# UNC Nutrient Management Study - In Situ Observational Study of Jordan Lake
## Final Report: 7/1/2017 – 6/30/2019

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## Background and Objectives

Jordan Lake receives water input from the Haw River, Upper New Hope and Lower New Hope watersheds. Associated with these water inputs are nutrients, sediments and, in some cases, significant debris. The Haw River watershed is mixed agricultural, rural and urban while the Upper and Lower New Hope watersheds are principally urban. The primary outflow from the lake occurs over the Jordan Lake Dam and comprises the starting point of the Cape Fear River. The Haw River drains the Haw River watershed and discharges into the southern, Haw River arm of Jordan Lake approximately 5 miles upstream of the Jordan Lake Dam. The Haw River provides 70 – 90 percent of the annual flow into the lake. The Upper and Lower New Hope watersheds drain into the New Hope Creek arm of Jordan Lake which extends approximately 17 miles upstream from the Dam. The Haw River arm and the New Hope Creek arm are naturally separated by a narrow channel referred to as the "s-bends" or "narrows". The New Hope Creek arm is further subdivided by two causeways with relatively narrow bridge openings, one where NC Highway 64 crosses the lake and the other where Farrington Road crosses the lake.

Jordan Lake has been a highly productive reservoir since its creation, and the upper New Hope arm of the lake above the Highway 64 causeway consistently violates NC standards for chlorophyll a (>40 $\mu g\ L^{-1}$). High phytoplankton biomass levels threaten the quality of lake water for use by the Town of Cary, and may also negatively impact aquatic life (e.g. due to hypoxia, poor water clarity etc.).

As part of the UNC Nutrient Management Study, we initiated a multi-part observational and experimental program in January, 2017 to help clarify the impacts of watershed input on key processes controlling water quality in Jordan Lake and to help inform management actions designed to improve water quality in the lake. This observational and experimental program was continued during project year 2 (July 2017 to June 2018) with three specific objectives

1.) to identify water circulation and exchanges in the lake, in particular, the extent to which the large volume of nutrient and sediment laden Haw River water affects water quality of the New Hope Creek arm of the lake;

2.) to better quantify the response of important water quality parameters in the lake to a range of forcing conditions (variations in flow, seasonal variations of temperature and light, etc)

via high frequency (e.g., hourly) in situ observations to complement the less frequent (e.g., monthly) sampling done by the NC Division of Environmental Quality; and

3.) to better quantify phytoplankton dynamics, including effects of nutrient and light limitation on the production of high phytoplankton biomass levels that are causing the lake to be out of compliance with state water quality standards.

The Paerl laboratory focused on objective number three and utilized a series of bioassay experiments, field measurements, and laboratory analysis on Jordan Lake water to:

- determine the limiting nutrient/s (N, P, or N and P) for phytoplankton growth
- determine the level of nutrient reductions necessary for reducing algal biomass in the lake
- determine the significance of light limitation in constraining algal growth by directly measuring the photosynthesis versus irradiance relationship
  provide laboratory and field measurements in support of future water quality modeling work

## Methods:

A total of seven nutrient addition/ nutrient dilution bioassay experiments have been conducted during project years 1 and 2. Six of the seven experiments were conducted on water collected near NCDEQ station CPF086F on the upper New Hope R. arm. In May 2018, an experiment was conducted on water from station CPF055C on the Haw R. arm of the lake.

Water for the bioassays was collected using a diaphragm pump into ten - 20L polyethylene carboys. The carboys were quickly transferred to a darkened truck bed and transported to UNC-IMS. Upon arrival (approximately 14:30) the carboys were placed in the outdoor incubation ponds to maintain ambient temperature and light conditions. The following morning water from the carboys was homogenized in a 300L fiberglass tub prior to dispersing into 4L Cubitainers ® and performing experimental nutrient addition and dilution treatments.

Nutrient additions consisted of a full factorial design of N and P additions, including a control with no nutrients added, an N treatment (0.63 mg $L^{-1}$ $N\text{-}NO_3^-$ plus 0.07 mg $L^{-1}$ $N\text{-}NH_4^+$), a P treatment (0.155 mg $L^{-1}$ $P\text{-}PO_4^{-3}$) and a N plus P treatment (0.63 mg $L^{-1}$ $N\text{-}NO_3^-$ plus 0.07 mg $L^{-1}$ $N\text{-}NH_4^+$ plus 0.155 mg $L^{-1}$ $P\text{-}PO_4^{-3}$). For the first six experiments, addition treatments were made to whole lake water as well as to lake water diluted by 10, 30, and 50 percent with a major ion solution that contained all the major salts of Jordan Lake water less N and P. The last experiment in April 2019 contained no dilution treatments.

Total phytoplankton biomass and biomass of the dominant phytoplankton classes, and nutrient concentrations were measured on days 0, 1, 3, and 6. Total phytoplankton biomass was fluorometrically measured as chlorophyll *a* was measured (Peierls et al. 2012), and class-specific

accessory pigments were measured by high pressure liquid chromatography (HPLC) (Pinckney et al. 1998). Nutrient concentrations (nitrate+nitrite, ammonium, total dissolved nitrogen, phosphate, silicate, total P, and total N) were measured colorimetrically (Peierls et al. 2012). During the last three bioassay experiments, rates of nitrogen fixation were measured via acetylene reduction according to Piehler et al. (2002) and assuming an acetylene to $N_2$ reduction ratio of 4:1.

Phytoplankton growth in the nutrient addition and dilution bioassays was tracked by measurements of chlorophyll *a* (Chl a) and accessory photopigments on day 0, day 1, day 3, and day 6 of the experiment. Chlorophyll *a* (Chl *a*), representing total phytoplankton biomass was measured fluorometrically (Peierls et al. 2012), and accessory photopigment data representative of the dominant phytoplankton classes were measured by HPLC. Phytoplankton growth rates (μ in units $d^{-1}$) at each time point during the experiment were calculated as:

Equation 1. $$\mu = ln(C_t/C_0)/t$$

where *ln* is the natural logarithm, t is the length of time elapsed since the beginning of the experiment, $C_t$ is the *Chl a* concentration at time t, and $C_0$ is the initial Chl *a* concentration. Using growth rates rather than biomass allows determination of the phytoplankton growth response that is not impacted by the reduction of biomass in the dilution treatments. Growth expressed in this manner is also consistent with the phytoplankton growth rates in many eutrophication models and therefore will facilitate incorporation of experimental results into such models. At each time point in the experiment, the effects of treatments on phytoplankton growth rates were analyzed using a three way ANOVA with dilution treated as a continuous variable and the addition or omission of N or P treated as categorical variables.


Figure 1. Photosynthetron set up for measuring the light vs. photosynthesis relationship. Note light increases from left to right within rows and also varies within columns.

Water samples collected for each bioassay and water collected opportunistically by Dr. Richard Luettich's lab (UNC-CH IMS ) during excursions to maintain AVP and ADCP equipment were used to measure the relationship between light availability and phytoplankton photosynthesis. Immediately upon delivery of lake water to UNC-CH IMS (~ 4.5 hours after collection), aliquots of water were dispensed into 20 mL borosilicate glass incubation vials. Photosynthesis was measured by $^{14}CO_2$ incorporation at 42 different light levels that span the range of light levels known to limit phytoplankton photosynthesis. The light gradient was produced using two photosynthetrons (Lewis and Smith 1987) which consist of a white light

source, and a range of light reducing filters to produce 21 light levels per photosynthetron, and an aluminum heat sink that surrounds each vial to control temperature (Figure 1). Light delivery to water samples within each vial was measured using a Biospherical Instruments Model QSL-100 irradiance meter with a QSL-101 4 π sensor. Water was circulated through the heat sink to a temperature-controlled water bath to maintain the water temperature present at the time of collection (8-31 °C). Samples were incubated in the photosynthetron for 1 hour and photosynthesis was determined by the amount of $^{14}CO_2$ incorporated according to standard methods. Photosynthesis was normalized by Chl *a* to express observed productivity in units of carbon produced per unit of phytoplankton biomass. Photosynthesis and light measurements were fit to a hyperbolic tangeant model (Equation 2) developed by Jassby and Platt (1976)

$$P^b = P^b_{max} \tanh(\alpha I / P^b_{max}) \qquad \text{Eq. 2}$$

where $P^b$ is the biomass normalized photosynthetic rate, $P^b_{max}$ is the light-saturated photosynthetic rate, α is the initial light limited slope, and I is photosynthetically active radiation (PAR) in units µmol photons $m^{-2}$ $s^{-1}$.

On 15 May 2019, an experiment was conducted to test the influence of nitrogen limitation on the relationship bewteen photosynthesis and irradiance. A surface water sample from Farrington was sampled at ~12:00 EST on 15 May 2019 and returned to IMS at ~16:30 EST. The sample was split into two 1 L cubitainers that are 80% transparent to PAR. To one cubitainer, ammonium and nitrate were added to a final concentration of 20 µmol/L each, for a total of 40 mmol dissolved inorganic nitrogen addition. The other cubitainer served as a control. The cubitainers were then placed in the IMS pond under three layers of neutral density screening and were retrieved at 12:00 EST the next day (16 May 2019) to measure the relationship between photosynthesis and irradiance. Subsamples from each treatment were incubated simultaneously using one photosynthetron (21 light levels) for each treatment. Bootstrapping with replacement was used to generate 95% confidence intervals around the $P^b_{max}$ and α values and non-overlapping confidence intervals indicate a low likelihood that observed differences were by chance.

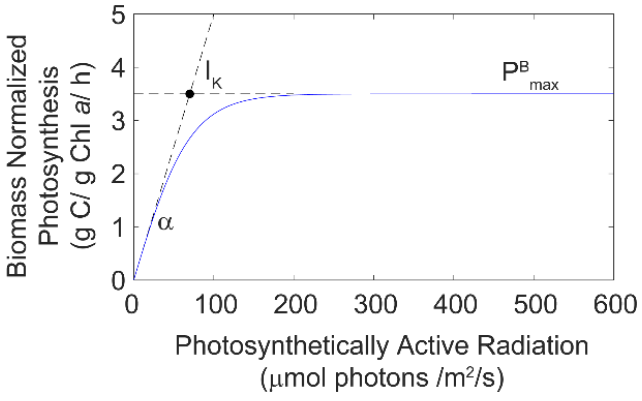


*Figure 2. Graphical description of an empirical model formulation of the relationship between photosynthetically active radiation and biomass normalized photosynthetic rate.*

Depth profiles of important water quality parameters were collected at the time and location of the bioassay water samples and during servicing of the *in situ* moorings. In each case, temperature, conductivity, pH, dissolved oxygen, in vivo fluorescence, and PAR were measured at 0.5 m depth intervals. Discrete water samples were also collected at 1 m depth intervals during the bioassay

sampling and at surface, mid-depth and near bottom during the servicing trips. Upon return to IMS, these were analyzed in the laboratory for dissolved nutrients and chlorophyll *a*.

**Nutrient budgets:** Daily average loads of total N and total P for the major tributaries to Jordan Lake and the Haw River outflow were calculated using the weighted regressions on time, discharge, and season (WRTDS) model (Hirsch et al. 2010) on USGS gaged discharge and monthly concentration data collected by NC DEQ's Ambient Monitoring System. Table 1. gives site information for gages and DEQ's monitoring program sites. For each station, the full record of flow and nutrient data of at least 15 years of data were used to fit the WRTDS model but only the period 2011 – 2016 was used to construct the nutrient budget. Daily data from this period were averaged to generate daily load estimates of N and P into and out of Jordan Lake. Loads from ungaged tributaries (~15% of watershed area) were calculated by assuming that flow and nutrient loading per watershed area were identical to the Morgan Creek watershed (TetraTech 2002). Atmospheric deposition of N was estimated based on 2016 annual averages of nitrate and ammonium from National Atmospheric Deposition Program site NC41. Atmospheric P deposition was not measured but was assumed negligible. The nutrient budgets were used to calculate total maximum daily loads of N and P to establish acceptable water quality conditions according to (Havens and Schelske 2001), and described in detail with the results below.

**Table 1. List of water quality, flow gage, and atmospheric deposition stations used in constructing a nutrient mass balance for Jordan Lake for the period 2011-2016.**

| Water Body | Parameter Type | Station ID | Site Description | Latitude | Longitude |
|---|---|---|---|---|---|
| Lake | Water quality | CPF086C | Morgan Cr. | 35.82 | -79.00 |
| | Water quality | CPF086F | above Farrington Rd. | 35.79 | -79.01 |
| | Water quality | CPF081A1C | New Hope Cr. | 35.82 | -78.99 |
| | Water quality | CPF087B | below Farrington Rd. | 35.79 | -79.02 |
| | Water quality | CPF087D | above HWY 64 | 35.74 | -79.02 |
| | Water quality | CPF0880A | above narrows | 35.71 | -79.03 |
| | Water quality | CPF055C | Haw R. arm | 35.69 | -79.08 |
| | Water quality | CPF055D | Haw R. arm | 35.67 | -79.08 |
| | Water quality | CPF055E | near dam | 35.66 | -79.07 |
| Tributaries | Water quality | B405 | Haw River outflow | 35.65 | -79.07 |
| | Water quality | B210 | Haw River inflow | 35.77 | -79.14 |
| | Water quality | B367 | B367 Northeast Cr. | 35.86 | -78.94 |
| | Water quality | B304 | B304 New Hope Cr. | 35.88 | -78.97 |
| | Water quality | B390 | B390 Morgan Cr. | 35.86 | -79.01 |
| | Gaged flow | USGS 2098197 | Haw River outflow | 35.65 | -79.07 |
| | Gaged flow | USGS 2096960 | Haw River inflow | 35.77 | -79.14 |
| | Gaged flow | USGS 2097314 | New Hope Cr. | 35.88 | -78.97 |
| Atmospheric | N deposition | NADP NC41 | Finley Farm | 35.73 | -78.68 |

## Results:

**Nutrient limitation:** The initial growth rate response from the first to the second day of the experiments is likely most representative of the in situ nutrient limitation status of the phytoplankton community in Jordan Lake (Figure 3).

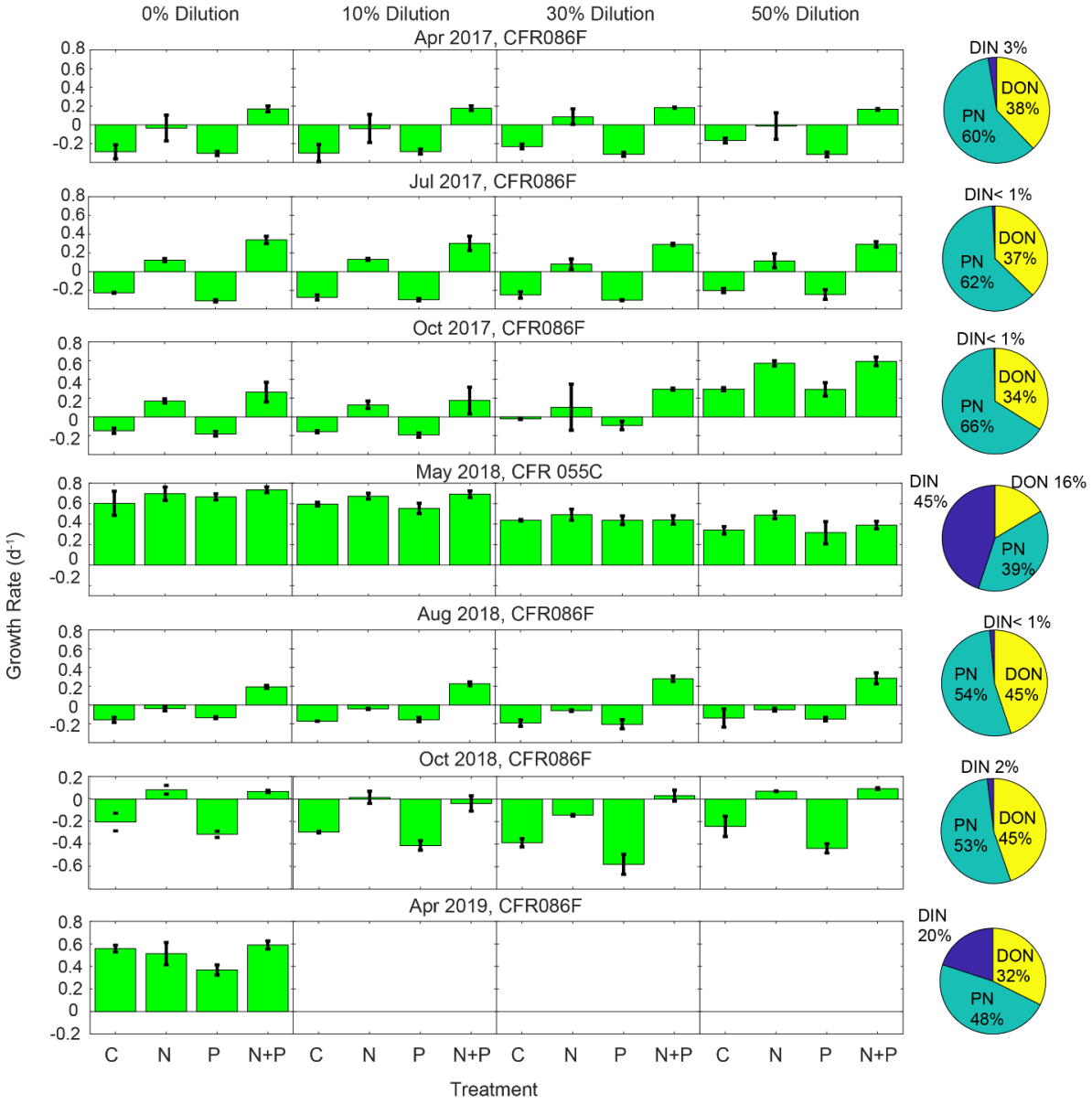


*Figure 3. Figure 3. Initial phytoplankton growth responses from day one to day two of seven bioassay experiments with nutrient addition and dilution treatments in seven bioassays. C = control. N = N addition, P = P addition, N + P = both N and P addition. Figure columns represent the different dilution levels from whole water (0%) to 50% dilution with a major ion solution. Bars and error bars are means and standard deviations of triplicate values for each treatment. Pie graphs to the right indicate the composition of the N pool at the beginning of each experiment.*

The initial growth response of five of the seven nutrient addition bioassays clearly indicated that N was the primary nutrient limiting phytoplankton growth during the spring through fall in Jordan Lake. In the absence of N addition, growth was negative during five of the seven experiments. Addition of nitrogen prevented biomass loss but did not allow biomass to increase without also adding P, indicating that N and P were close to "co-limiting". With the exception of the May 2018 and April 2019 experiments, in treatments with only P additions, biomass declined at a similar rate to the controls that received no nutrient additions. During the May 2018 and April 2019 experiments, nutrients were replete and phytoplankton growth was strong in all treatments including the controls.

In five of the six experiments that included nutrient dilutions, diluting the nutrient pools had either no significant effect or even a stimulatory effect on phytoplankton growth. The lack of a significant negative response to nutrient dilution during the 2017 experiments was likely because at the time and location in the lake during those experiments the vast majority of nutrients were contained within the phytoplankton or as recalcitrant dissolved organic forms rather than in bioavailable dissolved forms within the water. Nutrient concentrations measured at the beginning of the 2017 experiments confirmed that dissolved inorganic nitrogen (DIN) levels were low (~0.1 mg/L) and phosphate concentrations were below the limits of detection. Nearly all (97-99 %) nitrogen was either in the particulate pool, likely as phytoplankton, or in the dissolved organic N pool with only 1-3% of nitrogen as bioavailable dissolved inorganic nitrogen. Such low inorganic nutrient but high phytoplankton biomass conditions are typical of long residence time water bodies with high nutrient loads such as the upper New Hope River arm of Jordan Lake (Swaney et al. 2008).

The primary source of nutrients sustaining phytoplankton growth was likely remineralization of phytoplankton derived organic matter, which can maintain constant biomass levels over time, but cannot lead to an increase in biomass without a corresponding decrease in cell quota (Sunda and Shertzer 2012). Despite very low initial phosphate concentrations, it appeared that the supply of recycled N exerted a primary control on phytoplankton growth. The ability to maintain high growth under low phosphate conditions results from the ability of phytoplankton to strongly modulate their internal stores of P in response to decreases in availability (Flynn 2010), and due to the relatively faster rates of cycling of P compared to N (Clark et al. 1998; Monteiro and Follows 2012). In several of the experiments but particularly noticeable in October 2017, dilutions actually increased phytoplankton growth rates. This is likely because diluting out the nutrient pools also dilutes out the grazer populations and releases grazing pressure proportionally to the dilution factor (Landry and Hasset 1982). In the April 2017 and August 2018, N additions alone do not produce a positive growth response without additionally adding P. This indicates that the phytoplankton growth requirements for N and P were very close to being balanced (i.e., N and P co-limitation), and that by adding N, the phytoplankton were forced to P limitation. This is consistent with the strong positive growth achieved in the N plus P treatments and is common

when the biomass of a phytoplankton community is fueled by nutrient cycling mediated by grazing (Flynn 2010).

In contrast, during the May 2018 and April 2019 experiments appreciable fractions, ~50 and 20%, of the N pool were in the form of bioavailable, dissolved inorganic N (Figure 3). These nutrient conditions are typical during periods of high spring runoff as occurred prior to both experiments. Growth rates were strongly positive regardless of whether or not nutrients were added, and it was the only experiment where growth was negatively impacted by diluting the ambient nutrient pool. Diluting the ambient nutrients by 50% resulted in an approximate 40% decrease in growth within the control treatments where no nutrients were added. Nutrient limitation imposed by the 50 % dilution was partially alleviated by N addition which indicates again that N was the primary growth-limiting nutrient.

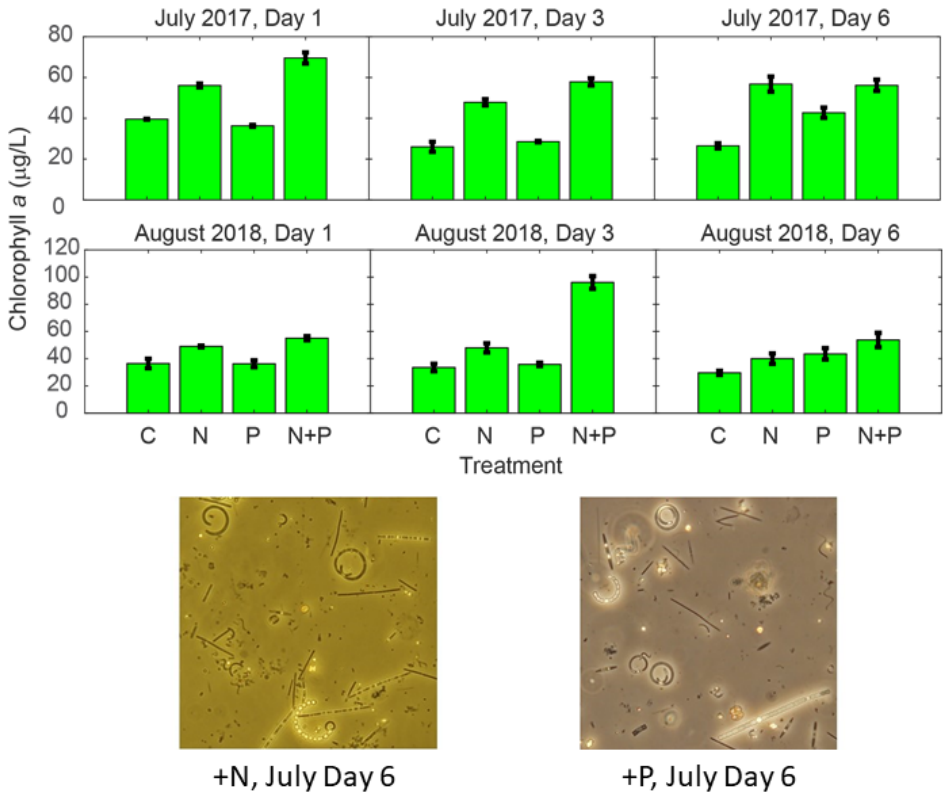


*Figure 4. Evidence for stimulation of nitrogen fixing cyanobacteria by experimental P additions during the July 2017 and August 2018 nutrient addition bioassay experiments. Bars and error bars represent means and standard deviations of triplicates.*

Although N was the primary nutrient limiting in situ growth, after a period of six days, P additions did increase the phytoplankton growth rate in the July 2017 and August 2018 experiments (Figure 4). Even in an N limited phytoplankton community, stimulation by P alone can occur when P additions stimulate the growth of cyanobacteria that are capable of using atmospheric nitrogen via the process of $N_2$-fixation. Although $N_2$-fixation was not measured during the July 2017 experiment, microscopic

examination of samples from the experiment did indicate that P addition had stimulated N-fixing, cyanobacteria having obvious heterocysts (specialized non-photosynthetic cells for $N_2$-fixation).

N fixation was directly measured during the August 2018, October 2018, and April 20109 bioassay experiments and confirmed that additions of P stimulated N fixation (Figure 5). Surprisingly, N fixation was stimulated regardless of whether P was added in concert with N. The N fixation rates measured during the August 2018 P addition were more than an order of magnitude higher than the highest rates observed during the October 2018 and April 2019 experiments. Based on a typical chlorophyll *a* to N ratio for phytoplankton of 1000 nmol N per µg chlorophyll *a* (Li et al. 2010) the measured rate of ~400 nmol/L/h multiplied by a 14 h light period could produce enough bioavailable N to sustain

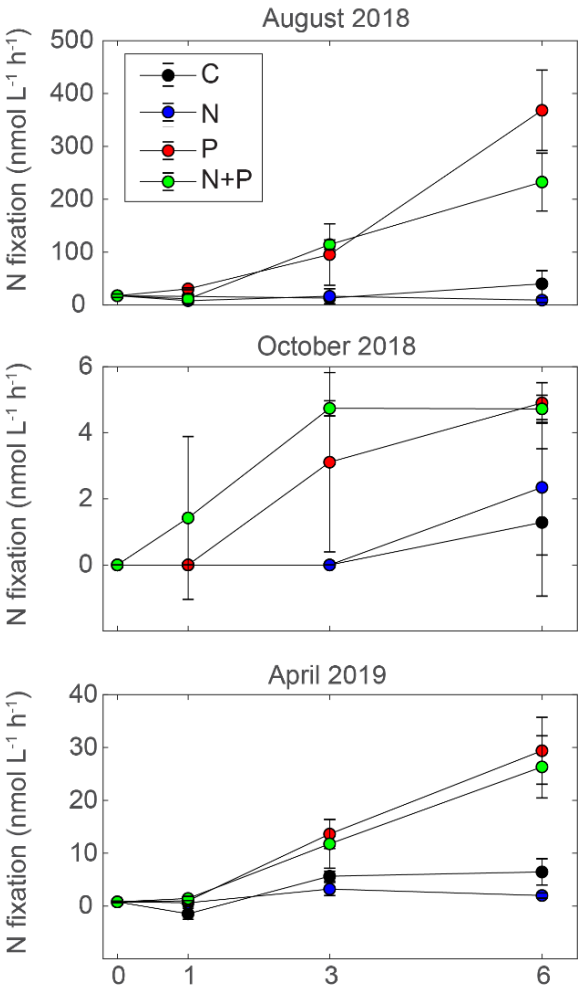


*Figure 5. Nitrogen fixation rates measured during nutrient addition bioassays in August 2018, October 2018, and April 2019 from water collected at Farrington. Symbols and error bars represent means and standard deviations of triplicates. C = control, N = N addition, P = P addition, and N + P = both N and P addition.*

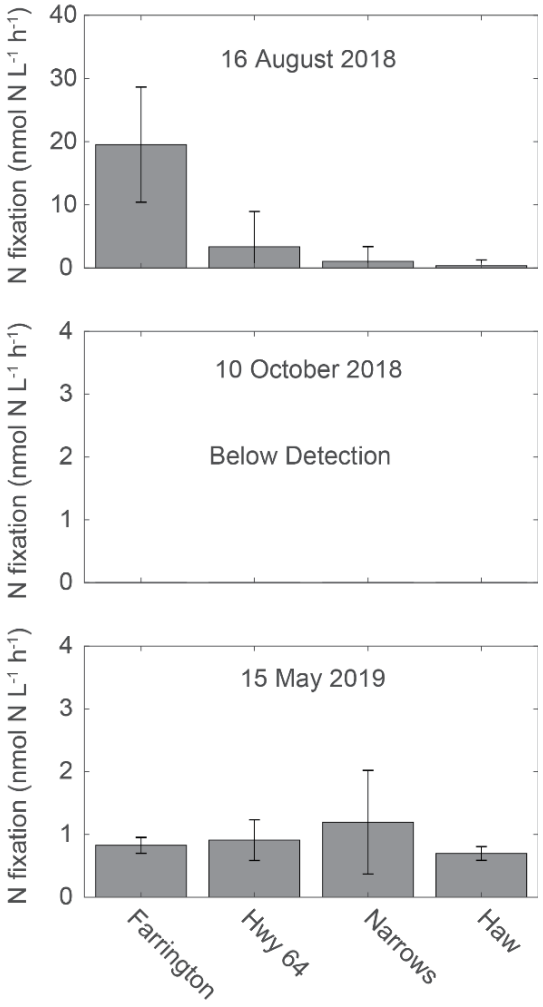


*Figure 6. Nitrogen fixation rates measured on unmanipulated water collected from four sites in Jordan Lake. Bars and error bars represent means and standard deviations of triplicates.*

growth of ~ 6 µg/L chlorophyll *a* per day. Therefore, the measured rate of N fixation can more than explain the ~8 µg/L growth of chlorophyll *a* in the P treatment during the last three days of the experiment.

N fixation measurements were additionally made on three dates from unmanipulated water samples collected opportunistically by Dr. Luettich's laboratory at the four temperature profile/ ADCP sites. On 16 Aug 2018, the highest N fixation measurement on a natural, unmanipulated sample (~ 20 nmol N/l/h) was observed at the Farrington bridge site (Figure 6). N fixation during at other stations and dates was less than 5 nmol N/l/h. Given the aforementioned typical N to chlorophyll ratio in phytoplankton, the measured rate of ~20 nmol/L/h extrapolated to a daily rate with a 14 h light period could only sustain the production of 0.28 µg/L chlorophyll *a* per day, or about 1 % of the typical chlorophyll *a* stock.

Species level phytoplankton community composition data collected by NCDEQ over the past eight years indicate that N fixing cyanobacteria is usually less than ten percent of total phytoplankton biomass (Figure 7). However, during summer N fixing cyanobacteria can episodically constitute half or more of the phytoplankton biomass at locations throughout the lake. Given the episodicity of peaks in N fixing biomass, we caution that our three ambient measurements cannot adequately constrain the importance of N fixation to the N budget, phytoplankton production, and phytoplankton community composition of Jordan Lake.

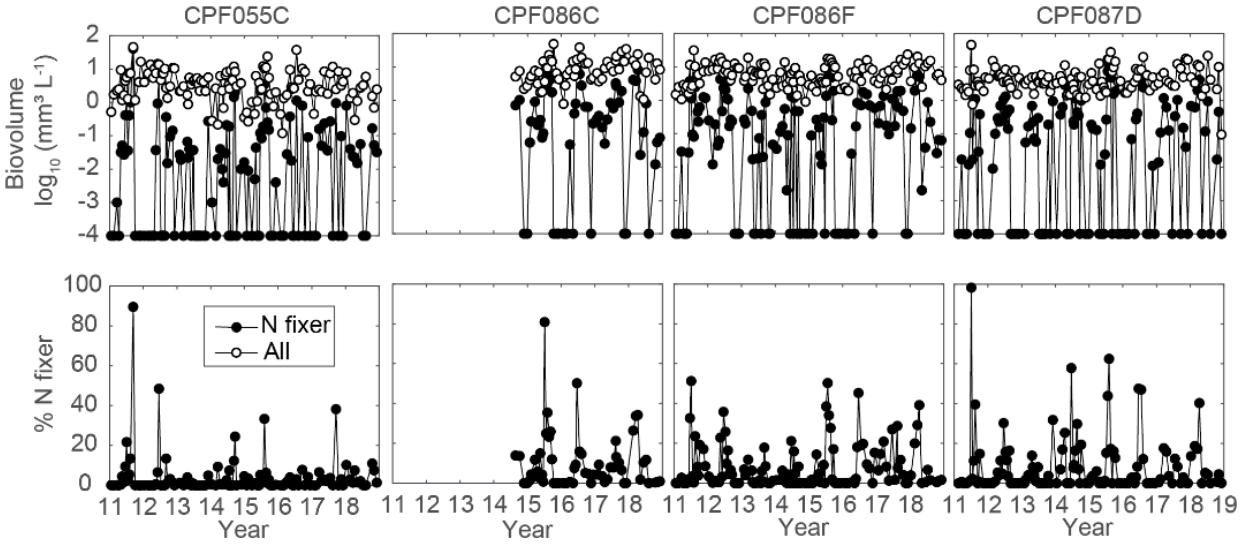


*Figure 7. Time series of total phytoplankton and N-fixing cyanobacteria biomass as biovolume at four stations in Jordan Lake from 2011-2018.*

**Establishing nutrient reduction targets using a simple mass balance approach:**

Inputs of total N and total P from tributaries and due to atmospheric deposition are presented in Table 2. The Haw River comprises about 90% of the flow to Jordan Lake but only about two thirds of the nutrient loading due to the higher nutrient concentrations of the smaller tributaries

of the New Hope arm. Water inflow estimates over the six year period were ~6% less than gaged outflow, and about a third of the difference can be explained by the ~15 million gallon per day withdrawal by the Town of Cary. Atmospheric deposition contributed only about 2% of the TN load. Total N and P loads to the lake ($M_{in}$) totaled 6682 kg N/d and 741 kg P/d. Losses due to the Haw River outflow were calculated based on gaged outflows multiplied by lake-wide average nutrient concentrations and ($M_{out}$) totaled 4821 kg N/d and 189 kg P/d. The much higher fraction of retained P (74%) compared to retained N (28%) is typical of reservoirs due to the high fraction of sediment bound P loaded to the reservoir and to the highly particle reactive nature of soluble P forms (e.g. *o*-phosphate) within the lake. The difference between loads in and losses due to outflow defines the net sedimentation term ($M_{sed}$) according to equation 3, and equaled 1861 kg N/d and 552 kg P/d.

Table 2. Components of the nutrient mass balance for N and P over the period 2011-2016.

| Tributary | Total N | Total P |
|---|---|---|
| Haw R. load | 4505 (kg/d) | 559 (kg/d) |
| Morgan Cr. load | 191 (kg/d) | 16 (kg/d) |
| Northeast Cr. load | 153 (kg/d) | 18 (kg/d) |
| New Hope Cr. load | 304 (kg/d) | 43 (kg/d) |
| Other creeks load | 1297 (kg/d) | 105 (kg/d) |
| Atmospheric load | 145 (kg/d) | 0 (kg/d) |
| Total Load In ($M_{in}$) | 6682 (kg/d) | 741 (kg/d) |
| Haw R. Load Out ($M_{out}$) | 4821 (kg/d) | 189 (kg/d) |
| Net Sedimentation ($M_{sed}$) | 1861 (kg/d) | 552 (kg/d) |
| Lake Area (A) | 53 x$10^6$ ($m^2$) | 53 x$10^6$ ($m^2$) |
| Lake-wide Average Concentration | 1.03 (mg/L) | 0.064 (mg/L) |
| Sedimentation Rate | 0.03 (m/d) | 0.16 (m/d) |

Net sedimentation encompasses all processes that lead to removal of N and P from the water column including sedimentation of particulate forms, but also transfer to higher trophic levels, and for N removal as gaseous forms via denitrification. The net sedimentation is assumed proportional to the average nutrient concentration in the lake ($C_{lake}$) scaled by lake surface area (A) and a net sedimentation velocity ($K_{net}$) (Equation 4). Lake area and sedimentation velocity are assumed constant so that only changes in the average lake nutrient concentration drive changes in net sedimentation. If flow into the lake is assumed equal to flow out, then substituting Q x C (Equations 5 and 6) for loads in and out and $C_{lake} \times A \times K_{net}$ for $M_{sed}$ in Equation 3 and rearranging yields Equation 8. Thus, the change in nutrient concentration in the lake is proportional to the average incoming nutrient concentration scaled by a term ($Q/(Q+AK_{net})$) that represents the relative importance of losses due to river flow versus net sedimentation. When sedimentation is small, the concentration in the lake responds strongly to changes in external loads, but as the relative importance of sedimentation increases, increasingly large changes in external loading are required to effect a change in lake-wide average nutrient concentrations. If a target lake-wide average nutrient concentration can be determined, then Equation 8 can be used to determine the external loading ($M_{in}$) necessary to achieve that concentration.

Equations that define a nutrient mass balance

$M_{in} = M_{out} + M_{sed}$ Eq 3.

$M_{sed} = C_{lake} \times A \times K_{net}$ Eq 4.

$M_{in} = Q_{in} \times C_{in}$ Eq 5.

$M_{out} = Q_{in} \times C_{lake}$ Eq 6.

*or*

$M_{out} = Q_{out} \times C_{out}$ Eq 7.

$C_{lake} = C_{in} \times Q_{in} / (Q_{in} + A \times K_{net})$ Eq. 8

Target nutrient concentrations were determined based on the relationships between TN and TP with chlorophyll *a* measured at stations throughout the lake by NC DEQ's Ambient Monitoring Program. The current water quality standard for NC surface waters states that the concentration of chlorophyll *a* should not exceed 40 µg/L in greater than 10% of collected samples. Based upon this standard and the allowed 10% exceedance, quantile regressions for the 90$^{th}$ quantile of chlorophyll *a* were used to determine the average in lake total N and total P concentrations that will maintain the 90$^{th}$ quantile of chlorophyll *a* below 40 µg/L (Figure 8). For TN and TP, the target concentrations identified were 0.78 mg N/L and 0.04 mg P/L. To reach these targets, the average inflowing TN concentration would need to be reduced by 24% from 1.71 mg/L to 1.30 mg/L. Average inflowing TP concentration would need to be reduced by 33% from 0.19 to 0.13 mg/L. These results are similar to conclusions drawn from the nutrient dilution bioassays that indicated reductions of 30-50% would be needed to significantly reduce phytoplankton biomass levels.

This implementation of the mass balance approach assumed that the concentration at the outfall was equal to the average lake-wide nutrient concentration (Equation 4; Havens and Schelske 2001). In reality, significant gradients occur within the lake due to the spatial distribution of sources and the sedimentation processes that occur as water is transported toward the dam. As a result, TN is about 30% higher and TP is 10% lower in the outflowing Haw River than the lake-wide average. Nutrient reductions calculated using measured Haw River outfall loads (Equation 6) instead of the lake-wide average concentration increased the TN reduction necessary to meet the chlorophyll *a* standard to 41% but decreased the necessary TP reduction to 28% of the current load.

A second critical assumption is the constancy of the sedimentation velocity. As nutrient inputs change the structure of the ecosystem including ratios of dissolved and particulate fractions, buoyancy of dominant phytoplankton groups, and ratios of benthic to water column production can all change with impacts on the net sedimentation velocity (Havens and Schelske 2001). Fortunately, in the long term, oligotrophication resulting from nutrient reductions should lead to increases in the net sedimentation velocity, and therefore, help maintain acceptable water quality conditions once they are established (Jeppesen et al. 2005; Havens and Schelske 2001). However, internal loading from legacy nutrients deposited in the sediments may delay water quality improvement for years to decades following load reductions (Jeppesen et al. 2005).

Overall, the bioassay experiments indicated that N was the primary limiting nutrient with secondary co-limitation by P, a condition observed in numerous eutrophic freshwater lakes and reservoirs (Elser et al. 2007; Paerl et al., 2016). Although N is the primary limiting nutrient, four of the seven experiments indicated that increasing the availability of P could stimulate cyanobacterial N fixation and partially compensate for N deficits. Although this process has been shown to occur when P is added, it is currently unclear to what extent external reductions in N loading might be compensated for by N fixation (Paerl et al. 2016). Both N and P should be considered when developing nutrient reduction strategies to reduce phytoplankton biomass of Jordan Lake. The May 2018 nutrient dilution experiment and nutrient mass balance approach indicated that reductions of N and P in the range 25-40% will likely be required to control phytoplankton growth.

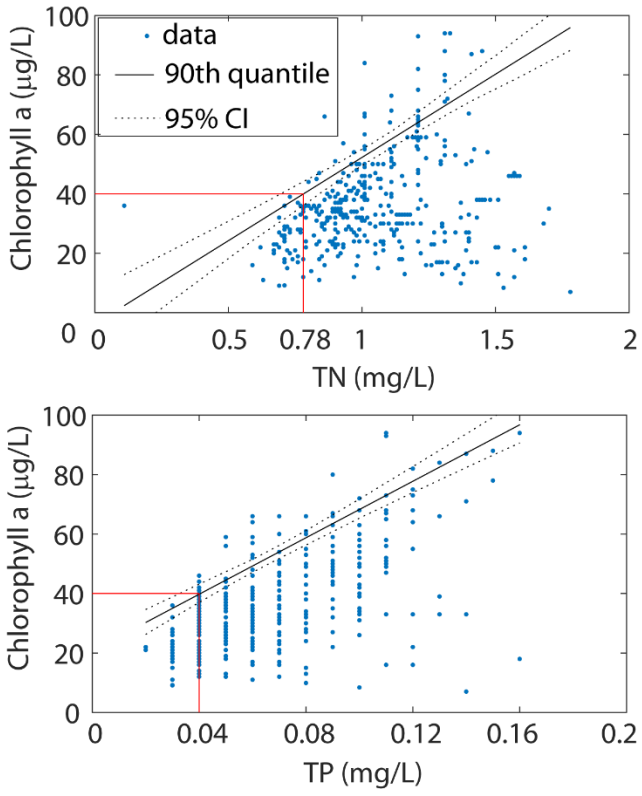


*Figure 8. Quantile regressions of chlorophyll a on total N and total P from NC DEQ's Ambient Monitoring System data from 2011-2016. Red lines indicate the TN or TP concentration that corresponds to when the 90th percentile of chlorophyll a equals the state standard of 40 µg/L.*

**Light limitation:** For all twelve photosynthesis versus irradiance assays, photosynthesis normalized to Chl *a* ($P^b$) increased rapidly with PAR up to 20-80 µmol photons/ $m^2$/s and began to saturate at higher values as $P^b_{max}$ was reached (Figure 9). $P^b_{max}$ is proportional to the maximum phytoplankton specific growth rate which can be readily calculated by division by the carbon to Chl *a* ratio (Cloern et al. 1995). $P^b_{max}$ ranged by more than an order of magnitude from 0.42 to 4.95 g C/ g chl *a*/ h (Table 3) and averaged 2.9 g C/ g chl *a*/ h, typical values for natural, temperate-climate phytoplankton assemblages (Gallegos 2012). $P^b_{max}$ displayed a seasonal cycle (Figure 10) with a maximum in the late spring to summer and minimum in the late fall and winter. The seasonal cycle and strong correlation of $P^b_{max}$ with temperature (Figure 11) are typical for natural phytoplankton assemblages and are driven by the temperature dependence of enzymatic reaction rates (Figure 11) (Gallegos 2012). The slope of the light limited portion of the curve ($\alpha$) varied from 0.05 to 0.11 and averaged 0.079 g C/ g chl *a*/ h/(µmol photons $m^2$/s/). These $\alpha$ values are on the high end of the expected range for natural phytoplankton samples (Boyer et al. 1993; Lewis et al. 1995) and indicate that Jordan Lake phytoplankton are highly efficient at utilizing the low levels of light present in its turbid,

phytoplankton rich waters.

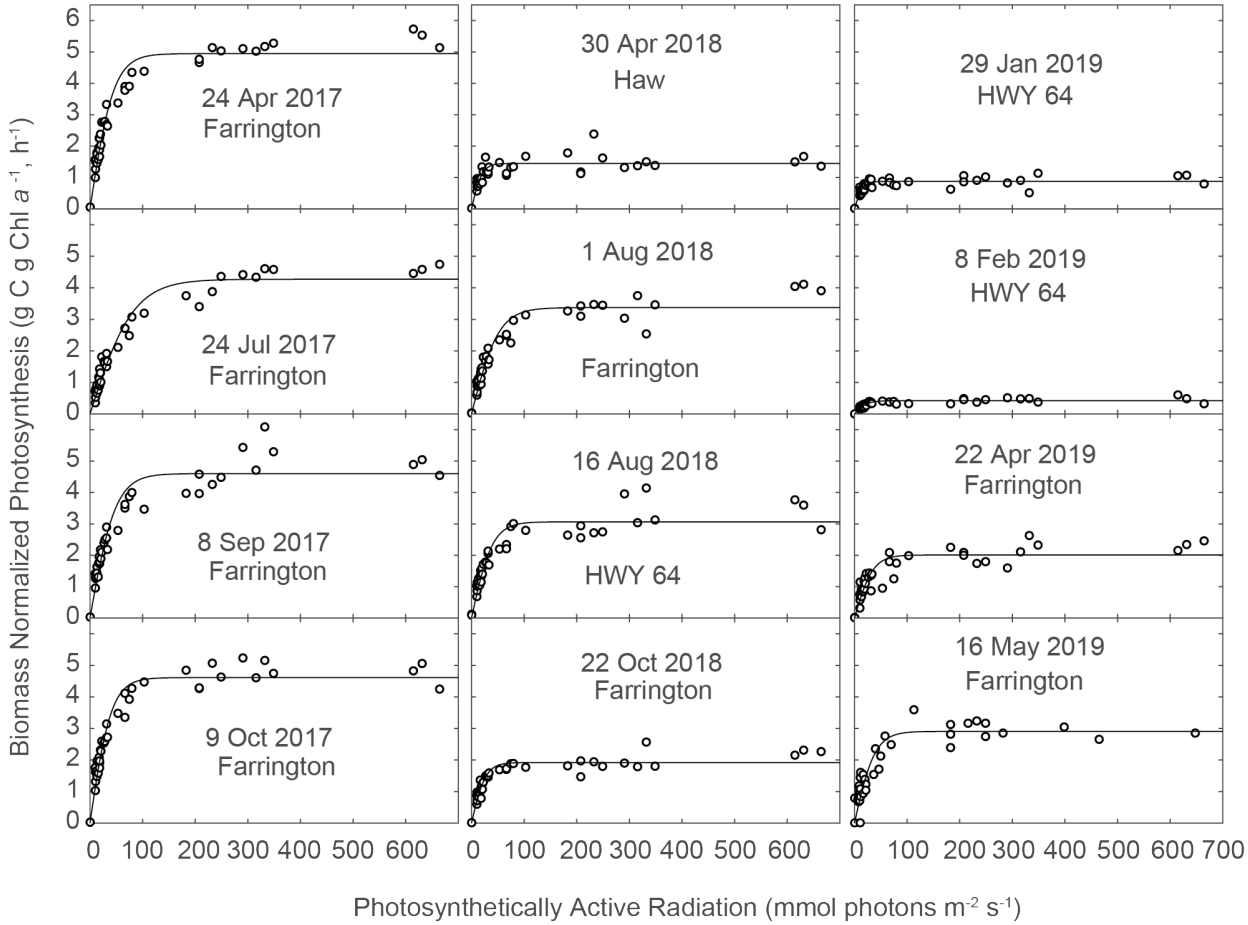


*Figure 9.. Results from twelve experimental measurements of phytoplankton photosynthetic rate versus light intensity. Data for 16 May 2019 are pooled data from the N addition and control treatments of the N addition experiment.*

The value of $P^b_{max}$ divided by α, commonly called $I_k$ , provides the irradiance at which photosynthesis is nearly saturated. $I_k$ ranged from 20 to 82 µmol photons/$m^2$/s and averaged 39 µmol photons/$m^2$/s (Table 3). As a daily average, incident irradiance is generally between about 500 µmol photons/$m^2$/s during the summer to 200 µmol photons/$m^2$/s during the winter (Figure 12). Based on the wealth of temperature profiles and light attenuation data collected at the four thermistor chain stations, significant temperature gradients often occur in the upper 2- 3 m of the water column (Figure 13) and the PAR extinction coefficient at different areas in the lake ranges from -1.5 to -2/m (see Figure 22 of report by Luettich, Whipple, Seim, and Gilchrest). The average light level of the upper mixed layer can be calculated according to equation 9

$$I_{avg} = I_0(1\text{-}exp(\text{-}k*Z_{uml}))/(k*Z_{uml}) \qquad \text{Equation 9}$$

where $I_{avg}$ is the average light level within the upper mixed layer of depth ($Z_{uml}$) based on the incident irradiance ($I_0$) and light extinction coefficient ($k$). Approximating the depth of the upper mixed layer as 2.5 m, and the extinction coefficient as -1.75/m, equation 9 gives a daily average upper mixed layer average that ranges from about ~45 µmol photons/$m^2$/s in the winter to ~110 µmol photons/$m^2$/s in the summer. These average upper mixed layer irradiance values are higher than $I_k$ and indicate that despite strong light limitation, phytoplankton in the upper mixed layer should be capable of photosynthesizing at near maximum rates.

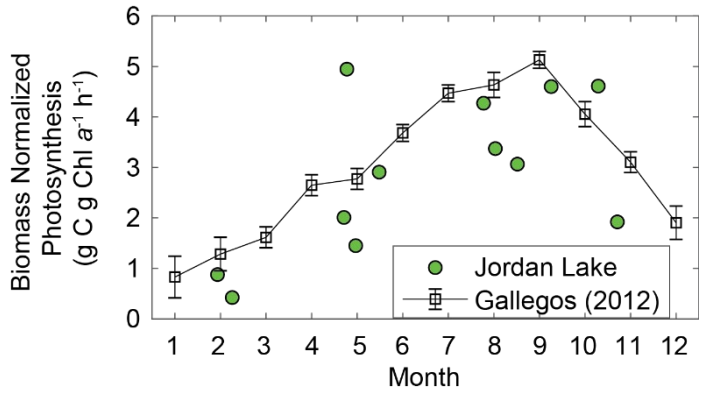


*Figure 10. Seasonality of the light saturated photosynthetic rate ($P^b_{max}$) in Jordan Lake in comparison to results from Gallegos (2012).*

What selective forces would generate a phytoplankton community that is adapted to significantly less light than they routinely experience? The answer may be two-fold. First, nutrient loading events are also accompanied by high sediment loads and the resulting turbidity can greatly increase light attenuation (see Figure 16 of report by Luettich, Whipple, Seim, and Gilchrest). Phytoplankton capable of growth under very low light levels would have a significant growth advantage allowing them to exploit these new inputs and out compete less shade adapted taxa. Secondly, while the shallow upper mixed layer is well lit, the lower layer is only very dimly lit. The ability to maintain modest photosynthetic rates below the thermocline provides a mechanism for survival until cells are re-entrained into the upper mixed layer (Richardson et al. 1983).

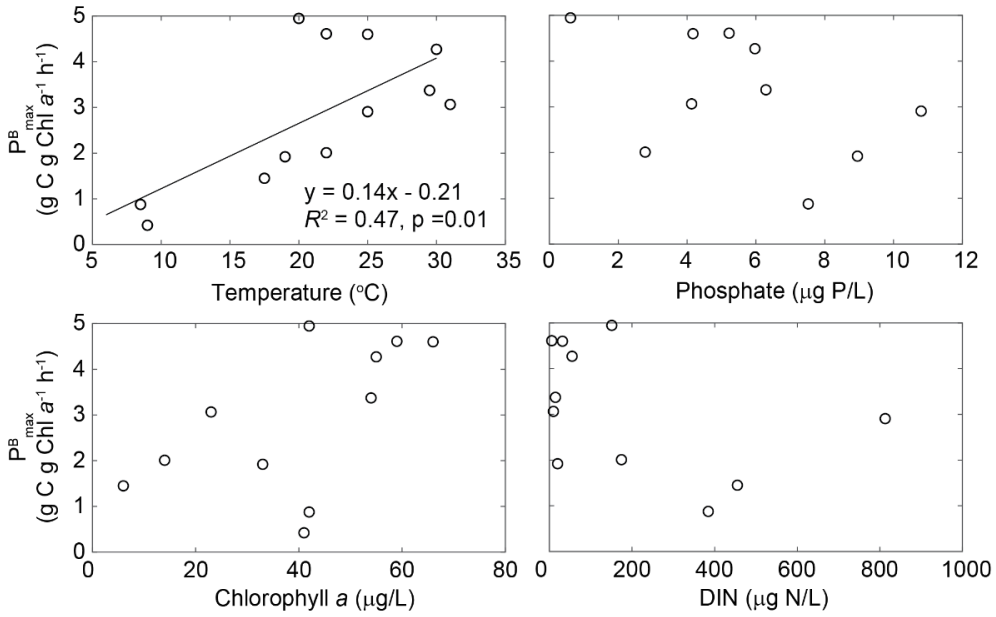


*Figure 11. Scatterplots of the light saturated maximum photosynthetic rate ($P^B_{max}$) against temperature, inorganic nutrients, and chorophyll a.*

Table 3. Parameters describing the hyperbolic tangeant relationship between phytoplankton photosynthesis and light intensity, and environmental parameters relevant to phytoplankton growth.

| Location | Date | $P^b_{max}$ | $\alpha$ | $I_k$ | Temp | Chl a | DIN | $PO_4$ |
|---|---|---|---|---|---|---|---|---|
| Farrington | 4/24/2017 | 4.95 | 0.11 | 45 | 20 | 42 | 150.8 | < 0.6 |
| Farrington | 7/24/2017 | 4.27 | 0.05 | 83 | 30 | 55 | 54.7 | 6.0 |
| Farrington | 9/8/2017 | 4.60 | 0.09 | 49 | 25 | 66 | 31.4 | 4.2 |
| Farrington | 10/9/2017 | 4.61 | 0.11 | 41 | 22 | 59 | 5.4 | 5.2 |
| Haw | 4/30/2018 | 1.45 | 0.07 | 20 | 17.5 | 6 | 455 | 23.2 |
| Farrington | 8/1/2018 | 3.37 | 0.07 | 51 | 29.5 | 54 | 14.3 | 6.3 |
| Hwy 64 | 8/16/2018 | 3.06 | 0.08 | 40 | 31 | 23 | 9.4 | 4.1 |
| Farrington | 10/22/2018 | 1.92 | 0.07 | 27 | 19 | 33 | 19.6 | 9.0 |
| Hwy 64 | 1/29/2019 | 0.88 | 0.05 | 16 | 8.5 | 42 | 384 | 7.5 |
| Hwy 64 | 2/8/2019 | 0.42 | 0.02 | 24 | 9 | 41 | NA | NA |
| Farrington | 4/22/2019 | 2.01 | 0.06 | 35 | 22 | 14 | 174.2 | 2.8 |
| Farrington | 5/16/2019 | 2.91 | 0.07 | 39 | 25 | 35* | 813 | 10.8 |

$P^b_{max}$ is the light saturated maximum biomass normalized photosynthetic rate (g C/ g Chl a/h), $\alpha$ is the slope of the light limited portion of the curve (g C/ g Chl *a*/h/ mmol photons/$m^2$/s), $I_k$ is the light level at which phytoplankton start to become saturated (μmol photons/$m^2$/s). Temp is water temperature (°C), and Chl *a*, DIN and $PO_4$ are all in units μg/L. NA = not assessed. * indicates that this Chl a measurement was the average from the N addition (37 μg/L) and control treatment (33 μg/L) for the N addition experiment on 5/15/2019.

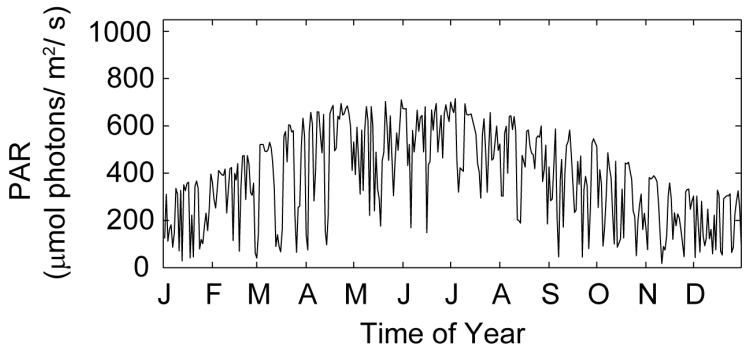


*Figure 12. Daily average incident photosynthetically active radiation during 2018 measured by the US Forestry Service at the Duke Forest, Remote Automated Weather Service (RAWS) site DKFN7.*

The Jordan Lake Nutrient Response Model developed by TetraTech (2002) is based on the Water Quality Analysis and Simulation Program (WASP) and assumes an $I_k$ near 500 μmol photons/ $m^2$/s. Therefore, the current water quality model has strongly overestimated the degree of light limitation experienced by phytoplankton in Jordan Lake.

Using parameter values that are specific to the phytoplankton community of Jordan Lake will provide a much more accurate representation of light limitation of phytoplankton growth than the literature values used in the current water quality model and can greatly affect model outcomes. As an example, consider a simple model of phytoplankton growth following a pulse loading event has raised the nitrate concentration to 1 mg/L but has also increased the light attenuation to an extinction coefficient of -5/m. The water column is assumed 3.5 m deep to approximate the deeper areas of the upper lake above Farrington Rd. The

relationship between irradiance and photosynthesis is modeled in two ways: 1) using the Jassby and Platt (1976) formulation from Equation 2 with average values for $P^b_{max}$ = 2.9 and $\alpha$ = 0.079 determined during this study, and 2) equation 10 using the formulation by Di Toro et al. (1971) utilized by the WASP model that forms the basis of the Jordan Lake Nutrient Simulation Model (Tetra Tech 2002)

$P^b = P^b_{max} *(I/I_{sat}).*\exp(1-I/I_{sat})$ Equation 10

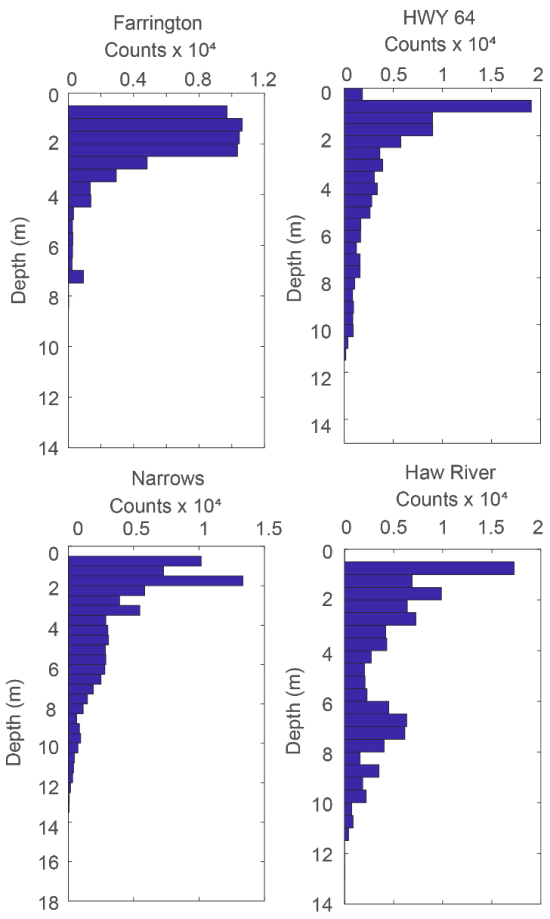


*Figure 13. Histograms of the depth of the upper mixed layer at the four thermistor chain locations in Jordan Lake. Upper mixed layer depth was defined as occurring from the surface to the first depth at which a temperature gradient greater than 0.5 °C/m occurred.*

where $I_{sat}$ is the light level at which photosynthesis saturates. $I_{sat}$ was set to 500 to match the parameterization of the Jordan Lake Nutrient Simulation Model (Tetra Tech 2002), and $P^b_{max}$ was assumed equal to the average measured value from this study. The principal difference between the two formulations is that the Di Toro et al. (1971) formulation decreases modeled photosynthesis at light levels higher than $I_{sat}$ to simulate photoinhibition. However, this effect of formulation on modeled productivity is insignificant compared to the effect of modeling severely overestimating $I_{sat}$. Nitrate uptake is determined by Monod uptake kinetics with a half saturation for nitrate of 28 µg/L. Rates of both productivity and nutrient uptake were scaled by dividing by respective maximum rates to give the productivity ($P^b_{scaled}$) and nutrient uptake rate ($V_{scaled}$) on a scale from 0 to 1 (Equations 11 and 12). Maximum growth rate ($\mu_{max}$) was assumed equal to 0.058/h based on dividing $P^b_{max}$ with a reasonable C: Chl a ratio of 50 (Cloern et al. 1995). Growth rate was then determined as the product of the maximum growth rate, the scaled photosynthetic, and nutrient uptake rates according to equation 13. Change in Chl a was modeled according to first order growth kinetics according Equation 14, and loss of nitrate occurred at the rate of Chl *a* growth multiplied by a stoichiometric conversion factor of 14 (Equation 15). The system of equations was

solved using the Euler method. Though simple, the principles of the model are the same as in the Jordan Lake Nutrient Simulation Model.

$P^b_{scaled} = \tanh(\alpha I / P^b_{max})$ *or* $(I/I_{sat})\exp(1-I/I_{sat})$ Equation 11

$V_{scaled} = N/(N + K_N)$ Equation 12

$\mu = \mu_{max} \times P_{scaled} \times V_{scaled}$ Equation 13

$dChl\ a/dt = \mu \times Chl\ a$ Equation 14

$dN/dt = -14 \times \mu \times Chl\ a$ Equation 15

Results demonstrate the large difference in model response governed by how light limitation is parameterized (Figure 14). Using a parameterization that accounts for strong adaptation leads to a much faster growth rate with Chl *a* concentration exceeding the NC water quality standard of 40 µg/L in about 6 d. With the current parameterization, biomass develops and nitrate is consumed much more slowly and it takes about twelve days to exceed the standard. Beyond a few weeks, the models converge as nutrients are consumed and the developing bloom is increasingly nutrient rather than light limited. It is clear that large improvements in modeling such events can be gained by more accurately portraying the shade adaptation of phytoplankton. However, more accurately portraying the productivity in the deeper areas of the lake may also lead to new insights into phytoplankton production, nutrient assimilation, and regeneration patterns in the lake.

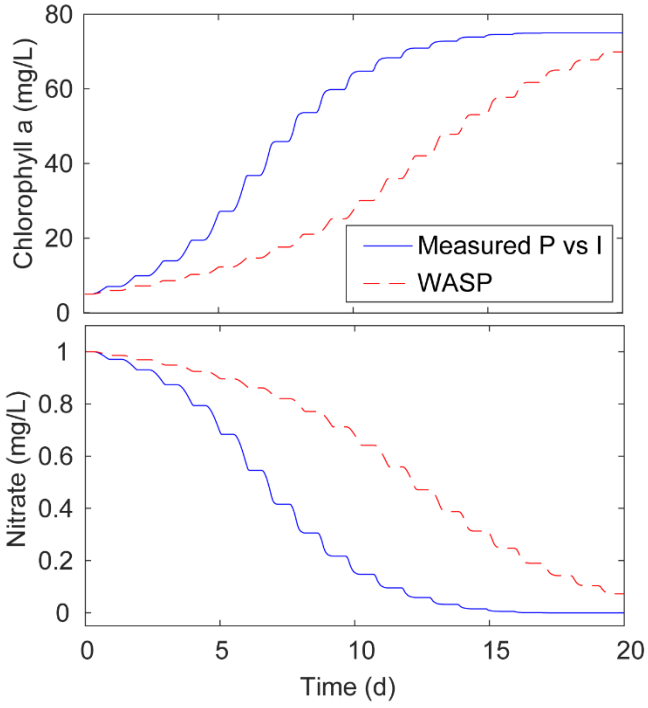


*Figure 14. Model output using parameterizations of the relationship between photosynthesis and irradiance measured in this study versus the parameterization currently assumed in the WASP model upon which the current Jordan Lake Nutrient Simulation Model is built.*

**Interactions of light and nutrient limitation:** The experiment designed to test the impact of nitrogen limitation on the shade adaptation of phytoplankton failed to reveal any significant differences in photosynthetic response between phytoplankton that received an N addition and a control treatment (Figure 15). However, inorganic nitrogen concentrations of the water sampled for the experiment were very high (>0.8 mg/L, Table 3). With such high DIN concentrations already present it is highly

unlikely that the phytoplankton were N limited, and a response to an additional (0.56 mg/L = 40 µmol/L) would not be expected. This experiment has been repeated in August 2019 but the results were not available at the time of writing this report. There were no observed significant relationships between maximum photosynthetic rate and nutrient concentrations across the twelve dates when both nutrients and light/photosynthetic response was measured (Figure 11).

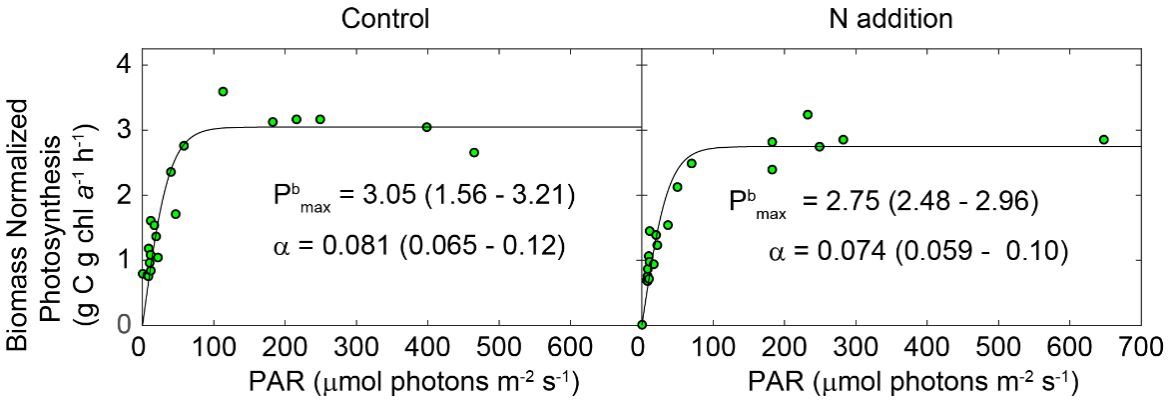


*Figure 15. Photosynthesis versus irradiance curves with and without the addition of 40 µmol/L dissolved inorganic nitrogen. Values in parentheses for $P^b_{max}$ and $\alpha$ are the 95% confidence intervals.*

**Important management implications of our work include:**

- Nutrient addition bioassays indicate that phytoplankton in the upper New Hope Creek arm of the lake and the Haw arm are primarily N limited from spring through fall. Additional stimulation by P was common, and during summer stimulation by P alone is possible due to stimulation of N-fixing cyanobacteria likely co-limited by N and P. This suggests that efforts to reduce phytoplankton biomass will need to address both N and P input reductions.

- Based on the dilution bioassays and nutrient mass balance approaches, reductions in the range of 25-40 % for N and about 30 % for P will be necessary to reduce chlorophyll *a* levels that will meet the current standard of 40 mg/L with a 10 % allowable exceedance frequency.

- Jordan Lake phytoplankton are much better adapted to growing in low light than represented by the current Jordan Lake Nutrient Response Model. The robust parameterization of the light versus photosynthesis relationship will greatly improve future water quality modeling efforts. This will enable more robust predictions of questions such as how fast can phytoplankton respond to pulses of nutrients from the tributaries given that such pulses are also associated with high loads of suspended particulates and color.

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