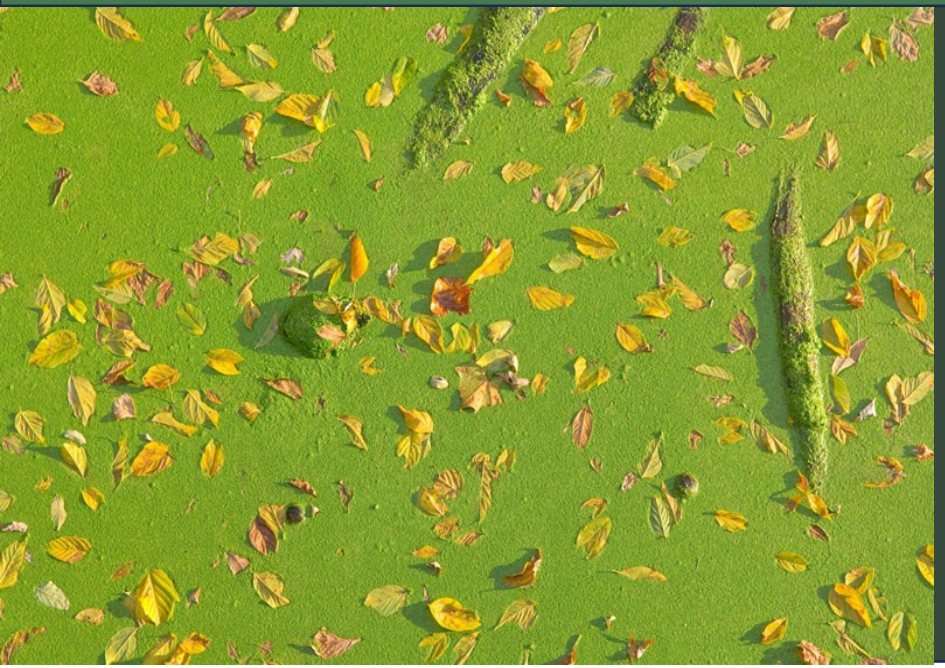
EPA United States Environmental Protection Agency

# Harmful Algal Blooms, Hypoxia, and Nutrients Research Webinar Series



**November 20, 2024, from 2:00 to 3:00 p.m. EST**

# Health Effects and Ecology of Anatoxin-Producing Cyanobacteria

A certificate of attendance will be offered for this webinar

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**Webinar Support:** Send email to _____.

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**Harmful Algal Blooms, Hypoxia, and Nutrients Research Webinar Series:**
https://www.epa.gov/water-research/harmful-algal-blooms-hypoxia-and-nutrients-research-webinar-series

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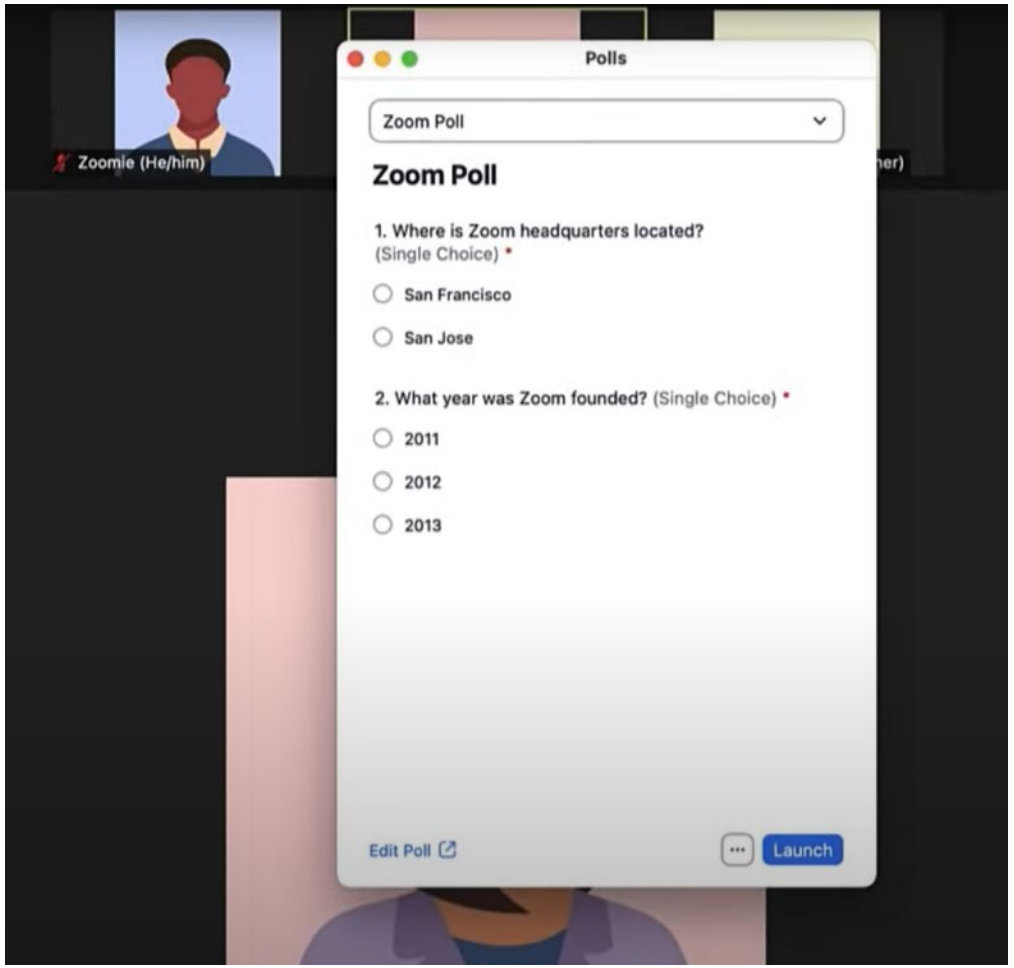

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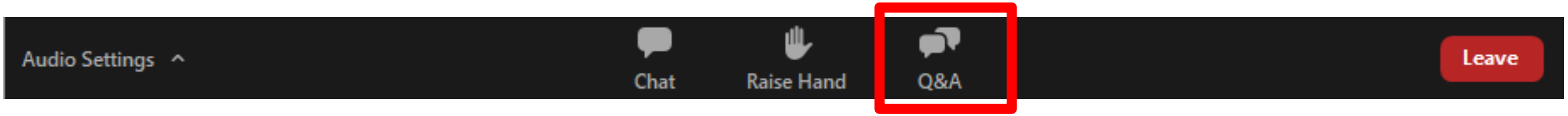

1. You must be registered with your Full Name and Email.
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EPA hosts webinar series dedicated to delivering the latest information and training on our cutting-edge research addressing environmental and public health issues.

If you are interested in upcoming webinars, stay up-to-date with email notifications:

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## Small Drinking Water Systems

**December 3:** *Lead Reduction Updates and Lead Service Line Identification (LSLID) and Replacement*

Registration and Additional Information

## Tools and Resources Training

**December 5:** *ECOTOX Knowledgebase and PFAS Updates*

Registration and Additional Information

## Healthy and Resilient Communities Research

**December 10:** *Allostatic Load and Epigenetic Age Acceleration as Measures of Cumulative Health Impacts*

Registration and Additional Information

## Emergency Response Research

**December 11:** *Regional Research Partnerships to Address High Priority, Near-Term Research Needs: Splash Pads & COTS Flight Simulator to Support Aerial Recon Training*

Registration and Additional Information

## Computational Toxicology and Exposure Communities of Practice

**December 12:** *Updates to the Web-based Interspecies Correlation Estimation (Web-ICE) application*

Registration and Additional Information

# Introduction

*John Ravenscroft, EPA Office of Water*

EPA's Harmful Algal Blooms, Hypoxia, and Nutrients Research Webinar Series

https://www.epa.gov/water-research/harmful-algal-blooms-hypoxia-and-nutrients-research-webinar-series

# Presenters

## Donna Hill, *EPA* (Hill.Donna@epa.gov)

Donna Hill works in the Office of Research and Development at US EPA in Research Triangle Park, North Carolina. For twelve years, she has been studying the health effects of cyanotoxins in mice which include projects with cylindrospermopsin, microcystins, and most currently, anatoxin-a. Presently, Donna also serves on the Health Institutional Animal Care and Use Committee (IACUC) at EPA.

## Susie Wood, *Lincoln University, NZ* (Susie.Wood@lincoln.ac.nz)

Susie Wood is co-director of the Waterways Centre, and a Professor at Lincoln University (New Zealand). She has worked for nearly two decades on both planktonic and benthic cyanobacteria ecology and toxin production.

# Evaluation of Anatoxin-a Health Effects in Mice- November 2024 Update

Donna Hill

Research Triangle Park, NC
hill.donna@epa.gov

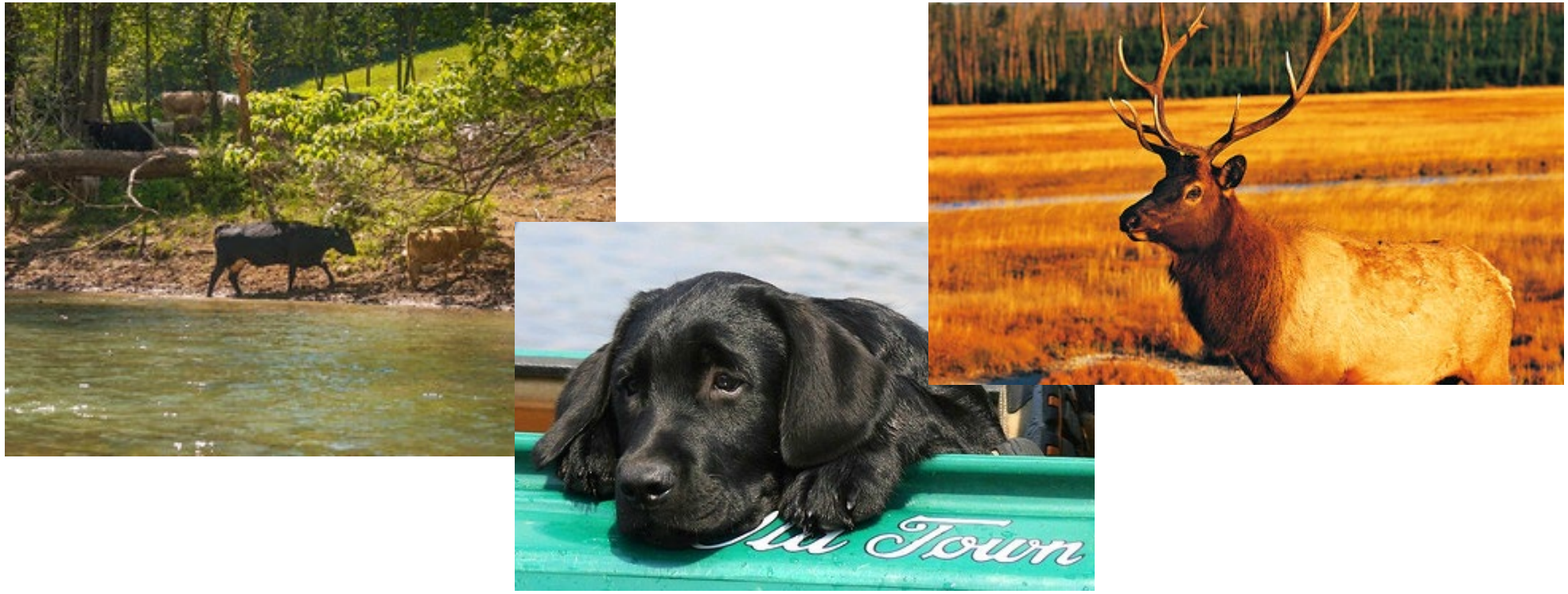
**Office of Research and Development**
Center of Public Health and Environmental Assessment
Public Health and Integrated Toxicology Division

# *Disclaimer:*

- *This presentation should not be construed to represent any Agency determination or policy, or promotion of a product.*

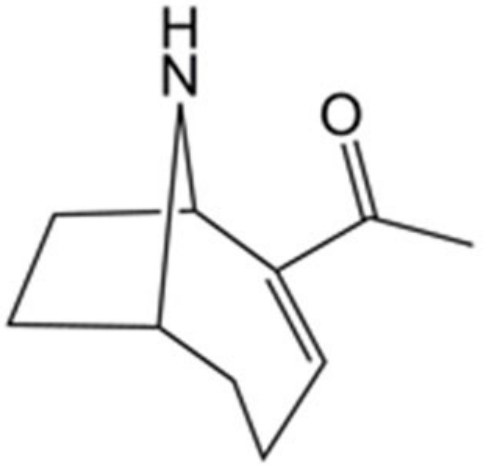
- *Data presented is not final.*

# Confirmed reports of lethality in dogs, livestock, and wildlife

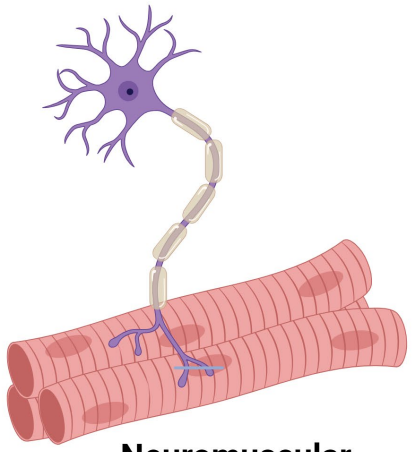


# Characteristics of Anatoxin-a Molecule

- Cyanotoxin found in fresh and marine waters
- 165 Daltons
- Hydrophilic
- Occurs naturally as (+) isomer
- Alkaloid
- Rapidly absorbed after ingestion
- Target: nervous system

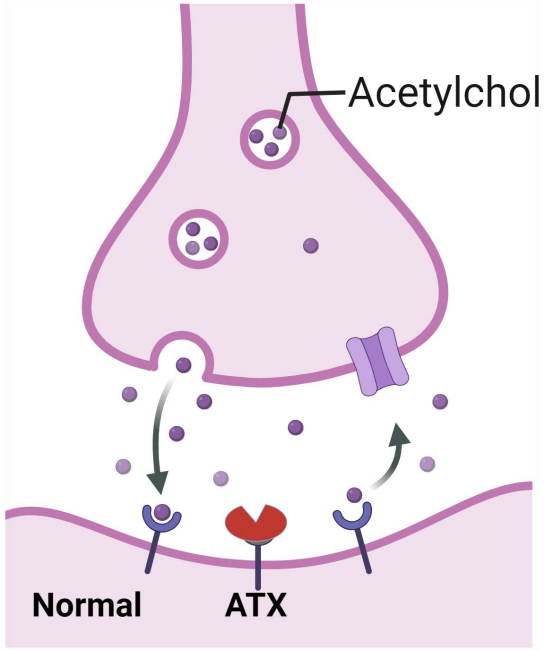


# Nicotinic Acetylcholine Receptors (nAChR)



Neuromuscular Junction

**Anatoxins bind irreversibly to nicotinic acetylcholine (Ach) receptors to keep muscle stimulated to point of fatigue and then paralysis.**




**Neuron Synapse with Muscle Cell**

Created in https://BioRender.com

# Nicotinic Acetylcholine Receptors (nAchR)

- These receptors are found throughout the central and peripheral nervous systems (including auditory pathway)
- Main receptor in muscle for contraction
- ATX has higher affinity to receptor than Ach and nicotine

# Symptoms with Exposure (usually animals)

- Weakness/Collapse
- Incoordination
- Tremors/Fasciculations
- Respiratory distress
- Seizures
- Death

# Route of Exposure

Oral* from recreational & drinking water

Possible with aerosol

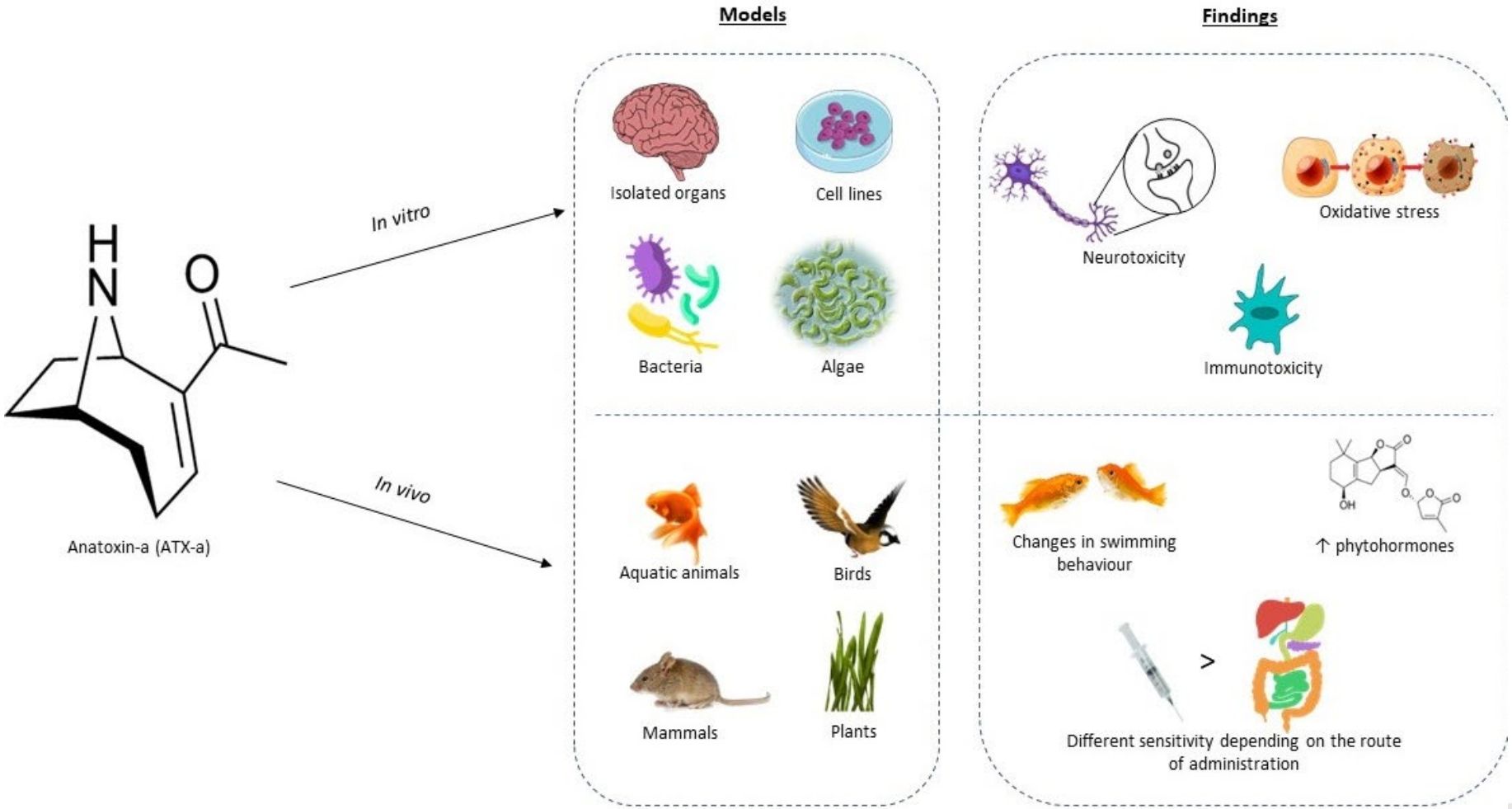
And bioaccumulation in food

No evidence for dermal absorption

# Reference value for anatoxin-a

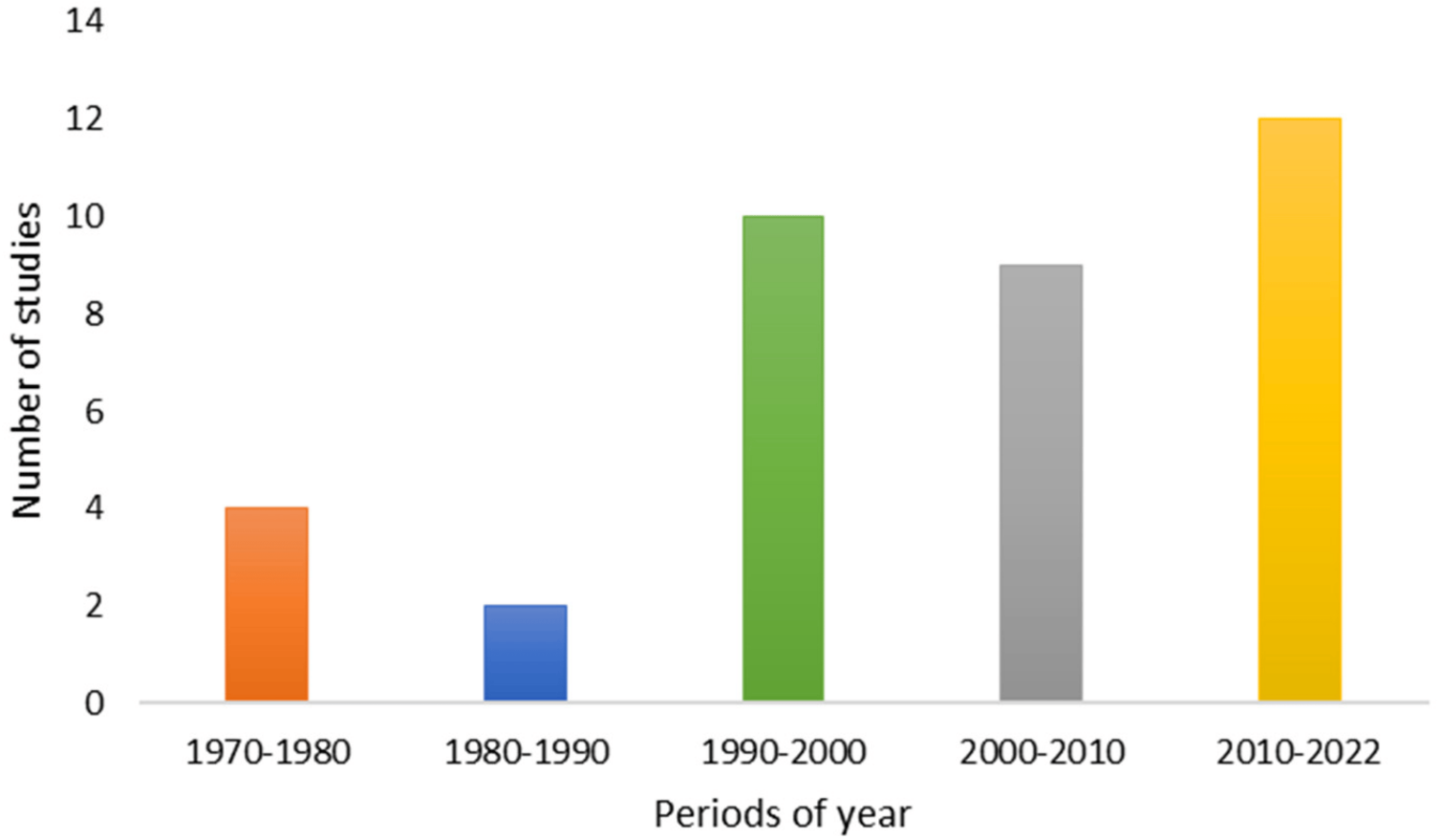
| Entity | Drinking Water (µg/L) | Recreational (µg/L) |
|---|---|---|
| WHO | 30 | 60 |

# Anatoxin Research Overview




Plata-Calzado, C.; Prieto, A.I.; Cameán, A.M.; Jos, A. Toxic Effects Produced by Anatoxin-a under Laboratory Conditions: A Review. *Toxins* **2022**, *14*, 861. https://doi.org/10.3390/toxins14120861

# Number of *in vivo* Anatoxin Studies

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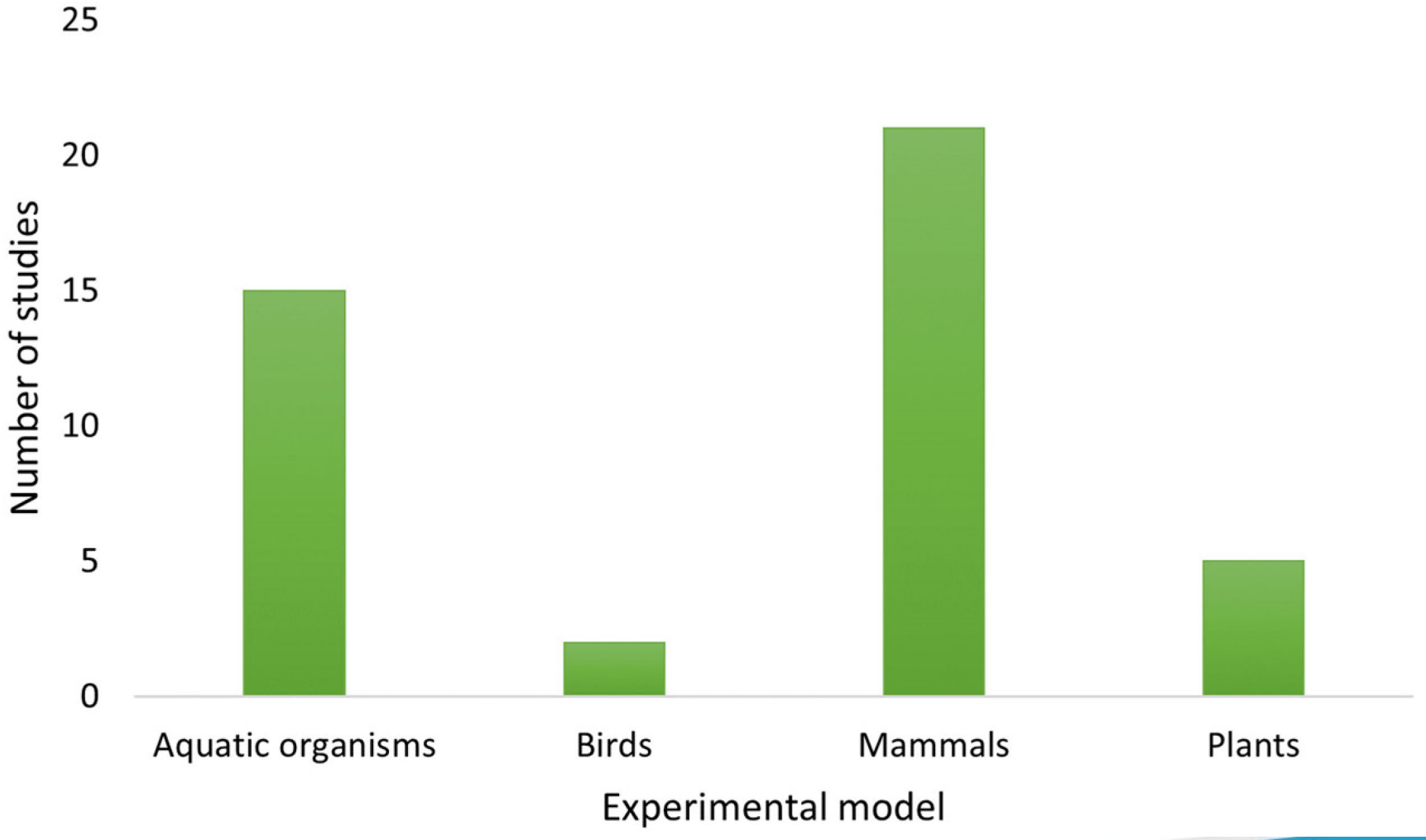
# In vivo Models Represented

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# Toxicity Studies on Anatoxin-a

- Acute toxicity to assess LD50
- 28-day oral ATX in mice (0.098, 0.49 or 2.46 mg/kg)
- 54-day drinking water study in rats (0.05 and 0.5 mg/kg bwt)
- 2 Reproductive studies in pregnant mice did not show adverse effects to offspring
- Seven daily injections caused decreased sperm counts in mice

**Missing factors limiting the use of previous repeat exposure studies for health guidelines :**

- Form, source, or purity of toxin
- Confirmation of ATX stability in dosing solutions
- Appropriate route of administration

# Zion National Park

## Toxic bacteria detected in several of Zion National Park's waterways

Health watches and warnings have been issued for certain bodies of water in the park due to the presence of cyanotoxins.

NPS.gov / Plan Your Visit / Safety / Toxic Cyanobacteria

### Toxic Cyanobacteria Bloom in the Virgin River and the Streams of Zion National Park

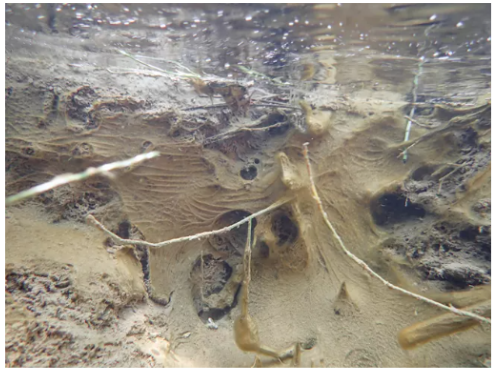
Zion National Park is monitoring for the presence of toxic cyanobacteria and cyanotoxins in water throughout the park. We share information so you can make informed decisions about whether and how to recreate. We work with the Utah Department of Environmental Quality to share recreational advisory levels for still and flowing water throughout the park. Recreational advisory levels are:

- Health Watch
- Warning
- Danger

Learn more about how the Utah Department of Environmental Quality sets these levels. The National Park Service shares current recreational advisory levels for Zion on this page.

**Need to know:**

Do not drink stream water anywhere in the park. Carry water or filter directly from a spring



Cyanobacteria can grow on surfaces like rocks, sticks, and sand. The yellow/brown, vein textured material in this photo is cyanobacteria.
NPS

# Summary of Cyanotoxin Results from Zion NP 2020

| Collection Date | Test Date | Testing Lab | Sample Origin | Toxin Identified | Toxin Quanity ug/L |
|---|---|---|---|---|---|
| 7/9/2020 | 7/10/2020 | R8 | CYCRA2 | ATX | 1130 |
| 7/9/2020 | 7/10/2020 | R8 | CYFLA4 | ATX | 163 |
| 7/9/2020 | 7/10/2020 | R8 | CYFLA4 | ATX | 126 |
| 7/9/2020 | 7/10/2020 | R8 | CYDOG4 | ATX | 338 |
| 7/9/2020 | 7/10/2020 | R8 | CYDOG4 | HTX | 45.7 |
| 7/9/2020 | 7/10/2020 | R8 | CYNAR4 | ATX | 0.054 |
| 7/9/2020 | 7/10/2020 | R8 | CYZVC2 | ATX | 139 |
| 7/9/2020 | 7/10/2020 | R8 | CYZVC2 | ATX | 96.8 |
| 10/6/2020 | 10/14/2020 | GW | CYTEM4 | ATX | 805 |
| 10/6/2020 | 10/14/2020 | GW | CYTEM4 | dhATX | 30,000 |
| 10/6/2020 | 10/14/2020 | GW | CYTEM4 | HTX | 0.56 |
| 10/6/2020 | 10/14/2020 | GW | CYCRA3 | ATX | 10.5 |
| 10/6/2020 | 10/14/2020 | GW | CYCRA3 | dhATX | 180 |
| 10/6/2020 | 10/14/2020 | GW | CYCRA3 | HTX | 0.13 |
| 10/6/2020 | 10/14/2020 | GW | CYFLA27 | ATX | 10.9 |
| 10/6/2020 | 10/14/2020 | GW | CYFLA27 | dhATX | 430 |
| 10/6/2020 | 10/14/2020 | GW | CYFLA27 | HTX | 0.08 |
| 7/9/2020 | 10/21/2020 | GW | CYCRA2 | ATX | 1800 |
| 7/9/2020 | 10/21/2020 | GW | CYCRA2 | dhATX | 9100 |
| 7/9/2020 | 10/21/2020 | GW | CYFLA4 | ATX | 470 |
| 7/9/2020 | 10/21/2020 | GW | CYFLA4 | dhATX | 270 |

# Areas Investigated with Anatoxin Study

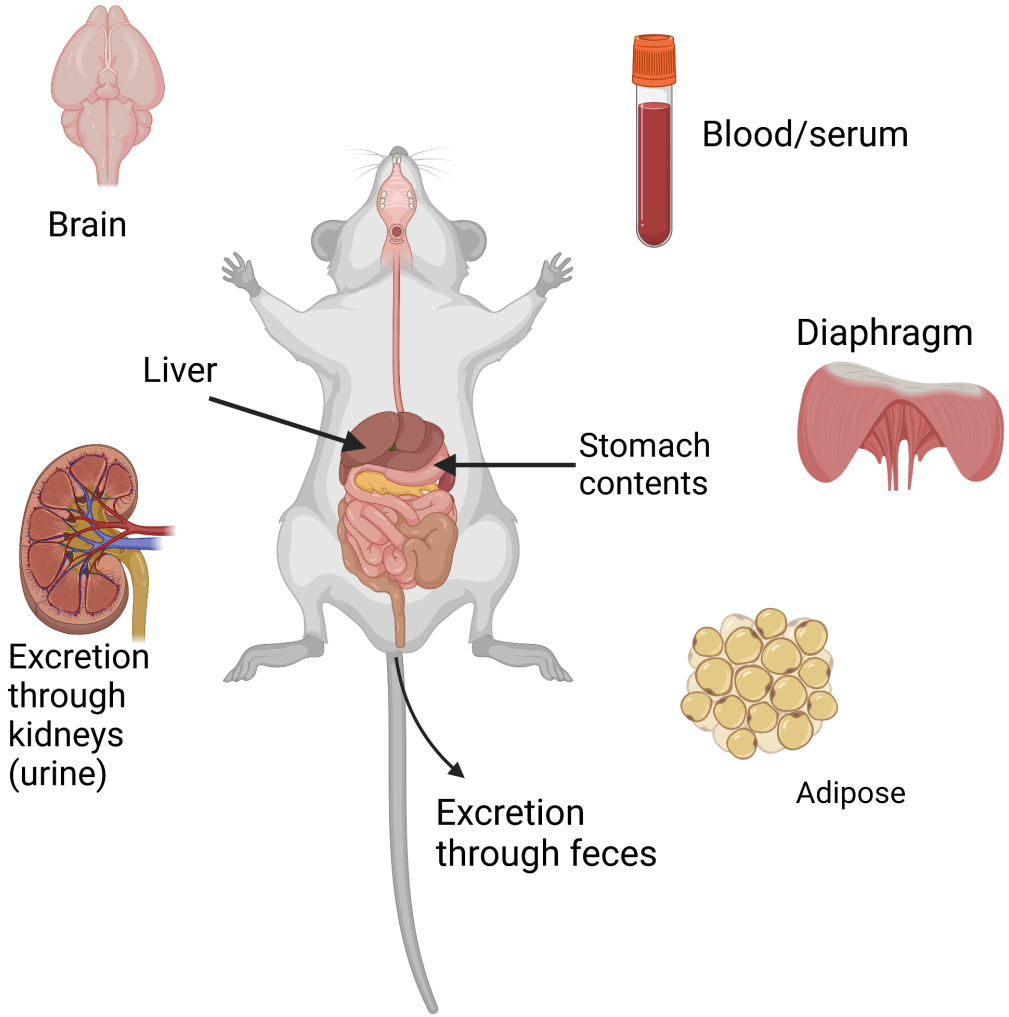
- Toxicokinetics
- Neurotoxicology- behavior, brainstem RNA
- Auditory toxicology
- Subacute exposures (5 days)

# Step 1: Dose-finding for transient neurologic endpoints

## Single, oral dose with anatoxin-a

- Start with dose range between NOAEL and LOAEL of Fawell's study (2.46-6.15 mg/kg)
- Observe for 24 hours
- Goal is to determine non-lethal dose that produces transient signs of anatoxin toxicity

# Step 2: Toxicokinetics of Anatoxin-a

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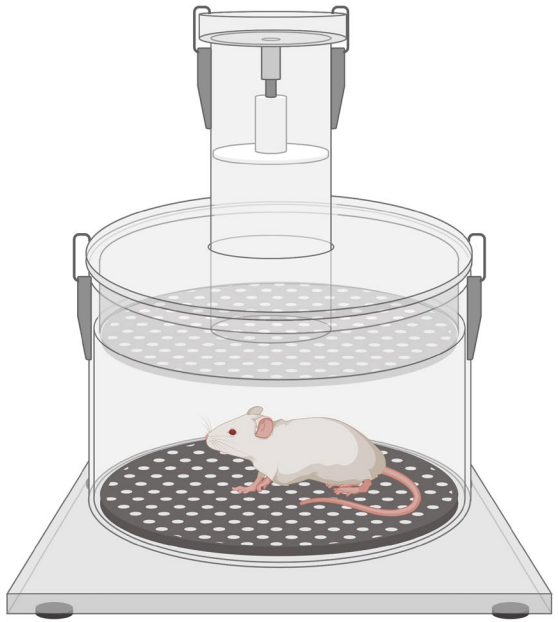
# Addition to the Toxicokinetics (TK) Study

- Whole body plethysmography to assess anatoxin effects on respiration



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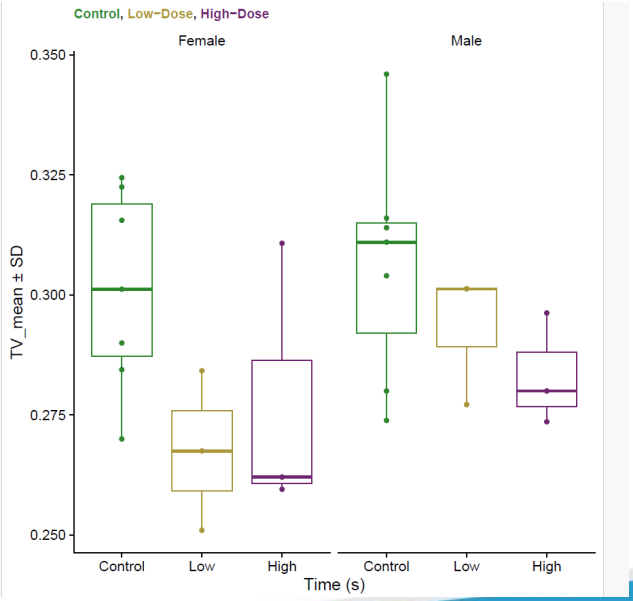
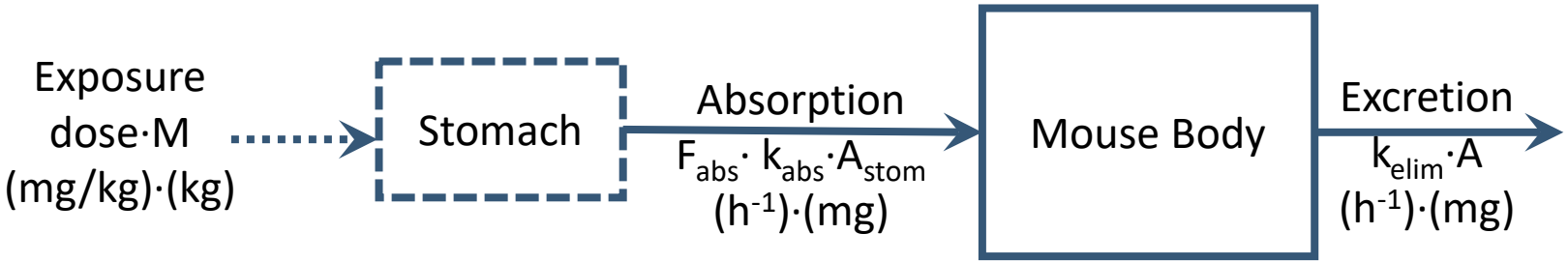


# Toxicokinetics (TK)

- Analyzed with pharmacokinetic modeling to maximize use of data
- Done with a 2-stage pilot informing the larger TK study
- Will aid risk assessment
- New information for anatoxin-a
- Bioavailability to be determined

# PK Model- *What the body does to the substance*

- A simple, “one-compartment” PK model was used for preliminary analysis of the ATX PK data.



# ATX Distribution to Tissues

- The PK analysis suggests high distribution to some tissues, but which ones?
- Data were collected for brain, fat, liver and muscle.
- Brain- and fat-to-blood concentration ratios do not indicate high distribution to those tissues.

# ATX Distribution Tissues (2)

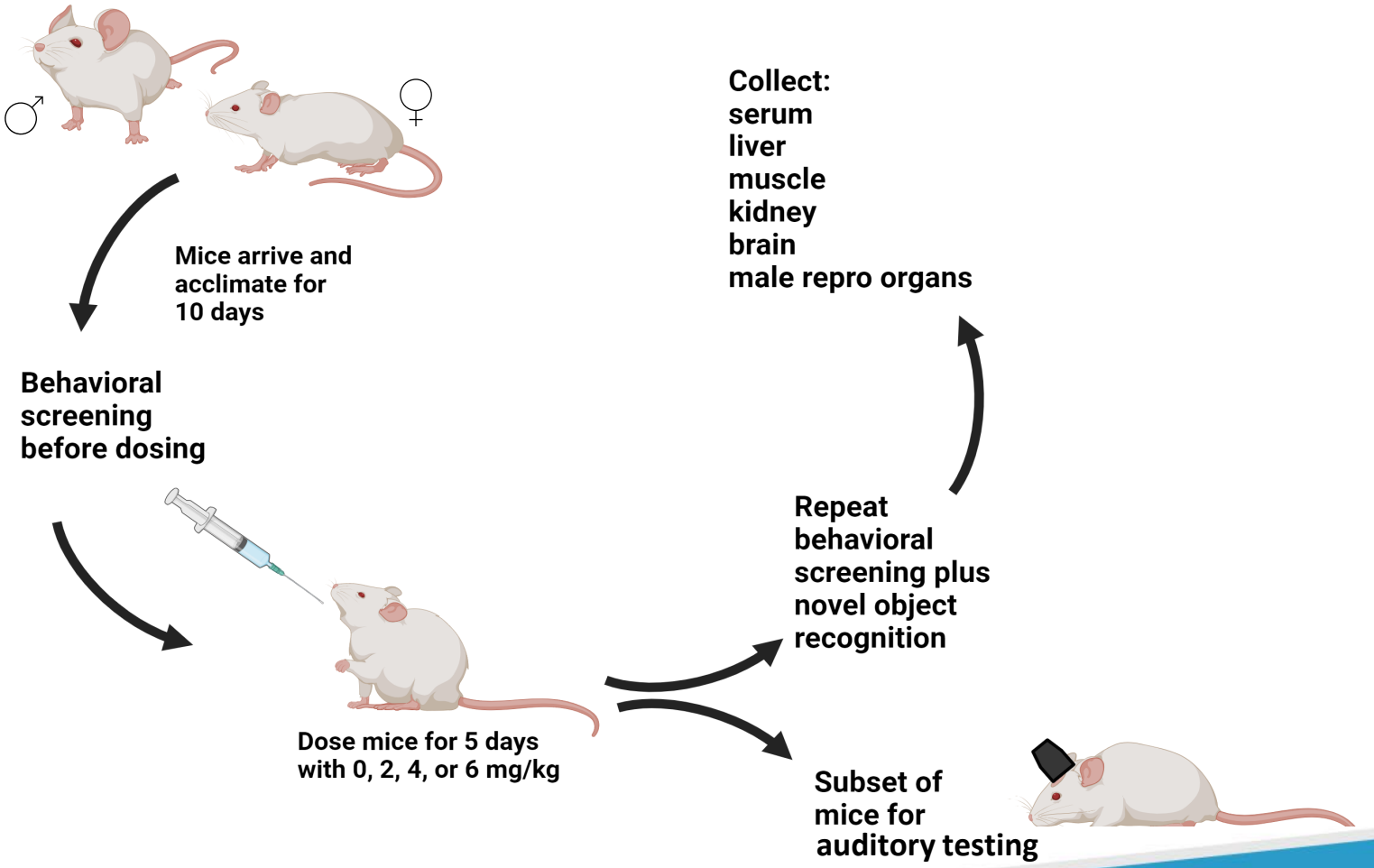
- However, in some mice distribution to liver and muscle were very high.
- ATX concentrations in muscle were as much as 10x blood.
- Liver concentrations were ~ 4x blood.

Step 3: 5-day oral exposure

## Key Endpoints

- Screening for ototoxicity
- Assessment of basic neurobehavioral effects
- Male reproduction
- Transcriptomics
- Metabolomics

# Study Design for 5-Day Oral Anatoxin

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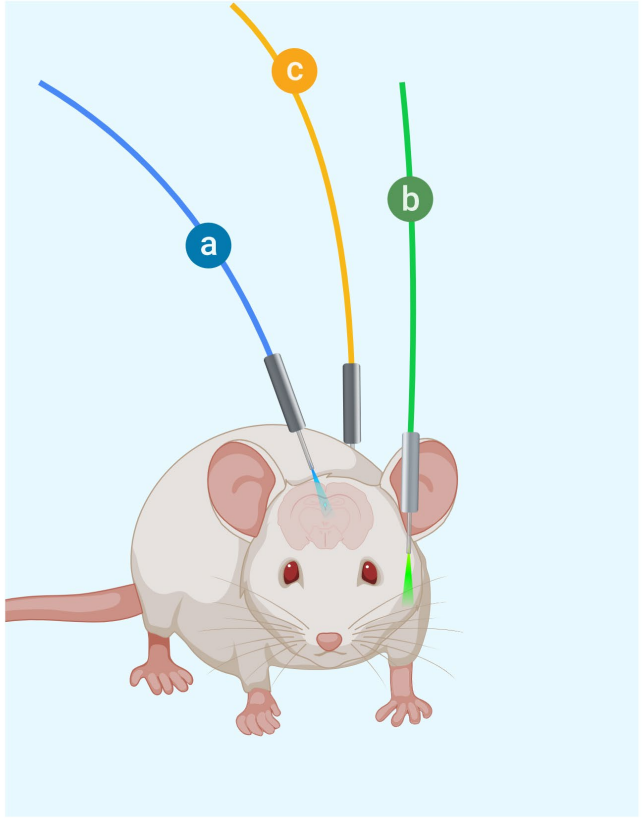
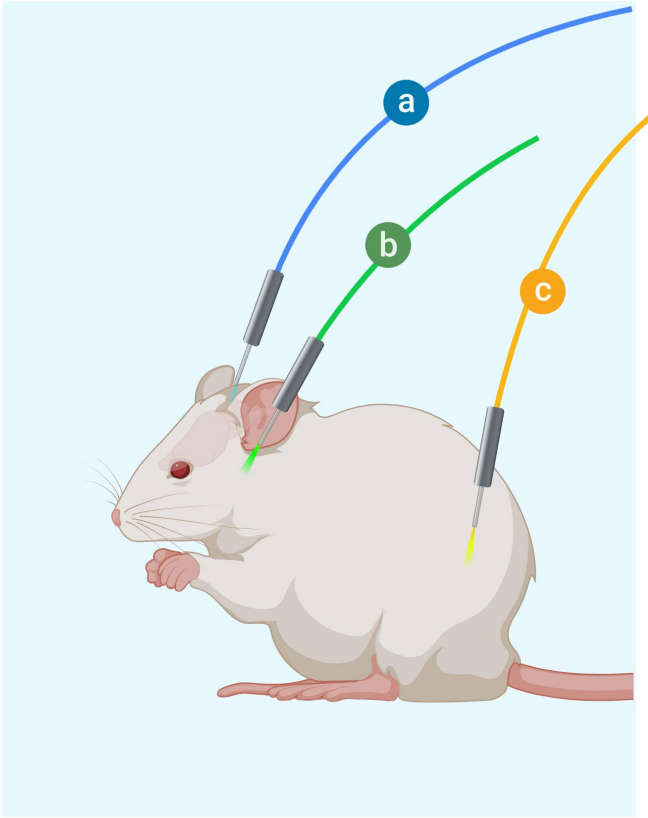
# Brainstem Auditory Evoked Response

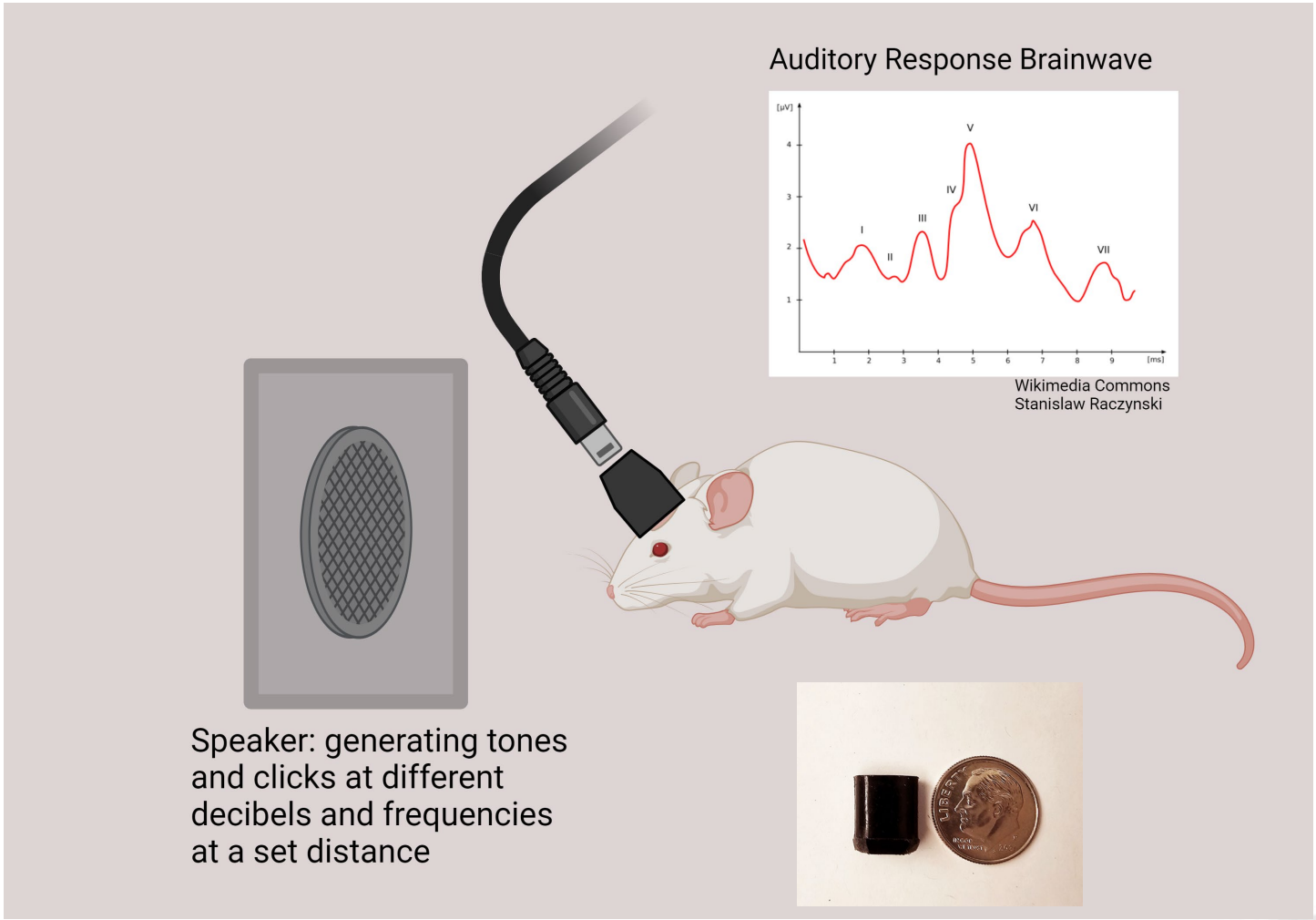
Anterior view




Lateral view



# Auditory Testing- Phase 2

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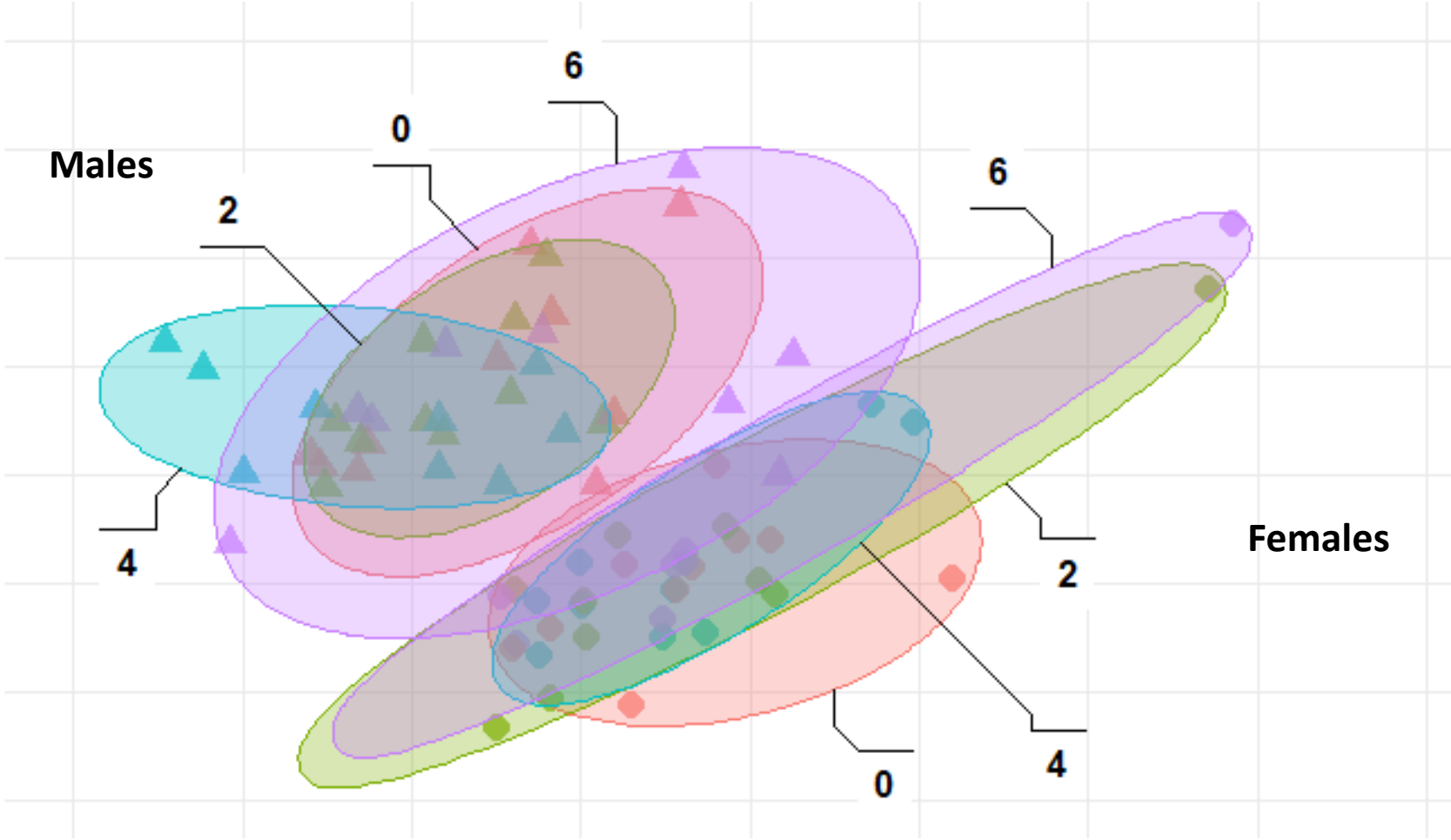
# Status of Endpoints

| Endpoint | In process or complete | Data Status |
|---|---|---|
| Serum Chemistry | complete | complete |
| Proteomic Assays | complete | To be analyzed |
| Neurobehavioral | complete | complete |
| Metabolomics | complete | Being analyzed |
| Male repro effects | In process | |
| Transcriptomics | In process | |

# Metabolomic Initial Analyses by Sex/Dose



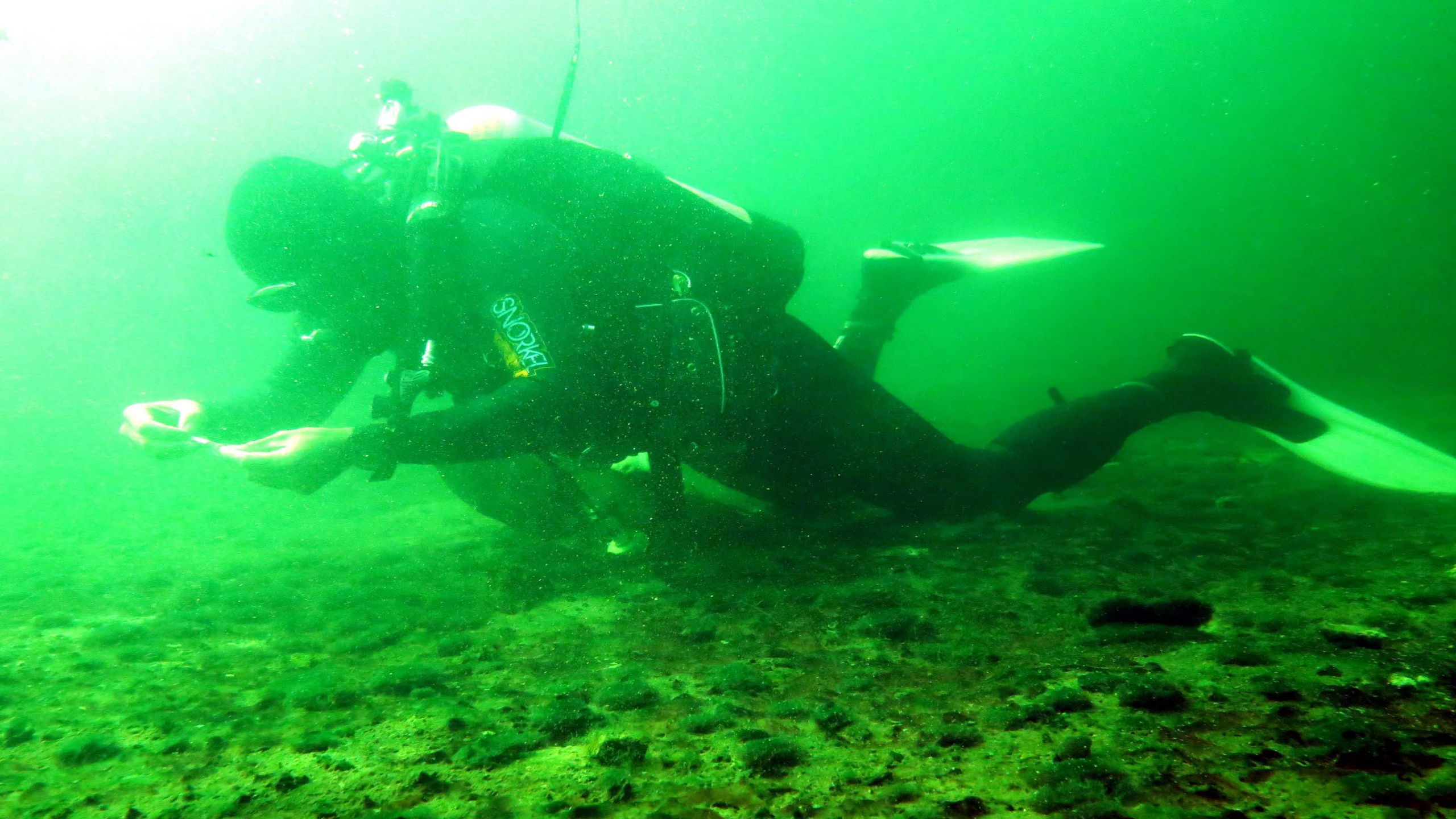

**No clear separation by dose**

# CDC’s HABS Reporting Site

KEY POINTS

- CDC's One Health Harmful Algal Bloom System (OHHABS) collects data about harmful algal blooms and the human and animal illnesses they cause.
- State and territorial public health departments voluntarily report to OHHABS.
- OHHABS is a reporting system that gathers information to better understand harmful algal blooms and prevent associated illnesses.

# Insights into Benthic Cyanobacteria Ecology and Anatoxin Production


SNORKEL

Harmful Algal Bloom Found in Zion National Park, UT River When Dog Died After Swimming

Anatoxins from benthic cyanobacteria responsible for dog mortalities in New Brunswick, Canada

**Beaches closed in Neuchâtel after dogs die from poisoning**

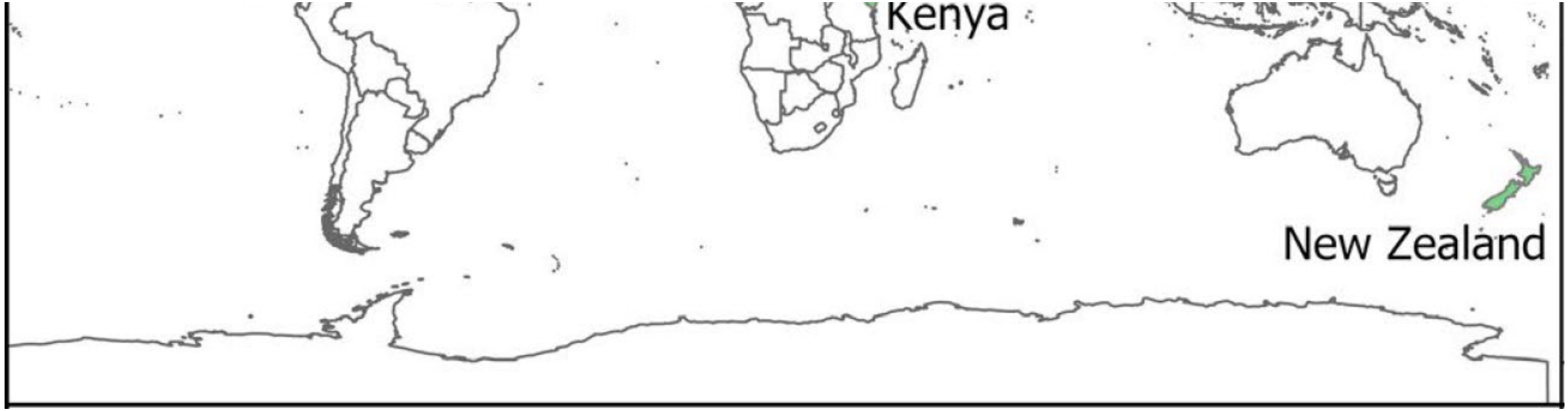
Cyanobacteria identified as killer of bald eagles

Cyanotoxins associated with macrophytes in Berlin (Germany) water bodies – Occurrence and risk assessment

**Cyanobacterial Harmful Algal Mats (CyanoHAMs) in tropical rivers of central Mexico and their potential risks through toxin production**


**Blue-green algae potentially harmful to dogs found in 10 spots along Lake Travis, LCRA says**


# Time to move beyond discovery phase…but how?



# *Microcoleus autumnalis*

NEWS 5

## Toxic algae may have killed three Hutt dogs

BY SIMON EDWARDS

## Algae can be deadly

# Dog killed by toxic algae in Hutt River

COLIN PATTERSON

A DISTRAUGHT Wellington dog owner performed cardiac massage on his dog in a desperate attempt to revive the animal when it collapsed after a swim in the Hutt River.

Despite his efforts, Richard Nicholls' six-year-old Labrador cross, Maggie, died after a dip in the river on Sunday.

Dog owners had been warned to keep their animals out of the water after tests revealed high levels of a bacterial alga.

**Warning too late:** Labrador cross Maggie died after swimming in the Hutt River last Sunday. Owner Richard Nicholls saw no warnings.

while his wife drove to a vet. The vet put Maggie into an incubator and gave her adrenaline.

However, effor... failed.

Both Mr Nichol... upset at the loss of...

"It's like losing one of your kids. My wife said she will refuse to go to the Hutt River ever again."

The Nicholls' other three dogs had their stomachs pumped.

Mr Nicholls believed Maggie's death would have been prevented had there been better signage. He saw no signs when he arrived at the river, though later saw one "relatively inconspicuous" sign. There were other dogs in the water with their owners.

Hutt City Council spokesman John Pepper said the council had erected more than 70 signs along the river.

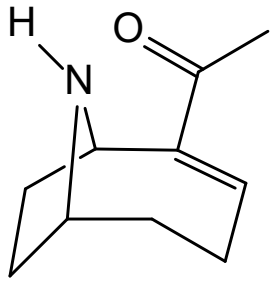
The council had also used the media to inform owners. Animal control staff and vets in the area had been alerted.

Upper Hutt City Council had also put signs by the river and had told dog owners about the problem.

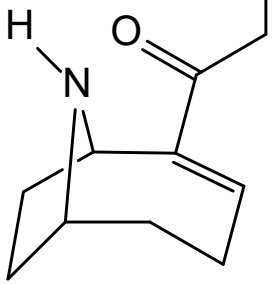
Health officials said yesterday that the...

# Council looks into dog 'poisonings' from river visits

## Fears killer algae may



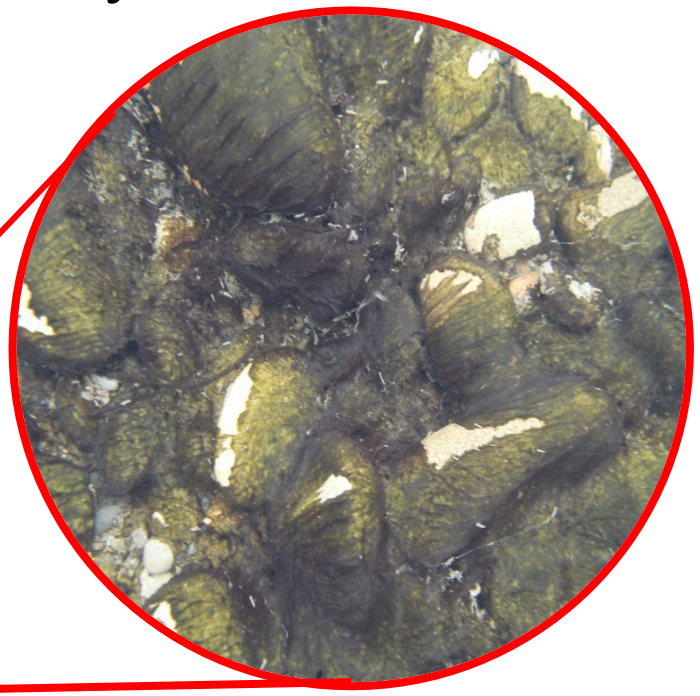

**Anatoxin-a (ATX)**




**Homoanatoxin-a (HTX)**

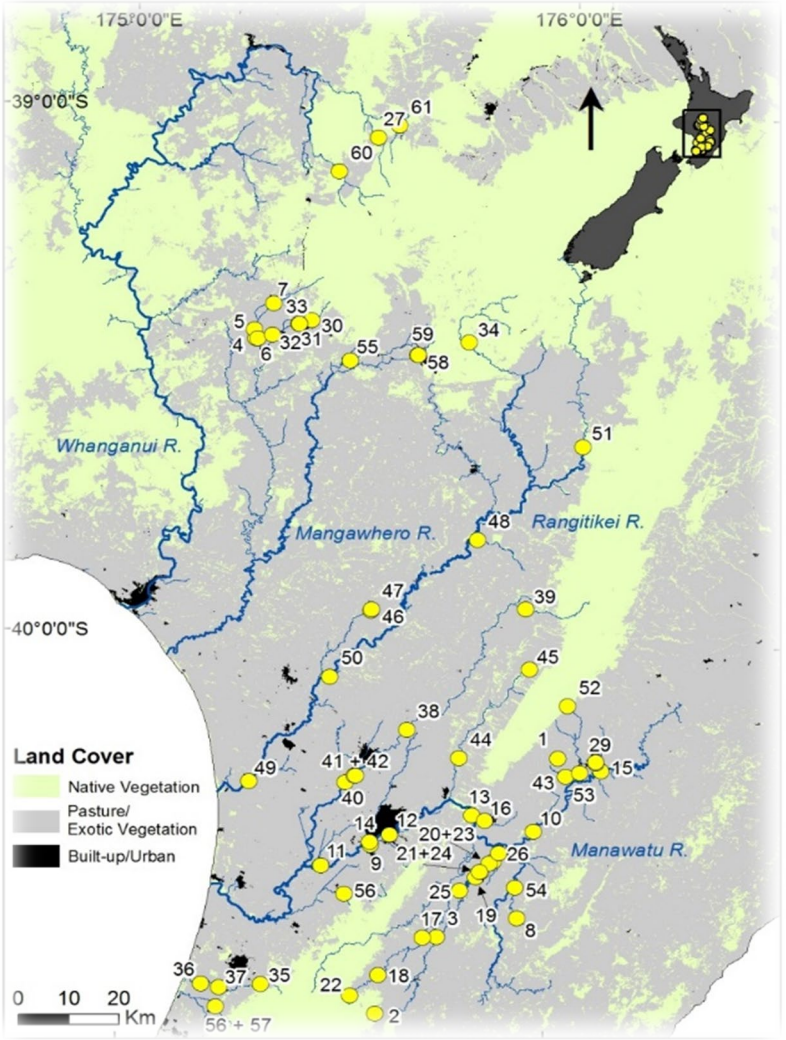
# Field studies

- Nutrients
- Metals
- Temperature
- River flow
- Physical characteristics

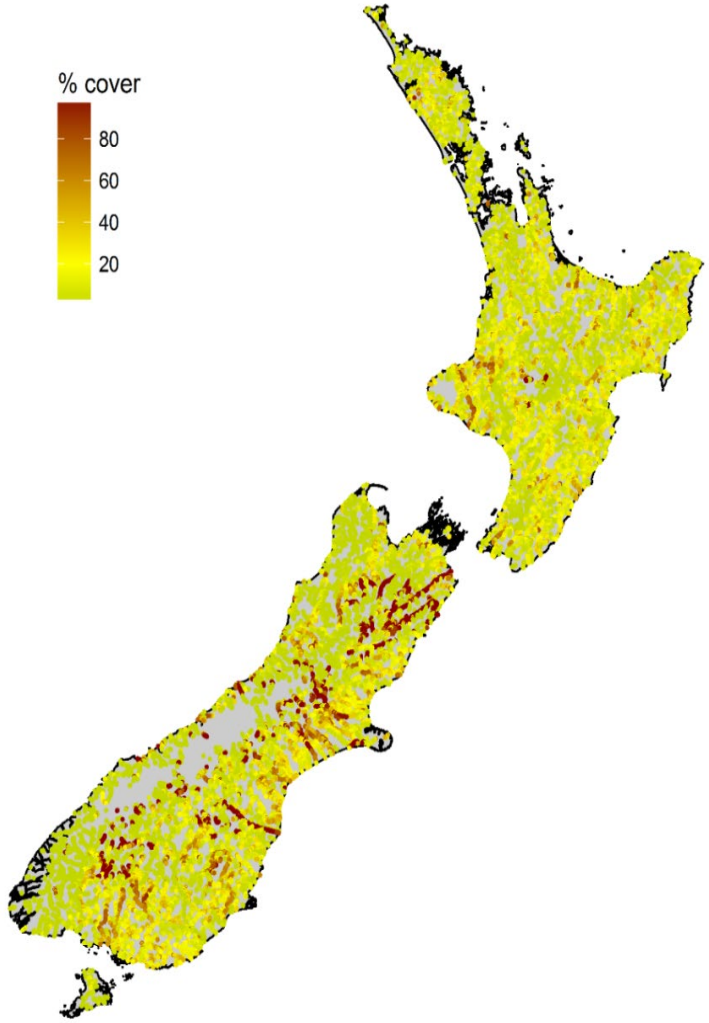


# Field studies

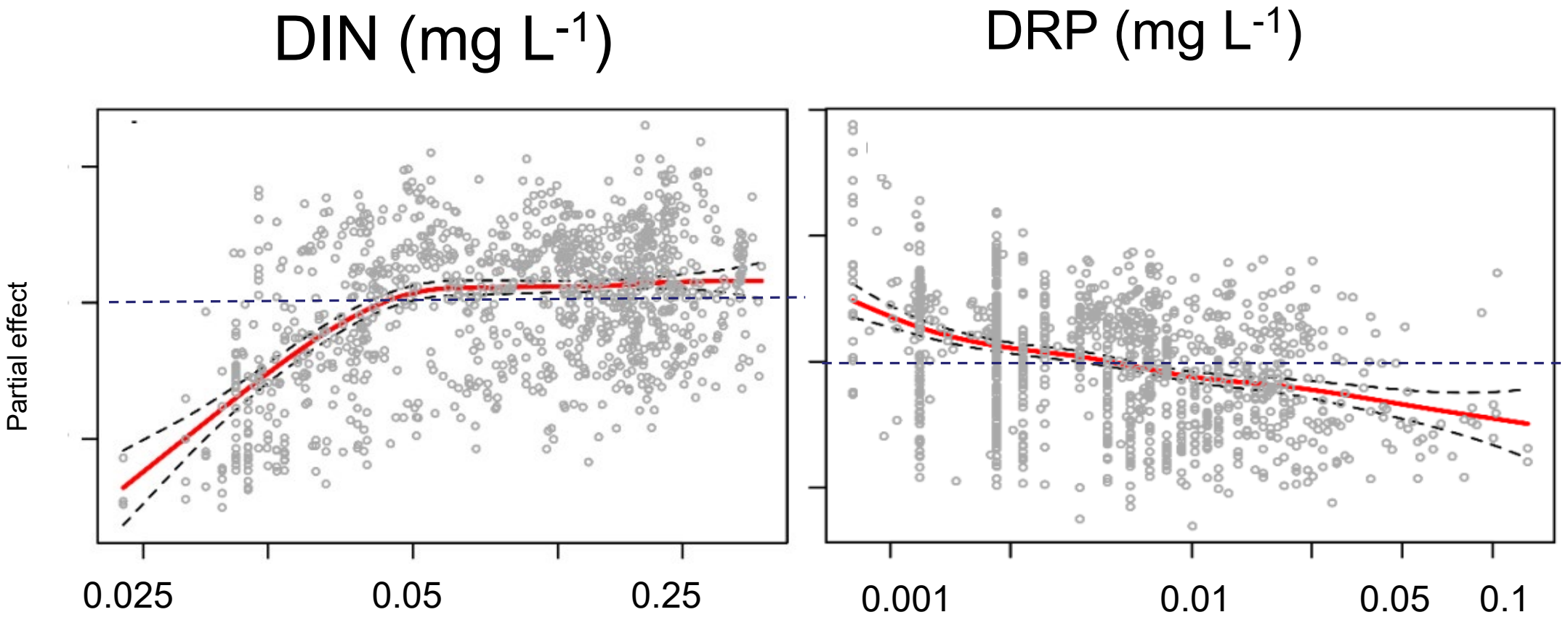
21 sites
Weekly, 2 years




64 sites
Monthly, 4 years




492 sites
Various, < 5 years



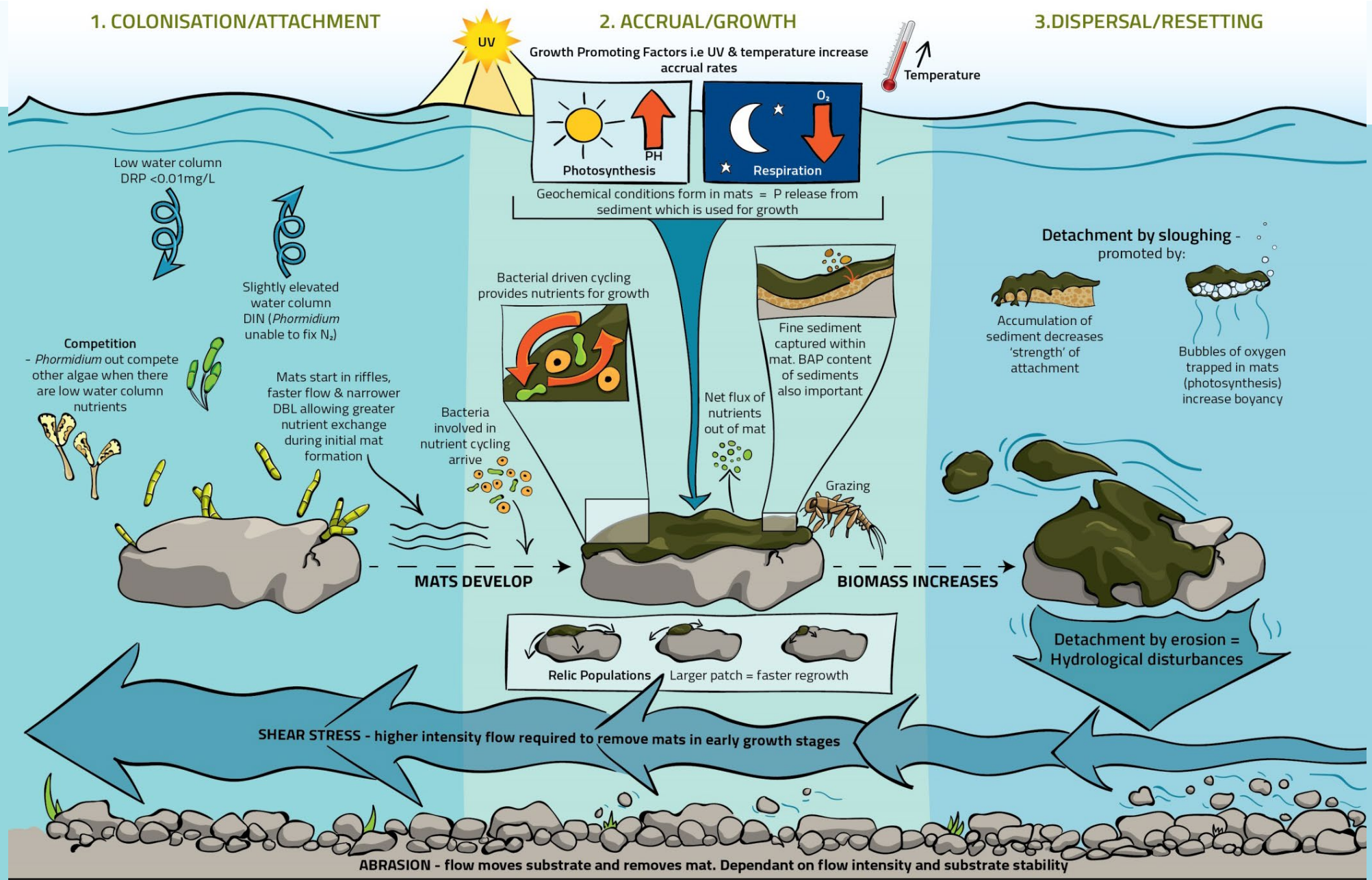

Strong patterns - DRP (<0.01 mg $L^{-1}$)
- elevated DIN

**Generally weak correlations…why?**

# Generally weak correlations…why?

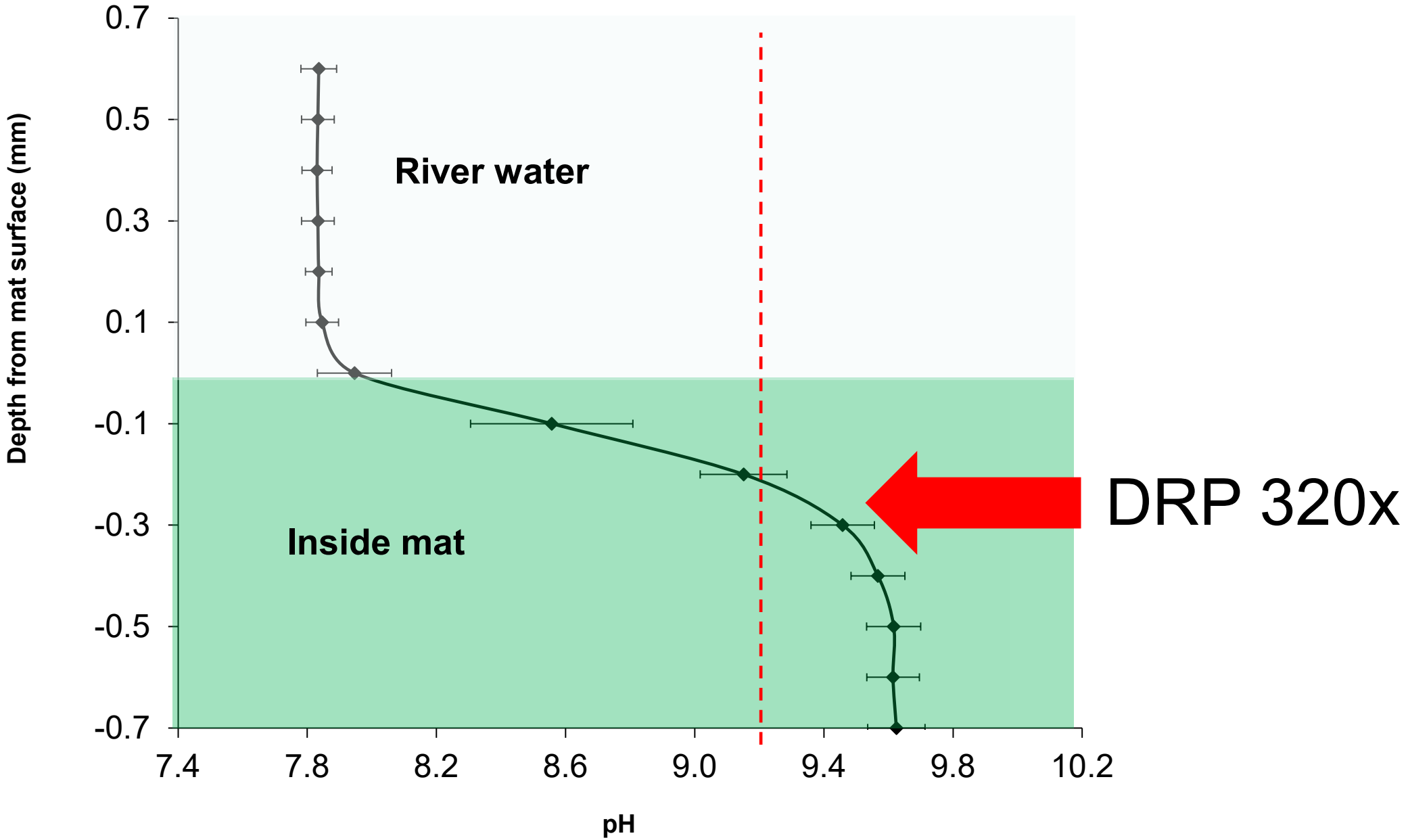
1. Each site is unique – hierarchy of importance
2. Need to consider accrual stage
3. Life inside a mat

# 2. Different divers – different part of accrual cycle

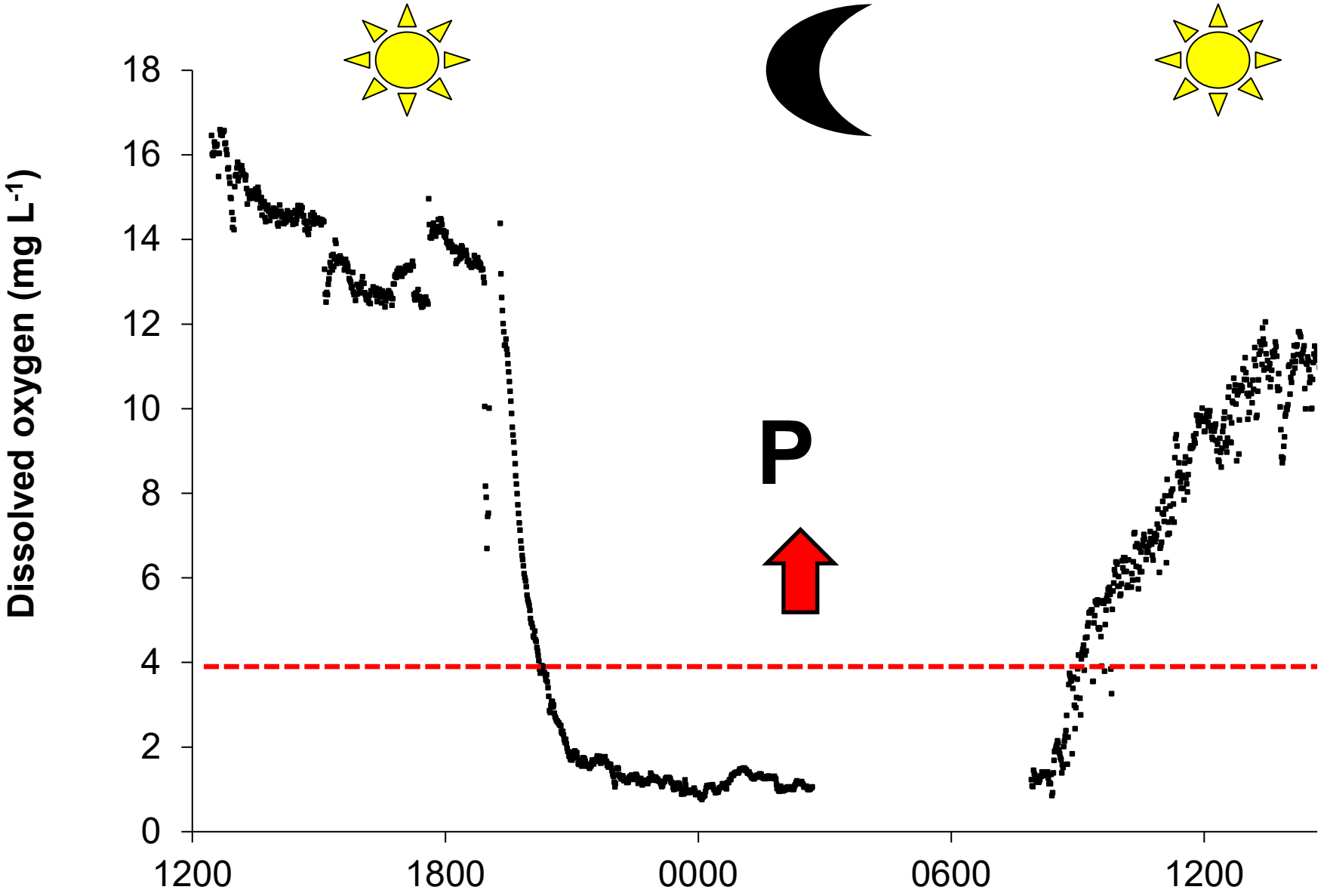


# 3. What happens inside the mats….

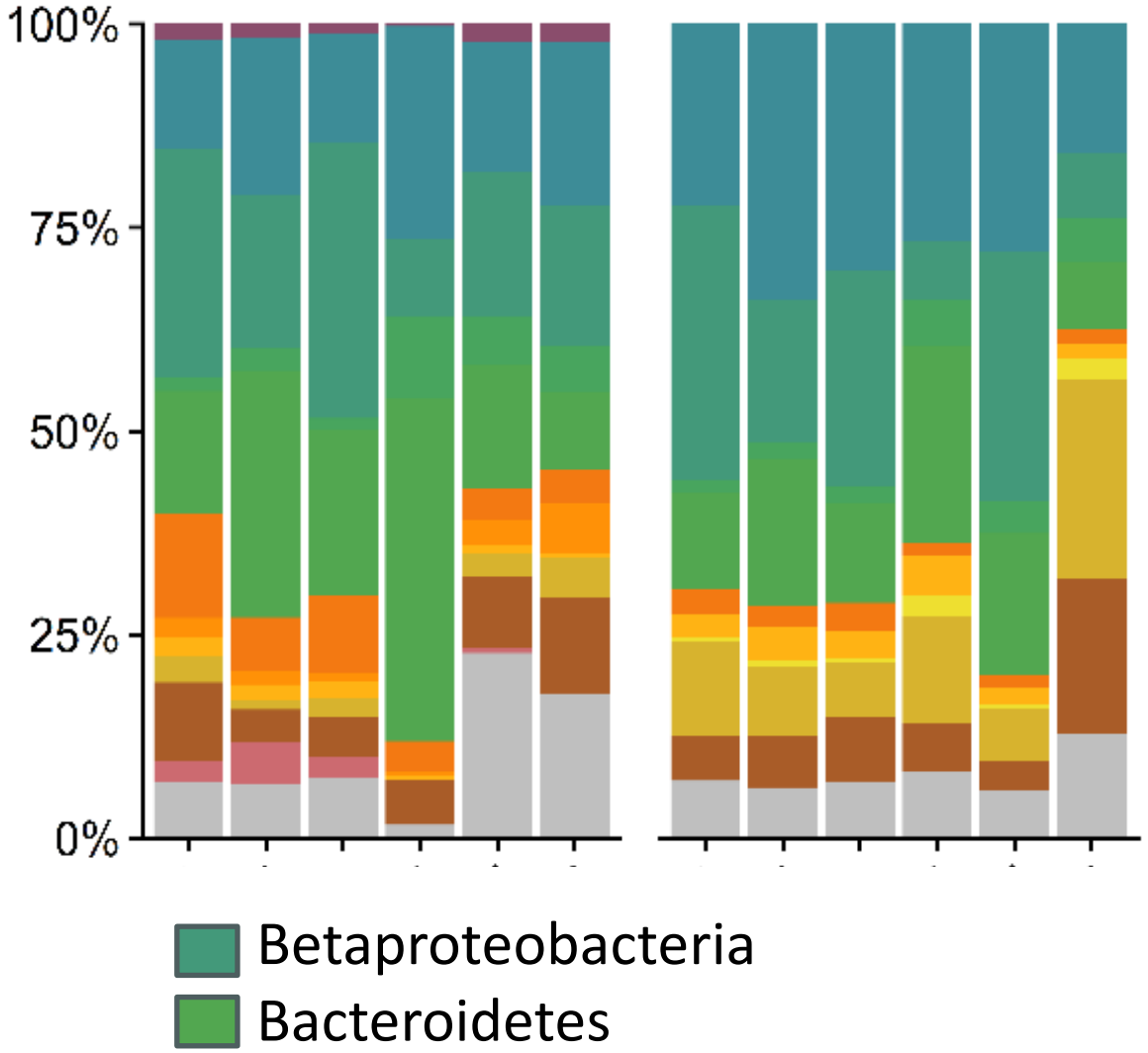
# pH within mats




DRP 320x


Dissolved oxygen (mg L-1)
18
16
14
12
10
8
6
4
2
0
1200
1800
0000
0600
1200
P

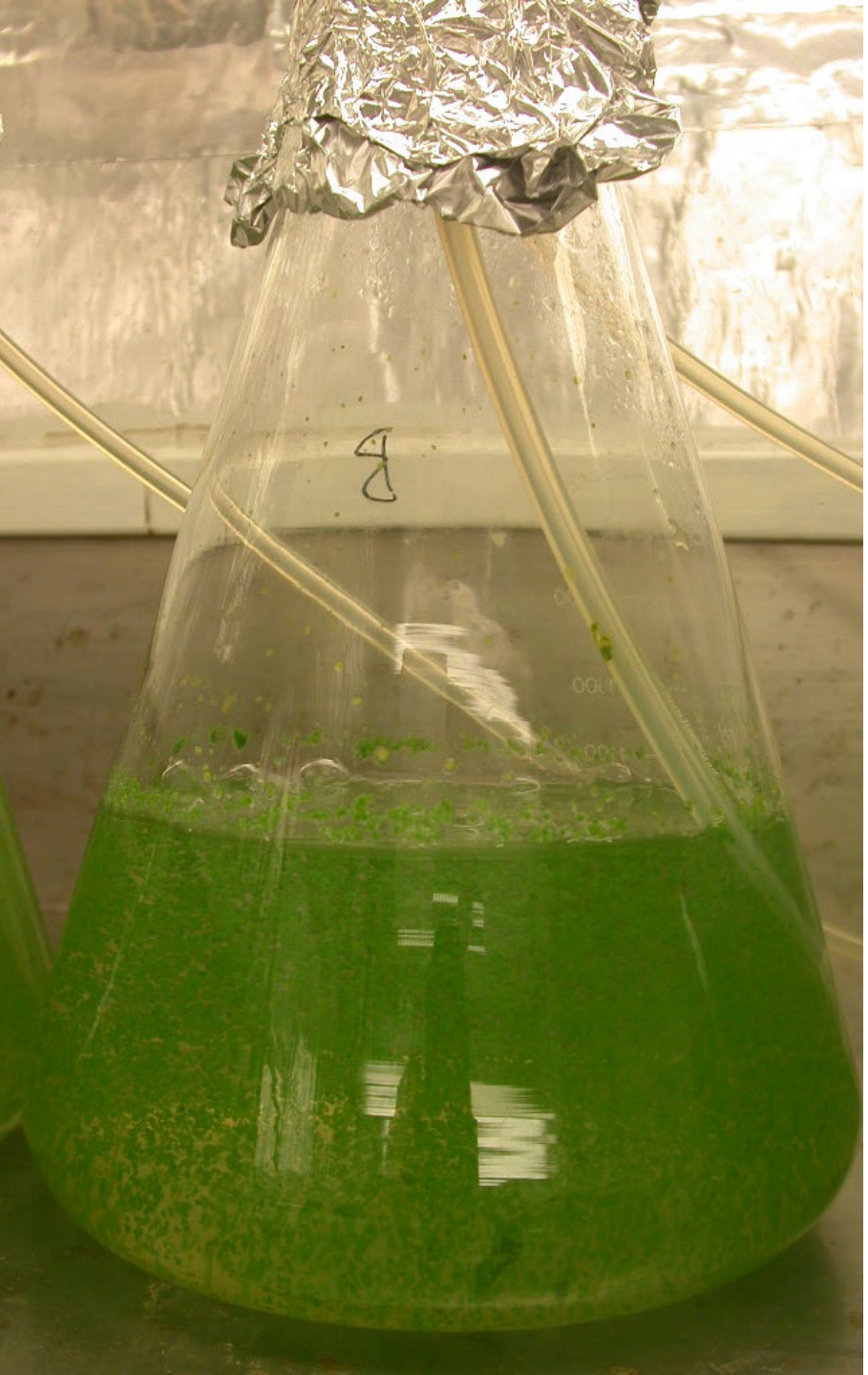
# Microbial consortiums - Metagenomes

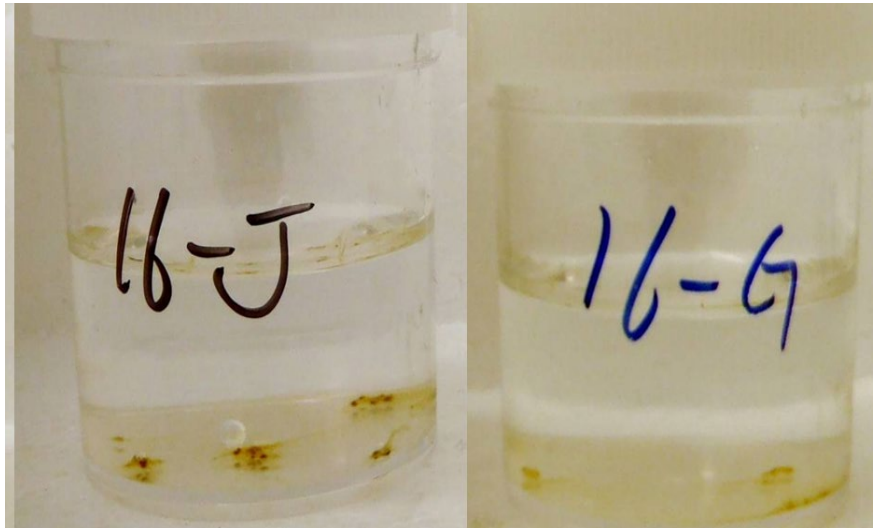


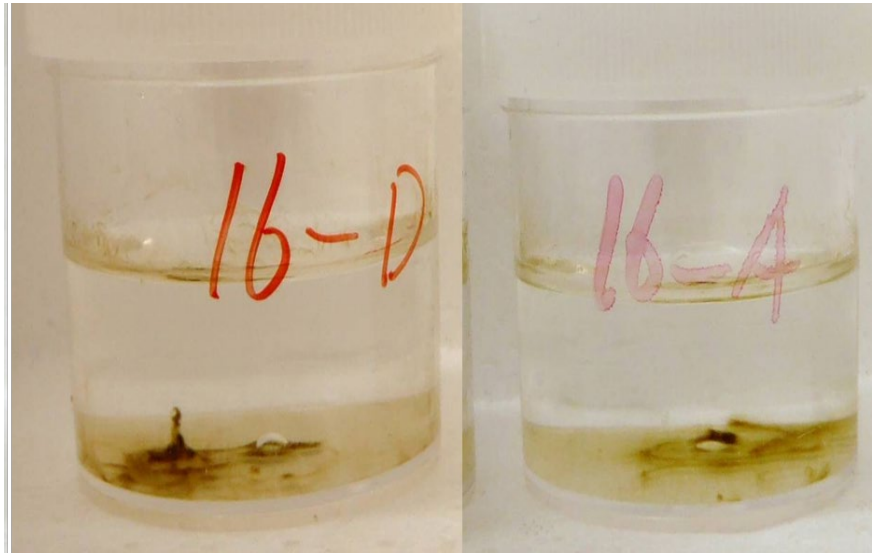

- Lack ability to fix $N_2$
- Dissolved nitrogen - urea & nitrate
- Cyanophycin - internal C & N storage

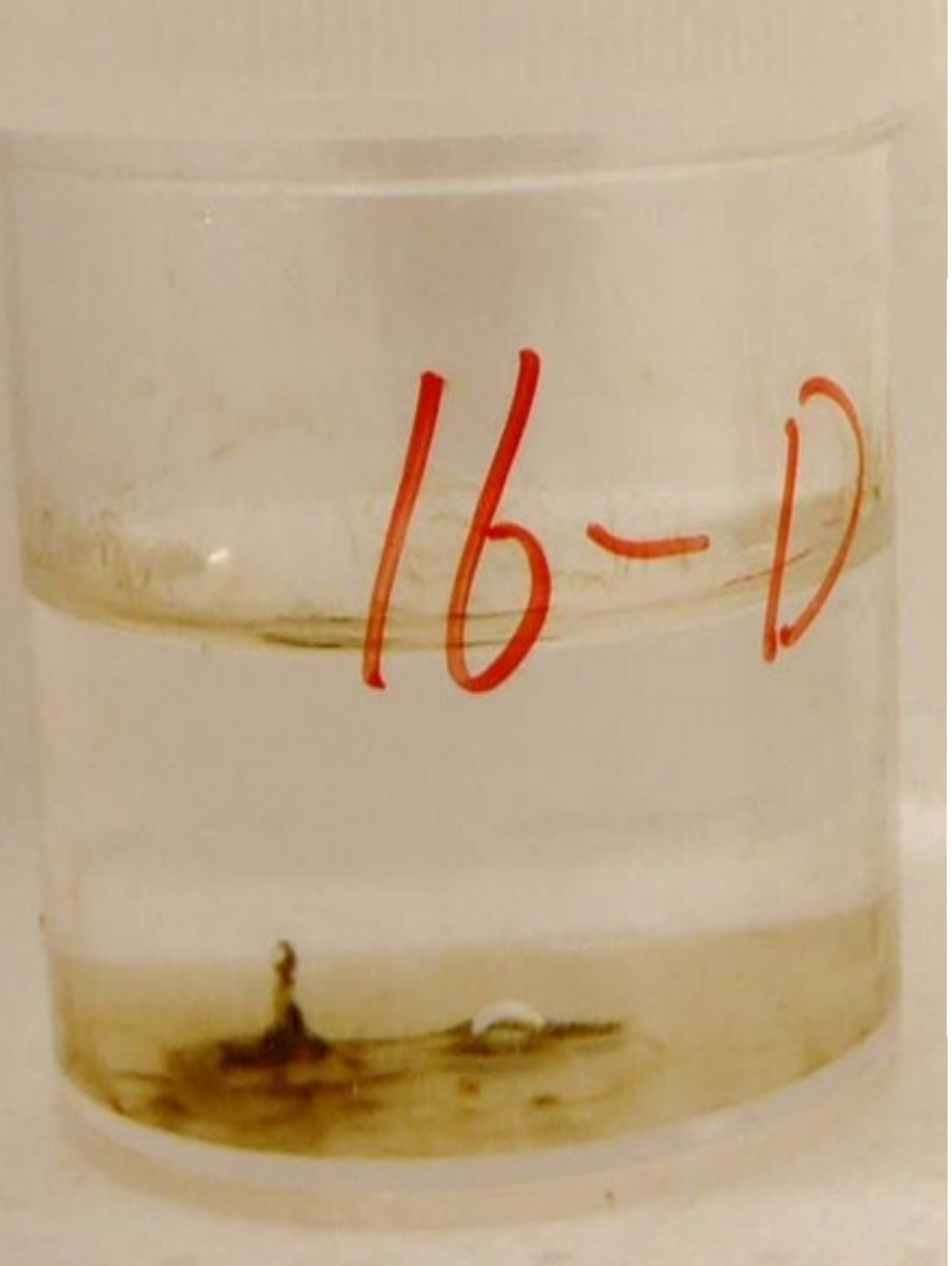
- <u>Multiple mechanisms:</u>
- Inorganic phosphate transport
- Three mechanisms - organic phosphorus mineralisation

Correlative ➡ causative




16-J
16-G


16-D
16-4


16-D

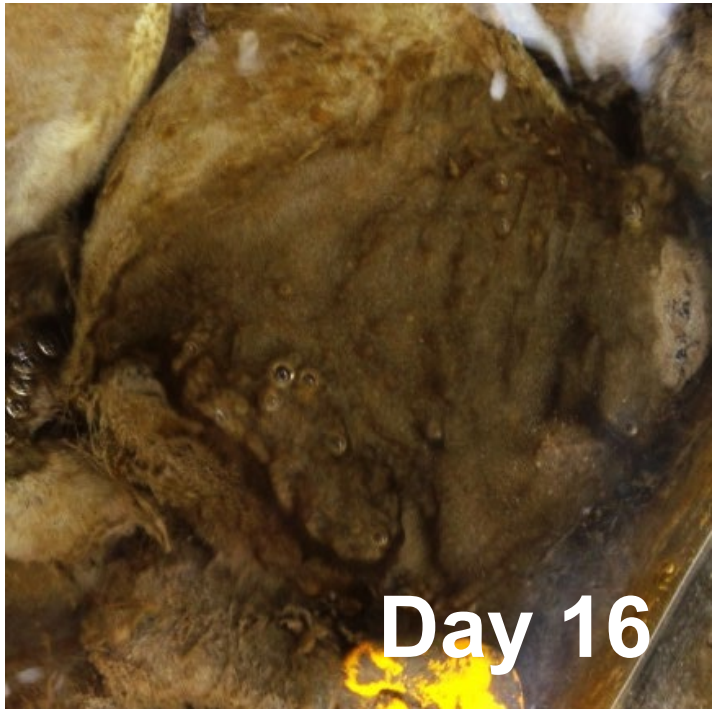
≠


Day 2

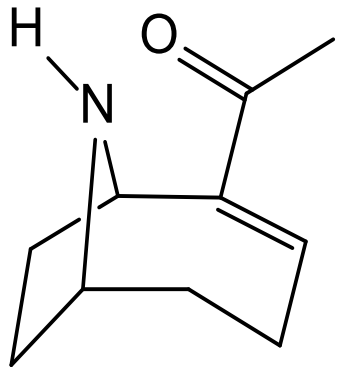

Day 4


Day 8

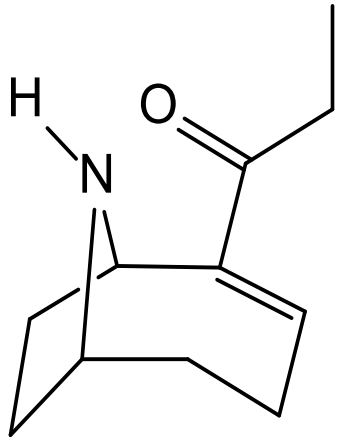

Day 13


Day 16

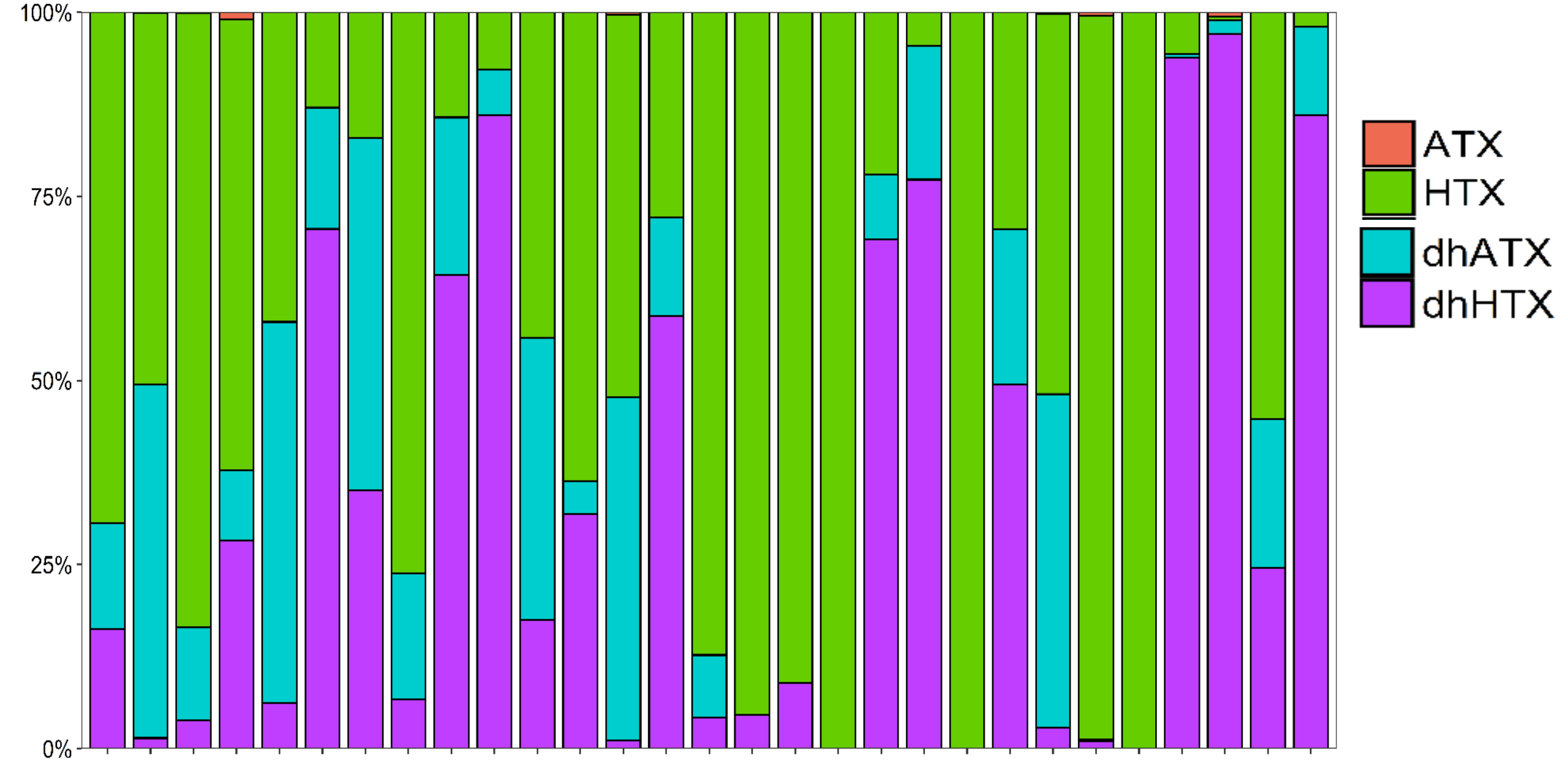
# Anatoxins

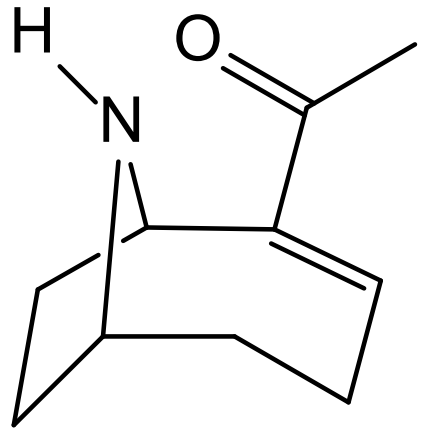



**Anatoxin-a (ATX)**



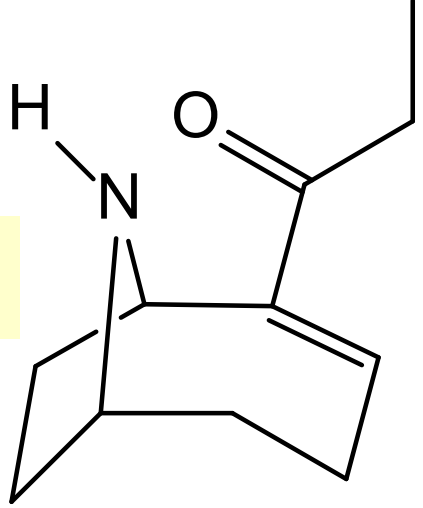

**Homoanatoxin-a (HTX)**


100%
75%
50%
25%
0%
ATX
HTX
dhATX
dhHTX

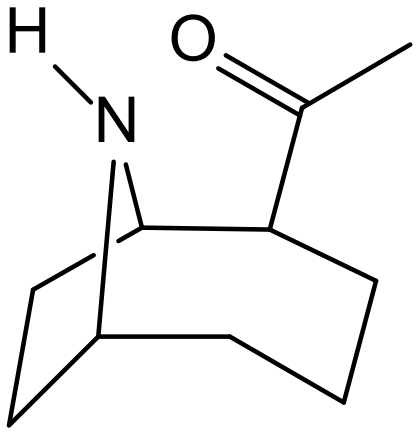



Anatoxin-a (ATX)

**$LD_{50}$ 200-400 µg/kg (i.p.)**

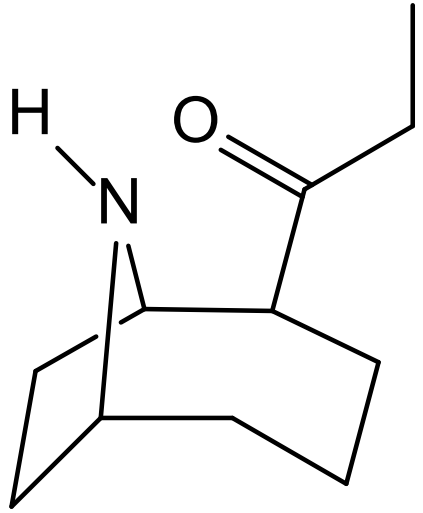



Homoanatoxin-a (HTX)




Dihydro-anatoxin-a (dhATX)

**$LD_{50}$ = ?? µg/kg**




Dihydro-homoanatoxin-a (dhHTX)

# Toxicity Project

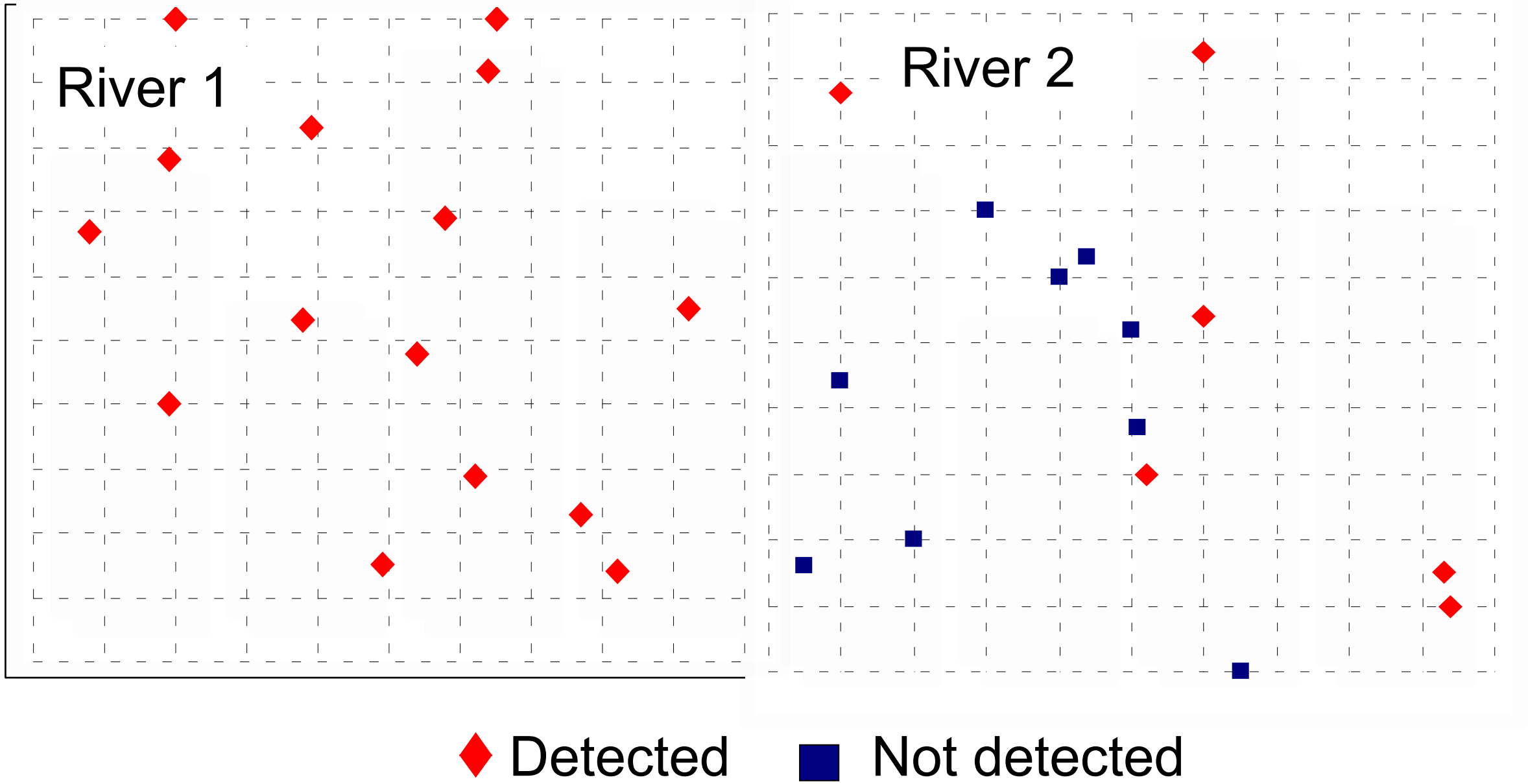
- **Determine accurate acute toxicity values for:**
  - **ATX**
  - **HTX**
  - **dhATX**
  - **dhHTX**

- **Three methods of administration:**
  - **Intraperitoneal (i.p.) injection**
  - **Gavage**
  - **Voluntary Feeding**


LC-01A
LC-02A
LC-03A
LC-04A

| | ATX (LD50 mg/kg) | dhATX (LD50 mg/kg) | |
|---|---|---|---|
|  | 0.23 | 0.73 | 3x less toxic |
|  | 10.6 | 2.5 | |
|  | 25 | 8 | 3-4x more toxic |

# Spatial Variability



| mg/kg |
| --- |
| 67 |
| 0 |
| 29 |
| 31 |
| 152 |
| 115 |
| 81 |
| 33 |
| 0 |
| 0 |
| 5 |
| 75 |
| 74 |

# Evolution of response in New Zealand

High risk sign – Greater Wellington Regional Council


# Recreational guidelines - Three-tier Risk Management Framework

## Surveillance Level / Green Mode

- Cyanobacteria levels are low:
  - Status quo

Up to 20% benthic cover

## Alert Level / Amber Mode

- Cyanobacteria are present in a waterbody:
  - Regular monitoring

20-50% benthic cover

## Action Level / Red Mode

- Cyanobacteria are present at health-adverse levels:
  - Restrict access to the waterbody to protect human health
  - Accurately assess the risk level through toxin testing

50+% cover OR detached mats

# Toxic Algae Communications

Case Study

## Greater Wellington's Evolution in Toxic Algae Communications

In November 2005, a dog that had been swimming in Te Awa Kairangi / Hutt River suffered a sudden and unexpected death. This was followed by four more dog deaths in the Hutt Valley during the summer period. These dog deaths were the catalyst of Greater Wellington's toxic algae communications journey that has evolved over the course of the last 18 years.

### The Power of Collaboration

These dog deaths shocked the local community and led to the formation of an inter-agency group to respond to cyanobacteria poisoning cases in the region, and better manage the risk to human and animal health. The inter-agency group included Greater Wellington, Upper Hutt City Council, Hutt City Council, Masterton District Council, Kāpiti Coast District Council and the Public Health Service of Te Whatu Ora. In 2007, the group established a traffic light framework and a monitoring programme – which was also later adopted in the interim 'recreational cyanobacteria guidelines'.

During the early stages of the response, the communications component relied predominantly on warning signs at rivers, press releases and traditional media (newspapers and radio; although [illegible] of warnings was minimal). and the public were uneasy about the situation. Feedback from the Wellington public included that:

- People weren't aware of health warnings for the river – they didn't hear about them in the media or they didn't see the warning signs.
- The current warning signs weren't effective – people ignored them because they were up all the time.
- People were avoiding using the river – even when it was safe to swim and walk dogs.
- In general, people had little understanding about toxic algae and what to look for.

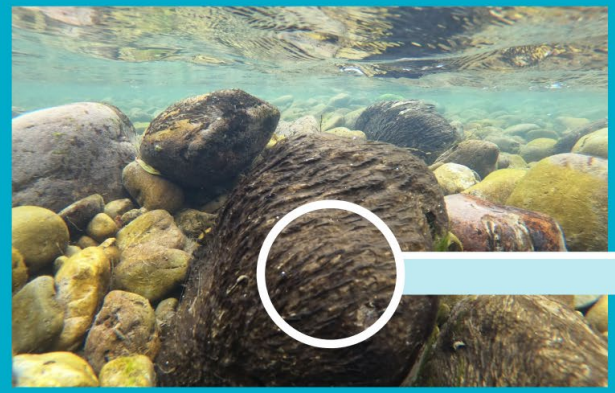
To address this feeling of unease, protect the community and encourage safe association with rivers in the region, the inter-agency group developed a targeted Toxic Algae Strategy and Communications Plan which continued to evolve over the following decade. The Strategy and

# STAY SAFE IN OUR WATERWAYS

## Water quality information

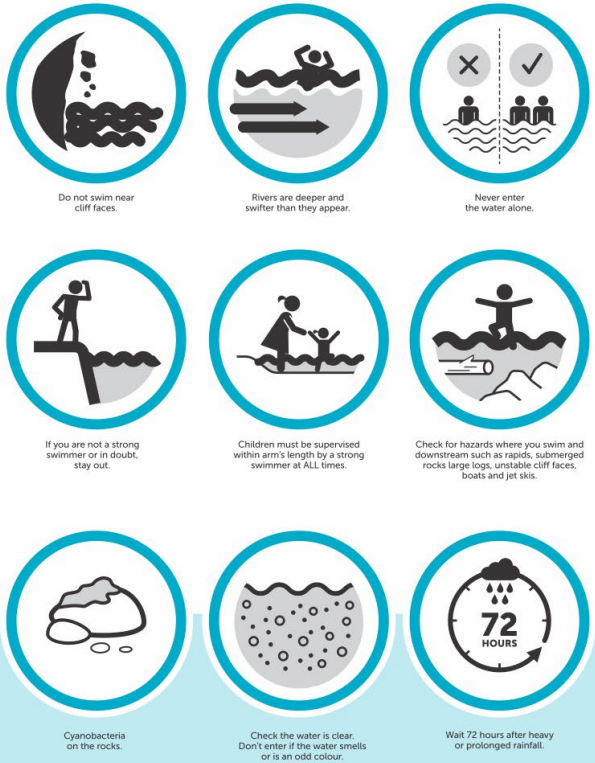
The water at this spot can have high levels of bacteria and/or potentially toxic algae at times. You can check the latest water quality information collected by Horizons Regional Council on the 'Can I Swim Here?' map at:

**www.lawa.org.nz**



Potentially toxin producing algae (cyanobacteria). Cyanobacteria can be very toxic to animals and small children so avoid contact where levels are high. In rivers and streams, look out for black mats attached to rocks in or above the water.

**In an emergency, call 111**

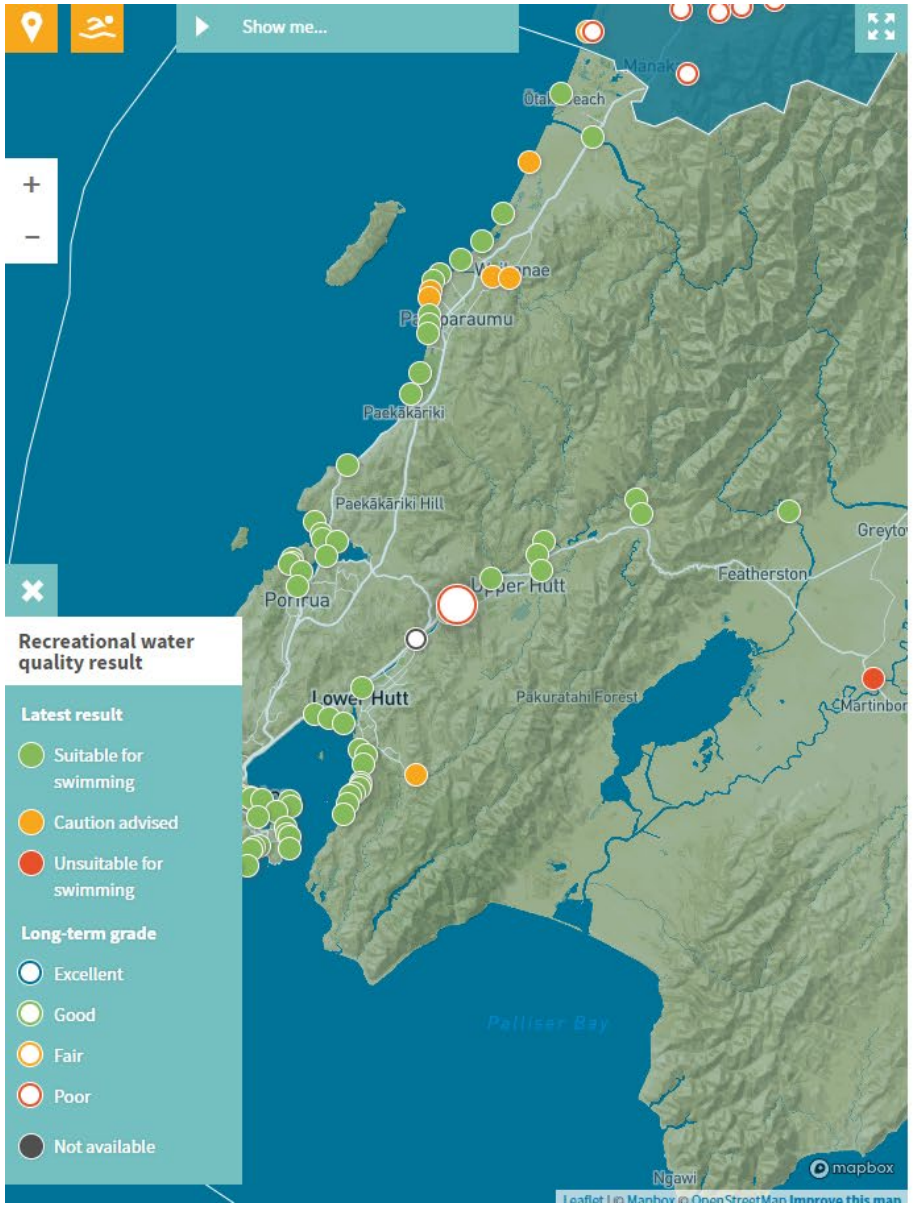



**Rivers change** | **Rivers are powerful** | **Rivers can be unpredictable**

horizons.govt.nz

horizons REGIONAL COUNCIL

# Toxic Algae Communications




**Toxic Algae sampling graph**


## What do the monitoring results mean?

**Suitable for swimming** – The monitoring result met national water quality guidelines at the time of testing.

**Caution advised** – The monitoring result for *E. coli* was slightly elevated at the time of testing. Water quality generally suitable for swimming, but young children, elderly or those with compromised health may be at increased risk of illness.
For sites where toxic algae is routinely monitored, the monitoring result exceeded the surveillance criteria. Avoid contact where toxic algae is present.

**Unsuitable for swimming** – The monitoring result did not meet the national guidelines at the time of testing.
For sites where toxic algae is routinely monitored, the monitoring result exceeded the alert level criteria. Avoid contact with the water and with toxic algae along the edge of the water.

## Long-term grade information

**Long-term grade based on five years of data**

Ministry for the Environment, MBIE – Catalyst fund, Regional Councils, Laura Kelly, Jonathan Puddick, Mark Heath, Katie Brasell, Francine Smith, Sally Gaw, Ken Ryan, Javier Atalah, Annika Wagenhoff, Tara McAllister, Kim Handley, Sze Tee, Ian Hawes, Keith Bouma-Gregson, Catherine Quiblier, Janani, Marcus, Roel van Ginkel, Andy Selwood, Iain McGrail, Sam Murray, Laura Biessy, Carrie Page, Hannah Greenhough, Joel Bowater, Penelope Truman, Sarah Munday, Rex Munday, Jean François Humbert