

Diel metatranscriptomes capture cyanobacteria-dominated Lake Erie community response to episodic events

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ABSTRACT Here, we report on the raw and coassembled metatranscriptomes of 39 Lake Erie surface (1.0 m) water samples collected over a 2-day diel period encompassing episodic weather and bloom events. Preliminary taxonomic annotations and read mappings revealed that *Microcystis* spp. accounted for up to ~47% of the transcriptionally active community.

KEYWORDS harmful algal blooms, phytoplankton, diatoms, summer, climate change

Climate change has been discussed in the context of long-term shifts in precipitation (1), pCO₂/pH (2, 3), temperature (4, 5), and ice cover (6, 7) in the Laurentian Great Lakes. Yet, few studies address how phytoplankton respond to short-term episodic events (i.e., storms), which are coincidentally exacerbated by climate change (8, 9). Even fewer studies investigate how algae respond to diel fluctuations within the water column. To address these research gaps, we sampled the Lake Erie planktonic community from a geostatic location for 2 days—capturing diel variation and episodic weather events. This time series captured ephemeral cyanobacterial pulses of *Aphanizomenon* and *Microcystis* (Table 1) (Fig. 1). We present this dataset as a tool to investigate how planktonic communities respond to episodic events across a diel period.

Diel sampling spanned 18–20 July 2023 aboard the NOAA RV *Laurentian* on the Diel Assessment of Life in Lake Erie (DALLE) cruise. The vessel anchored in the western basin at NOAA station WE12: 83.2686°W, 41.7336°N (depth: 6.7 m). Water column sampling was performed using the ship's conductivity, temperature, and depth (CTD) profiler with deployments made at 2:00 a.m., 8:00 a.m., 2:00 p.m., 5:00 p.m., and 9:00 p.m. daily. Depth-discrete water column samples (1.0 m) were collected with Niskin bottles and immediately processed. Biomass was collected on 0.22 µm pore-size Sterivex filters (120–400 mL whole water), flash frozen, and stored at –80°C. RNA extractions were performed using an acid phenol chloroform protocol, followed by ethanol precipitation (10). Residual DNA was removed using the Turbo DNA-free Kit (Ambion). Samples were confirmed to be DNA-free via PCR amplification of 16S rRNA gene fragments (11). RNA quantity was determined via Qubit RNA HS Assay (Invitrogen). Sequencing was performed at the University of Michigan Advanced Genomics Core. rRNA was depleted (NEBNext rRNA Depletion Kit), and libraries (*n* = 39) were prepared with the NEBNext Ultra II Directional RNA Library Prep Kit). Subsequent 150 bp paired-end sequencing on an Illumina NovaSeqX 10B 300 cycle flow-cell generated ~30–60 million reads per sample.

Bioinformatic processing was performed on the UTK ISAAC-NG Cluster. Default parameters were used, except where otherwise noted. Reads were quality-assessed using FastQC (v.0.12.1) and filtered/trimmed with Trimmomatic (v.0.39) (12). Paired-end reads were interleaved with reformat.sh in the BBTools suite (v.38.86) (13), and residual rRNA and contaminants were removed using bbmap.sh (minid = 0.93). Libraries were

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TABLE 1 Metadata and sequencing statistics for metatranscriptomic libraries ($n = 39$) and the concatenated assembly (coassembly)^a

SRA	Library ID	Date sampled	Time sampled	Episodic event	#Mapped reads	%MIC_g	% APH_f
SRS21235944	LE_1	7/15/23	8:00	Severe storm last night, overcast	89440147	46.59	7.69
SRS21235945	LE_2	7/18/23	2:00	Clear sky, wind, no algal scum	10460348	12.90	1.97
SRS21235957	LE_3	7/18/23	2:00	Clear sky, wind, no algal scum	14378200	11.23	1.50
SRS21235968	LE_4	7/18/23	2:00	Clear sky, wind, no algal scum	16865374	14.69	2.56
SRS21235978	LE_5	7/18/23	8:00	Sunny, slight wind, no algal scum	14136909	12.72	1.97
SRS21235979	LE_6	7/18/23	8:00	Sunny, slight wind, no algal scum	93782783	27.78	5.49
SRS21235980	LE_7	7/18/23	8:00	Sunny, slight wind, no algal scum	22701386	10.49	1.37
SRS21235981	LE_8	7/18/23	14:00	Sunny, calm, <i>Microcystis</i> bloom	18590909	22.86	3.47
SRS21235982	LE_9	7/18/23	14:00	Sunny, calm, <i>Microcystis</i> bloom	25007192	20.60	5.06
SRS21235983	LE_10	7/18/23	14:00	Sunny, calm, <i>Microcystis</i> bloom	24825762	26.96	3.58
SRS21235946	LE_11	7/18/23	14:00	Sunny, calm, <i>Microcystis</i> bloom	12765970	16.31	2.18
SRS21235949	LE_12	7/18/23	17:00	Sunny, <i>Aphanizomenon</i> bloom	1921983	13.70	5.14
SRS21235948	LE_13	7/18/23	17:00	Sunny, <i>Aphanizomenon</i> bloom	18269401	15.11	4.66
SRS21235950	LE_14	7/18/23	17:00	Sunny, <i>Aphanizomenon</i> bloom	19263607	12.06	5.15
SRS21235951	LE_15	7/18/23	17:00	Sunny, <i>Aphanizomenon</i> bloom	25257130	14.77	4.46
SRS21235952	LE_16	7/18/23	21:00	Clear, <i>Aphanizomenon</i> bloom	16314070	12.50	3.35
SRS21235953	LE_17	7/18/23	21:00	Clear, <i>Aphanizomenon</i> bloom	18266544	13.02	3.71
SRS21235954	LE_18	7/18/23	21:00	Clear, <i>Aphanizomenon</i> bloom	17632471	13.64	4.13
SRS21235956	LE_19	7/18/23	21:00	Clear, <i>Aphanizomenon</i> bloom	13886618	9.97	2.40
SRS21235955	LE_20	7/19/23	2:00	Calm, no wind, Mayfly swarm	43653450	9.51	1.86
SRS21235958	LE_21	7/19/23	2:00	Calm, no wind, Mayfly swarm	16067801	12.17	1.35
SRS21235959	LE_22	7/19/23	2:00	Calm, no wind, Mayfly swarm	15957545	9.08	1.81
SRS21235961	LE_23	7/19/23	8:00	Sunny, calm, <i>Aphanizomenon</i>	27871283	10.76	0.95
SRS21235960	LE_24	7/19/23	8:00	Sunny, calm, <i>Aphanizomenon</i>	17213578	11.74	1.51
SRS21235962	LE_25	7/19/23	8:00	Sunny, calm, <i>Aphanizomenon</i>	14977200	10.60	1.26
SRS21235963	LE_26	7/19/23	8:00	Sunny, calm, <i>Aphanizomenon</i>	16253880	13.00	1.17
SRS21235964	LE_27	7/19/23	14:00	Sunny, calm, <i>Microcystis</i>	3934239	17.63	2.21
SRS21235967	LE_28	7/19/23	14:00	Sunny, calm, <i>Microcystis</i>	33413600	15.50	1.67
SRS21235965	LE_29	7/19/23	14:00	Sunny, calm, <i>Microcystis</i>	16059662	14.38	1.50
SRS21235966	LE_30	7/19/23	17:00	Sunny, slight wind, <i>Microcystis</i>	8283383	15.88	1.47
SRS21235970	LE_31	7/19/23	17:00	Sunny, slight wind, <i>Microcystis</i>	55906891	17.07	0.79
SRS21235969	LE_32	7/19/23	17:00	Sunny, slight wind, <i>Microcystis</i>	19094231	21.55	1.03
SRS21235972	LE_33	7/19/23	17:00	Sunny, slight wind, <i>Microcystis</i>	20139429	17.90	0.88
SRS21235971	LE_34	7/19/23	21:00	Wind picking up, <i>Microcystis</i>	15727918	12.08	1.33
SRS21235974	LE_35	7/19/23	21:00	Wind picking up, <i>Microcystis</i>	21965755	14.03	0.69
SRS21235973	LE_36	7/19/23	21:00	Wind picking up, <i>Microcystis</i>	17635988	14.29	0.95
SRS21235975	LE_37	7/20/23	2:00	Cloudy, windy, waves	16601697	14.21	1.23
SRS21235977	LE_38	7/20/23	2:00	Cloudy, windy, waves	14406948	14.47	0.85
SRS21235976	LE_39	7/20/23	2:00	Cloudy, windy, waves	17709918	9.36	1.04
Coassembly ID				N50/N90 contig length	Length (bp)	#Contigs	GC (%)
LE_2023_diel_coassembly.fa				N50 = 1066/N90 = 565	1,067,995,868	1049738	51.55

^aEach library was mapped to the coassembly with the total number of mapped reads reported (#Mapped reads). The percent of mapped reads to *Microcystis* genera (%MIC_g) and Aphanizomenonaceae family (%APH_f) was reported. Biological replication ($n = 3$ or 4) was performed for each sample save for the out-group sample (LE_1).

concatenated and assembled using MEGAHIT (v.1.2.9) (14). Statistical assessment of coassembly quality was performed via QUAST (v.5.2.0) (15) (Table 1).

Preliminary mappings of libraries to the coassembly via bbmap.sh indicated that *Microcystis* spp. genera comprised $\leq 47\%$ of the transcriptionally active community (taxonomic annotations via Kraken2) (v.2.1.3) (16) (Table 1). The Aphanizomenonaceae family (containing *Aphanizomenon* and *Dolichospermum* genera, which are taxonomically unresolved) comprised $\leq 8\%$ (APH). Blastp of APH proteins against the NCBI nr database identified 4.3% as *Aphanizomenon*, 21% as *Dolichospermum*, and 74.7% as cyanobacterial/environmental. Considering that climate change is exacerbating both

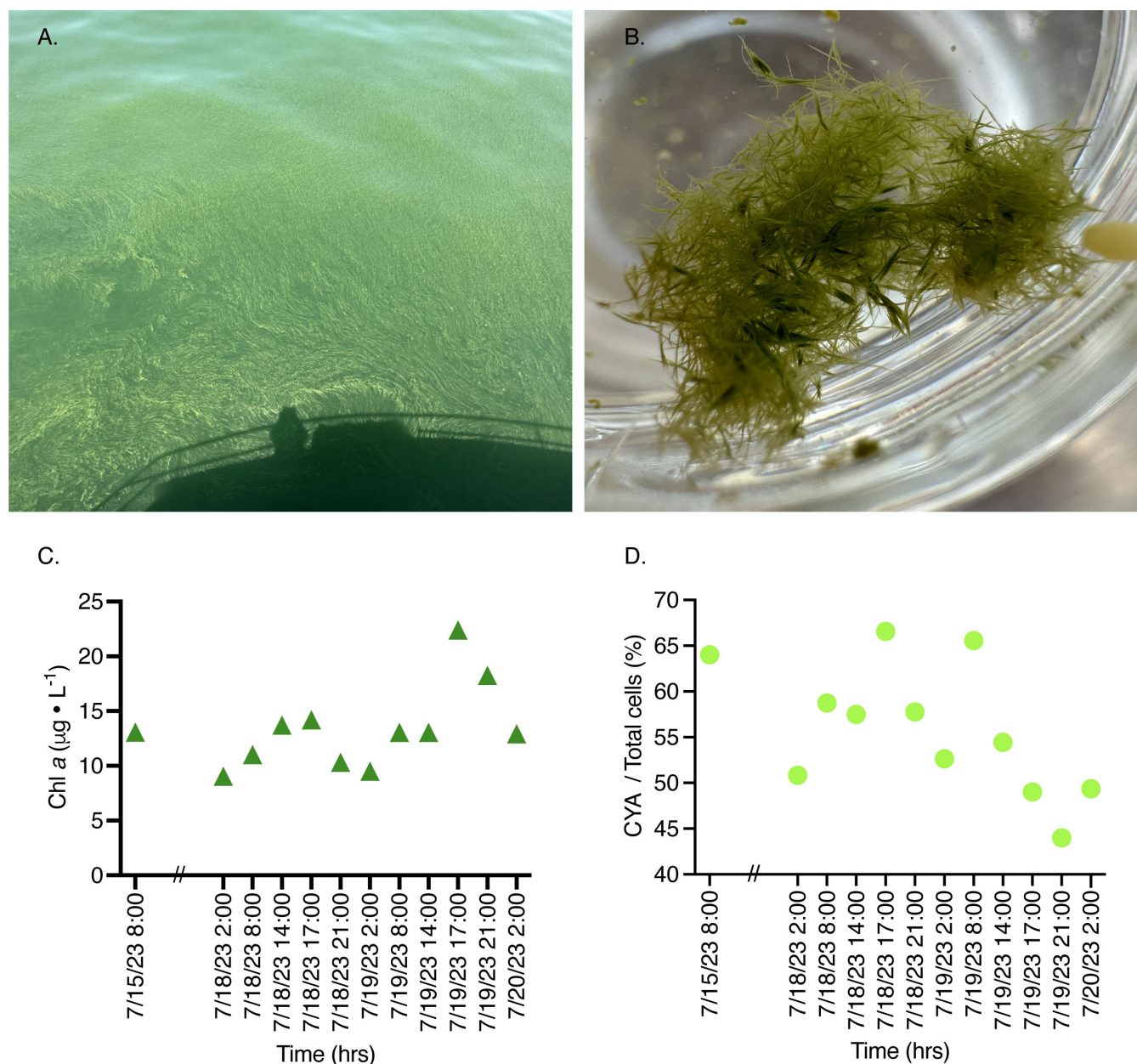


FIG 1 Cyanobacterial pulse conditions coinciding with diel sampling conducted during the cruise. (A) Cyanobacterial surface scum (largely comprised of *Microcystis* spp.) at 12:45 p.m. on 18 July 2023. Photo credit: Brittany N. Zepernick. (B) *Aphanizomenon* spp. colony collected at 7:15 p.m. on 18 July 2023. Photo credit: Brittany N. Zepernick. (C) Chlorophyll *a* ($\mu\text{g} \cdot \text{L}^{-1}$) in 1.0 m-depth whole water samples throughout the sampling period. (D) Proportion of cyanobacterial cell counts (CYA: cells mL^{-1}) compared to total Chl *a* autofluorescing cell counts (cells mL^{-1}) in 1.0 m-depth whole water samples throughout the sampling period. Data presented in panels C and D were analyzed *in vivo* via PhycoLabAnalyser (bbe-moldaenke GmbH).

cyanobacterial blooms (17, 18) and episodic events (8, 19), this data set serves as a resource to investigate how cyanobacterial communities will respond to a climatically altered future.

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DATA AVAILABILITY

Raw sequences are available on the NCBI SRA under [BioProject PRJNA1105990](#) and BioSamples [SAMN41114708-41114746](#). The concatenated assembly (coassembly) is available on the lead author's [Zenodo](#) (20). Physiochemical data are available on the NOAA National Centers for Environmental Information ([NCEI](#)) database.

REFERENCES

- McBean E, Motiee H. 2008. Assessment of impact of climate change on water resources: a long term analysis of the Great Lakes of North America. *Hydrol Earth Syst Sci* 12:239–255. <https://doi.org/10.5194/hess-12-239-2008>
- Phillips JC, McKinley GA, Bennington V, Bootsma HA, Pilcher DJ, Sterner RW, Urban NR. 2015. The potential for CO₂-induced acidification in freshwater: a Great Lakes case study. *Oceanography* 28:136–145. <https://doi.org/10.5670/oceanog.2015.37>
- Rowe MD, Errera RM, Rutherford ES, Elgin AK, Pilcher DJ, Day J, Guo T. 2020. Great Lakes Region acidification research
- Dobiesz NE, Lester NP. 2009. Changes in mid-summer water temperature and clarity across the Great Lakes between 1968 and 2002. *J Great Lakes Res* 35:371–384. <https://doi.org/10.1016/j.jglr.2009.05.002>
- Mason LA, Riseng CM, Gronewold AD, Rutherford ES, Wang J, Clites A, Smith SDP, McIntyre PB, Smith SD. 2016. Fine-scale spatial variation in ice cover and surface temperature trends across the surface of the Laurentian Great Lakes. *Clim Change* 138:71–83. <https://doi.org/10.1007/s10584-016-1721-2>
- Wang J, Kessler J, Hang F, Hu H, Clites A, Chu P. 2017. Great Lakes ice climatology update of winters 2012–2017: seasonal cycle, interannual variability, decadal variability, and trend for the period 1973–2017, in 2017: NOAA technical memorandum
- Zepernick BN, Chase EE, Denison ER, Gilbert NE, Truchon AR, Frenken T, Cody WR, Martin RM, Chaffin JD, Bullerjahn GS, McKay RML, Wilhelm SW. 2024. Declines in ice cover are accompanied by light limitation responses and community change in freshwater diatoms. *ISME J* 18:wrad015. <https://doi.org/10.1093/ismejo/wrad015>
- Rodell M, Li B. 2023. Changing intensity of hydroclimatic extreme events revealed by GRACE and GRACE-FO. *Nat Water* 1:241–248. <https://doi.org/10.1038/s44221-023-00040-5>
- Rhodes J, Hetzenauer H, Frassl MA, Rothhaupt K-O, Rinke K. 2017. Long-term development of hypolimnetic oxygen depletion rates in the large Lake Constance. *Ambio* 46:554–565. <https://doi.org/10.1007/s13280-017-0896-8>
- Martin RM, Wilhelm SW. 2020. Phenol-based RNA extraction from polycarbonate filters. *protocio*. <https://doi.org/10.17504/protocols.io.bivuke6w>
- Zepernick BN, Denison ER, Chaffin JD, Bullerjahn GS, Pennacchio CP, Frenken T, Peck DH, Anderson JT, Niles D, Zastepa A, McKay RML, Wilhelm SW. 2022. Metatranscriptomic sequencing of winter and spring planktonic communities from Lake Erie, a Laurentian Great Lake. *Microbiol Resour Announc* 11:e0035122. <https://doi.org/10.1128/mra.00351-22>
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>
- Bushnell B. 2014. BBTools software package. Available from: <http://sourceforge.net/projects/bbmap>
- Li D, Liu C-M, Luo R, Sadakane K, Lam T-W. 2015. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics* 31:1674–1676. <https://doi.org/10.1093/bioinformatics/btv033>
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>

16. Wood DE, Lu J, Langmead B. 2019. Improved metagenomic analysis with Kraken 2. *Genome Biol* 20:257. <https://doi.org/10.1186/s13059-019-1891-0>
17. Zepernick BN, Wilhelm SW, Bullerjahn GS, Paerl HW. 2023. Climate change and the aquatic continuum: a cyanobacterial comeback story. *Environ Microbiol Rep* 15:3–12. <https://doi.org/10.1111/1758-2229.13122>
18. Wells ML, Karlson B, Wulff A, Kudela R, Trick C, Asnaghi V, Berdalet E, Cochlan W, Davidson K, De Rijcke M, Dutkiewicz S, Hallegraeff G, Flynn KJ, Legrand C, Paerl H, Silke J, Suikkanen S, Thompson P, Trainer VL. 2020. Future HAB science: directions and challenges in a changing climate. *Harmful Algae* 91:101632. <https://doi.org/10.1016/j.hal.2019.101632>
19. Rohde MM. 2023. Floods and droughts are intensifying globally. *Nat Water* 1:226–227. <https://doi.org/10.1038/s44221-023-00047-y>
20. Zepernick BN. 2024. Lake Erie diel coassembly (metatranscriptomic). Zenodo.