Nutrient-Controlled Niche Differentiation of Western Lake Erie Cyanobacterial Populations Revealed via Metatranscriptomic Surveys

Matthew J. Harke‡, Timothy W. Davis§, Susan B. Watson§, and Christopher J. Gobler*†

†School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, New York 11794, United States
‡NOAA Great Lakes Environmental Research Laboratory, 4840 S. State Road, Ann Arbor, Michigan 48108, United States
§Canadian Centre for Inland Waters, Environment Canada, Burlington, Ontario L7R 4A6, Canada

ABSTRACT: Although toxic cyanobacterial blooms in western Lake Erie threaten drinking water supplies and are promoted by nutrient loading, the precise nutrient regime that selects specific cyanobacteria populations is poorly understood. Here, we assess shifts in cyanobacterial abundances and global gene-expression patterns in response to natural and manipulated gradients in nitrogen and phosphorus to identify gene pathways that facilitate dominance by different cyanobacteria. Gradients in soluble reactive phosphorus shaped cyanobacterial communities and elicited the largest transcriptomic responses. Under high-P conditions (closest to the mouth of the Maumee River), *Anabaena* and *Planktothrix* were the dominant cyanobacterial populations, and experimental P and ammonium enrichment promoted nitrogen fixation gene (*nifH*) expression in *Anabaena*. For *Microcystis*, experimental additions of P up-regulated genes involved in phage defense, genomic rearrangement, and nutrient acquisition but led to lower abundances. Within offshore, low-P regions of the western basin of Lake Erie, *Microcystis* up-regulated genes associated with P scavenging (*psSCAB, phoX*) and dominated cyanobacterial communities. Experimental additions of ammonium and urea did not alter *Microcystis* abundances but did up-regulate protease inhibitors (*aer* and *mcn* gene sets) and microcystin synthetase genes (*mcy*), with urea enrichment yielding significant increases in microcystin concentrations. Our findings suggest that management plans that reduce P loads alone may not significantly reduce the risk of cyanobacterial blooms in western Lake Erie but rather may promote a shift among cyanobacterial populations (*Microcystis, Anabaena*, and *Planktothrix*) toward a greater dominance by toxic strains of *Microcystis*. The biochemical pathways potentially important for facilitating shifts in cyanobacteria taxa during blooms remain poorly understood.

Molecular studies of *Microcystis* have resolved multiple aspects of this cyanobacterium’s ecology. Genetic studies of *Microcystis* populations in Lake Erie have documented strain diversity, microcystin dynamics, and genetic connectivity among toxic strains. Metagenomic comparisons between cyanobacterial populations in Lake Erie and other large lakes have revealed functional differences between communities. Transcriptional analyses of *Microcystis* cultures have identified biochemical pathways up-regulated under N and P limitation and the relationship between exogenous N-supply and microcystin synthetase, whereby N-deprived *Microcystis* cells display reduced transcription of microcystin synthetase genes.

Received: August 13, 2015
Revised: December 10, 2015
Accepted: December 10, 2015
Published: December 10, 2015

DOI: 10.1021/acs.est.5b03931

© 2015 American Chemical Society
and decreased microcystin content as compared to replete N cells.\textsuperscript{18} Transcriptomic studies of Microcystis have also explored the diurnal regulation of gene expression\textsuperscript{19} and the role of nutrients in influencing transposase transcription.\textsuperscript{20} Few studies have explored the transcriptomic responses of Microcystis in an ecosystem setting, and none have considered how altered nutrient regimes affect its dominance among cyanobacterial populations.

Here we present an in situ evaluation of the transcriptomic responses of Microcystis and other potentially toxic cyanobacteria to natural and manipulated nutrient gradients in western Lake Erie (Figure 1). Cyanobacteria-dominated plankton assemblages in regions close to and away from the primary nutrient source (i.e., the Maumee River) were investigated, and incubation experiments were conducted to evaluate responses to N and P enrichment. Concurrent evaluation of cyanobacterial abundances and differential gene expression via whole-transcriptome sequencing permitted the identification of gene pathways important for facilitating shifting dominance among genera.

\section*{MATERIALS AND METHODS}

During August of 2013, MODIS satellite images depicted the development of a large bloom of cyanobacteria in the western basin of Lake Erie, extending from the mouth of the Maumee River to the Bass Islands (Figure S1). This bloom persisted through October. On September 12th of 2013, a mid-day sampling transect was conducted through the bloom aboard the R/V Erie Monitor (The Ohio State University) from the mouth of the Maumee River to the Lake Erie Islands that separate the western and central basins of the lake (Figure 1). The stations visited during each transect represented a nutrient gradient in which both N and P typically decline with distance from the mouth of the Maumee River (high-N and -P region). At each station (n = 6), subsurface samples (~0.25 m) were collected to assess phytoplankton abundance and diversity using a fluoroprobe (BBE Moldenke) to differentiate cyanobacteria from other phytoplankton groups on the basis of photosynthetic accessory pigments.\textsuperscript{21} Dissolved (filtered through a combusted EMD Millipore APFB glass-fiber filter) and total nutrient samples were collected, together with water for analysis of photosynthetic efficiency (\(F_v/F_m\)), total microcystins, alkaline phosphatase activity (APA), and plankton community composition (preserved with Lugol’s iodine solution; see Sample Analyses section below). At a station with elevated cyanobacterial biomass (as indicated via the fluoroprobe), 1 L of water was filtered on 20 \(\mu\)m polycarbonate filters for transcriptomic analysis of colonial cyanobacteria, immediately immersed in liquid N, and stored at \(-80^\circ\) C. An additional 60 L of water was collected for a nutrient amendment experiment.

On October 8th of 2013, as part of the Lake Erie harmful algal bloom monitoring program conducted by Environment Canada aboard the Canadian Coast Guard ship Limnos, seven...
Table 1. Number of Differentially Expressed Genes in Each Category Up-Regulated (↑) and Down-Regulated (↓) for Planktothrix agardhii NIVA-CYA 15 and Anabaena sp. PCC7108 Relative to the Reference Station (LET7) for LET6 through LET1 or the Control (for +P, +NH4, and +Urea).a

<table>
<thead>
<tr>
<th>Planktothrix agardhii NIVA-CYA 15</th>
<th>LET6</th>
<th>LET5</th>
<th>LET4</th>
<th>LET3</th>
<th>LET2</th>
<th>LET1</th>
<th>+P</th>
<th>+NH4</th>
<th>+urea</th>
</tr>
</thead>
<tbody>
<tr>
<td>long-chain fatty acid CoA ligase</td>
<td>↑</td>
<td>↑</td>
<td>7↑</td>
<td>7↓</td>
<td>9↓</td>
<td>8↓</td>
<td>1↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypothetical protein</td>
<td>3↑</td>
<td>7↓</td>
<td>2↑</td>
<td>21↓</td>
<td>1875↓</td>
<td>1083↓</td>
<td>207</td>
<td>470↓</td>
<td>798↓</td>
</tr>
<tr>
<td>photosynthesis</td>
<td>15↓</td>
<td>3↓</td>
<td>2↓</td>
<td>55↓</td>
<td>55↓</td>
<td>49↓</td>
<td>6↓</td>
<td>3↓</td>
<td>2↓</td>
</tr>
<tr>
<td>alkaline phosphatase</td>
<td>↓</td>
<td>15↓</td>
<td>1↓</td>
<td>16↓</td>
<td>1↓</td>
<td>15↓</td>
<td></td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>transporters</td>
<td>↑</td>
<td>3↑</td>
<td>3↓</td>
<td>83↓</td>
<td>3↓</td>
<td>43↓</td>
<td>3↓</td>
<td>77↓</td>
<td></td>
</tr>
<tr>
<td>mobile elements</td>
<td>↑</td>
<td>4↑</td>
<td>3↓</td>
<td>19↓</td>
<td>31↓</td>
<td>24↓</td>
<td>2↓</td>
<td>13↓</td>
<td>17↓</td>
</tr>
<tr>
<td>cell division</td>
<td>20↓</td>
<td>20↓</td>
<td>13↓</td>
<td>19↓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anabaena sp. PCC7108</th>
<th>LET6</th>
<th>LET5</th>
<th>LET4</th>
<th>LET3</th>
<th>LET2</th>
<th>LET1</th>
<th>+P</th>
<th>+NH4</th>
<th>+urea</th>
</tr>
</thead>
<tbody>
<tr>
<td>nitrogenase</td>
<td>2↑</td>
<td>1↑</td>
<td></td>
<td>4↑</td>
<td>5↑</td>
<td>2↑</td>
<td>2↑</td>
<td></td>
<td>1↓</td>
</tr>
<tr>
<td>photosystem II</td>
<td>3↑</td>
<td>5↑</td>
<td>4↑</td>
<td>2↓</td>
<td>4↑</td>
<td>1↓</td>
<td>2↑</td>
<td>1↓</td>
<td></td>
</tr>
<tr>
<td>phycobilisome</td>
<td>3↓</td>
<td>3↓</td>
<td>4↑</td>
<td>5↓</td>
<td>6↓</td>
<td>7↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>transposase</td>
<td>↑</td>
<td>1↑</td>
<td>2↑</td>
<td></td>
<td>2↑</td>
<td>1↑</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aFunctions were binned on the basis of keyword searches in the PATRIC database.

stations were sampled at mid-day across the western basin of Lake Erie (Figure 1). At each station, samples were collected for the analysis of dissolved and total nutrients (see above), total microcysts, and plankton communities (see the Sample Analyses section below). Furthermore, at each site, ~1 L of surface water was filtered through replicate Millipore 0.22 μm Sterivex filters for transcriptomic analysis; filters were sealed, immediately immersed in liquid N, and stored at −80°C. Although these filters had a smaller pore size than our experimental samples (see below), our information analyses of transcriptome samples specifically targeted three populations of colonial cyanobacteria (>20 μm): Microcystis, Planktothrix, and Anabaena. Further, transcriptomes collected on different filter types were not compared to each other but rather to other transcriptomes collected at the same time using the same filter type.

Effect of Nutrients on Cyanobacteria. To further assess how nutrients affect the growth and physiology of algal communities in Lake Erie, we filled duplicate 1.1 L bottles (n = 8 for each experimental set) with water collected at station L16 (see above) and were left unamended to serve as a control or amended with nitrogen (2.5 μM urea (5 μM N) or 5 μM ammonium) or inorganic phosphorus (0.3 μM KH2PO4) at the start of the experiment and again after 24 h. Concentrations were chosen to be slightly above background during summer in the western basin of Lake Erie (<2-fold above ambient; Table 1), while the daily delivery was representative of nutrient pulses that can emanate from point sources in the region such as the Maumee River. Bottles were placed in a wire submersible rack at 0.25 m in Fishery Bay near the Franz Theodore Stone Laboratory (The Ohio State University) to provide ambient temperature and light incubation conditions. After 48 h, at mid-day, bottles were processed for the analysis of in vivo chlorophyll a, the phycoplancton abundance was measured with a fluoroprobe, and bulk APA, Fv/Fm, and dissolved and total nutrients (see Sample Analyses section below) were measured. Samples for total microcystin analysis were collected and frozen at −20°C (see Sample Analyses section below). The remaining volume was then filtered onto 20 μm polycarbonate filters and immediately flash-frozen in liquid N for the transcriptomic analysis of colonial cyanobacteria.

Sample Analyses. Nitrate was analyzed by reducing the nitrate to nitrite using spongy cadmium as per Jones. Ammonium and phosphate were analyzed using techniques from Parsons et al. Total dissolved N and P were analyzed using persulfate digestion techniques from Valderrama. These nutrient analyses provided 100 ± 10% recovery of standard reference material (SPEx CertiPrep) for nitrate, ammonium, phosphate, total dissolved N, and total dissolved P. Maximum quantum efficiency of photosystem II (PSII) was estimated from in vivo (Fv) and DCMU (3,4-dichlorophenyl-1,1-dimethyleurea)-enhanced in vivo fluorescence (Fm) of each sample measured on a Turner Designs TD-700 fluorometer (EM filter of >665 nm and EX filter of 340–500 nm). All readings were blank-corrected using 0.2 μm of filtered lake water. DCMU blocks electron transfer between PSII and PSI and yields maximal fluorescence, and previous studies have demonstrated that Fv/Fm can be diagnostic of nutrient limitation. Bulk APA was measured on a Turner Designs TD-700 fluorometer (EM filter of 410–600 nm and EX filter of 300–400 nm) using 4-methylumbelliferone phosphate (250 mM concentration) as the substrate. APA measured by this assay has been shown to be significantly correlated with the expression of the gene-encoding alkaline phosphatase (phoX) in cultures of Microcystis aeruginosa clone LE-3.

Whole water samples were analyzed for total microcysts by freezing samples at −20°C for >24 h and then lysing cells using an Abraxis QuikLyse Cell Lysis kit. Microcystin concentrations within lysed samples were quantified on a SpectraMax Plus 384 plate-reading spectrophotometer using the Abraaxis microcystins/nodularins (ADDA) ELISA colormetric immunoassay according to the manufacturer’s instructions. This assay is congener-independent, being sensitive to the ADDA moiety that is found in almost all microcysts, and provided an analytical precision of ±2% and a 96 ± 2% recovery of spiked samples.

For samples collected in September, Microcystis biomass was measured with an inverted microscope equipped with a Nikon DS-Vi1 camera using Lugol’s preserved samples. An entire gridded Sedgewick–Rafter chamber (1 mL) was visually

DOI: 10.1021/acs.est.5b03931

scanned, and *Microcystis* colonies, as well as *Planktothrix* and *Anabaena* colonies, were quantified and measured using NIS-Elements imaging software (version 3.22.11). The area of each *Microcystis* colony was converted to cells per milliliter according to Watzin et al.\(^{30}\) For samples collected in October, samples were enumerated with the Utermöhl technique for algal biomass and taxonomic composition.\(^{31}\) Depending on sample density, subsamples of 2–5 mL were settled for 24 h and counted at 100× or 400× using a Leica DM inverted-phase microscope, enumerating a minimum of 100 settled units for the most abundant taxa. Cell counts were converted to biomass from average measured cell volumes, and taxa were identified to genus or species levels according to major taxonomic sources. Colonial and filamentous forms were measured individually and biomass calculated as a function of average cell density per average biovolume. Although cyanobacterial quantification methods differed, the September and October sample sets were not compared to each other but rather to other samples collected at the same time and thus quantified in the same manner.

**RNA Isolation and Sequencing.** RNA was extracted from duplicate biological replicate samples using the RNeasy Mini Kit with RNAProtect bacteria reagent (Qiagen) as outlined by Ilikchyan et al.\(^{32}\) Briefly, a polycarbonate filter containing filtered cells was transferred to a 15 mL Falcon tube, and 2 mL of TE buffer was added to wash cells from the filter. Then, 4 mL of RNAProtect bacteria reagent was added, and the mixture was allowed to incubate for 5 min at room temperature. Following centrifugation for 10 min at 2000g, the supernatant was transferred out of the tube, and 200 μL of TE buffer with lysozyme (Sigma) and proteinase K (QIAGEN) was added. The pellet was resuspended by pipetting and transferred to a 1.5 mL microcentrifuge tube and allowed to incubate for 10 min at room temperature. The remainder of the RNeasy Mini Kit protocol was then followed according to the manufacturer’s instructions. Extraction of RNA from Sterivex filters followed Ilikchyan et al.,\(^{32}\) with an added 5 min incubation before passing RNAProtect through the filter and the RNA lysis buffer incubation being reduced to 30 min. For both polycarbonate filter and Sterivex filter samples, on-column DNase digestion was performed on RNA samples using RNase-free DNase (Qiagen) according to the manufacturer’s instructions. Ribosomal RNA was removed from total RNA (~3 μg) using an Epicenter Ribo-Zero magnetic kit (bacteria) and Ribo-Zero magnetic kit (plant leaf) according to the manufacturer’s instructions. Both kits were combined by mixing the Ribo-Zero RNA Removal Solutions at a 50:50 concentration per manufacturer recommendation. After RNA depletion, samples were purified using a Qiagen RNeasy MinElute cleanup kit according to the instructions outlined by the Epicenter Ribo-Zero magnetic kit. Post-digested RNA was assessed for quantity with a Qubit 2.0 fluorometer and quality with an Agilent Bioanalyzer. Samples were stored at −80°C until sequencing. Sequencing libraries were prepared using a TruSeq RNA Sample Preparation Kit v2 (Illumina) according to the manufacturer’s instructions, skipping the poly-A pull-down step. Library prep and sequencing of 100 bp single-end reads per library was performed by the JP Sulzberger Columbia Genome Center (New York, NY) on an Illumina HiSeq 2000 system.

**Read Mapping and Analysis.** Raw reads were initially characterized with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Trimming ambiguous reads slightly improved alignment rates (+~1%) but decreased the number of unique alignments. As such, ambiguous or low-quality reads were not trimmed prior to read mapping. Reads were mapped to the *M. aeruginosa* NIES-843 genome\(^{33}\) using RSEM v1.2.11\(^{34}\) with Bowtie 2\(^{35}\) and parameters recommended by the RSEM authors. We used Bowtie 2 because it is generally faster than Bowtie 1, supports reads longer than 50 bp, and provides alignments based on a quantitative scoring scheme rather than mismatch rate.\(^{36}\) Differential expression between treatments and the reference condition was computed with EBseq\(^{36}\) within RSEM using the soft-threshold option and median normalization. EBseq uses an empirical Bayesian method that has increased power of detection over most count-based methods, and true positive rates are generally independent of sample size and outliers.\(^{36,37}\) Genes with zero-read counts across all comparisons were not considered in differential expression analysis. In addition, because rRNA removal methods do not completely remove all rRNA, transcripts mapping to 5S, 16S, or 23S rRNA genes were removed prior to differential expression analysis. Comparisons were accepted as significant, with the false discovery rate (FDR) controlled at 0.05 (in EBseq PPDE ≥ 0.95). For the field samples, differential expression was calculated by comparing the station closest to the mouth of the Maumee River (LET7) to other stations (LET1–6). For the bottle incubation experiments, all treatments were compared to the control treatment. When there were only a small number of genes responding in bottle incubation experiments, an additional comparison to the initial as the reference condition was performed. Taxonomic profiling of each sequenced sample was conducted with MetaPhlAn v1.7.7\(^{38}\) that maps raw sequence reads to a database of predefined clade-specific microbial marker genes. MetaPhlAn identified to the order level two more abundant cyanobacteria: Oscillatoriales and Nostocales. Microscopy allowed us to assign a genus to these orders, and as such, we mapped to two genomes representing significant fractions of the microbial community, as identified by these two methods (*Planktothrix* and *Anabaena*). Of the *Anabaena* genomes examined (*Anabaena* sp. 90, *Anabaena variabilis* ATCC 29413, and *Anabaena* sp. PCC 7108), *Anabaena* sp. PCC7108 had the largest overall alignment rates (0.02–0.57; Table S4). Similarly, among the *Planktothrix* genomes examined (*Planktothrix agardhii* NIVA-CYA 563 and NIVA-CYA 15), *Planktothrix agardhii* NIVA-CYA 15 had the largest fraction of mapped transcripts (0.06–2.74%; Table S5) and was chosen to elicit genetic responses of this group. Differentially expressed genes for *Microcystis* were assigned functional categories on the basis of categories found in Cyanobase for the *Microcystis* NIES-843 genome (http://genomes.microbedb.jp/cyanobase/Microcystis). The blastp suite (http://blast.ncbi.nlm.nih.gov) was used to elucidate putative functions of hypothetical genes using an E-value cutoff of 1 × 10−5. The Phyre2 server was used to predict function on the basis of protein structure for some genes.\(^{39}\) The PATRIC Web site (http://patricbrc.org/portal/portal/patric/Home) was used to identify genes and annotations for the *Planktothrix* and *Anabaena* genomes.\(^{40}\) The Illumina sequences reported in this paper have been deposited in the National Center for Biotechnology Information’s Sequence Read Archive (accession no. SRP047492). One-way analysis of variance (ANOVA) and regression analyses of parameters measured during experiments or across transects were performed using SigmaPlot version.
Environmental Science & Technology

11.0 (build 11.1.0.102). Post-hoc multiple comparisons were performed with Tukey tests.

**RESULTS**

Lake Properties. Abundance of Microcystis was low at the mouth of the Maumee River but increased within the western basin of Lake Erie, while both alkaline phosphatase activity (APA) and photosynthetic efficiency ($F_v/F_m$) increased with distance from the mouth of the Maumee River (Table S1). Dissolved nutrient concentrations (nitrate, ammonium, and phosphate) were highest near the Maumee River and lower east of this region (Table S2). Microcystin levels were generally higher and more variable in September (3.8 ± 0.27 μg L⁻¹) compared to levels in October (0.96 ± 0.27 μg L⁻¹; Table S1).

Transcriptomic Sequencing. Transcriptomic sequencing yielded, on average, 31 million 100 bp reads per sample (Table S3). Taxonomic profiling of transcripts with MetaPhlAn indicated that the microbial community was largely dominated by Microcystis (>70%) in the open waters of western Lake Erie (stations LET5 to LET1), whereas regions closer to the Maumee River were dominated by Anabaena (>70%; Figure 2A). A total of 20% of transcripts aligned to the reference Microcystis genome (Table S3), with the exception of three October samples that displayed lower alignment rates (2% on average; Table S3). Of the putative protein-encoding genes in the Microcystis genome (6312), between 3% and 20% displayed significant differential expression (Figure S2). Planktothrix and Anabaena displayed lower alignment rates (up to 13% and 1%, respectively; Table S4, S5). MetaPhlAn also identified a Flavobacterium representing a low percentage of expressed transcripts (<4%) at all stations but LET3 (>13%), with other individual prokaryotes representing <9% of all transcripts (Figure 2A).

Transcriptomic Responses across Western Lake Erie. Microcystis. The number of significant, differentially expressed Microcystis genes increased with distance from the reference location (LET7) moving east along the cruise track (Figure S2A). Populations within midtransect (LET3) displayed the largest number of significant differentially expressed genes (445) with stations farther to the east having a slightly lower number (Figure S2A). Several genes involved in N transport and metabolism displayed significant increases in expression relative to the Maumee River station (LET7). For example, at station LET5 genes encoding a nitrate–nitrite transport permease protein (nrtB), ferredoxin–nitrite reductase (nirA), and ammonium permease (amt1) displayed large increases in expression (>2 log₂ fold change; Figure S3). In contrast, only one gene related to N metabolism, encoding glutamate dehydrogenase (gdhA), displayed lower transcript abundance at all stations compared to the reference site (Figure S3).

Microcystis genes related to the transport and metabolism of P displayed strong gradients in expression across Lake Erie. A gene encoding a periplasmic phosphate-binding protein (pstS, MAE18310) had large increases in transcript abundance relative to LET7 at all stations but LET6 (the closest station to LET7), with log₂ fold changes ranging from 3.36 to 6.87 and being inversely correlated with levels of SRP (p < 0.01; Figure 2B,C; Figure S4). At the only station with levels of SRP below detection limits (<0.1 μM; station LET4; Figure 2B), a number of additional phosphate transport genes displayed increased transcript abundance, including one phosphate permease (pstA) and three phosphate-binding proteins (pstB, pstB2, and sphX) with log₂ fold change values ranging from 1.84 to 6.87 (Figure 2C, Figure S4).

Expression of genes involved in the production of microcystin and other secondary metabolites also changed across the western basin of Lake Erie. At all stations, genes responsible for the production of the protease inhibitors, aeruginosin (aer gene set) and cyanopeptolin (mcn gene set) displayed large decreases in transcript abundance moving away from the mouth of the Maumee River (Figure 3). Several microcystin synthetase genes were differentially expressed; mcyD and mcyG displayed decreased transcript abundance at station LET2, while mcyA and mcyD had increased transcript abundance at LET3 (Figure 3). A large number of genes encoding transposases (genes involved in mediating the rearrangement of transposable elements within the genome) were differentially expressed at all stations, with the largest number observed in the middle part of the western basin at station LET3 (70; Figures 4A and S5). A similar pattern was observed with the expression of genes putatively involved in phage defense with the highest number of differentially expressed genes being observed at stations LET4 and LET3 (Figure 4B).

**Planktothrix.** Planktothrix decreased in abundance moving east from the Maumee River (Figure S6) and displayed a significant down-regulation of genes related to photosynthesis, cell division, and hypothetical proteins at stations farthest from the Maumee (stations LET1–4; Table 1, Figure S7A). Of the genes with increased transcript abundance (numbering <50),
many were annotated as long-chain fatty-acid CoA ligases, which may be involved in lipid recycling (Table 1).44 There were also multiple mobile elements, three transporters, and numerous hypothetical proteins with increased transcript abundance at all stations (Table 1).

Anabaena. Although the abundance of Anabaena decreased moving east from the Maumee River (Figure S6), its number of significant differentially expressed genes increased (Figure S8A). Numerous genes encoding nitrogenase (nifH) were up-regulated in stations farthest from the reference station (LET2 and LET1) as were several transposases (LET1–5; Table 1). Anabaena displayed a mixed response with regards to photosynthesis genes, with phycobilisomes being down-regulated and photosystem II genes being up-regulated (Table 1).

Nutrient Amendment Experiments. Relative to the unamended control, Microcystis abundances significantly decreased in response to P enrichment (p < 0.001) but did not change in response to urea or ammonium (Figure S5A). In contrast, total cyanobacterial biomass displayed significant increases in response to ammonium, urea, and P (p < 0.05; Figure S5D) relative to the control. Bulk APA was significantly higher in treatments amended with N relative to the P treatment, averaging 0.29 ± 0.01 (nmol mL⁻¹ h⁻¹) (p < 0.001; Figure S5B). Urea enrichment yielded a significant increase in microcystin concentrations (from 3.87 ± 0.40 to 10.90 ± 0.49 (µg L⁻¹ MC-LR equivalents)) relative to the control and initial conditions (p < 0.001; Figure SC). Nitrate, urea, and SRP were at submicromolar concentrations in all treatments by the end of the experiment (Table S6), whereas ammonium levels were highest in the + P treatment (1.66 ± 0.18 µM) and lower but similar in all other treatments (~1 µM, Table S6).

Transcriptomic Responses to Nutrient Amendments. Microcystis. The largest transcriptional response in Microcystis was observed in the P amendment treatment, with more than 1300 genes differentially expressed relative to the control. In contrast, treatments amended with ammonium or urea, the transcriptional response of Microcystis was smaller (579 and 524 genes, respectively; Figure S2B). Among identified genes, many were involved in photosynthesis, respiration, and translational functions (Table S7). Regarding N metabolism, P additions increased transcription of genes encoding a nitrate and nitrite reductase as well as genes within the nitrate–nitrite transport system (nrtABCD; Figure S3) but decrease transcription of glutamate synthase (glnA, glnB, gltB, gltD) genes (Figure S3). Urea additions increased the abundance of transcripts for genes encoding cyanase, an enzyme involved in the degradation of cyanate, a product of urea degradation (MAE10370; cynS), and evidencing active use of this compound by Microcystis. Urea additions also up-regulated nitrate and nitrite reductases (MAE53960, narB and MAE18410, nirA; Figure S3). Additions of ammonium enhanced transcription of a gene encoding glutamate–ammonia ligase (MAE19270, glnA; Figure S3) that is involved in the production of L-glutamine from ammonia. A gene encoding cyanophycinase (MAE29150, cphB), which degrades cyanophycin (a N-rich reserve material),45 decreased in transcript abundance in the urea and ammonium treatments, while a second gene annotated as cphB (MAE27450) decreased in transcript abundance when P was added. The cyanophycin
The expression of transposase genes in *Microcystis* was sensitive to nutrient amendments. In response to P enrichment, 141 transposases were differentially expressed, with 89 increasing in transcript abundance, whereas there was a smaller response to N (urea or ammonium; <50 genes, >50% increasing in transcript abundance; Figure 6B, Figure S9). A parallel response was observed among phage defense genes, whereby P enrichment promoted an order of magnitude larger differential response than N (Figure 6C). P enrichment also promoted the largest response in genes involved in DNA replication, restriction, modification, recombination, and repair with 12 genes in this functional category being up-regulated (Figure S10). The addition of ammonium increased transcript abundance of three genes within the *mcy* cassette (*mcyB*, *mcyD*, and *mcyG*), while the addition of urea increased the transcript abundance of the *mcyB* gene only (Figure 3). The expression of other secondary metabolites responded similarly, with two genes involved in aeruginosin and cyanopeptolin synthetase (protease inhibitors that may be involved in grazer defense; *aerA* and *mcnG*, respectively) displaying increases in transcript abundance when ammonium was added and with urea additions eliciting the up-regulation of *mcnF* (Figure 3).

**Planktothrix.** It was found that *Planktothrix* became less abundant during the experiment, declining from 4% to <3% of expressed transcripts (Figure S11). Relative to the initial or control, P enrichment produced the largest number of differentially expressed genes (6 and 27, respectively; Figure S7B, C) with the majority of these down-regulated. No genes were differentially expressed with urea enrichment relative to the control, although a similar number of genes to P enrichment were differentially expressed relative to the initial condition (Figure S7B, C). As in the field transect, many of the up-regulated genes were involved in fatty-acid metabolism, whereas the down-regulated genes were involved in photosynthesis, regardless of treatment (Table 1). In a response similar to that of *Microcystis*, P enrichment elicited a decrease in *Planktothrix* alkaline phosphatase transcripts (Table 1).

**Anabaena.** *Anabaena* became less abundant during the experiment, declining from 14% to <4% of expressed transcripts (Figure S11). Relative to the control, enrichment with ammonium produced the largest number of differentially expressed genes, whereas the down-regulated genes were involved in photosynthesis, regardless of treatment (Table 1).

![Figure 5](image-url) (A) *Microcystis* cell densities, (B) bulk alkaline phosphatase activity, (C) microcystin concentrations, and (D) cyanobacterial abundances as measured with a Fluoroprobe at the time of harvest for the nutrient amendment experiments. Error bars represent the standard deviation between biological replicates (n = 2). Star indicates significant difference relative to the control treatment (A, C, and D) or +P treatment (B) (p < 0.001). Microcystins were measured as MC−LR equivalents.
This study concurrently evaluated the abundances and transcriptomes of cyanobacteria as a function of nutrient gradients across western Lake Erie, providing novel insight regarding key physiological pathways that shape the niche of populations within this system. Changing SRP concentrations elicited the largest transcriptomic response among cyanobacteria. In *Microcystis*, expression of P-scavenging genes was heightened in the presence of low-P and decreased with higher P (Figures 2C, 4, and 5A). Furthermore, the station with the lowest SRP levels in Lake Erie (LET4, Figure 2B) displayed the largest up-regulation of five of these P scavenging genes (*pstSCAB*, Figure 2C). The degree of up-regulation (1.84–6.87 log, fold change) of these genes was similar to that observed in P-limited cultures of *Microcystis* (0.83–5.63 log, fold change), suggesting that within Lake Erie, *Microcystis* populations recruit these proteins to adapt to P limitation and persist within the lake despite very low levels of SRP. Unlike *Microcystis*, *Planktothrix* down-regulated its alkaline phosphatase genes in response to decreased SRP, and no P-scavenging genes were differentially expressed in *Anabaena* populations (Table 1).

Nitrogen had a smaller but broad effect on transcript abundance in *Microcystis*. Urea enrichment did not alter abundance in *Microcystis* expression of genes encoding urea transporters (*ureABCDEF*), demonstrating these populations were not N-limited given the strong response of these genes to N limitation in culture. However, it is possible urease is constitutively expressed in *Microcystis*, as has been reported for other microbes or the added urea was quickly metabolized and the expression of urease had already ceased when cells were harvested. A gene encoding cyanase (MAE10370, *cynS*) displayed elevated transcript abundance during urea enrichment (Figure S3). Cyanate can be produced from the decomposition of urea, and because it is toxic at high levels, some cyanobacteria express a cyanase to detoxify this compound. Therefore, *cynS* expression may serve as a marker for use of urea by *Microcystis* populations.

Some microcystin synthetase genes were up-regulated when exposed to higher ammonium and urea levels, and there was a significant increase in microcystin concentrations when natural populations of cyanobacteria were provided urea (Figure 5C). Intracellular levels of microcystin, a N-rich compound in *Microcystis*, generally increase under high concentrations of bioavailable N (ammonium or urea) and urea has been shown to increase microcystin concentrations in freshwater systems. Beyond microcystin, the synthesis of other secondary metabolites in *Microcystis* seems to be promoted by high N levels. The genes responsible for the production of the protease inhibitors, aeruginosin (*aer*) gene set) and cyanopeptolin (*mcn* gene set), two N-rich compounds, displayed significant decreases in transcript abundance moving away from the high nitrogen levels near the mouth of the Maumee River and significant increases in gene expression when nitrogen levels were experimentally enhanced. Therefore, nitrogen loading may promote *Microcystis* blooms both directly via the enhancement of growth as well as indirectly via the synthesis of protease inhibitors that discourage zooplankton grazing and thus facilitate bloom proliferation.

In the presence of high concentrations of nutrients, microbes often synthesize storage products that can serve as reserve material during nutrient stress. A pair of such storage products in cyanobacteria, cyanophycin (for N) and polyphosphate (for P), are formed through cyanophycin synthetase and polyphosphate (polyP) kinase, respectively. Additions of N elicited a decrease in transcript abundance of the genes involved in the breakdown (MAE29150, *cphA*) and synthesis (MAE27460, *cphA*) of cyanophycin. This response was partly unexpected because additions of N have been shown to stimulate synthesis of cyanophycin in some cyanobacteria, including *Aphanocapsa* and *Planktothrix*. However, N additions in these previous studies were more than 1000-fold higher than the environmentally relevant N additions used in this study, which were seemingly large enough to slow the use of cyanophycin (lower *cphB* expression) but not high enough to induce cyanophycin synthesis (*cphA*). Regarding polyP, the addition of ammonium significantly increased transcripts of polyphosphate kinase (MAE01300, *ppk1*), whereas P additions had the opposite effect. This response, although seemingly counterintuitive, is consistent with studies of other phytoplankton and cultured *Microcystis*, where under P stress there is an increase in polyphosphate production, and the addition of P causes a decrease in polyP levels. Under low-P conditions, therefore, *Microcystis* up-regulates P transporters (*e.g.*, *ps*), which shunt orthophosphate to polyP that is likely utilized only under periods of extreme P stress. Such a mechanism for carefully titering polyP levels likely facilitates the persistence of...
Microcystis blooms within low- (but dynamic) P environments such as western Lake Erie.

Transposases are thought to be the most abundant genes in nature, and are involved in mediating the movement of transposable elements within a genome, and can enhance a cell’s ability to adapt to changing environmental conditions. Microcystis contains more transposable elements than other cyanobacteria, comprising >10% of the genome in some strains of this genus, providing a high degree of genome plasticity, and potentially contributing toward its dominance in freshwater systems. In this study, during P enrichment, 141 genes encoding transposases were differentially expressed, with 89 displaying increases in transcript abundance (Figures 6B and S9). P enrichment also induced the up-regulation of the largest number of genes involved in DNA repair (Figure S10), further suggesting active genome rearrangement and repair under high-P conditions. When N was added (urea or ammonium), a smaller yet significant number of transposase genes responded (38 total, 28 with increased transcript abundance; Figure 6B, Figure S9), as did a smaller number of DNA repair genes (Figure S10). A similar response was observed in our previous culture-based transcriptomic study, in which N limitation up-regulated a larger number of transposase genes than did P limitation (21 versus 9; Figure S12). Another recent culture study also suggested that nutrient limitation regulates transposase expression, with transposases from the family IS200/IS605 responding most frequently.

Similarly, the majority of transposases with increased transcript abundance in this study were also within the IS605 family (Figures 4A,B and S9). The present study affirms the role of nutrients in influencing the expression of transposase genes and their potential importance in adapting to dynamic biogeochemical conditions in aquatic environments. Some of the genes identified as transposases have also been putatively assigned roles in phage defense, and it has been hypothesized that phages may act as donors of transposases, thereby contributing to the plasticity of the Microcystis genome. Viruses play a central role in structuring microbial communities and nutrient cycling in aquatic environments. Although cyanophages have been implicated as potential drivers of Microcystis bloom demise, investigations of viral infection in Microcystis have reported dramatic initial cell lysis but eventual recovery and resistance to future infections, suggesting resistance among subpopulations and whole-population adaptation. The Microcystis genome contains a remarkably large number of phage defense systems. Of more than 1000 genomes surveyed, Microcystis contained 492 defense genes, or 80% more than Cyanothece PCC8802 or Roseiflexus RS-1 (the next highest), with 29% of its genome assigned to defense islands. Furthermore, the presence of a large diverse number of CRISPR (clustered regularly interspaced short palindromic repeats) spaces within the Microcystis NIES-843 genome suggests this cyanobacterium is frequently infected by viruses. The large and diverse array of viral defense genes is likely an important factor in the continued dominance of this species in freshwater ecosystems. In the current study, when water was amended with P, we observed a large transcriptional response (>250 genes with 167 increasing in transcript abundance) in these phage defense genes, whereas additions of ammonium or urea elicited a smaller response (<70; Figure 6C). Given that viruses have an enhanced ability to replicate when host cells are P-replete, this transcriptomic response by Microcystis suggests P loading increases the frequency with which it is exposed to viruses. Further, these findings indicate that within high-P environments, Microcystis must dedicate more energy toward viral defense and thus may be less likely to dominate cyanobacterial communities.

During this study, a small fraction of transcripts aligned to non-Microcystis cyanobacteria in Lake Erie, likely due to the numerical dominance by Microcystis within most samples as well as genomic mismatches between the organisms found in Lake Erie and available reference genomes. Despite lower mapping rates, transcriptomic trends within Anabaena and Planktothrix genomes evidenced their niche within Lake Erie. For instance, abundance of cells and expressed transcripts for both groups decreased away from the mouth of the nutrient-enriched Maumee River, suggesting the conditions close to the Maumee (high N and P levels) were ideal for these cyanobacteria (Figure 2A; Figure S6). Consistent with these trends, numerous genes involved in photosynthesis and cell division were down-regulated by Anabaena and Planktothrix in the regions away from the Maumee River (a common response to nutrient stress in cyanobacteria), evidencing the physiological basis for their lower abundances (Table 1). Furthermore, the up-regulation of genes involved in degradation of fatty acids by Planktothrix under low-P conditions evidenced a specific energy-sparing mechanism invoked by this genera to subsist in a nutrient-poor environment. Transcriptomic profiling of Anabaena populations revealed that a gene-encoding nitrogenase (nifH) had increased transcript abundance in all stations but LET6 (closest to the high N, Maumee River), indicating its need to fix nitrogen away from high-N regions of Lake Erie (Table 1). During nutrient-amendment experiments, nifH was up-regulated with additions of P, suggesting that additions of P pushed the community further into N limitation. Ammonium also promoted nifH expression. Although high ammonium levels can repress N-fixation, previous work has shown that nitrogenase activity will continue if environmental nitrogen concentrations do not meet cellular N demands. This is likely the case given the low concentrations of ammonium used during the experiment (5 μM) and the relatively large biomass-associated cyanobacterial blooms.

GENE PATHWAYS FACILITATING DIFFERENTIAL DOMINANCE OF CYANOBACTERIA

Field surveys, experiments, and transcriptomic profiling performed during this study collectively provide insight regarding the specific niches of cyanobacteria across western Lake Erie as well as the genes pathways that facilitate their ability to exploit those niches and form blooms. While Planktothrix and Anabaena dominated the high nutrient environment near the Maumee River, these groups became scarce in more distal regions of the lake and during experiments with lower P concentrations, a pattern also observed across P gradients of the Baltic Sea, where nitrogen-fixing filamentous cyanobacteria dominate high-P regions. The ability of Anabaena to dominate high-P environments is likely related, in part, to its ability to fix N, consistent with the enhanced expression of nifH genes by this taxon when enriched with nutrients. In contrast, enhanced expression of transposases and viral defense genes by Microcystis following P enrichment suggests that synthesis of these proteins, which occupy a substantial portion of the Microcystis genome, represents a significant energy expenditure that may slow its growth within high-P regions. In contrast, Microcystis was the most abundant
cyanobacteria in the regions of western Lake Erie with low-P levels and in low-P experimental treatments (i.e., controls or N amendments). The ability of Microcystis to up-regulate genes to efficiently scavenge and store P within P-impoverished zones seemingly provide it with a competitive advantage over other cyanobacteria, allowing it to bloom. Given that Microcystis displayed a gene expression pattern more consistent with N-replete than N-limited conditions, the dominance of Microcystis may be further promoted in particular cases by the enhanced synthesis of grazing deterrents (protease inhibitors) under the relatively higher N levels present in Lake Erie. Rather, the reduction of P loads may promote a shift from one cyanobacteria to another and facilitate dominance by more toxic forms (Microcystis). Therefore, managerial schemes that also restrict N inventories may be required to limit the proliferation of nondiazotrophic cyanobacteria such as Microcystis.81

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b03931.

Tables showing physical, chemical, and community characterizing parameters measured at each station during September and October transects of the western basin of Lake Erie; nutrient concentrations [μM] for each station of the September and October transects of the western basin of Lake Erie; transcriptomic sequencing results; nutrient concentrations [μM] for each treatment for the nutrient amendment experiment; and the number of significantly differentially expressed genes for Microcystis within each functional category relative to the control. Figures showing a composite of MODIS Cyanobacterial Index images from the NOAA Experimental Lake Erie Harmful Algal Bloom Bulletin for 2013; the number of significant differentially expressed genes for Microcystis at each station relative to station LET7 and relative to the control after 48 hr; heat map of genes involved in nitrogen and phosphorous transport and metabolism and their significant differential expression at each station relative to LET7 and under each treatment relative to the control for Microcystis; heat map of transposase genes and their significant differential expression at each station relative to LET7 for Microcystis; cyanobacterial abundance during the October transect of the western basin of Lake Erie for the three genera discussed; the number of significant differentially expressed genes in Planktothrix agardhii NIVA-CYA 15; the number of significant differentially expressed genes in Anabaena sp. PCC7108; heat map of transposase genes and their significant differential expression relative to the control for Microcystis; heat map of genes involved in DNA replication, restriction, modification, recombina-

tion, and their significant differential expression at each station relative to LET7 and under each treatment relative to the control for Microcystis; community analysis via Metaphlan displaying the average percent abundance across two biological replicates for the nutrient enrichment experiments; the number of significant differentially expressed transposase genes. (PDF)

AUTHOR INFORMATION

Corresponding Author

*Phone: (631) 632-5043; fax: (631) 632-5070; e-mail: christopher.gobler@stonybrook.edu.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Jennifer Goleski for analytical assistance, the captains and crew of the research vessels R/V Erie Monitor and CGS Limnos, and the support staff of The Ohio State University Franz Theodore Stone Laboratory. This work was supported by the NOAA-ECOHAB program being funded by the National Oceanic and Atmospheric Center for Sponsored Coastal Ocean Research under award no. NA10NOS4780140 to Stony Brook University. This study is GLERL contribution number 1790 to the EC Great Lakes Nutrient Initiative (GLNI) and ECOHAB contribution number ECO841.

REFERENCES

(6) Han, H.; Allan, J. D.; Bosch, N. S. Historical pattern of phosphorus loading to Lake Erie watersheds. J. Great Lakes Res. 2012, 38, 289–298.


