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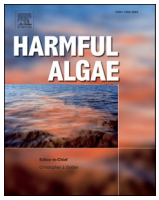

Unprecedented toxic blooms of *Microcystis* spp. in 2019 in the Chowan River, North Carolina

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ABSTRACT

The Chowan River flows from southern Virginia through northeastern North Carolina and into the Albemarle Sound, a part of the second largest U.S. estuary. The Chowan, which serves as an important recreational area and provides critical nursery habitat for multiple vulnerable species, has garnered much attention in recent years due to recurrent cyanobacterial harmful algal blooms (cHABs) associated with microcystins (MCs). Here we document unprecedented toxic blooms of *Microcystis* spp. during summer and fall of 2019 with MC concentrations two to three orders above the recreational guidelines of the Environmental Protection Agency (EPA, 2019). Based on 16S sequencing results in this study and previously published reports, the genus *Microcystis* emerged as a primary concern within the region. Shifts in assemblage composition, including relative abundance of *Microcystis* spp. and contributions from potential MC-degraders, linked to overall toxin concentrations and bloom stage. Congeners of varying toxicity, mainly MC-RR and MC-LR, were the most prevalent, corroborating that congeners other than MC-LR should be considered as health risk guidelines are developed. Downstream toxin transport was indicated based on changes in accumulated dissolved MC within the western Albemarle Sound which matched toxin dynamics in the Chowan River. This study provides important novel data on bacterial community composition, MC dynamics, and spatial connectivity for the Chowan River region that can aid monitoring approaches and management strategies for the protection of public health along the Chowan River and within the western Albemarle Sound.

1. Introduction

Cyanobacterial Harmful Algal Blooms (cHABs) are a major concern within coastal waters of North Carolina (NC) including the largest lagoonal estuary in the United States, the Pamlico-Albemarle Sound System and its tributaries (~7800 km^2) (NC Division of Water Resources, 2023). CHABs can adversely affect water quality and ecosystem health in varying ways, from blocking sunlight required by benthic vegetation to oxygen depletion that may kill resident fish and shellfish (Huisman et al., 2018; Loftin et al., 2016). Moreover, cyanobacterial toxins pose numerous threats to animal and human health through consumption of contaminated water, toxin-laden seafood, or breathing microbial aerosols in the vicinity of blooms (Hilborn and Beasley, 2015; Lang-Yona et al., 2014; Plaas et al., 2022).

Microcystins (MCs) are the most commonly occurring freshwater toxins in estuarine systems across the globe (Falconer et al., 1999). Exposure to MCs has been linked to liver toxicity and there is emerging evidence from rodent models and human epidemiological studies that these hepatotoxins might also be a cause for non-alcoholic fatty liver disease and liver cancer in humans (Clarke et al., 2019; Sarkar et al.,

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2020; Zhang et al., 2015). Assessing the risks that are associated with MC exposure is a difficult challenge since MCs include over 200 different structural variants (congeners) of unknown or varying toxicity (Catherine et al., 2017; Chernoff et al., 2021; Du et al., 2019). While there are currently limited data available on congener-specific concentrations for blooms or bloom stage-specific MC profiles, commonly reported congeners include MC-LR, MC-LA, MC-RR, and MC-YR (de Figueiredo et al., 2004; Rinehart et al., 1994; Watanabe et al., 1988). Single congener exposure studies using mouse models suggest that MC-LR and MC-LA are more toxic than MC-YR and even more so than MC-RR (Chernoff et al., 2021, 2020).

Toxin production and congener composition vary over space and time, and the latter has been linked to which toxin producers are present (Chorus et al., 2021; Monchamp et al., 2014; Wang et al., 2013). For instance, Monchamp et al. (2014) found that two different *Microcystis* species were associated with the presence of different MC congeners. It is noteworthy that even species level information may not be enough to make meaningful management decisions and more detailed genotype and gene expression data are needed (Bramburger et al., 2023). Since resolution at that level, for either cyanobacterial community composition or MC variants, is not routinely afforded through water quality and HAB monitoring programs, MC exposure risk guidelines are commonly based on chlorophyll-a (chl-a) and cyanobacteria cell counts (World Health Organization, 2020); metrics that are acquired with relative ease and often included in monitoring approaches. Guidelines for drinking water and recreational use are also directly based on MC concentrations but the studies that led to these thresholds are primarily based on MC toxicity studies that examined the effects of only a single congener, MC-LR (Chorus, 1999). For now, knowledge gaps on toxin mixtures and genotype composition make monitoring, predicting, and managing toxin exposure risks a complex challenge, particularly within highly dynamic riverine and estuarine systems (Geyer et al., 2018; Howard et al., 2022; Kudela et al., 2017).

The Chowan River, an approximately 50-mile-long river that begins in southern Virginia and flows through northeastern NC before draining into the Pamlico-Albemarle Sound System, the largest lagoonal sound in the United States, has garnered much attention due to recurrent cHABs (Burgess, 2018). In the early 1980s, management strategies implemented to address eutrophication problems that led to nuisance algal blooms and fish kills resulted in significant improvements to overall water quality (Stanley and Hobble, 1981). However, this period was followed by the re-emergence of high-biomass events since approximately 2013; the repeated detection of potential toxin-producing genera, such as *Dolichospermum* and *Microcystis*; and the confirmation of MC presence when selected bloom samples were screened (NC DEQ, 2021a). Congruently, remote sensing analyses indicate that algal bloom spatial extent and frequency has been increasing in the Chowan River since 2016 (EPA, 2023). The Chowan River system provides critical nursery habitat for several important aquatic organisms including herring, shad, and freshwater mussels (Lichti et al., 2017; O'Rear, 1983). The freshwater mussel populations have led to 100 miles of the Chowan and its associated tributaries being designated as a significant aquatic natural heritage site (Burgess, 2018). In addition, the Chowan River basin serves approximately 61,000 recreational users annually (U.S. Census Bureau, 2010). Since eutrophic systems like the Chowan are expected to experience increased severity of cHABs in response to climate change (Chorus et al., 2021; Glibert, 2020; Griffith and Gobler, 2020), questions about the degree to which the emergence of toxic cHABs will impact ecosystem health and ecosystem resources has become a pressing issue for local stakeholders, from recreational and commercial fisheries to resident communities.

The aim of this study is to compare bacterial community composition and MC profiles for six bloom assemblages collected from the Chowan River during summer and early fall of 2019 and discuss these highly toxic events considering previous and subsequent records on toxin presence. We also explore potential connectivity to downstream environments within the western Albemarle Sound. The study is meant to provide important novel data on toxin profiles and their associated bacterial communities in context of available information on toxin presence within a system prone to cHABs and to investigate the relationship between bacterial communities and MC dynamics to identify potential targets for monitoring approaches and future mitigation strategies.

2. Methods

2.1. Study site and sample collection

Surface water samples were obtained from the shoreline to target bloom events within the Chowan River reported as biomass accumulation or water discoloration by the Chowan Edenton Environmental Group, NC DEQ, and other local partners. Either a 20 L PC carboy or 2 L sampling bottles were gently immersed allowing them to fill slowly (0–0.5 m) for a total volume of at least 3 L per bloom event. When multiple bottles were used to sample at the same location for an individual bloom event, the samples were gently recombined prior to further processing. Samples were kept at ambient temperature and protected from direct sunlight during transport and were filtered within six hours of collection. Overall, a total of six discrete events were sampled between July and September of 2019 along the Chowan River (Table 1; Fig. 1). The Colerain (CR) bloom was associated with the formation of a surface scum several cm in thickness. For this event, surface scum was collected separately (uppermost layer) in addition to an integrated sample that included the surface down to 0.5 m. To explore possible downstream effects across the region, surface water samples were analyzed for toxins and bacterial assemblages at a site ~25–35 km downstream from the bloom events along the southern bank of the western Albemarle Sound (site 129S, Fig. 1). These samples were collected on a weekly to monthly basis from late May through early October of 2019 ($n = 9$, Table 1) within the framework of a study on toxin food web transfer in the region (Schnetzer et al., in prep). Sample collections and processing followed the same protocols, and all samples were analyzed to determine chl-a, particulate MCs (pMCs), dissolved MCs (dMCs), and bacterial community composition. Bloom samples were collected in triplicates while downstream information was based on single observations at the same site (Table 1). For the upstream bloom samples, total toxin analyses were further complemented by in-depth characterizing of toxin profiles for common congeners. Passive *in situ* toxin samplers or Solid Phase Adsorption Toxin Tracking (SPATT) units were deployed at site 129S downstream (average deployment = 18 days; range = 13 - 28 days). SPATTs were constructed, activated, and extracted following previously published protocols with the exception that elutes were combined into one sample prior to analysis (Howard et al., 2018; Kudela, 2011). Environmental parameters including average daily air temperature, average daily wind speed and direction, and total daily precipitation were accessed through the North Carolina

Table 1
Site names and coordinates sampled along the Chowan River (bloom events) and downstream in the western Albemarle Sound. Bloom events were sampled on specific dates in different locations while 129S was a site revisited 9 times throughout the study period. For the full list of dates for sampling at 129S see Figs. 6 and 7.

Site	Full Name	Date	Latitude	Longitude
AH	Arrowhead	07/16/2019	36.23	−76.71
CR	Colerain	07/16/2019	36.20	−76.75
IR	Indian River	07/31/2019	36.23	−76.70
LL	Leary's Landing	07/31/2019	36.14	−76.75
MC	Modoc Canal	08/13/2019	36.22	−76.71
CP	Charlton Pier	09/11/2019	36.14	−76.75
129S	Western Albemarle Sound	05/29/2019 – 10/08/2019	35.94	−76.61

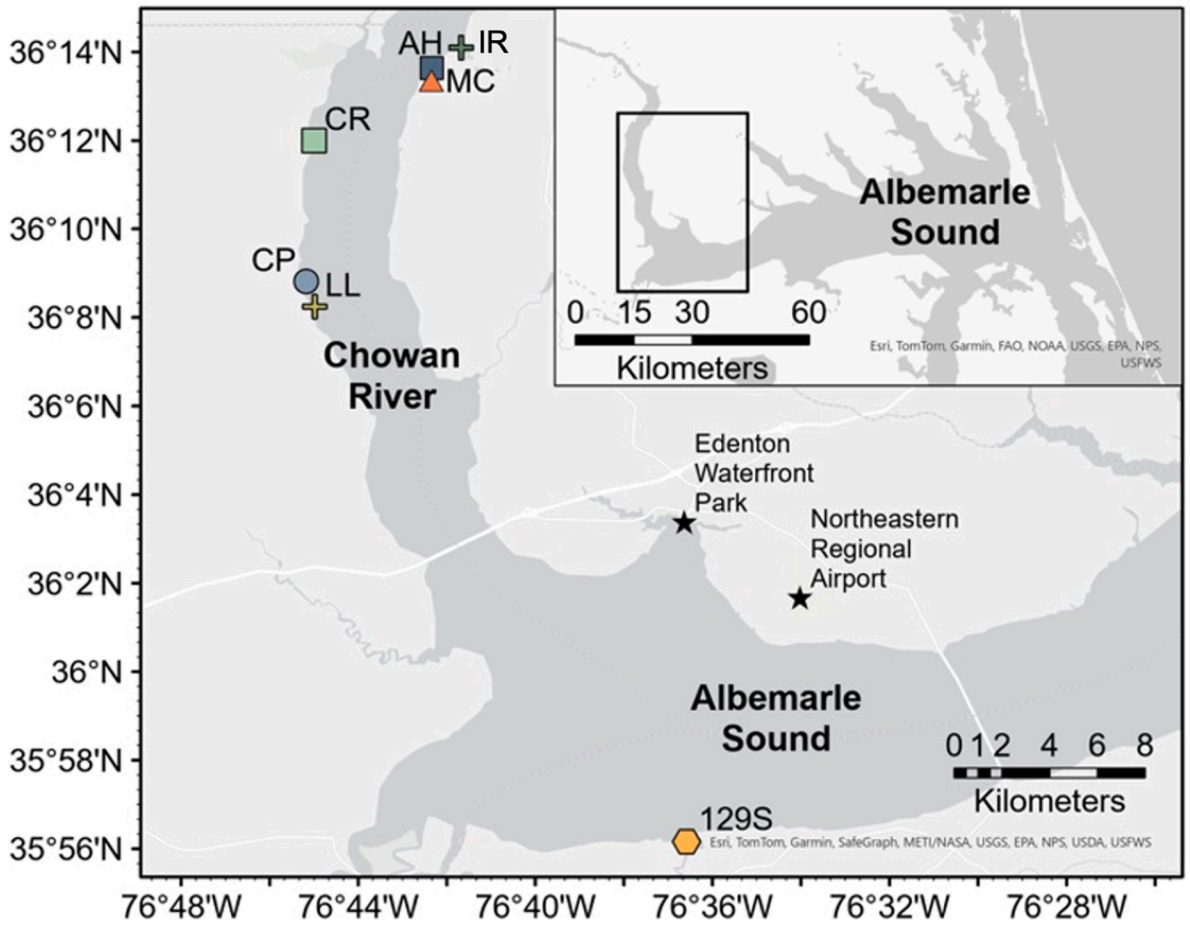

Fig. 1. Map of study sampling sites (AH = Arrowhead (blue square); CR = Colerain (green square); IR = Indian River (green cross); LL = Leary's Landing (yellow cross); MC = Modoc Canal (orange triangle); CP = Charlton Pier (blue circle), 129S = Western Albemarle Sound, see Table 1). Ancillary environmental data on water level was collected at Edenton Waterfront Park and for temperature, precipitation, and wind at Northeastern Regional Airport (locations marked with stars).

State Climate Office for a site located at the Northeastern Regional Airport in Edenton, NC (approximately 22–28 km from the Chowan River bloom locations and 9 km from the downstream sampling site, Fig. 1). Data on Chowan River water level was collected at Edenton Waterfront Park and accessed through the North Carolina Flood Inundation Mapping and Alert Network.

2.2. Chlorophyll analysis

Approximately 1–10 mL of water were filtered onto 0.7 μm Whatman GF/Fs and the filters stored at −20 °C until analysis. Filters were thawed, suspended in 7 mL of 100 % acetone, sonicated for 5 s at 50 % intensity (Fisher Scientific, Hampton, NH, USA, Model 120 Sonic Dismembrator) and extracted in the dark at −20 °C for 24 h (Strickland and Parsons, 1972). The chl-a concentration (μg L^{-1}) for each sample was then determined fluorometrically (Turner Designs Trilogy, San Jose, CA, USA) using the method detailed by Welschmeyer (1994).

2.3. MC analysis by ELISA

For pMC analysis, approximately 1–10 mL were filtered onto 0.7 μm Whatman GF/Fs and 1.5 mL of each filtrate collected in 2 mL glass autosampler vials for the analyses of dMC. For the CR bloom, pMC samples were also collected to analyze the top layer of surface scum (n = 5). Filters and filtrate were stored at −20 °C until analysis. Filters were then extracted through one freeze/thaw cycle in 3 mL of Milli-Q water followed by a 30 second sonication at 50 % intensity. . All samples were analyzed for MC concentrations using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Gold Standard Diagnostics, Westminster, PA, USA; Product #520,011; sensitive to MC-LR, -YR, -LF, -RR, LW, and nodularin; LDL = 0.10 μg L^{-1}). When samples returned MC concentrations outside of the range established by the ELISA standards, multiple dilutions using the provided sample diluent were run to make sure the concentration fell within the quantitative range. For the bloom samples, these dilution factors ranged from 10 to 10,000. Absorbances were read at 450 nm using a BioTek ELx800 Absorbance Microplate Reader (BioTek, Winooski, VT, USA). Resulting values for samples that required dilution were multiplied by the dilution factor to determine *in situ* MC concentrations. Values below detection were treated as zeros for analysis. PMC and dMC were calculated as μg MC L^{-1} and SPATT concentrations as ng MC g $resin^{-1}$ day^{-1}. SPATT concentrations were normalized by length of deployment in days to account for variability in deployment periods across SPATT samples.

2.3. Congener-specific MC analyses

Filters from the Chowan River bloom samples were further analyzed using liquid-chromatography mass spectrometry (LC-MS) to derive information on the presence and concentration of MC congeners (MC-RR, -LR, -LA, and -YR) by the Molecular Education, Technology and Research Innovation Center (METRIC) at NC State University using an Orbitrap Exploris 480 mass spectrometer. For pMC structural composition, 1–10 mL triplicate samples were filtered onto 0.7 μm Whatman GF/Fs. Filters were extracted in an 80 % MeOH solution for 12–24 h with sonication, dried in vacuo, and resuspended in a 50 % MeCN solution of varying volume (1–10x concentration) dependent on expected MC

Table 2
Historical record of MC presence within the Chowan River and the western Albemarle Sound. Note that only blooms for which MC testing was conducted are listed. Note: differences in methodology such as sampling approach, time of sampling, collection of ancillary data (chl-a levels, cell densities), or replication exist between the different studies.

Year	MM-DD	Site	Total MC (μg L^{-1})			Chl-a (μg L^{-1})	Dominant taxa[a]	ID[b]	Lat N	Lon W	Source
			Ave	Range	n	Ave					
2013	Aug-13	Near Leary's Landing	68	–	1	–	–	–	36.13	−76.75	(Fitzgerald and Gurley, 2017)
2013	Aug-29	Near Mount Gould	2.2	–	1	–	–	–	36.29	−76.7	
2018	Jun-27	Near Leary's Landing	0.44	–	1	5400	*Doli., Aphan.*	M	36.13	−76.75	(NC DEQ, 2021)
	Jul-17	Near Rockyhock	0.7	–	1	28	–	M	36.2	−76.72	
	Aug-7	Near Colerain	1.4	–	1	120	*Doli., Micro.*	M	36.21	−76.73	
	Aug-7	Near Wharf Landing	14	–	1	NA	*Doli., Micro.*	M	36.05	−76.68	
	Aug −27	Near Wharf Landing	6.4	–	1	120	*Pseudan., Doli.*	M	36.05	−76.68	
2019	July-16	Near Arrowhead Beach	55	54 - 55	3	455	*Micro.*	D	36.23	−76.71	This Study
	July-16	Near Colerain	997	849 - 1146	3	2772	*Micro.*	D	36.2	−76.75	
	July-16	Scum near Colerain	[c]11,669	6179 - 15,989	4	21,320	*Micro.*	D			
	Jul-31	Near Indian Creek	79	49 - 135	3	120	*Micro.*	D	36.23	−76.7	
	Jul-31	Near Leary's Landing	38	25 - 56	3	859	*Micro.*	D	36.14	−76.75	
	Aug-19	Near Modoc Canal	166	–	3	1283	*Micro.*	D	36.22	−76.71	
	Sep-11	Near Charlton Pier	1.4	0.7 - 2.5	3	889	*Micro.*	D	36.14	−76.75	
	May-Oct	Near Batemans Beach (S129)	0.14	BD - 0.31	9	17	*Cyano.*	D	35.94	−76.61	
	July-17	Near Arrowhead Beach	310	–	1	984	*Micro.*	M	36.22	−76.71	(NC DEQ, 2021) - Overlapping timeline with this study
	July-23	Near Arrowhead Beach	21	–	1	72	*Micro.*	M	36.22	−76.71	
	July-29	Near Leary's Landing	190	–	1	630	*Micro.*	M	36.14	−76.75	
	July-31	Near Colerain	3.3	–	1	9	–	–	36.21	−76.73	
	Aug-13	Near Indian River	620	–	1	–	*Micro.*	M	36.23	−76.69	
	Aug-19	Near Indian River	9.3	–	1	32	*Micro.*	M	36.23	−76.69	
2020	June - Oct	Near Arrowhead Beach	[c]0.003	BD - 0.01	8	–	*Cyano.*	D	36.21	−76.72	Plaas et al. (2022)
	June - Oct	Near Edenton	[c]0.004	BD - 0.006	8	–	*Cyano.*	D	36.06	−76.62	
2021	Jul-28	Near Arrowhead Beach	350		–	1580	*Micro., Doli.*	M	36.22	−76.71	(NC DWR, 2023)
2023	Jul-12	Near Rockyhock	0.30	0.05–0.54	2	1623	*Aphan.*	M	36.18	−76.72	(NC DWR, 2023)
	Jul-18	Near Arrowhead Beach	0.04	0.04	2	55	*Aphan.*	M	36.24	−76.69	
	Jun-22	Bennett's Mill Pond	BD	BD	2	266	*Doli.*	M	36.14	−76.67	A. Schnetzer (Unpublished)
	Jul-18	Near Indian River	0.22	0.21–0.23	2	2694	*Mixed: Doli., Aphan., Micro.*	–	36.23	−76.7	
	Aug-16	Bennett's Mill Pond	0.02	0.01–0.04	2	160	*Doli.*	M	36.14	−76.67	

[a] Dominant genera included *Dolichospermum* (*Doli.*), *Aphanizomenon* (*Aphan.*), *Microcystis* (Micro.), *Pseudanabaena* (*Pseudan.*), and *Cyanobium* (*Cyano.*).
[b] Taxonomic analysis was completed with light microscopy (M) or amplicon sequencing (D).
[c] These values were determined using LC-MS approaches whereas all other were determined using ELISA.

concentration for LC-MS analysis. The resuspension was centrifuged at 15,000 rpm at 4 °C for 10 min. A calibration curve was created for each congener using standards from Cambridge Isotope Laboratories (Tewksbury, MA, USA) with a linear range of 1 pg mL^{-1} to 2.5 ng mL^{-1}.

2.4. Community composition analysis

Bacterial composition of the natural assemblages was characterized based on 16S rDNA gene sequencing. Approximately 5–10 mL of samples were filtered onto 0.7 μm Whatman GF/F filters and frozen at −80 °C until further processing within 12 months. For the time-series downstream (129S) single filters were analyzed for each timepoint, whereas upstream bloom samples were analyzed in triplicates with the exception of site CR, where only one filter for the integrated surface sample (0–0.5 m) could be located. Additional filters containing concentrated surface scum were utilized to derive community composition analysis, producing a total of four composition profiles for site CR. Filters were extracted using the DNeasy PowerWater Kit (Qiagen, Germantown, MD, USA). DNA was quantified using the Invitrogen Qubit 1X dsDNA high sensitivity assay kit and a Qubit 2 fluorometer (Thermo Fisher Scientific, Waltham, MA). Amplification and sequencing were done through the Integrated Microbiome Resource (IMR) center using their standard protocol (Comeau et al., 2017) and universal V4-V5 primers (Parada et al., 2016; Walters et al., 2015) on an Illumina Miseq machine (paired-end, 300×300 bp). Sequences were analyzed using the DADA2 pipeline (Callahan et al., 2016) implemented in R v.4.1.3 using the Silva v138.1 taxonomic reference database (Quast et al., 2013). Mitochondrial and chloroplast sequences were removed from the database before analysis. Amplicon sequence variants (ASVs) were grouped at the genus level for downstream analysis using the tax_glom function of the phyloseq package in R (McMurdie and Holmes, 2013).

2.4.1. Satellite data processing

The National Aeronautics and Space Administration (NASA) obtained Sentinel-3A/B Ocean and Land Colour Instrument (OLCI) imagery from the European Space Agency under a data sharing agreement, and the data were processed by the NASA Ocean Biology Processing Group (OBPG; https://oceancolor.gsfc.nasa.gov/about/projects/cyan/). This study retrieved weekly maximum satellite detections over

the contiguous United States CONUS from the OLCI sensors, covering May 2019 through October 2019, at a spatial resolution of 300 m using version 5.0 of the Cyanobacteria Assessment Network (CyAN) processing. The OBPG utilized the standard satellite ocean color software package (l2gen), which is distributed publicly within the SeaWiFS Data Analysis System (SeaDAS; http://seadas.gsfc.nasa.gov). The data were processed using a Shuttle Radar Topography Mission (SRTM)-derived 60 m land mask (Urquhart and Schaeffer, 2020). Satellite imagery was corrected for cloud presence and shadow, and the presence of land pixels at the water's edge causing adjacency effects (Urquhart and Schaeffer, 2020; Wynne et al., 2018). Cyanobacterial biomass was quantified from the cyanobacteria index (CI_{cyano}) calculated using a spectral shape algorithm developed by Wynne et al. (2008), revised and updated by Lunetta et al. (2015), based on work from Matthews et al. (2012). A fully detailed description of the algorithm evolution is documented in Section 2.1 of Coffer et al. (2020). Briefly, cyanobacterial biomass is assessed using the 665 nm, 681 nm, and 709 nm spectral bands, while non-cyanobacterial blooms are excluded using the 620 nm, 665 nm, and 681 nm bands. For each week, a composite was generated using the maximum CI_{cyano} value for each pixel occurring during that 7-day period. The CI_{cyano} algorithm was extensively field validated (Seegers et al., 2021; Whitman et al., 2022).

2.5. Statistical analyses

Statistical analysis was completed using RStudio (RStudio Team, 2020). Any below detection (BD) values for MC were treated as zeros for statistical analysis and visualization. Data visualizations were produced

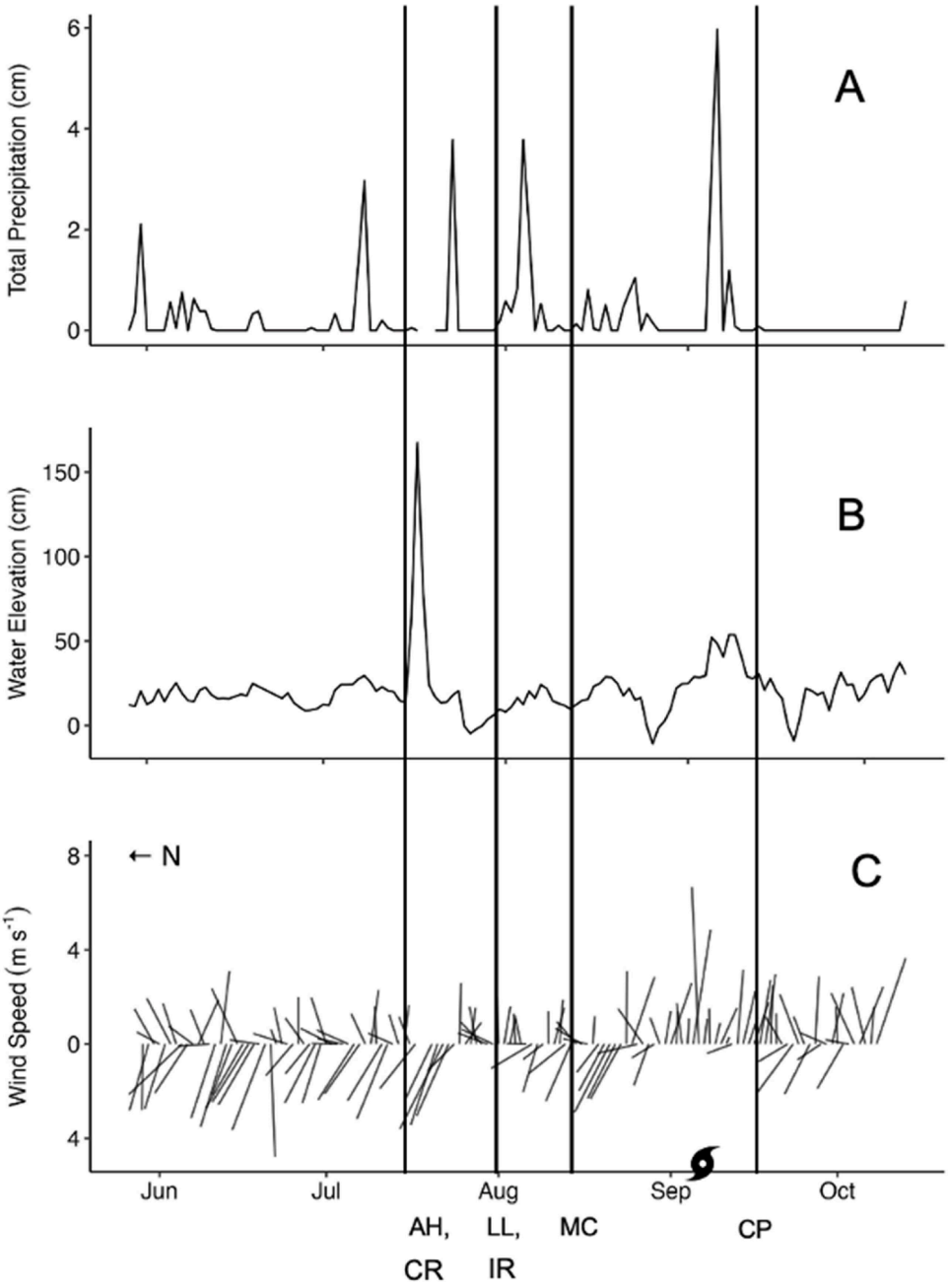

Fig. 2. Water level at Edenton Waterfront Park (A), total precipitation (B) and wind speed and direction (C) at Northeastern Regional Airport during the sampling period in 2019 (see further details on locations in Fig. 1). Vertical lines are plotted to indicate timepoints of upstream bloom sampling and are labelled under the x-axis (see details in Fig. 1). The storm symbol indicates the timepoint in which Hurricane Dorian reached the study region. All values are daily averages.

using the ggplot2 package (Wickham, 2011). Given the small and sometimes unequal sampling size across bloom events, non-parametric methods were used for analysis. A Kruskal-Wallis rank sum test from the built-in stats package was used to assess differences between upstream bloom algal biomass to compare upstream and downstream algal biomass, pMC, and dMC. A Spearman's rank correlation test was used to assess for correlations between algal biomass and both pMC and dMC across upstream bloom events. Kruskal-Wallis tests were also used to compare relative congener abundances across bloom events and to compare absolute ELISA and LC-MS-based total MC concentrations. Bacterial composition data were visualized in an nMDS plot using the phyloseq package (McMurdie and Holmes, 2013). An ANOSIM test within the vegan package was used to assess for similarity between bacterial communities across bloom events (Oksanen et al., 2022).

3. Results

3.1. Study site conditions

Average daily air temperatures ranged from 18.7 °C to 33.4 °C (average = 25.7 °C) during the period of the study. Total daily precipitation ranged from zero to 5.97 cm (average = 0.29 cm) and water elevation from zero to 168 cm (average = 21 cm, Fig. 2A and B). Wind speeds ranged from 0.4 to 6.7 m second^{-1} (average = 2.7 m sec^{-1}), with winds primarily blowing from the southeast (average wind direction of 132°, Fig. 2C). Hurricane Dorian, a Category 2 hurricane, was the only ranked storm to pass over the study area during the sampling period on September 6th (Fig. 2).

3.1. Chlorophyll a

Chl-a concentrations associated with the bloom events in the Chowan River ranged between 101 and 2772 μg L^{-1} with an average concentration of 849 μg L^{-1} and a median value of 680 μg L^{-1} (Fig. 3). The highest concentrations occurred at site CR in mid-July 2019, and minimum concentrations occurred at site IR in late July 2019. Average chl-a levels differed across upstream blooms ($X^2 = 13.3$, $df = 5$, $n = 16$, $p = 0.02$; Kruskal-Wallis test). At the downstream site, chl-a concentrations ranged from 3.6 – 29.2 μg L^{-1} with the highest concentration detected on July 16th (Fig. 3). Notably, chl-a concentrations downstream remained several orders of magnitude below those observed at the upstream bloom sites (Fig. 3) ($X^2 = 16.6$, $df = 1$, $n = 25$, $p = 4.6 \times 10^{-5}$; Kruskal-Wallis test). Satellite imagery derived weekly maximum cyanobacterial chl-a concentrations (Fig. 4) throughout the Chowan River and western Albemarle Sound reached up to 395 μg L^{-1}. Imagery-derived weekly maximum cyanobacterial chl-a concentrations (Fig. 4) rose above 95 μg L^{-1} in several regions of the Chowan River during several different weeks of summer 2019, as early as the week of May 26th, before bloom sampling was conducted.

3.2. MC concentration and partitioning

PMC concentrations, associated with the high-biomass events in the Chowan River, ranged from below detection (BD) to 1140.9 μg L^{-1} (average = 185.7 μg L^{-1}, Fig. 5A). Downstream, pMC concentrations for surface water collections ranged from BD to 0.12 μg L^{-1} (average = 0.02 μg L^{-1}). DMC concentrations ranged from 0.6 to 4.8 μg L^{-1} (average = 1.8 μg L^{-1}, Fig. 5B) for upstream sites and from BD to 0.22 μg L^{-1} (average = 0.12 μg L^{-1}, Fig. 5B) for the downstream site. Downstream dMC was significantly less than upstream bloom dMC ($X^2 = 16.6$, $df = 1$,

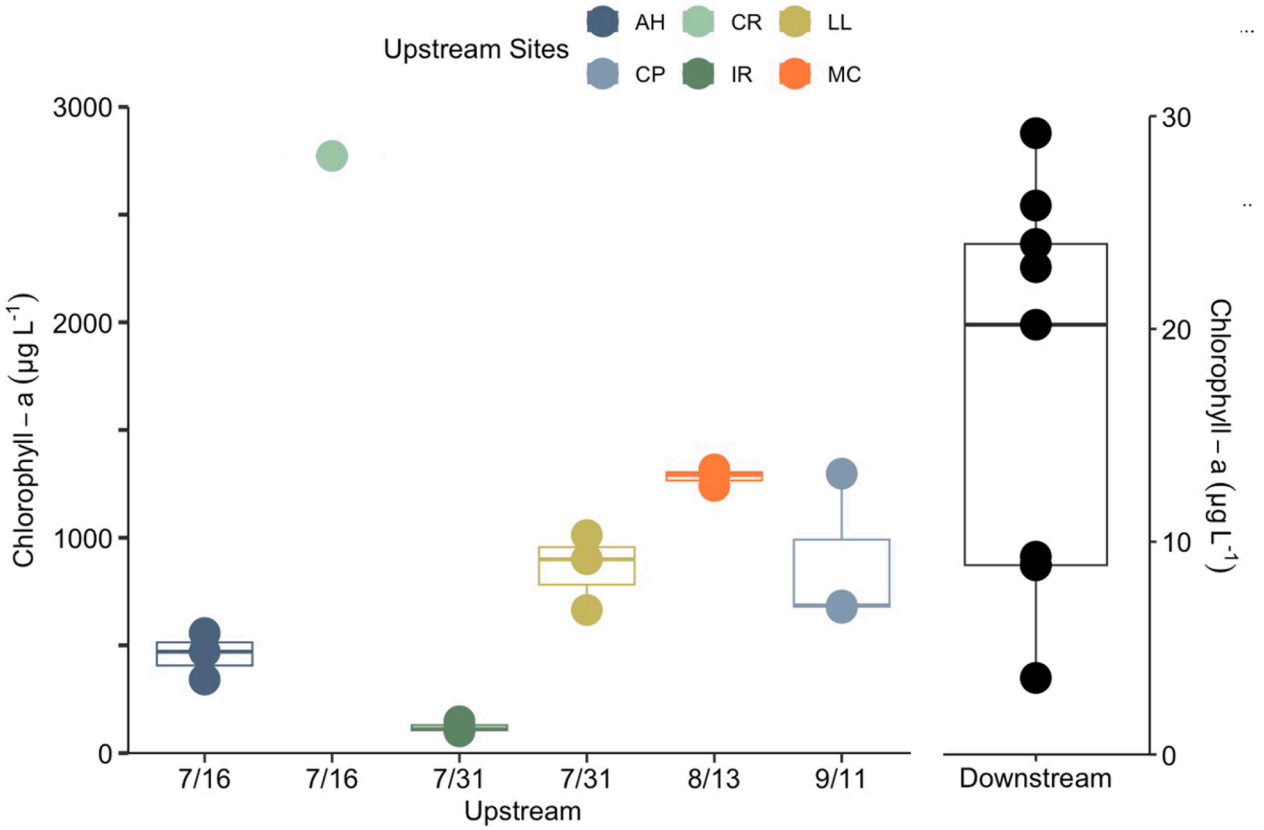

Fig. 3. Chl-a bloom concentrations ($n = 3$ except for CR with $n = 1$) and downstream chl-a over 9 consecutive sampling events from 5/29–10/8 ($n = 9$). Averages are shown in the corresponding boxplots with their standard error (±SE) on the y-axes. Note some of the blooms were sampled on the same day but in different locations upstream (see sampling location details in Table 1 and Fig. 1.). Note differences in range on y-axes.

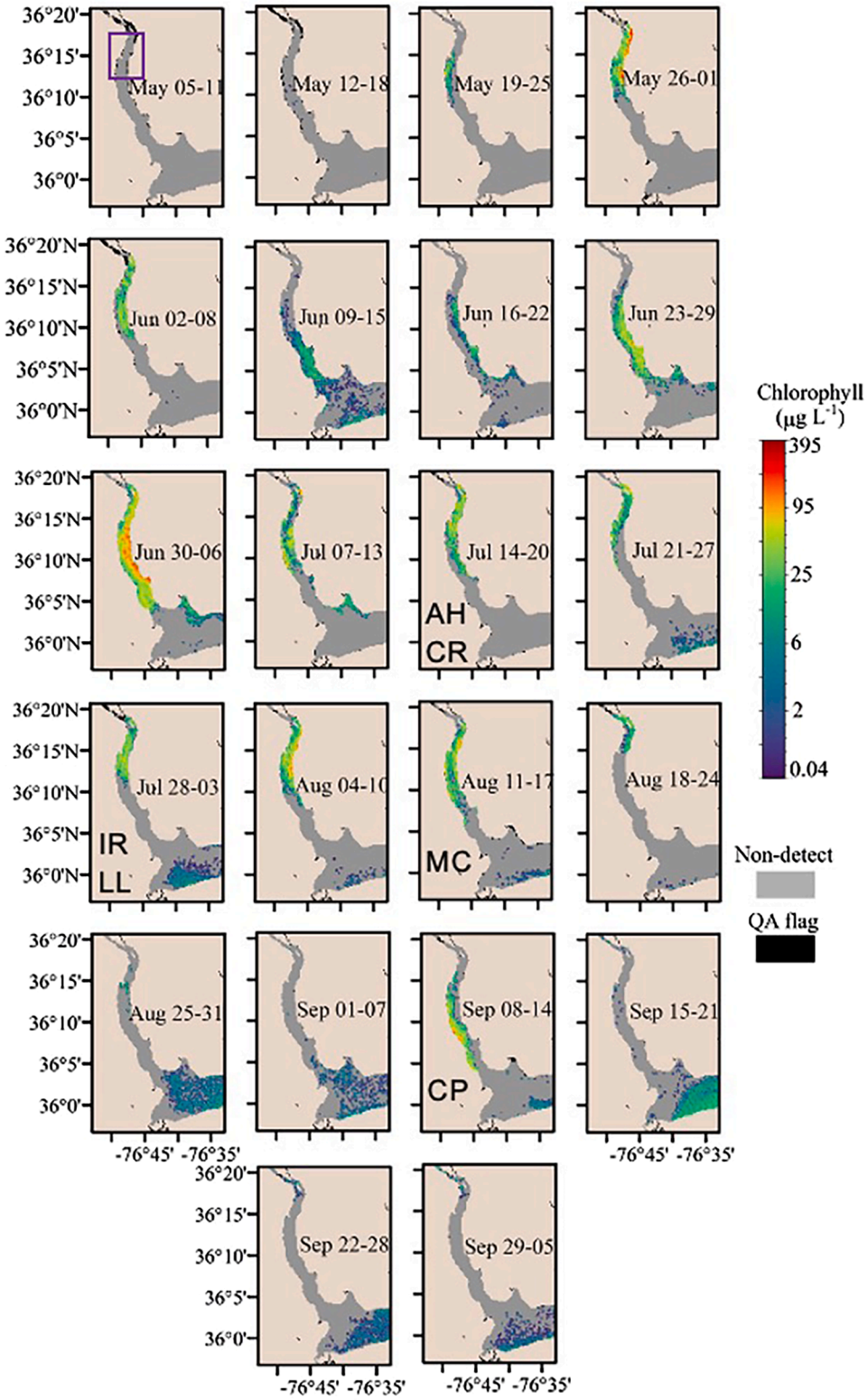

Fig. 4. Time series of weekly maximum CI_{cyano} satellite measures from May 5, 2019 through October 5, 2019 converted to chlorophyll from the slope coefficient reported in Seegers et al. (2021) national validation. The purple box in the first image contains the area of upstream bloom sampling and site abbreviations are shown in the panels for the weeks in which they were sampled.

$n = 25$, $p = 4.5 \times 10^{-5}$; Kruskal-Walis test). While the magnitude of the difference was smaller, presumably due to the high variability in pMC associated with the varying bloom events, downstream pMC was also significantly less than upstream pMC ($X^2 = 12.0$, $df = 1$, $n = 21$, $p = 0.0005$; Kruskal-Wallis test). Neither pMC nor dMC levels correlated with chl-a across the upstream bloom events ($p > 0.05$, Spearman's rank correlation test). Additional samples to analyze surface scum at site CR yielded an average pMC concentration of 11,669 μg L^{-1} (range from 6179 μg L^{-1} to 15,989 μg L^{-1}, $n = 5$, Table 2). Accumulated dMC (SPATT deployments) for the downstream site ranged from 12.3 to 77.4 ng g^{-1}

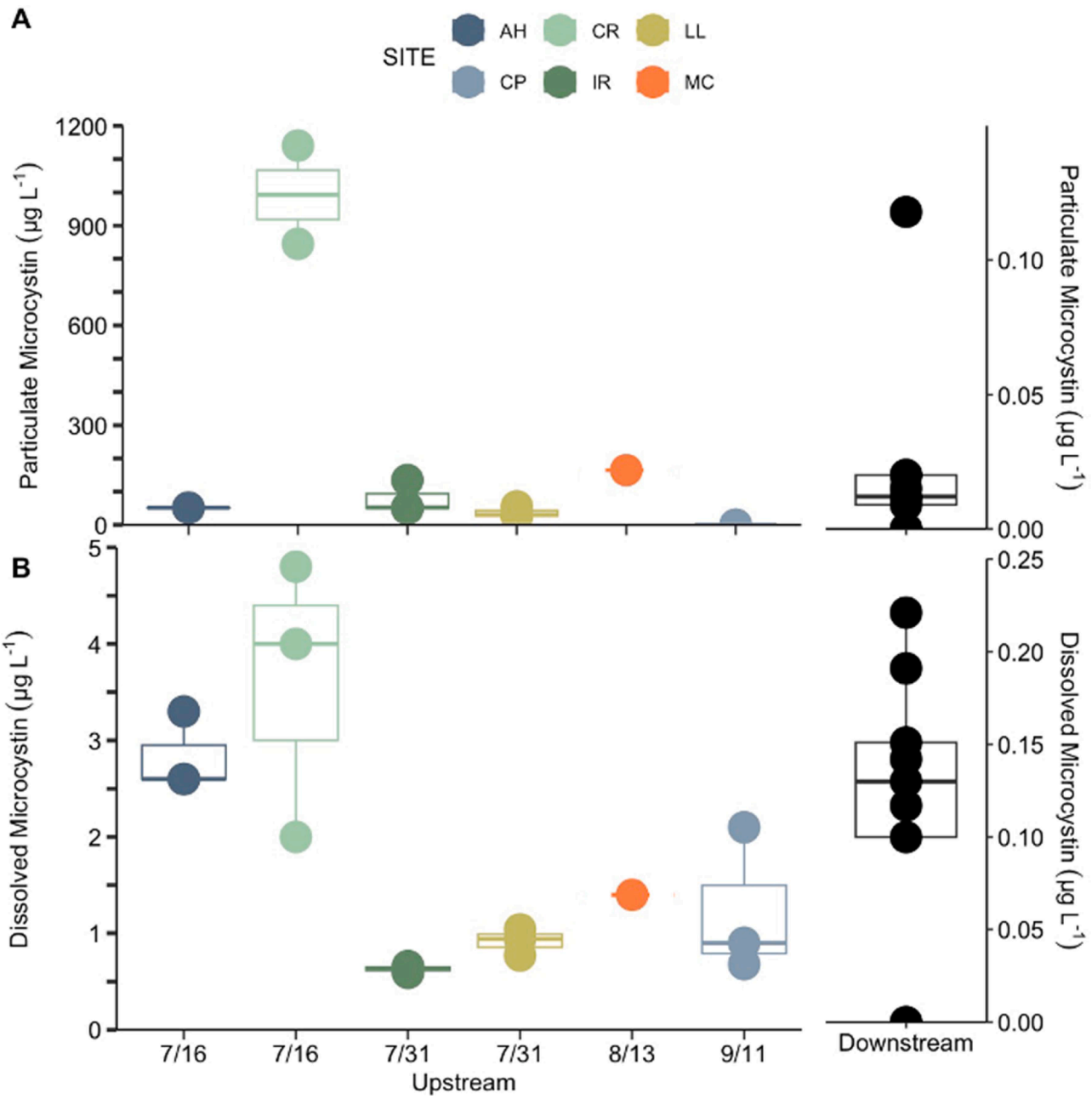

Fig. 5. pMC (A) and dMC concentrations (B) per bloom event ($n = 3$ except for MC $n = 1$) and downstream average over 9 consecutive sampling events from 5/29–10/8 ($n = 9$) with corresponding boxplot ±SE (see sampling location details in Table 1 and Fig. 1.). Note differences in range on y-axes. All values plotted were derived from ELISA measurements.

day^{-1} with an average value of 42.4 ng g^{-1} day^{-1} ($n = 8$, Fig. 6, Table 3). Relative changes in these accumulated dMC concentrations matched the changes in dMC, but not pMC, based on discrete sampling at 129S (Fig. 6). Furthermore, accumulated downstream dMC values followed similar patterns to dMC concentrations at the upstream bloom locations (Fig. 7).

3.3. MC congener profiles

Congener relative contributions in the particulate form did not vary across bloom sites ($X^2 = 0.05$, $df = 5$, $n = 24$, $p = 1$; Kruskal-Wallis Test). During each event, MC-RR was the most abundant congener, followed by MC-LR, MC-YR, and MC-LA (Fig. 8; Supp. Table 1). Summed absolute congener concentrations agreed with MC concentrations from ELISA methods, indicating the four congeners tested did account for the majority of MC present ($X^2 = 0.87$, $df = 1$, $n = 42$, $p = 0.35$; Kruskal-Wallis Test).

3.4. Bacterial community composition

Across all samples, upstream and downstream, 6528 ASVs were detected ($n = 28$). Bacterial communities at the bloom sites in the Chowan River were significantly different from those at the downstream site (129S) ($R = 0.21$, $p = 0.01$, ANOSIM) sharing between 52 and 72 % of their overall assemblage members (Fig. 8). *Sediminibacterium* was the only genus within the 10 most common taxa for both regions (Fig. 10). Bloom communities varied across the Chowan River upstream bloom events ($R = 0.99$, $p = 0.001$, ANOSIM) (Figs. 9 and 10). Communities from CR in mid-July and those from CP in September were the most distinct from all other bloom assemblages (Fig. 9). Bacterial

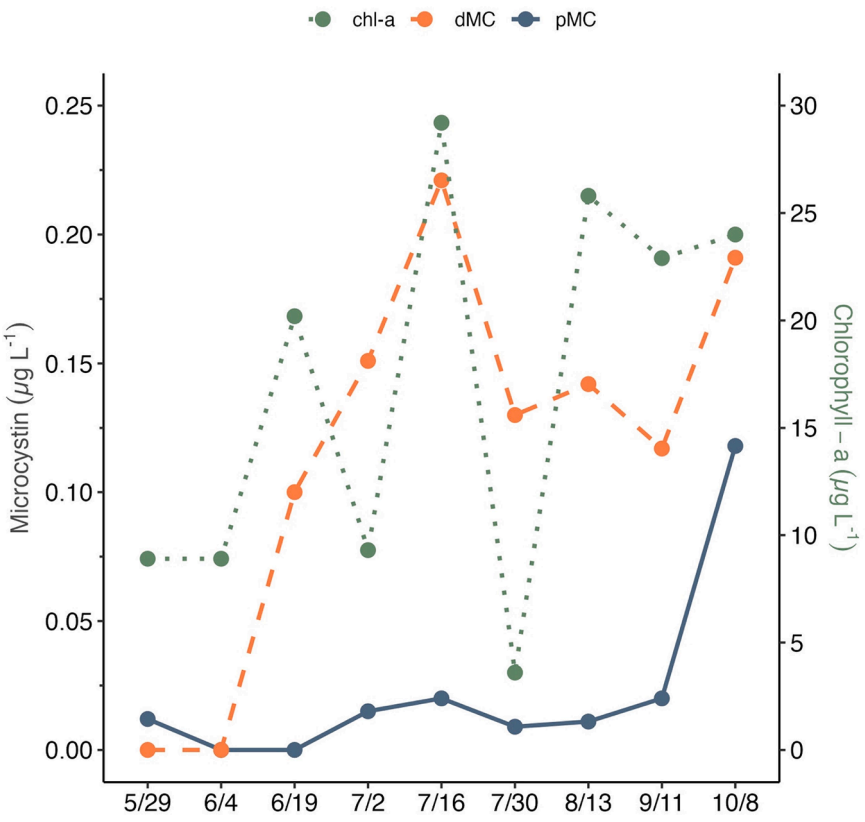

Fig. 6. Temporal changes in dMC (orange dashed line), and pMC (blue solid line) concentrations (μg L^{-1}) on the primary y-axes and in chl-a (green dotted line) on the secondary y-axes for all sampling timepoints at the downstream site within the western Albemarle Sound (129S in Fig. 1).

Table 3
Accumulated dissolved (Diss Accum.) toxin (MC = microcystin, CYN = cylindrospermopsin, ANA = anatoxin-a) values from SPATTs in the Chowan River (CR) and Albemarle Sound (AS).

Year / MM	Area	Site	Diss Accum MC			Diss Accum CYN			Diss Accum ANA			Lat	Lon
			ng g resin^{-1} day^{-1}			ng g resin^{-1} day^{-1}			ng g resin^{-1} day^{-1}			N	W
			Ave	Range	n	Ave	Range	n	Ave	Range	n		
2016 - 2017 Jul-Dec[a]	mid CR	near CR	29	2–72	6	0.10	BD-0.29	3	0.00	BD	6	36.20	−76.74
		near AH	29	2–148	11	0.10	BD-0.20	6	–	–	–	36.18	−76.72
		CP	39	4–120	11	0.04	BD-0.24	9	0.00	BD	5	36.14	−76.75
	lower CR	Edenhouse Bridge	39	13–84	4	0.08	BD-0.24	3	0.93	BD-5.58	8	36.02	−76.70
		Edenhouse Bridge	29	9–53	14	0.03	BD-0.16	14	4.86	1.77–7.96	2	36.04	−76.70
	Edenton area	Edenton Bay	24	11–46	3	0.03	BD-0.06	2	0.00	BD	5	36.04	−76.61
		Edenton Town Pier	25	3–92	14	0.03	BD-0.14	14	0.81	BD-2.42	3	36.06	−76.62
2019 May-Oct[b]	Western AS	Near Batemans Beach (129S)	42	12–77	9	–	–	–	–	–	–	35.94	−76.61

[a] Data from A. Schnetzer (unpublished). SPATT deployment periods were 24 – 34 days.
[b] Data from this study. SPATT deployment periods were 13 – 28 days. This study did not measure CYN or ANA.

communities sampled in September and October from both upstream blooms and the downstream site were distinct from communities sampled earlier in the summer period, suggesting temporal shifts may have been shaping community composition across both locations (Fig. 9). The bacterial community from the IR bloom event sampled in late July was similar to downstream communities sampled in June and August; however, the downstream communities sampled in July clustered together and appeared more similar with the bloom community from MC sampled in mid-August (Fig. 9). Given that the most abundant taxa were not shared across upstream and downstream sites, these similarities may be driven by rarer taxa and could potentially demonstrates community shifts towards a shared, non-bloom assemblage. The 10 most common taxa shared across all blooms made up ≥ 60 % of the community across the samples (range = 60 % - 93 %) and of those, two were known and potential cyanobacterial toxin producers, namely *Microcystis* and *Pseudanabaena* respectively (Fig. 10A). *Microcystis* spp. was most abundant within 4 of the 6 samples (AH and CR in mid-July, IR in late-July, and MC in mid-August) and was at least the third most abundant at LL in late-July (range = 9.9 – 70 %, Fig. 10A). At CP, *Microcystis* was in the top 10 but contributed <1 % to the overall assemblage. The genus *Pseudanabaena* was also detected in all bloom samples, with the exception of one sample from CP. However, *Pseudanabaena* always contributed much less to the overall assemblage than *Microcystis* (range = 0 – 10 %, Fig. 10A). While toxin production by *Pseudanabaena* is lesser studied, the genus has been detected in association with microcystin (Maršálek et al., 2003; Oudra et al., 2001). Other noteworthy functional groups within the top 10 taxa included genera associated with MC degradation like *Paucibacter, Flavobacterium,* and *Vogesella* (Berg et al., 2009; Le et al., 2022; Van Le et al., 2022; Zhao et al., 2017). There were no additional MC producing or known MC

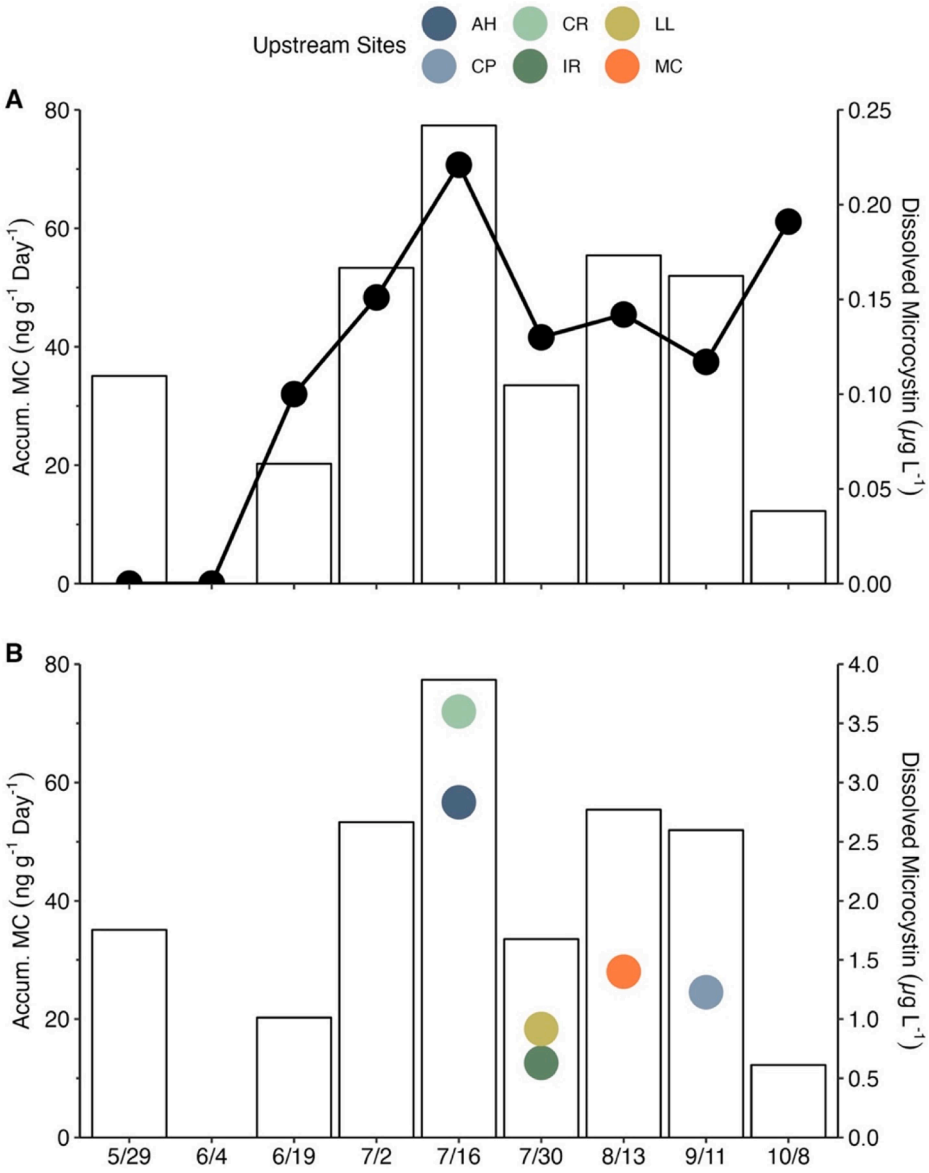

Fig. 7. Accumulated dMC concentrations from SPATTS (ng g^{-1} day^{-1}) at the downstream site on the primary y-axis (columns) compared to discrete dMC concentrations (µg L^{-1}) from the downstream site (A) and upstream sites (B) on the secondary y-axis (points).

degrading taxa dominant in any of the individual samples (categorized as "other") that were not also reflected in the top 10.

For site 129S, the 10 top taxa made up between 19 and 69 % of the overall assemblage, with the cyanobacterial genus *Cyanobium* ranging from 16 to 34 % (Fig. 10B). *Cyanobium* was observed as the most abundant taxa across dates with only one exception on September 11th when *Rhodoferax*, a beta-proteobacteria, was the most abundant at 7 % (not shown within the 10 most common taxa in Fig. 10B). Together with *Cyanobium*, other potential MC producers detected downstream included genera *Dolichospermum, Pseudanabaena, Microcystis*, and *Raphidiopsis* (formerly *Cylindrospermopsis)* though they respectively did not make up >1.5 %, 3.3 %, 0.3 %, and 1.2 % of the community at any time. There was no correlation between *Cyanobium* relative abundance and pMC or dMC ($p > 0.05$, Spearman's rank correlation test) at the downstream site. Also detected in the top 10 downstream was *Cuspidothrix*, a genus that together with *Microcystis* may be linked to anatoxin production (Christensen and Khan, 2020; Jiang et al., 2015) but testing for anatoxin was outside the scope of this study.

A strong, positive relationship was observed between the relative abundance of *Microcystis* and the natural log transformed total MC concentrations (dMC + pMC) in upstream bloom samples (Fig. 11A; $R^2 = 0.72$). This relationship held up, despite being slightly weakened if the highest MC concentration datapoint from the CR bloom event was removed ($R^2 = 0.57$). In-depth analyses yielded 10 unique ASVs within the genus *Microcystis* based on the V3-V4 region and the use of the general 16S primers used in this study. Only one ASV was further classified beyond the genus level as *Microcystis wesenbergii* in 4 of the 19 samples (sites AH and CR) never contributing >1.5 % of total *Microcystis* ASVs. The two most common ASVs, distinguished by a single nucleotide difference, made up between 94 −100 % of the *Microcystis* assemblage but no correlation between their relative contributions and total MC concentrations was found.

4. Discussion

4.1. Toxic blooms in the Chowan river

Several locations along the Chowan River constituted hot spots for MC throughout the 2019 bloom season, with record-breaking MC levels not previously reported anywhere across the Pamlico-Albemarle Sound

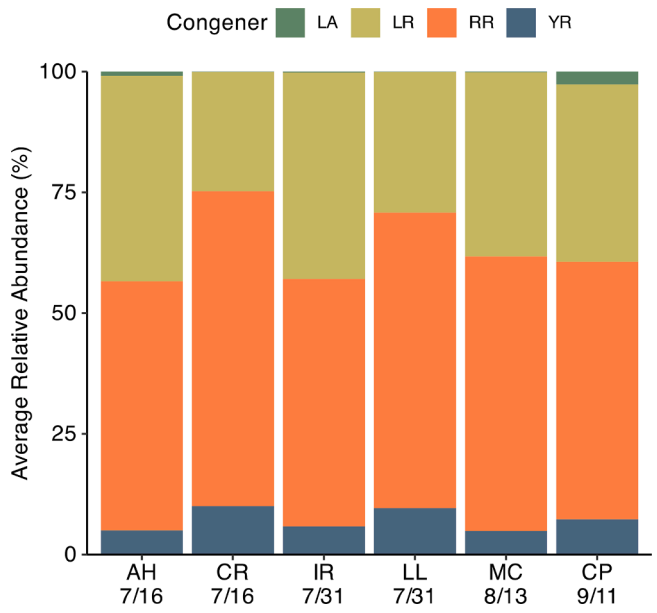

Fig. 8. Average relative abundance of the four MC congeners quantified with standards (-LA, -LR, -RR, -YR) for each bloom assemblage. Note, no congener information was collected for the downstream site. See sampling location details in Table 1 and Fig. 1.

System (Table 2). Maximum concentrations of 1141 μg L^{-1} in the upper surface water (< 0.5 m) and 15,989 μg L^{-1} within cyanobacterial surface scums, exceeded recreational thresholds recommended by the EPA (8 μg L^{-1}) (EPA, 2019), by 3 and 4 orders of magnitude, respectively. Satellite imagery (Fig. 4) suggests that bloom activity was ongoing throughout various river regions in the Chowan during the summer of 2019, even before study sampling began, and thus these bloom samples are snapshots of a bloom activity with a larger, variable spatiotemporal extent. Blooms of cyanobacteria are often patchy and heterogeneous (Kutser, 2009; Vander Woude et al., 2019), as a result, the maximum threat of toxin exposure associated with this ongoing bloom activity may be underestimated through the discrete, shoreline sampling approaches used here. However, shorelines are important access points where humans and animals are likely to interact with the water, and thus the values presented in this study, while they might not fully characterize blooms across large sections of the Chowan River, do provide useful insights related to exposure risk.

Previous reports from the Chowan River indicated the presence of known cyanotoxin producers, and a limited number of reports also confirmed the production of cyanotoxins (Table 2). A pilot study for which passive toxin trackers were deployed for the first time within the system was conducted throughout 2015 and 2016 and the results indicated that cyanotoxins were present year-round (Schnetzer, unpublished; Table 3). These previous observations, which spanned varying time periods at several locations within the mid and lower Chowan River, including the Edenton area, confirmed the presence of MC, anatoxin, and cylindrospermopsin (Table 3). Accumulated dMC concentrations exceeded those for other toxins by 1 to 2 orders of magnitude and agreed well with the SPATT ranges detected for the western Albemarle Sound in this study during 2019 (Table 3). In contrast to this earlier pilot study, event-driven sampling in 2019 clearly demonstrated that MC-producing blooms may reach toxin concentrations orders of magnitude higher than recommended EPA recreational guidelines, posing a significant threat to human health and impeding water uses in the Chowan River. These findings alert to an urgent need for continued regular and event-driven monitoring of the system, especially during more active bloom periods like summer and fall.

Concerns about toxin production in association with visible, high biomass blooms in the Chowan River had been voiced for more than a decade and before that in the 70s and 80s (Sauer and Kuenzler, 1981; Stanley and Hobble, 1981; Witherspoon et al., 1979). Toxin screening, however, has not been regularly conducted and assessing risk by monitoring agencies is typically based on chl-a levels and cyanobacterial abundance ranges as limited resources do not afford additional toxin testing or screening for multiple toxin types (Loftin et al., 2016; Wiltsie et al., 2018; World Health Organization, 2020). Metrics like chl-a biomass and cell densities alone are generally considered to lead to an overestimation of MC related exposure risks compared to actual toxin information (Loftin et al., 2016). This is due in part to the fact that not all cyanobacteria are toxin producers and known toxic species may not produce toxins continuously. In this study, excess algal biomass levels (chl-a) and total MC concentrations for 5 out of 6 bloom events both indicated a high MC exposure risk based on WHO guidelines. Only the CP bloom metrics disagreed; a high-risk exposure would have been suggested based on biomass and a low risk for humans based on MC concentrations. We presume that this was due to sampling during a late bloom stage when contributions from potential toxin producers were low and MC had either been degraded, potentially consumed, or exported from the system.

4.2. *Bloom assemblages*

Previous testing of blooms for MC had been conducted through NC DEQ primarily, on a case-by-case basis when reports from staff and citizens were received. These examinations rarely yielded MC levels above the EPA recreational guideline of 8 μg L^{-1} within the study area (since 2012, see Table 2). Based on microscopy analyses conducted by NC DEQ, recurrent known and potential MC-producing genera had mostly included *Dolichospermum, Pseudanabaena* and at times *Microcystis* and *Aphanizomenon* (Table 2). In 2019, independent observations, based on microscopy and DNA sequencing, agreed that *Microcystis* was the dominant genus during high biomass events in the Chowan River and in association with unprecedented high MC concentrations. The presence of *Microcystis*, albeit concurrent with *Dolichospermum*, was also linked to both high chl-a and high MC concentrations with 1580 μg L^{-1} and 350 μg MC L^{-1}, respectively, on July 28th of 2021 (Table 2). In contrast, the dominance of the genera *Dolichospermum* and/or *Aphanizomenon* yielded chl-a concentrations of > 4000 μg L^{-1} but toxin levels of ≤ 1 μg MC L^{-1} (June 27th of 2018 and July 12th of 2023; Table 2). Contextualizing these results further supports the powerful potential for molecular analyses as a risk assessment tool in addition to biomass or cell density measurements. The use of DNA based community composition analysis in this study allowed for the relative quantification of *Microcystis* abundance and the detection of lesser abundant and pico-sized taxa, all of which are difficult to document when using traditional microscopy to analyze dense bloom samples (MacKeigan et al., 2022; T-Krasznai et al., 2022). We propose, based on the strong link between *Microcystis* relative abundance and MC concentrations in this study, that the employment of more quantitative genetic tools to assess for *Microcystis* abundance (e.g., qPCR) could be a key tool to pinpoint areas for increased MC monitoring within the Chowan system. This notion agrees with previously published studies that emphasize the importance of genotype-specific resolution of bloom communities to distinguish toxin-producing and non-toxic members (Bramburger et al., 2023; Chorus et al., 2021; Rinta-Kanto et al., 2009).

One of the most striking differences observed during 2019 was the contrast in *Microcystis* relative abundance and MC concentrations for the CR and CP events. Bloom metrics indicated that the CR event was still developing or near peaking while the bloom at the CP site seemed to have entered a stage of decline by the time sampling occurred. The coloration of the water also indicated different bloom stages, as the bright green observed at the CR bloom was associated with active *Microcystis* spp. growth whereas a milky blue color, like the one observed at the CP bloom, has previously been correlated with

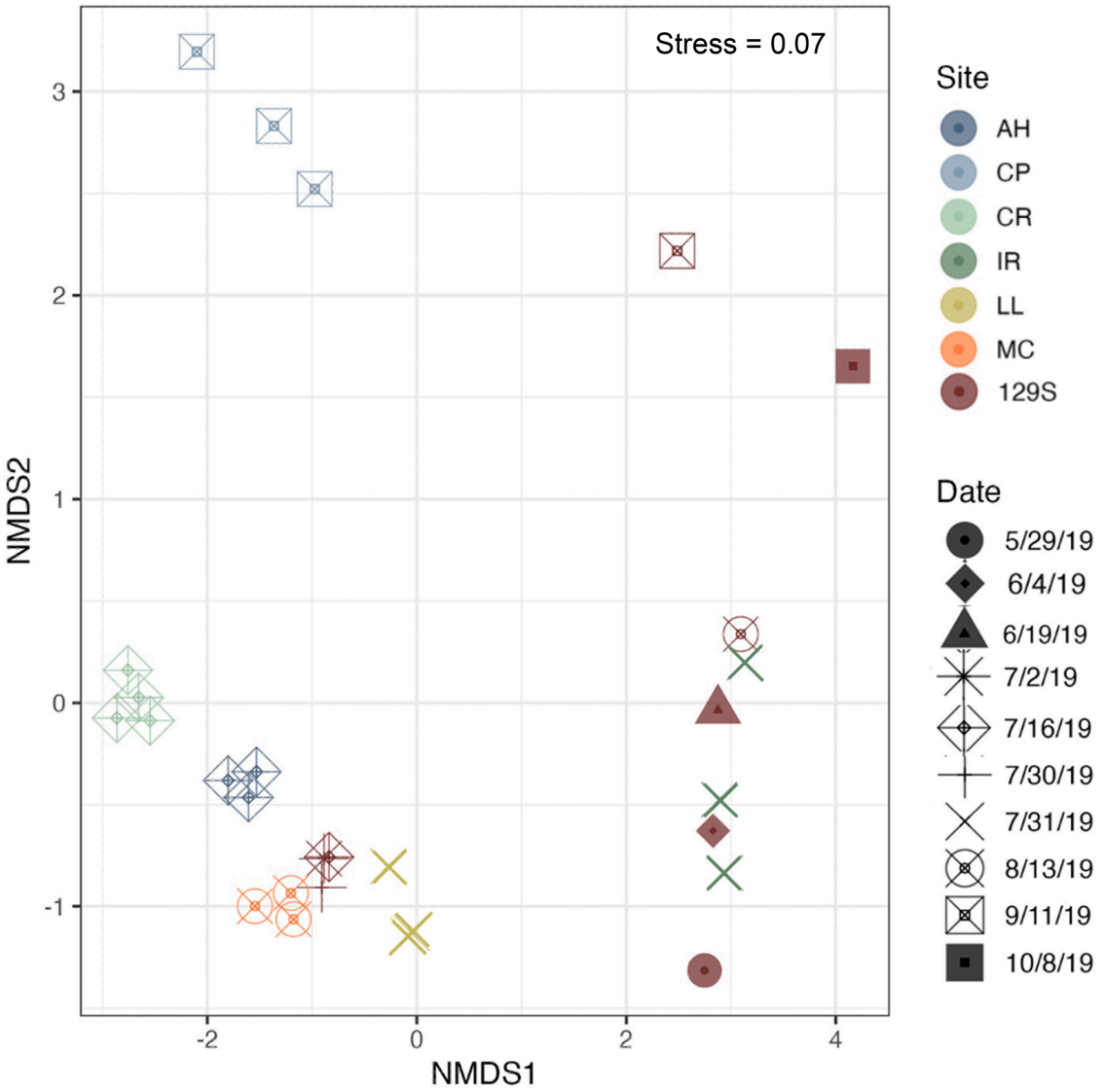

Fig. 9. NMDS plot produced from Bray-Curtis distance matrix illustrating similarities in bacterial community composition. See sampling location details in Table 1 and Fig. 1.

cyanobacterial lysis (Fig. 11B-D) (Arii et al., 2015; Fallon and Brock, 1979). The assumption that the CP bloom was in a declining stage was further supported by indicators of biotic loss processes such as higher contributions from genera such as *Paucibacter, Flavobacterium*, and *Vogesella* which have been associated with bloom decline (Harris et al., 2024). Species of *Paucibacter* had previously been linked to MC degradation and *Microcystis* algicidal activity (Le et al., 2023; Le et al., 2022; Rapala et al., 2005; Van Le et al., 2022). *Flavobacterium* was observed to exert control over *Microcystis* cell densities, paired with potential MC degrading capabilities (Berg et al., 2009; Cai et al., 2014; Maruyama et al., 2003). And finally, *Vogesella* was identified as a predominant genus in water and sediment samples during the decomposition of *Microcystis* biomass (Zhao et al., 2017). Several other taxa may also be indicative of distinct bloom stages in agreement with our results (e.g., *Roseomonas*, Sphingobacteriales) (Mankiewicz-Boczek and Font-Nájera, 2022). Two families of the order Sphingobacteriales were present across the blooms, most abundantly at site AH on July 16th and at site LL on July 31st. Sphingobacteriales has been previously associated with cyanobacterial blooms, especially during the early period of bloom decline because they thrive on cyanobacterial exudates (Berg et al., 2009; Parulekar et al., 2017). *Roseomonas*, a nitrogen transforming bacteria, was also present across blooms and most abundant at site CR on July 16th and site LL on July 31st. *Roseomonas* has been observed previously to increase in abundance when toxin producing *Microcystis* were beginning to bloom (Qian et al., 2017). As such, determining the relative contributions from toxin producers in combination with additional functional groups such as MC degrading bacteria throughout bloom progression may be key in deciphering toxin dynamics and fate, and in yielding a better understanding of how long blooms pose a risk. A laboratory study of MC dynamics using incubations of bloom water from the same blooms described in this study found that pMC concentrations declined exponentially, but at differing rates for different assemblages (average half-life = 10.2 days), and that dMC remained detectable for up to 100 days (Pierce and Schnetzer, 2023).

4.3. Congener composition matters

Current guidelines for human exposure are based primarily on the more toxic MC-LR congener, which may indicate that the exposure risks of the Chowan River blooms analyzed in this study may be overestimated using that framework (World Health Organization, 2020). In the Chowan River, MC congener profiles were predominantly made up of MC-RR and MC-LR where MC-RR has been deemed far less toxic than MC-LR based on rodent models (Chernoff et al., 2021, 2020). While

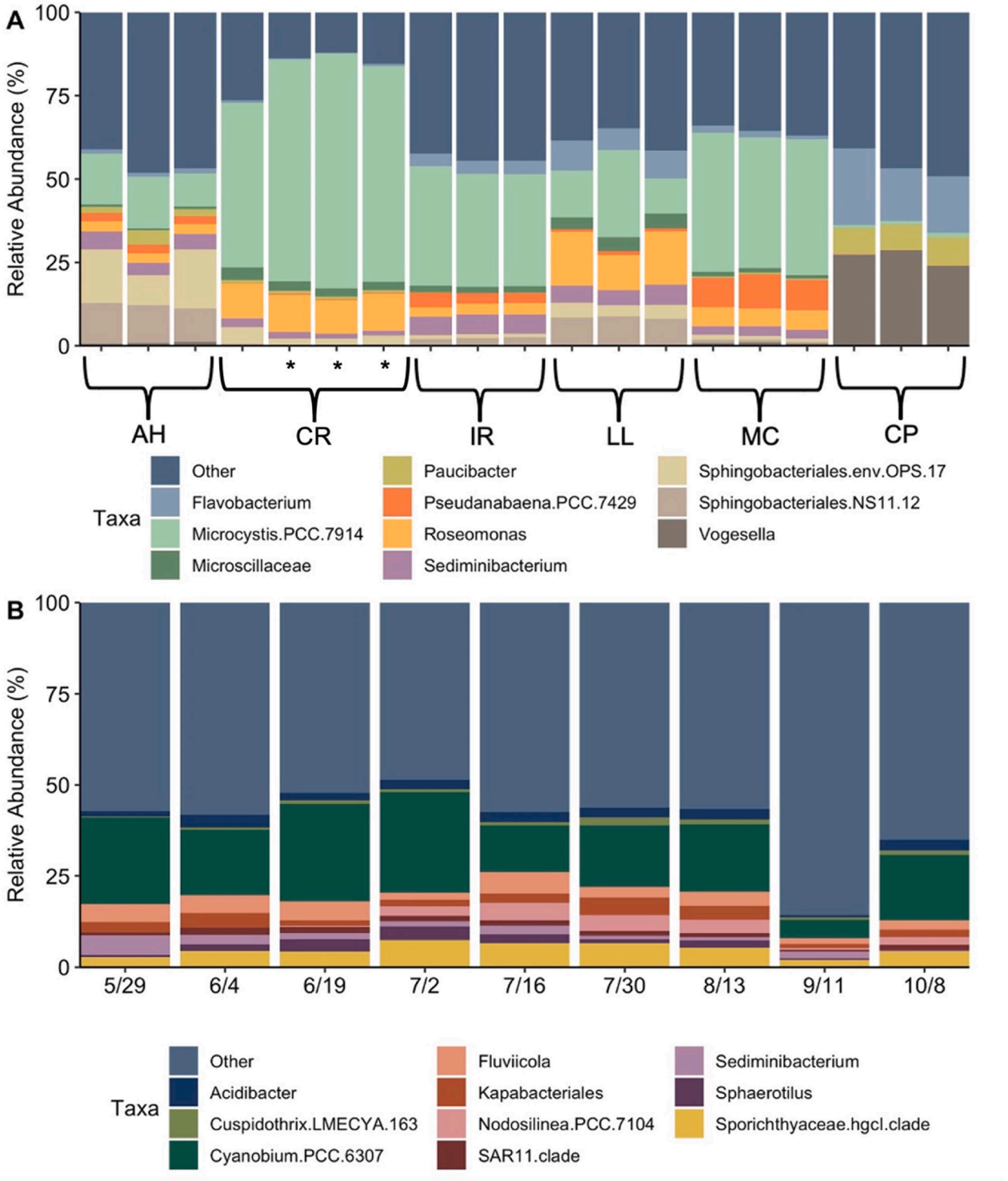

Fig. 10. (A) Relative abundance of the shared top 10 taxa across bloom samples in the Chowan River region. Taxonomic affiliations were assigned on the genus level for all but three members. Microscillaceae were assigned at the family rank and Sphingobacteriales.env.OPS.17 and Sphingobacteriales.NS11.12 at the order level. Samples are grouped by site and displayed in chronological order of sampling. Each site was analyzed using triplicates and CR as quadruplicate (1 water sample and three scum samples marked with *). (B) Top 10 taxa shared across the sampling time series at 129S downstream shown in chronological order (B). See sampling location details in Table 1 and Fig. 1. Note that the same color-coding was used for both graphs and only the category "other" and the taxa Sediminibacterium were shared.

studies on single congener exposure can support foundational knowledge of MC toxicity and risk, health impacts due to synergistic effects from environmentally relevant MC congener mixtures have yet to be addressed and this major knowledge gap impedes comprehensive management strategies for protecting public health. The four congeners examined in this study were chosen because they represent a range of toxicities, are commonly detected variants, and have been observed to co-occur to some degree in multiple other systems (Hotto et al., 2008; Howard et al., 2017; MacKeigan et al., 2023). MC structural profiles, based on these four common congeners, did not vary significantly across

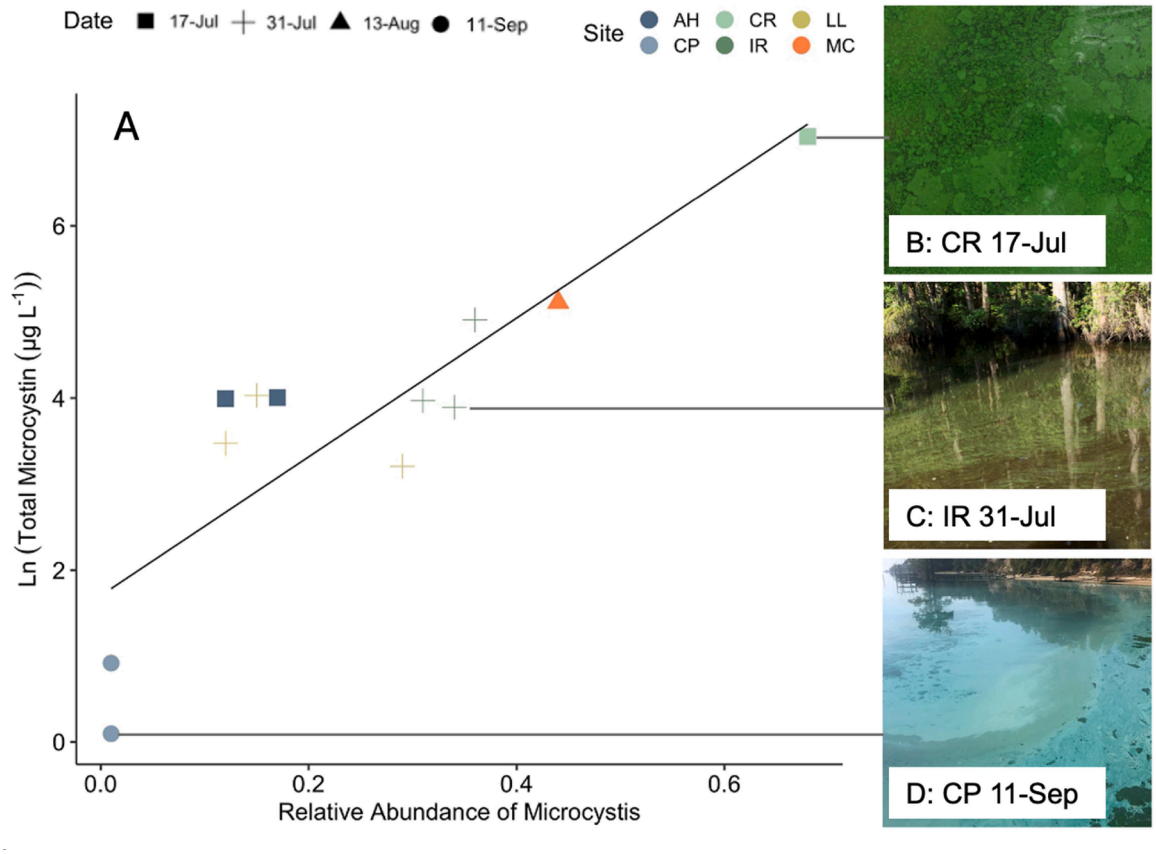

Fig. 11. Linear relationship between relative abundance of *Microcystis* spp. (%) and natural-log transformed total MC concentration across the upstream bloom sites (A) ($\ln(y) = 1.7 + 8.1x$, $R^2 = 0.72$) along with visual differences in bloom presentation from sites CR (B), IR (C), and CP (D) shown in connection with the associated points on the curve.

sites (Fig. 8), potentially due to the persistent dominance of the genus *Microcystis*. Research into the main drivers of congener composition is ongoing; recent studies have begun to link congener composition to strain-level genotype differences or in other instances to environmental or meteorological drivers (Dyble et al., 2008; Mikalsen et al., 2003; Monchamp et al., 2014; Puddick et al., 2016; Taranu et al., 2019). An observational study of congener composition of the same four congeners in this study assessed that warm, nutrient rich waters may correlate with MC-RR and MC-LR co-dominance (Taranu et al., 2019). While our findings on MC-RR and MC-LR dominance during an especially hot 2019, based on annual average air temperature (NOAA, 2020), in a nutrient replete Chowan River (NC DEQ, 2021b) generally corroborates such a proposition, much more information is needed to decipher these complex relationships. A study of Canadian lakes found that the presence of different *Microcystis* species was related to dominance of different congeners (Monchamp et al., 2014), and while there were multiple unique *Microcystis* ASVs in the Chowan River blooms, there was not enough taxonomic reference data to elucidate species or strain-level information to compare to congener composition. The 16S gene is regarded as insufficient for strain level identification within the genus *Microcystis*, but there is ongoing research into methods for resolving strain-level dynamics (Dick et al., 2021). Continuing to assess drivers of MC profiles will be an important part of characterizing risk for areas like the Chowan River that are prone to cHABs.

4.4. Bloom connectivity and downstream toxin transport

An increasing number of studies have raised concerns that freshwater toxins may be produced in brackish systems or transported downstream and subsequently incorporated into estuarine or marine food webs (Howard et al., 2022, 2021; Straquadine et al., 2022; Tatters et al., 2021). Targeting marine sounds at the outskirts of the Pamlico-Albemarle Sound System (i.e., Bogue Sound and Nelson Bay) and implementing passive toxin tracking devices to measure MC during a recent five-year study confirmed that dMC transport also takes place along the freshwater-marine continuum in NC waters (Anderson et al., 2023). In this study, the inclusion of samples from the western Albemarle Sound was meant to provide insight into upstream connectivity based on genotype surveys and toxin presence during 2019. Although transport of toxin-producing *Microcystis* cells has been suggested over long distances, greater than 250 km in the Klamath and Kansas Rivers (Graham et al., 2012; Otten et al., 2015), the comparison of bacterial assemblages for site 129S with the upstream bloom events, approximately 25–35 km away, did not indicate the transport of viable *Microcystis* cells (Fig. 9). This was further corroborated by low cyanobacterial chl-a concentrations near the downstream site throughout the summer of 2019 based on satellite imagery (Fig. 4). Low to moderate chl-a concentrations (< 40 µg L^{-1}) and pMC concentrations < 1 µg L^{-1} downstream (Fig. 6) were linked to bacterial assemblages that were comprised of very different taxa (Fig. 10B), with *Cyanobium* being the most abundant. While the research on MC production in picocyanobacteria is ongoing (Jakubowska and Szeląg-Wasielewska, 2015; Jasser and Callieri, 2016), *Cyanobium* relative abundance did not correlate with pMC or dMC at the downstream site. Such a disconnect between up and downstream cHAB assemblages may be due to several factors including Chowan River inputs to the Albemarle Sound being attenuated by flow from the Roanoke, which can contribute significantly to freshwater inputs (Fitzgerald and Gurley, 2017). *Cyanobium* also contributed strongly to Chowan River assemblages under non-bloom conditions and when pMC concentrations are low (< 1 µg L^{-1}, Table 2) in 2020 (Plaas

et al., 2022), further supporting the significance of genotype data to inform the prediction of bloom toxicity.

Comparing dissolved toxin dynamics, independent of bacterial assemblage composition, highlighted that relative changes at site 129S, both based on grab samples and SPATTs, mirrored patterns in bloom activity in the Chowan River, indicating that dissolved toxin transport may have taken place (Fig. 7). Follow-up laboratory studies of the Chowan bloom assemblages discussed here found dMC to persist for up to 100 days (Pierce and Schnetzer, 2023). Additional laboratory and in-situ measurements have documented relatively long and ranging half-lives of dMC between 1.5 and 60 days (Zastepa et al., 2014), exhibiting the toxin's persistence. In a study of the Kansas River, dMC transport mechanisms were determined to be important contributors to toxin fate in the system (Graham et al., 2012). For the Chowan River further information on flow rate and residence times will be key to decipher dMC transport. With an increasing number of survey studies including in situ approaches to assess MC transport along the freshwater-marine continuum, SPATT data might become more easily interpretable and integrated in monitoring efforts to assess exposure risks and the potential for food web accumulation in specific locales (Anderson et al., 2023; Howard et al., 2022). As for 2019, MC was also detected in several commercially and recreationally harvested fish collected within the western Albemarle Sound emphasizing the importance of assessing the risk for human exposure via seafood consumption downstream from the Chowan River (Schnetzer et al., in prep). More information is also urgently needed on how the approximately 61,000 recreational users may be affected by such events in the future as a eutrophic Chowan River is prone to increasing cHAB activity with climate change (EPA, 2023). This study is meant to provide key information about toxic bloom events and potential monitoring targets that can aid in the assessment of exposure risks for recreational users and residents, and ultimately, improve early warning capabilities for cHABs within the Chowan River and western Albemarle region.

5. Conclusions

This study observed the highest concentration of MC to date in the Chowan River during the bloom period of late summer through early fall of 2019. Algal scum was observed with especially high MC concentrations, well above the WHO high risk threshold potentially representing a severe threat especially to pets and children which may be more prone to interacting with scum aggregates along the shoreline. There was high spatiotemporal variability, presumably related to different bloom phases, in algal biomass, MC concentration, and bacterial communities across upstream blooms that were sampled.. Composition shifts from *Microcystis* dominated communities to assemblages with high abundance of bacteria associated with *Microcystis* decline indicated bloom degradation had set in during some of the events. This supports the argument that cyanobacterial community assemblage data can be important in documenting bloom progression and can provide insights for bloom monitoring and management strategies. Specific to the Chowan River in 2019, *Microcystis* relative abundance was strongly related to MC concentrations at upstream bloom sites making the genus a preferable molecular target for assessing exposure risk. Notably, MC congener profiles were not different across blooms, suggesting that any of the genus level differences across bloom assemblages were not influencing differential congener production, but that the presence of *Microcystis* spp. may have been a dominant factor. MC-RR, as the most prevalent congener in this study, is generally considered far less toxic than MC-LR which guidelines are based on. This finding highlights that congener detection should be built into bloom monitoring to better understand exposure risks. Downstream accumulated dMC detection in the absence of notable bloom activity in the surrounding water, points towards the need of downstream testing when upstream blooms take place to better understand toxin exposure risks associated with transport along the freshwater-marine continuum. Overall, we believe that this study led to several key findings that can inform management approaches and ultimately strengthen bloom prediction and protection of public health along the Chowan River and within the western Albemarle Sound, regions of ecological and social importance threatened by cHAB activity. Finally, the timely documentation of each toxic event in 2019 was only made possible through partnerships with stakeholders along the Chowan River, the CEEG and NC DEQ. In agreement with an increasing number of research efforts (Howard et al., 2022), this study models the importance of collaborations with local experts, water quality agencies, and concerned citizens as an integral component of cHAB monitoring approaches to protect public health from the adverse effects of toxic blooms.

CRediT authorship contribution statement

Emily Pierce: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Conceptualization, Data curation. **Marco Valera:** Writing – review & editing, Data curation. **Mark Vander Borgh:** Writing – review & editing, Data curation. **Daniel Wiltsie:** Writing – review & editing, Data curation. **Elizabeth Fensin:** Writing – review & editing, Data curation. **Charlton Godwin:** Writing – review & editing, Data curation. **Jill Paxson:** Writing – review & editing, Data curation. **Gloria Putnam:** Writing – review & editing, Data curation. **Colleen Karl:** Writing – review & editing, Data curation. **Blake Schaeffer:** Writing – review & editing, Visualization, Formal analysis, Data curation. **Astrid Schnetzer:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hal.2024.102747.

Data availability

Sequences generated in this study can be accessed on NCBI under BioProject accession number PRJNA1164174. All other data are available upon request.

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