Welcome to this week's presentation and conversation hosted by the
Canadian Association for the Club of Rome,
a Club dedicated to intelligent debate and action on global issues.

Connectivity between freshwater and marine ecosystems.

Our speaker today is Dr. Robin Sleith, who is with the Maine environmental DNA project, a multi-institutional initiative that seeks to better understand Maine's coastal ecosystems. Using a combination of eDNA tools such as metabarcoding and quantitative PCR, we are testing ecological hypotheses around the boom-and-bust dynamics underlying harmful algal blooms in marine and freshwater ecosystems. Ultimately, we want to understand the factors that promote or impede formation of harmful algal blooms to forecast and manage these events better.

DESCRIPTION: This talk will explore the connectivity between freshwater and marine ecosystems, using the ongoing Maine eDNA project as a guide. Climate change and nutrient pollution in freshwater ecosystems have led to increased impacts on nearby marine ecosystems. Furthermore, anadromous species that rely on freshwater during their lifespan both impact and are impacted by freshwater systems. Emerging technologies like eDNA are able to increase the frequency and resolution of monitoring programs and help us understand these dynamic processes.

The presentation will be followed by a conversation, questions, and observations from the participants.

CACOR acknowledges that we all benefit from sharing the traditional territories of local Indigenous peoples (First Nations, Métis, and Inuit in Canada) and their descendants.

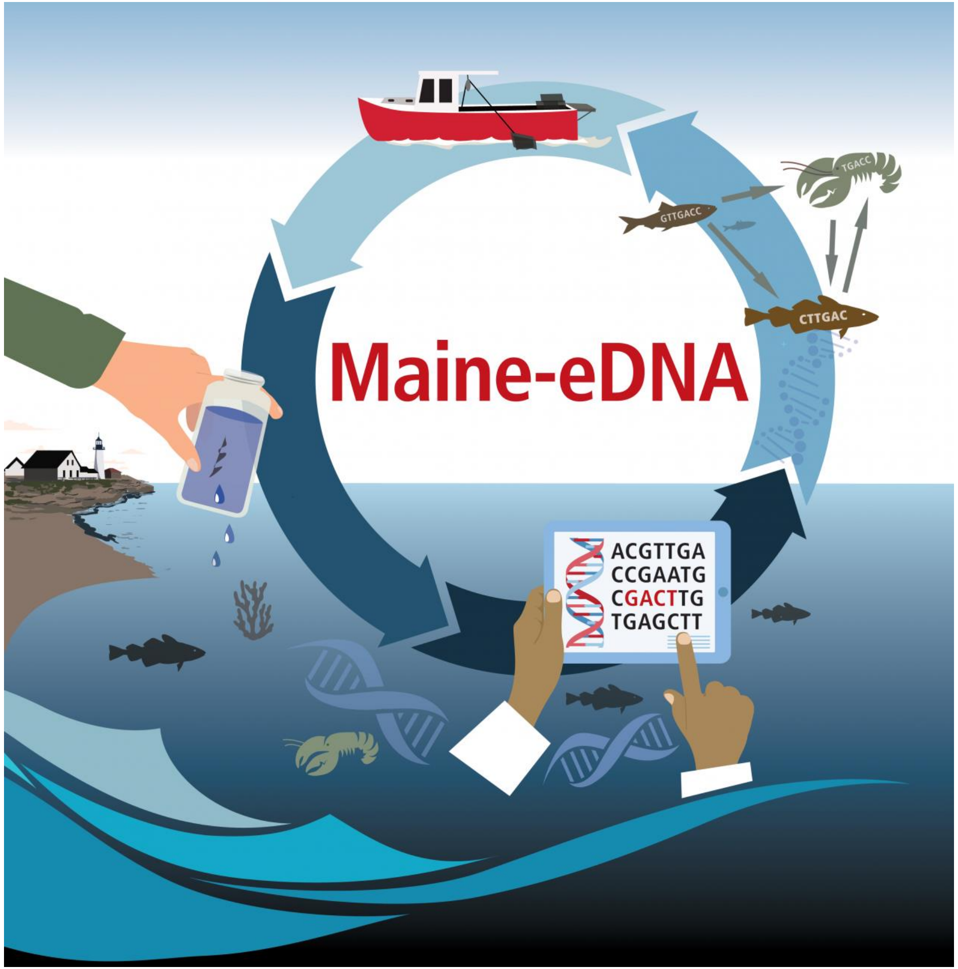
Canadian Association for the Club of Rome — Association canadienne pour le club de Rome

Website: canadiancor.com
Twitter: @cacor1968
YouTube: Canadian Association for the Club of Rome
2023 May 24 Zoom #148

Maine-eDNA

- Molecule to Ecosystem: Environmental DNA as a Nexus of Coastal Ecosystem Sustainability for Maine

- A $20 million NSF EPSCoR Research Infrastructure Improvement Track-1 Award



Genetic material obtained directly from environmental samples without any obvious signs of the biological source material (Thomsen and Willerslev, 2015)

Where does eDNA come from?

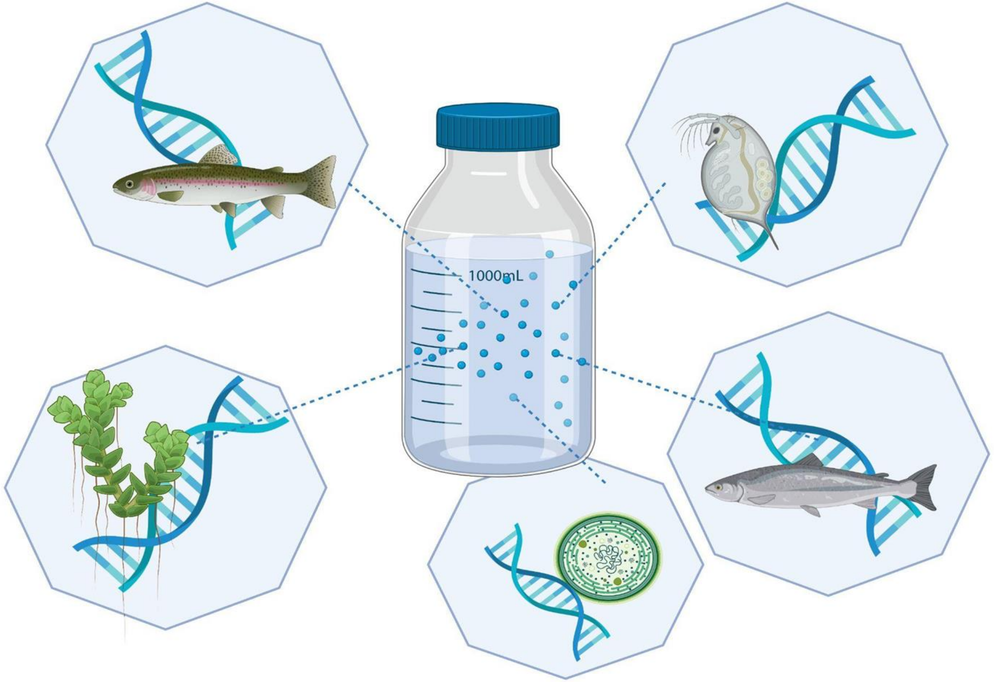
- Cellular decomposition
- Whole shed cells
- Whole microorganisms

Where is eDNA found?

- Water
- Soils
- Air

How is eDNA used?

- Community characterization (metabarcoding)
- Targeted detection & quantification (qPCR)



What is environmental DNA?

eDNA in the news

COVID-19

Science

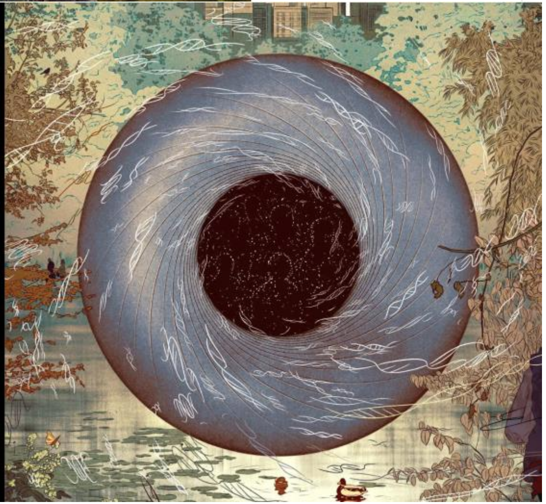
Lost world in northern Greenland conjured from DNA in ancient soil

Balmy ecosystem of mastodons and horseshoe crabs has no analog on modern Earth

The New York Times

Your DNA Can Now Be Pulled From Thin Air. Privacy Experts Are Worried.

Environmental DNA research has aided conservation, but scientists say its ability to glean information about human populations and individuals poses dangers.



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SHORT WAVE

SCIENCE

LISTEN & FOLLOW

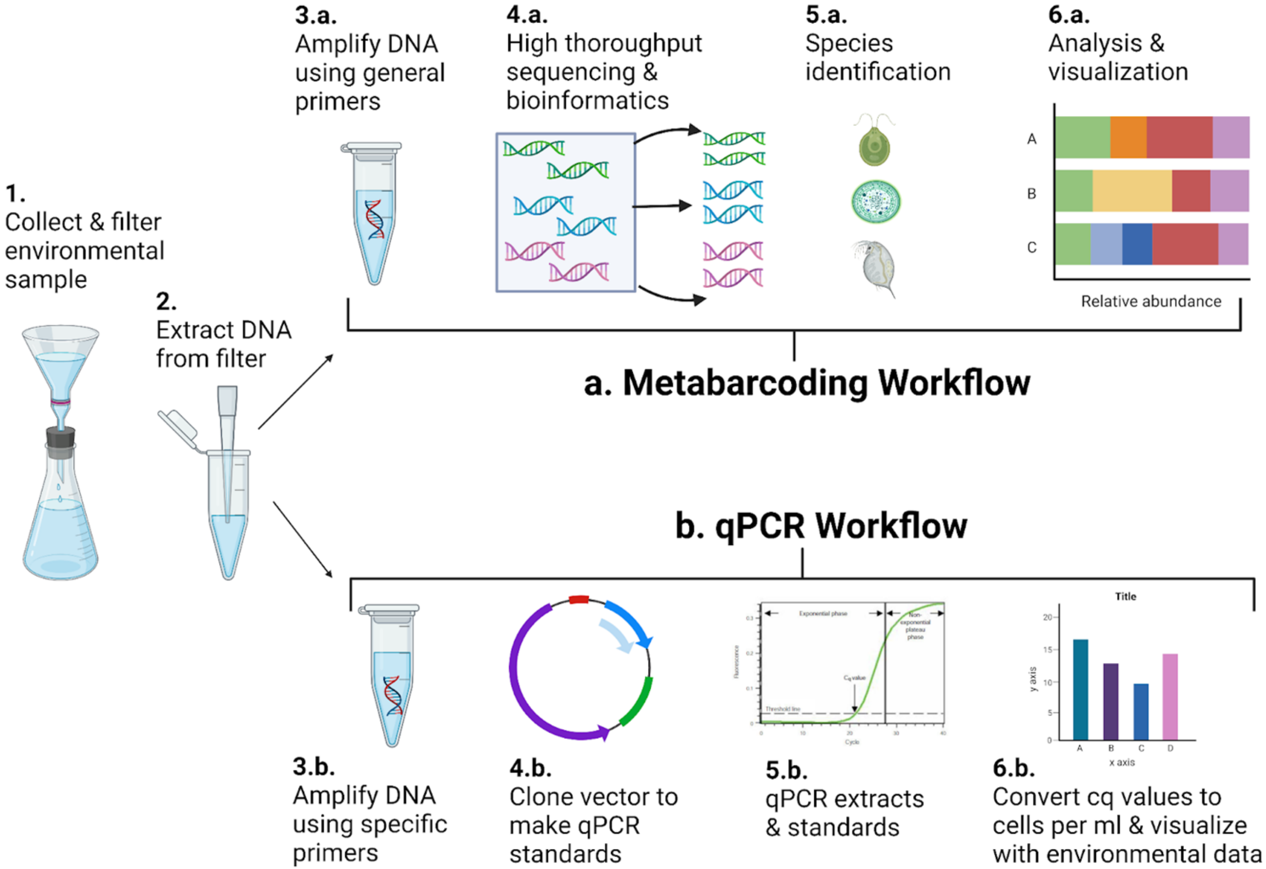
Scientists vacuum zoo animals' DNA out of the air

January 9, 2022 · 7:00 AM ET

Geoff Brumfiel

Researchers were able to detect DNA from elephants at the Copenhagen Zoo simply by sampling the air nearby.

Ida Marie Odgaard /Ritzau Scanpix/AFP via Getty Images


1.
Collect & filter environmental sample
2.
Extract DNA from filter
3.a.
Amplify DNA using general primers
4.a.
High thoroughput sequencing & bioinformatics
5.a.
Species identification
6.a.
Analysis & visualization
A
B
C
Relative abundance
a. Metabarcoding Workflow
b. qPCR Workflow
3.b.
Amplify DNA using specific primers
4.b.
Clone vector to make qPCR standards
5.b.
qPCR extracts & standards
6.b.
Convert cq values to cells per ml & visualize with environmental data
Title
y axis
x axis
A
B
C
D

eDNA tools

PROJECT ROADMAP

RESEARCH

Goal 1: Sustainable Fisheries

- Goal 1.1 Ecosystem Restoration
- Goal 1.2 Larval Black Box

Goal 2: Harmful Species

- Goal 2.1 Harmful Blooms
- Goal 2.2 Species on the Move

Goal 3: Macrosystem eDNA Integration

- Goal 3.1 Big Data Integration: Dynamics & Stability
- Goal 3.2 Microbial Communities as Biosensors of Change
- Goal 3.3 Comparative Study of eDNA Team Science

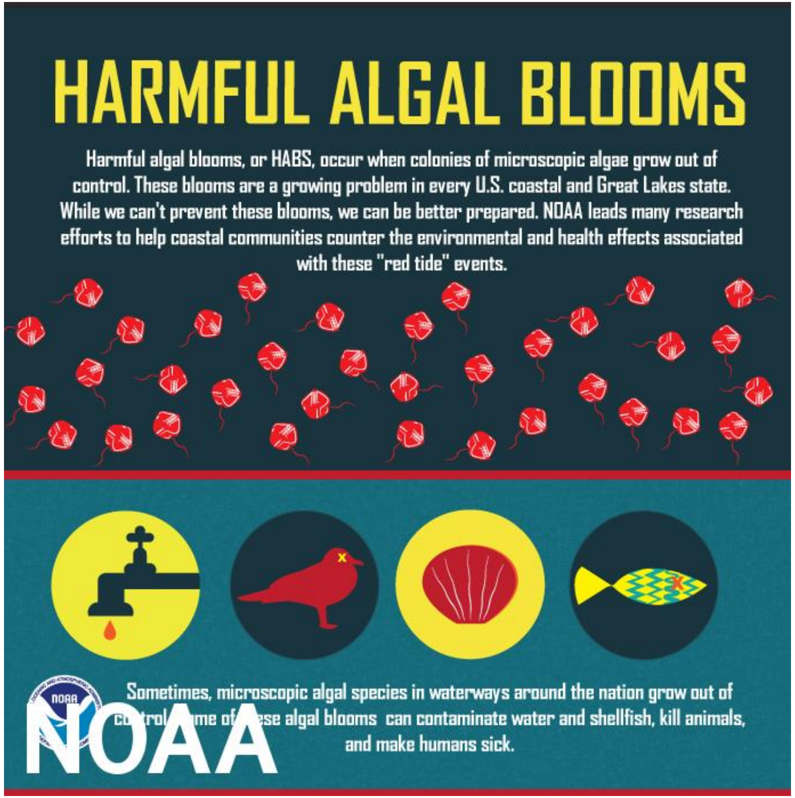
PROJECT ELEMENTS

- Goal 4.1 Education and Workforce Development
- Goal 4.2 Seed Funding and Emerging Areas
- Goal 4.3 Diversity
- Goal 4.4 Partnerships and Collaboration
- Goal 4.5 Communication and Dissemination
- Goal 4.6 Sustainability
- Goal 4.7 Management, Evaluation, and Assessment

Harmful Species Vignette 1: Lakes, Cyanobacteria, and Oysters

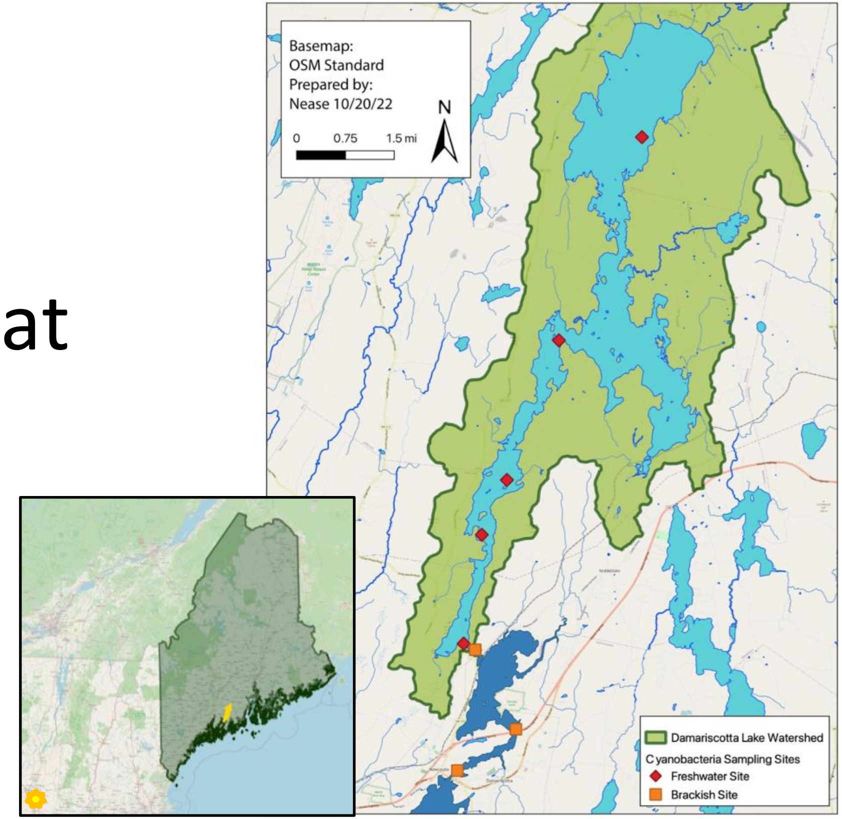
What is a HAB?

- “Out of control” growth of algae
- Impacts many sectors
- Commercial and recreational fisheries
- Aquaculture
- Tourism
- Wildlife
- Human health

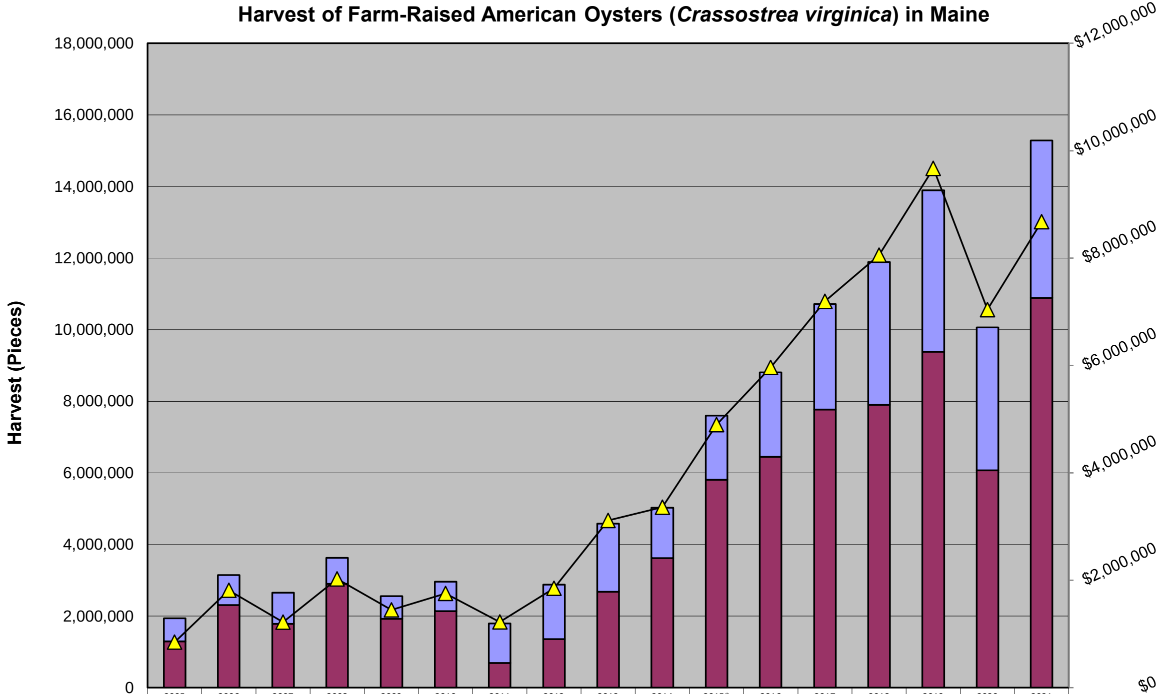


Damariscotta Lake Ecosystem

- 4,600 acres
- 22 miles of shoreline
- State park & public boat launch
- 3 distinct basins
- Large alewife run (>1 million fish!)



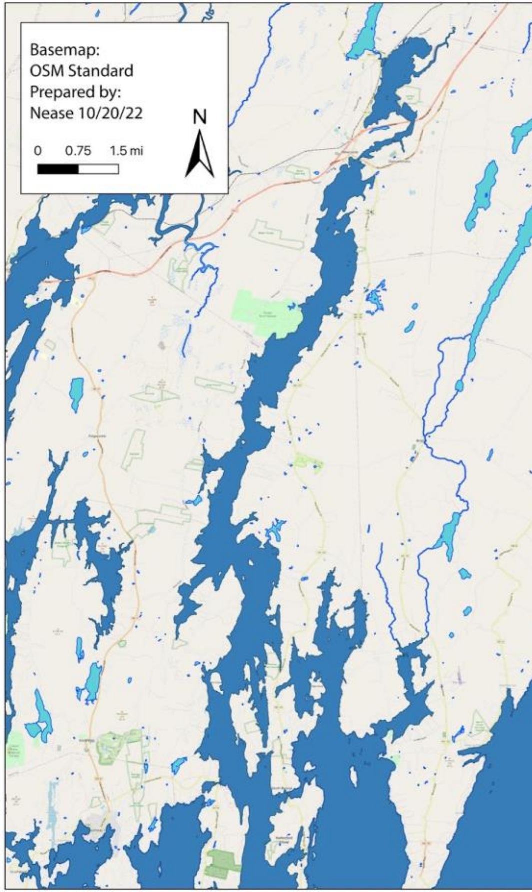
Damariscotta River Estuary



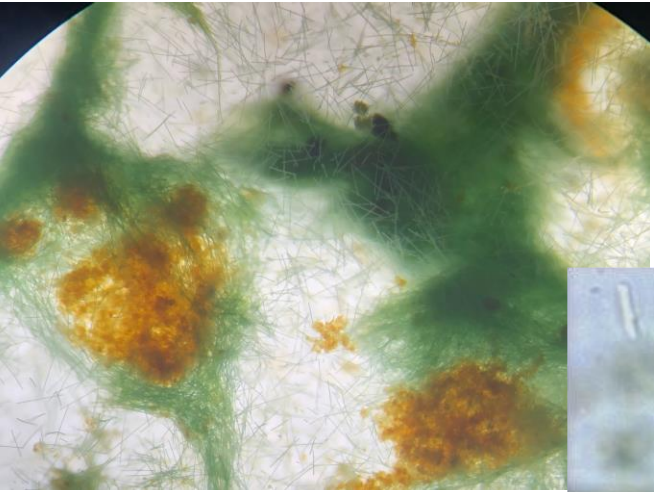
	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015*	2016	2017	2018	2019	2020	2021
Other Location	650,044	837,138	875,067	728,073	633,894	820,047	1,103,901	1,522,652	1,900,640	1,404,861	1,788,685	2,356,755	2,941,792	3,992,948	4,507,768	3,990,144	4,395,861
Damariscotta River	1,291,494	2,306,290	1,777,798	2,896,714	1,925,700	2,140,279	687,500	1,353,162	2,681,997	3,618,669	5,811,629	6,447,636	7,774,405	7,898,517	9,381,531	6,070,775	10,886,636
Total Harvest	1,941,538	3,143,428	2,652,865	3,624,787	2,559,594	2,960,326	1,791,401	2,875,814	4,582,637	5,023,530	7,600,314	8,804,391	10,716,197	11,891,465	13,889,299	10,060,919	15,282,497
Total Value ($)	$848,338	$1,812,677	$1,220,531	$2,024,575	$1,447,378	$1,754,744	$1,225,472	$1,851,331	$3,113,775	$3,359,665	$4,898,154	$5,964,214	$7,193,925	$8,054,957	$9,670,100	$7,041,070	$8,676,238

** DMR began collecting LPA harvest data in 2015.*
** 2021 data are preliminary and will be finalized when 2022 data are posted.*

ME-DMR 2022

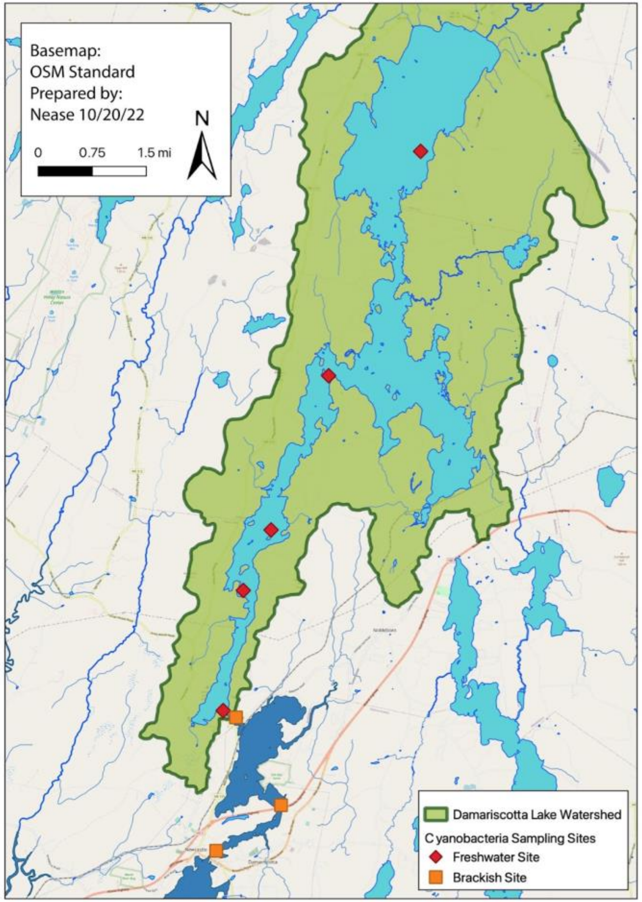


2020 *Planktothrix* Bloom

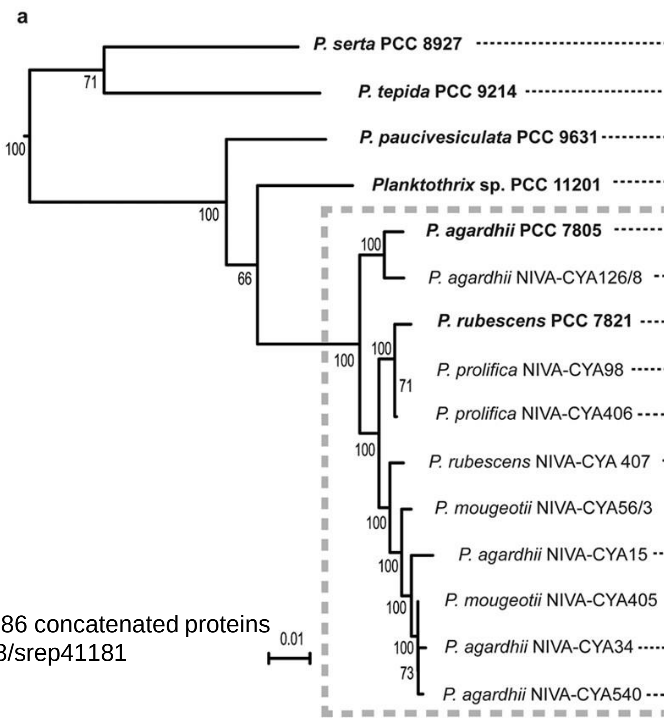


Research Questions

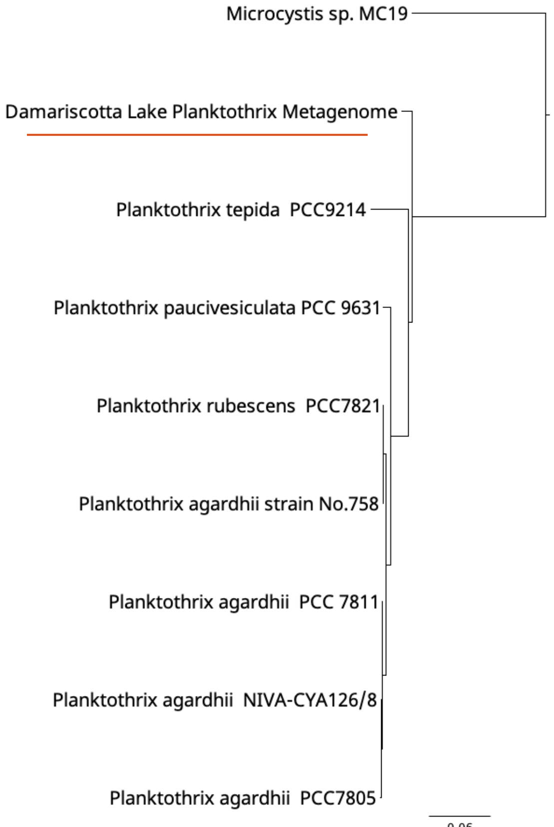
- What is the distribution and duration of the bloom
- Toxicity?
- Are cells/toxins moving downstream?
- What factors (biotic and abiotic) are associated with bloom formation and cessation

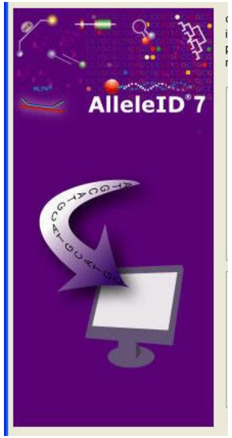


Planktothrix primer development



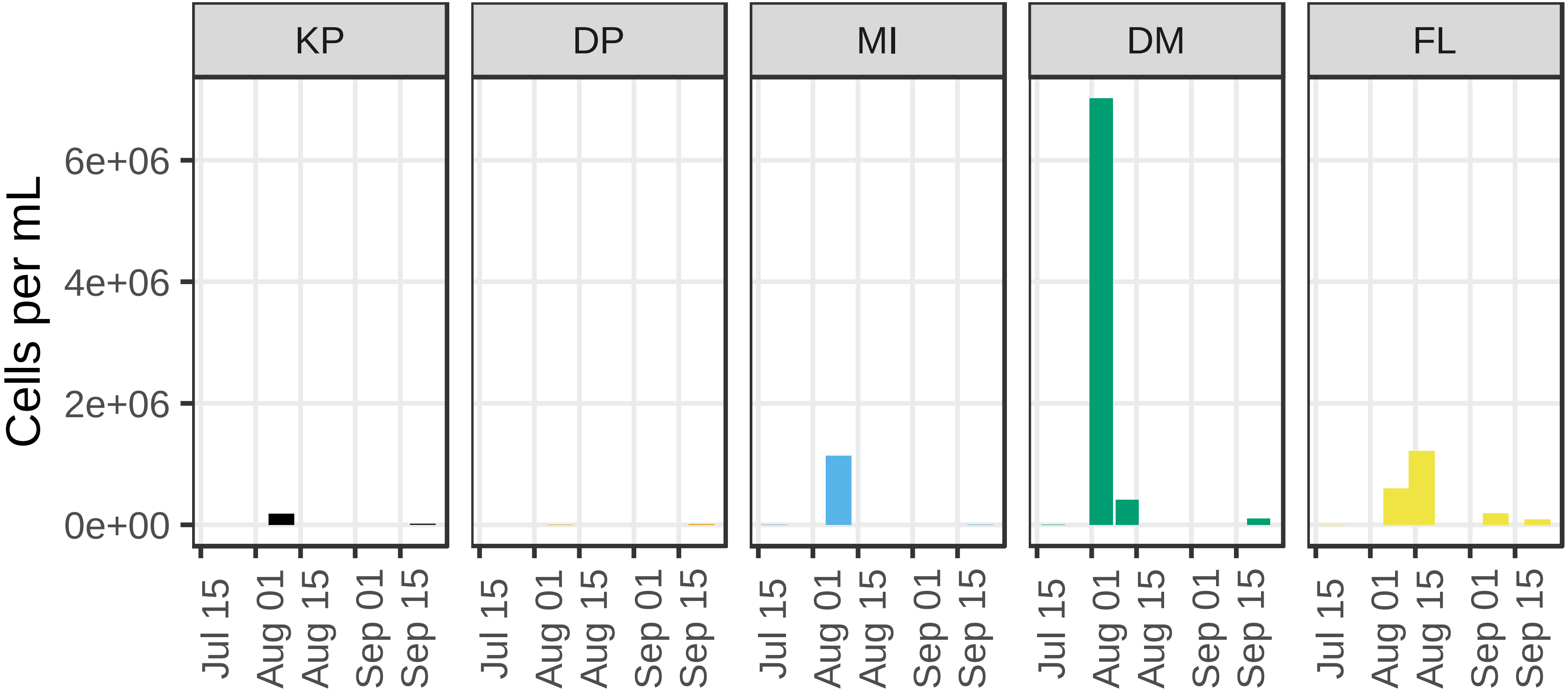
Pancrace et al 2017 586 concatenated proteins
https://doi.org/10.1038/srep41181





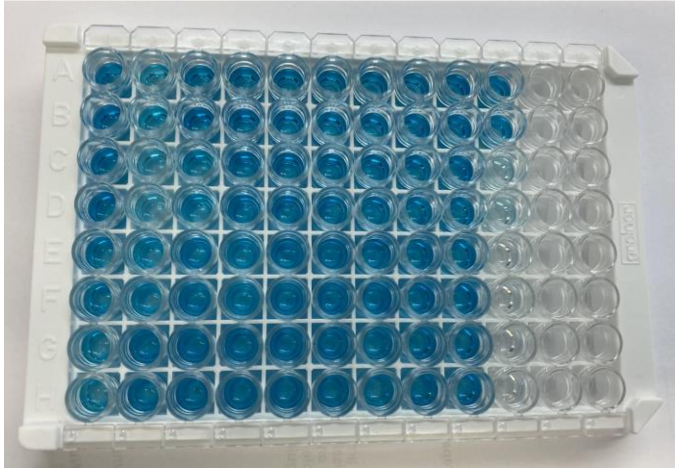
16S, ITS, 23S Alignment

2021 *Planktothrix* qPCR results



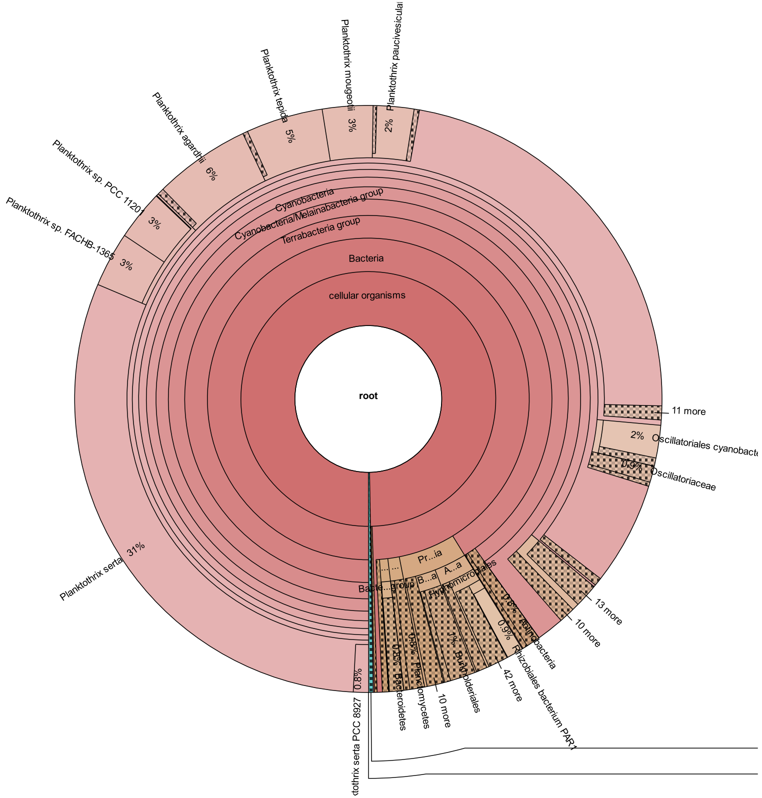
Planktothrix ELISA results

- Abraxis Microcystins/Nodularins (ADDA) kit
- No results above lowest threshold (0.3 ug/L)



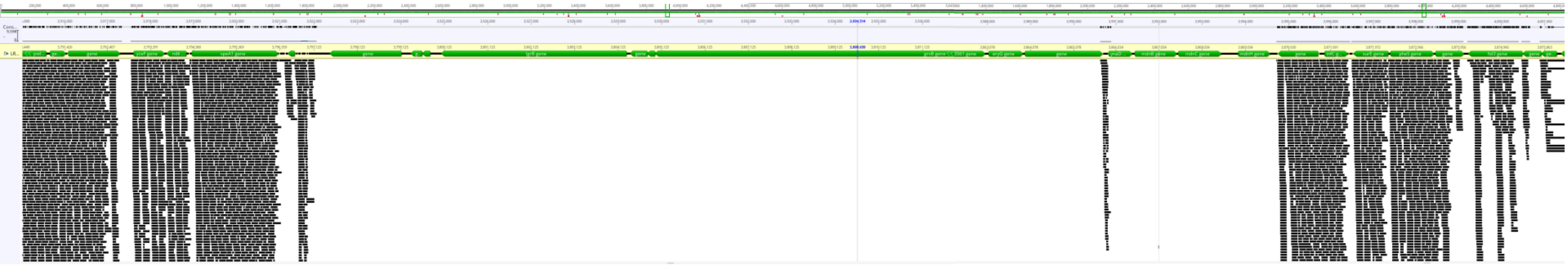
Planktothrix metagenome results

- 75% *Planktothrix*
- Antismash analysis
  - Cyanobactin: piricyclamid
  - Ladderane
  - Scytophycin



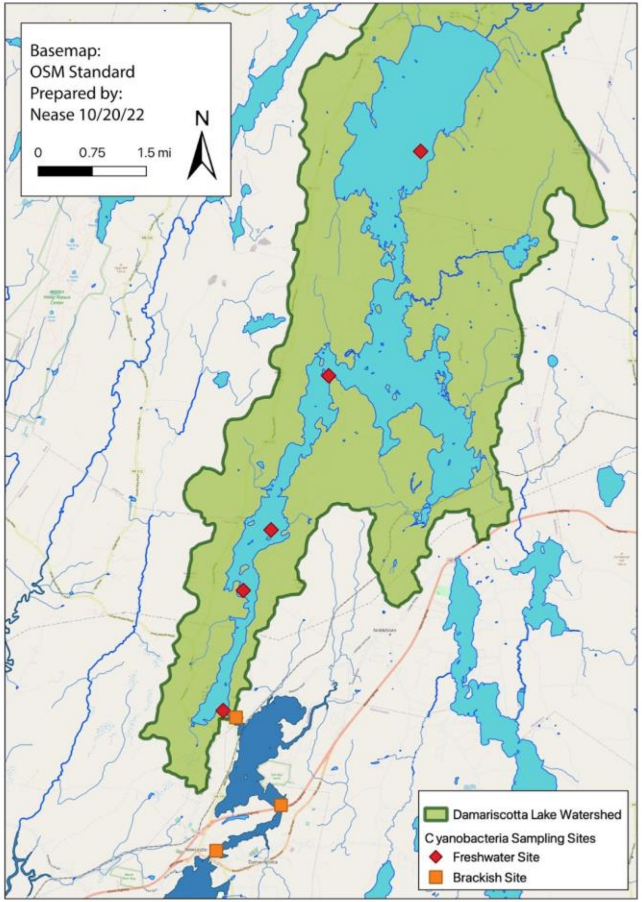
Planktothrix microcystin biosynthesis

- Damariscotta Lake *Planktothrix* lacks microcystin biosynthesis pathway

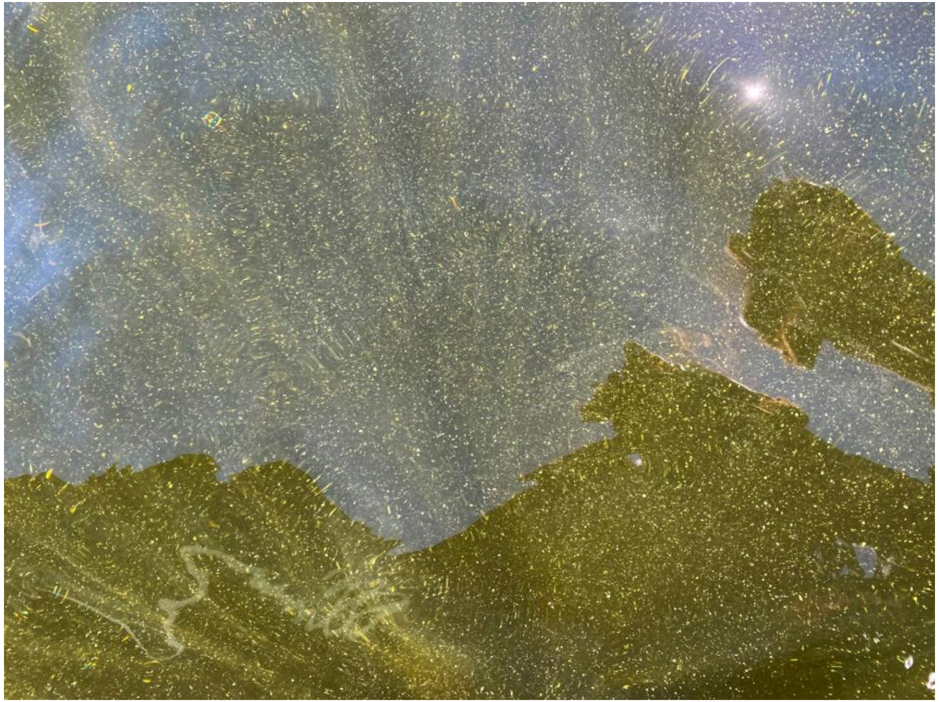


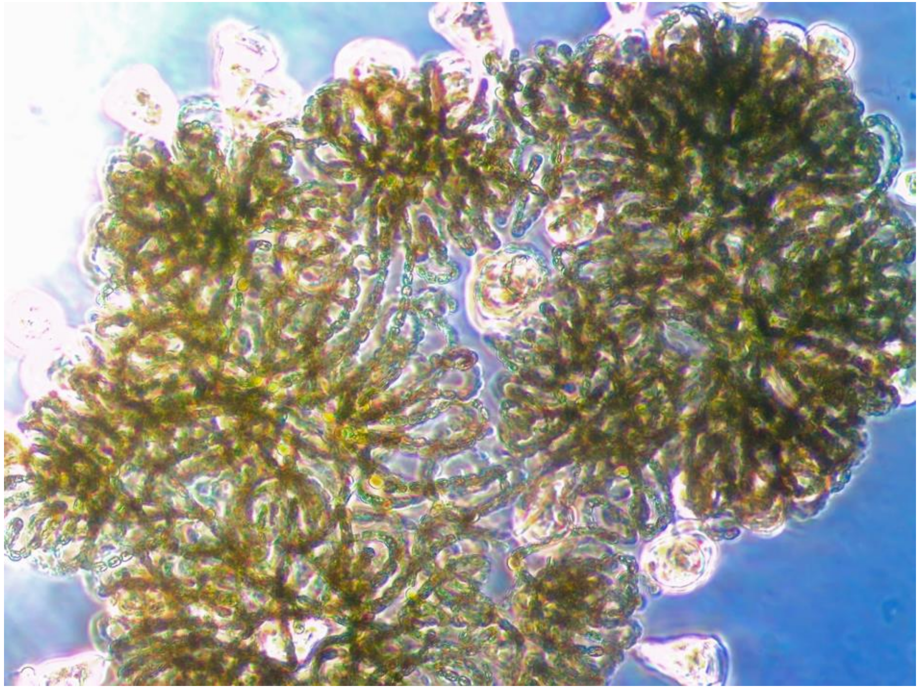
Plans for 2022

- Expand sampling further downstream
- Expand sampling window (June-October)

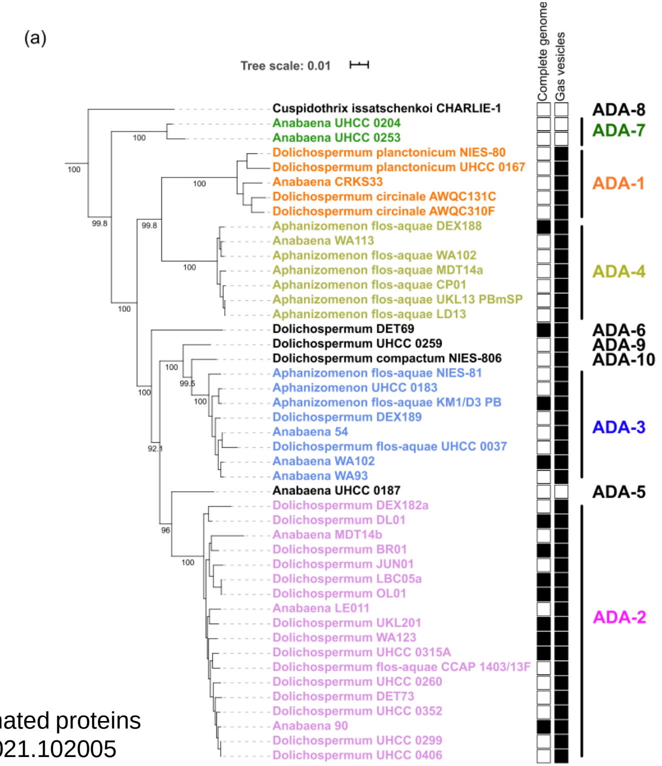


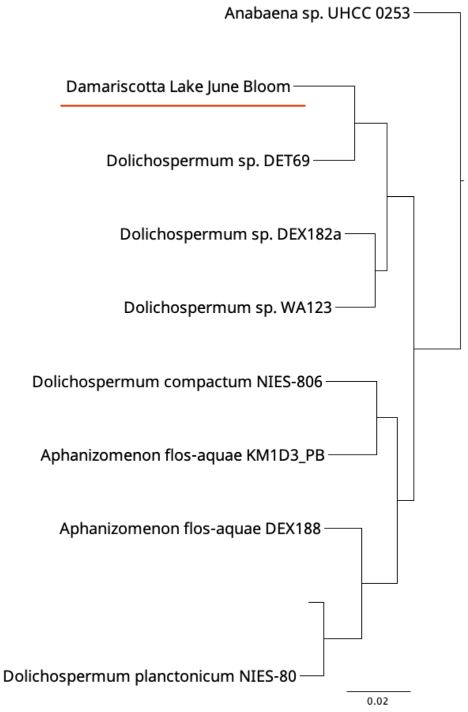
June *Dolichospermum*

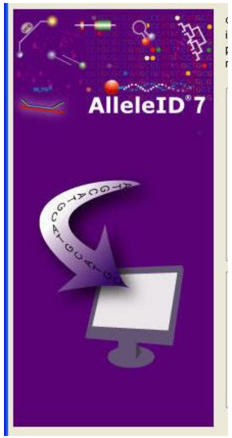




Dolichospermum primer development



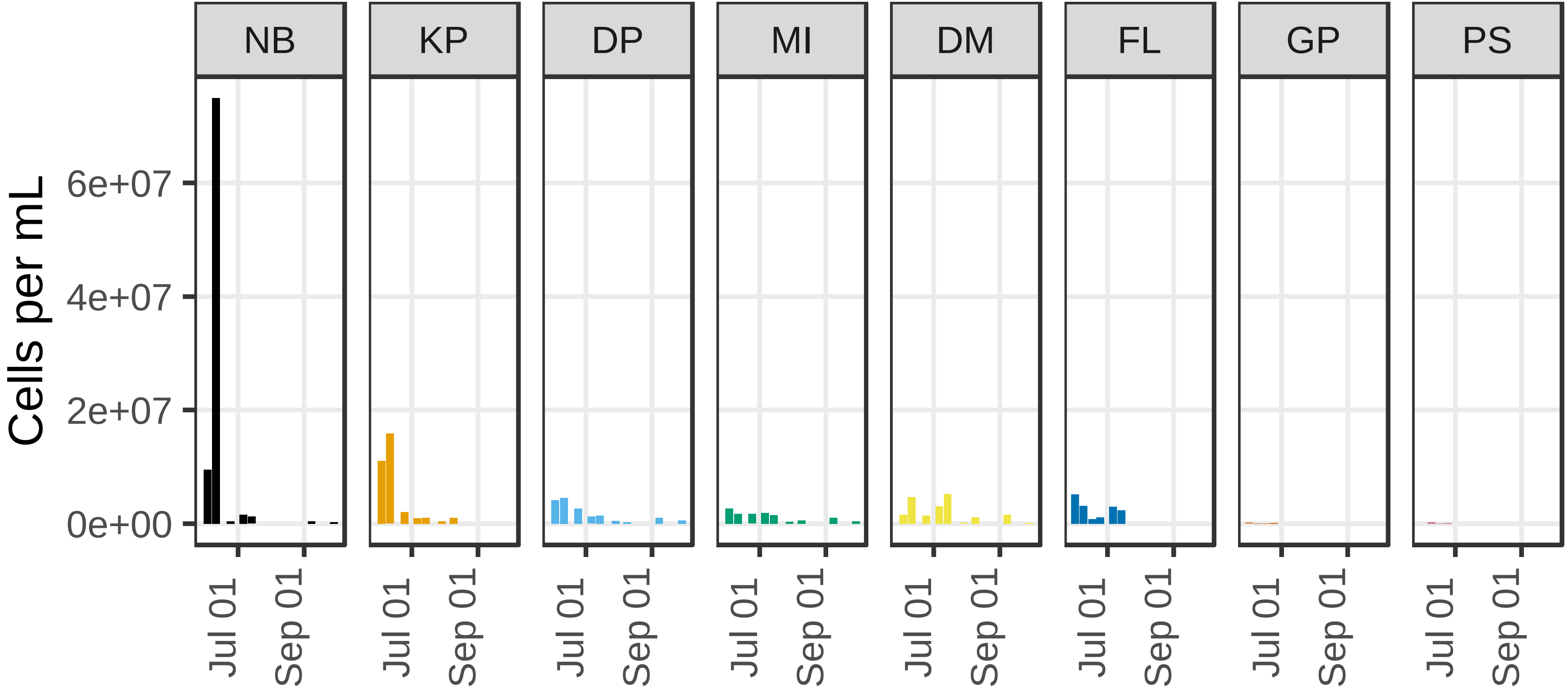




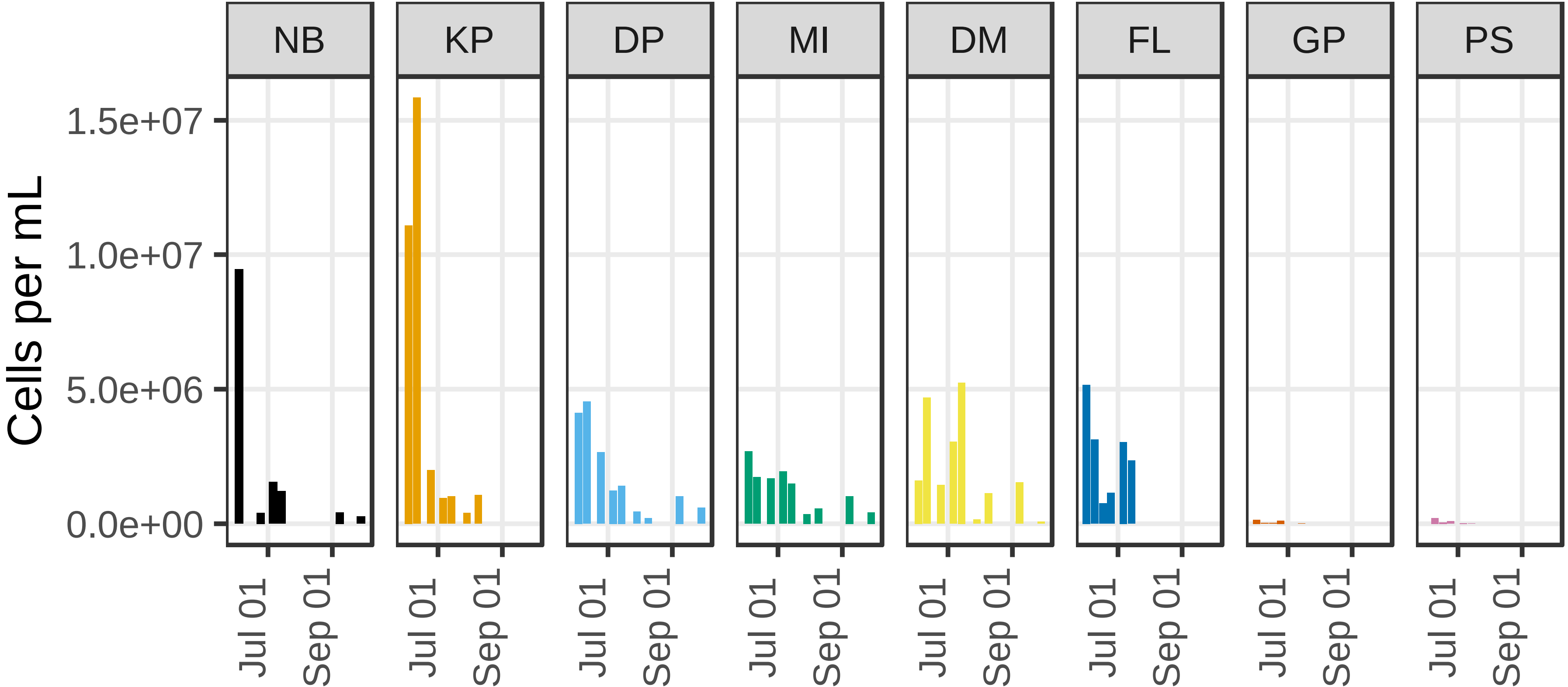
Dreher et al 2021 91 concatenated proteins
https://doi.org/10.1016/j.hal.2021.102005

16S, ITS, 23S Alignment

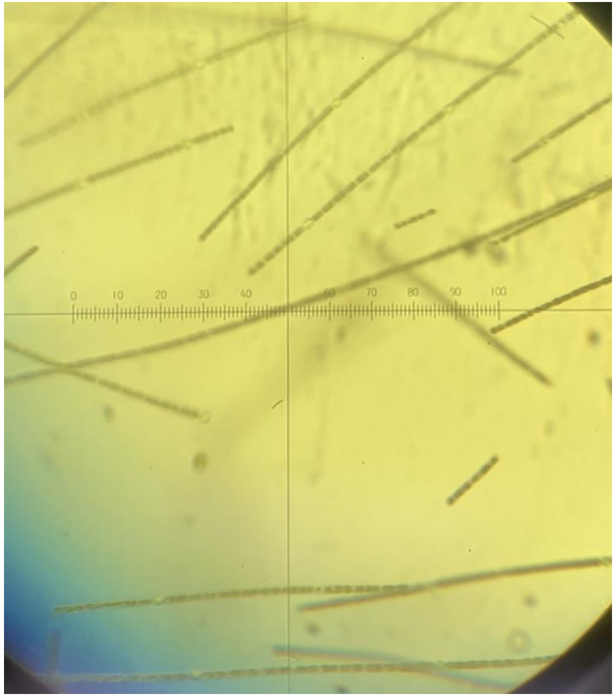
June *Dolichospermum* ddPCR counts



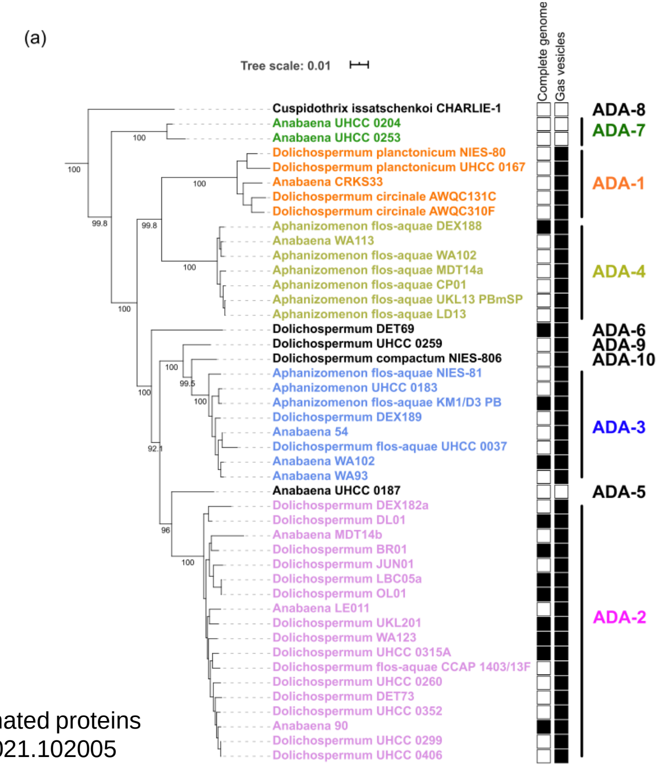
June *Dolichospermum* ddPCR counts



The Sequel: July *Dolichospermum*!



July *Dolichospermum* primer development



Dreher et al 2021 91 concatenated proteins
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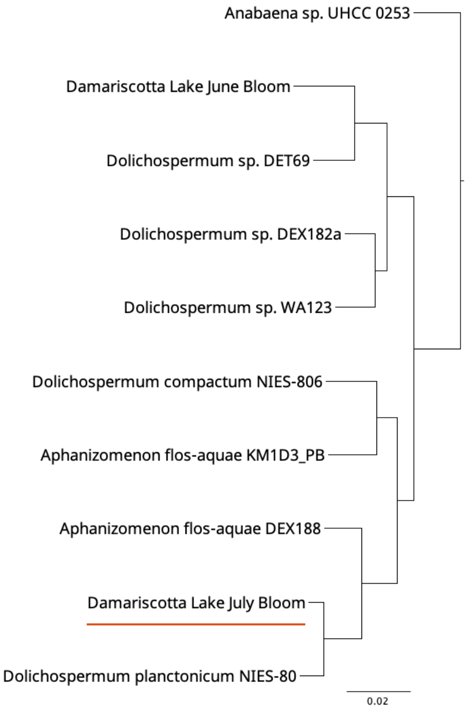
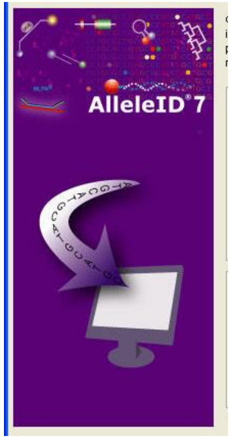
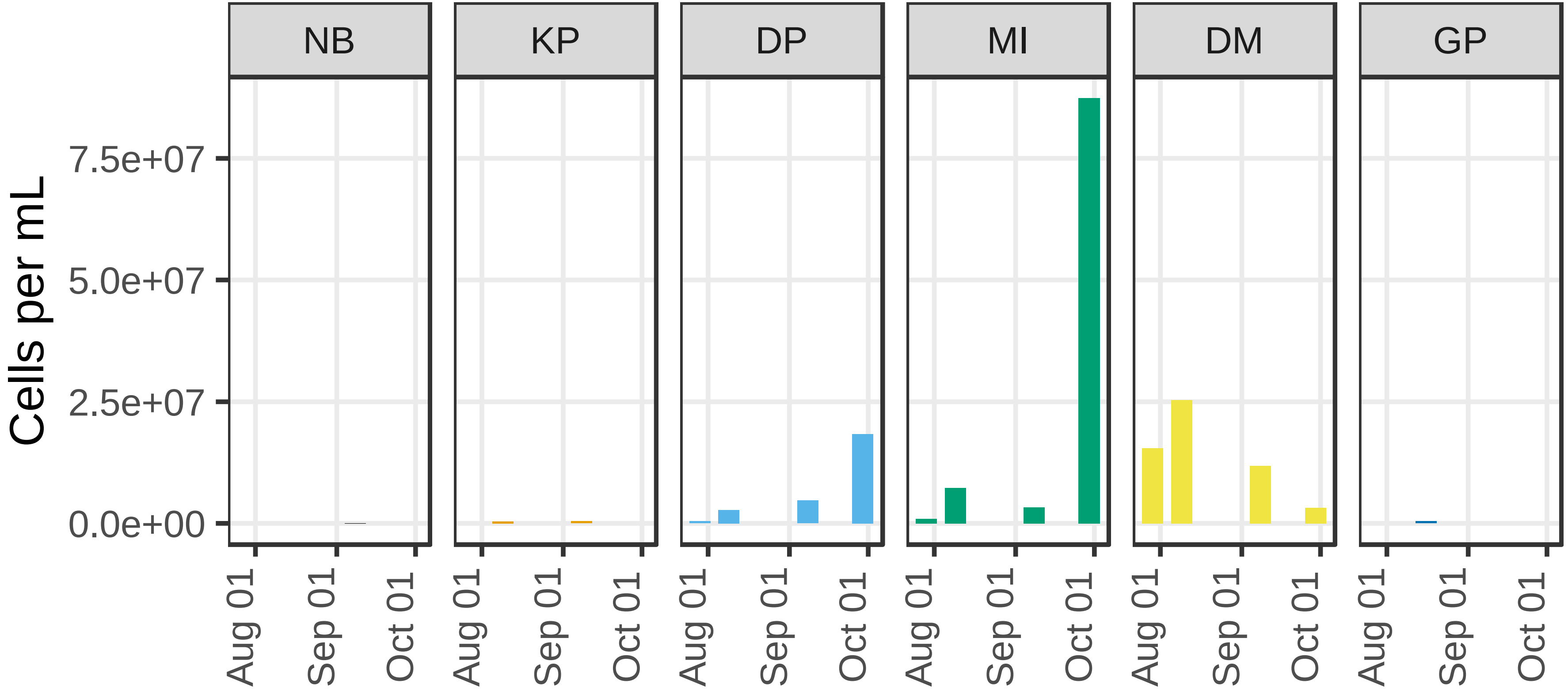




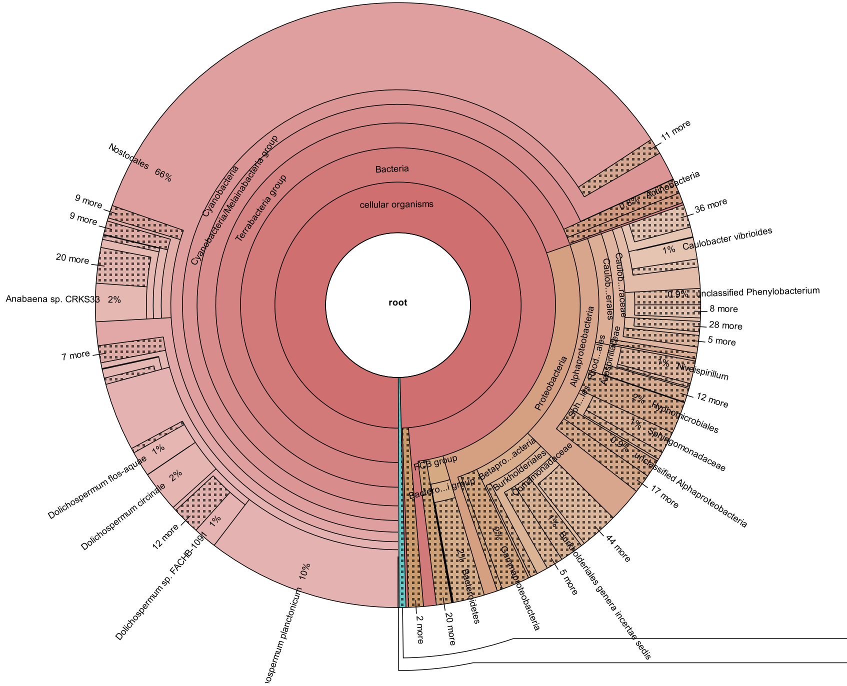
16S, ITS, 23S Alignment

July *Dolichospermum* ddPCR counts



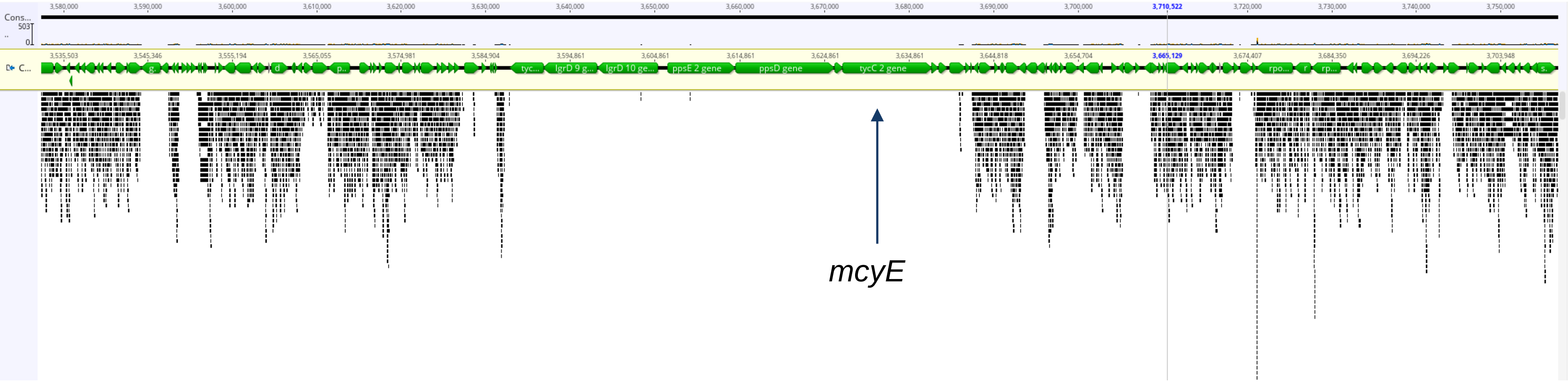
Dolichospermum metagenomes

- 66% Nostocales
- Antismash analysis
  - Anabaenopeptin
  - Cylindrospermopsin
  - Cyanobactin



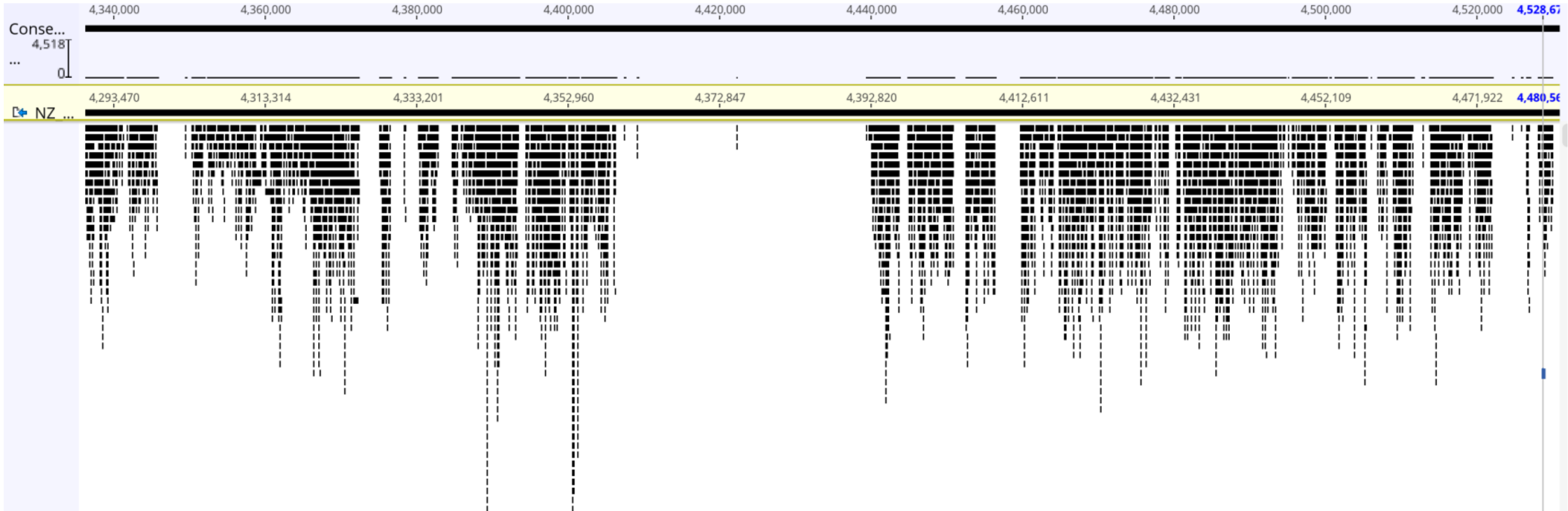
Dolichospermum microcystin biosynthesis

- Damariscotta Lake *Dolichospermum* lack microcystin biosynthesis pathway



Dolichospermum anatoxin biosynthesis

- Damariscotta Lake *Dolichospermum* lack anatoxin biosynthesis pathway

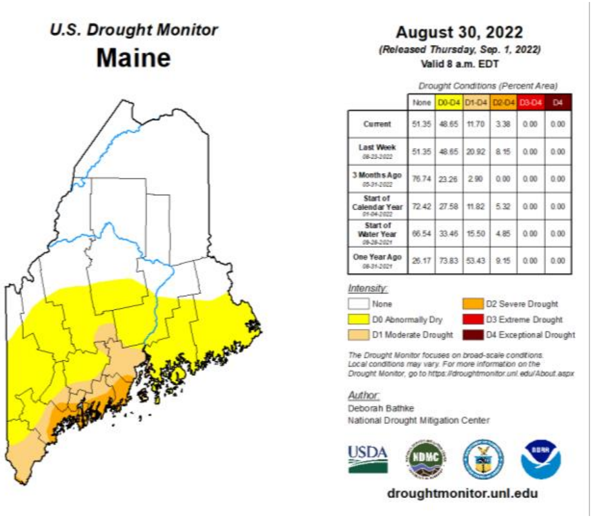


Don't forget about *Planktothrix!*

- Downgrading to nuisance bloom (for now)
- Restricted to upper reaches of South Basin in July

Conclusions and Next Steps

- Did a dry 2022 summer favor *Dolichospermum*?
- No evidence of survival in brackish water
- Unable to characterize microcystin transport (no microcystin detected)
- Incorporate nutrient and environmental data
- *mcyE* containing *Dolichospermum* genotype found 20 miles north, is it a threat?
- 2023 sampling oyster tissues just downstream for microcystin and anabaenopeptin



Precedent

- Freshwater *Microcystis* bloom
- Downstream transport
- Bioaccumulation of microcystin in shellfish
  - Up to 107x higher than adjacent seawater
- Acute liver failure for otters consuming shellfish

Sea otter deaths linked to toxin from freshwater bacteria

September 10, 2010
By Tim Stephens

SHARE THIS STORY:

A potent toxin produced by bright-green blooms of freshwater bacteria has been flowing into the ocean and poisoning sea otters, according to a team of investigators led by scientists at the California Department of Fish and Game (DFG) and the University of California, Santa Cruz.

In a paper published September 10 in *PLoS ONE*, the researchers reported that the deaths of at least 21 California sea otters (a federally listed threatened species) were linked to the toxin, called microcystin. First author Melissa Miller, a senior wildlife veterinarian at the DFG Marine Wildlife Veterinary Care and Research Center in Santa Cruz, will present the findings at the California and the World Ocean Conference in San Francisco on Friday, September 10.

The toxin is produced by a type of cyanobacteria (also known as "blue-green algae") called *Microcystis*. Warm, nutrient-rich water conditions favor large blooms of *Microcystis*, which can form thick green mats on the surface of the water.

"This study is significant because it is the first to establish a connection between freshwater contamination by microcystin and marine mammal mortality," said Miller, who is also affiliated with UCSC's Institute of Marine Sciences. "This land-to-sea link has important implications for marine life and human health."

Coauthor Raphael Kudela, professor of ocean sciences at UC Santa Cruz, said the team found high concentrations of microcystin in lakes bordering Monterey Bay and in rivers that flow into the bay.

"The toxin itself is extremely stable. In laboratory studies, we found that microcystin didn't degrade much even after three weeks in ocean water," Kudela said.

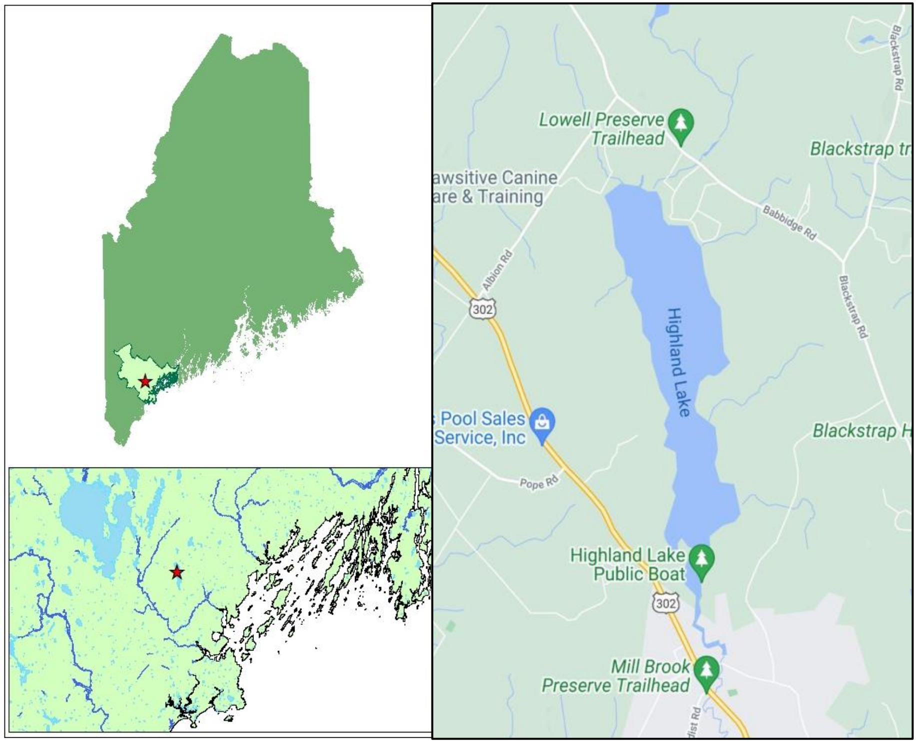
Microcystin poisoning can cause acute liver failure or damage other tissues and can be fatal. In 2007, Miller began seeing dead and dying sea otters recovered along the shore of Monterey Bay with evidence of acute liver failure. After tissue from the otters tested positive for microcystin, Miller teamed up with Kudela and DFG chemists to search for environmental

The toxic cyanobacteria *Mycrocystis* gives this water sample from Pinto Lake its bright green color. (Photo by R. Ketley)

California sea otters have been poisoned by the microcystin toxin. This sea otter pup is healthy. (Photo by R. Wilder, Monterey Bay Aquarium)

Harmful Species Vignette 2: Lakes, Algae, and Alewives

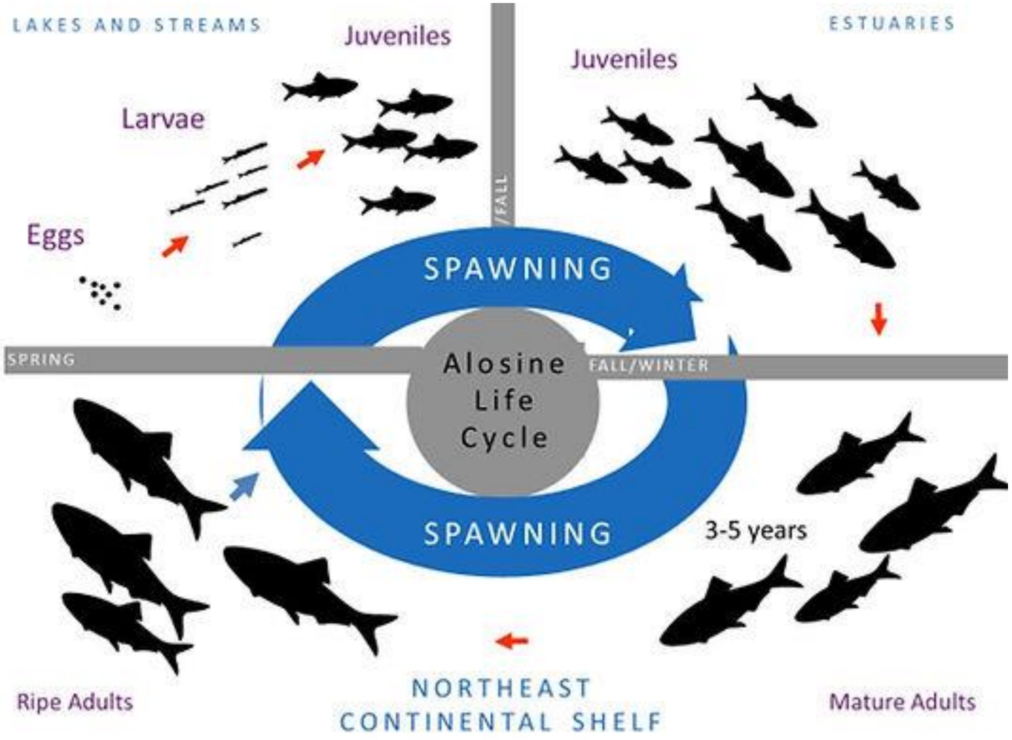
- Highly developed area
- Previously listed (1998-2010) as impaired by Maine-DEP due to deteriorating trophic state
- Active Lake Association water quality team
- Experienced an unusual nuisance bloom, coinciding with the first 4 years of high numbers of spawning anadromous alewife



Highland (Duck) Pond

Highland Lake experienced extended algae blooms from 2014-2017

Alewife life cycle



Cause(s) of algae bloom?

Excess nutrients?

Unusual phytoplankton species?

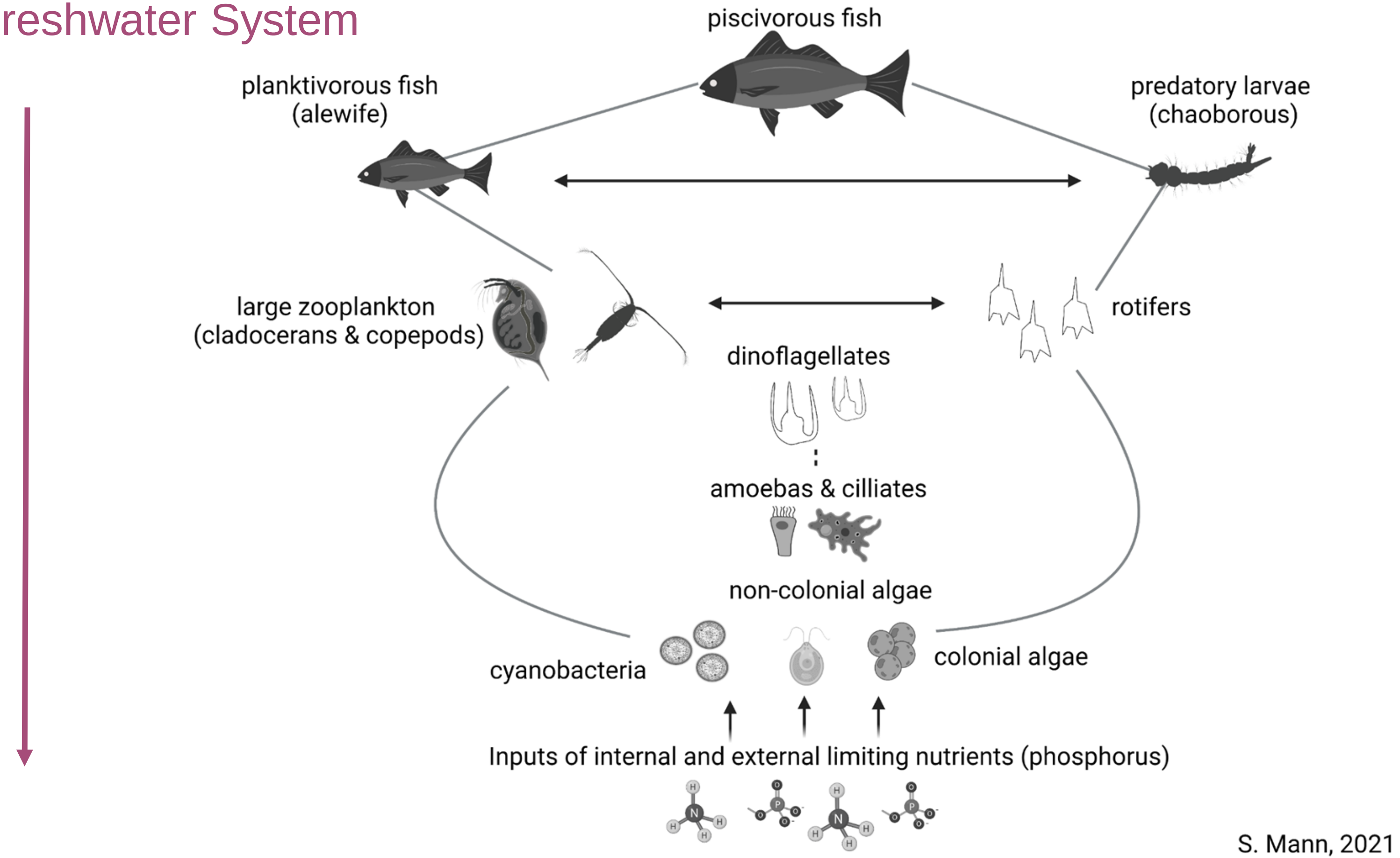
Trophic cascade triggered by consumption of herbivorous zooplankton by alewife?

Unlikely: TP levels lower than those associated with nuisance blooms

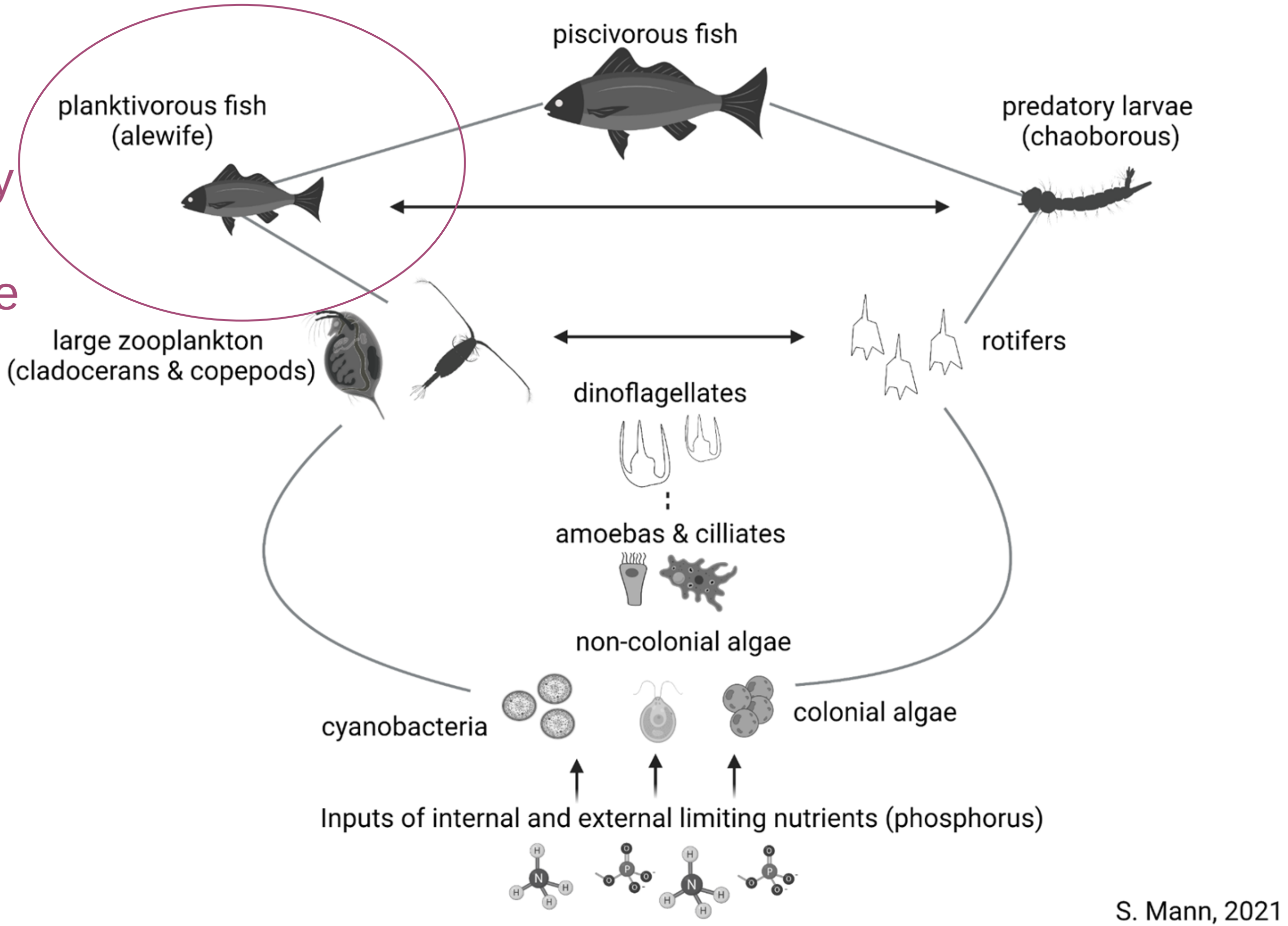
Preliminary observations suggested that the bloom was caused by a picocyanobacteria - Today’s talk!

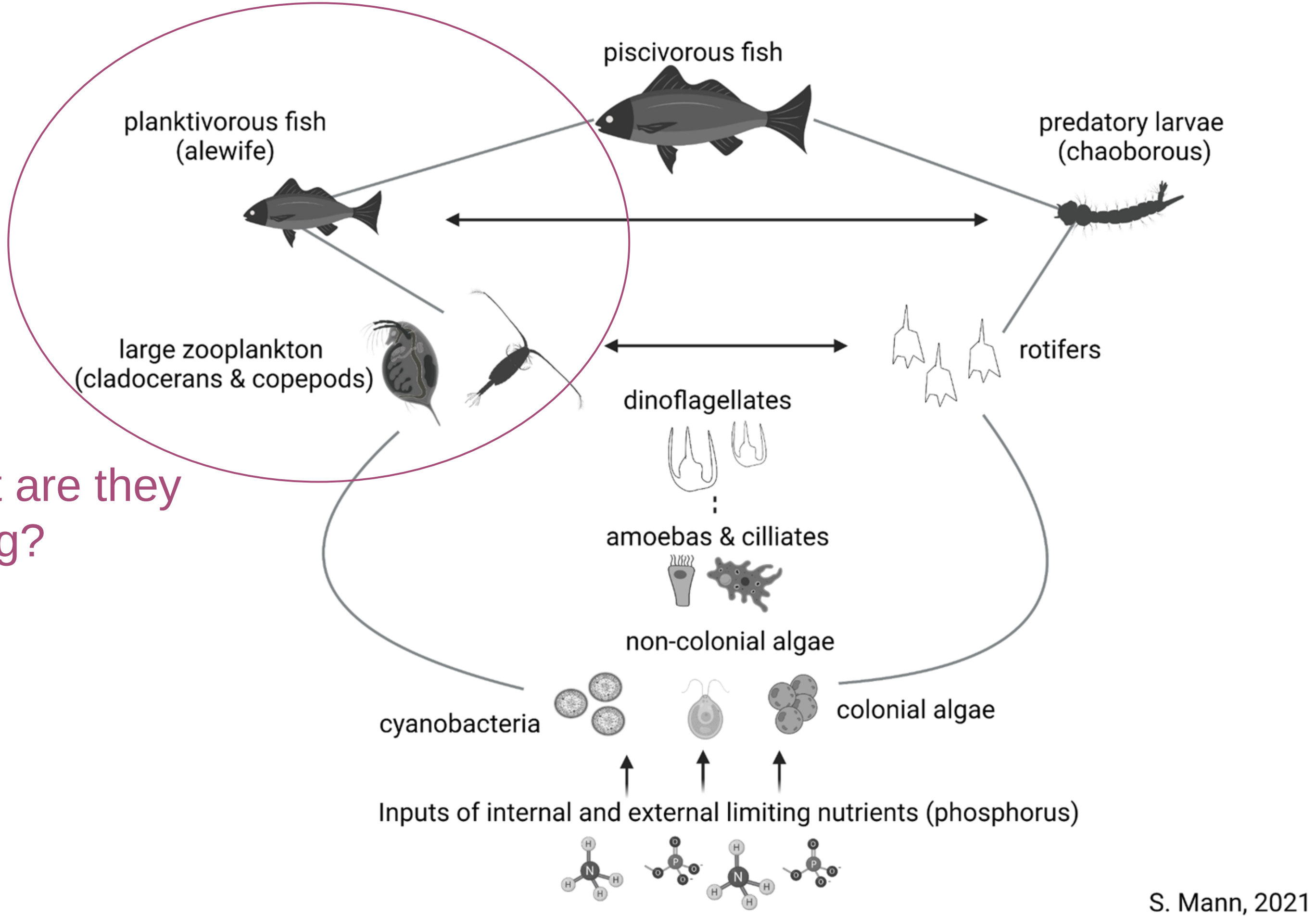
Coming soon!

Freshwater System

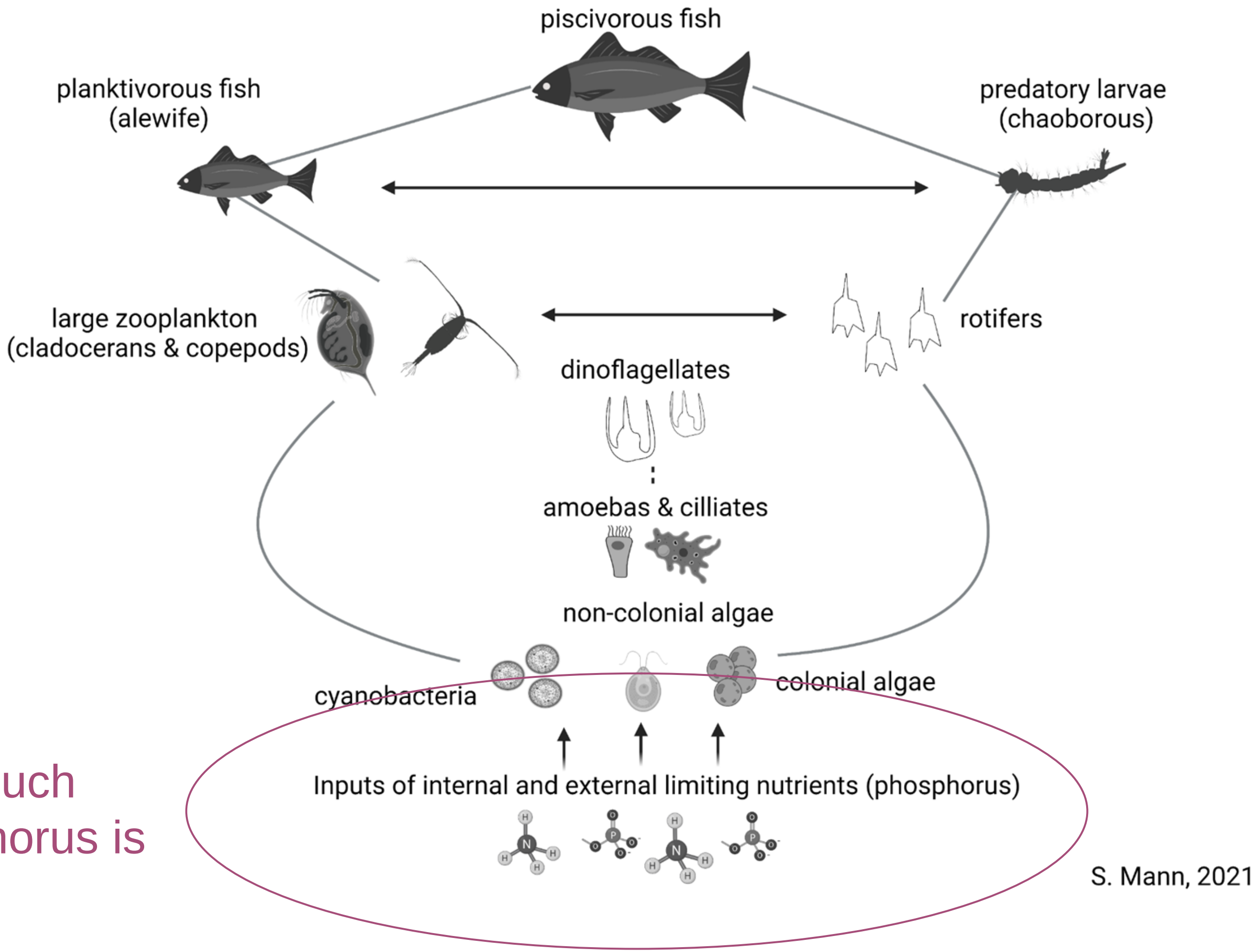


How many juvenile alewife are there?



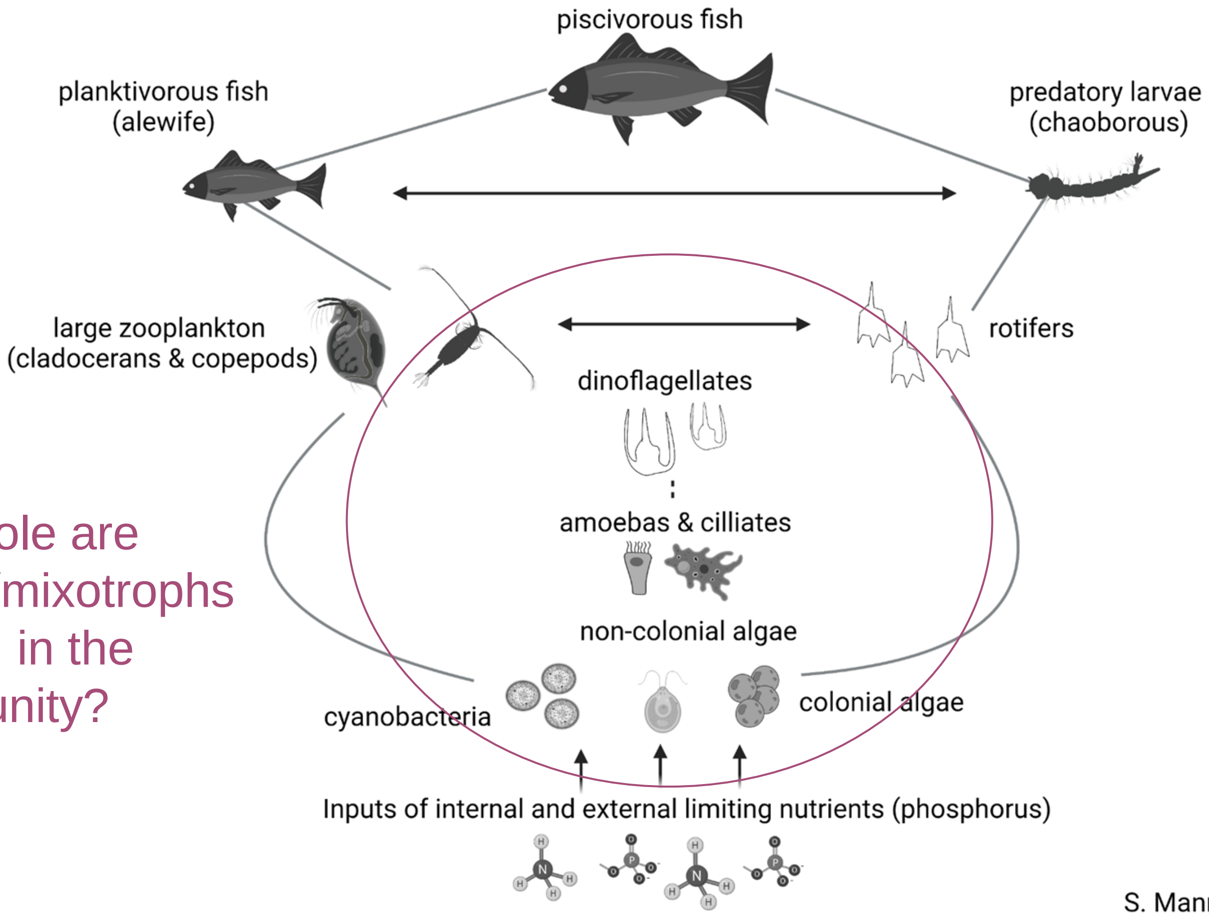


What are they eating?



How much phosphorus is there?

What role are hetero/mixotrophs playing in the community?



S. Mann, 2021

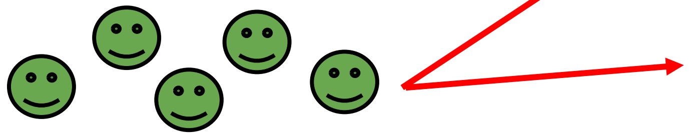
Why is this work needed?

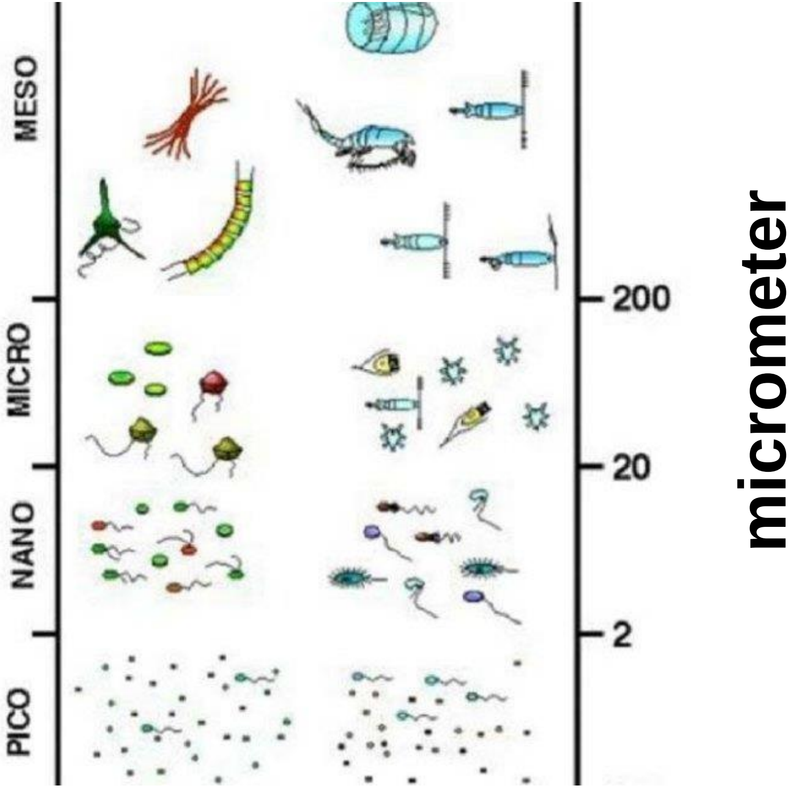
- Pico eukaryotes and pico cyanobacteria have different effects on ecosystems (cyanobacteria such as *Cyanobium spp.* can produce toxins)

Why use eDNA?

- Unable to identify the taxa responsible for decreasing water transparency using microscopy

L.G.B.

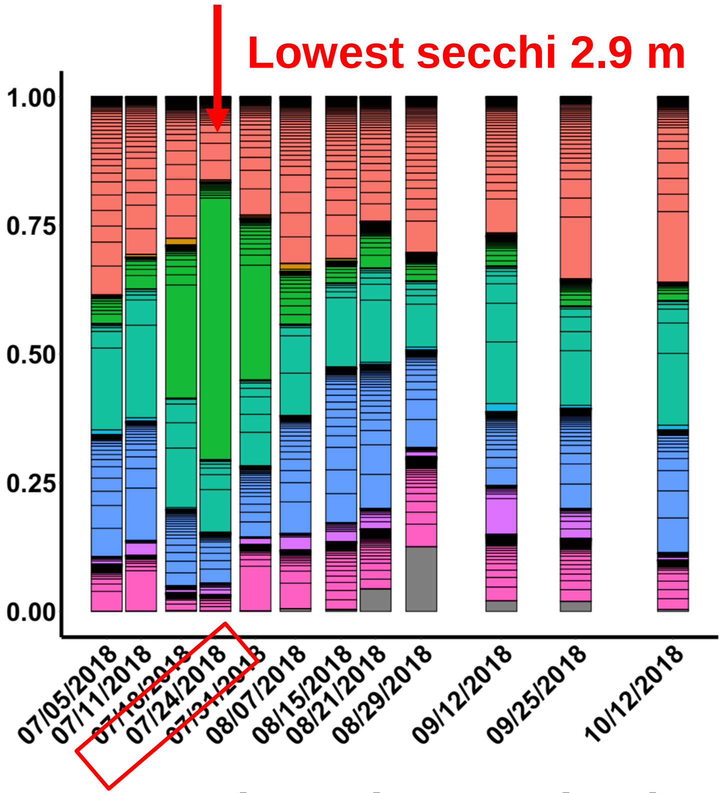




Alcaraz & Calbet (2003)

MAINE eDNA

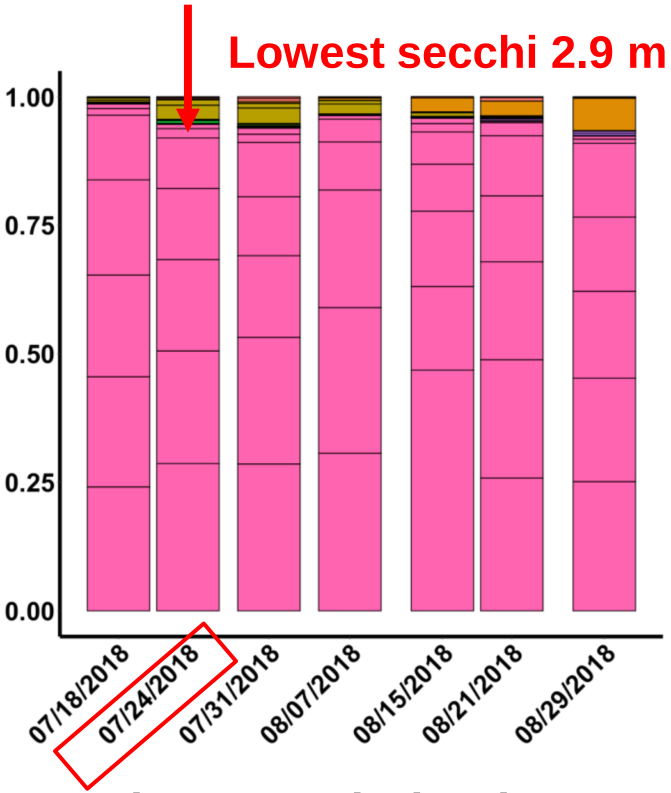
Pico and nano phytoplankton are difficult to identify



Super Order Eukaryote (18s)

- Alveolata
- Amoebozoa
- Apusozoa
- Archaeplastida
- Hacrobia
- Hacrobia:nucl
- Opisthokonta
- Rhizaria
- Stramenopiles
- NA

***Rhexinema spp.* (formerly known as *Helicodictyon*)**



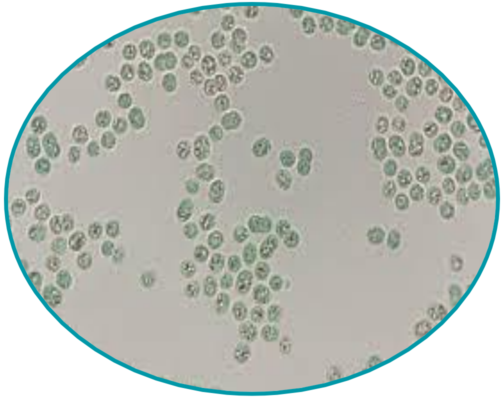
Order Bacteria (16s)

- Actinobacteria
- Chroococcales
- Cyanobacteriales
- Firmicutes
- Hyphomicrobiales
- Parcubacteria
- Planctomycetes
- Pseudanabaenales
- Sphingomonadales
- Synechococcales

***Cyanobium spp.* (pico-cyanobacteria)**

Figures provided by Robin Sleith

Rhexinema (genus of green algae)
One cell ≤ 10 μ m

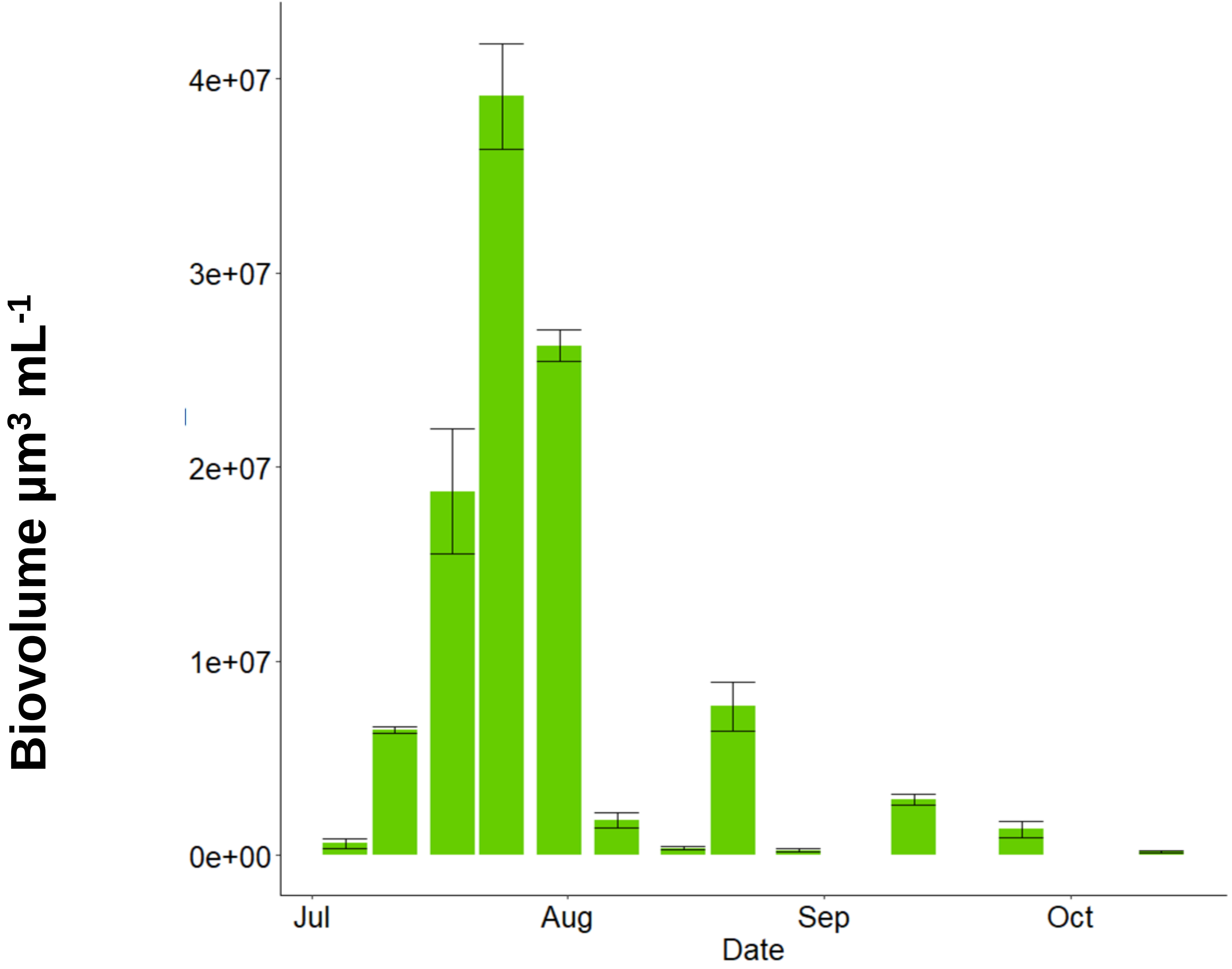


Cyanobium (genus of cyanobacteria)
One cell ≤ 2 μ m

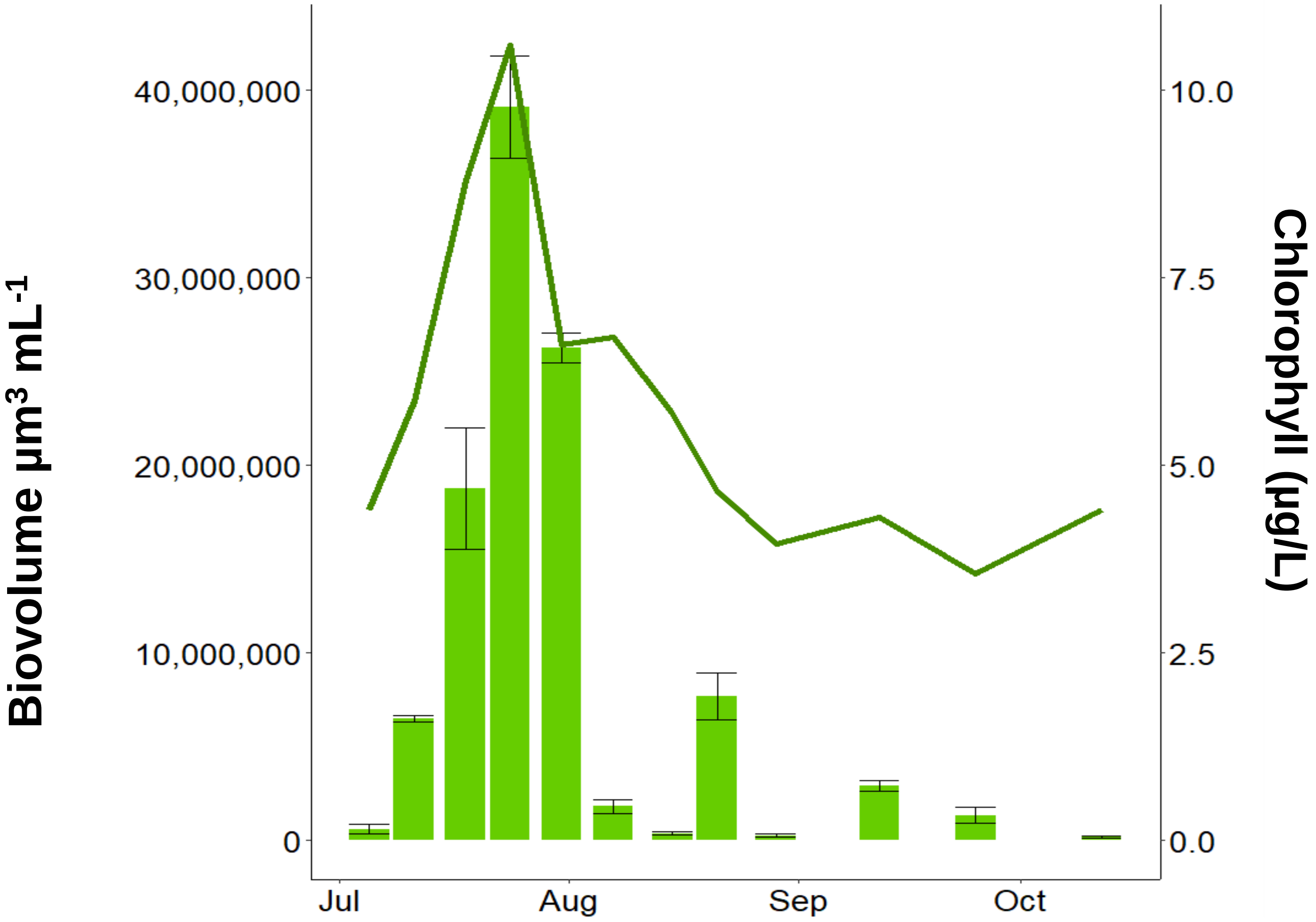
- Design qPCR assays
- Quantify cells volume in epilimnetic water samples

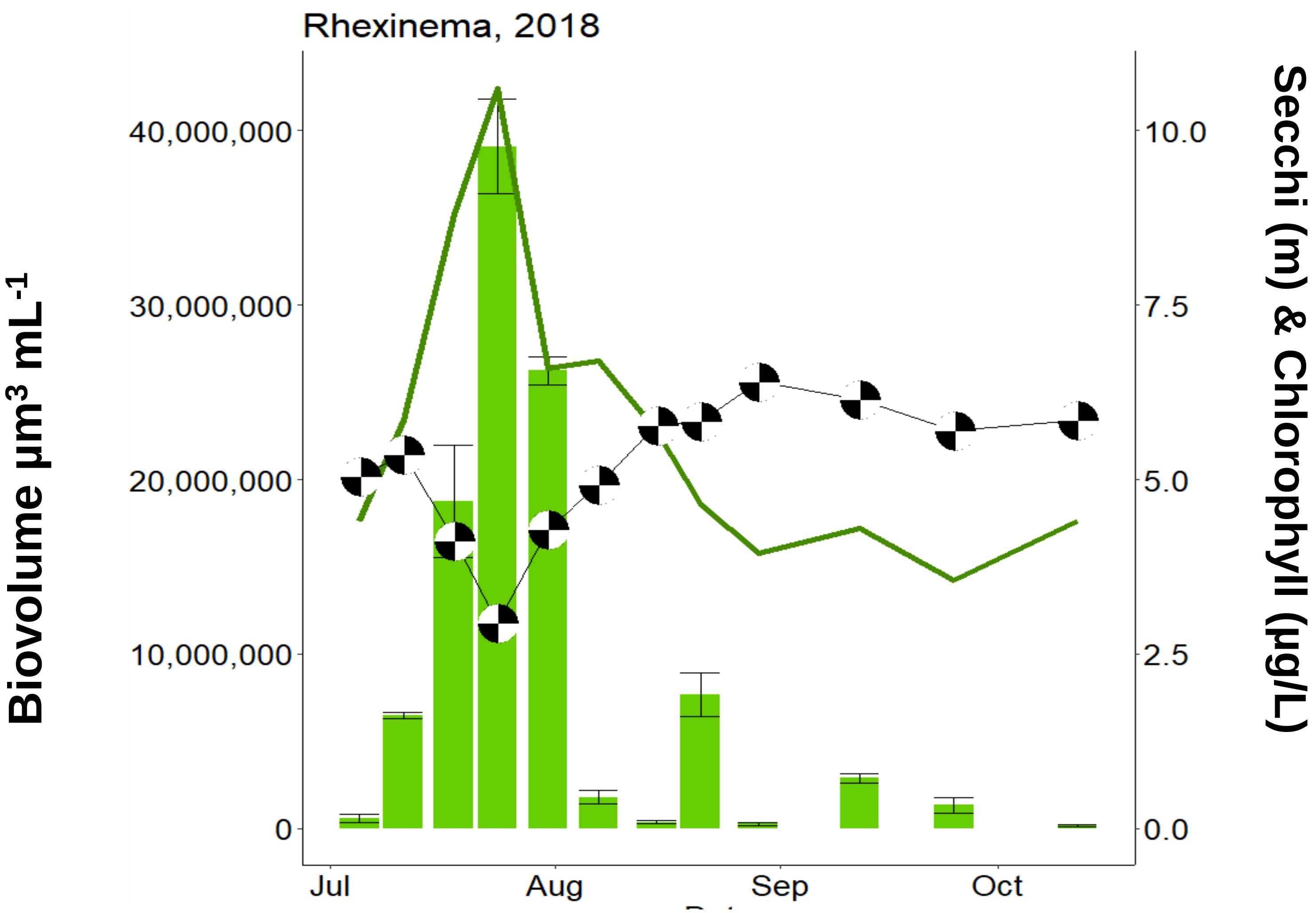
MAINE eDNA

Metabarcoding revealed two cryptic phytoplankton taxa

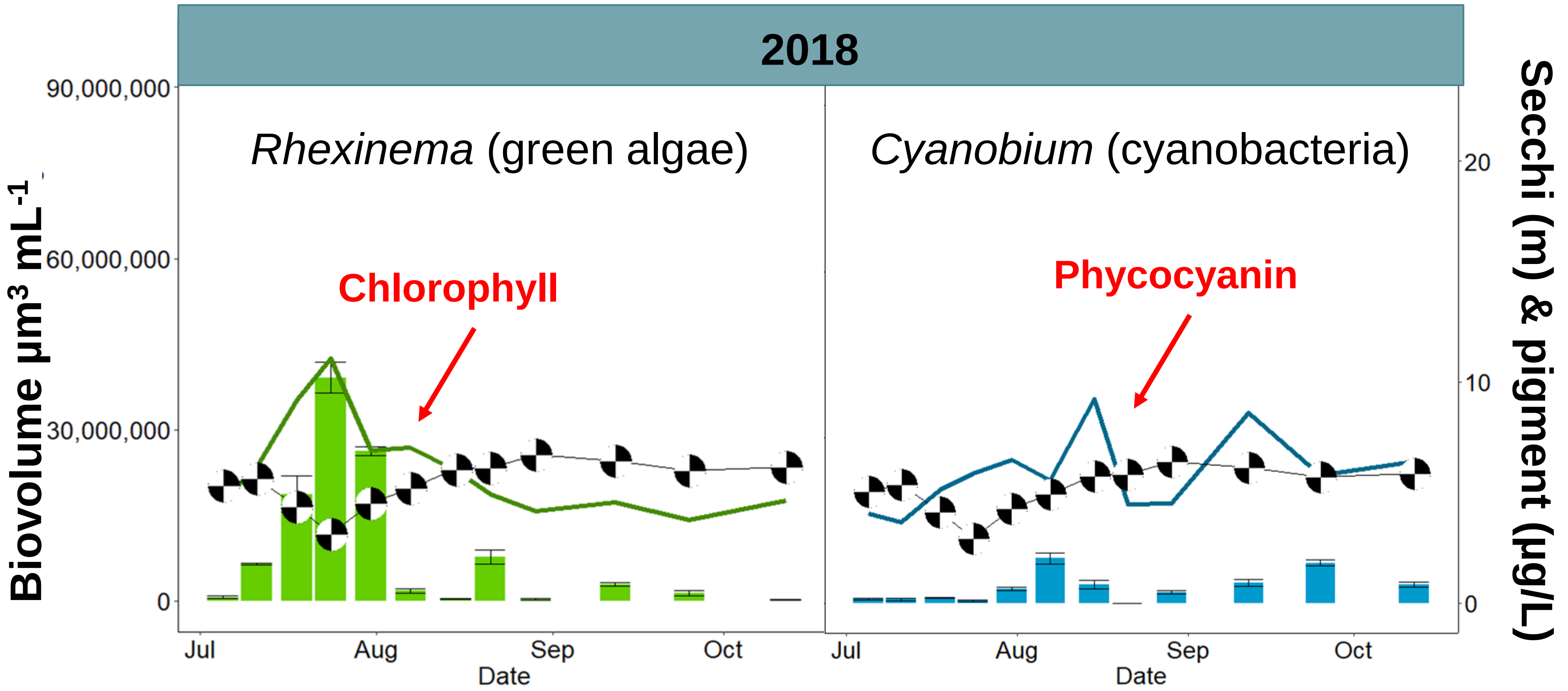


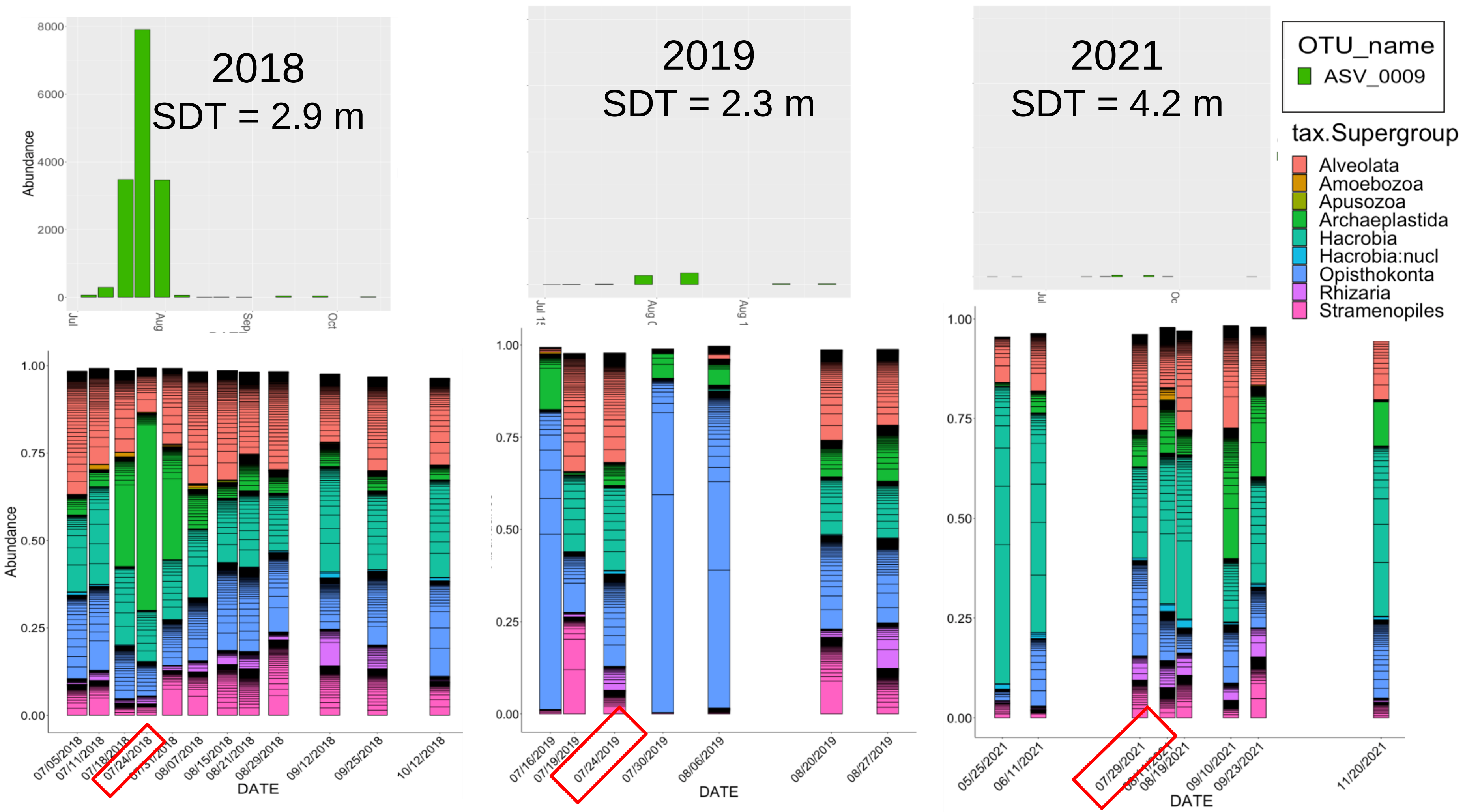
Biovolume µm3 mL-1
4e+07
3e+07
2e+07
1e+07
0e+00
Jul
Aug
Sep
Oct
Date


Biovolume μm³ mL⁻¹
40,000,000
30,000,000
20,000,000
10,000,000
0
Jul
Aug
Sep
Oct
Chlorophyll (μg/L)
10.0
7.5
5.0
2.5
0.0



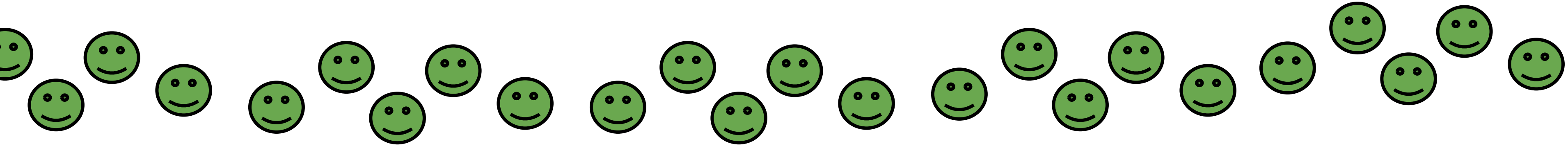
Rhexinema, 2018
Biovolume µm³ mL⁻¹
40,000,000
30,000,000
20,000,000
10,000,000
0
Secchi (m) & Chlorophyll (µg/L)
10.0
7.5
5.0
2.5
0.0
Jul
Aug
Sep
Oct


2018
Biovolume µm³ mL⁻¹
90,000,000
60,000,000
30,000,000
0
Rhexinema (green algae)
Chlorophyll
Cyanobium (cyanobacteria)
Phycocyanin
Jul
Aug
Sep
Oct
Date
20
10
0
Secchi (m) & pigment (µg/L)

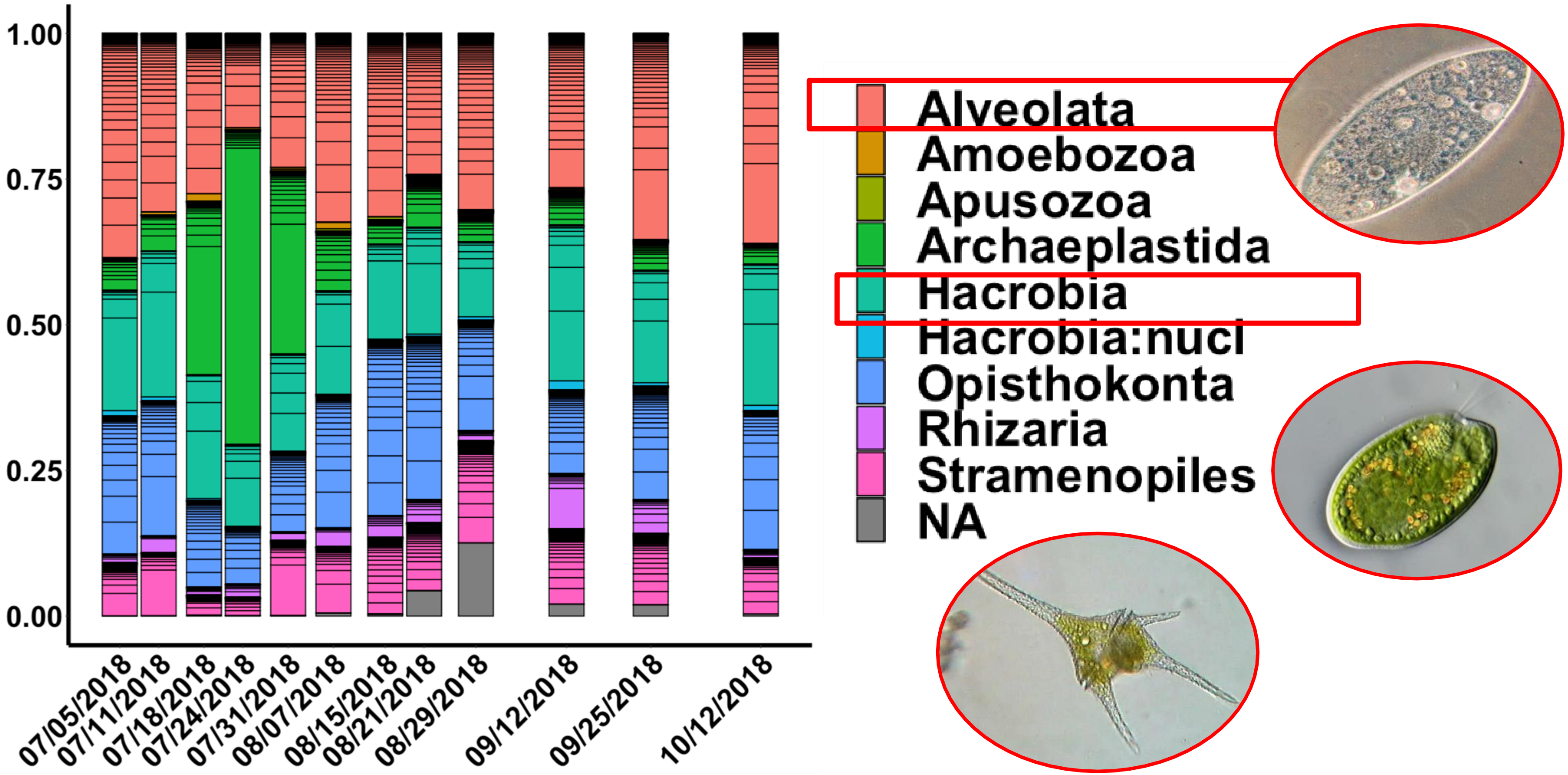


2018
SDT = 2.9 m
2019
SDT = 2.3 m
2021
SDT = 4.2 m
OTU_name
ASV_0009
tax.Supergroup
Alveolata
Amoebozoa
Apusozoa
Archaeplastida
Hacrobia
Hacrobia:nucl
Opisthokonta
Rhizaria
Stramenopiles
Abundance
DATE
07/05/2018
07/11/2018
07/24/2018
08/07/2018
08/15/2018
08/21/2018
08/29/2018
09/12/2018
09/25/2018
10/12/2018
07/16/2019
07/19/2019
07/24/2019
07/30/2019
08/06/2019
08/20/2019
08/27/2019
05/25/2021
06/11/2021
07/29/2021
08/19/2021
09/10/2021
09/23/2021
11/20/2021

Next Steps



- Culture Rhexinema
- Sequence the culture’s DNA
- Send culture isolates to a taxonomist


1.00
0.75
0.50
0.25
0.00
07/05/2018
07/11/2018
07/18/2018
07/24/2018
07/31/2018
08/07/2018
08/15/2018
08/21/2018
08/29/2018
09/12/2018
09/25/2018
10/12/2018
Alveolata
Amoebozoa
Apusozoa
Archaeplastida
Hacrobia
Hacrobia:nucl
Opisthokonta
Rhizaria
Stramenopiles
NA

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MAINE eDNA

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