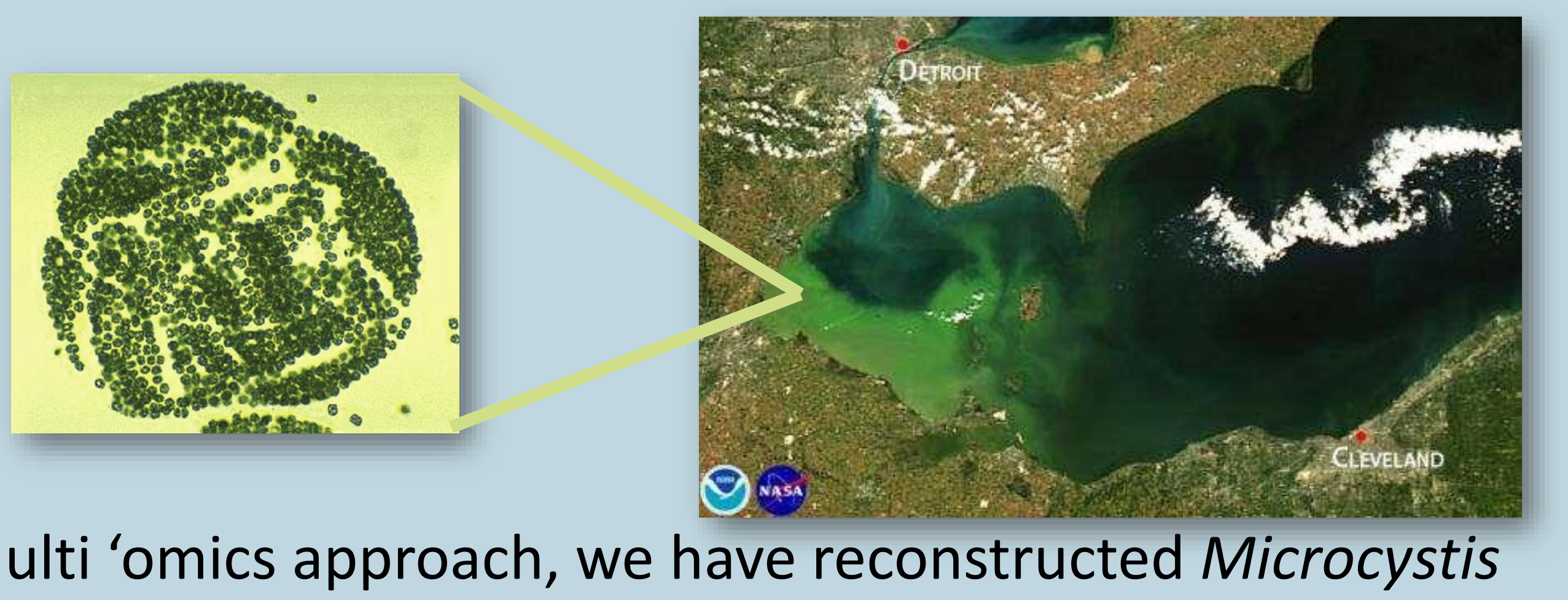
EXAMINING THE DIVERSE SUITE OF *MICROCYSTIS* NATURAL PRODUCTS IN WESTERN LAKE ERIE HARMFUL ALGAL BLOOMS

Colleen Yancey[1], Osama Mohamed[2,6], Fengan Yu[2,3,5], Ashootosh Tripathi[2,6], David Sherman[2,3,4,5,6], Greg Dick[1]
Earth and Environmental Sciences Department[1], Life Sciences Institute[2], Department of Medicinal Chemistry[3], Department of Microbiology and Immunology[4],
Department of Chemistry[5], Natural Products Discovery Core[6], University of Michigan, Ann Arbor MI 48109, United States
Contact: cyancey@umich.edu

OVERVIEW

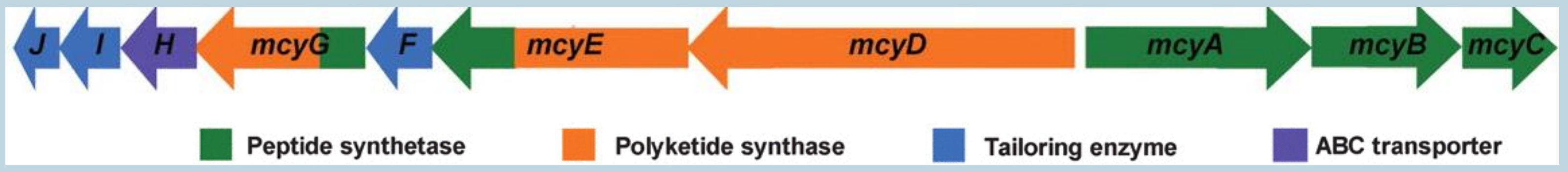
Cyanobacteria Harmful Algal Blooms (CHABs) are an annual occurrence in the Western Basin of Lake Erie. These globally ubiquitous blooms, which are dominated by the cyanobacterium *Microcystis aeruginosa* during peak phase, are responsible for the synthesis of toxic microcystin and other bioactive molecules, or natural products. While it is well understood that some of these natural products can have deleterious effects on human health, the majority are not well classified, and their function in the environment remains unclear.



Using a multi 'omics approach, we have reconstructed *Microcystis* Metagenome Assembled Genomes (MAGs) from the 2014 Lake Erie CHAB monitoring samples. *Mcy* gene structure and expression analysis as well as natural product mining was completed. Putatively identified novel natural product genes and compounds were identified and merit future study.

OBJECTIVES

- Characterize *mcy* gene **structure** and track **expression** throughout the bloom



Tooming-Klundred et al 2008

- Track *mcy* and natural product **spatial** and **temporal variation**
- Mine for **novel** natural products and putatively identify function

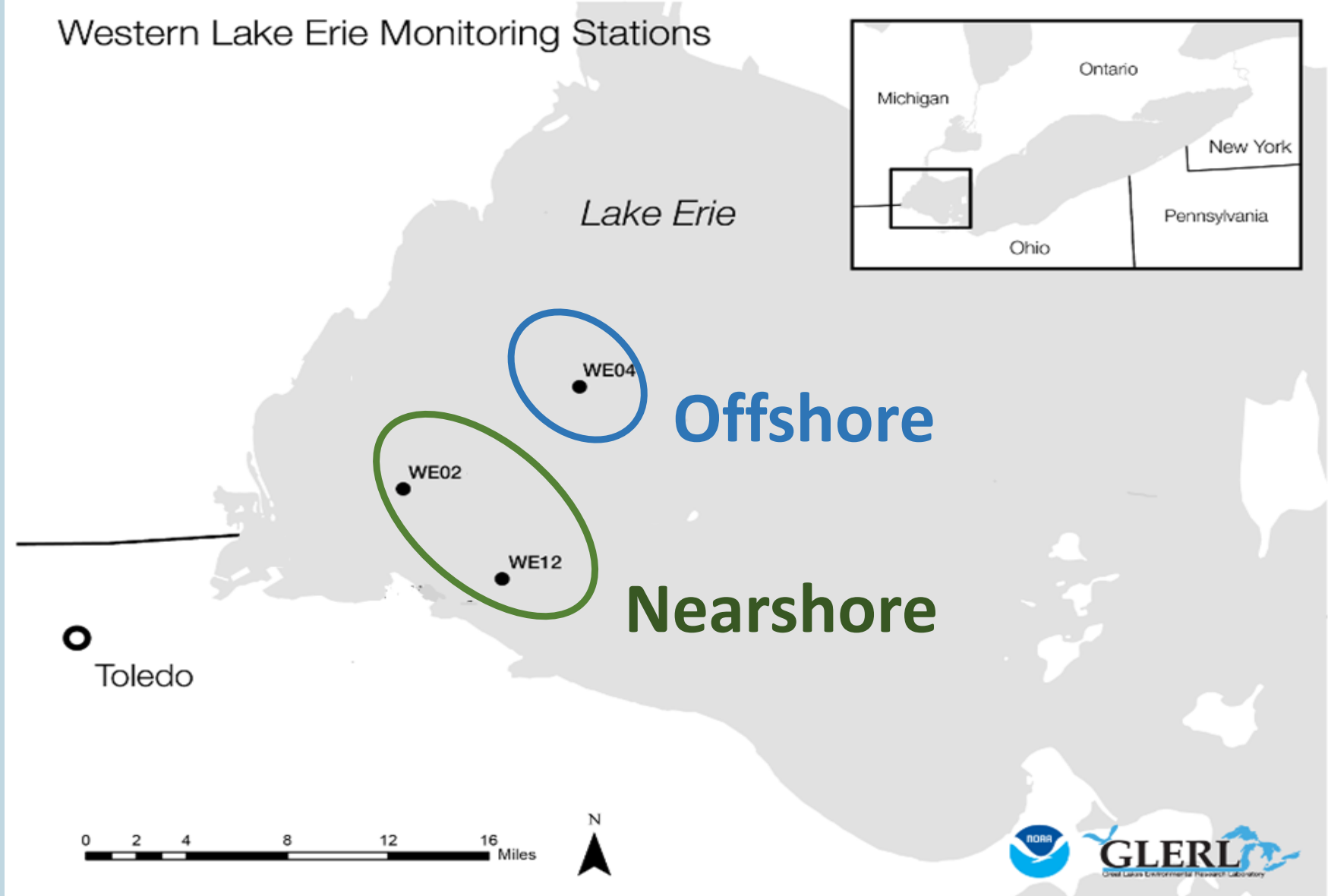
STUDY SITE

Location: Western Basin of Lake Erie

Stations: **WE2 + WE12 (Nearshore Site), WE4 (Offshore Site)**

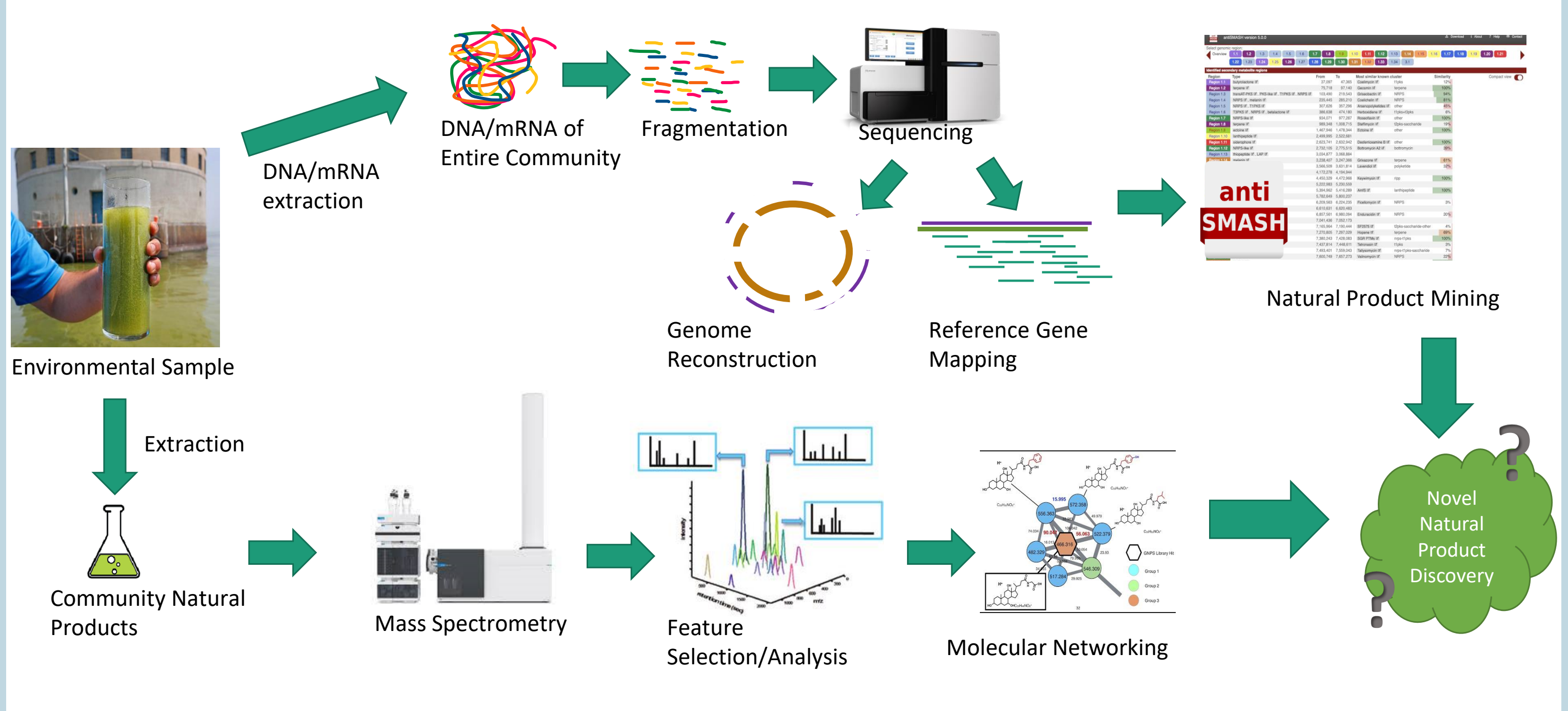
Sample Collection:

- Weekly Cruises **May-October 2014**
- Biomass collected on 100 µm filters for sequencing



Acknowledgements We thank the University of Michigan Omics Core for supporting the DNA sequencing, and the Cooperative Institute for Great Lakes Research (CIGLR) and NOAA Great Lakes Environmental Research Lab (NOAA-GLERL) for allowing us to take part in weekly sampling as a part of the HABs monitoring program and providing field support. We are also grateful to Derek Smith and Robert Hein for bioinformatics support.

METHODS



RESULTS

Table 1: Summary Statistics of Constructed *Microcystis* Metagenome Assembled Genomes (MAGs)

Sample ID	Station	Date	Completion (%)	Redundancy (%)	N50	Size (bp)	Number of Contigs	GC Content (%)
42896	WE12	Aug 4	40.29	2.16	2907	816 Kb	335	39.67
49629	WE12	Sep 29	93.53	10.23	2548	4.27 Mb	1888	43.4
49636	WE2	Oct 20	99.28	5.76	5472	4.69 Mb	1108	42.8
49637	WE12	Oct 20	96.4	12.95	4155	4.92 Mb	1461	42.83
53598	WE2	Jul 21	97.84	4.32	5000	4.27 Mb	1102	42.9
53599	WE4	Jul 29	92.81	20.86	1786	3.93 Mb	2234	43.53
53600	WE12	Aug 25	89.21	35.25	2353	5.99 Mb	2767	43.17
53601	WE4	Sep 8	94.96	9.67	2668	4.29 Mb	1797	43.49
53602	WE12	Sep 23	100	4.32	8601	5.31 Mb	903	42.79
53603	WE2	Oct 6	97.12	10.07	5061	5.39Mb	1404	43.28

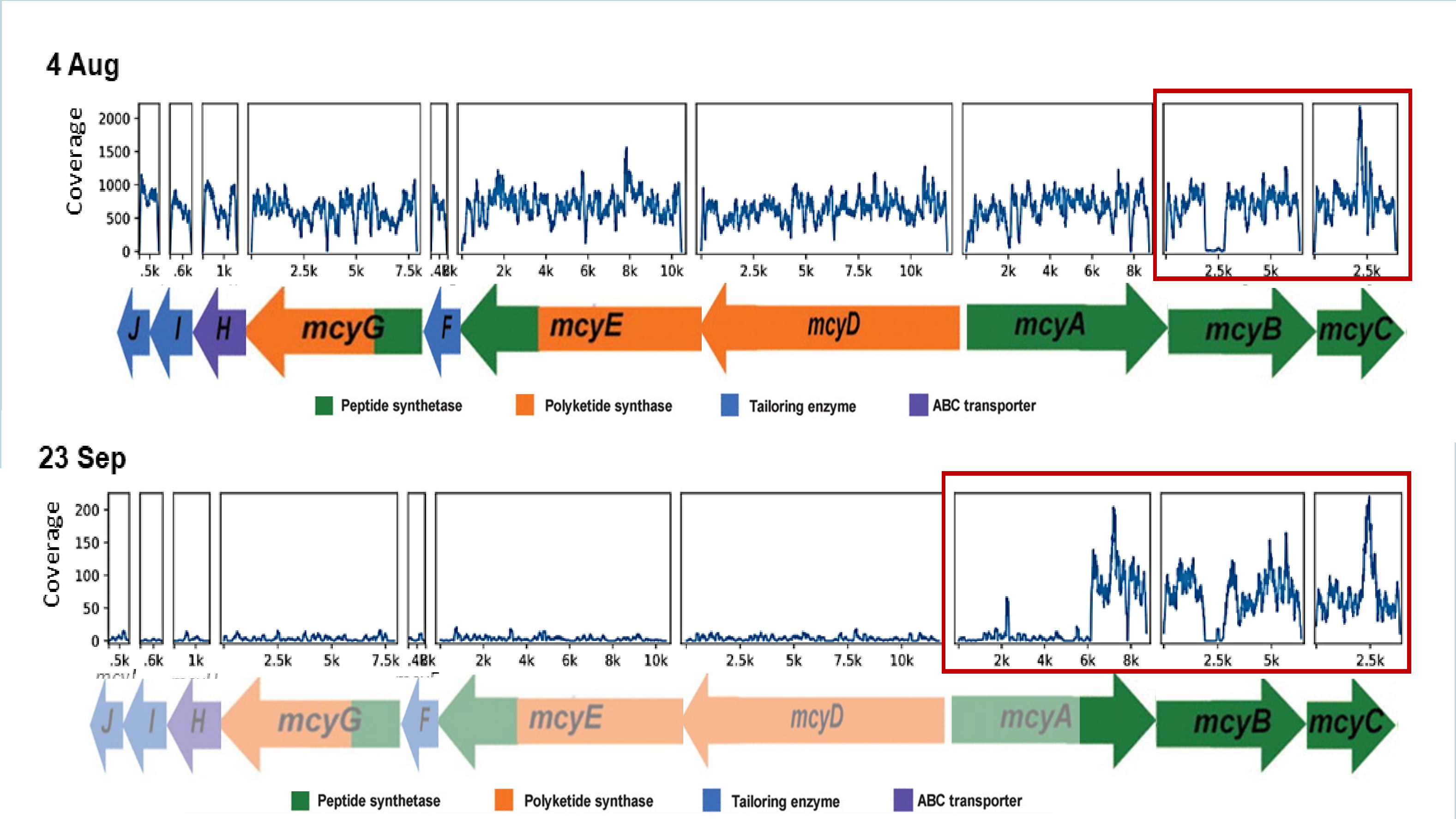


Figure 1 *Mcy* Operon Coverage as mapped to reference PCC 7806: Coverage patterns for representative for the peak phase of the bloom (4 Aug) and the late phase of the bloom (23 Sep). During both the peak and late phase there is an observed recombination between the *mcyB1* and *mcyC1* domain. During the late phase of the bloom, there is a dominant "non-toxic" genotype that contains a partial *mcy* operon.

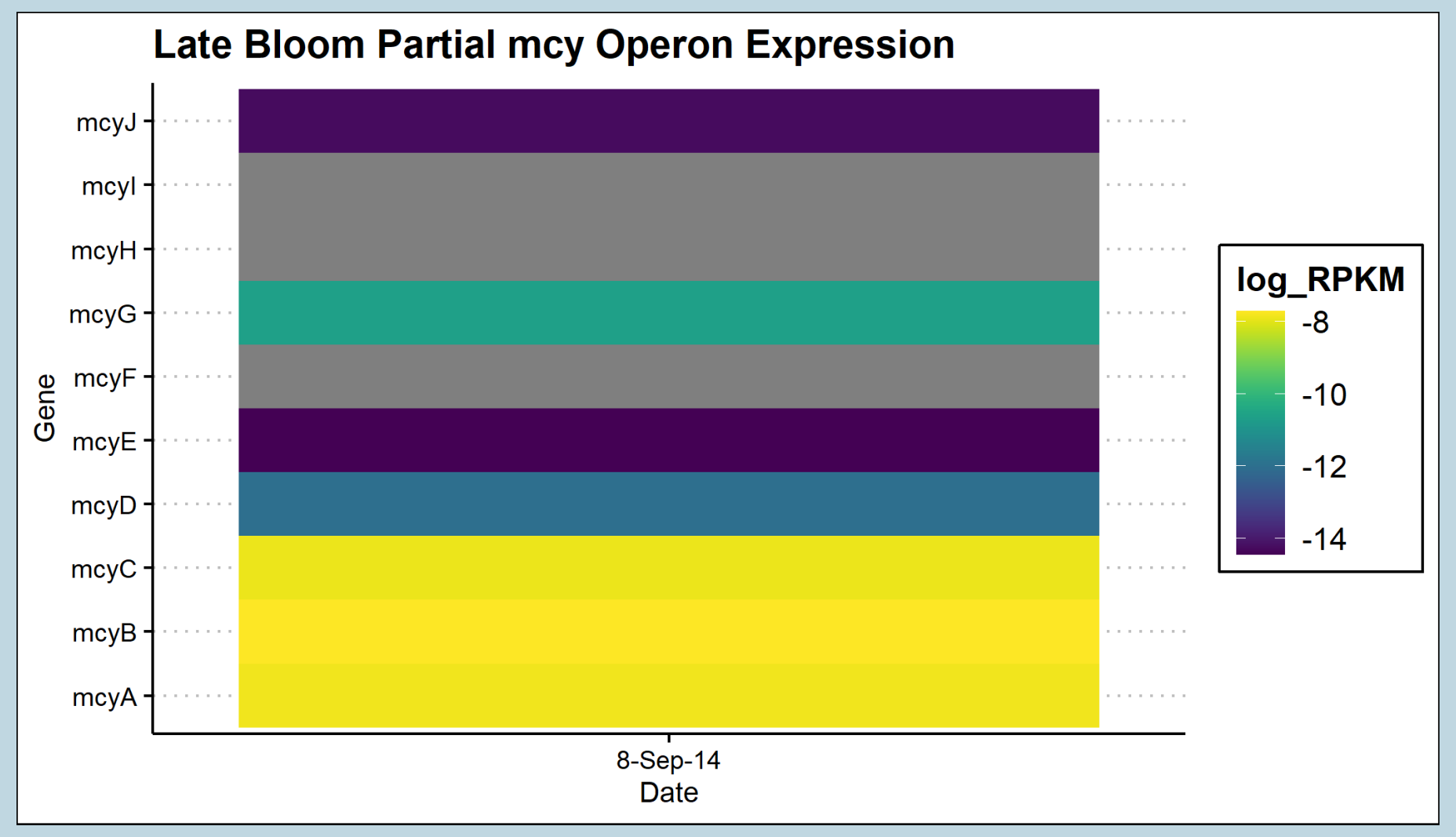


Figure 3 Partial *mcy* operons are expressed *in stiu:* Metatranscriptomic reads were mapped to the reference *mcy* operon from PCC 7806 and RPKM for each gene was calculated. Gray bars indicate an absence of any reads mapped to a particular gene Despite only possessing a partial operon during the late phase of the bloom, the *Microcystis* population appears to still be expressing *mcy* genes A-C which are present. Further work is needed to determined if a truncated microcystin product is being synthesized.

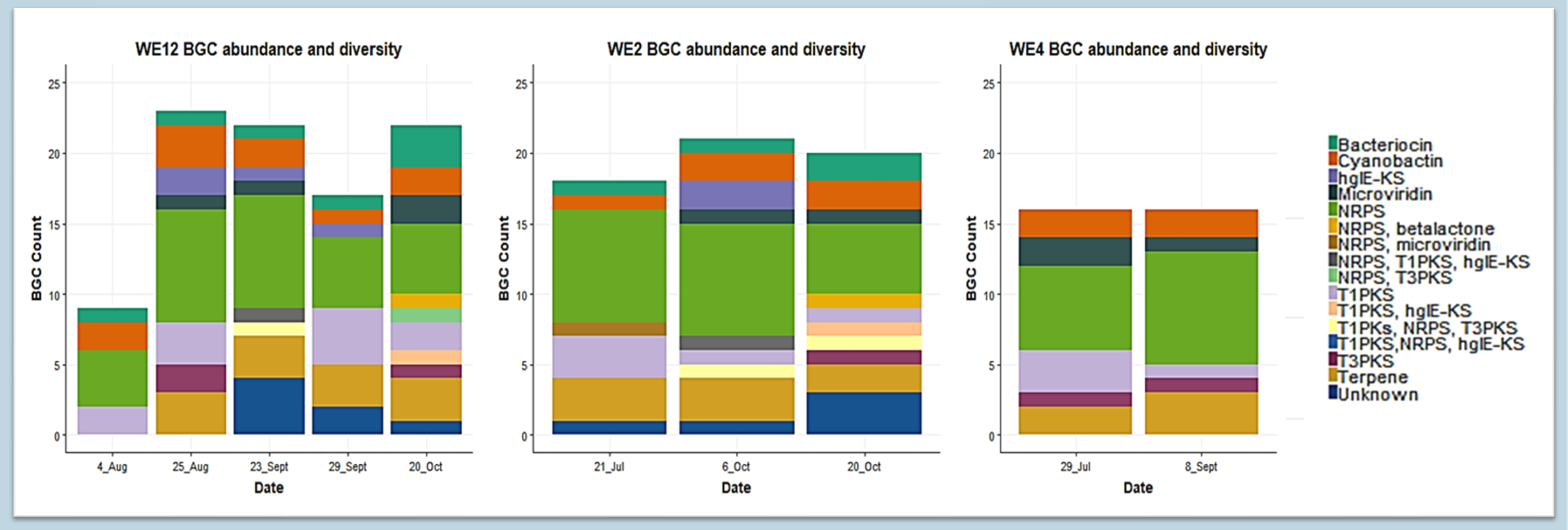


Figure 4: *Microcystis* MAG Biosynthetic Gene Cluster Content through time and space: Microcystis MAGs were run through AntiSMASH v.5.0 in order to annotate contigs for Biosynthetic Genes. The figure above illustrates that BGC content is relatively conserved throughout the bloom, but varies via location – particularly nearshore versus offshore. This likely reflects different strains of *Microcystis aeruginosa* observed as these stations (Berry et al. 2017 *Front. Microbiol.* 8: 365). Note: The sample collected from WE12 on Aug-4 only yielded a 40% complete MAG which likely explains low BGC content..

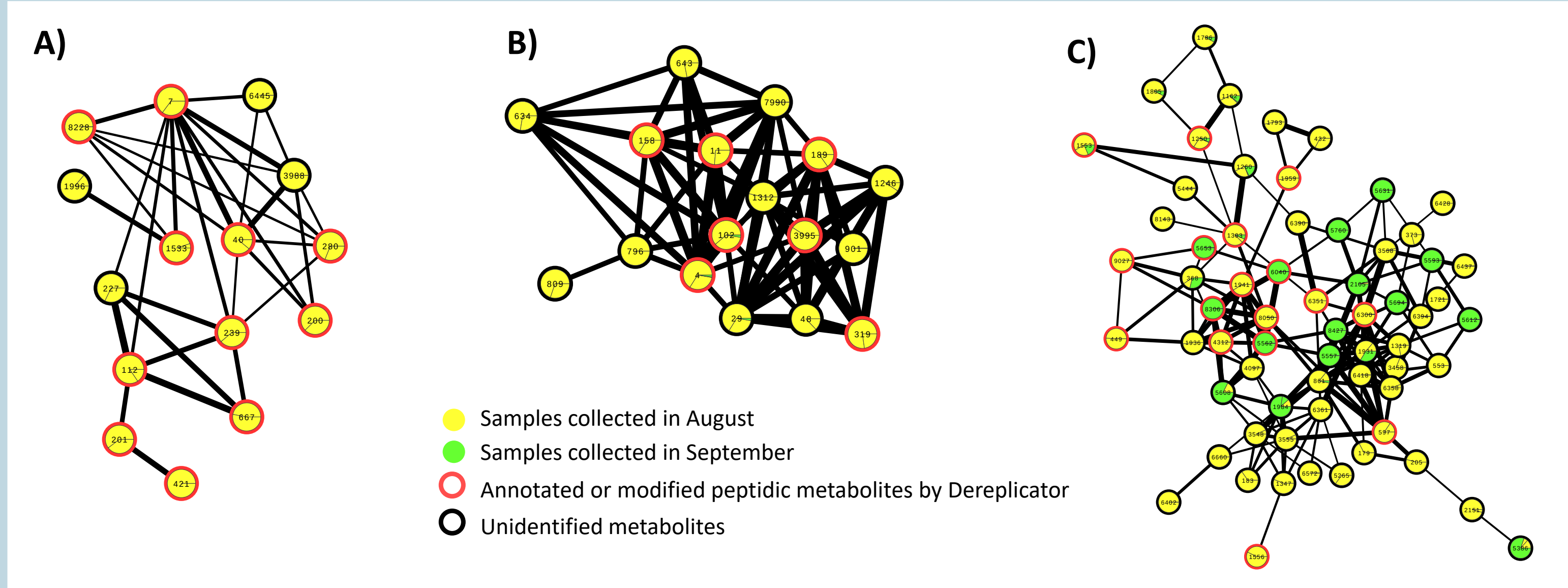


Figure 5: Molecular Networking of Chemical Compounds found in WLE HABs: Using GNPS, molecular networks were generated for peptidic compounds. Several novel or unidentified compounds were detected. A) Cluster of molecules that include microcystin and related compounds B) peptidic compound network for samples collected in August C) Network indicating distinct molecules between August and September s

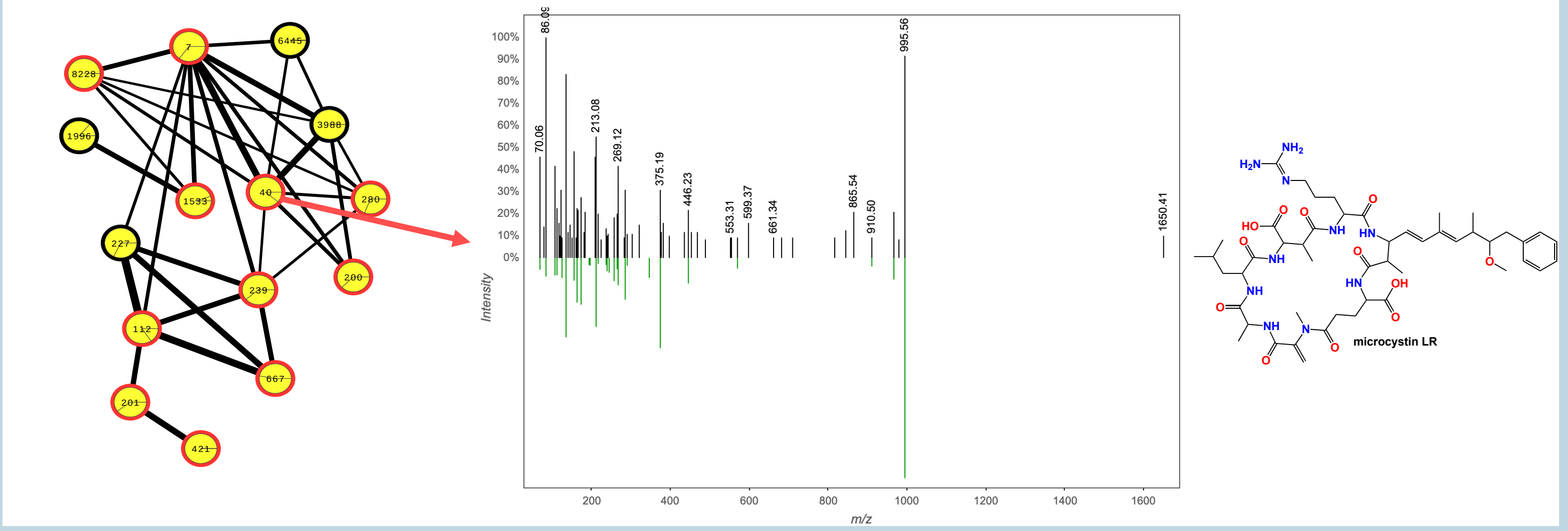


Figure 6: Spectral Data for Microcystin-L,R: Spectral analysis of Microcystin-L,R, the most common congener found in Western Lake Erie HABs.

CONCLUSION & FUTURE WORK

- ❖ *Microcystis* populations *mcy* operons in which the **B1** and **C1** domains have been **recombined** which produces microcystin-L,R and microcystin-R,R **Partial** *mcy* operons are still expressed, although future work is needed to determine **if truncated microcystin products** are being synthesized.
- ❖ W. Lake Erie *Microcystis* populations contain a **diverse range of BGCs and natural products**, some which are **novel**. **Linking** specific BGCs with specific natural product molecules will be critical future work

Funding: NIEHS-P01ES028939

Selected References

(1) Mikalsen, B. *et al.* Natural variation in the microcystin synthetase operon mcyABC and impact on microcystin production in Microcystis strains. *J. Bacteriol.* **185**, 2774–85 (2003)., (2)Steffen, M. M. *et al.* Ecophysiological Examination of the Lake Erie *Microcystis* Bloom in 2014: Linkages between Biology and the Water Supply Shutdown of Toledo, OH. *Environ. Sci. Technol.* **51**, 6745–6755 (2017)., (3) Tillett, D. *et al.* Structural organization of microcystin biosynthesis in Microcystis aeruginosa PCC7806: An integrated peptide-polyketide synthetase system. *Chem. Biol.* **7**, 753–764 (2000)., (4) Blin, K. *et al.* antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. *Nucleic Acids Res.* (2019). doi:10.1093/nar/gkz310, (5) Eren, A. M. *et al.* Anvi'o: an advanced analysis and visualization platform for 'omics data. *PeerJ* **3**, e1319 (2015). (6) Dittmann, E., Gugger, M., Sivonen, K. & Fewer, D. P. Natural Product Biosynthetic Diversity and Comparative Genomics of the Cyanobacteria. *Trends in Microbiology* **23**, 642–652 (2015).