Print version

CEE 697z
Organic Compounds in Water and Wastewater

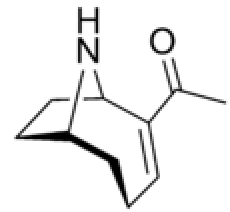
Cyanotoxins

Compounds, Toxicity and Occurrence

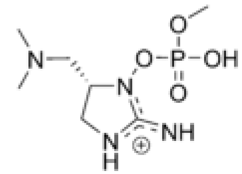
Lecture #27

Cyanotoxins

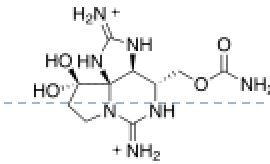
- **Neurotoxins**
  - Anatoxin-a
  - Anatoxin-as
  - Saxitoxin
  - β-Methylamino-L-alanine (BMAA)
- **Hepatotoxins**
  - Microsystins
  - Nodularins
- **Cytotoxins**
  - Cylindrospermopsin
- **Gastrointestinal and dermatotoxins**
  - Aplysiatoxin
  - Lyngbyatoxin a



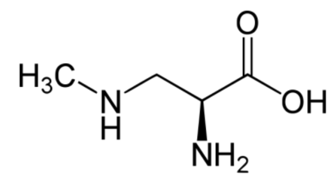
Anatoxin-a



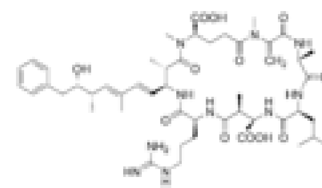
Anatoxin-as



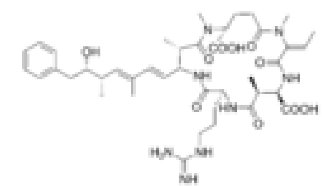
Saxitoxin



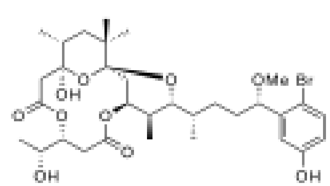
BMAA



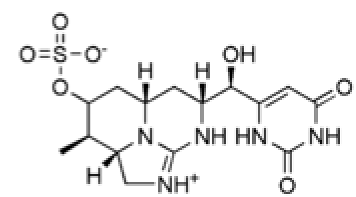
Microcystin LN



Nodularin R



Aplysiatoxin



Cylindrospermopsin

Neurotoxins

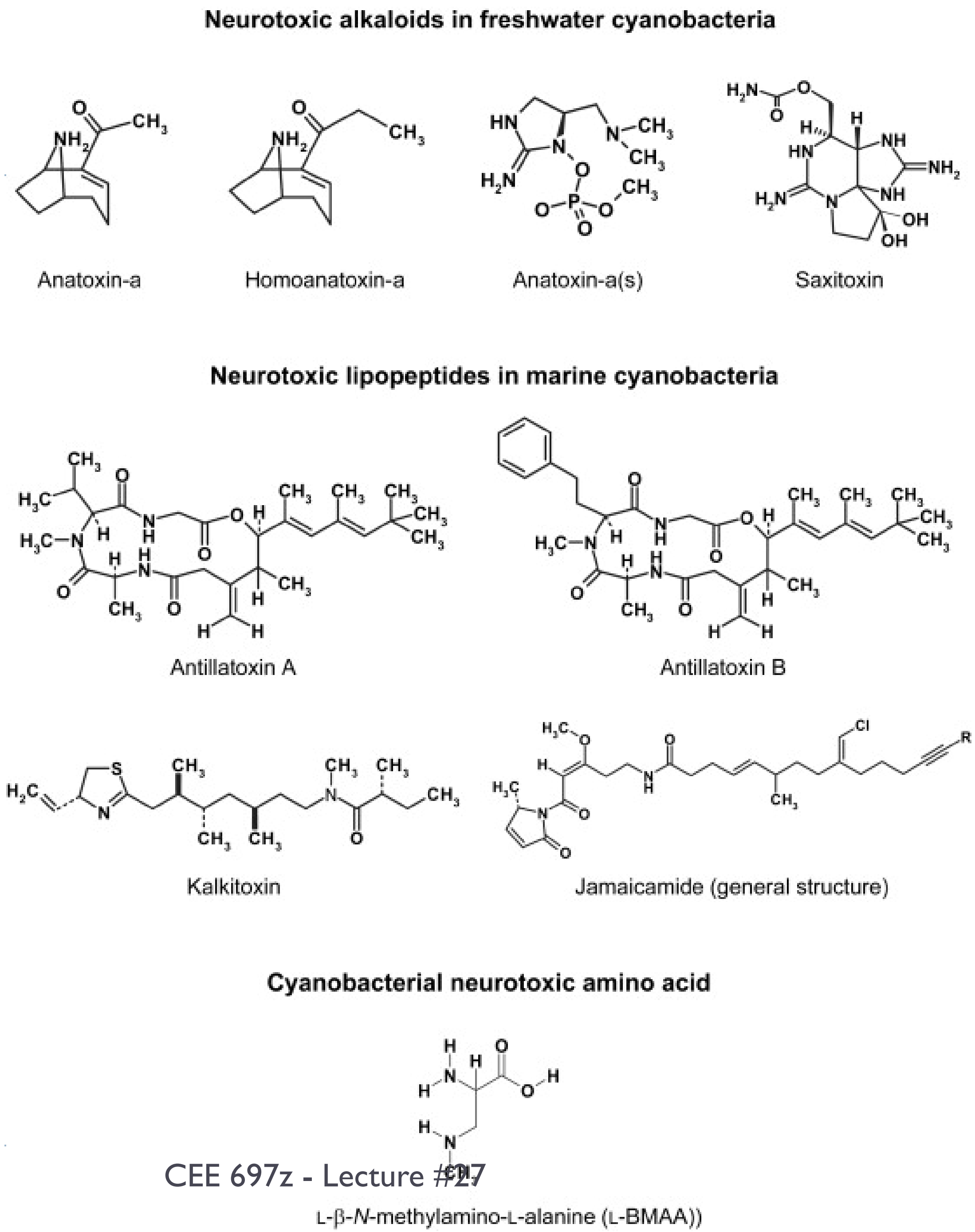
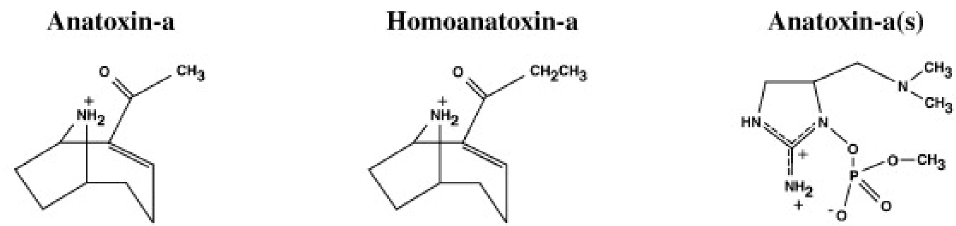


Fig. 1 Structure of the cyanobacterial neurotoxins.

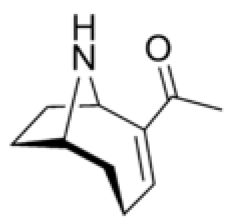
Araoz, R., Molgo, J. and de Marsac, N.T. (2010) Neurotoxic cyanobacterial toxins. Toxicon 56(5), 813-828.

Anatoxin

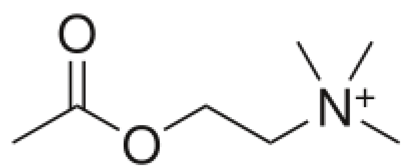
- An Alkaloid Neurotoxin
- 3 common variants
- Produced by Anabaena and o



- LD_{50} 200 ug/kg
- Anatoxin-a mimics acetylcholine (Nicotinic acetylcholine receptor agonists)



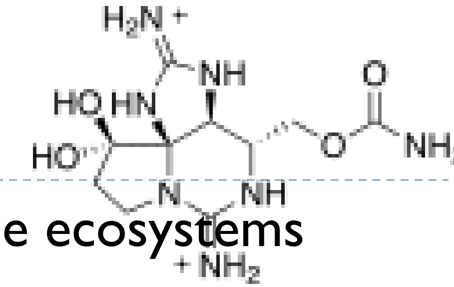
Anatoxin-a



Acetylcholine

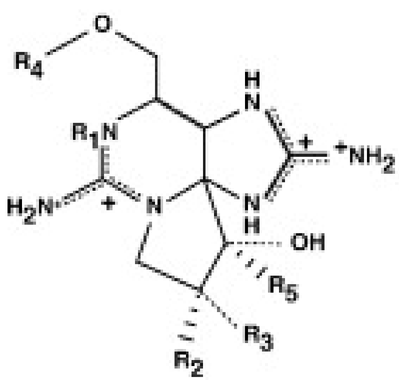
  - Residence of these toxins at post-synaptic cholinergic receptors results in nerve depolarisation
- Anatoxin-as is structurally different from Anatoxin–a and is highly toxic

Saxitoxins



- Saxitoxin is usually associated with red tides in marine ecosystems
  - Responsible for paralytic shellfish poisoning
  - Been detected in some freshwater species

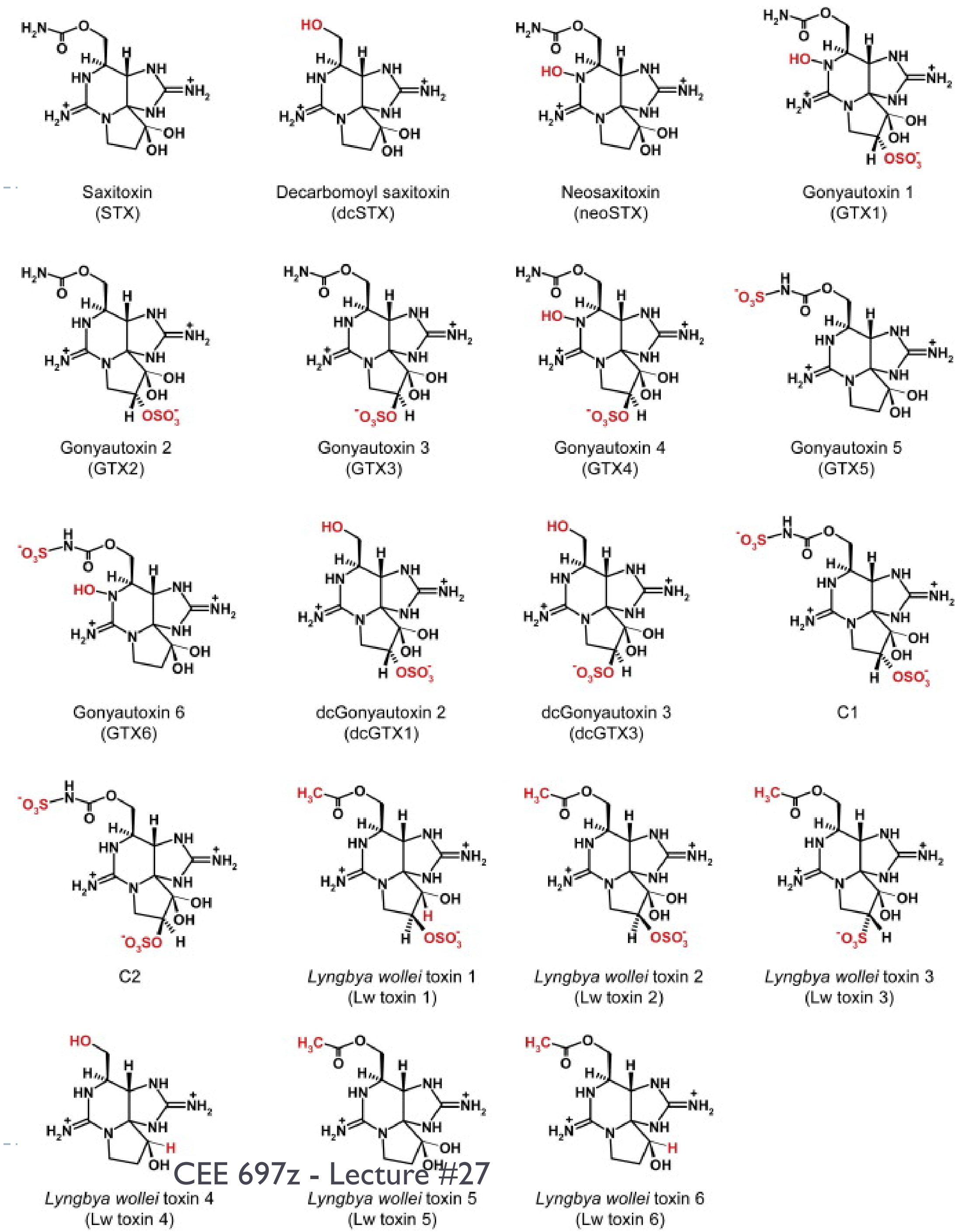
Saxitoxin



Toxin	R_1	R_2	R_3	R_4	R_5
STX	H	H	H	$CONH_2$	OH
GTX2	H	H	OSO_3^-	$CONH_2$	OH
GTX3	H	OSO_3^-	H	$CONH_2$	OH
GTX5	H	H	H	$CONHSO_3^-$	OH
C1	H	H	OSO_3^-	$CONHSO_3^-$	OH
C2	H	OSO_3^-	H	$CONHSO_3^-$	OH
C3	OH	H	OSO_3^-	$CONHSO_3^-$	OH
C4	OH	OSO_3^-	H	$CONHSO_3^-$	OH
neoSTX	OH	H	H	$CONH_2$	OH
GTX1	OH	H	OSO_3^-	$CONH_2$	OH
GTX4	OH	OSO_3^-	H	$CONH_2$	OH
GTX6	OH	H	H	$CONHSO_3^-$	OH
dcSTX	H	H	H	H	OH
dcneoSTX	OH	H	H	H	OH
dcGTX1	OH	H	OSO_3^-	H	OH
dcGTX2	H	H	OSO_3^-	H	OH
dcGTX3	H	OSO_3^-	H	H	OH
dcGTX4	OH	OSO_3^-	H	H	OH
LWTX1	H	OSO_3^-	H	$COCH_3$	H
LWTX2	H	OSO_3^-	H	$COCH_3$	OH
LWTX3	H	H	OSO_3^-	$COCH_3$	OH
LWTX4	H	H	H	H	H
LWTX5	H	H	H	$COCH_3$	OH
LWTX6	H	H	H	$COCH_3$	H

Saxitoxins

Fig. 4 Saxitoxin analogues produced by some members of different cyanobacteria genera.

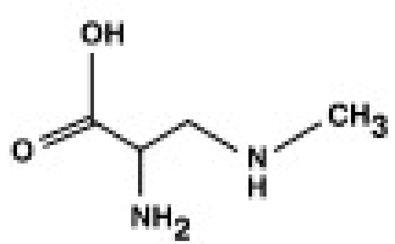


Araoz, R., Molgo, J. and de Marsac, N.T. (2010) Neurotoxic cyanobacterial toxins. Toxicon 56(5), 813-828.

BMAA neurotoxin

- Caused by over 30 species of cyanobacteria:
  - Ex. *Microcystis, Anabaena, Nostoc, Planktothrix*
- Can cause motor neuron disease or death
- Accumulates in brain tissue
- Found in Guam and linked to ALS

β-methylamino-L-alanine



Amyotrophic Lateral Sclerosis (ALS)

- Neurodegenerative disease
- About 2 per 100,000 people in US
- Can be caused by the neurotoxin BMAA
- Symptoms
  - Muscle weakness (including speech muscles)
  - Twitching and cramping of muscles
  - Trouble with speech
  - Shortness of breath, trouble swallowing
  - Death by suffocation

Parkinson's Disease (PD)

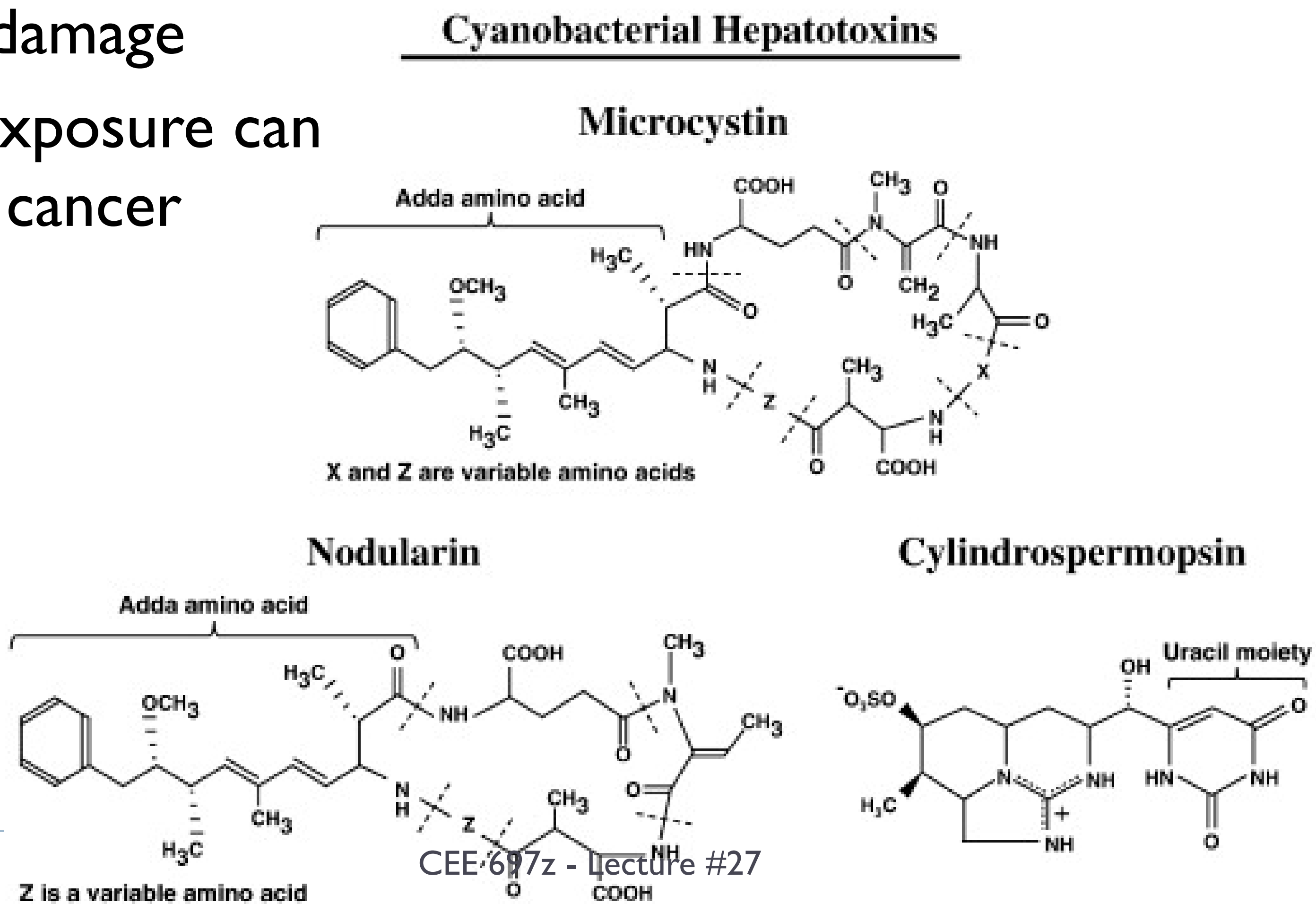
- Neurodegenerative Disease
- Symptoms
  - Rigidity of muscles, slowing of movement
  - Muscle spasms or tremors
  - Loss of smell, blinking, smiling
  - Speech changes (soft, monotone, repetition)
  - Dementia in later stages

Alzheimer's

- 7th leading cause of death
- Most common form of dementia
- Destroys brain cells leading to memory loss, confusion, changes in personality, mood, behavior, problems with language

Hepatotoxins

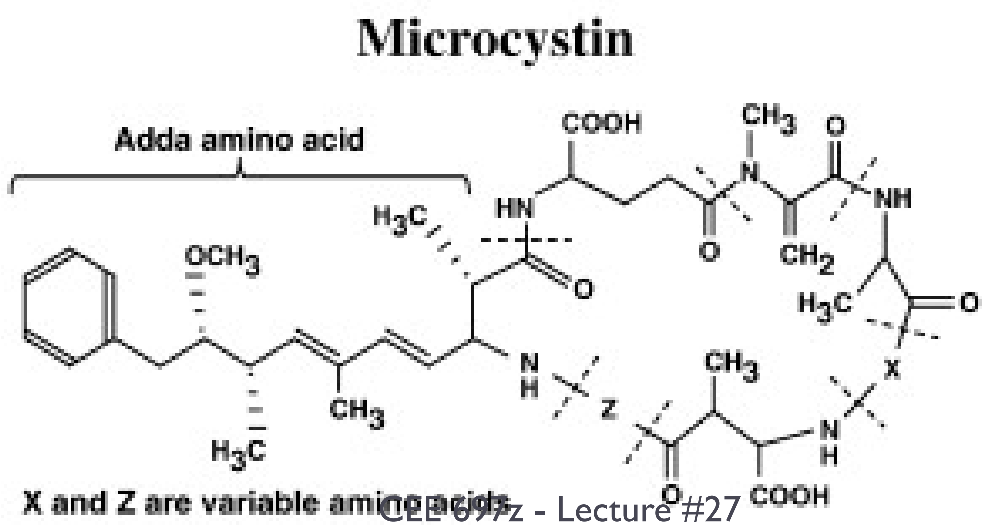
- Cyclic peptides
- Cause liver damage
- Long term exposure can lead to liver cancer



Merel, S., Walker, D., Chicana, R., Snyder, S., Baures, E. and Thomas, O. (2013) State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. Environment International 59, 303-327.

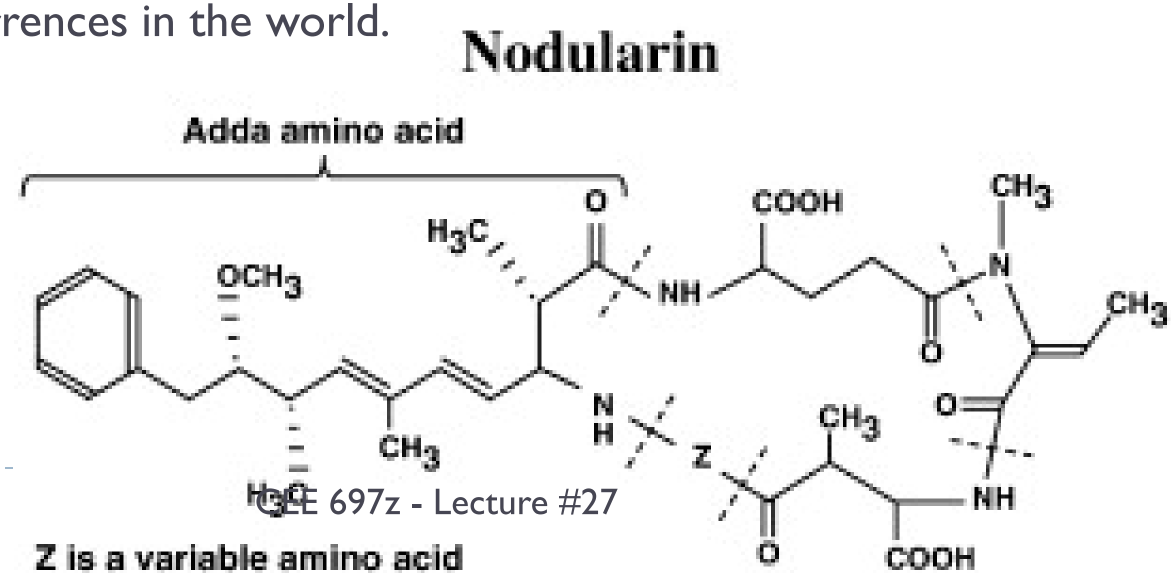
Microsystins

- Polypeptide produced by Microsystis & others
  - Adda is: 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid
- 90 congeners & 200 related compounds
- LD_{50} ~25-60 ug/kg (cyanide is 4,000 ug/kg)
- Hepatotoxin and tumor promoter



Nodularins

- Powerful hepatotoxins
- Cyclic nonribosomal peptide
  - Similar to microsystins, as both have 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda)
  - Difference is Nodularins have 2-(methylamino)-2-dehydrobutyric acid (Mdhb) where Microsystins have dehydroanaline
- Produced by Nodularia spumigena, a cyanobacterium
  - The late summer blooms of *Nodularia spumigena* are among the largest cyanobacterial mass occurrences in the world.
  - More in brackish waters

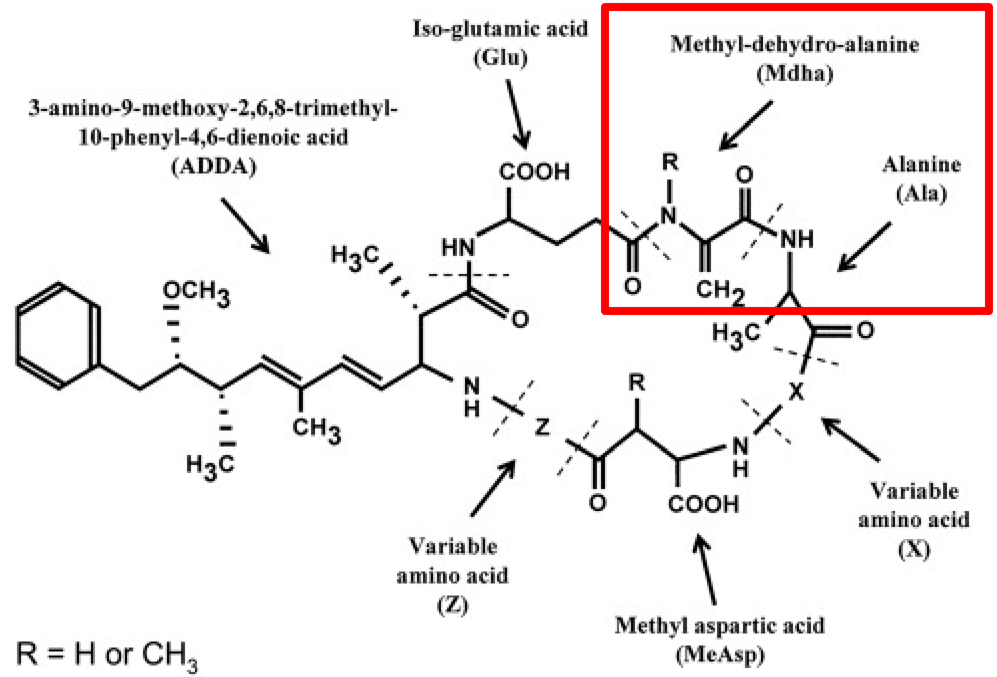


Very similar to microcystins, except that nodularins do not bind covalently to proteins in the body and thus move more easily throughout the body and cells

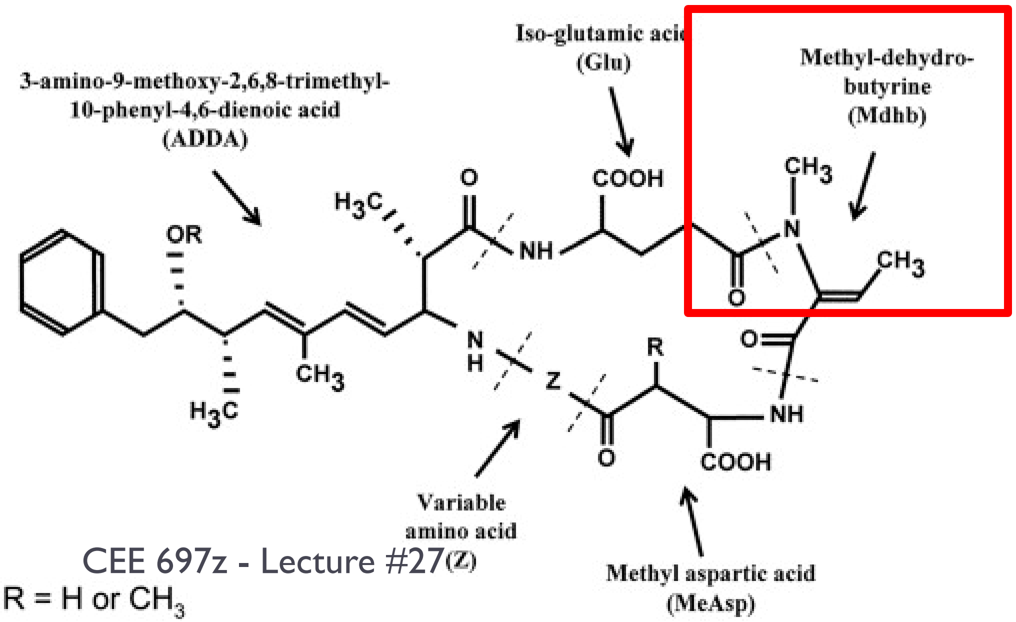
Fig. 1a Structure of microcystins and nodularins.

Merel, S., Walker, D., Chicana, R., Snyder, S., Baures, E. and Thomas, O. (2013) State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. Environment International 59, 303-327.

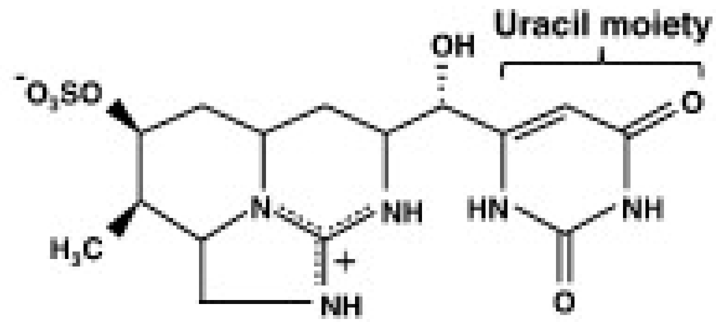
Microcystins



Nodularins



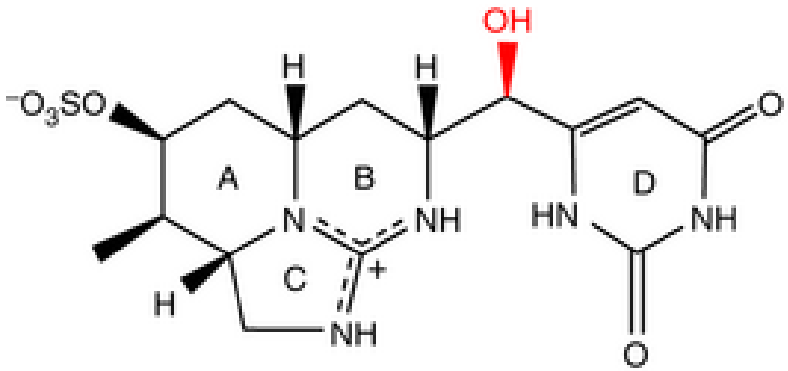
Cylindrospermopsin



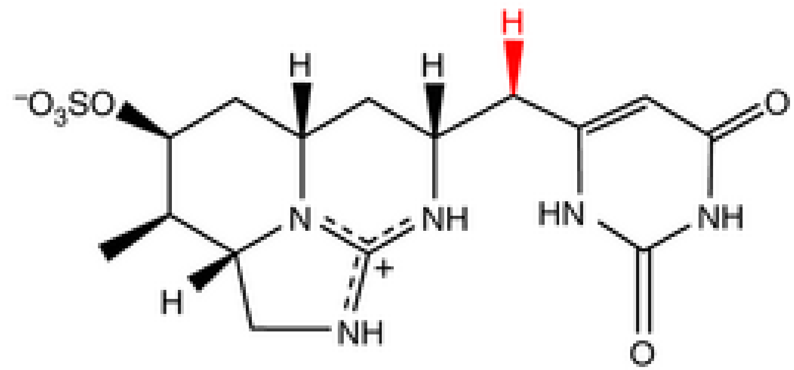
- Alkaloid
- Produced by Cylindrospermopsis
- LD_{50} 300 ug/kg
- Hepatotoxin and Neurotoxin
- Subtropical species recently reported in Michigan

Cylindrospermopsin: Variants

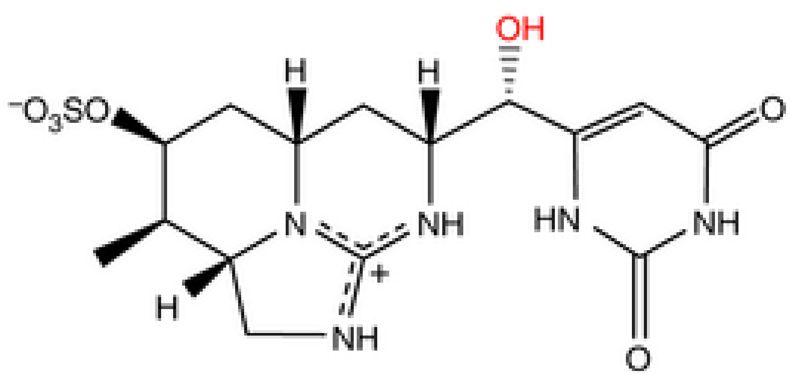
- The molecular structures of cylindrospermopsin (1) and its analogs 7-deoxy-cylindrospermopsin (2) and 7-epicylindrospermopsin (3).



(1)



(2)

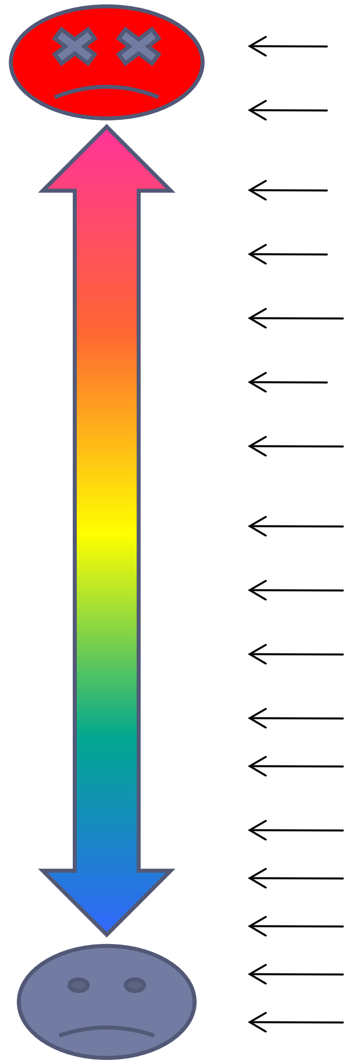


(3)

Journal of Applied Microbiology
Volume 114, Issue 3, pages 605-620, 19 NOV 2012 DOI: 10.1111/jam.12048
http://onlinelibrary.wiley.com/doi/10.1111/jam.12048/full#jam12048-fig-0001

Toxicity of Algal Toxins Relative to Other Toxic Compounds found in Water

- **Reference Dose = amount that can be ingested orally by a person, above which a toxic effect may occur, on a milligram per kilogram body weight per day basis.**



Toxin Reference Doses

- Dioxin (0.000001 mg/kg-d
- Microcystin LR (0.000003 mg/kg-d)
- Saxitoxin (0.000005 mg/kg-d)
- PCBs (0.00002 mg/kg-d)
- Cylindrospermopsin (0.00003 mg/kg-d)
- Methylmercury (0.0001 mg/kg-d)
- Anatoxin-A (0.0005 mg/kg-d)
- DDT (0.0005 mg/kg-d)
- Selenium (0.005 mg/kg-d)
- Botulinum toxin A (0.001 mg/kg-d)
- Alachlor (0.01 mg/kg-d)
- Cyanide (0.02 mg/kg-d)
- Atrazine (0.04 mg/kg-d)
- Fluoride (0.06 mg/kg-d)
- Chlorine (0.1 mg/kg-d)
- Aluminum (1 mg/kg-d)
- Ethylene Glycol (2 mg/kg-d)

US Regulatory Action

From: Cyanobacteria and Cyanotoxins: Information for Drinking Water Systems , USEPA , Ju

Table 1. Cyanotoxins on the Contaminant Candidate List (CCL)

Cyanotoxin	Number of known variants or analogues	Primary organ affected	Health Effects[1]	Most common Cyanobacteria producing toxin[2]
Microcystin-LR	80~90	Liver	Abdominal pain Vomiting and diarrhea Liver inflammation and hemorrhage Acute pneumonia Acute dermatitis Kidney damage Potential tumor growth promotion	*Microcystis* *Anabaena* *Planktothrix* *Anabaenopsis* *Aphanizomenon*
Cylindrospermopsin	3	Liver		*Cylindrospermopsis* *Aphanizomenon* *Anabaena* *Lyngbya* *Rhaphidiopsis* *Umezakia*
Anatoxin-a group[3]	2-6	Nervous System	Tingling, burning, numbness, drowsiness, incoherent speech, salivation, respiratory paralysis leading to death	*Anabaena* *Planktothrix* *Aphanizomenon* *Cylindrospermopsis* *Oscillatoria*

[1]Source: *Harmful Algal Research and Response National Environmental Science Strategy (HARRNESS)*
[2] Not all species of the listed genera produce toxin; in addition, listed genera are not equally as important in producing cyanotoxins.
[3]The anatoxin-a group does not include the organophosphate toxin anatoxin-a(S) as it is a separate group. In the US, the most common member is thought to be anatoxin-a, and thus this toxin is listed specifically.

Exposure

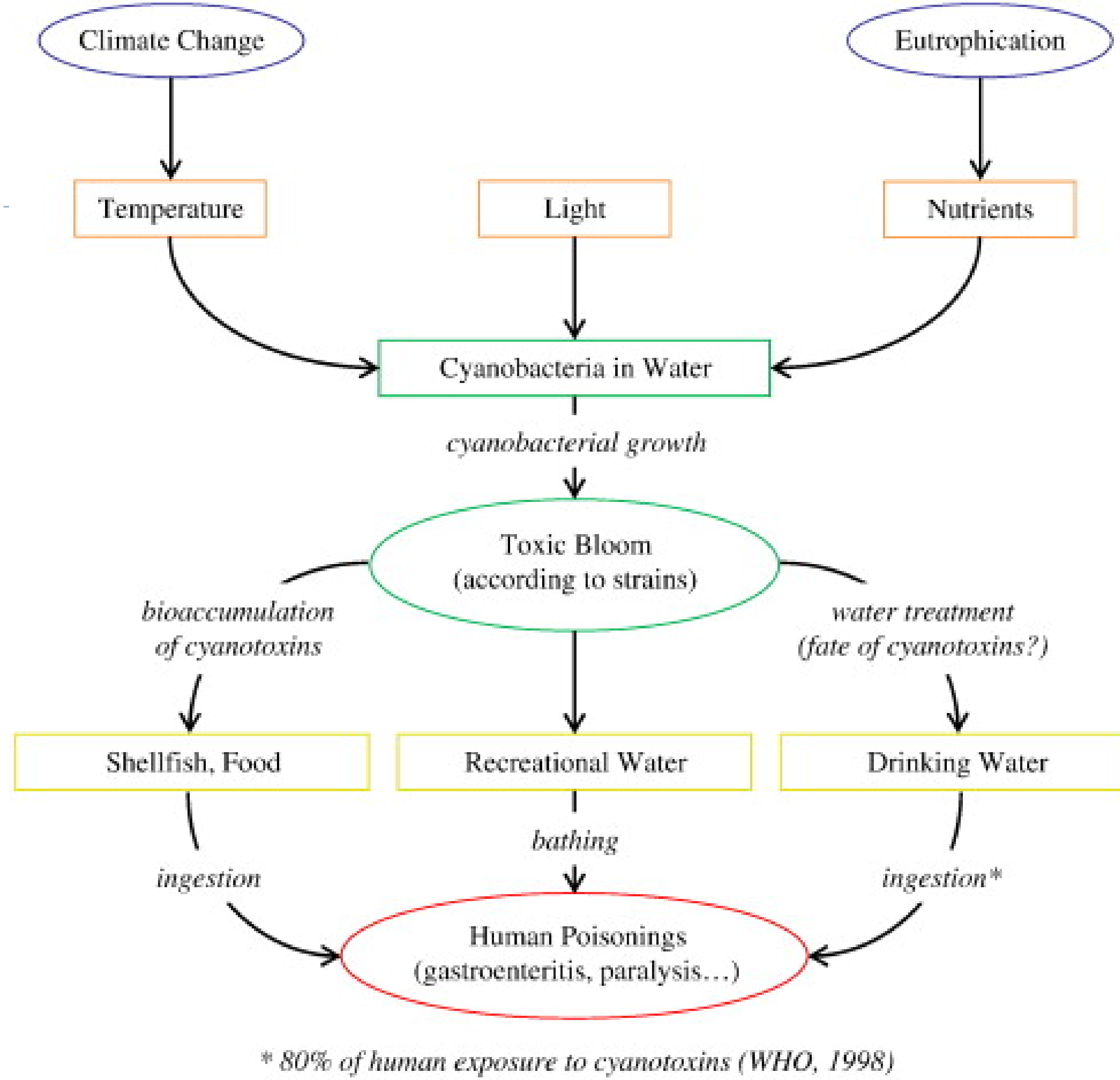


Fig. 1 Origin of toxic cyanobacterial blooms and human exposure.

Merel, S., Walker, D., Chicana, R., Snyder, S., Baures, E. and Thomas, O. (2013) State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. Environment International 59, 303-327.

TABLE 1
Toxigenic cyanobacteria from marine, brackish and freshwaters

Cyanotoxin	Main producing cyanobacteria	Bibliographic source
Microcystins	Most of *Microcystis* spp and *Planktothrix* spp, some *Anabaena*, *Nostoc* and *Synechocystis* and *Cyanobium bacillare*, *Arthrospira fusiformis*, *Limnothrix redekei*, *Phormidium formosum*, *Hapalosiphon hibernicus*	Sivonen and Jones, 1999; Cronberg et al., 2003; Odebrecht et al., 2002; Ballot et al, 2004; Gkelis et al., 2001; Steffensen et al., 2001; Prinsep et al., 1992
Nodularins	*Nodularia spumigena* (in transitional waters)	Rinheart et al., 1988
Cylindrospermopsin	*Cylindrospermopsis raciborskii*, *Umezakia natans*, *Aphanizomenon ovalisporum*, *Aphanizomenon flos-aquae*, *Rhaphidiopsis curvata*, *Anabaena lapponica*, *Anabaena bergii*	Ohtani et al., 1992; Harada et al., 1994; Banker et al., 1997; Schembri et al., 2001; Li et al., 2001; Fastner et al., 2007; Spoof et al., 2006
Anatoxin-a	Most of *Anabaena* spp., some *Aphanizomenon* (*A. flos-aquae*, *A. issatschenkoi*), *Cylindrospermum*, *Microcystis and Planktothrix* spp. *and Raphidiopsis mediterranea*	Edwards et al., 1992; Sivonen et al., 1989; Park et al., 1993; Namikoshi et al., 2003; Wood et al., 2007
Homoanatoxin-a	*Oscillatoria formosa*, *Raphidiopsis mediterranea*	Skulberg et al., 1992; Steffensen et al., 2001; Namikoshi et al., 2003
Anatoxin a-(s)	*Anabaena flos-aquae* and *A. lemmermannii*	Carmichael and Gorham, 1978; Henriksen et al., 1997
Saxitoxins (PSP)	*Aphanizomenon*, *Anabaena*, *Lyngbya* and *Cylindrospermopsis* spp.	Humpage et al., 1994
LPS endotoxins	All cyanobacteria	McElhiney and Lawton, 2005
Aplysiatoxin, Lyngbyatoxin Debromoaplysiatoxin	*Lyngbya majuscula* (marine waters), *Oscillatoria nigro-vridis*	Serdula et al., 1982; Mynderse et al., 1997
Microviridin J	*Microcystis* spp	Rohrlack et al., 2003
β-N-methylamino-L-alanine	*Microcystis*, *Anabaena*, *Nostoc* and *Planktothrix* spp and most of cyanobacteria symbionts tested	Cox et al., 2005

Funari E, Testai E. Toxigenic cyanobacteria from marine, brackish and freshwaters. Chart. *Critical Reviews in Toxicology*, Feb2008; 38(2):98 Available from: Academic Search Premier, Ipswich, MA. Accessed March 20, 2010.

TABLE 3
Main toxicological data of some cyanotoxins

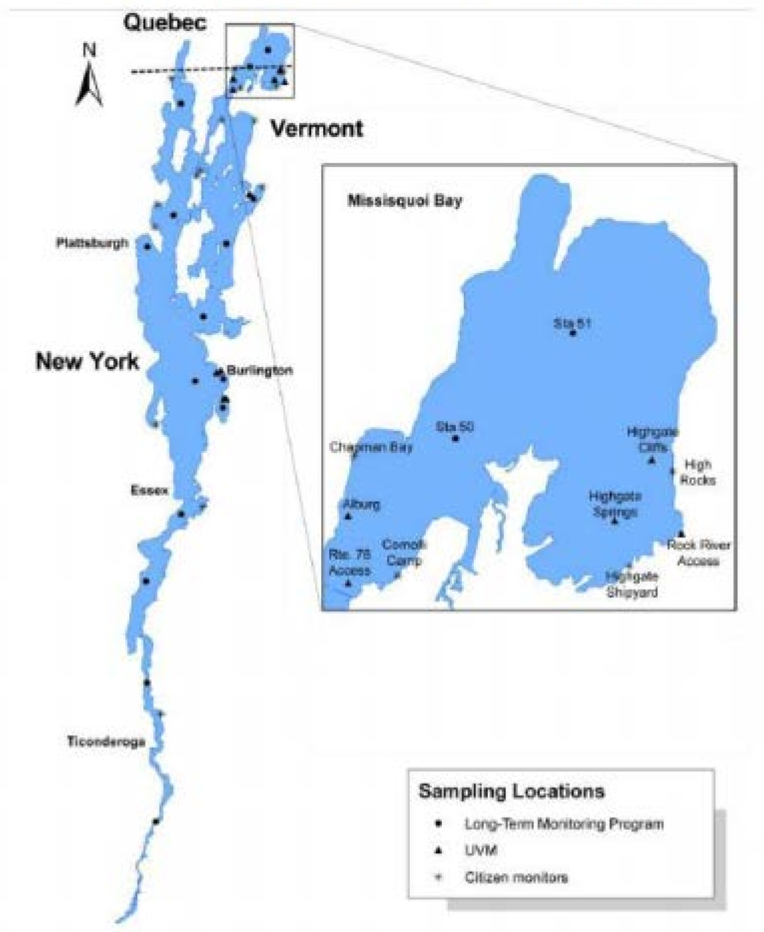
Cyanotoxin	i.p. LD_{50} (μg/kg b.w.)	Oral LD_{50} (μg/kg b.w.)	Target organ and mechanism of action	NOEL (μg/kg/d)	LOEL (μg/kg/d)	ADI/TDI (μg/kg/d)
Microcystin (MC)	50–1200	5000	Liver (PP1 and PP2A phosphatases inhibition-Tumor promoting activity)	40 (MC-LR; mice; 13 weeks; gavage) 330 (MC-LR in BGAS extracts; mice; 13 weeks; dietary)	200	0.04 (UF = 1000)
Nodularin (NOD)	50	ND	Liver (PP1 and PP2A phosphatases inhibition-Tumor promoting activity)	ND (refer to MC-LR)	ND	—
Cylindrospermopsin (CYN)	2100 (24 h) 200 (6 days)	4400–6900 (2–6 days)	Kidney, liver (Parent compound: protein synthesis inhibition; Metabolites: different but unknown mechanism; possible genotoxicity)	30 (Mice; 11 weeks; gavage) *C. raciborskii* extracts more toxic than pure CYN	60	0.03 (UF = 1000)
Anatoxin-a	375	5000	Neuromuscular system (Depolarizing effect due to binding to nicotinic Ach receptor)	>510 (mice; 54 days; drinking water) Limited chronic risk	ND	0.51 (UF = 1000)
Homoanatoxin-a	330	ND	Similar to anatoxin-a	ND Limited chronic risk	—	—
Anatoxin a-(s)	20–40		Peripheral nervous system (AChE inhibition; nerve hyper-excitability)	ND Limited chronic risk	—	—
Saxitoxin (STX)	10–20	263	Neuromuscular system (Membrane ion channel block) Human: 0.144–0.304 mg/person: mild symptoms 0.456–12 mg/person: from moderate symptoms up to paralysis and death	ND Acute risk > chronic	—	—
LPS Endotoxins	40–190 mg/kg bw	ND	Skin and mucosa (irritation, topic effects)	ND	—	—

i.p. = intraperitoneal; ND = Not determined; UF = uncertainty factor.
Note: bibliographic references are available within the text.

Funari E, Testai E. Toxigenic cyanobacteria from marine, brackish and freshwaters. Chart. *Critical Reviews in Toxicology*, Feb2008; 38(2): 101 Available from: Academic Search Premier, Ipswich, MA. Accessed March 20, 2010.

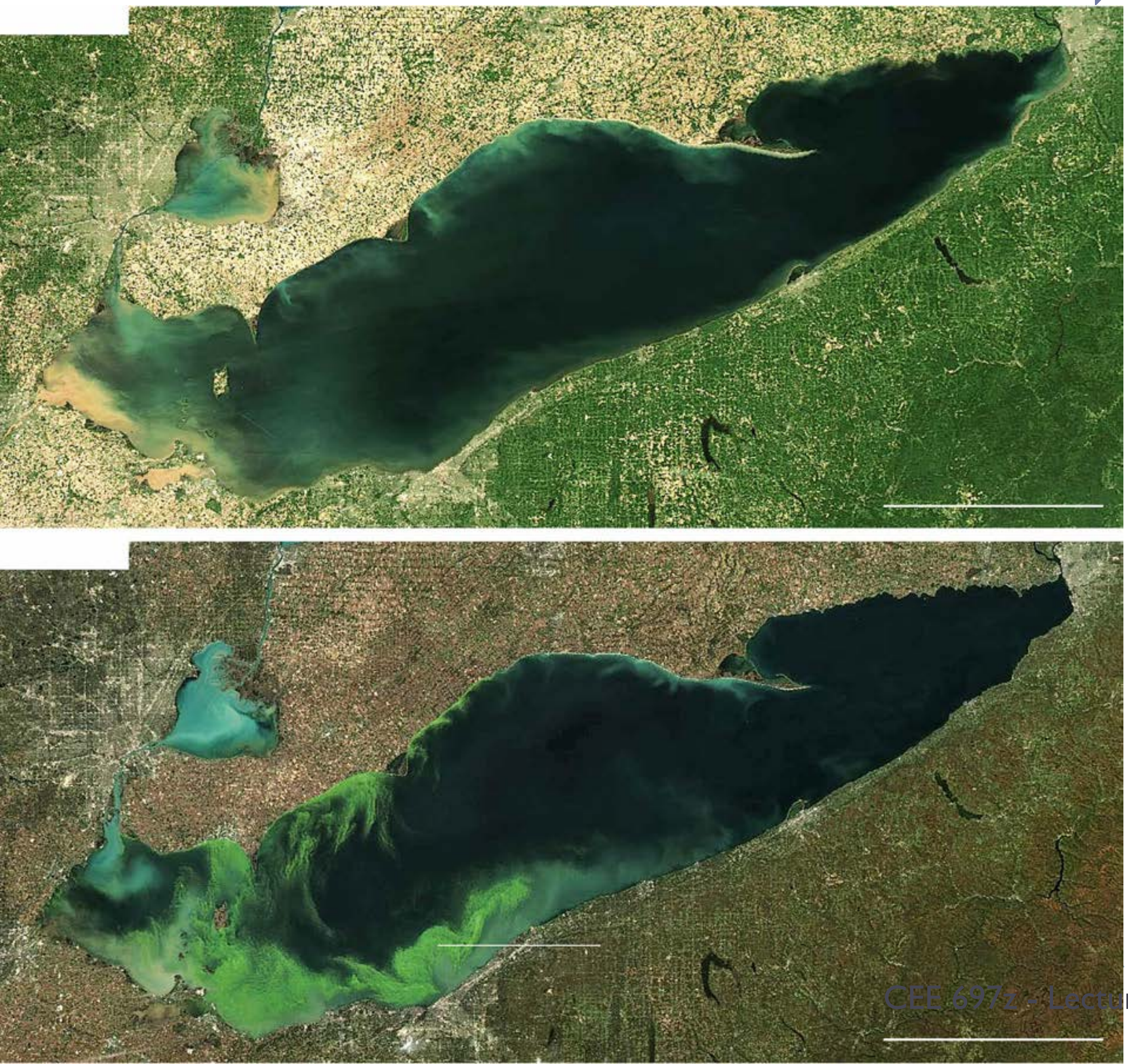
Lake Champlain

Figure 1. Location of the blue-green algae monitoring locations.

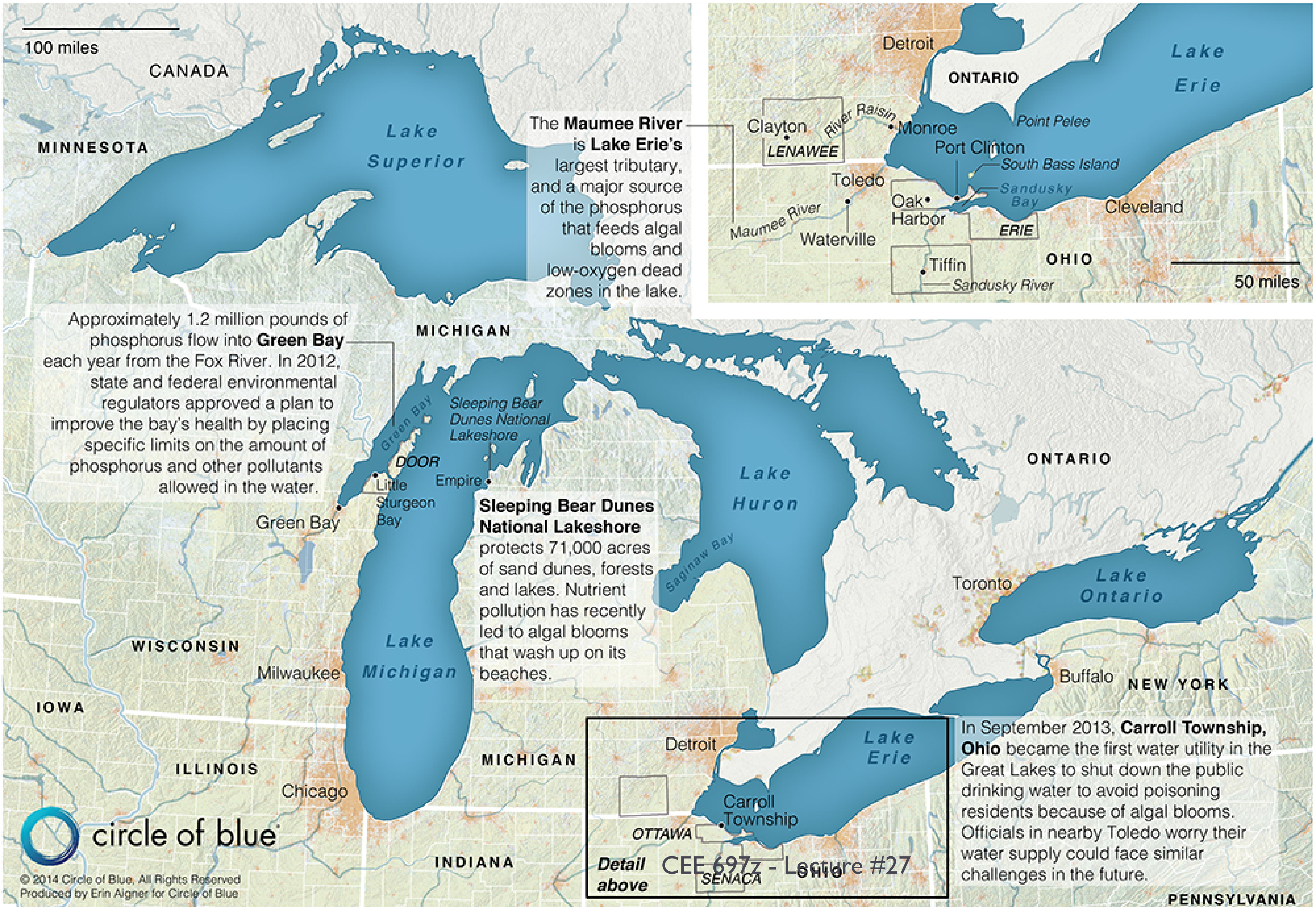


http://healthvermont.gov/enviro/bg_algae/bgalgae.aspx

Lake Erie; western basin

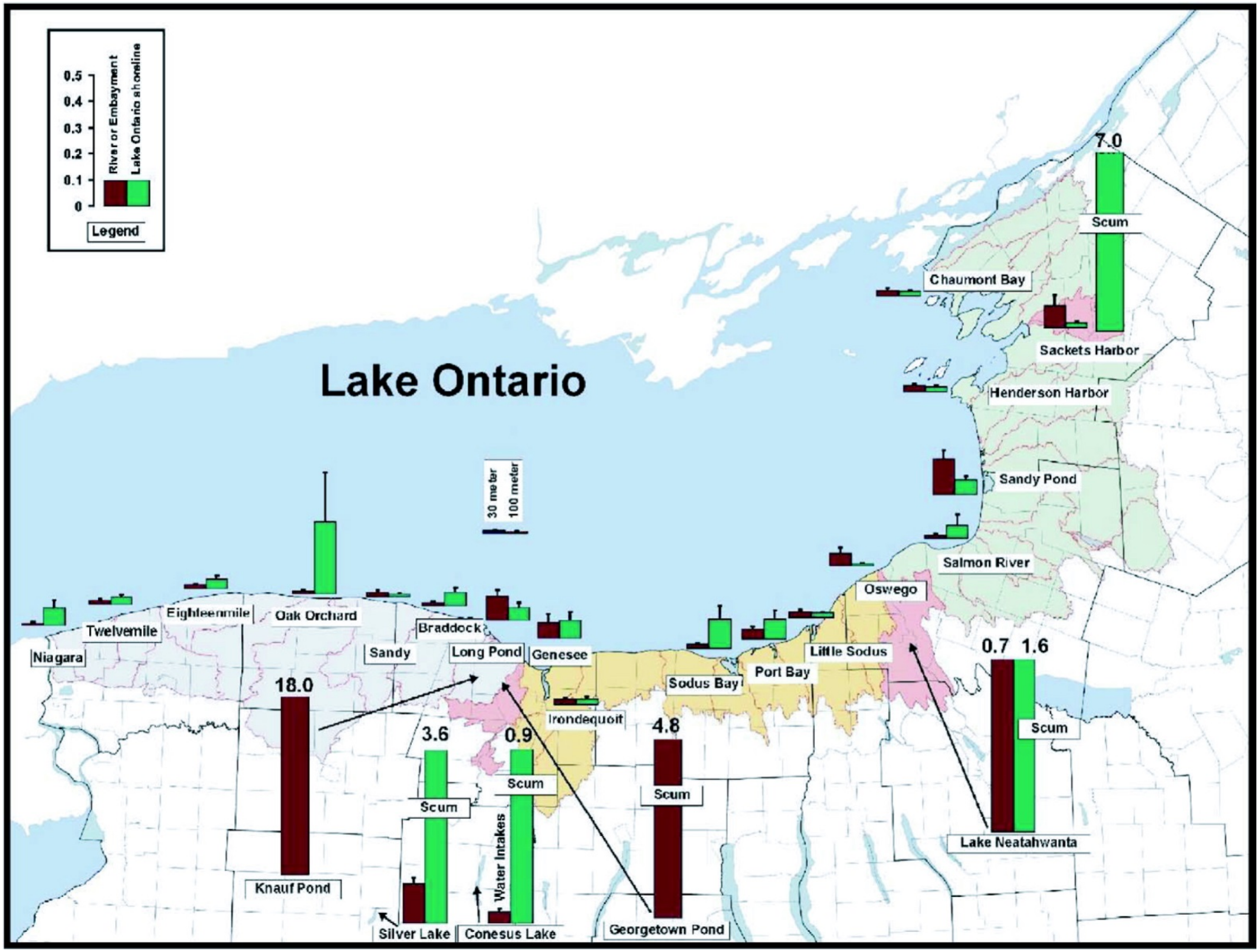


- The 2014 Toledo Ohio incident:
  - On-line reports
  - http://www.nytimes.com/2014/08/05/us/lifting-ban-toledo-says-its-water-is-safe-to-drink-again.html?_r=0
  - http://www.vox.com/2014/8/3/5963645/a-toxic-algae-bloom-has-left-400000-people-in-ohio-without-drinking

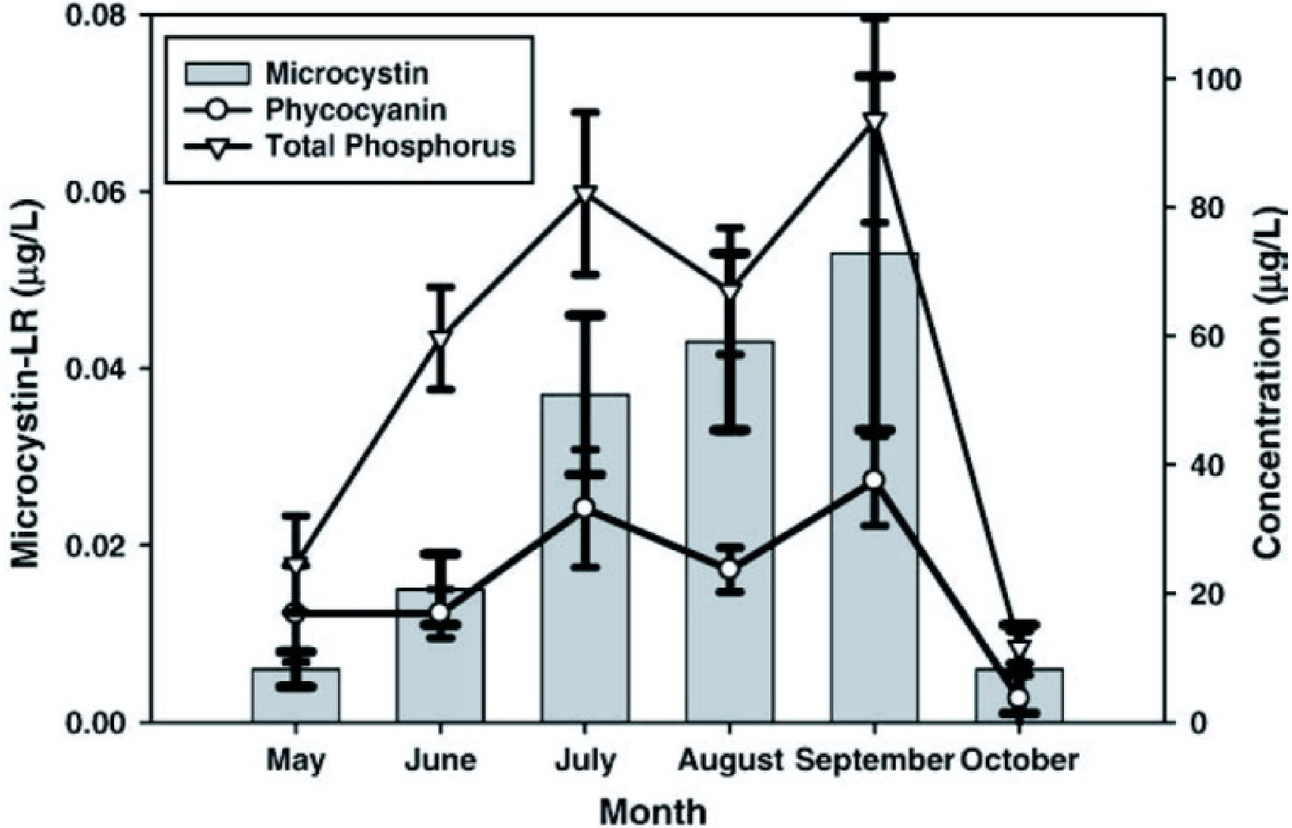

100 miles
CANADA
MINNESOTA
Lake Superior
The Maumee River is Lake Erie's largest tributary, and a major source of the phosphorus that feeds algal blooms and low-oxygen dead zones in the lake.
Detroit
ONTARIO
Lake Erie
Clayton
LENAWEE
River Raisin
Monroe
Point Pelee
Port Clinton
South Bass Island
Toledo
Sandusky Bay
Oak Harbor
Cleveland
Maumee River
Waterville
ERIE
OHIO
Tiffin
Sandusky River
50 miles
Approximately 1.2 million pounds of phosphorus flow into Green Bay each year from the Fox River. In 2012, state and federal environmental regulators approved a plan to improve the bay's health by placing specific limits on the amount of phosphorus and other pollutants allowed in the water.
MICHIGAN
Green Bay
Sleeping Bear Dunes National Lakeshore
DOOR
Empire
Little Sturgeon Bay
Green Bay
Sleeping Bear Dunes National Lakeshore protects 71,000 acres of sand dunes, forests and lakes. Nutrient pollution has recently led to algal blooms that wash up on its beaches.
Lake Huron
Saginaw Bay
ONTARIO
Toronto
Lake Ontario
WISCONSIN
Milwaukee
Lake Michigan
Buffalo
NEW YORK
IOWA
ILLINOIS
Chicago
MICHIGAN
Detroit
Lake Erie
Carroll Township
OTTAWA
Detail above
SENACA
OHIO
INDIANA
PENNSYLVANIA
In September 2013, Carroll Township, Ohio became the first water utility in the Great Lakes to shut down the public drinking water to avoid poisoning residents because of algal blooms. Officials in nearby Toledo worry their water supply could face similar challenges in the future.
circle of blue
© 2014 Circle of Blue, All Rights Reserved
Produced by Erin Aigner for Circle of Blue

Microcystin Concentrations

- **1 ppb WHO drinking water limit**
- **20 ppb WHO swimming limit**
- **60 ppb highest level for Lake Erie till 2011**
- **84 ppb highest level for Grand Lake St. Marys till 2010**
- **2000+ Grand Lake St. Marys 2010**
- **1200 Lake Erie Maumee Bay area 2011**
- **Carroll Water System, west of Davis-Besse, 4&5 Sept 2013, 1.4 and 3.5 ppb**



Sampling locations and microcystin-LR concentrations (average ± S.E., μg/L) along the Lake Ontario shoreline and the associated rivers, embayments and ponds. The green vertical bar at each Lake Ontario site represents the "shoreside" sampling site. The red vertical bar at each Lake Ontario site represents samples taken in the creek, river or embayment. Vertical bars are to scale. Bars for Knauf and Georgetown Ponds, Lake Neatahwanta, Sackets Harbor "scum" and the Conesus and Silver Lake "scum" concentrations are not to scale with concentration listed above the bar. The vertical bars for the nearshore and offshore of Lake Ontario are labeled "30 m" and "100 m"



Average monthly (±S.E.) microcystin-LR, total phosphorus and phycocyanin concentration (µg/L) at 37 sites in Lake Ontario from 2003–2006. Sites include streams, rivers, embayments, shoreside sites, and the nearshore and offshore zones. See Fig. 1 for location of sites.

- To next lecture