Spring 5-2013

Spatial Variation in Microcystin concentrations in Nebraska Reservoirs

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Introduction/ Literature Review

The purpose of this study was to see if there were spatial differences in microcystin concentrations within reservoirs and examine whether more than one sample needs to be collected to more accurately represent water quality conditions. The Nebraska Department of Environmental Quality (NDEQ) collects water samples from beach or shoreline areas where sampling is simple and people are more likely to be in direct contact with the water. I hypothesized that *Microcystis* concentrations would vary spatially in local reservoirs and that beach sampling may not accurately represent toxin conditions for the entire reservoir. This is significant at the time of sampling. For example, if prevailing winds blow towards the sampling site, toxin producing cyanobacteria may accumulate along the shoreline and give a false-positive reading and vice versa.

What is *Microcystis*? *Microcystis aeruginosa* is a single-celled blue green algae, or cyanobacteria, that occurs naturally in surface fresh waters. *Microcystis* can proliferate to form dense blooms and mats under certain conditions (reference). Many variants of these cyanobacteria produce multiple toxins, including the potent liver toxin, microcystin. When *Microcystis* die, their cells break open, releasing the *Microcystin* toxin into the water. Ingestion of water or algal cells containing *Microcystin* produces adverse effects in fish, dogs, cats, livestock, and humans and can cause death in cases of extreme exposure. The long-term risk associated with microcystin exposure is unknown. (WHO, 1997) With an introduction of excess nutrients, primarily phosphates, into a water system, algae quickly grow due to the high availability of nutrients present. Although this is short-lived, the result is a high concentration of dead organic matter which begins to decay. The decaying process consumes dissolved oxygen in

the water resulting in anoxic conditions; that limit oxygen for fish and invertebrates (Dodds, 2010) and can result in fish kills.

Microcystis blooms typically thrive in warm, nutrient-rich waters (OEHHA, 2009). Blooms producing very high biomass occur in reservoirs with high nitrogen and phosphorus availability, termed eutrophic waters. Nebraska's agricultural landscape is subjected to high rates of fertilizer application. Fertilizers contain nitrogen and phosphorus, which stimulate crop production. With continued use year after year, nitrogen and phosphorus eventually enter streams and downstream reservoirs. According to the National Oceanic and Atmospheric Administration (NOAA, Agriculture Land Use Issues) fertilizers are the primary cause for cultural eutrophication. *Microcystis* also require sufficient light intensity to conduct photosynthesis, which results in blooms. Also *Microcystis* may be present in the water in the absence of visible blooms because the mats can sink below the surface (OEHHA, 2009).

A eutrophic lake is characterized by an abundant accumulation of nutrients that support a dense growth of algae and other organisms, the decay of which depletes the deep waters of oxygen in summer (Dodds, 2010). This often results in algal blooms, which happen when the algae are stimulated by excess nutrients and grow rapidly. As cyanobacteria continue to grow, blooms expand and spread across lakes and reservoirs. Blooms form dense mats that attenuate sunlight rapidly, limiting photosynthetic activity to very shallow depths. Eventually, algae die and sink to the bottom where their decay depletes oxygen availability.

Materials and Methods

The Abraxis LLC Microcystins Enzyme-Linked Immunosorbent Assay (ELISA) laboratory test kits are used for analysis of total microcystins concentrations for NDEQ. I used data collected by NDEQ's beach monitoring program in 2011 and 2012. This program provides a public record of microcystin concentrations at beach locations for approximately 40 lakes and reservoirs. I also collected microcystin data collected by Dr. Steven Thomas in 6 local reservoirs. The UNL data is from multiple locations in each reservoir and include samples from the beach, 3 open water locations, and samples from the hypolimnion and epilimion from the central most open water site.

This past summer, I worked for NDEQ as a Surface Water Laboratory Technician and collecting the microcystin samples from around the state and analyzed those samples using the Abraxis ELISA method. We sampled each lake in their monitoring network weekly throughout 2012 growing season.

Microcystin samples were collected using a sterile, brown HDPE 125 mL bottle to collect the sample. Sample water was collected by wading out from the beach to about knee high water, inverting the bottle before submerging it about 10 inches into the water. Bringing the full bottle up, we would dump it out to rinse the bottle. After dumping the water out, the same technique is used to collect the final sample. After collecting the sample, we could put it in a cooler on ice to keep the sample fresh until we test them. Arriving back at the lab, the samples would be refrigerated right away until tested, usually Thursday of the same week. To prep day the microcystin for the tests, we would put 1 mL of the sample water into glass vials and use the "freeze and thaw" method to cause the *Microcystis* cells to break open, releasing their toxin.. We conducted three freeze and thaw cycles for each sample.

After the last cycle is complete, the cyanobacteria is ready for the tests to begin. The use of the ELISA kits provided a low cost, semi-quantitative analytical method for measuring concentrations of total microcystins, the most common toxin released by cyanobacteria. Additionally, analyzing water samples with ELISA kits provided for a quick turn- around time, which allowed weekly updates of lake conditions and public health alerts and advisories prior to each weekend's recreational activities. (NDEQ)

Results & Discussion

To examine spatial patterns in the cyanobacteria, I compared the data collected by Dr. Thomas and UNL and the samples collected by NDEQ. There were four reservoirs that overlapped in the UNL and NDEQ datasets: Bluestem, Conestoga, Pawnee, and Wagon Train.

I would have predicted that both, NDEQ and UNL, Beach concentrations would tend to follow the same trends and vary from the open water samples, which all be similar. The predicted order of highest concentrations would go, beach samples both NDEQ and UNL, then the Epilimnion (the top layer of the lake), and finally the Hypolimnion (the bottom layer of the lake). As the following figures show, that is not the case and the highest concentration of microcystin varied weekly.

The results of how the data turned out in graph form, caused some concern in the discrepancy between both NDEQ and UNL data. The results showed that the concentrations of microcystin present did vary weekly and also where in the water column the highest concentrations would show up. The results helped prove my first hypothesis saying that there are differences in microcystin concentrations within reservoirs. Although the data was not collected

on the same day, the difference of values could be caused by factors that I may have over looked or beyond my knowledge of Limnology and microcystin presence within the water column.

My second hypothesis of needing more than one sample to get an accurate representation was proven false. NDEQ while setting up their beach monitoring program had the same thought as I did and tested the multiple or single water sample. Statistical tests indicated microcystin concentrations were distributed similarly at all beach sampling locations and changes to NDEQ single sample beach sampling protocol were not warranted (NDEQ).

The World Health Organization (WHO) has addressed health hazards presented by cyanobacterial toxins as part of a continuing revision of the WHO Guidelines for Drinking Water Quality. With the main aim of protecting public health, a WHO Guideline Value (GV) for total (i.e. intracellular plus extracellular) microcystin-LR in drinking water has been derived. This GV of 1 μg/l, which is conditional, although derived for life-long exposure (WHO, 1997 and Chorus and Bartram, 1999), is already being used in some countries (e.g. Australia, UK) in the day-to-day management of water supply from sources affected by cyanobacterial blooms. Guidelines for cyanobacterial cell numbers in recreational waters, based on epidemiological studies (e.g. 20,000 cells per ml) or derived from the drinking water GV for microcystin, are also in use in the assessment and management of eutrophic recreational water bodies, e.g. in the UK and Australia. It would seem that the microcystin GV for drinking water, GVs still to be derived for other cyanobacterial toxins and exposure routes when sufficient data are available, and recreational water guidelines will be useful tools in the ranking of eutrophication among water quality problems, and in setting targets for eutrophication control according to water body use, in addition to drinking water treatment. (Codd, 2000)

Another factor to take into consideration, is the movement of the water in the water column. The internal waves of the water can transport the microcystin to other areas of the lake without being close to the shore. That could have greatly affected the differences in the data collected. The human error factor, the data could have been translated incorrectly from the test data into the computer data base; although it is highly unlikely.

The following figures are a graphical representation of the data collected by UNL and DEQ from the summer of 2011 and 2012.

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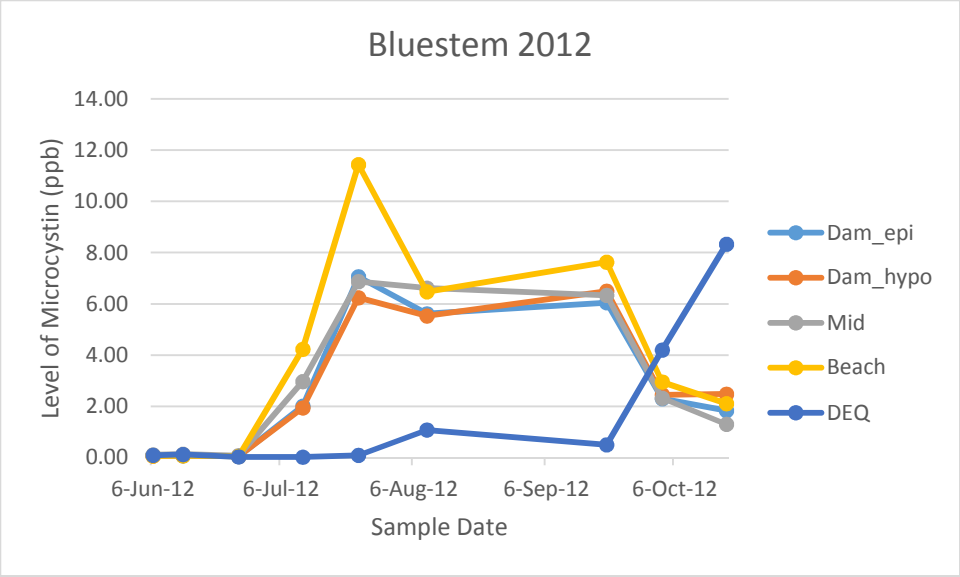

Figure 1- 2012 Bluestem Lake microcystin concentration

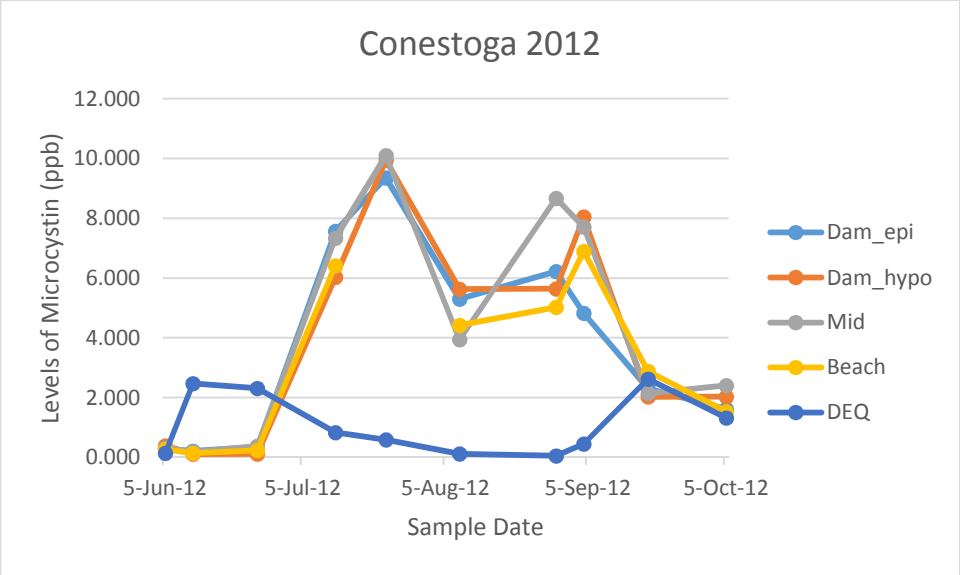

Figure 2- 2012 Conestoga Lake microcystin concentration

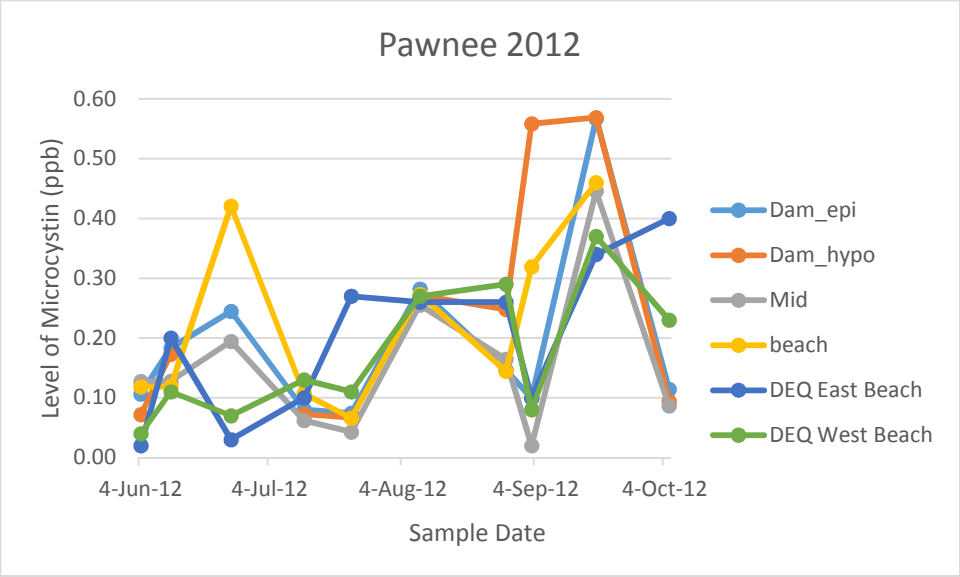

Figure 3- 2012 Pawnee Lake microcystin concentration

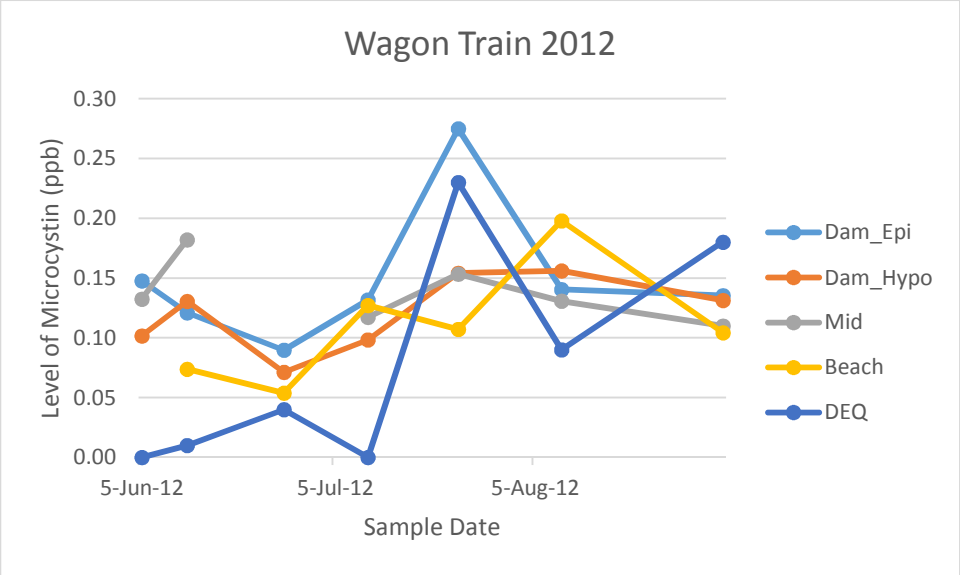

Figure 4- 2012 Wagon Train Lake microcystin concentration

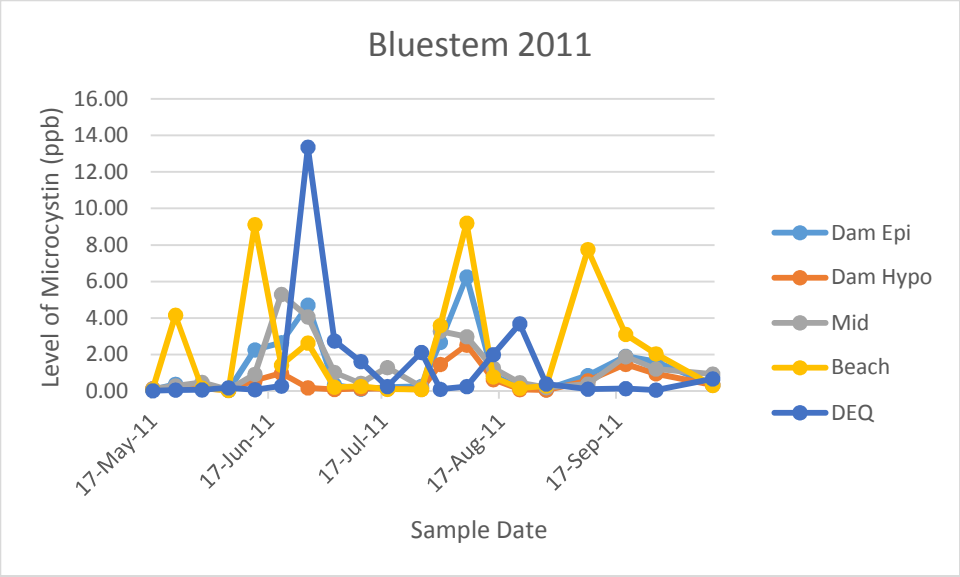

Figure 5- 2011 Bluestem Lake microcystin concentration

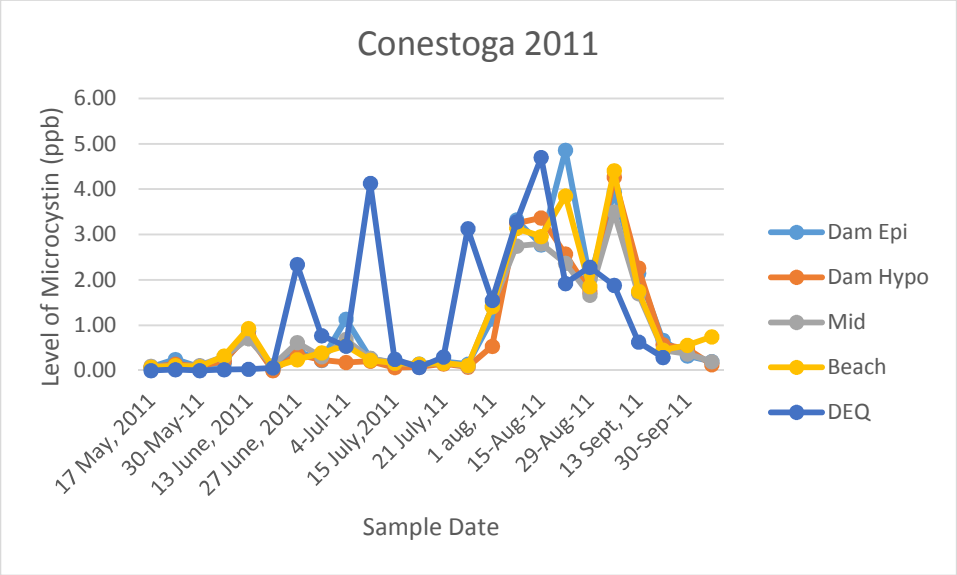

Figure 6- 2011 Conestoga microcystin concentration

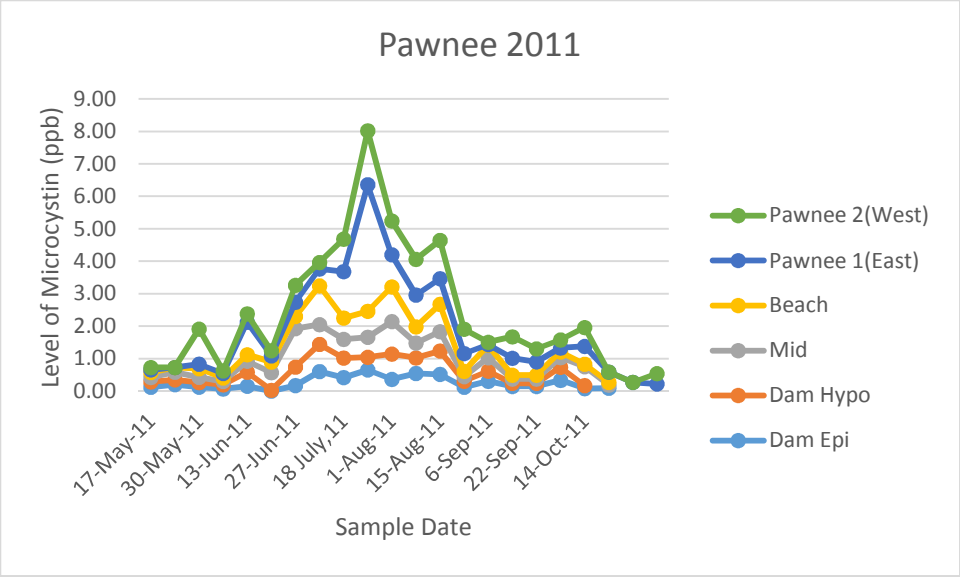

Figure 7- 2011 Pawnee microcystin concentration

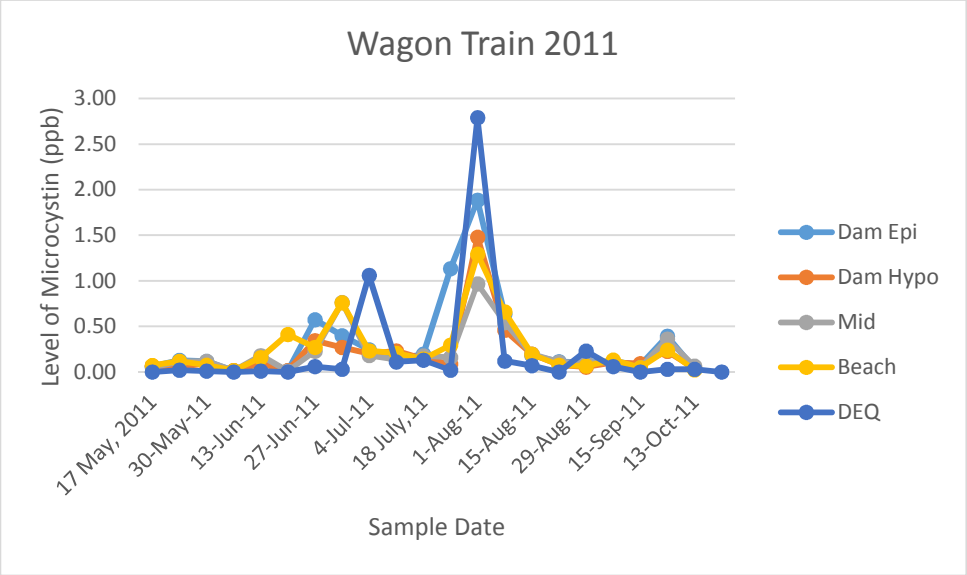

Figure 8- 2011 Wagon Train microcystin concentration