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ALGAL ASSAY STUDIES OF THE CHOWAN RIVER, NORTH CAROLINA

by

Mary M. Sauer* and Edward J. Kuenzler
Department of Environmental Sciences and Engineering
University of North Carolina at Chapel Hill

* Present address: Midwest Research Institute
4505 Creedmoor Road
Raleigh, North Carolina 27612

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ABSTRACT

Algal assays were conducted on water collected from three stations on the Chowan River to investigate the presence of limiting nutrients during the summer of 1980. Two types of algal inocula were used: the laboratory cultured green alga *Selenastrum capricornutum* and samples of the natural Chowan River phytoplankton. Phosphorus and nitrogen simultaneously limited total algal growth in most experiments; other nutrients did not significantly limit growth. Species-specific responses to nutrient enrichment occurred. Single additions of phosphorus increased numbers of nitrogen-fixing blue-green algae. Therefore, phosphorus is the more critical limiting nutrient to growth of the bloom-forming genera *Anabaena* and *Aphanizomenon*. Other algae required additions of both nitrogen and phosphorus for large increases in growth.

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CONCLUSIONS AND RECOMMENDATIONS

Algal assays performed upon water from the Chowan River during the summer of 1980 suggested the following conclusions:

1. Phosphorus and nitrogen simultaneously limited total algal growth in most experiments in May through September, 1980. Nutrients other than nitrogen and phosphorus did not limit algal growth in assays. In the river itself, however, physical factors may also limit phytoplankton growth.

2. Species-specific responses to nutrient enrichment occurred in the natural assays. Single additions of phosphorus increased numbers of nitrogen-fixing blue-green algae. Other algae required additions of both nitrogen and phosphorus for large increases in growth.

3. *Selenastrum* assays did not always accurately reflect responses of the natural population to nutrient enrichment. Experiments using natural algal populations worked well and provided information that would not be obtained from *Selenastrum* bioassays alone.

4. Phosphorus is the more critical limiting nutrient to growth of the nitrogen-fixing bloom-forming genera *Aphanizomenon* and *Anabaena*.

5. Reduction of nitrogen concentrations in the Chowan River without concomitant reduction of phosphorus levels could result in conditions that favor growth of nitrogen-fixing blue-greens over other

algae.

Based upon our investigation and upon other studies of the Chowan River we make the following recommendations for reducing the algal bloom problem:

1. The algal nutrients nitrogen and phosphorus are both implicated as contributors toward excessive abundance of phytoplankton most of the time and therefore any measure which substantially reduces the concentrations of nitrate, ammonium, or phosphate is desirable. It is clear that runoff to the river often contains undesirably high levels of nitrogen and phosphorus from both point sources and non-point sources. The contributions of nitrogen and phosphorus from sediment regeneration are presently being studied and their importance relative to runoff inputs will be of interest.

2. Because a large proportion of the algae constituting the nuisance summer bloom are nitrogen fixing blue-green algae, not entirely dependent upon nitrate and ammonium in the water, a reduction in the amounts of inorganic nitrogen in the water may not markedly reduce this algal problem. Phosphorus in solution, however, is critical for blue-green algal growth, as it is for other species, and substantial lowering of phosphorus inputs to the river may be expected to provide control of the blue-green bloom.

INTRODUCTION

The overenrichment of lakes, rivers, and coastal waterways with plant nutrients is an important factor behind deteriorating water quality. Increased nutrient inputs, expecially nitrogen and phosphorus, result in biological changes in the receiving water body; these include increased phytoplankton numbers and shifts in the biotic structure of the community. Watershed use determines the character and amount of nutrient loading to the water body. Municipal sewage treatment plants and industries are principal point sources of nutrients. Non-point sources include precipitation and runoff from agriculture, forests, and wetlands.

The nutrient in shortest supply, in respect to the requirements of the algae, will become the limiting factor, according to Liebig's law of the minimum. Nitrogen and phosphorus are nutrients required for phytoplankton growth. Other essential nutrients are present in sufficient concentrations at most times. Therefore, nitrogen and phosphorus are often found to limit algal growth. Additional limiting factors besides nutrient availability are light, temperature, and grazing.

This study was part of a larger project to investigate nutrient kinetics in relation to algal blooms in the Chowan River in northeastern North Carolina. Extensive algal blooms in the lower Chowan in the past decade have affected commercial and recreational use of the river. Non-point sources of nutrients, including agricultural runoff, are the principal inputs into the river. (N. C. Dept. of N.R.C.D. 1979a). A paper

mill and a nitrogen fertilizer plant are two important point sources. Nitrogen and phosphorus were the principal nutrients of interest in this research. Algal bioassays provided the experimental framework for investigating the presence of limiting nutrients. Identification of one or a combination of limiting nutrients from bioassay results can be useful in water quality assessment.

Two types of experiments were conducted, the *Selenastrum capricornutum* bioassay method developed by the Environmental Protection Agency, and enrichment experiments utilizing a sample of the natural population. Natural enrichment experiments provided a comparison to the *Selenastrum* method as well as an evaluation of species-specific responses to nutrient additions. Experiments were performed with water collected monthly from three stations on the Chowan River during the period May through September, 1980.

LITERATURE REVIEW

Chowan River

The Chowan River is formed at the confluence of the Blackwater and Nottaway Rivers, south of the Virginia-North Carolina border, and flows to the southeast into Albemarle Sound (Figure 1). Its drainage basin encompasses approximately 12,600 km^2 (Stanley and Hobbie 1977). Land use in the watershed is principally agricultural. River flow is generally highest in winter and lowest during summer months (Stanley and Hobbie 1977). The river widens downstream, and a substantial decrease in water velocity occurs. Lunar tides exert some influence on water movement; however, wind-induced tides are a more important mechanism effecting mixing in the river (Amein and Galler 1979). Long residence times for the phytoplankton in the lower river result when calm conditions last for an extended period.

Nutrient inputs into the river derive from several sources. According to recent estimates, agriculture contributes 48% of total nitrogen and 33% of total phosphorus entering the Chowan (N. C. Dept. N.R.C.D. 1979a). Inputs from forests and wetlands are estimated at 35% of total nitrogen and 47% of total phosphorus. Point sources account for other sizable inputs, 15% of total nitrogen and 18% of total phosphorus. Included in point sources are numerous domestic wastewater dischargers as well as industrial dischargers. Extensive algal blooms in the lower Chowan in 1972 brought much attention to one particular discharger, a nitrogen fertilizer plant at Tunis. Large amounts of nitrogen were dis-

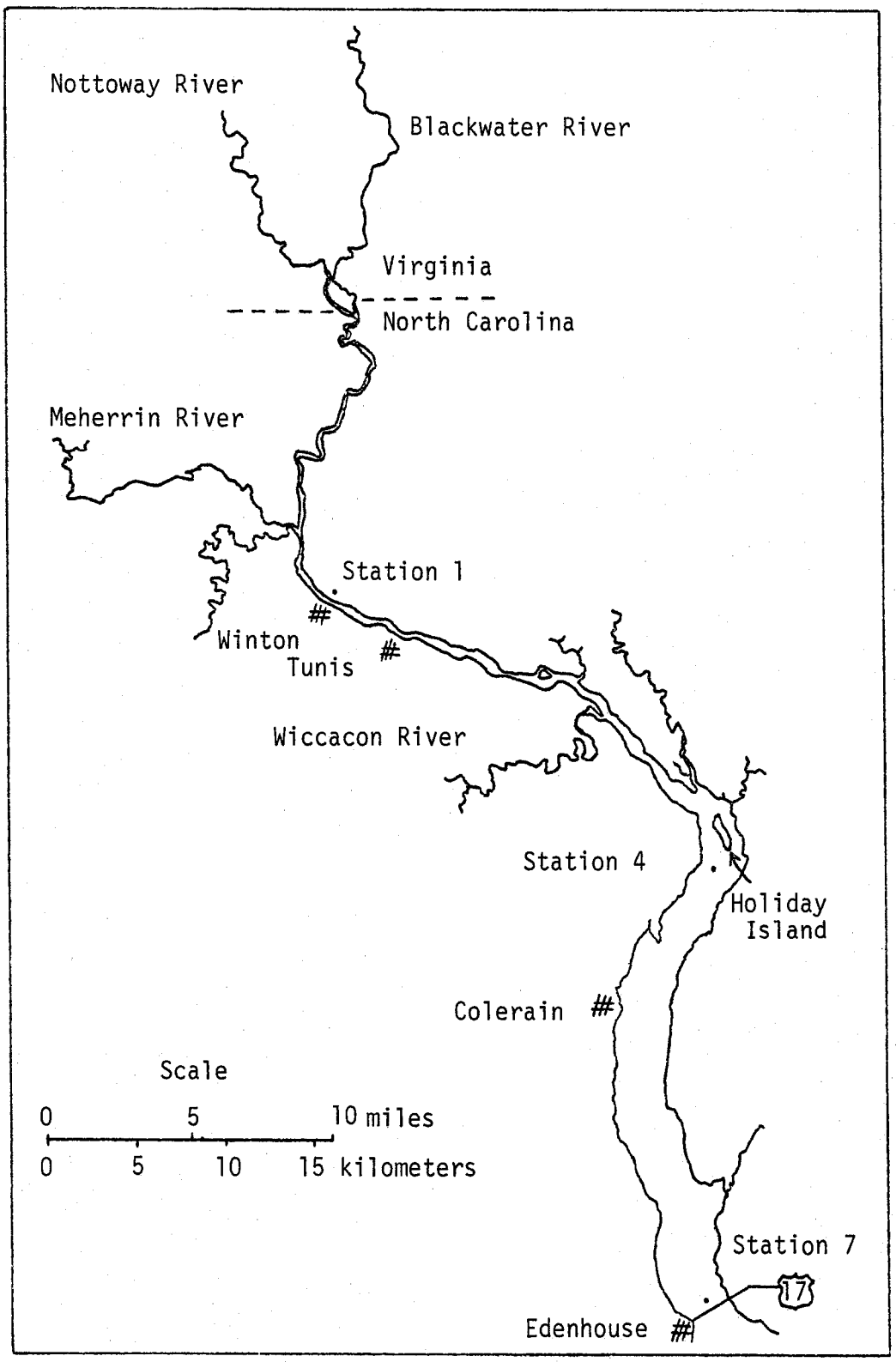

Figure 1. Map of the Chowan River and its major tributaries showing locations of sampling stations.

covered in the river at this site and were attributed to the plant (N. C. Dept. of N.R.C.D. 1979b).

In the winter, nitrogen loads were found to be higher than those in the summer (N. C. Dept. N.R.C.D. 1979b). Low values of nitrate and ammonia were found during the summer in the lower Chowan, where algal growth reached its maximum (Stanley and Hobbie 1977; N. C. Dept. N.R. C.D. 1979b). Stanley and Hobbie (1977) suggested that recycling of nitrogen from organic matter in the water column and sediments provided the nitrogen required for algal growth during summer months. Winter inputs of nutrients, although not directly supporting algal blooms at that time, might therefore play a role in sustaining summer blooms through recycling processes.

Serious bloom conditions were noted in the summers of 1972, 1976, and 1978 (Bond, Cook, and Howells 1977; N. C. Dept. N.R.C.D. 1979b). Losses in recreational values and damage to the fishing industry were blamed on nuisance algal growth (N. C. Dept. N.R.C.D. 1979b). Three genera of blue-greens have been identified as the principal bloom-forming algae: *Anabaena*, *Microcystis (Anacystis)*, and *Aphanizomenon* (Witherspoon *et al.* 1979). Diatoms were the dominant algae in the winter (Stanley and Hobbie 1977) and were present throughout the year, especially at upstream locations (Witherspoon *et al.* 1979). Stanley and Hobbie (1977) found that temperature and light limited algal growth throughout the year. Growth was limited by nitrogen only in the summer; effects of phosphorus were not investigated. During the study year, 1975, no significant blooms occurred.

Published reports of algal bioassays on the Chowan River are few. In a field evaluation of the algal assay procedure in North Carolina

surface waters, Weiss (1976) presented results of assays conducted on Chowan River water during the period September 1972 to August 1973. Although some samples appeared to be limited solely by nitrogen or phosphorus, the majority of significant increases in biomass occurred in response to combined nitrogen and phosphorus enrichment. Fisher and Witherspoon (unpublished manuscript) conducted bioassays on two streams in the Chowan River basin. Phosphorus was never limiting to growth while nitrogen was frequently potentially limiting. The results were expected in view of the low inorganic nitrogen to inorganic phosphorus ratios in the two streams. The streams studied, while rich in nutrients, supported little algal growth due to limitation by light and flushing rate.

Algal Blooms

The occurrence of nuisance algal blooms has increased steadily in response to man's intensive use of lake watersheds and river basins. Extensive use of fertilizers, channelization of streams (Kuenzler *et al.* 1977), increased detergent use and waste production, and expanded industrial development have contributed to high nutrient loading in many water bodies. Overenrichment with nutrients resulting in high biological productivity is termed eutrophication (Likens 1972). While many eutrophic water bodies have problem algal growth, actual blooms occur less frequently. Reynolds and Walsby (1975) defined a water bloom as the visible accumulation of algal material immediately below the surface. They attributed surface bloom formation to: (1) the existence of a substantial population of a bloom-forming species, (2) the algae being sufficiently gas-vacuolated to be bouyant, and (3) water turbulence too

weak to overcome the tendency for bouyant algae to float to the surface. Blue-greens are the principal bloom-forming algae. Their dominance in the biota of eutrophic water bodies is related to many factors. These include: possible ability to photosynthesize at lower light intensities than other algae, higher temperature range, growth favored by the onset of thermal stratification and decline in dissolved oxygen in the hypolimnion, formation of colonies and aggregation of trichomes that allow high flotation rates, and the ability to fix atmospheric nitrogen (Reynolds and Walsby 1975).

An upper limit to algal growth is set by nutrient availability, given optimum conditions of other growth factors. The concept of limiting nutrients was first described by Liebig's law of the minimum. That constituent present in the smallest quantity relative to the requirements for growth of organisms will become the limiting factor. The ratios of the major nutrients present in seawater and in the plankton were examined by Redfield (1958). He found that the ratio of nitrogen to phosphorus in the plankton was similar to the ratio of nitrogen to phosphorus available in the seawater, whereas carbon was available in great excess in relation to its algal requirement. Redfield's available nitrogen to available phosphorus ratio (N:P) of 15:1 (atoms) has been used extensively to express the optimal ratio of N:P for algal growth in many water bodies. Ratios departing from the ideal are sometimes interpreted as signifying nitrogen or phosphorus limitation.

Competitive interactions among algae depend in part on nutrient uptake. Tilman (1977) showed that *Asterionella* and *Cyclotella* stably coexisted when each was limited by a different resource. *Asterionella* dominated when both were limited by phosphorus. Silica limitation

allowed *Cyclotella* to dominate. Viner (1973), in natural enrichment experiments on tropical Lake George, separated the *Microcystis* fraction from the small plankton fraction. The two groups exhibited markedly different responses to nitrogen enrichment. In experiments on mixed blooms, Fitzgerald (1969) separated nitrogen-fixing from non-nitrogen-fixing algae. Non-N-fixers had higher surplus phosphorus; therefore, these algae were probably limited by nitrogen. The nitrogen of N-fixers and the phosphorus of non-N-fixers did not appear to be available to each other.

In coastal marine waters, nitrogen has been suggested as the critical limiting factor (Ryther and Dunstan 1971; Yentsch *et al.* 1977). Different combinations of nutrients limited diatom growth at different times of the year in bioassay studies on Narragansett Bay (Smayda 1974). Studies on freshwater systems have found phosphorus, nitrogen, or both to limit algal standing crops. Certain water bodies exhibit limitation by other elements. Schelske and Stoermer (1971) demonstrated silica limitation of Lake Michigan phytoplankton, which were dominated by diatoms. The importance of trace elements in limiting production, especially in oligotrophic lakes, was discussed by Goldman (1972).

Blue-green algae are a dominant component of the flora of many eutrophic water bodies; yet the appearance of blue-green blooms often coincides with very low nutrient concentrations in the water. Lund (1965) noted that phytoplankton in lakes often grow at nutrient levels far below their minimum requirements in culture. Where very large populations of phytoplankton exist, nutrient levels in the water body will be decreased to near limiting proportions (Droop 1973). Reynolds and Walsby (1975) stated that the concentration of a nutrient cannot be

interpreted as a true measure of availability, because it ignores nutrients tied up in cells and fluxes between plankton, water, and sediments. Upon phosphorus depletion of the water, phosphorus stored intracellularly by blue-green algae becomes available for growth (Stewart *et al.* 1978). Nutrients in the hypolimnion of a lake can be taken up and stored by blue-greens for use in the epilimnion (Lund 1965). Shapiro (1973) suggested that half-saturation constants for blue-greens for phosphate uptake were lower than those for greens, over a wide range of environmental conditions. The ability to fix atmospheric nitrogen and the utilization of certain organic compounds for growth are advantages when external nutrient levels are low (Lund 1965).

Some members of the Cyanophyceae exert toxic effects on other organisms, presumably through the production of extracellular substances (Lam and Silvester 1979). Probiosis and antibiosis can be important controlling factors in bloom sequence determination (Keating 1977). Cell-free filtrates of bloom-dominant algae produced only negative or neutral effects on growth of immediate predecessors in the bloom sequence. They produced only positive or neutral effects on their immediate successors. The competitive position of a bloom-forming species is thus enhanced by biotic interactions with the other principal bloom-forming algae (Keating 1977). Lam and Silvester (1979) studied interactions among *Anabaena oscillarioides*, *Microcystis aeruginosa*, and *Chlorella sp.* In mixed culture experiments, mutual antagonism was found between *Anabaena* and *Chlorella*, and between *Microcystis* and *Anabaena*. *Microcystis* inhibited *Chlorella* growth. U-tube culture experiments demonstrated the inhibitory effects of *Anabaena* and *Microcystis* on *Chlorella* and of *Microcystis* on *Anabaena*. Inhibition by *Microcystis* was attributed to

the production of inhibitory extracellular products. The inhibitory effect of *Anabaena* on the other algae was probably due to nutrient competition, with more efficient uptake of phosphate by *Anabaena*.

Algal Assay Studies

The National Eutrophication Research Program prepared a tentative procedure for a standardized algal assay in 1968. The procedure was designed to: (1) identify nutrients limiting to algal growth, (2) determine the biological availability of algal growth-limiting nutrients, (3) quantify biological response to changes in concentrations of limiting nutrients, and (4) develop a rational framework for application of quantified parameters to practical problems (Maloney and Miller 1975). The procedure was closely examined, and three types of tests were recognized: a static "bottle test," a continuous flow chemostat test, and an *in situ* test. In 1969, the procedures were published in the "Provisional Algal Assay Procedure" (Joint Industry/Government Task Force on Eutrophication 1969). Extensive laboratory evaluation of the tests followed. An interlaboratory precision test (Weiss and Helms 1971) demonstrated that algal assay experiments had relatively poor accuracy among different laboratories, but comparative algal assays performed in one laboratory were very precise. The test showed that algal growth response was linearly related to nutrient strength in the medium. The standard batch assay test was further refined, and *Selenastrum* was selected as the principal test organism in "The *Selenastrum capricornutum* Printz Algal Assay Bottle Test" (Miller, Greene, and Shiroyama 1978).

Since its establishment as a standard method, the algal assay bottle test has been widely used to study eutrophication problems. Miller and Maloney (1971) conducted bioassays on Shagawa Lake, Minnesota. From algal assay results and limnological data, the authors concluded that the installation of advanced waste treatment capable of high phosphorus removal would significantly retard eutrophication in the lake (Miller and Maloney 1971; Maloney *et al.* 1973). Algal assays were used to determine the effects of nitrogen, phosphorus, and carbon additions on growth rate in nine Oregon lakes of varying water quality (Maloney, Miller, and Shiroyama 1972). The authors found that, in those lakes determined to be phosphorus limited, increased phosphorus inputs would result in larger algal standing crops. Greene *et al.* (1975) used algal assays to study the effects of municipal, industrial, and agricultural wastewater effluents upon algal growth in the Snake River system. High concentrations of nitrogen and phosphorus in the Snake River basin had resulted in excessive algal growth. Phosphorus or nitrogen limited growth in the majority of water samples tested.

Natural phytoplankton populations have also been used in nutrient enrichment experiments. Assays in large plastic enclosures were conducted using the natural populations of Burntside River and Shagawa Lake, Minnesota (Powers *et al.* 1972). Growth response was measured by chlorophyll analyses. Phosphate and inorganic nitrogen concentrations were very low in Burntside River; therefore, a positive growth response was achieved only from combined nitrogen and phosphorus enrichment. Shagawa Lake water, in some experiments, responded significantly to single additions of phosphorus or nitrogen, as well as to the combined addition. The effect of phosphorus on growth was much greater than the effect of

nitrogen alone. The stimulatory effect of phosphorus occurred in spite of very low nitrogen concentrations in the water; this was attributed to the growth of *Anabaena*, a nitrogen-fixing alga. Results obtained by Powers *et al.* (1972) compared favorably to results of *Selenastrum* assays on the same water body (Miller and Maloney 1971). In a long-term bioassay study on water from Lake George, New York, Fuhs *et al.* (1972) found that the natural population was limited by both phosphorus and nitrogen. Schelske *et al.* (1975) enriched natural phytoplankton assemblages in Lake Michigan with nutrients. Subsequent carbon fixation rates and chlorophyll production were measured in plastic bottles floated in lake water. Phosphorus exhibited stimulatory effects at all stations while silica addition resulted in stimulation at some stations. Gerhart and Likens (1975) conducted several types of natural enrichment experiments on an oligotrophic, softwater lake in New Hampshire. Simultaneous growth limitation by nitrogen and phosphorus was demonstrated in experiments in large polyethylene enclosures, in continuous chemostat experiments, and in long-term ^{14}C bioassays. Species responding to nitrogen and phosphorus enrichment differed in the polyethylene enclosures from those in continuous culture experiments.

Bioassays have been conducted using several test species simultaneously. Shiroyama *et al.* (1976) pointed out the need to use more than one species if the possibility of a biological toxin existed. In their experiments on Shagawa Lake, *Selenastrum* exhibited nitrogen limitation in filtered and autoclaved-filtered water samples. Growth of *Anabaena flos-aquae* was not stimulated in filtered waters, but was stimulated by phosphorus in autoclaved water samples. The authors concluded that the lack of growth in filtered waters was probably due to a toxin in the

water. An *Aphanizomenon* bloom existed prior to the sampling time and may have produced the toxin. Lange's (1971) bioassays on filtered Lake Erie water, using four test organisms, also illustrated differences in species response. Following an *Aphanizomenon* bloom, lake water killed *Microcystis* and *Anabaena* cells, affected *Nostoc*, but had no effect on *Selenastrum* growth. Greene *et al.* (1978) compared assay results using *Selenastrum* and *Anabaena* with results using *Sphaerocystis*, a dominant green alga isolated from Long Lake, Washington. Due to zinc toxicity, *Selenastrum* and *Anabaena* did not grow well in control treatments. When the chelator EDTA was added, significant increases in growth occurred. *Sphaerocystis* did not require addition of EDTA for maximum growth. The authors concluded that zinc concentrations present in the lake had no adverse effects on growth of the indigenous phytoplankton.

Few authors have combined bioassays using laboratory cultures of algae with experiments on the natural populations. O'Brien and deNoyelles (1976) compared results from three types of bioassays. Experimental ponds responded to addition of nitrogen and phosphorus fertilizers, and the N:P ratio in the ponds suggested nitrogen as the principal limiting nutrient. Batch bioassays using *Pandorina*, isolated from the pond, demonstrated nitrogen limitation. Chemostat experiments on the natural phytoplankton, although not designed to show sole nitrogen or phosphorus limitation, showed stimulation due to their combined addition. Changes in species composition, similar to shifts in the enriched ponds, were noted. Short-term primary productivity experiments showed no stimulation of ^{14}C uptake upon nutrient enrichment. A similar result was obtained by Gerhart and Likens (1975). Schelske *et al.*

(1978) conducted three types of experiments on the Great Lakes: a spiked test using *Selenastrum*, a spiked test using the natural population, and a fixed-level test using the natural population. Results from the three experiments agreed for Lake Huron and Lake Michigan waters, which were limited by phosphorus. The authors concluded that enrichment experiments with natural populations could be used effectively to determine algal growth-limiting nutrients. Natural assays provided information on population dynamics that could not be obtained from bioassays using test species.

In order to use algal bioassay results to study eutrophication problems, findings must be related to limnological characteristics of the water body studied. Maloney, Miller, and Shiroyama (1972) found that algal growth rates in bioassays were strongly correlated to dissolved phosphorus concentrations in the Oregon lake waters studied. Weiss (1976) related results of *Selenastrum* assays to nitrogen to phosphorus (N:P) ratios in the water. Assays were placed into three categories of nutrient limitation. A mean ratio of inorganic nitrogen to soluble phosphorus (by weight) for all water samples was calculated for each group. P-limited samples exhibited N:P ratios greater than 13, whereas N-limited waters had N:P ratios in the range of 5 to 7. Samples limited by both N and P exhibited ratios in the range of 9 to 11. Chiaudani and Vighi (1975) determined that waters were P-limited when the inorganic nitrogen to phosphate ratio (by weight) was greater than 10. A ratio of less than 5 signified N-limitation. Miller *et al.* (1974) found a high degree of correlation between algal assay responses and the reported trophic state of lakes. Phosphorus limitation decreased as the reported trophic state increased. Payne (1976) determined that eutrophic

water bodies that exhibited high winter nutrient concentrations supported high standing crops in *Selenastrum* assays. High populations of phytoplankton in those water bodies were evident the following spring.

Nutrient enrichment studies are subject to procedural problems and difficulties in interpretation. In the *Selenastrum* assay, a choice must be made whether to pretreat the water by autoclaving followed by filtration or by filtration alone. Fitzgerald (1975) noted that membrane filtration removed all insoluble particles from the water sample. Upon filtration, all phytoplankton were removed completely, and nutrients released from recycling of the plankton were therefore not available for growth of the test algae. Autoclaving released available phosphorus, nitrogen, and iron from the natural algae. Weiss (1976) reported that autoclaving produced higher nutrient levels and greater control biomass in virtually all samples. He interpreted algal growth, following autoclaving as the pretreatment, as total growth potential. Growth in filtered samples was interpreted as available growth potential. Shiroyama *et al.* (1976) pointed out the importance of using both pretreatments when the presence of a biologically-produced toxin is suspected.

Problems inherent in long-term enrichment studies were discussed by Healey (1979). Factors such as light and grazing, that can limit algal growth, are altered. Natural loading and nutrient regeneration from sediments and plankton are eliminated or severely altered. Assay results are affected by long-term enclosure of small water volumes. Schindler (1971) noted the difficulty in extrapolating from nutrient enrichment experiments under selected conditions to the responses of natural phytoplankton. It is well documented that changes in species

composition occur in response to varying nutrient additions. Menzel *et al.* (1963) found that very different phytoplankton populations were produced from different enrichments of Sargasso Sea water. Relative ratios of different nutrients in the water provided an important selective mechanism for the phytoplankton. The greater success of diatoms in enrichment experiments, in relation to other groups, has been observed by several authors (Menzel *et al.* 1963; Thomas and Dodson 1974; Venrick *et al.* 1977).

METHODS

Field Sampling

Water samples were collected monthly from May through September, 1980 from Chowan River Stations 1, 4, and 7 (Figure 1). Samples were taken midstream at a depth of 0.5 m, using a Guzzler hand pump, and were stored in large carboys. These were shaded until returned to the laboratory at Edenton, several hours after collection. At each station, a Secchi disk depth reading was obtained. Temperature and conductivity readings were determined using a YSI Model 33 meter.

Laboratory Analyses

Samples for determination of filterable reactive phosphorus (FRP, will be referred to as phosphate (PO_4) in text), ammonia (NH_3-N), nitrate plus nitrite (NO_3+NO_2-N), and total phosphorus (Total P) were filtered soon after collection using Whatman glass microfibre filters (GF/C, 4.25 cm). In May and June, the same analyses were also performed on water filtered through 0.45 μm Metricel membrane filters (GA-6, 47 mm). Unfiltered water was used for Total Kjeldahl Nitrogen (TKN) and Total P analyses. Water was filtered through precombusted GF/C filters for particulate organic carbon (POC) and through GF/C filters for particulate organic nitrogen (PON) analyses. Analytic methods are outlined in Table 1. Water samples were preserved for all analyses except FRP and NH_3-N. Preservation methods used were: freezing in May, acidification, refrigera-

Table 1. Methods for nutrient analyses.

Nutrient	Method	Reference
Filterable Reactive Phosphorus (FRP)	Mixed Reagent Ascorbic Acid Reduction	Wetzel and Likens (1979), p. 90
Ammonia Nitrogen (NH_3-N)	Phenol-hypochlorite using Nitroprusside as Catalyst	Wetzel and Likens (1979), p. 83
Nitrate and Nitrite Nitrogen (NO_3+NO_2-N)	Automated Hydrazine Reduction	EPA (1979), Method 353.1
Nitrite Nitrogen (NO_2-N)	Automated Diazotization	EPA (1971), Method 353.1
Total Kjeldahl Nitrogen (TKN)	Block Digestor Automated	Technicon Autoanalyzer Methodology (1975), Industrial Method No. 329-74W; EPA (1979), Method 351.2 (for digestion mix)
Particulate Organic Nitrogen (PON)	Block Digester Automated	Technicon Autoanalyzer Methodology (1975), Industrial Method No. 329-74W; EPA (1979), Method 351.2 (for digestion mix)
Total Phosphorus (Total P)	Acid Persulfate Digestion, Automated Stannous Chloride	FWPCA (1969), p. 247
Particulate Organic Carbon (POC)	Wet Oxidation by Potassium Dichromate	Wetzel and Likens (1979), p. 131

tion, and subsequent neutralization in June, and addition of $HgCl_2$ and refrigeration in July through September.

Turbidity measurements were made on water samples brought back to Chapel Hill. A model 2100 Hach Turbidimeter was used. Samples for chlorophyll analyses were filtered through GF/C glass fiber filters in Edenton. Filters were frozen, and analyses were performed in Chapel Hill (Strickland and Parsons 1972). Subsamples of water from each station were preserved with Lugol's solution (Wetzel and Likens 1979) for later phytoplankton identification and enumeration. Appropriate volumes were settled in sedimentation chambers. Algae were examined through a Unitron inverted microscope and were identified utilizing the keys of Cocke (1967), Whitford and Schumacher (1969), Prescott (1962), and Smith (1950).

Pretreatment for Experiments

Water for use in *Selenastrum* bioassays was filtered as soon as possible after sample collection. Filters were washed with 10% hydrochloric acid and distilled water before sample filtration. Filtration for the May and June experiments used 0.45 μm Metricel membrane filters (GA-6, 47 mm). While preparing for the July experiment, increases in nitrogen, especially NO_3-N, were noted in membrane-filtered as compared to glass fiber-filtered samples. The increases were attributed to leaching from the membrane filters. Therefore, water for use in the July, August, and September experiments was filtered through Whatman glass microfibre filters (GF/C, 4.25 cm) only. This simplified the nutrient analyses required and still effectively removed the indigenous phytoplankton.

Filtered water samples were refrigerated at 4°C in completely filled plastic bottles until assays were conducted, in three weeks time or less.

Natural enrichment experiments required inocula of unfiltered water from the three stations. The unfiltered water was kept at room temperature for one to two days until assays were started. Water was also glass fiber-filtered (Whatman, GF/C, 4.25 cm) and kept refrigerated at 4°C for use in the natural assays. Experiments were begun as soon as possible upon return to Chapel Hill.

Selenastrum Assays

Methods for the *Selenastrum capricornutum* Printz algal assay test closely followed the procedure outlined by Miller, Greene, and Shiroyama (1978). A *Selenastrum* culture, obtained from the EPA laboratory in Corvallis, Oregon, was maintained by frequent transfers in synthetic nutrient medium (Table 2). Six to nine day cultures, centrifuged and resuspended in sterile distilled-deionized water several times, provided the inoculum. *Selenastrum* stock cultures were counted in hemacytometer counting chambers with a Spencer American Optical microscope. Appropriate dilutions were made to yield the desired inoculum concentration. Final *Selenastrum* concentrations in the flasks were 2.3×10^4 in May and ranged from 3.3×10^3 to 4.3×10^3 in June through September. Experimental treatments included medium and river controls, additions of phosphorus, nitrogen, phosphorus plus nitrogen, and complete (ALL) additions (Table 3). An algal inoculum of 1 mℓ was added to 59 mℓ of each treatment, in 250 mℓ Erlenmeyer flasks. Treatments were run in triplicate. Sterile technique was used whenever possible to cut down on bacterial

Table 2. Composition of synthetic algal nutrient medium showing final concentrations of compounds and final concentrations of elements.*

Macronutrients		Micronutrients	
Compound	Concentration (mg/ℓ)	Compound	Concentration (μg/ℓ)
$NaNO_3$	25.500	H_3BO_3	185.520
$MgCl_2 \cdot 6H_2O$	12.164	$MnCl_2 \cdot 4H_2O$	415.610
$CaCl_2 \cdot 2H_2O$	4.410	$ZnCl_2$	3.271
$MgSO_4 \cdot 7H_2O$	14.700	$CoCl_2 \cdot 6H_2O$	1.428
K_2HPO_4	1.044	$CuCl_2 \cdot 2H_2O$	0.012
$NaHCO_3$	15.000	$Na_2MoO_4 \cdot 2H_2O$	7.260
		$FeCl_3 \cdot 6H_2O$	160.000
		$Na_2EDTA \cdot 2H_2O$	300.000
Element	Concentration (mg/ℓ)	Element	Concentration (μg/ℓ)
N	4.200	B	32.460
Mg	2.904	Mn	115.374
Ca	1.202	Zn	1.570
S	1.911	Co	0.354
P	0.186	Cu	0.004
Na	11.001	Mo	2.878
K	0.469	Fe	33.051
C	2.143		

*Miller, Greene, and Shiroyama (1978).

Table 3. Experimental design for *Selenastrum* assays.*

Treatment	Description
Medium Control	Standard *Selenastrum* medium**
River Control (CNTL)	River water without nutrient additions
Phosphorus	River water plus: 0.05 mg-P/ℓ or 0.10 mg-P/ℓ
Nitrogen (N)	River water plus: 1.00 mg-N/ℓ or 2.00 mg-N/ℓ
Phosphorus plus nitrogen (P+N)	River water plus: 0.05 mg-P/ℓ + 1.00 mg-N/ℓ or 0.10 mg-P/ℓ + 2.00 mg-N/ℓ
All (ALL)	River water plus: 0.05 mg-P/ℓ + 1.00 mg-N/ℓ + all other nutrients as in standard medium or 0.10 mg-P/ℓ + 2.00 mg-N/ℓ + all other nutrients as in standard medium

*P added as K_2HPO_4 and N added as $NaNO_3$.

**See Table 2.

contamination. Experiments were carried out at 25 ± 1°C under continuous fluorescent lighting of 400 ± 100 foot-candles. Flasks were stoppered with cotton plugs and shaken at approximately ninety linear oscillations per minute. Algae in all flasks were counted at the end of the experiments, which lasted for ten days. Several counts over the ten day period were made for two of the experiments in order to establish the nature of the growth curves.

Natural Enrichment Experiments

Natural enrichment experiments were started within three days of sample collection. One part unfiltered water from each station was added to nine parts filtered water. The diluted water samples were then used in the natural assays. Subsamples were taken from the initial water for dry weight analyses, TKN and Total P determinations, and algal identifications. Nutrient treatments were identical to those in the *Selenastrum* assays (Table 3), with the exception of the media control. Conditions of light, temperature, and shaking were similar for the two experiments. Flasks contained 80 mℓ of control or treated water. Assays were ended after ten days and 10 mℓ of each sample were removed and preserved for algal identification and enumeration. In order to expedite counting, only composite samples of replicates were examined. Only algae present in large numbers were identified. Counts were very approximate as small transects were examined. Counting of samples with high algal concentrations was hindered by clumping, resulting in non-random distributions in the counting chambers.

Dry Weight Analyses

Algal dry weights were determined gravimetrically at the end of both *Selenastrum* and natural assays. Metricel membrane filters (0.8 μm, 47 mm) were soaked in distilled water for at least thirty minutes, dried at 70°C, dessicated, and weighed immediately prior to filtration. Known sample volumes were filtered. Filters were then dried, dessicated, and reweighed. Dry weights were calculated as the difference between final and initial filter weights. Filters lose weight in this process; therefore, distilled water alone was filtered through at least eight filters to provide a blank correction. The correction consisted of adding the mean loss in weight of the blanks to the sample dry weights. The corrected values were then converted to mg/ℓ dry weight. In some cases, negative values of dry weights were obtained. These were recorded as 0 mg/ℓ dry weight for purposes of data analysis. For *Selenastrum* assays, dry weights represented algal growth. However, adjustment was necessary in natural assays to account for different initial dry weights among the three stations. Mean initial dry weights for the stations were subtracted from the corresponding final dry weights. The adjusted numbers more accurately reflected algal growth.

Statistical Analyses

Multiple regression analyses were run for the eight experiments for the three sampling stations; the General Linear Models procedure (Barr *et al.* 1979) was used. Algal dry weights, in mg/ℓ, of all samples, were the dependent variables. Independent variables in the model were con-concentrations, in μg/ℓ, of phosphate (PO_4-P) and total soluble inorganic nitrogen (NH_3+NO_3+NO_2-N) (TSIN) in the samples. These values repre-

sented the sums of the concentrations of original nutrient in the river water sample plus any added nutrient spike. The model used in this procedure was:

$$E(y_{ij}) = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij},$$

where: $E(y_{ij})$ = expected value of algal dry weight for specified concentrations of PO_4-P and TSIN,

μ = overall mean dry weight,

α = effect of PO_4-P on dry weight,

β = effect of TSIN on dry weight,

$(\alpha\beta)$ = effect of the interaction of PO_4-P and TSIN on dry weight.

The interaction term was necessary because assay results consistently demonstrated an increase in biomass due to combined nitrogen and phosphorus addition, an increase above the sum of the two additions made separately. Partial F statistics were examined. These assess the significance of one variable given the other two already in the model. Significant effects on algal dry weight were attributed to variables whose F statistics were significant at the $\alpha = 0.05$ level.

Before dry weight values could be used in multiple regression models, transformation was necessary for data from all months but one. Multiple regression models assume homoscedasticity, equal variances for the dependent variable at all fixed combinations of independent variables. Plots of variances of treatment means *versus* actual means for each experiment showed an overall pattern of increasing variances with increasing means. The only exception was the May experiment; therefore, no transformations were required for that month. Square root and log-

arithmetic transformation were performed on dry weight data; the former appeared to stabilize the variances in relation to the magnitude of the means. The actual transformation was $\sqrt{y + 1/2}$ to account for dry weight values of zero (Sokal and Rohlf 1969). Residual analyses (Kleinbaum and Kupper 1978) of all experiments showed that using square-root transformed dry weights in the multiple regression models was appropriate.

RESULTS

Physical and Chemical Characteristics

Data on physical characteristics for Chowan River sampling Stations 1, 4, and 7 from May 1980 through September 1980 (Table 4) show increasing water temperatures. Conductivity measurements ranged from 66 to 89 μmho and showed no marked trends with station or month. Secchi depth varied between 0.4 and 1.15 m. Measurements of turbidity showed relatively little variation with time or station.

Several trends emerge from examination of NH_3-N, NO_3-N, and NO_2-N concentrations (Table 5). High concentrations of total soluble inorganic nitrogen (NH_3+NO_3+NO_2-N) were found consistently at Station 1. Concentrations of the three inorganic nitrogen forms tended to decrease from May to August. NH_3-N was always present in the water while NO_3-N was reduced to undetectable levels at several stations in July and September. Concentrations of NO_2-N were low for all stations throughout the summer. The dominant nitrogen form in the river was organic nitrogen (obtained by subtracting NH_3-N from TKN). Particulate organic nitrogen (PON) was higher at the downstream stations. PON values corresponded well with particulate organic carbon and chlorophyll values, indicating that most of the PON was tied up in algal biomass. Concentrations of dissolved organic nitrogen (DON, obtained by subtracting PON from total organic nitrogen) showed much less variation than was evident in the PON data. Comparison of DON and PON data for the May, June, and July sampling dates shows that more organic nitrogen was present in the dissolved rather

Table 4. Data on temperature, conductivity, Secchi depth, and turbidity for Chowan River Stations 1, 4, and 7 for the period May through September, 1980.

		5/12,13	6/10,11	7/8,9	8/5,6	9/5,6
Temperature (°C)	1	19.2	24.2	26.5	29.0	27.6
	4	19.8	24.8	28.5	30.0	29.0
	7	21.0	23.5	27.0	29.0	27.0
Conductivity (μmho)	1	75	70	66	71	74
	4	77	75	76	74	77
	7	75	70	82	84	89
Secchi depth (m)	1	1.1	1.1	0.8	0.8	0.9
	4	0.9	0.75	0.4	0.8	1.15
	7	1.2	0.75	0.75	0.8	1.0
Turbidity (JTU)	1	5	16	8	8	8
	4	4.5	8	9	4.5	4
	7	5	8	5	3	5

Table 5. Chemical data for Chowan River Stations 1, 4, and 7 for the period May through September, 1980.

		5/12,13*		6/10,11*		7/8,9	8/5,6	9/5,6
NH_3-N (µg N/ℓ)	1	74.5	(115)	60.6	(71)	50.5	13.0	59.5
	4	8.0	(6)	19.7	(18)	7.2	7.4	29.6
	7	7.0	(8)	35.5	(18)	11.3	5.8	10.1
NO_3-N (µg N/ℓ)	1	241	(232)	195.6	(179)	26.0	1.4	UD
	4	78	(60)	3.3	(3)	UD	4.5	UD
	7	15	(7)	8.2	(7)	UD	10.8	17.2
NO_2-N (µg N/ℓ)	1	11	(7)	4.3	(3)	3.6	3.8	2.9
	4	7	(2)	2.7	(3)	1.8	3.6	2.2
	7	3	(2)	2.6	(2)	2.1	1.6	2.0
FRP (µg P/ℓ)	1	38.8	(42)	33.0	(55)	42.1	41.5	43.8
	4	17.5	(12)	8.4	(13)	10.6	13.2	6.9
	7	6.7	(9)	4.7	(5)	7.8	6.4	2.8
TSIN:FRP	1	6.7	(8.4)	7.9	(4.6)	1.9	0.4	1.4
	4	5.3	(5.7)	3.1	(1.8)	0.8	1.2	4.6
	7	3.7	(1.9)	9.9	(5.7)	1.7	2.8	10.5
DON (µg N/ℓ)	1	840	(800)	810	(800)	700	130	480
	4	740	(740)	810	(810)	1090	430	430
	7	500	(500)	870	(890)	870	380	300
PON (µg N/ℓ)	1	50		190		90	180	140
	4	200		450		740	380	330
	7	130		660		600	340	280
Total P unfiltered (µg P/ℓ)	1	42.0		49.7		63.2	82.8	79.7
	4	54.0		31.0		79.0	64.7	45.3
	7	28.0		23.6		63.0	39.7	37.3
FUP (µg P/ℓ)	1	-	(M)	18.1	(14)	35.5	5.5	19.2
	4	-	(17)	8.2	(26)	20.7	9.5	22.1
	7	4.3	(4)	7.7	(17)	18.9	10.9	18.2
Particulate P (µg P/ℓ)	1	26.5	(M)	-	(-)	-	35.8	16.7
	4	45.0	(26)	14.4	(-)	47.8	42.0	16.3
	7	17.0	(15)	11.2	(2)	36.3	22.4	16.3
POC (µg C/ℓ)	1	150		910		320	780	910
	4	1370		2160		3950	2910	2210
	7	900		3160		1550	3390	1630
Chl. a (mg/m^3)	1	1.8		1.8		4.3	9.8	13.2
	4	25.0		28.6		66.8	29.3	21.0
	7	13.0		51.2		46.9	29.8	22.0

Table 5. (continued)

		5/12,13*	6/10,11*	7/8,9	8/5,6	9/5,6
TKN** (μg N/ℓ)	1		840	760		230
	4		810	940		550
	7		1020	760		300
Total P** (μg P/ℓ)	1		M	63.0		68.0
	4		M	47.3		33.3
	7		M	34.5		18.3

*May and June assay water was filtered through 0.45 μ filters; primary listing is for this water; values in parentheses are concentrations in glass fiber filtered water; water for July, August, and September assays was filtered only through glass fiber filters.

**TKN and Total P determined on initial water samples in natural enrichment assays (1 part unfiltered:9 parts filtered).

M missing value.

- negative value obtained.

than particulate form. In the late summer, concentrations of particulate and dissolved forms of organic nitrogen were similar except for Station 1 in September.

Concentrations of PO_4-P (approximately equal to FRP) showed little variation within one station over time, but showed marked variation between stations (Table 5). Highest values were found consistently at Station 1. Concentrations of filterable unreactive phosphorus [FUP, obtained by subtracting FRP from Total P (filtered)] were variable. Values for particulate phosphorus were obtained by subtracting Total P (filtered) from Total P (unfiltered). At Station 1, the dominant phosphorus form was PO_4-P throughout the summer. Particulate P was generally the dominant form at Stations 4 and 7. No overall trends emerged from examination of Total P (unfiltered) values, although variation among stations and sampling times was evident.

The ratio of NH_3+NO_3+NO_2-N to PO_4-P (TSIN:FRP, Table 5) was computed for all stations and sampling times. Ratios were generally higher during the first part of the summer, but definite trends were lacking.

Particulate organic carbon concentrations were consistently higher at Stations 4 and 7 as compared to Station 1 (Table 5). A similar pattern was observed for the chlorophyll data.

Concentrations of TKN and Total P (unfiltered) were measured for initial water samples in natural enrichment experiments (Table 5). As unfiltered water was diluted to obtain these initial samples, the values are lower than corresponding river concentrations.

Phytoplankton

Approximate cell counts and identifications were made on preserved

samples from the five sampling times. Higher algal concentrations were found at Stations 4 and 7 as compared to Station 1. Station 1 phytoplankton consisted mainly of diatoms and some green algae (Table 6). *Melosira spp.*, a diatom, was abundant in almost all river samples examined. The dominant algae found at Stations 4 and 7 were the blue-greens, including: *Aphanizomenon flos-aquae, Anabaena spiroides, Anabaena flos-aquae, Anabaena affinis, Anacystis incerta, Anacystis cyanea,* and *Oscillatoria spp. (?).* Some uncertainty exists concerning the identification of *Oscillatoria* in preserved samples. A significant portion of the filaments thus identified may have been filamentous bacteria. A large number of unidentified unicellular algae were present in some samples. Due to staining from the preservation method used, identification was not possible.

Experimental Results

Mean dry weights for all treatments for *Selenastrum* assays and natural enrichment experiments are listed with the corresponding sample concentrations of TSIN and PO_4-P (Table 7). Final mean cell counts from *Selenastrum* assays are also included. Cell counts were not used in data analyses because the ratio of dry weight to cell counts was not constant for different treatments. Cells in flasks spiked with phosphorus only were smaller than in other treatments. Therefore, an increase in cell counts did not necessarily signify a concomitant increase in total algal biomass. Typical *Selenastrum* growth curves from the May, Station 4 experiment show that most of the growth occurred in the first three days (Figure 2). In this experiment, *Selenastrum* cells did not grow in the control and N treatments. P appeared to stimulate growth, as did P+N and ALL treatments.

Table 6. Dominant phytoplankton in Chowan River samples for the period May through September, 1980.

	Station 1	Station 4	Station 7
5/12,13	Diatoms Green algae	*Melosira spp.** *Nitzschia spp.* *Anabaena spp.*	*Melosira spp.** *Aphanizomenon flos-aquae* *Nitzschia spp.* *Anabaena spp.*
6/10,11	*Nitzschia spp.* Other diatoms Green algae	*Melosira spp.* *Nitzschia spp.* *Aphanizomenon flos-aquae* *Anacystis incerta* *Oscillatoria spp. (?)*	*Aphanizomenon flos-aquae** *Melosira spp.* *Anacystis incerta*
7/8,9	*Melosira spp.* *Nitzschia spp.*	*Anabaena spiroides** *Melosira spp.* *Anabaena spp.* *Aphanizomenon flos-aquae* *Anacystis incerta*	*Anabaena spp.* *Melosira spp.* *Anacystis spp.* *Scenedesmus spp.* *Oscillatoria spp. (?)* *Aphanizomenon flos-aquae*
8/5,6	*Melosira spp.**	*Melosira spp.* *Oscillatoria spp. (?)* *Anabaena spp.* *Anacystis spp.* *Scenedesmus spp.*	*Melosira spp.* *Oscillatoria spp. (?)* *Anacystis spp.* *Anabaena spp.* *Scenedesmus spp.*
9/5,6	*Melosira spp.**	*Melosira spp.* *Anacystis spp.* *Anhistrodesmus falcatus* *Scenedesmus spp.*	*Melosira spp.* *Anhistrodesmus falcatus* *Anacystis spp.* *Aphanizomenon flos-aquae* *Scenedesmus spp.* *Oscillatoria spp. (?)*

*Alga is primary dominant in sample; algae are listed in decending order of importance.

Table 7. Data on concentrations of PO_4-P and TSIN in experimental flasks (ambient river concentration plus nutrient spike), mean algal dry weights ± one standard deviation for *Selenastrum* and Natural assays, and mean cell counts for *Selenastrum* assays for Chowan River Stations 1, 4, and 7.

Date	Station	Treatment	PO_4-P (µg/ℓ)	TSIN (µg/ℓ)	Mean Dry Wt. *Selenastrum* (mg/ℓ)	Mean Dry Wt. Natural (mg/ℓ)	Mean Cell Counts (cells/mℓ)
5/12,13	1	CNTL	39	327	3.6 ± 4.8		3.6×10^4
		P	89	327	21.8 ± 3.2		7.8×10^5
		N	39	1327	7.9 ± 1.0		2.1×10^4
		P+N	89	1327	52.7 ± 3.7		1.2×10^6
		ALL	89	1327	49.1 ± 0		1.0×10^6
	4	CNTL	18	93	3.6 ± 4.8		2.3×10^4
		P	68	93	9.1 ± 3.6		2.5×10^5
		N	18	1093	2.4 ± 2.8		1.9×10^4
		P+N	68	1093	40.6 ± 3.7		7.1×10^5
		ALL	68	1093	38.2 ± 3.2		6.6×10^5
	7	CNTL	7	25	2.4 ± 2.8		1.9×10^4
		P	57	25	6.7 ± 4.2		1.4×10^5
		N	7	1025	3.6 ± 3.7		2.4×10^4
		P+N	57	1025	43.6 ± 3.7		8.1×10^5
		ALL	57	1025	43.6 ± 0		8.5×10^5
6/10,11	1	CNTL	37	261	4.8 ± 3.6	20.1 ± 1.2	1.1×10^5
		P	133	261	12.5 ± 4.1	25.0 ± 2.3	4.6×10^5
		N	33	2261	19.3 ± 0.4	26.1 ± 4.1	2.9×10^5
		P+N	133	2261	95.5 ± 4.3	68.2 ± 6.1	2.8×10^6
		ALL	133	2261	93.6 ± 2.6	77.0 ± 11.1	2.9×10^6
	4	CNTL	8	26	0	9.4 ± 5.9	4.1×10^3
		P	108	26	0.9 ± 1.5	49.9 ± 1.9	1.5×10^4
		N	8	2026	3.3 ± 2.9	12.3 ± 1.0	2.6×10^3
		P+N	108	2026	67.7 ± 7.8	40.5 ± 1.4	2.1×10^6
		ALL	108	2026	80.3 ± 11.2	37.3 ± 11.4	2.6×10^6
	7	CNTL	5	46	1.0 ± 1.8	5.8 ± 2.5	3.3×10^3
		P	105	46	0.1 ± 0.1	27.5 ± 9.2	5.3×10^4
		N	5	2046	0.5 ± 0.9	3.7 ± 2.1	3.3×10^3
		P+N	105	2046	75.6 ± 6.4	34.2 ± 0.7	2.2×10^6
		ALL	105	2046	78.3 ± 4.4	39.6 ± 5.2	2.2×10^6

Table 7. (continued)

Date	Station	Treatment	PO_4-P	TSIN	Mean Dry Wt. *Selenastrum*	Mean Dry Wt. Natural	Mean Cell Counts
7/8,9	1	CNTL	42	80	7.1 ± 1.9	9.2 ± 2.8	1.7×10^5
		P	92	80		4.8 ± 1.4	
		P	142	80	11.2 ± 1.3	10.3 ± 1.0	2.7×10^5
		N	42	1080		20.0 ± 6.3	
		N	42	2080	17.1 ± 1.6	19.5 ± 3.5	2.7×10^5
		P+N	142	2080	74.2 ± 5.0	73.5 ± 12.5	3.2×10^6
	4	CNTL	11	9	5.7 ± 1.0	8.2 ± 2.6	4.7×10^4
		P	61	9		9.8 ± 2.5	
		P	111	9	8.5 ± 1.4	12.8 ± 5.3	1.6×10^5
		N	11	1009		12.1 ± 2.7	
		N	11	2009	5.7 ± 0.5	8.5 ± 3.4	3.4×10^4
		P+N	111	2009	67.0 ± 5.4	50.0 ± 8.7	2.0×10^6
	7	CNTL	8	13	0.2 ± 0.3	6.7 ± 2.8	6.3×10^3
		P	58	13		10.0 ± 0.3	
		P	108	13	2.0 ± 0.4	13.5 ± 4.0	3.4×10^4
		N	8	2013	2.0 ± 3.5	7.6 ± 1.2	4.1×10^3
		P+N	108	2013	57.5 ± 5.5	54.0 ± 4.4	1.8×10^6
8/5,6	1	CNTL	42	18	2.0 ± 1.4		9.1×10^4
		P	142	18	1.5 ± 1.0		5.8×10^4
		N	42	2018	5.4 ± 5.0		9.3×10^4
		P+N	142	2018	57.5 ± 2.5		2.0×10^6
		ALL	142	2018	78.1 ± 9.4		2.8×10^6
	4	CNTL	13	16	1.0 ± 1.7		2.7×10^3
		P	113	16	1.3 ± 1.5		5.9×10^4
		N	13	2016	0.5 ± 0.5		4.1×10^3
		P+N	113	2016	54.5 ± 4.4		2.0×10^6
		ALL	113	2016	66.8 ± 8.7		2.4×10^6
	7	CNTL	6	18	0.6 ± 0.7		1.8×10^3
		P	107	18	1.8 ± 1.4		5.5×10^3
		N	6	2018	1.6 ± 1.4		4.4×10^3
		P+N	107	2018	65.3 ± 3.1		2.2×10^6
		ALL	107	2018	70.3 ± 4.9		2.4×10^6
9/5,6	1	CNTL	44	62	2.6 ± 2.7	4.0 ± 1.6	3.4×10^5
		P	144	62	3.4 ± 2.0	3.9 ± 3.4	9.2×10^4
		N	44	2062	16.0 ± 3.1	25.3 ± 12.6	3.2×10^5
		P+N	144	2062	78.0 ± 8.0	58.9 ± 2.0	2.5×10^6
		ALL	144	2062	86.0 ± 3.0	49.8 ± 3.2	3.1×10^6
	4	CNTL	7	32	0.7 ± 0.6	2.5 ± 0.6	1.3×10^4
		P	107	32	2.2 ± 2.4	3.5 ± 1.3	5.8×10^4
		N	7	2032	1.5 ± 1.8	3.4 ± 1.4	6.7×10^3
		P+N	107	2032	45.4 ± 6.2	52.7 ± 4.5	1.5×10^6
		ALL	107	2032	73.2 ± 0.5	46.0 ± 5.7	2.5×10^6
	7	CNTL	3	29	1.7 ± 1.7	3.1 ± 2.5	2.0×10^4
		P	103	29	1.7 ± 1.2	3.7 ± 2.9	1.8×10^4
		N	3	2029	5.6 ± 3.4	13.0 ± 6.0	4.8×10^4
		P+N	103	2029	70.0 ± 5.5	61.7 ± 8.2	2.2×10^6
		ALL	103	2029	79.1 ± 1.3	47.1 ± 3.7	2.5×10^6

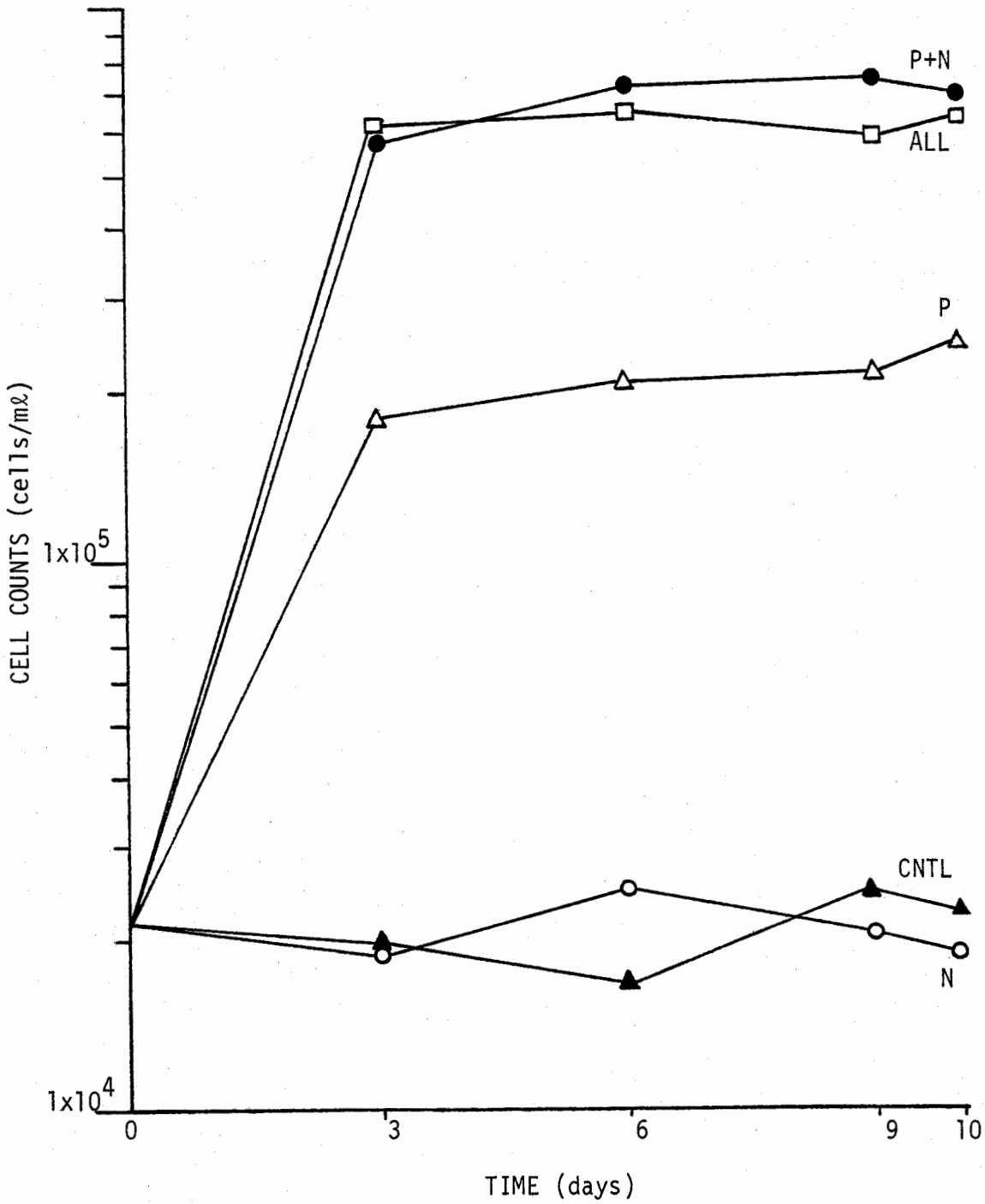

Figure 2. *Selenastrum* growth curves for 12, 13 May 1980 assay on Station 4.

May - *Selenastrum* Assay

Dry weight means ± one standard deviation are presented in bar chart form for the May, *Selenastrum* experiment (Figure 3). Growth in control and nitrogen treatments was similar for the three stations. The addition of phosphorus increased growth markedly at Station 1. Smaller increases were seen at Stations 4 and 7. P+N and ALL additions resulted in large dry weights at all stations.

June - *Selenastrum* Assay

All treatments of June, Station 1 water exhibited greater growth than those of Stations 4 and 7 (Figure 4.A). Single additions of P or N appeared to increase growth slightly. Similar additions to water from Stations 4 and 7 caused no real growth above that of the controls. The three stations showed large dry weight responses in the P+N and ALL treatments.

June - Natural Assay

Dry weights of the natural populations in June, Station 1 river control treatments were greater than the corresponding weights in samples from Stations 4 and 7 (Figure 4.B). Nitrogen-enriched samples exhibited dry weights similar to the controls. P+N and ALL additions augmented growth in water from all three stations, the effect was greatest at Station 1. The addition of P to Stations 4 and 7 samples resulted in biomass of similar magnitude to that of the P+N and ALL treatments.

Identification and enumeration of algae present at the end of the natural assay showed which algae responded to different nutrient treatments. Biomass increases at Station 1 in P+N and ALL treatments were due

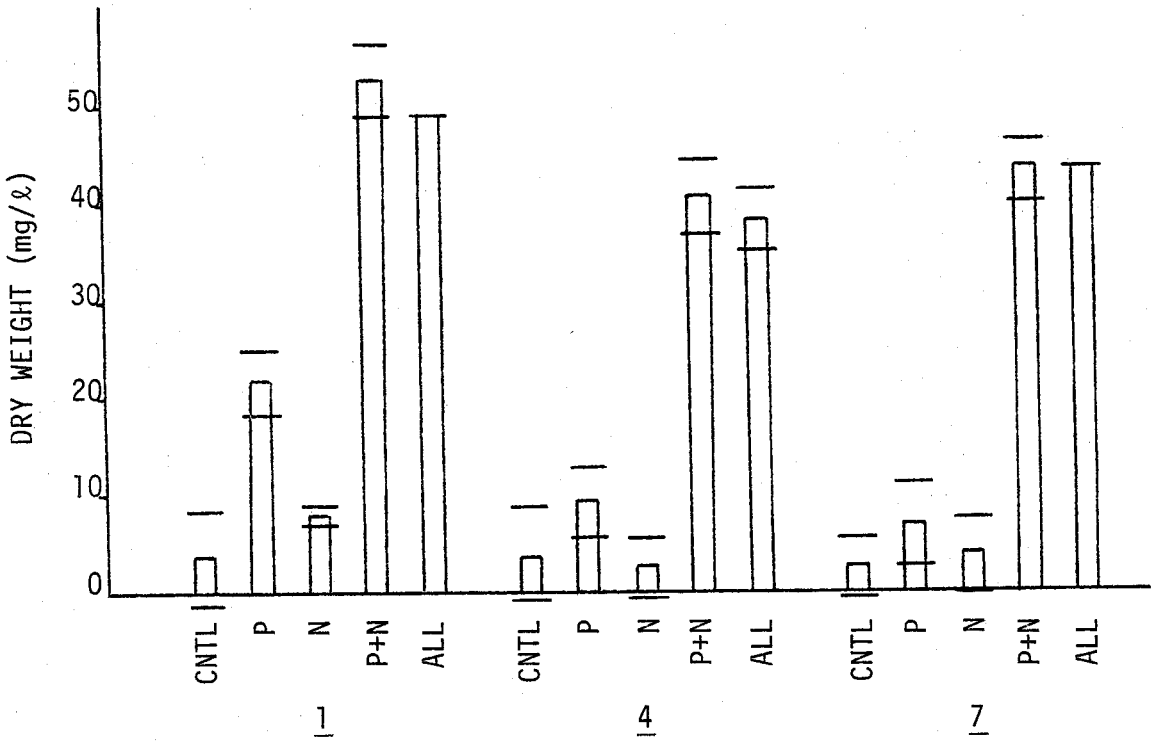

Figure 3. Mean algal dry weights ± one standard deviation (± SD) for *Selenastrum* assay on water collected from Stations 1, 4, and 7 on 12, 13 May 1980.

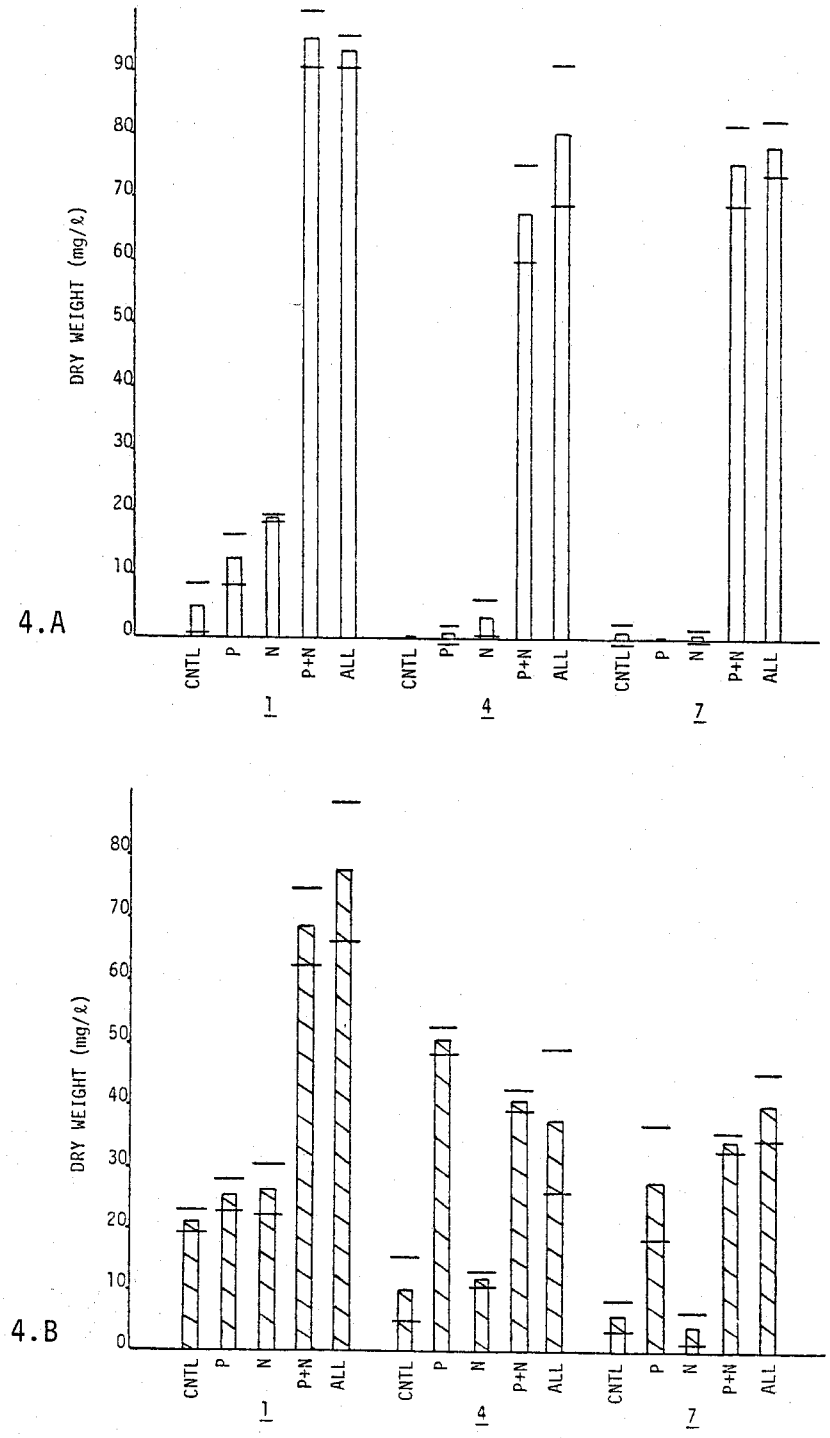

Figure 4. Mean algal dry weights ± SD for *Selenastrum* assay (4.A, unshaded) and Natural assay (4.B, shaded) on water collected from Stations 1, 4, and 7 on 10, 11 June 1980.

almost completely to growth of *Nitzschia spp.* Differential cell counts for Station 4 showed that increases in numbers of *Aphanizomenon flos-aquae* accounted for most of the dry weight in P, P+N, and ALL treatments (Figure 5). *Oscillatoria spp. (?)*,* *Nitzschia spp.*, and *Anacystis incerta* numbers also increased; the effect was more pronounced in P+N and ALL treatments than in the P treatment alone. Cell count data for Station 7 (Figure 6) showed that although *Aphanizomenon* was present in large numbers in the river sample, it was not the dominant species responding to P, P+N, and ALL additions. Increased growth in the P-enriched sample was due to *Aphanizomenon flos-aquae*, *Oscillatoria spp. (?)*. and *Anacystis incerta*. *Oscillatoria spp. (?)*, *Anacystis incerta*, and *Scenedesmus spp.* were the algae responsible for increased biomass in the P+N and ALL treatments.

July - *Selenastrum* Assay

In the July *Selenastrum* experiment, P+N additions increased growth well above the controls for the three stations (Figure 7.A). Sole phosphorus or nitrogen enrichment resulted in some growth stimulation.

July - Natural Assay

Two levels of nitrogen and phosphorus were included in this assay. P+N enrichment resulted in increased growth of the natural population at all stations (Figure 7.B). Nitrogen exerted some stimulatory effect at Station 1. Station 7 phytoplankton responded slightly to addition of P. Differential cell counts on phytoplankton from Station 1 (Figure 8) showed that the diatom *Nitzschia spp.* was the primary alga responding to N and P+N enrichment. *Anabaena spp.*, present in control and P treatments,

* See p. 32 regarding *Oscillatoria spp. (?)*.

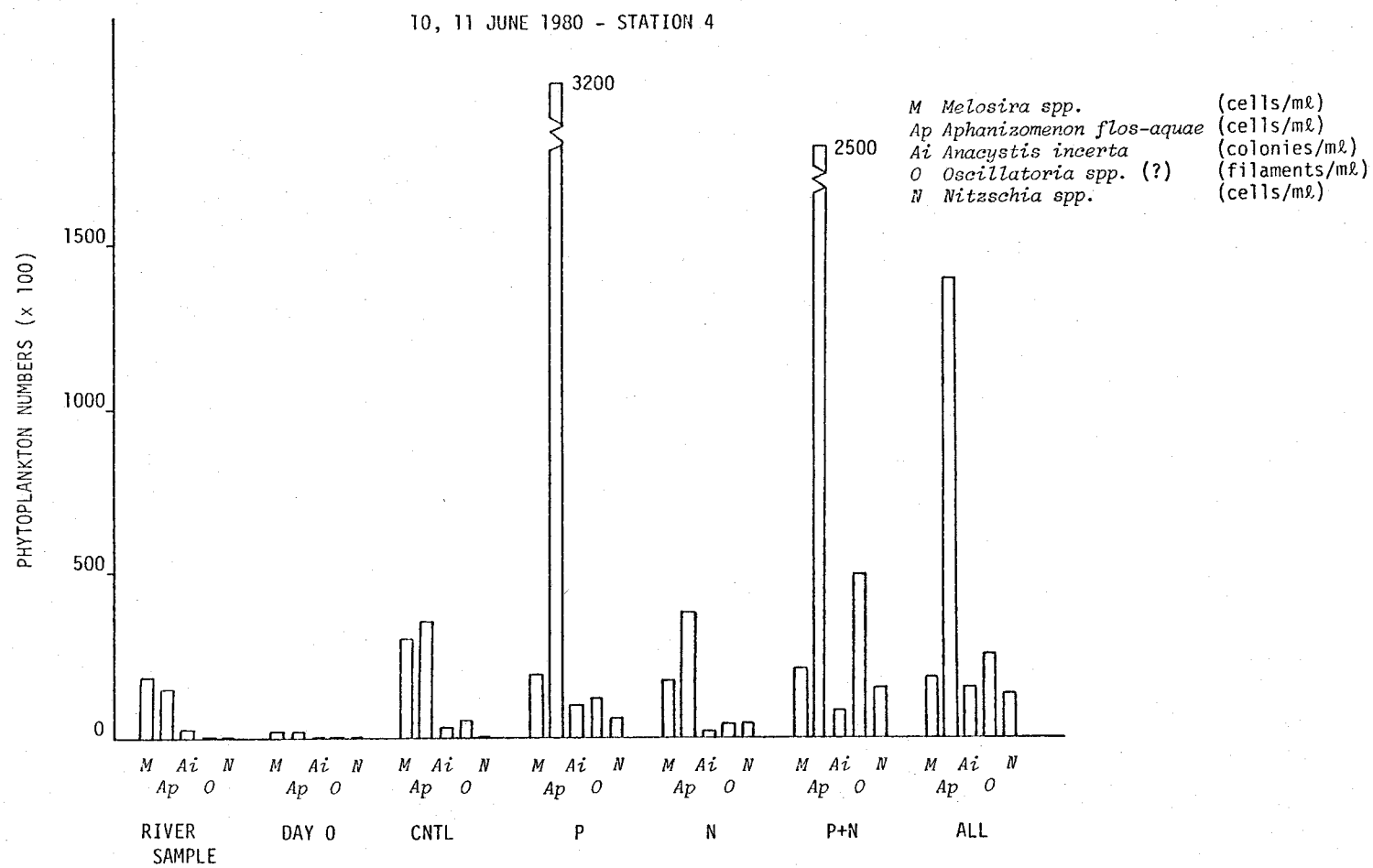

Figure 5. Differential cell counts for Natural assay on water collected from Station 4 on 10, 11 June 1980. River sample and Day 0 (algae from beginning of experiment) counts included for comparison with counts from end of assay in various treatment groups.

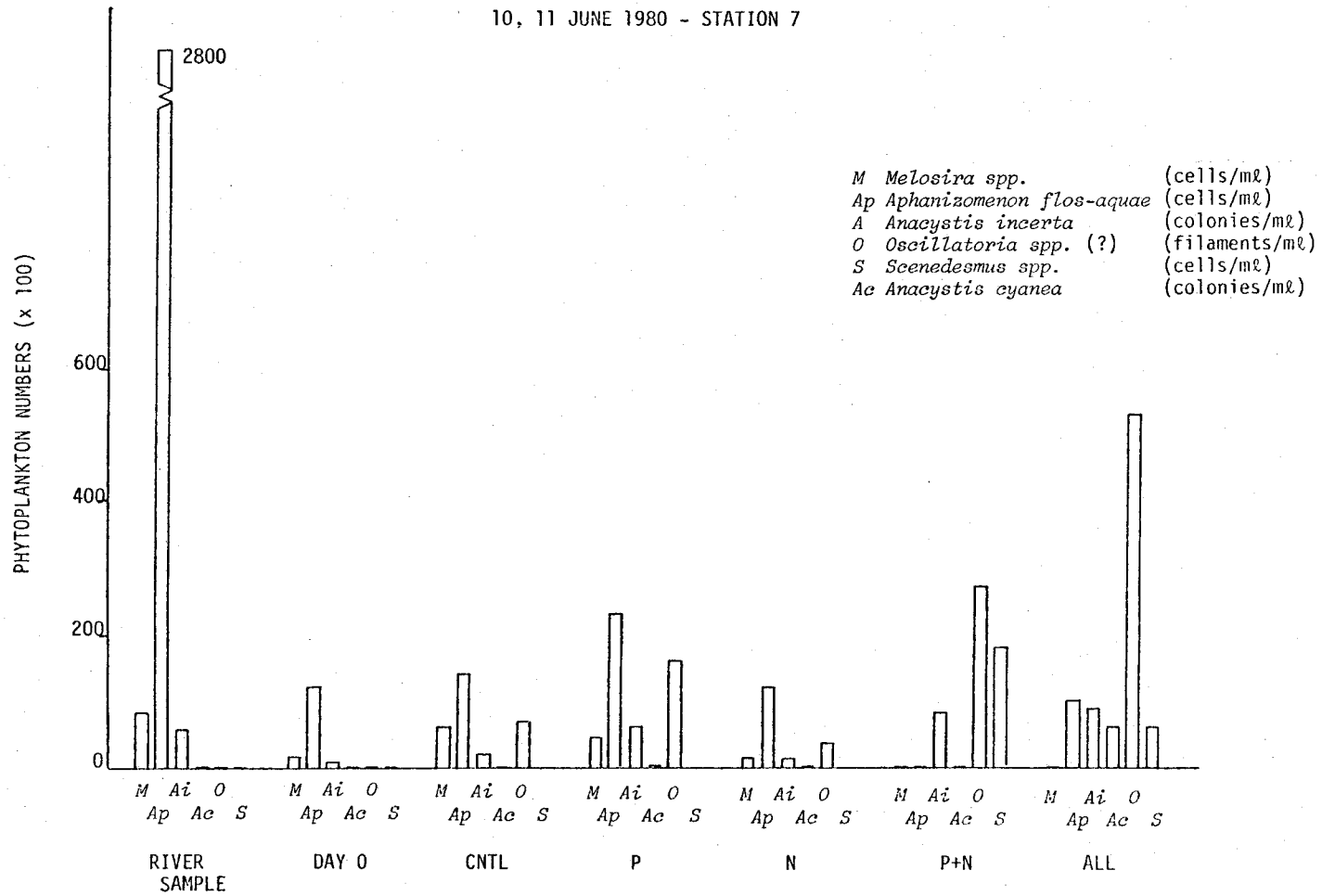

Figure 6. Differential cell counts for Natural assay on water collected from Station 7 on 10, 11 June 1980.

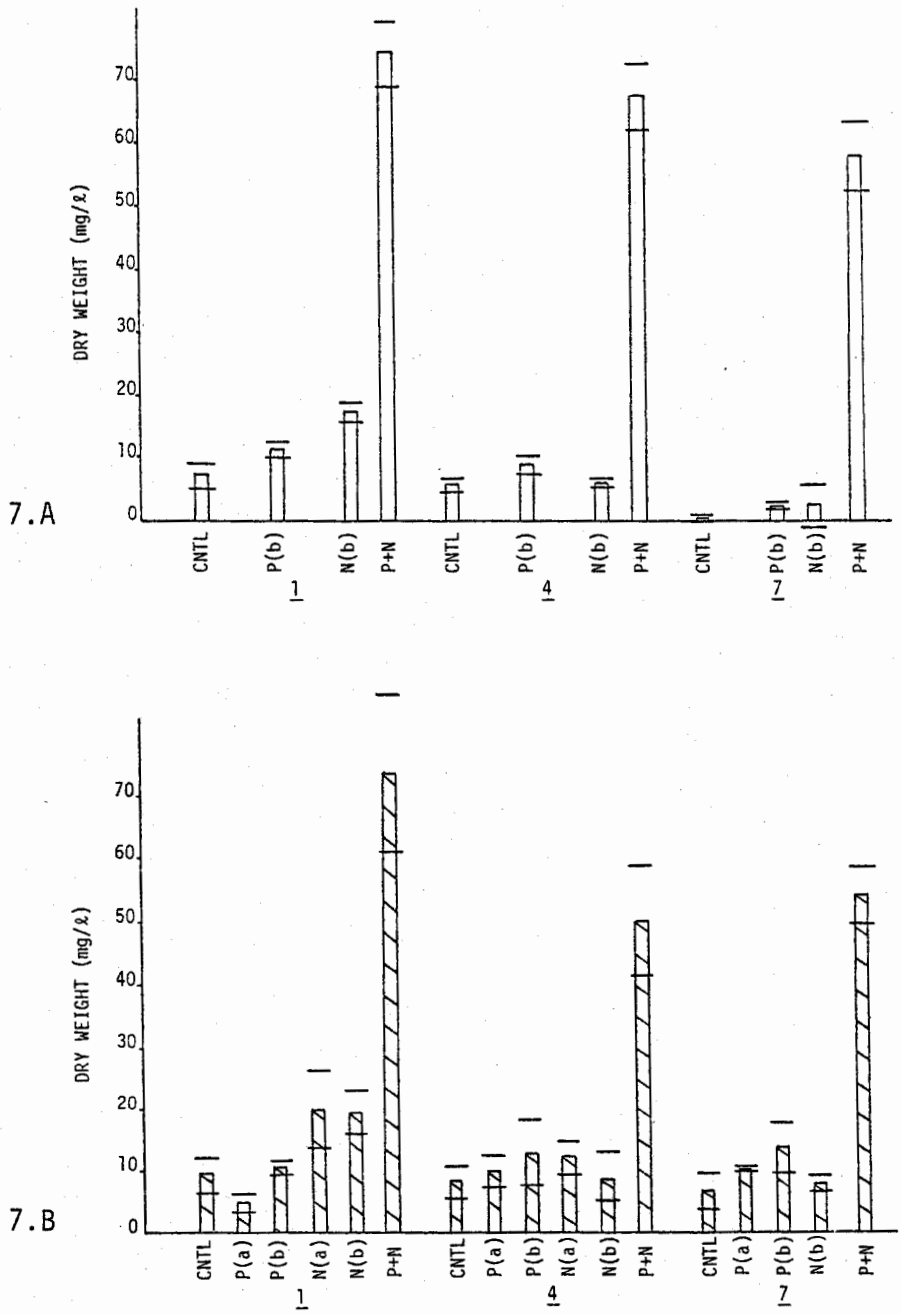

Figure 7. Mean algal dry weights ± SD for *Selenastrum* assay (7.A, unshaded) and Natural assay (7.B, shaded) on water collected from Stations 1, 4, and 7 on 8, 9 July 1980. P(a) = 0.05 mg P/ℓ; P(b) = 0.10 mg P/ℓ; N(a) = 1.0 mg N/ℓ; N(b) = 2.0 mg N/ℓ.

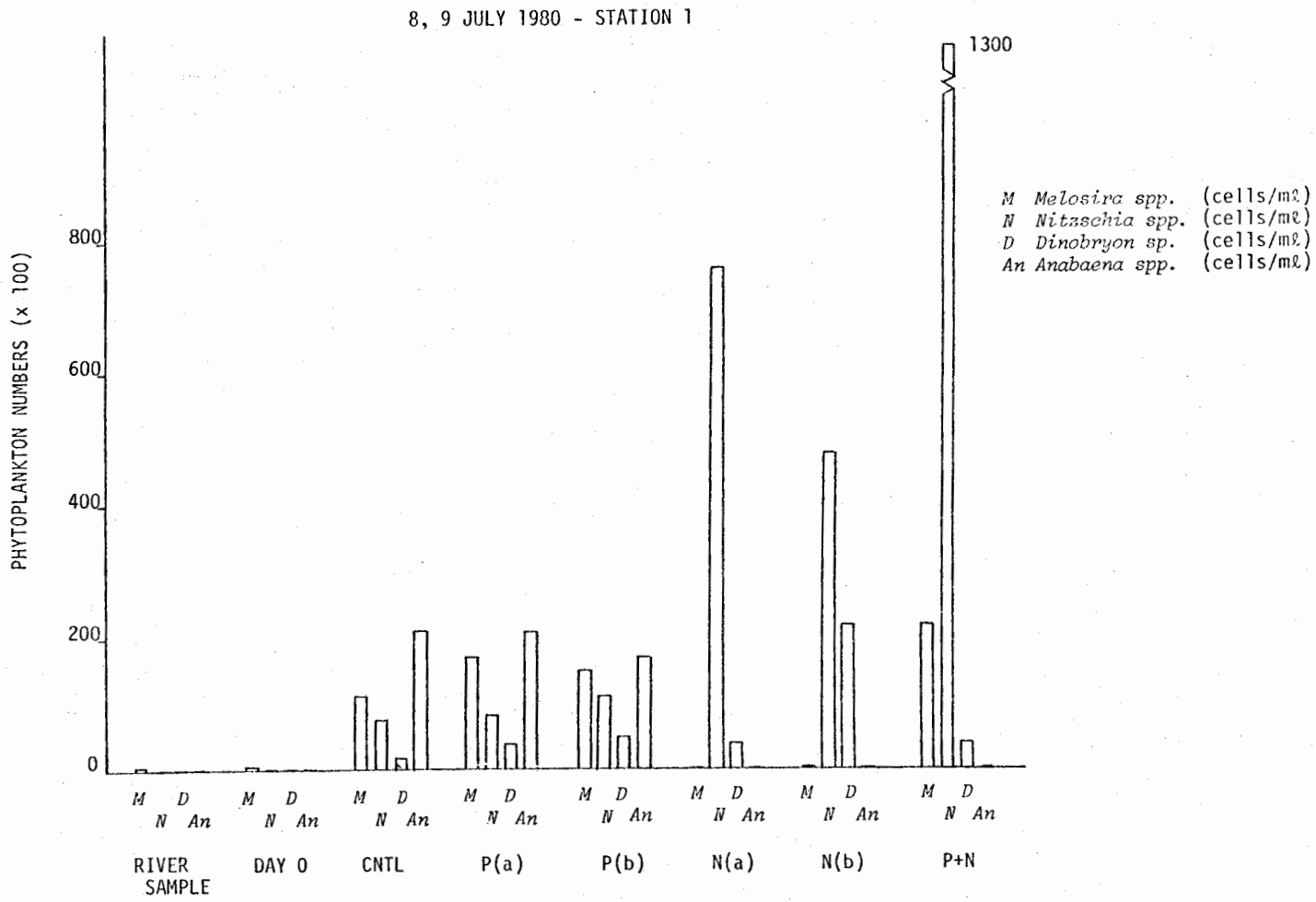

Figure 8. Differential cell counts for Natural assay on water collected from Station 1 on 8, 9 July 1980. P(a) = 0.05 mg P/ℓ; P(b) = 0.10 mg P/ℓ; N(a) = 1.0 mg N/ℓ; N(b) = 2.0 mg N/ℓ.

was not found in N and P+N additions. Increased numbers of unidentified unicellular algae were evident in N and P+N treatments. *Anabaena spiroides* was the dominant alga found in the Station 4 river sample (Figure 9). P enrichment stimulated its growth but did not result in overall increased dry weight. *Nitzschia spp.* grew well in N and P+N treatments only. The large algal biomass in the P+N treatment was caused mainly by increased numbers of *Oscillatoria spp. (?)* and *Scenedesmus spp.* Cell counts of Station 7 samples (Figure 10) showed increases in numbers of *Aphanizomenon flos-aquae* only in response to P additions. Higher numbers of *Anabaena spp.* were found in one of the P treatments and in the P+N treatment. Growth of *Nitzschia spp.* was stimulated slightly by P+N enrichment and markedly by sole N enrichment. High algal standing crop in the P+N treatment was due principally to growth increases of *Oscillatoria spp. (?)*, *Anabaena spp.*, and *Scenedesmus spp.*

August - *Selenastrum* Assay

Large biomass increases occurred in response to P+N and ALL enrichment in samples from the three stations in August (Figure 11). ALL additions produced higher *Selenastrum* dry weights. Single additions of P or N did not stimulate growth.

September - *Selenastrum* Assay

As in all algal assays conducted in this study, growth increases resulted from P+N and ALL enrichments (Figure 12.A). The effects of the ALL treatments were greater than those due to P+N additions, especially at Station 4. Sole P addition had little effect on *Selenastrum*. Enrichment with N stimulated growth somewhat at Stations 1 and 7.

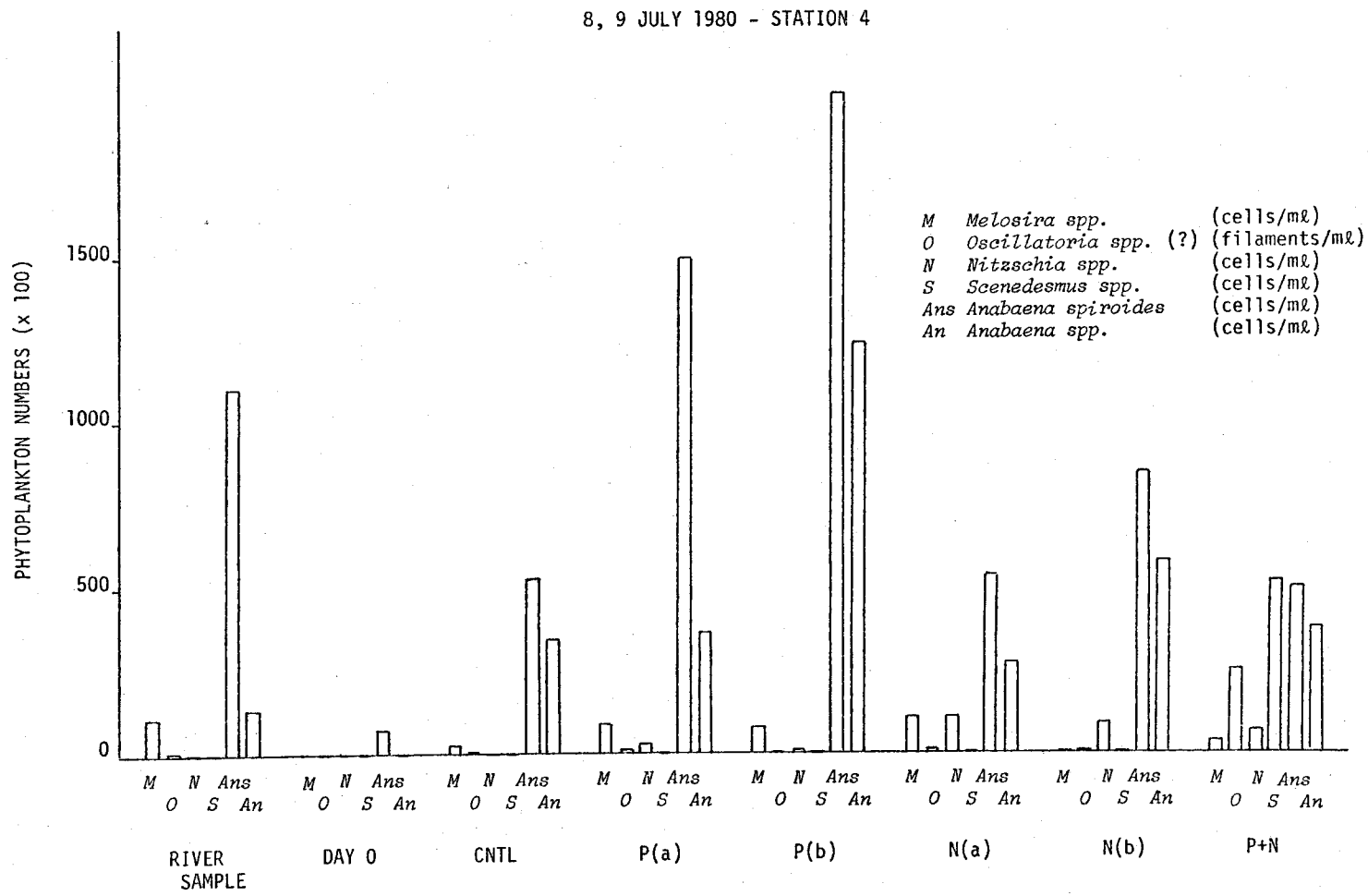

Figure 9. Differential cell counts for Natural assay on water collected from Station 4 on 8, 9 July 1980.

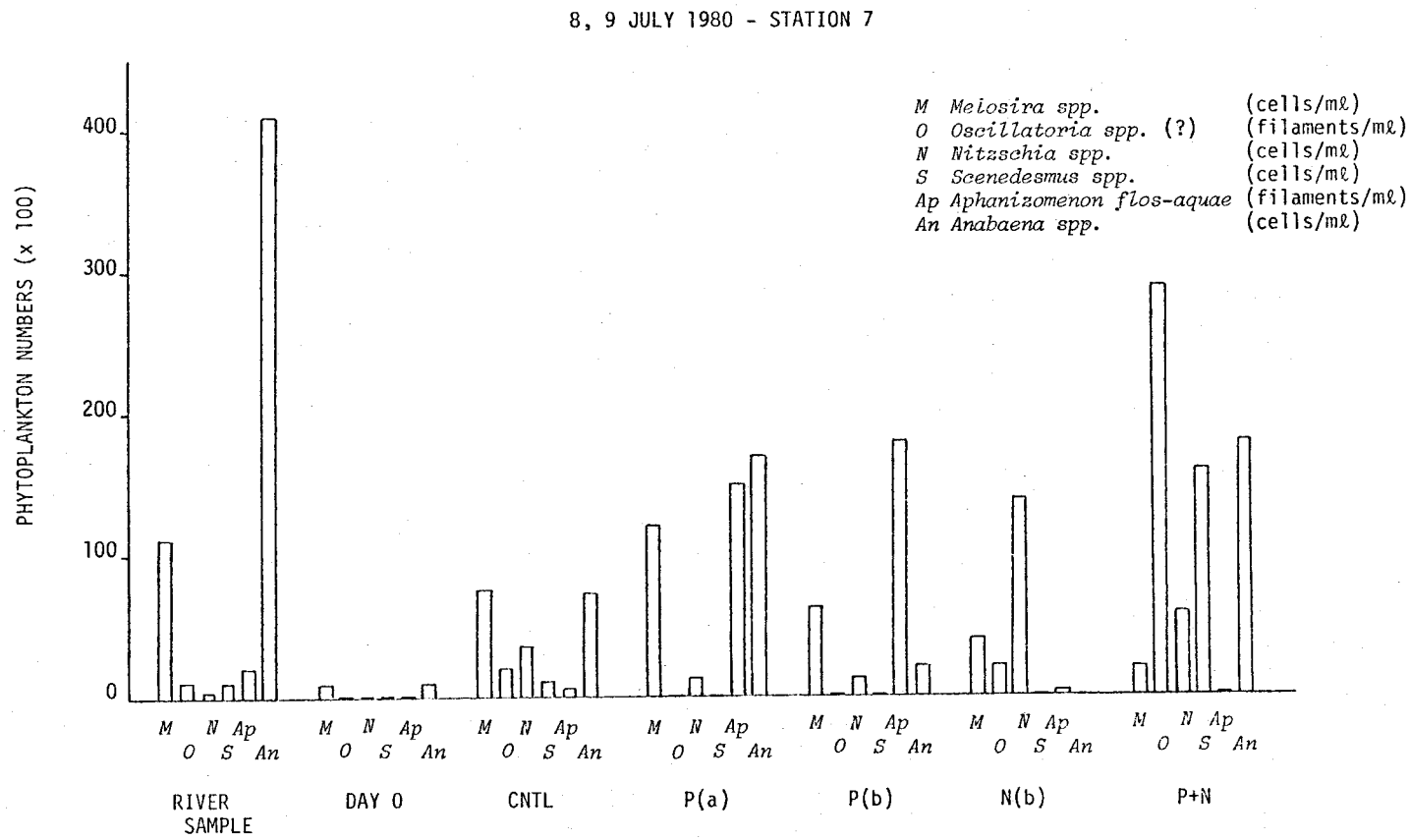

Figure 10. Differential cell counts for Natural assay on water collected from Station 7 on 8, 9 July 1980.

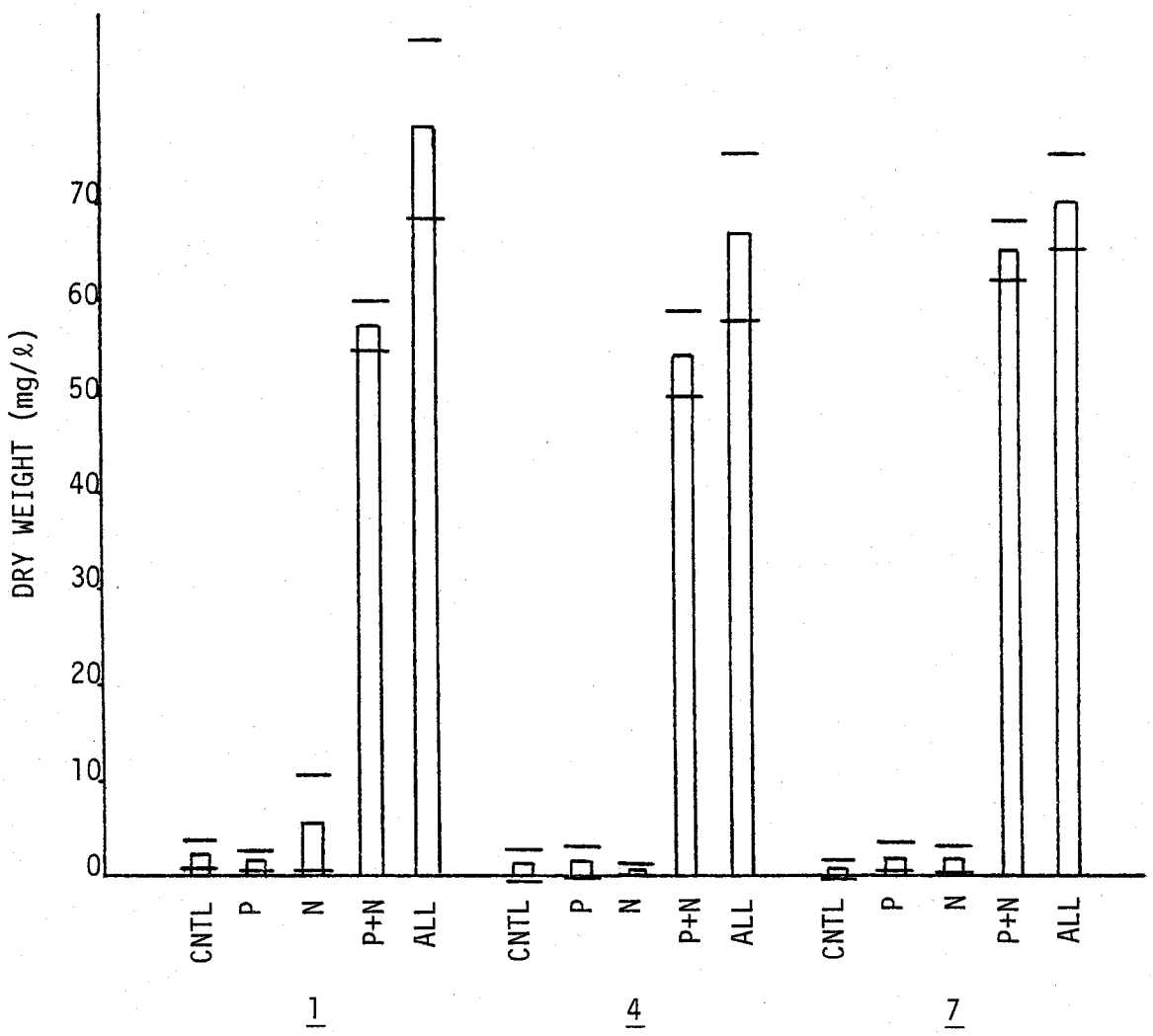

Figure 11. Mean algal dry weights ± SD for *Selenastrum* assay on water collected from Stations 1, 4, and 7 on 5, 6 August 1980.

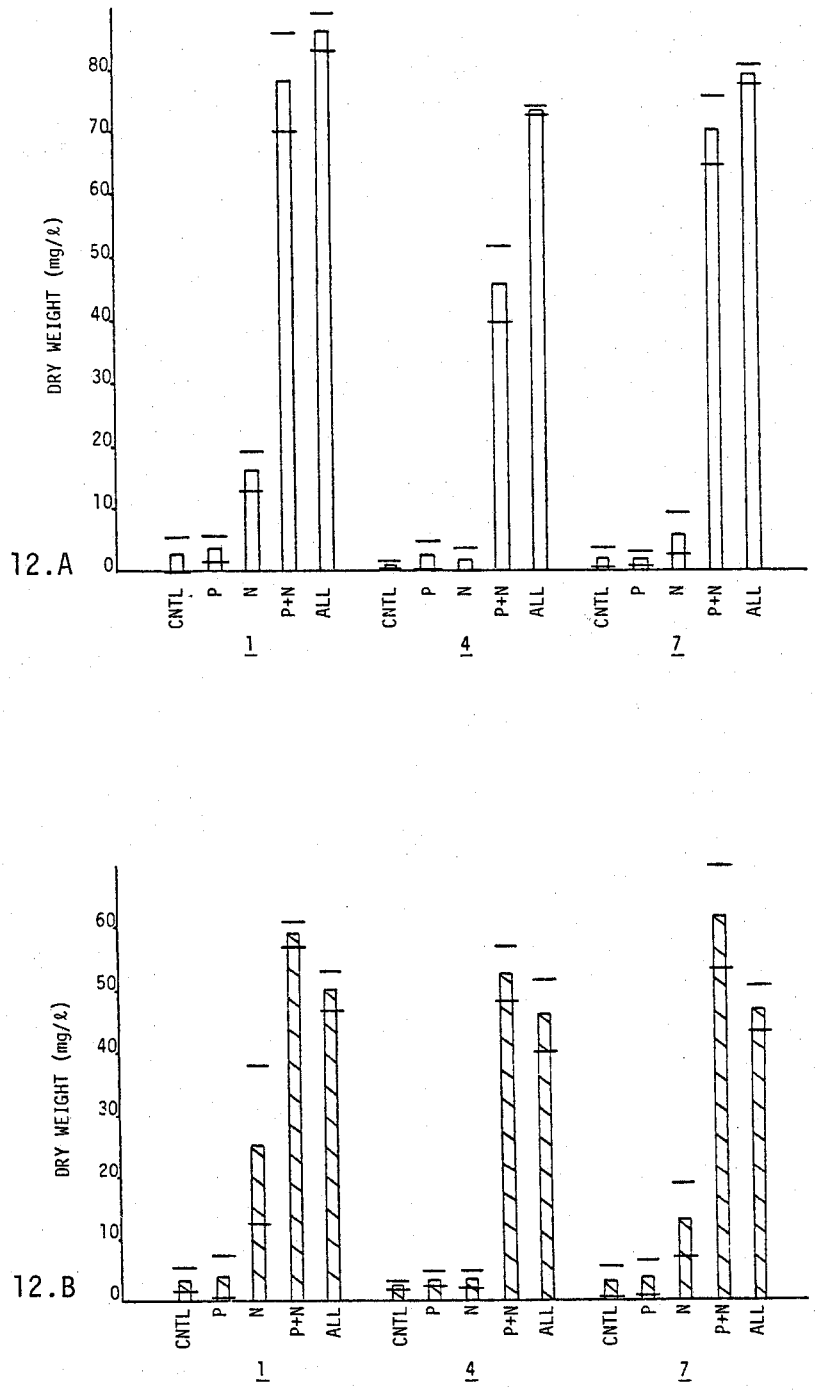

Figure 12. Mean algal dry weights ± SD for *Selenastrum* assay (12.A, unshaded) and Natural assay (12.B, shaded) on water collected from Stations 1, 4, and 7 on 5, 6 September 1980.

September - Natural Assay

Growth of the natural phytoplankton populations at Stations 1, 4, and 7 in the September study was increased sharply by P+N and ALL additions (Figure 12.B). Somewhat higher dry weights were noted in the P+N compared to ALL treatments. Addition of N exerted a stimulatory effect in samples from Stations 1 and 7. Phytoplankton biomass was not increased in P relative to control treatments. Differential cell counts of Station 1 algae showed that increases in numbers of *Nitzschia spp.*, *Oscillatoria spp. (?)*, *Scenedesmus spp.*, and unidentified unicellular algae accounted for increased biomass in N-enriched samples (Figure 13). Larger dry weights in P+N and ALL treatments, compared to sole N treatments, were due primarily to growth of *Nitzschia spp.* Cell counts of Station 4 samples (Figure 14) revealed an increase in *Oscillatoria spp. (?)* in response to N enrichment. Large dry weights in P+N and ALL enrichments resulted from increased growth of *Oscillatoria spp. (?)*, *Nitzschia spp.*, *Anacystis spp.*, *Scenedesmus spp.*, and unidentified unicellular algae. Enumeration of Station 7 phytoplankton (Figure 15) revealed increases in *Oscillatoria spp. (?)* and *Nitzschia spp.* in N-enriched samples. Stimulation in P+N and ALL treatments was attributed to growth of the following algae: *Oscillatoria spp. (?)*, *Melosira spp.*, *Nitzschia spp.*, *Scenedesmus spp.*, *Actinastrum spp.*, *Ankistrodesmus falcatus*, and unidentified unicellular algae.

Statistical Analyses

Multiple regression analyses were run for all experiments at the three stations to assess the relative importance of nitrogen and phosphorus levels on algal dry weight. Other nutrients were not considered

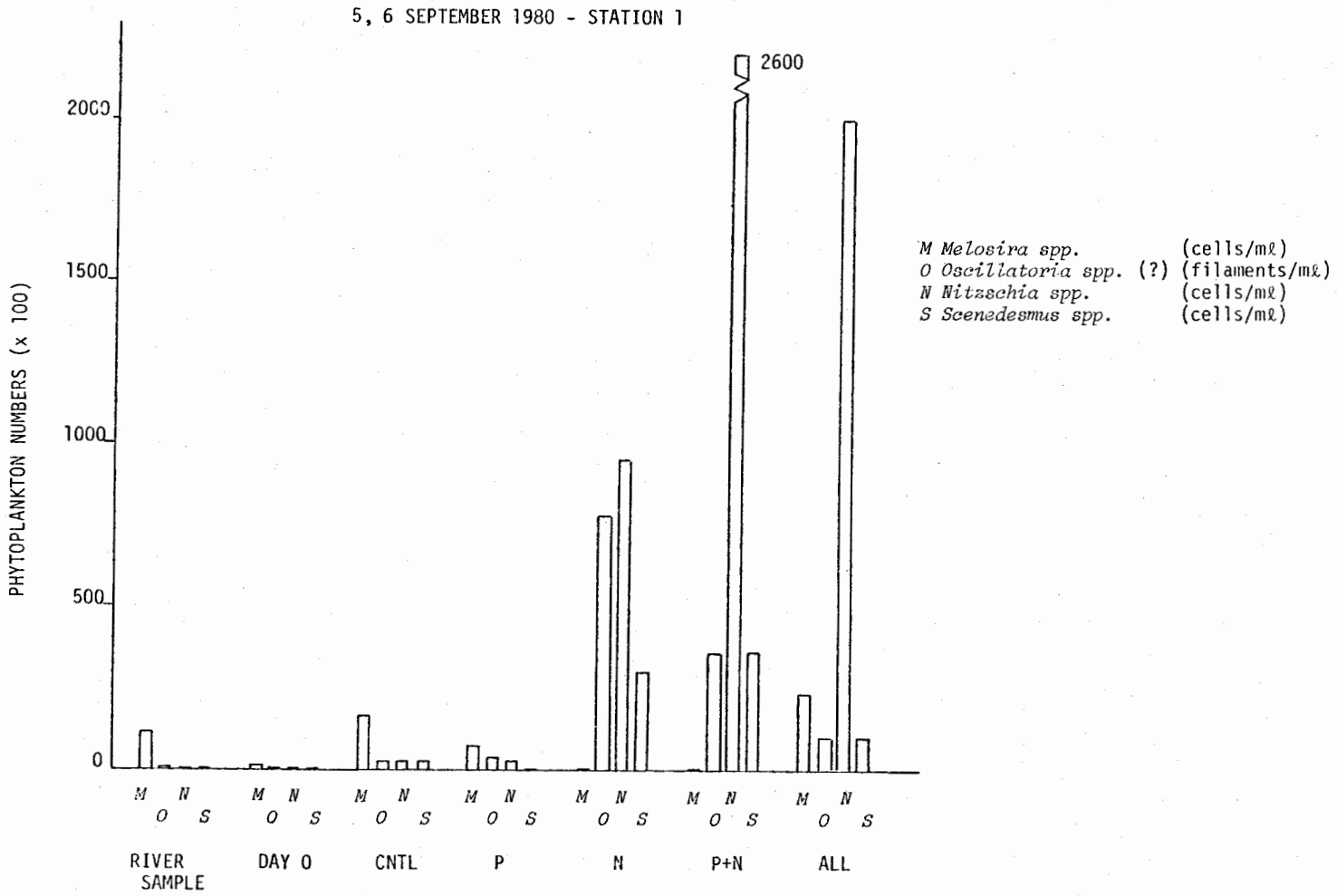

Figure 13. Differential cell counts for Natural assay on water collected from Station 1 on 5, 6 September 1980.

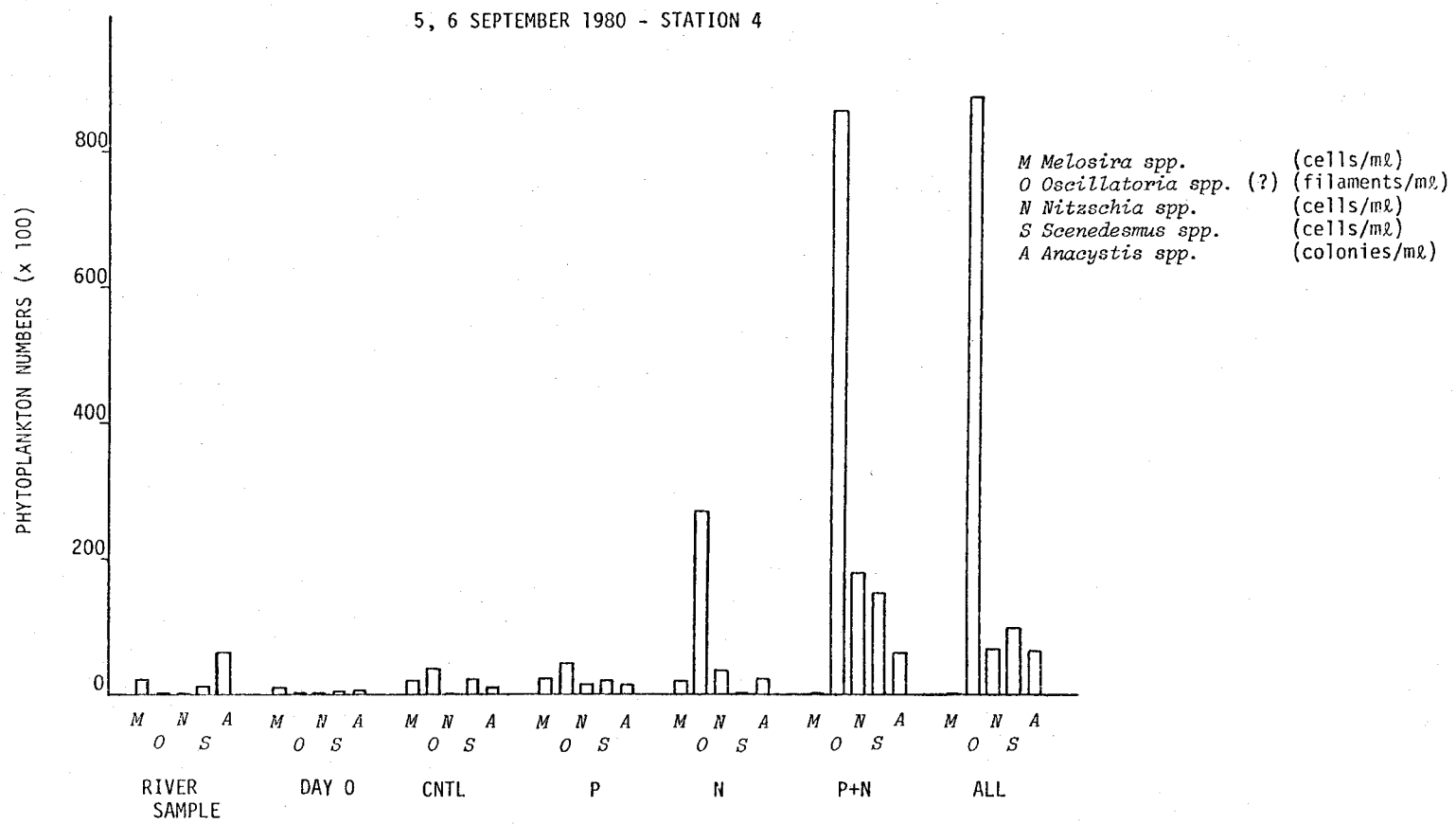

Figure 14. Differential cell counts for Natural assay on water collected from Station 4 on 5, 6 September 1980.

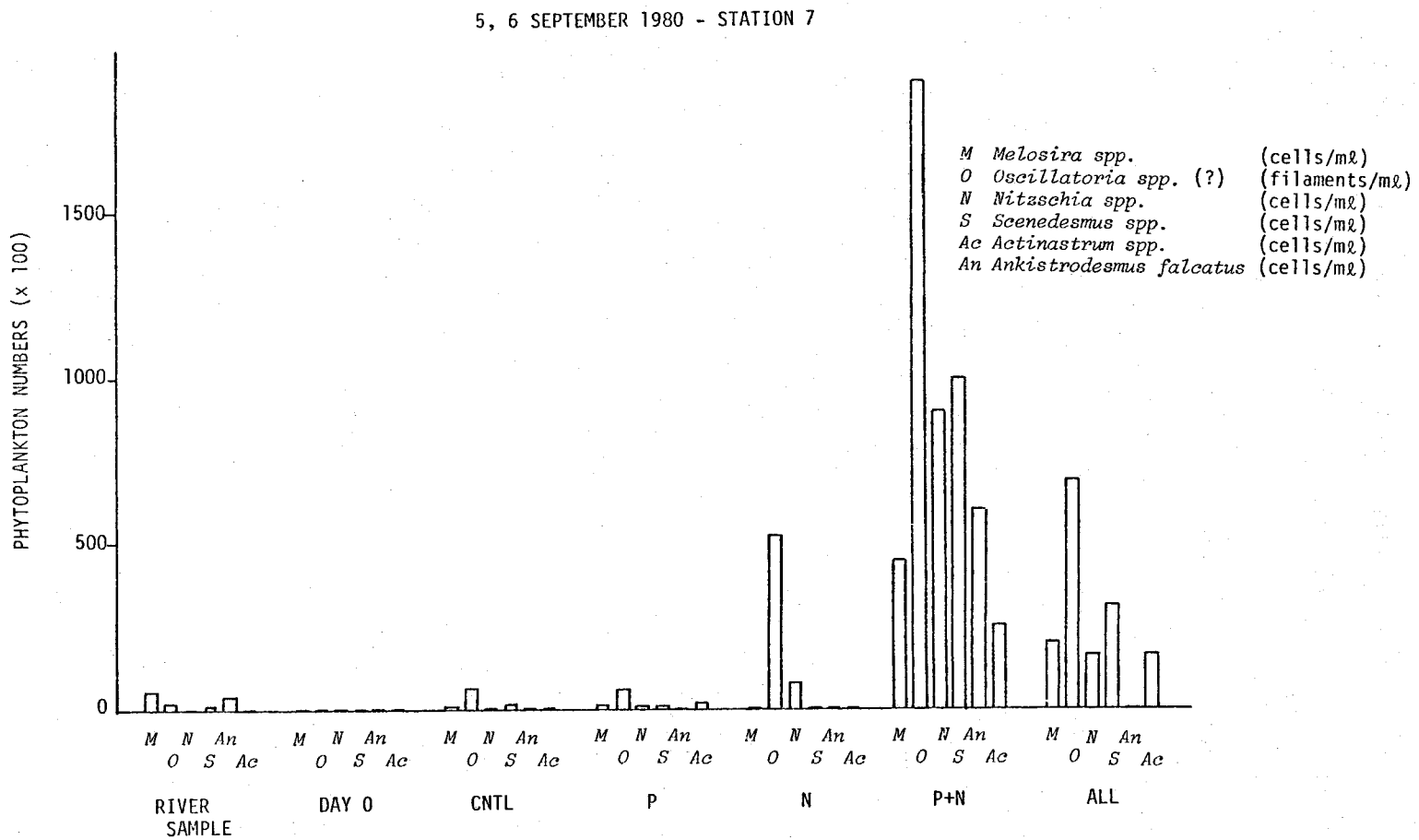

Figure 15. Differential cell counts for Natural assay on water collected from Station 7 on 5, 6 September 1980.

for purposes of statistical analyses because ALL and P+N treatments were similar for most experiments. Interpretation of analyses was based on the assumption that algal biomass was proportional to the concentration of limiting nutrient initially present in the water samples (Miller *et al.* 1978). One purpose of this research was to determine whether nitrogen or phosphorus was more important in limiting algal growth during the study period. Examination of statistical results (Table 8) shows that the interaction term is the most important factor affecting algal biomass. The multiplicative effect of phosphate and total soluble inorganic nitrogen was highly significant in most experiments. These factors considered separately in the model were important sources in relatively few assays. The effect of PO_4-P alone on dry weight was significant at the $p \leq .05$ level for the following experiments: May - Station 1 *Selenastrum*, June - Station 4 Natural, June - Station 7 Natural, July - Station 1 *Selenastrum*, July - Station 4 *Selenastrum*, and July - Station 7 Natural. Sample concentrations of TSIN considered alone in the model significantly affected algal biomass in the following assays: May - Station 1 *Selenastrum*, May - Station 4 *Selenastrum*, July - Station 4 *Selenastrum*, and September - Station 7 Natural.

Table 8. Results of multiple regression analyses showing the effects of phosphate-phosphorus (P), total soluble inorganic nitrogen (N), and their interaction (PxN) on dry weights in *Selenastrum* (*Sel.*) and Natural (Nat.) experiments. * = $p \leq .05$; ** = $p \leq .01$.

Date	Experiment	Station	Model R^2	Source	F	Pr>F	Significance
5/12,13	*Sel.*	1	0.98	P	7.9	0.0167	*
				N	8.9	0.0124	*
				PxN	50.4	0.0001	**
		4	0.97	P	0.6	0.4514	
				N	8.9	0.0126	*
				PxN	64.4	0.0001	**
		7	0.98	P	1.7	0.2222	
				N	1.6	0.2368	
				PxN	115.3	0.0001	**
6/10,11	*Sel.*	1	0.99	P	4.6	0.0554	
				N	3.9	0.0735	
				PxN	75.1	0.0001	**
		4	0.98	P	0.3	0.6201	
				N	0.9	0.3618	
				PxN	86.8	0.0001	**
		7	0.99	P	2.7	0.1299	
				N	2.4	0.1475	
				PxN	366.5	0.0001	**
6/10,11	Nat.	1	0.96	P	0.1	0.7318	
				N	0.6	0.4702	
				PxN	41.8	0.0001	**
		4	0.90	P	64.1	0.0001	**
				N	1.5	0.2420	
				PxN	4.4	0.0588	
		7	0.94	P	37.6	0.0001	**
				N	1.3	0.2792	
				PxN	5.3	0.0421	

Table 8. (continued)

Date	Experiment	Station	Model R^2	Source	F	Pr>F	Significance
7/8,9	*Sel.*	1	0.99	P	5.5	0.0465	*
				N	0.2	0.6984	
				PxN	159.5	0.0001	**
		4	0.99	P	8.2	0.0243	*
				N	7.6	0.0286	*
				PxN	391.1	0.0001	**
		7	0.97	P	2.4	0.1594	
				N	0.1	0.8085	
				PxN	68.5	0.0001	**
7/8,9	Nat.	1	0.92	P	0.2	0.6473	
				N	0	0.9297	
				PxN	26.5	0.0001	**
		4	0.90	P	1.0	0.3334	
				N	0.3	0.5924	
				PxN	28.9	0.0001	**
		7	0.97	P	12.7	0.0045	*
				N	0.1	0.8161	
				PxN	68.8	0.0001	**
8/5,6	*Sel.*	1	0.95	P	0.1	0.7441	
				N	3.9	0.0763	
				PxN	48.5	0.0001	**
		4	0.98	P	0.1	0.7927	
				N	3.7	0.0814	
				PxN	125.7	0.0001	**
		7	0.99	P	1.2	0.2990	
				N	0	0.9027	
				PxN	195.8	0.0001	**

Table 8. (continued)

Date	Experiment	Station	Model R^2	Source	F	Pr>F	Significance
9/5,6	*Sel.*	1	0.98	P	0.1	0.7296	
				N	0.3	0.5954	
				PxN	69.5	0.0001	**
		4	0.95	P	0.3	0.6071	
				N	0	0.8510	
				PxN	42.9	0.0001	**
		7	0.98	P	0	0.9501	
				N	3.6	0.0860	
				PxN	121.4	0.0001	**
9/5,6	Nat.	1	0.94	P	0.2	0.6825	
				N	3.9	0.0744	
				PxN	10.6	0.0078	**
		4	0.99	P	0.4	0.5295	
				N	0.1	0.7563	
				PxN	152.6	0.0001	**
		7	0.95	P	0.1	0.8351	
				N	7.3	0.0223	*
				PxN	21.0	0.0010	**

DISCUSSION

Identification of limiting nutrients from algal bioassays is relevant only when considered in the broader context of the aquatic environment. Results of enrichment experiments must be related to physical and chemical factors and to phytoplankton dynamics in the river.

Station Differences

Station 1 is located upstream near Winton (Figure 1). Higher concentrations of PO_4-P and TSIN were found at Station 1 compared to downstream stations (Table 5); the trend was similar to other findings in the river (N. C. Dept. N.R.C.D. 1979a). Concentrations of chlorophyll and particulate organic carbon were low (Table 5), indicating small algal populations. The generally faster water velocity at Station 1 relative to Stations 4 and 7 leads to short residence times for the algae. Few blue-green algae were found at Station 1. Blooms have not occurred this far upstream because conditions do not favor blue-green growth. Algal identification showed that diatoms, especially *Melosira spp.* and *Nitzschia spp.*, dominated the Station 1 phytoplankton. Findings were similar to those of Witherspoon *et al.* (1979). Concentrations of PO_4-P and TSIN remained high at Station 1 because algal populations were low. Algal dry weights in Station 1 assays were usually higher overall than corresponding treatments in Stations 4 and 7 samples because of the higher available nutrient concentrations. (Figure 3, 4, 7, 11, 12).

Concentrations of PO_4-P and NH_3-N, although lower at Stations 4 and 7, were always present in measurable quantities. However, NO_3-N was undetectable in July at Stations 4 and 7 and in September at Stations 1 and 4 (Table 5). Stanley and Hobbie (1977) and the North Carolina Dept. of Natural Resources and Community Development (1979b) also found low values of NO_3-N and NH_3-N at downstream stations on the Chowan. Chlorophyll values were high, especially in June and July (Table 5). Blue-greens were the dominant algal group while diatoms were present in large numbers also. The three blue-green genera identified as principal bloom-formers in the Chowan River by Witherspoon *et al.* (1979) were present at Stations 4 and 7. *Aphanizomenon flos-aquae* was the principal blue-green species in May and June samples. *Anabaena spp.* dominated the July sample. In August and September, *Anabaena spp. and Anacystis spp.* were present. The Chowan River widens downstream; slower water velocities result. The greater success of blue-greens in the lower Chowan compared to the upper river is related to the longer residence times downstream.

Response of Indigenous Phytoplankton to Nutrient Enrichment

Examination of differential cell count data for natural enrichment experiments provides insight into the nutrient dynamics of Chowan River phytoplankton. The diatom *Nitzschia spp.* was the dominant alga responding to N, P+N, and ALL additions for Station 1 experiments (Figures 8, 13). Although *Nitzschia spp.* was not present in large numbers in Stations 4 and 7 river samples, its growth was usually stimulated by N, P+N, and ALL treatments (Figures 9, 10, 14, 15). *Melosira spp.* was present in most samples from the three stations (Table 6), but did not usually grow well in experimental flasks.

Numbers of *Aphanizomenon flos-aquae* were greatly increased in the June, Station 4 experiment in response to P, P+N, and ALL treatments (Figure 5). *Aphanizomenon* is a nitrogen-fixing alga (Stewart 1973); therefore, it had a competitive advantage over other algae in the P treatment when the TSIN concentration was low. In P+N and ALL treatments, the addition of nitrogen allowed other algae to compete successfully with *Aphanizomenon*. Lower numbers of *Aphanizomenon* in the P+N and ALL treatments resulted. *Aphanizomenon* growth was stimulated by sole P additions in the June and July experiments on Station 7 water (Figures 6, 10). Members of the genus *Anabaena* are also capable of nitrogen-fixation (Stewart 1973). Numbers of *Anabaena spiroides*, the dominant alga at Station 4 in July, increased sharply in response to addition of P alone (Figure 9). However, algal dry weight was not increased much above the control weight (Figure 7.B). Growth of *Anabaena spiroides* in the P+N treatment was closer to control growth (Figure 9), but large increases in total dry weight were noted (Figure 7.B.).

Oscillatoria spp. (?)* *Anacystis incerta*, and *Anacystis cyanea* are blue-green algae that do not fix nitrogen. *Oscillatoria spp.* (?) exhibited a general pattern of increased growth from P+N and ALL additions (Figures 5, 6, 9, 10, 13, 14, 15). Single additions of nitrogen sometimes increased growth (Figures 13, 14, 15). For those experiments in which *Anacystis spp.* was an important component of the plankton, growth was stimulated by P, P+N, and ALL additions (Figures 5, 6, 14). Numbers of the green alga *Scenedesmus spp.* were markedly increased in P+N and ALL treatments at the downstream stations in most experiments (Figures 6, 9, 10, 14, 15). The September, Station 7 experiment showed increased growth of other green algae, *Actinastrum spp.* in response to

*See p. 32 regarding *Oscillatoria spp.* (?).

P+N and ALL addition and *Ankistrodesmus falcatus* in response to P+N addition (Figure 15).

Growth of algae that do not fix nitrogen was increased only in response to combined nitrogen and phosphorus enrichment in most experiments. Single additions of N or P generally did not increase growth because concentrations of the element not added were too small to support additional growth. The pattern exhibited by the nitrogen-fixing algae was one of marked stimulation of cell numbers by single additions of phosphorus. Enrichment with phosphorus and nitrogen increased overall algal growth and allowed other algae to successfully compete with blue-greens for available phosphorus. Species-specific responses to nutrient additions have been found in other studies (Menzel *et al.* 1963). Fitzgerald (1969), Viner (1973), and Shiroyama *et al.* (1976) also demonstrated that nutrient limitation characteristics differed between nitrogen-fixing and non-nitrogen-fixing algae. The importance of blue-green inhibition of other algae to phytoplankton succession in the Chowan River and in laboratory assays is not known. Keating (1977) found autoclave-labile inhibition of diatoms, which he attributed to blue-green algae.

Limiting Nutrients

The addition of an algal growth-limiting nutrient to a test water will result in greater growth of the test species. Final algal biomass should be closely related to initial concentrations of limiting nutrients. These assumptions provide the rationale for using bioassays to identify limiting nutrients. In this study, multiple regression analyses provided the means to assess which nutrients limited standing crops in test

samples. For most experiments, addition of nutrients other than N or P, in the ALL treatment, did not result in greater growth than in the P+N treatment (Figures 3, 4, 7, 11, 12). Therefore, nitrogen and phosphorus were identified as the only nutrients that potentially limited algal growth in the Chowan in the summer of 1980.

Examination of bar charts of mean dry weights (Figures 3, 4, 7, 11, 12) and of significant sources in multiple regression models (Table 8) leads to one general conclusion. The interaction effect of P and N addition on algal growth was much greater than the effect of single additions in most experiments. Simultaneous limitation by P and N has been found repeatedly in bioassay experiments in diverse water bodies (Schindler 1971; Powers *et al.* 1972; Weiss 1976; Shubert 1978). One explanation for the synergistic effect of P and N is that the ratio of available nitrogen to available phosphorus might be approximately balanced in respect to algal requirements. Addition of one of the two elements will quickly cause the other to become limiting. Weiss (1976) found N:P ratios in the range of 9 to 11 for test waters limited by both nitrogen and phosphorus. N:P ratios in the Chowan River samples (Table 5) were compared to assay results in order to determine whether such a relationship existed for this study. Ratios of TSIN:PO_4-P ranging from 0.4 to 10.5 were associated with significant P and N interaction. Some very low ratios in the river samples were evident in July and August but did not result in findings of nitrogen limitation. Thus N:P ratios were not sensitive indicators of nutrient limitation findings in algal assays conducted in this study.

The significance of combined P and N limitation might be due to low concentrations of available nutrients in the water samples. Concentra-

tions of PO_4-P, NH_3-N, NO_3-N, and NO_2-N were low at downstream stations at times during the summer (Table 5). Addition of nitrogen to a water sample, in which little phosphorus is available for growth, will not result in notable increases in dry weight. P addition will increase growth only if enough N is available, or if the test species are capable of nitrogen-fixation. In assays using natural populations, two groups of dominant algae might be limited by different nutrients (Powers *et al.* 1972). Results from this study showed that *Anabaena spp. and Aphanizomenon flos-aquae* were P-limited while *Nitzschia spp.* was N-limited in the same water sample (Figure 10).

In some experiments, nitrogen or phosphorus alone was significant in multiple regression analyses (Table 8). It is important to note that regression models used the initial concentrations of TSIN and PO_4-P in the water samples as the independent variables. Therefore, statistically significant sources of variation in the models might not relate exactly to bar chart presentation of the data. The only assay in which a single nutrient was more important than the P and N interaction was the natural assay in June. P alone was highly significant for Stations 4 and 7 (Table 8). No general trends emerge from examination of dry weight data and statistical analyses as to whether nitrogen or phosphorus is the more important nutrient limiting to total algal growth throughout the summer. Based on the relatively small quantity of data accumulated in this research, no definite patterns of limitation with sampling station or time were evident. It is apparent, however, that species-specific responses to nutrient enrichment occurred. Phosphorus addition increased growth of nitrogen-fixing blue-green algae in natural assays. Therefore, phosphorus should be considered the critical limiting nutrient in systems dominated by nitrogen-fixing algae.

Comparisons Between *Selenastrum* and Natural Assays

Natural enrichment experiments were conducted for three of the five sampling dates in order to provide a comparison method to the *Selenastrum* bioassay. An important criticism of the *Selenastrum* bottle test relates to its ability to accurately predict responses of natural phytoplankton. Comparison of results between the two methods provides some insight into this problem.

While procedures for the two types of experiments were similar, unavoidable differences could lead to dissimilar results. In *Selenastrum* experiments, cultures were inoculated into filtered water samples. The only nutrients available for *Selenastrum* growth were soluble forms. Natural enrichment experiments consisted of one-in-ten dilutions of unfiltered in filtered water. Nutrients tied up in algal cells could become available for growth in the assay. Many species of blue-greens and other algae can store phosphorus in excess of their needs (Stewart *et al.* 1978). Stored phosphorus may become available for growth in the assay test water as concentrations of PO_4-P are depleted.

Experimental results from the two types of assays reveal both similarities and differences. P+N and ALL additions increased algal dry weights markedly at all stations (Figures 4, 7, 12). Dry weight magnitude varied between the two methods, yet the station-to-station order was consistent. Higher control growth was evident in most experiments with the natural populations. The greatest disparity between results of the two experimental types was seen in the June assays. Statistical analyses showed that only the interaction between P and N was important in *Selenastrum* assays (Table 8). Natural experiments offered evidence for limitation by phosphorus at Stations 4 and 7. As previously dis-

cussed, the algal species primarily responsible for growth in P treatments was *Aphanizomenon flos-aquae*, a nitrogen-fixing blue-green (Figures 9, 10). For this sampling date, *Selenastrum* assays did not accurately reflect responses of the dominant river algal species. Results for the July and September experiments agreed fairly well between the two methods (Figures 7, 12). However, in September, *Selenastrum* responded better to the ALL than to the P+N treatment while the reverse was true of the natural population (Figure 12). *Anabaena*, also a nitrogen-fixing genus, was dominant in the July river sample at Stations 4 and 7 (Figures 9, 10). While *Anabaena* was selected for in P treatments, total algal biomass increased only slightly above the controls (Figure 7.B). This species-specific response is of interest ecologically but would be missed without differential cell count data.

Experiments on the natural Chowan populations provided important information that would not be obtained from *Selenastrum* bioassays alone. Few studies have conducted both types of experiments on the same water samples. Schelske *et al.* (1978) found close agreement between spiked tests using *Selenastrum* and those using samples of the natural population. It seems likely that results of comparisons between the two methods will vary depending on the type of nutrient limitation and the phytoplankton populations in the water body studied.

As shown in this study, phytoplankton response to nutrient enrichment can vary among different species. The response will depend, in part, on kinetic constants for nutrient uptake, ability to store nutrients, and ability to use alternate nutrient sources such as atmospheric nitrogen and organic compounds. The *Selenastrum* assay is a useful tool for investigating nutrient limitation. However, the use of one test

species to represent natural populations can be misleading. Natural assays provide a means to assess the composite response to nutrient enrichment; differential cell counts allow further evaluation at the population level. Therefore, when experiments can be initiated soon after sample collection, natural assays provide the better means for investigating nutrient limitation.

Possible Problems Relating to Algal Assay Studies

In light of the results presented in this study, several problems related to algal assay experiments merit discussion. Water samples were collected from three stations in the Chowan River on five sampling dates. River water was always well-mixed so samples were probably representative of the sampling stations on those dates. However, the river environment is constantly changing, and it is not known how well the samples represented true river conditions in the summer study period. Heterogeneity of plankton and nutrient concentrations with time and space is an important consideration. Examination of Chowan River data for 1978-1979 (N. C. Dept. of N.R.C.D. 1979a) reveals considerable variation in chlorophyll and nutrient concentrations from week to week at one station and between neighboring stations on one date. A problem often encountered in studies spanning relatively short time periods is representativeness of experimental results to long-term river conditions. The Chowan River has experienced widespread algal blooms in the past decade, but serious blooms have not occurred every summer. Severe bloom conditions were not encountered at the sampling times in this study. However, algal concentrations were high at downstream stations, especially in June and July, when nitrogen-fixing blue-greens dominated the plankton.

Phytoplankton in the river environment are exposed to continuous inputs of nutrients from upstream. Regeneration from the sediments can provide appreciable quantities of PO_4-P and TSIN to the overlying water in the Chowan (Albert 1980). Nutrients tied up in indigenous algae are constantly recycled in the water column (Stanley and Hobbie 1977). These potential sources of nutrients were largely ignored in the static bioassays conducted in this study. Water samples were enclosed in flasks and subjected to artificial conditions of light, temperature, and shaking. Temperature was kept at approximately 25°C, a value similar to the mean surface water temperature in the Chowan during the summer sampling period. In the natural environment, light and temperature levels and flushing rate often limit algal standing crops. Stanley and Hobbie (1977) found that light and temperature limited phytoplankton growth in the Chowan River throughout the year. Bottle tests eliminate these factors as growth limitors and, in effect, force nutrient limitation on the test algae. Additions of phosphorus and nitrogen in high enough concentrations relative to ambient levels will almost certainly result in increased algal growth. Thus, ambient concentrations of nutrients, while perhaps not limiting growth in the river, might be limiting under artificial conditions of the algal assay experiments.

Factors Affecting Algal Blooms in the Chowan River

Although algal assays are useful in investigating nutrient limitation, they provide limited information on the overall processes involved in algal blooms. Some of the factors that might affect algal growth in the Chowan are mentioned here.

Excessive algal growth in the Chowan River results from high nutrient loadings. Nitrogen and phosphorus enter the river from tributary inflow, precipitation, and point and non-point source discharges. A large number of interacting processes determine the fate of different forms of nutrients in the river. The overall picture is complex, but some processes are singled out here for consideration. Once in the river, nutrients can be simply transported downstream into Albermarle Sound. Particulate forms may settle, thus adding to the nutrient storage capacity of the sediments. Organic compounds in the water column and sediments undergo bacterial and fungal decomposition. Complete remineralization may occur or forms resistant to degradation may persist. Fluxes of dissolved nutrients in and out of the sediments occur under certain conditions. Nitrogen-fixing algae utilize atmospheric nitrogen, incorporating it into organic matter. River phytoplankton will take up available forms of nitrogen and phosphorus for growth. Dissolved organic compounds are utilized by some algae. Certain phytoplankton excrete dissolved organics. Upon senescence, algae are decomposed; the nutrients released then become available for further algal growth. Alternatively, algal cells can settle to the sediments or be carried downstream with the river flow.

In the winter, phytoplankton populations in the Chowan are low. Much of the available nutrient supply is not utilized. Water velocities are typically higher in the winter compared to the summer. A large portion of the nutrients entering the river are carried out with the downstream flow. Some portion of nutrients is probably trapped by the sediments, through settling of particulates and diffusion of soluble forms from the water column into the sediments. With the advent of spring, warmer temperatures and greater sunlight encourage phytoplankton growth,

especially diatoms. Spring algal growth in the river is probably very sensitive to rainfall and storm events. Extensive overland runoff of nutrients due to high rainfall could result in greater growth of spring algae. However, the opposite effect is also possible. Wind and rain can cause high river flow which flushes phytoplankton out of the river system. If large spring diatom blooms do occur, they could act to temporarily store nutrients in algal biomass. Decomposition of algal cells in the water column or settling and subsequent decomposition could provide large quantities of available nutrients for algal growth later on in the year. The temporary removal of available nutrients from the water column by spring blooms might result in conditions favoring growth of blue-green algae. When available forms of combined nitrogen are in short supply, nitrogen-fixing blue-green algae often dominate the plankton.

The severe algal problems encountered during the summer in the Chowan River are closely related to the winter and spring conditions just discussed. Algal growth depends on the amounts of nutrients available. Recycling from the water column and sediments, in addition to inputs into the river, provide available nutrients for summer blooms. As with spring blooms, summer algal growth is closely dependent on physical factors as well as nutrient availability. Rainfall will influence nutrient concentrations in the river through its effect on agricultural runoff and total flow. Wind direction and intensity affect bloom location and duration in the river. Water that is well-mixed and swift-flowing usually will not support surface algal scums. Serious blooms occur in the summer under conditions of calm weather and low flow. High algal biomass in itself is not always undesirable in a water body. The problem arises when species diversity decreases and nuisance algal predominate. Shifts

in the producer base of the food chain affect higher trophic levels. In general, blue-green algae are not a good food source for primary consumers, zooplankton and some fish. Decrease in zooplankton numbers and diversity will, in turn, affect fish populations.

Management Implications of Algal Assay Results

Results from algal assays showed simultaneous limitation of growth by nitrogen and phosphorus for most samples. The question arises: were the indigenous phytoplankton nutrient-limited in the river? Examination of chemical data (Table 5) shows that PO_4-P and TSIN were never reduced to undetectable levels. However, at downstream Stations 4 and 7, levels were low. Phytoplankton at Station 1 were probably limited in the river only by physical factors. At times during the summer, algae at Chowan Stations 4 and 7 were probably limited by nutrient concentrations in the river. Regardless of actual river conditions of nutrient limitation, bioassay results can be interpretated on the basis of potential limitation. Phosphorus and nitrogen, together or separately, would limit algal growth in the Chowan if physical conditions were optimal for growth, or if concentrations of these elements in the river were reduced substantially. Reduction of nutrients to limiting levels could result from extensive algal growth using up all available nitrogen and phosphorus or from decreased nutrient inputs into the Chowan.

High year-round inputs of nitrogen and phosphorus and favorable physical conditions sustain nuisance algal populations in the summer in the lower Chowan River. Phytoplankton growth is dependent on concentrations of nitrogen and phosphorus. Limitation by other nutrients was not shown in this study. It is usually impractical, if not often impossible,

to control physical conditions of light, temperature, and flow. Therefore, reduction of nitrogen and phosphorus concentrations in the river is the only practical way to reduce overall algal growth. Environmental managers are confronted with the scientific and political problem of which nutrient to attempt to control. Substantial reductions of both nitrogen and phosphorus would certainly reduce algal growth eventually by forcing nutrient limitation status on the plankton. Initially, however, sediment regeneration of nutrients might provide enough available nitrogen and phosphorus to support large algal populations.

Consideration of effects of nutrient enrichment at the population level is necessary to an understanding of nutrient limitation in the river. Assay results show that additions of phosphorus selected for nitrogen-fixing blue-green algae. Therefore, phosphorus is probably the more critical limiting nutrient to growth of the bloom-forming genera *Anabaena* and *Aphanizomenon*. Reduction of nitrogen in the Chowan River without concomitant reduction of phosphorus levels could lead to conditions that favor growth of nitrogen-fixing blue-greens over other algae.

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