

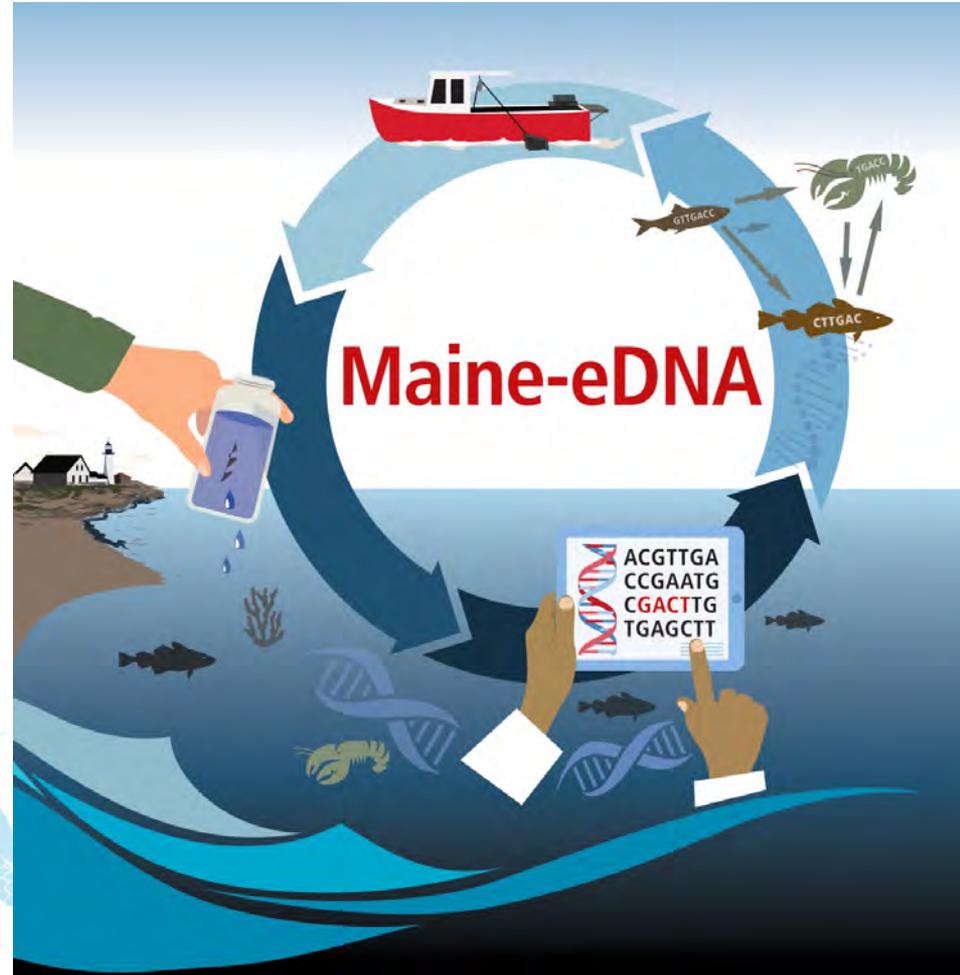
# The distribution of toxic *Dolichospermum* strains across Maine

Robin Sleith and Pete Countway



# 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

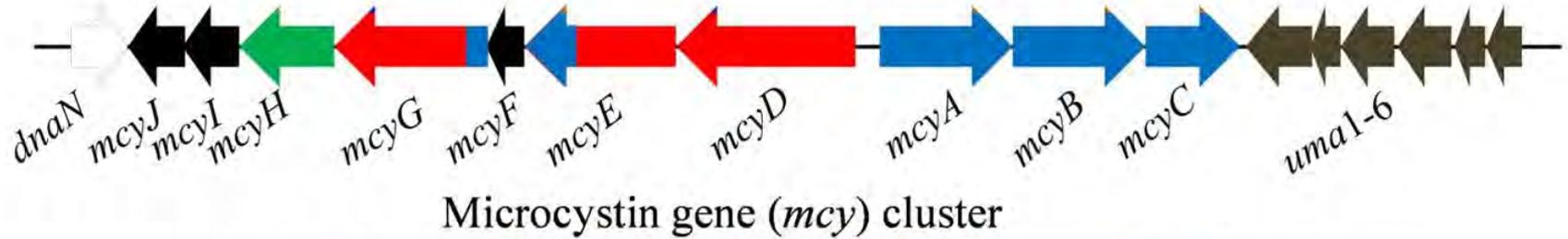


# Overview

- Toxin biosynthesis
- Toxic and non-toxic strains
- ddPCR of microcystin biosynthesis genes
- The road ahead



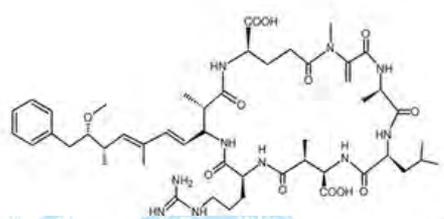
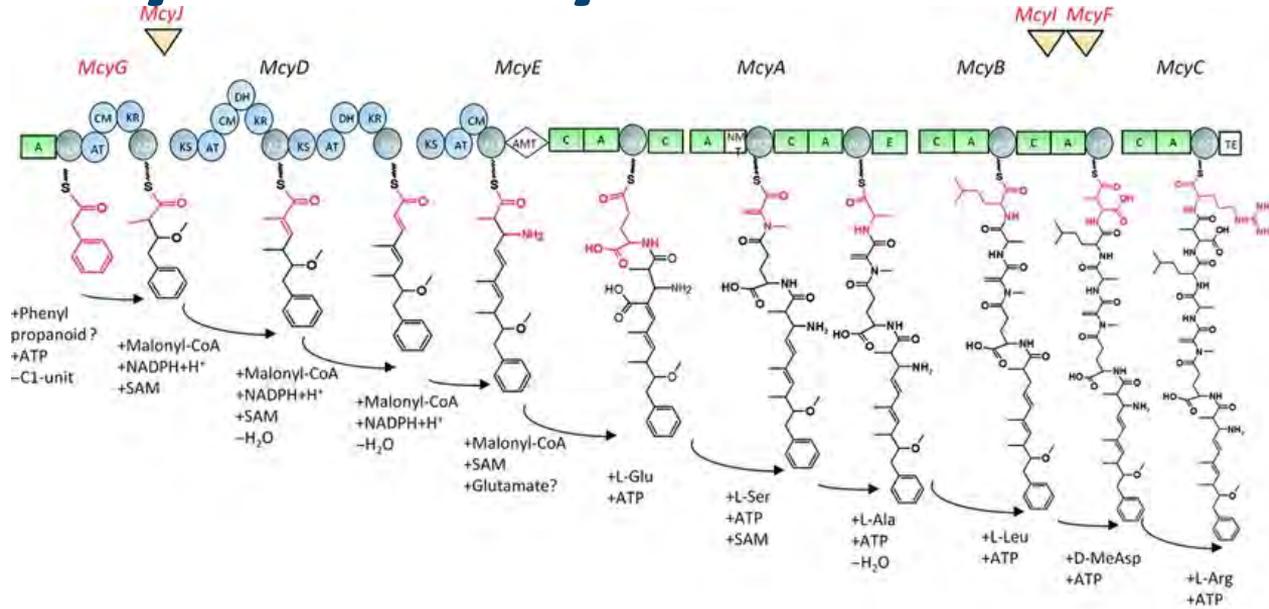
# Microcystin biosynthesis



Rastogi et al. 2015



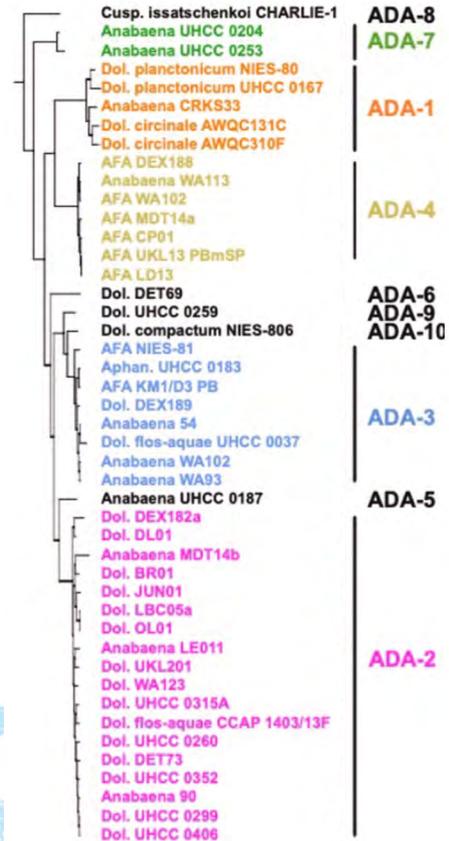
# Microcystin Biosynthesis



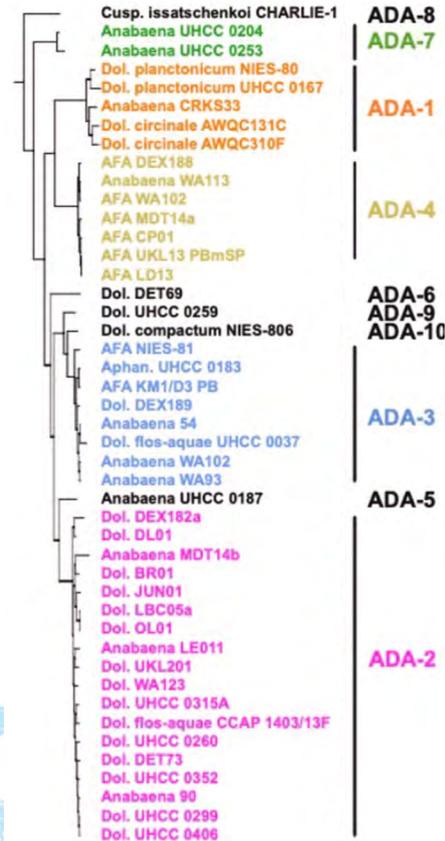
Cyclization (-H<sub>2</sub>O)

Dittmann et al. 2012

# Toxic strains of *Dolichospermum*



# Toxic strains of *Dolichospermum*

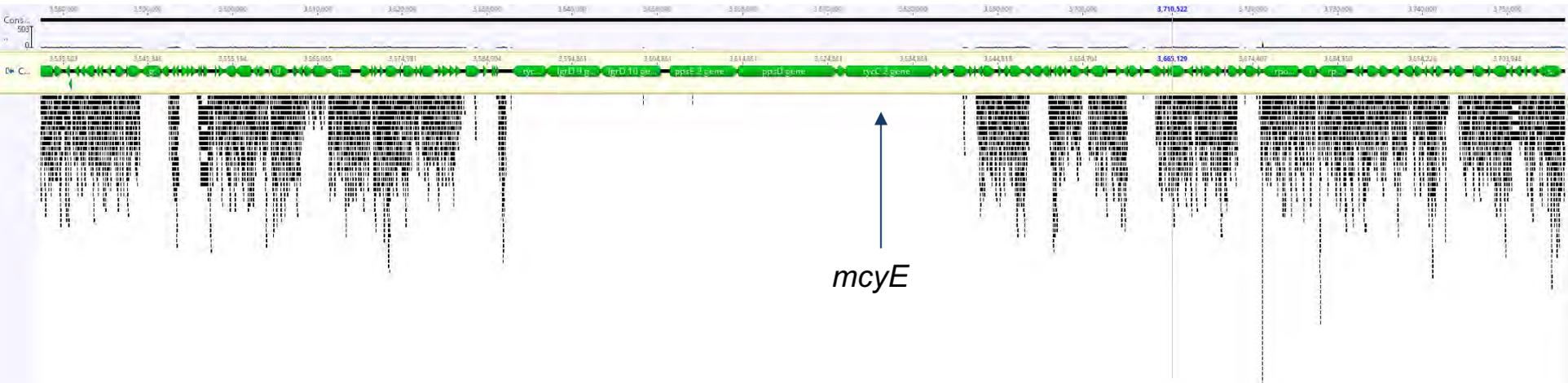


Damariscotta Lake  
China Lake  
North Pond

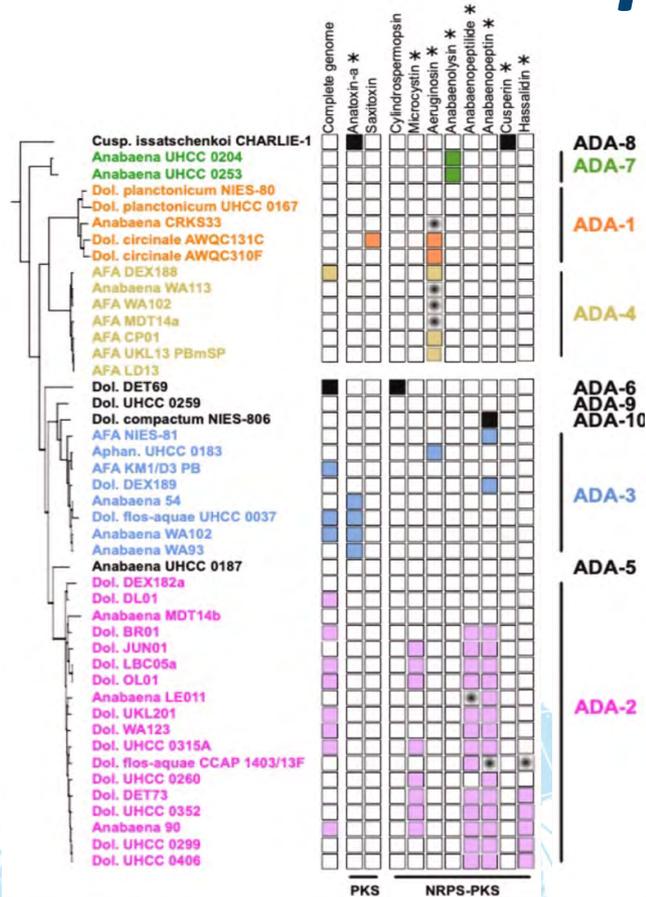
Damariscotta Lake  
Little Dyer Pond  
China Lake  
North Pond  
Lake Winnepesaukee

# Toxic strains of *Dolichospermum*

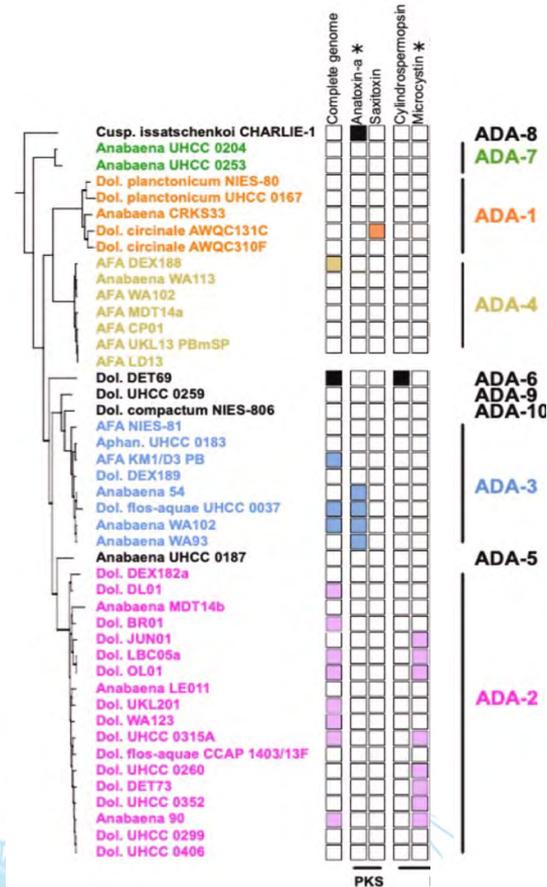
- Damariscotta Lake *Dolichospermum* lack microcystin biosynthesis pathway



# Toxic strains of *Dolichospermum*



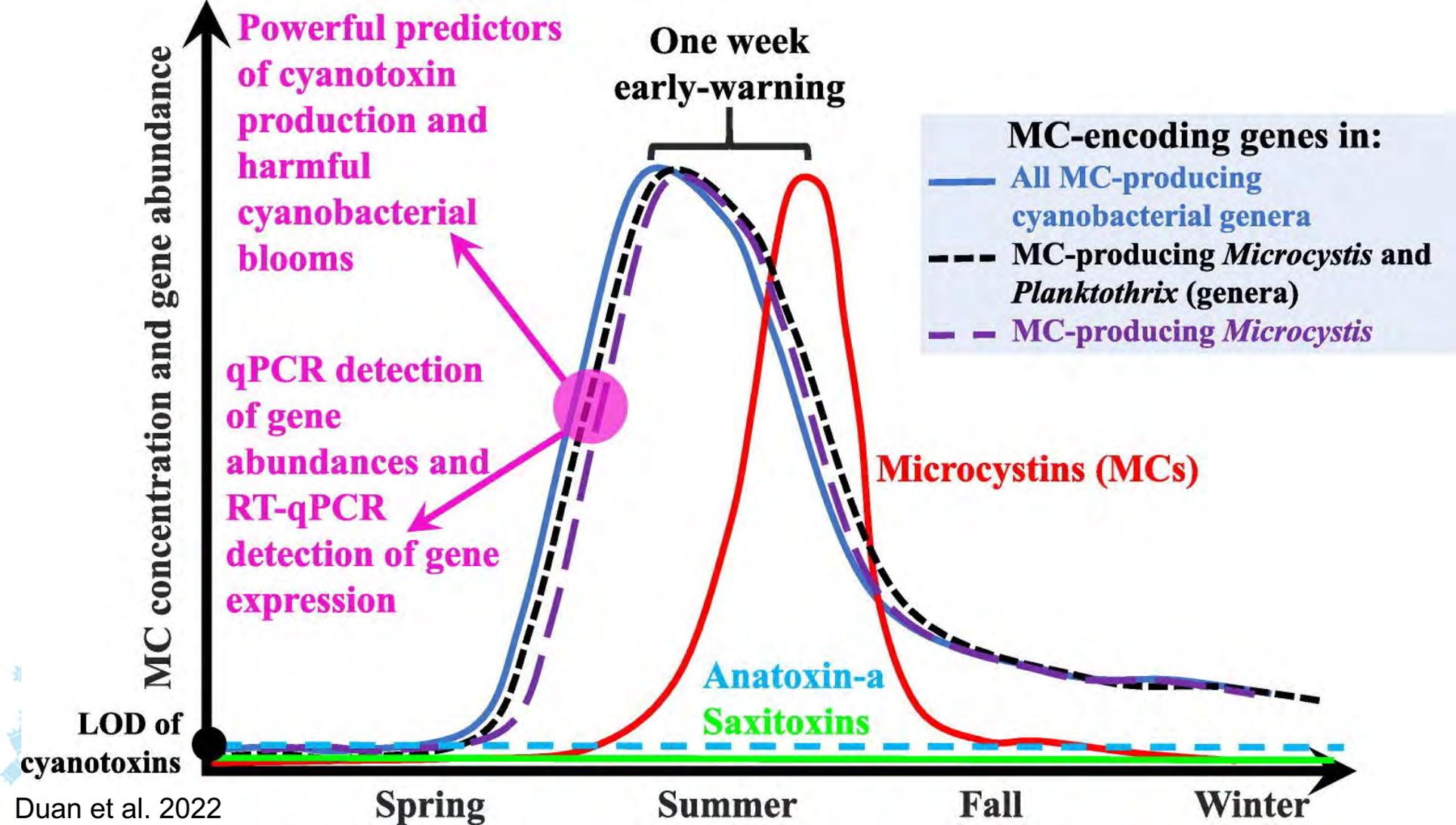
# Toxic strains of *Dolichospermum*

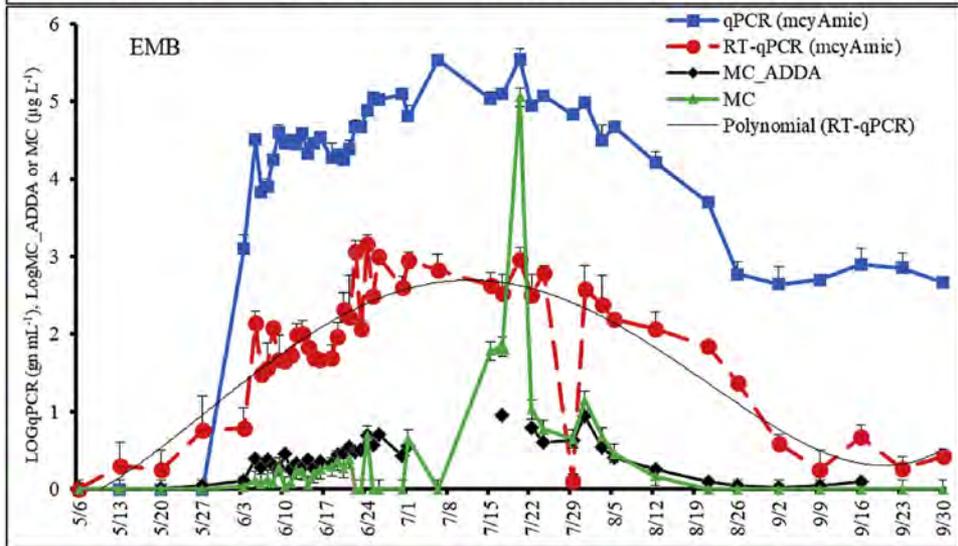
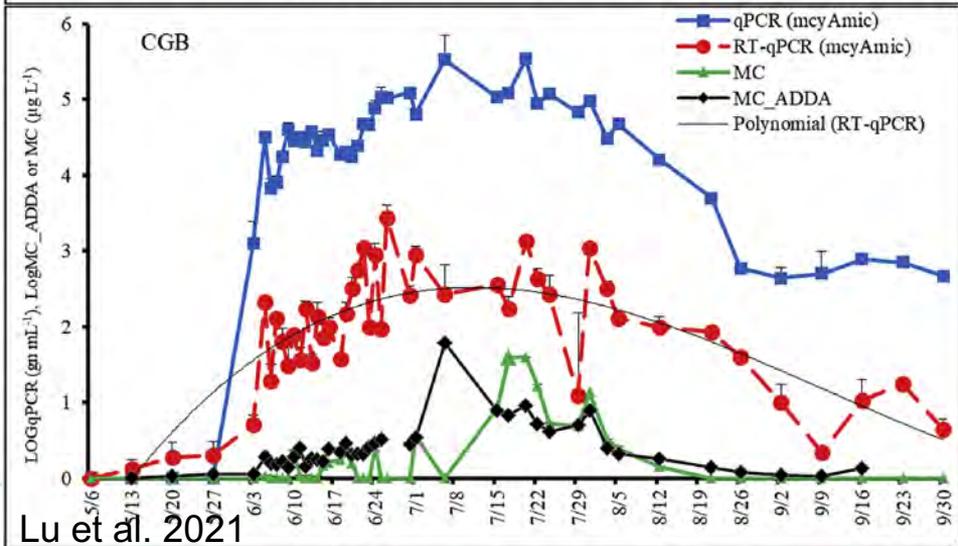
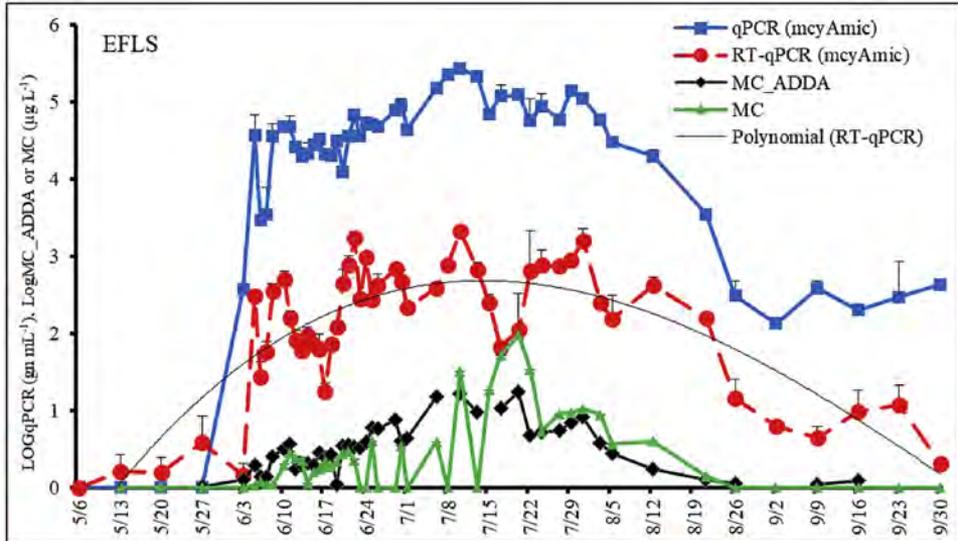
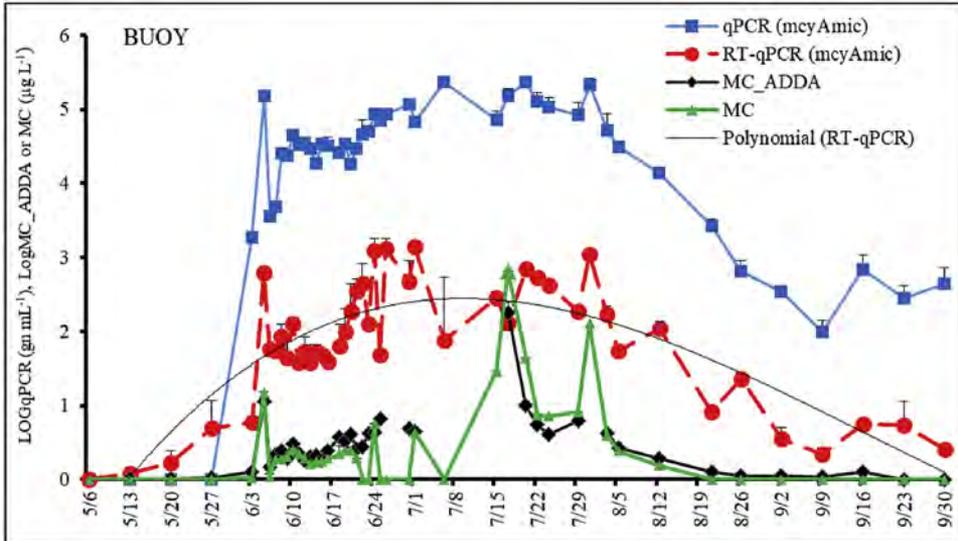


# Toxin genes as sentinels of change

- Primers and probes target key biosynthesis gene (e.g. *mcyE*)
- qPCR, ddPCR, RT-qPCR techniques track gene or transcript abundance over time







# Toxin genes as sentinels of change

- To link gene abundance to toxicity can be a challenge
  - Frequent sampling
  - Likely different lag times and patterns based on local conditions
- Toxin gene presence/abundance nevertheless an important piece of the HABS puzzle
- Species level resolution important as mitigation strategies are often species specific!



# Maine *mcyE* gene abundances

- 50 samples (triplicate filters) from lakes across Maine provided by Linda Bacon
- Qiagen PowerWater extractions
- Duplex ddPCR targeting *mcyE* in *Microcystis* and *Anabaena/Dolichospermum* (ADA2) from Ngwa et al. (2013)



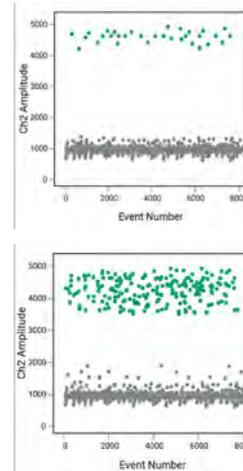
**Step 1:**  
Collect a  
water  
sample



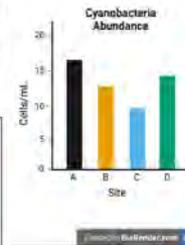
**Step 2:** Filter  
the water  
sample



**Step 3:**  
Extract DNA  
from filter



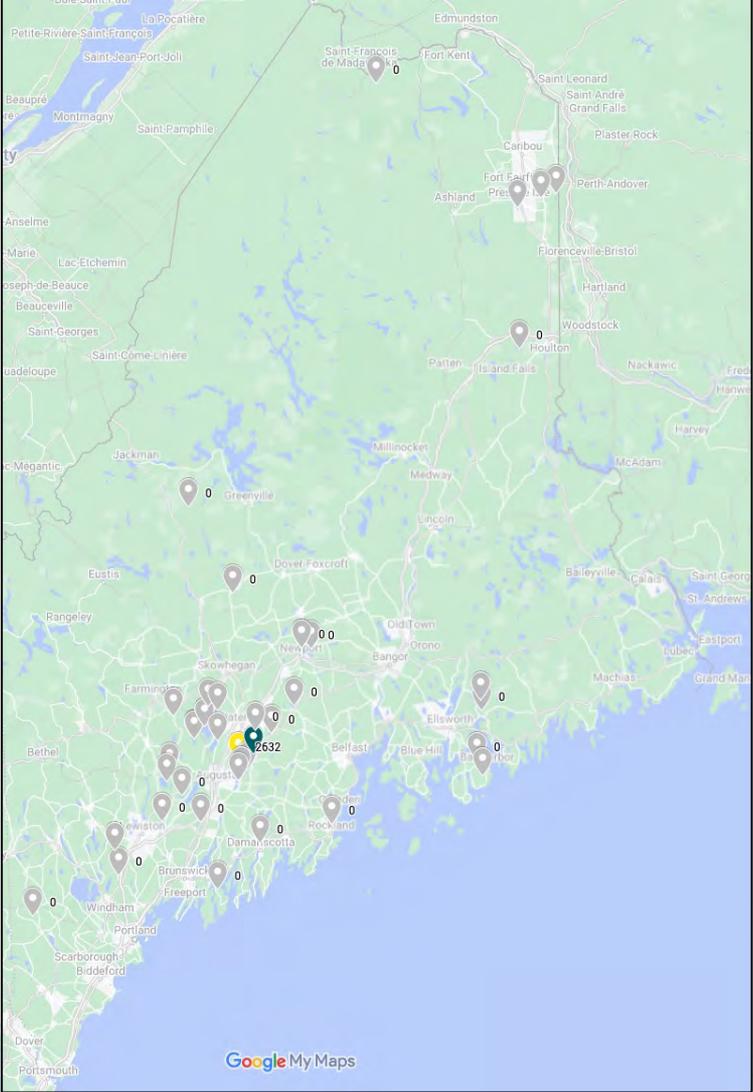
**Step 4:** Run  
ddPCR



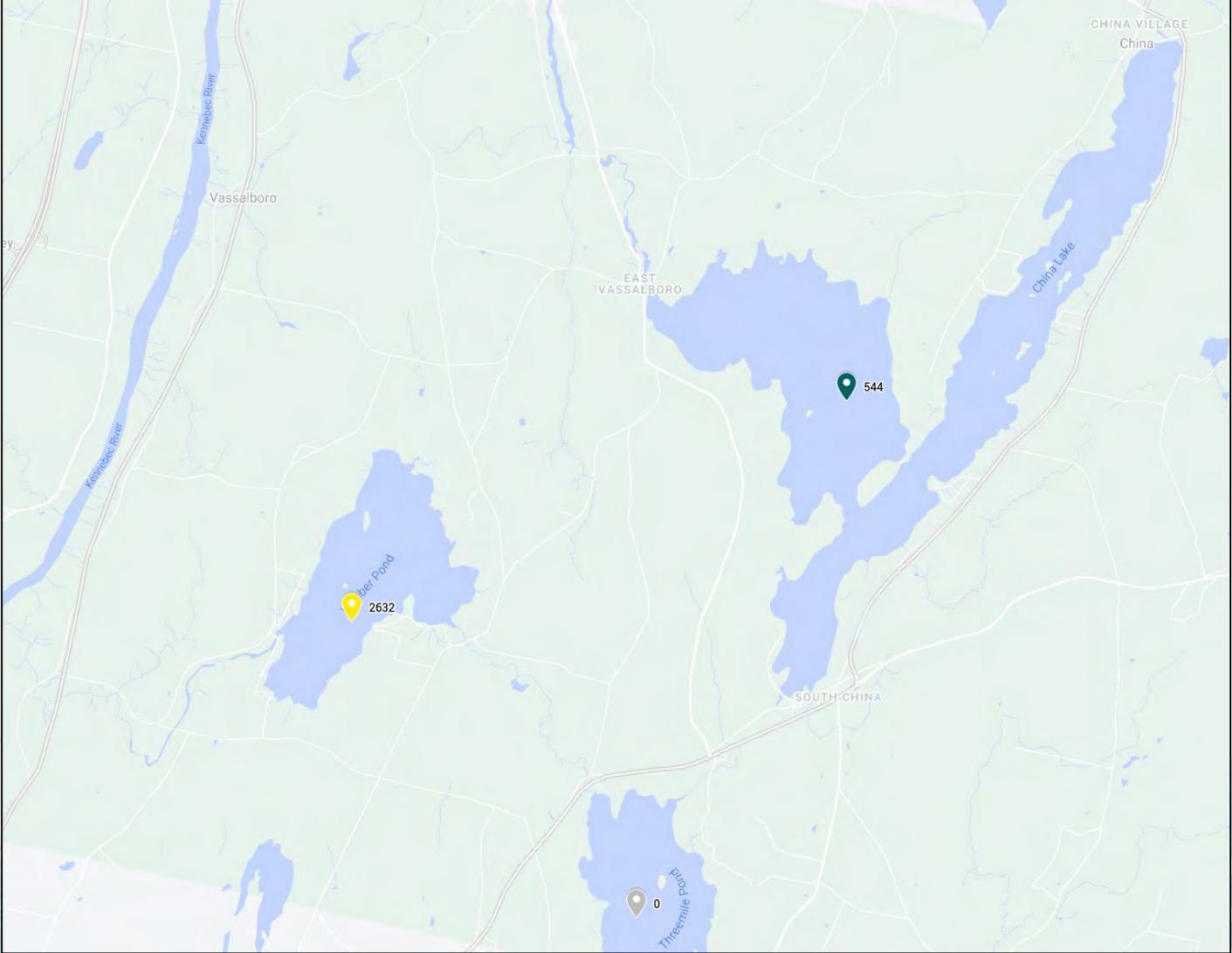
**Step 5:** Convert  
output to  
abundance



# ADA-2 *mcyE*



ADA-2 mcyE







# Conclusions and next steps

- Toxic ADA-2 distribution extremely limited, why?
- Toxic *Microcystis* widespread, is toxicity widespread, Linda?
- What controls the distributions of toxic genotypes?



# Acknowledgements



Pete Countway



MIDCOAST  
CONSERVANCY

Colby



Supported by the National Science Foundation under Grant  
No. 11A-1849227