Cyanobacteria & Cyanotoxins: A 2014 Update on Their Environmental Toxicology

Kim Ward
Division of Water Quality
State Water Resources Control Board

Presented To

Twenty-First Annual Meeting of the California Aquatic Bioassessment Workgroup

U.C. Davis, November 19, 2014

Overview

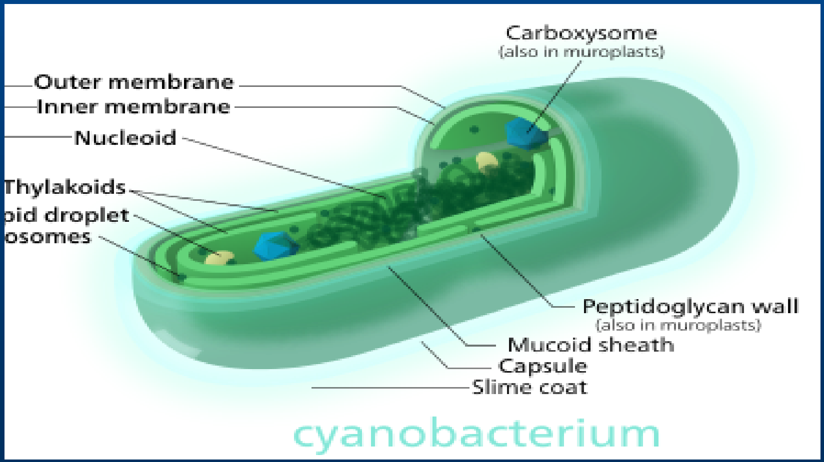
- CyanoHAB blooms have increased in freshwater habitats in the U.S. & globally (Paerl & Otten, 2013)

- 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)

- Toxin-producing taxa tend to be cosmopolitan in soil, fresh water, estuaries, marine water, as symbionts/endosymbionts associated with plants, cyanolichens, biological soil crusts, etc.

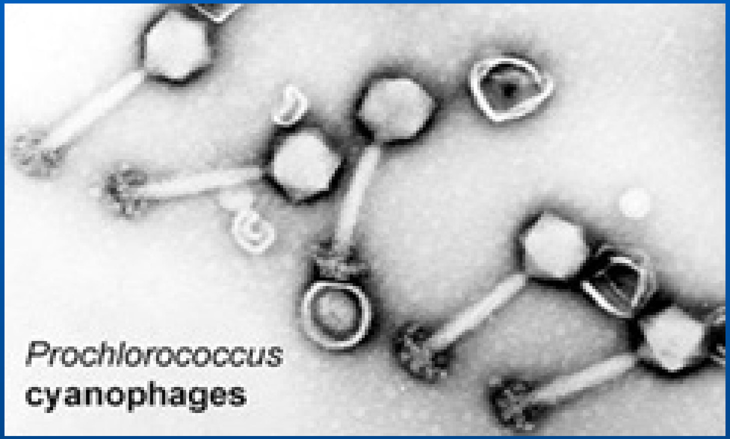
What are the Cyanobacteria?

- *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



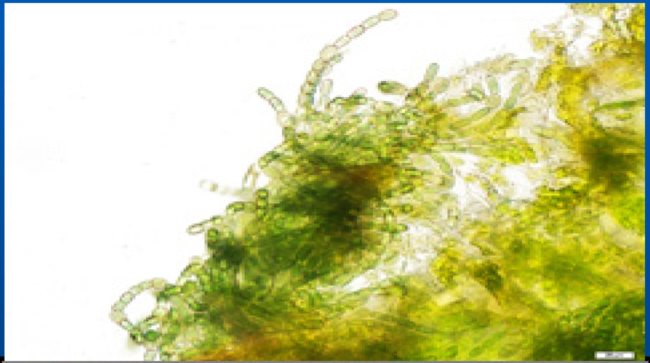
Horizontal Gene Transfer (HGT) Mechanisms Ensure Genetic Heterogeneity - Replacing The "Species" Concept With Unstable Genomes in Constantly Proliferating "Strains" in Bacterial Taxonomy

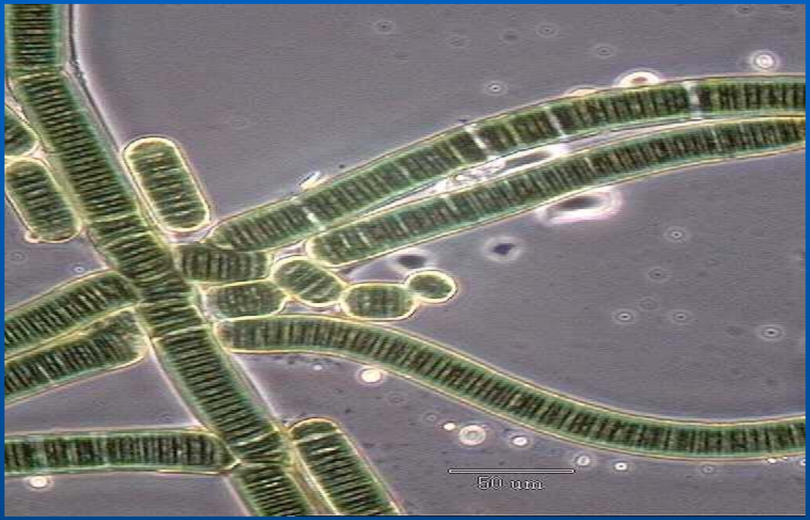
- Many types of bacterial genes are mobile, including at least *some* genes involved with biotoxin synthesis, N2-fixation, etc. (Olendzenski & Gogarten, 2009)
- Cyanophages (viruses which infect cyanobacteria) are numerous, varied, and often confer key gene sequences to infected hosts, e.g., genes for biotoxin synthesis and N2 fixation (e.g., mcy and nifH genes) in aqueous media, including fresh, brackish, and marine waters.
- It is therefore more useful to replace the concept of a fixed species with that of highly mutable strains which can contribute genes to other taxa in close proximity. Typically, a macroscopic freshwater "bloom" will contain numerous strains, and several genera.
- Example: Synechococcus strains have genomes whose phylogenetic histories are obscured by multiple, continuous HGT events (Huang & Gogarten, 2009).

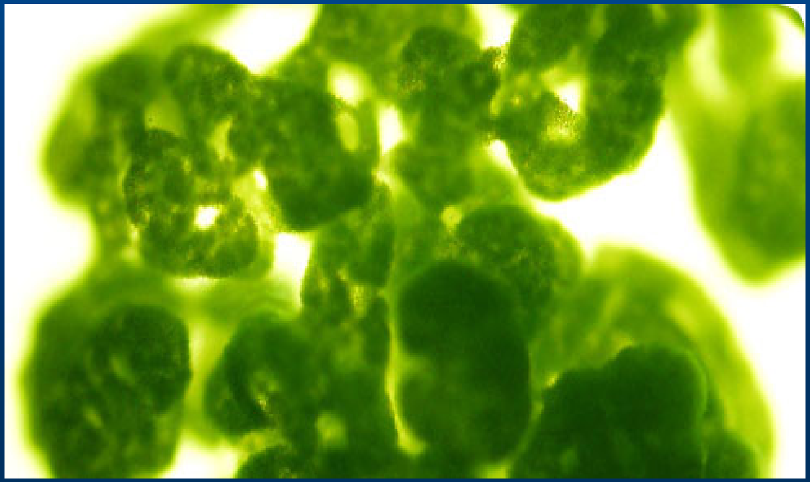


Where Are The Cyanotoxin Producers? – Part 1

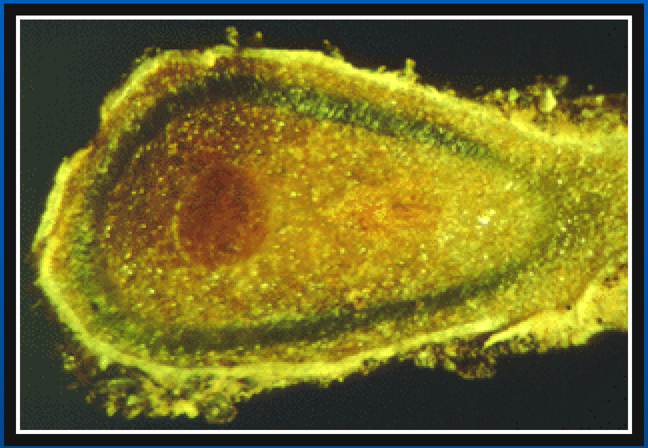
Some taxa are colonial & some are single cells: some display both “tendencies”, e.g. Microcystis. Some are also endosymbionts, e.g., in cycads. Some are present in cyanolichens and biological soil crusts. Both of the latter can also produce cyanotoxins (along with N2-fixation).

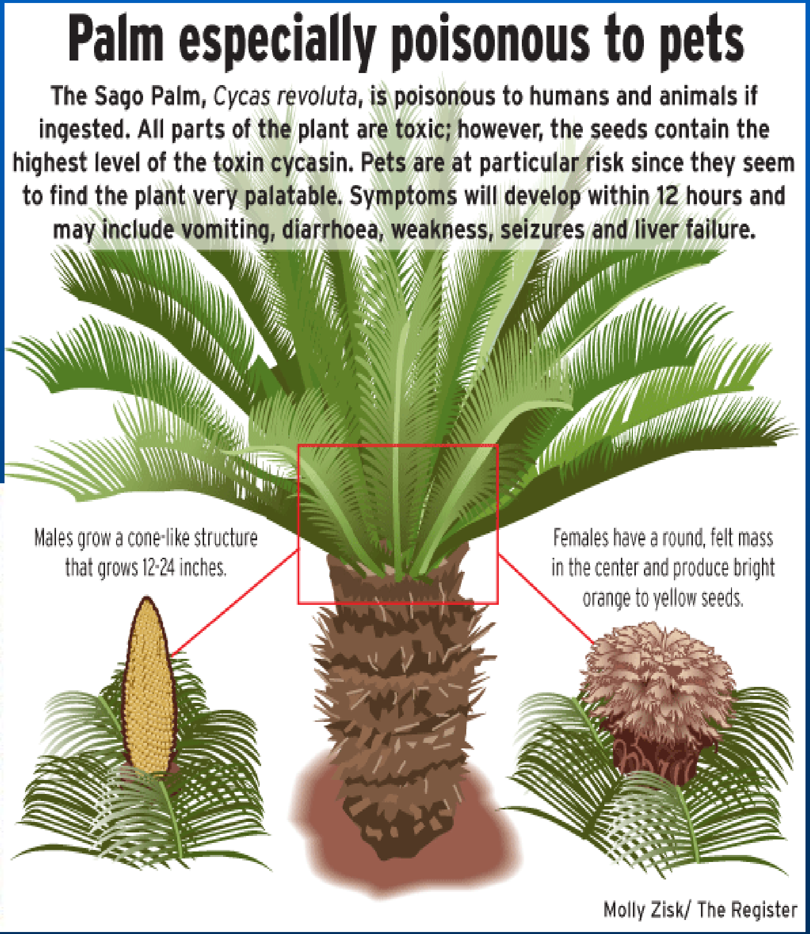






Where are the Cyanotoxin Producers? Part 2

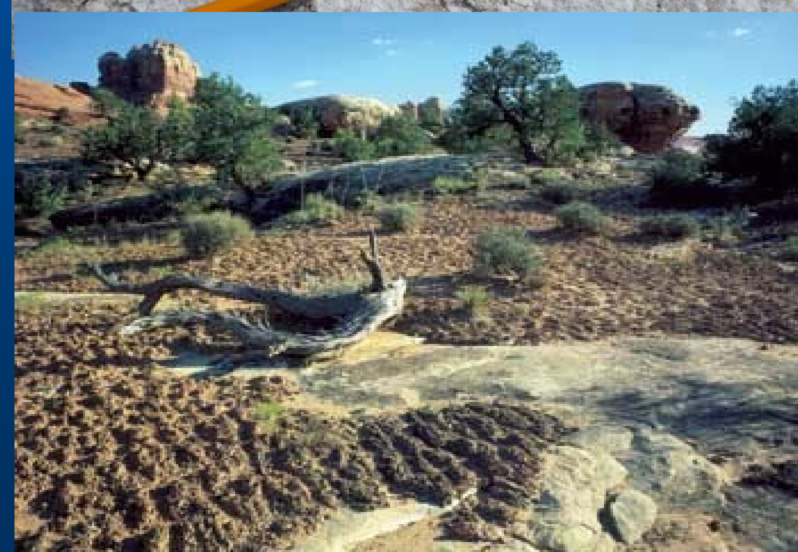



Palm especially poisonous to pets

The Sago Palm, *Cycas revoluta*, is poisonous to humans and animals if ingested. All parts of the plant are toxic; however, the seeds contain the highest level of the toxin cycasin. Pets are at particular risk since they seem to find the plant very palatable. Symptoms will develop within 12 hours and may include vomiting, diarrhoea, weakness, seizures and liver failure.

Where are the Cyanotoxin Producers? Part 3

- Biological soil “crusts” in a variety of environmental settings can produce cyanotoxins (Metcalf et al. 2012), e.g., dry streambeds & salt pans
- Some of these toxins can become airborne in dust & sandstorms
- Do cyanotoxins in soil crusts, cyanolichens, thalloid bryophytes, etc. “leach” into surface waters?
- Can they bioaccumulate in terrestrial/aquatic food webs?
- microcystin & anatoxin-a(s) detected in some species – these may bioaccumulate in food webs in arid regions, forests, etc.



Where are the Cyanotoxin Producers? Part 4

Where are the Cyanotoxin Producers? Part 5

Left: Aging Bloom in Freshwater Lake (PRC)
Right: Marine Bloom of Trichodesmium

Where are the Cyanotoxin Producers? Part 6

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**
  - ✓ Can 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 metabolites ("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.

*<u>*One Net Result Seems To Be A Nationwide Occurrence of Toxigenic Blooms*</u>*

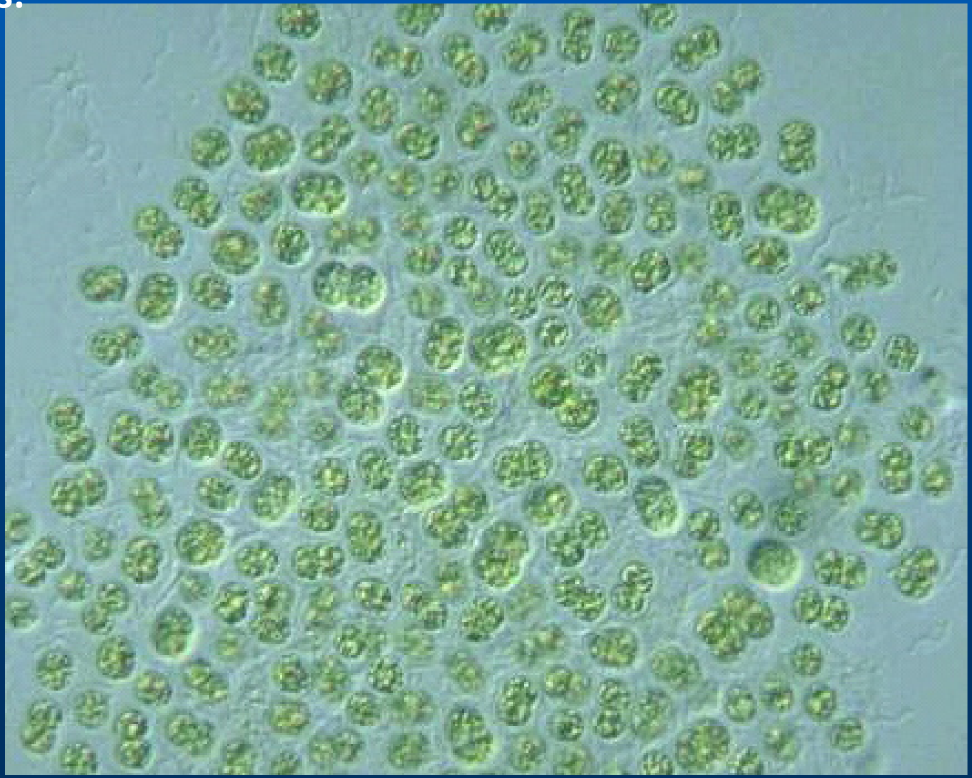
- U.S.A., 2007: National Lakes Assessment survey found 42% of samples exceeded microcystin concentrations of 10 ppb (WHO 1999 guidelines recommend 1 ppb for drinking water); 30% of lakes sampled had MCYNs
- Cyanotoxins found in all 48 states; most abundant genera identified were potential cyanotoxin 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

- Some are cytotoxins – toxins with cytotoxic (cellular) effects
  - ✓ Pharmacological potential – possible antibiotics, chemotherapeutic agents, etc.
- Effects can be acute &/or chronic: some bioaccumulate in tissues & food webs.
- An ever-increasing list of cyanotoxins: 90+ microcystins, 10 nodularins, 3 cylindrospermopsins & anatoxins, 27 members of "PSP" saxitoxin group, aplysiatoxins, lyngbyatoxins, palytoxins, etc. - a diverse assortment of cyclic peptides and alkaloids, including 1 organophosphate pesticide (ATX-a(s))
- 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 can be accounted for by known toxins present in the extracts



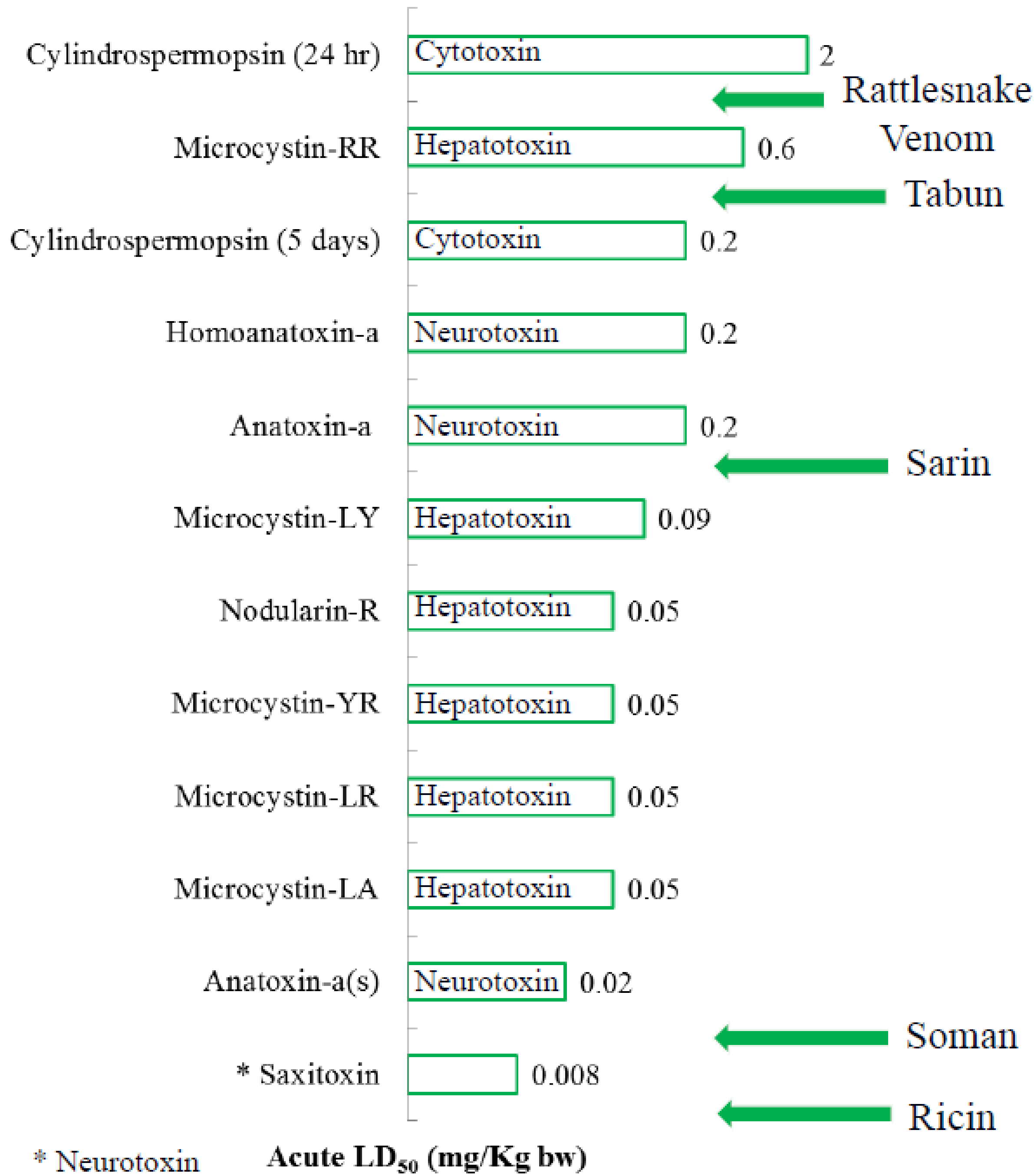
Cyanotoxins: The List Keeps Growing (Castle & Rodgers, 2009)
A *Diverse Assortment of Cyclic Peptides & Alkaloids*

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

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

Water Environment:

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

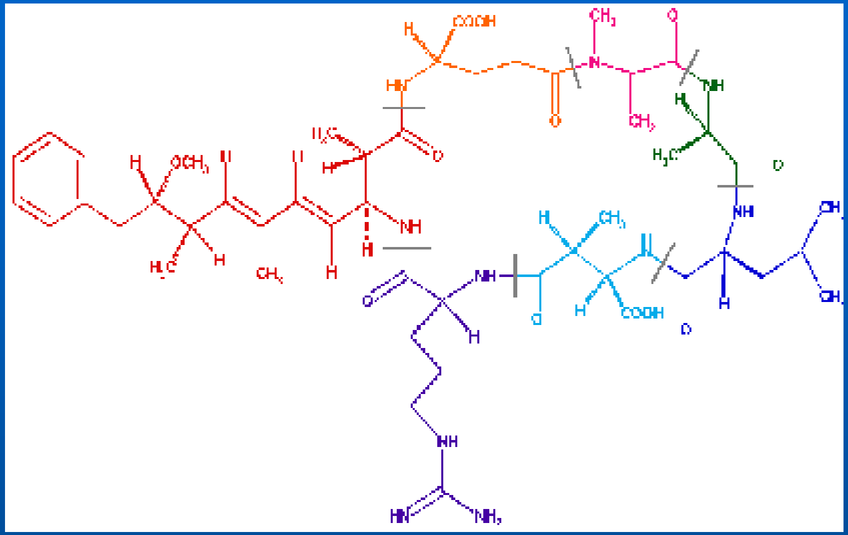
Water Users:

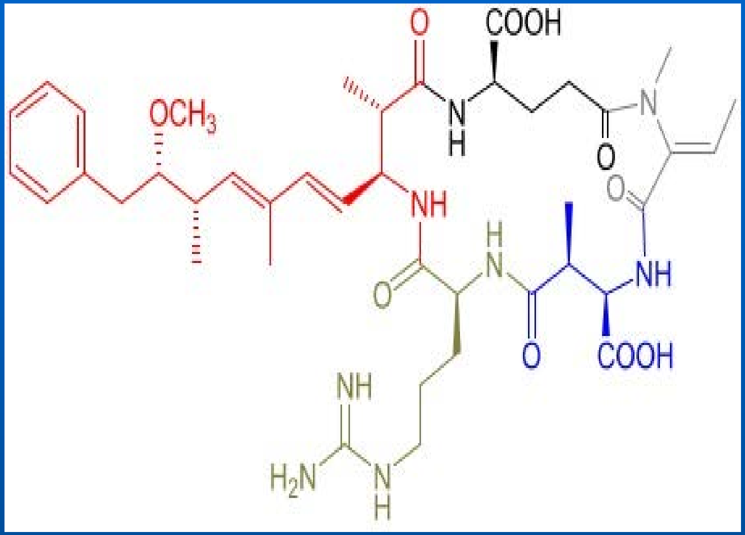
- Domestic & Wild Animals
- Humans
- Irrigated Crops






90+ Microcystins & 10 Nodularins

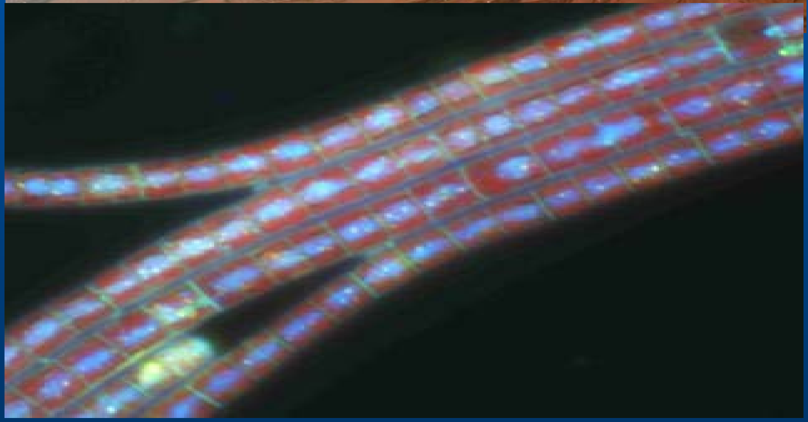




- Hepatotoxin + Tumor Promoter
- Can bioaccumulate in invertebrates
- Stable in water column - weeks
- Best studied groups cyanotoxins
- Global distribution of events
- Potential cardio and neurotoxins

Microcystis & Microcystins in Aquatic Food Webs

- Impact of cyanobacterial bloom masses on freshwater ecosystem services believed to be due to (1) low food quality, & (2) bioactive metabolites (e.g., microcystins)
- *Daphnia* spp. vary in sensitivity to blooms, but all suffer reduced fitness in response to *Microcystis* blooms – for at least *some* species, *some* microcystins do not exert a toxic effect (Sadler & von Elert, 2014).
- NZ: Some MCYNs appear to be stressors to juvenile crayfish & freshwater mussels @ concs > 11146 & 5500 ppb, respectively in MCYN-containing cell-free extracts from *Microcystis* (Clearwater et al., 2012)
- *Planktothrix* in a eutrophic Swiss Lake: 1st study to show direct transfer of MCYNs between zooplankton @ different trophic levels in a lake: *Daphnia*, *Bosmina* & *Chaoborus* larvae MCYN "vectors" in the salmonid whitefish (*Coregonus*) diet (Sotton et al., 2014)



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. ; Semaylo et al., 2009)

Some Newly-Identified/Suspected Cyanotoxins:

. Mercurio et al. (2014) Stigonematales strains produce toxin(s) causing vacuolar myopathy in North American painted turtles. Vacuolar myopathy also occurs in birds such as bald eagles, mallard ducks, buffleheads, & Canada geese
. Jiang et al. (2014) identified a new lyngbyatoxin in Hawaiian strain of *Moorea (formerly Lyngbya)*
. Tripathi et al. (2014) identified a new cytotoxic cyclic peptide, lagunamide C, in Lyngbya found in coastal waters of Singapore
. Vehovsky et al. (2014) found evidence of novel neurotoxic metabolite(s) of Cylindrospermopsis in a pond in Hungary.

"Beyond Mammalian Toxicity"

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

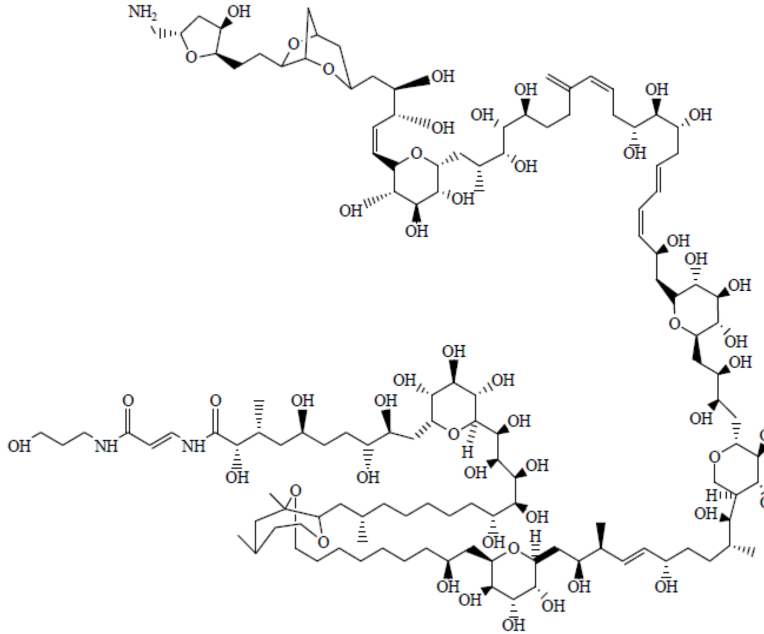
- Much remains unknown (Corbel et al., 2013), but recent studies show:
- Microcystins (MCYN) can impair plant physiology & metabolism
- Cylindrospermopsin can inhibit plant pollen germination
- Anatoxin-a as stressor for C. demersum (submerged macrophyte)
- MCYN can reduce activity of Photosystem II 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)

Some Toxins Produced By Marine Cyanobacteria: The Multiple Cyanotoxins Produced by Trichodesmium

- **Sporadic reports dating back to the 1990's have indicated the presence of unidentified toxin(s) in invertebrates, etc.**
- **Some strains produce palytoxins, aka PTXs (Kerbrat et al. 2011) & aplysiatoxins (Gupta et al., 2014)**
- **Some strains produce MCYN-LR (Ramos et al. 2005) & others produce STX, MCYN & CYN analogs (Proenca et al. 2009)**
- **PTXs have relatively low LD-50's in mammals & are problematic in many marine food webs (Ramos & Vasconcelos, 2014)**
- **Poorly characterized but common global occurrence of human GI, neurological & cardiac toxicity attributed to chronic "ciguatera" poisoning from seafood consumption may be due, in part, to a combination of *Trichodesmium* toxins bioaccumulating in marine food webs (Kerbrat et al. 2009)**

Table 1. Toxicity reference values of PTX and its analogs administered by several different routes on two model animals.

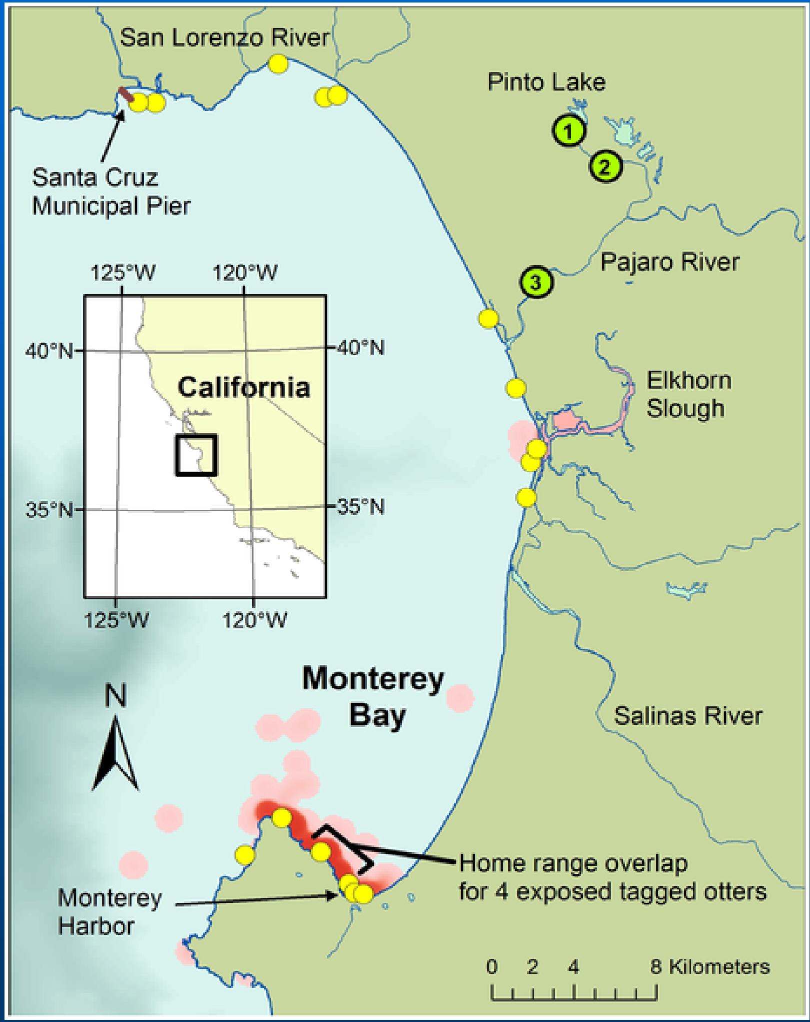
Test animal	Toxin type	Toxin source	Route of exposure	24 h LD_{50} (μg/kg)	References
Rat	Palytoxin	semi-purified material from *Palythoa* spp.	intravenous	0.089	[38,49]
	" "	" "	subcutaneous	0.4	[38,49]
	" "	" "	intragastric	>40.0	[38,49]
	" "	" "	intraperitoneal	0.63	[38,49]
	" "	" "	intratracheal	0.36	[38,49]
Mouse	Palytoxin	" "	intravenous	0.045	[38,49]
	" "	" "	subcutaneous	1.39	[38,49]
	" "	*P. caribaeorum*	oral	510	[29]
	" "	*P. tuberculosa*	" "	767	[53]
	" "	" "	intraperitoneal	0.295	[51]
	Ostreocin-D	*Ostreopsis siamensis*	" "	0.75	[7,52]
	Mascarenotoxin-A	*O. mascarenensis*	" "	900	[9,12]
	Ostreotoxin *	*O. lenticularis*	" "	32100	[12,54]
N.T.	Ovatoxin-A	*O. ovata*	-	-	[11]



Trichodesmium & Palytoxins (PTX)

- Human & animal poisoning cases reported in Polynesia (e.g., Hawaii), Mediterranean, etc.
- May be lethal @ 0.36 mg/kg/bw (rat, intratracheal)
- More toxicity via respiration of aerosols & IP injection than by ingestion, but ingestion is a better-studied exposure pathway
- Recent work by Kerbrat et al. (2011) confirms *Trichodesmium* strains as one source of palytoxins in marine food webs.
- PTXs are large, very complex molecules which can theoretically have more than 10-21st stereoisomers (Ramos & Vasconcelos, 2010)
- Much work remains to be done on identifying these biotoxins & studying their effects

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



The growing literature shows that a number of previously unrecognized cyanotoxins may be having effects on freshwater, estuarine, and marine ecosystems, and will continue to be invisible without more resources dedicated to exploring these many biotoxins and their complex environmental effects (KW).

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.
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0012576

PLOS ONE

California CyanoHAB Network (CCHAB)*

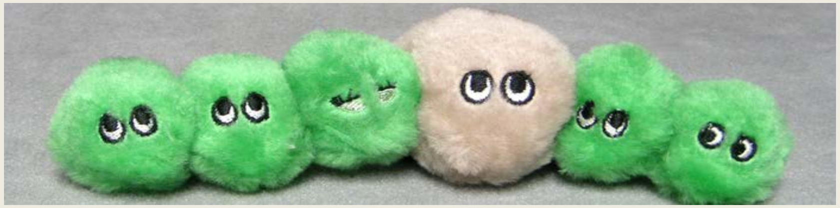
To develop a statewide framework to address CyanoHABs in California's freshwater and marine ecosystems



* Formally the Statewide Blue Green Algae Public Working Group

Goals

1. C**oordinate monitoring, and management** of CyanoHABs and effects in freshwater and marine ecosystems throughout California.
2. Develop **collaborative relationships** among entities responsible for addressing cyanobacteria concerns and impacts to beneficial uses.
3. Make **efficient use** resources to address cyanobacteria concerns by sharing information, avoiding duplicative efforts, promoting research, monitoring, and assessment, identifying technical and policy gaps, and communicating cyanobacteria concerns to the public.



Next Steps and Contact

- CCHAB Meeting
  - December 18, 2014 9 a.m. – 12 p.m.
- Contact
  - **Executive Sponsor** – Phil Crader, Division of Water Quality (DWQ) – Assistant Deputy Director
  - **Project Manager** – Zane Poulson, DWQ Inland Planning Standards & Implementation Unit Chief
  - **Project Staff Lead** – Johanna Weston, DWQ Ocean Standards Unit
    - Johanna.weston@waterboard.ca.gov

http://www.waterboards.ca.gov/water_issues/programs/bluegreen_algae/index.shtml