Cyanobacteria & Cyanotoxins: Recent Progress Toward Understanding Impacts on Water Quality

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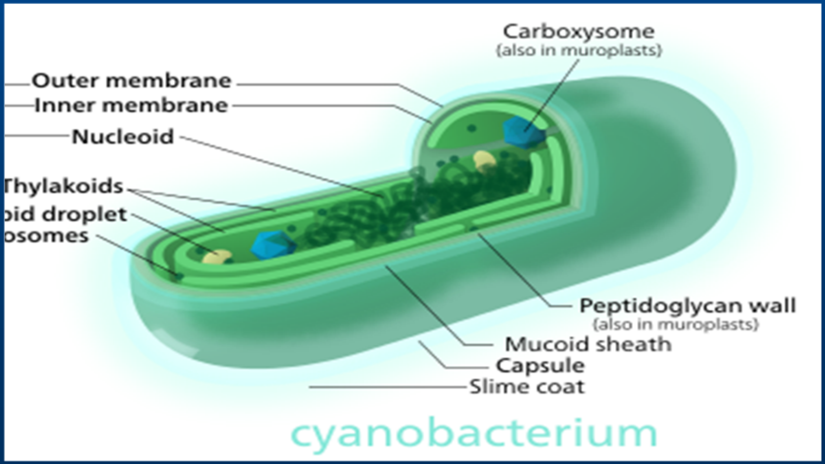
October, 2013

Overview

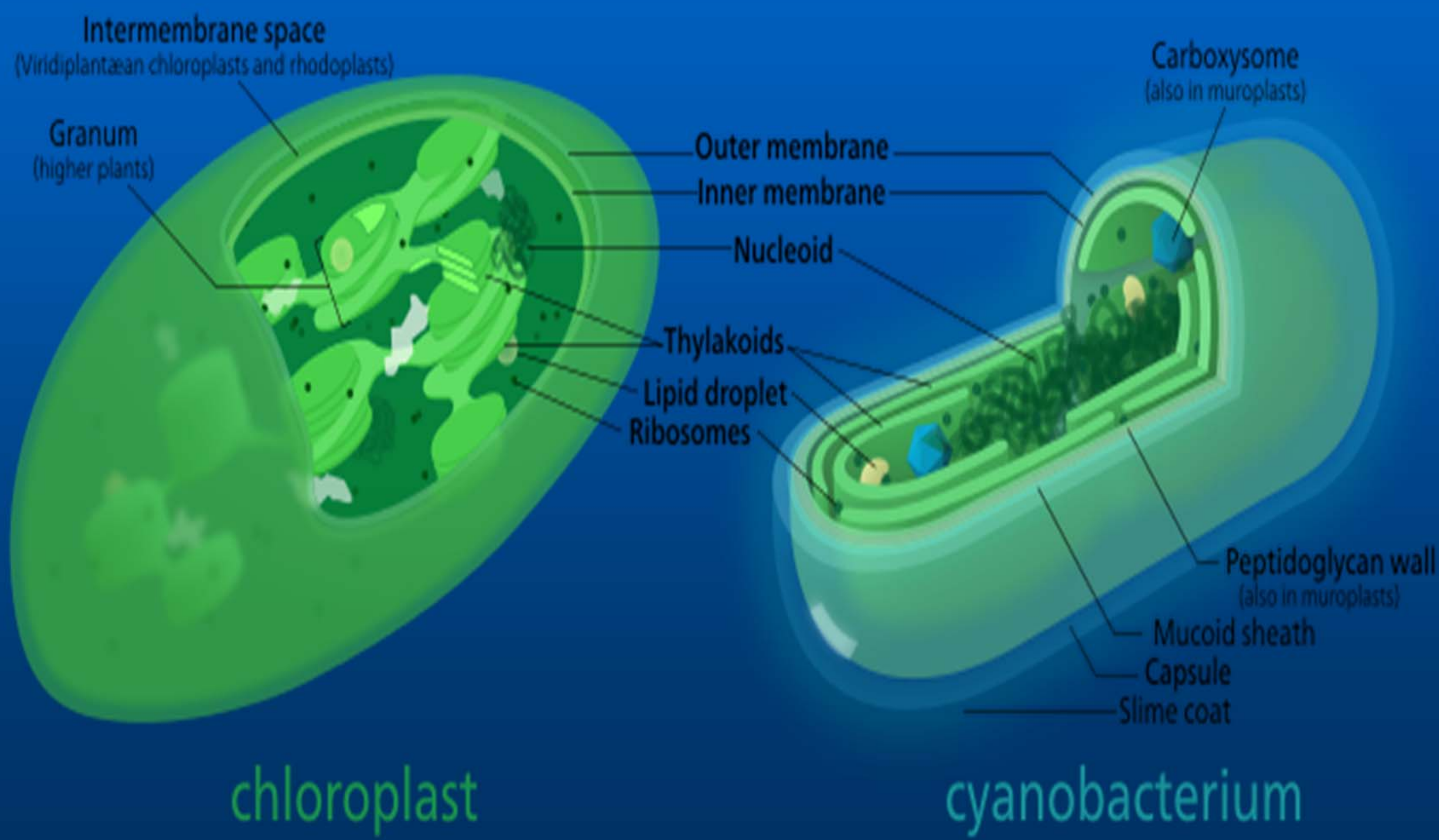
- CyanoHAB blooms have increased in freshwater habitats in the U.S. & globally (Paerl & Otten, 2013)
- Some effects include toxicity to humans, mammals, vertebrates, invertebrates, and some green algae and higher plants due to production of a growing list of known cyanotoxins, plus additional suspected cyanotoxins (Corbel et al., 2013)
- No national active surveillance program for monitoring/reporting on cyanoHABs in bloom-prone water bodies: information on recurring blooms in CA sporadic, beset by numerous types of resource limitations: nevertheless, some impaired CA water bodies have been listed due to impacts

What are the Cyanobacteria?

- *Not* “Blue Green Algae”: Not Eukaryotic Algae, & Not Always Blue-Green (Chromatic Adaptation, Multiple Accessory Pigments)
- Anaerobic Photosynthetic Bacteria: Common in Surface Soils & Surface Waters
- Tolerant of High pH, High Turbidity, Elevated Water Temps: Some “Extremophiles”
- Can Reduce N_2 & CO_2, Use NH3/NH4 as N-Source, N2-Fixation, Use NO3 & NO2 as N-Source
- Some Unicellular &/Or Colonial as Filaments, Hollow Balls, Mats, etc.: Some Endosymbionts /Symbionts(e.g., in lichens & higher plants [Azolla, Sago Palm])
- “Ecoservices”: Biogeochemical Cycling of N, Help Pump O2 Into Atmosphere, 50% of Marine Photosynthesis, Chloroplast “Inventors”
- 3.5 Billion Years of Horizontal Gene Transfer Events & Mutations



Chloroplasts As Descendants of Ancient Endosymbiotic Cyanobacteria (Goksoyr, 1967)



Color Variation: A "Hallmark" of Cyanobacterial Blooms

Left: Aging Bloom in Freshwater in PRC

Right: Marine Bloom of Trichodesmium

Mixed Cyanobacterial/Eukaryotic Algal Communities Are Common, Even In Extreme Environments:

Yellowstone's Geothermal Pools & Benthic Mats in Antarctic Lakes

Cyanobacteria Versus Other Phytoplankton

- *Eukaryotic algae grow faster largely because they can thrive in full sunlight & have more efficient aerobic metabolism (e.g., Cladophora)*
- **Cyanobacteria**
  - ✓ Out-compete green algae for nutrients (N,P)
  - ✓ Indifferent to high pH & low O2, utilize Ammonia
  - ✓ Photosynthesize more efficiently in turbid waters
  - ✓ Engage in “chemical warfare” with numerous bioactive compounds (“allelopathy”, other interactions)
  - ✓ Some can fix nitrogen from the atmosphere

CyanoHAB Genesis in Freshwater: Global Trends & Geographic Distribution in North America

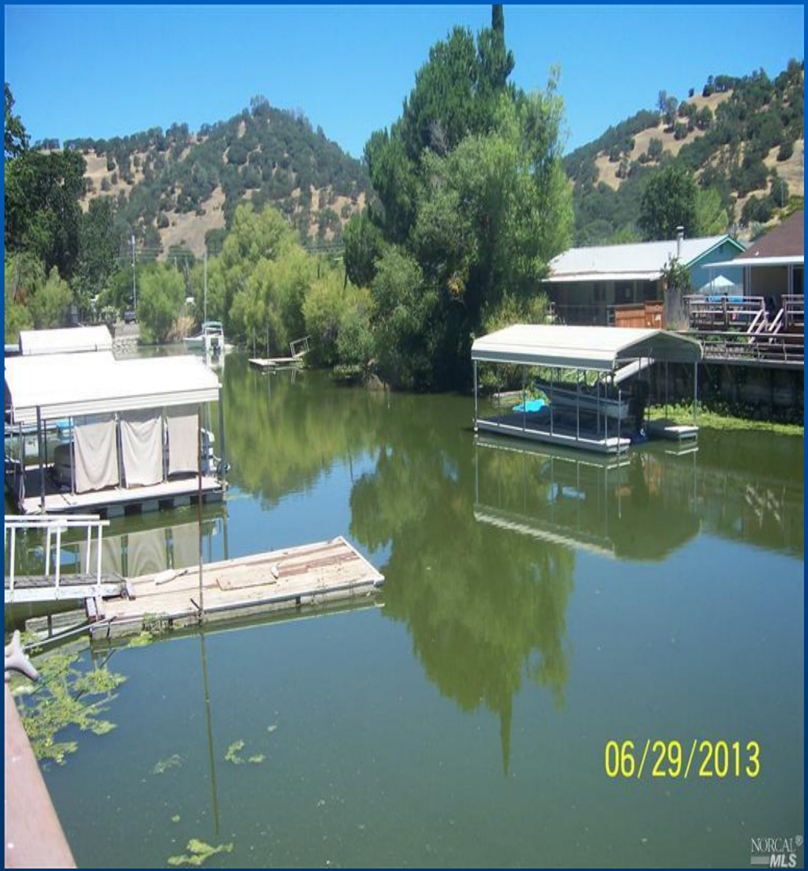
- 3.5 BY of adapting to geochemical & climatic change (Paerl & Otten, 2013)
- Anthropogenic modification of aquatic environments favor bloom formation, e.g., eutrophication, water diversions, alterations in watershed hydrology, and salinization: many cosmopolitan freshwater taxa exhibit optimal growth @ increased surface water temperatures – hence increased size, duration, and frequency of potentially toxigenic blooms
- CyanoHAB harmful environmental effects on ecosystems include: cyanotoxins, out-competing eukaryotic phytoplankton, DO depletion when blooms enter senescence.

One Net Result Seems To Be An Nationwide Occurrence of Toxigenic Blooms

- U.S.A., 2007: National Lakes Assessment survey found 42% of samples exceeded microcystin concentrations of 10 ppb (WHO 1999 guidelines recommend 1 ppb for drinking water); 30% of lakes sampled had MCYNs
- Cyanotoxins found in all 48 states; most abundant genera identified were potential cyanotoxin producers (*coastal Hawaii is subject to toxigenic Lyngbya blooms…)*
- Saxitoxins, cylindrospermopsin, anatoxin-a &/or nodularin found in 8%, 5%, 15, % 3.7% of samples, respectively

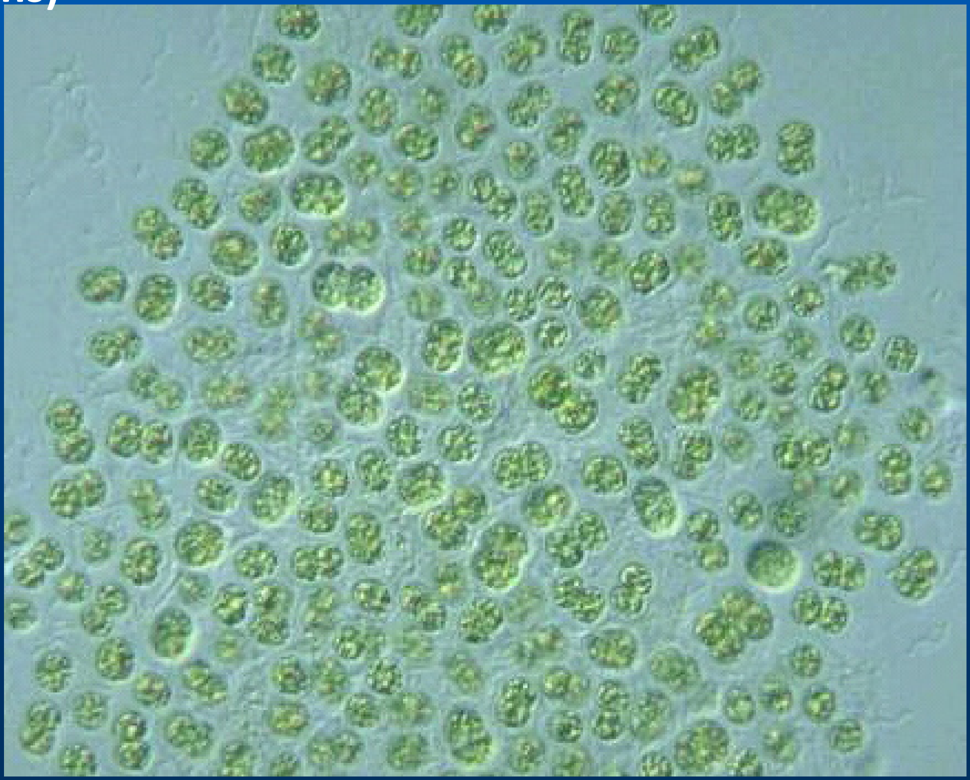
Elevated Nutrient Concs & Shallow Artificial Waterways = "Culture Flasks" For Cyanobacterial Blooms

Left: Clearlake Oaks Keys Right: Irrigation Canal in UK



Bioactive Metabolites of Cyanobacteria

- Cytotoxins – toxins with cytotoxic (cellular) effects
  - ✓ Pharmacological potential – possible antibiotics, chemotherapeutic agents, etc.
- Effects can be acute, acute-lethal, or chronic biological: some bioaccumulate (e.g., 90+ microcystins, nodularins, cylindrospermopsin, 17+ members of "PSP" saxitoxin group)
- Potential Human/Animal Exposure Pathways: Aerosols, Food, Water, Dermal, IV
- Colorless, Odorless, Tasteless (To Humans)
- Cyclic protein toxins resistant to heating & freezing
- Can be recalcitrant to conventional water treatment technologies
- Resistance develops with repeated treatment with $CuSO_4$, H_2O_2, etc.
- Toxin (& other) genes transferred among related taxa
- Toxins also found in marine taxa
- Whole-cell extracts almost invariably produce more toxicity than individual toxins



Cyanotoxins – 3 Major Modes of Action In Animals & Humans

- **Neurotoxins**
  - Anatoxins
  - PSP toxins
  - Anatoxin-a(S)
- **Hepatotoxins +**
  - Microcystins (& Neuro/Cardio?)
  - Cylindrospermopsins (& Kidney, Lymphatics….)
- **Dermatoxins**
  - Lyngbyatoxins (& GI Tract)
  - Aplysiatoxins

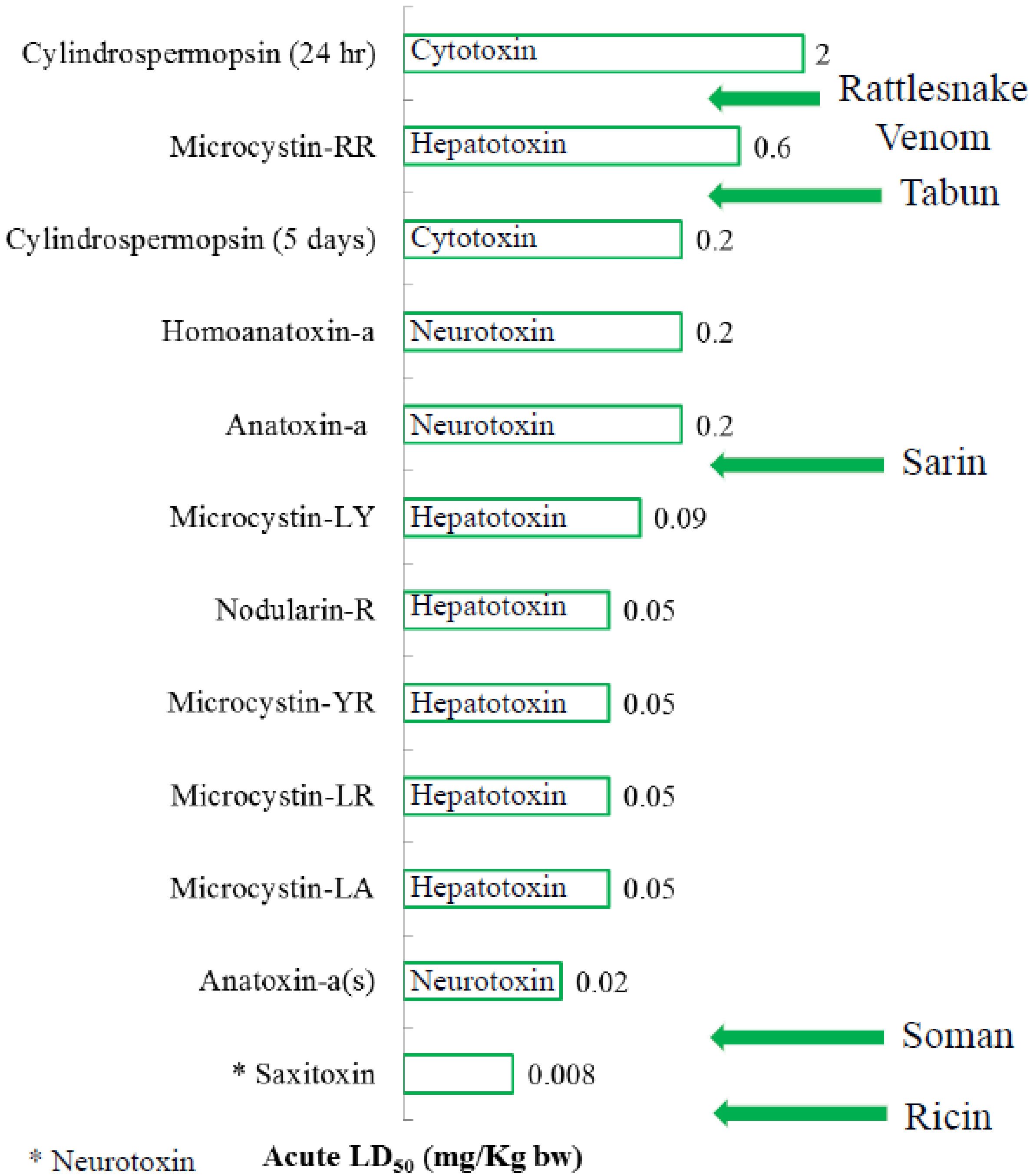
Cyanotoxins: The List Keeps Growing (Castle & Rodgers, 2009)
A *Diverse Assortment of Cyclic Peptides & Alkaloids*

Table 3. Toxins Produced by Modern Cyanobacteria

Toxin	General Characteristics	Taxa-Producing Toxin	Structure and Activity	References
Cyclic peptides				
Microcystins	Hepatotoxin, liver toxin	*Microcystis, Anabaena, Planktothrix (Oscillatoria), Nostoc, Haplosiphon, Anabaenopsis, Nodularia, Anacystis, Gloeocapsa, Synechococcus, Eucapsis, Aphanocapsa, Rivularia, Entophysalis, Schizothrix, Phormidium, Microcoleus*	Cyclic heptapeptides; hepatotoxic, protein phosphatase inhibition, membrane integrity, and conductance disruption, tumor promotors	Carmichael (1997), Falconer (1998), Codd et al. (2005)
Nodularins	Hepatotoxin, liver toxin	*Nodularia*	Cyclic pentapeptides; hepatotoxins, protein phosphatase inhibition, membrane integrity, and conductance disruption, tumor promoters, carcinogenic	Sivonen and Jones (1999)
Alkaloids				
Anatoxin-a (including homoanatoxin-a)	Neurotoxin-nerve synapsis	*Planktothrix (Oscillatoria), Anabaena, Plectonema, Aphanizomenon, Rhaphidiopsis, Hyella*	Alkaloids; postsymaptic, depolarizing neuromuscular blockers	Sivonen and Jones (1999), Namikoshi et al. (2003)
Anatoxin-a(S)	Neurotoxin-nerve synapsis	*Anabaena*	Guanidine methyl phosphate ester; inhibits acetylcholinestenase	Sivonen and Jones (1999), Namikoshi et al. (2003)
Aplyslatoxins	Dermal toxin, skin	*Lyngbya, Schizothrix, Planktothrix (Oscillatoria), Microcoleus*	Alkaloids; inflammatory agents, protein kinase C activators	Osborne et al. (2001), Mastin et al. (2002), Codd et al. (2005)
Cylindrospermopsins	Hepatotoxins, liver, kidney, and lymphoid tissue	*Cylindrospermopsis, Aphanizomenon, Umezakia, Raphidiopsis*	Guanidine alkaloids; liver necrosis (also kidneys, spleen, lungs, intestine); protein synthesis inhibitor, genotoxic	Lagos et al. (1999), Li et al. (2001), Schembri et al. (2001), Namikoshi et al. (2003)
Lyngbyatoxin-a	Skin, gastrointestinal tract	*Lyngbya, Schizothrix, Planktothrix (Oscillatoria)*	Alkaloids; inflammatory agents, protein kinase C activators	Codd et al. (1999, 2005), Sivonen and Jones (1999)
Saxitoxins	Neurotoxin, nerve axons	*Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis, Planktothrix (Oscillatoria)*	Carbamate alkaloids, sodium channel-blockers	Codd et al. (1999, 2005), Sivonen and Jones (1999)
Lipopolysaccharides				
G^- cyanobacteria	General irritant, affects any exposed tissue	"All" G^- cyanobacteria (prokaryotes)	Lipopolysaccharides; endotoxins, inflammatory agents, gastrointestinal irritants	Sivonen and Jones (1999)
Uncharacterized structure				
Neurotoxin	Brain, vacuolar mylinopathy	Unnamed *Stigonematales* species	Undescribed; avian vacuolar mylinopathy	Birrenkott et al. (2004), Wilde et al. (2005)

Toxicity of Known Cyanotoxins

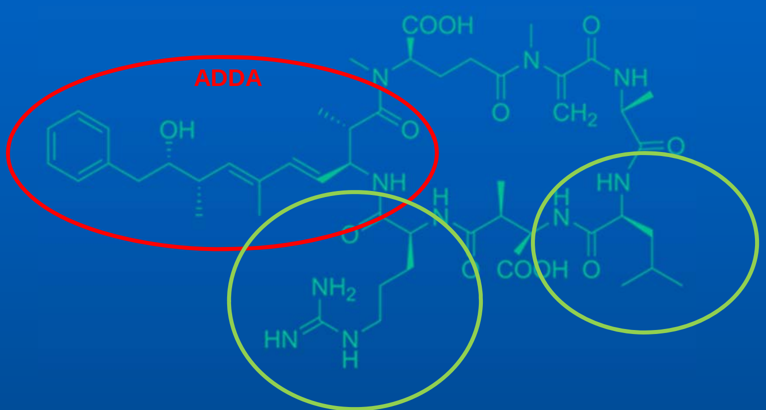
- **Acute Toxicity**
  - Cytotoxic
  - Neurotoxic
  - Hepatotoxic
  - Dermatoxic
  - Respiratory Distress
- **Chronic Toxicity**
  - Carcinogen
  - Tumor Promotion
  - Mutagen
  - Teratogen
  - Embryolethality



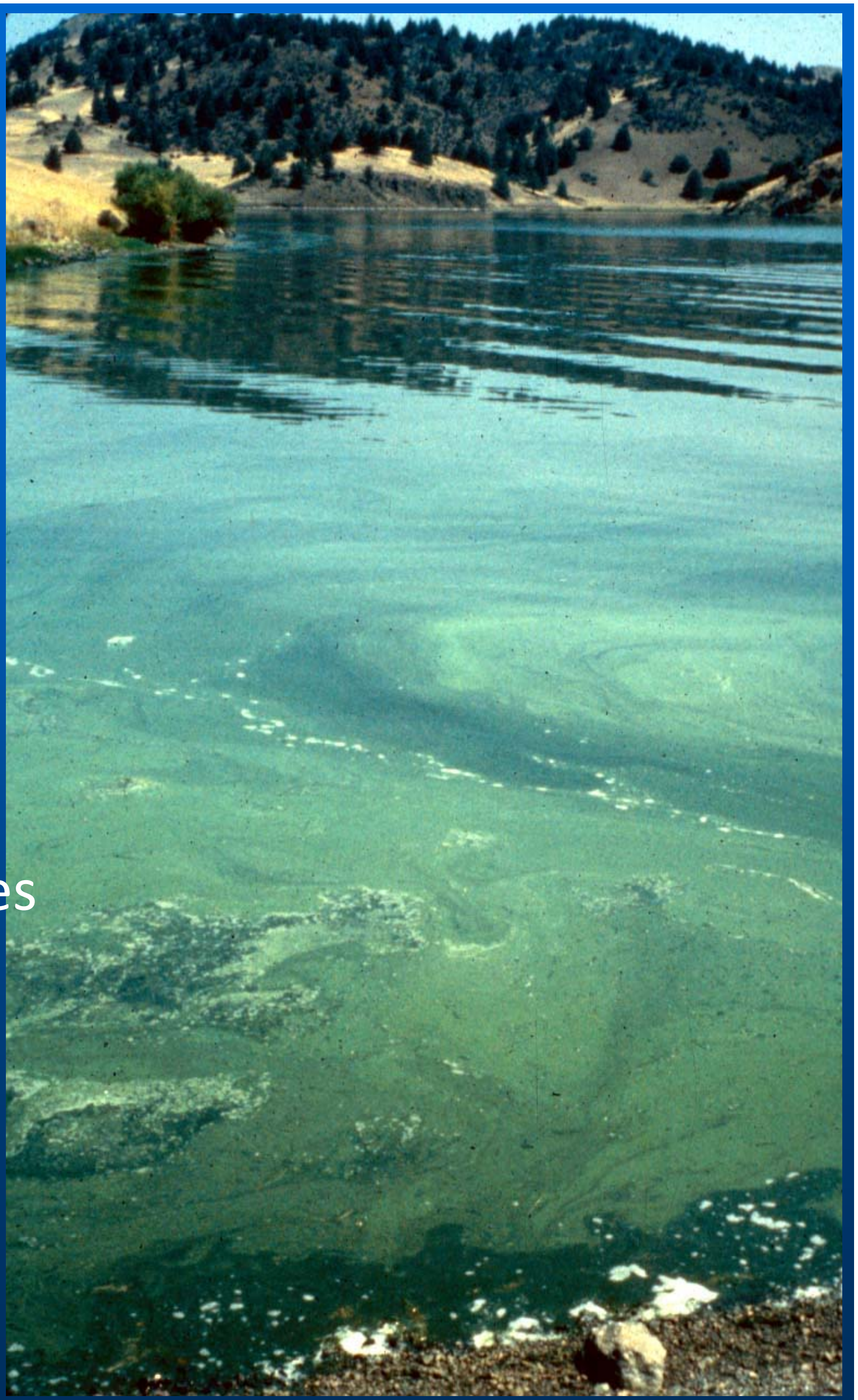
USGS science for a changing world

A Bit More About Microcystins

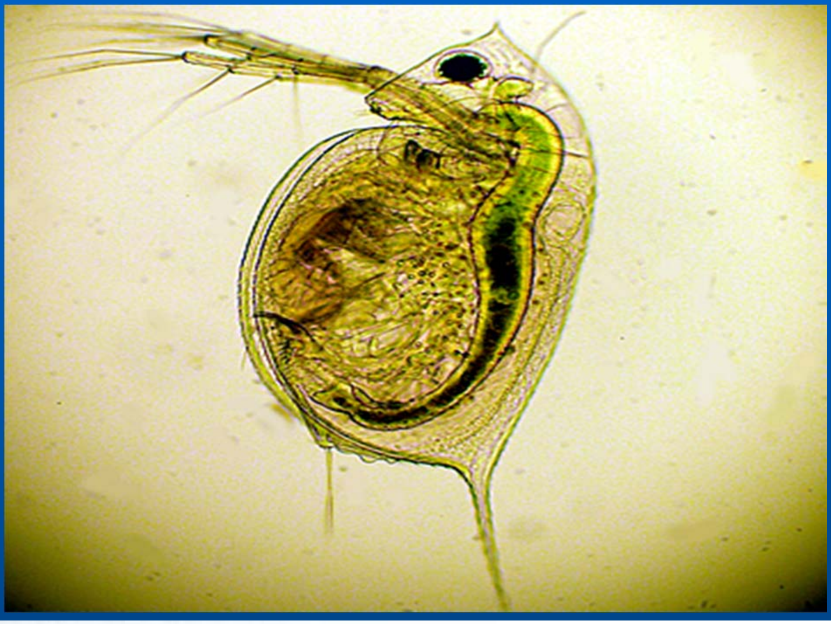
- Cyclic heptapeptides (90+)



- Hepatotoxin + Tumor Promoter
- Can bioaccumulate in invertebrates
- Stable in water column - weeks
- Best studied group cyanotoxins
- Global distribution of events
- Potential cardio and neurotoxin

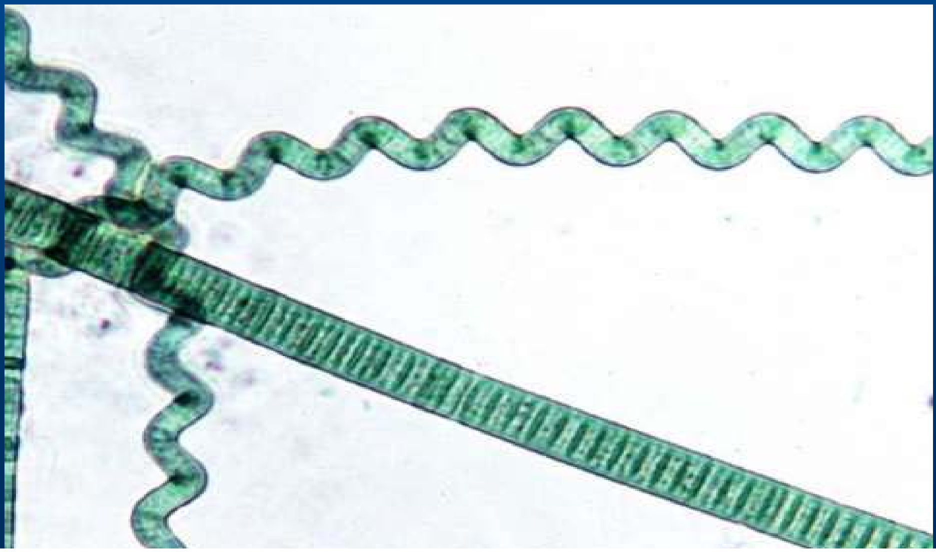


Cyanotoxins – Multiple Possible Effects on Multiple Plant & Animal Taxa, & Food Webs



Water Environment:

- Wild Birds & Fish
- Wild Invertebrates
- Aquacultured Fish & Inver-
- tebrates



Water Users:

- Domestic & Wild Animals
- Humans
- Irrigated Crops

TOXICOLOGICAL SUMMARY AND SUGGESTED ACTION LEVELS TO REDUCE POTENTIAL ADVERSE HEALTH EFFECTS OF SIX CYANOTOXINS

May 2012

Action levels for selected scenarios

	Microcystins[1]	Anatoxin-a	Cylindro-spermopsin	Media (units)
Human recreational uses[2]	0.8	90	4	Water (µg/L)
Human fish consumption	10	5000	70	Fish (ng/g) ww[3]
Subchronic water intake, dog[4]	2	100	10	Water (µg/L)
Subchronic crust and mat intake, dog	0.01	0.3	0.04	Crusts and Mats (mg/kg) dw[5]
Acute water intake, dog[6]	100	100	200	Water (µg/L)
Acute crust and mat intake, dog	0.5	0.3	0.5	Crusts and Mats (mg/kg) dw[5]
Subchronic water intake, cattle[7]	0.9	40	5	Water (µg/L)
Subchronic crust and mat intake, cattle[7]	0.1	3	0.4	Crusts and Mats (mg/kg) dw[5]
Acute water intake, cattle[7]	50	40	60	Water (µg/L)
Acute crust and mat intake, cattle[7]	5	3	5	Crusts and Mats (mg/kg) dw[5]

[1] Microcystins LA, LR, RR, and YR all had the same RfD so the action levels are the same.
[2] The most highly exposed of all the recreational users were 7- to-10-year-old swimmers. Boaters and water-skiers are less exposed and therefore protected by these action levels. This level should not be used to judge the acceptability of drinking water concentrations.
[3] Wet weight or fresh weight.
[4] Subchronic refers to exposures over multiple days.
[5] Based on sample dry weight (dw).
[6] Acute refers to exposures in a single day.
[7] Based on small breed dairy cows because their potential exposure to cyanotoxins is greatest. See Section VI for action levels in beef cattle.

CYANOTOXINS IN AQUATIC FOOD WEBS

Some cyanotoxins are toxic even to fish who graze on phytoplankton (e.g., Li et al., 2007). Zooplankton, oysters, mussels, & other shellfish/invertebrates can bioaccumulate some cyanotoxins, & may also exhibit signs of toxicity (e.g., Miller et al., 2010; Boltovsky et al., 2013; Papadimitriou, et al. 2012; Semaylo et al., 2009)

"Beyond Mammalian Toxicity"

Aquatic & Terrestrial Ecosystem Effects of Cyanobacterial Metabolites – Many Questions Remain

- Much remains unknown (Corbel et al., 2013)
- Microcystins (MCYN) can impair plant physiology & metabolism
- Cylindrospermopsin can inhibit plant pollen germination
- Anatoxin-a as stressor for C. demersum (submerged macrophyte)
- MCYN can reduce activity of Photosystem II in green algae (Perron, et al. 2012)
- MCYN reduces apple shoot growth in vitro @ .03 microgr/ml (Chen et al. 2010)
- Daphnia feeding study: Microcystis strain reduced growth & survival that was not due to microcystins (Semalyo et al., 2009)
- Planktothrix bloom extract acted as an endocrine disruptor on Medaka (fish);(Marie, et al. 2012)
- Anatoxin-a caused motor impairment in rainbow trout (Oswald et al., 2013)
- Reduced acetylcholinesterase activity in Canadian freshwater amphipods collected from Lyngbya mats (Perron et al., 2013)

CWA 303(d) Listings in California

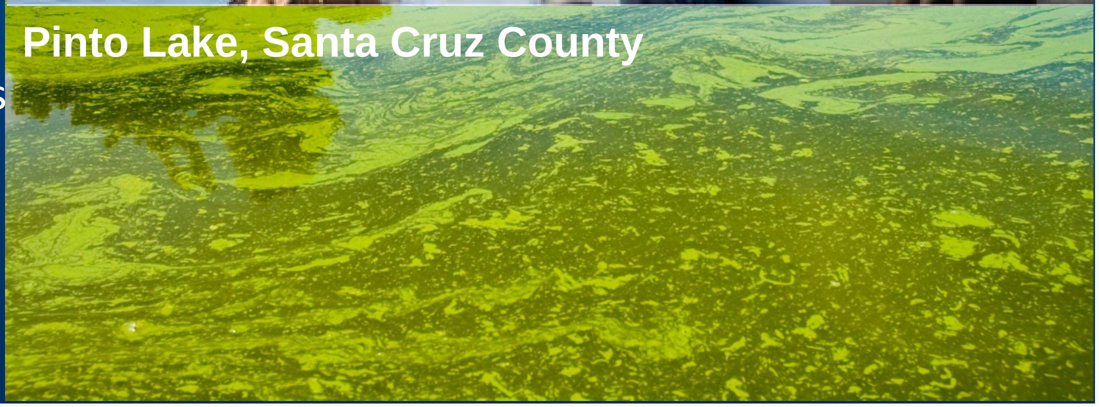
3 major reasons for listing waterbodies in California as impaired or threatened are derived from concerns about anthropogenic eutrophication

1. Alteration of Natural Watershed Hydrology, e.g., reservoirs, altered flow regimes, water diversions: net result is often reduced flow, warm, stagnant shallow water → "culture flask" for bloom formation

2. Multiple sources of anthropogenic N & P, e.g., urban and agricultural sources

3. Multiple anthropogenic sources of PO4 adhering sediments

Result: *Accelerated Eutrophication Processes in Watersheds*

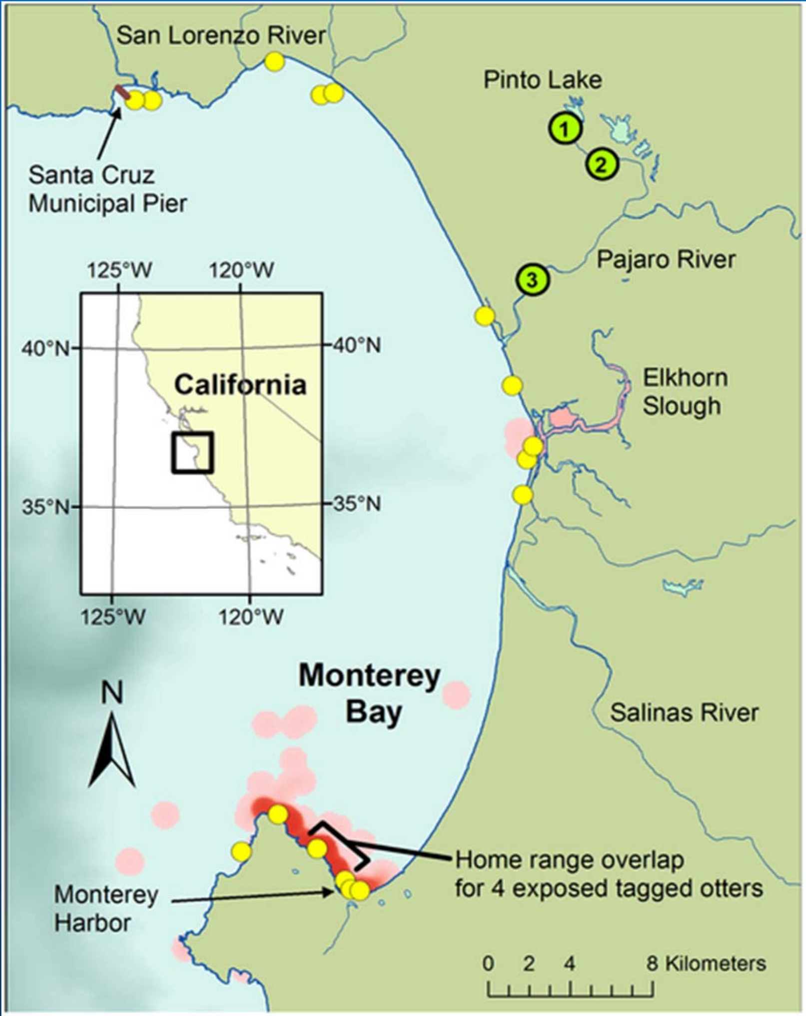


CWA 303(d) Impaired Waterbody Listings in California:
On the Road to TMDLs

- **Systematic survey data remains to be done, but regional information on microcystin is becoming more available**
  - Klamath watershed (MCYN)
  - Eel River
  - Big Lagoon
  - Lake Isabella
  - Salton Sea
  - Clear Lake
  - Sacramento/San Joaquin Delta & Estuary
  - Pinto Lake (Watsonville, Santa Cruz Co.)
  - Sea otter poisonings along Monterey Bay shoreline
  - Various southern CA reservoirs/lakes

- .

Figure 4. Map of Monterey Bay showing distribution of sea otters dying due to microcystin intoxication (yellow circles)

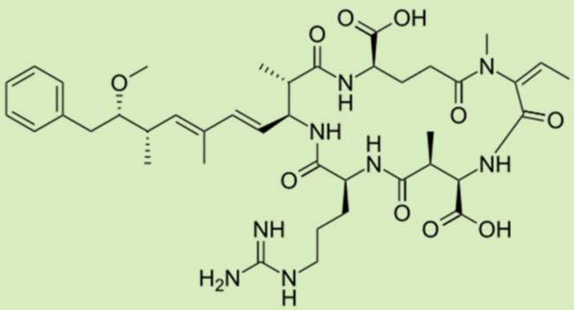


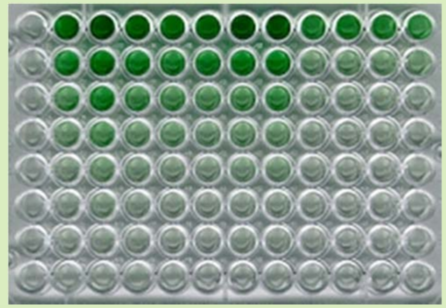
From: Miller MA, Kudela RM, Mekebri A, Crane D, et al. (2010) Evidence for a Novel Marine Harmful Algal Bloom: Cyanotoxin (Microcystin) Transfer from Land to Sea Otters. PLoS ONE 5(9): e12576. doi:10.1371/journal.pone.0012576
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0012576

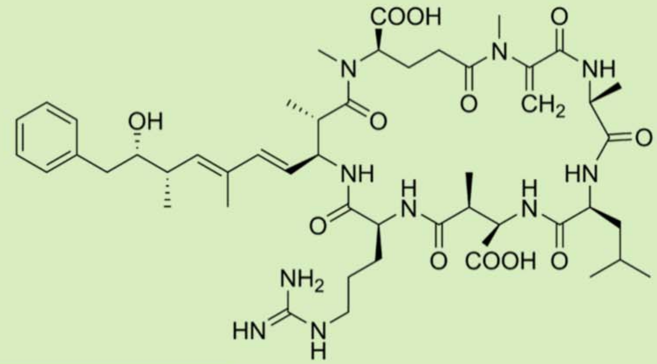
Examples of "CyanoHAB" Effects in CA

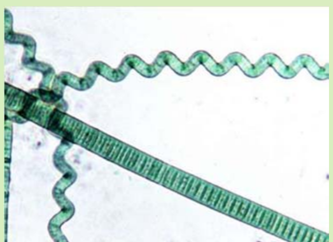
Impacts on "Beneficial Uses"	*Documented Effects on California Water Bodies And Biota*
Fishing/Invertebrate Harvesting/ Cultural/Recreational	- Klamath River postings - Pinto Lake, Santa Cruz County - Clear Lake - Sacramento/San Joaquin Delta - Lake Almaden/ City of San Jose (2010 postings)
Drinking Water	- Riverside County: Microcystin production in Metropolitan Water District reservoirs (Izaguirre et al. 2008) - Sacramento/San Joaquin Delta & Microcystins
Wildlife	Monterey Bay: 21 +Threatened Southern Sea Otter poisoning mortalities linked to coastal watershed sources of microcystins (Miller et al., 2010) -

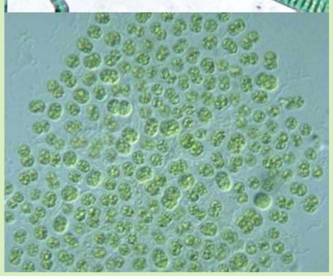
Laboratory Analysis of Cyanotoxins

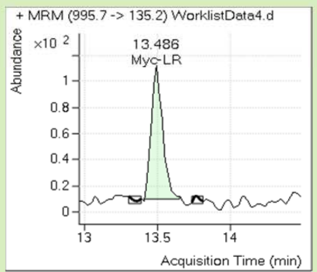


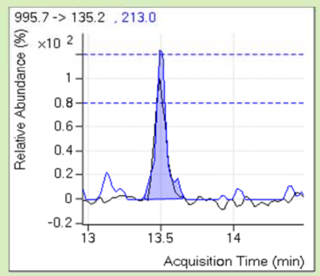


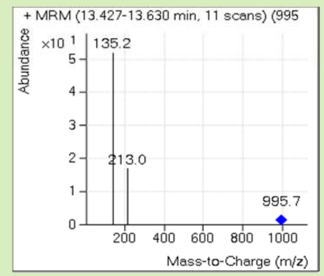


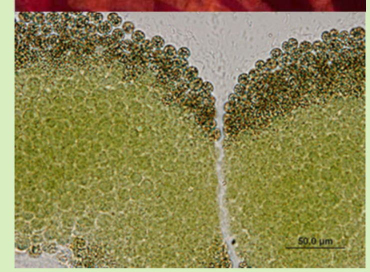




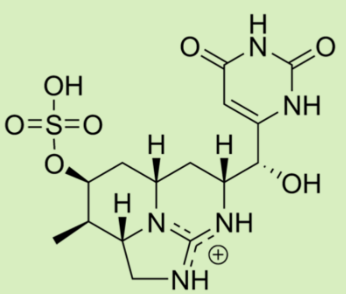


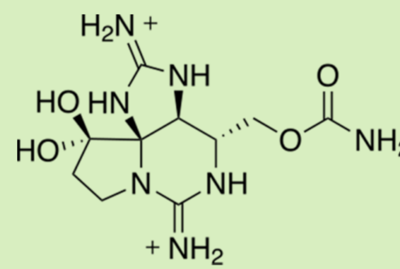






David Crane[1], Cindy Tsai[2] and Abdou Mekebri[2]
[1]CA Dept of Fish and Wildlife and
[2]San Jose State University Research Foundation
Fish and Wildlife Water Pollution Control Laboratory





Analytical Challenges

- An area of active research: over 90 microcystin variants known to exist[1]
- Few standardized analysis methods exist
- Need selective and sensitive methods
- Need low cost screening method(s) for large numbers of samples
- Analytical standards exist for only a few microcystin variants
- Toxin-producing genera generally produce more than one cyanotoxin[2]

[1] Walker and Von Dohren, 2006, FEMS Microbiology Reviews, v.30, p. 530-563
[2]Keith Loftin, USGS

Exposure risk and toxin concentration (how low do we need to go?)

WHO risk definitions (*Chorus and Bartram, 1999*):

— Low risk: less than 10 micrograms per liter (µg/L)

— Moderate risk: 10–20 µg/L

— High risk: 20–2,000 µg/L

— Very high risk: greater than 2,000 µg/L

WHO provisional guideline for drinking water

— 1 µg/L for microcystin-LR

Analytical reporting limit needed - (1 µg/L÷10)

— **0.1 µg/L (ppb)**

Recommended Sample Handling*

- Toxin samples - processed and shipped same day or within 24 hours @ 4^{o}C stored in the dark (*amber glass, Teflon® or polyethylene*)*
- Toxins may be stored frozen several months or years (*only <u>total</u> toxin concentrations can be measured after freezing*) *
- Toxin LC extracts - analyzed within 40 days

*Cyanobacteria in Lakes and Reservoirs: Toxin and Taste-and-odor Sampling Guidelines (ver. 1.0): USGS Techniques of Water Resources Investigations, Book 9, Chapt A7, Section 7.5, Sep 2008

Cyanotoxin Measurement

Water and scum:

Total Toxin = Dissolved-phase toxin + particulate/bound toxin (*analysis of total toxin requires **cell-lysis***)

Biological tissues:

Total Toxin = Free toxin + covalently bonded toxin

(*Most tissue analysis methods only measure **free** toxin*)

Analysis Methods Available

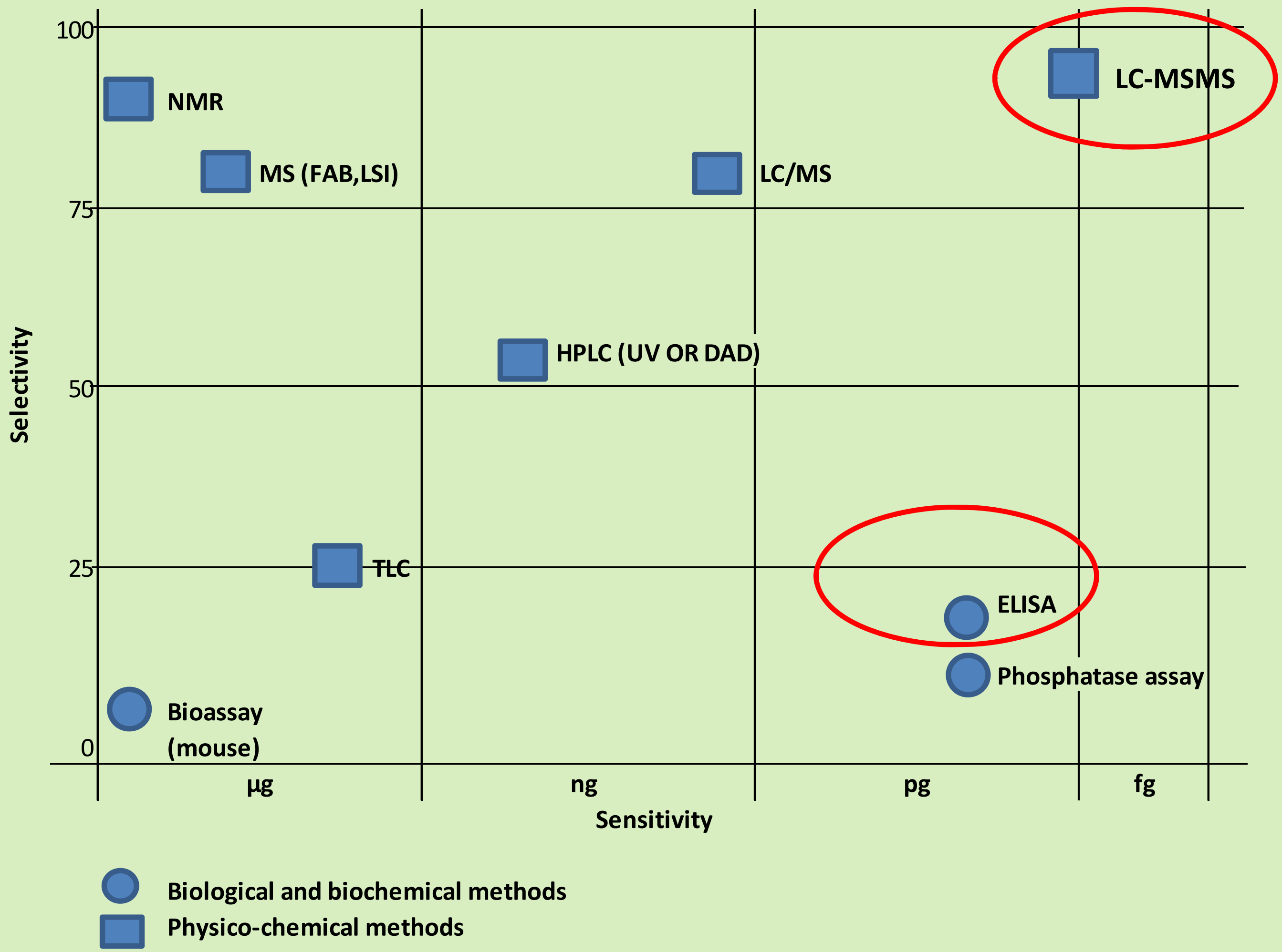
Methods Available for Cyanotoxin Detection

	Freshwater Cyanotoxins				
	Anatoxins	**Cylindrospermopsins**	**Microcystins**	**Nodularins**	**Saxitoxins**
Biological Assays (Class Specific Methods at Best)					
Mouse	Yes	Yes	Yes	Yes	Yes
PPIA	No	No	Yes	No	No
Neurochemical	Yes	No	No	No	Yes
ELISA	In progress	Yes	Yes	Yes	Yes
Chromatographic Methods (Compound Specific Methods)					
Gas Chromatography					
GC/FID	Yes	No	No	No	No
GC/MS	Yes	No	No	No	No
Liquid Chromatography					
LC/UV (or HPLC)	Yes	Yes	Yes	Yes	Yes
LC/FL	Yes	No	No	No	Yes
Liquid Chromatography combined with mass spectrometry					
LC/IT MS	Yes	Yes	Yes	Yes	Yes
LC/TOF MS	Yes	Yes	Yes	Yes	Yes
LC/MS	Yes	Yes	Yes	Yes	Yes
LC/MS/MS	Yes	Yes	Yes	Yes	Yes

Genetic – Quantitative polymerase Chain Reaction (qPCR) toxin gene identification (future)

K. Loftin, J. Graham, B. Rosen, USGS 2010

Relationship Between Sensitivity and Selectivity of Analytical Methods for Microcystins*



*Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management, Ch 13, WHO 1999

ELISA kits for microcystins and nodularin

PROS

- Sensitive for water (0.1 µg/L)
- Inexpensive ($20/sample)
- Good recoveries[1] –
  MC LR kit 73-93%
  %RSD 14-21%
- Analysis doesn't require multiple standards

[1]T. Triantis et al., Toxicon 55 (2010) 979-989.

CONS

- High %rec and RSD - Adda kit (133-189%, %RSD>28%) [1]
- False positives:
  17% MC LR kit and 6% Adda kit[1]
- False negatives:
  15% MC LR kit and **0%** Adda kit[1]
- Variable cross reactivity with other MC variants[1,2,3]
- Matrix interferences (***some severe***)

[1]T. Triantis et al., Toxicon 55 (2010) 979-989.
[2]F. Gurbuz et al. , Environmental Forensics, 13:105-109, 2012
[3]Lawrence et al., JAOAC, 84(4), 2001

ELISA kits for microcystins and nodularin - recommendations

- ELISA kits – should be systematically tested for performance to specific applications including matrix[1]
- Analyst - good technique is important!
- Use of second source standard solutions [1]
- All positive results and a percentage of negative results should be confirmed by LC-MS or LC-MSMS[1]
- LC-MSMS - preferred analysis method for quantitation of MCs (*may agree better with ELISA than LC-MS)* [2]

[1] T. Triantis et al., Toxicon 55 (2010) 979-989.
[2] Lawrence et al., JAOAC, 84(4), 2001

Summary

- There is no perfect analysis method
- Screening with ELISA followed by quantitative confirmation by LC-MSMS is a good approach
- (5%?) of ELISA negative results should be confirmed by LC-(DAD, MS, or MSMS)
- Future routine use of polymerase chain reaction (qPCR) to determine if potentially toxic organisms are present
- Clear communication w/laboratories required to ensure relevant results (*always the case!*)

Questions?