The Biology of HAB events – (lessons learned)

Steven Wilhelm
Kenneth & Blaire Mossman Professor
Department of Microbiology
The University of Tennessee

Your're glumping the pond where the Humming-Fish hummed!

No more can they hum, for their gills are all gummed.

So I'm sending them off. Oh their future is dreary.

They'll walk on their fins and get woefully weary

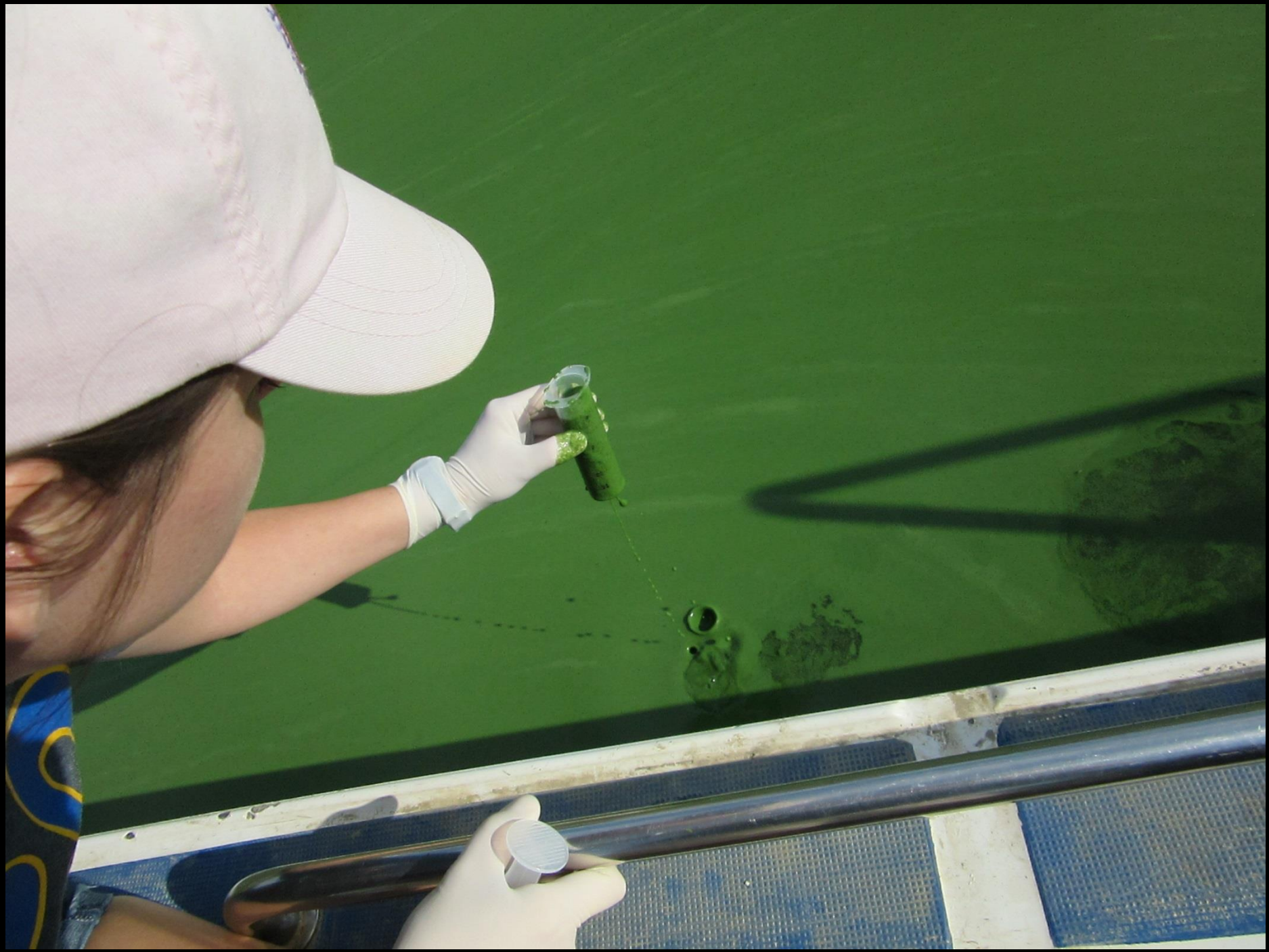
in search of some water that isn't too smeary

<u>I hear things are just as bad up in Lake Erie.</u>

(Seuss, 1971)

What are harmful algal blooms ???

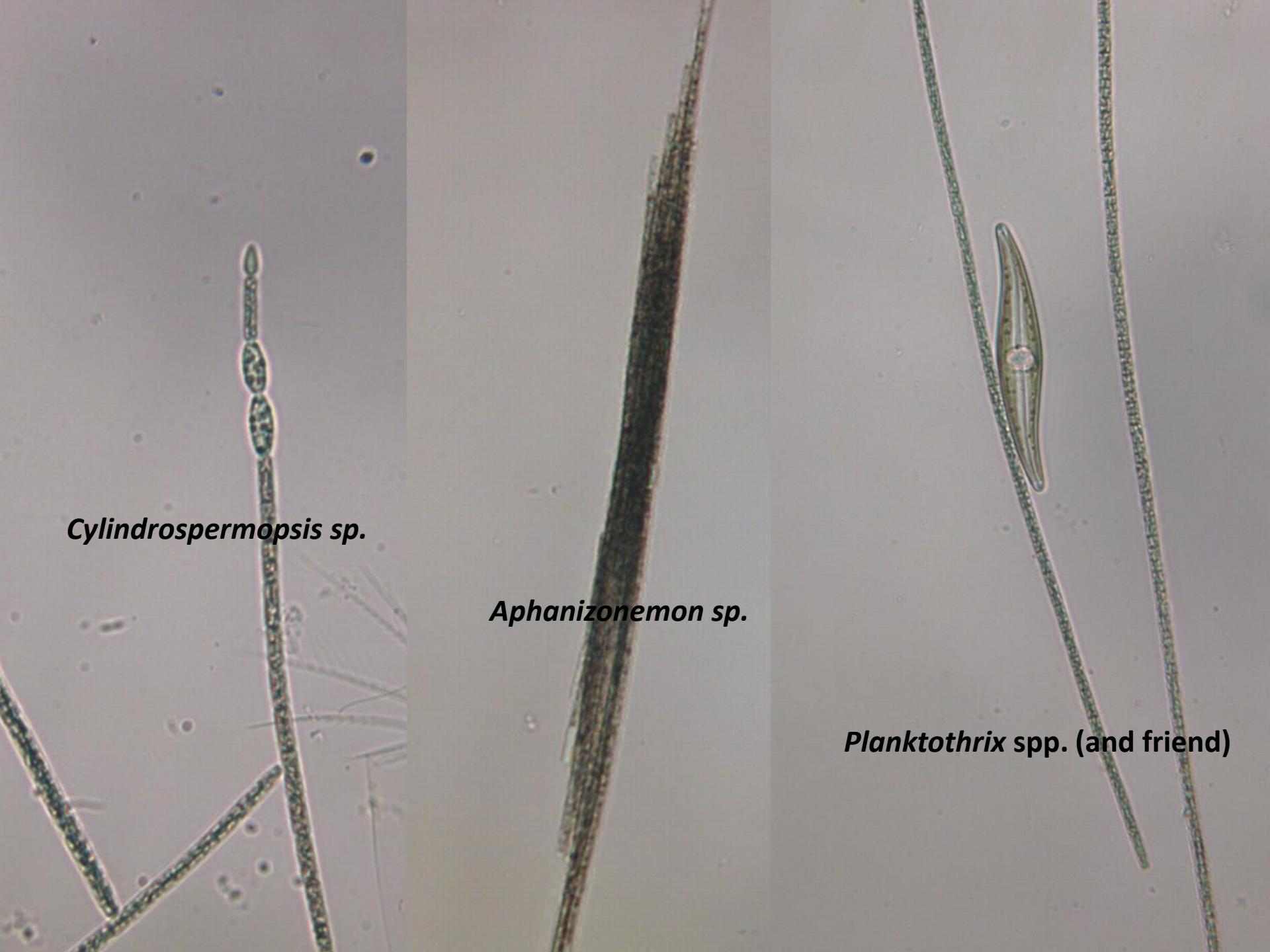
What are cyanobacterial harmful algal blooms?



Microcystis spp.

***Microcystis* full of ghost cells (empty cells)**

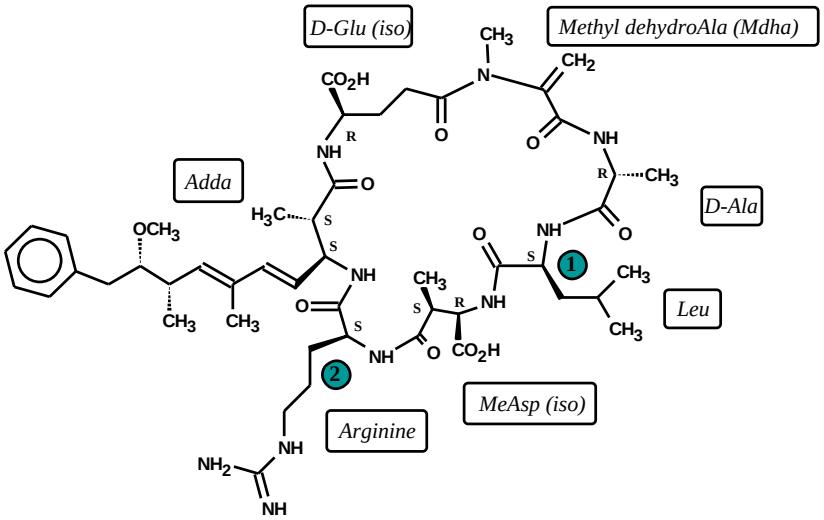
Anabaena / Dolichospermum spp.

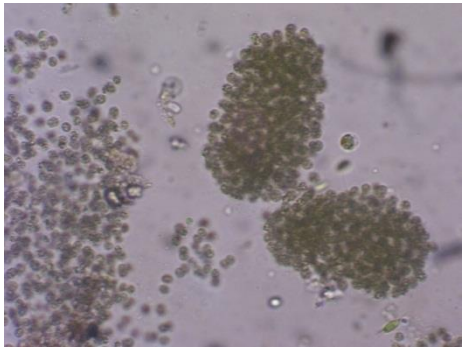

Cylindrospermopsis sp.
Aphanizonemon sp.
Planktothrix **spp. (and friend)**

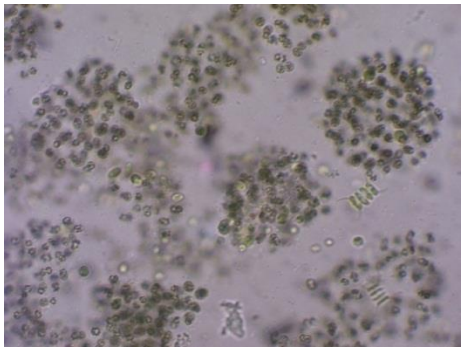
Microcystis spp.

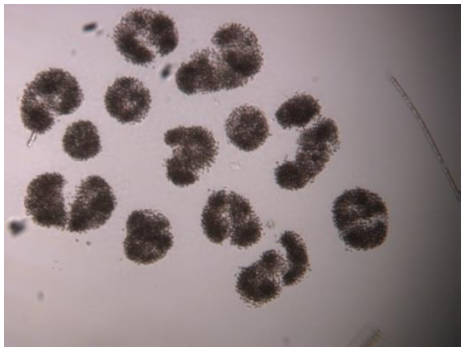
- **Globally distributed HAB species**
- **Reoccurring in Laurentian Great Lakes since 1995.** Brittain et al. (2000).
- **Produces a known secondary metabolite (microcystin) with hepatotoxic effects that was originally called**

  FAST DEATH FACTOR Bishop et al. (1959)
- **Produces an as-of-yet unidentified secondary metabolite with endocrine disrupting effects** Rogers et al. (2011)
- **Blooms to massive scales, causing disruption of food webs and water column physiochemistry.**









Photos by GL Boyer, J Dyble Bressie and SW Wilhelm

So what can we say we know

FOR SURE

about cyanobacterial harmful algal blooms ???

They are ancient organisms (R. Stumpf).

People do not like them

The mess up the environment and socioeconomics.

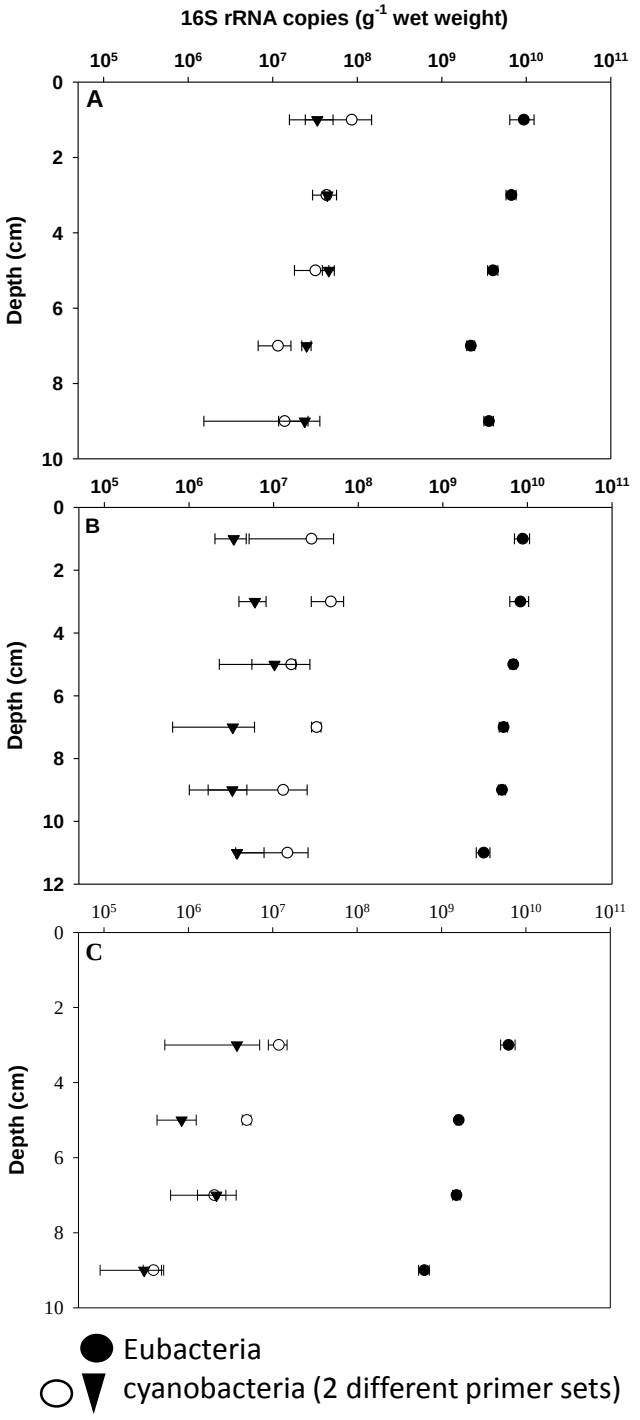
Blooms are tied to various environmental conditions

We have a long way to go before we can successfully and consistently "control" them

Microcystis **genotypes in the western basin of Lake Erie have been present since at least 1970 (based on *mcyA* sequencing, *Rinta-Kanto et al. 2009*).**

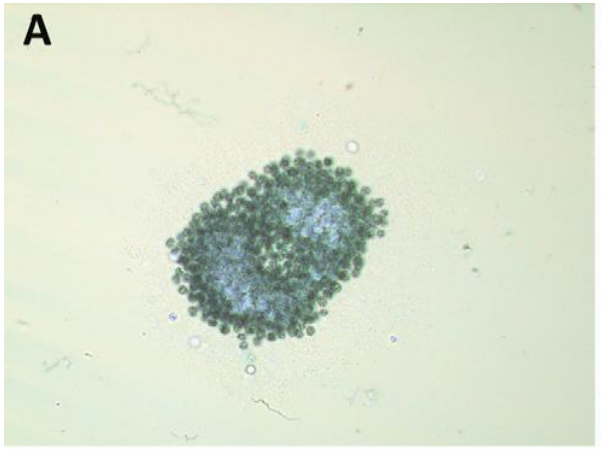
Microcystis **that has bloomed in Lake Erie is genetically consistent with cells in the sediments of Lake Erie (*Rinta-Kanto et al. 2009, Kutovaya et al 2012*) as well as with cells from Lake St. Clair (*Davis et al. 2014*).**

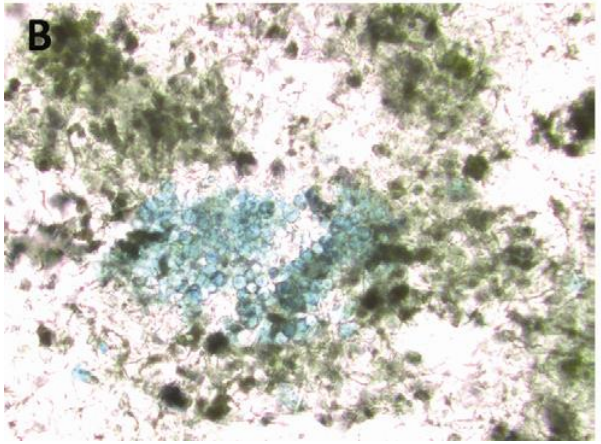
Microcystis **in the Maumee River has been (to date) genetically distinct from blooms in the western basin (*Rinta-Kanto et al. 2009, Kutovaya et al. 2012*)**



Rinta-Kanto et al. 2009 *Harmful Algae*

MICROCYSTIS HAS BEEN PRESENT FOR A LONG TIME BUT ONLY STARTED BLOOMING IN LAKE ERIE (THAT WE NOTICED) *CA* 1995.





The results generally support a classic model of microbial ecology, that "everything is everywhere but the environment selects" (De Wit and Bouvier, 2006; O'Malley, 2007) suggesting that populations may always be present but proliferate based on environmental conditions. In the case of the Laurentian Great Lakes, that principle could even be phrased as "everything is everywhere but human activity selects".

Rinta-Kanto et al. 2009 *Harmful Algae*

So what can we say we know

POTENTIALLY

about cyanobacterial harmful algal blooms ???

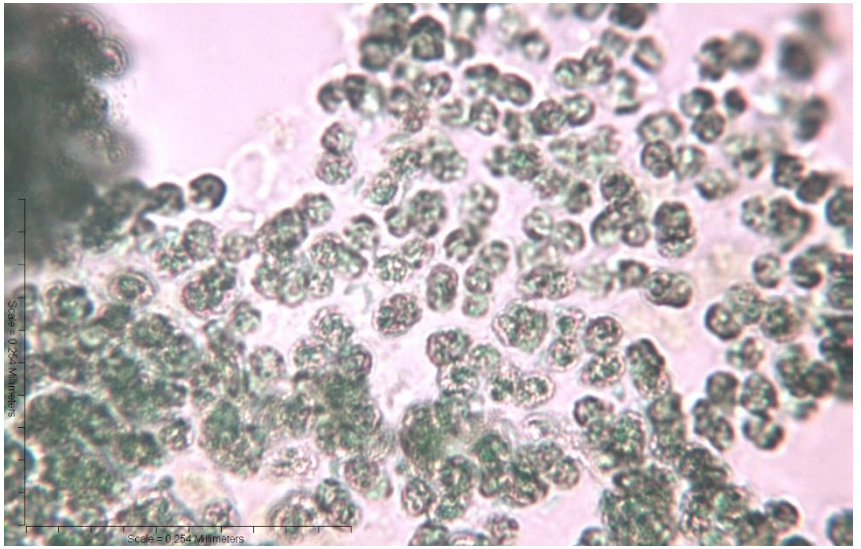
There is a linkage between CHABs in lakes and

- **Nutrient concentrations and chemistry**
- **Temperature**
- **Time of season**
- **Meteorology (rain, wind, *etc.*)**
- **Trophic status (*few to no blooms in oligotrophic systems* – *thanks Mark / Hans*)**
- **Agricultural chemicals (farms but also includes golf courses and urban green space)**

We also tend to think as a community that blooms are growing in frequency, severity and duration

So why can't we come to any sort of consensus about cyanobacterial harmful algal blooms ???

As a community we use a collection of very different tools



Data collection comes in all shapes and sizes.
From the remote to hands on.

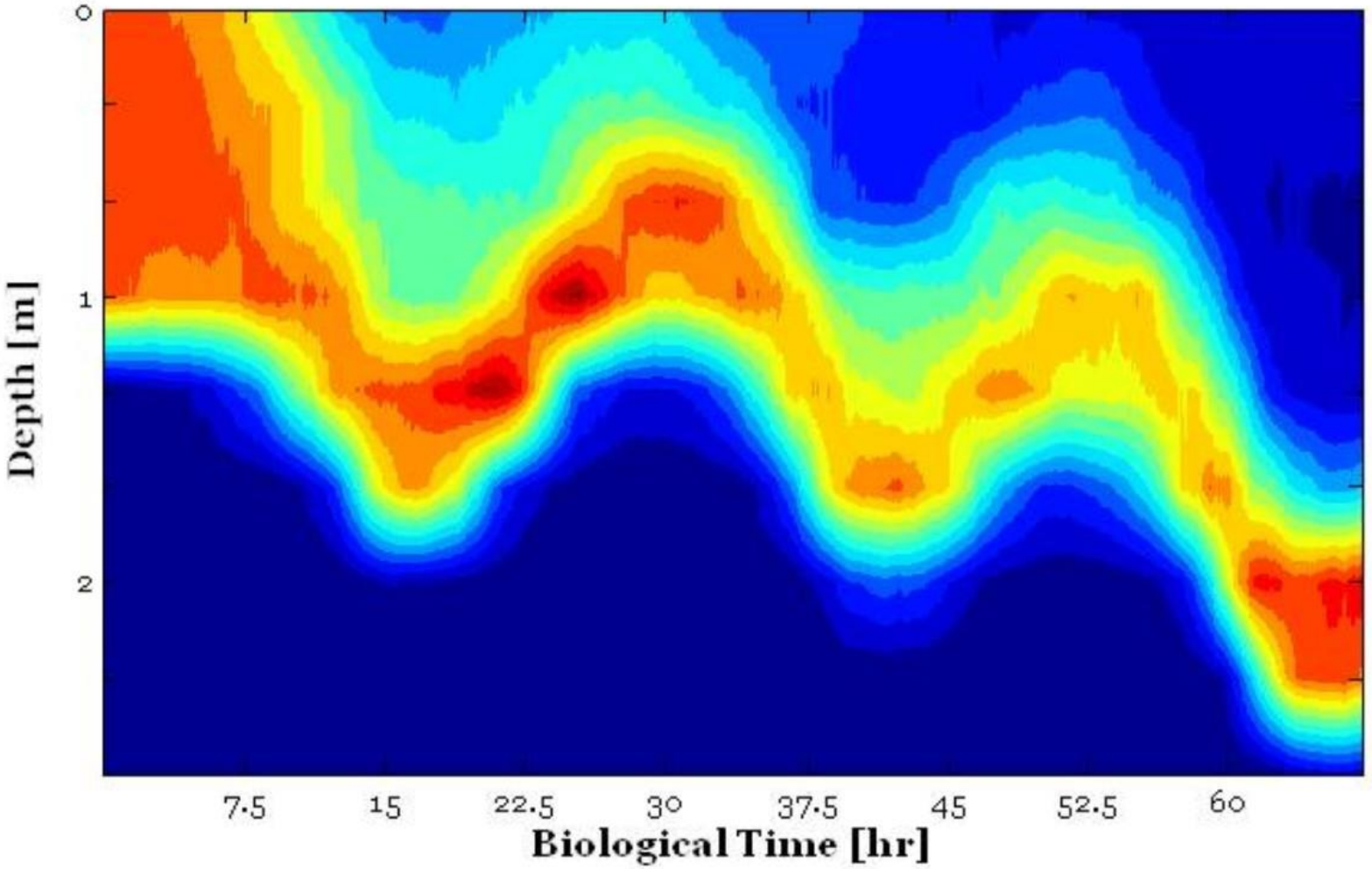
This often leads to different frames of reference

Are there metrics, parameters of functions that we over interpret?

That is, what do we think we know / what do we do that is confounding our observations and experiments?

BLOOMS CAN BE INCREDIBLE PATCHY!

Under conditions of low turbulence *Microcystis* vertical migration depends mainly on its buoyant status



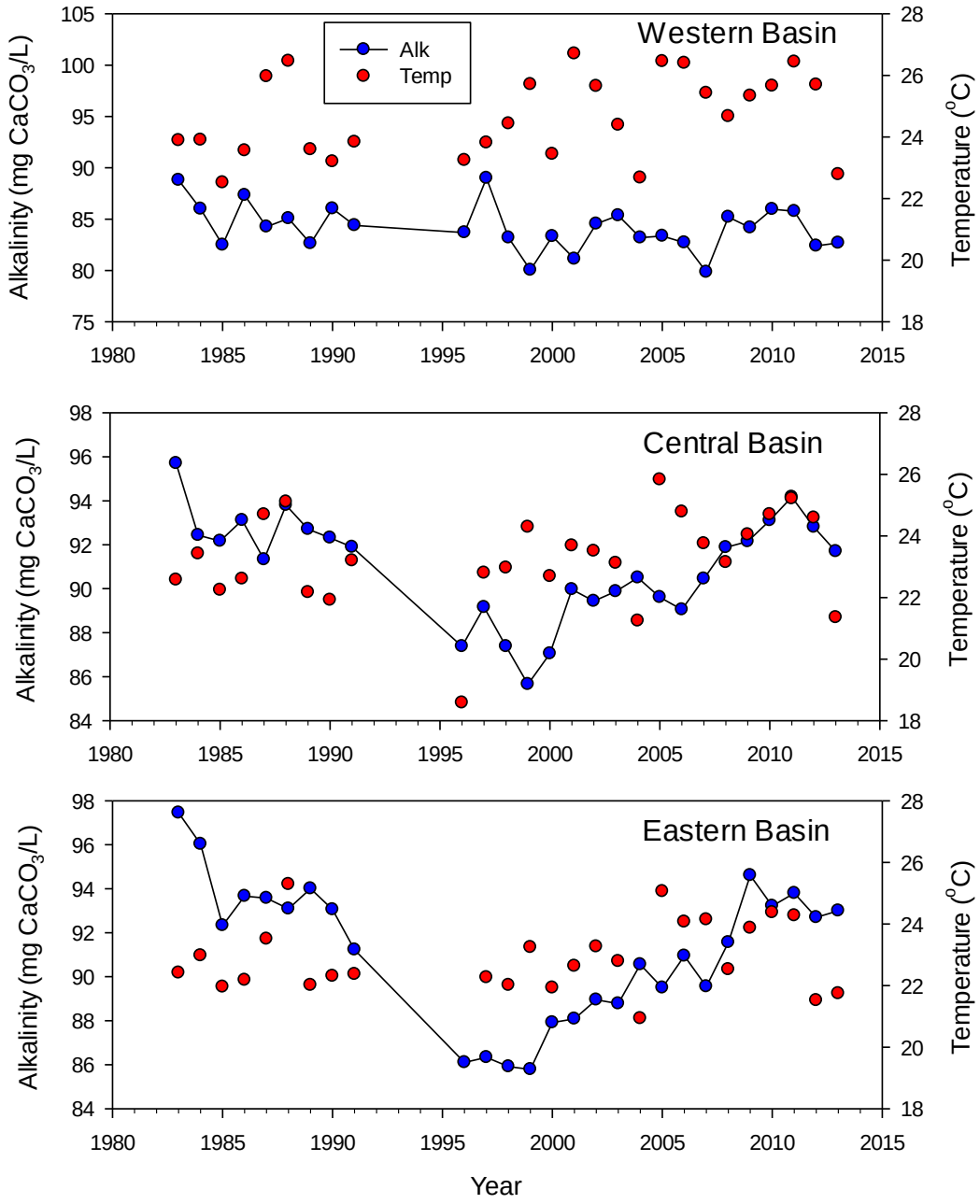
Aparicio Medrano, E., Uittenbogaard, R.E., Dionisio Pires, L.M., Wiel, B.J.H. van de & Clercx, H.J.H. (2013). *Coupling hydrodynamics and buoyancy regulation in Microcystis aeruginosa for its vertical distribution in lakes*. Ecological Modelling, **248**, 41-56.

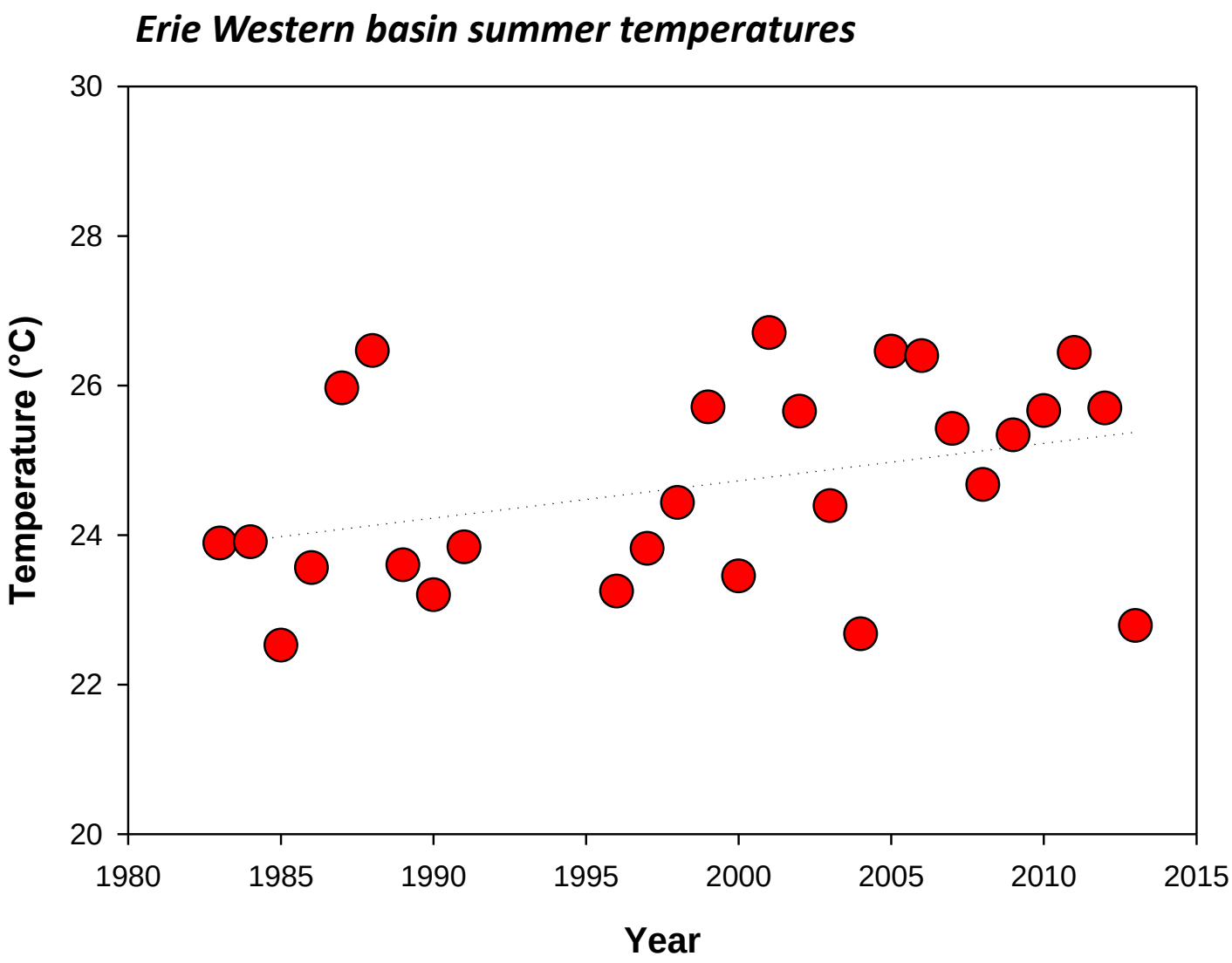
- **The way we use the term "Cyanobacteria"** ***(see Carrick and Shelske 1997)***
- **Chlorophyll – can vary 10-fold per cell with nutrient changes, varies with cell light history (ergo time of day). Must interpret carefully.**
- **Buoyancy – we ignore this but it is tightly tied to time of day**
- **"Bloom chasing" – bigger numbers do not equal better data. This is complicated by the fact that blooms move.**
- **Temporal disconnects – "the Heisenberg *stupidity* principle" : researchers measure nutrients and biomass at the same time, but they are not directly connected (one begets the other, but which?)**
- **Things are not always what they seem (*e.g.,* alkaline phosphatase in *Microcystis* is more strongly regulated by urea than P, *Steffen et al. 2014*). We need to be sure of our science**

What changes in the Great Lakes basin (or that could affect this basin) have occurred that can be potentially linked to the increased *Microcystis* events we are seeing ???

The physiochemistry of the Lake Erie is changing - mean alkalinity made a significant drop off around the time that *Microcystis* started to bloom but is now recovering (Western basin static but low)

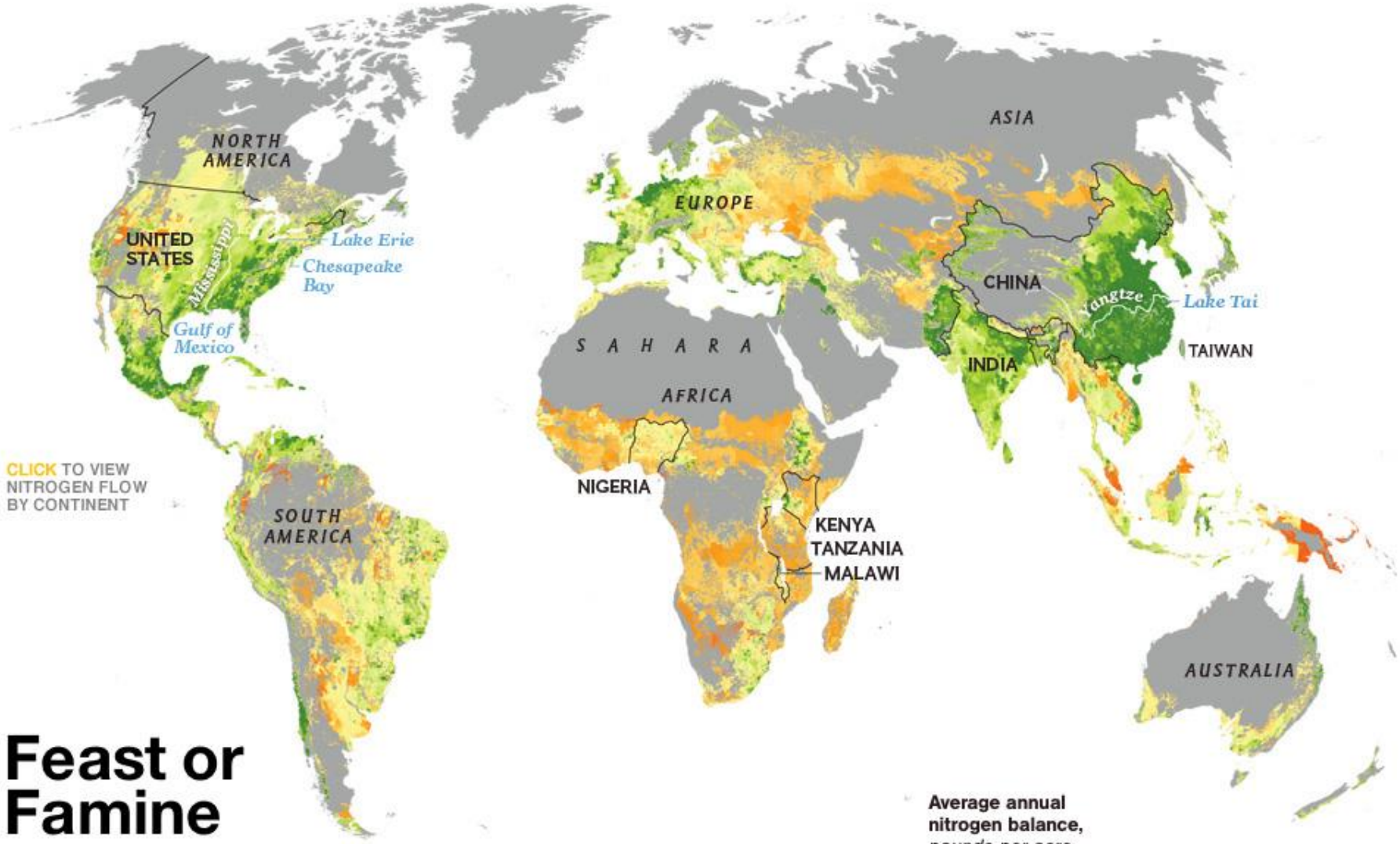
Data from GLNPO courtesy Rick Barbiero





There has been a slight increase in the Western Basin temperature in Lake Erie during the summer months – the relationship is weak ($R^2 = 0.127$)

Data from GLNPO courtesy Rick Barbiero



Feast or Famine

Nearly half the people on the planet wouldn't be alive if not for the abundant food made possible by nitrogen fertilizer. Yet its benefits have not reached everyone. In sub-Saharan Africa, where 239 million people go hungry in a year, crops fail as soil is stripped of nutrients, and farmers can't afford to buy fertilizer. Elsewhere overuse pollutes waterways and releases greenhouse gases.

JEROME N. COOKSON AND LAWSON PARKER, NGM STAFF
SOURCE: PAUL C. WEST, INSTITUTE ON THE ENVIRONMENT, UNIVERSITY OF MINNESOTA

Average annual nitrogen balance, *pounds per acre*

Deficiency — Excess

-200 0 220

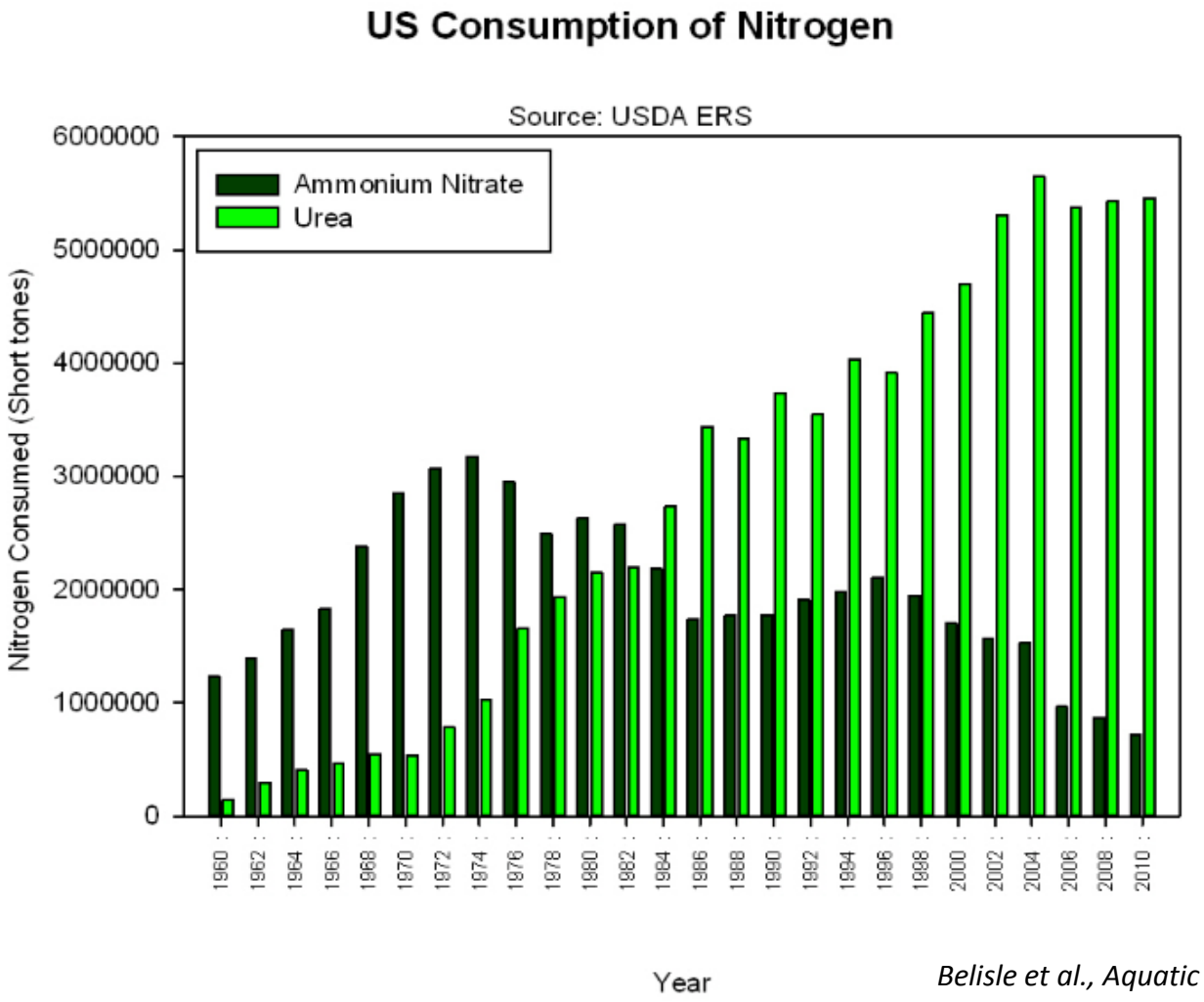
Little or no cropland

Zero means the crop used exactly the amount of nitrogen applied. The ideal range varies due to local conditions.

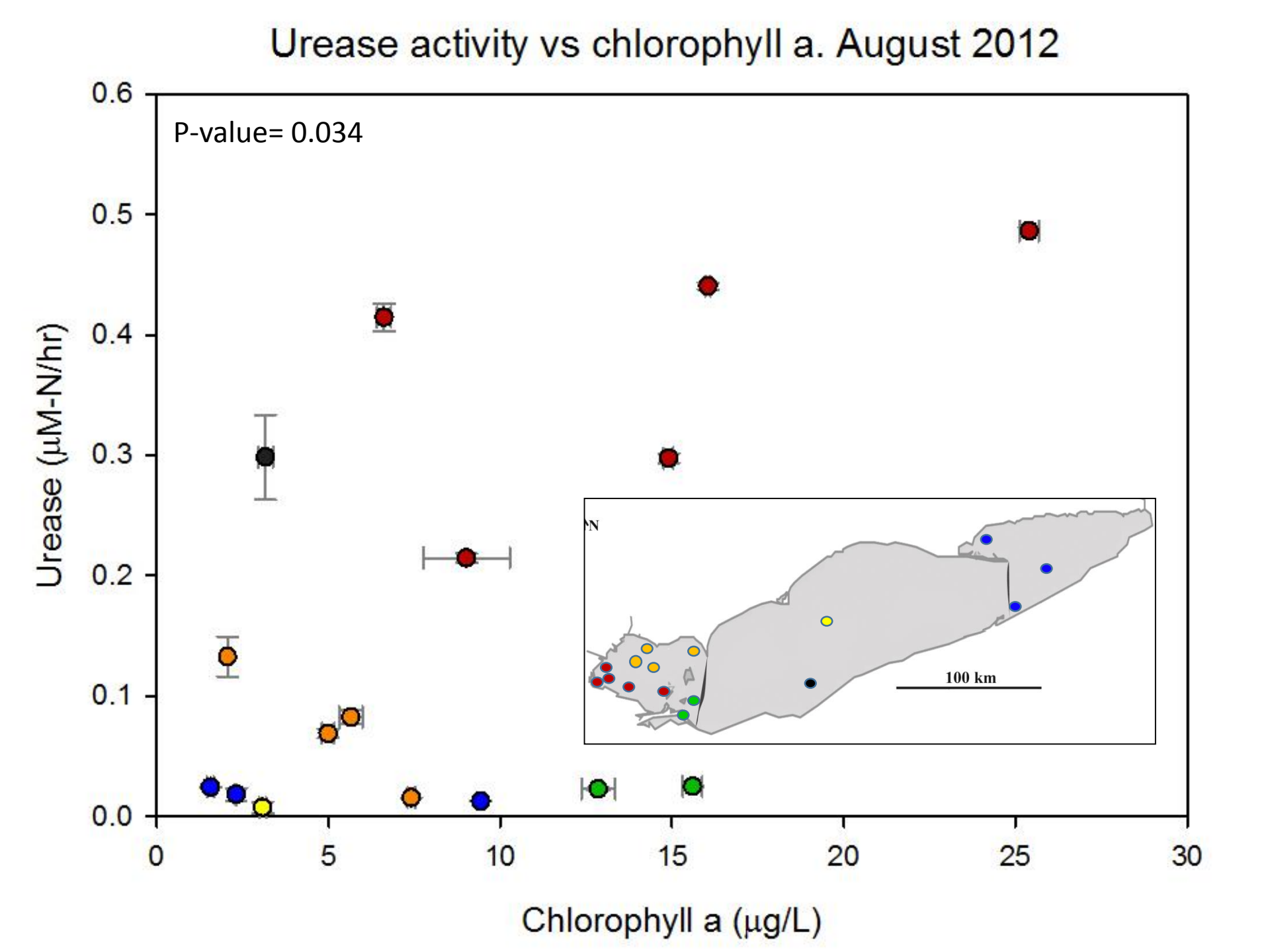
"Our Fertilized World" National Geographic, May 2013

Urea as a Nitrogen Source

- **>50% of Nitrogen in global fertilizer usage (Glibert *et al.*, 2007)**
  - **Use has increase more than 100-fold in the past 4 decades**

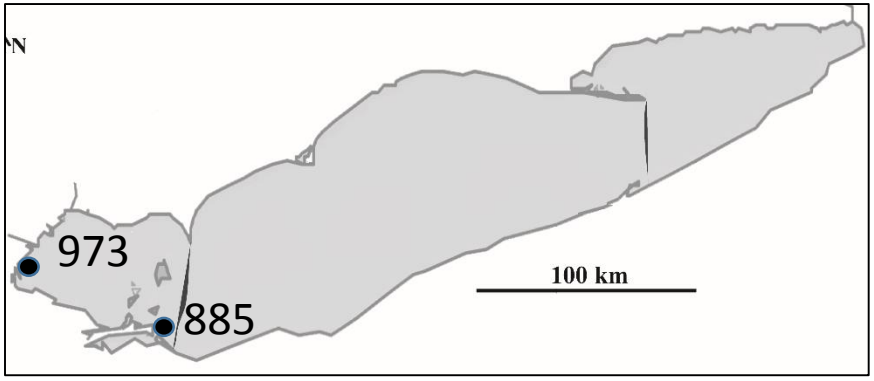


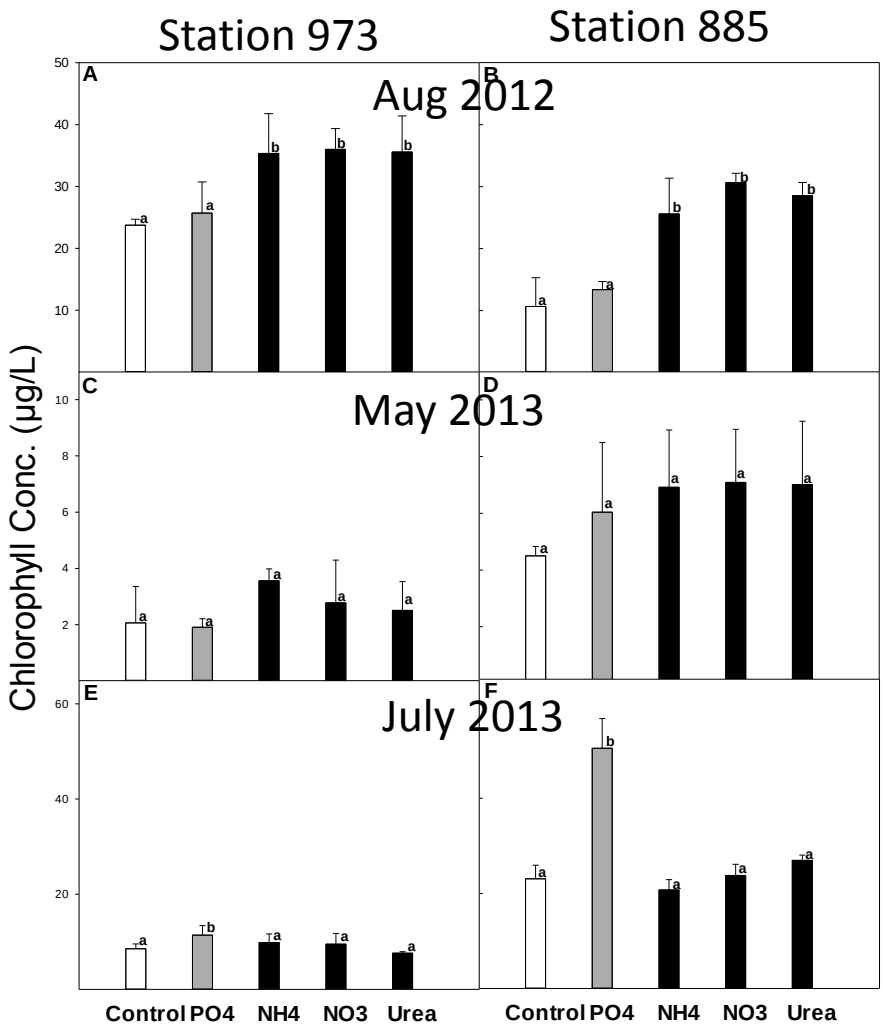
Belisle et al., Aquatic Microbial Ecology, in review


Urease activity vs chlorophyll a. August 2012
P-value= 0.034
Urease (µM-N/hr)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
5
10
15
20
25
30
Chlorophyll a (µg/L)
100 km

Does N really stimulate growth?

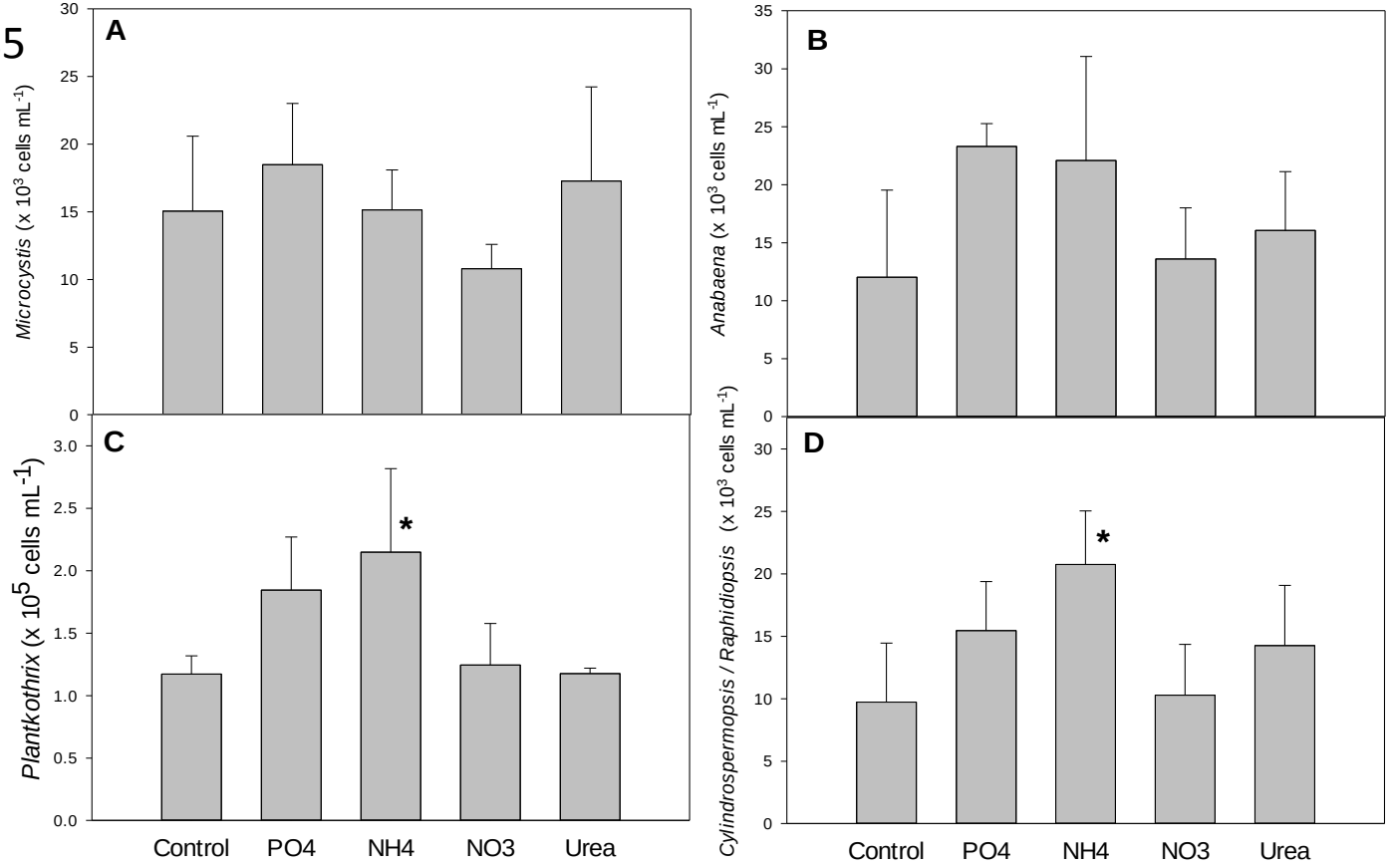
(N – 168 µM; P - 1 µM. *All treatments p < 0.05 relative to controls)*



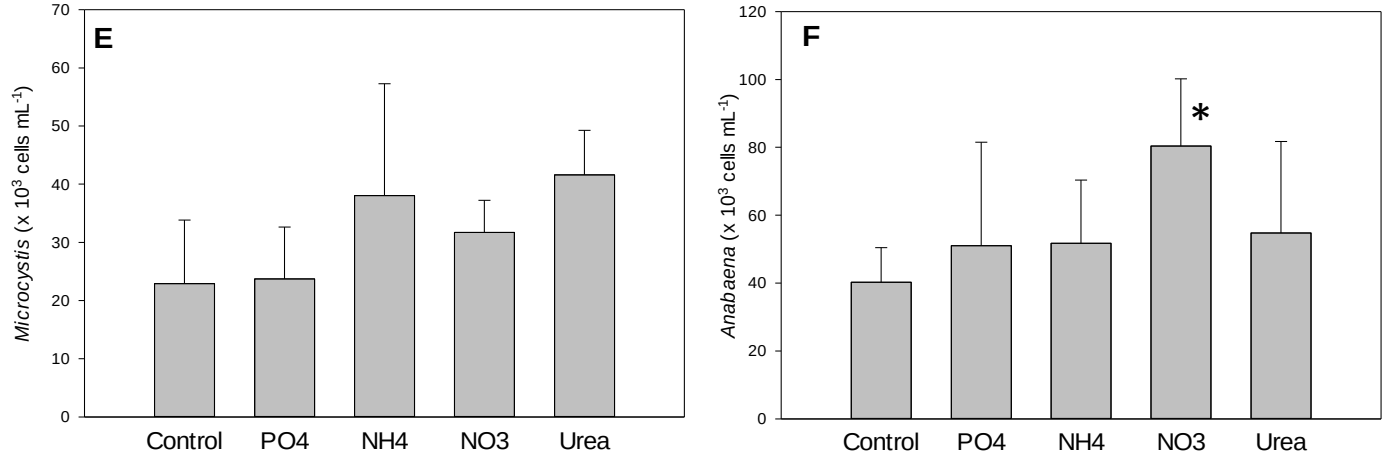


During late summer when we see *Microcystis* blooms, N stimulates production

Station 885

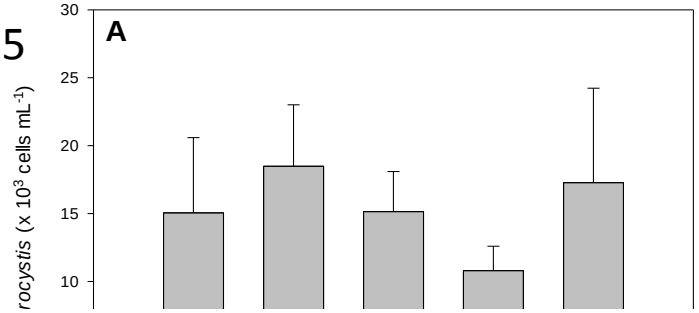


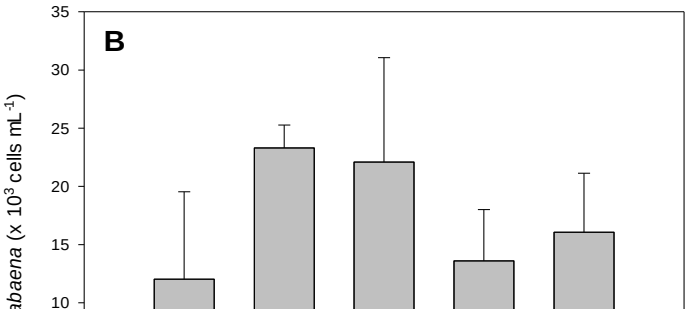
Station 973



AUG2012

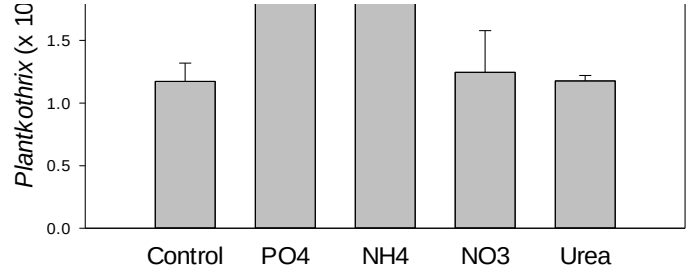
Station 885

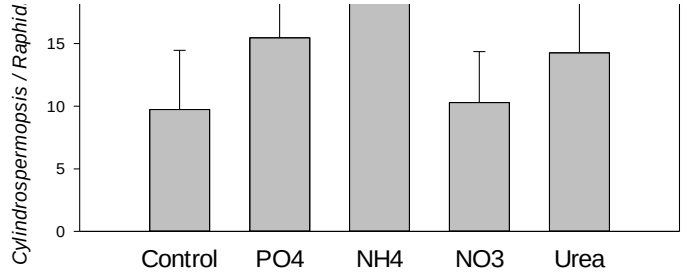




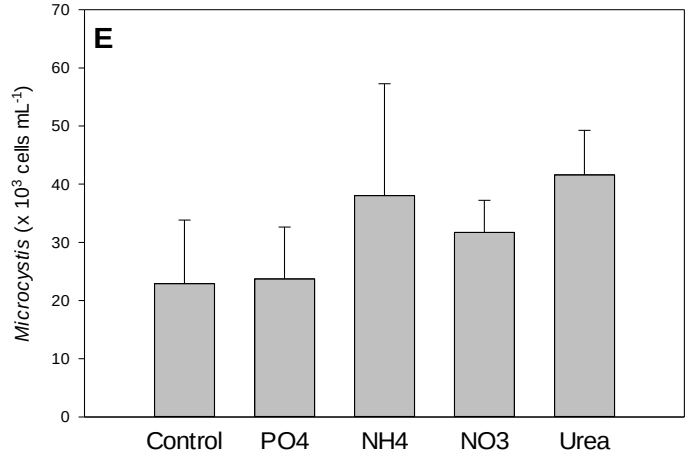
More chlorophyll but <u>not</u> more cells for most species.

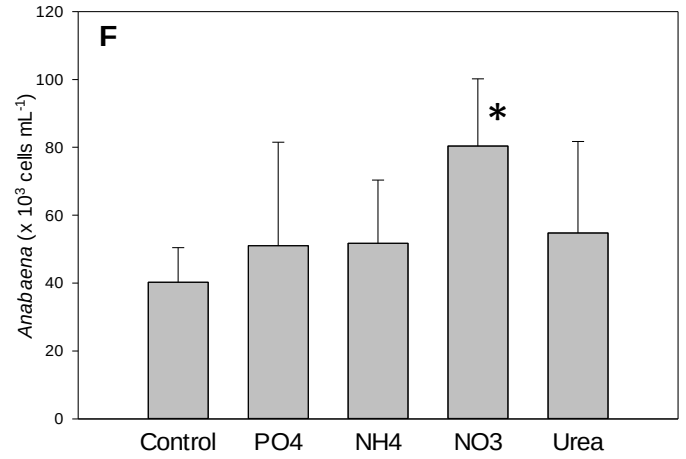
Are cells more robust and growing better?





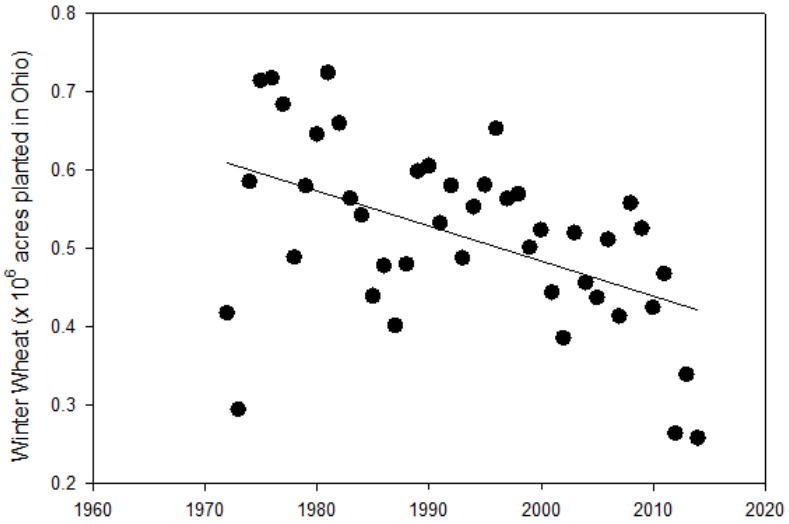
Station 973



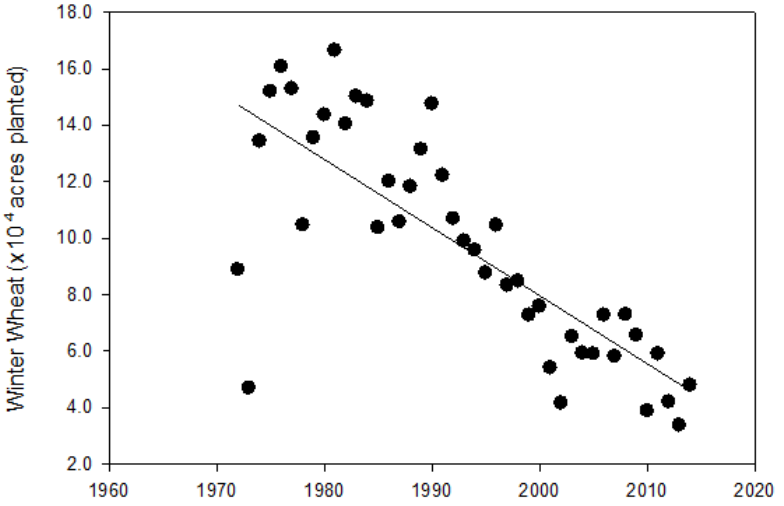


AUG2012

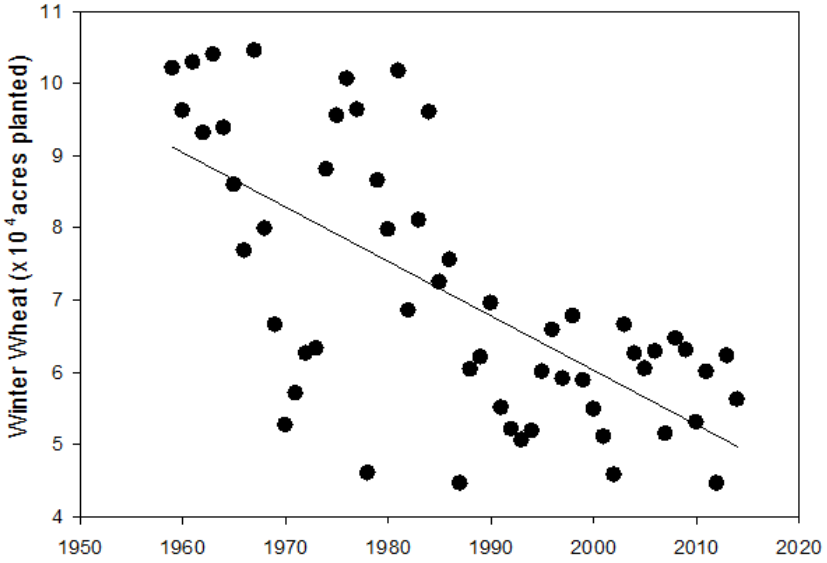
Winter wheat – acres plant in Ohio (Maumee Watershed)



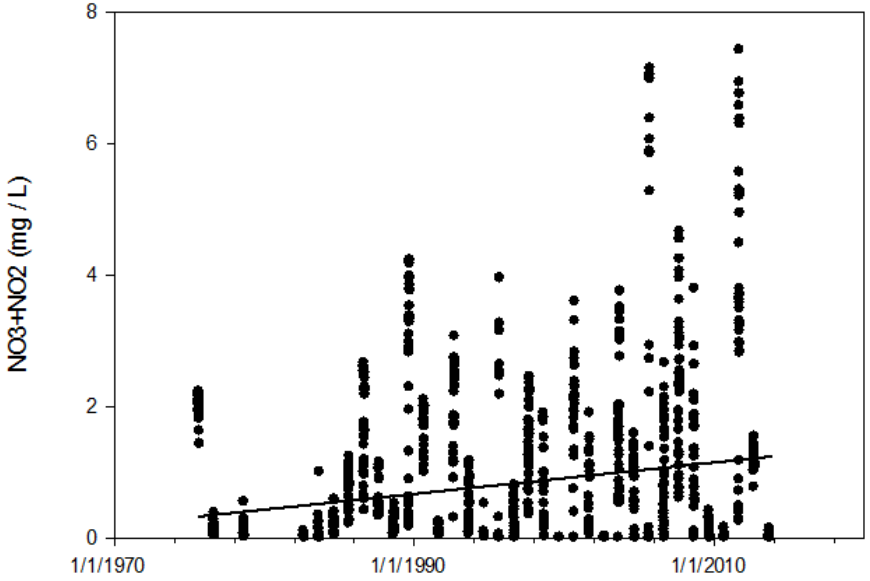
Winter wheat – acres plant in Indiana (Maumee Watershed)



Winter wheat – acres plant in Michigan (Maumee Watershed)



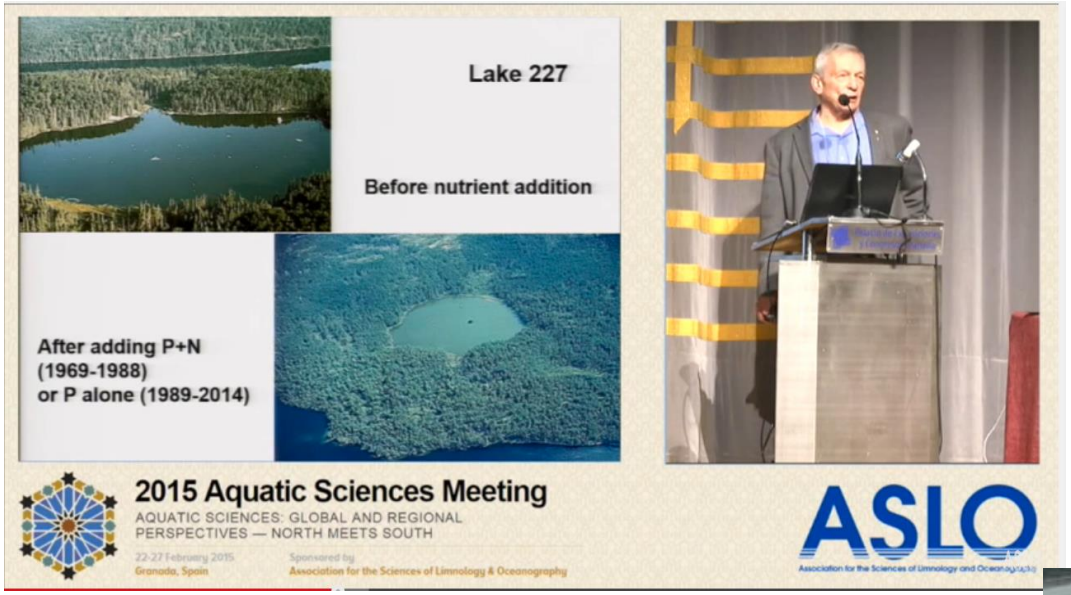
Nitrate plus nitrate flow in Septembers since 1975



Data from ers.usda.org and *National Center for Water Quality*

We are trying to see what cells are doing?

Why not ask the cells???



"The only way you can possible know what is going on in a lake is to manipulate it"

"bottle assays do not tell you about ultimately limiting nutrients, but only proximately limiting nutrients"

Why not ask the cells?

The '*Omics* tool box – "discovery tools"

Genomics

Tools to generate and analyze large of amounts of data are necessary to fully understand cell potential.
Targeted and shotgun approaches

Transcriptomics

Focused on mRNA sequences to begin to understand cell activity (what is the cell trying to do???)
Targeted and shotgun approaches

Proteomics

Like above but offers different perspectives. Much is still unknown.

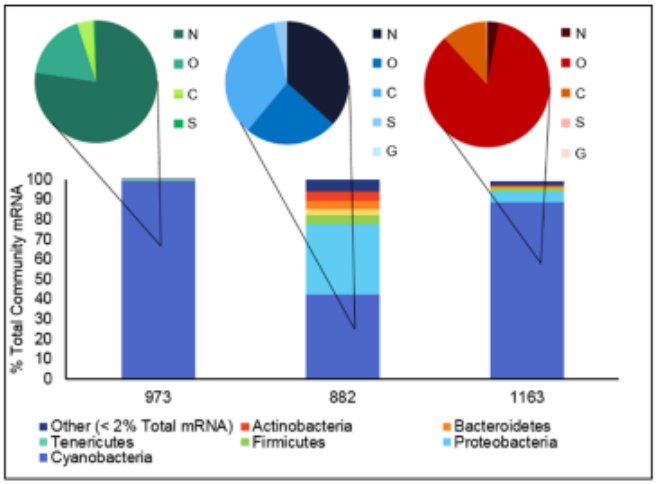
e.g., Escherichia coli:
~4,500 open reading frames (ORFS)
~1,000 genes are essential
~2,500 have assigned function

Metabolomics

The study of small molecules

- Shotgun: all molecules (excellent for discovery)
- Targeted: selective reaction monitoring:
- *E. coli* ~230 of the 500 metabolites can be detected in cell extract (~130 can be quantified)
- Most are conserved between microbial species

Active Bloom-Associated Community

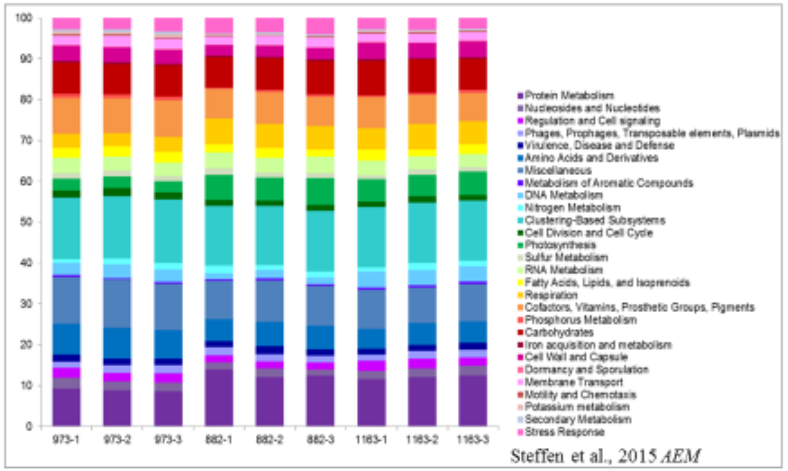


N- Nostocales
O- Oscillatoriales
C- Chroococcales
S- Synechococcales
G- Gloebacteria

Annotation package: MetaPhlAn (Segata et al., 2012)

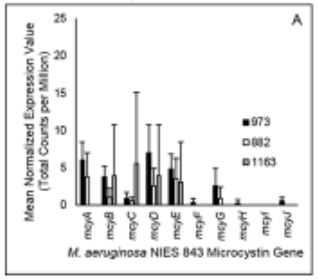
Steffen et al., 2015 *AEM*

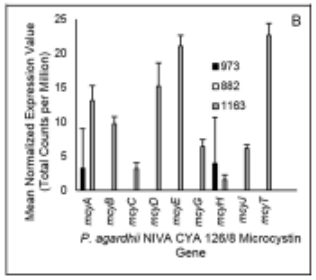
Community Function



Steffen et al., 2015 *AEM*

Microcystin

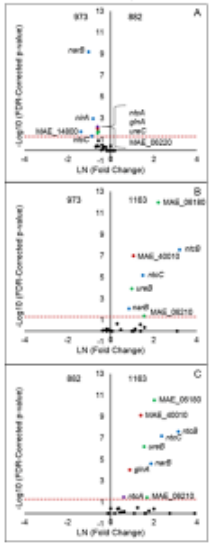




	Station 973	Station 882	Station 1163
Latitude (N)	41°47'30"	41°46'00"	41°28'10"
Longitude (W)	83°19'58"	83°18'30"	82°43'00"
Time of Sample Collection	08/15, 14:45	08/15, 11:25	08/14, 19:10
Surface Temperature (°C)	23.7	23.4	22.8
pH	8.51	8.32	8.06
Microcystin (µg/L)	0.37	0.11	0.61
Anatoxin-a (µg/L)	None detected	None detected	0.77
Cylindrospermopsin (ug/L)	None detected	None detected	None detected
Chlorophyll *a* (µg/L)	25.4 ± 0.3	16.1 ± 0.1	15.6 ± 0.3

Steffen et al., 2015 *AEM*

Comparing N Metabolism



Urea
ureA-G, MAE_06180-MAE_06220

Ammonia
glnA, glnN, MAE_12590, MAE_17690, MAE_40010, MAE_40020

Nitrate/Nitrite
narB, nirA, ntcB-D, MAE_14800

ntcA

Steffen et al., 2015 *AEM*

Conclusions (2012 bloom metatranscriptomics)

- Cyanobacterial bloom community function is conserved in spite of variable community structure
- The activities of different members of the community (*Microcystis, Anabaena, Planktothrix*) can be readily distinguished
- In August 2012, *Microcystis* in the western basin of Lake Erie were
  - Actively scavenging nitrogen
  - Actively scavenging phosphorus
  - Actively scavenging carbon dioxide
  - Actively fighting off an active virus infection

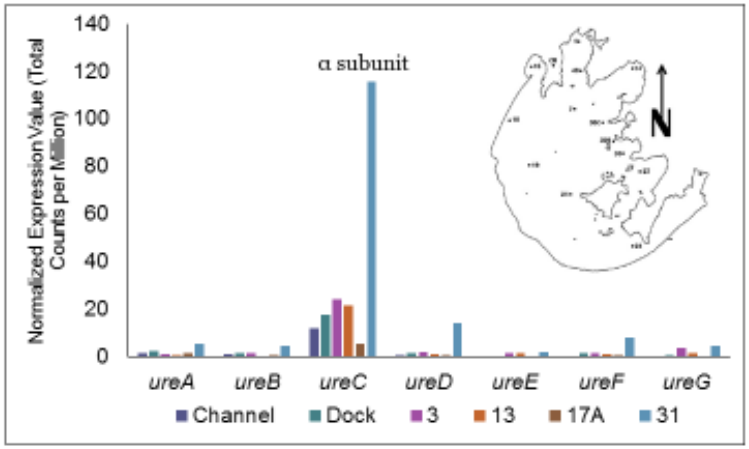
These are for just three sites. Data from 2013 and 2014 for > 60 sites in Taihu and Lake Erie currently being processed

Validation required!!

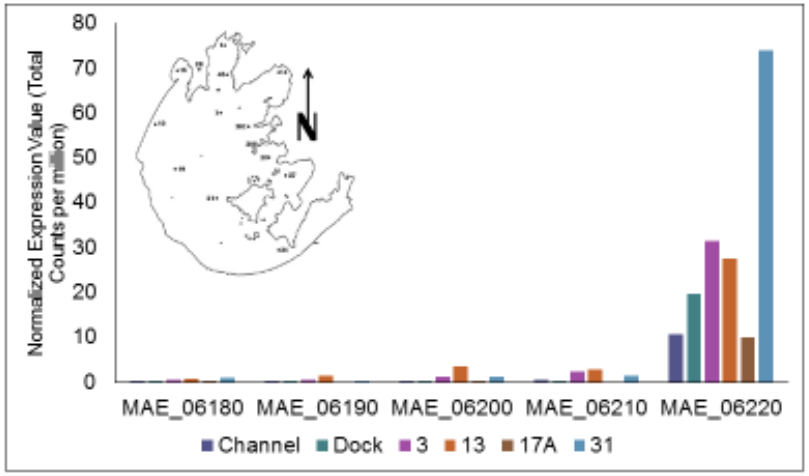
For Taihu in 2010 the UreC protein was massively upregulated

(Dill, Wilhelm and others unpublished).

Taihu 2013: Urease Genes



Taihu 2013: Urea Transport



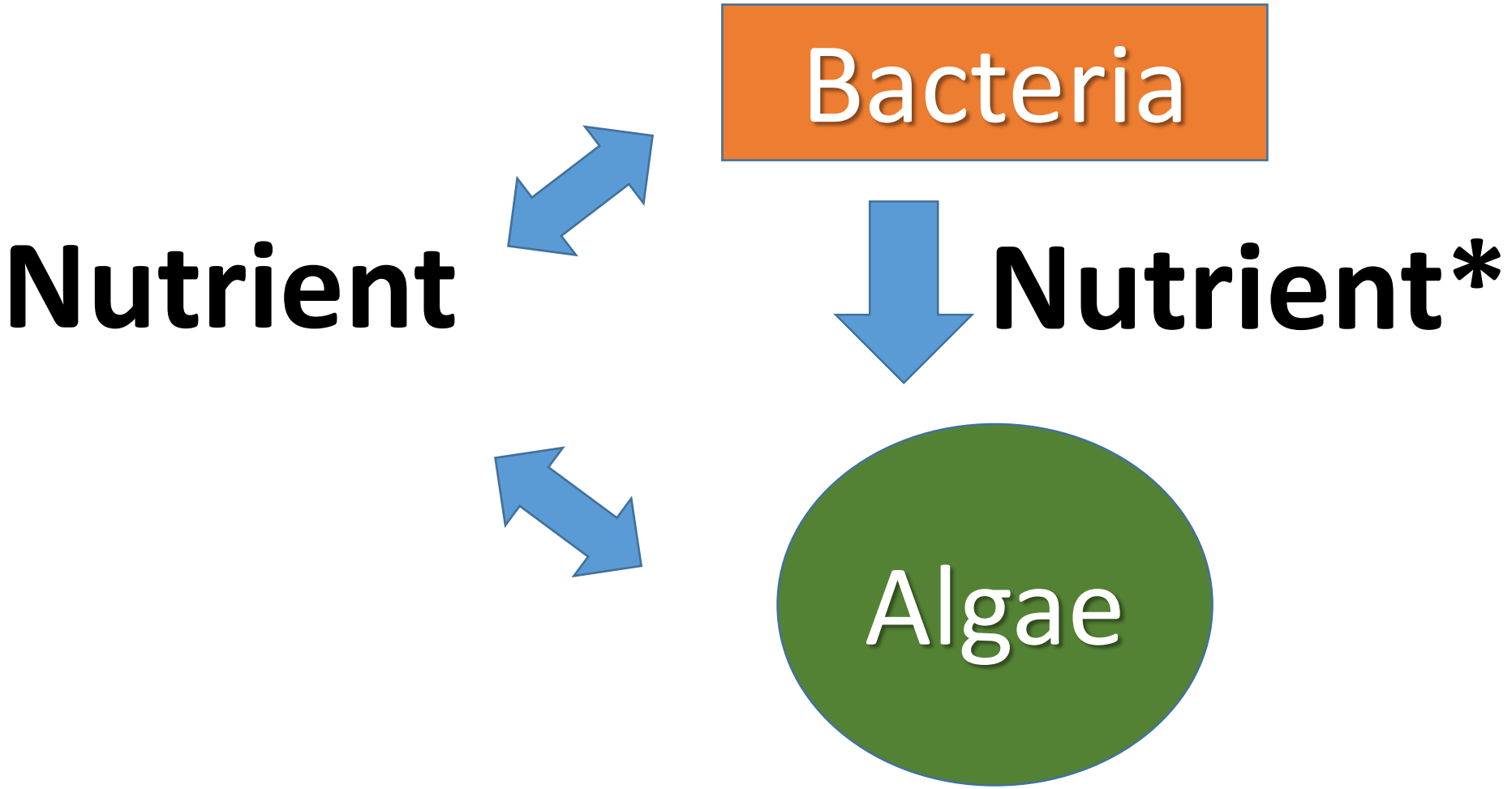
Taihu 2013

Currently quantifying transcripts and screening targeted libraries from Lake Erie to determine who is processing the urea.

So what are our grand challenges

(as one person sees them)…..

What is the roll of the "microbial community" ?



All nutrients are not created equal…..

"The data support the hypotheses that increases in PO_4-P would influence planktonic biomass, alter size class compositions and influence the abundance of bacterioplankton. The results also suggest that the phytoplankton community structure (based on size-class distributions) is strongly influenced by the biological availability (and not total concentration) of nitrogen in this system."
Wilhelm et al. Aquatic Microbial Ecology 2003

HOW MUCH NUTRIENT CHEMISTRY MATTER?

What are the appropriate scales??

What are the appropriate scales??

How do we integrate across these scales?

What are the appropriate scales??

What are the appropriate scales??

THE UNIVERSITY OF TENNESSEE KNOXVILLE

DEPARTMENT OF MICROBIOLOGY

Morgan Steffen
Matthew Saxton
Johanna Rinta-Kanto
Shafer Belisle
Brian Dill
Andy Ouellette
Lauren Krausfeldt
Robbie Martin

Gary LeCleir
Jennifer DeBruyn
Shawn Campagna
Stephen Dearth

Hans Paerl
Ferdi Hellweger
Wayne Gardner
Mark McCarthy
Silvia Newell

Boqiang Qin
Guangwei Zhu
Guang Gao
Xiangming Tang
Hai Xu
Mengyuan Zhu

Greg Boyer
Sue Watson
Rick Bourbonniere
Mike McKay
George Bullerjahn
Chris Gobler
Tim Davis

and many others…