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**Genetic Characterization of Saxitoxin Producing Cyanobacteria Associated with Western
Lake Erie Harmful Algal Blooms**

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Abstract

Saxitoxins (STXs) are a group of closely related neurotoxins and among the most potent natural toxins known. While genes encoding STX biosynthesis have been observed in Lake Erie, the organism(s) responsible for producing STXs in the region have not been confirmed. We used metagenomic tools to identify a full suite of STX biosynthesis genes in a high-quality metagenome-assembled genome (MAG) from the Anabaena-Dolichospermum-Aphanizomenon (ADA) clade of cyanobacteria. The order and sequence of *sxt* genes suggest the Lake Erie MAG is capable of producing STX as well as other STX congeners. The absence of a *sxtX* gene suggests an inability to produce neoSTX, one of the most potent congeners of STX. We also recovered highly similar ADA MAGs that did not contain STX genes, implying gene loss or horizontal gene transfer. The full suite of STX biosynthesis genes was observed in metagenomic datasets across 13 of 123 unique sampling dates between 2014 and 2022, with occurrences spanning from July 6 to September 19. Our results also reveal trends in the increasing abundance of ADA MAGs during late-season blooms. Collectively, this study provides a foundation for understanding a potential new front of threats to Lake Erie water quality.

Introduction

Saxitoxins (STXs) are primarily sourced from freshwater cyanobacteria and marine dinoflagellates and have been extensively studied as the causative agent for paralytic shellfish poisoning (PSP) in marine environments. Research on STXs in freshwater ecosystems is limited compared to other cyanotoxins like microcystin. Despite no reported human fatalities from STX exposure in freshwater, incidents of sheep mortality due to STX-contaminated cyanobacterial blooms highlight potential risks (Negri et al., 1995; Smith, 2000). Given the significance of many freshwater bodies as drinking water sources, recognizing STX presence during water treatment processes is imperative (Orr et al., 2004; Westrick, 2008). In freshwater, instances of STX exposure have been linked to recreational activities, with exposure able to occur through inhalation and skin contact (Rapala et al., 2005). STXs can also bioaccumulate in freshwater fish and shellfish, posing concerns for human consumption (J. P. Berry & Lind, 2010; da Silva et al., 2011; Galvão et al., 2009; Ibelings & Chorus, 2007; Negri & Jones, 1995). The proliferation of cyanobacteria harmful algal blooms (cyanoHABs) due to climate change further amplifies concerns about STX presence in freshwater environments (Paerl & Huisman, 2009).

More than 50 STX congeners have been identified, including non-sulfated (STX and neoSTX), mono-sulfated (gonyautoxins (GTxs 1-6)), dicarbamoyl (dcSTX, dc-neoSTX, dcGTxs 1-4) and di-sulfated (C1-4) forms (Christensen & Khan, 2020). STX congeners display variable toxicity, with STX, neoSTX, and gonyautoxins (GTx) consistently identified as the most potent (Ballot et al., 2016; Selwood et al., 2017; Suarez-Isla, 2014; United Nations Environment Programme, 1984). Due to its extreme toxicity, STX is classified as a bioweapon under the Chemical Weapons Convention (Al-Tebrineh et al., 2010; Sierra & Martínez-Álvarez, 2020).

Nine genes in cyanobacteria (*sxtA*, *sxtG*, *sxtB*, *sxtD*, *sxtS*, *sxtU*, *sxtI*, *sxtH*, and *sxtT*) have been proposed to play a direct role in STX biosynthesis (Kellmann et al., 2008). Additional genes have been hypothesized to alter STX into distinct congeners (*sxtL*, *sxtN*, *sxtX*) (Mihali et al., 2009). The *sxtA* gene takes a central role in initiating STX biosynthesis operon through catalyzing a unique Claisen condensation reaction. The crucial role played by the *sxtA* gene in

STX biosynthesis makes it an effective qPCR target for accurately quantifying STX producers in the environment (Al-Tebrineh et al., 2010; Pearson et al., 2010).

Western Lake Erie (WLE) is especially susceptible to toxic cyanoHABs, including those with the potential to produce saxitoxin (Watson et al., 2016). This vulnerability is underscored by the presence of several municipal water intakes, notably including Toledo, OH, where a nearly three day "do not drink" advisory was issued in 2014 as a result of microcystins persisting in finished water (Steffen et al., 2017; Wynne & Stumpf, 2015). The 2014 Toledo Water Crisis left over 400,000 residents without access to safe tap water. Additionally, the area relies heavily on recreation, tourism, and a significant commercial fishing industry, potentially heightening the exposure risks to cyanoHABs. While considerable efforts have been dedicated to monitoring and understanding the development, movement, and toxicity of cyanoHABs in WLE, research into cyanotoxins beyond microcystin in this region remains notably understudied. A recent investigation into the diversity of cyanobacterial biosynthetic gene clusters in WLE, which identified the presence and expression of cyanotoxins and cyanopeptides beyond microcystins, highlights the need for expanded research on this topic (Yancey et al., 2023).

Use of ELISA to identify saxitoxin and qPCR to detect *sxtA* genes in Lake Erie has confirmed the presence of both the toxin and key genetic markers responsible for STX production (Chaffin et al., 2019; Nauman et al., 2024; Ohio Environmental Protection Agency, 2022). Despite preliminary evidence from Chaffin et al. (2019) suggesting a potential association between the presence of the *sxtA* gene and the cyanobacterial genus *Dolichospermum* based on flowCAM observations, a confirmed source of STXs in WLE or the Great Lakes has not been established. STXs have been found to be produced by several cyanobacterial genus present in WLE. Potential STX producers include *Microcystis*, the dominant genus of WLE cyanoHABs, along with members of the *Anabaena*, *Dolichospermum*, and *Aphanizomenon* (ADA) cyanobacteria clade, as well as *Cylindrospermopsis*, and *Lyngbya* (Conroy et al., 2007; Naknaen et al., 2021; Wiese et al., 2010). STX genes have not been identified in any ADA-clade or *Microcystis* genomes characterized in the Great Lakes (Sheik et al., 2022; Yancey et al., 2023). The potency of STX paired with the potential for production by multiple, currently unidentified, species of cyanobacteria creates a situation of concern for cyanoHABs in WLE.

The existence of extensive monitoring efforts in WLE offers opportunities to investigate the presence of STXs. In this study, metagenomic sequence data collected from both routine and opportunistic cyanoHABs monitoring efforts (Boegehold et al., 2023) was used to assess the potential for STX production and its distribution across WLE. The DNA sequence database used in this study, the Great Lakes Atlas of Multi-omics Research (GLAMR, greatlakesomics.org), included more than 500 samples of sequenced metagenomic data collected in WLE between 2014 and 2022 (Chaffin et al., 2021; Colleen E. Yancey et al., 2022; Den Uyl et al., 2022; Koeppel et al., 2022; McKindles Katelyn M. et al., 2020). Incorporating GLAMR into this research provides a robust methodology for pinpointing neurotoxin-producing organisms and understanding their spatial and temporal distribution. This framework is essential for advancing knowledge and awareness of the associated risks of STXs in WLE.

Results

Identification of a saxitoxin (STX) source and additional ADA MAGs

Within GLAMR, *de novo* assembly of metagenomic reads from 570 WLE samples produced a total of 1,844 metagenome-assembled genomes (MAGs) with greater than or equal to

90% completeness and less than 10% contamination. Dereplication of MAGs using a 98% average nucleotide identity (ANI) threshold resulted in 850 representative MAGs generated. After dereplication, a single high-quality representative MAG belonging to the cyanobacterial ADA clade was identified possessing a full suite of biosynthetic genes for STX production (STX+). This MAG, designated LE20-WE8, was generated from a sample taken at the WLE routine monitoring station WE8 on July 6, 2020. LE20-WE8, classified as the species *Dolichospermum sp000312705*, has high completion (96.78%) and low contamination (2.56%) (Table 1) and is the only representative MAG in this study possessing the biosynthetic potential to produce STXs. Ten additional representative MAGs from the GLAMR database were identified in WLE belonging to the ADA clade (Table 1). Genes specific to STX biosynthesis were not detected (STX-) in these ten MAGs. Representative ADA MAGs were also examined for the presence of the cyanotoxin genes known to produce microcystin (*mcyE gene*), anatoxin A (*anaC, anaF*), cylindrospermopsin (*cyrA*), guanitoxin (*gntF*), and β-Methylamino-L-alanine or BMAA (*sbnA-B*) using blastn. None of these additional cyanotoxin genes were detected.

MAG	STX Potential	Date of collection	Latitude	Longitude	Station	GTDB-Tk species classification	Completeness	Contamination	Strain heterogeneity	N50	GC	Number of contigs	Size mb
LE20-WE8	+	6-Jul-20	41.8192	-83.3592	WE8	<i>Dolichospermum sp000312705</i>	96.78	2.56	58.82	7007	0.39	903	4.577
LE17-WE2	-	7-Aug-17	41.7633	-83.3303	WE2	<i>Dolichospermum sp000312705</i>	93.05	2.83	60	18103	0.38	348	4.338
LE17-WE12	-	11-Sep-17	41.7032	-83.254	WE12	<i>Dolichospermum circinale</i>	90.54	6.81	62.5	2791	0.38	1678	4.108
LE20-WE12	-	5-Oct-20	41.704	-83.2552	WE12	<i>Dolichospermum circinale</i>	95.2	7.85	82	3051	0.37	1664	4.352
LE21-WE12	-	21-Sep-21	41.7041	-83.2529	WE12	<i>Dolichospermum circinale</i>	90.7	1.08	85.71	11487	0.38	414	3.569
LE22-WE12	-	30-Sep-22	41.7058	-83.2556	WE12	<i>Dolichospermum circinale</i>	99.55	0.22	100	24272	0.37	342	4.773
LE21-ER30	-	7-Apr-21	42.4295	-81.2044	ER30	<i>Dolichospermum flosaqueae</i>	98.22	0.22	50	33828	0.37	190	3.905
LE16-WE8	-	12-Sep-16	41.8322	-83.3589	WE8	<i>Dolichospermum flosaqueae</i>	90.06	4.83	40.74	3373	0.37	1734	3.787
LE19-WE12	-	3-Sep-19	41.7034	-83.2558	WE12	<i>Dolichospermum flosaqueae</i>	98.91	0.78	50	20287	0.37	293	4.238
LE20-WE2-Aug	-	10-Aug-20	41.7614	-83.3329	WE2	<i>Cuspidothrix issatschenkoi</i>	97.33	4.19	80.95	4975	0.38	1200	4.345
LE20-WE2-Sep	-	9-Sep-20	41.7628	-83.3297	WE2	<i>Cuspidothrix issatschenkoi</i>	92.37	1.85	92.31	8046	0.38	598	3.602

Table 1. Metadata and summary statistics of representative ADA MAGs obtained from WLE samples using the GLAMR database (greatlakesomics.org).

Both LE20-WE8 (STX+) and LE17-WE2 (STX-) clustered into a distinct ADA branch that contains several strains isolated from the Great Lakes, including *Dolichospermum* sp. SB001 (Lake Superior metagenome), *Anabaena* sp. AL09 (Lake Huron culture), *Anabaena* sp. LE011-02 (Lake Erie culture) and LE14-WE4 (Lake Erie metagenome) (Driscoll et al., 2018; Sheik et al., 2022; Yancey et al., 2023) (Fig. 1). GTDBTK annotations designate individuals within this branch as *Dolichospermum sp000312705*, whereas earlier research has sometimes used the name *Dolichospermum lemmermannii* (Sheik et al., 2022). Additional ADA MAGs recovered here also fell into branches with previously identified species. LE20-WE2-Aug and LE20-WE2-Sep clustered with *Cuspidothrix issatschenkoi*. LE16-WE8, LE19-WE12, and LE21-ER30 were in a branch with *Dolichospermum flosaqueae* and are very closely related to LE14-WE12 (97.9% - 99.6% ANI) which came from WLE in August 2014 (Yancey et al., 2023). The largest diversity of MAGs identified in this study (LE17-WE12, LE20-WE12, LE21-WE12, and LE22-WE12) clustered in a branch with *Dolichospermum circinale*. These MAGs were closely related (96.8% - 97.1% ANI) to an STX producer, *D. circinale* AWQC131C isolated from Lake Cargelligo,

NSW Australia (Llewellyn et al., 2001). There does not appear to be any relationship between the genetic potential to produce STXs and strain placement within the phylogenomic tree of the ADA clade (Fig. 1).

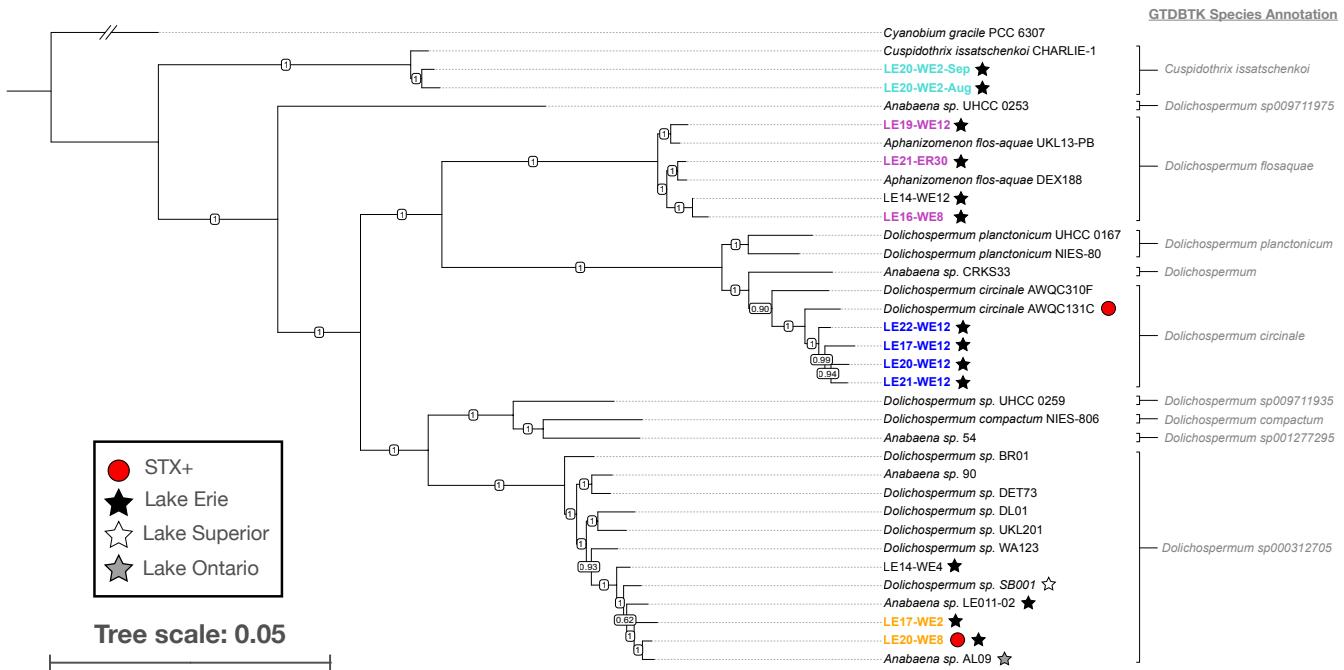


Figure 1. Phylogenomic tree of representative ADA clade members constructed using 251 housekeeping genes and a maximum likelihood approach through the GToTree workflow. The dataset incorporated ADA genomes from prior investigations (Dreher et al., 2021; Österholm et al., 2020; Sheik et al., 2022; Yancey et al., 2023). MAGs obtained from this study are highlighted in colored and bolded text. Colors reflect phylogeny and correspond to Figure 4 to aid comparison. Stars indicate ADA strains from the Great Lakes, sourced from: Lake Erie (black), Lake Superior (white), and Lake Ontario (grey). Red circles identify STX+ strains. Numbers on branches are bootstrap values, support metrics that reflect the robustness of relationships among taxa based on resampling.

24 additional MAGs from the ADA clade were identified using a stricter ANI threshold for dereplication compared to the criteria used for establishing representative MAGs (99% ANI instead of 98%) (Fig. S1). These 24 MAGs were also examined for the presence of cyanotoxin-encoding genes, but none were identified. Despite appearing directly adjacent to LE20-WE8 (STX+) on the phylogenomic tree, the MAG samp_2047_concoct_1911 lacked genes associated with STX production (STX-). We identified that the *sxt* genes were instead assigned to an *alphaproteobacteria* MAG (samp_2047_concoct_1969) during the binning process by CONCOCT. *sxt* genes were ultimately determined not to belong to the *alphaproteobacteria* bin after manual QC and genome refinement via Anvi'o (Eren et al., 2015). There is no record of *alphaproteobacteria* being able to produce STXs, further suggesting the contigs were incorrectly assigned. These contigs containing *sxt* genes were annotated to belong to *Dolichospermum* by kaiju. Binning by MaxBin also assigned these *sxt* genes to a MAG annotated as

Dolichospermum sp000312705, which matches the species annotation of LE20-WE8 (STX⁺). We conclude *sxt* genes contained within the MAG samp_2047_concoct_1969 were misassigned during the generation of bins using the automated binner CONCOCT. To confirm that STX biosynthesis genes weren't misassigned for other MAGs that were closely related to STX⁺ organisms, such as LE17-WE2 (STX⁻), metagenomic sample reads were examined using blastn for *sxt* genes. There were no *sxt* genes present in the sample reads from which LE17-WE2 (STX⁻) was generated. Further analysis of MAGs will be focused only on representatives determined using the ANI threshold of 98%, referred to hereafter as representative MAGs (n = 11, Table 1).

STX biosynthesis genes

The STX biosynthesis operon identified in LE20-WE8 was divided across three contigs (Fig. 2). Each contig contained *sxt* genes in nearly the same order and orientation as the STX biosynthesis operon identified in *Aphanizomenon* sp. NH-5, a culture isolated from a small pond near Durham, New Hampshire (Mihali et al., 2009). The most notable differences between the gene clusters identified in this study and *Aphan.* sp. NH-5 are the absence of *sxtX*, *sxtW*, and a partial length *sxtD* gene in LE20-WE8.

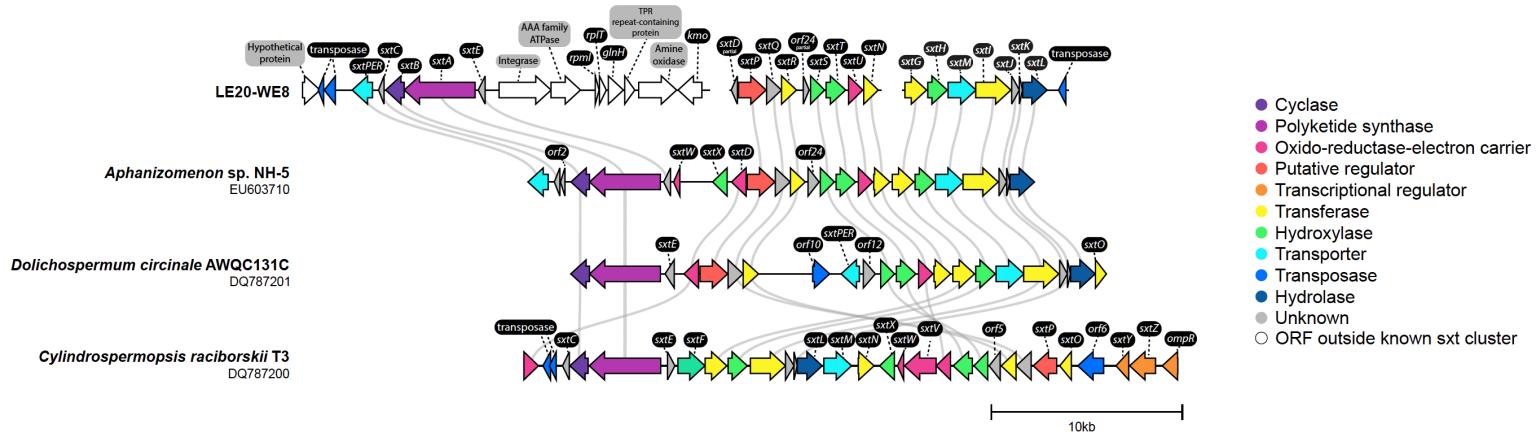


Figure 2. Comparison of STX gene cluster structure between LE20-WE8, *Aphanizomenon* sp. NH-5, *Dolichospermum* circinale AWQC131C, and *Cylindrospermopsis* raciborskii T3. GenBank accession numbers are listed below organism names. Gray lines indicate homologous genes sharing ≥80% nucleotide sequence identity. Gray labels show CoCoding Sequence (CDS) annotations. Figure adapted from Mihali et al., 2009.

Only 156bp of the 759bp *sxtD* gene sequence from *Aphan.* sp. NH-5 aligned with LE20-WE8 contigs (99.4% ID; 21% coverage) via blastn. An unassigned contig (402bp) from sample LE20-WE8 showcased high sequence similarity (blastn 99.0% ID; 100% coverage) to a distinct section of the *sxtD* *Aphan.* sp. NH-5 reference. The length of this contig (402bp) makes it too short to reliably bin using automated methods. Considering this additional fragment and the partial *sxtD* gene identified in LE20-WE8, 74% of the *sxtD* gene is accounted for with greater than 99% blastn ID.

To examine the distribution and diversity of STX biosynthesis genes across WLE, metagenomic reads were mapped back to the assembled *sxt* genes from the three LE20-WE8 contigs (Fig. S2). Metagenomic read mapping resulted in 22 samples having >60% coverage of each of the three reference contig sequences (Table S1). All results with >60% coverage of each

of the three reference sequences had at least 95% nucleotide identity between each mapped alignment and its complimentary reference sequence (Fig. S3-S5). The 22 samples with substantial read mapping alignment to *sxt* genes were collected across 13 unique sampling dates, encompassing a seasonal range from as early as July 6 to as late as September 19, spanning years 2016 to 2021.

ADA MAG metabolic pathways

KEGGDecoder was used to annotate metabolic pathways in the 11 representative ADA MAGs. Notable pathways of LE20-WE8 include ferrous iron transporter *feoB*, which was only found in the two representative *D. sp000312705* MAGs (Fig. 3). The pathway for synthesis of the carotenoid zeaxanthin diglucoside was also possessed by *D. sp000312705* MAGs, LE20-WE8 (STX+), LE17-WE2 (STX-) and one *C. issatschenkoi* MAG, LE20-WE2-Aug. Nitrogen fixation pathways were present in KEGGDecoder annotations for all *Dolichospermum* MAGs, ranging between 33-66% complete, but were absent in *Cuspidothrix* MAGs. The nitrogen fixation pathway for LE20-WE8 was identified by KEGGDecoder as 66% complete, with two out of the three enzymes required for nitrogen fixation, *nifK* (K02591) and *nifD* (K02586), present. Genome annotation using Bakta revealed the remaining *nif* gene, *nifH* (K02588) in LE20-WE8. Additional *nifH*, *D*, *K* genes, beyond those annotated in KEGGDecoder, were also identified in six additional *Dolichospermum* MAGs through Bakta annotation (Table S3). No additional *nif* genes were identified in *Cuspidothrix* MAGs by Bakta.

All representative MAGs exhibited pathways facilitating the uptake of additional nutrients via urea, phosphate, and phosphonate transporters. Urease subunits were identified in Bakta annotations for all eleven representative MAGs, including the alpha, beta, and gamma subunits in LE20-WE8 (Table S3). Additionally, all representative MAGs featured pathways involved in sulfolipid biosynthesis, as well as the production of carotenoids nostoxanthin, myxoxanthophylls, and astaxanthin (Fig. 3).

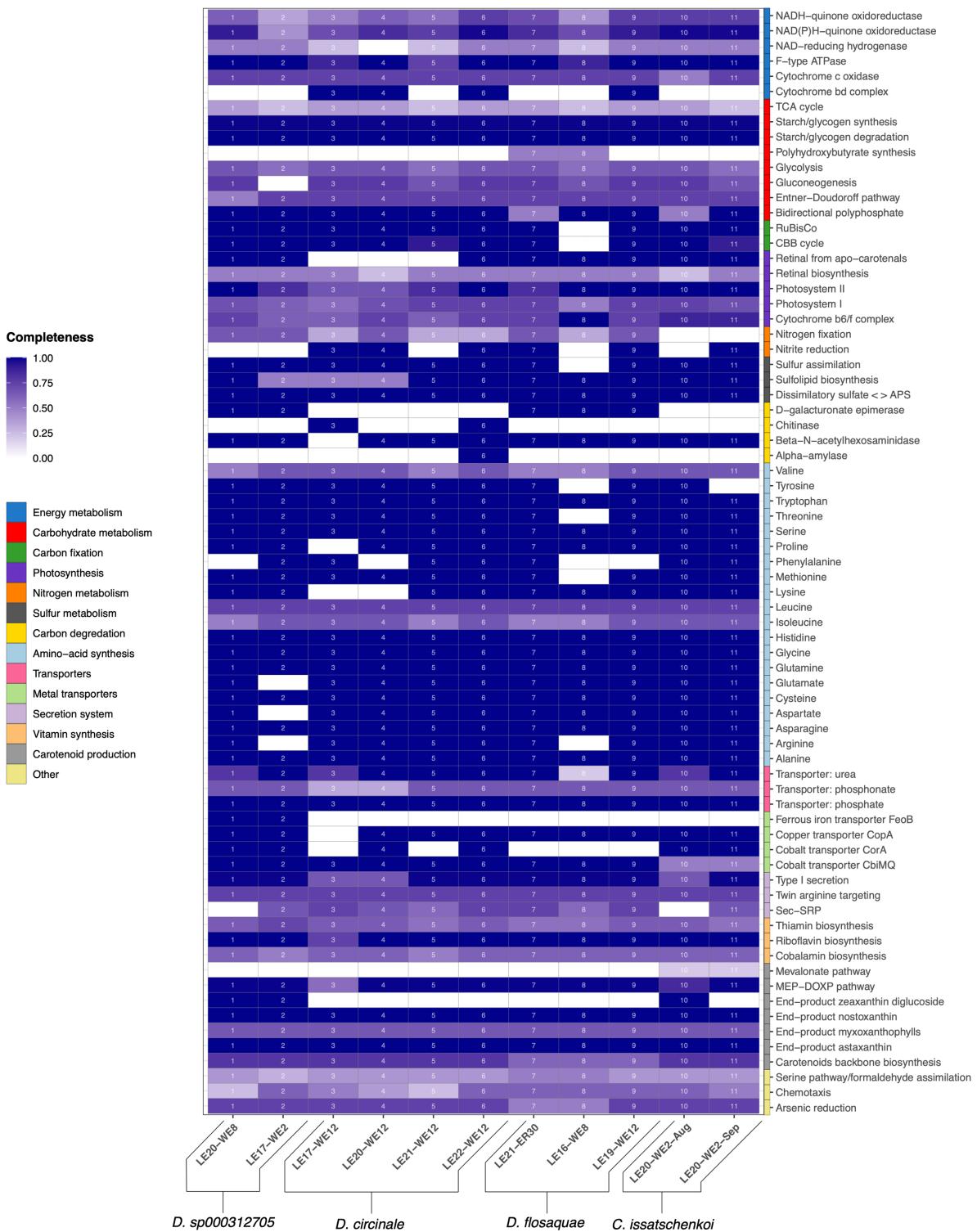


Figure 3. Heatmap visualization of KEGGDecoder functional analysis for representative MAGs

Temporal and spatial abundance of ADA MAGs

Metagenomic read mapping to representative ADA MAGs allowed for the calculation of relative organism abundance across stations and dates of sample collection (Fig. 4). Given the

difficulty of short read mapping to discriminate different strains within the same species, reads per kilobase million (RPKM) values were combined for MAGs sharing the same species annotation. Across the sampling season, ADA MAGs were consistently identified in metagenomes, indicating a persistent presence. However, their abundance and distribution displayed substantial variability between years. In years 2014, 2017, and 2018, ADA MAGs were detected for only 4 weeks, highlighting the dynamic nature of their occurrence. Multiple species often co-occurred in the same sample (37% of samples). When just one species was detected, it was most often *D. circinale* (42% of samples), followed by *D. flosaquae* and *D. sp000312705* (15% and 5% of samples, respectively).

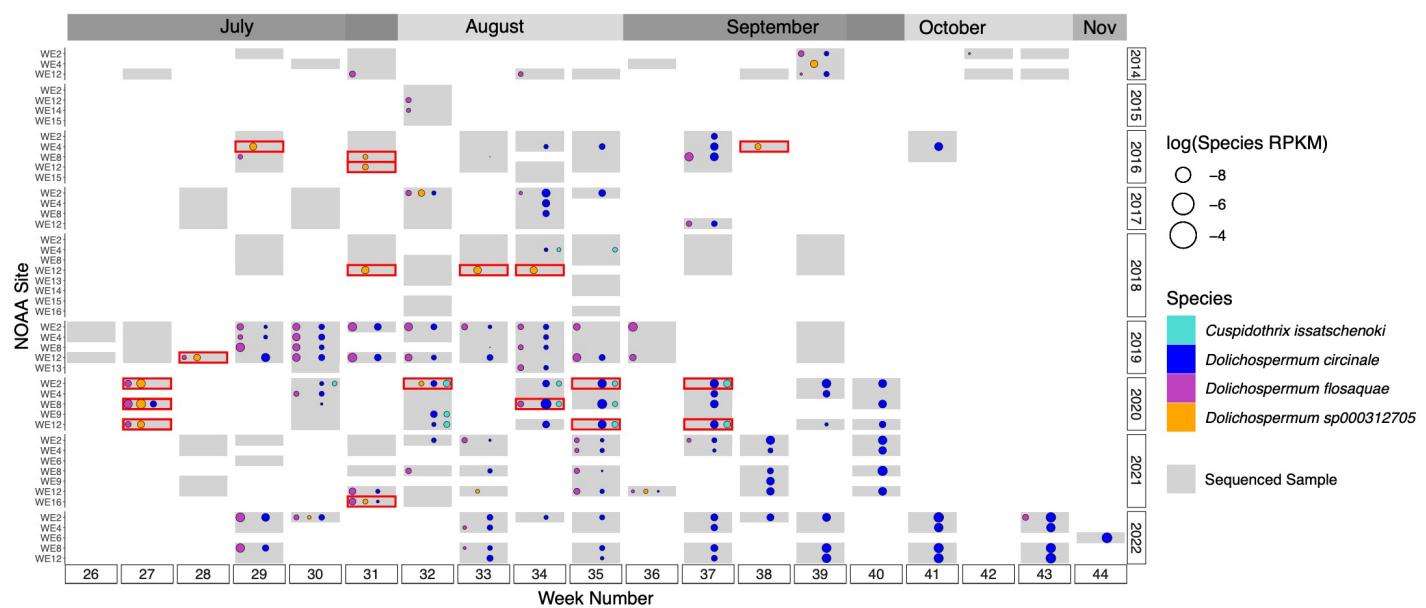


Figure 4. Reads per kilobase million (RPKM) determined from metagenomic read mapping of MAGs. Dot size determined from summing RPKM of MAGs that belong to the same species. WE20-WE8 (STX+) and LE17-WE2 (STX-) = *D. sp000312705*; LE17-WE12, LE20-WE12, LE21-WE12, and LE22-WE12 = *D. circinale*; LE21-ER30, LE16-WE8, and LE19-WE12 = *D. flosaquae*; and LE20-WE2-Aug and LE20-WE2-Sep = *C. issatschenkoi*. Only the highest RPKM value is shown for each unique species and week combination. Red outlines indicate the presence of a STX biosynthesis operon determined through read mapping to assembled *sxt* genes from LE20-WE8 contigs ($\geq 60\%$ coverage and $\geq 95\%$ nucleotide identity) (Fig. S3-S5). NOAA Site based off of routine WLE monitoring stations (Boegehold et al., 2023) and expanded to include samples collected within a 5 km radius of each station.

The presence of the species *D. sp000312705* is particularly significant due to the identification of the saxitoxin-producing capability of MAG LE20-WE08, a species member. The maximum RPKM belonging to species *D. sp000312705* reached 6.93e-03 on July 6, 2020, at WE8 (Table S2). This was the same sample that representative MAG LE20-WE8 was sourced. *D. sp000312705* prominently occurred at station WE12, being identified at that site on 8 out of 15 of the species detection dates. Dates of *D. sp000312705* observations are concentrated in the months of July (n=5), August (n=7), and September (n=3). In 2014, 2016, and 2018, whenever *D. sp000312705* appeared, it was consistently the only ADA species from this study identified in

a sample ($n = 10$). For other years where *D. sp000312705* appeared (2017, and 2019-2022) it co-occurs with other ADA species in 10 of 11 samples.

D. sp000312705 presence corresponds well with the occurrence of STX biosynthesis genes determined by metagenomic read mapping (15 of 22 samples). Species *D. circinale* and *C. issatschenoki* were both present in the seven instances where STX biosynthesis genes were identified through read mapping and *D. sp000312705* was not. *D. sp000312705* also occurs where there are no significant STX biosynthesis genes mapped on five occasions, including WE2 in week 32 (Aug, 7th) of 2017, the sample that representative MAG LE17-WE2 (STX-) was sourced.

D. circinale stood out as the species exhibiting the highest relative organism abundance in a sample, reaching 0.207 RPKM within a late-season cyanobacterial bloom in early November of 2022 (week 44, Figure 4). Notably, the same sample also recorded the highest individual MAG abundance, corresponding to LE22-WE12 at 0.167 RPKM. The nine highest species RPKM values identified were from late season 2022 cyanobacterial bloom(s) and belonged to *D. circinale*. LE22-WE12 was the most abundant *D. circinale* MAG in each of these occurrences. *D. circinale* appears to have an increased presence in years 2020-2022, particularly after week 37 each season. *D. circinale* is present in 4 of 12 sampling dates collected after week 37 between years 2014-2019, while it is present in 16 of 16 sampling dates after week 37 between 2020-2022.

The least frequently detected species from metagenomic read mapping was *C. issatschenoki*, observed only in samples collected during 2018 and 2020 sampling years. *C. issatschenoki* did emerge as the second most frequently observed species by date in 2020 (5 of 10 sampling dates, 13 of 49 samples). *D. flosaquae* showcased a variable presence in WLE. *D. flosaquae* stood out as the most frequently observed species in 2019, by both date and sample count (15 of 22 sampling dates, 37 of 120 samples), while it was absent in all samples collected in 2018.

Discussion

To identify the organism(s) responsible for producing potential STXs, a potent group of neurotoxins (Wiese et al., 2010) with supporting biochemical (Ohio Environmental Protection Agency, 2022), environmental (Watson et al., 2016), and genetic evidence (Chaffin et al., 2019; Nauman et al., 2024) in Lake Erie, we analyzed metagenomes from 570 samples collected across WLE from 2014-2022. We identified one MAG containing a full suite of STX biosynthesis genes, attributing it to *D. sp000312705* through phylogenomic analysis. Beyond identification of organisms responsible for STX production, these results offer insights into potential biosynthetic products of the identified *sxt* genes. Additionally, the metabolic potential and environmental distribution of this STX-producing organism and related non-STX-producing organisms is discussed in more detail below.

STX biosynthesis genes – potential STX products

Comparing *sxt* genes identified in the LE20-WE8 MAG obtained from WLE to the *sxt* genes of closely related organisms with characterized STX products provides crucial insights into STX biosynthesis and potential congeners produced. The conserved order and sequence similarity observed between contigs with *sxt* genes in MAG LE20-WE8 and the STX biosynthesis operon of *Aphan.* sp. NH-5 initially suggests a capacity to produce similar products

(Fig. 2, Table S4). *Aphan.* sp. NH-5's toxin profile is primarily composed of STX and neoSTX with minor constituents also identified as 12,12dido-dcSTX, 12 β do-doSTX, dcNEO, dcSTX, 12 β do-dcSTX (LWTX-4), 12 α do-doSTX, doSTX, M4, and 12 α do-dcSTX congeners (D'Agostino et al., 2019). neoSTX was identified to compose approximately 85% of the quantified toxin profile of *Aphan.* sp. NH-5 in culture (D'Agostino et al., 2019; Mahmood & Carmichael, 1986). STX and neoSTX have been characterized to have similar toxicity and be amongst the most toxic saxitoxin congeners (2008; Selwood et al., 2017; Suarez-Isla, 2014; Wiese et al., 2010).

A closer investigation into the *sxt* genes identified in LE20-WE8 MAG suggests that it is likely not capable of neoSTX production. *sxtX*, the gene putatively responsible for N-1 hydroxylation of STX, converting STX to neoSTX (Kellmann et al., 2008; Mahmood & Carmichael, 1986; Mihali et al., 2009; Soto-Liebe et al., 2010), is absent in LE20-WE8. *sxtX* is also absent in previously characterized STX biosynthesis gene clusters belonging to *Raphidiopsis brookii* D9, *D. circinale* AWQC131C, and ACMB13 (formerly referred to as species *Anabaena circinalis*), all of which have been shown to not synthesize any STX variants featuring a hydroxyl group at N-1, including neoSTX (Mihali et al., 2009; Soto-Liebe et al., 2010). Subsequently, the toxin profile of the Great Lakes MAG LE20-WE8 may be suggested to consist of STX and other congeners without a hydroxyl group at N-1 observed in *Aphan.* sp. NH-5's toxin profile (12,12dido-dcSTX, 12 β do-doSTX, dcSTX, 12 β do-dcSTX (LWTX-4), 12 α do-doSTX, doSTX, M4, and 12 α do-dcSTX).

The lack of a *sxtW* gene in MAG LE20-WE8 is also shared by *R. brookii* D9 and *D. circinale* AWQC131C (Mihali et al., 2009; Stucken et al., 2010). The putative function of *sxtW* is thought to be similar to a ferredoxin and perform electron transport required for hydroxylation of products encoded by *sxtT* and *sxtH*. Mihali et al. (2009) suggested an endogenous ferredoxin may compensate for the lost function from a missing *sxtW* gene, but no ferredoxin-like alternative was identified in LE20-WE8. Even without *sxtW*, *R. brookii* D9 and *D. circinale* AWQC131C and have been found to have toxin profiles that include STX and other STX, GTX, and C-toxin congeners (D'Agostino et al., 2019; Llewellyn et al., 2001; Soto-Liebe et al., 2010).

The direct involvement of *sxtD* in STX synthesis raises questions about its partial presence in LE20-WE8. *sxtD* encodes a putative desaturase and is suggested to introduce double bonds into carbon-carbon bonds within the STX biosynthetic pathway. It plays a crucial role alongside *sxtS* and *sxtU*, directly contributing to STX synthesis (Kellmann et al., 2008; Mihali et al., 2009; Pearson et al., 2010). The potential absence of certain genes, including *sxtD*, could be attributed to artifacts in metagenomic assembly and binning methods. To address this, manual analyses were conducted scrutinizing the presence of *sxtD*. It is asserted that the occurrence of *sxtD* has been adequately addressed through these analyses, particularly after identifying an additional partial *sxtD* fragment in an unassigned contig post-binning. This manual examination significantly bolsters confidence in affirming the presence of a *sxtD* gene in the MAG LE20-WE8.

With over 50 documented STX congeners and an incomplete understanding of the relationship between biosynthetic genes and products, there exists a pressing need for further investigation. This is crucial to unveil the complete range of STX congeners produced by LE20-WE8. Toxicity levels among these congeners vary significantly, with STX, neoSTX, and gonyautoxins (GTX) identified as the most potent variants (Wiese et al., 2010). Metabolic transformations of mixtures of STX congeners have also been observed in vectors like shellfish and humans (Munday & Reeve, 2013; Suarez-Isla, 2014). These transformations often escalate

toxicity rather than diminish it, emphasizing the critical importance of comprehensively understanding toxin profiles.

Metabolic potential and distribution

Exploring metabolic pathways of representative ADA MAGs can enhance our comprehension of cyanoHABs members and their potential interactions within WLE (Fig. 3, Fig. S6). Notably, the presence of nitrogen fixation genes in all nine *Dolichospermum* MAGs builds upon previous observations of nitrogen-fixing abilities within the WLE ADA clade (Yancey et al., 2023). This nitrogen-fixing potential in *Dolichospermum* is crucial for late-season or secondary blooms, potentially allowing *Dolichospermum* to thrive after *Microcystis* has depleted much of the available nitrogen (Chaffin, 2013; Michalak et al., 2013; Takamura et al., 1987; Wang et al., 2021). The occurrence of cyanoHABs with a large presence of ADA clade cyanobacteria, specifically *D. circinale*, are being increasingly observed later in the year (Fig. 4). Fortunately, these late-season blooms do not appear to possess the genetic potential to produce STXs. Further research is imperative to understand the evolving dynamics of late-season blooms in Lake Erie.

Mechanisms to compensate for phosphorus limitation are also present in representative ADA-clade MAGs. The presence of sulfolipid biosynthesis genes is identified in all eleven MAGs (Fig. 4). Substitution of sulfolipids for phospholipids in response to phosphorus limitation has been identified in *Microcystis* cultures, mesocosms, and *Microcystis*-dominated cyanoHAB samples from WLE (Martin et al., 2023). These non-phosphorus-containing lipids (sulfolipids) reduce the burden of phosphorus in cellular membrane construction as they can be used to substitute phosphorus containing phosphatidylglycerol.

The presence of urease genes and at least partial urea, phosphate, and phosphonate transporters across all representative MAGs highlights additional mechanisms for external nutrient acquisition in WLE ADA-clade cyanobacteria, including the use of organic nutrients. These pathways may be closely tied to agricultural practices in the Lake Erie watershed. Around 60% of land use in the Lake Erie watershed is allocated for agriculture, leading to significant contributions of nitrogen from prevalent inorganic fertilizers, commonly in the form of phosphate (Barnard et al., 2021; Mohamed et al., 2019). Increased use of organic urea in fertilizers in recent years further adds to the high nutrient setting in WLE (Belisle et al., 2016).

Transporters for phosphonate, conventionally perceived as a less bioavailable organic phosphorus form, also potentially serves as a nutrient source for ADA-clade cyanobacteria (Horsman & Zechel, 2017; McGrath et al., 2013). Glyphosate, commonly referred to as RoundUp Ready®, is a phosphonate that has seen increased use in recent decades and could promote ADA-clade cyanobacteria including strains capable of saxitoxin production (Spiese et al., 2023). The utilization of organic phosphonate by ADA-clade cyanobacteria is also relevant to the ongoing phosphorus management efforts by the United States and Canada, which rely on reduction in total phosphorus and soluble reactive phosphorus (SRP) and do not include phosphonate (Maccoux et al., 2016).

Comparing metabolic pathways in MAGs also demonstrated several potential factors governing spatial and temporal distribution of STX. The pathway for ferrous iron Fe(II) transporter *FeoB* is unique to *D. sp000312705* MAGs and may also be relevant to nitrogen fixation. The production of nitrogenase, the enzyme accountable for nitrogen fixation, relies heavily on significant quantities of iron (Larson et al., 2018). Therefore, the potential need for iron uptake to provide for nitrogenase function may support nitrogen fixation in low nitrogen

environments. STXs are also nitrogen rich and demand substantial nitrogen. It has been suggested that STX production in cyanobacteria may be regulated by environmental factors such as nitrogen and phosphate (Dias et al., 2002). The exclusive presence of the *FeoB* transporter in *D. sp000312705*, the species associated with LE20-WE8 (STX+), might be attributed to nitrogen demands for STX production. This transporter aids in the uptake of exogenous Fe(II), potentially supplementing the nitrogenase requirements for nitrogen fixation, crucial in supplying the necessary nitrogen for STX production.

Other notable metabolic pathways include zeaxanthin diglucoside synthesis unique to *D. sp000312705* MAGs LE20-WE8, LE17-WE2 and *C. issatschenoki* MAG, LE20-WE2-Aug. Zeaxanthin diglucoside is a carotenoid synthesized as a protective response to environmental stressors, including high light intensity and oxidative stress commonly experienced in cyanoHAB conditions (Hellweger et al., 2022; Latifi et al., 2009; Smith et al., 2022). All MAGs also have the pathways for additional carotenoids including nostoxanthin, myxoxanthophylls, and astaxanthin. Some missing pathways may be due to incomplete MAGs especially those with lower completeness such as LE17-WE12, LE21-WE12, and LE16-WE8 (~90% complete; Table 1).

Impact of STX source identification and *sxt* gene distribution

Identification of an STX producer in WLE emphasizes the current gaps in knowledge of toxin production within the region's cyanoHABs. This limitation significantly impacts the capacity to provide comprehensive guidance for general monitoring or risk assessments in the area. The characterization of STX biosynthesis genes in LE20-WE8 signify a critical advancement in ongoing efforts aimed at understanding and tracking potential cyanotoxin producers in WLE and the broader Great Lakes.

Specific observations of concern include the prevalence of LE20-WE8 around monitoring station WE12. This station is adjacent to the city of Toledo's water intake that supplies water to >400,000 residents. The occurrence of a full suite of STX biosynthesis genes near WE12 occurs across all summer months (July – September) and multiple years (2016, 2018 – 2020), suggesting reoccurring STX presence in the area. This occurrence of STX biosynthesis genes is also widespread across the western basin of Lake Erie, occurring at five of eight routine NOAA WLE monitoring stations. Four of these five stations (WE2, WE8, WE12, and WE16) can be described as nearshore, but are outside of more direct input from the Maumee River such as stations WE6 and WE9. The fifth NOAA station, WE4, has been characterized as offshore and receives more significant influence from the Detroit River along with station WE13, which did not have any occurrence of STX biosynthesis identified (Berry et al., 2017).

There is also a potential for other strains and species to produce STX in WLE. While the presence of LE20-WE8 corresponded well with the presence of *sxt* genes through read mapping analysis, there were several instances where complete STX operons were identified as present and the species belonging to MAG LE20-WE8, *D. sp000312705*, was not. Both species corresponding with STX genes in the instances where *D. sp000312705* was absent, *D. circinale* and *C. issatschenoki*, have strains capable of producing STXs in other environments (Mihali et al., 2009; Nogueira et al., 2004). Given the trends in late-season bloom occurrences of *D. circinale*, it is particularly important to acknowledge its ongoing potential for STX production (Fig. 4). *sxt* genes are variably present in closely related strains (Fig. 1) and do not correlate with overall organism phylogeny. This variability suggests frequent gene loss or horizontal gene

transfer of *sxt* genes within the ADA-clade and incites an additional concern in determining STX sources in WLE.

Potentially positive observations also came to light through the characterization and examined distribution of LE20-WE8. The absence of the *sxtX* gene responsible for neoSTX production suggests an inability to produce one of the most toxic STX congeners (Ballot et al., 2016). The current absence of STX genes within late season cyanoHAB blooms is also a positive indication. However, the presence of STX earlier in the season alongside microcystin may pose a risk from synergistic effects between the two cyanotoxins or other secondary metabolites (Metcalf & Codd, 2020).

Conclusions

This study underscores the significance of expanding the research scope of cyanoHAB bloom monitoring to encompass various potential cyanotoxins. The identification of a *Dolichospermum* MAG (LE20-WE8) possessing a full suite of STX biosynthesis genes is a significant and necessary stride forward in determining risks to both public health and ecosystem well-being associated with these toxins. The available infrastructure currently used to investigate WLE cyanoHABs, ranging from field monitoring efforts (Boegehold et al., 2023) to data processing tools (GLAMR, greatlakesomics.org), provides an opportunity to address many subsequent questions regarding STX production in WLE. Furthermore, the importance of employing these methods in the broader Great Lakes, where many areas experience cyanoHABs, may be valuable in determining potential remaining STX producers, as well as producers of other cyanotoxins and impactful secondary metabolites.

Materials and Methods

Database

In this study, the GLAMR database was utilized to access and analyze over 500 metagenomic samples from WLE collected between 2014-2022 (greatlakesomics.org). GLAMR serves as a comprehensive repository housing metagenomic, transcriptomic, and amplicon sequencing data alongside relevant metadata, including environmental measurements and processing details. This database boasts a user-friendly interface and standardized bioinformatics pipelines, streamlining the exploration and comparison of omics datasets.

Bioinformatic Analysis

Assembly, binning, and refinement

De novo assemblies were performed on single samples to recover MAGs using GLAMR pipelines for metagenomic assembly and binning (https://github.com/Geomics/GLAMR_omics_pipelines). Metagenomic assemblies were completed using MEGAHIT (D. Li et al., 2015). Multiple binning software were used to maximize MAG recovery, including CONCOCT, MetaDecoder, VAMB, MetaBAT 2, MaxBin 2.0, and SemiBin (Alneberg et al., 2014; Kang et al., 2019; Liu et al., 2022; Nissen et al., 2021; Pan et al., 2022; Wu et al., 2016). MAG refinement was manually performed on representative MAGs using Anvi'o v.7 utilizing differential coverage read mapping from bowtie2 (Eren et al., 2015; Langmead & Salzberg, 2012). Kaiju 1.6.2 annotated metagenomic contigs using its nr database (accessed March 23, 2023), with results imported into Anvi'o to aid in MAG assessment and curation (Menzel et al., 2016).

MAG identification

blastn was used to check GLAMR assemblies for the presence of *sxtA*, *G*, *H*, and *I*, four genes essential to produce STXs 5/13/24 3:09:00 PM. This step and all other uses of blastn in this study set an e-value of 1e-2 to reduce the chances of missing any potential targets of interest. Bins from assemblies with positive blastn hits to *sxtA*, *G*, *H*, or *I* were then examined for the presence of these *sxt* genes using a second blastn analysis.

Through GLAMR pipelines, all bins were assigned taxonomy using GTDB-Tk v2.1.1 with reference data from GTDB release207 v2 (Chaumeil et al., 2020) and completion, contamination, and strain heterogeneity was assessed using CheckM v.1.2.2 (Parks, 2014). Additional ADA bins were selected for further analysis if they were annotated belonging to the family *Nostocaceae*, regardless of STX gene presence. Ultimately, only bins with greater than or equal to 90% completeness and less than 10% contamination were retained for subsequent analyses. These bins, referred to as MAGs due to their high quality, were then run through dRep to identify the highest quality representatives at two different secondary ANI cutoffs, 98 and 99 (Olm et al., 2017).

Genetic analysis and visualization

To establish the phylogenetic context of representative ADA MAGs, GToTree was employed to construct a phylogenomic tree (Lee, 2019). Using GToTree, a maximum likelihood tree encompassing 251 marker genes was generated featuring ADA genomes sourced from prior studies and an outgroup represented by Cyanobium gracile PCC 6307 (NCBI: PRJNA158695) (Dreher et al., 2021; Österholm et al., 2020; Sheik et al., 2022; Yancey et al., 2023). ANI values between representative ADA MAGs were calculated using FastANI (Jain et al., 2018). The program “clinker” was utilized to visualize contigs of LE20-WE8 containing STX genes and compare them with previously characterized STX operons (Gilchrist & Chooi, 2021; Mihali et al., 2009).

Metagenomic read mapping

Metagenomic read mapping was performed using Minimap2 with presets for short genomic paired-end reads (Li, 2018). Minimap2 output was filtered using AUGUSTUS to retain reads with minimum query cover of 50% and minimum percent ID of 80% when mapping to *sxt* genes and a minimum query cover of 80% and minimum percent ID of 90% when mapping to representative ADA MAGs (Stanke et al., 2006). The determination of the final cutoff (>60% cover) for each of the three STX reference contig sequences, indicating the presence or absence of STX genes in a sample, was validated by plotting percent coverage and mean coverage depth of read mapping results for each reference. Rsamtools was utilized to aid in calculations of percent identity, coverage, and mean depth for read mapping results (Morgan et al., 2023). RPKM values were calculated in the R statistical platform (Equation 1) (Dick, 2018). RPKM values were aggregated for MAGs of the same species to reduce unambiguous read mapping between MAGs within a species. Results were visualized using ggplot2 (Wickham, 2016).

Equation 1.

$$\text{Relative Organism Abundance} = \text{RPKM per genomes} = \frac{\text{total # of reads mapped per MAG}}{(\text{length of MAG}) * (\text{total number of reads per sample})} * 10^6$$

Functional profiling

Representative ADA MAGs were annotated to predict protein-coding genes in microbial genomes using Prodigal v2.6.3 (Hyatt et al., 2010). KofamScan v1.3.0 annotated genes based on KEGG orthology (database accessed September 16, 2022). KEGGDecoder v1.3 was used to examine KofamScan output to determine the metabolic pathway completeness (<https://github.com/bjtully/BioData>). Results were visualized using ggplot2. To supplement the profiling of pathways that were either not included in the original analysis or found to be incomplete by KofamScan, Bakta v1.8.1 annotations were generated using database v5.0 (Schwengers et al., 2021).

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Supplemental Figures and Tables

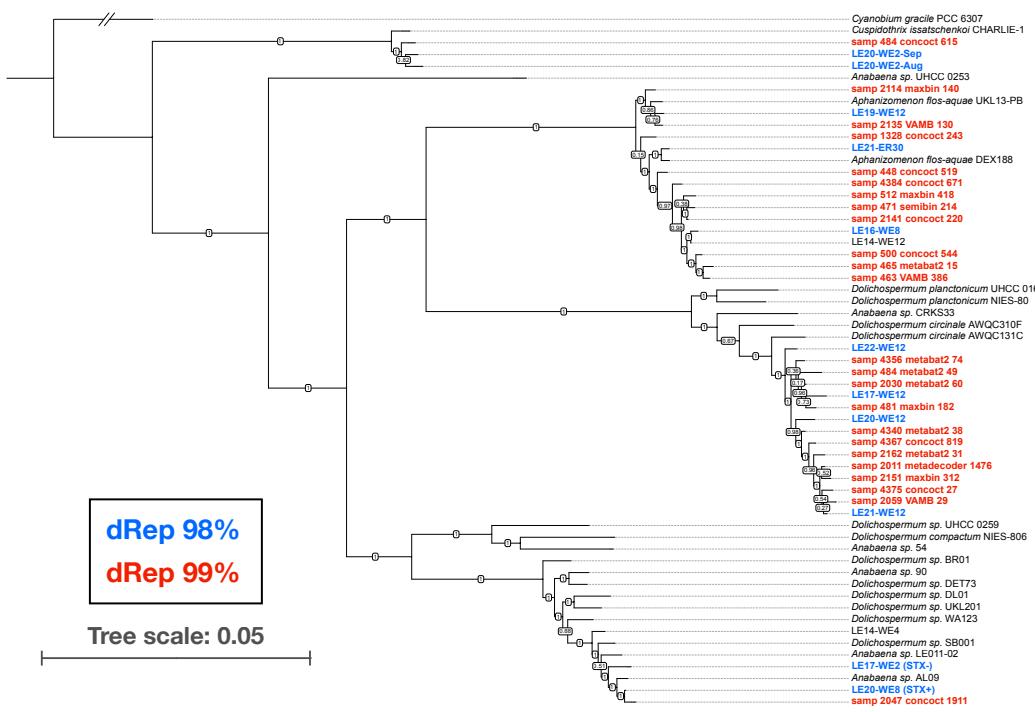


Figure S1. Phylogenomic tree comprised of ADA clade members constructed using a maximum likelihood approach, utilizing 251 housekeeping genes through the GToTree workflow. The dataset incorporated ADA genomes from prior investigations (Dreher et al., 2021; Österholm et al., 2020; Sheik et al., 2022; Yancey et al., 2023). MAGs obtained from this study are highlighted in colored and bolded text. The distinct colors represent the average nucleotide identity (ANI) thresholds (98 in blue and 99 in red) applied during MAG dereplication to select the respective strain representatives.

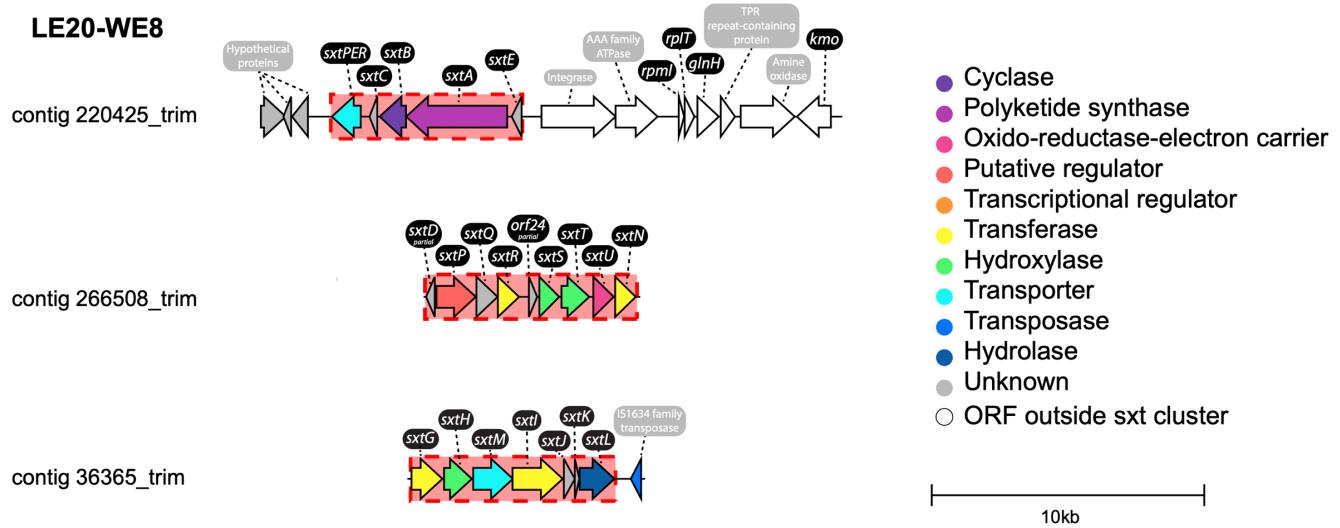


Figure S2. Sections of LE20-WE8 contigs highlighted (in red) indicate sequences utilized for genomic read mapping of *sxt* genes. This mapping serves to determine the presence or absence of STX biosynthesis potential in a sample.

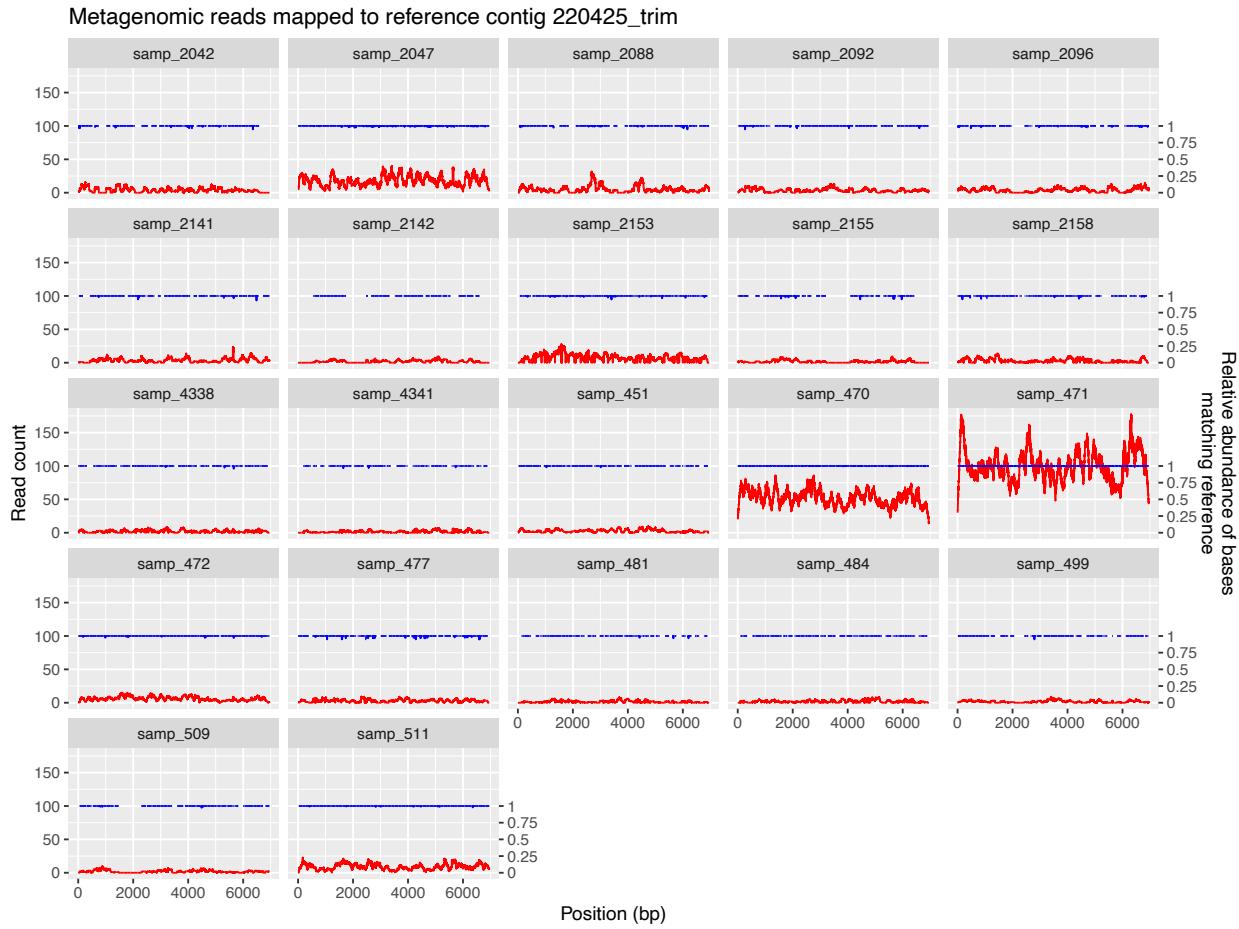


Figure S3. Read counts at each base position (red) and relative abundance of matching bases to reference averaged over 10bp intervals (blue) of for metagenomic read mapping results. Breaks in blue line and red line at 0 indicate no read mapping at that position. Reference contig 220425_trim only (Fig. S2).



Figure S4. Read counts at each base position (red) and relative abundance of matching bases to reference averaged over 10bp intervals (blue) of for metagenomic read mapping results. Breaks in blue line and red line at 0 indicate no read mapping at that position. Reference contig 266508_trim only (Fig. S2). Investigations into the 203bp sequence causing substantial peaks in read counts does not appear to be explicitly related to saxitoxin production, appearing in genomes not capable of saxitoxin production (STX-) via web based blastn (blast.ncbi.nlm.nih.gov).

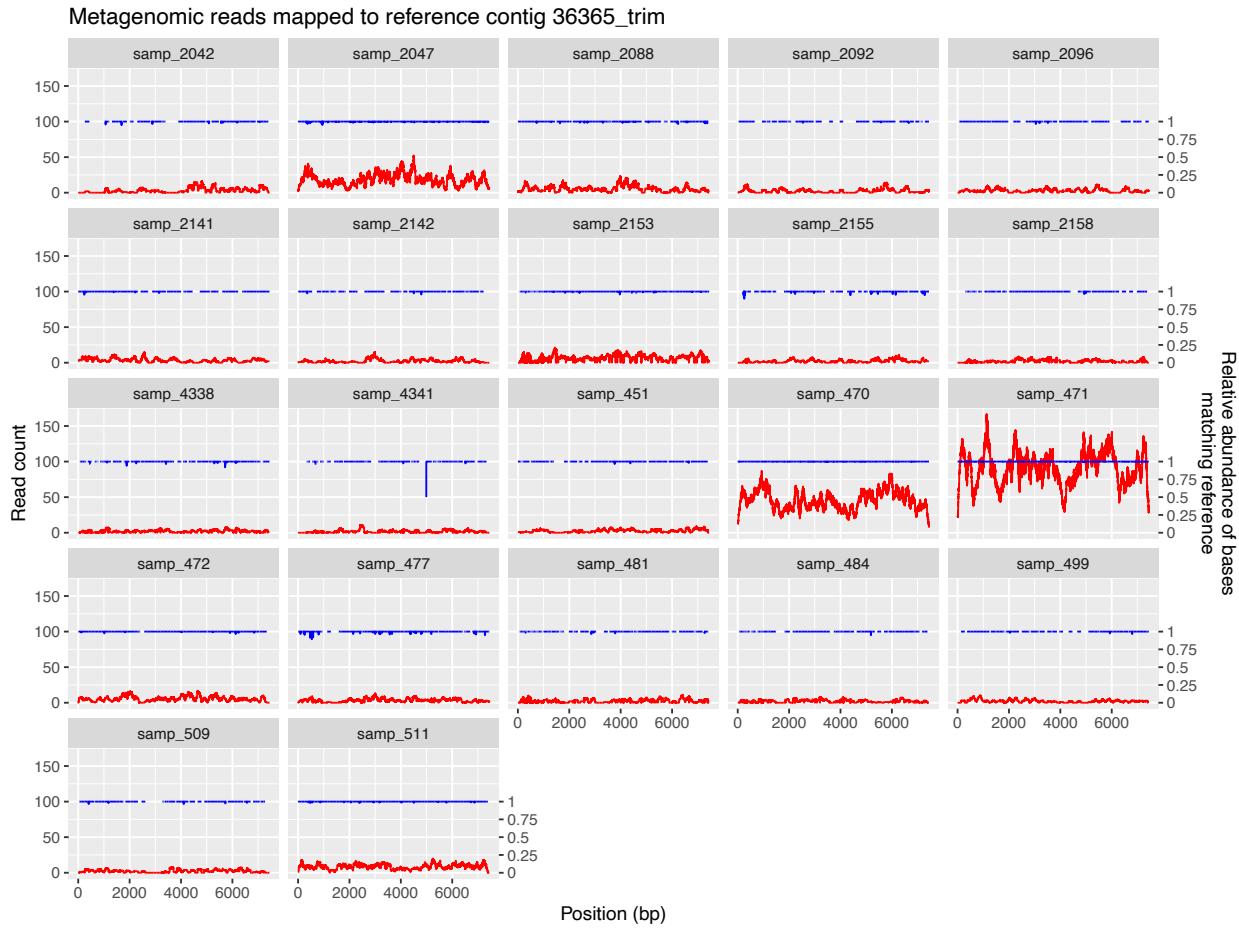


Figure S5. Read counts at each base position (red) and relative abundance of matching bases to reference averaged over 10bp intervals (blue) of for metagenomic read mapping results. Breaks in blue line and red line at 0 indicate no read mapping at that position. Reference contig 36365_trim only (Fig. S2).

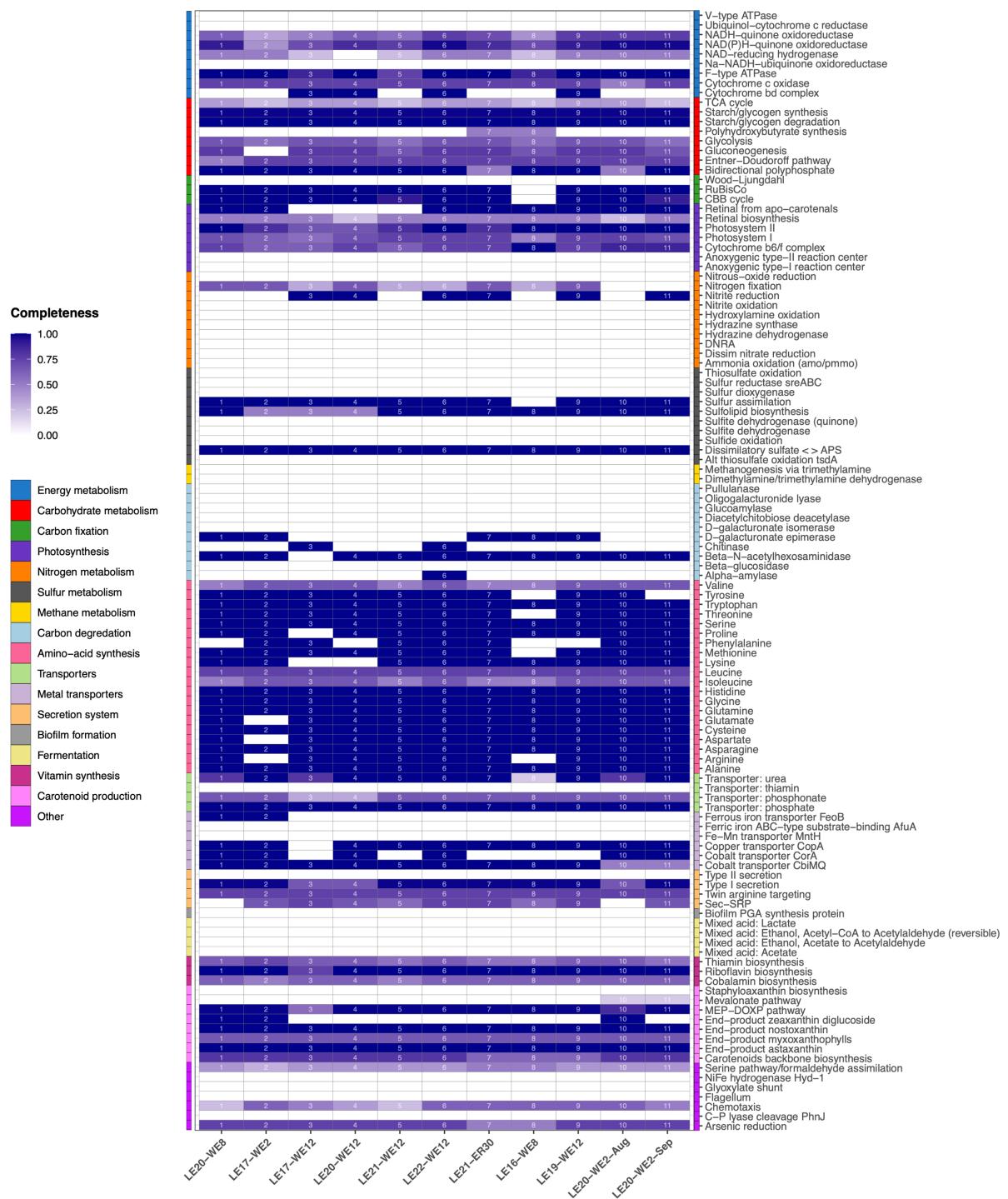


Figure S6. Heatmap visualization of KEGGDecoder functional analysis for representative MAGs, including pathways with zero completeness across examined MAGs.

SampleName	percent_cover all_ref	percent_id all_ref	lat	lon	collection date	NOAA_Site	contig_220425_trim percent_cover	contig_266508_trim percent_cover	contig_36365_trim percent_cover	contig_220425_trim percent_id	contig_266508_trim percent_id	contig_36365_trim percent_id	sample_id GLAMR
7-18-16WLE4	74.14	99.92	41.826	-83.1947	7/18/16	WE4	79.01	70.65	73.18	99.96	99.82	99.94	samp_2042
8-1-16WLE8	99.99	99.8	41.8346	-83.3565	8/1/16	WE8	100	100	100	99.91	99.52	99.91	samp_2047
7-30-18WLE12	84.07	99.91	41.7036	-83.2547	7/30/18	WE12	82.85	77.23	92.33	99.94	99.5	99.93	samp_2088
8-13-18WLE12	75.99	99.93	41.7032	-83.2536	8/13/18	WE12	89.05	69.78	70.17	99.96	99.69	99.98	samp_2092
8-20-18WLE12	83.12	99.95	41.7031	-83.2543	8/20/18	WE12	81.86	86.57	80.92	99.94	99.68	99.98	samp_2096
7-6-20WLE8	85.75	99.95	NA	NA	7/6/20	WE8	83.79	86.79	86.6	99.95	99.92	99.97	samp_2141
7-6-20WLE12	74.7	99.98	41.7035	-83.2552	7/6/20	WE12	63.09	80.57	79.65	99.98	99.97	99.98	samp_2142
8-24-20WLE8	95.49	99.4	41.8342	-83.3604	8/24/20	WE8	97.31	93.71	98.84	99.79	98.26	99.85	samp_2153
8-31-20WLE2	70.46	99.41	NA	NA	8/31/20	WE2	65.7	67.27	78.99	99.8	98.41	99.65	samp_2155
8-31-20WLE12	85.32	99.52	NA	NA	8/31/20	WE12	84.92	92.03	81.58	99.93	98.77	99.99	samp_2158
8761-SRC-7	85.59	98.99	41.7628	-83.3297	9/9/20	WE2	85.42	87.68	86.42	99.97	97.78	99.89	samp_4338
8761-SRC-10	74.59	99.03	41.7055	-83.2542	9/9/20	WE12	76.61	72.92	76.82	99.93	97.34	99.93	samp_4341
E20160030	85.08	99.95	41.7048	-83.2544	8/1/16	WE12	85.5	84.83	84.98	99.99	99.87	99.98	samp_451
E20200011	100	99.96	41.7634167	-83.3307	7/6/20	WE2	100	100	100	99.98	99.92	99.99	samp_470
E20200013	100	99.95	41.81916	-83.35921	7/6/20	WE8	100	100	100	99.98	99.9	99.98	samp_471
E20200015	98.12	99.96	41.7034833	-83.255167	7/6/20	WE12	99.24	99.27	95.93	99.97	99.93	99.98	samp_472
E20200047	92.32	99.05	41.76135	-83.33285	8/10/20	WE2	89.82	96.45	91.15	99.72	97.85	99.61	samp_477
E20200089	77.49	97.51	41.7627833	-83.32965	9/9/20	WE2	75.15	77.5	82.37	99.94	95.28	99.97	samp_481
E20200094	80.9	98.33	41.7054833	-83.254233	9/9/20	WE12	83.43	78.79	83.37	99.99	95.9	99.99	samp_484
E20212010	80.65	99.96	41.653433	-83.177667	8/3/21	WE16-WSW	75.02	86.13	80.55	99.99	99.73	99.98	samp_499
LE17	80.87	99.98	41.8265	-83.1893	9/19/16	WE4	78.14	86.39	77.89	99.97	99.99	99.96	samp_509
LE20	99.64	99.96	41.7035	-83.2537	7/15/19	WE12	99.64	99.97	99.3	99.99	99.92	99.97	samp_511

Table S1. Metagenomic read mapping results for LE20-WE8 *sxt* reference contig sequences (Figure S2). Column headers labeled with 'all_ref' represent the aggregated results of all three contig sections.

SampleName	mag	group	group_rpkm	mag_length_mb	id_ref_mag_percent	mag_cover_percent	rpkm	lat	lon	noaa_site	collection_date	clustered_site	study_id	year	month	day	yday	week	sample_id	GLAMR
E2014-0110-100LTR	LE20-WE8	D_item	0.00126443	4.57749	99.05	77.48	0.000873822	41.8268	-83.1947	WE4	9/29/14	WE4	set_17	2014	Sep	29	272	39	samp_40	
E20160030	LE20-WE8	D_item	0.000372243	4.57749	99.6	77.3	0.000261752	41.7048	-83.2544	WE12	8/1/16	WE12	set_35	2016	Aug	1	214	31	samp_451	
E20200011	LE20-WE8	D_item	0.003766677	4.57749	99.91	99.14	0.0003120784	41.7634167	-83.3307	WE2	7/6/20	WE2	set_35	2020	Jul	6	188	27	samp_470	
E20200013	LE20-WE8	D_item	0.006929648	4.57749	99.87	99.54	0.005769564	41.81916	-83.35921	WE8	7/6/20	WE8	set_35	2020	Jul	6	188	27	samp_471	
E20200015	LE20-WE8	D_item	0.000941888	4.57749	99.74	93.7	0.000561013	41.7034833	-83.255167	WE12	7/6/20	WE12	set_35	2020	Jul	6	188	27	samp_472	
E20200047	LE20-WE8	D_item	0.000217949	4.57749	99.57	60.86	0.000167038	41.76135	-83.33285	WE2	8/10/20	WE2	set_35	2020	Aug	10	223	32	samp_477	
E20210086	LE20-WE8	D_item	0.000129569	4.57749	99.64	52.31	8.37E-05	41.7026667	-83.25035	WE12	8/16/21	WE12	set_35	2021	Aug	16	228	33	samp_490	
E20212010	LE20-WE8	D_item	0.00022155	4.57749	99.27	78.56	0.000124514	41.653433	-83.177667	WE16-WSW	8/3/21	WE16	set_35	2021	Aug	3	215	31	samp_499	
LE17	LE20-WE8	D_item	0.000458414	4.57749	99.7	63.23	0.000337667	41.8265	-83.1893	WE4	9/19/16	WE4	set_35	2016	Sep	19	263	38	samp_509	
LE20	LE20-WE8	D_item	0.000702374	4.57749	99.84	94.24	0.000560901	41.7035	-83.2537	WE12	7/15/19	WE12	set_35	2019	Jul	15	196	28	samp_511	
SV_663	LE20-WE8	D_item	0.000114083	4.57749	98.39	54.9	5.33E-05	41.7574	-83.3325	T10	7/26/22	WE2	set_42	2022	Jul	26	207	30	samp_2016	
7-18-16 WLE 4	LE20-WE8	D_item	0.000932758	4.57749	99.89	56.26	0.000750993	41.826	-83.1947	WE4	7/18/16	WE4	set_41	2016	Jul	18	200	29	samp_2042	
8-1-16 WLE 8	LE20-WE8	D_item	0.000215746	4.57749	99.76	96.27	0.000164075	41.8346	-83.33565	WE8	8/1/16	WE8	set_41	2016	Aug	1	214	31	samp_2047	
8-7-17 WLE 2	LE20-WE8	D_item	0.000612251	4.57749	99.25	55.66	0.000130652	41.7633	-83.3303	WE2	8/7/17	WE2	set_41	2017	Aug	7	219	32	samp_2073	
7-30-18 WLE 12	LE20-WE8	D_item	0.001231645	4.57749	99.88	58.34	0.00099205	41.7036	-83.2547	WE12	7/30/18	WE12	set_41	2018	Jul	30	211	31	samp_2088	
8-13-18 WLE 12	LE20-WE8	D_item	0.001495235	4.57749	99.9	55.5	0.001199665	41.7032	-83.2536	WE12	8/13/18	WE12	set_41	2018	Aug	13	225	33	samp_2092	
8-20-18 WLE 12	LE20-WE8	D_item	0.001018504	4.57749	99.81	67.67	0.000797739	41.7031	-83.2543	WE12	8/20/18	WE12	set_41	2018	Aug	20	232	34	samp_2096	
7-6-20 WLE 8	LE20-WE8	D_item	0.000773275	4.57749	99.5	89.52	0.000583259	NA	NA	WE8	7/6/20	WE8	set_41	2020	Jul	6	188	27	samp_2141	
7-6-20 WLE 12	LE20-WE8	D_item	0.000441888	4.57749	99.7	75.73	0.000322172	41.7035	-83.2552	WE12	7/6/20	WE12	set_41	2020	Jul	6	188	27	samp_2142	
LE14	LE20-WE8	D_item	0.00016342	4.57749	98.88	58.75	9.70E-05	41.7026	-83.2547	WE12	9/7/21	WE12	set_35	2021	Sep	7	250	36	samp_3935	
E2014-0110-100LTR	LE17-WE2	D_item	0.00126443	4.337595	98.97	73.35	0.000390608	41.8268	-83.1947	WE4	9/29/14	WE4	set_17	2014	Sep	29	272	39	samp_40	
E20200011	LE17-WE2	D_item	0.003766677	4.337595	99.49	68.63	0.000645893	41.7634167	-83.3307	WE2	7/6/20	WE2	set_35	2020	Jul	6	188	27	samp_470	
E20200013	LE17-WE2	D_item	0.006929648	4.337595	99.45	74.44	0.001160084	41.81916	-83.35921	WE8	7/6/20	WE8	set_35	2020	Jul	6	188	27	samp_471	
E20200015	LE17-WE2	D_item	0.00941889	4.337595	99.69	87.33	0.000380876	41.7034833	-83.255167	WE12	7/6/20	WE12	set_35	2020	Jul	6	188	27	samp_472	
8-1-16 WLE 8	LE17-WE2	D_item	0.00215746	4.337595	99.07	50.89	5.17E-05	41.8346	-83.33565	WE8	8/1/16	WE8	set_41	2016	Aug	1	214	31	samp_2047	
8-7-17 WLE 2	LE17-WE2	D_item	0.000612251	4.337595	99.86	99.59	0.000481599	41.7633	-83.3303	WE2	8/7/17	WE2	set_41	2017	Aug	7	219	32	samp_2073	
E2014-0106-CNA	LE17-WE2	D_circ	0.000289378	4.108029	99.13	60.88	9.98E-05	41.7647222	-83.3335	WE2	9/29/14	WE2	set_17	2014	Sep	29	272	39	samp_14	
E2014-0106-100LTR	LE17-WE2	D_circ	0.00053057	4.108029	99.13	71.06	0.000186769	41.7647222	-83.3335	WE2	9/29/14	WE2	set_17	2014	Sep	29	272	39	samp_15	
E2014-0108-100LTR	LE17-WE2	D_circ	0.000371661	4.108029	99.33	67.66	0.000131044	41.7031	-83.2551	WE12	9/29/14	WE12	set_17	2014	Sep	29	272	39	samp_38	
E2014-0108-CNA	LE17-WE2	D_circ	0.000726368	4.108029	99.5	84.88	0.00025968	41.7031	-83.2591	WE12	9/29/14	WE12	set_17	2014	Sep	29	272	39	samp_39	
E20170045	LE17-WE2	D_circ	0.000205555	4.108029	99.7	73.84	0.000805534	41.76135	-83.33285	WE2	8/28/17	WE2	set_35	2017	Aug	28	240	35	samp_456	
E20190025	LE17-WE2	D_circ	0.003766682	4.108029	97.75	76.95	0.000828435	41.7624	-83.3306	WE2	8/5/19	WE2	set_35	2019	Aug	5	217	31	samp_462	
E20190026	LE17-WE2	D_circ	0.002921565	4.108029	98.32	82.95	0.000704012	41.703	-83.2544	WE12	8/5/19	WE12	set_35	2019	Aug	5	217	31	samp_463	
E20190028	LE17-WE2	D_circ	0.001933314	4.108029	97.91	72.12	0.000426497	41.7615	-83.3309	WE2	8/12/19	WE2	set_35	2019	Aug	12	224	32	samp_464	
E20190029	LE17-WE2	D_circ	0.001004902	4.108029	97.84	61.63	0.000221754	41.7021	-83.2551	WE12	8/12/19	WE12	set_35	2019	Aug	12	224	32	samp_465	
E202																				

SampleName	mag	group	group_rpkm	mag_length_mb	id_ref_mag_percent	mag_cover_percent	rpkm	lat	lon	noa_site	collection_date	clustered_site	study_id	year	month	day	yday	week	sample_id	GLAMR
8-17-WLE_2	LE20-WE12	D_circ	0.000412191	4.351921	99.42	58.47	7.54E-05	41.7633	-83.3303	WE2	8/7/17	WE2	set_41	2017	Aug	7	219	32	samp_2073	
8-21-17WLE_2	LE20-WE12	D_circ	0.00677139	4.351921	99.66	90.9	0.001308785	41.7624	-83.3295	WE2	8/21/17	WE2	set_41	2017	Aug	21	233	34	samp_2077	
8-21-17WLE_4	LE20-WE12	D_circ	0.003743202	4.351921	99.63	63.83	0.000720065	41.8265	-83.1978	WE4	8/21/17	WE4	set_41	2017	Aug	21	233	34	samp_2078	
8-21-17WLE_8	LE20-WE12	D_circ	0.001866484	4.351921	99.48	84.9	0.000364749	41.8337	-83.3583	WE8	8/21/17	WE8	set_41	2017	Aug	21	233	34	samp_2079	
7-22-19WLE_2	LE20-WE12	D_circ	0.000491693	4.351921	98.53	60.75	0.000101262	41.763	-83.3289	WE2	7/22/19	WE2	set_41	2019	Jul	22	203	29	samp_2109	
7-22-19WLE_4	LE20-WE12	D_circ	0.000531143	4.351921	97.28	54.78	0.000129564	41.8311	-83.195	WE4	7/22/19	WE4	set_41	2019	Jul	22	203	29	samp_2110	
7-29-19WLE_4	LE20-WE12	D_circ	0.002200593	4.351921	97.26	67.85	0.000519774	41.8261	-83.1946	WE4	7/29/19	WE4	set_41	2019	Jul	29	210	30	samp_2114	
7-29-19WLE_8	LE20-WE12	D_circ	0.000939711	4.351921	97.65	84.53	0.000206517	41.8328	-83.3625	WE8	7/29/19	WE8	set_41	2019	Jul	29	210	30	samp_2115	
7-29-19WLE_12	LE20-WE12	D_circ	0.002278847	4.351921	97.53	57.6	0.000522083	41.7028	-83.2563	WE12	7/29/19	WE12	set_41	2019	Jul	29	210	30	samp_2116	
8-19-19WLE_2	LE20-WE12	D_circ	0.000707756	4.351921	98.57	76.66	0.000148936	41.7621	-83.3303	WE2	8/19/19	WE2	set_41	2019	Aug	19	231	33	samp_2117	
8-19-19WLE_8	LE20-WE12	D_circ	0.000266800	4.351921	97.45	50.13	6.01E-05	41.8336	-83.3598	WE8	8/19/19	WE8	set_41	2019	Aug	19	231	33	samp_2119	
7-27-20WLE_2	LE20-WE12	D_circ	0.000619176	4.351921	98.43	64.78	0.000139861	NA	WE2	7/27/20	WE2	set_41	2020	Jul	27	209	30	samp_2143		
8-10-20WLE_2	LE20-WE12	D_circ	0.002007348	4.351921	98.95	82.55	0.000434908	41.7614	-83.3329	WE2	8/10/20	WE2	set_41	2020	Aug	10	223	32	samp_2147	
8-24-20WLE_2	LE20-WE12	D_circ	0.003888262	4.351921	99.22	85.89	0.000832171	41.7618	-83.331	WE2	8/24/20	WE2	set_41	2020	Aug	24	237	34	samp_2151	
8-24-20WLE_8	LE20-WE12	D_circ	0.06810198	4.351921	99.57	99.2	0.013609278	41.8342	-83.3604	WE8	8/24/20	WE8	set_41	2020	Aug	24	237	34	samp_2153	
8-24-20WLE_12	LE20-WE12	D_circ	0.002665071	4.351921	99.14	56.29	0.000565948	41.7031	-83.2557	WE12	8/24/20	WE12	set_41	2020	Aug	24	237	34	samp_2154	
8-31-20WLE_2	LE20-WE12	D_circ	0.01550335	4.351921	99.47	93.87	0.00322325	NA	WE2	8/31/20	WE2	set_41	2020	Aug	31	244	35	samp_2155		
8-31-20WLE_8	LE20-WE12	D_circ	0.032520372	4.351921	99.55	97.68	0.006664626	NA	WE8	8/31/20	WE8	set_41	2020	Aug	31	244	35	samp_2157		
8-31-20WLE_12	LE20-WE12	D_circ	0.012362757	4.351921	99.08	96.76	0.002724943	NA	WE12	8/31/20	WE12	set_41	2020	Aug	31	244	35	samp_2158		
9-14-20WLE_2	LE20-WE12	D_circ	0.010189501	4.351921	99.6	77.03	0.0002008196	NA	WE2	9/14/20	WE2	set_41	2020	Sep	14	258	37	samp_2159		
9-14-20WLE_8	LE20-WE12	D_circ	0.00965797	4.351921	99.52	82.86	0.00193478	NA	WE8	9/14/20	WE8	set_41	2020	Sep	14	258	37	samp_2161		
9-14-20WLE_12	LE20-WE12	D_circ	0.004791122	4.351921	99.46	73.49	0.000946782	NA	WE12	9/14/20	WE12	set_41	2020	Sep	14	258	37	samp_2162		
TWTPSurge_080219	LE20-WE12	D_circ	0.000507701	4.351921	97.82	65.22	0.000106491	41.700044	-83.25017	NA	WE2	8/2/19	WE12	set_18	2019	Aug	2	214	31	samp_2214
LE14	LE20-WE12	D_circ	0.000390983	4.351921	98.92	54.88	7.86E-05	41.7026	-83.2547	WE12	9/7/21	WE12	set_35	2021	Sep	7	250	36	samp_3935	
LE2	LE20-WE12	D_circ	0.000619176	4.351921	99.45	56.84	0.00138642	41.7032	-83.254	WE12	9/11/17	WE12	set_35	2017	Sep	11	254	37	samp_3937	
doli_UT_Erie_late_2022	LE20-WE12	D_circ	0.265590332	4.351921	99.83	70.57	0.011840857	NA	NA	WE6	set_35	2022	NA	NA	NA	44	sample_4333			
8761-SRC-7	LE20-WE12	D_circ	0.00932581	4.351921	99.45	96.21	0.001893382	NA	NA	WE2	9/9/20	WE2	set_41	2020	Sep	9	253	37	samp_4338	
8761-SRC-9	LE20-WE12	D_circ	0.007746412	4.351921	99.53	92.59	0.001539619	NA	NA	WE8	9/9/20	WE8	set_41	2020	Sep	9	253	37	samp_4340	
8761-SRC-10	LE20-WE12	D_circ	0.0087103	4.351921	99.49	94.78	0.001767362	NA	NA	WE12	9/9/20	WE12	set_41	2020	Sep	9	253	37	samp_4341	
8761-SRC-11	LE20-WE12	D_circ	0.008033088	4.351921	99.56	85.68	0.00138138	NA	NA	WE2	10/5/20	WE2	set_41	2020	Oct	5	279	40	samp_4342	
8761-SRC-13	LE20-WE12	D_circ	0.005412309	4.351921	99.66	88.29	0.000963899	NA	NA	WE8	10/5/20	WE8	set_41	2020	Oct	5	279	40	samp_4343	
8761-SRC-14	LE20-WE12	D_circ	0.003237957	4.351921	99.23	88.71	0.000913982	NA	NA	WE12	10/5/20	WE12	set_41	2020	Oct	5	279	40	samp_4344	
8761-SRC-24	LE20-WE12	D_circ	0.009401801	4.351921	99.62	90.36	0.0177516	NA	NA	WE2	9/20/21	WE2	set_41	2021	Sep	20	263	38	samp_4353	
8761-SRC-26	LE20-WE12	D_circ	0.001780052	4.351921	99.49	83.98	0.000344715	NA	NA	WE8	9/20/21	WE8	set_41	2021	Sep	20	263	38	samp_4355	
8761-SRC-27	LE20-WE12	D_circ	0.005748146	4.351921	99.52	92.37	0.001119933	NA	NA	WE12	9/20/21	WE12	set_41	2021	Sep	20	263	38	samp_4356	
8761-SRC-28	LE20-WE12	D_circ	0.00427285	4.351921	99.58	92.1	0.000806469	NA	NA	WE2	10/4/21	WE2	set_41	2						

SampleName	mag	group	group_rpkm	mag_length_mb	id_ref_mag_percent	mag_cover_percent	rpkm	lat	lon	noaa_site	collection_date	clustered_site	study_id	year	month	day	yday	week	sample_id	GLAMR
8761-SRC-22	LE21-WE12	D_circ	0.000173264	3.568765	99.5	78.77	6.85E-05	NA	NA	WE8	8/30/21	WE8	set_41	2021	Aug	30	242	35	samp_4351	
8761-SRC-23	LE21-WE12	D_circ	0.000188204	3.568765	99.23	60.58	6.78E-05	NA	NA	WE12	8/30/21	WE12	set_41	2021	Aug	30	242	35	samp_4352	
8761-SRC-24	LE21-WE12	D_circ	0.009401801	3.568765	99.69	99.42	0.002085465	NA	NA	WE2	9/20/21	WE2	set_41	2021	Sep	20	263	38	samp_4353	
8761-SRC-25	LE21-WE12	D_circ	0.00050037	3.568765	99.63	76.34	0.000236695	NA	NA	WE4	9/20/21	WE4	set_41	2021	Sep	20	263	38	samp_4354	
8761-SRC-26	LE21-WE12	D_circ	0.001780052	3.568765	99.71	98.75	0.000647797	NA	NA	WE8	9/20/21	WE8	set_41	2021	Sep	20	263	38	samp_4355	
8761-SRC-27	LE21-WE12	D_circ	0.005748146	3.568765	99.72	99.74	0.00200959	NA	NA	WE12	9/20/21	WE12	set_41	2021	Sep	20	263	38	samp_4356	
8761-SRC-28	LE21-WE12	D_circ	0.0042785	3.568765	99.68	99.67	0.001060734	NA	NA	WE2	10/4/21	WE2	set_41	2021	Oct	4	277	40	samp_4357	
8761-SRC-29	LE21-WE12	D_circ	0.004382093	3.568765	99.66	75.7	0.000942667	NA	NA	WE4	10/4/21	WE4	set_41	2021	Oct	4	277	40	samp_4358	
8761-SRC-30	LE21-WE12	D_circ	0.027606804	3.568765	99.7	99.9	0.004933266	NA	NA	WE8	10/4/21	WE8	set_41	2021	Oct	4	277	40	samp_4359	
8761-SRC-31	LE21-WE12	D_circ	0.008022129	3.568765	99.72	99.71	0.002405091	NA	NA	WE12	10/4/21	WE12	set_41	2021	Oct	4	277	40	samp_4360	
8761-SRC-33	LE21-WE12	D_circ	0.00582939	3.568765	99.4	99.79	0.001242405	NA	NA	WE2	7/18/22	WE2	set_41	2022	Jul	18	199	29	samp_4362	
8761-SRC-35	LE21-WE12	D_circ	0.001918135	3.568765	99.56	99.67	0.00061266	NA	NA	WE8	7/18/22	WE8	set_41	2022	Jul	18	199	29	samp_4364	
8761-SRC-37	LE21-WE12	D_circ	0.002836836	3.568765	99.74	96.95	0.000626485	NA	NA	WE2	8/17/22	WE2	set_41	2022	Aug	17	229	33	samp_4366	
8761-SRC-38	LE21-WE12	D_circ	0.001836272	3.568765	99.73	97.92	0.000655981	NA	NA	WE4	8/17/22	WE4	set_41	2022	Aug	17	229	33	samp_4367	
8761-SRC-39	LE21-WE12	D_circ	0.00135896	3.568765	99.64	98.54	0.000477768	NA	NA	WE8	8/17/22	WE8	set_41	2022	Aug	17	229	33	samp_4368	
8761-SRC-41	LE21-WE12	D_circ	0.001953536	3.568765	99.76	99.46	0.000544383	NA	NA	WE2	8/29/22	WE2	set_41	2022	Aug	29	241	35	samp_4370	
8761-SRC-43	LE21-WE12	D_circ	0.002178549	3.568765	99.76	99	0.000725817	NA	NA	WE8	8/29/22	WE8	set_41	2022	Aug	29	241	35	samp_4372	
8761-SRC-44	LE21-WE12	D_circ	0.00107359	3.568765	99.71	97.83	0.000299595	NA	NA	WE12	8/29/22	WE12	set_41	2022	Aug	29	241	35	samp_4373	
8761-SRC-45	LE21-WE12	D_circ	0.010036444	3.568765	99.76	96.98	0.001222558	NA	NA	WE2	9/12/22	WE2	set_41	2022	Sep	12	255	37	samp_4374	
8761-SRC-46	LE21-WE12	D_circ	0.005257714	3.568765	99.75	91.53	0.002047036	NA	NA	WE4	9/12/22	WE4	set_41	2022	Sep	12	255	37	samp_4375	
8761-SRC-47	LE21-WE12	D_circ	0.010050091	3.568765	99.77	99.26	0.001620955	NA	NA	WE8	9/12/22	WE8	set_41	2022	Sep	12	255	37	samp_4376	
8761-SRC-48	LE21-WE12	D_circ	0.00329929	3.568765	99.75	98.65	0.00144835	NA	NA	WE12	9/12/22	WE12	set_41	2022	Sep	12	255	37	samp_4377	
8761-SRC-49	LE21-WE12	D_circ	0.02605088	3.568765	99.75	65.15	0.002065696	NA	NA	WE2	9/30/22	WE2	set_41	2022	Sep	30	273	39	samp_4378	
8761-SRC-51	LE21-WE12	D_circ	0.059879046	3.568765	99.78	99.79	0.004633158	NA	NA	WE8	9/30/22	WE8	set_41	2022	Sep	30	273	39	samp_4379	
8761-SRC-53	LE21-WE12	D_circ	0.148223532	3.568765	99.56	99.19	0.010197157	NA	NA	WE2	10/11/22	WE2	set_41	2022	Oct	11	284	41	samp_4381	
8761-SRC-55	LE21-WE12	D_circ	0.097418149	3.568765	99.78	99.17	0.006677353	NA	NA	WE8	10/11/22	WE8	set_41	2022	Oct	11	284	41	samp_4382	
8761-SRC-56	LE21-WE12	D_circ	0.083122387	3.568765	99.79	99.78	0.005997857	NA	NA	WE12	10/11/22	WE12	set_41	2022	Oct	11	284	41	samp_4383	
8761-SRC-57	LE21-WE12	D_circ	0.142999268	3.568765	99.79	84.34	0.009374743	NA	NA	WE2	10/24/22	WE2	set_41	2022	Oct	24	297	43	samp_4384	
8761-SRC-58	LE21-WE12	D_circ	0.04847821	3.568765	99.82	76.31	0.0032265213	NA	NA	WE4	10/24/22	WE4	set_41	2022	Oct	24	297	43	samp_4385	
8761-SRC-59	LE21-WE12	D_circ	0.11365768	3.568765	99.8	93.82	0.007414863	NA	NA	WE8	10/24/22	WE8	set_41	2022	Oct	24	297	43	samp_4386	
8761-SRC-60	LE21-WE12	D_circ	0.169975815	3.568765	99.77	88.03	0.010907274	NA	NA	WE12	10/24/22	WE12	set_41	2022	Oct	24	297	43	samp_4387	
E2014-0106-100LTR	LE22-WE10	D_flos	0.00053057	4.773271	98.39	55.12	0.00181114	41.7647222	-83.335	WE2	9/29/14	WE2	set_17	2014	Sep	29	272	39	samp_15	
E2014-0108-100LTR	LE22-WE10	D_flos	0.000371661	4.773271	98.55	53.66	0.00121579	41.7031	-83.2591	WE12	9/29/14	WE12	set_17	2014	Sep	29	272	39	samp_38	
E2014-0108-CNA	LE22-WE10	D_flos	0.000726368	4.773271	99.12	76.07	0.002005077	41.7031	-83.2591	WE12	9/29/14	WE12	set_17	2014	Sep	29	272	39	samp_39	
E20170045	LE22-WE10	D_flos	0.00205555	4.773271	99.3	61.01	0.000551354	41.764	-83.3296	WE2	8/28/17	WE2	set_35	2017	Aug	28	240	35	samp_456	
E20190025	LE22-WE10	D_flos	0.003766682	4.773271	97.04	76.93	0.000876648	41.7624	-83.3326	WE2	8/5/19	WE2	set_35	2019	Aug	5	217	31	samp_462	
E20190026	LE22-WE10	D_flos	0.002921565	4.773271	97.3															

SampleName	mag	group	group_rpkm	mag_length_mb	id_ref_mag_percent	mag_cover_percent	rpkm	lat	lon	noaa_site	collection_date	clustered_site	study_id	year	month	day	yday	week	sample_id GLAMR
sv_639	LE21-ER30	D_flos	0.000467941	3.90547	99.66	69.09	0.00012834	41.7635	-83.3288	T10	8/17/21	WE2	set_42	2021	Aug	17	229	33	samp_2025
9-12-16 WLE 8	LE21-ER30	D_flos	0.004472647	3.90547	99.52	58.58	0.000936293	41.8322	-83.3589	WE8	9/12/16	WE8	set_41	2016	Sep	12	256	37	samp_2059
8-7-17 WLE 2	LE21-ER30	D_flos	0.000463084	3.90547	99.54	66.54	0.00010219	41.7633	-83.3303	WE2	8/7/17	WE2	set_41	2017	Jul	7	219	32	samp_2073
7-22-19 WLE 2	LE21-ER30	D_flos	0.001406048	3.90547	99.57	93.02	0.000453391	41.763	-83.3289	WE2	7/22/19	WE2	set_41	2019	Jul	22	203	29	samp_2109
7-22-19 WLE 8	LE21-ER30	D_flos	0.014755786	3.90547	99.58	55.73	0.002935736	41.8354	-83.3584	WE8	7/22/19	WE8	set_41	2019	Jul	22	203	29	samp_2111
7-29-19 WLE 2	LE21-ER30	D_flos	0.003999353	3.90547	99.58	80.28	0.000729401	41.7602	-83.3336	WE2	7/29/19	WE2	set_41	2019	Jul	29	214	30	samp_2113
7-29-19 WLE 4	LE21-ER30	D_flos	0.003999353	3.90547	99.58	95.67	0.001336861	41.8261	-83.1946	WE4	7/29/19	WE4	set_41	2019	Jul	29	214	30	samp_2114
7-29-19 WLE 8	LE21-ER30	D_flos	0.003109758	3.90547	99.55	97.52	0.000818504	41.8328	-83.3625	WE8	7/29/19	WE8	set_41	2019	Jul	29	210	30	samp_2115
7-29-19 WLE 12	LE21-ER30	D_flos	0.007113439	3.90547	99.58	92.76	0.001725159	41.7028	-83.2563	WE12	7/29/19	WE12	set_41	2019	Jul	29	210	30	samp_2116
8-19-19 WLE 2	LE21-ER30	D_flos	0.000366966	3.90547	99.37	76.96	0.000104146	41.7621	-83.3303	WE2	8/19/19	WE2	set_41	2019	Aug	19	231	33	samp_2117
9-3-19 WLE 12	LE21-ER30	D_flos	0.003446266	3.90547	99.67	67.12	0.000681879	41.7034	-83.2558	WE12	9/3/19	WE12	set_41	2019	Sep	3	246	36	samp_2138
7-6-20 WLE 2	LE21-ER30	D_flos	0.001248611	3.90547	99.53	79.13	0.000403482	41.7634	-83.3307	WE2	7/6/20	WE2	set_41	2020	Jul	6	188	27	samp_2139
7-6-20 WLE 8	LE21-ER30	D_flos	0.005559795	3.90547	99.58	97.88	0.001863517	NA	NA	WE8	7/6/20	WE8	set_41	2020	Jul	6	188	27	samp_2141
7-6-20 WLE 12	LE21-ER30	D_flos	0.000835268	3.90547	99.51	65.98	0.002565829	41.7035	-83.2552	WE12	7/6/20	WE12	set_41	2020	Jul	6	188	27	samp_2142
8-24-20 WLE 8	LE21-ER30	D_flos	0.001169967	3.90547	97.85	69.8	0.000350495	41.8342	-83.3604	WE8	8/24/20	WE8	set_41	2020	Aug	24	237	34	samp_2153
TWTPSurge_080219	LE21-ER30	D_flos	0.000383046	3.90547	99.33	77.42	0.000129331	41.70044	-83.250217	NA	8/2/19	WE12	set_18	2019	Aug	2	214	31	samp_2214
TWTPIntake_080219	LE21-ER30	D_flos	0.001809966	3.90547	99.6	95.55	0.000482774	41.70044	-83.250217	NA	8/2/19	WE12	set_18	2019	Aug	2	214	31	samp_2215
LE2	LE21-ER30	D_flos	0.000495575	3.90547	99.54	53.23	0.000131546	41.7032	-83.254	WE12	9/11/17	WE12	set_35	2017	Sep	11	254	37	samp_3937
8761-SRC-20	LE21-ER30	D_flos	0.000380035	3.90547	99.62	74.82	0.00017691	NA	NA	WE2	8/30/21	WE2	set_41	2021	Aug	30	242	35	samp_4349
8761-SRC-22	LE21-ER30	D_flos	0.00052983	3.90547	99.62	78.33	0.000156485	NA	NA	WE8	8/30/21	WE8	set_41	2021	Aug	30	242	35	samp_4351
8761-SRC-23	LE21-ER30	D_flos	0.000891071	3.90547	99.62	85.03	0.000277323	NA	NA	WE12	8/30/21	WE12	set_41	2021	Aug	30	242	35	samp_4352
8761-SRC-35	LE21-ER30	D_flos	0.007307129	3.90547	99.61	91.77	0.002439134	NA	NA	WE8	7/18/22	WE8	set_41	2022	Jul	18	199	29	samp_4362
8761-SRC-57	LE21-ER30	D_flos	0.00010291	3.90547	99.61	95.55	0.000129317	NA	NA	WE12	8/10/15	WE12	set_35	2015	Aug	10	222	32	samp_448
E2014-0106-100LTR	LE16-WEB	D_flos	0.000797091	3.786874	99.52	83.71	0.000316552	41.7647222	-83.335	WE2	9/29/14	WE2	set_17	2014	Sep	29	272	39	samp_15
E2014-0048-100LTR	LE16-WEB	D_flos	0.000718553	3.786874	99.59	95.78	0.000421657	41.7025	-83.26305556	WE12	8/4/14	WE12	set_17	2014	Aug	4	216	31	samp_22
E2014-0124-100LTR	LE16-WEB	D_flos	0.001436282	3.786874	99.46	50.57	7.14E-05	41.7638888	-83.3308333	WE2	10/20/14	WE2	set_17	2014	Oct	20	294	42	samp_35
E2014-0108-100LTR	LE16-WEB	D_flos	0.000173721	3.786874	99.45	52.18	7.68E-05	41.7031	-83.2591	WE12	9/29/14	WE12	set_17	2014	Sep	29	272	39	samp_38
E2014-0066-100LTR	LE16-WEB	D_flos	0.000417839	3.786874	99.38	59.92	0.000198859	41.705	-83.25638889	WE12	8/25/14	WE12	set_17	2014	Aug	25	237	34	samp_42
E2015030	LE16-WEB	D_flos	0.000656511	3.786874	99.65	75.2	0.000251933	41.7037	-83.2553	WE12	8/10/15	WE12	set_35	2015	Aug	10	222	32	samp_448
E2015032	LE16-WEB	D_flos	0.000392729	3.786874	99.62	63.14	0.0001454826	41.7172	-83.016	WE14	8/10/15	WE14	set_35	2015	Aug	10	222	32	samp_450
E2019025	LE16-WEB	D_flos	0.010823969	3.786874	99.66	96.53	0.000337208	41.7624	-83.3306	WE2	8/5/19	WE2	set_35	2019	Aug	5	217	31	samp_462
E2019026	LE16-WEB	D_flos	0.013620331	3.786874	99.72	98.01	0.004497454	41.703	-83.2544	WE12	8/5/19	WE12	set_35	2019	Aug	5	217	31	samp_463
E2019028	LE16-WEB	D_flos	0.007465809	3.786874	99.67	96.1	0.001979967	41.7615	-83.3309	WE2	8/12/19	WE2	set_35	2019	Aug	12	224	32	samp_464
E2019029	LE16-WEB	D_flos	0.0003531	3.786874	99.65	93.81	0.000955152	41.7021	-83.2551	WE12	8/12/19	WE12	set_35	2019	Aug	12	224	32	samp_465
E2019038	LE16-WEB	D_flos	0.002238698	3.786874	99.53	52.7	0.000252182	41.7034	-83.2558	WE12	9/3/19	WE12	set_35	2019	Sep	3	246	36	samp_468

SampleName	mag	group	group_rpkm	mag_length_mb	id_ref_mag_percent	mag_cover_percent	rpkm	lat	lon	noaa_site	collection_date	clustered_site	study_id	year	month	day	yday	week	sample_id	GLAMR
8-31-20 WLE 2	LE20-WE2-Aug	C_issa	0.000560606	4.344744	98.38	51.95	0.000289555	NA	NA	WE2	8/31/20	WE2	set_41	2020	Aug	31	244	35	samp_2155	
8-31-20 WLE 8	LE20-WE2-Aug	C_issa	0.000459211	4.344744	98.14	65.56	0.000234755	NA	NA	WE8	8/31/20	WE8	set_41	2020	Aug	31	244	35	samp_2157	
8-31-20 WLE 12	LE20-WE2-Aug	C_issa	0.001040414	4.344744	98.11	83.04	0.000532351	NA	NA	WE12	8/31/20	WE12	set_41	2020	Aug	31	244	35	samp_2158	
8761-SRC-7	LE20-WE2-Aug	C_issa	0.000801379	4.344744	98.65	93.09	0.000407065	NA	NA	WE2	9/9/20	WE2	set_41	2020	Sep	9	253	37	samp_4338	
8761-SRC-10	LE20-WE2-Aug	C_issa	0.000324033	4.344744	98.25	73.61	0.000170138	NA	NA	WE12	9/9/20	WE12	set_41	2020	Sep	9	253	37	samp_4341	
E20200026	LE20-WE2-Sep	C_issa	0.000367634	3.60207	99.28	52.42	0.00017542	41.7622	-83.331167	WE2	7/28/20	WE2	set_35	2020	Jul	28	210	30	samp_473	
E20200047	LE20-WE2-Sep	C_issa	0.001336614	3.60207	99.28	93.43	0.000551712	41.76135	-83.33285	WE2	8/10/20	WE2	set_35	2020	Aug	10	223	32	samp_477	
E20200051	LE20-WE2-Sep	C_issa	0.000728399	3.60207	99.16	88.95	0.000377167	41.7174667	-83.424367	WE9	8/10/20	WE9	set_35	2020	Aug	10	223	32	samp_479	
E20200052	LE20-WE2-Sep	C_issa	0.0008080706	3.60207	99.25	87.57	0.000379572	41.702	-83.254333	WE12	8/10/20	WE12	set_35	2020	Aug	10	223	32	samp_480	
E20200089	LE20-WE2-Sep	C_issa	0.000234427	3.60207	99.26	99.37	0.001215193	41.7627833	-83.32965	WE2	9/9/20	WE2	set_35	2020	Sep	9	253	37	samp_481	
E20200094	LE20-WE2-Sep	C_issa	0.001424058	3.60207	99.18	96.57	0.000694975	41.7054833	-83.254233	WE12	9/9/20	WE12	set_35	2020	Sep	9	253	37	samp_484	
SC17_50_Metagenome	LE20-WE2-Sep	C_issa	0.000249467	3.60207	98.89	54.52	0.000119392	41.85046938	-83.19494729	NF	8/31/18	WE4	set_36	2018	Aug	31	243	35	samp_791	
SC33_50_Metagenome	LE20-WE2-Sep	C_issa	0.000354827	3.60207	98.78	61.85	0.000169056	41.84968278	-83.19036052	NF	8/29/18	WE4	set_36	2018	Aug	29	241	35	samp_861	
SC50_50_Metagenome	LE20-WE2-Sep	C_issa	0.000247093	3.60207	98.82	52.16	0.000113841	41.82489782	-83.21184393	NF	8/24/18	WE4	set_36	2018	Aug	24	236	34	samp_924	
8-24-20 WLE 2	LE20-WE2-Sep	C_issa	0.000447967	3.60207	98.49	56.92	0.000214742	41.7618	-83.331	WE2	8/24/20	WE2	set_41	2020	Aug	24	237	34	samp_2151	
8-24-20 WLE 8	LE20-WE2-Sep	C_issa	0.000582846	3.60207	98.16	92.47	0.000300457	41.8342	-83.3604	WE8	8/24/20	WE8	set_41	2020	Aug	24	237	34	samp_2153	
8-31-20 WLE 2	LE20-WE2-Sep	C_issa	0.000560606	3.60207	98.67	53.45	0.000271052	NA	NA	WE2	8/31/20	WE2	set_41	2020	Aug	31	244	35	samp_2155	
8-31-20 WLE 8	LE20-WE2-Sep	C_issa	0.000459211	3.60207	98.47	67.26	0.000224456	NA	NA	WE8	8/31/20	WE8	set_41	2020	Aug	31	244	35	samp_2157	
8-31-20 WLE 12	LE20-WE2-Sep	C_issa	0.001040414	3.60207	98.26	85.64	0.000508062	NA	NA	WE12	8/31/20	WE12	set_41	2020	Aug	31	244	35	samp_2158	
8761-SRC-7	LE20-WE2-Sep	C_issa	0.000801379	3.60207	98.86	95.43	0.000394314	NA	NA	WE2	9/9/20	WE2	set_41	2020	Sep	9	253	37	samp_4338	
8761-SRC-10	LE20-WE2-Sep	C_issa	0.000324033	3.60207	98.57	76.06	0.000153895	NA	NA	WE12	9/9/20	WE12	set_41	2020	Sep	9	253	37	samp_4341	

Table S2. (Page 5 of 5) Reads per kilobase million (RPKM) calculations and sample metadata used in Figure 4.

mag	gtdbtk_species_classification	gene	contig	type	start	stop	strand	locus_tag	product	db_refs
LE16-WEB	Dolichospermumflosaqueae	nifH	samp_2059_2985	cds	1432	1833	-	BHEOGE_16726	Nitrogenase (Molybdenum-Iron) reductase and maturation protein NifH	S00001217,UniRefUniRef50_A04689R233,UniRefUniRef90_W6FTP0
LE16-WEB	Dolichospermumflosaqueae	ureA	samp_2059_47461	cds	27	329	+	BHEOGE_0600	urease subunit gamma	COG:CG00949,COG_E:ECs_3.5.1,GO:0005757,GO:0008909,Q00016151,GO:04341919,S0:0001217,UniParc:UP0007F8E4B8,UniRefUniRef100_A0A1B7X008,UniRefUniRef50_Q4K06,UniRefUniRef90_B2IT64
LE16-WEB	Dolichospermumflosaqueae	ureB	samp_2059_14083	cds	1291	1596	-	BHEOGE_2925	urease subunit beta	COG:CG00852,COG_E:ECs_3.5.1,GO:0005757,GO:0008909,Q00016151,UniParc:UP0007F8E4B8,UniRefUniRef50_A0A1B7X008,UniRefUniRef100_A0A1B7V277,UniRefUniRef50_A1TS25,UniRefUniRef90_K9ZN6
LE17-WEB	Dolichospermumflosaqueae	nifH	samp_3937_114453	cds	382	1000	+	CELJHL_1440C	Nitrogenase eATPase subunit NifH/coenzyme F430 biosynthesis subunit CfbC	COG:CG00948,COG_E:ECs_3.5.1,GO:0005757,GO:0008909,Q00016151,UniParc:UP0007F8E4B8,UniRefUniRef100_A0A1B7X008,UniRefUniRef50_A0A1B7V277,UniRefUniRef90_K9ZN6
LE17-WEB	Dolichospermumflosaqueae	ureB	samp_3937_676628	cds	112	414	-	CELJHL_0942C	urease subunit gamma	COG:CG00948,COG_E:ECs_3.5.1,GO:0005757,GO:0008909,Q00016151,UniParc:UP0007F8E4B8,UniRefUniRef100_A0A1B7X008,UniRefUniRef50_A0A1B7V277,UniRefUniRef90_K9ZN6
LE17-WEB	Dolichospermumflosaqueae	nifH	samp_3937_20761	cds	2897	2899	+	PHGANY_00510	Nitrogenase eATPase subunit NifH/coenzyme F430 biosynthesis subunit CfbC	COG:CG00948,COG_E:ECs_3.5.1,GO:0005757,GO:0008909,Q00016151,UniParc:UP0007F8E4B8,UniRefUniRef100_A0A1B7X008,UniRefUniRef50_A0A1B7V277,UniRefUniRef90_K9ZN6
LE17-WEB	Dolichospermumflosaqueae	nifH	samp_2073_767671	cds	15733	17298	-	PHGANY_07990	nitrogenase molybdenum-iron protein subunit beta	COG:CG00970,COG_E:HP_S00001217,UniRefUniRef50_P07329,D2CN98
LE17-WEB	Dolichospermumflosaqueae	ureA	samp_2073_915305	cds	564	886	-	PHGANY_09445	urease subunit gamma	COG:CG009831,COG_E:ECs_3.5.1,GO:0005757,GO:0008909,Q00016151,UniParc:UP0007F8E4B8,UniRefUniRef100_A0A1B7X008,UniRefUniRef50_Q4K06,UniRefUniRef90_B2IT64
LE17-WEB	Dolichospermumflosaqueae	ureA	samp_2073_58479	cds	528	528	-	PHGANY_01015	urease subunit beta	COG:CG00982,COG_E:ECs_3.5.1,GO:0005757,GO:0008909,Q00016151,UniParc:UP0007F8E4B8,UniRefUniRef50_A1TS25,UniRefUniRef90_W6FTP0
LE17-WEB	Dolichospermumflosaqueae	ureB	samp_2073_58478	cds	582	528	+	PHGANY_01020	urease subunit alpha	COG:CG00984,COG_E:SO0001217,UniRefUniRef50_Q7V3V2,UniRefUniRef90_A0A1B7Y2P7
LE19-WEB	Dolichospermumflosaqueae	nifH	samp_468_229190	cds	6587	7891	-	FOGGM_07890	nitrogenase molybdenum-iron protein alpha chain	COG:CG02710,COG_E:HP_S00001217,UniRefUniRef50_P07328,UniRefUniRef90_UP0001E496DE
LE19-WEB	Dolichospermumflosaqueae	nifH	samp_468_229190	cds	6023	6424	-	FOGGM_07885	Nitrogenase (Molybdenum-Iron) reductase and maturation protein NifH	S00001217,UniRefUniRef50_A04689R233,UniRefUniRef90_W6FTP0
LE19-WEB	Dolichospermumflosaqueae	nifH	samp_468_793005	cds	8582	10117	-	FOGGM_19175	nitrogenase molybdenum-iron protein subunit beta	COG:CG02710,COG_E:HP_RefSeqWP_190387296,1,SC0:0001217,UniRefUniRef50_A0A1B7X008,UniRefUniRef90_UP00021008,UniRefUniRef100_A0A1B7V277,UniRefUniRef50_Q4K06,UniRefUniRef90_B2IT64
LE19-WEB	Dolichospermumflosaqueae	ureA	samp_468_294592	cds	20666	21268	-	FOGGM_10037	urease subunit gamma	COG:CG009831,COG_E:SO0001217,UniRefUniRef50_Q7V3V2,UniRefUniRef90_A0A1B7Y2P7
LE19-WEB	Dolichospermumflosaqueae	ureB	samp_468_294592	cds	20638	20943	-	FOGGM_10070	urease subunit beta	COG:CG00982,COG_E:RefSeqWP_190386701,1,SC0:0001217,UniRefUniRef50_Q7V3V2,UniRefUniRef90_UP000801968D,UniRefUniRef100_A0A1B7V277,UniRefUniRef50_A1TS25,UniRefUniRef90_K9ZN6
LE19-WEB	Dolichospermumflosaqueae	ureA	samp_468_294592	cds	18880	20586	-	FOGGM_10069	urease subunit alpha	COG:CG00984,COG_E:SO0001217,UniRefUniRef50_Q7V3V2,UniRefUniRef90_A0A1B7Y2P7
LE20-WEB	Dolichospermumflosaqueae	nifH	samp_4344_89087	cds	5588	6514	-	AIOHN_04875	Nitrogenase M-Fe protein NifHd/coenzyme F430 biosynthesis subunit CfbD	COG:CG02710,COG_E:HP_RefSeqWP_190387296,1,SC0:0001217,UniRefUniRef50_A0A1B7X008,UniRefUniRef90_A0A1B7V277,UniRefUniRef50_Q4K06,UniRefUniRef90_K9ZN6
LE20-WEB	Dolichospermumflosaqueae	nifH	samp_4344_89087	cds	4566	5454	-	AIOHN_04870	nitrogenase iron protein	COG:CG01348,COG_E:EC-1.18.6.1,GO:0005524,GO:0008939,GO:0016163,GO:0018697,GO:0046872,GO:0051539,KEGG:K02588,RefSeqWP_028089570,1,SC0:0001217,UniParc:UP0004280ACB,UniRefUniRef100_A0A480ACX4,UniRefUniRef50_P22291,UniRefUniRef90_P0A3S1
LE20-WEB	Dolichospermumflosaqueae	nifH	samp_4344_179825	cds	305	1840	-	AIOHN_08975	nitrogenase molybdenum-iron protein subunit beta	COG:CG02710,COG_E:HP_S00001217,UniRefUniRef50_P07329,D2CN98
LE20-WEB	Dolichospermumflosaqueae	ureA	samp_4344_207048	cds	3244	3546	-	AIOHN_10135	urease subunit gamma	COG:CG00984,COG_E:SO0001217,UniRefUniRef50_Q7V3V2,UniRefUniRef90_A0A1B7Y2P7
LE20-WEB	Dolichospermumflosaqueae	ureB	samp_4344_117061	cds	1204	1509	-	AIOHN_06185	urease subunit beta	COG:CG00982,COG_E:RefSeqWP_190386701,1,SC0:0001217,UniRefUniRef50_Q7V3V2,UniRefUniRef90_UP0016801939,UniRefUniRef100_UP0016801939,UniRefUniRef50_A1TS25,UniRefUniRef90_K9ZN6
LE20-WEB	Dolichospermumflosaqueae	ureC	samp_4344_61552	cds	5296	7022	-	AIOHN_03775	urease subunit alpha	COG:CG00980,COG_E:SO0001217,UniRefUniRef50_Q7V3V2,UniRefUniRef90_A0A1B7Y2P7
LE20-WEB	Cuspidothrixissatschenkoi	ureA	samp_477_328402	cds	744	1046	-	FCGBEC_06260	urease subunit gamma	COG:CG00831,COG_E:ECs_3.5.1,GO:0005737,GO:0009039,Q00016151,GO:0043419,1,SC0:0001217,UniParc:UP000CEA16C,UniRefUniRef100_A0A256CYG2,UniRefUniRef50_Q4K06,UniRefUniRef90_B2IT64
LE20-WEB	Cuspidothrixissatschenkoi	ureA	samp_477_432475	cds	351	656	-	FCGBEC_07690	urease subunit beta	COG:CG00832,COG_E:ECs_3.5.1,GO:0005737,GO:0009039,Q00016151,GO:0043419,1,SC0:0001217,UniRefUniRef50_Q7V3V2,UniRefUniRef90_K9ZN6
LE20-WEB	Cuspidothrixissatschenkoi	ureC	samp_477_432475	cds	699	2405	-	FCGBEC_07695	urease subunit alpha	COG:CG00800,COG_E:SO0001217,UniRefUniRef50_Q7V3V2,UniRefUniRef90_A0A1B7Y2P7
LE20-WEB	Cuspidothrixissatschenkoi	ureA	samp_481_103302	cds	4655	4957	-	JAKCJU_05040	urease subunit gamma	COG:CG00831,COG_E:EC-1.18.6.1,GO:0005524,GO:0008939,GO:0016151,GO:0043419,1,SC0:0001217,UniParc:UP000CEA16C,UniRefUniRef100_A0A256CYG2,UniRefUniRef50_Q4K06,UniRefUniRef90_B2IT64
LE20-WEB	Cuspidothrixissatschenkoi	ureB	samp_481_103302	cds	2450	2755	-	JAKCJU_05050	urease subunit beta	COG:CG00832,COG_E:RefSeqWP_104368318,1,SC0:0001217,UniParc:UP000CEA16B8A,UniRefUniRef100_A0A256CY55,UniRefUniRef50_A1TS25,UniRefUniRef90_K9ZN6
LE20-WEB	Cuspidothrixissatschenkoi	ureA	samp_481_103302	cds	701	2407	-	JAKCJU_05050	urease subunit alpha	COG:CG00800,COG_E:RefSeqWP_104368318,1,SC0:0001217,UniParc:UP000CEA16B8A,UniRefUniRef100_A0A256CY55,UniRefUniRef50_A1TS25,UniRefUniRef90_K9ZN6
LE20-WEB	Dolichospermumflosaqueae	nifH	samp_000312705	nifH	471	4997	-	INKDDH_04075	nitrogenase molybdenum-iron protein alpha chain	COG:CG02710,COG_E:HP_S00001217,UniParc:UP0008014104,UniRefUniRef50_Q7V3V2,UniRefUniRef90_A0A1B7Y2P7
LE20-WEB	Dolichospermumflosaqueae	nifH	samp_000312705	nifH	471	4997	-	INKDDH_2109	nitrogenase molybdenum-iron protein alpha chain	COG:CG00984,COG_E:RefSeqWP_104368384,1,SC0:0001217,UniParc:UP000CEA16C,UniRefUniRef100_A0A1B7Y2P7,UniRefUniRef50_Q4K06,UniRefUniRef90_B2IT64
LE20-WEB	Dolichospermumflosaqueae	nifH	samp_000312705	nifH	471	716301	-	INKDDH_18800	nitrogenase molybdenum-iron protein alpha chain	COG:CG01346,COG_E:HP_S00001217,UniParc:UP0008014104,UniRefUniRef50_Q7V3V2,UniRefUniRef90_D2CN98
LE20-WEB	Dolichospermumflosaqueae	nifH	samp_000312705	nifH	471	100553	-	INKDDH_04438	nitrogenase molybdenum-iron protein subunit beta	COG:CG02710,COG_E:HP_S00001217,UniParc:UP0007F8E4B8,UniRefUniRef100_A0A1B7Y2P7,UniRefUniRef50_Q4K06,UniRefUniRef90_B2IT64
LE20-WEB	Dolichospermumflosaqueae	ureA	samp_471_293068	cds	771	8003	-	INKDDH_10965	urease subunit gamma	COG:CG00831,COG_E:ECs_3.5.1,GO:0005737,GO:0009039,Q00016151,GO:0043419,1,SC0:0001217,UniParc:UP0007F8E4B8,UniRefUniRef100_A0A1B7Y2P7,UniRefUniRef50_Q4K06,UniRefUniRef90_B2IT64
LE20-WEB	Dolichospermumflosaqueae	ureB	samp_471_293068	cds	8585	8963	-	INKDDH_10975	urease subunit beta	COG:CG00832,COG_E:RefSeqWP_104368320,1,SC0:0001217,UniRefUniRef50_Q7V3V2,UniRefUniRef90_K9ZN6
LE20-WEB	Dolichospermumflosaqueae	ureC	samp_471_293068	cds	9007	10173	-	INKDDH_10980	urease subunit alpha	S00001217,UniParc:UP0008014104,UniRefUniRef50_A0A1B7Y2P7,UniRefUniRef90_Q7V3V2,UniRefUniRef90_K9ZN6
LE21-E90	Dolichospermumflosaqueae	nifH	samp_470_756901	cds	3621	5015	-	FJKMMH_13010	nitrogenase molybdenum-iron protein alpha chain	COG:CG02710,COG_E:HP_S00001217,UniParc:UP0008014104,UniRefUniRef50_Q7V3V2,UniRefUniRef90_A0A1B7Y2P7
LE21-E90	Dolichospermumflosaqueae	nifH	samp_4305_756901	cds	3075	3454	-	FJKMMH_13005	nitrogenase molybdenum-iron protein alpha chain	COG:CG00984,COG_E:RefSeqWP_104368384,1,SC0:0001217,UniParc:UP0008014104,UniRefUniRef50_Q7V3V2,UniRefUniRef90_A0A1B7Y2P7
LE21-E90	Dolichospermumflosaqueae	nifH	samp_4305_143453	cds	56647	58182	-	FJKMMH_01615	nitrogenase molybdenum-iron protein subunit beta	COG:CG02710,COG_E:HP_S00001217,UniParc:UP00080099EB,UniRefUniRef100_A0A1B7X3U9,UniRefUniRef50_Q7V3V2,UniRefUniRef90_D2CN98
LE21-E90	Dolichospermumflosaqueae	nifH	samp_4305_486248	cds	63200	63502	-	FJKMMH_00855	urease subunit gamma	COG:CG00831,COG_E:ECs_3.5.1,GO:0005737,GO:0009039,Q00016151,GO:0043419,1,SC0:0001217,UniParc:UP0007F8E4B8,UniRefUniRef100_A0A1B7X008,UniRefUniRef50_Q4K06,UniRefUniRef90_B2IT64
LE21-E90	Dolichospermumflosaqueae	nifH	samp_4305_486248	cds	62872	63177	-	FJKMMH_00850	urease subunit beta	COG:CG00832,COG_E:SO0001217,UniRefUniRef50_Q7V3V2,UniRefUniRef90_A0A1B7UFO
LE21-E90	Dolichospermumflosaqueae	nifH	samp_4305_486248	cds	61114	6280	-	FJKMMH_00840	urease subunit alpha	COG:CG00800,COG_E:SO0001217,UniParc:UP0007F8E531,UniRefUniRef50_Q7V3V2,UniRefUniRef90_A0A053TDX2
LE21-E90	Dolichospermumflosaqueae	nifH	samp_507_981320	cds	1503	3038	-	FLNQOI_11290	nitrogenase molybdenum-iron protein subunit beta	COG:CG02710,COG_E:HP_S00001217,UniParc:UP0007F8E532,UniRefUniRef50_Q7V3V2,UniRefUniRef90_K9ZN6
LE21-E90	Dolichospermumflosaqueae	nifH	samp_507_468850	cds	14369	14671	-	FLNQOI_03475	urease subunit gamma	COG:CG00831,COG_E:ECs_3.5.1,GO:0005737,GO:0009039,Q00016151,GO:0043419,1,SC0:0001217,UniParc:UP028090420,1,SC0:0001217,UniParc:UP0004144060,UniRefUniRef100_A0A1B7X008,UniRefUniRef50_Q4K06,UniRefUniRef90_B2IT64
LE21-E90	Dolichospermumflosaqueae	nifH	samp_507_468850	cds	16572	16877	-	FLNQOI_03490	urease subunit beta	COG:CG00832,COG_E:RefSeqWP_104368384,1,SC0:0001217,UniParc:UP00080099EB,UniRefUniRef100_A0A1B7X3U9,UniRefUniRef50_Q7V3V2,UniRefUniRef90_K9ZN6
LE21-E90	Dolichospermumflosaqueae	nifH	samp_507_468850	cds	16843	18627	-	FLNQOI_03495	urease subunit alpha	COG:CG00800,COG_E:RefSeqWP_104368384,1,SC0:0001217,UniRefUniRef50_Q7V3V2,UniRefUniRef90_A0A1B7Y2P7
LE22-WEB	Dolichospermumflosaqueae	nifH	samp_4380_175307	cds	9160	10587	-	DPCBHP_09890	NDI element site-specific recombinase	S00001217,UniRefUniRef50_Q7V3V2,UniRefUniRef90_W7K7W50
LE22-WEB	Dolichospermumflosaqueae	nifH	samp_4380_175307	cds	11291	12217	-	DPCBHP_09900	Nitrogenase M-Fe protein NifHd/coenzyme F430 biosynthesis subunit CfbD	COG:CG02710,COG_E:HP_S00001217,UniParc:UP0007F8E457,UniRefUniRef50_A0A1B7X008,UniRefUniRef90_A0A1B7V277,UniRefUniRef50_Q4K06,UniRefUniRef90_K9ZN6
LE22-WEB	Dolichospermumflosaqueae	nifH	samp_4380_175307	cds	10743	11159	-	DPCBHP_09885	Nitrogenase eATPase subunit NifH/coenzyme F430 biosynthesis subunit CfbC	COG:CG01348,COG_E:HP_S00001217,UniRefUniRef50_A0A1B7X008,UniRefUniRef90_A0A1B7V277,UniRefUniRef50_Q4K06,UniRefUniRef90_K9ZN6
LE22-WEB	Dolichospermumflosaqueae	nifH	samp_4380_175307	cds	12316	2651	-	DPCBHP_21475	nitrogenase molybdenum-iron protein subunit beta	COG:CG02710,COG_E:HP_S00001217,UniRefUniRef50_A0A1B7X008,UniRefUniRef90_W6FTP0
LE22-WEB	Dolichospermumflosaqueae	nifH	samp_4380_38824	cds	8413	8715	-	DPCBHP_14045	urease subunit gamma	COG:CG00831,COG_E:ECs_3.5.1,GO:0005737,GO:0009039,Q00016151,GO:0043419,1,SC0:0001217,UniParc:UP0004144060,UniRefUniRef100_A0A1B7X008,UniRefUniRef50_Q4K06,UniRefUniRef90_B2IT64
LE22-WEB	Dolichospermumflosaqueae	nifH	samp_4380_38824	cds	10301	10606	-	DPCBHP_14060	urease subunit beta	COG:CG00832,COG_E:RefSeqWP_028090420,1,SC0:0001217,UniParc:UP00042296D1,UniRefUniRef100_UP00042296D1,UniRefUniRef50_A1TS25,UniRefUniRef90_K9ZN6
LE22-WEB	Dolichospermumflosaqueae	nifH	samp_4380_38824	cds	10649	12355	-	DPCBHP_14065	urease subunit alpha	COG:CG00804,COG_E:SO0001217,UniRefUniRef50_Q7V3V2,UniRefUniRef90_A0A1B7Y2P7

Table S3. Urease and nif gene annotations from Bakta for all eleven representative MAGs.

query	gene	subject	percent_identity	length	mismatch	gapopen	query_start	query_end	subject_start	subject_end	evalue	bitscore	qcovs
samp_471_220425_trim	sxtPER	sxtPER_Aphanizomenon sp. NH-5_EU603710.1	99.43	1059	6	0	5899	6957	1	1059	0.00E+00	1923	15
samp_471_220425_trim	sxtC	sxtC_Aphanizomenon sp. NH-5_EU603710.1	99.65	285	1	0	5294	5578	1	285	1.33E-149	521	4
samp_471_220425_trim	sxtB	sxtB_Aphanizomenon sp. NH-5_EU603710.1	99.17	969	8	0	4239	5207	1	969	0.00E+00	1746	14
samp_471_220425_trim	sxtA	sxtA_Aphanizomenon sp. NH-5_EU603710.1	99.19	3705	30	0	529	4233	1	3705	0.00E+00	6676	53
samp_471_220425_trim	sxtE	sxtE_Aphanizomenon sp. NH-5_EU603710.1	99.45	363	2	0	1	363	1	363	0.00E+00	660	5
samp_471_266508_trim	sxtD	sxtD_Aphanizomenon sp. NH-5_EU603710.1	99.36	156	1	0	1	156	156	1	7.36E-78	283	2
samp_471_266508_trim	sxtP	sxtP_Aphanizomenon sp. NH-5_EU603710.1	99.52	1443	7	0	219	1661	1	1443	0.00E+00	2627	19
samp_471_266508_trim	sxtQ	sxtQ_Aphanizomenon sp. NH-5_EU603710.1	98.84	777	9	0	1686	2462	1	777	0.00E+00	1386	10
samp_471_266508_trim	sxtR	sxtR_Aphanizomenon sp. NH-5_EU603710.1	99.61	777	3	0	2473	3249	1	777	0.00E+00	1419	10
samp_471_266508_trim	sxtS	sxtS_Aphanizomenon sp. NH-5_EU603710.1	98.63	729	10	0	3993	4721	1	729	0.00E+00	1291	10
samp_471_266508_trim	sxtT	sxtT_Aphanizomenon sp. NH-5_EU603710.1	99.61	1020	4	0	4805	5824	1	1020	0.00E+00	1862	14
samp_471_266508_trim	sxtU	sxtU_Aphanizomenon sp. NH-5_EU603710.1	98.8	750	9	0	5973	6722	1	750	0.00E+00	1336	10
samp_471_266508_trim	sxtN	sxtN_Aphanizomenon sp. NH-5_EU603710.1	97.32	747	14	1	6775	7515	1	747	0.00E+00	1264	10
samp_471_36365_trim	sxtG	sxtG_Aphanizomenon sp. NH-5_EU603710.1	99.12	1134	10	0	6310	7443	1134	1	0.00E+00	2039	15
samp_471_36365_trim	sxtH	sxtH_Aphanizomenon sp. NH-5_EU603710.1	99.31	1020	7	0	5229	6248	1020	1	0.00E+00	1845	14
samp_471_36365_trim	sxtM	sxtM_Aphanizomenon sp. NH-5_EU603710.1	98.97	1458	15	0	3725	5182	1458	1	0.00E+00	2610	20
samp_471_36365_trim	sxtI	sxtI_Aphanizomenon sp. NH-5_EU603710.1	98.48	1839	28	0	1899	3737	1839	1	0.00E+00	3241	25
samp_471_36365_trim	sxtJ	sxtJ_Aphanizomenon sp. NH-5_EU603710.1	99.75	405	1	0	1453	1857	405	1	0.00E+00	743	5
samp_471_36365_trim	sxtK	sxtK_Aphanizomenon sp. NH-5_EU603710.1	99.39	165	1	0	1289	1453	165	1	7.24E-83	300	2
samp_471_36365_trim	sxtL	sxtL_Aphanizomenon sp. NH-5_EU603710.1	95.54	1278	57	0	1	1278	1278	1	0.00E+00	2045	17

Table S4. Blastn results from comparison between LE20-WE8 *sxt* genes and *Aphanizomenon* sp. NH-5 reference.