Tug Lake Internal Phosphorus Loading Study 2018: Summary Report

Todd Miller

University of Wisconsin – Milwaukee, Joseph J. Zilber School of Health, Milwaukee, WI 53211

Tug Lake Task Force Study Team: Reid Badeau, Mary Badeau, Glenn Hartley, Barb Hartley, Phil Hill, Judy Hill, Jon Malm, Don Olson, Sarah Olson, and John Redmann

Outline

Tables

Appendices

Introduction

Tug Lake is a 151- acre lake located in Irma, WI (Lincoln County). It is within the Lilly Hay Meadow Creek watershed (HUC-10 # 070700020305) draining an area of approximately 12 square miles consisting of primarily forest (54%), wetland (22%), and agriculture (10%). Over 60 residential homes are built on its shores with a mix of cottage and year around dwellings. Tug is a highly recreational lake used for boating fishing and swimming. Over the last several years, surveillance by the Tug Lake Task Force has shown that the lake occasionally experiences large accumulations of cyanobacteria (a.k.a. blue- green algae) otherwise known as cyanobacterial harmful algal blooms (cyanoHABs) primarily during Summer and Fall. While several types of cyanobacteria have been identified in Tug Lake the dominant form was one of several species of cyanobacteria within the genus *Microcystis*. These species are distributed globally and are responsible for most cyanoHABs observed in temperate lakes including Lake Erie, Green Bay and other well- known lake systems (De Stasio and Richman 1998; Ouellette et al. 2006; Xiao et al. 2018). Most, but not all *Microcystis* species are capable of producing the potent liver toxin microcystin in addition to a number of other less toxic substances (Welker and Von Döhren 2006). Over the last two years (2017 and 2018) the Tug Lake Task Force in collaboration with the Miller Laboratory at the University of Wisconsin – Milwaukee and Cason and Associates, LLC. (Berlin, WI) have engaged in water quality research to answer two questions 1) do microcystin toxins in Tug Lake occur at hazardous levels, and 2) what is the source of nutrients feeding cyanoHABs in Tug Lake.

In 2017 weekly water sampling was conducted by the Tug Lake Task Force and samples were sent to the Miller Laboratory for toxin and phosphorus analysis. Samples were also taken ad hoc from areas where blooms were observed. Details of results from 2017 toxin testing are

Box 1: Nutrients feed algal blooms
Most algal blooms in lakes are formed as a result of excess nutrients that enter the lake from snowmelt, storm runoff or seepage. These nutrients include various forms of nitrogen such as nitrate, nitrite, and ammonia or phosphorus as phosphate or phosphorus contained within organic molecules (e.g. DNA). Both nitrogen and phosphorus are required by algae for growth. Normally the availability of one or both of these nutrients is what limits growth of algae in lakes. When these nutrients are provided in excess and if all other conditions are conducive for growth (e.g. water temperature, pH, sunlight) cyanobacteria and other algae can grow and divide rapidly producing blooms. Some cyanobacteria have the ability to take nitrogen from air in a process called nitrogen fixation. In many cases the nitrogen these nitrogen- fixing cyanobacteria take in can be released for other organisms to use for growth. Thus, when nitrogen is supplied through nitrogen fixation then phosphorus becomes the only nutrient that is in limited supply for algal growth. Nitrogen fixing cyanobacteria have been identified in Tug Lake previously, however, rates of nitrogen fixation have not been studied.

available in the 2017 summary report in Appendix I. The major toxins detected were several different microcystin variants in 37 of 39 samples. At that time the United States Environmental Protection Agency had established draft guidelines for recreational environments which stated that swimming or other in- water activities should be avoided when microcystin concentrations exceed 4 micrograms per liter (µg/L) (United States Environmental Protection Agency 2016). As of 2018 those limits have now been raised to 8 µg/L. Of the 39 samples analyzed in 2017 a total of 22 samples or 56% exceeded this threshold. The US EPA considers any lake with greater than 10% of samples exceeding this threshold to be impaired for recreation. These results suggest that microcystin toxins do occur at hazardous concentrations in Tug Lake and that further research is necessary to understand what factors are instigating the cyanoHABs that produce these toxins.

Cyanobacteria like *Microcystis* in Tug Lake need sunlight, warmer water temperatures and nutrients in the form of nitrogen and phosphorus to form blooms (see Box 1). Since nitrogen- fixing cyanobacteria have been identified in Tug Lake it is likely that nitrogen is not a limiting nutrient for growth of cyanobacteria in Tug Lake, although it may be limiting at certain times. Phosphorus is most likely the most limiting nutrient for algal growth in Tug Lake for at least the major part of the algal growing season. Therefore, excess phosphorus supplied to the lake from an unknown source is likely at least one of the major factors instigating cyanoHABs.

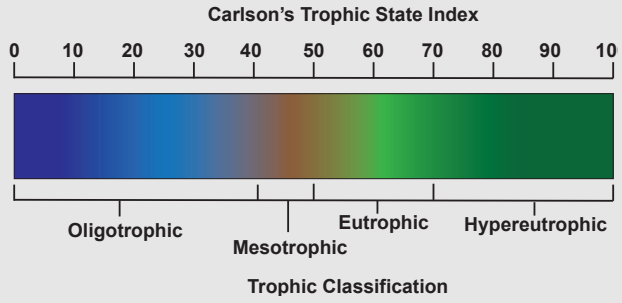
In 2017 Cason and Associates conducted baseline water quality monitoring for nutrients including phosphorus. The concentration of total phosphorus at the stream inlet to Tug Lake, the deep hole (center basin of the lake) and stream outlet of Tug Lake ranged from 25 – 100 µg/L at the surface with a median value of 32 µg/L based on samples collected at four time points in April, July, August, and October. A value greater than 24 µg/L means Tug Lake is nutrient rich and is defined as a eutrophic lake environment under the Carlson trophic state index classification system (Carlson 1977). However, the range of values for phosphorus are essentially at the division between a eutrophic lake and a mesotrophic lake. We conclude from this data that Tug Lake can be classified as a mesotrophic lake possibly transitioning to a eutrophic lake.

Box 2: Carlson's Trophic State Index

Lakes can be classified by the level of nutrients they receive which is often correlated with the amount of algae that can grow in them (i.e. chlorophyll) and water clarity. Lakes fall on a spectrum from nutrient poor lakes called oligotrophic lakes to slightly nutrient enriched called mesotrophic lakes and nutrient rich to very nutrient rich lakes called eutrophic and hypereutrophic lakes, respectively. In 1977 Robert Carlson proposed a classification method for lakes that describes their trophic status based on either phosphorus, chlorophyll or water clarity as measured by a Secchi disk.

Index	Phosphorus (µg/L)	Chlorophyll (µg/L)	Secchi Disk Depth (m)	State
<40	0 - 12	0 - 2.6	>4	O
40-50	12 - 24	2.6 - 20	4 - 2	M
50-70	24 - 96	20 - 56	2 - 0.5	E
>70	>96	>56	<0.5	H

O = oligotrophic, M = mesotrophic, E = eutrophic, H = hypereutrophic



Cason and Associates also measured phosphorus at the bottom of Tug Lake at the deep hole location on four occasions. Total phosphorus at the deep hole was relatively higher than the surface ranging from 24 – 230 µg/L and a median value of 50 µg/L. In all samples taken concentrations of phosphorus were highest in July and August (i.e. the middle of summer). These data suggested, but did not ultimately prove that internal phosphorus from bottom waters of Tug Lake could provide a source of phosphorus to support the formation of cyanoHABs in

Tug Lake. This would require sufficient lake mixing into deeper waters in July and August to entrain bottom water phosphorus into sunlit waters. Mixing down to 4 m did occur in 2017 down to 4 m at the end of July, which could have possibly entrained phosphorus from the metalimnion into surface waters triggering blooms in September.

To determine if internal phosphorus from the bottom of Tug Lake is a sufficient source of phosphorus feeding algal blooms, the Tug Lake Task Force in collaboration with the Miller Laboratory performed a field study to characterize phosphorus concentrations by depth in the main central basin of Tug Lake. We then compared its association with thermal stratification, bottom water anoxia, the development of cyanoHABs, and toxin production. This report is a summary description of our observations with minimal statistical analysis at this time. Further modeling and water quality monitoring work should continue.

Methods

Sampling. Water sampling was conducted at four sites within the central basin of the lake (Figure1, Table 1). Water was collected at discrete depths using a Van Dorn sampler and transferred to certified cleaned amber glass bottles filling the bottle half way, and stored frozen within two hours. Samples were then shipped frozen to the Miller Laboratory at the University of Wisconsin – Milwaukee in batches throughout the summer. At each location and during each sampling event a data sonde (In Situ Aqua Troll 600) was used to measure water temperature, dissolved oxygen, pH, oxidative-reductive potential, and conductivity at multiple depths. A detailed sampling protocol written by the Tug Lake Task Force (Reid Badeau) is provided in Appendix II.

Water Quality Monitoring Buoy. A water quality monitoring buoy (Nexsens CB-450 Data Buoy, Fondriest Environmental) was deployed at site B (Figure 1) on June 28th, 2018 equipped with sensors measuring water temperature every meter from the surface to a depth of 6 m, photosynthetic active radiation (light at wavelengths that can be used by algae) at 0.5 m, algal pigments chlorophyll and phycocyanin at 0.5 m, dissolved oxygen at depths of 0.5 m and 4 m and wind speed approximately 0.5 meters above the lake surface (Table 2). Measurements were made every minute.

Algal Toxin Analysis. Research in 2017 sought to determine which algal toxins, if any, were present in Tug Lake. A total of 17 toxin types were targeted in 2017 and the liver toxins microcystins were the primary toxins detected. As such, in 2018, we focused on quantifying 11 of the most commonly measured structural chemical variants of microcystin liver toxins in Tug Lake (Table 3). Microcystins were quantified in thawed water samples using liquid chromatography tandem mass spectrometry on a Sciex 4000 Qtrap mass spectrometer equipped with a Shimadzu Prominence HPLC following chemical extraction in acidified methanol as previously described (Miller et al. 2019).

Soluble Reactive Phosphorus. Soluble reactive phosphorus (SRP) was measured in thawed, filtered samples using the ascorbic acid method as previously described (Valderrama 1981a).

Results

Algal Blooms. In 2018 the occurrence of cyanoHABs was low compared to previous years, as observed anecdotally by Tug Lake residents and the sampling team. Data from the in situ chlorophyll and phycocyanin fluorometric sensors on the buoy indicated one major bloom event

occurred from approximately August 2 – August 17 (Figure 2). This bloom was large enough to cause saturation of the fluorometric sensors. The sensors were set to a gain setting of 10X and according to the manufacturer (Turner Designs, San Jose, CA) at this setting the chlorophyll sensor saturates at 50 µg/L of chlorophyll. This concentration of chlorophyll is a typical average concentration found in eutrophic lakes like Lake Mendota, for example (Beversdorf et al. 2015). Blooms in eutrophic lakes may contain much higher concentrations of chlorophyll. Thus, this bloom was likely of low to moderate intensity. Anecdotally, sampling team members did not note any observations of a bloom during this time.

Phycocyanin is primarily only present in cyanobacteria and therefore an increase in phycocyanin relative to chlorophyll is an indication of a cyanobacteria dominated bloom. The August bloom in Tug Lake appears to have been a mixture of cyanobacteria and other algal species as the phycocyanin-to-chlorophyll ratio was on average 0.5 (Figure 2). This ratio increased to above 1.0 as the season progressed indicating increasing cyanobacterial dominance later in the season. Small increases in chlorophyll phycocyanin fluorescence occurred in early September and again in late September when the buoy was removed. A late season near-shore bloom was observed by a member of the sampling team (Read Badeau) on October 26th near his home (Figure 3). A south wind may have blown the bloom into his bay. Given this observation, it is possible that conditions (nutrients, light, temperature etc) continued to be favorable for cyanobacterial growth after the buoys were removed well into October.

Algal toxins. A total of 195 samples were analyzed for the liver toxin microcystin. The overall average concentration of microcystin in 2018 at all sites (0.7 µg/L) was much lower compared with 2017 (7.2 µg/L). The only microcystin variant that was detected was microcystin with

leuicine and alanine (MC-LA) in the 'X' and 'Y" variable positions of the molecule. Other microcystin variants were detected in 2017, but the MC-LA variant was the most abundant. It is somewhat unusual for MC-LA to be the dominant variant, but other studies in Canadian lakes have made similar observations. Concentrations of MC-LA at all sites was well below the U.S. EPA threshold for recreational environments of 8 µg/L. At the deep hole location concentrations of MC-LA were elevated at the beginning of sampling in early July, as well as on August 30th and at the end of the season in mid- October (Figure 4). In fact, the highest concentration detected at the deep hole was on the last day of sampling on October 17th. Concentrations of MC-LA at the north, south, east, and west sites showed a similar trend with brief increases in MC-LA at the beginning of the sampling period, at the end of August or early September, and at the end of the sampling period in October (Figure 5).

Extra samples were taken by Reid Badeau of the near shore bloom in late October. One sample was taken near the shore in heavy bloom conditions, one off the dock 18 feet from shore where very little bloom was showing, and at the beach on the north end where no bloom was showing. Concentrations of MC-LA in the heavy bloom sample was 130 µg/L, over 16 times the safe recreational level. Concentrations of MC-LA was approximately 0.5 µg/L at both of the other sites where little or no bloom was evident.

Water Temperature and Thermal Stratification. Water temperature was near its maximum of almost 30 °C for the season when sampling started in early July (Figure 6). At this time thermal stratification, or the difference in temperature from the surface to six meters was at its greatest. The amount of energy required to completely mix the water column can be estimated by the Schmidt stability index. Schmidt stability reached 56 joules/m^2 in early July suggesting relatively

high thermal stratification. It then decreased to 20 joules/m^2 by July 23rd during a brief mixing event down to 3 m, likely due to higher wind speeds. The lake completely re-stratified again by August 13th where Schmidt stability reached 50 joules/m^2. At the end of August the lake mixed to 4 m before re-stratifying again in mid- September where Schmidt stability reached 30 joules/m^2. By the end of September the lake had almost completely turned over where Schmidt stability was negligible. These data indicate that while Tug Lake is a dimictic lake that completely mixes twice per year, partial mixing events occur during the summer prior to fall mixis (i.e. complete turnover).

Thermal stratification produces density gradients in lakes that sets up well defined layers in the water column. The epilimnion is the only later that receives sunlight, it is the warmest, and least dense layer. Algal blooms are generally confined to this layer of the lake. In Tug lake the depth of the epilimnion was limited to 2 m for most of the season until September when the lake entered fall mixis (Figure 7). A shallow epilimnion in Tug is partially due to high light attenuation by tannic acids in the lake. The hypolimnion at the bottom of the water column is the most- dense layer where nutrients such as nitrogen and phosphorus are concentrated. The hypolimnion was significantly more dense than the epilimnion in Tug Lake (Figure 8) and they were well separated from each other except for September when the lake entered fall mixis (Figure 7). The metalimnion is a transition zone between the epilimnion and hypolimnion. The metalimnion could be an important source of nutrients feeding algal blooms if even brief, partial mixing events deliver nutrients to the epilimnion. This has been shown to occur in other lakes including Lake Mendota (Stauffer and Lee 1973). In addition, cyanobacteria (blue- green algae) can vertically migrate through the water column using proteinaceous gas vesicles. In some cases they may even migrate to metalimnion depths (Ibelings et al. 1991).

Dissolved Oxygen. Surface dissolved oxygen saturation showed diel trends as expected and averaged 69% saturation during the sampling period. It was highest in early July at approximately 80% and decreased to 50% saturation in mid- August before increasing to 85% in early to mid - September. Surface dissolved oxygen fell to its lowest levels when it was mixed with hypoxic/anoxic bottom waters during fall mixis in mid- to late September.

The water column at 4 m was anoxic with undetectable dissolved oxygen from the time the buoy was deployed on June 28th until the end of August. Starting on August 30th brief pulses of dissolved oxygen were detected. This was coincident with thermal mixing of the water column down to 3 meters (note, in 2017 this occurred a month earlier). Increases in dissolved oxygen to 65% occurred on September 1st when the water column mixed to 4 meters briefly and then re-stratified. Accordingly re-stratification of the water column resulted in a dramatic decrease in dissolved oxygen saturation at 4 meters to non-detectable levels. Complete or nearly complete mixing of the water column during fall mixis in late September resulted in re-oxygenation at 4 meters to 65%, equal to surface dissolved oxygen saturation.

Sonde casts measuring dissolved oxygen at north, south, east, and west locations showed that the water column was anoxic from 3 meters and below for much of the season (Figures 11 – 13) and followed similar trends measured by the buoy dissolved oxygen sensors. Therefore, the anoxic zone below 3 meters was not limited to the deep hole location. This is important since the absence of oxygen can encourage the release of phosphorus from sediments.

Phosphorus. Soluble reactive phosphorus (SRP) at the deep hole was below 20 µg/L in the upper 3 m of the water column for most of the season except for August 13 – 16 when it briefly

increased to approximately 30 µg/L (Figure 6, *note this data is shown with water temperature data for comparison*). This was coincidental with peak water column stratification, bottom water anoxia, and a weak algal bloom as detected by an increase in signal from chlorophyll and phycocyanin in situ sensors on the buoy (Figure 2). SRP at 4 m was rarely below 30 µg/L and from approximately July 30 – August 19 increased to over 40 µg/L.

Concentrations of SRP at the east, north, south, and west locations was significantly higher at 4 m compared to the surface ($p<0.01$, Figure 14). Surface SRP reached eutrophic conditions at two of the sites in August, but was otherwise below mesotrophic conditions for most of the sampling period. Bottom water SRP was between mesotrophic and eutrophic conditions for most of the sampling period at three locations and above eutrophic conditions except for at the south location. Taken altogether, these data suggest that the bottom waters of Tug Lake may contain a sufficient amount of phosphorus to drive periodic algal blooms if entrained in the epilimnion by mixing events.

Estimating Internal Phosphorus Loads. The anoxic zone occurred at 3- 4 meters and deeper at all locations within the central basin of Tug Lake. Using the documented bathymetry of Tug Lake (Figure 15) we estimate the volume of anoxic water in the center basin to be 2.8×10^{10} liters. This assumes the center basin is cone shaped. This is a reasonable assumption given that there is a linear slope in bathymetric area with depth (Figure 16). The average SRP at all locations at 4 meters was 18.7 µg/L. Assuming a homogenous distribution at 4 m and below to 6 m then the total mass of SRP in the anoxic bottom waters of Tug averaged an estimated 5.3 kg. At peak SRP in mid- August the total mass of SRP is estimated to be 36 kg in anoxic bottom waters. If all of the mass of SRP in bottom waters was available for growth of *Microcystis* species then it is

estimated that it could result in the growth of 7 x 10^{15} cells at a minimum and 5.2 x 10^{16} at a maximum. It is unlikely that all of this SRP would have been entrained into shallow waters and upon re- oxygenation of the bottom waters some of the phosphorus could have become bound to sediments. These estimates are preliminary and are only an approximation. Further modeling work is necessary to estimate the contribution of internal phosphorus loading to algal blooms.

Conclusions

Bottom water anoxia in Tug Lake during peak stratification of the water column in July and August has likely caused the release of sediment derived phosphorus into the hypolimnion and possibly into the metalimnion. Partial mixing events in late summer and early fall may entrain phosphorus into the epilimnion where it can be used by algae for growth. The degree to which this happens depends on the frequency, depth, and duration of partial mixing events. In addition, the timing of partial mixing events is important. If later in the season then water temperatures may not be conducive for rapid growth of algae. In 2018, partial mixing down to 4 meters where phosphorus was most abundant did not occur until water temperature was well below 20°C. Optimal growth temperature for *Microcystis* species is generally above 22°C. If the partial mixing event down to phosphorus rich water layers had occurred earlier in the season then it is likely that the bloom observed in late October would have been larger and earlier in the season. In 2017, mixing down to nearly 4 m occurred at the end of July and several times thereafter in August down to 3 m. This may be why blooms were larger in 2017 and occurred earlier starting at the beginning of September.

The source of phosphorus contributing to the weak bloom observed in August of 2018 was unlikely caused by internal phosphorus from bottom waters since no mixing events had

occurred to entrain that phosphorus up to surface waters. It is more likely that this weak August bloom occurred as a result of external nutrient loading.

I recommend the following:

1. Public health and safety is of utmost importance. No mitigation strategy will completely stop blooms in the short term short of draining the lake. Therefore I recommend that the Tug Lake Task Force purchase strip test kits for microcystins from Abraxis (part# 520022) and use these to qualitatively estimate whether toxin levels are above or below safe recreation thresholds as dictated by the US EPA. Furthermore, I advise against in-water activities and boating when blooms are observed.
2. If there is interest in investing in technologies for the mitigation of algal blooms then I would suggest that the Tug Lake Task Force begin research on technologies to control internal phosphorus loading. This may include, but not limited to dredging, aeration, or the application of clays to lock phosphorus into sediments. A full review of these technologies is beyond the scope of this report. However, the data presented here suggest that reductions in internal phosphorus loading would help to reduce algal blooms. Furthermore, given the physics of Tug Lake (depth and high light attenuation characteristics) aeration may be an approach worth researching further. An excellent review on this topic is given by Visser et al. (Visser et al. 2016).

References

Beversdorf LJ, Chaston SD, Miller TR, McMahon KD. 2015. Microcystin mcyA and mcyE gene abundances are not appropriate indicators of microcystin concentrations in lakes. PLOS ONE 10:e0125353.

Carlson RE. 1977. A trophic state index for lakes. Limnol Oceanogr 22:361-369.

De Stasio BT, Richman S. 1998. Phytoplankton Spatial and Temporal Distributions in Green Bay, Lake Michigan, Prior to Colonization by the Zebra Mussel (Dreissena polymorpha). J Gt Lakes Res 24:620-628.

Ibelings BW, Mur LR, Walsby AE. 1991. Diurnal changes in buoyancy and vertical distribution in populations of Microcystisin two shallow lakes. J Plankton Res 13:419-436.

Miller TR, Bartlett SL, Weirich CA, Hernandez J. 2019. Automated subdaily sampling of cyanobacterial toxins on a buoy reveals new temporal patterns in toxin dynamics. Environmental Science & Technology 53:5661-5670.

Ouellette A, Handy S, Wilhelm S. 2006. Toxic *Microcystis* is widespread in Lake Erie: PCR detection of toxin genes and molecular characterization of associated cyanobacterial communities. Microb Ecol.

Stauffer RE, Lee GF. 1973. The role of thermocline migration in regulating algal blooms. (Modeling the Eutrophication Process: Workshop Proceedings). Utah State University Merril-Cazier Library.

United States Environmental Protection Agency. 2016. Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin.

Valderrama JC. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters Mar Chem 10:109-122.

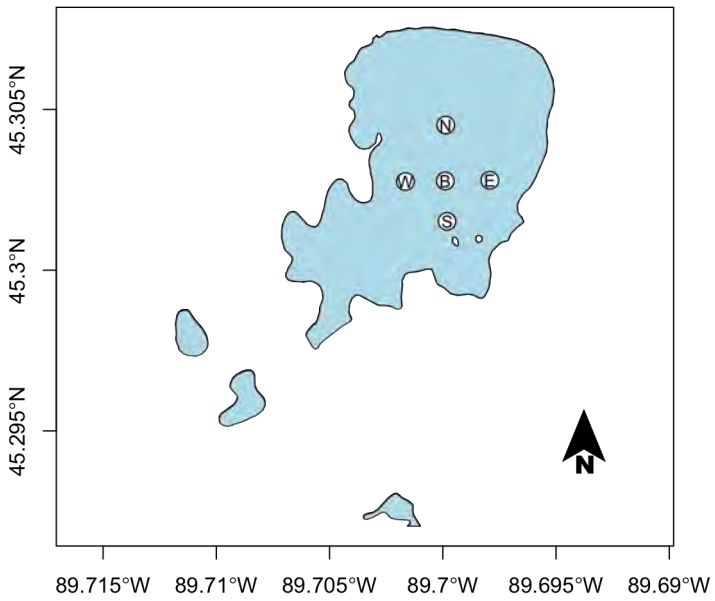
Visser PM, Ibelings BW, Bormans M, Huisman J. 2016. Artificial mixing to control cyanobacterial blooms: a review. Aquat Ecol 50:423-441.

Welker M, Von Döhren H. 2006. Cyanobacterial peptides — Nature's own combinatorial biosynthesis. FEMS Microbiol Rev 30:530-563.

Xiao M, Li M, Reynolds CS. 2018. Colony formation in the cyanobacterium Microcystis. Biological Reviews 93:1399 - 1420.

Figures

A



B

Figure 1. Study site. A). Tug Lake sampling sites. N = north, W = west, S = south, E = east, B = buoy location. B) Water quality monitoring buoy in Tug Lake

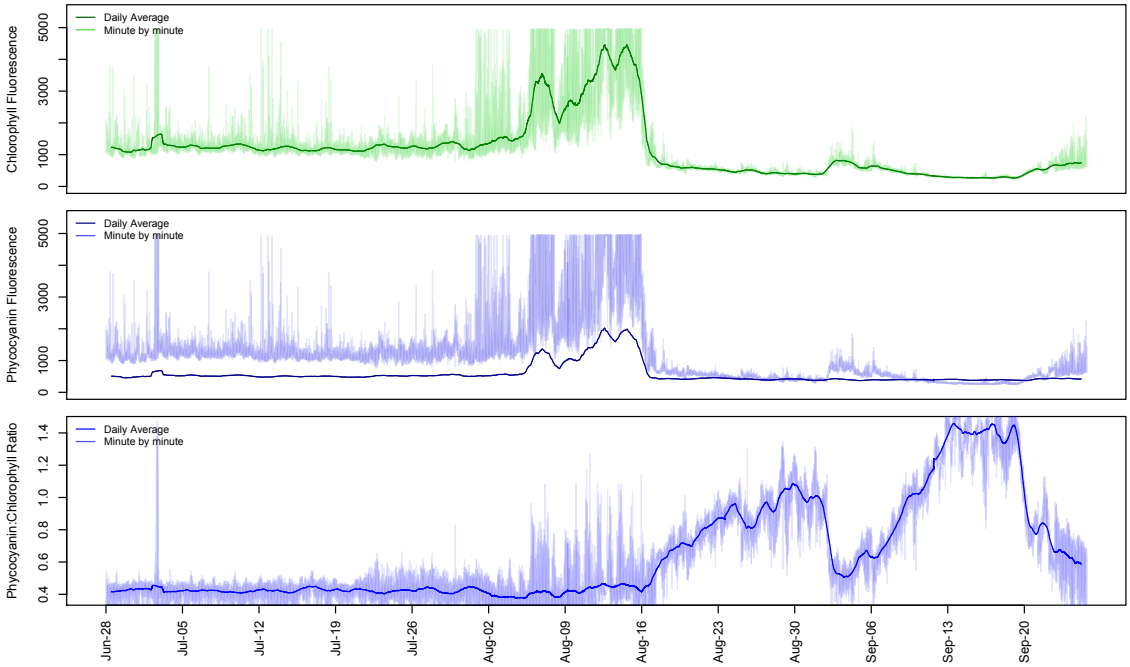


Figure 2. Algal blooms in 2018. (Top) Chlorophyll fluorescence, (Middle) phycocyanin fluorescence, and (Bottom) the ratio of phycocyanin to chlorophyll.

Figure 3. A late October bloom. Near shore bloom late in the season on October 26th (photo credit: Reid Badeau)

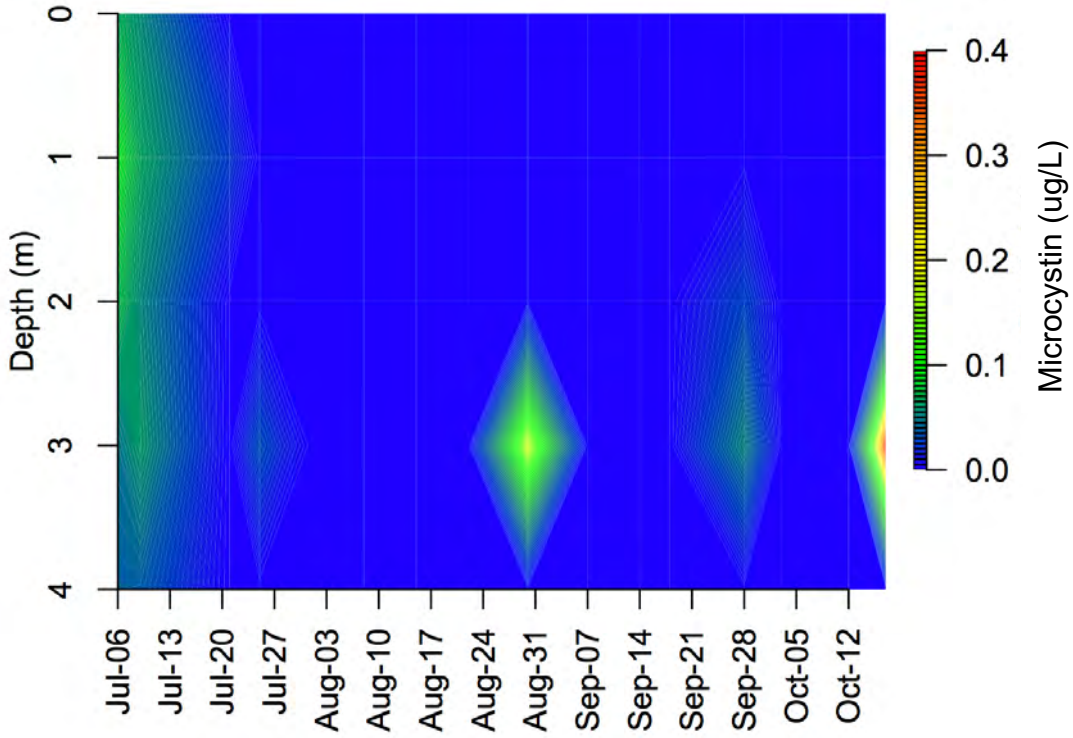


Figure 4. Microcystin toxins at the deep hole. Concentration of microcystins by depth at the deep hole of Tug Lake (location B).

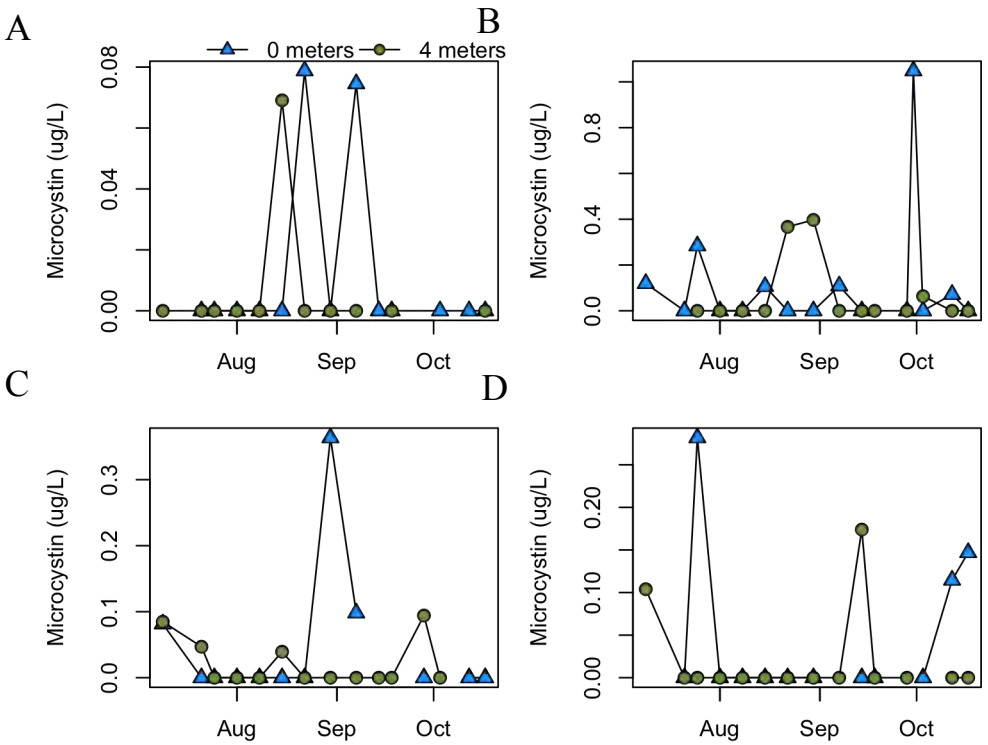


Figure 5. Microcystin toxins at other locations. Concentration of microcystins at north (A), south (B), east (C), and west (D) locations

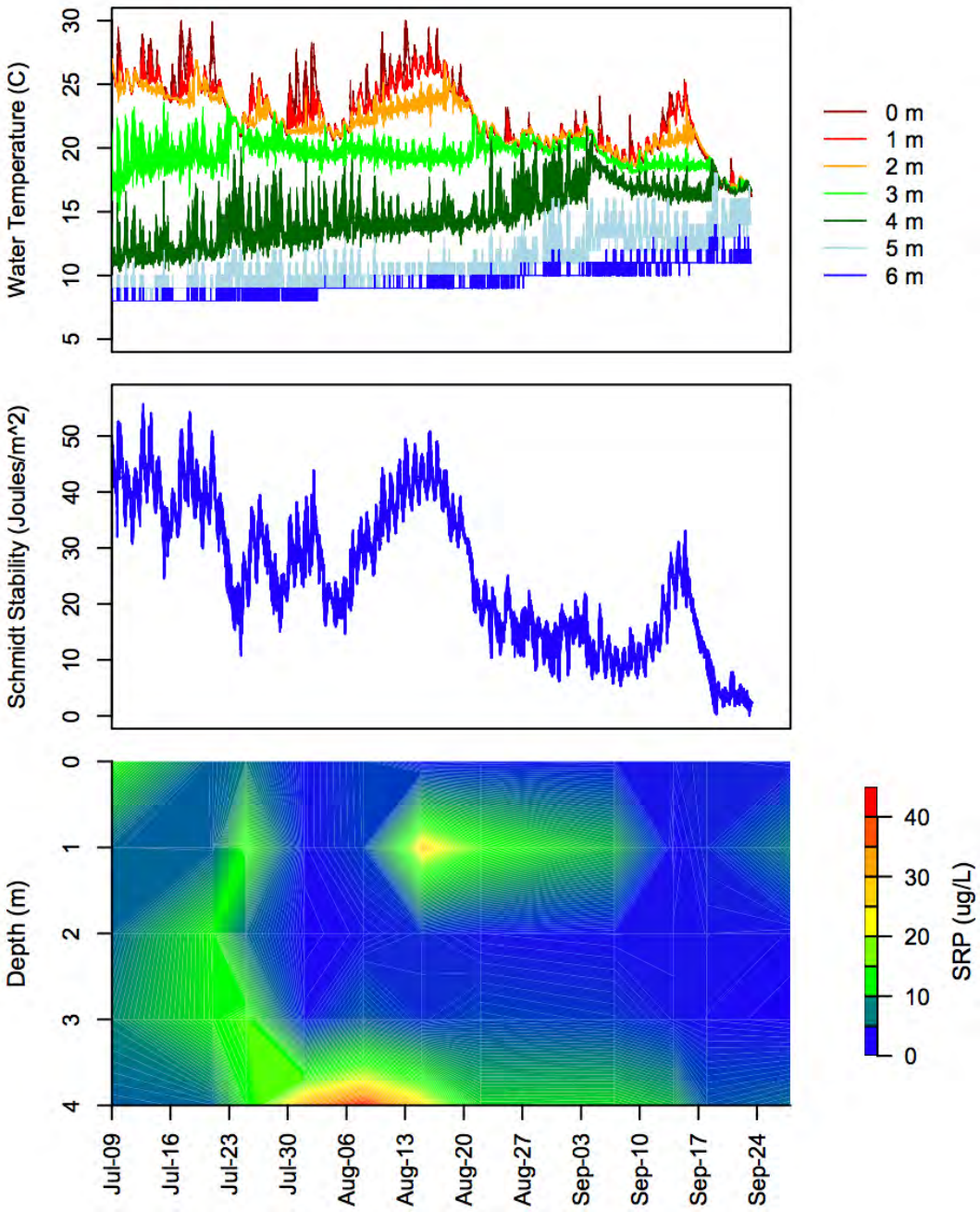


Figure 6. Thermal stratification and phosphorus at the deep hole. (Top) Water temperature by depth measured by the buoy. (Middle) Schmidt stability. (Bottom) Concentration of soluble reactive phosphorus by depth.

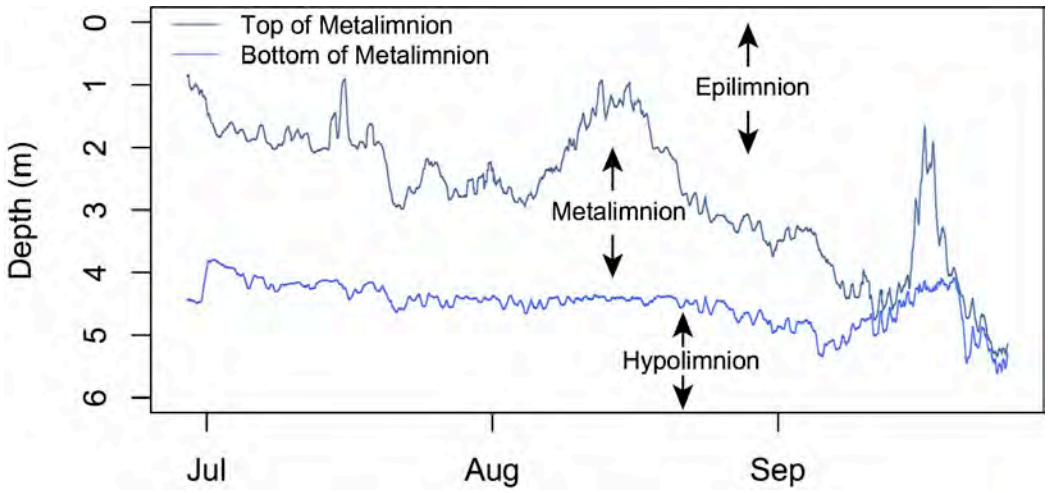


Figure 7. Water layers. Depths of the epilimnion, metalimnion, and hypolimnion in Tug Lake in 2018.

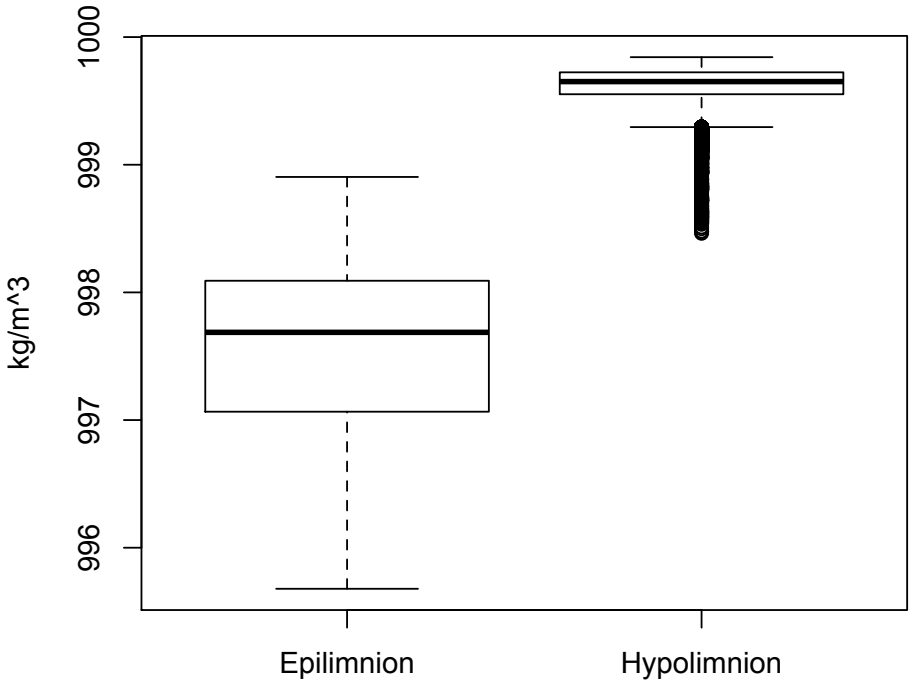


Figure 8. Water density. Box plot of water density of the epilimnion and hypolimnion in Tug Lake in 2018. The dark line in the center of each box is the median, edges of each box are the uopoper and lower quartiles, the whiskers represent scores above and below the middle 50%. Circles are outliers. The average density in epilimnion and hypolimnion are significantly different at an alpha of $P<0.01$ based on a rank sum test.

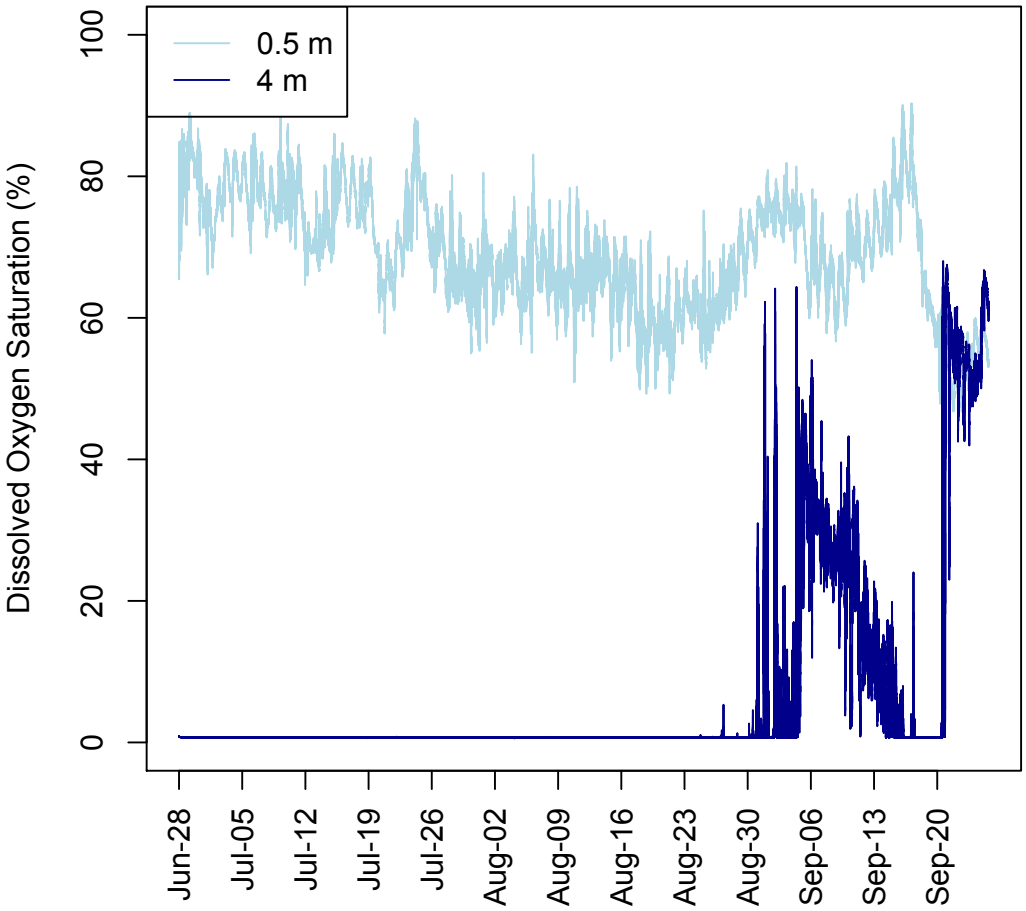


Figure 9. Dissolved oxygen at the deep hole. Dissolved oxygen saturation in Tug Lake 2018 near the surface and at 4 m

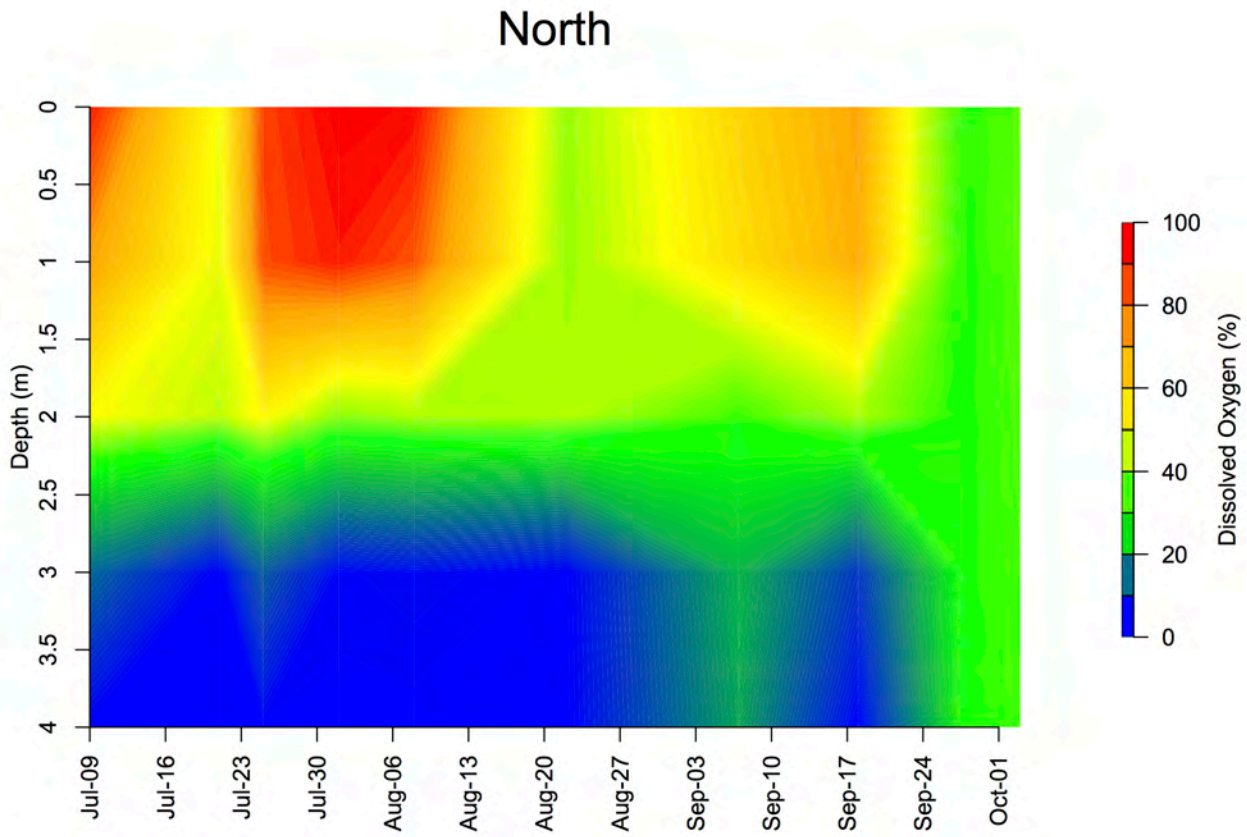


Figure 10. Dissolved oxygen by depth at the north location.

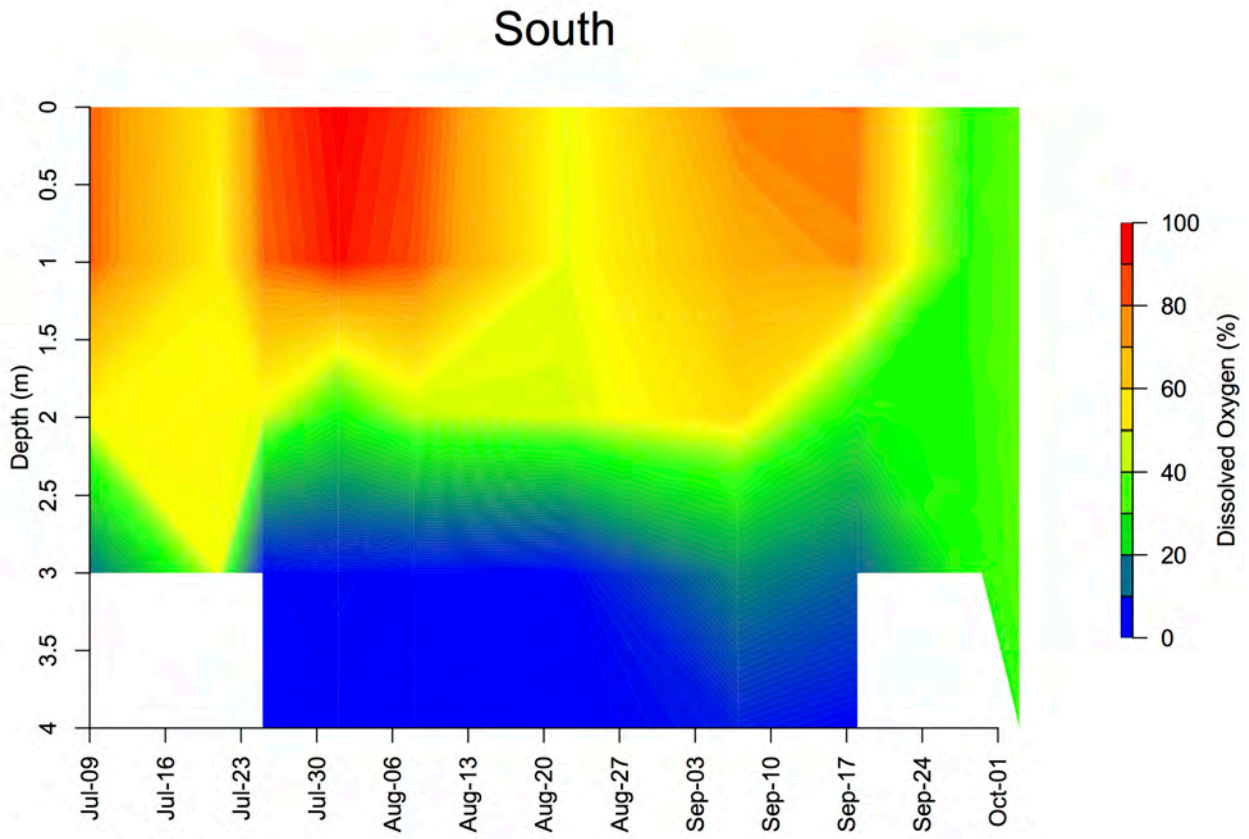


Figure 11. Dissolved oxygen by depth at the south location.

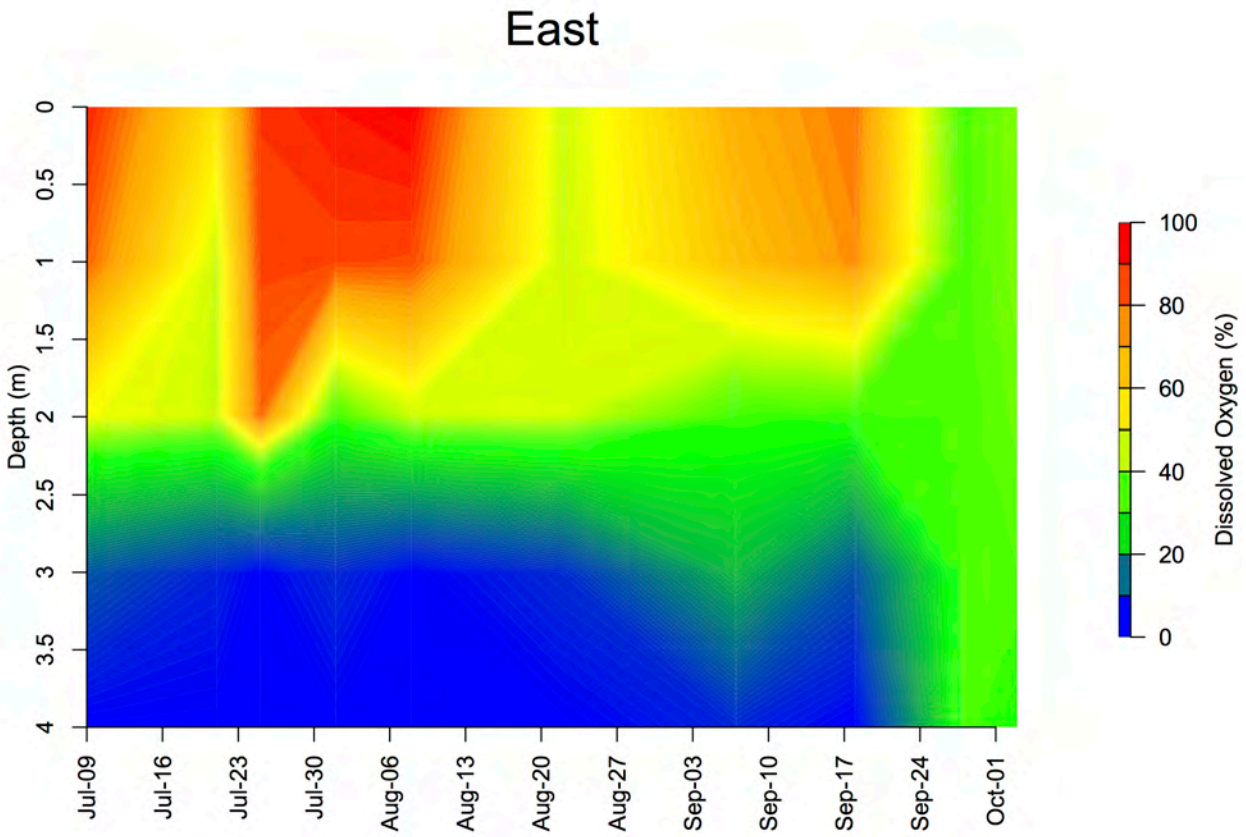


Figure 12. Dissolved oxygen by depth at the east location.

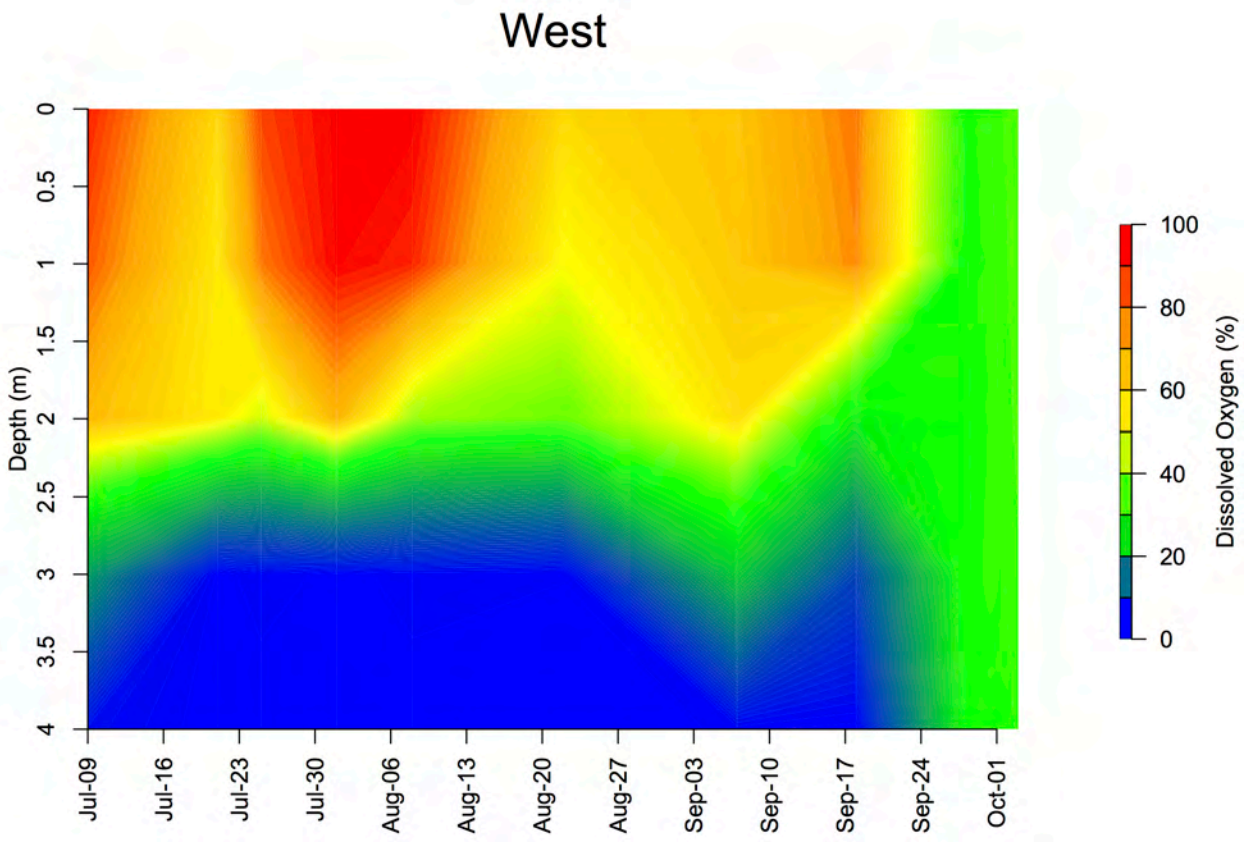


Figure 13. Dissolved oxygen by depth at the west location.

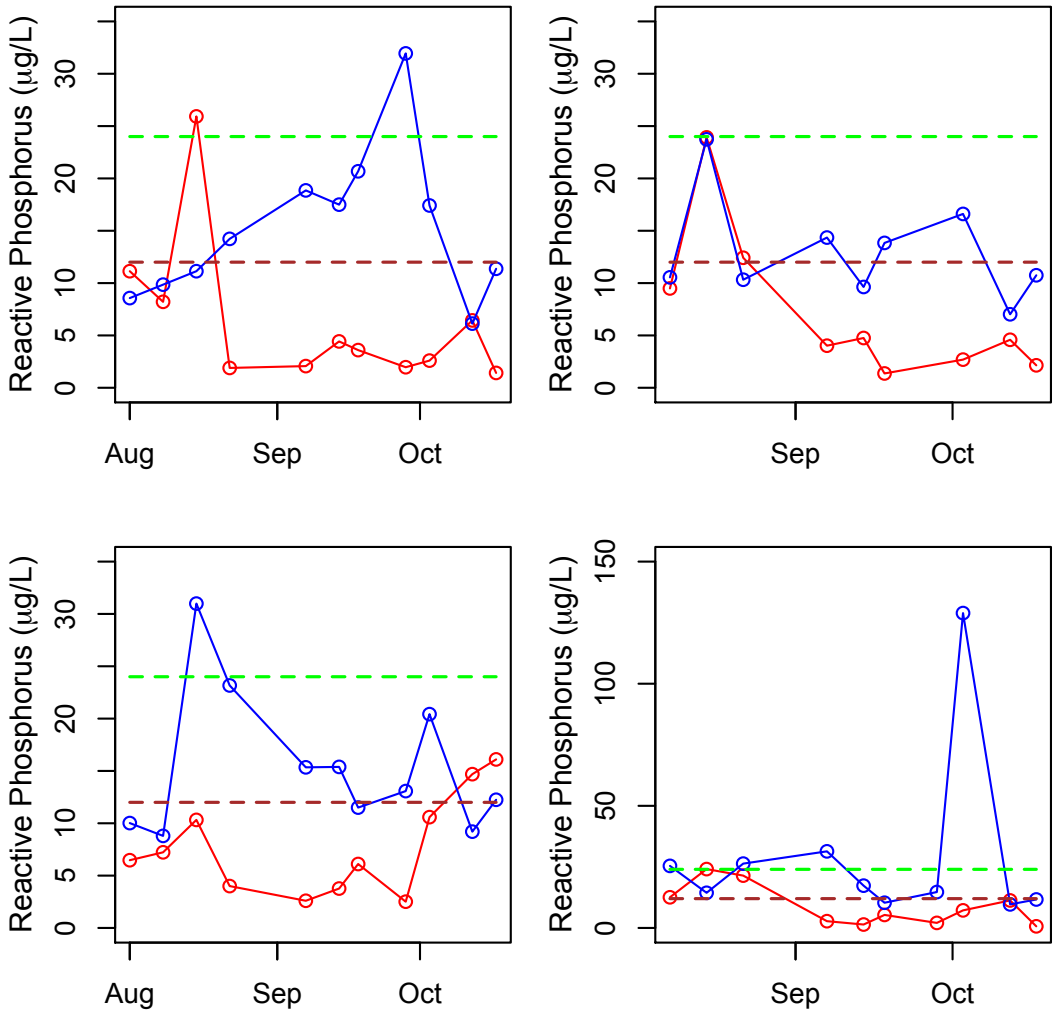


Figure 14. Soluble reactive phosphorus at other locations. Concentrations of soluble reactive phosphorus at the east (A), west (B), north (C), and south (D) locations. Blue lines are 4 m and red lines at the surface. The green and red dotted lines indicate thresholds for eutrophic and mesotrophic lakes, respectively.

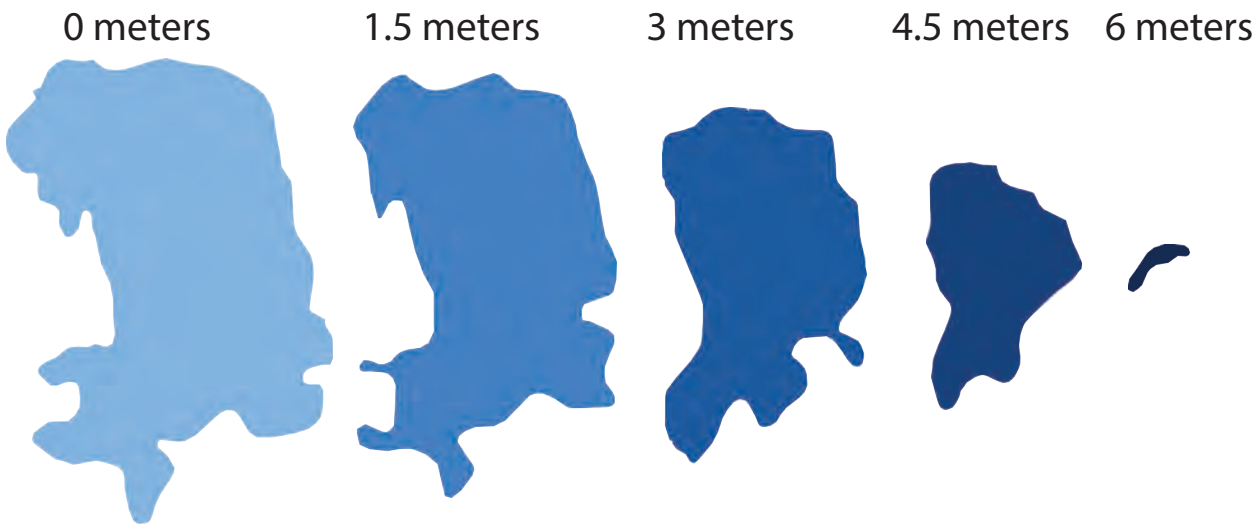


Figure 15. Bathymetry of Tug Lake. Based on information from the Wisconsin Department of Natural Resources

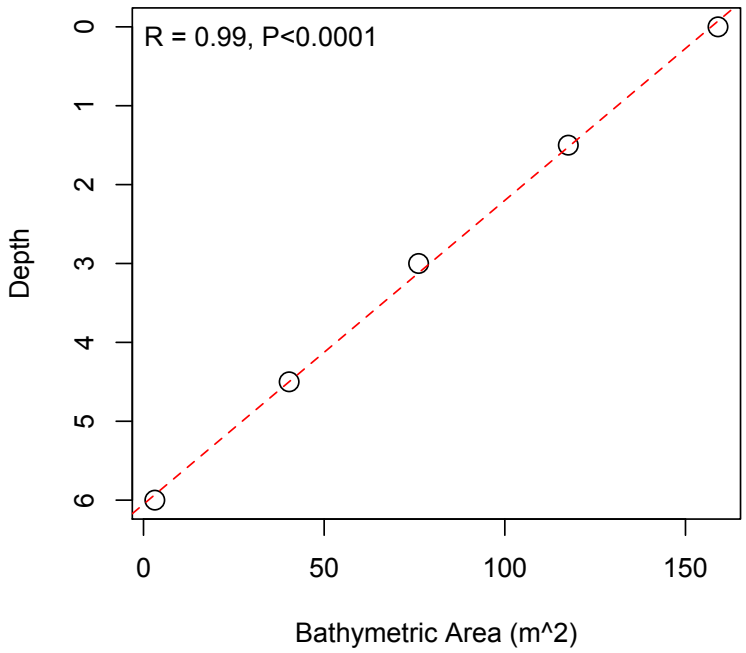


Figure 16. Bathymetry of Tug Lake is cone shaped. Relationship between bathymetric area of Tug Lake and depth in meters is linear.

Table 1. Tug Lake sampling sites in 2018

Site	Lat	Lon	Max Depth (m)	Sampling Depths (m)
Buoy	45.30278	-89.69989	6.4	0, 1, 2, 3, 4
East	45.3028	-89.69792	5.7	0 and 4
West	45.30276	-89.70165	5.3	0 and 4
North	45.30452	-89.69987	4.8	0 and 4
South	45.30152	-89.69981	5.7	0 and 4

Table 2. Parameters measured by the water quality monitoring buoy

Parameter	Depths (m)	Instrument
Water Temperature	0, 1, 2, 3, 4, 5, 6	UWM Sensor
Dissolved Oxygen	0.5, 4	In Situ RDO
Photosynthetic Active Radiation	0.5	Licor LI-192
Chlorophyll	0.5	Turner Cyclops 7F
Phycocyanin	0.5	Turner Cyclops 7F
Water Color	0.5	UWM Sensor
Wind Speed	-0.5	R.M. Young 5106

Table 3. Microcystin chemical variants targeted in 2018

Microcystin	Parent Mass (m/z)	Quantitative Daughter Ion (m/z)	Retention Time	Standard Source (Purity %)
[Dha^7]MC-LR	981.5	135.3	8.53	NRC (95%)
MCHilR	1009.5	135.3	8.63	Enzo (95%)
MCHtyR	1059.5	135.3	8.39	Enzo (95%)
MCLA	910.6	776.4	9.79	Sigma (95%)
MCLF	986.5	135.3	10.45	Enzo (95%)
MCLR	995.6	135.3	8.49	NRC (95%)
MCLW	1025.5	135.3	10.25	Enzo (95%)
MCLY	1002.5	135.3	9.79	Enzo (95%)
MCRR	520.1	70.1	7.62	Enzo (95%)
MCWR	1068.5	135.3	8.68	Enzo (95%)
MCYR	1045.6	135.3	8.39	Enzo (95%)

NRC = National Research Council Canada Biotoxins Program (Ottawa, Ontario), Enzo = Enzo Life Sciences (Ann Arbor, MI), Sigma = Sigma – Aldrich (Milwaukee, WI)

Appendix I
Summary of Results from the 2017 Citizen- Based Water Quality Monitoring Campaign in Tug Lake, WI

Principal Investigator - Todd Miller, PhD, Zilber School of Public Health, University of Wisconsin – Milwaukee

Co- investigator – Matthew Smith, PhD School of Freshwater Sciences, University of Wisconsin – Milwaukee

Introduction

Tug Lake is a 151- acre lake located in Rock Falls, WI (Lincoln County). It is within the Lilly Hay Meadow Creek watershed (USGS hydrologic unit # 070700020305) draining an area of approximately 12 square miles consisting of primarily forest (54%), wetland (22%), and agriculture (10%) (Figure 1). Over 60 residential homes are built on its shores with a mix of cottage and year around dwellings. Tug is a highly recreational lake used for boating, fishing and swimming. Over the last several years, homeowners have raised concerns over deteriorating water quality and the occurrence of large accumulations of algae or algal blooms primarily during summer and fall. Tug Lake has been listed as an impaired waterway by the Wisconsin Department of Natural Resources since 2010 due to mercury contamination of fish (Wisconsin Department of Natural Resources 2018). It remains a low priority lake in the Total Maximum Daily Loads program for mercury contamination. The Wisconsin DNR also considers it a eutrophic lake.

A meeting was convened with members of the Tug Lake Task Force and the Miller Laboratory in the spring of 2017 in order to formulate a citizen- based monitoring campaign for

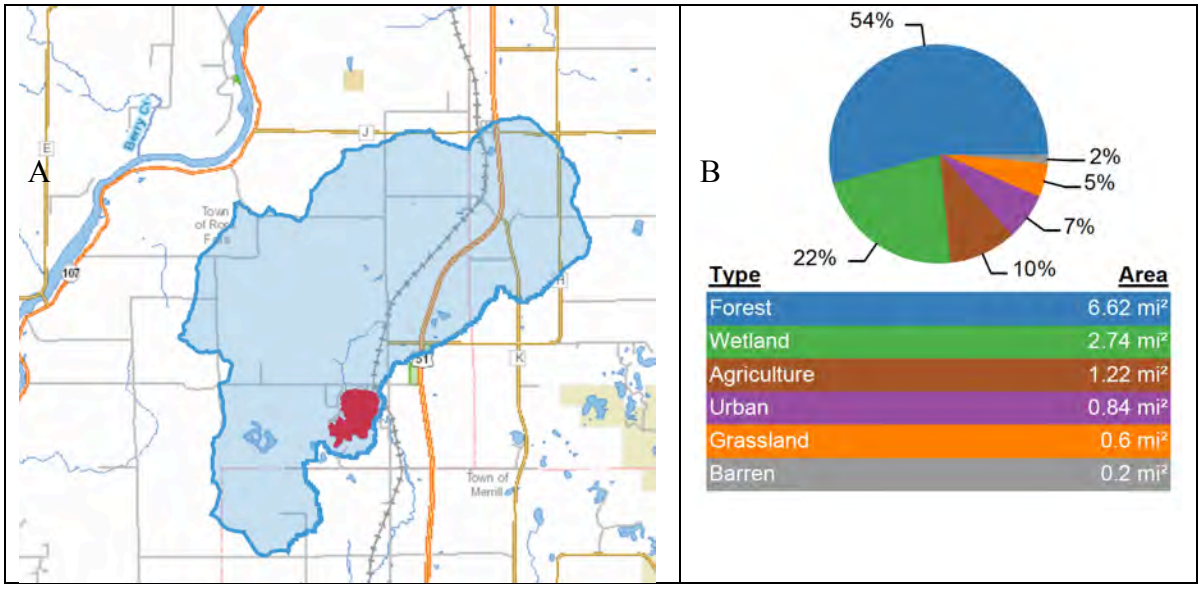


Figure 1. A) Tug Lake (red) and its watershed (blue). B) Land types in Tug Lake's watershed.

the summer of 2017. These activities were planned in conjunction with a larger water quality study conducted by Cason & Associates (Berlin, WI). Our primary goals for the field season were to 1) determine the species of algae causing blooms in Tug Lake, 2) quantify the temporal and spatial distribution of any toxins associated with the algae, and 3) deploy a continuous monitoring station capable of measuring water quality parameters in near- real time. Results from these project goals were to be compared with other water quality monitoring activities conducted by Cason & Associates.

Methods

Sampling. Water sampling was conducted at the deep hole (DH) on a weekly basis and at other locations when algal blooms were present (Figure 2). Water was collected from the surface ("glug") in certified cleaned amber glass bottles filling the bottle half way, and stored frozen within two hours. Samples were then shipped frozen to the Miller Laboratory at the University of Wisconsin – Milwaukee in batches throughout the summer.

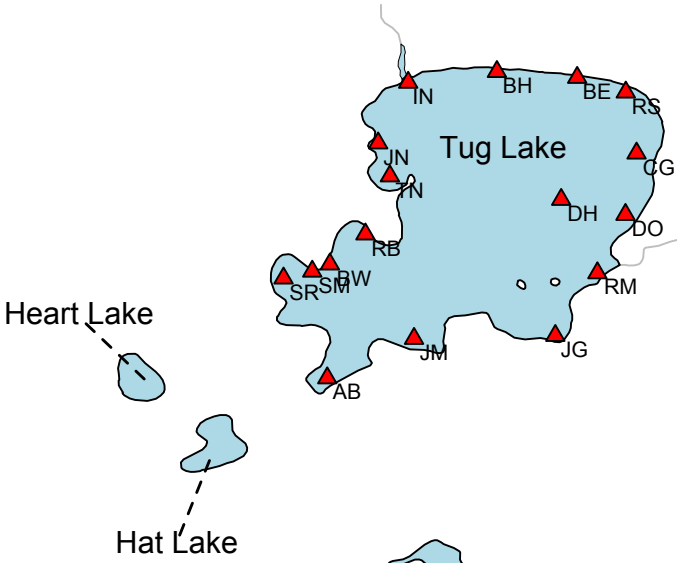


Figure 2. Sampling locations in Tug Lake

Identification of Bloom Species. Frozen samples were thawed at 4°C and qualitatively inspected under the microscope to identify the most abundant algae. While freezing is not an ideal preservation method for algae, given limited resources this allowed for identifying the most likely species causing blooms.

Algal Toxin Analysis. The most common species capable of producing toxins of human health concern in freshwater lakes

Table 1. Algal toxins targeted in this study

Cyanobacterial Toxins	Target Organ	Acute Effects	Chronic/Sub-Acute Effects
Microcystins (11 different types), Nodularin	Liver	Diarrhea, vomiting, rash, joint pain	Tumor promoter/cum. liver damage
Anatoxin-a/ & homoanatoxin-a, Saxitoxin & Neosaxitoxin	Nerve Synapse	Limb twitching, paralysis	Unknown
Cylindrospermopsin	Liver/ Kidneys	Diarrhea,vomiting, kidney failure	Mutagen and possible tumor initiator

are cyanobacteria or "blue- green algae." As such 17 different toxins produced by cyanobacteria were quantified in thawed water samples using liquid chromatography tandem mass spectrometry (LC-MS/MS) following chemical extraction in methanol as previously described (Beversdorf et al. 2017). Toxins targeted included the liver toxins microcystins, neurotoxins including saxitoxin, anatoxin-a and homoanatoxin-a, and the kidney toxin cylindrospermopsin (Table 1).

Total Phosphorus. Total phosphorus (TP) was measured in thawed samples using the ascorbic acid method after digestion with Valderrama's reagent (Valderrama 1981b). TP was only measured in samples from the DH location.

Water Quality Monitoring Buoy. A continuous monitoring buoy (Nexsens CB-450 Data Buoy, Fondriest Environmental) equipped with water quality sensors was deployed at the DH location on July 7th, 2017. The sensors include a custom- made thermistor chain measuring water temperature at five depths (0 – 4 m), chlorophyll and phycocyanin algal pigment sensors (Turner C7), and an air temperature sensor. Water temperature data from the thermistor chain was recorded by a custom- made data logger and transmitted to a CR1000 Campbell Scientific data logger. All data was then sent to a computer in the Smith Laboratory at the University of Wisconsin – Milwaukee via cellular communication (Verizon network). All electronics are powered by a 55 amp-hour marine battery, which is charged daily by three 10- watt solar panels.

Figure 3. Algal bloom caused by *Dolichospermum* in AB ("Airplane Bay") on June 13, 2017.

Results

Sampling effort. Sampling officially began on June 15 at site DH, however, a large bloom of *Dolichospermum* (formerly known as *Anabaena*) was observed two days earlier on June 13 with highest biomass located in and around site AB and JM (Figure 3). As such a sample of this bloom was taken from these sites on June 13th. A total of 39 samples were taken for laboratory analyses. Of these, 26 were weekly samples from the DH location. Others were near shore bloom samples at sites indicated in Figure 2, however not all sites were sampled. These sites may be sampled in future studies.

Table 2. Summary of Microcystin Detections at Sites in Tug Lake 2017 (red = detected)

Site	#	Date(s)	Mean or Amount (µg/L)	Microcystin Types Detected									
				dm LR	LA	LF	LR	RR	LY	LF	LW	YR	Hil R
DH	26	6/15 – 10/11	27										
AM	1	6/13	17.7										
BH	3	6/16/, 6/19, 7/6	10.0										
JG	1	6/13	52.0										
AB	1	6/13	6.3										
Dock	1	8/29	4,261										
CG	1	9/13	44.6										
JM	1	9/10	3,905										
DO	1	9/7	2,860										
TN	1	8/29	42.7										
BayRd	1	6/21	52.0										

Toxins Detected. Of the 17 toxins targeted, 9 were detected in at least one sample and all were one of several types of microcystin liver toxins (Table 2). Total microcystin concentrations ranged from 6 to >4,000 µg/L. The greatest diversity and highest concentration of toxins was detected in bloom samples from three near shore sites (Dock, JM, and DO). The majority of samples were taken from the DH location, which allows for a temporal analysis at this site. Concentrations of microcystin at the DH were relatively low when sampling began in early June (Figure 4). The mean concentration from June through mid- August was approximately 2.5 µg/L. In mid- August the concentration of microcystin at the DH location increased rapidly over a two week period to over 20 µg/L and remained relatively high over 4 µg/L for the remainder of the sampling season until sampling ended on October 11th, 2017. One sample during this period was an outlier at over 500 µg/L microcystin on September 9th.

The United States Environmental Protection Agency's draft recreational water quality limits for microcystin in lakes is 4 µg/L. Swimming is not recommended in waters above this limit and any lake with concentrations above this limit for 10% or more of the recreational season (generally Memorial Day to Labor Day in temperate lakes) are considered impaired for recreation. During the 2017 recreational season 34% of sampling days in Tug Lake had greater than 4 µg/L microcystin and the average concentration of microcystin at all locations was above 4 µg/L indicating that Tug Lake is impaired for recreation due to the presence of algal toxins. As such in-water activities where accidental ingestion may occur (e.g. swimming, water skiing, jet skiing) is not warranted.

Results from the Data Buoy. The buoy was deployed and recording data from July 7th until October 2nd and made 125,517 sampling events resulting in over 3 million data points (Figure 5). During the initial period when the buoy was deployed the lake was strongly stratified for several weeks from July 7th until the end of July. The lake rapidly mixed between August 3rd and August 4th. Soon afterwards the lake quickly stratified again on August 5th and remained weakly stratified for much of August until a strong mixing event occurred again on September 6th. The

lake slowly re-stratified throughout much of September before decreasing air temperatures resulted in the fall mixing event beginning on September 29th.

Chlorophyll and phycocyanin algal pigments as recorded by the buoy fluorometer sensors indicated that approximately 4 bloom events occurred. One bloom was in progress when the buoy was deployed and algal pigments were decreasing. Dolichospermum likely caused this bloom. A small brief bloom of *Microcystis* was recorded on or around July 28th. A larger and longer lasting bloom of *Microcystis* occurred August 18 – September 4th before the largest bloom of *Microcystis* beginning on approximately September 22nd.

Microcystin toxin concentrations largely vary with the algal pigment data. However, there were some notable discrepancies. For example, toxins were nearly undetectable on July 13th, but algal pigments were still moderately high and as pigments were decreasing on July 21st microcystin toxin concentrations increased to nearly above the EPA recreational limit. Perhaps most importantly, the highest recorded microcystin toxin concentration at the DH location occurred during a non- bloom period.

Total Phosphorus. TP at the DH location was highly variable. For the majority of sampling dates TP was less than 0.1 mg/L. However, on three occasions TP increased to well above 0.5 mg/L on August 13th, August 29th, and September 7th. All three of these occasions occurred after the first mixing event was recorded by the buoy. This may indicate that mixing played a role in delivering phosphorus from bottom waters to the surface waters at the DH location.

Discussion

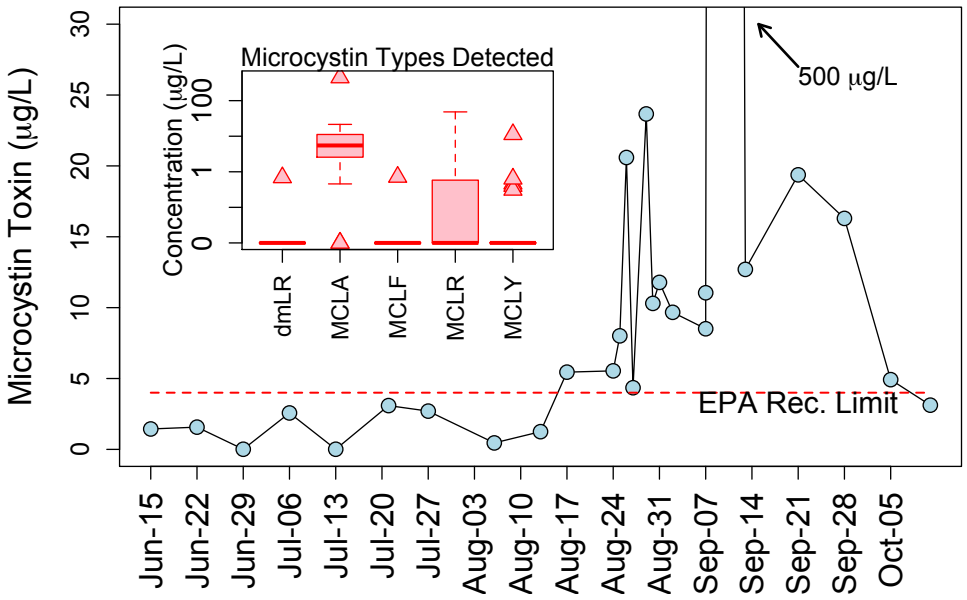


Figure 4. Trends in microcystin concentrations in Tug Lake at the Deep Hole location. Results indicate the sum of all microcystin types detected. The inset

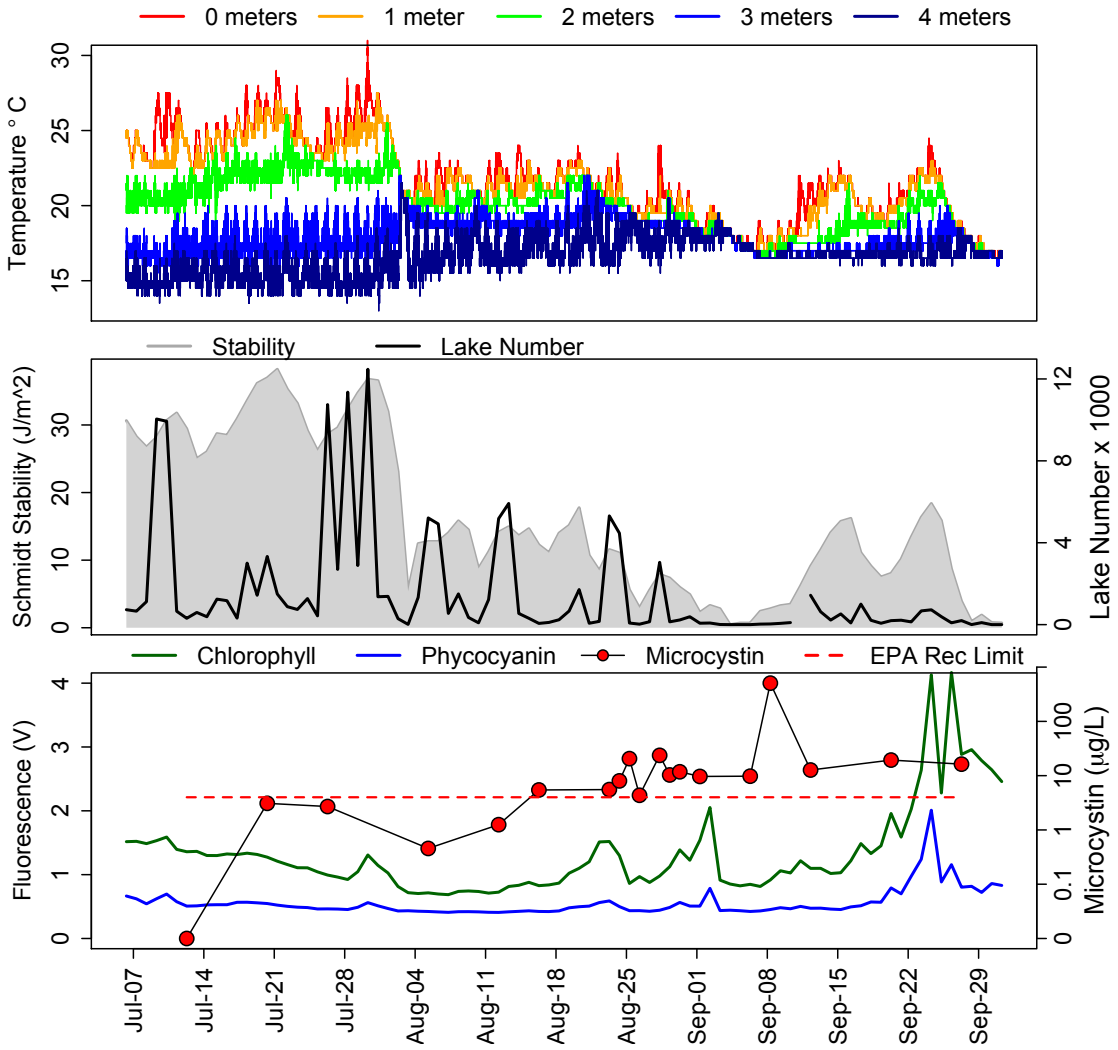


Figure 5. Top) Water temperature at five depths, Middle) Stability indices Schmidt Stability or energy required to mix the lake and Lake Number, which indicates if the lake is mixing. Higher numbers for both indicate lake stratification, Bottom) Algal pigment fluorescence and microcystin toxin concentrations.

The occurrence of cyanobacterial toxins is often related to other factors occurring in the lake including the amount of algae and type of species present, water temperature, water temperature by depth or water column stratification, and the presence of nutrients for algal growth such as nitrogen and phosphorus. Some of these environmental factors may change rapidly over the course of minutes to hours. These changes would typically be missed when manually sampling by boat. The data buoy was deployed in an attempt to measure some of these environmental factors in near – real time, continuously.

Water temperature measured at depths from the surface down to below the thermocline indicate the degree to which the lake is stratified or mixed. When lake water is warmer at the surface compared to deeper waters the lake is said to be thermally stratified setting up a density gradient. When there is a strong temperature difference, particularly in eutrophic lakes or stained lakes like Tug, bottom waters (i.e. the hypolimnion) may become oxygen depleted (hypoxic), anoxic (no oxygen), or anaerobic (no oxygen and no nitrate). In order for the lake to become

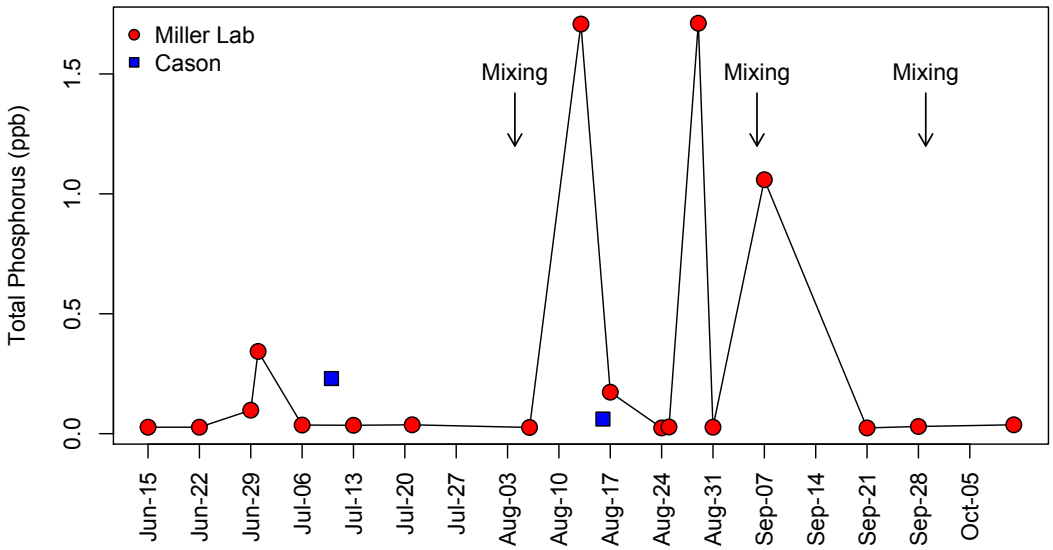


Figure 6. Trends in total phosphorus concentrations at the Deep Hole location.

mixed and de-stratified energy must be applied, usually in the form of wind and waves, or rapid cooling of surface waters, such as occurs in the fall. The amount of energy required to mix the lake can be expressed in units as Joules per cm squared and is given by the Schmidt Stability equation (see Figure 5).

The results of the buoy thermistor data indicate that Tug Lake is a polymictic lake, mixing more than two times per year. Most lakes in temperate environments undergo mixing events in spring due to ice and snowmelt whereas the fall mixing event occurs due to decreasing sunlight and air temperatures. Lake mixing that occurs between the spring and fall mixing events could be due to one of many factors including strong wind speed, waves, and rainfall. Neither wind speed nor storm events or precipitation correlate with the two mixing events recorded by the buoy thermistor chain in Tug Lake in 2017. However, several strong thunderstorms did occur during the deployment period with heavy rains and wind. It's possible mixing occurred due to a surge in groundwater seepage, but only after aquifers are fully recharged. In general groundwater seepage into lakes is correlated with rainfall (Downing and Peterka 1978). Alternatively, it's also possible that changes in cloud cover and solar irradiation may be important drivers of these mixing events.

Mixing may be a mechanism to bring nutrients from the bottom waters in the lake up into sunlit layers where they feed algae. Insufficient quantities of nutrients including nitrogen and phosphorus are most often responsible for limiting algal growth in lakes. Since some cyanobacteria have the ability to obtain nitrogen from air, their growth is most often limited by a lack of phosphorus. If sufficient quantities of phosphorus enter the lake then algal growth may ensue assuming other conditions are optimal (such as water temperature). If enough phosphorus is supplied and conditions are optimal then an algal bloom may occur. Since increases in TP occurred after the first mixing event it is possible that the large spikes in TP (Figure 6) originated from the bottom waters of Tug Lake. The lake had been well stratified for several weeks or longer. The data from Cason & Associates suggests this resulted in bottom water oxygen depletion and increases in phosphorus release from sediments due to diagenesis.

Conclusions:

1. *Microcystis* and *Dolichospermum* were the dominant species present in algal blooms in Tug Lake. While Tug Lake is stained with tannic acids, the pH data recorded by Cason & Associates is still within an optimal range for growth of these cyanobacteria. Given this and the eutrophic nature of the lake the occurrence of blooms of these two species is not remarkable.
2. The liver toxin, microcystin was the dominant toxin detected. Concentrations varied from 6 - >4,000 µg/L, well above the EPA recreational guideline value of 4 µg/L. Over 10% of sampling days had concentrations above this limit. As such Tug Lake is impaired for in-water recreational activities. Fish consumption may also carry some risk, but this has not been evaluated.
3. The lake mixed at least three times during the open water season indicating that it is a polymictic lake.
4. Total phosphorus spiked to above 0.5 ppb on at least three occasions after the first mixing event recorded by the buoy.

Recommendations:
The data from Cason & Associates study does not indicate that phosphorus from surface runoff in spring is a significant source of eutrophication in the lake. Under-sampling in the Cason study likely underestimated chlorophyll concentrations and the full extent of algal bloom activity in Tug Lake, which is not uncommon since blooms can form rapidly and decay. Photos of algal blooms in the lake taken by residents (Figure 3) in conjunction with the very high microcystin concentrations are evidence that toxic algal blooms in the lake are problematic, despite a lack of high spring phosphorus input. It should be noted that nitrogen concentrations reported by the Cason & Associates study does not suggest that cyanobacterial growth would be limited by nitrogen. Thus, since phosphorus in this case is the key nutrient fueling algal blooms, it is likely that another source of phosphorus is entering the lake at times other than during the spring snowmelt and from sources other than runoff.

There are two other obvious sources of phosphorus to investigate. These include 1) internal phosphorus release from sediments during periods of thermal stratification of the water column, and/or 2) phosphorus within groundwater seepage into the lake. The former was detected in the Cason study but not fully characterized. It is not clear what percentage of algal growth can be attributed to phosphorus release from sediments. In the latter, seepage may contain phosphorus that entered groundwater from various surface water sources. In the absence of intense agricultural land usage in the watershed (Figure 1) the most obvious source of phosphorus in groundwater seepage is from residential activities around the lake (e.g. lawn fertilizers, grey water input) or from wastewater as a result of faulty septic systems. Given these conclusions the following recommendations are offered:

Recommendation 1: Characterize the extent of bottom water oxygen depletion and phosphorus release from sediments in combination with the mixing regime of the lake.

Recommendation 2: Quantify the concentration of wastewater indicators in Tug Lake relative to a nearby control lake lacking a significant amount of septic systems such as Heart or Hat Lake.

The goal of these recommendations is to identify potential sources of nutrients to the lake that are responsible for causing toxic algal blooms.

References Cited:

Beversdorf, L.J., Weirich, C.A., Bartlett, S.L. and Miller, T.R. (2017) Variable cyanobacterial toxin and metabolite profiles across six eutrophic lakes of differing physiochemical characteristics. Toxins (Basel) 9(2).

Downing, J.A. and Peterka, J.J. (1978) Relationship of rainfall and lake groundwater seepage. Limnol. Oceanogr. 23(4), 821-825.

Valderrama, J.C. (1981) The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Mar. Chem. 10(2), 109-122.

Wisconsin Department of Natural Resources (2018) 2018 Draft Impaired Waters List. Water Evaluation Section, http://dnr.wi.gov/topic/impairedwaters/2018IR_IWList.html.

Appendix II. Detailed Sampling Protocols Used for the 2018 Tug Lake Study

Tug Lake Sampling Protocol 2018

Materials

1. Amber 250 ml sample containers (13 containers needed on each sampling event)
2. In-Situ Aqua Troll Sonde
3. Turner Handheld Optical Brightener Fluorometer
4. Van Dorn Sampler

Table 1. Site locations and sample depths

Site	Lat		Lon		Max Depth (m)	Sample Depths (m)
Buoy	45.30278	N45°18'10.0"	-89.69989	W89°41'59.6"	6.4	0, 1, 2, 3, 4
East	45.3028	N45°18'10.08"	-89.69792	W89°41'52.51"	5.7	0 and 4
West	45.30276	N45°18'9.935"	-89.70165	W89°42'5.94"	5.3	0 and 4
North	45.30452	N45°18'16.271"	-89.69987	W89°41'59.532"	4.8	0 and 4
~~South~~	~~45.30152~~	~~N45°18'5.471"~~	~~-89.69981~~	~~W89°41'59.315"~~	~~5.7~~	~~0 and 4~~
South	45.30181	N45°18'6.516"	-89.69961	W89°41'58.596"	4.80	0 and 4

Sampling Frequency

All sites weekly or at least once every two weeks

Procedure at all sites

1. Sample water at the surface (0 meters). Place bottle just under water surface and fill completely. Cap bottle, shake and empty back into lake, Repeat. Fill empty bottle approximately ¾ full. Cap the bottle and place into cooler or shaded away from sunlight. Once on dry land store at minus 20 Celsius in freezer on its side within 12 hours.

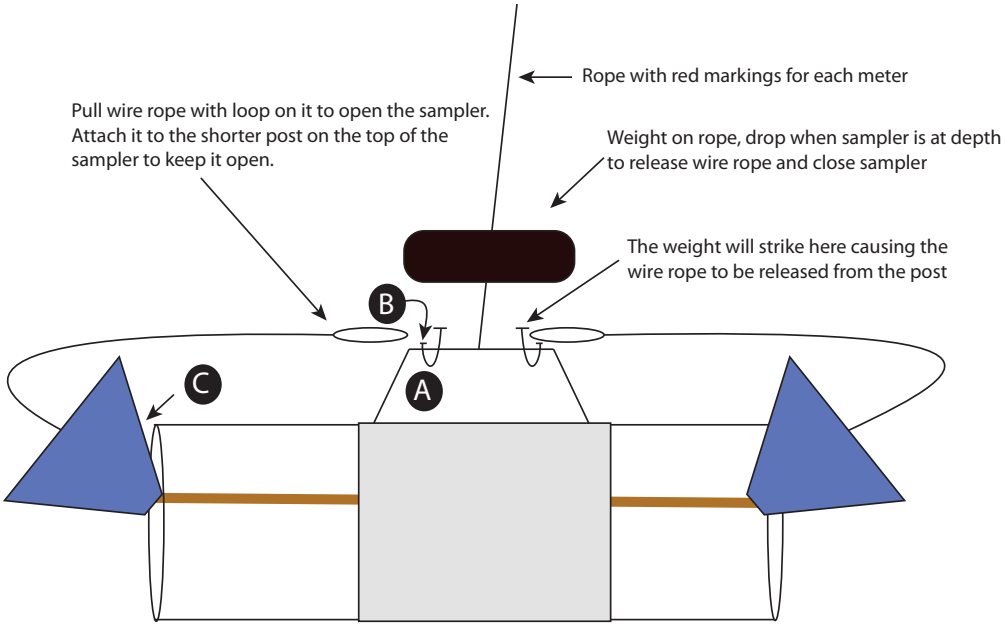
2. Sample water at the sample depths indicated in table 1 using the Van Dorn sampler. Refer to Van Dorn sampler instructions (appendix A).
3. Take In-Situ Sonde measurements at **surface and every meter to 4 meter depth**. Hold the Sonde at each depth for 5 to 10 measurements. Refer to Sonde instructions (appendix B and Table 2).
4. Take a reading of optical brighteners at the surface using Turner DataBank handheld instrument (yellow looks like spotlight with cable attached). See instructions (appendix C).

Procedure at impromptu bloom sightings

1. If surface scums are spotted then take a water sample using any available beverage container (water, vitamin water, Gatorade, juices), no oils.
2. Fill container in water near scum. Cap container and shake. Empty container into lake.
3. Repeat step 2 five times.
4. Fill container ½ full, at least 4 ounces.
5. Freeze once on land within 12 hours.

Appendix A. Using the Van Dorn Sampler

1. With your left hand grab the sampler by the top handle near one of the posts and push up on the post (A). Attach wire rope to the smaller post (B). The suction cup should be pinched on its side, not on the bottom of the suction cup (too far).
2. Repeat for the other suction cup on the other side of the sampler.
3. You should now have the sampler with both suction cups open, held open with wire rope loops attached to small posts on top of the sampler.



4. Lower the sampler into the water to the desired sampling depth keeping the weight above the water (or in the boat).
5. When sampler is at the desired depth (indicated by markings on rope) drop the weight (messenger) to trigger closing of the sampler.
6. Bring sampler back up out of the water and empty into the sample bottle. Rinse the sample bottle with one volume of sample water then fill ¾ full, cap and label sample.

Sample Schedule	Buoy (Map A)	East (Map B)	South (Map C)	North (Map D)	West (Map E)
Surface (Glug)	**X**	**X**	**X**	**X**	**X**
1 Meter	**X**				
2 Meter	**X**				
3 Meter	**X**				
4 Meter	**X**	**X**	**X**	**X**	**X**

TABLE 1

Appendix B. Using the In-Situ Sonde (excerpts are from the In-Situ manual)

1. Open the Sonde storage box.
2. Carefully remove the sensor with guard ("restrictor"), two attached coils of sensor cable, and the blue "Bluetooth" transmitter from the storage box and set down.

   NOTE: Refer to the laminated In-Situ instruction pages from the In-Situ manual in the test kit for additional information. (Pages 15 through 36)

3. Unscrew the silver guard ("restrictor") from the sensor and set the guard down. (laminate page 18 item 1)
4. Remove the orange storage plug and place in the storage box. (laminate page 18 item 5)
5. Remove the pH/ORP sensor from the storage bottle. (laminate page 18 item 6)
6. Use the alignment marks to properly align the pH/ORP sensor with the port connection, and press firmly into place. (laminate page 18 item 7)
   IMPORTANT: Push until the sensor is completely inserted into the port.
7. Twist the silver guard ("restrictor") onto the sensor and set down. (laminate page 18 item 8)
8. Remove the single Velcro strap from the smaller coil of cable and straighten the cable.

TAKING SAMPLES:

Sample Schedule	Buoy (Map A)	East (Map B)	South (Map C)	North (Map D)	West (Map E)
Surface	**X**	**X**	**X**	**X**	**X**
1 Meter	**X**	**X**	**X**	**X**	**X**
2 Meter	**X**	**X**	**X**	**X**	**X**
3 Meter	**X**	**X**	**X**	**X**	**X**
4 Meter	**X**	**X**	**X**	**X**	**X**

TABLE 2

1. Turn on the 'Testers iPad'. Log in with tltf00.
2. Select (tap) the iSitu app.

3. In the upper left corner tap Sites.
4. Select your present testing site by tapping 'Set'
5. The app will return to the "readings" screen. At the bottom it will display "Turn on Battery Pack"
6. At the Bluetooth device connected to the sensor cable press the ON/OFF power button.
7. Observe the power Status light. It will be red and then go to green. When it is green the sensor is communicating with the iPad and testing can begin.

8. Lower the sensor into the water to the desired depth. The depth is marked at one meter intervals by red tape.
9. Watch the RDO indications as well as the other readings for stability. The unit is on a ten second update. It may take over one minute for the reading to stabilize.
10. When RDO is stable press RECORD on the upper right corner. A little stopwatch icon will appear in the upper right corner. It will also have a number incrementing up. When the number switches from 5 to 6 press STOP.
11. Repeat step 10 for each depth readings are taken. (See TABLE 2)
12. The Bluetooth device has an internal timer. If too much time occurs without a change of data the unit will power down. Go to step 6 and start over.

STOWING THE In-Situ UNIT

1. At the Bluetooth device connected to the sensor cable press the ON/OFF power button. The Status light will go out.
2. Unscrew the silver guard ("restrictor") from the sensor and set the guard down. (laminate page 18 item 1)
3. Wipe the pH/ORP sensor sides with paper towel. This will make removal easier.
   CAUTION: Do Not Touch the sensor tip with fingers or paper towel.
4. Pull the pH/ORP sensor from the main sensor port connection. (laminate page 18 item 7)
5. Remove the cover and washer from the storage bottle. Place the washer over the narrow insertion end of the sensor and slide half way. Place the cover over the narrow insertion end of the sensor and onto the washer. Place the pH/ORP sensor into the bottle. Verify the sensor tip is not touching the sponge. Screw the cap onto the bottle. Carefully slide the sensor until it contacts the sponge. Stow the bottle in the box.
   NOTE: The sensor tip must not bottom out in the bottle. (laminate page 18 item 6)
6. Insert the orange storage plug. (laminate page 18 item 5)
7. Twist the silver guard ("restrictor") onto the sensor. (laminate page 18 item 8)
8. Carefully wind the cable in loops and wrap with the single Velcro strap.
9. Stow the cables, Blue Tooth and sensor in the Sonde storage box.
10. When back at Reid's garage remove the cables, Blue Tooth and sensor from the Sonde storage box and set on the floor to dry. Reid will stow when dry.

Appendix C. Using the handheld Turner DataBank optical brightener sensor.

1. Remove the single Velcro strap from the cable.
2. Press the Power button to turn on. The unit will power up and say “Not Calibrated”. Ignore this comment.
3. Place the sensor at the test depth. Wet the sensor to the top. The deep reading is the red tape just below the hand held unit.
4. Wait for the reading to stabilize. (Stable is reading +/- 100)
5. Estimate the average reading and record on the data sheet provided.
   NOTE: Between sampling stations power down the unit to conserve the battery.

Sample Schedule	Buoy (Map A)	East (Map B)	South (Map C)	North (Map D)	West (Map E)
Surface	**X**	**X**	**X**	**X**	**X**
1 Meter					
2 Meter					
3 Meter					
4 Meter	**X**	**X**	**X**	**X**	**X**

Sampling Locations and Frequency

Due to confusion I have added this page for clarity.

Water Sample					
Sample Schedule	Buoy (Map A)	East (Map B)	South (Map C)	North (Map D)	West (Map E)
Surface (Glug)	**X**	**X**	**X**	**X**	**X**
1 Meter	**X**				
2 Meter	**X**				
3 Meter	**X**				
4 Meter	**X**	**X**	**X**	**X**	**X**

In-Situ Sampler with iPad					
Sample Schedule	Buoy (Map A)	East (Map B)	South (Map C)	North (Map D)	West (Map E)
Surface	**X**	**X**	**X**	**X**	**X**
1 Meter	**X**	**X**	**X**	**X**	**X**
2 Meter	**X**	**X**	**X**	**X**	**X**
3 Meter	**X**	**X**	**X**	**X**	**X**
4 Meter	**X**	**X**	**X**	**X**	**X**

DataLogger Optical Brightener Test – battery dead					
Sample Schedule	Buoy (Map A)	East (Map B)	South (Map C)	North (Map D)	West (Map E)
Surface	**X**	**X**	**X**	**X**	**X**
1 Meter					
2 Meter					
3 Meter					
4 Meter	**X**	**X**	**X**	**X**	**X**

TUG LAKE TASK FORCE
COORDINATES

Location	GPS Name	Function	Time	Latitude	Longitude	Depth (Ft)	Depth (M)	John Depth
Buoy	Buoy 1	At Buoy	11:11 AM	N45° 18' 10.0"	W089° 41' 59.6"	20.9'	6.37	
Buoy	Buoy 2	Verify	11:13 AM	N45° 18' 10.1"	W089° 41' 59.6"	20.9'	6.37	
Sample East	Buoy East Tst	East Test Point	11:50 AM	N45° 18' 09.8"	W089° 41' 56.6"	18.7'	5.7	
Sample South	Buoy South Tst	South Test Point	11:53 AM	N45° 18' 07.5"	W089° 41' 59.1"	18.7'	5.7	
Sample West	Buoy West Tst	West Test Point	11:56 AM	N45° 18' 11.4"	W089° 42' 03.4"	17.4'	5.3	
Sample North	Buoy North Tst	North Test Point	11:59 AM	N45° 18' 14.8"	W089° 41' 59.2"	15.6'	4.75	
Miller Coords	We will use yours					Transducer 12" below water		
Buoy	Map A			N45.30278	W89.69989	20.9'		----
			Deg/min	N45° 18' 10.0"	W89° 41' 59.6"			
East	Map B			N45.3028	W89.69792	17.7'		17.0'
			Deg/min	N45° 18' 10.08"	W89° 41' 52.51"			
South	Map C			N45.30152	W89.69981	13.1'		11'
			Deg/min	N45° 18' 5.47"	W89° 41' 59.315"			
West	Map E			N45.30276	W89.70165	19.6'		17.4'

			Deg/min	N45° 18’ 9.935”	W89° 42’ 5.94”			
North	Map D			N45.30452	W89.69987	16.7’		-------
			Deg/min	N45° 18’ 16.271”	W89° 41’ 59.532”			
Miller Coordinates	Miller Map Location					Transducer 12” below water		John
Buoy	A			N45.30278	W89.69989	20.9’		----
			Deg/min	N45° 18’ 10.0”	W89° 41’ 59.6”			
East	B			N45.3028	W89.69792	17.7’		17.0’
			Deg/min	N45° 18’ 10.08”	W89° 41’ 52.51”			
South	C			N45.30152	W89.69981	13.1’		11’
			Deg/min	N45° 18’ 5.47”	W89° 41’ 59.316”			
North	D			N45.30452	W89.69987	16.7’		-------
			Deg/min	N45° 18’ 16.272”	W89° 41’ 59.532”			
West	E			N45.30276	W89.70165	19.6’		17.4’
			Deg/min	N45° 18’ 9.936”	W89° 42’ 5.94”			

Optical Brightener Test
SAMPLE

Identity	Map Reference	Surface	Range	Comment Surface	5 Meter	Range	Comment 5 Meter
Deep Hole	Map A	2630	Low		2850	Low	
East	Map B	2680	Low		2830	Low	
South	Map C	2675	Low		2830	Low	at 4.5 M - Depth locater was reading approx. 12 feet. I will retake depths and verify reading against actual measurement.
North	Map D	2700	Low		2840	Low	
West	Map E	2600	Low		2790	Low	

Name of Tester: Date:

Identity	Map Reference	Surface	Range	Comment Surface	5 Meter	Range	Comment 5 Meter
Deep Hole	Map A						
East	Map B						
South	Map C						
North	Map D						
West	Map E						

SUGGESTED LABELING BY TODD MILLER (Provided to us 2017)
For a serial label Dr. Miller would like to use the following example:
TG0515171515DH00A
TG = Tug Lake
05 = month May
15 = 15th day of May
17 = year
15 = hour in military time. (1500 = 3 PM)
15 = minutes (1515 = 3:15 PM)
DH = two digit description for location. DH = Deep Hole
00 = depth in meters.
A = replicate. If you want to do two replicate samples on each sampling date then you will need to indicate a replicate in the label (e.g. A,B,C)

The most important thing is to keep the serial number the same length by using ”0” place holders (e.g. for one digit months) and be sure the label gives enough information so we can distinguish between samples. This makes sample tracking and analyses much easier.
On July 9, 2018 John Redmann and I used the following identifiers
DH = DEEP HOLE – Map location A
EA = EAST – Map location B
SO = South – Map location C
NO = North – Map location D
WE = West = Map Location E
e.g. TG0709181424SO02

LAKE	MONTH	DAY	YEAR	HOUR	MINUTE	MAP LOCATION	DEPTH
TG	07	09	18	14	24	SO	02
TUG LAKE	JULY	09	2018	2:PM	2:24 PM	SOUTH (MAP C)	02 METERS

If people sample in other sites then they will have to come up with a two digit code for those sites. Then have a separate list maintained by someone that gives the approximate GPS coordinates of those locations linked to the two digit code. It would be best for samplers to just used their cell phones to get the GPS location while out sampling. (some apps for this, Android https://play.google.com/store/apps/details?id= com.woozilli.gpscoordinates&hl=en. iPhone, https:itunes.apple.com/us/app/compass-for-iphone-6/id930467975?mt=8). Otherwise they can get approximate GPS coordinates using google maps, or google earth.