8-2022

Gaging Downstream Transport of Cyanobacteria in the Raritan River Basin

Kyle R. Clonan

Abstract:

Cyanobacteria dominated Harmful Algal Blooms (HABs) have the potential to release toxic compounds harmful to human health. Few studies have examined the potential for cyanobacteria travelling from lacustrine HAB sources through fluvial systems used for potable water supply. The Raritan Basin Water Supply Complex (RBWSC) sources potable water for several utilities serving more than 1.5 million people in central New Jersey. The RBWSC features three lentic waterbodies with persistent HABs; discharges from these waterbodies all reach the downstream drinking water intakes. The objectives of this study were to 1) review the persistence of cyanobacteria during fluvial transport from upstream, cyanobacteria-dominated lakes and reservoirs to downstream drinking water intakes in the RBWSC, and 2) find which physiological parameters influence the downstream transport of cyanobacteria. This study found cyanobacteria persistence downstream was primarily influenced by discharge, with periods of higher discharge resulting in greater persistence of cyanobacteria travelling downstream. Factors frequently associated with lentic cyanobacteria growth—such as higher concentrations of nitrogen and phosphorous, warm water temperatures, and lower turbidity—did not show evidence of aiding lotic cyanobacteria travel. It is unclear why some cyanobacteria genera showed greater persistence than others. Drinking water managers in the RBWSC should know that an increased, but diluted, amount of cyanobacteria may reach their intakes during periods of increased discharge. Downstream transport of cyanobacteria from HABs should be investigated on a site-specific basis, as transport mechanisms, impoundments, trappings, and phytoplankton composition are specific to each water basin.

Keywords: Cyanobacteria, Harmful Algae Blooms, HABs, drinking water.

MONTCLAIR STATE UNIVERSITY

Gaging Downstream Transport of Cyanobacteria in the Raritan River Basin

by

Kyle R. Clonan

A Master's Thesis Submitted to the Faculty of

Montclair State University

In Partial Fulfillment of the Requirements

For the Degree of

Master of Science

August 2022

College of Science and Mathematics

Department of Earth and Environmental Studies

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Acknowledgements

I would like to my thesis sponsor, Dr. Meiyin Wu for welcoming me into her lab, as well as for her relentless work ethic in research, investigation, and team management. Dr. Wu's diligence in following the scientific method and her thoroughness in logistical planning was instrumental to the success of this study. I value Dr. Wu's endless knowledge and hope to continue working with her throughout my career.

I would also like to thank my committee members Dr. Galster & Dr. Deng for their continual oversight, comments, and thoughts during this thesis process. Thank you, Pam Reilly, for your thoroughness in securing the grant funding for this project, for consistently aiding in last-minute field work requests, and necessary perspective on the application of this research.

My thesis is a small part of a much larger study focusing on the downstream fate and transport of cyanobacteria and cyanotoxins in the Raritan Basin, which is funded by a USGS Cooperative Matching Funds Grant and is a collaboration between the United States Geological Survey (USGS), Montclair State University (MSU), the New Jersey Water Supply Authority (NJWSA) and the New Jersey Department of Environmental Protection (NJDEP). I could never enjoy working on this project more than I did, and I owe that to this incredible team. Since joining the NJWSA, Heather Desko, has been instrumental in my career development; I have been allowed incredible job opportunities and learned so much because of her work. Heather Heckathorn of USGS has done a tremendous job coordinating many meetings and overseeing logistics between project partners. Rob Newby, Bob Schuster and Eric Ernst of NJDEP have each had the patience to teach me about water quality, nutrients and cyanobacteria and I value their thoughts and experience so much. Finally, this project would not have been anywhere near as fun without Molly Hillenbrand and Melissa Mazzaro at my side for each sampling event. Their preparation and thoroughness were instrumental to the completion of the study, I have continued to lean on their experience while writing this thesis, and I hope we can continue to bounce ideas off each other for years to come.

Lastly, I would like to thank my family, who continues support me and put perspective on life. I owe both my parents everything for placing me into a position to succeed in life and helping me to get the most out of myself. I have always valued my special connection with my mother over our interests in math and science; I am so happy to have had her encouragement and support when picking this career path.

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1. Introduction

1.1 The Dangers of Cyanobacteria and Harmful Algal Blooms in Drinking Water

Cyanobacteria compose a diverse group of microorganisms commonly included with algae in phytoplankton assemblages. This is despite cyanobacteria's phylogenetic separation from true algae due to their bacterial, prokaryotic cell structure (Moreira et al., 2022; Wehr et al., 2015). Cyanobacteria can be characterized by their predominate chlorophyll-a and phycocyanin pigmentation, resulting in a "blue-green" color and the ability to photosynthesize (Wehr et al., 2015). Under certain conditions, physiological adaptations allow cyanobacteria to outcompete other algal families, resulting in rapid population growth and the formation of blooms, often referred to as Harmful Algal Blooms (HABs) (Al-Tebrineh et al., 2012; Casamatta and Hasler, 2016; Graham, 2012; Ho et al., 2019; Moreira et al., 2022; Paerl and Otten, 2013; Wehr et al., 2015; Wood et al., 2011). These adaptations include the ability of some cyanobacteria species to control their buoyancy through the use of gas vesicles. This allows the cyanobacterial cell to position itself towards the water surface, maximizing its exposure to sunlight necessary for photosynthesis. Some cyanobacteria can outcompete other algae in areas of low nitrogen availability by using specialized nitrogen-fixing cells called "heterocytes". A third competitive advantage includes certain cyanobacteria's possession of asexual "akinete" cells, which feature a thick cell wall and help these cyanobacteria withstand harsh environmental conditions (Baker et al., 2000; Brookes, 2002; Paerl and Otten, 2013; Wehr et al., 2015).

Episodic intense HAB events can materialize in eutrophic lakes and reservoirs during prolonged periods of calm winds and intense sunlight (Brookes, 2002; Paerl and Otten, 2013; Wehr et al., 2015). Depending upon the cyanobacteria species present in the HAB, a cyanobacteria bloom may appear to the naked eye as surface scum, a dense blue-green coloration in the water, be concentrated several meters below the surface, or not be visible at all (Paerl and Otten, 2013; Wehr et al., 2015). Over the last 40 years, HAB frequency has increased across the planet, which has been often attributed to a mix of both local and global factors (Cha et al., 2017; Ho et al., 2019). Increases in global temperature have resulted in prolonged periods of waterbody thermal stratification, a lake condition which favors the formation of HABs due to cyanobacteria's ability to regulate their buoyancy, as well as cyanobacteria's preference for a warmer water temperatures relative to other families of phytoplankton (Baker et al., 2000; Cha et al., 2017; Graham, 2012; Paerl and Otten, 2013). The increased rate of HAB formation has also been attributed to local factors amplifying waterbody eutrophication, such as watershed urbanization, direct sewer discharges, septic tank effluent inputs, and agricultural runoff (Cha et al., 2017; Conroy et al., 2017; Copetti, 2021; Ho et al., 2019; Paerl and Otten, 2013).

This increase in HAB presence has become a human health issue as some cyanobacteria can produce toxic compounds known as cyanotoxins. These include saxitoxins and anatoxins, which are neurotoxic and dermatoxic; while microcystins and cylindrospermopsins are hepatoxic (Cirés et al., 2017; Gibble and Kudela, 2014; Graham, 2012; Graham et al., 2008; Wehr et al., 2015). Cyanotoxin production depends upon the species and life stage of the cyanobacteria cell. Cyanotoxins can be stored within the cell, during which time they are known as endotoxins or released into the surrounding water as dissolved cyanotoxins periodically during the cell's life cycle. Most cyanotoxins exist in the environment as endotoxins. During cell lysis, all remaining intracellular cyanotoxins are released to the water column (Copetti, 2021; Graham, 2012; Walker, 2017).

Understanding cyanobacteria and cyanotoxin presence in raw source water is an increased focused for drinking water resource managers as they work to reduce potential toxin exposure and human health impacts (Al-Tebrineh et al., 2012; Deng et al., 2017; Dunlap et al., 2015; Graham, 2012; Graham et al., 2018; Iva et al., 2017; Lawton and Robertson, 1999; Walker, 2017). Even if dissolved cyanotoxins are not detected in the raw source water, lysing cyanobacteria cells during drinking water treatment processes can release cyanotoxins. In addition to their ability to produce cyanotoxins, cyanobacteria add other drinking water treatment concerns. Higher cell counts can increase turbidity and the likelihood for the presence of taste and odor compounds, such as geomsim and 2-methyl isobomeol (MIB) (Deng et al., 2017; Graham, 2012; Walker, 2017). To combat the high cell densities and presence of cyanotoxins, drinking water treatment costs can rapidly increase during a HAB (Dunlap et al., 2015; Walker, 2017). This may be due to treatment responses such as greater electricity needs, increased filter screen cleanings, or additional treatments such as the use of Powder Activated Carbon (PAC) or other methods (Walker, 2017). Removal and degradation of cyanotoxins during the drinking water treatment process can differ widely based upon the treatment plant and treatment chain specifics (Walker, 2017).

Most HAB research has targeted the causes, prevention, monitoring and management of intense, acute lacustrine bloom events. Few studies have examined movement of lacustrine cyanobacteria from persistent HAB sources through fluvial systems used for potable water supply (Graham, 2012). There are significant knowledge gaps in understanding the spatial and temporal persistence of cyanobacteria during fluvial transport, which may hold ramifications for downstream waterbody users and potable water intakes (Cha et al., 2017; Graham, 2012; Schmidt et al., 2014). Additionally, most existing HAB literature has also focused on the health effects of acute, high-concentration cyanotoxin exposure, while chronic, low-level cyanotoxin exposure is less understood (Graham, 2012). Therefore, understanding the potential year-round transport of cyanobacteria from upstream lakes and reservoirs to downstream drinking water purveyors can help water managers and public health agencies understand the persistence of HABs and the potential chronic exposure of their associated cyanotoxins (Graham, 2012).

Reservoir and lake outflows can have a significant effect on downstream phytoplankton composition (Cha et al., 2017; Graham, 2012; Reif, 1939). Previous studies on lotic phytoplankton assemblages consistently identify discharge as the primary factor governing downstream phytoplankton composition; but these studies differ amongst themselves on the relative importance of secondary parameters such as nutrient availability, sunlight, seasonality, travel time and distance from lacustrine source origin (Baker et al., 2000; Brookes, 2002; Cha et al., 2017; Conroy et al., 2017; Eddy, 1931; Graham, 2012; Graham et al., 2020; Moss and Balls, 1989; Reif, 1939; Reinhard, 1931; Reinl et al., 2020; Taylor et al., 2020; Wehr et al., 2015; Wiebe, 1928). While these secondary factors have been documented as influential on downstream phytoplankton transport, it seems how these parameters influence riverine cyanobacteria persistence, transport, and lotic production can differ between river basins and locations within the same river basin (Al-Tebrineh et al., 2012; Baker et al., 2000; Brookes, 2002; Graham, 2012). As such, the potential for lentic cyanobacteria to travel to lotic drinking water intakes needs to be investigated on a basin-specific basis to create an accurate risk assessment for drinking water managers.

The objectives of this study are to 1) review the persistence of cyanobacteria during fluvial transport from upstream, cyanobacteria dominated lakes and reservoirs to downstream drinking water intakes the Raritan Basin Water Supply Complex, and 2) find which physiological parameters may influence the downstream transport of cyanobacteria.

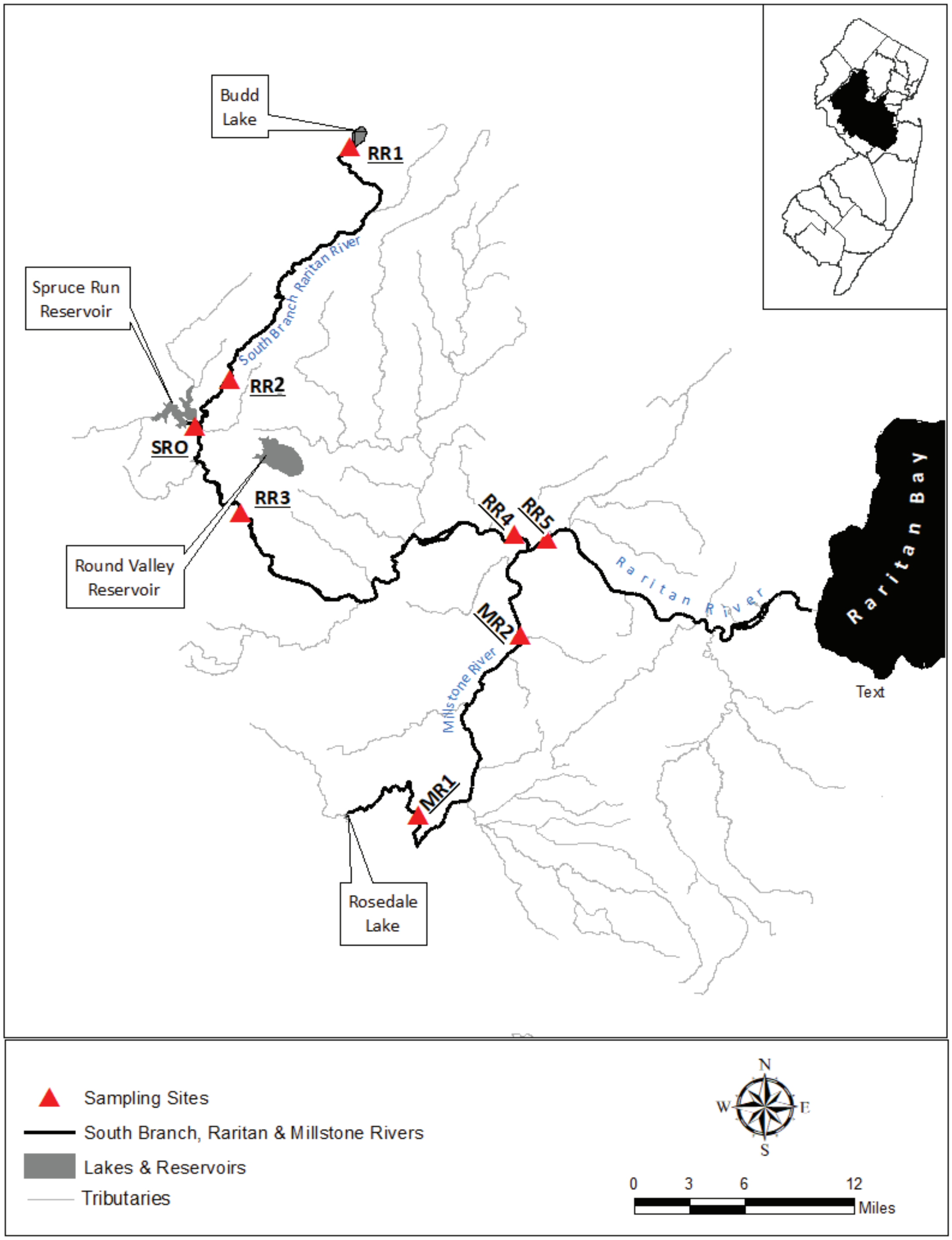
1.2 Raritan Basin Water Supply Complex

The Raritan Basin Water Supply Complex (RBWSC); illustrated in Figure 1, is the potable water source for utilities serving more than 1.5 million people in central New Jersey (Shallcross, 2002). The RBWSC consists of the entire Raritan River watershed, draining 1,100 square miles spread across three sub-watersheds: 1) The South Branch of the Raritan River (the South Branch) watershed, beginning at the outflow of Budd Lake and later absorbs the outflow of the Spruce Run Reservoir; 2) the North Branch of the Raritan River (the North Branch) watershed, which drains several prominent tributaries including the controlled, intermittent outflow of Round Valley Reservoir, and 3) the Stony-Brook Millstone watershed, composed of the Stony Brook and Millstone River (Shallcross, 2002). The Stony Brook-Millstone rivers together drain several small lakes and ponds, the most prominent of which, Rosedale Lake, intermittently discharges to the Stony Brook through a boxed weir. The Raritan River itself begins at the confluence of the North and South Branches. Shortly downstream of the North and South Branch confluence is the Raritan River's confluence with the Millstone River, with the first of several drinking water intakes on the Raritan River in short spatial proximity thereafter. Each of these rivers feature numerous historical low-flow dams, some of which have been removed in recent years.

HABs have been persistent across upstream lakes and reservoirs of the Raritan Basin from 2018-2020, leading to this drinking water basin' selection as the study location. In the South Branch watershed, Budd Lake, whose drainage begins the South Branch of the Raritan River, frequently featured HABs during the spring and fall from 2018-2020, which often impeded recreation and resulted in closure of the bathing beach. Spruce Run Reservoir, a 1,290-acre, 11-billion-gallon run-of-the-river reservoir had confirmed HABs in the fall of 2018 and an extensive bloom from June 2019 through December 2020 and has not re-opened its swimming beach since 2019. In the

Stony Brook-Millstone watershed, Rosedale Lake also features frequent HABs and recorded the highest cell count in New Jersey during the 2019 HAB season (NJDEP, 2022a). Other small ponds and lakes in the Raritan watershed have also occasionally featured HAB conditions during the last several years.

Figure 1 Map of Raritan Basin Water Supply Complex (RBWSC) with sampling locations



2. Materials and Methods

2.1 Discrete Water Quality Sampling Regime

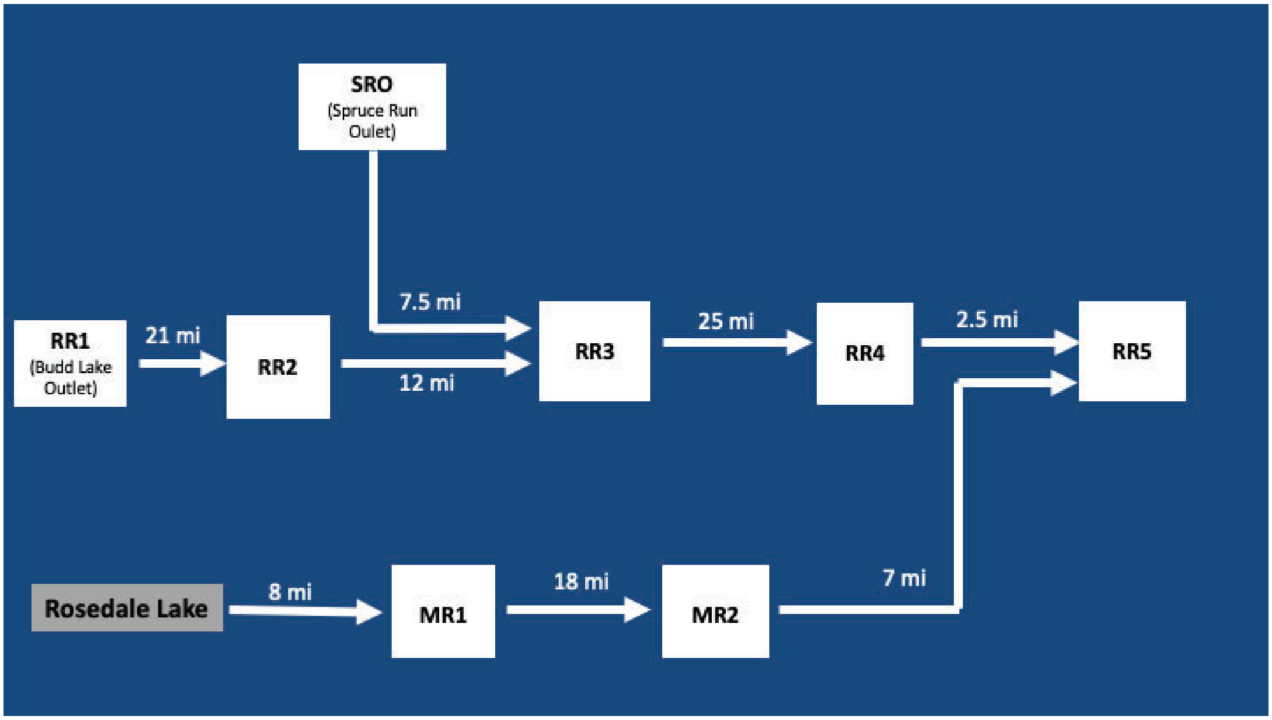
Following the discharge from Budd Lake, Spruce Run Reservoir, and Rosedale Lake downstream to the beginning of the RBWSC drinking water intakes, eight discrete sampling locations were chosen based upon existing United States Geological Survey (USGS) surface water sampling stations. The three most upstream sites, referred to as the “headwater sites” included the Spruce Run Reservoir outlet stream, Budd Lake outlet stream, and Stony Brook at Princeton, which is just downstream Rosedale Lake and was used to capture Rosedale Lake’s intermittent discharge into the Stony Brook. A description of the three headwater sites and all five downstream sites, as well as the abbreviations used hereafter, are listed in Table 1. A map of the entire basin is included as Figure 1, with a schematic of locations visible in Figure 2. The most downstream site, RR5, is positioned near the beginning of several drinking water intakes on the Raritan River and served as a proxy for their raw source water.

For simplicity throughout this narrative, discrete sample sites located along the South Branch of the Raritan River and the Raritan River itself are referred to as “Raritan River mainstem” sites and abbreviated RR-1 through RR-5. Sample sites located in the Stony Brook-Millstone watershed are referred to as tributary sites to the Raritan River mainstem and are denoted MR-1 and MR-2. The Spruce Run Reservoir Outlet site, itself located on a short tributary to the South Branch, is abbreviated “SRO”.

Table 1 Information on discrete sample site locations, draining areas (NJDEP, 2022b), and stream order (NJDEP, 2022b).

Site Name	Site Description	USGS Station	River Miles Upstream of RR5 (miles)	Drainage Area (square miles) (NJDEP, 2022b)	Stream Order of Site (NJDEP, 2022b)
MR1	Stony Brook at Princeton	014010000	25.7	45	5
MR2	Millstone River Blackwells Mills	01402000	7.4	258	7
RR1	South Branch of Raritan River near Manor House Road at Budd Lake	01396085	59.5	5	3
RR2	South Branch near High Bridge	01396500	38.7	65.3	5
SRR	Spruce Run at Clinton [1]	01396800	34.4	42	5*
RR3	South Branch at Stanton	0139700	26.9	143	6
RR4	Raritan River at Manville [2]	01400500	2.6	490	7
RR5	Raritan River below Calco Dam [3]	01403060	0	785	8

Figure 2: Schematic of sampling sites (white boxes) and headwater lakes and reservoirs (grey boxes). Arrows between sites are labeled with river miles.



Twenty discrete sampling events were conducted from August 2020 to August 2021, generally once a month except twice a month during September through November 2020 and May through July 2021. During each event, the upstream sites of RR1, RR2, SRO, RR3 and MR1 were sampled on day 1, while MR2, RR4 and RR5 were sampled on day 2. While no full time of travel study exists for the Raritan Basin, the observations of hydrographs during storms and releases by the NJWSA from Spruce Run Reservoir have provided an estimate of 24 hour travel time from Spruce Run Reservoir to RR5 (Shallcross, 2002).

During each event, discrete water samples were collected for all laboratory parameters while in-situ readings for physiochemical parameters were logged at each site. The physiochemical parameters water temperature, specific conductance, dissolved oxygen, and pH were measured using an in-situ YSI Professional Plus multiparameter instrument (Yellow Springs Instrument Incorporated, Yellow Springs, Ohio). In-situ physiochemical parameters were recorded as the median of the readings taken at three equally distributed points along a cross-sectional transect. Discrete water-quality samples were collected via a composite of three surface grabs, each taken these same three equally distributed points along the site’s cross-sectional transect.

This process was altered for safety reasons during periods of high discharge. During periods of unwadable flow, grab samples and in-situ readings were taken from a bridge if one was present at the site using a Van Dorn sampler at approximately 0.5m below the surface or a 1L plastic bottle secured inside a weighted basket. In-situ readings were then taken by lowering the YSI cable from the bridge. If no bridge was present at the site, samples and readings were taken from the bank. These approaches were only occasionally necessary and confined to RR4 and RR5.

All sample bottles were triple rinsed with site water before filling. Amber glass bottles were used to collect phytoplankton samples while two 45 mL plastic vials were used to collect water nutrients at each site. Samples were stored on ice and delivered back to their respective laboratory of analysis daily for preservation, filtration, or analysis, depending upon parameter and method.

2.2 Laboratory Methods

2.2.1 Phytoplankton Identification and Enumeration

Phytoplankton samples were analyzed by the New Jersey Center for Water Science and Technology (NJCWST) at Montclair State University. NJCWST provided phytoplankton identification and enumeration to the genus or otherwise lowest recognizable taxonomic level.

2.2.2 Chlorophyll-a Extraction:

Water samples were analyzed by NJCWST. NJCWST provided the results used for this analysis.

2.2.3 Water Nutrients

Water samples were analyzed for total nitrogen, ammonia, nitrate plus nitrite, total phosphorous and orthophosphate by the NJDEP Division of Water Monitoring and Standards laboratory in Leeds Point, NJ (Certified Laboratory #01179).

2.2.4 Turbidity

Samples were analyzed for turbidity at NJCWST using an Oakton Turbidity Meter (Model TN-100) following the Meter's Standard Operating Procedure. Three readings were taken for each sample, with the median reading recorded. All samples were analyzed within 48 hours of sampling.

2.2.5 Quality Control

Using a random number generator matched to site names, a random site was selected to serve as a trip duplicate for physiochemical readings and water sample collection during each sampling event and evaluated in the laboratory along with all other samples. All laboratory analyses performed lab duplicates, relative percent difference (RPD) and quality assurance checks in line with their methods' requirements.

2.3 Discharge Data

All sites except for RR1 were located at USGS stations outfitted with in-situ equipment for monitoring continuous (15-minute intervals) discharge. USGS personnel oversaw the equipment's calibration, maintenance, and logging of data according to published USGS methods (Sauer and Turnipseed, 2010; Turnipseed and Sauer, 2010). Discharge data was downloaded from the USGS National Water Information System (NWIS) online database. Each discrete water sample at each site was time-matched with the respective site's closest 15-minute discharge reading.

Without a continuous discharge gage, discharge at RR1 was estimated by matching past manually-measured discharge readings taken by USGS at the site location with continuous gage readings from the next site downstream, RR2. The paired data was log-transformed, and a linear regression produced an equation relating discharge between the two sites. This equation was then used to estimate RR1 discharge during each of the 20 sampling events based upon continuous discharge readings from RR2.

2.4 Data Analysis

All statistics, calculations and graphing included hereafter were completed using the statistical software R and R Studio (Team, 2022) utilizing the packages dplyr (Wickham et al., 2022), tidyr (Wickham and Girlich, 2022), and ggplot2 (Wickham, 2016). Discharge data from the NWIS online database was downloaded directly to R using the dataRetrieval package (De Ciccio et al., 2022). All correlations values are based upon Pearson's correlation coefficients using the p value of 0.05 as a significance test. When used, the "Percentage of the median discharge" was calculated by dividing the sample's discharge reading by the site-specific median discharge reading during the periods of study (8/1/20-8/31/21). This was done to control fo r

2.4.1 Trophic State Index

Trophic state index (TSI) values were calculated for each sample from laboratory results for extracted chlorophyll-a as described by Carlson (Carlson, 1977) according to the equation:

$$\boldsymbol{TSI(Chl) = 10(6 - \frac{2.04 - 0.68\, ln(Chl)}{ln(2)}})$$

Interpretation was aided by guidelines outlined by Carlson and Simpson (Carlson and Simpson, 1996).

2.4.2 Dilution Model

Expected total cyanobacteria abundance due to dilution at downstream sites (RR2, RR3, RR4, RR5, MR2) was calculated according to the equation below:

$$\frac{\boldsymbol{m_1 Q_1}}{\boldsymbol{Q_2}} = \boldsymbol{m_2}$$

Where:
m_1 = measured cyanobacteria abundance (cells/mL) at origin site—SRO, RR1, or MR1. If a downstream site received water from more than 1 of these sites, the expected abundance due to each individual upstream site were summed.
m_2 = expected cyanobacteria abundance at site due to dilution.
Q_1 = instantaneous discharge at time of sample collection at origin site.
Q_2 = instantaneous discharge at time of sample collection at downstream site.

This method of dilution modelling allowed for comparison between measured and expected cyanobacteria abundance was based on Graham (Graham, 2012), whose simple dilution model additionally incorporated gains and losses due to groundwater. Groundwater data was not available at the study area; inputs and losses of discharge due to groundwater were not considered for this study as it was outside the scope of this project; hence groundwater exchange was excluded from data analysis. Cyanobacteria recovery rates were calculated by dividing the measured cell abundance by the expected cell abundance due to dilution, and then multiplying by 100 to create a percentage.

2.4.3 Phytoplankton Summary Statistics

Summary statistics regarding phytoplankton samples were calculated and are summarized in the following tables and figures. Phytoplankton richness at each site was measured by genera, as identification did not include species level. The "Most Common Genera" at each site were defined as genera present in at least 40% of site's samples, regardless of abundance. Genera classified as "Dominant Genera" composed at least 50% of the relative abundance at the site during any sampling event.

3. Results

3.1 Downstream Transport of Cyanobacteria

Downstream transport of cyanobacteria was reviewed by comparing the measured total cyanobacteria cell abundance in cells/mL at each downstream site (RR2, RR3, RR4, RR5, MR2) to each site's expected cell abundance. This expected abundance was calculated by taking the total measured cyanobacteria abundance at each headwater site(s) (RR1, SRO, and/or MR1) discharging to the downstream site and diluting this abundance based upon the site's discharge reading, according to the equation in section 2.5.2. This expected abundance is based only off the total cyanobacteria abundance at the upstream and outlet sites and assumes cyanobacteria population sizes stayed constant while travelling downstream, this means the expected abundance excludes population changes due to cyanobacterial life cycle (i.e., cell reproduction, mortality, etc.), predation, as well as additional inputs from the catchment basins. This method follows Graham and others (Graham, 2012), though unlike Graham it does not take into account water lost to groundwater.

Figure 3 illustrates these expected cyanobacteria abundances (light red circles) compared to the measured cyanobacterial abundance in each sample (black triangles) at downstream Raritan River mainstem for the first sampling date of each month. The green vertical line indicates the location of SRO as a tributary to the Raritan River mainstem and the green triangle marks SRO's measured cyanobacteria abundance, which is then loaded into the Raritan River mainstem. The purple line indicates the location of MR2 as a tributary to the mainstem, along with MR2's measured cyanobacteria abundance (triangle) and expected abundance (circle) diluted from MR1.

Figure 3 Expected (Red Circles) and measured (Black Triangles) cyanobacteria abundance (y axis) travelling downstream in the Raritan Water Supply Complex. Position on X axis indicates the site's location in terms of distance from RR5. Inputs from tributaries are marked as vertical lines showing the location of the tributary joining the mainstem —the green line and triangle indicates the measured abundance at SRO, while the purple indicates the location of MR2 along with its expected cyanobacteria abundance from MR1 and measured abundance. Due to sizing, only the first sample of each month is shown.

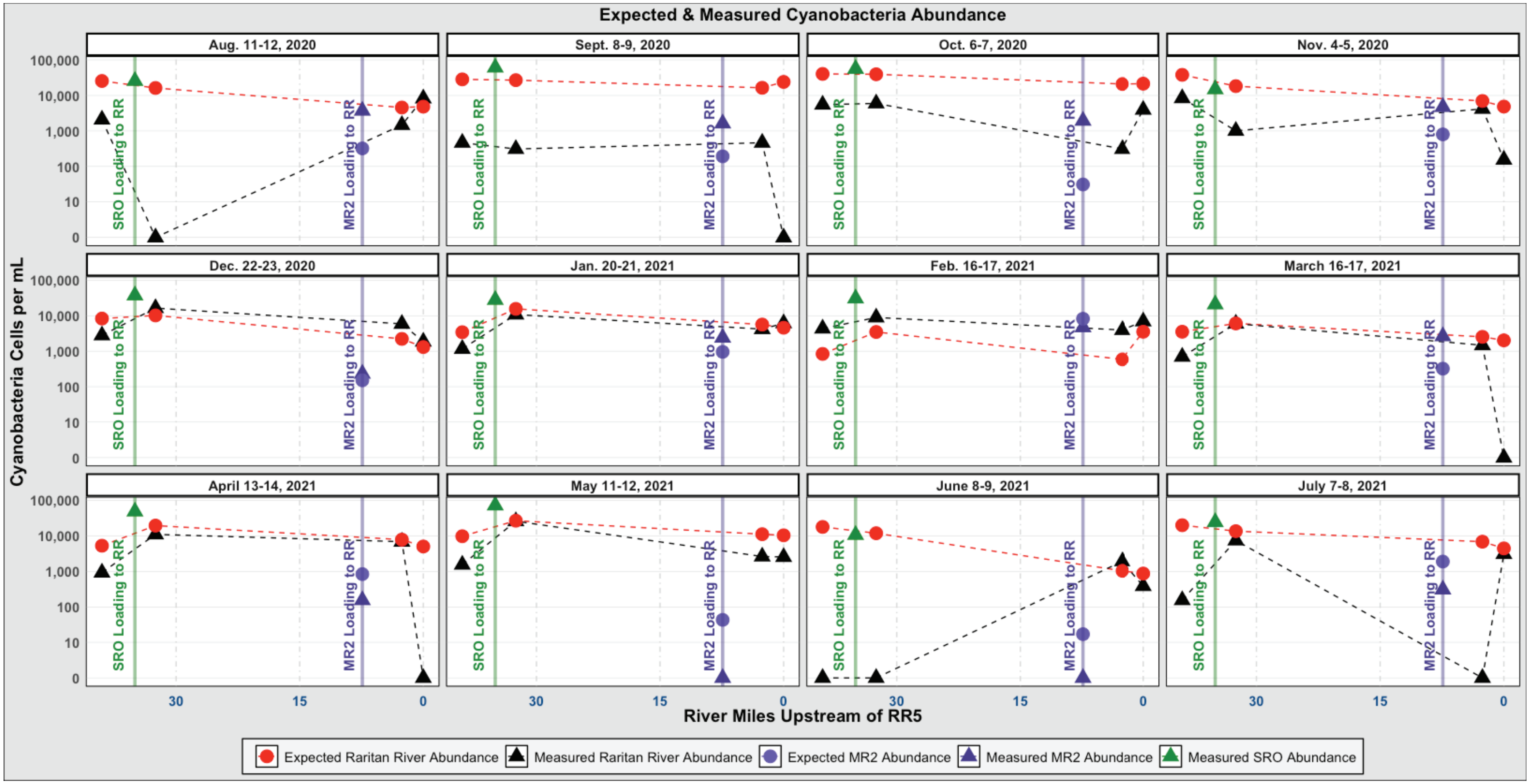


Table 2: Average total cyanobacteria recovery rate at each downstream site. Recovery rate was calculated by dividing the measured cyanobacteria abundance by the expected cyanobacteria abundance.

Downstream Site	Average Recovery Rate
RR2	37%
RR3	90%
RR4	75%
RR5	50%
MR2	1,215%

Measured cyanobacteria abundance in the Raritan River mainstem was normally lower than expected abundance. On average, cyanobacteria recovery was lowest at RR2, indicating most cyanobacteria likely perished between RR1 and RR2. The Raritan mainstem sites below SRO—RR3, RR4, and RR5—all featured higher average recovery rates, indicating Spruce Run Reservoir may have a larger influence on the downstream cyanobacteria community. However, cyanobacteria recovery difference could fluctuate widely between sites and events, as viewed in Figure 3. For instance, during the July 7-8, 2021 event, measured abundance nearly resembled expected abundance at RR3 and RR5 but was much lower than expected abundance at RR2 and RR4. Measured Raritan River mainstem cyanobacterial abundance most resembled expected abundance during the winter and early spring sampling events dating December 22-23, 2020 through May 11-12, 2021; though RR5 was well below expected values on March 17 and April 14, 2020. Nearly all these sampling events were during the winter and early spring.

In the Stony Brook-Millstone tributary, MR2, found measured abundance above the expected abundance during three-quarters of events, largely from August 2020 through March 2021. Overall, it averaged a 1,215% recovery, much higher than any site on the Raritan mainstem. This notably high recovery rate indicates the influence of cyanobacteria growth between MR1 and MR2, or significant loading from an unsampled source of cyanobacteria. However, while growth or loading was apparent between MR1 and MR2 due to the extremely high recovery rate, it did not appear this phenomenon continued between MR2 and RR5, as RR5 featured recovery rates more aligned with the other Raritan mainstem sites, where recovery was highest during the winter and spring months and rarely exceeded 100%.

As noted, measured cyanobacteria abundance at MR2 was nearly equal to or higher than expected abundance from August 2020 through March 2021, while measured cyanobacteria in the Raritan River mainstem most resembled expected abundance from December 2020 through April 2021, but rarely exceeded it. Both rivers featured measured abundance lower than expected abundance from Mary 2021 through August 2021. As RR5 receives the discharges from both the Raritan River mainstem and the Millstone River tributary, recovery of cyanobacteria at RR5 followed a mixture of the trends observed in the Millstone tributary and the Raritan River mainstem. RR5 also had 5 events where no cyanobacteria were measured, despite an expectation of 10,00 cells/mL on nearly all these dates.

3.2 Cyanobacteria Assemblages

While the previous section found the best evidence of downstream transport of cyanobacteria in the Raritan River mainstem during the winter and early spring, reviewing the composition of cyanobacteria genera in each sample may show if certain genera survive lotic transport better than others or act as source specific signatures, marking the influence of an upstream source on downstream sites. Additionally, the downstream presence of genera that were absent in sampled upstream sites could indicate the loading of cyanobacteria from other, unsampled inputs between sites sampled as part of this study.

Carlson trophic level values (Carlson, 1977) were calculated for each site utilizing extracted chlorophyll-a values (Table 3). RR1 and SRO were eutrophic across all four seasons, while MR1, the most upstream site in the Stony Brook-Millstone watershed which aimed to capture the intermittent outflow of Rosedale Lake, was oligotrophic to mesotrophic. All downstream sites in the Raritan River mainstem and the Stony-Brook Millstone tributary varied between oligotrophic and mesotrophic depending upon the season. Median genera richness, cyanobacterial abundance and relative abundance of cyanobacteria all decreased downstream.

Table 3 Site cyanobacteria assemblage summary: trophic state, mean chlorophyll-a (ug/L), median & tange of cyanobacteria abundance, median & range of cyanobacteria relative abundance, percentage of samples exceeding the NJDEP Recreational Advisory threshold of 20,000 cells/mL, median genera richness, common genera (genera present in at least 40% of samples from site); and dominant genera (genera composing at least 50% of overall cell abundance in any one sample from site).

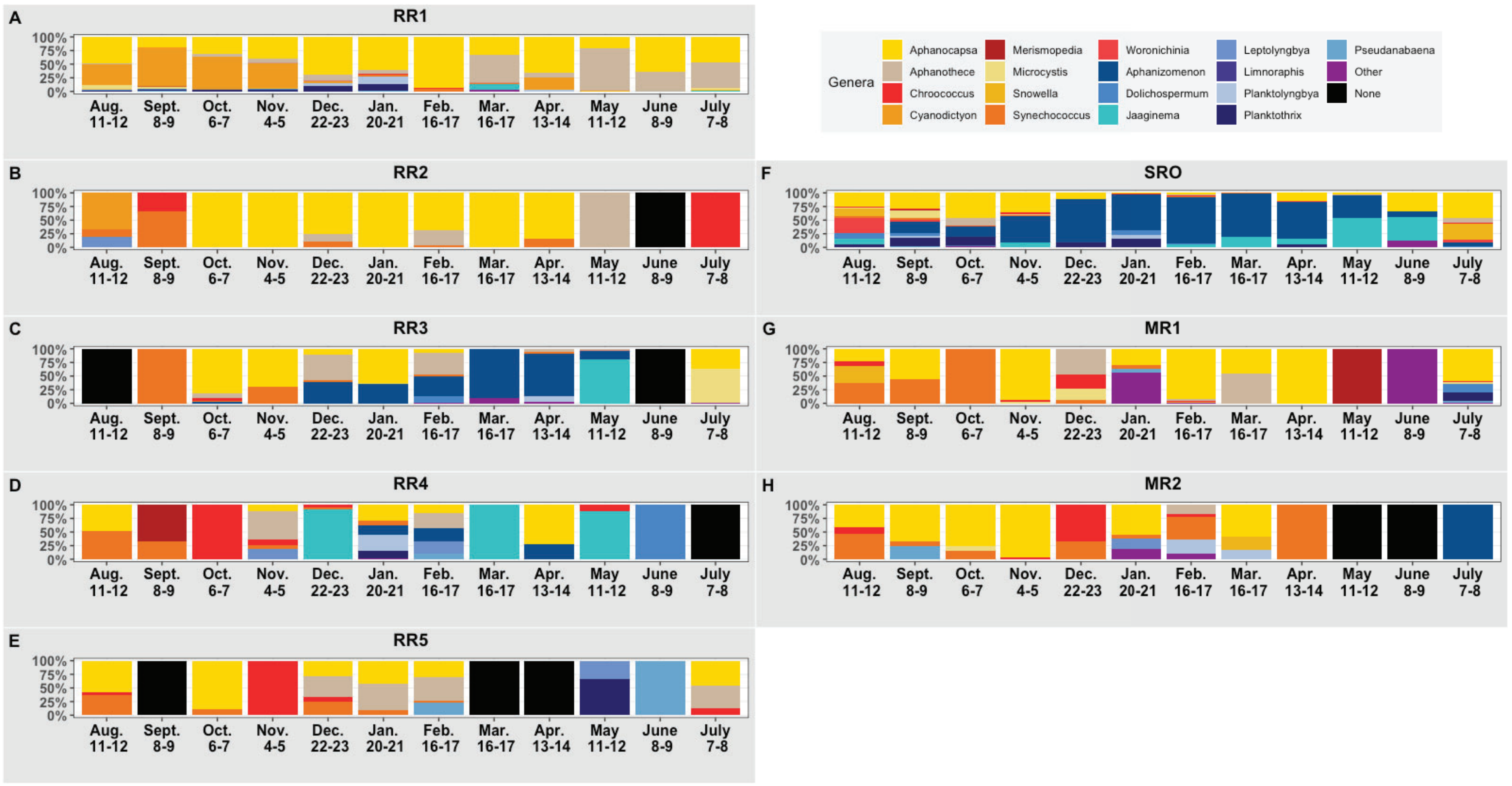
Site	Trophic Level	**Mean Chl-a (ug/L)**	Median Cyano. Abundance (cells/mL)	Range of Cyano. Abundance (cells/mL)	Percentage of Samples exceeding NJDEP Recreational Threshold	Median Cyano. Relative Ab.	Range Cyano. Relative Ab.	Median Richness	Common Genera	Dominant Genera
RR1	Eutrophic	33.40	245,235	18,123-1,074,641	95%	92%	46-99%	8	*Aphanocapsa, Aphanothece, Chroococcus, Cyanodictyon, Dolichospermum, Microcystis, Planktothrix, Pseudanabaena*	*Aphanocapsa, Aphanothece, Cyanodictyon,*
RR2	Mesotrophic	3.95	1,826	0-8,364	0%	28%	0-94%	1	*Aphanocapsa*	*Aphanocapsa*
SRO	Eutrophic	16.20	33,999	155-99,829	75%	81%	11-93%	7	*Aphanocapsa, Aphanizomenon, Aphanothece, Chroococcus, Dolichospermum, Jaaginema, Microcystis, Planktothrix, Synechococcus, Woronichinia*	*Aphanizomenon, Aphanocapsa, Cyanogranis,*
RR3	Mesotrophic-Eutrophic	5.62	6,699	0-42,673	10%	65%	0-93%	3	*Aphanocapsa, Aphanizomenon, Aphanothece, Synechoccus*	*Aphanizomenon, Aphanocapsa,*
RR4	Mesotrophic	4.29	774	0-6,893	0%	34%	0-92%	2	*Synechococcus*	*Jaaginema, Leptolyngbya*

RR5	Mesotrophic	7.41	542	0-9913	0%	28%	0-96%	1	*Aphanocapsa, Synechococcus*	*Aphanocapsa, Dolichospermum, Merismopedia*
MR1	Oligotrophic-Mesotrophic	6.75	1,665	0-42,518	5%	43%	0-89%	3	*Aphanocapsa, Chroococccus, Synechococcus*	*Aphanocapsa*
MR2	Oligotrophic-Mesotrophic	8.81	1,200	0-6,583	0%	44%	0-95%	2	*Aphanocapsa Synechoccus*	*Aphanocapsa,*

Twenty-six (26) cyanobacterial genera, including 24 known potential cyanotoxin producers (Chapman and Foss, 2019; Ibelings et al., 2021; Pearson et al., 2010) were present across the 160 total samples taken at the eight sites. Across all 160 samples, the most frequently present cyanobacteria were *Aphanocapsa* (present in 60% of samples), *Synechococcus* (41%), *Chroococcus* (39%), and *Aphanothece* (33%). These four were also the only genera continually present in assemblages at the most downstream sites (RR4 & RR5), regardless of changes in their relative abundance at the headwater sites (RR1, SRO, and MR1). These are all coccid cyanobacteria known to have mucus sheaths. *Aphanocapsa* and *Aphanothece* form spherical colonies featuring any number of cells, while *Chroococcus* and *Synechococcus* can form small colonies or exist in pseudo-filaments as conjoined cells which do not fully separate after cell division (Wehr et al., 2015). *Aphanocapsa* is also the only genera consistently linking RR1 to RR2 and RR3. Filamentous genera (shaded by blue hues in Figure 4) were often present in small relative abundances at RR1 and larger relative abundances SRO—especially *Aphanizomenon* and *Jaaginema*. These two genera showed evidence of passing to the next sites, RR3 (Figure 4, C) and RR4 (Figure 4, D), where they contributed a large proportion of the cyanobacteria community during winter and spring sampling events. Filamentous genera were rare in the Stony-Brook Millstone tributary, and generally only present at SRO, RR3 and RR4.

Many other genera were frequent at the headwater sites but rare if ever present at downstream sites. These include the coccid colonial (Wehr et al., 2015) genera *Microcystis*, *Merismopedia, Cyanodictyon, Snowella,* and *Woronichinia* and the filamentous genera *Dolichospermum, Pseudanabaena, Leptoplyngya, Planktolyngbya,* and *Planktothrix.*

Figure 4 Cyanobacteria genera relative abundance. Coccid genera are shades of yellow, red and orange while filamentous genera are shades of blue. For visual simplicity, only the first sample of each month is displayed.



Relative abundances of genera often changed between sites during each sampling event and date. This is just as true during the winter and spring events, when observed total cyanobacteria abundance nearly matched expected abundance, as it is during the fall and summer events, when measured abundances were much lower than expected. There were even events when zero cyanobacteria were present at a site, but present at adjacent sites upstream and downstream (eg., zero cyanobacteria cells measured at RR3 on Aug.11-12). It is unclear if these differences are due to sampling variability, growth of certain cyanobacteria genera between sites, uneven persistence of genera between sites, or from the contributions of cyanobacteria from unsampled areas of the river basin.

While genera richness continually decreased downstream (Table 2), nearly all cyanobacteria genera in downstream sites could be found present at upstream sites on the same sampling date: on a presence/absence basis throughout the entire study, 72% of genera present at downstream sites were present at an upstream site during the same event. It was especially rare for a cyanobacteria genus to be detected at RR5 that was not present at RR1, SRO or MR1 during the same sampling event. Most of the remaining unexplained genera were found at MR2, which often featured small abundances of cyanobacteria genera absent at MR1. These genera were often filamentous genera such as *Planktolyngbya* and *Psuedanabaena* and did not show evidence of successful travel to RR5.

3.2 Discharge Patterns

Discharge at all Raritan mainstem sites were highest during the winter and early spring sampling events (Figure 5), overlapping the period of highest cyanobacteria recovery during the winter and early spring. Discharge at SRO is manually controlled by NJWSA as part of Spruce

Run Reservoir operations and did not follow the seasonal discharge patterns observed at other Raritan River sites. MR1 and MR2, the two sites in the Stony Brook-Millstone tributary, featured more even discharge throughout the year. This also aligned with the previously reviewed rates of cyanobacteria recovery, where measured cyanobacteria abundance met or exceeded expected abundance at equal rates throughout the year in the Millstone tributary.

Pearson's correlation values were calculated to quantify the relationship between downstream cyanobacteria recovery and discharge level in both the Raritan mainstem sites and the Stony Brook-Millstone sites. Discharge level, expressed as a percentage of measured discharge at time of sample divided by the sample site's median discharge during the sampling year (8/1/20 through 8//31/21), was positively correlated with cyanobacteria recovery rate in the Raritan Mainstem ($r^2 = 0.36$, $p < 0.001$). There was no significant relationship between discharge level and cyanobacteria recovery in the Stony Brook-Millstone watershed.

Figure 5 Site Discharge during discrete sample collection at each site, A) RR1, B) RR2, C) RR3, D) RR4, E) RR5, F) SRO, G) MR1, and H) MR2. Changes in discharge at SRO are reflective of the controlled release from Spruce Run Reservoir. RR3 discharge is highly influenced by SRO, while other sites reflect more seasonal flows. Discharge at Raritan mainstem sites peaked during the winter and early spring sampling dates (December 2020 through April 2021).

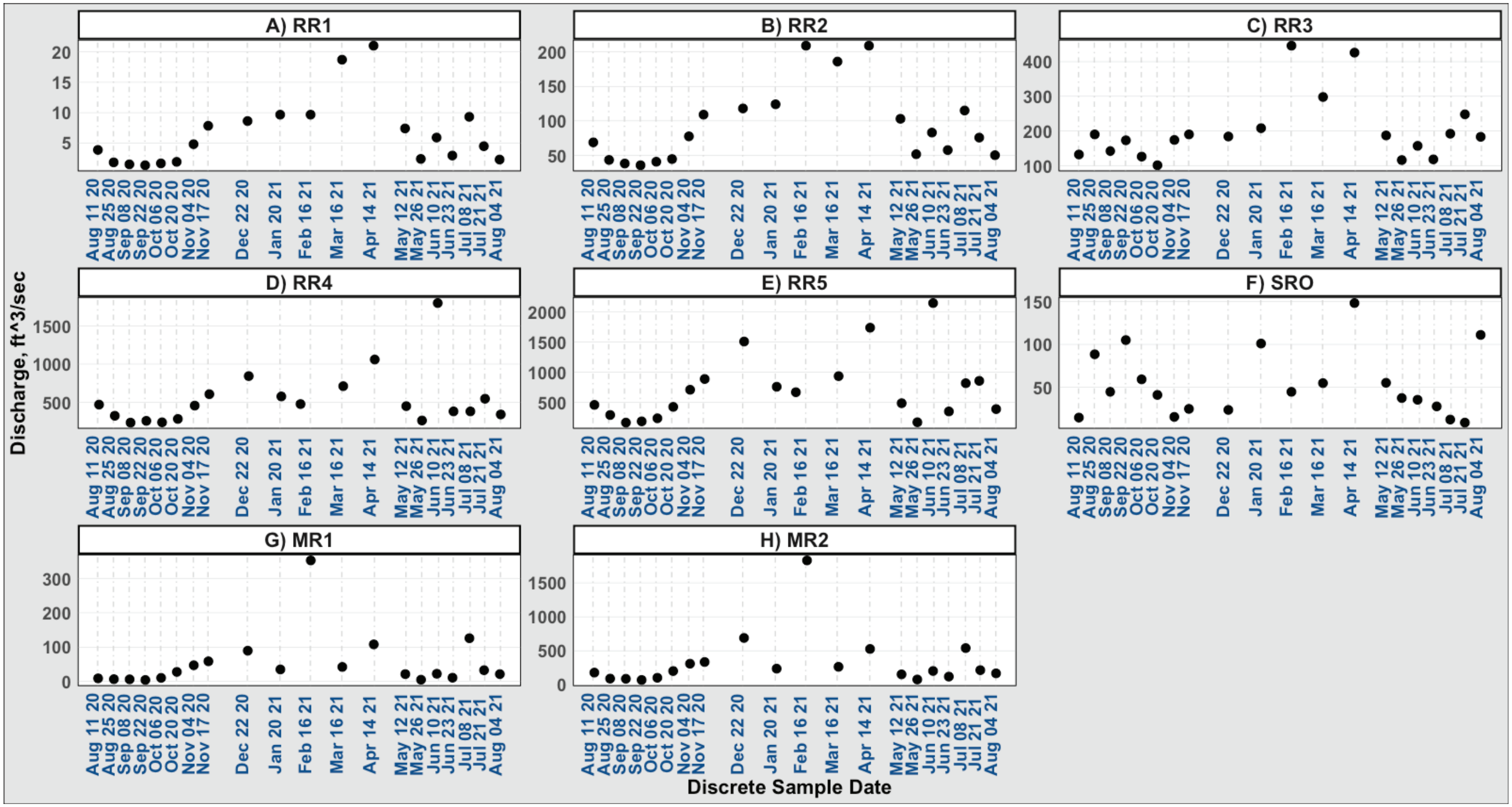
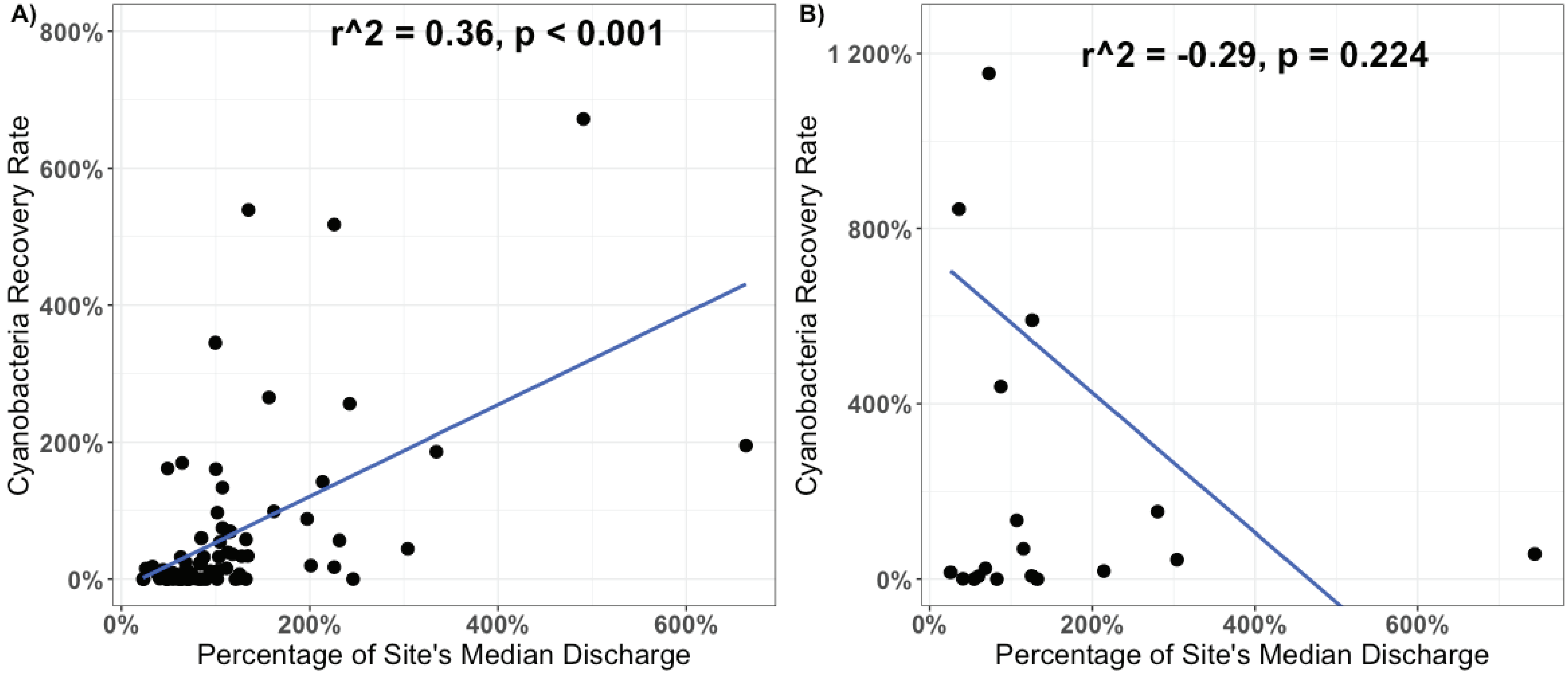


Figure 6: Downstream cyanobacteria recovery rate correlated with discharge level in the Raritan mainstem sites (left) and the Stony-Brook Millstone (right). Across the Raritan mainstem sites, cyanobacteria recovery correlated positively with discharge level (r^2 = 0.36, $p < 0.001$). There was no significant relationship observed between discharge level and cyanobacteria recovery in the Stony Brook-Millstone (r^2 = -0.29, p = 0.224).



3.3 Contribution of Headwater Sites (RR1, SRO, MR1) Discharge to Downstream Sites

Graham and others (Graham, 2012) found higher abundances of cyanobacteria at downstream sites in the Kansas River system during periods when upstream reservoirs contributed greater percentages of downstream discharge. Table 4 (below) summarizes the mean percentage and range of discharge contributed by headwater sites (RR1, SRO, and MR1) to all downstream sites during the sample period. On average, the lacustrine outlet sites RR1, SRO and MR1 constituted 0.7%, 9% and 5% of the RR5 discharge, for a combined average of 15% during sampling events. This indicates that water at RR5 included a significant amount of input from unsampled sources. This dilution likely accounts for the diminishing cyanobacteria abundance while going downstream in the Raritan mainstem.

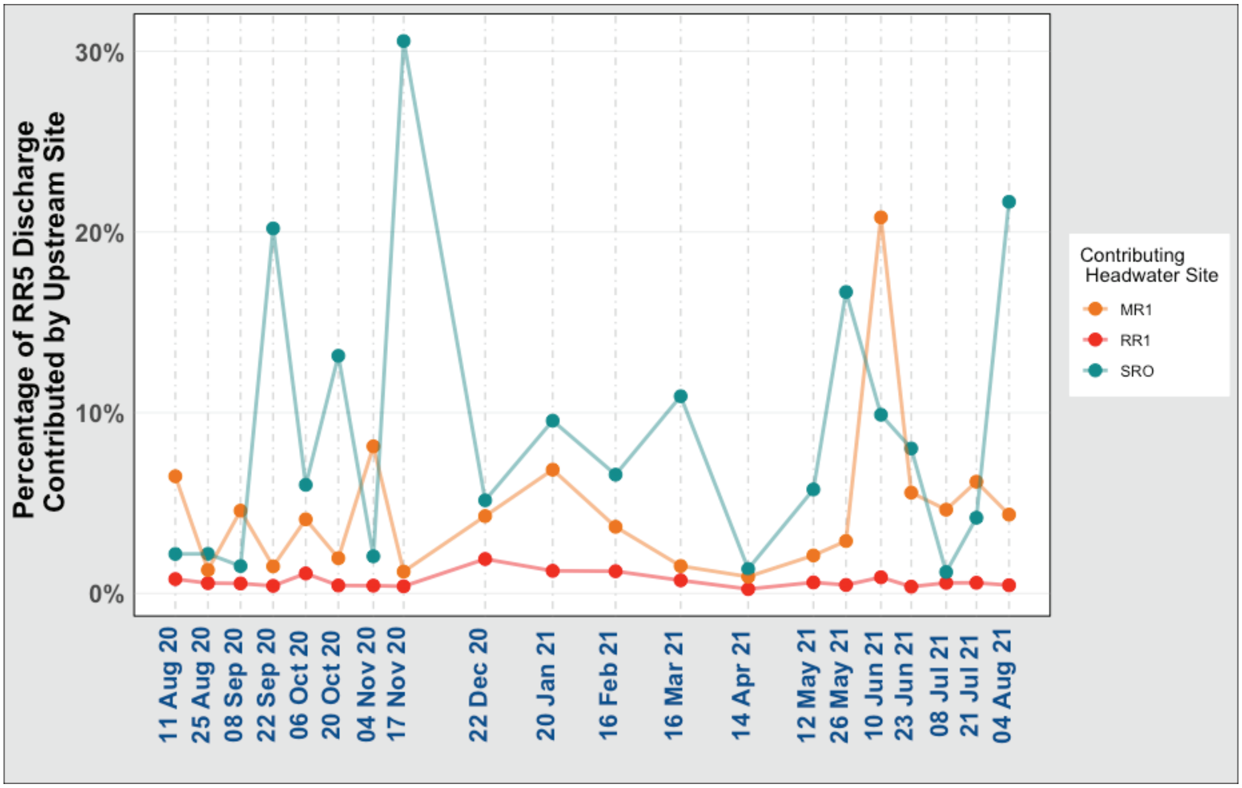
Figure 5 displays the three headwater sites' contributions to the discharge at RR5 during each sampling event. Outside of a spike in contribution from MR1 on February 16, 2021, the winter to early spring period did not feature any overwhelming trends that differentiated RR5's source water from the rest of the sample period. SRO had its lowest influence on RR5 discharge during the winter and early spring when cyanobacteria recovery was the highest; SRO was responsible for a higher percentage of RR5's discharge during the summer and fall of 2020. On average, water discharged from MR1 composed 13% of RR5's discharge and was steady throughout the study period, as was the contribution of RR1, which averaged 0.7% of RR5's discharge.

Comparing this data with the previously reviewed cyanobacteria genera relative abundance data, RR3's assemblages appear to be more influenced by RR2 than SRO during the summer and fall of 2020, even though this is the period when SRO's discharge contribution was the largest. RR3 and RR4 instead displayed SRO signatures during the winter and spring of 2020 to 2021, as evidenced by the presence of *Aphanizomenon*—this was also the period when total cyanobacteria recovery was the greatest in the Raritan mainstem. RR5 relative abundance assemblages rarely reflected RR4's assemblages regardless of the combined discharge contribution levels of SRO and RR1. RR5 assemblages showed increased resemblance to MR2 assemblages during two sampling events (Oct. 6-7, 2020 and Dec. 22-23, 2020), but these did not reflect any sudden spikes in MR1's contribution of discharge to RR5. RR2 showed its greatest reflectance of RR1 during the winter and early spring as well, when flows were the highest and when RR1's discharge contribution was the highest.

Table 4 Mean and range percentage of discharge at each downstream site contributed by upstream headwater sites (*indicates upstream, not applicable to proportion calculation)

Site	Mean RR1	Range RR1	Mean SRO	Range SRO	Mean MR1	Range MR1
RR2	6%	4-10%	*	*	*	*
RR3	3%	1-6%	30%	7-61%	*	*
RR4	1%	0.3-3%	14%	1-38%	*	*
RR5	0.7%	0.2-2%	9%	1-31%	5%	0.9-21%
MR2	*	*	*	*	13%	6-35%

Figure 7 Percentage of discharge contributed by upstream sites (RR1, SRO, MR1) to the most downstream site, RR5, across all sampling dates. SRO was the largest contributor on 70% of sampled dates, while MR1 was the highest contributor on all other dates. RR1 discharge is an estimation based upon RR2's discharge, as described in the Methods section.



3.3 Water Quality Parameters

During discrete sampling across all sites, seasonal water temperature averaged 14.4ºC in the fall, 2.79ºC in the winter, 12.9ºC in the Spring and 24.4ºC in the Summer. Water temperatures were within the 18-25ºC range that is optimal for phytoplankton growth (Fernández-González and Marañón, 2021) from August-September 2020 as well as mid-May through August 2021. It is notable that water temperatures were lowest, and below the normal temperature to support cyanobacterial growth, during the period of highest downstream cyanobacteria recovery observed in the winter and early spring. During any individual sampling event, water temperatures only differed by 1-2ºC between sites, except during summer and fall events when RR2 could register 4-7ºC colder than other sites. RR2's lower temperature could be due to its location with heavy tree cover located just downstream of a gorge.

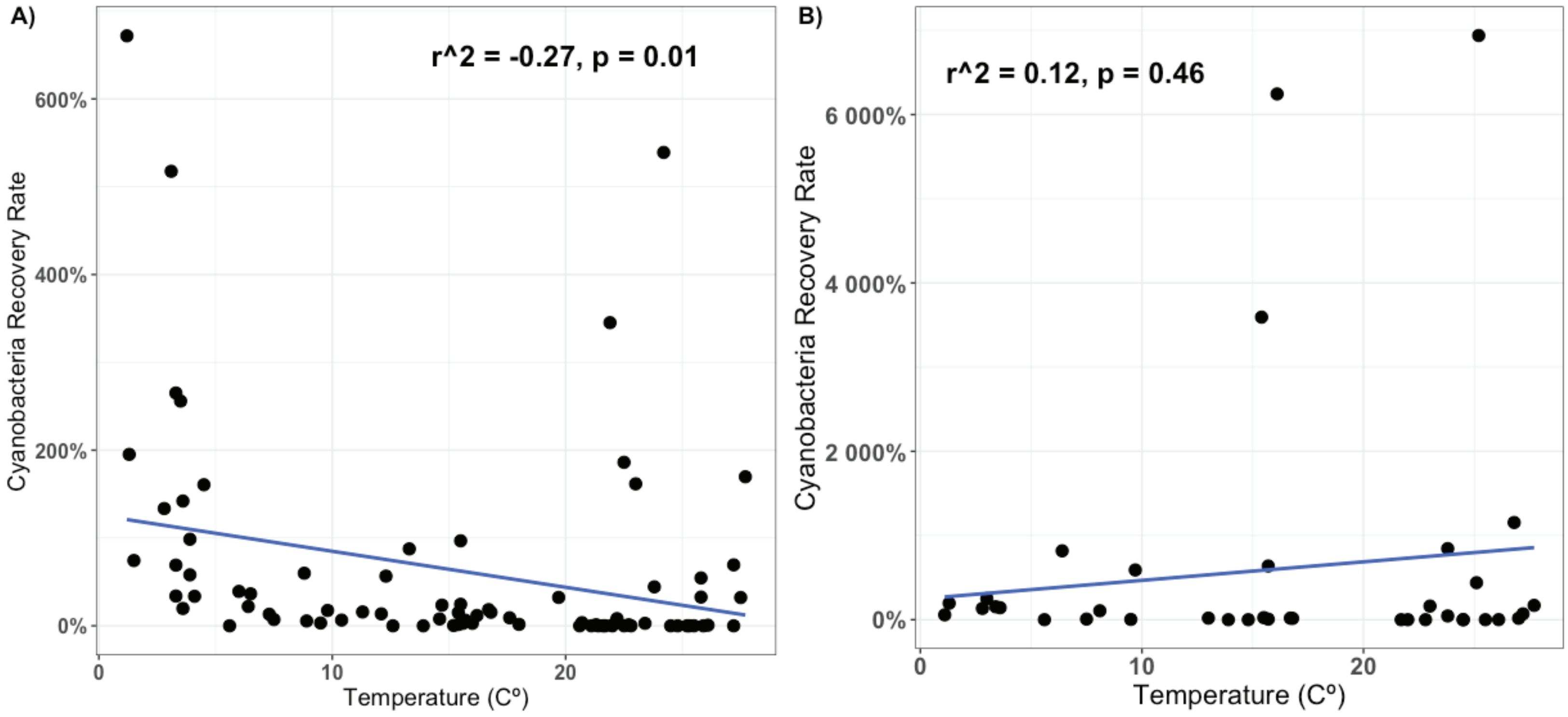
Table 5 displays site-specific averages and ranges of several water quality parameters tied to phytoplankton production. Mean site pH ranged between 7.12-8.3 while mean dissolved oxygen ranged 7.42-11.6 mg/L, all within levels to support phytoplankton production. MR2 featured both the lowest average pH (7.12) and lowest DO (7.42 mg/L). Mean turbidity ranged from a low of 2.45 NTU at RR2 to a high of 12.5 NTU at RR1, while the sites with highest discharge-RR4 and RR5-averaged 11.0 and 10.7 NTU, respectively. The high turbidity at RR1, where discharge is nearly stagnant, is likely reflective of the site's higher chlorophyll-a pigmentation levels from its high average cyanobacteria abundance. Turbidity at RR4 and RR5 are more likely influenced by suspended sediment. Neither pH, DO, nor Turbidity displayed seasonal fluctuations coinciding with the increased winter to spring downstream cyanobacteria recovery previously observed. However, correlation tests between temperature and cyanobacteria recovery rate (Figure 8) resulted in a significant ($p = 0.01$) negative correlation ($r^2 = 0.27$)

between water temperature and cyanobacteria recovery rate among Raritan mainstem sites. This coincides with the previously observed results, where cyanobacteria recovery was highest during the winter and early spring months, when discharge was highest and water temperatures were coldest. No significant correlation was observed between water temperature and downstream cyanobacteria recovery in the Stony-Brook Millstone.

Table 5 Mean and range of discharge, temperature, pH, dissolved oxygen (DO mg/L) and Turbidity (NTU) at each site during sample collection. * RR1's mean discharge is a calculated approximation, whereas all other discharge data is from continuous in-situ gages.

Site	Mean Water Temp. (C^{o})	Water Temp. Range (C^{o})	Mean pH	pH range	Mean DO (mg/L)	DO Range (mg/L)	Mean Turbidity (NTU)	Turbidity Range (NTU)
RR1	15.7	0.6-26.7	7.66	6.98-8.91	8.52	4.49-15.1	12.5	3.19-53.7
RR2	13.1	3.1-22.2	8.19	7.86-8.69	11.1	8.05-15.3	2.45	0.78-6.58
SRO	16.8	2.4-27.3	8.11	7.53-9.03	10.1	7.36-15.2	6.05	1.17-13.0
RR3	16.0	3.3-25.9	8.3	7.76-8.87	11.6	7.88-15.8	4.84	1.55-19.0
RR4	15.7	1.2-27.5	7.71	7.15-8.26	10.0	6.16-14.9	11.0	2.52-100
RR5	16.6	1.3-27.7	7.35	6.94-7.74	9.89	6.59-14.9	10.7	3.22-54.9
MR1	16.7	0.6-28.7	7.98	7.32-9.39	11.4	7.21-18.3	7.95	1.94-45.3
MR2	16.2	1.1-27.0	7.12	6.78-7.57	7.42	3.38-14.6	8.84	4.03-19.2

Figure 8 Correlations between cyanobacteria recovery rate and water temperature among A) the Raritan River sites and B) the Stony Brook-Millstone Sites.



3.4 Water Nutrient Concentrations

Figure 9 illustrates concentrations of the water nutrients Ammonia (A), Nitrate plus Nitrite (B), Orthophosphate (C), Total Nitrogen (D) and Total Phosphorous (E) during the period of study. Ammonia concentrations (A) saw spikes at SRO, RR1and MR2 during the winter but were otherwise highest at each site during the Summer and Fall sampling events. Seasonal fluctuations for nitrate plus nitrite, orthophosphate, total nitrogen and total phosphorous were most evident in the downstream sites RR5 and MR2 which each featured their highest levels of nitrate plus nitrite, orthophosphate, total nitrogen and total phosphorous during the Summer and Fall of 2020 and the late Spring and Summer of 2021. MR1, MR2, RR4 and RR5 often exceeded the NJDEP total phosphorous surface water standard of 0.10 mg/L (NJDEP, 2016). Outside of the mentioned winter spikes in ammonia, most sites either kept a relatively stable concentration of nutrients throughout the study or featured their highest available nutrient concentrations during the summer and fall seasons.

At all sites, total nitrogen was primarily composed of nitrate + nitrite, while total phosphorous was primarily composed of orthophosphate. This indicates that most nitrogen and phosphorous where in forms available for cyanobacteria uptake and supporting phytoplankton production (Carlson and Simpson, 1996; Kudela, 2011). Concentrations of both phosphorous and nitrogen were higher in the river sites (MR1, MR2, RR2, RR3, RR4, RR5) than in the direct lacustrine outlet sites (SRO, RR1), yet cyanobacteria abundance was much higher in the outlet sites compared to the river sites (Figure 10).

Cyanobacteria recovery rate did not significantly correlate with total phosphorous or total nitrogen concentrations in the Raritan mainstem (Figure 11 A, C). However, in the Stony Brook-Millstone, total nitrogen was positively correlated with cyanobacteria recovery (Figure 11B).

Total Phosphorous was also positively correlated with cyanobacteria recovery in the Stony Brook-Millstone (Figure 11D) but this was not a significant result ($p = 0.07$).

Figure 9 Concentrations of A) ammonia, B), nitrate + nitrate, C) orthophosphate, D) total nitrogen and E) total phosphorous by site throughout the study period. Mainstem Raritan sites are different shades of blue, Stony Brook-Millstone tributary sites are shades of yellow, while Spruce Run Outlet is red.

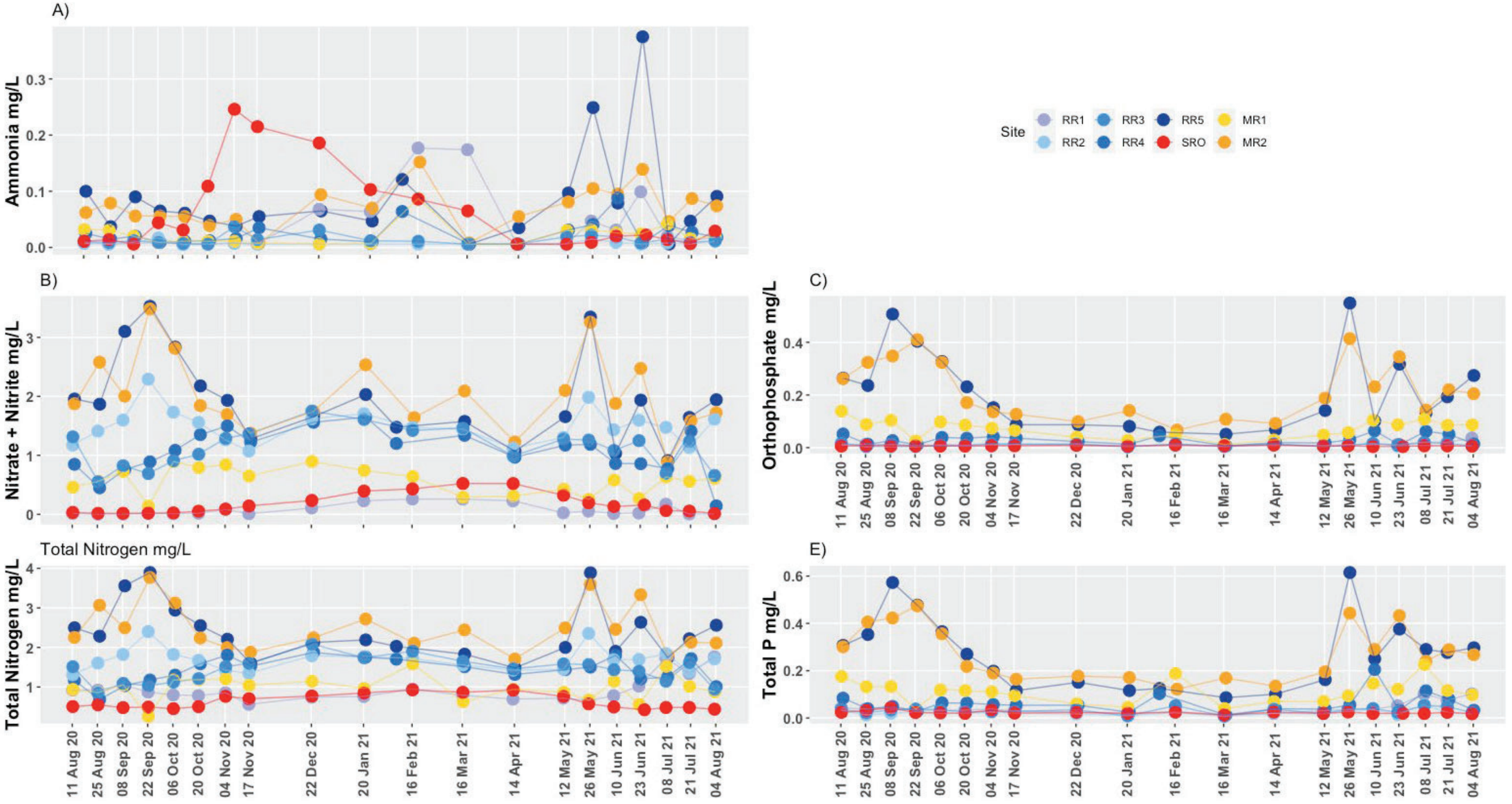


Figure 10 Plots of A) Total cyanobacteria and total phosphorous and B) total cyanobacteria and total nitrogen. As circled, two groups emerge in each plot: The direct lake outlet sites (RR1 and SRO) each regularly had the lowest phosphorous and nitrogen concentrations but the highest total cyanobacteria abundances, while the remaining riverine sites regularly had higher nutrient concentrations but lower overall cyanobacteria abundances.

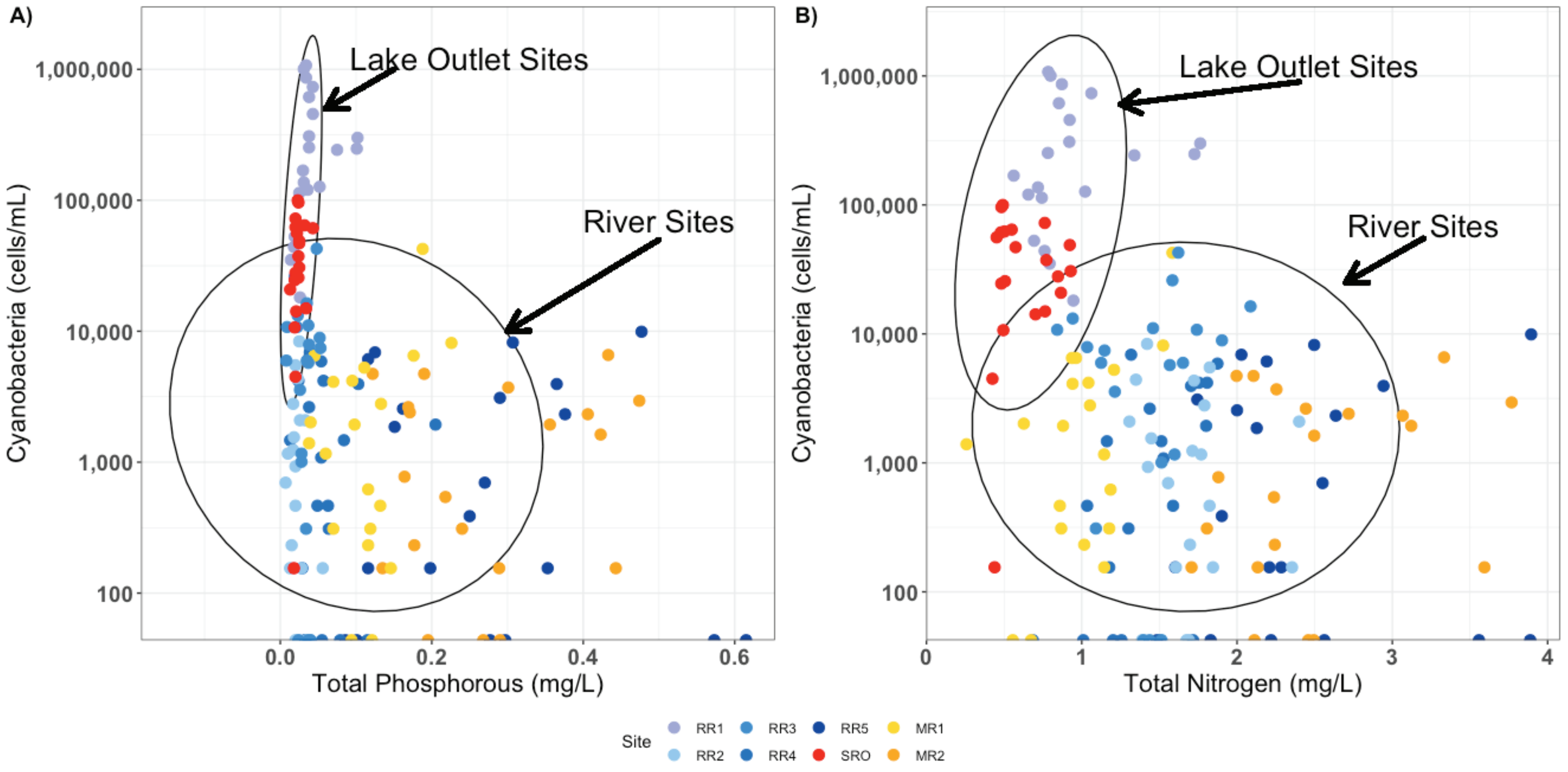
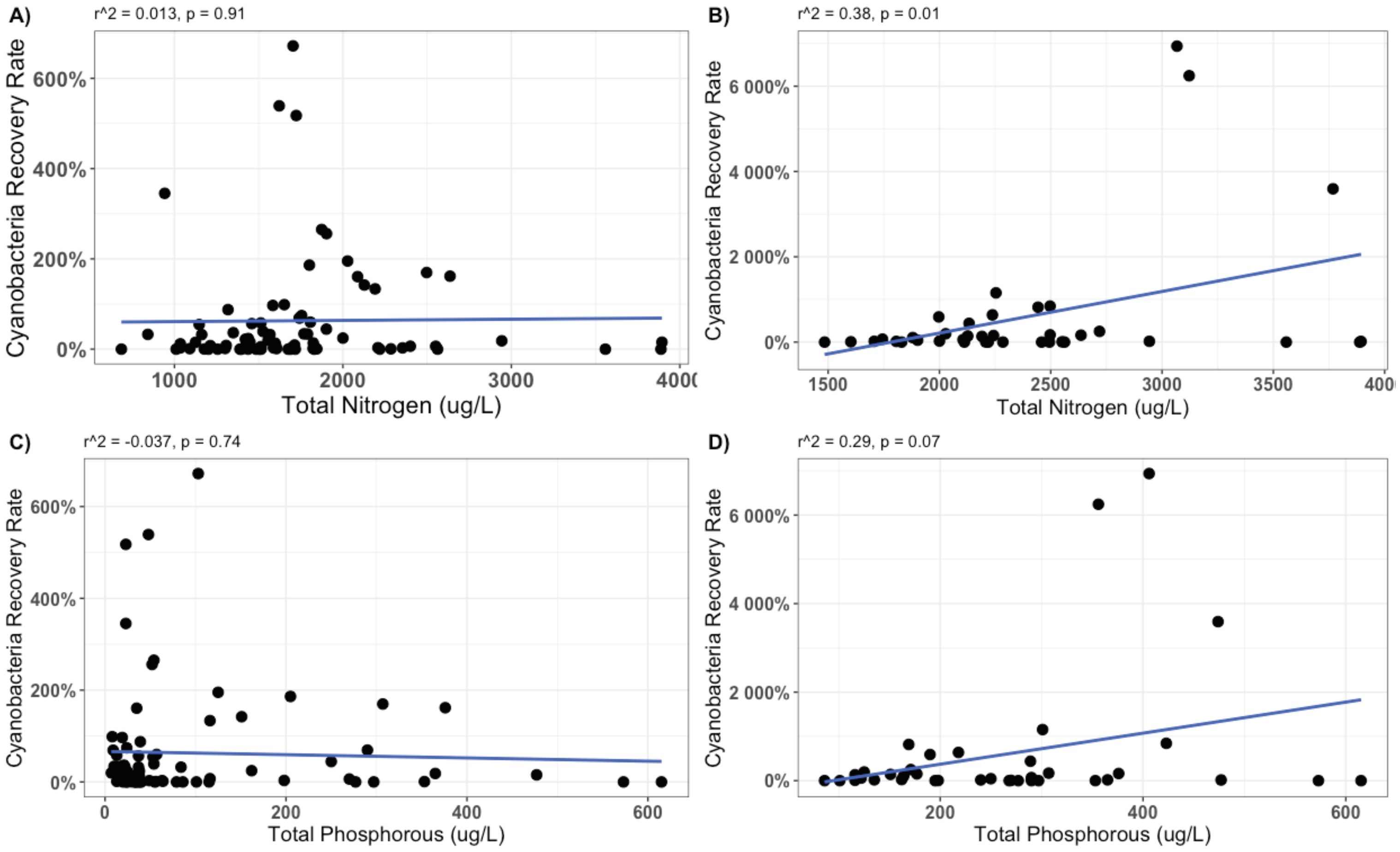


Figure 11 Correlation graphs depicting the relationships between nutrient levels and cyanobacteria recovery rates in among mainstem Raritan River sites and Stony Brook-Millstone sites. In the Raritan mainstem, were no significant relationships between cyanobacteria recovery rate and A) total nitrogen or C) total phosphorous concentrations. The Stony Brook-Millstone sites did display a significant, positive correlation between cyanobacteria recovery rate B) total nitrogen and a weak relationship with D) total phosphorous.



4. Discussion

While tracking the fluvial transport of cyanobacteria from HAB sources to downstream drinking water intakes in the RBWSC, different trends were evident between the Raritan River mainstem and the Stony Brook-Millstone tributary. In the Raritan mainstem, cyanobacteria persistence downstream was more likely during periods of increased discharge. The Stony Brook-Millstone showed evidence of both cyanobacteria loading from an unsampled source, as well as cyanobacteria growth in between MR1 and MR2. Cyanobacteria genera did not persist at the same rates between sites in either watershed.

The published literature consistently cites discharge as the primary factor governing lotic phytoplankton production, composition, and abundance (Baker et al., 2000; Brookes, 2002; Cha et al., 2017; Conroy et al., 2017; Eddy, 1931; Graco-Roza et al., 2020; Moss and Balls, 1989; Reif, 1939; Wehr et al., 2015). While pooling and decreased discharge are generally found to favor lotic phytoplankton production (Al-Tebrineh et al., 2012; Baker et al., 2000; Cha et al., 2017; Wehr et al., 2015), increases in stream velocity and discharge have been found to shorten travel time and increase the downstream transport of phytoplankton (Reif, 1939; Somma et al., 2022). Increased discharge may also allow for cyanobacteria communities originating upstream to overcome trapping while travelling downstream, which otherwise could lead to cell degradation (Eddy, 1931; Reif, 1939; Reinhard, 1931; Wehr et al., 2015). Meanwhile, other factors often associated with lentic phytoplankton production, including seasonality, precipitation, turbidity, water temperature, nitrogen and phosphorous concentrations have shown varied evidence of influence on lotic cyanobacteria abundance, community composition, and downstream transport (Baker et al., 2000; Brookes, 2002; Casamatta and Hasler, 2016; Cha et al., 2017; Conroy et al., 2017; Eddy, 1931; Graham, 2012; Graham et al., 2020; Moss and Balls, 1989; Reif, 1939; Reinhard, 1931; Reinl et al., 2020; Taylor et al., 2020; Wehr et al., 2015; Wiebe, 1928).

Previously published literature indicated most lotic phytoplankton may originate from upstream lentic sources (Cha et al., 2017; Eddy, 1931; Moss and Balls, 1989; Reif, 1939; Reinhard, 1931; Somma et al., 2022; Wehr et al., 2015). Several studies also note a lack of evidence for lotic phytoplankton production outside of slow-moving river stretches where pooling waters feature warmer temperatures, possible stratification, decreased turbidity and increased light penetration, providing an inoculum for cyanobacteria production (Al-Tebrineh et al., 2012; Baker et al., 2000; Cha et al., 2017; Wehr et al., 2015). While there are multiple low-flow dams throughout the RBWSC, there was little evidence of cyanobacteria production between sites in this study, as it was rare for measured cyanobacteria abundance to exceed expected cyanobacteria abundance.

In this study, sites along the Raritan River mainstem displayed increased cyanobacteria downstream persistence during sampling events featuring increased discharge levels and colder temperatures. It is unlikely that colder temperatures favor downstream cyanobacteria persistence, rather this relationship is more likely due to the fact that discharge levels and cyanobacteria persistence were highest during the winter, when water was coldest. Cyanobacteria recovery in the Raritan mainstem rarely exceeded 100%, indicating most cyanobacteria was sourced by the sampled upstream sources (Spruce Run Reservoir and Budd Lake) and that there was no substantial cyanobacteria production during lotic travel. Outside of periods of increased flow, cyanobacteria at RR2 was low, and then would increase in the sites RR3, RR4 and RR5. For instance, measured cyanobacteria abundance at RR2 averaged 71% of expected abundance during the winter to early spring dates, compared to averaging just 4% during summer and fall dates. This indicates that outside of periods of high discharge, most cyanobacteria would perish between RR1 and RR2, and that most cyanobacteria downstream in the Raritan was sourced by Spruce Run Reservoir. In the Raritan mainstem, parameters normally attributed with phytoplankton production—such as nutrient levels and turbidity—were not significantly correlated with downstream cyanobacteria persistence.

Previous literature reported that during high discharge, headwater streams and lacustrine outlets see increased stream velocity; in turn this increased stream velocity shortens travel time, allowing phytoplankton to travel further distances than during slower, low discharge periods (Reif, 1939; Somma et al., 2022). The results of this study observed similar trends, finding periods of higher discharge overlapped with periods of greater cyanobacteria recovery downstream. This indicates that the likelihood of cyanobacteria from Budd Lake (RR1) persisting downstream was greatest during the seasons featuring higher baseflow.

After leaving a lentic setting, it is well documented that increases in discharge continually select for small algal species with rapid growth rates and competitive advantages surviving flowing conditions (Baker et al., 2000; Casamatta and Hasler, 2016; Reif, 1939; Wehr et al., 2015; Wiebe, 1928). Reif (1939) and Wiebe (1928) suggest these competitive advantages favor strongly structured and passive phytoplankton, such as coccid, spherical, mucus-sheath lined cyanobacteria. This could indicate that *Aphanocapsa, Aphanothece, Synechoccocus*, and *Chroocococcus*, the four most common genera throughout the study sites, are better suited to travel downstream in the Raritan Basin compared to filamentous genera, which did not show evidence of travelling as far. Al-Tebrineh et al. (2012) found that algal assemblage composition could be grouped by river section defined by proximity to river impoundments—various site-specific characteristics of each separate dam or weir selected for different cyanobacteria species.

As these assemblages then travelled downstream, the next impoundment would select for a different cyanobacteria species, accounting for different relative abundances between sites (Al-Tebrineh et al., 2012). While there was no consistent trend of specific genera dominating a downstream site during multiple events, there are numerous low-flow dams in the South Branch of the Raritan River, the Stony-Brook Millstone, and the Raritan River. This phenomenon could partly explain the consistent persistence of *Aphanocapsa, Aphanothece, Synechoccocus*, and *Chroocococcus* downstream over other genera which started with greater population densities upstream.

While the genera *Aphanocapsa, Aphanothece, Synechoccocus*, and *Chroocococcus* were the most common genera throughout the study sites, it is difficult to ascertain if these genera are truly more persistent than other genera during downstream transport from that data gathered in this study. This is because their densities, diluted population sizes and relative abundances were inconsistent between sites and sampling events. While it is possible these four genera have adaptations that help them survive lotic pressures better than other genera during downstream transport, it is also possible that they are simply the most common genera across the RBWSC and their downstream abundances were inflated due to their addition from unsampled inputs.

It was especially rare for a cyanobacteria genus to be detected at RR5 that was not present at RR1, SRO or MR1 during the same sampling event. This shows that even though most cyanobacteria genera seemed to perish before reaching RR5, most cyanobacteria present at RR5 can be attributed to the outlets of the upstream perennial HAB sources targeted as part of this study. It also indicates that cyanobacteria loading to the Raritan mainstem from unsampled inputs are not substantial.

Conversely, in the Stony Brook-Millstone watershed, MR2 frequently featured genera not present upstream at MR1; and MR2's measured total cyanobacteria abundance was often above its expected abundance. There is evidence that this both due to loading into the Stony Brook-Millstone River from an unsampled source, and due to lotic cyanobacteria production in between MR1 and MR2. Cyanobacteria recovery rates at MR2 averaged 1,200% during the entirety of the study and were positively correlated with higher concentrations of total nitrogen. One possible source of unsampled cyanobacteria between MR1 and MR2 is the Delaware & Raritan Canal which runs parallel to the Millstone between MR1 and MR2 and continually outlets into the Millstone River at spillways and controlled outlets. There are also various impoundments along the Millstone River between MR1 and MR2 which could allow for pooling and the growth of cyanobacteria, including Carnegie Lake, a 262-acre run-of-the-river reservoir formed on the Millstone River by two low flow dams.

Drinking water managers of the RBWSC should be advised that increased levels of cyanobacteria might be seen from the upstream HAB sources during period of increased discharge in the Raritan River. The maximum cyanobacteria abundance at RR5 near the first RBWSC drinking water intake was approximately 9,913 cells/mL, still well below the NJDEP freshwater health advisory levels. For drinking water management purposes, it is convenient that these periods of increased discharge and increased cyanobacteria travel potential will likely dilute the cyanobacteria, and the seasonal periods of high discharge in the winter and early spring are often the periods of lowest cyanobacteria abundance in the upstream lakes and reservoirs.

Future studies in the RBWSC should give a greater focus to the Stony Brook-Millstone subwatershed. These results indicated that there is likely an additional source of cyanobacteria loading into the Stony Brook-Millstone in between MR1 and MR2, and that cyanobacteria may be growing between sites. This potential growth may be supported by the high nitrogen and phosphorous concentrations found at MR1 and MR2. Additionally, sites should be added around Carnegie Lake, the Delaware and Raritan Canal's discharges to the Millstone River, the Millstone River above Carnegie Lake, and the Stony Brook Wastewater Treatment Authority's discharge.

The North Branch of the Raritan River is the third major subwatershed of the RBWSC and joins the South Branch between RR3 and RR4 to compose the Raritan River. While the North Branch was not directly sampled as part of this study, the lack of unexplained genera or growth in cyanobacteria abundance between RR3 and RR4 indicates the North Branch of the Raritan River as a significant loader cyanobacteria. However, a more complete study would at least sample at the confluence of the South and North Branches to better account for any loadings from the North Branch watershed.

There were several other limitations of this study which may limit the correct interpretation of the data, or the applicability of the study's findings. During periods of high discharge, RR4 and RR5 were occasionally sampled only from the bank instead of a full composite to include the center of flow. Graham and others (2012) found phytoplankton assemblages differed along river transects, indicating a bank sample may not be indicative of the rest of the water column. However, dates of bank sampling did not seem to affect the recovery of cyanobacteria or any other results based upon observed trends. Since the discharge at Budd Lake (RR1) was only an approximation based upon previously observed relationships between RR1 and RR2, it may not be truly indicative of current discharge rates and could have affected the calculated expected cyanobacteria abundances at RR2, and the observed recovery rates and trends. Inputs and losses of discharge due to groundwater exchange were outside the scope of this study but certainly affect accuracy of dilution rates used to calculate the expected cyanobacteria abundances downstream.

5. Conclusion

Cyanobacteria presence in raw source water are an increasing concern for drinking water resource managers. Existing literature on HABs has primarily focused on lentic cyanobacteria production, HAB prevention, and the monitoring and management of intense, acute lacustrine bloom events. Understanding the persistence of lacustrine-sourced cyanobacteria travelling through fluvial systems used for potable water supply holds ramifications for downstream potable water intakes.

This study found cyanobacteria persistence downstream was influenced by discharge, with periods of higher discharge resulting in greater persistence of cyanobacteria travelling downstream in the Raritan Basin Water Supply Complex (RBWSC). Factors frequently associated with lentic cyanobacteria growth—such as higher concentrations of nitrogen and phosphorous, warm water temperatures, and lower turbidity—did not show evidence of aiding lotic cyanobacteria travel. Richness of cyanobacteria assemblages continually decreased downstream, and the relative abundances of surviving genera often changed between sites during all sampling events. It is possible certain genera are selected for while travelling downstream in the RBWSC, due to site and date specific pressures including multiple low-flow dams located along the Raritan River and its tributaries.

The results of this study suggest that the Spruce Run Reservoir, Budd Lake and the Millstone River are responsible for most of the cyanobacteria travelling to the drinking water intakes on the Raritan River. This is for two reasons: 1) after accounting for dilution from these sources, downstream cyanobacteria abundances were rarely larger than expected abundances;

and 2) it was rare for cyanobacteria genera to be present downstream that were not present at these headwater sites during the same sampling event.

The potential of lentic HAB cyanobacteria travelling to downstream drinking water intakes should be investigated on a site-specific basis, as transport mechanisms, impoundments, trappings, and phytoplankton composition are specific to each water basin. Future studies are needed to ascertain why certain cyanobacteria genera display greater persistence to lotic pressures during fluvial transport. Drinking water managers in the RBWSC should know that most cyanobacteria reaching their intakes originate from the Spruce Run Reservoir, Budd Lake and the Millstone River, and that an increased, but diluted, amount of cyanobacteria may reach their intakes during periods of increased discharge.

6. References

Al-Tebrineh, J., Merrick, C., Ryan, D., Humpage, A., Bowling, L., Neilan, B.A., 2012. Community composition, toxigenicity, and environmental conditions during a cyanobacterial bloom occurring along 1,100 kilometers of the Murray River. Applied and Environmental Microbiology 78(1), 263-272.

Baker, P., Brookes, J., Burch, M., Maier, H., Ganf, G., 2000. Advection, growth and nutrient status of phytoplankton populations in the lower River Murray, South Australia. Regulated Rivers-research & Management - REGUL RIVER 16, 327-344.

Brookes, J.B., P.; Burch, M. , 2002. Ecology and management of cyanobacteria in rivers and reservoirs, Blue-green algae: their significance and management within water systems. Cooperative Research Center for Water Quality and Treatment, pp. 33-42.

Carlson, R., 1977. A Trophic State Index for Lakes. Limnology and Oceanography - LIMNOL OCEANOGR 22, 361-369.

Carlson, R.E., Simpson, J., 1996. A Coordinator's Guide to volunteer lake Monitoring methods. North American Lake Management Society, p. 96.

Casamatta, D., Hasler, P., 2016. Blue-Green Algae (Cyanobacteria) in Rivers, pp. 5-34.

Cha, Y., Cho, K., Lee, H., Kang, T., Kim, J.H., 2017. The relative importance of water temperature and residence time in predicting cyanobacteria abundance in regulated rivers. Water Research 124.

Cirés, S., Casero, M.C., Quesada, A., 2017. Toxicity at the edge of life: A review on cyanobacterial toxins from extreme environments. Mar Drugs 15(7), 233.

Conroy, J.D., Kane, D.D., Quinlan, E.L., Edwards, W.J., Culver, D.A., 2017. Abiotic and biotic controls of phytoplankton biomass dynamics in a freshwater tributary, estuary, and large lake ecosystem: Sandusky Bay (Lake Erie) chemostat. Inland Waters 7(4), 473-492.

Copetti, D.M., R.; Bresciani, M.; Guzzella, L., 2021. Integration of in situe and remote sensing measurements for the mangement of harmful cyanobacteria blooms. A lesson from a strategic multiple-uses reservoir (Locke Occhito, South Italy). Water 13.

De Ciccio, L.A., Lorenz, D., Hirsch, R.M., Watkins, W., Johnson, M., 2022. dataRetrieval: R packages for discovering and retrieving water data available from Federal hydrologic web services, 2.7.11 ed.

Deng, Y., Wu, M., Zhang, H., Zheng, L., Acosta, Y., Hsu, T.-T.D., 2017. Addressing harmful algal blooms (HABs) impacts with ferrate(VI): Simultaneous removal of algal cells and toxins for drinking water treatment. Chemosphere 186, 757-761.

Dunlap, C.R., Sklenar, K.S., Blake, L.J., 2015. A costly endeavor: addressing algae problems in a water supply. Journal: American Water Works Association 107(5), E255-E262.

Eddy, S., 1931. The plankton of the Sangamon River in the summer of 1929. Illinois Natural History Survey Bulletin 19(1-6), 469-486.

Fernández-González, C., Marañón, E., 2021. Effect of temperature on the unimodal size scaling of phytoplankton growth. Scientific Reports 11(1), 953.

Gibble, C.M., Kudela, R.M., 2014. Detection of persistent microcystin toxins at the land–sea interface in Monterey Bay, California. Harmful Algae 39, 146-153.

Graco-Roza, C., Santos, J.B.O., Huszar, V.L.M., Domingos, P., Soininen, J., Marinho, M.M., 2020. Downstream transport processes modulate the effects of environmental heterogeneity on riverine phytoplankton. Science of The Total Environment 703, 135519.

Graham, J.L., Dubrovsky, N.M., Foster, G.M., King, L.R., Loftin, K.A., Rosen, B.H., Stelzer, E.A., 2020. Cyanotoxin occurrence in large rivers of the United States. Inland Waters 10(1), 109-117.

Graham, J.L., Foster, G.M., Williams, T.J., Mahoney, M.D., May, M.R., Loftin, K.A., 2018. Water-quality conditions with an emphasis on cyanobacteria and associated toxins and taste-and-odor compounds in the Kansas River, Kansas, July 2012 through September 2016, Scientific Investigations Report, Reston, VA, p. 66.

Graham, J.L., Loftin, K.A., Ziegler, A.C., Meyer, M.T., 2008. Chapter A7. Section 7.5. Cyanobacteria in lakes and reservoirs: Toxin And taste-and-odor sampling guidelines, Techniques of Water-Resources Investigations, Reston, VA, pp. 1-65.

Graham, J.L., Ziegler, Andrew C., Loving, Brian L., Loftin, Keith A., 2012. Fate and transport of cyanobacteria and associated toxins and taste-and-odor compounds from upstream reservoir releases in the Kansas River, Kansas, September and October 2011: U.S. Geological Survey Scientific Investigations Report 2012, In: Interior, D.o.t. (Ed.), p. 65.

Ho, J.C., Michalak, A.M., Pahlevan, N., 2019. Widespread global increase in intense lake phytoplankton blooms since the 1980s. Nature 574(7780), 667-670.

Ibelings, B., Kurmayer, R., Azevedo, S., Wood, S., Chorus, I., Welker, M., 2021. Understanding the occurrence of cyanobacteria and cyanotoxins, pp. 213-294.

Iva, S., Pavel, B., Ondřej, A., Alla, A., Volodymyr, T., Brad Luther, U., Luděk, B., 2017. Chlorination and ozonation differentially reduced the microcystin content and tumour promoting activity of a complex cyanobacterial extract. Advances in Oceanography and Limnology(1).

Kudela, R.M., 2011. Characterization and deployment of solid phase adsorption toxin tracking (SPATT) resin for monitoring of microcystins in fresh and saltwater. Harmful Algae 11, 117-125.

Lawton, L.A., Robertson, P.K.J., 1999. Physico-chemical treatment methods for the removal of microcystins (cyanobacterial hepatotoxins) from potable waters. Chemical Society Reviews 28(4), 217-224.

Moreira, C., Vasconcelos, V., Antunes, A., 2022. Cyanobacterial Blooms: Current knowledge and new perspectives. Earth 3(1), 127-135.

Moss, B., Balls, H., 1989. Phytoplankton distribution in a floodplain lake and river system. II Seasonal changes in the phytoplankton communities and their control by hydrology and nutrient availability. Journal of Plankton Research 11(4), 839-867.

NJDEP, 2016. N.J.A.C. 7:9B Surface Water Quality Standards. New Jersey Department of Environmental Protection, New Jersey, pp. 27-28. Retrieved April 10, 2022, from https://www.nj.gov/dep/standards/njac7_9b.pdf

NJDEP, 2022a. NJDEP Harmful Algal Bloom (HAB) Data Retrieval. New Jersey Department of Environmental Protection, New Jersey. Retrieved March 22, 2022 from https://njdep.maps.arcgis.com/apps/dashboards/49190166531d4e5a811c9a91e4a41677

NJDEP, 2022b. NJDEP Open Data. New Jersey Department of Environmental Protection, New Jersey. Retrieved July 14, 2022 from https://gisdata-njdep.opendata.arcgis.com

Paerl, H.W., Otten, T.G., 2013. Harmful Cyanobacterial Blooms: Causes, consequences, and controls. Microbial Ecology 65(4), 995-1010.

Pearson, L., Mihali, T., Moffitt, M., Kellmann, R., Neilan, B., 2010. On the chemistry, toxicology and genetics of the cyanobacterial toxins, microcystin, nodularin, saxitoxin and cylindrospermopsin. Mar Drugs 8(5), 1650-1680.

Reif, C.B., 1939. The effect of stream conditions on lake plankton. Transactions of the American Microscopical Society 58(4), 398.

Reinhard, E.G., 1931. The plankton ecology of the Upper Mississippi, Minneapolis to Winona. Ecological Monographs 1(4), 396-464.

Reinl, K.L., Sterner, R.W., Lafrancois, B.M., Brovold, S., 2020. Fluvial seeding of cyanobacterial blooms in oligotrophic Lake Superior. Harmful Algae 100, 101941.

Sauer, V.B., Turnipseed, D.P., 2010. Stage measurement at gaging stations: U.S. Geological Survey Techniques and Methods, book 3, chap. A7, 45p. Reston, VA.

Schmidt, J., Wilhelm, S., Boyer, G., 2014. The fate of microcystins in the environment and challenges for monitoring. Toxins 6(12), 3354-3387.

Shallcross, A.L., Stanuikynas, Tom J. , 2002. Raritan Basin: Portait of a watershed. New Jersey Water Supply Authority, New Jersey.

Somma, A., Bonilla, S., Aubriot, L., 2022. Nuisance phytoplankton transport is enhanced by high flow in the main river for drinking water in Uruguay. Environmental Science and Pollution Research 29(4), 5634-5647.

Taylor, J., Rodman, A., Scott, J., 2020. Stream algal biomass response to experimental phosphorus and nitrogen gradients: A case for dual nutrient management in agricultural watersheds: TAYLOR et al. Journal of Environmental Quality 49.

Team, R.C., 2022. R: A language and environment for statistical computing, Vienna, Austria.

Turnipseed, D.P., Sauer, V.B., 2010. Discharge measurements at gaging stations, U.S. Geological Survey Techniques and Methods, book 3, chap. A8, 87p. Reston, VA.

Walker, H.W., 2017. Harmful algae blooms in drinking water: Removal of cyanobacterial cells and toxins. CRC Press, Boca Raton, Florida.

Wehr, J., Sheath, R., Kociolek, P., 2015. Freshwater algae of North America: Ecology and classification.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Wickham, H., Fraçois, R., Henry, L., Müller, K., 2022. dplyr: A Grammar of Data Manipulation.

Wickham, H., Girlich, M., 2022. R package version 1.2.0 ed.

Wiebe, A.H., 1928. Biological survey of the upper Mississippi River with special reference to pollution. US Government Printing Office.

Wood, S.A., Rueckert, A., Hamilton, D.P., Cary, S.C., Dietrich, D.R., 2011. Switching toxin production on and off: intermittent microcystin synthesis in a Microcystis bloom. Environmental Microbiology Reports 3(1), 118-124.