

PLANKTON DYNAMICS IN MESOTROPHIC HIGHLAND RESERVOIRS OF THE  
COLORADO RIVER SYSTEM, TEXAS

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San Marcos, Texas  
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PLANKTON DYNAMICS IN MESOTROPHIC HIGHLAND RESERVOIRS OF THE  
COLORADO RIVER SYSTEM, TEXAS

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## **ABSTRACT**

# **PLANKTON DYNAMICS IN MESOTROPHIC HIGHLAND RESERVOIRS OF THE COLORADO RIVER SYSTEM, TEXAS**

by

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Texas State University-San Marcos

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**SUPERVISING PROFESSOR: ALAN GROEGER**

Various ecological and limnological factors affect plankton dynamics and nutrient processing in clear, hard water subtropical reservoirs where temperature and light usually are conducive to year-round phytoplankton growth. Selected reservoirs in the Highland Lakes system that are currently under a point-source discharge ban, yet are slated for future wastewater discharges, were studied by collecting natural plankton assemblages from the surface-mixed layer at various uplake to near-dam sites in the larger reservoir, Lake Lyndon B. Johnson (LBJ), and from the near-dam sites in the two smaller reservoirs, Inks Lake and Lake Marble Falls. My questions were: how do the nutrients, nitrogen (N) and phosphorus (P), affect phytoplankton growth; and to what extent, seasonally, does zooplankton grazing impact phytoplankton, since phytoplankton mortality is a major loss factor of productivity and biomass from the photic zone?

Nutrient bioassays were prepared by initially pre-sieving the water to remove adult zooplankton (>150 micron) and with the following treatments: control (no nutrients added); nitrogen (N); phosphorus (P); and N+P. The monthly bioassays were incubated 5-7 d in a growth chamber set at reservoir temperature and seasonal photoperiods. Phytoplankton growth was measured using daily *in vivo* fluorescence, and by initial and final extracted chlorophyll (Chl a). Phytoplankton growth rates differed statistically among treatments and generally were negligible in the controls and increased four-fold in the N+P treatments. In the single nutrient treatments (N or P), significant interactions occurred with N-limitation during the warmer, stratified months, and P-limitation during the cooler months when the vertical water column was fully mixed.

Phytoplankton mortality due to zooplankton grazing was assessed seasonally with natural zooplankton assemblages that were collected by vertical net tows at the near-dam sites of each reservoir. Once in the lab, a density gradient was prepared by adding progressively higher numbers of zooplankton (grazers) to each treatment (0-3X) and incubated from 24-48 h with the ambient phytoplankton assemblages collected from the near-surface mixed layer at various reservoir sites. The 0X treatment had no grazers added to the water and served as a control to assess the phytoplankton growth rate in the absence of grazers. The 1X treatment had about 7-10 grazers, the 2x had about 20 grazers, and the 3X had about 30 grazers added to the ambient phytoplankton assemblages. The grazing rate was determined by the change in phytoplankton growth between 0 and 3X treatments, with phytoplankton growth rate plotted as a linear function of zooplankton density. N and P were added to all treatments at approximately half the concentration of the [NP] in the nutrient bioassays to allow for phytoplankton growth and to dampen the effects of nutrient regeneration by zooplankton.

Phytoplankton mortality due to zooplankton grazing was significant by the reservoir copepod-dominated assemblages. In post-analysis, the phytoplankton growth rates in the controls were compared to the overall grazing rates to determine seasonal patterns of growth versus grazing, by season and site. In spring, phytoplankton growth exceeded zooplankton grazing from all sites except one site, and at all but two sites in the late summer. In the fall, winter, and early summer, zooplankton grazing rates were equal to or exceeded growth rates at most sites.

Currently, the Highland Lakes' phytoplankton communities are constrained by both ambient nutrient limitation and zooplankton grazing. This study serves as an important assessment of primary producers and secondary consumers in subtropical reservoirs, prior to potential system eutrophication. The ecological and limnological processes studied here also may be useful as indicators to assess ecosystem health, climate, and anthropogenic changes within the context of aquatic food web ecology, limnology, and biogeochemical cycling.

## CHAPTER 1

### GENERAL INTRODUCTION

#### *Background*

What are the dominant factors controlling phytoplankton growth in aquatic systems? Two major approaches to study this question are to explore limiting resources such as nutrients as *bottom up*, and mortality by herbivorous zooplankton (grazing) as *top down* mechanisms, respectively. I studied both nutrients and grazing in reservoirs that currently have fairly low ambient nutrients and low to medium-high chlorophyll biomass. The reservoirs are located in subtropical latitudes such that water temperatures are conducive to year-round phytoplankton growth that is to some extent similar to tropical lakes. Conversely, temperate lakes exhibit strong periodicity in their plankton dynamics as cold water temperatures, ice coverage, and subsequent low light penetration limit phytoplankton growth. This pronounced seasonality results in pulsed timing of zooplankton grazing and subsequent phytoplankton succession (Gerten and Adrian 2000; Vasseur et al. 2005; Reynolds 2006).

#### Reservoir Plankton Studies

Using natural assemblages of phytoplankton, an experimental approach was taken to explore the effects of nutrients on their growth dynamics. Near-surface phytoplankton



were collected monthly from reservoir sites, and enriched and grown with nutrients (N and P) from 5-7 days in a plant growth chamber that was programmed to reservoir water temperatures and seasonal light cycles. I postulated that the phytoplankton communities in this system were susceptible to increasing biomass through higher community-level growth rates from enrichment of either, or both, N and P, and the extent of either response would be site-specific and vary monthly. Indeed, phytoplankton bioassay results showed significant nutrient responses, and the extent to which the phytoplankton growth rates changed, relative to each treatment, yielded both temporal and spatial nutrient limitation trends within each reservoir (see Ch. 2).

To what extent do zooplankton communities regulate phytoplankton? Zooplankton communities exert both negative and positive influences on phytoplankton through the processes of grazing (predation) and nutrient regeneration (Glibert 1998; Queimalinos et al. 1998; Wassman 1998). I studied the direct effects of zooplankton grazing on phytoplankton and postulated that grazing rates would be significant and the extent of grazing impact on phytoplankton biomass may fluctuate seasonally. The zooplankton grazing experiments contained natural zooplankton assemblages that were collected from each reservoir on a bimonthly to quarterly basis to reflect the naturally occurring seasonal dynamics. Indeed, phytoplankton mortality by zooplankton grazing was significant, and the extent to which this process impacted biomass varied spatially and seasonally (see Ch. 3).

Although plankton ecology is dynamic in this reservoir system and therefore inherently fascinating, why should we care from an aquatic resources perspective? These studies are aimed toward a better understanding the potential ecosystem level effects of

eutrophication of mesotrophic Highland reservoirs on the mid-Colorado River system in central Texas, an area of rapid urban development. Eutrophication is the process where a waterbody becomes enriched with nutrients, which typically are human-induced, through agricultural and stormwater runoff, and wastewater discharges for examples, with estimated economic impacts to the U.S. of \$2.2 billion annually (Dodds et al. 2009).

Eutrophication can lead to persistent and toxic cyanobacterial blooms (Paerl et al. 2011), and once established are difficult to remediate in aquatic systems. Cyanobacteria produce taste and odor compounds that are expensive to treat in drinking water as well (Dzialowski et al. 2009). Ultimately, these studies may be used in part for modeling ecosystem health as a goal for environmental management of these reservoirs (Xu et al. 2001), and their connectivity to the Colorado River system.

In a broader context, the reservoirs showed patterns of nitrogen limitation and co-limitation of N and P that adds evidence of similar multiple nutrient limitation patterns of other lakes that are not ‘naturally oligotrophic’ (Sterner 2008). Nutrient co-limitation has been documented recently for marine and terrestrial systems as well (Elser et al. 2007). Sterner (2008) proposed that co-limitation and multiple limitation patterns may occur on smaller time scales, and that ultimately P-limitation may occur over multi-annual scales in freshwater systems. An alternative mechanism for occurrences of N-limitation is increased N-deposition, proposed by Bergstrom (2010).

### Project Purpose

The four Highland reservoirs studied were Lakes Inks, Lyndon B. Johnson (LBJ), Marble Falls, and Travis. Demographic and associated land use changes in the lower Colorado River watershed have placed increasing demands on Lake Travis for

water supply. For example, in 1999 an agreement was placed between the City of Austin and the Lower Colorado River Authority (LCRA) for 325,000 acre-feet of water for municipal use to meet projected needs through 2050 (LCRA 2007). In addition, there are pending wastewater discharge permits by the cities of Burnet and Leander that may affect the water quality through increased nutrient loadings for the Highland Lakes. Point source discharges into the reservoirs are prohibited since the late 1980's (Texas Administrative Code 30.1.311). In 2009, the cities of Burnet and Leander filed a petition with the Texas Commission on Environmental Quality (TCEQ) to end the ban on the Highland Lakes discharges (Price 2009a). Although the petition was denied, TCEQ decided to discuss the watershed rules during proposed public stakeholder meetings (Price 2009b). The research presented in subsequent chapters supports a larger project called Colorado River Environmental Models (CREMS), sponsored by the LCRA. The CREMS model scenarios are designed to provide water resources managers with a better understanding of potential changes related to watershed urbanization and wastewater discharges.

#### Project Description

The LCRA manages the Highland Lakes water resources mainly for flood control, drinking water supply, hydropower, and recreation. In 2002, LCRA began collecting reservoir water quality and stormwater runoff data specifically for two different models, CE-QUAL-W2 and SWAT, respectively. The CREMS project goals are to address changing water uses, demands, and quality. Examples of the changing water resources for the Colorado River include decreasing demand for agricultural water supply near the coast, increasing municipal water allocations in the central Texas region, and the need to

predict possible outcomes of pending wastewater discharges in the mid Colorado River region. The Lake Travis water quality model final report is currently available (Anchor QEA, LLC and Parsons Water and Infrastructure, Inc. 2009). I performed monthly phytoplankton nutrient bioassays from six sites in Lake Travis (2005–06) and enumerated four major groups of phytoplankton from preserved samples that were used in the water quality model referenced above.

### Doctoral Timeline and Research Scope

I joined the Biology Department's aquatic resources doctoral program at Texas State University–San Marcos in 2003 and became involved with the cooperative research project between the Lower Colorado River Authority (LCRA) and U.S. Geological Survey (USGS) as a biologist student intern in 2005. I became a doctoral candidate in June 2007 and expanded the research scope with an additional funding agreement for Lakes Inks, LBJ, and Marble Falls later that year. The scientific contribution of these studies is that they represent a system level baseline study of plankton dynamics and ecosystem health, prior to proposed changes that potentially may drive the system towards higher chlorophyll biomass, lowered water clarity, and perhaps ultimately to eutrophication.

Water for the bioassays was collected by the LCRA in addition to water column physicochemical characterization from a total of 11 sites in four of the Highland reservoirs during 2005–08. All of the experiments were conducted at the USGS Water Science Center in Austin, TX. These studies include an experimental approach of phytoplankton growth and zooplankton grazing to explore plankton ecological and limnological processes. I tested bottom up effects of nutrients on

phytoplankton growth with nutrient bioassays. I tested top down effects of phytoplankton mortality through zooplankton grazing. I collected and performed the grazing experiments with natural zooplankton assemblages from each of the near-dam sites in the reservoirs. In addition, I enumerated phytoplankton and zooplankton collections from all sites. Three reservoirs studied in 2007-08 that comprise five sites are presented in this dissertation.

### Dissertation Organization

The following chapters feature the plankton dynamics in a linked reservoir system on the Colorado River in central Texas, USA. A general introduction is provided here, in Chapter 1. The phytoplankton nutrient bioassay studies from five sites in three reservoirs are included in Chapters 2, followed by zooplankton grazing studies in Chapter 3. Last, Chapter 4 explores relationships among particulate carbon, nitrogen, and phosphorus ratios relative to the chlorophyll-a and phytoplankton growth rates discussed in Chapter 2.

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## **CHAPTER 2**

### **NUTRIENT-DEPENDENT PHYTOPLANKTON GROWTH RESPONSES IN MESOTROPHIC RESERVOIRS, COLORADO RIVER SYSTEM, CENTRAL TEXAS**

#### *Introduction*

Anthropogenic eutrophication is a global problem in surface waters (Schindler 2006; Smith et al. 2006) and can lead to water quality degradation. Some biophysical characteristics of poor water quality are algal blooms, increased turbidity, decreased light penetration, and lower dissolved oxygen (Wetzel 2001). Algal blooms can increase both human health risks and water treatment costs, especially when cyanobacteria blooms occur and become toxic or produce taste and odor compounds in drinking water supply (Wang et al. 2005). Community phytoplankton growth rates, composition, and primary production are important factors in considering how an aquatic system potentially may be altered through nutrient loadings (Horvatic et al. 2009), particularly in lakes and reservoirs requiring good water quality for drinking water supply and contact recreation (Grover et al. 1999; Dzialowski et al. 2005).

The traditional view of P-limitation in freshwater is becoming revised to an understanding of the importance of multiple and co-limitation (Guildford and Hecky 2000; Chrzanowski and Grover 2001; Elser et al. 2007; Sterner 2008). A few examples ranging in trophic status follow. N-limitation with the addition of trace



metals was found in Crater Lake, Oregon (Groeger 2007), and both N- and P-limitation was found in 21 and 11 taxa, respectively, in two northern Texas reservoirs (Grover et al. 1999). Multiple limitation (i.e. N, P and co-limitation) occurred 26, 60 and 23%, respectively, in Joe Poole Lake, Texas (Sterner 1994). In selected Kansas reservoirs the frequency of N, P, and co-limitation occurred 16, 8, and 63% (Dzialowski et al. 2005), and 13, 24, and 63%, respectively, in United Kingdom lakes (Maberly et al. 2002). Other examples of nitrogen limitation in freshwater systems include: in the Danube River (Horvatic et al. 2009); in high altitude lakes in both Europe (Bergstrom 2010) and North America (Lafrancois et al. 2003); and in a Tennessee reservoir (Groeger and Kimmel 1988). The experimental approach presented here addresses the potential for eutrophication in three of the Highland Lakes by measuring phytoplankton growth responses to nutrients on both spatial and temporal scales. Eutrophication criteria is specified for this study as the potential enrichment of a water body with nutrients, typically N and/or P that results in accelerated phytoplankton growth rates and subsequent production of phytoplankton biomass (Ging 1999; Wetzel 2001).

### Site Description

A total of five sites were studied including the near-dam site of Inks Lake, three sites along the longitudinal axis of Lake Lyndon B. Johnson (LBJ), and the near-dam site of Lake Marble Falls (Fig. 2.1), as part of the Highland Lakes on the mid Colorado River system in central Texas. The surrounding watershed is characterized by limestone karst hydrogeology and the dominate land cover is rangeland (Raines and Rast 1999). The climate is characterized by short, mild winters, and long, moderately hot summers with

moderate to high humidity and southerly prevailing winds. The mean annual rainfall in Austin is about 33 inches, and typically occurs in the spring and early fall with widely scattered thunderstorms that produce rainfall during the summer (<http://web2.airmail.net/danb1/annualrainfall.htm>).

The storage capacities of the three reservoirs vary widely at highest elevation. At full capacity, Lake LBJ holds 138,000 acre-feet (AF) of water. The two smaller reservoirs, Inks Lake and Lake Marble Falls, hold 17,300 and 8,050 AF, respectively (Wallace and Kiesling *in prep*). Water residence times (WRT) in the reservoirs vary from days to months with inflow and storage volume. The mean WRTs for each reservoir from August 2007–September 2008 were as follows: 139.4 days (d) for Lake LBJ; and 93.3 and 7.2 d, for Inks Lake and Lake Marble Falls, respectively (A.W. Groeger, personal commun. 2011).

## *Methods*

Monthly sampling occurred at all sites for 14 consecutive months (August 2007–September 2008), and included physicochemical depth profiles, and Chl a and nutrient chemistry (data are available at <http://waterquality.lcra.org>). Whole water samples were collected from the near-surface (0.3m) in 3-6 1L pre-washed and rinsed poly-bottles for the nutrient bioassays. The 1L whole water samples were stored in coolers at near-ambient lake temperature to avoid excessive heat and exposure to sunlight.

### Phytoplankton nutrient bioassays

Lab-based nutrient bioassays were initiated within 12-14 h of collection. The 1L whole-water samples from each site were gently mixed and passed through 153  $\mu\text{M}$  Nitex® to remove zooplankton from the bioassay fraction ( $<153\mu\text{M}$ ). The  $<153\mu\text{M}$  fraction was pooled and transferred into four separate 500 ml pre-rinsed poly-bottles, and assigned to the following treatments: (1) controls with no added nutrients; (2) +N,  $\sim 235 \mu\text{M}$  final  $[\text{NO}_3]$ ; (3) +P,  $\sim 30 \mu\text{M}$  final  $[\text{PO}_4]$ ; and (4) NP,  $[\text{NO}_3 + \text{PO}_4]$  with the same amounts as in (2) and (3). Each treatment was then mixed and split aseptically into four replicate pre-sterilized borosilicate culture tubes ( $\sim 35 \text{ ml}$  each). The bioassay sample tubes were then placed in racks and into a growth chamber, equipped with full spectrum lighting and set to the monthly temperature recorded from the surface depth of the Lake LBJ near-dam site, ranging from  $21^\circ\text{C}$  (winter) to  $27^\circ\text{C}$  (summer). The incubation photoperiods were programmed to approximate the seasonal fluctuation in daylight (L) and dark (D) hours, thus the number of hours for light and dark were: 10L:14D (winter); 12L:12D (spring); and

14L:10D (summer). The bioassays were incubated for 5-7d and growth responses were monitored and recorded daily.

#### Phytoplankton growth rates

*In vivo* fluorescence (IVF) was used to monitor daily phytoplankton growth responses (Leboulanger et al. 2006). Each day, the culture tubes were removed from the incubator and acclimated to room temperature. Each replicate was gently mixed using a Genie® vortex mixer prior to recording IVF, using a Turner® 10-AU digital fluorometer equipped with an optical filter set for Chl a (Turner® kit #10-037R). Refer to Fig. 2.2 as an example plot showing daily Chl a (IVF) growth from one site and one experimental treatment (N=4).

Phytoplankton growth rates ( $r \text{ day}^{-1}$ ) were determined with the following exponential equation:

$$N_t = N_o e^{(rt)} \quad (1)$$

where,

$N_t$  is phytoplankton biomass, [Chl a IVF], after time  $t$ ;

$N_o$  is initial phytoplankton biomass [Chl a IVF];

$e$  is base of natural logarithms;

$r$  is phytoplankton growth rate, per day, and;

$t$  is time, in days.

Taking the natural logarithm ( $\ln$ ) of both sides of equation 1 and solving for 'r' results in

$$r = (\ln[N_t] - \ln[N_o])/t. \quad (2)$$

### Data analysis and interpretation

After plotting and computing the phytoplankton growth rates resulting from the four treatments using daily IVF as in Fig. 2, a factorial analysis of variance (ANOVA) was used to compare least square mean (LSM) growth rates with site, treatment, and month as main effects. To determine the overall growth rate differences between sites, the growth rates means obtained by daily IVF per site were compared (i.e. 4 treatments x 4 replicates per treatment x 5 sites x 14 m; total N = 1120). Bonferroni post-hoc comparisons of all effects and interactions between effects were tested to determine statistically significant differences among growth rates, relative to site, month, and treatment using STATISTICA (2008) software.

The seasonal patterns of nutrient limitation were assessed by computing the frequency of significant treatment responses per month by combining the growth rate means for all five sites per treatment and comparing each treatment by month (m). N or P limitation was determined by significant growth responses in either treatment relative to the controls. Two types of co-limitation for both nutrients were determined based on the following interpretation by Arrigo (2005). Simultaneous co-limitation occurred when both of the N and P treatments were significant and positive relative to the controls. Biochemical co-limitation occurred when the NP treatment was significant and positive relative to the controls.

Next, the three nutrient treatments (N, P, and NP) were compared individually to the controls and nutrient limitation frequencies were computed as follows. For each month, the number of significant nutrient treatment responses relative to the controls were added together and expressed as a percentage of the total number of

months in the study. The NP treatment contained equal concentrations of both the single nutrient treatments, thus assigned the value of 0.50, as compared to the two single nutrient treatments with an assigned value of 0.25 each, such that when all three nutrient treatments yielded higher growth rates than the control treatment, the total = 1.0. For all nutrient combinations, i.e. N, P, and/or NP, the significant nutrient responses were multiplied by the number of months (m) that any one of the combinations of nutrient responses occurred, and divided by the total (14m), and expressed as percent (%) frequency N, P, simultaneous co-limitation, and/or biochemical co-limitation (NP).

For example, in five months of the study, the P and NP treatments yielded positive, significant growth rates relative to both the control and N treatments. To compute the frequency of occurrence, the assigned values of P and biochemical co-limitation are 0.25 and 0.50, respectively. Multiply the combined assigned value (0.75) by 5m, divide by 14m, and express the result as %: thus, the nutrient limitation is 26.8%. In other words, P and NP co-limitation occurred at a frequency of 26.8% during the study.

The relative strength of nutrient limitation was determined by calculating the ratio between the ambient and maximum growth rates ( $\mu:\mu_{\max}$  in Sterner 1994) using the controls and the NP growth rates, respectively, and designated here as  $[r_{\text{CON}}:r_{\text{NP}}]$ . In other words, when the NP and control growth rates are equal, the ratio is 1; and the ambient condition of the phytoplankton is assumed to be nutrient sufficient. When  $[r_{\text{CON}}:r_{\text{NP}}] \leq 0.05$ , the phytoplankton communities are considered to be nutrient limited.

## *Results*

The near-surface temperatures ranged from 11.94 to 30.94 °C (Fig. 2.3) and Chl *a* ranged from 1.6 to 26.0 µg L<sup>-1</sup> across all sites (August 2007–September 2008). The phytoplankton communities responded to one or more nutrient treatments in the bioassays throughout the entire study from all sites. A total of 1120 phytoplankton growth rates were analyzed as least square means (LSM) (N=280). Significant differences occurred for all main effects and their interactions ( $p < 0.00$ ). The overall phytoplankton growth rate means by treatment and site are presented, and then followed by the results of the interaction effects between treatment and site and between month and treatment.

### Treatment comparisons

In this comparison, the main effect of treatment was analyzed, with all sites and months combined per treatment. The LSM growth rates were lowest in control and highest in the NP treatments, (-0.039 and 0.464 d<sup>-1</sup>), respectively (Table 2.1). The treatment means differed significantly from each other in the post-hoc Bonferroni analysis ( $p < 0.00$ ).

### Site comparisons

In this comparison, the main treatment effect of site was analyzed, with all treatments and months combined per site. The overall growth rate LSMs were lowest from the two smaller reservoirs, Lakes Inks and Marble Falls (0.093 and 0.094 d<sup>-1</sup>, respectively), and highest from the Lake LBJ near-dam site (0.193 d<sup>-1</sup>) (Table 2.2 & Fig. 2.4). Post-hoc site comparisons were significant ( $p \leq 0.001$ ), with the exception of LSM growth rates comparisons between Lakes Inks and Marble Falls near-dam sites.

### Treatment and site comparisons

All of the monthly growth rates were combined per month and analyzed for treatment and site interactions. The LSM growth rates were highest in the NP treatment from the Lake LBJ near-dam site ( $0.559 \text{ d}^{-1}$ ) and negligible in the control treatments from Marble Falls and Lakes Inks (Table 2.3 & Fig. 2.5). All of the growth responses between treatments comparisons were significant with the exception of the N and P treatments from the Lake LBJ uplake site.

### Month and treatment comparisons

All of the sites were combined per treatment and analyzed by month. The NP treatment LSM growth rates were highest throughout the study, and peaked in October 2007, and again in June and August 2008 (Fig. 2.6). Negligible growth rates occurred consistently in the controls, and in either of the single-nutrient treatments throughout the study, depending on season.

The phytoplankton communities responded to one or more of the nutrient treatments compared to the control treatment in all study months (Table 2.4). Recall that the % nutrient limitation frequency was calculated by the assigned value for all three nutrient treatments set equal to 1.0; multiplied by the number of months that yielded that particular combination of significant responses, and divided by the total number of months, and expressed as %. In three study months, all three nutrient treatments were significantly different and positive relative to the controls, indicating that both forms of co-limitation occurred at a frequency of 21.4% during the summer months of both study years (August–September 2007 & July 2008 in Table 2.4). In five study months, the N and NP treatments were significant and positive relative to



the control and P treatments, indicating that N- and biochemical co-limitation occurred at a frequency of 26.8% during fall 2007 and summer 2008. Five separate months yielded P- and biochemical co-limitation, also at a frequency of 26.8%, yet these nutrient limitation responses occurred during the winter and early summer months (except February). In February 2008, only the NP treatment yielded a significant growth response over controls: a frequency of 3.6% of biochemical co-limitation only.

## *Discussion*

This study, which began in August 2007, was preceded by rainfall and flooding that occurred in early July 2007 (Fig. 2.3) resulting in the inundation of the reservoirs with stormwater runoff and precipitation. As the study progressed into fall, high growth rates were achieved in October 2007, which is usually the month when the reservoir mixes vertically, which results in ‘reservoir turnover’. Reservoir turnover may also bring pulses of nutrients into the upper mixed layer from depth, as shown by the high phytoplankton growth rate potential in the NP treatment that occurred during October 2007 (Fig. 2.6). Nutrient pulses may continue to occur through vertical mixing or through horizontal advection during winter with localized wind mixing, or by storm events, as shown by high phytoplankton growth rate potential in the P treatment during the winter months.

The seasonal progression of nutrient-dependent growth was highly variable, and switched from single (either N or P) to multi-nutrient responses (Fig. 2.6) throughout the study. While the phytoplankton communities responded to all three nutrient treatments in the both summer periods, which indicate both forms of nutrient co-limitation, there also was a significant and sustained growth response to P in the winter and to N during the second summer. Meanwhile, the phytoplankton communities responded to NP ( $p \leq 0.01$ ) during all study months, which indicates strong biochemical co-limitation. Both forms of co-limitation occurred at a frequency of 21.4% during the summer months of both study years (August–September 2007 & July 2008 in Table 2.4). N- and biochemical co-limitation occurred at a frequency of

26.8% during fall 2007 and summer 2008, while P- and biochemical co-limitation occurred at the same frequency during the winter and early summer months.

Phytoplankton growth rate ratios in the controls and NP treatments ( $r_{\text{CON}:\text{rNP}}$ ) that are equal to 1.0 indicate no nutrient limitation, and  $r_{\text{CON}:\text{rNP}} \leq 0.5$  indicate nutrient limitation, based on Sterner (1994). Nutrient limitation occurred at all sites during the study since none of the ratios ( $r_{\text{CON}:\text{rNP}} = 1.0$ ) (Fig. 2.7). One ratio that approached the value of 1.0 ( $r_{\text{CON}:\text{rNP}} = 0.92$ ) was from Inks Lake in February 2008, which coincides with the same month with the lowest percentage (3.6%) of significant nutrient effects in the study.

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Table 2.1 Phytoplankton growth least square means (LSM) summarized for treatment effect, with all sites and months combined per treatment (total N=280). Each treatment LSM differed significantly from all other treatments ( $p \leq 0.00$ ).

Treatment	Growth (LSM)	N
Control	-0.039	70
Nitrate	0.028	70
Phosphorus	0.054	70
N+P	0.464	70

Table 2.2 Phytoplankton growth least square means (LSM) per site (N = 56) with confidence intervals (-/+ 95%). Each site comparison differed significantly ( $p \leq 0.00$ ), except for Lakes Inks and Marble Falls, thus underlined.

Lake Site	Growth rate $d^{-1}$ (LSM)	Confidence Interval (-/+95%)
Inks near-dam	<u>0.093</u>	(0.090, 0.099)
LBJ Uplake	0.117	(0.189, 0.197)
LBJ Midlake	0.138	(0.134, 0.142)
LBJ near-dam	0.193	(0.113, 0.122)
Marble Falls near-dam	<u>0.094</u>	(0.088, 0.097)

Table 2.3 Phytoplankton growth rate least square mean (LSM) with confidence intervals (-/+ 95%). Bonferroni post-hoc comparisons of treatment by site were significant ( $p \leq 0.00$ ), except for Lake LBJ uplake N and P treatments, which are underlined. [TRT, treatment]

Site	TRT	LSM growth rate ( $r\ d^{-1}$ )	Confidence interval (-/+ 95%)
Lake Inks Dam	Control	-0.056	(-0.065, -0.047)
	N	0.024	(0.015, 0.033)
	P	-0.003	(-0.012, 0.006)
	NP	0.406	(0.397, 0.414)
Lake LBJ Uplake	Control	-0.055	(-0.063, -0.046)
	N	<u>0.029</u>	(0.021, 0.039)
	P	<u>0.032</u>	(0.023, 0.041)
	NP	0.462	(0.453, 0.470)
Lake LBJ Mid-lake	Control	-0.037	(-0.046, -0.028)
	N	0.029	(0.021, 0.038)
	P	0.069	(0.060, 0.078)
	NP	0.490	(0.482, 0.499)
Lake LBJ Dam	Control	0.022	(0.013, 0.031)
	N	0.055	(0.047, 0.064)
	P	0.136	(0.128, 0.145)
	NP	0.559	(0.550, 0.567)
Lake Marble Falls Dam	Control	-0.067	(-0.076, -0.059)
	N	0.003	(-0.005, 0.012)
	P	0.037	(0.029, 0.046)
	NP	0.404	(0.395, 0.413)



Table 2.4 Treatment comparisons of phytoplankton growth means with all sites combined per month. Significant comparisons are not underlined ( $P \leq 0.05$ ). Non-significant treatment comparisons are underlined. [C, control; N, nitrogen; P, phosphorus; NP, nitrogen & phosphorus]

Month	Month	Year	Treatment growth rate mean comparisons (lowest to highest)			
8	August	2007	C	N	P	NP
9	September		C	N	P	NP
10	October		<u>C</u>	<u>P</u>	N	NP
11	November		<u>C</u>	<u>P</u>	N	NP
12	December		<u>C</u>	<u>N</u>	P	NP
13	January	2008	<u>C</u>	<u>N</u>	<u>P</u>	<u>NP</u>
14	February		<u>N</u>	<u>C</u>	P	NP
15	March		<u>N</u>	<u>C</u>	P	NP
16	April		<u>N</u>	<u>C</u>	P	NP
17	May		<u>C</u>	<u>N</u>	P	NP
18	June		<u>C</u>	<u>P</u>	N	NP
19	July		C	P	N	NP
20	August		<u>C</u>	<u>P</u>	N	NP
21	September		<u>P</u>	<u>C</u>	N	NP

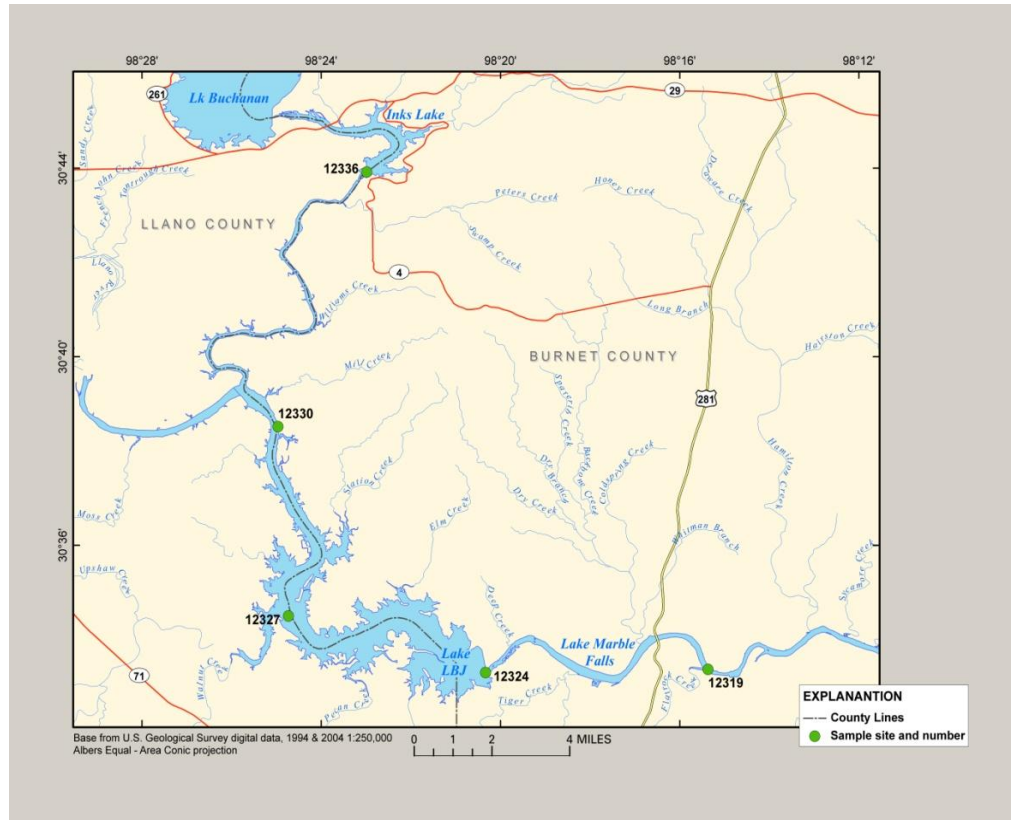


Figure 2.1 Study sites in Lakes Inks, LBJ, and Marble Falls, mid-Colorado River system, central Texas. The near-dam sites are 12336 (Inks Lake), 12324 (Lake LBJ), and 12319 (Lake Marble Falls).

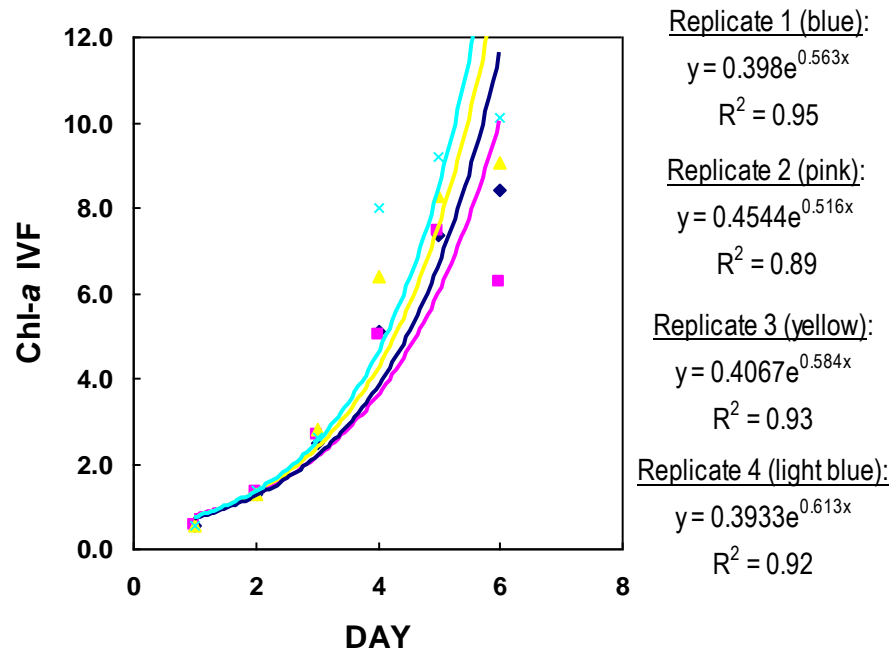


Figure 2.2 Phytoplankton bioassay, NP treatment (N=4), as measured by daily Chl a IVF for Lake LBJ near-dam site (12324) in September 2008. The exponential slope is the growth rate ( $r \text{ day}^{-1}$ ) for each replicate. For example, replicate 1 phytoplankton growth rate is  $0.563 \text{ day}^{-1}$ ,  $R^2 = 0.95$ .

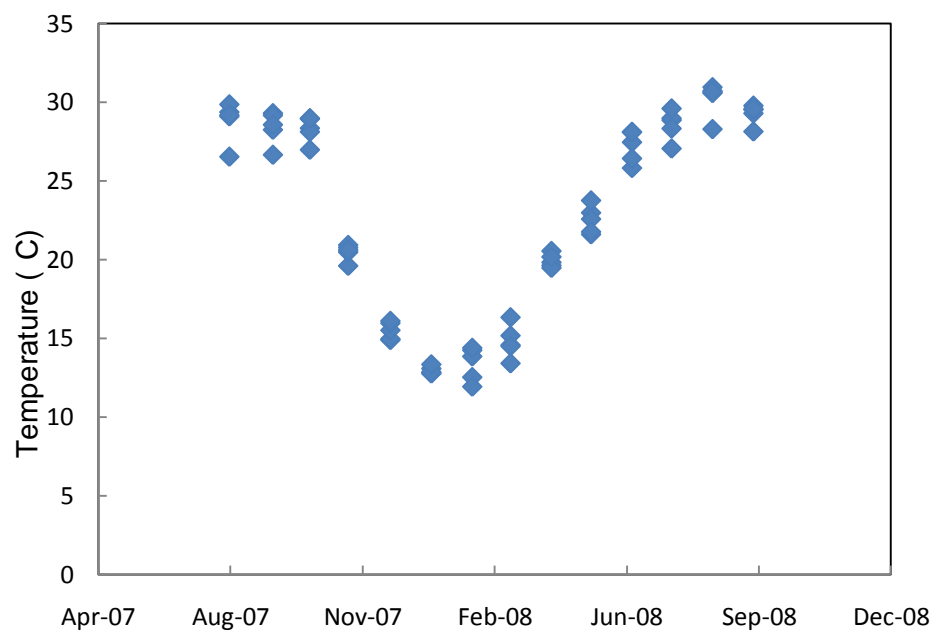


Figure 2.3 Near-surface (0.33 m) monthly temperatures (°C) from five sites in Lakes Inks, LBJ and Marble Falls, August 2007–September 2008.

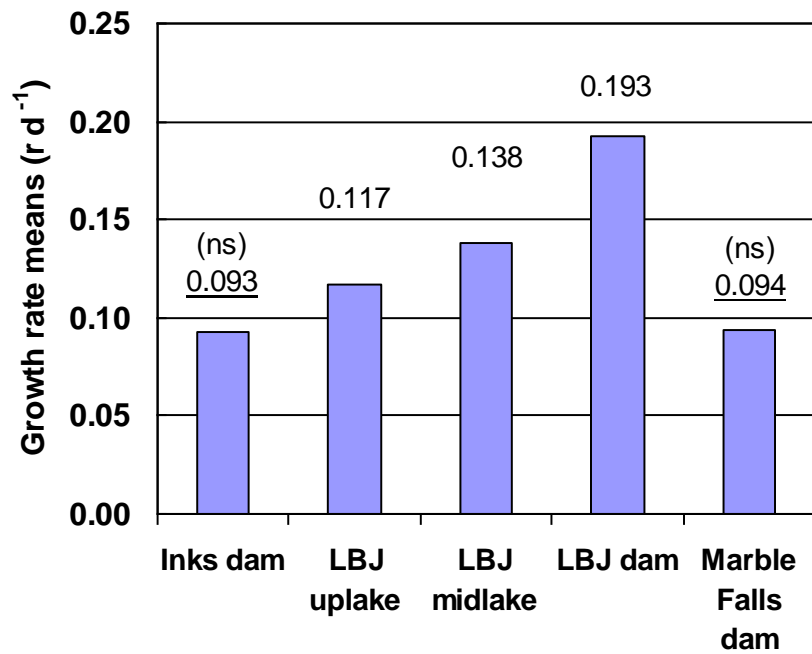


Figure 2.4 Phytoplankton growth rate ( $r\ d^{-1}$ ) least square means (LSM), all treatments combined ( $N = 56$ ) from five sites in Lakes Inks, LBJ and Marble Falls, arranged in downstream order (August 2007–September 2008).

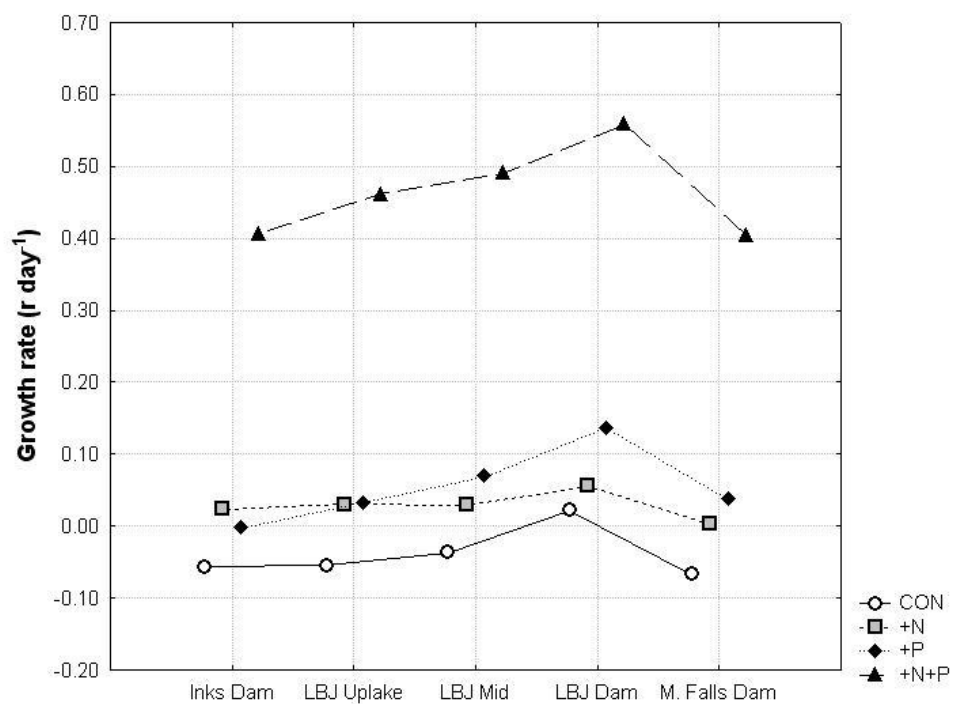


Figure 2.5 Phytoplankton growth rates ( $r \text{ d}^{-1}$ ) least square means (LSM) by site (N=56), arranged in downstream order, for each treatment with all months combined per treatment (August 2007–September 2008).

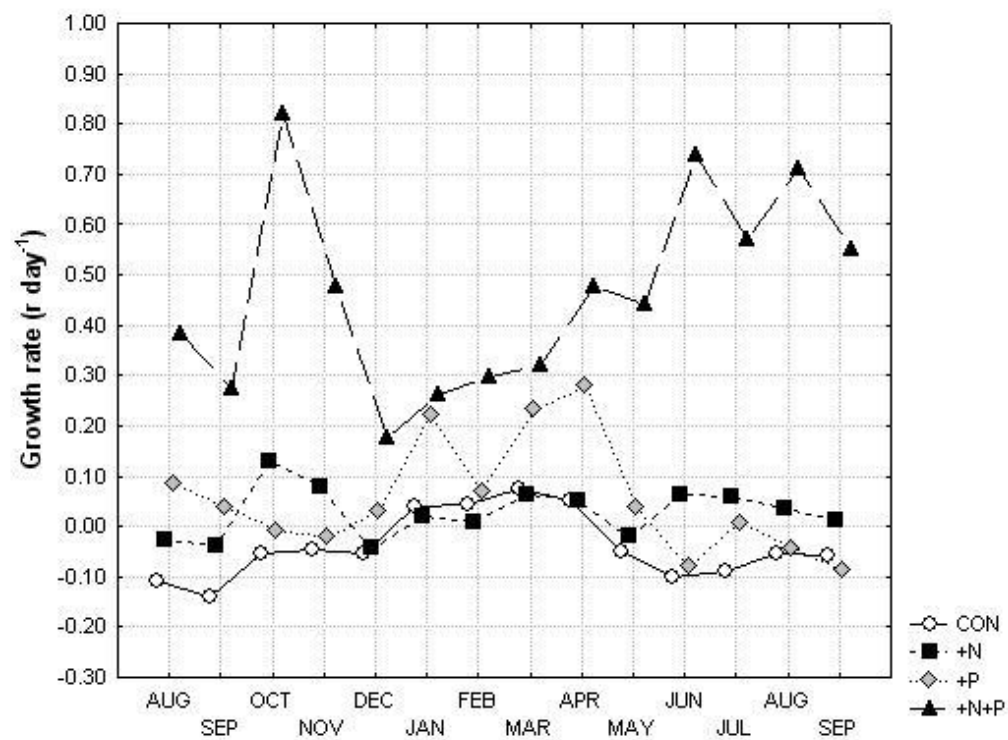


Figure 2.6 Monthly phytoplankton growth ( $r$  day<sup>-1</sup>) least square means (LSM) by treatment (August 2007–September 2008).

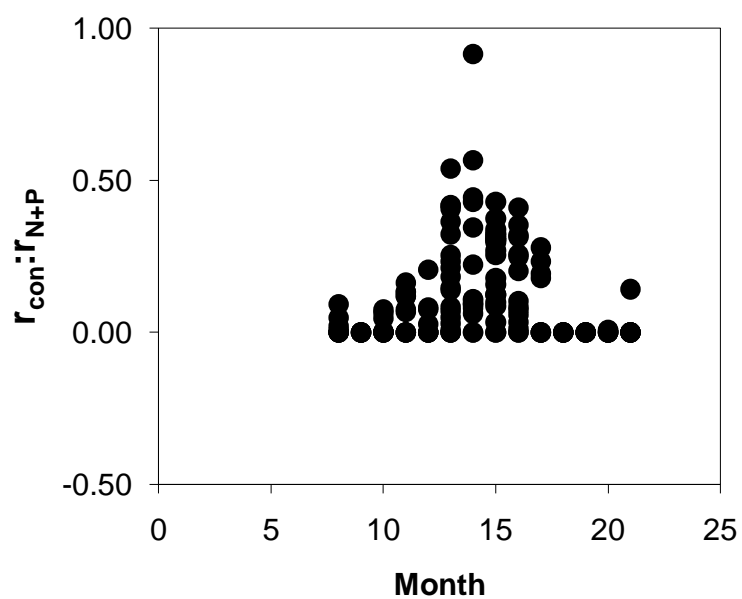


Figure 2.7 Phytoplankton growth rate ratios of the control and NP treatments ( $r_{con}:r_{NP}$ ) by month from all sites in Lakes Inks, LBJ and Marble Falls.



## CHAPTER 3

### SEASONAL ZOOPLANKTON GRAZING IMPACTS ON PHYTOPLANKTON GROWTH IN MESOTROPHIC RESERVOIRS, COLORADO RIVER SYSTEM, CENTRAL TEXAS

#### *Introduction*

Zooplankton communities shape in-lake processes directly through phytoplankton predation and selective grazing that result in loss of chlorophyll biomass from the photic zone (Quimaliños et al. 1998; Hulsmann et al. 2011) and ultimately can result in trophic status alteration. Seasonally, zooplankton grazing pressure can cause clear water phases (Lehman et al. 2007), affect phytoplankton succession (Brett et al. 1994; Adrian and Schneider-Olt 1999; Hambright et al. 2007), and contribute to nutrient regeneration (Sterner 1986; Glibert 1998; Wassman 1998; Vanni 2002; Pilati and Wurtsbaugh 2003), as well as to indirectly affect N- and P-limitation among algal communities (Elser et al. 1988 and 2007) and to alter resource competition dynamics between phyto- and bacterioplankton (Chrzanowski and Grover 2001).

A seasonal zooplankton grazing study was conducted using natural plankton assemblages from three hydrologically linked reservoirs of the Colorado River system in central Texas as part of a larger study to better understand reservoir plankton dynamics relative to potential changes in trophic status. After obtaining the seasonal grazing

estimates, I assessed grazing pressure by plotting the phytoplankton growth rates achieved in the controls against each respective experimental grazing rate by site and season. This approach is based on microzooplankton grazing studies from coastal marine and open ocean systems (Brainard 1996; Strom et al. 2001). Further, I discuss the results of my study relative to the concepts of *coherent* and *compensatory* phytoplankton dynamics (Vasseur et al. 2005). Coherent dynamics occurs during winter and spring when phytoplankton biomass loss factors are primary physically driven by light limitation and/or water column mixing, whereas compensatory dynamics occurs during summer and fall when nutrient limitation and/or grazing pressure are the dominant mechanisms for biomass loss from the photic zone (Vasseur et al. 2005).

## *Methods*

### Study site description

Three reservoirs were studied from August 2007–September 2008 and included five sites total: the near-dam site in Lake Inks, three sites in Lake Lyndon B. Johnson (LBJ) that span the reservoir's longitudinal axis, and the near-dam site in Lake Marble Falls, located within the mid-Colorado River system, in central Texas (Fig. 3.1).

### Natural phytoplankton assemblages

The grazing experiments were performed on the natural phytoplankton assemblages collected from each of the five sites. The surface water from each site initially was pre-sieved to remove the adult zooplankton, and is referred to as the <153 $\mu$ M phytoplankton assemblages.

### Live zooplankton field collection

Numerous quantities of zooplankton were needed for the experiments, yet they generally migrate to depth during the day to avoid predation by fish. Therefore, the deeper, near-dam sites of each reservoir were chosen for the collections, assuming that the relative proportions of dominant zooplankton species would be present at mid- and up-lake sites of Lake LBJ. Sequential non-quantitative vertical net tows were lowered to ~10m depth using a 76 $\mu$ M mesh conical plankton net with a 50cm diameter opening and a modified cod-end to prevent escape of the fast swimming copepods. The zooplankton collections were transferred into a carboy filled with surface reservoir water from each respective site to ensure adequate oxygen. The carboys were kept in the dark in the field, transported to the lab, then placed in a growth chamber with loosened caps to allow gas exchange, and

covered to reduce irradiance. The growth chamber temperature was set to the monthly surface Lake LBJ temperature (Table 3.1); and to daylight (L) and dark (D) hours of: 10L:14D (winter); 12L:12D (spring); and 14L:10D (summer).

#### Zooplankton density-gradient preparation

The grazing experiments were initiated within 24-48 hours of collection and based on a grazer density method, which assumes that phytoplankton mortality is directly proportional to zooplankton density (Lehman and Sandgren 1985). To prepare the density gradient for each reservoir, zooplankton were transferred from the appropriate field carboy and concentrated onto a 153 $\mu$ M sieve. The sieve was suspended in 0.45  $\mu$ m-filtered reservoir water, and 5-15ml aliquots of the concentrated zooplankton were added into ~25ml starting volume of <153 $\mu$ M phytoplankton assemblages from each reservoir site. A few experiments were performed in 125ml poly-bottles to compare with the results obtained from the smaller containers, with approximately 75ml starting volume and with proportionately more grazers added in each treatment.

The following treatments were prepared per site with 2-4 replicates each: control with ambient surface water containing phytoplankton in the <153 $\mu$ M fraction that contained no large zooplankton; '1X' with ~10 grazers added; '2X' with ~20 grazers added; and '3X' with ~30 grazers added. The 1-3X treatment designations are typically used in grazer-density arrays (Queimaliños et al. 1998). Nutrients were added as ~100  $\mu$ mol L<sup>-1</sup> [NO<sub>3</sub>] and ~15  $\mu$ mol L<sup>-1</sup> [PO<sub>4</sub>], respectively, to suppress phytoplankton nutrient uptake through regeneration.

Final zooplankton samples from each treatment (1-3X) were collected on a 80 $\mu$ M mesh sieve, backwashed into a vial with tap water and preserved with 4% formalin (final

volume). Experiments were performed in 50 ml sterile, loosely capped culture tubes, which enabled direct readings in the Turner 10-AU fluorometer for daily *in vivo* fluorescence (IVF), and allows the non-destructive growth measurements of chlorophyll a (Chl a). Additional initial and final extracted Chl a samples were determined as described below.

#### Phytoplankton biomass and growth rate determination

Initial and final Chl a samples were collected and analyzed to measure phytoplankton growth and to verify IVFs. The initial and final sample volumes, 125 and 50ml, respectively, were filtered by minimal vacuum pressure through 47mm (initial controls only) and 25mm (final) glass fiber filters (GF/Fs, 0.7µM pore size) for each final treatment. All filters were folded and stored in foil packets at -20°C for < 30d prior to analysis. The filters were thawed in the dark and placed into 7mm glass tubes containing 10ml reagent-grade methanol to extract the Chl a. The Chl a extracts were placed in racks and covered to prevent light degradation prior to analysis. A Turner TD700 fluorometer, equipped with specific excitation and emission filters, was used to determine total Chl a (Welschmeyer 1994).

Phytoplankton apparent growth rates (i.e. growth minus grazing losses expressed as  $r$  day<sup>-1</sup>) were calculated as in Adrian and Schneider-Olt (1999):

$$r = (\ln(N_f / N_i)) / T$$

Where  $N_f$  and  $N_i$  are the final and initial IVF or Chl a in the controls with no grazers >153 µM present, and in the presence of grazers (1-3X), assuming that phytoplankton growth

was the same across all treatments regardless of the presence or absence of grazers.  $T$  is the duration of the experiment in days.

#### Data analysis

Phytoplankton growth rates obtained from the initial and final IVFs were plotted for each experiment using linear regression analysis. The regression slope determines the community zooplankton grazing rate (Lehman and Sandgren 1985), which is negative when grazing occurs.

What defines a season in Texas? For this study, the author designated seasons based on reservoir water temperature changes (Table 3.1). The following months were grouped together accordingly: late summer, September–October 2007; fall, November 2007; winter, January 2008; spring, April 2008; early summer, May 2008; and late summer, August–September 2008. Simple regressions were performed on each individual experiment to determine the grazing rate and are discussed relative to the phytoplankton growth rates achieved in all of the control treatments (0X) by season and site.

## *Results*

### Ambient reservoir conditions

Reservoir temperature ranged from 12.8 to 30.6°C and light from 33 to 1858  $\mu\text{Em}^2\text{s}^{-1}$ ; ambient Chl a ranged from 4.0 to 25.7  $\mu\text{g L}^{-1}$  across all sites during the study period (Table 3.1). Two broad groups of zooplankton, cladocerans and copepods, were present from all sites throughout the study and were also the dominant members of the zooplankton in all of the grazing experiments (Table 3.1).

### Zooplankton grazing estimates

Zooplankton grazing estimates by simple linear regression analysis were significant ( $p < 0.05$ ) at all but two sites throughout the study period (Table 3.2). The zooplankton grazing rates analyzed together by a multiple linear regression and subsequent analysis of variance (ANOVA) were significant.

### Seasonal growth and grazing comparisons

#### *Late summer season, 2007*

Paired growth rates from the control treatment ( $r \text{ d}^{-1}$ ) and grazing rates ( $G \text{ d}^{-1}$ ) (table 3.1) were nearly equal in the first summer season as shown by the proximity of the data to the 1:1 diagonal line (Fig. 3.2), including Lake LBJ midlake site (0.52 and 0.53  $\text{d}^{-1}$ , respectively) and Inks Lake near-dam site (0.18 and 0.20  $\text{d}^{-1}$ , respectively).

#### *Fall and winter seasons, 2007-08*

The grazing rates were generally higher than phytoplankton growth rates in the fall. Two exceptions were from Lake LBJ mid- and uplake sites, where growth equaled and exceeded grazing, respectively (Fig 3.3). In winter, zooplankton grazing was either

equal or exceeded phytoplankton growth from most sites except from Inks Lake (Fig 3.4).

*Spring and early to late summer, 2008*

Phytoplankton growth exceeded zooplankton grazing from most sites in spring (Fig. 3.5). By early summer, grazing exceeded phytoplankton growth rates from most sites (Fig. 3.6). By late summer, phytoplankton growth exceeded grazing from all sites except for the mid- and near-dam Lake LBJ sites in August 2008 (Fig. 3.7).



## *Discussion*

In a total of 35 experiments, zooplankton grazing rates ranged from 0.05 to 0.52 day<sup>-1</sup>, and control (with no grazers added) phytoplankton growth rates ranged from -0.04 to 0.53 day<sup>-1</sup>. Grazing rates exceeded or equaled phytoplankton growth in 60% of the experiments (Table 3.2). The zooplankton grazing rates analyzed together by a multiple linear regression were significant from all sites and dates.

The plankton dynamics changed seasonally. In the first summer season phytoplankton growth and zooplankton grazing were equal. During the following spring, phytoplankton growth exceeded grazing, which was followed by substantial grazing the following summer. This indicates alternating seasonal plankton dynamics: tight coupling between primary producers and consumers and substantial top-down control of phytoplankton biomass by zooplankton; and intermittent pulses when phytoplankton growth exceeds grazing.

In terms of grazing dynamics in a reservoir system that is strongly nutrient-limited, the seasonal alternation seen in this study corroborates the concept of coherent and compensatory growth and grazing dynamics (Fig. 1 in Vasseur et al. 2005). Although light usually is not limiting in the upper mixed layers of these reservoirs, strong wind-mixing events occur that may influence coherent dynamics from a physical perspective. From a biological perspective, relaxed grazing pressure may influence this dynamic as well.

In the first summer season, growth and grazing rates were equal from two sites (in Fig. 3.2, Lakes Inks & LBJ's midlake sites), also which occurred from Lake LBJ's midlake site in fall and winter (Fig. 3.3 & 3.4, respectively). The seasons when growth

exceeded grazing may be interpreted as coherent dynamics, which prevailed in spring and late summer (Figs. 5 & 7, respectively). Alternatively, compensatory dynamics prevailed in fall, winter, and early summer when the zooplankton grazing rate exceeded the community phytoplankton growth rate (Figures 3.3, 3.4 & 3.6, respectively).

### Study significance

Copepod-dominated zooplankton communities significantly regulated phytoplankton biomass through strong grazing pressure in the Highland reservoirs from fall through early summer as compared to spring and the following late summer when grazing pressure relaxed relative to the control phytoplankton growth rates in the absence of grazers  $>153\mu\text{M}$ . Grazing pressure influenced phytoplankton growth dynamics, and I propose that the alternation between compensatory and coherent growth may be seen in differences among dominant phytoplankton species throughout the year as well. This idea can be tested with further analysis of the preserved phytoplankton samples collected from these reservoirs and upon termination of the nutrient bioassays in a related study by the author (Wallace Ch. 2).

Significant seasonal zooplankton grazing impacts on phytoplankton growth occurred in this study that demonstrates that the phytoplankton is not influenced solely by bottom-up factors in mesotrophic reservoir systems. In lakes of contrasting trophic status, i.e. either oligo- or eutrophic, the opposite trend is seen (Zhang et al. 2007 and references therein), based on physical forcing as well as zooplankton-phytoplankton interactions.

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Table 3.1 Reservoir near-surface (0.33 m) temperature, light, and biomass<sup>1</sup>.

Zooplankton groups, cladoceran and copepod, were collected from vertical tows<sup>2</sup>.

Designated seasons separated by shaded line (Example: Season 1 included Sep.-Oct. 2007).

[Temp., temperature; uE, microEinstein; m, meter; s, second; #, number; ug, microgram; l, liter]

Date	Site	Temp. (°C)	Light (uE m <sup>-2</sup> s <sup>-1</sup> )	Cladoceran (#)	Copepod (#)	Chl a (ug l <sup>-1</sup> )
9/11/07	LBJ near-dam	29.10	33.3	10	41	9.5
10/3/07	LBJ midlake	28.35	1000.0	10	31	14.4
10/3/07	Inks near-dam	26.98	570.7	44	64	15.6
11/7/07	Marble Falls dam	20.61	243.4	118	315	4.3
11/6/07	LBJ near-dam	20.93	117.7	54	164	12.1
11/6/07	LBJ midlake	20.48	106.7	27	124	5.9
11/6/07	LBJ uplake	20.77	84.7	37	98	4.9
11/7/07	Inks near-dam	19.61	130.1	63	132	26.0
1/9/08	Marble Falls dam	13.35	1406.0	229	12	2.3
1/8/08	LBJ near-dam	12.87	113.4	290	190	2.2
1/8/08	LBJ midlake	12.79	464.8	143	38	2.4
1/8/08	LBJ uplake	13.09	299.1	320	93	3.2
1/9/08	Inks near-dam	12.78	1162.0	126	9	19.6
4/2/08	Marble Falls dam	20.18	1540.0	650	312	2.9
4/1/08	LBJ near-dam	20.55	1096.0	317	301	2.0
4/1/08	LBJ midlake	19.48	348.4	880	188	3.1
4/1/08	LBJ uplake	19.83	566.1	198	270	3.0
4/2/08	Inks near-dam	19.63	187.7	188	84	7.2
5/7/08	Marble Falls dam	23.76	1858.0	955	71	2.5
5/6/08	LBJ near-dam	21.77	382.5	699	454	2.7
5/6/08	LBJ midlake	22.58	482.1	1185	145	5.7
5/6/08	LBJ uplake	22.98	791.1	357	25	7.8
5/7/08	Inks near-dam	21.61	83.6	333	191	8.8
8/6/08	Marble Falls dam	30.64	1552.0	78	146	15.5
8/5/08	LBJ near-dam	30.58	725.2	44	246	12.2
8/5/08	LBJ midlake	30.72	720.6	56	139	10.7
8/5/08	LBJ uplake	30.95	1509.0	55	38	7.4
8/6/08	Inks near-dam	28.29	885.7	145	202	10.1
9/3/08	Marble Falls dam	29.58	1216.0	67	119	15.9
9/2/08	LBJ near-dam	29.52	541.9	59	149	10.3
9/2/08	LBJ midlake	29.78	1161.0	58	20	13.4
9/2/08	LBJ uplake	29.29	1566.0	138	26	8.0

<sup>1</sup> Physical data & zooplankton collected and provided by the Lower Colorado River Authority.

<sup>2</sup> Chl a determined by author (see Methods). Zooplankton were counted by author & are raw totals, comprised of 3 to 5 vertical tows, 2-18 m, using a 153uM mesh Wisconsin® net.

Table 3.2 Phytoplankton growth in control treatment and zooplankton grazing rates in Lakes Inks, LBJ, and Marble Falls (2007-08). Seasons are separated by colored lines. [#, number; con, control; G, grazing; d, day; df, degrees of freedom; reg., regression; sig, significant]

Date	Site	control (r d <sup>-1</sup> )	grazing (G d <sup>-1</sup> )	total df	reg. sig. F	reg. R <sup>2</sup>
9/21/07	LBJ near-dam	0.10	0.05	4	0.11	0.63
10/3/07	LBJ midlake	0.53	0.52	7	0.01	0.70
10/3/07	Inks near-dam	0.18	0.20	7	0.00	0.86
11/17/07	Marble Falls dam	0.03	0.11	15	0.00	0.75
11/17/07	LBJ near-dam	0.07	0.09	13	0.00	0.66
11/17/07	LBJ midlake	0.10	0.10	11	0.00	0.82
11/17/07	LBJ uplake	0.16	0.13	11	0.00	0.67
11/17/07	Inks near-dam	0.08	0.18	15	0.00	0.67
1/13/08	Marble Falls dam	0.22	0.36	5	0.03	0.75
1/13/08	LBJ near-dam	0.24	0.26	7	0.03	0.58
1/13/08	LBJ midlake	0.16	0.15	7	0.01	0.73
1/13/08	LBJ uplake	0.07	0.13	7	0.01	0.68
1/13/08	Inks near-dam	0.48	0.23	5	0.03	0.58
4/4/08	Marble Falls dam	0.23	0.20	6	0.03	0.65
4/4/08	LBJ near-dam	0.28	0.16	5	0.00	0.93
4/4/08	LBJ midlake	0.16	0.18	7	0.00	0.90
4/4/08	LBJ uplake	0.28	0.10	7	0.00	0.79
4/4/08	Inks near-dam	0.29	0.15	7	0.01	0.75
5/11/08	Marble Falls dam	0.01	0.44	7	0.00	0.97
5/14/08	LBJ near-dam	0.15	0.27	5	0.04	0.71
5/11/08	LBJ midlake	0.25	0.18	4	0.01	0.90
5/14/08	LBJ midlake	0.06	0.27	7	0.00	0.87
5/11/08	LBJ uplake	0.13	0.18	7	0.00	0.79
5/14/08	LBJ uplake	-0.04	0.37	5	0.01	0.81
5/11/08	Inks near-dam	0.13	0.51	7	0.00	0.87
5/14/08	Inks near-dam	0.10	0.25	7	0.01	0.73
8/7/08	Marble Falls dam	0.09	0.05	7	0.04	0.54
9/6/08	Marble Falls dam	0.30	0.06	6	0.06	0.54
8/7/08	LBJ near-dam	0.05	0.18	7	0.00	0.85
9/6/08	LBJ near-dam	0.24	0.14	7	0.01	0.68
8/7/08	LBJ midlake	0.07	0.15	7	0.01	0.68
9/6/08	LBJ midlake	0.33	0.15	7	0.00	0.81
8/7/08	Inks near-dam	0.09	0.05	7	0.02	0.65
8/7/08	LBJ uplake	0.35	0.22	5	0.03	0.73
9/6/08	LBJ uplake	0.44	0.07	7	0.04	0.54

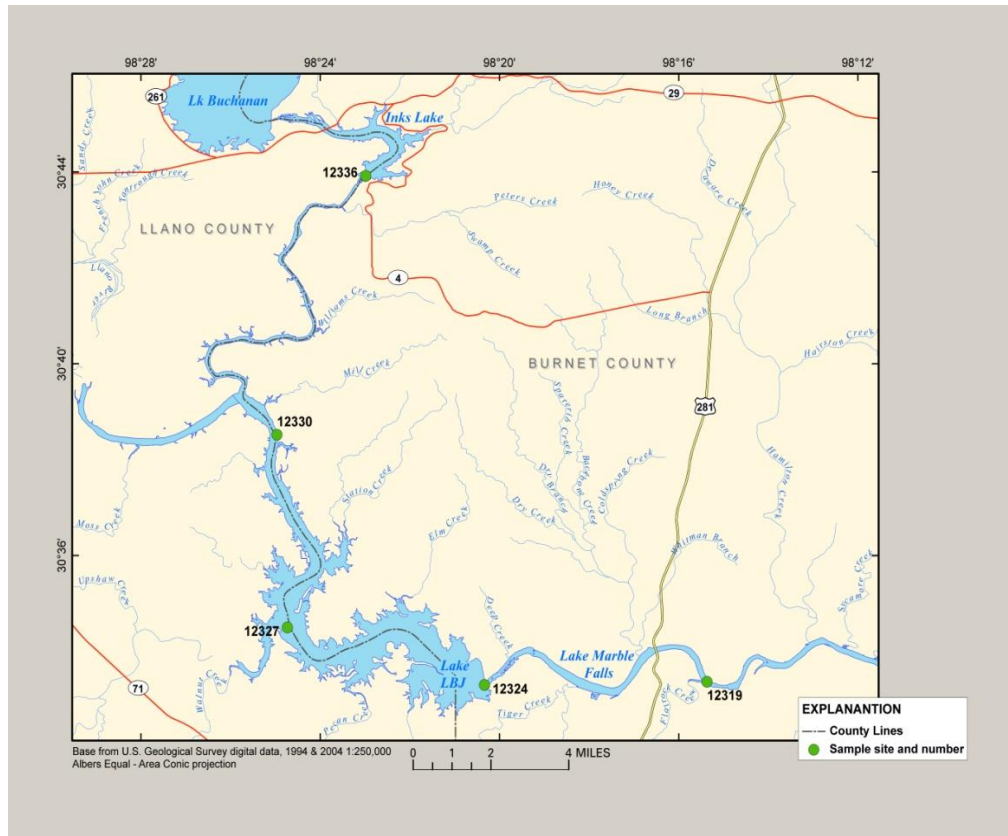


Figure 3.1 Study sites in Inks Lake, Lake Lyndon B. Johnson (LBJ), and Lake Marble Falls, mid-Colorado River system in central Texas. [Lake sites: Inks near-dam, 12336; LBJ uplake, 12330; LBJ midlake, 12327; LBJ near-dam site is 12324; Marble Falls near-dam, 12319.]



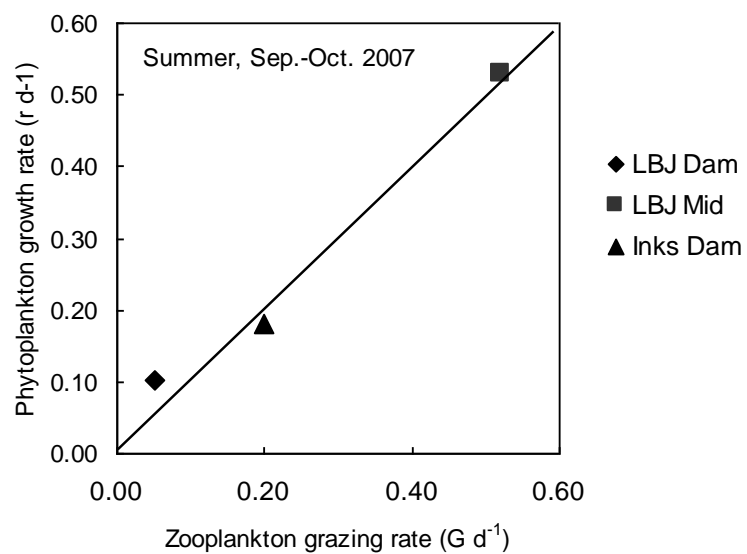


Figure 3.2 First summer season with phytoplankton growth rate ( $r \text{ d}^{-1}$ ) in the control treatment compared to each respective grazing rate ( $G \text{ d}^{-1}$ ) by site (September–October 2007).

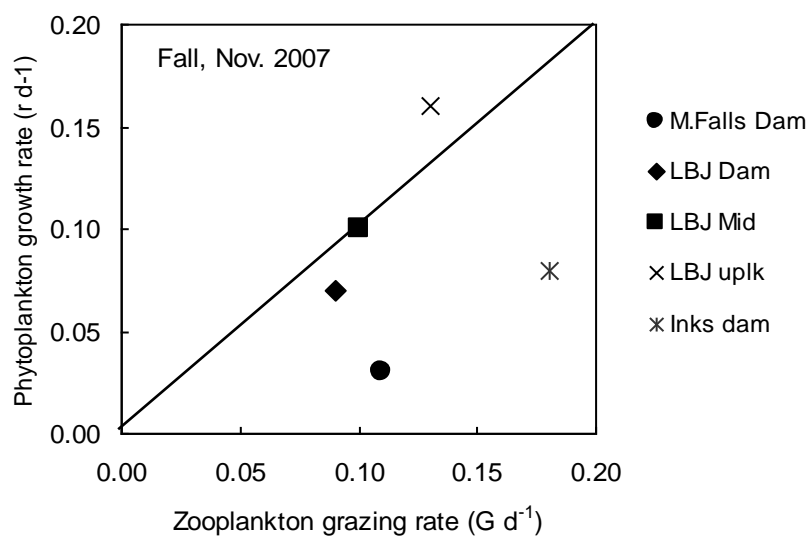


Figure 3.3 Fall season with phytoplankton growth rate ( $r \text{ d}^{-1}$ ) in the control treatment compared to each respective grazing rate ( $G \text{ d}^{-1}$ ) from all five sites (November 2007).

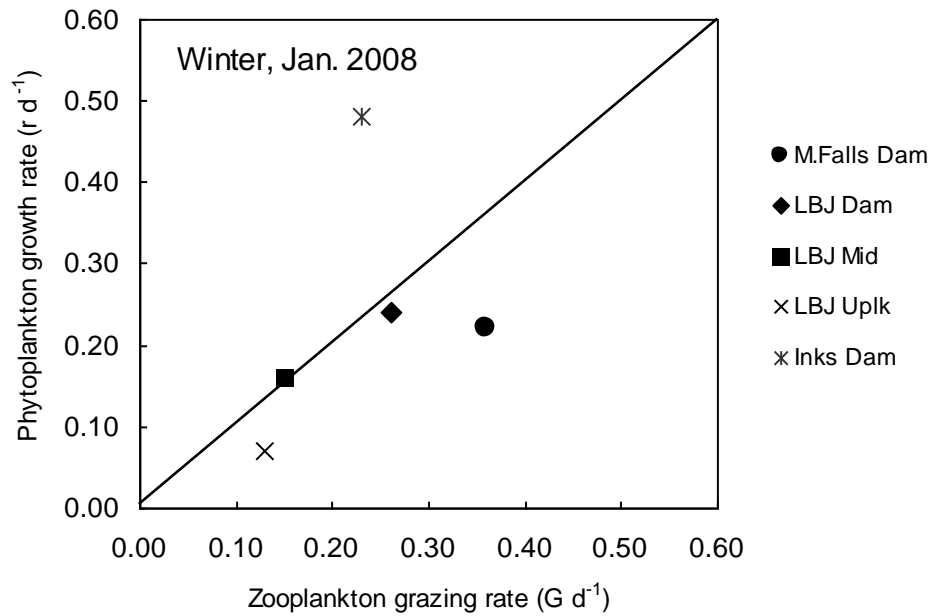


Figure 3.4 Winter season with phytoplankton growth rate ( $r \text{ d}^{-1}$ ) in the control treatment compared to each respective grazing rate ( $G \text{ d}^{-1}$ ) from all five sites (January 2008).

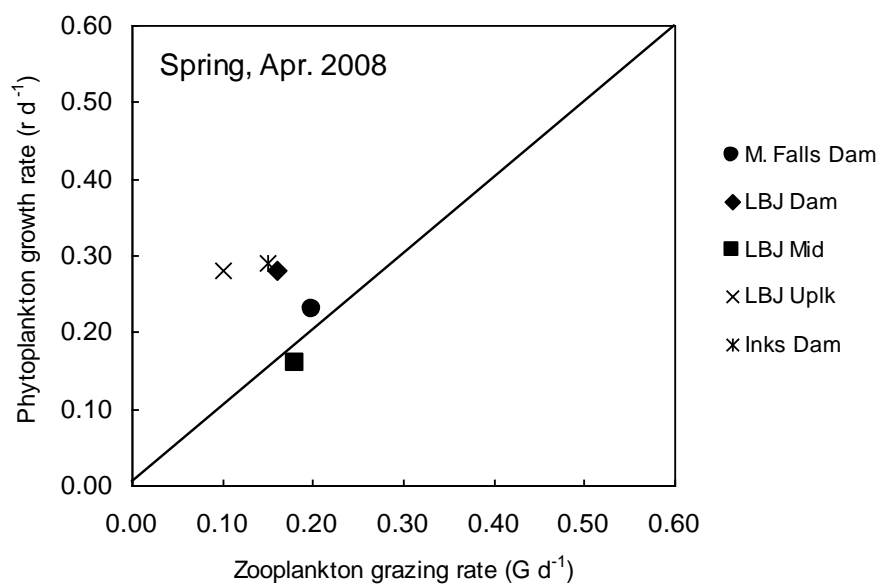


Figure 3.5 Spring season with phytoplankton growth rate ( $r \text{ d}^{-1}$ ) in the control treatment compared to each respective grazing rate ( $G \text{ d}^{-1}$ ) from all five sites (April 2008).

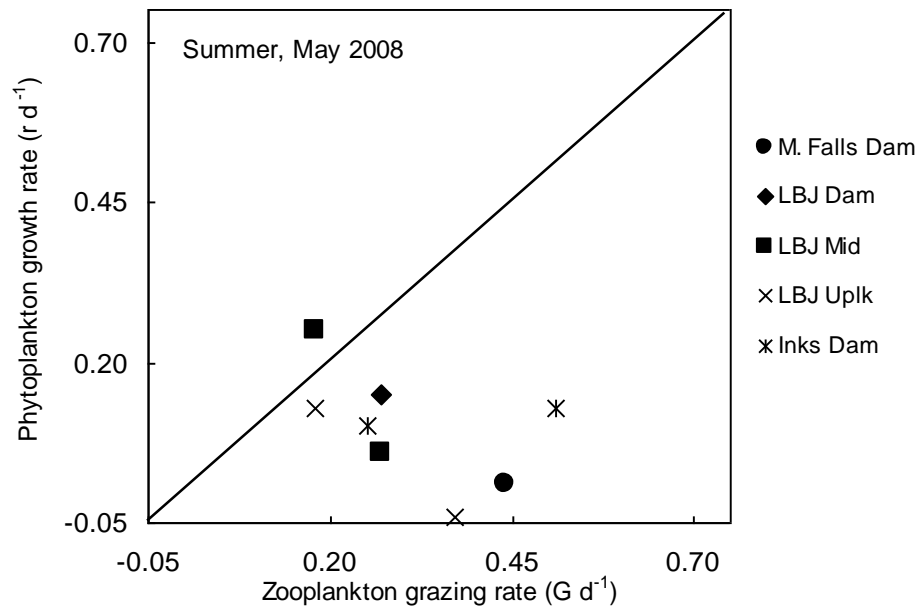


Figure 3.6 Summer season with phytoplankton growth rate ( $r \text{ d}^{-1}$ ) in the control treatment compared to each respective grazing rate ( $G \text{ d}^{-1}$ ) from all five sites (May 2008).

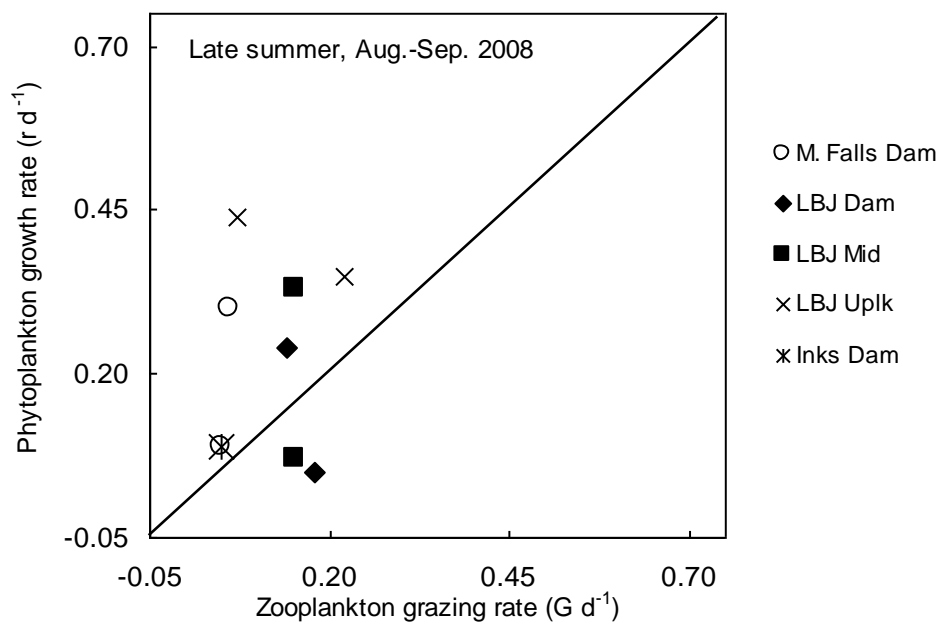


Figure 3.7 Late summer season with phytoplankton growth rate ( $r \text{ d}^{-1}$ ) in the control treatment compared to each respective grazing rate ( $G \text{ d}^{-1}$ ) from all five sites (August–September 2008).

## CHAPTER 4

### PHYTOPLANKTON AND RESERVOIR NUTRIENT STOICHIOMETRY, COLORADO RIVER SYSTEM, CENTRAL TEXAS

#### *Introduction*

The nitrogen to phosphorus ratio (N:P) similarities between marine phytoplankton from the upper ocean and dissolved inorganic seawater samples collected from depth have inherently linked oceanic biological and geochemical processes with primary producers in the ocean system: a biogeochemical concept first proposed by Redfield (1934 and 1958 in Falkowski 2000). Further, the Redfield ratio of  $C_{106}:N_{16}:P_1$  has become an indicator of optimal cell growth under nutrient-replete conditions. Recently, a physiological mechanism was proposed (Loladze and Elser 2011) to explain the intracellular conservation of Redfield ratios, essentially as a surrogate for protein: rRNA ratios (Raven 2011), such that N-limitation constrains protein synthesis rates, and P-limitation constrains RNA production that result in N:P ratios below or above Redfield, respectively. Either form of limitation results in decreased growth rates in phytoplankton. This mechanistic view of Redfield ratio departures helps us to understand potential consequences of nutrient imbalances in aquatic food webs as a form of constraint upon the phytoplankton as primary producers that consequently affects the

secondary consumer's nutritional requirements through poor prey quality (Acharya et al. 2004; Siuda & Dam 2010).

Optimal phytoplankton N:P ratios now include a range of values among community-level measurements of phytoplankton as additional aquatic studies have emerged (Hecky et.al 1993; Guildford and Hecky 2000; Bergstrom 2010). Many individual species of marine phytoplankton showed significant departures in Redfield N:P ratios (Geider and La Roche 2002). For this study's purposes, Heckey et al. (1993) is utilized as freshwater guideposts.

The question explored here is *what are the ranges of particulate nutrient ratios in a nutrient co-limited freshwater system?* Here, the relational changes of ambient and nutrient-enriched phytoplankton chlorophyll a (Chl a), as an indicator of biomass, as well as phytoplankton growth rates, were compared with the particulate nutrient ratios determined from a low nutrient subtropical reservoir system that exhibited a general pattern of N-limitation during stratified months (summer), P-limitation during months (winter, early spring) when the vertical column was full mixed, and also included monthly patterns of co-limitation for both N and P (year-round).



## *Methods*

### Field sampling and ambient Chl a and particulate analyses

Near surface (0.3 m), whole water samples were collected in 1L pre-rinsed polypropylene containers on a monthly basis (August 2007–September 2008). The 1L samples were stored at ambient temperature and out of direct sunlight during transport to the lab within 12 h of collection. After gentle mixing, 50-100ml aliquots were filtered for ambient Chl a under low vacuum pressure ( $\leq 10$ psi) through 25mm GF-F filters (0.7mm nominal pore size). The filters were then folded in half, placed in foil packets, and frozen ( $-20^{\circ}\text{C}$ ) prior to analysis. Upon analysis, Chl a filters were extracted using in 10ml methanol, kept in the dark, and determined using a Turner TD-700® fluorometer equipped with a blue lamp and excitation (436nm) and emission (680nm) filters designed to fluoresce and detect Chl a (Welschmeyer 1994). Calibration was performed with Chl a range of standards ( $1$  to  $50\mu\text{g L}^{-1}$ ) and verified using a Turner® solid Chl a standard.

For total particulate C (TPC) and N (TPN), three replicate aliquots (50ml) were filtered through pre-baked GF-F filters with 0.7mm nominal pore size. Filters were folded, placed in foil packets and frozen ( $-20^{\circ}\text{C}$ ) prior to shipping on ice to the USGS National Water Quality Laboratory (NWQL) for analysis (US EPA 1997). Total particulate P (TPP) was calculated by subtracting total dissolved P from total P. Total P concentrations that were less than the minimum detection limit were estimated as the value of half of the reporting limit. Particulate C, N, and P concentrations were used to calculate stoichiometric (elemental) molar ratios of C:N, N:P, and C:P by dividing the

concentration of the first constituent by the concentration of the second constituent for each ratio.

#### Final experimental Chl a and particulate analyses

Chl a was analyzed from all of the nutrient bioassay experiments as described previously (Wallace Ch. 2). An additional subset of particulate C and N were analyzed from all five sites upon termination of the nutrient bioassays from five dates throughout the study: collected quarterly to represent seasons, and referred to as *final* Chl a and particulate CN ratios (N=25 per parameter). The dates of the final samples were in October and December 2007, which corresponded to fall and winter seasons; and in March, May, and August 2008, which corresponded to spring, and early and late summer, respectively.

#### Ambient post-data analyses

The Chl a and particulate ratios were removed from the data set of five dates and three sites, respectively. The reason is that the N:P and C:P ratios were extreme outliers due to the low concentration of P, and the excluded data were: Lake LBJ's uplake site (December 2007 & June 2008); Lake LBJ's midlake site (November 2007); and Lake Marble Falls near-dam site (November and December 2007). Thus, total N=65 for each parameter: Chl a; C:N; N:P; and C:P. The data were tested for normality and linear regressions were performed.

#### Final post-data analyses

Calculated phytoplankton growth rates from the four treatment groups (Wallace Ch. 2) were compared to the particulate C:N ratios. These values were analyzed by both

near regression and by analysis of variance (ANOVA). The Bonferroni test was used for post-hoc comparisons between sites and seasons for significant ANOVAs.

## *Results*

### Ambient Chl a and C:N:P ratios

Normality plots of each parameter showed that the data were slightly negatively skewed (Appendix 4.1 a-d). Of the ambient Chl a, 72 % of the values ranged between 1.7–10.7  $\mu\text{g L}^{-1}$  (Appendix 4.1 a). The majority of carbon to nitrogen (C:N) ranged between 7.4–9.8 and the nitrogen to phosphorus (N:P) molar ratios ranged between 17–36 (Appendix 4.1 b-c, respectively); and the majority of C:P molar ratios ranged between 175–295 (Appendix 4.1 d).

Ambient Chl a concentrations differed between sites by nonparametric ANOVA ( $p < 0.05$ ; Fig. 4.1). The lowest and highest median Chl a were from Lake LBJ and Lake Inks near-dam sites (4.45 & 10.27  $\mu\text{g L}^{-1}$ , respectively). Lakes LBJ uplake and Marble Falls sites' median Chl a were 7.66 and 7.00  $\mu\text{g L}^{-1}$ , respectively, which both were approximate to the overall median of 7.8  $\mu\text{g L}^{-1}$ .

Ambient, median particulate nutrient ratios did not differ significantly between sites (Figures 4.2-4.4). Median C:N ratios ranged between 8.6–9.6 among sites (Fig. 4.2). Median N:P ratios ranged between 27.5–32.3 (Fig. 4.3) and the median C:P molar ratios ranged between 222–273 (Fig. 4.4). Linear regression results were significant ( $p < 0.05$ ) for each ratio as a function of Chl a (Figures 4.5-4.7), with a slight negative trend shown for C:N with increasing Chl a (Fig. 4.5), and slight positive trends shown for both N:P and C:P (Figures 4.6-4.7, respectively). Also, none of the particulate results presented here were corrected for detrital contribution, based on the underlying assumption that [C:Chl a] is constant among samples from each site, according to Hecky et al. (1993).

### Final Chl a and CN ratios

The final Chl a and CN ratios are plotted in upstream order by site and date (Fig. 4.8). With nutrient enrichment, the Chl a biomass reached eutrophic concentrations, ranging from 49–109  $\mu\text{g L}^{-1}$ , in the NP treatment during the fall and summer seasons of 2007-08 (Fig. 4.8).

The final C:N ratios differed significantly between months (Fig. 4.9), but not between sites (Appendix 4.1 d). By month, the post-hoc comparisons of each treatment yielded significantly different C:N ratios in three of the four nutrient bioassay treatments as follows. The control treatment's final mean C:N ratios were higher from winter (December 2007) through spring and early summer (March & May 2008, respectively) as compared to the late summer season (Fig. 4.9). The NP treatment's final mean C:N ratio of 10.3 in spring was higher than in all other seasons; as well as the N treatment's final mean C:N ratio of 11.2 in spring that was higher compared to the fall and summer seasons (Fig. 4.9).

### Phytoplankton growth rates and C:N ratios

Ambient phytoplankton growth rates and their respective C:N ratios are summarized by site and month in Appendix 4.1 f. The control treatment growth rates were plotted as a linear function of the ambient C:N ratios for each site, and significant relationships were found in only two near-dam sites, Lakes LBJ and Marble Falls (N=14, each site;  $p < 0.01$ ; Fig. 4.10). Subsequent linear regressions were performed on the seasonal, final phytoplankton growth rates from each of the bioassay treatments and their respective final C:N ratios (N=25 each treatment): only the +N treatment yielded a significant relationship ( $p < 0.05$ ) (Fig. 4.11).

## *Discussion*

Although the Chl a and particulate nutrient ratios were slightly negatively skewed, the author chose to analyze the data directly without transformation because the majority of the data were normally distributed (Appendix 4.1 a-d). The majority of the Chl a values indicate that all three reservoirs are of mesotrophic status (US EPA 2010).

The correlation coefficients ( $r$ ) in each of the regressions (Figs. 4.5-4.7) for the particulate ratios as a function of Chl a concentrations were low, indicating that the variation in these nutrient ratios is independent of phytoplankton biomass. One plausible explanation is that the nutrients in each of the ratios are partitioned differently among dominant phytoplankton species on either temporal or spatial scales, as all sites and dates were included in figs. 4.5-4.7, and Chl a differed spatially and with nutrients (Figs. 4.1 & 4.10, respectively), whereas the C:Ns differed temporally and with nutrients (Fig. 4.9).

The overall means of the particulate C:N, N:P, and C:P ratios were 9.3, 35.3, and 317, respectively, and were higher than Redfield averages, which may be attributed mainly to water residence time (WRT) that affects nutrient cycling (Hecky et al. 1993): e.g. shallow lakes with short WRT typically have N:P and C:P ratios less than 26 and 350, respectively, which is reflective of riverine influence. Only one site, Lake Marble Falls near-dam site had ratios that were riverine-like, with N:P and C:P means equal to 25.5 and 230, respectively. Conversely, the other four sites in this study had mean ratios comparable to nutrient ratios in lakes with longer residence times, with N:P and C:P ratios greater than 30 and 400, respectively. One of four sites was Inks Lake, a smaller reservoir, yet one with a longer mean WRT than in Lake Marble Falls (Table 4.1).

In terms of nutrient limitation, C:N ratios between 8.3–14.6 indicate moderate N limitation; and N:P ratios >22, as well as C:P ratios >258, both indicate severe P limitation (Table 4.1 in Hecky et al. 1993), which corroborate the nutrient limitation patterns documented in these reservoirs, as shown here in the particulate nutrient ratios, and also by the nutrient-dependent growth responses during the same study (Table 2.4 in Wallace Ch. 2). Furthermore, the seasonal fluctuation of the final C:N ratios from each of the bioassay nutrient treatments (Fig. 4.9) indicates the temporal variability inherent in the analysis of community-level phytoplankton dynamics, relative to the potential of increasing nutrient loadings into these reservoirs.

One may test whether or not the growth rate hypothesis (GRH) (Sterner and Elser 4.11 corroborate the GRH in a general sense, with significant trends relative to growth rate and C:N ratio. The near-dam site in Lake LBJ had the highest growth rate for that reservoir and the lowest C:N ratio (Appendix 4.1 d), which follows the expected GRH trend: as growth rates increase, the C:N ratios typically should decrease.

Of greater interest to the author is to measure particulate CNP ratios on secondary consumers (zooplankton), before and after grazing on ambient versus nutrient replete phytoplankton assemblages, to further elucidate nutritional requirements among consumers in nutrient limited systems, and the amount of omnivory and prey selection that is occurring among the copepod-dominated communities in mesotrophic reservoirs (Wallace Ch. 3).

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- Wallace, M. A. Chapter 3. Seasonal zooplankton grazing impacts on phytoplankton growth in mesotrophic reservoirs, Colorado River system, central Texas. Texas State University-San Marcos. Dissertation.

Table 4.1 Theoretical water residence time (WRT) by reservoir ('--' for multiple sites in one reservoir), and ambient, mean C:N, C:P, N:P ratios ( $\mu\text{M}$ ) by site from upstream to downstream order in the mid-Colorado River, Texas (Aug. 2007–Sep. 2008).

Site	WRT (d)	C:N	C:P	N:P
Inks Dam	93.3	8.9	549	61
LBJ uplake	--	9.4	494	49
LBJ midake	--	9.6	1258	133
LBJ Dam	139.4	9.3	548	61
Marble Falls Dam	7.2	9.3	230	26

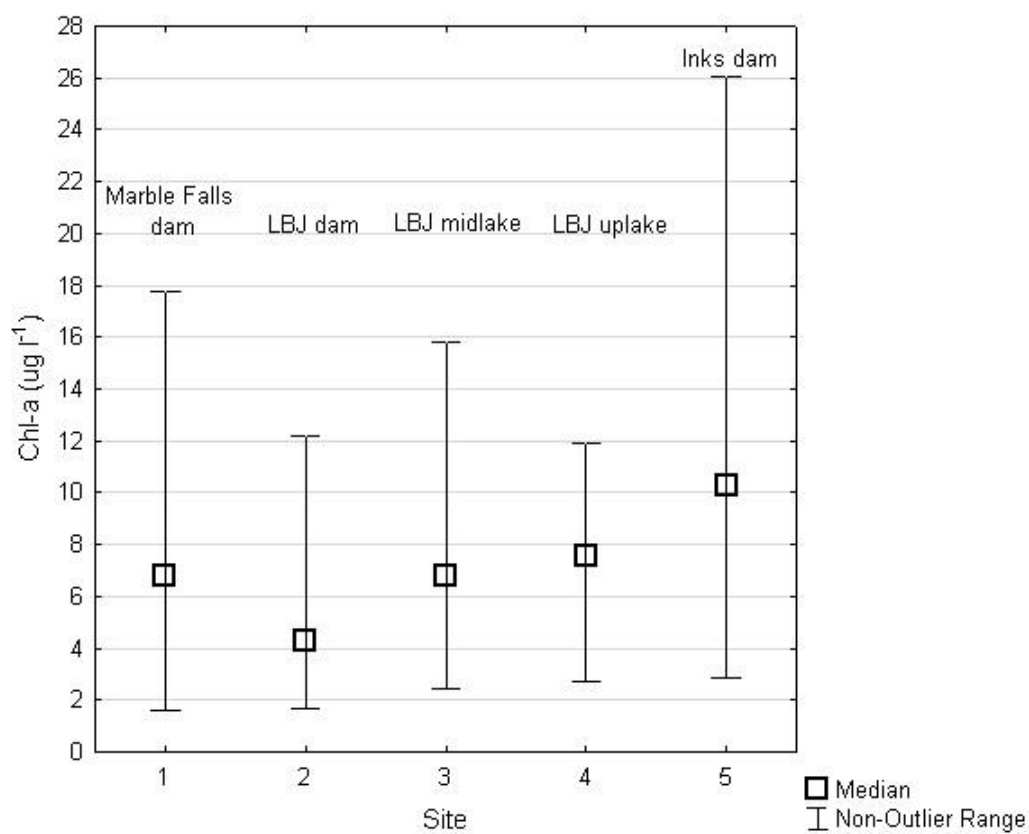


Figure 4.1 Ambient, median Chl a ( $\mu\text{g L}^{-1}$ ) from five sites in three reservoirs arranged (left to right) in upstream order, Colorado River system, Texas (August 2007–September 2008:  $N=65$ ;  $F(4,60)=3.2$ ;  $p<0.05$ ).

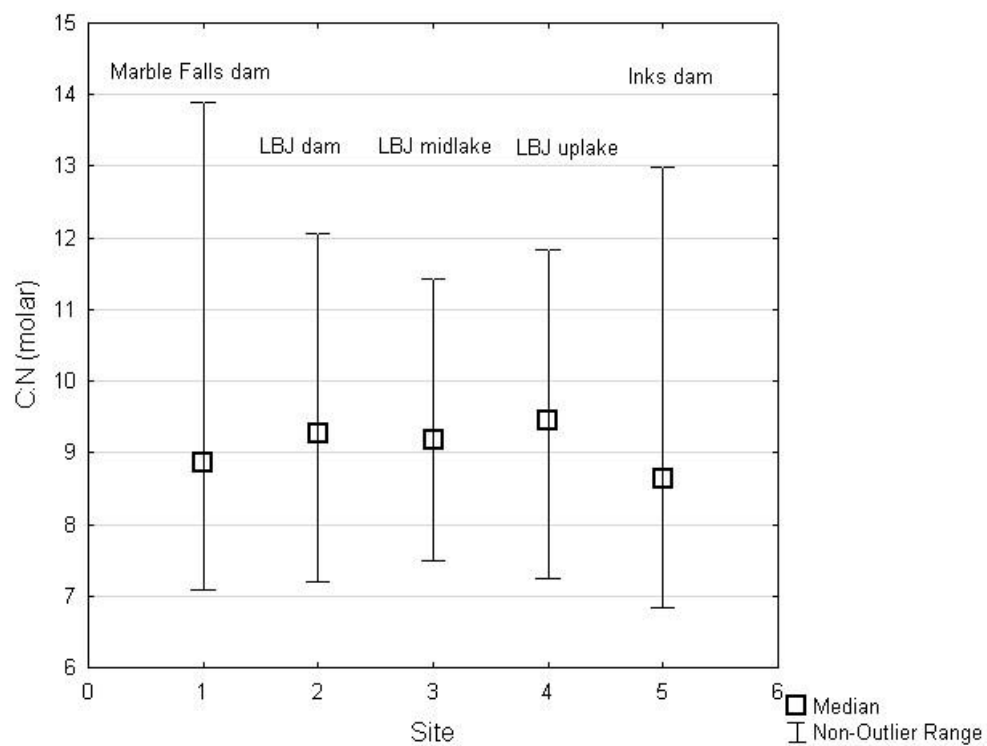


Figure 4.2 Ambient, median C:N (molar) ratios from five sites in three reservoirs arranged in upstream order, Colorado River system, Texas (August 2007–September 2008:  $N=65$ ;  $F(4,60)=0.32$ ;  $p>0.05$ ).

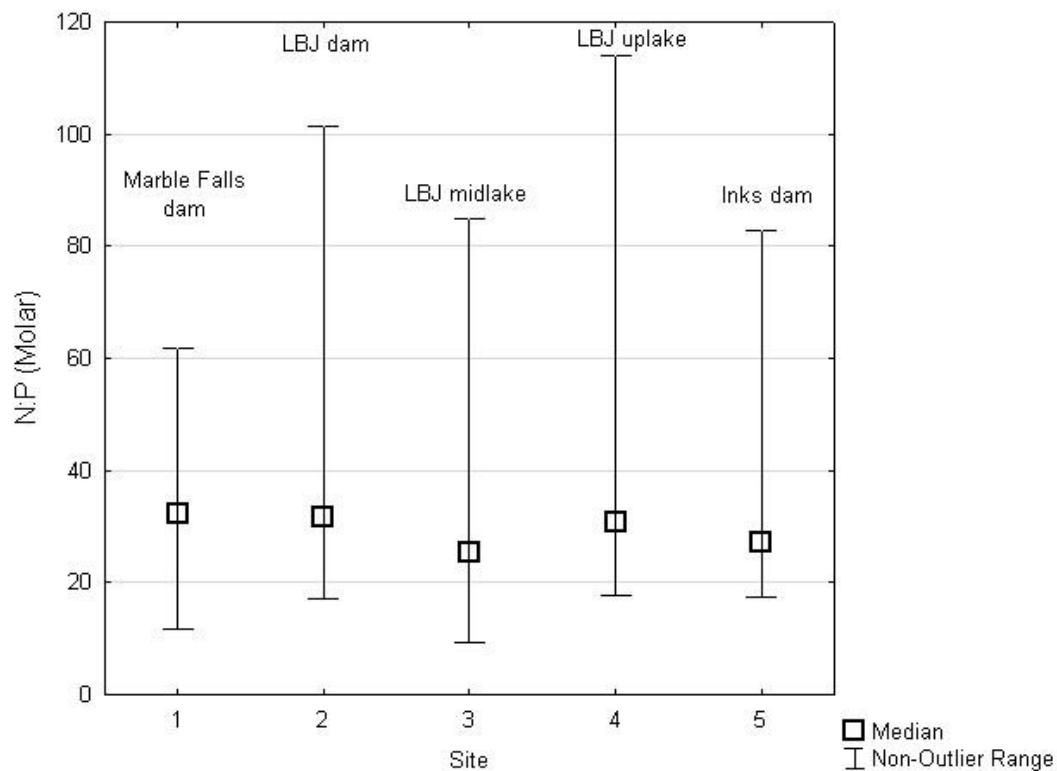


Figure 4.3 Ambient, median N:P (molar) ratios from five sites in three reservoirs arranged in upstream order, Colorado River system, Texas (August 2007–September 2008:  $N=65$ ;  $F(4,60)=0.60$ ;  $p>0.05$ ).

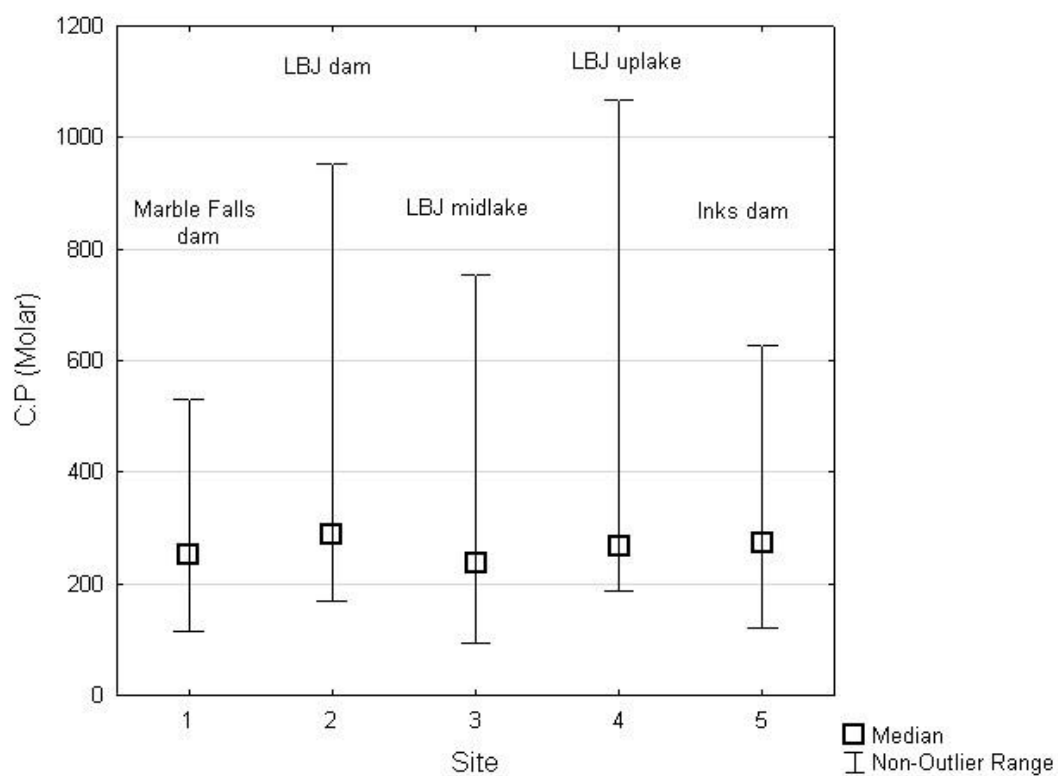


Figure 4.4 Ambient, median C:P (molar) ratios from five sites in three reservoirs arranged in upstream order, Colorado River system, Texas (August 2007–September 2008: N=65;  $F(4,60)=0.78$ ;  $p>0.05$ ).

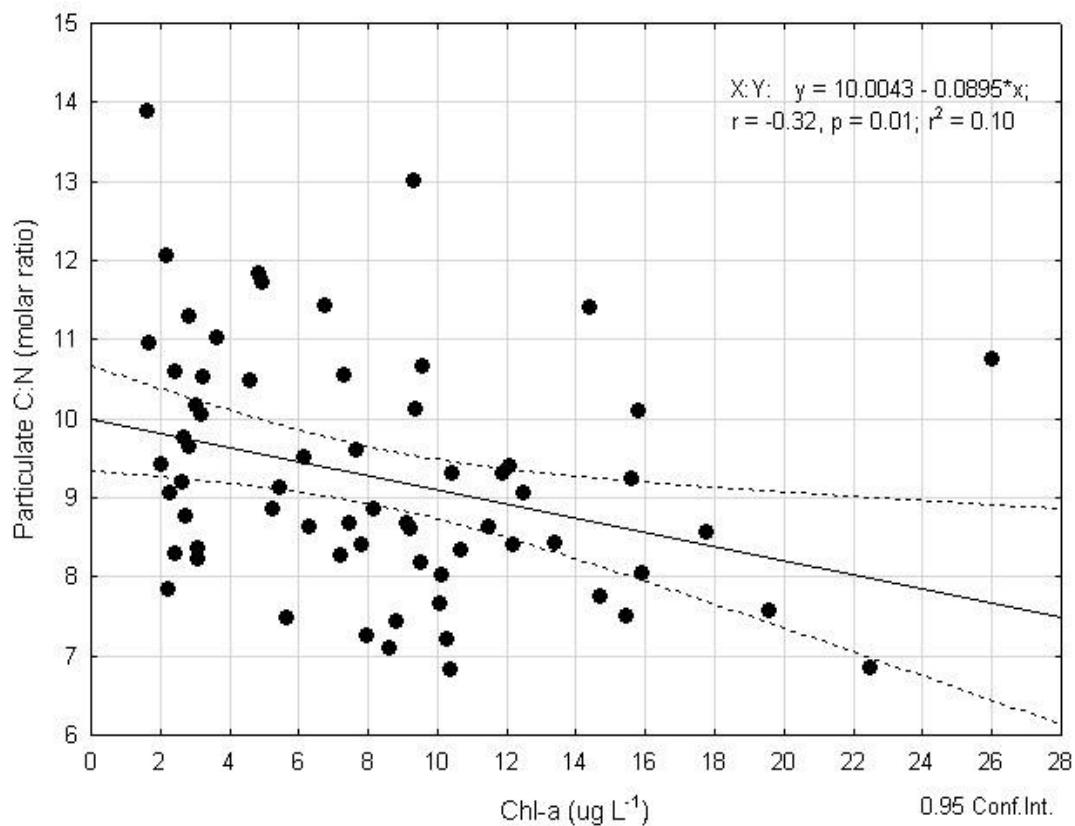


Figure 4.5 Particulate C:N ratios as a linear function of Chl a ( $p \leq 0.01$ ) from all five sites in three reservoirs, Colorado River system, Texas (August 2007–September 2008).

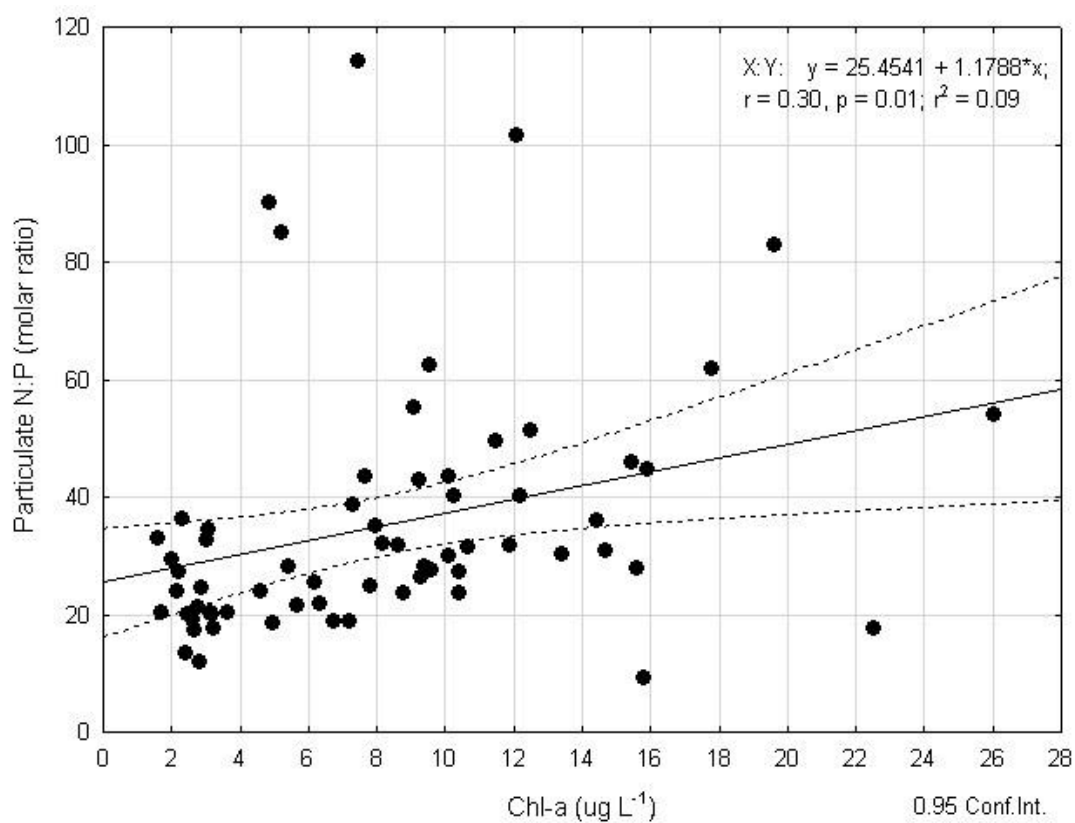


Figure 4.6 Particulate N:P ratios as a linear function of Chl a ( $p \leq 0.01$ ) from all five sites in three reservoirs, Colorado River system, Texas (August 2007–September 2008).



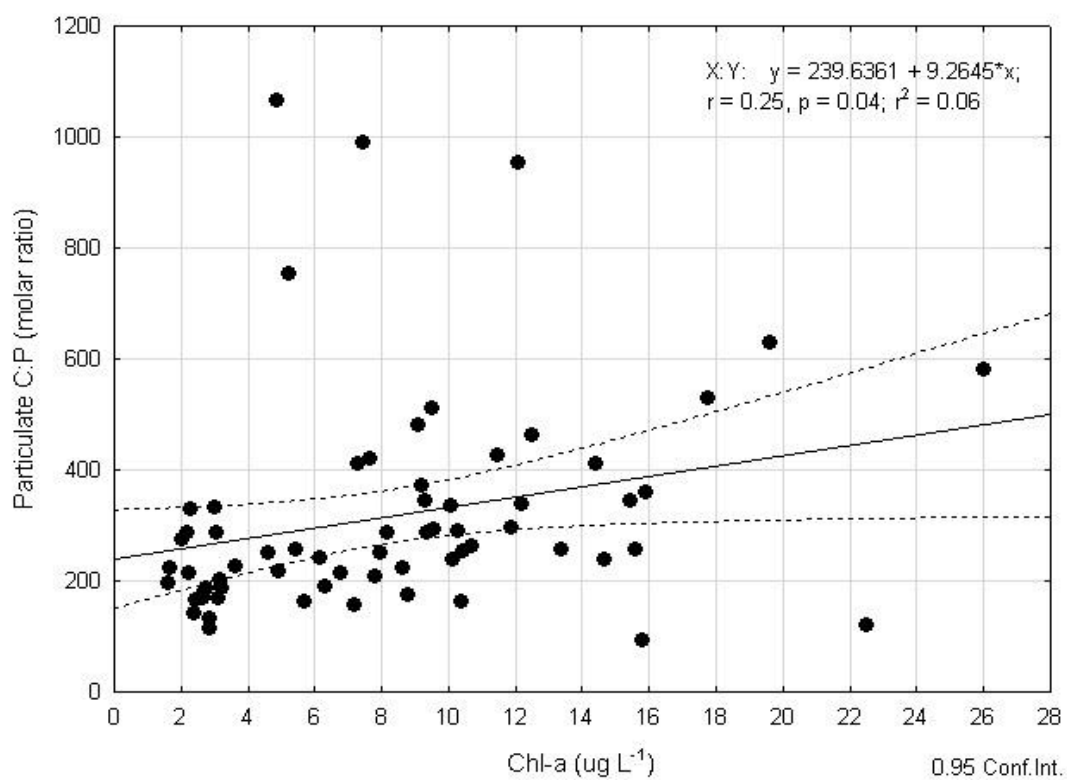


Figure 4.7 Particulate C:P ratios as a linear function of Chl a ( $p < 0.05$ ) from all five sites in three reservoirs, Colorado River system, Texas (August 2007–September 2008).

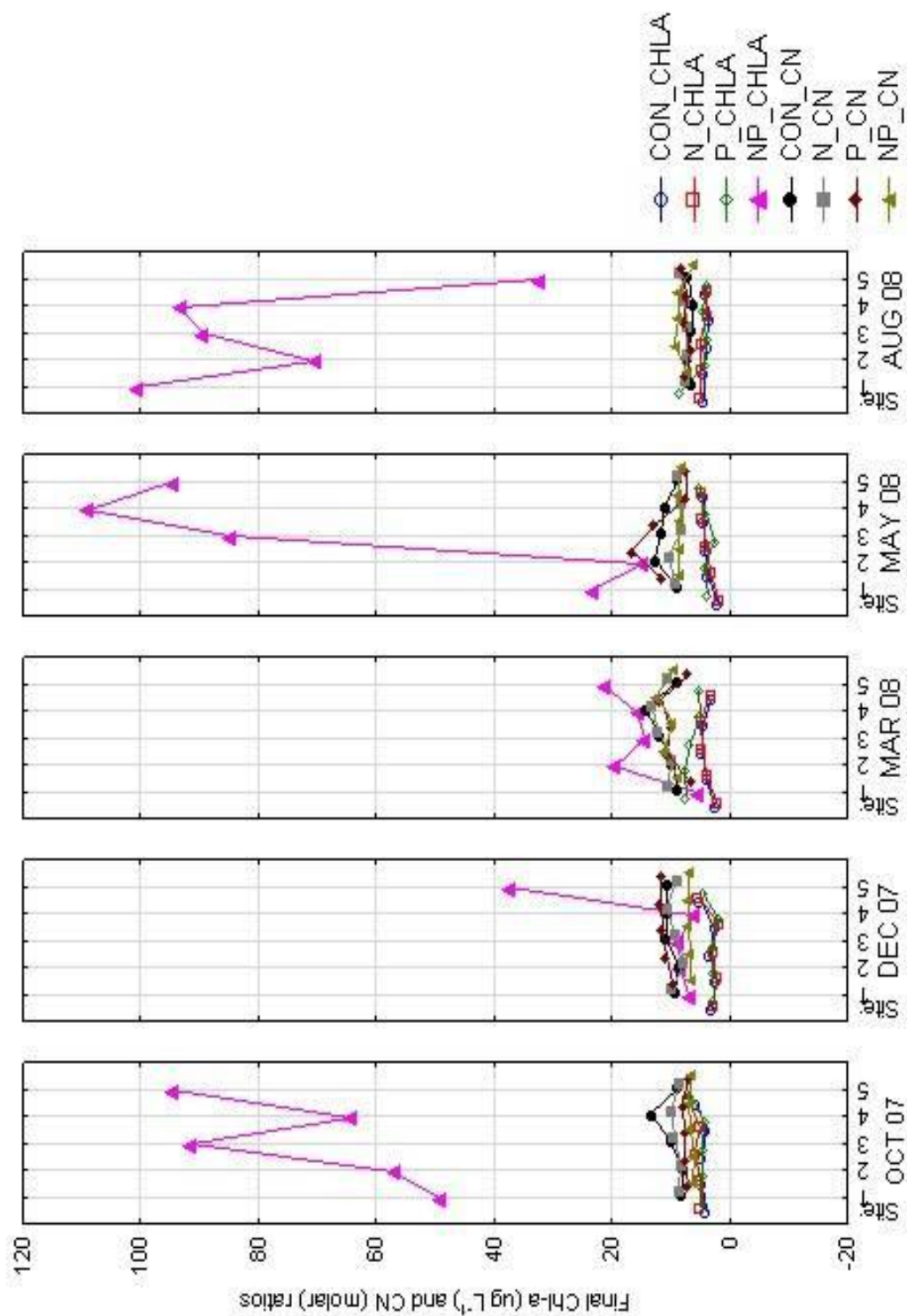


Figure 4.8 Final Chl a and molar CN ratios of nutrient bioassay treatments for five sites in upstream order by date.

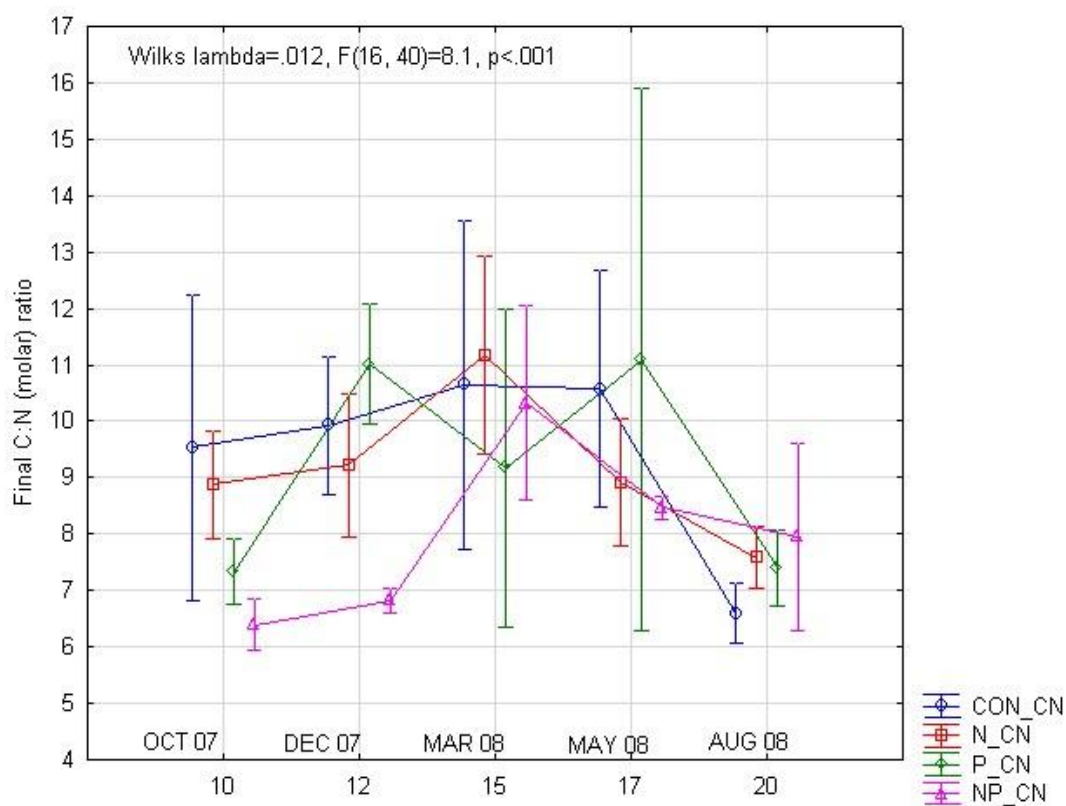


Figure 4.9 Weighted means of final molar CN ratios of nutrient bioassays with all five sites in each treatment combined by date.

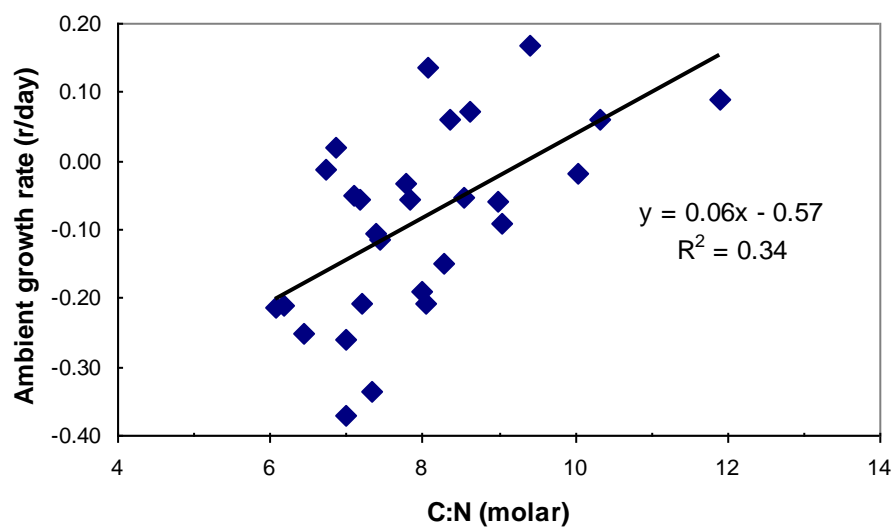


Figure 4.10 Ambient phytoplankton growth rate ( $r \text{ day}^{-1}$ ) as a function of C:N molar ratio ( $N=28$ ) from two near-dam sites in Lakes LBJ and Marble Falls (August 2007–September 2008),  $p<0.01$ .

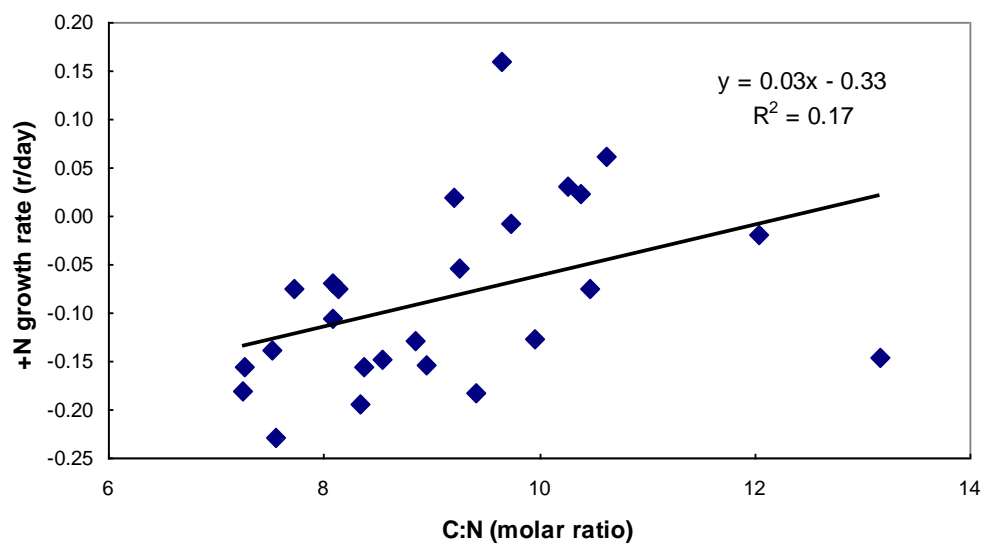


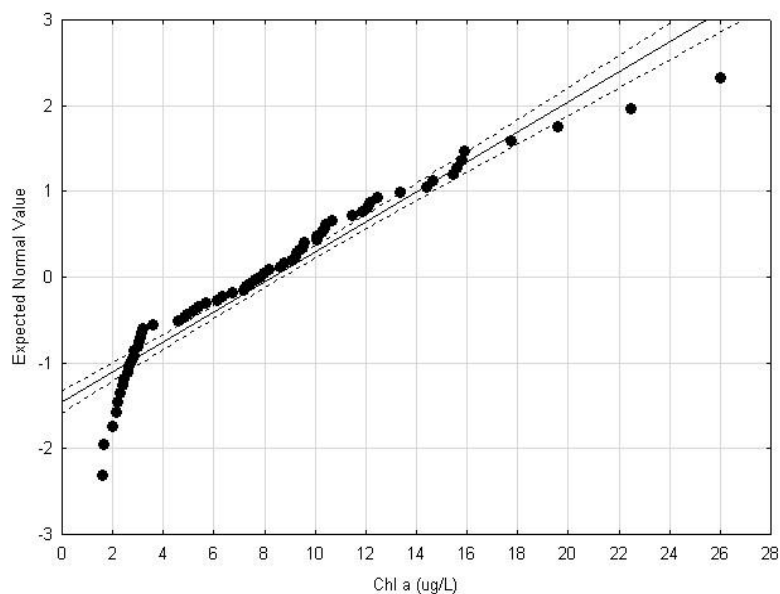
Figure 4.11 Phytoplankton growth rate ( $r \text{ day}^{-1}$ ) and final C:N molar ratios from the +N treatment (N=25) from all sites in Lakes LBJ and Marble Falls (August 2007–September 2008),  $p < 0.05$ .

## APPENDIX

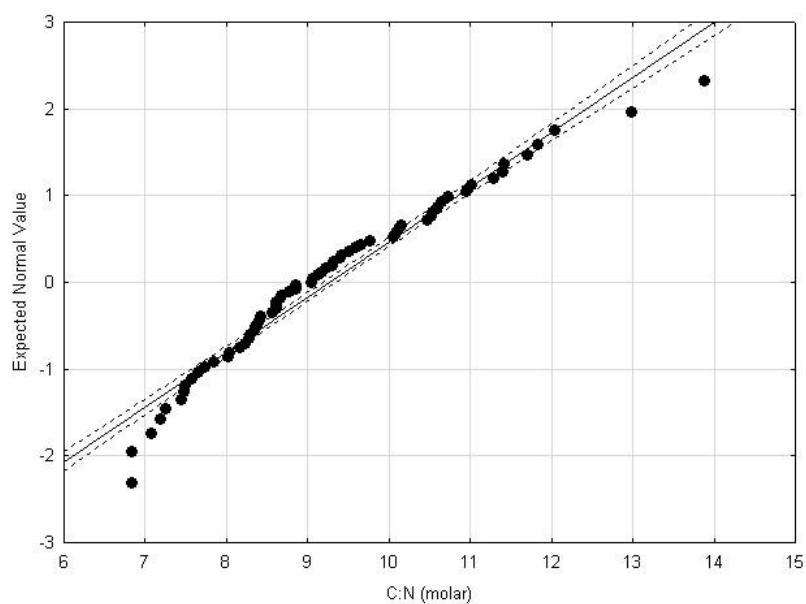
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Appendix 4.1 Normality plots of ambient parameters (a) Chl a ( $\mu\text{g L}^{-1}$ ), (b) C:N (molar ratio), (c) N:P (molar ratio), and (d) C:P (molar ratio); and (e) Seasonal, final C:N (molar ratio) by site and nutrient bioassay treatment ( $N = 5$  dates per symbol); and (f) ambient (control) growth rates ( $r$  day $^{-1}$ ) and C:N (molar ratio) by site (in upstream order) and by month.

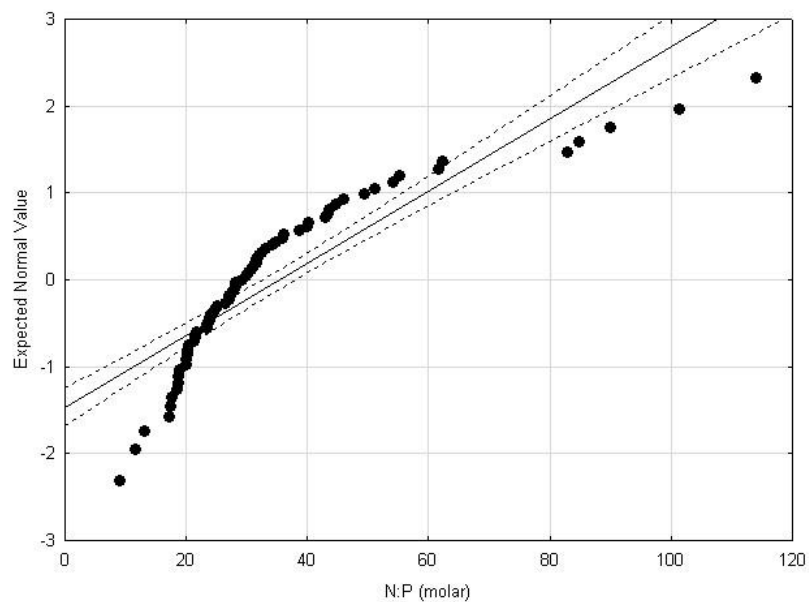
#### 4.1 (a) Chl a ( $\mu\text{g L}^{-1}$ )



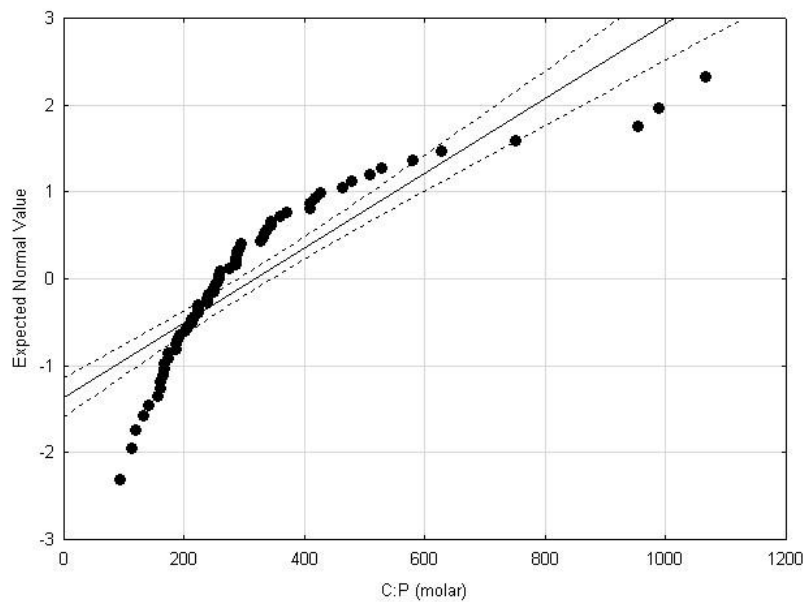
#### 4.1 (b) C:N (molar ratio)



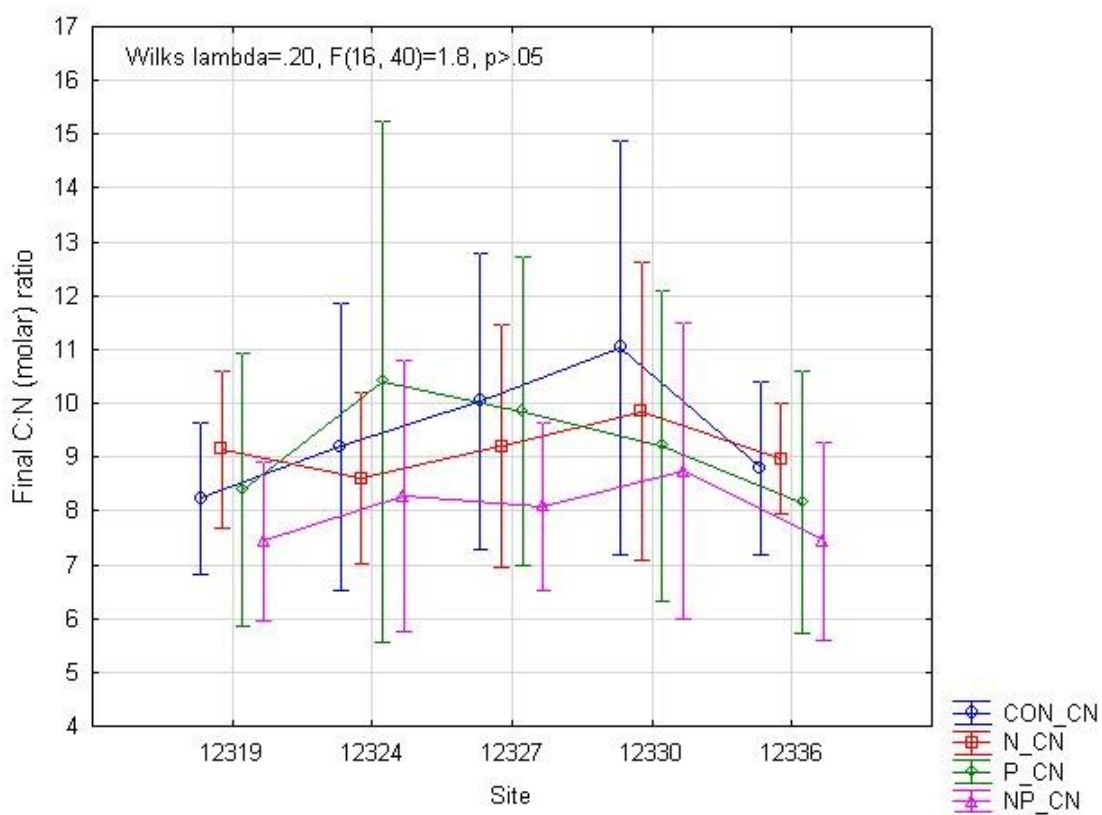
4.1 (c) N:P (molar ratio)



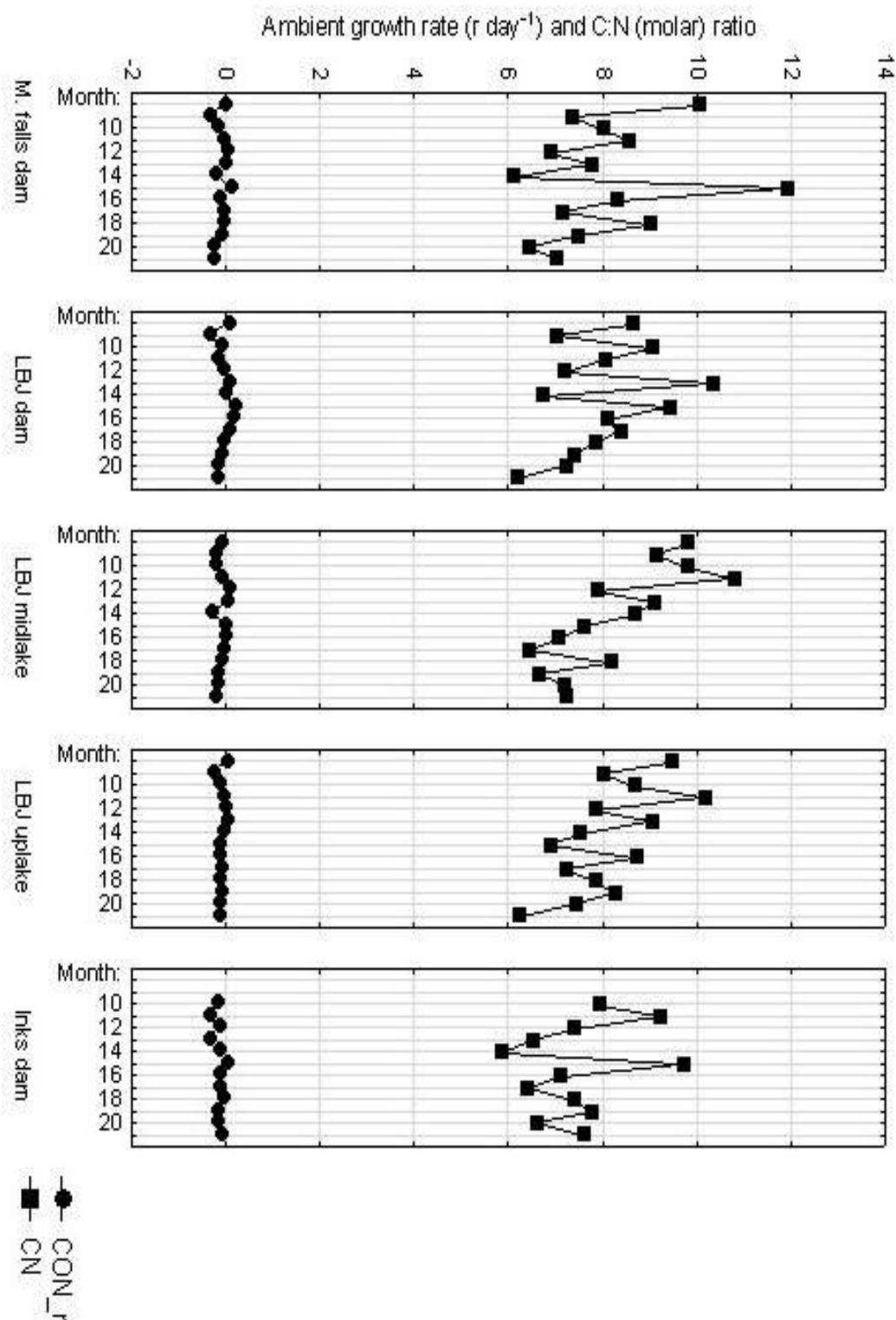
4.1 (d) C:P (molar ratio)







4.1 (e) Seasonal, final C:N (molar ratio) by site and nutrient bioassay treatment (N = 5 dates per symbol).



4.1 (f) Ambient (control) growth rates ( $r$  day<sup>-1</sup>) and C:N (molar ratio) by site (in upstream order) and by month.

## **VITA**

Mary Anne (Mimi) Wallace was born in Houston, Texas, the daughter of Raymond Richard Brown and Janet Adair Brown. She holds a Bachelor of Science degree in Biology (1986) from Stephen F. Austin State University, Nacogdoches, Texas, and a Master of Science degree (1996) in Biology, marine and estuarine science, from Western Washington University, Bellingham, Washington. Between degrees, she worked in various research positions from molecular genetics of humans, Hawaiian monk seal studies, and salmon (post Baccalaureate), then on to planktonic nitrogen cycling in the mid-Atlantic Ocean and harmful algae research in the Gulf of Maine and Mexico (post M.S.). Her Master's thesis entitled, "Seasonal Microzooplankton Grazing in North Puget Sound, Washington," ultimately inspired her to enter the aquatic resources program at Texas State University–San Marcos in 2003, and led to an internship with U.S. Geological Survey (2005-11) where she has studied plankton–reservoir dynamics for her dissertation.

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