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Seasonal Gene Expression and the Ecophysiological Implications of Toxic *Microcystis aeruginosa* Blooms in Lake Taihu

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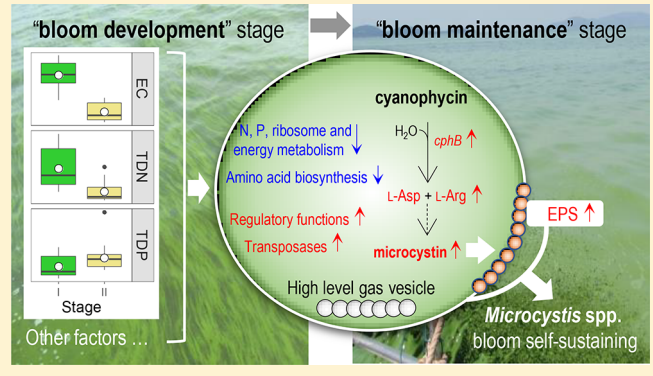
Supporting Information

ABSTRACT: Harmful cyanobacterial blooms represent an increasing threat to freshwater resources globally. Despite increased research, the physiological basis of how the dominant bloom-forming cyanobacteria, *Microcystis* spp., proliferate and then maintain high population densities through changing environmental conditions is poorly understood. In this study, we examined the transcriptional profiles of the microbial community in Lake Taihu, China at 9 stations sampled monthly from June to October in 2014. To target *Microcystis* populations, we collected metatranscriptomic data and mapped reads to the *M. aeruginosa* NIES 843 genome. Our results revealed significant temporal gene expression patterns, with many genes separating into either early or late bloom clusters. About one-third of genes observed from *M. aeruginosa* were differentially expressed between these two clusters. Conductivity and nutrient availability appeared to be the environmental factors most strongly associated with these temporal gene expression shifts. Compared with the early bloom season (June and July), genes involved in N and P transport, energy metabolism, translation, and amino acid biosynthesis were down-regulated during the later season (August to October). In parallel, genes involved in regulatory functions as well as transposases and the production of microcystin and extracellular polysaccharides were up-regulated in the later season. Our observation indicates an eco-physiological shift occurs within the *Microcystis* spp. transcriptome as cells move from the rapid growth of early summer to bloom maintenance in late summer and autumn.



INTRODUCTION

In recent decades, toxin-producing cyanobacterial harmful algal blooms (CyanoHABs) driven by anthropogenic eutrophication and climate change have become a major threat to fresh waters worldwide,[1−4] potentially impacting human health and many drinking water supplies.[5−8] *Microcystis* is one of the most common CyanoHAB genera in freshwater ecosystems. The blooms of *Microcystis* have been recorded in at least 108 countries, and researchers have reported the presence of hepatotoxic microcystins (MCs) in 79 countries.[9,10] *Microcystis* spp. are notorious producers of MCs, originally known as the "*fast death factor*".[11] These secondary metabolites are potent agents resulting in animal and human poisonings[12,13] as well as liver cancer in regions affected by blooms.[14]

MCs are encoded by the microcystin synthetase genes (*mcyA-J*) and are nitrogen (N)-rich secondary metabolites (10 N atoms per molecule) which can reach up to 2% of cellular dry weight of *Microcystis*.[15] In fresh waters, MC concentrations have been found to be positively correlated with the biomass of CyanoHAB species.[16] Since numerous studies have demonstrated the importance of both N and phosphorus (P) in the development and proliferation of CyanoHABs,[17−20] nutrient availability has been thought of as a major factor regulating MC production.[21] Yet while many lakes receive external N and P

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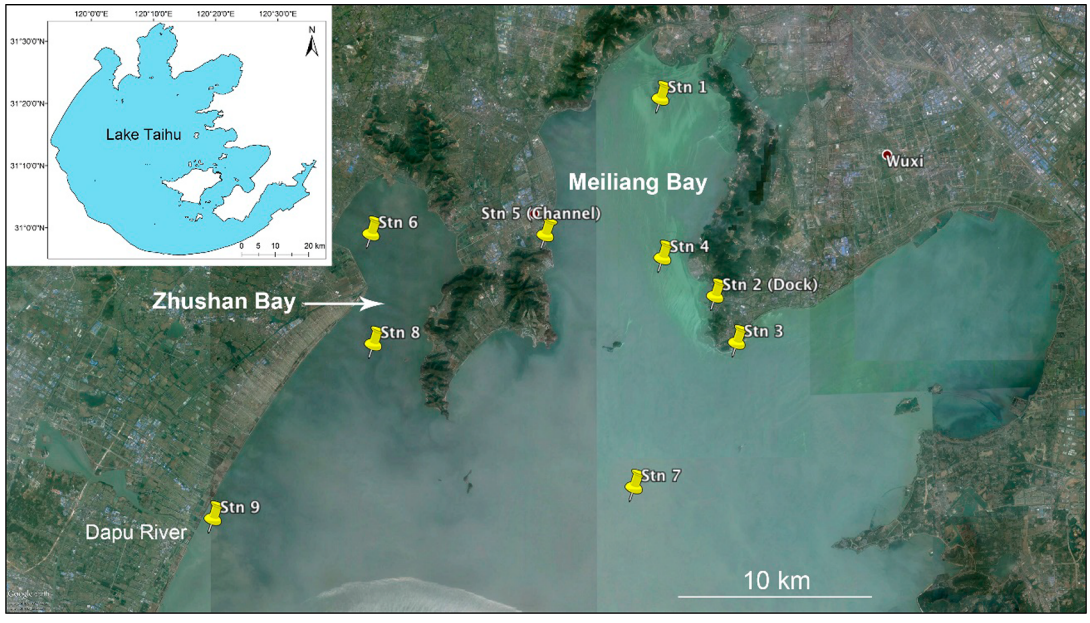

Figure 1. Sample stations in Lake Taihu (captured and modified using Google Earth; Map data: Google, DigitalGlobe). Stn 2 is located at the dock of the Taihu Laboratory for Lake Ecosystem Research (TLLER). Stn 3 is near the Nanquan Water Plant. Stn 5 is located in a channel, which connects Meiliang Bay and Zhushan Bay. Other stations (Stn 1, 4, 6, 7, 8, and 9) belong to long-term monitoring stations (i.e., THL1, 4, 16, 7, 17, and 10, respectively) conducted by TLLER monthly.

loads, seasonal nutrient limitation characteristics[6,22,23] may have effects on both the physiology (including MC synthesis) and the ecology of these bloom-forming organisms.

Lake Taihu is China's third largest freshwater lake (2338 km^2) and serves as a drainage basin for ~40 million residents.[24] In this lake, cyanobacterial blooms are dominated by the non-N_2 fixing *Microcystis*. Biovolumes of this genus can, on occasion, exceed 90% of the total phytoplankton biomass during summer.[25−27] P-limitation (in spring) and N-limitation (during summer and fall) have been documented in Lake Taihu.[23,27,28] Field surveys have shown concentrations of MCs in northern Lake Taihu as high as 870 $\mu g\ g^{-1}$ (dry weight) during the summer to autumn period.[29] Since the synthesis of N-rich MCs requires N, understanding how and why CyanoHABs produce MCs during the N-limited component of the growth season is important.

Although Lake Taihu has been plagued for more than three decades by *Microcystis* blooms,[8,30,31] the seasonal shifts in cellular physiology that allow for *Microcystis* to first bloom and then to persist for long periods are poorly understood.[32] Generally, cyanobacterial blooms in Lake Taihu can be classified into three stages: (1) "bloom development" during late spring to early summer: *Microcystis* accumulates biomass and form bloom "scum"; (2) "bloom maintenance" during middle summer to middle autumn: *Microcystis* are broadly distributed over the surface waters of much of the lake; (3) "bloom decay" in late autumn: blooms disaggregate and decline. While many researchers have asked questions concerning bloom initiation, adaptations by the cyanobacterial community across the fluctuating bloom season environment remain poorly resolved.

At the onset of this study, we hypothesized that *Microcystis* must transition its physiology during different bloom initiation and maintenance stages to preserve its competitive advantage. Here, we evaluated the spatiotemporal dynamics of the metatranscriptomic response of *Microcystis* over a 5-month period (encompassing the "bloom development" and "bloom maintenance" stages) in Lake Taihu (Figure 1). Our observations provide an integrated understanding of within season shifts in *Microcystis* spp. physiology relative to fluctuating environmental conditions and how these changes may allow for bloom maintenance.

MATERIALS AND METHODS

Sample Collection and Survey of Environmental Conditions. Surface water samples were collected monthly from nine stations in northwestern Lake Taihu during June to October 2014 (Figure 1) as described previously.[33,34] To avoid diel variations in gene expression and cell physiology,[35] all samples were collected during daylight hours (Table S1). Water temperature (WT), dissolved oxygen (DO), pH, electrical conductivity (EC), turbidity (NTU), and phycocyanin (PC, an indicator of cyanobacterial abundances) were measured *in situ* using a multiparameter water quality sonde (YSI 6600 V2). Total nitrogen (TN), total dissolved nitrogen (TDN), ammonium (NH_4^+), total phosphorus (TP), total dissolved phosphorus (TDP), orthophosphate (PO_4^{3-}), and chlorophyll *a* (Chl-*a*) were measured according to standard methods (see Supporting Information).[36]

For quantification of cellular (particulate) toxin, a 50−500 mL volume (depending on sample density) of surface water from each station was filtered onto GF/F (Whatman, UK) filters and then dried using a vacuum freeze-dryer (Christ Alpha 1-2 LD, Germany). Intracellular microcystin concentrations were determined without preconcentration using HPLC coupled with single quadrupole mass spectroscopy and photodioarray spectroscopy (see Supporting Information).[34,37] Total MC concentrations are reported as the sum of all congeners.

For RNA samples collected for transcriptomics, a 20−120 mL volume (volumes adjusted based on water column particulate load) of surface water from each station was immediately

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(within 4 min) filtered through a 0.22 μm filter cartridge (Sterivex, Millipore) using a sterile 60 mL syringe. Filters were preserved immediately using RNAlater (Invitrogen, Waltham, MA) and stored at −80 °C prior to RNA extraction.

RNA Extraction and Sequencing. RNA extraction was completed as previously described.[38] Briefly, total RNA was extracted using the MoBio PowerWater DNA Isolation kit modified and optimized for RNA isolation.[39] Genomic DNA contamination was removed using the On-Spin Column DNase I kit (MoBio), and samples were checked by PCR amplification using 16S rDNA primers 27F and 1522R. Total RNA concentration and quality were assessed with a spectrophotometer (NanoDrop ND-1000, ThermoFisher Scientific). Reverse transcription, library preparation, and rRNA removal were performed using an Epicenter Ribo-Zero rRNA removal kit by the Hudson Alpha Genomic Services Laboratory (Huntsville, AL, USA) prior to 125 bp paired-end sequencing on their Illumina HiSeq 2500. To test sequencing consistency, four samples were submitted as technical replicates (duplicates of the same extracted sample) and included in blind format in the sample series.

RNA-seq Data Processing and Phylogenetic Analysis. Low quality reads, based on default settings for quality scores (limit = 0.02) and the presence of two or more ambiguous nucleotides, were removed using CLC Genomics Workbench version 8.5.1 (CLC Bio, Qiagen, Denmark). Ribosomal RNA (rRNA) reads that passed through sequencing were removed *in silico* using SortMeRNA v2.0,[40] and the non-rRNA reads were reimported into the CLC Genomics Workbench. To conservatively examine the transcriptomic patterns of *Microcystis* populations, reads were mapped onto the genome of *M. aeruginosa* NIES 843 (GenBank accession number NC_010296.1). Mapping was performed as previously reported.[38] Expression values were calculated as reads per kilobase per million (RPKM).[41,42] Raw reads from this study were also imported into the MG-RAST v.3.6 online server and can be download freely using the deposition IDs provided in Table S2. Taxonomic composition was annotated using the MG-RAST automated processing pipeline with default settings (see Supporting Information).[43]

Statistical Analyses. Principal components analyses (PCA) were performed with the PRIMER-E (v7) software package[44] to discern variability of environmental parameters among months and sampling stations in Lake Taihu. NTU, TN, TDN, NH_4^+, TP, TDP, PO_4^{3-}, and Chl-*a* were square root transformed due to right-skewed data distributions. PRIMER-E was also used to compare RPKM values between samples. All RPKM values were standardized to the total number of reads per library (proportional abundances). The standardized relative RPKM abundances were square-root-transformed, and a Bray−Curtis similarity matrix was constructed to perform clustering, metric multidimensional scaling (mMDS), and nonmetric multidimensional scaling (nMDS) analyses for visualization of gene expression patterns among samples. After removing the strongly correlated variables, the remaining 10 environmental parameters (EC, WT, pH, NTU, DO, TN, TDN, NH_4^+, TDP, and Chl-*a*) were log $(x + 1)$ transformed and normalized. Then, the nonparametric BEST (Bio-Env + Stepwise) test using stepwise search and spearman rank correlation method with 999 permutations and LINKTREE (linkage tree analysis) were used to investigate relationships between gene expression patterns and environmental parameters.[44]

On the basis of results from the mMDS and nMDS, statistical comparisons of *Microcystis* genes (RPKM values) between the different clusters were performed using the empirical analysis of DGE tool.[45] For more stringent statistical test results, the default total count filter cutoff was increased from the default value of 5 to 1000, while a false-discovery rate (FDR) adjusted *P* value <0.05 and an absolute fold change ≥1.5 was used as a statistical cutoff. In addition, differentially expressed genes for *Microcystis* were assigned functional categories on the basis of the Cyanobase assignments for the *Microcystis* NIES-843 genome (http://genome.microbedb.jp/cyanobase/). Metabolic pathway analyses were conducted using KEGG (Kyoto Encyclopedia of Genes and Genomes) database (http://www.kegg.jp).

RESULTS

Spatial and Temporal Variation in Environmental Parameters. Detailed environmental parameters are listed in Table S1. Briefly, WT, averaged across all stations sampled for a given date, ranged from 20.7 °C (in October) to 28.3 °C (in September). The highest EC was recorded in June (712 μS cm^{-1}) and the lowest, in October (467 μS cm^{-1}). Across sample times and locations, the pH ranged from about 8 to 10. Chl-*a* concentrations spanned 3 orders of magnitude (from 15 to 46 872 μg L^{-1}) with the highest observed at the channel location (Stn 5, Figure 1) in October (T10_5; T indicates Lake Taihu, the number behind T represents sampling month, and the last number represents sampling station; similarly hereinafter) and the lowest at Stn 9 in July (T07_9). Microcystin-LR, -RR, and -YR comprised ∼85−100% of the total MC in all toxic samples (data not shown). MC concentrations ranged from undetectable (ca. 0.1 μg L^{-1}) to over 6300 μg L^{-1} (T10_5) and were positively correlated with Chl-*a* (Spearman's correlation $\rho = 0.77$, $P < 0.001$). TN and TP ranged from 1.10 to 98.47 mg L^{-1} and 0.077 to 42.835 mg L^{-1}, respectively. TN, TP, pH, and NTU were all positively correlated with algal biomass (i.e., Chl-*a*).

Compared with the early bloom season (Stage I: June and July), EC and TDN decreased significantly ($P < 0.001$) in the late bloom season (Stage II: August to October), while pH, TP, TDP, and PO_4^{3-} increased significantly (Figure S1). The average concentrations of Chl-*a* and MCs also showed an increasing trend from Stage I to Stage II although the statistical tests were not significant ($P > 0.05$). PCA showed that the first four components explained 86.2% of the total variation of the environmental parameters. In the first principal component, EC, pH, and TP have the highest loads. In the second principal component, TDP, PO_4^{3-}, and NH_4^+ have the highest loads (Figure 2). The PCA showed distinct separation between samples according to EC.

Summary of Sequencing Output. A total of 39 metatranscriptomic libraries representing 35 samples and 4 blind technical replicates were generated from the 45 samples (10 samples were excluded due to either poor quality or quantity of the extracted RNAs; see Table S3). Using CLC Genomics Workbench and the parameters described previously, a total of 850 million reads (average length = 115 bp) passed QC, representing 85.3% of the raw reads (Table S2). Of post-QC reads, 83.8% were non-rRNA. Each library contained, on average, 18.3 million reads after rRNA removal.

Active Bloom Community Structure. On average, MG-RAST assigned ∼80% of the total RNA reads to the bacterial domain (Figure S2a). Twenty-seven phyla were detected in the

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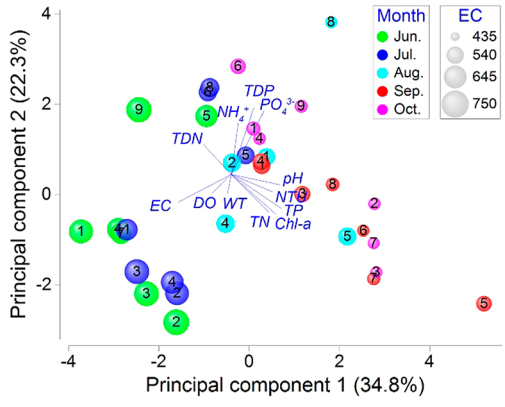

Figure 2. Principal components analysis (PCA) of the environmental parameters in Lake Taihu. Sampling station numbers (see Figure 1) are listed within the symbols. Principal components 1 and 2 accounted for 57.1% of variance between samples. The sizes of the bubbles are scaled to electrical conductivity (EC) values in water samples. TDN: total dissolved nitrogen; NH_4^+: ammonium; TDP: total dissolved phosphorus; PO_4^{3-}: orthophosphate; NTU: turbidity; TP: total phosphorus; Chl-*a*: chlorophyll *a*; TN: total nitrogen; WT: water temperature; DO: dissolved oxygen. Note: samples failed in metatranscriptomic sequencing were excluded.

bacterial reads with cyanobacteria accounting for, on average, 89.7% (range 27.0−96.9%) of all bacterial sequences (Figure S2b). The bacterial community structures of samples T07_9 and T08_9 were different from other samples, with *Proteobacteria* comprising 45.5% and 42.0% of the communities, respectively, and *Cyanobacteria* comprising 27.0% and 39.0%, respectively. MG-RAST results suggested more than 50 genera of *Cyanobacteria* were transcriptionally active during our study. At the genus level, *Microcystis* was dominant in all samples except T07_9 and T08_9, accounting for 42.9% of all cyanobacterial reads (Figure S2c). *Cyanothece*, *Synechocystis*, *Anabaena* (including *Dolichospermum*), *Nostoc*, *Synechococcus*, and *Trichodesmium*-like cell lines were also present, averaging 2.6% to 17.5% of the total cyanobacterial read pool.

Read Recruitment to *M. aeruginosa* NIES 843 Genome. Because most of the cyanobacterial RNA sequences belonged to genus *Microcystis*, we mapped the QC filtered reads to the genome of *M. aeruginosa* NIES 843. On average, 34.8% (ranging from 0.2% to 48.9%) of the total reads recruited non-redundantly to the genome of *M. aeruginosa* NIES 843 (Table S2). This value was substantially reduced in samples T07_9 and T08_9, with only 0.2% and 0.9% of reads, respectively.

Cluster analysis showed that samples T07_9 and T08_9 were different from other samples (Figure S3). T07_9 and T08_9 were both sampled from the river mouth of Dapu River (Figure 1), which was affected by riverine input heavily. In July and August, there was no bloom here and the active communities in this station were dominated by *Proteobacteria* (see Figure S2 and ref 34). Considering the low number of reads recruiting to *M. aeruginosa* NIES 843 genome, the two samples were excluded from further analysis. The four technical replicates showed >97.7% similarity with each other (Figure S3), indicating good reproducibility of our RNA sequencing procedures.

The mMDS bootstrap average analysis showed a clear temporal pattern in *Microcystis* gene expression (Figure 3a). Although there were no significant differences between June and July samples, comparisons between late months revealed shifts

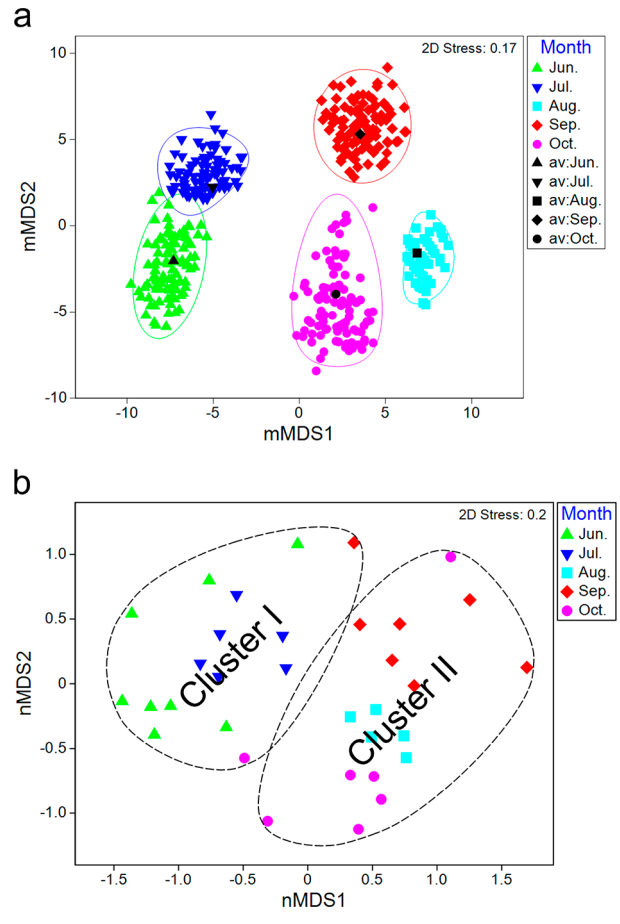

Figure 3. (a) Metric multidimensional scaling plot (mMDS) of bootstrap averages (100 repetitions) for *M. aeruginosa* NIES 843 recruited transcripts showing monthly variations (June to October 2014) of overall gene expression in Lake Taihu. The smooth envelopes contain more than 95% confidence intervals for each month with the centroid of each month in black. The dots inside the colored area represent the individual bootstrap values. The overlap of 95% confidence intervals between June and July indicates the differences are not significant. (b) Nonmetric multidimensional scaling plot (nMDS) comparing all samples. Gene compositions of *M. aeruginosa* NIES 843 with similarities >86% are circled with dashed lines. Similarity lines were automatically drawn on the basis of results from a hierarchical cluster analysis performed within PRIMER-E v7. At the similarity of 86%, the samples can be divided into two clusters: Cluster I (mostly June and July samples) and Cluster II (August, September, and October samples). Note: the red diamond from September (T09_3) and the pink dot from October (T10_9) were classified into Cluster I.

in *M. aeruginosa* gene expression. This pattern was confirmed by an analysis of similarity with global (R = 0.54, P = 0.0001) and pairwise tests (Table S4). However, no significant differences in overall *M. aeruginosa* gene expression profiles were found between stations (R = 0.08, P = 0.17).

An nMDS analysis also confirmed temporal differences in *M. aeruginosa* transcript profiles (Figure 3b). Generally, samples clustered into two groups: samples from June and July clustered together (Cluster I) and samples from August, September, and October clustered into a separate cluster (Cluster II). Statistical comparisons of RPKM demonstrated that there were 1970 genes expressed differently between the two clusters (Cluster II has 545 genes under-represented and 1425 genes over-represented compared with Cluster I; Figure S4). Many of the

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observed differentially expressed genes were involved in regulation of transposable elements, translation, regulatory functions, transport and binding proteins, photosynthesis, and respiration (Tables 1 and S5). Across all samples, some of the most highly expressed genes were related to gas vesicle production and photosynthesis (Figure 4).

Table 1. Number of Significantly Differentially Expressed Genes for *Microcystis* within Each Functional Category Based on Cyanobase in Late Bloom Months (Aug. to Oct. 2014, Cluster II in Figure 3b) Compared with Earlier Months (June and July, Cluster I in Figure 3b)[a]

category name	up-regulated	down-regulated	total
amino acid biosynthesis	1	9	10
biosynthesis of cofactors, prosthetic groups, and carriers	3	10	13
cell envelope	3	3	6
cellular processes	6	12	18
central intermediary metabolism	2	6	8
energy metabolism	1	10	11
fatty acid, phospholipid, and sterol metabolism	1	2	3
photosynthesis and respiration	5	14	19
purines, pyrimidines, nucleosides, and nucleotides	1	2	3
regulatory functions	23	13	36
DNA replication, restriction, modification, recombination, and repair	6	2	8
transcription	3	2	5
translation	3	42	45
transport and binding proteins	13	20	33
other categories[b]	423	132	555
hypothetical	634	188	822
unknown	297	78	375
sum	1425	545	1970

[a]See http://genome.microbedb.jp/cyanobase/. [b]Among "other categories", 189 and 13 genes encoding transposase (genes involved in mediating the rearrangement of transposable elements within the genome) were significantly up-regulated and down-regulated significantly, respectively.

Nitrogen Metabolism. To examine active nutrient-associated metabolism of *Microcystis* across seasons, a series of genes involved in N (55 genes) and P (30 genes) metabolism were selected for expression analysis between the two clusters. Compared with Cluster I, three genes involved in N transport and metabolism displayed significant increases in Cluster II (Figure 5a): the *cphB* gene (MAE_29150) involved in cyanophycin (a N-rich reserve material) degradation increased 5.66-fold; genes encoding ammonium permease (*amt1*) and amino acid adenylation (MAE_27820) increased 2.80- and 2.86-fold, respectively. In contrast, transcript abundances for genes related to glutamate metabolism (*gln A*, *gltBDSX*), nitrate transport and metabolism (*nrtACD*, *nirA*), and urea transport (*urtA*) decreased in late bloom season (Figure 5a and Table S6).

Phosphorus Metabolism. Compared with Cluster I, expression of a phosphate ABC-transporter periplasmic phosphate-binding protein (MAE_38290) and alkaline phosphatase (MAE_16640) increased in Cluster II. Half of the phosphate transport genes within the *pstSCAB* gene set and the gene encoding alkaline phosphatase (*phoX*, MAE_30190) decreased in Cluster II (Figure 5b and Table S7).

Toxin Metabolism. Genes involved in toxin metabolism (i.e., microcystin, aeruginosin, cyanopeptolin, and microviridin) showed no consistent spatial expression patterns (Figure 5c and Table S8). Compared with Cluster I, transcripts involved in synthesis of microcystin (*mcyCBAD*) up-regulated significantly in Cluster II. Many genes involved in synthesis of the protease inhibitors: aeruginosin (*aer* gene set) and microviridin (*mdnABCDE*) were down-regulated significantly (Figure 5c). Genes involved in synthesis of cyanopeptolin (*mcn* gene set) did not exhibit significantly different expressions.

EPS Metabolism. Extracellular polysaccharide (EPS) has an important role in the process of colony formation in *Microcystis*. Hence, we checked the genes potentially involved in EPS production (glycosyl transferases and sugar modification enzymes) and export.[46] Among the 42 significantly expressed genes, 34 (81%) of them were up-regulated in Cluster II compared with Cluster I (Table S9).

Correlation of Environmental Variables with *Microcystis* Gene Expression Patterns. A BEST analysis showed that the best 3-variable solution (EC, pH, and TDN) might be preferred as a simple "explanatory" set of abiotic variables for the overall *M. aeruginosa* gene expression patterns (global test: Spearman's rank correlation (ρ) = 0.564, P = 0.001). In line with results from mMDS and nMDS (Figure 3), the LINKTREE analysis also indicated a similar monthly division of *M. aeruginosa* gene expression (Figure S5). The samples separated into two clusters on the basis of EC. TDN, TDP, DO, and WT were the main environmental factors separating samples into smaller subclusters.

DISCUSSION

We investigated spatial and temporal variations in the transcriptome of the *Microcystis* populations in the surface waters of Lake Taihu. About one-third of the *Microcystis* genes detected during the late bloom season (August to October) were differently expressed compared with early months (June and July). These patterns in transcription suggested a transition from active growth of *Microcystis* cells ("bloom development" stage) to a self-sustaining physiology ("bloom maintenance" stage) from early to late bloom seasons, which we discuss below (briefly summarized in Figure 6). While these new observations need further experimental and field validation, the findings provide new insights toward understanding the *in situ* ecophysiology of the toxic *Microcystis* in eutrophic freshwater lakes, demonstrating that not just the cause but the maintenance of the bloom community is an important consideration.

N and P Metabolism Genes of *Microcystis* Responding to Temporal Shifts. Previous studies have linked bottom-up controls, like N and P concentrations, to massive changes in *M. aeruginosa* gene expression.[38] P-limitation in spring and N-limitation during summer and fall have been shown to have recurring trends in Lake Taihu.[23,27,28] Generally, it has been observed that, under N-limited conditions, *Microcystis* genes involved in N acquisition and transport (i.e., the global N regulatory gene *ntcA*, the ammonium related *amt* gene set, the nitrate/nitrite related *nrt* gene set, and the urea related *ure* and *urt* gene sets) increase in transcript abundance.[7,22,38] In this study, the concentration of TDN in northwest Lake Taihu where blooms occurred was lower during August to October (average ± sd, 1.0 ± 0.6 mg L^{-1}) compared with June and July (2.5 ± 1.2 mg L^{-1}). In addition, the ratios of TN/TP and TDN/DTP decreased from an average of 23.2 and 70.9 in June and July, respectively, to 9.8 and 17.4, respectively, during August to

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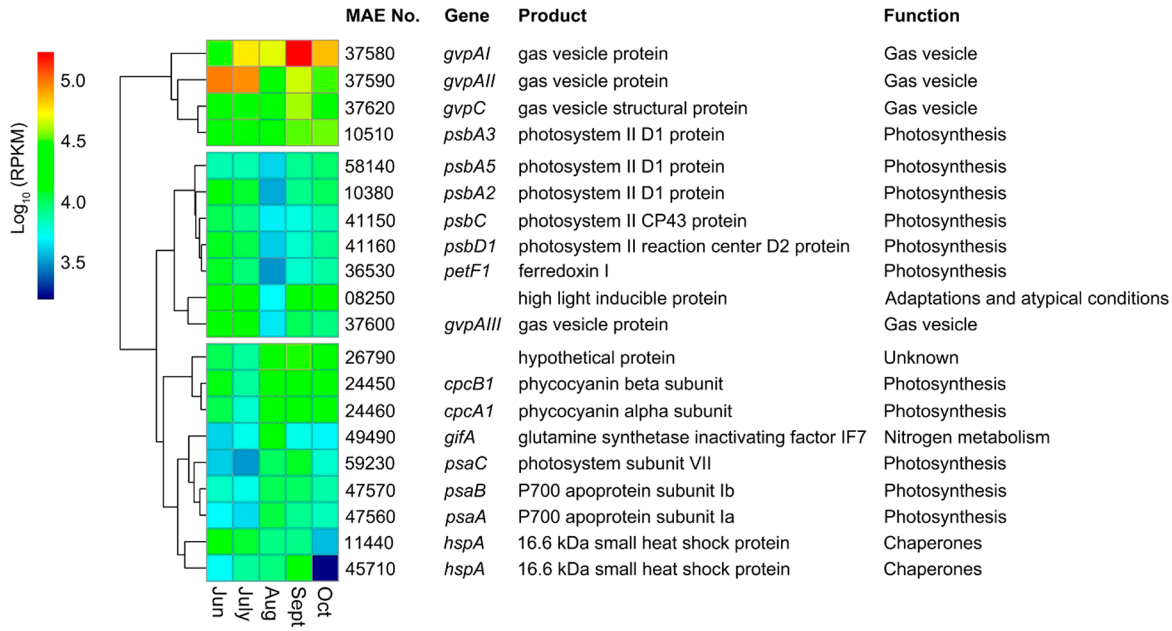

Figure 4. Heatmap of 20 genes with the most abundant transcripts expressed as $\log_{10}$-transformed mean RPKM (reads per kilobase per million) values in each month.

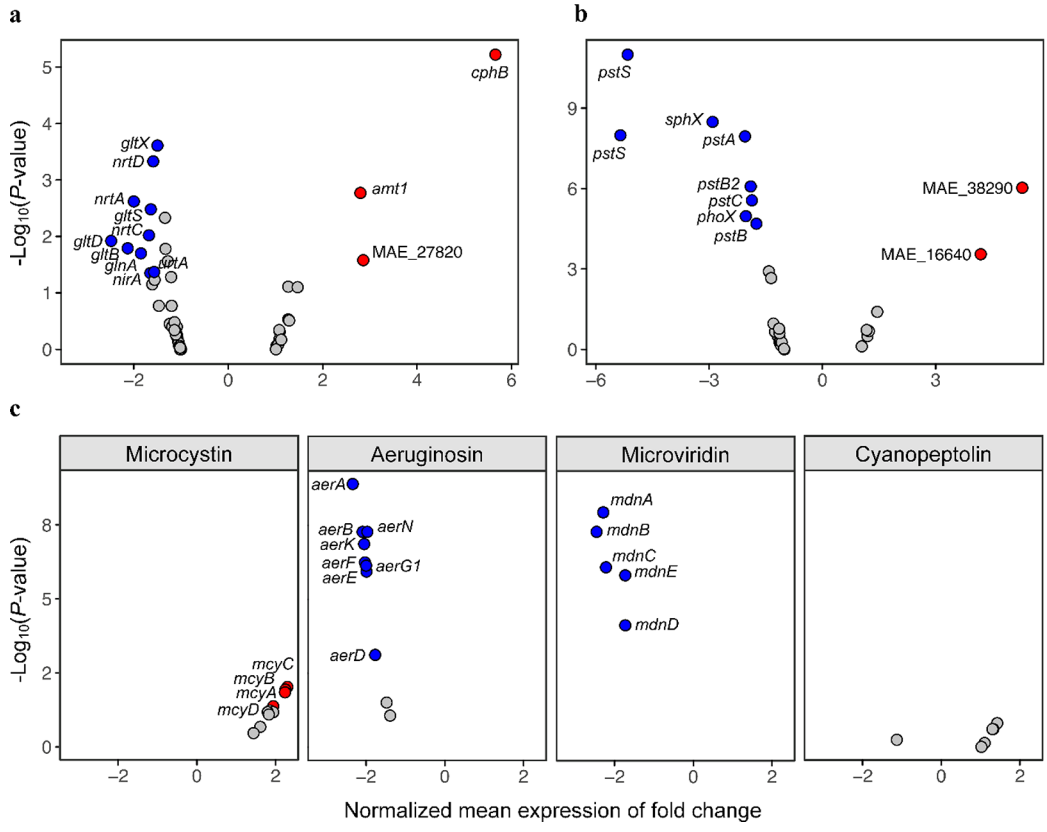

Figure 5. Volcano plots depicting normalized mean expression (RPKM) of fold changes and *P*-values (corrected for false discovery rate [FDR]) for statistical comparison between Cluster I (samples from June and July) and Cluster II (samples from August to October) for *M. aeruginosa* NIES 843 expressed genes involved in nitrogen (a), phosphorus (b), and toxin (c) transport and metabolism. Red and blue points represent those genes expressed significantly (|fold change| ≥ 1.5 and $P < 0.05$) up-regulated and down-regulated, respectively, while gray points represent insignificant expressions of genes between clusters. Detailed information on genes involved in nitrogen, phosphorus, and toxin transport and metabolism was listed in Tables S6–S8.

October. Monthly monitoring conducted by TLLER showed that the concentration of nitrate (NO_3^-) in the late bloom season (August to October; mean = 0.21 mg L^{-1}) was much lower than that in the early bloom season (June to July; mean = 0.65 mg L^{-1}) (Figure S6). On the basis of the results of *in situ* microcosm nutrient dilution bioassays and mesocosm nutrient

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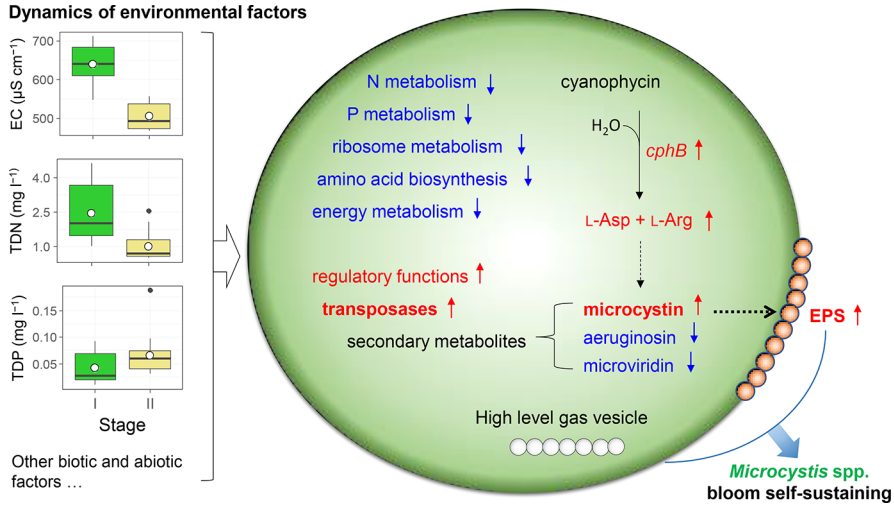

Figure 6. Schematic diagram describing natural *Microcystis* spp. transcriptomic responses of main functional categories and metabolisms to environmental variations in Lake Taihu. Compared with earlier months (Stage I, June and July) of the bloom season, electrical conductivity (EC) and the concentrations of total dissolved nitrogen (TDN) were decreased (Kruskal−Wallis rank sum test, $P < 0.001$) during late months (Stage II, August to October), while the concentration of total dissolved phosphorus (TDP) was increased ($P < 0.05$). Under such circumstances (N-limitation accompanied with changes of other environmental parameters), transcript abundances of many genes involved in N, P, ribosome, energy metabolism, and amino acid biosynthesis were down-regulated, while many genes involved in regulatory functions, transposases, and microcystin were up-regulated, suggesting low growth rate and efficient environmental adaptation through genomic rearrangement. In addition, the biodegradation product of cyanophycin (up-regulated of *cphB*) may contribute to the increased expression of microcystin, which promoted the transcripts of extracellular polysaccharide (EPS). Meanwhile, *Microcystis* spp. had abundant transcripts for gas vesicle genes, which enhanced the buoyancy of *Microcystis* aggregates. Collectively, all these transcriptomic responses indicated the ecophysiological changes of the toxic *Microcystis* spp. from rapid growth to bloom maintenance and duration.

addition experiments, Xu et al.[23] found that N-availability controlled summer−fall blooms and predicted 0.80 mg L^{-1} as the limiting N concentration for intrinsic growth of *Microcystis* in Lake Taihu. In the current study, compared with early bloom season, the relatively low concentration of TDN and low N/P ratio in late bloom season suggest that the *Microcystis* population was at least partially N-limited. However, expression of genes encoding the urease enzyme (*ureABCDEFG*) and urea transporters (*urtBCDE*) were not altered significantly (Table S6), and expression of genes involved in nitrate transport (*nrtACD*) and glutamate synthesis (*gltBDSX*) decreased significantly (Figure S7a). In contrast, a gene involved in the breakdown of cyanophycin (*cphB*) displayed large increases in expression (Figure 5a), indicating degradation of cyanophycin and utilization of stored N by *Microcystis* cells. Cyanophycin serves as a putative reservoir for newly assimilated N when cyanobacteria are exposed to an excess of N in the environment:[47] they then consume that cyanophycin when exogenous N is depleted.[9,48] This process separates environmental supply from the metabolic demands within the cyanobacterial cells.[49] Early in the bloom season, *Microcystis* appears to be scavenging N for rapid growth and storage as cyanophycin. However, as the bloom progresses, *Microcystis* cells begin utilizing stored N (cyanophycin degradation) for survival and bloom maintenance as surrounding N becomes limiting. These observations would explain why genes involved in urease enzyme, urea transporters, nitrate transport, and glutamate synthesis had not up-regulated (Figure 5a; Table S6 and Figure S7a).

Previous studies have demonstrated that *Microcystis* is highly effective at sequestering and storing P as polyphosphate bodies, even in low P environments.[50−52] In this study, we found higher concentrations of TDP from August to October (average = 65 $\mu g\ L^{-1}$) compared with June and July (average = 42 $\mu g\ L^{-1}$). Accordingly, P-scavenging genes (*pstSCAB*) and an alkaline phosphatase gene (*phoX*) decreased with higher TDP, suggesting P-limitation in June and July.[23,27] This is consistent with a report that the largest up-regulation of the P-scavenging gene set *pst* was observed at stations with the lowest soluble reactive phosphorus (SRP) level in Lake Erie.[53] However, another putative alkaline phosphatase gene (MAE_16640; a classic marker for P-limitation)[21,38] increased in transcript abundance (fold change = 4.19, Figure 5b) during late bloom season. Steffen et al.[54] have shown that this putative alkaline phosphatase (MAE_16640) was more strongly regulated by urea rather than phosphate, with increased expression under low urea concentrations (further indication of N-limitation in the late bloom season), illustrating the complicated regulation mechanisms of P-related genes under the dynamic nutrient concentrations *in situ*.

MC Genes of *Microcystis* Responding to Temporal Shifts and Ecological Function. MCs are cyclic heptapeptides which can cause liver disease as well as nephrotoxicity.[37] N uptake is thought to be one of the most important modulators controlling cellular MC quota.[22,55,56] As a N-rich secondary metabolite, the concentration of MCs generally increases under high concentrations of ammonium or urea.[57,58] In this study, we found highly up-regulated microcystin synthase genes (*mcyABCD*) during late bloom season (Figure 5c), suggesting higher levels of MC correspond with trends in actual measurements (Figure S1). This response was unexpected because the average concentration of TDN in the water column was lower than in June and July. However, along with cyanophycin biodegradation, [cyanophycin + $n H_2O \rightarrow n$[L-Asp (4-L-Arg)] dipeptide],[59] indicated by the up-regulation of *cphB* (Figure 5a), degradation products L-Asp and L-Arg should accumulate. A gene encoding aspartate racemase (*racD*), i.e., *mcyF*,[60] increased (fold change = 1.94) though the *P* value ($P = 0.067$) did not reach our chosen significance level. Under these

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circumstances, the cellular materials for MC synthesis (i.e., L-Arg and D-Asp) may come from cyanophycin biodegradation.

MC may trigger EPS as a way to maintain the bloom later in the season. Gan et al.[61] found that MC exposure can trigger up-regulation of polysaccharide biosynthesis-related genes and subsequently the production of EPS, which has been shown to enhance colony formation in *Microcystis*. In addition, the expression of gas vesicle genes (*gvp* gene set) was found to be positively related to buoyancy of *Microcystis*, thus facilitating bloom formation.[62] During this study, we observed a simultaneous increase in transcript abundance for genes involved in MC synthesis (Figure 5c) and EPS production (Table S9). Furthermore, high expression of gas vesicle genes (*gvpAI-AIII* and *gvpC*) were also observed (Figure 4). These results, as well as the high concentration of Chl-*a* (a proxy for cyanobacterial biomass) during August to October (Figure S1), suggest that the higher concentration of MCs in later bloom season in Lake Taihu may play an important role in maintaining a large scale *Microcystis* bloom by means of influencing enhanced EPS production. This agrees with previous reports highlighting the positive relationship between MCs and *Microcystis* colony size[61,63] and Chl-*a* concentration, respectively.[16,32]

An important caveat to our results is the "snapshot" nature of the sampling. The collection of samples at single time points during a bloom season that extends over 5 months captures only a small subset of cyanobacterial physiology and does so in the background of other short-term variations (e.g., diel cycles). Additional consideration should be given to variations within *Microcystis* populations over time and space in Lake Taihu. Toxic *Microcystis* genotypes in Lake Taihu have varied from 12.5% to 65.6%, with an average value of 27.9%.[32] Chen et al.[64] found that the high concentrations of MCs in Lake Taihu were consistent with the presence of *Microcystis aeruginosa* and *Microcystis flos-aquae*, while low concentrations of MCs were associated with *Microcystis wesenbergii* (nontoxic). While our study is a first step toward providing an overall understanding of ecophysiology of this toxic, bloom-forming *Microcystis* spp., future simulated experiments *in situ* providing better temporal resolution of the complete blooming process will be useful for verifying patterns observed in this study.

Effects of Environmental Factors on Global Gene Expression and the Physiology of *Microcystis*. In this study, EC was found to be one of the most strongly correlated environmental factors with temporal variation in gene expression of the *Microcystis* population. In Lake Taihu, EC peaked in June, was positively correlated with rainfall in the catchment (Figure S8a), and predictably dissolved anions such as Na^+, K^+, Cl^-, and SO_4^{2-} (Figure S8b). Nonpoint sources of pollution, such as agricultural fields, have become the main contributors to the eutrophication of Lake Taihu.[65] The dynamics of EC affected by the fluxes of agricultural nonpoint source pollution along with rainfall runoff may drive shifts of *Microcystis* gene expression in Lake Taihu. As an integrated index of dissolved inorganic ions, EC can affect cyanobacterial physiological and biochemical processes, such as Na^+ and K^+ transport,[66] self-organization behavior of EPS,[67] and cyanobacterial community succession.[68] In Lake Taihu, Na^+ concentrations were also correlated with the relative abundance of MC-RR and MC-LR,[69] and total dissolved solids (highly correlated to EC) contributed significantly to lytic−lysogenic cycle genes of *Microcystis* phage.[33]

The transcription of rRNA and ribosomal proteins is generally related to the growth rate of prokaryotes.[70] A recent culture study also suggested that nutrient limitation resulted in reduced growth rate of *M. aeruginosa* LE-3 and a subsequent large-scale decreased transcription of genes involved in ribosomal synthesis and amino acid biosynthesis.[21] Similarly, we observed more than half of the genes involved in ribosomal protein synthesis and modification were down-regulated in transcript abundance and none were up-regulated in the late bloom season in this study (Figure S7b), suggesting depressed growth of *Microcystis* spp. in the late bloom season responding to the dynamics of environmental factors, including nutrients.

Along with growth, the evolution and adaptation of populations of *Microcystis* may be critical in its survival after bloom peak occurs. Transposases are thought to be the most ubiquitous genes in nature and play beneficial roles in environmental adaptation by means of offering selective advantage to the host genomes.[71,72] Transposase genes encode DNA-binding enzymes that catalyze "cut/copy-and-paste" reactions promoting the movement of DNA segments (i.e., insertion sequences, ISs) to new sites.[73] Previous studies have found extremely high content of mobile ISs (>10% of the genome) in *M. aeruginosa* NIES 843[74,75] and have linked their expression to the nutrient sources the cells were consuming.[38,54] In *Microcystis*, these transposases are thought to provide a higher degree of genomic plasticity and potentially stronger adaptation compared with other cyanobacteria in freshwater ecosystems. During this study, we observed 202 genes encoding transposases that were differentially expressed in the late bloom season, of which 189 of them were up-regulated with a majority of them belonging to the IS605 family (Table S5). This suggests active genomic rearrangement to adapt to the changed environment of the late bloom months. These observations provide support for the idea that nutrient sources not only drive biomass accumulation but also may alter the genomic architecture (and thus evolutionary trajectory) of *Microcystis* populations.

Our observations point to key changes in microbial physiology (via cellular transcriptomes) that occur throughout the bloom season as cells shift from active bloom formation to population maintenance. These same observations and the information provided in this study also highlight the complicated nature of bloom dynamics with respect to *Microcystis* populations and toxin production: it is becoming increasingly clear that no single variable promotes or constrains these populations. Moving forward, understanding why and how blooms occur will require data collected not only at the level of individual species but also at the level of environmental populations and likely in consideration of the entire microbial community within individual systems.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b01066.

Raw environmental parameters; summary of RNA sequence information; amount of RNA yielded; comparison tables of genes involved in transposase, N, P, toxin, and EPS; dynamics of main environmental parameters; community makeup; cluster analysis of metatranscriptomics mapped to the genome of *M. aeruginosa* NIES 843; volcano plot of differentially expressed genes;

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linkage tree; boxplot of NO_3^- during June to October 2014; KEGG maps of N and ribosome pathways; relationships between rainfall, dissolved anions, and EC (PDF)

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