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COMPOSITION AND PHYSIOLOGICAL CHANGES OF THE UNIVERSITY LAKE
ECOSYSTEM PHYTOPLANKTON COMMUNITY: IMPACTS OF SEASONAL
AND EPISODIC EVENTS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Environmental Sciences

by
Brienne Norris
B.A. University of Colorado, 2010
August 2012

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Table of Contents

ACKNOWLEDGMENTS	ii
ABSTRACT	iv
CHAPTER 1: INTRODUCTION	1
1.1 HISTORY.....	1
1.2 HYDROLOGY.....	3
1.3 URBAN WATER BODIES.....	4
CHAPTER 2: LITERATURE REVIEW	7
2.1 NUTRIENT LOADING.....	7
2.2 EUTROPHICATION.....	8
2.3 PRIMARY PRODUCTION.....	9
2.4 LIMITING NUTRIENTS.....	10
2.5 DIAGNOSTIC PIGMENTS.....	12
2.6 COMMUNITY COMPOSITION.....	13
CHAPTER 3: METHODS	15
3.1 STUDY SITE.....	15
3.2 IN-SITU SAMPLING.....	17
3.3 MEASUREMENTS OF INORGANIC NUTRIENTS.....	19
3.4 MEASUREMENTS OF PHOTOSYNTHETIC AND RESPIRATION RATES.....	20
3.4.1 CHANGES IN DISSOLVED OXYGEN USING THE “LIGHT AND DARK BOTTLE METHOD”.....	20
3.4.2 CHANGES IN DISSOLVED OXYGEN USING A YSI.....	22
3.5 MEASUREMENT OF DIAGNOSTIC PIGMENTS.....	22
CHAPTER 4: RESULTS	25
4.1 ANALYSIS OF INORGANIC NUTRIENTS.....	25
4.2 ANALYSIS OF PHOTOSYNTHETIC AND RESPIRATION RATES.....	34
4.3 DIAGNOSTIC PIGMENT ANALYSIS.....	41
CHAPTER 5: DISCUSSION	54
5.1 INORGANIC NUTRIENTS AND VARIABILITY.....	54
5.2 DISCUSSION OF GROSS ASSIMILATION NUMBERS AND RESPIRATION RATES.....	58
5.3 DISCUSSION OF THE PHYTOPLANKTON COMMUNITY COMPOSITION.....	59
CHAPTER 6: CONCLUSION	64
REFERENCES	66
APPENDIX	72
VITA	73

Abstract

This research determined the changes in phytoplankton community composition in a shallow subtropical lake, influenced by urban surroundings. Specifically this research focused on the effects of seasonal progression and episodic events on the changing inorganic nutrient dynamics and the effects those dynamics had on the phytoplankton community composition and productivity. This research quantified gross primary production and respiration to determine if seasonality or episodic events were acting as forcing functions of the phytoplankton community composition. Water samples were collected weekly at three sites on the lake's perimeter, as well as following episodic events, to monitor nitrate (NO_3), phosphate (PO_4^{-3}), ammonium (NH_4), and silicate (Si) concentrations, and diagnostic pigment concentrations. Gross primary production and respiration was measured following a four-hour incubation period. Results show that seasonality was not significant in affecting the inorganic nutrient concentrations, but episodic events were significant in influencing the concentrations of NO_3 and PO_4^{-3} . Gross primary production existed at a mean rate of 3.45 gram carbon/gram chlorophyll *a*/ hour (g C/g chl/hr), and the median respiration rate was 0.66 g C/g chl/hr. Primary production and respiration were not significantly affected by seasonal progression, but gross assimilation of oxygen was significantly increased following episodic events, and dependent on phosphate and ammonium concentrations. The phytoplankton community composition was determined to be 51% chlorophyceae, 30% cyanobacteria, 10% diatoms, and 3.4% cryptophyceae, Chlorophytes and diatoms significantly affected by seasonality and episodic events, but only at particular test sites, and the cyanobacteria and diatom populations experience a negative linear growth relationship with one another. The changes in community composition were the result of both seasonality and episodic events, and fluctuations of ammonium and phosphates, while productivity was

influenced solely by the occurrence of episodic events. Low biodiversity within the phytoplankton community exists in this lake as a result of urban runoff and eutrophication.

1. Introduction

1.1 History

The University Lake ecosystem in Baton Rouge, Louisiana, is a man-made drainage system consisting of six lakes: University Lake, City -Park Lake, Campus Lake, Crest Lake, Lake Erie, and College Lake (Figure 1.1.1). University Lake is located approximately three kilometers east of the Mississippi River and 105 km north of the Gulf of Mexico.

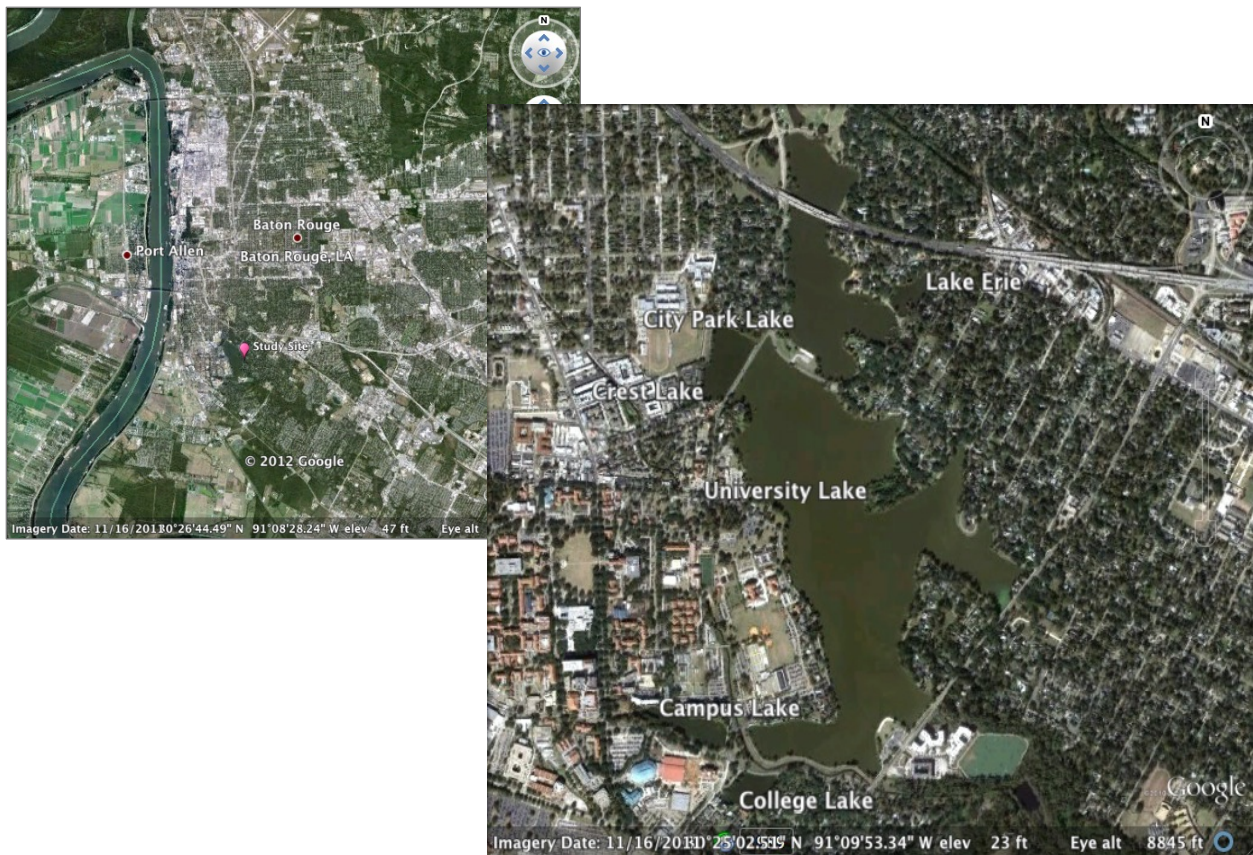


Figure 1.1.1 University Lake System, Baton Rouge, LA

The natural state of this region is a low-lying, sub-tropical drainage bayou. The man-made lakes were constructed in the early 1930's when the existing cypress swamps were timbered and dammed. Construction of the lakes coincided with expansion and development of the Louisiana State University campus and the surrounding residential areas. This increased development of infrastructure and drainage systems, which further divided the lake into the six lakes present today (U.S. ACE 2004). Further modifications to the infrastructure and drainage systems due to human activity have impacted the hydrology of the lakes, limiting freshwater inflow and circulation. Other concerns from anthropogenic modifications include eutrophication, sewage infiltration, sedimentation, retreating bank edges, collapsing drainage infrastructure, and inadequate depth. Such water quality concerns are often limiting to the health of the ecosystem and could limit biodiversity as well.

A restoration effort was made in 1977 by the U.S. Environmental Protection Agency, the State of Louisiana, and the City of Baton Rouge. During this period fecal coliform levels were very high, fish kills were common, bank erosion was becoming very dangerous, and the overall water quality for the six lakes was in a non-attainment status (City-Parish of Baton Rouge 1977). Fish kills in particular were extremely frequent between 1957 and 1978 because of oxygen depletion from algal decomposition (Knaus and Malone 1984). The plan for restoration included deepening the lakes by dredging in order to remove phosphorus-saturated sediments, increasing the retention time of the lake, and improving the oxygen levels in the lake, which were severely low due to decomposition of organics in the sediments. The areas that were dredged can be seen in Figure 1.1.2. Sewer system lines that were damaged and/or broken were identified by smoking the lines and repaired during the restoration. In 1990, post-restoration monitoring results indicated that fecal coliform levels had been reduced from pre-restoration levels but were still

above attainment concentrations. The frequency of fish kills has decreased since the 1980's, although fish kills have still been occasionally occurring in the summer months. Erosion of the banks has continued to be an issue for the City of Baton Rouge and is compromising the adjacent infrastructure (Malone et al. 1991). Today there are new plans for lake maintenance and restoration designed by the New Orleans District Army Corps Of Engineers, including another dredging of the four largest lakes, but the plans are very costly, and there has been no progress to date.

1.2 Hydrology

The Baton Rouge climate is subtropical, with a 30-year normal annual precipitation of 155 cm (Ruley and Rusch 2002). Damming of Bayou Duplantier began in the 1920's and flooded the cypress swamp. Completion of the lakes and the 1977 restoration effort has yielded a total water surface area of 120 hectares (ha). The watershed of the system is approximately 486 ha of land that was historically part of the Mississippi River floodplain before the levee system was constructed (Malone et al. 1985). Approximately 140 outflows enter the lakes from storm drains in the watershed. Corporation Canal was built during the 1930's as a means to reroute runoff from the urban areas of Baton Rouge surrounding the lakes. The canal is located on the south and west sides of University Lake, and drains into Bayou Duplantier downstream of the system. Campus Lake and College Lake still drain into Corporation Canal and are not connected to the rest of the system; the remaining four lakes are connected through a series of culverts and risers that outflow from University Lake into Bayou Duplantier through a spillway (Malone et al. 1985). The fetch of the system is oriented from north to south. University Lake is the largest lake in the system at approximately 80 ha. Its watershed includes the watersheds of the smaller

City Park Lake, Crest Lake, and Lake Erie, a total area of 425 ha. University Lake has an average hydraulic retention time (HRT) of approximately 50 days (Malone et al. 1985). This average HRT is very similar to the rest of the lakes in the system, with the exception of Crest Lake, which has an average HRT of 561 days (Malone et al. 1991). The average depth of University Lake is 0.86 m (Mesmer 2008). Depth and volume vary slightly throughout the year because of changes in precipitation and evapotranspiration. The University Lake system is shallow and eutrophic. Many characteristics commonly seen in shallow lake environments are apparent in the University Lake system. Shallow lakes tend to have highly variable physical and chemical characteristics (Petaloti et al. 2004). They lack a stable thermocline, experience frequent mixing of the entire water column, experience re-suspension of sediments, and in urban environments are likely to receive large inputs of allochthonous nutrients (Petaloti et al. 2004). Shallow lakes have water quality conditions that reflect these characteristics. Phosphorous concentrations, turbidity, chlorophyll *a* concentrations, and algal blooms display a much different pattern in shallow lakes compared to deeper lakes in the same locality (Petaloti et al. 2004). All of these factors are dependent on the external nutrient loads, which are influenced by the surrounding environment.

1.3 Urban Water Bodies

University Lake is surrounded by a portion of Baton Rouge that is primarily residential with respect to development. An interstate highway bridge passes over City Park Lake roughly 1 km north of the juncture of University Lake and City Park Lake. The surrounding urbanized area very likely subjects the University Lake system to higher levels of pollution runoff than a more isolated water body might experience (Lund 1972).



Figure 1.1.2 Corporation Canal and the University Lake drainage system, Lakeshore Drive, Baton Rouge

April, 12 2012

Photo A: Corporation Canal and drainage to Bayou Duplantier

Photo B: Culvert between University Lake and Corporation Canal

Photo C: Culvert draining into Corporation Canal

Urban environments are much more prone to be associated with non-point source pollution because of the large percentage of impervious surfaces present. Impervious surfaces prevent

infiltration into the ground, and surface rainfall and waters become contaminated with nutrients, heavy metals, pesticides, and toxic organic pollutants. Polluted runoff can eventually reach local water bodies and alter the ecosystem (Paul and Meyer 2001). Such alterations include effects on the decomposition of leaf-litter, the natural cycling of carbon, removal of nutrients from the water column, primary and secondary production, and algal community composition. Algal community composition has been shown to be a sensitive measure of water quality where non-point source pollution is concerned (Johnson et al. 2011).

2. Literature Review

2.1 Nutrient Loading

External nutrient loads are vital to the health and functionality of a lake ecosystem. Inputs of nutrients and recycling thereof sustain the growth of aquatic plants in a natural lake. Nitrogen and phosphorous often limit the productivity of phytoplankton. Compared to other nutrients, nitrogen and phosphorus are generally in short supply relative to cell growth requirements (Dodds et al. 2002). Increases in nitrogen and phosphorous to an otherwise oligotrophic water body will result in increases in phytoplankton biomass (Vanni 1987). The link between nutrient concentrations and algal biomass can have obvious effects on the health and community composition of a water body. Without tertiary treatment, sewage effluent contains high concentrations of nitrogen and phosphorus, which can lead to algal blooms and have major impacts on the ecology of a lake (Edmondson and Lehman 1981). The amount of phosphorous present in a water body is closely related to the quantity of phytoplankton. Seasonality of a lake is reflected in primary productivity levels, and in temperate lakes there is typically a high correlation between phytoplankton levels in the spring/summer and the concentration of phosphorous during the winter (Edmondson and Lehman 1981). Ammonium is a form of nitrogen available to all phytoplankton, and nitrate can be used as a nitrogen source by any species with the ability to carry out assimilatory nitrate reduction. Silicates are an important essential nutrient for diatoms. Discharges of these nutrients, along with phosphorus, may have dramatic effects on phytoplankton biomass and community composition. The relative abundance of nutrients can affect phytoplankton species composition. Phytoplankton communities respond to both nutrient supply as well as ratios of essential nutrients. For example, nitrogen-fixing cyanobacteria are dominant at low N:P ratios, and diatom species are dominant at high Si:P

ratios (Vanni 1987). The relative supply of nutrients will influence phytoplankton community composition because of the nutritional preferences of different groups of algae.

2.2 Eutrophication

Over time, nutrients are assimilated by plants. In some cases herbivores are unable to keep pace with algal production, and a phytoplankton bloom occurs. When nutrients are exhausted and/or conditions become unfavorable for algal growth, the algae may die and decay. When this happens, the decomposition process may strip virtually all oxygen from the water, the result being fish kills and septic conditions. Eutrophication, or the enrichment by plant nutrients, is a natural process, but it can be accelerated by anthropogenic activities (Lund 1972).

The University Lake watershed is in an urbanized area, and the lakes experience nutrient inputs from point and non-point pollution sources. Due to the shallowness of the University Lake system, its resident organisms are much more vulnerable to loading of nutrients and pollutants. The system is highly eutrophic, most likely from a combination of street runoff and leaky sewer lines. The former may in part reflect use of fertilizer on lawns and gardens. Eutrophication can significantly alter productivity, nutrient cycling, water quality, biodiversity, and health of a water body (Paerl et al. 2007). Phytoplankton are especially responsive to eutrophication of a lake with a long enough residence time to permit the development of algal blooms. In streams and fast-moving waters, phytoplankton are not as much of a concern as are periphyton and rooted aquatic plants, which cannot be flushed out of the system. Chlorophyll is a reliable indicator of nutrient enrichment because of the fact that it is found in all phytoplankton (Cottingham and Carpenter 1998). In relation to eutrophication, increases in chlorophyll could be used to determine changes in nutrient availability and inputs of excess nutrients. Sewage and

fertilizers contain basic nutrients vital to plant growth. When large quantities of nitrogen and phosphorous enter a water body, whether from natural or anthropogenic sources, a spike in algal growth often occurs, particularly in tropical and sub-tropical climates. These algal blooms can be potentially harmful to the fauna of a lake if the algae release toxins into the water (Van Dolah 2000) or if the subsequent decomposition of the algal bloom reduces oxygen concentrations to stressful levels, a condition referred to as hypoxia (Paerl et al. 1998). The virtual absence of oxygen (anoxia) will likely have devastating effects (Paerl et al. 1997) and is associated with a shift from aerobic to anaerobic respiration. This can lead to fish kills and undesirable consequences for the lake. The relationship of nutrient availability to algal biomass is quite strong in a limnetic environment, and knowledge of that relationship can be used to manage problems caused by eutrophication and to monitor water quality (Dodds et al. 2002).

2.3 Primary Production

Phytoplankton are responsible for the majority of primary production in most aquatic environments. The energy stored during primary production moves through the food chain and supports secondary production at higher trophic levels. Nutrient loading can have noticeable impacts on the phytoplankton community, whose biomass, composition, and productivity will likely have major effects on organisms at higher trophic levels, on ecosystem processes, and on the ecosystem overall (Paerl et al. 2007). An addition of nitrogen, phosphorous, or both nutrients has been demonstrated to stimulate algal growth and alter the primary production cycle (Francouer 2001). Growth may also be accelerated in shallow waters because microbial activity in the sediments can maintain nutrient levels for algal growth (Eppley 1972). In temperate and boreal latitudes seasonality will also affect the primary production cycle (Heinrich 1962). The

seasonal increase in temperature and irradiance in the spring and summer months seen in temperate and boreal latitudes leads to the vernal blooming of phytoplankton and an increase in primary production (Eppley 1972). Seasonal variations in temperature, turbulence, and environmental disturbances can put stress on phytoplankton and thus temporally alter the community composition. Under more severe conditions, certain algal species may be outcompeted by other more adaptive species that will come to dominate the community composition (Aubry and Acri 2004). Seasonal progression of phytoplankton will also be dependent on the particular environment. The ability of a body of water to develop a seasonal thermocline and stratified layers encourages particular algal forms and alters the planktonic community composition (Proulx et al. 1996).

2.4 Limiting Nutrients

Two main factors that control progression of phytoplankton growth are light and nutrients. Nutrient deprivation inhibits growth in certain phases of the phytoplankton cell cycle, and nutrient limitation may also lead to a difference in duration of growth phases (Pascual and Caswell 1997). Even in a constant environment the structure of the phytoplankton population varies over time (Pascual and Caswell 1997). Interaction of light and nutrients and the effect those factors have on different algal groups can greatly affect the community composition. In a natural environment, the length of daylight and angle of the sun will vary seasonally, and effects on primary production rates can be documented. Other factors, such as the grazing of herbivorous zooplankton, sedimentation, water column stability, environmental disturbances, and benthic organism activity may also have effects on the phytoplankton community structure (Mallin and Paerl 1994, Lewis 1990). Species richness and diversity of the phytoplankton

community are generally greater than the number of limiting resources, and it has been shown that disturbances can increase diversity and species richness within the community structure (Interlandi and Kilham 2001). Although disturbances can increase species richness, the stability of the water column, and the health of the environment must be taken into account. Limiting resources and disturbances are both factors that influence the diversity of phytoplankton species. Frequency and intensity of disturbance may increase or decrease diversity, depending on nutrient characteristics of the water body. Frequent disturbances can cause dominance by a few species and decrease the diversity of the community composition (Grover and Chrzanowski 2004). The impacts of external forcing factors are important considerations when assessing the phytoplankton community composition of a water body, but characteristics of the phytoplankton themselves must also be taken into account. The size of particular phytoplankton species may have an effect on the transfer efficiency of energy. In eutrophic systems, the energy generated during primary production is the basis for the energy that will flow up through higher trophic levels. Average size of phytoplankton generally increases with community biomass, and larger algae tend to be better competitors in dense communities, whereas the smaller varieties have the competitive advantage in more sparse communities (Duarte et al. 1990). For example, a shallow, eutrophic lake without a thermocline would be better suited to large forms because increased vertical mixing prevents them from sinking out of the water column (Eppley 1972). Dense communities are more likely to be seen in a eutrophic environment, so it is reasonable to expect that slightly larger algal species represent a larger portion of the algal community in a eutrophic environment as opposed to an oligotrophic environment. Eutrophic environments experience many water quality issues, most of which reflect an imbalance between algal production and

consumption. Identifying the phytoplankton community structure of a water body helps one to understand the natural and anthropogenic conditions and forces at work in an ecosystem.

2.5 Diagnostic Pigments

A pigment profile is useful for identifying the phytoplankton groups present in a water body, as certain pigments are representative of specific taxa. The composition of the algal community is a function of environmental conditions. In eutrophic lakes with dense algal communities, cyanobacteria tend to be dominant (Duarte et al. 1992). Particular community structures develop as a result of trophic energy transfer efficiency, nutrient availability, and the current environmental conditions. The combination of these natural factors determines which algal groups will dominate a particular water body. Since certain pigments, or combinations of pigments, are unique to particular algal groups, an analysis of the concentrations of those particular pigments can be used to estimate the biomass of that algal group. Chlorophyll *a* is the major light-harvesting pigment for photoautotrophs and can be used as a measure of total algal biomass. Pigments can be used as biomarkers of phytoplankton groups and even determine community composition with seasonal progression (Deydier-Stephan et. al. 2003). An example of the use of a pigment biomarker would be using chlorophyll b, lutein, violaxanthin, and β -carotene concentrations to identify Chlorophyta (green algae). This particular pigment combination is generally in high concentration when green algae are present. Diagnostic High pressure liquid chromatography (HPLC) pigment markers are excellent not only for measuring biomass, but for environmental monitoring of phytoplankton as well. HPLC is the preferred technique for determining phytoplankton community composition (Paerl et al. 2003).

2.6 Community Composition

The algal community composition of a lake environment can be dependent on many natural factors. Nutrient concentrations can greatly influence the success or demise of particular algal species. For example, it has been shown that the high success of cyanobacteria can often inhibit growth of diatoms. In eutrophic lakes in particular, it is thought that cyanobacterial dominance is due to allelopathic effects (Keating 1987). The initial cyanobacterial growth leaves behind allelopathic substances, which inhibit future diatom growth. Nutrient levels and circulation patterns are known to have an effect on the phytoplankton community structure of a body of water, but there are more factors to take into account. Interspecies competition always exists in a natural environment but is not the only factor that affects community composition of phytoplankton. Zooplankton as well as planktivorous fish can be responsible for changes in phytoplankton community composition. Changes in terms of a trophic cascade can also be responsible for altering the phytoplankton community composition as well as changes to population size. Zooplankton health is often a direct result of phytoplankton health, and large numbers of zooplankton do not always lead to a decline in phytoplankton (Kerfoot et al. 1988). Of course, predation does play a part in the regulation of phytoplankton concentrations. Carnivorous large-bodied zooplankton do have the ability to clear the water column, although in shallow, eutrophic environments, phytoplankton growth is so rapid that this cannot generally be accomplished. The presence of fish can often result in higher phytoplankton populations, especially when zooplankton populations are high, as the fish will feed on the larger zooplankton (Proulx et al. 1996). The impact of high-trophic-level fish on phytoplankton biomass is determined by whether their presence increases or decreases the concentration of herbivores (Brett and Goldman 1996). Predatory fish will influence the abundance of planktivorous fish,

which will determine the size and composition of the zooplankton community. The zooplankton community composition, particularly whether the community has a large representation of carnivorous zooplankton, will in turn influence the abundance, community composition, and productivity of the phytoplankton community (Carpenter et. al 1987). An example of this trophic control can be seen in the highly eutrophic Lake Washington. Phytoplankton would be considered trophic level 1, and any increase of biomass on an even numbered trophic level would be expected to reduce the phytoplankton population. It can be assumed that any increase of biomass on an odd numbered trophic level would be expected to increase the phytoplankton population. In the 1970's, Lake Washington's smelt population reduced the population of predatory *Neomysis mercedis*. Low neomysis population allows for an increase in daphnia population, thereby reducing the phytoplankton population (Edmondson and Litt 1982).

Disturbances may also be able to affect the variations in quantity of certain algal forms, as well as presence or absence. Disturbances are generally measured in intensity and frequency. High frequency and low intensity has been linked to higher biological diversity in a natural water body than low frequency, high intensity disturbances (Sommer 1995). Diversity in relation to disturbance is calculated based on the assumption of an existing negative relationship between disturbance intensity and frequency (Gaedeke 1986). Most phytoplankton communities naturally exhibit a high level of species diversity. It is thought that disturbances allow an intermediate period for species succession to occur. Disturbances of medium intensity are also favorable for the maintenance of high species diversity (Hambright and Tamar 2000).

3. Methods

3.1 Study Site

University Lake is located adjacent to the Louisiana State University campus in Baton Rouge, Louisiana (Latitude 30°24'N, Longitude 91°10'W). It is the largest lake of the system and is surrounded by five smaller lakes. The lake perimeter is 6.7 km, and the combined shoreline perimeter of all six lakes is approximately 10 km. The climate in this area is considered subtropical, with a long, hot summer period, and a short, mild winter. Long-term climate data (1931–2000) collected by the National Weather Service (station ID# 160549) at the Baton Rouge Ryan Airport shows a historical mean annual temperature of 19.9 °C. The highest historical monthly mean (27.9 °C) occurred in July, and the lowest historical monthly mean (19.9 °C) occurred in January. Historical average annual temperature trends can be seen in Figure 3.1.1, and the climate normals are displayed in Figure 3.1.3.

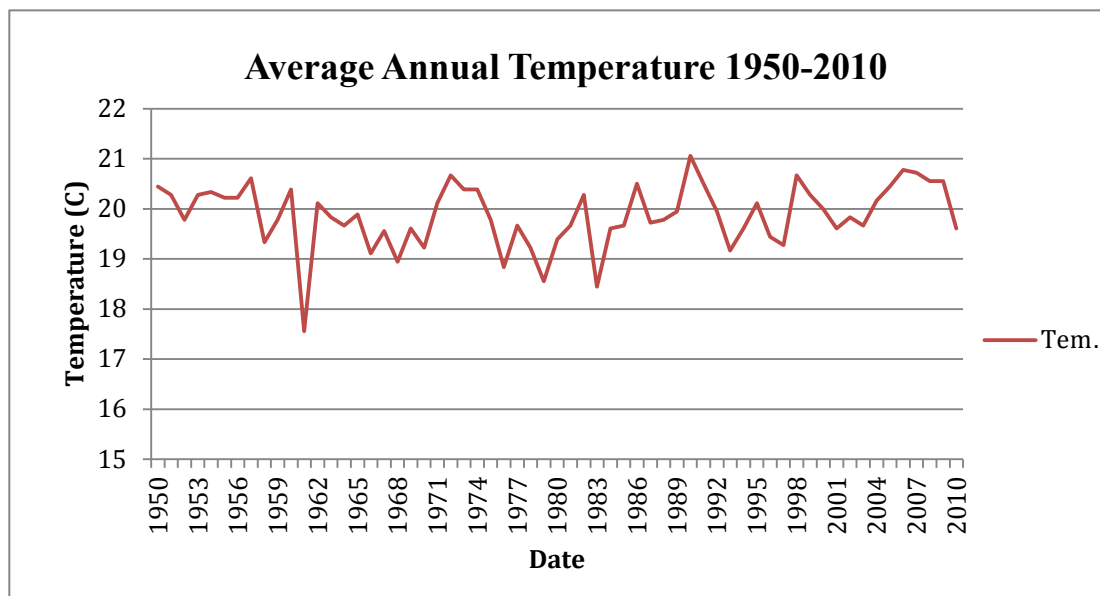


Figure 3.1.1 Average Annual Temperature

Historically, the greatest monthly precipitation levels have occurred in the month of June. These high June precipitation levels reflect the rainy season that has existed in the past sixty years during the summer months, and a decrease in precipitation experienced throughout the fall. Average annual accumulated precipitation from 1950–2010 was 148 cm (Fig. 3.1.2). Weather data for the sampling period from February 2011–February 2012 were collected at a residential station on the lake’s perimeter (ID LA-EB-2), operated by an employee of the Southern Regional Climate Center.

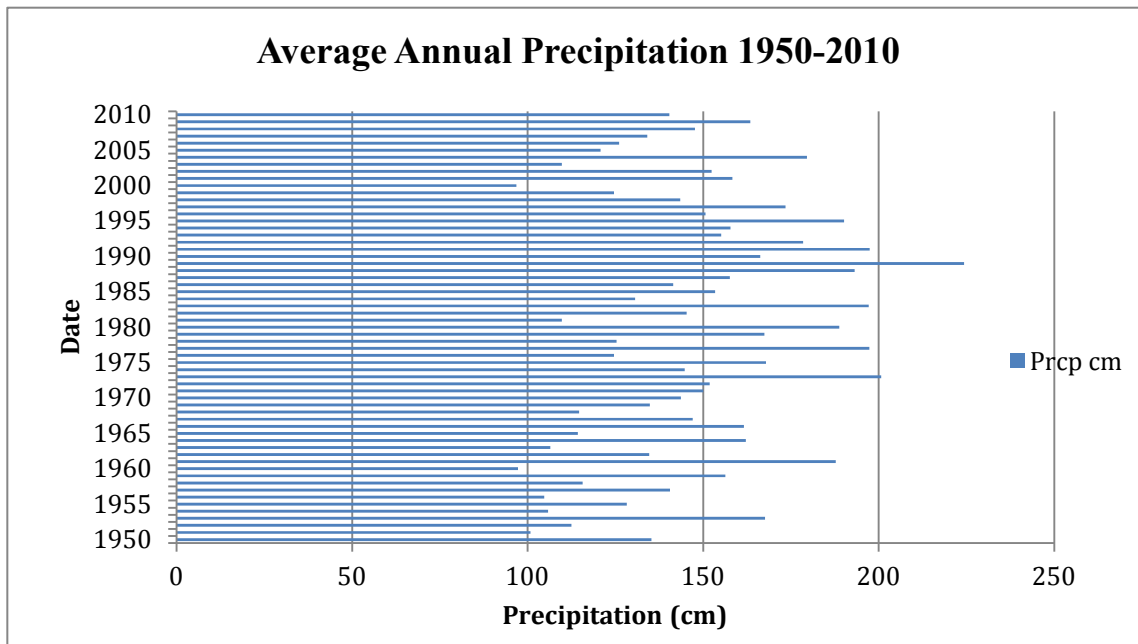


Figure 3.1.2 Average Annual Precipitation

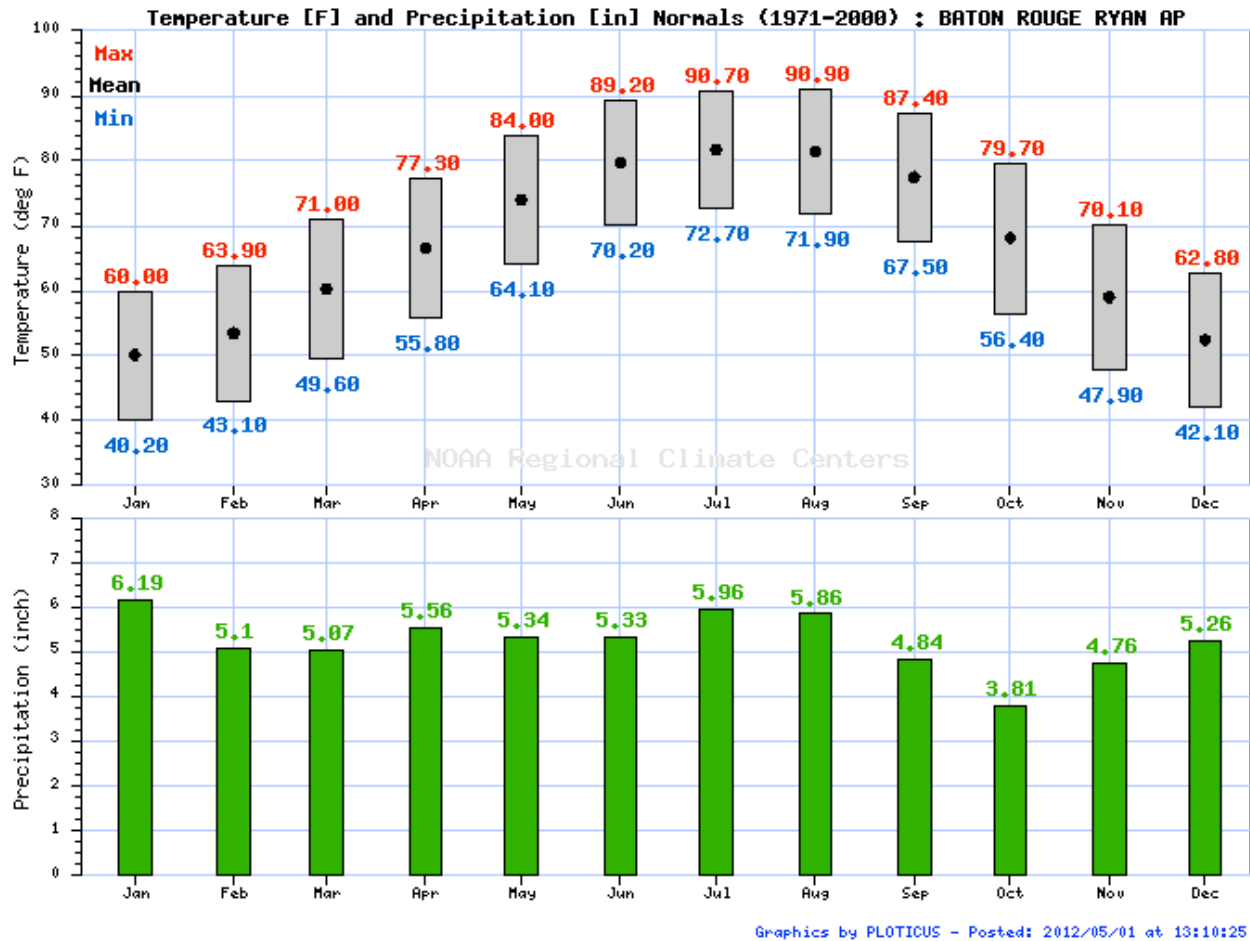


Figure 3.1.3 Temperature and Precipitation Normals for Baton Rouge, LA, Courtesy of the Southern Regional Climate Center, LSU

3.2 In-situ Sampling

Water samples for this project were taken from three locations on the lake’s perimeter. Each site is in an area of the lake that experiences heightened human-environment interaction. Sites can be seen in Figure 3.2.1. Site 1: Dalrymple is located on Dalrymple Drive, a few meters from the intersection of Dalrymple Dr. and Lakeshore Dr. This site is located on a land bridge that divides University Lake and Crest Lake. A railed bridge area provided a convenient sampling point for collecting water. Site 2: Campus is located on West Lakeshore Drive, where

South Campus Drive becomes West Lakeshore Drive. This site is adjacent to the LSU dormitories. The bank edge at this location was fairly stable and facilitated taking water samples. Site 3: Stanford is located on Stanford Street, at what is called the Stanford Beach. It is a popular area for recreation and has a large parking lot as well as a boat launch. The boat launch docks were used for water collection. These sites were chosen because they each represent a different fraction of University Lake and experience a unique input and output hydrology in comparison to the other sites.

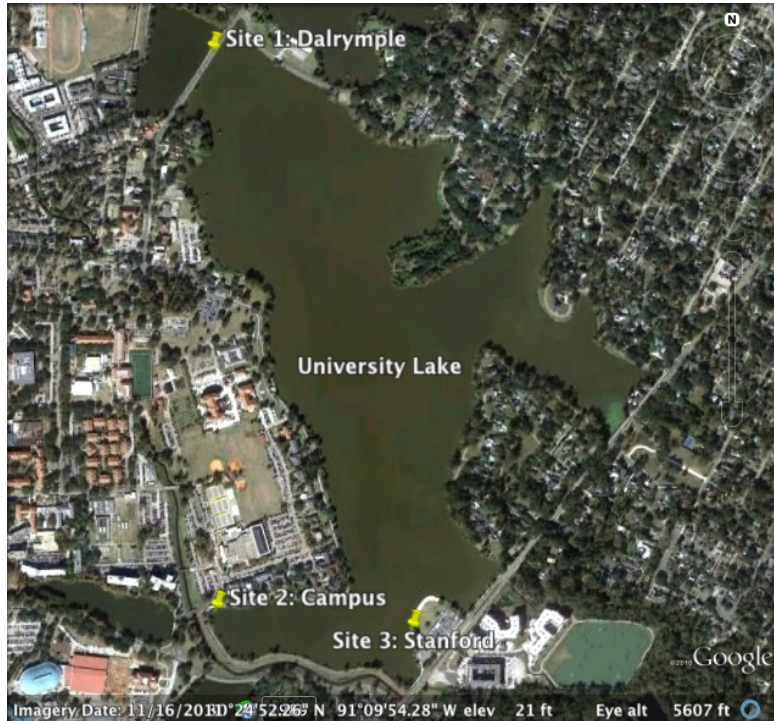


Figure 3.2.1 Test Sites

Water samples were taken on Wednesday of each week. These regular samples act as the control samples for the set. Samples were also taken two days after an episodic event occurred to allow enough time for the development of a phytoplankton community response. An episodic

event was defined as any precipitation event in which more than one centimeter of precipitation fell in a one-hour period. On every sample date, two liters of water were collected at each site for analysis of diagnostic pigments, inorganic nutrients, and photosynthetic and respiration rates assayed by changes in dissolved oxygen. Measurements of water temperature were also taken at each location with a thermometer. Water was collected using a homemade Niskin bottle. The Niskin bottle was a 500 mL volume bottle, supported by a PVC pipe casing. A length of rope was attached on both sides of the PVC pipe, and a metal clamp marker was used to ensure that samples would be collected at a uniform depth of 30 cm. Immediately after water collection, the samples were returned to the lab for initial analysis.

3.3 Measurement of Inorganic Nutrients

Nutrient analysis for phosphates, silicates, and ammonium were carried out according to the methods described by Strickland and Parsons (1972). The concentrations of inorganic nutrients are detected beginning with the limit of detection for each method. The limit of detection when referring to inorganic nutrients is the smallest concentration that can be measured with reasonable certainty, based on the method of analysis (Thomsen et al. 2003).

Silicate analysis is accomplished by allowing the water sample to react with molybdate under conditions that result in the formation of silicomolybdate, phosphomolybdate, and arsenomolybdate complexes. A reducing solution of metol and oxalic acid is added to reduce the silicomolybdate complex, yielding a reduction compound. The reducing solution also decomposes the phosphomolybdate and arsenomolybdate to prevent any phosphate or arsenate interference. The extinction was measured at 8100 Å using a Varian Cary 50 WinUV spectrophotometer.

Phosphate analysis allows the water sample to react with a composite reagent of molybdcic acid, ascorbic acid, and trivalent antimony. The resulting complex heteropoly acid is reduced to create a blue solution whose extinction was measured at 8850 Å.

Nitrate analysis is conducted by reducing the nitrate in a water sample to nitrite by passing the water sample through a column of granulated copper-cadmium filings. This process allows detection of nitrate plus nitrite. The nitrite produced by this oxidation-reduction reaction was determined by diazotizing sulphanilamide and combining it with N-(1-naphthyl)-ethylenediamine to form a pink solution whose extinction was measured at 5430 Å.

Determination of ammonia-ammonium was conducted by treating the water sample in an alkaline citrate medium with sodium hypochlorite and phenol. Sodium nitroprusside acts as a catalyst to form a blue solution. The extinction was read at 6400 Å.

Thomas Blanchard of Analytical Services in the Department of Oceanography and Coastal Sciences at LSU, using an OI Analytical Flow Solutions IV autoanalyzer, performed an additional nutrient analysis for all four measured inorganic nutrients.

3.4 Measurement of Photosynthetic and Respiration Rates

3.4.1 Changes in Dissolved Oxygen Using the “Light and Dark Bottle” Method

Photosynthetic and respiration rates measured from February 23, 2011 through October 12, 2011 were analyzed using the “light-and-dark-bottle” method. Since the majority of aquatic photosynthesis is carried out by phytoplankton, a glass BOD (biochemical oxygen demand) bottle can be a tool for creating a representative environment to determine the approximate photosynthetic and respiration rates of an area. When photosynthesis or respiration occurs, the associated changes in the concentrations of oxygen and carbon dioxide in the bottle provide

estimates of the photosynthetic and respiration rates (Strickland and Parsons 1972). BOD bottles were used to measure the initial oxygen concentration at each of the sites by immediately adding the required reagents according to the procedure developed by Strickland and Parsons (Strickland and Parsons 1972). BOD bottles were also used for the light and dark incubations. The “light” bottles were placed in front of fluorescent lights in the lab at a standard irradiance of $400 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$. The use of a standard irradiance allows for a more accurate assessment of the physiological condition of the algae. This irradiance is more than adequate to saturate photosynthetic rates (Davis and McIntire 1983). Naturally varying light has obvious effects on metabolic rates because light is the most basic requirement for photosynthesis. Using a constant light source provides a consistent reflection of the physiological condition of the algae. The “light” bottles were placed in front of the fluorescent lights for four hours to allow photosynthesis and oxygen liberation. “Dark” bottles were immediately wrapped in aluminum foil to protect the samples from any light infiltration and to allow respiration and oxygen uptake to occur. The dark incubation also lasted four hours. Both light and dark bottles were incubated in the Environmental Microbiology lab, kept at 19-20°C.

The procedure used to determine oxygen concentrations following the incubations was a modification of the Winkler method, where a divalent manganese solution, followed by strong alkali is added to the sample. The precipitated manganous hydroxide is dispersed evenly throughout the water sample. Any dissolved oxygen oxidizes an equal amount of divalent manganese to basic hydroxides of higher valency states. Once the solution is acidified in the presence of iodide, the oxidized manganese reverts to the divalent state and iodine is liberated. The iodine is then titrated with a standardized thiosulphate solution (Strickland and Parsons 1972). This method has a precision at the 0.7 mg-at/liter level. The assimilation numbers, or

photosynthetic rate per unit chlorophyll, can then be determined, as well as the photosynthesis to respiration ratio (P/R).

3.4.2 Changes in Dissolved Oxygen Using a YSI

Photosynthetic and respiration rates measured between October 12, 2011 and February 15, 2012 were analyzed using a YSI 5905 BOD Probe dissolved oxygen meter. The YSI was calibrated before every use with de-ionized water from the Environmental Microbiology lab at LSU. Dissolved oxygen concentrations were recorded from an initial bottle, light bottle, and dark bottle for each site. Water samples from University Lake were saturated with oxygen, and required de-gassing. De-gassing the “light” and “dark” bottles with nitrogen gas prevented them from becoming super-saturated with oxygen so photosynthesis and respiration could be measured accurately. The light and dark bottles were incubated for four hours. Once measurements of dissolved oxygen in mg/mL were taken, the assimilation number could again be determined.

3.5 Measurement of Diagnostic Pigments

High performance liquid chromatography (HPLC) pigment analyses were carried out at the University of Hawaii at the Center for Marine Microbial Ecology and Diversity (CMMED), according to the following method described by Bidigare et al. (2005). Filters for pigment analyses were extracted in 3 mL of HPLC-grade acetone in culture tubes with 50 μ L of an internal standard (canthaxanthin) at 4 °C for 24 hours. Filters were then hand ground in acetone using a glass-glass tissue homogenizer to ensure complete extraction of all pigments from phytoplankton cells. The extracts were then vortexed and centrifuged for five minutes to remove

cellular and filter debris. Mixtures of 1-mL extracts plus 0.3-mL HPLC grade water were prepared in opaque auto-sampler vials and 200 μ L injected onto a Varian 9012 HPLC system equipped with a Varian 9300 auto-sampler, a Timberline column heater (26 $^{\circ}$ C), and a Waters Spherisorb[®] 5- μ m ODS-2 analytical (4.6 x 250 mm) column and corresponding guard cartridge (7.5 x 4.6 mm). Pigments were detected with a ThermoSeparation Products UV2000 detector ($\lambda_1 = 436$, $\lambda_2 = 450$). A ternary solvent system was used for pigment analysis: Eluent A (methanol:0.5 M ammonium acetate, 80:20, v/v), Eluent B (acetonitrile:water, 87.5:12.5, v/v), and Eluent C (100 % ethyl acetate). Solvents A and B contained an additional 0.1 % 2,6-di-*tert*-butyl-*p*-cresol (0.01 % BHT, w/v; Sigma-Aldrich) to prevent the conversion of chlorophyll *a* into chlorophyll *a* allomers. The linear gradient used for pigment separation was a modified version of the Wright et al. (1991) method: 0.0' (90 % A, 10 % B), 1.00' (100 % B), 11.00' (78 % B, 22 % C), 27.50' (10 % B, 90 % C), 29.00' (100 % B), 30.00' (100 % B), 31.00' (95 % A, 5 % B), 37.00' (95 % A, 5 % B), and 38.00' (90 % A, 10 % B) (Bidigare et al., 2005). Eluent flow rate was held constant at 1.0 mL min⁻¹.

Chlorophyll *a* is the major light harvesting pigment for photosynthesis, but accessory pigment compounds can extend an organism's optical collection window and prevent cellular damage at high growth irradiances (Christensen 2011). Use of an HPLC to simultaneously determine the concentrations of several carotenoids and chlorophylls and their degradation products allows for the isolation of particular pigments. Certain diagnostic pigments are representative of particular algal groups. A table of major freshwater algal classes and their diagnostic pigments can be seen in the Appendix. Those pigment signatures can then be matched to specific algal groups. Accessory pigments make up an alga's antenna (Falkowski and Raven 2007), which is used to capture wavelengths of light not effectively trapped by chlorophyll *a*.

The ratio of accessory pigments to chlorophyll provides the key to identifying what types of algae are present in the University Lake system. A list of pigments to be measured by HPLC can be seen in Table 3.5.1.

Table 3.1.5 Pigments to be Measured by HPLC

Xanthophylls	Apocarotenoids	Carotenes	Chlorophylls	Chlorophyll breakdown product
Lutein	Peridinin	α carotene	Chlorophyll <i>a</i>	Chlorophyllide
Violaxanthin		β carotene	Divinyl	
Diadinoxanthin			Chlorophyll <i>b</i>	
Diatoxanthin				
Alloxanthin				
Fucoxanthin				
19'-butanoyl-oxyfucoxanthin				
19'-hexanoyl-oxyfucoxanthin				
Prasinoxanthin				
Zeaxanthin				

4. Results

4.1 Analysis of Inorganic Nutrients

The development of algal blooms and changes in algal community composition are heavily influenced by nutrient inputs. An input of allochthonous nutrients will allow for an increased rate of photosynthesis and growth of phytoplankton. Many factors contribute to the co-existence of multiple species during a bloom, and certain factors provide an advantage for particular species. When nutrient supplies fluctuate, multiple species are able to emerge and bloom, based on their nutrient preferences (Ebenhoh 1987). Changing mortality rates during a time of fluctuating nutrient availability can cause fluctuations in species abundance, but can contribute to the dominance of certain algal species in that period of time (Kishimoto 1990).

This analysis focused on the fluctuation of inorganic nutrient levels, and whether seasonality or episodic events had a greater influence on those fluctuations. A summary of the nutrient concentrations can be viewed in Table 4.1.1. Nitrate levels remained low throughout the experiment, and the three sites experience different peak levels on different dates. Phosphate levels were expected to be low over the course of the experiment because University Lake is phosphate limited (Mesmer 2010). Ammonium, when compared to the other inorganic nutrients measured in University Lake, had the largest swings in concentration levels, reaching very high values as well as values below the limit of detection. Silicates in University Lake have been shown to be abnormally high when compared to marine water bodies, but more comparable to silicate levels in other similar sub-tropical freshwater lakes (Pacheco et al 2010). Each site's individual nutrient profiles can be seen in Figure 4.1.1-4.1.4.

Table 4.1.1 Summary of Nutrient Concentrations (μM) at Sites 1–3. * $<\text{LoD}$ = levels below the limit of detection

Site	Nitrate			Phosphate			Ammonium			Silicate		
	mean	min	max	mean	min	max	mean	min	max	mean \pm	min	max
1	0.85 \pm	<	9.6	0.74 \pm	0.27	5.2	5.8 \pm	<	33	70 \pm 26	24	140
	1.7	LoD		0.85			6.5	LoD				
2	0.74 \pm	<	4.9	0.84 \pm	0.18	2.4	4.2 \pm	0.31	21	150 \pm	23	521
	1.3	LoD		0.6			5.1			106		
3	1.5 \pm	<	8.2	0.81 \pm	0.24	8.0	7.1 \pm	<	32	74 \pm 30	29	125
	2.1	LoD		1.3			7.0	LoD				

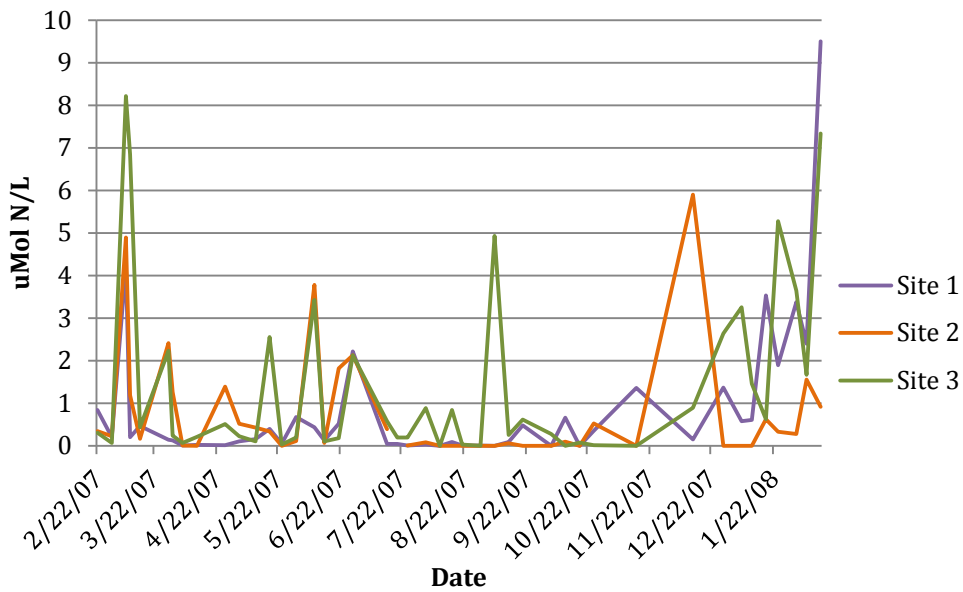


Figure 4.1.1 Concentration of Nitrates by Test Site

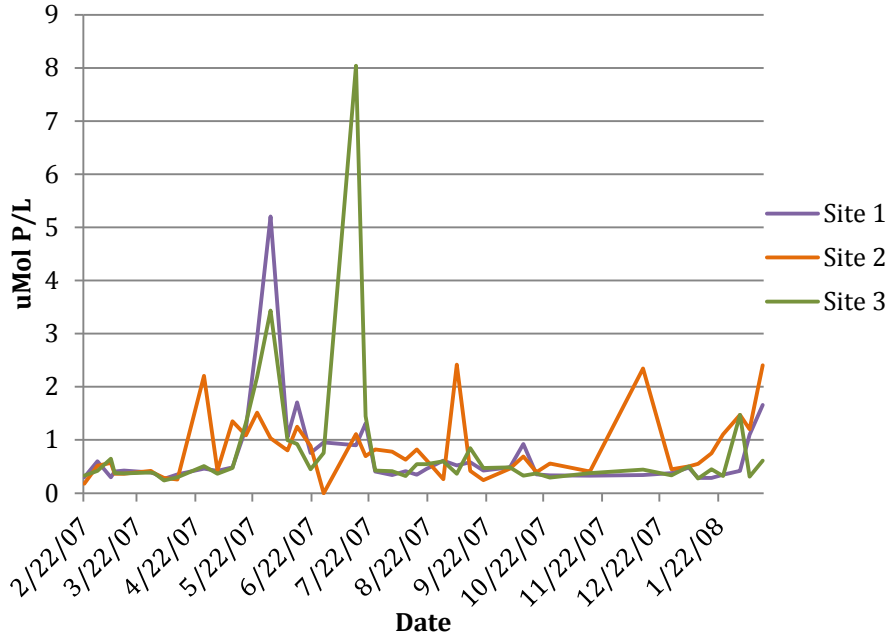


Figure 4.1.2 Concentration of Phosphates by Test Site

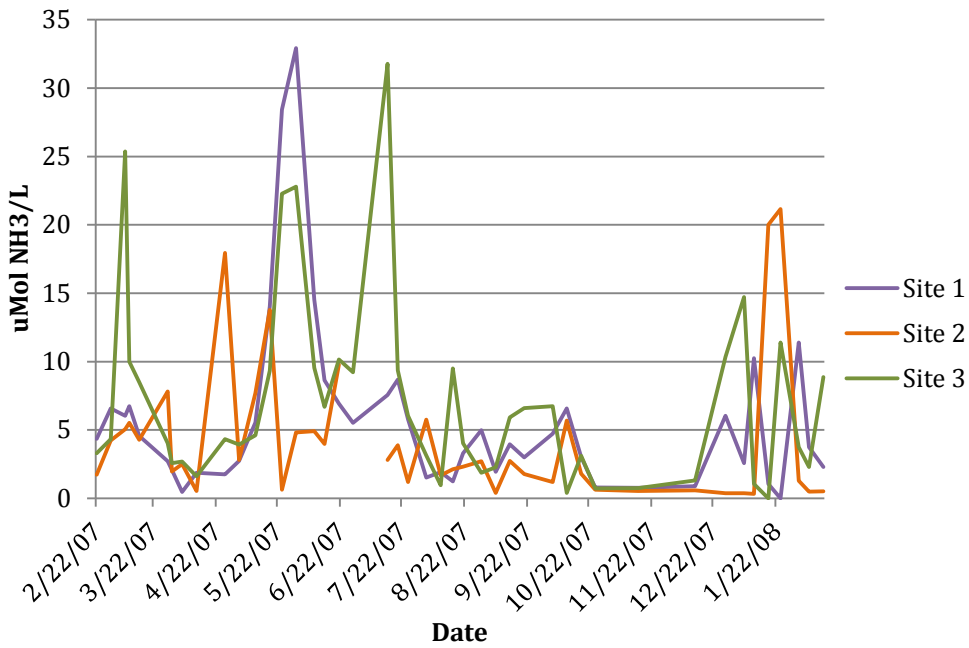


Figure 4.1.3 Concentration of Ammonium by Test Site

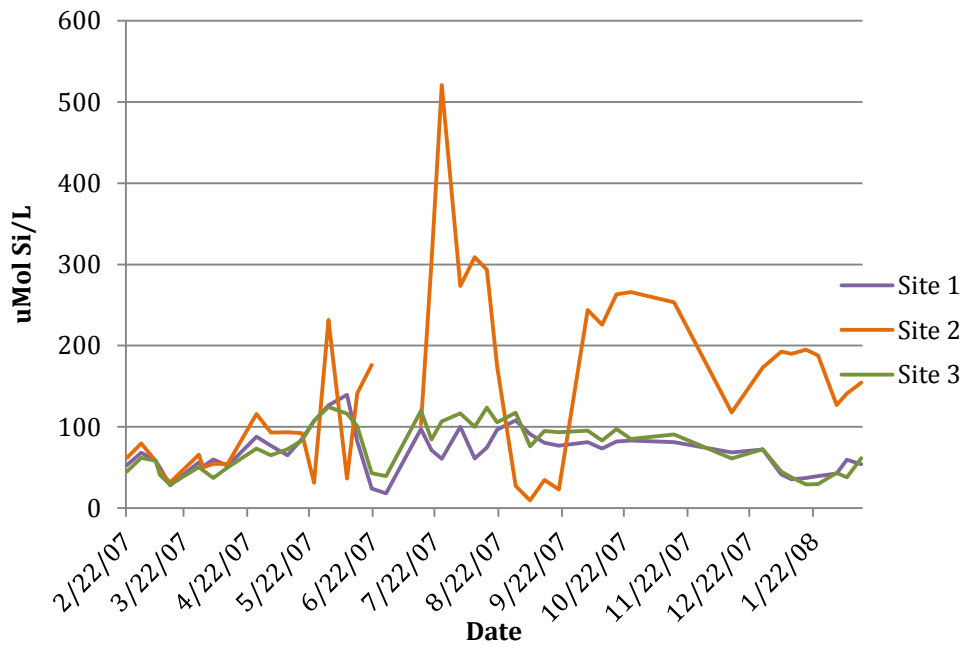


Figure 4.1.4 Concentration of Silicates by Test Site

Analysis of variance (ANOVA) was performed to determine if concentrations of inorganic nutrients were affected by seasonal progression, with the null hypothesis being that there would exist no seasonal pattern in the concentrations of inorganic nutrients. A one-way ANOVA, or the non-parametric Kruskal Wallis counterpart, was conducted to test the effects of seasonality on each inorganic nutrient measured at the three test sites. The unique hydrology of each site required that inorganic nutrient levels be considered individually by site. Seasonality in this case can be considered the independent variable and nutrient concentrations the dependent variable.

To filter out the effects of episodic events, the sampling dates following an event were excluded from the data. The nutrient concentrations were then assigned to one of four groups based on the season of the year when the samples were taken. The null hypothesis was that the

variance between groups was no bigger than the variance within groups. The results were considered significant if the type I error rate was less than 0.1. Tests for normality were performed to determine whether parametric or non-parametric testing would be used, and the results of those analyses are summarized in Table 4.1.2. There was no evidence of seasonality affecting the variance in nitrate concentrations at any of the sites.

Table 4.1.2 Statistical Results for Effects of Seasonality on Nitrate Concentrations

Site	Data Distribution	Test Performed	Resulting p Value
1	Normal	ANOVA	0.17
2	Non-normal	Kruskal Wallis	0.74
3	Non-normal	Kruskal Wallis	0.15

The procedure conducted for the effects of seasonality on variance in nitrate concentrations was repeated to test for the effects of seasonality on the variance of phosphate concentrations. These results are summarized in Table 4.1.3. There is no evidence that seasonality is affecting the phosphate concentrations at University Lake.

Table 4.1.3 Statistical Results for Effects of Seasonality on Phosphate Concentrations

Site	Data Distribution	Test Performed	Resulting p Value
1	Non-normal	Kruskal Wallis	0.15
2	Normal	ANOVA	0.20
3	Non-normal	Kruskal Wallis	0.23

Ammonium concentrations at each site were analyzed for effects of seasonality using the same procedures for nitrates and phosphates. Similarity of the variances of the ammonium concentrations justified the use of ANOVA for those tests, the results of which are summarized in Table 4.1.4. Overall, there is no evidence to conclude that seasonality is affecting the ammonium concentrations.

Table 4.1.4 Statistical Results for Effects of Seasonality on Ammonium Concentrations

Site	Data Distribution	Test Performed	Resulting p Value
1	Normal	ANOVA	0.13
2	Normal	ANOVA	0.35
3	Normal	ANOVA	0.10

Silicates were analyzed for effects of seasonality using ANOVA, due to the similarity of the variances of silicate concentrations. The results of these tests are summarized in Table 4.1.5. There was no evidence that seasonality affects the silicate concentrations at Sites 1 and 2,

however the ANOVA for the effects of seasonality on silicate concentrations at Site 3 indicated that the silicate concentrations are being significantly affected at Site 3.

Table 4.1.5 Statistical Results for Effects of Seasonality on Silicate Concentrations

Site	Data Distribution	Test Performed	Resulting <i>p</i> Value
1	Normal	ANOVA	0.50
2	Normal	ANOVA	0.37
3	Normal	ANOVA	0.02

Because seasonality was concluded to be significantly affecting only the silicate concentrations at Site 3, it seems reasonable to conclude that seasonal phenomena are not primarily responsible for changes in inorganic nutrient levels in University Lake. The nutrient concentrations may not be driven by seasonal progression, but it could be possible that episodic events are having a significant effect on the availability of nutrients. All sampling dates were included to test for a difference in inorganic nutrient concentrations due to episodic events. These events were defined as storms in which more than one centimeter of precipitation fell in one hour, weather in which wind speeds caused visible surface disturbance, and fish kills.

Lilliefors tests for normal distribution were again performed on each set of data by site, to determine whether parametric or non-parametric testing was to be used. The results of these tests are summarized in Table 4.1.6. Episodic events did have a significant affect on the nitrate concentrations at Sites 1 and 3, as the nitrate concentrations following episodic events were significantly different from the background data, but there is no evidence to conclude that

episodic events are significantly affecting the nitrate concentrations at Site 2. An additional ANOVA test also provides evidence that the nitrate concentrations are not significantly different between test sites ($p=0.129$).

Table 4.1.6 Statistical Results for the Effects of Episodic Events on Nitrate Concentrations

Site	Data Distribution	Test Performed	Resulting p Value
1	Normal	ANOVA	0.074
2	Non-normal	Kruskal Wallis	0.11
3	Normal	ANOVA	0.035

The procedures used for statistical testing for the effects of episodic events on nitrate concentrations were repeated for phosphates, the results of which are summarized in Table 4.1.7. There was no evidence to suggest that phosphate concentrations after episodic events were significantly different from background phosphate concentrations at Sites 1 and 3, but Site 2 did experience a significant difference. An additional KW to test for differences in phosphate concentrations between sites was performed, but it did not provide sufficient evidence that the concentrations were significantly different between sites ($p=0.068$).

Table 4.1.7 Statistical Results for the Effects of Episodic Events on Phosphate Concentrations

Site	Data Distribution	Test Performed	Resulting p Value
1	Non-normal	Kruskal Wallis	0.34
2	Normal	ANOVA	0.031
3	Non-normal	Kruskal Wallis	0.90

There was no evidence to suggest that the occurrence of episodic events significantly affects the ammonium concentrations in University Lake. A summary of the statistical tests can be seen in Table 4.1.8. An ANOVA to test for differences in the ammonium concentrations between sites was also performed, and the sites were not significantly different ($p= 0.11$).

Table 4.1.8 Statistical Results for Effects of Episodic Events on Ammonium Concentrations

Site	Data Distribution	Test Performed	Resulting p Value
1	Normal	ANOVA	0.22
2	Normal	ANOVA	0.44
3	Normal	ANOVA	0.68

Silicate concentrations, like ammonium, were not significantly affected by the occurrence of episodic events; and the concentrations of silicate following episodic events were not different from the background silicate concentrations. The results of the statistical tests are summarized in

Table 4.1.9. An additional ANOVA did provide evidence that the silicate concentrations between the three test sites are significantly different ($p=4 \times 10^{-8}$). Site 2 had a mean rank that was significantly different than Sites 1 and 3.

Table 4.1.9 Statistical Results for Effects of Episodic Events on Silicate Concentrations

Site	Data Distribution	Test Performed	Resulting p Value
1	Normal	ANOVA	0.4761
2	Normal	ANOVA	0.8193
3	Normal	ANOVA	0.121

4.2 Analysis of Photosynthetic and Respiration Rates

Growth and development of phytoplankton are functions directly related to the carbon gained during photosynthesis and the carbon lost during respiration. Photosynthetic and respiration rates of phytoplankton are indicators of the health and physiology of the phytoplankton community, as well as environmental indicators. An average of photosynthetic to respiration ratios in freshwater environments are generally within a range of 10:1 to 12:1 (Groeger and Kimmel 1989).

Oxygen gain and loss was recorded in samples collected between September of 2011 and February of 2012. During these months, the median gross assimilation number was 3.45 gram carbon/gram chlorophyll *a*/ hour (g C/g chl/hr), and the median respiration rate was 0.66 g C/g chl/hr. These numbers are quite reasonable, and within the range of average photosynthetic to respiration ratios that are expected in a limnetic environment (Geider and Osborne 1989).

However, there were dates when the gross assimilation number was above 10 g C/g chl/hr, and dates when no oxygen was lost during respiration. The highest gross assimilation number was reached at Site 2 on January 12, 2012, and gains of oxygen during dark respiration occurred in 23% of the respiration samples. The changes in gross assimilation number and respiration can be seen in Figure 4.2.1-4.2.3.

The changes in gross assimilation number and respiration over time reflect the health and productivity of the phytoplankton community. Changes in the productivity of the community are likely a sign of changes to their environment, which could include episodic events and the flux of inorganic nutrients. To determine if the gross assimilation numbers were different on sampling dates following an episodic event compared to the regular sampling dates, a one-way ANOVA was conducted. The results were significant ($p = 0.035$), indicating that the assimilation numbers on sampling dates following episodic events were higher than the assimilation numbers on regular sampling dates. The results of that analysis can be seen in Figure 4.2.4.

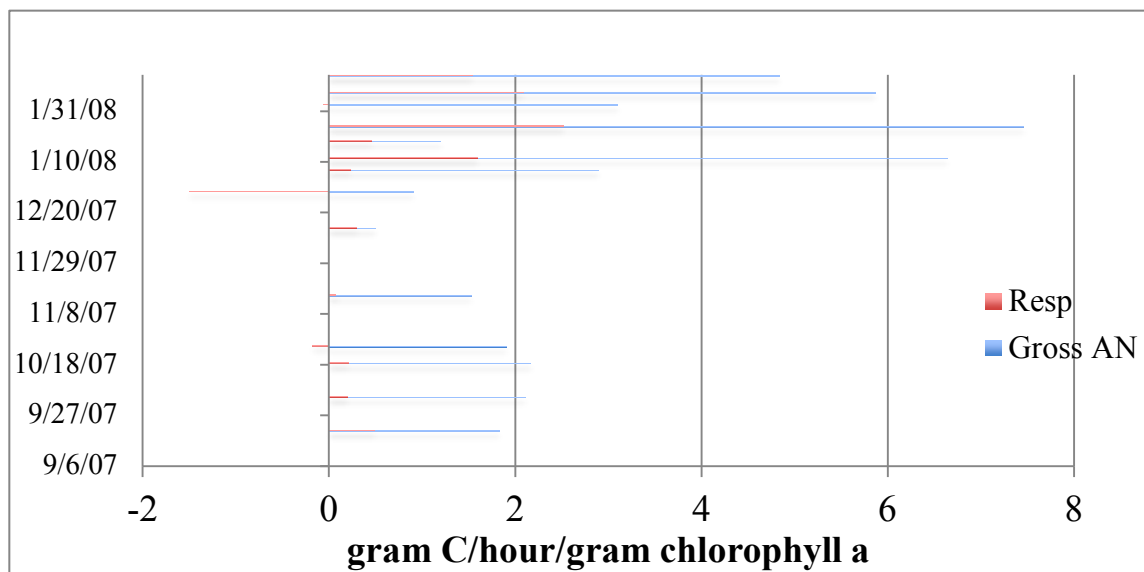


Figure 4.2.1 Gross Assimilation Number and Respiration at Site 1 *Negative respiration values indicate that respiration did not occur during the incubation period, rather the bottles continued to assimilate oxygen

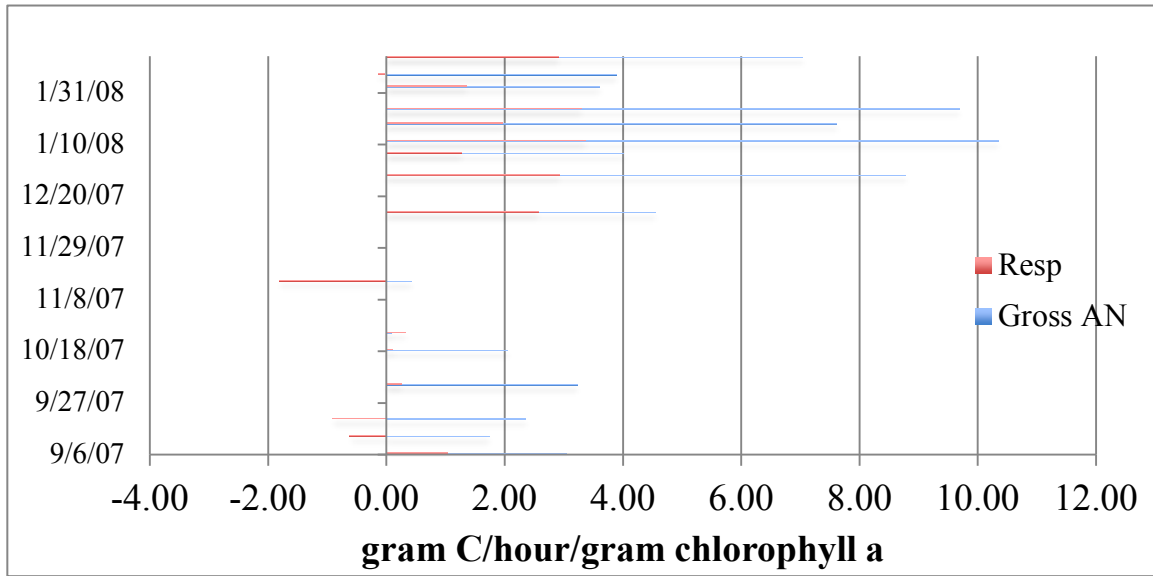


Figure 4.2.2 Gross Assimilation Number and Respiration at Site 2

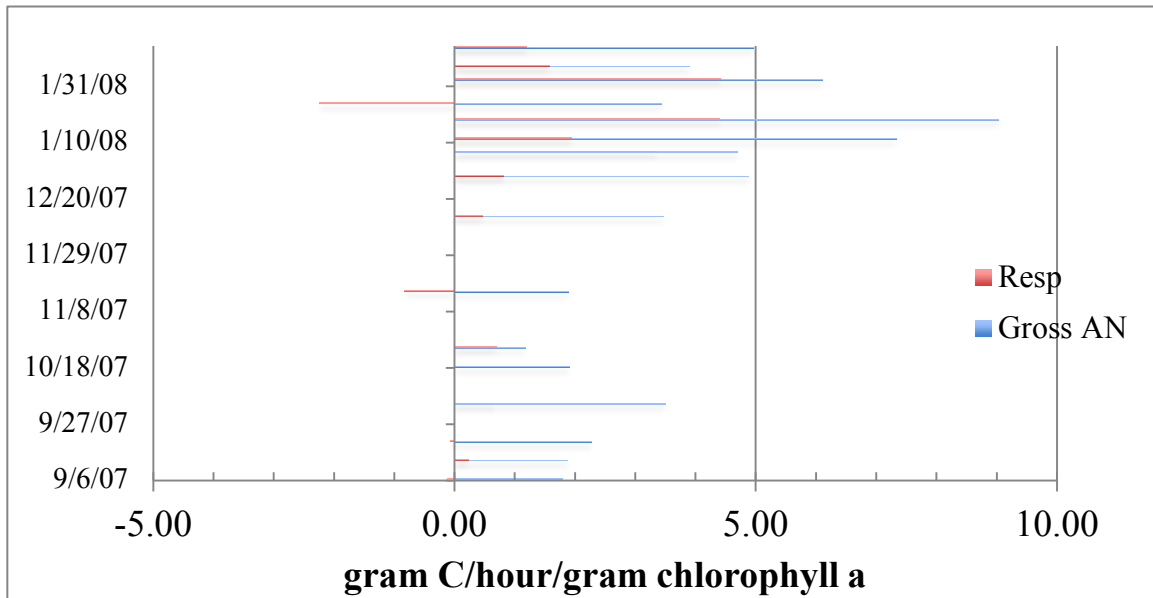


Figure 4.2.3 Gross Assimilation Number and Respiration at Site 3

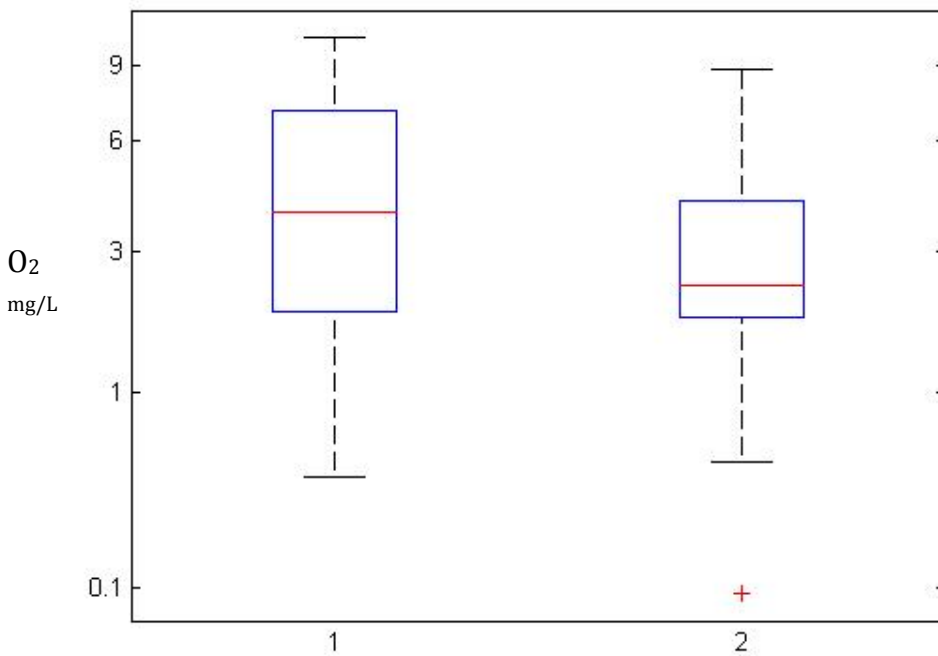


Figure 4.2.4 Results of Statistical Testing for Differences in Assimilation Numbers for Episodic Events vs. Regular Sampling *Group 1: Samplings following episodic events, Group 2: Regular samplings

The results of this analysis could indicate that the presence of a particular nutrient could be affecting the rates of photosynthesis and respiration.

Tests for correlation between gross assimilation numbers and individual inorganic nutrients were performed to determine if a particular nutrient was affecting productivity. In the case of gross assimilation numbers, the Pearson's correlation coefficient between assimilation and ammonium was significant, demonstrating that the presence of ammonium has an effect on assimilation. The results of this correlation analysis can be seen in Table 4.2.1, and an example

figure of the relationship between gross assimilation number and ammonium concentration can be viewed in Figure 4.2.5.

Table 4.2.1 Correlation Coefficients Between Gross Assimilation Numbers and Inorganic Nutrients *Correlation is significant at the 0.05 level (2-tailed). AN= assimilation number

		Gross AN	Silicates	Nitrates	Ammonium	Phosphates
Gross AN	Pearson Correlation	1	-.014	.185	.329*	.243
	Sig. (2-tailed)		.927	.208	.022	.096
	N	48	48	48	48	48

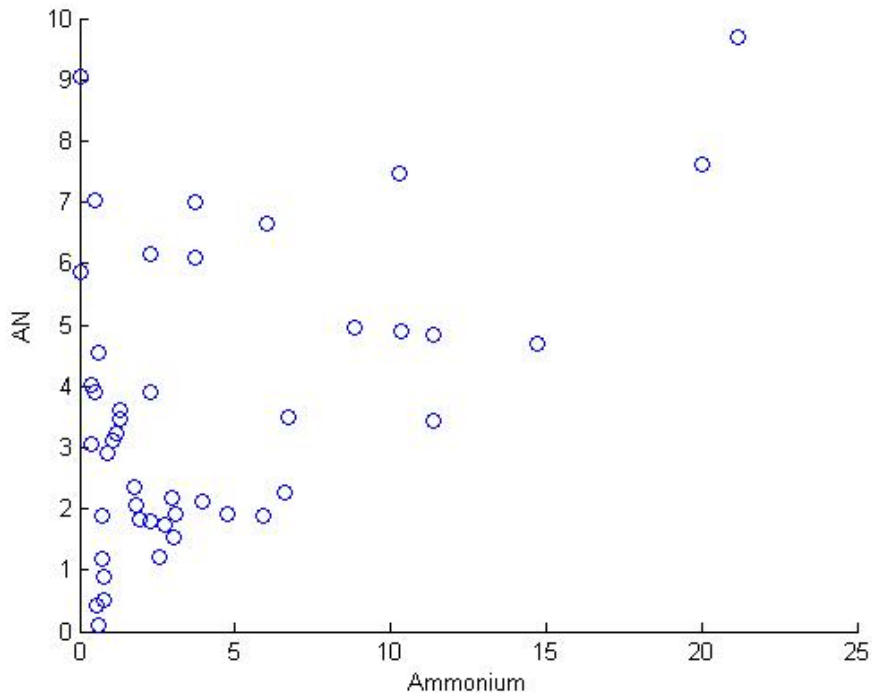


Figure 4.2.5 Gross Assimilation Number versus Ammonium Concentration, *AN units= gC/g chl a/hr, Ammonium units= μ Mol/L

A similar correlation test was performed to determine if phytoplankton respiration was being affected by the presence or absence of inorganic nutrients. In the case of respiration rates, the Pearson's correlation coefficient between respiration and phosphates was significant, and a visualization of the relationship between respiration rates and phosphate concentrations can be viewed in Figure 4.2.6. The results of this analysis can be seen in Table 4.2.2.

Table 4.2.2 Correlation Coefficients Between Respiration Rates and Inorganic Nutrients

*Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). Resp.= respiration rates

		Silicates	Nitrates	Ammonium	Phosphates	Resp
Resp	Pearson Correlation	-.045	.171	.184	.393**	1
	Sig. (2-tailed)	.761	.245	.210	.006	
	N	48	48	48	48	48

While the positive correlations were quite strong in the cases of ammonium concentrations and gross assimilation number, and phosphate concentrations and respiration, the correlations do appear to be more prevalent in the lower bounds. At high nutrient levels, assimilation and respiration is accordingly higher, but at low nutrient levels, assimilation of oxygen and respiration seems to be subject to variance. An approximation of these bounds can be seen in Figures 4.2.7 and 4.2.8.

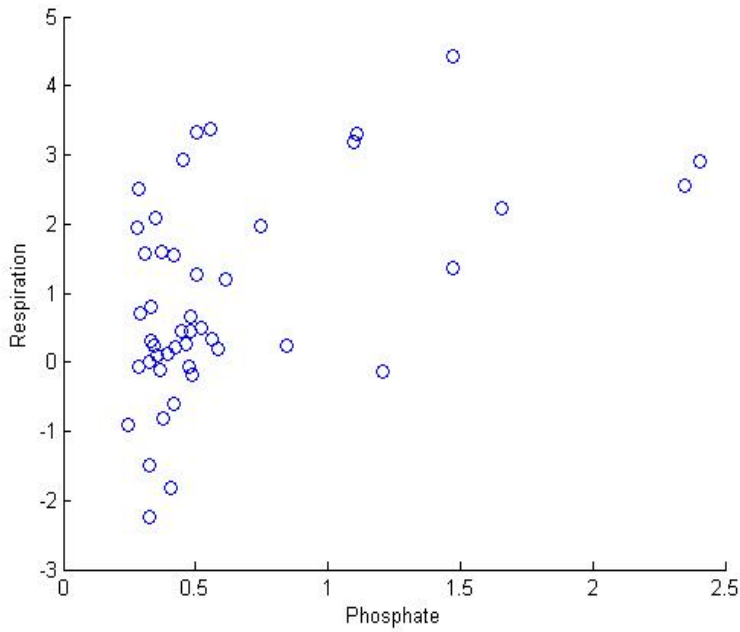


Figure 4.2.6 Respiration Rates versus Phosphate Concentrations, *Resp units= gC/g chl a/hr, Phosphate units= μ Mol/L

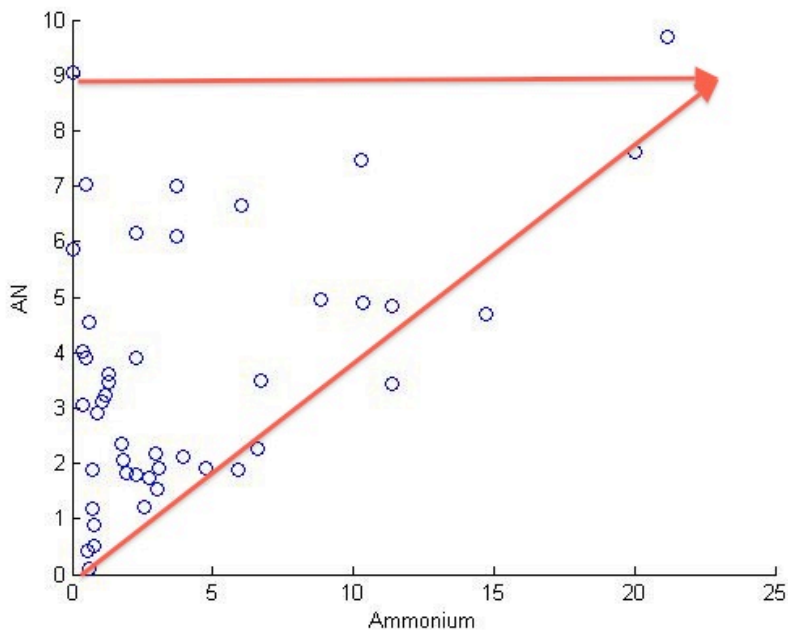


Figure 4.2.7 Confidence Bounds for the Correlation Between Ammonium Concentrations and Gross Assimilation Numbers *AN units= gC/g chl a/hr, Ammonium units= μ Mol/L

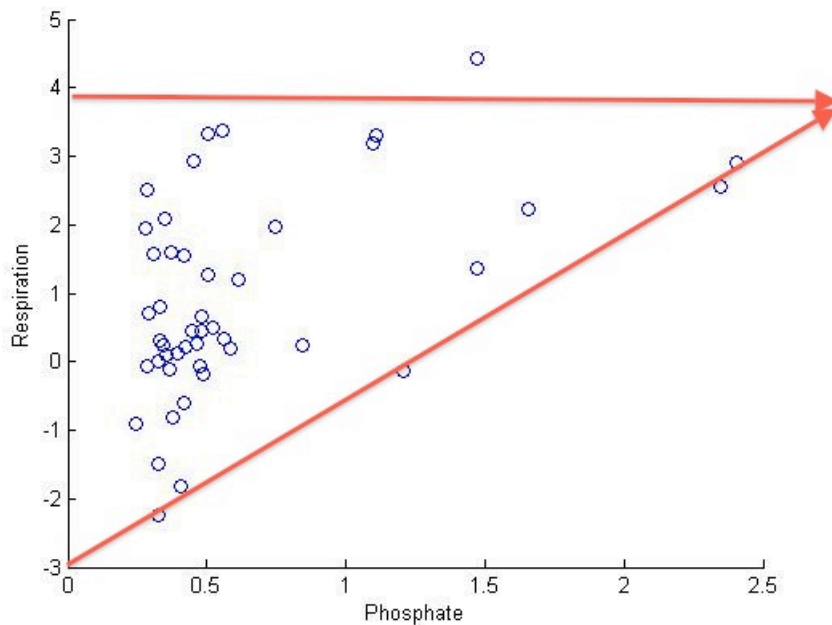


Figure 4.2.8 Confidence Bounds for the Correlation Between Phosphate Concentrations and Respiration Rates *Resp units= gC/g chl a/hr, Phosphate units= μ Mol/L

4.3 Diagnostic Pigment Analysis

Chlorophyll *a* is commonly known as the light harvesting pigment for photosynthetic organisms. However, there are many additional pigments that allow an organism to maximize photosynthetic capabilities, and these pigments vary between algal groups. By analyzing concentrations of marker pigments present in a water sample, the algal community composition can be discovered. The community composition of any body of water is variable to the parameters of its environment, which include effects of seasonality as well as disturbances.

Sixteen diagnostic pigments were measured for this analysis. A table of algal classes and their major diagnostic markers can be seen in Appendix X. The pigments peridinin,

prasinolaxanthin, 19'-butanoyloxyfucoxanthin, and 19'-hexanoyloxyfucoxanthin were not detected at any time over the course of the yearlong study. The implication is that dinoflagellates, prasinophytes, pelagophytes, and prymnesiophytes are not part of the phytoplankton community of University Lake. Once the absent groups were eliminated, analyses to explain the variance in chlorophyll *a*, and analysis for the effects of seasonality and episodic events were carried out. The procedure used during the inorganic nutrient analysis was repeated to determine whether or not seasonality was a factor in the variations of monovinyl chlorophyll *b*, lutein, alloxanthin, fucoxanthin, zeaxanthin, and violaxanthin concentrations.

Marker pigments can be used to identify and quantify classes of phytoplankton. However, quantification is based on the ratio of marker pigment:chlorophyll *a*, which is not a fixed number (Woitke et al. 1996). Variations of marker pigment:chlorophyll *a* ratios may occur as a result of change in the marker cell content or chlorophyll *a* cell content, or from changes in both (Descy et al. 2009). To determine community composition, the concentration of chlorophyll *a* present at a given time must be explained by the presence of multiple algal species. The ratio of a marker pigment:chlorophyll *a* allows quantification of the amount of chlorophyll *a* associated with each class of phytoplankton that is present in the water.

A multiple regression analysis to explain as much as possible of the variance in chlorophyll *a* concentrations was conducted. All detected diagnostic pigments were used as independent variables. Violaxanthin, alloxanthin, and lutein were not significantly related to chlorophyll *a* in University Lake. The final regression model included fucoxanthin, monovinyl chlorophyll *b*, and zeaxanthin (marker pigments for diatoms, chlorophytes, and cyanobacteria, respectively) as the independent variables. Variations in the concentrations of these three

pigments accounted for 87% of the variance of the chlorophyll *a* concentrations. The regression line can be seen in Figure 4.3.1.

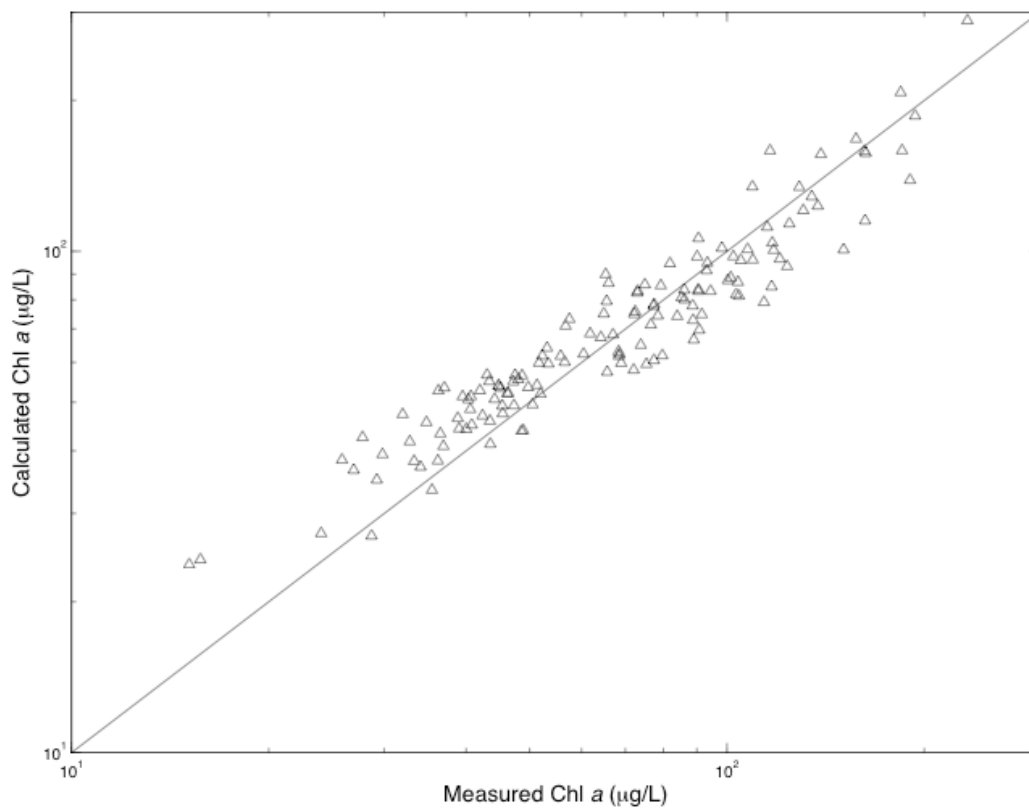


Figure 4.3.1 Multiple Regression Explaining Variance of Chlorophyll *a*. The regression equation is $\text{Chl } a = 6.5 + 4.2 \cdot \text{fucoxanthin} + 2.0 \cdot \text{zeaxanthin} + 5.8 \cdot \text{chl } b$

Based on this multiple regression analysis, the regression coefficients for fucoxanthin (4.2), monovinyl chlorophyll *b* (5.8), and zeaxanthin (2.0) were multiplied by the concentration of each pigment to determine the percentage of chlorophyll *a* accounted for by diatoms,

chlorophytes, and cyanobacteria on each sampling date. This calculation indicated that approximately 10% of the phytoplankton community consisted of diatoms, 51% was chlorophytes, and 30% was cyanobacteria. These percentages fluctuated over the course of the experiment, and the changes in community composition can be seen in Figures 4.3.2-4.3.4.

Although lutein is a diagnostic pigment for chlorophytes, it did little to improve the goodness of fit of the model as long as monovinyl chlorophyll *b* (also diagnostic for chlorophytes) was also included. Because no other diagnostic pigments associated with chlorophyll *b* were detected, it can be assumed that the monovinyl chlorophyll *b* present during this analysis reflected the presence of chlorophytes in the phytoplankton community.

Variations in alloxanthin explained an insignificant percentage of the variance in chlorophyll *a*, but alloxanthin was consistently present in low concentrations throughout the year. Alloxanthin is a marker pigment only for cryptophytes, so it is clear that a small percentage of the phytoplankton community consisted of cryptophytes. Descy et al. (2009) estimated the ratio of cryptophytes:chlorophyll *a* in freshwater to be 0.3. The average concentration of alloxanthin in University Lake was 765 ng/L. When that ratio is applied to the average concentration of alloxanthin in University Lake, 2.6 µg/L of chlorophyll *a* can be explained by the presence of cryptophytes, which accounts for 3.4% of the total chlorophyll *a* or about 40% of the 6.5 µg/L of chlorophyll not explained by the multiple regression model (i.e., the intercept of the regression line) and indicates that cryptophytes represent approximately 3.4% of the phytoplankton biomass of University Lake.

Violaxanthin, like alloxanthin, accounted for an insignificant percentage of the variance of chlorophyll *a*, but is also present in low concentrations throughout the year. Unfortunately, the

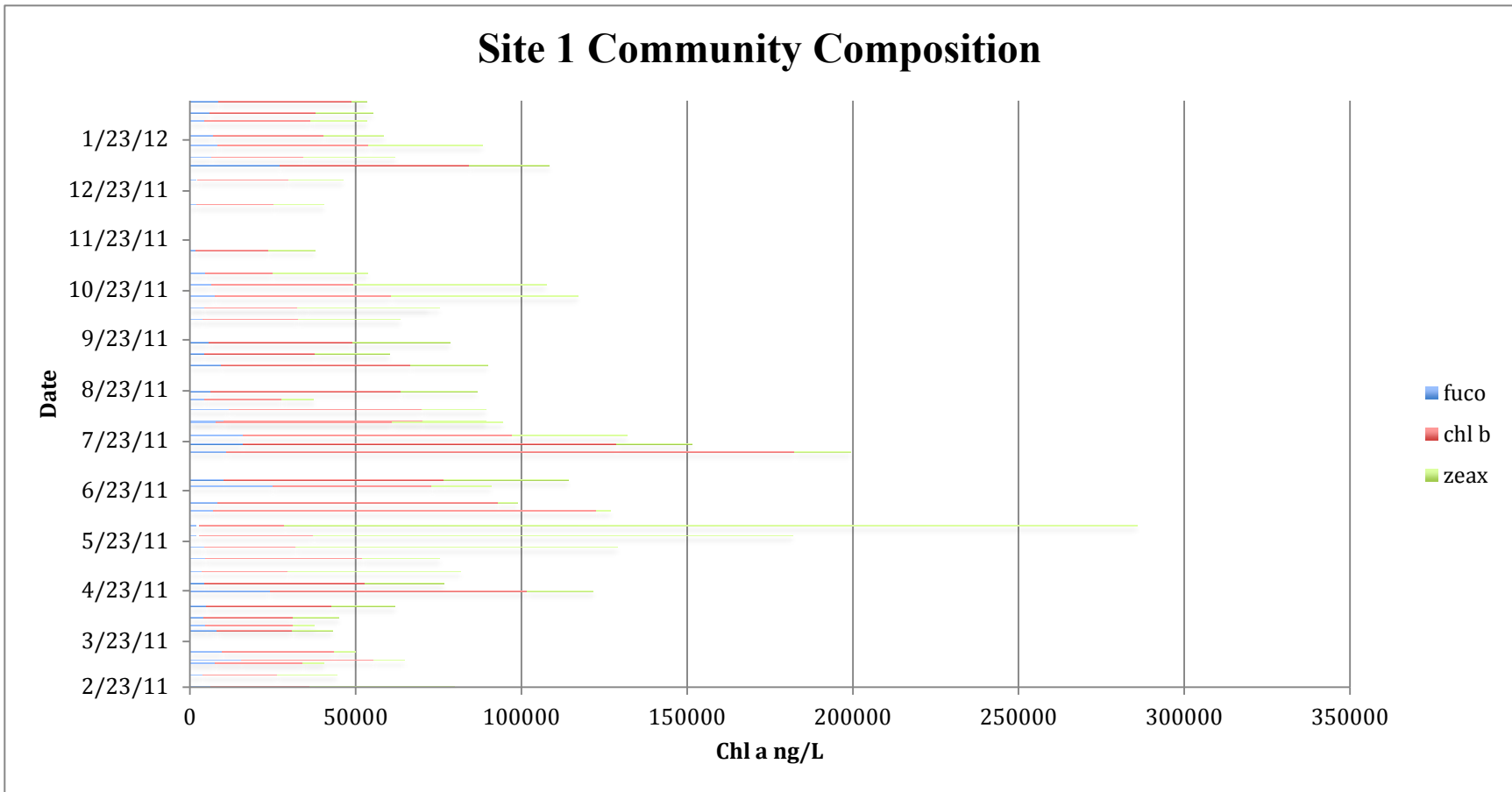


Figure 4.3.2 Phytoplankton Community Composition Changes at Site 1. Fuco=fucoxanthin, marker pigment of diatoms. Chl b=monovinyl chlorophyll *b*, marker pigment of chlorophytes. Zea=zeaxanthin, marker pigment of cyanobacteria.

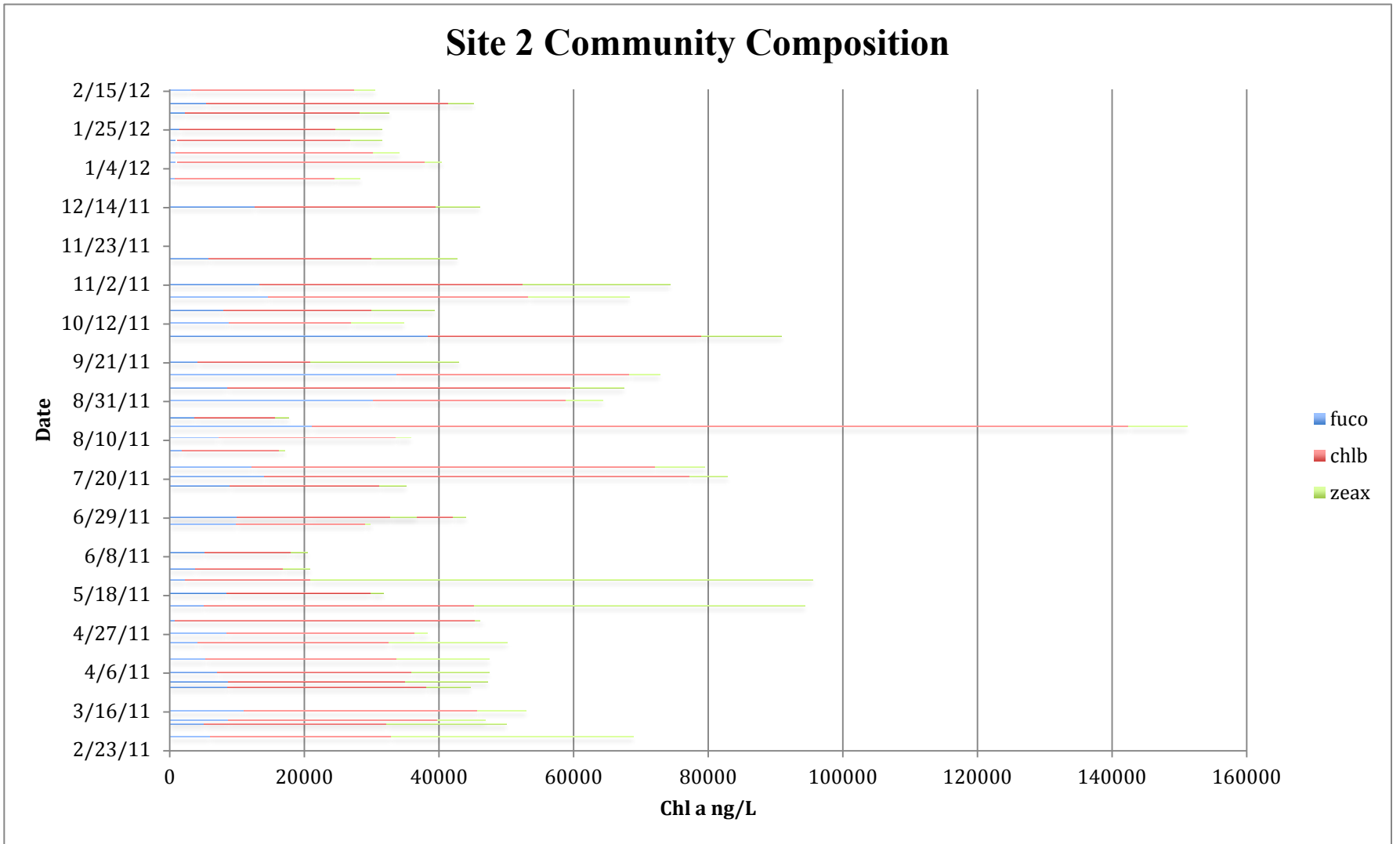


Figure 4.3.3 Phytoplankton Community Composition Changes at Site 2

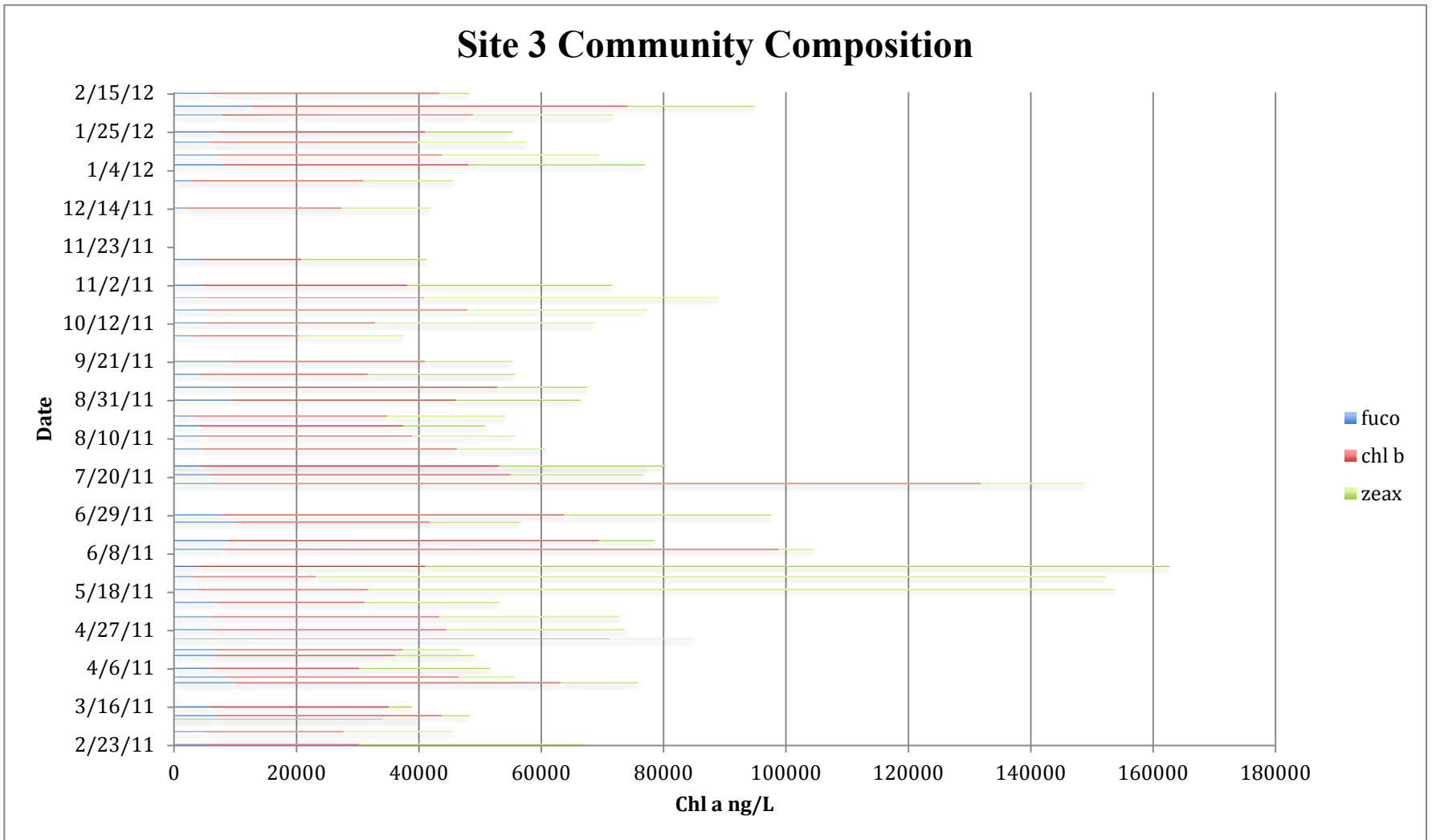


Figure 4.3.4 Phytoplankton Community Composition Changes at Site 3

fact that no established ratio of violaxanthin:chlorophyll *a* exists makes an estimation of the biomass that contains violaxanthin extremely difficult. With this multiple regression analysis, only 6% of the total phytoplankton population is unaccounted for.

A principal component analysis was performed to determine variance about the diagnostic pigments. The first two principal components accounted for approximately 75% of the variance about the pigments. Principal component one was comprised of chlorophyll *b*, violaxanthin, and lutein, all of which represent chlorophytes and is consistent with the results of the multiple regression analysis. The second component was largely comprised of zeaxanthin, but also larger, negative contributions from fucoxanthin and diadinoxanthin. Component two also supports the conclusions of the multiple regression analysis, but is also indicative of a relationship between the cyanobacteria and diatom populations of the lake. The relationship between cyanobacteria and diatoms can be seen in Figure 4.3.5. It seems that when fucoxanthin and/or zeaxanthin concentrations are low, any concentration of the other pigment is possible. However, high fucoxanthin concentrations only occur when zeaxanthin is low, and high zeaxanthin concentrations only occur when fucoxanthin is low. This indicates that cyanobacteria are only able to bloom when the diatom population is low, and vice versa.

Seasonal progression can be expected to have an effect on phytoplankton growth and development, because more irradiance in summer months allows for increased productivity. Lilliefors tests for normality and equality of variance were performed on each set of pigment data identified during the multiple regression analysis, as well as for alloxanthin. The results of the ANOVAs are summarized in Table 4.3.1. Overall, the statistical testing did not provide evidence to suggest that seasonality significantly affects the phytoplankton community

composition of University Lake. Fucoxanthin concentrations were significantly different between seasons at Site 2, with summer and winter having significantly different mean concentrations. Chlorophyll *b* concentrations were also significantly affected by seasonality at Site 2, with spring and summer having significantly different mean concentrations.

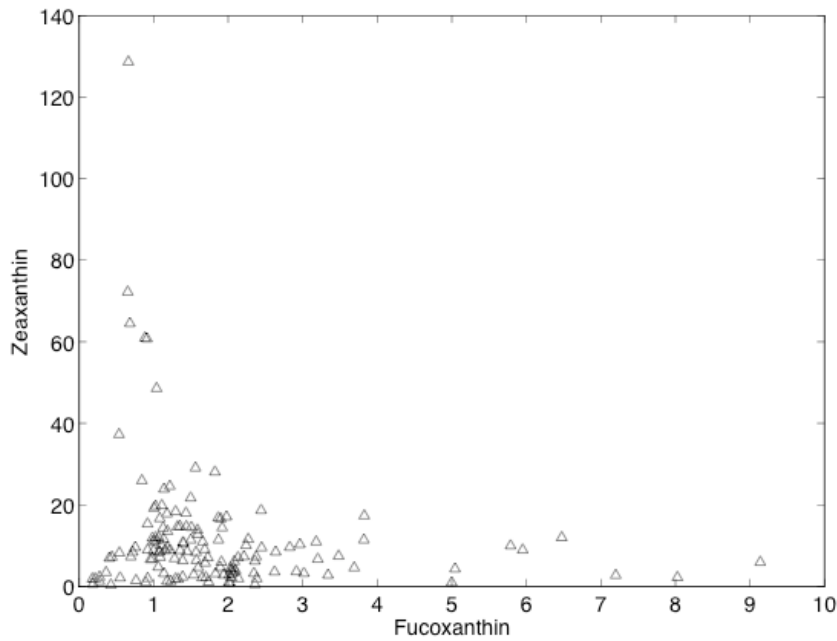


Figure 4.3.5 Relationship Between Fucoxanthin and Zeaxanthin Concentrations *units are ng/L

Table 4.3.1 Statistical Results for Effects of Seasonality on Phytoplankton Community Composition

Pigment	Site 1	Site 2	Site 3
Fucoxanthin	$p = 0.52$	$p = 0.014$	$p = 0.44$
Zeaxanthin	$p = 0.11$	$p = 0.14$	$p = 0.69$
Monovinyl Chl <i>b</i>	$p = 0.14$	$p = 0.01$	$p = 0.12$
Alloxanthin	$p = 0.12$	$p = 0.46$	$p = 0.64$

Episodic events have been shown to affect phytoplankton community composition (White 1976, Thomas and Gibson 1990, Tynan 1993, Truscott 1995). The severity of an event may provide opportunity for enhanced interspecies competition, or a flux of nutrients that may favor the development of one algal class over another. One-way ANOVAs were run to determine if episodic events were significantly altering the phytoplankton community composition. The results of these tests are summarized in Table 4.3.2. The ANOVAs again returned mostly insignificant results, indicating that episodic events are not a significant factor in the changing phytoplankton community composition. Fucoxanthin concentrations at Site 1 were found to be significantly different, with the concentrations of fucoxanthin following episodic events significantly higher than the concentrations found in background samples. Chlorophyll *b* concentrations were also significantly increased following episodic events at Site 1.

Table 4.3.2 Statistical Results for the Effects of Episodic Events on Phytoplankton Community Composition

Pigment	Site 1	Site 2	Site 3
Fucoxanthin	$p = 0.026$	$p = 0.56$	$p = 0.58$
Zeaxanthin	$p = 0.491$	$p = 0.47$	$p = 0.61$
Monovinyl Chl <i>b</i>	$p = 0.042$	$p = 0.21$	$p = 0.77$
Alloxanthin	$p = 0.41$	$p = 0.41$	$p = 0.70$

The tests for effects of seasonality and episodic events did not yield results significant enough to signify that either forcing function is impacting the phytoplankton community composition. ANOVAs to determine if the community composition varied between the three test

sites returned extremely significant results (fucoxanthin, $p = 9.31 \times 10^{-7}$; zeaxanthin, $p = 8.48 \times 10^{-5}$; monovinyl chlorophyll *b*, $p = 4 \times 10^{-4}$; alloxanthin, $p = 0.08$), with the exception of alloxanthin, which was not significant at the 95% confidence level, but was at the 90% level. These differences in pigment concentrations by site can be seen in Figures 4.3.5-4.3.8.

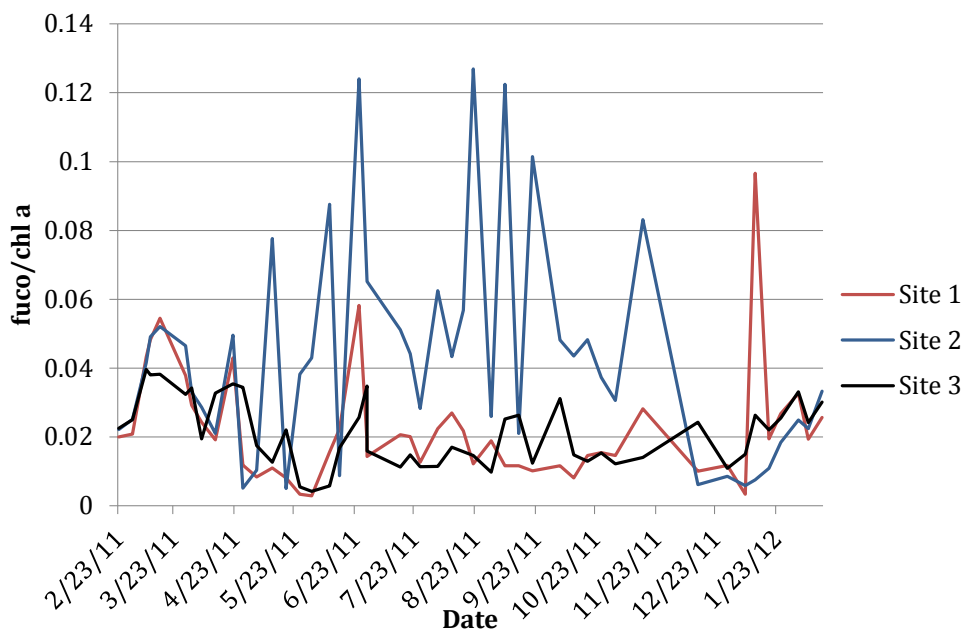


Figure 4.3.5 Fucoxanthin Concentration by Site *fucoxanthin measured in ng/L

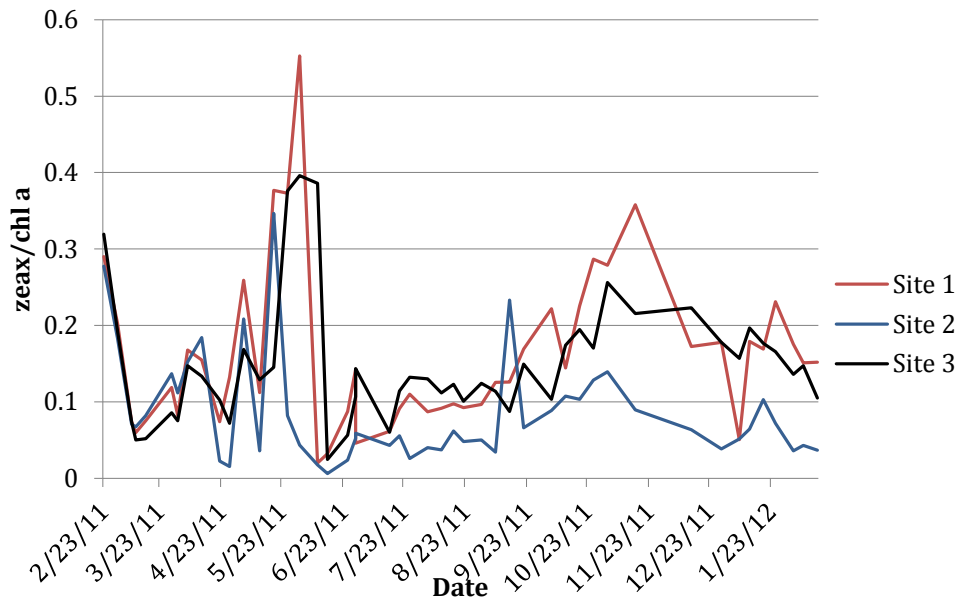


Figure 4.3.6 Zeaxanthin Concentration by Site *zeaxanthin measured in ng/L

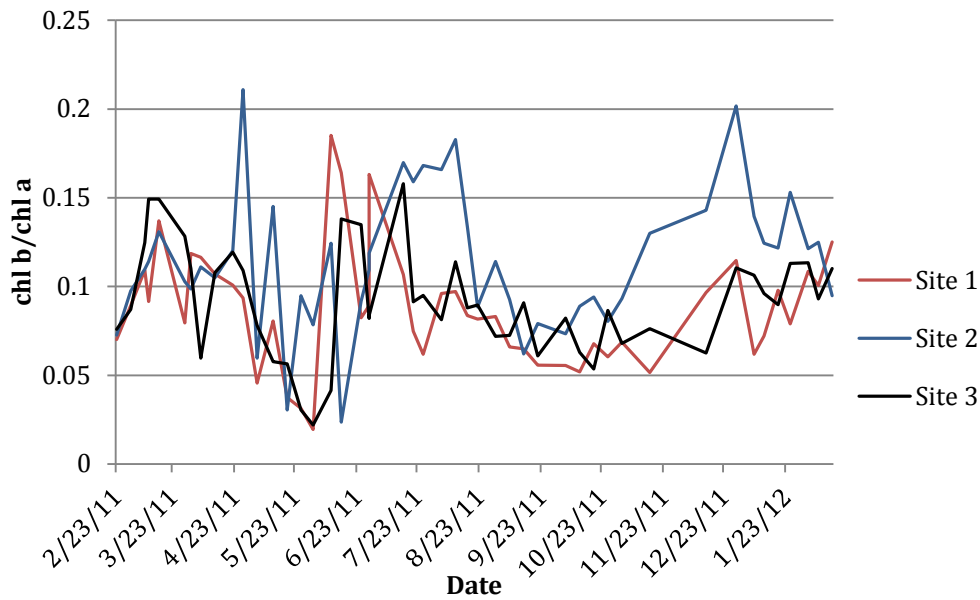


Figure 4.3.7 Monovinyl Chlorophyll *b* Concentrations by Site *chl b measured in ng/L

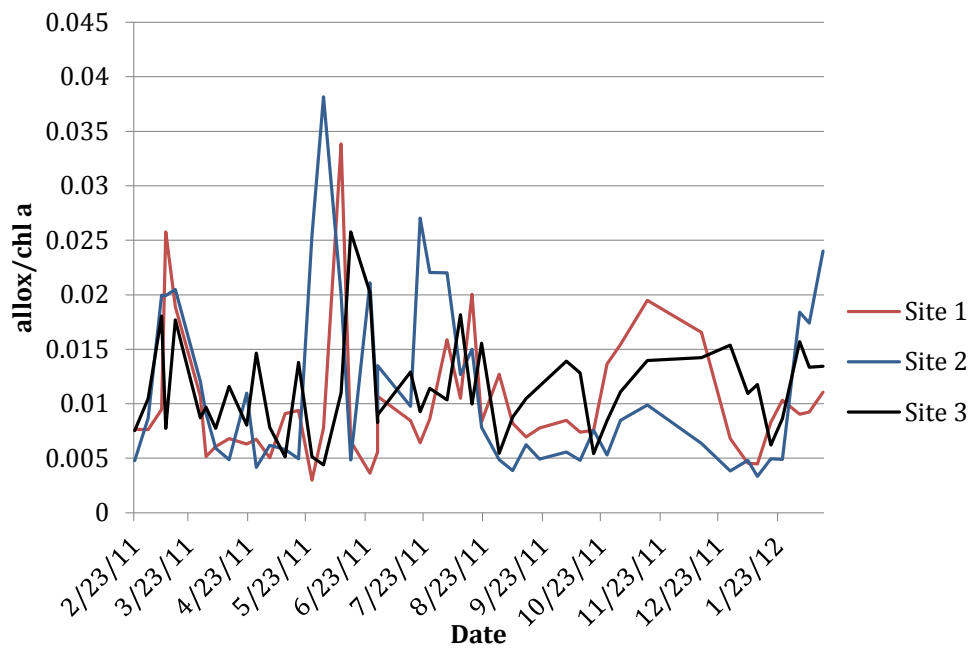


Figure 4.3.8 Alloxanthin Concentrations by Site *alloxanthin measured in ng/L

5. Discussion

5.1 Inorganic Nutrients and Variability

University Lake is a highly eutrophic, shallow lake, located in an urban setting. Urban water bodies tend to experience increased input of inorganic nutrients due to the large amount of impervious surfaces surrounding them. This addition of nutrients can alter the hydrology of the drainage systems because excess nutrients will speed up the eutrophication process. Urban lakes in particular are also more susceptible to the accumulation of nutrients as their hydrologic retention time is much longer than a stream or river.

Nutrient availability is crucial for algal development, but other environmental characteristics (e.g., temperature, irradiance) may have a greater influence on the type of species that will become dominant. It is necessary to consider the effects of seasonality in relation to the flux of inorganic nutrients, as well as the occurrence of episodic events. Turbulence is a factor that greatly affects the phytoplankton community composition of a water body and the potential for a bloom. Turbulent mixing can control the scale of nutrient availability, average water column irradiance, and phytoplankton growth. While turbulence can be beneficial for the phytoplankton population overall, laboratory experiments have shown that turbulent conditions depress dinoflagellate growth by reducing dinoflagellate cell division capabilities (White 1976, Thomas and Gibson 1990, Tynan 1993). Dinoflagellates seem to react poorly to episodic disturbances, while diatoms seem to be stimulated by the increased movement (Thomas and Gibson 1990). Stratification of a water body is often affected by turbulence, as well as modifiable by heat, wind, and runoff. Variations in microhabitats from changes in the stratification of a water body influences phytoplankton population growth. Fresh water from a combination of rainfall, runoff, and irradiance, creates improved zones for population growth

(Smayda 1997). These zones of improved growth exist only temporarily and are dependent on nutrient availability and algal utilization, but can result in a bloom. Eventually, horizontal dispersal of phytoplankton and nutrients will hinder bloom development (Kierstead and Slobodkin 1951).

University Lake is phosphate limited, with runoff being the primary factor affecting phosphate levels (Mesmer 2010). Measurable nitrate and phosphate levels have been recorded at extremely low levels in the past, while silicate levels are far in excess of concentrations that would be associated with nutrient limitation (Neale et al. 1991, Tilman et al. 1982, Hutchinson 1957). Elevated levels of silicate in a water body indicate that diatoms should be present because the primary distinguishing feature of diatoms is the highly silicified cell wall composed of two overlapping halves (Smith 1950).

Overall, the nutrient concentrations in University Lake from February 2011 to February 2012 echoed the expectations formulated from previous studies. The nitrate and phosphate concentrations remained at low levels throughout the year, with some prolonged spikes. Ammonium and silicate remained at high levels throughout the year, and the lowest levels experienced were still relatively high when compared with the concentrations found in other comparable lakes (Kilham 1971). It is likely that the nitrate levels remain low because the sediments of the lake are anoxic, and nitrate is being denitrified in the sediments. Seasonality was shown to be an insignificant forcing factor in terms of variations of inorganic nutrient concentrations, with the exception of silicate levels at Site 3. Site 3 is located at a busy intersection in Baton Rouge, and the sampling location was very close to the culvert connecting University Lake with Crest Lake. The hydrology of this site may be playing a bigger role in the control of silicate concentrations than seasonality. This sampling period also took place during a

warm year with a hot summer and mild winter. It is possible that the unusually warm temperatures prevented effects of seasonality on inorganic nutrient concentrations during this experiment.

Episodic events are often responsible for an influx of nutrients through runoff. In the case of University Lake, episodic events did have a significant effect on the nitrate concentrations at Sites 1 and 3, as well as the phosphate concentrations at Site 2. Extraneous inputs of nitrates are to be expected with a storm, especially in urban areas. Site 1 is adjacent to a large road in Baton Rouge, and the site is also the lake's beach, with a boat launch, large parking lot, and restroom facilities. Sites 1 and 3 are more vulnerable to non-point source pollution and runoff than Site 2, which could explain why Site 2 did not experience such an effect from episodic events.

However, the phosphate concentrations at site 2 were altered by episodic events. University Lake is extremely shallow, with an average depth of 0.83 m (Mesmer 2010), and shallow lakes are more susceptible to changes of biogeochemistry from bottom mixing (Carrick et al. 1993). An episodic event such a severe rainstorm or windstorm may have caused bottom mixing and the release of phosphates from the sediments at Site 2. The rapid uptake of nutrients by the lake phytoplankton community may have also prevented accurate measurement of the true phosphorous content entering the lake. Certain phytoplankton have been shown to take up phosphates at rates two to three orders of magnitude greater than normal conditions following substantial inputs of phosphates to their environments (Laws et al. 2011). Particularly in a phosphate-limited system, this mechanism provides a competitive advantage. Rapid uptake for storage could explain why the phosphorous levels at Sites 1 and 3 were not significantly affected by episodic events.

Ammonium and silicate concentrations were not significantly affected by episodic events. Although the storm events did not significantly alter the concentration of ammonium, a different type of event did result in markedly high ammonium levels: a fish kill that occurred on July 15, 2011. The ammonium levels peaked in the lake on July 16, 2011, and never reached concentrations as high as this sample again. This event can be attributed to the eutrophication of the lake, and increased nutrient inputs, which resulted in amplified phytoplankton productivity followed by increased bacterial respiration, and depletion of oxygen. University Lake is a man-made system that is constantly in EPA non-attainment status for fecal coliforms. The lake conditions were improved after the dredging in 1977, but the sedimentation and reversion of the lakes to a drainage bayou is worsening those conditions once more. This reversion could be contributing to the abnormally high levels of ammonium, but the ammonium content in University Lake may be a product of crumbling infrastructure and leaky sewage pipes. Broken sewage pipes were repaired in the 1977 restoration effort made by the City, and the systems are still maintained by the City, but it is likely that sewage effluent is escaping from the large sewage drainage infrastructure underneath and surrounding the lake.

The high silicate concentrations in University Lake are more difficult to account for. Eutrophic waters experience increased input of nitrogen compounds and phosphate, which can alter other elemental cycles. Other eutrophic systems have been shown to be silicate limited because the nitrate and phosphate inputs stimulate the growth of non-silicate requiring phytoplankton, causing a silicate limit, and limiting the growth of silicate-requiring phytoplankton (Admiraal and Van Der Vlugt 1990). This is obviously not the case in University Lake, since diatoms represent a significant portion of the variance in chlorophyll *a*. The lake has high levels of silicate and a stable diatom population. Hydrologic studies of the region have

found that in the inner shelf of the Atchafalaya Bay, rivers inject high concentrations of silicate into nearby surface waters (Sahl et al. 1993). It is possible the reversion of the lake to a drainage bayou, in combination with a hydraulic retention time more comparable to that of a lake, is trapping silicate deposits in University Lake.

5.2 Discussion of Gross Assimilation Numbers and Respiration Rates

Inorganic nutrients are vital to the growth and development of phytoplankton. When the inorganic nutrient levels in a body of water fluctuate, the availabilities of nutrients may select for certain algal forms. There are also many algal classes capable of using multiple forms of nutrients, which allows for some leeway in their nutritional requirements. For example, many classes are able to utilize nitrates, nitrites, or ammonium compounds equally as well to satisfy their nitrogen requirements (Smith 1950).

The assimilation of oxygen was significantly affected following episodic events, which caused enhanced photosynthetic activity (ref. Figure 4.2.4). Nutrient loading from storm runoff provides the additional nutrients required to increase phytoplankton biomass and increased productivity. A study done on Kaneohe Bay in Hawaii reported that nutrient loading via runoff caused an increase in algal biomass and productivity. The rapid depletion of nutrients by the enlarged phytoplankton population allowed the growth peak to last only a few days, before rapidly declining (Ringuet and Mackenzie 2005). The phytoplankton population of University Lake is experiencing a similar phenomenon following storms, but the high availability of ammonium is likely preventing a rapid decline of the phytoplankton population. The significant Pearson's correlation coefficient between gross assimilation numbers and ammonium supports this conclusion.

Studies indicate that a positive relationship between respiration rates and growth rates exists (Geider and Osborne 1989, Myers and Graham 1971). Dark respiration rates increase with growth rates, and are directly proportional to photosynthetic rates. The ratio of respiration to growth should only increase in suboptimal growth conditions (Geider and Osborne 1989). The significant Pearson's correlation coefficient between respiration rates and phosphates suggests increased metabolic activity as a result of the presence of phosphates, which implies that gross assimilation of oxygen also increased in the presence of phosphates. During the course of this experiment, numerous samples from all three sites experience negative respiration. Most of the negative respiration was very near zero, but there were a few samples that experienced substantial negative respiration. When using BOD bottles for dissolved oxygen analysis, a common problem is that bubbles of oxygen remain after the bottle is sealed, and the oxygen bubbles break and release the oxygen into the sample during incubation. Additional inorganic matter from the unfiltered samples could also be trapping oxygen in a manner similar to the additional bubbles. It is likely that the negative respiration experienced in these samples is the result of trapped oxygen in the sample, and is preventing the respiration of the phytoplankton from being detected.

5.3 Discussion of the Phytoplankton Community Composition

The multiple regression analysis successfully identified 87% of the variance in chlorophyll *a* levels in University Lake. It was clear that roughly 51% of the phytoplankton population is chlorophytes, 30% is cyanobacteria, and 10% is diatoms. An additional estimation made from the established alloxanthin:chlorophyll *a* ratio (Descy et al. 2009), allowed for the quantification of the cryptophyte population, accounting for another 3.4% of the chlorophyll *a*,

meaning cryptophytes are 3.4% of the phytoplankton community. That left only 6% of the chlorophyll *a* unaccounted for. Violaxanthin was present throughout the year, but it was not significant in explaining the variance about the chlorophyll *a*. Violaxanthin is a marker pigment for chrysophytes, but is also part of the xanthophyll cycling of chlorophytes. The xanthophyll cycle acts as a measure of protection for phytoplankton from chlorophyll *a* in its excited form, or damage from harsh light. Since University Lake is so shallow, it would be reasonable to assume that the phytoplankton community requires the use of the xanthophyll cycle to protect them from harsh UV rays (Demmig-Adams and Adams 1996). Chlorophytes account for over half the phytoplankton biomass in University Lake, so it is likely that the violaxanthin detected during this study is associated with chlorophytes rather than chrysophytes (Masojidek et al. 2008).

Chlorophytes make up the majority of the phytoplankton community in University Lake (51%). Freshwater chlorophytes outnumber the combined species of all other algae in the United States. Samples taken from semi-permanent to permanent freshwater bodies often consist wholly of chlorophytes (Smith 1950). Chlorophytes have been documented to be most abundant during late spring and early fall, but the chlorophyte population in University Lake was only significantly affected by seasonality at Site 2. If the University Lake population of chlorophytes is a representative sample of a typical chlorophyte in the United States, seasonality should have played a greater role in the fluctuation of the chlorophyte population. The lack of seasonality experienced here may have been a result of the unseasonably warm temperatures in Baton Rouge between February 2011 and February 2012, which would have resulted in the absence of a traditional spring and fall turnover event. The larger factor to consider is the ecology of shallow lakes. University Lake has an average depth of less than one meter, and probably experiences

daily overturning of the water column. Either factor could be limiting the effects of seasonality on the fluctuations in chlorophytes present in University Lake.

Cyanobacteria make up the next largest algal portion of the phytoplankton community in University Lake (30%). Cyanobacteria are found in a variety of habitats, but are always present in freshwater bodies. The proportion of the community composition cyanobacteria comprise is known to fluctuate based on seasonality and the chemical composition of the water, and they are usually the most abundant during warm months (Smith 1950). The cyanobacteria population of University Lake was not significantly affected by seasonality or episodic events, which may be a consequence of the unusually warm temperatures experienced throughout this experiment. Although the statistical testing for effects of seasonality on the fluctuation of the cyanobacteria population was insignificant, a clear spike in zeaxanthin can be seen at all three sites during late May, which coincides with the seasonal growth patterns typically observed for cyanobacteria.

Diatoms make up 10% of the University Lake phytoplankton community. These unicellular algae are unique when compared to chlorophytes and cyanobacteria. Diatoms are encased in a unique cell wall comprised of silica, called the frustule (Smith 1950). Diatoms require silicates to produce frustules, and the copious amounts of silicate found in University Lake sustain the diatom population, without any major blooms or population crashes. Seasonality was a significant effect on the diatom population at Site 2, likely as a result of the unseasonably warm year during this project. Diatoms are stimulated by increased turbidity, and heightened growth periods have been recorded following disturbances (Thomas and Gibson 1990). However, the diatom population of University Lake only fluctuated as a result of episodic events at Site 1. Site 1 is much more unprotected by vegetation and urban structures than Sites 2 and 3, and may be more vulnerable to the effects of episodic events.

Cryptophytes make up only 3.4% of the phytoplankton community of University Lake. Certain species of cryptophytes are known to grow in water rich with organic material and nitrogenous material, and others can grow in nutrient poor waters (Smith 1950). Cryptophytes are one of the least researched classes of phytoplankton, and this population did not experience significant fluctuations in population from seasonality or episodic events.

The phytoplankton community composition of University Lake does not exhibit much diversity at the class level. However, the three major representatives of the phytoplankton community experience unique relationships between one another. The inability of cyanobacteria to bloom during times when the diatom population is high, and the inability of diatoms to bloom while the cyanobacteria population is high, could be indicative of an allelopathic relationship between the cyanobacteria and diatoms. Either class may be excreting a substance that prevents growth of the other, but toxic cyanobacteria are much more common in freshwater environments than toxic diatoms, so it is likely that a University Lake cyanobacteria species is excreting a toxin. For example, cyanobacteria have been reported to excrete toxins that can inhibit growth of other phytoplankton, plants, and microbes in their ecosystem (Inderjit and Dakshini 1994). However, it may be possible that some other environmental parameter, such as pH, temperature, or micronutrients, is limiting all three classes from blooming simultaneously. Macronutrients like nitrogen and phosphorous play a vital role in the growth and development of all phytoplankton, but the presence or absence of essential micronutrients may be a contributing factor to this limited diversity. Trace metals such as iron, copper, cobalt, nickel, and zinc can be crucial to the development of certain algal forms, but these metals in precise combination and ratios can also be toxic (Chakraborty et al. 2010). The classes of phytoplankton that make up the University

Lake phytoplankton community may have developed a tolerance for the different metal concentrations in the lake, or may be subject to allelopathic relationships within the system.

6. Conclusion

University Lake is a shallow, highly eutrophic, man-made lake system. Its urban setting allows for inputs of superfluous inorganic nutrients, which leads to excessive algal growth. The biogeochemical cycling of nutrients in this lake may be affected by the anthropogenic alterations to its hydrology, as well as the polluted inputs from anthropogenic activities.

Inorganic macronutrients were found to be unaffected by seasonality, and only affected by the occurrence of episodic events in the cases of nitrate and phosphate concentrations, on a site-by-site basis. Since nitrogen is a nutrient crucial to the growth and development of all phytoplankton, this fluctuation as a result of episodic events should be having a greater effect on the phytoplankton community of the lake. However, ammonium levels were measurably high throughout the year in the lake, and many phytoplankton classes prefer ammonium as their nitrogen source, and are able to utilize that form just as well, if not better, than nitrates. Excessive ammonia, perhaps from leaking sewer pipes, was positively correlated with gross assimilation of oxygen, and phytoplankton growth and productivity. With an excess of ammonia, low phosphate concentrations are the only factor restricting phytoplankton growth in the lake. Phosphate levels were positively correlated with phytoplankton respiration rates with extreme significance, which allows the conclusion that the phytoplankton community will respond to increased phosphate levels with increased growth and productivity. Silicate concentrations in University Lake are very high, which permits a relatively large and sustained population of diatoms.

Inorganic nutrients and the environmental parameters of University Lake have selected for a phytoplankton community composition of low diversity. Three algal classes represent 87% of the phytoplankton community. Seasonal progression is typically a considerable forcing

function for explaining changes in phytoplankton community composition, however the effects of seasonality were only experienced at one of the test sites, and not by the entire phytoplankton community. The year of 2011-2012 was extremely mild, and may have lessened the effects of temperature on the algal community. Mild temperatures and the extremely shallow nature of the lake prevent substantial fluctuations of representative phytoplankton class populations. Episodic events were also not a major forcing function for explaining the changes in phytoplankton community composition. The tremendous availability of ammonia and silicate is likely sustaining the phytoplankton community composition, and maintaining the low diversity.

Further studies of University Lake should include an analysis photosynthetic and respiration rate analysis with measurement of oxygen as well as carbon dioxide assimilation, measurement of zooplankton biomass, sample cell counts to gain a better understanding of the density of the phytoplankton community, and an analysis of essential micronutrients. It is possible that micronutrients are playing a role in the low phytoplankton biodiversity, however, the poor health of University Lake and the ongoing eutrophication processes likely explain the phenomena observed with the phytoplankton community during this experiment.

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Appendix

Summary of the Characteristic Pigment Biomarkers Used for the Identification of the Main Phytoplankton Phyla

Algal Group	Major Pigment Biomarkers
Prochlorophytes	Divinyl chlorophylls <i>a</i> and <i>b</i>
Cyanobacteria	Zeaxanthin, phycocyanin, monovinyl chlorophyll <i>a</i>
Diatoms	Fucoxanthin, diadinoxanthin
Prymnesiophytes	19'-hexanoyloxyfucoxanthin
Pelagophytes	19'-butonoyloxyfucoxanthin
Chrysophytes	Fucoxanthin, violaxanthin
Cryptophytes	Alloxanthin, monovinyl chlorophyll <i>a</i>
Dinoflagellates	Peridinin, monovinyl chlorophyll <i>a</i>
Prasinophytes	Prasinoxanthin, monovinyl chlorophyll <i>a</i>
Chlorophytes	Monovinyl chlorophyll <i>a</i> and <i>b</i> , lutein, violaxanthin

VITA

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