Methodological analysis of algal toxins

Comparing ELISA and LC/MS methods for toxin production

Project Summary

Overview

Collaborators

Megan L. Larsen and Daniel D. Snow *University of Nebraska-Lincoln Water Sciences Center*

Project questions

1. What are the temporal patterns of microcystins in the reservoir systems? – Focus on microcystins with coupled lake monitoring data available in Storet
2. What are the temporal patterns in physical, chemical, and biological characteristics of Nebraska Lakes? – Incorporate information from the Ambient Lake Monitoring programs with NDEQ with microcystins – make note of lake treatments
3. How do different analytical methods (ELISA vs. LC/MS) compare for the detection of algal toxins? – Total concentration – Specificity (ELISA = broad; LC/MS = analyte specific) – detection of other cyanotoxins: Many programs have regulations for other toxins (Ohio)

List of abbrevations

NDEQ: Nebraska Department of Environmental Quality

USEPA: United States Environmental Protection Agency

USACOE: United States Army Corp of Engineers

USGS: United States Geological Survey

NOAA: National Oceanic and Atmospheric Administration

Contents

Materials and Methods

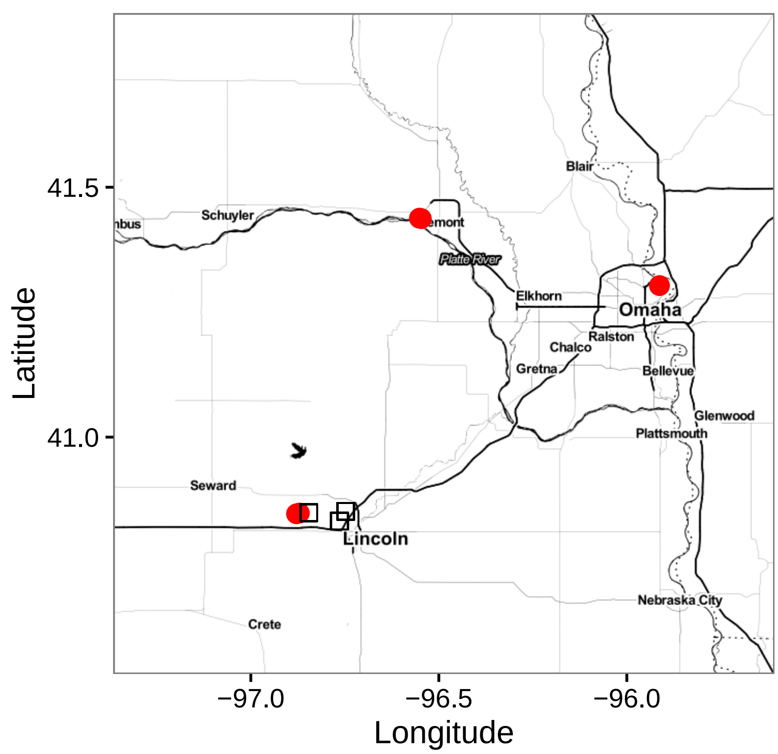
Sample collection and analysis

Reservior and monitoring programs

Fifty-four lakes and reservoirs (herein referred to as lakes) across the state of Nebraksa were sampled for total microcystin concentration as part of the Nebraska Department of Environmental Quality (NDEQ) Beach Monitoring program (BMP) between 2004 - 2014. These ecosystems are part of several other lake monitoring programs conducted by the NDEQ (Ambient Lake Monitoring Program), United States Army Corp of Engineers (USACOE), United States Geological Survey (USGS), and United States Environmental Protection Agency (USEPA). For our methodological comparision of microcystin concentrations, we focused on samples collected from Pawnee Lake (Lancaster County), Carter Lake (Douglas County), and Fremont 20 Lake (Dodge County).

Pawnee Lake (Lancaster County, Nebraska) is one of X freshwater ecosystems consisently monitored for algal toxins and coliform bacteria by the NDEQ over the last decade as part of the Ambient Lake Monitoring Program. Pawnee Lake was formed as an irrigation reservoir (?) in 19XX by the GROUP by adding an earthen dam at the southeast corner. Chemical and physical traits of Pawnee Lake are monitored by the NDEQ, USCOE, and USGS with data publically available from the National Water Quality Database Climatic data for the area was obtained from NOAA.

[More information about Fremont 20 and Carter]



Algal toxin analysis

NDEQ personnel collected surface samples from lake swimming beaches [details to be added]; samples were processed for total/dissolved (?) microcystin concentration with enzyme-linked immunosorbent assays (ELISA; Abraxis -adda specific). ELISA "is non-specific and reported concentrations potentially include multivariante, degradates, and precursors; thus, the entire toxin class is referred to when discussing ELISA results" (From Grahm et al 2010). [ELISA method: four paramter curve fit?]

[Who collected samples for LC/MS/MS] collected [sample location] samples were processed for specific cyanotoxins (microcystin-LA, microcystin-LF, microcystin-LR, microcystin-RR, microcystin-LW, anatoxin-a, cylindrospermopsin) were quantified by direct-inject multianalyte liquid chromatography/tandem mass spectrometry (LC/MS/MS) method. [Detection limit/specifics]. We report total microcystin concentrations as the sum of the five individual microcystin congeners following correction for molecular cross-reactivity (Loftin et al 2008).

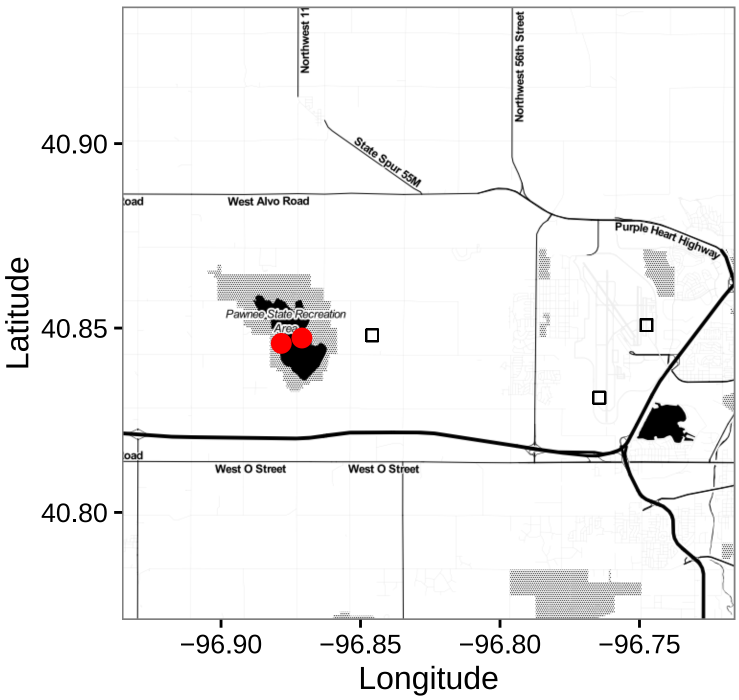
We evalutated the relationship between analytical methods for microcystin quanitification with Spearman-Rank correlation in R version 3.2.5 (2016-04-14).

2005 Nebraska Landuse and watersheds

TO BE ADDED FROM GIS ANALYSIS

Results

Pawnee Lake Reservoir



Temporal Trends

Air and water temperatures for the Pawnee Lake region from 1996 - 2016

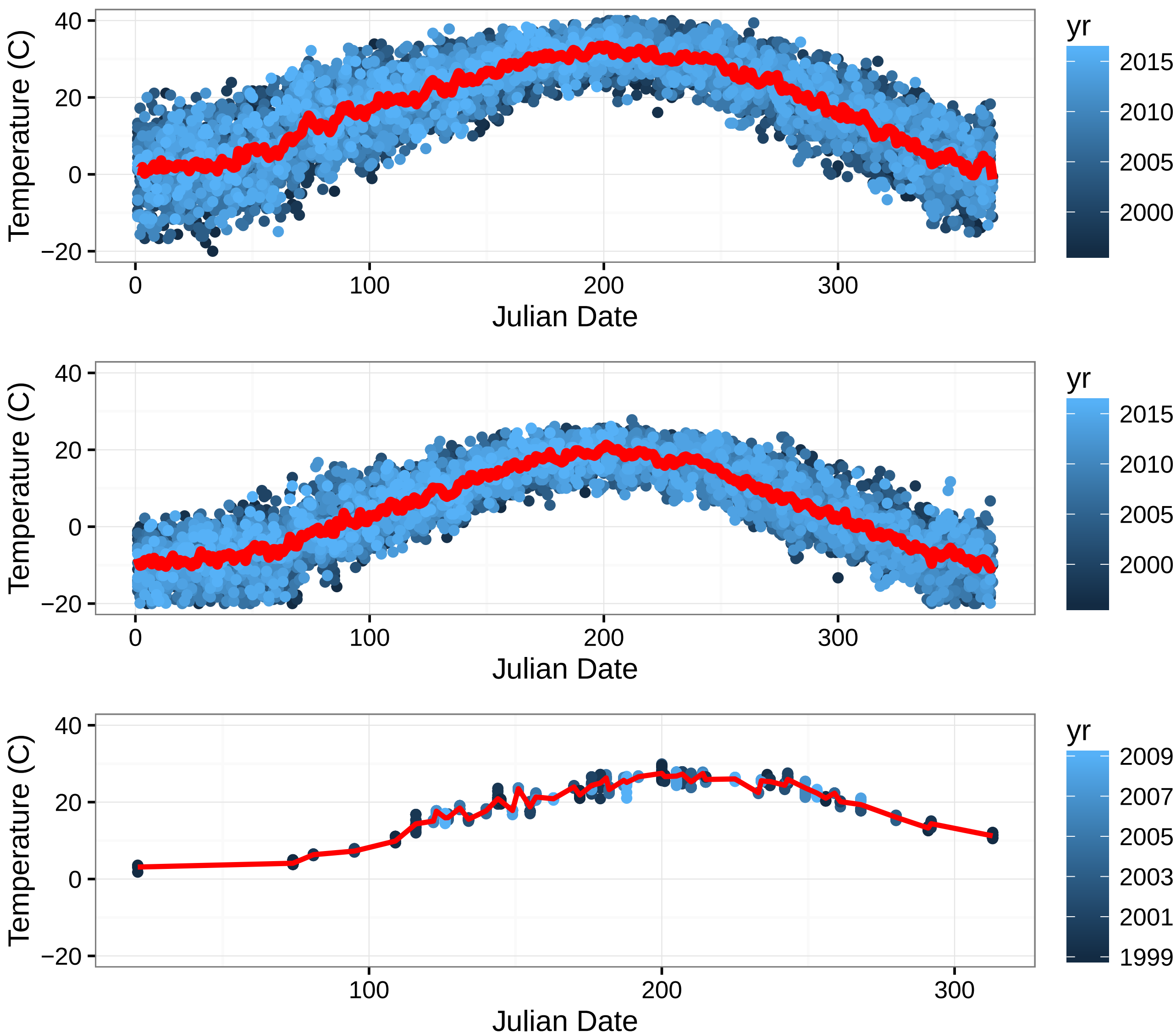


Figure 1: Air temperatures and water temperature for the Pawnee Lake region and reservoir.

Observattions

1. Air temperature (minimum and maximum) data were obtained from NOAA; Water temperature readings were obtained from USACOE.
2. Both air and water data follow similar temporal trends with no significant climatic deviations between years.

Temporal Reservior Characteristics 2001 - 2009

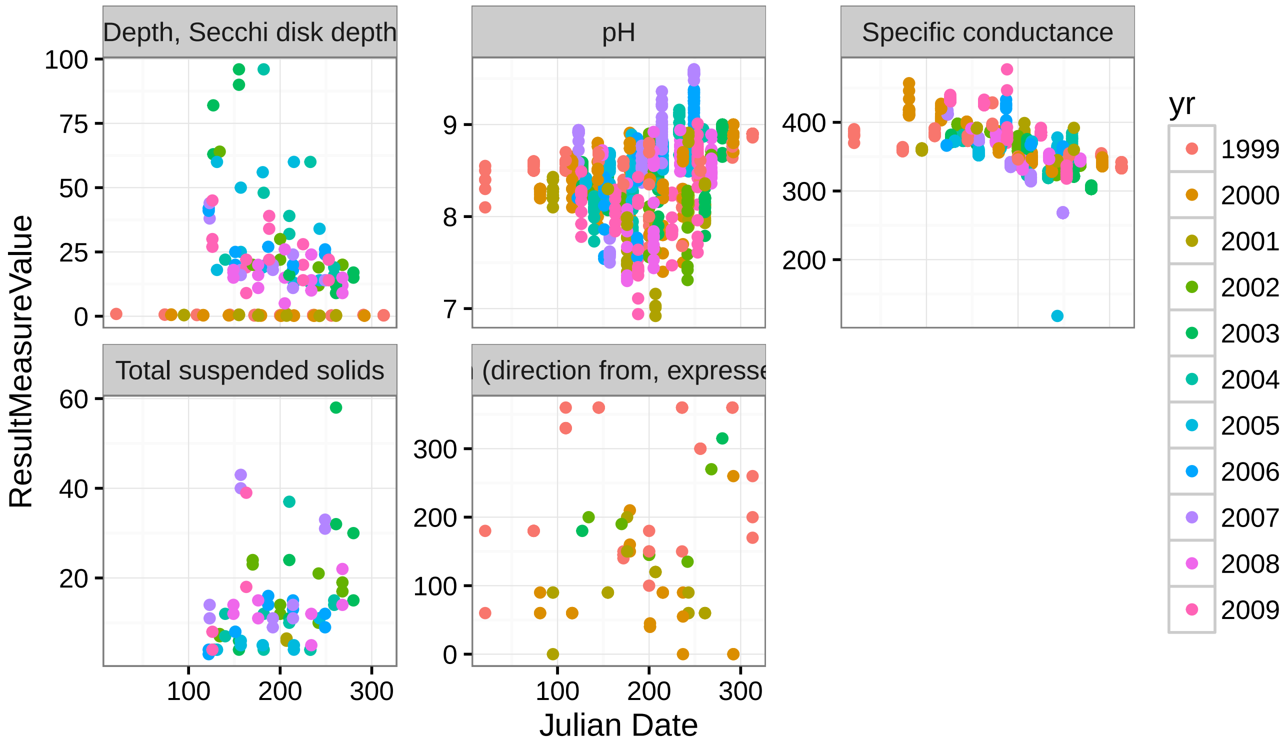


Figure 2: Measured physical characteristics of Pawnee Lake.

Observations

1. Data used in these plots were obstained from the National Water Quality Portal and contain information from projects at the NDEQ, USACOE, USGS, and USEPA.
2. Only notable temporal trend in the collected physical data is a decline in Specific conductance through the year.
3. pH highly variable in summer months, which is likely due to rainfall events

Annual trends

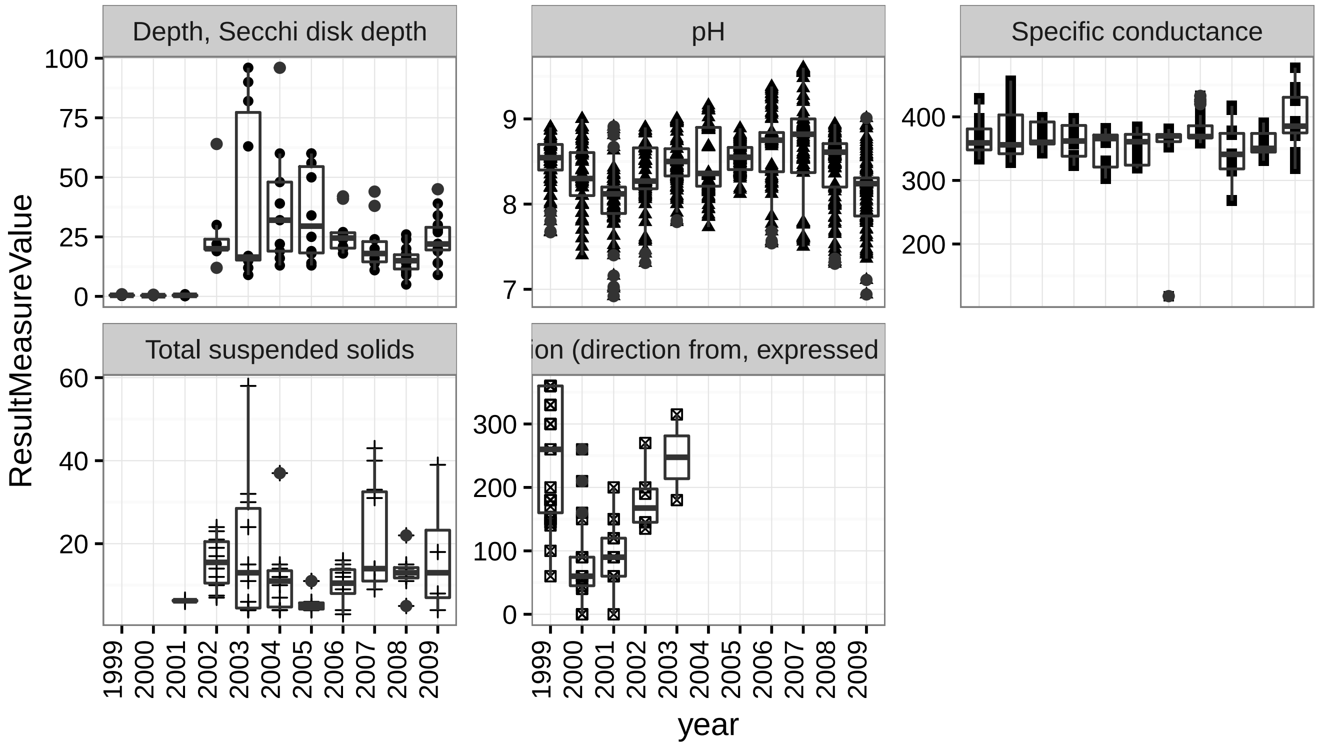


Figure 3: Average annual trends from 2001-2009

Observations

1. NOTE: The Secchi depth measurements are in both meters and inches. These values need to be converted to a standardized unit.

Nutrients

Nitrogen

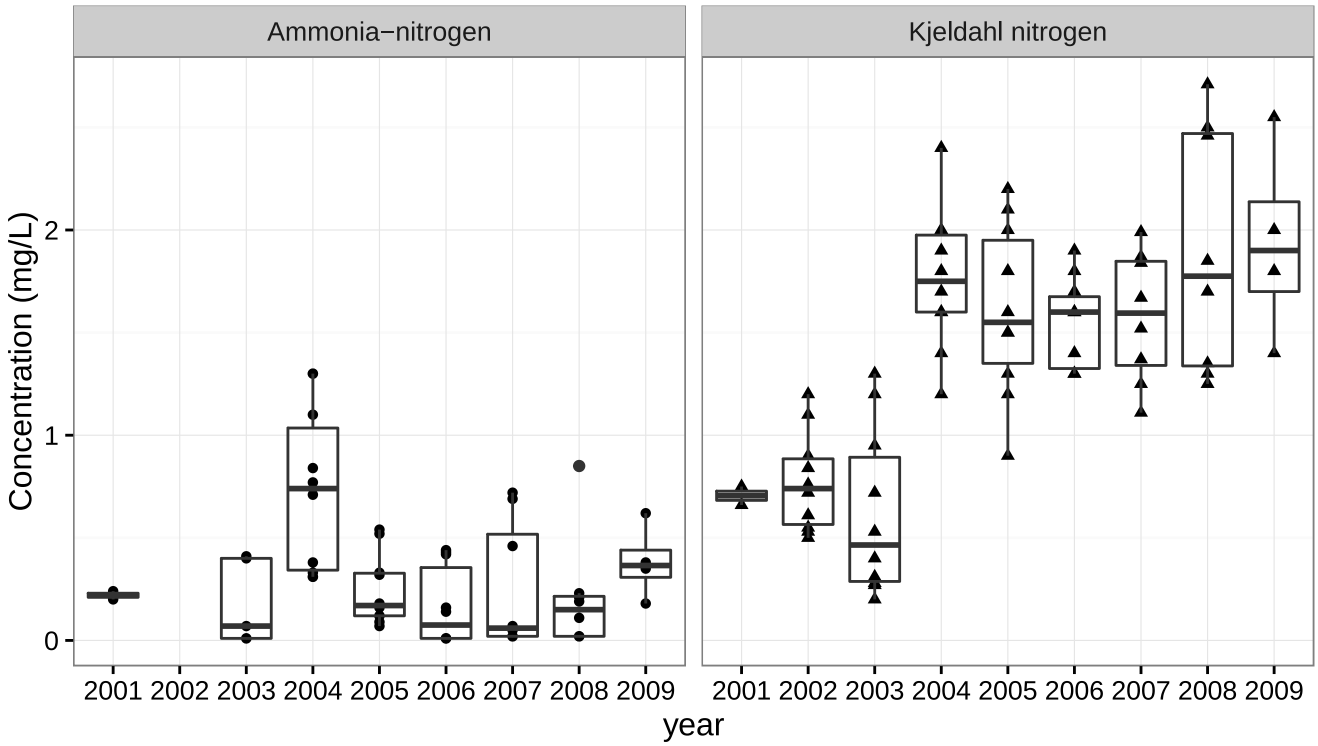


Figure 4: Nitrogen-related data from Pawnee Lake Reservoir between 2001-2009

Observations

1. Total nitrogen (as measured by Kjedahl digestion) drastically increased between 2003 and 2004.

Phosphorus

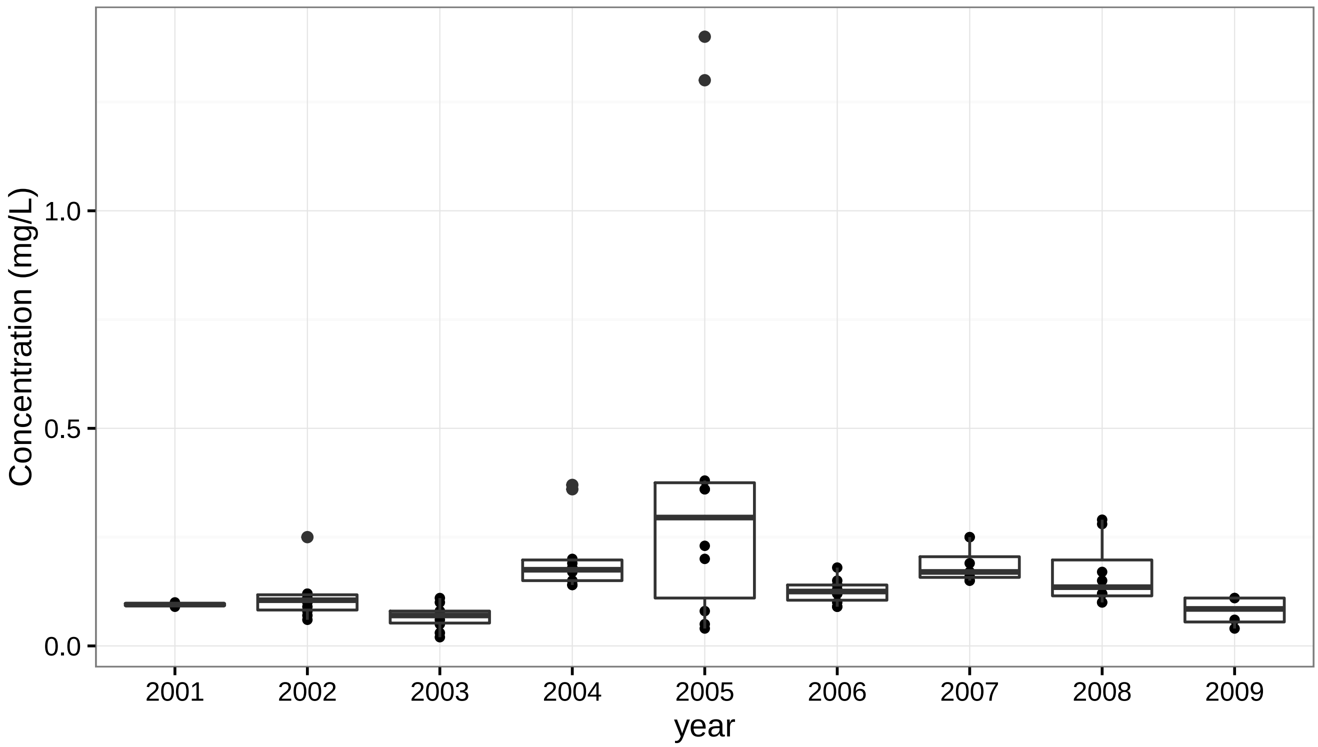


Figure 5: Phosphorus-related data from Pawnee Lake Reservoir between 2001-2009

Observations

1. Total phosphorus remained relatively similar between 2001-2009. However, phosphorus concentrations in 2005 were much more variable than any other year.

Toxins

NDEQ Total microcystins

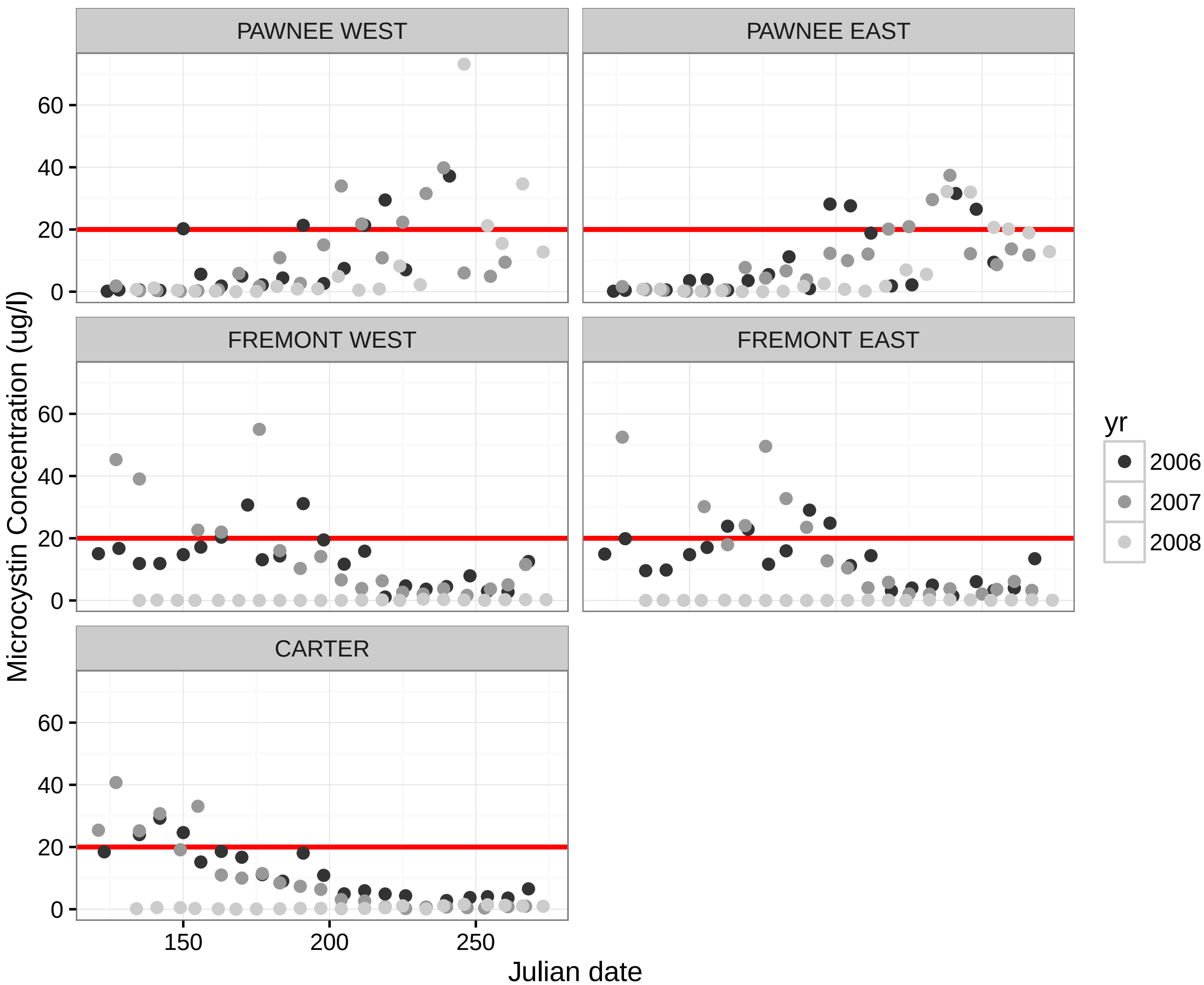


Figure 6: Total microcystin concentration (ug/L) as measured by ELISA in Fremont, Pawnee, and Carter Lakes (2006-2008). Data provided by the Nebraska Department of Environmental Quality as part of the beach monitoring program. Concentrationis greater than 20 ug/L (red line) result in beach closings.

Observations

1. Fremont and Carter Lakes show a loss of microcystin detection between 2007 and 2008. Fremont Lake was treated with alum at the end of the 2007 season and Carter in 2010.

WSL Algal toxins

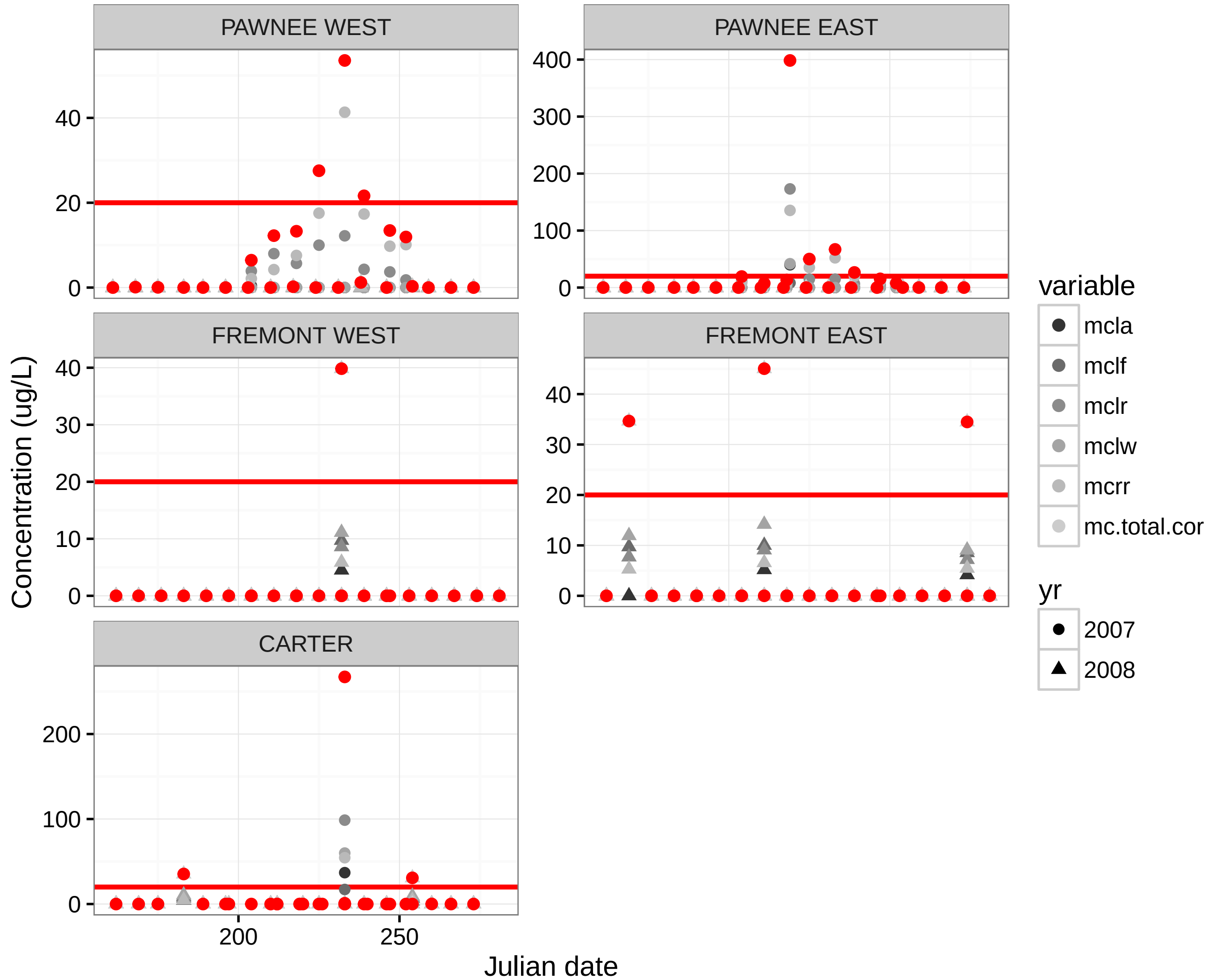
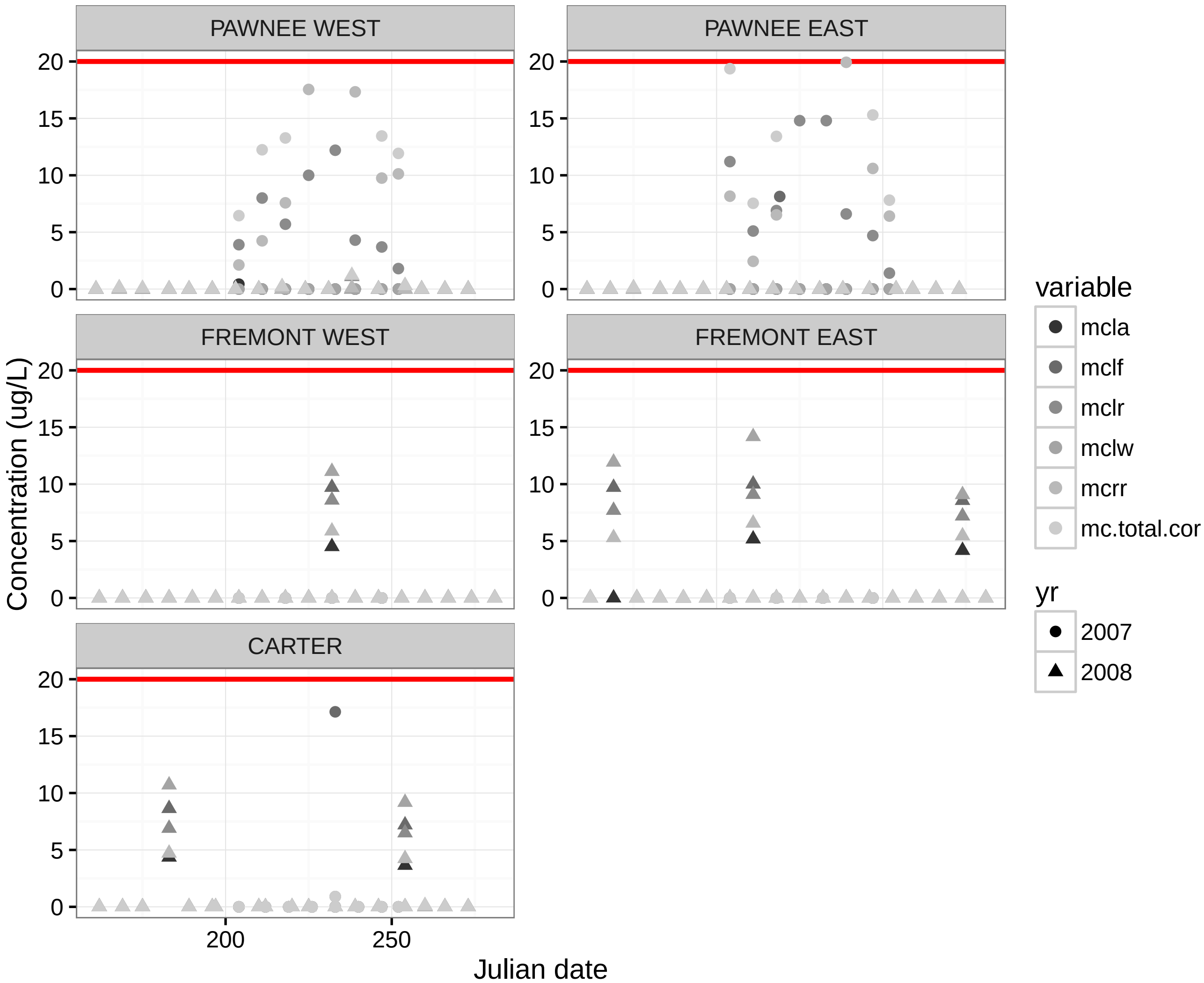


Figure 7: Corrected total microcystin concentration (red, ug/L) and individual microcystin congener concentrations as measured by LC/MS/MS in Fremont, Pawnee, and Carter Lakes (2006-2008). Concentrationis greater than 20 ug/L (red line) result in beach closings.


PAWNEE WEST
PAWNEE EAST
FREMONT WEST
FREMONT EAST
CARTER
Concentration (ug/L)
Julian date
variable
mcla
mclf
mclr
mclw
mcrr
mc.total.cor
yr
2007
2008

Other algal toxins

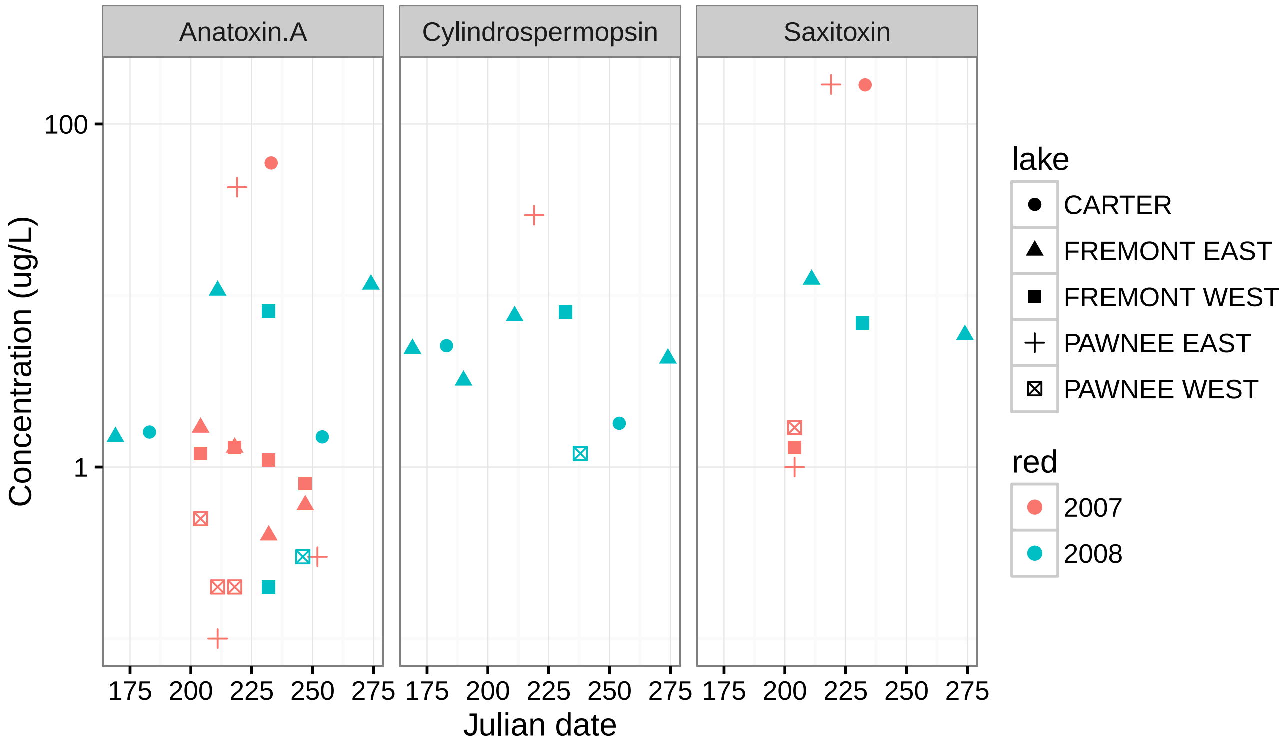


Figure 8: Other cyanotoxins detected by LC/MS. Only concentrations greater than the detection limit have been included on log base 10 y scale.

Observations

1. The State of Nebraska only monitors the concentration of microcystins as an aggregate group and issues health advisories for recreational water bodies as a total toxin concentrations greater than 20 ug/L. Other states issue both *public health advisories* (PHA) and *no contact advisories* (NCA) depending on the type of cyanobacterial toxin. For example, Ohio regularly tracks microcystin-LR, anatoxin-a, saxitoxin, and cylindrospermopsin and issues both PHA and NCA throughout the season (Table 1, next page).
2. **Anatoxin a**: The OH PHA advisory for anatoxin a is 80 ug/L; In this study, 0 samples (~ 0 %) contained an anatoxin concentration greater than the PHA advisory (mean =1.1 ug/L). The OH NCA advisory is 300 ug/L; In these samples, 0 samples (~ 0 %) contained a anatoxin concentration greater than the NCA advisory.
3. **Cylindrospermopsin**: The OH PHA advisory for cylindrospermopsin is 5 ug/L; In this study, 4 samples (~ 3.1 %) contained a cylindrospermopsin concentration greater than the PHA advisory (mean = 0.5 ug/L). The OH NCA advisory for cylindrospermopsin is 20 ug/L; In these samples, 1 samples (~ 0.8 %) contained a cylindrospermopsin concentration greater than the NCA advisory.
4. **Saxitoxin**: The OH PHA advisory for saxitoin is 0.8 ug/L; In this study, 8 samples (~ 6.2 %) contained a saxitoxin concentration greater than the PHA advisory (mean = 2.9 ug/L). The OH NCA advisory for saxitoin is 3 ug/L; In these samples, 5 samples (~ 3.9 %) contained a saxitoxin concentration greater than the NCA advisory.

Table 1: Selected state regulations for cyanotoxins.PHA = Personal Health Advisory; NCA = No Contact Advisory

State	Compound	PHA	NCA
Nebraska	Microcystin aggregate	NA	20
Ohio	microcystin-LR	6	20
	anatoxin-a	80	300
	saxitoxin	0.8	3
	cylindrospermopsin	5	20
California	microcystin	0.8	NA
	anatoxin-a	90	NA
	cylindrospermopsin	4	NA
Indiana	microcystin-LR	4	20
	cylindrospermopsin	NA	5
Vermont	microcystin-LR	NA	6
	anatoxin-a	NA	10

Methodological Comparison

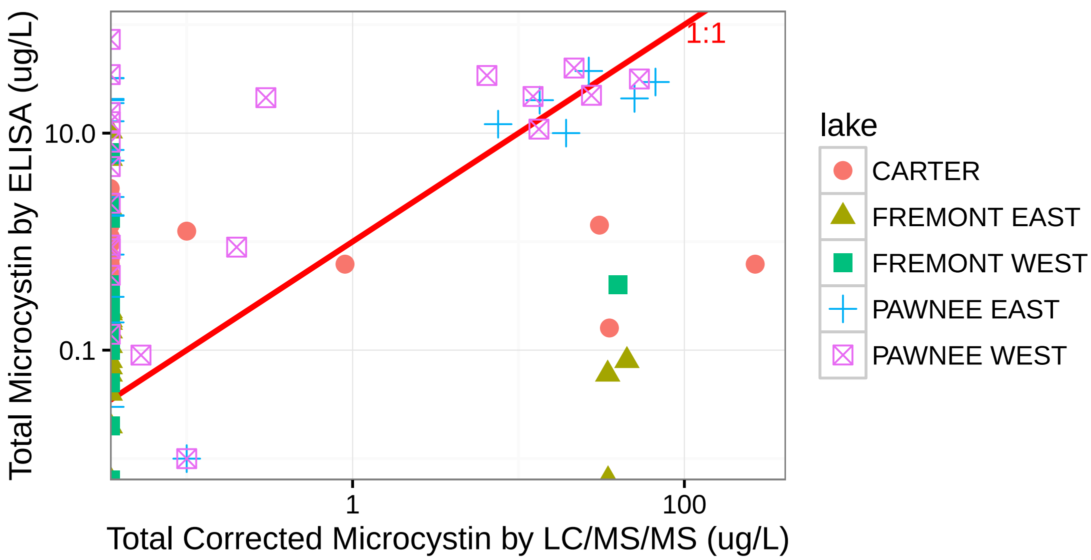


Figure 9: Relationship between total microcystin concentration as measured by LC/MS/MS and ELISA methods (n = 110). LC/MS/MS concentrations were calculated as the cross-reactivity corrected sum of each microcystin congener (-LA, -LF,-LR,-LW,-RR)

Observations

1. Large number of measurements where LC/MS did not detect microcystins above the detection limit. We tested for five of the most common microcystin congeners which have standards currently available for the LC/MS/MS analysis. To date, there are over 80 congeners of microcystin that have been reported. ELISA ADDA kits have a broad specificity that may either be detecting other microcystin congers or providing false positive results.
2. Seven of the samples (isolated from Fremont and Carter Lakes) contained higher concentrations of microcystins than detected by ELISA. These samples also contained high concentrations of anatoxin a, cylindrospermopsin, and saxitoxin.

Lake	Sample Date	ELISA	LC/MS	Anatoxin A	Cylindro	Saxitoxin
CARTER	2007-08-21	0.62	267.2	59.3	0	169.2
CARTER	2008-07-01	0.16	35.34	1.6	5.1	0
CARTER	2008-09-10	1.42	30.77	1.5	1.8	0
FREMONT EAST	2008-06-17	0	34.65	1.5	4.9	0
FREMONT EAST	2008-07-29	0.08	45.04	10.7	7.6	12.4
FREMONT EAST	2008-09-30	0.06	34.48	11.6	4.3	5.9
FREMONT WEST	2008-08-19	0.4	39.83	8.1	8	6.9

3. ELISA and cross-reactivity corrected LC/MS/MS concentrations were strongly correlated (rho = 0.2308405, p = 0.0152543, n = 110).
4. Nineteen samples (17.3) contained more than one microcystin congener.