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**PHYTOPLANKTON NUTRITIONAL ECOLOGY IN A PIEDMONT
RESERVOIR**

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Phytoplankton Nutritional Ecology in a Piedmont Reservoir

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Abstract

Many potable source-waters in the U.S. are sustaining cultural eutrophication from elevated nutrient supplies concomitant with major shifts in nutrient ratios away from healthy conditions as indicated by Redfield proportions (16:1, molar). Falls Lake is a representative eutrophic impoundment (reservoir; length ~38 km, width ~0.2-2.9 km) in a rapidly urbanizing watershed in the southeastern U.S. The shallow upper reservoir is especially prone to algal blooms in comparison to the deeper lower reservoir (mean depth 5 m and 14 m, respectively).

Using a long-term water quality dataset provided by the Center for Applied Aquatic Ecology (CAAE; 2011-2019, biweekly to monthly samples), we compared the upper and lower reservoir over time for nitrogen (N) and phosphorus (P) nutrient regimes, phytoplankton biomass (as chlorophyll *a* [*chl**a*] concentrations), and phytoplankton assemblage composition during bloom conditions defined as > 40 µg *chl**a*/L, the state water quality standard. In the upper reservoir, TP and inorganic N (N_i) were chronically elevated and *chl**a* commonly exceeded the state standard, with noxious cyanobacteria dominant in cell number. In contrast, the lower reservoir was generally characterized by moderate nutrient regimes except for elevated NH_4^+ (up to ~400 µg/L) in surface waters during fall-winter.

Short-term experiments were conducted to gain further insights about assemblage responses to changing nutrient regimes during two summers that were planned to be replicate seasons; however, one summer had average precipitation (considering the past decade; 127 mm) whereas the other had much higher precipitation (170 mm). Microcosm experiments in situ (duration, 5 days) were used to assess reservoir phytoplankton assemblage responses to inorganic N form (N_i) ± inorganic P (P_i) enrichment, and to $N_i:P_i$ ratios. The abundance and composition of phytoplankton functional groups were similar reservoir-wide in both summers, except for higher relative abundance of cyanobacteria in the upper region under average precipitation. In both summers, there was a positive relationship between *chl**a* and N_i concentrations (both forms), with or without P_i enrichment, and a stronger relationship between *chl**a* and NH_4^+ than between *chl**a* and NO_3^- . Also in both summers, the eutrophic lower region assemblage responded more strongly to nutrient enrichment. Maximal final phytoplankton biomass as *chl**a* was attained, for the upper region assemblage, with $N_i + P_i$ enrichment as NO_3^- . For the lower region assemblage, maximum *chl**a* occurred with $N_i + P_i$ enrichment as NH_4^+ . The toxigenic cyanobacterium *Cylindrospermopsis raciborskii* was the most abundant taxon initially reservoir-wide; it was stimulated by enrichment with either N_i form, especially along with P_i enrichment. Similar responses in the two summers likely occurred because the precipitation/dilution/washout regimes would only have affected the phytoplankton before they were placed into closed microcosms in the experiments. The precipitation differential during the two summers, although substantial, probably was not enough to cause major changes in these resilient reservoir.

Overall, this study contributed species-level insights about seasonal influences of chronic cultural eutrophication on reservoir phytoplankton blooms. The findings indicate that reservoir assemblages are well-adapted to variable precipitation/hydrologic changes, and that cyanobacteria will continue to be favored under warming temperatures and high N/P enrichment. This study also supports co-managed reductions of both N and P supplies, as in the Falls Lake Rules, to minimize noxious algal blooms in this important, representative potable source-water reservoir of the North Carolina Piedmont.

Introduction

Cultural eutrophication, the nutrient over-enrichment of surface waters by human activity (Rothenberger et al. 2009a), has degraded surface freshwaters, decreased aquatic biodiversity, disrupted ecosystem functioning, and caused high-biomass outbreaks of harmful algae (He et al. 2015, Huisman et al. 2018). Freshwater harmful algal “blooms” (HABs) generally consist of toxigenic cyanobacteria in warmer months and golden-brown taxa and/or diatoms in colder periods (Sommer et al. 1986, Grover and Chrzanowski 2006, Wang et al. 2007). These blooms commonly cause high diel variation in dissolved oxygen, degrade habitat, and adversely alter food webs across trophic levels (Reynolds 1984). They also form disinfection byproducts, and produce taste-and-odor compounds in drinking water (Liu et al. 2017).

Across geographic regions, freshwater HABs have become more frequent, severe, and persistent within the past few decades (e.g., Harke et al. 2016, Ho et al. 2019), concomitant with increasing nutrient inputs and warming temperatures (O’Neil et al. 2012). The adverse impacts of these conditions are expected to worsen as with continued human population growth (Boretti and Rosa 2019). Increasing potable water demands are being met by use of reservoirs, or run-of-river impoundments (Zarfl et al. 2015), but many reservoirs are sustaining chronic cultural eutrophication (Williamson et al. 2009). Long-term dynamics of reservoir phytoplankton assemblages have seldom been tracked in efforts to assess the environmental factors regulating seasonal bloom composition, dynamics, and stability as eutrophication progresses (Znachor et al. 2020).

Reservoir ecosystems are poorly understood in comparison to natural lakes (Hayes et al. 2017). Reservoirs have much shorter water residence times, higher flow rates, more frequent sedimentation or mixing events, extreme water level fluctuations, and larger watersheds and basins (Kennedy et al. 1985). Lack of a littoral zone or a terrestrial riparian zone leaves these systems more open to sediment and nutrient inputs from stormwater runoff (Wetzel 2001). Besides controlling light and nutrients, hydrological conditions also act directly upon phytoplankton (Reynolds 2006, Vanni et al. 2006). For survival and success in reservoirs, phytoplankton must adapt quickly to both flooding and drought events, low light availability, polymictic conditions in some areas, and frequent pulses of nutrients and sediments (Burkholder 1992, Wetzel 2001). With larger watersheds than natural lakes, reservoirs are especially vulnerable to increased nutrient and sediment loading; high precipitation events result in nutrient and sediment pulses that can either stimulate or inhibit phytoplankton growth (Vanni et al. 2006, Hayes et al. 2017). Stimulation of phytoplankton growth occurs when nutrient inputs alleviate N or P limitation; inhibition occurs when increased suspended sediments decrease light availability (Vanni et al. 2006). In addition, high precipitation events can physically remove phytoplankton from the water column through flushing (Kimmel et al. 1990, Vanni et al. 2006). While nutrient inputs can stimulate phytoplankton biomass, phytoplankton species and strains have varying preferences and requirements for nutrient supplies and forms (Glibert et al. 2016). In addition to quantity and form, the ratio of N and P supplies influences both phytoplankton abundance and assemblage composition (Smayda 1990, Sterner and Elser 2002).

Strengthened insights about the effects of increasing nutrient supplies and ratios, nutrient forms, and climatic variation on phytoplankton growth in potable source-water reservoirs is an identified need for improved forecasting of HABs and successful mitigation of their impacts (Ho

et al. 2019). This long-term (nine-year) study of a turbid eutrophic reservoir focused where possible beyond phytoplankton groups to the species level, recommended to advance understanding about long-term relationships between harmful species and environmental factors (Reynolds et al. 2000). We examined reservoir phytoplankton bloom composition by season and upper versus lower reservoir region over time. Ordination techniques (Kent and Coker 1992) were used to assess changes in bloom composition and response to environmental conditions over time, and to influences of weather variation. To gain further insights about nutrient regime controls on phytoplankton bloom taxa, summer microcosm experiments in situ (duration, 5 days) were used to assess responses to enriched inorganic nitrogen (N_i) form \pm enrichment with inorganic phosphorus (P_i) across a gradient of $N_i:P_i$ ratios.

Materials and Methods

Study Area

The study was conducted in the southeastern U.S., where shallow, turbid reservoirs are a major landscape feature (Touchette et al. 2007, Sun et al. 2013). Falls “Lake” (Falls of the Neuse Reservoir, 1983-; hydrologic unit 03020201; classification Water Supply IV, Nutrient Sensitive Water NSW since the late 1980s, Critical Area) is a relatively long, narrow impoundment (dimensions ~ 38.1 km x 0.2- 2.9 km at conservation pool capacity of 252 m³; dam location – lat 36°02’75”N, long 78°71’93”W; maximum depth 15 m) in the Piedmont region within the upper Neuse River Basin (hydrologic code 8; watershed area 1,994 km²) (U.S. Army Corps of Engineers [USACE] 2007, 2021) (Fig. 1). Two sites, one each in the old river channel of the upper and lower regions, were selected for study. The upper polymictic, hypereutrophic region is much shallower and wider than the lower eutrophic region (mean depth during the study, 5 m and 14 m in the upper and lower regions, respectively). The lower region is dimictic, mixing fully in spring and fall.

Falls Lake is designated for use as potable water supply, recreation, fish and wildlife habitat, and flood control (NC DEQ 2021). This potable-source water is depended upon by more than 0.6 million people in the City of Raleigh and some smaller population centers in the watershed, excluding the City of Durham which also lies partially in the watershed near the upper region. The urbanizing watershed is characterized by rapid population growth (population as of 2019, 200,000; U.S. Census data; 2.2% increase per year in the past \sim decade; U.S. Census Bureau data). For the past two decades, Falls Lake has been partly assessed by the state as impaired from excessive algal biomass or turbidity (NC Department of Environment and Natural Resources [DENR] 2009a,b; NC DEQ 2019; and see Carle et al. 2005, Rothenberger et al. 2009b) (Fig. 1).

The hydrologic regime was highly variable over the long-term study (Fig. 2). A total of 18 major storms passed through or near the state during the study, half of which especially affected the Falls Lake watershed with strong winds and high precipitation including tropical storms Claudette (August 2015) and Julia (September 2016); and hurricanes Irene (August 2011), Sandy (October 2012), Arthur (July 2014), Matthew (October 2016), Florence and Michael (August and October 2018, respectively), and Dorian (September 2019). Reservoir water level was maintained by the USACE (2021) (median 76.7 m; range 76.1 to 77.0 m; Fig. 2), with the greatest outflow matching periods of high precipitation in summers of 2016 and 2018, and

winters of 2014, 2016, and 2018 -2019. The study area also sustained droughts in summers of 2011 and 2015, and throughout 2012 and 2017.

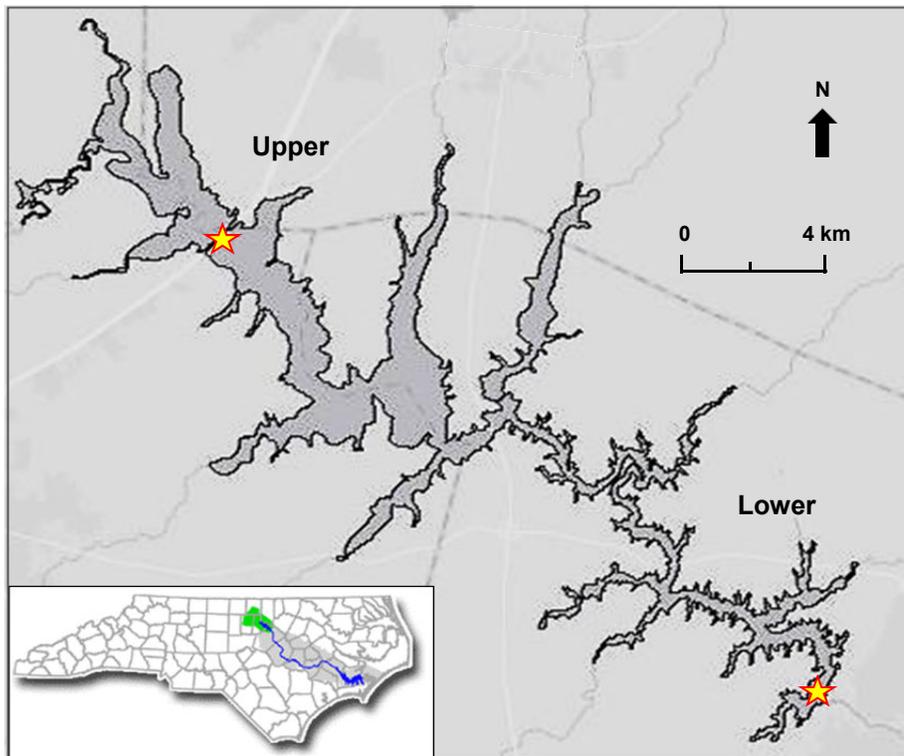


Figure 1. Map showing the long, narrow reservoir, Falls Lake, including the two sampling stations (stars) in the upper and lower regions. Insert map – Neuse River watershed (grey), which includes the Falls Lake watershed (green) comprising the upper river basin.

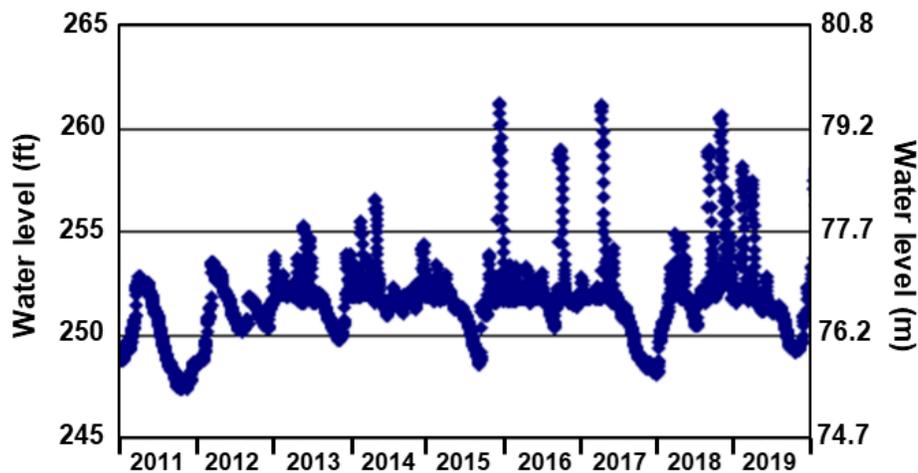


Figure 2. Water level at the Falls Lake dam, measured by the U.S. Army Corps of Engineers (Wilmington District, NC; available at <http://epec.saw.usace.army.mil/fallrept.txt>, last accessed in July 2020).

Environmental measurements and water quality sampling/analyses

Reservoir water level data for the study duration were available, for the lower region only, from the USACE (2021). Discharge data, of limited use, were available only from the U.S. Geological Survey - Bahama, NC station 0208550, ~6 km upstream from the upper reservoir. Precipitation data at the RDU airport were provided by the National Centers for Environmental Information at the National Oceanic and Atmospheric Administration (NOAA).

A long-term dataset on environmental measurements and water quality conditions, compiled by the Center for Applied Aquatic Ecology (CAAE, College of Agriculture and Life Sciences, North Carolina State University) was used for this study of reservoir surface waters (depths ≤ 2.5 m and ≤ 3 m in the upper and lower regions, respectively). Environmental measurements and water quality data were taken biweekly (April-October) to monthly (November-March). Data for environmental conditions (temperature, pH, dissolved oxygen [DO] as concentration and percent saturation) were collected from hydrocasts (Pro1020 Dissolved Oxygen and pH Meter, Yellow Springs Instrument, Yellow Springs, Ohio, USA) at ~0.5 m depth increments. Water-quality nutrients, total suspended solids (TSS), and phytoplankton biomass as chlorophyll *a* (*chl*_{*a*}) were sampled at depths 1 and 2.5 m in the upper region and 1 m and 3 m in the lower region. All physical measurements and samples for nutrient analyses were collected at discrete depths using a lab-line water sampler (model #CS-30, Medora Corporation, Dickinson, North Dakota, USA). Nutrient forms analyzed included total P (TP), total organic carbon (TOC), and N as total ammonia (tNH₃), nitrate+nitrite (hereafter, NO_x), and total Kjeldahl N, TKN (used to calculate TN as TKN + NO_x). Silica and soluble reactive phosphorus (SRP) were infrequently measured. Abundant alumino-silicate clays characteristic of the Piedmont (Weil and Brady 2017, Daniels et al. 1999) were reflected by high silica (several dates: 4,107 to 4,811 $\mu\text{g SiO}_2/\text{L}$, $n = 45$) and by SRP reservoir-wide (means $12 \pm 4 \mu\text{g P/L}$, $n = 18$).

Samples for nutrient and total suspended solids data were analyzed using standard methods from the U.S. Environmental Protection Agency (EPA) (1993, 1997) and the American Public Health Association (APHA) et al. (2017). Samples for all N and P forms were analyzed on a QuAAtro segmented flow autoanalyzer (Seal Analytical, Inc., Mequon, Wisconsin, USA). Samples for TOC analysis were analyzed on a Teledyne Torch Combustion TOC analyzer (Teledyne Tekmar, Mason, Ohio, USA). Samples for silica analysis were analyzed on a TrAACs 800 segmented flow analyzer (Bran +Luebbe, Buffalo Grover, Illinois, USA). Samples for *chl*_{*a*} analysis were analyzed following U.S. EPA (1997).

Phytoplankton assemblages

The environmental, nutrient, and *chl*_{*a*} data from the CAAE were used for retrospective statistical analyses to characterize bloom conditions in upper and lower Falls Lake during 2011-2019. Samples for analysis of phytoplankton assemblages had been preserved in the field with acidic Lugol's solution and stored in darkness at 4.4°C (Vollenweider 1974). Bloom assemblage samples were defined as having been taken at the same time/site as samples wherein *chl*_{*a*} equaled or exceeded the state standard for acceptable water quality ($\geq 40 \mu\text{g/L}$; NC DEQ 2019). Preserved samples taken during bloom conditions were analyzed by the Utermöhl method (Lund et al. 1958), using an Olympus IX70 inverted microscope (Olympus Scientific Solutions

Americas Corp., Waltham, Massachusetts, USA) at 200x to 400x. Cells were identified to the lowest possible taxonomic level (generally to genus or species). At least 400 cells and at least 100 cells of abundant taxa (present in $\geq 75\%$ of seasonal bloom samples; the 5 most abundant taxa each contributed $\sim 5\%$ or more of the total phytoplankton cells per sample) were counted from each sample (Lund et al. 1958, Brierley et al. 2007). Taxa with large variation in cell size (maximum dimension 30 to 150 μm) were sub-divided into size categories and biovolumes were estimated using geometric shape formulae that most closely matched the cell shapes (Burkholder and Wetzel 1989, Hillebrand et al. 1999). Biovolumes were calculated by multiplying cell densities by the average biovolume for each taxon (basis: prior measurement of at least 20 cells per size category per taxon).

In situ summer experiments (2017, 2018)

The original intent was to conduct short-term experiments during two summers considered as replicates, to assess the response of phytoplankton from the upper and lower regions to varied nutrient regimes. However, the two summers varied substantially in precipitation. Precipitation in the first summer was similar to the historic average for summers over the past decade (127 mm; NOAA data), whereas precipitation in the second summer was much higher (170 mm). This difference afforded the opportunity for an additional interesting weather comparison.

During the week before each experiment, arithmetic means, medians and ranges were given for data describing ambient environmental conditions in the upper versus lower regions during spring and summer, separately considered, in both years (Table 1). These data were used as background to calculate ammonia, nitrate+nitrite, and/or phosphate treatments for experiments (duration 5 days; Table 2), which were conducted over a two-week period in each summer. Experiments were initiated with water collected below the surface (depth ~ 0.5 m) from the upper and lower regions (Fig. 1). The water was filtered through a nylon screen (pore size 160 μm) to remove larger zooplankton, and then was added to fill 3.8-L cubitainers.

Experiments were initiated with water collected below the surface (depth ~ 0.5 m) from the upper and lower reservoir regions. The water was filtered through a nylon screen (pore size 160 μm) to remove larger zooplankton, and then was added to fill 3.8-L cubitainers. The cubitainers were placed within floating frames (depth 0.25 m) at a site minimally disturbed by boat traffic to maintain equal incubation depth for controls and treatments. At 1100 h on Day 1, $\text{N}_i \pm \text{P}_i$ were added to impose treatments. Before each experiment, background environmental conditions were recorded, including weather and air temperatures; and at depth 0.5 m, water temperature, pH, and dissolved oxygen [DO] as percent saturation and concentration. Samples were taken for initial (ambient) phytoplankton biomass as *chl**a* (corrected for pheopigments) and assemblage composition, and for nutrient concentrations including total ammonia (here referred to as NH_4^+ , assuming negligible unionized ammonia); nitrate+nitrite, NO_x (here referred to as NO_3^-); total Kjeldahl nitrogen, TKN (used to calculate TN as $\text{TKN} + \text{NO}_3^-$); total phosphorus, TP; soluble reactive phosphorus, SRP; total organic carbon, TOC; and silica, SiO_2 .

The imposed replicated treatments ($n = 3$) were selected to mimic common conditions during chronic cultural eutrophication, and included the maximum combination that logistically could be assessed. The experimental design was similar in both summers, and included a treatment with

Table 1. Ambient conditions in the two regions (means \pm 1 standard error [SE], range; $n = 3$) collected 5-7 days before the in situ experiments (precip = precipitation).

Upper region		
Parameter	1 st Summer (avg precip)	2 nd Summer (high precip)
Air Temperature (°C)	26 \pm 1 (24 – 27)	26 \pm 0.9 (26 – 29)
Water Temperature (°C)	29 \pm 0.2 (28 – 31)	28 \pm 0.1 (26 – 30)
pH	7.8 \pm 0.1 (7.9 – 8.1)	7.5 \pm 0.1 (7.3 – 7.6)
Dissolved oxygen (DO, mg/L)	9.5 \pm 0.2 (8.3 – 14.2)	7.4 \pm 0.2 (6.5 – 7.6)
Dissolved oxygen (DO, % saturation)	124 \pm 3 (108 – 185)	106 \pm 3 (88 – 140)
PAR ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)	1010 \pm 126 (818 – 1363)	1097 \pm 101 (715 – 1313)
Chl a ($\mu\text{g/L}$)	46 \pm 3 (30 – 59)	36 \pm 6 (17 – 52)
NH $_4^+$ ($\mu\text{g/L}$)	16 \pm 3 (11 – 23)	18 \pm 2 (15 – 24)
NO $_3^-$ ($\mu\text{g/L}$)	7 \pm 0.3 (7 – 8)	12 \pm 0.3 (12 – 13)
TN ($\mu\text{g/L}$)	1006 \pm 1 (1005 – 1006)	1048 \pm 20 (1007 – 1089)
SRP ($\mu\text{g/L}$)	6 \pm 2 (2 – 11)	4 \pm 1 (2 – 5)
TP ($\mu\text{g/L}$)	88 \pm 2 (81 – 91)	96 \pm 12 (34 – 127)
TN : TP	6 \pm 1 (4 – 7)	10 \pm 2 (8 – 13)
N $_i$: P $_i$	5 \pm 2 (2 – 8)	5 \pm 2 (2 – 7)
TOC (mg/L)	7.9 \pm 0.1 (7.1 – 8.7)	7.6 \pm 0.1 (6.4 – 9.5)
SiO $_2$ ($\mu\text{g/L}$)	8717 \pm 302 (3366 – 11180)	-----
Lower region		
Parameter	1 st Summer (avg precip)	2 nd Summer (high precip)
Air Temperature (°C)	26 \pm 1 (24 – 27)	26 \pm 0.9 (26 – 29)
Water Temperature (°C)	29 \pm 0.2 (28 – 31)	29 \pm 0.2 (29 – 30)
pH	7.9 \pm 0.5 (7.1 – 8.5)	7.4 \pm 0.1 (7.1 – 7.9)
Dissolved oxygen (DO, mg/L)	7.7 \pm 1.8 (6.8 – 8.0)	8.1 \pm 0.6 (7.6 – 8.4)
Dissolved oxygen (DO, % saturation)	98 \pm 2 (88 – 104)	105 \pm 2 (101 – 117)
PAR ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)	1011 \pm 126 (897 – 1176)	1281 \pm 101 (978 – 1484)
Chl a ($\mu\text{g/L}$)	16 \pm 1 (14 – 22)	16 \pm 1 (13 – 22)
NH $_4^+$ ($\mu\text{g/L}$)	10 \pm 1 (7 – 11)	3 \pm 5 (6 – 32)
NO $_3^-$ ($\mu\text{g/L}$)	5 \pm 1 (3 – 4)	4 \pm 0.3 (3 – 4)
TN ($\mu\text{g/L}$)	676 \pm 13 (663 – 289)	472 \pm 27 (445 – 526)
SRP ($\mu\text{g/L}$)	1 \pm 0.3 (0.3 – 2)	2 \pm 1 (1 – 3)
TP ($\mu\text{g/L}$)	21 \pm 0.2 (21 – 22)	23 \pm 2 (13 – 29)
TN : TP	5 \pm 2 (14 – 16)	23 \pm 4 (14 – 34)
N $_i$: P $_i$	4 \pm 1 (1 – 3)	5 \pm 2 (1 – 8)
TOC (mg/L)	7.7 \pm 0.1 (7.1 – 8.4)	7.4 \pm 0.1 (6.6 – 8.5)
SiO $_2$ ($\mu\text{g/L}$)	5266 \pm 256 (2078 – 10400)	-----

Table 2. Inorganic N and P additions and N_i:P_i ratios (by mass) used in treatments (*n* = 3; controls had ambient conditions as shown in Table 2).

Treatment	Concentration (µg/L)			Concentration (µg/L)		
	1 st Summer (avg precip)			2 nd Summer (high precip)		
Upper region	NH ₄ ⁺	NO ₃ ⁻	SRP	NH ₄ ⁺	NO ₃ ⁻	SRP
4:1 Ratio	---	---	---	ambient	ambient	15
14:1 Ratio	---	---	---	800	800	50
50:1 Ratio		300	ambient		400	ambient
72:1 Ratio	150	150	ambient	150	150	ambient
150:1 Ratio	800		ambient	800		ambient
Enriched Redfield (7:1)	1600	1600	150	---	---	---
Lower region	1 st Summer (avg precip)			2 nd Summer (high precip)		
4:1 Ratio	---	---	---	ambient	ambient	10
14:1 Ratio	---	---	---	800	800	50
50:1 Ratio		300	ambient		400	ambient
72:1 Ratio	150	150	ambient	150	150	ambient
150:1 Ratio	800		ambient	800		ambient
Enriched Redfield (7:1)	1600	1600	150	---	---	---

co-enrichment of both N_i and P_i (avg-precip summer – Redfield, 7:1 by mass; high-precip summer, 14:1 by mass); and a P_i alone treatment (N_i : P_i, 4:1) was added in the high-precip summer. Filtered reservoir water in cubitainers was amended with N_i as ammonium (NH₄⁺, using NH₄Cl) or nitrate (NO₃⁻, using KNO₃). Additions of P_i (soluble reactive phosphorus (SRP) were made using K₂HPO₄.

Nutrient concentrations and both TN:TP and N_i:P_i ratios and phytoplankton biomass as *chl a* were tracked daily, analyzed as described above. At depth 0.25 m, water temperature and photosynthetically active radiation (PAR) were recorded daily at 1100 h, and pH and DO were measured within each cubitainer. Phytoplankton assemblage samples were preserved and analyzed as described above.

Data Analyses

Environmental drivers of phytoplankton assemblages – Two seasons were emphasized in the long-term field study as summer (here, June through September) and winter (December through mid-March), based on monthly mean temperatures. Descriptive statistics (means, medians, ranges) were calculated for environmental variables (temperature, pH, DO, nutrients, TSS) and phytoplankton biomass as *chl_a* for the two designated seasons, reservoir-wide and separately for the upper and lower reservoir regions. Analysis of variance (ANOVA; Sokal and Rolff 2012) was used to assess statistically significant seasonal differences ($p < 0.05$ and $p < 0.1$) between the two reservoir regions considering all water quality variables.

Relationships between phytoplankton abundance as *chl_a* and environmental variables were preliminarily evaluated using scatter plots (U.S. EPA 2010). Correlations between *chl_a* and environmental data were then assessed using Pearson's correlation analysis ($p < 0.05$; version 4.0.0; R Development Core Team 2020). Correlations were considered as strong ($r \geq 0.7$), moderate ($0.7 > r \geq 0.5$), or weak ($r < 0.5$) following Moore et al. (2013). Non-metric multidimensional scaling (NMDS) was used to examine the similarity (or dissimilarity) in bloom assemblage composition or abundance as cell number and environmental variables considering reservoir region or season, using the metaMDS function from the “vegan” package in R (999 permutations; version 4.0.0; R Development Core Team 2020; Oksanen et al. 2019). Based on a scree-plot of stress and dimensionality, a two-dimensional plot was selected and stress was confirmed to be < 0.2 for all ordinations (Clarke 1993). A Shepard plot was used to assess the fit of the ordination using the “stressplot” function from the “vegan” package in R (version 4.0.0; R Development Core Team 2020; Oksanen et al. 2019). Prior to conducting NMDS, rare taxa (defined as present in $< 5\%$ of bloom samples) were removed (McCune and Grace 2002). Remaining phytoplankton abundances were compiled into a matrix of mean cell number by season and year (55 columns of taxa, 98 rows of bloom samples). Phytoplankton abundance data were then $\log_{10}(x + 1)$ transformed before estimating Bray-Curtis similarities (Bray and Curtis 1957). In NMDS ordinations, bloom samples were plotted based on their similarity (points closer together) or dissimilarity (points farther apart) in assemblage composition. In addition to its application to the full data matrix, NMDS was applied to summer and winter seasons separately to assess relationships between assemblage structure and environmental gradients and phytoplankton abundance within each season.

After data from samples were plotted, vectors representing environmental and species gradients were superimposed on the ordination biplots using the “Envfit” function in R (version 4.0.0; R Development Core Team 2020). These vectors were fit to represent the strength of correlations of environmental variables (vector cutoffs; $r^2 = 0.1$) and taxa (vector cutoffs; $r^2 = 0.4$) to bloom assemblage samples according to NMDS. Environmental variables in NMDS analyses included temperature, DO, nutrients, pH, TSS, reservoir water level (by the dam; thus, data for the lower region only), and discharge (data available from a U.S. Geological Survey gaging station ~6 km upstream from the upper region).

Indicator species analysis was also performed on the phytoplankton assemblage data (Dufrene and Legendre 1997), using the “Indicspecies” package in R (version 4.0.0; R Development Core Team 2020). Indicator taxa were defined as those consistently identified and most strongly

related to a specific group, as indicated by a significant indicator index value ($p < 0.05$; Dufrene and Legendre 1997). Indicator taxa were determined by running the indicator species analysis for year groupings that divided the study period into thirds (2011-2013, 2014-2016, 2017-2019); the two seasons of focus in this study (summer and winter as defined above); discharge ($\leq 15 \text{ m}^3/\text{sec}$, $\geq 45 \text{ m}^3/\text{sec}$) water level ($< 77 \text{ m}$, $\geq 77 \text{ m}$), temperature ($< 10^\circ\text{C}$, $> 25^\circ\text{C}$), and TSS ($< 10 \text{ mg/L}$, $10\text{-}30 \text{ mg/L}$). The delineations were based on the upper and lower data quartiles for discharge, from daily discharge measurements that coincided with sampling dates; the conservation pool capacity for water level; summer and winter medians for temperature; and relatively clear vs. turbid conditions for TSS (sensu Spruill et al. 2006).

Indicator taxa analysis was used to identify taxa associated with different concentration ranges or ratios of nutrients, as tNH_3 ($< 20 \text{ }\mu\text{g/L}$, $20\text{-}40 \text{ }\mu\text{g/L}$, $> 40 \text{ }\mu\text{g/L}$); NO_x ($\leq 200 \text{ }\mu\text{g/L}$, $> 200 \text{ }\mu\text{g/L}$); TN ($< 600 \text{ }\mu\text{g/L}$, $600\text{-}1200 \text{ }\mu\text{g/L}$, $> 1200 \text{ }\mu\text{g/L}$); TP ($< 50 \text{ }\mu\text{g/L}$, $50\text{-}100 \text{ }\mu\text{g/L}$, $> 100 \text{ }\mu\text{g/L}$); TN:TP (< 7 , $7\text{-}14$, > 14 by mass); TOC ($< 6 \text{ mg/L}$, $\geq 6 \text{ mg/L}$). Delineations for nitrogen were selected based on the following considerations: tNH_3 – U.S. EPA’s (1976) description of historic background levels generally at $\leq 20 \text{ }\mu\text{g/L}$; and NO_x and TN – approximating minimally impacted conditions ($177 \text{ }\mu\text{g NO}_x/\text{L}$ and $615 \text{ }\mu\text{g TN/L}$) for human-related nutrient inputs in rivers within level-III ecoregion IX, numeric sub-ecoregion 45 which includes the study area. The TP delineation greatly exceeded the U.S. EPA (2000a,b) minimally impacted condition for rivers ($30 \text{ }\mu\text{g/L}$; also see Dodds 2006) and reservoirs ($22.5 \text{ }\mu\text{g/L}$) in the study area, and was closer to Wetzel’s (2001) description of general eutrophic conditions in natural lakes ($> 80 \text{ }\mu\text{g TP/L}$). The TN:TP ratio delineations represented above versus below Redfield conditions by mass (Redfield 1958). The TOC cutoff was the City of Raleigh’s level (6 mg TOC/L) above which additional, costly treatment is required for removal of disinfection byproducts in potable water treatment (Hazen and Sawyer 2009).

Summer experiments – For data from the experiments, considering each summer and region, nutrient and *chl a* means were compared and significance ($p < 0.05$) was determined by day (Day 1 = initial, Day 2, Day 3, Day 4, Day 5) and treatment using two-factor ANOVAs followed by Tukey HSD tests (Helsel et al. 2020). Figures were created using GraphPad Prism version 9.0.0 for Windows (GraphPad Software, San Diego, California, USA). Linear regression analysis was used to assess relationships between final phytoplankton biomass as *chl a* and $\text{N}_i \pm \text{P}_i$ enrichment, using GraphPad Prism version 9.0.0 for Windows (GraphPad Software, San Diego, California, USA). Significance was assessed using a coefficient of determination ($R^2 \geq 0.5$ and $p < 0.1$).

Results

Long-Term Field Data: Environmental Conditions and Blooms

Reservoir seasonal conditions – The surface waters of Falls Lake were generally turbid; TSS concentrations in the upper region ranged from 19 to 72 mg/L over the nine-year study, versus 3 to 27 mg/L in the lower region (Fig. 3). High turbidity especially characterized summer conditions, when TSS concentrations were more than ten-fold higher in the upper region than in the lower region. Seasonal mean surface water temperatures ($\leq 3 \text{ m}$) varied from $26 \pm 0.3^\circ\text{C}$ (means ± 1 standard error [SE]; range, $25\text{-}30^\circ\text{C}$) in summer to $11 \pm 0.8^\circ\text{C}$ (range, $7\text{-}15^\circ\text{C}$) in winter (Fig. 3). Rainfall was highly variable, usually with monthly precipitation highest in summer and lowest in winter. Summer precipitation ranged from $8 \pm 1 \text{ cm}$ to $15 \pm 2 \text{ cm}$, whereas

mean precipitation was consistent throughout most winters (12 ± 2 cm). Dissolved oxygen concentrations in surface waters during summer seasons ranged from 4.9 to 8.2 mg/L, averaging 6.6 ± 0.2 mg/L (mean percent saturation $83 \pm 3\%$, range 21 to 119%), and the reservoir sustained hypoxic conditions throughout the water column in both regions for days to weeks in some years.

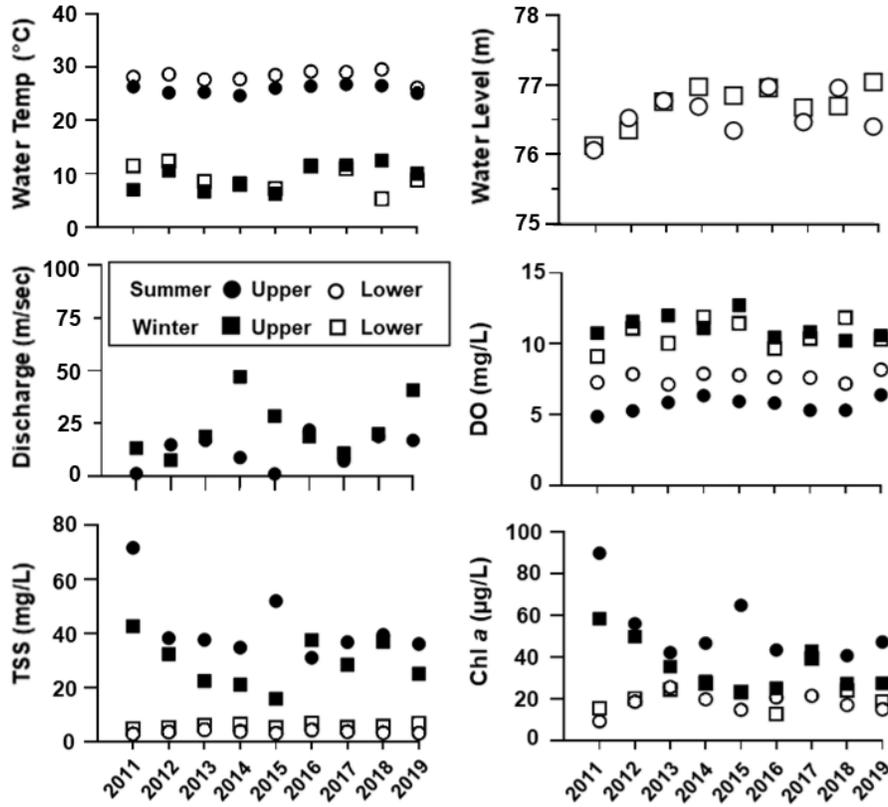


Figure 3. Environmental conditions (water temperature, temp; water level, lower reservoir only, in meters above mean sea level; discharge, upper reservoir only; dissolved oxygen, DO; total suspended solids, TSS) and algal biomass as corrected chlorophyll *a* concentrations (chl*a*) during summer and winter seasons (2011 through 2019) in surface waters (depth ≤ 3 m) of the upper and lower reservoir. Data are given as means ± 1 SE.

During drought years (2011, 2015, 2017), higher TSS and TP levels were observed in summers (Fig. 4). The upper region was characterized by more than two-fold higher mean TN concentration and more than five-fold higher mean TP (1030 ± 72 $\mu\text{g TN/L}$, 110 ± 10 $\mu\text{g TP/L}$) in comparison to the lower region (630 ± 20 $\mu\text{g TN/L}$, 30 ± 2 $\mu\text{g TP/L}$). Lower-region TN levels were depressed in the latter third of the study (2017-2019). Maximal TN in both seasons was measured from the upper region (1910 $\mu\text{g/L}$ in summer 2011, 1290 $\mu\text{g/L}$ in winter 2012). Summers were associated with highest TP concentrations: In the upper region, TP maxima were measured in summer 2011 (230 $\mu\text{g/L}$), whereas in the lower region, maximal TP occurred in summer 2016 (50 $\mu\text{g/L}$). TN:TP ratios in both seasons were lower in the upper region (Fig. 4). $\text{N}_i:\text{P}_i$ ratios (based on limited SRP data) in both regions were lower in summers than in winters ($4:1 \pm 0.2$ and 10.7 ± 3.3 , respectively).

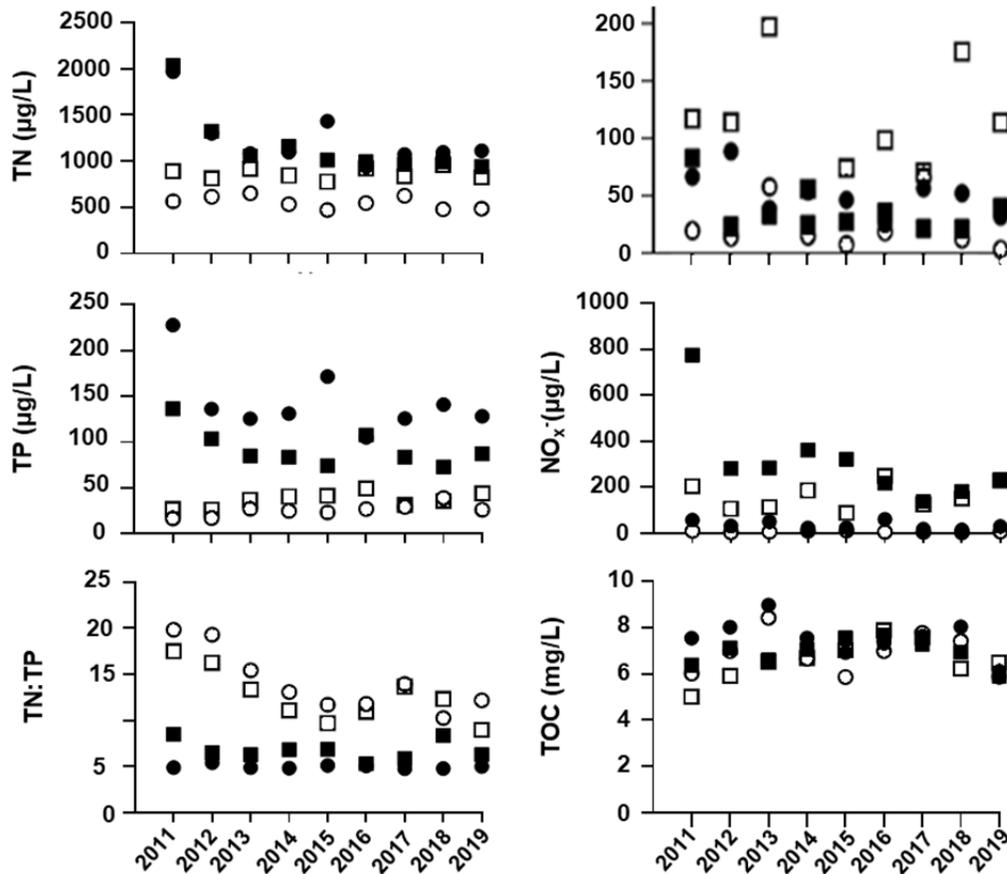


Figure 4. Nutrient concentrations and ratios during summer and winter seasons (2011 through 2019) in surface waters (depth ≤ 3 m) of the upper and lower reservoir, including total nitrogen, TN; ammonium, NH_4^+N , total phosphorus, TP; nitrate+nitrite-N, here NO_xN ; the TN:TP ratio by mass; and total organic carbon, TOC. Data are given as means ± 1 SE.

The dominant inorganic N (N_i) form changed depending on the season, with NO_x dominant in winter and tNH_3 more abundant in summer. During winter blooms, mean NO_x was often higher in the upper region (all winters: 260 ± 50 $\mu\text{g/L}$ and 160 ± 20 $\mu\text{g/L}$ in the upper and lower regions, respectively), and was often below 30 $\mu\text{g/L}$ in both regions during summers. Mean winter NO_xN concentrations were elevated in drought years (140 ± 34 $\mu\text{g/L}$ to 330 ± 160 $\mu\text{g/L}$), and maximal NO_xN was measured from the upper region (640 $\mu\text{g/L}$). Mean NH_4^+N during summer was similar in both regions (50 ± 6 $\mu\text{g/L}$ and 70 ± 13 $\mu\text{g/L}$ in the upper and lower regions, respectively), and was generally below 30 $\mu\text{g/L}$ during winters in the upper region. In contrast, winters within the lower region were characterized by highly variable. Maximal NH_4^+N (200 $\mu\text{g/L}$) occurred in the lower region during summer 2017, indicative of substantial internal loading. The two regions had similar TOC concentrations in both seasons (5.01 to 8.98 mg/L).

Phytoplankton blooms – A total of 98 bloom samples (82 in the summer and winter seasons), comprised of 55 taxa, were analyzed during the study, mainly including green algae (24 chlorophyte and streptophyte taxa), cyanobacteria (12 taxa), and diatoms (6 taxa) (Fig. 5). Dinoflagellates, cryptophytes, euglenoids, golden-brown flagellates, and yellow-green algae

were also well-represented during some seasons/years. Most blooms occurred in the upper region; in summers there were, on average, ~4 blooms and ~2 blooms in surface waters of the upper and lower regions, respectively. Winter bloom frequency ranged from 1 to 3 blooms in the upper region, and 1 to 4 blooms per month in the lower region.

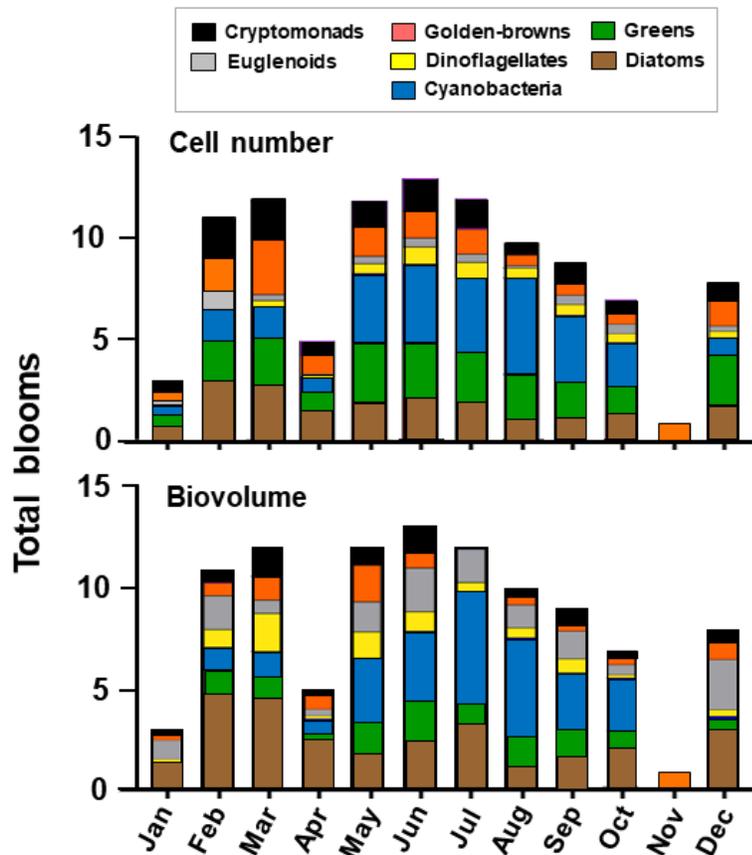


Figure 5. Total number of algal blooms per month over the study duration (2011-2019), and mean relative abundance of algal groups by (upper panel) cell number and (lower panel) biovolume, with blooms defined as *chl a* concentrations > 40 $\mu\text{g/L}$ in violation of the North Carolina state water quality standard (NC DEQ 2019).

Reservoir-wide, phytoplankton biomass as *chl a* was weakly to moderately negatively correlated with discharge ($p < 0.1$) (Table 3). Throughout the study in both seasons, there were significant positive correlations between algal biomass as *chl a* and TN, TP, TN:TP and TSS. *chl a* was positively correlated with tNH_3 in summers, but negatively correlated with tNH_3 in winters. *Chl a* also was positively correlated with NO_x in winters reservoir-wide and in summers within the lower region. In the upper region during summers, and in the lower region during both seasons, *chl a* was positively correlated with TOC, indicating that algal biomass was an important component of TOC throughout each year.

Blooms were larger in the upper region during both seasons. Summer blooms in the upper region varied over the study in total cells from 5 to 35 $\times 10^4$ cells/mL (overall mean $\sim 15 \times 10^4$ cells/mL) and in biovolume from 2 to 17 $\times 10^4$ $\mu\text{m}^3/\text{mL}$ (overall mean $\sim 6 \times 10^4$ $\mu\text{m}^3/\text{mL}$). In the lower

Table 3. Significant correlations involving *chl a* in the upper region, lower region, and reservoir-wide by season (winter, summer) over the study period (Pearson correlation coefficient *r*; significant at $p < 0.1$ (*)).

Parameters	<u>Upper Region</u>		<u>Lower Region</u>		<u>Reservoir-wide</u>	
	Summer	Winter	Summer	Winter	Summer	Winter
<i>Chla</i> vs. tNH ₃	0.34	----	----	----	0.50	-0.59
<i>Chla</i> vs. NO _x	----	----	----	0.78	----	0.57
<i>Chla</i> vs. TN	0.86	0.66	----	0.51	0.40	----
<i>Chla</i> vs. TP	0.77	0.76	0.47	----	0.83	0.40
<i>Chla</i> vs. TN:TP	0.44	----	----	0.62	0.70	0.84
<i>Chla</i> vs. TOC	0.45	----	0.45	0.57	0.49	0.76
<i>Chla</i> vs. TSS	0.44	0.62	0.53	0.44	0.85	0.88
<i>Chla</i> vs. discharge	-0.43	-0.49	----	----	-0.32*	-0.62

region, the overall means were 8.5×10^4 cells/mL and $4.5 \times 10^4 \mu\text{m}^3/\text{mL}$. The winter bloom overall mean and maximum were about twice as high in cell density in the upper region (maxima 25×10^4 cells/mL and 13×10^4 cells/mL in the upper and lower regions, respectively). Mean phytoplankton abundance in winter blooms was often comparable, however, in the two regions, as reflected by overall means of 10×10^4 cells/mL and 8.7×10^4 cells/mL in the upper and lower regions, respectively.

The onset and frequency of phytoplankton blooms varied by reservoir region and season. Over the study, 10 or more blooms occurred in the months of February, March, May, June, July, and August, whereas 5 or fewer blooms occurred in November (minimum of 1), January, and April (Fig. 5). Notably, cyanobacteria were major contributors to total phytoplankton cell number year-round. Moreover, although many blooms in the freshwater harmful algal literature have been described as monospecific (Huisman et al. 1999, Wynne et al. 2010, and references therein), blooms in this turbid reservoir were comprised of a mix of taxa.

Summers in both regions were characterized by higher algal biomass as *chl a* as well as higher bloom frequency. The upper region sustained higher *chl a* concentrations ($52 \pm 5 \mu\text{g/L}$, maximum $90 \mu\text{g/L}$) than the lower region ($18 \pm 2 \mu\text{g/L}$) in summers, often exceeding the state standard of $40 \mu\text{g/L}$ (Fig. 3). Blooms in the upper region were characterized by a higher percentage of toxigenic cyanobacteria by cell number ($38 \pm 4\%$) than blooms in the lower region ($20 \pm 2\%$). In winters, *chl a* averaged $34 \pm 3 \mu\text{g/L}$ and $23 \pm 3 \mu\text{g/L}$ in the upper and lower regions, respectively, and the maximum ($40 \mu\text{g/L}$) occurred in the lower region.

In summer blooms of the upper region, cyanobacteria were dominant or co-dominant with green algae by cell number, whereas diatoms were dominant or co-dominant with a mix of green algae or other groups in winter blooms. In terms of biovolume, diatoms were dominant or co-dominant in summer blooms of the first four years; thereafter, cyanobacteria were most abundant in two years, and a mix of cyanobacteria, euglenoids, and/or dinoflagellates were most abundant in the other three years. Winter blooms in the upper region were also variable in composition by biovolume. Diatoms predominated in four years. In the other five years, diatoms were co-dominant with euglenoids (two years), golden-brown algae (1 year), or dinoflagellates (1 year); and in the remaining year, euglenophytes were the most abundant group.

In the lower region, blooms did not occur in 2011 (either season) or in winter of 2016. During the other seven years, cyanobacteria were generally co-dominant by cell number with green algae in summers, whereas various taxa among diatoms, green algae, and golden-brown algae were the major bloom formers in winters. In terms of biovolume, cyanobacteria predominated in the lower region during six of eight summers, and were mixed with euglenophytes, green algae, and diatoms in the other two summers. Winter blooms were dominated by diatoms in three of seven years. Other winter blooms consisted mostly of diatoms with cyanobacteria, dinoflagellates, or golden-brown algae.

Abundant taxa reservoir-wide in summer blooms by cell number included *Dolichospermum flos-aquae*, *Cylindrospermopsis raciborskii*, *Raphidiopsis curvata*, naviculoid diatoms, and the cryptophyte *Plagioselmis nannoplanctica*. In the upper region, higher abundances of *C. raciborskii*, *R. curvata*, and *D. flos-aquae* occurred during drier years (2011, 2015, 2017). Whereas the abundance of most winter taxa remained fairly consistent or decreased, *C. raciborskii* increased in abundance over the study duration. This toxigenic cyanobacterium first became abundant in winter bloom samples during 2014 (lower region) – 2015 (upper region). For the remainder of the study, winter blooms contained abundant *C. raciborskii*, *R. curvata*, and *Plagioselmis nannoplanctica* along with centric diatoms, the *Synedra* complex, and filamentous centric diatoms *Aulacoseira* spp.

NMDS ordination and indicator taxa – NMDS ordination indicated different phytoplankton bloom assemblages during the two seasons, based on the general separation between summer and winter bloom points (Fig. 6). In the upper region in particular, the distance between summer and winter bloom points indicates that blooms seasonally differed in taxa composition. Blooms in the lower region were more mixed in taxa composition, indicated by the lack of taxa vectors. In NMDS ordination of the full data matrix, no taxa were associated with high discharge (≥ 45 m³/sec). Winter blooms occurred in the upper region under periods of low discharge (≤ 15 m³/sec), with abundant taxa including chlamydomonad-like taxa, cryptophytes, and diatoms within the *Synedra* complex (Fig. 6). Discharge did not meet the vector cutoff for analysis of phytoplankton assemblages in summers. Low water level periods at the dam were related to both winter and summer blooms in the lower region, including small chlorophytes and diatoms *Aulacoseira* spp. in both seasons. In the lower region, *Aphanizomenon* sp. was abundant in high-water periods during summers.

Reservoir-wide, the strongest associations to summer bloom assemblages throughout the study were to TN and TP concentrations in the upper region, and to TN:TP in the lower region (Fig. 6).

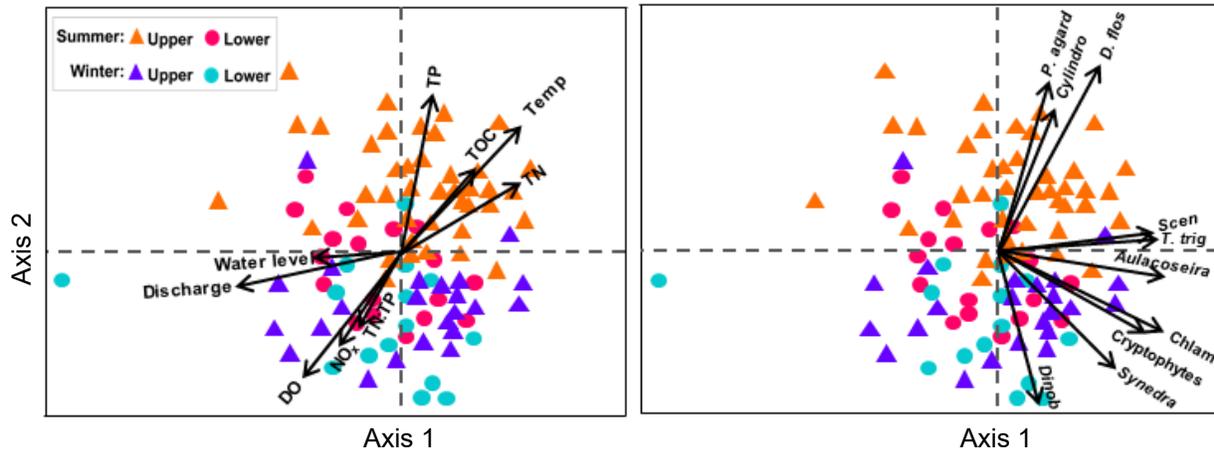


Figure 6. NMDS ordination of all summer and winter bloom samples, reservoir-wide: Analysis of all summer and winter samples by phytoplankton data, showing differences in environmental conditions and phytoplankton species composition by season and reservoir region. (A) Vectors indicate the strength and direction of environmental gradients (vector r^2 cutoff value = 0.1; Temp = temperature). (B) Vectors indicate strength and direction of species gradients, (vector r^2 cutoff value = 0.4). Algal taxa: *Planktothrix agardhii* (*P. agard*), *Cylindrospermopsis raciborskii* (*Cyllindro*), *Dolichospermum flos-aquae* (*D. flos*), *Scenedesmus* spp. (*Scen*), *Tetraedon trigonum* (*T. trig*), *Aulacoseira* spp., chlamydomonads (*Chlam*), cryptophytes other than *Plagioselmis nannoplanctica*, the *Synedra* complex, and *Dinobryon divergens* (*Dinob*).

In warmer summer temperatures, the filamentous cyanobacteria *Dolichospermum flos-aquae*, *Planktothrix agardhii*, and *Cylindrospermopsis raciborskii* were abundant, associated with high TOC, TN, and TP concentrations and low TN:TP ratios and NO_x . During the cooler temperatures of winter seasons, blooms were associated with DO and were characterized by a mix of mostly motile taxa such as the golden-brown flagellate *Dinobryon divergens*. Dominant summer bloom formers included cyanobacteria *D. flos-aquae*, *A. elenkinii*, and *P. agardhii*, especially in the upper region with higher tNH_3 concentrations as well as TSS, TN, and TP. In addition, during recent years (2017 through 2019), tNH_3 , TP, and TSS were strongly associated with summer bloom assemblages in the upper region and water level, and TN:TP in the lower region. These blooms also contained abundant colonial cyanobacteria *Merismopedia* spp. and *Aphanocapsa delicatissima* under conditions of high tNH_3 and high discharge. In the lower region, summer blooms consisted of abundant dinoflagellates, cryptophytes, and cyanobacteria at higher pH, DO, water level, and TN:TP ratios. Small chlorophytes and diatoms *Aulacoseira* spp. were abundant under conditions of lower water level and elevated TN. Winter blooms from more recent years (2017-2019) were more strongly related to TN, TSS, and TP than in recent years, and more weakly related to NO_x and TN:TP ratios.

Indicator taxa analysis supported the NMDS ordinations. Phytoplankton bloom assemblages shifted from abundant diatom and chlorophyte taxa earlier in the study (2011-2013) to dominance of cyanobacteria in more recent years (2015-2019). Indicator taxa for high discharge ($\geq 45 \text{ m}^3/\text{s}$) in the upper reservoir included *Merismopedia* spp., whereas many taxa were abundant under low discharge conditions ($\leq 15 \text{ m}^3/\text{s}$; *Aulacoseira* spp. and other centric diatoms, pennate diatoms within the *Synedra* complex, the chlorophyte *Chlorogonium* sp., the cyanobacteria *Dolichospermum* spp., and euglenophytes). In the lower reservoir, elevated water

levels (> 77 m) were indicated only by cyanobacteria. At low turbidity (TSS < 10 µg/L), colonial cyanobacteria, small chlorophytes, and euglenophytes *Phacus* spp. were identified as indicator taxa. TSS concentrations often exceeded 30 µg/L in the upper region (Fig. 3), coinciding with higher stormwater runoff. Taxa present under these conditions more likely were responding to these unfavorable conditions rather than indicating them. Diazotrophic cyanobacteria *Cylindrospermopsis raciborskii* and *Dolichospermum flos-aquae* were indicator species for tNH₃ concentrations below those recorded two decades ago in North Carolina Piedmont rivers (median 40 µg/L; Spruill et al. 2006). Falls Lake TP often exceeds 100 µg/L, and indicator taxa at such P levels included a subset of taxa from the 50-100 µg TP/L grouping.

Summer Short-Term Field Experiments (2017, 2018)

Ambient environmental conditions – In the week before the experiments, mean air (26°C - 27°C) air temperatures, water temperatures (29°C), and PAR were similar in both summers. As mentioned, the upper region was characterized by sustained high N and P concentrations (means > 1,000 µg TN/L, ~90-100 µg TP/L), whereas nutrient supplies were more variable in the lower region (means ~470-675 µg TN/L, ~20-25 µg TP/L) (Table 1). Prior to the experiments, TN and TP concentrations indicated excess nutrient supplies (sensu Bannister 1974, Wetzel 2001). In both summers, the upper region was characterized by significantly lower ambient TN:TP ratios (mean TN:TP 6:1, 10:1) and higher phytoplankton biomass (means 36 to 43 µg chl_a/L) than in the lower region (mean TN:TP 15, 23; mean chl_a ~16 µg/L) (Table 2). The chl_a of the upper region frequently exceeded the state standard for acceptable water quality (> 40 µg/L; NC DEQ 2019), indicative of excess algal biomass of “bloom” conditions. Prior to the experiments, TN and TP concentrations were suggestive of excess nutrient supplies (sensu Bannister 1974, Wetzel 2001). In contrast, concentrations of both N_i forms were relatively low, with NH₄⁺ about twice as high than NO₃⁻ (means in both summer and both regions, 10-18 µg NH₄⁺/L) (Table 1). Ambient SRP concentrations were also very low, below the PQL (≤ 6 µg/L) in both summers and regions. Mean N_i:P_i ratios were 4:1 to 5:1 in both summers and regions.

Upper and lower reservoir phytoplankton assemblages – In summer 2017-2018 phytoplankton assemblages, as in other years as described above, the most abundant algal groups were cyanobacteria, green algae, and diatoms. These three groups comprised more than 90% of the initial reservoir assemblages (Fig. 7). During both summers, the two regions had similar phytoplankton taxa, but varied in initial total cell number and relative abundance of algal groups. Although cyanobacteria dominated the phytoplankton assemblages from both regions, they initially comprised a higher proportion of the assemblage

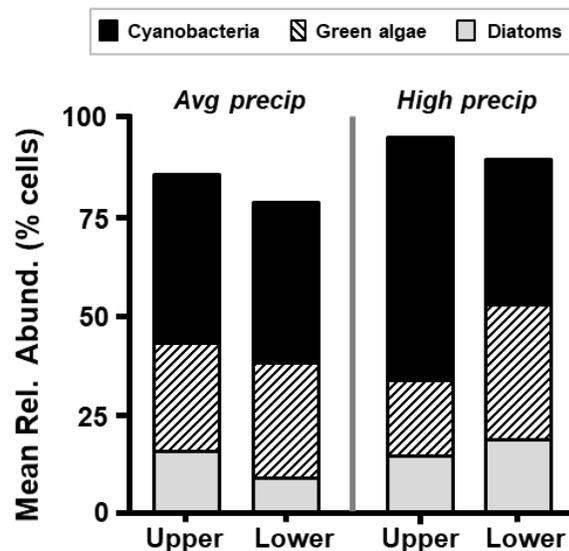


Figure 7. Initial relative abundance of cyanobacteria, green algae, and diatoms in natural phytoplankton assemblages from the upper and lower regions of the Falls Lake reservoir during the two summers.

from the upper region. During both summers reservoir-wide, cyanobacteria represented 40-60% of the relative abundance of assemblages in both regions, whereas green algae and diatoms contributed ~20% and ~15%, respectively. The most abundant taxon, toxigenic cyanobacterium *Cylindrospermopsis raciborskii*, comprised ~12% and ~21% of the natural phytoplankton assemblages from the upper and lower regions, respectively.

Phytoplankton response to nutrient treatments – Phytoplankton biomass as *chl a* was higher in treatments with $N_i + P_i$ co-enrichment than in treatments with N_i or P_i enrichment alone (Fig. 8). During both summers, maximum biomass of assemblages from both reservoir regions occurred in the co-enriched ratio treatments with N_i as NH_4^+ . During the avg-precip summer, phytoplankton from the upper region increased in biomass in the 150:1 ratio treatment (N_i as NH_4^+ ; Days 2-4, $p < 0.05$). Biomass was almost three-fold higher than initial levels by Day 3 in the co-enriched treatment (Redfield, 7:1; N_i as $NH_4^+ + P_i$; $p = 0.0024$). During the high-precip summer, biomass of phytoplankton from the upper region significantly increased in the 14:1 ratio treatment (N_i as $NH_4^+ + P_i$; Day 3; $p < 0.001$), whereas maximum biomass occurred on Day 5 in the 14:1 treatment with N_i added as NO_3^- ($p < 0.0001$). The final biomass exceeded the state standard for *chl a* when enriched with NO_3^- alone (Fig. 8).

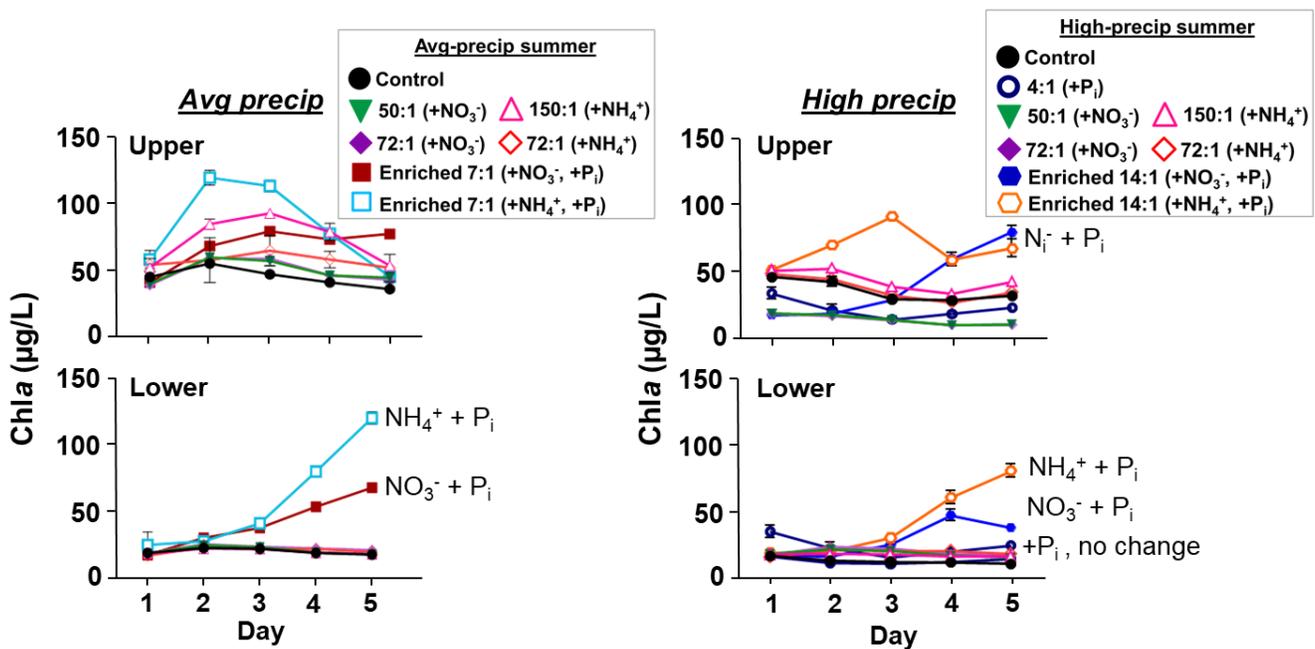


Figure 8. Chlorophyll *a* (*chl a*) concentrations (means \pm 1 SE) from Day 1 to Day 5 in treatments enriched with NH_4^+ (open symbols) or NO_3^- (closed symbols), with or without P_i enrichment, for phytoplankton assemblages from the upper and lower regions during the two summers. See text for discussion of statistically significant differences.

Biomass of the lower region assemblage in both summers responded more strongly to nutrient enrichment than the upper region assemblage, especially in the co-enriched treatments with either N_i form (Days 3-5; $p < 0.05$). In the avg-precip summer, maximum biomass (as *chl a*) occurred in the co-enriched treatment (Redfield, 7:1, Day 5; $p < 0.0001$) (Fig. 8). During the high-precip summer, biomass increased four-fold under $NO_3^- + P_i$ co-enrichment (14:1 ratio treatment (Days 3-5; $p < 0.0001$) (Fig. 8). Thus, P_i enrichment generally enhanced phytoplankton

response to N_i forms in both summers; moreover, P_i rapidly decreased via apparent drawdown when P_i was added alone or with N_i , such that P_i concentrations were negligible by Day 2.

Discussion

Long-Term Field Data - Environmental Controls on Phytoplankton Blooms

Seasonal dynamics of bloom assemblages – This study yielded new insights about the seasonality of phytoplankton bloom assemblages at functional group and taxa level in shallow turbid reservoirs such as Falls Lake. The long-term dataset on reservoir environmental conditions and phytoplankton blooms revealed interesting differences in bloom assemblages depending on season and reservoir region, with bloom assemblages in winters and summers more similar in the eutrophic lower region than in the hypereutrophic upper region. Ordination techniques indicated that blooms were dissimilar in the two reservoir regions, which differed in characteristics of water-column stability, nutrient supplies/stoichiometry, and light availability (as indicated by TSS; Kimmel et al. 1990). Bloom assemblage structure also varied temporally, driven by fluctuations in discharge (inflow) and water level, temperatures, turbidity (suspended solids concentrations), and shifting nutrient regimes. While blooms were more frequent in summers as expected, winter blooms were also common, especially in the upper region during most winter months.

Cyanobacteria, especially toxigenic filamentous species (*C. raciborskii*, *Dolichospermum flos-aquae*, and *Planktothrix agardhii*), dominated upper region bloom assemblages in cell number from May through September, extending into October in some years. Their dominance in the upper region increased in the latter half of the study considering both cell number and biovolume, indicating that cultural eutrophication is progressing in the upper region. In the lower region, cyanobacteria were one of several sub-dominant groups; they were variable in relative contribution to biovolume and remained ~stationary in cell number across years. The toxigenic filamentous cyanobacterium *C. raciborskii* was stimulated under variable N supplies and forms, likely contributing to its success in both winter and summer conditions. Diatoms were abundant in summer as well as winter blooms. NMDS indicated that the centric, chain-forming diatom, *Aulacoseira* spp., was associated with summers and mild winters. Naviculoid pennate diatoms, also abundant in Falls Lake summer bloom samples, can interfere with filtering in potable water treatment processes (Bellinger and Sigeo 2010). Naviculoid diatoms were summer indicators at temperatures above 25°C and low water level when increased mixing/turbulence of shallow waters likely assisted in maintaining naviculoid taxa in suspension (Lomas and Glibert 1999, Andersen et al. 2019).

Environmental drivers – Run-of-river impoundments receive higher nutrient and suspended sediment inputs when compared to natural lakes, due to their construction along a river course and to larger catchment-to-surface-area ratios (Vanni et al. 2006, Hayes et al. 2017). Following urbanization of watersheds, as is rapidly occurring in the Falls Lake basin, the loss of vegetation, high slopes, and low soil permeability increase transport of nutrients and sediments through increased stormwater runoff (Dabrowska et al. 2018 and references therein). High precipitation events supply additional nutrients while also contributing to less available light through increased suspended sediments, and/or lower phytoplankton biomass through physical removal (Kimmel et al. 1990, Vanni et al. 2006). Light availability in turbid reservoirs is often controlled

by suspended sediments, and increased sediment inputs typically occur under higher tributary discharge (Kimmel et al. 1990). Decreased light availability favors cyanobacteria and various mixotrophic flagellates, many of which can increase their concentrations of accessory pigments to photosynthesis under low light (Graham et al. 2016 and references therein).

Among the most important conditions known to affect reservoir phytoplankton are hydrologic characteristics such as water residence time and internal flow dynamics, mixing, main tributary discharges, and water level (Kimmel et al. 1990, Wetzel 2001, Vanni et al. 2006). Discharges commonly cause dilution and washout of phytoplankton blooms, and sinking out of encysted cells of various taxa to the sediments (Wetzel 2001, Vanni et al. 2006, Graham et al. 2016 and references therein). Changes in water level alter mixing dynamics and can either suppress or stimulate phytoplankton (Naselli-Flores and Barone 2000; Naselli-Flores 2000). Frequent, abrupt water level changes have promoted ecosystem destabilization symptoms such as proliferation of noxious species, including more frequent cyanobacteria blooms, and increased internal nutrient loading (Zohary and Ostrovsky 2011). In this study, increased flows negatively affected bloom assemblages: Phytoplankton biomass in the upper region was negatively related to discharge of a main upstream tributary to Falls Lake in both seasons, more notably in winter. In the lower region, elevated water level was associated with both summer and winter blooms. Elevated water level supported more cyanobacteria in winters, whereas many taxa thrived at low water level including diatoms, chlorophyte flagellates, filamentous cyanobacteria, and euglenoids.

Despite the hypereutrophic environment of the upper region, the reservoir bloom assemblages seasonally were strongly related to some nutrient forms. This finding lends support to Vanni et al.'s (2006) observation that in reservoirs affected by high abiotic turbidity, primary resource limitation switches between light (during/after episodic sediment loading events from storms) and nutrients (in periods of elevated water clarity). Phosphorus has long been recognized as a “master” nutrient controlling freshwater phytoplankton (Vollenweider 1968). In this study, bloom biomass (as *chl*_a) was positively related to TP supplies reservoir-wide in both seasons, moreso in summers. In summers TP was related to filamentous cyanobacterial abundance, whereas in winters TP supplies were associated with larger, more slowly growing taxa including diatoms, dinoflagellates, streptophyte greens, and euglenophytes. Phytoplankton biomass was positively related to tNH₃ concentrations, expected since ammonia or ammonium uptake requires less energy than NO₃⁻ uptake (Syrett and Lefty 1976, Flores and Herrero 2005). Indicator species for low to moderate tNH₃ concentrations (< 20 to ≤ 40 μg/L) included di-nitrogen-fixing cyanobacteria such as *C. raciborskii*. Green algal abundance frequently coincided with higher tNH₃ supplies, and the only indicator species for elevated tNH₃ (> 40 μg/L) were the green algae *Coelastrum* spp. and chlamydomonads. Biomass of winter blooms (as *chl*_a) was positively related to NO_x reservoir-wide. Diatoms have been reported to prefer NO₃⁻ over ammonia, linked to more NO₃⁻ transporters and enhanced ability to release excess NO₃⁻ in comparison to other algae (Lomas and Glibert 1999, Glibert et al. 2016).

Nutrient (N:P) ratios have been shown to be important influences on phytoplankton assemblage composition in waters across the salinity gradient worldwide (Burkholder and Glibert 2013 and references therein). In this study, assemblage abundance and composition were more weakly related to N:P ratios than to N and P concentrations or inorganic N forms, suggesting that the amount and forms of these nutrients were more important in influencing blooms.

Influences of Nutrient Regime: Insights from Short-Term in situ Experiments

The generally similar responses of the phytoplankton assemblages in the two summers likely occurred because the precipitation/ dilution/washout regimes would only have affected the phytoplankton before they were placed into closed microcosms in the experiments. The precipitation differential during the two summers, although substantial, probably were not enough to cause major changes in these resilient reservoir phytoplankton assemblages. Reservoir phytoplankton are well-acclimated to frequent, sudden changes in discharge and water level, and are adapted to thrive under the associated changing nutrient regimes (sensu Vanni et al. 2006). Cyanobacteria with high nutrient optima and adaptations to low light and mixing can thrive under such conditions until affected by appreciable washout (Jeppesen et al. 2011, Haakonsson et al. 2017). For example, they can withstand episodic sediment loading events because their sheaths protect the cells from direct contact with clay particles; they settle out, adsorbed to the clays, but the benthic population can serve as an inoculum for subsequent blooms when the water column clears (Burkholder 1992 and references therein).

Storm events alter nutrient supplies, light availability, and create turbulence that can mix and re-suspend microalgae within the water column (Tsai et al. 2008, Kuha et al. 2016), either stimulating or inhibiting phytoplankton growth and shifting assemblage composition (Naselli-Flores and Barone 2000, Schindler 2006). Precipitation varies in its effects on reservoirs and lakes, depending on the water residence time, depth, and catchment area-to-surface area (Søballe and Kimmel 1987, Sobek et al. 2007). Land use and cover also influence rainfall effects because urban lands have more runoff and nutrient/sediment inputs per unit land area (Fraterrigo and Downing 2008). Increased nutrient supplies can sustain elevated phytoplankton biomass concurrent with higher resource use efficiency and lower rates of cell turnover (Reynolds 2006).

Both the total assemblage and the cyanobacteria group were more stimulated by co-enrichment of N_i and P_i than by either N_i form or P_i alone. Filamentous cyanobacteria were dominant both in the reservoir and throughout the experiments. Of these, *C. raciborskii* showed the strongest stimulation by $N_i + P_i$ enrichment, supporting previous research in temperate and subtropical reservoirs (Burford et al. 2018 and references therein). In the assemblage from the hypereutrophic upper region, *C. raciborskii* increased with co-enrichment by either N_i form + P_i , whereas *C. raciborskii* from the eutrophic lower region was more strongly stimulated by $NH_4^+ + P_i$ co-enrichment. Thus, despite elevated ambient nutrient supplies, the biomass (as chl a) of assemblages from both regions increased under N_i or $N_i + P_i$ co-enrichment. Phytoplankton from the lower region responded more strongly to nutrient enrichment than did the upper region assemblage. Similar findings have been reported from eutrophic versus hypereutrophic natural lakes (e.g., Brookes and Carey 2011). Ambient conditions for Falls Lake suggest that the upper reservoir assemblage was more nutrient-replete (Vanni et al. 2006). Nevertheless, there was a rapid decrease of the additional N_i and P_i supplies, inferred as drawdown, even by the hypereutrophic assemblage, as has been reported from other surface waters (Glibert et al. 2016). The assemblages from both regions attained maximum biomass under N_i and P_i co-enrichment, suggesting synergistic interaction of the two nutrients are in affecting phytoplankton growth (Elser et al. 2007). Increased P availability indirectly promotes N_i uptake and phytoplankton growth through the increased availability of adenosine triphosphate (ATP) and the activation of enzymes for the uptake and assimilation of N_i (Flores and Herrero 2005).

Conclusions

This work contributes information on long-term changes in phytoplankton bloom composition in temperate shallow, eutrophic reservoirs sustaining chronic cultural eutrophication. NMDS ordination and indicator taxa analysis provided insights about phytoplankton bloom dynamics, including toxigenic taxa within two algal groups, along physical/chemical gradients in winter and summer seasons. Phytoplankton blooms in this turbid reservoir varied by region and season, and toxigenic cyanobacteria as well as toxigenic euglenophytes were often abundant in both summer and winter assemblages of the upper region. The southeastern U.S. is projected to continue to warm (Harned et al. 2010, Terando et al. 2014), as are many regions of the world, and filamentous, toxigenic cyanobacteria are expected to increase, displacing diatoms and chlorophytes (Staehr and Sand-Jensen 2006, Zohary et al. 2021).

The dynamics of reservoir phytoplankton bloom assemblages have seldom been characterized during the eutrophication process using enough data to assess relationships between environmental factors and harmful taxa over a long-term period. As Rothenberger et al. (2009a) noted using a similar approach in an estuary, identification of the environmental factors that drive phytoplankton [assemblage] change using long-term data series will improve predictions about the probability of phytoplankton blooms and the most likely causal taxa given a specific combination of environmental factors. Building from the field data in this study, future work can strengthen insights about phytoplankton blooms by experimentally testing assemblage responses to nutrient enrichment and stoichiometric imbalance under warming conditions and anticipated more extreme weather events, in comparison to their responses under historic average conditions.

This long-term field study of Falls Lake phytoplankton blooms and the short-term in situ summer experiments underscore the increasing importance of reducing supplies of *both* N and P to improve water quality by decreasing noxious algal growth in freshwaters such as shallow turbid reservoirs. The findings support both the Falls Lake Rules (NC DEQ 2021) and the U.S. EPA (2015). The data also illustrate the often-overlooked importance of N_i in stimulating phytoplankton (Gobler et al. 2016 and references therein). In the short-term experiments where N_i was added to all treatments except the 4:1 ratio treatment (enriched with P_i alone), phytoplankton biomass was more strongly stimulated as nutrient enrichment increased. As expected, phytoplankton responded more strongly to concentrations rather than ratios, and biomass as the *chl a* indicator showed more of a response than species composition, likely due to the initially elevated phytoplankton biomass, excess nutrient supplies, abundance of relatively few species, and the short experimental period (Smayda 1990, Edwards et al. 2011, Dolman et al. 2012).

The toxigenic cyanobacterium *Cylindrospermopsis raciborskii* dominated the Falls Lake phytoplankton assemblages both initially and throughout these short-term experiments with varying nutrient supplies, N_i forms, and both N_i and P_i supplies and ratios. Aside from its plasticity in response to N and P enrichment, the geographic range of this cyanobacterium appears to be expanding, as it recently has been found more frequently in temperate climates (Bonilla et al. 2016 and references therein). Projected warming trends for the Southeast (Terando et al. 2014) are expected to favor increased cyanobacterial dominance (e.g., O'Neill et al. 2012). Increased precipitation events and associated nutrient enrichment, along with climate warming

(Sun et al. 2013), are also expected to favor cyanobacteria, many of which have high temperature optima and can withstand increased disturbance (Burkholder 1992, Jöhnk et al. 2008). The data indicate that some filamentous cyanobacteria can rapidly respond to enrichment with either N_i form, and that filamentous cyanobacteria will increase production under varying nutrient regimes once established in the phytoplankton assemblage.

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