



Dear Participants:

Thank you for your cooperation on our Michigan Harmful Algal Bloom (HAB) study. We appreciate your help as we try to better understand one of the new concerns to our Michigan lakes; the presence of cyanotoxins due to HABs. The map shows the participation in our study.

We made many measurements over the 2017 season, but the focus is on understanding the occurrence of a group of naturally occurring toxins called microcystins (MC), which are a toxic to the liver. The same group of toxins were responsible for the “Do Not Drink” order issued by the Toledo, OH water treatment authority in 2014. In addition, our study looked for other, less common toxins called, anatoxin-a, and cylindrospermopsin.

Over the course of our survey, we sampled the lake water, did an ecosystem and land use review, set-up monitoring equipment, and did four months of analytical testing. Our data included the levels of toxin found, water conditions, the presence (or absence) of zebra mussels, and the types of toxin producing cyanobacteria (also called blue-green algae) present in the lake.

Through the participation of the many lake associations, the Michigan Department of Environmental Quality, Oakland University, Lumigen Instrument Center at Wayne State University, and the Environmental Analysis Lab at Lake Superior State University, and you, our volunteers, we have gained a better understanding of the conditions that lead to HABs. Preliminary data confirm warm, phosphorus rich environments are at the greatest risk of developing a bloom. Through further data analysis and an additional survey year we hope to learn even more.

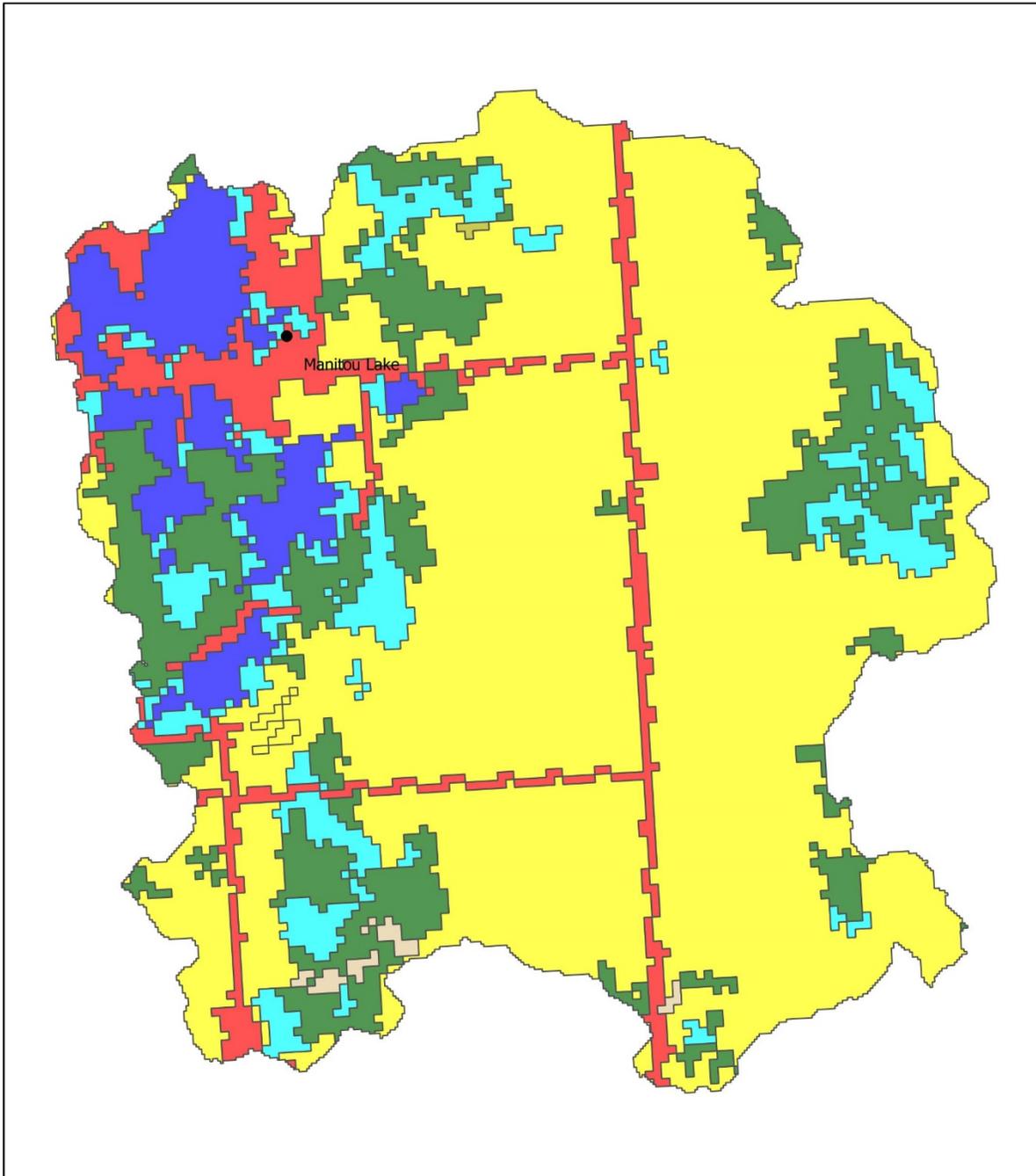
Thank you very much for your assistance and we look forward to working with you in 2018.

David C. Szlag and the HAB research team

Synopsis

We measure microcystins by two methods: Adda ELISA and Liquid Chromatography Mass spectrometry (LC/MS/MS). The LC/MS/MS method is more sensitive and less likely to generate a false positive. It is much more time consuming to run. Grab samples were taken in July, August, and October. The microcystin levels in Lake Manitou were well below the Environmental Protection Agency's draft guidance level of 4 ppb (parts per billion) using both methods. We also analyze samples for anatoxin-a and cylindrospermopsin. There were no detections of cylindrospermopsin, or anatoxin-a. The phosphorus levels in the lake were indicative of a "Eutrophic" lake signifying a moderate amount of phosphorus throughout the sampling season. The lake's watershed is mostly urban and agriculture. It is important to understand that these cyanobacteria are often present in Michigan Lakes.

Manitou Lake



Legend

- Land Use in Lake Watershed
- Water
 - Urban
 - Barren
 - Forest
 - Shrub
 - Herbaceous
 - Agriculture
 - Wetlands
 - Sampling point

Lake Reachcode: 4050010000000

Lake Area: 0 sq Km

Lake Max Depth: 0 meters

Zebra mussels were not present.



INTRODUCTION

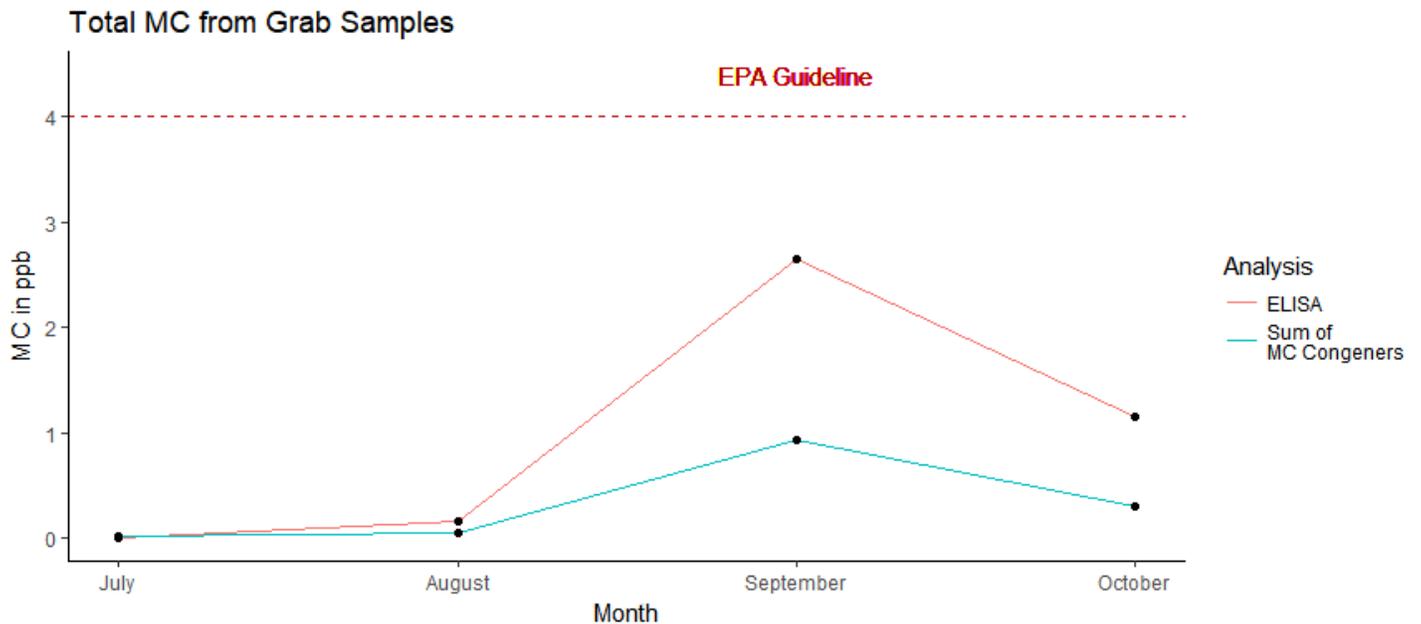
Once each month from July to October of 2017 we surveyed 32 Michigan lakes. At the start of each month, water conditions were recorded, samples were taken, and Solid Phase Adsorption Toxin Tracking (SPATT) bags were deployed and collected. Analysis of the water samples included analysis of nutrients, qPCR for cyanobacteria genes, and microcystins and other toxins. SPATTs are a new way of tracking toxins that provide a time weighted relative measure.

MICROCYSTIN AND AQUATIC TOXIN SUMMARY

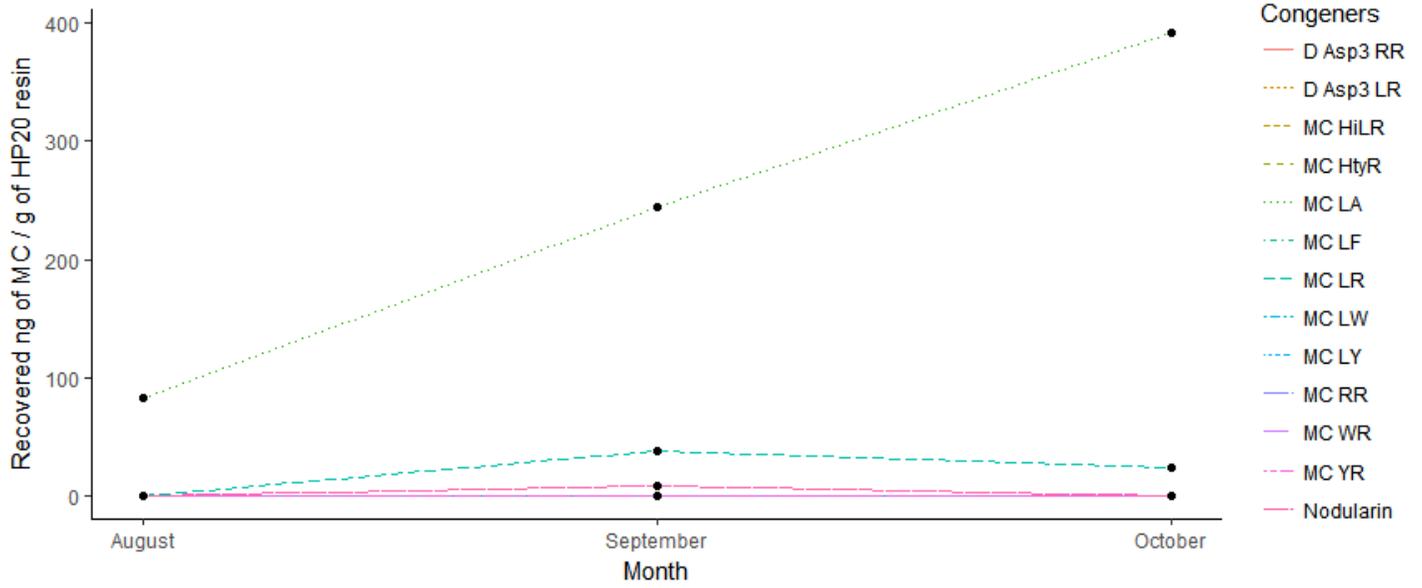
Enzyme Linked Immunosorbent Assay (ELISA) is a most commonly used analytical method for microcystin (MC) detection. Further data will demonstrate the validity of the test across multiple aquatic environments. We will be ultimately comparing ELISA with Mass Spectrometry (MS) results because it is the most precise and accurate method of quantification. Different MC congeners were measured and added as a sum (Sum of MC Congeners) to be compared with ELISA.

The information collected reflects the conditions at the time of sampling, but toxins recovered from the SPATT bags are indicative of toxin levels during the weeks between sampling. The SPATT bags were deployed for the month-long period between sampling events and then collected. SPATT data are represented by the total toxins collected during the period. The reported concentrations are from the analytical procedures used on the SPATT bags and are not directly indicative of average toxin levels. There is no USEPA guidance for this procedure yet and we will use the 2017 and 2018 data to provide interim guidance.

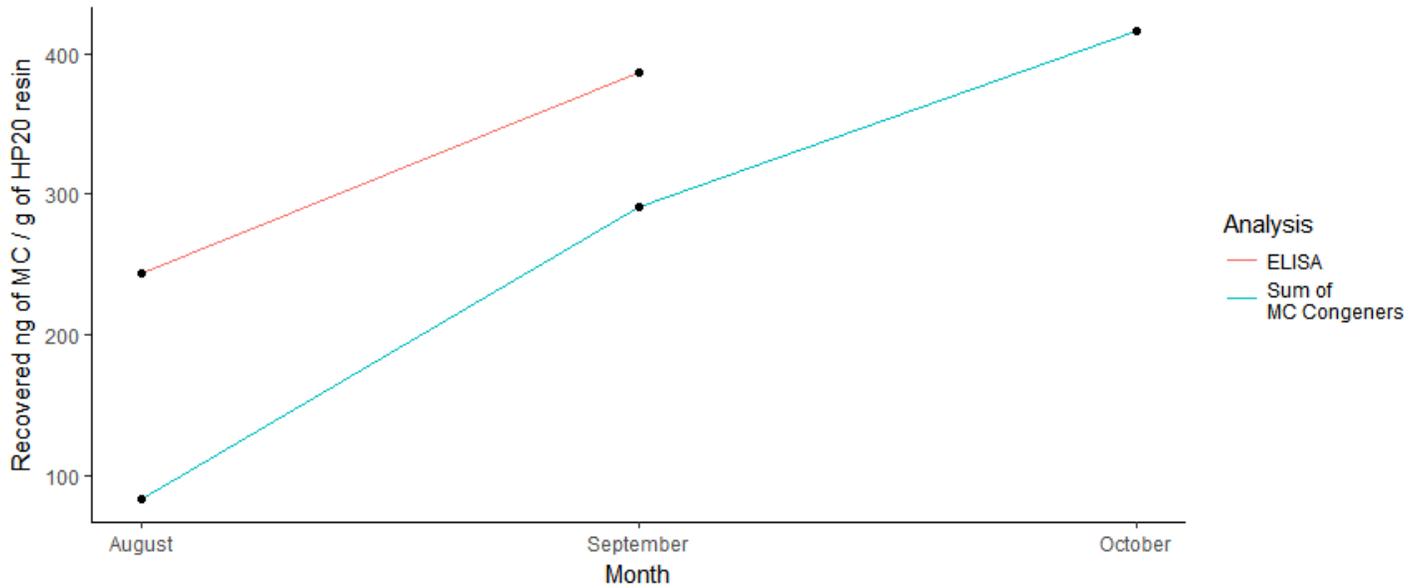
GRAB SAMPLE



MC Congeners from SPATTS



Total MC from SPATTS



QPCR

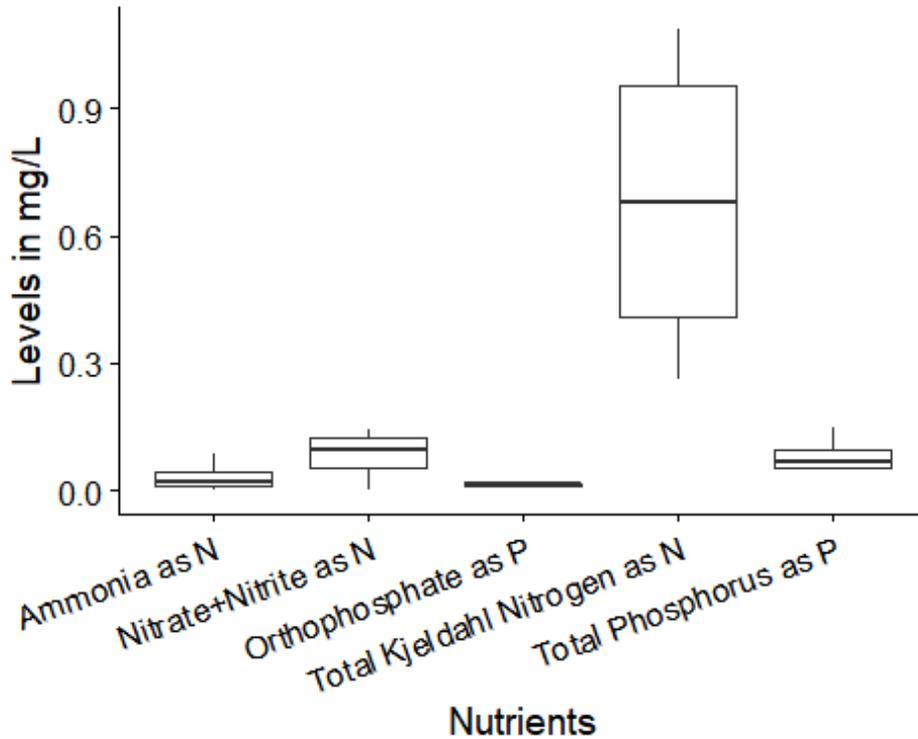
QPCR is a DNA test to rapidly measure the amount of total cyanobacteria (16s rRNA) and toxin genes (mcyE) present. Phytoxigene™ CyanoDtec test was performed with Applied Biosystem StepOnePlus PCR. Total Cyanobacteria 16s rRNA and toxin gene assay were analyzed in parallel for each month of grab samples. Data for each month is listed in table below. CyrA and SxtA were not detected for this year. The calculated values are expressed as “GeneCopies/mL”.

16s rRNA is a ubiquitous gene which is found in mostly all cyanobacteria. The 16s rRNA gene copies is used to measure relatively how much cyanobacteria is found in your lake. McyE gene is one of the few genes responsible of producing microcystin. Presence of these genes do not indicate that the toxins are present. Detection of McyE gene would indicate that the lake has the potential to produce microcystin and is advised to continue monitoring the lake. We do not become concerned until total cyanobacteria (16s rRNA) are above 200,000 and toxin genes (McyE) are above 10,000.

Month	16s rRNA (copies/mL)	mcyE (copies/mL)
July	53103	7
August	169977	99
September	234084	351
October	45339	464

NUTRIENT SUMMARY

The chart and table show the nutrient levels below. The key nutrients we measure are the forms of nitrogen and phosphorous. Phosphorus has long been known to increase the algal and cyanobacteria growth.



Month	Orthophosphate (mg P/L)	Nitrate + Nitrite (mg N/L)	Ammonia (mg N/L)	Total Phosphorus (mg P/L)	Total Kjeldahl Nitrogen (mg N/L)
July	0.0139	0.143	0.0865	0.077	0.907
August	0.0127	0.116	0.0000	0.051	0.263
September	0.0044	0.000	0.0271	0.056	0.455
October	0.0214	0.074	0.0132	0.149	1.085

WATER PARAMETERS

Month	Temperature (°C)	Turbidity (NTU)	pH	Dissolve Oxygen (mg/L)	Conductivity (µS)	Chlorophyll-a (RFU)	Phycocyanin (RFU)
July	26.18	6.5	8.24	7.02	430	1.03	0.09
August	24.68	10.3	8.46	9.94	434	1.17	0.17
September	19.10	16.9	8.35	8.98	368	0.90	0.82
October	18.68	12.1	8.45	9.86	291	0.90	0.37