

Profiling Microcystin Toxins in the Red Cedar Watershed

Authored by Kellen Doerr, facilitated by Dr. Jennifer Grant

1. The Applied Science Program, University of Wisconsin at Stout, Menomonie, WI 54751.
2. The Biology Department, University of Wisconsin at Stout, Menomonie, WI 54751.

1 Abstract

Chronic exposure to microcystin-contaminated water can have long-lasting negative effects on human health for those living and recreating in an affected area. Microcystin levels can become very high during the summer month when cyanobacteria density increases, and cells rupture, releasing their contents into the water. The World Health organization has indicated that above concentrations of 1 ug/L, microcystin levels are dangerous to human health.

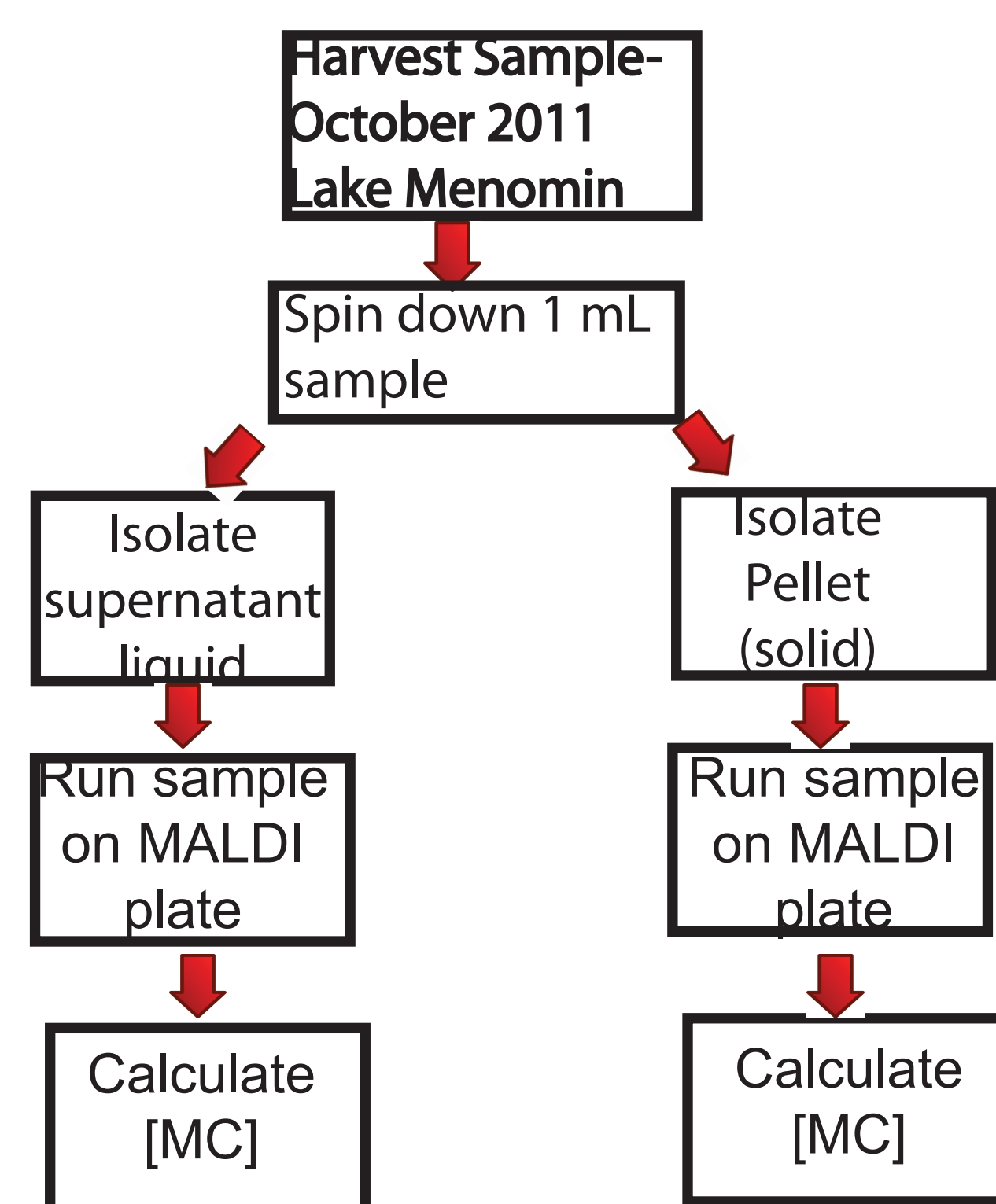
We implemented a technique to quantify the amount of microcystin present in lakewater collected from Lake Menomin (Menomonie, Wisconsin, using Matrix Assisted Laser Desorption Ionization Mass Spectrometry. This method is straightforward, robust and requires a minimum amount of sample processing or analysis time.

This poster describes this assay.

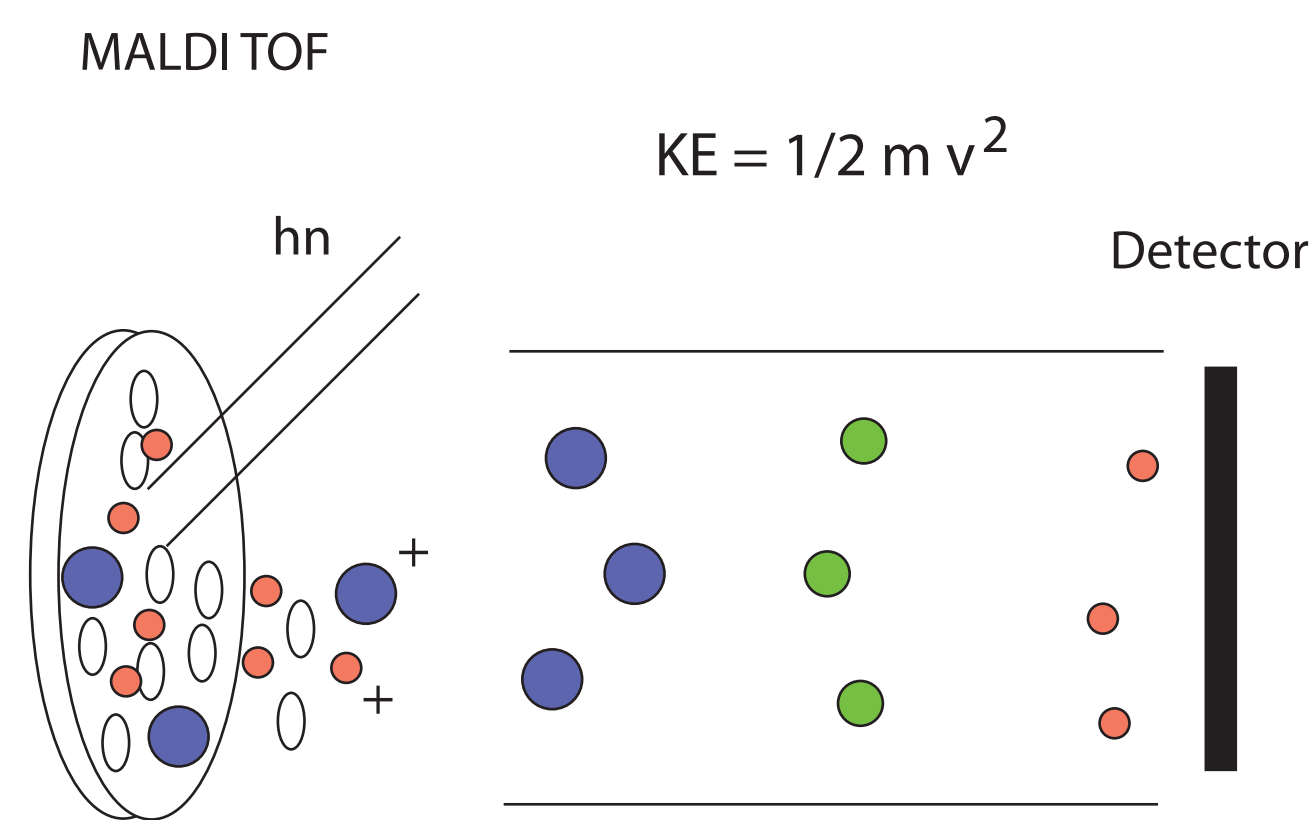
2 Introduction

There are 70 microcystin peptide isoforms, which vary widely in toxicity. Of these, the LR isoform is the most toxic.⁵ The precise distribution of microcystin in water depends on the strain of the cyanobacterium, growth rate, and local environmental conditions⁶ and cannot be predicted based on colony size and shape. For this reason, the use of Matrix Assisted Laser Desorption Ionization (MALDI) mass spectrometry to visualize and semi-quantify microcystin content of lakewater has become of increasing importance.

3 Sample Collection and Processing

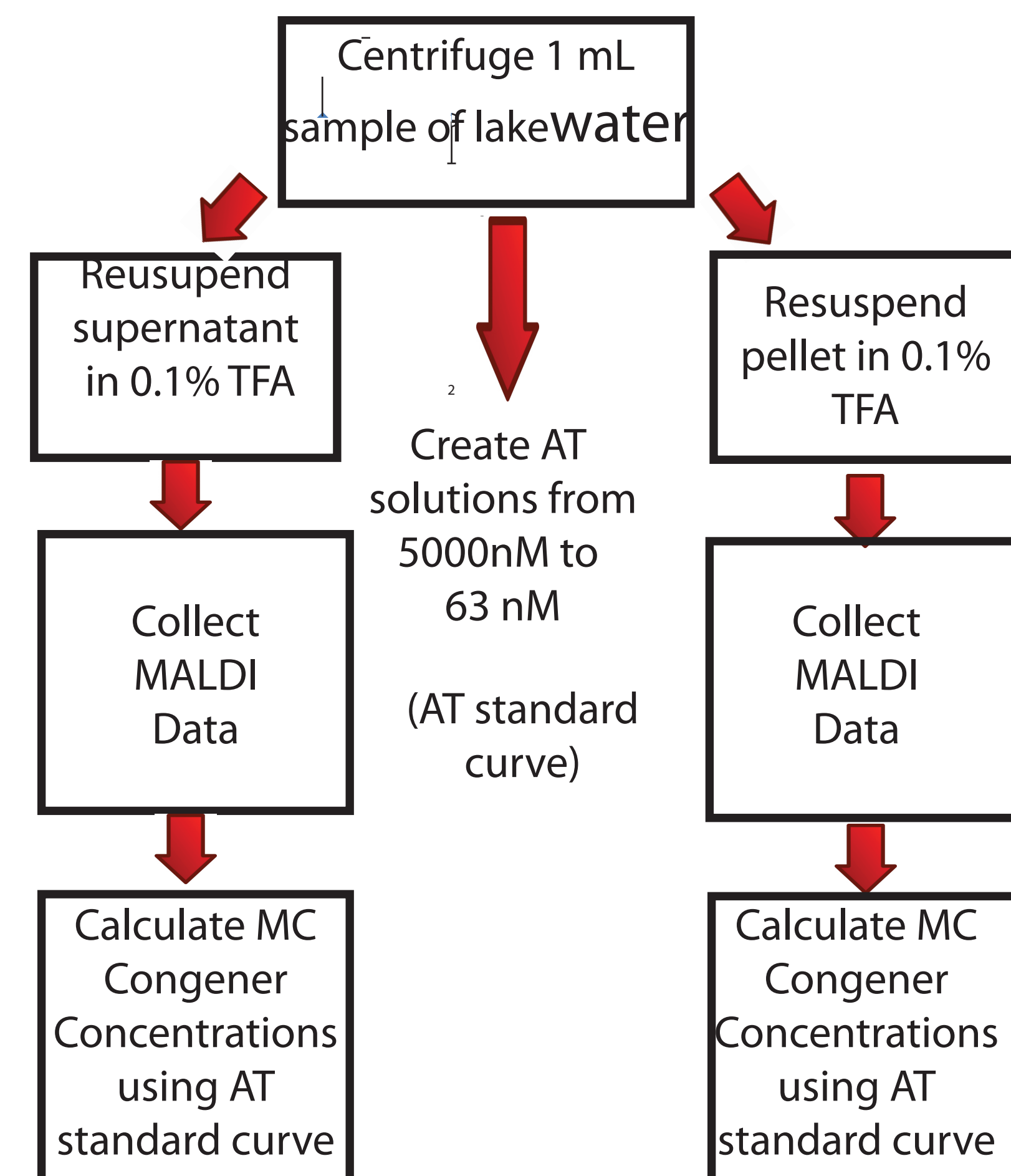


4 MALDI-TOF Mass Spectrometry



Mass spectra was collected using a Bruker Microflex Linear Time of Flight Mass Spectrometer (MALDI-TOF), using data collection methods compatible with the measurement of peptides. Data representing the intensity of different ion species was collected in the mass-to-charge (m/z) range of 300 to 2000 Daltons (Da)

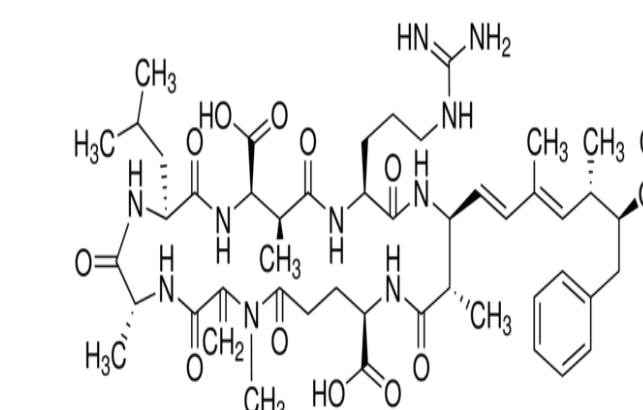
5 Analysis of Microcystin Toxins



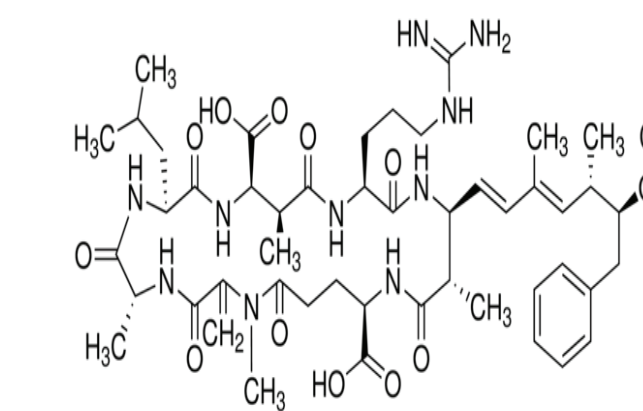
The Sample concentrations for the soluble and insoluble MC's were recorded in the figure in section 7 and the AT standard curve used to quantify the microcystin congeners in the lakewater samples is shown in section 8. The concentration for the MC congeners was as calculated and is shown in section 9.

6 Microcystin Congeners

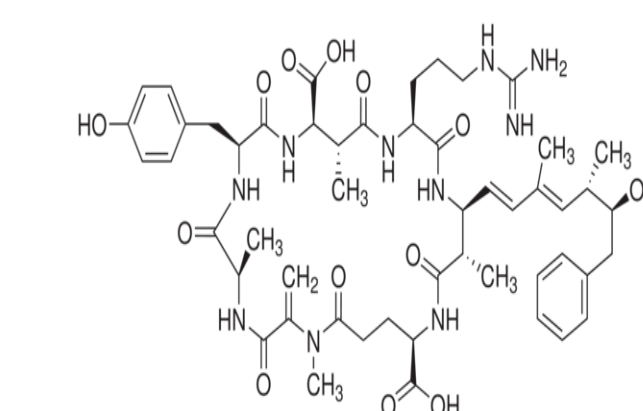
LR Congener
MW +1=
996.17 Da



RR Congener
MW+1=
1039.2 Da



YR Congener
MW+1=
1046.19 Da



9 Quantification of Microcystin Congeners

Microcystin	Molecul massht (Daltons)	Molecul massht +1 (weight of hydrogen)	Concentration of MC's in 1mL lakewater sample(µM)	Standard Deviation Of 2 samples
LR	995.17	996.17	2.06	0.374
RR	1038.2	1039.2	3.39	0.332
YR	1045.19	1046.19	1.49	0.387
FR	1015	1016	2.65	0.3

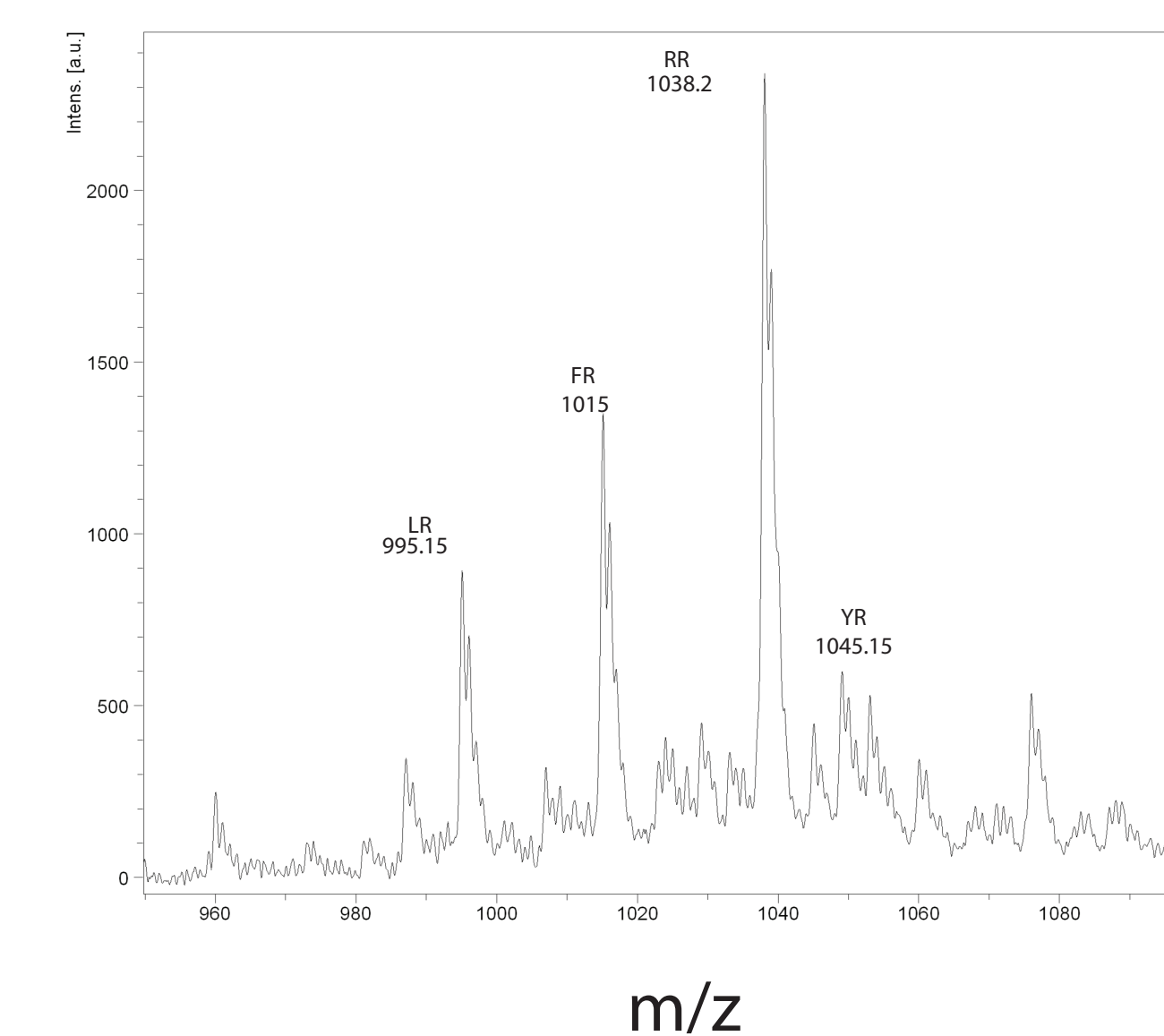
10 Conclusion

The data collected confirm that the LR congener of Microcystin is the most prevalent in Lake Menomin on the day this water was sampled.

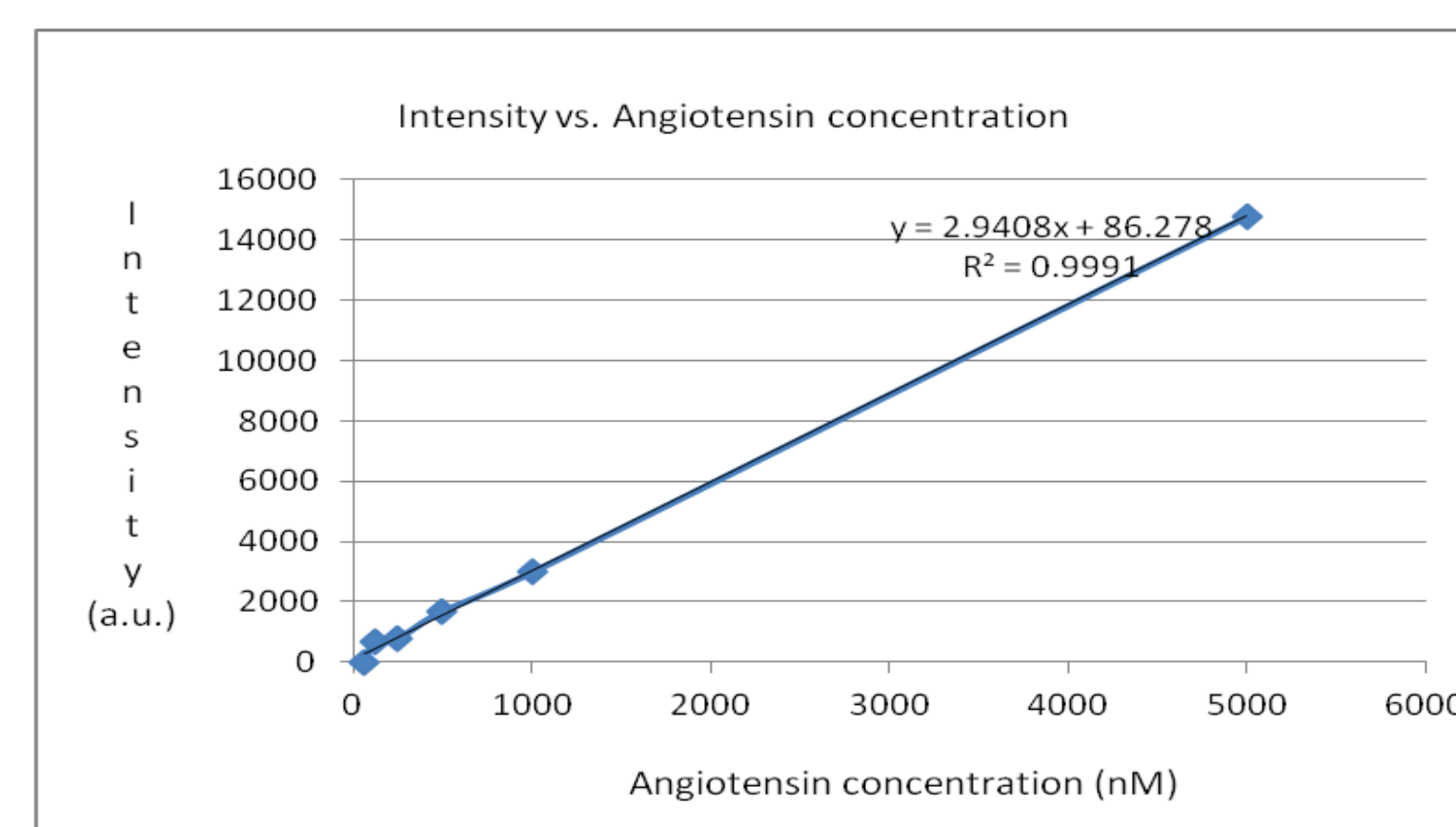
MALDI TOF MS provides a rapid, simple method for measuring the levels of microcystin congeners in lakewater.

The future goals of this project are to:
-Map seasonal variations in microcystin concentrations.
-Measure the ability of specific oxidizing agents to destroy microcystins.

7 Microcystins in Lake Menomin



8 Quantification of Microcystin Congeners



11 References

1. Li, L., Xu, J. (2009). First Identification of the Hepatotoxic Microcystins in the Serum of a Chronically Exposed Human Population Together with Indication of Hepatocellular Damage. *Toxicol Sci*, 108, 81-89
2. Jochimsen, E. M., Carmichael, W. W., An J. S., Cardo D.M., Cookson S. T., Holmes C. E. M., Antunes M. B. D., Demelo D. A., Lyra T. M., Barreto, V. S. T., Azevedo S. M. F. O., Jarvis, W. R. (1998). Liver failure and death after exposure to microcystin at a hemodialysis N. *Engl.J. Med.* 338, 873-878
3. Rinehart, K. L., M. Namikoshi, and B. W. Choi. (1994). Structure and biosynthesis of toxins from blue-green algae (cyanobacteria). *J. Appl. Phycol.* 159-176
4. Sivonen, K. (1996). Cyanobacterial toxins and toxin production. *Phycologia*, 35, 12-24.
5. Chorus I, Bartram J, ed. (1999) Toxic cyanobacteria in water. A guide to their public health consequences, monitoring and management. Published by E & FN Sponsered on behalf of the World Health Organization.
6. Kurmayer, R., and Christensen, G. (2009). The genetic basis of toxin production in Cyanobacteria. *Freshwater Reviews*, 2, 31-50
7. Hillenkamp, F., and Karas, M. (1991). Matrix-assisted laser desorption/ ionization mass spectrometry of biopolymers. *Anal Chem*, 63, 1193A-1203A
8. Via-Ordorika L, Fastner J, Kurmayer R, Hisbergues M, Dittmann E, Komarek J, Erhard M, Chorus I. (2004). Distribution of microcystin-producing and non-microcystin-producing Microcystis sp. in European freshwater bodies: detection of microcystins and genes in individual colonies. *Syst Appl Microbiol*, 27, 592-602
9. Howard, K.L., and Boyer, G.L. (2007). Quantitative analysis of cyanobacterial toxins by matrix-assisted laser desorption ionization mass spectrometry. *Anal Chem*, 79, 5980-5986
10. Gregson, B.P., Millie, D.F., and Cao, C., et al (2006). Simplified enrichment and identification of environmental peptide toxins using antibody-capture surfaces with subsequent mass spectrometry detection. *J Chromatogr A.*, 1123, 228-233

12 Acknowledgements

-We thank Dr. Scott McGovern for his contributions in lending his expertise in the field of water ecology.
-We thank the UW-Stout Biology Department for the use of it's facilities, equipment, and work environment.
-This project was supported by a UW-Stout Student Research Grant.