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EFFECT OF ORGANIC AND INORGANIC NUTRIENT LOADING ON PHOTOSYNTHETIC AND HETEROTROPHIC PLANKTON COMMUNITIES IN BLACKWATER RIVERS

by

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ABSTRACT

Blackwater stream systems are abundant in the Coastal Plain of North Carolina. These streams have historically not been considered to be at risk from nutrient based eutrophication problems. However, the Coastal Plain is also the major production area for intensive livestock operation industries (swine and poultry) in the state. These operations are documented sources of acute and chronic nutrient loading to receiving waters. Some public and private point source dischargers have also been shown to introduce high nutrient loads to blackwater streams. The objective of this research was to use experimental bioassays to examine the effect of organic and inorganic nutrient loadings on stream planktonic communities in the Black and Northeast Cape Fear Rivers. Various nitrogen (N) and phosphorus (P) treatments were used at levels of 1.0 milligrams per liter (mg/L) as N or P. Phytoplankton response to nutrient loading was measured over a six day period by chlorophyll *a* production, while general photoautotrophic plus heterotrophic planktonic response was measured by adenosine triphosphate (ATP) analysis. Nutrient additions in late fall and winter did not significantly stimulate chlorophyll biomass. However, additions of ammonia, N+P, and urea were capable of producing high algal growth during the bioassays in spring and summer. The organic compound urea often yielded significant chlorophyll production comparable to that of ammonia treatments, while the N+P combination usually produced the strongest response of all. Neither organic nor inorganic phosphorus additions produced significant phytoplankton responses. In contrast, both orthophosphate and glycerophosphate treatments significantly stimulated ATP production year-round, with particularly high yields during summer. Phytoplankton in the Northeast Cape Fear River tended to be more nitrogen limited than in the Black River, while the heterotrophic community in the Black River was more phosphorus limited than that of the Northeast Cape Fear River. The results of this research support the hypothesis that nutrient additions to Coastal Plain blackwater streams can support algal blooms during selected periods and will also stimulate the growth of heterotrophic microflora which then become sources of labile biochemical oxygen demand (BOD) in these waters.

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SUMMARY AND CONCLUSIONS

The Coastal Plain of North Carolina is characterized by extensive blackwater stream and river systems. Blackwater systems have historically not been considered to be at risk from eutrophication problems, and are thus not normally considered nutrient sensitive waters by the State of North Carolina. However, in recent years there has been a dramatic rise in numbers of intensive livestock operations (ILO's) in the Coastal Plain, particularly swine and poultry. Waste from large scale swine operations is concentrated in lagoons and later sprayed on vegetated fields, while poultry waste is often land applied as dry manure. These wastes are highly concentrated sources of nitrogen and phosphorus, which have been shown to enter neighboring water bodies either acutely through lagoon accidents or chronically from leakage, spray field runoff, or illegal discharges. Additionally, some public and private point source dischargers in the Coastal Plain contribute high levels of nitrogen and phosphorus in wastewater effluents to blackwater systems. We conducted a series of nutrient bioassay experiments designed to assess the effect of nutrient inputs on the photosynthetic and heterotrophic plankton communities in two blackwater systems, the Black and the Northeast Cape Fear Rivers.

We used cubitainer bioassays to investigate the effect of ammonia, orthophosphate, N+P, urea, and glycerophosphate treatments at 1.0 mg/L as N or P. This level was chosen as a conservative measure of nutrient inputs to streams from swine effluent spray fields and other documented sources such as wastewater treatment plants. Phytoplankton response was measured by chlorophyll *a* production and general microbial biomass accumulation was measured by ATP analysis. Responses were averaged over three collections during a six day period. In addition, numerous physical, chemical and biological parameters were measured at our sampling stations on a monthly basis.

The two blackwater rivers were characterized by low turbidity and low dissolved oxygen during the summer months. BOD_5 concentrations were typically low, however - about 1.0 mg/L. Nitrogen and phosphorus concentrations were dominated by the organic forms. The low turbidity and dominance of organic over inorganic nutrients demonstrates the important role of the extensive streamside wetlands as filters and transformers of pollutants in these coastal plain ecosystems. Chlorophyll *a* concentrations were low at all times, possibly as a result of light limitation in the deep, well mixed river channels.

Chlorophyll *a* showed no significant response to nutrient inputs in the bioassay experiments from November through March. From late spring through summer, however, chlorophyll readily responded to inputs of ammonia, urea, and especially the combined N+P treatment. The organic nitrogen treatment, urea, often yielded chlorophyll production comparable to that of the inorganic treatment, ammonia, and usually the same phytoplankton taxa (mainly green algae, cryptomonads and diatoms) were stimulated in both treatments. Glycerophosphate did not stimulate chlorophyll production at any time, and orthophosphate inputs significantly stimulated chlorophyll production on only one occasion.

Orthophosphate, and especially glycerophosphate, inputs caused significant ATP increases during all seasons. The magnitude of the responses was much higher during summer. These significant ATP responses to phosphorus often occurred at the same time that significant photosynthetic responses to nitrogen inputs occurred. Thus, it is evident that two components of the planktonic microbial community are limited by two different nutrients during the same period. The N+P combination treatment caused significant stimulation of both components, and usually to a degree exceeding that of any individual treatment.

Comparing watersheds, the phytoplankton community in the Northeast Cape Fear River was more often limited by nitrogen than was the Black River. Also, the non-photosynthetic community of the Black River was more often limited by phosphorus than that of the Northeast Cape Fear River.

Coastal Plain blackwater streams often are under low dissolved oxygen stress during summer because of swamp water inputs. Thus, any additional inputs of BOD further stresses these water bodies. Our data demonstrate that both organic and inorganic phosphorus inputs cause significant increases of heterotrophic microbial biomass. This material is a source of BOD to streams and rivers. Phytoplankton produce oxygen while alive, but upon death and senescence also become labile sources of BOD. Therefore it is likely that nutrient loading to coastal plain blackwater streams will lead to BOD increases and lowering of stream dissolved oxygen concentrations.

Algal blooms in blackwater are likely to occur only in shallow streams. Higher order rivers such as the Black and Northeast Cape Fear are deep and well mixed, and phytoplankton production is low under any nutrient conditions because algal cells spend most of their time entrained below the narrow photic zone of these highly colored waters. Streams containing algal blooms will enter the rivers, and the phytoplankton will likely rapidly senesce in the poor light conditions. As a result, algal blooms in shallow streams are likely to become additional sources of BOD in larger rivers.

Our results indicate that the importance of nutrient loading to blackwater systems should not be ignored. One of the principal water quality problems in these systems is low dissolved oxygen. It is apparent that nutrient loading, through the stimulation of heterotrophic and photosynthetic plankton growth, can contribute to BOD stress in blackwater systems. It is also clear that both nitrogen and phosphorus are contributors to this problem and together have a synergistic effect that can be more problematic than either by itself.

RECOMMENDATIONS

Nutrient discharge limits in North Carolina have previously only been used for water bodies that are highly susceptible to algal bloom formation (called nutrient-sensitive waters). It is clear from our experiments that nutrient inputs to blackwater streams can lead to algal blooms under appropriate conditions. What is possibly even more important is that nutrient loading can cause significant increases in heterotrophic microbiota in all seasons, but especially during summer. Heterotrophic microbiota, as well as senescing phytoplankton, can become highly labile sources of BOD. Therefore we recommend that steps be taken to determine appropriate nitrogen and phosphorus discharge limits for public and private dischargers, as well as agricultural and livestock operations in coastal blackwater drainage basins. It is evident that both inorganic and organic forms are important. We thus suggest that ambient nutrient concentration standards be set for coastal blackwater systems in an effort to help alleviate the low dissolved oxygen problems that occur in many of these systems.

INTRODUCTION

The Coastal Plain region of North Carolina is characterized by extensive blackwater stream and river systems. Historically, land use in these Coastal Plain watersheds has been characterized by agriculture and relatively diffuse human population, with generally low impacts to water quality. In recent years, however, there has been a major shift in land use in some coastal watersheds toward concentrated animal operations, especially swine production.

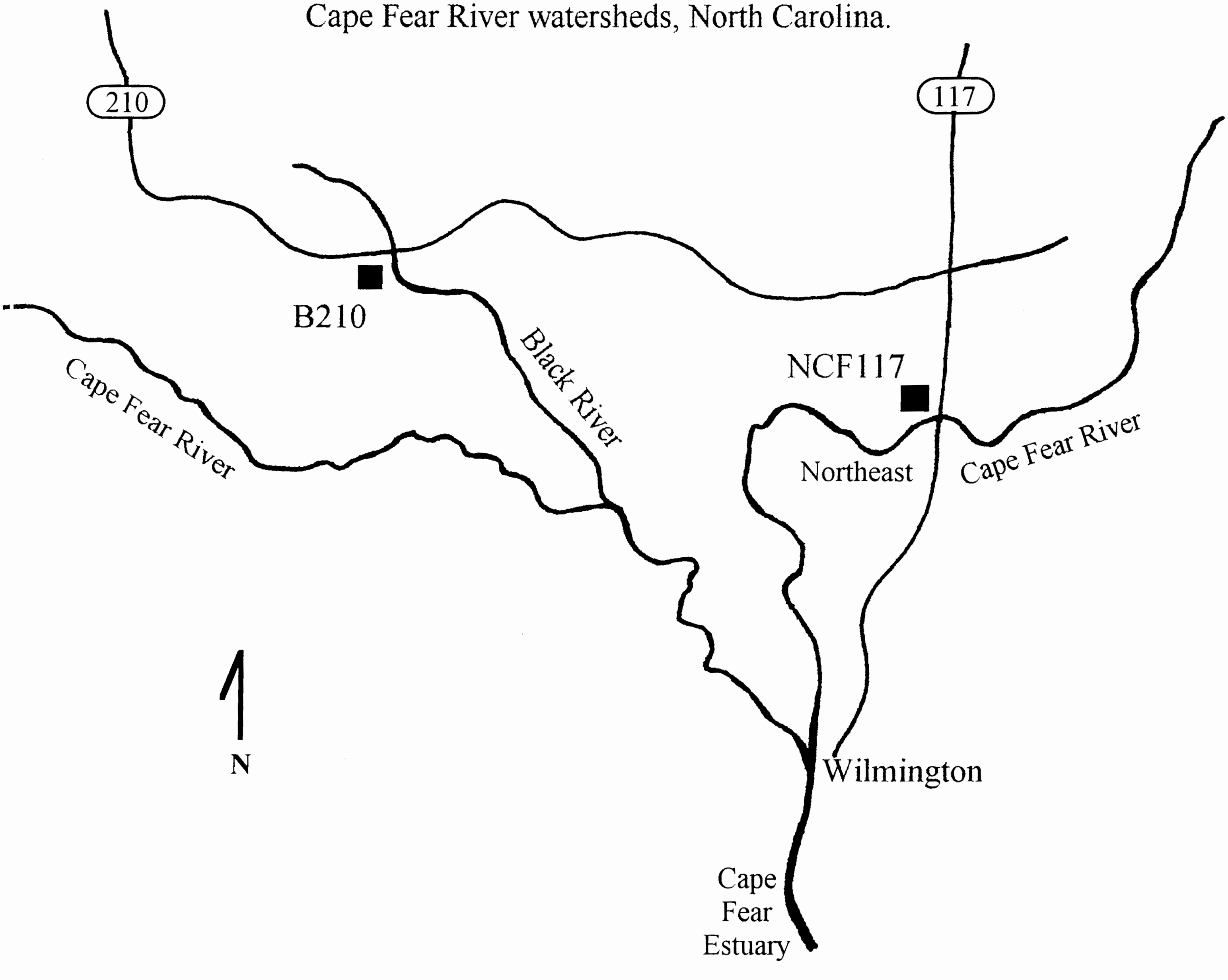
North Carolina's swine population expanded from 2.7 million in 1990 to over 9.8 million in 1997 (North Carolina Department of Agriculture 1997). The poultry industry also expanded rapidly in the period between 1985 and 1990. This extremely rapid growth occurred with little foresight as to potential environmental problems. Unlike human waste, swine waste does not normally receive secondary treatment. Swine waste is pumped into lagoons, where it is stored and later periodically sprayed onto nearby fields (Evans et al. 1984; Westerman et al. 1985a). Animal waste lagoons contain large quantities of both organic and inorganic nitrogen and phosphorus, with inorganic nutrients primarily in the form of ammonia and orthophosphate (Westerman et al. 1985b; Westerman et al. 1990; Mallin et al. 1997a). Neighboring water bodies have been subject to large acute nutrient loads from these lagoons. During 1995 at least six major swine and poultry waste lagoon ruptures occurred in eastern North Carolina, ranging from 750,000 to 22,000,000 gallons of waste released into area water bodies (North Carolina Division of Water Quality records). Three of the animal waste spill events were extensively sampled during summer 1995. These events caused inorganic nitrogen and phosphorus in receiving waters to exceed 46 mg/L and 11.5 mg/L, respectively (Burkholder et al. 1997; Mallin et al. 1997a).

Release of inorganic nutrients can cause stimulation of primary producers, often in the form of algal blooms. At least two of the sampled spill events caused massive algal blooms in receiving waters. In the New River, Onslow County, a 22 million gallon spill near Richlands caused chlorophyll *a* levels in the blackwater river 32 km downstream near Jacksonville to reach 50-75 μg/L, and when the nutrient plume reached the head of the estuary (Wilson Bay), chlorophyll *a* levels rose as high as 330 μg/L (Burkholder et al. 1997). Fish kills occurred in both the freshwater river and upper estuary during this event. A two million gallon hog waste lagoon spill in Brunswick County in August 1995 also produced algal blooms exceeding 100 μg/L chlorophyll *a* in a freshwater tidal creek complex. What is particularly notable is that those algal blooms occurred in blackwater creeks, an environment not normally considered conducive to excess phytoplankton growth (Herlong and Mallin 1985).

In addition to acute spill incidents, nutrients can enter receiving waters chronically through subsurface movement (Evans et al. 1984; Huffman and Westerman 1995), and overland runoff from sprayed fields during rain events (Westerman et al. 1985a). In a North Carolina study of hog lagoon waste sprayed on Bermudagrass fields, average nitrate concentrations in subsurface flow ranged from 7 to 30 mg/L (Evans et al. 1984). In another study, average nitrate concentrations in surface runoff from Bermudagrass fields ranged from 2.8 - 6.4 mg/L, depending on application rate (Westerman et al. 1985a). In a third study (Stone et al. 1995) a stream draining a subwatershed containing swine production facilities contained total inorganic nitrogen levels of 6.0-8.0 mg/L, and orthophosphate levels of 0.7-1.3 mg/L. Inorganic nutrient load to a nearby mainstem stream increased 3X for nitrogen and 6X for phosphorus due to inputs from the stream draining the swine facility (Stone et al. 1995). Gilliam et al. (1996) found an average of 7.7 mg/L nitrate-N in a stream draining a swine waste spray field. In addition to agricultural concerns it is clear that industrial and municipal point sources can also discharge excessive organic and inorganic nutrients to blackwater streams. For example, in a stream below a textile mill we have documented average total nitrogen concentrations of 5.6 mg/L (range 2.2-14.9 mg/L), and total phosphorus averaging 1.1 mg/L with a range of 0.5-2.3 mg/L (Mallin et al. 1997c). In a stream below a municipal wastewater treatment plant we have recorded average total nitrogen of 3.5 mg/l (range 1.4-7.6 mg/L) and average total phosphorus of 0.6 mg/l with a range of 0.2-1.5 mg/l (Mallin et al. 1997c). Regardless of sources, excess nutrient loading may represent an important cause of degradation to blackwater stream systems. Our primary objective was to assess the response of the algal and microbial primary producer communities in representative receiving waters to inputs of organic and inorganic nutrients.

The highest concentrations of hog and poultry operations in North Carolina are in Sampson and Duplin Counties (Cahoon et al. 1998), most of which drain into the Cape Fear watershed, which includes the mainstem Cape Fear, Northeast Cape Fear, and Black Rivers (Fig. 1). These two counties import large quantities of nitrogen and phosphorus in animal feed (Cahoon et al. 1998) and produce high loads of excess available plant nutrients (Barker and Zublena 1995). Thus, besides the documented effects of acute lagoon spills, there exists a potential for surface water quality effects from chronic nutrient buildup and loading to receiving waters. One important system is the Black River, a 5th order stream that has been designated by the State of North

Figure 1. Location of sampling sites in the Black and Northeast Cape Fear River watersheds, North Carolina.



Carolina as Outstanding Resource Waters. This river represents the primary drainage system for Sampson County. Another receiving watercourse is the Northeast Cape Fear River (NECFR) a 5th order stream draining Duplin and Pender Counties. Thus, these two watercourses were appropriate locations for research concerning the potential effect of nutrient loading on blackwater streams.

The Black and Northeast Cape Fear Rivers are coastal plain blackwater streams and such systems operate somewhat differently than clearwater systems (Meyer 1990). Under normal circumstances phytoplankton production is generally low due to physical factors such as light limitation (Mallin 1984; Herlong and Mallin 1985; Meyer 1992; Carlough 1994). However, we have demonstrated that unusual nutrient loading events caused dense algal blooms in blackwater receiving streams during two of the three waste lagoon breaches analyzed during the summer of 1995 (Burkholder et al. 1997; Mallin et al. 1997a). Thus, our first major objective was to investigate the potential for algal bloom formation under controlled conditions in these watersheds. A bioassay approach was used to test the growth response of natural phytoplankton communities to inputs of ammonia, orthophosphate, and organic N and P at concentrations typical of spills and runoff. Phytoplankton response to nutrient additions was measured by chlorophyll *a* production. Similar methods have been used successfully in North Carolina coastal waters such as the Neuse, Cape Fear, and New River Estuaries (Rudek et al. 1991; Paerl et al. 1995; Mallin et al. 1997b).

Blackwater rivers are naturally high in bacterial abundance and associated protozoa (heterotrophic activity), due to inputs of dissolved organic material from surrounding wetlands (Meyer 1990; Carlough 1994). Animal waste lagoons contain high concentrations of organic compounds (Westerman et al. 1990; James Barker, North Carolina State University, unpublished data), which can enter streams during spills (Mallin et al. 1997a). Bacteria can utilize both inorganic and organic substrates for growth (Parker et al. 1975; Ford 1993; Bjorkman and Karl 1994) and grazers such as protozoans and dinoflagellates can respond and reproduce rapidly to this increase in prey biomass (Pace 1982; Beaver and Crisman 1989; Sanders 1991). Increases in the biomass of the heterotrophic community degrade stream water by increasing the BOD load (Parker et al. 1975; Clark et al. 1977; Wetzel 1983), particularly in Southeast blackwater systems (Meyer 1990; Meyer 1992). Therefore, our second major objective was to assess the impact of inorganic and organic nutrient additions on the non-photosynthetic community (bacteria, heterotrophic algae, rotifers, and protozoa). To accomplish this, our bioassay experiments were enhanced by the addition of the sensitive ATP analysis, which provides an estimate of production of both heterotrophic biota biomass as well as photoautotrophs. ATP makes an excellent measure of microbial biomass because it is ubiquitous in all living organisms; the ratio of ATP to cellular organic carbon is fairly uniform across bacteria, phytoplankton, and zooplankton; ATP levels are similar in light and dark, and adsorption of ATP by detrital material is insignificant (Vollenweider 1974; Pridmore et al. 1984). Since seasonal runoff patterns can strongly influence nutrient loading and productivity patterns (Mallin et al. 1993; Mallin et al. 1997b) bioassays were conducted in all seasons.

UTILITY OF THE EXPERIMENTAL BIOASSAYS

An essential factor in watershed management planning is to assess the nutrient carrying capacity of the system. Whether nutrient inputs are acute or chronic, from point sources or non-point sources, these inputs to blackwater systems will have ecological ramifications. This research was designed to determine which nutrients most stimulate photoautotrophic and heterotrophic producers, which forms (organic or inorganic) are most important, and how seasons and background parameters (physico-chemical and biological) affect biotic responses.

Freshwater systems are generally believed to be phosphorus-limited (Hecky and Kilham 1988). However, research demonstrating this has occurred mainly in clearwater lakes and reservoirs. Study of the ecology of the abundant blackwater lotic systems in the southeast U.S. is a relatively new field (Meyer 1990), and studies of nutrient limitation are lacking. Because of the high heterotrophic capacity of blackwater systems, the growth stimulation of bacteria and protozoans (and BOD increase potential) from organic and inorganic nutrient loading needs attention, particularly with these Coastal Plain systems currently at risk from dissolved oxygen problems.

OBJECTIVES

1) Experimentally determine the impact of addition of nutrients common in swine and poultry waste effluent on the growth of phytoplankton in water from the Northeast Cape Fear River and the Black River. Use bioassay data and ambient nutrient concentrations to determine if impacts remain constant or change on a seasonal basis.

2) Utilize an experimental ATP-production bioassay approach to investigate the potential for responses of the heterotrophic microbial community to organic and inorganic nutrient loading in these blackwater systems.

3) Assay the sample site water monthly for nutrient concentrations (total nitrogen, nitrate, ammonia, dissolved organic N, total phosphorus, phosphate, dissolved organic phosphorus), biochemical oxygen demand, fecal coliform bacterial abundance, and phytoplankton biomass (as chlorophyll *a*).

4) Coincident with sample collection, collect pertinent water quality data while on station, including water temperature, dissolved oxygen, conductivity, turbidity, and solar irradiance for light attenuation data.

MATERIALS AND METHODS

SAMPLING STATIONS

Water for the experiments was collected at 2 stations in the targeted region. One was in the Black River near Highway 210 near the Pender-Sampson County line, the other was in the Northeast Cape Fear River near Castle Hayne at Highway 117 (Fig. 1). These stations are located in watersheds containing heavy swine and poultry lagoon concentrations, and at least eight documented large lagoon breaches occurred in these watersheds during the summers of 1995 and 1996 (Mallin et al. 1997a; Mallin et al. In press; North Carolina Department of Environment, Health and Natural Resources records).

WATER QUALITY

Water temperature, dissolved oxygen, conductivity and turbidity data were collected monthly on site using YSI Model 55 or 85 dissolved oxygen meters and a LaMotte turbidity meter. Vertical solar irradiance data was collected at NCF117 using a Li-Cor LI-1000 integrator interfaced with a LiCor LI-193S spherical quantum sensor, and the light attenuation coefficient (k) was determined from these data following the procedure in Mallin and Paerl (1992).

NUTRIENT ADDITION EXPERIMENTS

We tested the hypothesis that both organic and inorganic nutrients will stimulate algal growth in the Northeast Cape Fear and Black River water by the use of nutrient addition bioassays. Similarly, we tested the hypothesis that additions of organic nutrients will stimulate heterotrophic production in these blackwater systems. The basis of these experiments was to add the experimental nutrient(s) to replicated river water samples and determine if the phytoplankton and heterotrophic communities in the samples show a positive response (i.e. a chlorophyll or ATP production increase). A replicated set of control samples was incubated to serve as a baseline. The specific design was as follows: Water was collected on station in 25-L carboys, returned to the CMSR, and dispensed into 1 gallon cubitainers (3 L per cubitainer). Nutrient treatments were added as follows (expressed as final concentration): no additions (controls), phosphate alone (1.0 mg/L or 32.2 μM as P), ammonia alone (1.0 mg/l or

71.4 μM as N), combination N+P (1.0 mg/L ammonia and 1.0 mg/L phosphate), an organic nitrogen treatment (the common waste product urea, featuring a low C:N ratio) and an organic phosphorus compound (glycerophosphate). Addition concentrations of these were in molar equivalent amounts to the other N and P treatments. The nutrient addition concentrations chosen for our experiments were conservative, based on measured field data from spills (Burkholder et al. 1997; Mallin et al. 1997a) and swine farm runoff and infiltration studies (Evans et al. 1984; Westerman et al. 1985a; Huffman and Westerman 1995; Stone et al. 1995; Gilliam et al. 1996). All treatments were conducted in triplicate. Nutrient bioassays using 1.0 mg/L concentrations were run in July, August, October and November of 1996, and January, March, April, May and June of 1997. Two additional bioassays using high nutrient concentrations (10 mg/L) were conducted in February and July of 1997.

Cubitainers were floated on flow-through ponds near the CMSR at ambient river temperatures. The cubitainers were covered by 2 layers of neutral density screening to allow solar irradiance penetration of about 30% of that reaching the water surface to prevent photostress to the phytoplankton (Mallin and Paerl 1992). The cubitainers were kept in motion by constant circular agitation of the pond water using a submerged bilge pump (Mallin et al. 1997b). The cubitainers were incubated for six days and sampled on Days 1, 3 and 6 for chlorophyll *a* content (measured by the fluorometry method), and ATP production, using the luciferin-luciferase photometric technique (Holm-Hansen and Booth 1966; Vollenweider 1974). A LumiTec Photometer was used for the ATP analysis (Cahoon et al. 1990). A standard BOD_5 measurement (APHA 1995) was made on the contents of the July 1997 bioassay upon termination of the experiment.

STATISTICAL ANALYSIS

Statistical analyses of nutrient limitation test results were performed using the Statistical Analysis System (SAS) procedure of Analysis of Variance (ANOVA). This test utilizes the means and standard deviations of the response data (chlorophyll and ATP concentrations) and determines if there exists a significant difference ($p < 0.05$) among the response means of the various nutrient treatments. If a difference in response means exists among the treatments, the ANOVA test is followed by treatment ranking by Fisher's Least Significant Difference procedure. This statistical test compares each treatment response mean with the others, shows which of the different treatments (nutrient additions) elicited the greatest chlorophyll or ATP response, and ranks the treatment responses in descending order. This provides a sound statistical basis for reporting which nutrient(s) are most limiting to phytoplankton growth in the system being tested (i.e., which nutrient additions evoked the greatest phytoplankton response and in which months this occurred). This statistical treatment is recommended in Day and Quinn (1989), and examples of its successful use in similar studies in North Carolina are provided in Paerl et al. (1990) and Rudek et al. (1991).

NUTRIENTS

The water used for the bioassays was assessed for a suite of nutrient parameters including total Kjeldahl nitrogen (TKN), nitrate, ammonium, total phosphorus (TP), orthophosphate and reactive silicate (Si). TKN, TP, nitrate, and ammonia were measured using Standard Methods (APHA 1995). Total nitrogen (TN) was computed as TKN + nitrate. Orthophosphate was measured on a Technicon AutoAnalyzer at the UNCW Center for Marine Science Research using standard techniques (Parsons et al. 1984). Reactive Si was measured using the method described in Parsons et al. (1984). To obtain background information on the microbial community both fecal coliform analysis (membrane filtration method) and BOD were analyzed (APHA 1995).

CHLOROPHYLL AND PHYTOPLANKTON

Triplicate water samples were collected on site in amber plastic containers, placed on ice, and returned to the laboratory for chlorophyll *a* analysis. Subsamples (50 mL) were filtered through previously combusted glass fiber filters (Gelman A/E, nominal pore size = 1 μm), the filters were wrapped in foil, and frozen. After at least 24 hr the filters were unwrapped, placed in 15 mL centrifuge tubes containing 10 mL of 90% acetone, and extracted for 24 hr. A Turner Model 10-AU fluorometer was used for chlorophyll *a* analysis of the extract. This method utilizes very narrow excitation and emission bandwidths to provide a fluorescent measurement of chlorophyll *a* that minimizes interferences from chlorophyll *b* and phaeopigments (Welschmeyer 1994). Consequently this method reports chlorophyll *a* conservatively compared to previously used fluorometric methods. Upon completion of each bioassay samples of each treatment were collected and preserved with Lugol's solution. Treatments demonstrating phytoplankton bloom formation were examined for taxonomic composition with an Olympus BX50 research microscope.

RESULTS

PHYSICAL PARAMETERS

Water temperature over the year at the Black River station B210 averaged 18.5°C with a minimum of 7.5°C during December and a maximum of 29.2°C during July 1997(Table 1). Temperatures at the Northeast Cape Fear River station NCF117 averaged 19.1°C with a minimum of 9.5°C during December and a maximum of 28.5°C during July 1997.

Dissolved oxygen (DO) at B210 demonstrated distinct decreases in August and September 1996, following Hurricanes Bertha and Fran (Fig. 2). Concentrations rose to a maximum in February 1997, and decreased gradually through July of 1997. Dissolved oxygen at NCF117 followed a similar pattern but the post-hurricane decreases were more pronounced than those at B210, becoming nearly anoxic in September 1996 (Fig. 2).

Turbidity was generally low year-round at both stations (Table 1). There were increases to a maximum of 9.5 NTU in September following Hurricane Fran, but turbidity was always well below the state standard of 50 nephelometric units (NTU).

Light attenuation coefficient *k* values are only available for NCF117. The average *k* during the study was 3.72/m, ranging from 2.89 to 4.70/m (Table 1). The *k* values in general are high, indicating strong light-limiting conditions below the first 0.5 m of the water column. There was no particular seasonality demonstrated by these values, but *k* values in summer 1996 were especially elevated (relative to summer 1997) following inputs of swamp water in the aftermath of two hurricanes (Fig. 3). Since turbidity at NCF117 is generally low, the strong light attenuation can be attributed to inputs of highly colored swamp water to the main river channel.

Table 1. Mean ± standard deviation and (range) of parameter concentrations for Black River Station B210 and Northeast Cape Fear River Station NCF117, July 1996 - July 1997. NA = no data available.

Parameter	B210 mean (range)	NCF117 mean (range)
Temperature (°C)	18.5 ± 7.1(7.5-29.2)	19.1± 6.5 (9.5-28.5)
Dissolved oxygen (mg/L)	6.3 ± 2.3 (2.0-10.0)	5.8 ± 2.4 (0.4-9.5)
Turbidity (NTU)	4.0 ± 1.4 (1.5-7.0)	4.4 ± 1.9 (2.4-9.5)
Light attenuation	NA	3.72 ± 0.65 (2.89-4.70)
Nitrate (µg/L)	265 ± 143 (50-520)	273 ± 134 (60-480)
Ammonia (µg/L)	28 ± 24 (10-80)	44 ± 38 (10-140)
Total nitrogen (µg/L)	1317 ± 234 (1000-1750)	1352 ± 378 (940-2360)
Orthophosphate (µg/L)	31 ± 19 (10-84)	40 ± 23 (15-88)
Total phosphorus (µg/L)	98 ± 41 (50-200)	128 ± 86 (50-380)
Silica (µg/L)	1285 ± 685 (475-2716)	1360 ± 459 (784-2247)
Chlorophyll *a* (µg/L)	0.9 ± 0.6 (0.3-2.1)	1.6 ± 1.1 (0.4-4.2)
Fecal coliforms (cfu/100 mL)	33 ± 18 (4-67)	54 ± 51 (14-200)
BOD_5 (mg/L)	0.75 ± 0.32 (0.25-1.35)	1.47 ± 2.10 (0.25-8.05)
BOD_{20} (mg/L)	2.51 ± 0.99 (1.70-5.55)	3.07 ± 1.60 (1.65-8.05+)

Figure 2. Dissolved oxygen concentrations at Black River Station B210 and Northeast Cape Fear River Station NCF117, July 1996 - July 1997.

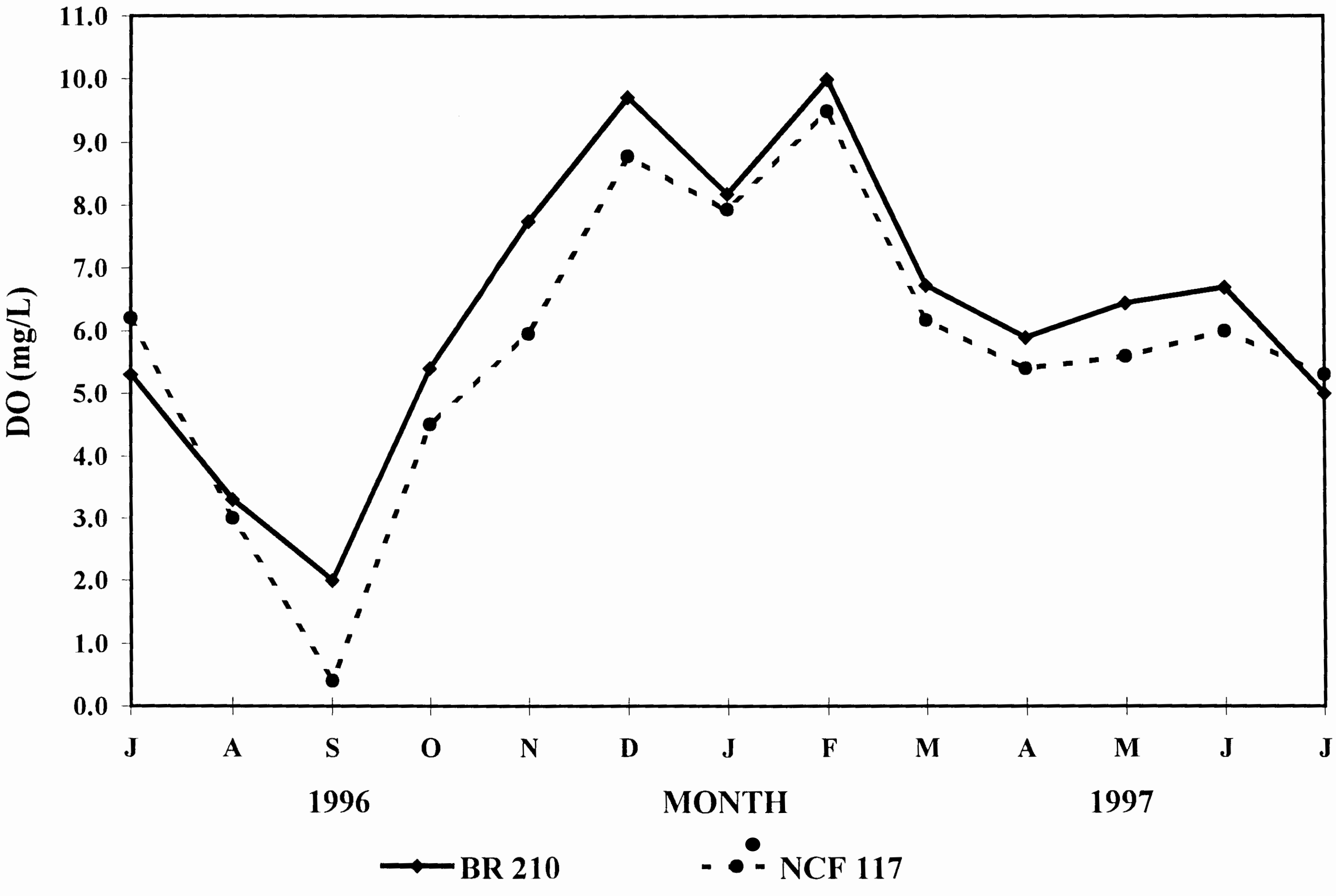
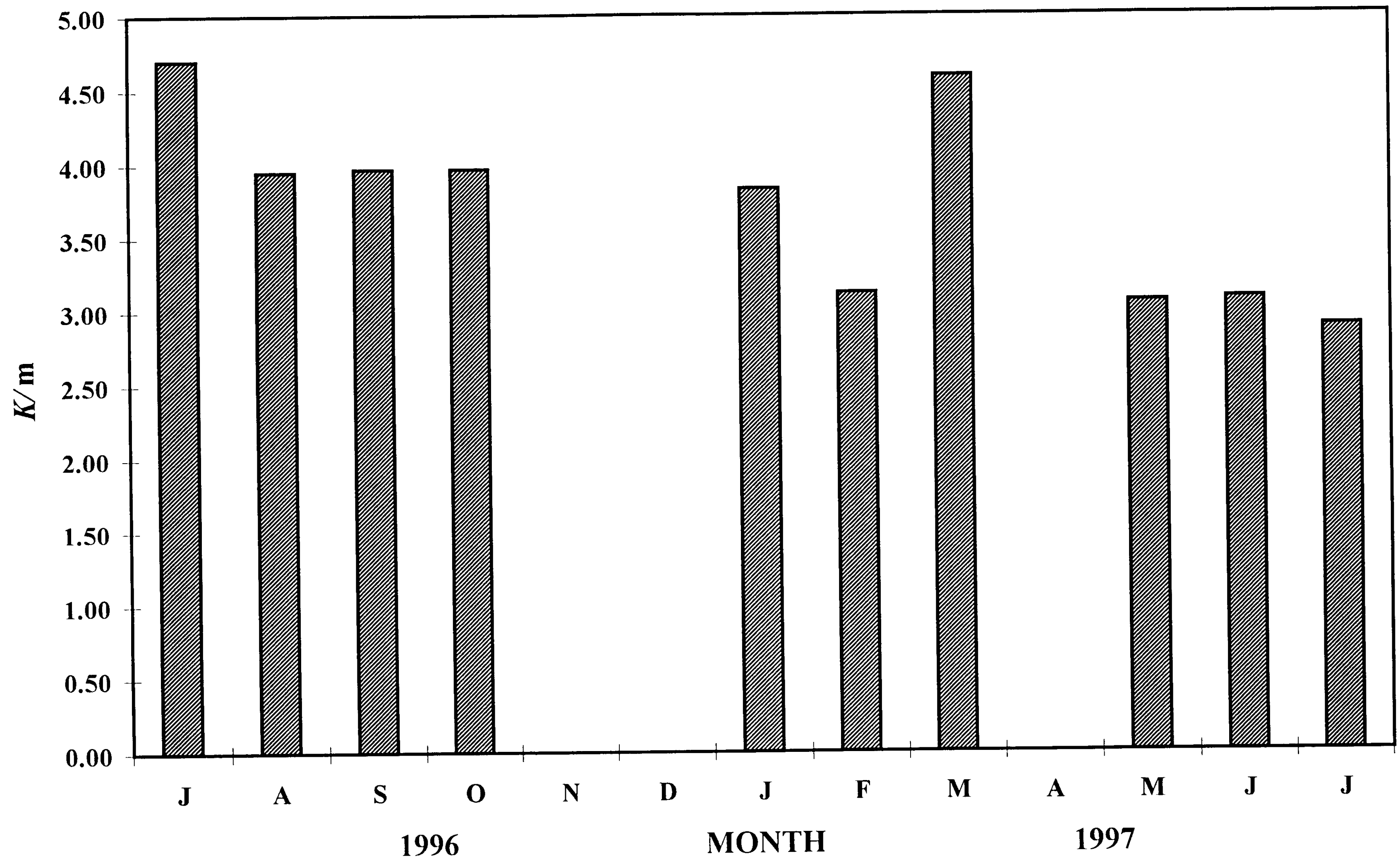


Figure 3. Light attenuation coefficient (k) at Northeast Cape Fear River Station NCF117, July 1996 - July 1997.



CHEMICAL PARAMETERS

Nitrate concentrations were very similar between the two rivers (Table 1; Fig. 4). Maximum concentrations ranged up to approximately 500 µg/L during January-February and May-June 1997 and reached minimal concentrations of about 50 µg/L in September following Hurricane Fran. In contrast, ammonia concentrations were highest in September, peaking at 80 µg/L at B210 and 140 µg/L at NCF117 (Fig. 5). Ammonia concentrations were low in both rivers from December 1996 through March 1997 (Fig. 5). Total nitrogen concentrations were similar between both rivers, and displayed little seasonality in general (Table 1; Fig. 6). The exception was September 1996, when NCF117 reached an unusually high peak of 2400 µg/L following Hurricane Fran (Fig. 6). In general organic nitrogen comprised the greatest proportion of TN, averaging 77% at B210 and 80% at NCF117.

Orthophosphate concentrations showed a seasonal pattern of highest values in summer and lowest in winter (Fig. 7). Maximum concentrations were between 85 and 90 µg/L for both rivers and occurred in September 1996 (Table 1; Fig. 7). Throughout most of the year total phosphorus concentrations were very similar between the two rivers and displayed little seasonality (Table 1; Fig. 8). A major peak occurred in September 1996, when maximal concentrations were reached at B210 (200 µg/L) and NCF117 (380 µg/L). Organic phosphorus averaged 68% of TP at B210 and 69% of TP at NCF117.

Reactive silicate concentrations displayed a seasonal pattern of lowest values in winter and highest in summer (Fig. 9). There was a distinct drop in September 1996, possibly a result of dilution from post-hurricane surface runoff and high river flow. Minimum Si concentrations were never below 475 µg/L on any sampling date during the study (Table 1; Fig. 9).

BIOLOGICAL PARAMETERS

Chlorophyll *a* concentrations were very low throughout the year for both rivers, and displayed little seasonality (Table 1; Fig. 10). The maximum concentration at NCF117 was 4.2 µg/L in September 1996 and at B210 was 2.1 µg/L in April 1997 (Fig. 10).

Fecal coliform concentrations displayed no particular seasonality, although concentrations at NCF117 were greater on average than at B210 (Table 1; Fig. 11). Fecal coliform levels at B210 remained below 80 colony-forming units (CFU)/100 mL

Figure 4. Nitrate concentrations at Black River Station B210 and Northeast Cape Fear River Station NCF117, July 1996 - July 1997.

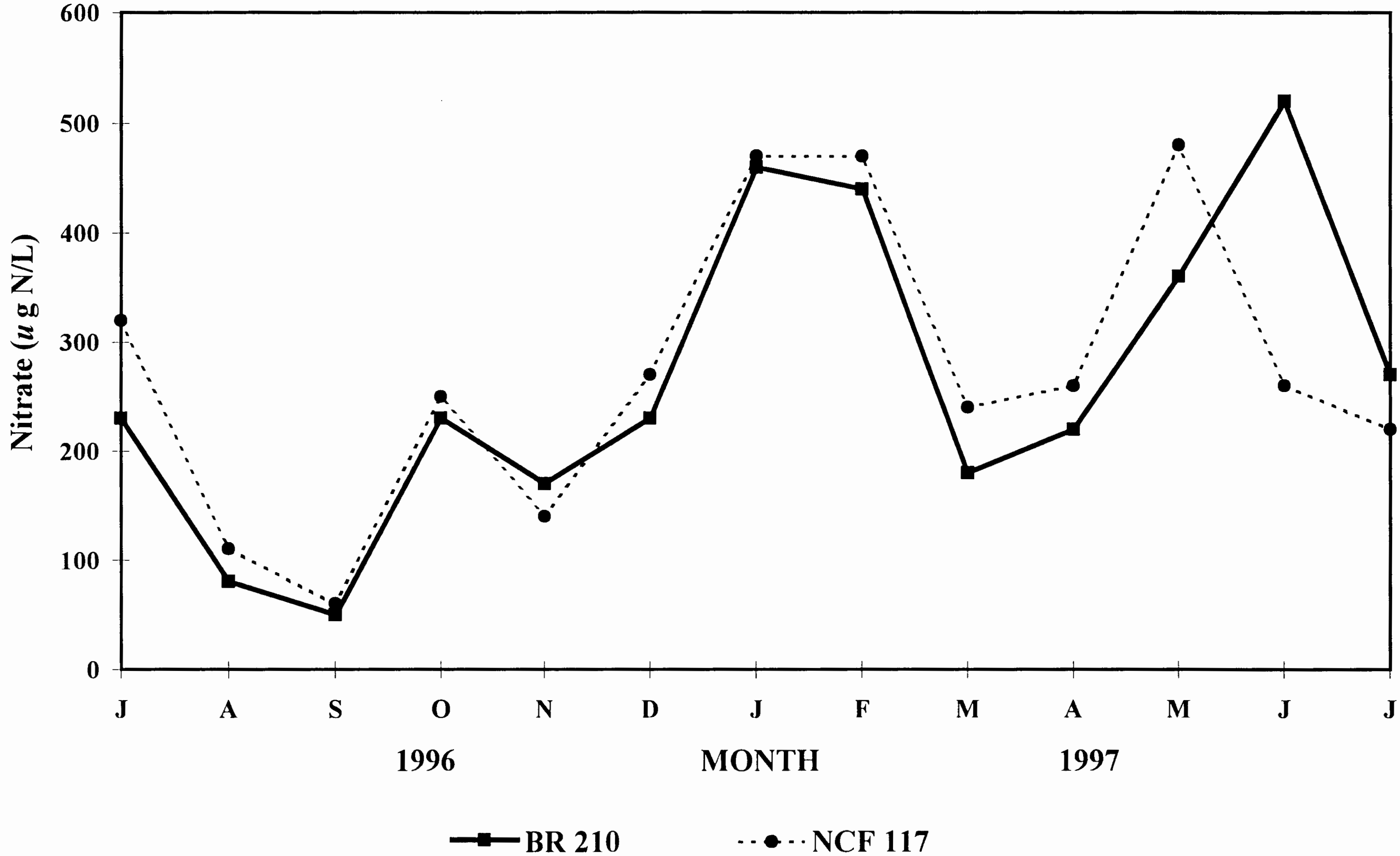


Figure 5. Ammonia concentrations at Black River Station B210 and Northeast Cape Fear River Station NCF117, July 1996 - July 1997.

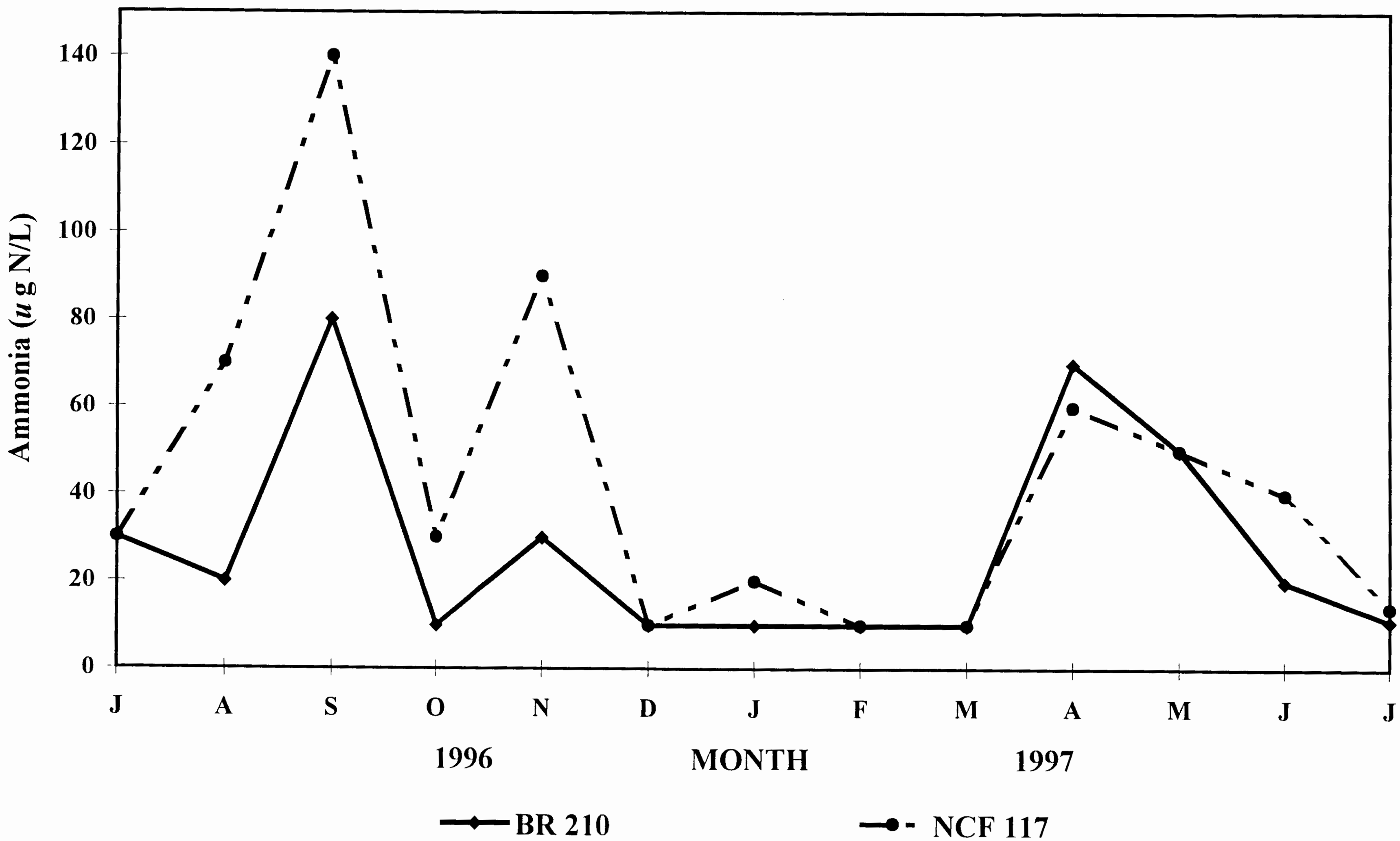


Figure 6. Total nitrogen concentrations at Black River Station B210 and Northeast Cape Fear River Station NCF117, July 1996 - July 1997.

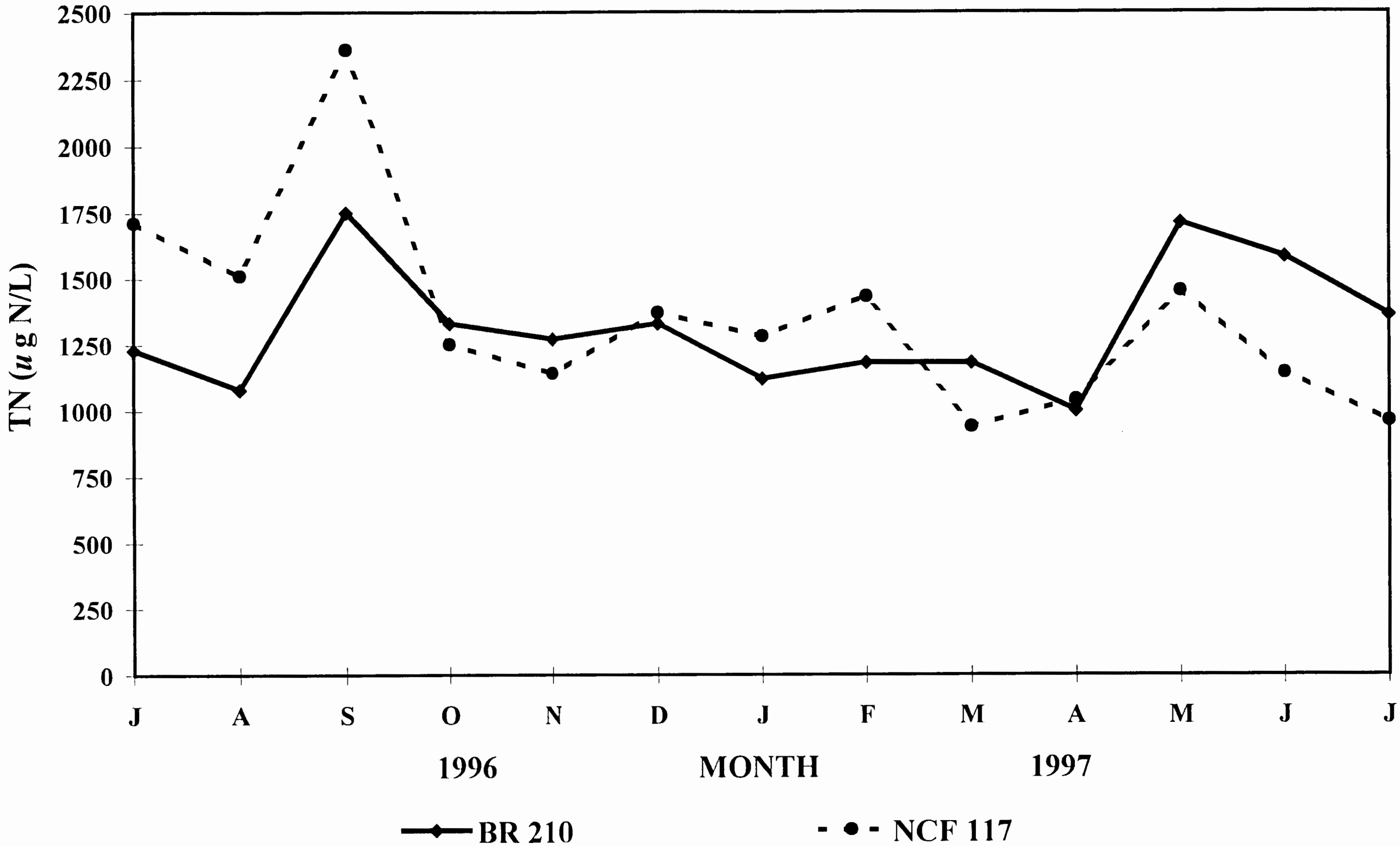


Figure 7. Orthophosphate concentrations at Black River Stations B210 and Northeast Cape Fear River Station NCF117, July 1996 - July 1997.

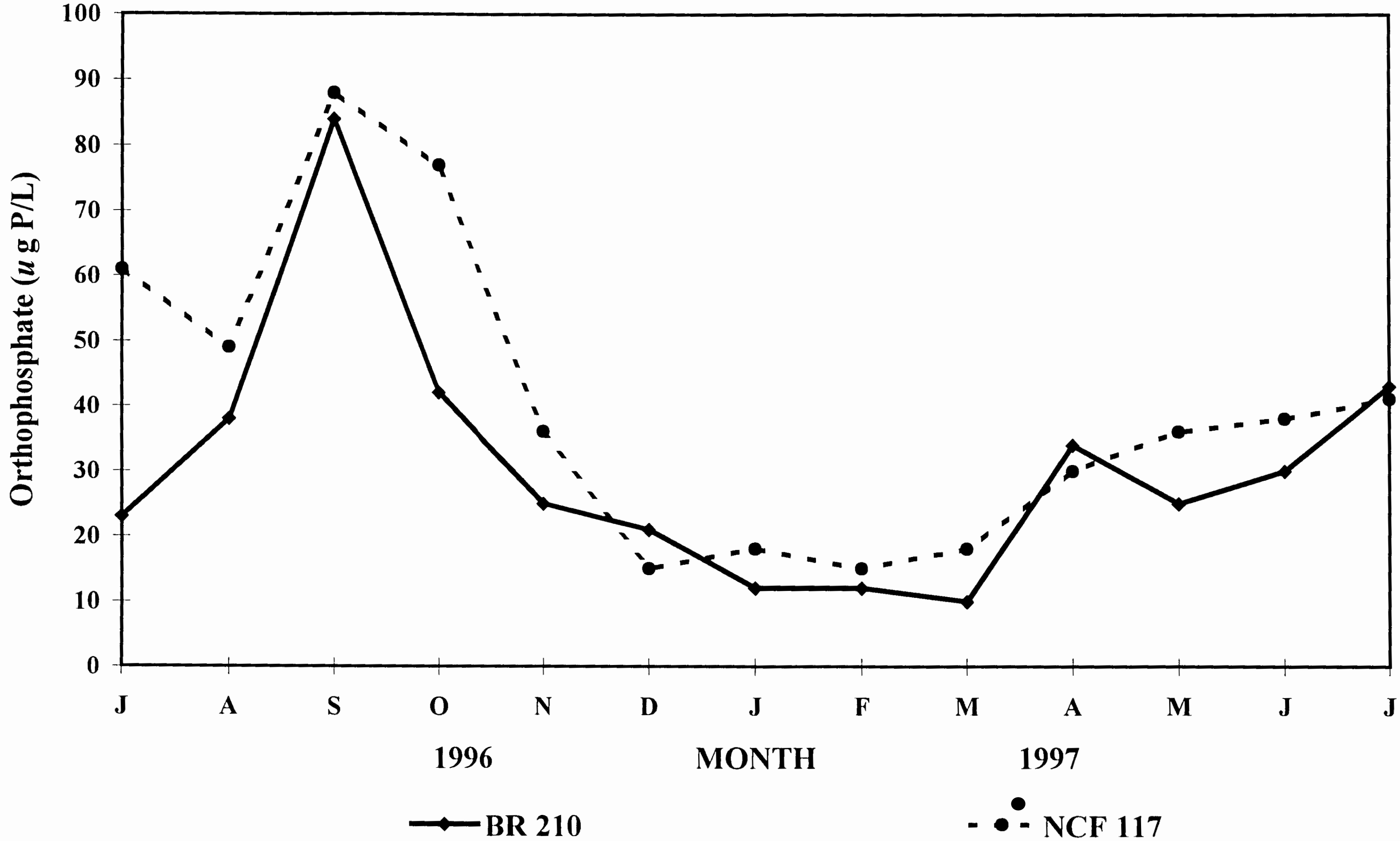


Figure 8. Total phosphorus concentrations at Black River Station B210 and Northeast Cape Fear River Station NCF117, July 1996 - July 1997.

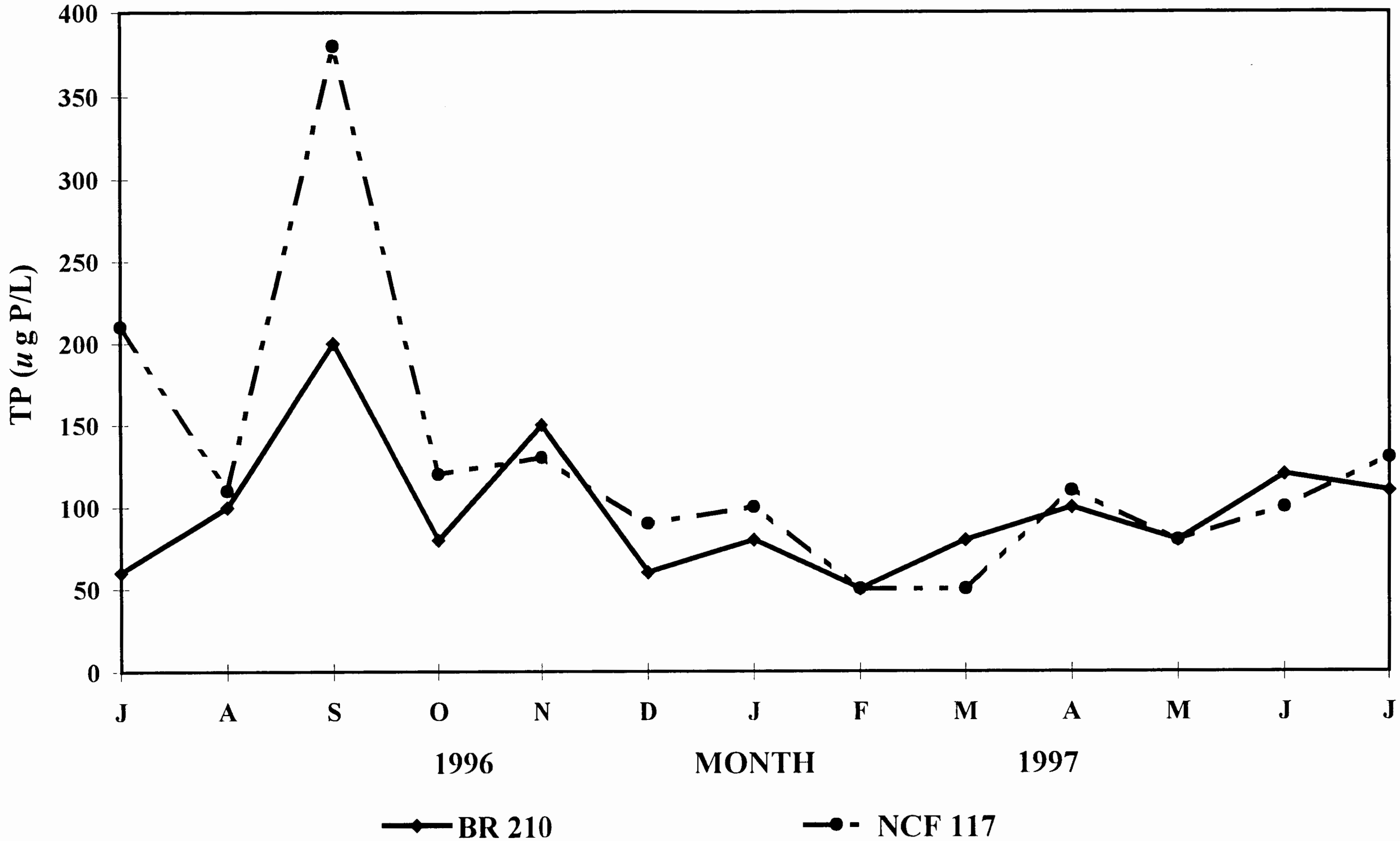


Figure 9. Reactive silicate concentrations at Black River Station B210 and Northeast Cape Fear River Station NCF117, July 1996 - July 1997.

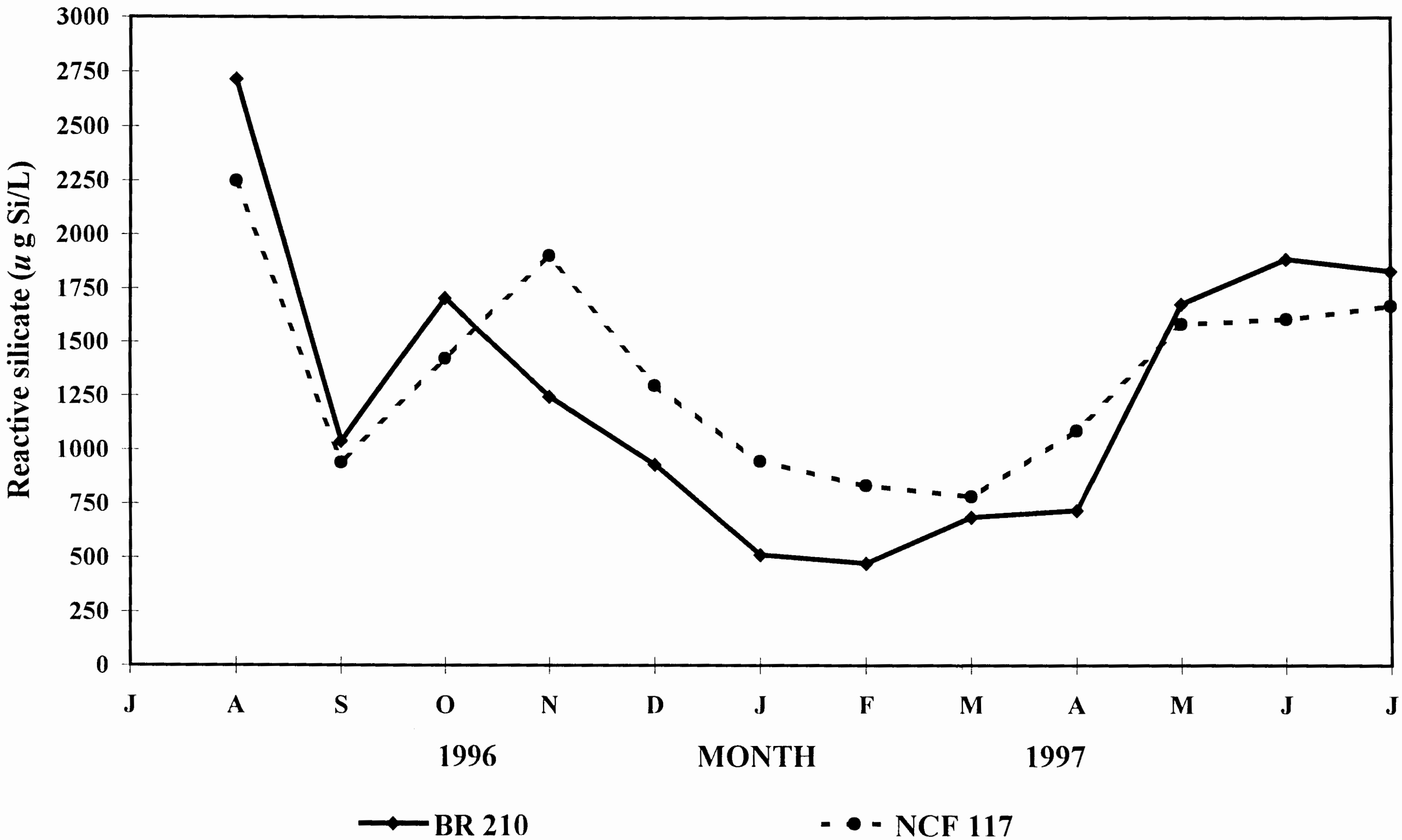


Figure 10. Chlorophyll *a* concentrations at Black River Station B210 and Northeast Cape Fear River Station NCF117, July 1996 - July 1997.

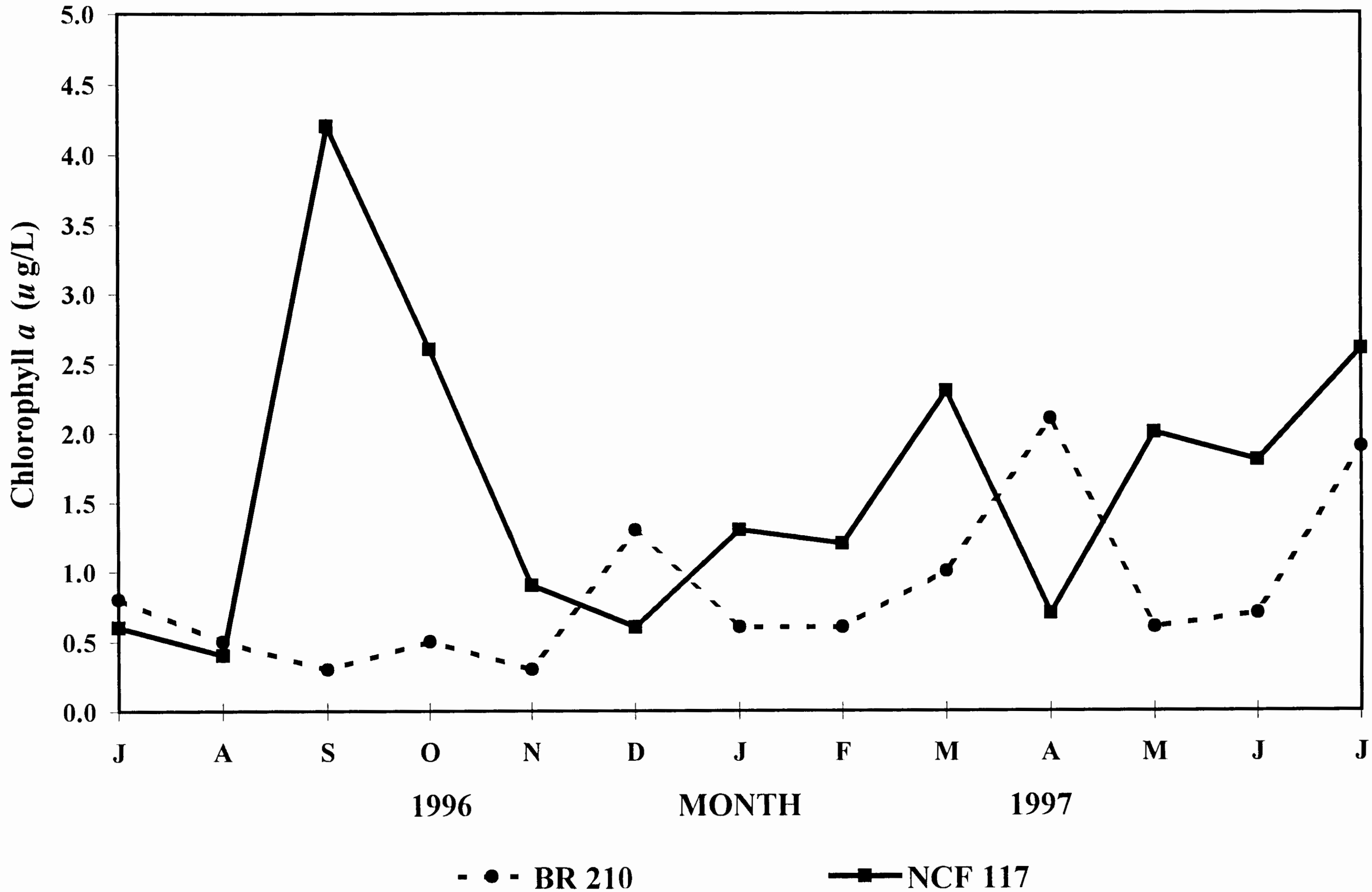
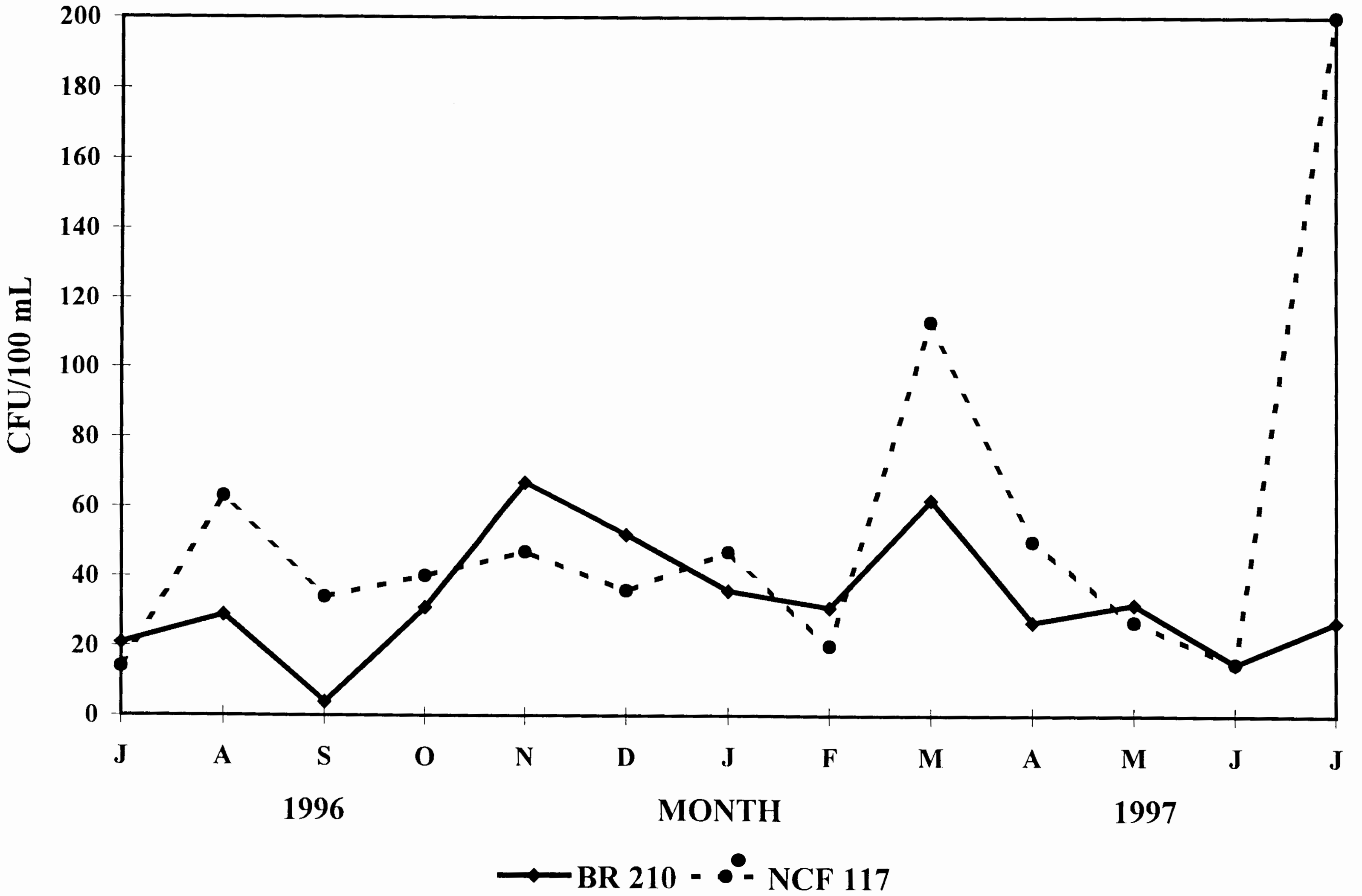


Figure 11. Fecal coliform concentrations at Black River Station B210 and Northeast Cape Fear River Station NCF117, July 1996 - July 1997.



year-round, while NCF117 reached peaks of 120 CFU/100 mL in March and 200 CFU/100 mL in July 1997 (Fig.11).

BOD_5 was generally low in these blackwater rivers, averaging 0.75 mg/L at B210 and 1.47 at NCF117 (Table 1). BOD_5 remained below 1.5 mg/L at all times and displayed little seasonality except for September 1996, when BOD_5 in the Northeast Cape Fear River rose to 8.05 mg/L (Fig. 12). There was no unusual BOD_5 peak in September at B210. A number of swine waste lagoons breached, overtopped or were flooded in the Northeast Cape Fear watershed following Hurricane Fran, contributing large amounts of labile BOD to this river (Mallin et al. In press). BOD_{20} ranged from 2.0 to 4.0 mg/L during most of the year for both rivers, with concentrations at NCF117 generally higher than at B210 (Table 1; Fig. 13). Peak concentration at B210 was 5.5 mg/L in September. September BOD_{20} at NCF117 would have likely been considerably greater than 8.05 mg/L but all DO in the BOD sample was exhausted by Day 5 of the incubation (Figs. 12; 13).

NUTRIENT ADDITION BIOASSAYS

Figures 14-24 represent average chlorophyll and ATP responses for Days 1, 3 and 6 of the bioassays. The July 1996 bioassay at B210 demonstrated strong inorganic N+P stimulation of both chlorophyll *a* and ATP (Fig. 14). Urea also caused significant chlorophyll *a* stimulation (Fig. 14). At NCF117 ammonia, N+P and urea all caused significant chlorophyll *a* stimulation, while ammonia and urea both caused significant ATP stimulation (Fig. 15). The August 1996 bioassay used river water which was augmented considerably by highly-colored swampwater inputs following Hurricane Bertha. There was very low chlorophyll *a* yield in B210 water, although there was statistically significant stimulation by N+P and statistically significant stimulation of ATP production by ammonia, N+P, and glycerophosphate (Fig. 14). There was also little chlorophyll *a* yield at NCF117 in August, although ammonia and orthophosphate additions both produced statistically significant increases (Fig. 15). No additions proved stimulatory to ATP yield at NCF117.

Because of high variability among replicates there was no statistically significant nutrient stimulation of chlorophyll *a* production at B210 in October, although both urea and glycerophosphate caused significant ATP production over control (Fig. 16). At NCF117 in October, N+P and urea both significantly stimulated chlorophyll *a* production, and ammonia and urea both stimulated ATP production (Fig. 17). In November no treatment stimulated chlorophyll *a* production in either river. There was

Figure 12. BOD_5 concentrations at Black River Station B210 and Northeast Cape Fear River Station NCF117, July 1996 - July 1997.

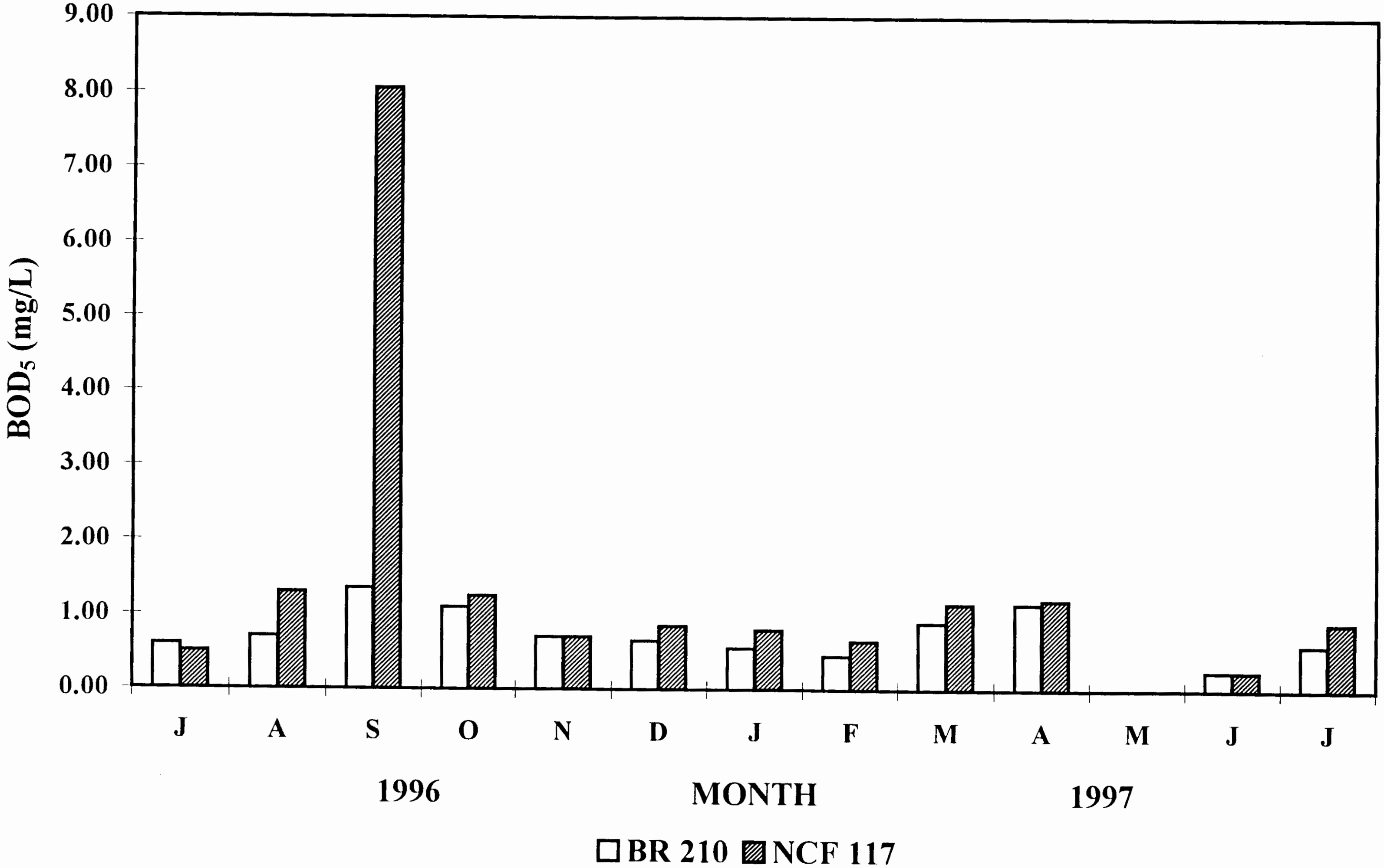


Figure 13. BOD_{20} concentrations at Black River Station B210 and Northeast Cape Fear River Station NCF117, July 1996 - July 1997.

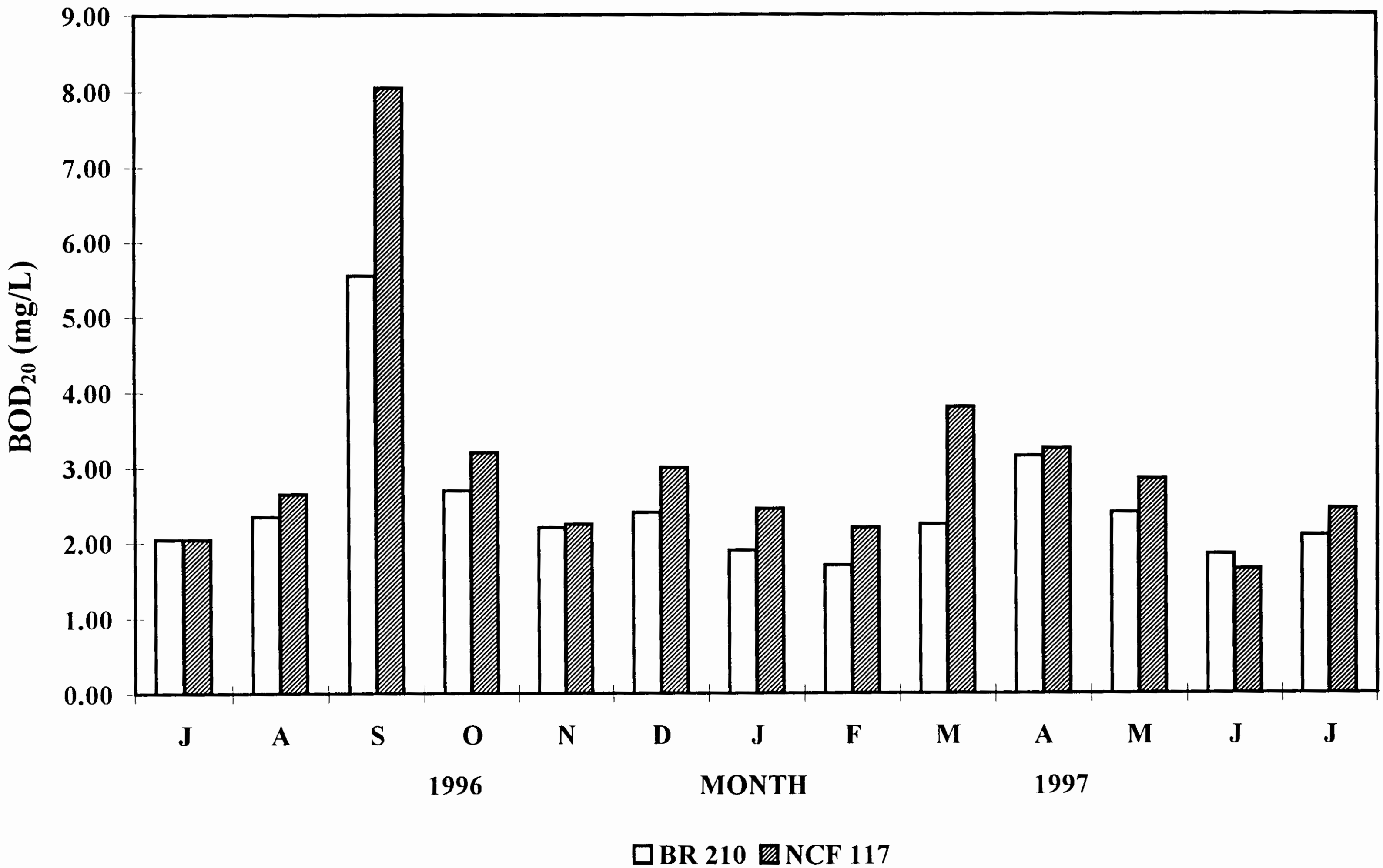


Figure 14. Response of Black River water to nutrient treatments, July (left) and August (right) 1996. Top panel - chlorophyll *a*, bottom - ATP response.
* denotes significantly greater than control

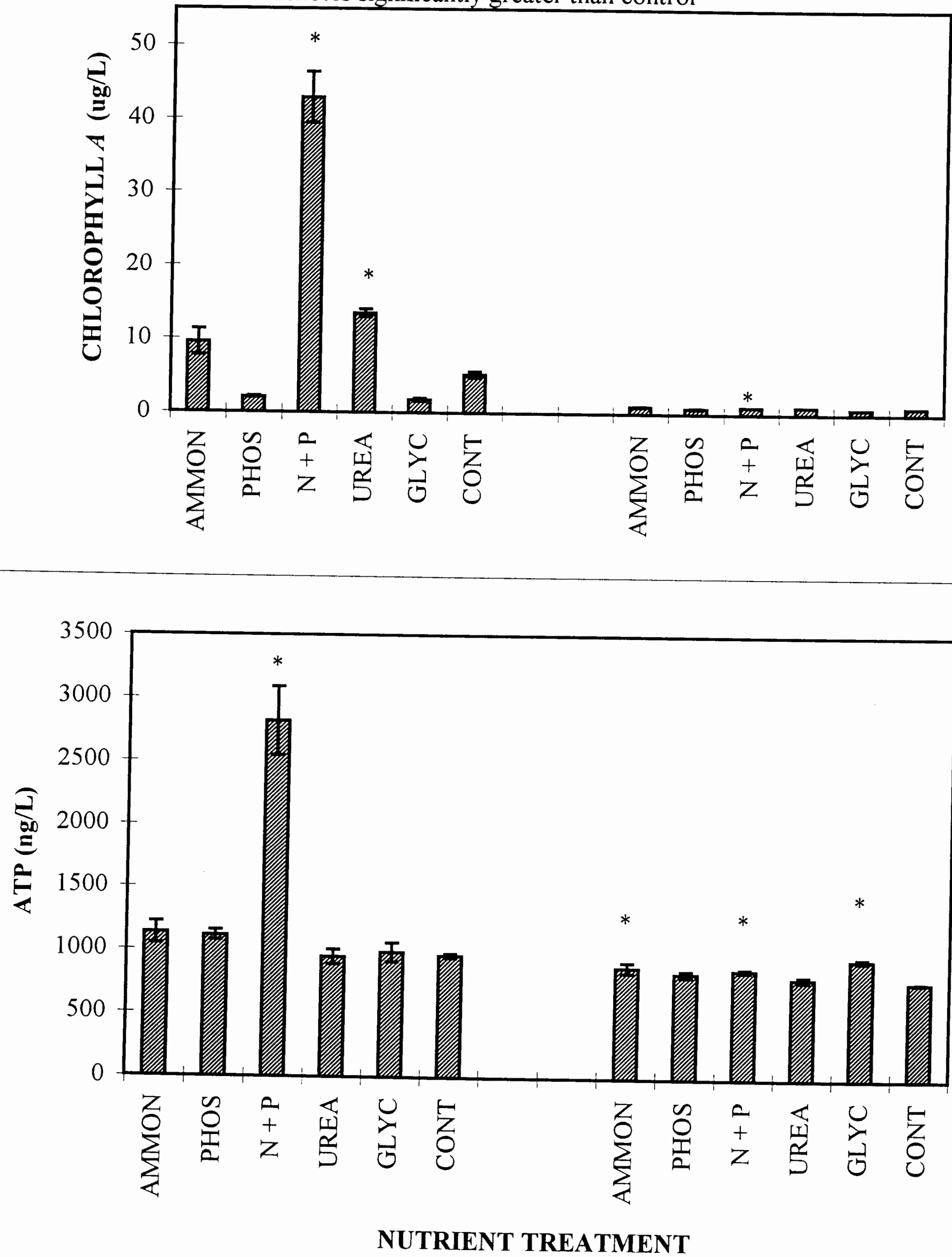


Figure 15. Response of Northeast Cape Fear River water to nutrients, July (left) and August (right) 1996. Top - chlorophyll *a*, bottom - ATP.
* denotes significantly greater than control

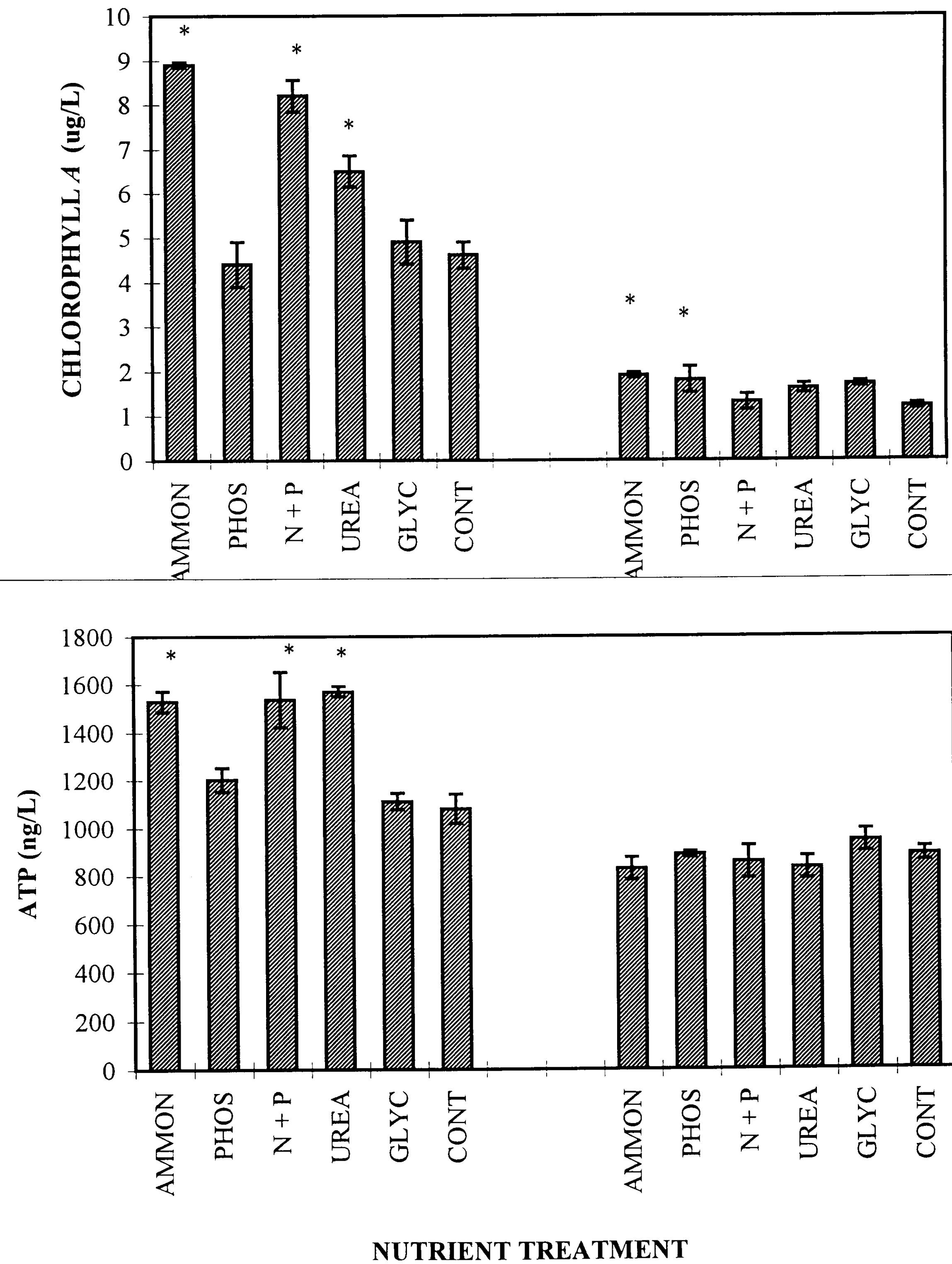


Figure 16. Response of Black River water to nutrient treatments, October (left) and November (right) 1996. Top panel - chlorophyll a, bottom - ATP response.
*denotes significantly greater than control

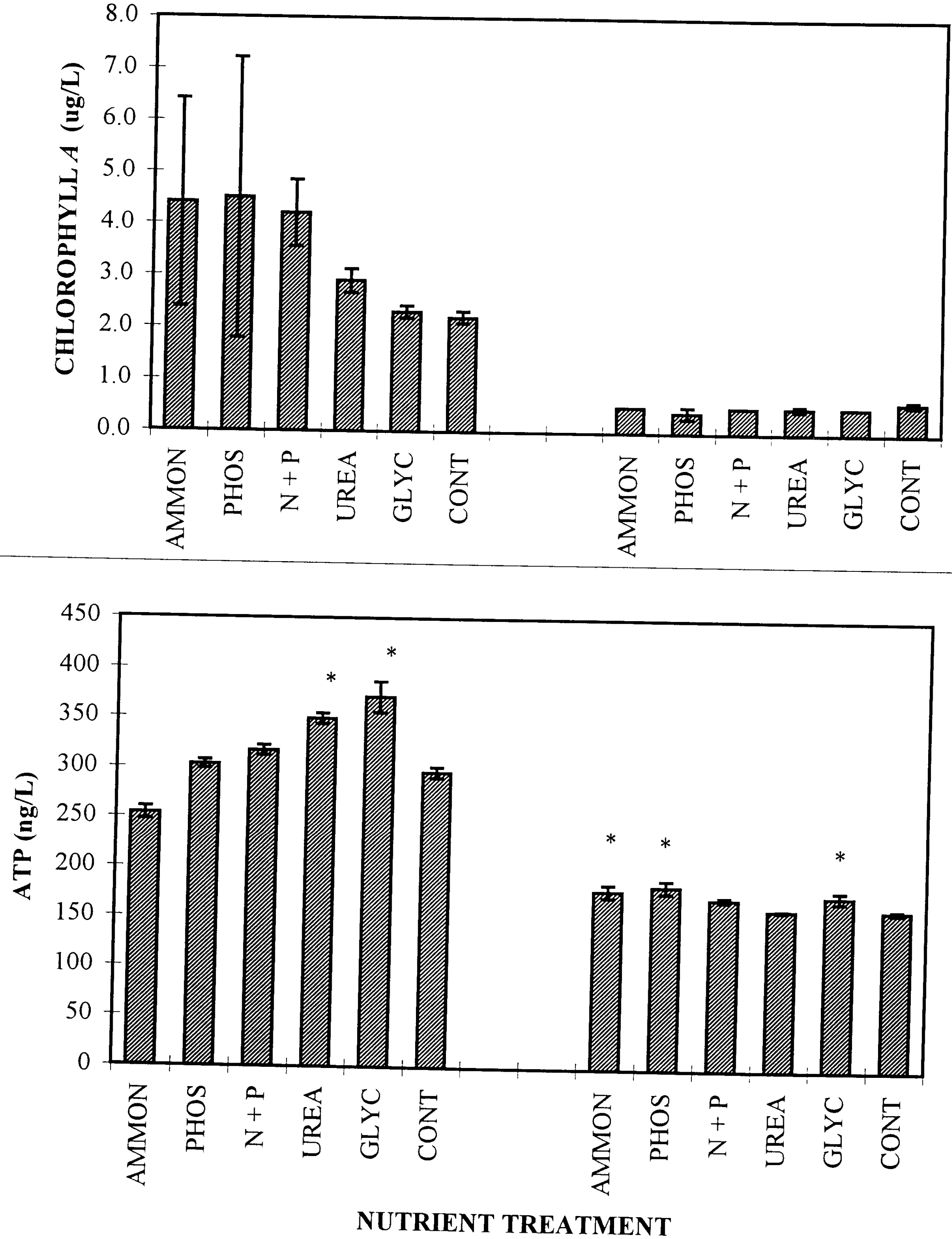
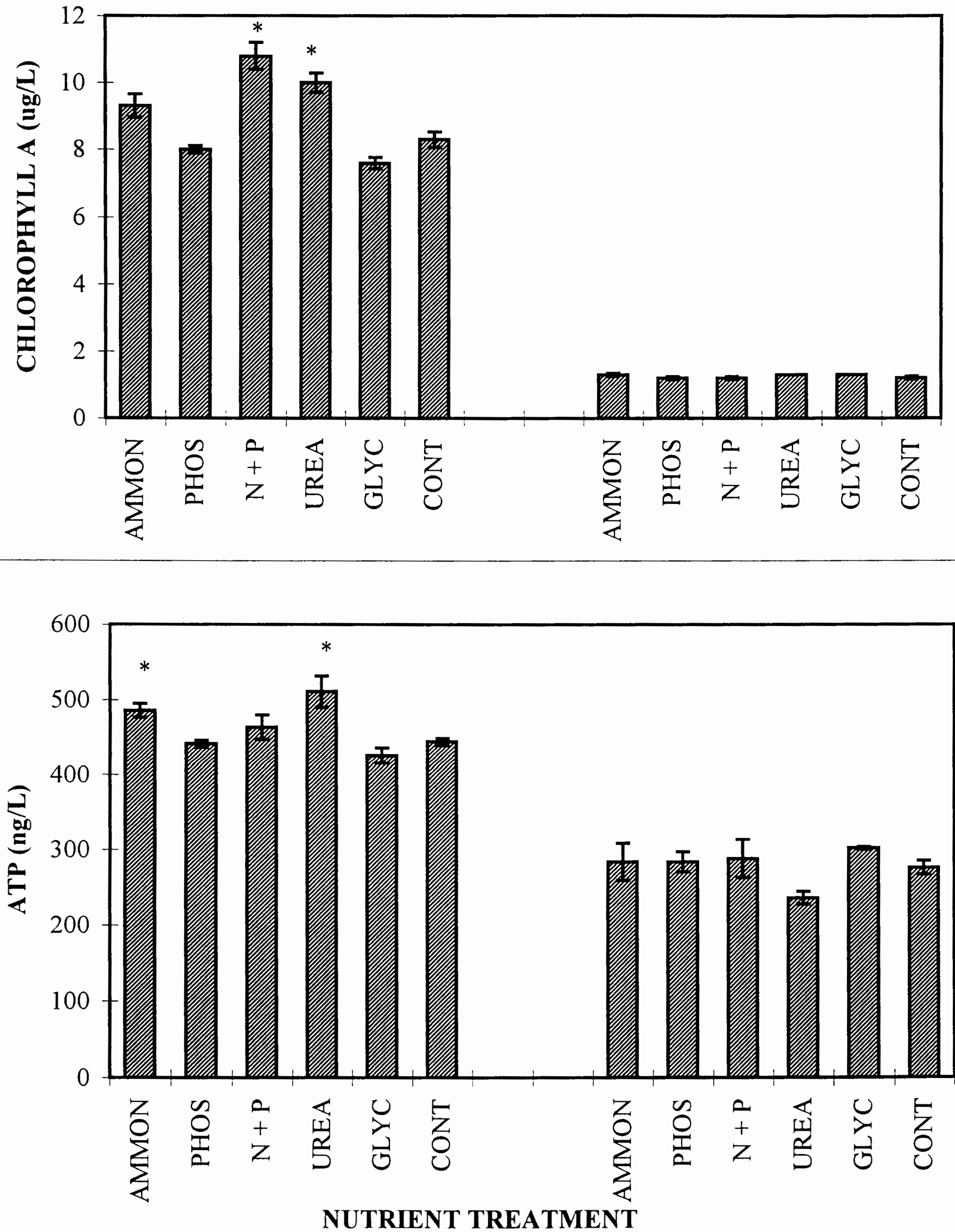


Figure 17. Response of Northeast Cape Fear River water to nutrients, October (left) and November (right) 1996. Top panel - chlorophyll, bottom - ATP.
* denotes significantly greater than control



slight, but significant stimulation of ATP by ammonia and glycerophosphate at B210 (Fig. 16), but no stimulation of ATP at NCF117.

Chlorophyll *a* remained low and was not significantly stimulated by nutrient additions during January and February at either B210 or NCF117. ATP production was low during those months as well, with significant stimulation by ammonia only in January at B210, glycerophosphate at B210 in January, and N+P and glycerophosphate at NCF117 in February (Figs. 18 and 19).

Chlorophyll *a* levels increased somewhat in March, although there was no significant stimulation by nutrient treatments in either river. ATP was significantly stimulated by orthophosphate, N+P, urea and glycerophosphate at B210, and N+P at NCF117 in March (Figs. 20 and 21). In April at B210 chlorophyll *a* was significantly stimulated by ammonia, N+P and urea, and ATP was significantly stimulated by N+P and glycerophosphate (Fig. 20). Chlorophyll *a* at NCF117 was stimulated by ammonia, N+P and urea, and ATP was stimulated by glycerophosphate only (Fig. 21).

A strong summer pattern was initiated with the May bioassays. Both chlorophyll *a* and ATP yield increased considerably (an order of magnitude or more). Chlorophyll *a* production was usually stimulated by both organic and inorganic nitrogen treatments, as well as the N+P treatment (Figs. 22, 23 and 24). In contrast, ATP production was usually stimulated by organic and inorganic phosphorus treatments, as well as N+P (Figs. 22, 23 and 24). High chlorophyll *a* levels were induced by nitrogen in the cubitainers, as opposed to the very low levels during winter. The same winter-summer contrast was evident with ATP and phosphorus additions. The 10 mg/L nutrient additions in July produced large responses, but they were no greater than the 1 mg/L treatment responses in June, probably indicating that after a certain nutrient load other factors, such as self shading by accumulated phytoplankton, may limit growth.

Summary data (Tables 2 and 3) show that phytoplankton growth at NCF117 tended to be more N limited than at B210, while heterotrophic growth was more P limited at B210 than at NCF117. It was also notable that there was no chlorophyll *a* stimulation by P additions at B210 and only one occasion of chlorophyll stimulation by P at NCF117. Urea additions proved to be as stimulatory as ammonia additions in both rivers, while the N+P combination additions caused both frequent and strong stimulation of both chlorophyll *a* and ATP.

Figure 18. Response of Black River water to nutrient treatments, January (left) and February (right)1997. Top panel - chlorophyll *a* reponse, bottom - ATP.
* denotes significantly greater than control

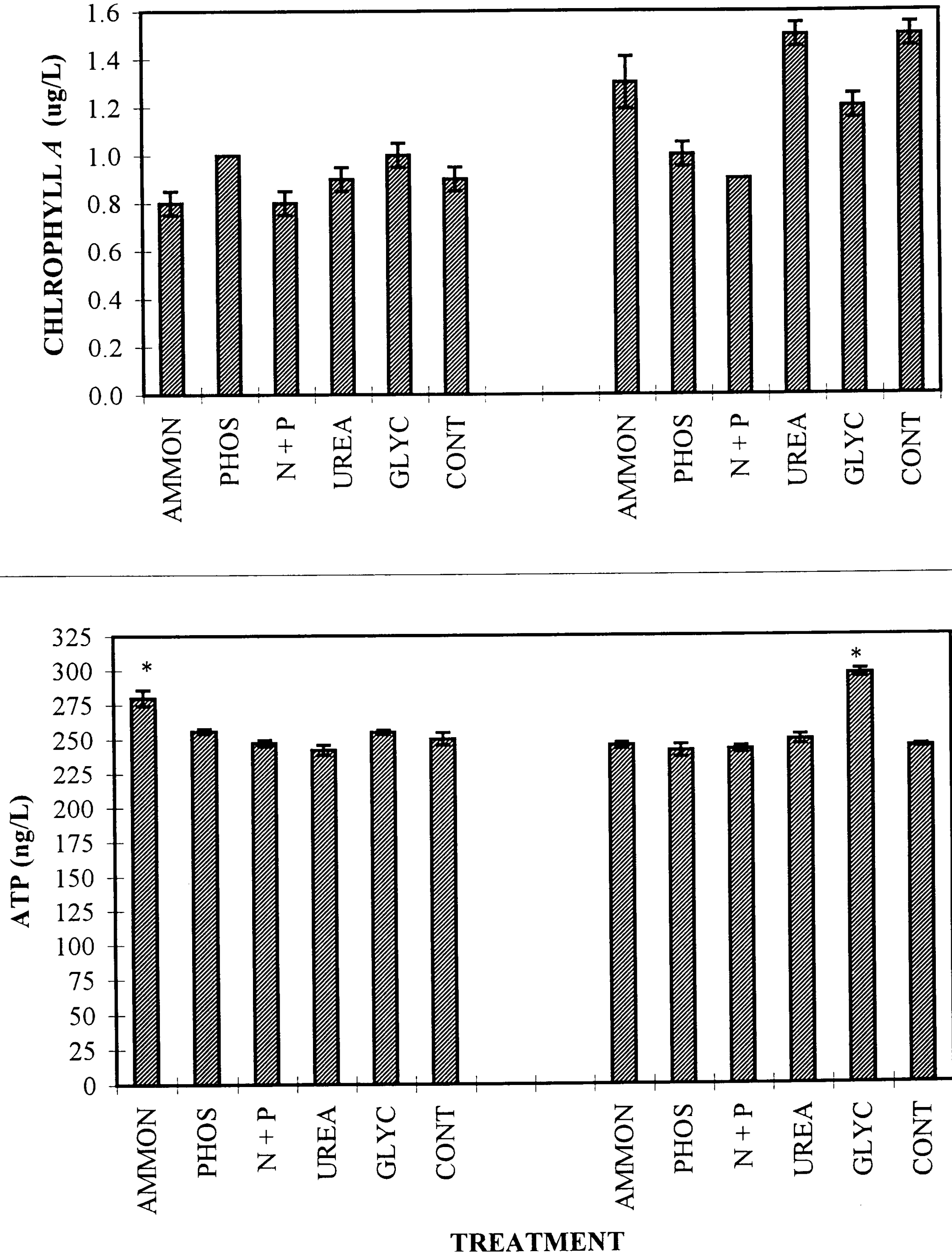


Figure 19. Response of Northeast Cape Fear River water to nutrient additions, January (left) and February (right) 1997. Top - chlorophyll *a*, bottom - ATP.
* denotes significantly greater than control

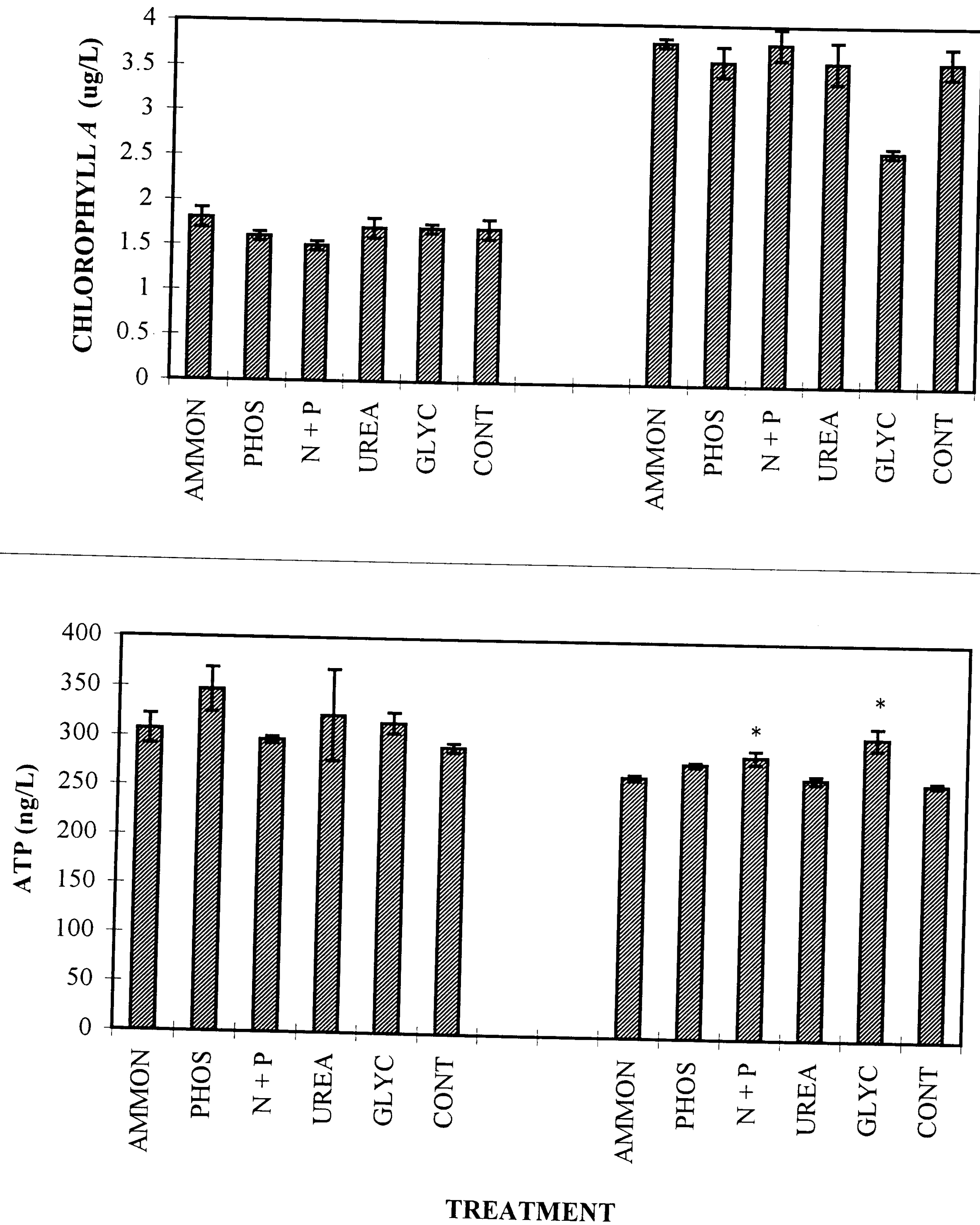


Figure 20. Response of Black River water to nutrient additions, March (left) and April (right) 1997. Top panel - chlorophyll *a*, bottom - ATP response.
* denotes significantly greater than control

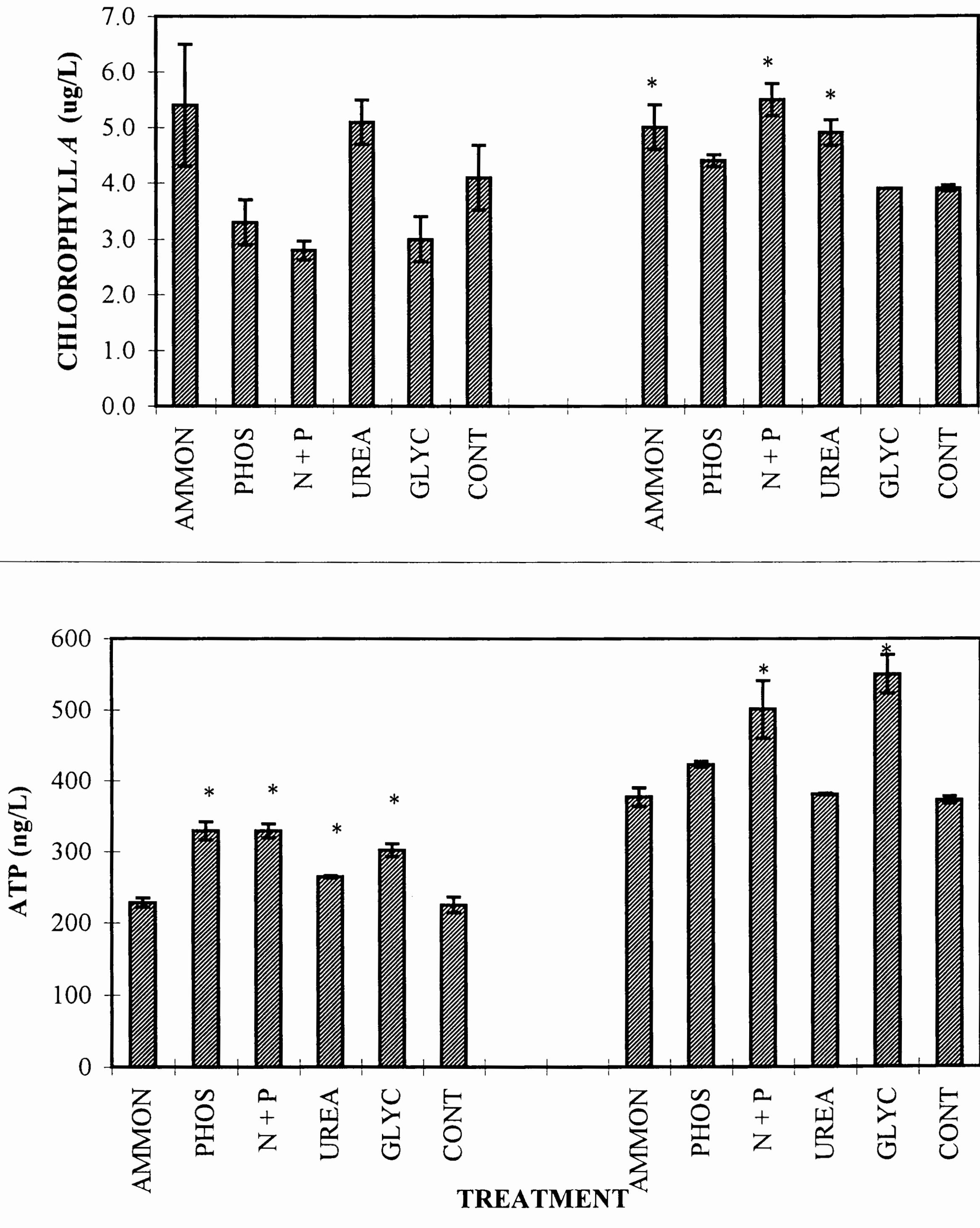


Figure 21. Response of Northeast Cape Fear River water to nutrient additions, March (left) and April (right) 1997. Top panel - chlorophyll *a*, bottom - ATP .
* denotes significantly greater than control

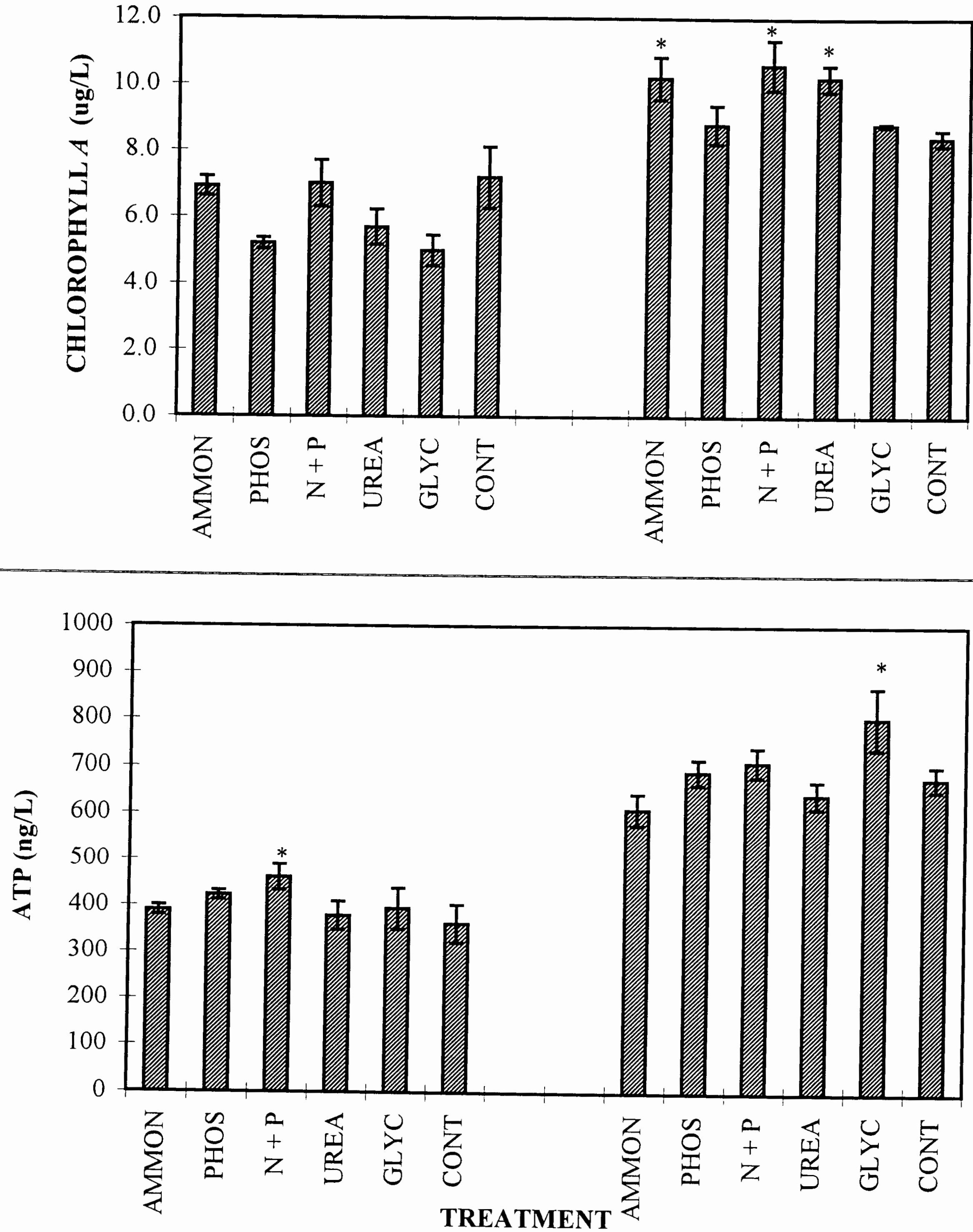


Figure 22. Response of Black River water to nutrient additions, May (left) and June (right) 1997. Top panel - chlorophyll a, bottom - ATP response.
* denotes significantly greater than control

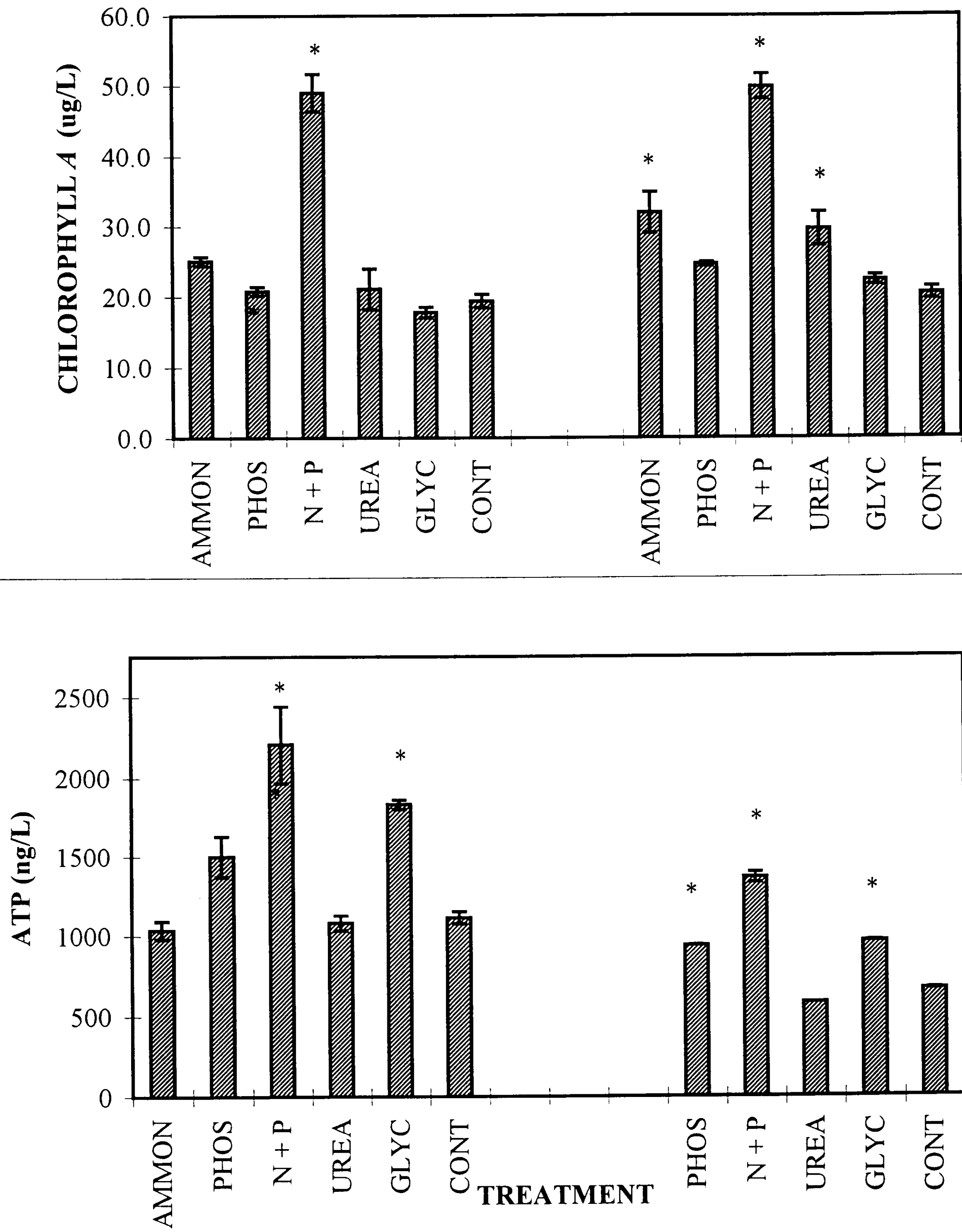


Figure 23. Response of Northeast Cape Fear River water to nutrient additions, May (left) and June (right) 1997. Top panel - chlorophyll *a*, bottom - ATP. * denotes significantly greater than control.

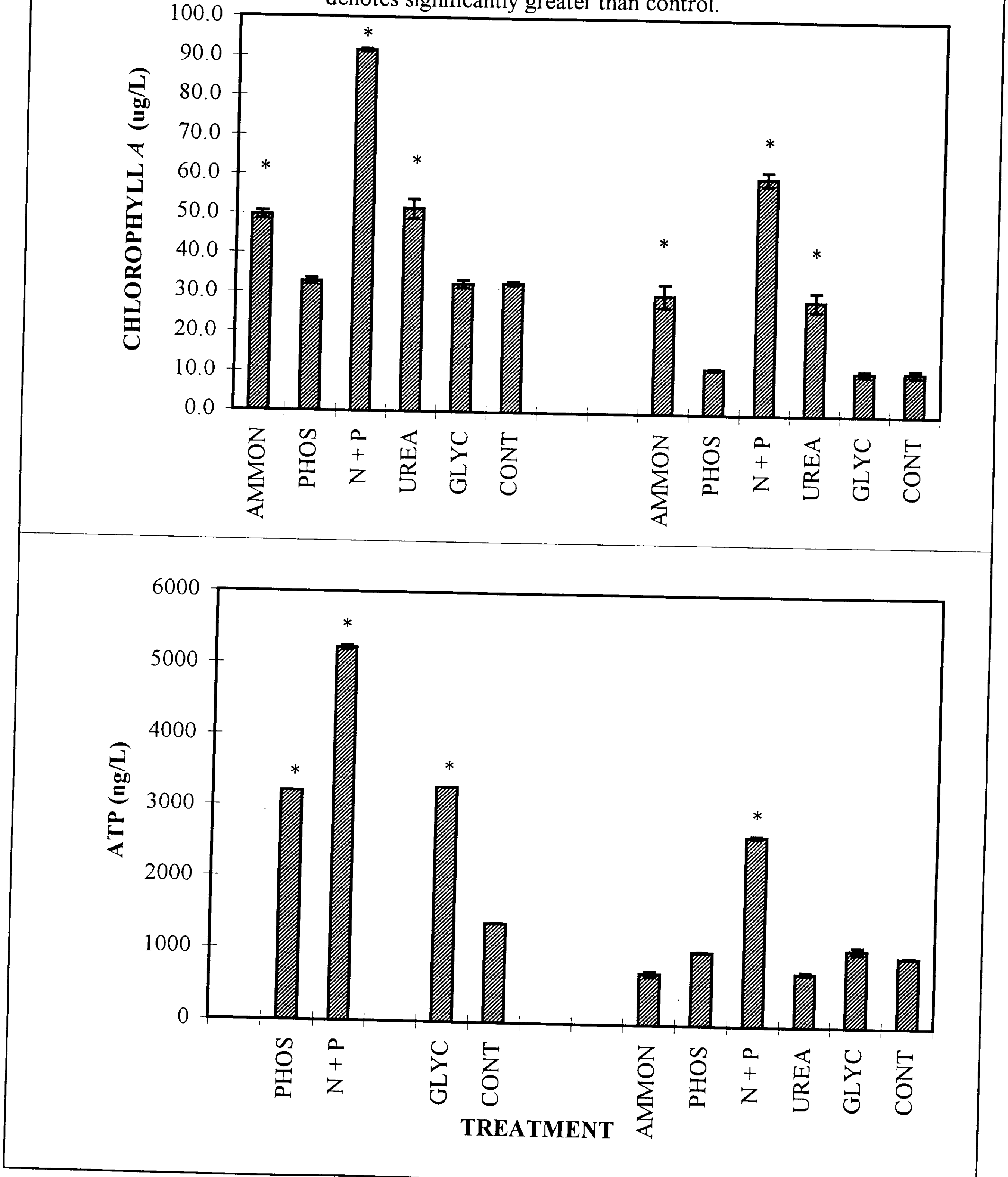
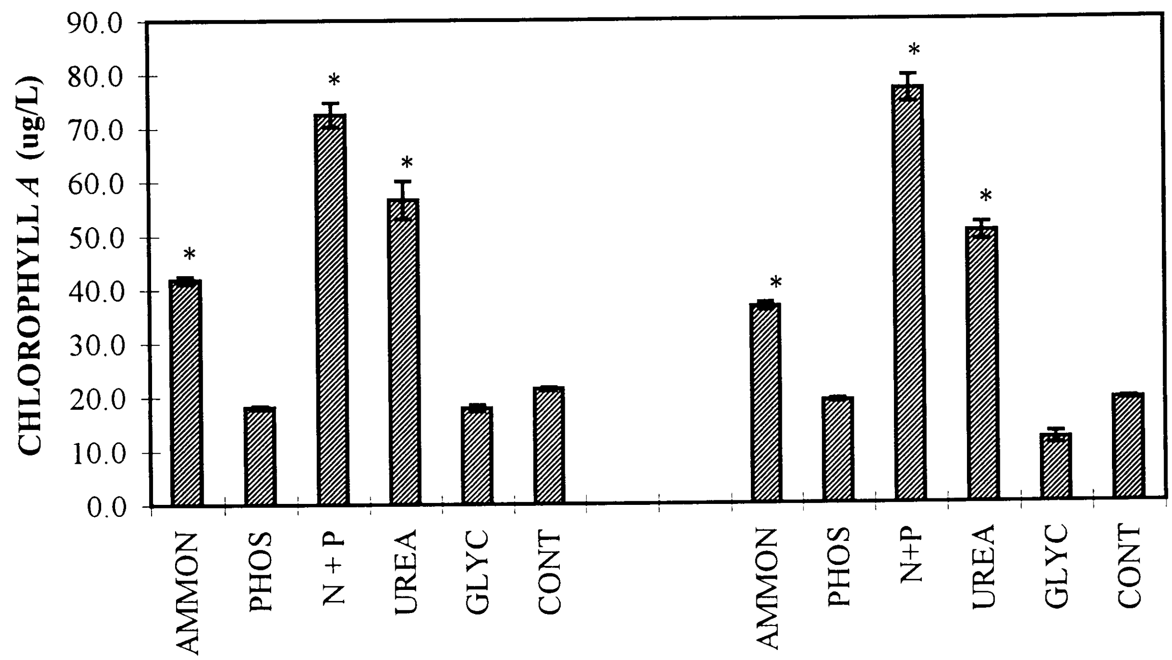


Figure 24. Response of Black River (left) and Northeast Cape Fear River (right) water to nutrient additions of 10.0 mg/L, July 1997.
* denotes significantly greater than control.



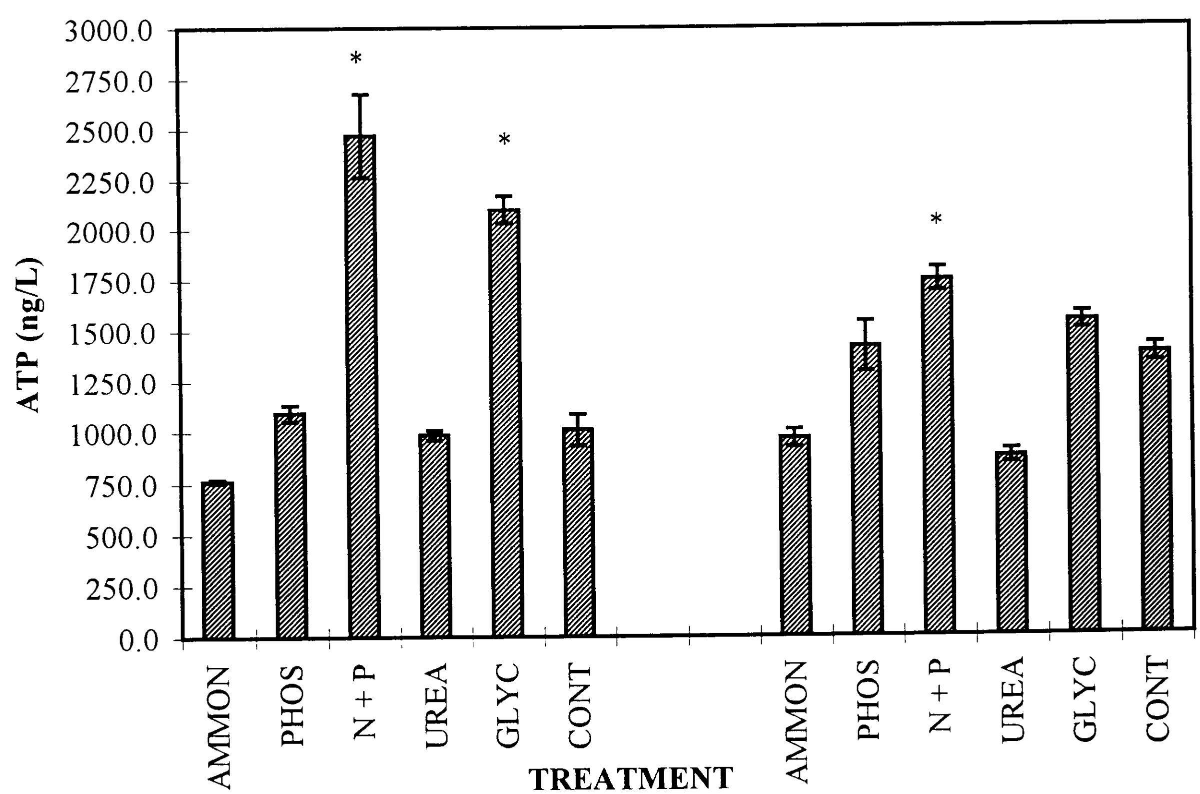


Table 2. Occurrence of significant stimulation over control of either chlorophyll *a* or ATP by organic or inorganic nutrients in the Black and Northeast Cape Fear Rivers (out of 11 bioassays).

Treatment	Black River		Northeast Cape Fear River	
	Chl *a*	ATP	Chl *a*	ATP
Ammonia	4	3	6	2
Orthophosphate	0	4	1	1
N+P	6	7	6	6
Urea	4	2	6	2
Glycerophosphate	0	9	0	4

Table 3. Percent of bioassays demonstrating stimulation by various nutrient treatments (out of 11 bioassays).

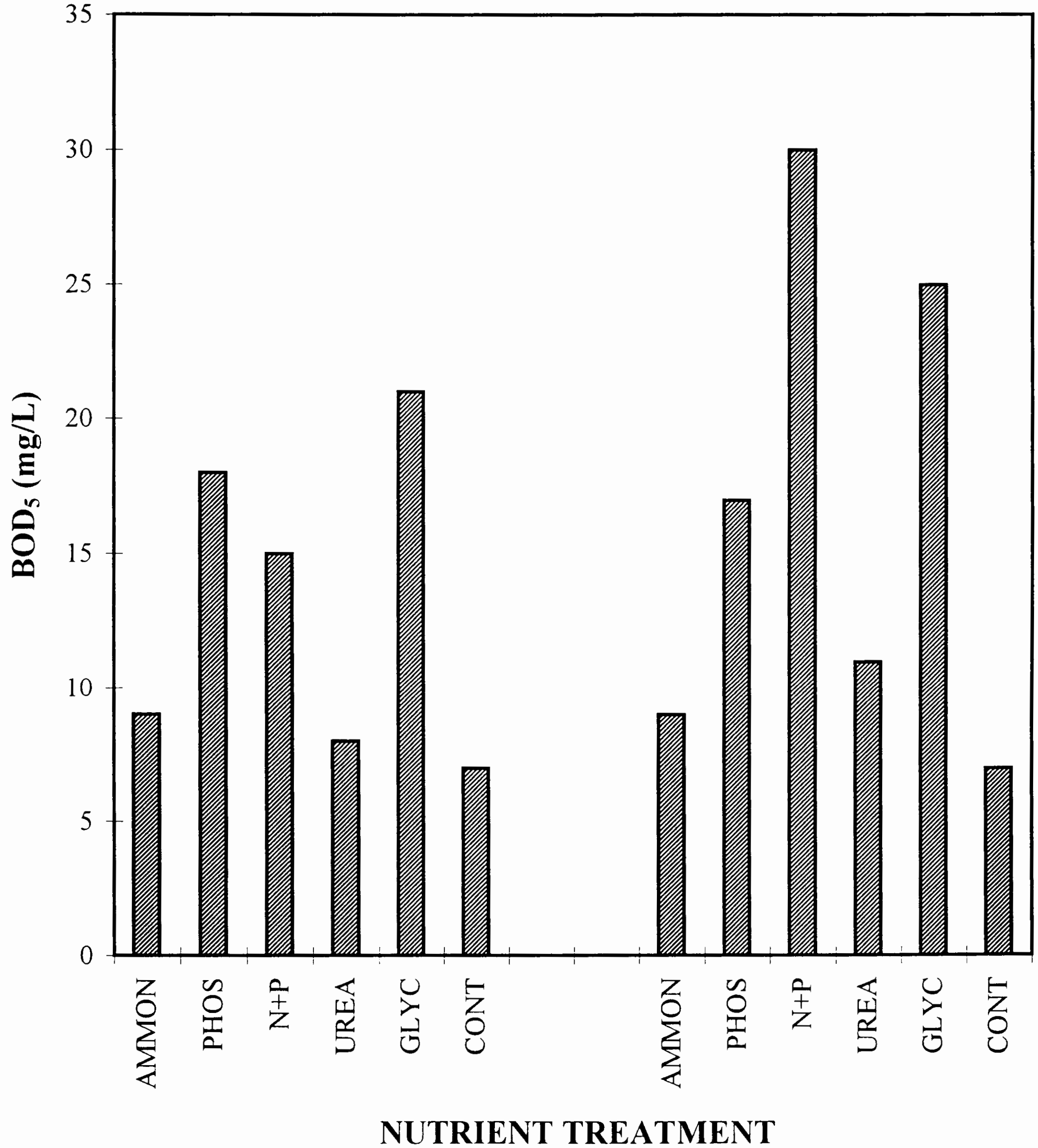
Percent of bioassays showing ammonia or urea stimulation.		
B210	chlorophyll *a*	45%
B210	ATP	45%
NCF117	chlorophyll *a*	64%
NCF117	ATP	18%
Percent of bioassays showing orthophosphate or glycerophosphate stimulation.		
B210	chlorophyll *a*	0%
B210	ATP	82%
NCF117	chlorophyll *a*	9%
NCF117	ATP	36%
Percent of bioassays showing N+P stimulation		
B210	chlorophyll *a*	55%
B210	ATP	64%
NCF117	chlorophyll *a*	55%
NCF117	ATP	55%

PRINCIPAL PHYTOPLANKTON TAXA

Taxonomic analysis of phytoplankton was performed on treatments demonstrating high chlorophyll *a* production. The N+P combination in July 1996 at B210 produced a peak well above the other treatments. This was due to stimulation of several species of green algae (*Selenastrum westii*, *Scenedesmus quadricauda*, *Spermatozoopsis* sp., *Actinastrum Hantschii* and *Microspora* sp.), several cryptomonads, and naviculoid diatoms. A large chlorophyll *a* peak occurred in the N+P treatment for B210 in May 1997 as well. Dominant flora consisted of *Selenastrum minutum*, *S. westii*, *Scenedesmus abundans*, *S. quadricauda*, mixed cryptomonads and pennate diatoms. May 1997 samples from NCF117 yielded high chlorophyll in the ammonia, urea, and N+P treatments. The flora was very diverse, but the same suite of species were dominant in all three of these treatments. Abundant taxa were *Scenedesmus acuminatus*, *S. bijuga*, *S. quadricauda*, *Ankistrodesmus falcatus*, *Coelastrum* sp., mixed cryptomonads and pennate diatoms. June samples from NCF117 also displayed considerable similarity among the three treatments inducing significant chlorophyll production. Dominant chlorophytes included *Selenastrum westii*, *Scenedesmus quadricauda*, *S. bijuga*, *S. dimorphus*, and *Coelastrum sphaericum*. Also abundant were cryptomonads and mixed pennate diatoms, with the N+P treatment inducing abundant centric diatoms as well. The three chlorophyll-stimulating treatments for B210 in June induced *Selenastrum westii, Scenedesmus quadricauda, S. bijuga* among the chlorophytes, abundant naviculoid diatoms, and cryptomonads. *Oocystis borgei* was also abundant in the ammonia treatment and *C. sphaericum* in the N+P treatment. In July at N117 the ammonia treatment yielded an extremely diverse flora, dominated by the chlorophytes *S. quadricauda, A. falcatus, Closterium* sp. and *Scroederia setigera*, naviculoid diatoms and the centric diatom *Cyclotella* sp., and cryptomonads. The urea treatment yielded a less diverse flora dominated by *S. quadricauda, Closterium* sp., *Cyclotella* sp. and naviculoid diatoms, and the N+P treatment yielded primarily *S. quadricauda*, *S. setigera, A. falcatus* and naviculoid diatoms. All three of those treatments yielded a very diverse flora at B210 in July 1997. Dominant chlorophyte species were *S. quadricauda, S. bijuga, A. falcatus, S. westii, Actinastrum Hantschii* and *Dictyosphaerium pulchella*, along with *Navicula* spp. of the diatoms and cryptomonads. In summary, the dominant phytoplankton taxa in these blackwater samples were chlorophytes, diatoms and cryptomonads; some dinoflagellates, euglenoids and blue-green algae were also present, but in low numbers. Nutrient additions did not stimulate only one or two taxa; rather, a suite of several taxa were stimulated together in all cases. Additionally, most of the same organisms stimulated by ammonia loading were also stimulated by urea inputs.

We ran a preliminary experiment to test the potential effect of nutrient inputs on biochemical oxygen demand in blackwater rivers. At the end of the July 1997 bioassay BOD_5 tests were conducted on the cubitainer contents. Results (Fig. 25) demonstrated that both organic and inorganic nutrient inputs caused considerable growth of labile oxygen-demanding microbiota. Organic P caused a 200% BOD_5 increase over control at B210 and a 260% increase over control at NCF117, whereas orthophosphate inputs caused increases of 160% and 140% over control at B210 and NCF117, respectively. Urea inputs caused BOD_5 increases of 14% and 57% at B210 and NCF117, respectively, and ammonia increased BOD_5 by 30% at both B210 and NCF117. The combination N+P treatment led to 114% and 330% increases over control at B210 and NCF117, respectively.

Figure 25. BOD$_5$ concentrations for Day 6 cubitainer bioassays for stations B210 (left) and NCF117 (right), July 1997.



DISCUSSION

Under normal circumstances these blackwater rivers have low chlorophyll *a* biomass year-round, with moderate concentrations of nutrients that are primarily in the organic form. BOD_5 concentrations are generally quite low, with organic materials apparently largely refractory as opposed to labile. These observations are consistent with those for blackwater rivers studied in Georgia (Meyer 1990; 1992). Our observations also indicated that light attenuation is generally high year-round, and is likely a major constraint on phytoplankton productivity in deeper areas. Dissolved oxygen concentrations under normal circumstances range from high in winter to approximately 5.0 mg/L in summer, the water quality DO standard for the State of North Carolina (North Carolina Department of Environment, Health and Natural Resources 1994). Thus, in summer any additional DO stresses are likely to cause a significant negative impact to the system.

Hurricane Fran had a major impact on water quality of these blackwater rivers during the study, especially in the Northeast Cape Fear River. The hurricane's impact led to very low DO, an unusual peak in BOD_5 at NCF117, and elevated concentrations of ammonia, total nitrogen, orthophosphate, and total phosphorus (September TP concentrations at NCF117 were the highest in 27 years of recorded N.C. Division of Water Quality data). Natural processes can partially account for these decreases in water quality, as large quantities of swampwater flooded into the river mainstem. However, human practices greatly increased the deleterious water quality effects, particularly in the Northeast Cape Fear River (Mallin et al. In press). Power outages caused wastewater treatment plants and pump stations to divert raw or partially treated human sewage into the rivers, with diversions totaling approximately 1.4 million gallons in the Black River and 1.0 million gallons in the Northeast Cape Fear River (North Carolina Division of Water Quality 1996). One swine waste lagoon accident was reported in the Black River watershed but at least four swine waste lagoon breaches, overtoppings, or inundations were reported in the Northeast Cape Fear watershed (North Carolina Division of Water Quality 1996). Swine waste lagoon contents are very labile, with chemical oxygen demand (COD) of lagoon liquid approximately 1,840 mg/L (Westerman et al. 1990) and COD of lagoon sludge approximately 67,430 mg/L (James Barker, North Carolina State University, unpublished data). Nutrient concentrations are also very high in swine waste lagoons (Westerman et al. 1990; Burkholder et al. 1997; Mallin et al. 1997a). These lagoon accidents contributed to severe water quality problems, benthic invertebrate mortality, and fish kills in the Northeast Cape Fear River

(Mallin et al. In press). Had animal waste lagoons been prohibited from river floodplains, and had alternate backup generators been widely available for sewage treatment facilities, water quality damage associated with Hurricane Fran would likely have been less severe and long-lasting.

Our experiments have demonstrated that nutrient loading at moderate concentrations can cause phytoplankton blooms to form in blackwater streams. When this would occur in nature would likely dependent on meteorology and stream physical characteristics. Shallow streams under low flow conditions would be most susceptible to increases in phytoplankton biomass. This was demonstrated during 1995 in a tidally driven system of blackwater streams following a swine waste lagoon rupture, when chlorophyll *a* concentrations exceeded 100 μg/L and blooms persisted for over three weeks until an extensive rain event washed them out (Mallin et al. 1997a). We have also found algal bloom conditions (chlorophyll *a* concentrations up to 140 μg/L) at stations in small blackwater streams during the low-rainfall summer of 1997 (unpublished data). Blooms are not likely to occur in deep, well mixed blackwater rivers of greater than 4th or 5th order, such as the Black or Northeast Cape Fear Rivers. Monitoring efforts demonstrate low chlorophyll *a* in these systems (Mallin et al. 1997c), where entrained algal cells likely spend very little time exposed to near surface irradiance, and are mainly confined to light limited conditions.

The question of what becomes of blooms in creeks when the creeks join larger rivers is important. We hypothesize that much of the phytoplankton biomass would senesce in the light limited river, and become converted to oxygen consuming BOD. Since creek blooms are only likely to appear in late spring and summer (when dissolved oxygen is low in the rivers), bloom senescence may exacerbate low riverine DO conditions.

We noted that on several occasions urea additions stimulated significant phytoplankton growth at levels as high or higher than ammonia treatments. Some ammonia uptake may have occurred in phytoplankton passing through the 1.0 μm filters; however, in marine waters Wheeler and Kirchman (1986) found very little urea uptake associated with particles < 1.0 μm in size. The use of urea as a nutrient source by numerous phytoplankton species has been well documented (see Antia et al. 1991). Since urea uptake occurs in the dark as well as the light this ability would aid in phytoplankton survival under the light limited conditions present in blackwater rivers. Our taxonomic analysis demonstrates that the species (chlorophytes, diatoms and cryptomonads) which responded best to ammonia loading likewise responded well to urea, indicating a flora well adapted to light limited blackwater conditions

Anthropogenic nutrient inputs to streams are generally interpreted to be inorganic (ammonia, nitrate, orthophosphate). However, there are potential and realized organic nutrient sources to blackwater streams as well. As mentioned, swine waste lagoon liquid is commonly sprayed on fields surrounding concentrated animal operations in coastal North Carolina and other states (Evans et al. 1984; Westerman et al. 1985b). Ammonia comprises over 80% of total nitrogen and orthophosphate over 80% of total phosphorus in lagoon liquid (Westerman et al. 1990, Mallin et al. 1997a). However, with average total nitrogen and phosphorus concentrations in lagoon liquid of 615 mg/L and 100 mg/L, respectively (Westerman et al. 1990) these would still be considerable organic nutrient loads available for runoff during rain events. Other sources of organic nutrient loading to streams would be public or private wastewater treatment systems, feedlots, and runoff from fields harboring grazing animals.

Stimulation of ATP production by a given organic or inorganic nutrient without corresponding stimulation of chlorophyll *a* was demonstrated on a number of occasions, especially throughout the fall and winter months. No attempt was made to analyze this group of heterotrophs taxonomically, but potential heterotrophs in these systems would include bacteria, fungi, and protozoa, and to a lesser extent rotifers and other zooplankton. Bacteria readily utilize dissolved organic and inorganic nutrients (Morris and Lewis 1992; Bjorkman and Karl 1994; Chrzanowski et al. 1995), and fungal activity increases in nitrogen-rich environments (Mitsch and Gosselink 1986; Suberkropp 1995; Suberkropp and Chauvet 1995). Protozoans are a critical component of the microbial community in blackwater systems and will likely respond to increases in microbial food supplies rapidly (Carlough and Meyer 1989). Rotifers and other zooplankton are not abundant in blackwater streams (Herlong and Mallin 1985) but within a period of several days may increase significantly given sufficient prey and calm water conditions. In late spring and summer both orthophosphate and glycerophosphate stimulated ATP production without stimulating chlorophyll production. It has been demonstrated that phytoplankton can readily take up both inorganic and dissolved organic P, particularly when P concentrations increase above ambient (Cotner and Wetzel 1992), thus, it does not appear that the phytoplankton were outcompeted for P by bacteria.

Our experiments were not designed to measure total autotrophic or heterotrophic biomass; rather, for consistency both chlorophyll *a* and ATP were sampled from 1.0 μm filters. We compared ATP content collected on both 0.22 μm and 1.0 μm filters during one of the blackwater experiments. Ratios ranged widely, from approximately 15:1 to 1:1, with the largest ratios from Day 1and the smallest ratios from

Day 6. Thus, varying amounts of heterotrophic material were not reflected in the ATP concentrations. Likewise, the concentration of picoplankton passing the filters was not reflected in the chlorophyll *a* values.

Another potential contribution to heterotrophic activity may come from increased grazing on fine particulate organic matter (FPOM) and ultrafine particulate organic matter (UFPOM), some of which would be entrained in the plankton. Heterotrophic activity upon these small particles increases significantly with nitrogen content of this material (Fuss and Smock 1996). Enrichment of these particles by the adsorption of organic or inorganic nutrients further increases heterotrophic activity in the plankton.

Significant heterotrophic biomass increases occurred with nutrient additions year round, but most prominently during summer. Increased water temperature is known to be positively correlated with increased heterotrophy in streams (Fuss and Smock 1996). Thus, the maximum heterotrophic stimulation by nutrients occurs when the waters are warmest and capable of holding the least oxygen (Wetzel 1983). Our preliminary July BOD_5 tests showed significant increases of labile organic material over control following nutrient loading. Nutrient loading in summer would likely cause the highest seasonal stimulation of the BOD load in already oxygen-stressed systems.

An interesting question is the importance of heterotrophic biomass increases in cooler months. The decreased water temperature should retain sufficient dissolved oxygen under moderate nutrient loading (although acute, large releases will still be problematic). Some of the increased heterotrophic biomass will undoubtedly pass up the food chain into biomass of higher orders. However, some of this biomass after death may accumulate as organic matter in or on the sediments, and become available as a BOD source in spring or summer.

The inorganic N/P ratio of 24:1 of the Black River led us to hypothesize that our bioassays would show that the phytoplankton community of this river was P limited. This was not the case, as neither orthophosphate nor glycerophosphate stimulated significant chlorophyll *a* growth on any occasion. However, significant planktonic heterotrophic growth was stimulated by glycerophosphate during 82% of the bioassays and by orthophosphate during 36% of the bioassays. Thus, control of phosphorus should be a key strategy for improvement of stream water quality in the Black River watershed. Both inorganic and organic N stimulated heterotrophic growth periodically in the Black River as well. The combination inorganic N+P treatment caused an apparent synergistic effect, yielding more frequent and often greater chlorophyll stimulation than any individual treatment. Thus nitrogen inputs, while second to phosphorus in this system, also require control to ensure acceptable water quality improvement in the Black River basin.

The inorganic N/P ratio of the Northeast Cape Fear River was 17:1, close to the Redfield ratio for phytoplankton, thus prediction of a limiting nutrient for phytoplankton growth would be inappropriate. Our bioassays demonstrated a clear tendency for chlorophyll *a* and, to a lesser extent ATP growth to be significantly stimulated by inorganic and organic N as opposed to P. Again, the inorganic N+P combination proved to be more stimulating than any single nutrient treatment.

We chose our two watershed stations for several reasons, including availability of comprehensive background data, having a representative for each of the two large blackwater basins, and collection at a point that integrated numerous tributary streams in the watershed. Thus, our results are generalizations for each watershed. Doubtless the many individual lower order streams each have varying N/P ratios and background nutrient loads, and each will respond somewhat differently to nutrient inputs. Regardless, our experiments have demonstrated that both organic and inorganic nutrients can cause significant heterotrophic community increases year-round in blackwater systems, and significant phytoplankton growth in summer. Further, we suspect that phytoplankton biomass in shallow feeder streams is likely converted to BOD upon entering the deep, well-mixed and light limited blackwater rivers.

Monitoring data demonstrates that dissolved oxygen concentrations in these blackwater rivers are low during summer (Mallin et al. 1997c). Suggested reasons for low DO in blackwater rivers include inputs from oxygen-poor swamp water, high background bacterial populations, high respiration rates from inputs of particulate and dissolved organic carbon, and low, light limited phytoplankton biomass (Meyer 1992). Exacerbating these natural stresses were acute anthropogenic incidents in recent years, including animal waste lagoon ruptures in 1995 (Mallin et al. 1997a) and animal waste lagoon accidents combined with human sewage bypasses following Hurricane Fran in 1996 (Mallin et al. In press). Our experiments demonstrate the potential water quality degradation caused by chronic anthropogenic nutrient loading in blackwater streams. Potential non-point sources of nutrients in these watersheds include swine lagoon spray fields, row crop fertilization and grazing animals. Nutrient inputs from these sources could best be controlled by source reduction, mandated vegetated buffer zones near watercourses, enhancement of denitrification, use of constructed wetlands and preservation of existing riparian wetlands. Potential point sources of nutrients include municipal or industrial wastewater treatment systems that are either faulty, lack tertiary treatment for nutrients, or do not have total N or total P discharge limits. These sources fall under the NPDES permitting system and can and should be controlled by regulatory means. Without increased attention paid to controlling nutrients, deterioration of water quality can be expected in the extensive blackwater systems characterizing the Coastal Plain.

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