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# Reservoir Management Techniques to Enhance Biological Productivity and Protect Water Quality

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Reservoir Management Techniques to Enhance Biological Productivity and Protect Water Quality

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By

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## ABSTRACT

Three reservoirs of similar size, watershed land use, and qualitative characteristics in northwest Arkansas, USA were selected to compare the effects of chemical fertilization and pulsed artificial-upwelling on whole-lake productivity, specifically primary production and phytoplankton biomass. Numerous water quality parameters were quantified over a two year period (2011-13) with the goal of understanding how each management technique would stimulate productivity. This experiment was the first step towards a larger goal to ultimately enhance sport fish production. The first year of monitoring occurred in 2011 and served as a control year for the three lakes. Treatments were initiated in two of the lakes (Lakes Rayburn and Norwood) in the second year, as one lake (Lake Brittany) remained a control. Due to difficulty in scaling and manipulation cost, small-scale microcosms experiments were used in 2012 directly prior to each whole-lake experiment. Microcosm experiments were used to derive appropriate whole-lake fertilization rates and make predictions for whole-lake responses to nutrient additions. Both microcosm and whole-lake phytoplankton responses varied seasonally with water temperature and initial dissolved nutrient concentrations. Few interannual effects from whole-lake manipulation were observed in the treatment lakes due to the 'pulse' nature of nutrient additions. However, the short-term data revealed increased concentrations of total phosphorus (TP), total dissolved P (TDP), chlorophyll *a* (chl *a*), and <80 µm chl *a* in Lake Rayburn and, in Lake Norwood, total dissolved nitrogen (TDN), TP, TDP, chl *a*, and < 80 µm chl *a* concentrations were elevated. Few whole-ecosystem aquatic studies have been conducted in the manner of this experiment. Preliminary results from these data suggest that periodically pulsed nutrient additions can result in short-term increases in biological productivity without affecting interannual water quality patterns.

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## 1. Introduction

Strikingly, few surface waters worldwide remain undisturbed, directly or indirectly, by human activities (Smith et al. 2006). Eutrophication, the excessive nutrient enrichment of aquatic ecosystems, is a primary problem facing many surface waters today (Smith and Schindler 2009). Eutrophication has resulted in significant alterations in biogeochemical cycling in aquatic systems over both space and time (Smith et al. 2006). Some of the common effects of eutrophication are: rapid increases in the rate of biological production, significant reductions in water column transparency, undesirable water quality changes, shifts in phytoplankton species composition, an increase in the frequency and intensity of harmful algal blooms (HABs) which are typically dominated by odor and toxin producing cyanobacteria, increased oxygen demand, and changes in the composition and structure of aquatic food webs (Boynton 2000, Downing et al. 2001, Huisman and Hulot 2005, Fisher et al. 2006).

Nitrogen (N), which is required for protein synthesis, and phosphorus (P), a critical component in DNA and RNA construction as well as the transfer of energy, are both required to support aquatic plant growth and are the key limiting nutrients in most aquatic ecosystems (Conley et al. 2009, Sterner and Elser 2002). Phytoplankton production represents a major basal component of the aquatic food web and primary producer growth continues as long as there is sufficient N, P, light energy for photosynthesis, and suitable temperatures. The availability of dissolved inorganic carbon (DIC) and micronutrients can limit phytoplankton productivity as well (Goldman et al. 1974). However, dissolved inorganic P (DIP) and/or dissolved inorganic N (DIN) are typically the limiting nutrient(s) in freshwater lakes (Elser et al. 2007). As a result, phytoplankton productivity typically increases as more DIP and DIN enter a lake either naturally or from anthropogenic sources (Posselt et al. 2009).

While excessive N and P are commonly recognized as pollutants in eutrophic waterways, societal awareness of the positive effects of these nutrients in oligotrophic ecosystems and their central role in regulating biological productivity are limited (Anders and Ashley 2007). Managing N and P load reductions into freshwater lakes has led to measured decreases in trophic status (i.e. eutrophy to oligotrophy) of some lakes (Anderson et al. 2005, Conveney et al. 2005, Romo et al. 2005). This managed reversal of eutrophication has been termed 'cultural oligotrophication' or the human-induced reduction of excess nutrients in aquatic systems (Stockner et al. 2000). A classic example of this shift from eutrophy to oligotrophy is Lake Washington near Seattle, where wastewater diversion greatly decreased algal biomass and productivity, and changed algal community composition (Edmondson 1994). A goal when managing lakes solely for drinking water and/or aesthetics is to manage the systems toward relatively unproductive nutrient states. However, the designated use of many lakes includes recreational fishing and harvestable fish populations, and oligotrophic systems often lack the nutrients required to support a robust biological community. Studies of eutrophic, lentic systems shifting to oligotrophy have shown both immediate (Yurk and Ney 1989) and delayed (Jeppesen et al. 2005, Sondergaard et al. 2005) responses in production and nutrient concentration due to reduced external nutrient loading.

### 1.1 Nutrients and Fisheries Management

Numerous studies show the positive relationship between total P (TP) concentration, chlorophyll *a* (chl *a*) concentration, and fish biomass (Jones and Hoyer 1982, Ney et al. 1990, Maceina et al. 1996). It is common practice throughout the southeastern United States and elsewhere for lake and fisheries managers to apply fertilizers to raise biological productivity in unproductive waters (Vaux et al. 1995, Buyank et al. 2001, Perrin et al. 2006, Boyd et al. 2008).

However, this management technique has sometimes been misused or overused to the point where water quality concerns caused fertilization activities to cease (Figure 1.1). In cases where chemical fertilization was discontinued and nutrient loading reductions made, shifts from eutrophic to oligotrophic conditions have been observed in naturally nutrient-depleted waters (Vollenweider and Dillion 1974, Anderson et al. 2005, Jeppesen et al. 2005). Neither eutrophic nor oligotrophic conditions are desirable in most multi-use lakes. In multi-use lakes, a balance must be stuck between the lower nutrient concentrations needed to avoid undesirable algal production and higher nutrient concentrations to sustain good fishing (Ney 1996). Mesotrophic conditions are often the target 'middle ground' for many managers.

Mesotrophic systems typically support optimal biological production and consequently robust food webs (Stockner et al. 2000). An important component in the maintenance of productive food webs in lakes managed for mesotrophy is to maintain moderate nutrient availability, a well-balanced N:P supply ratio optimal for the growth of "edible" phytoplankton (< 35 μm), and zooplankton populations suitable for plantivorous fish predation (Sarnelle 1992, Stockner and Maclsaac 1996, Stemberger and Miller 1998). However, implementation of lake fertilization practices requires good limnological knowledge and an understanding of how nutrient additions and N:P ratios effect the ecosystem services associated with multi-use lakes (Stockner et al. 2000).

## 1.2 Lake Productivity and Environmental Controls

Nitrogen and P loading, lake morphometry, and hydraulic residence time all are factors controlling primary production potential in lake ecosystems (Vollenweider 1968, Shannon and Brezonik 1972, Dillion 1974). Numerous phytoplankton bioassays have shown N, P, or both limit algal productivity in lakes (Dillon and Rigler 1974, Elser et al. 2007). Phosphorus loading

rates of 3.0 kg P $ha^{-1}$ $yr^{-1}$ and N loading rates of 50 kg N $ha^{-1}$ $yr^{-1}$ have been shown to create eutrophic conditions in lakes, whereas P loading rates of 1.0 kg P $ha^{-1}$ $yr^{-1}$ and N loading rates of 30 kg N $ha^{-1}$ $yr^{-1}$ have resulted in oligotrophic conditions (Vollenweider 1968, Shannon and Brezonik 1972). Eutrophication can occur when the nutrient supply rate saturates the demand of primary producers. If the supply rate of the limiting nutrient is controlled, then primary production is controlled pending no other factor (e.g. light or temperature) is limiting (Vollenweider 1968).

Optimal N:P ratios of phytoplankton have been documented ranging from 3.2 to 20.4 (mass ratios throughout) across numerous phytoplankton species (Rhee and Gotham 1980). In natural phytoplankton communities many species are present at any given time and, therefore, represent a wide spectrum of individual requirements for N and P (Suttle and Harrison 1988). Over such a wide range of N:P supply requirements, it is common for some species to be N limited, while others are P limited. However, measured inorganic nutrient concentrations do not specifically denote nutrient availability to phytoplankton, but merely represent what is left over by lake production processes. Total N (TN) and total P (TP) concentrations more appropriately reveal the condition of lakes by taking into consideration the nutrients incorporated in biomass as well (Shannon and Brezonik 1972). Additionally, a TN:TP range for balanced growth conditions has been suggested to be between 9-22.5 for phytoplankton (Smith 1979, Guildford and Hecky 2000, Dzialowski et al. 2005).

The effects of light and temperature on phytoplankton growth are inseparable due to the interrelationship between metabolism and light saturation (Wetzel 2000). Light availability effects phytoplankton biomass and community composition. For example, some cyanobacteria are able to grow at lesser light intensities than many other phytoplankton species, while other

cyanobacteria have gas vesicles that allow them to rise to the surface to reach abundant light (Smith 1986, Klemer 1991). Light conditions optimal for phytoplankton photosynthesis are specific to water temperature and exist for different phytoplankton species (photosynthetically active radiation (PAR) 200-400 µmol $m^{-2}$ $s^{-1}$; Wetzel 2000). Additionally, warmer water temperatures (≥ 24°C) have been shown to favor cyanobacteria species in competition studies (Tilman and Kiesling 1986, Robarts and Zohary 1987). Recognizing that cofactors often regulate phytoplankton biomass is critical to understanding nutrient availability in lake ecosystems. For example, low light and low N:P ratios can interactively support cyanobacteria dominance in phytoplankton assemblages, particularly since the optimal N:P supply ratios of phytoplankton can vary with light intensity (Rhee and Gotham 1981, Healey 1985). Additionally, food web structure can affect pelagic primary producers at a wide range of nutrient levels (Kitchell 1992, Carpenter et al. 2001).

### 1.3 Understanding Nutrient-Productivity Relationships at Multiple Scales

There are numerous small-scale field and laboratory fertilization experiments that examine nutrient limitation (McDiffett 1980, Prepas and Trimbee 1988, Dzialowski et al. 2005, Elser et al. 2007, Abell et al. 2010). However, despite the amount of small-scale studies present, there are still data needed for whole-ecosystem responses to nutrient additions because responses to nutrient additions typically are taxon and lake specific (Carpenter et al. 2001). Opponents of small-scale experiments contend that these results have limited relevance to whole-lake processes because they do not account for long-term changes in community dynamics and biogeochemical processes (Carpenter et al. 1995, Schindler 1998, Schindler et al. 2008). However, other studies have shown that small-scale experiments spanning several orders of magnitude and experiments lasting longer in duration can be more informative to phytoplankton

growth responses than routine *in situ* monitoring (Spivak et al. 2010, Xu et al. 2010). Still, there are some opinions that only whole-lake studies are relevant to inform scientists and mangers of lake responses to nutrient additions and limitations (Schindler 2012). Employing both small-scale and whole-lake experiments in tandem could provide more information to address this issue.

### 1.4 Study Objectives

The objective of this study was to explore the effects of N and P additions on primary production using both microcosm and whole-lake fertilization experiments collectively. Specifically, the goals were to: 1) assess the effects of chemical fertilizer additions on whole-lake productivity in one lake, 2) evaluate the effects of a novel alternative management technique to increase whole-lake productivity in a second lake, 3) determine how much nutrient concentrations must be increased to enhance edible phytoplankton bloom formation, 4) characterize nutrient limitation and how limitation varies seasonally in a third, reference lake, and 5) determine if nutrient pulses could increase production on the short-term with negligible effects on interannual lake water quality and downstream waterbodies. The data collected to achieve these goals included whole-lake nutrient and biomass data as well as microcosm biomass data.

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## 1.6 Figure Legend

Figure 1.1. Annual fertilizer application rates as N and P applied to Lake Brittany, Bella Vista, Arkansas. Lake fertilization was halted in 2002 due to citizen concern over accelerated eutrophication.

## 1.7 Figures

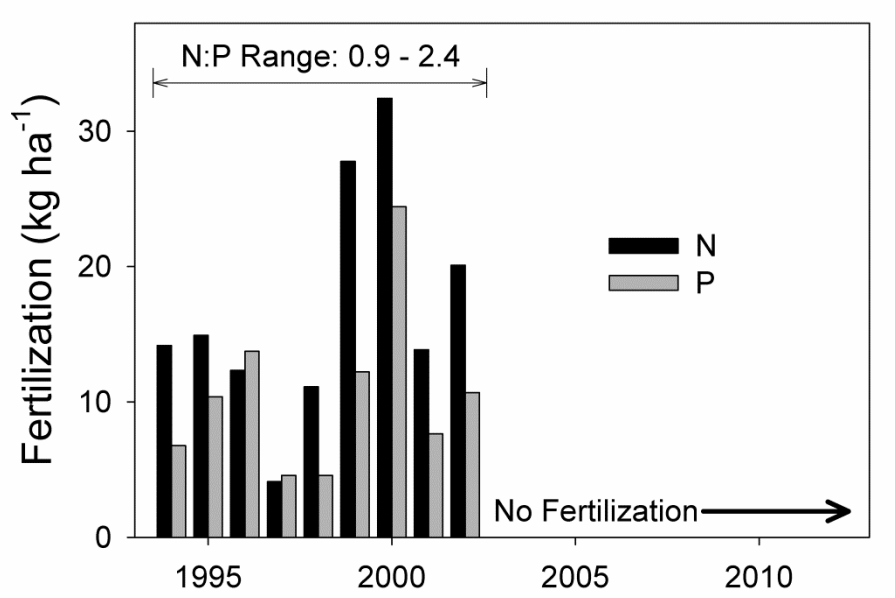


Figure 1.1

## 2. Chemical Fertilization as a Reservoir Management Technique to Enhance Biological Productivity

### 2.1 Introduction

It has been well-established that two principal nutrients (N and P) regulate aquatic primary productivity and biomass in most lake ecosystems (Smith 1982, Hecky and Kilham, 1988, Downing and McCauley 1992). However, the actual response of primary producers to N and P enrichment can be modified by factors such as light limitation, temperature, hydrology, and grazing (Smith et al. 2006). Successful eutrophication management has been primarily based on the restriction of N and P inputs to water bodies. This reduction in nutrient loading has been accomplished by a wide variety of external and internal controls (Cooke et al. 2005). In most cases, simultaneous reduction of both N and P has been required to effectively mitigate eutrophication (Conley et al. 2009). Attenuating the effects of eutrophication remains one of the greatest challenges presented to limnologists and lake managers around the globe (Schindler 2006).

Eutrophication typically results in undesirable water quality perturbations, specifically an increase in the frequency and abundance of cyanobacteria that can produce harmful odors, toxins, and are relatively unpalatable to zooplankton (Burns 1987, Haney 1987, Lampert 1987, de Bernardi and Giussani 1990, Paerl et al. 2001, Paerl and Huisman 2008). In contrast, oligotrophic conditions persist in lakes that receive relatively low amounts of N and P. Low nutrient concentrations can be ideal if the designated use of the lake is to provide a particular ecosystem service, such as a drinking water supply, that is optimal at low nutrient concentrations (Conley et al. 2009, Paerl and Huisman 2009, Hudnell 2010). However, low nutrient concentrations are undesirable in lakes in which designated uses include fisheries production

(Boyd and Sowles 1978). Thus, multi-use lakes create a management paradox for managers seeking aesthetics and a productive fishery (Anders and Ashley 2007).

Primary production is a rate (e.g. amount of C $L^{-1}$ $d^{-1}$) that is controlled by the ecosystem supply rates of N and P. Supply rates in lakes are primarily controlled naturally by stream inflows and food web recycling, and anthropogenically by pollution or intentional fertilization. Eutrophication can occur when the nutrient supply rate saturates the demand of primary producers. If the supply rate of the limiting nutrient is controlled, then primary production is controlled pending no other factor (e.g. light or temperature) is limiting primary production (Vollenweider 1968). Oligotrophication can occur when decreasing nutrient supply rates limit primary producer growth rates. This nutrient limitation can transfer up the food web by limiting the food quantity and quality for higher trophic levels, including fish (Stockner and Shortreed 1985, Ney 1996, Pieters et al. 2003). In oligotrophic lakes, where fisheries production is a primary management endpoint, chemical fertilization is often used as a management strategy to increase fish biomass and productivity (Hyatt and Stockner 1985, Axler et al. 1988, Meyer et al. 1993, Hyatt 2004). However, multi-use lake managers are challenged by the need to enhance fisheries without over stimulating primary production to the point that increased production affects other ecosystem services, such as contact recreation and drinking water supply. Therefore, in multi-use lakes managing nutrient inputs to achieve mesotrophy is a goal of many fisheries managers today (Stockner et al. 2000).

In addition to controlling the amount of primary production, most managers are also interested in stimulating the growth of the most edible phytoplankton groups, such as green algae, in order to increase zooplankton and fish growth. In particular, cyanobacteria are inedible to many zooplankton, specifically large-bodied cladocerans (Haney 1987, Lampert 1987, Paerl et

al. 2001). Nitrogen limitation in particular favors the growth of certain cyanobacteria in aquatic environments because these organisms have the capacity to fix atmospheric $N_2$ into a biologically available form (Whitton 2012). Optimal N:P supply ratios have been documented ranging from 3.2 to 20.4 for several species of freshwater phytoplankton (Rhee and Gotham 1980). Total Nitrogen:TP ratios have been identified as suitable predictors of phytoplankton biomass because the TN:TP ratio reflects the total pool available to phytoplankton, and balanced phytoplankton growth has been identified at a TN:TP ratio range of 9 to 22.5 (Guildford and Hecky 2000, Dzialowski et al. 2005). Additionally, a threshold of 29 TN:TP was shown for cyanobacteria. Above the TN:TP of 29 cyanobacteria were rare, and below 29, cyanobacteria were shown to dominate phytoplankton assemblages (Smith 1983). Knowledge of current nutrient conditions as well as an appropriate fertilizer N:P is critical to enhance edible phytoplankton growth when adding nutrients to aquatic systems. The key is to have nutrients translate into increased fish biomass rather than phytoplankton (i.e. trophic efficiency; Hecky 1984).

There are numerous small-scale field and laboratory fertilization experiments that examine nutrient limitation (Lin and Schelske 1981, Dodds and Priscu 1990, Elser et al. 2007, Maberly et al. 2002, Symons et al. 2012). However, despite the amount of small-scale information present, there are still data needed for whole-ecosystem responses to nutrient additions**.** Additionally, there is debate on the validity and extent one can deduce from bioassays in regard to ecosystem processes (Schindler 1998, Spivak et al. 2010). There are strong interactions between nutrients and food webs that still need investigation to better understand the ecosystem-level alterations that occur with changes in the extent of external nutrient loading to lakes (Edmondson 1993, Carpenter et al. 1995). This study assessed the effects of nutrient

addition using both small-scale and whole-ecosystem experiments collectively, to determine if bottle experiment results were similar to whole-lake results.

The objective of this study was to explore the effects of N and P additions on primary production with data collected from both microcosm and whole-lake fertilization experiments. The goals were to: 1) assess what nutrient(s) limited productivity, 2) how much nutrients were needed to maximize productivity without oversaturating growth, and 3) what the effect of the selected fertilization rate was at the whole-ecosystem scale. Based on existing literature, it was predicted that the fertilized lake would: 1) exhibit increased short-term productivity proportional to the fertilization rate and 2) exhibit no change in interannual water quality conditions including phytoplankton biomass and nutrient concentrations.

## 2.2 Methods

### 2.2.1 Study Sites

The study was conducted in two monomictic recreational, sport fishing reservoirs (< 0.2 $km^2$ surface area) in the Springfield Plateau region of northwest Arkansas. Lake Brittany (36°28'08"N, 94°12'04"W) and Lake Rayburn (36°27'43"N, 94°14'21"W) are steeply sloped lakes with mean depths of 7.6 to 8.8 m and maximum depths of 21.0 to 23.3 m. Both lakes have similar watershed characteristics with primarily forest (64 – 68%) and urban (23 – 25%) land cover, resulting in moderately low nutrient concentrations in both lakes. The inflowing streams to both lakes were exclusively ephemeral. In the past, augmentation with chemical fertilizers was employed to raise biological productivity of the lakes, but this management technique was not used in the decade preceding this study.

### 2.2.2 Experimental Design

#### 2.2.2.1 Phase I – Microcosm Fertilization Experiments

A series of in-lake microcosm experiments were conducted in Lake Rayburn to inform the whole-ecosystem manipulations. The goal of this component was to determine an appropriate fertilization rate for the whole-lake experiments and to evaluate multiple forms of nutrient additions and their effects on primary production. Microcosm experiments were conducted in May, June, and July 2012 to estimate the potential effect of whole-lake fertilization in each month. Vertically integrated water samples were collected from the photic zone at five sites in Lake Rayburn using a 4-L Van Dorn horizontal sampler (Alpha water sampler, Wildco, Yulee, FL) and pooled in a bucket to create a whole-lake composite (WLC) sample. The WLC sample was equally divided into 21 semi-transparent 4-Lcubitainers (2 L of WLC water per cubitainer). The cubitainers were randomly grouped into sets of three replicates that were amended as follows to test the effect of fertilizer rate and form on phytoplankton biomass production: 1) Control (no fertilizer addition), 2) 0.7 kg P $ha^{-1}$ as Sportmax® (10-52-4 ($N-P_2O_5-K_2O$); lake and pond grade fertilizer), 3) 0.7 kg P $ha^{-1}$ as Sportmax® plus calcium nitrate (6.3 kg N $ha^{-1}$), 4) 0.7 kg P $ha^{-1}$ as Sportmax® plus urea (6.3 kg N $ha^{-1}$), 5) 0.7 kg P $ha^{-1}$ as Sportmax® plus ammonium nitrate (6.3 kg N $ha^{-1}$), 6) 0.35 kg P $ha^{-1}$ as Sportmax® plus calcium nitrate (3.15 kg N $ha^{-1}$), and 7) 1.05 kg P $ha^{-1}$ as Sportmax® plus calcium nitrate (9.45 kg N $ha^{-1}$). This treatment combination allowed comparisons between low (0.35 kg P $ha^{-1}$), medium (0.7 kg P $ha^{-1}$), and high (1.05 kg P $ha^{-1}$) P fertilization rates with and without calcium nitrate additions. Further, the treatments allowed comparisons between calcium nitrate, urea, and ammonium nitrate at the medium fertilization rate. Nitrogen-amended treatments always received 9x more N than P (by mass).

Cubitainers were incubated in Lake Rayburn for 4 to 8 days. *In-vivo* fluorescence of samples from each cubitainer was measured every 1 to 2 days during microcosm experiments. Experiments were halted when phytoplankton biomass saturated in each microcosm. Water from

each cubitainer was then completely mixed and filtered for seston biomass as particulate organic carbon (PC) and chlorophyll-a (chl *a*). Particulate C was measured using an elemental analyzer (NC Soil Analyzer, Flash 2000 Organic Elemental Analyzer, Thermo Scientific, Lakewood, NJ) (APHA 2005) and chl *a* was measured fluorometrically (Turner Designs Trilogy Benchtop) following 90 % acetone extraction in a dark freezer for 24 hours. Chlorophyll *a* concentrations were phaeophytin corrected (Marker et al. 1980, APHA 2005). The effect of fertilization rate and form on PC, Chl *a*, and Chl *a*:PC ratio were tested using a one-way analysis of variance for each experiment in SAS 9.3 (SAS Institute Inc. Cary, N.C.). Post-hoc comparisons were conducted using the REGWQ multiple comparison test when the omnibus F test was statistically significant at alpha = 0.05.

**2.2.2.2 Phase II – Whole-Lake Fertilization Experiments**

A before/after control/impact paried (BACIP) design was used to test the effect of whole-lake fertilization on a variety of limnological endpoints associated with primary production. Whole-lake experiments were conducted with a heavy emphasis placed on the growing season when the lakes were stratified and the lowest epilimnion nutrient concentrations were likely to limit primary production. Sampling of Lake Brittany and Rayburn occurred approximately weekly for two years, with some sampling occurring at even finer intervals. In 2011 (before year), no fertilization (impact) occurred and only monitoring data were collected in both Lake Brittany (control lake) and Lake Rayburn (impact lake). In 2012 (after year), monitoring continued in both lakes, but Lake Rayburn received three pulses of chemical fertilizers, while Lake Brittany received no treatments. Commercial fertilizer in the form of Sportmax® (10-52-4) plus calcium nitrate (15-0-0) was applied to Lake Rayburn based on the fertilizer recommendations derived from microcosm experiments. Fertilizer was applied to Lake Rayburn

at the following rates: 2.91 kg N $ha^{-1}$ plus 0.281 kg P $ha^{-1}$ on May 4 2012, 4.18 kg N $ha^{-1}$ plus 0.422 kg P $ha^{-1}$ on June 12 2012, and 2.51 kg N $ha^{-1}$ plus 0.227 kg P $ha^{-1}$ on July 6 2012.

Whole-lake monitoring on Lakes Brittany and Rayburn was conducted weekly during the growing season of both years and approximately monthly during the non-growing season. Additionally, more intense monitoring was conducted on both lakes in the two weeks following each fertilization event. Whole-lake composite photic zone samples were collected on each sample date. In addition to the WLC sample collected at each of the five sites, a 6-m vertical haul with an 80 µm Wisconsin tow-net (Wisconsin sampler, Wildco, Yulee, FL) was collected and combined into a single, WLC sample for >80 µm biomass on each sample date. All water samples were collected in amber bottles to prevent photodegradation.

A sediment trap was deployed at a central (pelagic) location and left for a period of 7-14 days. Each sediment trap was deployed 6-m from the surface so that the opening was located at the top of the metalimnion. Sediment deposition rates (g $m^{-2}$ $day^{-1}$) were calculated from sediment trap samples using the surface area of the trap opening (0.0064$m^2$), depth of the trap (6 m), and duration of deployment (7-14 days). A hypolimnion sample was collected weekly from this central location. Light extinction, resulting in photic depth, was measured with a Licor quantum sensor (LI-250A Light Meter/Photometer, LI-193 bulb, LI-COR®, Lincoln, NE), and water transparency was measured with a 20-cm diameter secchi disk at this location as well. Multi-parameter profile data, including depth, water temperature (WT), pH, specific conductance (SPC), dissolved oxygen (DO), and oxidation-reduction potential (ORP), were routinely collected (600 XLM YSI, Yellow Springs, OH) from the centrally located site to characterize the physical structure of each lake.

A multiparameter datasonde (600 XLM YSI, Yellow Springs, OH) was also deployed in both Lake Brittany and Lake Rayburn for continuous measurements. Continuous logging data sondes were deployed at the central location at a depth of 2 m. The depth of deployment was chosen to capture the production and consumption of oxygen in the photic zone of each lake as well as to minimize perturbations caused by wind. Water temperature, DO, pH, and SPC were logged every 15 minutes in each lake. Diurnal DO concentrations were converted into whole-ecosystem primary production and respiration rates (mg C $m^{-3}$ $h^{-1}$) according to the method described by Wetzel (2000). Briefly, the rate of oxygen concentration increase during daytime hours was converted into net primary production. Respiration rates were calculated in a similar manner based on nighttime decreases in DO concentrations. It was assumed that production and respiration were consistent throughout the volume of each lake's photic zone, the 2 m sonde deployment depth was representative of the entire lake's photic zone, and gas exchange with the atmosphere in any 24 hour period was minimal compared to the change in $O_2$ concentration. Units were converted between $O_2$ and C based on a photosynthetic quotient of one.

Whole-lake composite and >80 µm WLC samples were filtered onto pre-combusted (4 h at 450 °C) 25 mm Whatman GF/F glass fiber filters for PC and particulate N (PN), an acid-washed GF/F filter for particulate P (PP), and an untreated GF/F filter for chl *a*. Sediment trap samples were filtered similarly for particulate C, N, P, and total suspended solids (TSS). Filtrate for WLC and hypolimnion samples was collected after passing through 47 mm GF/F filters and preserved by freezing for later analyses of dissolved nutrients. Whole-lake composite samples were analyzed for total dissolved P (TDP), total dissolved N (TDN), ammonium ($NH_4^+$), and nitrate ($NO_3^-$). Hypolimnion samples were analyzed for $NH_4^+$, $NO_3^-$, TDN, and soluble reactive P (SRP).

Filters for PC and PN analysis were stored frozen and then oven-dried prior to elemental analysis as described previously. Particulate P filters were autoclave-digested in a 1% acid-persulfate solution. After digestion, the samples were analyzed by colorimetry produced by the ascorbic acid method to determine PP concentrations (APHA 2005). Chlorophyll *a* samples were analyzed by fluorometry as described previously.

Ammonium was analyzed by colorimetry produced using the Hach method (Turner Designs Instrument Model 7200 Trilogy™, Sunnyvale, CA; APHA 2005), while $NO_3^-/NO_2^-$ were quantified colorimetrically using the cadmium reduction method (Turner Designs Instrument Model 7200 Trilogy™, Sunnyvale, CA; APHA 2005). Total dissolved N was analyzed using a TOC-TN analyzer (Shimadzu Scientific Instruments TOC-$V_{CSH}$ and TNM-1 analyzer, Columbia, MD; APHA 2005). Total dissolved P was analyzed spectrophotometrically via the ascorbic acid method following a 1 % persulfate digestion (Agilent Technologies, Cary 300 UV-Vis, Foster City, CA; APHA 2005).

A WLC subsample was preserved with $M^3$ phytoplankton fixative to determine community composition on five sampling dates throughout the impact summer (2012). Phytoplankton were enumerated with a Wilde M40 inverted microscope at 400 x using a 5 mL or 10 mL fixed Utermohl tube, following the methods described by Utermohl (1958) in Britton and Greeson (1987). Briefly, phytoplankton cells or colonies (natural counting units) were identified and enumerated across the diameter of the settling chamber (transect) then multiplied by the focal width of the field of view, rather than the sum of individual fields of view, until a minimum of 300 counting units were obtained (APHA, 2005).

### 2.2.3 Statistical Analysis

Whole-lake data were compared among lakes using a Before-After-Control-Impact design with paired data (BACIP; Underwood 1994, Benedetti-Cecchi 2001, Stewart-Owten and Bence 2001, Smith 2002). The before year (2011) was compared to the after year (2012) and the control lake (Brittany) was compared to the impact lake (Rayburn). The BACIP design employs a two-way repeated measures analysis of variance (ANOVA) to analyze the interactive effect of time (Before-After) and treatment (Control-Impact) for each water quality parameter tested. The interaction of Before-After (BA) and Control-Impact (CI) takes into account both the temporal variations in the data (e.g. seasonal trends) as well as the site differences in the impact lake compared to the control lake. This BA x CI interaction measures if the tested impact or treatment caused a differential change to occur. Each lake was sampled within 1 to 2 hours on the same day creating paired samples to analyze. The BACIP analyses were conducted on the annual data and also a truncated data set that only included data from the growing season in each year (May-August) since whole-lake manipulations were only conducted in the growing season. The BACIP analyses were conducted on raw data which were normally distributed or data which were log- or square-root-transformed to meet the assumption of normality.

Data from three individual pulse events (14 day duration after fertilization) from 2012 were also analyzed using analysis of covariance (ANCOVA) to compare the slopes of response variables over time between Lake Rayburn and Lake Brittany. Statistical differences in the slopes indicated that the lakes differed in their short-term (2 week) responses to fertilization. All statistical analyses were conducted using SAS 9.3 (SAS Institute Inc., Cary, N.C.).

## 2.3 Results

### 2.3.1 Microcosm Fertilization Experiments

Chemical fertilization stimulated phytoplankton growth in all three months in 2012 (Figure 2.1). The initial conditions for the May experiment contained TN concentrations of 0.8062 mg $L^{-1}$, TP concentrations of 0.0235 mg $L^{-1}$, and chl *a* concentrations of 22.1 µg $L^{-1}$. In May, the increase in chl *a* was proportional to the fertilization rate where chl *a* increased from 22.5 µg $L^{-1}$ in the control to 66.4 µg $L^{-1}$ in the 0.7 kg P $ha^{-1}$ plus 6.3 kg N $ha^{-1}$ treatment, with no further increase in chl *a* observed at the greatest fertilization rate. The initial conditions for the June experiment contained TN concentrations of 0.6485 mg $L^{-1}$, TP concentrations of 0.0330 mg $L^{-1}$, and chl *a* concentrations of 17.4 µg $L^{-1}$. In June, chl *a* increased proportionally across all fertilization rates with the greatest increase in the 1.05 kg P $ha^{-1}$ plus 9.45 kg N $ha^{-1}$ treatment where chl *a* increased from 5.28 µg $L^{-1}$ in the control to 48.7 µg $L^{-1}$ in the high fertilization treatment. The initial conditions for the July experiment contained TN concentrations of 0.7750 mg $L^{-1}$, TP concentrations of 0.0360 mg $L^{-1}$, and chl *a* concentrations of 29.3 µg $L^{-1}$. In July, a similar trend was measured with the greatest increase in the 1.05 kg P $ha^{-1}$ plus 9.45 kg N $ha^{-1}$ treatment where chl *a* increased from 7.58 µg $L^{-1}$ in the control to 96.9 µg $L^{-1}$ in the high fertilization treatment.

Particulate C increased equally among all fertilizer treatments in May. In June, the increase in PC was proportional to the fertilization rate up to 0.7 kg P $ha^{-1}$ plus 6.3 kg N $ha^{-1}$, with no more increase in PC observed at the greatest fertilization rate. Particulate C increased from 1.59 mg $L^{-1}$ in the control to 11.7 mg $L^{-1}$ in the 0.7 kg P $ha^{-1}$ plus 6.3 kg N $ha^{-1}$ fertilization treatment. In July, a similar trend was measured with the greatest increase in PC was measured in the 0.7 kg P $ha^{-1}$ plus 6.3 kg N $ha^{-1}$ treatment where PC increased from 1.90 mg $L^{-1}$ in the control to 12.6 mg $L^{-1}$ in the medium fertilization treatment.

When comparing similar fertilization rates, the N + P treatment always yielded greater chl *a* concentrations than the P-only treatment at the same rate. Also, less PC was measured in the P-only treatment in June and July when compared to the same fertilization rate with added N, but there was no difference in PC between the various fertilization rates in May. The form of N never effected the amount of phytoplankton biomass as chl *a* or PC in any experiment in any month. The ratio of chl *a*:PC varied substantially across experiments, but less within experiments. Fertilization increased the chl *a*:PC in May and July, but had little effect in June.

### 2.3.2 Whole-Lake Fertilization Experiments

Nutrient concentrations (Figure 2.2.) and phytoplankton biomass (Figure 2.3) exhibited typical seasonal distributions in both lakes, with concentrations generally greater in Lake Rayburn than Lake Brittany. For example, Lake Rayburn's interannual mean TN concentrations ranged from 0.72 to 0.74 mg $L^{-1}$ whereas Lake Brittany's TN concentrations ranged from 0.49 to 0.55 mg $L^{-1}$. Interannual mean TP concentrations in Lake Rayburn ranged from 0.033 to 0.035 mg $L^{-1}$ while TP concentrations in Lake Brittany ranged from 0.016 to 0.021 mg $L^{-1}$. Additionally, interannual mean chl *a* concentrations ranged from 19.6 to 23.3 µg $L^{-1}$ in Lake Rayburn, and Lake Brittany's chl *a* concentrations ranged from 5.27 to 7.33 µg $L^{-1}$. Springtime P limitation and summer N limitation was observed in the lakes which is characteristic of many southeastern U.S. lakes. Nutrient limitation was relieved via three chemical fertilizations in Lake Rayburn during summer stratification.

The BACIP analysis on interannual data revealed that there were no differential changes for any of the 18 parameters tested, with the exception of TP (Table 2.1). Annual mean TP in Lake Brittany decreased from 0.021 to 0.016 mg $L^{-1}$ from 2011 to 2012, but increased in Lake Rayburn from 0.033 to 0.035 mg $L^{-1}$ during that same time, which resulted in the differential

change of 0.007 mg $L^{-1}$ ($p$ =0.0017) measured by the BACIP analysis. This small increase in Lake Rayburn TP concentrations along with a corresponding decrease in Lake Brittany TP concentrations resulted in a significant differential change measured by the BACIP analysis.

Before-After-Control-Impact analysis on truncated data (Table 2.2) indicated many more differential changes, including nutrient concentrations and phytoplankton biomass (Figure 2.4), due to the impact of the three chemical fertilization pulse events in summer 2012. Truncated TP concentrations decreased in Lake Brittany from 0.019 to 0.016 mg $L^{-1}$ between summer 2011 and summer 2012, whereas TP concentrations in Lake Rayburn increased between summer 2011 and summer 2012 from 0.031 to 0.037 mg $L^{-1}$ resulting in a differential change of 0.009 mg $L^{-1}$ ($p$ =0.0005). Truncated TDP concentrations decreased in Lake Brittany from 0.009 to 0.006 mg $L^{-1}$ and TDP concentrations in Lake Rayburn increased from 0.008 to 0.009 mg $L^{-1}$ between summer 2011 and summer 2012 resulting in a differential change of 0.004 mg $L^{-1}$ ($p$ =0.0009).

The effect of treatment did not change summer TN concentrations in Lake Rayburn. However, truncated TDN concentrations decreased by 0.127 mg $L^{-1}$ in Lake Brittany from summer 2011 to summer 2012. By contrast, TDN concentrations in Lake Rayburn decreased only by 0.028 mg $L^{-1}$ from summer 2011 to summer 2012, resulting in a statistically significant differential change of 0.099 mg $L^{-1}$ ($p$ =0.0013; Figure 2.4D). A differential change ($p$ <0.0001) was also measured on the truncated time-scale for phytoplankton biomass measured as chl *a*. Lake Rayburn's chl *a* concentrations increased from 14.2 µg $L^{-1}$ to 24.7 µg $L^{-1}$ between summer 2011 and summer 2012, whereas chl *a* concentrations in Lake Brittany decreased from 6.20 µg $L^{-1}$ to 5.44 µg $L^{-1}$ between summer 2011 and summer 2012 (Figure 2.4A).

Phytoplankton community composition data were analyzed for five sample dates during stratification from May-September in 2012 to compare the fertilized Lake Rayburn to non-

fertilized Lake Brittany (Figure 2.5). Cyanobacteria always comprised less than 50% of the phytoplankton biomass in Lake Brittany, but comprised as much as 80% of the phytoplankton biomass in Lake Rayburn. In particular, $N_2$-fixing cyanobacteria dominated Lake Rayburn over most of the summer. However, the proportion of $N_2$-fixing cyanobacteria, and total cyanobacteria, decreased following the fertilization events indicated by the dashed lines in Figure 2.5B.

Analysis of covariance was used to test the differences between Lakes Brittany and Rayburn during 'pulse' sampling events. Pulse sampling was conducted in both lakes during the two weeks following fertilization of Lake Rayburn 2012, but also twice in 2011 after neither lake was manipulated. The differences between two-week responses of the lakes are shown in Table 2.3. Statistically significant positive values indicate that the slope of the 14-day pulse event for a parameter was greater for Lake Rayburn than for Lake Brittany. Statistically significant negative values indicate that Lake Brittany's slope was greater than Lake Rayburn's. Although there were some differences in two-week nutrient concentration patterns in 2011, there were no differences in phytoplankton biomass or productivity in the control year. Conversely, there were few differences in nutrient concentrations in 2012, but phytoplankton biomass, measured as chl *a*, almost always increased in Lake Rayburn compared to Lake Brittany in the two weeks following fertilization.

## 2.4 Discussion

The objective of this study was to derive appropriate whole-lake fertilization rates based on data from microcosm experiments. Furthermore, the effects of chemical fertilizer additions on whole-lake productivity were assessed. Results showed that both N and P fertilizer were needed to stimulate primary productivity in the Lake Rayburn photic zone during summer stratification.

Additionally, these experiments revealed that even moderate (0.35 kg P $ha^{-1}$ and 3.15 kg N $ha^{-1}$) fertilization could result in a significant increase in phytoplankton biomass, although phytoplankton biomass did increase proportionally with fertilization rate up to 0.7 kg P $ha^{-1}$ and 6.3 kg N $ha^{-1}$. There was no effect of varying fertilizer type (i.e. calcium nitrate, urea, ammonium nitrate) on phytoplankton biomass or quality, indicating that the most inexpensive fertilizer is likely the best choice for management. However, the samples were not analyzed to assess the effect of the different fertilizers on the phytoplankton community composition and other studies have suggested that the less-edible cyanobacteria prefer and are more competitive for $NH_4^+$ rather than $NO_3^-$ (Blomqvist et al. 1994).

The fertilization of Lake Rayburn had little effect on nutrient concentrations and no effect phytoplankton biomass and productivity when considered on an annual scale. Mean annual total P concentration in Lake Rayburn increased by 0.002 mg $L^{-1}$ from 2011-2012, while mean annual total P concentration in Lake Brittany decreased by 0.005 mg $L^{-1}$ over that same time (Table 2.1). This pattern was not surprising because the impact was a 'pulse' and large datasets 'wash out' the effects seen from a pulse-type of impact (Smith 2002). This is opposed to a press-type of impact (e.g. waste water treatment effluent continually discharging into a stream), in which the effects should be seen on an annual time-scale. Nutrient concentrations and phytoplankton biomass increased as a result of fertilization when data were limited only to the growing season (Table 2.2). These results indicate that conservative fertilization events can stimulate ecosystem productivity on a seasonal scale without influencing the trophic state of the waterbody over longer timescales.

Microcosm experiments may not translate accurately to predictable whole-ecosystem expectations due to food-web (Carpenter et al. 2010) and sediment-water interactions (Carpenter

et al. 1995, Carpenter et al. 1996, Schindler 1998, Schindler et al. 2008) that are not captured in bottle experiments. However, smaller, 'closed' experiments spanning several orders of magnitude in size have provided similar phytoplankton growth responses to that of whole-ecosystem studies (Spivak et al. 2010). Although the data are limited to three whole-ecosystem fertilization events, two of those three responded as predicted based on microcosm results.

Both chl *a* and PC are proxies for phytoplankton biomass. Chlorophyll *a* is more frequently used as a proxy for phytoplankton biomass, as it is easier to measure (Felip and Catalan 2000). Direct PC measurements are less common, and most phytoplankton C content is derived via microscopic observation where imprecise calculations based on geometric shapes of specific species are used to derive biovolumes (Hillebrand et al. 1999). Particulate C measurements inherently incorporate detritus, primarily from recently deceased planktonic organisms, making PC a better total measurement of phytoplankton biomass, but not as useful when assessing growth for a short-term experiment.

Chlorophyll *a* was the most sensitive predictor of phytoplankton biomass in both the microcosm and whole-lake experiments. The fertilization rates tested in Lake Rayburn's microcosm experiments were expected to yield different maximum phytoplankton biomasses at different times throughout the growing season. In May, the 0.70 kg P $ha^{-1}$ plus 6.3 kg N $ha^{-1}$ yielded the greatest chl *a*, whereas in June and July, chl *a* increased proportionally with fertilization across all treatments. The lesser rate used in May was due to greater ambient nutrient concentrations in the epilimnion at the onset of stratification (Figure 2.2A-D). As stratification persisted, N was always a primary or co-limiting nutrient. This was corroborated by the P-only treatment yielding a consistent similar response to the control unit.

The effects of different N sources on phytoplankton abundance, composition, and cyanobacteria toxicity have been tested in several field and laboratory studies. At the coarse taxonomic level, $NH_4^+$ has been shown to favor cyanobacteria (Blomqvist et al. 1994), $NO_3^-$ tends to favor diatoms and eukaryotes, and urea has been shown to favor non-heterocystous cyanobacteria (Reynolds 2006, Donald et al. 2011). In the second part of Lake Rayburn's microcosm experiment, phytoplankton counts were to be measured to test the effects of N form on phytoplankton community composition in addition to biomass determinations. Both proxies for biomass (chl *a* and PC) showed no differences in growth between N forms (Figure 2.1A-B). Samples for phytoplankton community composition were kept from the microcosm experiments, but the analysis of those samples was beyond the scope of this thesis. Analysis of those samples will reveal, however, if forms of N in chemical fertilizer shifted the phytoplankton to cyanobacteria. If not, then fertilizer selection could be expanded to include ammonium nitrate or urea, which are consistently less expensive than calcium nitrate.

Chlorophyll *a*:PC ratios have been used to evaluate the quality (physiological condition) and potential growth rate of phytoplankton populations in various studies (Cloern et al. 1995). Causes for deviations of the chl *a*:PC ratio have not been adequately studied, but have been attributed to nutrient and light limitation (Laws and Bannister 1980). An interesting trend in chl *a*:PC was observed in the microcosm experiments. The P-only treatment in each experiment had the lowest chl *a*: PC ratio, indicating that N may have limited the capacity of phytoplankton to add N-rich chlorophyll and thereby limited their ability to grow and reproduce (Cloren et al. 1995, Schindler et al. 2008).

### 2.4.1 Conclusion

No effects of fertilization were detected on the annual timescale, with the exception of TP. However, increased nutrient and biomass concentrations were observed on the short timescale (truncated and pulse). This work has shown that increases in primary production can be achieved for a short time when nutrient limitations are greatest and production increases are desired. These small fertilization events provided needed nutrients to the biota without affecting annual and interannual patterns in water quality. This finding is consistent with the idea that limited fertilization can be used to manage multi-use lakes for mesotrophic conditions without increasing nutrients or algal biomass in downstream sensitive waters.

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## 2.6 Tables

Table 2.1 Annual time-scale data from May 2011 to April 2013 is shown for Lakes Brittany and Rayburn. For chl *a*:PC ratio values, chl *a* units are $\mu g\ L^{-1}$, and PC units are $mg\ L^{-1}$. Less than 80 µm chl *a* concentrations are in $\mu g\ L^{-1}$ and <80 µm PC concentrations are in $mg\ L^{-1}$. TN:TP, seston C:P, and seston N:P values are reported as mass ratios. Transformations of the data conducted to meet assumptions for normality are indicated, but actual raw mean ± standard deviation (SD) values are reported for each lake (control/impact) and for each year (before/after).

| Parameters | Trans- | Annual BACI | | Control Brittany (x ± SD) | | Impact Rayburn (x ± SD) | |
|---|---|---|---|---|---|---|---|
| | formation | *F* value | *p* value | Before (2011) | After (2012) | Before (2011) | After (2012) |
| TN ($mg\ L^{-1}$) | none | 3.020 | 0.3325 | 0.55 ± 0.12 | 0.49 ± 0.15 | 0.72 ± 0.15 | 0.74 ± 0.13 |
| TP ($mg\ L^{-1}$) | none | 10.17 | 0.0017 | 0.021 ± 0.008 | 0.016 ± 0.004 | 0.033 ± 0.008 | 0.035 ± 0.005 |
| TDN ($mg\ L^{-1}$) | none | 1.590 | 0.4269 | 0.418 ± 0.126 | 0.353 ± 0.141 | 0.445 ± 0.154 | 0.438 ± 0.145 |
| TDP ($mg\ L^{-1}$) | none | 5.060 | 0.2663 | 0.009 ± 0.005 | 0.006 ± 0.003 | 0.008 ± 0.003 | 0.007 ± 0.002 |
| TN:TP | $\log_{10}$ | 1.990 | 0.1601 | 28.4 ± 9.93 | 30.5 ± 7.84 | 22.6 ± 6.11 | 21.5 ± 4.60 |
| chl *a* ($\mu g\ L^{-1}$) | $\log_{10}$ | 0.350 | 0.6583 | 5.27 ± 3.07 | 7.33 ± 4.01 | 19.6 ± 12.7 | 23.3 ± 11.1 |
| PC ($mg\ L^{-1}$) | $\log_{10}$ | 2.660 | 0.3503 | 0.82 ± 0.30 | 0.76 ± 0.20 | 1.62 ± 0.40 | 1.79 ± 0.33 |
| chl *a* : PC | $\log_{10}$ | 3.080 | 0.3295 | 6.47 ± 2.97 | 9.86 ± 5.21 | 12.08 ± 7.10 | 13.06 ± 5.76 |
| <80 $\mu$m chl *a* | $\log_{10}$ | 3.700 | 0.0565 | 3.92 ± 2.68 | 6.99 ± 4.05 | 19.2 ± 12.9 | 23.1 ± 11.2 |
| <80 $\mu$m PC | $\log_{10}$ | 1.730 | 0.2257 | 0.672 ± 0.282 | 0.71 ± 0.21 | 1.97 ± 0.43 | 1.72 ± 0.33 |
| Seston C:P | none | 1.730 | 0.1918 | 68.1 ± 25.2 | 77.1 ± 25.6 | 66.2 ± 16.6 | 67.1 ± 15.2 |
| Seston N:P | $\log_{10}$ | 4.730 | 0.2743 | 11.0 ± 3.2 | 13.7 ± 4.0 | 11.2 ± 2.6 | 11.3 ± 2.6 |
| Secchi (m) | $\log_{10}$ | 0.650 | 0.4231 | 3.87 ± 1.42 | 4.04 ± 1.09 | 2.48 ± 0.73 | 2.34 ± 0.62 |
| Photic (m) | none | 0.910 | 0.3455 | 7.82 ± 2.10 | 8.11 ± 1.28 | 5.15 ± 0.84 | 5.08 ± 1.03 |
| Sed N ($mg\ m^{-2}d^{-1}$) | $\log_{10}$ | 4.970 | 0.2685 | 40.3 ± 24.6 | 41.9 ± 14.8 | 45.8 ± 12.0 | 71.4 ± 28.4 |
| Sed C ($mg\ m^{-2}d^{-1}$) | $\log_{10}$ | 10.13 | 0.1938 | 279.1 ± 161.9 | 241.6 ± 104.3 | 296.4 ± 94.3 | 481.3 ± 229.5 |
| PP ($mg\ C\ m^{-3}\ h^{-1}$) | none | 1.020 | 0.3163 | 9.58 ± 6.02 | 8.66 ± 4.30 | 19.8 ± 14.9 | 15.5 ± 10.6 |
| P/R | $\log_{10}$ | 2.460 | 0.3614 | 1.38 ± 0.650 | 1.50 ± 0.441 | 1.40 ± 0.800 | 1.14 ± 0.578 |

Table 2.2 Truncated time-scale data from May 13 to August 4, 2011 and May 4 to July 26, 2012 are shown for Lakes Brittany and Rayburn. For chl *a*:PC ratio values, chl *a* units are $\mu g\ L^{-1}$, and PC units are $mg\ L^{-1}$. Less than 80 µm chl *a* concentrations are in $\mu g\ L^{-1}$ and <80 µm PC concentrations are in $mg\ L^{-1}$. Total N:TP, seston C:P, and seston N:P values are reported as mass ratios. Transformations of the data conducted to meet assumptions for normality are indicated, but actual raw mean ± standard deviation (SD) values are reported for each lake (control/impact) and for each year (before/after).

| Parameters | Trans-formation | Truncated BACI | | Control Brittany (x ± SD) | | Impact Rayburn (x ± SD) | |
|---|---|---|---|---|---|---|---|
| | | *F* value | *p* value | Before (2011) | After (2012) | Before (2011) | After (2012) |
| TN ($mg\ L^{-1}$) | none | 62.43 | 0.0801 | 0.58 ± 0.11 | 0.44 ± 0.09 | 0.72 ± 0.10 | 0.72 ± 0.07 |
| TP ($mg\ L^{-1}$) | sqrt | 13.00 | 0.0005 | 0.019 ± 0.007 | 0.016 ± 0.004 | 0.031 ± 0.006 | 0.037 ± 0.004 |
| TDN ($mg\ L^{-1}$) | $\log_{10}$ | 39.71 | 0.0013 | 0.448 ± 0.109 | 0.321 ± 0.084 | 0.430 ± 0.114 | 0.402 ± 0.038 |
| TDP ($mg\ L^{-1}$) | $\log_{10}$ | 12.08 | 0.0009 | 0.009 ± 0.006 | 0.006 ± 0.003 | 0.008 ± 0.003 | 0.009 ± 0.002 |
| TN:TP | $\log_{10}$ | 1.060 | 0.3078 | 31.9 ± 6.5 | 30.0 ± 8.8 | 24.0 ± 5.0 | 19.9 ± 3.1 |
| chl *a* ($\mu g\ L^{-1}$) | none | 43.77 | < 0.0001 | 6.20 ± 3.01 | 5.44 ± 3.08 | 14.2 ± 5.75 | 24.7 ± 10.6 |
| PC ($mg\ L^{-1}$) | $\log_{10}$ | 13.46 | 0.1694 | 0.85 ± 0.28 | 0.70 ± 0.19 | 1.70 ± 0.37 | 1.90 ± 0.30 |
| chl *a* : PC | $\log_{10}$ | 2.400 | 0.1256 | 7.35 ± 2.69 | 8.44 ± 5.60 | 8.41 ± 3.05 | 13.4 ± 5.81 |
| <80 $\mu$m chl *a* | $\log_{10}$ | 1.230 | 0.2701 | 4.06 ± 2.86 | 4.97 ± 3.0 | 13.92 ± 5.89 | 24.46 ± 10.71 |
| <80 $\mu$m PC | $\log_{10}$ | 1.230 | 0.2718 | 0.65 ± 0.26 | 0.63 ± 0.20 | 1.60 ± 0.386 | 1.82 ± 0.292 |
| Seston C:P | none | 0.040 | 0.8494 | 82.1 ± 26.3 | 79.0 ± 30.0 | 74.9 ± 18.0 | 69.3 ± 17.3 |
| Seston N:P | $\log_{10}$ | 1.410 | 0.4453 | 12.7 ± 2.8 | 13.8 ± 4.4 | 12.6 ± 2.3 | 11.7 ± 2.9 |
| Secchi (m) | $\log_{10}$ | 18.05 | < 0.0001 | 3.23 ± 0.69 | 4.31 ± 1.06 | 2.57 ± 0.60 | 2.21 ± 0.46 |
| Photic (m) | none | 22.42 | < 0.0001 | 6.70 ± 1.26 | 8.72 ± 1.45 | 5.18 ± 0.77 | 4.43 ± 0.40 |
| Sed N ($mg\ m^{-2}d^{-1}$) | $\log_{10}$ | 9.570 | 0.1990 | 50.0 ± 35.4 | 46.5 ± 12.9 | 44.9 ± 13.2 | 84.1 ± 15.8 |
| Sed C ($mg\ m^{-2}d^{-1}$) | $\log_{10}$ | 18.48 | 0.1455 | 351.5 ± 228.4 | 277.7 ± 118.8 | 314.3 ± 97.6 | 632.0 ± 142.1 |
| PP ($mg\ C\ m^{-3}\ h^{-1}$) | none | 0.050 | 0.8636 | 11.5 ± 8.2 | 6.6 ± 2.4 | 26.2 ± 17.8 | 23.6 ± 11.4 |
| P/R | $\log_{10}$ | 3.350 | 0.3184 | 1.12 ± 0.44 | 1.47 ± 0.40 | 1.59 ± 0.98 | 1.07 ± 0.57 |

Table 2.3 Pulse time-scale data for Lakes Brittany and Rayburn are shown. The 2011 pulse events were simulated with no actual fertilization. Values reported are the slope of the line from the 14 day pulse sampling for Lake Rayburn minus (-) Lake Brittany sampled in the same manner. A single * indicates $p < 0.05$ and two** indicate $p < 0.01$. Thirteen of the total 18 water quality parameters were measured using the general linear model to analyze covariance in SAS. Photic depth, sediment trap PC, PN, estimated PP rates, and P/R ratio were not used in SAS because they were measured weekly and not on the same frequency of the other parameters and therefore are not reported here.

| **P Fertilization Rate** | **0 kg ha$^{-1}$** | **0 kg ha$^{-1}$** | **0.28 kg ha$^{-1}$** | **0.42 kg ha$^{-1}$** | **0.23 kg ha$^{-1}$** |
|---|---|---|---|---|---|
| Parameter | 2011 June | 2011 July | 2012 May | 2012 June | 2012 July |
| TN (g L$^{-1}$ day$^{-1}$) | 14.1* | 10.8* | -17.8* | 3.57 | 3.60 |
| TP (g L$^{-1}$ day$^{-1}$) | -0.85 | 0.51** | 0.23 | 0.53 | 0.61* |
| TDN (g L$^{-1}$ day$^{-1}$) | 13.6** | 3.70 | -5.50 | 6.15 | 0.51 |
| TDP (g L$^{-1}$ day$^{-1}$) | -0.76 | 0.18 | -0.22 | 0.22 | 0.11 |
| TN:TP (day$^{-1}$) | 2.76 | -0.09 | -0.30 | -1.51 | -0.66 |
| Chl *a* ($\mu$g L$^{-1}$) | -0.30 | 0.98 | 1.13** | 1.60** | -2.49** |
| PC (g L$^{-1}$ day$^{-1}$) | -36.9 | 45.6 | -71.3 | -12.8 | 1.24 |
| Chl *a* **:** PC | -0.01 | 0.37 | 0.98* | 0.58 | -1.55** |
| <80 $\mu$m Chl *a* ($\mu$g L$^{-1}$ day$^{-1}$) | 0.18 | 0.89 | 1.17** | 1.67** | -2.44** |
| <80 $\mu$m PC (g L$^{-1}$ day$^{-1}$) | -27.8 | 46.6 | -65.3 | -13.8 | 3.10 |
| Seston C:P | -4.02 | -0.18 | -8.05 | -6.36 | -2.82 |
| Seston N:P | 0.01 | 0.01 | -1.33 | -1.11 | -0.31 |
| Secchi (m day$^{-1}$) | 0.149** | 0.012 | 0.214** | -0.101 | -0.006 |

## 2.7 Figure legends

Figure 2.1 Lake Rayburn 2012 microcosm study. Vertical bar graphs show the relationship between added nutrients and stimulated phytoplankton biomass. One-way ANOVA statistics were conducted in SAS v. 9.3 for chl *a*, PC, and chl *a*:PC. Different letters within each month's experiment indicate mean differences. Chlorophyll *a* (A) May: $F = 6.44$, $p = 0.0020$; June: $F = 41.88$, $p = <0.0001$; July: $F = 92.4$, $p = <0.0001$. PC (B) May: $F = 6.51$, $p = 0.0019$; June: $F = 72.45$, $p = <0.0001$; July: $F = 28.47$, $p = <0.0001$. Chl *a*:PC (C) May: $F = 7.60$, $p = 0.0009$; June: $F = 4.33$, $p = 0.0112$; July: $F = 21.62$, $p = <0.0001$.

Figure 2.2 Annual Lake Rayburn, (impact lake) and Lake Brittany, (control lake) nutrient data from before (2011) and after (2012) the impact of chemical fertilization. Total N (A), total P (B), total dissolved N (C), and total dissolved P (D) nutrient concentrations are shown. A BACIP (before-after-control-impact-paired) design was used to evaluate if annual differences occurred due to the impact of chemical fertilization. Solid vertical lines represent the beginning of each study year. The data located between the solid and dashed vertical lines represents the truncated study period.

Figure 2.3 Annual Lake Rayburn, (impact lake) and Lake Brittany, (control lake) biomass data from before (2011) and after (2012) the impact of chemical fertilization. Annual chlorophyll *a* (A), particulate C (B), chlorophyll *a*:particulate C (C), and primary production (D) data are shown. A BACIP (before-after-control-impact-paired) design was used to evaluate if annual differences occurred due to the impact of chemical fertilization. Solid vertical lines represent the

beginning of each study year. The data located between the solid and dashed vertical lines represents the truncated study period.

Figure 2.4 Truncated Lake Rayburn, (impact lake) and Lake Brittany, (control lake) biomass and nutrient data from before (2011) and after (2012) the impact of chemical fertilization. Truncated chlorophyll *a* (A), chlorophyll *a*:particulate C (B), primary production rates (C), and total dissolved N (D) data are shown. A BACIP (before-after-control-impact-paired) design was used to evaluate if annual differences occurred due to the impact of chemical fertilization. Solid vertical lines represent the beginning of each study year.

Figure 2.5 Lake Brittany (A) and Rayburn (B) phytoplankton community composition data for May to September 2012. Percentages of total phytoplankton biomass of the five major taxa groups are shown. The two dashed vertical lines are the June and July fertilization events. The May fertilization event occurred a week prior to the first phytoplankton sample date shown.

## 2.8 Figures

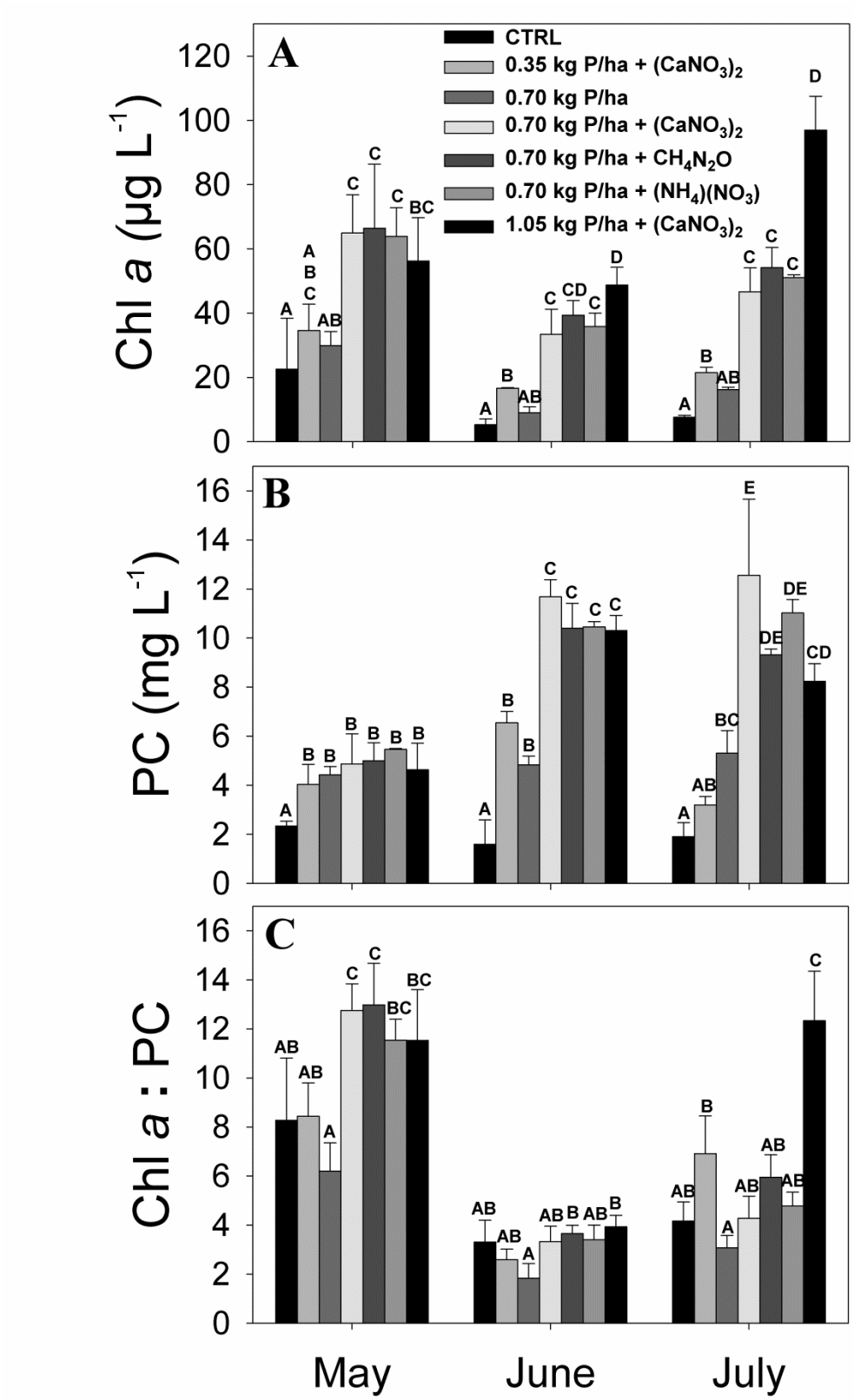


Figure 2.1

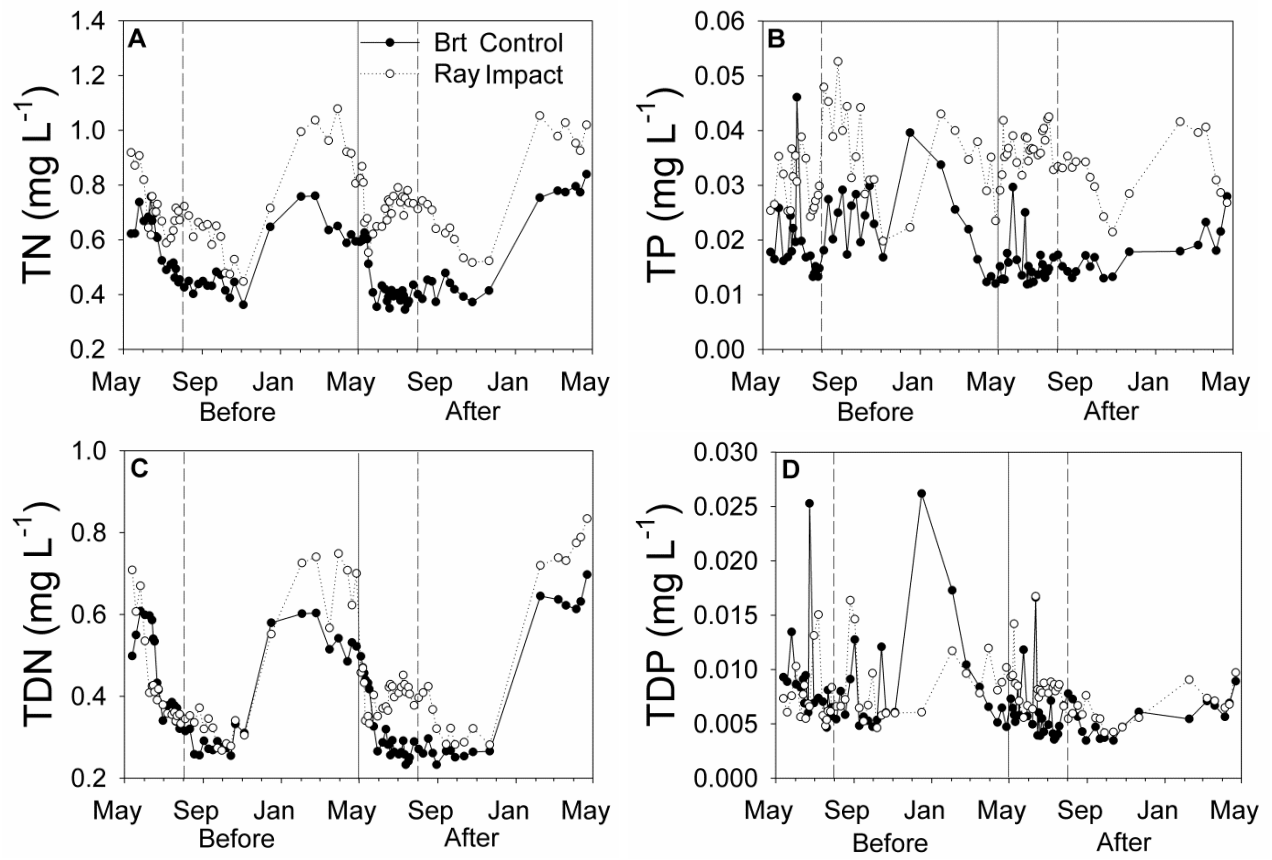
A
Brt Control
Ray Impact
TN (mg L-1)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
B
TP (mg L-1)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
C
TDN (mg L-1)
1.0
0.8
0.6
0.4
0.2
D
TDP (mg L-1)
0.030
0.025
0.020
0.015
0.010
0.005
0.000
May Sep Jan May Sep Jan May
Before
After

Figure 2.2

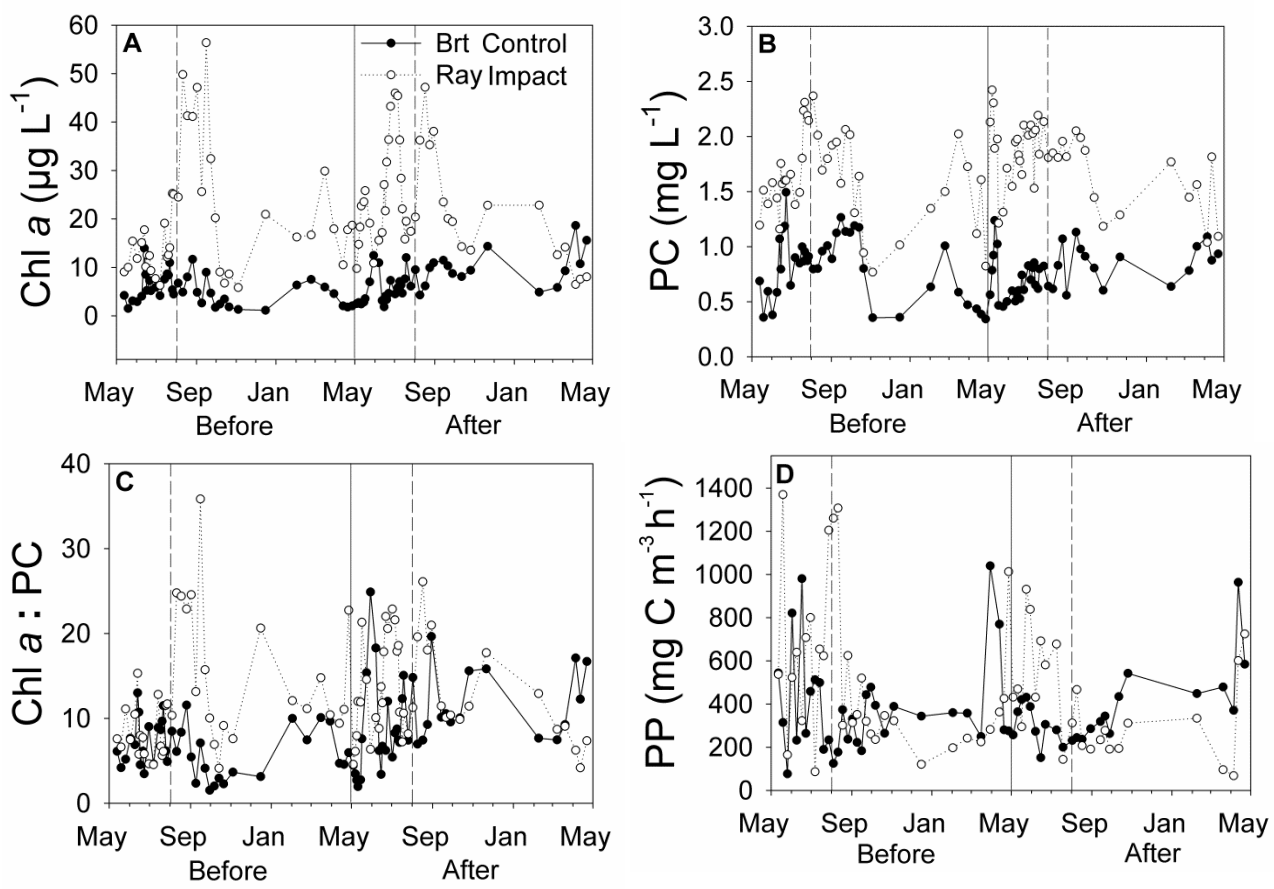
A
Brt Control
Ray Impact
Chl a (µg L-1)
B
PC (mg L-1)
C
Chl a : PC
D
PP (mg C m-3 h-1)
May Sep Jan May Sep Jan May
Before
After

Figure 2.3

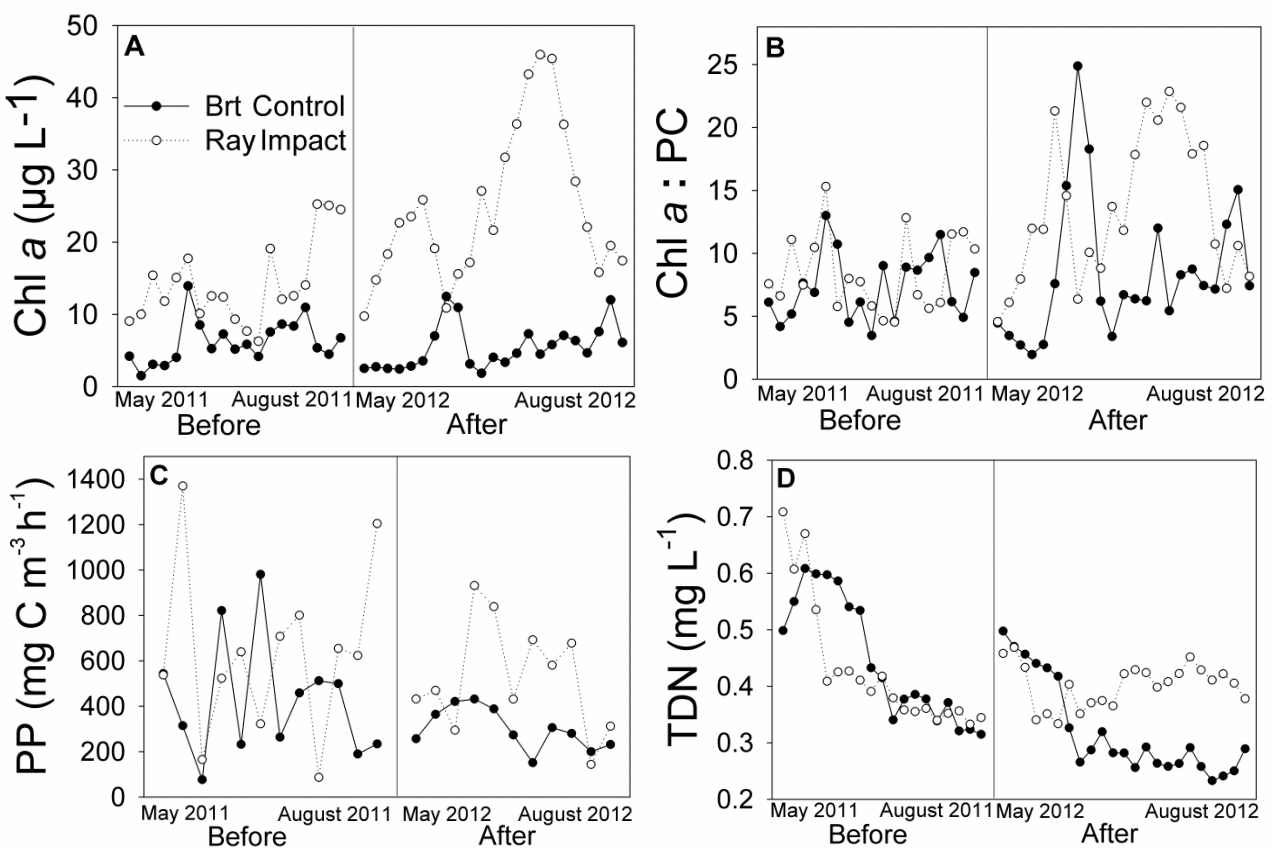
A
Brt Control
Ray Impact
Chl a (µg L-1)
May 2011
August 2011
May 2012
August 2012
Before
After
B
Chl a : PC
C
PP (mg C m-3 h-1)
D
TDN (mg L-1)

Figure 2.4

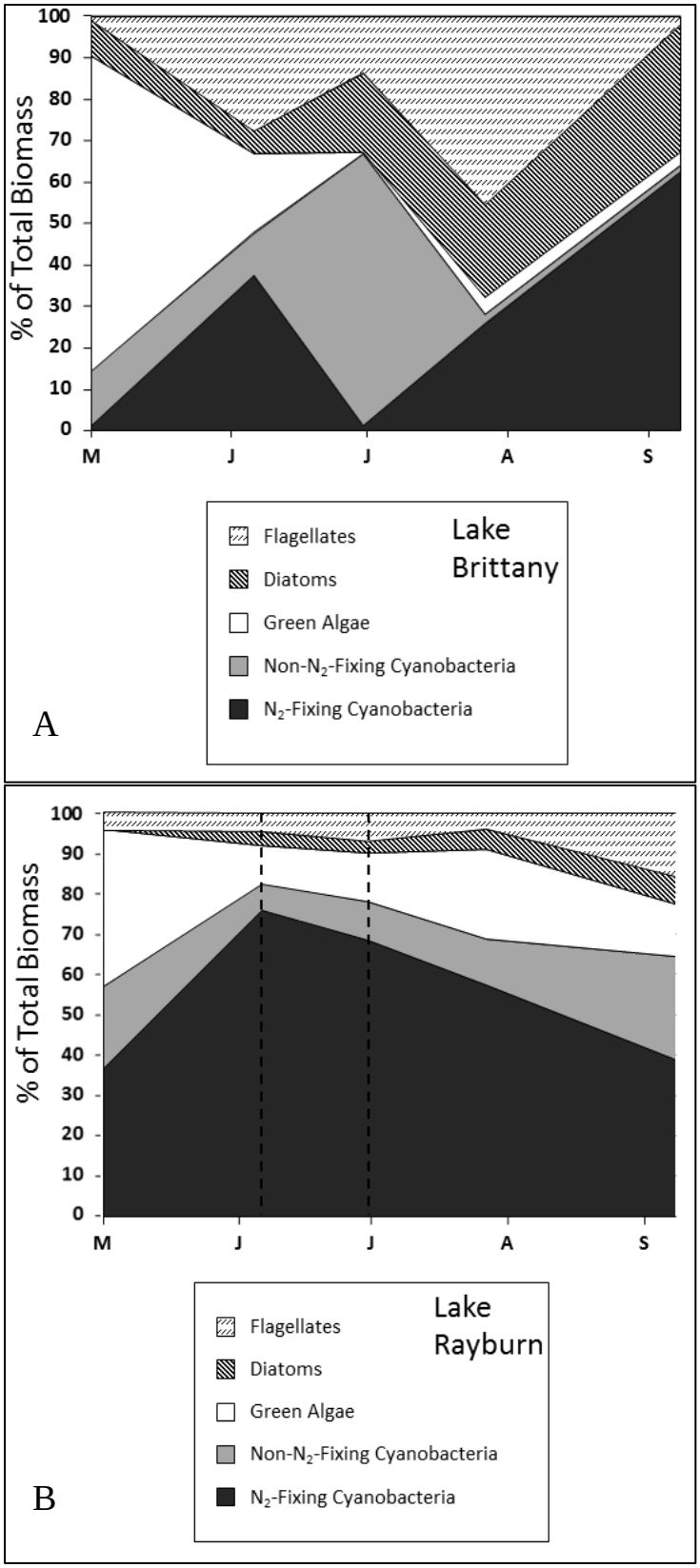
% of Total Biomass
100
90
80
70
60
50
40
30
20
10
0
M
J
J
A
S
Flagellates
Diatoms
Green Algae
Non-N2-Fixing Cyanobacteria
N2-Fixing Cyanobacteria
Lake Brittany
A
% of Total Biomass
100
90
80
70
60
50
40
30
20
10
0
M
J
J
A
S
Flagellates
Diatoms
Green Algae
Non-N2-Fixing Cyanobacteria
N2-Fixing Cyanobacteria
Lake Rayburn
B

Figure 2.5

# 3. Pulsed Artificial-Upwelling as a Reservoir Management Technique to Enhance Biological Productivity

## 3.1 Introduction

Nutrient enrichment of terrestrial and aquatic ecosystems from anthropogenic sources has caused eutrophication to be one of the major problems facing freshwater environments today (Hasler 1947, Carpenter et al. 1998, Smith et al. 1999), and a common problem associated with eutrophic conditions is freshwater harmful algal blooms (FHABs; Paerl et al. 2001). Eutrophication not only affects the biota present within lakes, but the human use of these systems as well (e.g. recreational fishing; Smith et al. 1999). Nitrogen, which is required for protein synthesis, and P, a critical component in DNA and RNA construction as well as the transfer of energy, are considered the key limiting nutrients in lake ecosystems (Sterner and Elser 2002, Conley et al. 2009). Employing single nutrient management approaches (e.g. P-only mitigation) for eutrophic lakes have produced undesirable results (Findlay et al. 2013). Therefore, in most cases, simultaneous control of both N and P is required to effectively mitigate eutrophication (Conley et al. 2009).

The effective management of N and P reduction has shifted some lakes from eutrophic to oligotrophic nutrient states. This is known as cultural oligotrophication, or the human-induced reduction of excess nutrients in aquatic systems (Stockner et al. 2000). Phytoplankton production represents a major basal component of the pelagic aquatic food web and primary producers will grow as long as they have access to sufficient N, P, light energy for photosynthesis, and suitable temperatures (Posselt et al. 2009). Oligotrophic conditions occur primarily when phytoplankton growth is limited due to low water column concentrations of N and P.

One designated use of many lakes is recreational fishing, and therefore they have a need for a robust biological community, something oligotrophic systems often lack. In these nutrient-

deficient lakes, the use of chemical N and P fertilizers have been shown to enhance productivity both experimentally and as a management strategy (Schindler 1974, Vaux et al. 1995, Cottingham and Carpenter 1998, Welcomme and Bartley 1998, Buyank et al. 2001, Boyd et al. 2008). Nitrogen:P supply ratios for phytoplankton ranging from 3.2 to 20.4 (Rhee and Gotham 1980), and TN:TP ratios ranging from 9 to 22.5 (Guildford and Hecky 2000, Dzialowski et al. 2005) have been shown as optimal ranges for phytoplankton growth.

Therefore, it is important to maintain an N:P supply ratio optimal for the growth of edible phytoplankton and consequently healthy zooplankton populations (Stockner and Maclsaac 1996, Stemberger and Miller 1998). Properly administered nutrient additions to lakes needing increased biological production can be viewed as restoration of ecosystem production rather than pollution (Stockner et al. 2000). The key is to have nutrients accumulate in fish biomass rather than in phytoplankton biomass (i.e. trophic efficiency; Hecky 1984). Nutrients do not degrade water quality when channeled to the highest trophic levels, but rather they become valuable resources for human consumption and commercial application (Hudnell 2010). High rates of nutrient addition are not necessarily undesirable considering the greatest natural rates of primary production and fish production occur in naturally enriched upwelling areas (Hecky and Killham 1988).

The greatest rates of primary production in oceans occur in costal upwelling zones (Kokkinakis and Wheeler 1987, Feidler et al. 1991, Olivieri and Chavez 2000). Additionally, studies in polymictic lakes have shown increased phytoplankton growth following nutrient pulses from the anoxic zone during brief mixing events (Wilhelm and Adrian 2008). Therefore, nutrient-rich hypolimnetic water may be a suitable alternative to chemical fertilizers, which are increasingly expensive (USDA 2013). The hypolimnia of many stratified lakes contain nutrients,

specifically DIN and DIP, which are unavailable to phytoplankton present within the photic zone during summer stratification. Although rare, there has been one instance where hypolimnetic water was used to increase nutrient concentrations within the photic zone of a lake. The single study was performed in a small Michigan trout lake via pumping hypolimnion water to the surface to increase phytoplankton production (Hooper 1953). Although not widely tested, using hypolimnetic nutrients within stratified lakes could be a potential solution to increase nutrient concentrations in lakes needing greater primary production levels.

There are few data available on the effects of hypolimnion water stimulating phytoplankton biomass and alleviating nutrient limitation, but there are numerous data from small-scale field and laboratory fertilization experiments that examine nutrient limitations to primary production (Suttle and Harrison 1988, Philips 1997, Elser et al. 2007). However, there are a number of issues associated with bioassay experiments aimed at assessing phytoplankton responses to nutrient addition, including the absence of environmental heterogeneity and the removal of natural sources of nutrient recycling (Dzialowski et al. 2005, Schindler 1998). This creates the need for whole-ecosystem experiments to be performed as they can be useful for examining the importance of bottom-up control of aquatic food webs (Schindler 1974, Schindler and Fee 1974, Carpenter et al. 1985). Additionally, the responses of lakes to nutrient additions vary and are affected by the trophic status, structure, and the physical and chemical condition of the water and lake sediments (Schindler 1974, Carpenter et al. 1985, Elser et al. 1990). Therefore, both microcosm and whole-lake fertilization experiments were conducted to evaluate the effects of hypolimnetic water on phytoplankton growth.

A solar-powered pump (SolarBee©) was deployed in the study lake (Lake Norwood) and after stratification, hypolimnion water was pumped in pulses to the photic zone to assess the

effects of hypolimnetic nutrients on phytoplankton production. The "pulsed" nature of the artificial upwelling was used to provide short-term increases in nutrient concentrations and to avoid major changes in the natural stratification and water temperatures of Lake Norwood. Additionally, two SolarBee© epilimnion long distance circulators (LDC) were installed in Lake Norwood to create a mixing current within the epilimnion to remove the advantage of cyanobacteria that can regulate their location in the water column and better compete for light and nutrients.

The central research objective of this study was to test the effects of pulsed artificial-upwelling as a nutrient source for increasing primary production. This was achieved using data collected from both microcosm and whole-lake fertilization experiments. The goals were to: 1) assess what nutrient(s) limited productivity, 2) evaluate how much nutrient additions were needed to maximize productivity without oversaturating growth, and 3) determine the effect of the selected upwelling rate at the whole-ecosystem scale. Based on the small amount of existing literature and preliminary studies available, it was predicted that the pulsed artificial-upwelling lake would: 1) exhibit increased short-term productivity proportional to the upwelling/pumping rate and 2) exhibit no change in interannual water quality conditions including phytoplankton biomass and nutrient concentrations.

## 3.2 Methods

### 3.2.1 Study Sites

The study was conducted in two monomictic recreational, sport fishing reservoirs (surface area < 0.2 $km^2$) in the Springfield Plateau region of northwest Arkansas. Lake Brittany (36°28'08"N, 94°12'04"W) and Lake Norwood (36°28'45"N, 94°14'44"W) are steeply sloped lakes with mean depths of 7.6 to 8.8 m and maximum depths of 21.0 to 23.3 m, respectively.

Both lakes have similar watershed characteristics with primarily forest (64 to 78%) and urban (14 to 23%) land cover, resulting in moderately low nutrient concentrations in both lakes. The inflowing streams to both lakes were exclusively ephemeral. In the past, augmentation with chemical fertilizers was employed to raise biological productivity of the lakes but this management technique was not used in the decade preceding this study.

### 3.2.2 Experimental Design

#### 3.2.2.1 Phase I – Microcosm Fertilization Experiments

A series of in-lake microcosm experiments were conducted in Lake Norwood in order to inform the whole-ecosystem manipulations. The goal of this component was to evaluate a method of increasing nutrient concentrations using a novel form of nutrient addition and to test its effects on primary production biomass. Specifically, the efficacy and viability of hypolimnetic water as a nutrient source for phytoplankton growth stimulation was tested. Also, the addition of N was assessed to determine if it was needed in succession with P-rich hypolimnion water to stimulate phytoplankton biomass.

Microcosm experiments were conducted in May, June, and July 2012 to estimate the potential effect of whole-lake pulsed artificial-upwelling in each month. Vertically integrated water samples were collected from the photic zone at five sites in Lake Norwood using a 4-L Van Dorn horizontal sampler (Alpha water sampler, Wildco, Yulee, FL) and pooled in a bucket to create a WLC sample. The WLC water was equally divided into 21 semi-transparent 4-L cubitainers (2 L of WLC water per cubitainer). The cubitainers were randomly grouped into sets of three replicates in which a portion of the volume was replaced with hypolimnetic water to test the effect of pulsed artificial-upwelling duration. Some treatments also received chemical fertilization with a calcium nitrate ($Ca(NO_3)_2$) (15-0-0; N-P-K) to test the effect of supplemental

N because hypolimnetic waters were expected to have a low N:P ratio. The experimental design included: 1) Control (no hypolimnion addition), 2) 5% hypolimnion addition (5% H) , 3) 5% hypolimnion addition plus N (5% H + N), 4) 10% H addition, 5) 10% H + N addition, 6) 20% H addition, and 7) 20% H + N addition. A mean SRP concentration of 200 µg $L^{-1}$ P was expected in the hypolimnion based on previous data, and N-amended treatments always received 9x more N than P (by mass) assuming a hypolimnetic SRP concentration of 200 µg $L^{-1}$.

Cubitainers were incubated in Lake Norwood for 4-8 days. *In-vivo* fluorescence samples from each cubitainer were measured every 1-2 days during microcosm experiments. Experiments were halted when phytoplankton biomass saturated in each microcosm. Water from each cubitainer was then completely mixed and filtered for seston biomass as PC and chl *a*. Particulate C was measured using an elemental analyzer (NC Soil Analyzer, Flash 2000 Organic Elemental Analyzer, Thermo Scientific, Lakewood, NJ; APHA 2005) and chl *a* was measured fluorometrically (Turner Designs Trilogy Benchtop) following 90 % acetone extraction in a dark freezer for 24 hours. Chlorophyll *a* concentrations were pheophytin corrected (Marker et al. 1980, APHA 2005). The effect of upwelling duration with and without added N on PC, chl *a*, and chl *a*:PC values were tested using a one-way analysis of variance for each experiment in SAS 9.3. Post-hoc comparisons were conducted using the REGWQ multiple comparison test when the omnibus F test was statistically significant at alpha = 0.05.

**3.2.2.2 Phase II – Whole-Lake Fertilization Experiments**

A before/after control/impact paired (BACIP) design was used to test the effect of whole-lake pulsed artificial-upwelling on a variety of limnological endpoints associated with primary production. Whole-lake experiments were conducted with a heavy emphasis placed on the growing season when the lakes were stratified and lowest epilimnion nutrient concentrations

were likely to limit primary production. Sampling of Lake Brittany and Norwood occurred approximately weekly during the growing season over two years, with some sampling occurring at even finer intervals. In 2011, (before year) no upwelling (impact) occurred and only monitoring data were collected in both Lake Brittany (control lake) and Lake Norwood (impact lake). In 2012, (after year) monitoring continued in both lakes, but Lake Norwood received three pulses of hypolimnetic water via pulsed artificial-upwelling while also experiencing epilimnion circulation and Lake Brittany received no treatments.

Two solar-powered SolarBee© SB10000 units and one SB5000 unit were installed in Lake Norwood in February 2012 (see Figures 3.1 and 3.2). The SB10000 units each consisted of three pontoons supporting a platform comprised of above water, near surface, and under water components. A 0.914 m diameter, flexible, intake hose was attached to the base of the impeller. Intake hoses were set to a depth of 3.7 m. A steel plate suspended 0.305 m beneath the hose caused water in that density layer to be drawn in radially with near-laminar flow. The units transported approximately 38,000 L $min^{-1}$ of water to the surface. Approximately 12,000 L $min^{-1}$ of direct flow were transported through the hose, and another 26,000 L $min^{-1}$ of induced flow were transported external to the hose (Hudnell et al. 2010; SolarBee©.com). The two SB10000 units began circulating the upper surface layer of Lake Norwood continually from February 2012 to the end of the experiment in April 2013.

The SB5000 unit was an artificial upweller that had a similar above-water setup to the SB10000 units. However, the intake hose reached 21.5 m to the lake bottom. The SB5000 supplied hypolimnetic water at an approximate rate of 2,650 L $min^{-1}$ of direct flow to the lake's surface. No induced flow was supplied to the surface water due to the length of transport (SolarBee©.com). Dissolved inorganic N and DIP rich hypolimnion water was artificially

upwelled in pulses into the photic zone of Lake Norwood, based on the recommendations derived from microcosm experiments. Measured hypolimnetic nutrient concentrations and the SB5000 pump rate were used to derive the amount of applied nutrients to Lake Norwood at the following rates: 1.14 kg N $ha^{-1}$ plus 0.090 kg P $ha^{-1}$ from May 4 to 7 2012, 1.90 kg N $ha^{-1}$ plus 0.170 kg P $ha^{-1}$ from June 12 to 17 2012, and 8.46 kg N $ha^{-1}$ plus 1.990 kg P $ha^{-1}$ from July 6 to 23 2012.

Whole-lake monitoring on Lakes Brittany and Rayburn was conducted weekly during the growing season of both years and approximately monthly during the non-growing season. Additionally, more intense monitoring was conducted on both lakes in the two weeks following each fertilization event. Whole-lake composite photic zone samples were collected on each sample date. In addition to the WLC sample collected at each of the five sites, a 6-m vertical haul with an 80 µm Wisconsin tow-net (Wisconsin sampler, Wildco, Yulee, FL) was collected and combined into a single, WLC sample for >80 µm biomass on each sample date. All water samples were collected in amber bottles to prevent photodegradation.

A sediment trap was deployed at a central (pelagic) location and left for a period of 7-14 days. Each sediment trap was deployed 6-m from the surface so that the opening was located at the top of the metalimnion. Sediment deposition rates (g $m^{-2}$ $day^{-1}$) were calculated from sediment trap samples using the surface area of the trap opening (0.0064$m^2$), depth of the trap (6 m), and duration of deployment (7-14 days). A hypolimnion sample was collected weekly from this central location. Light extinction, resulting in photic depth, was measured with a Licor quantum sensor (LI-250A Light Meter/Photometer, LI-193 bulb, LI-COR®, Lincoln, NE), and water transparency was measured with a 20-cm diameter secchi disk at this location as well. Multi-parameter profile data, including depth, water temperature (WT), pH, specific conductance

(SPC), dissolved oxygen (DO), and oxidation-reduction potential (ORP), were routinely collected (600 XLM YSI, Yellow Springs, OH) from the centrally located site to characterize the physical structure of each lake.

A multiparameter datasonde (600 XLM YSI, Yellow Springs, OH) was also deployed in both Lake Brittany and Lake Rayburn for continuous measurements. Continuous logging data sondes were deployed at the central location at a depth of 2 m. The depth of deployment was chosen to capture the production and consumption of oxygen in the photic zone of each lake as well as to minimize perturbations caused by wind. Water temperature, DO, pH, and SPC were logged every 15 minutes in each lake. Diurnal DO concentrations were converted into whole-ecosystem primary production and respiration rates (mg C $m^{-3}$ $h^{-1}$) according to the method described by Wetzel (2000). Briefly, the rate of oxygen concentration increase during daytime hours was converted into net primary production. Respiration rates were calculated in a similar manner based on nighttime decreases in DO concentrations. It was assumed that production and respiration were consistent throughout the volume of each lake's photic zone, the 2 m sonde deployment depth was representative of the entire lake's photic zone, and gas exchange with the atmosphere in any 24 hour period was minimal compared to the change in $O_2$ concentration. Units were converted between $O_2$ and C based on a photosynthetic quotient of one.

Whole-lake composite and >80 µm WLC samples were filtered onto pre-combusted (4 h at 450 °C) 25 mm Whatman GF/F glass fiber filters for PC and particulate N (PN), an acid-washed GF/F filter for particulate P (PP), and an untreated GF/F filter for chl *a*. Sediment trap samples were filtered similarly for particulate C, N, P, and total suspended solids (TSS). Filtrate for WLC and hypolimnion samples was collected after passing through 47 mm GF/F filters and preserved by freezing for later analyses of dissolved nutrients. Whole-lake composite samples

were analyzed for total dissolved P (TDP), total dissolved N (TDN), ammonium ($NH_4^+$), and nitrate ($NO_3^-$). Hypolimnion samples were analyzed for $NH_4^+$, $NO_3^-$, TDN, and soluble reactive P (SRP).

Filters for PC and PN analysis were stored frozen and then oven-dried prior to elemental analysis as described previously. Particulate P filters were autoclave-digested in a 1% acid-persulfate solution. After digestion, the samples were analyzed by colorimetry produced by the ascorbic acid method to determine PP concentrations (APHA 2005). Chlorophyll *a* samples were analyzed by fluorometry as described previously.

Ammonium was analyzed by colorimetry produced using the Hach method (Turner Designs Instrument Model 7200 Trilogy™, Sunnyvale, CA; APHA 2005), while $NO_3^-/NO_2^-$ were quantified colorimetrically using the cadmium reduction method (Turner Designs Instrument Model 7200 Trilogy™, Sunnyvale, CA; APHA 2005). Total dissolved N was analyzed using a TOC-TN analyzer (Shimadzu Scientific Instruments TOC-$V_{CSH}$ and TNM-1 analyzer, Columbia, MD; APHA 2005). Total dissolved P was analyzed spectrophotometrically via the ascorbic acid method following a 1 % persulfate digestion (Agilent Technologies, Cary 300 UV-Vis, Foster City, CA; APHA 2005).

A WLC subsample was preserved with $M^3$ phytoplankton fixative to determine community composition on five sampling dates throughout the impact summer (2012). Phytoplankton were enumerated with a Wilde M40 inverted microscope at 400 x using a 5 mL or 10 mL fixed Utermohl tube, following the methods described by Utermohl (1958) in Britton and Greeson (1987). Briefly, phytoplankton cells or colonies (natural counting units) were identified and enumerated across the diameter of the settling chamber (transect) then multiplied by the

focal width of the field of view, rather than the sum of individual fields of view, until a minimum of 300 counting units were obtained (APHA, 2005).

### 3.2.3 Statistical Analysis

Whole-lake data were compared among lakes using a Before-After-Control-Impact design with paired samples (BACIP; Underwood 1994, Benedetti-Cecchi 2001, Stewart-Owten and Bence 2001, Smith 2002). The before year (2011) was compared to the after year (2012) and the control lake (Brittany) was compared to the impact lake (Norwood). The BACIP design employs a two-way repeated measures analysis of variance (ANOVA) to analyze the interactive effect of time (Before-After) and treatment (Control-Impact) for each water quality parameter tested. The interaction of Before-After (BA) and Control-Impact (CI) takes into account both the temporal variations in the data (e.g. seasonal trends) as well as the site differences in the impact lake compared to the control lake. This BA x CI interaction measures if the tested impact or treatment caused a differential change to occur. Each lake was sampled within 1 to 2 hours on the same day creating paired samples to analyze. The BACIP analyses were conducted on the annual data and also a truncated data set that only included data from the growing season in each year (May-August). The BACIP analyses were conducted on raw data which were normally distributed or data which were log- or square-root-transformed to meet the assumption of normality.

Data from three individual pulse events (14 day duration after upwelling) from 2012 were also analyzed using analysis of covariance (ANCOVA) to compare the slopes of response variables over time between Lake Norwood and Lake Brittany. Statistical differences in the slopes indicated that the lakes differed in their short-term (2 week) responses to upwelling. All statistical analyses were conducted using SAS 9.3 (SAS Institute Inc., Cary, N.C.).

## 3.3 Results

### 3.3.1 Microcosm Fertilization Experiments

Additions of hypolimnion water (% H) and hypolimnion water + N (% H + N) stimulated phytoplankton growth in all three months (Figure 3.3). The initial conditions for the May experiment contained TN concentrations of 0.7966 mg $L^{-1}$, TP concentrations of 0.0403 mg $L^{-1}$, and chl *a* concentrations of 2.01 µg $L^{-1}$. In May, the increase in chl *a* concentration was similar between the 20% H and the 5% H + N treatments, and the 5% H and 10% H treatments did not differ from the control. The greatest increase in chl *a* was produced by the 10% H + N, 20% H, and 20% H + N treatments where chl *a* increased from 2.39 µg $L^{-1}$ in the control to 20.1 µg $L^{-1}$ in the 10% H + N treatment, 17.7 µg $L^{-1}$ in the 20% H treatment, and 20.4 µg $L^{-1}$ in the 20% H + N treatment.

The initial conditions for the June experiment contained TN concentrations of 0.6774 mg $L^{-1}$, TP concentrations of 0.0220 mg $L^{-1}$, and chl *a* concentrations of 8.08 µg $L^{-1}$. In June, there was no difference between the 5% H, 10% H, and 20% H treatments, whereas the 20% H + N treatment yielded the greatest increase in chl *a* concentration where chl *a* increased from 5.20 µg $L^{-1}$ in the control to 31.4 µg $L^{-1}$ in the 20% H + N treatment. The initial conditions for the July experiment contained TN concentrations of 0.5600 mg $L^{-1}$, TP concentrations of 0.0230 mg $L^{-1}$, and chl *a* concentrations of 8.34 µg $L^{-1}$. In July, there was no difference between the control, 5% H, 10% H, and 20% H treatments, and the 5% H + N and 10% H + N treatments increased chl *a* concentrations more than the 20% H treatment did. Again the 20% H + N treatment yielded the greatest increase in chl *a* concentration where chl *a* increased from 3.4 µg $L^{-1}$ in the control to 12.7 µg $L^{-1}$ in the 20% H + N treatment. In both June and July experiments, the 10% H + N treatment yielded greater chl *a* concentrations than the 20% H treatment.

In the May and June experiments, N addition did not increase PC concentrations at each % H level in all treatments except in the June 20% H and 20% H + N treatments. In July, the 5% H + N treatment increased PC concentration to the same level as the 10% H treatment. Additionally, the 10% H + N treatment increased PC concentration to the same level as the 20% H treatment, and the 20% H + N treatment increased PC concentrations greater than all other treatments. In both June and July experiments, the 20% H + N treatment increased chl *a* and PC concentrations greater than all other treatments.

Phytoplankton quality, measured as chl *a*:PC, showed differences among treatments in June and July, but not in May; however, the greatest chl *a*:PC measurements were observed in the May experiment. With each successive experiment, chl *a*:PC steadily decreased and the July experiment had the lowest chl *a*:PC values. The decreasing trend in chl *a*:PC was driven by a decline in hypolimnetic N:P through summer stratification. Hypolimnion N:P in May was 13.0, in June was 10.8, and in July was 4.25. Hypolimnetic addition rates had no effect on the chl *a*:PC in May but greater rates had a negative effect on chl *a*:PC in June and July. In June, the N-amended treatments yielded greater chl *a*:PC values compared to the same addition rate without N added.

### 3.3.2 Whole-Lake Fertilization Experiments

Nutrient concentrations (Figure 3.4) and phytoplankton biomass (Figure 3.5) exhibited typical seasonal distributions in both lakes, with concentrations typically greater in Lake Norwood than Lake Brittany. For example, Lake Norwood's annual mean TN concentrations ranged from 0.69 to 0.70 mg $L^{-1}$ whereas Lake Brittany's annual TN concentrations ranged from 0.49 to 0.55 mg $L^{-1}$. Annual mean TP concentrations in Lake Norwood ranged from 0.029 to 0.032 mg $L^{-1}$ while annual TP concentrations in Lake Brittany ranged from 0.016 to 0.021 mg $L^{-}$

[1]. Additionally, annual mean chl *a* concentrations ranged from 12.9 to 20.3 µg $L^{-1}$ in Lake Norwood, and Lake Brittany's annual chl *a* concentrations ranged from 5.27 to 7.33 µg $L^{-1}$. Spring P limitation and summer N limitation was observed in the lakes which is common in many southeastern U.S. lakes. In Lake Norwood nutrient limitation was relieved to some degree by three pulsed artificial-upwelling events.

The annual BACIP analysis revealed that there was no differential change for any of the eighteen water quality parameters tested (Table 3.1). However, BACIP analysis on truncated data indicated several differential changes in nutrient concentrations due to the impact of three pulsed artificial-upwelling events in summer 2012 (Table 3.2).Truncated TP concentrations decreased in Lake Brittany from 0.019 to 0.016 mg $L^{-1}$ between summer 2011 and summer 2012, whereas TP concentrations in Lake Norwood increased between summer 2011 and summer 2012 from 0.025 to 0.027 mg $L^{-1}$ resulting in a differential change of 0.005 mg $L^{-1}$ ($p$ =0.0262). Truncated TDP concentrations decreased in Lake Brittany from 0.009 to 0.006 mg $L^{-1}$ and TDP concentrations in Lake Norwood increased from 0.007 to 0.009 mg $L^{-1}$ between summer 2011 and summer 2012 resulting in a differential change of 0.005 mg $L^{-1}$ ($p$ =0.0009). Truncated TDN concentrations decreased from 0.448 mg $L^{-1}$ to 0.321 mg $L^{-1}$ in Lake Brittany between summer 2011 and summer 2012 and TDN concentrations increased from 0.414 mg $L^{-1}$ to 0.452 mg $L^{-1}$ in Lake Norwood between summer 2011 and summer 2012 resulting in a differential change of 0.165 mg $L^{-1}$ ($p$ =0.0500).

Phytoplankton community composition data were analyzed on five sample dates during stratification from May-September in 2012 to observe natural phytoplankton succession in Lake Brittany and responses to pulsed artificial-upwelling in Lake Norwood (Figure 3.7). In Lake Brittany, a low percentage of total biomass early in the season consisted of cyanobacteria.

Conditions improved for cyanobacteria (e.g. high water temperatures and increased nutrient limitation) later in the summer resulting in $N_2$-fixing cyanobacteria composing more than 50 % of the total phytoplankton biomass. Lake Norwood's phytoplankton community composition in May was initially composed primarily with species of green algae and flagellates. After the May upwelling event, $N_2$-fixing cyanobacteria biomass increased to 45 % of the total biomass. After the June upwelling event, $N_2$-fixing cyanobacteria biomass decreased to 35 %. Throughout the remaining sample dates, $N_2$-fixing cyanobacteria biomass steadily increased reaching 70 % of the total phytoplankton biomass in September.

Analysis of covariance was used to test the differences between Lakes Brittany and Norwood during 'pulse' sampling events. Pulse sampling was conducted in both lakes during the two weeks following pulsed artificial-upwelling of Lake Norwood in 2012, but also twice in 2011 after neither lake was manipulated. The difference between two-week responses of the lakes is shown in Table 3.3. Statistically significant positive values indicate that the slope of the 14-day pulse event for a parameter was greater for Lake Norwood than for Lake Brittany. Statistically significant negative values indicate that Lake Brittany's slope was greater than Lake Norwood's. Although there were some differences in two-week nutrient concentration and water transparency patterns in 2011, there were no differences in phytoplankton biomass or productivity in the control year. Conversely, there were few differences in nutrient concentrations in 2012 with the exception of TP for July in Lake Norwood when compared to Lake Brittany in the two weeks following upwelling. Phytoplankton biomass (as chl *a*, < 80 µm chl *a*, and chl *a*:PC) increased for May in Lake Norwood when compared to Lake Brittany in the two weeks following upwelling. Additionally, the secchi depth decreased less drastically in Lake Norwood when compared to Lake Brittany for May. Nutrient ratios (TN:TP, N:P, and C:P)

decreased for July in Lake Norwood when compared to Lake Brittany in the two weeks following upwelling.

### 3.4 Discussion

The objective of this study was to test the effects of pulsed artificial-upwelling as a nutrient source for increased primary production. Furthermore, assessing the effects of pulsed artificial-upwelling on whole-lake productivity and determining how much nutrient concentrations must be increased (duration of upwelling) to enhance edible phytoplankton bloom formation was a goal. Some of the results support the general prediction that the impact lake would respond to pulsed artificial-upwelling in the form of increasing phytoplankton biomass, that nutrient limitation in the photic zone could be controlled by pulsed artificial-upwelling events, and that nutrient concentrations would only need to be increased minimally to enhance phytoplankton growth. These finding are consistent with the goal of managing this multi-use lake for mesotrophic nutrient status (Stockner et al. 2000).

The pulsed artificial-upwelling of Lake Norwood had no effect on nutrient concentrations, phytoplankton biomass, and productivity when considered on an annual scale (Table 3.1). However, nutrient concentrations increased as a result of pulsed artificial-upwelling when data analysis was limited only to the growing season (Table 3.2). An increase in truncated TN and TP concentrations were predicted due to the amounts of N and P upwelled during each event. An increase in mean-truncated TP concentrations was observed, but only by 0.002 mg $L^{-1}$ and no change in TN concentrations was observed in Lake Norwood. However, TDN concentrations increased from summer 2011 to summer 2012. These results indicate that conservative upwelling events can increase nutrient concentrations in the upper water column of the lake. However, there were fewer observed increases in phytoplankton biomass and primary

production. Thus, the increased whole-lake nutrient concentrations did not always stimulate ecosystem productivity. Nevertheless, some increases in whole-lake phytoplankton biomass were observed in the truncated and pulse data, indicating that pulsed artificial-upwelling can be effective on a seasonal scale without influencing the trophic state of the waterbody over longer timescales.

Given that pulsed artificial-upwelling increased nutrient concentrations, a measureable increase in phytoplankton biomass and decrease in water clarity was expected. However, mean truncated secchi disk transparency increased from 1.7 m to 3.9 m (Figure 3.6D), and photic depth increased from 4.3 m to 6.5 m from summer 2011 to summer 2012, a possible result from the two epilimnion SB10000 units that continually circulated the upper 3.7 m of Lake Norwood (Figure 3.6). The SB10000 units were used to artificially circulate the nutrient-rich upwelled hypolimnetic water throughout the epilimnion of Lake Norwood as well as to suppress cyanobacteria from dominating because cyanobacteria have been shown to regulate their position in the water column via specialized gas vacuoles (Klemer 1991, Beaulieu et al. 2013). Therefore, the lack of new phytoplankton production measured could have been related to light limitations associated with continuous artificial circulation within the epilimnion. Additionally, temperature in the epilimnion was monitored to see if upwelling cold hypolimnetic water would lower the epilimnion water temperatures; the data revealed no changes in epilimnion water temperature.

The microcosm experiments were designed to test different upwelling durations and whether or not additional N could stimulate phytoplankton since hypolimnion N:P was expected to be low. Results show that an N source was needed to stimulate greater levels of phytoplankton production, especially later in the summer. In May, the 10% H + N, 20% H, and 20% H +N treatments yielded a similar response. In June, the lowest hypolimnion addition rate with

amended N (5% H + N) yielded the same increase in chl *a* concentrations as the greatest addition rate without N (20% H), indicating possible N limitation in Lake Norwood in June 2012. In July, the greatest yielding treatments were N amended, and the treatments without added N produced similar results to the control. Additionally, phytoplankton community composition data suggested that the upwelled hypolimnetic water earlier in the season reduced N limitation. As stratification persisted and hypolimnion N:P decreased with each successive upwelling event, the potential to relieve N limitation in the epilimnion of Lake Norwood was diminished later in the summer (Figure 3.7). These results indicated that the effect of upwelling could be enhanced by providing some chemical N fertilizer.

Microcosm and other similar bioassay experiments have associated assumptions and limitations similar to mathematical or simulation models (Scheffer and Beets 1994). Like models however, microcosms can be an inexpensive method to gain insight about more complex systems or the mechanisms that drive these systems. The hope was that performing microcosm experiments across a gradient of time scales and testing different hypolimnetic additions would provide insight about the effects of upwelled hypolimnion water during stratification on these relatively unstudied lakes. The limitations of extrapolating microcosm results to whole-ecosystem processes were acknowledged. Those limitations include complex food-web interactions (Carpenter et al. 2010) and long-term biogeochemical processes (Carpenter et al. 1995, Carpenter et al. 1996, Schindler 1998, Schindler et al. 2008) that cannot be accounted for. However, smaller, 'closed' experiments spanning several orders of magnitude in size have provided similar phytoplankton growth responses to that of whole-ecosystem studies (Spivak et al. 2010). Further, bottle experiments repeated through time, as was done in this research, provide seasonal patterns in phytoplankton response to fertility, which are informative.

Therefore, these results provide confidence to relate the findings from these small-scale experiments to the whole-lake scale.

Both chl *a* and PC were used as proxies for phytoplankton biomass. Chlorophyll *a* is more frequently used proxy for phytoplankton biomass as it is easier to measure (Felip and Catalan 2000). Direct PC measurements are less common, and phytoplankton biomass as C content is often derived via microscopic observation where imprecise calculations based on geometric shapes of specific species are used to derive biovolumes (Hillebrand et al. 1999). Alternatively, PC measurements of whole seston inherently incorporate detritus, primarily from recently deceased planktonic organisms, and non-photosynthetic organisms into the measurement. Thus, PC can be a useful measurement because it is more quantitative than microscopic data, but may also be influenced by seasonal or spatial differences in detritus accumulation.

Chlorophyll *a*:PC ratios have been used to evaluate the quality of phytoplankton populations in various studies (Cloern et al. 1995). Causes for deviations of the chl *a*:PC ratio have not been adequately studied, but have been attributed to nutrient and light limitation (Laws and Bannister 1980). The May experiment had the highest chl *a*:PC values and a steady decrease in June and July chl *a*:PC values occurred. This was most likely due to the decreasing N:P of the hypolimnion water. The overall quality of the phytoplankton community was highest in May, and lowest in July.

No measureable impact on water quality parameters were seen on an annual time-scale. This was expected because the impact was a 'pulse' and large datasets 'wash out' the effects seen from a pulse-type of impact (Smith 2002). This is opposed to a press-type of impact (e.g. waste-water treatment effluent continually discharging into a stream) in which the effects should

be seen on an annual time-scale. However, the intense sampling throughout the two year experiment produced a large dataset for biomass and nutrients in each lake. The trend of nutrient limitation increasing as summer stratification persists can be seen in these data.

In Lake Brittany, nutrient concentrations were greatest in the epilimnion at the onset of stratification. As summer progressed, the nutrients steadily declined as no additional inputs occurred with the lowest nutrient concentrations occurring in early November just before fall mixing (Figure 3.4 A-D). The biomass data presented as chl *a*, PC, and chl *a*:PC demonstrated the biological responses to nutrient limitation in both Lakes Brittany and Norwood in 2011 and upwelled nutrients in Lake Norwood in 2012. Coupled nutrient and biomass data indicated the fate of added nutrients (particulate or dissolved) in Lake Norwood in 2012. For example, more than 60 % of TP was in the form of PP (Figure 3.4 B and D). Particulate data provided insight into whether the nutrients were incorporated into phytoplankton biomass (Figure 3.6A and B) after upwelling. Lake Norwood chl *a* concentrations and sedimentation rates of PC were elevated for a longer duration during stratification in 2012 than in 2011 (Figure 3.6C) due to pulsed artificial-upwelling.

Hypolimnion waters in many stratified lakes contain nutrients sufficient to elicit a phytoplankton bloom (Hooper 1953, unpublished data). However, there are very few bioassay or whole-lake studies that have tested hypolimnion water as a nutrient source to increase phytoplankton biomass. Entrainment of the dense, hypolimnion water in the warm surface layer was not expected to be completely efficient. However it was expected that some of the water and nutrients would be incorporated into the warm surface layer for durations where phytoplankton could uptake the upwelled nutrients.

### 3.4.1 Conclusion

No effects of pulsed artificial-upwelling were observed on the annual time-scale, but seasonal and pulse effects were noted for several parameters. This work has shown that increases in nutrient concentration and subsequent primary production levels can be achieved for a short time when nutrient limitation is greatest and production increases are desired. These upwelling events provided desired nutrients to the biota with no long-term effects on nutrient and biomass concentrations. Lastly, these data provide support, consistent with the goal to manage multi-use lakes for mesotrophic conditions, that production can be increased without harming downstream sensitive waterbodies.

Future studies could be aimed at modeling the fate of upwelled hypolimnetic water. Also, developing methods to increase the efficiency of entraining the dense, nutrient-rich water in the warm photic zone for longer durations could be addressed. Additionally, with phytoplankton samples collected weekly in 2011, before SolarBee© installation, and in 2012, after SolarBee© installation, analysis of the epilimnion circulators' effect on cyanobacteria suppression could be evaluated. Last, collecting measurements to determine the extent of methyl mercury incorporation into the food web after upwelling could be evaluated for sake of human health.

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### 3.6 Tables

Table 3.1 Annual time-scale data from May 2011 to April 2013 is shown for Lakes Brittany and Norwood. For chl *a*:PC ratio values, chl *a* units are $\mu g\ L^{-1}$, and PC units are $mg\ L^{-1}$. Less than 80 µm chl *a* concentrations are in $\mu g\ L^{-1}$ and <80 µm PC concentrations are in $mg\ L^{-1}$. Total N:TP, seston C:P, and seston N:P values are reported as mass ratios. Transformations of the data conducted to meet assumptions for normality are indicated, but actual raw mean ± standard deviation (SD) values are reported for each lake (control/impact) and for each year (before/after).

| Parameters | Trans-formation | Annual BACI *F* value | *p* value | Control Brittany (x ± SD) Before (2011) | After (2012) | Impact Norwood (x ± SD) Before (2011) | After (2012) |
|---|---|---|---|---|---|---|---|
| TN ($mg\ L^{-1}$) | none | 2.05 | 0.1542 | 0.55 ± 0.12 | 0.49 ± 0.15 | 0.69 ± 0.14 | 0.70 ± 0.15 |
| TP ($mg\ L^{-1}$) | $\log_{10}$ | 7.54 | 0.2224 | 0.021 ± 0.008 | 0.016 ± 0.004 | 0.032 ± 0.011 | 0.029 ± 0.008 |
| TDN ($mg\ L^{-1}$) | none | 1.63 | 0.4229 | 0.418 ± 0.126 | 0.353 ± 0.141 | 0.444 ± 0.161 | 0.447 ± 0.145 |
| TDP ($mg\ L^{-1}$) | none | 3.22 | 0.0748 | 0.009 ± 0.005 | 0.006 ± 0.003 | 0.009 ± 0.006 | 0.008 ± 0.005 |
| TN:TP | $\log_{10}$ | 0.41 | 0.6359 | 28.4 ± 9.93 | 30.5 ± 7.84 | 23.7 ± 8.00 | 25.5 ± 7.10 |
| chl *a* ($\mu g\ L^{-1}$) | $\log_{10}$ | 0.19 | 0.6677 | 5.27 ± 3.07 | 7.33 ± 4.01 | 12.9 ± 8.13 | 20.3 ± 13.1 |
| PC ($mg\ L^{-1}$) | $\log_{10}$ | 0.05 | 0.8549 | 0.82 ± 0.30 | 0.76 ± 0.20 | 1.39 ± 0.44 | 1.41 ± 0.82 |
| chl *a* : PC | sqrt | 0.76 | 0.3842 | 6.47 ± 2.97 | 9.86 ± 5.21 | 9.01 ± 4.35 | 14.03 ± 5.42 |
| <80 $\mu$m chl *a* | $\log_{10}$ | 0.45 | 0.6254 | 3.92 ± 2.68 | 6.99 ± 4.05 | 12.8 ± 8.21 | 19.8 ± 12.6 |
| <80 $\mu$m PC | $\log_{10}$ | 1.18 | 0.2796 | 0.672 ± 0.282 | 0.71 ± 0.21 | 1.27 ± 0.47 | 1.31 ± 0.08 |
| Seston C:P | $\log_{10}$ | 0.41 | 0.5254 | 68.1 ± 25.2 | 77.1 ± 25.6 | 63.0 ± 21.4 | 67.1 ± 28.0 |
| Seston N:P | $\log_{10}$ | 0.89 | 0.3480 | 11.0 ± 3.2 | 13.7 ± 4.0 | 11.2 ± 3.5 | 12.3 ± 4.10 |
| Secchi (m) | none | 2.66 | 0.1052 | 3.87 ± 1.42 | 4.04 ± 1.09 | 2.30 ± 1.19 | 3.09 ± 1.29 |
| Photic (m) | $\log_{10}$ | 0.07 | 0.7967 | 7.82 ± 2.10 | 8.11 ± 1.28 | 5.31 ± 1.96 | 5.47 ± 1.73 |
| Sed N ($mg\ m^{-2}d^{-1}$) | $\log_{10}$ | 1.11 | 0.4835 | 40.3 ± 24.6 | 41.9 ± 14.8 | 44.6 ± 15.1 | 57.5 ± 23.6 |
| Sed C ($mg\ m^{-2}d^{-1}$) | $\log_{10}$ | 5.54 | 0.2557 | 279.1 ± 161.9 | 241.6 ± 104.3 | 278.2 ± 107.7 | 362.6 ± 153.5 |
| PP ($mg\ C\ m^{-3}\ h^{-1}$) | $\log_{10}$ | 0.26 | 0.6158 | 9.58 ± 6.02 | 8.66 ± 4.30 | 16.4 ± 10.1 | 15.7 ± 9.3 |
| P/R | $\log_{10}$ | 2.20 | 0.1456 | 1.38 ± 0.650 | 1.50 ± 0.441 | 1.60 ± 0.74 | 1.50 ± 0.52 |

Table 3.2 Truncated time-scale data from May 13 to August 4, 2011 and May 4 to July 26, 2012 is shown for lakes' Brittany and Norwood. For chl *a*:PC ratio values, chl *a* units are $\mu g\ L^{-1}$, and PC units are mg $L^{-1}$. Less than 80 µm chl *a* concentrations are in $\mu g\ L^{-1}$ and <80 µm PC concentrations are in mg $L^{-1}$. Total N:TP, seston C:P, and seston N:P values are reported as mass ratios. Transformations of the data conducted to meet assumptions for normality are indicated, but actual raw mean ± standard deviation (SD) values are reported for each lake (control/impact) and for each year (before/after).

| Parameters | Trans-formation | Annual BACI | | Control Brittany (x ± SD) | | Impact Norwood (x ± SD) | |
|---|---|---|---|---|---|---|---|
| | | *F* value | *p* value | Before (2011) | After (2012) | Before (2011) | After (2012) |
| TN (mg $L^{-1}$) | none | 2.05 | 0.1542 | 0.55 ± 0.12 | 0.49 ± 0.15 | 0.69 ± 0.14 | 0.70 ± 0.15 |
| TP (mg $L^{-1}$) | $\log_{10}$ | 7.54 | 0.2224 | 0.021 ± 0.008 | 0.016 ± 0.004 | 0.032 ± 0.011 | 0.029 ± 0.008 |
| TDN (mg $L^{-1}$) | none | 1.63 | 0.4229 | 0.418 ± 0.126 | 0.353 ± 0.141 | 0.444 ± 0.161 | 0.447 ± 0.145 |
| TDP (mg $L^{-1}$) | none | 3.22 | 0.0748 | 0.009 ± 0.005 | 0.006 ± 0.003 | 0.009 ± 0.006 | 0.008 ± 0.005 |
| TN:TP | $\log_{10}$ | 0.41 | 0.6359 | 28.4 ± 9.93 | 30.5 ± 7.84 | 23.7 ± 8.00 | 25.5 ± 7.10 |
| chl *a* ($\mu g\ L^{-1}$) | $\log_{10}$ | 0.19 | 0.6677 | 5.27 ± 3.07 | 7.33 ± 4.01 | 12.9 ± 8.13 | 20.3 ± 13.1 |
| PC (mg $L^{-1}$) | $\log_{10}$ | 0.05 | 0.8549 | 0.82 ± 0.30 | 0.76 ± 0.20 | 1.39 ± 0.44 | 1.41 ± 0.82 |
| chl *a* : PC | sqrt | 0.76 | 0.3842 | 6.47 ± 2.97 | 9.86 ± 5.21 | 9.01 ± 4.35 | 14.03 ± 5.42 |
| <80 µm chl *a* | $\log_{10}$ | 0.45 | 0.6254 | 3.92 ± 2.68 | 6.99 ± 4.05 | 12.8 ± 8.21 | 19.8 ± 12.6 |
| <80 µm PC | $\log_{10}$ | 1.18 | 0.2796 | 0.672 ± 0.282 | 0.71 ± 0.21 | 1.27 ± 0.47 | 1.31 ± 0.08 |
| Seston C:P | $\log_{10}$ | 0.41 | 0.5254 | 68.1 ± 25.2 | 77.1 ± 25.6 | 63.0 ± 21.4 | 67.1 ± 28.0 |
| Seston N:P | $\log_{10}$ | 0.89 | 0.3480 | 11.0 ± 3.2 | 13.7 ± 4.0 | 11.2 ± 3.5 | 12.3 ± 4.10 |
| Secchi (m) | none | 2.66 | 0.1052 | 3.87 ± 1.42 | 4.04 ± 1.09 | 2.30 ± 1.19 | 3.09 ± 1.29 |
| Photic (m) | $\log_{10}$ | 0.07 | 0.7967 | 7.82 ± 2.10 | 8.11 ± 1.28 | 5.31 ± 1.96 | 5.47 ± 1.73 |
| Sed N (mg $m^{-2}d^{-1}$) | $\log_{10}$ | 1.11 | 0.4835 | 40.3 ± 24.6 | 41.9 ± 14.8 | 44.6 ± 15.1 | 57.5 ± 23.6 |
| Sed C (mg $m^{-2}d^{-1}$) | $\log_{10}$ | 5.54 | 0.2557 | 279.1 ± 161.9 | 241.6 ± 104.3 | 278.2 ± 107.7 | 362.6 ± 153.5 |
| PP (mg C $m^{-3}\ h^{-1}$) | $\log_{10}$ | 0.26 | 0.6158 | 9.58 ± 6.02 | 8.66 ± 4.30 | 16.4 ± 10.1 | 15.7 ± 9.3 |
| P/R | $\log_{10}$ | 2.20 | 0.1456 | 1.38 ± 0.650 | 1.50 ± 0.441 | 1.60 ± 0.74 | 1.50 ± 0.52 |

Table 3.3 Pulse time-scale data for lakes' Brittany and Norwood are shown. 2011 pulse events were simulated with no actual fertilization. Values reported are the slope of the line from the 14 day pulse sampling for Lake Norwood minus (-) Lake Brittany sampled in the same manner. A single * indicates $p < 0.05$ and two** indicate $p < 0.01$. Thirteen of the total 18 water quality parameters were measured using the general linear model to analyze covariance in SAS. Photic depth, sediment trap PC, PN, estimated PP rates, and P/R ratio were not used in SAS because they were measured weekly and not on the same frequency of the other parameters and therefore are not reported here.

| **Upwelling Duration** | **0 hours** | **0 hours** | **72 hours** | **120 hours** | **408 hours** |
|---|---|---|---|---|---|
| Parameter | June 2011 | July 2011 | May 2011 | June 2012 | July 2012 |
| TN (g $L^{-1}$ $day^{-1}$) | 0.774 | 6.046 | -4.55 | -3.77 | -4.12 |
| TP (g $L^{-1}$ $day^{-1}$) | -1.08 | 0.267 | -0.19 | 0.447 | 0.469* |
| TDN (g $L^{-1}$ $day^{-1}$) | 7.452* | 6.262 | -1.55 | -1.82 | -0.75 |
| TDP (g $L^{-1}$ $day^{-1}$) | -0.82 | -0.10 | -0.26 | 0.571 | -0.11 |
| TN:TP ($day^{-1}$) | 1.70 | -0.06 | 0.83 | -1.70 | -0.88* |
| Chl *a* ($\mu$g $L^{-1}$) | -0.20 | 0.78 | 0.54** | -0.10 | 0.48 |
| PC (g $L^{-1}$ $day^{-1}$) | -44.8 | -3.46 | -20.0 | -17.3 | -16.0 |
| Chl *a* **:** PC | 0.04 | 0.62 | 1.12** | -0.09 | 0.48 |
| <80 $\mu$m Chl *a* ($\mu$g $L^{-1}$ $day^{-1}$) | 0.13 | 0.69 | 0.53** | -0.05 | 0.53 |
| <80 $\mu$m PC (g $L^{-1}$ $day^{-1}$) | -40.1 | 3.00 | -18.3 | -13.8 | -15.1 |
| Seston C:P | -4.23 | -4.92 | -1.98 | -3.43 | -5.62* |
| Seston N:P | -0.58 | -0.58 | -0.38 | -0.39 | -1.01** |
| Secchi (m $day^{-1}$) | 0.06** | -0.03 | 0.27** | -0.08 | -0.01 |

### 3.7 Figure legends

Figure 3.1 Areal depth and topography map of Lake Norwood showing the location of where SolarBee© units were deployed in 2012.

Figure 3.2 SolarBee© deployment in Lake Norwood. The two SB10000 epilimion circulators were continuously operated from February 2012 until the experiment's end, and the single SB5000 unit was turned on for each of the three (May, June, and July) pulsed artificial-upwelling events in 2012.

Figure 3.3 Lake Norwood 2012 microcosm study. Vertical bar graphs of the relationship between nutrients added and stimulated phytoplankton biomass. One-way ANOVA statistics were conducted in SAS v. 9.3 for chl *a*, PC, and chl *a*:PC. Different letters within each month's experiment indicate mean differences. Chlorophyll *a* (A) May: $F = 14.51$, $p = <0.0001$; June: $F = 107.36$, $p = <0.0001$; July: $F = 34.51$, $p = <0.0001$. PC (B) May: $F = 9.02$, $p = 0.0004$; June: $F = 45.91$, $p = <0.0001$; July: $F = 65.94$, $p = <0.0001$. Chlorophyll *a*:PC (C) May: $F = 1.68$, $p = 0.1983$; June: $F = 15.27$, $p = <0.0001$; July: $F = 21.27$, $p = <0.0001$. In the figure legend 5% H represents the treatments in which 5% hypolimnion water was added to the 2 L of whole-lake composite photic water (i.e. 100 mLs of hypolimnion water). The 5% H + N designation represents the 5% hypolimnion water addition plus a calcium nitrate $(CaNO_3)_2$ addition at an N:P of 9 assuming a hypolimnion SRP concentration of 200 µg $L^{-1}$.

Figure 3.4 Annual Lake Norwood, (impact lake) and Lake Brittany, (control lake) nutrient data from before (2011) and after (2012) the impact of pulsed artificial-upwelling. Total N (A), total

P (B), total dissolved N (C), and total dissolved P (D) data are shown. A BACIP (before-after-control-impact-paired) design was used to evaluate if annual differences occurred due to the impact of pulsed artificial-upwelling and epilimnion long distance circulation (LDC). Solid vertical lines represent the beginning of each study year. The data located between the solid and dashed vertical lines represents the truncated study period.

Figure 3.5 Annual Lake Norwood (impact lake) and Lake Brittany (control lake) biomass data from before (2011) and after (2012) the impact of pulsed artificial-upwelling. Chlorophyll *a* (A), particulate C (B), chlorophyll *a*:PC (C), and sedimentation rate of PC (D) data are shown. A BACIP (before-after-control-impact-paired) design was used to evaluate if annual differences occurred due to the impact of pulsed artificial-upwelling and epilimnion long distance circulation (LDC). Solid vertical lines represent the beginning of each study year. The data located between the solid and dashed vertical lines represents the truncated study period.

Figure 3.6 Truncated Lake Norwood (impact lake) and Lake Brittany (control lake) biomass data from before (2011) and after (2012) the impact of pulsed artificial-upwelling. Chlorophyll *a* (A), chl *a*:PC (B), sedimentation rata of PC (C), and secchi depth (D) data are shown. A BACIP (before-after-control-impact-paired) design was used to evaluate if annual differences occurred due to the impact of pulsed artificial-upwelling and epilimnion long distance circulation (LDC). Solid vertical lines represent the beginning of each study year.

Figure 3.7 Lake Brittany (A) and Norwood (B) phytoplankton community composition data for May to September 2012. Percentages of total phytoplankton biomass of the five major taxa

groups are shown. The two shaded bars indicate the June (120 hours) and July (408 hours) pulsed artificial-upwelling events. The May upwelling event occurred a week prior to the first phytoplankton sample date shown.

## 3.8 Figures

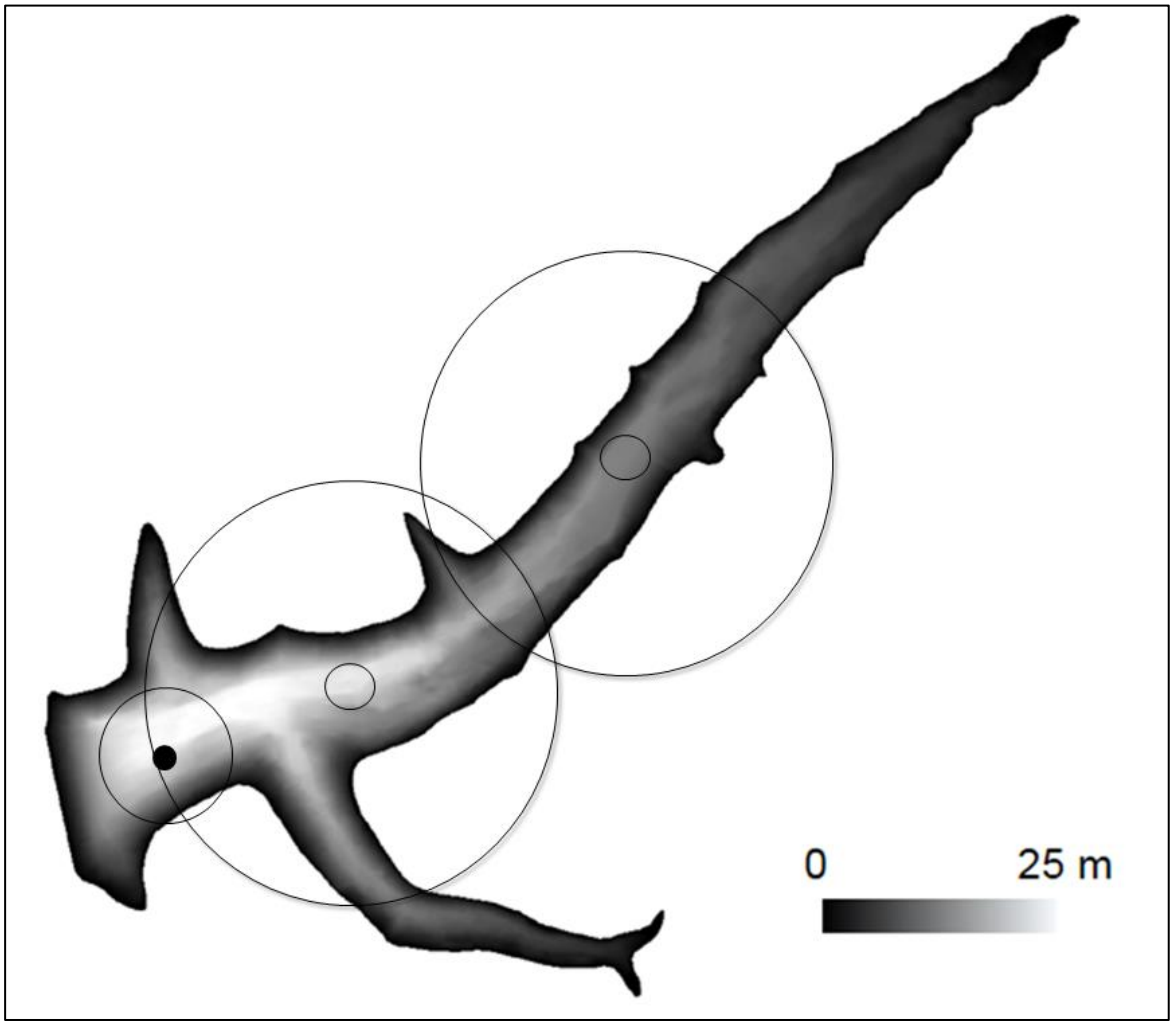



Figure 3.1

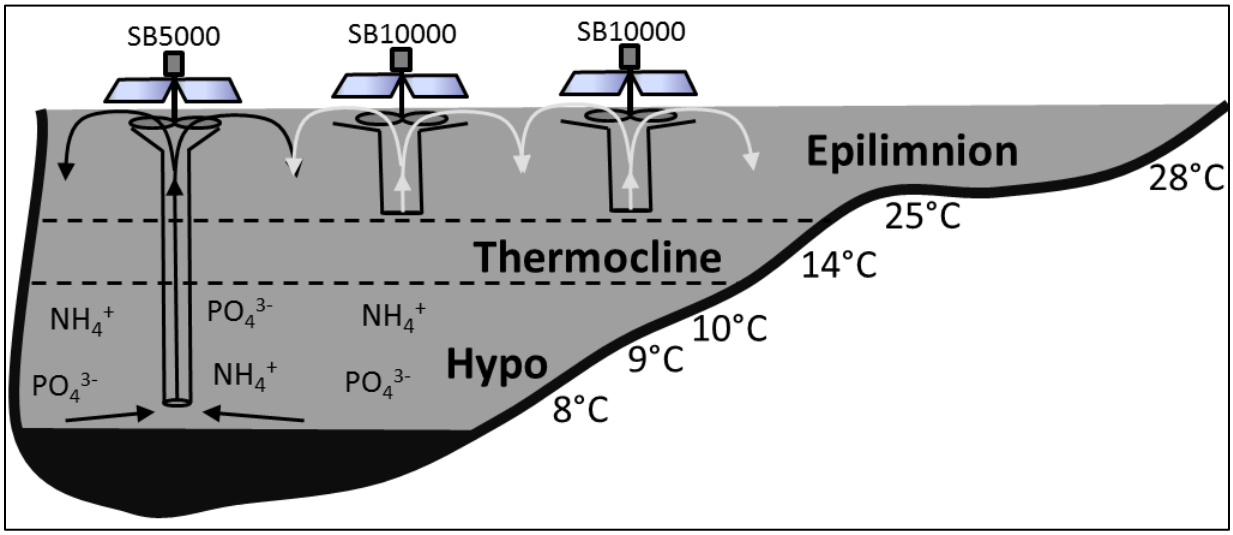

SB5000
SB10000
SB10000
Epilimnion
28°C
25°C
Thermocline
14°C
$NH_4^+$
$PO_4^{3-}$
$NH_4^+$
10°C
$PO_4^{3-}$
$NH_4^+$
$PO_4^{3-}$
Hypo
9°C
8°C

Figure 3.2

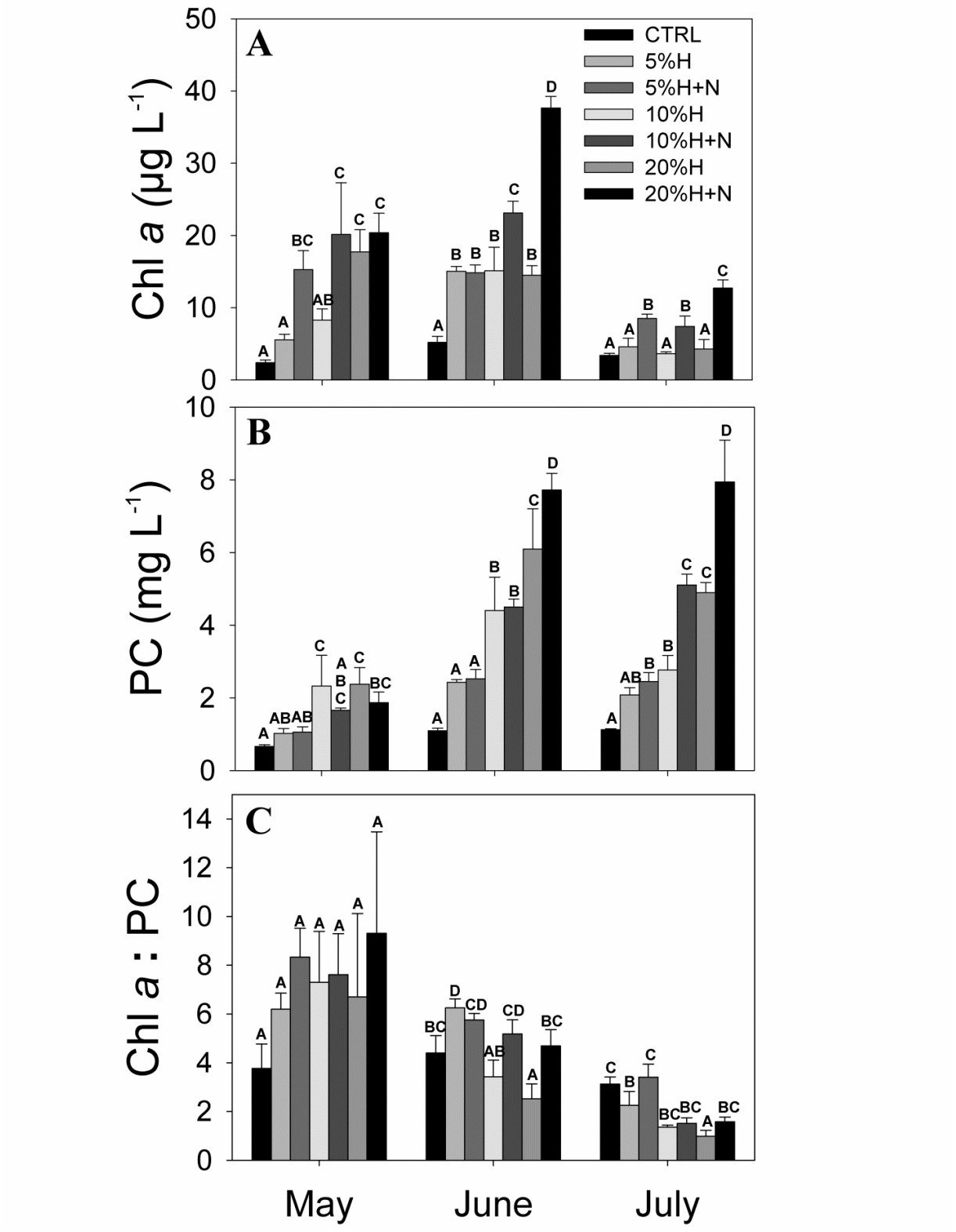

A
B
C
CTRL
5%H
5%H+N
10%H
10%H+N
20%H
20%H+N
Chl a (µg L-1)
PC (mg L-1)
Chl a : PC
May
June
July

Figure 3.3

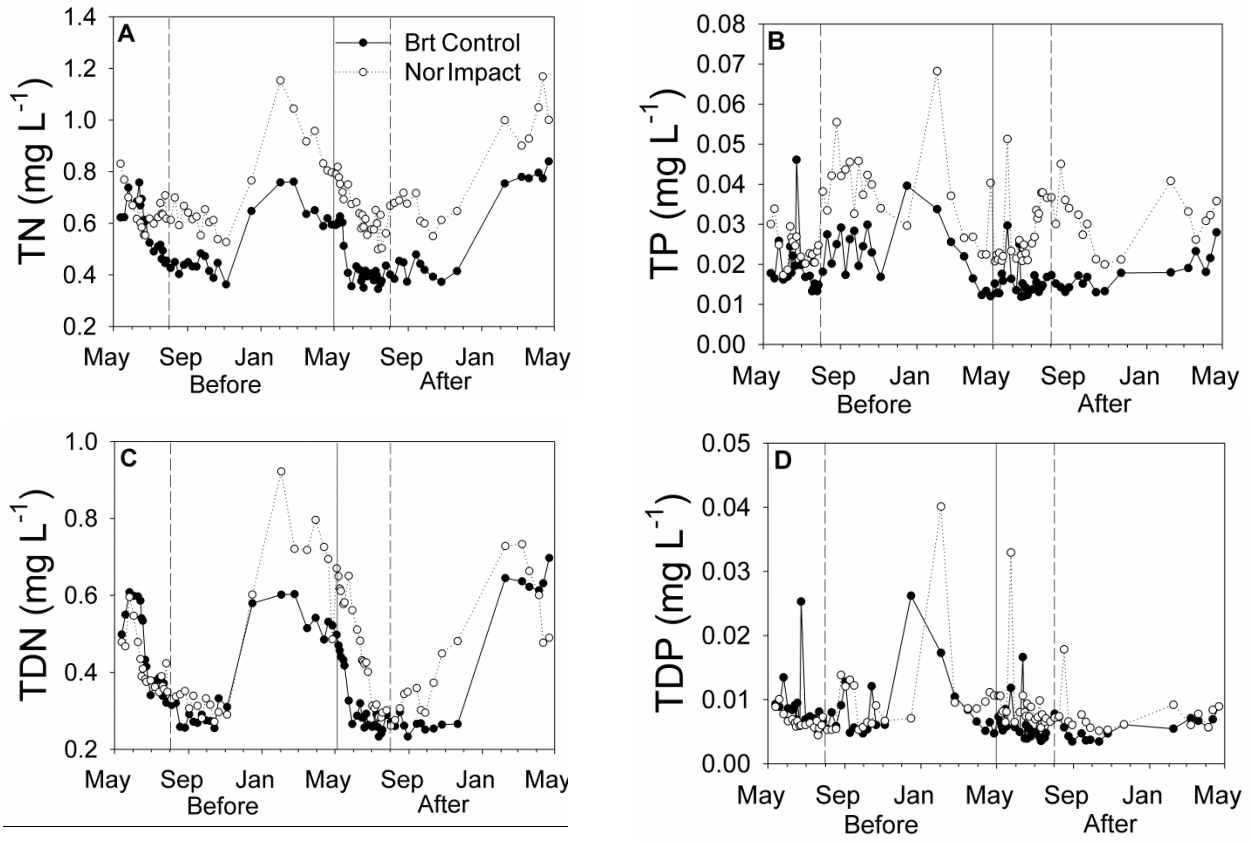

A
Brt Control
Nor Impact
TN (mg L-1)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
May Sep Jan May Sep Jan May
Before
After
B
TP (mg L-1)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
May Sep Jan May Sep Jan May
Before
After
C
TDN (mg L-1)
1.0
0.8
0.6
0.4
0.2
May Sep Jan May Sep Jan May
Before
After
D
TDP (mg L-1)
0.05
0.04
0.03
0.02
0.01
0.00
May Sep Jan May Sep Jan May
Before
After

Figure 3.4

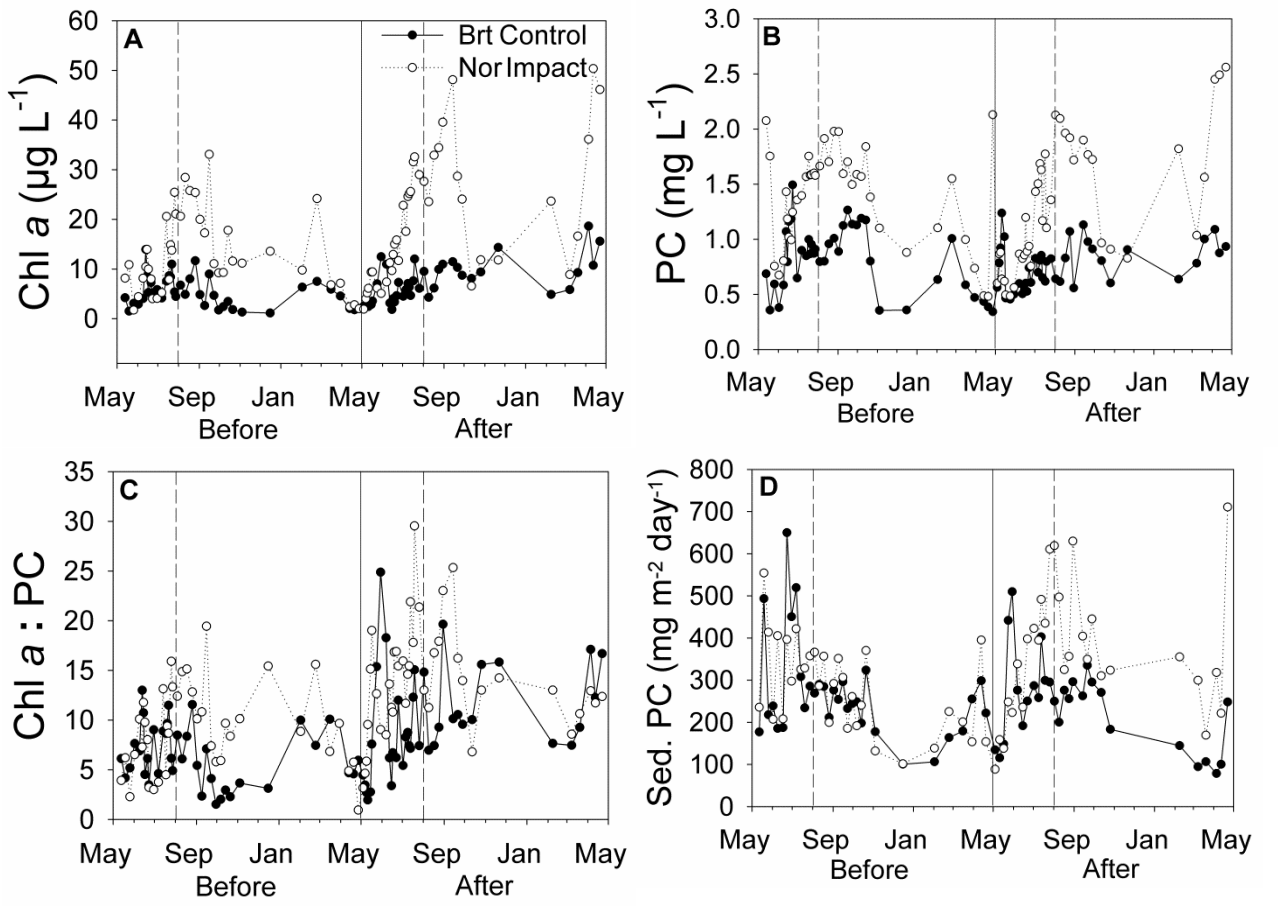

A
Brt Control
Nor Impact
Chl a (µg L-1)
B
PC (mg L-1)
C
Chl a : PC
D
Sed. PC (mg m-2 day-1)
May Sep Jan May Sep Jan May
Before
After

Figure 3.5

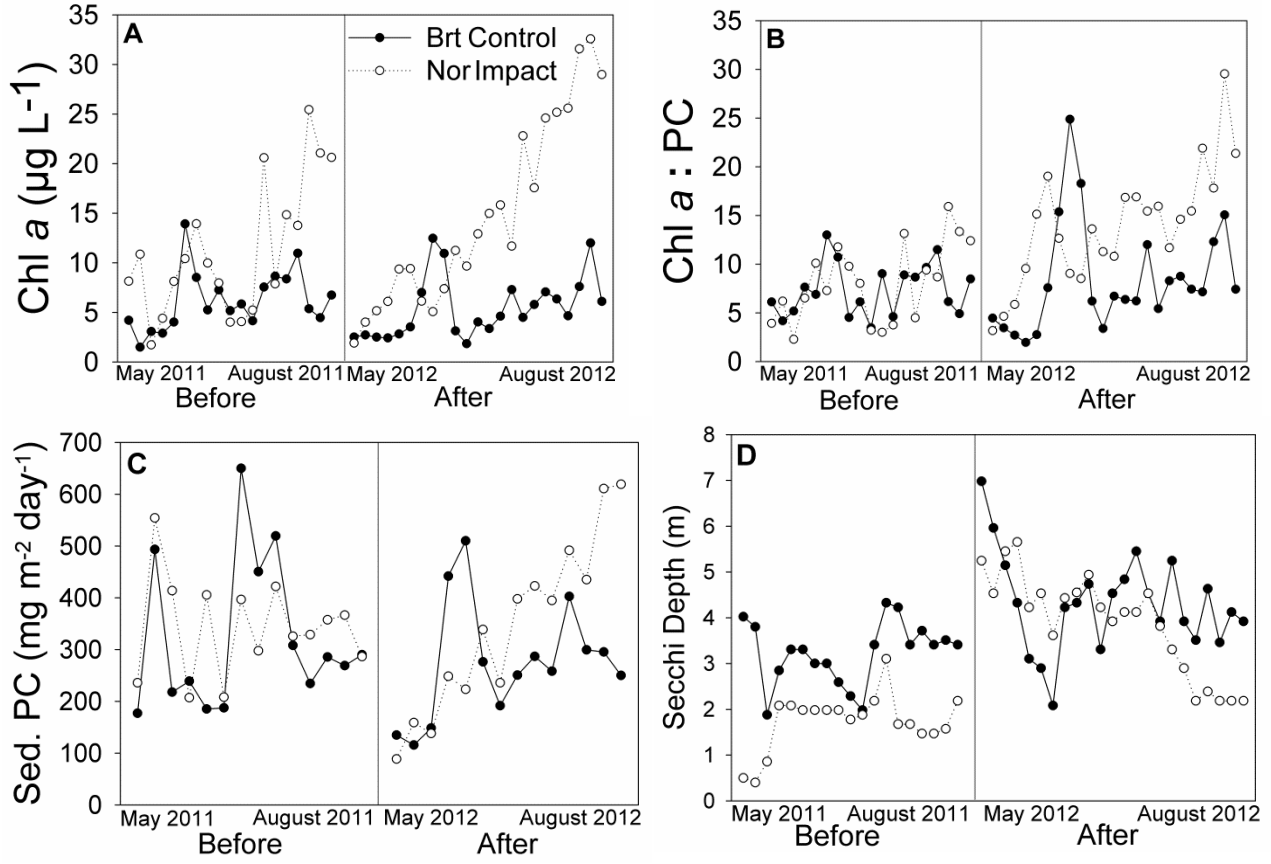
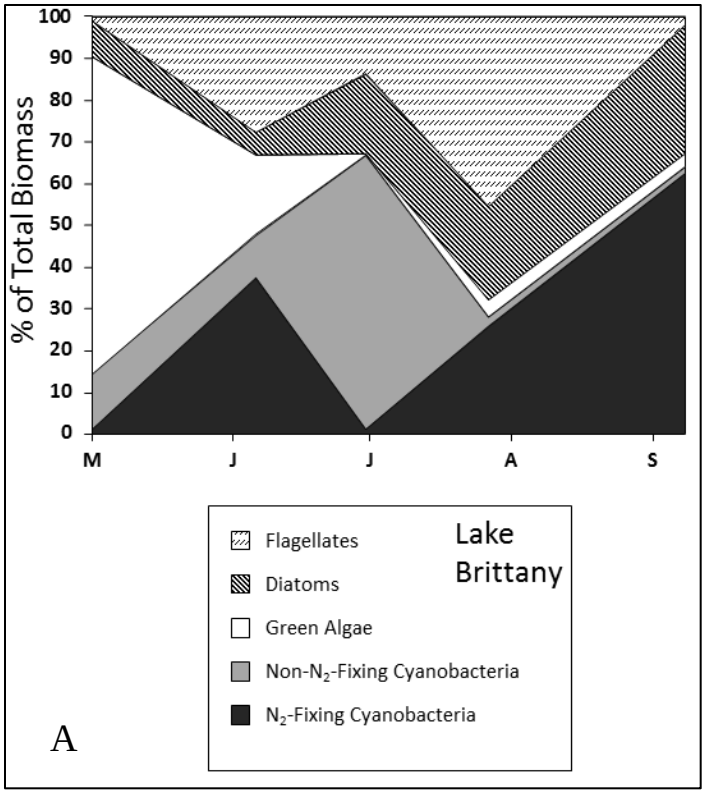

A
Brt Control
Nor Impact
Chl a (µg L-1)
May 2011
August 2011
May 2012
August 2012
Before
After
B
Chl a : PC
C
Sed. PC (mg m-2 day-1)
D
Secchi Depth (m)

Figure 3.6


% of Total Biomass
100
90
80
70
60
50
40
30
20
10
0
M
J
J
A
S
Flagellates
Diatoms
Green Algae
Non-N2-Fixing Cyanobacteria
N2-Fixing Cyanobacteria
Lake Brittany
A

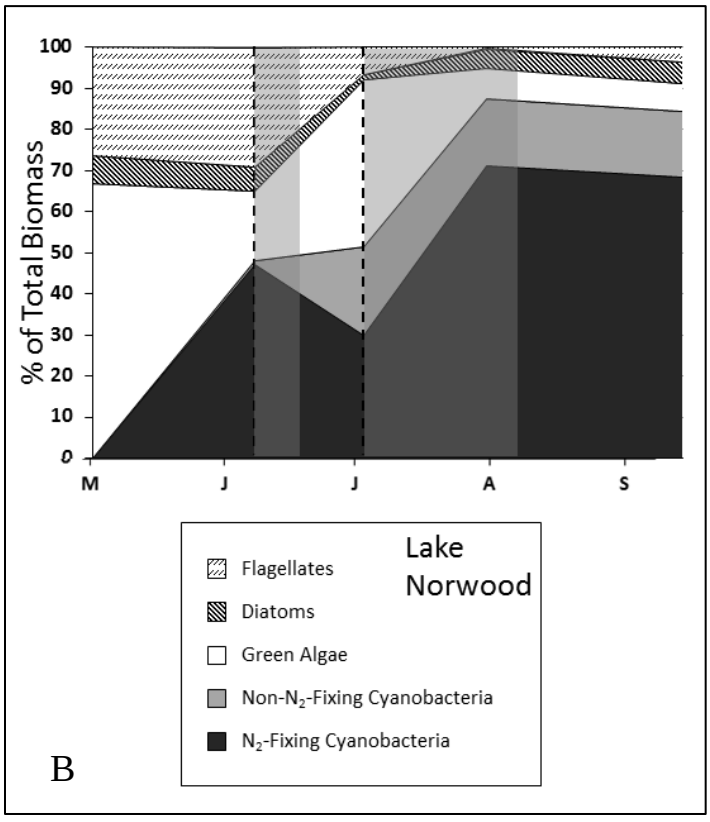

% of Total Biomass
100
90
80
70
60
50
40
30
20
10
0
M
J
J
A
S
Flagellates
Diatoms
Green Algae
Non-N2-Fixing Cyanobacteria
N2-Fixing Cyanobacteria
Lake Norwood
B

Figure 3.7

## 4. Overall Conclusion

The objectives of this study were to explore the effects of N and P additions on primary production using both microcosm and whole-lake fertilization experiments. Specifically, this study: 1) assessed the effects of chemical fertilizer additions on whole-lake productivity in Lake Rayburn, 2) evaluated the effects of pulsed artificial-upwelling on whole-lake productivity in Lake Norwood, 3) characterized nutrient limitation and how limitation varied seasonally in Lake Brittany, 4) determined via microcosms how much nutrient concentrations must be increased to enhance phytoplankton bloom formation, and 5) determined if nutrient pulses could increase production on the short-term with negligible effects on interannual lake water quality conditions as well as and downstream waterbodies. The data collected to achieve these goals included whole-lake nutrient and biomass data as well as microcosm biomass data.

Numerous studies have demonstrated the response of freshwater lakes to nutrient enrichment in both laboratory and field bioassays as well as in whole-lake experiments. However, few studies have included experiments conducted similarly to those in this study. Very few studies used small-scale experiments prior to whole-ecosystem manipulations to provide some expected outcome on the whole-ecosystem scale (Table 4.1). Additionally, the use of hypolimnion water as a nutrient source for increasing phytoplankton production is unique to this study, with the exception of Hooper (1953).

Both chemical fertilization and pulsed artificial-upwelling increased nutrient concentrations and phytoplankton biomass, to various degrees, on the seasonal time-scale. Further, there were negligible interannual differences which indicated that the nutrient addition effects were muted on the annual time scale (Figure 4.1). Specifically, chemical fertilization in Lake Rayburn increased phytoplankton biomass (measured as chl *a*) during the summer of 2012

on a short-term basis, but only moderately effected TP concentrations and had no effect on other nutrient concentrations on an annual timescale. This suggests that added nutrients were effectively incorporated into biomass. The phytoplankton community composition data suggested that fertilization not only increased phytoplankton biomass but decreased N-fixing cyanobacteria.

In Lake Norwood, pulsed artificial-upwelling events during the summer of 2012 increased nutrient concentrations (measured as TP, TDP, and TDN) and phytoplankton biomass (measured as chl *a*) on a short-term basis, with no interannual differences measured. Pulsed artificial-upwelling was selected over continuous upwelling because it was preferred for stratification and epilimnion water temperatures to remain unchanged. The epilimnion long distance circulators (LDC) proved somewhat effective at increasing springtime water transparency compared to previous years with no SolarBee© influence (Hudnell 2010), but our study did not test this effect directly.

Lake Brittany exhibited springtime P limitation and summer N limitation. Treatment lakes (Rayburn and Norwood) were augmented with nutrients and reduced this seasonal pattern in P to N limitation. Nutrient limitation existed in the lakes due to low nutrient inputs from their forested watersheds. The streams flowing into the lakes were ephemeral, further exacerbating nutrient limitation. In the past, augmentation with chemical fertilizers was used to raise biological productivity of the lakes in these unproductive watersheds. The amount of nutrients applied to these lakes from 1994 to 2002 resulted in eutrophic conditions, but in 2003 chemical fertilization was halted, and from 2003 to 2011 no nutrient additions were applied to the three lakes (Figure 4.2 A and B). The fertilization rates used in the 1990s and early 2000s likely saturated phytoplankton nutrient demand and caused eutrophic conditions, and the relative

amount of N and P in the fertilizer was imbalanced so that N-fixing cyanobacterial blooms were favored. Fertilization rates used in this study were much lower, and an N:P ratio was used to better match the demand of diverse a phytoplankton community (Smith 1979, Guildford and Hecky 2000, Dzialowski et al. 2005). As a result, this study suggested that desired short-term changes on water quality could be produced without undesirable interannual changes in-lake or deleterious effects on downstream waterbodies (Figure 4.1).

Neither eutrophic nor oligotrophic conditions are desirable in most multi-use lakes. In these multi-use lakes where fishing and contact recreation occur, a balance is needed between low nutrient concentrations to avoid undesirable algal production and high concentrations to sustain good fishing (Ney 1996). Mesotrophic conditions are often the target 'middle ground' for many managers (Stockner et al. 2000). The results from this study are the first step in addressing how to increase fish production in these multi-use lakes (Anders and Ashley 2007). Although our study did not include data on fish biomass or production, many previous studies have demonstrated the link between nutrients and fish (Jones and Hoyer 1982, Ney et al. 1990, Vaux et al. 1995, Maceina et al. 1996, Buyank et al. 2001, Perrin et al. 2006, Boyd et al. 2008). We presume that any increase in phytoplankton production will have a positive impact on the fish community. More data is needed to quantify this link directly, but this study provides a firm foundation for managing nutrients in a useful and sustainable way for multi-use reservoirs.

## 4.1 References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## 4.2 Tables

Table 4.1 Nutrient addition schedule, type, and amount (areal rates) of nutrients applied in the upper mixed layer to both Lakes Rayburn and Norwood in 2012.

| Lake | Month | Fertilizer Type (N-P-K) | Quantity N (kg) | Quantity P (kg) | N Application Rate (kg $ha^{-1}$) | P Application Rate (kg $ha^{-1}$) | N:P mass ratio |
|---|---|---|---|---|---|---|---|
| Rayburn | May | 15-0-0 | 51.0 | 0.00 | 2.91 | 0.28 | 10.4 |
| | | 10-52-4 | 2.27 | 5.14 | | | |
| Rayburn | June | 15-0-0 | 73.1 | 0.00 | 4.18 | 0.42 | 9.92 |
| | | 10-52-4 | 3.40 | 7.71 | | | |
| Rayburn | July | 15-0-0 | 42.5 | 0.00 | 2.51 | 0.23 | 11.1 |
| | | 15-42-4 | 3.40 | 4.15 | | | |
| Norwood | May | Artificial Upwelling | 14.9 | 1.15 | 1.14 | 0.09 | 13.0 |
| Norwood | June | Artificial Upwelling | 24.8 | 2.29 | 1.90 | 0.17 | 10.8 |
| Norwood | July | Artificial Upwelling | 110.3 | 25.9 | 8.46 | 1.99 | 4.25 |

## 4.3 Figure legends

Figure 4.1 Time-scale relationship between sensitivity of measuring statistical differences from the three impact pulses applied to each impact lake.

Figure 4.2 A and B Historic annual fertilization rates of N and P applied to Lake Rayburn (Figure 4.1 A) and Norwood (Figure 4.1 B) during 1994-2002. The high amounts of N and P were applied at a low N:P ratio ranging from 0.9-2.0 in Lake Rayburn and 0.9-2.7 in Lake Norwood. No fertilization has taken place since the end of 2002. In 2012, three chemical fertilization events occurred in Lake Rayburn and the mean N:P of the added nutrients was 10.3. In 2012, pulsed artificial-upwelling increased the rate on nutrient cycling in Lake Norwood. The mean N:P of upwelled hypolimnetic water in 2012 was 6.6.

## 4.4 Figures

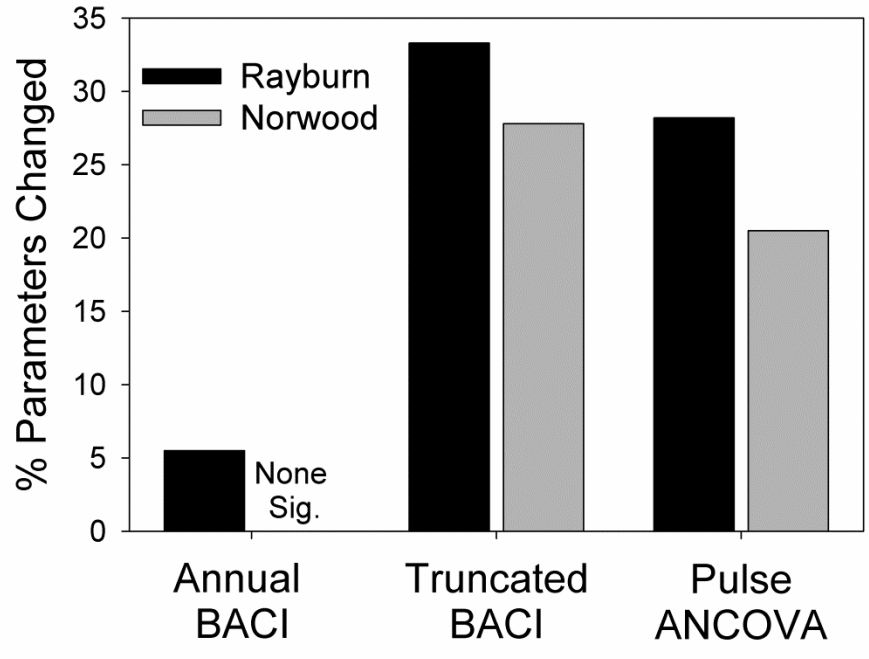
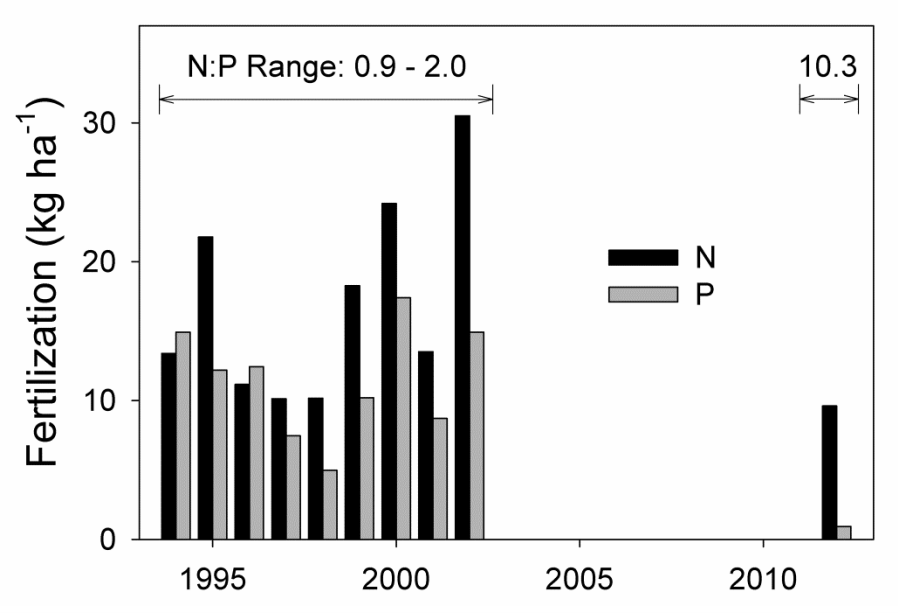
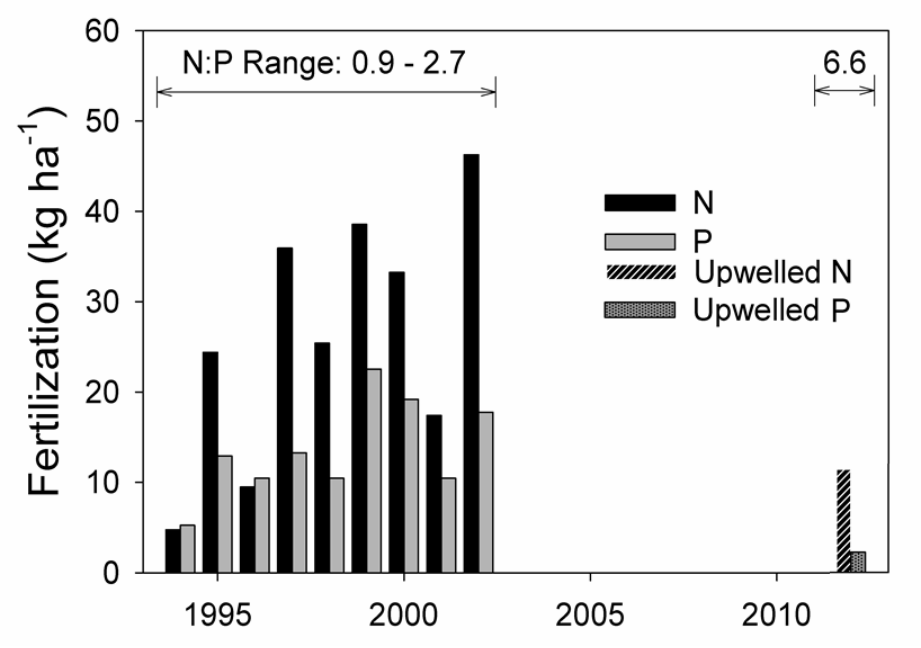



Figure 4.1




**A**




**B**

Figure 4.2 A and B