# US EPA BENTHIC HABS DISCUSSION GROUP WEBINAR

## DECEMBER 2, 2022, 9:30am-11:00am Pacific Daylight Time

### Webinar registration:

https://zoom.us/webinar/register/WN IQIwxP4QcOL-I5Ndtvoow




**GUEST SPEAKERS:**

**KURT D. CARPENTER, RESEARCH HYDROLOGIST, U.S. GEOLOGICAL SURVEY, OREGON WATER SCIENCE CENTER**

**ELVIRA PERONA, PROFESSOR, UNIVERSIDAD AUTÓNOMA DE MADRID & ALBANO DÍEZ-CHIAPPE, PHD CANDIDATE, UNIVERSIDAD AUTÓNOMA DE MADRID**

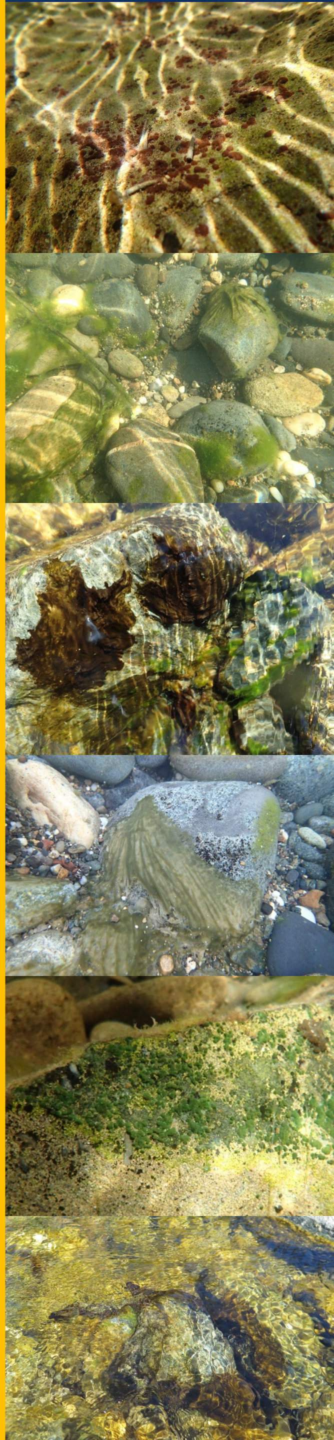
# I. AGENDA

I **Welcome, Agenda Overview, Announcements, and Introductions**
*Keith Bouma-Gregson & Margaret Spoo-Chupka, Janice Alers-Garcia*

II **Presentation: Benthic HAB Surveys in Oregon's Cascade Rivers Using Hyperspectral Technology**
*Guest Speaker – Kurt Carpenter*

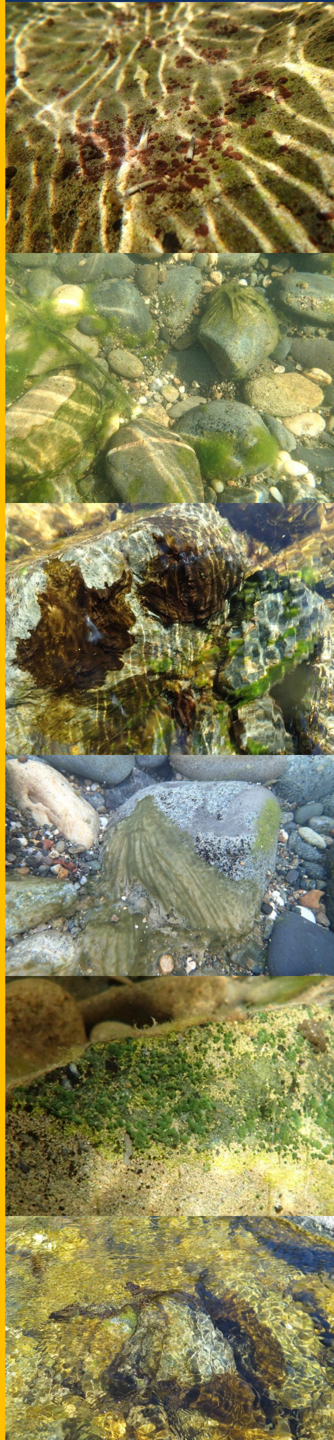
III **Presentation: Assessing Risks of Benthic Cyanobacterial Communities in National Parks**
*Guest Speakers – Elvira Perona & Albano Díez-Chiappe*

IV **Wrap Up & Next Steps**
*Facilitators & Benthic HAB members*



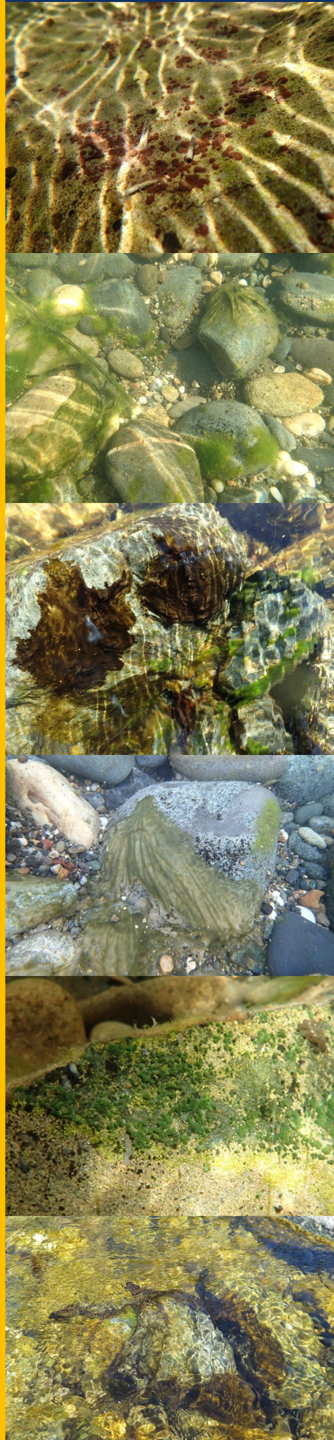
# I. INTRODUCTIONS

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



# I. ANNOUNCEMENTS

- REQUEST: Looking for cultures of benthic toxin producing cyanobacteria to aid in the development of a sandwich hybridization assay for cyanotoxin production (Project lead: Bowling Green State University). Please contact Keith Bouma-Gregson (kbouma-gregson@usgs.gov) if you have any culture material you could share
- Articles of Interest:
  - Schulte, N.O., Carlisle, D.M., and Spaulding S.A. (2022). Natural and anthropogenic influences on benthic cyanobacteria in streams of the northeastern United States. *Science of the Total Environment*
  - Putnam, S.P., Smith, M.L., Metz, T.T., Womer, A.M., Sellers, E.J., McClain, S.J., Crandell, C.A., Scott, G.I., Shaw, T.J., and Ferry J.L. (2022). Growth of the harmful benthic cyanobacterium Microseira wollei is driven by legacy sedimentary phosphorous. *Harmful Algae* Vol. 17: 102263

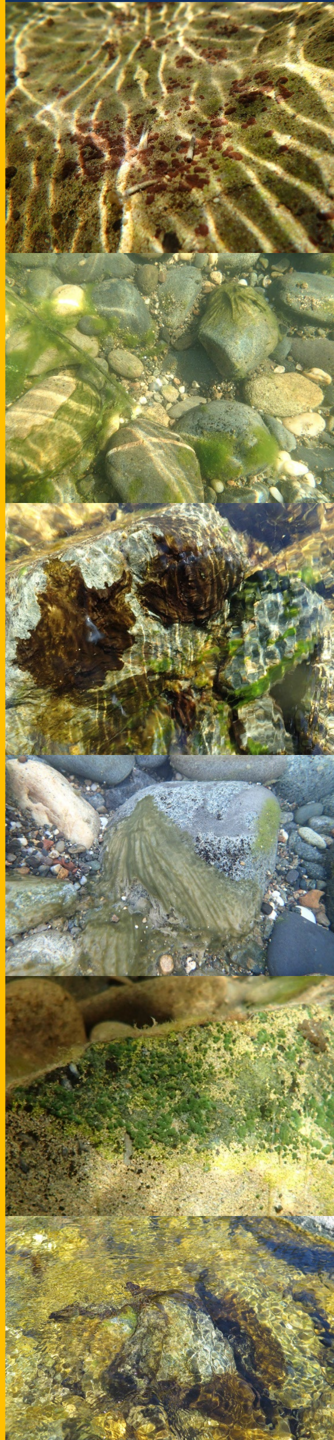


# ITEM II

# GUEST PRESENTATION:

# BENTHIC HAB SURVEYS IN OREGON’S CASCADE RANGE RIVERS USING HYPERSPECTRAL TECHNOLOGY

*Kurt D. Carpenter, Research Hydrologist, U. S. Geological Survey, Oregon Water Science Center*



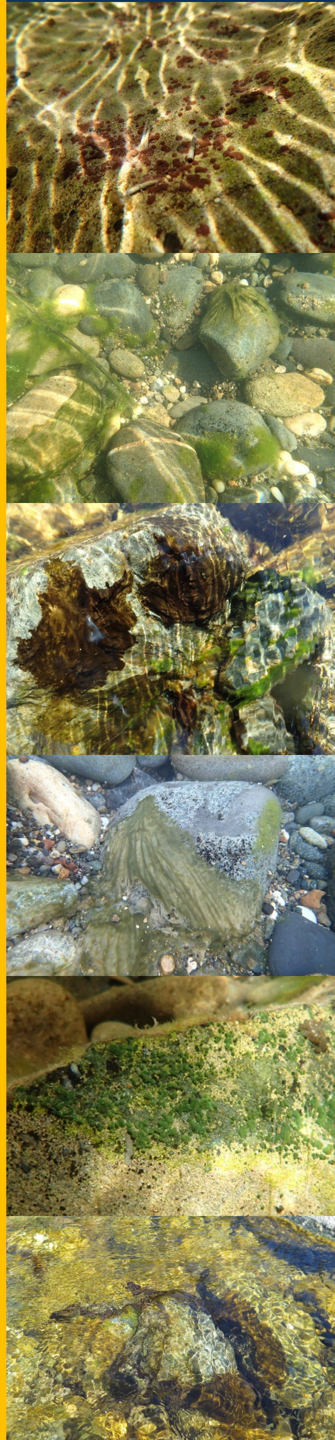
# ITEM III

# Guest Presentation: Assessing Risks of Benthic Cyanobacterial Communities in National Parks

*Elvira Perona, Professor, Universidad Autónoma de Madrid*

*&*

*Albano Díez-Chiappe, PhD Candidate, Universidad Autónoma de Madrid*



# ITEM IV
# Wrap Up & Next Steps

*Facilitators & Benthic HAB members*

| Name | Affiliation | Contact Information |
|---|---|---|
| Eric Zimdars | U.S. Army Corps of Engineers | Phone: 206-764-3506<br>Email: Eric.S.Zimdars@usace.army.mil |
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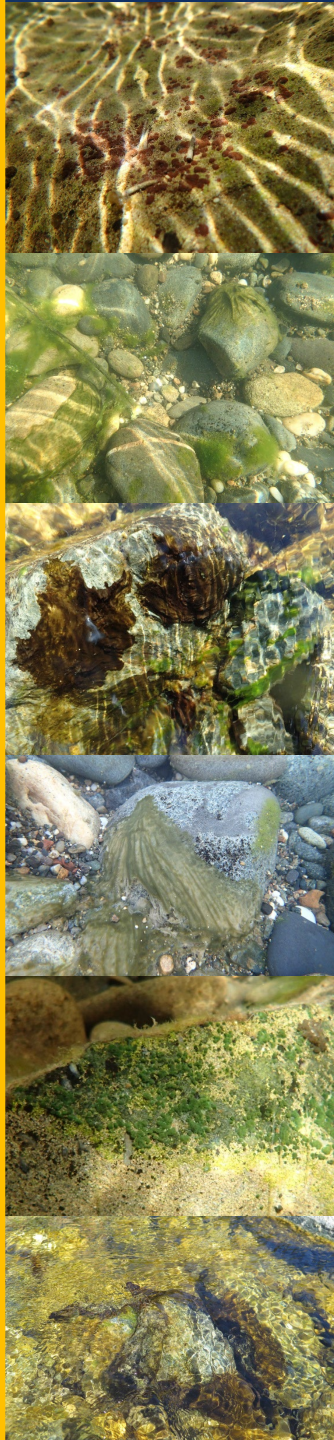
| Janice Alers-Garcia | US EPA, Washington, DC | Phone: 202-566-0756<br>Email: Alers-Garcia.Janice@epa.gov |



# Benthic HAB surveys in Oregon's Cascade Range Rivers Using Hyperspectral Technology

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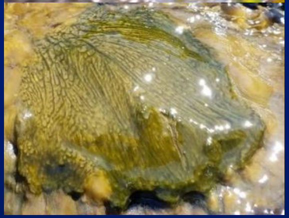
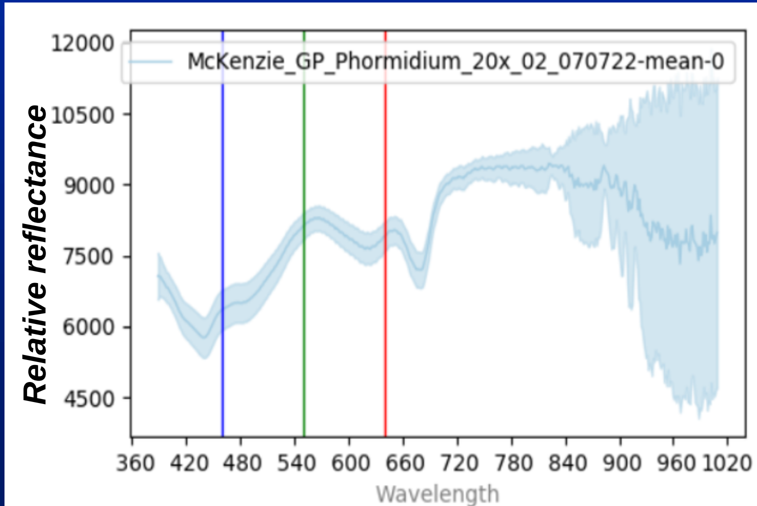
science for a changing world




Kurt Carpenter, Brandon Overstreet,
Paul Diaz, Will Long, and Wesley Noone (OR WSC)
Carl Legleiter (WMA-OSD)
Tyler King (ID WSC)
Natalie Hall (MD-DE-DC WSC)
Adam Mumford (MD-DE-DC WSC)
Victoria Scholl and Matthew Burgess (NUSO)
Terry Slonecker (USGS NCAC)
Barry Rosen (Florida Gulf Coast University)


**Benthic HAB Discussion Group**
**December 2, 2022**




*--Preliminary Information-Subject to Revision. Not for Citation or Distribution--*

U.S. Department of the Interior
U.S. Geological Survey

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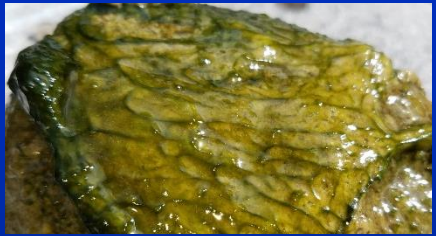
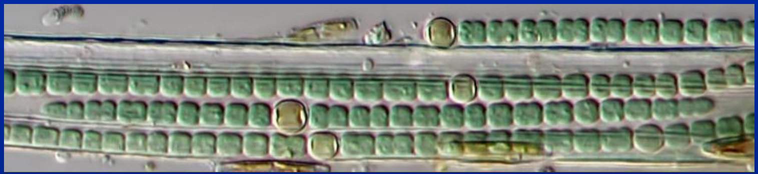
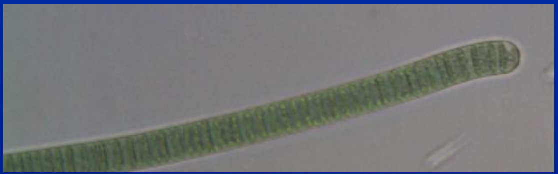
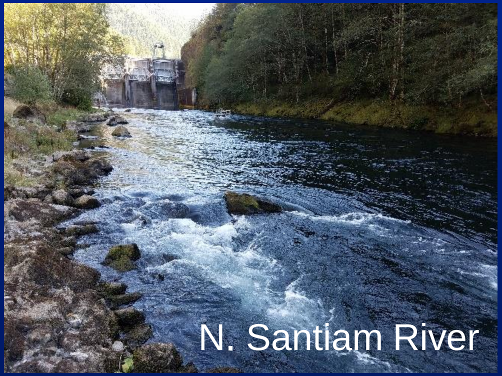
# 2016-18 USGS Study Finds Cyanotoxins Common in Oregon's Clackamas, N. Santiam, and McKenzie Rivers







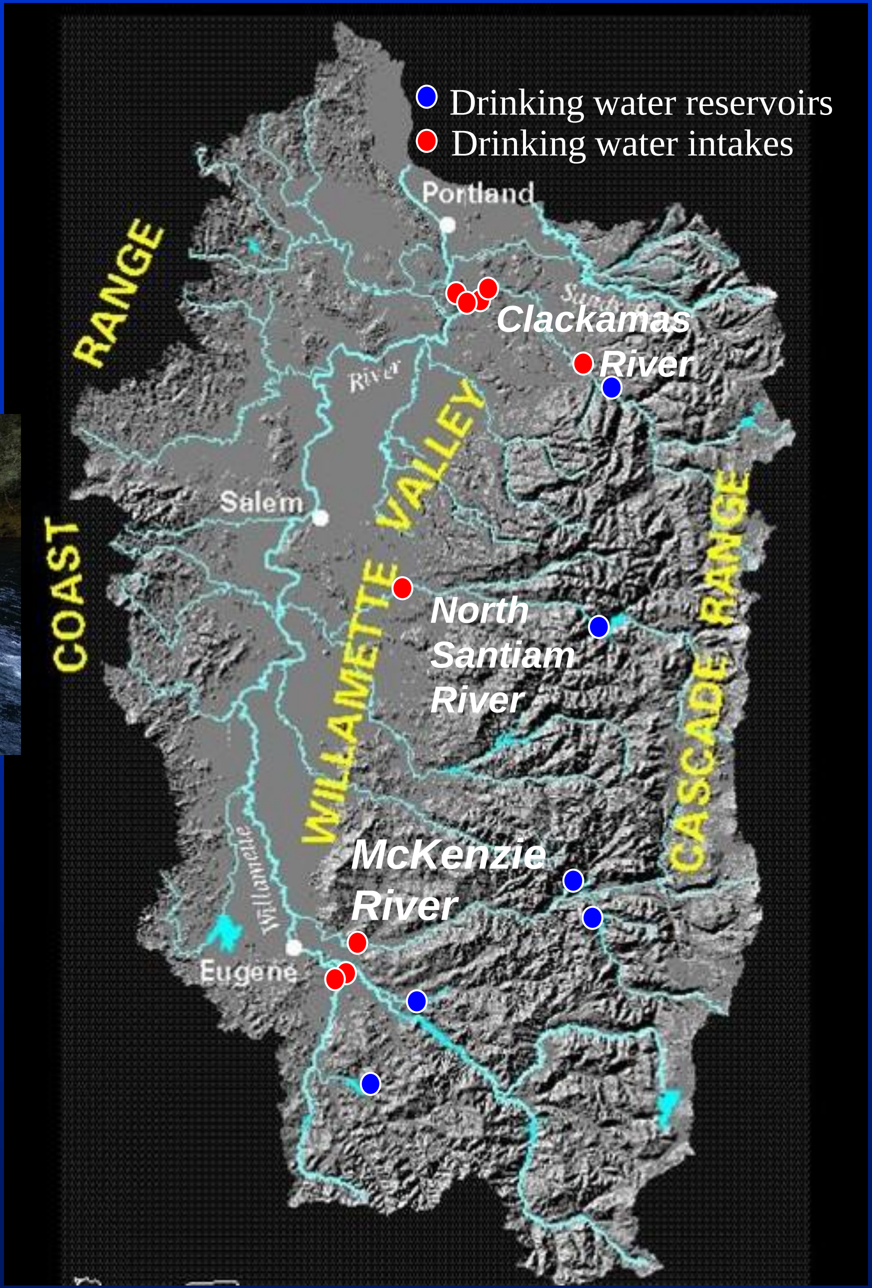





U.S. Department of the Interior
U.S. Geological Survey

# 59 Sampling Sites

- Clackamas R. / tributaries / upstream reservoir
- N. Santiam R. / tributaries / upstream reservoir
- McKenzie R. / tributaries / upstream reservoirs
- Middle Fork Willamette R. / upstream reservoir
- Coast Fork Willamette River
- Upper Willamette River

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# “Multiple Lines of Evidence” Approach

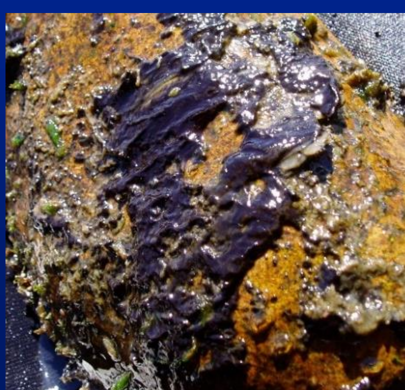
- ***Cyanobacteria colonies and mats*** (n=78) hand-picked during visual surveys
- ***Plankton net tows*** (n=84) from reservoirs and riverine sites to identify the types of cyanobacteria and potential cyanotoxins in transport to downstream DWTP intakes
- ***SPATTs*** - *S*olid-phase adsorption toxin trackers (n=122) in drinking-water intakes, tributaries, main-stem & reservoir sites
- **ELISA** tests for microcystins, cylindrospermopsins, anatoxin-*a* & saxitoxin

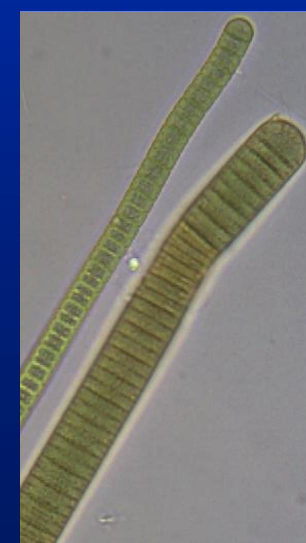
# Cascade River Periphyton Includes Toxin-Producing Cyanobacteria

*Nostoc*

*Microcoleus*

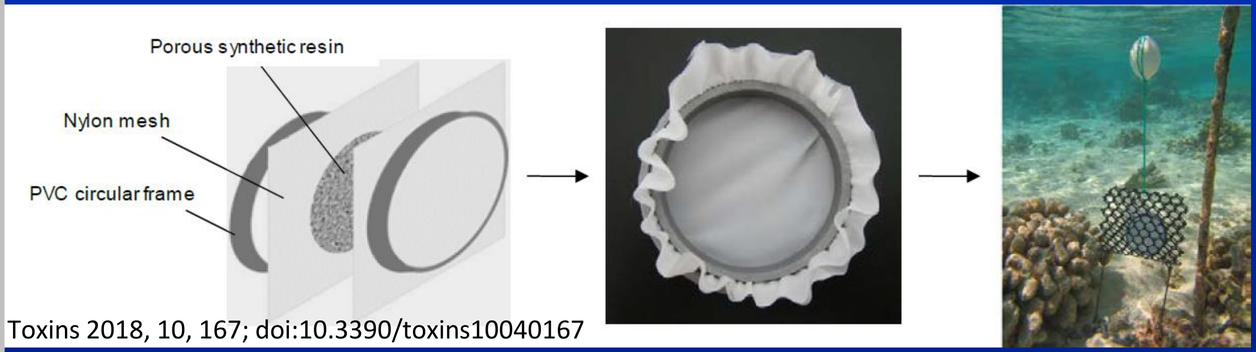
*Oscillatoria*





# SPATTs - Solid Phase Adsorption Toxin Tracking

- Time-integrating (hours to weeks) passive sampler
- HP20 “Diaion” microbead resins adsorb dissolved toxins
- Toxins extracted off resins using 50% methanol
- Extracts evaporated and reconstituted
- Concentrations of four cyanotoxins determined by ELISA
- Units: nanograms per gram of resin per day



Toxins 2018, 10, 167; doi:10.3390/toxins10040167

# Cyanotoxin Detections

- 544 cyanotoxin detections in 289 samples from 59 sites
- Anatoxin-*a* and microcystins were detected in 63% and 60% of SPATTs
- All 4 cyanotoxins detected in 8% of samples (all sample types)

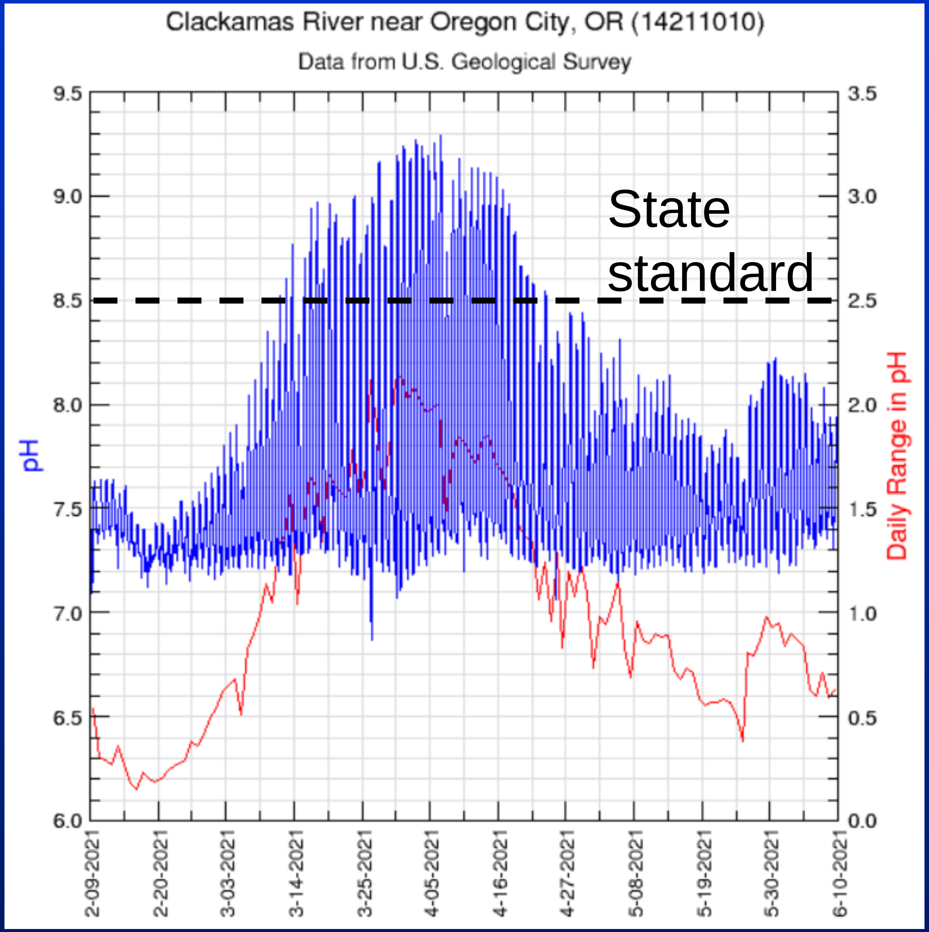
| | | Total (ADDA) Microcystins/ Nodularins | Cylindro-spermopsin | Anatoxin-*a* | Saxitoxin |
|---|---|---|---|---|---|
| All 289 samples | Detections | 202 | 78 | 135 | 129 |
| | % detection | 70% | 27% | 47% | 45% |
| 84 net tows | Detections | 66 | 21 | 23 | 66 |
| | % detection | 79% | 25% | 27% | 79% |
| 122 SPATTs | Detections | 73 | 21 | 77 | 32 |
| | % detection | 60% | 17% | 63% | 26% |
| 78 Cyanobacteria colonies/mats | Detections | 59 | 32 | 34 | 31 |
| | % detection | 76% | 41% | 44% | 40% |
| 5 Planktonic cyanobacteria | | 4 | 4 | 1 | 0 |
| | | 80% | 80% | 20% | 0% |

| Color Legend: | > 50% | 40-50% | 15-30% | 0% |
|---|---|---|---|---|

*--Preliminary Information-Subject to Revision. Not for Citation or Distribution--*

# Periphyton-Photosynthesis-Induced Diel Swings in pH

- pH up to 9.3 units
- Diel swings up to 2.0 units




https://waterdata.usgs.gov/monitoring-location/14211010

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# Post-Fire Nutrient Enrichment

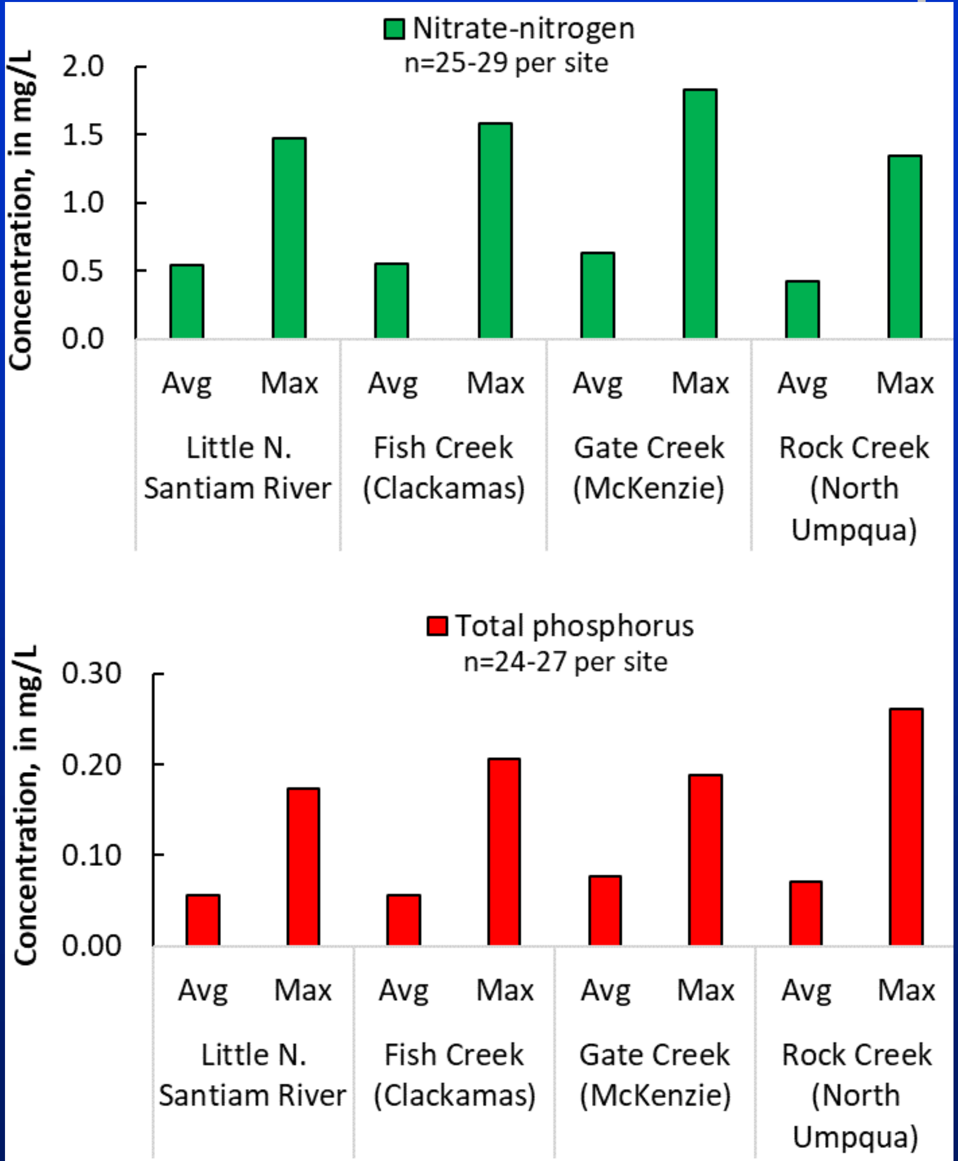
**Nitrate Concentrations**

- Max concentrations of 1.8 mg/L in fire affected tributaries during storms
- ~ 0.25-0.5 mg/L in mainstems

**Nitrogen + geologic phosphorus** = algae growth

Harmful algal blooms (HABs) & nuisance growths of "Periphyton"

**Increases in Benthic HABs and cyanotoxins?**

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# New Study Uses Hyperspectral Data at Multiple Scales to Characterize Periphyton

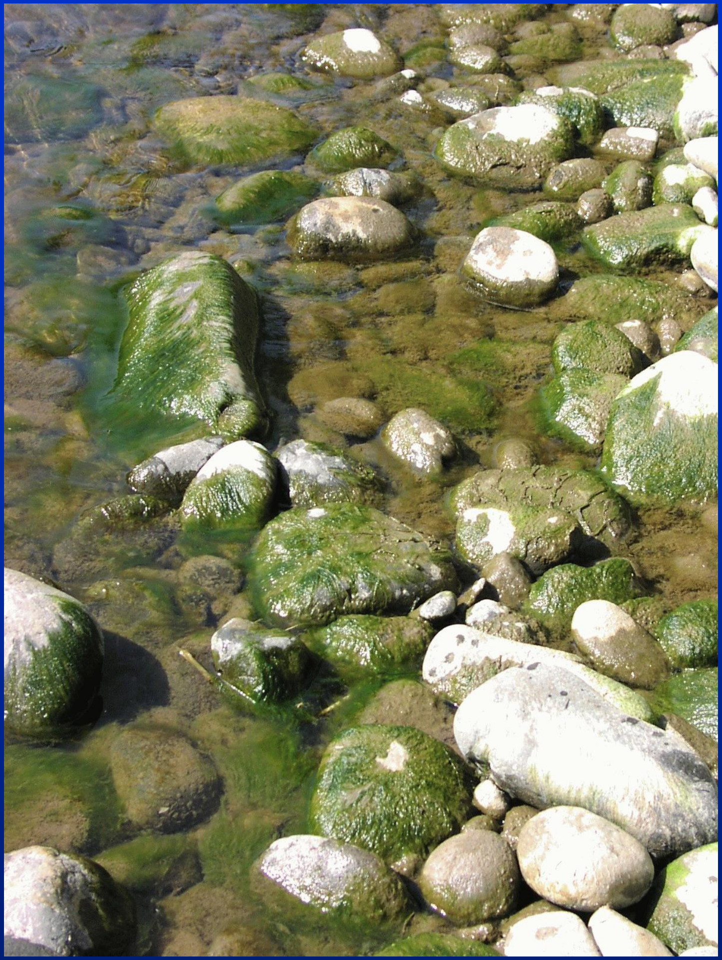
Goals

- Collect hyperspectral profiles of periphyton
- Expand reference database of algal reflectance “signatures” with hyperspectral microscope
- Utilize hyperspectral data to monitor periphyton cover, biomass, and possibly major types over time

# Challenges with Sampling Periphyton

- Many rivers only partially wadable
- Most often riffle scale at best
- Difficult/expensive to sample
- Can we monitor with remote sensing?





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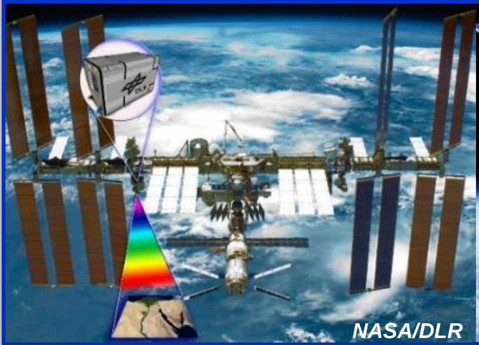
# Multi-Scale Hyperspectral Instruments







Collecting periphyton spectra,
North Santiam River

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# Hyperspectral Sensors

| Sensor | Platform | Wavelength range (nm) | Spectral sampling interval (nm) | Number of bands |
|---|---|---|---|---|
| **DESIS** | ISS | 400-1000 | 2.55 | 235 |
| **Other satellites (Sentinel, etc.)** | TBD | TBD | TBD | TBD |
| **Resonon Pika L** | UAS/Airplane | 400-1000 | 1.3 | 900 |
| **Headwall Nano** | UAS | 400-1000 | 2.2 | 640 |
| **ASD HandHeld2 Pro** | Field | 325-1075 | 1 | 750 |
| **Ocean Insight Flame** | Field | 350-1000 | 1.3 | 485 |
| **Resonon Pika XC2** | Microscope | 400-1000 | 1.3 | 447 |

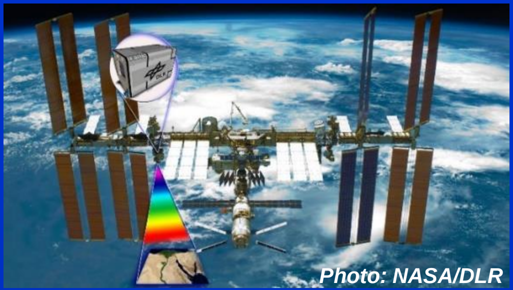


Photo: NASA/DLR

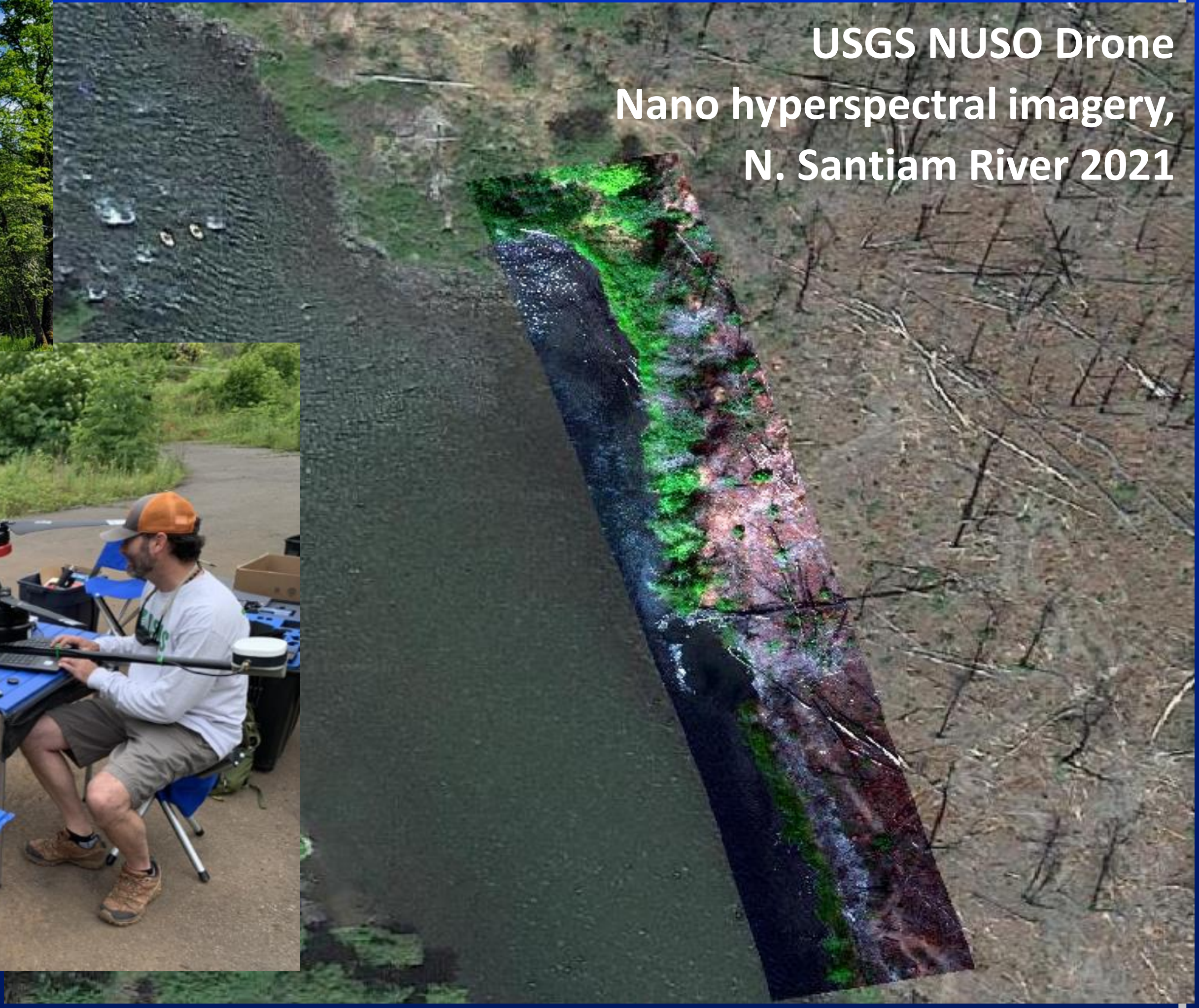
Photo: Civil Air Patrol

https://resonon.com/Pika-XC2



# Drones

- M600 drone w HS camera (Pika L)
- Parrot Anafi drone (high-res RGB)
- FLIR




# Field Spectra

- Tripod-mounted FLAME & ASD HS Cameras
- Above and below water measurements
- Targeted periphyton types (green algae, diatoms, cyanobacteria)
- Transect based measurements

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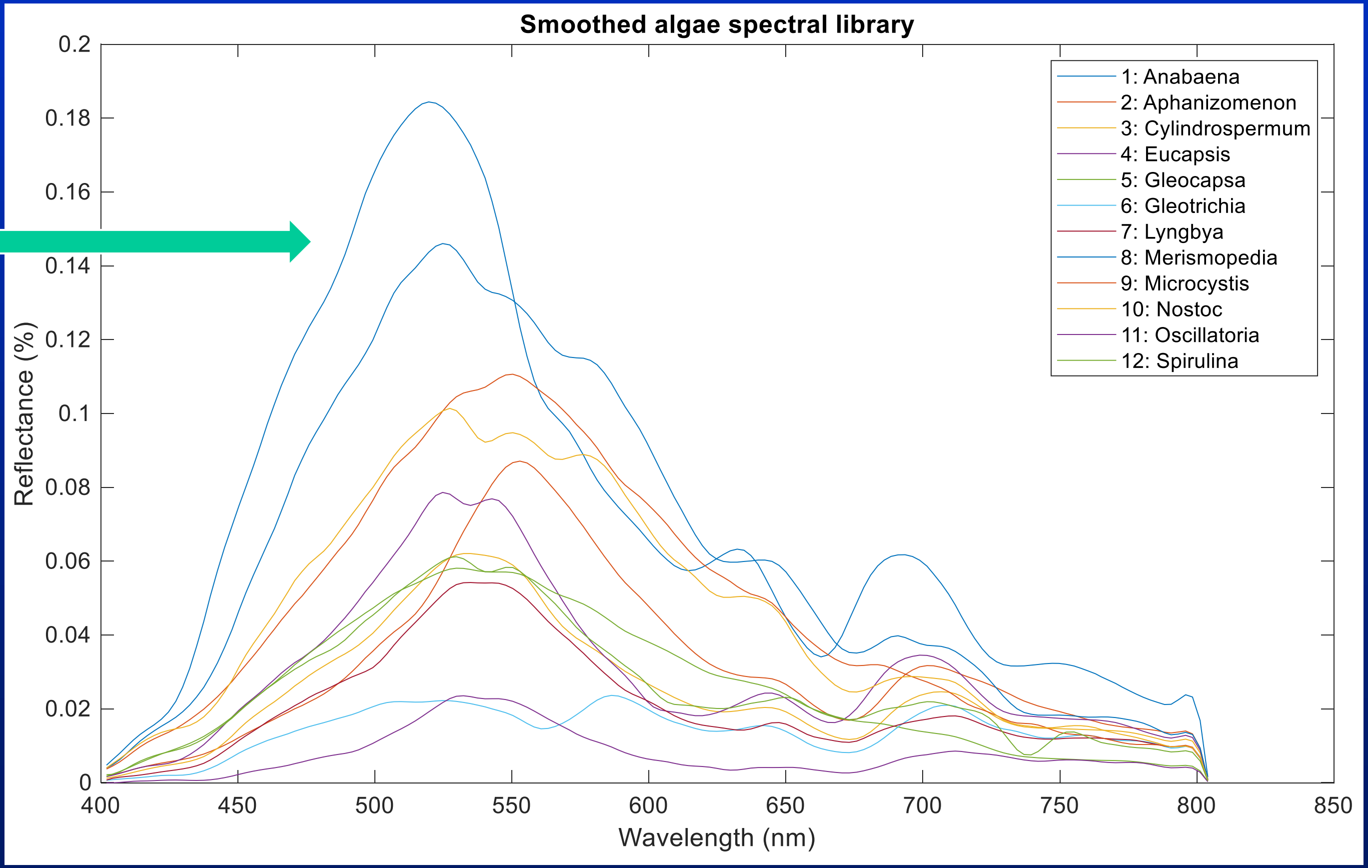
# Hyperspectral Microscope

- Reference Database of Spectral Reflectance "signatures"
- Added green algae, diatoms & 3 types of cyanobacteria


https://www.sciencebase.gov/catalog/item/get/5d5d6b3de4b01d82ce91e4dc

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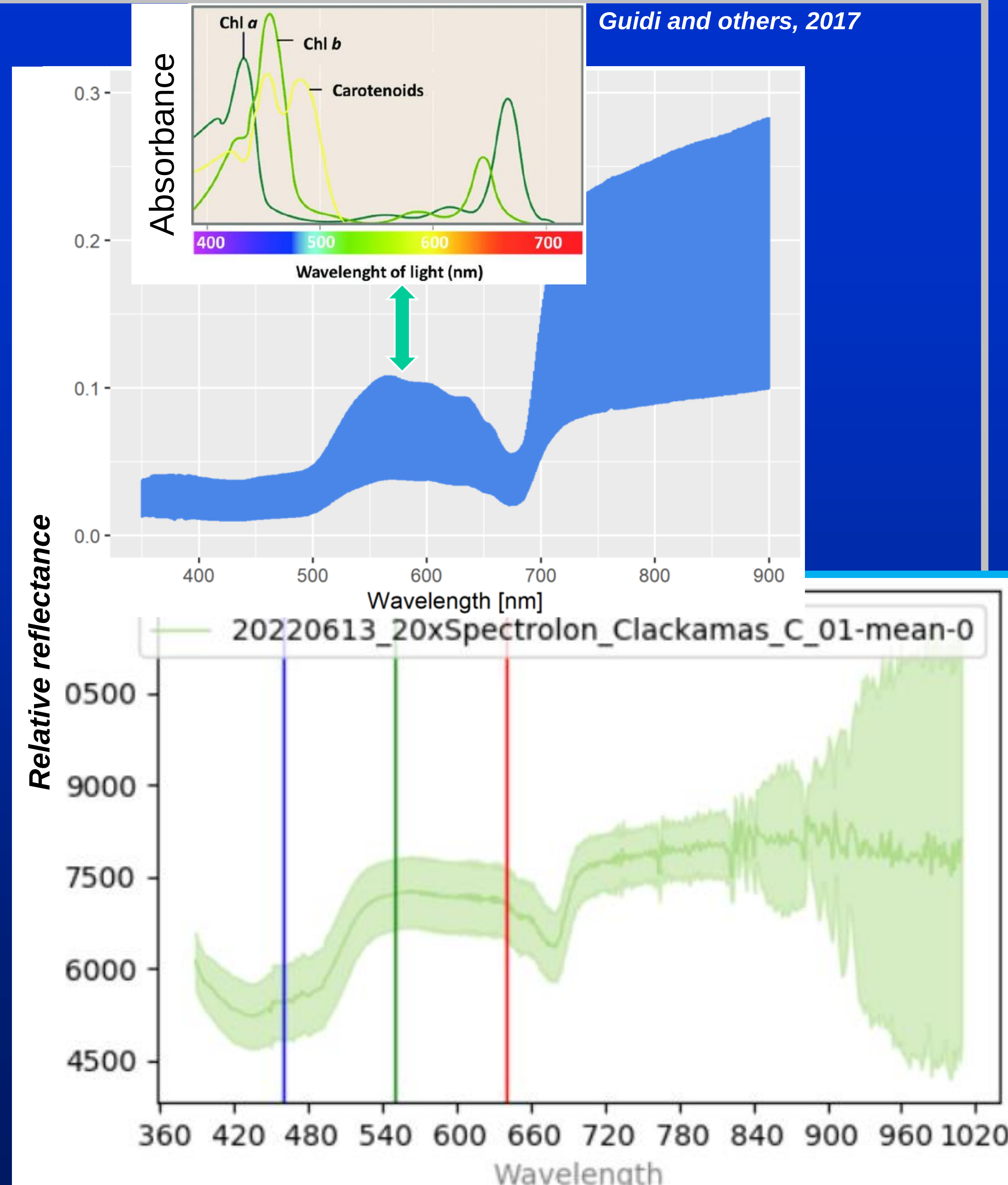
# Green Algae Spectra

*Ulothrix*

- **Rise at 500-650 nm**
- **Corresponds to inverse of the absorbance spectrum of chlorophylls a and b**

*Guidi and others, 2017*

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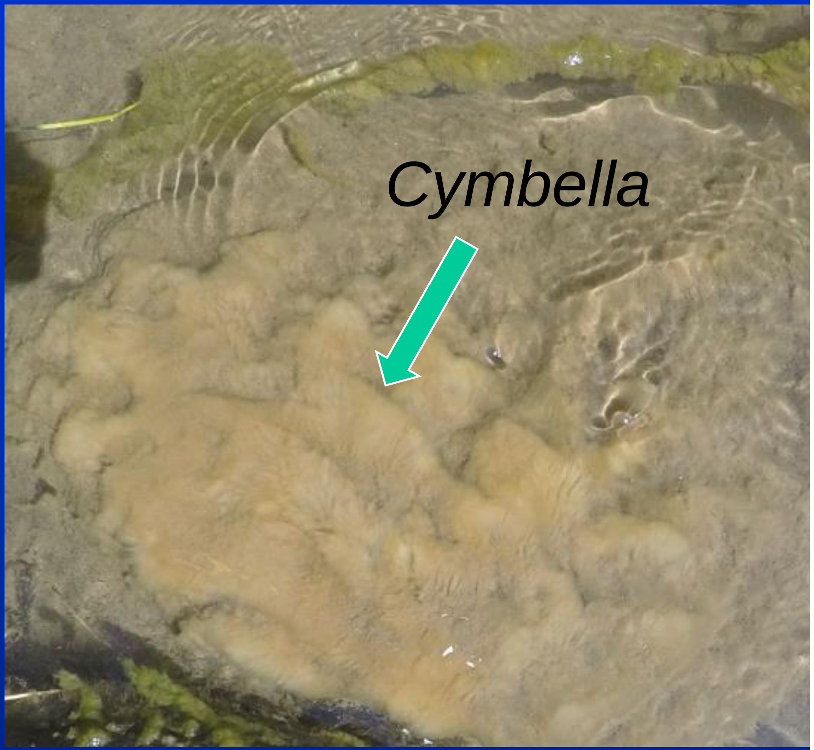
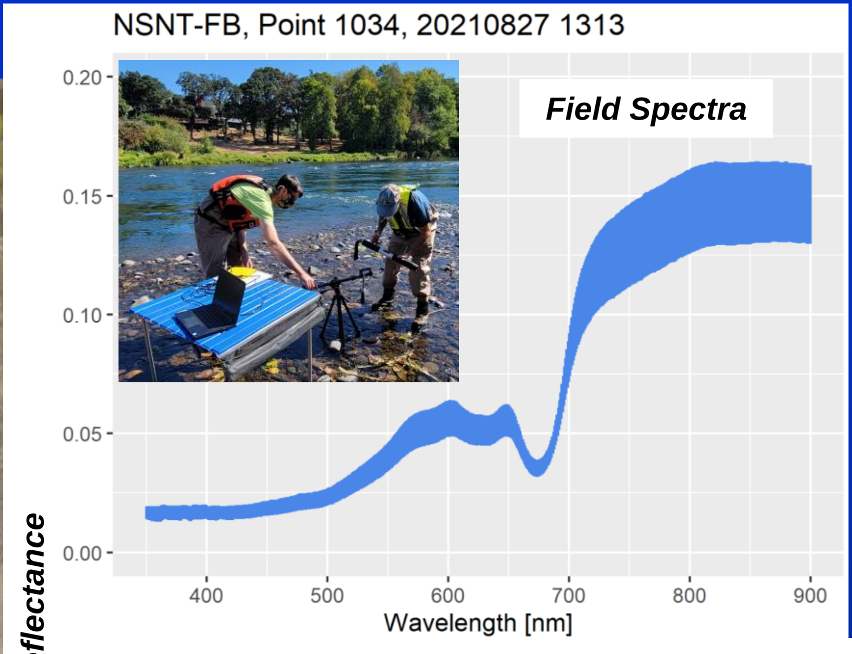
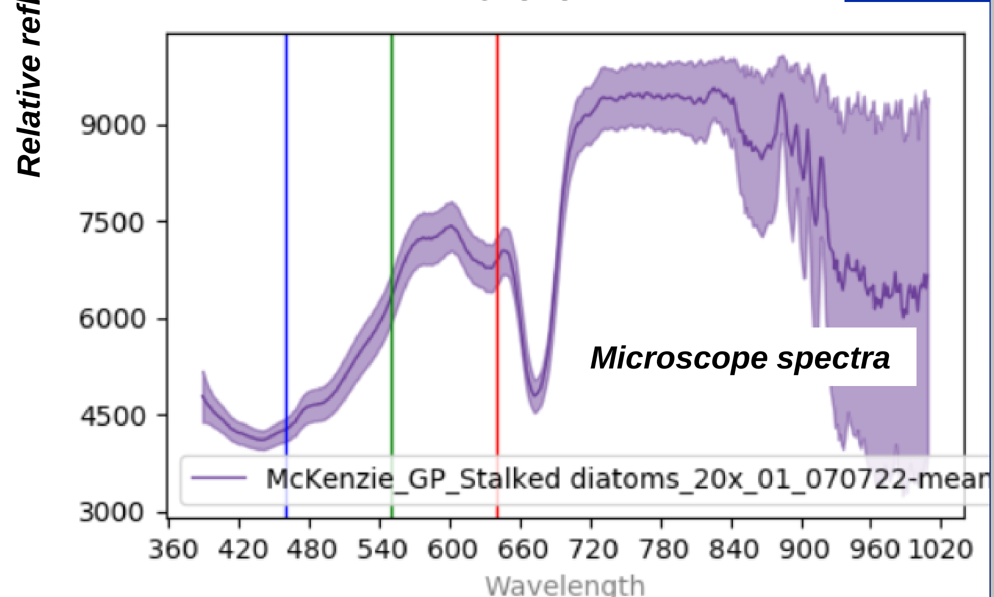
# Diatom Spectra




- Similar rise at 500-650 nm
- Good agreement between field spectra and micro-scope spectra

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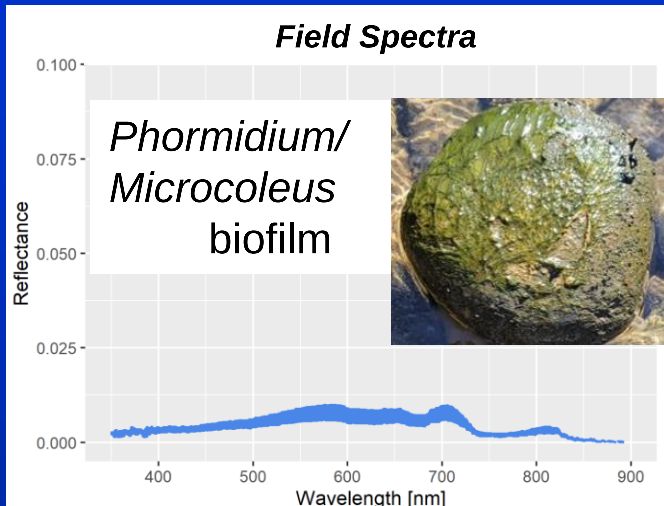
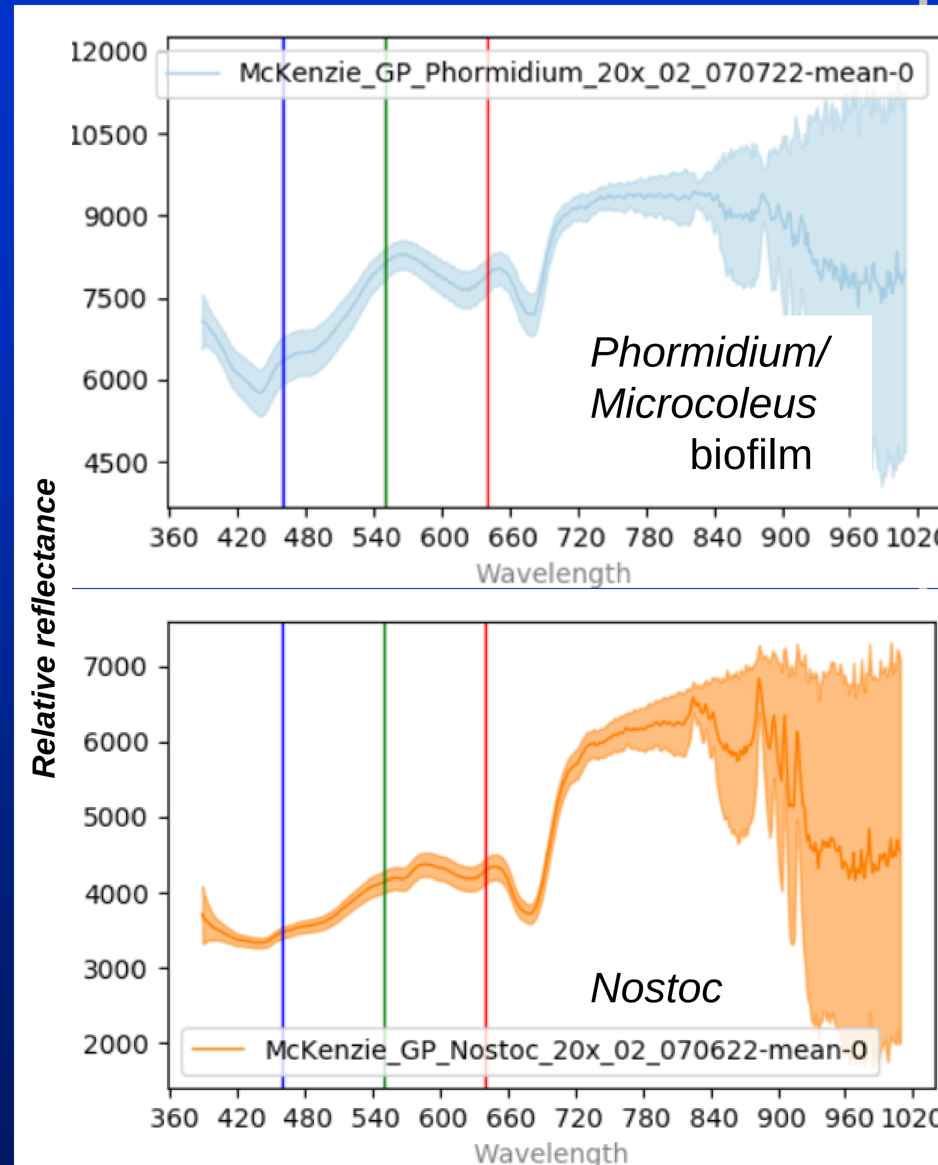
# Cyanobacteria Spectra




Microscope spectra




--Preliminary Information-Subject to Revision.
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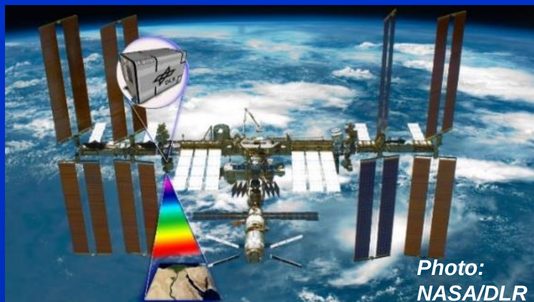
- Pigments: chlorophyll-*a*, carotenoids, phycocyanin & phycoerythrin
- Efficient light harvesters

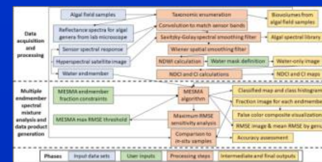
# Upper Klamath Lake

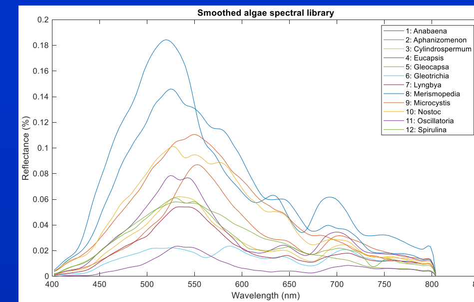



## Satellite Data & In situ Algal Field Sample Data

Photo: Justin Von Tungeln

## "SMASH"






## Spectral Data from Microscope

## Multiple Lines of Evidence

*Aphanizomenon*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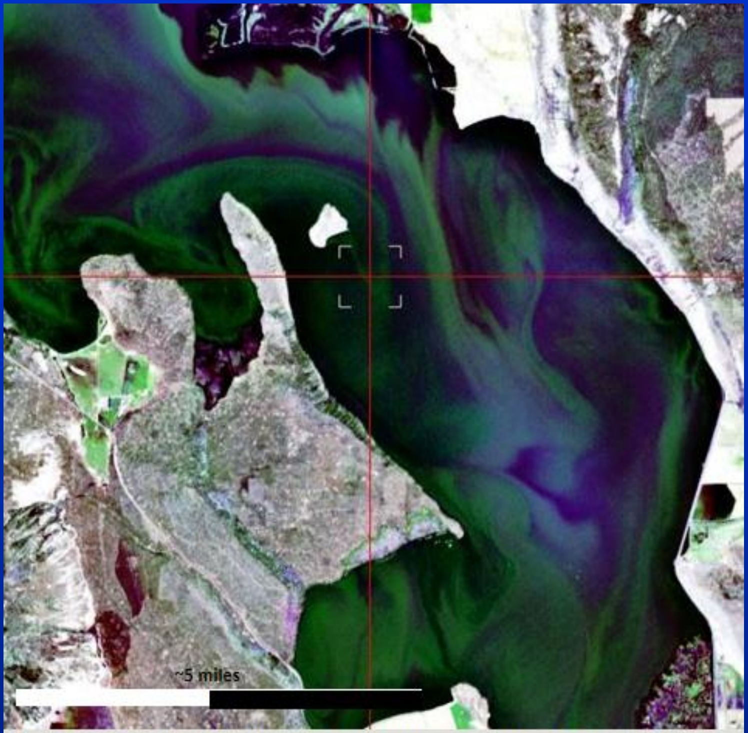
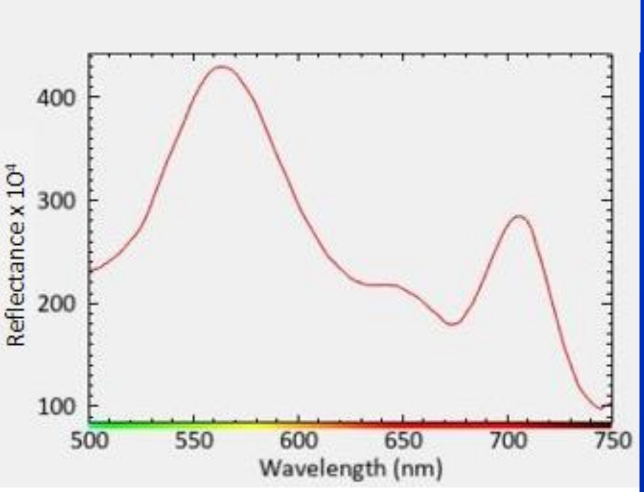
Legleiter et al., 2022 (https://doi.org/10.1016/j.rse.2022.113089).

# DESIS vs Microscope-Derived Spectra

DESIS

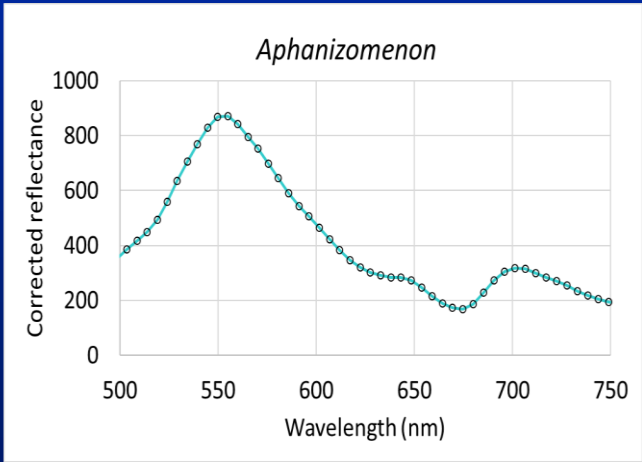



Upper Klamath Lake, Oregon DESIS image August 10, 2020




DLR

Lab


*Slonecker and others (2021)*
*Remote Sensing, v. 10 p. 66*

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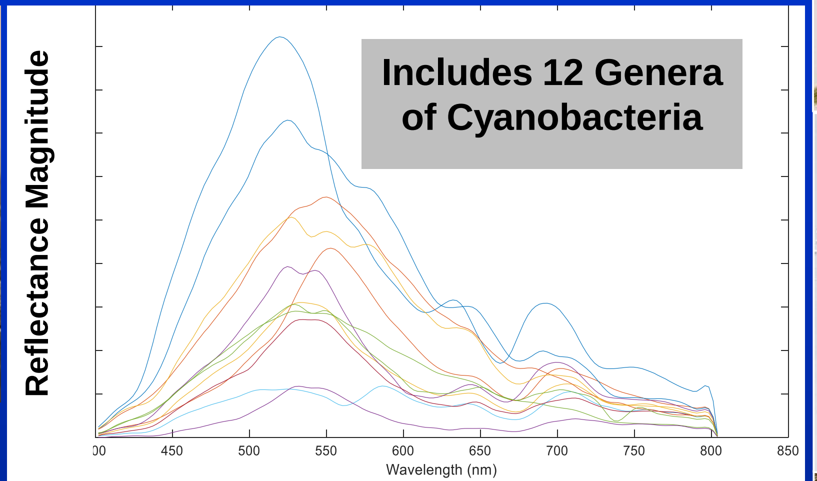
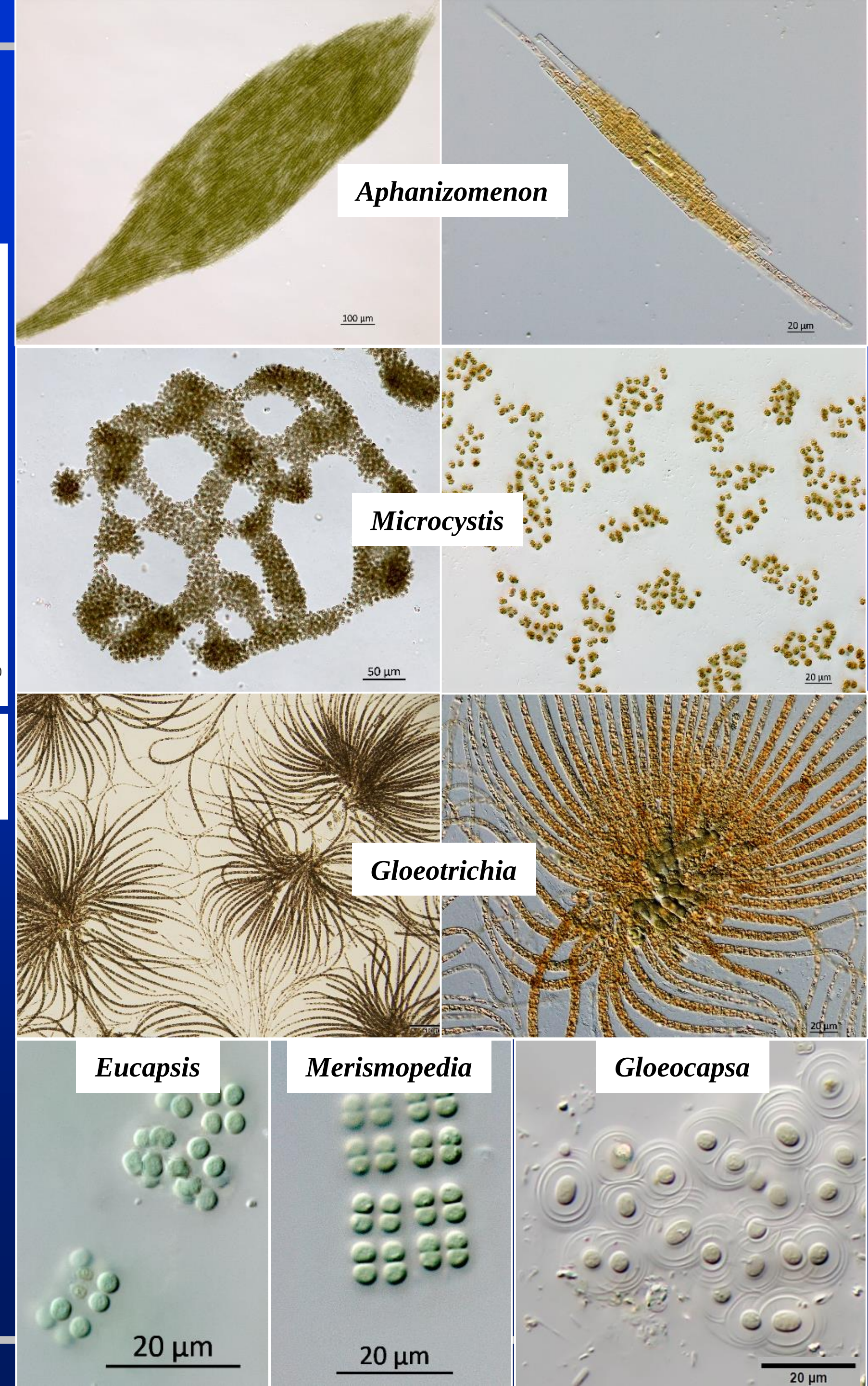
# Microscope-Derived Reflectance Spectra



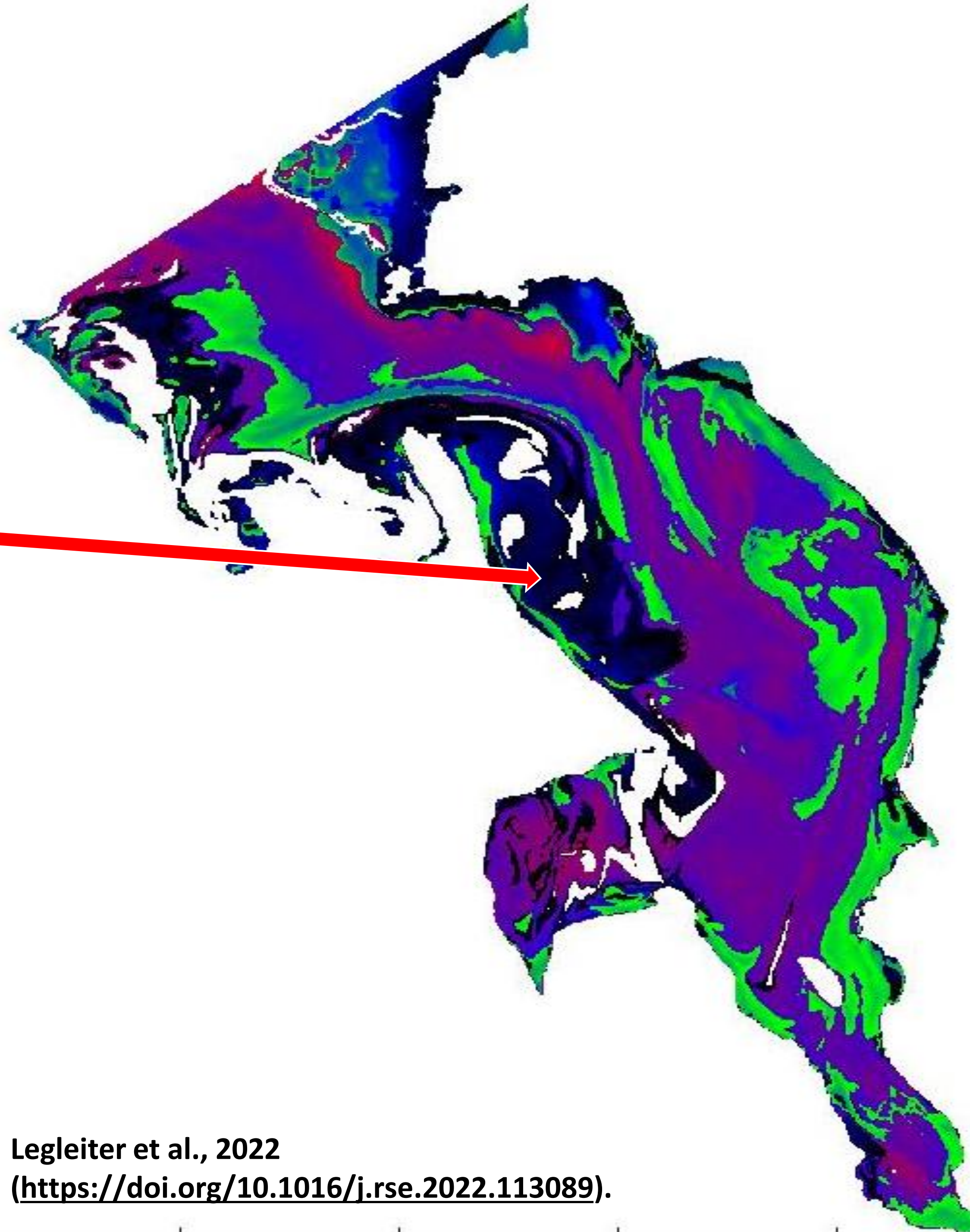

Legleiter et al., 2022 (https://doi.org/10.1016/j.rse.2022.113089).

- Reflectance Spectra Reference Dataset
- Mostly Planktonic Cyanobacteria, but includes some benthic taxa (*Oscillatoria*)
- *Phormidium*, *Nostoc* and *Dolichospermum* imaged in 2022



# Reflectance Signature Upper Klamath Lake

- Red = *Aphanizomenon*
- Green = *Gloeotrichia*
- Blue = *Water*
- Black = *Oscillatoria* (Benthic)



Legleiter et al., 2022 (https://doi.org/10.1016/j.rse.2022.113089).

# Potentially Toxin Producing Cyanobacteria along the Columbia River at Steigerwald Lake Wildlife Refuge, WA

Oct. 10, 2022 Indigenous
Peoples Day Field Report

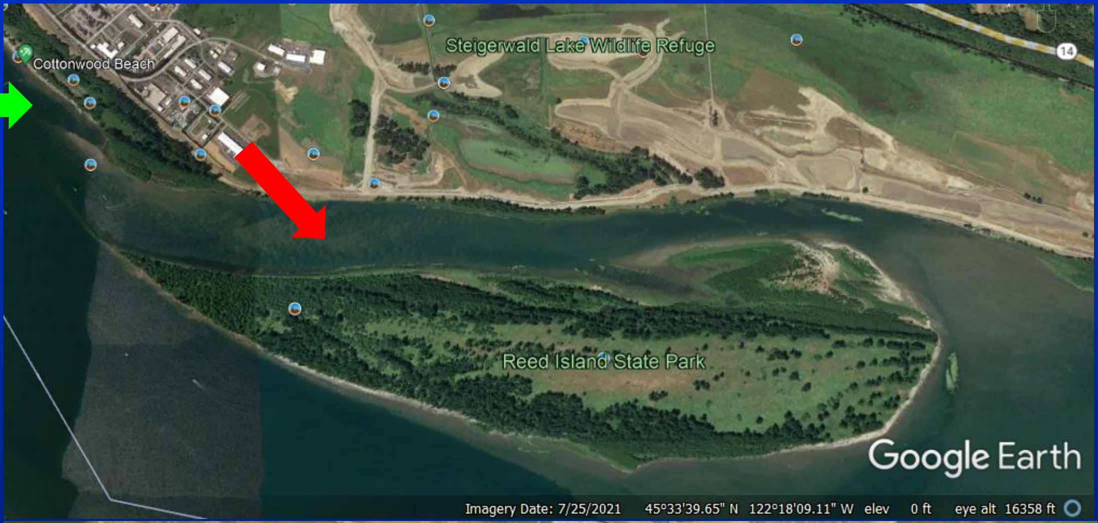
Kurt Carpenter, ORWSC

****USGS Provisional Data –
Not for Release or Distribution****

U.S. Department of the Interior
U.S. Geological Survey

# NEW Wildlife Refuge @ Steigerwald Lake

- Cyanobacteria mats suspected in Google Earth imagery along margin in channel north of Reed Island (red arrows)
- Photos taken from green arrow (Cottonwood Beach), a popular area to exercise dogs




- Close up of cyanobacteria mats in the "dunes"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# NEW Wildlife Refuge @ Steigerwald Lake

- Close up of cyanobacteria mats

# Filamentous Cyanobacteria

- Widespread growths in the shallow and sandy margin habitat
- Rooted macrophytes also common
- Boat/barge traffic causing much disturbance of shoreline

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# Filamentous Cyanobacteria

- Small mats were flaking off the bottom and floating where dogs can ingest – based on the intense wave action, boat/barge traffic likely exacerbates this sloughing

# Filamentous Cyanobacteria

- Productive mats showing oxygen bubble formation from active photosynthesis

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# Filamentous Cyanobacteria

- **Dried mats were prolific along the shoreline above the tidal line**
- **It would be good to test the mats, live and dead, for cyanotoxins, and deploy SPATTs in these highly recreated areas**
- **Also found live cyano mats (*Phormidium*) in Big Tree Creek, Lewis River, WA, a Columbia R. tributary this summer**

USGS

# Thanks to My Colleagues Hyperspectral-Periphyton Research Team

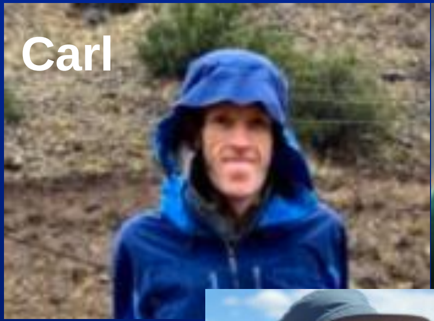
## Oregon





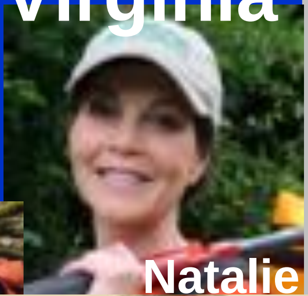

## Idaho


## Colorado



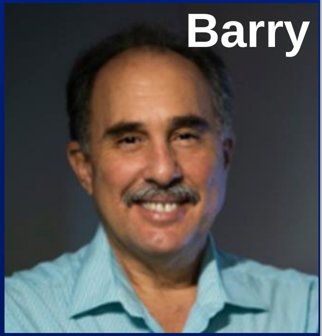



## Virginia




## Florida



# Assessing risks of benthic cyanobacterial communities in National Parks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UAM Universidad Autónoma de Madrid


US EPA BENTHIC HABS DISCUSSION GROUP

# OUTLINE

Assessing risks of benthic cyanobacterial communities in National Parks

**1) Benthic cyanobacteria and cyanotoxins**

**2) Benthic Mats and toxicity in Spain**

- *Microcoleus/Phormidium* benthic mats
- *Phormidium* species distribution
- Toxicity of *Microcoleus* mats
- Increasing abundance in time relation with temperature

**3) Toxigenic benthic mats in Spanish National Parks**

- Guadarrama Mountains National Park: rivers
- Monfragüe National Park: rivers and ponds

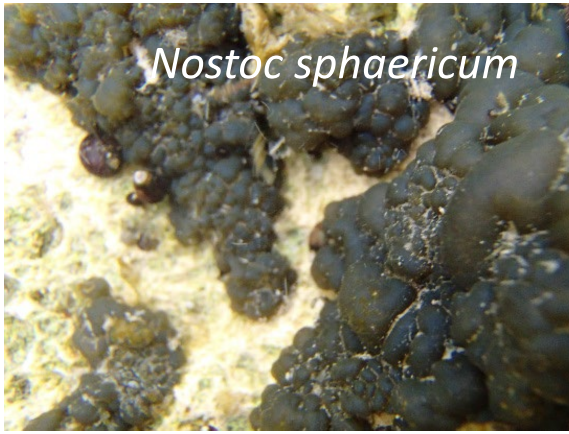
**4) Future directions**

**5) Take-home messages**

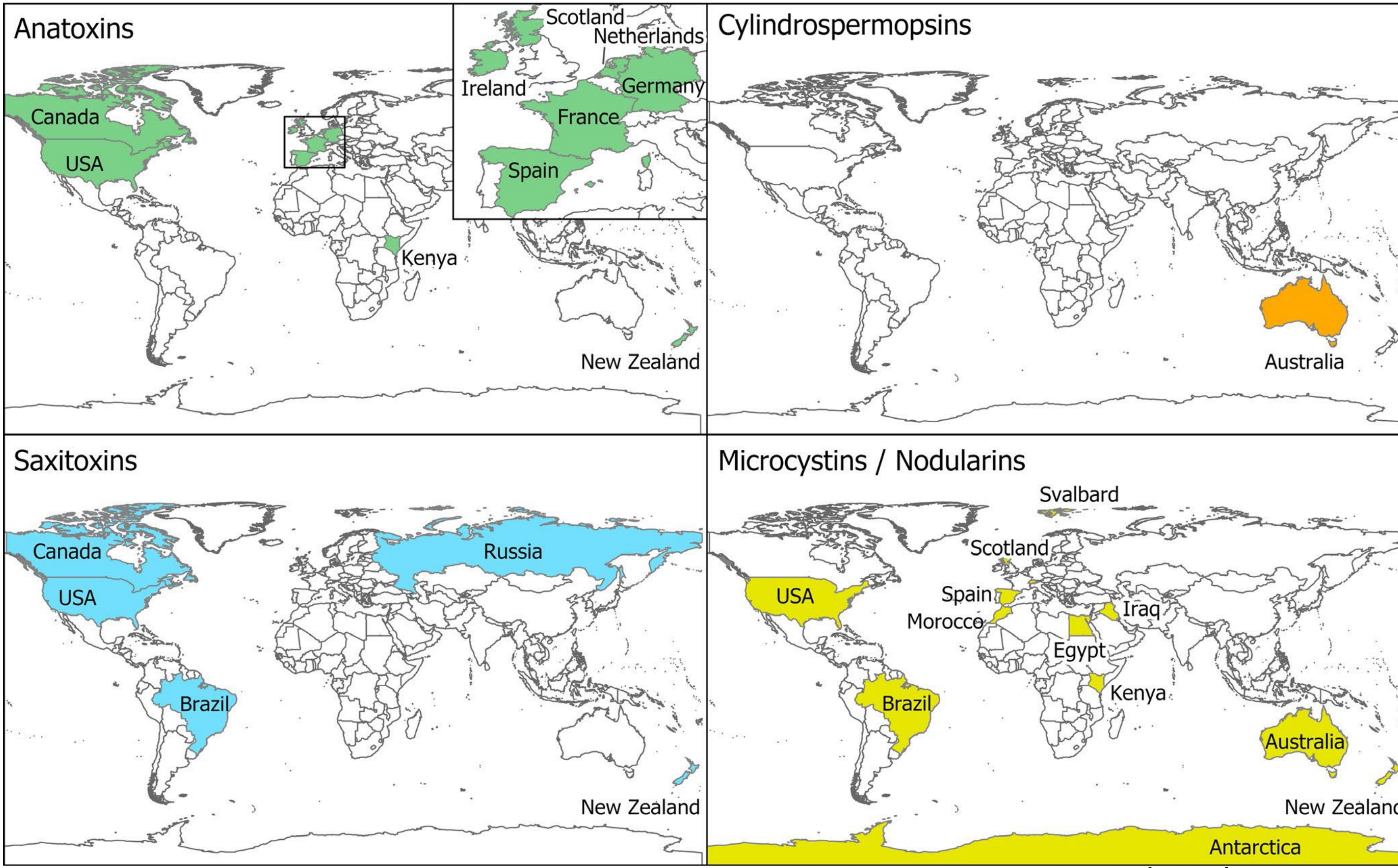
# Benthic cyanobacteria and cyanotoxins

Benthic cyanobacterial communities :

- are important in rivers and are associated to eutrophic in lentic habitats
- may become dominant in rivers, showing **high biomass and species richness** even in oligotrophic conditions
- can grow macroscopically forming colonies and/or mats
- Due to the intense and varied secondary metabolisms can synthetize toxins: microcystins, anatoxins, saxitoxins….
- have caused health hazards to animals and human


Toxigenic benthic mats have been reported in 19 countries (6 in Europe)
More information is needed.




*Wood et al, 2020*

# *Microcoleus/Phormidium*-mats and Anatoxin

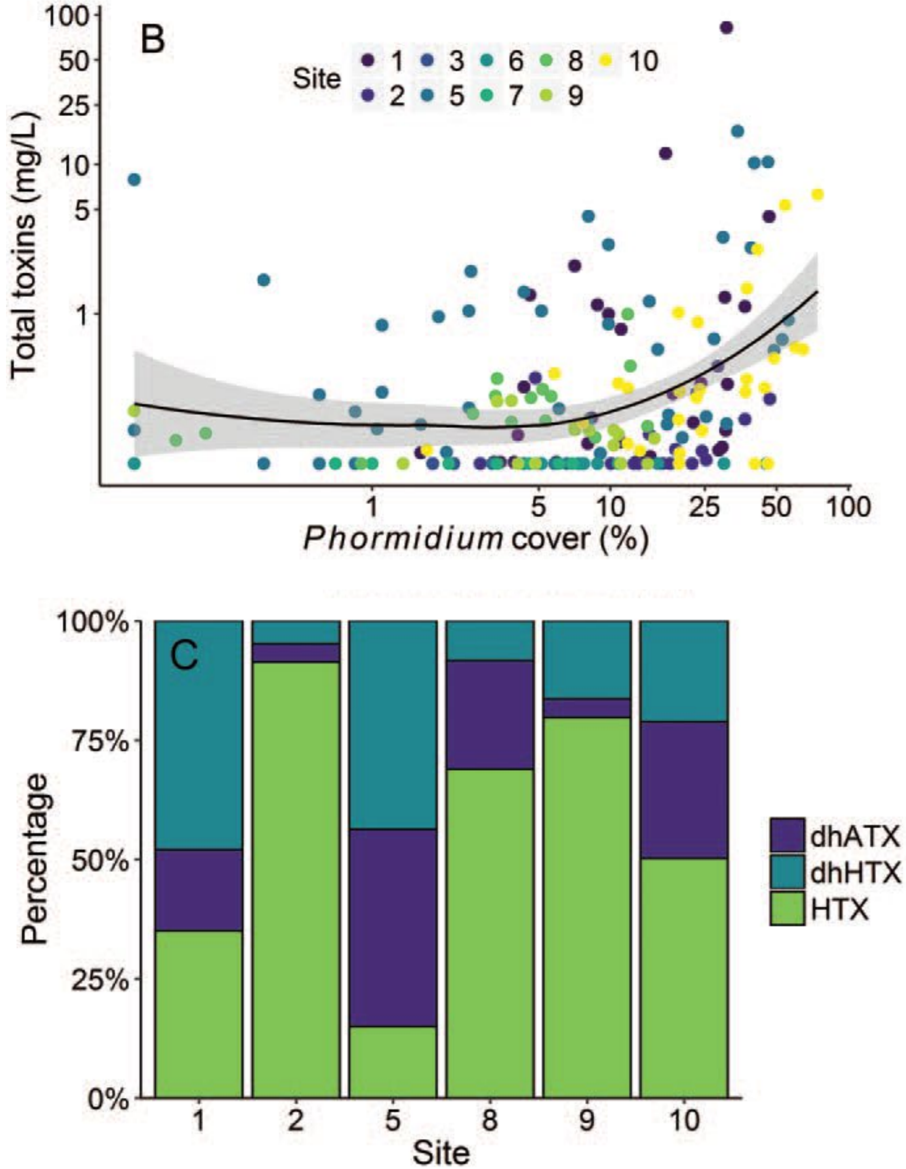
- *Microcoleus/Phormidium* mats are toxic by ingestion
- Can kill dogs > 100 dogs, in last 5-10 years (ATX or HTX in their stomach)
- High percentage of toxicity (67 % mats with ATX in New Zealand)
- Health warning advisors

- Drivers affecting *Microcoleus/Phormidium* proliferation

- Hydrological regime, up to 0.4
- Temperature >15 ºC
- DIN < 0,2 mg/L,
- PRS, 0,01 mg/l
- Light

# *Microcoleus/Phormidium*-mats and Anatoxin

- Spatial and temporal distribution of proliferation and toxin production
- More toxins at higher biomass
- Anatoxin and homoanatoxin more toxic and abundant
- High toxin concentration at low conductivities
- Low toxin concentration with > 0,2 mg /L DIN



# OUR PREVIOUS RESEARCH

To study and identify the presence of toxigenic mats in Spanish rivers

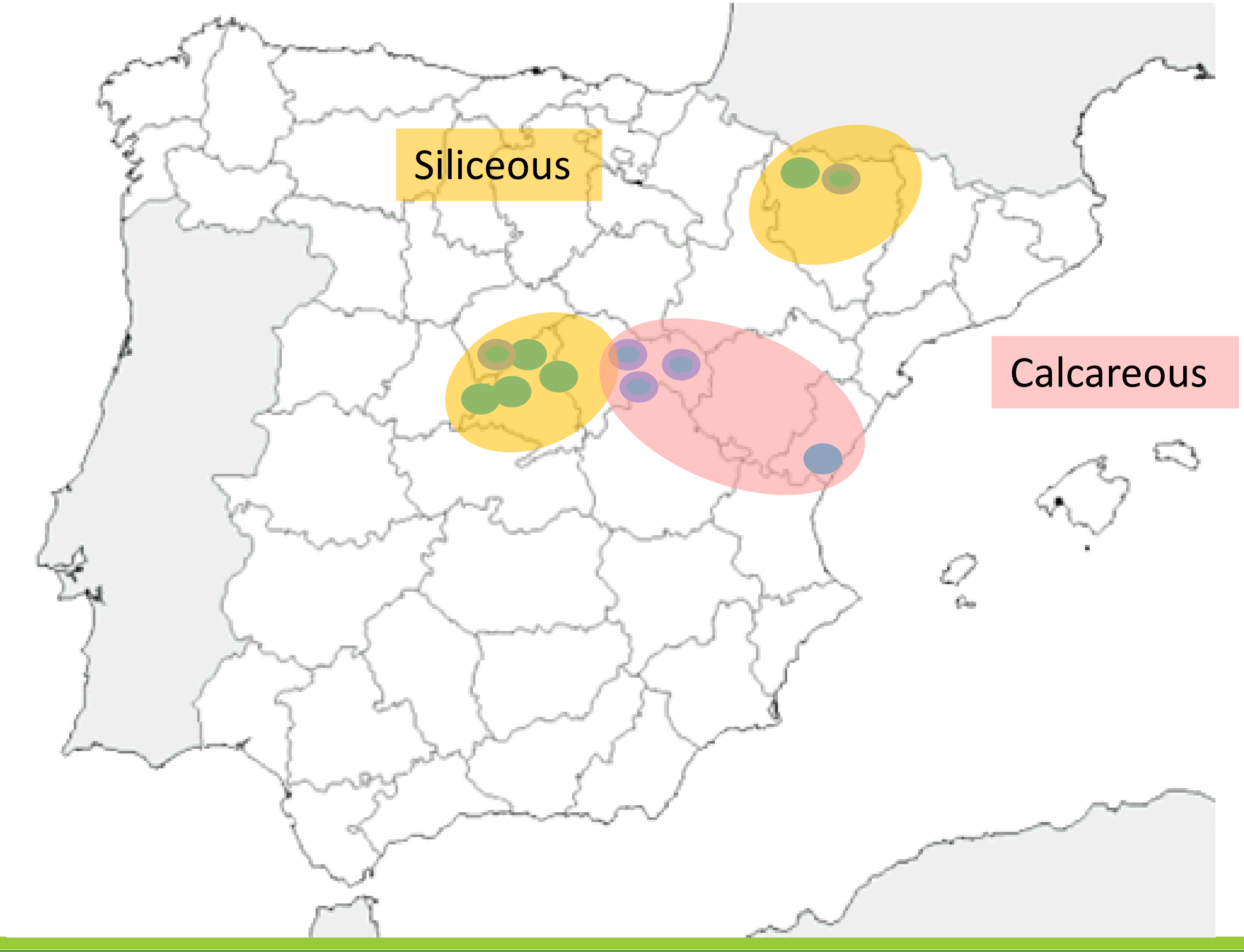
## by means of

- Characterization of mats :
  - Phenotype
  - Genotype
  - Structure
- Analysing the environmental parameters
- Screening the presence of one of the anatoxin genes (*Polyketide synthetase gene, PKS)*

# Rivers sampling

11 Mountain Rivers (oligotrophic to eutrophic)

**+30 individual mats**
***Microcoleus/Phormidium***




R. Caldarés
R. Brazato

R . Guadiela
R. Tajo
R. Escabas
R. Mijares

R. Manzanares
R. Eresma
R. Lozoya
R. Jarama
A. Mediano,

# *Microcoleus/Phormidium* abundance

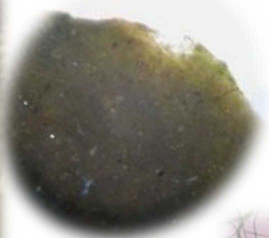
In most cases mats proliferations were not highly abundant

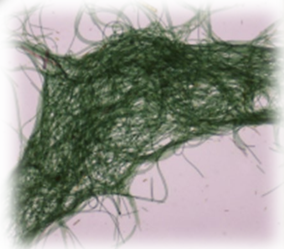
| Site | lithology | Trophic status (P) | *Phormidium*-like Cover (%) |
|---|---|---|---|
| Mediano | Siliceous | Oligo-mesotrophic | 10-20 |
| Manzanares | Siliceous | Oligo-mesotrophic | 5-10 |
| Lozoya | Siliceous | Mesotrophic | 2 |
| Eresma | Siliceous (high conductivity) | Mesotrophic | 2 |
| Jarama | Siliceous | Mesotrophic | <1 |
| Escabas | Calcaerous | Eutrophic | 2-5 |
| Tajo | Calcareous | Mesotrophic | 30 |
| Guadiela | Calcareous | Mesotrophic | 5 |
| Brazato | Siliceous | Oligo-mesotrophic | 20-25 |
| Caldares | Siliceous (high mountain) | Oligotrophic | 2-5 |
| Mijares | Calcareous | Mesotrophic | 5 |

# *Phormidium* like-mats in Spain

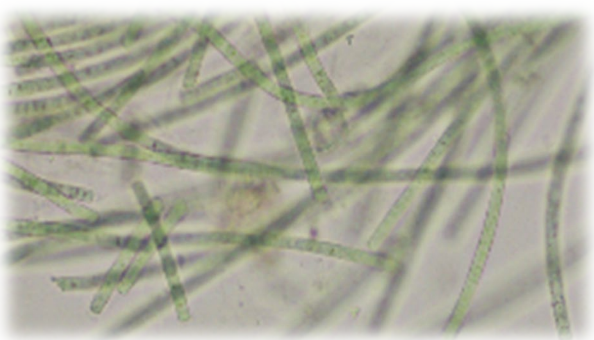
## Brown Mats

## Green Mats



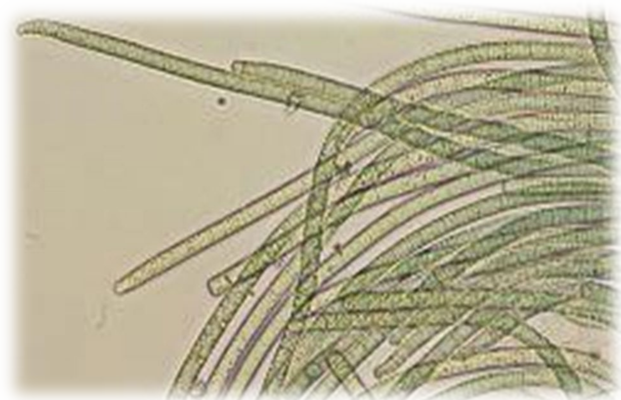


## Dominance



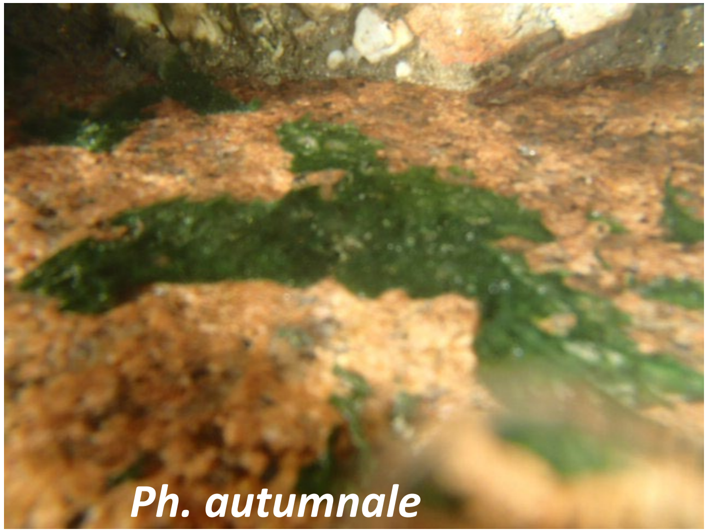
*Ph. corium*

*Ph. aerugineo-caeruleum*

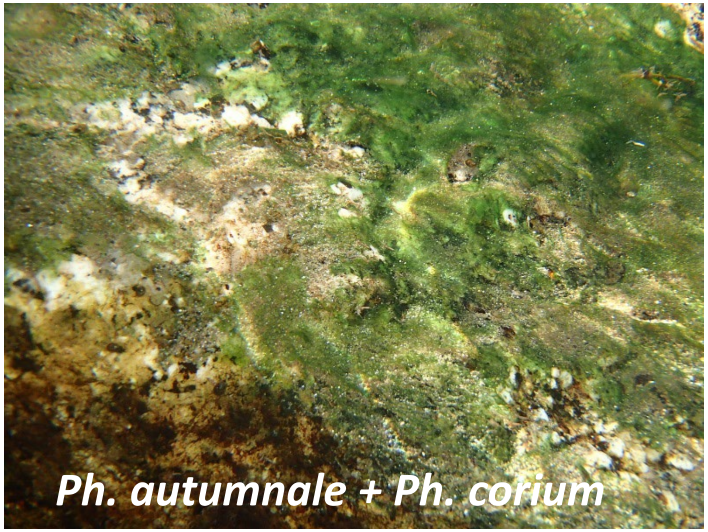


*Ph. autumnale*

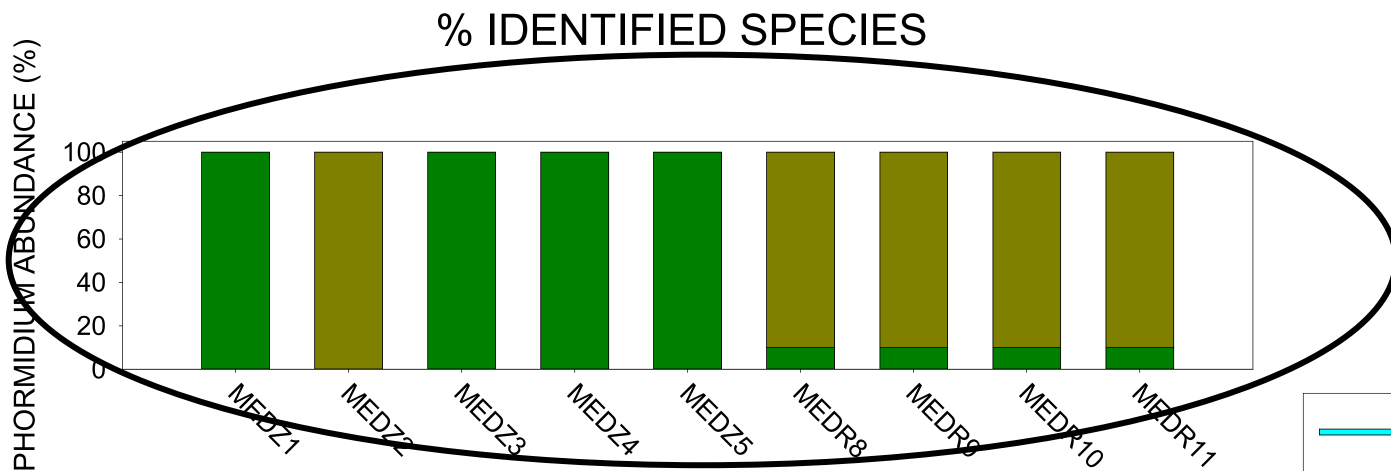
# Communities

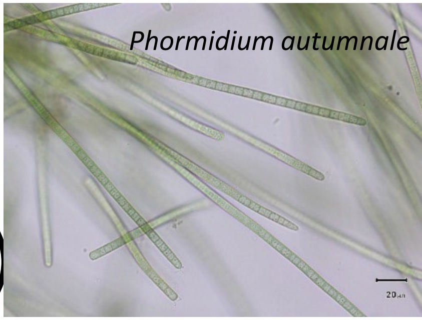


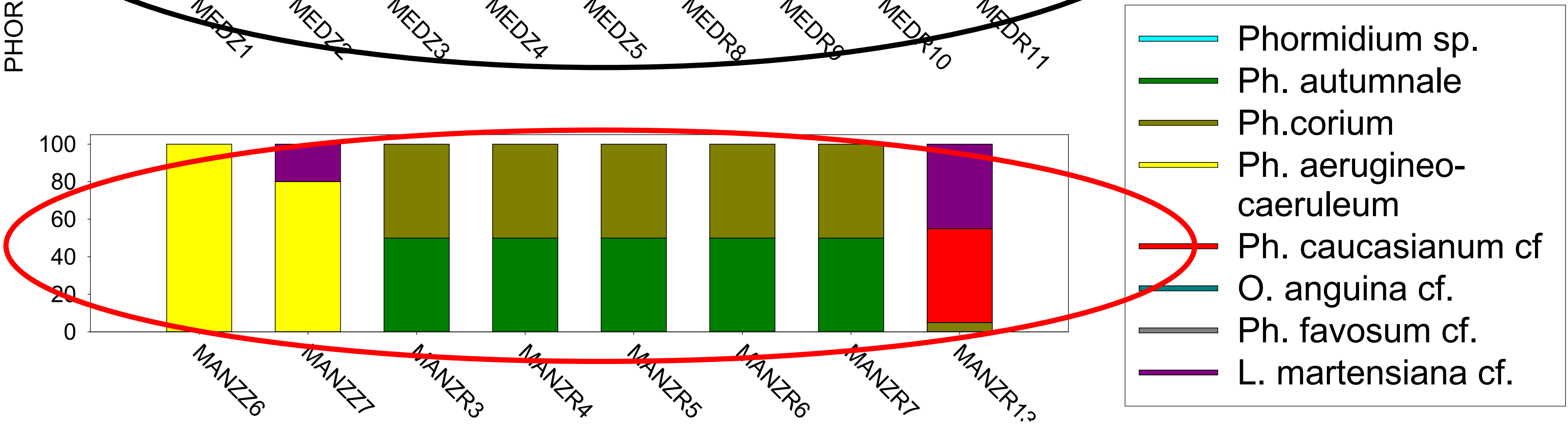


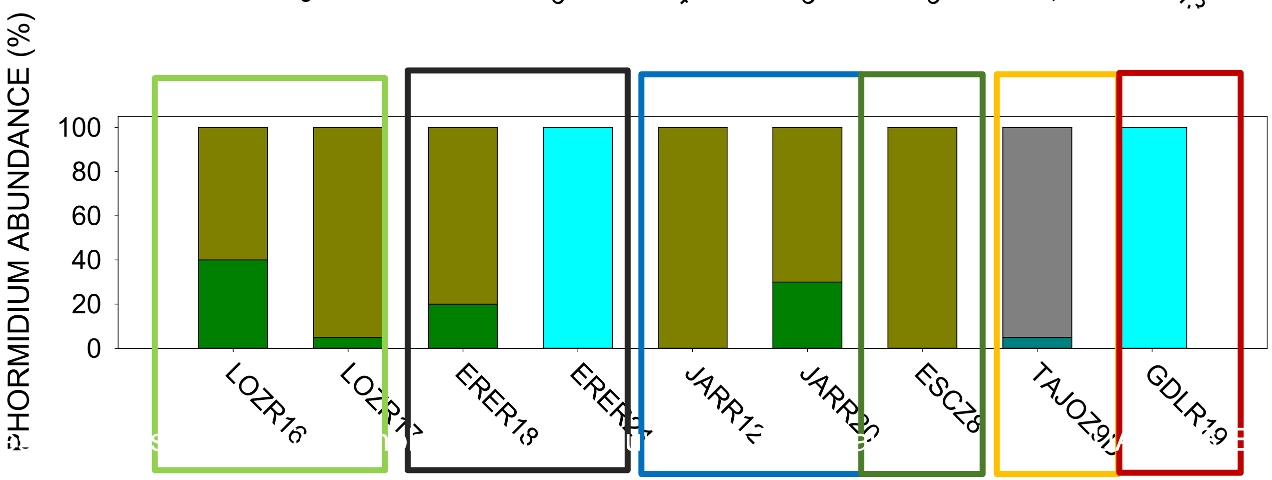


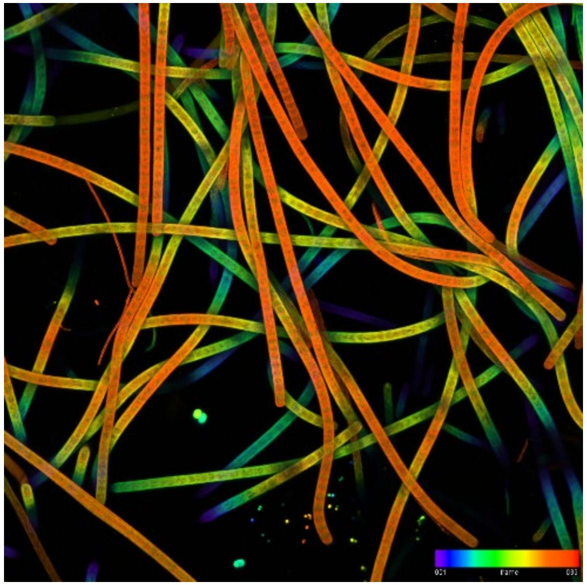

# Community structure is variable- *Ph autumnale* is present /dominant



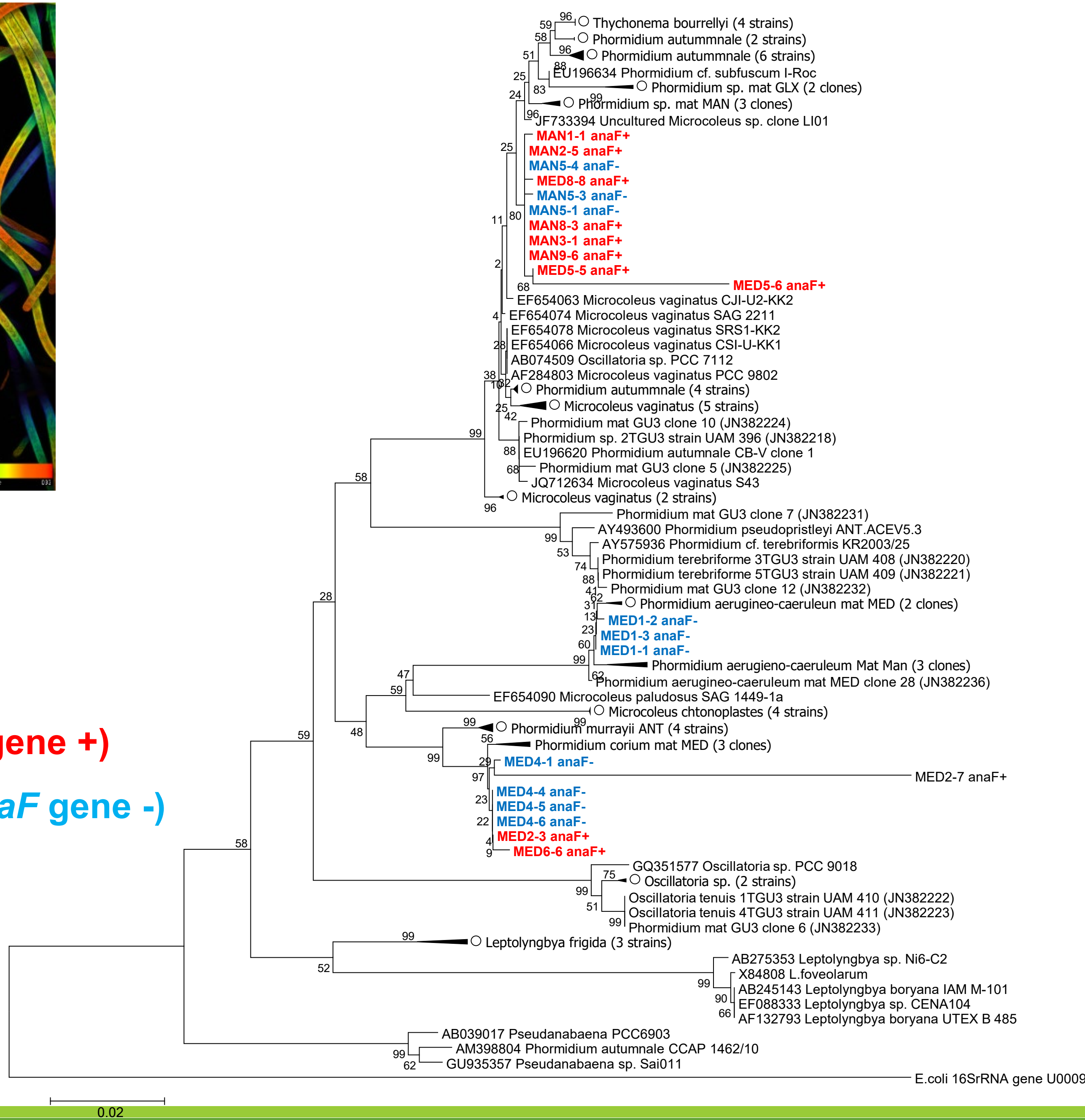



*Perona et al (2022)*

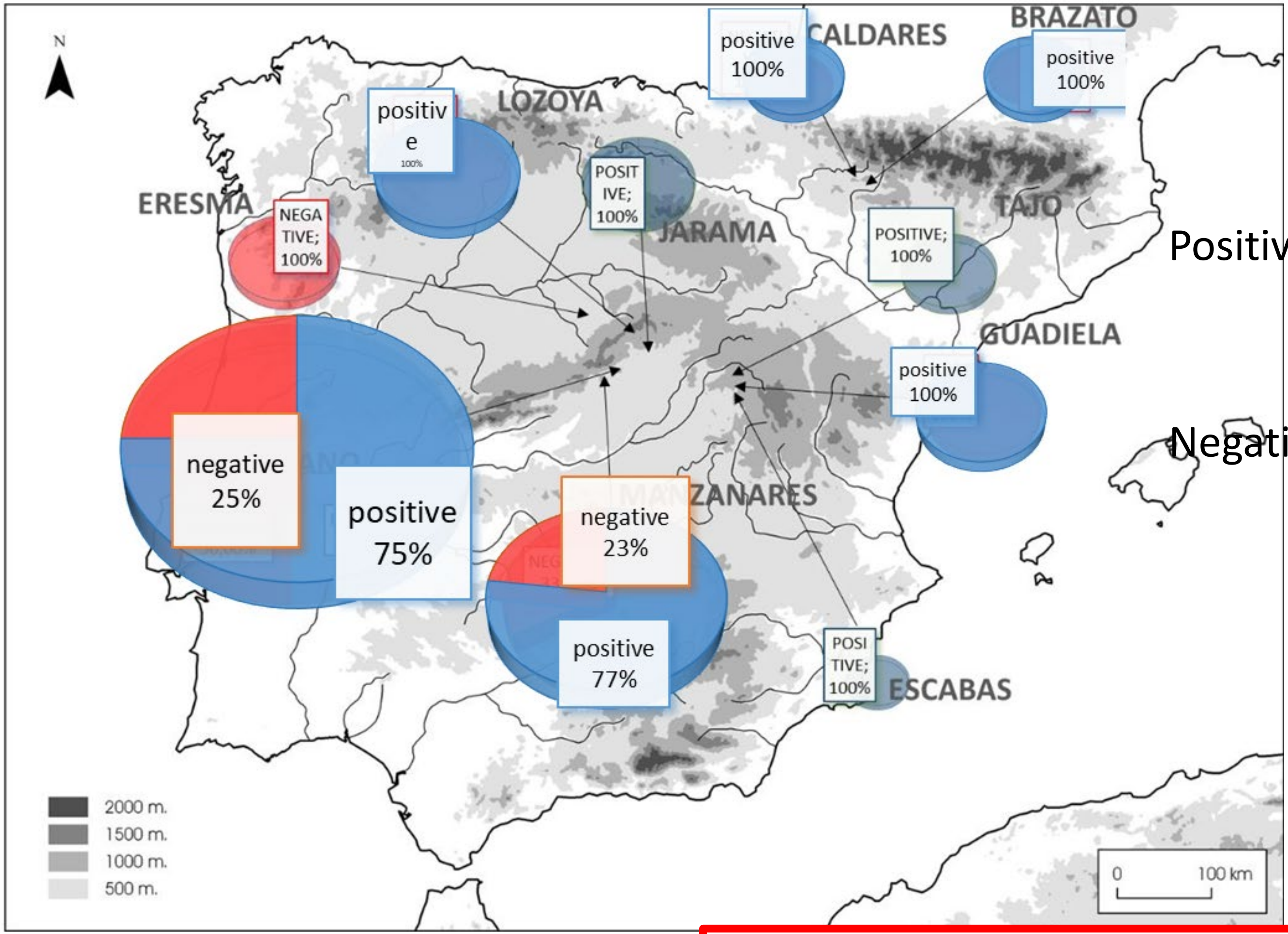



## 16S-rRNA gene

**Potentially toxic (*anaF* gene +)**

**NO Potentially toxic (*anaF* gene -)**

# Potential toxicity *(anaF* gene occurrence)



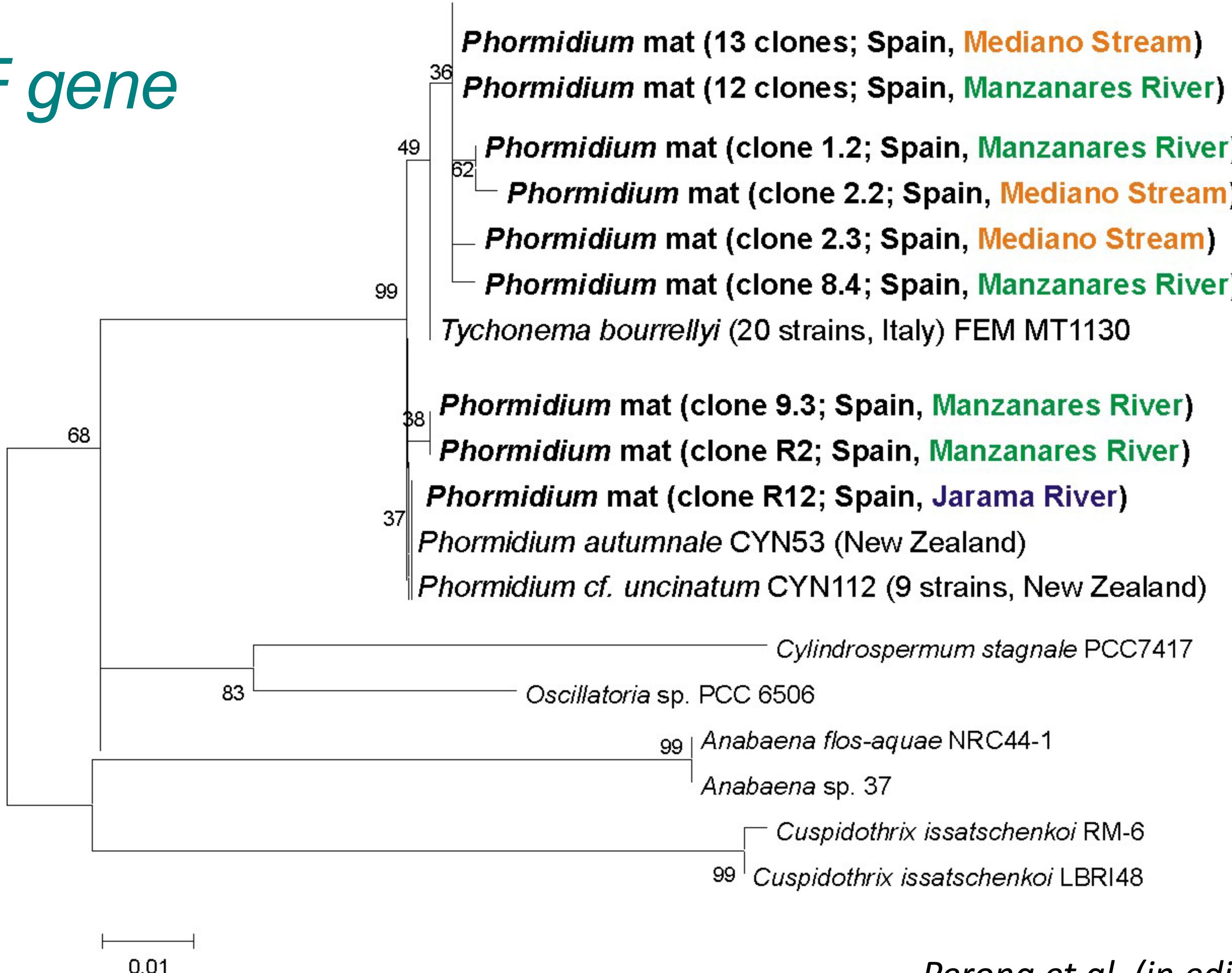

By PCR

Positive *anaF* presence

Negative *anaF* presence

**80 % positive anaF**

# *anaF gene*




*Perona et al, (in edition)*

# TOXIN CONCENTRATION

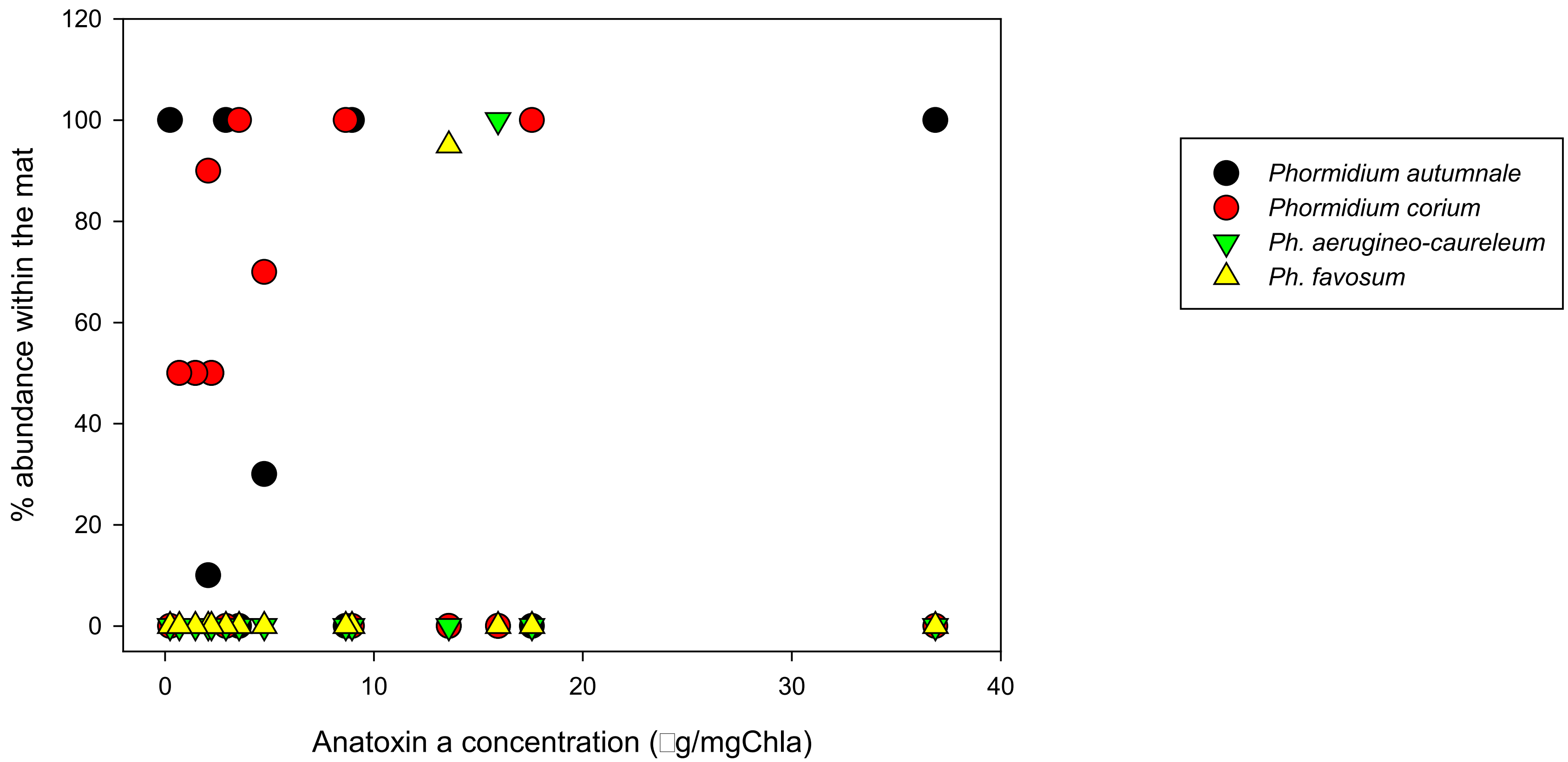
- Analysed using RBA and ELISA and ecotoxicologial bioassays
- Anatoxin is present at all mats analysed and highly variable

| BIOFILM SAMPLE | µg ANTX-A / mg chla | µg ANTX-A /$cm^2$ |
|---|---|---|
| MEDZ1 | 36.87 | 0.85 |
| MEDZ2 | 3.54 | 0.03 |
| MEDZ3 | 8.94 | 0.18 |
| MEDZ4 | 2.91 | 0.07 |
| MEDZ5 | 0.24 | 0.002 |
| MEDR9 | 2.06 | 0.07 |
| MANZZ6 | 15.94 | 0.24 |
| MANZZ7 (*) | nd. | nd. |
| MANZR2 | 2.22 | 0.03 |
| MANZR3 | 1.46 | 0.04 |
| MANZR5 | 0.69 | 0.01 |
| MANZR6 | 17.56 | 0.43 |
| JARR12 | 4.75 | 0.09 |
| JARR20 | 1.56 | 0.04 |
| ESCZ8 | 35.01 | 0.61 |
| TAJOZ9 | 8.65 | 0.31 |
| CALR14 | 13.58 | 0.34 |

*M. autumnale*
*Pot. aeurgineo-caeruleum*
*Ph. favosum*

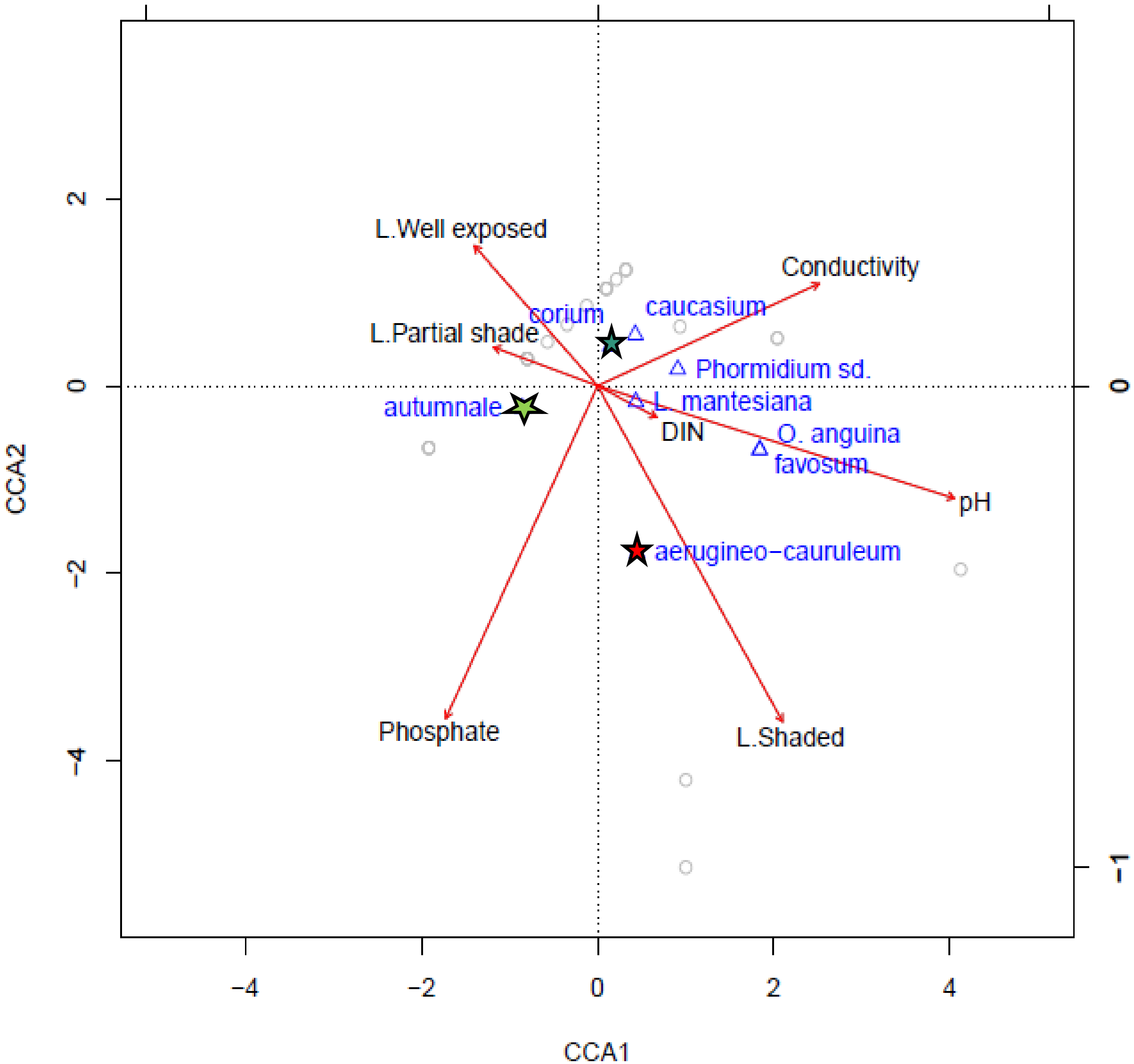
*Munnoz et al,*
*Sci tot environ 2021*

# Anatoxin-*a* concentration *vs*. community structure




- *M. autumnalis* seem to be related with toxicity (> 30 % biofilm??)
- Other species

# Relationships between environmental conditions and spp distribution



# Alarming persistence during spring and summer

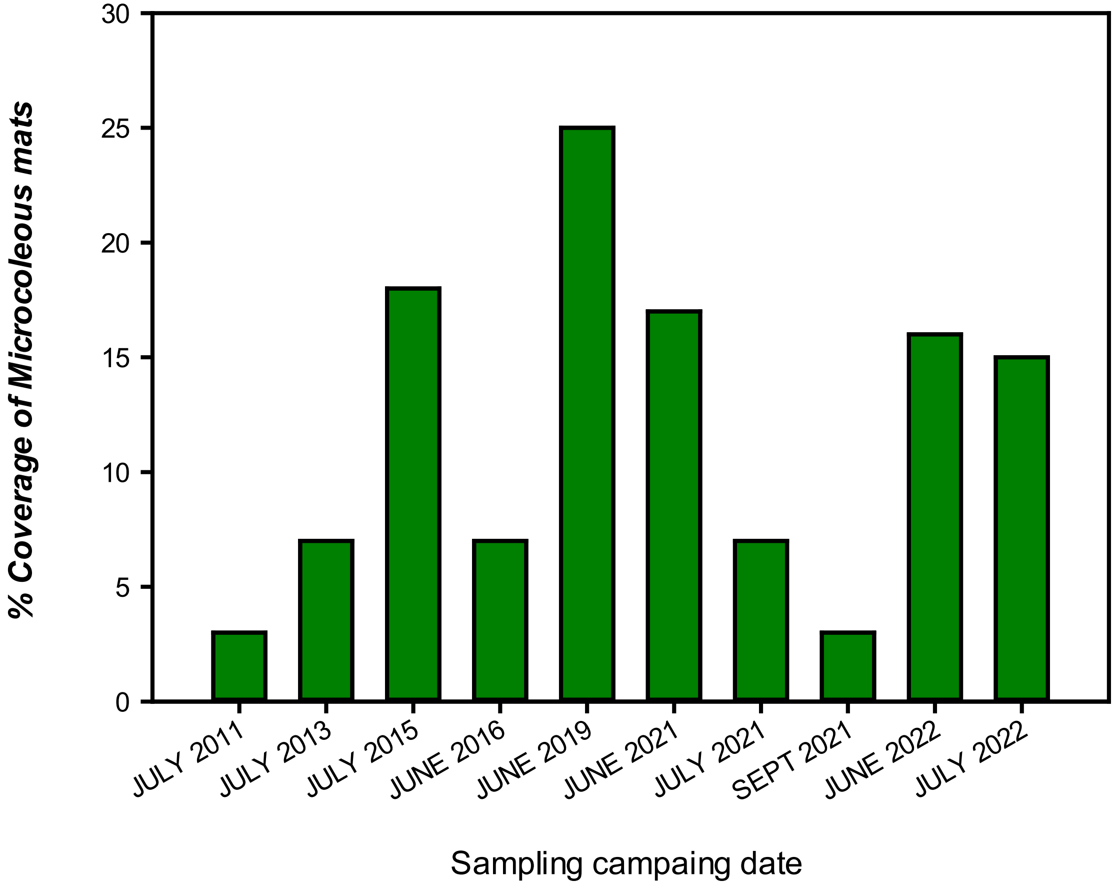



Figure 2: Temporal evolution of *Phormidium*-Like abundance mats in Manzanares river

# Toxic mats in Spain

- *Toxigenic mats  are present and widely distributed at all rivers studied*
- *Microcoleus/Phormidium* mats seem to be  abundant and dangerous
- They are increasing their abundance
- There are no clear relationships with environment
- Investigate the problem in Spain is interesting.

**more studies were needed to identify potential risks**

# New Projects Talgentox and Cyanopark

**to identify and characterize the cyanobacterial** communities from rivers and reservoirs within protected ecosystems

Ecologically → Taxonomically → Toxicologically

**to evaluate the potential ris**k to visitors, workers and protected fauna

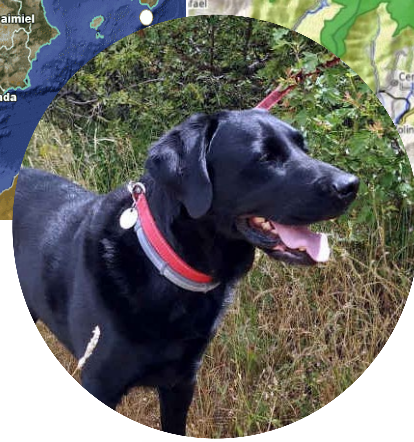

2 NATIONAL PARKS

- MONFRAGÜE
- GUADARRAMA MOUNTAINS

# Guadarrama Mountains National Park

- Ecological relevance
- Protected fauna
- High number of visitors
- Areas used for livestock

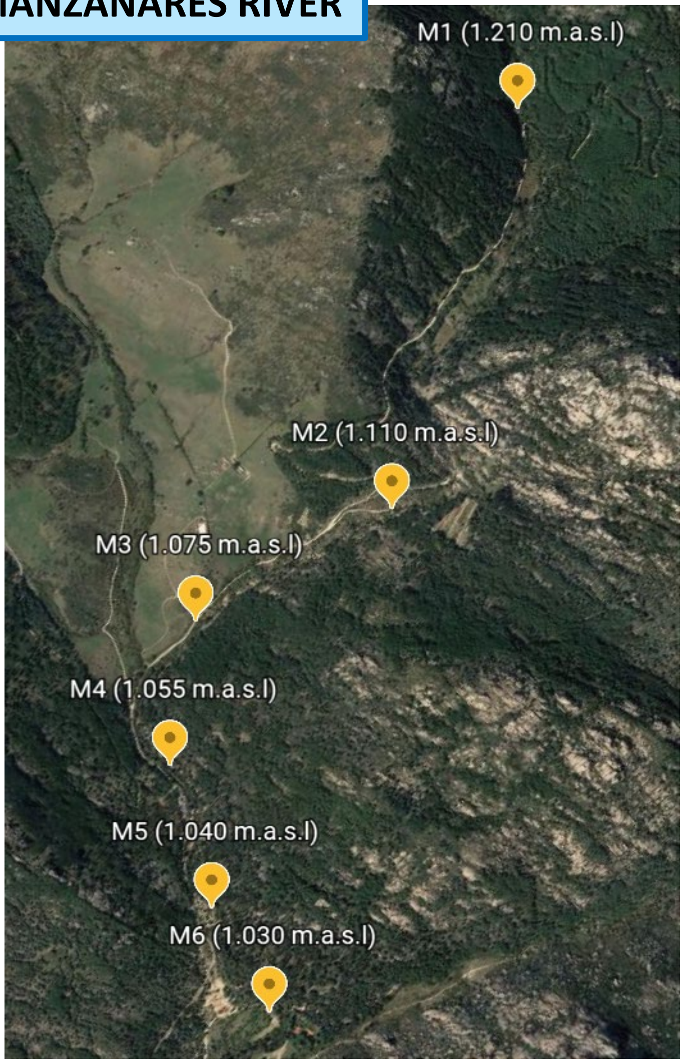




# SAMPLING POINTS:

Using maps, presential visits and presence of potential risks for users

MANZANARES RIVER



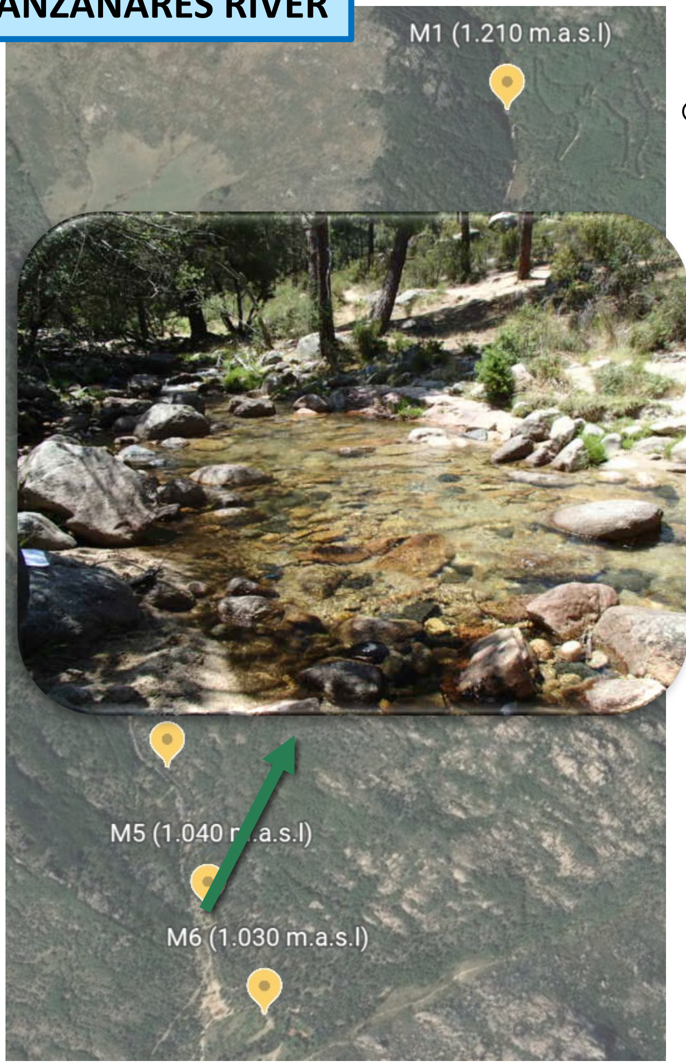

- High-mountain rivers
- Oligotrophic
- Low conductivity


LOZOYA RIVER

Guadarrama Mountains N.P

# SAMPLING POINTS:

**MANZANARES RIVER**



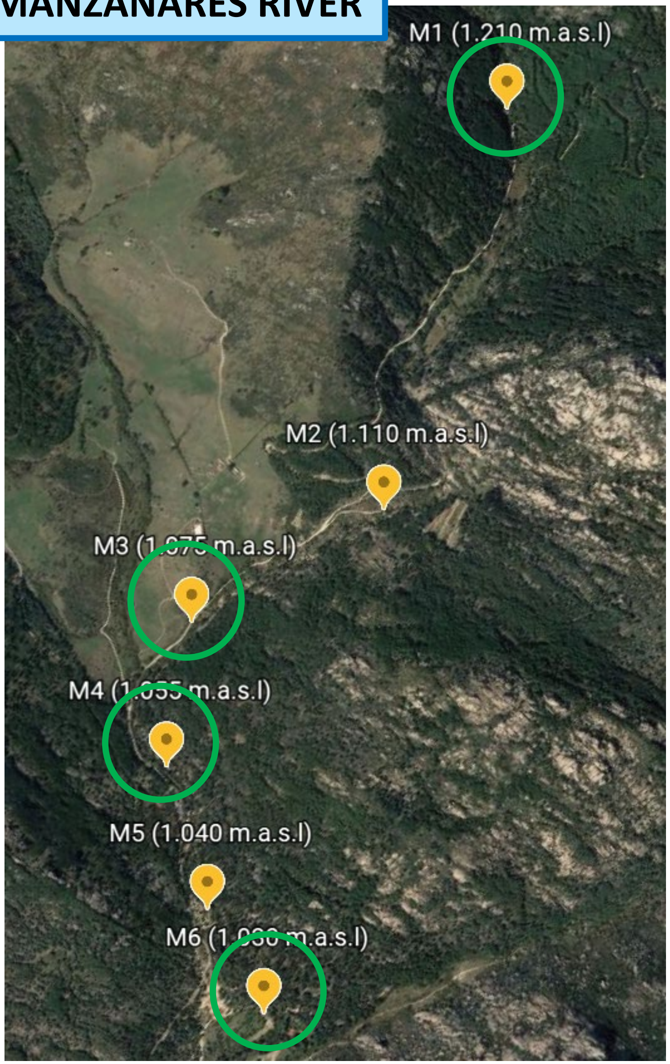

- **Highly frequented by visitors and livestock**

**LOZOYA RIVER**

Guadarrama Mountains N.P

# SAMPLING POINTS:

**MANZANARES RIVER**




**MATS CHARACTERIZATION (ECO/TAX/TOX):**

**Sample collection**
**june/july/september 2021**

**MATS MONITORING:**

**Visual estimation of mat cover (%)**
**Summer 2021**
**Summer 2022→ PRESENT**

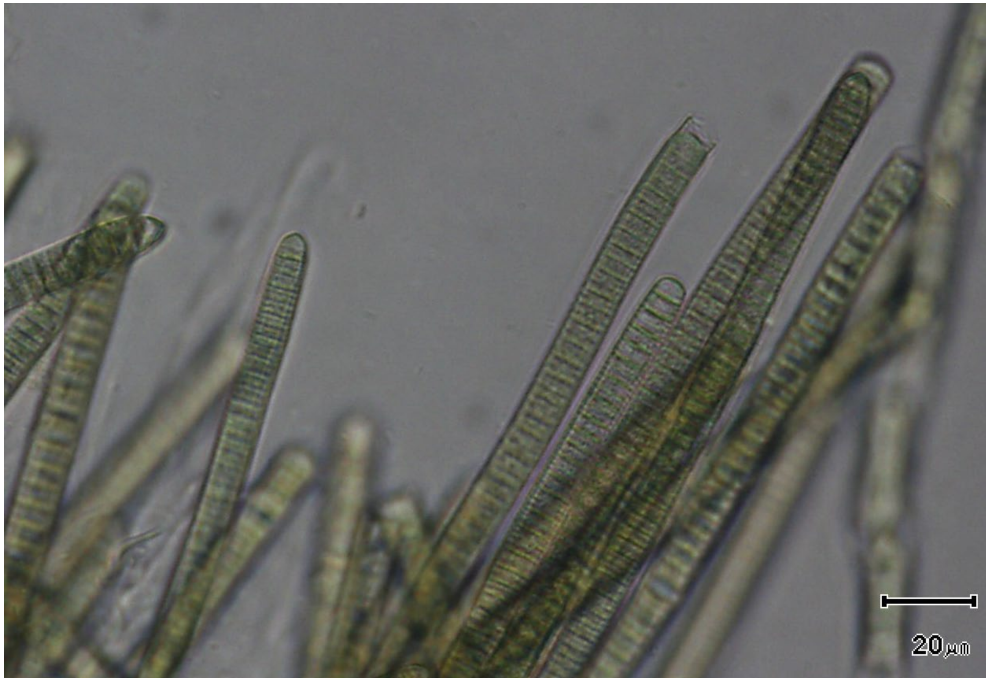
Water flow

**LOZOYA RIVER**

# RESULTS: cyanobacterial diversity

- *Microcoleus autumnalis*-dominated mats in both rivers (*16S* cloned and sequenced)

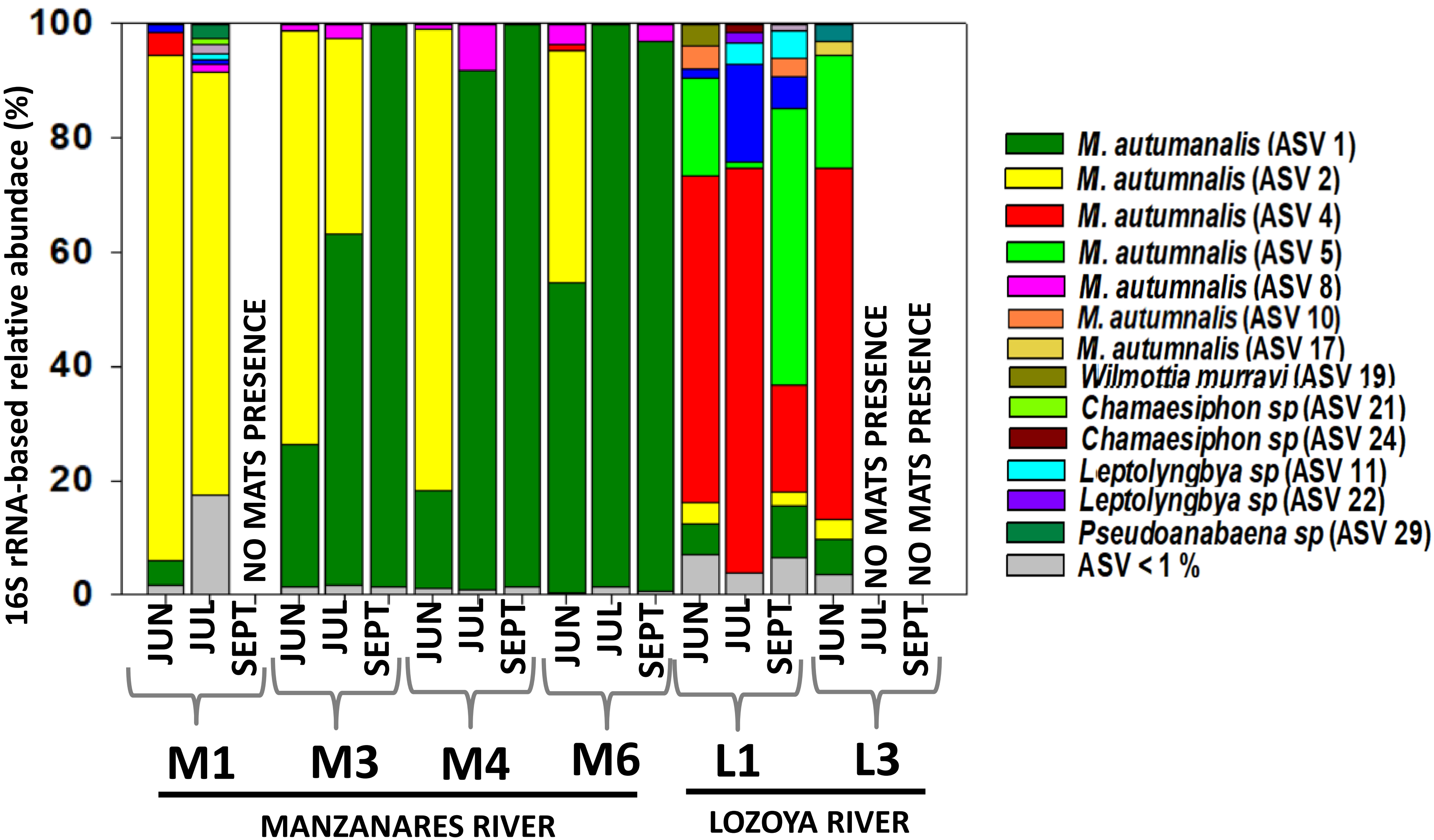




**RESULTS:** cyanobacterial diversity in sampling points

Guadarrama Mountains N.P

- Representative sample
- Dominance of *M. autumnalis*

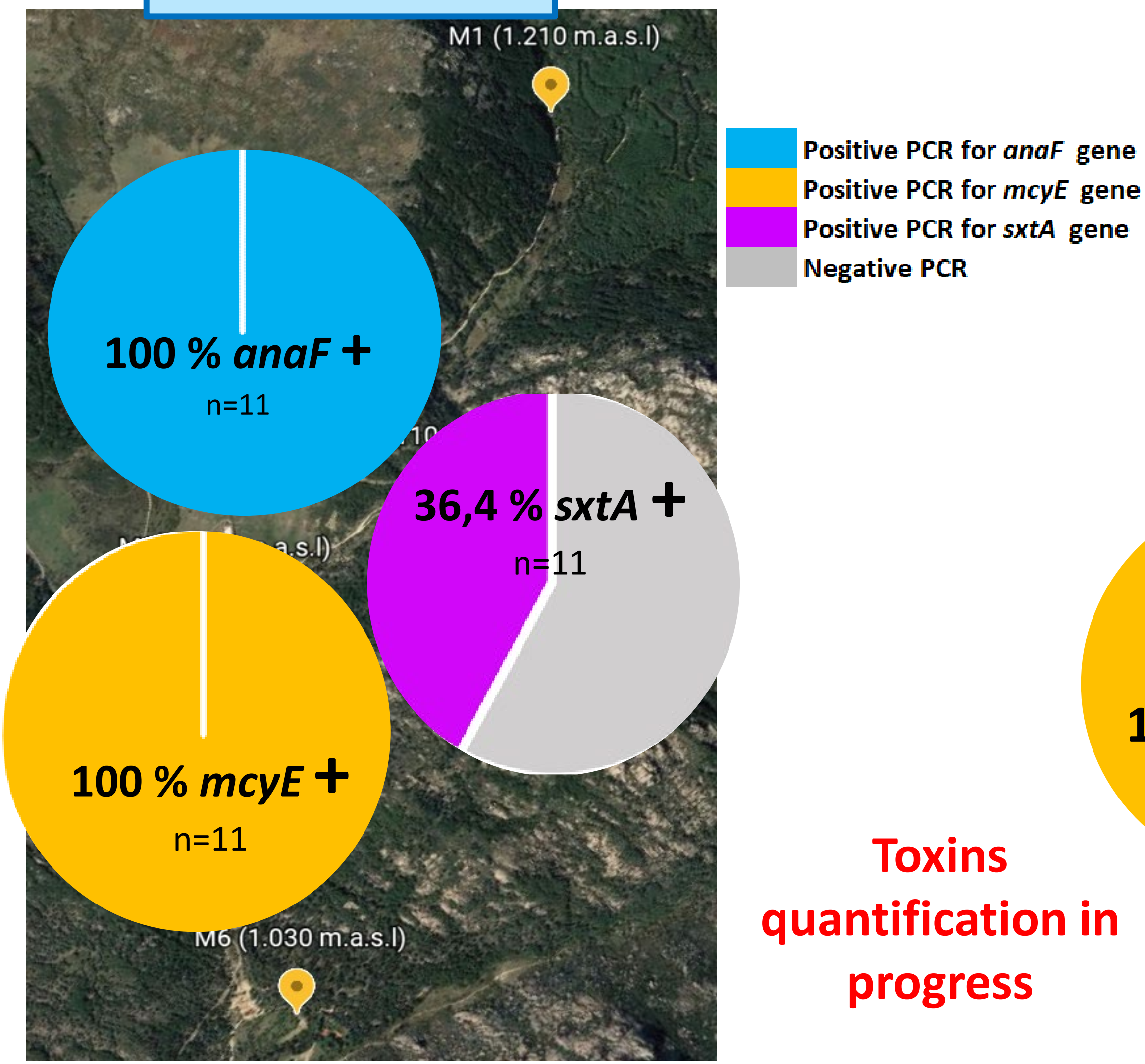


## RESULTS: potential toxicity in sampling points

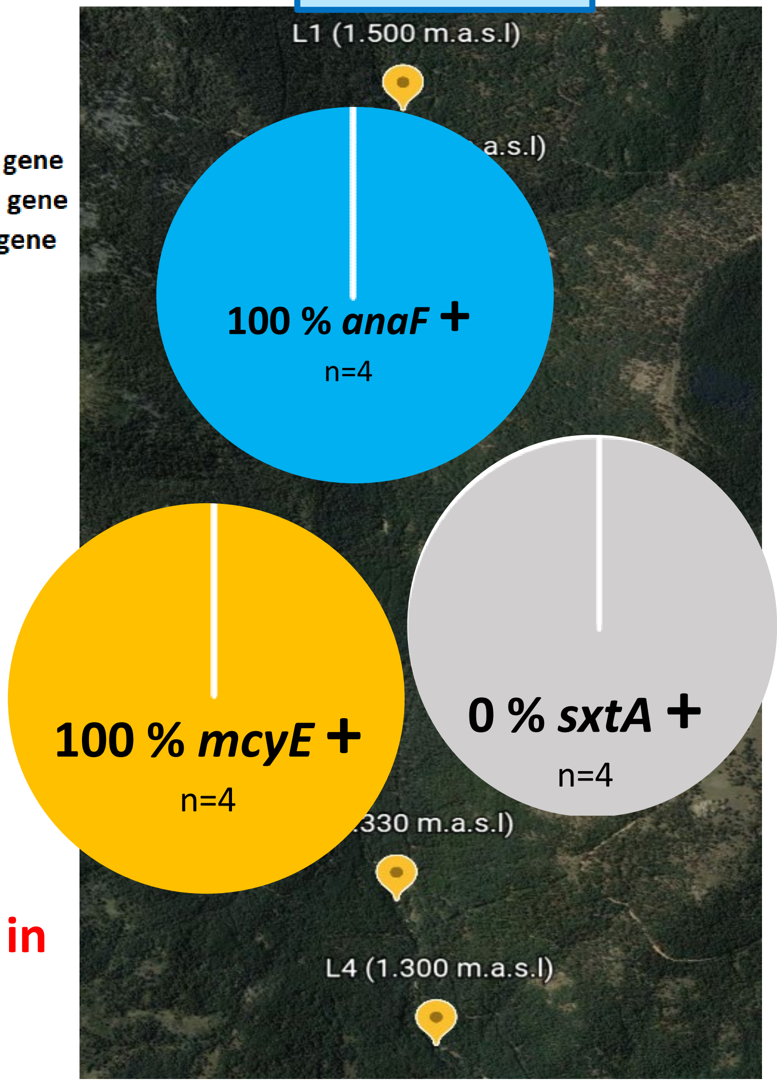
Guadarrama Mountains N.P

- Potential presence of ANATOXINS, MICROCYSTINS and SAXITOXINS
- Screening by PCR (*anaF*, *mcyE* and *sxtA*)

MANZANARES RIVER




LOZOYA RIVER



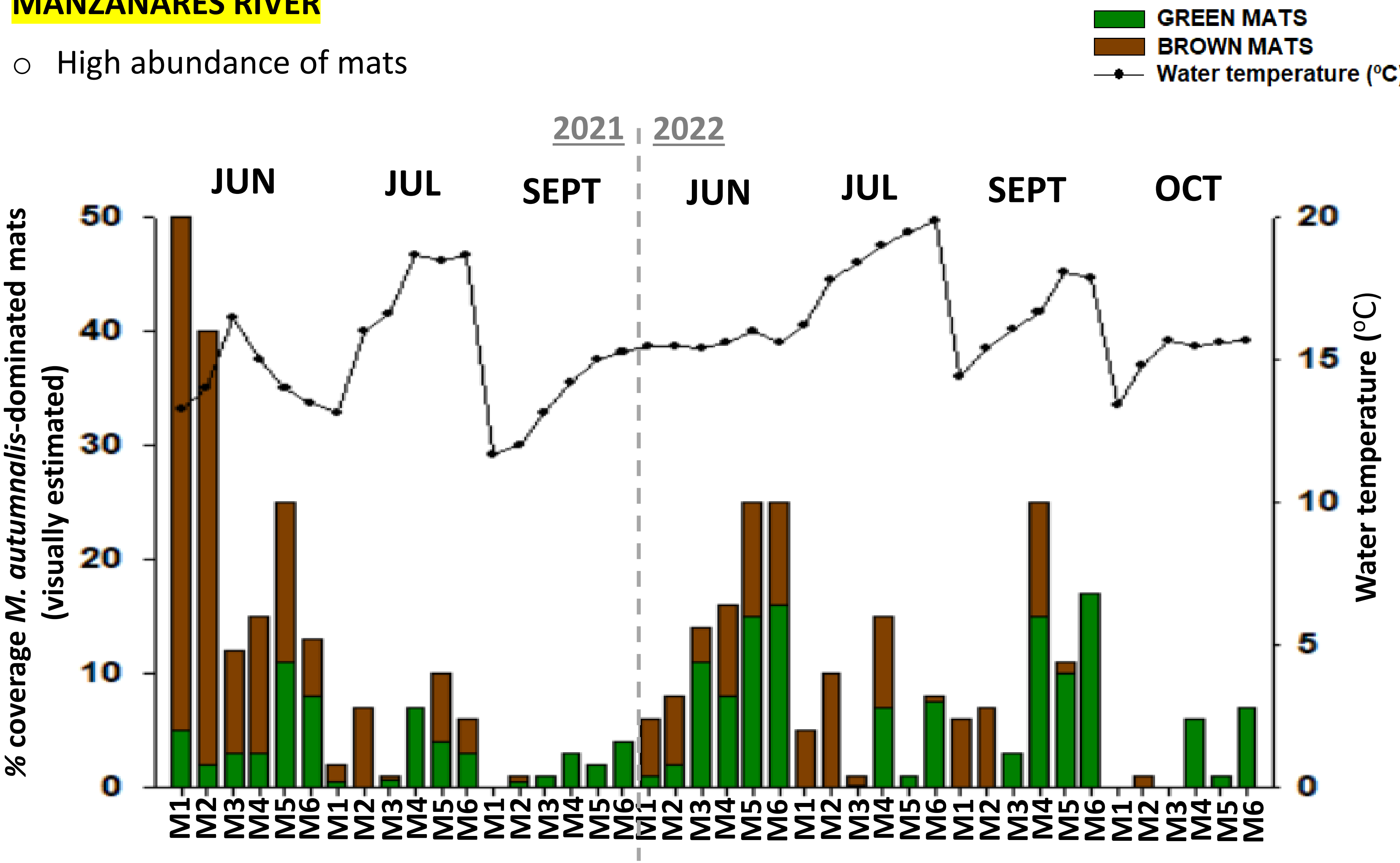

**Toxins quantification in progress**

**RESULTS:** mats monitoring

Guadarrama Mountains N.P

# MANZANARES RIVER

- High abundance of mats

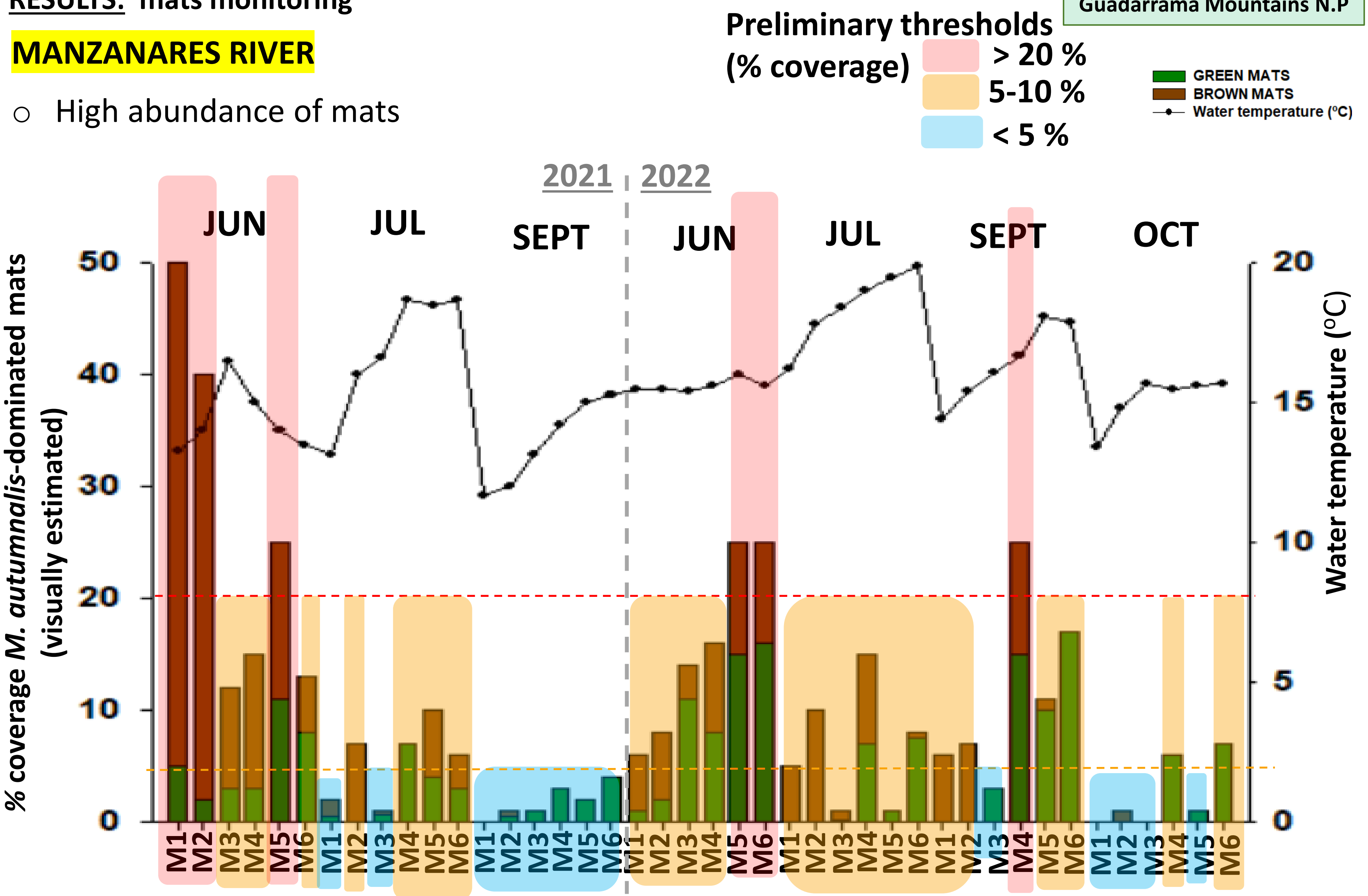


**RESULTS:** mats monitoring

**MANZANARES RIVER**

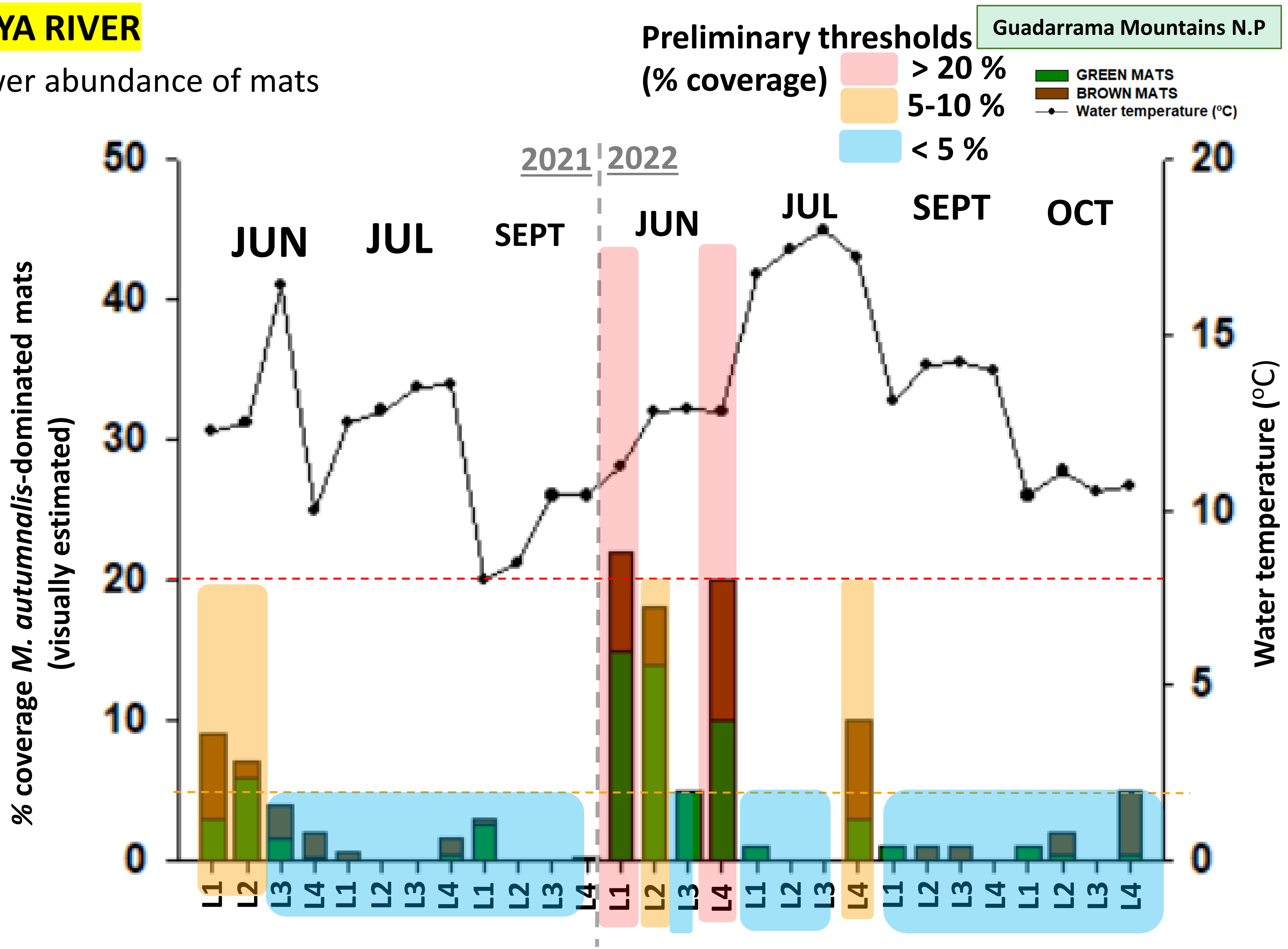
- High abundance of mats





**LOZOYA RIVER**

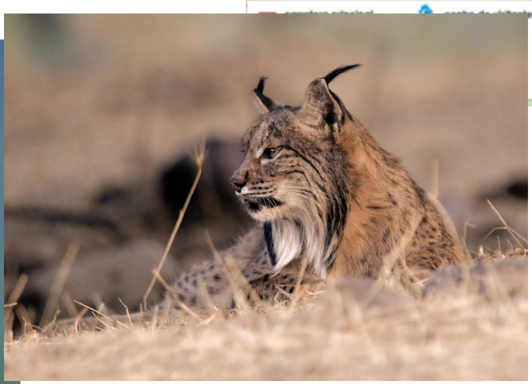
- Lower abundance of mats

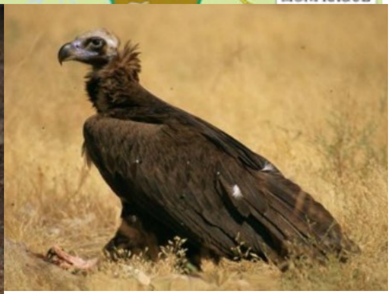


# Monfragüe National Park

- Ecological relevance
- Protected fauna
- High number of visitors







# SAMPLING POINTS:

**2 RIVERS:** MALVECINO RIVER and BARBAÓN RIVER (JUNE/JULY/OCTOBER 2021)

**4 RESERVOIR SHORES** (JUNE/JULY/OCTOBER 2021)

**8 NATURAL PONDS** (JUNE 2022)

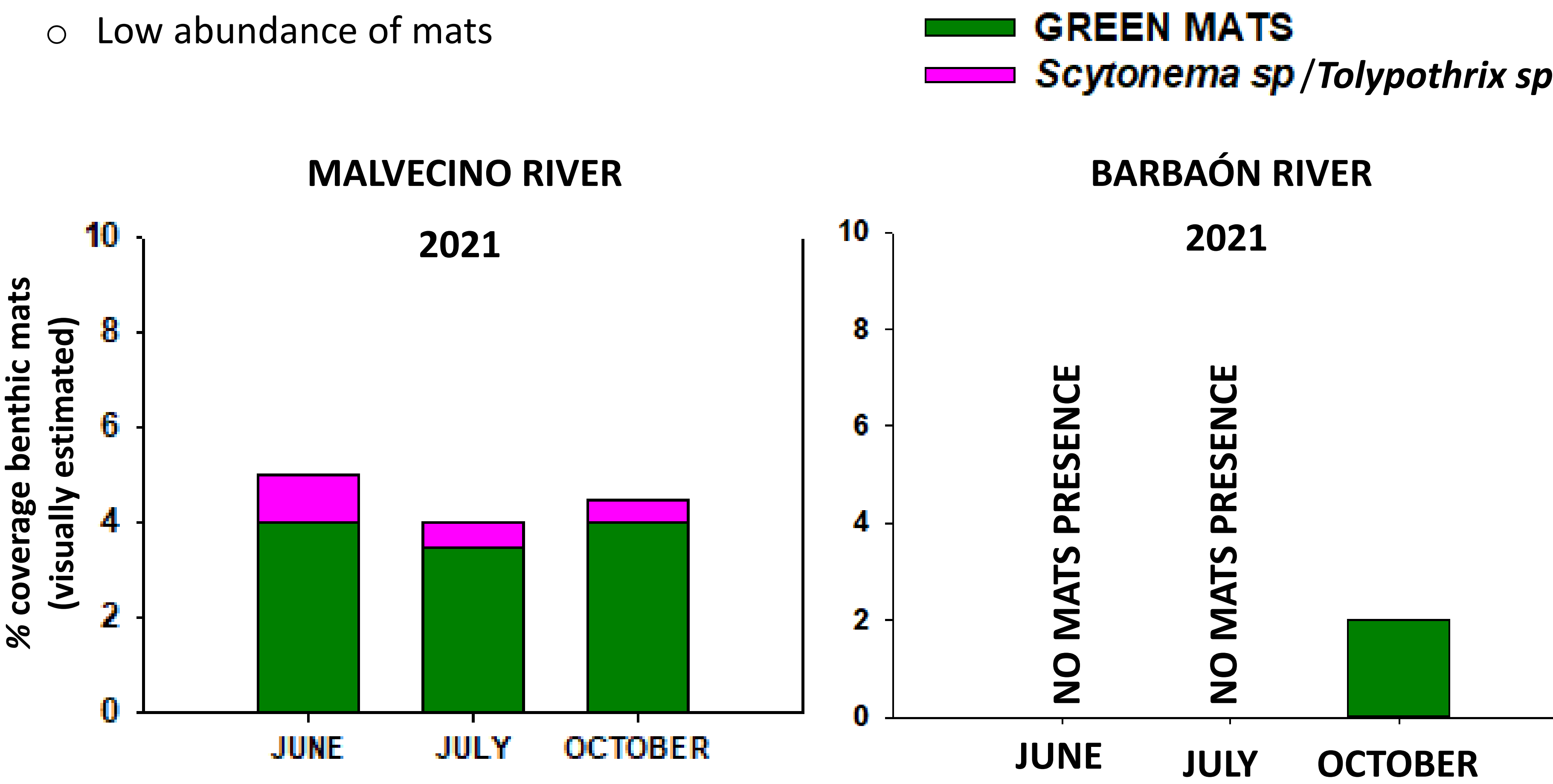
# RESULTS: cyanobacterial ocurrence

- 2 RIVERS **Benthic cyanobacteria confirmed**
- 4 RESERVOIR SHORES **NO benthic cyanobacteria**
- 8 NATURAL PONDS **Benthic cyanobacteria confirmed**

Monfragüe N.P

## RIVERS RESULTS: monitoring mats

- Low abundance of mats



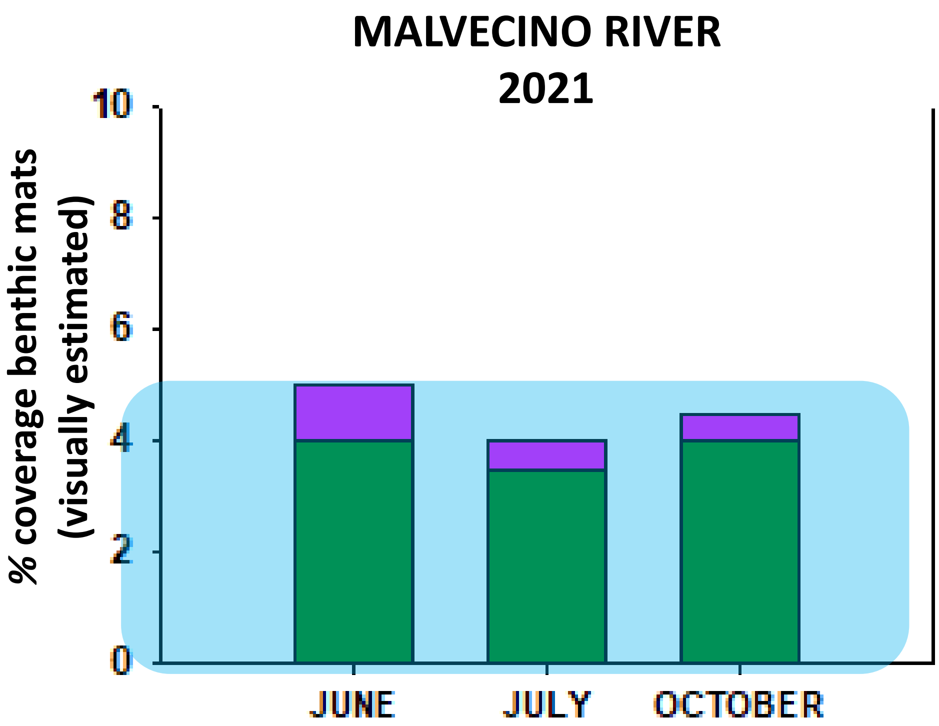
Monfragüe N.P

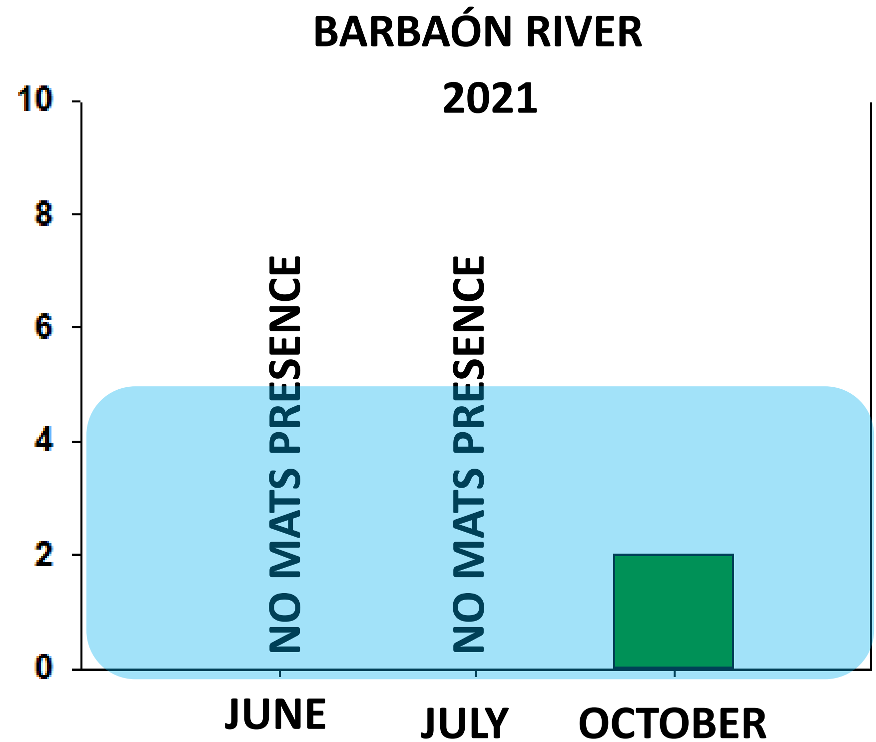
## RIVERS RESULTS: monitoring mats

- Low abundance of mats

**Preliminary thresholds (% coverage)**
- > 20 %
- 5-10 %
- < 5 %

GREEN MATS
*Scytonema sp* /*Tolypothrix sp*

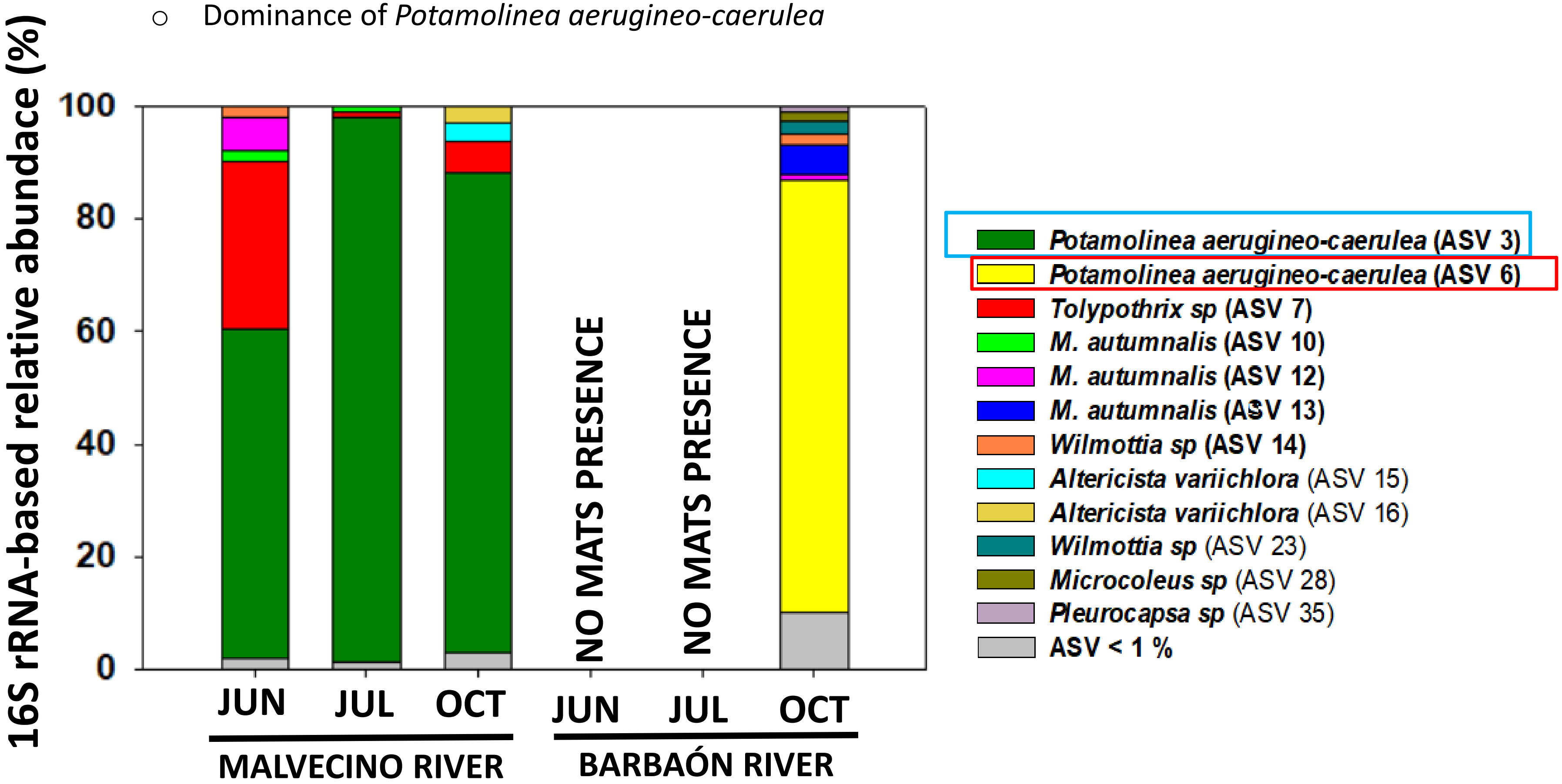


## RIVERS RESULTS: cyanobacterial diversity

Monfragüe N.P

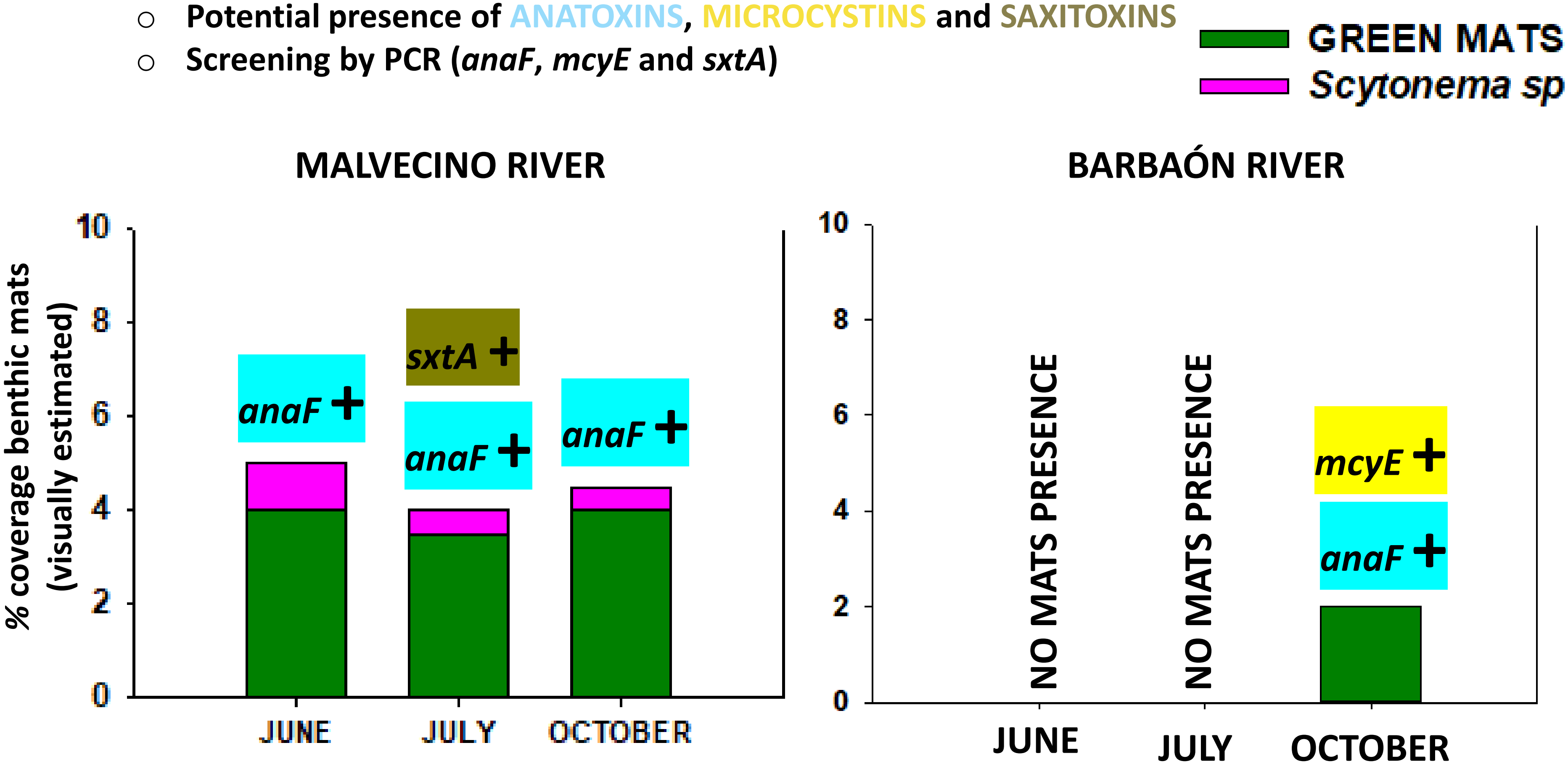
- Representative sample
- Dominance of *Potamolinea aerugineo-caerulea*



Monfragüe N.P

# RIVERS RESULTS: cyanobacterial diversity/potential toxicity

- Potential presence of ANATOXINS, MICROCYSTINS and SAXITOXINS
- Screening by PCR (*anaF*, *mcyE* and *sxtA*)




**Toxins quantification in progress**

## NATURAL PONDS RESULTS: cyanobacterial diversity

8 ponds sampled: **benthic/epiphytic communities only in 3 ponds**

### POND 1: *Tychonema sp*-like mats

## NATURAL PONDS RESULTS: cyanobacterial diversity

**POND 2: *Phormidium/Microcoleus*-like mats**

## NATURAL PONDS RESULTS: cyanobacterial diversity

### POND 3: *Gloeothrichia sp*

# <u>NATURAL PONDS RESULTS</u>: cyanobacterial diversity

- Presence of genera described as toxin producers
- Characterization **in progress**
- **Highly frequented by protected fauna**

# Future directions

- To characterize mats individually and some isolated cultures
- Risk assessment
- Create protocols to asses/manage that risk
- Citizen science

# Take-Home Messages

- Presence of anatoxin-a in benthic mats from Spanish rivers
- Presence of potentially toxin-producing benthic communities in two Spanish National Parks
- Alarming presence (high abundance) of potentially toxin-producing benthic mats dominated by *M. autumnalis* in Guadarrama Mountains National Parks
- Presence of genera described as toxin producers in natural ponds from Monfragüe National Park
- Results will be use to create protocols to asses/manage risks in these National Parks

# Acknowledgments:

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**TALGENTOX (CYTED 2019-P919PTE0047)**

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## Assessing risks of benthic cyanobacterial communities in National Parks

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