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Mussels as Biomonitors of Lake Water Microcystin:

A Final Report for the Summer 2000 Microcystin Monitoring Study

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May 2001

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Acknowledgments

I would really like to thank all of the volunteer monitors from the New Hampshire Lakes Lay Monitoring Program that participated in the mussel and lake water sampling for this study. Your willingness to help and go beyond your normal LLMP sampling is greatly appreciated. If you have any questions about this report or would like additional information, please feel free to contact me at the phone number or email address on the cover.

Introduction

Cyanobacteria (blue-green algae) are among the group of primary producers within an aquatic ecosystem that utilize sunlight to convert carbon dioxide into energy through the process of photosynthesis. Cyanobacteria have historically been associated with nutrient-enriched systems, where explosive summer growth can create green-tainted water, surface films, and shoreline scums. These dense concentrations of cyanobacteria can affect consumption and recreational usage of waters by their unsightliness, taste, and odor. In addition to these problems, certain species of bloom-forming cyanobacteria also pose health risks because of their ability to produce several types of toxins.

There are two main types of toxins produced by cyanobacteria – neurotoxins and hepatotoxins. Neurotoxins interfere with nerve impulses within the body, quickly affecting muscle coordination and breathing. The hepatotoxins are much more slow-acting, disrupting the structure and function of the liver. Although neurotoxins have been blamed in several incidents within North America, Great Britain, Australia, and Scandinavia, the hepatotoxins are more widespread and have been implicated across much of the world in cases of toxicosis involving wildlife, pets, and humans.

Our laboratory at the University of New Hampshire has been working with the hepatotoxin, microcystin, for several years. Much of our most recent work has examined microcystin accumulation throughout the aquatic food web. When organisms consume microcystin-containing

cyanobacteria either directly or indirectly (while drinking or feeding on other substances), the toxin concentrates in the liver and can accumulate to high levels over time as more and more microcystin is consumed. Past studies from our lab have identified microcystin accumulation in several aquatic organisms, including several species of zooplankton, snails, and mussels. For my thesis research, I was interested in further analyzing and understanding microcystin accumulation in two very common benthic (sediment-dwelling) invertebrates – crayfish and mussels.

Crayfish are found worldwide in a wide range of habitats (lakes, rivers, swamps, ditches) and nutrient conditions (nutrient-poor to nutrient-rich). Crayfish are common invertebrates in New Hampshire lakes, inhabiting the littoral (shallow) zone around and under rocks and debris. As omnivores, crayfish feed on a variety of substances, including vegetation, carrion, animal protein, detritus, and algae. Through direct consumption of algae or indirect algal uptake while ingesting plants and detritus, crayfish probably ingest cyanobacteria and may accumulate associated microcystin. Mussels are also found worldwide in a range of habitats and nutrient conditions. In New Hampshire lakes, mussels are extremely common inhabitants of shoreline zones where sand or mud is the dominant substrate. As filter feeders, mussels siphon in lake water between gill filaments and strain out particles for consumption. Algae are a primary food source and cyanobacterial consumption can lead to microcystin accumulation.

During the summer of 1999, I examined microcystin accumulation in crayfish and mussels from lakes throughout New Hampshire. Although the lakes varied in location, size, morphometry, and nutrient status, I found detectable microcystin levels in the livers of all the crayfish and mussels sampled from the different lakes. One very interesting finding in this study was that lakes with higher concentrations of microcystin in the lake water generally had mussels with higher concentrations of microcystin in the mussels. The mussels concentrated microcystin to levels that were much higher than the levels in the lake water. This is important because lake water microcystin concentrations are typically low and near the limits of detection for the analytical procedures used.

Although lakes with low microcystin levels may not currently pose a direct threat to animals and humans, assessing trends in these systems may allow us to identify escalating problems that can be proactively remedied through preventative management before harmful cyanobacterial blooms occur.

The study that identified the relationship between lake water microcystin and mussel liver microcystin only analyzed levels within the lake and organisms on a single date and did not include a time element to take into account changes in microcystin levels over time in a lake. The study that you and seven other Lakes Lay Monitoring Program volunteer groups participated in during the summer of 2000 was designed to test whether this relationship holds true throughout the summer season. By concurrently sampling mussels and lake water on a weekly time scale in eight different New Hampshire lakes, microcystin levels could be compared over time to determine if changes in mussel microcystin levels reflect corresponding changes in the lake water microcystin. We wanted to test whether monitoring programs could use mussel sampling to track microcystin levels in the lake water.

Study Methods

The lakes chosen for this study (Table 1) were selected to represent a range of trophic condition (i.e. chlorophyll α and total phosphorus concentrations, transparency), size, and location (Figure 1). Lakes were sampled weekly for 8-10 weeks between June and August of 2000 by volunteer monitors in the New Hampshire Lakes Lay Monitoring Program (NHLLMP). During normal weekly NHLLMP sampling, triplicate integrated water samples were taken of the epilimnion using a weighted garden hose. From each of these samples, 20 mL were poured into a vial and stored frozen for whole lake water (WLW) microcystin analysis. Following this sampling, six to ten mussels were collected from a predetermined shoreline site and stored frozen for microcystin

analysis. In our laboratory, the WLW and mussel liver samples were analyzed for microcystin concentration using a highly-sensitive ELISA procedure (enzyme-linked immunosorbent assay).

Results and Discussion

Microcystin Concentrations in the Lakes

Microcystin was detectable in the mussels and lake water from all eight lakes (Figure 2). Mussels from North River Lake and Squam Lake Squaw Cove contained the highest average concentration of microcystin in their liver tissue (Figure 2A). Baboosic Lake had the highest lake water microcystin level, with nearly twice the microcystin concentration of the other lakes (Figure 2B). The range of microcystin concentrations found in the lake water (~2.5 to 25 ng L^{-1}) were 40 to 400 times lower than the 1 μg L^{-1} (1000 ng L^{-1}) safe limit suggested by the World Health Organization for drinking and recreational waters. Although these lake water concentrations are low, microcystin could still pose a threat if the toxic cyanobacteria aggregate in a surface or shoreline bloom. These aggregations, seen as localized bright green water and/or green shoreline scums, could concentrate the microcystin to levels that could be harmful if consumed.

Mussels as Microcystin Biomonitors

The mussels from all lakes accumulated microcystin in their liver tissues. Mussel liver microcystin concentrations (presented as nanograms of microcystin per gram of liver) were higher than levels in the lake water (presented as nanograms of microcystin per liter of lake water), which is important because higher levels of microcystin are easier to detect with the ELISA analysis procedure we use.

The main goal of this study was to determine if changes in mussel microcystin levels reflect corresponding changes in lake water microcystin. To examine this, mussel liver microcystin and lake water microcystin concentrations were plotted for each lake (Figure 3). Looking at these graphs, one

can see that microcystin levels in both the mussels and lake water did change over the 8-10 week sampling period in each lake. However, the important question is whether the changes in the mussels and lake water occurred at the same time (same sampling date) and in the same direction (both increasing or decreasing). To answer this question, a statistical analysis known as regression or correlation analysis was used. In this analysis, which was performed separately for each lake, the mussel and lake water microcystin levels from each sampling date were plotted on a single graph (Figure 4). Once all 8-10 points were placed on each graph representing the 8-10 sampling dates, a statistical calculation was used to determine whether the points lie along a relatively straight line and, if so, a line was drawn representing the relationship (correlation) found between the mussel liver microcystin and lake water microcystin concentrations. In some cases, however, the points were so scattered that no significant relationship was found.

For the eight lakes in this study, correlations were identified for only two of the lakes (Lovell Lake and Squam Lake Squaw Cove). For these two lakes, as the microcystin concentration in the lake water increased, microcystin levels in the mussels also increased in a predictable (linear) manner. The presence of this relationship suggests that, at least for some lakes, mussels can be utilized in monitoring programs to assess and track microcystin levels in the lake water.

The points for the other lakes, however, were very scattered and did not show a straight-line relationship, indicating that changes in microcystin accumulation in the mussels did not match the changes in the lake water microcystin levels. This brings into question why the relationship existed in some lakes but not in others, and whether mussels can be utilized to track lake water microcystin levels. There is a possible explanation as to why the relationships were not seen in all of the lakes. Mussels are primarily known as filter feeders and would presumably accumulate the microcystin by feeding on toxic cyanobacteria in the lake water. However, in another study done for my thesis, I found stronger relationships between mussel microcystin and lake water microcystin when the concentrations of microcystin in the sediments was included, which indicates that the mussels may

also be getting some microcystin by ingesting the surrounding sediments and any toxic cyanobacteria inhabiting those sediments. Several studies have shown that toxic forms of cyanobacteria can live in the upper sediments of lakes, and work by our lab has found that these sediment concentrations of microcystin can be 5 to 100 times higher than lake water levels. Although most known for filtering feeding, several types of mussels have been shown to feed on sediments through a process called pedal feeding, in which the mussels extend their foot muscle outside of the shell and use small hairs on the foot to pull sediment inside the shell to be ingested. It is likely that the mussel species in New Hampshire can also utilize pedal feeding. The lack of lake water microcystin-mussel microcystin relationships in six of the eight lakes could be because the mussels are getting some portion of their microcystin from the sediments and we did not measure or take into account changes in sediment microcystin levels in our study and graphs. In the two lakes where the relationship was significant, the sediments may not be a major source of microcystin for the mussels, either because the mussels are not utilizing pedal feeding or toxic cyanobacteria do not exist in the sediments around the mussels for whatever reason.

The next step in this study would be to repeat the lake water and mussel monitoring, but in addition either include sediment sampling for microcystin analysis or isolate the mussels away from the sediments to prevent them from ingesting microcystin from the sediments. Sediment sampling in lakes is very difficult and time consuming and requires the use of very demanding coring equipment. This does not favor the inclusion of sediment sampling in volunteer monitoring programs. On the other hand, the latter idea of isolating the mussels away from the sediments is very promising and would be relatively easy to do. Mussels could be placed in cages or minnow traps which could be suspended from buoys or docks so that the mussels would not be in contact with the sediments. The mussels would only be able to ingest microcystin by filter feeding the toxic cyanobacteria from the lake water. This may allow us to establish relationships in all or most of the lakes since the problem of sediment microcystin ingestion would be excluded.

Practical Implications

Although the microcystin concentrations in the lake water of all eight lakes were low and below the suggested World Health Organization safe level, the mere presence of the toxic cyanobacteria indicates that each lake has the potential for increased problems. The cyanobacteria that produce microcystin are generally associated with more nutrient-enriched systems and so the problem of lake eutrophication can bring about increased occurrences of toxic blooms. If any of the eight study lakes were to receive nutrient enrichment, the summer levels of microcystin could increase as the toxic cyanobacteria become more prominent in the plankton.

The ability of the mussels to accumulate microcystin could pose problems for the rest of the aquatic food web components. Studies have shown that several aquatic invertebrates, including mussels and crayfish, can accumulate microcystin without adverse effects. Most mammals, birds, and other terrestrial organisms appear to be very sensitive to the toxin. Although humans generally do not eat freshwater mussels, some birds and mammals, such as muskrats, otters, raccoons, and mink, do consume mussels. If these predators were to consume large enough amounts of mussels containing high levels of accumulated microcystin, the toxin could be transferred to them and potentially cause harm to the predator.

Summary

Microcystin was detectable in the lake water and mussels from all eight study lakes. Lake water microcystin concentrations were 40 to 400 times lower than the safe limit suggested by the World Health Organization for drinking and recreational waters. Microcystin concentrations in the mussels were generally higher than levels in the lake water, demonstrating the mussels' ability to concentrate the toxin in its liver tissue. A relationship between lake water microcystin and mussel microcystin was identified for two of the study lakes, suggesting that mussels may be useful as microcystin biomonitors to track lake water levels of the toxin. However, the lack of a relationship

for the other six lakes suggests the need to modify the procedure used in any future mussel monitoring. Pedal feeding by the mussels on the surrounding sediments may explain the absence of a relationship in the six lakes, since microcystin concentrations in the sediment have been found to be 5 to 100 times higher than levels in the lake water. Elevating the mussels above the sediments would exclude the problem of sediment ingestion and may allow relationships to be established in all of the lakes so that mussel sampling can be used in monitoring programs to track microcystin levels in the lake water.

Table 1. Morphometric data of the lakes sampled for the Mussel MC Monitoring Study. Data were compiled from the New Hampshire Department of Environmental Services Lakes Inventory Database (1998). * - Data derived from maps produced in Delorme's 3-D TopoQuads (Yarmouth, ME).

Lake	Abbrev.	Town	County	Mean Depth (m)	Maximum Depth (m)	Lake Area (Ha)	Lake Volume (Ha-m)	Watershed Area (Ha)	Hydraulic Retention Time [if 0.5 m Runoff] (yr)
Baboosic Lake	BBL	Amherst	Hillsborough	4.1	8.8	89.8	368.2	787.8	0.8
Depot Pond	DP	Milton	Strafford	5.5	16.8	72.8	400.4	29571.6	0.0
Little Squam Lake	LSQ	Holderness	Grafton	10.1	21.9	165.2	1668.5	9324	0.4
Lovell Lake	LVL	Wakefield	Carroll	5.1	11.9	217.7	1113.6	1219.2	0.6
North River Lake	NRL	Nottingham	Rockingham	2.4	6.7	32.4	77.8	377	0.4
Northeast Pond	NEP	Milton	Strafford	2.7	14.9	228.5	617.0	29597.5	0.0
Squam Lake - Squaw Cove	SQSC	Holderness	Grafton	N/A	5*	39.8*	N/A	18112*	N/A
Townhouse Pond	TP	Milton	Strafford	3.3	10.1	48	158.4	29525.9	0.0

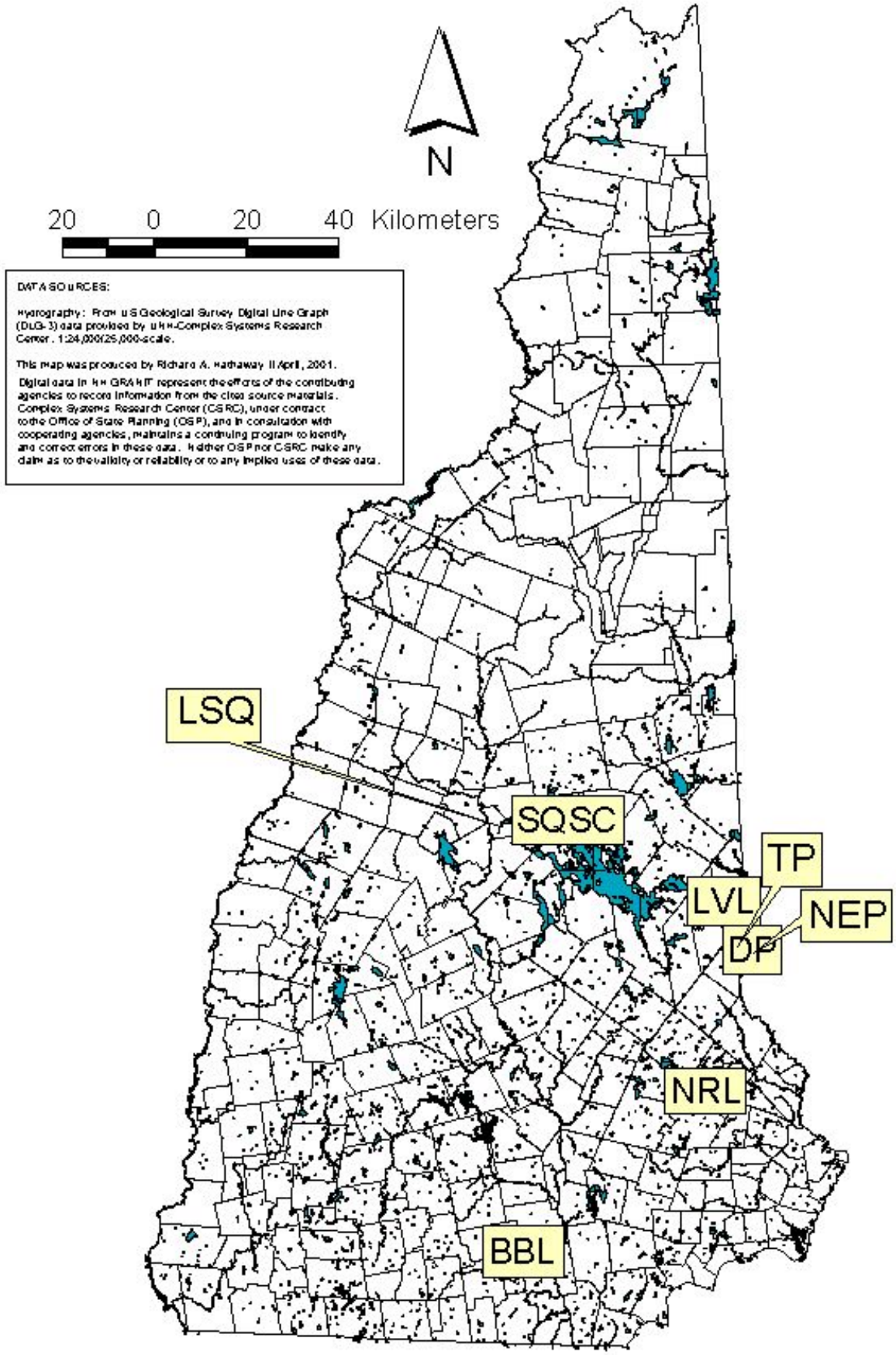
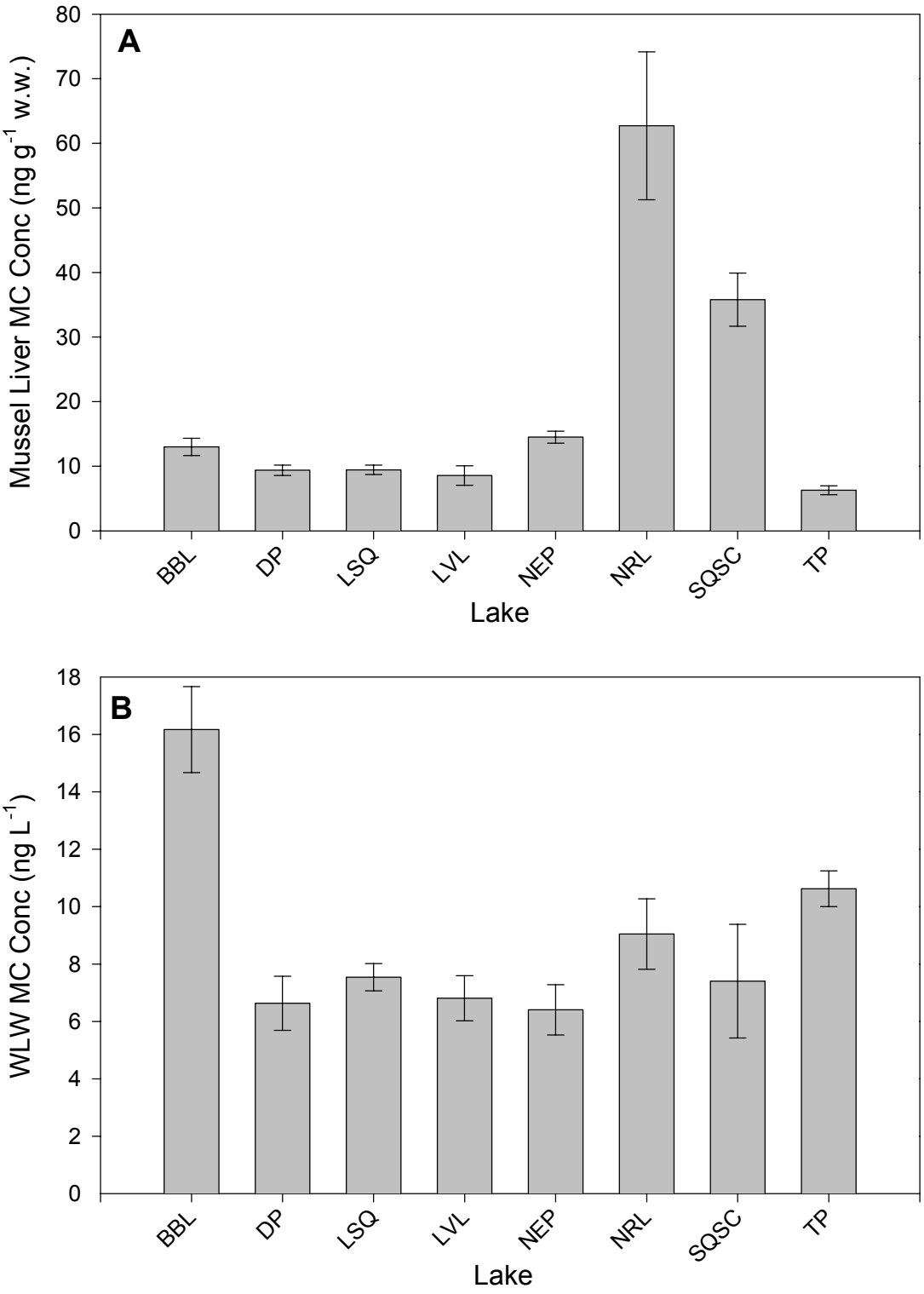


Figure 1. Lakes sampled for the Microcystin Monitoring Study.



Figures 2 A-B. Average (A) mussel liver and (B) whole lake water (WLW) microcystin concentrations for the eight lakes sampled in the Microcystin Monitoring Study. Error bars represent standard errors. w.w. - wet weight

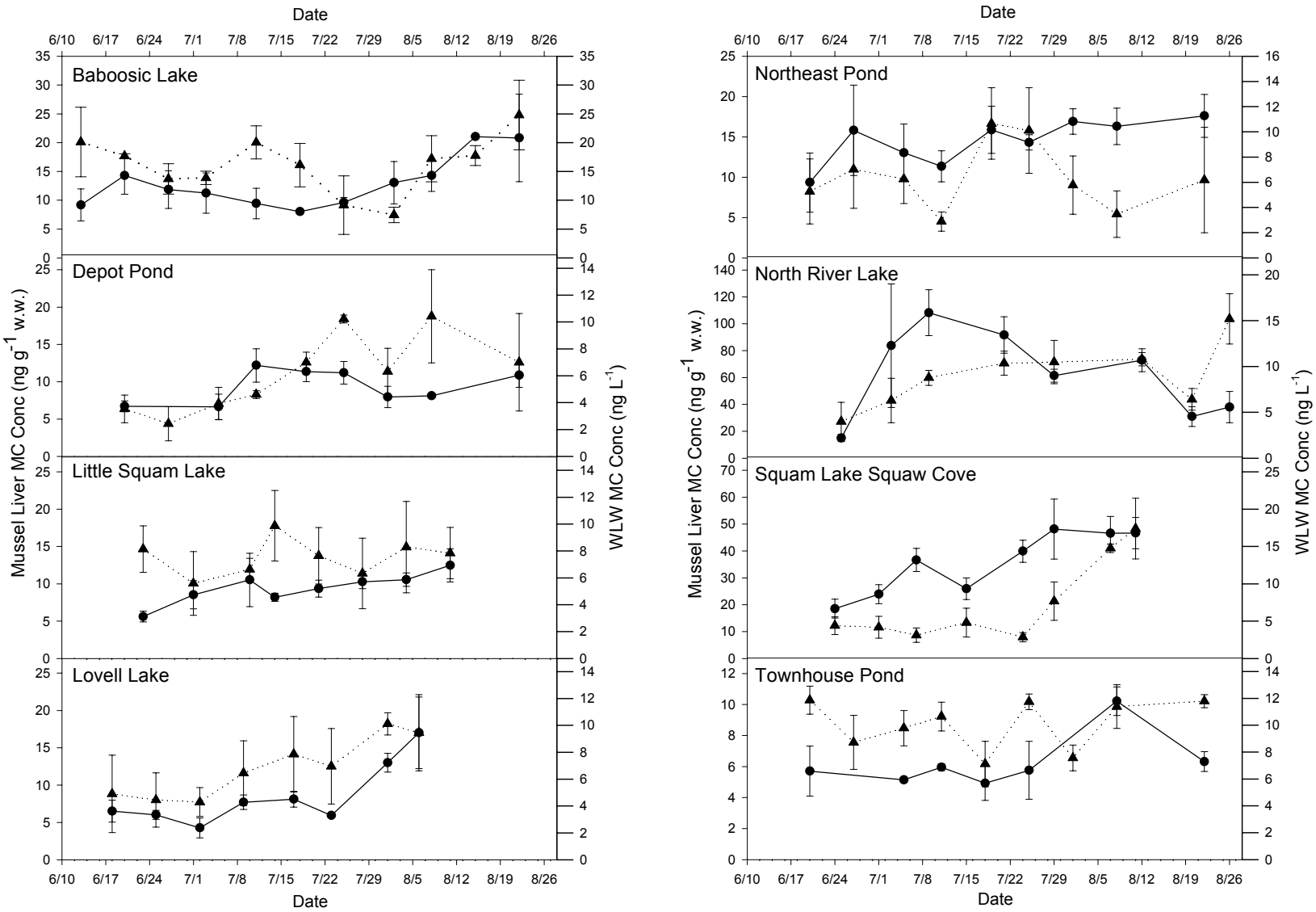


Figure 3. Temporal changes in mussel liver microcystin and whole lake water (WLW) microcystin concentrations for the eight lakes sampled in the Microcystin Monitoring Study. Error bars represent standard errors. ● -Mussel Liver microcystin ▲ -WLW microcystin. Note that mussel liver microcystin (left axis) and WLW microcystin (right axis) axes are in different units and on different scales.

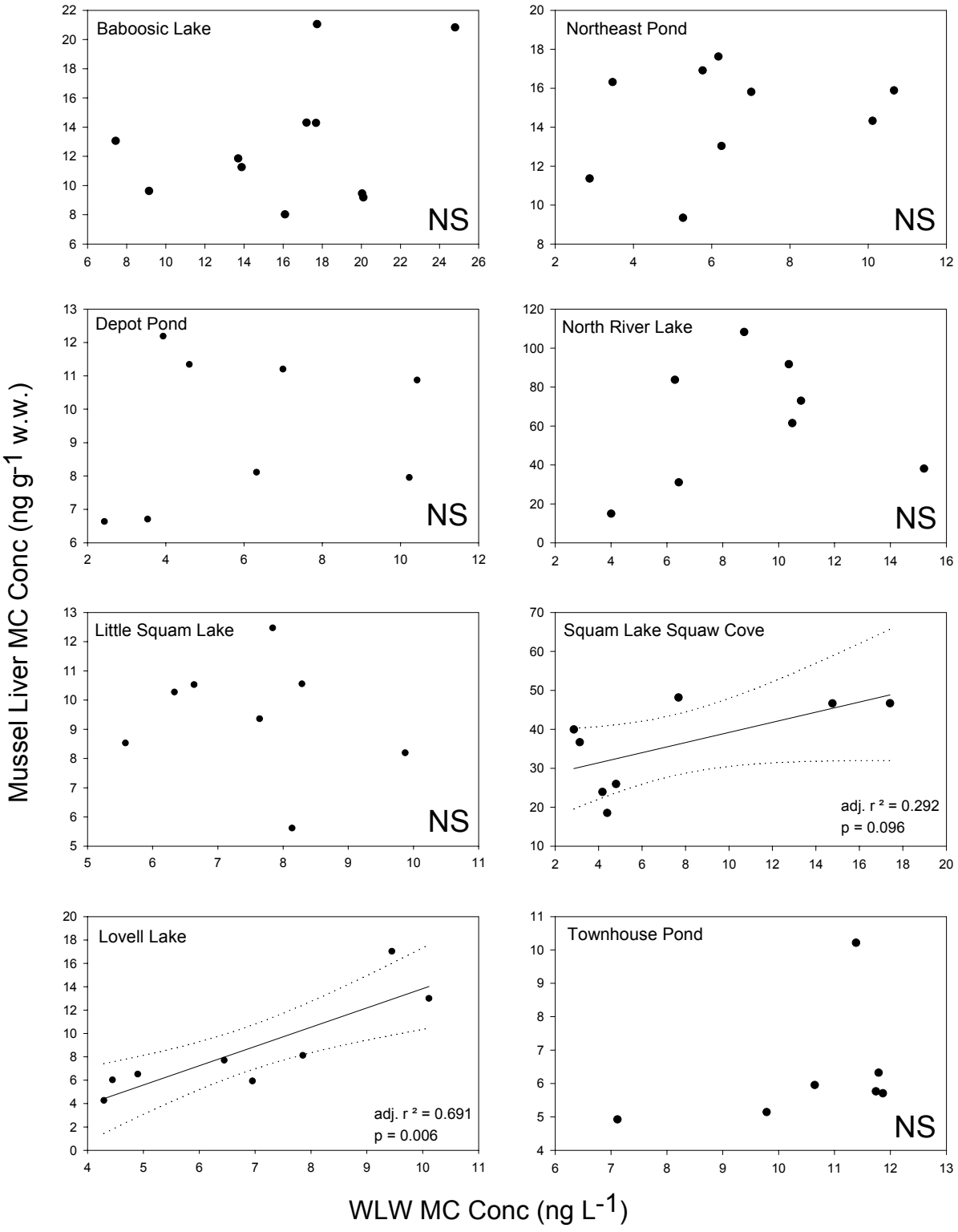


Figure 4. Correlations between mussel liver microcystin concentration and whole lake water (WLW) microcystin concentrations for the eight lakes sampled in the Microcystin Monitoring Study. Adjusted r^2 alues (adj. r^2) indicate the percent variability in mussel liver microcystin that can be explained by ıe WLW microcystin. Higher adj. r^2 values indicate a stronger fit between the two variables. Plots with "NS" indicate no significant correlation. Dotted lines represent 95% confidence intervals.

Additional Acknowledgments

I would like to thank Dr. James Haney (Professor of Zoology, University of New Hampshire) for his guidance and assistance in designing this study and formulating this report. I would also like to thank Robert Craycraft (Cooperative Extension Program Coordinator, University of New Hampshire) for his assistance in selecting the study lakes, and Juliette Nowak (Graduate Student, University of New Hampshire) for her help in performing the ELISA analyses. This report was reviewed by Dr. James Haney and Robert Craycraft. Funding for this study was provided by the University of New Hampshire Agricultural Experiment Station Hatch 205 Grant. For additional assistance, you can contact:

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