Algae Metabolites in Arizona Surface Waters

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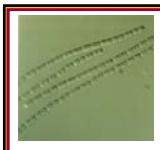
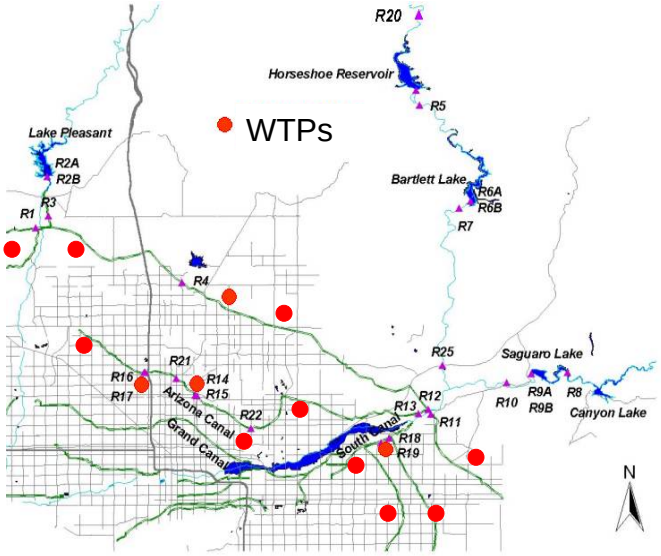


How can things so small cause such big problems?

- What do you say if a customer asks:
  - "My water smells, is it safe to drink?"
  - "Why should I drink tap water if it smells like dirt?"
  - "If you can't make it smell and taste good why should I pay for it?"
- What do you say if a WTP operator asks:
  - "What do I do to control in-plant algae growth that causes short circuiting and short filter run times now that you convinced me that our previous practice of prechlorinating may kill people?
- What do you say when a utility manager asks:
  - "What is the best way to monitor and prevent algae related problems?"

All those questions have been asked recently in Central Arizona



Presentation Outline

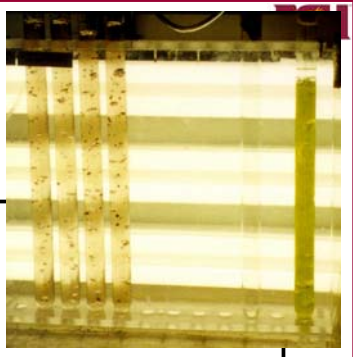
- Methods
- Algae Metabolites
  - Bulk organic matter
  - Taste and odors
  - Cyanotoxins
- Ongoing algae activites
  - Genetic fingerprinting of culprit algae
  - Managing nuisance algae
- Conclusions

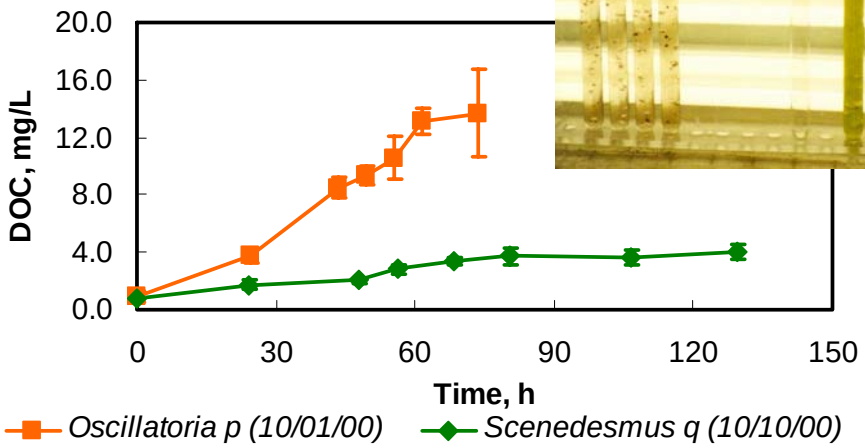
Methods

- DOC & DON: Shimadzu TOC-V
- DIN: Ion chromatography & phenate method
- T&O: SPME with GC/MS
- Algal toxins:
  - ELISA
  - Protein Phosphatase Inhibition Assay (PPIA)
  - Anatoxin-a/Saxatoxin – HPLC after fluorescent derivatization
  - Cylindrospermopsin – HPLC using a photodiode array detector
- PCR for 16s-RNA genetic fingerprinting

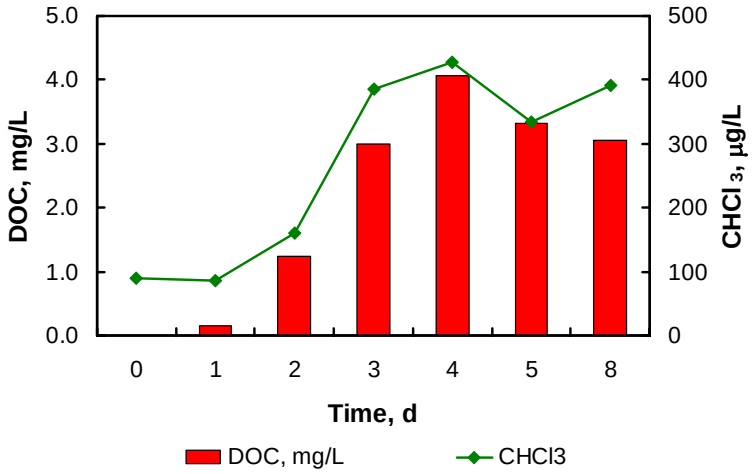
Bulk Organic Matter Production by Algae

Algae release extracellular material (bulk DOC)



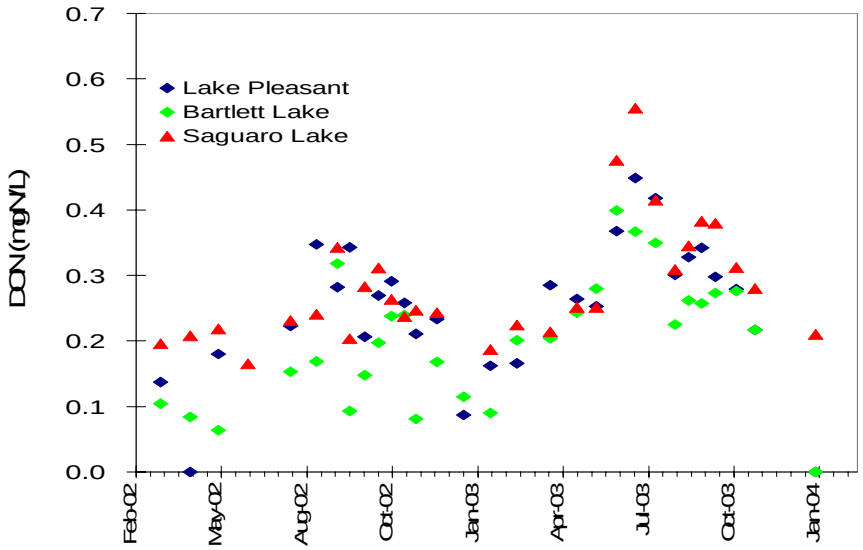


DOC production & DBP formation potential with DISSOLVE metabolites from *Scenedesmus quadricauda*



THM formation potential: 118µg $CHCl_3$/mgDOC

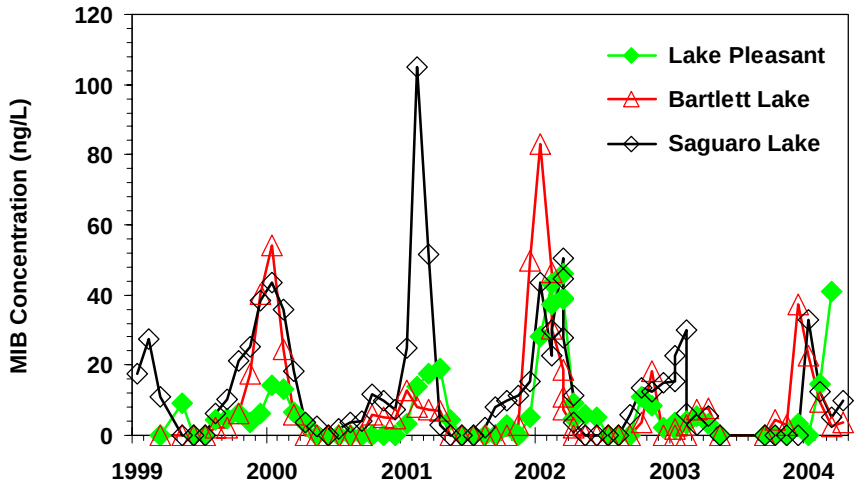
Many Algae Metabolites Contain Dissolved Organic Nitrogen



Algae Influence Watersheds Produce NOM (mg/L)

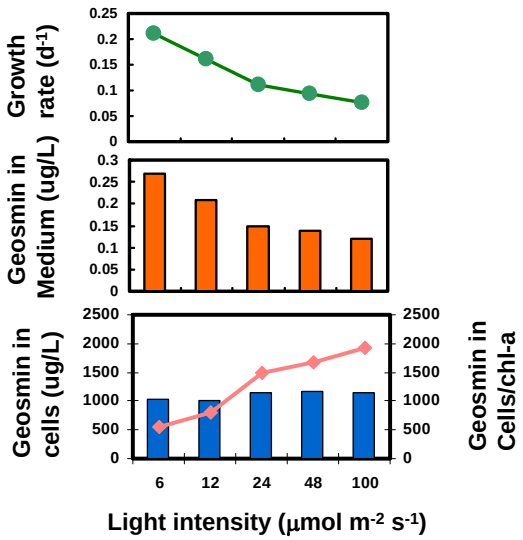
- Laboratory results showed that only 55 to 70% of algae-produced DOC were removable by biodegradation.
- Long-term reservoir storage accumulates refractory algal DOC causing a net gain (~10%) in DOC budgets.
- In an arid region where terrestrial DOC input is low (<0.3 g/m^2-yr), controlling reservoir hydraulic retention time (HRT) is key to reducing the amount of DOC production and export to downstream.

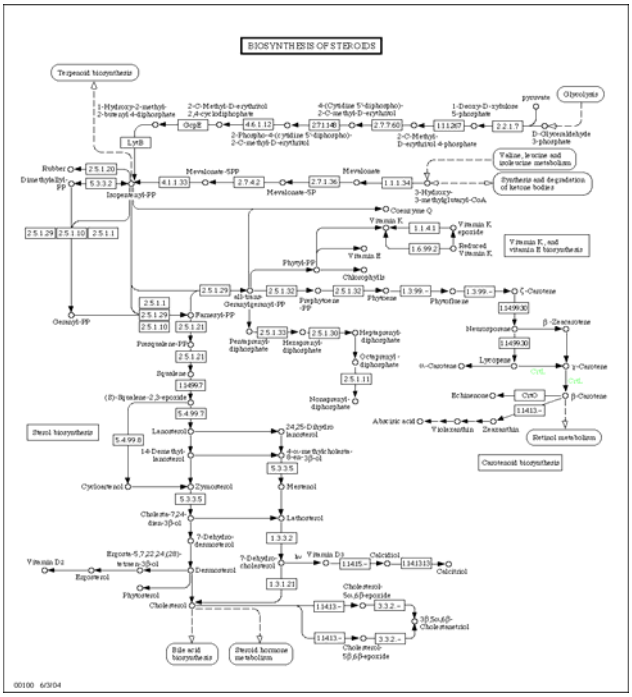
Western Watersheds Produce T&O (ng/L)



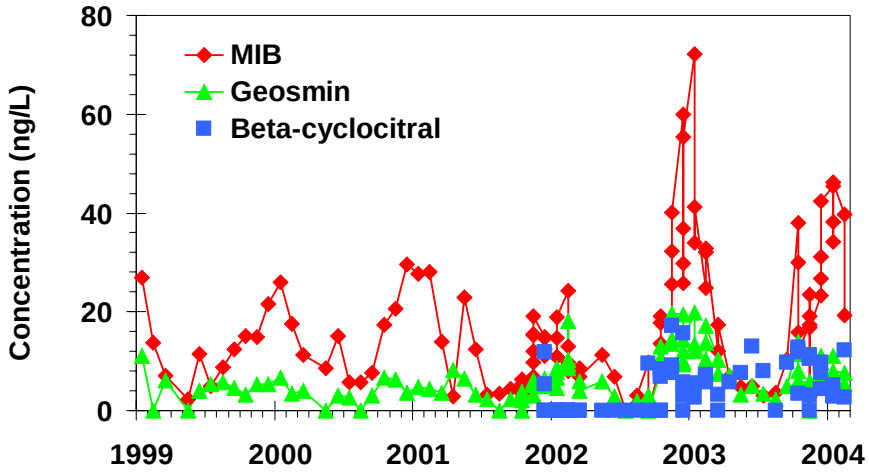
Culprit Algae can be cultured in the lab to study environmental factors

- Increasing light intensity affects biomass production and yield of T&O compounds
- Intracellular MIB and geosmin levels are much higher than concentrations in aqueous media

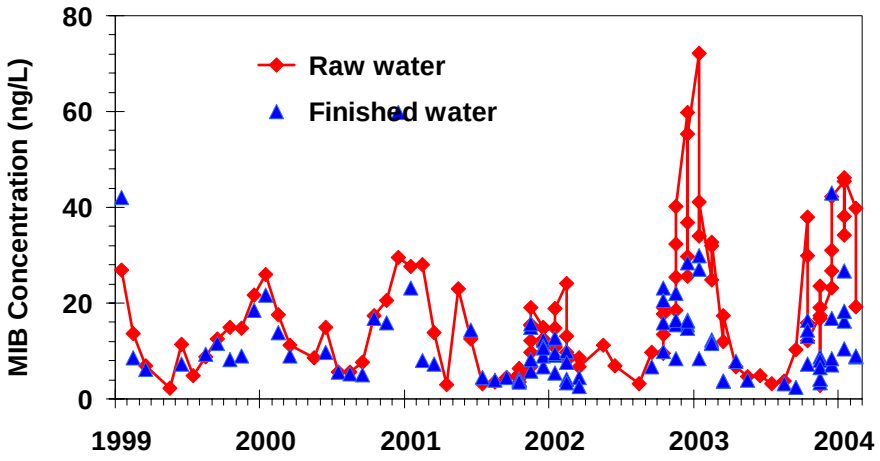




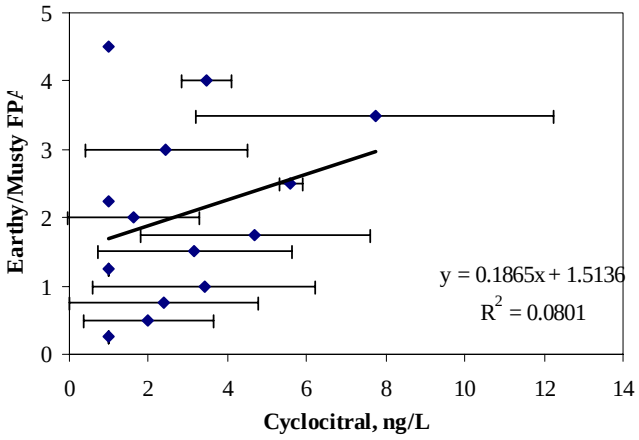
Raw water entering 24th Street WTP from a concrete lined canal



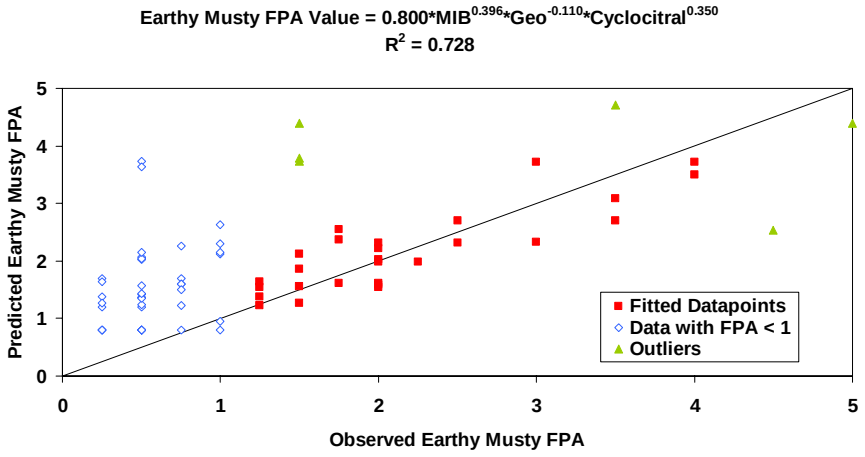
Powder Activated Carbon can remove T&O compounds within WTPs



Relationships between FPA and GC/MS Analysis



Predicting Expected FPA Intensity based upon GC/MS data



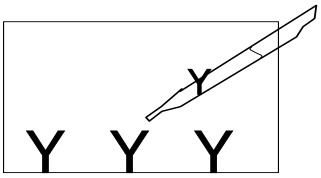
FPA= 2 when MIB=5, Geosmin=3, Cyclocitral=3 ng/L

Cyanotoxins are also present (ng/L to ug/L)

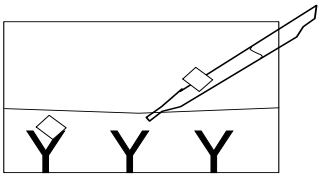
How can we make toxin analysis WTP friendly?

Toxin groups	Chemical Structure	Primary target organ in mammals	Cyanobacterial genera
Microcystins	Cyclic peptides	Liver	*Microcystis, Anabaena, Oscillatoria, Nostoc*
Nodularin	Cyclic peptides	Liver	*Nodularia*
Cylindrospermopsin	Alkaloid	Liver	*Cylindrospermosis* *Anabaena* *Aphanizomenon* *Umezakia*
Anatoxin-a, Anatoxin-a (S)	Alkaloid	Nerve synapse	*Anabaena, Planktothrix, Aphanizomenon, Cylindrospermopsis*
Saxitoxins	Alkaloid	Nerve axons	*Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis*

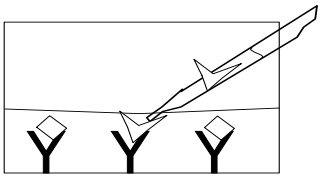
ELISA
(enzyme-linked immunosorbent assay)



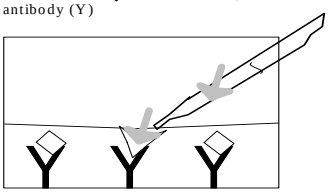
Step #1 Well is coated with antibody (Y)



Step #2 Sample is added and microcystin (◇) is bound by the antibody (Y)

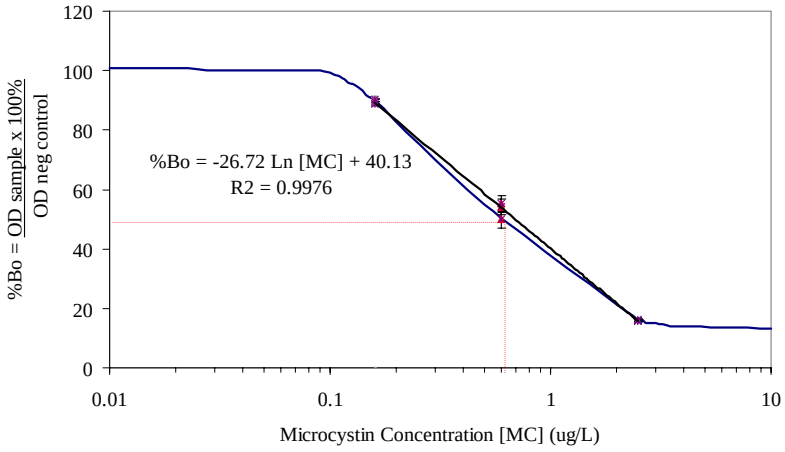


Step #3 Enzyme linked antigen (⩛) is added and binds to the remaining unoccupied sites on the antibody (Y)



Step #4 Enzyme substrate ( ) is added to produce color reaction

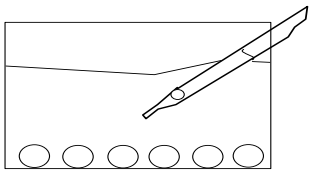
ELISA Calibration Curve



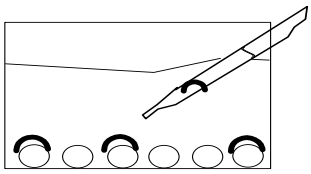
ELISA Assay Results

Assay No.	Date	Kit No.	50%Bo (µg/L)	Slope	R^2	Acceptable	Range (µg/L)	Comments
1	11/27/02	1	0.4	-28.8	0.957	Yes	0.16-1.6	Calibrators less than recommended %Bo ranges. Near expiration date
2	1/22/02	1	0.2	-22.3	0.947	Yes	0.16-1.6	
3	2/02/02	2	0.2	-22.7	0.961	Yes	0.16-1.6	
4	5/15/02	2	0.2	-24.3	0.947	Yes	0.16-1.6	
5	6/25/02	2	0.5	-28.3	0.982	Yes	0.16-1.6	
6	8/28/02	3	0.6	-27.0	0.993	Yes	0.16-1.6	
7	9/12/02	3	0.6	-24.1	0.975	Yes	0.16-2.5	
8	9/19/02	4	0.6	-22.6	0.989	Yes	0.16-2.5	
9	10/10/02	5	0.6	-27.6	0.992	Yes	0.16-2.5	
10	10/17/02	6	0.6	-26.7	0.997	Yes	0.16-2.5	
11	10/25/02	7	0.6	-26.8	0.987	Yes	0.16-2.5	

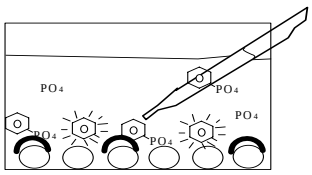
PP2A
(protein phosphatase-2A assay)



Step #1 Well is coated with enzyme PP1 or PP2A (⬭)



Step #2 Sample is added and microcystin (◠) inhibits enzyme



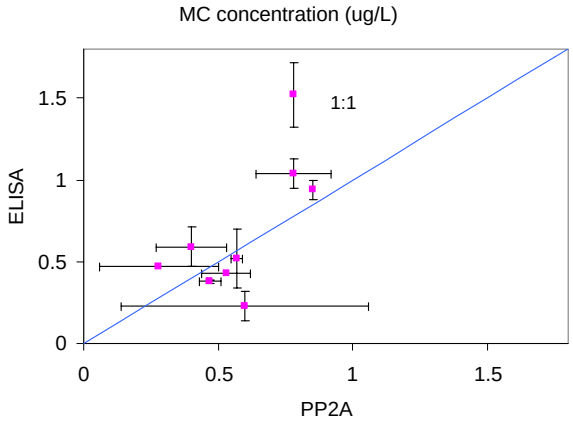
Step #3 Substrate (⬡ PO4) is added.

Overtime unihibited enzyme reacts with substrate releasing phosphate (PO4) resulting in yellow color. Enzymes inhibited by microcystin do not react with substrate and produce no color.

PP2A Assay Results

Assay No.	Date	Enzyme No.	V/Vo= 50% µg/L	Slope	R^2	Acceptable	Assay Range (µg/L)	Comments
1	4/26/02	1	0.15	-3.12	0.9988	Yes	0.05-0.30	Enzyme at room temperature
2	6/06/02	1	0.15	-3.36	0.9415	Yes	0.05-0.25	
3	6/13/02	1	0.15	-3.03	0.9143	Yes	0.05-0.30	
4	6/20/02	2	0.15	-2.93	0.7110	Yes	0.05-0.25	Poor R^2 value
5	6/27/02	2	NA	NA	0.2489	No	NA	New enzyme used, calibrators
6	6/27/02	2	NA	NA	0.3110	No	NA	not in range of Vi/Vo < 1
7	7/10/02	2	NA	NA	NA	No	NA	
8	7/12/02	2	0.6	-0.54	0.9276	Yes	0.2-1.2	
9	7/12/02	3	0.8	-0.55	0.9741	Yes	0.2-1.2	
10	7/24/02	3	0.6	-0.52	0.9656	Yes	0.2-1.2	
11	7/24/02	3	0.7	-0.53	0.8232	Yes	0.2-1.5	
12	7/24/02	3	0.7	-0.52	0.9626	Yes	0.2-1.5	
13	7/24/02	3	0.9	-0.51	0.9306	Yes	0.2-1.5	
14	8/02/02	3	NA	NA	0.0085	No	NA	Enzyme was inactive from overuse at room temperature
15	8/02/02	4	1.1	-0.51	0.9027	Yes	0.2-1.6	
16	8/08/02	4	0.6	-1.03	0.9297	Yes	0.2-0.9	
17	8/21/02	4	1.4	-0.43	0.9588	Yes	0.3-1.8	
18	8/28/02	4	1.0	-0.47	0.9917	Yes	0.2-1.5	
19	9/12/02	4	1.0	-0.53	0.9310	Yes	0.4-1.5	
20	9/19/02	5	1.5	-0.19	0.7516	No	0.5-2.5	Inconsistent calibrator results
21	9/25/02	5	NA	NA	NA	No	NA	No color produced
22	9/26/02	4	1.2	-0.36	0.9416	Yes	0.5-2.1	
23	10/03/02	4	1.4	-0.35	0.9077	Yes	0.5-2.5	

Assay Comparison



Assay Comparison

(measure of…)	ELISA (presence)	PP2A (toxicity)
MDL (mg/L)	0.147	0.05, 0.24
Range (ug/L)	0.147-2.5	0.05-0.25 0.24-2.5
% acceptable	100%	74%
Cost per well	$4.12	~$1.00

- Recommend ELISA for monitoring

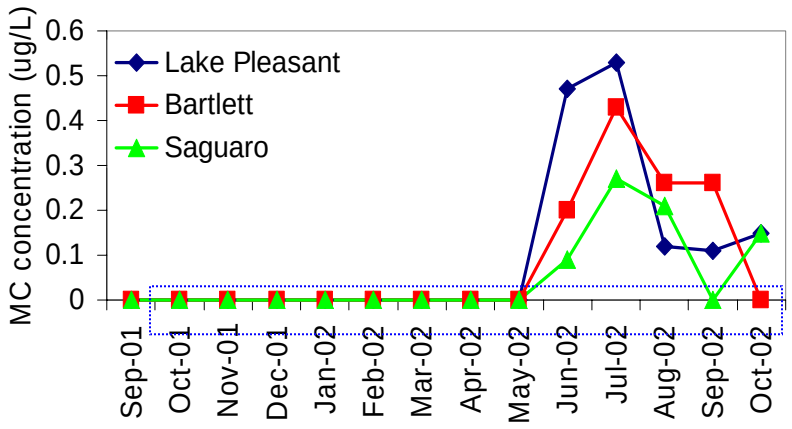
Intracellular Extraction

Sample Number	*Microcystis aeruginosa* (µg-MC/OD_{730})			
	24 hrs in MtOH + Beadbeating		24 hrs in MtOH	
	10 day	29 day	10 day	29 day
1	0.662	1.042	0.437	>1.036
2	0.732	1.076	0.380	>1.036
3	0.634	0.991	0.408	>1.036
4	0.915	0.762	0.775	0.949
5	0.887	0.758	0.648	0.990
6	0.859	0.275	0.592	0.966
7		0.242	1.056	0.680
8		0.275	0.944	0.874
9			0.958	0.829
Mean	0.782	0.926	0.689	0.881
Standard Deviation	0.121	0.154	0.257	0.116

Culture Growth = 10 days
Extracellular conc. = >2.5µg-MC/L
Intracellular conc. = 56 µg-MC/L

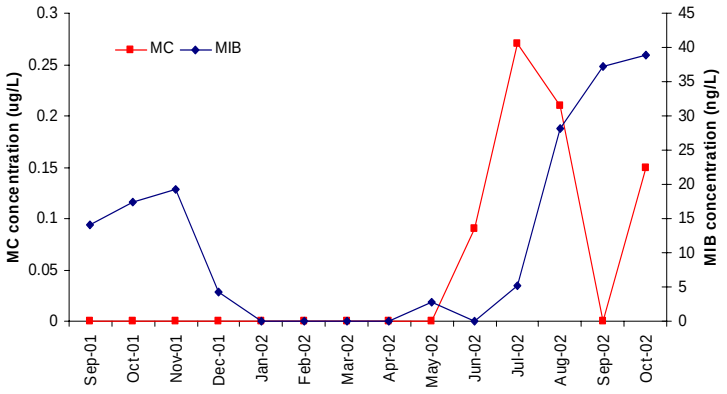
Culture Growth = 29 days
Extracellular conc. = >25 µg-MC/L
Intracellular conc. = 838 µg-MC/L

Microcystin Monitoring

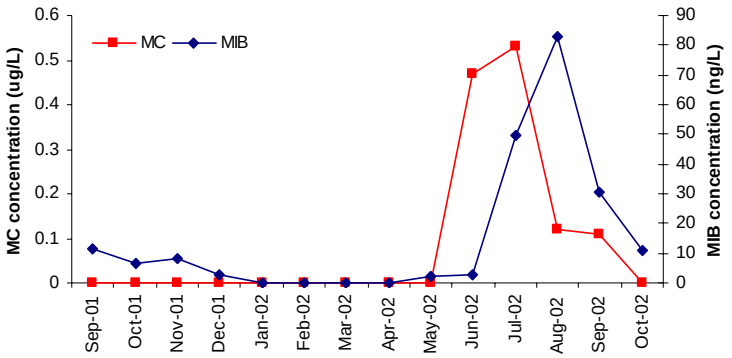


Intracellular levels always slightly higher than extracellular (dissolved) MC concentrations

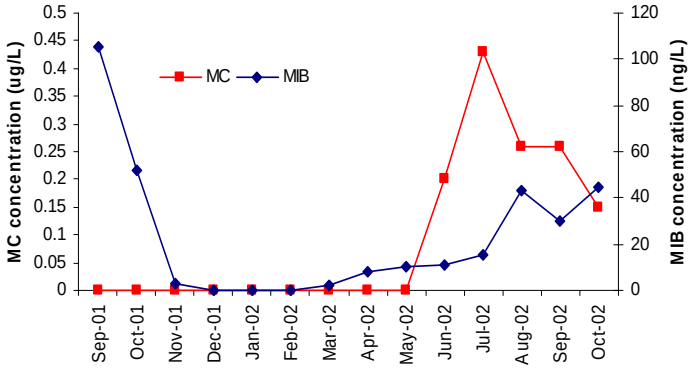
MIB and MC Lake Pleasant



MIB and MC
Bartlett Lake



MIB and MC
Saguaro Lake

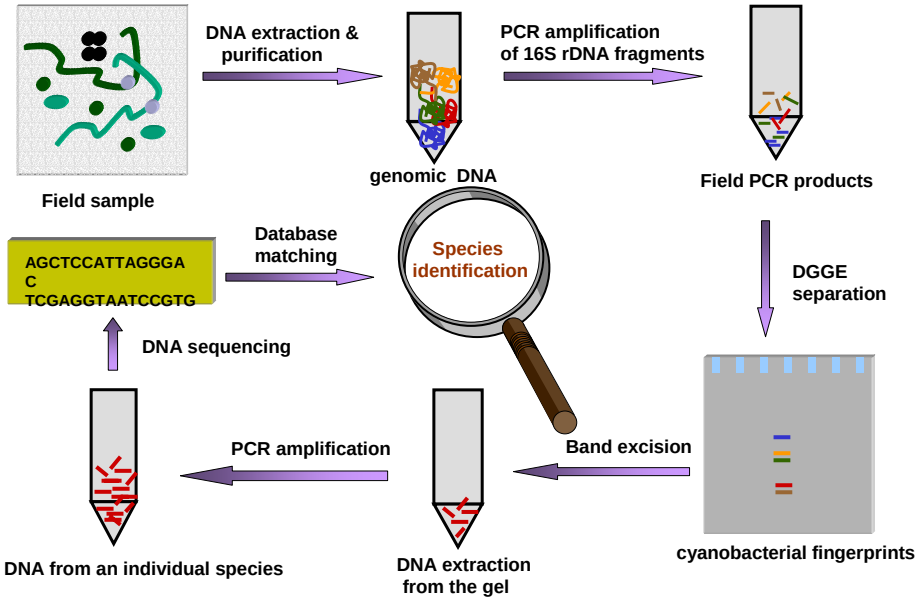


Cyanotoxin Summary

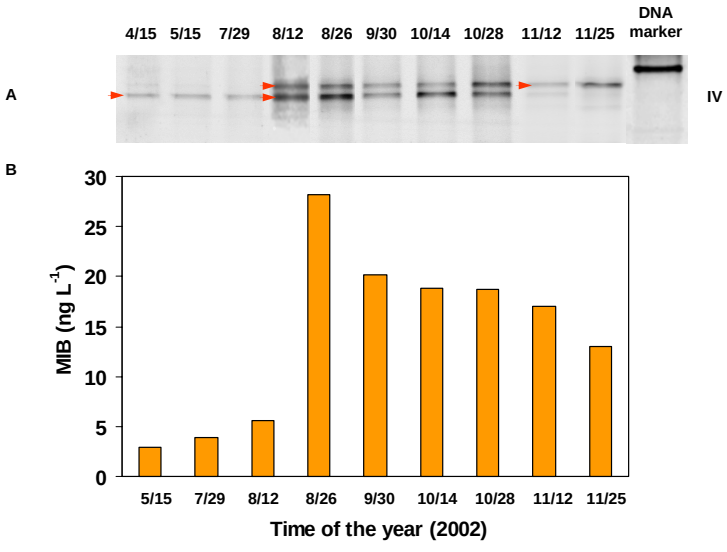
- Relatively large background levels of microcystin found in all wastersheds.
- Anatoxin-a only found in fish stomach samples, however, the likely cause of large fish kills.
- Fast degradation rates of anatoxin-a make this neurotoxin extremely difficult to detect in surface water.
- While numbers of *C. raciborskii* have, at times, dominated the phytoplankton cylindrospermopsin has been found in very low levels and only in concentrated samples (plankton tows).

Early warning indicators for culprit algae

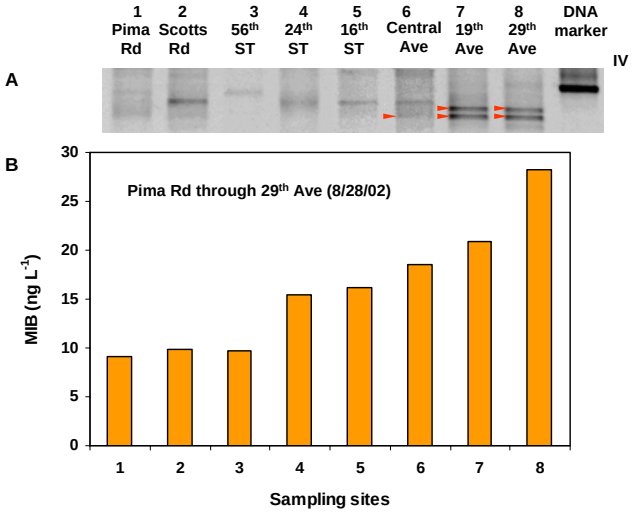
General strategy

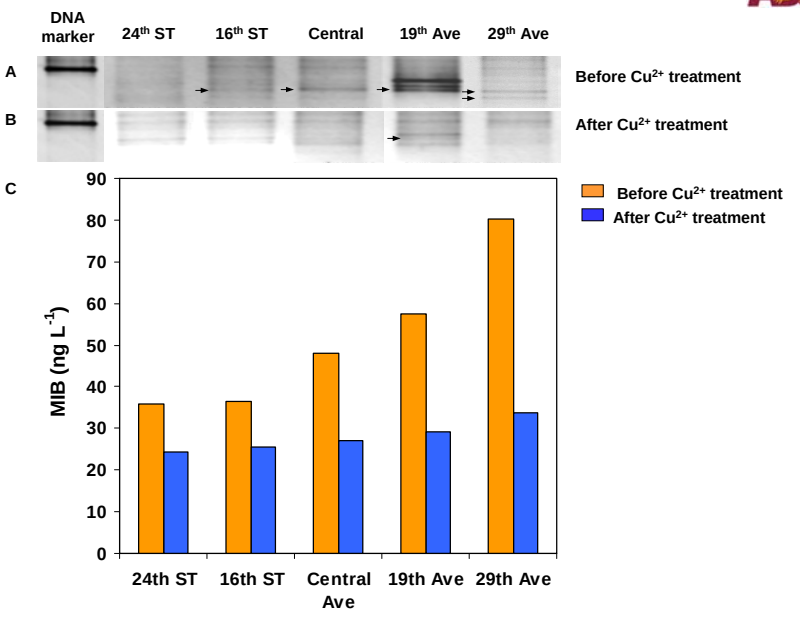


Relation between DNA profiles and MIB throughout the seasons

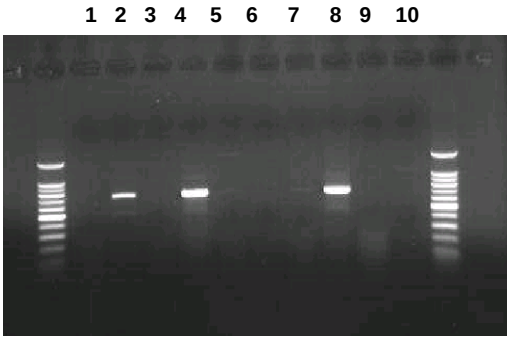


Relation between DNA profiles and MIB at selected sites





Detection of *Cylindrospermopsis* in Saguaro Lake samples using a specific gene probe

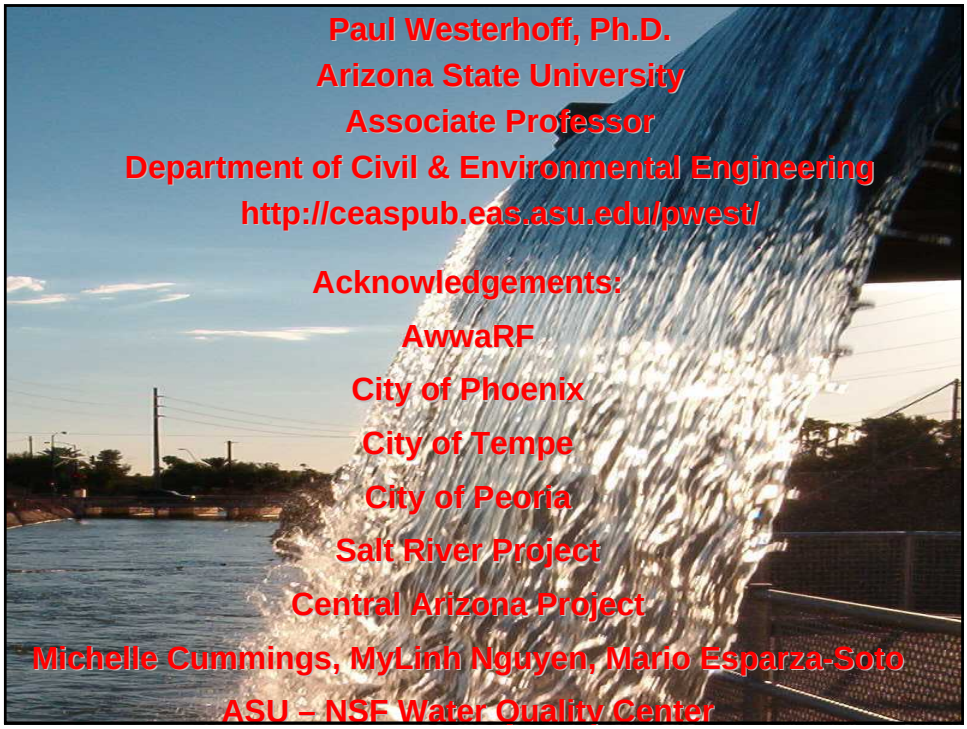


1: ***Anabaena*** **TAC426**
2: **Saguaro lake sample (6/15/04)**
3: ***Nodularia*** **strain 575**
4: ***Cylindrospermopsis*** **AWT205**
5: ***Plankothrix*** **PCC7811**
6: ***Microcystis*** **LE-3**
7: ***Nostoc*** **PCC73102**
8: **Saguaro lake sample (6/22/04)**
9: ***Aphanizomenon*** **strain Zayi**
10. **No DNA sample**

100 bp DNA ladders used at both sides of the sample lanes.

Summary

- Algae metabolites include:
  - Bulk OM (mg/L) that produces THMs and is enriched in organic nitrogen
  - T&O terpenoids (ng/L) with high intracellular levels. Produced by cyanobacteria with specific genetic fingerprints, but which represent a small percentage of the total algae biomass
  - Cyanotoxins (ug/L) are present, have lead to fish kills (makes news headlines), and has raised concern by many WTPs
  - 16S rDNA primers have resolved 16 different cyanobacterial sequences from the AZ Canal by PCR and DGGE techniques
- Variable hydrologic conditions, reservoir management, and water conveyance will affect magnitude for the significance of algae metabolites in Arizona
- T&O and cyanotoxins can be removed in WTPs, but provide added motivation for WTPs to install GAC
- #1 problem reported by each WTP related to algae: how to control inplant algae growth without prechlorination. So this is our challenge for 2005
- Arizona watersheds are representative of many others throughout the western US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