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Factors Regulating Nuisance Blue-Green Algal Bloom Potentials in the Lower Neuse River, N.C.

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DISCLAIMER STATEMENT

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Abstract

Bi-weekly examinations of physical, chemical and biotic factors suspected of playing a role in the establishment and proliferation of blue-green algal (Microcystis aeruginosa) blooms in the lower Neuse River have helped us focus on several causative agents. Included are: 1) excessive (for algal growth) concentrations of both nitrogen and phosphorus nutrients throughout much of the year and 2) periods of low flow and decreased turbulence (vertical mixing), leading to thermal stratification of the water column. Periods of thermal stratification, even lasting only a day or two, are instrumental in promoting dominance by surface-dwelling Microcystis populations, thereby increasing overall bloom potential and resulting water quality degradation. A combination of these physical and chemical agents leads to maximal bloom development. This was directly observed in the summer of 1981, when dry, sunny weather, combined with excess nitrogen (mainly as NO_3^-) and phosphorus (as PO_4^{3-}) concentrations led to several bloom periods in summer months. In contrast, 1982, which witnessed abundant rainfall in spring and summer months, resulted in high river-flow velocities. Despite accompanying excess nitrogen and phosphorus loading, bloom development was not observed. The consistently high flow periods severely hampered the ability of Microcystis to become a nuisance bloom organism during 1982.

The deployment of small-scale (1-liter volume Cubitainers) and large-scale (1,500-liter hydrocorrals) bioassay enclosures demonstrated: 1) a constant excess of nitrogen and phosphorus nutrients needed to produce blooms and 2) the importance of physical constraints (turbulence, thermal stratification) on bloom potential. In fact, even though Microcystis blooms were not observed in the lower Neuse River during 1982, they could be produced in hydrocorrals placed in the river for only four days. The hydrocorrals producing blooms received no nutrient additions and were isolated from bottom sediments, further proof that nutrient sufficiency is a common and troublesome feature of the lower Neuse River.

During the summer of 1982, nutrient-dilution bioassays were developed and tested in order to obtain initial assessments as to which nutrients were closest to being present in "limiting" concentrations. Preliminary experiments indicate that nitrogen, largely as NO_3^-, cutbacks (of at least 30%) are more effective than phosphorus cutbacks in arresting bloom potential. These findings dictate

an immediate concern for stemming nitrogen loading. The formulation of specific magnitudes of nitrogen-loading constraints must await research to be conducted during 1983. Even though phosphorus-loading constraints may not have yielded as direct an impact as nitrogen constraints in reducing bloom potentials, parallel decreases in phosphorus loading would be desirable in the long run. This is due to the fact that nitrogen constraints without parallel phosphorus constraints may lead to the promotion of N_2-fixing nuisance blue-green algae (Anabaena, Aphanizomenon), as opposed to the non-N_2-fixing Microcystis. All three genera have been implicated in causing rapid water quality degradation. Accordingly, recommended magnitudes for phosphorus cutbacks will be formulated during 1983 dilution bioassay studies.

Because both nitrogen and phosphorus levels are currently in vast excess, and due to naturally occurring low levels of dissolved inorganic carbon, it appears that inorganic carbon limitation of algal production may, at times, exist in the lower Neuse River. Both Cubitainer and hydrocorral bioassays point to this possibility. Research during summer bloom periods of 1983 will investigate this possibility. If inorganic carbon limitation exists, it could prove to be a complicating factor in our understanding of nitrogen and phosphorus limitations of algal production. Such complicating factors must be recognized over time and space in order to properly establish nutrient constraints and priorities and to predict the long-term impact of such constraints on bloom potential.

At present it can be concluded that the periodic salinity intrusions into the lower Neuse River are ineffective in arresting Microcystis bloom potentials, particularly in surface waters where bloom intensity is often most severe. The effects of pulp mill effluent, from the Weyerhaeuser plant at Vanceboro, on algal production and bloom potentials are currently under investigation. At present, these effects are not well understood and must be addressed by additional experimental work. Such work is planned for 1983 and 1984.

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Summary and Major Conclusions

Segments of the lower Neuse River between Goldsboro and New Bern, N.C. have, over the past five years, shown accelerated rates of eutrophication. The three main symptoms of eutrophication are: 1) generally high rates of primary productivity and standing stocks of algal biomass, often in excess of 100 $\mu gC \cdot l^{-1} \cdot h^{-1}$ and 25 μg chlorophyll *a* per liter respectively; 2) periodic spring and summer blooms of nuisance blue-green algae, particularly the surface-dwelling colonial species *Microcystis aeruginosa*; and, 3) algal nutrient levels of both nitrogen (NH_4^+ and NO_3^-) and phosphorus (PO_4^{3-}) greatly exceeding levels which are considered to be growth-limiting to nuisance species. Because nitrogen and phosphorus levels exist in excess for much of the year, other factors, such as temperature, light penetration, water turbulence (either wind-driven or due to river flow) and dissolved inorganic carbon (DIC) levels may act to control phytoplankton growth, and thus bloom potential, of the lower Neuse River.

In this initial year of monitoring and research, a large proportion of our time and effort was spent on identifying the environmental factors most directly regulating, and hence controlling, bloom intensity. Since little previous data exist on either the biological or chemical characteristics of the lower Neuse River, an attempt was made to establish "baseline" data on various relevant biotic and chemical parameters. Included under biotic parameters were: *in situ* primary productivity (CO_2 fixation); chlorophyll *a* content (as an indicator of algal biomass); nitrogen (N_2) fixation, using the acetylene-reduction technique, and collection of river water followed by Lugol's iodide preservation (for algal identification and enumeration by Dr. A. M. Witherspoon, N.C.S.U.). Physical characteristics of the water column examined included: *in situ* temperature and salinity profiles and drogue measurements of surface flow, as well as *in situ* determinations of photosynthetically active radiation (PAR). PAR is that portion of the visible light spectrum used for photosynthetic production (400-700 nm).

Chemical measurements included monitoring of what are commonly considered to be growth-limiting nutrients, including NH_4^+, NO_3^- and PO_4^{3-}. Periodic measurements of Si as SiO_3^{2-}, and Fe as Fe^{2+} were also made. It was determined early in

the study that Si and Fe were abundant enough throughout the lower Neuse River to never constitute growth-limiting concentrations. Additionally, dissolved inorganic carbon (DIC), oxygen and pH measurements were made both on vertical profiles and horizontal transects.

Much of our effort was focused on the lower Neuse River segment between New Bern and Vanceboro, N.C. (Fig. 1), where blue-green algal blooms have previously been reported (Tedder et al. 1980). Three major activities were completed: 1) bi-weekly in situ monitoring of primary productivity and the above-listed biotic, physical and chemical factors at a vertical profile station 4 km upstream from New Bern and simultaneous sampling of seven transect stations for a variety of biotic and chemical parameters (these stations were located between New Bern and Vanceboro); 2) a series of weekly in situ bioassays conducted during spring and summer 1981-1982 at a location just upstream from the Weyerhaeuser pulp mill at Vanceboro (to avoid obtaining river water containing pulp mill effluent), and 3) a set of large-enclosure experiments designed to test the effects of river stagnation and nutrient loading on blue-green algal bloom potential. A set of 1-m diameter, 2-m deep translucent Fiberglas cylinders (hydrocorrals) was placed in the Neuse River at the same location where bioassays were conducted. The hydrocorrals served as experimental large-volume enclosures in an attempt to minimize "bottle" or "container effects," which often complicate interpretations of bioassays. Hydrocorrals were also open to the atmosphere, thereby allowing gaseous exchange between the air and water column.

The following pages constitute a report on preliminary findings obtained from an initial year of study on the lower Neuse River. Again, a significant portion of the initial year's work included identification of relevant parameters, followed by refinements of measuring techniques in order to make them applicable to the waters of the lower Neuse River. Much of the work and results discussed lay the groundwork for a more focused (on specific parameters) effort, which we hope to undertake during 1983-1984. Using the information on relevant parameters obtained during 1981-1982, we intend to quantitatively determine nutrient and physical "threshold" levels. These are the levels below which continued degradation of water quality through blue-green algal nuisance blooms can be avoided through proper management of the Neuse River watershed.

Major Conclusions

From work thus far completed, the following conclusions can be presented with respect to factors promoting and regulating algal bloom potentials.

1. Both biologically available nitrogen (mainly as NO_3^-) and phosphorus (as PO_4^{3-}) are in excess of algal growth requirements during much of the year in the lower Neuse River. Nutrient excesses also occur during blue-green algal blooms. The need for substantial reductions in both is an inevitable conclusion. Hence, reductions must be considered immediately. The formulation of the magnitudes of nutrient cutbacks is a priority research objective for 1983-1984.

2. Dilution bioassays indicate that biologically available nitrogen (mainly as NO_3^-) is most stimulating to algal growth in the lower Neuse River. Accordingly, we predict that cutbacks in nitrogen loading will be the most effective step in lowering bloom potential. A comprehensive, long-term management plan must ultimately consider decreased phosphorus loading as well, since drastic cutbacks in nitrogen without parallel phosphorus removal may lead to dominance by N_2-fixing blue-green algae. The recommended magnitudes of nitrogen and phosphorus cutbacks will be determined in field studies to be conducted in 1983-1984.

Dilution bioassays thus far have shown trace-metal sufficiency in the lower Neuse River.

3. Because both biologically available nitrogen and phosphorus are often in excess, the potential exists that dissolved inorganic carbon (DIC) depletion may, at times, limit algal production. Preliminary bioassay and hydrocorral data indicate that DIC limitation can occur during stagnant, low-flow periods, particularly if calm, sunny weather prevails. Such conditions are common during mid- to late-summer months.

4. Periods of low river flow, which prevailed during 1981 but not during 1982, increase the potential for bloom development and proliferation. As mentioned in Conclusion 3, sunny weather further increases the degree of bloom proliferation. Since nutrient concentrations largely remain in excess during

bloom development, the physical environment, including sunlight, low turbulence (stagnation) and warm temperatures, strongly dictates the magnitude of bloom development.

5. Although temperature can regulate the growth rates of bloom organisms such as Microcystis, the minimal temperature needed for bloom development, 23°C, is usually established and surpassed by late spring. Therefore, bloom regulation by temperature seems secondary to regulation by other physical factors and nutrient sufficiency.

6. Because the brown coloration of the Neuse River (from humic substances) greatly decreases transparency, surface-dwelling nuisance species such as Microcystis are favored over algae more evenly distributed throughout the water column. The latter group of algae are considered to be more desirable food items for zooplankton and juvenile fish. Results show that Microcystis is readily adapted to thriving in surface waters. Hence, as long as excess nutrient loading and favorable climatic conditions exist, blooms will proliferate in such waters.

7. Correlation analyses reveal positive relationships between nutrient levels, algal biomass and primary production. These results show both a direct relationship between nutrient loading and algal growth potential, and the presence of excess (for growth) nutrient levels in the Neuse River. During 1981, when Microcystis blooms were commonplace, there proved to be a strong, positive relationship between DIC and algal biomass. In that year, the correlation value as well as confidence levels exceeded those for nitrogen and phosphorus correlations with algal biomass. In 1982, when Microcystis blooms were rare, a much lower direct correlation between DIC and algal biomass was observed. These findings are taken as additional evidence that DIC limitation of algal growth can occur in the lower Neuse River. Redfield ratios, used to predict the type of nutrient limitation in natural waters, can also be used to argue for periods of DIC limitation. The periodicity and extent of DIC limitation need to be investigated in order to predict its likelihood. Furthermore, the hypothesis that excess nitrogen and phosphorus loading leads to DIC limitation must be substantiated in next year's research efforts.

8. When humic substances present in Weyerhaeuser's pulp mill effluent

were added to Neuse River water at current discharge rates, algal growth was negatively affected during specific periods, but at other times no significant effects were observed. The nature of these stimulatory differences needs to be investigated prior to assessing the overall effects of Weyerhaeuser's effluent on algal production and bloom events.

9. Salinity intrusions into the lower Neuse River above New Bern are not substantial enough to arrest the growth of known blue-green algal nuisance species, including Microcystis aeruginosa, Anabaena spiroides and Aphanizomenon flos aquae. Even during 1981, a very dry year, salinity wedges reaching upstream from New Bern seldom led to elevated salinities (above 1.5 ppt) in surface waters. At this salinity, growth of the above species can still proceed.

Sampling and Experimentation Locations, Methods and Materials

The lower Neuse River was examined on a bi-weekly basis. One station, marked as Number 1 in Figure 1, was designated as a water column profile station, where physical, chemical and biotic parameters discussed below were examined over close-interval horizontal samplings. This station was located approximately 4 km upstream from New Bern. In addition to this location, six upstream locations, constituting a transect, were sampled as well. The most upstream location on this transect was 3 km above the Vanceboro bridge and Weyerhaeuser's pulp-processing plant. The transect stations, including Station 1, were sampled for surface nutrient, chlorophyll a, dissolved inorganic carbon, pH and current characteristics. At each transect station, vertical O_2, temperature and salinity profiles were also monitored. Weather conditions, river flow conditions and photosynthetically active radiation (PAR) transparency of the water column (at Station 1) were also routinely recorded.

Sampling was conducted as follows. First, Station 1 was sampled. An 8-depth profile was sampled using a clean, horizontal 3-l PVC Van Dorn water sampler. Horizontal positioning assured close-interval sampling, which was particularly useful at near-surface depths during bloom periods. Because of the high degree of brown coloration attributed to humic organic constituents in the lower Neuse River, PAR transparency was greatly limited. Accordingly, sampling depths were

concentrated near the surface, where optimal photosynthetic production rates were expected. Sampling depths included 0, 0.25, 0.5, 0.75, 1, 1.5, 2 and 3 meters.

Nutrient, chlorophyll a, primary productivity and N_2-fixation samples were obtained from the same bottle cast at each depth. Following sample collection and incubation of primary productivity and N_2-fixation samples, O_2, temperature, salinity and PAR profiles were determined. Then the remaining transect stations were sampled (surface chemistry and chlorophyll a and vertical profiles for O_2, temperature and salinity). Following transect sampling, we returned to Station 1 to collect primary productivity and N_2-fixation samples, which were left to incubate *in situ* at this location for 3 hours.

Station 1 was chosen as a representative station for the assessment of aerial (per m^2) primary production, chlorophyll a and physical-chemical characteristics of the lower Neuse River. The station is located in approximately 3 to 4 m of water, typifying the average depth of the lower Neuse River. This location has previously experienced massive blue-green algal blooms. It is largely dominated by freshwater inputs (only in extremely dry years, such as 1981, were elevated salinity levels detected in surface waters) and exhibits typical riverine flow regimes during dry and wet seasons. A sampling location upstream from New Bern was also useful for assessing trophic status and the extent of water quality degradation attributable to any upstream sources suspected of potentially altering the quality of river water available for fishing, recreational, agricultural and municipal use in the New Bern area.

Cubitainer and hydrocorral bioassays: Their location and descriptions

Location Number 2 (shown in Figure 1) was chosen as a site for bioassay and hydrocorral experiments. This location is just upstream from Weyerhaeuser Corporation's pulp-processing plant (both intake and discharge points). Hence, the effects of pulp mill effluent as well as nutrients on algal production potentials could be independently assessed at this location. The exact location is 0.5 km downstream from the Vanceboro bridge. At this location the river was wide enough for placement of a bioassay rig and set of hydrocorrals in the

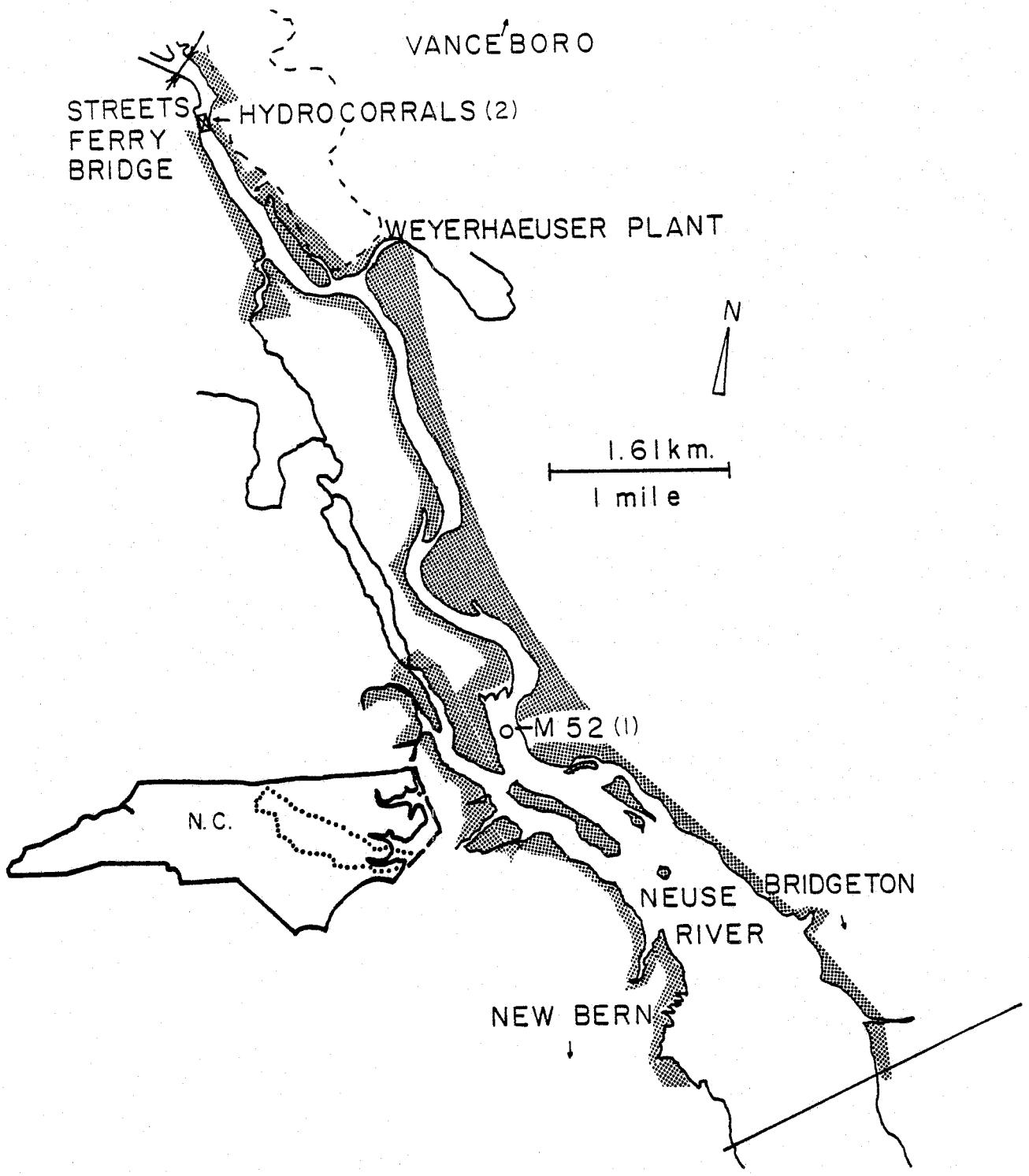

Figure 1. Sampling stations: (1) 4 km upstream from New Bern and (2) just upstream from the Weyerhaeuser pulp mill. The location of New Bern and watershed limits of the Neuse River system are indicated on the inserted map of North Carolina.

the effects of pulp mill effluent as well as nutrients on algal production potentials could be independently assessed at this location. The exact location is 0.5 km downstream from the Vanceboro bridge. At this location the river was wide enough for placement of a bioassay rig and set of hydrocorrals in the river without interfering with boat traffic in the main channel. Furthermore, reasonably firm muddy bottom sediments provided an excellent substrate for anchoring and staking of hydrocorrals, allowing them to remain in place regardless of river flow stages or water levels.

Both container and hydrocorral column bioassays were conducted during the summer of 1982. Because they are chemically inert, unbreakable and virtually transparent to PAR, polyethylene Cubitainers, varying from 1 to 4 ℓ in volume, were routinely used as containers to assess nutrient limitation. Cubitainers were filled and stored with 0.1 N HCl prior to use in order to avoid chemical contamination and microbial surface growth. They were exhaustively rinsed with control (untreated) river water prior to their use for bioassays. All Cubitainer bioassays employed $^{14}CO_2$ assimilation and chlorophyll a content of algae as growth-response parameters (see p 26 for more details on field procedures). Cubitainer bioassays were incubated in situ on an anchored rectangular incubation frame floating at the river's surface.

During the spring of 1982, a set of 9 translucent Fiberglas cylinders ("hydrocorrals") was placed in the river at a point 1 km upstream from Weyerhaeuser's effluent discharge point. The cylinders measured 1 m in diameter and 2 m long, with flanges to allow for a flotation collar to orient the columns in a vertical position regardless of various river flood stages. The bottoms of columns were closed with heavy-duty polyethylene sheeting. Approximately 1.75 m^3 of river water was contained in this manner for experimental purposes.

Preliminary bioassay tests indicated that the Fiberglas material (1 layer of woven roven and 2 layers of polyester resin) was non-toxic to Neuse River algae. Furthermore, PAR-light transparency measurements made inside columns compared favorably with in-river measurements. Columns caused a decrease in vertical PAR penetration of approximately 15%. A major benefit of using these large enclosures was the minimal "bottle effect" often plaguing small-container bioassays. Furthermore, by having the cylinder tops open, atmospheric gaseous

exchange, which is restricted in closed bioassay containers, is allowed to continue uninterrupted.

Primary productivity measurements

In situ primary productivity was determined by the use of the ^{14}C method originally described by Steeman-Nielsen (1952) and subsequently modified for limnological use by Goldman (1963). Duplicate 125-ml clear and single opaque, acid-cleaned, distilled water-rinsed Pyrex reagent bottles, fitted with ground-glass stoppers, were filled at each sampling depth in the field. During sampling, all filled bottles were stored in a light-tight box on board. A 0.3 ml aliquot of ^{14}C-$NaHCO_3$ solution (Amersham Corp.) containing 2.2 μCi of ^{14}C was then added to each bottle. The original batch of ^{14}C-$NaHCO_3$ obtained from Amersham (25 mCi) was diluted with pH 7.5 deionized water, ampulated and sterilized by the principal investigator. Specific as well as total activity of this diluted batch was determined by liquid scintillation counting (Paerl 1982a).

All vertical profile samples were suspended on an incubation line at respective sampling intervals. The incubation line was then anchored and marked with a surface buoy. Incubation periods averaged three hours and were conducted on sunny days. At the end of each incubation period, bottles were collected, immediately placed in a light-tight box and filtered within approximately 1-1½ hours at the Institute of Marine Sciences. A 25-ml subsample was removed from each bottle and filtered at gentle vacuum pressure (200 Torr) on 25-mm-diameter Whatman GFC glass fiber filters, followed by air drying of filters. Laboratory trials proved that GFC filters were as effective as previously used HA Millipore filters (0.45-μm retention characteristics) in retaining radioactive phytoplankton. We preferred using GFC filters because of their lower cost (approximately half the cost of Millipore filters) as well as rapid filtration characteristics.

Dried filters were fumed in a concentrated HCl atmosphere for 20 minutes to remove abiotically precipitated ^{14}C. Filters were then air-dried for at least 1 hour and analyzed for ^{14}C content by placing them in 7-ml mini-scintillation vials containing 5 ml of Fisher Scintillene^{R} cocktail

and counting them in a Beckman LS 7000 microprocessor-controlled liquid scintillation counter. Counting efficiencies, as determined by the use of internal ^{14}C hexadecane standards, ranged from 92 to 96% for all filters.

Dissolved inorganic carbon (DIC) content of samples run for primary productivity (as well as transect samples) was determined by infra-red analyses. A 0.25 ml water sample was injected into a 4-ml reservoir of 50% H_2SO_4, which was continually purged with CO_2-free argon. The argon (carrier gas) was then dried through a "tell-tale" $CaSO_4$ desiccant column and passed through a Beckman 864 infra-red analyzer. All DIC standards were made up from reagent-grade Na_2CO_3 dissolved in deionized water.

Primary productivity was determined using the following equation:

$$^{12}C \text{ fixed} = \frac{^{14}C \text{ fixed (I)}}{^{14}C \text{ available}} \times {}^{12}C \text{ available} \times AxBxC$$

where: A = Correction for the aliquot filtered $\frac{125}{25}$ ml

B = Correction for incubation time to an hourly rate

C = Conversion factor to µg/ℓ or mg/m^3

(I) = Isotope effect (of ^{14}C over ^{12}C) = 1.06

Standard integration techniques were used to determine primary productivity rates per m^2.

N_2 fixation measurements:

Since known N_2 fixing blue-green algal genera (Anabaena, Aphanizomenon) were observed in the lower Neuse River, N_2-fixing potentials were investigated in situ.

For estimation of N_2 fixation, the acetylene reduction assay was used (Dilworth

1966; Stewart et al. 1967). This technique is based on the fact that the enzyme complex responsible for N_2 fixation, nitrogenase, will also reduce acetylene, which, like N_2, contains a triple covalent bond. Nitrogenase reduces acetylene (C_2H_2) to ethylene (C_2H_4) in a manner similar to reducing N_2 to NH_3.

1. Nitrogen fixation:

$$N\equiv N \xrightarrow{\text{nitrogenase}} 2\ NH_3$$

$$6e^- \nearrow$$

2. Acetylene reduction:

$$HC\equiv CH \xrightarrow{\text{nitrogenase}} H_2C = CH_2$$

$$2e^- \nearrow$$

For each molecule of N_2 reduced, 6 electrons are required, while each C_2H_2 molecule reduced to C_2H_4 requires only 2 electrons. The theoretical molar ratio for equating N_2 fixation to C_2H_2 reduction is 1:3. Thus, C_2H_2 reduction values must be divided by 3 in order to approximate N_2 fixation.

Acetylene reduction assays can be routinely used without prior removal of N_2, since acetylene is approximately 65 times more soluble than N_2 in water and hence can easily outcompete N_2 for active sites on the nitrogenase complex. The acetylene-reduction technique is far easier, cheaper, quicker and less troublesome to perform in the field than $^{15}N_2$ assimilation techniques.

We conducted in situ acetylene reduction assays in parallel with ^{14}C incubations. At each sampling depth, 40-ml subsamples were dispensed in triplicate clear and single dark 60-ml serum bottles at each depth. Pre-filtered (H.A. Millipore 0.45-µm porosity) water samples served as blanks (to check on ethylene contamination of acetylene). Samples were sealed with serum caps and injected with 4.4 ml of high-purity acetylene (Matheson Co.). Following incubation alongside primary productivity bottles, ethylene (C_2H_4) production was determined. Samples were withdrawn from incubation depths and shaken vigorously to equili-

brate aqueous and vapor C_2H_2 phases. A 3-ml sample was then withdrawn from the vapor phase (head space) and immediately dispensed into an evacuated Vacutainer tube, which acted as a storage vessel for gasses produced during the incubation. Samples stored in Vacutainers were analyzed within a few days at the Institute of Marine Sciences.

To measure C_2H_4 production from C_2H_2, 0.3-ml gas samples were injected into a Carle AGC-311 FID gas chromatograph, set at 80°C and fitted with a 1.0-m stainless steel column packed with Poropak-T. Purified N_2 served as a carrier gas.

On occasions, parallel $^{15}N_2$-fixation analyses were run to confirm that the acetylene-reduction method quantitatively represented N_2 fixation. The $^{15}N_2$ assimilation assays were conducted in accordance with procedures outlined by Kanazawa and Yoneyama (1976), using an emission-spectrophotometry technique. Previous $^{15}N_2$ studies have employed mass spectrometry. This latter technique is very time-consuming, requires a large sample (up to 500 μg N) and necessitates the addition of high concentrations of $^{15}N_2$ to obtain suitable sensitivity. For good analytical sensitivity with mass spectrometry, long incubation periods (sometimes up to 24 hours) are required. Often, sensitivity is further reduced by having to use a mass spectrometer that is set up and designed for multi-purpose use. The emission-spectrophotometry procedure is approximately one-tenth as time-consuming as mass-spectrometric procedures.

Following incubation, 100-ml samples were filtered on precombusted GFF filters, dried and placed in Pyrex discharge tubes. Quartz wool plugs were placed at each end of the central constriction of each tube to prevent samples from entering the constriction. Calcium oxide, preheated to 800°C for 8 hours, was placed in the tubes. This served to absorb H_2O and CO_2. Cuprox and CuO were added. The tubes were then evacuated to 10^{-5} Torr and excited during the evacuation with a Tesla coil to dislodge adsorbed gases. Tubes were then sealed under vacuum and heated in a box furnace at 590°C for 16 h. Tubes were then cooled for 24 hours and analyzed for $^{15}N_2$, $^{15}N^{14}N$ and $^{14}N_2$ emission ratios using a JASCO NIA-1 emission spectrophotometer. Duplicate samples were analyzed and each sample was scanned three times. The average $^{14}N_2$ ratios proved to be the

most useful ratios for calculating ^{15}N enrichment. Normally 15 µg N per sample proved to be an optimum amount of N for adequate emissions.

To calculate the ratio of C_2H_4 produced to N_2 fixed, the following formula was used:

$$\frac{\mu g\ C_2H_4}{\mu g\ N} =$$

$$\frac{n \text{ moles } C_2H_4 \times g/\text{mole } C_2H_4}{\text{atom \% excess } ^{15}N \text{ in samples } (-) \text{ background } ^{15}N} \times \frac{mg\ ^{15}N \text{ in samples}}{\text{atom \% excess } ^{15}N (-)\ N_2 \text{ in headspace}}$$

Total particulate N content of samples was determined with a Carlo Erba Model 1101 C, H, N analyzer housed at the National Marine Fisheries Services Laboratory, Beaufort, N.C.

Transect primary productivity and N_2 fixation measurements

Due to the time involved in collecting transect samples, incubation of these samples was conducted the following morning at the Institute of Marine Sciences. All transect samples were collected at the surface, using 4ℓ acid-cleaned polypropylene bottles for overnight storage at ambient temperatures. Samples were dispensed in primary productivity and N_2-fixation bottles identical to those used for *in situ* incubations. Likewise, identical quantities of ^{14}C and acetylene were added. All samples were incubated for 3-4 hours on an orbital shaker under 250 µEinsteins·m^{-2}·sec cool white fluorescent illumination. Filtration steps and measurements of ^{14}C fixation as well as acetylene reduction were similar to those for field samples. Transect primary productivity characteristics were examined on 5 separate occasions during 1981.

Monitoring of physical factors:

O_2/temperature measurements: During the course of *in situ* primary productivity and N_2 incubations, O_2 and temperature measurements were conducted at

various river locations. A profile was normally examined at the incubation station. A YSI 54 ARC O_2/temperature meter, employing a model 5775 probe, was used. Similar measurements were made at all transect stations. Values obtained were in mg/ℓ (or ppm) dissolved O_2 content, which could be converted to % saturation according to the nomogram described by Mortimer (1955).

Photosynthetically active radiation measurements

In situ photosynthetically active radiation (PAR), which is that portion of the visible light spectrum available for photosynthetic utilization (400-700 nm), was determined in the water column during incubation periods. PAR measurements were utilized to determine both the irradiation available as well as extinction properties of the water column. Extinction varied substantially during the year. This variation was attributable to pulp mill effluent discharge, surface runoff and sedimentation as well as algal bloom periodicity. A Li-Cor 185A Quantum radiometer-photometer having a Li-192S underwater sensor was used to determine PAR. A sensor correction factor of 1.34 was used when submersed PAR readings were converted to absolute μEinsteins$\cdot m^{-2} \cdot sec^{-1}$ values (Li-Cor Inc. Calibration Services). Continuous PAR measurements at the Institute of Marine Sciences were recorded with a Li-Cor LI-550B Printing Integrator coupled to a Li-190SB Quantum Sensor. This instrument allowed for incident PAR correction during reduced irradiation on cloudy days. Fortunately, this correction was not needed during approximately 80% of the incubation periods, since most incubations were performed on cloudless days.

Salinity measurements

Salinity intrusions were a common feature of the lower Neuse River, particularly during the drought year 1981. At both the in situ incubation station and the transect stations, salinity was routinely measured using a YSI model 33 salinometer-temperature-conductivity meter and probe. The salinometer was calibrated against known NaCl solutions made up in the laboratory.

Chemical techniques and measurements

Nutrient analyses: Soluble phytoplankton growth nutrients were routinely

examined during the course of this study. Nutrient measurements were made during vertical profile and transect samplings. Prior to analyses, freshly sampled water was collected in HCl-cleaned, 1-l Nalgene bottles and transported to shore. Once there, samples were vacuum filtered (within 2 hours) through 47-mm-diameter Whatman GFC filters, which had been pre-cleaned with dilute HCl and deionized water to remove potential nutrient contamination. The filtrate was collected in Nalgene bottles for nutrient analyses at the Institute of Marine Sciences. Nutrient analyses were normally conducted the following day. All filtered samples were refrigerated overnight.

The GFC filters used for obtaining soluble particle-free nutrients were also used for chlorophyll a determinations. From 500 to 750 ml of water was normally filtered, followed by a coating of saturated $MgCO_3$ solution on each filter prior to wrapping filters in foil and freezing. Filtrate samples prepared for nutrient analyses were completed prior to $MgCO_3$ additions. The $MgCO_3$ was added to ensure alkaline conditions on frozen filters, counteracting degradation of chlorophyll a during storage in a freezer at -20°C. Filters were routinely assayed for chlorophyll a within 3 weeks of collection. Chlorophyll a was extracted by sonic disruption of filtered material in 8 ml of 90% acetone. Chlorophyll a concentrations were derived from spectrophotometric (Bausch and Lomb 710 or 2000 spectrophotometers) readings at 663, 647 and 630 as well as 750 nm (turbidity determinations) in a trichromatic formula described by Burnison (1980).

Ammonia determinations: The phenol-hypochlorite method of Solorzano (Strickland and Parsons 1968) was employed. This method proved sensitive and highly reliable down to 5 µgN/ℓ as ammonia, which as well within the range of ammonia levels encountered during this study. A 50-ml sample was used for this determination. Color development was read at 640 nm in a 5-cm spectrophotometer cell 2-3 hours after initiating the assay. A calibration solution of NH_4Cl made up in double-deionized distilled water was used as a standard.

Nitrate determinations: The cadmium-reduction technique, as described by Strickland and Parsons (1968), was used to determine nitrate and nitrite concentrations. This method proved highly sensitive and precise down to 1 µgN/ℓ as NO_3^{-1}, far below the lowest levels encountered in water samples. A 100-ml

sample was used for each determination. Color development was read at 543 nm, using a 1-cm spectrophotometer cell. Nitrite consistently proved to be less than 2% of nitrate-nitrite concentrations during this study.

Orthophosphate determinations: Reactive phosphorus (orthophosphate) was analyzed according to the molybdate blue method of Murphy and Riley (1962). The lowest level of detection was 2 µg P/ℓ as PO_4^{-3}, which proved adequate for detecting orthophosphate levels during this study. Color development was monitored at 885 nm, using a 5-cm spectrophotometer cell. Turbidity and water color blanks were determined in parallel with orthophosphate determinations and were subtracted from absorbence values obtained for each sample.

pH measurements: All samples were analyzed for pH immediately after collection. A Fisher model 750 ion analyzer was used. Calibrations were made against Fisher certified buffers having pH values of 4.00, 7.01 and 9.00.

Research Findings

Water column characteristics of the lower Neuse River 1981-1982: Integrated aerial(per m^2) measurements

Results of bi-weekly samplings for biological and chemical characteristics of the water column at marker 52A (4 km upstream from New Bern) have been graphically presented in integrated form (Figs. 2,3,4). A striking feature of all parameters is the high degree of variability between sampling periods. Although in general both chlorophyll a content and primary productivity rates are minimal during winter months (Jan-March), summer and fall growth periods are often interrupted by fluctuations in both biotic and chemical parameters. Such fluctuations are most likely due to periodic flushings of the lower Neuse River by rainfall events. A good example of this can be observed during mid-August 1981, when tropical storm Dennis came ashore in eastern North Carolina, causing "washout" of a Microcystis aeruginosa nuisance bloom which had proliferated since June (Figs. 2,3,4). Fortunately, close-interval (bi-weekly) sampling allowed us to detect and assess the impacts of such climatological events on biotic and chemical parameters. It is obvious that bi-weekly sampling is necessary to observe, in fine detail, quantitative characteristics of algal production as

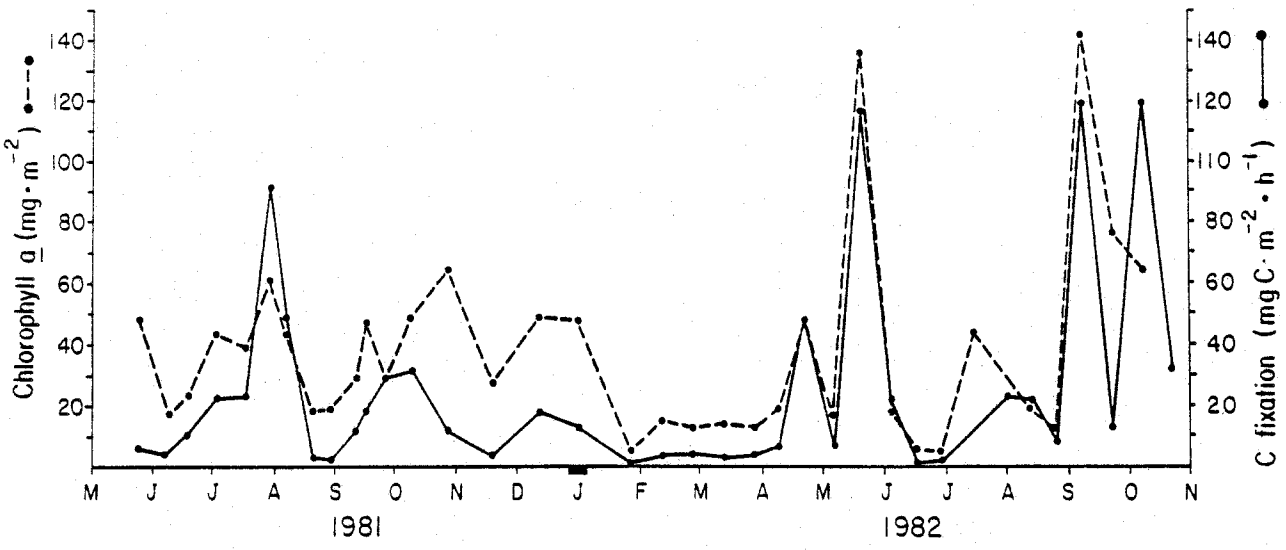

Figure 2. Aerial primary productivity (in mgC fixed·m^{-2}·h^{-1}) and chlorophyll a levels at marker 52A (designated as Station 1 in Figure 1). Biweekly sampling was conducted. Data for each sampling point are integrated over an 8-depth sampling profile. In general, a good relationship in time and space was observed between these biological parameters, representing algal biomass and photosynthetic growth.

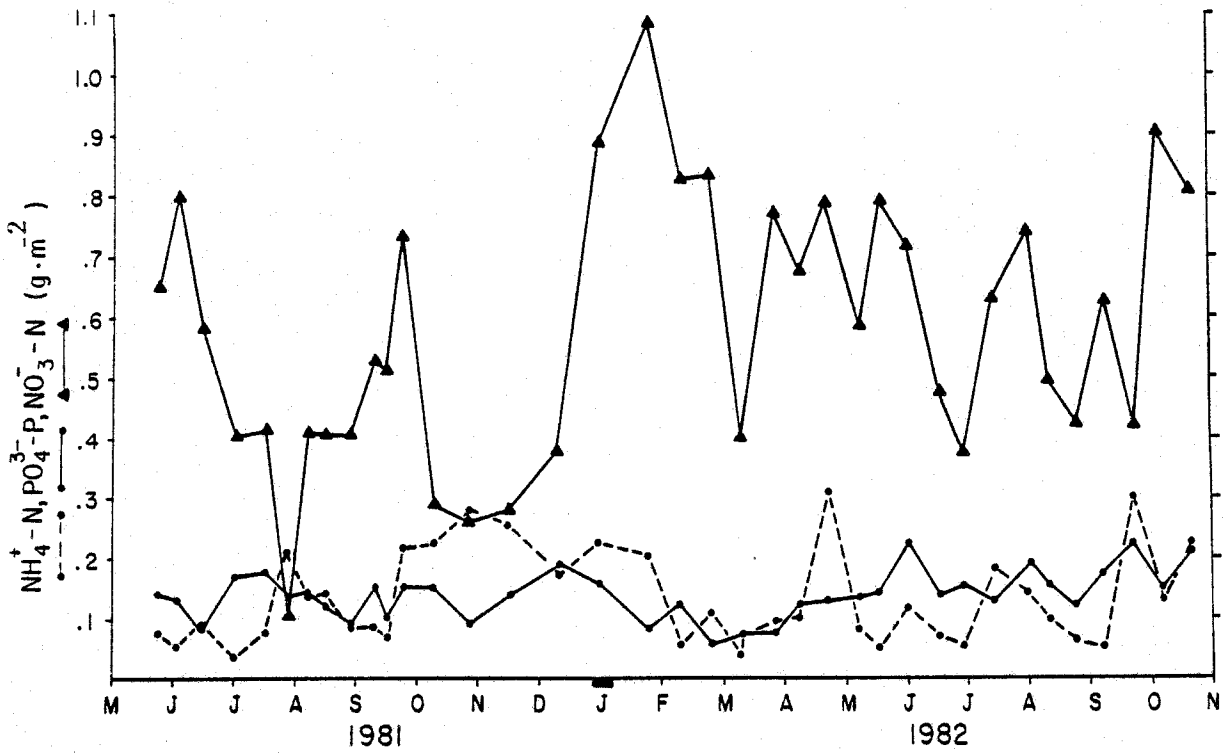

Figure 3. Aerial algal nutrient characteristics derived from an 8-sample depth water column at marker 52A. Note the generally high values for all nutrients, while nitrate, NO_3^--N, loading reveals dramatic pulses during specific periods of the year. These periods mostly represent high precipitation and, hence, elevated runoff events. Note also that nutrient depletion, that is, complete utilization of nutrients by algal biomass, is not apparent in the lower Neuse River at any time of the year. These findings indicate that large excesses of nutrients are present year-round.

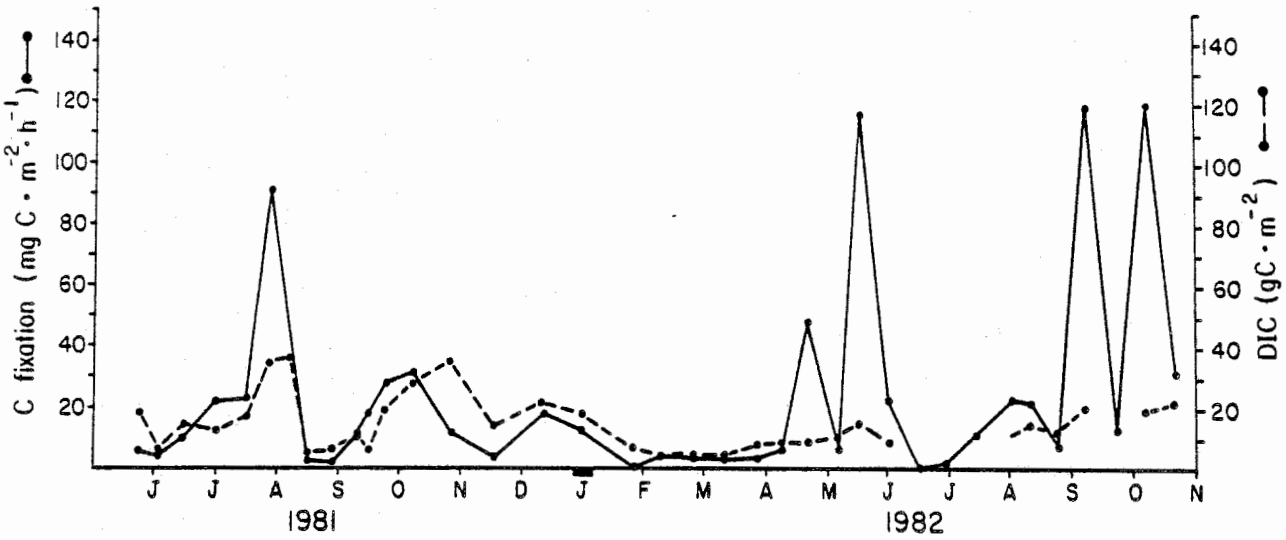

Figure 4. The relationships, over time, between aerial dissolved inorganic carbon (DIC) concentrations and algal primary productivity rates in the lower Neuse River (Station 5). A very close association between these parameters was observed in 1981, when surface Microcystis blooms were present, while the association was less distinct in 1982, when Mycrocystis blooms were undetected.

well as major fluctuations in production rates, biomass and nutrients.

Despite the fact that 1981 proved to be a "bloom year" for blue-green algal nuisance species, periods of maximum overall primary production as well as algal biomass occurred during 1982, which turned out to be a "non-bloom year" (Figs. 2,3,4). How is this possible? The ecological characteristics of nuisance blooms as well as physical characteristics of the water column play a major part in explaining this apparent paradox. Blue-green algal blooms tend to proliferate near the water's surface. Sub-surface waters are severely shaded when surface blooms proliferate, thereby restricting photosynthetic potentials of underlying algae. During periods not dominated by blue-green algal surface blooms, increased tranparency occurs throughout the water column. As a result, PAR penetrates deeper into the water column and underlying algae are able to photosynthesize. With a larger proportion of the water column receiving PAR, total aerial primary productivity as well as chlorophyll a can exceed levels recorded during surface blue-green algal blooms. During May and early September 1982, increased water column transparency combined with nutrient sufficiency led to aerial primary production rates as well as chlorophyll a levels in excess of levels recorded during blue-green algal blooms in 1981.

In general, PAR transparency is greatly restricted due to heavy coloration of river water (Fig. 5). This coloration is largely attributable to the presence of humic substances released during the breakdown of leaf matter and soil organic constituents as well as humic constituents in pulp mill effluent. Decreased PAR transparency restricts the zone in the water column where photosynthetic growth can take place (Fig. 5). If poor PAR transparency is combined with stagnation, or a lack of vertical mixing, a significant proportion of the algal biomass can be entrained in the non-photosynthetic "aphotic" deeper waters (Fig. 5). PAR can be extinguished over the first 1-1/2 m of the water column, leaving the lower 2/3 of the column devoid of photosynthetic activity and, hence, of net algal growth. Vertical mixing, either due to high river flow or wind mixing, can help alleviate entrainment problems by periodically mixing deep-living algae into surface waters (Fig. 5). During stagnant periods, algal entrainment in aphotic waters can last from hours to days.

Blue-green algal genera such as Microcystis and Aphanizomenon are able

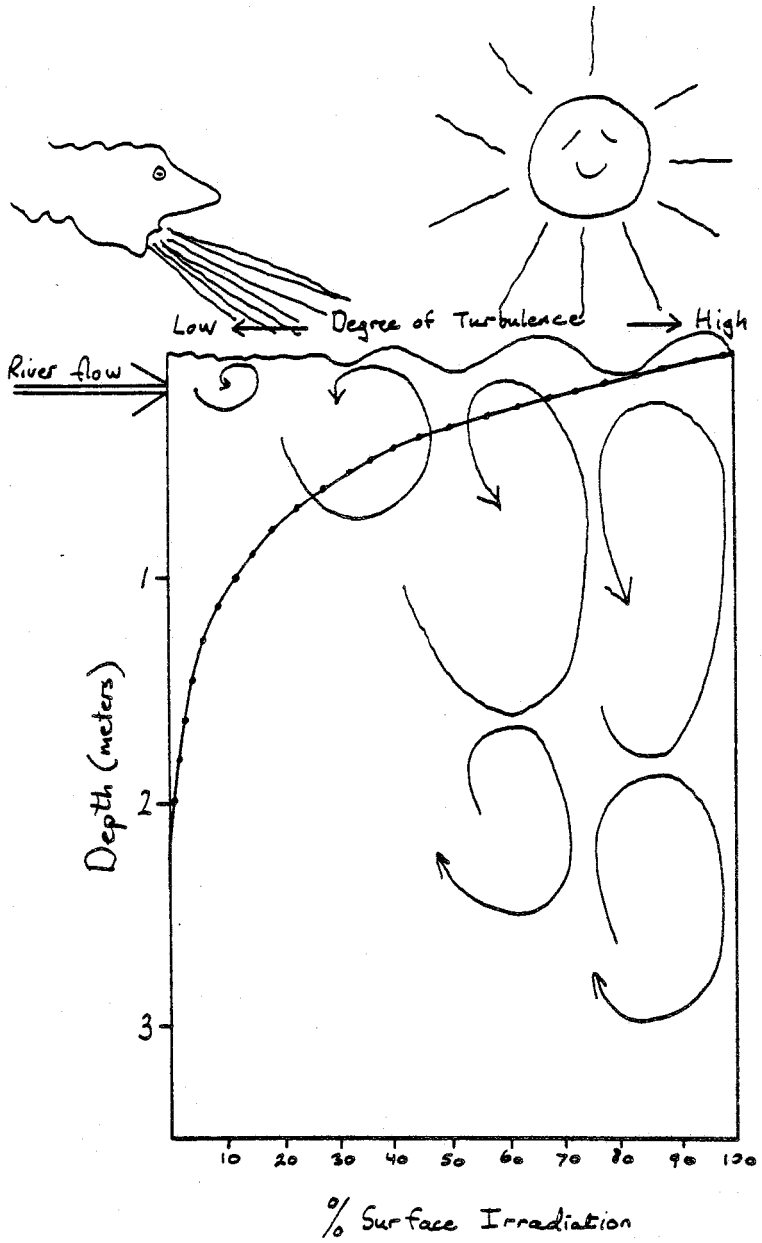

Figure 5. Interacting physical characteristics of the lower Neuse River and the potential role they play in regulating nuisance algal bloom formation. Represented is a profile of PAR transparency over degrees of turbulence. In the upper left portion of the column is the combination of low turbulence (caused by reduced river flow and no wind mixing) and high PAR availability. Such are the ideal conditions for bloom formation. Subsurface-dwelling algae are entrained in the lower, PAR-poor waters during such events. In the lower right (in fact, throughout all the water column) portion of this representation is the combination of dominance by the more desirable, non-bloom species. With high degrees of turbulence, the subsurface algal populations are periodically mixed to the surface, thereby enhancing their photosynthetic potentials and resulting growth.

to circumvent entrainment by altering their buoyancy through the formation of cellular gas vacuoles. Accordingly, when stagnant periods predominate during low-flow and calm-wind conditions, blue-green algae are favored to dominate the algal flora of the lower Neuse River. Such conditions are commonplace from late spring through summer months. Since nutrient sufficiency (of both nitrogen and phosphorus) exists throughout much of the year, physical limitation of blue-green algal bloom formation appears to outweigh nutrient limitation during summer months.

Ambient nutrient levels appear to parallel trends in algal production as well as chlorophyll a, particularly during summer bloom periods. All three major algal growth nutrients, nitrate (NO^-_3), ammonium (NH^+_4) nitrogen and phosphate (PO_4^{3-}) phosphorus revealed positive correlations when aerial (per m^2) concentrations were compared to both chlorophyll a levels and primary productivity rates. Correlation coefficients and confidence levels for 1981-1982 were as follows: aerial chlorophyll a vs. NO_3^- = +0.12, p=0.47; aerial chlorophyll a vs. NH_4^+ = +0.24, p = 0.14; aerial chlorophyll a vs. PO_4^{3-} = +0.37, p = 0.02. Because periodic pulses of NO_3^-, far in excess of algal growth requirements, occurred after storm (high-flow) events, the correlations between chlorophyll a (biomass) and NO_3^- proved to be low, although positive. Phosphate, which is more tightly bound to soil materials, is accordingly less likely to be a product of terrestrial runoff following heavy rainfall. As a result, proportionally less PO_4^{3-} than NO_3^- enters the river following rainfall and terrestrial runoff, and a better correlation between PO_4^{3-} and algal biomass (both PO_4^{3-} and algae biomass are "washed out" or are in abundance simultaneously during storm and drought periods respectively) results. Ammonia correlations with chlorophyll a assumed an intermediate value. This presumably occurred because: 1) ammonia is more tightly bound to soil particles than NO_3^- during runoff events, and 2) excessive NH_4^+ inputs following storm events did not occur as they had for NO_3^-. It should be mentioned that nitrification, if significant in the lower Neuse River, would also increase NO_3^- concentrations at the expense of NH_4^+ (D. Stanley, personal communication).

Strong positive correlations were also obtained between DIC and both chlorophyll a and primary productivity. Correlation coefficients and confidence

levels for 1981-1982 were as follows: chlorophyll *a* vs. DIC = +0.38, p = 0.03, and primary productivity vs. DIC = +0.381, p = 0.03. Particularly during 1981, when *Microcystis aeruginosa* blooms dominated during summer months, DIC content of the water column was well-linked to both chlorophyll *a* (biomass) and primary productivity; chlorophyll *a* vs. DIC = +0.74, p = 0.006; primary productivity vs. DIC = +0.67, p = 0.003. Similar correlations for 1982 yielded the following values: chlorophyll *a* vs. DIC = +0.32, p = 0.24, and primary productivity vs. DIC = +0.36, p = 0.1715. During 1981, low flow rates and a general lack of precipitation led to stagnant conditions in the lower Neuse River (Fig. 5). These conditions, combined with surface water temperatures in excess of 23°C and ample sunlight, provided optimal conditions for *Microcystis aeruginosa* bloom development. During these conditions, both nitrogen and phosphorus supplies remained in excess in the lower river. DIC levels, however, are characteristically low in this segment of the river, ranging from 2 to 7 mg $C \cdot l^{-1}$. Combined with observed pH rises during 1981 (Fig. 6), the possibility of DIC limitation, and particularly CO_2 limitation, of algal photosynthesis became a distinct possibility. Intense photosynthetic CO_2 demands by near-surface blooms of *Microcystis*, in the face of limited DIC supplies, could have led to CO_2 limitation. Furthermore, atomic ratios of biologically available carbon (calculated as total DIC), nitrogen and phosphorus measured during 1981 bloom periods also provide a basis for suspecting DIC limitation as playing an important role in controlling algal growth. Ratios of measured C:N:P levels ranged from 35:15:4 to 55:15:3 during 1981 as well as 1982. The generally accepted ratio for C:N:P sufficient for algal growth is approximately 105:15:1. A first-hand comparison of required nutrient ratios versus those measured in the lower Neuse River would also lead to the suspicion that DIC may have limited algal growth during both 1981 and 1982. When taking huge ambient excesses of nitrogen and phosphorus into consideration, the above lines of evidence strongly suggest a high degree of susceptibility to DIC limitation in the lower Neuse River. DIC limitation may be most profound when low river flow and calm days coincide, thereby minimizing introduction of atmospheric CO_2 into the water column by vertical mixing. Bioassay data presented later in this report substantiate this suspicion.

Two sets of observations throughout the sampling period suggest that both nitrogenous and phosphorous nutrient concentrations were consistently in excess of algal requirements. The first of these are the repeated instances of ex-

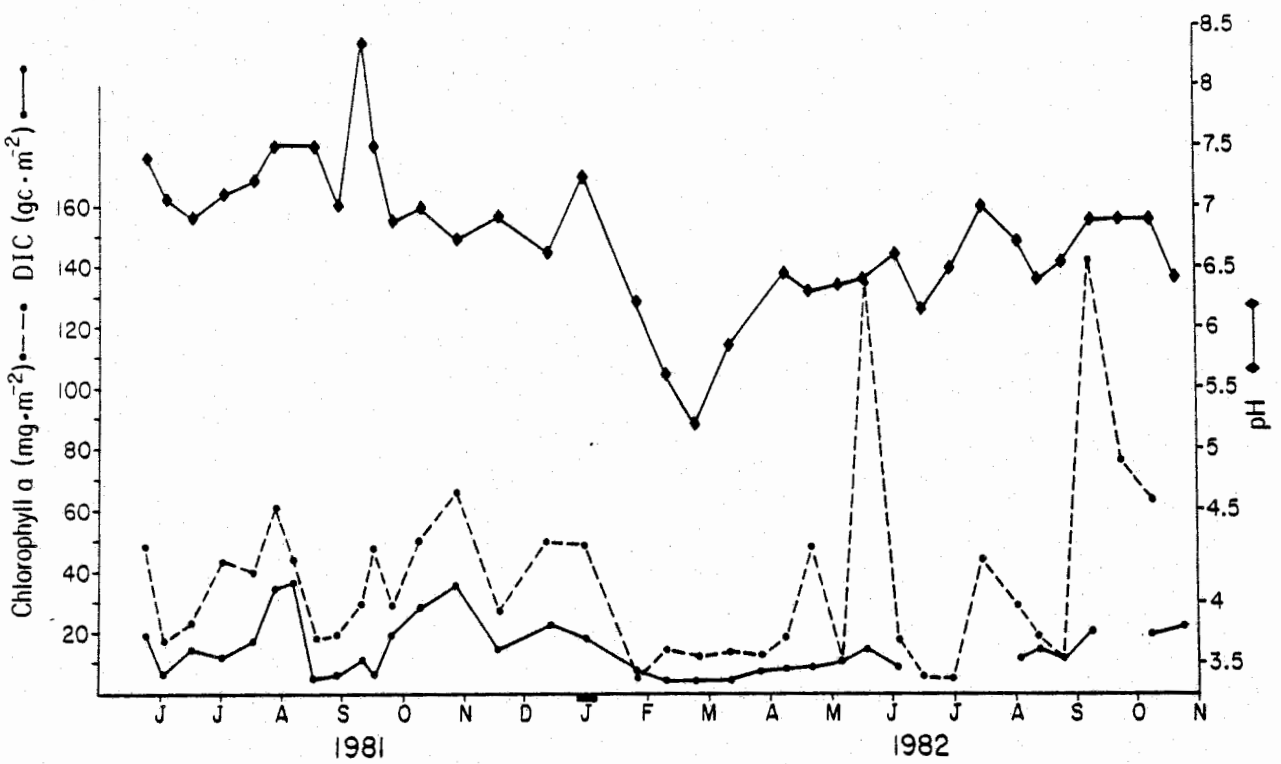

Figure 6. Combined measurements of dissolved inorganic carbon (DIC), pH and chlorophyll a (biomass) made on integrated water samples (8) on a biweekly basis at marker 52A. A close relationship between DIC and chlorophyll a is apparent in 1981; a rise in pH during this period is also noted. Such a pH rise could further decrease DIC availability, because at elevated pH values (algae 7), proportionally less CO_2 and more CO_3 would result, particularly in the face of naturally low DIC levels occurring in the Neuse River. Algal bloom genera such as Microcystis have been shown to prefer CO_2 over CO_3 as a carbon source in photosynthesis. The scumming behavior during the low CO_2 availability periods is thought to be a mechanism by which Microcystis can alleviate CO_2 limitations (by exposure to atmospheric CO_2 at the air-water interface).

tremely high nutrient values, in excess of several hundred ppb for each element, residing in the water column even during periods of maximum bloom intensity. If nitrogen and/or phosphorus content were limiting algal growth, one would expect to observe diminishing, and at times undetectable, nutrient levels during bloom episodes. Such events were never observed during 1981-1982. Secondly, periodic pulses of extremely high nitrate and phosphate concentrations proved to be a common feature of the water column throughout 1981-1982. Such pulses were associated with periods of accelerated river flow, strongly suggesting terrestrial runoff to be the source of nutrient enhancement. In particular, very high (in excess of 0.5 mg $N \cdot l^{-1}$) nitrate concentrations were observed during accelerated river flow, especially in 1982 when precipitation was abundant. Such concentrations were far in excess of known algal nutrient requirements for any of the bloom species inhabiting the lower Neuse River. By national and international limnological standards, such nitrate concentrations are alarmingly high. Even highly eutrophic aquatic systems such as the lower Mississippi River, Lake Erie and a variety of farm ponds seldom attain such high nitrate levels. Lastly, N_2 fixation was not detected at any time of the year, pointing to N sufficiency, even among the blue-green algal genera.

Vertical water column characteristics of the lower Neuse River: The potential for stratification

In several riverine-estuarine environments recently examined in eastern North Carolina (including the Chowan, Pamlico and Neuse River systems), periods of vertical stratification have been detected in respective water columns (Paerl 1982a; Kuenzler 1982; N. C. Department of Natural Resources and Community Development, Division of Environmental Management). Stratification in these systems is often an ephemeral event, lasting from several hours to several days (Paerl and Haibach 1983,unpublished results). The lower Neuse River appears to fit this description, largely being a polymictic system (Hutchinson 1957) and occasionally exhibiting thermal and/or chemical (salinity gradients) stratification. The duration of stratification events is largely unknown and will be investigated during 1983-1984 in the form of diurnal studies.

Despite the absence of diurnal data revealing temporal aspects of stratification, evidence gathered during our field studies of 1981-1982 implicates

climatic factors as dictating the frequency as well as duration of such stratification events. Rainfall appears to be an overriding determinant with respect to stratification potentials. Periods of reduced rainfall, as witnessed during spring and summer months of 1981, greatly promote the potential for stratification by reducing horizontal flow rates, thereby enhancing the ability of the water column to thermally stratify (to the extent that thermal stratification can overcome periodic surface and wind-mediated turbulent events). In addition, lower freshwater flow rates lead to periods of upstream salinity intrusion from the Neuse Estuary. When salinity intrusions became a feature of the lower Neuse River as they did during the late summer of 1981, strong vertical stratification was evident, particularly throughout the bottom third of the water column (2.5-4 m depth). Conversely, when periods of abundant precipitation characterized the Neuse River watershed as in 1982, elevated river flow rates minimized the opportunities for both thermal and salinity stratification. The contrasting impacts of dry (1981) versus wet (1982) hydrological years on vertical stratification are illustrated in Figure 7. While it appears that the amount of freshwater throughput is of central importance in dictating potential stratification (Fig. 7), ambient air and water temperatures are of consequence in enhancing summer stratification only when low flow rates predominated. Hence, calm (low horizontal velocity) waters were a necessary prerequisite for vertical stratification potentials, regardless of air temperatures. Minimal water (and air) temperatures conducive to stratification appeared to be 21-22°C. Since such temperatures are normally already present by late April, it is felt that river flow becomes a regulatory factor beyond this period. Since blue-green algal blooms are not initiated until after this period, late spring and summer river flow rates are likely to determine the magnitude and duration of blooms.

During stratification periods rapid decreases in dissolved oxygen became apparent in non-mixed layers (Fig. 7). The combination of salinity intrusions with very calm sunny weather in 1981 led to extensive periods of oxygen depletion in the lower third of the water column between New Bern and the Streets Ferry bridge. At times, and particularly in wide stagnant portions of this river region, dissolved oxgyen levels at the sediment-water interface dropped close to 0 ppm, indicating near-anoxic conditions. Most often, sediment-water interface oxygen readings of 0.1-0.3 ppm were recorded during stratified periods. More extensive surveys are planned during 1983-1984 to obtain detailed infor-

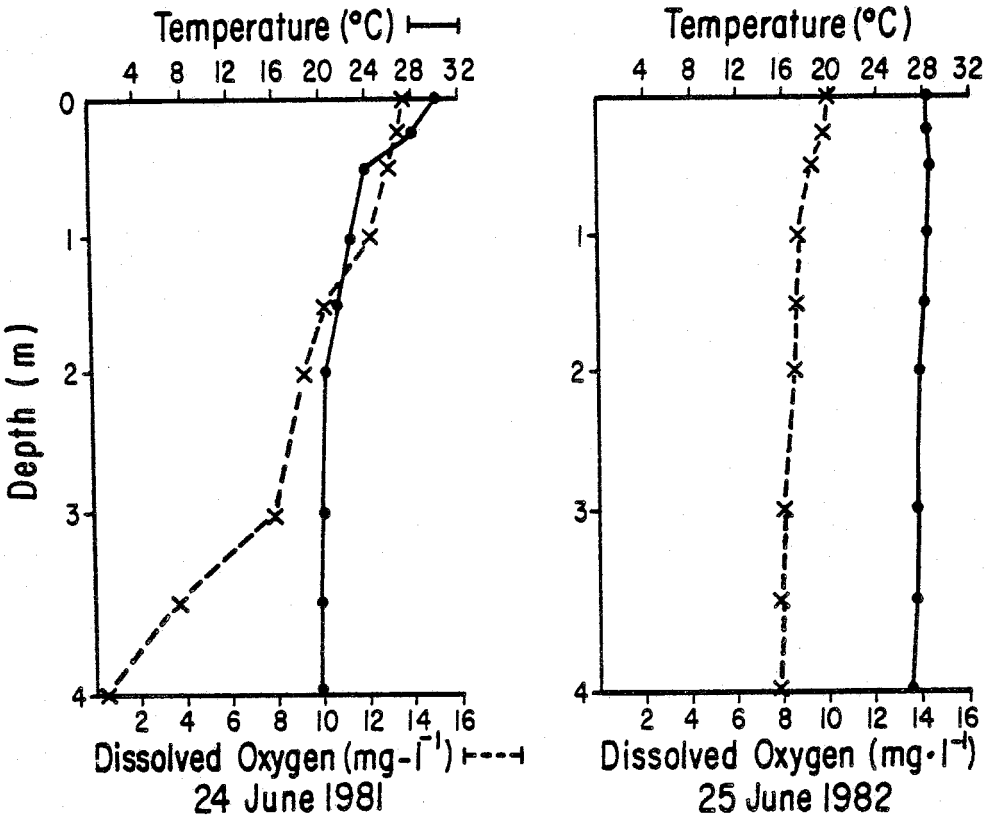

Figure 7. A comparison of stratified conditions in 1981 versus non-stratified conditions in 1982 on temperature and dissolved oxygen profiles measured at marker 52A during similar times of the year. In 1981 deoxygenated conditions were observed at the sediment-water interface (bottom), while fully oxygenated conditions occurred throughout the water column in 1982.

mation on deoxigenation potentials of bottom waters in the lower Neuse River. At this stage of our research it appears likely that deoxygenation events are a distinct possibility during low-flow summer periods. The probability of deoxygenation events is considerably enhanced during algal bloom periods when maximal amounts of algal biomass, coupled with minimal PAR transparency, increase the non-photosynthetic aphotic zone where O_2 usage through mineralization processses will greatly exceed O_2 production by photosynthesis. It follows that increased bloom occurrences will lead to higher chances for bottom water deoxygenation.

Salt water intrusions are particularly effective in promoting deoxygenation events, since density differences imparted by differing salinity layers lead to strong stratification which can resist wind mixing in the lower Neuse River. This was the case during late summer 1981, when a salt wedge proved to be a consistent feature of the river's bottom water between New Bern and the Weyerhaeuser effluent point near the Streets Ferry bridge (Fig.8). On numerous occasions no detectable oxygen was found at the sediment-water interface covered by the salt wedge.

The potential for a river or lake to deoxygenate at or above the sediment-water interface is considered to be an ominous symptom of advanced eutrophication. Fauna and flora dependent on the presence of dissolved oxygen in their ambient environment are often excluded from their habitat during deoxygenation events. The sediment-water interface represents an important source of food for deposit feeders, including a variety of crustaceans, mollusks (commercial shellfish species such as oysters, scallops, clams) and bottom feeding fish (both juvenile and adult individuals). Organisms normally adapted to oxygen-rich environments would, upon deoxygenation, find both their habitat and sources of food greatly curtailed, if not eliminated. This potentially harmful scenario may likely be occurring in the spawning and feeding grounds represented by the oligohaline waters of the lower Neuse River. There accordingly exists a distinct need to understand and evaluate impacts of bloom events, and associated enhancement of deoxygenation, on invertebrate fish and shellfish fecundity and productivity in the lower Neuse River.

Hypolimnetic oxygen depletion, including complete deoxygenation, also leads

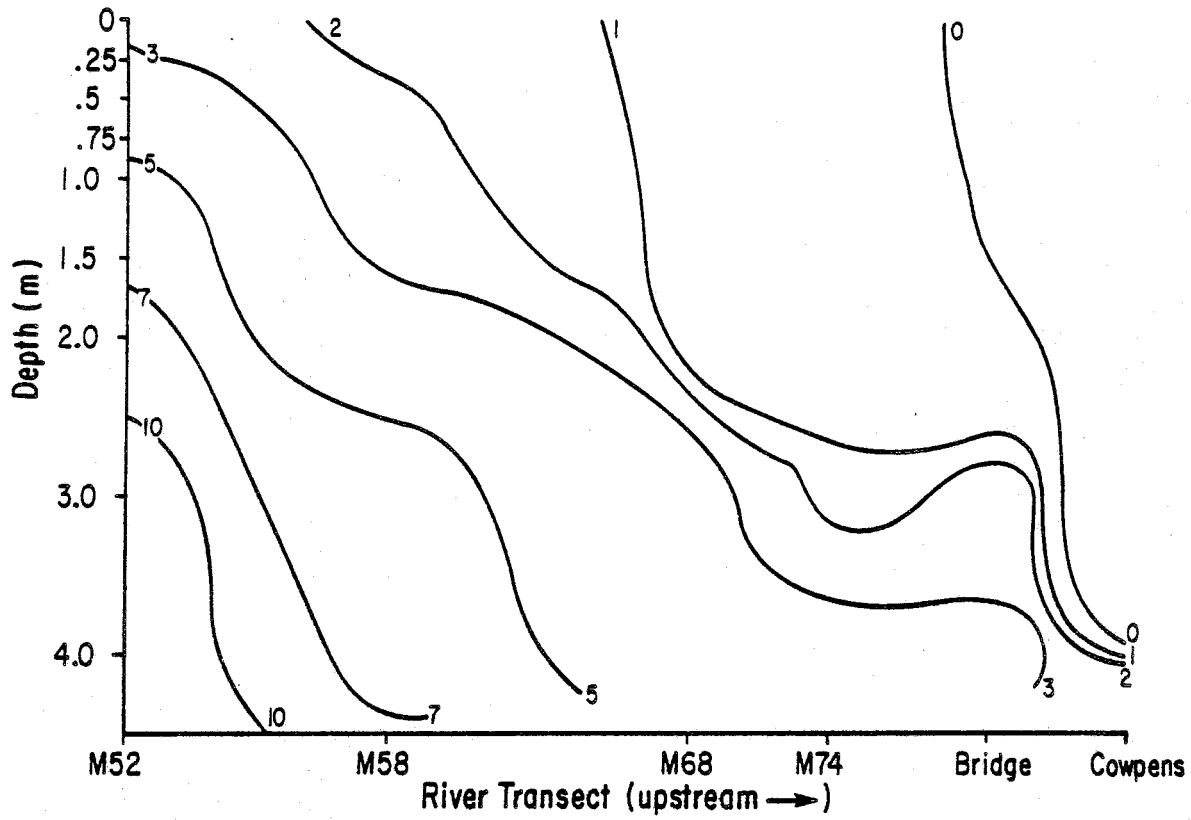

Figure 8. An isopleth illustration of a salinity wedge extending upstream from marker 52A to the Cowpens location near Vanceboro, N.C. during fall (October) 1981. Lines are marked with respective salinity levels (in ppt) determined from vertical profiles measured during a river transect.

to enhanced nutrient (largely as NH_4^+ and PO_4^{3-}) release from sediments as well as decomposing suspended organic matter above the sediments (Hutchinson 1957; Golterman 1975; Wetzel 1975). Hence, instead of acting as a nutrient sink, which typifies oxygenated sediments, oxygen depletion can lead to a net release of biologically available nutrients, thereby potentially aggravating eutrophication problems downstream. The lower Neuse River is a likely site for such nutrient release events, particularly in regions where our dissolved oxygen measurements have indicated levels <0.5 mg $O_2 \cdot \ell^{-1}$. Such regions were commonplace during summer stratification and bloom periods in 1981.

Salinity intrusions were evident throughout the late summer of 1981, starting in mid-June and ending in late November (Fig. 9). A similar intrusion period was confined from mid-August to mid-December in 1982 (Fig. 9). Clearly, periodic salinity intrusions into the Neuse River upstream from New Bern are an integral feature of hydrodynamics of the upper Neuse Estuary. An expanded presentation of the dynamics of salinity regimes in the lower Neuse River - upper Neuse Estuary will be given elsewhere (Paerl in preparation); however, major features of salinity intrusion will be summarized here.

Salt water intrusions are largely in the form of salt wedges, which move upstream along the bottom 0.5 m to 1.5 m of river water. Very little mixing of freshwater moving downstream and salt water wedges is apparent in the lower Neuse River section between New Bern and Vanceboro. As a result, discrete high-salinity wedges remain a stable feature of the bottom waters, while low-salinity surface waters penetrate the Neuse River Estuary down to New Bern. During the peak drought period in late September 1981, vertical salinity gradients typically appeared as follows: surface = 0 ppt (as NaCl), 0.25 m = 0 ppt, 0.5 m = 1.5 ppt, 0.75 m = 2.0 ppt, 1 m = 3.0 ppt, 2 m = 6.0 ppt, 2.5 m = 7.0 ppt, 3 m >7.0 ppt and the bottom at 3.2 m >7.0 ppt. The furthest upstream penetration of the salt wedge in 1981 was to the Weyerhaeuser discharge point, approximately 12 km upstream from New Bern (Fig. 8). Downstream from New Bern, salinity wedges are more diffusely distributed in the water column, and as a result surface salinities in excess of 1 ppt are more commonly encountered (Paerl, 1983a). It is largely due to wind mixing and resultant total destratification of the water column that salt water is introduced into freshwater-dominated surface layers. The segment of the river upstream from New Bern is well sheltered from either

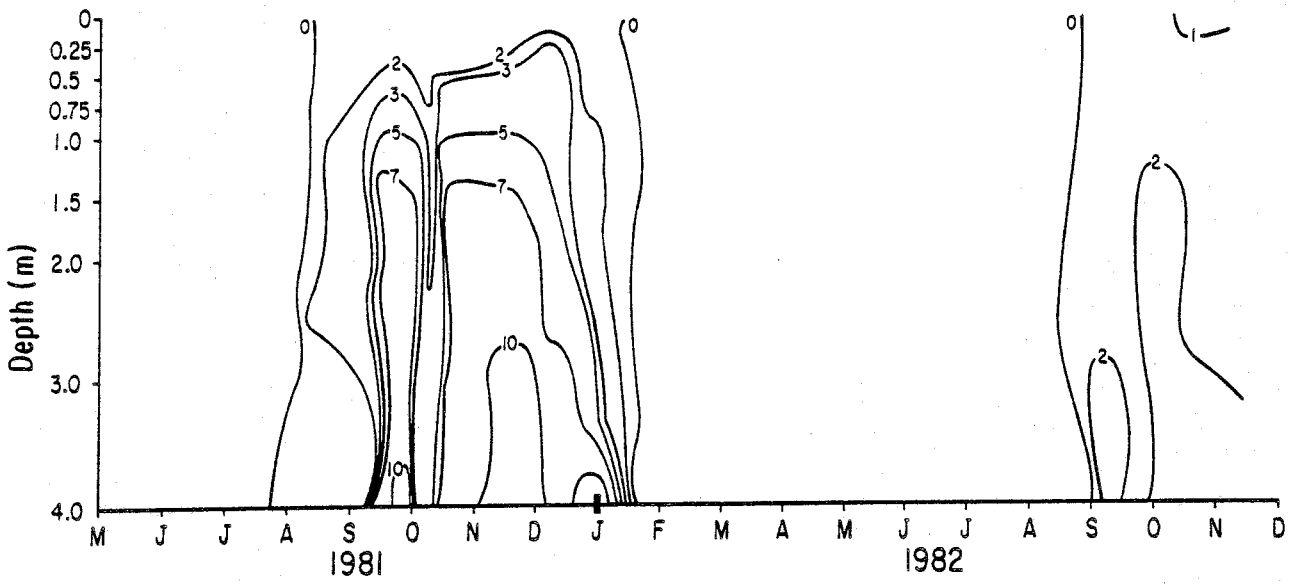

Figure 9. An isopleth illustration of periodic salinity intrusions recorded at marker 52A. Note that during a dry year (1981) salinity intrusions were more profound than during the following wet year (1982). Lines are marked with respective salinity levels (in ppt).

extensive north-south or east-west fetches, thereby minimizing the ability of windy periods to destratify the strong salinity gradients. As a result of minimal wind mixing and destratification, the surface waters encountered between Vanceboro and New Bern seldom reveal salinities in excess of 1.5 ppt, which is well below salinity levels considered to be inhibitory to growth of the three major bloom-forming blue-green algal genera, Anabaena, Aphanizomenon and Microcystis (Paerl 1982a). Hence, salinity is not expected to play a major role in arresting bloom activity or potentials in the lower Neuse River, since a bulk of the bloom organisms are located in the well-illuminated, near-surface, low-salinity waters. The possibility exists that activation of blue-green algal reproductive spores (akinetes) may be inhibited in sediments covered by salt wedges (Witherspoon et al. 1979). However, the question of whether or not akinete activation can occur in saline sediments of the lower Neuse River is not germane to potential penetration of blooms in the overlying freshwater surface layers, since blue-green algal cell division and akinete activation can take place in these uninhibited surface waters. In this manner, blue-green algal populations, which originate well upstream from Vanceboro, can maintain viable, buoyant populations, extending into the estuarine environment near New Bern.

In summary, although salinity wedges do penetrate the lower Neuse River upstream from New Bern, these wedges do not mix significantly enough with overlying low-salinity waters to cause growth-inhibiting salinity concentrations in surface waters supporting blue-green algal blooms. Hence, it must be concluded that salinity is of little consequence in altering or modifying bloom potentials in the lower Neuse River.

The combination of excess nitrogen and phosphorus loading, combined with relatively low DIC levels, makes the lower Neuse River susceptible to DIC limitation of algal growth. When climatic conditions prove optimal (spring and summer months), this nutrient combination leads to the promotion of blue-green algal blooms. Preliminary research results on blue-green algal bloom characteristics indicate that surface scums may, in part, be attributable to relatively low DIC but also high N and P levels (Paerl and Ustach 1982). This work has thus far shown that blue-green algae become more buoyant in the face of DIC limition. Increased buoyancy causes accumulations of blue-green algal in surface waters. It is hypothesized at this point that blue-green algae accumu-

late in surface waters to: 1) obtain CO_2 supplies from the atmosphere to alleviate DIC limitation, and 2) reside in a section of the water column rich in PAR, needed to power photosynthetic production of biomass. Hence, surface bloom formation appears to be a product of both excessive nutrient enrichment and naturally occurring low DIC levels in the lower Neuse River (Paerl 1983b).

Because of the heavy brown coloration (due to humic substances) as well as periods of enhanced algal growth typifying the lower Neuse River, PAR availablity is often confined to the upper 1-1½ meters of the water column. Figure 10 shows typical PAR, chlorophyll a and primary productivity profiles characteristic of both spring (diatom and green algae dominated) and summer (blue-green algae dominated) periods. In both cases PAR availability is restricted to the upper portions of the water column, but this trend is particularly evident during summer Microcystis blooms (Fig. 10). PAR shading due to the presence of surface scums of Microcystis adds significantly to PAR transparency decreases. Because of poor PAR transparency, primary production is usually maximal in the upper 0.25 meter of the water column. Slight inhibition of photosynthesis is, at times, apparent at the surface during spring diatom and green algal blooms, presumably due to extremely high incident PAR radiation and relatively good PAR transmittance characteristics. In contrast, summer Microcystis blooms reveal a distinctly different vertical profile of photosynthesis. Microcystis is not readily inhibited by the high surface PAR levels which proved inhibitory to spring algal populations. Microcystis generally reveals adaptability to high PAR levels, making optimal use of surface irradiation for photosynthetic growth. In part this adaptability has been attributed to enhanced cellular carotenoid pigment synthesis. Carotenoids are beneficial for optimal photosynthetic production in 2 ways: 1) they help protect cellular compounds and processes from photooxidation, and 2) they function as accessory pigments, channelling captured PAR in the 400-550 nm region (maximum PAR absorbence region for carotenoids) to chlorophyll a and ultimately to photoreductive processes (such as CO_2 fixation) (Paerl et al. 1983).

The ability of Microcystis to make efficient use of surface PAR gives it a distinct advantage over non blue-green algae which appear to be inhibited by surface PAR levels. Resultant surface dominance by Microcystis forces less-dominant diatoms and green algae into the underlying shaded waters, where sub-optimal

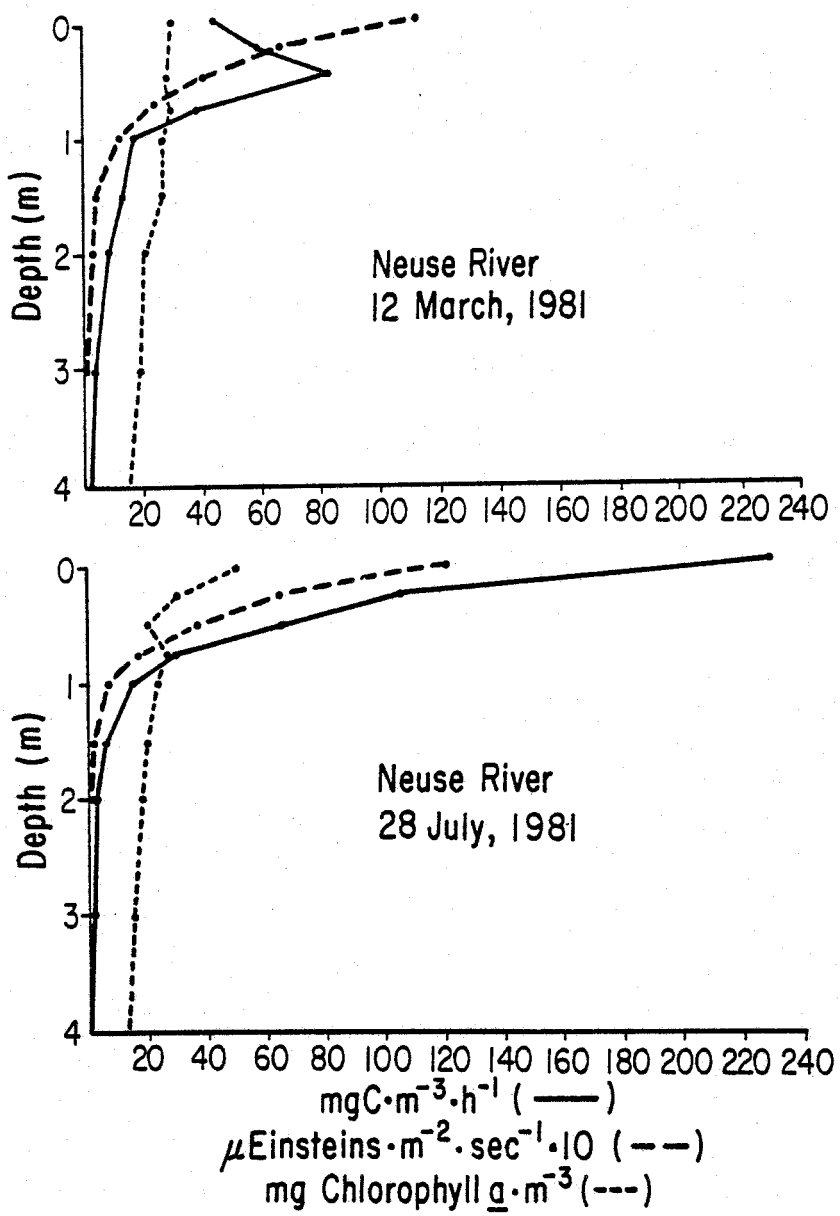

Figure 10. Vertical profile characteristics of primary productivity (in $mgC \cdot m^{-3} \cdot h^{-1}$), PAR transmittance ($\mu Einsteins \cdot m^{-2} \cdot sec^{-1} \cdot 10$) and chlorophyll a (in $mg \cdot m^{-3}$) during spring versus summer phytoplankton growth periods at marker 52A. Spring phytoplankton communities were dominated by diatoms and green algae, while summer communities were dominated by surface scums of the blue-green alga (Microcystis aeruginosa.

PAR levels exist. As long as calm, clear weather conditions and some degree of stratification (including low river flow and salinity stratification) persist and nutrient sufficiency prevails (which appears to be true on a year-round basis), Microcystis blooms will develop, proliferate and dominate the surface waters of the lower Neuse River. Only major rainfall events (causing river flushing) and generally poor weather (reduced PAR inputs due to cloudiness,for example) conditions will interrupt bloom events.

In conclusion, given the combination of nutrient excesses, heavy coloration of river water and periods of DIC insufficiency, conditions are ideal (particularly in warm, calm, clear weather) for the initiation, and eventual dominance, of nuisance blooms by blue-green algal genera capable of buoyancy regulation. Microcystis is one such genus.

Bioassay Experiments

In situ bioassays employed natural algal communities sampled at Vanceboro upstream from the Weyerhaeuser pulp mill discharge point. The previously stated objective of these experiments was severalfold: 1) to determine which major nutrient may be limiting algal growth, 2) to detect potential trace-metal deficiencies and 3) to assess the influence of pulp mill effluent on algal primary productivity. Bioassays were conducted during the summer of 1981 as well as spring and summer of 1982 using clear polyethylene Cubitainers as incubation vessels. A detailed description of bioassay techniques is given in Paerl (1982b). River water was collected in 25-liter pre-cleaned polyethylene carboys, followed by distribution of subsamples in 1-liter Cubitainers. Following nutrient, humic substance (prepared by column chromatography and dialysis in order to remove nutrients associated with pulp mill effluent) and trace metal additions, ^{14}C-$NaHCO_3$ was added to Cubitainers in order to measure photosynthetic growth responses. Final volume of river water plus nutrient additions was 900 ml per 1-liter Cubitainer. All treatments were conducted in triplicate or quadruplicate. Growth responses in terms of ^{14}C incorporation and chlorophyll a accumulation were measured 4-5 days after placement of the bioassay on a rectangular incubation frame anchored in the Neuse River near Vanceboro. Initially, bioassays were conducted in 1-liter, 4-liter and 10-liter Cubitainers to assess the effect of Cubitainer size on biostimulatory responses. These trials revealed that identical responses took

place regardless of Cubitainer size. Therefore, the smaller-volume 1-liter Cubitainers were chosen in order to optimize replication and space on the incubation rack.

During 1981, the effects of nitrogen as NO_3^-, phosphorus as PO_4^{3-}, iron and a trace-element mixture (Cu) were tested on three occasions. A set of representative results is shown in Figure 11. When compared to controls (no additions), neither nitrogen nor phosphorus was able to stimulate primary production or chlorophyll a production. Iron, as Fe^{3+}, marginally revealed stimulation of these parameters, while a trace-element mixture failed to stimulate algal growth. In all three experiments, both nitrogen and phosphorus failed to stimulate algal growth above control levels (Fig.11). These findings pointed to factors other than these two major algal nutrients as controlling growth.

During 1982, the same bioassay experiments were repeated in spring and summer months. Again, out of 4 bioassays conducted, neither nitrogen, phosphorus or a trace-element mixture were able to stimulate algal growth significantly above control levels. On one occasion, nitrogen stimulated growth marginally, but not significantly. Overall, however, nutrient additions of any type failed to enhance algal growth above control conditions. These findings collectively indicated nutrient sufficiency, at least during a 4-5 day enclosure period in Cubitainers.

In order to prove nutrient sufficiency, we chose to dilute several treatments with deionized water and glass fiber-filtered (GFC filters used) river water, followed by measurements of algal growth in comparison to undiluted controls. All dilution bioassays were supplied with 7 mg $C \cdot l^{-1}$ as $NaHCO_3$, in order to have identical initial DIC concentrations. The rationale behind dilution efforts was as follows. If nutrient sufficiency (in excess of algal growth requirements) was occurring, then by diluting river water with nutrient-free deionized water, nutrient limitation of growth should ensue at some dilution point. Similar dilution with filtered river water should dilute the test algae but not the nutrients. Hence, comparisons could be made between nutrient-poor and nutrient-rich conditions at similar starting densities of river algae. Control bioassays contained both undiluted densities of algae as well as nutrients.

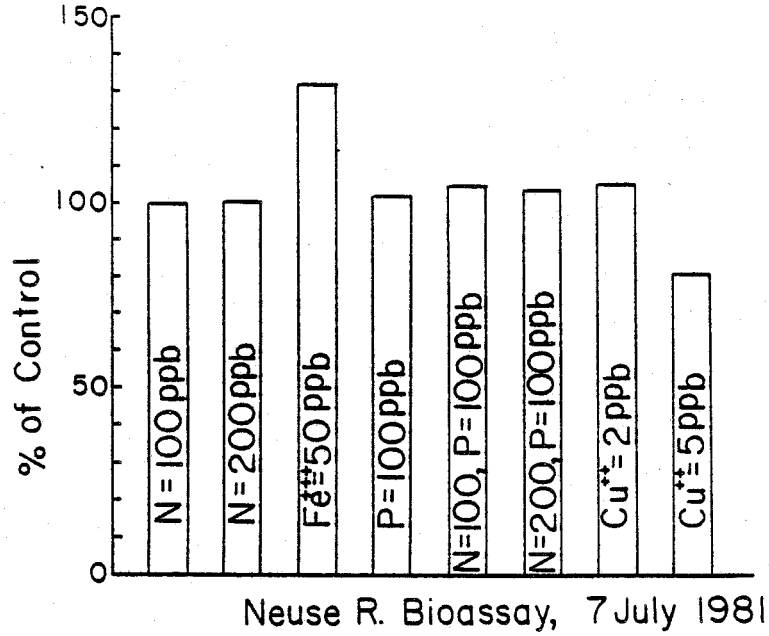

Figure 11. Results from an in situ $^{14}CO_2$ assimilation bioassay in which nitrogen (N), phosphorus (P), iron (Fe^{3+}) and Copper (Cu^{2+}) were added to river water sampled near Vanceboro, N.C. No nutrients, except Fe, were able to stimulate $^{14}CO_2$ assimilation above control (no-additional) levels.

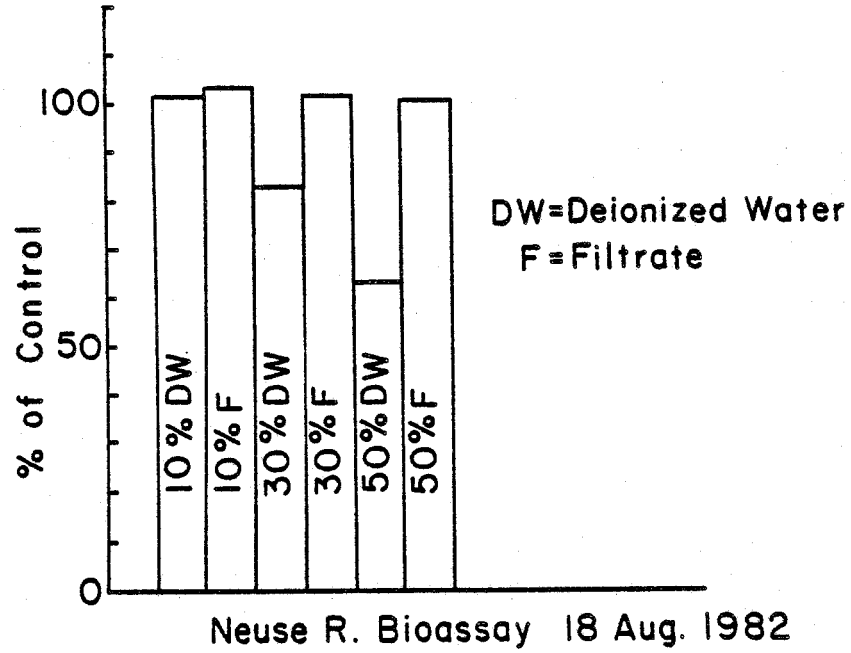

Figure 12. An in situ dilution bioassay showing the effects of dilution with either deionized water or filtered river water on $^{14}CO_2$ assimilation. Beyond 10% dilution, deionized water inhibited $^{14}CO_2$ assimilation, while filtered river water (at all dilutions) failed to inhibit $^{14}CO_2$ assimilation.

The results of these bioassays were very informative in that they pointed to both: 1) nutrient sufficiency under undiluted conditions and 2) a high potential for DIC limitation of algal growth. Figure 12 demonstrates typical results of dilution bioassays when deionized water dilutions are compared to filtrate dilutions. Deionized water (DW) dilutions of 10% failed to lower algal growth rates significantly. At 30% DW, approximately 80% of control growth rate was reported, while at 50% DW, approximately 60% of control growth rate was observed. Filtered river water dilutions up to 50% failed to lower overall growth. Hence, biostimulation by filtrate is high enough to circumvent an initial dilution of 50% of natural algal flora within 5 days. Algal biomass increases due to the high nutrient content of filtrate, which can overcome initial algal biomass dilution. This was not true if a 50% DW dilution was attempted. In this case, algal production could not recover to control levels, indicating either nutrient depletion or toxic effects of a 50% addition of DW. To test for nutrient depletion and to eliminate the possibilities of DW toxicity (either due to osmotic shock or major ion dilution), NO_3^- and PO_4^{3-} were added to 30% and 50% DW diluted samples, both individually and combined. The results showed a strikingly differential response to nitrogen and phosphorus (Fig. 13). In the case of nitrogen additions, supplementation of 50% DW dilutions with 200 ppb of N as NO_3^- overrode the inhibitory effects of dilution. When P as PO_4^{3-} was added at 100 (or 200) ppb, no such recovery of algal growth was observed. Together, NO_3^- (at 200 ppb) and PO_4^{3-} (at 100 ppb) led to a recovery of algal growth identical to NO_3^- additions alone. These results point to two conclusions which can be summarized at present: 1) nutrient (N and P) sufficiency, that is, nutrient concentrations in excess of algal requirements, appear to be commonplace in the lower Neuse River, and 2) nitrogen limitation is more readily attainable than phosphorus limitation, as shown by the fact that nitrogen additions could override the inhibitory effect of DW dilution. Putting this in a management-oriented perspective, constraints on nitrogenous inputs will have more immediate impacts on reducing algal growth, and bloom potential, than phosphorus cutbacks. Nitrogen, therefore, appears to be the most important, and most attainable, limiting nutrient.

Clearly, phosphorus also exists in vast excess for algal requirements. In fact, based on a comparison of commonly accepted nutrient ratios desirable for the maintenance of algal growth (Redfield ratio - C:N:P = 106:15:1), phos-

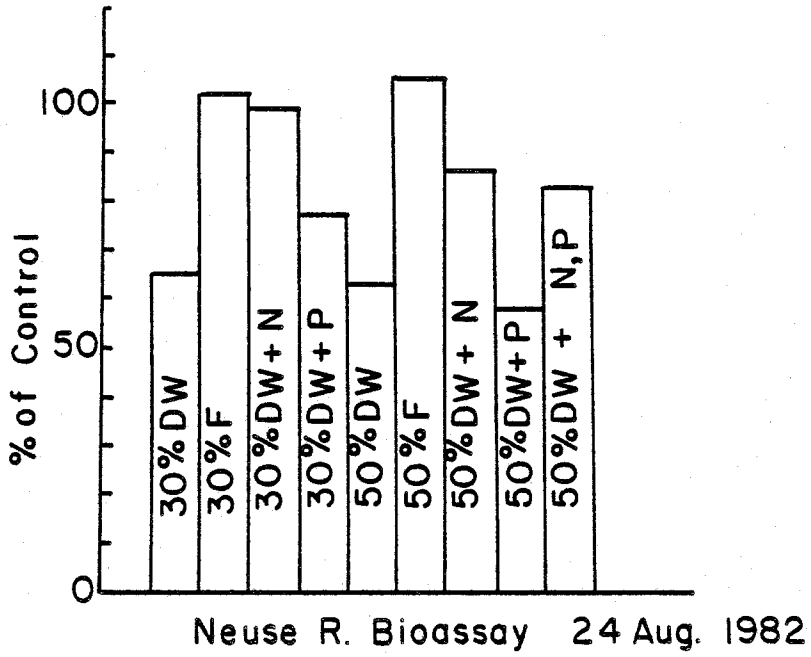

Figure 13. Dilution bioassay in which deionized water (DW) and river water filtrate were used to dilute water sampled near Vanceboro, N.C. Both 30% and 50% DW dilutions inhibited $^{14}CO_2$ assimilation in river algae, while filtrate dilutions of either 30% or 50% failed to inhibit $^{14}CO_2$ assimilation. The addition of 200 ppb N (as NO_3^-) overrode inhibition, while 100 (and 200) ppb P (as PO^{3-}) addition to override inhibition. Combined N and P additions had the same effect as N addition alone.

phorus appears to reveal the most inflated relative concentration in the Neuse River (C:N:P 35:15:3.5). Relative to DIC, approximately 9 times the desirable phosphorus concentrations would appear to exist in the lower Neuse River, based on largely theoretical grounds (Redfield ratio). In future management actions for the lower Neuse River, a 9-fold reduction is in all likelihood unattainable. A practical and realistic formulation to reduce nutrient loading can be accomplished largely through the development of dilution bioassays and their application to specific nutrient sensitivities in the lower Neuse River. We have spent the first year of bioassay research developing this technique in order to have a method of assessing relative nutrient sensitivities. We hope to apply the technique during 1983-1984 in order to address crucial questions as to what magnitudes of specific nutrient cutbacks should be sought in the Neuse River basin in order to assure cessation of nuisance bloom activities and restoration of acceptable water quality levels for recreational and commercial purposes (Figs. 14, 15).

One footnote with regard to the application of bioassay techniques in the lower Neuse River. This river represents a scientific challenge in terms of nutrient-sensitivity assessments. Since the river appears to experience excessive (with regard to algal growth needs) nutrient concentrations, conventional nutrient-addition bioassay techniques are not applicable. We have found it necessary to develop alternative technology to assess which, if any, nutrients are most likely to restrict algal growth when their loading rates are decreased. The application of dilution bioassay techniques is a necessary step in setting proper priorities and target levels for constraints on nutrient inputs in the Neuse River basin.

Hydrocorral Enclosure Experiments

The purpose for employing hyrocorrals in this project was threefold: 1) hydrocorrals were suitable enclosures for testing the potential effects of stagnation on blue-green algal bloom development, 2) by closing off the bottom of hydrocorrals, nutrient sufficiency (for promoting blue-green algal blooms) in the water column alone could be tested, and 3) large-container DIC, nitrogen and phosphorus enrichment experiments could be conducted on an isolated water column.

Much time was spent during April, May and June of 1982 designing proper

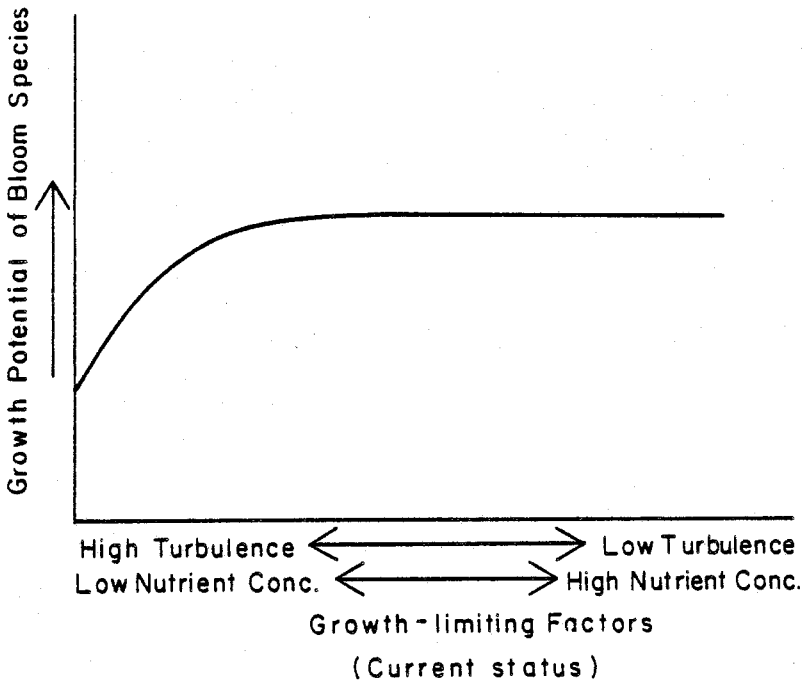

Figure 14a. Conceptual relationships between nutrient loading, water turbulence and growth potential of algal bloom species in the lower Neuse River. The above figure represents the current status where nutrient "overloading" is occurring. In this case, even a dramatic increase in turbulence cannot eliminate the presence, and at times dominance, of bloom species.

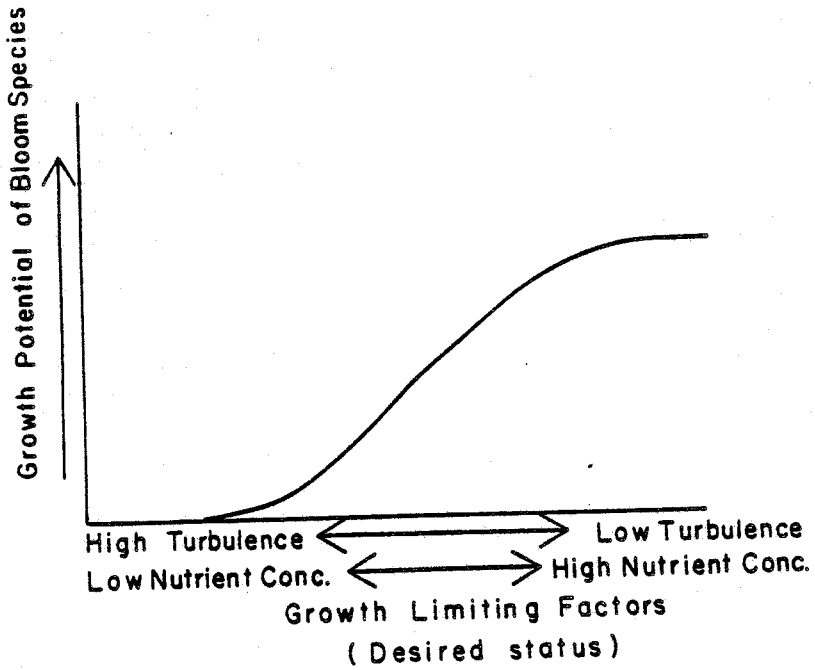

Figure 14b. A representation of the conditions desired in the lower Neuse River. In this case, when nutrient levels are reduced enough, no degree of turbulence will influence the growth potential of bloom species. Such conditions can only be attained if drastic cuts in nitrogen, followed by phosphorus, loading are achieved. The magnitudes of such cutbacks will be formulated in the next stage of research (1983-1984).

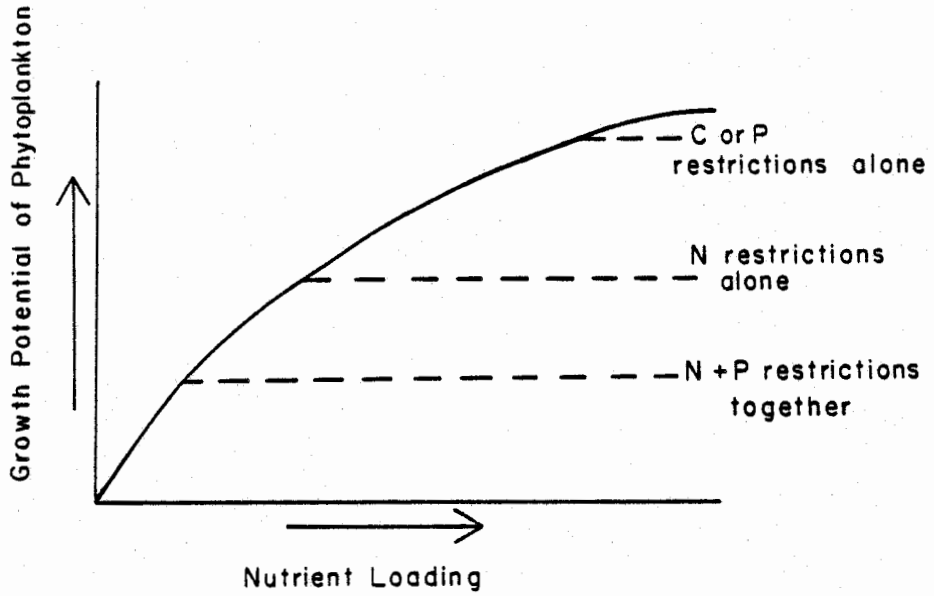

Figure 15. A representation of the most likely results of nutrient loading constraints on growth of phytoplankton in the lower Neuse River. Carbon, as DIC, and phosphorus restrictions alone will have little effect in lowering growth potential, while nitrogen alone, and particularly combined with phosphorus constraints, will be the most effective means of restricting accelerated eutrophication.

anchoring, flotation and bottom-closure devices. During late June, however, acceptable solutions were found to these problems and several experiments were conducted, which are reported below.

Initially, a line of 9 columns was placed in the river. No nutrients were added. At the time, no blue-green algal blooms were present in the river. However, 4 days later, large Microcystis colonies had developed in 6 of the 9 columns. Temperature and oxygen profiles measured in the columns revealed non-mixed, weakly stratified conditions. In 2 of the 3 columns not supporting Microcystis it was discovered that the bottom enclosures had broken loose, thereby destratifying those columns. These results indicated that physical stratification of the water column alone was an important factor implicated in promoting Microcystis bloom activity. This experiment also proved that nutrient concentrations present in the river at the initiation of stratification were adequate for supporting bloom activity. After 6 days of stratification, noticeable surface blooms of Microcystis prevailed in the columns, while the river proper remained free of Microcystis colonies. No significant temperature differences were recorded between columns and flowing river water (Fig. 16); however, supersaturated near-surface oxygen concentrations testified to the high rates of photosynthesis attributed to Microcystis blooms. Subsequent laboratory tests revealed that Microcystis was able to grow luxuriantly above 23°C, which was surpassed by both river and column water temperatures (Fig. 16). In conclusion, it appeared that physical stratification of the water was of prime importance in initiating bloom activity. Low river flow and calm weather conditions, as experienced in 1981, provided such favorable conditions. In contrast, consistently high river flow rates negated these conditions in 1982. Despite the absence of blooms in the river, they could be induced in the columns within several days.

In a second experiment, nutrient additions were made to the hydrocorrals. Corrals 1, 2 and 3 were designated as controls, having no nutrients added. Corrals 4, 5 and 6 received DIC additions (to test for DIC limitation of algal growth) in the form of $NaHCO_3$ at a final concentration of 7 mg $C \cdot l^{-1}$. Columns 7, 8 and 9 received DIC (7 mg $C \cdot l^{-1}$) as well as nitrogen (200 µg $N \cdot^{-1}$ as NO_3^-) and phosphorus (100 µg $P \cdot l^{-1}$ as PO_4^{3-}) additions. As a measurement of algal growth response, chlorophyll a was monitored in surface waters. Dissolved oxygen,

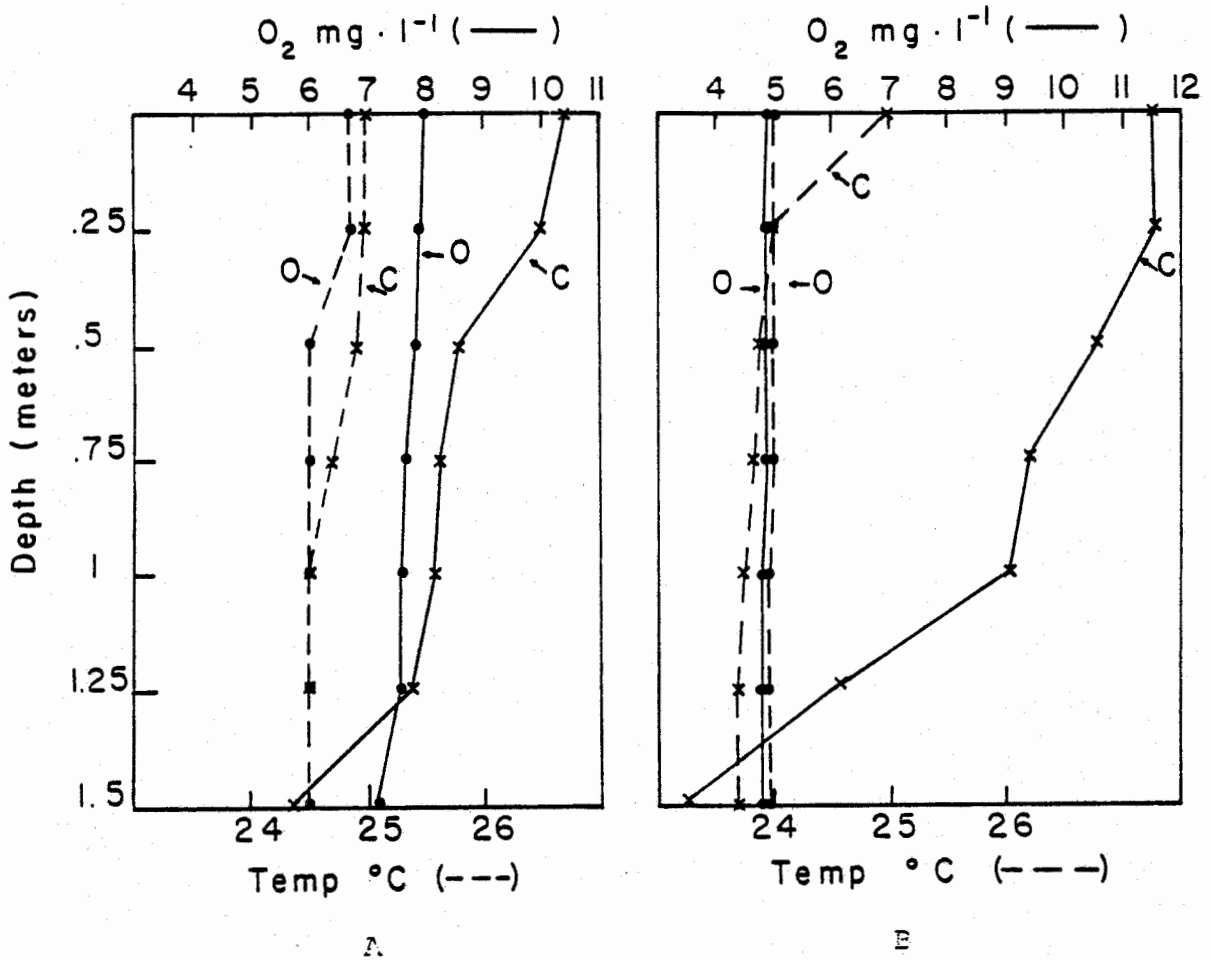

Figure 16. Results of temperature and oxygen measurements made in both open (running) Neuse River water and hydrocorrals on 2 separate occasions. Figure 16a shows results after 2 days of column emplacement, while Figure 16b shows results after 4 days of emplacement. Open river water measurements are denoted as "O," while column measurements are denoted as "C." In both instances, temperature measurements remained similar between column and open river water. However, due to the rapid establishment of Microcystis blooms in the columns, supersaturated oxygen concentration appeared in surface waters, while bottom waters assumed oxygen concentrations below those recorded in open river water. These 1982 column results mimic the 1981 open-water results when, due to low river flow, similar oxygen stratification could be seen in the water column. In 1981, such stratification was associated with Microcystis blooms.

temperature and light (PAR) transparency profiles were also measured in all columns as well as outside river waters. Figure 17 shows the results of this bioassay. On 21 July, the experiment was initiated by filling columns with river water followed by nutrient additions. Fairly uniform oxygen, chlorophyll a and temperature conditions were recorded in all 9 columns at this time. Values presented for each treatment are the average of triplicate column results. Two days later, on 23 July, some similarities as well as differences were apparent among treatments. All columns, including controls, revealed developing Microcystis colonies (indicated as a + in Figure 17). DIC treatments supported the most profound development of colonies (indicated as ++). The magnitudes of O_2 super-saturation also followed the development of Microcystis blooms; good agreement between the density of Microcystis and O_2 content of surface waters in columns was apparent throughout the experimental period. Both DIC and DIC plus nitrogen and phosphorus treatments revealed O_2 enrichment as well as elevated chlorophyll a levels. An important finding was the fact that DIC treatments alone led to elevated (over control) algal growth. The same scenario proved true on 30 July, 9 days after the initiation of this experiment. Elevated O_2 and chlorophyll a levels were again apparent in DIC as well as DIC plus nitrogen and phosphorus treatment (Fig. 17). Microcystis bloom development was also most noticeable in these treatments. From 30 July onward, O_2 and chlorophyll a enrichment proved to be most profound in DIC plus nitrogen and phosphorus treatments. Although DIC initially stimulated algal growth within 2 to 3 days, further nutrient (nitrogen and phosphorus) enhancement led to maximum development of algal blooms in the hydrocorrals (indicated as +++).

These initial experiments illustrate the usefulness of hydrocorrals in gaining an understanding of the complex interplay of physical and chemical factors influencing the development and proliferation of nuisance algal blooms in the lower Neuse River. The deployment of hydrocorrals as well as Cubitainer bio-asays has allowed us to focus on several key parameters to be more precisely assessed and formulated in future research. These parameters include: 1) the role of DIC in bloom development; 2) the role of nitrogen, followed by phosphorus, cutbacks, and recommended magnitudes of such cutbacks needed to arrest bloom potential; 3) proper turbulent regimes, including river flow, wind exposure and micro-stratification needed to minimize bloom potential, and 4) tolerable

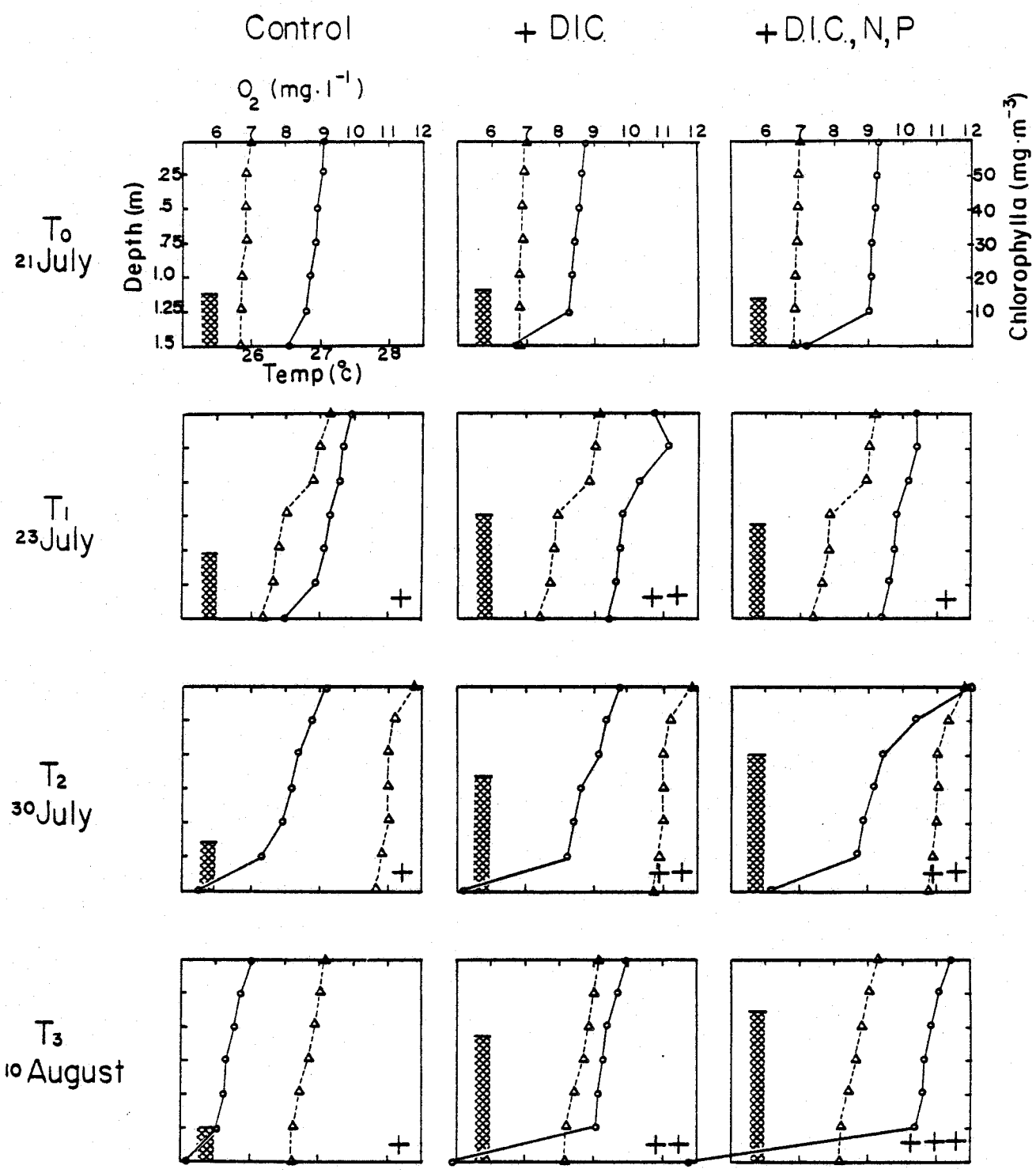

Figure 17. Results of a hydrocorral nutrient-addition experiment initiated 21 July, 1982. Three sets of triplicate columns were run: controls (no addition), a DIC (7 mg C·l^{-1}) addition and a DIC plus nitrogen 200 µg N·l^{-1}) and phosphorus (100 µg P·l^{-1}) addition. Resulting integrated temperature, oxygen and surface chlorophyll a levels are illustrated at initial and subsequent sampling intervals. A+ indicates the presence of Microcytis colonies, while ++ and +++ indicate increased dominance by Microcystis. Bars represent chlorophyll a values, temperature is shown as Δ----Δ and dissolved oxygen is shown as o——o.

levels of pulp mill effluent (considering both nutrient and humic characteristics of effluent) which can be discharged into the lower Neuse River. Our objectives in 1983-1984 are to resolve the above crucial water quality questions by developing predictable relationships between the above parameters and nuisance bloom potentials in the lower Neuse River.

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