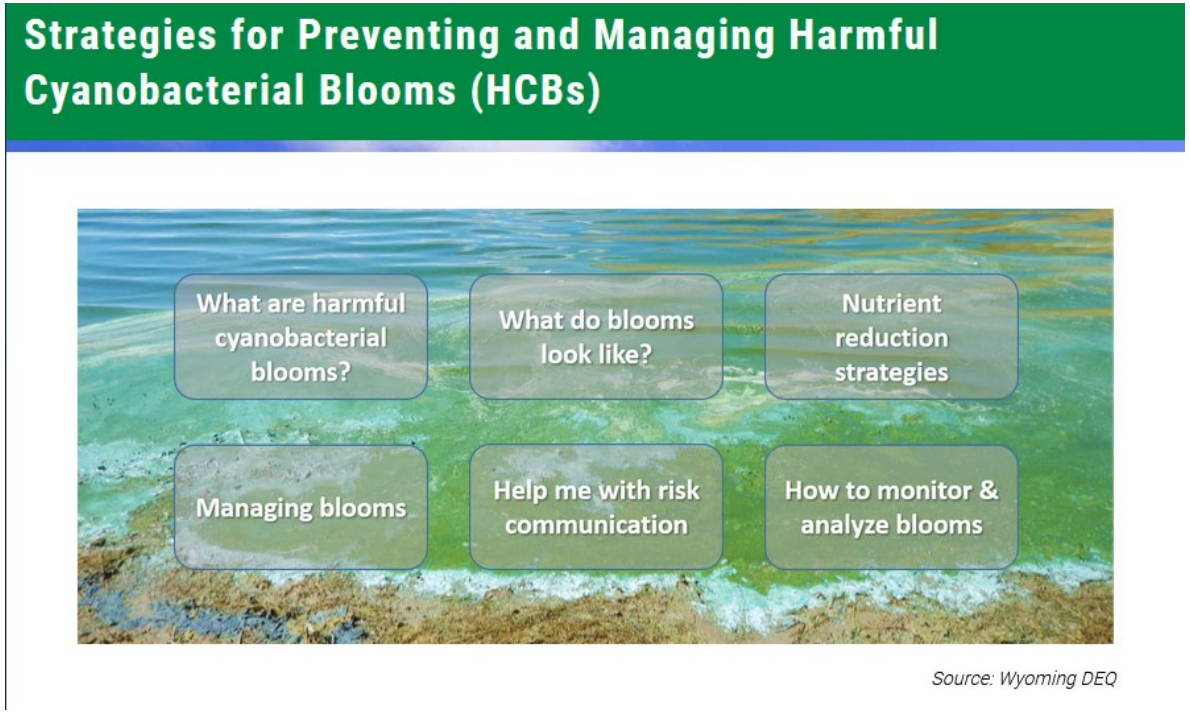
Team Goal

- **Background**
  - Benthic cyanobacteria have unique characteristics compared to planktonic cyanobacteria.
  - Benthic cyanobacteria-specific resources are limited for:
    - Field screening methods
    - Sampling and analytical methods for mat samples
    - Thresholds for cyanotoxins in mat samples
    - Thresholds for neurotoxins or dermal toxins in mat or water samples
    - Advisories
    - Prevention and management and control measures

- **Team Goal**
  - To enhance the ITRC HCB technical and regulatory guidance document with more detailed information focused on benthic cyanobacteria.

Companion To New ITRC HCB Guidance

- ITRC HCB Guidance now live (http://hcb-1.itrcweb.org/)
  - Benthic cyanobacteria are briefly mentioned throughout the HCB guidance
  - ITRC HCB training (April 29th)
- Proposed Benthic HCB Guidance
  - Not totally stand alone and do not need to duplicate information already included
  - Will share some resources (tools, Visual Guide)
  - Follow same general framework and primary audience (water body manager)



Source: Wyoming DEQ

Team Deliverables

- Companion web-based technical regulatory guidance and training focused on:
  - Introduction to benthic cyanobacteria and connection to existing HCB document
  - Field screening and sampling for benthic cyanobacteria
  - Analytical toxin testing methods for mat samples
  - Toxin Thresholds
    - All cyanotoxins in mats
    - Neurotoxins and dermal toxins in water
  - Communication and Response Planning
    - Specific advisory signage and messaging
  - Specific considerations for Prevention and Management and Control Strategies

Benthic HCB Team Activities

- Monthly team calls
- Bi-monthly sub-group calls
  - Introduction
  - Methods (field and lab)
  - Toxin thresholds
  - Communication and signage
  - Management strategies
- State survey (ongoing to April 2nd)
- Virtual spring meeting April 14-15
- External review (Fall 2021)
- Final document and training (Spring 2022)

Benthic HCB Team – Get Involved!

- Join us! Visit www.itrcweb.org and select 2021 Team Registration
  - Don't have a lot of time? Sign up as an Interested Party
  - Ready to get to work with us? Sign up as an Active Member
- Welcome email will provide details on our team's next steps

Thank You!

Stay Updated on ITRC's Activities

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ITEM IV

GUEST PRESENTATION: Mass Occurrence of Anatoxin-a- and Dihydroanatoxin-a-Producing *Tychonema* sp. in Mesotrophic Reservoir Mandichosee (River Lech, Germany) as a Cause of Neurotoxicosis in Dogs

Toxins 2020: https://doi.org/10.3390/toxins12110726

Dr. Franziska Bauer, Dr. rer. nat.,

Limnological Research Station Iffeldorf, Germany, Aquatic Systems Biology Unit, Technical University of Munich, Hofmark

Limnological Research Station Iffeldorf
Aquatic Systems Biology Unit
TUM School of Life Sciences
Technical University of Munich

Mass occurrence of anatoxin-a and dihydroanatoxin-a-producing *Tychonema* sp. in mesotrophic reservoir Mandichosee (River Lech, Germany) as a cause of neurotoxicosis in dogs

Dr. Franziska Bauer, Limnological Research Station, Iffeldorf (Germany)

Benthic HABs Discussion Group, Webinar, 30th March 2021

funded by
Bavarian State Ministry of the Environment and Consumer Protection
Bavarian State Ministry of Health and Care

Structure of the talk

1. The Limnological Research Station in Iffeldorf
2. The incidents at reservoir Mandichosee
3. Sampling campaign 2019
4. The agencies involved
5. Publication of the results
6. Follow-up study 2020
7. Future plans

Limnological Research Station in Iffeldorf

Technical University of Munich (TUM)

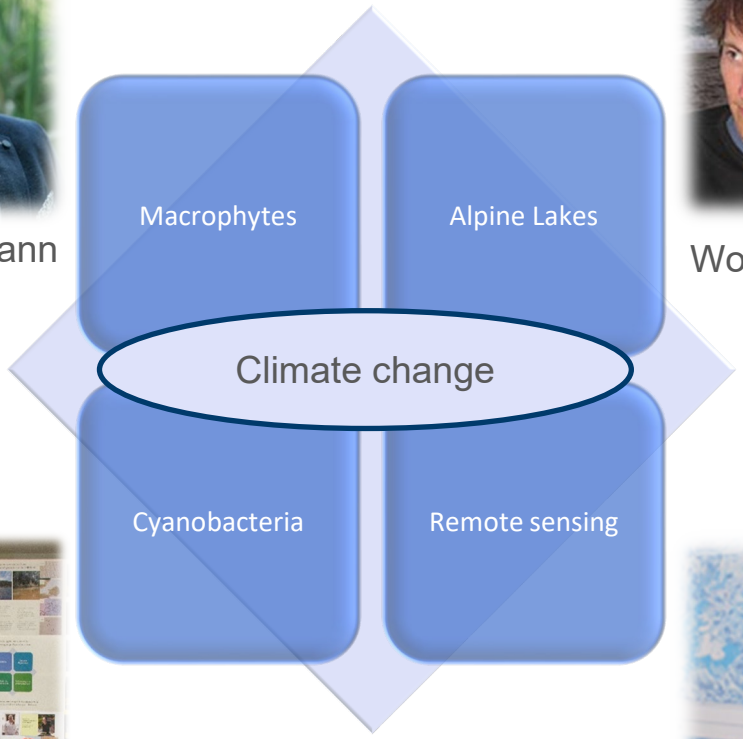
Dr. Uta Raeder

Research groups

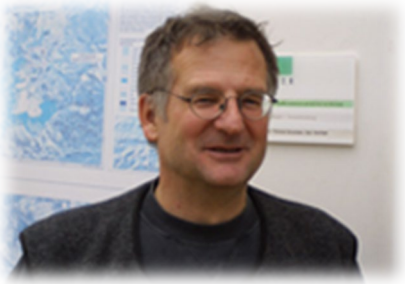
Limnological Research Station Iffeldorf

Dr. Markus Hoffmann

Wolfgang Küfner



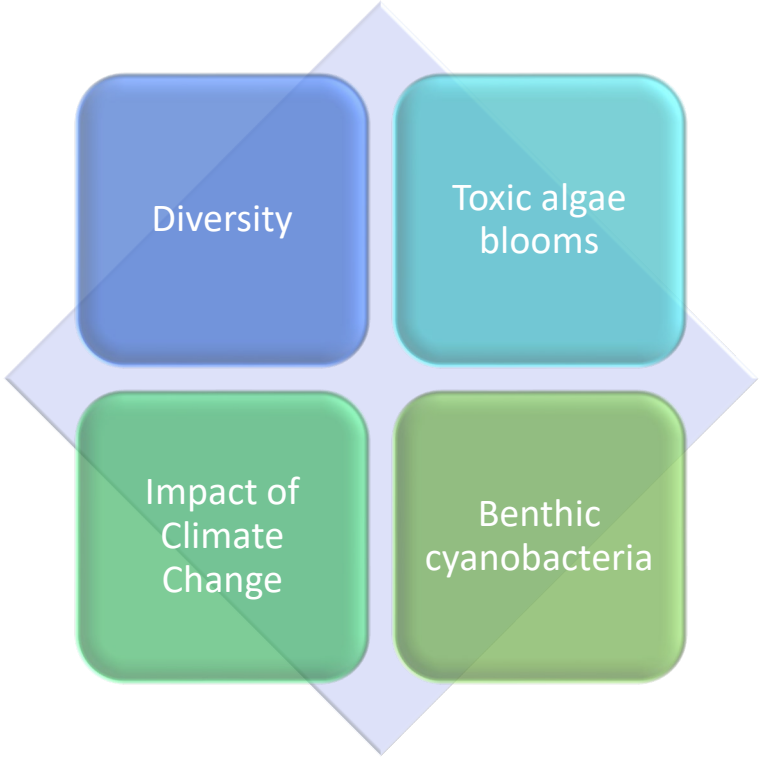
Dr. Franziska Bauer



Dr. Tomi Schneider

Cyanobacteria working group

Limnological Research Station Iffeldorf



In general, only **pelagic** cyanobacteria have been studied before August 2019

Current project

Cyanobacteria and Climate Change – Cyanotoxin genes in Bavarian lakes

Joint project: *Climate change and Health*

funded by
Bavarian State Ministry of the Environment and Consumer Protection
Bavarian State Ministry of Health and Care

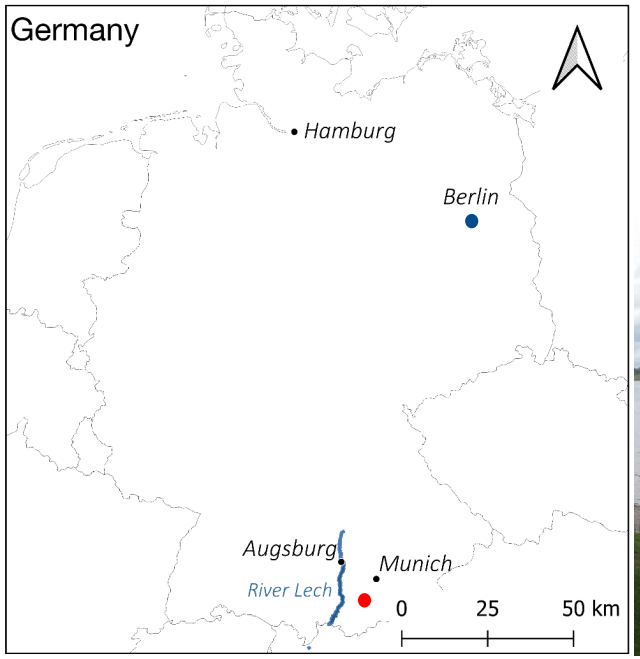
→ Regular exchange with a cyanobacteria expert/senior scientist

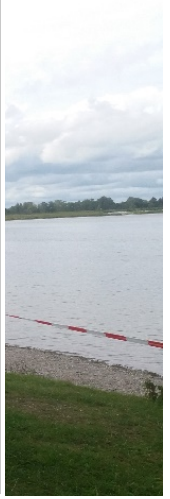
Dr. Jutta Fastner / Cyanocenter / German Environment Agency, Berlin

Incidents with dead dogs

Reservoir Mandichosee, August 2019

- Problems with toxic cyanobacteria at reservoir Mandichosee, reservoir 23, River Lech
- Intoxication of dogs caused by anatoxin-a producing *Tychonema* sp.



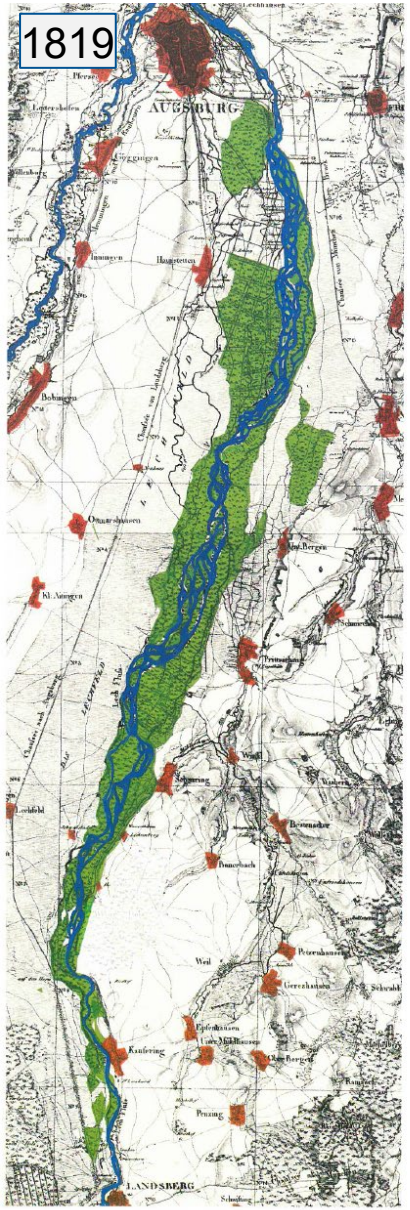


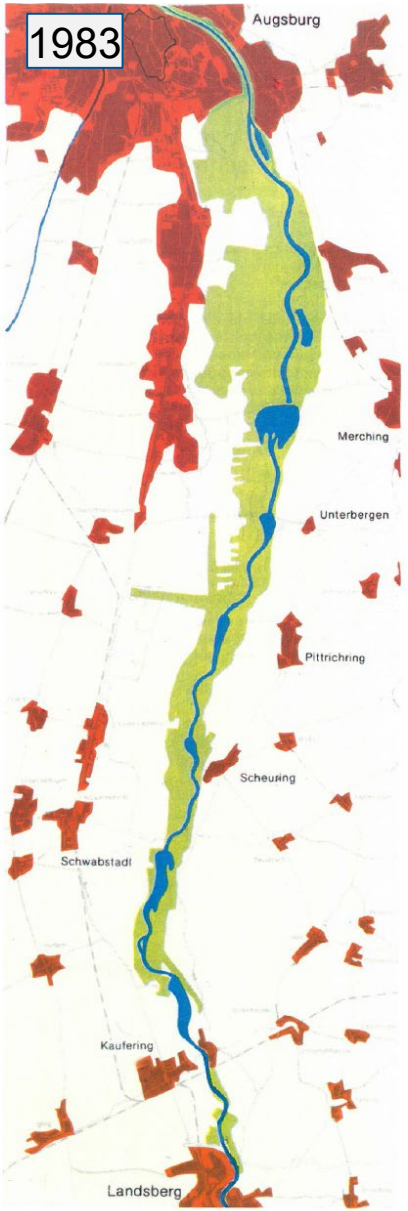
Closure of the bathing area by the responsible authority (LGL)

Limnological Research Station Iffeldorf
Aquatic Systems Biology Unit
TUM School of Life Sciences
Technical University of Munich

River Lech

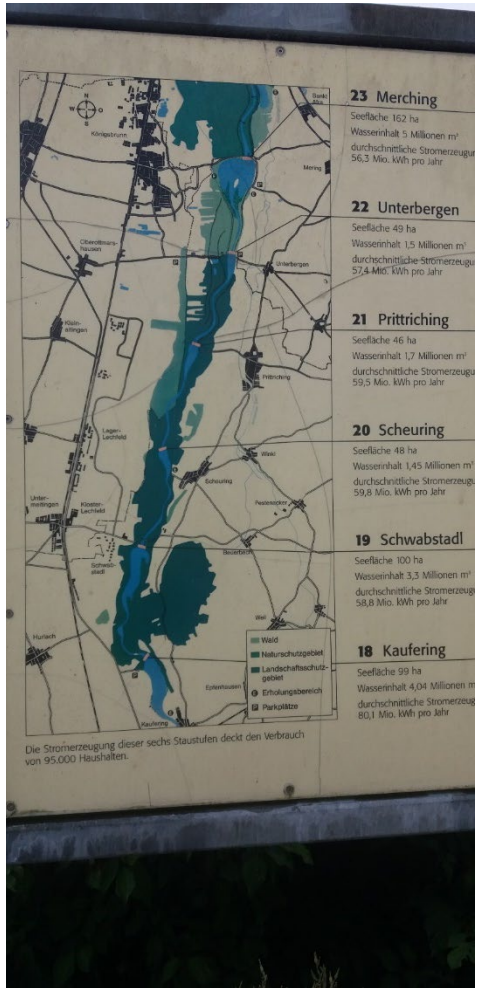
a tamed river

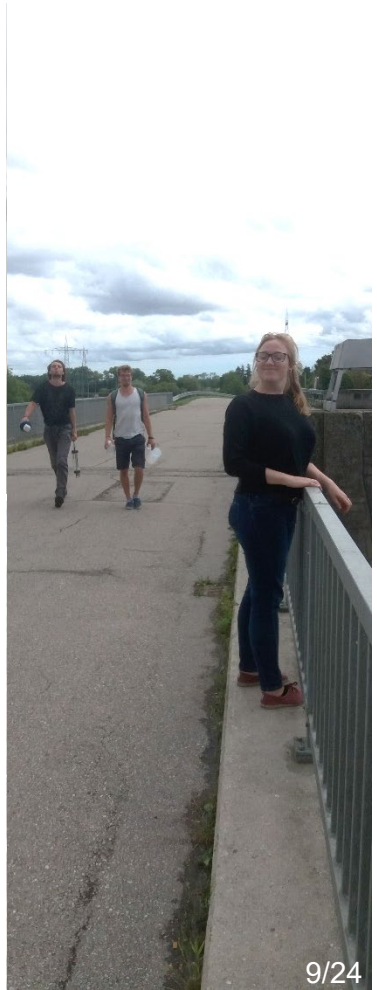




Assessment of the situation on site

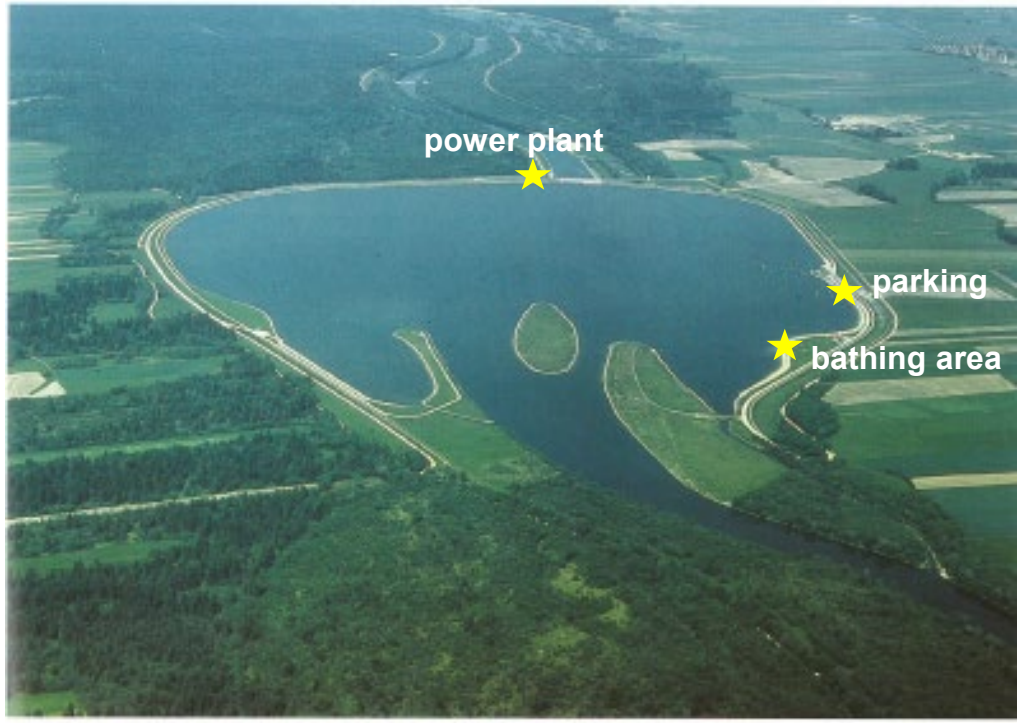
Reservoir Mandichosee, 19th August 2019





Sampling campaign

Reservoir Mandichosee



Sampling dates (Mandichosee)

- 19.08.2019
- 21.08.2019
- 10.09.2019

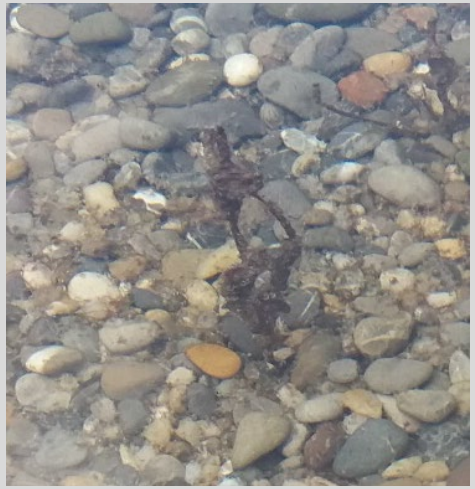
Sampling site „parking“, 19th Aug. 2019
mat sample

Sampling site „parking“, 19th Aug. 2019
growing on washed up macrophytes

Example images

benthic *Tychonema* mats and biofilm

Mandichosee (23), 21.08.2019



Example images

floating *Tychonema* clumps

Mandichosee (23), 21.08.2019

Schwabstadl (19), 12.09.2019

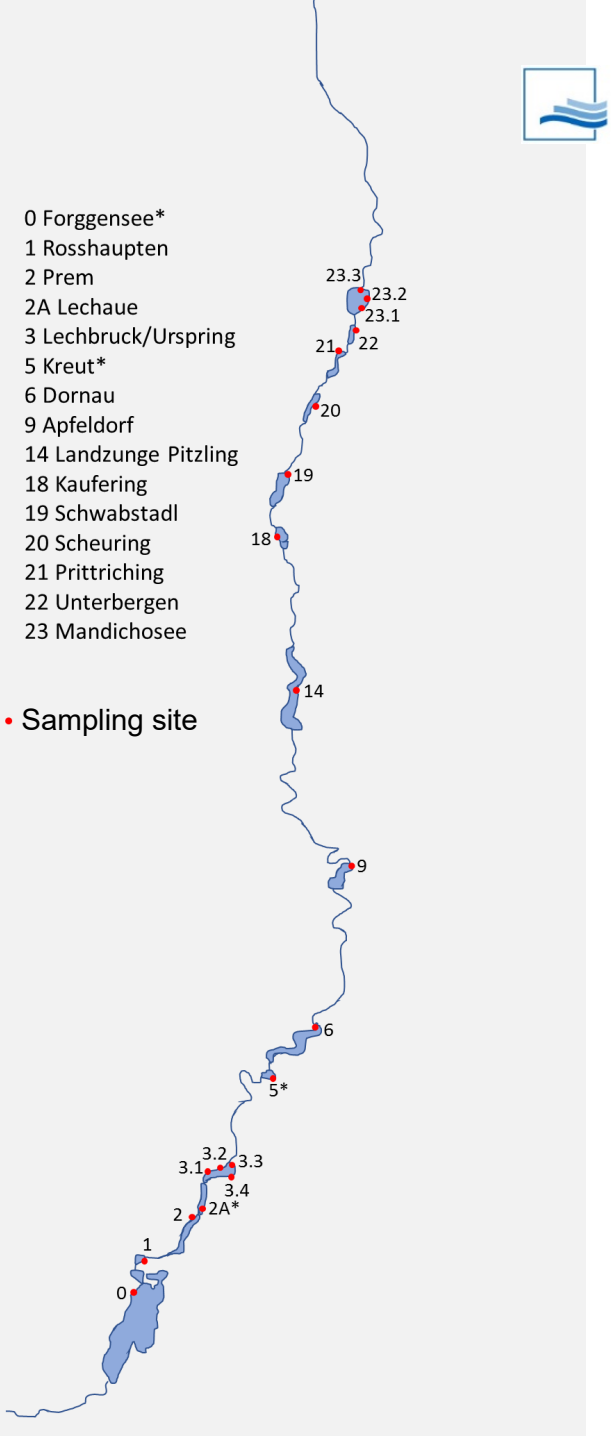
Sampling campaign 2019

River Lech

Reservoirs 18-22:
12.09.2019

Southern Reservoirs (between Forggensee and Pitzling):
19.09.2019

→ a total of 20 sampling sites



2a Lechaue

Macroscopic: *Tychonema* suspected (on wood and sediment)
Microscopy: *Tychonema* positive
Genetics: no *anaC genes* detected

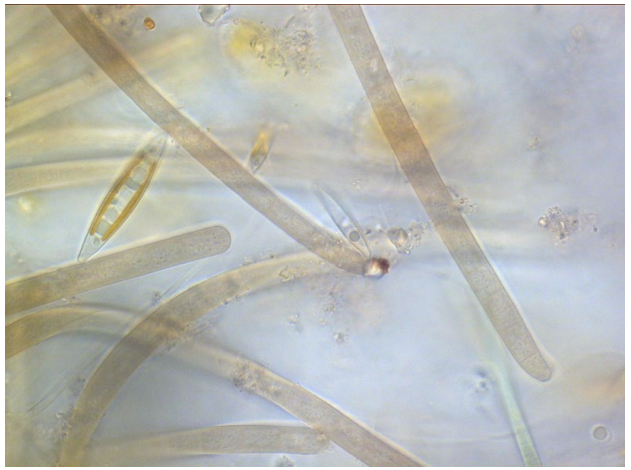
3.2 Lechbruck- camping site

Macroscopic: *Tychonema* suspected (uncertain)
Microscopy: *Tychonema* positive
Genetics: no *anaC genes* detected

3.4 Lechbruck-Urspring

Macroscopic: *Tychonema* positive
Microscopy: *Tychonema* positive
Genetics: no *anaC genes* detected



Data collection and analysis

German Environment Agency (UBA)

Jutta Fastner
Cyanocenter

Toxin analyses

Bavarian Health and Food Safety Agency (LGL)

Christiane Höller, Stefanie Huber, Bernadett Bartha-Dima

Bathing water control

Limnological Research Station (LSI)

Franziska Bauer, Uta Raeder
Sampling, DNA analyses (PCR, sequencing), physical and chemical characterization

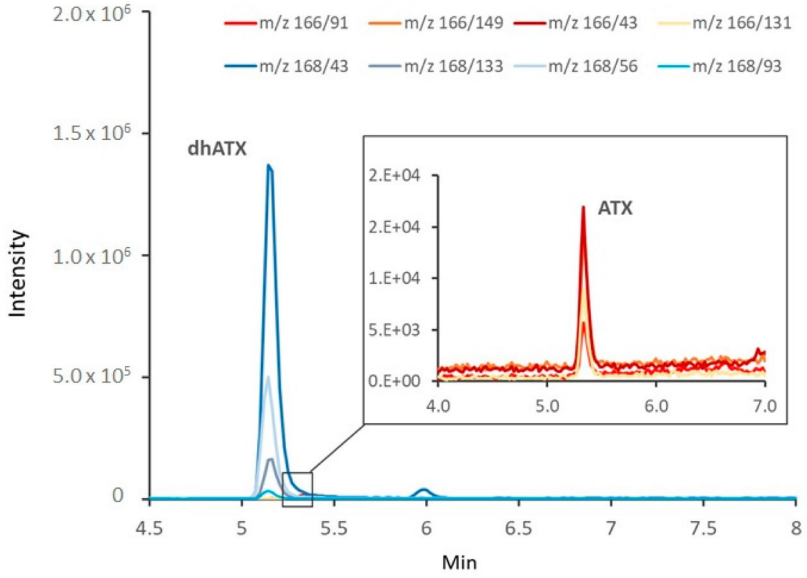
LGL in-house pathologists
Wolfram Breuer

Institute of Veterinary Pathology (Ludwig Maximilian University of Munich)
Almuth Falkenau & Christian Mayer
Necroscopies

Toxin analyses

- Anatoxin-a / dihydroanatoxin-a → mats, macrophytes covered with *Tychonema*, sediments, stomachs of the dogs (not found in open water)
- Homoanatoxin-a / dihydrohomoanatoxin-a → not detected in any sample
- Cylindrospermopsin / deoxy-Cylindrospermopsin → not detected in any sample

Method: LC-MS/MS



Anatoxins: up to 68,000 µg/L in samples containing a very large amount of mat material

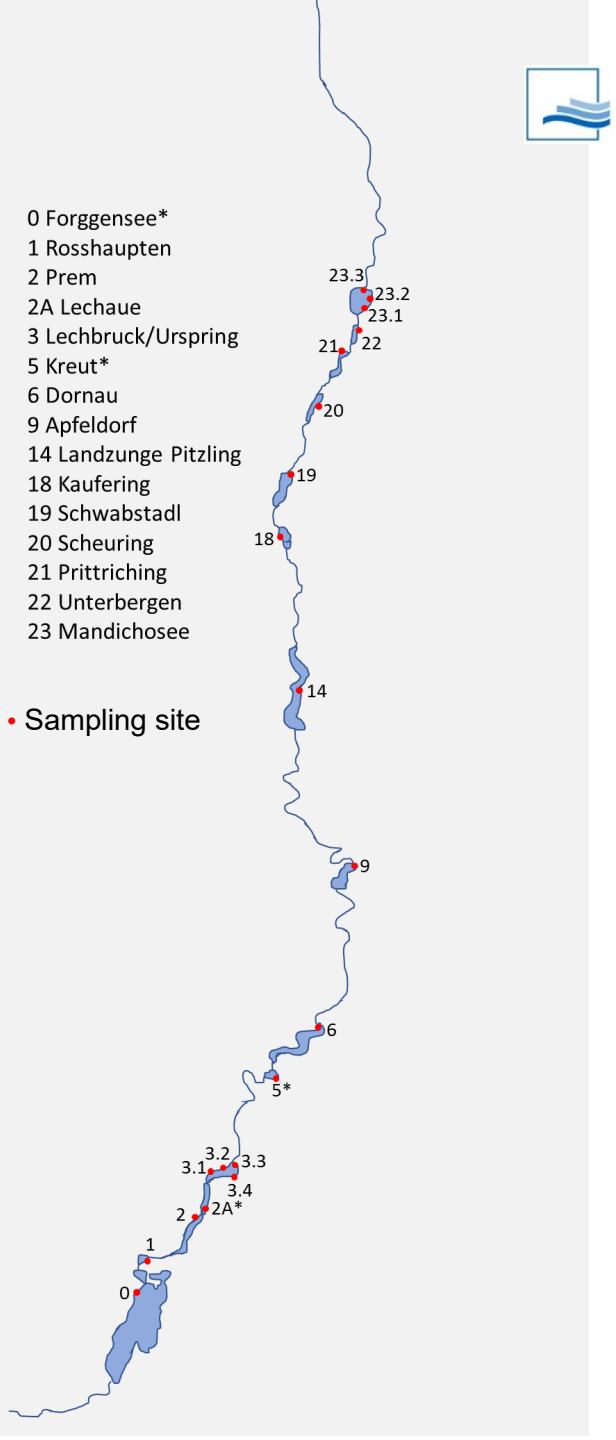
Molecular analyses

PCR

- to detect target genes for anatoxin-a, microcystin and saxitoxin
- *anaC* detected in several samples of Lake Mandichosee and in samples of the reservoirs Schwabstadl (19) and Prittriching (21)

Illumina MiSeq sequencing (*16S rRNA gene*)

- Mandichosee: *Tychonema* detected in all samples except the open water sample
- River Lech upstream: *Tychonema* detected in all samples except Forggensee (0), Roßhaupten (1) and Pitzling (14)



Summary results 2019

Reservoir Mandichosee

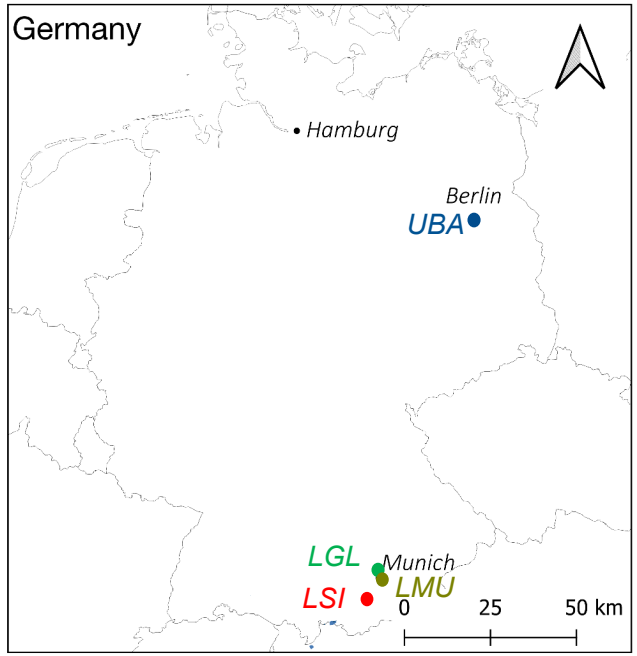
- *Tychonema* detected by microscopy and genetics in all samples
- Anatoxin-a genes detected in many samples (PCR)
- Sequencing confirmed the classification as *Tychonema* (Illumina MiSeq)
- Toxin range: up to 68,000 µg/l (floating mat material)

River Lech upstream

- In the South of River Lech *Tychonema* sp. was macroscopically and microscopically detected at several reservoirs (Prem, Lechaue, Kaufering, Schwabstadl, Scheuring, Prittriching)
- Sequencing confirmed results
- Anatoxin-a genes were detected in reservoir 19-Schwabstadl und 21-Prittriching (PCR)

Cooperation between the institutes

German environment agency/Cyanocenter (UBA)
Dr. Jutta Fastner



Bavarian Health and Food Safety Agency (LGL)

Limnological Research Station Iffeldorf (LSI)

Ludwig Maximilian University of Munich (LMU)

Publication

Open Access Article

Mass Occurrence of Anatoxin-a- and Dihydroanatoxin-a-Producing *Tychonema* sp. in Mesotrophic Reservoir Mandichosee (River Lech, Germany) as a Cause of Neurotoxicosis in Dogs

by Franziska Bauer [1,*], Jutta Fastner [2], Bernadett Bartha-Dima [3], Wolfram Breuer [3], Almuth Falkenau [4], Christian Mayer [4] and Uta Raeder [1]

[1] Limnological Research Station Iffeldorf, Aquatic Systems Biology Unit, Technical University of Munich, Hofmark 1-3, 82393 Iffeldorf, Germany

[2] German Environment Agency, Schichauweg 58, 12307 Berlin, Germany

[3] Bavarian Health and Food Safety Authority, Veterinärstraße 2, 85764 Oberschleißheim, Germany

[4] Center for Clinical Veterinary Medicine, Institute for Veterinary Pathology, Ludwig-Maximilians-University, Veterinärstraße 13, 80539 Munich, Germany

* Author to whom correspondence should be addressed.

Toxins **2020**, *12*(11), 726; https://doi.org/10.3390/toxins12110726

Received: 28 October 2020 / Revised: 17 November 2020 / Accepted: 17 November 2020 / Published: 20 November 2020

(This article belongs to the Special Issue Cyanotoxins in Bloom: Ever-Increasing Occurrence and Global Distribution of Freshwater Cyanotoxins from Planktic and Benthic Cyanobacteria)

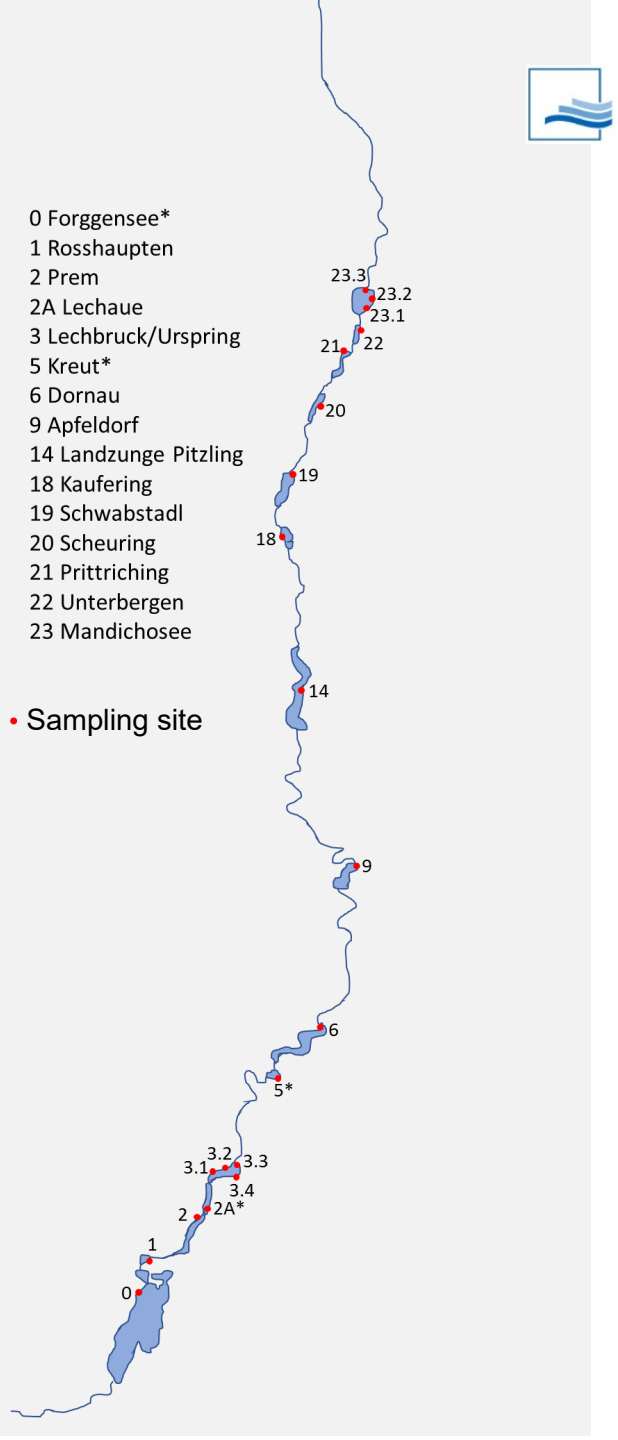
Follow up study – monitoring 2020 in river Lech

"Detection of the occurrence of the anatoxin-a-producing cyanobacterium *Tychonema* at the reservoirs of river Lech in the vegetation period 2020"

- Biweekly sampling in reservoir Mandichosee
- Monthly sampling at all sampling sites

Michael Stix

Robert Kagerer



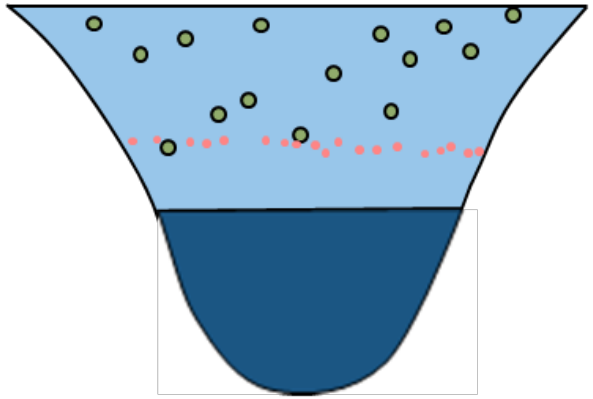
Findings 2020

- *Tychonema* spread all over River Lech
- *Tychonema* present throughout the sampling period (April-November)
- There are probably two species
- Measured anatoxin-a values (ELISA) between 0.1 and 220 µg/L, highest values in reservoir Mandichosee in autumn
- Mass occurrence Apfeldorf, 5.5.2021, toxin value 1.8 µg/L

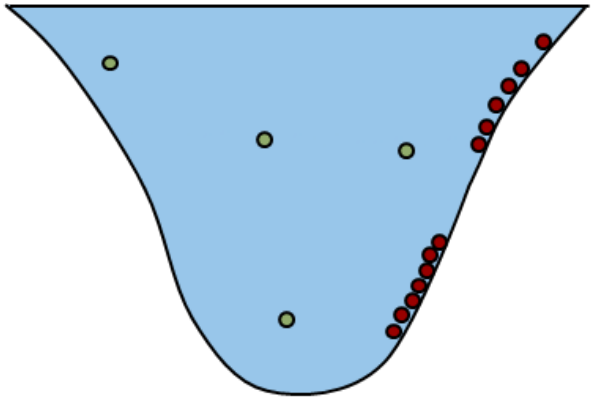

Apfeldorf, 05.05.2020


Mandichosee bathing area, 15.09.2020

Future plans – monitoring 2021



- lakes divided in two zones: euphotic zone and aphotic zone
- Nutrient content: medium to high
- Pelagic cyanobacteria dominant



- lakes flooded with light in wide areas or down to the bottom
- Nutrient content: low to medium
- benthic cyanobacteria dominant

species, like *Microcystis aeruginosa*, which occur in the light-flooded area of the pelagial zone

species, like *Planktothrix rubescens*, which stratify at a certain depth

species, like *Tychonema bornetii*, which grow benthic at the bottom of the water bodies

→ **Microscopy, molecular biology (genera and toxin genes), Toxin analyses** (in cooperation with Jutta Fastner)

Reservoir Mandichosee, sampling site next to the power plant, 09.06.2020

References of the images

Slide 7

Husky: https://www.purina.de/hunde/hunderassen/siberian-husky (26.03.2021)
Yorkshire Terrier: https://hundeinfoportal.de/hundethemen/hunderassen/kleine-hunderassen/yorkshire-terrier/ (26.03.2021)
Jack Russel Terrier: https://wamiz.de/hund/rassen/12/jack-russell-terrier (26.03.2021)

Slide 8

Augsburger Ökologische Schriften, Der Lech – Wandel einer Wildflusslandschaft, Stadt Augsburg, Referat für Umwelt und Kommunales, Amt für Grünordnung und Naturschutz, 1991

All other images: LSI

WRAP UP

- PRESENTATION MATERIAL & RECORDING 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 PRESENTERS
- IF YOU'D LIKE TO BE ADDED TO THE BENTHIC HAB WORKGROUP DISTRIBUTION LIST, SEND AN EMAIL TO THE BENTHIC HAB FACILITATORS.
- WANT TO BE A PRESENTER? CONTACT US!
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