Microcystin Research in Southern Ontario: Some Recent Results

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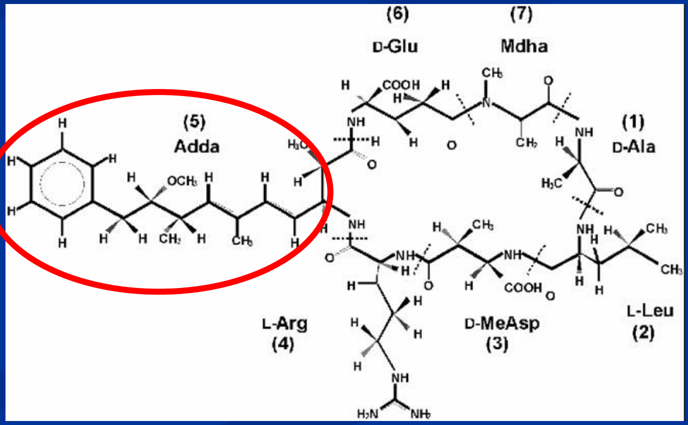
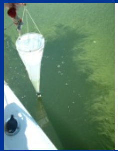
What is Microcystin?

- Toxin produced by selected genera of Cyanobacteria (ex.: *Microcystis*, *Anabaena*)
  - Molecule has >70 variants
  - Variants have different toxicity

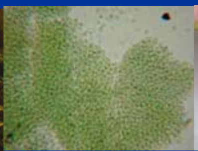
- “Adda” amino acid responsible for most toxicity by binding protein phosphatase enzyme and preventing its normal activity (Falconer 2005)

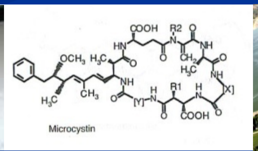








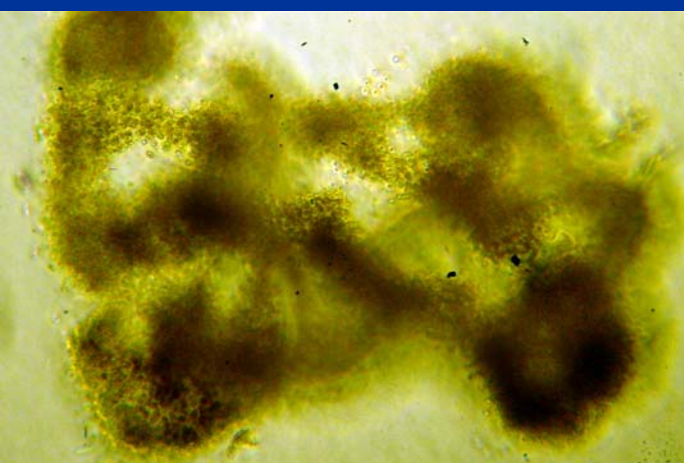


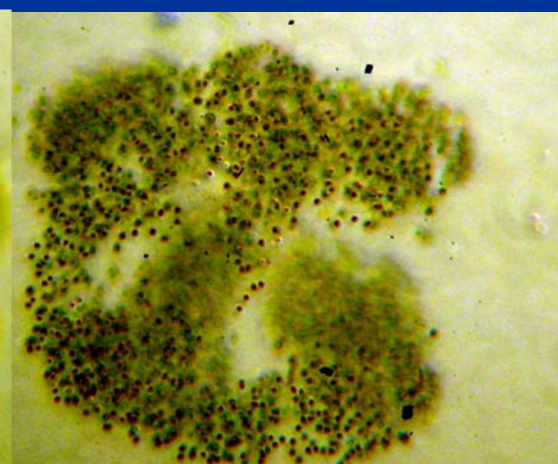


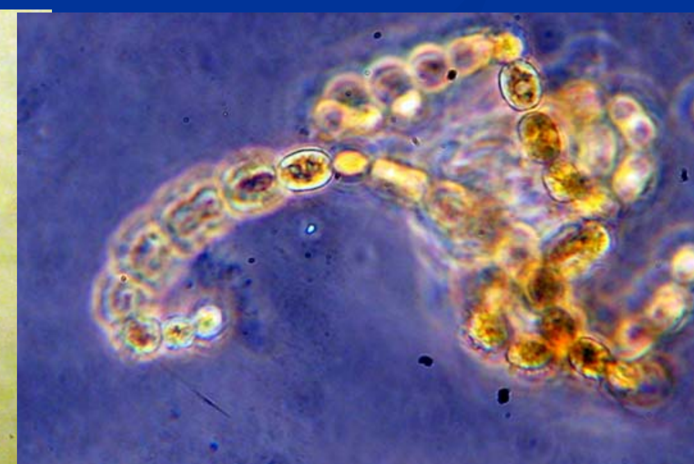
Effects of Microcystin

Protein phosphatase inhibition

Bioaccumulation







Bay of Quinte July 06

M.ichthyoblabe *M. aeruginosa* *Anabaena lemmermanii*

Production of Microcystin

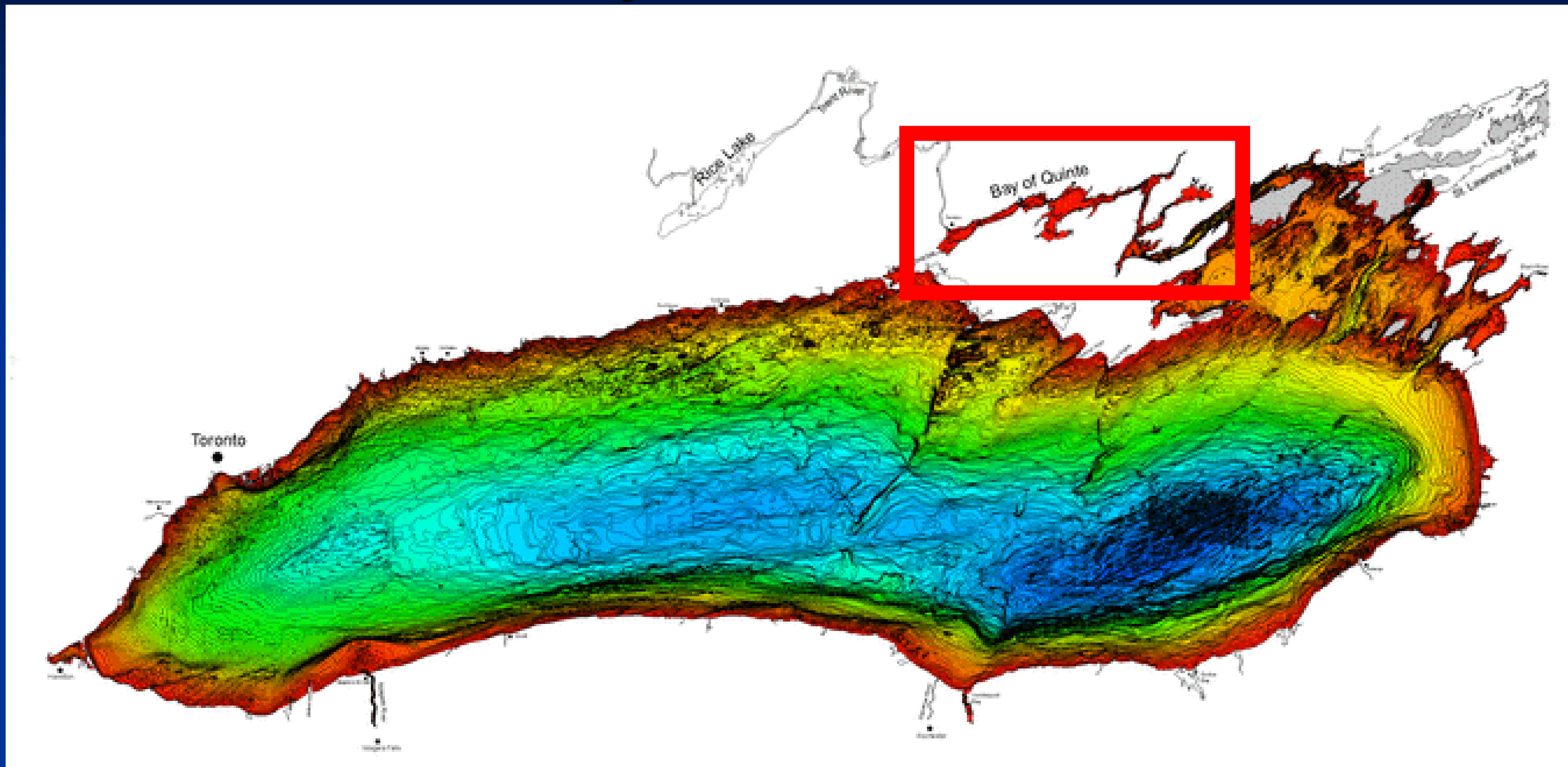
- Mcy genes responsible for production
- Variable expression and abundance of toxic strains affect microcystin levels
- Production hypothesized to be greatest under ideal growth conditions

Environmental conditions associated with microcystin production

- High phosphorus
- Low N:P
- Warm water
- Stable water column
- Presence of dreissenid mussels

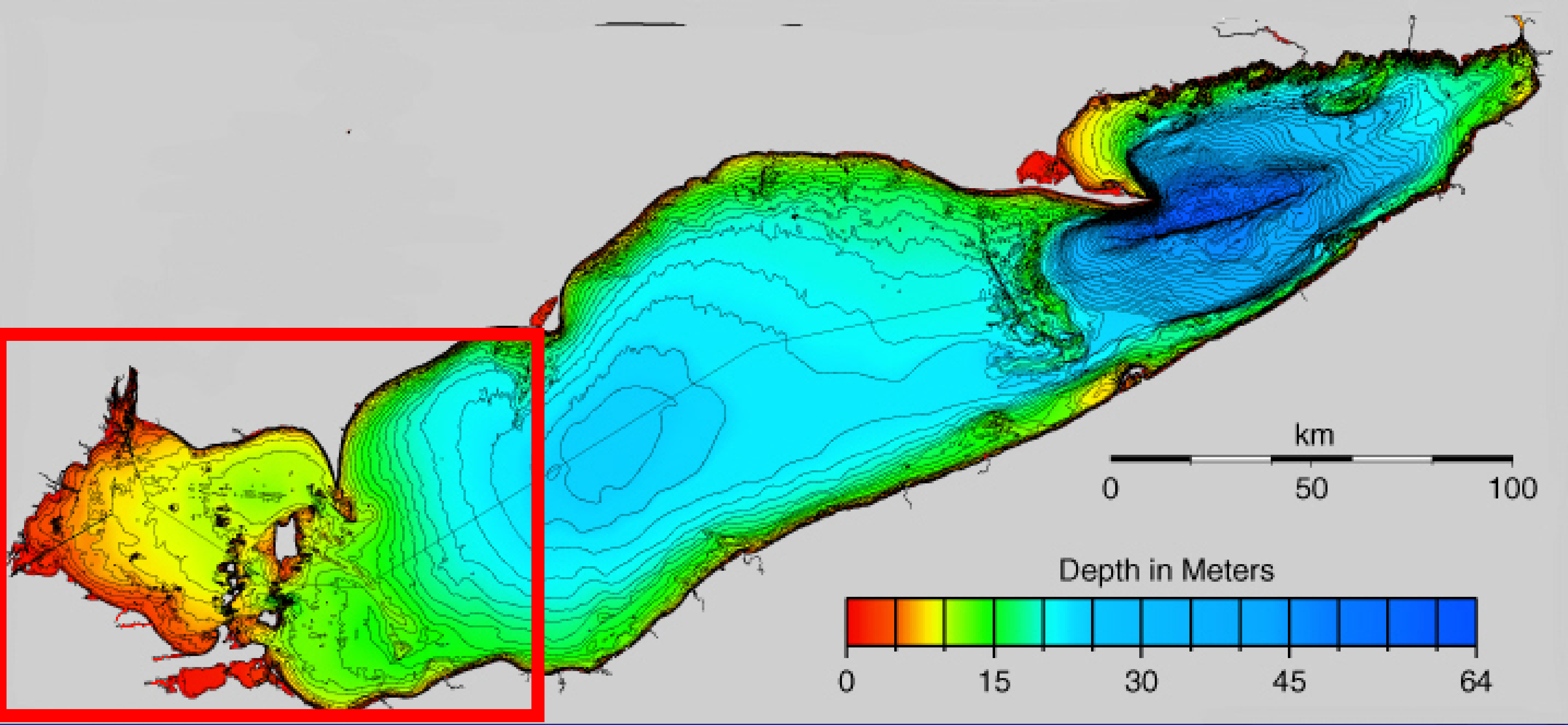
Study Sites

Bay of Quinte



Meso to eutrophic, warm and often stratified in summer, dreissenids, several water intakes for small towns and city of Belleville, important recreational fishery

Maumee Bay

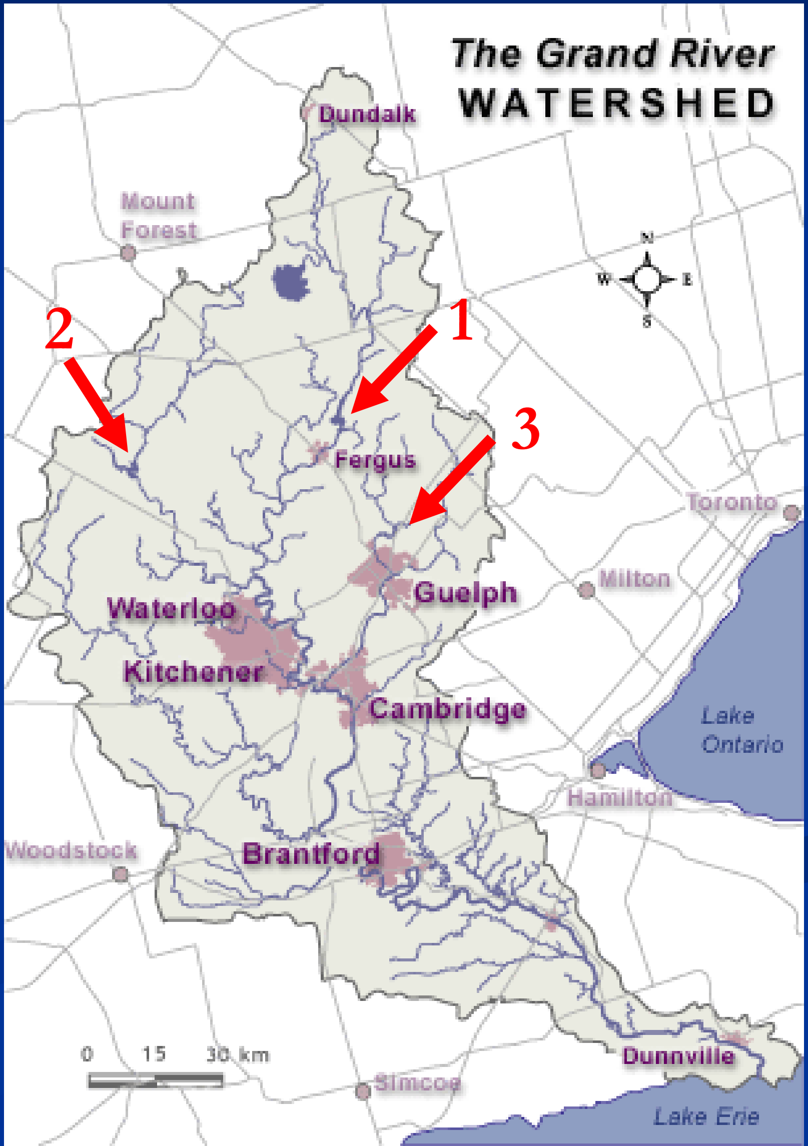


Western end of Lake Erie, meso-eutrophic, high population, warm, some stratification in summer, dreissenids, recreational and commercial fishery

Grand River Conservation Area Reservoirs

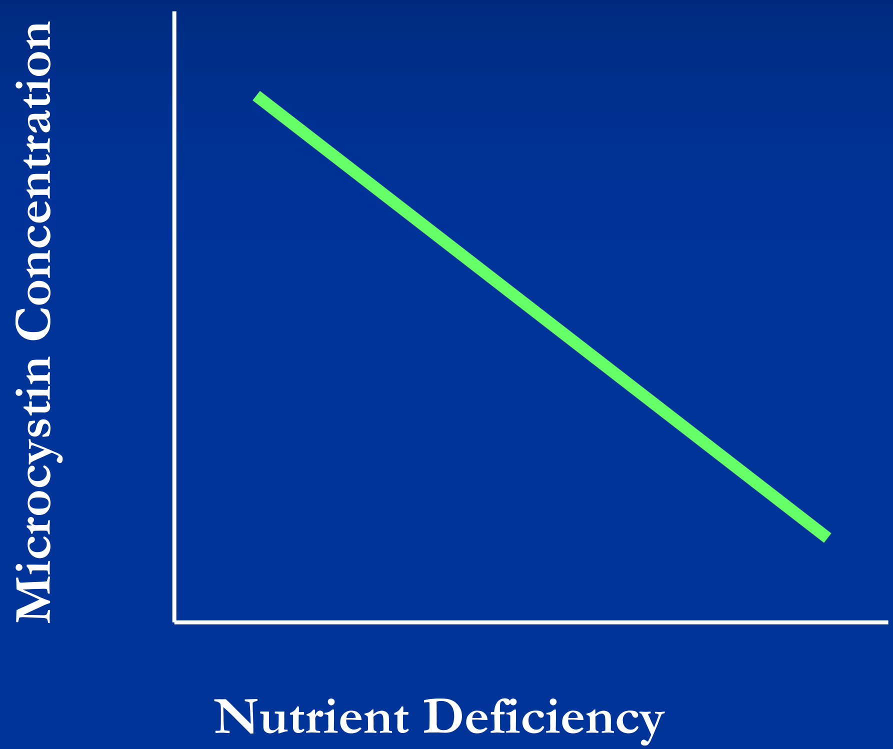
- 1. Belwood Lake
  - Grand River dammed 1942
- 2. Conestogo Lake
  - Conestogo River dammed 1958
- 3. Guelph Lake
  - Speed River dammed 1975

Mesotrophic, flood control, recreation



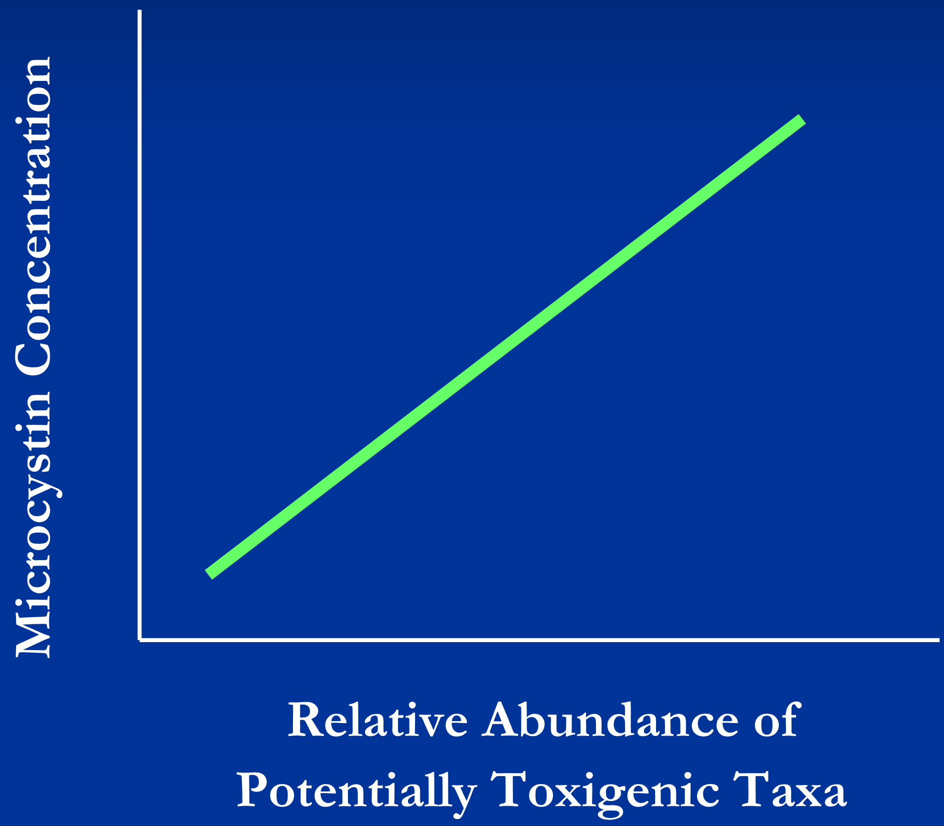

Belwood
Reservoir

Nutrient Status Hypothesis



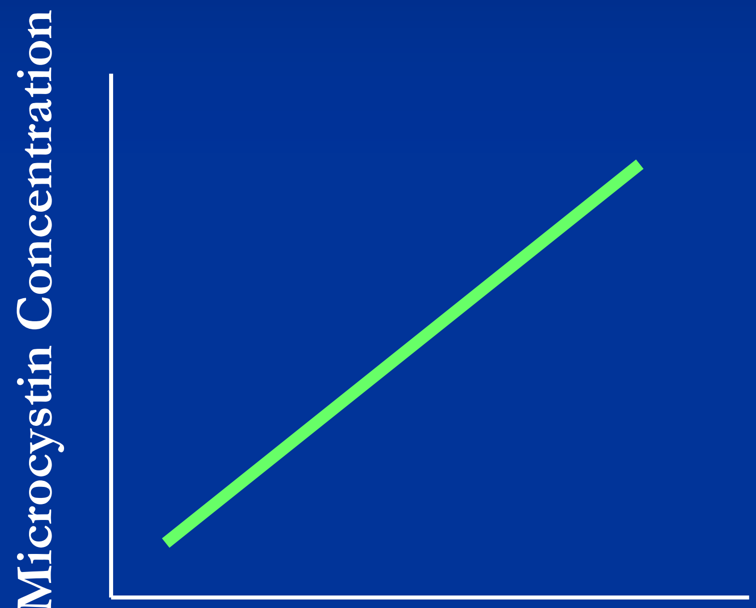
- Cells in better physiological condition will produce more toxin.

Toxic Biomass Hypothesis

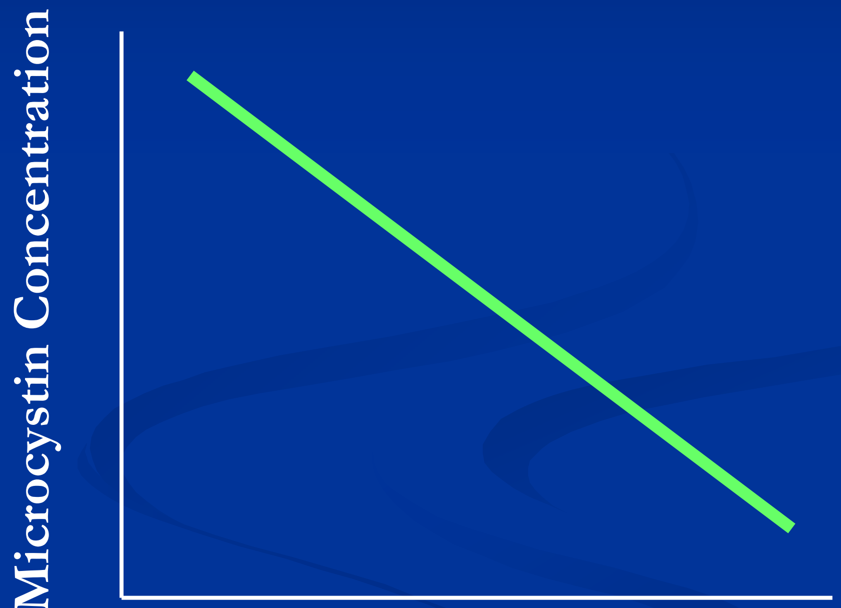


- More microcystin will occur when there are more potential toxin producers.

Indirect Effects Hypotheses



1) Water column stability
2) Soluble Reactive P
3) Total P
4) Dreissenid Presence



1) TN:TP
2) Water Transparency

Methods

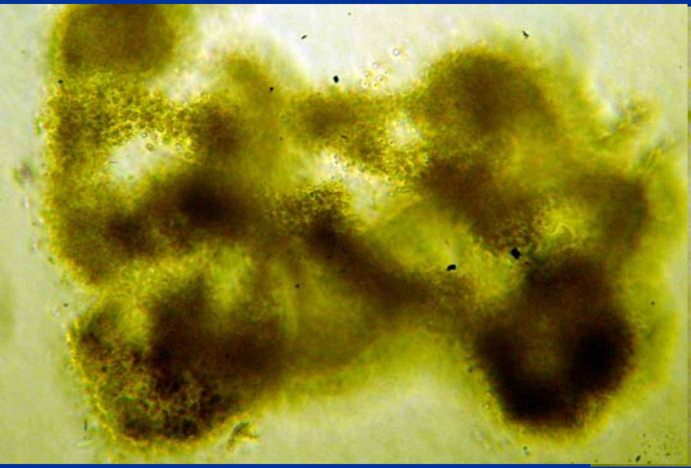
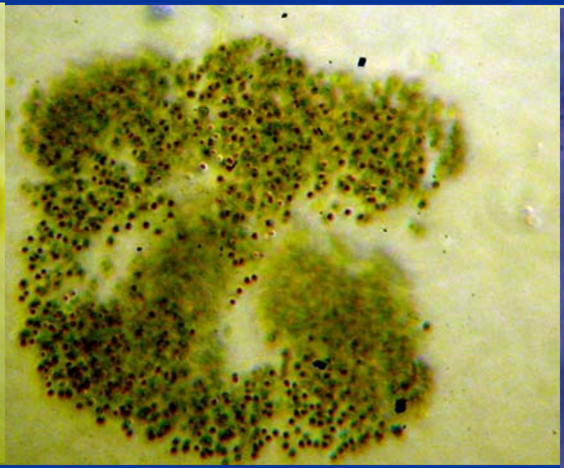
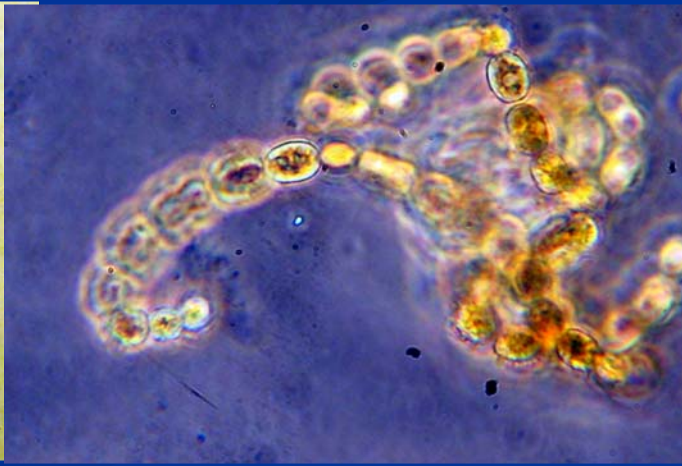
- Typical limnological measurements
  - Light
  - Chemistry
  - Temperature
  - Chlorophyll
- Fluoroprobe
- Variable Fluorescence
- Microcystin


Waterloo
3-128
Biology


GAST
PALL Gelman Laboratory

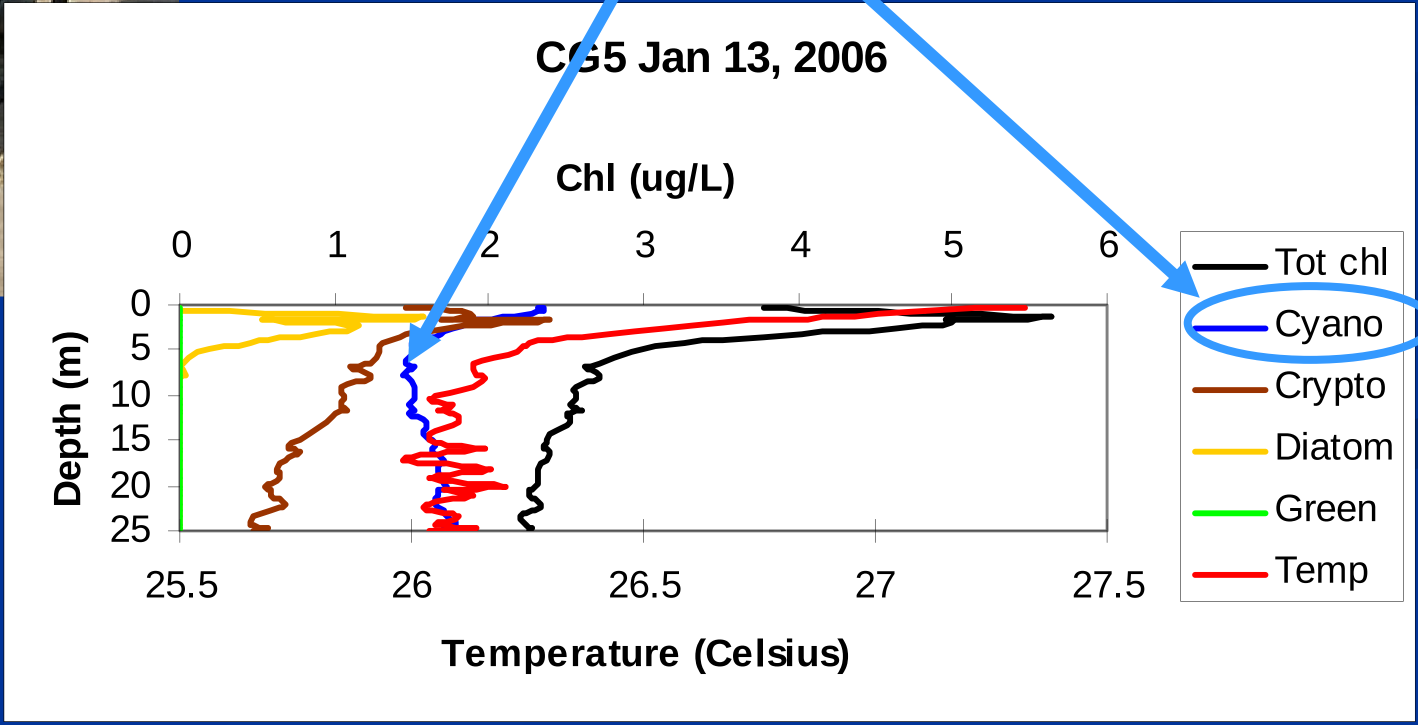

Microcystin

- Protein Phosphatase Inhibition assay
- Quantitative polymerase chain reaction (qPCR) mcy e

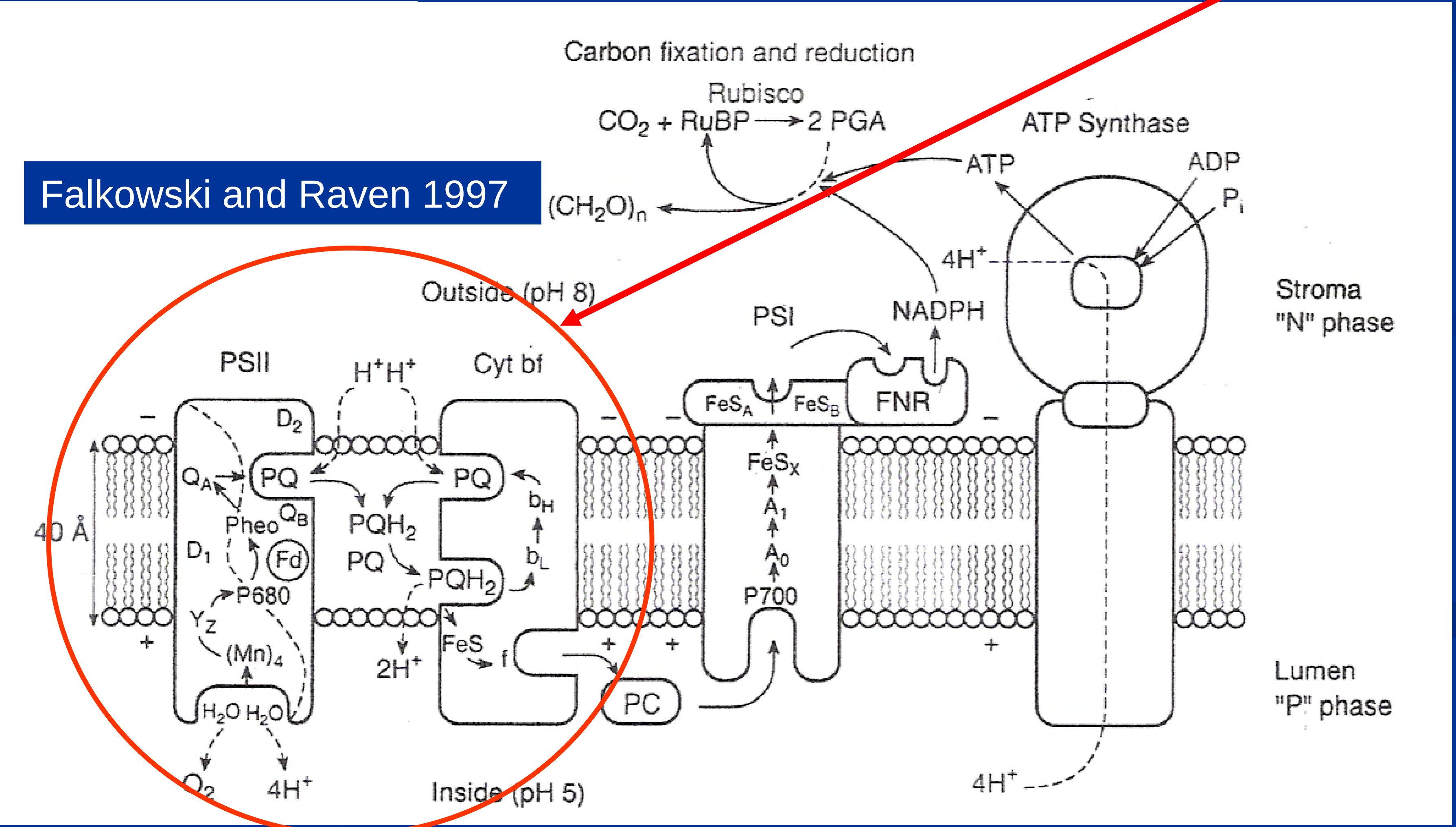




FluoroProbe

Information about phytoplankton groups based on photosynthetic pigments

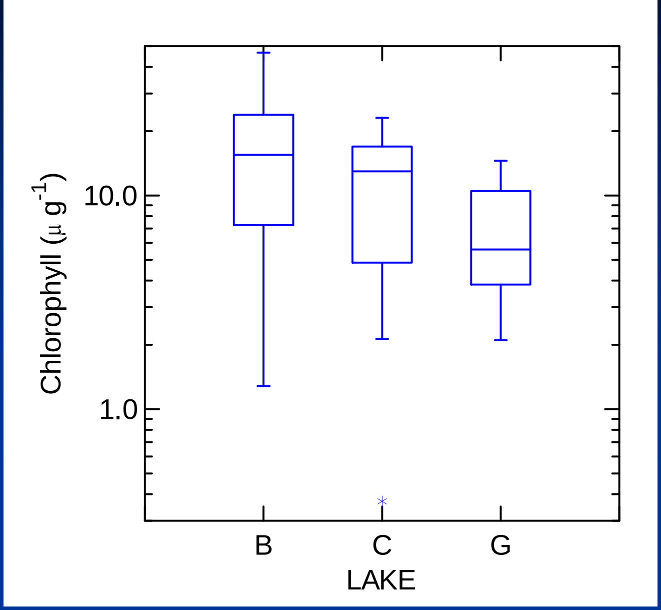


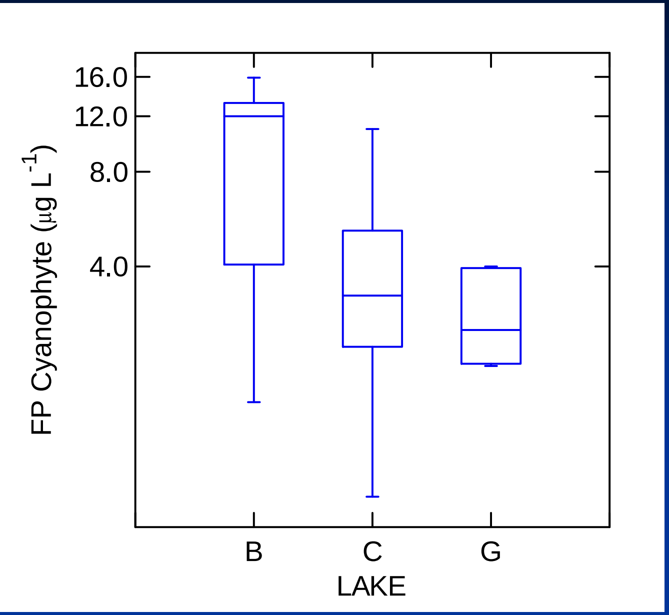
PAM fluorometer Variable fluorescence (Fv/Fm) information about phytoplankton physiological status based on yield of chlorophyll fluorescence when electron transport is temporarily blocked.

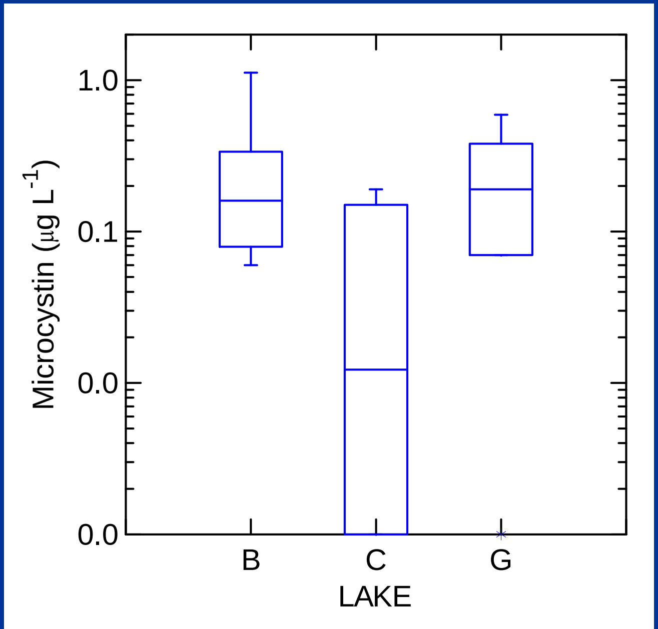


Falkowski and Raven 1997

GRCA Reservoirs

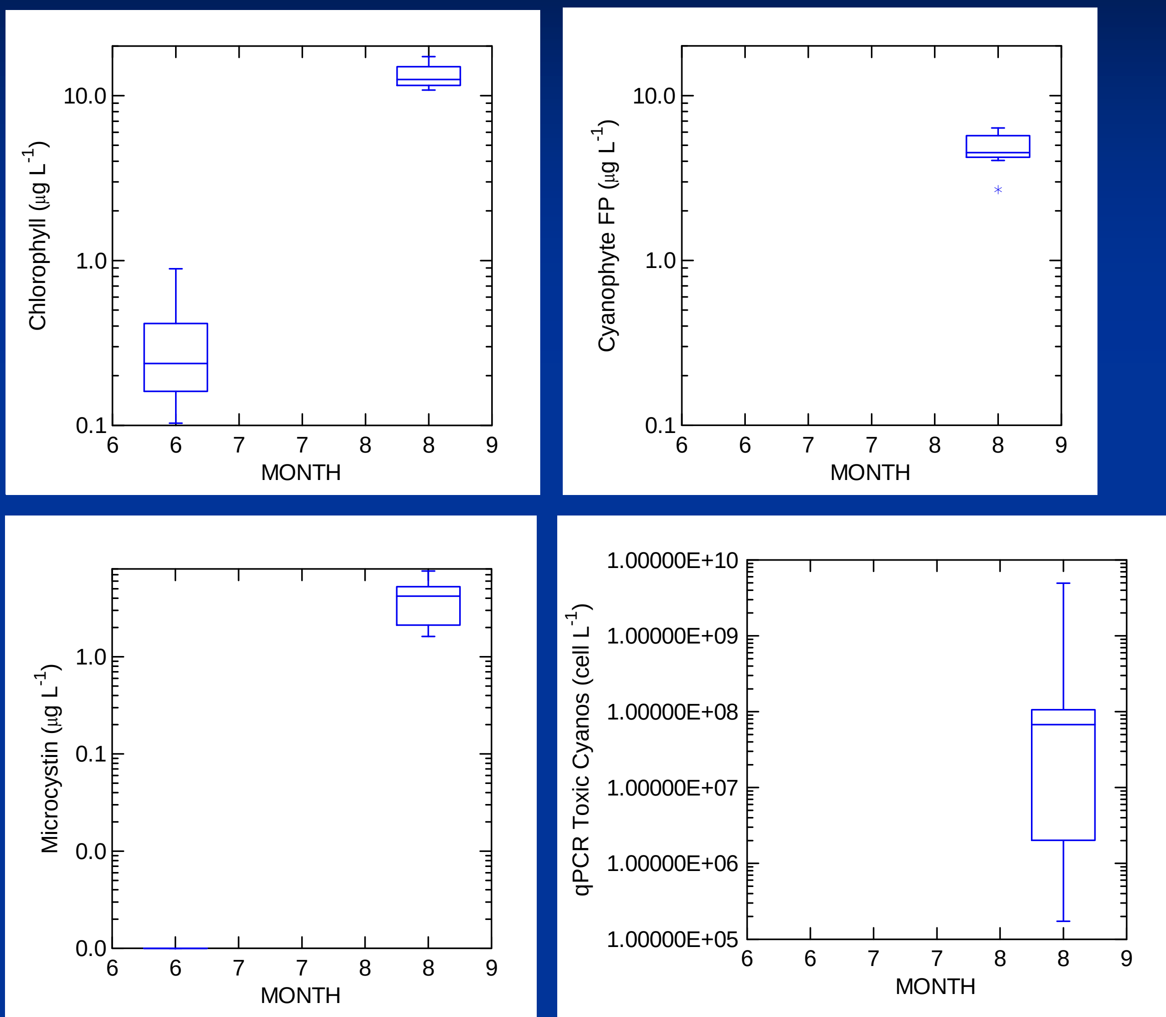






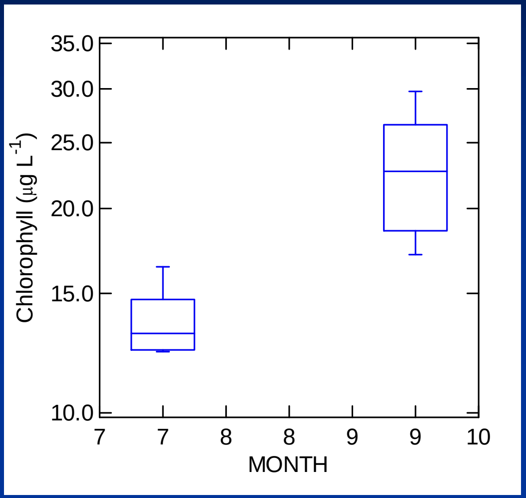
- Microcystin concentration low
- Microcystin not correlated to Cyanobacteria or total chlorophyll
- Species not toxin producers?

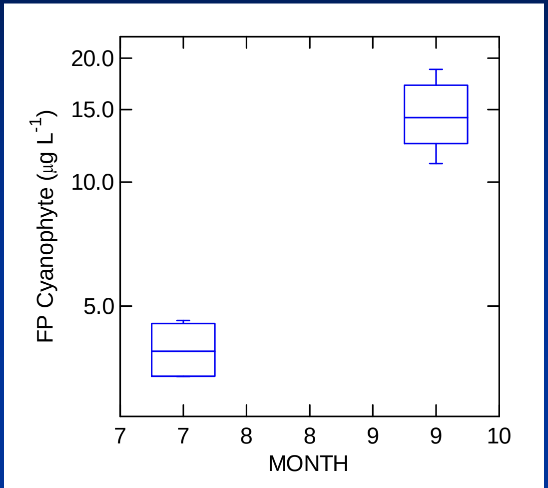
Maumee Bay Lake Erie

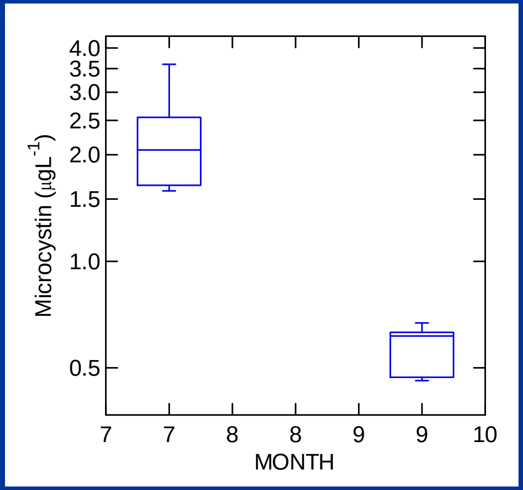


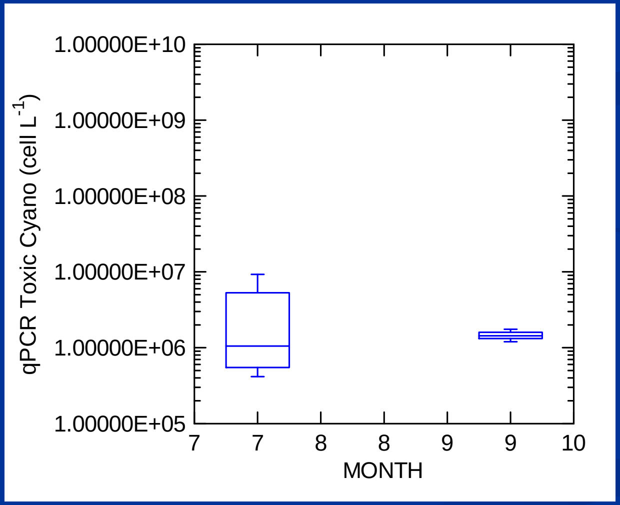
- Clear water in June
- Cyanobacteria in August
- Microcystin above WHO in August
- qPCR indicates mcy gene detected

Bay of Quinte Lake Ontario



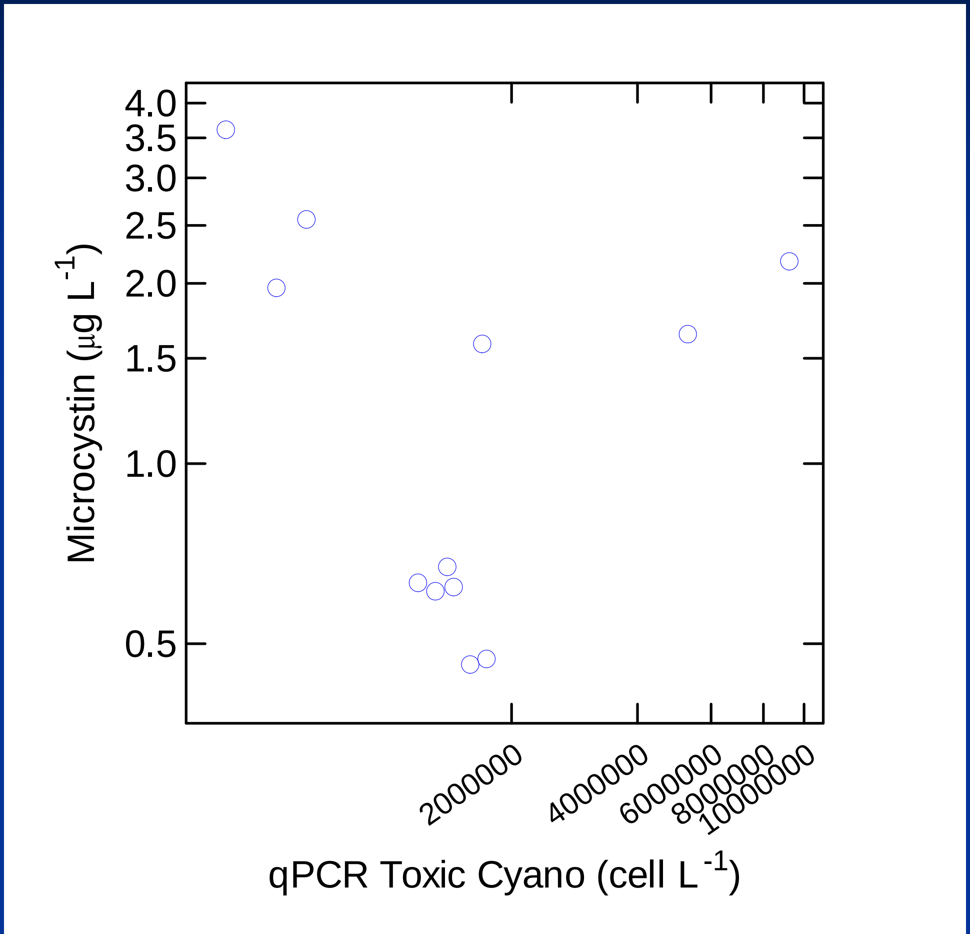






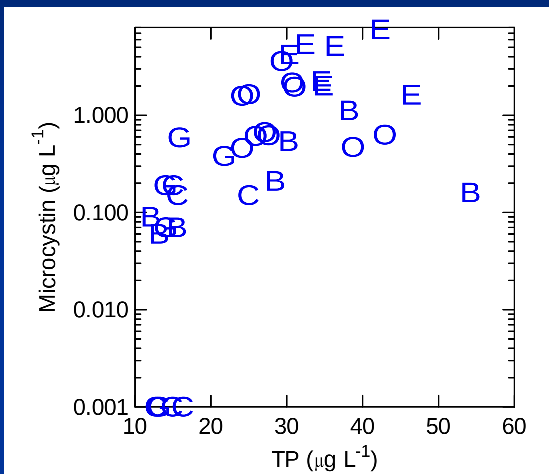
- Microcystin not correlated to chlorophyll or Cyanobacteria
- Microcystin high in July lower in Aug
- qPCR detected mcy gene at same concentration in both months

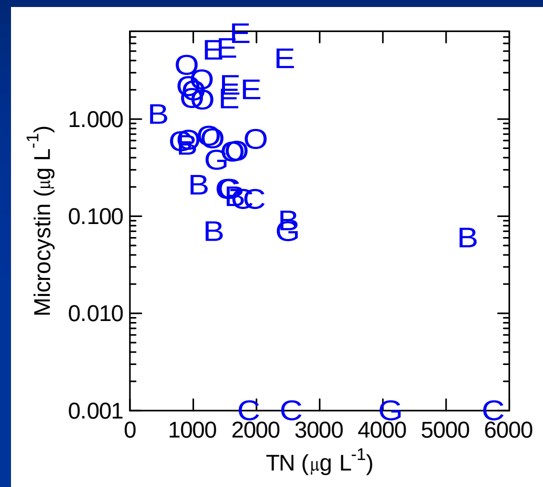
qPCR mcy gene vs microcystin

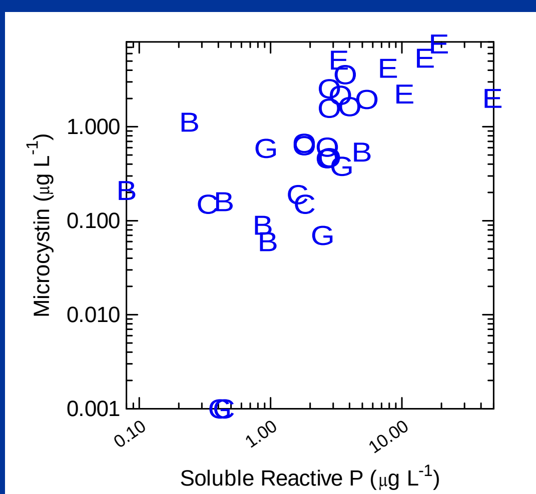


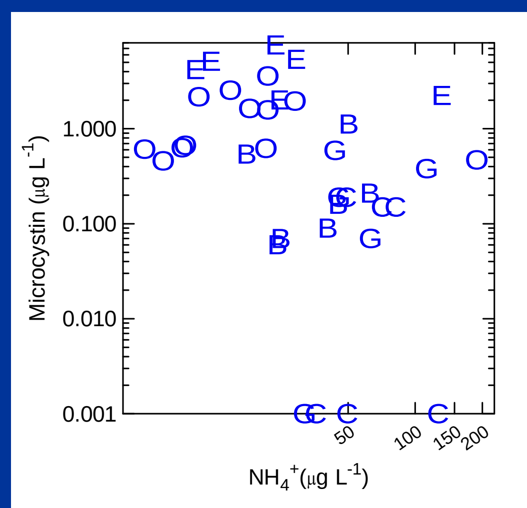
- No correlation between mcy e gene and microcystin production
- Presence of gene does not always mean it is expressed

Microcystin and environmental variables



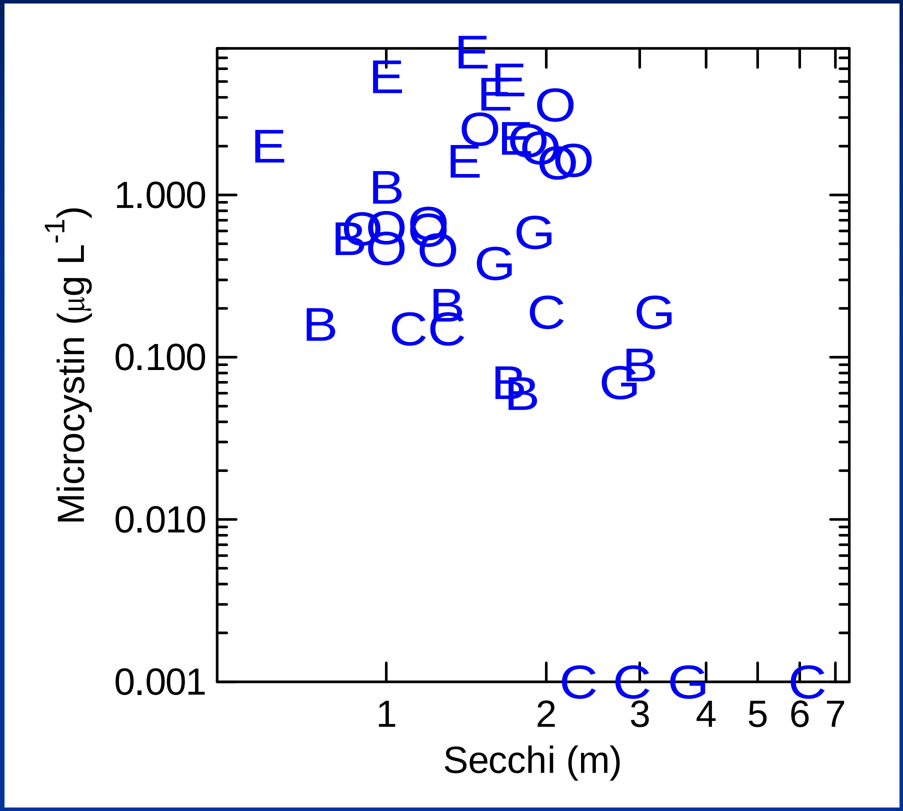




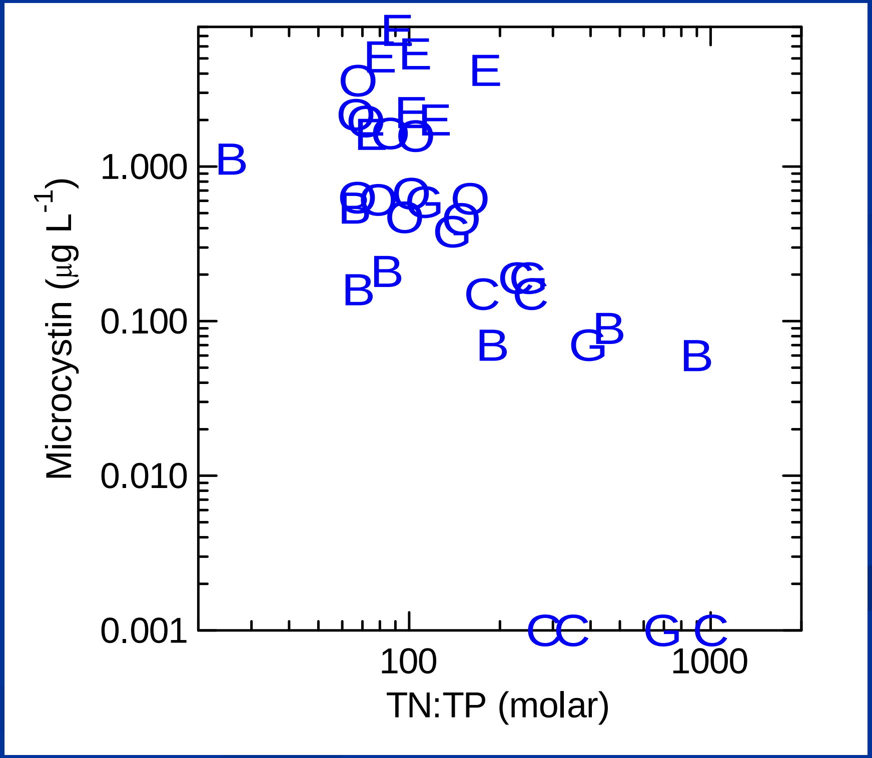


- Microcystin is positively correlated to TP and SRP
- Microcystin is slightly negatively corelated to TN and NH_4^+

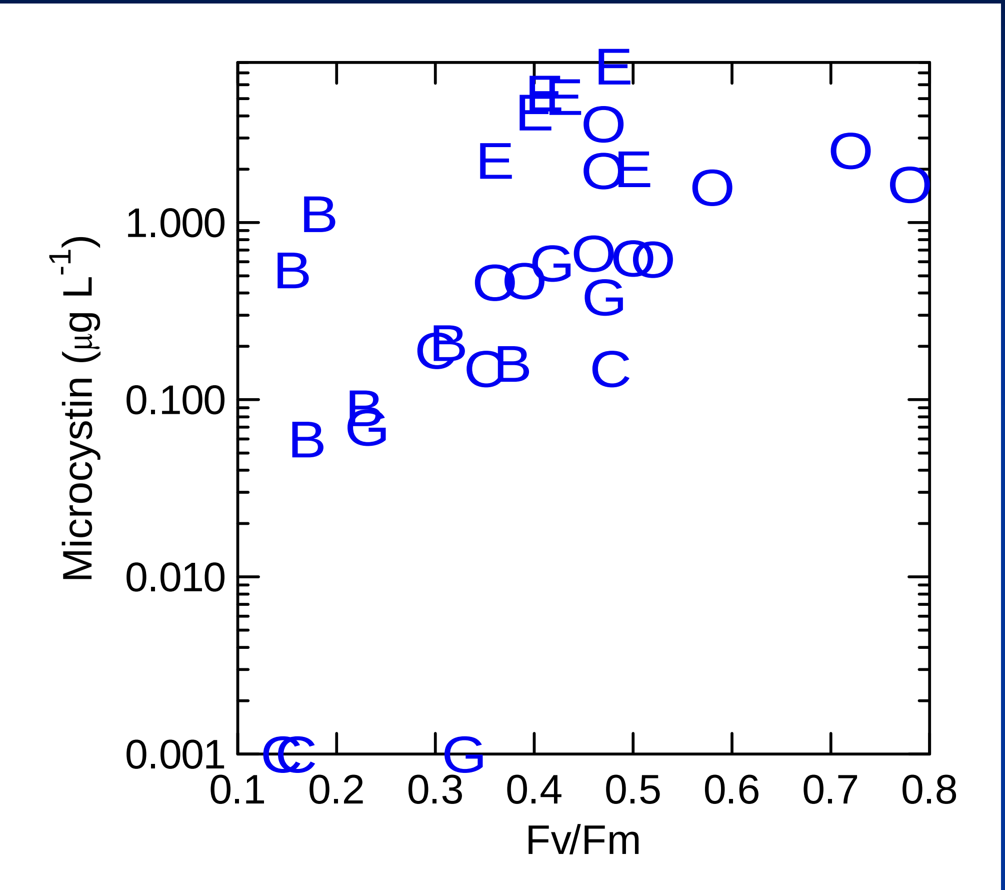
Microcystin and environmental variables



- Microcystin highest at low light and low TN:TP



Microcystin and Physiological condition



- Microcystin concentrations were highest when Fv/Fm indicated cells were in better physiological condition

These data suggest the production of microcystin is linked to favourable growth conditions for toxic species

Summary

- Microcystin is positively correlated to TP
- Microcystin is negatively correlated to TN:TP
- Microcystin is generally correlated to Cyanobacteria biomass
- However even when present the gene for microcystin is not always expressed
- Growth rate may be important to microcystin production

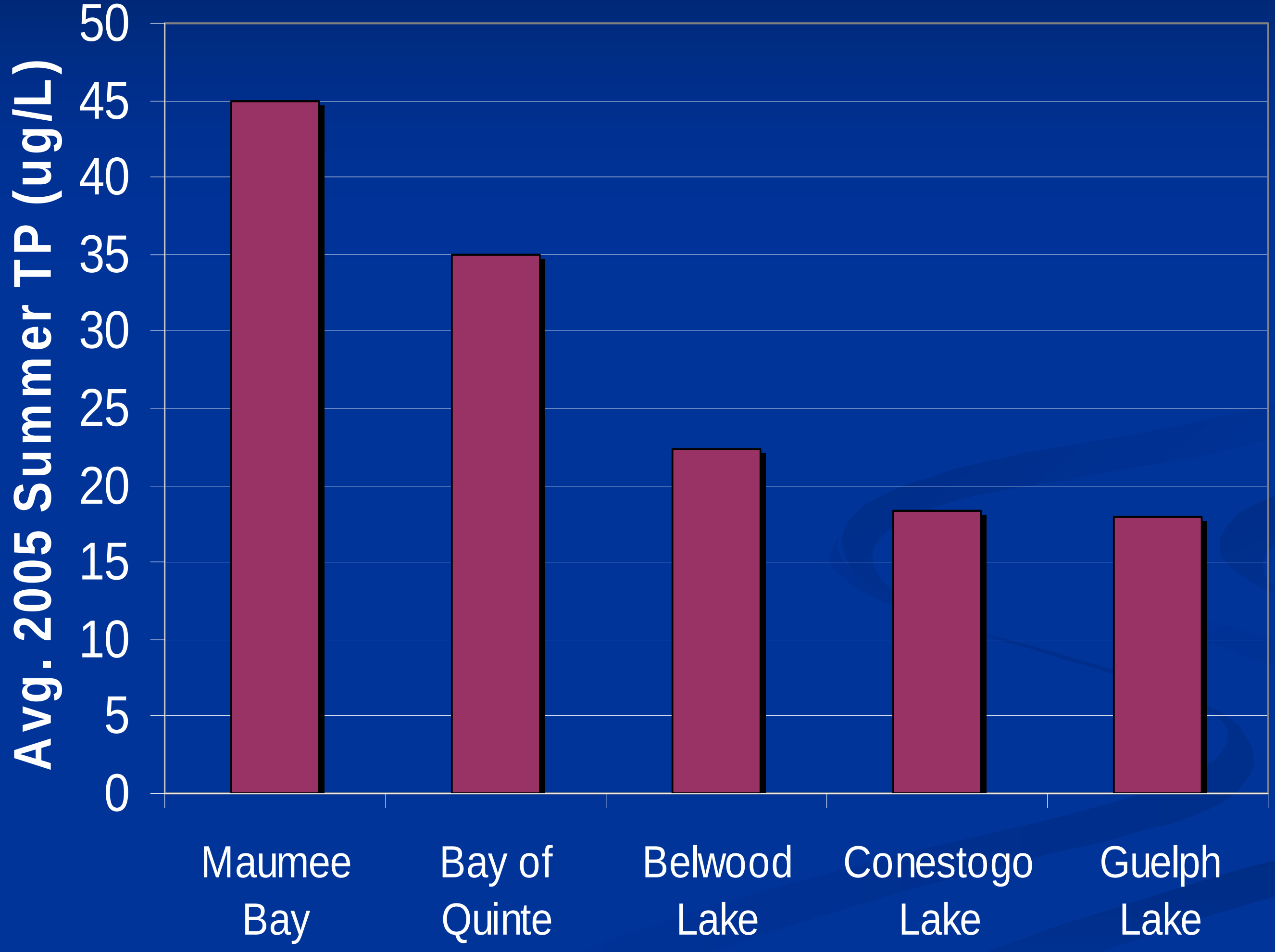
Managing Microcystin Risk

- Manage Phosphorus to reduce Cyanobacteria abundance
- High TN:TP reduces microcystin concentration
- Both these conditions if satisfied would lead to strong P deficiency which may be the mechanism for reduced mcy gene expression
- Dreissenids may increase risk by selective grazing and increasing growth rate (Hypothesis)

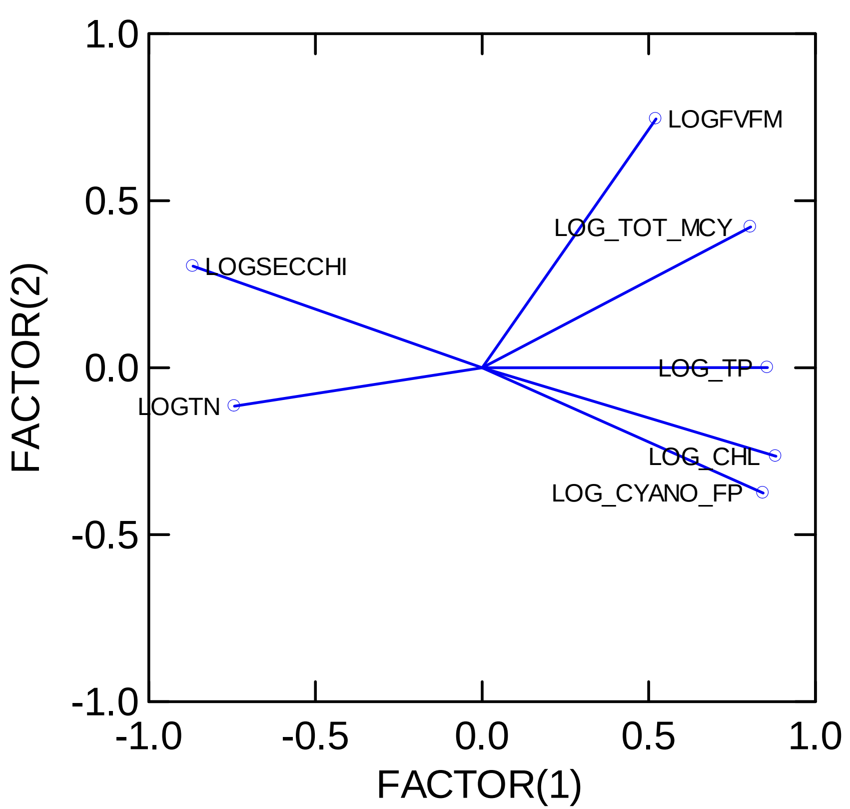
Acknowledgements

Dave Depew (CN), Dr. Yuri Kozlov (some GRCA chemistry), Zing-Ying Ho and Janet Ma (lab assistance), Ann Balasubramaniam and Justin Lorentz (field assistance), Ontario Graduate Scholarship program, Ontario Ministry of the Environment, Grand River Conservation Authority, Great Lakes Fishery Commission, NSERC (funding)

Bloom-Prone Study Sites



Multivariate approach to explain Microcystin?



Component loadings

	1	2
LOGFVFM	0.522	0.745
LOG_TOT_MCY	0.806	0.421
LOG_CYANO_FP	0.843	-0.375
LOGSECCHI	-0.867	0.304
LOG_CHL	0.881	-0.265
LOGTN	-0.743	-0.115
LOG_TP	0.856	0.000

Percent of Total Variance Explained

1	2
63.519	14.986

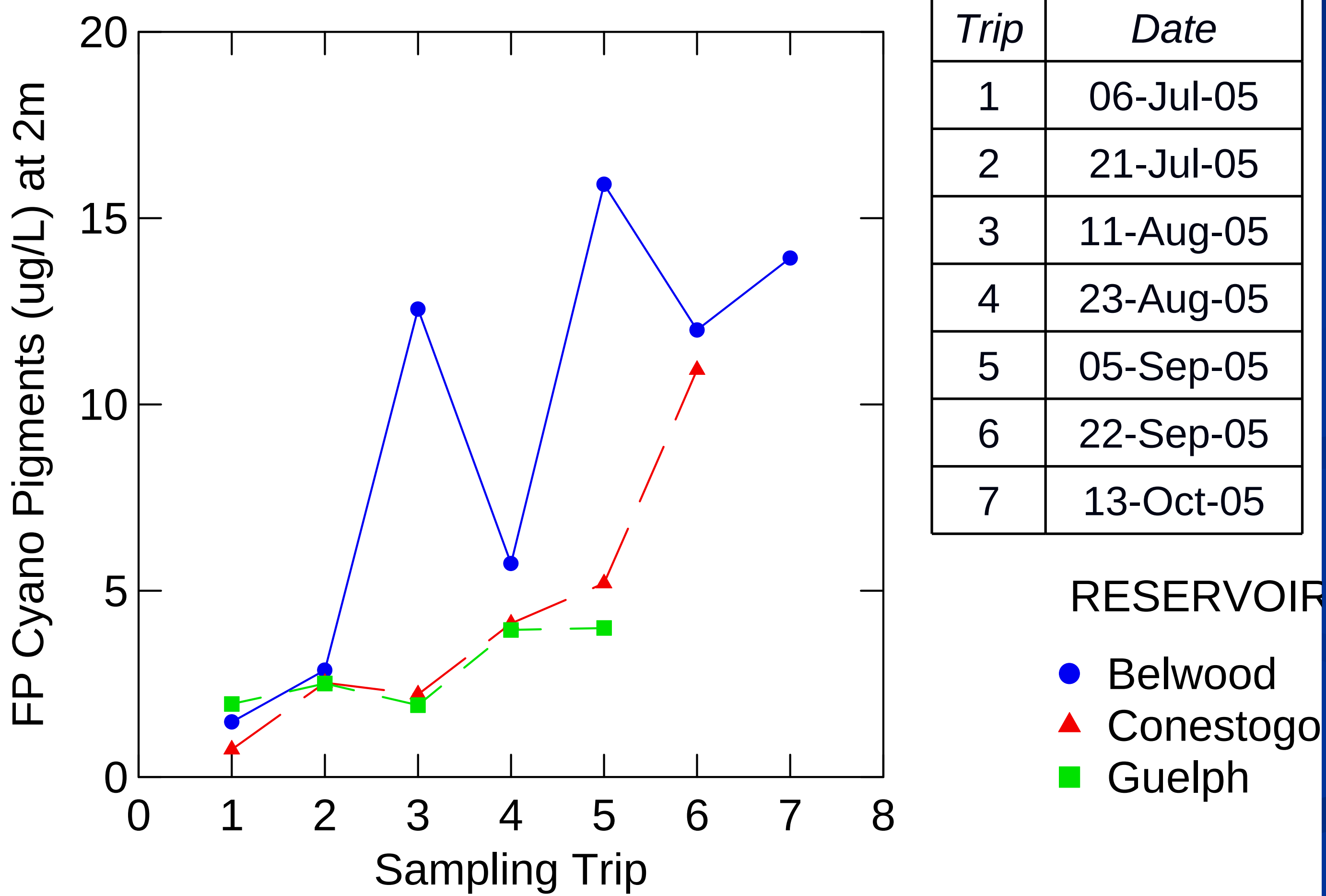
TP important predictor and Fv/Fm a measure of growth rate provides additional predictive capability

Microscopic information from 1 location in Bay of Quinte

	July 4, 2006	Sept. 22, 2006
Total Cyano Biomass	1615 mg/m^3	15397 mg/m^3
Cyanos as % of Total Phyto. Biomass	81%	94%
Microcystis as % of Cyanos	97%	8%
Anabaena spiroides as % of Cyanos	2%	83%
Heterocysts as % of Producers	1.7%	0.3%

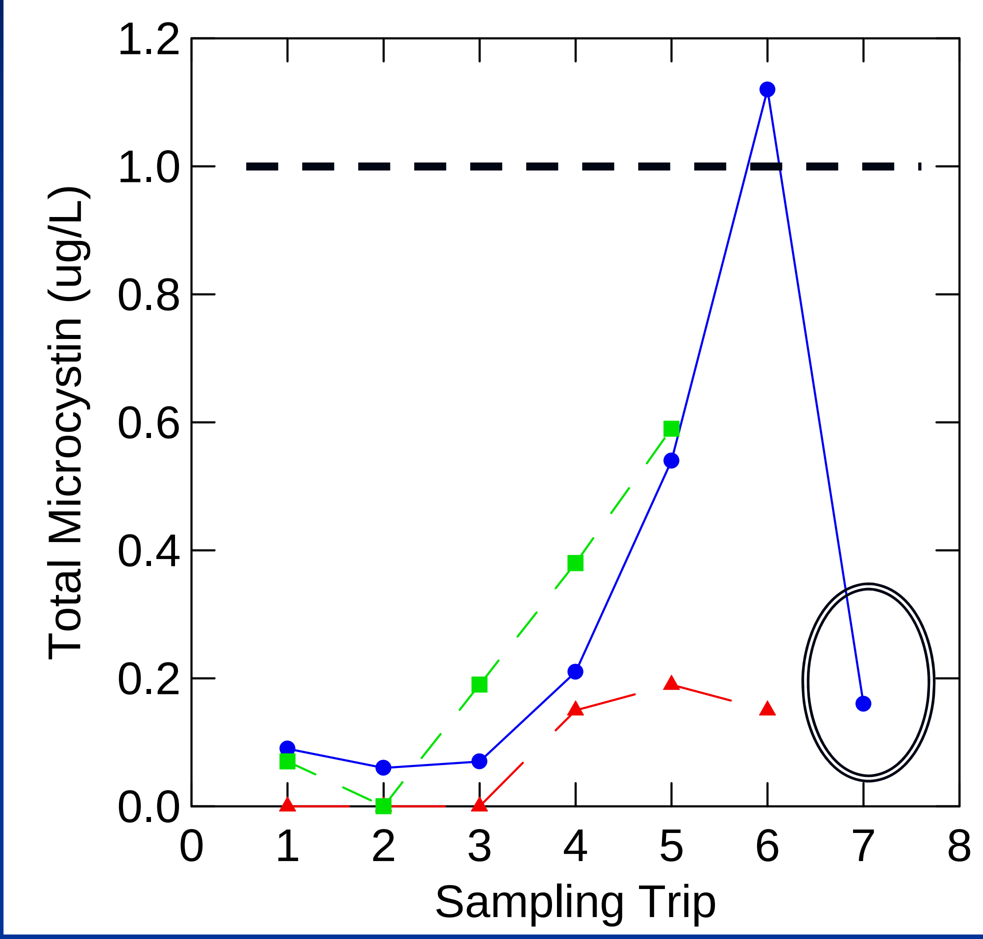
Higher microcystin levels were not associated with greater potentially toxic cyanobacterial biomass as indicated by microscopy or by qPCR

Cyanobacteria at 2m



Trip	*Date*
1	06-Jul-05
2	21-Jul-05
3	11-Aug-05
4	23-Aug-05
5	05-Sep-05
6	22-Sep-05
7	13-Oct-05

GRCA Microcystin



Trip	*Date*
1	06-Jul-05
2	21-Jul-05
3	11-Aug-05
4	23-Aug-05
5	05-Sep-05
6	22-Sep-05
7	13-Oct-05

August Cyanobacteria

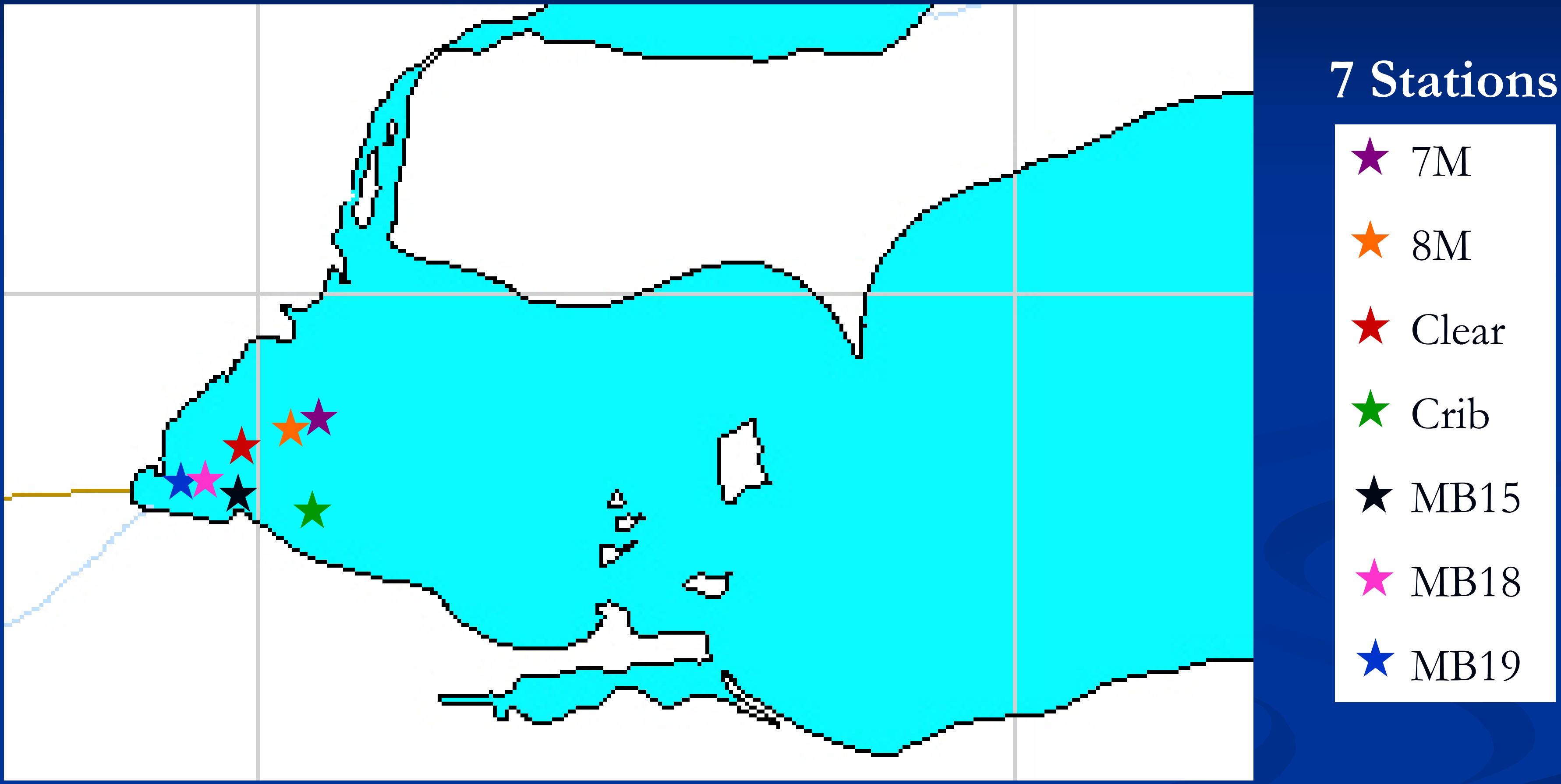
	Aug. 21, 2006
Total Cyano Biomass	1354 mg/m3
Cyanos as % of Total Phyto. Biomass	76%
Microcystis as % of Cyanos	22%
Aphanizomenon flos aquae as % of Cyanos	48%

- Relatively small *Microcystis* biomass produced 5.9 µg/L microcystin.
- Potentially toxic biomass was positively associated with microcystin levels.

Depths of 6 Stations: 1.2 m to 6.1 m

- Fully sampled July 4-5 and Sept. 22, 2006
- Microcystin measured from 4 dates in 2005

Stations in Maumee Bay



Station Depths: 1.3 m to 6 m.

Sampled June 20, 2006 and Aug. 22, 2006

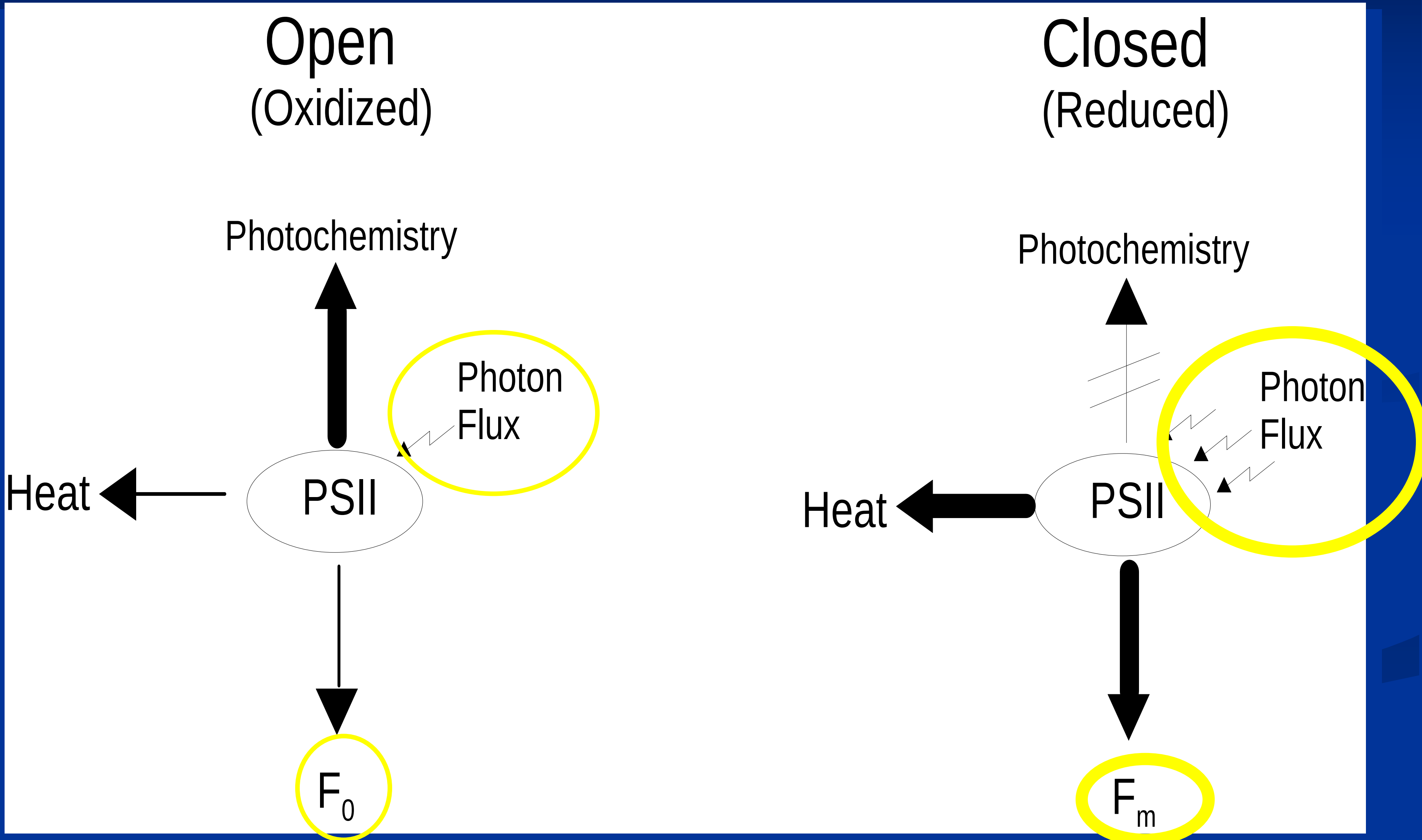
GRCA

- Sampled biweekly from July to end of September 2005

- Belwood Lake sampled on Oct. 13, 2005 due to cyanobacterial bloom

At very low photon flux, all light energy is absorbed and used for photochemistry

At very high photon flux the PSII reaction centre is saturated and light energy is reemitted as fluorescence



Thank-you!

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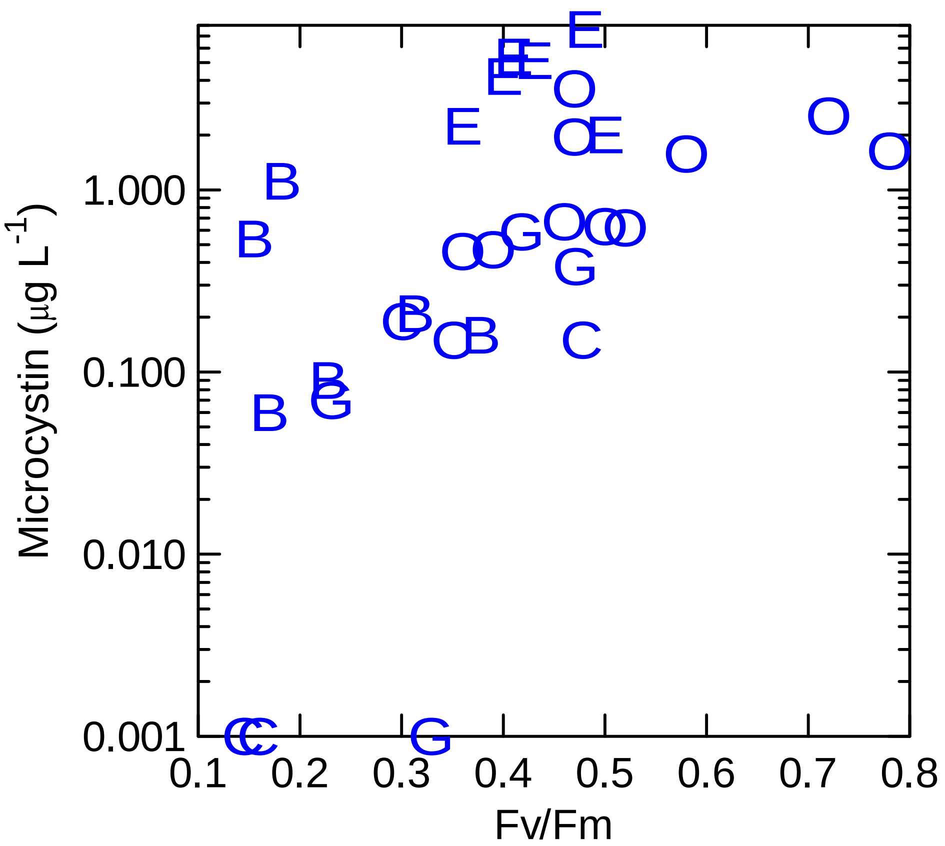
Grand River Conservation Authority

Ontario Graduate Scholarship Program

Assistance and Support:

UWAEG



Microcystin (μg L-1)
1.000
0.100
0.010
0.001
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Fv/Fm