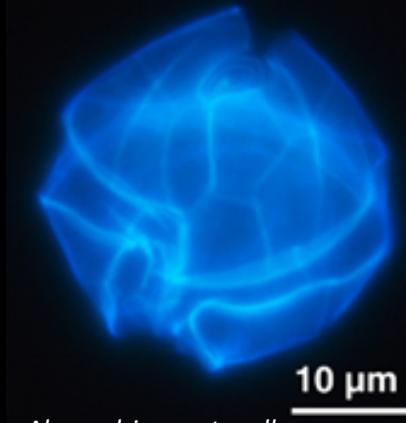


*Alexandrium tamarensis*



*Alexandrium catenella*



*Pseudonitzschia*



*Microcystis*

# What Controls Microcystis Bloom & Toxicity in the San Francisco Estuary? (Summer/Fall 2008 & 2009)

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# Acknowledgements

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## *Postdoctoral Fellowship*

*California Bay-Delta Authority Agreement No. U-04-SC-005*

## *Postdoctoral Fellowship*

*Sacramento Regional County Sanitation District Agreement No. 93597*

# Outline

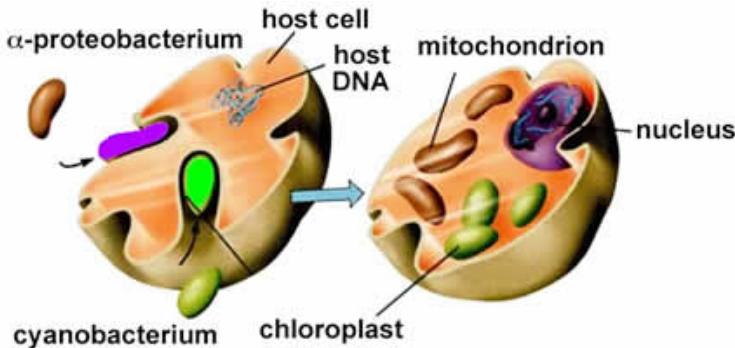
- **Background**
  - ✓ CyanoHABs Formation and Distribution
  - ✓ Routes of exposure
  - ✓ Health effects
  - ✓ Microcystis/Microcystins
- **CyanoHABs in the San Francisco Estuary (2009)**
  - ✓ Study Area – San Francisco estuary
  - ✓ Results
- **Future strategies and Prospective work**

# Background – CyanoHABs, Cyanotoxins



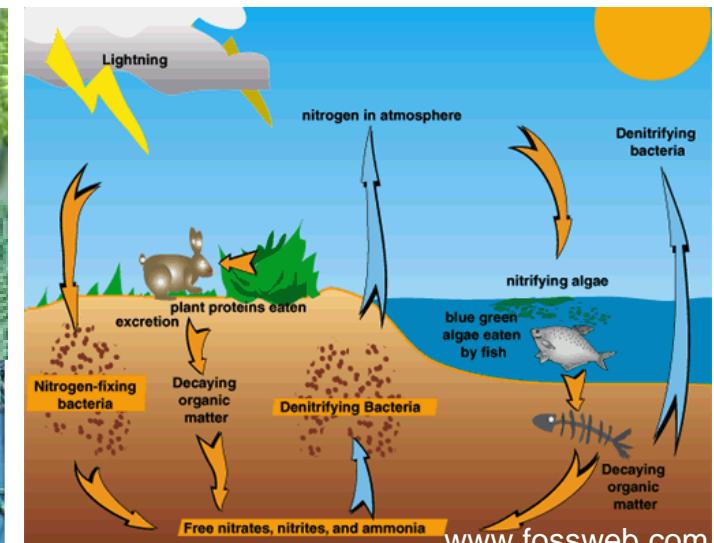
Courtesy: NASA

# The Good...



## Cyanobacteria (blue-green algae):

- Oldest oxygenic phototrophs (>3.5 billion yo)
- Instrumental in the evolution of life (oxygen)
- Origin of Plants (chloroplast/endosymbiosis)
- Important in the Nitrogen Cycle ( $N_2$  fixation)



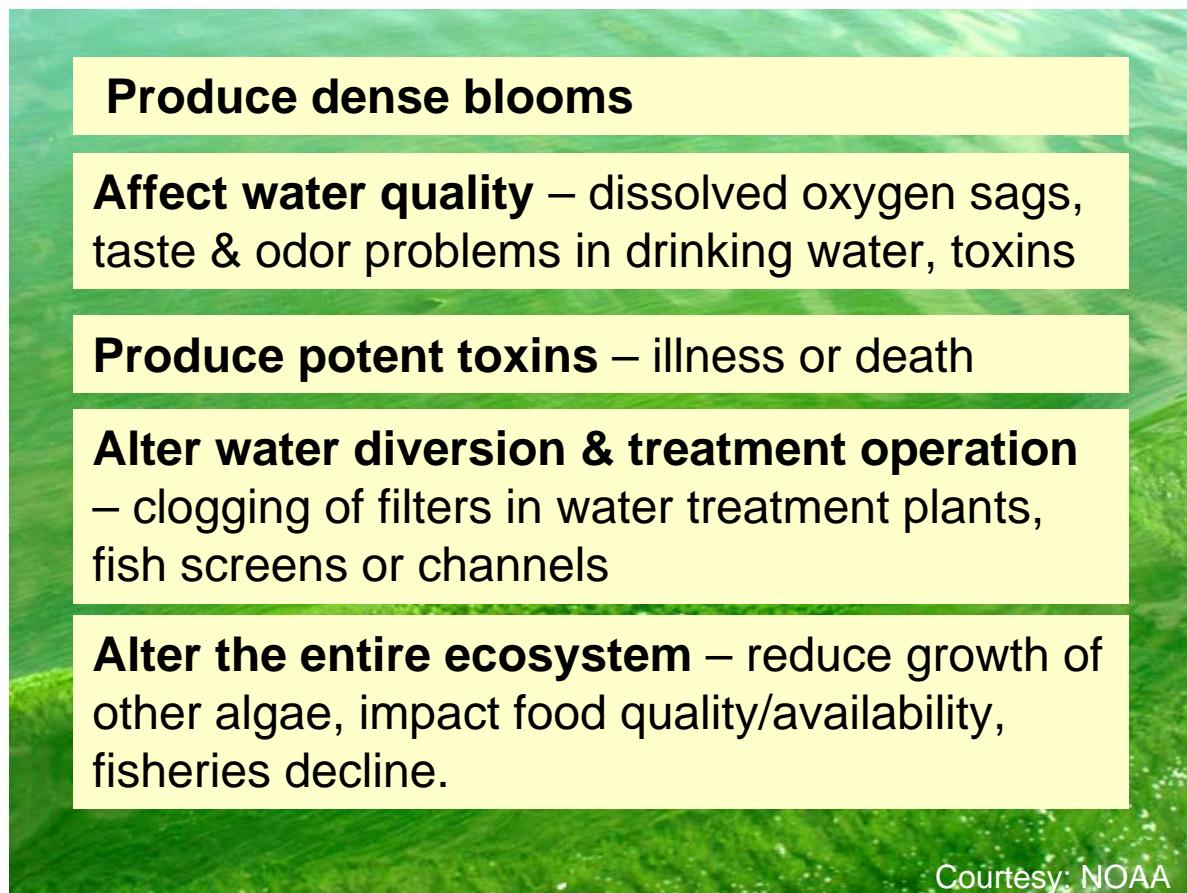
# ...the bad and the ugly



- 1000 years ago – Earliest report of cyanobacteria poisoning (soldiers) in Southern China  
General Zhu Ge-Ling
- 1878 – “Poisonous Australian Lake”  
(sheep poisoning, Lake Alexandrina)  
George Francis. *Nature*. May 2, 1878
- Similar early documented mass algae poisonings in the US in 1883, 1887, 1925.

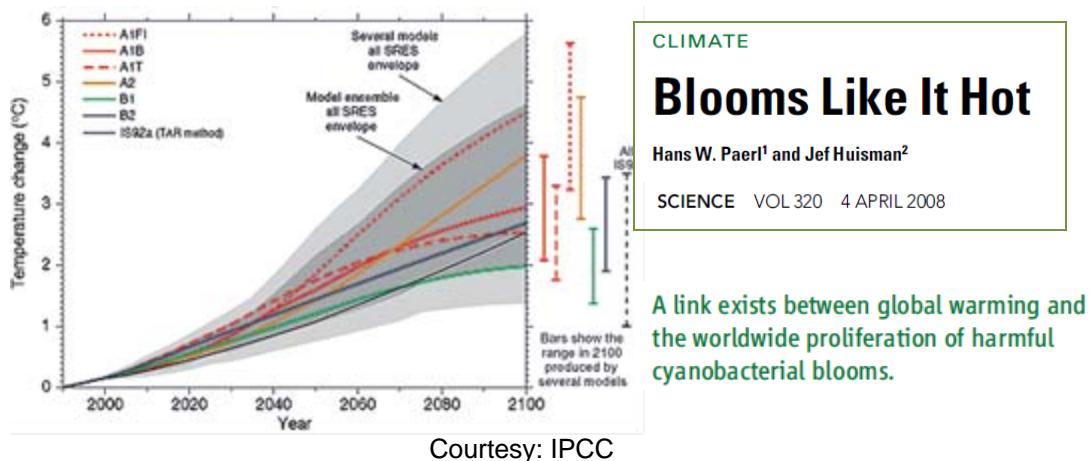
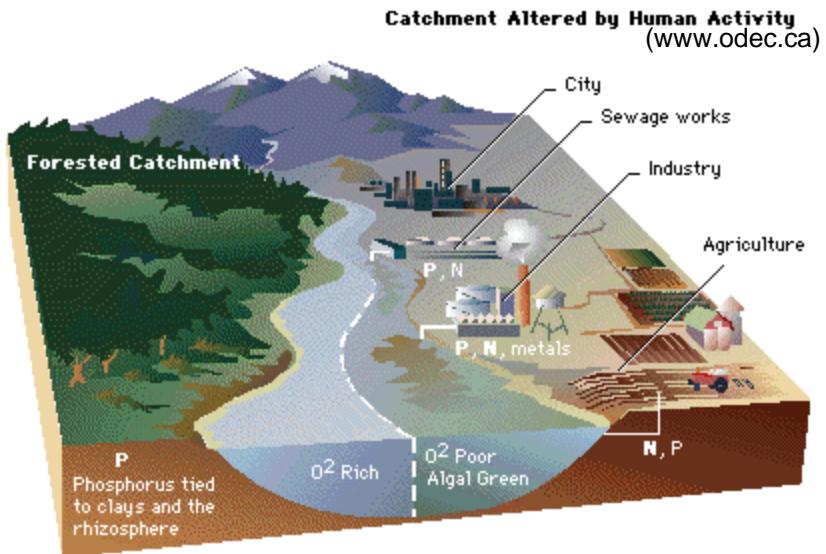
# Harmful Cyanobacteria Blooms

## aka CyanoHABs



Courtesy: NOAA

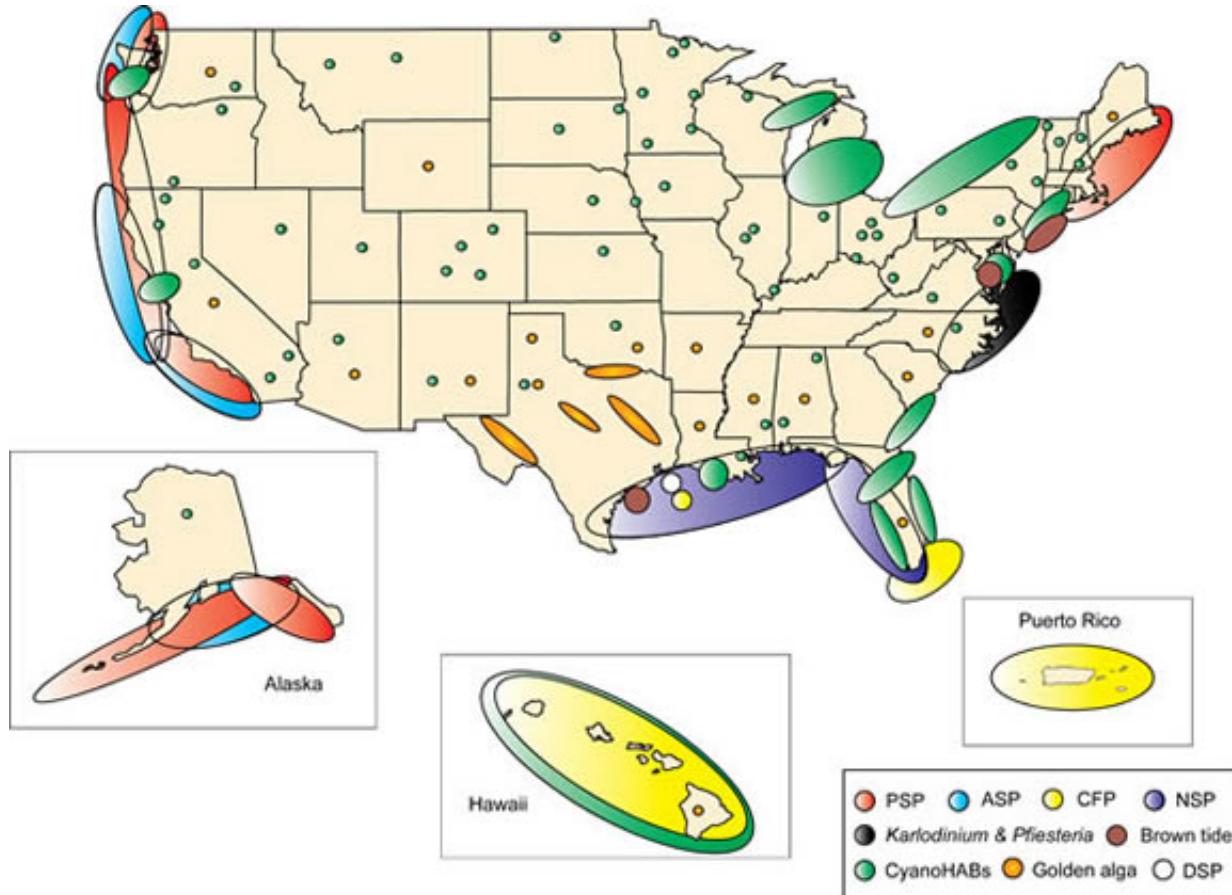
# CyanoHABs – Formation



## DRIVERS:

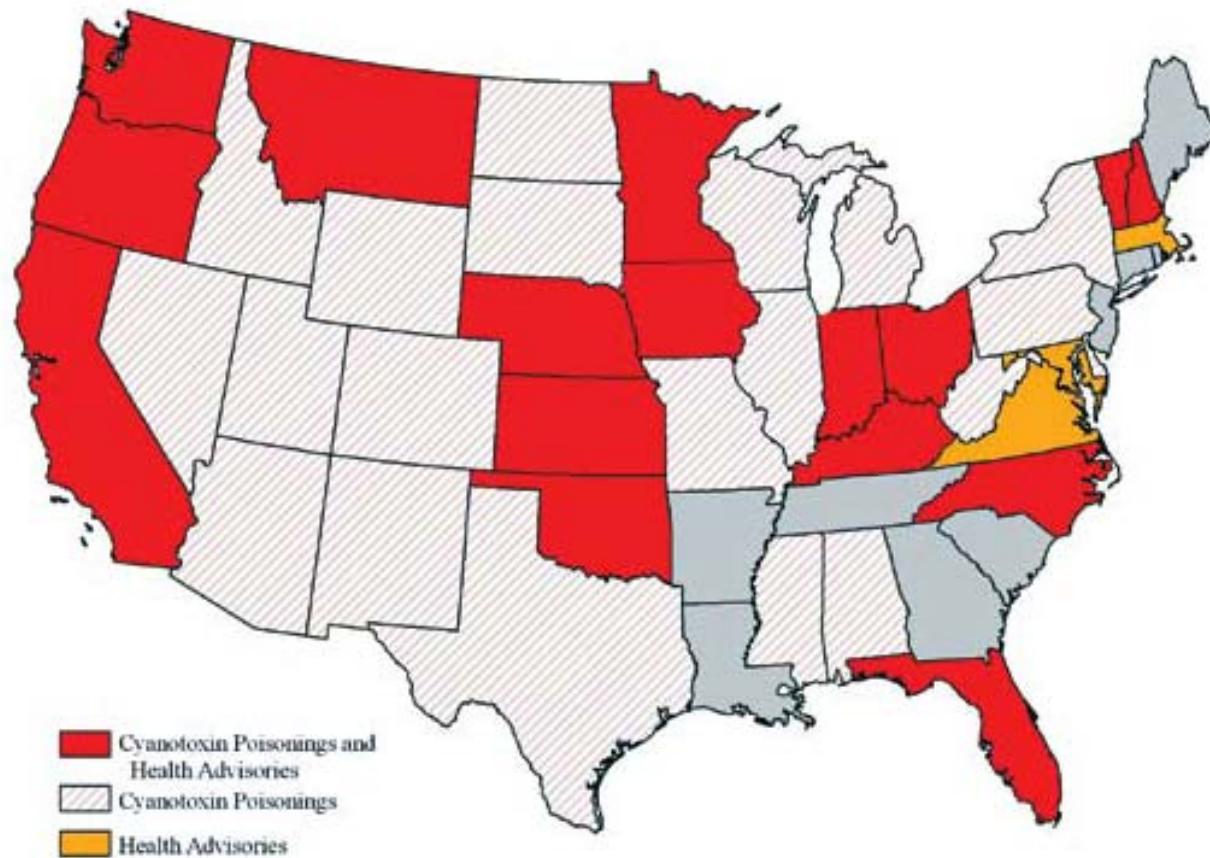
- **Land use** – Urban, agricultural & industrial expansion
- **Nutrient loading (N, P)**
- **Water use & hydrologic changes** – reduced water flow & mixing
- **Climate** (temperature increase)
  - Blooms become more common, more intense and of longer duration.

# Harmful Algae Bloom in the US



(from U.S. National Office for Harmful Algal Blooms).

# Reports of acute cyanotoxin poisonings of animals and/or humans



From Graham et al. 2009

# Countries Exhibiting one or more documented CyanoHAB Events



(adapted from W. Carmichael)

# Cyanotoxins – Routes of Exposure

## Human

Recreational

Aerosols

Drinking water

Food

Medical (dialysis)

### WARNING



Harmful bacteria is present in this water

Contact may cause serious harm to humans and animals.



Human intoxication  
by microcystins  
during renal  
dialysis treatment  
in Caruaru—Brazil

(Feb. 1996)

## Animals

Aerosols

Drinking water

Food

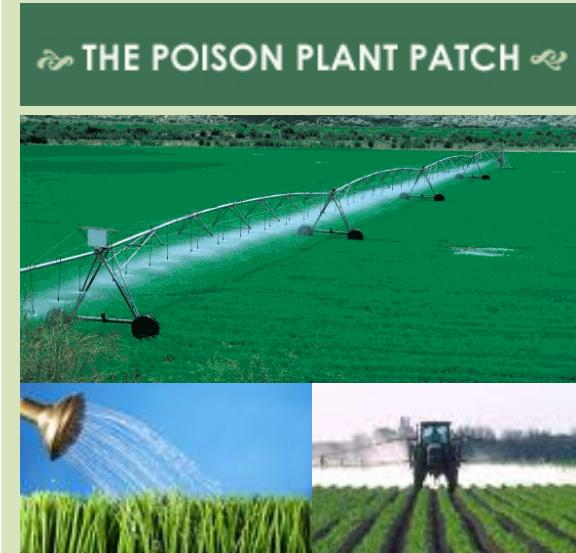


W. Carmichael



## Plants

Irrigation



# Cyanotoxins – Health Effects

## Dermatologic effects

- Lyngbyatoxins (“swimmer itch”)



## Neurotoxic

- Anatoxins
- Paralytic shellfish poisoning toxins (saxitoxins)



## Hepatotoxic

- Microcystins
- Cylindrospermopsins



# Microcystis

## Microcystis

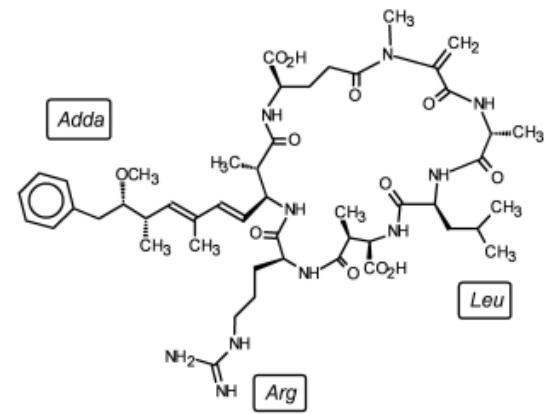
- Small planktonic cells (3 - 5  $\mu\text{M}$ )
- Unicellular, Colonial
- Embedded in gelatinous matrix
- Buoyant (gas vesicle)
- Fresh and brackish water
- Warm, stable, nutrient enriched



## Microcystins

(<http://oehha.ca.gov/ecotox/documents/Microcystin031209.pdf>)

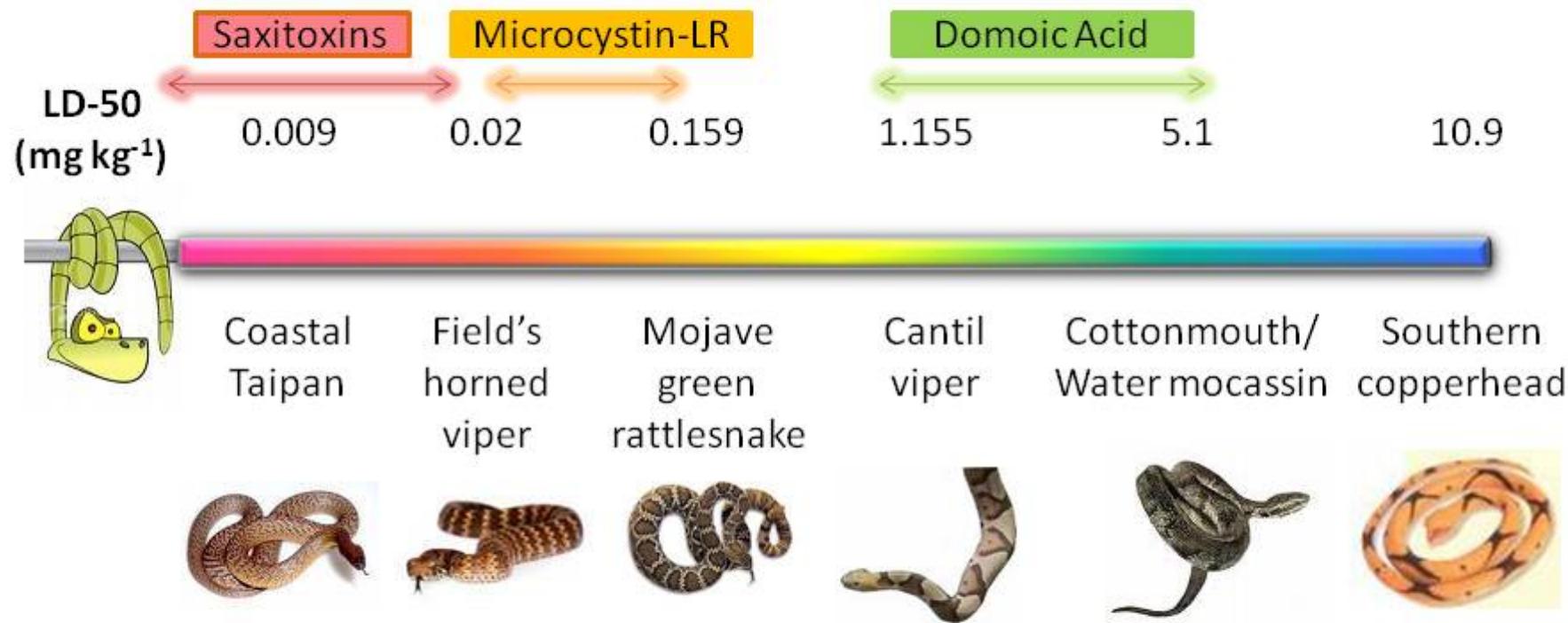
- Most numerous of the cyanotoxins
- Over 80 variants
- Extremely stable (half life = 10 weeks)
- Hepatotoxins: inhibit protein phosphatases
- Carcinogenic : Liver, colon (Grosse et al. 2006)



Microcystin-LR

# The Snake Scale

Comparison of Algal Toxins with some of the most venomous snakes in the world



Note: this comparison based on route of exposure (intraperitoneal). LD-50 can differ among different exposure routes.

(Source of LD-50s: <http://www.venomdoc.com/LD50/LD50men.html>)

# Microcystis – Adverse Impacts

- Death & cancer in human and wildlife  
(<http://oehha.ca.gov/ecotox/documents/Microcystin031209.pdf>)
- Hypoxia/anoxia (Carmichael, 1995)
- Bad taste and odor (Carmichael, 1995)
- Reduced zooplankton & fish feeding success (Rohrlack et al., 2005; Malbrouck & Kestemont, 2006)
- Fish food quality (Ger et al. 09)
- Total carbon production – shift from large to small zooplankton species (Smith & Gilbert, 1995)
- Marine species (Melissa Miller, pers. com)
- Kill crops  
(Allen.Milligan@science.oregonstate.edu)



H. Paerl, Lake Taihu



Bloom 2008, SFE

# Guidance Values

**Table 1.** World Health Organization guidance values for the relative probability of acute health effects during recreational exposure to cyanobacteria and microcystins, based on information presented in Chorus and Bartram 1999.

Relative Probability of Acute Health Effects	Cyanobacteria <sup>1</sup> (cells/mL)	Microcystin-LR <sup>2</sup> ( $\mu$ g/L)	Chlorophyll- $a$ <sup>3</sup> ( $\mu$ g/L)
Low	< 20,000	< 10	< 10
Moderate	20,000-100,000	10-20	10-50
High	100,000-10,000,000	20-2,000	50-5,000
Very High	>10,000,000	>2,000	>5,000

CA Environmental Protection Agency Department of Health Local agencies	Cell counts <sup>1</sup> Microcystin <sup>2</sup> Visual Assessment	40,000 to 100,000 cells/ml Microcystin $\geq$ 8 $\mu$ g/L Scum associated with toxicogenic species	Advisory/Closure
--	---	--	------------------

Brazil is the only country to have CynoHABs mandatory regulations  
Other countries have guidelines (WHO guideline for drinking water = 1 $\mu$ g/L)

# Is 1 $\mu$ g/L enough?

## \*\*Chronic Human Health Effects from drinking water: Liver and colorectal cancers\*\*

- “Microcystins from tap water could be a risk factor for liver and colorectal cancer: A risk intensified by global change”  
(Martínez Hernández et al., 2009)
- 0.61  $\mu$ g/L in areas of high incidence of liver cancer in China (Yu, 1994)



# Microcystis in the San Francisco Estuary



# SF Estuary: territory of outsize importance

## SF Estuary/Delta

- Water diverted for agricultural, industrial & domestic uses
- Recreational area (fishing, water contact sports)
- Habitat of vulnerable species (Delta Smelt, Striped Bass, Threadfin Shad, Chinook Salmon)

## Goals:

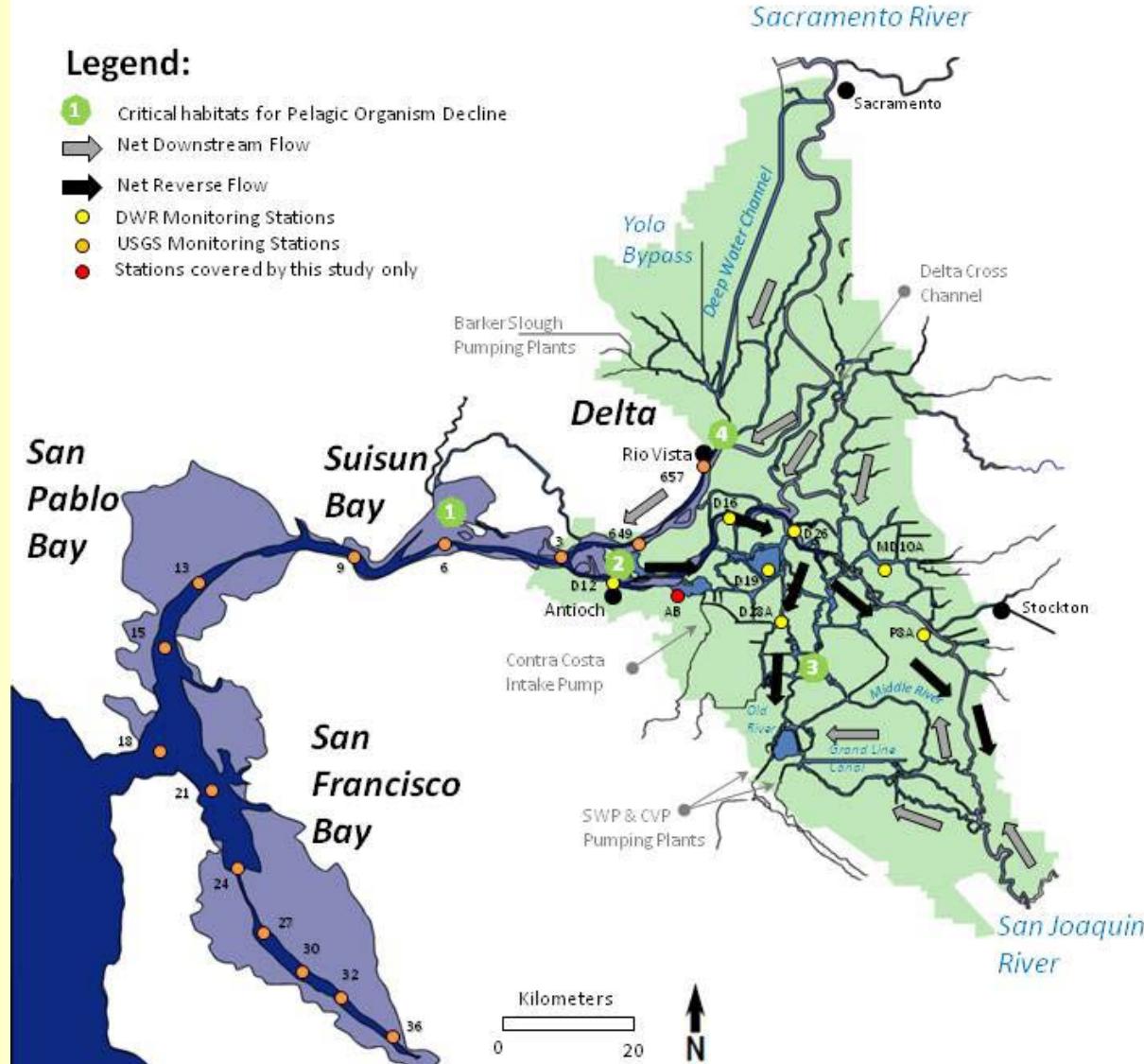
- Establishing a baseline
- Identifying HAB drivers

## Strategy:

- Monthly monitoring @ 21 stn
- Variables: toxins, HA cells abundance, DOC, nutrients, ...

### Legend:

- 1 Critical habitats for Pelagic Organism Decline
- Net Downstream Flow
- Net Reverse Flow
- DWR Monitoring Stations
- USGS Monitoring Stations
- Stations covered by this study only



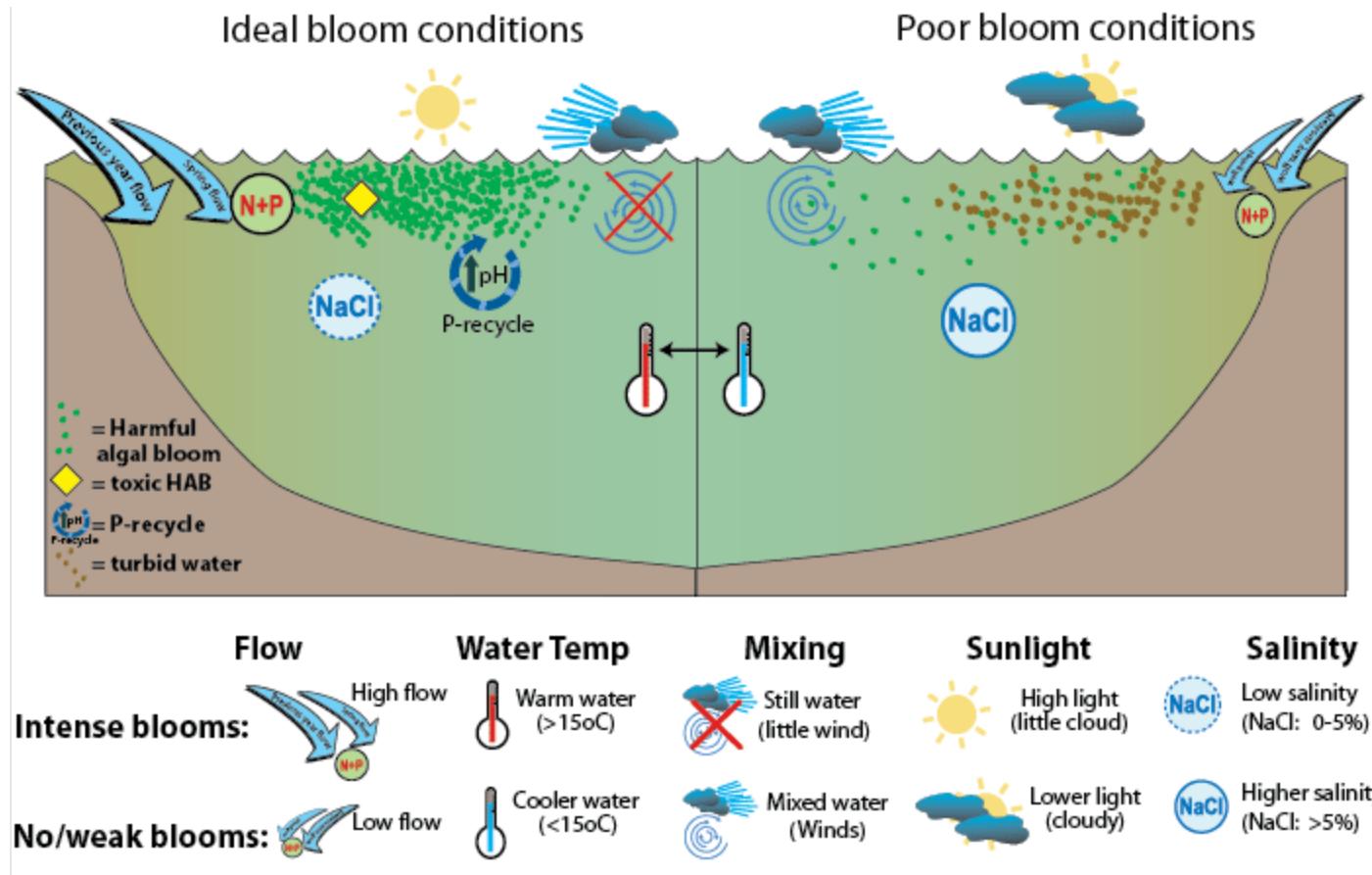
# Microcystis in the Delta

- 1980's: Increase in cyanobacteria biomass, decline of diatoms (Lehman & Smith, 1991)
- 1999: First Microcystis bloom (Lehman, 2000)
- Now: present throughout Delta (Lehman et al., 2005, 2008, 2010)
- Coincided with Pelagic Organism Decline (Delta Smelt, Striped Bass, Threadfin Shad, Chinook Salmon, Copepods)
- Coincided with environmental changes:
  - Increased water transparency
  - Increased temperature
  - Increased salinity (specific conductance)
  - Increased residence time

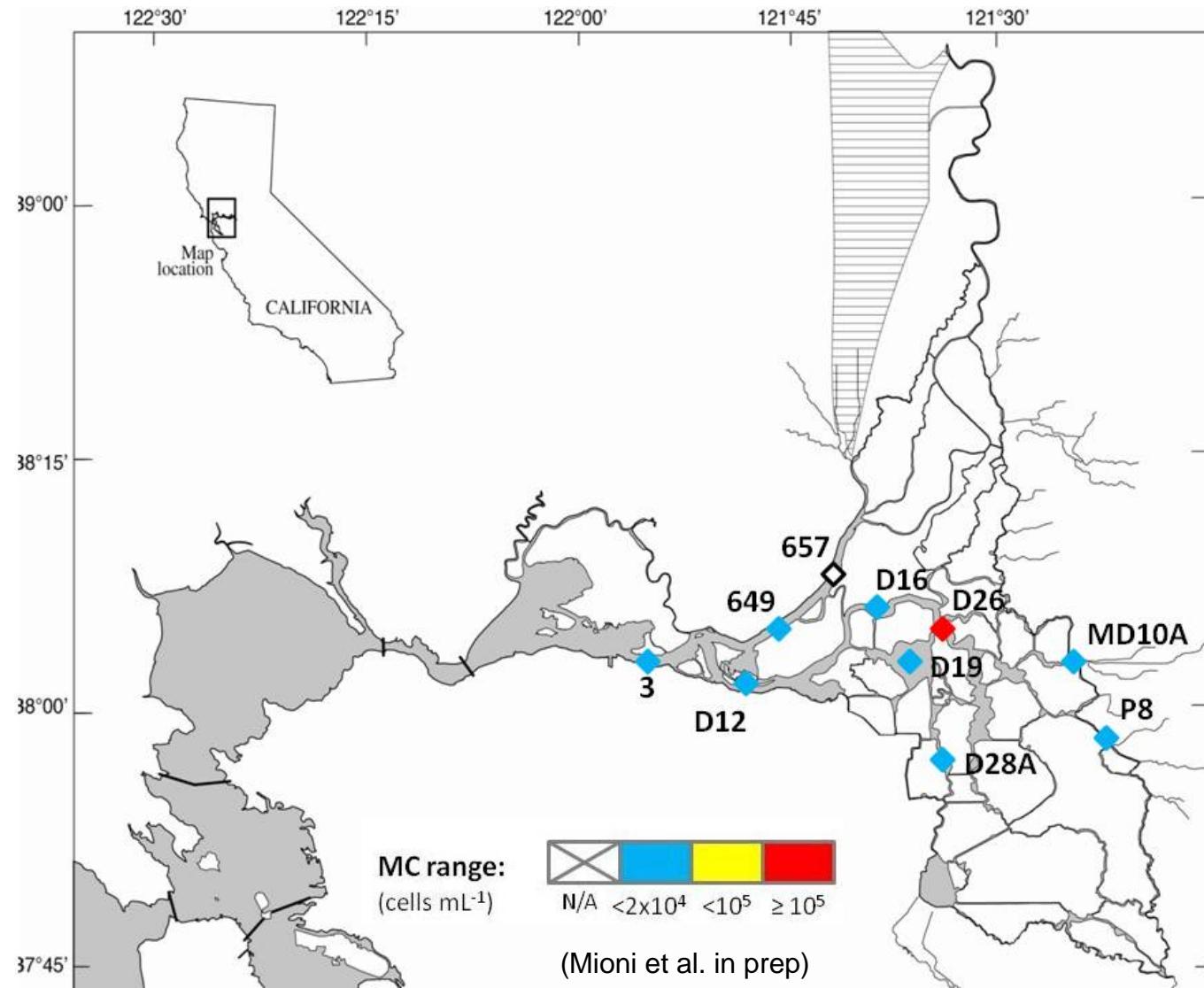


Microcystis Bloom 2008  
[www.water.ca.gov/ssr/microcystis.cfm](http://www.water.ca.gov/ssr/microcystis.cfm)

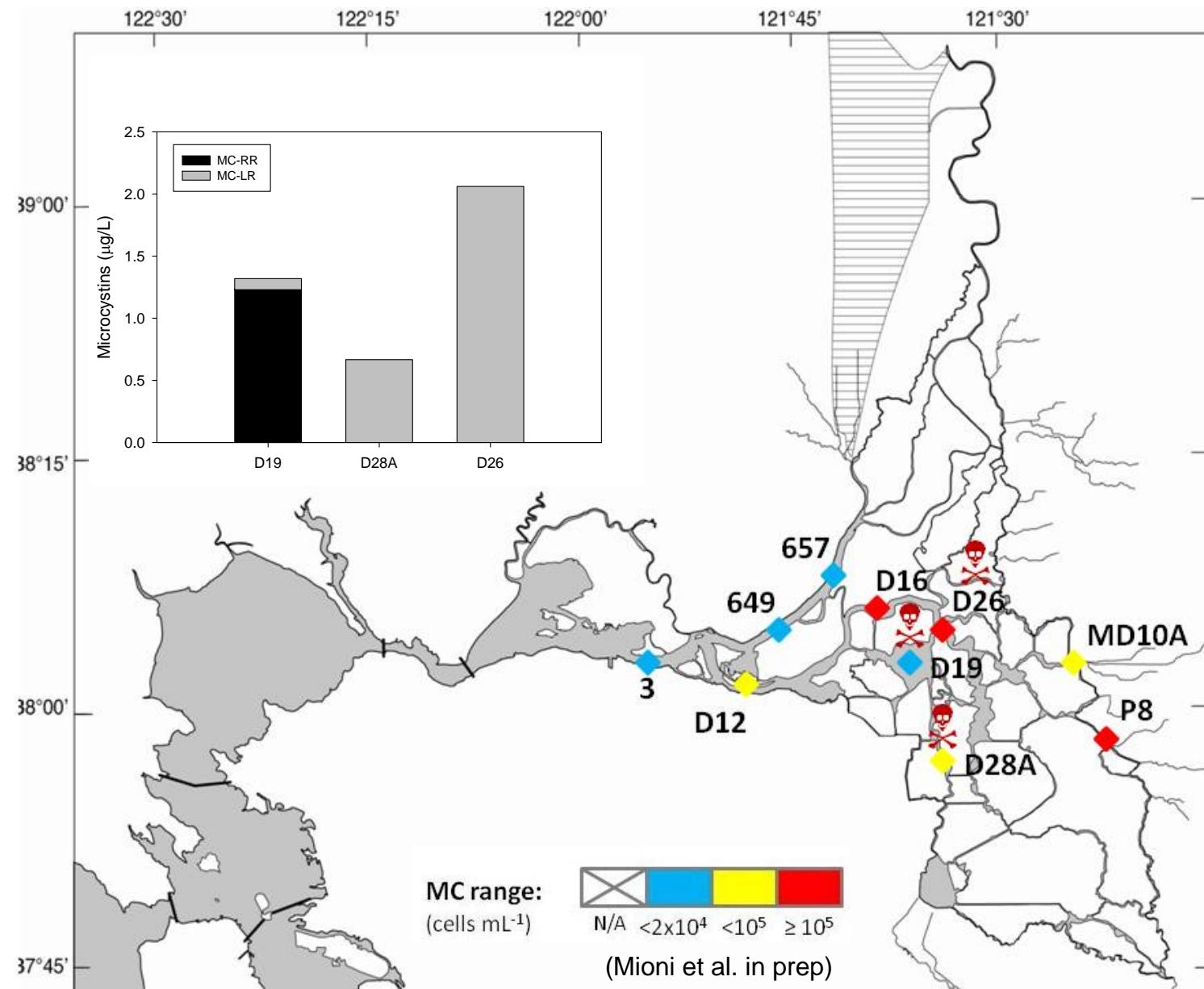
# Microcystis blooms : Environmental drivers



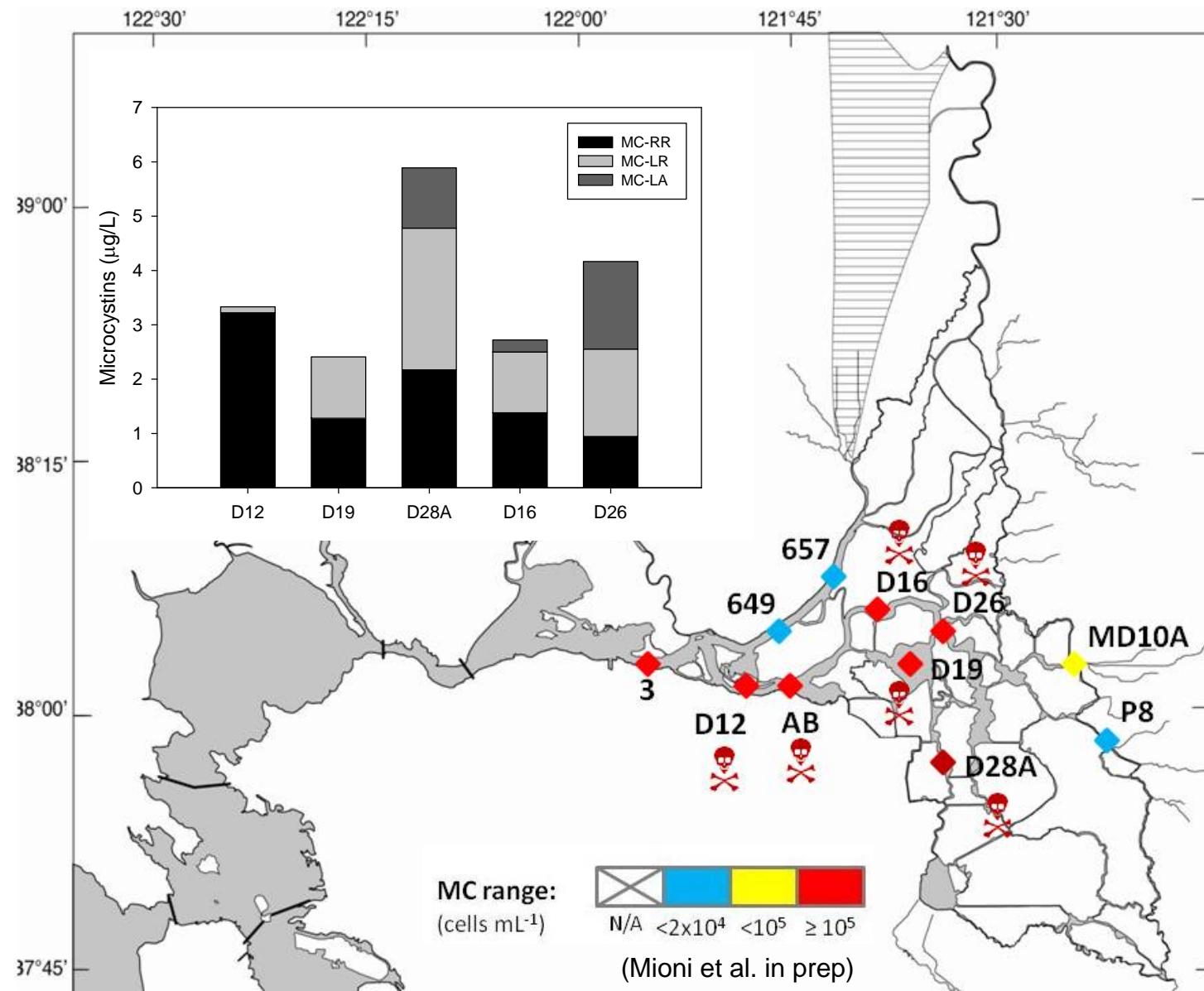
# June 2009



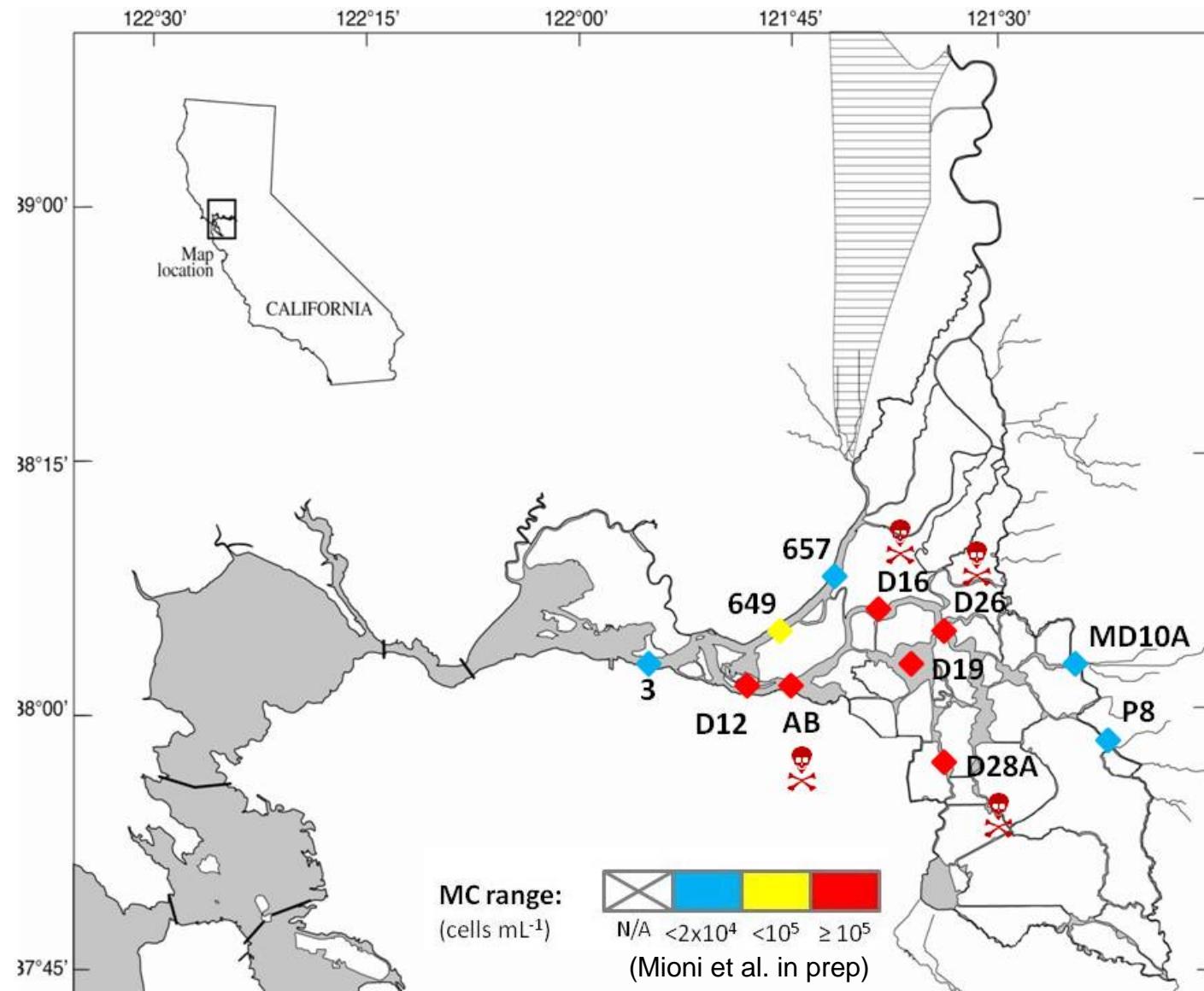
# July 2009



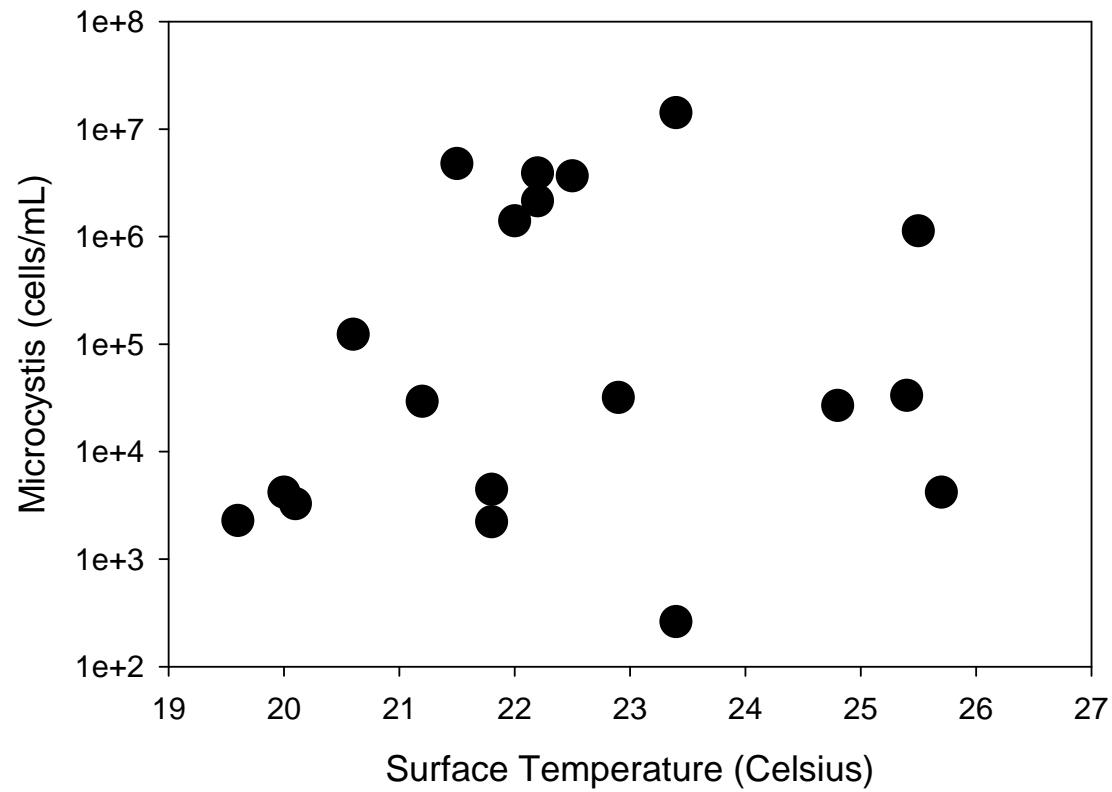
# August 2009



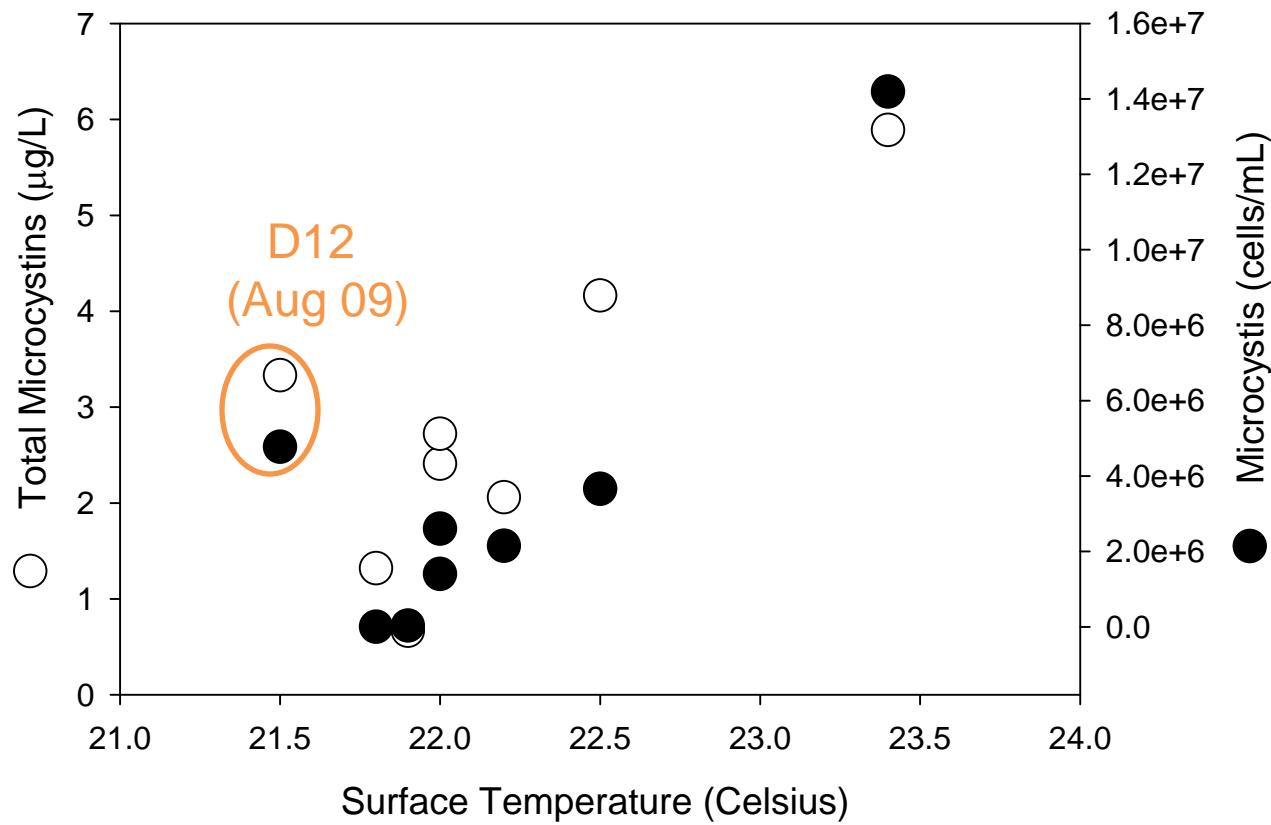
# September 2009



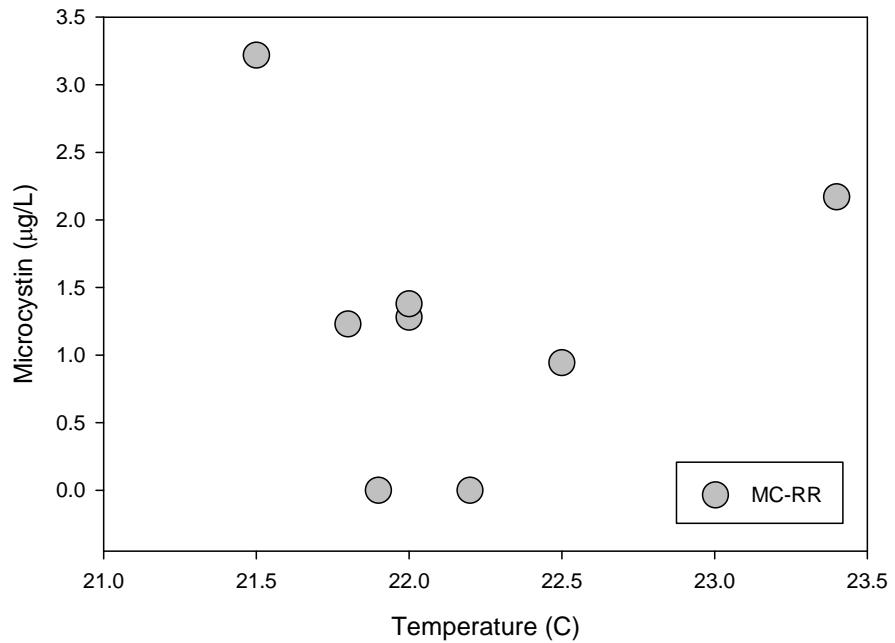
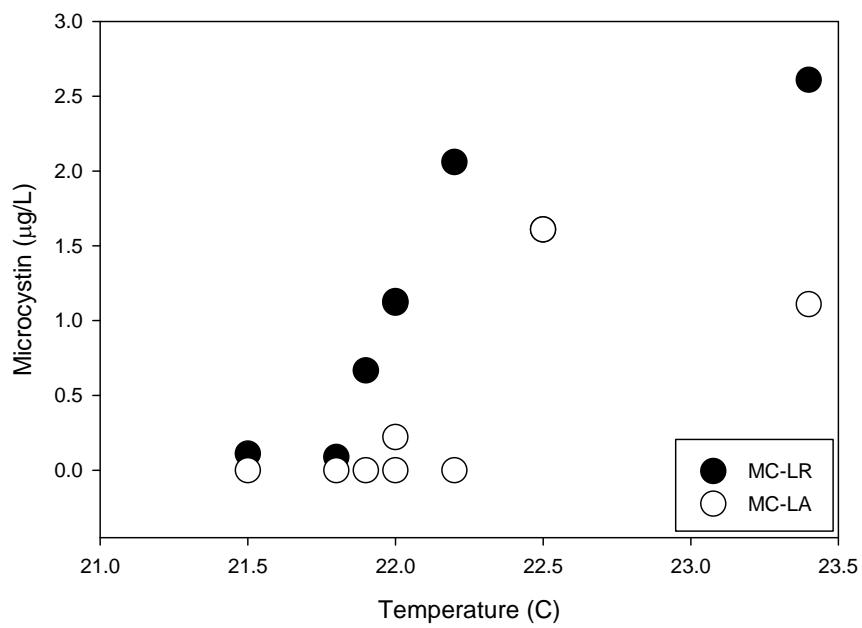
# Temperature (all stations)



# Temperature (only toxic stations)

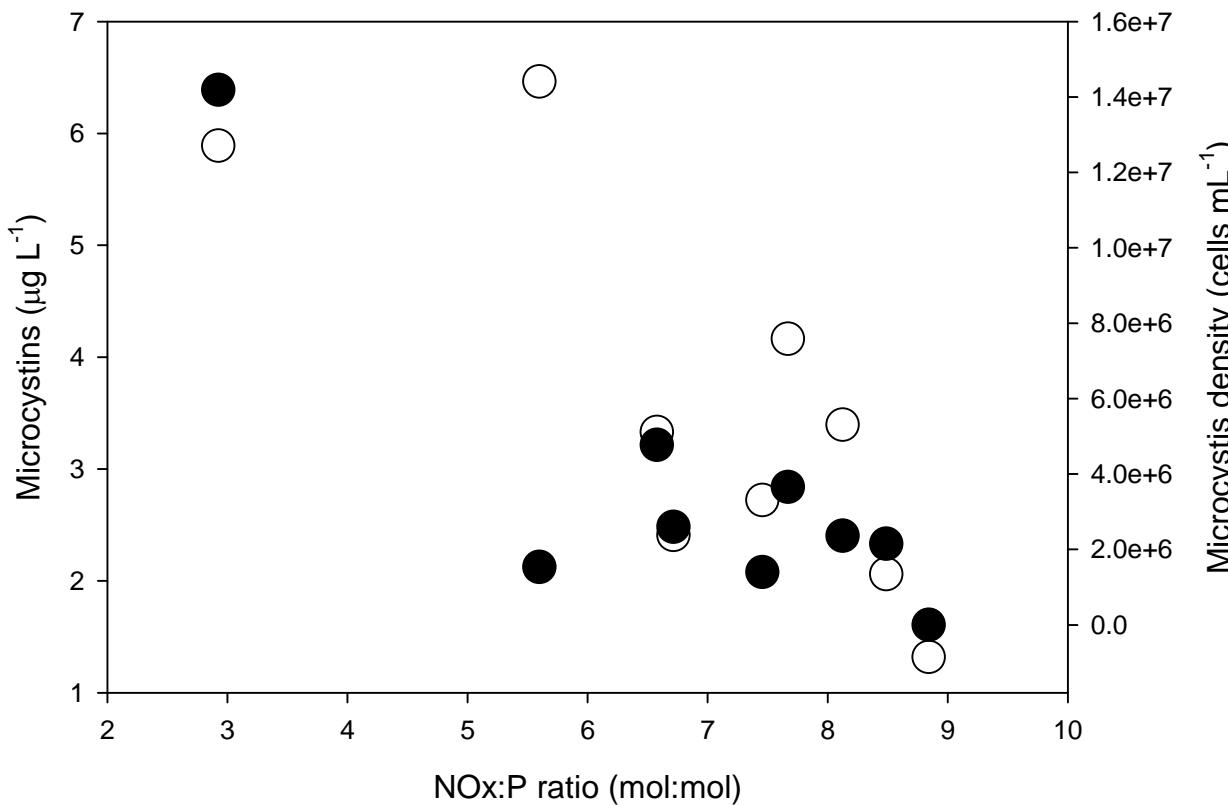


# MC-variants vs Temperature

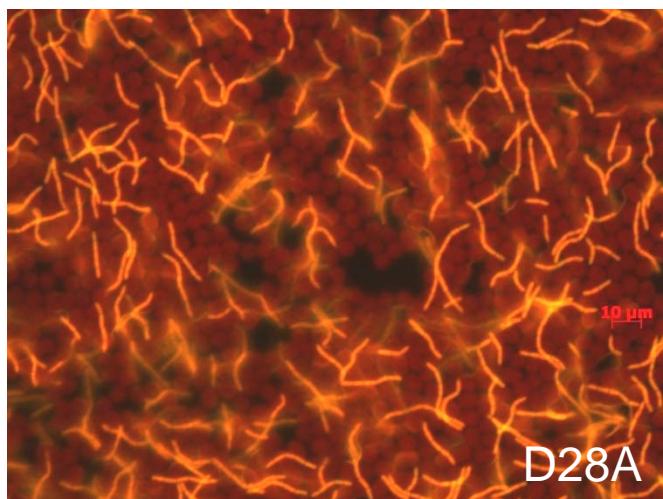
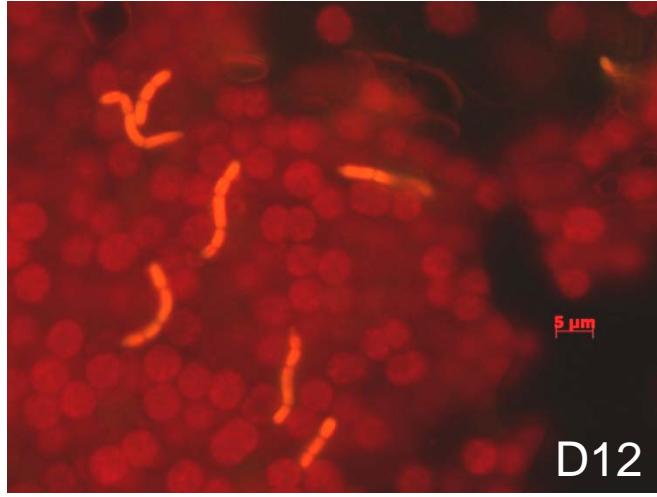


Different strains/variants? Different requirements?

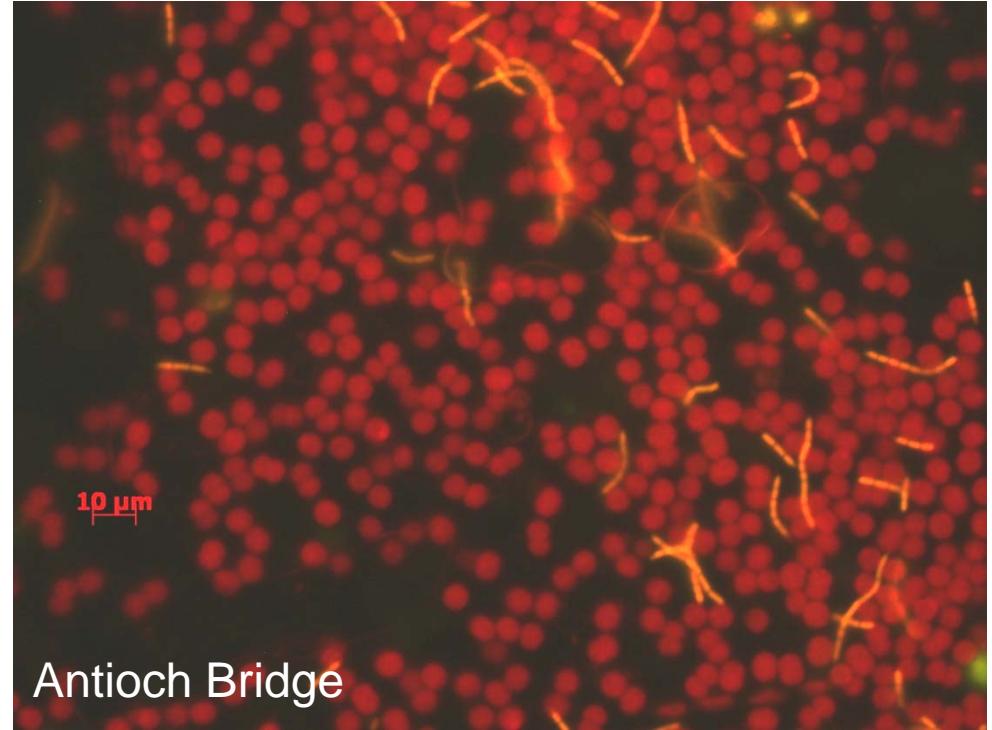
# The lower the NOx:P ratio, the greater the toxicity?



# Microbial Associations?



(x40, blue: 450-490nm)



(x40, blue: 450-490nm)

- Single *Microcystis* cells vs. Colonies
- Associated filamentous cyanobacterium

(Mioni et al., in prep.)



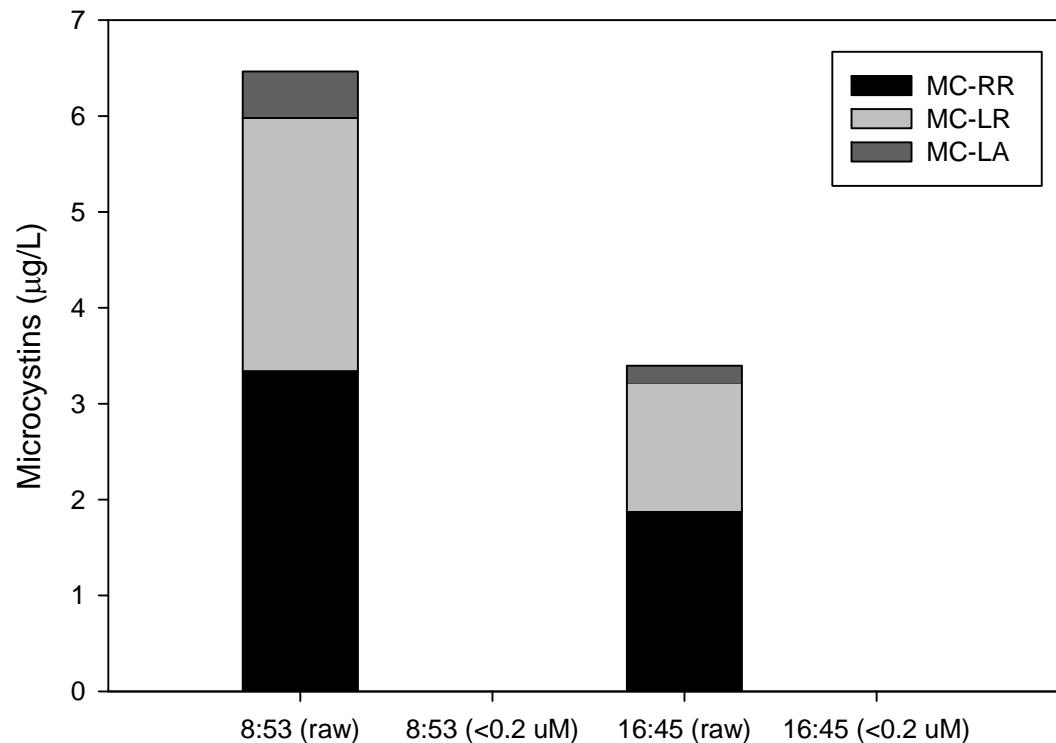
Ashley Mendoza © 2009



# DIURNAL VARIATIONS

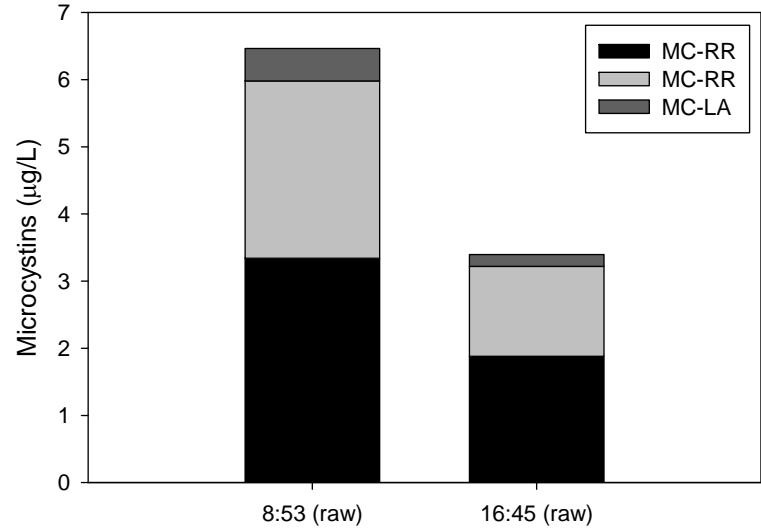
# MC variations with time

Antioch Bridge - 08/13/2009

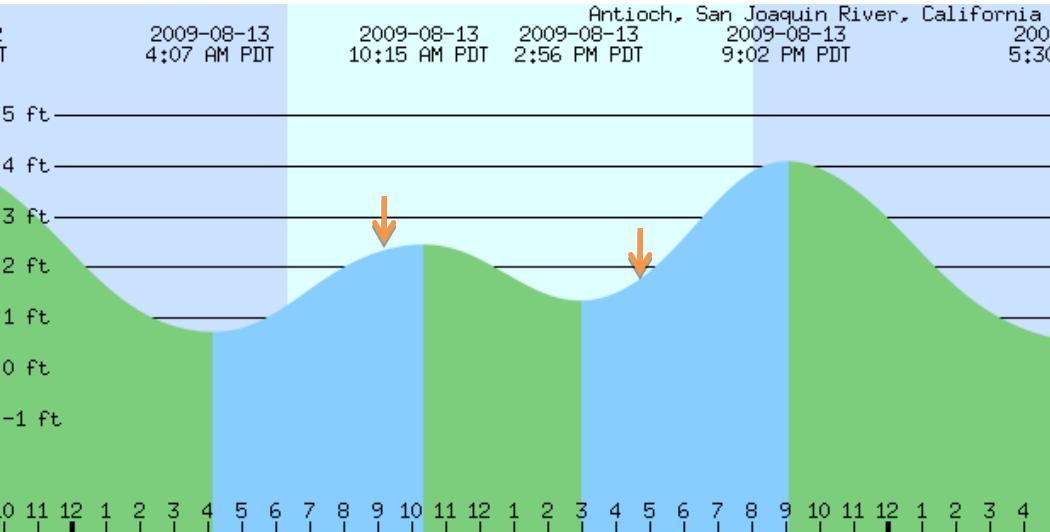


# Why these diurnal variations?

Time	NH <sub>4</sub> <sup>+</sup> (μM)	NO <sub>3</sub> <sup>-</sup> (μM)	PO <sub>4</sub> <sup>3-</sup> (μM)	N/P
<b>8:53</b>	0.48	12.58	2.25	5.81
<b>16:45</b>	0.23	15.23	1.87	8.25

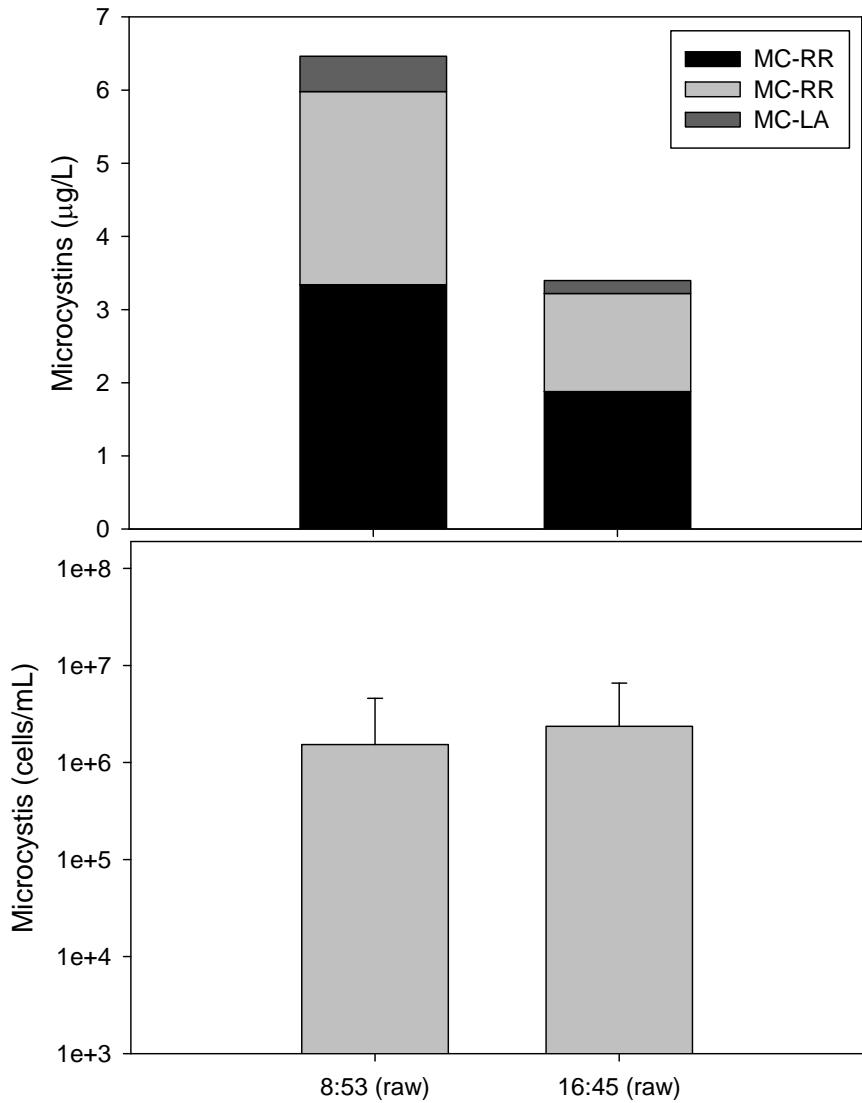
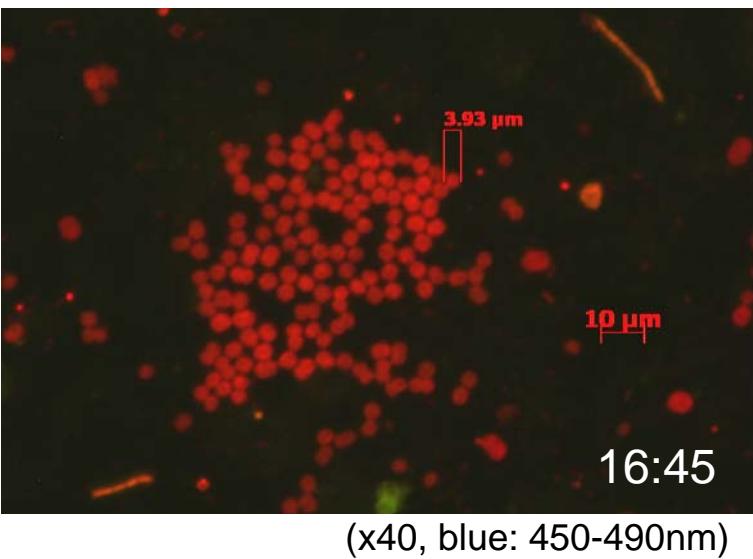
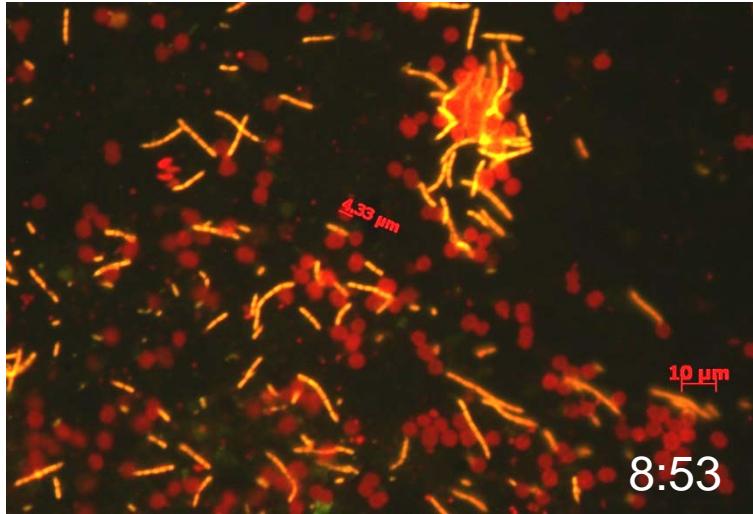


(Mioni et al., in prep.)



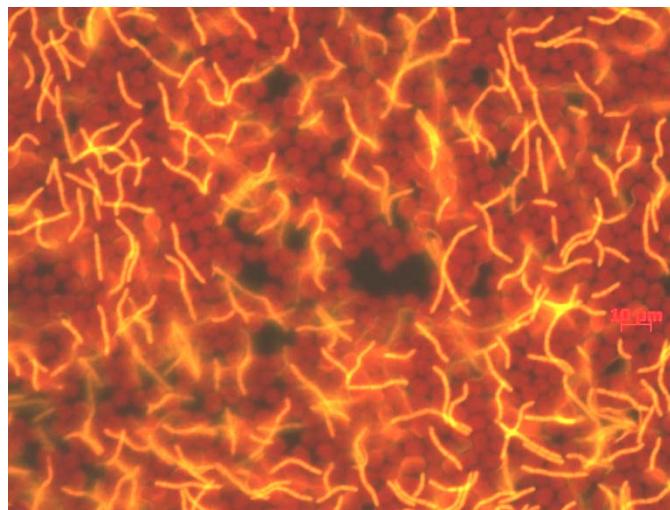
- Different water mass signatures
  - Different microbial community?
  - Horizontal transport?

# Why these diurnal variations?

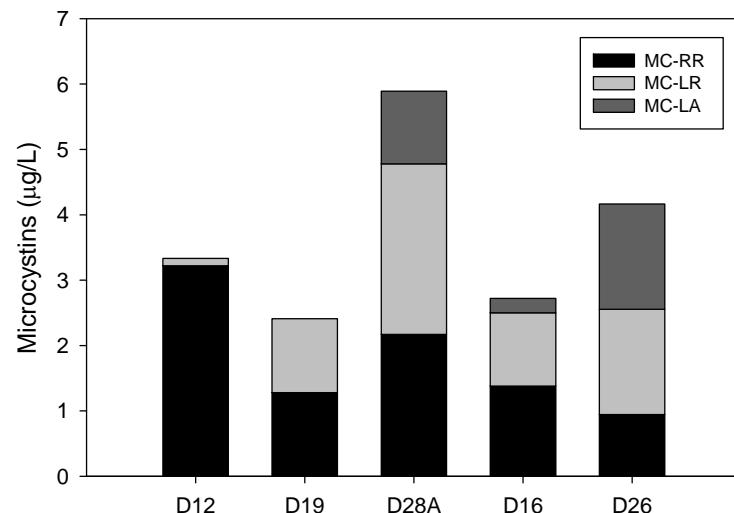


(Milioni et al., in prep.)

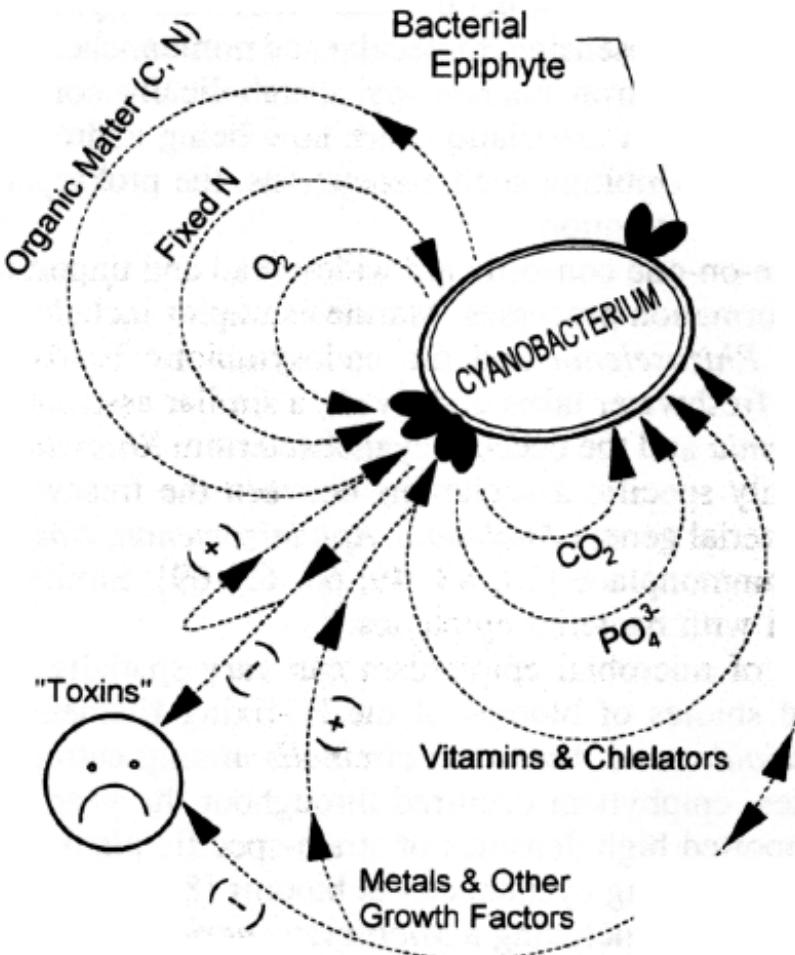
# They were at D28A in Aug. 09 too!



August 2009



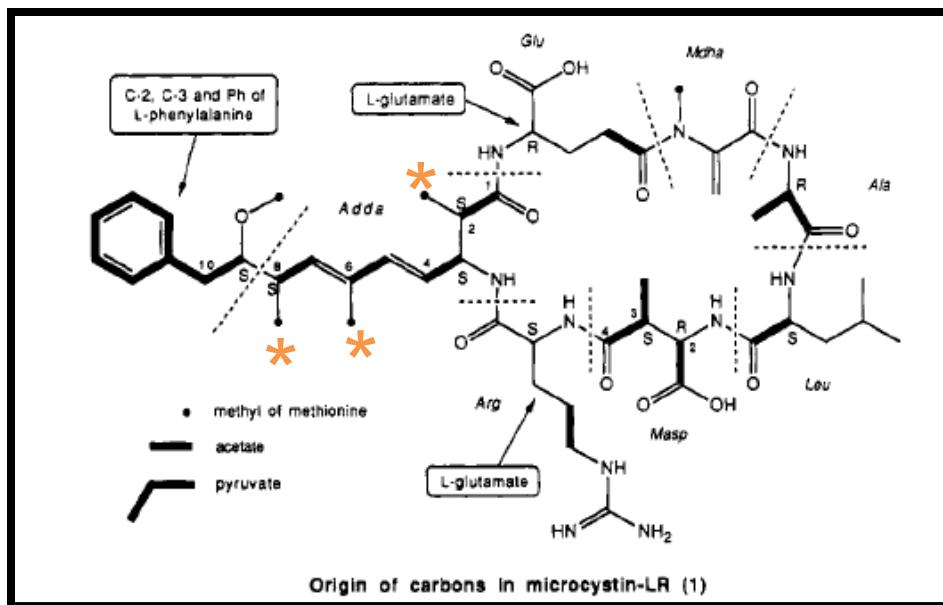
# Role of biological drivers in Microcystin toxin production?



(Pearl & Pinckney, 1996)

# Methionine

## Role in Microcystin Production



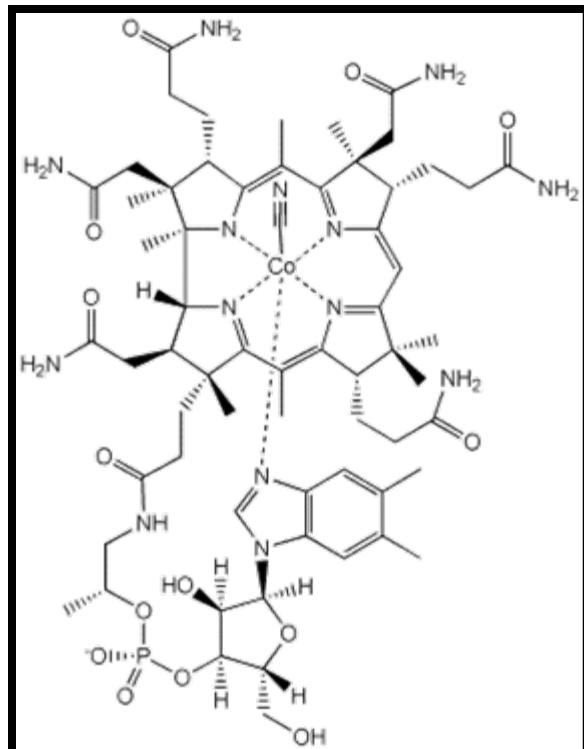
(Moore, Chen et al. 1991)

**Methionine: important source of methyl groups for microcystins and cyanotoxins**

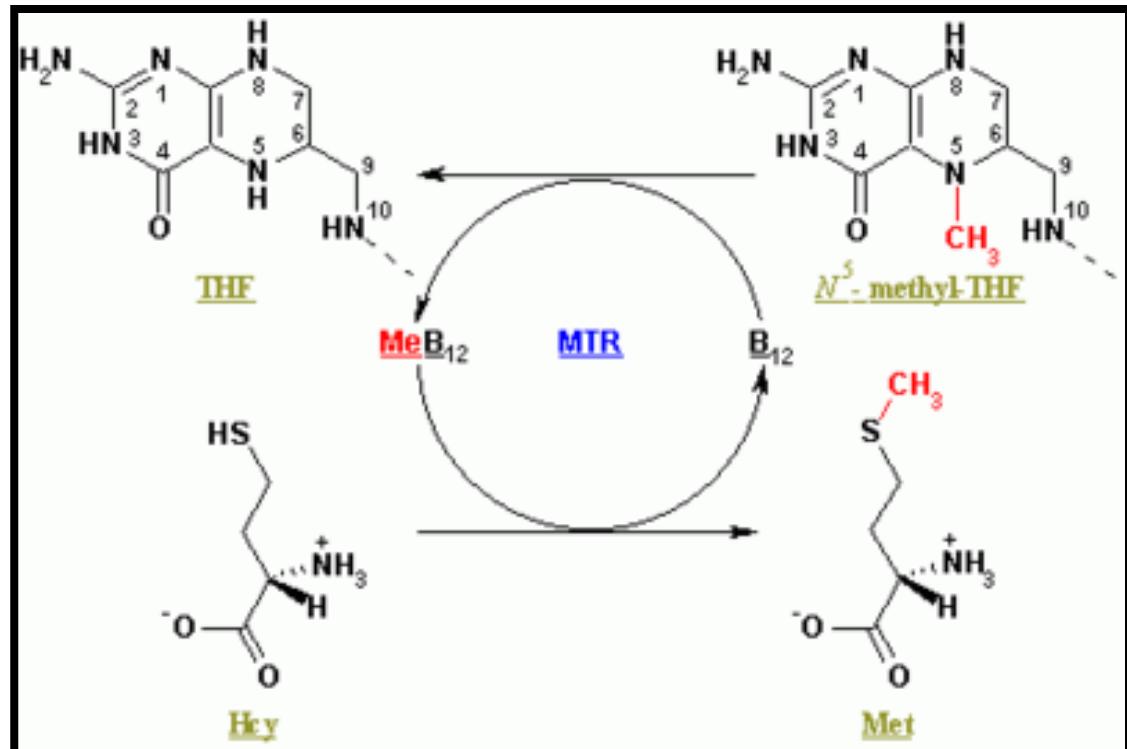
The methyl groups on C-2, C-6 and C-8 of the ADDA portion of microcystin derive from methionine.

# Cobalt and Vitamin B12

## Drivers of Community Structure and Function?



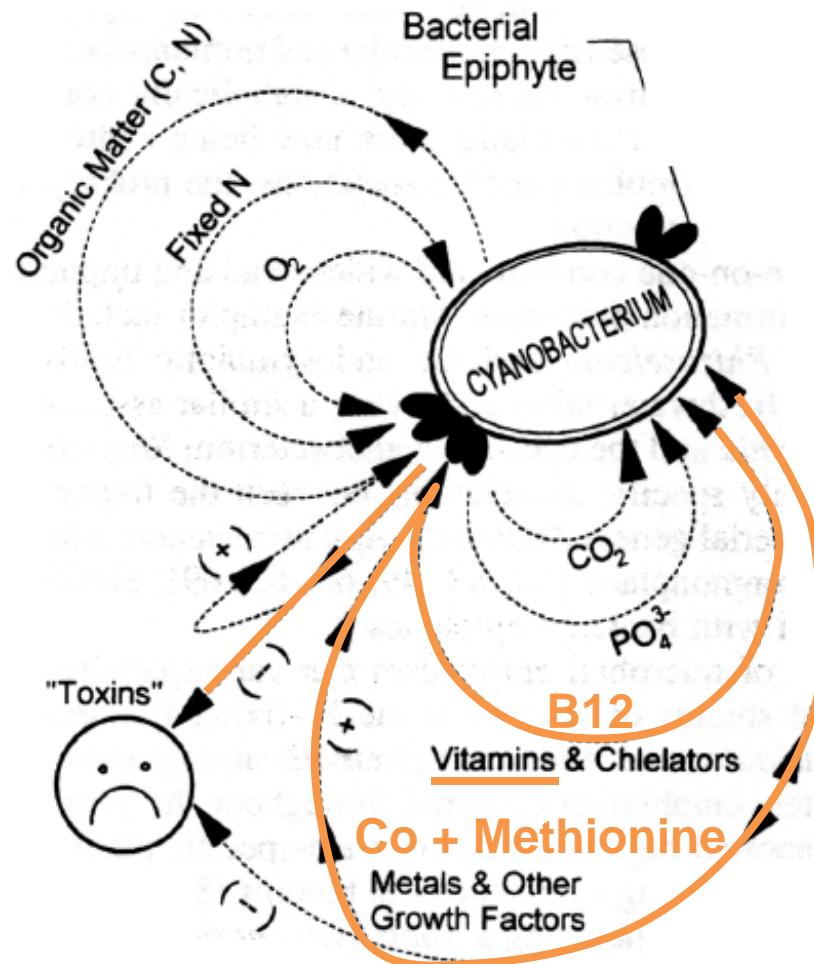
**Vitamin B12**  
(Cobalamin/Cyanocobalamin)



**Methionine Biosynthesis**

(source: wikipedia)

# Role of biological drivers in Microcystin toxin production?



(Pearl & Pinckney, 1996)

# Conclusions

- Microcystins levels > 1µg/L at most stations during bloom.
- *Microcystis* abundance : high risk to very high risk levels.

Date	Microcystis (cell/mL)	Microcystis (µg/L)	Method	Source
2008/9	0 – 1.4x10 <sup>7</sup>	0 – 6.46	LCMSMS	This study
2007	7.7x10 <sup>4</sup> – 9.9x10 <sup>7</sup>	0.007 – 10.81	PPIA	Baxa et al. 2010
2005	0 – 32 x 10 <sup>9</sup>	0 – 60 (ng/L)	PPIA HPLC	Lehman et al. 2010

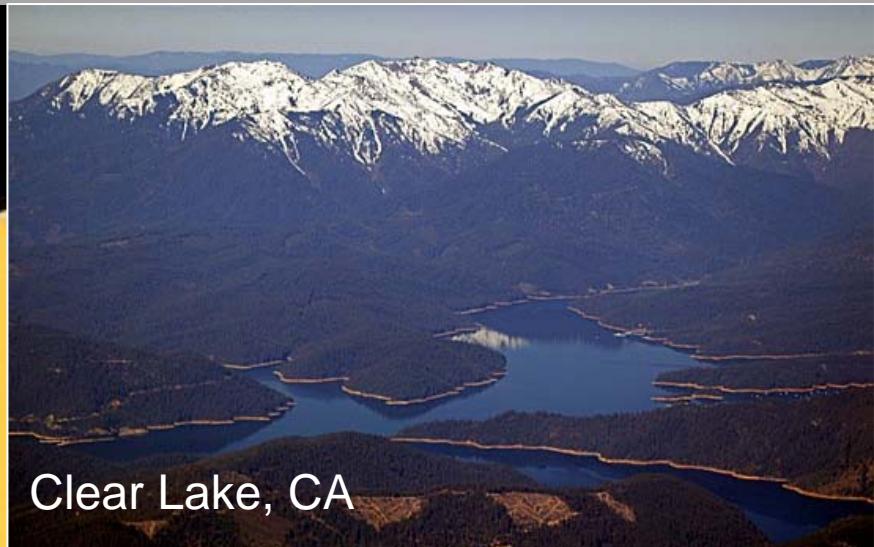
- Total microcystins concentrations correlate with surface water temperature. Different variants? Different needs?

# Problems to solve

- Integrate strong short-time scale variations.
  - Vertical transport
  - Horizontal transport
  - Microbial consortia
- Identify associated filamentous cyanobacteria.
- Determine the Function of these cyanobacterial consortia.

# Future work

We can do it!



Clear Lake, CA

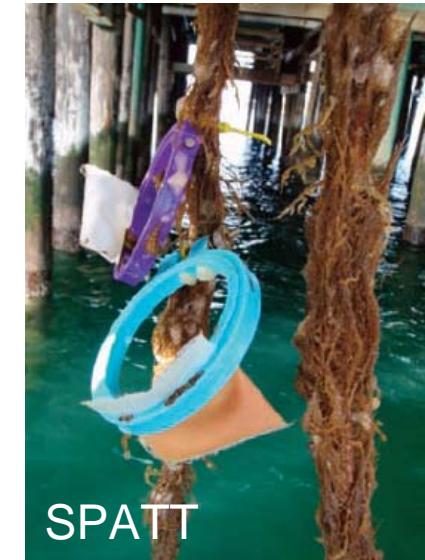


The Delta, CA

# New Strategies

- **Integrating temporal variations:**

- ✓ SPATT = Solid Phase Adsorption Toxins Tracking  
(Raphael Kudela, UCSC)



- **Microbial community in a snapshot:**

- ✓ PhyloCHIP : DNA barcoding microchip  
(Gary Andersen, LBNL).
- ✓ Distinguish 50,000 microbial species simultaneously  
(353 cyanobacteria, 184 algae).
- ✓ Microbial interaction (association, allelopathy)





# Thank you!

