Technical Publication SJ2021-02

ASSESSMENT OF FACTORS INFLUENCING CYANOBACTERIAL BLOOM DEVELOPMENT IN THE LOWER ST. JOHNS RIVER BASIN

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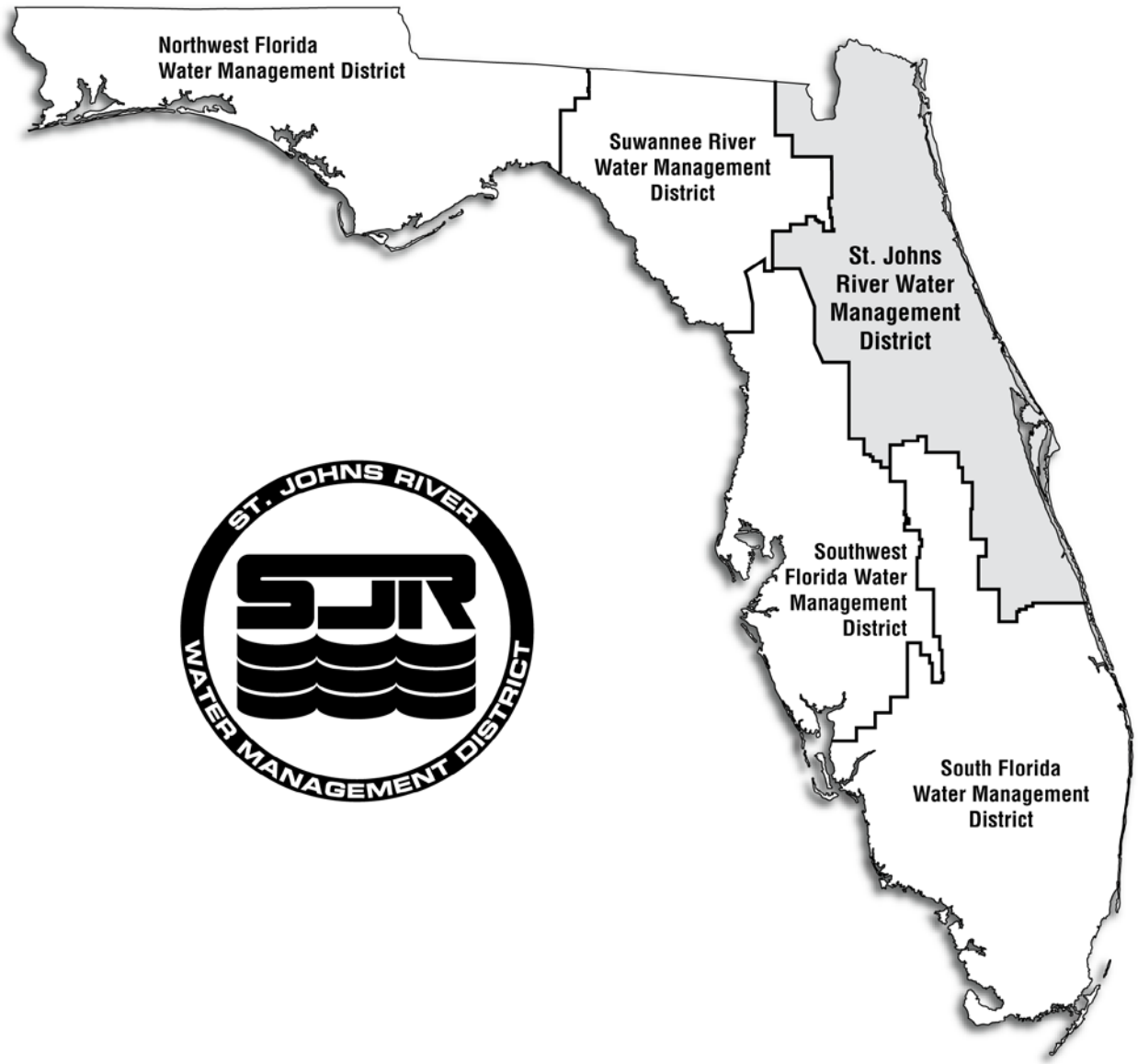
2021



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EXECUTIVE SUMMARY

The St. Johns River Water Management District has monitored phytoplankton in the Lower St. Johns River Basin (LSJRB) since the early 1990s. The LSJRB experiences severe cyanobacterial blooms in its freshwater and oligohaline reaches. Analyses were conducted of relationships between cyanobacteria and hydrologic and water quality factors for six sites in the LSJRB. The most severe cyanobacterial blooms were associated with relatively low discharges and long water residence times, often following a period of higher discharges, and high concentrations of total nitrogen (TN) and total phosphorus (TP). Internal sources often appeared to be the proximate sources of increases in TP and TN associated with cyanobacterial blooms; in several cases nitrogen-fixation was probably a major reason for the increases in TN concentrations. Statistical relationships between cyanobacteria and environmental factors were often weak, particularly for taxa that tend to form conspicuous surface blooms and are known to produce cyanotoxins, *Microcystis aeruginosa* and the nitrogen-fixing cyanobacterial genera *Dolichospermum* and *Aphanizomenon*. However, these statistical analyses also showed relationships of cyanobacteria with discharges and nutrients. The nature of the relationships with discharges varied among sites. In Lake George and the river as far downstream as Mandarin Point, high river discharges strongly reduce water residence time, resulting in strong negative relationships of discharge and cyanobacterial biovolumes. However, in Doctors Lake, an embayment off the oligohaline portion of the mainstem St. Johns River, water residence time is always long, and discharges are positively associated with cyanobacterial biovolumes, probably because they bring in nutrients in watershed runoff, and reduce conductivity to concentrations tolerable by cyanobacteria. At the St. Johns River at Piney Point, the most downstream station in the river, there is also a positive relationship between discharges and cyanobacteria. High river discharges may serve to transport cyanobacteria downstream into the oligohaline zone and reduce conductivity to concentrations tolerable by cyanobacteria. Crescent Lake, an embayment off the freshwater portion of the mainstem St. Johns River with intermediate water residence times, is also intermediate in the relationship between discharges and cyanobacteria. Because discharges in the LSJRB cannot be managed, control of cyanobacterial blooms depends primarily on reduction in nutrient concentrations. Total maximum daily loads (TMDLs) adopted for the oligohaline lower St. Johns River and for Doctors Lake require reduction only in TN. Although the statistical analyses often indicated stronger relationships of cyanobacteria with TN, the scientific literature supports reduction of TP as well as TN to control cyanobacterial blooms in both the freshwater and oligohaline sections because of the prominence of nitrogen-fixers in the LSJRB. Apparent threshold concentrations above which cyanobacterial blooms have occurred in the LSJRB are about 0.05 mg TP/L and 1 mg TN/L. Effective monitoring of cyanobacterial bloom development and ascertaining the causes of blooms in the LSJRB are difficult because of high temporal and spatial variability. Alternative or complementary methods that may improve monitoring of cyanobacterial blooms include continuous monitoring probes, satellite remote sensing, and biochemical or molecular identification/quantification.

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INTRODUCTION

BACKGROUND

Harmful algal blooms (HABs) occur in lakes, reservoirs, rivers, estuarine, and coastal waters. These algae occur naturally, but bloom development is exacerbated by eutrophication and other anthropogenic activities. Cyanobacteria tend to be the dominant HAB-formers in freshwater systems, but some species can also occur in more saline waters (Moisander et al. 2002; Paerl et al. 2018). Cyanobacterial blooms can have several undesirable effects, including unsightly surface scums, foul odor and tastes, depletion of dissolved oxygen, food web alterations and toxicity. These effects can lead to fish and wildlife mortality events. Toxins produced by cyanobacteria can affect animal and human health in waters used for recreational and drinking purposes (Paerl et al. 2001).

Cyanobacterial blooms can occur in large or regulated rivers, in which water residence time can be long (Sherman and Webster 1998; Bormans et al. 1997; Mitrovic et al. 2003; Kim et al. 2019; Graham et al. 2020). Cyanobacterial blooms commonly occur in the lower St. Johns River (Phlips et al. 2007; Havens et al. 2016, Nelson et al. 2018). *Microcystis aeruginosa* is the most conspicuous bloom former in the lower St. Johns River (Figure 1), although blooms of other cyanobacterial taxa have also occurred.

A number of factors are thought to contribute to development of cyanobacterial HABs. Water temperatures above 20^{o} C favor growth of cyanobacteria (O'Neil et al. 2012; Kim et al. 2019). High concentrations of nutrients, particularly phosphorus (P) and nitrogen (N), favor cyanobacterial dominance. If the ratio of N:P is low, then nitrogen-fixing cyanobacteria, such as *Dolichospermum*, *Aphanizomenon*, or *Cylindrospermopsis*, may be favored. However, if concentrations of both phosphorus and nitrogen are high then non-nitrogen-fixing species, such as *Microcystis*, may be favored (Paerl et al. 2001; Paerl and Fulton 2006; O'Neil et al. 2012). Joung et al. (2011) reported a strong relationship between total phosphorus (TP) and *Microcystis* in a Korean reservoir. Vertical stratification can favor development of cyanobacterial booms, especially of buoyant species that can form surface blooms, such as *Microcystis*, *Dolichospermum*, or *Aphanizomenon* (Paerl et al. 2001; Kim et al. 2019). Long water residence times also favor blooms of cyanobacteria (Paerl et al. 2001; Mitrovic et al. 2003; Cha et al. 2017). Long residence times also favor growth by zooplankton grazers, but cyanobacteria are often toxic, unpalatable, or of poor nutritional value to zooplankton grazers, leading to decreases in zooplankton populations, particularly of large cladocerans, which tend to be the most efficient grazers (Leonard and Paerl 2005; Paerl and Fulton 2006).

The St. Johns River Water Management District has been monitoring water quality and phytoplankton at several sites in the Lower St. Johns River Basin (LSJRB) since the early 1990s. The objective of this report is to examine relationships of cyanobacterial biovolumes to environmental factors, with particular focus on factors associated with HABs of *Microcystis aeruginosa* and the nitrogen-fixing genera *Dolichospermum* and *Aphanizomenon*, which tend to form conspicuous surface blooms and are notorious as

producers of cyanotoxins. Another report examines cyanotoxin occurrence in the LSJRB (Trent et al. 2019).

STUDY AREA

The St. Johns is the largest river in the southeastern United States, draining a 24,780-km^2 watershed in the temperate/subtropical Atlantic Coastal Plain of northeast Florida, extending 500 km from its headwaters to the Atlantic Ocean, near the city of Jacksonville (Phlips et al. 2007). It is a low-head river, with an average fall of only 2.2 cm/km (Toth 1993). Because of the shallow slope and basin morphology, the course of the St. Johns River includes several lakes and impoundments, including Lake George, Florida's second largest lake. The shallow slope also results in long water residence times during periods of low flows, permitting the development of cyanobacterial blooms. Salinity from the estuary and Atlantic sources can also encroach a long way upstream during low flow periods and reverse flow events. The average annual maximum upstream encroachment of estuarine salinity is to river km 74 (near the upstream end of the Oligohaline zone, Figure 2), with the maximum measured upstream extent to river km 100 (near Racy Point).

Analyses of cyanobacterial blooms were conducted for six sites in the LSJRB (Figure 2):

- Lake George – located in the freshwater portion of the mainstem St. Johns River, it can receive high flows from upstream, and has an estimated water replacement time averaging 68 days, with a minimum of 22 days. For Lake George, data were combined from multiple stations located from the center to the outlet of the lake.
- Crescent Lake – an embayment off the freshwater portion of the mainstem St. Johns River, which can receive relatively high flows from its watershed. It has an estimated water replacement time averaging 128 days, with a minimum of 7 days. For Crescent Lake, data were combined from multiple stations located from the center to the outlet of the lake.
- St. Johns River at Racy Point – in the freshwater portion of the mainstem St. Johns River downstream from Lake George.
- Doctors Lake – an embayment off the oligohaline portion of the mainstem St. Johns River, which receives relatively low flows from its watershed. It has an estimated water replacement time averaging 547 days, with a minimum of 246 days. These estimated water replacement times do not consider tidal flushing of the lake. We do not have sufficient data to calculate the effects of tidal flushing on water residence time, but we believe that even with tidal flushing the water residence time of Doctors Lake would be longer than for the mainstem river sites. Data were collected from a single station near the center of Doctors Lake.
- St. Johns River at Mandarin Point – in the oligohaline portion of the mainstem St. Johns River, near the outlet from Doctors Lake.
- St. Johns River at Piney Point– in the oligohaline portion of the mainstem St. Johns River, further downstream from Mandarin Point.

Mandarin Point, August 19, 2005

Shands Pier Ramp October 1, 2013

September 14, 2005

Doctors Lake, October 3, 2013

August 19, 2005

SE Doctors Lake, October 1, 2013

Figure 1. Lower St. Johns River *Microcystis aeruginosa* blooms.

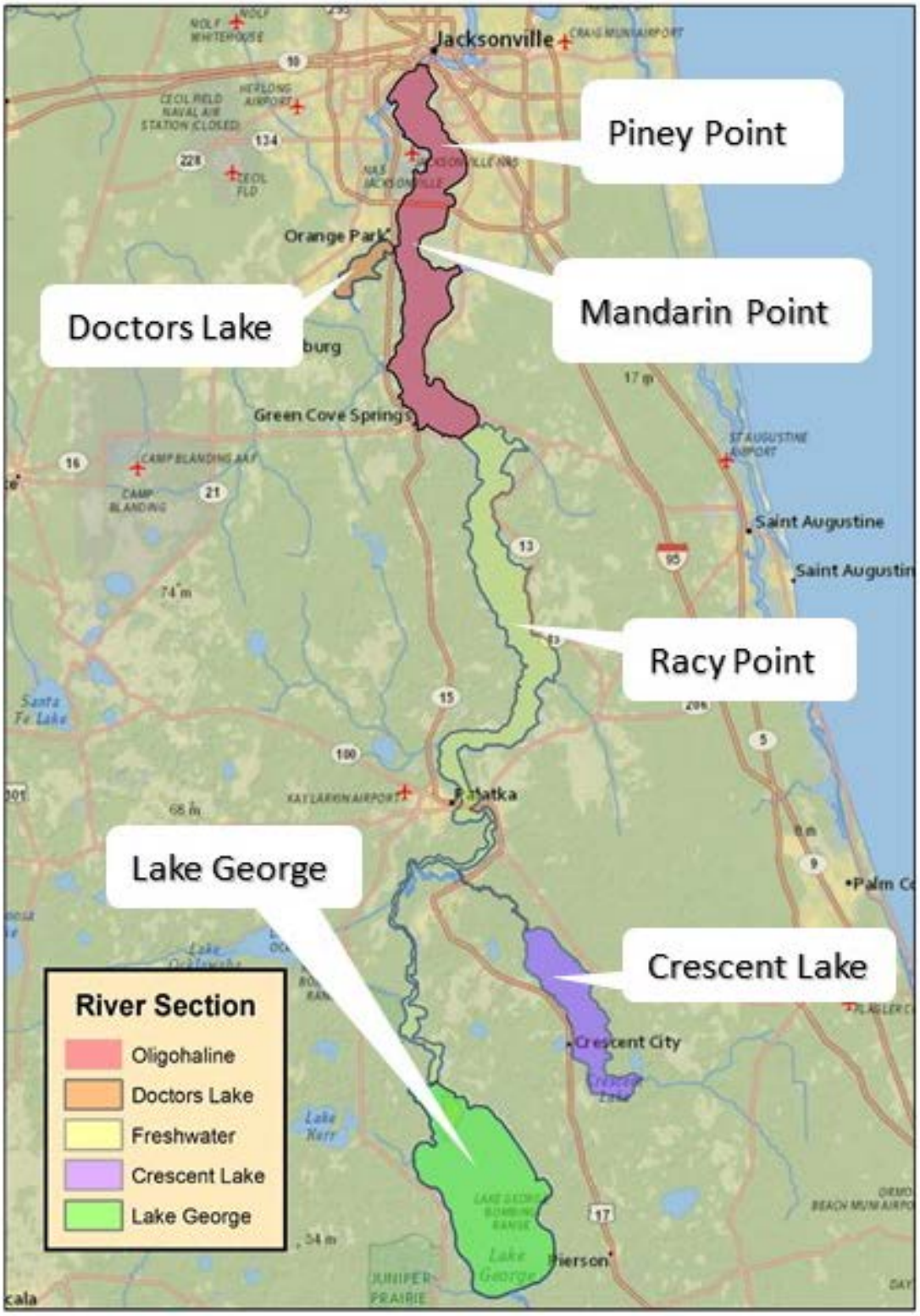


Figure 2. Study sites in the lower St. Johns River basin.

METHODS

WATER QUALITY AND PHYTOPLANKTON SAMPLING AND ANALYSES

Samples for water chemistry measurements and phytoplankton taxa identifications were collected from the top 2.5 m of the water column with a vertical integrating tube at scheduled intervals, usually monthly or twice monthly. Field measurements, including conductivity and water temperature, were measured with a multi-parameter sonde, usually at a range of depths over the top 2.5 m, although only at a single depth prior to 1997-1998. A suite of chemical analyses were performed on water samples using U.S. E.P.A. approved methods, including TP (EPA Method 365.4), nitrate/nitrite (NO_x) (EPA 353.2), ammonium (NH_4) (EPA 350.1), orthophosphate (PO_4) (EPA 365.1), pheophytin-corrected chlorophyll-*a* (EPA Method 10200 H), and water color (SM 2120B or 2120C). Total nitrogen (TN) was calculated from the sum of total Kjeldahl nitrogen (EPA Method 365.4) plus NO_x (EPA Method 353.2). See Kopp & McKee (1983) for EPA methods.

For NH_4 and NO_x generally only dissolved forms were measured after 2005, but before then either both dissolved and total forms or only total forms were measured. NH_4-D tended to be measured throughout the period of record, but NO_x-D tended to be measured only after the late-1990s. We compared measurements of the total and dissolved forms over periods of overlap (paired comparisons t-tests). NH_4-T was always significantly higher than NH_4-D, although differences in means were small. There were generally no significant differences between NO_x-T and NO_x-D, except for the St. Johns River at Racy Point where NO_x-D was higher, although the difference in means was small.

Phytoplankton samples were collected with a 1-liter amber bottle, preserved with 6 mls of Lugol's solution, and transported to the Phytoplankton Ecology Laboratory at the University of Florida. Samples were typically analyzed within 3 months of collection using the Utermöhl (1958) method. Phytoplankton were settled and enumerated using an inverted microscope. An aliquot of Lugol's-preserved sample was placed directly into settling chambers, and phytoplankton were classified and enumerated in random grids until a count of 100 counting units was attained or five grids were examined at 400X. Uncommon forms were enumerated in 30 grids. Large forms were enumerated at 100X utilizing the entire bottom of the chamber. Phytoplankton cell counts and biovolumes were determined for the lowest identifiable taxonomic units. Phytoplankton biovolumes were estimated using the closest geometric shapes and the appropriate volumetric formulae.

Two different phytoplankton analysts were used by the University of Florida laboratory. Analyst 1 covered most of the period of record, through 2011 for the St. Johns River at Piney Point and through 2015 for all other sites. Analyst 2 covered samples beginning in 2013 for the St. Johns River at Piney Point and in 2016 for the other sites. For both analysts, we conducted regression analyses to examine the relationships of estimated phytoplankton biovolume to corrected chlorophyll-*a* concentrations in the same samples.

Phytoplankton were divided into several groups for further analyses:

1. *Microcystis aeruginosa*
2. *Microcystis pulverea incerta*
3. *Microcystis* spp. (if present)
4. *Cylindrospermopsis* + *Raphidiopsis* (combined because recent genetic studies indicate they should be considered the same genus, Aguilera et al. 2018). This combined group will be referred to as *Cylindrospermopsis* in figures, since that genus had much higher reported biovolumes than *Raphidiopsis*.
5. Other Nitrogen-fixers (primarily *Dolichospermum* [previously called *Anabaena*] and *Aphanizomenon*). These were separated from *Cylindrospermopsis* because they tend to form conspicuous surface blooms, while *Cylindrospermopsis* does not.
6. Other Cyanobacteria
7. Other algae (in some statistical analyses these were further divided into Bacillariophyta, Chlorophyta, Pyrrophycophyta, and Other Phytoplankton).

Three general types of data summaries and analyses were used to examine changes in cyanobacterial biovolumes and relationships to other environmental factors:

1. Phytoplankton time series – plots of biovolumes and relative biovolumes of cyanobacterial and other phytoplankton, and summaries of highest biovolumes for each of the six study sites
2. Assessment of major bloom events
3. Statistical analyses of cyanobacterial-environmental factor relationships

ASSESSMENT OF MAJOR BLOOM EVENTS

We used time series plots to examine changes in biovolumes and environmental factors during major bloom events. Bloom events examined included:

M. aeruginosa:

- Lake George and downstream St. Johns River 1996
- Lake George 2016
- Crescent Lake 2017
- Doctors Lake 2007
- Doctors Lake 2008
- Doctors Lake 2017
- St. Johns River at Mandarin Point and Piney Point 1998
- St. Johns River at Piney Point 2013

Several of these apparent *M. aeruginosa* blooms occurred after the change in phytoplankton analysts (those in 2013-2017).

Other Nitrogen-fixers:

- Lake George, Crescent Lake, and St. Johns River at Racy Point 2010

- Doctors Lake 2001

Table 1 shows environmental variables considered in assessment of cyanobacterial bloom events. Several of these variables require further explanation:

- NO_x – for bloom events during 1996, in which NO_x-T or NO_x-D were measured on different dates, figures combine those forms and show NO_x in the labels. Figures for later bloom events during periods in which NO_x-D was measured on all dates show that in the labels.
- DDays – this was a function cumulating daily water temperatures for the 30-day period prior to the phytoplankton sample date. Daily water temperatures were available for most of the period of record from four continuous recorders in the LSJRB. For early periods in which continuous records were not available, we did linear interpolation between measurements taken on water quality sample dates.
- TempRange1.5m or TempRange2m – vertical range in water temperatures, measured over a depth range of 1.5 or 2 m – this was used as a measure of water column stability, with a high temperature range indicating a stratified water column.
- CondRange1.5m or CondRange2m – vertical range in conductivity, measured over a depth range of 1.5 or 2 m – this was used as a measure of water column stability, with a high range indicating a stratified water column.
- Discharge – average discharge at the closest measurement site for the 30-day period prior to the sample date.
- Wind-D or Wind-P – composite functions combining wind speed and fetch. Daily wind directions were taken from either the NOAA Daytona weather station (D) or the Pierson FAWN weather station (P). The Pierson station is closer to the sites but had a shorter period of record. Wind speeds were also available for a 3-year period for Lake George; these wind speeds were higher than measured at the weather stations. We developed a quadratic regression equation to predict Lake George daily wind speeds from Daytona daily wind speed data (R^2=0.61) and used those predicted wind speeds for all sites. Fetch lengths were estimated for each site for a range of different directions, and from those data polynomial equations were developed to predict fetch length as a function of wind direction. Composite wind functions were calculated as wind speed (m/s) x square root (fetch[m]). This is based on a commonly used equation relating significant wave height to wind speed and square root of the fetch (Smith 1991). Daily wind functions were averaged for a period of time prior to samples (the time period varied among sites from 1 to 14 days, we chose time periods which generally had highest correlations with cyanobacteria.

Table 1. Environmental variables considered in bloom event assessment.

TP – total phosphorus	DDays – degree days
TN – total nitrogen	Cond – conductivity
TNTP – ratio of TN to TP	Color – water color
PO_4-D – dissolved phosphate	TempRange1.5m or TempRange2m
NH_4 – ammonium	CondRange1.5m or CondRange2m
NO_x – nitrate-nitrite	Discharge
Temp – water temperature	Wind-D or Wind-P – Daytona or Pierson wind function

STATISTICAL ANALYSES OF CYANOBACTERIAL-ENVIRONMENTAL FACTOR RELATIONSHIPS

The samples we used in analyses included only the warm season (April-October), the period in which cyanobacterial blooms primarily occur. Initial analyses combined untransformed data for all sites. First, plots and regressions were developed to assess relationships between chlorophyll-*a* and phytoplankton biovolumes for the two phytoplankton analysts. Further analyses to evaluate relationships of cyanobacteria to environmental variables used only data from the first phytoplankton analyst, who covered most of the period of record, through 2011 for the St. Johns River at Piney Point and through 2015 for all other sites. Next, plots were constructed to visualize relationships across sites of TP and TN with cyanobacterial biovolumes and with chlorophyll-*a*.

Multivariate statistical analyses were used to further assess relationships between cyanobacteria and environmental factors at individual sites. Environmental variables used in the analyses included those listed in Table 1. Several other variables were also included in the statistical analyses:

- NH_4 and NO_x – For statistical analyses, we combined the total and dissolved forms for the entire period of record.
- Discharge – average discharge at the closest measurement site for a period of time prior to samples (the time period varied among sites from 30 to 360 days; we chose durations that generally had highest correlations with cyanobacteria).
- WintPrevDis – average discharge for the preceding winter (November-March).
- WintSummDis – average preceding winter discharge divided by the average for a period of time before samples (the time period varied among sites from 30 to 270 days; we chose durations that generally had highest correlations with cyanobacteria).
- Rain – Beginning in 1995, Nexrad Doppler radar data were used to determine average daily rainfall over the drainage basin for the phytoplankton sample sites. Prior to then

we used average rainfall from several rain gauges in the drainage basin. Daily rainfall was cumulated over a period of time before samples (the time period varied among sites from 60 to 360 days; we chose durations that generally had highest correlations with cyanobacteria).

- Secchi depth – included in multivariate ordinations, but it was not included in regression analyses because of missing records and because we thought its variation was more of a response to cyanobacterial biomass changes than a cause of them.

The multivariate statistical analyses could only be run for samples that had no missing records for the environmental variables. Some of the environmental variables had a substantial number of missing records. We initially performed analyses using the full suite of environmental variables, but in later iterations dropped variables that were not significant, had missing records, or were highly correlated with other environmental variables, to allow an increase in sample sizes. The environmental variables were $\log_{10}$ transformed prior to analyses if they had strongly skewed distributions.

We used three types of multivariate data analyses to evaluate relationships of cyanobacteria to environmental variables. The first analysis was nonmetric multidimensional scaling (NMDS), using the R package "vegan" (Oksanen et al. 2018). This is a nonparametric multivariate ordination. Phytoplankton biovolumes were Wisconsin standardized plus square root transformed prior to analysis – this is the default in vegan if the values are large enough, and the vegan manual notes these transformations generally improve the results. The Wisconsin is a double standardization; first by columns (divides biovolume for each taxon in one sample by the maximum biovolume for that taxon among all the samples), then by rows (divides biovolume for each taxon in one sample by the total biovolume for that sample). Wisconsin standardized values would be highest if a taxon's biovolume is high relative to both the period of record for that taxon and to other taxa in that sample, which would be the case in bloom situations. We tested other transformations and found that the Wisconsin plus square root generally provided the best separation among taxa.

We used the Bray-Curtis dissimilarity index for the ordination, the default in vegan. We used the "envfit" function in vegan, which performs linear regression between environmental variables and the ordination axes. The regressions are independent for each environmental variable. We also used the "ordisurf" function, which fits contours of environmental variables to the ordination plot. These contours are useful for visualizing relationships between variables and the ordination, particularly whether the relationships are nonlinear.

We also performed parametric and nonparametric multiple regression analyses. We also used the Wisconsin standardization plus square root transformation of the phytoplankton biovolumes in the regression analyses, for consistency with the NMDS ordination. The Wisconsin standardization plus square root transformation also tended to reduce the strong skewness in the distributions of the biovolume data.

The parametric analysis was stepwise multiple linear regression, using the Minitab program. The Stepwise option was used, with $p<0.05$ for a term to enter or be removed

from the model. All environmental variables were standardized (subtract the mean and divide by the standard deviation) before analysis. Variance Inflation Factors (VIF) were low (<3) in all the final regression models, indicating relatively little multicollinearity. A VIF value greater than 5 suggests that the regression coefficient is poorly estimated due to severe multicollinearity (Minitab 18 support).

We also used random forests with recursive feature elimination (RFE), a nonparametric alternative to multiple regression with variable selection. Random forests can be used for classification or regression, creating a "forest" of decision trees using subsets of observations and variables. RFE is helpful in analyses with correlated predictors, when assessing the relative importance of the predictors is desired. In random forests with RFE, the predictor with the lowest importance is removed at each iteration until a specified number of variables remain, and the model with the best performance is selected. To better estimate performance when using RFE (Kuhn 2009), we used 10-fold cross-validation repeated 5 times, meaning 90% of the observations were used for training and the remaining 10% for testing, with resampling at each iteration. Each tree in a random forest is fit to a different bootstrapped sample of the training data set, and predictions are made by running cases through all trees and taking the average for regression. The main parameters in the analysis are the number of trees in the forest (in our case, 500), and the number of predictors randomly selected for each split in the tree (in our case, the number of predictors divided by 3, rounded down, the default). We used the R package "caret" (Kuhn 2020), which also uses the R package "randomForest" (Liaw and Wiener, 2002), and recomputed the relative importance values of the predictors at each iteration. The lowest RMSE was the performance metric used to select the optimal set of predictors for the final model for each group of phytoplankton at each site. The measure of importance used for predictors in the RFE Models is the %IncMSE. This is the increase in mean square error of predictions (estimated with the test subset) as a result of the variable being permuted (values randomly shuffled). If a predictor is important in the model, then assigning other values for that predictor by permuting this predictor's values over the dataset should have a negative influence on prediction, i.e. using the same model to predict from data that is the same except for the one variable, should give worse predictions.

RESULTS

PHYTOPLANKTON TIME SERIES

Lake George

Total cyanobacteria were always present in Lake George, although they dominated the phytoplankton biovolume primarily in the warmer months (Figure 3, Figure 4). The Other Cyanobacteria tended to dominate the phytoplankton biovolume, always present, averaging 3.8 mm^3/L, 42% of total biovolume, and reaching a maximum of 91%. Common genera in this group included *Limnothrix*, *Planktolyngbya*, *Pseudanabaena*, and *Chroococcus*.

Microcystis aeruginosa was sporadic in occurrence, being absent on 82% of sample dates, and overall average biovolume of 0.12 mm^3/L, averaging 1.1% of total biovolume. Highest biovolumes of *M. aeruginosa* were in September 2016 (12.8 mm^3/L, 48% of total biovolume), and October 2016 (10.1 mm^3/L, 70% of total biovolume). Hurricane Matthew occurred between the September and October sample dates. Other relatively high biovolumes of 1 – 2 mm^3/L occurred in June-July 1995, July-October 1996, and June 2010.

Microcystis pulverea incerta was absent on 17% of sample dates and had an average biovolume of 0.21 mm^3/L, averaging 3.7% of total biovolume. Maximum biovolume was 3.6 mm^3/L (48% of total biovolume) in June 2000.

Cylindrospermopsis + *Raphidiopsis* were absent on 21% of sample dates and had an average biovolume of 0.75 mm^3/L, averaging 7.4% of total biovolume. Maximum biovolume was 6.9 mm^3/L (47% of total biovolume) in May 1997. Other high biovolumes of *Cylindrospermopsis* + *Raphidiopsis* occurred in April 2017 (6.8 mm^3/L, 35% of total biovolume), May 2004 (6.1 mm^3/L, 40% of total biovolume), April 2011 (5.7 mm^3/L, 42% of total biovolume), September 2007 (5.5 mm^3/L, 13% of total biovolume), June 2009 (5.3 mm^3/L, 39% of total biovolume), and April 1999 (5.1 mm^3/L, 29% of total biovolume).

Other Nitrogen-fixers were absent on 29% of sample dates and had an average biovolume of 0.53 mm^3/L, averaging 5.7% of total biovolume. Maximum biovolume was 48.7 mm^3/L (96% of total biovolume) in May 2010. This was a large bloom of *Aphanizomenon*, which continued through the next sample in June 2010 (15.5 mm^3/L, 87% of total biovolume). Other high biovolumes of Other Nitrogen-fixers occurred in April 2017 (6.9 mm^3/L, 36% of total biovolume), May 2015 (6.0 mm^3/L, 67% of total biovolume), May 2009 (4.5 mm^3/L, 55% of total biovolume), and July 1996 (4.2 mm^3/L, 33% of total biovolume).

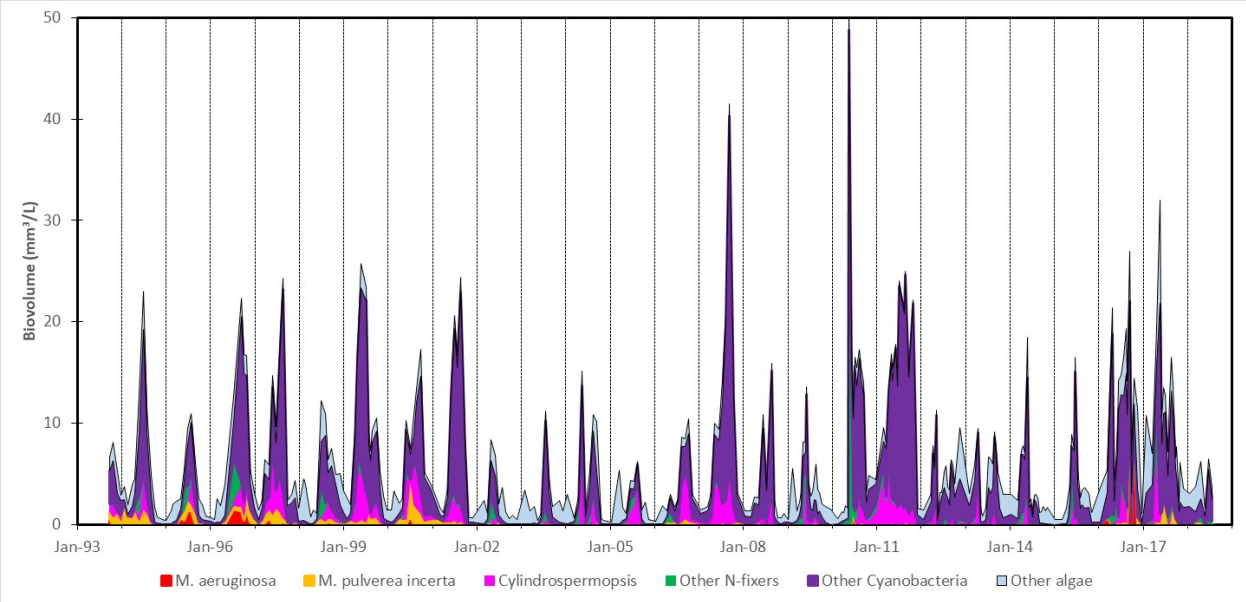


Figure 3. Lake George phytoplankton biovolumes.

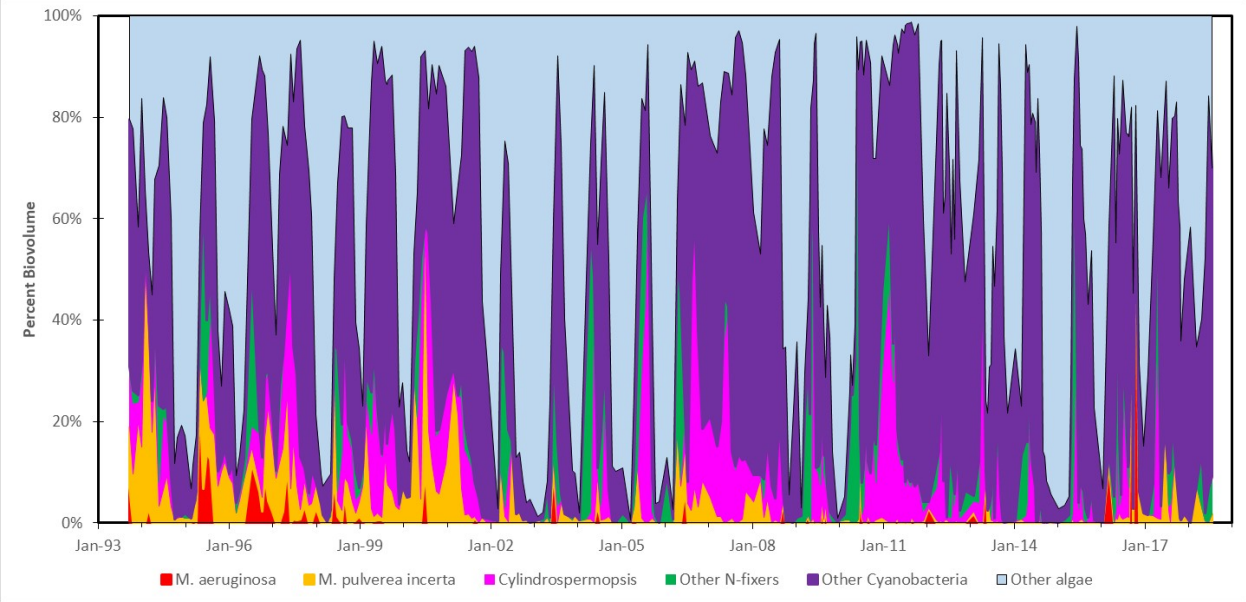


Figure 4. Lake George phytoplankton percent biovolumes.

Crescent Lake

Phytoplankton composition in Crescent Lake was broadly similar to that in Lake George. Total cyanobacteria were always present in Crescent Lake, although they dominated the phytoplankton biovolume primarily in the warmer months (Figure 5, Figure 6). The Other Cyanobacteria tended to dominate the phytoplankton biovolume, always present, averaging 1.6 mm^3/L, 32% of total biovolume, and reaching a maximum of 89%. Common genera in this group included *Limnothrix*, *Planktolyngbya*, *Pseudanabaena*, *Oscillatoria*, and *Chroococcus*.

Microcystis aeruginosa was sporadic in occurrence, being absent on 70% of sample dates, and overall average biovolume of 0.15 mm^3/L, averaging 3.6% of total biovolume. Highest biovolume of *M. aeruginosa* was in August 2017 (16.9 mm^3/L, 42% of total biovolume). Other relatively high biovolumes of 1 – 3 mm^3/L occurred in 1997, 2005, 2007, and 2010.

Microcystis pulverea incerta was absent on 26% of sample dates and had an average biovolume of 0.045 mm^3/L, averaging 1.8% of total biovolume. Maximum biovolume was 0.7 mm^3/L (18% of total biovolume) in October 2017. Unidentified *Microcystis* sp. occurred at low biovolumes on only two dates in 2017.

Cylindrospermopsis + *Raphidiopsis* were absent on 36% of sample dates and had an average biovolume of 0.20 mm^3/L, averaging 3.3% of total biovolume. Maximum biovolume was 4.5 mm^3/L (44% of total biovolume) in April 2013. Biovolumes also exceeded 3 mm^3/L in 1999 and 2007.

Other Nitrogen-fixers were absent on 22% of sample dates and had an average biovolume of 0.33 mm^3/L, averaging 9.4% of total biovolume. Maximum biovolume was 4.9 mm^3/L (95% of total biovolume) in June 2010. This bloom persisted from May through June, dominated by *Aphanizomenon* on the first two sample dates, and *Dolichospermum* on the third date. A similar bloom of *Aphanizomenon* occurred during the same time period in Lake George. Biovolumes of Other Nitrogen-fixers also exceeded 2 mm^3/L in 1999, 2007, 2009, 2011, and 2015.

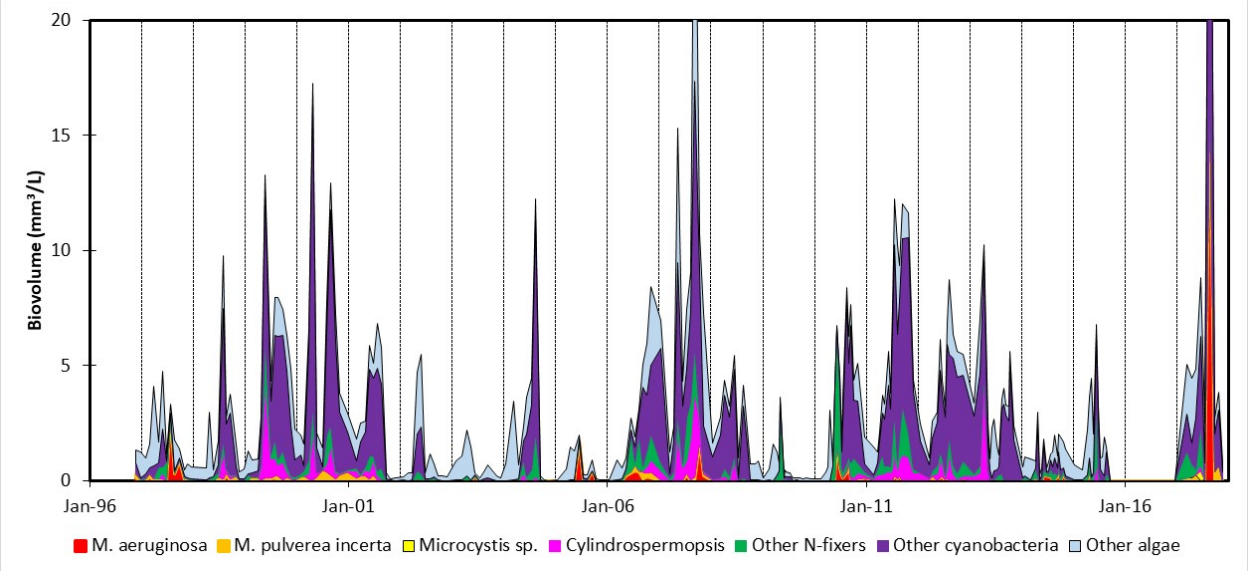


Figure 5. Crescent Lake phytoplankton biovolumes.

(no samples between August 2015 and March 2017)

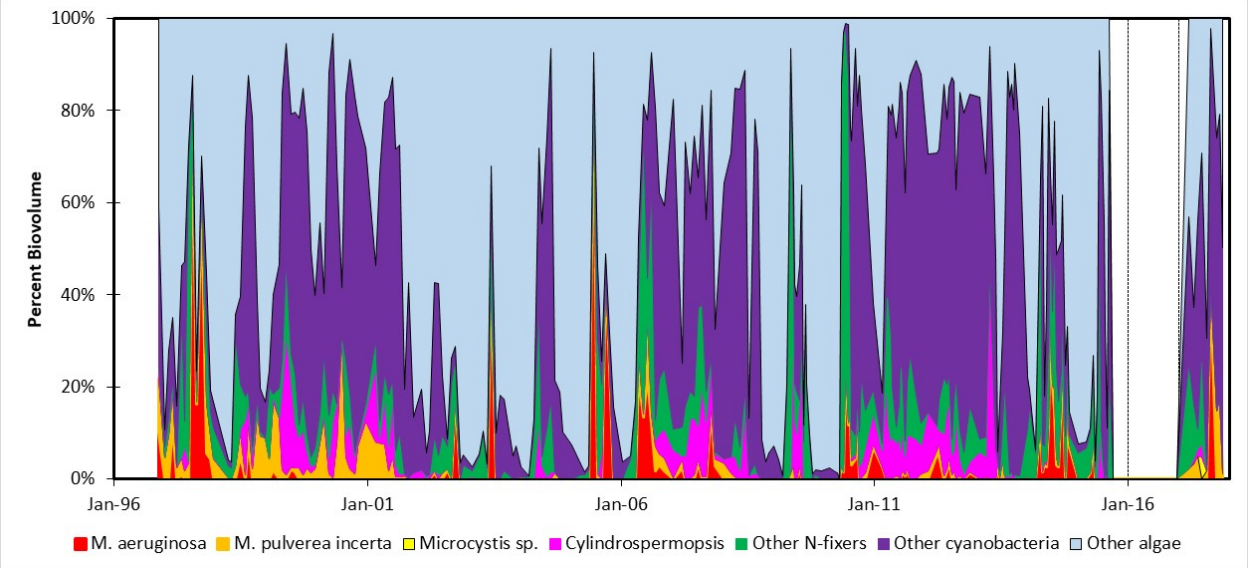


Figure 6. Crescent Lake phytoplankton percent biovolumes.

(no samples between August 2015 and March 2017)

St. Johns River at Racy Point

Phytoplankton composition was broadly similar to Lake George in the St. Johns River at Racy Point. Total cyanobacteria were always present at Racy Point, although they dominated the phytoplankton biovolume primarily in the warmer months (Figure 7, Figure 8). The Other Cyanobacteria tended to dominate the phytoplankton biovolume, always present, averaging 1.7 mm^3/L, 30% of total biovolume, and reaching a maximum of 84%. Common genera in this group included *Limnothrix*, *Planktolyngbya*, *Pseudanabaena*, and *Chroococcus*.

Microcystis aeruginosa was sporadic in occurrence, being absent on 76% of sample dates, and overall average biovolume of 0.055 mm^3/L, averaging 1.1% of total biovolume. Highest biovolume of *M. aeruginosa* was in July 1996 (2.0 mm^3/L, 23% of total biovolume). Biovolume exceeded 1 mm^3/L on only one other date, in November 1996.

Microcystis pulverea incerta was absent on 16% of sample dates and had an average biovolume of 0.094 mm^3/L, averaging 2.6% of total biovolume. Maximum biovolume was 1.2 mm^3/L (14% of total biovolume) in July 1996, same date as the *M. aeruginosa* peak. Biovolume also exceeded 1 mm^3/L in July 1995.

Cylindrospermopsis + *Raphidiopsis* were absent on 28% of sample dates and had an average biovolume of 0.30 mm^3/L, averaging 3.8% of total biovolume. Maximum biovolume was 8.4 mm^3/L (82% of total biovolume) in June 2007. Biovolumes also exceeded 3 mm^3/L twice in 1999, in 2001, 2007, and 2016.

Other Nitrogen-fixers were absent on 31% of sample dates and had an average biovolume of 0.44 mm^3/L, averaging 5.1% of total biovolume. Maximum biovolume was 52.6 mm^3/L (95% of total biovolume) in May 2010. This was a large bloom of *Aphanizomenon*, which continued through the next sample in June 2010 (7.3 mm^3/L, 87% of total biovolume). A similar bloom of *Aphanizomenon* occurred during the same time period in upstream Lake George and Crescent Lake. Biovolumes of Other Nitrogen-fixers also exceeded 2 mm^3/L twice in 1999, and once in 1998, 2000, 2006, and 2011.

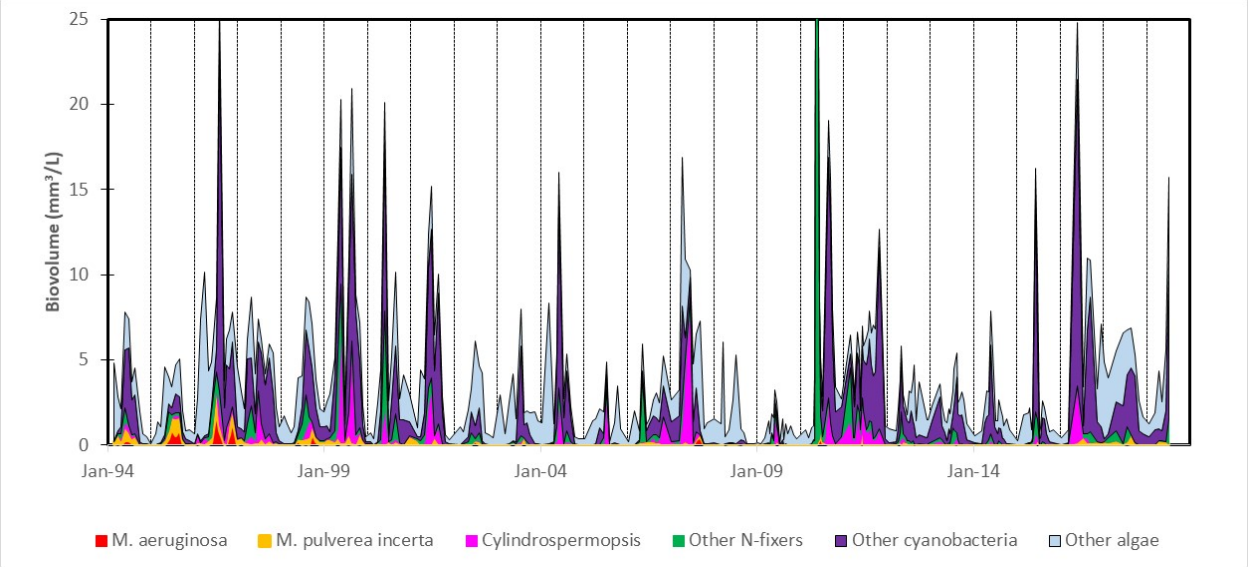


Figure 7. St. Johns River at Racy Point phytoplankton biovolumes.

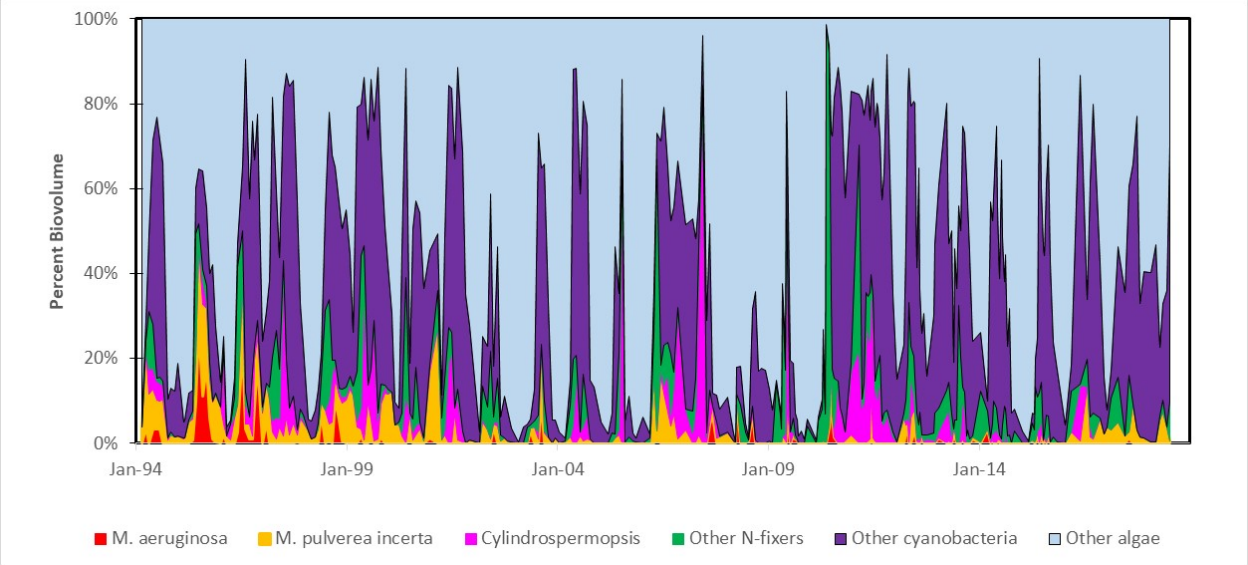


Figure 8. St. Johns River at Racy Point phytoplankton percent biovolumes.

Doctors Lake

Total cyanobacteria were less prevalent in Doctors Lake than in the upstream freshwater sites, but were always present in Doctors Lake, and often dominated the phytoplankton biovolume in the warmer months (Figure 9, Figure 10). The Other Cyanobacteria again tended to be most prominent, always present, averaging 0.83 mm^3/L (substantially lower than in the freshwater sites), 22% of total biovolume, and reaching a maximum of 85%. Common genera in this group included *Limnothrix*, *Planktolyngbya*, *Oscillatoria*, *Chroococcus*. and *Synechococcus*.

Microcystis aeruginosa was again sporadic in occurrence, being absent on 73% of sample dates, and overall average biovolume of 0.23 mm^3/L, averaging 3.0% of total biovolume. This average biovolume exceeded that in the freshwater sites in the LSJRB. *M. aeruginosa* blooms were more frequent than in the freshwater sites. Highest biovolume of *M. aeruginosa* was in June 2017 (15.1 mm^3/L, 90% of total biovolume). Other high biovolumes included 7.9 mm^3/L in 2007, 3.3 mm^3/L in 2008, 2.9 mm^3/L in 2016, and over 1 mm^3/L in several other years.

Microcystis pulverea incerta was absent on 20% of sample dates and had an average biovolume of 0.13 mm^3/L, averaging 3.1% of total biovolume. Maximum biovolume was 1.8 mm^3/L (16% of total biovolume) in June 2005. Biovolumes also exceeded 1 mm^3/L in 1998, 2007, 2009, and 2017. Unidentified *Microcystis* sp. was reported on only 2 dates, at 1.3 mm^3/L in June 2016 (on the same date *M. aeruginosa* had biovolume of 1.9 mm^3/L and *M. pulverea incerta* had biovolume of 0.8 mm^3/L), and at low biovolume in May 2018.

Cylindrospermopsis + *Raphidiopsis* were absent on 49% of sample dates and had an average biovolume of 0.057 mm^3/L, averaging 2.2% of total biovolume. Maximum biovolume was 0.97 mm^3/L (26% of total biovolume) in June 2005. *Cylindrospermopsis* + *Raphidiopsis* biovolumes were substantially lower in Doctors Lake than in the freshwater sites.

Other Nitrogen-fixers were absent on 35% of sample dates and had an average biovolume of 0.45 mm^3/L, averaging 9.1% of total biovolume. Maximum biovolume was 8.0 mm^3/L (71% of total biovolume) in April 2001. Biovolumes of Other Nitrogen-fixers also exceeded 2 mm^3/L twice in 1997, in 1999, July 2001, 2003, twice in 2006, 2008, three times in 2011, twice in 2016, and in 2017.

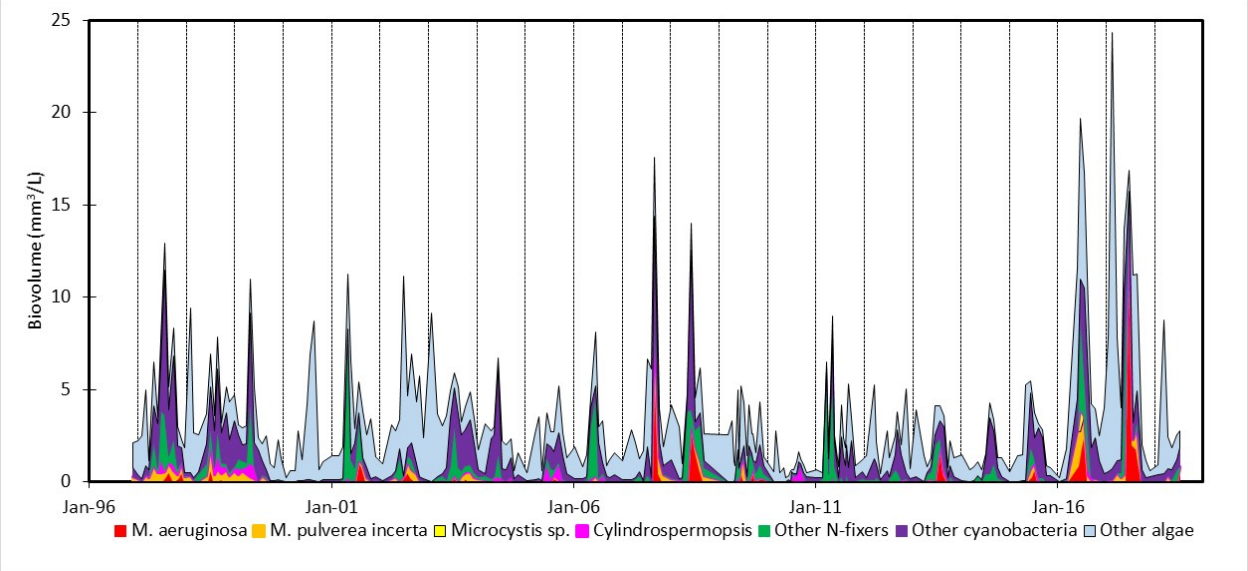


Figure 9. Doctors Lake phytoplankton biovolumes.

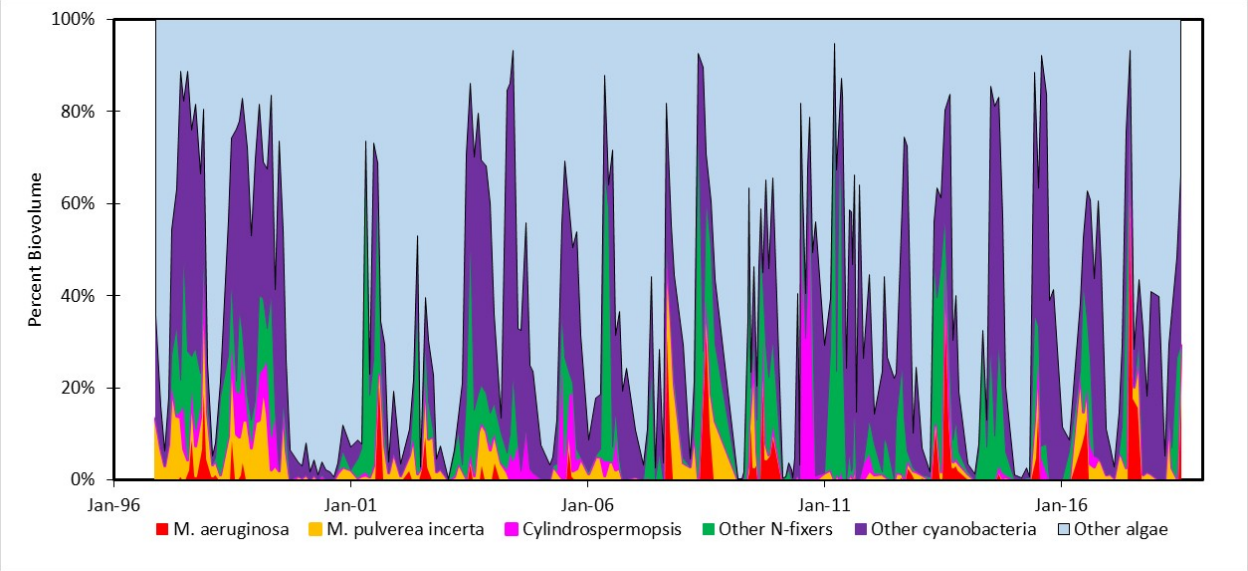


Figure 10. Doctors Lake phytoplankton percent biovolumes.

St. Johns River at Mandarin Point

Total cyanobacteria were less prevalent in the St. Johns River at Mandarin Point than in the upstream freshwater sites or in Doctors Lake. Total cyanobacteria were always present at Mandarin Point, but dominated the phytoplankton biovolume in the warmer months in less than half of the years (Figure 11, Figure 12). The Other Cyanobacteria again tended to be most prominent, always present, averaging 0.13 mm^3/L (much lower than in Doctors Lake or the freshwater sites), 10% of total biovolume, and reaching a maximum of 65%. Common genera in this group included *Limnothrix*, *Planktolyngbya*, *Chroococcus*, and *Synechococcus*.

Microcystis aeruginosa was again sporadic in occurrence, being absent on 81% of sample dates, and overall average biovolume of 0.038 mm^3/L, averaging 1.9% of total biovolume. Highest biovolume of *M. aeruginosa* was in August 1998 (2.3 mm^3/L, 28% of total biovolume). Biovolumes also exceeded 1 mm^3/L in 1995, twice in 1996, and 1998. *M. aeruginosa* on several occasions reached a higher percentage of total biovolume at Mandarin Point, including 71% in January 2014 (biovolume 0.3 mm^3/L); 69% in September 2005 (biovolume 0.6 mm^3/L); 52% in August 1996 (biovolume 1.2 mm^3/L).

Microcystis pulverea incerta was absent on 20% of sample dates and had an average biovolume of 0.037 mm^3/L, averaging 2.5% of total biovolume. Maximum biovolume was 0.9 mm^3/L (20% of total biovolume) in June 1994.

Cylindrospermopsis + *Raphidiopsis* were absent on 63% of sample dates and had an average biovolume of 0.017 mm^3/L, averaging 0.8% of total biovolume. Maximum biovolume was 1.6 mm^3/L (53% of total biovolume) in July 2005. *Cylindrospermopsis* + *Raphidiopsis* also exceeded 1 mm^3/L in August 1998. *Cylindrospermopsis* + *Raphidiopsis* biovolumes were substantially lower at Mandarin Point than in the upstream freshwater sites.

Other Nitrogen-fixers were absent on 56% of sample dates and had an average biovolume of 0.036 mm^3/L, averaging 2.1% of total biovolume. Maximum biovolume was 1.4 mm^3/L (18% of total biovolume) in August 1998. There were no other exceedances of a biovolume of 1 mm^3/L. Other Nitrogen-fixer biovolumes were substantially lower at Mandarin Point than in the upstream freshwater sites or in Doctors Lake. Other Nitrogen-fixers on several occasions reached a higher percentage of total biovolume at Mandarin Point, including 41% in April 2011 (biovolume 0.3 mm^3/L); 38% in June 2008 (biovolume 0.8 mm^3/L); 36% in May 2011 (biovolume 0.1 mm^3/L), 30% in May 1995 (biovolume 0.5 mm^3/L).

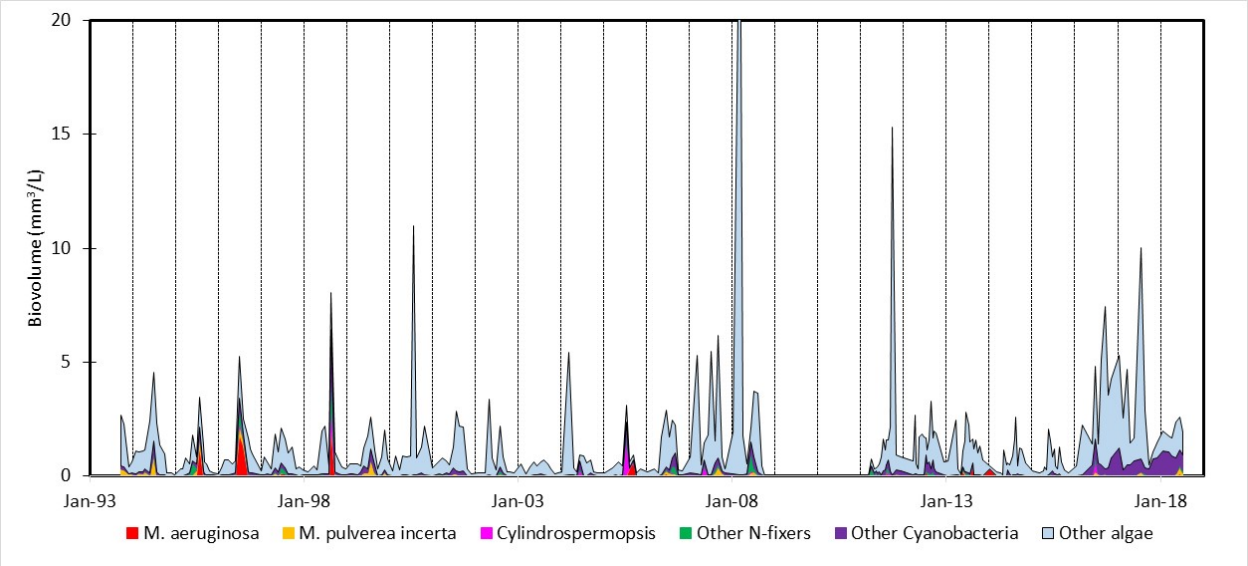


Figure 11. St. Johns River at Mandarin Point phytoplankton biovolumes.

No samples between Oct 2008 and April 2011.

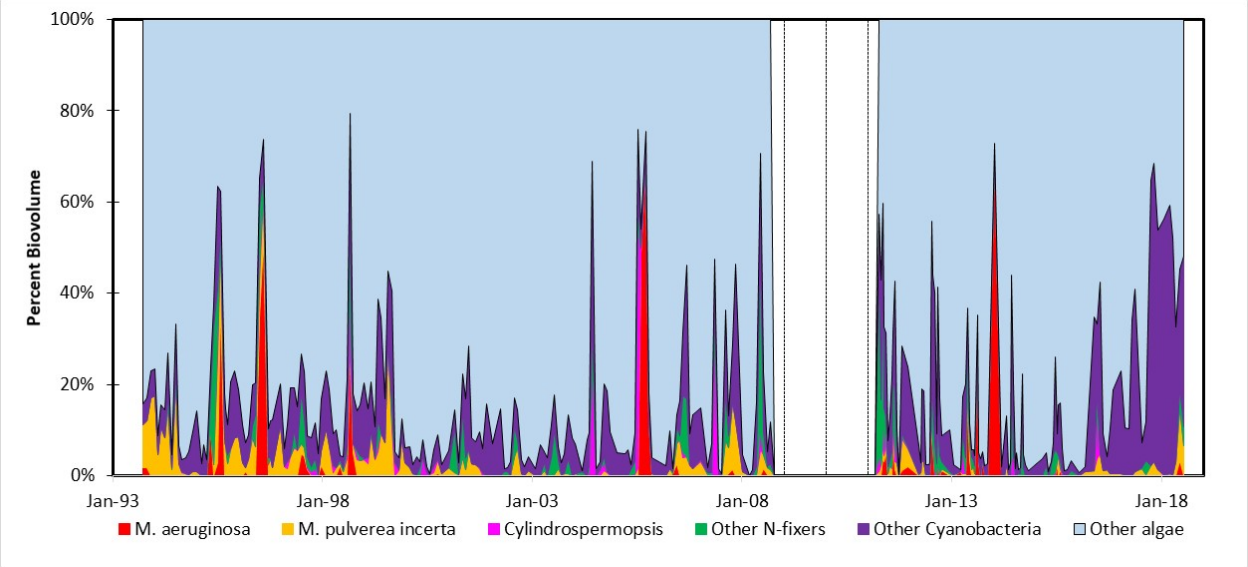


Figure 12. St. Johns River at Mandarin Point phytoplankton percent biovolumes.

No samples between Oct 2008 and April 2011.

St. Johns River at Piney Point

Total cyanobacteria were less prevalent in the St. Johns River at Piney Point than in the upstream freshwater sites or in Doctors Lake. Total cyanobacteria were nearly always present at Piney Point, but dominated the phytoplankton biovolume in the warmer months in only some years (Figure 13, Figure 14). The Other Cyanobacteria again tended to be most prominent, always present, averaging 0.096 mm^3/L (much lower than in Doctors Lake or the freshwater sites), 10% of total biovolume, and reaching a maximum of 85%. Common genera in this group included *Limnothrix*, *Chroococcus*, *Merismopedia*, and *Synechococcus*.

Microcystis aeruginosa was again sporadic in occurrence, being absent on 88% of sample dates, and overall average biovolume of 0.084 mm^3/L, averaging 2.0% of total biovolume. Highest biovolume of *M. aeruginosa* was in October 2013 (12.6 mm^3/L, 99% of total biovolume). However, chlorophyll-*a* concentration in this sample was only 4.5 μg/L, well below the average of 8.2 μg/L for this site. The next highest biovolume was 0.36 mm^3/L in 1998. The next highest percent biovolume was 54% in 2005 (biovolume 0.24 mm^3/L).

Microcystis pulverea incerta was absent on 39% of sample dates and had an average biovolume of 0.015 mm^3/L, averaging 1.5% of total biovolume. Maximum biovolume was 0.5 mm^3/L (38% of total biovolume) in July 1999.

Cylindrospermopsis + *Raphidiopsis* were absent on 81% of sample dates and had an average biovolume of 0.019 mm^3/L, averaging 0.9% of total biovolume. Maximum biovolume was 1.5 mm^3/L (19% of total biovolume) in August 1998. *Cylindrospermopsis* + *Raphidiopsis* also exceeded 1 mm^3/L in July 2005. *Cylindrospermopsis* + *Raphidiopsis* biovolumes were substantially lower at Piney Point than in the upstream freshwater sites.

Other Nitrogen-fixers were absent on 75% of sample dates and had an average biovolume of 0.021 mm^3/L, averaging 1.4% of total biovolume. Maximum biovolume was 1.6 mm^3/L (20% of total biovolume) in August 1998. There were no other exceedances of a biovolume of 1 mm^3/L. The highest percent biovolume was 52% in 2011 (biovolume 0.16 mm^3/L). Other Nitrogen-fixer biovolumes were substantially lower at Piney Point than in the upstream freshwater sites or in Doctors Lake.

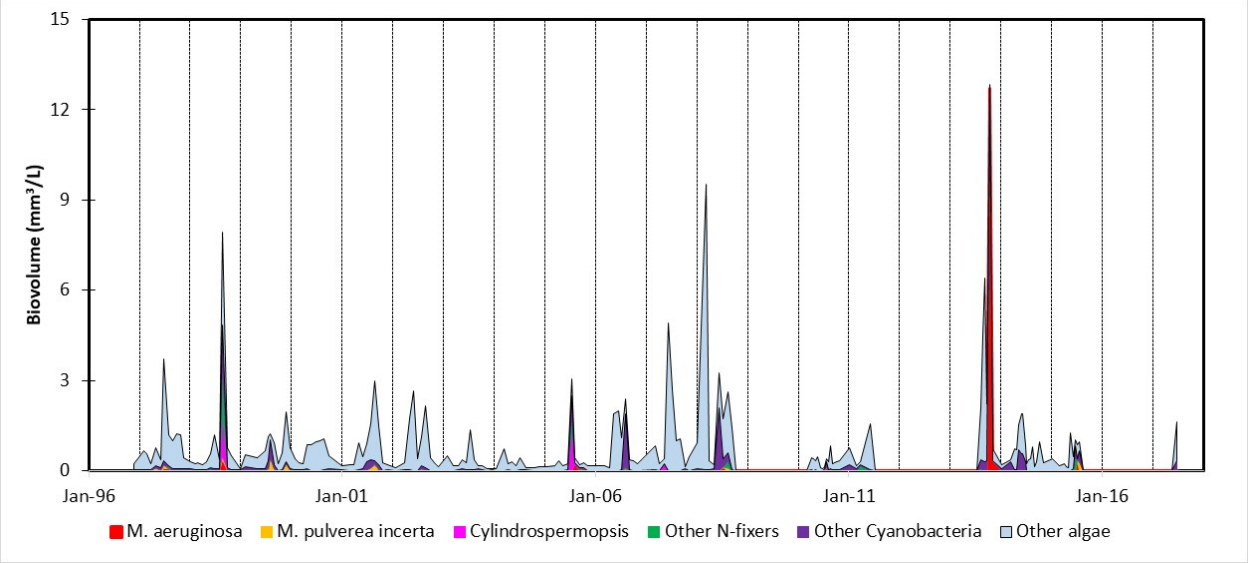


Figure 13. St. Johns River at Piney Point phytoplankton biovolumes.

No samples between September 2008 and April 2010, between June 2011 and August 2013, or between July 2015 and June 2017.

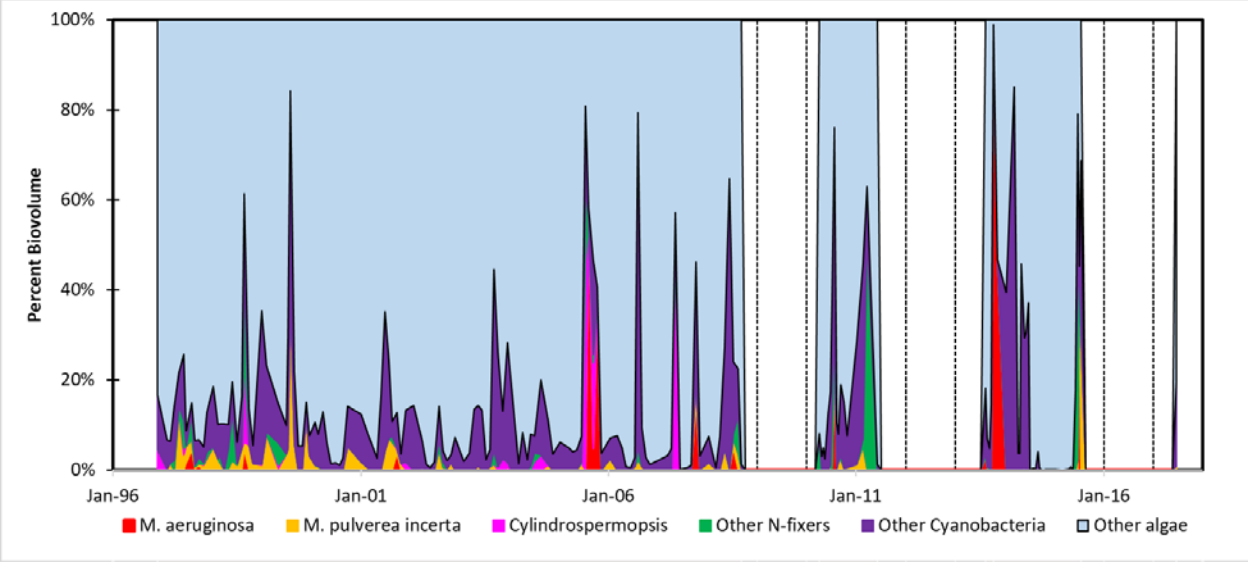


Figure 14. St. Johns River at Piney Point phytoplankton percent biovolumes.

No samples between September 2008 and April 2010, between June 2011 and August 2013, or between July 2015 and June 2017.

ASSESSMENT OF MAJOR BLOOM EVENTS

Lake George and downstream St. Johns River bloom, 1996

This was a multi-species bloom. *M. aeruginosa* was never the dominant taxon in Lake George or in the St. Johns River at Racy Point during this period, but it did have among its highest biomasses for the period of record at these sites. Further downstream at Mandarin Point, *M. aeruginosa* was the dominant taxon during this period.

Lake George

In Lake George, *M. aeruginosa* had high biovolumes for an extended period: 1.5 mm^3/L, 12% of total biovolume on July 9, 1996, 1.4 mm^3/L; 6% of total biovolume on September 12, 1996 (no August sample); and 1.5 mm^3/L, 9% of total biovolume on October 24, 1996 (Figure 15A). There was a lower *M. aeruginosa* biovolume on October 1. July 9 was also a peak for Other Nitrogen-fixers, primarily *Dolichospermum* spp. (4.2 mm^3/L, 33% of total biovolume). *Cylindrospermopsis* + *Raphidiopsis* reached biovolume peaks from September 12 through October 24 of 1.3 – 2.1 mm^3/L, 8 – 12% of total biovolume. Other Cyanobacteria (primarily *Chroococcus* and *Limnothrix*) were dominant from September 12 through October 24, comprising 59 – 76% of total biovolume.

Other measures of algal biomass or suspended solids were high on July 9; chlorophyll-*a* was 80 µg/L, TSS was 25 mg/L, turbidity was 11.8 NTU (all well above average for that site). These measures gradually decreased over the summer but remained above average through early October. These other biomass measures were highest at the July peak of *M. aeruginosa* and *Dolichospermum* spp., not during the September – October peak of total phytoplankton biovolume.

St. Johns River at Astor 30-day average discharges decreased from high levels over 6,000 cfs in spring 1996 to below 2,000 cfs in June. Throughout the bloom period, discharges ranged from about 1,200 to 3,400 cfs (Figure 15A), which brackets the average (3,200 cfs) discharge for this site. Average discharges for the previous winter (November-March) were above normal. Subsequent to the blooms, 30-day average discharges increased to about 4,800 cfs in November.

The July 9 first peak of *M. aeruginosa* and peak of *Dolichospermum* spp. had an increase in TN concentration from May (no June samples) to 2.2 mg/L, well above the long-term average of 1.5 mg/L (Figure 15B). TP concentration also increased to the highest level in 1996 on July 9 (0.1 mg/L), and above the long-term average of 0.07 mg/L (Figure 15C). Concentrations of both TN and TP remained relatively high into September. Inorganic nutrient concentrations were low through the bloom period. Water temperature and DDays were near annual highs during the bloom period. Water color was relatively low during the bloom period (decreasing from 100 cpu on July 9 to 50 cpu in early October). There were no vertical profile data to assess vertical stratification during the bloom period. The calculated 14-day wind function was near average on July 9 but below average later in the summer.

Racy Point

On the St. Johns River at Racy Point, *M. aeruginosa* had high biovolume on July 2, 1996 (2.0 mm^3/L, 23% of total biovolume) (Figure 16A). *M. pulverea incerta* also had relatively high biovolume on July 2 (1.2 mm^3/L, 14% of total biovolume). After decreasing through the rest of the summer, *M. aeruginosa* had another late season biovolume peak on November 21 (1.5 mm^3/L, 19% of total biovolume). The July 2 peak was preceded by and coincident with high biovolume of Other Nitrogen-fixers (primarily *Dolichospermum* spp.), 1.6 mm^3/L (32% of total biovolume) on May 28 and 1.1 mm^3/L (12% of total biovolume) on July 2. Similar to Lake George, there was a later summer increase in Other Cyanobacteria (primarily *Limnothrix*) and *Cylindrospermopsis + Raphidiopsis*, peaking on August 6.

Other measures of algal biomass or suspended solids were high through the bloom period at Racy Point. Chlorophyll-*a* was highest on August 6 (103 μg/L), consistent with highest biovolume on that date, while on July 2 it was 54 μg/L, both well above average for the site. TSS and turbidity were 23 mg/L and 9.1 NTU on August 6, and 16 mg/L and 8.8 NTU on July 2, all well above average for that site.

St. Johns River at Buffalo Bluff 30-day average discharges decreased from high levels over 10,000 cfs in spring 1996 to below 4,000 cfs in June. Through the bloom period discharges ranged from about 4,000 to 7,000 cfs (Figure 16A), which brackets the average (4,800 cfs) discharge for this site. Average discharges for the previous winter (November-March) were above normal. In early November 30-day average discharges increased to about 7,300 cfs but decreased to 5,200 cfs by the late-season *M. aeruginosa* peak on November 21.

TN concentration peaked at 1.9 mg/L on May 28, coincident with the peak biovolume of *Dolichospermum* spp., and a secondary peak of 1.8 mg/L occurred in late-August, soon after the peak biovolume of *Cylindrospermopsis + Raphidiopsis*, both above the long-term average of 1.3 mg/L (Figure 16B). TP concentration was also relatively high from May – August, above the average concentration for the site of 0.08 mg/L, peaking at 0.12 mg/L on August 6 (Figure 16C). Inorganic nitrogen concentrations were low through the bloom period, but PO_4-D did have increases in July and October. Water temperature and DDays were near annual highs during July – August; they had decreased by November 21, but still water temperature was 19.8ºC on that date. Water color was generally below 100 cpu from May – September, other than one measurement of 200 cpu on July 16. There were no vertical profile data to assess vertical stratification during the boom period. The calculated 14-day wind function was below average on the May, July, and August sample dates, but near average on November 21.

Mandarin Point

At Mandarin Point the period of high cyanobacterial biovolume was shorter than the upstream sites and was dominated by *M. aeruginosa*. *M. aeruginosa* had high biovolume on July 2, 1996 (1.8 mm^3/L, 35% of total biovolume) and August 6, 1996 (1.3 mm^3/L, 52% of total biovolume) (Figure 17A). On July 2, peaks were reached by both Other Nitrogen-fixers (0.66 mm^3/L, 13% of total biovolume) and *Cylindrospermopsis +*

Raphidiopsis (0.17 mm^3/L, 3% of total biovolume), but were much less prominent than upstream.

Other measures of algal biomass or suspended solids were high through the bloom period at Mandarin Point. Chlorophyll-*a* was 44 µg/L on July 2 and 25 µg/L on August 6, both well above average for the site. TSS and turbidity were 10.5 mg/L and 6.4 NTU on July 2, and 11 mg/L and 6.9 NTU on August 6, all above average for that site.

St. Johns River at Buffalo Bluff 30-day average discharges decreased from high levels over 10,000 cfs in spring 1996 to below 4,000 cfs in June. Through the bloom period discharges ranged from about 4,000 to 7,000 cfs (Figure 17A), which brackets the average (4,800 cfs) discharge for this site. Average discharges for the previous winter (November-March) were above normal.

TN concentration increased to 1.5 mg/L on July 2, coincident with the peak biovolumes of *Dolichospermum* spp. and *Cylindrospermopsis* + *Raphidiopsis*, above the long-term average of 1.2 mg/L, but decreased to 0.85 mg/L on August 6 (Figure 17B). TP concentration was 0.07 mg/L on July 2 and 0.1 mg/L on August 6; below and close to the average concentration for the site of 0.1 mg/L (Figure 17C). Inorganic nitrogen concentrations were low through the bloom period, but PO_4-D did increase on August 6. Water temperature and DDays were near annual highs during July – August. Water color was 100 cpu in July and 70 cpu in August. There were no vertical profile data to assess vertical stratification during the boom period. The calculated 14-day wind function was below average on the July and August sample dates.

The 1996 blooms

The 1996 blooms appear to have a similar timing, at least as far as can be determined from the rather infrequent sampling. There is no evidence that the blooms started first in upstream Lake George and appeared later at the downstream sites. At all three sites the *M. aeruginosa* blooms first became evident in early July. At Racy Point a *Dolichospermum* spp. bloom began in late May. We don't know if this also occurred in upstream Lake George because no sample was collected there at that time, although it seems likely as *Dolichospermum* spp. was blooming at both sites in early July. TN peaks in Lake George in July and at Racy Point in late May coincided with *Dolichospermum* spp. blooms, suggesting that nitrogen fixation contributed to that increase. *Cylindrospermopsis* + *Raphidiopsis* and Other Nitrogen-fixers were less prominent at Mandarin Point, although present in early July. Increased TN at Mandarin Point at that time may have been primarily transported from upstream. Nutrient concentrations at Racy Point and Mandarin Point could be influenced by runoff from the Tri-County Agricultural area (TCAA), which borders Crescent Lake and the St. Johns River downstream to near Green Cove Springs (Figure 2). The increased TN concentrations and generally high TP concentrations at all three sites likely promoted the blooms of *M. aeruginosa* and subsequently of Other Cyanobacteria.

Other conditions that favored bloom development were relatively low discharges and water color. For Lake George, a discharge range of 1,200 to 3,400 cfs would result in estimated water replacement times for an average lake volume of 220 days to 76 days.

The period of high cyanobacterial biovolume was most prolonged in Lake George. The river sites have greater depths, which may allow deeper mixing that limits cyanobacterial growth – mean site depths were 2.9 m at Lake George, 7.5 m at Racy Point, and 9.2 m at Mandarin Point. Phlips et al. (2000) estimated light availability partially as a function of water depth for stretches of the St. Johns River. They concluded that light availability in the stretch including Mandarin Point was below a threshold for light limitation during summer. They concluded light availability was higher in Lake George and the stretch of the river including Racy Point, but light was somewhat lower at Racy Point than in Lake George, and it is downstream of a river stretch with considerably lower light availability. Those estimates of light availability assume complete water column mixing. The abundance of surface-blooming taxa *M. aeruginosa* and *Dolichospermum* suggest there may have been stratification during the bloom period.

One obvious difference among sites was the lower biovolumes of cyanobacteria other than *M. aeruginosa* at Mandarin Point. One possible explanation may be intolerance of the other cyanobacteria to higher conductivity further downstream. However, measured conductivities were not higher during this period at Mandarin Point than at the upstream sites (generally below 1,000 μmhos/cm). It is possible that conductivities were higher lower in the water column. There is incomplete information on depths of site and collection during this period for Mandarin Point, but for dates that those were reported, the water samples were taken over the top 2.5 – 3 m, while the site depth was 9 – 10 m.

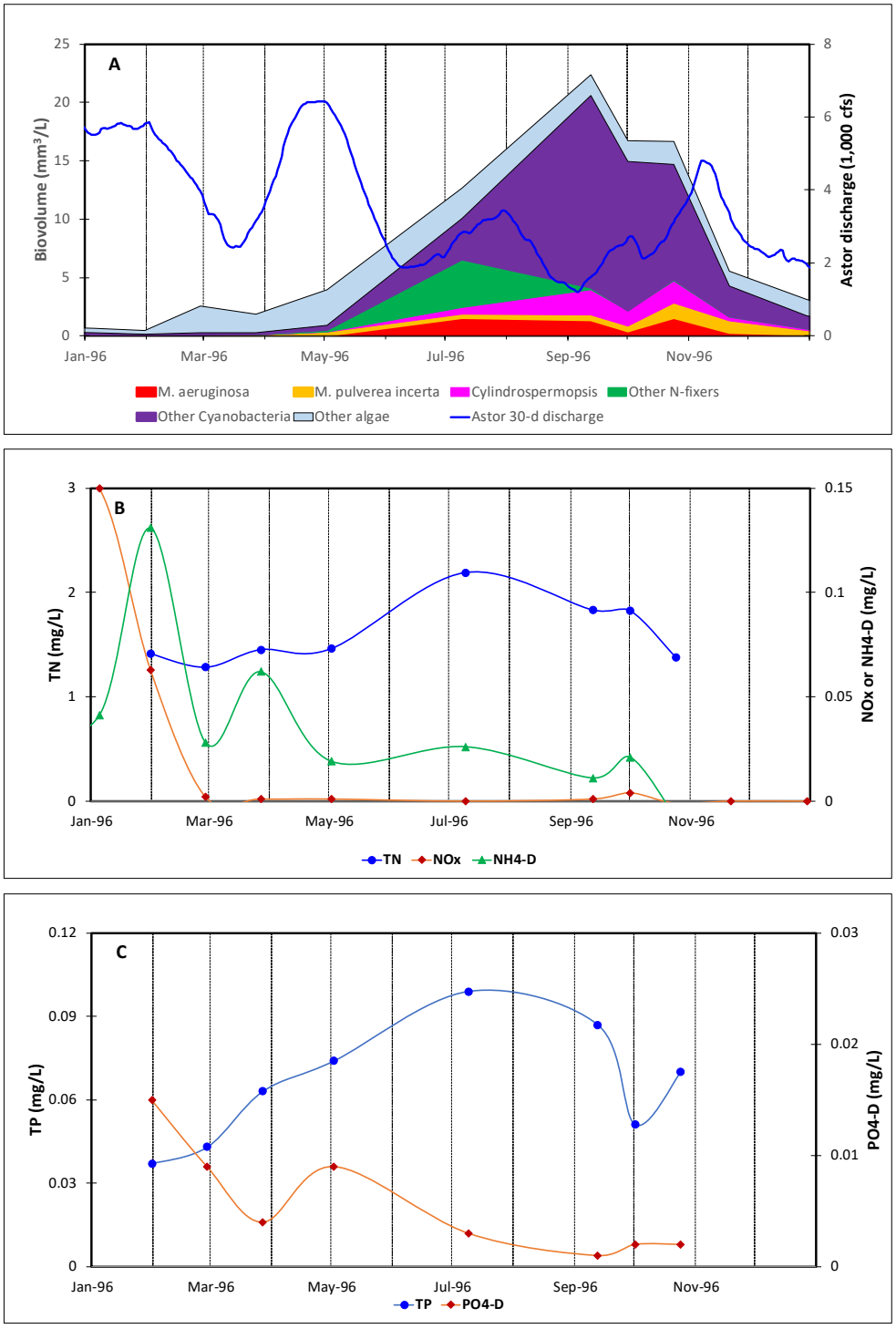


Figure 15. Lake George 1996 bloom event.

A. Lake George 1996 phytoplankton biovolumes and St. Johns River discharges at Astor.
B. Lake George 1996 nitrogen concentrations.
C. Lake George 1996 phosphorus concentrations.

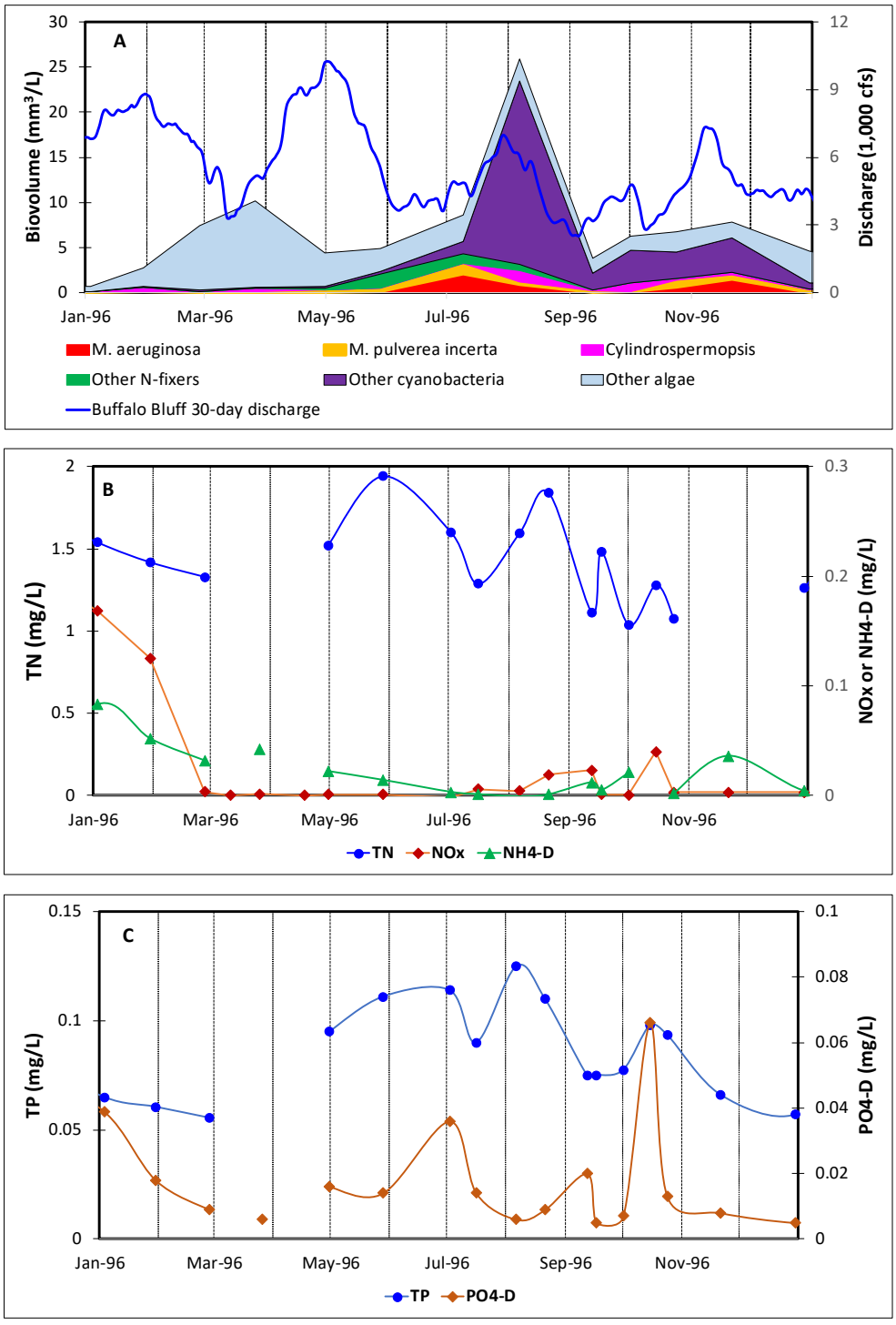


Figure 16. St. Johns River at Racy Point 1996 bloom event.

A. St. Johns River at Racy Point 1996 phytoplankton biovolumes and St. Johns River discharges at Buffalo Bluff.
B. St. Johns River at Racy Point 1996 nitrogen concentrations.
C. St. Johns River at Racy Point 1996 phosphorus concentrations.

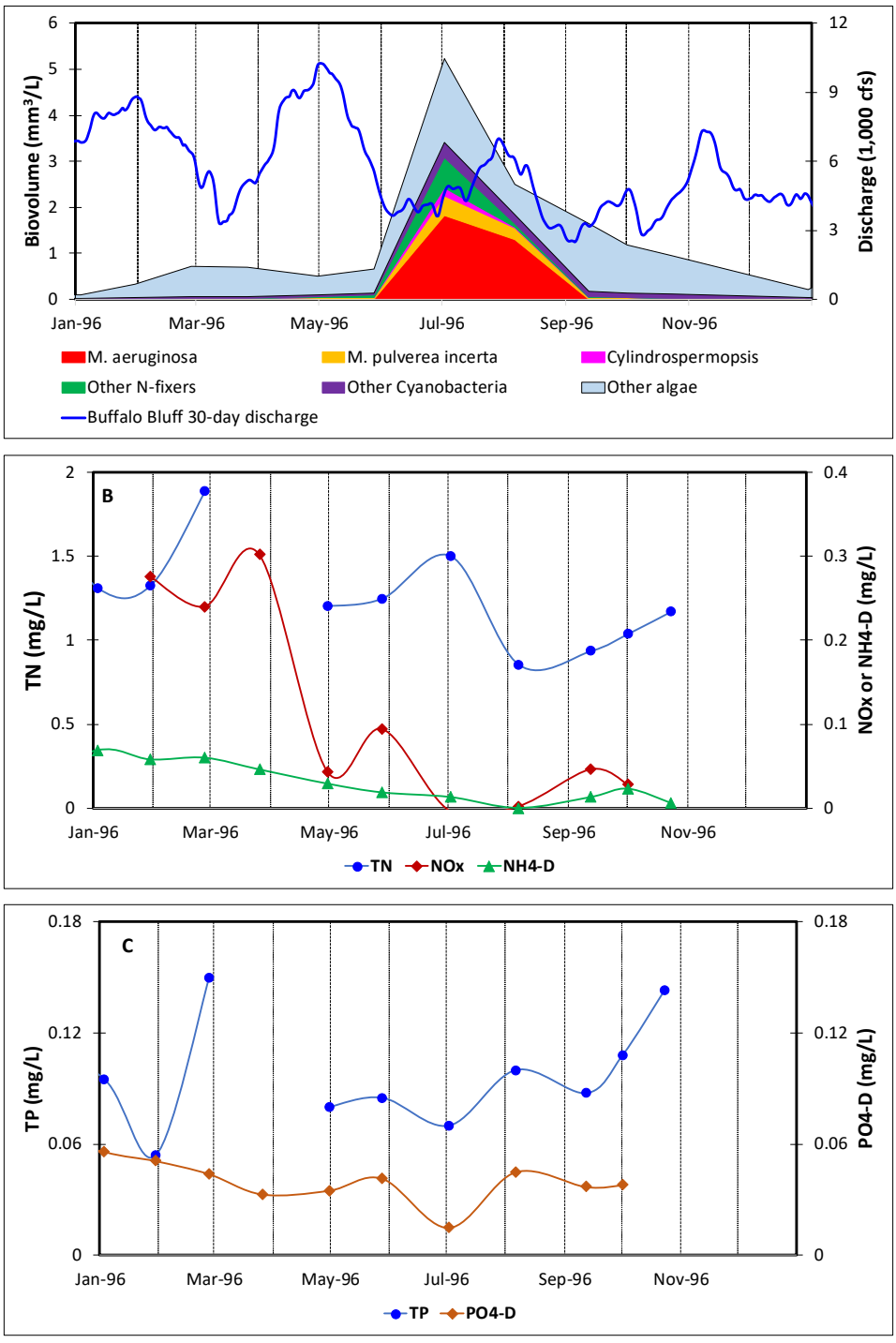


Figure 17. St. Johns River at Mandarin Point 1996 bloom event.

A. St. Johns River at Mandarin Point 1996 phytoplankton biovolumes and St. Johns River discharges at Buffalo Bluff.
B. St. Johns River at Mandarin Point 1996 nitrogen concentrations.
C. St. Johns River at Mandarin Point 1996 phosphorus concentrations.

Lake George *Microcystis aeruginosa* bloom, 2016

The highest biovolumes of *M. aeruginosa* in the period of record for Lake George were on September 7, 2016 (12.8 mm^3/L, 48% of total biovolume), and October 17, 2016 (10.1 mm^3/L, 70% of total biovolume) (Figure 18A). Between those peaks there was a low *M. aeruginosa* biovolume on September 27, 2016. Other measures of algal biomass or suspended solids were generally high on September 7; chlorophyll-*a* was 154 μg/L (although J-coded, indicating an estimated value with some quality control problems), TSS was 30.1 mg/L, turbidity was 10.0 NTU (all well above average for that site). However, on October 17, all these other measures of algal biomass or suspended solids were below average.

St. Johns River at Astor 30-day average discharges were around 2,000 cfs (Figure 18A), below the average (3,200 cfs) and median (2,450 cfs) discharges for this site. Average discharges for the previous winter (November-March) were also below normal. Subsequent to the blooms, 30-day average discharges increased to about 6,000 cfs in November. The Lake George *M. aeruginosa* blooms on September 7 and October 17, 2016 bracketed Hurricane Matthew. Available data indicate the primary effects of the hurricane were October 6-7. Radar rainfall for the Lake George basin totaled 4.4 inches on those days. Mean daily winds peaked on October 7 at 12.3 m/s at Daytona, 11.8 m/s at Jacksonville, and 11.8 m/s at Hastings. Daily discharges at Astor were negative (water flow going upstream) from October 5-October 8, peaking at –8,900 cfs on October 7. After that daily discharges gradually increased to +6,460 cfs on October 17.

TN concentration increased to the highest concentration in 2016 on September 7 (2.8 mg/L), and well above the long-term average of 1.5 mg/L (Figure 18B). By October 17 TN concentration had decreased to 1.3 mg/L. Inorganic nitrogen concentrations were low on September 7 but increased in late September and October; although some of that increase occurred before Hurricane Matthew, the NO_x-D peak on October 17 could reflect storm-induced watershed runoff. TP concentration also increased to the highest concentration in 2016 on September 7 (0.11 mg/L), and above the long-term average of 0.07 mg/L (Figure 18C). By October 17, TP concentration had decreased to 0.05 mg/L. PO_4-D concentrations were low during the bloom period. Water temperature and DDays were gradually decreasing from mid-summer highs during the bloom period. Water color was relatively low during the bloom period (below 60 cpu). There was little indication of vertical stratification on the bloom dates of water temperature (TempRange2m <0.1°C) or conductivity (CondRange2m < 2 μmhos/cm). The calculated 14-day wind function was above average on the bloom dates, suggesting those conditions were not conducive to vertical stratification.

Overall, it appears that the September 7, 2016 Lake George *M. aeruginosa* bloom was promoted by high TN and TP concentrations. Other conditions that favored bloom development were relatively low discharges and water color. For a discharge of 2,000 cfs, estimated water replacement time for an average lake volume would be about 130 days. The low discharges prior to Hurricane Matthew suggest that nutrient loading from upstream was relatively low during this period, so the nutrient source may have been internal, likely regeneration from the bottom sediments. Biovolumes of

Cylindrospermopsis + *Raphidiopsis* and Other Nitrogen-fixers were relatively low during this period (Figure 18A), suggesting that nitrogen fixation was not a major factor in the increase in TN concentrations. The apparent rebound in *M. aeruginosa* biovolume on October 17 is more puzzling. The short-term wind speeds and longer-term discharges related to Hurricane Matthew might be expected to be deleterious to *M. aeruginosa* growth. Possibly the storm-induced flows and turbulence injected a nutrient pulse, although that is not apparent from the available nutrient data, other than the increase in NO_x on October 17. Also, the inconsistency of the October 17 biovolume measurement with other measures of algal biomass or suspended solids raises questions about the accuracy of that biovolume measurement. The increased discharges in late October-November, as well as decreasing water temperatures, likely terminated the *M. aeruginosa* bloom.

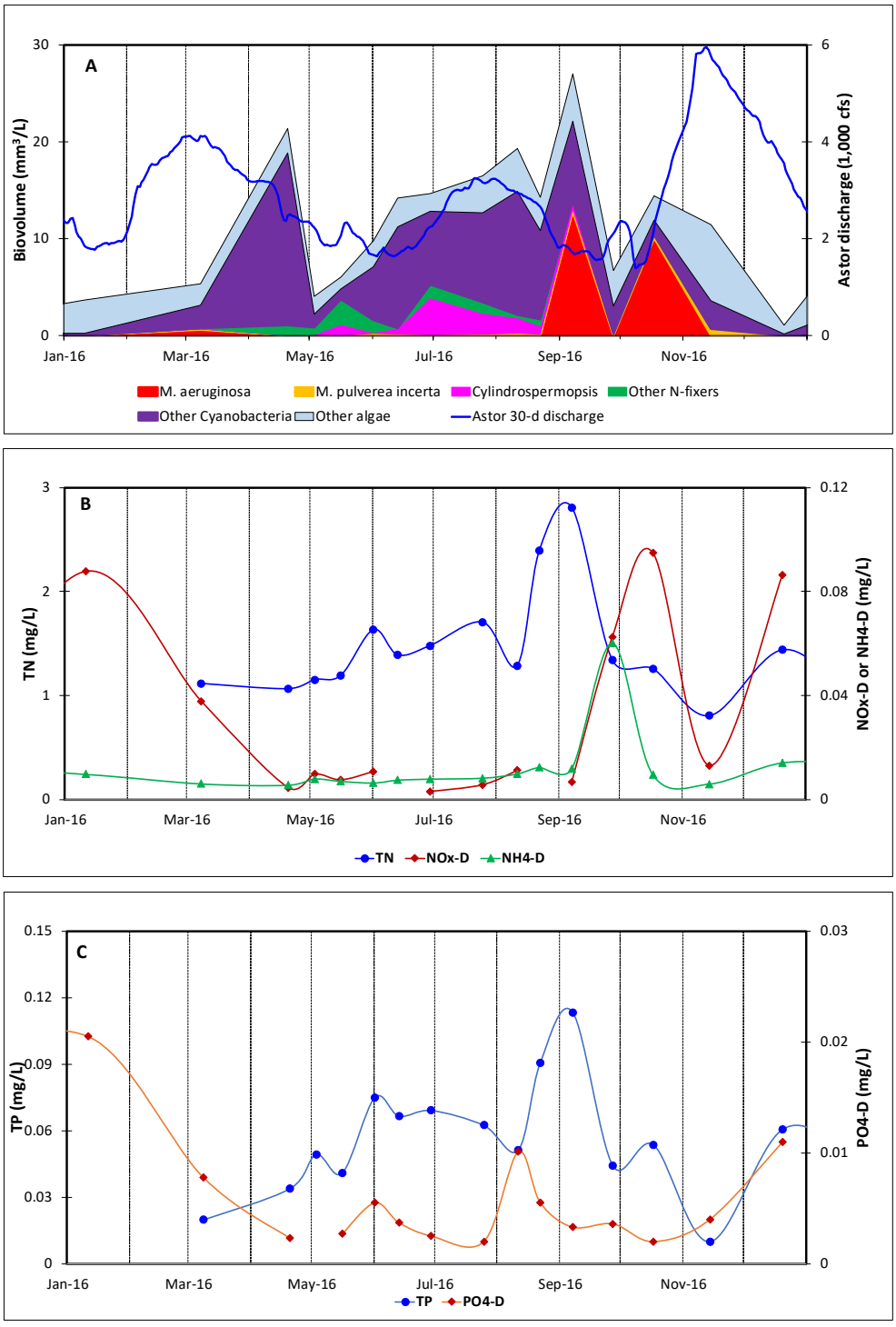


Figure 18. Lake George 2016 bloom event.

A. Lake George 2016 phytoplankton biovolumes and St. Johns River discharges at Astor.
B. Lake George 2016 nitrogen concentrations.
C. Lake George 2016 phosphorus concentrations.

Crescent Lake *Microcystis aeruginosa* bloom, 2017

The highest biovolume of *M. aeruginosa* in the period of record for Crescent Lake was on August 16, 2017 (16.9 mm^3/L, 42% of total biovolume) (Figure 19A). Biovolume of Other Cyanobacteria also reached a peak on that date (primarily *Pseudanabaena*, *Lyngbya* and *Limnothrix*), but biovolumes of nitrogen-fixers were low throughout 2017. Other measures of algal biomass or suspended solids were generally above average for that site on August 16. Chlorophyll-*a* was well above average at 35.1 µg/L, TSS (9.6 mg/L), and turbidity (6.8 NTU) were slightly above average. However, these other measures had similar concentrations in samples from May – July 2017, although phytoplankton biovolume measurements were much lower.

Dunns Creek (outflow from Crescent Lake) 30-day discharges were around 600 cfs (Figure 19A), higher than the average (441 cfs) and median (267 cfs) discharges for this site. Average discharges for the previous winter (November-March) were below normal. Subsequent to the bloom, discharges increased to over 4,000 cfs in October. TN concentration was relatively low on August 16 (1.2 mg/L). TN concentration was somewhat higher in July, but still not much above the long-term average of 1.4 mg/L (Figure 19B). Inorganic nitrogen concentrations were low on and before August 16. TP concentration also was relatively low on August 16 (0.067 mg/L), below a long-term average of 0.088 mg/L (Figure 19C). PO_4-D concentrations were also low during the bloom period. Water temperature and DDays were near mid-summer highs during the bloom period. Water color was relatively low before and during the bloom period (below 125 cpu vs. an average of 279 cpu). There was little indication of vertical stratification on the bloom date of water temperature (TempRange1.7m 0.1^oC) or conductivity (CondRange1.7m 1 µmhos/cm). The calculated 14-day wind function was slightly below average on the bloom date, suggesting those conditions were not conducive to vertical stratification.

Overall, it is not clear why such a strong *M. aeruginosa* bloom occurred in Crescent Lake in August 2017. Nutrient concentrations were relatively low, although evidently high enough to support growth. Factors that were conducive to cyanobacterial growth were high temperature, relatively low water color, and long water replacement time, although these factors were also favorable earlier in the summer. Although the discharge from the lake was higher than average, for a discharge of 600 cfs estimated water replacement time for an average lake volume would be about 110 days. The fact that other measures of algal biomass or suspended solids on August 16 were not substantially higher than in previous months raises questions about the accuracy of that biovolume measurement. High flows and water instability associated with Hurricane Irma in early September likely terminated the *M. aeruginosa* bloom.

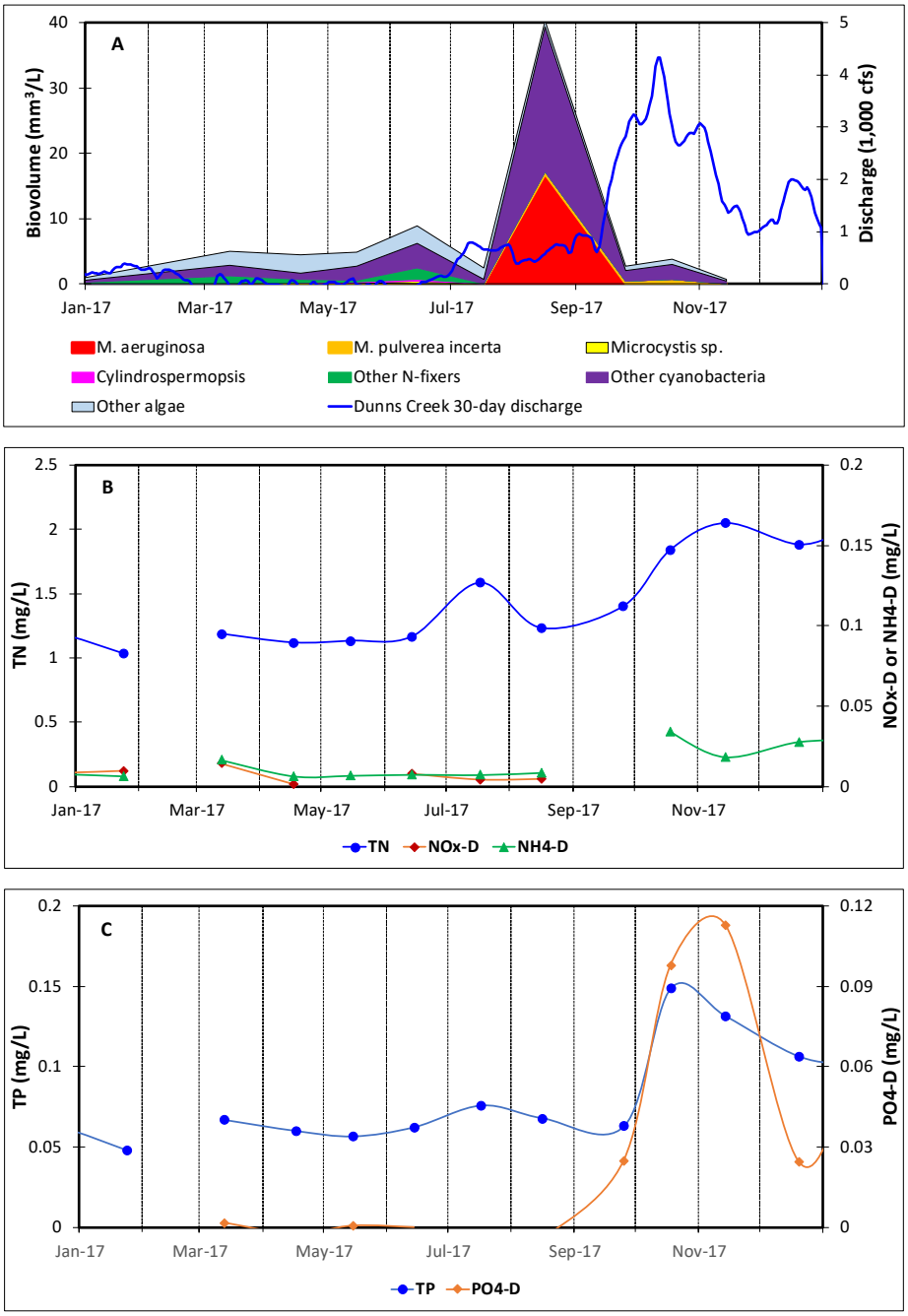


Figure 19. Crescent Lake 2017 bloom event.

A. Crescent Lake 2017 phytoplankton biovolumes and Dunns Creek discharges.
B. Crescent Lake 2017 nitrogen concentrations.
C. Crescent Lake 2017 phosphorus concentrations.

Doctors Lake *Microcystis aeruginosa* bloom, 2007

The second highest biovolume of *M. aeruginosa* in the period of record for Doctors Lake was on September 4, 2007 (7.9 mm^3/L, 45% of total biovolume) (Figure 20A). Other measures of algal biomass on September 4 were generally well above averages for the site and well above concentrations earlier in summer 2007, including chlorophyll-*a* (58.9 µg/l), TSS (18.4 mg/L), and turbidity (14.2 NTU).

Thirty-day discharges at nearby Black Creek were about 51 cfs (Figure 20A), well below the average (304 cfs) and median (182 cfs) discharges for this site. Average discharges for the previous winter (November-March) were also below normal. Subsequent to the blooms, 30-day discharges increased to about 500 cfs in October. TN concentration increased to the highest concentration in 2016 on September 4 (1.8 mg/L), and above the long-term average of 1.4 mg/L (Figure 20B). NO_x-D concentrations also increased somewhat on September 4, but were below the long-term average of 0.068 mg/L. TP concentrations were at a high concentration of about 0.2 mg/L from July-September, well above the long-term average of 0.1 mg/L (Figure 20C). PO_4-D concentrations were also relatively high during this period, well above the long-term average of 0.024 mg/L. Water temperature and DDays were near mid-summer highs during the bloom period. Water color was relatively low during the bloom period (50 cpu). Conductivity was relatively high (10,224 µmhos/cm), but this was a decrease from values twice as high earlier in the summer. There was little indication of vertical stratification on the bloom date of water temperature (TempRange1.5m 0.11°C) but some stratification in conductivity (CondRange1.5m 159 µmhos/cm). The calculated 14-day wind function is close to average on the bloom date, suggesting those conditions were not conducive to vertical stratification.

Overall, it appears that the 2007 Doctors Lake *M. aeruginosa* bloom was promoted by high nutrient concentrations. Other conditions that favored bloom development were a decrease in conductivity, and low discharges and water color. The two highest *M. aeruginosa* biovolumes in the period of record for Doctors Lake were both at conductivities of about 10,000 µmhos/cm, but there were no significant biovolumes at higher conductivities, so that may be close to the conductivity tolerance limit. Black Creek discharges cannot be used to estimate water residence time in Doctors Lake, but we do have simulated watershed inflows to Doctors Lake for that period from an HSPF watershed runoff model. For average simulated watershed inflows for the 30-day period prior to September 4, 2007 a water replacement time would be about 2,300 days (this is probably an overestimate since it does not consider tidal flushing of the lake, but we believe the water replacement time was very long during this period). The low discharges and simulated inflows suggest that nutrient loading from the watershed was relatively low during this period, so the nutrient source may have been internal or from downstream tidal inflows. Biovolumes of all nitrogen-fixers were low during this period (Figure 20A), suggesting that nitrogen fixation was not a major factor in the increase in nitrogen concentrations.

Increased discharge may have been a major factor in the disappearance of the *M. aeruginosa* bloom by the next sample on October 8. TP and TN measurements were

missing on that date, but subsequent measurements indicate only a modest decrease. Conductivity decreased moderately and water color increased moderately by October. Both Black Creek discharges and simulated inflows to Doctors Lake increased substantially in early October. Estimated water replacement time based on the average simulated watershed inflow for the first 8 days in October would be only 38 days.

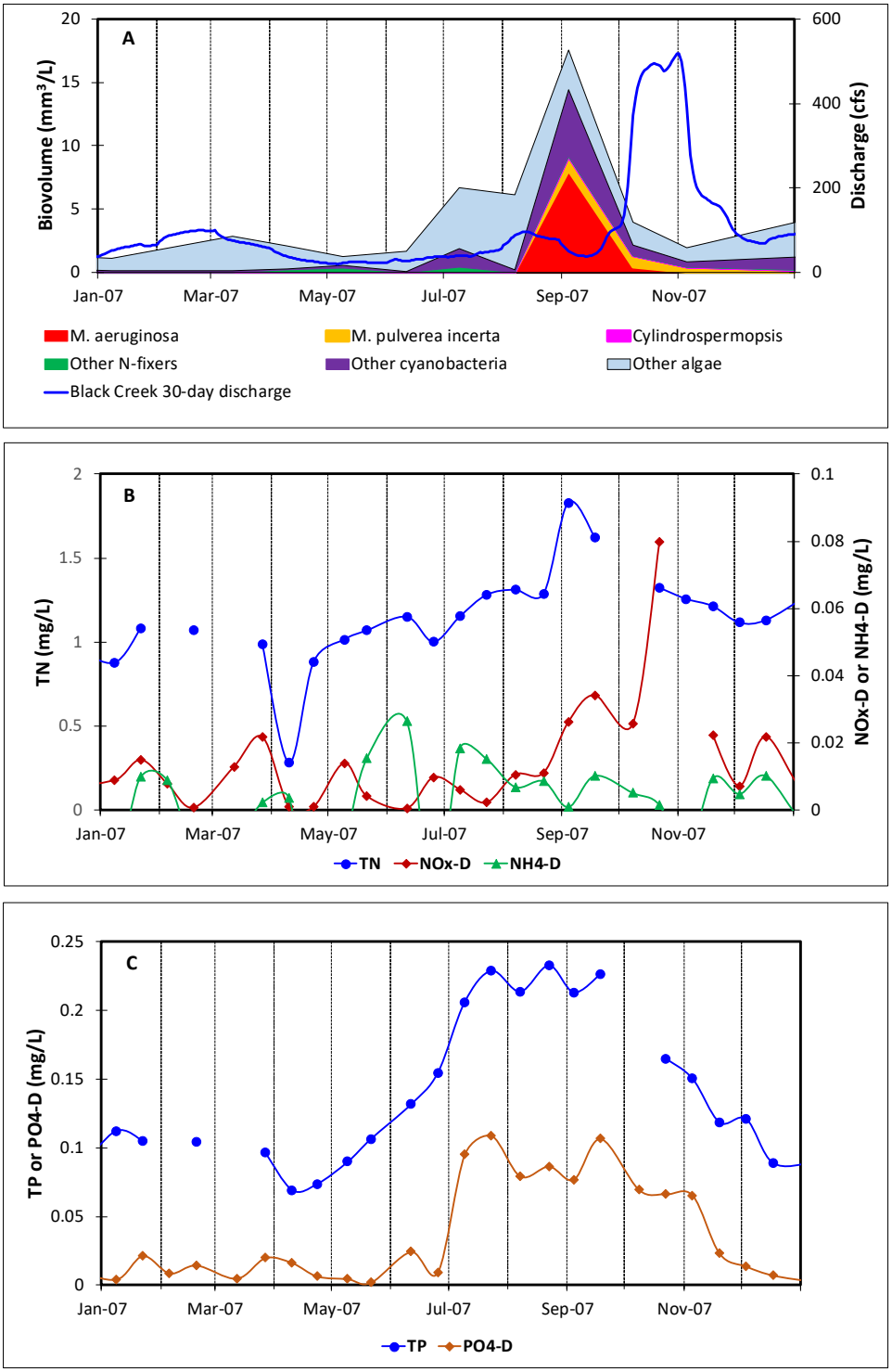


Figure 20. Doctors Lake 2007 bloom event.

A. Doctors Lake 2007 phytoplankton biovolumes and Black Creek discharges.
B. Doctors Lake 2007 nitrogen concentrations.
C. Doctors Lake 2007 phosphorus concentrations.

Doctors Lake *Microcystis aeruginosa* bloom, 2008

The third highest biovolume of *M. aeruginosa* in the period of record for Doctors Lake was on June 9, 2008 (3.2 mm^3/L, 24% of total biovolume) (Figure 21A). There was also a peak of Other Cyanobacteria on that date, dominated by *Limnothrix*. Relatively high biovolume of *M. aeruginosa* continued on the next sample date July 8, 2008 (1.8 mm^3/L, 40% of total biovolume). The *M. aeruginosa* peak was preceded by a relatively high biovolume of Other Nitrogen-fixers (primarily *Dolichospermum* spp.) on May 5, 2008 (3.8 mm^3/L, 80% of total biovolume). Other measures of algal biomass on June 9 were generally well above averages for the site, including chlorophyll-*a* (47.9 µg/l), TSS (28.4 mg/L), and turbidity (9.4 NTU). Chlorophyll-*a* concentrations were above average from May – August.

Thirty-day discharges at nearby Black Creek in early June were about 30 cfs (Figure 21A), well below the average (304 cfs) and median (182 cfs) discharges for this site. Average discharges for the previous winter (November-March) were near normal. Subsequent to the blooms, 30-day discharges increased to over 1,500 cfs in late August. TN concentration increased in May to about 1.4 mg/L (near average for the lake), coincident with the increase in Other Nitrogen-fixers, and remained around 1.5 mg/L through the bloom period (Figure 21B). NO_x-D and NH_4-D concentrations were low through this period. TP concentrations were above 0.15 mg/L from during June-July, well above the long-term average of 0.1 mg/L (Figure 21C). PO_4-D concentrations increased to 0.05 mg/L in late-June, well above the long-term average of 0.024 mg/L, although this was after the *M. aeruginosa* peak biovolume. Water temperature and DDays were near mid-summer highs during the bloom period. Water color was relatively low during the bloom period (50 – 60 cpu). Conductivity was around 9,000 µmhos/cm, relatively high but lower than the level observed during the 2007 bloom. There was little indication of vertical stratification of water temperature through the bloom period (TempRange1.5m <0.9°C) but some stratification in conductivity, particularly on May 5 (CondRange1.5m 1,108 µmhos/cm, decreasing to 189 µmhos/cm on June 9). The calculated 14-day wind function was close to average on the May 5, then gradually decreased through mid-July, suggesting wind conditions were growing more conducive to vertical stratification.

Overall, it appears that the 2008 Doctors Lake *M. aeruginosa* bloom was promoted by high TP and moderately high TN concentrations. Other conditions that favored bloom development were tolerable conductivity, and low discharges and water color. Black Creek discharges cannot be used to estimate water residence time in Doctors Lake, but we do have simulated watershed inflows to Doctors Lake for that period. For average simulated watershed inflows for the 30-day period prior to June 9, 2008, a water replacement time would be about 1,300 days (this estimate does not consider tidal flushing of the lake). The low discharges and simulated inflows suggest that nutrient loading from the watershed was relatively low during this period, so the nutrient source may have been internal or from downstream tidal inflows. Vertical stratification may have contributed to the Other Nitrogen-fixer increase in early May and nitrogen fixation may have contributed to the increase in TN at that time.

Although *M. aeruginosa* gradually decreased from its June 9 peak, cyanobacteria remained abundant through early August. Increased discharges in late August probably ended the cyanobacterial bloom for that year.

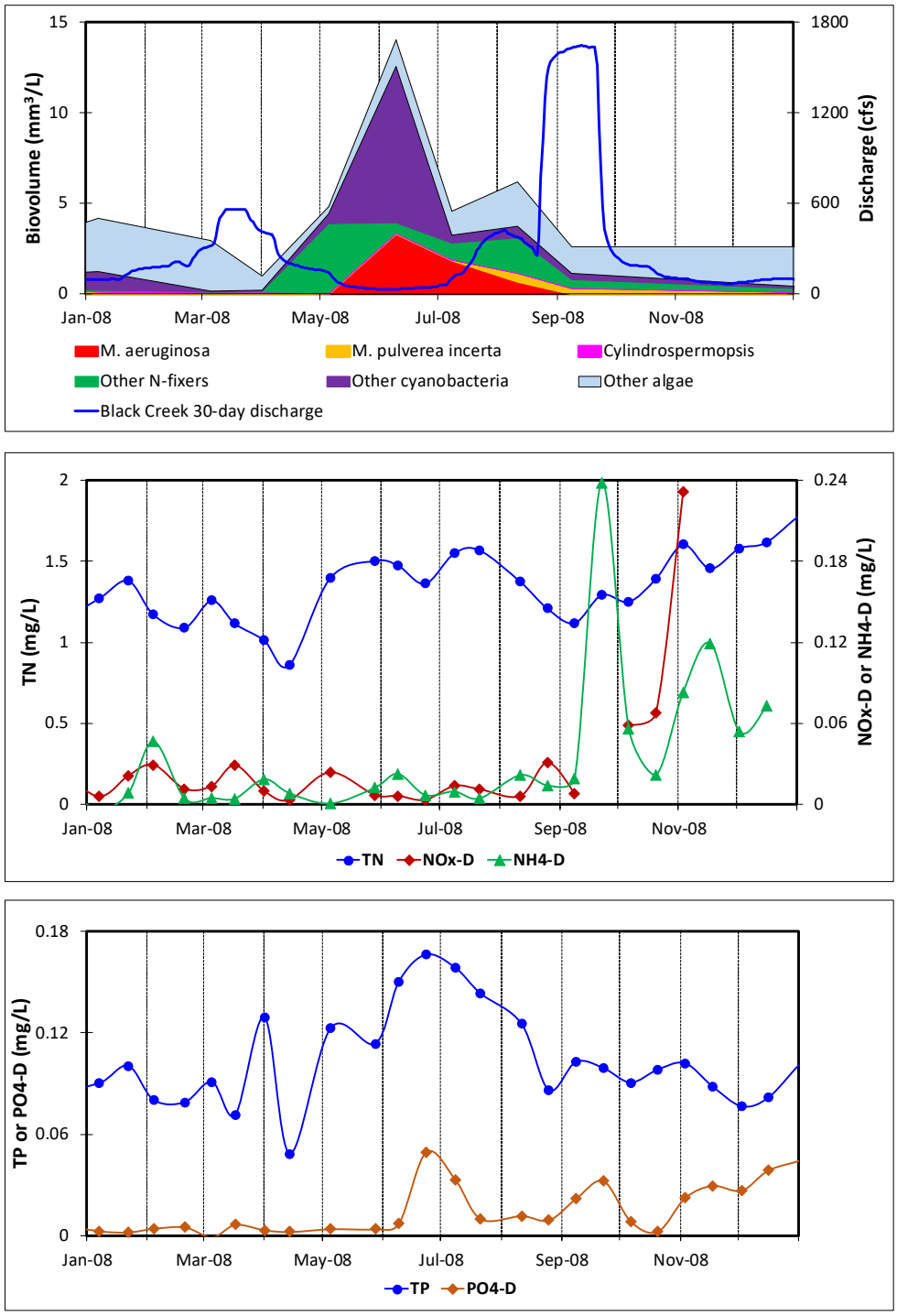


Figure 21. Doctors Lake 2008 bloom event.

A. Doctors Lake 2008 phytoplankton biovolumes and Black Creek discharges.
B. Doctors Lake 2008 nitrogen concentrations.
C. Doctors Lake 2008 phosphorus concentrations.

Doctors Lake *Microcystis aeruginosa* bloom, 2017

The highest biovolume of *M. aeruginosa* in the period of record for Doctors Lake was on June 19, 2017 (15.1 mm^3/L, 90% of total biovolume) (Figure 22A). Other measures of algal biomass and suspended solids are somewhat inconsistent with this biovolume peak. Chlorophyll-*a* (39.5 µg/L) was above average for the site but substantially lower than the measurement in May 2017. TSS (13.2 mg/L) was slightly higher than the average and the measurement in the previous month. Turbidity (12.9 NTU) was well above average, and slightly higher than the previous month.

Thirty-day discharges at nearby Black Creek were about 327 cfs (Figure 22A), above the average (304 cfs) and median (182 cfs) discharges for this site. Average discharges for the previous winter (November-March) were below normal. TN concentration increased in the previous month and remained high on June 19 (1.6 mg/L), above the long-term average of 1.4 mg/L (Figure 22B). NH_4-D concentration increased on June 19, although it was still below the long-term average. TP concentration was 0.11 mg/L on June 19, slightly above the long-term average of 0.1 mg/L (Figure 22C). NO_x-D and PO_4-D concentrations were low on June 19. Water temperature and DDays were increasing during the bloom period. Water color was relatively low during the bloom period (57 cpu). Conductivity was relatively high (10,037 µmhos/cm), similar to the level during the 2007 *M. aeruginosa* bloom. There was little indication of vertical stratification on the bloom date of water temperature (TempRange2m 0.1°C) or conductivity (CondRange2m 12 µmhos/cm). The calculated 14-day wind function is close to average on the bloom date, suggesting those conditions were not conducive to vertical stratification.

As with several other apparent major blooms after the change in phytoplankton analysts, inconsistency with other measures of algal biomass or suspended solids raises questions about the magnitude of the reported bloom biovolume. Overall, it appears that the 2017 Doctors Lake *M. aeruginosa* bloom was promoted by high TN and TP concentrations. Other conditions that favored bloom development were low water color, a tolerable conductivity, and long water residence time. The two highest *M. aeruginosa* biovolumes in the period of record for Doctors Lake were both at conductivities of about 10,000 µmhos/cm, but there were no significant biovolumes at higher conductivities, so that may be close to the conductivity tolerance limit. Although Black Creek discharges were above normal, for average simulated watershed inflows for the 30-day period prior to June 19, 2017 a water replacement time would be about 480 days (this estimate does not consider tidal flushing of the lake). The relatively high Black Creek discharges suggest that there may have been significant nutrient loading from the watershed during this period. Also, biovolume of Other Nitrogen-fixers (*Dolichospermum* spp.) was relatively high in May, 5.5 mm^3/L, 40% of total biovolume (Figure 22A), suggesting that nitrogen fixation may have been a factor in the increase in TN concentrations.

M. aeruginosa biovolume decreased later in the summer but remained at a relatively high concentration in the July and August samples (over 1.7 mm^3/L). The decreased biovolume may have been due to decreased TN and TP concentrations, since other environmental factors remained favorable (long water replacement times, low water color, decreasing conductivity, increasing temperature). The disappearance of *M.*

aeruginosa in September was probably related to high discharges and water instability related to Hurricane Irma. Both Black Creek discharges and simulated inflows to Doctors Lake increased substantially at that time. Estimated water replacement time based on the average simulated watershed inflow for the 3 days in September around the hurricane was only 20 days, indicating a substantial, but short-term flushing event.

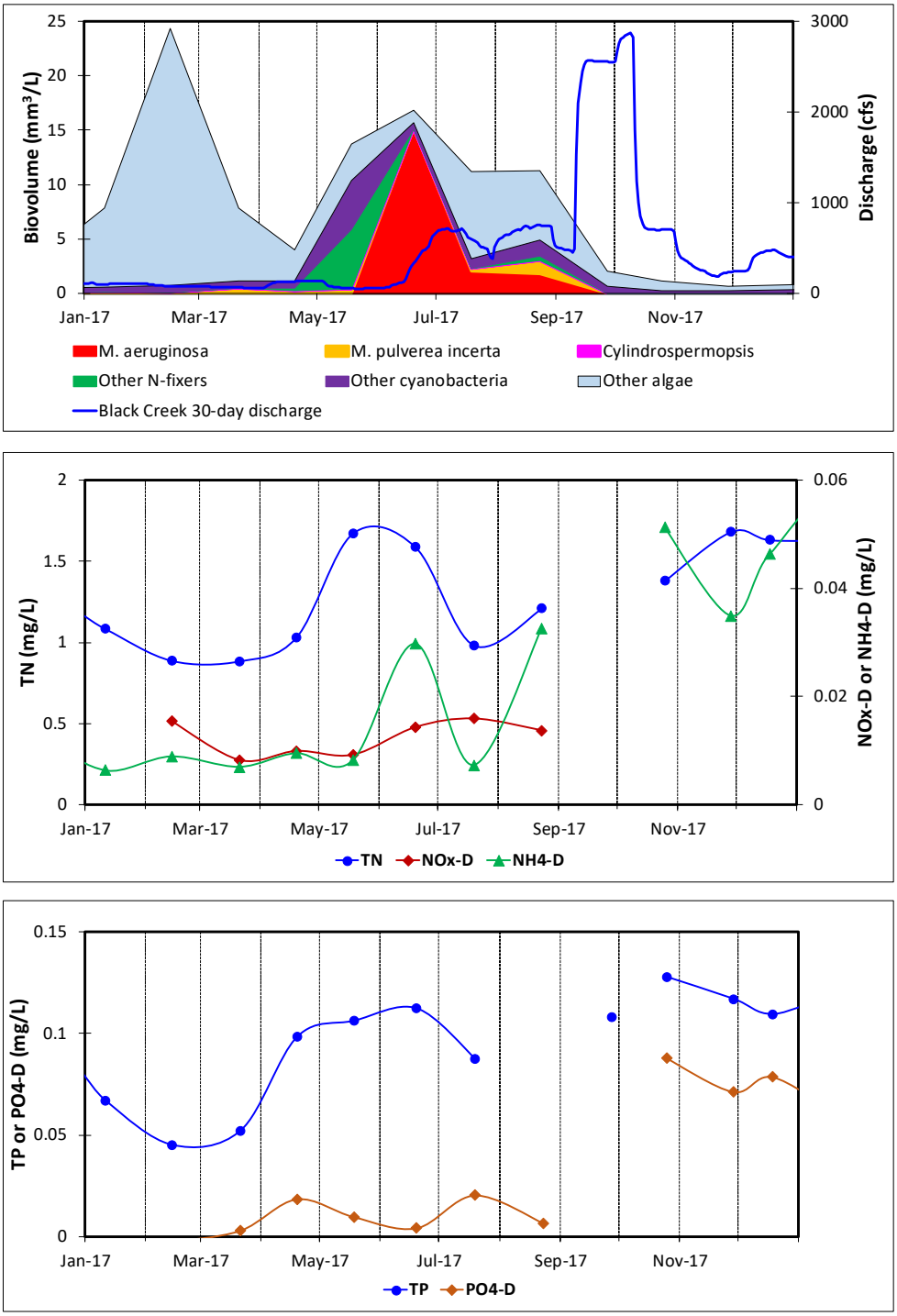


Figure 22. Doctors Lake 2017 bloom event.

A. Doctors Lake 2017 phytoplankton biovolumes and Black Creek discharges.
B. Doctors Lake 2017 nitrogen concentrations.
C. Doctors Lake 2017 phosphorus concentrations.

St. Johns River at Mandarin Point and Piney Point bloom, 1998

This was a brief mixed-species bloom on August 24, 1998, in which *Microcystis aeruginosa* was most prominent at Mandarin Point, but *Cylindrospermopsis* + *Raphidiopsis* and Other Nitrogen-fixers were more prominent at Piney Point.

Mandarin Point

On August 24, 1998, *M. aeruginosa* had a biovolume of 2.3 mm^3/L (28% of total biovolume) (Figure 23A), Other Nitrogen-fixers (primarily *Dolichospermum* spp.) had a biovolume of 1.4 mm^3/L (18% of total biovolume), *Cylindrospermopsis* + *Raphidiopsis* had a biovolume of 1.1 mm^3/L (13% of total biovolume), and Other Cyanobacteria (primarily *Limnothrix*) had a biovolume of 1.6 mm^3/L (20% of total biovolume). None of these taxa were prominent before or after that date at Mandarin Point. There were similar or higher biovolumes of Other Nitrogen-fixers, *Cylindrospermopsis* + *Raphidiopsis*, and Other Cyanobacteria at upstream sites in August, suggesting an upstream source for these taxa. However, biovolumes of *M. aeruginosa* were much lower upstream. Chlorophyll-*a* was also sharply higher on August 24 (62 µg/L), well above average for the site, although TSS and turbidity were near their averages.

Thirty-day discharges in the St. Johns River at Buffalo Bluff were high the previous winter but had decreased to below average by July (Figure 23A). On August 24, discharges were 2,254 cfs, well below the long-term average (4,797 cfs), and median (3,918 cfs). Discharges did not increase to above average until October, well after the end of the bloom.

TN concentration on August 24 was the highest for the year, 1.9 mg/L, above a site average of 1.2 mg/L (Figure 23B). Similarly, TP was also at the high for the year, 0.14 mg/L, above a site average of 0.10 mg/L (Figure 23C). Inorganic nitrogen concentrations were low, although NO_x-D was higher on August 3 (0.129 mg/L, near average for the site). PO_4-D was near average on August 24.

Water temperature and DDays were near seasonal highs on the bloom date. Water color was relatively low (100 cpu). Conductivity was also relatively low (1,602 µmhos/cm), decreased from 4,802 µmhos/cm on August 3. Field measurements were taken at only one depth on the bloom date, so vertical stratification of water temperature or conductivity could not be assessed. The calculated 14-day wind function was below average on the bloom date, suggesting those conditions may have been conducive to vertical stratification.

Piney Point

On August 24, 1998, at Piney Point Other Nitrogen-fixers (primarily *Dolichospermum* spp.) had a biovolume of 1.6 mm^3/L (20% of total biovolume) and *Cylindrospermopsis* + *Raphidiopsis* had a biovolume of 1.5 mm^3/L (18% of total biovolume) (Figure 24A), both similar to those at upstream Mandarin Point. Other Cyanobacteria (primarily *Limnothrix*) had a biovolume of 1.2 mm^3/L (16% of total biovolume), also similar to upstream. However, *M. aeruginosa* biovolume was lower than upstream (0.4 mm^3/L (5% of total biovolume). As at Mandarin Point, none of these taxa were prominent before or after that

date at Piney Point. There were similar or higher biovolumes of Other Nitrogen-fixers, *Cylindrospermopsis* + *Raphidiopsis*, and Other Cyanobacteria at upstream sites in August, suggesting an upstream source for these taxa. However, biovolumes of *M. aeruginosa* were much lower upstream of Mandarin Point. Chlorophyll-*a* was also sharply higher on August 24 (61 µg/L), well above average for the site, although TSS and turbidity were near their averages.

Thirty-day discharges in the St. Johns River at Buffalo Bluff were high the previous winter but had decreased to below average by July (Figure 24A). On August 24, discharges were 2,254 cfs, well below the long-term average (4,797 cfs), and median (3,918 cfs). Discharges did not increase to above average until October, well after the end of the bloom.

TN concentration on August 24 was 1.4 mg/L, slightly above a site average of 1.2 mg/L (Figure 24B). Inorganic nitrogen concentrations were low, although NO_x-D was higher on August 3 (0.23 mg/L, near average for the site). TP was at the high for the year, 0.14 mg/L, above a site average of 0.12 mg/L (Figure 24C). PO_4-D was below average on August 24.

Water temperature and DDays were near seasonal highs on the bloom date. Water color was relatively low (80 cpu). Conductivity was also relatively low (3,207 µmhos/cm), decreased from 16,381 µmhos/cm on August 3. Field measurements were taken at only one depth on the bloom date, so vertical stratification of water temperature or conductivity could not be assessed. The calculated 14-day wind function was below average on the bloom date, suggesting those conditions may have been conducive to vertical stratification.

The 1998 blooms

The August 24, 1998 blooms appear to have been promoted by high TP and TN concentrations in the St. Johns River at Mandarin Point and Piney Point. Due to the relatively low flows during this period, the nutrients may have been internally generated. Nitrogen-fixation may have contributed to the increased TN concentrations. However, runoff from the TCAA could also influence nutrient concentrations at these sites. NO_x-D was higher earlier in the month. *Cylindrospermopsis* + *Raphidiopsis* and Other Nitrogen-fixers were not abundant at these stations in early August, although they were more abundant further upstream.

Other factors that may have contributed to the bloom were high water temperatures, relatively low water color, a substantial decrease in conductivity, and possibly water column stratification.

It is not clear what led to the rapid collapse of the bloom, other than a decrease in TP concentrations. Discharges, TN, water temperature, conductivity, water color, and wind conditions remained relatively stable through September.

This bloom was somewhat different from the more common pattern of *M. aeruginosa* becoming more prominent further downstream and under higher conductivity conditions. In this case *M. aeruginosa* was more prominent upstream at Mandarin Point and decreasing further downstream at Piney Point, while *Cylindrospermopsis* + *Raphidiopsis*, Other Nitrogen-fixers, and Other Cyanobacteria remained prominent at the downstream site.

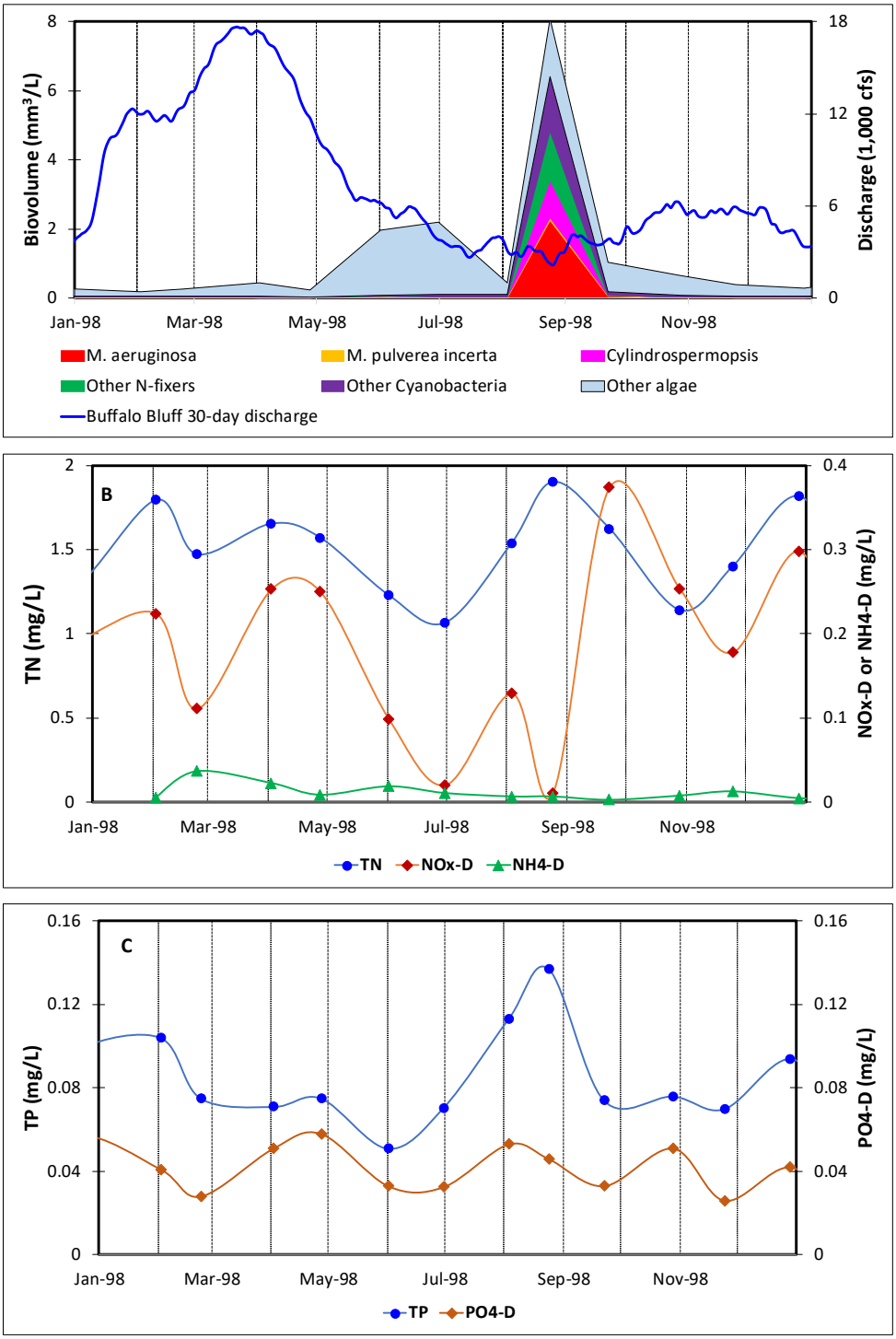


Figure 23. St. Johns River at Mandarin Point 1998 bloom event.

A. St. Johns River at Mandarin Point 1998 phytoplankton biovolumes and St. Johns River at Buffalo Bluff discharges.
B. St. Johns River at Mandarin Point 1998 nitrogen concentrations.
C. St. Johns River at Mandarin Point 1998 phosphorus concentrations.

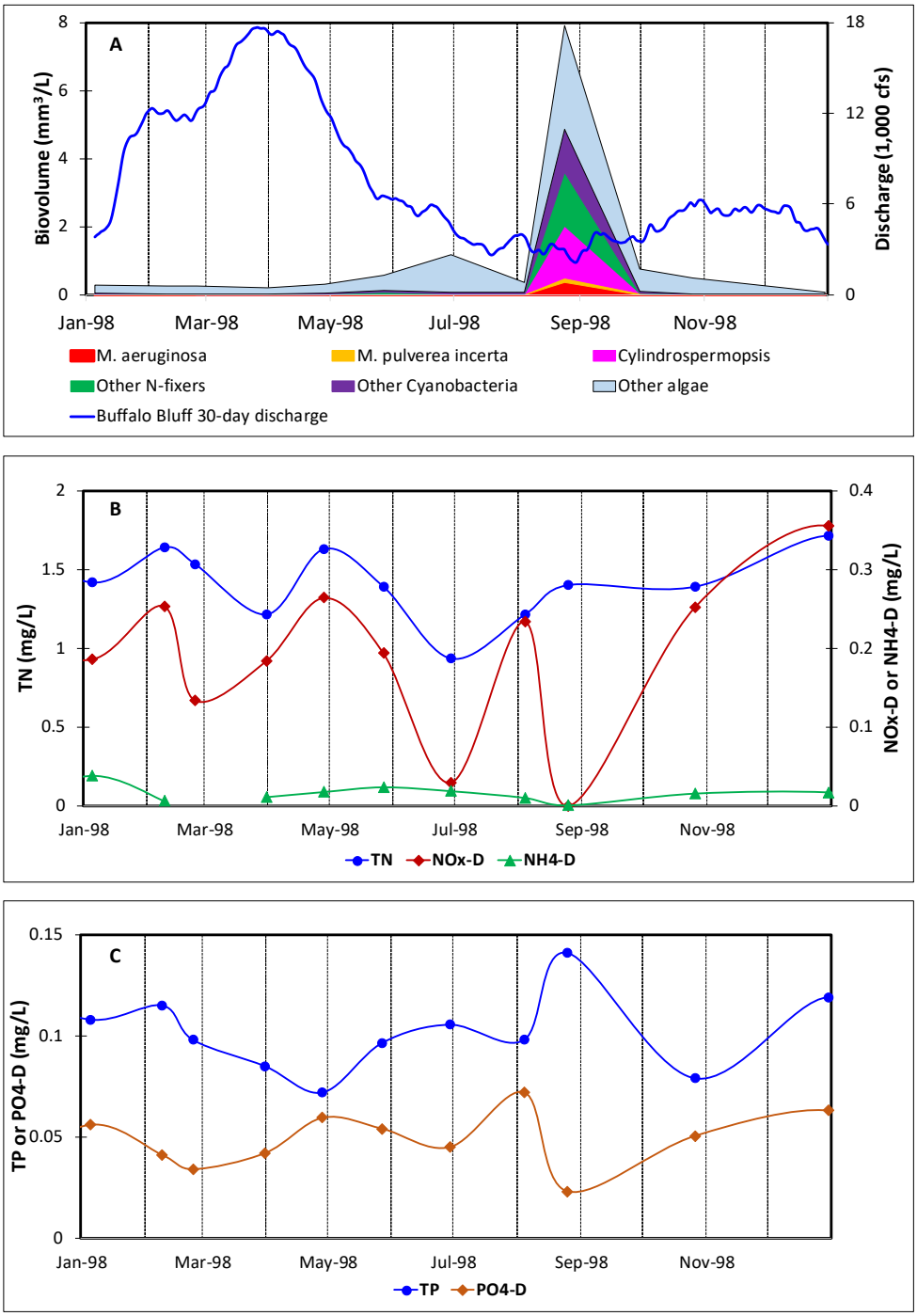


Figure 24. St. Johns River at Piney Point 1998 bloom event.

A. St. Johns River at Piney Point 1998 phytoplankton biovolumes and St. Johns River at Buffalo Bluff discharges.
B. St. Johns River at Piney Point 1998 nitrogen concentrations.
C. St. Johns River at Piney Point 1998 phosphorus concentrations.

St. Johns River at Piney Point *Microcystis aeruginosa* bloom, 2013

The highest biovolume of *M. aeruginosa* in the period of record for the St. Johns River at Piney Point was on October 10, 2013 (12.6 mm^3/L, 99% of total biovolume) (Figure 25A). This biovolume measurement was inconsistent with other measures of phytoplankton biomass or suspended solids. Chlorophyll-*a* (4.5 μg/L) and TSS (6.4 mg/L) were well below averages of 8.2 μg/L and 12.7 mg/L for this site, and also below measurements earlier in the summer. Turbidity (6.2 NTU) was somewhat below average and similar to or somewhat below measurements earlier in the summer.

Thirty-day discharges in the St. Johns River at Buffalo Bluff were 4,217 cfs (Figure 25A), somewhat lower than the long-term average (4,797 cfs), but higher than the median (3,918 cfs). There were higher discharges in the previous month, up to 6,200 cfs. Discharges the previous winter were slightly below average. TN concentration decreased on the bloom date to 0.8 mg/L, less than the long-term average of 1.2 mg/L (Figure 25B). NO_x-D was not available on October 10, but prior and subsequent measurements bracketed the long-term average of 0.18 mg/L. TP concentration was 0.09 mg/L on October 10, below the long-term average of 0.12 mg/L (Figure 25C). Water temperature and DDays were decreasing from summer levels but still relatively high. Water color was relatively low during the bloom period (75 cpu). Conductivity was 3,532 μmhos/cm, well below the average for that site and the concentrations during the 2007 and 2017 Doctors Lake *M. aeruginosa* blooms. There was little indication of vertical stratification on the bloom date of water temperature (TempRange2m 0.11°C) but there was a substantial stratification of conductivity (CondRange2m 1,164 μmhos/cm). The calculated 14-day wind function (279) is somewhat lower than the average (306) on the bloom date, suggesting those conditions may have been conducive to vertical stratification.

Overall, it is not clear why such a strong *M. aeruginosa* bloom occurred at Piney Point in October 2013. The discrepancy with other measures of phytoplankton biomass or suspended solids again raises questions about the magnitude of this bloom. Nutrient concentrations were relatively low, although probably high enough to support growth. Factors that were conducive to cyanobacterial growth were relatively high temperature, and relatively low water color and conductivity. The conductivity stratification indicates a fresher surface layer overlying more saline water. The higher discharges in September may indicate that the bloom was transported from upstream. None of the upstream sites had bloom concentrations of *M. aeruginosa* in September or October, but there are pictures documenting *M. aeruginosa* blooms in Doctors Lake and in the St. Johns River at the Shands Bridge (near Green Cove Springs, Figure 2) in early October (Figure 1). The blooms could have developed between sample dates at the upstream sites, or if the blooms were concentrated in nearshore areas, they may not have been apparent at the upstream sample sites located near the middle of the water body.

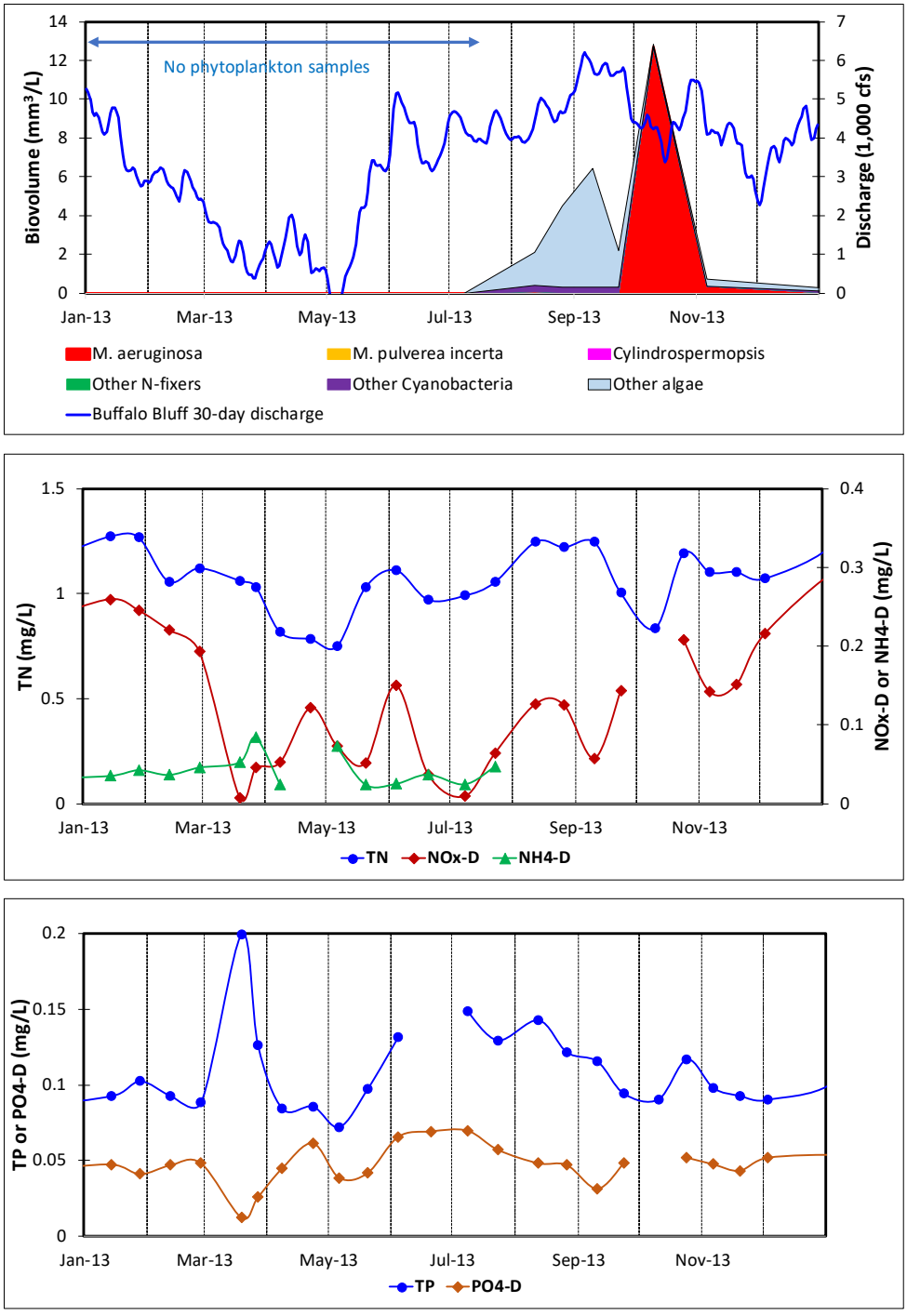


Figure 25. St. Johns River at Piney Point 2013 bloom event.

A. St. Johns River at Piney Point 2013 phytoplankton biovolumes and St. Johns River at Buffalo Bluff discharges.
B. St. Johns River at Piney Point 2013 nitrogen concentrations.
C. St. Johns River at Piney Point 2013 phosphorus concentrations.

Lake George, Crescent Lake, and St. Johns River at Racy Point Other Nitrogen-fixer bloom, 2010

This was a prolonged and widespread bloom of Other Nitrogen-fixers in May-June 2010.

Lake George

The highest biovolume of Other Nitrogen-fixers in the period of record for Lake George was 48.7 mm^3/L (96% of total biovolume) on May 20, 2010 (Figure 26A). This was a large bloom of *Aphanizomenon*, which continued through the next sample on June 7, 2010 (15.5 mm^3/L, 87% of total biovolume). There was some consistency with other measures of phytoplankton biomass. Chlorophyll-*a* concentrations were well above average on the bloom dates (109 µg/L on May 20 and 77 µg/L on June 7), although similarly high concentrations occurred later in the summer. Other measures of suspended solids, TSS, and turbidity were all above average on the bloom dates, but similar concentrations occurred later in the summer.

The bloom occurred as St. Johns River 30-day discharges at Astor were decreasing from around 5,000 cfs in April-early May (Figure 26A). Discharges had decreased to about 4,400 cfs on May 20 and 2,900 cfs on June 7, bracketing the average (3,200 cfs) and exceeding the median (2,450 cfs) discharges for this site. Average discharges for the previous winter (November-March) were near normal. TN concentration increased to 2.0 mg/L on May 20, above the long-term average of 1.5 mg/L (Figure 26B). NO_x-D concentrations were low during the bloom period. TP concentration increased to 0.11 mg/L on May 20, above the long-term average of 0.07 mg/L (Figure 26C). PO_4-D concentrations were above average in late April but decreased to below average during the bloom period. Water temperature and DDays were gradually increasing from winter lows during the bloom period; water temperature reached 28^{o}C on May 20. Water color was 150 cpu in May, 100 cpu on June 7; average for the site is 119 cpu). There was some vertical stratification on May 20 of water temperature (TempRange2m 1.6^{o}C) and conductivity (CondRange2m 57 µmhos/cm); less stratification on June 7. The calculated 14-day wind function was near average on the bloom dates, suggesting those conditions were not conducive to vertical stratification.

Overall, it appears that the 2010 Lake George *Aphanizomenon* bloom was promoted by an increase in nutrient concentrations and decreasing discharge and increasing water temperature. The increase in TP concentration may have been the primary trigger and could have had an external or internal source. The increase in TN concentration could have been due to nitrogen fixation. For a discharge of 4,400 cfs, estimated water replacement time for an average lake volume would be about 59 days. It is not clear what led to the decline of the *Aphanizomenon* bloom. Nutrient concentrations remained relatively high through September and other environmental factors remained favorable. The *Aphanizomenon* bloom was succeeded by a fairly typical summer biovolume of Other Cyanobacteria, dominated by *Limnothrix.*

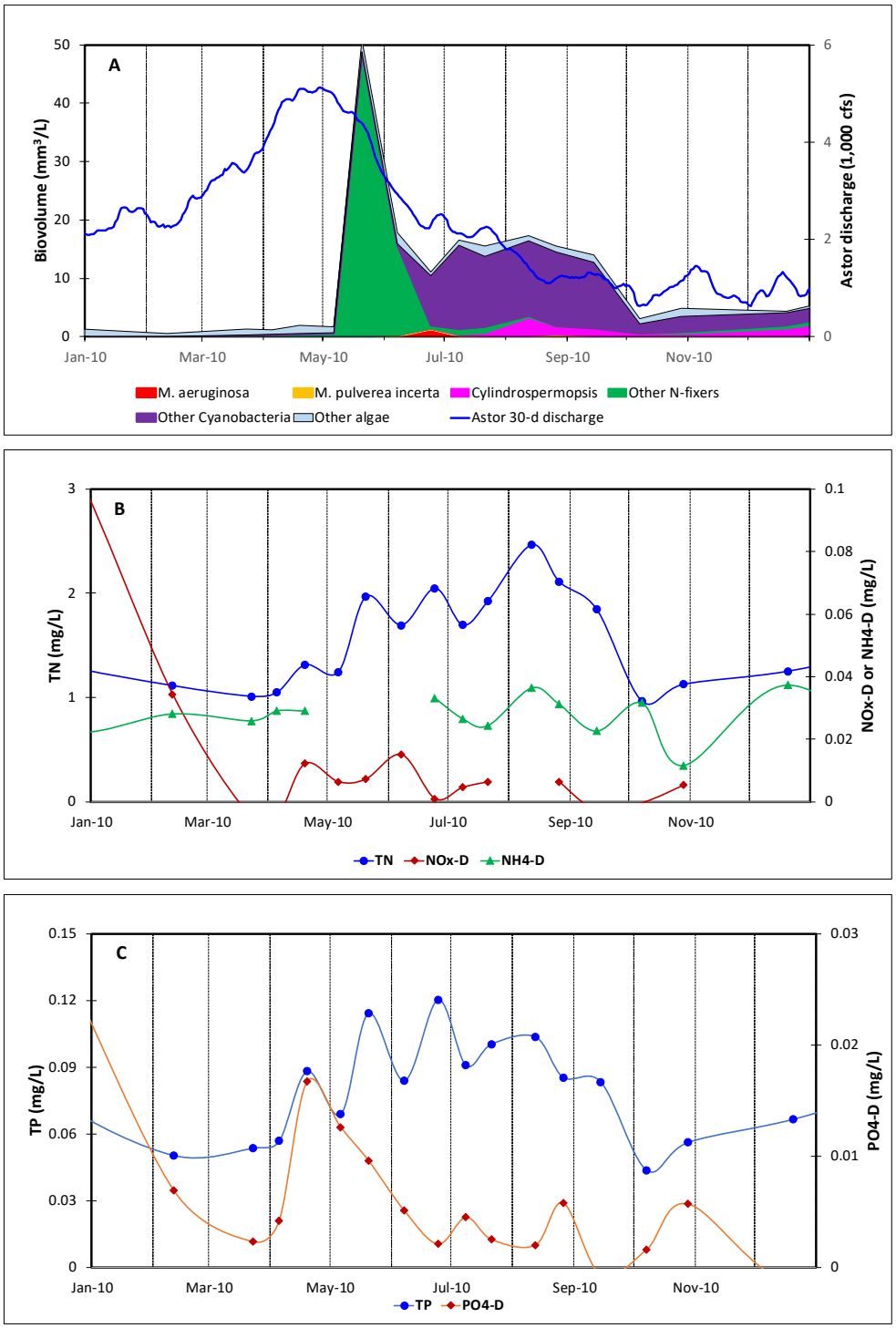


Figure 26. Lake George 2010 bloom event.

A. Lake George 2010 phytoplankton biovolumes and St. Johns River at Astor discharges.
B. Lake George 2010 nitrogen concentrations.
C. Lake George 2010 phosphorus concentrations.

Crescent Lake

An Other Nitrogen-fixer bloom persisted on Crescent Lake from May 20 through June 24, 2010, dominated by *Aphanizomenon* on the first two dates, and *Dolichospermum* on the third date (Figure 27A). Maximum biovolume in the period of record for Other Nitrogen-fixers in Crescent Lake was 4.9 mm^3/L (95% of total biovolume) on June 7, 2010; this was only about 10% of the maximum biovolume reached in the 2010 Lake George bloom. There was also a relatively high biovolume of *M. aeruginosa* on June 7. Chlorophyll-*a* ranged widely during the bloom period, from 16 to 171 μg/L; the higher measurements were well above both the average for the lake and other measurements later in the summer. TSS and turbidity measurements were also well above averages on the same dates that chlorophyll-*a* was high.

Dunns Creek 30-day discharges had decreased from about 1,200 cfs in April to below 200 cfs during the bloom period, well below the average discharge of 440 cfs (Figure 27A). For much of the bloom period discharges were negative, indicating a net flow into Crescent Lake from the St. Johns River. Average discharges for the previous winter (November-March) were near normal.

TN concentration was relatively high during the bloom period, above the long-term average of 1.4 mg/L (Figure 27B). Inorganic nitrogen concentrations were low during the bloom period. TP concentration also was relatively high during the bloom period, above a long-term average of 0.088 mg/L (Figure 27C). PO_4-D concentrations were slightly above average during the first part of the bloom period. Water temperature and DDays were gradually increasing from winter lows during the bloom period; water temperature reached 28°C on May 20. Water color was relatively high during the bloom period (decreasing from 400 to 250 cpu vs. an average of 279 cpu). There was some indication of vertical stratification of water temperature on May 20 (TempRange2m 1.1°C) and June 24 (TempRange2m 3.2°C). The calculated 14-day wind function was slightly below average during the bloom period, suggesting those conditions were not conducive to vertical stratification.

Similar to Lake George, it appears that the 2010 Crescent Lake Other Nitrogen-fixer bloom was promoted by an increase in nutrient concentrations and deceasing discharge and increasing water temperature. The increase in TP concentration may have been the primary trigger and could have had an external or internal source. The increase in TN concentration could have been due to nitrogen fixation. Runoff from the TCAA also could affect nutrient concentrations at this site. For a discharge of 200 cfs, estimated water replacement time for an average lake volume would be about 330 days. It is not clear what led to the decline of the Other Nitrogen-fixer bloom. TP concentrations remained relatively high through September, although TN concentrations decreased, and other environmental factors remained favorable. After a brief biovolume decline in early July the Other Nitrogen-fixer bloom was succeeded by a fairly typical summer biovolume of Other Cyanobacteria, dominated by *Limnothrix.*

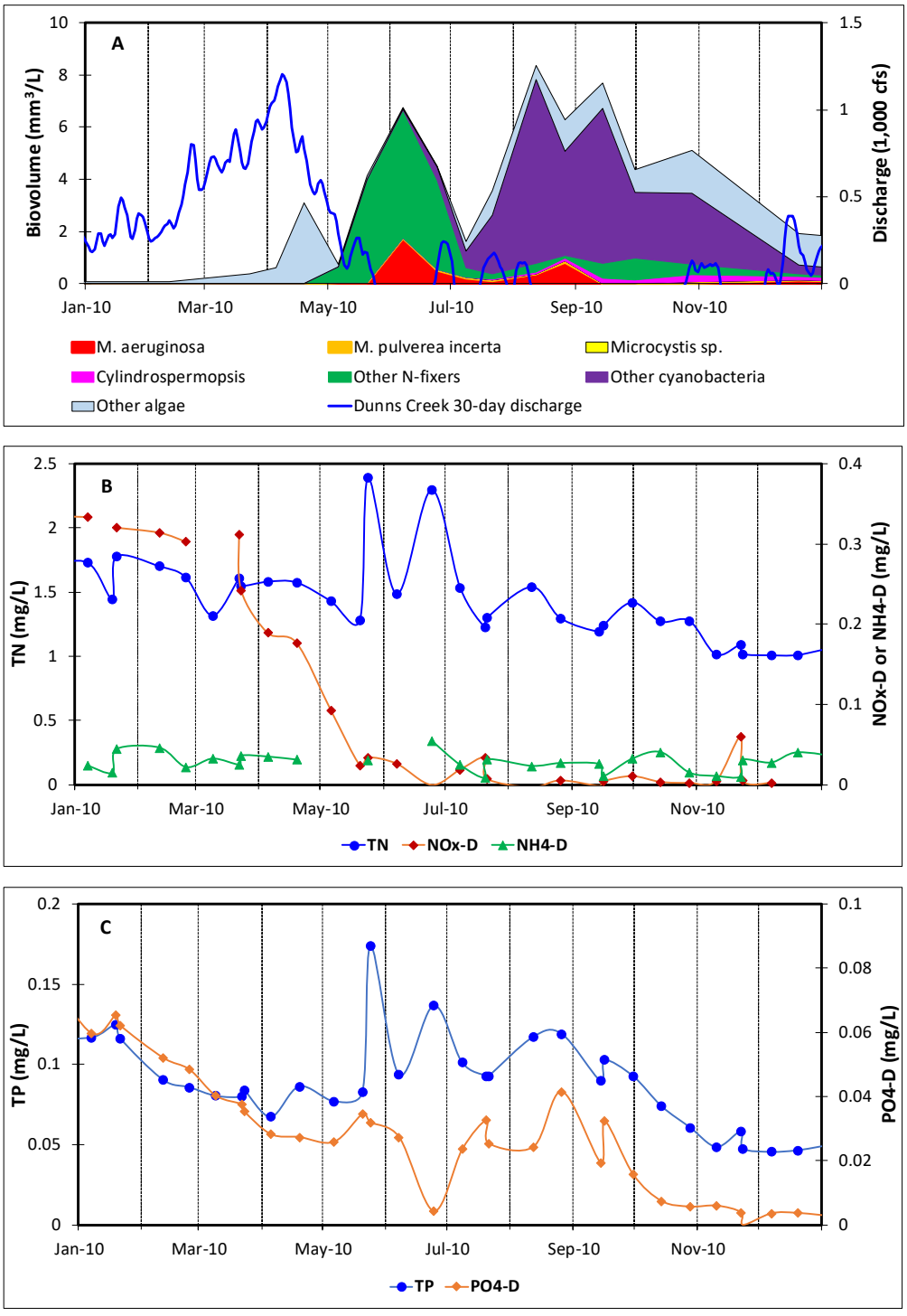


Figure 27. Crescent Lake 2010 bloom event.

A. Crescent Lake 2010 phytoplankton biovolumes and Dunns Creek discharges.
B. Crescent Lake 2010 nitrogen concentrations.
C. Crescent Lake 2010 phosphorus concentrations.

St. Johns River at Racy Point

The highest biovolume of Other Nitrogen-fixers in the period of record for the St. Johns River at Racy Point was 52.6 mm^3/L (95% of total biovolume) on May 19, 2010 (Figure 28A). A similar biovolume occurred at Lake George at this time. This was a large bloom of *Aphanizomenon*, which continued through the next sample on June 9, 2010 (7.3 mm^3/L, 87% of total biovolume). There were also very high values for other measurements of phytoplankton biomass and suspended solids on May 19; chlorophyll-*a* was 290 μg/L, TSS was 31 mg/L, and turbidity 42 was NTU, all much above averages and other measurements that summer.

The bloom occurred as St. Johns River 30-day discharges at Buffalo Bluff were decreasing from around 8,000 cfs in April (Figure 28A). Discharges had decreased to about 6,300 cfs on May 19 and 3,800 cfs on June 9, bracketing the average (4,800 cfs) and median (3,920 cfs) discharges for this site. Average discharges for the previous winter (November-March) were near normal.

TN concentration increased to 3.5 mg/L on May 19, above the long-term average of 1.3 mg/L (Figure 28B). NO_x-D concentrations were low during the bloom period. TP concentration increased to 0.17 mg/L on May 19, above the long-term average of 0.085 mg/L (Figure 28C). PO_4-D concentrations were low during the bloom period. Water temperature and DDays were gradually increasing from winter during the bloom period; water temperature reached 27°C on May 19. Water color was 100 cpu in May, 80 cpu on June 7; average for the site is 136 cpu). There was little vertical stratification on May 19 of water temperature (TempRange2m 0.1°C) and conductivity (CondRange2m 1 μmhos/cm). The calculated 14-day wind function was slightly below average on the bloom dates, suggesting those conditions were not conducive to vertical stratification.

As with Lake George and Crescent Lake, it appears that the 2010 *Aphanizomenon* bloom at Racy Point was promoted by an increase in nutrient concentrations and improving discharge and water temperature conditions. The increase in TP concentration may have been the primary trigger and could have had an external or internal source. The increase in TN concentration could have been due to nitrogen fixation. Runoff from the TCAA also could affect nutrient concentrations at this site. No estimates of water replacement time are available for the St. Johns River at Racy Point, but replacement time estimated at this time for upstream Lake George was about 59 days. The similarity of timing and magnitude of the *Aphanizomenon* bloom at the two sites indicates this was a single event that extended downstream from Lake George. It is not clear what led to the decline of the *Aphanizomenon* bloom. Nutrient concentrations decreased at Racy Point after May but remained relatively high through the summer and other environmental factors remained favorable. The *Aphanizomenon* bloom was succeeded by a relatively high summer biovolume of Other Cyanobacteria, dominated by *Limnothrix.*

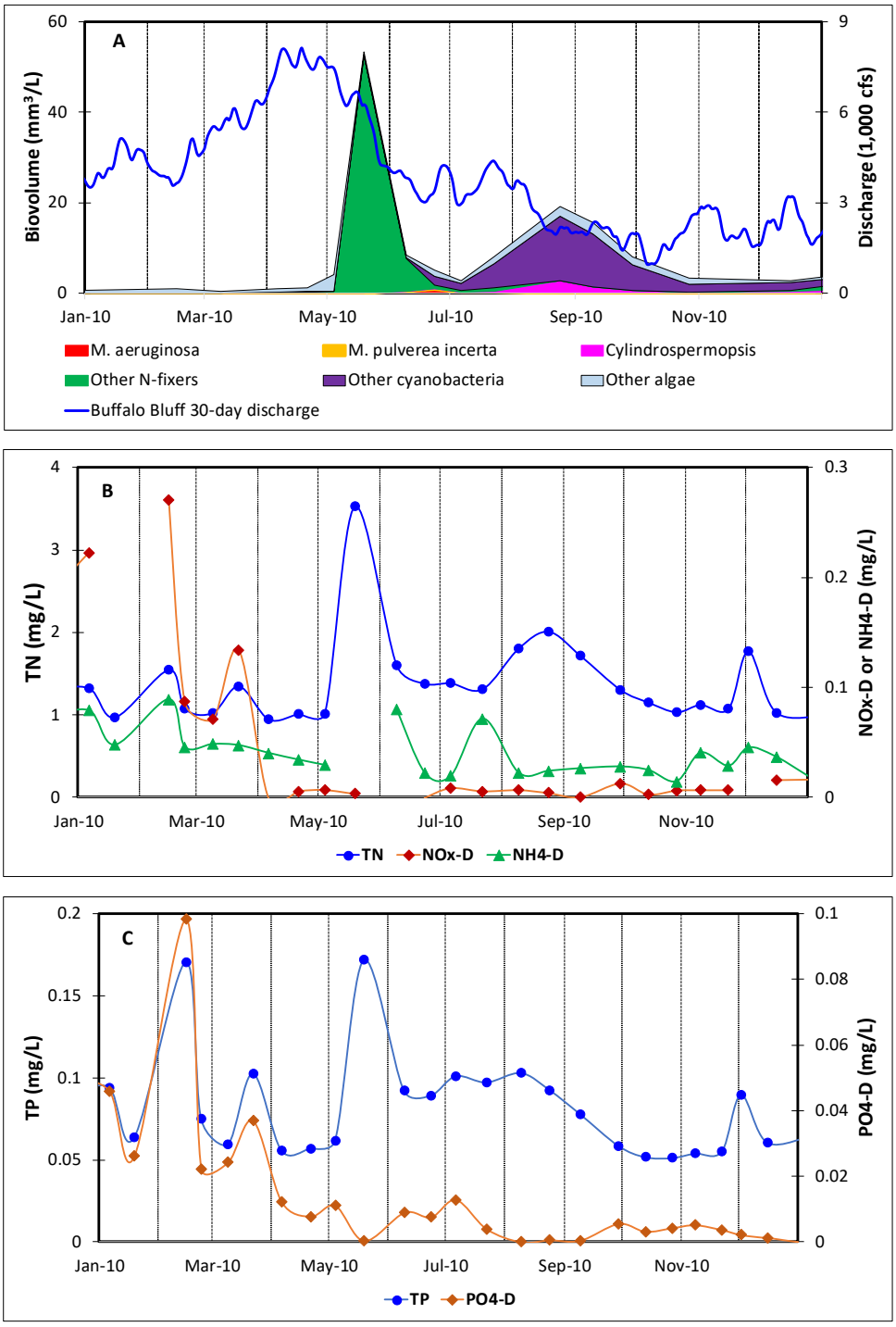


Figure 28. St. Johns River at Racy Point 2010 bloom event.

A. St. Johns River at Racy Point 2010 phytoplankton biovolumes and St. Johns River at Buffalo Bluff discharges.
B. St. Johns River at Racy Point 2010 nitrogen concentrations.
C. St. Johns River at Racy Point 2010 phosphorus concentrations.

Doctors Lake Other Nitrogen-fixer bloom, 2001

The highest biovolume of Other Nitrogen-fixers in the period of record for Doctors Lake was 8.0 mm^3/L (71% of total biovolume) on April 30, 2001 (Figure 29A). This bloom was dominated by *Dolichospermum*. Chlorophyll-*a* concentration was also very high on April 30 (147 µg/L), well above the average and other measurements that summer. TSS (29 mg/L) and turbidity (23.7 NTU) were also well above average on that date, although there were some similarly high measurements later in the summer.

Thirty-day discharges at nearby Black Creek were about 68 cfs (Figure 29A), below the average (304 cfs) and median (182 cfs) discharges for this site. Average discharges for the previous winter (November-March) were below normal. TN concentration increased to 3.1 mg/L on April 30, above the long-term average of 1.4 mg/L (Figure 29B). Inorganic nitrogen concentrations were low on this date. TP concentration increased to 0.18 mg/L on April 30, above the long-term average of 0.1 mg/L (Figure 29C). PO_4-D concentration was near the long-term average on April 30. Water temperature and DDays were increasing during the bloom period; temperature was 23°C on April 30. Water color was relatively low during the bloom period (50 cpu). Conductivity was relatively high (6,174 µmhos/cm), but lower than concentrations during the 2007 and 2017 *M. aeruginosa* blooms in Doctors Lake. There was little indication of vertical stratification on the bloom date of water temperature (TempRange2m 0.04°C) or conductivity (CondRange2m 1 µmhos/cm). The calculated 14-day wind function is above average on the bloom date, suggesting those conditions were not conducive to vertical stratification.

Overall, it appears that the 2001 Doctors Lake *Dolichospermum* bloom was promoted by high TN and TP concentrations. Other conditions that favored bloom development were low water color, a tolerable conductivity, and long water residence time. Black Creek discharges were low. Average simulated watershed inflows are not available for this time period, but a modeled water age for Doctors Lake on April 30, 2001 is about 290 days, well above the average of 179 days. The low Black Creek discharges suggest that the source for the nutrient increases may have been internal; regeneration from the sediments and nitrogen fixation.

The decreased *Dolichospermum* biovolume after April 30 may have been due to decreased TP and TN concentrations and an increase in conductivity, since other environmental factors remained favorable (low discharges, low water color, increasing temperature). Conductivity increased to the 8,000-11,000 µmhos/cm range in June and July. In late July there was a secondary Other Nitrogen-fixer bloom, dominated by *Anabaenopsis*, and a relatively high biovolume peak for *M. aeruginosa*. This event was associated with secondary peaks in nutrient concentrations and conductivity of 9,604 µmhos/cm.

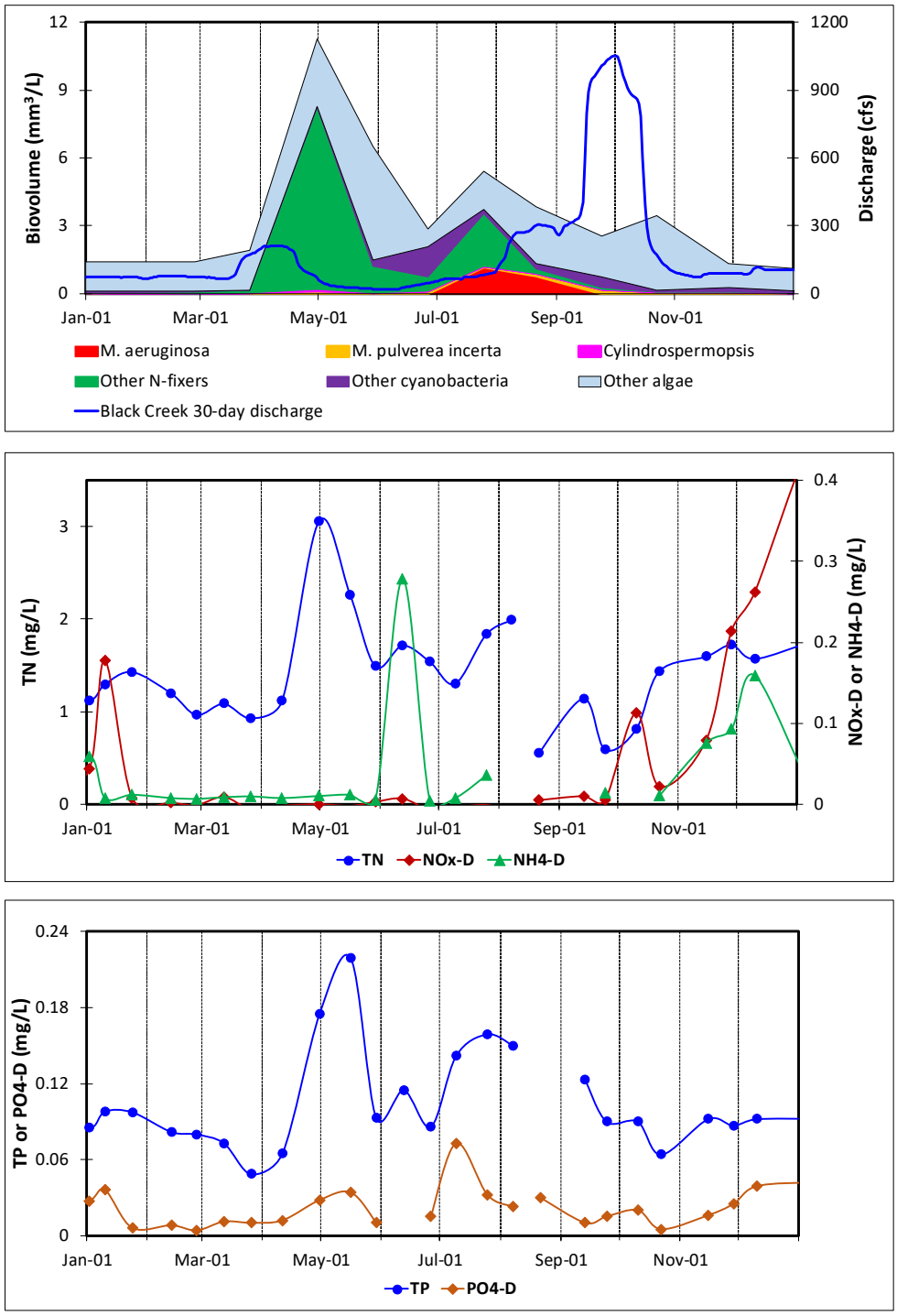


Figure 29. Doctors Lake 2001 bloom event.

A. Doctors Lake 2001 phytoplankton biovolumes and Black Creek discharges.
B. Doctors Lake 2001 nitrogen concentrations.
C. Doctors Lake 2001 phosphorus concentrations.

Summary of bloom events

Environmental factors associated with the bloom events are summarized in Table 2. In this summary, blooms observed at multiple sites during the same time period are considered as separate events, and the apparent blooms observed in Lake George before and after Hurricane Matthew in 2016 are treated as separate events. All of the bloom events analyzed by Analyst 1 were associated with high concentrations of other measures of phytoplankton biomass, long discharges or long water replacement times, higher than normal TP and TN concentrations, and relatively high water temperatures. Most blooms analyzed by Analyst 1 had relatively low conductivity and low water color. Nearly half had higher than average discharges the previous winter, and few had high concentrations of inorganic nutrients. Substantial vertical stratification of conductivity or temperature was observed in only two bloom events, but in only 6 of the bloom events were measurements taken over 2/3 of the water column depth, and for the other 5 events measurements were taken only at a single depth.

Only one of the bloom events analyzed by Analyst 2 were associated with unusually high concentrations of other measures of phytoplankton biomass, raising questions about the accuracy of the biovolume measurements during these apparent blooms. All of the apparent blooms had high water temperature and low water color, and most had long discharges or long water replacement times and relatively low conductivity. Few had unusually high concentrations of total or inorganic nutrients, and only one had substantial vertical stratification.

Table 2. Summary of environmental factors associated with bloom events.

Environmental factor	**Analyst 1**	**Analyst 2**
Number of bloom events	11	5
High chlorophyll-*a*, TSS, turbidity	11	1
Low discharges/long water replacement time	11	4
High discharges previous winter	5	0
High TP	11	2
High TN	11	2
High PO_4-D	4	0
High inorganic nitrogen	0	1
Water temperature > 20^{o}C	11	5
Conductivity < 5,000 μmhos/cm	8	4
Water color low	10	5
Vertical stratification (vertical profiles over 2/3 of water depth)	2 (6)	1 (5)

STATISTICAL ANALYSES OF CYANOBACTERIAL-ENVIRONMENTAL FACTOR RELATIONSHIPS

Chlorophyll vs. biovolume

Due to apparent discrepancies between chlorophyll-*a* and biovolume measurements in some of the major bloom events, we examined the relationships between chlorophyll-*a* and total phytoplankton biovolume for the two analysts. These data sets combine data for six sites, Lake George, Crescent Lake, the St. Johns River at Racy Point, Doctors Lake, the St. Johns River at Mandarin Point, and the St. Johns River at Piney Point. There were positive relationships for both analysts, but the relationship was stronger for Analyst 1 (Figure 30). If the outlier chlorophyll-*a* measurement of 296 μg/L is removed from the Analyst 1 data set, R^2 is reduced to 0.612, still higher than that for Analyst 2. The slope and intercept for the trend line were also lower for Analyst 1, so Analyst 1 tended to measure a lower phytoplankton biovolume per unit chlorophyll than Analyst 2.

Hendrickson et al. (2003) reported a strong relationship between chlorophyll-*a* and biovolume in the freshwater zone of the river, but a poor relationship in the oligohaline zone, for a shorter period of record during which Analyst 1 was the sole analyst. For our longer period of record, for Analyst 1 there was also a stronger relationship between chlorophyll and biovolume for the freshwater sites (R^2=0.692) than for the oligohaline sites (R^2=0.485). However, for Analyst 2 there were opposite results, a stronger relationship between chlorophyll and biovolume for the oligohaline sites (R^2=0.608) than for the freshwater sites (R^2=0.354).

Because of these differences and other concerns about inconsistency in taxa identification and biovolume determinations between the two analysts, we used only data from Analyst 1, who covered most of the period of record, in statistical analyses of cyanobacterial-environmental factor relationships.

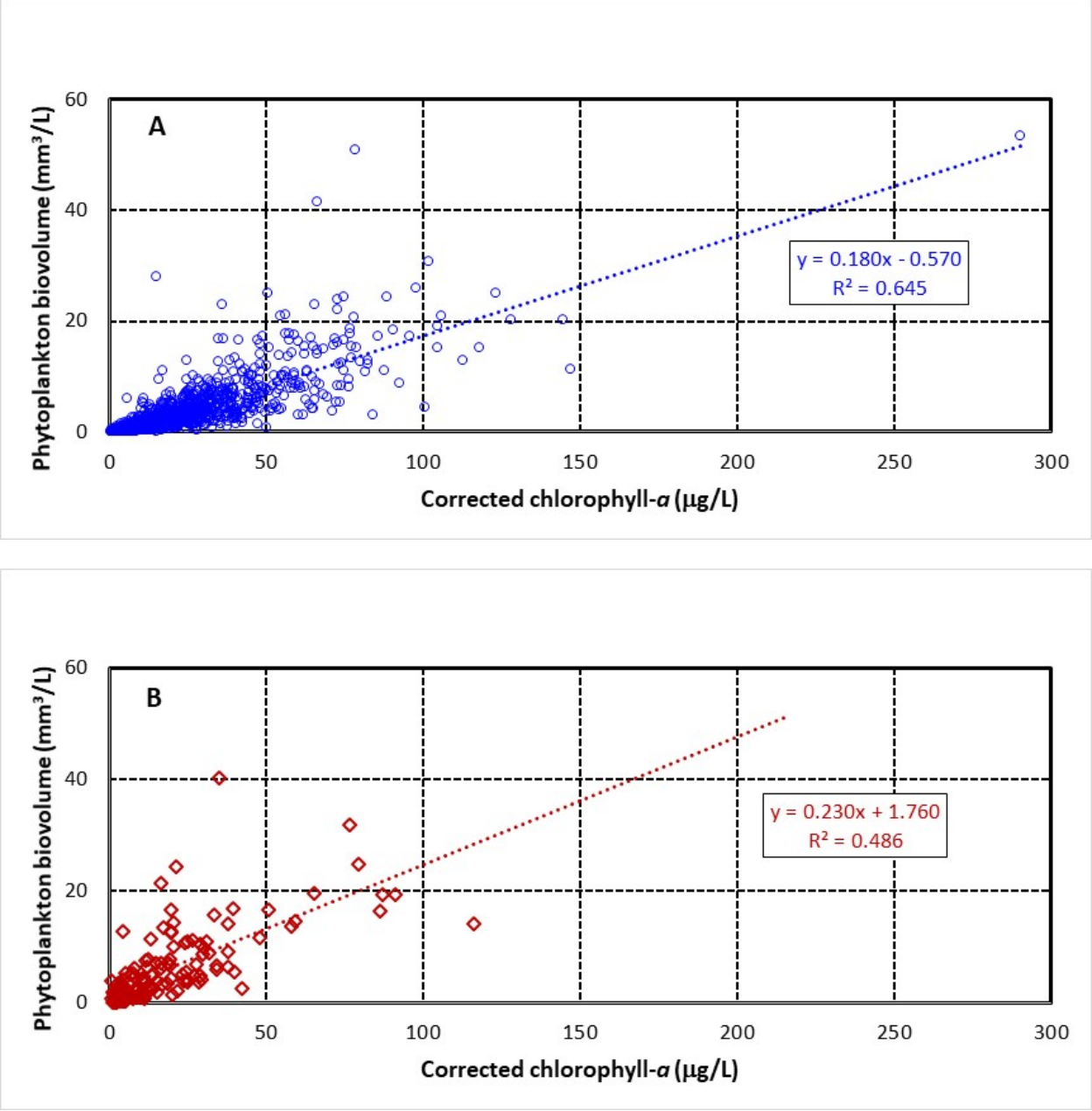


Figure 30. Lower St. Johns River basin chlorophyll vs. phytoplankton biovolumes, by analyst.

A. Analyst 1
B. Analyst 2

Cyanobacterial-nutrient relationships across sites

Combining data from all the sites (only using data from phytoplankton Analyst 1), there are weak relationships of cyanobacterial biovolume with TP and TN (Figure 31, Figure 32). However, there do appear to be threshold nutrient concentrations above which high biovolumes can occur. For TP, the threshold generally appears to be about 0.05 mg/L, with some variability among the cyanobacteria groups. The threshold appears to be lower for *M. pulverea incerta* and perhaps higher for *M. aeruginosa* and Other Nitrogen-fixers.

For TN, the threshold generally appears to be about 1.0 mg/L, again with some variability among the cyanobacteria groups. The threshold perhaps may be lower for *M. aeruginosa* and higher for Other Nitrogen-fixers. For the *Cylindrospermopsis* + *Raphidiopsis* and Other Nitrogen-fixer taxa, the relationships with TN may reflect nitrogen fixation enhancing nitrogen concentrations at higher biovolumes.

Chlorophyll-*a* concentrations are weakly related to TP, but high concentrations of chlorophyll-*a* also increase substantially above a TP concentration of about 0.05 mg/L (Figure 33). High concentrations of chlorophyll-*a* tend to steadily increase as TN increases, with perhaps a step change close to a concentration of 1.5 mg/L (Figure 33).

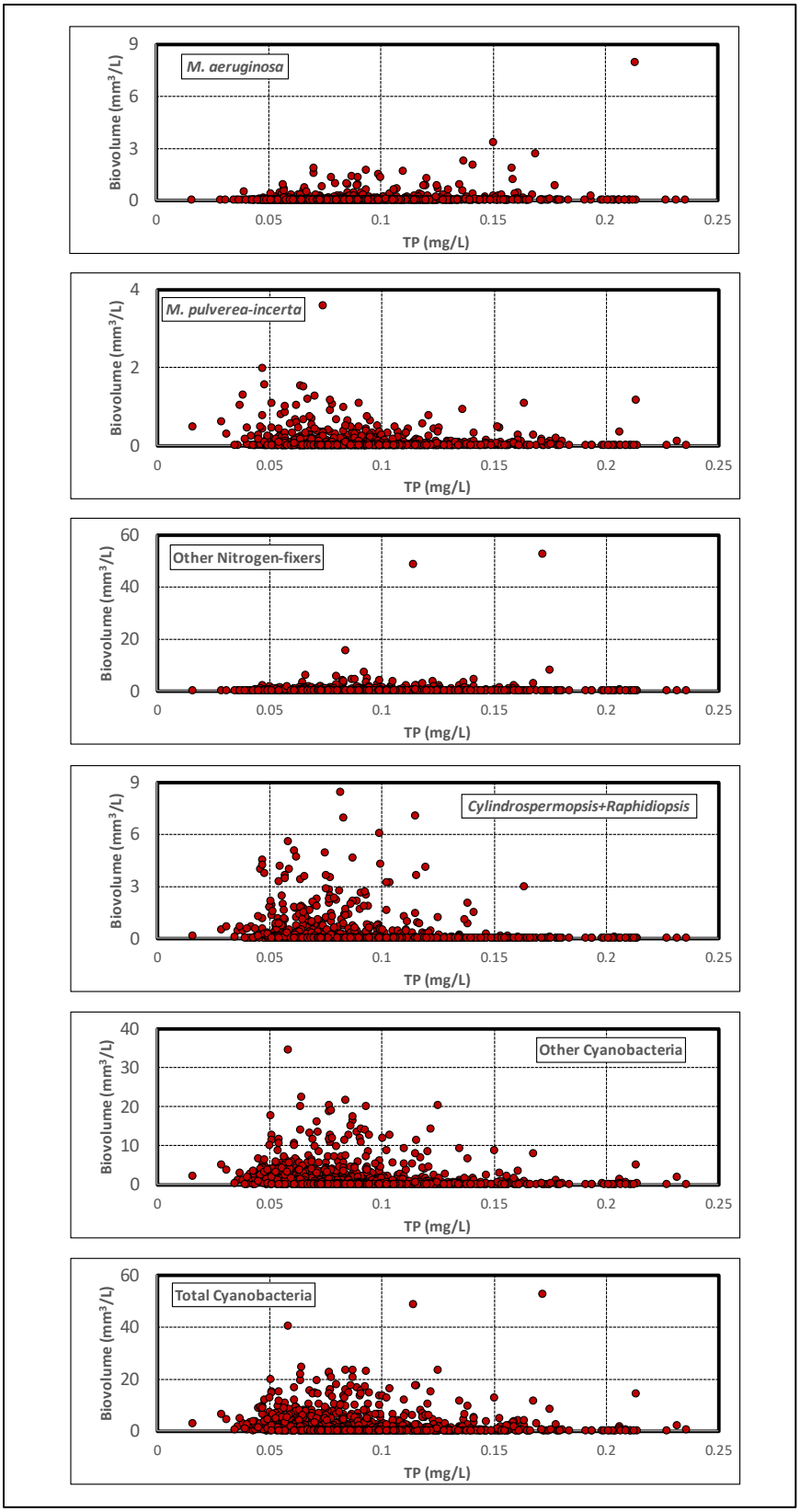


Figure 31. Cyanobacterial biovolume vs. total phosphorus, all sites combined.

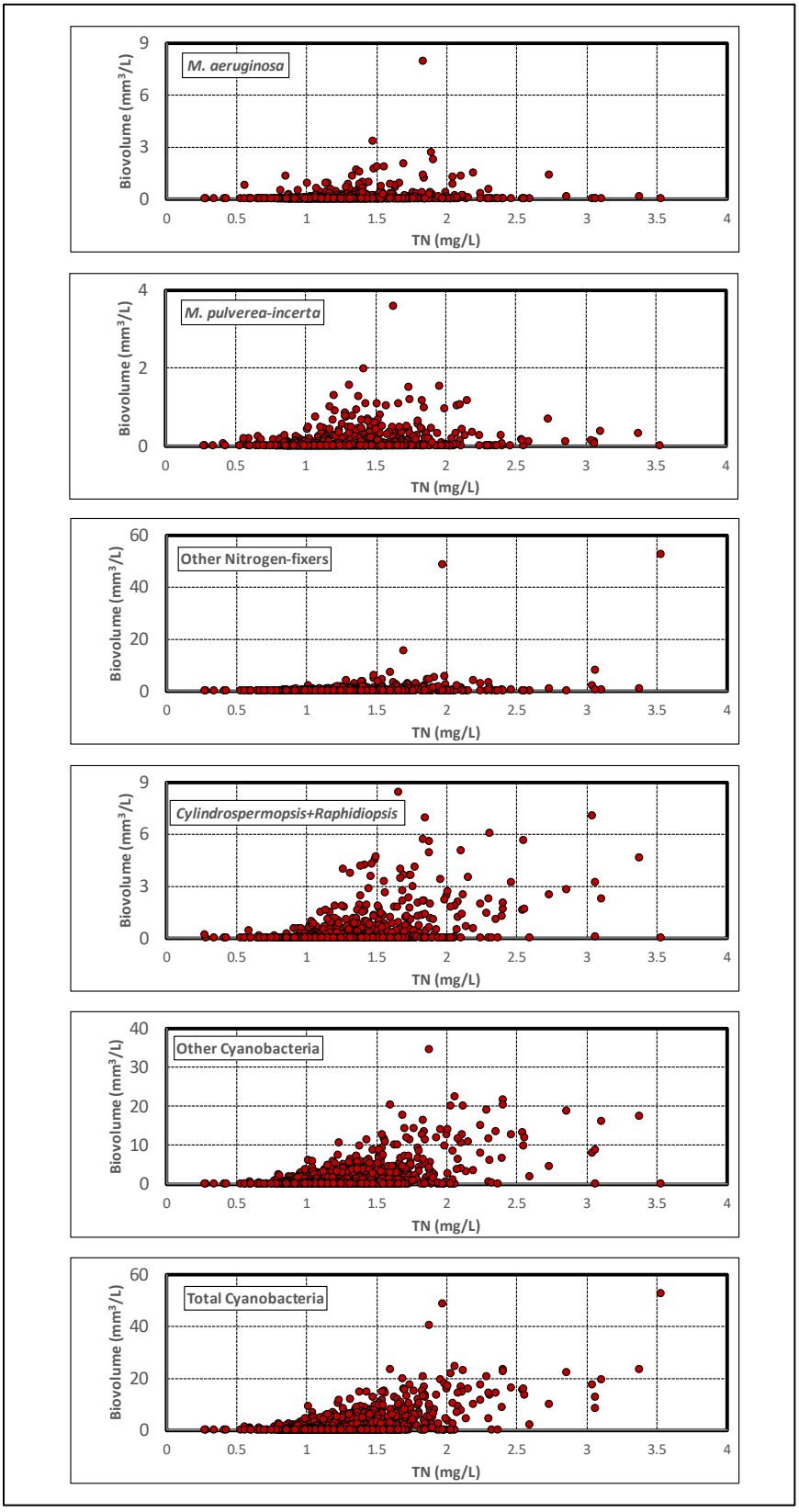


Figure 32. Cyanobacterial biovolume vs. total nitrogen, all sites combined.

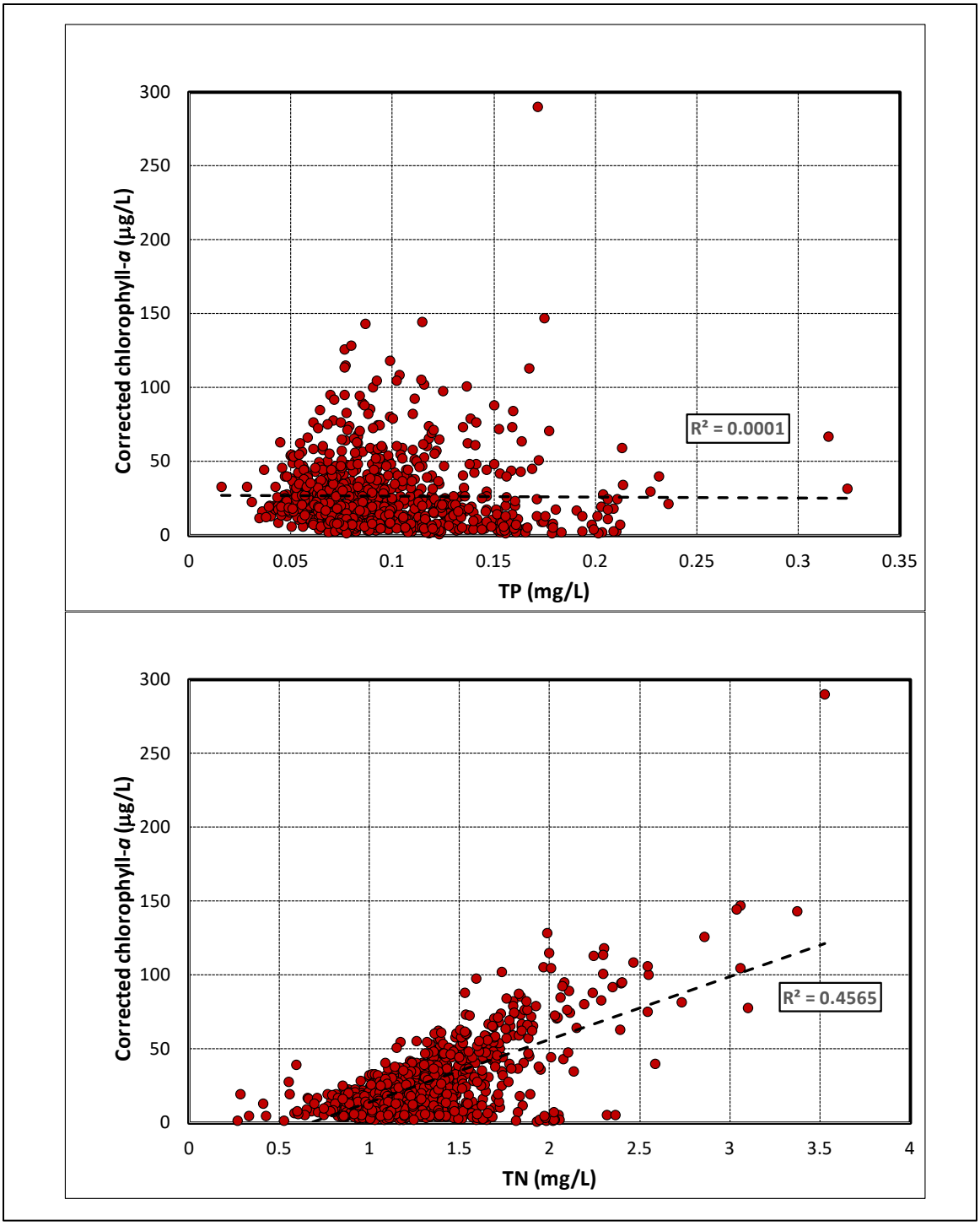


Figure 33. Corrected chlorophyll-*a* vs. total phosphorus and total nitrogen, all sites combined.

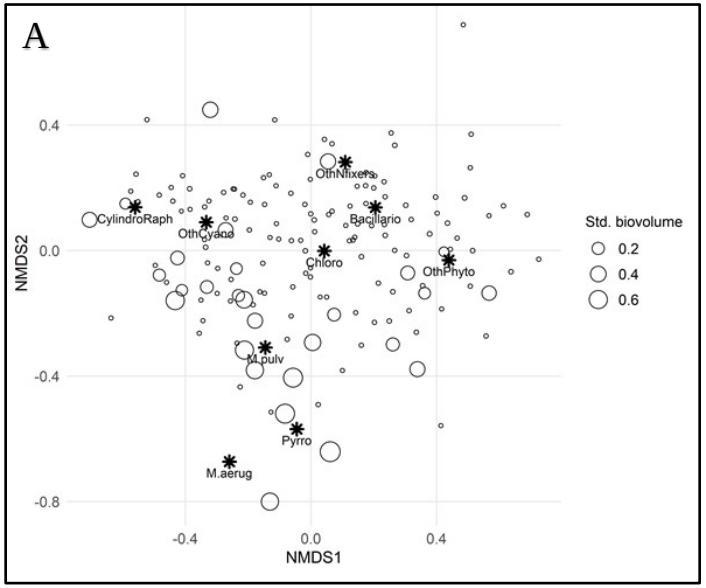
Lake George

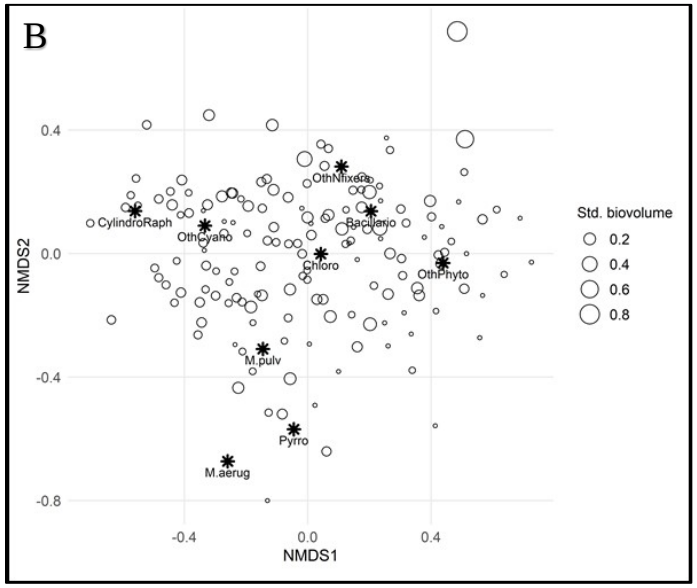
Results of the NMDS ordination for Lake George are shown in Figure 34 and Figure 35. Figure 34A is a bubble plot for *M. aeruginosa*. Each bubble represents a sample and the size of the bubbles is proportional to the standardized biovolume in that sample. The smallest bubbles represent zero biovolume. Also shown is the centroid for each of the phytoplankton groups in the ordination space. The centroid for *M. aeruginosa* is in the lower left quadrant, and most of the highest biovolumes are also in that quadrant. That centroid seems to be more on the periphery of the plot than would be expected from the bubble plot, which seems to often occur for taxa with many zero values. The centroid for *M. pulverea incerta* is also in the lower left quadrant, while the centroids for *Cylindrospermopsis-Raphidiopsis* and Other Cyanobacteria are in the upper left quadrant. The centroid for Other Nitrogen-fixers is in the upper right quadrant, reflecting the location of a few high biovolumes (Figure 34B). Otherwise, Other Nitrogen-fixer biovolumes don't seem to be aggregated in any area of the ordination plot.

Figure 34C superimposes the environmental vectors on the ordination plot, all of which are statistically significant, and Figure 35 shows ordisurf contour plots for selected environmental factors. The vectors point in the direction of increasing values of the factor and the lengths of the vectors are proportional to the correlations between the vector and the ordination axes. The vector plot and the discharge contour plot indicate that the first NMDS axis is primarily a discharge axis, with samples collected during higher discharge periods on the right. Other vectors that are associated with high discharges are water color, inorganic nutrients, and TP, all of which might reflect high watershed runoff during those periods. Secchi depths also increase toward the right, probably reflecting low phytoplankton biomass during those high flow periods. Conductivity, TN, and TNTP, Temp, and DDays increase toward the left, under low discharge conditions.

The cyanobacterial centroids are located on the left side of the ordination plot (except Other Nitrogen-fixers), indicating an association with low discharges. The increase in TN and TNTP toward the left may reflect nitrogen fixation by *Cylindrospermopsis-Raphidiopsis*. Although conductivity increases under low discharge conditions, it is unlikely to get high enough at these freshwater sites to significantly affect cyanobacteria. The centroid for *M. aeruginosa* seems most associated with high WintSummDis, TNTP, and TN. The association with high WintSummDis may indicate *M. aeruginosa* blooms are favored by high winter discharges followed by low summer discharges. The date vector increases in the opposite direction to the *M. aeruginosa* centroid, indicating that most of its highest biovolumes were early in the period of record. The ordination doesn't provide a clear explanation for the few blooms of Other Nitrogen-fixers. They occurred late in the period of record (2010 and 2015), and occurred during the spring and early summer, when water temperatures were relatively low. It is surprising that the highest Other Nitrogen-fixer biovolumes appear to be associated with low TN and TNTP. However, examination of the ordisurf contours shows nonlinearity with TN, in particular the highest Other Nitrogen-fixer biovolume at the upper right is in an area near a relatively high TN contour, although not as high as the contours in the lower left side of the plot (Figure 35). The TP ordisurf contours also show nonlinearity, with lowest

concentrations near the center of the plot, and increasing toward the periphery, including moderately high contours near the cyanobacterial centroids.





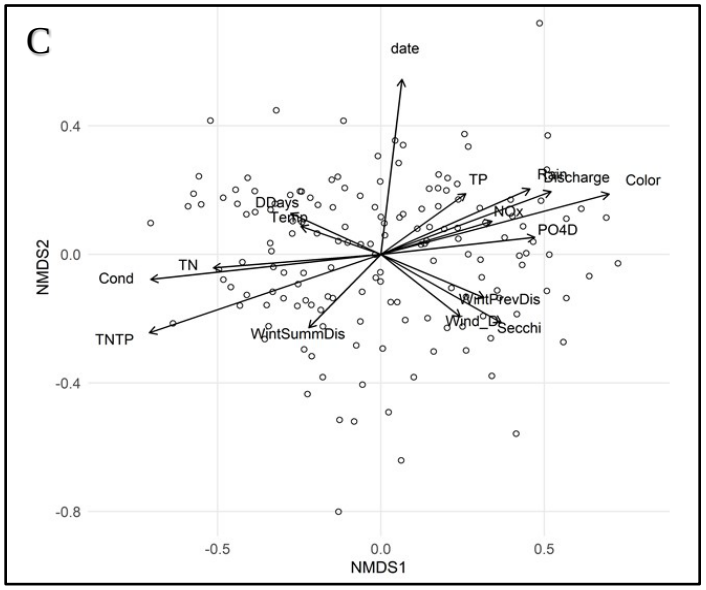


Figure 34. NMDS ordination for Lake George.

A. *Microcystis aeruginosa* bubble plot and taxa centroids.
B. Other Nitrogen-fixer bubble plot and taxa centroids.
C. Ordination plot with statistically significant environmental vectors.

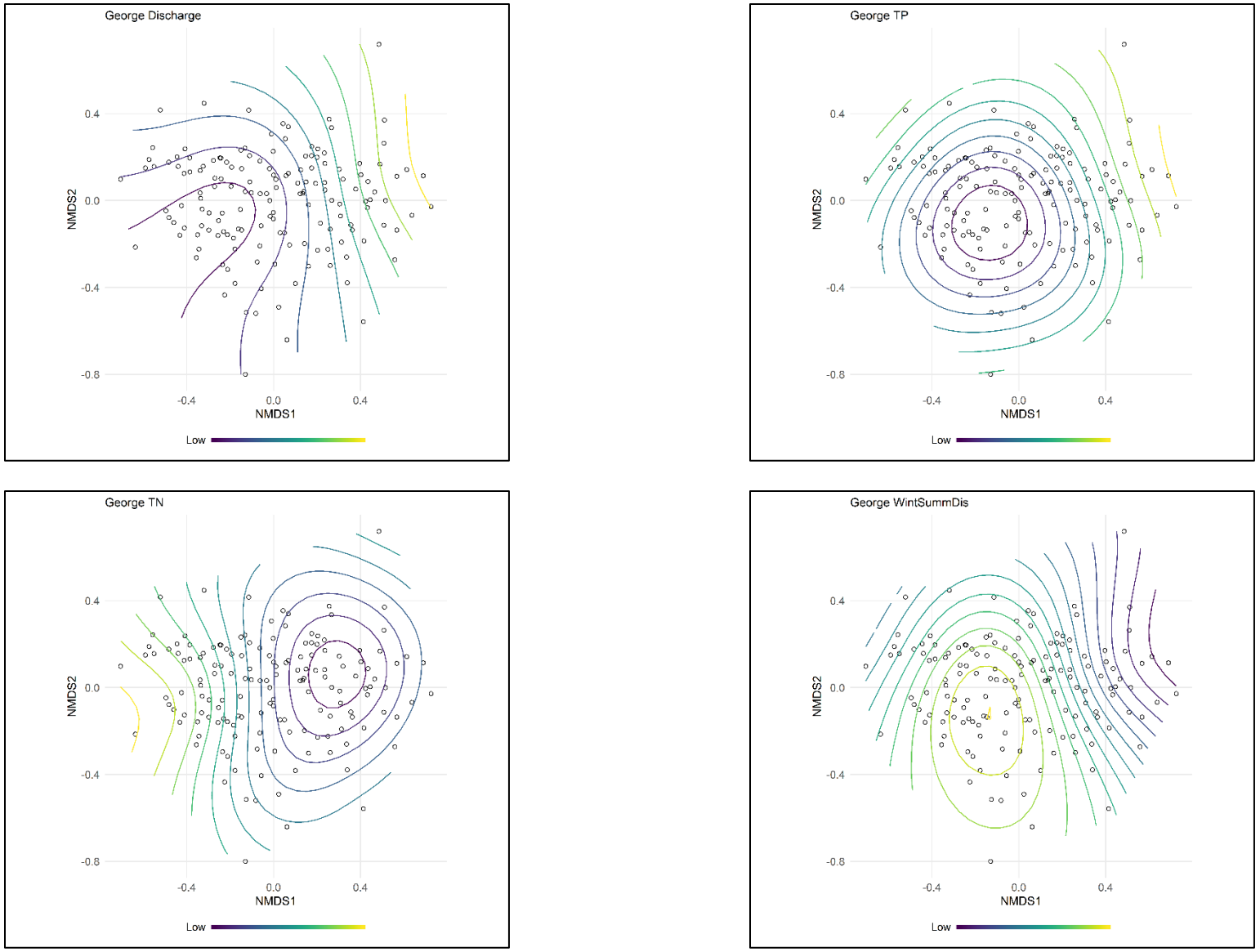


Figure 35. Surface plots for Lake George NMDS ordination.

Results of the multiple regression and Random Forest RFE models for Lake George are summarized in Table 3. For the multiple regression, statistically significant parameters are shown; the sign in front of the R^2 indicates whether the relationship is positive or negative for that parameter. Unlike the regression models, the variables included in Random Forest models are not those that are statistically significant; rather they are the set of variables that result in the lowest error in the model predictions. Unlike the multiple regression R^2 values, the Importance values do not give the direction of the relationship, which may not be relevant since the relationship modeled may be nonlinear.

The regression and Random Forest models only accounted for a small part of the variability for *M. aeruginosa* in Lake George. TN appeared in both models, consistent with the NMDS ordination. Both models also included WintPrevDis, which does not seem as consistent with the ordination, although the vector for WintPrevDis did increase toward the lower part of the ordination plot, where most of the highest *M. aeruginosa* biovolumes were. Both models also included Rain, which appeared negatively associated with *M. aeruginosa* in the ordination. WintSummDis appears in the Random Forest model, consistent with the ordination.

The regression and Random Forest models accounted for more of the variability for *M. pulverea incerta***.** TNTP was important in both models, consistent with the NMDS ordination. Color and WintPrevDis also appear in both models. It is interesting that the association was negative for color but positive for WintPrevDis, although both vectors increase toward the right in the NMDS ordination (Figure 34). Rain appears in the Random Forest model, appearing to be a negative factor from the ordination.

The regression and Random Forest models only accounted for a small part of the variability for Other Nitrogen-fixers (a negative R^2 for the Random Forest model, which we interpret as not explaining any of the variability). NO_x was relatively important in the multiple regression model, a negative relationship. DDays was negatively related in the multiple regression model, perhaps reflecting the tendency for blooms of Other Nitrogen-fixers to occur in spring-early summer. TP also appeared in the multiple regression model, a positive relationship.

The regression and Random Forest models accounted for more of the variability for *Cylindrospermopsis-Raphidiopsis*, and even more for Other Cyanobacteria and Total Cyanobacteria. There were some similarities in the models for these three taxa. TN and TNTP (positive relationship) and water color (negative relationship) were key factors for all these taxa, consistent with the ordination. WintPrevDis also appeared in several of the models, although at lower importance.

It is interesting that Discharge did not appear important in any of the regression or Random Forest models, although that appeared to be a key factor organizing the NMDS ordination. Instead the regression models selected other variables that appeared associated with high or low discharges.

Overall, these analyses suggest that low discharges are key conditions for development of high biovolumes in Lake George for all the cyanobacterial groups except for Other

Nitrogen-fixers. High TN is associated with high biovolumes of all the cyanobacterial taxonomic groups. These high TN concentrations may at least partially reflect nitrogen-fixation and may promote growth of the non-nitrogen-fixing cyanobacteria. Other factors that appear of lesser importance in promoting cyanobacterial blooms include high WintSummDis, high WintPrevDis, and high temperatures (by restricting analyses to the warm season, temperatures were almost always high enough for cyanobacterial growth). It is interesting that TP did not show up as very important in most of these analyses, although in the more qualitative assessment of bloom events TP appeared to be a key promoter. The NMDS ordisurf contour plots indicate substantial nonlinearity or even non-monotonicity for both TP and TN, which may explain their low contribution to the regression models. Also, TP concentrations are relatively high in Lake George (average 0.073 mg/L). These concentrations may be high enough that they are often not limiting to cyanobacterial growth, so cyanobacteria are not very responsive to variations in TP concentrations.

Table 3. Lake George multiple regression analysis and Random Forest RFE results. Only included the 6 highest importance parameters for the RFE models.

Multiple Regression		**Random Forest RFE**	
Taxon: *Microcystis aeruginosa*			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	19.4%	Model	9.4%
TN	+ 6.7%	WintPrevDis	7.2
WintPrevDis	+ 6.4%	TN	5.8
Rain	+ 3.3%	WintSummDis	5.8
Wind_D	− 3.1%	TP	5.5
		Rain	5.4
		NO_x	5.1
Taxon: *Microcystis pulverea incerta*			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	32.6%	Model	35.0%
Color	− 12.1%	TNTP	10.7
WintPrevDis	+ 9.1%	WintPrevDis	8.1
TNTP	+ 5.8%	Rain	7.7
Wind_D	+ 3.4%	Color	5.8
PO_4-D	+ 2.3%	NO_x	5.4
Taxon: Other Nitrogen-fixers			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	17.0%	Model	− 12.4%
NO_x	− 10.4%	Rain	9.5
DDays	− 4.3%	TNTP	8.4
TP	+ 2.3%		

Table 3 (continued).

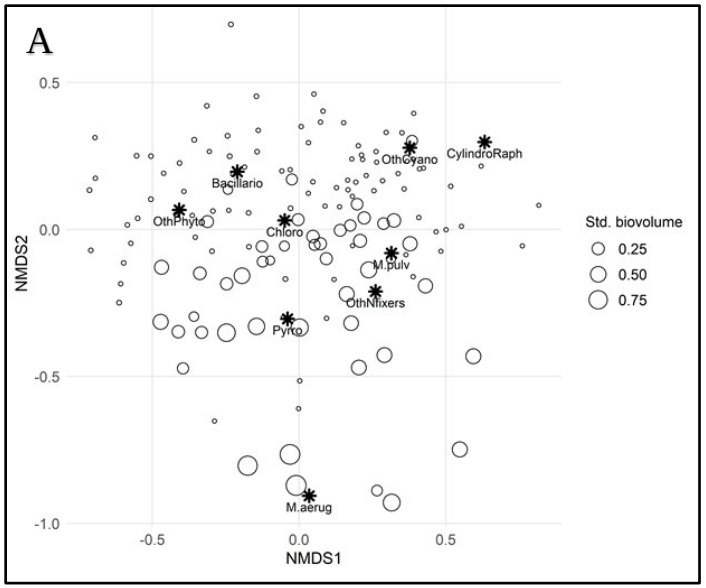
Multiple Regression		**Random Forest RFE**	
Taxon: *Cylindrospermopsis-Raphidiopsis*			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	38.0%	Model	43.0%
Color	− 20.2%	TNTP	17.0
TN	+ 15.5%	TN	16.2
TNTP	+ 2.4%	Rain	9.9
		NO_x	9.3
		TP	8.9
		Color	7.9
Taxon: Other Cyanobacteria			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	59.0%	Model	63.3%
Color	− 33.4%	TN	22.5
TN	+ 11.5%	Color	16.1
Wind_D	− 5.7%	TNTP	15.2
WintPrevDis	− 4.2%	WintPrevDis	12.4
Rain	+ 2.4%	TP	9.8
PO_4-D	− 1.8%	Wind_D	9.6
Taxon: Total Cyanobacteria			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	59.1%	Model	50.5%
Color	− 32.8%	TN	22.2
TN	17.4%	TNTP	10.5
PO_4-D	− 4.3%	Color	8.9
Wind	− 2.9%	NO_x	8.6
WintPrevDis	− 1.8%	PO_4-D	7.6
		Rain	6.0

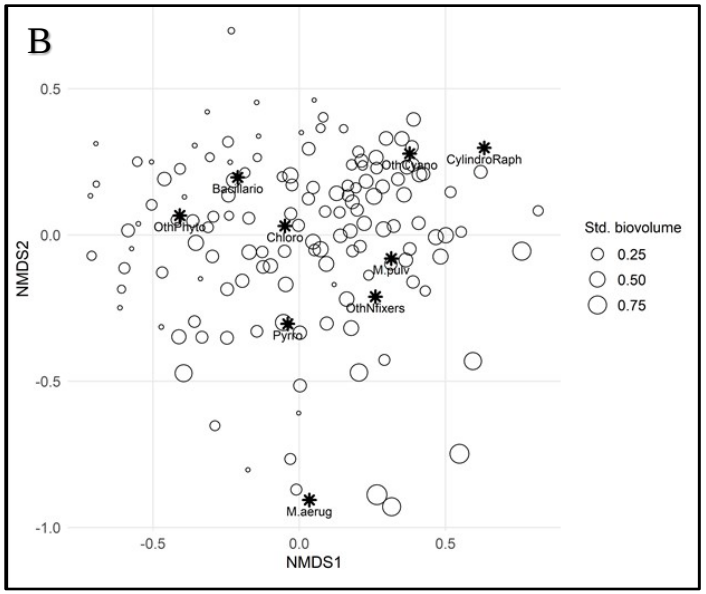
Crescent Lake

Results of the NMDS ordination for Crescent Lake are shown in Figure 36 and Figure 37. The centroid for *M. aeruginosa* is in the lower center part of the ordination plot, and most of the highest standardized biovolumes are also in the lower half. The centroid for Other Nitrogen-fixers is in the lower right quadrant, as are the highest standardized biovolumes; moderate biovolumes are spread more widely through the plot, but mostly on the right side. Centroids for the other cyanobacterial taxa are all in the right side of the plot.

The plots of the environmental vectors (Figure 36C) and ordisurf contours (Figure 37) indicate that, similar to Lake George, the first NMDS axis is primarily a discharge axis, with samples collected during higher discharge periods on the left. Other vectors that are associated with high discharges are Rain, water color, inorganic nutrients, and TP, all of which might reflect high watershed runoff during those periods. Conductivity, TNTP, Temp, DDays, WintSummDis, and Secchi depths increase toward the left, under low discharge conditions. The TN vector was not statistically significant, but the TNTP vector increasing to the upper right is opposite in direction to the TP vector, probably indicating primarily that the TNTP ratio increases as TP decreases. The ordisurf plots indicate substantial nonlinearity for both TN and TP, with lowest values near the center and increasing levels to the right and left, under both low and high discharge conditions. Other vectors that were not statistically significant are WintPrevDis, Wind-D, and date.

The *M. aeruginosa* centroid seems most closely associated with high TP and water temperatures. The distribution of moderately high biovolumes across the lower half of the plot suggests tolerance of a wide range of discharge conditions. As an embayment off the mainstem St. Johns River, Crescent Lake has substantially longer average water replacement time than Lake George (see **Study Area**). The other cyanobacterial centroids are located on the right side of the ordination plot indicating an association with low discharges, and relatively high TN and TP contours, as well as with high temperatures, WintSummDis (high previous winter discharges followed by low summer discharges), and Secchi depth. A positive association between cyanobacteria and Secchi depth is the opposite to that seen in Lake George. This suggests that factors other than cyanobacterial biomass are more limiting to Secchi transparency in Crescent Lake, such as color and nonalgal suspended solids. Average water color in Crescent Lake is substantially higher than in Lake George.





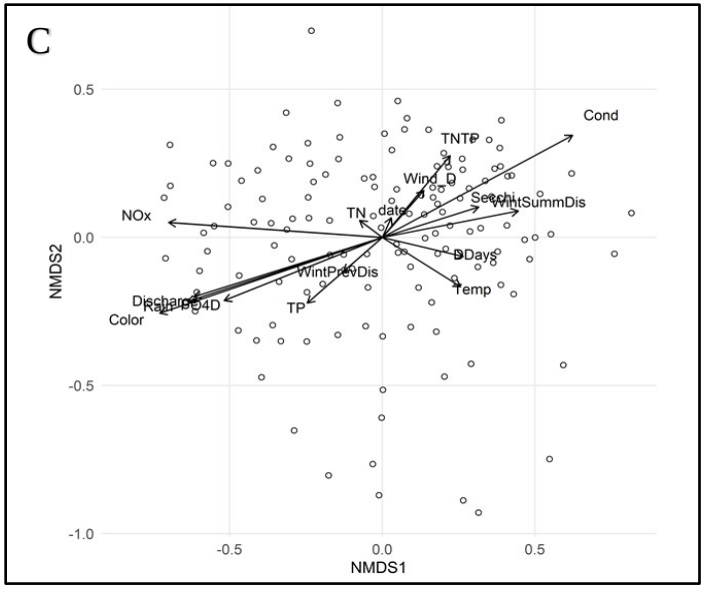


Figure 36. NMDS ordination for Crescent Lake.

A. *Microcystis aeruginosa* bubble plot and taxa centroids.
B. Other Nitrogen-fixer bubble plot and taxa centroids.
C. Ordination plot with statistically significant environmental vectors.

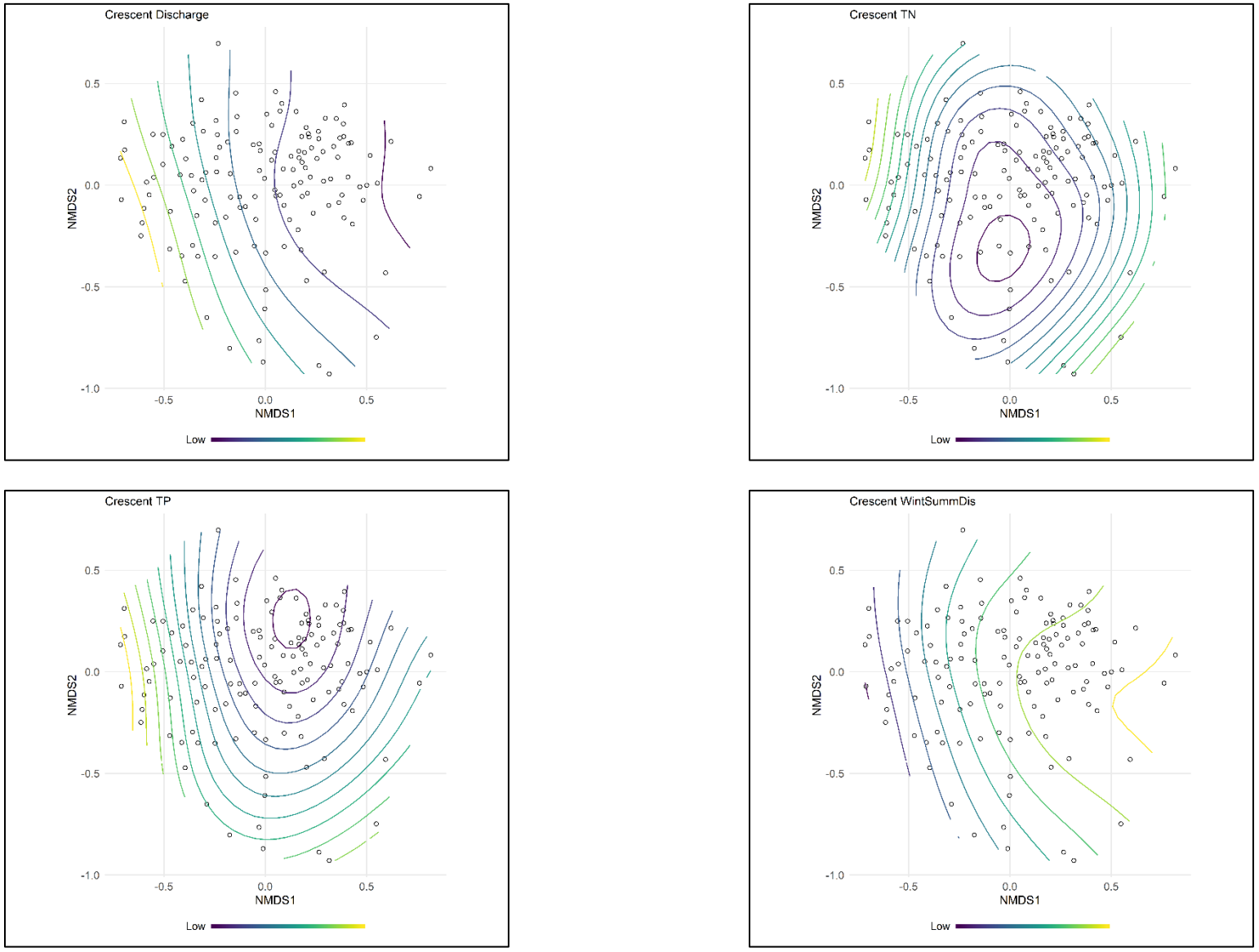


Figure 37. Surface plots for Crescent Lake NMDS ordination.

The regression and Random Forest models only accounted for a small part of the variability for *M. aeruginosa* in Crescent Lake (Table 4). NO_x appeared in both models, negatively associated in the multiple regression model, consistent with the ordination. Rain and DDays appeared in the regression model, positively associated with *M. aeruginosa*, which is consistent with the NMDS ordination. PO4-D, Discharge, and WintSummDis appeared in the Random Forest model; the first two likely positively associated from their vectors in the ordination, while WintSummDis may be negatively associated.

The regression and Random Forest models accounted for more of the variability for *M. pulverea incerta***.** Rain was negatively associated and WintSummDis positively associated in both models, consistent with the NMDS ordination.

The models accounted for little of the variability for Other Nitrogen-fixers. NO_x appeared in both models, negatively associated in the multiple regression model, again consistent with the ordination. Of the other variables appearing in the Random Forest model, PO4-D, Rain, and Discharge would appear to be negatively related from the NMDS ordination; TN and WintSummDis may be positively related.

The regression and Random Forest models accounted for more of the variability for *Cylindrospermopsis-Raphidiopsis*, and even more for Other Cyanobacteria and Total Cyanobacteria. There were some similarities in the models for these three taxa. The major environmental factors were negatively associated with these taxonomic groups, including Rain, Discharge, and inorganic nutrients. Positive factors that appeared in some of the models included TN and DDays.

Overall, these analyses suggest that low discharges are key conditions for development of high biovolumes in Crescent Lake for all the cyanobacterial groups except for *M. aeruginosa*. Crescent Lake has a longer average water replacement time than Lake George, which may explain why *M. aeruginosa* did not appear to respond to discharge changes in Crescent Lake but was negatively associated with them in Lake George. The NMDS ordination indicated a positive association of *M. aeruginosa* with TP but that term did not appear in the regression analyses. As with Lake George, the NMDS ordisurf contour plots indicate substantial nonlinearity or even non-monotonicity for both TP and TN, which may explain their low contribution to the regression models. For the cyanobacterial taxa other than *Microcystis* spp., a consistent positive association was with TN, particularly in the Random Forest analyses, which may be detecting nonlinear relationships of TN with the cyanobacterial ordination (Figure 37). There were also consistent positive associations of WintSummDis and DDays with cyanobacteria.

Table 4. Crescent Lake multiple regression analysis and Random Forest RFE results. Only included the 6 highest importance parameters for the RFE models.

Multiple Regression		**Random Forest RFE**	
Taxon: *Microcystis aeruginosa*			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	15.5%	Model	10.3%
Rain	+ 9.3%	NO_x	14.9
DDays	+ 4.9%	PO_4-D	12.1
NO_x	− 1.3%	Discharge	7.4
		WintSummDis	6.9
Taxon: *Microcystis pulverea incerta*			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	27.8%	Model	55.1%
Rain	− 15.7%	Rain	22.7
WintSummDis	+ 12.1%	WintPrevDis	20.9
		WintSummDis	18.4
Taxon: Other Nitrogen-fixers			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	6.2%	Model	11.1%
NO_x	− 6.2%	NO_x	9.0
		PO_4-D	8.4
		Rain	7.7
		TN	6.8
		Discharge	6.0
		WintSummDis	4.7
Taxon: *Cylindrospermopsis-Raphidiopsis*			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	33.8%	Model	24.1%
PO_4-D	− 19.6%	TN	8.6
Rain	− 12.4%	Rain	7.8
TN	+ 1.8%	PO_4-D	7.3
		Discharge	6.4
		WintSummDis	5.9
		DDays	5.0

Table 4 (continued).

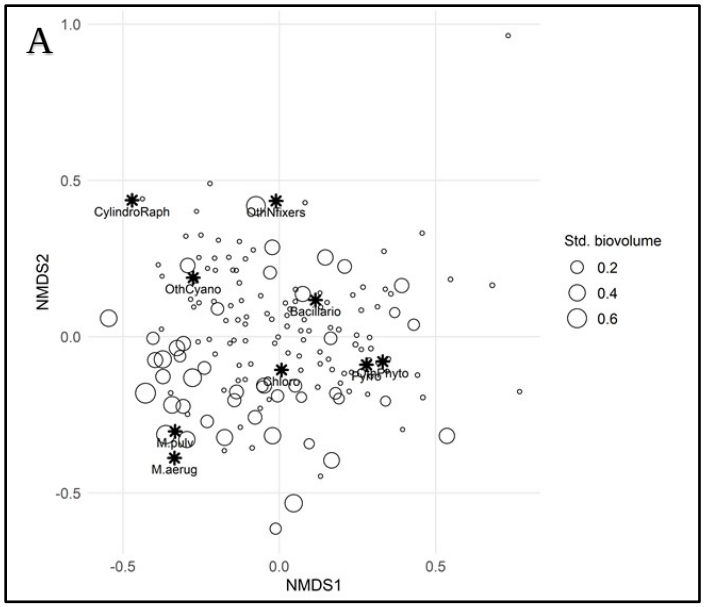
Multiple Regression		**Random Forest RFE**	
Taxon: Other Cyanobacteria			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	46.0%	Model	49.5%
Discharge	− 28.4%	Rain	19.8
PO_4-D	− 6.5%	TN	14.8
Rain	− 6.2%	PO_4-D	14.5
DDays	+ 5.0%	NO_x	12.5
		Discharge	7.9
		WintPrevDis	6.1
Taxon: Total Cyanobacteria			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	46.8%	Model	49.9%
NO_x	− 35.8%	Rain	17.9
DDays	+ 4.0%	NO_x	15.9
PO_4-D	− 3.9%	TN	15.9
Rain	− 3.0%	PO_4-D	9.7
		Discharge	7.5
		DDays	6.4

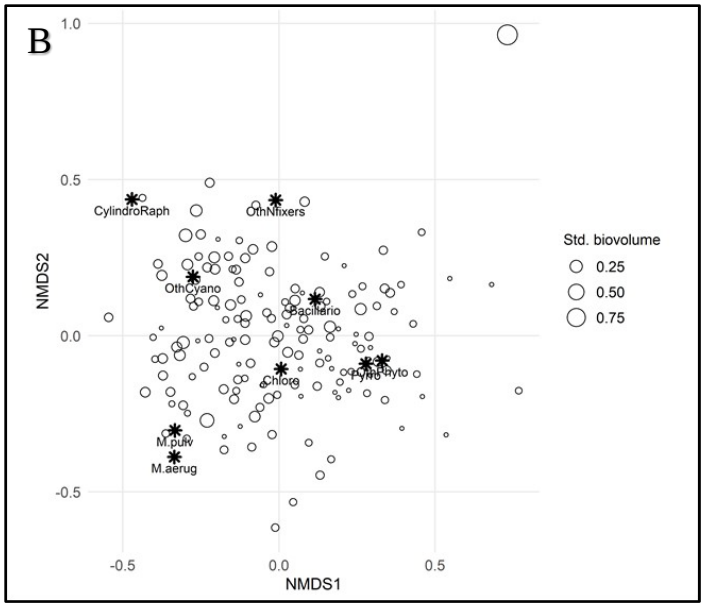
St. Johns River at Racy Point

Results of the NMDS ordination for the St. Johns River at Racy Point are shown in Figure 38 and Figure 39. The centroid for *M. aeruginosa* is in the lower left quadrant, as are most of its relatively high standardized biovolumes. Centroids for the other cyanobacterial taxa are also on the left side, except that for Other Nitrogen-fixers, which is upper center. That position for the Other Nitrogen-fixer centroid seems to be influenced by the highest biovolume, which is at the upper right, but most of the other relatively high biovolumes are on the left side.

The vector and ordisurf plots show Discharge increasing to the lower right, along with associated parameters Rain, water color, and inorganic nutrients. Conductivity, TN, and TNTP, Temp, and DDays increase toward the upper left, under low discharge conditions. There is less nonlinearity in the TN and TP ordisurf contour plots at Racy Point, both tending to increase most toward the upper right. Wind-D was the only vector that was not statistically significant.

The cyanobacterial centroids are located on the left side of the ordination plot (except Other Nitrogen-fixers), indicating an association with low discharges. The increase in TN toward the upper part of the plot may reflect nitrogen fixation by Other Nitrogen-fixers and *Cylindrospermopsis-Raphidiopsis*. The centroid for *M. aeruginosa* seems most associated with high WintSummDis, possibly indicating *M. aeruginosa* blooms are favored by high winter discharges followed by low summer discharges. The TP vector increases in the opposite direction to the *M. aeruginosa* centroid, although there is some indication in the ordisurf plot of a secondary increase in TP toward the lower left. The date vector also increases in the opposite direction to the *M. aeruginosa* centroid, indicating that most of its highest biovolumes were early in the period of record. The Other Nitrogen-fixer centroid is most closely associated with high TN and TP; in particular the highest biovolume point at the upper right is in the area of highest TN and TP contours.





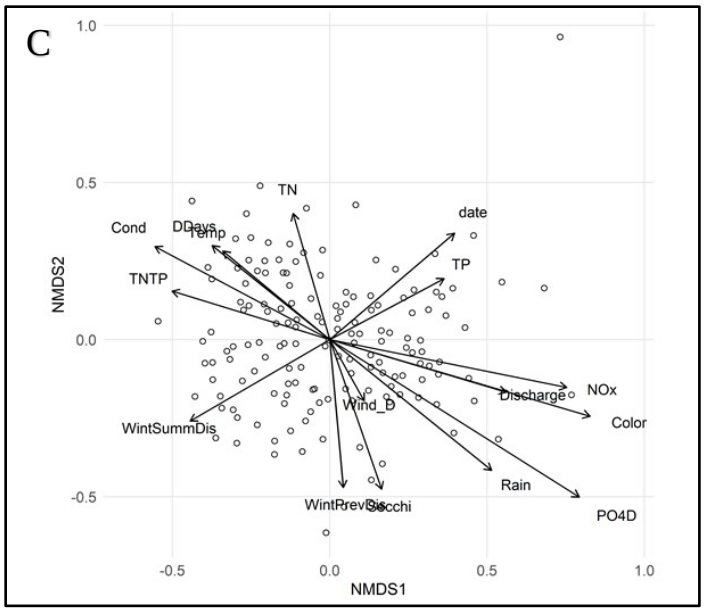


Figure 38. NMDS ordination for St. Johns River at Racy Point.

A. *Microcystis aeruginosa* bubble plot and taxa centroids.
B. Other Nitrogen-fixer bubble plot and taxa centroids.
C. Ordination plot with statistically significant environmental vectors.

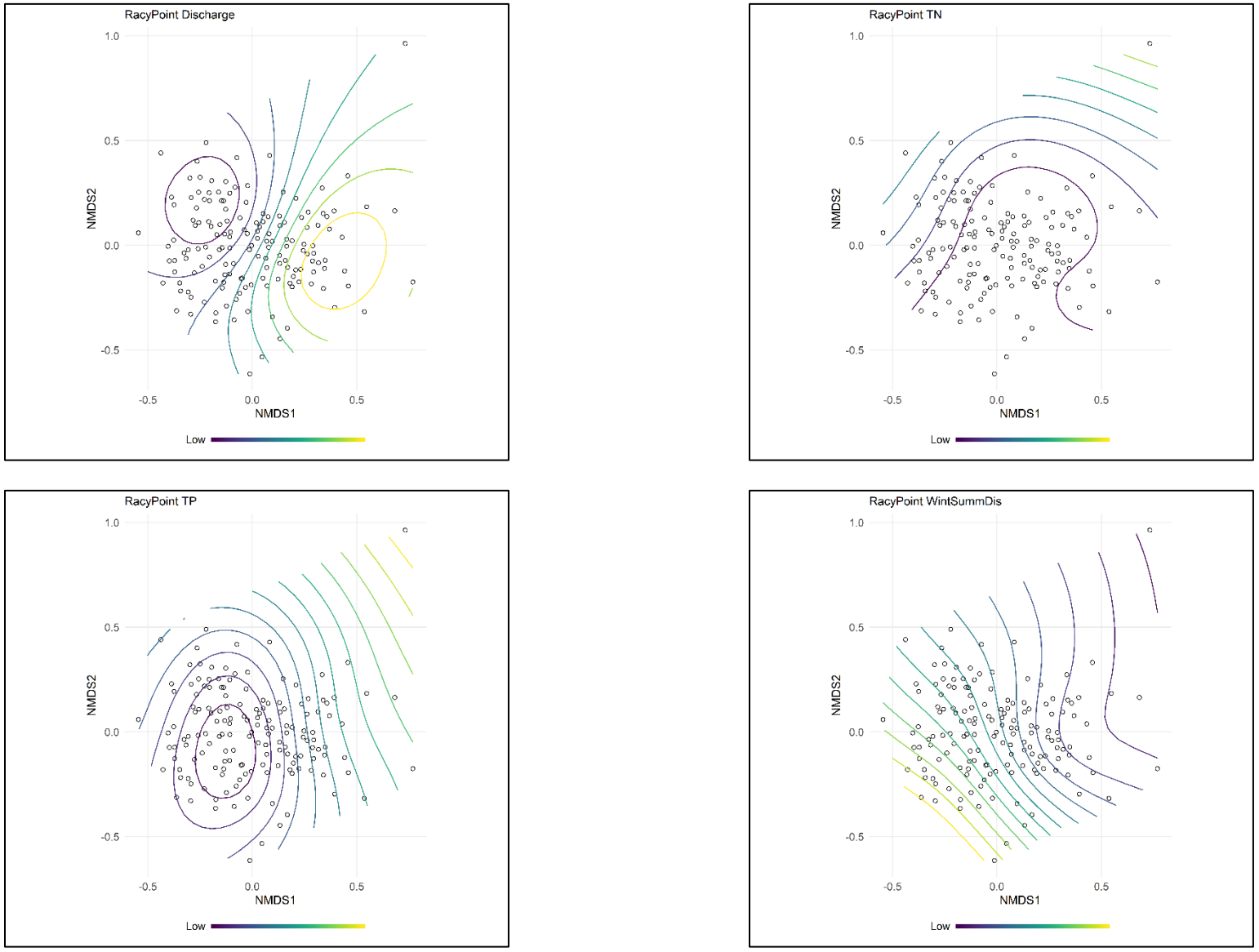


Figure 39. Surface plots for St. Johns River at Racy Point NMDS ordination.

Results of the multiple regression and Random Forest RFE models for the St. Johns River at Racy Point are summarized in Table 5.

The regression and Random Forest models only accounted for a small part of the variability for *M. aeruginosa* at Racy Point. Both models included WintPrevDis, and the Random Forest model included WintSummDis, which is consistent with the NMDS ordination.

The regression and Random Forest models accounted for more of the variability for *M. pulverea incerta***.** Both models included WintPrevDis, and the Random Forest model included WintSummDis, which is consistent with the NMDS ordination. NO_x was negatively associated with *M. pulverea incerta*, again consistent with the ordination. Both models also included TNTP, more weakly related, consistent with the vector increasing towards the right side of the NMDS ordination.

The models only accounted for a small part of the variability for Other Nitrogen-fixers, although regression did better than Random Forest. Although the Random Forest model included several factors, the negative model R^2 indicated they did not explain a significant part of the variability. TP was positively associated, and PO_4-D was negatively associated in the regression model, again consistent with the ordination.

The regression and Random Forest models accounted for more of the variability for *Cylindrospermopsis-Raphidiopsis*, and even more for Other Cyanobacteria and Total Cyanobacteria. There were some similarities in the models for these three taxa. Water color and PO_4-D were negatively related to these taxa. TP and TN appeared in several of the models, although TP was more important in the regression models, but TN was more important in the Random Forest models. TP was positively related in regression models for *Cylindrospermopsis-Raphidiopsis* and Total Cyanobacteria.

Similar to the upstream lakes, these analyses suggest that low discharges are key conditions for development of high biovolumes in at Racy Point for all the cyanobacterial groups. High TN and TP are associated with high biovolumes most of the cyanobacterial taxa, but not with *Microcystis* spp. These high TN concentrations may at least partially reflect nitrogen-fixation. Runoff from the TCAA also could affect nutrient concentrations at this site. High WintSummDis and high WintPrevDis were most closely associated with higher biovolumes on *Microcystis* spp. As at the upstream sites, the multiple regression and Random Forest models accounted for relatively little of the variability for *M. aeruginosa* and Other Nitrogen-fixers.

Table 5. St. Johns River at Racy Point multiple regression analysis and Random Forest RFE results.

Only included the 6 highest importance parameters for the RFE models.

Multiple Regression		**Random Forest RFE**	
Taxon: *Microcystis aeruginosa*			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	6.2%	Model	20.1%
WintPrevDis	+ 6.2%	WintPrevDis	13.5
		WintSummDis	9.4
		NO_x	6.7
		PO_4-D	5.8
		TNTP	5.0
		Rain	3.8
Taxon: *Microcystis pulverea incerta*			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	20.4%	Model	43.9%
NO_x	− 9.6%	WintPrevDis	18.4
WintPrevDis	+ 7.9%	WintSummDis	13.1
TNTP	+ 2.9%	NO_x	9.9
		TNTP	8.5
		PO_4-D	6.1
		Rain	5.5
Taxon: Other Nitrogen-fixers			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	24.0%	Model	− 4.6%
TP	+ 11.7%	TN	10.6
PO_4-D	− 10.8%	WintSummDis	2.2
DDays	− 1.5%	Color	2.1
		NO_x	1.8
		PO_4-D	1.6
		TP	0.9

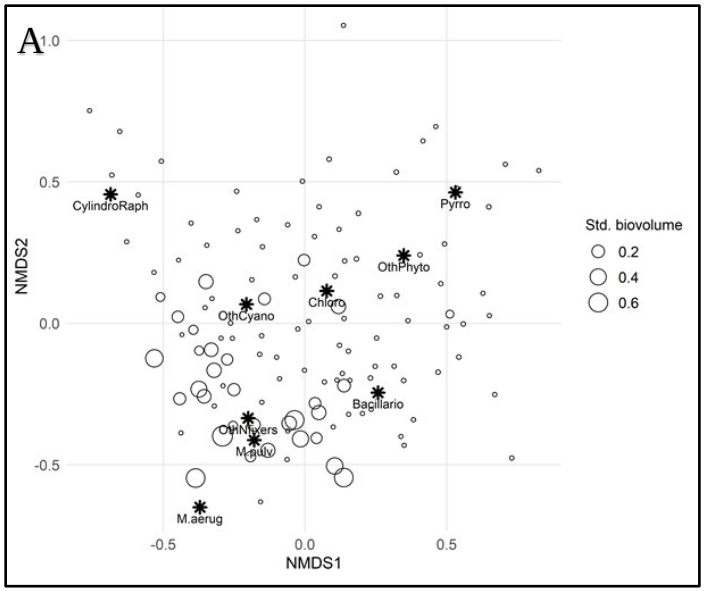
Table 5 (continued).

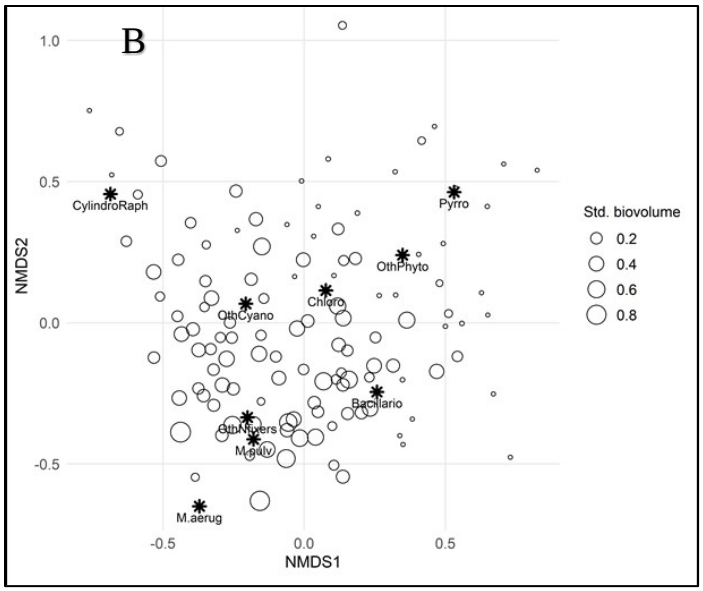
Multiple Regression		**Random Forest RFE**	
Taxon: *Cylindrospermopsis-Raphidiopsis*			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	36.9%	Model	28.1%
Color	− 15.0%	TN	6.4
PO_4-D	− 7.7%	Discharge	5.5
TP	+ 7.7%	PO_4-D	5.3
WintPrevDis	− 3.4%	TP	5.3
TN	+ 3.1%	TNTP	4.4
		WintPrevDis	4.4
Taxon: Other Cyanobacteria			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	42.8%	Model	47.9%
PO_4-D	− 22.5%	TN	12.5
DDays	+ 14.8%	Color	11.1
Rain	− 5.5%	TNTP	9.9
		PO_4-D	9.3
		NO_x	6.9
Taxon: Total Cyanobacteria			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	60.2%	Model	37.1%
Color	− 23.6%	PO_4-D	13.2
TP	+ 14.7%	TN	12.5
PO_4-D	− 11.5%	TNTP	8.7
TN	+ 4.6%	TP	8.1
NO_x	− 3.3%	NO_x	7.0
WintSummDis	− 2.4%	Color	5.5

Doctors Lake

Results of the NMDS ordination for Doctors Lake are shown in Figure 40 and Figure 41. The centroid for *M. aeruginosa* is in the lower left quadrant, as are most of its relatively high standardized biovolumes. Centroids for Other Nitrogen-fixers and *M. pulverea incerta* are also in the lower left quadrant, while those for the other cyanobacterial taxa are in the upper left quadrant.

Discharges increase toward the lower left in the Doctors Lake ordination. Unlike the freshwater sites previously discussed, Discharge seems positively associated with both *M. aeruginosa* and Other Nitrogen-fixers, as well as with TN. The close association of the TN vector with Other Nitrogen-fixers, suggests that they are more responsible for nitrogen-fixation than *Cylindrospermopsis-Raphidiopsis* in Doctors Lake; this is consistent with the substantially lower biovolumes of *Cylindrospermopsis-Raphidiopsis* in Doctors Lake and the other oligohaline sites. Rain and WintPrevDis also appear positively associated with these taxa. The TP vector was not statistically significant, but the ordisurf contours showed nonlinearity for TP, with higher contours toward both the upper right and lower left, so the centroids for *M. aeruginosa* and Other Nitrogen-fixers appear positively associated with higher concentrations of TP (Figure 40). *Cylindrospermopsis-Raphidiopsis* and Other Cyanobacteria appear most closely associated with higher temperatures and TNTP. Environmental variables that appear most negatively associated with cyanobacteria include conductivity, which is also opposite the trend found with the freshwater sites. Other environmental variables negatively associated with cyanobacteria are Secchi transparency and PO_4-D. The vector for Wind-D was also not statistically significant.





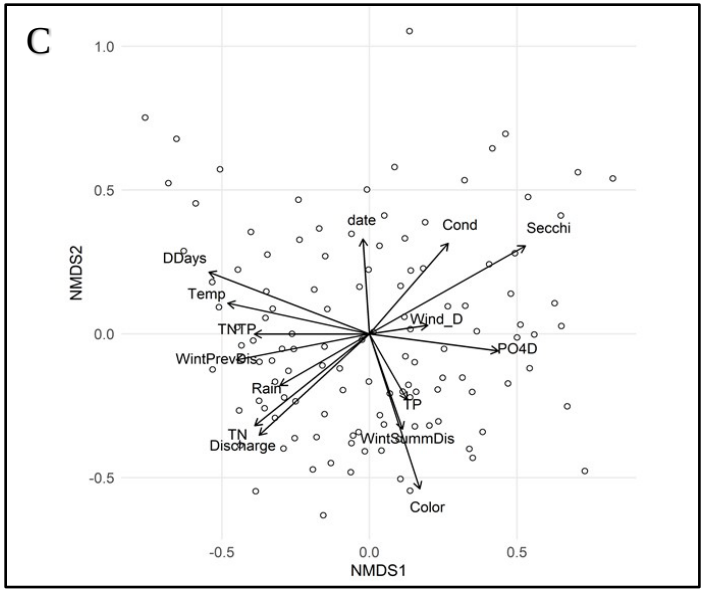


Figure 40. NMDS ordination for Doctors Lake.

A. *Microcystis aeruginosa* bubble plot and taxa centroids.
B. Other Nitrogen-fixer bubble plot and taxa centroids.
C. Ordination plot with statistically significant environmental vectors.

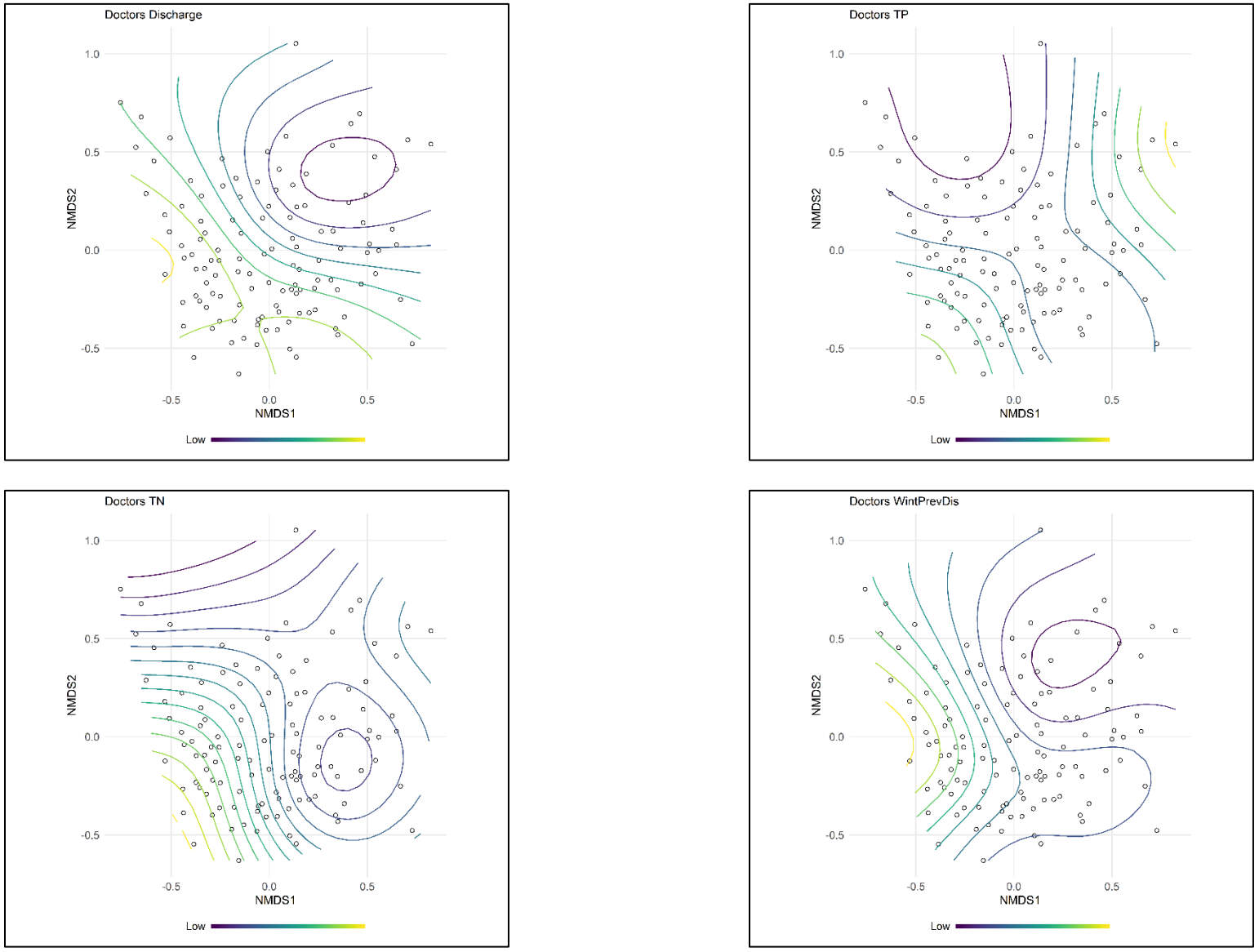


Figure 41. Surface plots for Doctors Lake NMDS ordination.

Results of the multiple regression and Random Forest RFE models for the Doctors Lake are summarized in Table 6. The Random Forest model had a negative R^2, not accounting for any significant part of the variability for *M. aeruginosa* in Doctors Lake, but the multiple regression did somewhat better. The multiple regression model included Rain and TP as positively associated, which is consistent with the NMDS ordination. The Random Forest model also included TP, but Discharge instead of Rain.

The regression and Random Forest models accounted for more of the variability for *M. pulverea incerta***.** Both models included TN, NO_x, and Color. NO_x was negatively associated with *M. pulverea incerta* but was not used in the final ordination (deleted because it was not significant in an earlier ordination and had missing records). The Random Forest model had WintPrevDis as the most important variable, which is consistent with the NMDS ordination.

The models only accounted for a small part of the variability for Other Nitrogen-fixers. TN had a positive influence in both models, consistent with the ordination. The Random Forest model also included Rain, Discharge and WintPrevDis, presumably as positive influences.

The regression and Random Forest models accounted for less of the variability for the other cyanobacterial groups than occurred for the freshwater sites. The regression models were stronger than the Random Forest models for these groups. For *Cylindrospermopsis-Raphidiopsis*, DDays was the most important factor in both models, consistent with the ordination. TP (negative factor) also appeared in both models, also consistent with the ordination. For Other Cyanobacteria and Total Cyanobacteria, TN (positive) and PO_4-D (negative) were among the most important variables in both models. Discharge, Rain, and WintPrevDis also appeared in the Random Forest models for these taxa.

The analysis results for Doctors Lake show some similarities and some differences from the results for the freshwater sites. Similar to the freshwater sites, these analyses indicate that TN and TP are positively associated with *M. aeruginosa* and Other Nitrogen-fixers in Doctors Lake. Nitrogen-fixation probably contributes to the association of TN with Other Nitrogen-fixers. Contrary to the freshwater sites, Discharge was positively associated, and conductivity was negatively associated with these cyanobacterial taxa in Doctors Lake. Doctors Lake has a much longer water replacement time than the freshwater lakes; under high discharge conditions the water residence time in the freshwater sites may be too short to allow bloom development. However, residence time is always long in Doctors Lake, and high discharges may supply additional nutrients to support algal growth. In the freshwater sites, higher conductivity is associated with low discharges, but it probably never gets high enough there to inhibit cyanobacteria. However, in oligohaline Doctors Lake the conductivity probably does get high enough to inhibit at least some of the cyanobacterial taxa. Also different in Doctors Lake was that PO_4-D appeared positively associated with conductivity and negatively associated with Discharge, but the opposite trends occurred in the freshwater sites. At the freshwater sites PO_4-D appears associated with watershed runoff, but in Doctors Lake it may be associated with saltwater intrusion.

Table 6. Doctors Lake multiple regression analysis and Random Forest RFE results. Only included the 6 highest importance parameters for the RFE models.

Multiple Regression		**Random Forest RFE**	
Taxon: *Microcystis aeruginosa*			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	19.0%	Model	− 5.7%
Rain	+ 9.6%	WintPrevDis	3.0
TP	+ 5.4%	TN	2.8
Wind_D	− 3.9%	DDays	2.5
		Discharge	1.9
		NO_x	1.6
		TP	1.2
Taxon: *Microcystis pulverea incerta*			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	25.8%	Model	19.7%
NO_x	− 9.7%	WintPrevDis	10.6
TN	+ 8.4%	TN	9.5
Color	+ 7.7%	NO_x	7.9
		Color	6.4
		DDays	4.2
		Rain	4.1
Taxon: Other Nitrogen-fixers			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	13.5%	Model	14.6%
TN	+ 5.6%	Rain	9.9
PO_4-D	− 4.8%	TN	8.8
DDays	− 3.2%	Discharge	5.8
		WintPrevDis	4.5

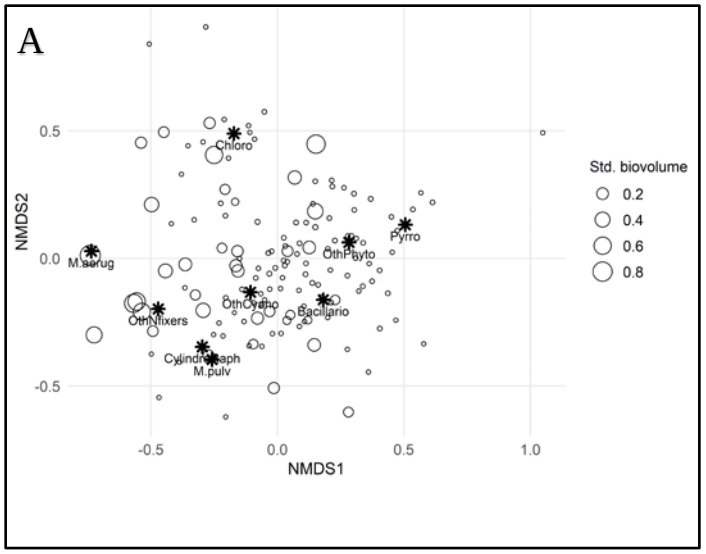
Table 6 (continued).

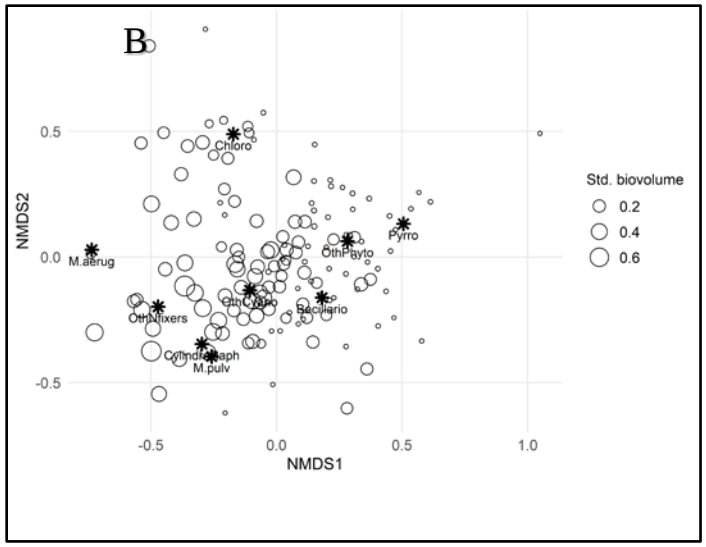
Multiple Regression		**Random Forest RFE**	
Taxon: *Cylindrospermopsis-Raphidiopsis*			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	23.4%	Model	12.3%
DDays	+ 19.9%	DDays	8.6
TP	− 3.4%	WintPrevDis	5.9
Wind_D	+ 0.1%	TP	4.8
		Rain	4.4
		PO_4-D	2.9
		Color	2.9
Taxon: Other Cyanobacteria			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	26.7%	Model	13.5%
PO_4-D	− 11.2%	TN	10.8
TN	+ 9.8%	Color	4.6
DDays	+ 5.8%	WintPrevDis	4.4
		PO_4-D	3.8
		NO_x	3.7
		Discharge	3.1
Taxon: Total Cyanobacteria			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	33.8%	Model	26.6%
TN	+ 16.9%	TN	15.8
PO_4-D	− 12.6%	Color	6.2
Wind_D	− 4.4%	Discharge	6.1
		WintPrevDis	5.8
		PO_4-D	4.8
		NO_x	4.7

St. Johns River at Mandarin Point

Results of the NMDS ordination for the St. Johns River at Mandarin Point are shown in Figure 42 and Figure 43. The centroid for *M. aeruginosa* is on the far-left side. Relatively high standardized biovolumes appear more widely scattered than at previously discussed sites, but most are on the left side of the plot. Centroids for other cyanobacterial taxa are in the lower left quadrant.

The ordination plot is similar to the upstream freshwater sites in some respects. Discharge increases to the upper right, the opposite direction to the cyanobacterial centroids, and associated with that are NO_x and Water color. Environmental factors that seem most positively associated with cyanobacteria are WintSummDis and water temperature. There are fewer significant relationships of environmental vectors to the ordination plot at Mandarin Point. Vectors that were not significantly linearly related here, but often were upstream, include TN, TP, TNTP, and WintPrevDis. However, both TN and TP ordisurf plots show substantial nonlinearity with some high contours in the lower left quadrant near cyanobacterial centroids (Figure 43). Conductivity increases to the lower right, away from the cyanobacterial centroids, somewhat similar to Doctors Lake. The vectors for DDays and Wind-D were also not statistically significant.





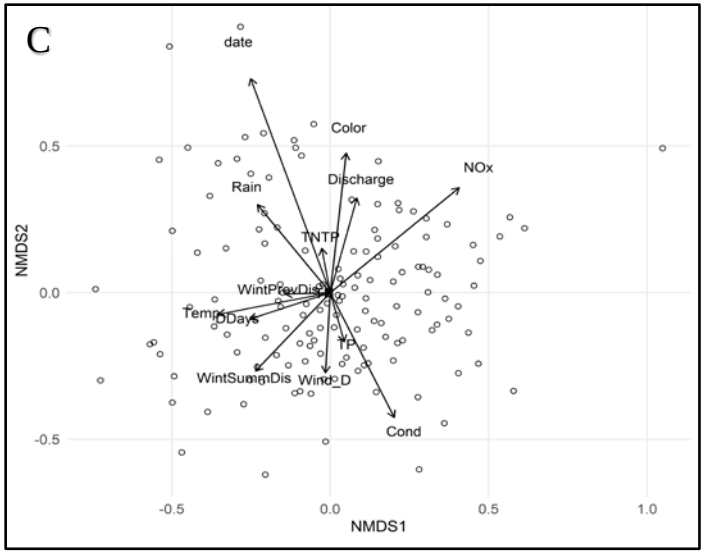


Figure 42. NMDS ordination for St. Johns River at Mandarin Point.

A. *Microcystis aeruginosa* bubble plot and taxa centroids.
B. Other Nitrogen-fixer bubble plot and taxa centroids.
C. Ordination plot with statistically significant environmental vectors.

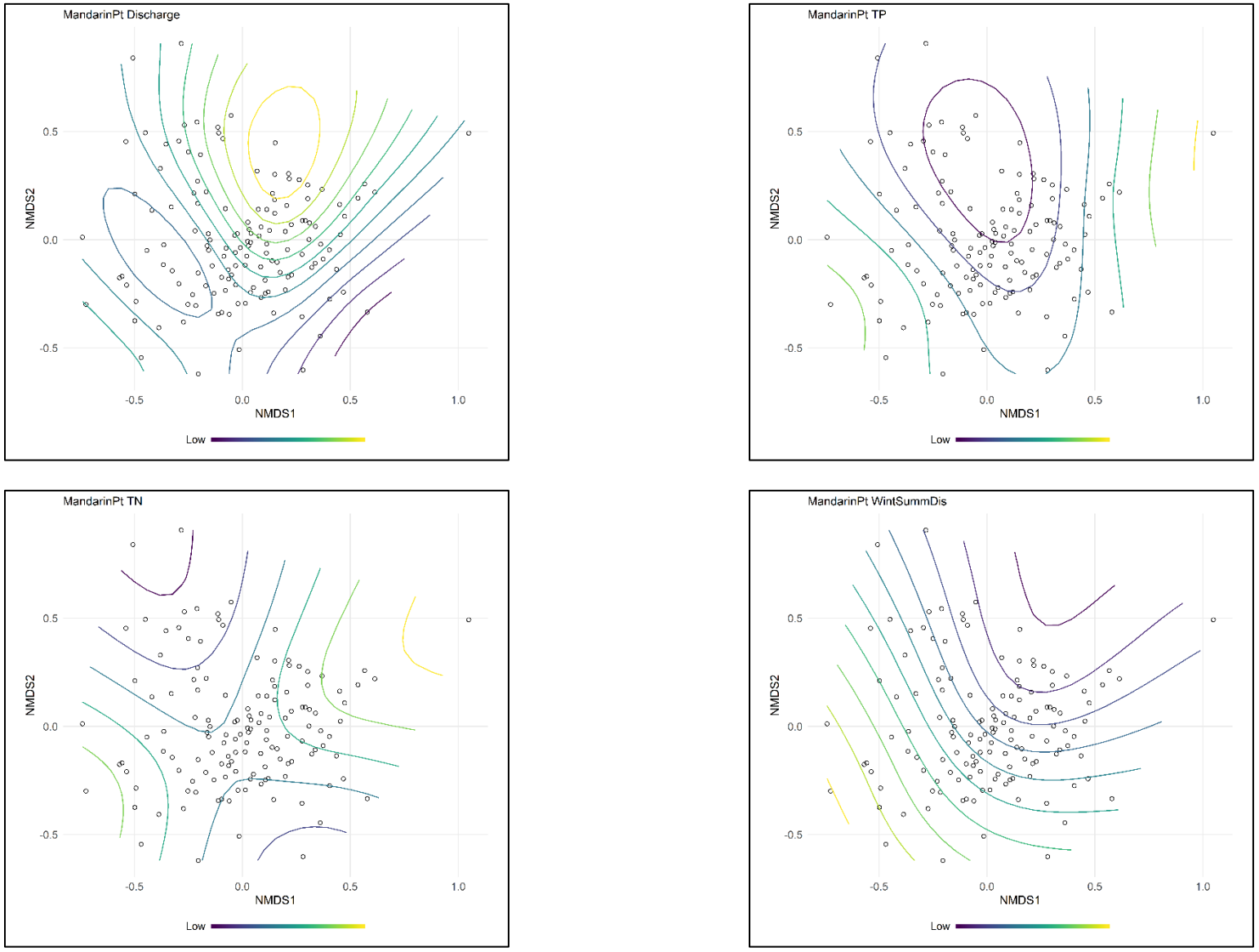


Figure 43. Surface plots for St. Johns River at Mandarin Point NMDS ordination.

Results of the multiple regression and Random Forest RFE models for the St. Johns River at Mandarin Point are summarized in Table 7. Both the regression and Random Forest models included WintPrevDis as positively related to *M. aeruginosa*, although that was not significant in the ordination. The Random Forest model had NO_x as the strongest factor, presumably a negative factor. Rain was positively associated in the regression model. The Random Forest model included Discharge as a relatively low importance factor; appearing to be a negative relationship from the ordination.

For *M. pulverea incerta***,** both models included TN as the most important positive factor. Although TN was not a significant vector in the ordination, some of the high contours are near the *M. pulverea incerta* centroid. Both models included NO_x and Color, which appeared negatively related in the ordination.

Both the regression and Random Forest models accounted for very little of the variability for Other Nitrogen-fixers. Both models included NO_x as a negative factor. The R^2 was negative for the Random Forest model (which we interpret as not explaining any of the variability).

Both the regression and Random Forest models accounted for very little of the variability for *Cylindrospermopsis-Raphidiopsis* (again a negative R^2 for the Random Forest model). For Other Cyanobacteria the regression model accounted for little of the variability, while the Random Forest model accounted for somewhat more. NO_x appeared as a negative factor in both models. Conversely, for Total Cyanobacteria the Random Forest model the regression model did somewhat better, including TN and WintSummDis as positive factors. WintSummDis and WintPrevDis appeared as factors in the models for both Other and Total Cyanobacteria.

Overall, relationships between cyanobacteria and environmental factors at Mandarin Point appear weak and not very consistent in the different analyses. As with the upstream sites, cyanobacteria appear negatively related to Discharge. TN, TP, WintSummDis, and WintPrevDis show some weak positive relationships with cyanobacteria. As with Doctors Lake, the NMDS ordination suggests a negative relationship of cyanobacteria with conductivity at this oligohaline site.

Table 7. St. Johns River at Mandarin Point multiple regression analysis and Random Forest RFE results.

Only included the 6 highest importance parameters for the RFE models.

Multiple Regression		**Random Forest RFE**	
Taxon: *Microcystis aeruginosa*			
Parameter	R^2	Parameter	R^2 / Importance
Model	22.9%	Model	17.2%
WintPrevDis	+ 10.3%	NO_x	9.3
Rain	+ 6.2%	WintPrevDis	6.8
Wind_D	− 5.6%	TNTP	5.2
NO_x	− 0.7%	Wind_D	5.0
		Discharge	4.6
Taxon: *Microcystis pulverea incerta*			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	27.4%	Model	30.7%
TN	+ 13.2%	TN	12.9
Color	− 4.5%	NO_x	12.7
NO_x	− 4.0%	Color	10.1
Wind_D	+ 3.1%		
WintSummDis	+ 2.5%		
Taxon: Other Nitrogen-fixers			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	9.4%	Model	− 4.6%
NO_x	− 9.4%	TP	6.3
		WintPrevDis	5.5
		TNTP	5.3
		NO_x	4.4
		Discharge	2.0

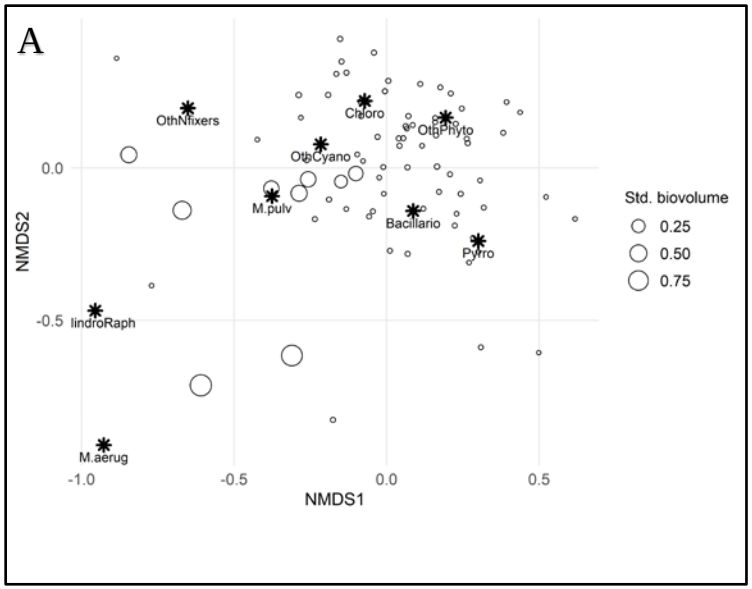
Table 7 (continued).

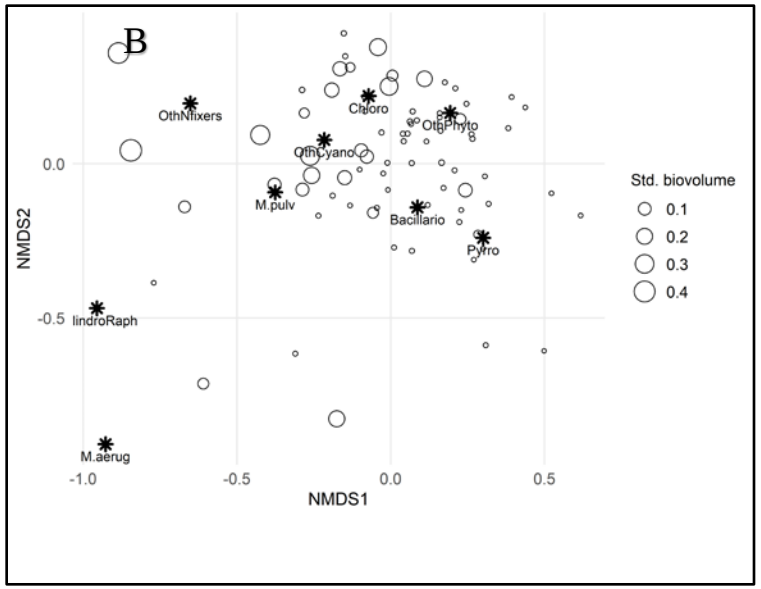
Multiple Regression		**Random Forest RFE**	
Taxon: *Cylindrospermopsis-Raphidiopsis*			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	4.1%	Model	− 5.4%
Color	− 4.1%	TP	7.2
		WintPrevDis	7.1
		TNTP	7.0
		NO_x	3.6
Taxon: Other Cyanobacteria			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	5.9%	Model	18.8%
NO_x	− 5.9%	NO_x	10.3
		WintPrevDis	8.3
		WintSummDis	7.1
		TP	5.1
Taxon: Total Cyanobacteria			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	20.5%	Model	15.4%
PO_4-D	− 10.4%	NO_x	9.5
TN	+ 7.1%	WintPrevDis	6.0
WintSummDis	+ 3.0%	TP	4.4
		DDays	3.6

St. Johns River at Piney Point

Results of the NMDS ordination for the St. Johns River at Piney Point are shown in Figure 44 and Figure 45. JAXSJR40, referenced in Figure 45, is the station name. The centroid for *M. aeruginosa* is at the bottom left. As in other cases with many zero values, that centroid seems to be more on the periphery of the plot than would be expected from the bubble plot, although nearly all the higher biovolumes are in the lower left quadrant. The centroid for Other Nitrogen-fixers is in the upper left quadrant, as are most of the higher biovolumes for that taxon. Centroids other cyanobacterial taxa are also on the left side of the ordination plot.

The environmental vector plot shows some differences from the upstream sites. Discharge increases to the upper left, in the same direction as some of the cyanobacterial centroids, and opposite to the direction of inorganic nutrients. Rain, TN, TNTP, and WintPrevDis also increase to the upper left. A close association of the TN vector with Other Nitrogen-fixers, suggests that they are more responsible for nitrogen-fixation than *Cylindrospermopsis-Raphidiopsis* at Piney Point. Conductivity increases to the lower right, away from the cyanobacterial centroids. In these respects, the vector plot is similar to that for Doctors Lake. The environmental factor that seems most positively associated with *M. aeruginosa* is DDays. The TP vector is not significantly linearly related to the ordination axes, but the TP ordisurf plot shows nonlinearity, with some moderately high contours near the *M. aeruginosa* centroid (Figure 45). Other vectors that were not statistically significant are Temp, WintSummDis, Wind-D, and date.





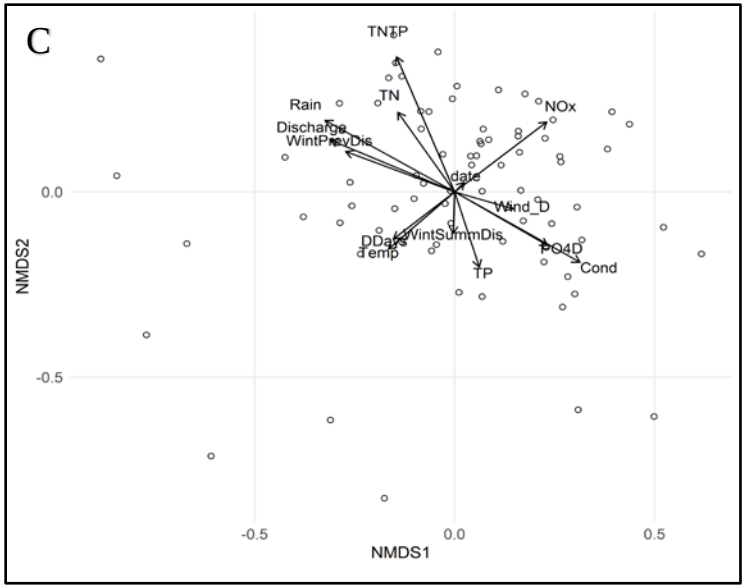


Figure 44. NMDS ordination for St. Johns River at Piney Point.

A. *Microcystis aeruginosa* bubble plot and taxa centroids.
B. Other Nitrogen-fixer bubble plot and taxa centroids.
C. Ordination plot with statistically significant environmental vectors.

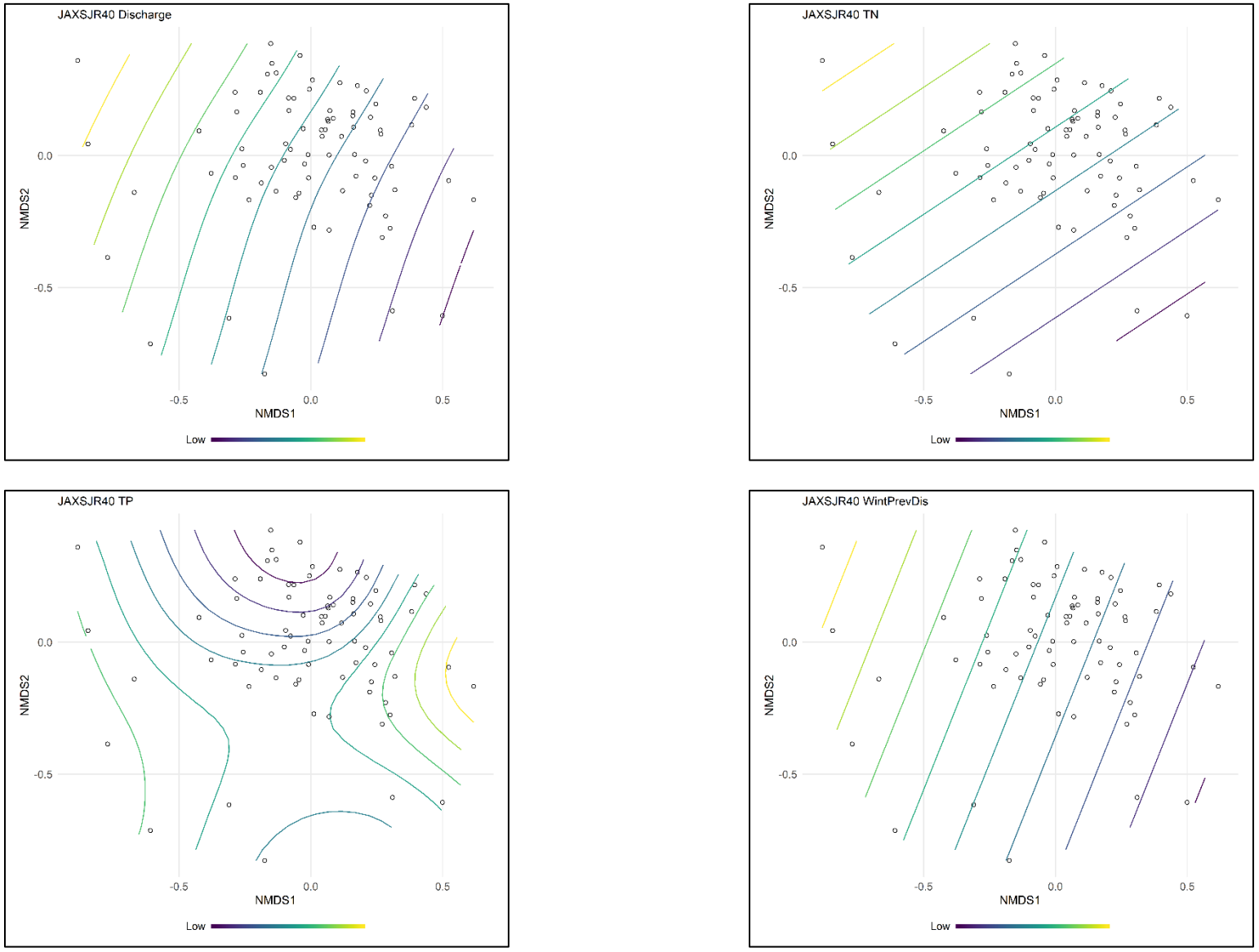


Figure 45. Surface plots for St. Johns River at Piney Point NMDS ordination.

Results of the multiple regression and Random Forest RFE models for the St. Johns River at Piney Point are summarized in Table 8. Both the regression and Random Forest model accounted for little of the variability for *M. aeruginosa*. The regression model included only Rain as a positive factor, which was not consistent with the ordination. The Random Forest model included TN and TNTP, which from the ordination would appear negatively related to *M. aeruginosa*. TP and DDays may be positively related.

For *M. pulverea incerta***,** the regression model accounted for more of the variability than the Random Forest model and included TN as the most important positive factor. TN also appeared in the Random Forest model. Both models included NO_x as a negative factor, consistent with the ordination. Both models included PO_4-D as a positive factor, which does not appear consistent with the ordination. TP and TNTP also appeared in the Random Forest model.

For Other Nitrogen-fixers the regression model included WintSummDis as a positive factor, which was not significant in the ordination, although the ordisurf plot showed nonlinearity for WintSummDis, with some relatively high contours toward the left side. The Random Forest model had a negative R^2 for Other Nitrogen-fixers. Both models showed PO_4-D as the most important negative factor, consistent with the ordination.

For *Cylindrospermopsis-Raphidiopsis*, most of the selected factors were negative for both models. The negative relationship with TNTP again suggests that *Cylindrospermopsis-Raphidiopsis* contributed little to nitrogen-fixation at Piney Point. For Other Cyanobacteria and Total Cyanobacteria, the models accounted for little of the variability, and most of the important factors were inorganic nutrients negatively related to both taxa. Both Random Forest models included TP (probably as a negative factor) and TN (likely a positive factor). Rain appeared as a positive factor for Total Cyanobacteria in the regression model.

The analysis results for the St. Johns River at Piney Point are most similar to those for Doctors Lake. There are a few similarities of both this site and Doctors Lake with the freshwater sites. Similar to the freshwater sites, high TN is positively associated with Other Nitrogen-fixers, and TP may show an association with *M. aeruginosa*. Nitrogen-fixation probably contributes to the association of TN with Other Nitrogen-fixers. Contrary to the upstream sites other than Doctors Lake, Discharge was positively associated, and conductivity was negatively associated, with these cyanobacterial taxa at both Piney Point and Doctors Lake. The reason for the positive relationship with discharges may be somewhat different between these two sites. As previously discussed, residence time is always long in Doctors Lake, and high discharges may supply additional nutrients to support algal growth. At Piney Point high river discharges may serve to transport cyanobacteria downstream into the oligohaline zone and reduce conductivity allowing them to thrive if residence time is sufficient. This appears to have been the case with the previously discussed 2013 *M. aeruginosa* bloom at Piney Point, which occurred after a period of relatively high discharges. In the freshwater sites, higher conductivity is associated with low discharges, but it probably never gets high enough there to inhibit cyanobacteria. However, in oligohaline St. Johns River at Piney Point and in Doctors Lake the conductivity probably does get high enough to inhibit at least

some of the cyanobacterial taxa. Another similarity between Piney Point and Doctors Lake was a negative association between PO_4-D and Discharge, while the freshwater sites had a positive association. At the freshwater sites PO_4-D appears associated with watershed runoff, but in oligohaline Doctors Lake and Piney Point it may be associated with tidal mixing.

Table 8. St. Johns River at Piney Point multiple regression analysis and Random Forest RFE results.

Only included the 6 highest importance parameters for the RFE models.

Multiple Regression		**Random Forest RFE**	
Taxon: *Microcystis aeruginosa*			
Parameter	R^2	Parameter	R^2 / Importance
Model	9.9%	Model	11.3%
Rain	+ 9.9%	TN	7.3
		TNTP	6.8
		Color	5.8
		DDays	5.4
		TP	3.9
Taxon: *Microcystis pulverea incerta*			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	30.6%	Model	7.7%
TN	+ 12.6%	NO_x	8.6
NO_x	− 11.2%	TP	5.4
PO_4-D	+ 6.8%	TN	4.4
		PO_4-D	4.1
		TNTP	3.9
Taxon: Other Nitrogen-fixers			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	22.4%	Model	− 5.3%
PO_4-D	− 14.4%	PO_4-D	5.2
WintSummDis	+ 7.9%	NO_x	3.5
		TNTP	1.2
		Color	0.9
		WintPrevDis	0.5
Taxon: *Cylindrospermopsis-Raphidiopsis*			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	23.6%	Model	18.3%
TNTP	− 12.1%	PO_4-D	8.4
PO_4-D	− 11.4%	TN	7.6
TN	+ 0.1%	NO_x	6.6

Table 8 (continued).

Taxon: Other Cyanobacteria			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	8.8%	Model	8.9%
NO_x	− 8.8%	TP	8.6
		NO_x	8.3
		PO_4-D	6.4
		TN	6.4
Taxon: Total Cyanobacteria			
Parameter	**R^2**	**Parameter**	**R^2 / Importance**
Model	18.6%	Model	10.7%
NO_x	− 11.2%	NO_x	8.8
Rain	+ 7.4%	PO_4-D	6.5
		TN	6.1
		TP	5.2
		DDays	4.4

DISCUSSION

CYANOBACTERIA DIFFERENCES AMONG SITES

There were substantial differences in cyanobacterial biovolumes and composition among the study sites. The oligohaline sites (Doctors Lake, and St. Johns River at Mandarin Point and at Piney Point) differed in having substantially lower average biovolumes and percent biovolumes of *Cylindrospermopsis-Raphidiopsis* and of Other Cyanobacteria than the freshwater sites (Lake George, Crescent Lake, and St. Johns River at Racy Point), although their biovolumes were higher at Doctors Lake than at the other oligohaline sites. However, *Microcystis aeruginosa* and Other Nitrogen-fixers representation was not always lower at oligohaline sites. At Doctors Lake *M. aeruginosa* had its highest average biovolume and Other Nitrogen-fixers average biovolume was within the range of the freshwater sites. Biovolumes of *M. aeruginosa* and Other Nitrogen-fixers were lower at the oligohaline river sites than at the freshwater sites, however the average percent biovolume of *M. aeruginosa* at the oligohaline river sites was similar to that at the freshwater sites.

One likely reason for these changes is differences in tolerance to higher salinity at the oligohaline sites. Average conductivity at the freshwater sites was around 1,000 μmhos/cm (salinity near 0.5 ppt). Conductivity averaged about 5,000 μmhos/cm (salinity near 2.7 ppt) at Doctors Lake and Mandarin Point and about 9,000 μmhos/cm (salinity near 5.2 ppt) at Piney Point. The among-site biovolume differences suggest higher salinity tolerance by *M. aeruginosa* and Other Nitrogen-fixers. The two highest *M. aeruginosa* biovolume peaks at Doctors Lake occurred at a conductivity of about 10,000 μmhos/cm (salinity near 6 ppt). The 2001 Doctors Lake Other Nitrogen-fixer bloom (dominated by *Dolichospermum*) occurred at a conductivity of 6,174 μmhos/cm (salinity near 3.6 ppt). These apparent salinity tolerances are not completely consistent with a recent compilation of cyanobacterial tolerances. Paerl et al. (2018) reported *Microcystis* and *Cylindrospermopsis* were tolerant to a low salinity range (0-4 ppt), but *Aphanizomenon*, *Dolichospermum*, and *Raphidiopsis* were tolerant to a low-moderate salinity range (0-16 ppt). However, other studies have found higher salinity tolerances of *Microcystis* and Other Nitrogen-fixers. Another literature summary reported salinity tolerances of *Microcystis* up to 25 ppt and *Anabaena* up to 15 ppt (Preece et al. 2017). A recent report found *M. aeruginosa* from Lake Okeechobee was tolerant to salinities up to 18 ppt, while *Dolichospermum circinale* was tolerant to 7.5 ppt (Rosen et al. 2018).

Several factors may contribute to the generally higher cyanobacterial biovolumes at Doctors Lake than at the other oligohaline sites. Doctors Lake has a shallower depth (about 3 m at the sample site) than at Mandarin Point (9 m) or Piney Point (5.5 m), resulting in higher light availability if the water column is mixed to the bottom. Doctors Lake may also tend to have more water column stratification due to a shorter estimated fetch length (average 4 km over a range of wind directions, as opposed to 12 km for Mandarin Point and 9 km for Piney Point). Doctors Lake also has a very long water replacement time (average 547 days not considering tidal flushing), allowing a long time for accumulation of phytoplankton biomass. We don't have water replacement time

estimates for the oligohaline river sites, but they are likely to be shorter due to high flows from upstream and greater tidal flushing.

Among the freshwater sites, Lake George had the highest average biovolume and percent biovolume of *Cylindrospermopsis-Raphidiopsis* and of Other Cyanobacteria, and the highest average biovolume Other Nitrogen-fixers. However, Crescent Lake had a higher average biovolume and percent biovolume of *M. aeruginosa* and a higher median and percent biovolume of Other Nitrogen-fixers. The contrast between higher average biovolume of Other Nitrogen-fixers in Lake George, but higher percent biovolume and median in Crescent Lake, is probably because of the extremely high biovolumes in Lake George during the May-June 2010 bloom. The taxa most prominent in Lake George, *Cylindrospermopsis-Raphidiopsis* and Other Cyanobacteria, are primarily filamentous species that do not tend to form surface blooms and are tolerant to low light levels (Havens et al. 1998; Burford et al. 2016). In contrast, the taxa that were more prominent in Crescent Lake, *M. aeruginosa* and Other Nitrogen-fixers, do tend to form surface blooms. Lake George likely has a more turbulent water column, due to high river flows and a longer fetch length (estimated as 15 km vs. 11 km in Crescent Lake), making it less suitable for development of surface blooms.

The St. Johns River at Racy Point had lower average biovolumes of all cyanobacterial groups than in Lake George. Phlips et al. (2000) estimated a somewhat lower light availability for the stretch of river including Racy Point than for Lake George, and that river stretch is downstream of a river section with considerably lower light availability (light availability estimates based on water column depths). The St. Johns River at Racy Point also had lower average biovolume of *M. aeruginosa* than in Crescent Lake, but similar average biovolumes for the other cyanobacteria groups. The two sites have similar average fetch, but higher river flows at Racy Point may result in a more turbulent water column, less suitable for surface blooms. Water depth at the Crescent Lake sites average considerably lower (about 3 m) than at Racy Point (7 m). Conversely, Crescent Lake has considerably average higher water color (279 cpu) than Racy Point (136 cpu).

BLOOM EVENT ASSESSMENT

Several of the apparent cyanobacterial blooms seem questionable because of discrepancies with simultaneous measurements of chlorophyll-*a*, and environmental conditions that did not seem particularly conducive for bloom formation (Table 2). These included the second 2016 peak in *M. aeruginosa* in Lake George after Hurricane Matthew (Figure 18), the 2017 Crescent Lake *M. aeruginosa* bloom (Figure 19), the 2017 Doctors Lake *M. aeruginosa* bloom (Figure 22), and the 2013 Piney Point *M. aeruginosa* bloom (Figure 25). Analyst 2 did the biovolume measurements for all these apparent blooms. Overall, there was a weaker relationship between chlorophyll-*a* and phytoplankton biovolume for Analyst 2 (Figure 30). There are some reasons why measurements of biovolume may be more inaccurate than those of chlorophyll-*a*. Chlorophyll-*a* measurements are based on a much larger sample volume, often several hundred milliliters, while biovolume estimates are based on counting cells in a fraction of a milliliter. Biovolume estimates are also approximations based on similarity of the cells

to standard geometrical shapes. Biovolume estimates may be particularly difficult for colonial or filamentous species, which are among the dominant taxa in the lower St. Johns River during these bloom events. Potentially only a few large colonies of *M. aeruginosa* in a count could result in a high biovolume estimate. On the other hand, the chlorophyll-*a* content of algal cells can be highly variable (Reynolds, 2006). Although both biovolume and chlorophyll-*a* are imperfect measures of algal biomass, there is more confidence in data interpretation when the two measures are consistent.

In the other bloom events, a common feature was low discharges and long water replacement times (Table 2, Figure 15, Figure 17, Figure 18 [first *M. aeruginosa* bloom], Figure 20, Figure 21, Figure 23, Figure 24, Figure 26 – Figure 28, Figure 29). Often the discharges had decreased from higher levels earlier in the year or during the previous winter. Also, in several cases the termination of the blooms seemed associated with a substantial increase in discharges. Phlips et al. (2007), Srifa et al. (2016), and Nelson et al. (2018) previously found negative relationships between phytoplankton biovolume and discharges in the lower St. Johns River.

Another common feature of the bloom events was an increase in TP and TN concentrations (Table 2, Figure 15, Figure 16, Figure 18 [first *M. aeruginosa* bloom], Figure 20, Figure 21, Figure 23, Figure 24, Figure 26 – Figure 28, Figure 29). Since the blooms tended to occur during periods of low discharge, the proximate source of the nutrients was probably primarily internal, although they could have entered the system during earlier periods of higher discharge. Runoff from the TCAA also could affect nutrient concentrations at Crescent Lake and the downstream St. Johns River. A relatively small runoff volume from the agricultural area could potentially carry a large nutrient load.

Blooms of *Dolichospermum* and *Aphanizomenon* tended to occur in the spring-early summer, preceding later blooms of *M. aeruginosa* or of Other Cyanobacteria, for example, Lake George 1996 (Figure 15), Doctors Lake 2008 (Figure 21), Doctors Lake 2001 (Figure 29), and the widespread 2010 bloom (Figure 26 –Figure 28). In other cases, blooms of *M. aeruginosa* appeared simultaneous with *Cylindrospermopsis+Raphidiopsis* or Other Nitrogen-fixers (e.g. Mandarin Point and Piney Point 1998, Figure 23, Figure 24), although it is possible that a higher sampling frequency could have detected earlier development of the nitrogen-fixers. These occurrences of earlier or simultaneous blooming by nitrogen-fixers suggests that nitrogen fixation was a primary source of the increased TN concentrations, facilitating the later blooms of *M. aeruginosa* or of Other Cyanobacteria.

Another common feature of the bloom events was high water temperature; in all events above the 20^{o}C level that favors growth by cyanobacteria (O'Neil et al. 2012; Kim et al. 2019). Srifa et al. (2016) and Nelson et al. (2018) have previously noted increased cyanobacterial biovolume at higher temperatures in the LSJRB. The spring-early summer blooms of *Dolichospermum* and *Aphanizomenon* had relatively low temperatures, suggesting a lower temperature optimum for those taxa. In the multiple regression analyses, temperature or DDays sometimes entered as significant negative factors for Other Nitrogen-fixers (Table 3, Table 5, Table 6), probably reflecting the

spring-early summer peaks for these taxa. In the multiple regression analyses for the other cyanobacterial groups, temperature or DDays entered as only positive factors.

A final factor common to the bloom events was relatively low water color, including the 1996 blooms in Lake George and downstream, the 2016 Lake George bloom, the 1998 bloom at Mandarin Point and Piney Point, and the Doctors Lake blooms in 2001, 2007, and 2008. Phlips et al. (2007) and Nelson et al. (2018) have previously noted the importance of water color as a factor limiting light availability to phytoplankton in the LSJRB.

STATISTICAL ANALYSES

The NMDS ordinations were useful in visualizing relationships among cyanobacteria and environmental factors. They also help in interpreting the often-poor performance of the multiple regression and Random Forest modeling. TN and TP often showed relationships with cyanobacteria in the ordination plots, but those relationships were often nonlinear or non-monotonic. For the freshwater sites, high concentrations of TN and TP occurred under both high discharge conditions, in which water residence time may have been insufficient for cyanobacterial bloom development, and under low discharge situations in which residence time was sufficient for bloom development (e.g. Figure 35, Figure 37). In the oligohaline sites, Doctors Lake and St. Johns River at Piney Point, there were also some non-monotonic relationships between discharges and nutrients (e.g. Figure 41, Figure 45), although at these sites cyanobacteria were positively related to discharges. These non-monotonic relationships of nutrients to cyanobacteria may partially explain the weak relationships in the linear regression and even the Random Forest models.

The performance of the multiple regression and Random Forest models was generally poor for *M. aeruginosa* and Other Nitrogen-fixers at all sites, and for all cyanobacterial groups at the oligohaline sites. Part of the reason for the poor performance may be the sporadic occurrence of the cyanobacterial taxa in these situations. Generally, the percent of variability explained by the models decreased as the fraction of samples with zero biovolume increased (Figure 46). When a large faction of the samples has zero biovolume, there is no variability to relate to changes in environmental factors. Model performance similarly declined as average and median cyanobacterial biovolumes decreased.

The poor model performance could also be due to temporal and spatial variability. Samples have been taken 1-2 times monthly; cyanobacterial doubling times can be much shorter than that, so sampling may not be frequent enough to track wax and wane of populations, and phytoplankton may be responding to environmental conditions between samples. *M. aeruginosa* and some of the Other Nitrogen-fixers tend to float at the water surface, and wind/waves result in concentrations near shorelines (e.g. Figure 1). The vertically-integrated samples we take near center of the water body may not well characterize these surface blooms. The other cyanobacterial groups do not tend to form surface blooms, and the models generally explained more variability for them.

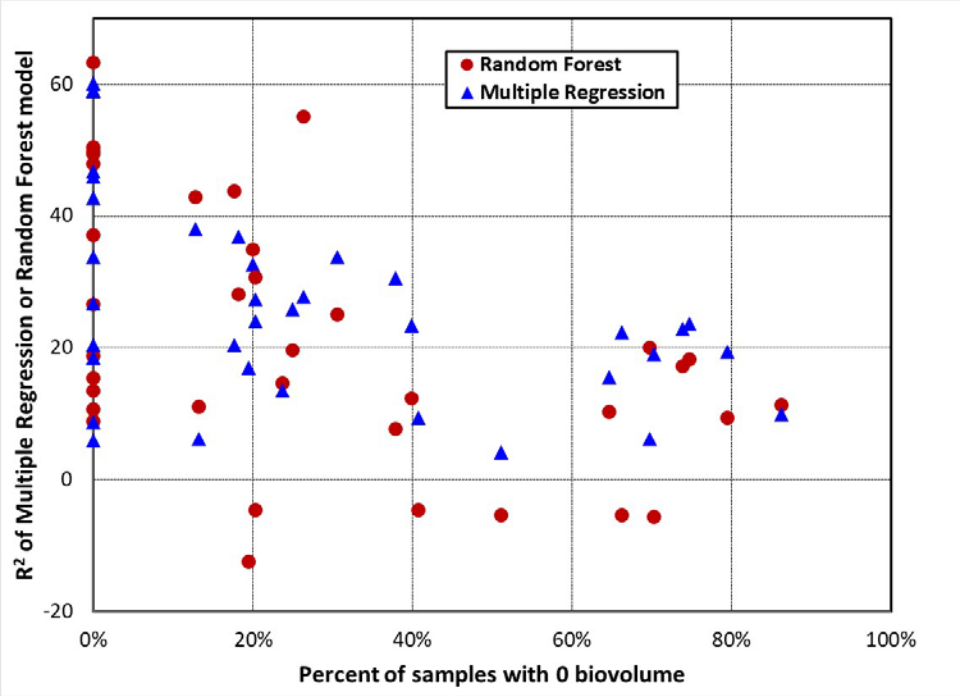


Figure 46. Relationship between regression and Random Forest model performance and percent occurrence of cyanobacteria.

GENERAL DISCUSSION

Roles of hydrology and nutrients in control of cyanobacterial bloom development in the LJSRB

These analyses point to hydrology and nutrients as primary factors controlling cyanobacterial bloom development in the LSJRB. Assessment of bloom events showed cyanobacterial blooms consistently occurred during periods of low discharge. Often the bloom periods followed an earlier period of higher discharges, which may have introduced nutrients in watershed runoff. However, the NMDS analyses showed different relationships with discharges in different parts of the basin. In Lake George and the river as far downstream as Mandarin Point, high river discharges strongly reduce water residence time, resulting in strong negative relationships of discharge and cyanobacterial biovolumes. Factors associated with high discharges which also limit cyanobacterial growth include high water color (also noted by Phlips et al. 2007 and Nelson et al. 2018), and possibly disturbance of a stagnant, stratified water column (Havens et al. 2019). However, in offline Doctors Lake, water residence time is always long, and discharges are positively associated with cyanobacteria, probably because they bring in nutrients in watershed runoff, and reduce conductivity to concentrations tolerable by cyanobacteria. Crescent Lake, another offline lake with intermediate water residence

times, is also intermediate in the relationship between discharges and cyanobacteria. There, *M. aeruginosa* showed a positive relationship with discharges, but the other cyanobacterial groups were negatively related (Figure 36). In the St. Johns River at Piney Point, the most downstream station in the river, there was also a positive relationship between discharges and cyanobacteria. We speculate that high river discharges may serve to transport cyanobacteria downstream into the oligohaline zone and reduce conductivity in that area to concentrations tolerable by cyanobacteria.

Bloom events were consistently associated with increased TN and TP concentrations. Since these events occurred during low discharge situations, the proximate source of the nutrients was likely internal. In several cases coincident increases in nitrogen-fixing cyanobacteria indicate that the increased TN concentrations were primarily due to nitrogen fixation. Runoff from the TCAA also could affect nutrient concentrations at Crescent Lake and the downstream St. Johns River. A relatively small runoff volume from this agricultural area could potentially carry a large, bioavailable nutrient load. At two long-term TCAA water quality monitoring sites, bioavailable phosphorus ranged from 57%-83% and bioavailable nitrogen ranged from 23%-39%. (P. Way, personal communication). The NMDS analyses also showed positive associations between TN and TP concentrations and cyanobacterial biovolumes. However, high nutrient concentrations occurred during both low discharge periods conducive to cyanobacterial growth and high discharge periods that prevented bloom development. As there is little that can be done to manage discharges in the LSJRB beyond limiting water withdrawals, control of cyanobacterial blooms depends primarily on reduction in nutrient concentrations.

In contrast to TN and TP, cyanobacteria were negatively related to inorganic nutrients, NO_x, NH_4 and PO_4. Concentrations of inorganic nutrients were generally low during bloom events. The NMDS and regression analyses also showed negative relationships of inorganic nutrients with cyanobacteria. One probable reason for this is that high discharge events bring inorganic nutrients into the system in watershed runoff, and also prevent cyanobacterial populations from growing to the point that they deplete the available nutrients. Even in Doctors Lake, which always has long water residence times, inorganic nutrients were negatively related to cyanobacteria (Figure 40, Table 6). In Doctors Lake inorganic nutrients may be associated with saltwater intrusion which increases conductivity to concentrations deleterious to cyanobacteria. During periods in which cyanobacterial populations bloom, uptake quickly depletes the inorganic nutrients and nearly all of the measurable nutrients are organic.

Nutrient control for management of cyanobacterial blooms in the LSJRB

There is considerable debate whether control of cyanobacterial blooms requires reductions of only TP or of both TP and TN. Advocates of control by TP reduction point to studies showing long-term effectiveness of TP control, the nitrogen-fixing ability of some cyanobacteria, and the greater cost and difficulty of controlling TN (Schindler et al. 2016). Advocates of dual nutrient control point to studies of TP-TN co-limitation, evidence that nitrogen-fixation may not meet ecosystem nitrogen requirements, and the

need to limit nitrogen transport to estuarine and coastal systems, which are generally considered to be primarily nitrogen limited (Lewis and Wurtsbaugh 2008; Paerl et al. 2016, 2018).

TMDLs adopted for the freshwater lower St. Johns River and for Crescent Lake require reductions in both TP and TN (US EPA 2008; FDEP 2017), but TMDLs adopted for the oligohaline lower St. Johns River and for Doctors Lake require reduction only in TN (US EPA 2008; Magley 2009). Although the statistical analyses often indicated stronger relationships of cyanobacteria with TN, the scientific literature supports reduction of TP as well as TN to control cyanobacterial blooms in both the freshwater and oligohaline area in the LSJRB. *M. aeruginosa* is not a nitrogen-fixer, so requires both phosphorus and nitrogen. *M. aeruginosa* may be the taxon of most concern because of its propensity to form conspicuous blooms and produce cyanotoxins. However, reductions of TN alone could cause shifts in the cyanobacterial community to dominance by nitrogen-fixers, some of which also form conspicuous surface blooms and produce cyanotoxins.

The apparent threshold TP and TN concentrations above which cyanobacterial blooms have occurred (about 0.05 mg TP/L and 1 mg TN/L, Figure 31, Figure 32), are very similar to the target concentrations specified in the Crescent Lake TMDL (0.05 mg TP/L and 1.16 mg TN/L, FDEP 2017). The other adopted TMDLs for the lower St. Johns River do not specify target nutrient concentrations. Higher concentrations of chlorophyll-*a* also tend to occur at nutrient concentrations above about 0.05 mg TP/L and 1 mg TN/L (Figure 33). In the lower St. Johns River TMDL, a chlorophyll-*a* target was set at 40 μg/L, not to be exceeded more than 10% of the time, because above that level cyanobacteria tend to dominate and zooplankton decline (Hendrickson et al. 2003; US EPA 2008). Highest concentrations of chlorophyll-*a* exceed that target at TP concentrations below 0.05 mg/L, and TN concentrations slightly above 1 mg/L (Figure 33).

Monitoring for cyanobacteria in the LSJRB

SJRWMD has discontinued quantitative measurements of phytoplankton biovolumes and composition in the LSJRB, although water quality monitoring, including measurements of chlorophyll-*a*, continue at a monthly or twice-monthly frequency. In addition, contingency samples are collected when phytoplankton blooms are observed; analyses of these samples include a qualitative assessment of the dominant phytoplankton species and measurement of cyanotoxins.

There are strong relationships between chlorophyll-*a* and cyanobacterial biovolumes in both the freshwater and oligohaline zones (Figure 47 A, B). Cyanobacterial relative biovolume is variable and often low for chlorophyll-*a* concentrations below about 50 μg/L. Above this concentration cyanobacteria usually represent more than 60% of the phytoplankton community biovolume (Figure 47 C, D). Hendrickson et al. (2003) found similar trends in cyanobacterial relative composition in the LSJRB. Of the few cases in the oligohaline sites with very low percent cyanobacteria at chlorophyll-*a* concentrations

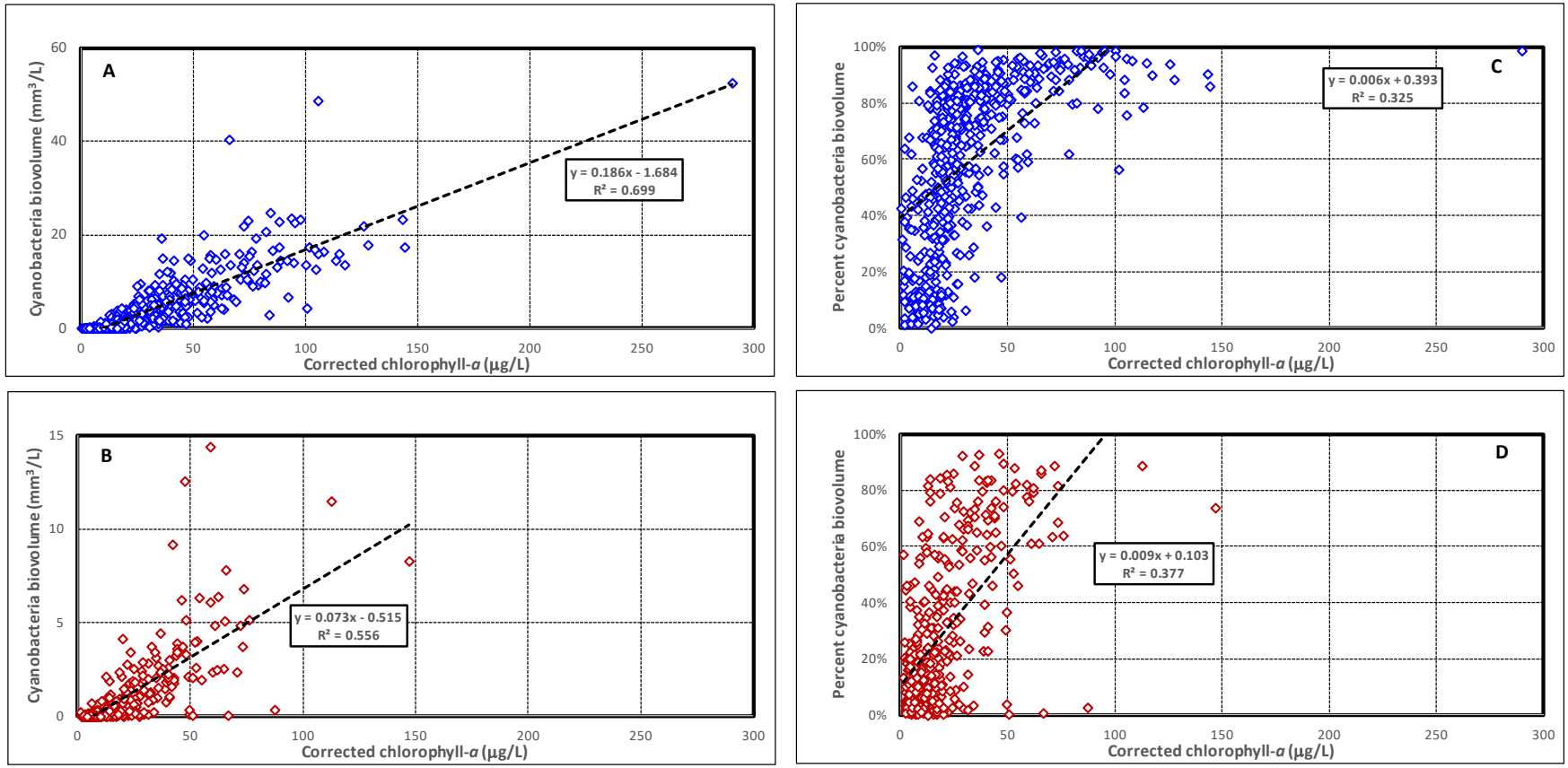


Figure 47. Lower St. Johns River basin chlorophyll vs. cyanobacteria biovolumes and percent biovolumes, warm season samples, Analyst 1.

A and C. Freshwater sites
B and D. Oligohaline sites

above 50 µg/L, three were samples with conductivity exceeding 12,000 µmhos/cm and one was late in the warm season (October 22) during a period of high discharges. Thus, chlorophyll-*a* measurements provide a good indication of when cyanobacterial biovolumes are high and likely to dominate the phytoplankton assemblage. However, they do not provide information on which cyanobacterial species are present.

Effective monitoring of cyanobacterial bloom development and ascertaining the causes of blooms in the LSJRB are difficult because of high temporal and spatial variability. Cyanobacterial doubling times can be much shorter than our sampling frequency, so monthly sampling may not be frequent enough to track wax and wane of populations, and phytoplankton may be responding to environmental conditions between sample collection dates. *M. aeruginosa* and some of the Other Nitrogen-fixers (*Dolichospermum*, *Aphanizomenon*) tend to float at the water surface, and wind/waves result in concentrations near shorelines (e.g. Figure 1). The vertically-integrated samples we take near center of the water body may not well characterize these surface blooms.

Continuous monitoring at a few locations may be able to better track cyanobacterial bloom development, particularly for those taxa that do not tend to form surface blooms, because these taxa are likely to be more uniformly distributed through a water body. These taxa include many of the Other Cyanobacteria and *Cylindrospermopsis* + *Raphidiopsis*, which often dominate phytoplankton biovolumes (Figure 3 - Figure 14), and some of which produce cyanotoxins (Trent et al. 2019). Potential sites for continuous monitoring include Lake George, Crescent Lake, St. Johns River at Racy Point, and Doctors Lake. The oligohaline river sites would be less suitable because of the more sporadic occurrence of cyanobacterial blooms in that area. Parameters that would be useful to monitor include chlorophyll and phycocyanins (pigments specific to cyanobacteria, Brient et al. 2008, Zamyadi et al. 2012). Vertical profiles of dissolved oxygen and conductivity may be useful to assess stratification, which may promote low sediment redox and subsequent phosphorus release, contributing to bloom formation. Continuous measurement of phosphate or nitrate would be less useful because of the general negative relationships of these inorganic nutrients with cyanobacteria in the LSJRB. It is possible that in some cases continuous monitoring of inorganic nutrients could show nutrient releases from the TCAA or other sources, or a decrease in inorganic nutrients as cyanobacteria increase. These monitoring probes are not capable of determining the cyanobacterial species composition.

Satellite remote sensing also has potential for monitoring of cyanobacterial blooms, Data from the Medium Resolution Imaging Spectrometer (MERIS) and the Sentinel-3 Ocean and Land Color Instrument (OLCI) satellites have been used to quantify chlorophyll and cyanobacteria in lakes (Tomlinson et al. 2016; Coffer et al. 2020; Cyanobacteria Assessment Network (CyAN) | Water Research | US EPA). Imagery from these satellites has been available at 2-3 day intervals, and in the future will become available near daily (Coffer et al. 2020), potentially allowing high-frequency assessments of cyanobacterial bloom development. However, cloud cover frequently interferes with the satellite imagery. Another issue is spatial resolution. Minimum resolution (pixel size) for these satellites is 300 m, but a 3 x 3 pixel block (900 m) is considered more robust for analyses (Tomlinson et al. 2016; Schaeffer 2017). Also, land cover can confound the satellite

signal, so pixels containing land or adjacent to land are usually eliminated from analyses (Tomlinson et al. 2016; Schaeffer 2017; Coffer et al. 2020). This is a problem for detecting surface blooms, which are often concentrated near shorelines. Again, satellite imagery cannot determine the cyanobacterial species composition. The Florida Department of Environmental Protection (FDEP) currently distributes a weekly blue-green algal update which includes summaries of satellite observations for the LSJRB (Weekly Updates and Subscription | Florida Department of Environmental Protection).

Microscopic identification and quantification of phytoplankton is time consuming and requires high expertise. Our experience has found a lack of consistency between different analysts. This study found some differences between two analysts working in the same laboratory (Figure 30). Another study found large differences in cyanobacterial identification and quantification between different laboratories (Fulton 2021). Molecular or biochemical approaches should be considered as an alternative to microscopic analyses. Coyne (2015; 2016) reviewed several molecular and biochemical approaches that may be used to evaluate community composition of microbial species in environmental water samples, and recommended next generation sequencing (NGS) of microbial rRNA gene sequence amplicons as a comprehensive and cost effective method for investigating species composition and their relative abundance. Most of the examples included in this review dealt with bacteria. One cited study compared microscopy cell counts and biovolume estimates to data generated by NGS sequencing for bacteria and phytoplankton in three freshwater lakes (Eiler et al. 2013). Before molecular or biochemical or molecular approaches are implemented for monitoring a study should be conducted to evaluate their feasibility, cost-effectiveness, and comparability to traditional microscopic phytoplankton analyses.

REFERENCES

Aguilera, A., E.B. Gómez, J. Kaštovskỳ, R.O. Echenique, and G.L. Salerno. 2018. The polyphasic analysis of two native *Raphidiopsis* isolates supports the unification of the genera *Raphidiopsis* and *Cylindrospermopsis* (Nostocales, Cyanobacteria). Phycologia 57 (2): 130–146.

Bormans, M., H. Maier, and M. Burch. 1997. Temperature stratification in the lower River Murray, Australia: Implication for cyanobacterial bloom development. Marine and Freshwater Research 48: 647–654.

Brient, L., M. Lengronne, E. Bertrand, D. Rolland, A. Sipel, D. Steinmann, I. Baudin, M. Legeas, B. Le Rouzica, and M. Bormans. 2008. A phycocyanin probe as a tool for monitoring cyanobacteria in freshwater bodies. Journal of Environmental Monitoring 10: 248–255.

Burford, M.A., J. Beardall, A. Willis, P.T. Orr, V.F. Magalhaes, L.M. Rangel, S.M.F.O.E. Azevedo, and B.A. Neilan. 2016. Understanding the winning strategies used by the bloom-forming cyanobacterium Cylindrospermopsis raciborskii. Harmful Algae 54: 44–53.

Cha, Y., K.H. Cho, H. Lee, T. Kang, and J.H. Kim. 2017. The relative importance of water temperature and residence time in predicting cyanobacteria abundance in regulated rivers. Water Research 124: 11-19.

Coffer, M.M., B.A. Schaeffer, J.A. Darling, E.A. Urquhart, and W.B. Salls. 2020. Quantifying national and regional cyanobacterial occurrence in US lakes using satellite remote sensing. Ecological Indicators 111: 105976. https://doi.org/10.1016/j.ecolind.2019.105976.

Coyne, K.J. 2015. Contract 28191 – Biochemical procedure recommendation services. Task 1: Literature review. Report to St. Johns River Water Management District, Palatka, Florida.

Coyne, K.J. 2016. Contract 28191 – Biochemical procedure recommendation services. Task 2: Recommendations. Report to St. Johns River Water Management District, Palatka, Florida.

Eiler, A., S. Drakare, S. Bertilsson, J. Pernthaler, S. Peura, C. Rofner, K. Simek, Y. Yang, P. Znachor, and E. S. Lindstrom. 2013. Unveiling Distribution Patterns of Freshwater Phytoplankton by a Next Generation Sequencing Based Approach. Plos One 8. https://doi.org/10.1371/journal.pone.0053516.

FDEP. 2017. Final TMDL report. Nutrient TMDLs for Crescent Lake (WBID 2606B). Division of Environmental Assessment and Restoration, Florida Department of Environmental Protection, Tallahassee, Florida.

Fulton, R.S. III. 2021. External nutrient loading and water quality trends in the upper Ocklawaha basin lakes, 1980s through 2019-2020. Technical Publication SJ2021-01. St. Johns River Water Management District, Palatka, Florida.

Graham, J.L. N.M. Dubrovsky, G.M. Foster, L.R. King, K.A. Loftin, B.H. Rosen, and E.A. Stelzer. 2020. Cyanotoxin occurrence in large rivers of the United States. Inland Waters 10: 109-117. DOI: 10.1080/20442041.2019.1700749

Havens, K.E., E.J. Phlips, M.F. Cichra, and B.-L. Li. 1998. Light availability as a possible regulator of cyanobacteria species composition in a shallow subtropical lake. Freshwater Biology 39: 547–556.

Havens, K., H. Paerl, E. Phlips, M. Zhu, J. Beaver, and A. Srifa. 2016. Extreme weather events and climate variability provide a lens to how shallow lakes may respond to climate change. Water 8: 229. doi:10.3390/w8060229

Havens, K.E., G. Ji, J.R. Beaver, R.S. Fulton III, and C.E. Teacher. 2019. Dynamics of cyanobacteria blooms are linked to the hydrology of shallow Florida lakes and provide insight into possible impacts of climate change. Hydrobiologia 829: 43–59. doi 10.1007/s10750-017-3425-7

Hendrickson, J., E.F. Lowe, D. Dobberfuhl, P. Sucsy, and D. Campbell. 2003. Characteristics of accelerated eutrophication in the Lower St. Johns River Estuary and recommended targets to achieve water quality goals for the fulfillment of TMDL and PLRG objectives. Department of Water Resources, St. Johns River Water Management District, Palatka, Florida.

Joung, S.-H., H.-M. Oh, S.-R. Ko, and C.-Y. Ahn. 2011. Correlations between environmental factors and toxic and non-toxic *Microcystis* dynamics during bloom in Daechung Reservoir, Korea. Harmful Algae 10:188–193. doi:10.1016/j.hal.2010.09.005.

Kim, S., S. Chung, H. Park, Y. Cho, and H. Lee. 2019. Analysis of environmental factors associated with cyanobacterial dominance after river weir installation. Water 11: 1163, doi:10.3390/w11061163.

Kopp, J.F. and G.D. McKee. 1983. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020, U.S. Environmental Protection Agency, Washington, DC.

Kuhn, M. 2009. Variable Selection Using the caret Package. https://r-forge.r-project.org/scm/viewvc.php/*checkout*/pkg/caret/inst/doc/caretSelection.pdf?revision=77&root=caret&pathrev=90

Kuhn, M. 2020. caret: Classification and Regression Training. R package version 6.0-86. https://CRAN.R-project.org/package=caret

Leonard J.A., and H.W. Paerl. 2005. Zooplankton community structure, microzooplankton grazing impact, and seston energy content in the St. Johns river system, FL as influenced by the toxic cyanobacterium *Cylindrospermopsis raciborskii*. Hydrobiologia 537:89–97.

Lewis, W.M. Jr. and W.A. Wurtsbaugh. 2008. Control of lacustrine phytoplankton by nutrients: erosion of the phosphorus paradigm. Internationale Revue gesamten Hydrobiologie 93: 446–465.

Liaw, A. and M. Wiener. 2002. Classification and Regression by randomForest. R News 2/3: 18-22. https://cogns.northwestern.edu/cbmg/LiawAndWiener2002.pdf

Magley, W. 2009. FINAL TMDL Report. DO and Nutrient TMDLs for Swimming Pen Creek (WBID 2410) and Nutrient TMDL for Doctors Lake (WBID 2389). Florida Department of Environmental Protection, Bureau of Watershed Restoration, Tallahassee, Florida.

Mitrovic, S.M., R.L. Oliver, C. Rees, R. Bowling, and T. Buckney. 2003 Critical flow velocities for the growth and dominance of *Anabaena circinalis* in some turbid freshwater rivers. Freshwater Biology 48: 164–174.

Moisander, P.H., E. McClinton, and H.W. Paerl. 2002. Salinity effects on growth, photosynthetic parameters, and nitrogenase activity in estuarine planktonic cyanobacteria. Microbial Ecology 43: 432–442.

Nelson, N.G., R. Muñoz-Carpena, E.J. Phlips, D. Kaplan, P. Sucsy, and J. Hendrickson. 2018. Revealing biotic and abiotic controls of harmful algal blooms in a shallow subtropical lake through statistical machine learning. Environmental Science and Technology 52: 3527−3535.

Oksanen, J., F.G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P.R. Minchin, R.B. O'Hara, G.L. Simpson, P. Solymos, M.H.H. Stevens, E. Szoecs, and H. Wagner, 2018. Package 'vegan'. https://cran.r-project.org, https://github.com/vegandevs/vegan.

O'Neil, J.M., T.W. Davis, M.A. Burford, and C.J. Gobler. 2012. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. Harmful Algae 14: 313–334.

Paerl, H.W., and R.S. Fulton III. 2006. Ecology of harmful Cyanobacteria. pp. 95-109 In: E. Granéli and J.T. Turner (Eds.) Ecology of Harmful Algae. Ecological Studies, Vol. 189. Springer-Verlag, Berlin Heidelberg.

Paerl H.W., R.S. Fulton III, P.H. Moisander, and J. Dyble. 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. The Scientific World 1:76–113.

Paerl, H.W, J.T. Scott, M.J. McCarthy, S.E. Newell, W.S. Gardner, K.E. Havens, D.K. Hoffman, S.W. Wilhelm, and W.A. Wurtsbaugh. 2016. It takes two to tango: when and where dual nutrient (N & P) reductions are needed to protect lakes and downstream ecosystems. Environmental Science and Technology 50:10805−10813.

Paerl, H.W, T.G. Otten, and R. Kudela. 2018. Mitigating the expansion of harmful algal blooms across the freshwater-to-marine continuum. Environmental Science and Technology 52: 5519−5529.

Phlips, E.J., M. Cichra, F.J. Aldridge, J. Jembeck, J. Hendrickson, and J. Brody. 2000. Light availability and variations in standing crop in a nutrient-rich blackwater river. Limnol. Oceanogr. 45(4): 916-929.

Phlips, E. J., J. Hendrickson, E. L. Quinlan, and M. Cichra. 2007. Meteorological influences on algal bloom potential in a nutrient-rich blackwater river. Freshwater Biology 52: 2141–2155. doi:10.1111/j.1365-2427.2007.01844.x.

Preece, E.P., F.J. Hardy, B.C. Moore, and M. Bryana. 2017. A review of microcystin detections in estuarine and marine waters: Environmental implications and human health risk. Harmful Algae 61: 31–45.

Reynolds, C.S. 2006. Ecology of phytoplankton. Cambridge University Press, Cambridge, U.K.

Rosen, B.H., K.A. Loftin, J.L. Graham, K.N. Stahlhut, J.M. Riley, B.D. Johnston, and S. Senegal. 2018. Understanding the effect of salinity tolerance on cyanobacteria associated with a harmful algal bloom in Lake Okeechobee, Florida: U.S. Geological Survey Scientific Investigations Report 2018–5092, 32 p., https://doi.org/10.3133/sir20185092.

Schaeffer, B. 2017. EPA Tools and Resources webinar: Monitoring cyanobacteria with satellites, November 15, 2017. https://www.epa.gov/research/epa-tools-and-resources-webinar-series.

Schindler, D.W., S.R. Carpenter, S.C. Chapra, R.E. Hecky, and D.M. Orihel. 2016. Reducing phosphorus to curb lake eutrophication is a success. Environmental Science and Technology 50: 8923−8929.

Sherman, B.S. and I.T. Webster. 1998. Transitions between *Aulacoseira* and *Anabaena* dominance in a turbid river weir pool. Limnology and Oceanography 43: 1902–1915.

Smith, J.M. 1991. Wind-wave generation on restricted fetches. Misc. Paper CERC-91-2, USAE Waterways Experiment Station, Vicksburg, MS.

Srifa, A., E.J. Phlips, M.F. Cichra, and J.C. Hendrickson. 2016. Phytoplankton dynamics in a subtropical lake dominated by cyanobacteria: cyanobacteria 'Like it Hot' and sometimes dry. Aquatic Ecology 50:163-174. doi 10.1007/s10452-016-9565-4.

Tomlinson, M.C., R.P. Stumpf, T.T. Wynne, D. Dupuy, R. Burks, J. Hendrickson, and R S. Fulton III. 2016. Relating chlorophyll from cyanobacteria-dominated inland waters to a MERIS bloom index. Remote Sensing Letters 7(2): 141–149.

Toth, D. J. 1993. Volume 1 of the lower St. Johns River basin reconnaissance: Hydrogeology. Palatka, FL.

Trent, T., J. Hendrickson, C. Harris, and C. Akers. 2019. Cyanotoxin occurrence in the lower St. Johns River estuary, 2005–2017. Technical Memorandum 60. St. Johns River Water Management District, Palatka, Florida.

US EPA. 2008. Total Maximum Daily Load (TMDL) for nutrients. Lower St. Johns River. US EPA Region 4, Atlanta, Georgia.

Utermöhl, H. 1958. Methods of collecting phytoplankton for various purposes are discussed. Internationale Vereinigung für Theoretische und Angewandte Limnologie: Mitteilungen 9:1–38. doi:10.1080/05384680.1958.11904091.

Zamyadi, A., N. McQuaid, M. Prévost and S. Dorner. 2012. Monitoring of potentially toxic cyanobacteria using an online multi-probe in drinking water sources. Journal of Environmental Monitoring 14:579-588.