GROWTH OF NATURAL PHYTOPLANKTON POPULATIONS OF WILSON BAY: A NUTRIENT BIOASSAY APPROACH

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ABSTRACT

Wilson Bay is a shallow estuarine embayment found within the New River Estuary. Previous work sponsored by NC WRRI examined phytoplankton bloom species composition, rainfall and nutrient levels within the New River Estuary. This study focused on the influence of nutrients in forming blooms using a bioassay format measuring phytoplankton growth over a 7 day incubation period. Growth rates were measured by fluorometric readings and visual eye counts performed on natural populations that were incubated and treated with nutrient addition and exclusions. Additions consisted of sodium nitrate ($NaNO_3$), ammonium chloride (NHCl), urea ($CO(NH_2)_2$) and a complete control, including phosphate, silicate, vitamins and trace metals. Exclusions minus nitrogen, minus phosphate, minus silicate and an unenriched control were also measured. Ambient nutrient levels were measured in sample water before nutrients were added. Previous work done on Wilson Bay showed to often be nitrogen limiting. The unexpected amounts of rainfall during the study period provided data that showed that Wilson Bay was nitrogen limited with nitrogen additions giving the greatest stimulation. The study period (March – September 2003) had abundant amounts of rainfall compared to the previous (2002) which was a drought years. Wilson Bay blooms are strongly affected by local weather and the rate and types of nutrient delivery.

ACKNOWLEDGMENTS

I would like to express my thanks to my advisor, Dr. Carmelo Tomas, for passing on his knowledge to methroughout my years here as a student. Thanks also go out to Dr. Martin Posey and Dr. Jack Manock for their assistance in my research and for agreeing to serve on my committee.

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My thanks also go out to my husband, Michael, my son, Dylan, and the rest of my family for their support throughout what seemed to be my never-ending journey in graduate school.

DEDICATION

I would like to dedicate this thesis to my husband, Michael Brousseau. Without his support and understanding for continuing my education, this would not have been possible. His love helped me through the rough days when I never thought I would finish. This paper is for you.

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INTRODUCTION

Blooms of single-celled algae, known as phytoplankton were observed throughout history. Increases in toxic or noxious phytoplankton populations were often referred to as red tides and more recently as harmful algal blooms (HABs). The HAB phenomenon is increasing in frequency and duration in coastal areas throughout the world (Smayda, 1997a). Approximately 7% of the totalphytoplankton species are considered harmful and while some harmful blooms are transient (i.e. occurs once and disappears), a bloom species can become persistent in a particular area by virtue of resting stages or cysts. The presence of these cells or their toxin can reduce competition by predators, inhibit the growth of non-harmful species (Smayda, 1997b), produce toxic aerosols and through accumulation of toxins in shellfish and fishes serve as a threat to human health.

Coastal regions of the world have become increasingly stressed in recent years often due to increases in pollution imposed by proximity of highly populated and industrialized areas near the oceans (Smayda, 1997b). For the past few decades bloom events also appear to be increasing which is directly correlated to the fact that approximately 60% of the world's population now lives within a 100 miles of the coast. This demographic drives the increased pollution associated with coastal eutrophication and presumably the rise in the frequency and intensity of phytoplankton blooms. One of the best examples demonstrating this linkage is that of Tolo Harbor, Hong Kong (Lam and Ho, 1989) where increased occurrences of "red tides" accompanied marked increases in population. In addition to stressors posed by increased population densities, numerous factors such as the degree of stratification, tidal dispersal and predation can affect the

intensity of algal blooms. One obvious factor influencing phytoplankton blooms is the availability of nutrients (Ault et al, 2000).

The decline in water quality and rise in primary productivity and HAB events are a result of increased organic loading to marine environments contributed by point and non-point sources including waste from livestock and domestic sources through discharge from major treatment plants. For example, nutrient loading in Hong Kong from sewage doubled from 1976 to 1985 (Lam and Ho, 1989). This semi-enclosed bay whose nutrient enrichment is poorly flushed has become a concern over the past two decades (Lee and Arega, 1999).

A bloom is defined as a considerable increase in biomass over what is considered a baseline level. The abundance of a bloom and its fluctuations are generally viewed in terms of community abundance often expressed as chlorophyll *a* levels, a surrogate for algal biomass. A "harmful" algal bloom (HAB) is also defined as one having negative impacts caused by the presence of a toxic or noxious species within the bloom community. The descriptors "toxic," "noxious," and "nuisance" for HAB species convey the degree of impact as defined by Smayda (1997a).

The mere presence of a toxic species does not necessarily lead to a damaging impact; such effects require threshold populations and toxin levels to influence the ecosystem. Certain harmful algal species are more potent than others requiring modest population levels (not "red tide" levels) in order to cause a significant impact. Mortality modes and impact mechanisms of harmful blooms include anoxia, mechanical impairment of the gills, phycotoxicity, and allelopathy (Smayda, 1997b). The effects of a HAB event on humans are many and widespread. Regional mariculture crop can become

tainted by the presence of a HAB and massive fish kills can also occur due to toxins and low oxygen levels (Anonymous, 1999). Shellfish-vectored poisonings can have devastating effects on human health. Florida, Texas and California have recently experienced catastrophic losses of populations of marine mammals, pelicans and cormorants due to a HAB occurrence (Anonymous, 1999). Economic losses due to a HAB event can reach into the hundreds of millions of dollars (Smayda, 1997a). Nutrients are a major factor involved in the development of HAB's. In studies on nutrient limitation in marine systems, nitrogen is thought to be the nutrient that most commonly limits growth of phytoplankton in coastal and oceanic waters as opposed to phosphorus that is considered limiting in freshwater systems (Bernhard and Peele, 1997).

Estuarine systems differ from the open ocean partially due to the constant changes and varying mixtures of fresh and saline waters. Tides, winds, sediment mixing, and run-off from urban areas all have an influence on estuarine systems. Semi-enclosed waters thus become threatened by deterioration of water quality exacerbated by urbanization and a lack of sufficient exchange with the open ocean (Okay et al, 1996). If human induced alterations of the natural world (such as increased pollution levels) continue to occur, the nutrient ratios established by Redfield (1958) may change drastically.

It is important for management purposes to know the nutrient that is limiting in a particular system. For inshore waters where the input of various nutrients can be variable, the nutrients limiting phytoplankton growth may change with time and nitrogen in particular is difficult to manage due to the fact that it can enter a system in many different forms and from many different sources including sewage, agricultural run-off, nitrogen fixing organisms and lightning (not usually considered to be a significant

source). Understanding how a system responds to nutrient levels is important in forecasting trends and beginning to testlimitations withing a system.

An effective method used to assess a system response to nutrients is the use of algal bioassays to make predictions concerning species selection and standing crop (Parker, 1997). It is of utmost importance to have indicators of nutrient limitation and identification of the limiting nutrient in order to manage aquatic systems (Holmboe et al, 1999). These concerns make it necessary to develop experimental procedures as tools for monitoring levels of eutrophication and algal bioassays are but one of many tools used for the evaluation of water quality (Parker, 1997). To expect this outcome, other outside factors, such as light and temperature, must be optimal for growth of phytoplankton in a bioassay (Ryther and Dunstan, 1971).

Within the various formats, the use of native populations in algal bioassays can give more realistic responses than a single test organism alone. This is most likely due to the fact that manipulations of nutrient additions will not only cause changes in algal biomass but also shifts in species dominance (Lopez and Davalos-Lind, 1998). In order to examine algal growth potential and species composition, it is essential to compare bioassays between a test organism and native algal population. For this study, natural algal populations were employed. The use of natural phytoplankton populations in these bioassays allowed more realistic responses to nutrient additions and exclusions as well as to discern the cause of changes in algal biomass along with shifts in species composition.

Nitrogen makes up approximately one-half the mass in urea ($CO(NH_2)_2$). Urea is best metabolized by the enzyme, urease. If a particular species does not contain urease, then they still grow well on a urea substrate due to the fact that they may contain a urea

carboxylase and also allophanate hydrolase (Syrett, 1980). Nitrate is the most difficult nutrient for phytoplankton to assimilate. Once uptake of nitrate has occurred within a phytoplankton cell, it undergoes a two-step reduction process involving nitrate and nitrite reductase. Nitrate must be reduced to nitrite and then to ammonium before an algal cell can use it. Once it is in the form of ammonium, it is assimilated in the same manner as any ammonium that might be taken up.

Wilson Bay (34° 45' N, 77° 24' W) is a shallow estuarine embayment found within the New River Estuary (Figure 1). This bay is located at the head of the New River estuary and is adjacent to the city of Jacksonville, North Carolina. The New River is considered a blackwater system with tidal influences found predominately below Wilson Bay (Mallin et al, 2000). Blackwater system is used here to define streams, lakes and rivers that are stained a dark, tea-like color due to humic acids that leach from swamp vegetation, but is harmless to humans and animals (Mallin et al, 2001).

A residential community, a recreational park and the United States Marine Corps Base Camp Lejeune also surround the bay. This site once was the recipient of treated sewage from the Wilson Bay Wastewater Treatment Plant in of the city of Jacksonville. The treatment plant is no longer in operation and was converted into an aquaculture facility for Sturgeon in 1998. Oysters are currently being placed throughout Wilson Bay as a means of a biological control for phytoplankton blooms. These bivalves are being used in the bay to increase natural biological filtration system by their filtering out phytoplankton through their gills. (Dr. Jay Levine, personal correspondence). Periodic algal blooms were recorded in the northern half of the New River, Wilson Bay (Tomas,

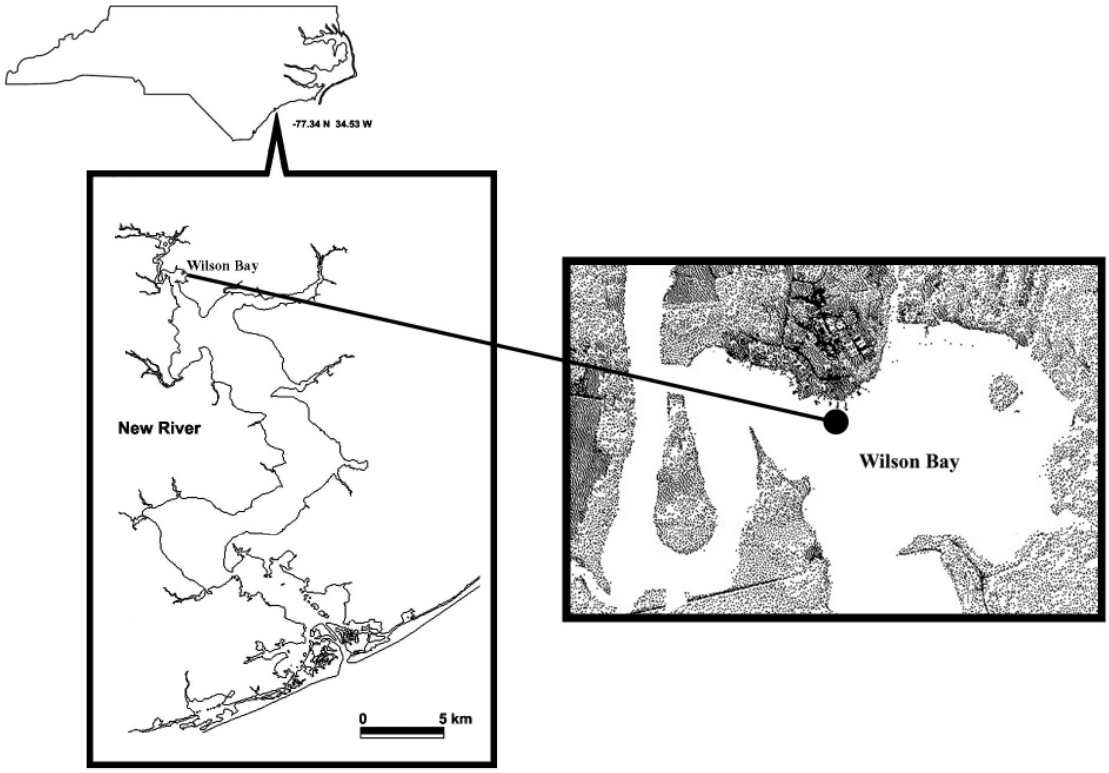

Figure 1. Study site, Wilson Bay, New River in North Carolina.

2003) with more than eighteen HAB species identified. Due to the elevated levels of phytoplankton and organic loads within the water column, low levels of oxygen were at times recorded throughout the bay (Dr. Jay Levine, personal correspondence). The New River is monitored for water quality on a monthly basis by the North Carolina Department of Natural Resources Division of Water Quality.

Historical nutrient data collected for Wilson Bay showed abundant levels of nitrate-nitrite and ammonium before the closure of Wilson Bay Wastewater Treatment Plant (Mallin et al, 2005). Only ammonium showed a clear decrease in abundance within Wilson Bay following 1998. Looking at maximum bloom events based on historical data and the highest levels of nutrients available, most major events occurred during late spring through early fall. High levels of nitrogen sources point to phosphorus being limiting within Wilson Bay. Historical temperature and precipitation data for 2002 indicated highest temperatures and lowest amounts of rainfall during the months falling in the late spring through early fall (Figure 2). These conditions were accompanied by extreme blooms.

My objectives for this thesis project included the following:

- To ascertain the role of nitrogen in limiting growth and bloom development in Wilson Bay, New River, North Carolina.
- To determine if the form of nitrogen supplied made any difference to natural populations by stimulating growth and species composition.
- To determine how nitrogen species influenced harmful algal species composition in Wilson Bay.

- To determine the effect of each of the nitrogen sources tested to produce a change in biomass from natural algal populations from Wilson Bay.

METHODS AND MATERIALS

Study Site

Based on previous studies on species composition of phytoplankton blooms in the New River, North Carolina (Tomas, 2003), Wilson Bay was selected as the study site. Natural algal populations were collected and used throughout these experiments from Wilson Bay, located at 34° 45' N, 77° 24' W, is found at the head of the New River Estuary (Figure 1). This bay is a shallow estuarine embayment located at the head of the New River and is adjacent to the city of Jacksonville, North Carolina. Up until 1998, this embayment was the site of a municipal sewage treatment discharge for the city.

Sampling

Surface water samples were taken from a public dock at Wilson Bay in a two liter plastic bottle a total of nine times from March through September 2003 (Table 1). This time period was selected as that having the majority of bloom events during HAB study of 2002-2003 (Tomas, 2003). Temperature was taken at time of collection using a mercury thermometer and salinity was measured using a refractometer previously calibrated against standard seawater, upon returning to the University of North Carolina at Wilmington's Center for Marine Science. Samples were screened through a 120 μm mesh net to remove large phytoplankton grazers and detritus. In small volumes of bioassay experiments, grazers can strongly influence the phytoplankton through the removal of selected species and excretion of wastes containing nitrogen.

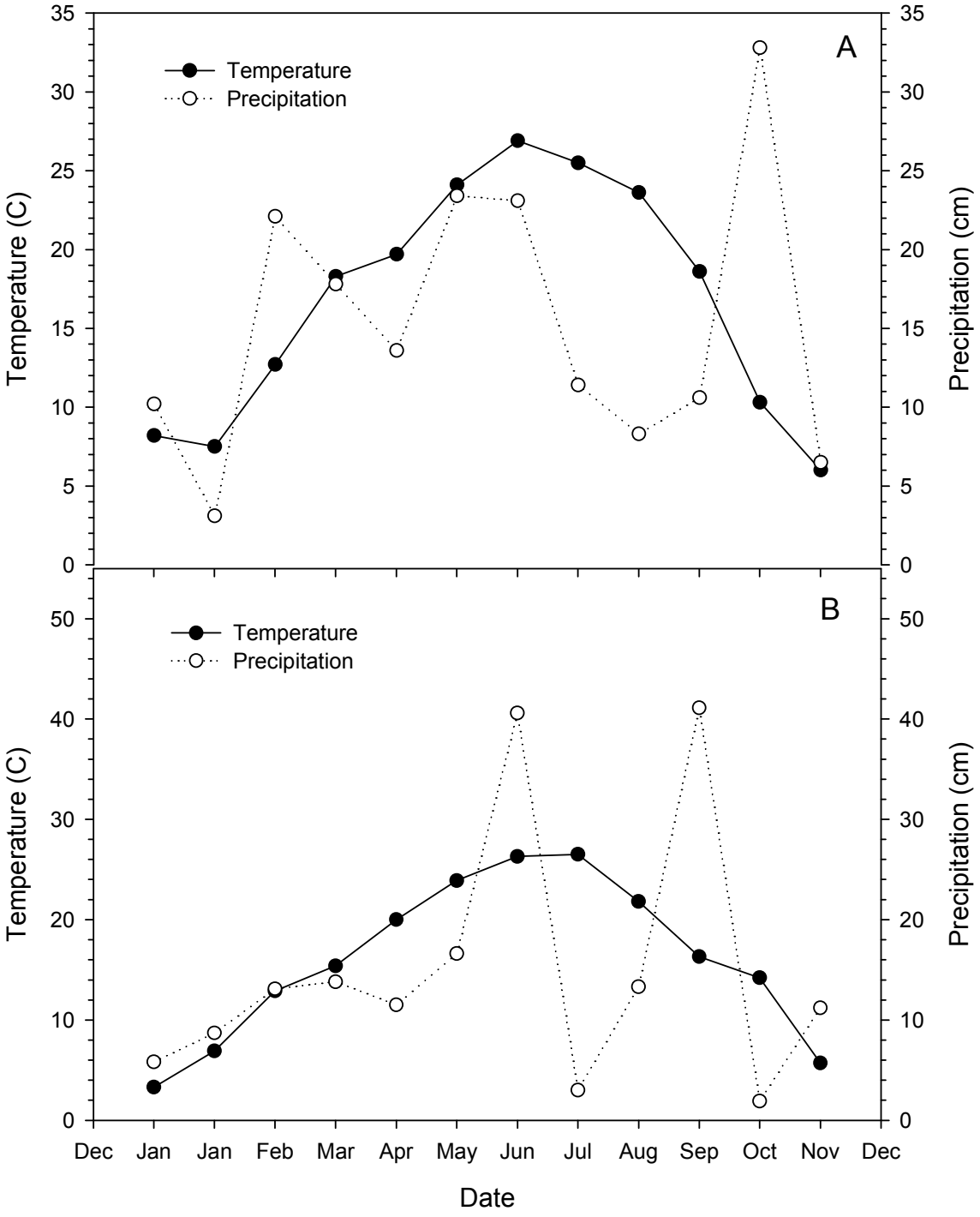

Figure 2. Historical monthly measurements of temperature and precipitation data for (A) 2002 and (B) 2003 for Wilson Bay, New River, North Carolina.

Experiment	Dates	Temperature (°C)	Salinity
1	11-17 Mar 2003	12	10
2	28 Mar-3 Apr	19	3
3	22-28 Apr 2003	22	4
4	7-13 May 2003	26	4
5	13-19 Jun 2003	28	1
6	15-21 Jul 2003	29	0
7	29 Jul-4 Aug 2003	29	0
8	27 Aug-2 Sept 2003	31	0
9	16-22 Sept 2003	26	6

Table 1. Temperature and Salinity measurements for bioassay experiments performed on samples from Wilson Bay, New River, North Carolina, March - September 2003.

Cultures and Growth Measurements

Aliquots of 40 mL of the net-screened sample water were placed into thirty-two 60 mL Pyrex screw cap culture tubes to which specific nutrients were added for the bioassays (Table 2). In addition 250 mL of each sample was filtered through a 45mm nucleopore filter, frozen at -20° C in plastic bottles and analyzed for subsequent nitrate + nitrite, ammonium, and urea concentration. Sample water taken from the beginning and end of each experiment was filtered through a 25 mm GG/F glass filter under reduced light conditions and frozen for chlorophyll *a* analysis. Forty mLs of net screened water was taken from the beginning and end of each bioassay treatment and preserved in a 2% Lugols solution to determine species composition and abundance.

Two types of bioassays were performed for each of the nine experiments. These included bioassays by addition and exclusion. Bioassay tubes were incubated in a constant temperature water bath maintained within ± 0.5° C of the ambient water temperature at time of collection and with a constant 100µE/m^2/s of light provided by cool white fluorescent lamps. Sodium nitrate, ammonium chloride and urea were added in replicate culture tubes (n = 4 for each treatment) using an Eppendorf repeat pipetter. Sodium phosphate, f/2 vitamins and trace metals (Guillard, 1973) were also added to each tube (Table 2). The nitrogen levels added were selected to be ecologically realistic. The assays by exclusion had replicate tubes with all nutrients added minus one. A complete control was used containing all additions and an unenriched control was also employed, which contained no nutrient additions.

Growth in the form of fluorescence readings were recorded twice daily at six hour intervals over each of the 7-day experimental periods by measuring *in vivo* fluorescence

Table 2. Enrichment and values used for each seven bioassays on Wilson Bay, North Carolina water for experiments 1-9, March – September 2003.

Additions	Nitrate	Ammonium	Urea	Complete	- Nitrogen	- Phosphate	- Silicate	Unenriched
Sodium Nitrate	100 µL	0 µL	0 µL	33 µL	0 µL	33 µL	33 µL	0 µL
Ammonium Chloride	0 µL	100 µL	0 µL	33 µL	0 µL	33 µL	33 µL	0 µL
Urea	0 µL	0 µL	100 µL	16 µL	0 µL	16 µL	16 µL	0 µL
Sodium Phosphate	100 µL	100 µL	100 µL	100 µL	100 µL	0 µL	100 µL	0 µL
Sodium Silicate	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	0 µL	0 µL
Trace Metals	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	0 µL
Vitamins	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	0 µL

sodium nitrate: 100 µL = 1.2 µM/100 mL; 33 µL = 0.4µM/100mL; ammonium chloride: 100 µL = 1.2 µM/100 mL; 33 µL = 0.4µM/100mL ; urea: 100 µL = 0.6 µM/100mL; 16 µL = 0.2µM/mL; sodium phosphate: 100 µL = 0.4 µM/100mL; sodium silicate: 100 µL = 1.2 µM/100 mL; 33 µL = 0.4µM/100mL; trace metals: 100 µL = 1.2 µM/100 mL; vitamins: 100 µL = 1.2 µM/100 mL

on a Turner 10 AU fluorometer. Fluorescent based growth rates calculated are the linear growth phase according to Guillard (1973). Species composition and abundance were determined at the beginning and end of each 7-day experiment using a Nikon Diaphot inverted microscope and Utermöhl settling counting chambers (Sournia, 1978). Forty-milliliters of preserved (2% Lugols) sample from the beginning and end of each bioassay were gently agitated by hand and ten milliliters of each was then placed in Utermöhl counting chambers and allowed to settle for least twelve to twenty-four hours prior to examination and counting. Each chamber was then examined for dominant species and counted using the methods described in Sournia (1978).

Nutrient Analysis:

Ammonium

Ammonium analysis from each sample collection was filtered through a 25mm, GF/F Whatman glass fiber filter under vacuum. Fifty milliliters of filtered sample was then placed into a sixty milliliter plastic bottle and frozen – 20° C. Aliquots (2.5 mL) of the filtered sample were pipetted with an automatic Eppendorf pipette into scintillation vials with Teflon caps in triplicate for each of the nine experiments and analyzed using protocol B in Holmes et al (1999). Protocol A is used in samples with less than 0.5 μM/L due to a reduced sensitivity in protocol B. Reagents were added to each vial, vortexed and placed in the dark for two hours. Standards, blanks and samples were read using a Turner Designs TD-700 fluorometer.

Nitrate

Frozen samples were thawed and analyzed using a Bran + Luebbe Autoanalyzer III at the Center for Marine Science at UNCW which had a sensitivity of 0.104 μM/L for

this procedure. An automated gas segmented continuous flow colorimetric method for the analysis of nitrate concentrations was used. Nitrate in the samples was reduced to nitrite in a buffer solution. A color dye produced was linearly proportional to the concentrations of nitrate + nitrite in the sample. Standards and blanks were also part of this analysis. There was no significant salt error in this method (EPA, 1997).

Urea

The urea assay followed the Koreleff method (Grasshoff, 1983) and analysis of the samples was completed on a Spectrophotometer (Shimadzu UV-1601) at 520nm. Assuming a absorbance of 0.010 as the detection limit, 0.1 μM dm^{-3} = 1.4 μg · dm^{-3}N can be observed. Twenty-five mL aliquots in triplicate of the filtered sample were poured into 50ml Pyrex screw cap test tubes. Reagent blanks consisted of 25 mL aliquots of reagent grade deionized water in triplicates. Standards were made up in 100 mL volumetric flasks with appropriate dilution of primary urea stock (100/mL) in reagent grade deionized water and added to the test tubes in 25mL aliquots. Measurements of 5.5 +/- 0.1 g of sodium chloride were added to each test tube and all were placed in a water bath for ninety minutes at a temperature of 70°C.

Phosphate

An automated colorimetric method was used to determine ambient levels of orthophosphate in the samples. Frozen samples were thawed and run on a Bran + Luebbe Autoanalyzer III at the Szmant lab at the Center for Marine Science at UNCW which had a sensitivity of 0.016 μM/L for this method. Standards and blanks were also part of this analysis. Ammonium molybdate and antimony potassium tartrate react in an acidic

medium to form a complex which produces an intense blue color. The color produced was directly proportional to the level of phosphate in the sample (EPA, 1997).

Chlorophyll *a*

Chlorophyll *a* analyses was conducted on sample prescreened, filtered onto a 25 mm Whatman GF/F glass fiber filters under reduced light conditions. Each filter was folded and placed in individual 1.8 mL cryogenic vials and frozen in liquid nitrogen. The frozen samples were stored in a freezer at -80° C until analysis could be performed. Upon analysis, frozen filters were individually placed into 15 mL Corning centrifuge tubes along with ten mLs of 90% acetone. Samples were cooled by ice and sonicated for three 20 second bursts and placed in a darkened freezer at -4°C for one hour. The tubes were then centrifuged for 10 minutes at 3000 rpm. The supernatant from each vial was then read in a Turner Design 10 AU Fluorometer previously calibrated using chlorophyll *a* standard following the protocol as described by Jeffrey (1997).

RESULTS

Historical Data

Monthly data for salinity and temperature for Wilson Bay during the years 2002-2003 (Table 3) show subtle but evident differences. During the year prior to this study, temperatures varied from 6.0 to 26.9 ºC while precipitation of 3.1 to 32.8 cm was recorded. For the following year (2003), slightly lower temperatures were found from 3.3 ºC and a similar maximum to that of 2002 of 26.5 ºC was recorded. The patterns for both variables (Figure 2), had similar patterns for temperature with the maxima for both years found in July - August of each year. Temperatures steadily increased from January

Date	Temperature (ºC)	Precipitation (cm)
1/1/2002	8.2	10.2
2/1/2002	7.5	3.1
3/1/2002	12.7	22.1
4/1/2002	18.3	17.8
5/1/2002	19.7	13.6
6/1/2002	24.1	23.4
7/1/2002	26.9	23.1
8/1/2002	25.5	11.4
9/1/2002	23.6	8.3
10/1/2002	18.6	10.6
11/1/2002	10.3	32.8
12/1/2002	6.0	6.5
1/1/2003	3.3	5.8
2/1/2003	6.9	8.7
3/1/2003	12.9	13.1
4/1/2003	15.4	13.8
5/1/2003	20.0	11.5
6/1/2003	23.9	16.6
7/1/2003	26.3	40.6
8/1/2003	26.5	3.0
9/1/2003	21.8	13.3
10/1/2003	16.3	41.1
11/1/2003	14.2	1.9
12/1/2003	5.7	11.2

Table 3. Monthly average water temperature (ºC) and precipitation (cm), Wilson Bay, North Carolina for 2002 and 2003.

to the maxima in mid summer and then declined slowly to the annual minima in December. Precipitation varied more between the two years. Spring rainfall (March) had elevated precipitation values exceeding 20 cm that were found again in July and August and again in November (Figure 3). High spring rainfall levels were not seen in the study year (2003) and peak values exceeding 30 cm were recorded in July and October with the remainder at or below 10 cm.

Experimental Data

Ambient temperatures of surface waters of Wilson Bay, measured at the time of collection (Table 1), varied from 12 – 31 ºC. From March through June 2003, temperatures increased to a peak values of 29 – 31 ºC found in July and August. September declined slightly to 26 ºC. Salinity showed an inverse pattern with temperature and measured at the time of sample collection for the bioassays varied from 10 to 0 with the majority of values at 4 or less for the experimental period (Table 1).

Ambient nutrients measured for each experiments date are listed in Table 4 and Figure 4 and 5. The most inorganic nitrogen source was nitrate + nitrite with values varying from 4.28 to 50.26 µM/L. For 7 of the 9 sampling dates, this nutrient exceeded 20 µM/L (Figure 5A). Ammonium had values from 6.0 to 19.8 µM/L with values above 8 µM/L for 7 of the 9 samples (Table 4 and Figure 5A). Urea, reported for the first time for this region, varied between 0.72 and 3.32 µM/L with standing stock exceeding 1.0 µM/L for all but one of the samples (Figure 5B). Dissolved orthophosphate varied from a low of 0.13 to a high of 2.41 µM/L and exceeded 1.0 µM/L for all but three of the sample dates (Figure 5B). Using the sum of nitrate + nitrite and ammonium for dissolved inorganic nitrogen (DIN) and the N: P ratio (atoms) calculated by dividing DIN by

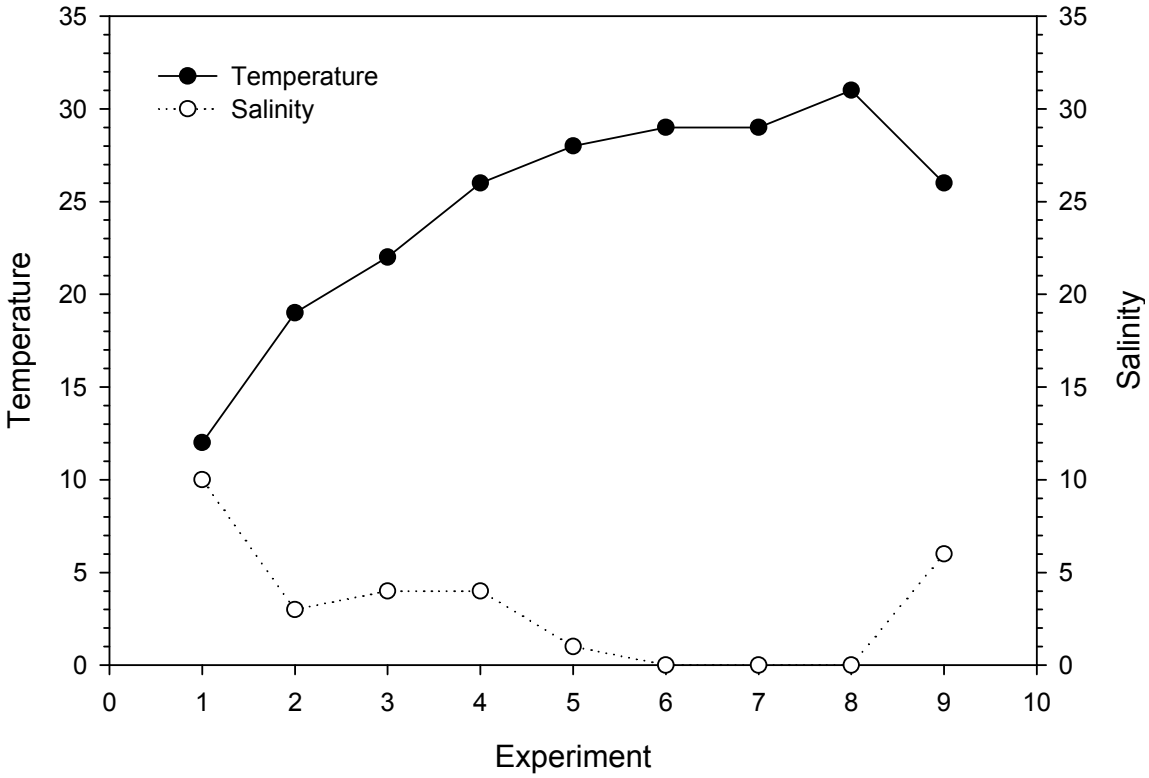

Figure 3. Variations in temperature (^{o}C) and salinity for the samples used for the bioassay experiments 1-9.

Table 4. Summary of sample date, ambient nitrogen [(NO_3^- + NO_2^-), $NH4^+$, Urea], phosphorus, dissolved inorganic nitrogen, nitrogen: phosphorus ratios by atoms and Chlorophyll *a* for Wilson Bay, New River, March - September 2003.

Experiment	Date	NO3 + NO2 (a)	NH4+ (a)	Urea (a)	Phosphate (a)	DIN (b)	N:P (c)	Chl *a* (d)
1	3/11	36.50	15.60	2.87	0.84	52.10	62.02	7.64
2	3/28	35.11	19.80	3.32	2.23	54.91	24.62	19.92
3	4/22	17.99	8.20	1.95	0.86	26.19	30.45	39.92
4	5/7	25.99	10.70	2.57	1.75	36.69	20.97	23.26
5	6/13	27.79	9.30	1.53	2.41	37.09	15.39	57.40
6	7/15	50.26	13.60	1.83	1.84	63.86	34.71	30.56
7	7/29	49.07	8.30	1.16	1.70	57.37	33.75	31.88
8	8/27	27.73	7.80	1.42	1.52	35.53	23.38	34.24
9	9/16	4.28	6.00	0.72	0.13	10.28	79.10	67.80

a) Units for nutrients are μM/L b) DIN = NO_3^- + NO_2^- + NH_4^+ c) N: P ratio by atoms d) Chl *a* as mean of two repetitions

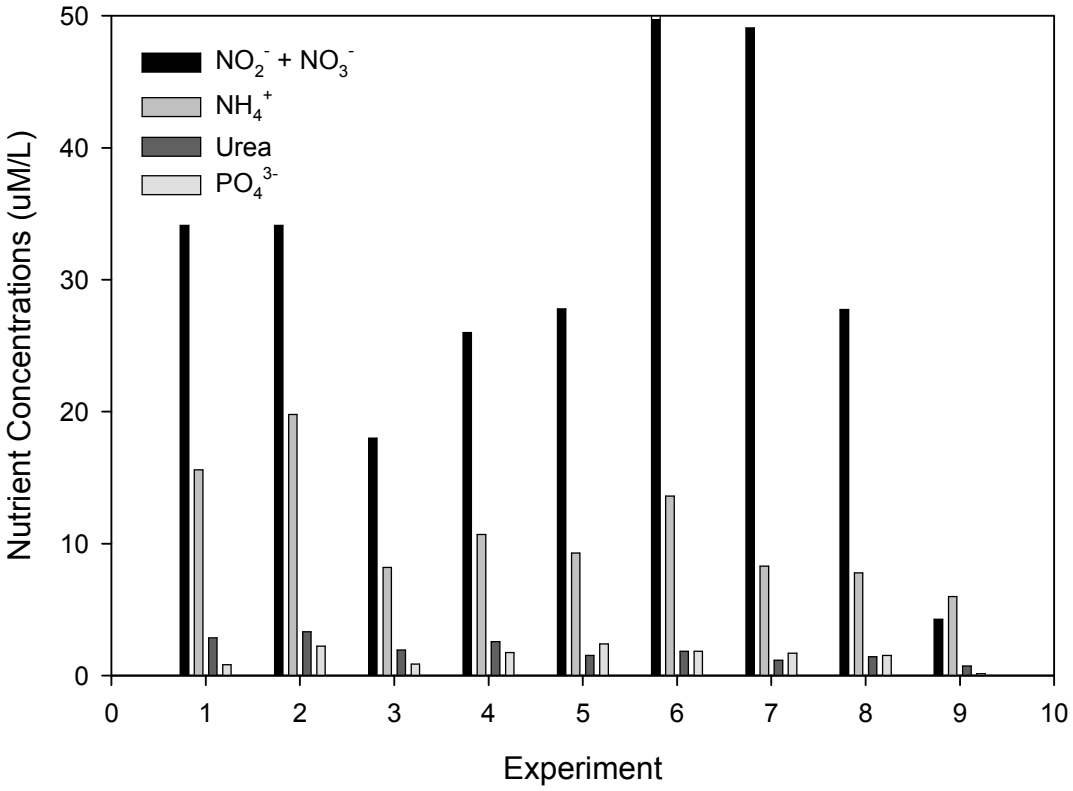

Figure 4. Nutrient composition as (NO_3^- + NO_2^-), NH_4^+, Urea and PO_4^{3-} as expressed as µM/L for experiments 1-9 on Wilson Bay, New River water (March – September 2003).

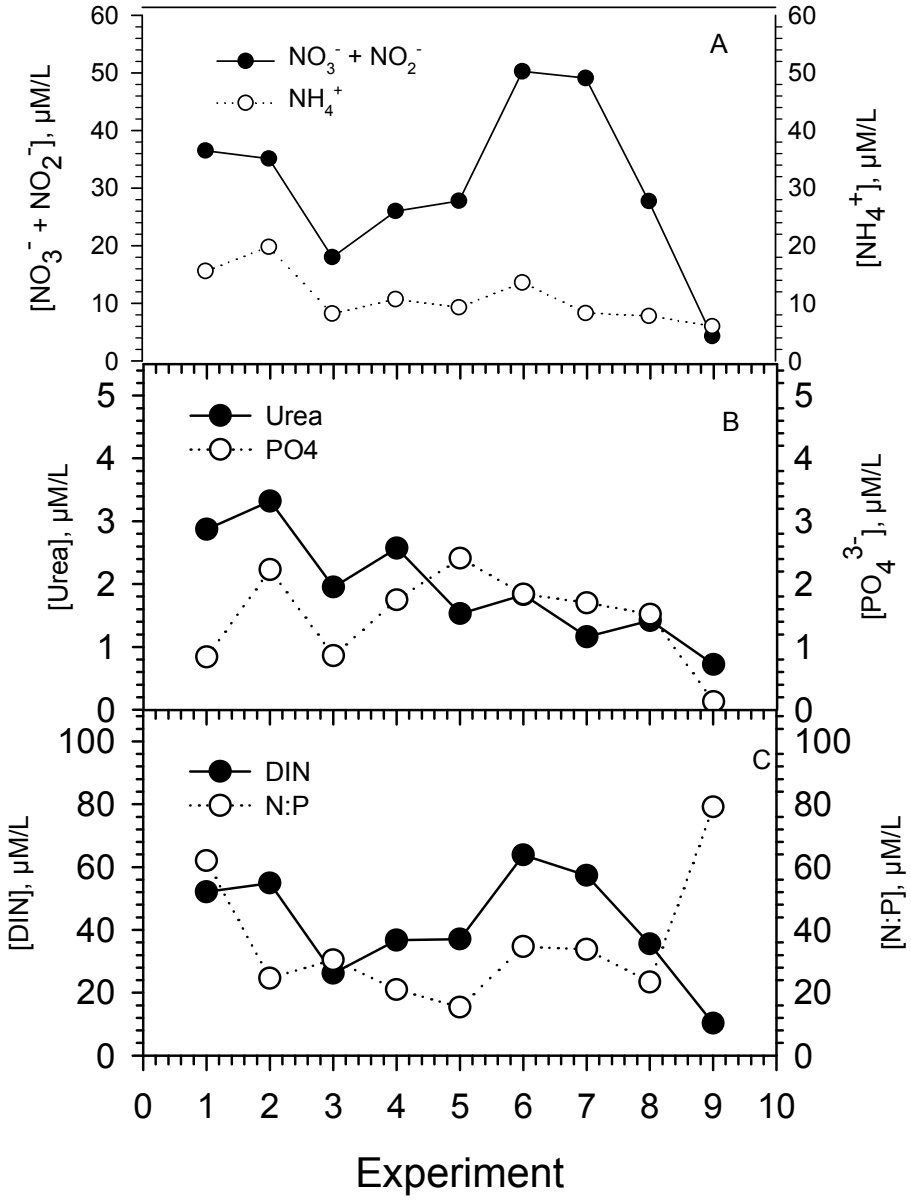

Figure 5. Ambient nutrient concentrations for Wilson Bay bioassays, March – September 2003. A) Nitrate + Nitrite and Ammonium B) Urea and Phosphate C) DIN (total dissolved inorganic nitrogen) and N:P (DIN: Phosphate) ratio.

phosphate concentrations (Figure 5C), DIN values consistently exceeded 20 µM/L for all but the last sampling date. The lowest N: P ratio was 15.4 while all others exceeded 20. The highest N: P occurred in September during a bloom event with a value of 79.1 (Figure 5C).

The chlorophyll *a* biomass (Figure 6) suggests the eutrophic nature of the station for each sample date by having never less than 7.0 µg/L. The highest value of 67.8 µg/L occurred during the final sampling date when a dinoflagellate bloom occurred. Initial chlorophyll *a* values were greater than all bioassay values for experiment 9 which was a bloom event (Figure 6). All nitrogen additions produced values at or below 90 µg/L for all experiments excluding experiment 2. That yielded values greater than 100 µg/L for nitrate and urea and 80.6 µg/L for the ammonium addition. The complete control only produced significantly higher values in comparison with nitrogen additions in experiment 8. For exclusions, experiment 2 continued to produce high values which exceeded 90 µg/L for the three exclusions and greater than 70 µg/L for the unenriched control (Figure 7). Experiment 1 also yielded high chlorophyll *a* values which exceeded 60 µg/L. The remainder of the experimental exclusions produced values lower than 50 µg/L with the exception of the minus silicate exclusion in experiment 8 which had a high value of 96.4 µg/L (Figure 7).

Terminal chlorophyll *a* values expressed as a percent of the initial values for nutrient addition bioassays showed values less than 200% for all but the first two. Experiment 1 nitrogen additions yielded values greater than 150% while the complete had a value distinctly lower at around 90%. Experiment 2 had values greater than 400%

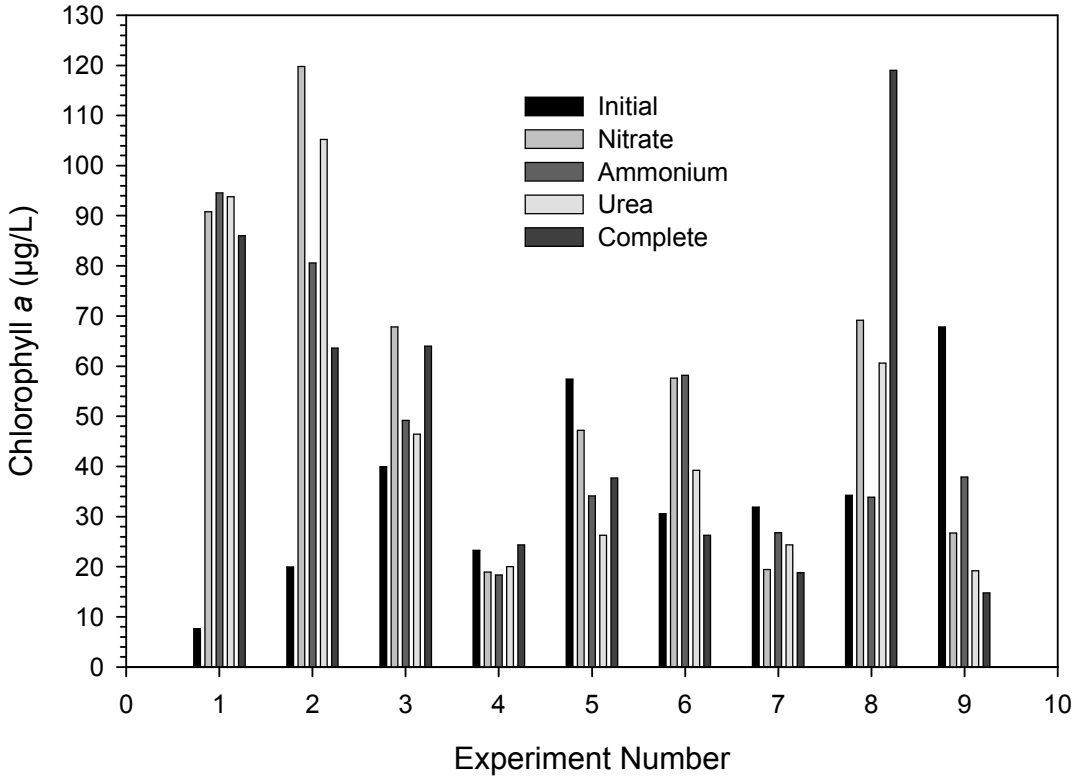

Figure 6. Initial and terminal chlorophyll *a* values for nutrient addition bioassays for Wilson Bay, New River, NC experiments 1-9.

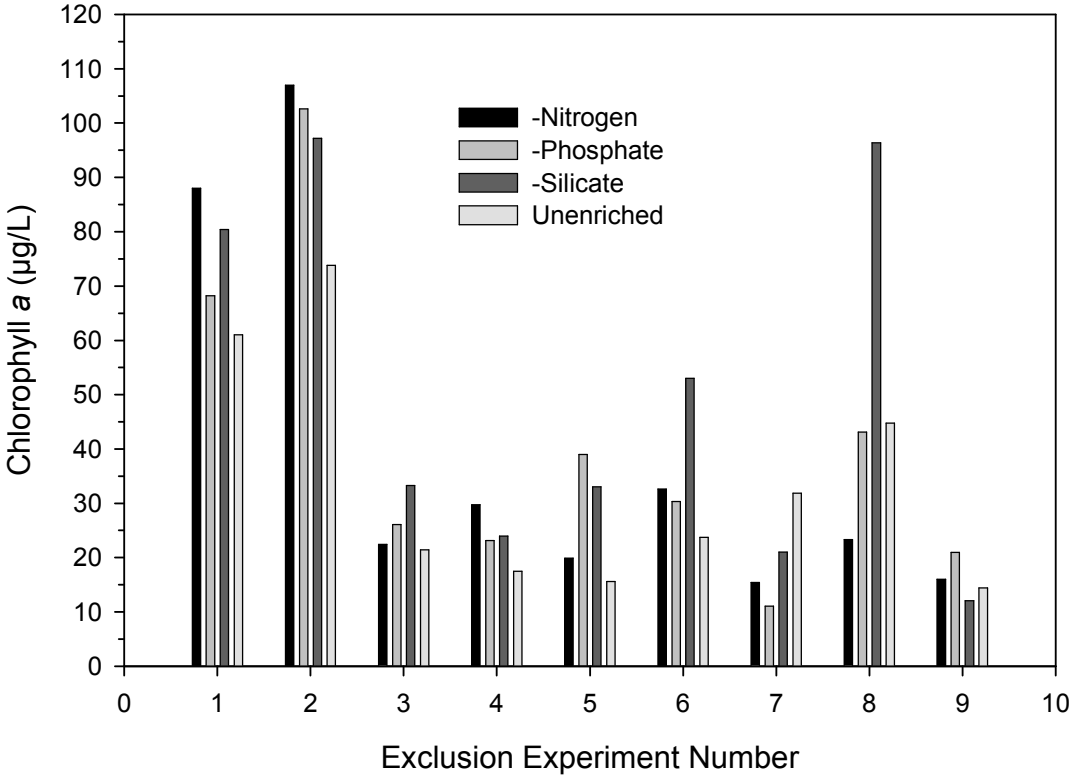

Figure 7. Terminal density as Chl *a* for the nutrient exclusion experiments conducted on Wilson Bay, New River, NC experiments 1-9.

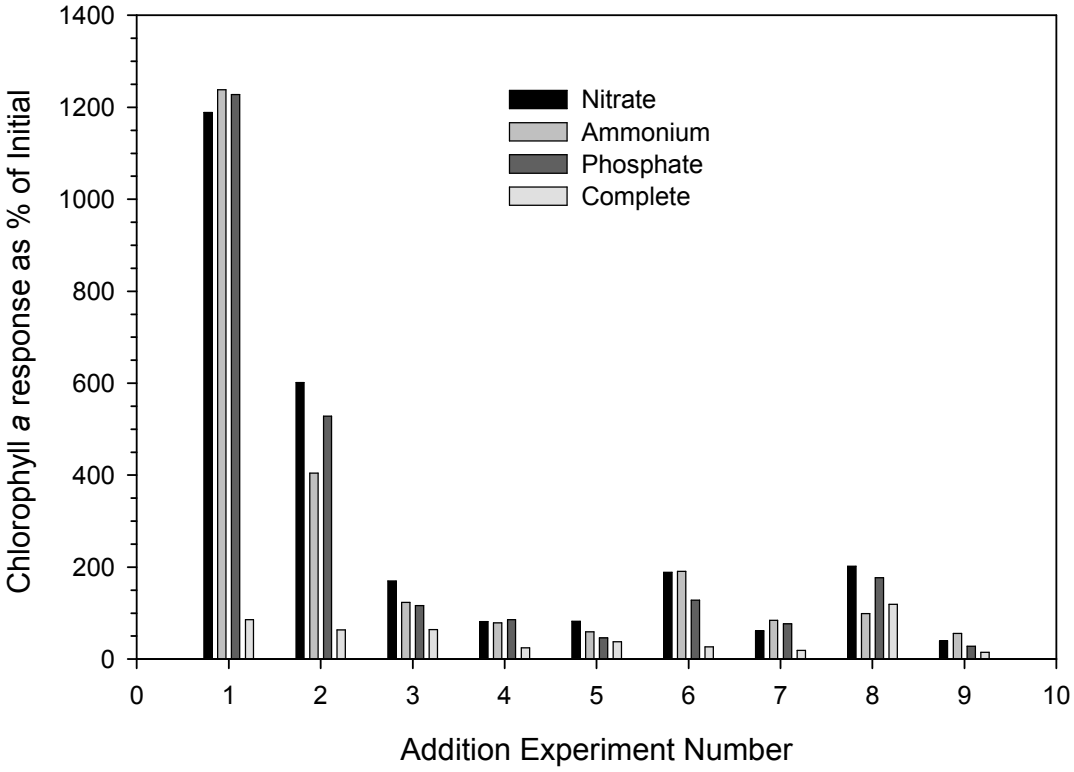

Figure 8. Terminal Chlorophyll *a* values expressed as percent of initial values for nutrient addition bioassay experiments conducted on Wilson Bay, New River, NC, experiments 1-9.

for nitrogen additions with the value for the complete similar to that of experiment 1 (Figure 8).

Nitrogen additions as a percent of the complete control were calculated and can be seen on Figure 9. Lowest values were seen in experiment 9 which had a bloom. Experiment 8 had the lowest values with all three nitrogen additions with values less than 75% of the complete control. Percentage of the three exclusions as a percent of the unenriched control can be seen in Figure 10. All experiments with the exception of the last three had percentages that exceeded 100% for all exclusions. Experiment 7 was the lowest with values less than 80%. Experiment 8 had a lone high percentage with the minus silicate exclusion that had a value over 200%.

Summarizing this information, Figure 4 clearly shows that nitrate + nitrite exceeded all other nitrogen sources for all but one time with ammonium, the next abundant nitrogen source rarely exceeded one half that of nitrate + nitrite and that urea and phosphorus were present at all samplings.

With the exception of experiments 4, 5 and 7, nutrient additions resulted in greater growth as terminal chlorophyll *a* with nitrogen additions. Experiment 9, a period of bloom formation, had declining populations throughout the nutrient experiments. For all others, nitrate and urea gave the best growth consistently . Ammonium additions gave the next greatest stimulation to growth. Only one experiment, number 8, did the complete control overwhelm the nitrogen treatments (Figure 6).

For the nutrient exclusion experiments, those done in 1, 2 and 4 showed the exclusion of nitrogen did not impede the assay for producing a higher chlorophyll *a*

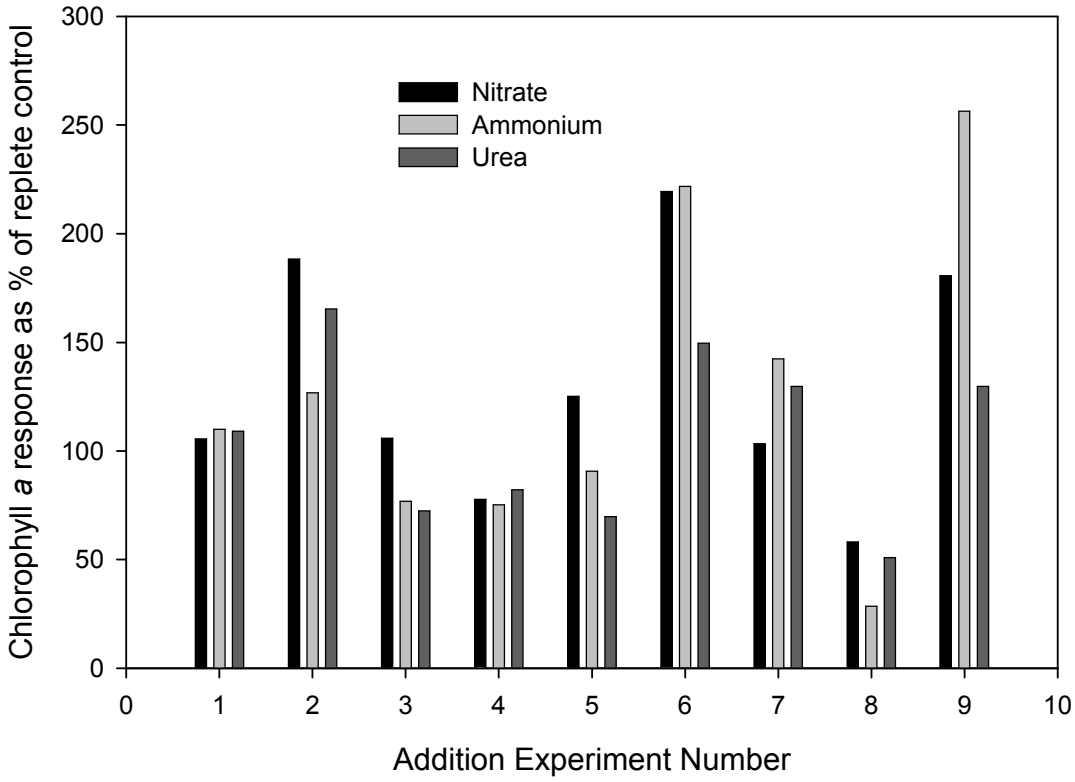

Figure 9. Terminal density expressed as percent of the nutrient complete control Chl *a* for nutrient addition bioassay experiments, Wilson Bay, New River, NC experiments 1-9.

biomass that other exclusions (Figure 7). Phosphorus appeared to be abundant throughout the experimental period giving terminal chlorophyll *a* values at or above the complete control. For all but experiment 9, the lack of silica did not impede the growth of the populations. Experiment 9 again showed a diminished response in relation to the others.

When terminal chlorophyll *a* was expressed as percent change from the initial, the greatest percent change was observed in experiment 1 and 2 where all additions exceeded 400% of the initial (Figure 8). Experiments 3, 6 and 8 were observed with uneven increases between 100% and 200%. Experiment 9 was the least responsive of them all. When comparing the terminal chlorophyll *a* density of each treatment to the complete control, five of the nine experiments (1, 2, 6, 7, and 9) showed increases greater than 100% of the complete control. Only experiment 8 had less than 50% of the control. Seven of the nine experiments showed values greater than 100% of the complete control for at least one nitrogen addition (Figure 8, 9).

In all exclusion experiments except 7 and 8 did the lack of nitrogen result in less than 100% chlorophyll *a* terminal densities. The minus phosphate treatments nearly always equaled or exceeded the terminal chlorophyll *a* values of those treated as minus nitrogen. Experiments 3, 6 and 8 had higher chlorophyll *a* densities when silica was not added (Figure 10).

Growth bioassay treatments of single nutrient additions and exclusions and appropriate control of nutrient complete and unenriched treatments were measured as *in vivo* fluorescence. Chlorophyll *a* based growth rates were calculated from each treatment

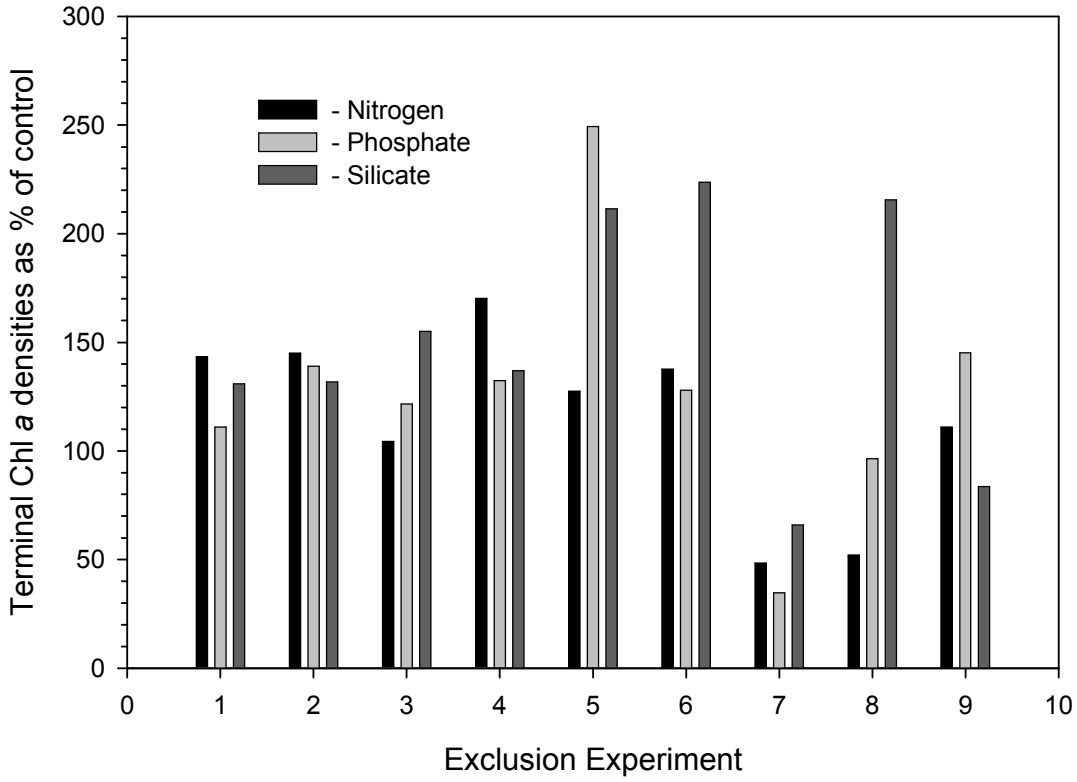

Figure 10. Terminal density expressed as percent of the unenriched nutrient control of Chl *a* for nutrient exclusion bioassay experiments, Wilson Bay, New River, NC, experiments 1-9.

and the mean (n = 4) of each treatment is found in Table 5. A mean growth rate (K) exceeded 1.0 divisions/day was found with each of the nitrogen sources tested. For the 20 March 2003 sample, K rates values exceeding 1.0 divisions/day occurred in all treatments except the unenriched control indicating a population poised to exploit the nutrient additions equally well. Nitrate gave the best growth during experiments in March through May and again in early July with values of >0.7 divisions/day. Lowest growth measured with nitrate was in September during a bloom event. In contrast, ammonium consistently gave mean growth of 1.12 divisions/day, matching those of 1.08 for nitrate and 1.12 for urea. The lowest growth rate recorded with ammonium additions occurred on the last sampling date with a rate of 0.03 divisions/day.

Urea addition showed growth often similar to that of ammonium. This substrate was quickly assimilated by the natural population. The nutrient exclusion bioassays indicated low or negative growth for nitrogen and silica on the last two sampling dates (Table 5). These results mirrored the nutrient additions and unenriched control suggesting other factors beyond nutrients in regulating growth at that time. The mean growth rates measured from the unenriched control were less than the nitrogen additions.

The K values and r^2 regression values were calculated from *in vivo* growth curves for all bioassays in each of the nine experiments and are listed in Table 6. Maximum growth rates for all nitrogen additions were found in experiment 2. The K values of 1.08, 1.12 and 1.12 divisions/day. for nitrate, ammonium and urea respectively and had r^2 regression values greater than 0.9542. Community doubling densities greater than 1 division/day represent a strong response to nitrogen additions (Figure 11). Intermediate

Table 5. Initial and terminal densities measured as mean chlorophyll *a* (µg/L) for bioassay experiments for Wilson Bay, New River, North Carolina, March - September 2003.

Treatment	E1	E2	E3	E4	E5	E6	E7	E8	E9
Initial	7.64	19.92	39.92	23.26	57.40	30.56	31.88	34.24	67.80
Nitrate	90.80	119.80	67.80	18.96	47.20	57.60	19.44	69.14	26.70
Ammonium	94.60	80.60	49.20	18.34	34.16	58.20	26.80	33.88	37.90
Urea	93.80	105.20	46.40	20.00	26.28	39.24	24.40	60.62	19.18
Complete	86.00	63.60	64.00	24.38	37.70	26.24	18.82	119.00	14.78
- Nitrogen	88.00	107.00	22.40	29.76	19.92	32.64	15.40	23.30	16.00
- Phosphate	68.20	102.60	26.08	23.12	38.96	30.34	11.06	43.10	20.94
- Silicate	80.40	97.20	33.26	23.94	33.02	53.00	21.00	96.40	12.06
Unenriched	61.40	73.80	21.44	17.48	15.62	23.70	31.84	44.72	14.42

Table 6. Fluorescence based growth rates (K) and regression coefficient (r^2) for log phase portion of nutrient bioassays for March-September 2002 on Wilson Bay, New River, North Carolina sample.

	Nitrate		Ammonium		Urea		Complete		Minus N		Minus P		Minus Si		Unenriched	
	K	r^2	K	r^2	K	r^2	K	r^2	K	r^2	K	r^2	K	r^2	K	r^2
11 Mar 03	0.59	0.922	0.57	0.956	0.56	0.947	0.47	0.984	0.61	0.991	0.48	0.982	0.55	0.966	0.41	0.954
28 Mar 03	1.08	0.954	1.12	0.970	1.12	0.969	1.15	0.968	1.00	0.928	1.14	0.976	1.16	0.970	0.63	0.963
22 Apr 03	0.77	0.977	0.83	0.993	0.79	0.992	0.83	0.985	0.65	0.989	0.65	0.996	0.70	0.994	0.57	0.996
7 May 03	0.70	0.877	1.00	0.962	0.94	0.938	0.99	0.940	0.76	0.982	0.94	0.949	0.94	0.949	0.52	0.952
13 Jun 03	0.39	0.963	0.46	0.986	0.43	0.975	0.41	0.979	0.25	0.961	0.46	0.990	0.52	0.993	0.36	0.934
15 Jul 03	0.81	0.977	0.98	0.998	0.97	0.999	0.95	0.999	0.87	0.998	0.84	0.973	0.95	0.996	0.81	0.958
29 Jul 03	0.38	0.993	0.56	0.987	0.48	0.994	0.55	0.995	0.53	0.969	0.50	0.988	0.55	0.992	0.42	0.992
27 Aug 03	0.30	0.998	0.36	0.991	0.18	0.991	0.34	0.995	-0.39	1.000	0.11	0.966	-0.21	0.972	-0.73	1.00
16 Sep 03	0.23	0.972	0.30	0.823	0.15	0.836	0.17	0.788	0.05	0.999	0.07	0.999	-0.12	0.733	-0.60	1.00

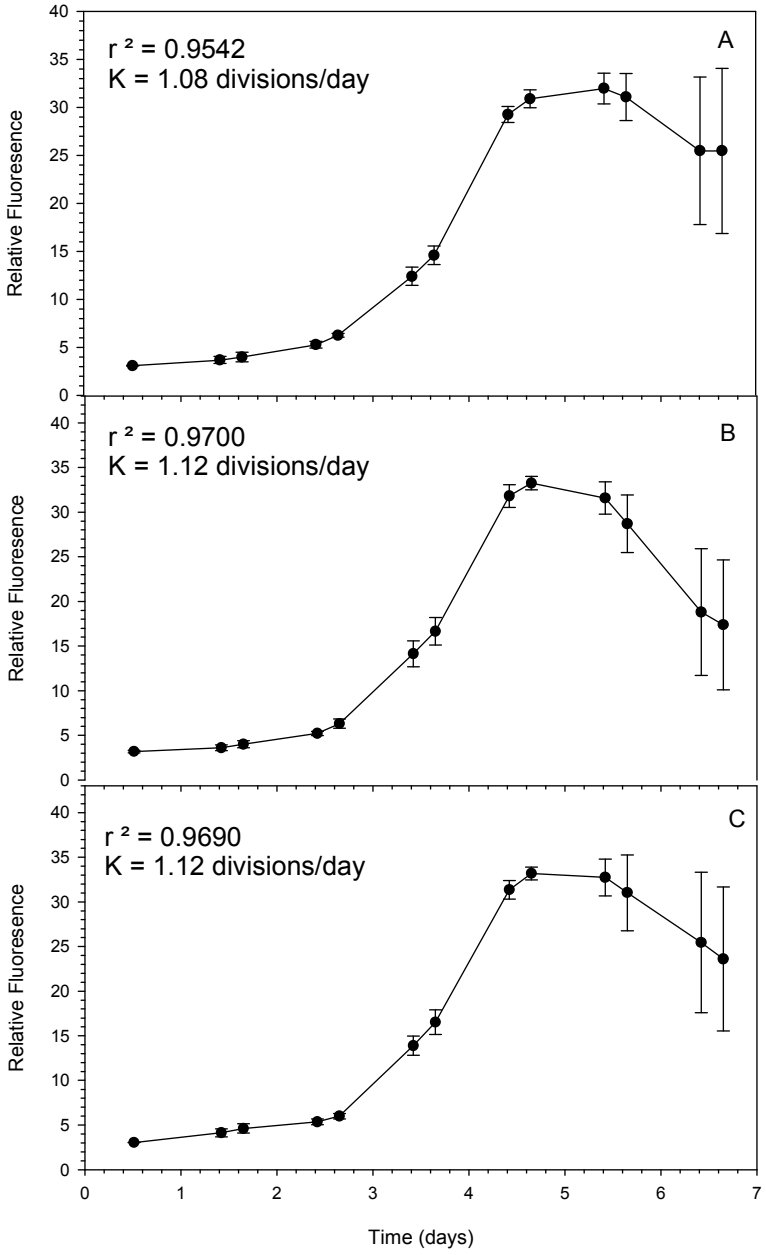

Figure 11. Growth curves for experiment 2 having nitrogen additions exhibiting rapid growth: A) Nitrate B) Ammonium C) Urea.

growth rates were observed in experiment 3. The K values of 0.77, 0.83 and 0.79 divisions/day for nitrate, ammonium and urea respectively had r^2 regression values greater than 0.9776. The K values seen in experiment 3 are intermediate in comparison to experiment 2which had the highest values (Figure 12). The lowest growth rates were seen in experiment 9 which has K values of 0.23, 0.30 and 0.15 divisions/day for nitrate, ammonium and urea respectively and had r^2 regression values greater than 0.8237. The lowest growth rates seen in experiment 9 are in comparison to experiment 2 which had the highest growth rates (Figure 13).

Cellular eye counts varied from each experiment due to the different algal species represented in each collection. Experiment 1 (Table 7) had three different algal species represented and one ciliate species, tintinnid. *Selenastrum* and *Cyclotella* both had high cell counts which were observed in the nitrate addition. Chlorococcum also had a high in the nitrate addition. The zooplankton, tintinnid, had a small population represented in most bioassays. It had the highest number in the complete addition. The following (Experiment 2, Table 7) had five different species in the sample, which were *Selenastrum*, flagellate species, Euglena, *Heterocapsa* and *Chloroccum*. *Heterocapsa* and *Chloroccum* were only found in the initial sample and had disappeared from the bioassays for final counts. *Selenastrum* was represented in every bioassay.

The next experiment conducted, number 3 (Table 7), had six species represented. These included *Pseudo-nitzschia*, tintinnid, *Selenastrum*, *Scenedesmus, Chloroccum* and *Rhizosolenia*. *Pseudo-nitzschia* and tintinnid were only found in the initial counts and had vanished by the final counts. Alternately *Selenastrum, Scenedesmus, Chloroccum* and *Rhizosolenia* were not found in the initial count. *Scenedesmus* did not appear in the

minus phosphate exclusion, but was highest in the complete addition and lowest in the unenriched exclusion. *Chloroccum* was not present in the minus nitrogen nor was it in the unenriched. It had the most cells in the ammonium and the fewest in the complete control. *Rhizosolenia* was not found in the final count of the minus phosphate exclusion.

Four species were found in experiment 4 (Table 7). *Pseudo-nitzschia, Scenedesmus, Chloroccum*and stellate ameba. *Pseudo-nitzschia* appeared in all except the minus phosphate exclusion. *Scenedesmus* did not appear in the initial, nitrate addition, minus silicate exclusion and the unenriched treatment. *Chloroccum* appeared in all except the unenriched treatment. The stellate appeared in all but the initial which indicates that nutrient additions may have stimulated growth.

The next sample (experiment 5) contained seven different species (Table 7). Tintinnid did not appear in the initial sample or in the ammonium, urea and complete additions. Its highest value was in the unenriched exclusion and its lowest in the minus silicate. *Pseudo-nitzschia* was not found in the ammonium and complete additions. Its highest numbers was in the urea addition and its lowest in the minus phosphate exclusion. *Scenedesmus* was not in the initial sample or in the nitrate addition, minus nitrogen and minus silicate exclusions. *Selenastrum* was not present in the initial sample nor in the urea and complete additions and in the minus nitrogen exclusion. Flagellate was not found in the initial sample nor in the nitrate and complete additions and minus nitrate exclusion. Stellate ameba was not in the initial sample nor in the nitrate and ammonium additions nor in the minus phosphate exclusion. *Rhizosolenia* was only found in the minus nitrogen.

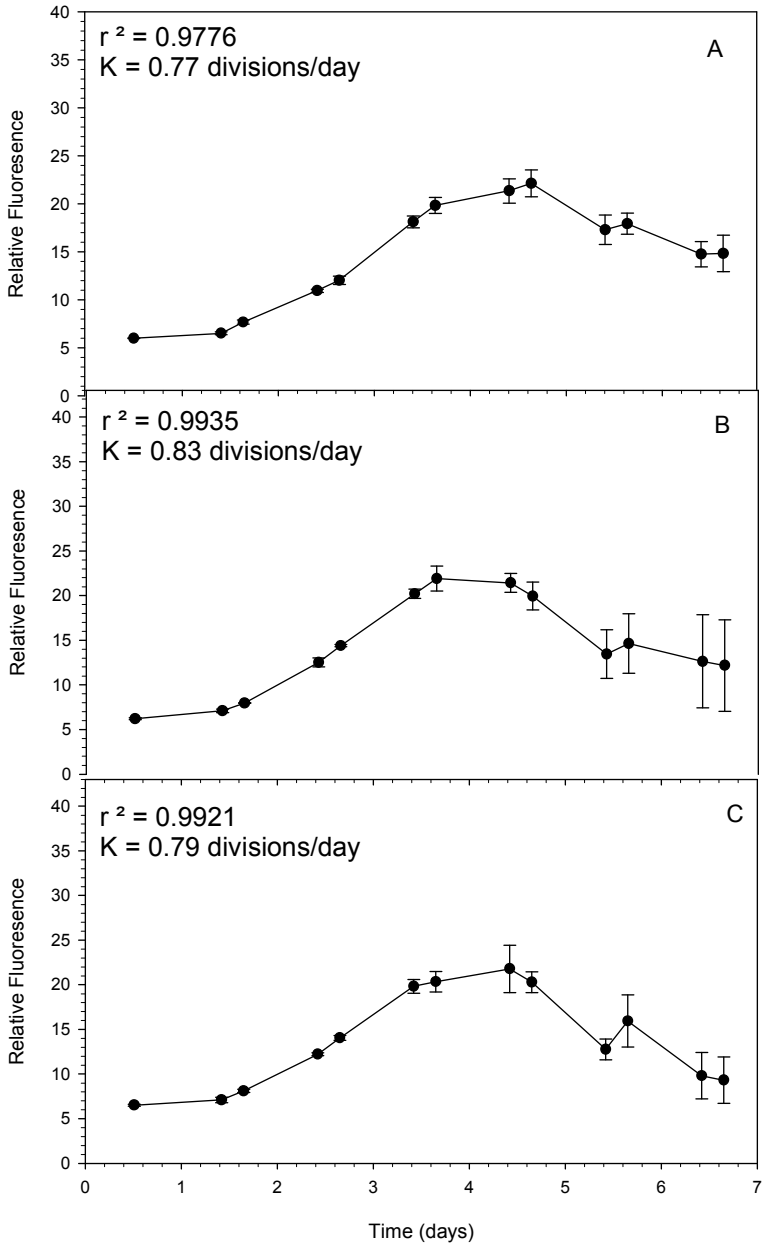

Figure 12. Growth curves for experiment 3 having nitrogen additions exhibiting intermediate growth as compared to experiment 2: A) Nitrate B) Ammonium C) Urea.

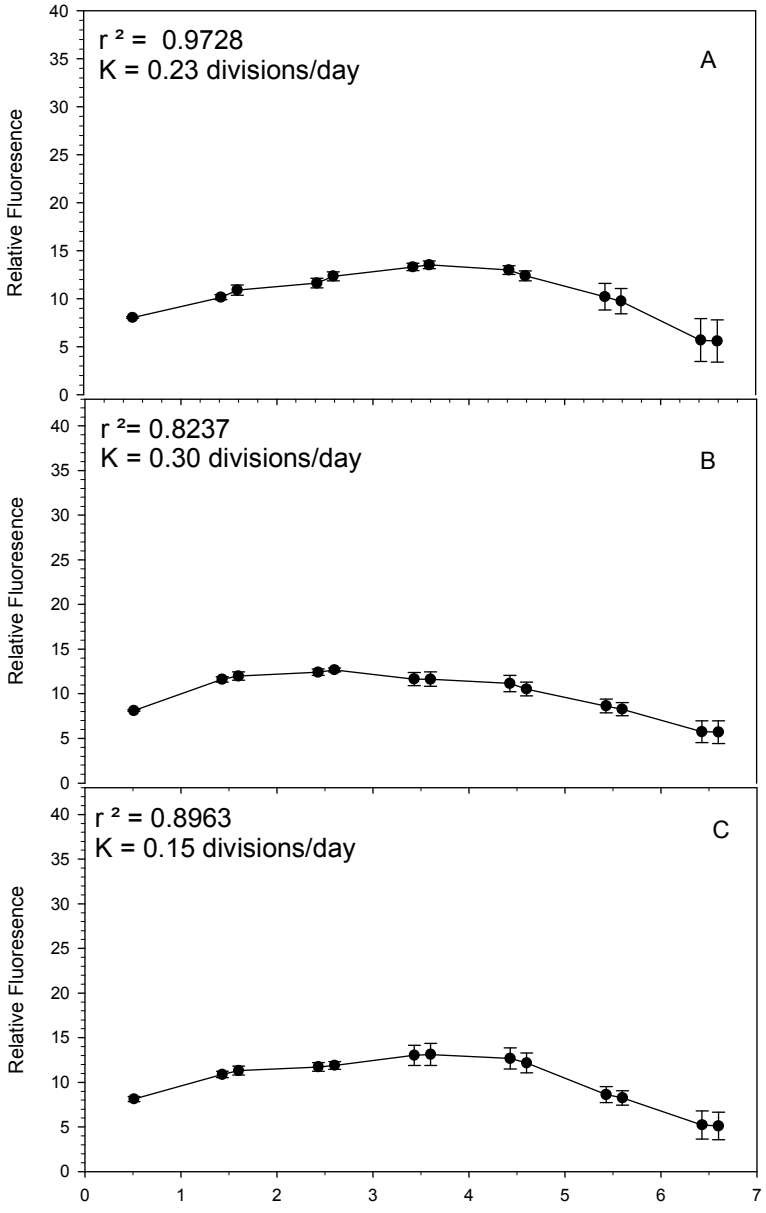

Figure 13. Growth curves for experiment 9 having nitrogen additions exhibiting lowest growth as compared to experiment 2: A) Nitrate B) Ammonium C) Urea.

Table 7. Dominant taxa observed during the nutrient bioassay experiments of natural populations taken from Wilson Bay, New River, North Carolina during the period from 11 March through 16 September 2003.

	Experiment #								
Major Taxa	1	2	3	4	5	6	7	8	9
Algae									
*Selenastrum**	+	+	+		+				
*Chlorococcum**	+		+	+		+	+		
Cyclotella	+								
Flagellate sp.		+			+				
Euglena*		+				+			
Cryptomonas		+							
Heterocapsa		+							
Pseudo-nitzschia			+	+	+				
*Scenedesmus**			+	+	+	+	+		
Rhizosolenia			+		+	+			
*Kirchneriella**						+	+		
Synedra						+	+	+	+
Protoperidinium								+	+
Gyrodinium								+	+
Protozoa									
tintinnids	+		+		+	+	+	+	+
stellate ameba				+	+	+		+	+
lobate ameba								+	+

* = denotes freshwater species, all others are brackish or marine

With the sample collected on 29 July (experiment 6, Table 7), it had eight species represented. Euglena was the only species in experiment 6 that was found in the initial sample. Tintinnid was not found in the initial sample and had the highest numbers in the unenriched exclusion. *Scenedesmus* was not in the urea addition or in the minus silicate exclusion. *Chloroccum* was not initial, urea addition or in the minus nitrogen, minus phosphate and unenriched exclusions. It had the greatest numbers in the minus silicate exclusion. *Rhizosolenia* was only found in the nitrate addition and the ammonium addition with. Stellate ameba was only found in three bioassays which were the urea addition, complete addition and the minus silicate exclusion. *Kirchneriella* was not found in the initial, nitrate and urea additions nor in the minus nitrogen exclusion. *Synedra* was not found in any of the addition bioassays and was only found in the exclusions.

For experiment 7 (Table 7) five species were represented. *Synedra* was found in the initial and all bioassays. It had the highest values in the nitrate addition. Tintinnid was not found in the nitrate addition nor in the minus nitrogen exclusion. *Scenedesmus* was not found in the initial sample. *Chloroccum* was not found in the initial, urea addition, silicate and unenriched exclusions. *Kirchneriella* was not found in the initial, minus phosphate and unenriched exclusions. Tintinnid was not found in the nitrate addition and the minus nitrogen exclusion.

Next followed experiment 8 (Table 7) which had five species. *Synedra* was found in the initial and all bioassays. It had the highest values in the minus phosphate exclusion. Tintinnid was not found in the nitrate addition nor in the minus nitrogen exclusion. *Scenedesmus* was not found in the initial sample. *Chloroccum* was not found

in the urea addition and unenriched exclusions. *Kirchneriella* was not found in the initial sample and the unenriched treatment.

The final experiment (experiment 9, Table 7) had six species represented. *Protoperidenium* was found neither in the ammonium and complete additions nor in the minus nitrogen, minus phosphate and unenriched exclusions. *Gyrodinium* was only found in the initial count and the unenriched exclusion. *Synedra* was not found in the minus silicate exclusion. Stellate ameba was only found in the nitrate addition and the minus nitrogen exclusion. Lobate ameba was neither found in the initial sample nor in the minus nitrogen exclusion. Tintinnid was found in the initial sample, ammonium addition and the unenriched exclusion.

DISCUSSION

Wilson Bay, located in the New River Estuary, North Carolina, was chosen as the study site due to its historical importance for algal blooms. Repeated bloom events and fish kills were reported through water quality monitoring efforts done by the North Carolina Department of Environmental Natural Resources, the EPA and the Tomas Lab, Center for Marine Science of UNCW (Tomas, 2003). Prior to 1999 this area was heavily influenced by receipt of treated sewage from the city of Jacksonville, Wilson Bay Wastewater Treatment Plant. This input presumably left the bay with unknown amounts of nutrients which could impact the area for years to come particularly with the disturbance of sediments. Before the closure, high levels of nitrate-nitrite, ammonium and phosphorus were measured. Since the closure of the plant, ammonium showed the greatest abatement (Mallin et al, 2005).

Comparing two years of ambient conditions and nutrient availability in Wilson Bay (Table 4, Mallin et al, 2005) showed that the period of this study behaved differently when compared to the previous year. Rainfall during the previous year and bloom occurrence was markedly different. During the previous summer (2002) a drought was accompanied by a high number of algal blooms within Wilson Bay (Tomas, unpublished data). Bloom events typically occurred during the warm weather months of March through September of previous years. The experimental year of 2003 was one of high amounts of rainfall and a low level of bloom occurrence with only one significant bloom occurring during the final week of sampling. Another factor to be considered is the fact that all historical data prior to 1999 would have been heavily influenced by the discharge from the Jacksonville Wastewater Treatment Plant and prior to its closure (Mallin et al, 2005).

Wilson Bay is a partially enclosed, poorly flushed, shallow embayment that is susceptible to tides, wind mixing and run-off variations influencing the ratio of salt to fresh water which complicates the use of Redfield ratio's in predicting bloom. The validity of the ratio of 16:1 (atoms) as defined by Redfield must be used with caution within this system. Fluxes in coastal regions, such as Wilson Bay, are thus a function of amounts of rainfall, tidal changes, disturbance of bottom sediments and influx of nutrients caused by various sources, such as runoff (Mallinet al , 2005). These components can make a system such as Wilson Bay difficult to understand and must therefore be looked at as a unique system not easily defined by nutrient standing stock only. While the amounts of nutrients that flow into Wilson Bay are variable depending on any one day, the cycling of nutrients will remain a critical measurements required to

give insight as to how the system functions. The purpose of using natural populations in bioassays as opposed to a unialgal test species was to tell us about the nutrients impact their growth.

Algal bioassays can be executed in different ways. One way is to perform "bottle experiments" and using a culture of a single "indicator" algal species. These assays can be replicated and assume that results from laboratory studies can be applied to events in the field. This type of bioassay can also be performed using a phytoplankton community, containing a mixture of natural populations (Hecky and Kilham, 1988). Replications and assumptions can also be made by using data obtained from laboratory results, but extrapolation from bioassays can be risky due to the fact that elements of the ecosystem, such as grazers or turbidity, may be missing or altered. Mesocosm and field experiment (in situ) bioassays can also be performed. These are often able to incorporate factors that are omitted from bottle bioassays. Problems can also arise in these experiments such as founder effects. These effects are the establishment of a new population by a few original founders, which carry only a small fraction of the total genetic variation of the parental population creating a difficulty in understanding the ecosystem (Hecky and Kilham, 1988).

Algal bioassays, while informative, have limitations in that each experiment is normally of a short duration yet are also time consuming. Each bioassay has numerous analyses that must be performed and some results cannot be determined for at least one week. In dealing with natural populations as opposed to using a unialgal test species, taxonomic expertise is necessary as well. Dominant species must be properly identified in order to determine its impact on the bioassay. Another limitation to using natural

populations in bioassays is grazers. Grazers sometimes manage to slip through the screening process and their impact is not known until the bioassay in complete. Their presence can skew the final species composition by lowering the numbers of certain populations that might not otherwise be.

The idea that either nitrogen or phosphorus being the limiting nutrient was initially suggested by Redfield with his ratios (1958) and it was later debated by Ryther and Dunstan (1971) and by Hecky and Kilham (1988). Natural water sources have many nutrients available to phytoplankton, but it is often nitrogen and/or phosphorus that can be quickly depleted, hence becoming a limiting factor on growth. The ratio of nitrogen to phosphorus in marine environments is thought to be an important factor in regulating algal bloom events (Hodgkiss and Ho, 1997). The levels of nitrogen and phosphorus in a natural coastal marine environment can fluctuate rapidly depending on numerous factors such as rain, runoff and bottom disturbances. It is difficult to predict how anthropogenic input of nutrients would affect algal growth because the additions to a system could alter the N:P ratio, thereby changing the limiting factor for a particular species.

The growth of phytoplankton measured by in vivo fluorescence in each experiment depended in large part on the species present and the amount of nutrients already available in sample water. The N:P ratio varied greatly in each experiment as did the growth of algal biomass (Table 3). The amounts of total nitrogen (both organic and inorganic) available in the waters of Wilson Bay were routinely high so the amounts added for each bioassay, although environmentally realistic, did not seem to make much of a difference in terms of growth overall. The algal populations grew rapidly in each bioassay due to the continuous light exposure. Populations in each tube increased greatly

in number and crashed after the midpoint of each 7-day experiment. The fact that these natural populations were forced to grow in an enclosed space (wall effects) with a limited amount of nutrients contributed to the population crash and the response will depend greatly on the scale. A microcosm, such as this, will produce different results compared to that of a meso- or macrocosom.

Species composition showed throughout the nine bioassay experiments to have a majority of freshwater algal species despite the fact that Wilson Bay is a brackish embayment. The unusually high amounts of rainfall during the experimental period raised the amounts of freshwater in the system resulting in salinities of zero or close to zero (Table 1). This differed markedly from the previous year when a drought occurred and samples taken showed more salt water algal species and higher salinities due to the reversal of fresh to salt water dominance.

One of the goals of this experiment was to see how various nitrogen sources stimulated growth over a short duration of time. At the conclusion of the nine algal bioassays, nitrogen clearly stimulated growth nearly all the time. Nitrogen, both from review of historical data and from bioassays, has proven to indeed be limiting in nearly all cases and the addition of the various forms (nitrate, ammonium and urea) caused significant changes. These changes were also noted in the exclusion bioassays.

Nitrate and urea in particular were both highly stimulatory to growth as seen in the terminal chlorophyll *a* values (Figure 5) and nutrient exclusion treatments. In particular, experiment 2 clearly shows the dramatic effect nitrogen additions have on growth. The growth is so dramatic and rapid that it produced an equally dramatic population crash (Figure 10). The role of urea in Wilson Bay had not been addressed in

previous water quality studies or had levels been monitored. Because its impact was unknown and since preliminary analysis showed it to be present, urea was used as a nitrogen addition. This study clearly showed that urea had a significant impact particularly on growth as seen in experiments 1, 2 and 9. It was present in significant amounts throughout this study (Figure 5). This is not surprising considering that Wilson Bay was the former recipient of treated sewage continuously receiving inputs of nitrogen including urea.

Despite the fact that numerous algal bloom events were not seen during this study period, it is important to note that nutrients that enter Wilson Bay regulate the dynamics of phytoplankton species that grow there even if high levels of rainfall may dilute the populations present for a period of time. High amounts of rainfall equate to high amounts of run-off which can increase the amounts of nitrogen entering into Wilson Bay. Out of nine experiments performed in this study period, five had salinities of one or zero which would influence the species represented.

The role of silica in the bioassay experiments did not appear to be significant based on the growth measurements recorded. The exclusion of sodium silicate from natural samples did not fail to hinder growth nor did its presence stimulate growth. On the contrary, the lack of silicate in the exclusion performed in each of the nine experiments showed that growth was not affected which can be clearly seen especially in experiments 1, 2, 6 and 8. In these, the lack of silicate did not impede high terminal chlorophyll *a*.

Phosphate as examined through these experiments indicated that it was limiting only on one occasion. This may have been due to the species present at the time the

experiments were performed whereas a different set of species may not have been limited in terms of growth. Phosphate was abundant in Wilson Bay throughout the entire study period. The role of phosphorus should be addressed to see if its presence has any impact on the growth of species that are found in brackish waters and that might have been found in higher numbers in Wilson Bay had it not been for the high levels of rainfall.

While this experiment was a good starting point, it is begs for more questions to investigated. Bioassays are useful but do not always offer very sensitive measurements. Cellular eye counts, while able to give an overview of the cells present, it cannot give detail of the smaller species within a sample that are unable to be identified. Electron microscopy would provide that detail and would help determine if these smaller species are significant. This would add to the processing time needed for the bioassays. While not studied as in depth as nitrogen in this experiment, it was noted that the role of phosphate within Wilson Bay is important. The abatement of nitrogen will do little unless problems regarding phosphate are resolved. The role of phosphate and mitigating its impact should be looked at closely to determine the role it plays on algal bloom formation.

The bioassays despite their shortcomings are able to give an integrated biological response to the conditions and help put into perspective the impact of supply and utilization of the common N and P macronutrients. This begs the question of looking at the phytoplankton populations in a dynamic way. This might involve assays investigating nitrogen and phosphorus assimilative enzymes, gene expression for these enzymes (such as nitrate and nitrite reductase, urease, glutamine synthethase and alkaline phosphatases). Using the rates measured by the enzyme measurements, knowing which

nitrogen assimilative genes are up regulated as well as the standing stocks of all the nitrogen and phosphorus sources will give a better understanding of the bloom process. As with the forms of nitrogen used in this experiment, both inorganic and organic sources of phosphorus should be studied.

Summary Conclusions

These bioassays showed the following:

1. The experimental results suggest nitrogen could limit phytoplankton biomass within Wilson Bay.
2. Despite abatement of ammonium, nitrate and urea are both readily assimilated to support blooms.
3. Urea plays an important part of the nitrogen budget. It needs to be monitored along with other forms of nitrogen.
4. Phosphorus was found as particularly limiting once signifying that reduction in phosphate within Wilson Bay is equally important as nitrogen.
5. Silicate was not important in influencing blooms as based on these studies.
6. The year chosen for the bioassay study was different from previous years when blooms were much more common.
7. In addition to the static nutrient standing stock nutrient measurements, rate measurements of assimilation by the phytoplankton and bacterial populations are necessary to define the dynamics observed supporting blooms.

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