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NUTRIENT LIMITATION OF PHYTOPLANKTON IN LAKE WATEREE, SOUTH
CAROLINA: IMPLICATIONS FOR FUTURE WATER QUALITY MANAGEMENT

by

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ABSTRACT

Cultural eutrophication is a primary contributor to phytoplankton production in freshwater lakes from excess anthropogenic nutrient inputs, and resulting impacts on water quality, aquatic ecosystems, human and animal health are increasing worldwide. Understanding the factors that limit phytoplankton growth is an important strategy for identifying and managing nutrient sources and successfully controlling the over-enrichment of nitrogen (N) and/or phosphorus (P) in freshwater lakes. The Redfield molecular ratio of carbon (C), N and P maintains that the C:N:P molar ratio is 106:16:1 when nutrients are not limiting, and the ecosystem is balanced. A N:P molar ratio > 20:1 tends to be indicative of P limitation in freshwaters, while a N:P molar ratio < 10:1 usually indicates N limitation, regardless of freshwater or seawater. Historically, studies on freshwater lakes have emphasized control of P, but more recent studies have challenged the P limitation paradigm and emphasized control of N or duel control of N and P.

Surveillance monitoring and ambient water quality and nutrient data in Lake Wateree, South Carolina indicate elevated symptoms of excess nutrients including decreased dissolved oxygen and water clarity, elevated pH and increasing phytoplankton blooms, both spatially and temporally. This study aimed to define the nutrient limitation indicator(s) to predict phytoplankton growth in Lake Wateree using nutrient enrichment bioassays. A series of four factorial design experiments were conducted during the

summer and fall (August through October 2017) for the evaluation of both separate and interactive roles of N and P during *in situ* incubation periods of 48 hours within the lake environment at ambient conditions. The four treatments included a control (deionized water), + N (NH_4^+), + P (PO_4^{3-}) and + NP (NH_4^+ + PO_4^{3-}) additions and their effects on phytoplankton growth using chlorophyll-*a* fluorescence as the response variable.

Nutrient relationships were determined from the twenty categorical responses, and despite exceedances in P water quality criteria, the bioassays produced no P limitation or serial P limitation responses. Instead, twelve of the experimental responses (60%) were co-limitation, four responses (20%) were serial N limitation, three responses (15%) were N limitation, and one response (5%) was not significant. The results of this study are valuable in identifying the importance of each nutrient factor (N and P) and achieving successful lake management goals by reducing excess nutrients and improving water quality in Lake Wateree.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Eutrophication is the process of natural aging and nutrient enrichment in lakes, streams and estuaries that normally occurs over hundreds or thousands of years. As organic matter and sediments from surrounding terrestrial areas flow into an aquatic ecosystem, waterbody characteristics including depth, oxygen levels, biological productivity and water clarity become altered (SCDHEC 2014). Cultural eutrophication occurs when natural eutrophication is accelerated by anthropogenic activities. Pollution sources may include the discharge of nutrients such as nitrogen (N) and phosphorous (P) from waste water treatment plants (WWTPs) and septic systems, urban and agricultural nonpoint source runoff, concentrated animal feeding operations (CAFOs), atmospheric deposition, industry discharges, and fertilizers from lawns and golf courses. Primary symptoms of cultural eutrophication may include decreased biodiversity, changes to dominant species, and stimulated growth of aquatic autotrophs including both beneficial and harmful algae as well as aquatic vascular plants, hence increasing primary productivity in aquatic ecosystems. (Lewis et al. 2011). Secondary symptoms of nutrient enrichment from eutrophication include water quality degradation, increased risk of harmful algal blooms (HABs), excessive decomposition of organic matter, oxygen depletion (hypoxia, anoxia), decreased water clarity, and taste and odor issues. Other

secondary symptoms are water treatment impairment, loss of recreational services and tourism, alterations in aquatic community compositions, and fish kills. Identifying the relationships between ecological status and anthropogenic pressures are important in determining and quantifying the impacts of excess nutrients on phytoplankton communities (Phillips et al. 2008).

Trophic state indices (TSI) are used to define eutrophication and measure water quality in aquatic environments. Waters are classified as oligotrophic, mesotrophic, eutrophic and hypereutrophic using surrogate measurements of Secchi transparency, total phosphorus (TP) and chlorophyll-*a* (Chl-*a*) (Kuehl et al. 2013). The relationships between surrogate measurements and lake productivity may be influenced by external factors and can be regionally variable (Kuehl et al. 2013). The South Carolina Department of Health and Environmental Control (SCDHEC) has classified Lake Wateree as eutrophic, using Chl-*a* numeric criterion for trophic state classifications that maintains Chl-*a* for lakes (> 40 acres) in the Piedmont is not to exceed 40 µg/L (SCDHEC 2014).

Table 1.1: SCDHEC trophic state and Chl-*a* numeric criteria for lakes (> 40 acres) in the Piedmont is not to exceed 40 µg/L (or 0.04 mg/L). Note that Lake Wateree is classified as eutrophic. (Source: SCDHEC 2014)

TSI	Classification Criteria	Chl-*a* (µg/L)
Oligotrophic	Clear waters with little organic matter or sediment, and minimum biological activity.	< 5
Mesotrophic	Waters with more nutrients and more biological productivity.	5-20
Eutrophic	Waters extremely rich in nutrients, with high biological productivity, episodes of algal blooms and low oxygen. Some desirable species may be eliminated.	20-60
Hypereutrophic	Very high nutrient loading, highly productive waters, frequent algal blooms, turbid, low oxygen, fish kills.	> 60

Chl-*a* concentrations are commonly used to measure phytoplankton biomass and are significantly correlated to total nitrogen (TN) and TP (Phillips et al. 2008). Chl-*a* is the most predominant photosynthetic pigment for oxygenic photosynthesis, and it absorbs red, blue and violet wavelengths in the electromagnetic spectrum, and reflects green and yellow light. Accessory pigments, including chlorophylls *b* and *c*, carotenes and xanthophylls, transfer energy to Chl-*a* so it can be used. Chl-*a* is highly correlated with phytoplankton biomass and can be used as a proxy for measuring direct algal cell counts or indirectly quantifying phytoplankton pigment concentrations. The quantity of fluorescence emitted from Chl-*a* is inversely proportional to the energy expended for photochemical exertion, and is representative of the energy metabolism in photosynthetic cells (Beardall et al. 2001).

The abundance and availability of nutrients can limit primary productivity and alter ecosystem processes in lakes, especially in surface waters (Beardall et al. 2001). N and P have been significant factors in many historical investigations of nutrient impacts on aquatic ecosystems, including nutrient limitation (Lewis et al. 2011). The limiting nutrient of phytoplankton growth in inland waters, under natural or anthropogenic conditions, can be determined by measuring increases in algal biomass in the presence of a particular nutrient (N, P or both N and P), and understanding nutrient roles and availability in natural populations (Beardall et al. 2001). Therefore, the limiting nutrient in an aquatic ecosystem is the nutrient available in the shortest supply relative to phytoplankton demand that regulates phytoplankton growth. Liebig's Law of the Minimum is a historical conceptual explanation of nutrient limitation that maintains the theory of single-nutrient limitation (Liebig 1842, Harpole et al. 2011). However, Liebig's

Law was developed for crop responses to growth factors, and its relevance to naturally diverse communities has been debated (Harpole et al. 2011, Müller et al. 2015). The Redfield molecular ratio of carbon (C), N and P maintains that a balanced ecosystem will have a molecular ratio of 106C: 16N: 1P when nutrients are not limiting, and is one of the most frequently applied stoichiometric references for nutrient limitation of phytoplankton biomass (Ptacnik et al. 2010). A N:P molar ratio > 20:1 usually indicates P limitation in freshwaters, while a N:P molar ratio < 10:1 is typically indicative of N limitation, regardless of freshwater or seawater (Redfield 1934). The control of anthropogenic N and P inputs in freshwater ecosystems is a primary strategy for controlling cultural eutrophication. Historically, studies on freshwater lakes have emphasized control of P, but more recent studies have challenged the P limitation paradigm and emphasized control of N or a combination of N and P equally (co-limitation) (Lewis et al. 2011). Consequently, existing paradigms and limited understanding of nutrient roles across diverse aquatic ecosystems have made it difficult for ecologists to make recommendations to watershed managers for nutrient controls (Elser et al. 2007). Understanding the N and P biogeochemical cycles and the factors that limit phytoplankton growth is important in the protection of aquatic ecosystems and public health.

1.1 Nitrogen as a Water Pollutant

Nitrogen is an essential nutrient for growth of all living organisms and exists as a solid, liquid and gas. Major anthropogenic sources of N in the United States include industry, agriculture and transportation (Davidson et al. 2012), and excess N inputs to aquatic ecosystems may occur from localized sources such as ground and surface waters

and septic systems, though the relative importance of N sources depends on location and climate (Kanter et al. 2016). N pollution can cause adverse ecological impacts including hypoxia and anoxia, HABs, loss of biodiversity, and disease and die-offs of impacted species (Davidson et al. 2012). Excess N in soils may be converted to nitrates by bacteria, which can infiltrate groundwaters and contaminate drinking water sources (Davidson et al. 2012). In addition, increasing anthropogenic N inputs have impacted the global carbon cycle, causing elevated primary production and carbon dioxide (CO_2) fixation (Davidson et al. 2012).

The various forms of N can have specific effects on nutrient limitation in lakes (Sterner 2008). The dominant source of N for phytoplankton growth in inland waters is usually dissolved inorganic nitrogen (DIN) (Lewis et al. 2011), and its most abundant forms include nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+) (Collos and Berges 2003). Concentrations of NO_3^- can have considerable spatial and temporal variability and are usually the predominant form of N in enriched rivers (Durand et al. 2011), while NO_2^- is usually a minor component of TN relative to NO_3^-. Ammonium concentrations may also vary widely spatially and temporally (Kumar et al. 2007) and are usually higher in hypoxic and anoxic environments such as sewage and wastewater inputs, agricultural runoff, and areas of high benthic biomass. The preferred form of N for phytoplankton is NH_4^+, as it requires less energy (Collos and Berges 2003). Dissolved organic nitrogen (DON) is nitrogen combined with carbon that is composed from compounds such as amino acids, nucleic acids and urea (Lewis et al. 2011). DON is characterized as either labile compounds which are easily broken down, or refractory compounds that are not easily broken down.

Nutrient biogeochemical cycles can limit aquatic ecosystems (Khangaonkar et al. 2012), and the complex global N cycle consists of several processes by which N is interconverted between its different chemical forms. N fixation is a process in which prokaryotes including bacteria and archaea convert atmospheric nitrogen (N_2) to NH_4^+ using the enzyme nitrogenase to catalyze the reduction, and N fixation can be inhibited by elevated NH_4^+ and oxygen (O_2) (Howarth et al. 1988). Some cyanobacteria species have herterocysts with thick cellular walls that protect the nitrogenase from exposure to O_2, enabling N fixation and often allowing cyanobacteria to thrive under certain environmental conditions. Nitrification and denitrification are coupled microbial processes. Nitrification (new production) is the oxidation of NH_4^+ to NO_2^- to NO_3^- and can cause O_2 depletion in aquatic ecosystems, while denitrification is the reduction of NO_3^- to N_2 and can only occur under anoxic conditions (Durand et al. 2011). Ammonification (regenerated production) is the conversion of NO_3^- to NH_4^+, and though certain phytoplankton species can tolerate high levels of NH_4^+, this compound may be toxic at low concentrations to other species such as dinoflagellates (Collos and Berges 2003).

1.2 Phosphorus as a Water Pollutant

Phosphorus (P) is an essential nutrient for all organisms and is a major element in organic matter. Unlike N, P has no significant gaseous component (Schlesinger and Bernhardt 2013). Phosphates are slowly dissolved from rocks by natural weathering, but the anthropogenic mining of P containing rock formations accelerates the P cycle. Phosphorous is a common component in fertilizers, industrial effluent, manure and organic sewage wastes (Metson et al. 2016), and it has important implications for

transport mechanisms, either in dust or dissolved in water. Soil erosion is a major contributor of impacted surface water quality when P attaches to soil particles and is transported into aquatic ecosystems in land surface runoff (USEPA 2019). Excess P can accelerate eutrophication in lakes and rivers, and cause harmful ecological impacts including phytoplankton blooms, alterations to food webs, loss of species diversity and hypoxia (USEPA 2019). Phosphorous in groundwaters may also contaminate drinking water sources, causing public health concerns (USEPA 2019).

Phosphorus exists as dissolved inorganic phosphorus (DIP), dissolved organic phosphorus (DOP) and particulate phosphorus (PP) (Kaiser et al. 2011). Particulate P concentrations tend to be the most dominant forms, while DIP concentrations are usually more minor contributors and are the most bioavailable (Kaiser et al., 2011). In aerobic environments, P occurs almost exclusively as orthophosphate (PO_4) which is widely bioavailable in the environment to phytoplankton and other plants (Boström et al. 1988). Phosphates can form insoluble compounds with certain metals and can be limiting in calcium-rich environments. The reduction of ferric iron (Fe_3^+) to ferrous iron (Fe_2^+) from the combination of bacteria and hydrogen sulfide (H_2S) can result in the greater availability of dissolved P. Phosphonates were historically thought to be unavailable to phytoplankton, but cyanobacterial diazotrophs have been observed to use phosphonate and fix N when DIP is low (Wu et al. 2003).

1.3 Study Area

Lake Wateree is a eutrophic reservoir that spans approximately 240 miles of shoreline in Kershaw, Lancaster, Fairfield and Chester counties in the Piedmont of South Carolina (SC) (Figure 1.1). It was named after The Wateree, a Native American tribe that

once inhabited the area near present-day Camden, SC. Lake Wateree was formed when the Wateree Hydroelectric Station was completed in 1919, and is one of the oldest man-made reservoirs in SC. Owned and managed by the Duke Energy Corporation, Lake Wateree has 13,025 acres of surface water, an average depth of 6.9 feet, an average hydraulic residence time of 27 days, and a maximum elevation of about 225.5 feet above mean sea level (SCDNR 2019). It is a major drinking water source for both Lugoff-Elgin and Camden, SC, a recreational resource for contact recreation, and it supports diverse fish and wildlife habitats. Lake Wateree is home to 18 public access locations, the Lake Wateree State Park, the Shaw Air Force Base Recreation Center and a 1,628-acre nature preserve.
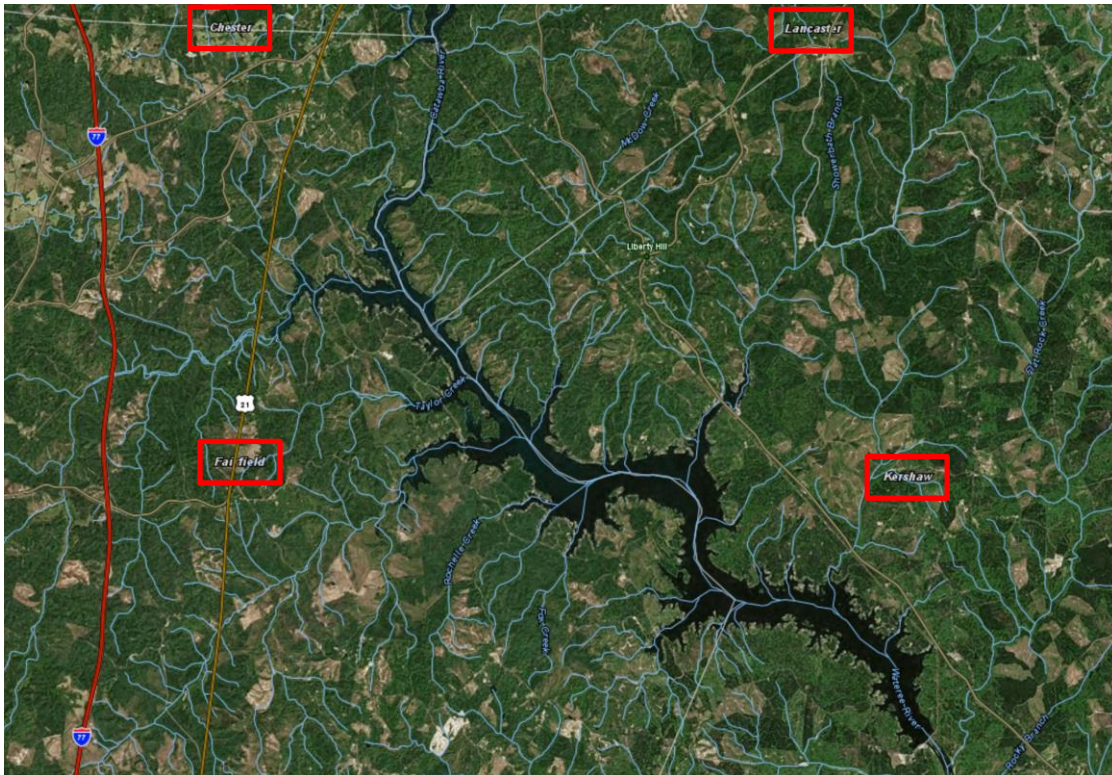
Figure 1.1: Lake Wateree spans Kershaw, Lancaster, Fairfield and Chester counties in South Carolina, as indicated by the *red rectangles*. (Source: United States Department of Agriculture, Web Soil Survey, 2019)

Lake Wateree is downstream from ten other major reservoirs in the Catawba-Wateree River Basin (Catawba Riverkeeper 2019). The Catawba-Wateree River originates in the Blue Ridge Mountains of western North Carolina (NC) and flows through metropolitan Charlotte, NC and into Lake Wateree before discharging into the Congaree National Park in Hopkins, SC and meandering to the coast (Figure 1.2). It supplies drinking water to approximately two million people and is one of the most rapidly developing areas in the United States (Catawba Riverkeeper 2019). The Catawba-Wateree River was named the most endangered river in the United States in 2008 by American Rivers, and was noted as the third most endangered river in the Southeast United States in 2012 by the Southern Environmental Law Center. In 2011, the Union of Concerned Scientists named the Catawba-Wateree River the fourth most stressed river in the United States from power production. It was estimated that the river loses 75 million gallons of water each day from power generation, and unknown quantities of water are lost daily from cooling processes. In addition to being threatened by power generation from coal and nuclear energy production, the Catawba-Wateree River is increasingly stressed from point and nonpoint source pollution discharges from urbanization, municipal wastewater treatment plants, industrial waste, agriculture, stormwater discharge, CAFOs, increased imperviousness and climate change (Catawba Riverkeeper 2019).
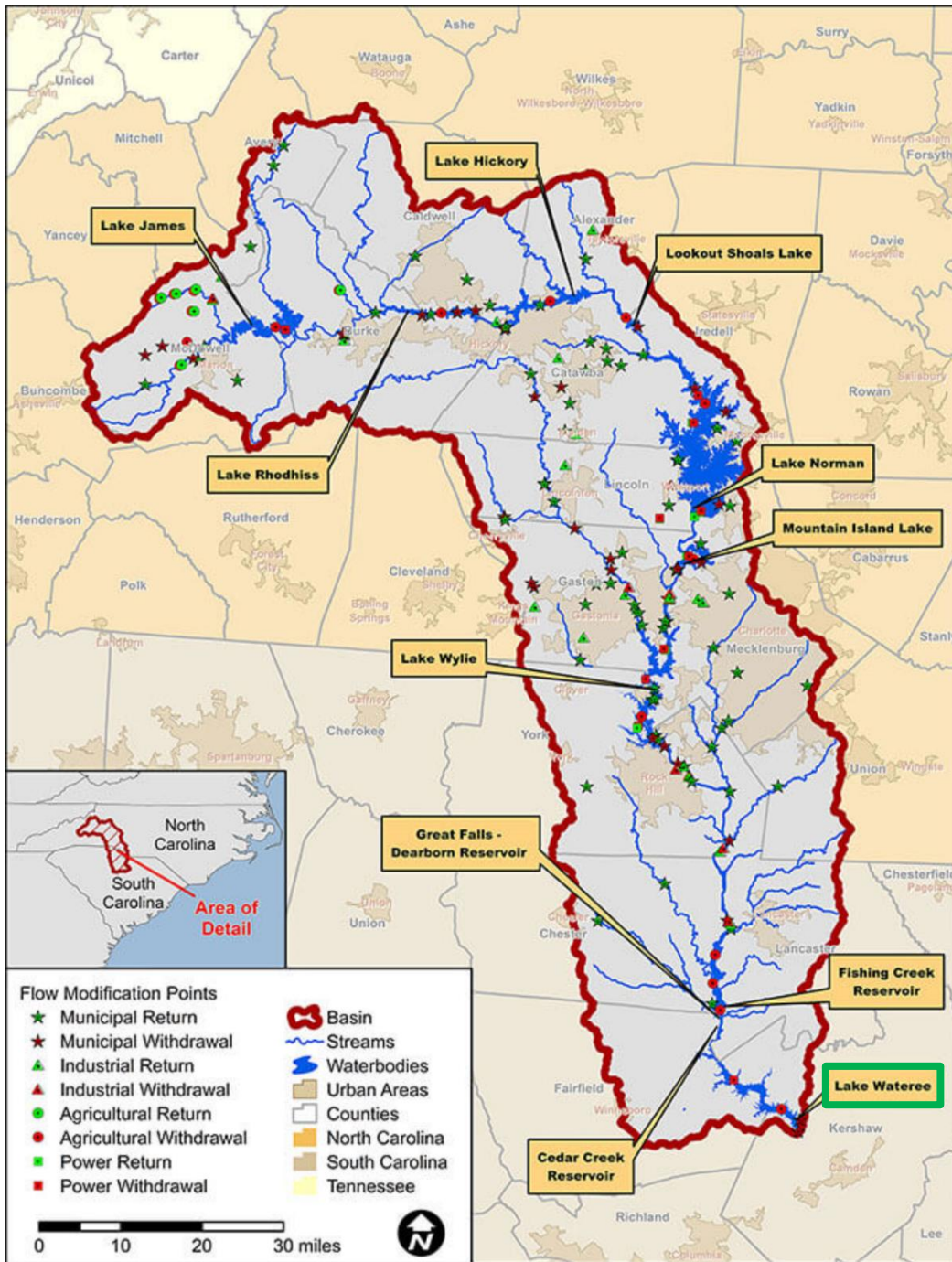
Figure 1.2: Water withdrawals and returns surrounding the eleven major reservoirs in the Catawba-River Basin. Lake Wateree is indicated by the *green rectangle*. Note the significant number of municipal WWTPs and industrial and agricultural permitted discharges into the watershed, as well as the significant water withdrawals for drinking water, agricultural and industrial production. (Source: Duke Energy, 2007)

In recent years, phytoplankton blooms have increased in prevalence, duration and magnitude in Lake Wateree, which have the potential to harm ecosystem and human health, domesticated animal, livestock and wildlife health, ecosystem function and sustainability, recreational resources and tourism. Researchers, stakeholders and citizen scientists from Lake Wateree Water Watch (WW) have been tracking phytoplankton growth and blooms, as well as the environmental factors that may cause these blooms, through the collection and analysis of ambient water quality and nutrient data, phytoplankton species identification, biannual filamentous cyanobacterial HAB shoreline mapping, and data sharing. WW has been a collaborative water quality monitoring and algal program between the University of South Carolina (USC), the Lake Wateree Association (LWA) and the Wateree Home Owners Association (WHOA) since 2008. WW has formed partnerships with the Baruch Marine Field Laboratory (BMFL) at the Belle W. Baruch Institute for Marine and Coastal Sciences in Georgetown, SC, the Catawba Riverkeeper Foundation (CRF), the South Carolina Department of Natural Resources (SCDNR), and the National Oceanic and Atmospheric Administration (NOAA). Bimonthly and annual reports are published with exceedances, analysis and communications of water quality and other environmental monitoring data. Scientific results are then communicated with partners, stakeholders, residents and local communities. Surface water quality criteria, excess nutrients, elevated pH, turbidity and HABs of *Lyngbya wollei* are all significant issues identified by WW (Lake Wateree Annual Report 2017, 2018).

WW also collaborates with SCDHEC for the development of nutrient Total Maximum Daily Load (TMDL) determinations for the Lower Catawba River Basin in

accordance with the Clean Water Act (CWA) and 40 Code of Federal Regulations Part 130. A TMDL for nutrients will address the impairments from excess nutrients by calculating the maximum amount of N and/or P Lake Wateree and upstream impoundments can receive while still meeting water quality standards (SCDHEC 2014). The allowable nutrient loads will include quantification of nutrients from point and nonpoint pollution sources, considering seasonal variations (SCDHEC 2018). There are more than 30 ambient monitoring stations in the Catawba-Wateree River system that are included in the 2018 South Carolina 303 (d) List of Impaired Waters, and many are included for TN, TP and CHL-*a* (SCDHEC 2018). Lake Wateree is currently listed as impaired due to excessive nutrients and microscopic algal growth (SCDHEC 2018).

1.4 Objectives and Hypotheses

The objective of this study was to identify the effects of limiting nutrient(s) on phytoplankton in Lake Wateree, SC. First, a comprehensive literature review of N and P limitation of phytoplankton in freshwater lakes was conducted, and relevant findings were interpreted and applied to the experimental design in Lake Wateree. Next, four nutrient enrichment bioassay experiments were conducted *in situ* during the growing season for the evaluation of separate and interactive roles of N and P, and relationships were determined. Finally, the experimental results of these nutrient enrichment bioassays were compared to historical Chl-*a* and nutrient data in Lake Wateree that was compiled, reviewed and analyzed to provide greater insight of spatial and temporal patterns that may have affected nutrient bioassay results from different locations throughout the lake. Results from this study determined if Lake Wateree is limited by N, P, or a combination of N and P.

Three hypotheses were tested in this study, as both Null (H_0) and Alternative (H_A) hypotheses:

H_O1: Lake Wateree is not P limiting

H_A1: Lake Wateree is P limiting

H_O2: Lake Wateree is not N limiting

H_A2: Lake Wateree is N limiting

H_O3: Lake Wateree is not co-limited by N and P

H_A3: Lake Wateree is co-limited by N and P

These three hypotheses were designed to test what is consistent with historical scientific literature investigations of nutrient limitation of phytoplankton studies in freshwater lakes, and with the historical Chl-*a* and nutrient data from Lake Wateree.

CHAPTER 2

MATERIALS AND METHODS

2.1 Analysis of Published Literature

A comprehensive literature review was conducted on N and P limitation of

phytoplankton in freshwater lakes using key terms "nutrient limitation," "nutrient

enrichment," "eutrophication," "lakes," "phytoplankton," "nitrogen" and "phosphorus."

The review focused on studies that included nutrient enrichment bioassay experiments

with additions of N, P or +NP to samples from freshwater lakes. The published literature

reviewed used Chl-*a* as the most common response variable for phytoplankton biomass.

Responses to nutrient additions in the selected studies identified in the search were

obtained and reviewed, and the presented data and figures were used for comparisons

with experimental results from this study.

2.2 Historical Data Analysis

Two decades of historical ambient water quality and nutrient data from twenty

fixed sites in Lake Wateree were analyzed to provide insight of spatial and temporal

patterns (Figure 2.1). Calibrated YSI multiparameter sondes were used to take vertical

profiles of depth (ft), temperature (°C), dissolved oxygen (DO in mg/L), pH, specific

conductivity (mS/cm) and turbidity in Nephelometric Turbidity Units (NTU) from four

channels, eight embayments and eight headwater sites bimonthly (Figure 2.1). In the

channels and embayments, measurements were taken at one, four, seven and ten feet, and

then in increments of ten feet until the lake floor was reached, and ambient water quality

parameters were measured at one-foot depths in the headwater sites. Turbidity was also measured using a Secchi Disk in the channels and embayments.
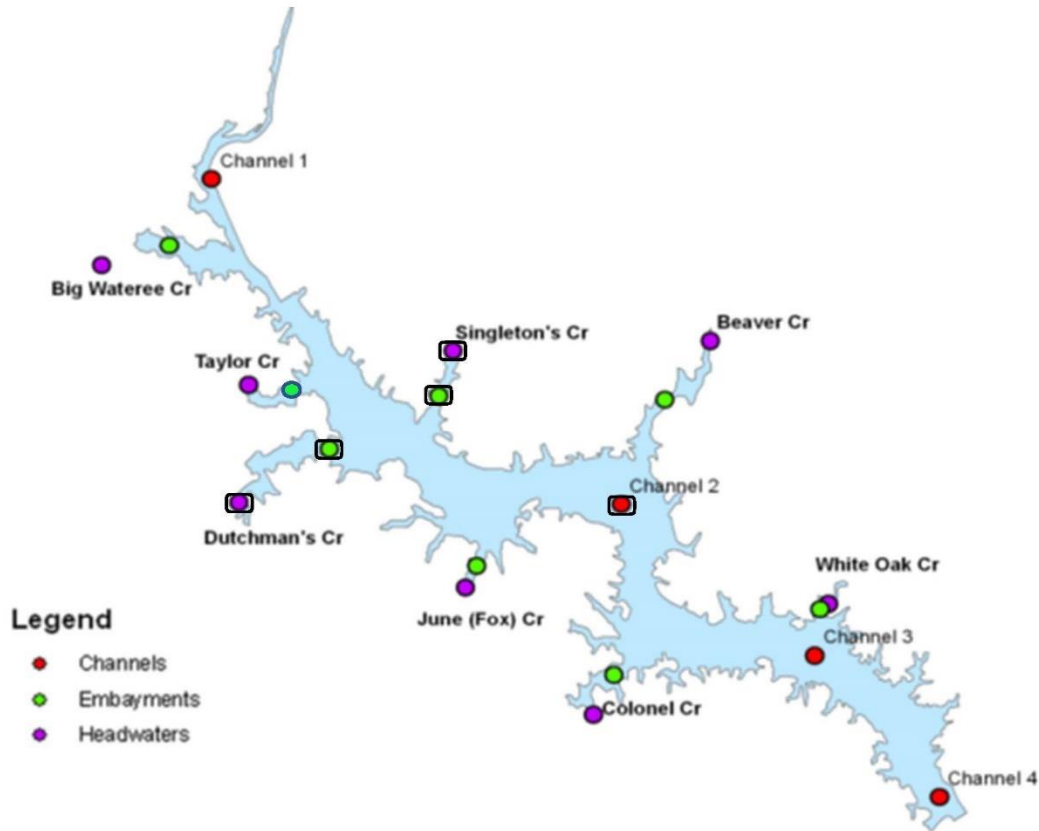
Figure 2.1: Location of the 20 routine monitoring stations in Lake Wateree. *Black rectangles* indicate the experimental study sites used for the nutrient addition bioassays in this project.

Nutrient samples (Chl-*a*, TN, TP, NO_3^-, NO_2^-, NH_4^+ and PO_4^{3-}) were collected in the months of April, June, August and October at four fixed sites including Channel Two (CH2), Channel Four, (CH4) Dutchman's Creek Embayment (DC-E) and Singleton's Creek Embayment (SC-E). Whole (unfiltered) water samples were taken in triplicates from one-foot depths at each site using 500 mL wide-mouth Nalgene bottles that were acid-washed in a 10% hydrochloric acid bath. Triplicate subsamples were collected from

each of the whole water samples using a BD 60 mL Luer-Lok Tip Syringe, and filtered

into acid-washed 60 mL wide-mouth Nalgene bottles using Pall Acrodisc 25-millimeter

filters with 0.45 micron (µm) pore size membranes. Unfiltered and filtered samples were

stored on ice and immediately transported to the BMFL for processing of Chl-a, TN, TP,

NO_3^-, NO_2^-, NH_4^+ and PO_4^{3-} fractions. The nutrient historical data were statistically

analyzed and comparisons were made of N:P ratios, as either TN:TP or DIN:DIP.

2.3 Nutrient Addition Bioassays

Four controlled nutrient addition bioassay experiments were conducted in three-

week intervals from August through October 2017 to study the relationships between

nutrients and phytoplankton communities in Lake Wateree. To account for spatial and

temporal variation, five sites with historical and current ambient water quality and

nutrient data were selected, including CH2, Singleton's Creek headwater (SC-H), SC-E,

Dutchman's Creek headwater (DC-H) and DC-E (Figure 2.1). Incubation water was

collected from the surface at each of the five sites in acid-washed 2.5-gallon potable

water carboys. One 60 mL wide-mouth Nalgene bottle of whole water was taken from

each carboy for unfiltered N+P fractions, and two 60 mL wide-mouth Nalgene bottles

were filtered for inorganic N+P fractions using Pall Acrodisc 25-millimeter filters with

0.45 µm pore size membranes. The nutrient samples were immediately transported on ice

to the laboratory for processing and analysis.

The four experimental treatments included a control (deionized water), + N

(NH_4^+), + P (PO_4^{3-}) and + NP $(NH_4^+ + PO_4^{3-})$, and the final concentrations of N and P

used were 50 µm, and 5 µm, respectively. To maximize replicability and control, each of

the carboys were subsampled into twelve clear 500 mL acid-washed Nalgene bottles for

each of the five sites. Nutrient spikes of each treatment were added as 1 mL volume additions to each of the 500 mL bottles, with triplicates of each treatment. Chl-*a* was selected as the phytoplankton community response variable, and initial Chl-*a* samples were collected on glass fiber filters (Whatman GF/F, nominal pore size of 0.7 µm) from each of the sixty 500 mL bottles, transported on dry ice, and stored at -80°C. The twelve clear 500 mL Nalgene bottles for each site were placed in separate crates, covered in gray screen to reduce irradiance, and incubated in the lake for forty-eight hours. After 48 hours, each crate was systematically removed from the lake, and final Chl-*a* sample measurements were taken. Each bioassay experiment was repeated four times throughout the summer and early fall to account for temporal and seasonal variation.

2.4 Chlorophyll Processing and Analysis

Initial (day 0) and final (48 h) Chl-*a* samples were processed at the BMFL and had a hold time no longer than 28 days. Samples were filtered onto glass fiber filters (Whatman GF/F, nominal pore size of 0.7 µm) and the Chl-*a* was extracted in 90% acetone for 24 hours at -4 C. The extracted Chl-*a* concentrations were quantified fluorometrically using a Turner Trilogy fluorometer using the non-acidification technique according to EPA Method 445 (Arar and Collins 1997).

2.5 Nutrient Processing and Analysis

Concentrations of dissolved inorganic nutrients (NO_2^-, NO_3^-, NH_4^+, PO_4^{3-}) in filtered samples were determined colorimetrically using an AutoAnalyzer (TechniCon Systems, Inc.) following TechniCon procedures (158-71 W/B, 155-71W). Whole water samples for the determination of TN and TP concentrations received an additional persulfate oxidation step prior to analysis (Gilbert and Loder 1997).

2.6 Statistical Analysis

      Statistical tests of treatment differences within each individual experiment (station and date) were determined by a one-way analysis of variance (ANOVA), followed by a comparison of all means using the Tukey-Kramer HSD test at the $p < 0.05$ level of significance using JMP 14.0 statistical software (SAS Institute, Cary, North Carolina).

CHAPTER 3

RESULTS

3.1 Historical Nutrient Data

Samples for nutrient fractions were collected by WW during the routine sampling events in June, August and October from 2012-2015. The average TN (mg/L) in CH2, CH4, DC-E and SC-E had little variance between stations, and the lowest concentrations were seen in CH4 (Figure 3.1). For each site, the average TN did not exceed the SCDHEC numeric nutrient criteria threshold of 1.50 mg/L for lakes in the Piedmont and Southeastern Plains ecoregions, which indicates P limitation in Lake Wateree (Figure 3.1).
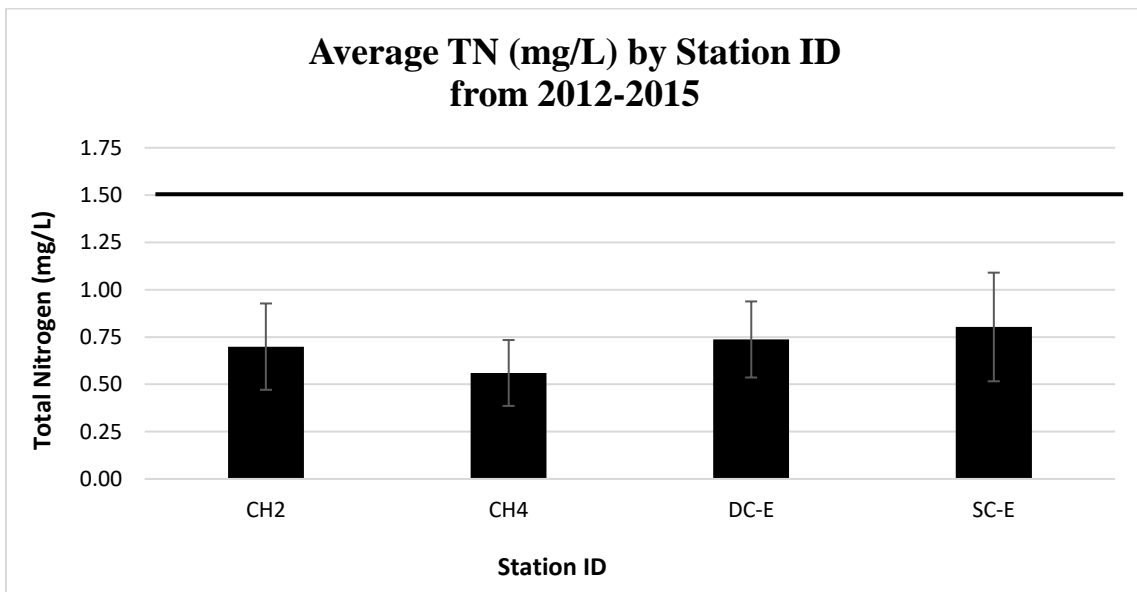
Figure 3.1: Average TN (mg/L) concentrations in Lake Wateree from 2012-2015 were below the SCDHEC standard threshold for TN (*Black horizontal line*). There was little variance noted between stations.

There was little variance in the average TP (mg/L) between sites CH2, CH4, DC-E and SC-E from 2012-2015 (Figure 3.2). The average TP was in exceedance of the SCDHEC numeric nutrient criteria threshold of 0.06 mg/L for lakes in the Piedmont and Southeastern Plains ecoregions in CH2, DC-E and SC-E, and was slightly below the threshold in CH4, which is also indicative of P limitation (Figure 3.2).
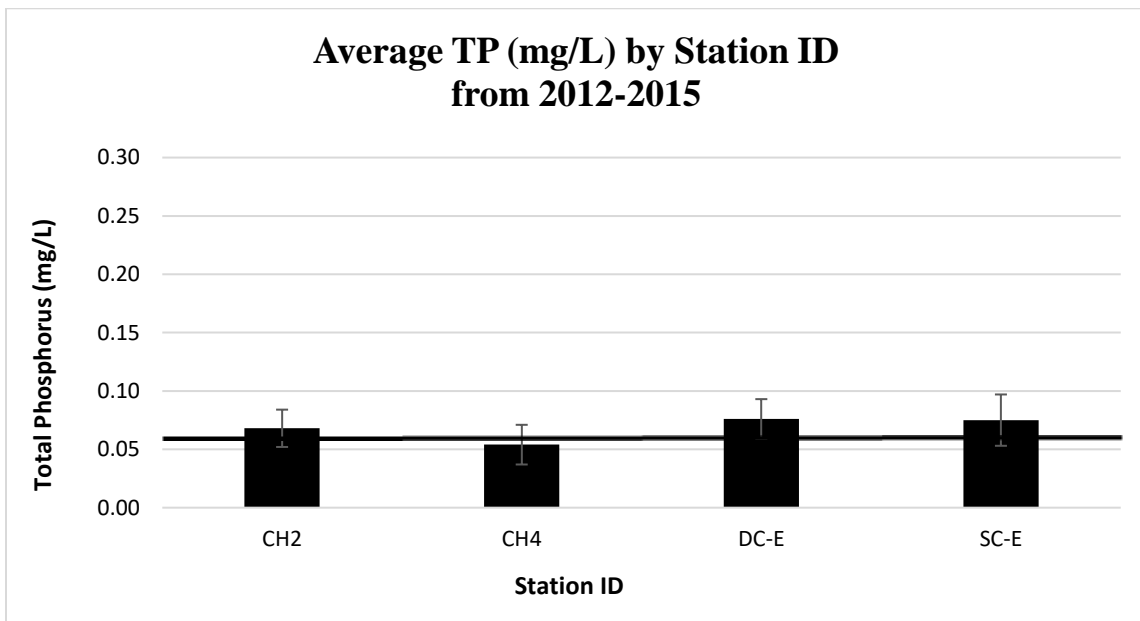
Figure 3.2: Average TP (mg/L) concentrations in Lake Wateree from 2012-2015 were above the SCDHEC standard threshold for TP (*Black horizontal line*) at sites CH2, DC-E and SC-E. There was little variance noted between stations.

High temporal variability of TN:TP and DIN:DIP was observed at sites CH2, CH4, DC-E and SC-E from 2012-2015 (Figures 3.3, 3.4). The vast majority of TN:TP and DIN:DIP ratios were well above the Redfield Ratio of 16:1, which indicates P limitation (Figures 3.3, 3.4).
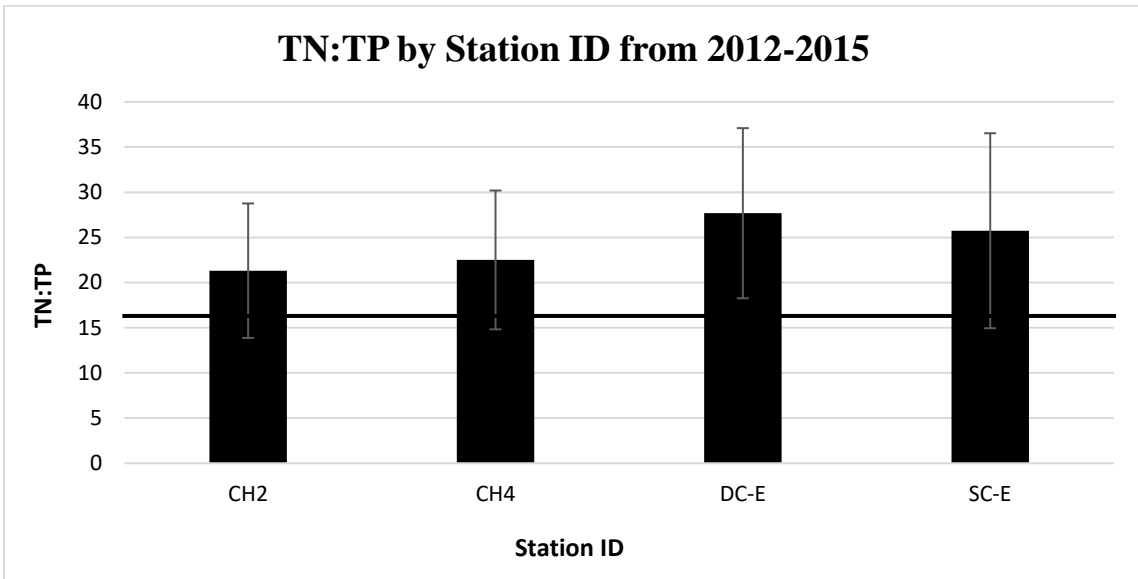
Figure 3.3: TN:TP in Lake Wateree from 2012-2015 was above the Redfield Ratio (*Black horizontal line*) for each station. High temporal variability was noted between stations.
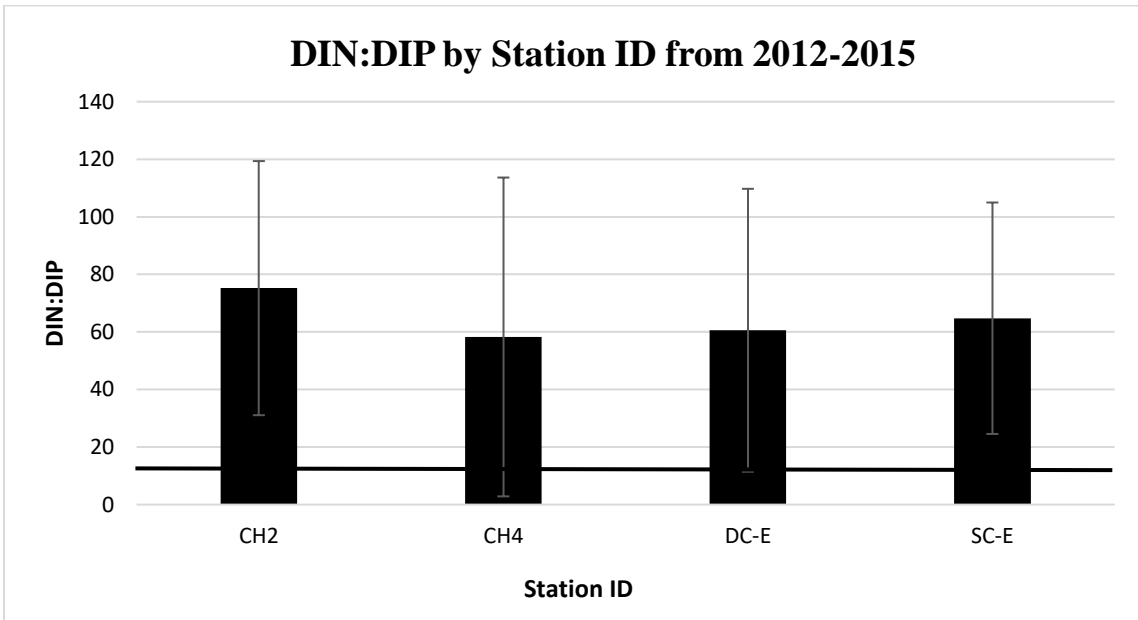
Figure 3.4: DIN:DIP in Lake Wateree from 2012-2015 was above the Redfield Ratio (*Black horizontal line*) for each station. High temporal variability was noted between stations.

3.2 Nutrient Addition Bioassays

The factorial nutrient addition bioassays provided several possible categorial responses, including the following (Harpole et al. 2011, Kolzau et al. 2014):

1. No significant treatment effects (no nutrient limitation): no change in algal biomass across the four treatments after 48 hours.

2. Exclusive nitrogen limitation: significantly higher response to the +N and +NP treatments, and no response to the +P treatment relative to the control.

3. Serial nitrogen limitation: response to the +N treatment, significantly higher response to the +NP treatment, and no response to the +P treatment relative to the control. N is the primary limiting nutrient until consumed, and the system becomes P limited.

4. Exclusive phosphorus limitation: significantly higher response to the +P and +NP treatments, and no response to the +N treatment relative to the control.

5. Serial phosphorus limitation: response to the +P treatment, significantly higher response to the +NP treatment, and no response to the +N treatment relative to the control. P is the primary limiting nutrient until consumed, and the system becomes N limited.

6. Co-limitation: some response to +N and +P treatments with a significant response only to the +NP treatment. The system is balanced, and both N and P are required for phytoplankton grow. The primary limiting nutrient is indicated by the single treatment with the higher response.

On August 9, 2017, co-limitation was the categorical response at sites CH2, SC-H, SC-E and DC-E. The control, +N and +P treatments were not statistically different (p > 0.05) but were significantly different from the +NP treatment (Figure 3.5). Though the four sites are co-limited, the higher response of the single additions was to the +N treatment, indicating N as the primary limiting nutrient. In Dutchman's Creek headwater (DC-H), the categorical response was serial N limitation, as shown by the response in algal biomass in the +N treatment and significantly higher algal biomass in the +NP treatment. The responses resulted in statistical differences in the +N and +NP treatments relative to the control and +P treatments (Figure 3.5).
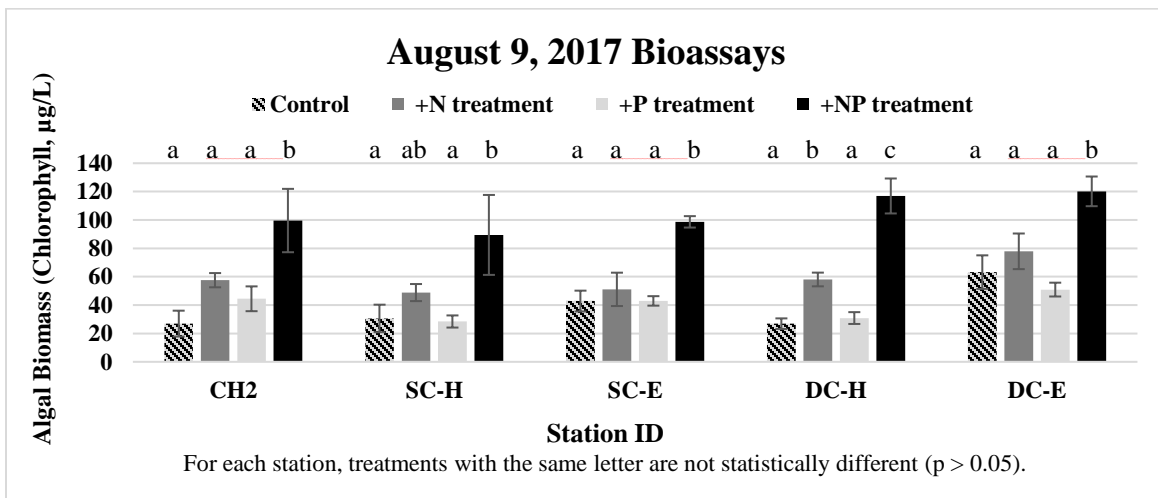
Figure 3.5: Algal biomass (Chlorophyll-*a*, µg/L) by station in Lake Wateree on August 9, 2017. Treatments with different letters (a, b, c) were significantly different.

The nutrient enrichment bioassays on August 29, 2017 produced the categorical responses of serial N limitation at sites CH2 and SC-H, co-limitation at sites SC-E and DC-E, and N limitation at site DC-H (Figure 3.6). At sites CH2 and SC-H, serial N limitation was indicated by a response to the +N treatment, a significantly higher response to the +NP treatment, and no response to the +P treatment relative to the

23

control. Therefore, there were statistical differences between the +N and +NP treatments relative to the control and +P treatment (Figure 3.6). At sites SC-E and DC-E, the control, +N and +P treatments were not statistically different from each other, but were significantly different from the +NP treatment, indicating co-limitation (Figure 3.6). The higher responses of single nutrient additions were to the +N treatment in both SC-E and DC-E (Figure 3.6). At site DC-H, the categorical response was N limitation, as the +N and +NP treatments had significantly higher algal biomass, and there was no change in algal biomass in the +P treatment relative to the control (Figure 3.6).
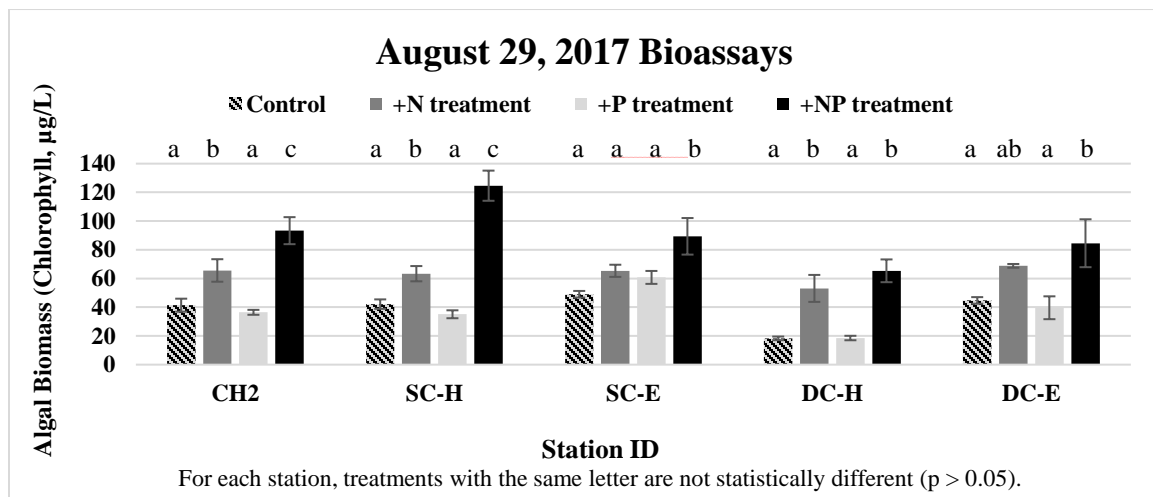
Figure 3.6: Algal biomass (Chlorophyll-*a*, µg/L) by station in Lake Wateree on August 29, 2017. Treatments with different letters (a, b, c) were significantly different.

The categorical response to nutrient additions at site CH2 on September 19, 2017 was N limitation, as indicated by a significantly higher response to the +N and +NP treatments, and no response to the +P treatment relative to the control (Figure 3.7). Sites SC-H, SC-E and DC-E had some response to +N and +P treatments, with a significant response only to the +NP treatment, which indicates co-limitation. The single treatment with the higher response for each of these three sites was the +N treatment, which also

suggests that N is the primary limiting nutrient in each of the three co-limited sites. At site DC-H the categorical response was serial N limitation, as there was a response to the +N treatment, a significantly higher response to the +NP treatment, and no response to the +P treatment relative to the control (Figure 3.7).

Figure 3.7: Algal biomass (Chlorophyll-*a*, μg/L) by station in Lake Wateree on September 19, 2017. Treatments with different letters (a, b, c) were significantly different.

The October 10, 2017 nutrient enrichment bioassay results indicated co-limitation at sites CH2, SC-E and DC-H, as there was some response to the +N and +P treatments and a significant response to the +NP treatment (Figure 3.8). The higher response to a single treatment for each of the co-limited sites was +N, indicating N as the primary limiting nutrient. At site DC-E the categorical response was N limitation, as there was significantly higher algal biomass in the +N and +NP treatments, and no algal growth in the +P treatment relative to the control (Figure 3.8). The categorical response at site SC-H was no significant response (ns), or no nutrient limitation (Figure 3.8).
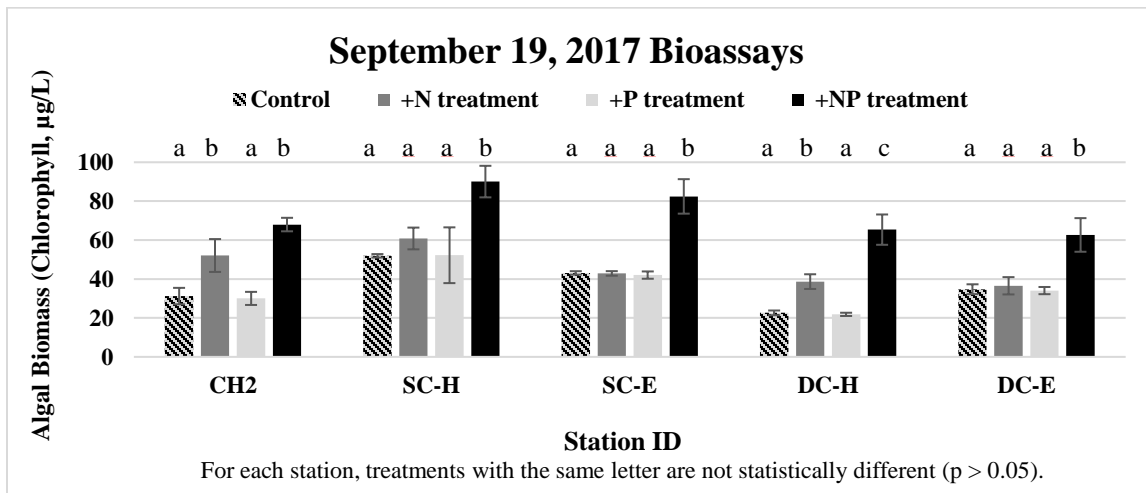
25
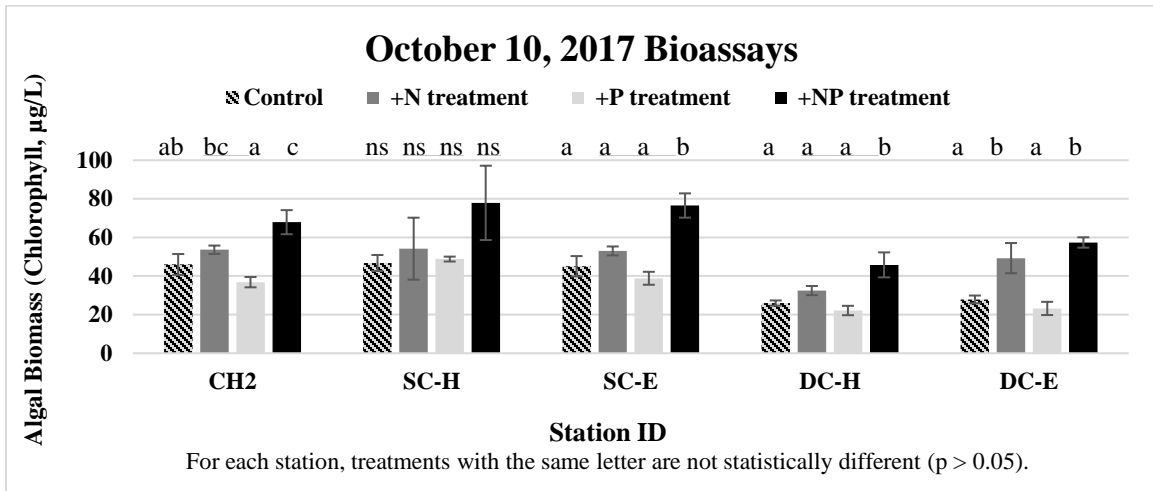
Figure 3.8: Algal biomass (Chlorophyll-*a*, ug/L) by station in Lake Wateree on October 10, 2017. Treatments with different letters (a, b, c) were significantly different.

The four nutrient enrichment bioassay experiments at the five selected sites in Lake Wateree produced twenty categorial responses (Figure 3.9). Twelve (60%) of the bioassay results suggested co-limitation in Lake Wateree, four responses (20%) indicated serial N limitation, and three responses (15%) suggested N limitation (Figure 3.9). None of the categorial responses to nutrient additions were indicative of serial P limitation or P limitation, and one response (5%) was not significant (ns) (Figure 3.9).

Summary of Experimental Responses to Nutrient Additions

(Chart: Treatment vs. Number of Statistically Significant Responses)

- N Limitation: ~3
- Serial N Limit.: ~4
- Co-Limitation: ~12
- Serial P Limit.: 0
- P Limitation: 0
- No Response: ~1
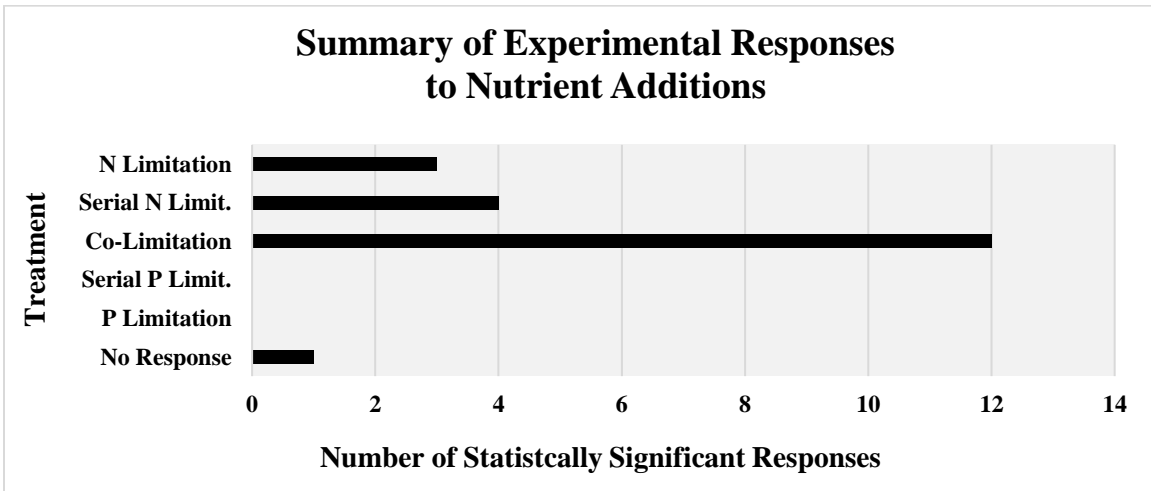
Figure 3.9: Summary of the 20 nutrient addition bioassay categorical responses in Lake Wateree from August through October 2017. Note the absence of serial P or P limitation despite exceedances in the P water quality criteria, and the N, serial N, and co-limitation observed.

CHAPTER 4

DISCUSSION

The objective of this study was to identify the nutrient limitation status in Lake Wateree. In nutrient enrichment experiments with natural populations, stimulation of phytoplankton biomass from a specific nutrient addition is usually suggestive of that particular nutrient being a limiting factor (Beardall et al. 2001). Conversely, if phytoplankton biomass is similar in the control and experimental samples without a particular nutrient addition, it is usually indicative that the nutrient is not limiting (Beardall et al. 2001).

Nutrient enrichment influenced phytoplankton biomass across the four different factorial experiments in Lake Wateree from August through October 2017, and results from this study showed that phytoplankton growth in the lake is limited by N or co-limited by both N and P. The most frequent significant community response (60%) to nutrient additions in Lake Wateree occurred when two nutrients (N and P) were simultaneously added, which is classified as co-limitation by both nutrients (Harpole et al. 2011). This response was observed at sites CH2, DC-E, SC-E and SC-H on August 9 and in DC-E and SC-E on August 29. Co-limitation was also observed at sites DC-E, SC-E and SC-H on September 19 and in CH2, DC-H and SC-E on October 10. This predominant phytoplankton community response to nutrient additions in Lake Wateree is consistent with the paradigm that co-limitation is frequent in aquatic ecosystems (Harpole

et al. 2011), and that greater primary production occurs by additions of N and P (Elser and Kimmel 1985, Elser et al. 1990). Results from a meta-analysis of 653 freshwater, 243 marine and 173 terrestrial nutrient enrichment field experiments, and the largest study of its nature, concluded that simultaneous additions of N and P yielded higher significant responses than single additions across all ecosystems (Elser et al. 2007). Results from the same meta-analysis also found individual responses to N and P to be generally equivalent ($P = 0.222$). It concluded that phytoplankton in freshwater lakes are equally responsive to N or P, but benthic autotrophs (primarily attached algae) demonstrated weak synergistic responses and were more strongly limited by P than N (Esler et al. 2007). In another analysis of 641 studies of nutrient limitation in primary producer communities, 28% of the responses were co-limitation of phytoplankton, as either simultaneous (response to only N and P combined) or independent, and were commonly found in ecosystems with lower TN and TP levels (Harpole et el. 2011). The mechanism for co-limitation is likely that single additions of N or P stimulate limitation from the alternative nutrient, indicating that N and P supplies are relatively balanced in many aquatic ecosystems (Elser et al. 2007), but due to the wide range of possible individual, community and biogeochemical processes, underlying mechanisms are not easily understood (Harpole et al. 2011). Though co-limitation is more common than early studies suggested, some studies have shown that this category may be more difficult to predict (Ptacnik et al. 2010), and it is not yet understood if growth of individual taxa can also be co-limited (Müller et al. 2015). According to the resource competition theory that assumes co-existing species cannot exceed the number of limiting resources, co-limitation may be valuable in understanding diversity (Sterner et al. 2008). These results clearly support the

hypothesis that co-limitation of N and P plays a major role on phytoplankton in Lake Wateree.

Twenty percent of the nutrient enrichment experiments in Lake Wateree were classified as serial N limiting and had a significant community response to the +NP treatment coupled with a significant response to an addition of N (Harpole et al. 2011). Serial N limitation was observed at sites DC-H on August 9; CH2 and SC-H on August 29; and DC-H on September 19. The response in 15% of the nutrient addition experiments in Lake Wateree was N limitation, and this response was observed at sites DC-H on August 29; CH2 on September 19; and DC-E on October 10. These results support the evidence of widespread distributions of the N-fixing cyanobacteria species *Lyngbya wollei* in Lake Wateree. The serial N limitation and N limitation results in Lake Wateree are consistent with results from a study by Downing and McCauley (1992) that indicated frequent N limitation in eutrophic lakes and showed that P limitation is more likely in oligotrophic lakes (Kolzau et al 2014). Also, serial and single limitation categories are usually found in studies that have higher levels of TN and TP (Harpole et al. 2011). Results from nutrient enrichment experiments globally indicate that N limitation status in freshwater lakes is becoming as common as P limitation (Lewis et al. 2011). Furthermore, an investigation of eight nutrient enrichment studies on 17 New Zealand lakes showed significant responses in phytoplankton growth after N additions in 19 (86.4%) out of 22 experiments and a significant response of 11 (52.4%) out of 21 experiments following P additions (Abell et al. 2010). The Pearson correlation analysis indicated TN as a better predictor of Chl-*a* than TP in New Zealand Lakes (Abell et al.

2010). These results clearly support the hypothesis that N plays a major role on phytoplankton populations in Lake Wateree.

Single additions of P did not lead to any significant increases in phytoplankton biomass in this study, as there were no community responses classified as serial P limitation or P limitation. Therefore, the experimental results did not support the hypothesis that Lake Wateree is P limited. Currently, water quality management in Lake Wateree is focused on TP, and significant P reductions are likely from improvements to better control point source pollution upstream. The control of P is also important for toxic N-fixing cyanobacteria and should be continued (Müller et al. 2015). In addition, a review of published literature on nutrient limitation status in freshwater lakes did not support the hypothesis that Lake Wateree is P limited. Finally, no significant response was observed at site SC-H on October 10.

There are limitations to nutrient enrichment of phytoplankton experiments. Some investigations suggest that only nutrient enrichment experiments conducted long-term and at the ecosystem scale provide accurate inferences as to the nutrient limitation status of phytoplankton communities in a whole lake (Sterner et al. 2008, Schindler and Hecky 2009), but such experiments are rare and can often have short-term damaging impacts on an aquatic ecosystem (Abell et al. 2010). Results from nutrient enrichment studies in natural environments and mesocosms have important implications for management decisions of eutrophication controls in freshwater lakes. In a study investigating the relationships between mesocosm volume and shape and nutrient enrichment on phytoplankton responses, phytoplankton responses to nutrient additions were not

significantly modified and a low variance in Chl-*a* among standard field treatments was observed (Spivak et al. 2011). Shape and volume effects were temporary and were variable by day, which emphasizes the importance of experimental duration (Spivak et al. 2011). These mesocosm results suggest that scale does not significantly influence phytoplankton responses to nutrient enrichment, and implications from field mesocosm experiments can be extended to large scale, natural aquatic environments (Spivak et al. 2011). A meta-analysis by Elser et al. (2007) of 1,060 nutrient enrichment experiments determined that additions of N and P increased phytoplankton biomass in freshwater, marine and terrestrial habitats (Spivak et al. 2011). Spivak et al. (2011) selected 359 freshwater pelagic experiments from the 1,060 experiments that had an average duration of 7 days (1-71 day range) in mesocosms between 0.02-3200 L in size, and were treated with additions of N, P or both. The 359 freshwater experiments were pooled with results from the Spivak et al. (2011) mesocosm experiments, which showed that phytoplankton responses were similar. Combined results suggest that volumes of <1 L produced greater responses to nutrient enrichment and intermediate-sized mesocosms may have underestimated phytoplankton biomass. Responses to nutrient additions were comparable in larger volume mesocosms (>20 L) (Spivak et al. 2011).

The limitation of nutrients other than N or P may be induced with the addition of one macronutrient such as C, O, or hydrogen (H) to certain species compositions of the phytoplankton sample, which can further complicate nutrient addition experiments (Beardall et al. 2001). In addition, chemical speciation of micronutrients such as Fe and trace metals within the water column of freshwater lakes can convolute the relationship between measured concentrations and bioavailability for phytoplankton (Beardall et al.

2001). The availability of macronutrients interacts with metal availability which may be important in freshwater ecosystems (Sterner et al. 2008). Furthermore, physical enclosures during incubation periods can isolate the natural population from key nutrient sources such as particulate matter, regenerated nutrients and sediments, and invoke changes in the species composition of phytoplankton (Venrick et al. 1977, Healey 1979, Beardall et al. 2001). The elimination of grazing pressures from zooplankton can stimulate phytoplankton biomass until a nutrient eventually becomes limiting in all tested nutrient additions including the control, and Liebig limitation occurs (Cullen et al. 1992, Beardall et al. 2001).

The Redfield elemental composition of phytoplankton can provide evidence of Liebig limitation of phytoplankton populations, but can be variable and less indicative of nutrient limitations in a given population at a specific time (Beardall et al. 2001). Comparing TN:TP to the established Redfield stoichiometry can be inconclusive, as ratios may be variable and have different thresholds among lakes, and may be dependent on phytoplankton assemblages and their physiological and nutritional status (Sterner and Hessen 1994, Abell et al. 2010). The determination of the relationships between TN:TP may provide understanding of the processes that control N:P in lakes of various trophic status (Downing and McCauley 1992), with TN:TP being commonly positively correlated (Sterner et al. 2008, Harpole et al. 2011). Furthermore, complex biogeochemical processes such as photosynthesis, respiration, N fixation, nitrification, denitrification and sedimentation can cause variability in Redfield stoichiometry, primarily on short temporal scales and small spatial scales, and may also influence TN and TP levels in lakes (Downing and McCauley 1992). Freshwater lakes with balanced

responses of N:P intermediate ratios (Elser et al. 2009) and higher rates of N deposition

may shift from N limitation to P limitation (Harpole et al. 2011). In addition, variances in

mean light levels have been observed to influence nutrient enrichment bioassays, both in

mesocosms and in the natural environment (Müller et al. 2015).

An investigation of the species composition of assemblages in Lake Wateree and

their responses to other limiting variables including other nutrients, pH, light, microbial

function and temperature should be considered (Harpole et al. 2011). The elemental

composition of phytoplankton is not fixed, and certain genera have been observed to

demonstrate a delay in response to nutrient additions, while other taxa within

phytoplankton communities may exhibit different growth rates and have diverse

nutritional needs. Some generas of cyanobacteria can fix atmospheric N (N_2) and may use

this to their advantage in the P treatments. Furthermore, low N:P ratios may result in

dominance of the phytoplankton community by N-fixing cyanobacteria (Müller et al.

2015). Conversely, some genera of phytoplankton may utilize internal P storages, and

become capable of changing from NP treatment response to the N treatment (Müller et al.

2015). The results of Shindler et al. (2008) indicated that Chl-*a* and TN concentrations

decreased without N fertilization and were interpreted by Scott and McCarthy (2010) to

suggest that N- fixing cyanobacteria cannot compensate for N limitation (Abell et al.

2010, Lewis et al. 2011, Kolzau et al. 2014). However, results from a subsequent study

on the same lake indicated increasing N-fixation rates and high Chl-*a* concentrations

without N fertilization (Paterson et al. 2011, Kolzau et al. 2014). Cyanobacteria have

diverse responses to N and P, and studies of how functional groups of cyanobacteria shift

along eutrophication and N and P gradients are limited (Dolman et al. 2012).

Seasonal nutrient enrichment bioassays would be valuable in understanding phytoplankton seasonal succession (Elser et al. 1990) and determining if the categorical responses to nutrient additions prevail throughout the year in Lake Wateree. Lower algal growth in the winter may suggest a combination of nutrient, light and/or temperature limitation (Müller et al. 2015). Also, sampling frequency and experimental duration may influence phytoplankton response to nutrient enrichment in Lake Wateree, as longer duration experiments have important implications for quantifying the effects of nutrients on ecosystem function, microbial nutrient cycling and community composition (Clark and Tilman 2010, Harpole et al. 2011). In addition, further research is required to better understand how the drivers of biogeochemical and ecological processes in Lake Wateree may influence nutrient concentrations, as the N, P, C, O and Fe biogeochemical cycles are strongly linked. Sub-habitat variability should also be considered, as a predominance of lake benthos have been observed to demonstrate P limitation while pelagic species have patterns of more balanced N and P limitation (Elser et al. 2007). Finally, understanding the role and impacts of other nutrients such as Fe, silica (Si), sulphur (S) and potassium (K) in addition to N and P will benefit both ecologists and watershed managers in Lake Wateree (Elser et al. 2007).

Investigations of ecoregions with similar hydrology and morphology, trophic state and nutrient loading characteristics as Lake Wateree would be valuable in providing a spatial framework for determining numeric criteria. It is important to continue collecting and analyzing nutrient data in Lake Wateree, as it provides annual mean nutrient ratios that are indicative of the relative abundance of nutrients over long durations. High intra-

annual variations are possible in TN:TP ratios, and as nutrient enrichment experiments provide insight to the nutrient limitation status of phytoplankton at one particular time, there may be variations between results and analysis of nutrient stoichiometry (Barica 1990, Abell et al. 2010).

Most of the P in lakes is available to phytoplankton as total dissolved P (TDP) that includes DIP, particulate P (PP) from internal P stores, and dissolved organic P (DOP), and this may be supportive of using TP as an indicator of P availability (Lewis et al. 2011). In lakes, DIN, some DON and the particulate fraction of N consisting primarily of phytoplankton are also considered to be bioavailable (Lewis et al. 2011). Therefore, water quality standards for P can be based on TP and have high feasibility and moderate cost, but the standards for N should be adjusted (Lewis et al. 2011). Subtracting the refractory (unavailable) DON from TN would reduce the cost and increase the feasibility of N control (Lewis et al. 2011).

Findings from the literature review, historical data analysis and nutrient enrichment bioassays in Lake Wateree have important implications for understanding and mitigating current and future eutrophication issues. Results from this study strongly imply that duel inputs of both N and P should be controlled to prevent excessive phytoplankton growth, and this is consistent with recent data from many natural lakes (Sterner et al. 2008). Reducing P inputs from fertilizer use and controlling developments and disturbances in the watershed will likely reduce N inputs in Lake Wateree (Elser et al. 1990). Also, regulation of effluents from WWTPs and septic systems, decreasing fertilizer, manure and pesticide applications to lawns and agricultural fields, following

proper procedures in septic system operations and maintenance, and upgrading sewage treatment plants and stormwater systems will all reduce excess nutrients in both the lake and upstream in the Catawba-Wateree River Basin.

Further assessments of limiting factors in the Catawba-Wateree River Basin and Lake Wateree are needed to identify the causes of increasing N concentrations and determine the point and non-point sources of N loading. The predominance of co-limitation from the nutrient enrichment results indicates that both quantitative and qualitative changes are important for nutrient controls (Elser et al. 2007). Results from this study showed that the simultaneous increase of both N and P in Lake Wateree induced significantly higher phytoplankton biomass; therefore, a balanced approach should be used for lake management and conservation efforts. The control of both N and P in Lake Wateree and upstream will be a significant strategy in reducing the symptoms of cultural eutrophication and potential harmful impacts on biota, ecosystem services and public health.

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