Exhibit 3

Chapter xx: Phytoplankton in Lake Mohegan

INTRODUCTION

Planktonic algae tend to dominate pelagic primary productivity in shallow turbid lakes (Liboriussen and Jeppesen 2003). Having low algal densities in a lake can reduce overall system productivity (Carpenter et al. 2001). Similarly, high algal densities which is a result from overly successful growth processes (i.e., sufficient nutrients, light, temperature, etc.) and insufficient loss processes (i.e., grazing, mortality, hydraulic washout, etc; Kalff 2003 (Chapter 21), may lead to insufficient processing of energy by higher trophic levels (Carpenter et al. 2001). Consequently, high algal densities can reduce system productivity by promoting greater energy flow to benthic and detrital pathways, which can lead to greater oxygen demands. Also, high algal densities can lead to a variety of water quality issues including high suspended solids, low water clarity, fluctuating dissolved oxygen and pH levels, taste and odor, and possible toxicity (Kalff 2003; Havens 2008). These conditions can make water bodies uninhabitable for many aquatic organisms and restrict water use leading to resource and economic losses. Managing algal densities to be at an intermediate level is often the goal for water resource managers since productivity is likely greatest at an intermediate biomass (Carpenter et al. 2001) and water impairment would be restricted.

Since algae respond to varying environmental changes, their assemblage can provide insight into the condition of a lake. Water resource managers have used algal assemblages as water quality indicators, where certain taxa have been ranked based off their tolerance to pollution or lake features (i.e., mixing regimes and trophic status) (Heinonen et al. 2008 (Chapter 2.1); Bellinger and Sigee 2015). For example, lakes that contain *Oscillatoria* and *Euglena* species of algae are indications of high organic pollution while lakes containing *Anacystis* and *Melosira* are typically associated with low organic pollution (Palmer 1969). The presence of colonial cyanobacteria, also called blue-green algae, are typical of high nutrient status lakes (Heinonen et al. 2008).

Harmful algal blooms (HABs), which is any concentration of algae that causes impacts to an aquatic system that can be documented as hazardous to human or ecological health have been demonstrated on Lake Mohegan. Surveys evaluating public perception on the lake found the greatest management concern reported by Lake Mohegan stakeholders was nuisance algae, and water clarity see Appendix xx. Thus, identifying the risks associated with the HABs occurring on Lake Mohegan and the factors influencing their growth should be of great importance and value to the MLID. The objective of this chapter was to evaluate the phytoplankton community on Lake Mohegan and to provide a literature overview of the risks associated with the HABs occurring on Lake Mohegan.

METHODS

Much of the phytoplankton monitoring on Lake Mohegan did not begin until 2011-2012, which is when CSLAP volunteer began recording percent composition of alga, dominant taxa (to genus level), and testing for cyanotoxins (microcystin-LR, anatoxin-a, and cylindrospermposin) in downwind shoreline areas and in one surface water sampling site as indicated in Chapter xx. Much of the prior CSLAP data is incomplete regarding sampling consistency. The phytoplankton monitoring described was only done in full in 2014 and 2016, while the rest of the years had partial monitoring. Two other phytoplankton assessments before 2012, included summer sampling in 1993 by Robert W. Kortmann (1993) and in 2004 by Martin R. Michael (2012), both of which reported percent composition of alga and dominant taxa (to genus level) with the latter also recording biovolume. No detailed methods were provided in both assessments, only that an open water surface sample was taken. It's important to note not having methods for algal enumerations makes results somewhat complicated or impossible to compare and in some cases incomparable. For example, counting the total number of cells (total cell count) vs. counting cells and colonies and recording them as units (typically called natural units) makes results incomparable.

In the fall of 2015, a more detailed assessment of Lake Mohegan's phytoplankton community and abundance was undertaken as part of the state of this lake report. Surface phytoplankton samples were taken using a Kemmerer water sampler (z = 0.5m) at the same location as outlined in chapter xx. Phytoplankton samples were preserved with Lugol's iodine solution, using a dilution of 5 mL of Lugol's solution per 100 mL of lake water (5%; Bellinger and Sigee 2015). All quantitative analyses were accomplished following protocols outlined in Bellinger and Sigee (2015), involving the use of settling chambers and an inverted light microscope. The preserved samples were subsampled respectively (10ml) and settled for > 18 hours in a 10 mL settling chamber. Many of the samples had to be diluted (1 ml preserved sample in 9 ml of deionized water), due to high amounts of phytoplankton. Microscopic analysis was done with the use of a Carl Zeiss Axio Observer.A1 with phase contrast. Each 10 mL chamber was then examined, where all algal cells were enumerated in each settling chamber. In some instances, cell counts for colonial algal forms were approximated by extrapolating either an average cell density or cell per unit length depending on aggregation type. Identification of each of the major algal groups was recorded, and the dominant taxa for each sample were identified to the genus level.

RESULTS

The phytoplankton community on Lake Mohegan has been dominated by cyanobacteria in 6 of the 7 sampling years, apart from 2015. In 1993, cyanobacteria represented 34 – 71 % of the phytoplankton community and were dominated by *Dolichospermum spp.* (*Anabaena sp.*) early in the season and then switched to *Oscillatoria spp.* later during the summer. In 2004, cyanobacteria represented 62-95% of the phytoplankton community and consisted of mainly *Coelosphaerium spp.* and *Aphanizomenon spp.* throughout the growing season. Biovolumes for 2004, ranged from 28,245 to 153,265 cells / ml cyanobacteria. From 2012 to 2014, cyanobacteria dominated the phytoplankton community never documented below 80% throughout the growing season. There were no dominant taxa recorded for 2012. However, 2013 and 2014 *Microcystis sp.* was the dominant taxa. In 2015, the percent cyanobacteria dropped less than 7 %, and chlorophytes (*Pediastrum spp.*) and bacillariophytes (*Fragilaria spp.)* dominated. In 2016, a switch back to cyanobacteria was observed with *Woronichinia spp.* dominating early in the season and *Microcystis spp.*, dominating later in the growing season (Figure xx). Cyanobacteria biovolumes for 2016, ranged from 174 cells / ml (3/18/2016) to 490,625 cells / ml (10/2/2016), respectively (Figure xx). Though, biovolumes reached > 25,000,000 cells / ml in downwind areas where phytoplankton accumulated. No cyanobacteria were found on sample dates 5/12/2016 and 5/25/2016 (Figure xx). Both chlorophytes (*Pediastrum spp.*) and *bacillariophytes* (*Fragilaria spp.*) were present during each sampling event in 2016.

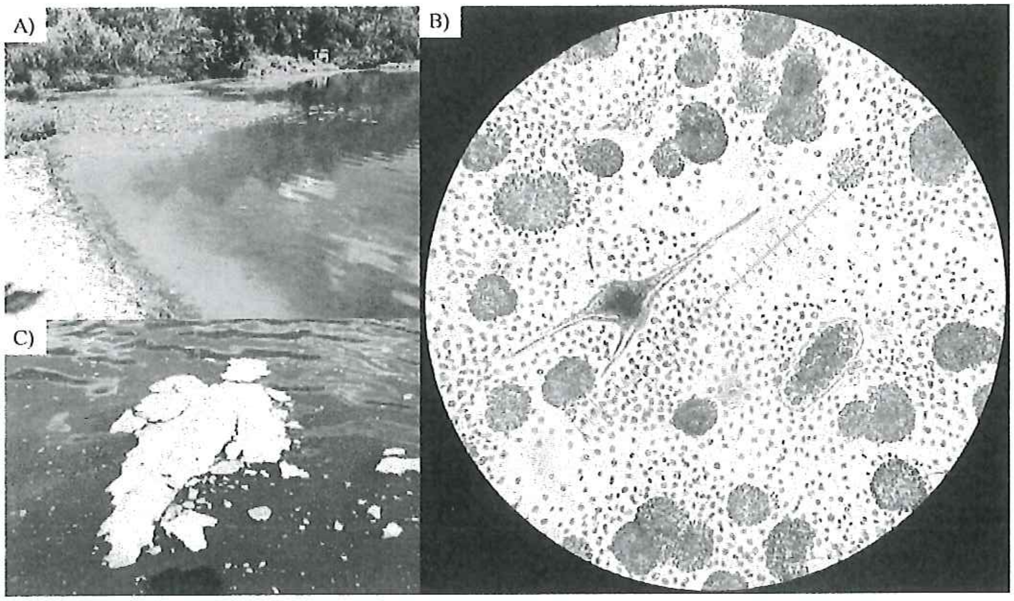

Figure xx. Harmful algal blooms (HABs) occurring on Lake Mohegan in 2016. Picture A) shows the first observable bloom during the 2016 growing season (6/17/2016) and B) shows the microscopic view (40x) of that bloom. Note the dominant alga *Woronichinia spp.* Picture C) shows a late season (10/2/2016) HAB of mixed cyanobacteria with *Microcystis spp.* dominating.

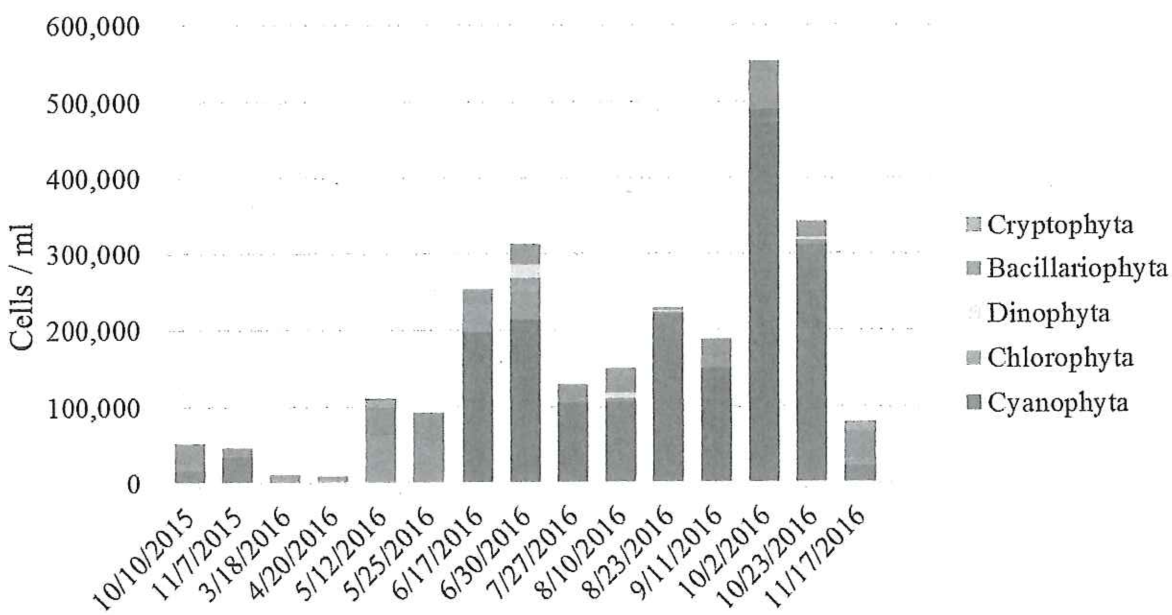

Figure xx. Open water algal enumeration results (Cells / ml) for fall 2015 and 2016.

Of the three major cyanotoxins tested through CSLAP (microcystin-LR, anatoxin-a, and cylindrospermposin), microcystin-LR was the only one to go above the New York Department of Environmental Conservation (NYDEC; 2017) bloom criteria. According to the NYDEC (2017), a "confirmed bloom" threshold of > 4 μg / L microcystin in the open water sampling sites occurred in 2011 and 2012. The "confirmed with high toxin bloom" level of > 10 μg / L microcystin in the open water sampling site was observed in 2013 and 2014, and the threshold of > 20 μg / L for shoreline/beach sampling was confirmed in 2014 (NYDEC 2017). Though, there is only shoreline/beach sampling data for 2014. Average microcystin levels were 960 μg / L with a maximum value of 7,032 μg / L in 2014.

DISCUSSION

HABs in 2016 began on Lake Mohegan in the middle of June and continued until late October. The algal assemblage during the 2016 HAB was predominantly made up of cyanobacteria. In general, cyanobacterial blooms tend to dominate in lakes with excess P (Watson et al. 1997; Downing et al. 2001) and when total algal biomass becomes high (> 100 mg / L algal biomass; Canfield Jr et al. 1989), both of which occurred during the summer of 2016. The dominant cyanobacteria (*Dolichospermum spp., Coelosphaerium spp., Aphanizomenon spp., Woronichinia spp., and Microcystis spp.*) found on Lake Mohegan over the sampling years, are all indicators of nutrient-rich lakes (Heinonen et al. 2008). Even the green algae dominating in 2015 (*Pediastrum spp.* and *Fragilaria spp.*) are also indicators of high nutrient statues lakes (Heinonen et al. 2008). Though, cyanobacteria have dominated the phytoplankton community on Lake Mohegan for most sampling years. Other potential reasons why cyanobacteria dominant over other phytoplankton have been outlined by Shapiro (1990) and include: 1) superior energetics in high water temperatures (typically > 20 °C), 2) better adapted to lower light levels over other phytoplankton, 3) ability to buoyant themselves in the water column to obtain nutrients from bottom waters or more light in surface waters, 4) reduced zooplankton grazing, 5) ability to thrive in high pH and low CO_2 environments and 6) better adapted to low N environments. Taken together, cyanobacterial blooms will likely continue each summer on Lake Mohegan as long as the current nutrient status persists.

The timing of Lake Mohegan's HAB's also coincides with the recreational use of the lake, increasing the probability of human and animal exposure to cyanotoxins. Although of the three major toxins tested, microcystin was the only toxin to go above threshold levels set by the NYDEC and the World Health Organization (WHO). A variety of cyanobacteria can produce the cyanotoxin microcystin. Though, the species that have been documented to produce microcystin that also occur on Lake Mohegan are *Oscillatoria spp.*(Meriluoto et al. 1989), *Microcystis spp.* (Reynolds 1984), and *Woronichinia spp.* (Heinonen et al. 2008). Microcystin has roughly 70 structural analogs, each of which varies in toxicity (Microcystin-LR being the most toxic; Rinehart et al., 1994; Sivonen & Jones, 1999). Microcystin is a hepatotoxin, which causes hemorrhaging of the liver by blocking protein phosphatases 1 and 2A with an irreversible covalent bond (MacKintosh et al. 1990). Microcystin has also been linked to tumor production (Fitzgeorge et al. 1994). Human exposure to microcystin on Lake Mohegan would likely be from oral consumption and/or inhalation of water during recreational use (i.e., swimming and boating). Microcystin may also accumulate in certain fish (Papadimitriou et al. 2012), thus consuming fish caught on Lake Mohegan may pose another pathway for microcystin exposure.

The risks associated with microcystin exposure varies substantially. For example, the acute health risks associated cyanobacterial blooms on Lake Mohegan varied over time and space with "low" probable risks in open water samples to "very high" in shoreline downwind areas of the lake (World Health Organization 1999; Table xx). Variation in animal species sensitivity; amount consumed by the animal; age and sex of the animal; and the amount of other food in the animal's gut will also contribute to toxicity (Carmichael 2001). Fitzgeorge et al. (1994) found that microcystin toxicity is cumulative, where continual low dose exposures can lead to chronic liver damage and/or tumor production. Thus, as cyanobacterial blooms continue to occur on Lake Mohegan so will the potential short and long-term health risks.

Table xx. Guidance values for the relative probability of health effects resulting from exposure to cyanobacteria blooms and microcystin taken from the World Health Organization (1999).

Relative Probability of Acute Health Effects	Cyanobacteria (cells / mL)	Microcystin-LR (μg / L)	Chlorophyll-a (μg / L)
Low	< 20,000	< 10	< 10
Moderate	20,000-100,000	10-20	10-50
High	100,000-10,000,000	20-2,000	50-5,000
Very High	> 10,000,000	> 2,000	> 5,000

Predicting toxin levels has been described by Havens (2008) as less predictable than predicting cyanobacteria occurrence. This unpredictability in cyanotoxin production during a bloom is likely because there are distinct toxin and non-toxin producing strains of cyanobacteria, which can grow together during blooms and vary over time and space (World Health Organization 1999; Carmichael 2001). Worldwide, about 60 % of cyanobacterial blooms contain toxic cyanobacterial strains (World Health Organization 1999). While the risks of having a known toxin producer and at high concentrations is likely high, one cannot assume toxicity. Because of this, monitoring cyanotoxin concentrations is preferred rather than surrogates like algal biovolume or chl. *a*.

The ecological impact of cyanobacteria blooms can also be devastating. For simplicity, the ecological impacts of cyanobacterial blooms have been arranged in a model taken from Havens (2008):

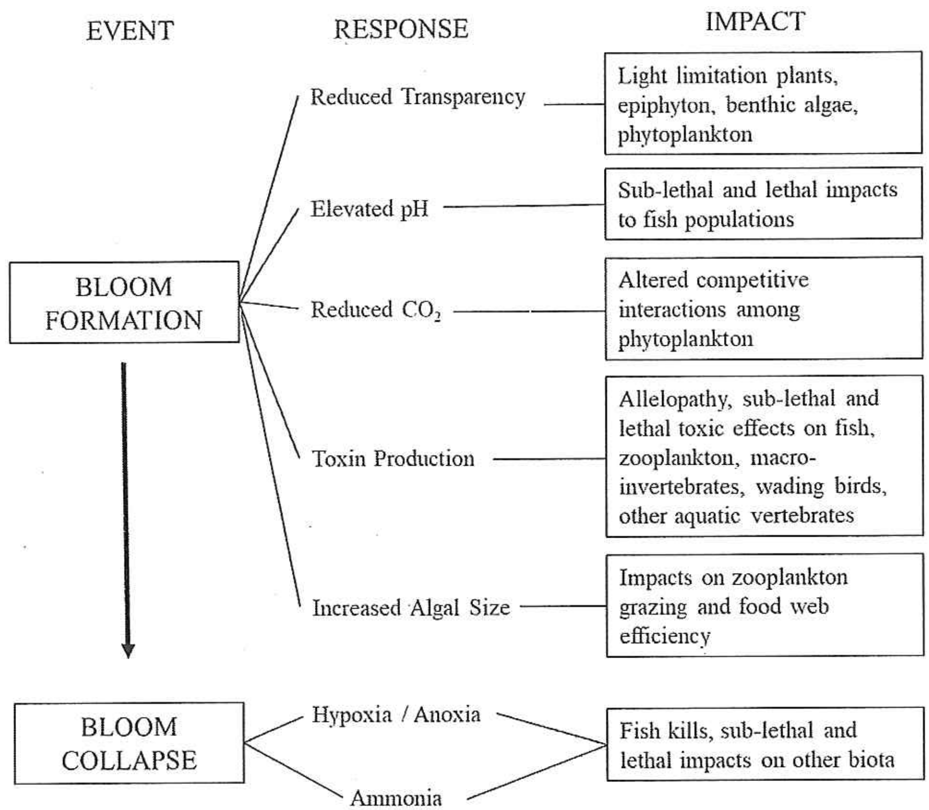

Figure xx. Summary of the ecological responses and impacts associated with cyanobacterial blooms. Taken from Havens (2008).

SUMMARY POINTS

- Cyanobacteria were the dominant taxa among all sample years except for 2015.
- The relative probability of health effects resulting from exposure to cyanobacteria blooms and microcystin ranged from low to very high.
- Microcystin-LR in downwind areas of the lake can reach as high as 7,032 µg / L.
- Continued HABs on Lake Mohegan will likely increase the potential for health risks both short and long-term.

WORK CITED

Bellinger, E. G., and D. C. Sigee. 2015. Freshwater algae: identification and use as bioindicators, John Wiley & Sons.

Canfield Jr, D. E., E. Phlips, and C. M. Duarte. 1989. Factors influencing the abundance of blue-green algae in Florida lakes. Can. J. Fish. Aquat. Sci. **46**: 1232–1237.

Carmichael, W. W. 2001. Health effects of toxin-producing cyanobacteria:"The CyanoHABs." Hum. Ecol. Risk Assess. Int. J. **7**: 1393–1407.

Carpenter, S. R., J. J. Cole, J. R. Hodgson, and others. 2001. Trophic cascades, nutrients, and lake productivity: whole lake experiments. Ecol. Monogr. **71**: 163–186.

Fitzgeorge, R., S. Clark, and C. Keevil. 1994. Routes of intoxication. Spec. Publ. R. Soc. Chem. **149**: 69–74.

Havens, K. E. 2008. Cyanobacteria blooms: effects on aquatic ecosystems. Cyanobacterial Harmful Algal Blooms State Sci. Res. Needs 733–747.

Heinonen, P., G. Ziglio, and A. Van der Beken. 2008. Hydrological and limnological aspects of lake monitoring, John Wiley & Sons.

Kalff, J. 2003. Limnology: Inland Water Ecosystems, Prentice Hall.

Liboriussen, L., and E. Jeppesen. 2003. Temporal dynamics in epipelic, pelagic and epiphytic algal production in a clear and a turbid shallow lake. Freshw. Biol. **48**: 418–431.

MacKintosh, C., K. A. Beattie, S. Klumpp, P. Cohen, and G. A. Codd. 1990. Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. FEBS Lett. **264**: 187–192.

Martin R. Michael. 2012. Mohegan Lake Diagnostic Report.

Meriluoto, J. A. O., A. Sandström, J. E. Eriksson, G. Remaud, A. G. Graig, and J. Chattopadhyaya. 1989. Structure and toxicity of a peptide hepatotoxin from the cyanobacterium Oscillatoria agardhii. Toxicon **27**: 1021–1034.

NYDEC. 2017. Harmful Algal Blooms (HABs) Program Guide.

Palmer, C. M. 1969. A composite rating of algae tolerating organic pollution. J. Phycol. **5**: 78–82.

Papadimitriou, T., I. Kagalou, C. Stalikas, G. Pilidis, and I. D. Leonardos. 2012. Assessment of microcystin distribution and biomagnification in tissues of aquatic food web compartments from a shallow lake and evaluation of potential risks to public health. Ecotoxicology **21**: 1155–1166.

Reynolds, C. S. 1984. The ecology of freshwater phytoplankton, Cambridge University Press.

Robert W. Kortmann. 1993. Lake Mohegan 1993 Diagnostic Report.

Shapiro, J. 1990. Current beliefs regarding dominance by blue-greens: the case for the importance of CO_2 and pH. Verh Intern. Ver. Limnol **24**: 38–54.

World Health Organization. 1999. Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management. Lond. E FN Spon.