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**STABLE ISOTOPE TRACERS OF NITROGEN SOURCES TO THE  
NEUSE RIVER, NORTH CAROLINA**

by

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## ABSTRACT

Stable nitrogen isotopic analyses ( $\delta^{15}\text{N}$ ) were conducted on dissolved nitrate in surface water samples collected over a two year period from the lower Neuse River, North Carolina, to assess temporal variation of nitrate sources to the watershed. Nitrate samples collected at the Raleigh and Durham Municipal Sewage Treatment Plants (MSTP) have nitrogen isotopic values in the +9 to +14 per mil range. Nitrate samples collected from agricultural drainage areas in the lower portion of the Neuse River basin have nitrogen isotopic values in the +4 to +9 per mil range. These nitrogen isotopic values correspond well to literature values published for point and non-point nitrate sources, respectively, and confirm that there is a well defined isotopic difference between point and non-point nitrate inputs to the Neuse River Basin. The nitrogen isotopic values of nitrate from the lower Neuse, which integrates the nutrient loading trends over the entire basin, show an annual cycle. Surface water nitrate during low discharge periods is isotopically enriched and is in the point source range of +9 to +14 per mil. Surface water nitrate collected during high discharge periods is isotopically depleted and is in the non-point source range of +4 to +9 per mil. Over the entire annual hydrological cycle, surface water nitrate nitrogen isotopic values are exponentially related to river discharge rate. This relationship suggests that a residence time delay factor in an intermediate reservoir (groundwater, wetlands) plays an important role in the transfer of non-point source nitrate to the riverine system during the falling discharge (spring) period. The enhanced fertilizer loading in wet years during the critical spring period can influence the biological species succession and would therefore play a significant role in enhancing nuisance algae bloom potential in the late summer.

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$${}^2\delta\text{R} = (\text{R}_{\text{sam}} - \text{R}_{\text{std}} / \text{R}_{\text{std}}) \times 10^3 \quad \text{R} = {}^{15}\text{N}/{}^{14}\text{N} \quad \text{R}_{\text{std}} = \text{Atmospheric Nitrogen}$$

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## SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Point and non-point nitrogen sources sampled in the Neuse River Basin have distinct nitrogen isotope signals similar to published values from other watersheds. The nitrogen isotopic composition of nitrate, which is the dominant form of nitrogen entering the Neuse River, can be employed to distinguish between point and non-point source inputs. The nitrogen isotopic composition of surface water nitrate collected above New Bern has a bimodal annual trend. This indicates that there is a temporal separation of nitrate nutrient sources during the annual nitrogen loading cycle in the Neuse River Basin. Nitrate isotopic compositions similar to point source values are observed during the low discharge summer months. Nitrate isotopic compositions similar to non-point source values occur during the high discharge winter months. The nitrogen isotopic composition of nitrate is exponentially related to river discharge rate. This indicates that the mixing of point and non-point source nitrogen reservoirs is not strictly controlled by wet period surface water runoff of agricultural fertilizers and excess soil nitrate into the river. The non-point source nitrate must pass through an additional reservoir that modulates the mixing with a residence time delay factor. This is most likely the result of the leakage of non-point source nitrate out of the surface saturated groundwater reservoir or wetlands systems in the basin as the river goes into low flow conditions. Therefore, although the dominant form of nitrate in the river during summer nuisance blooms is of point source origin, the potential for nuisance algal blooms established during the initial spring bloom period is also influenced by non-point source loading. The late winter/early spring nitrogen fertilizer loading that occurs in the Neuse River Basin, coupled with the character of the annual river discharge trend, is therefore critical to the development of late summer blooms by enhancing nuisance bloom potential during the spring bloom initiation period.

Management strategies that attempt to limit nuisance bloom development in the lower Neuse River must address spring excessive non-point loading. It is clear that the concentration of non-point source agricultural nitrogen in the late winter/early spring discharge has profound impacts on inorganic nitrogen loading and  $\text{NO}_3$  nutrient enrichment well beyond the wet winter period. The crucial transitional period of May-June when phytoplankton growth accelerates as a result of the optimization of physical factors (light and temperature) is when bloom potentials develop. We suggest that these blooms can be influenced by residual non-point source  $\text{NO}_3$  emanating from the earlier (February to April) high discharge runoff that enters the river in exponentially diminishing concentrations after transiting through the groundwater and/or swamp and wetland systems as the river changes from high to low flow conditions. As shown by the exponential relationship between  $\delta^{15}\text{N-NO}_3$  and discharge, this large agricultural non-point nitrogen source has clearly not exited the lower Neuse by the time that the critical late spring transitional period arrives. The duration of the spring/summer period of influence of the agricultural non-point source on the river nitrate reservoir is directly related to the shape of the annual hydrograph and the amount of water delivered to the basin during the wet winter season. During drought years and years when the

normally wet winter period is relatively dry, non-point source nitrogen is not flushed into the river system, but rather remains in the areas to which the fertilizer has been applied. In years when the winter months are very wet, this non-point source nitrogen is flushed through the groundwater reservoir and/or the wetland systems, and continues to "leak" into the river system until low flow conditions are stabilized in during the late summer months. By this time physical and biological conditions are well established for the development of nuisance blooms and algal species succession patterns amenable to nuisance bloom development and growth. At this point only large flushing events such as tropical storms or persistent thunderstorm activity can rid the lower Neuse River of nuisance blooms (Paerl, 1987).

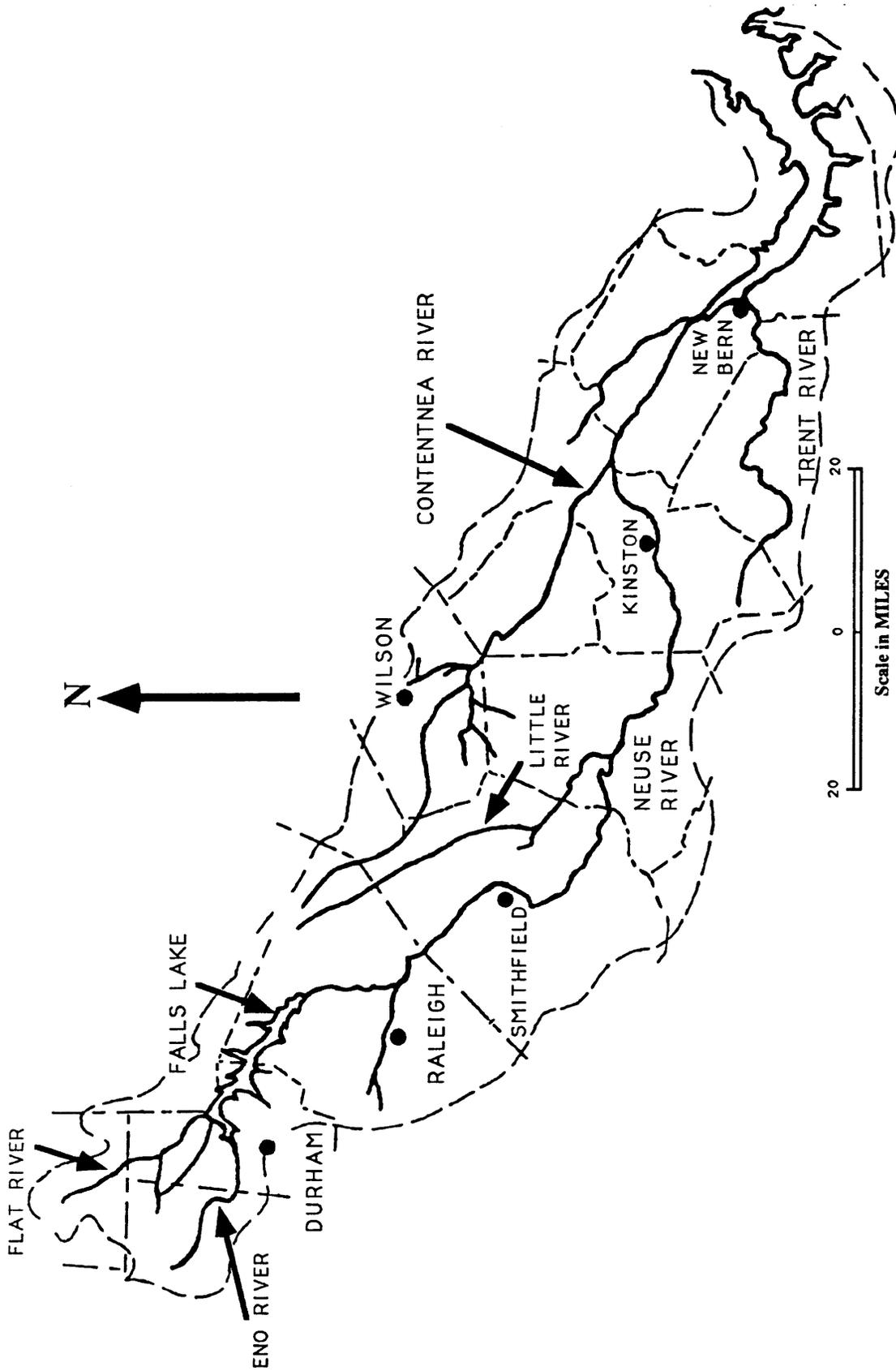
It would therefore appear crucial to reduce the amount of non-point source nitrogen loading during the early spring (March-April) period, so that conditions that favor nuisance bloom development (slower moving water, increased light and temperature) are not augmented by the exponentially decreasing non-point source nitrogen entering the lower Neuse as the river flow state changes into low flow conditions. This would require a reduction in the non-point source nitrogen loading rates in the March/April period closer to May/June loading rates, which would not allow the establishment of potential bloom conditions during the critical transition period. Such a management strategy will not be easy to attain. However, if there is to be a definitive, consistent, and effective manner to arrest nuisance blooms in wet and dry years, such a strategy is inevitable. The recommended temporal constraints on spring nitrogen loading will yield additional parallel benefits of reducing some phosphorus loading originating from agricultural activities. In the long term, additional phosphorus loading reductions may be beneficial as the river becomes more nitrogen depleted and susceptible to N<sub>2</sub> fixing cyanobacterial blooms (*Anabaena*, *Aphanizomenon*) as opposed to the current dominance of the non-N<sub>2</sub> fixing cyanobacteria (*Microcystis*, *Oscillatoria*). The recommended reduction of spring non-point loading will undoubtedly lead to improved trophic and aesthetic conditions in bloom-sensitive water of the lower Neuse River.

## INTRODUCTION

### EUTROPHICATION AND NUISANCE ALGAE IN THE LOWER NEUSE RIVER

The commercially and recreationally important Neuse River drains approximately 25% of North Carolina's Piedmont and Coastal Plain regions. The Neuse River is the second largest river basin contained entirely within the state of North Carolina and flows over 220 miles from the confluence of the Flat and Eno Rivers near Durham, N.C., to below New Bern, N.C, where it widens to become an estuary draining into Pamlico Sound (DEM, 1983). In the upper portion of the watershed, the Neuse River is a typical Piedmont river system that drains urban centers (Raleigh-Durham, Chapel Hill-Hillsborough, Kinston, Goldsboro). In the lower portion of the watershed, from Smithfield to New Bern, the Neuse River is a typical coastal river that supports agricultural and industrial centers (DEM, 1983; Figure 1). During the past decade this watershed has experienced accelerated nutrient inputs accompanied by an alarming rate of eutrophication. The most serious symptoms of enhanced eutrophication are nuisance blue-green algae blooms which have led to unacceptable water quality conditions (DEM, 1983, 1984, 1985; Paerl, 1983). During certain years when a high spring runoff period is followed by dry low flow summer conditions, nuisance algae blooms (*Microcystis*, *Oscillatoria*) can coat the river with green paint-like scums (Paerl, 1987, 1988; DEM, 1984). On average, such blooms occur with a frequency of every 2-3 years. When nuisance blooms are present, they frequently persist for several months.

During the summer of 1983, much of the lower Neuse River was plagued with *Microcystis* blooms for 4 months (DEM, 1984). Blooms of this magnitude can lead to long lasting alterations in water quality and trophic characteristics (Paerl, 1988). Periods of dissolved oxygen depletion (anoxia) in river bottom waters and sediments can exist under surface blooms in stagnant sections of the Neuse River, and leave portions of the river uninhabitable for desirable fauna and flora (Paerl, 1983). Paerl (1983, 1988) has suggested that both nitrogen and phosphorus loadings exceed algal growth requirements throughout much of the year in the Neuse River, particularly during the initial spring bloom period. Enhanced anthropogenic nutrient loading has led to hypereutrophic conditions in years when nutrient inputs exceeded algal nutrient demands (Paerl and Bowles, 1987). Nitrogen appears to be the limiting nutrient during intense bloom years (Paerl, 1983). The Neuse River receives approximately a 25:75 ratio of nitrogen from point and non-point sources on an annual basis (DEM, 1985). However the relative proportions of the source inputs may vary over the annual hydrological cycle, and nitrogen loading characteristics are thought to be seasonally variable in the Neuse River Basin (NRCD, 1983). Although excessive nutrient loading enhances the bloom potential of the lower Neuse River, physical factors such as high spring discharge coupled with low flow conditions during the summer months are also of importance with respect to the promotion and persistence of summer nuisance blooms (Paerl, 1983, 1988). In particular, the timing of the nitrogenous inputs into the Neuse River is critical



**FIGURE 1.** The Neuse River Basin drainage area. The upper portion of the basin is dominated by urban areas, while the lower portion of the basin is dominated by rural areas.

with respect to the enhancement of spring-summer algal growth and bloom potentials. Characterization of different nitrogenous sources can be determined by measuring the natural abundances of stable nitrogen isotopes by ratio mass spectrometry (Heaton, 1986).

### STABLE ISOTOPE DISCRIMINATION OF NITROGENOUS SOURCES

Differences exist between the natural abundances of stable nitrogen isotopes ( $^{15}\text{N}/^{14}\text{N}$ ) in dissolved and particulate matter from terrestrial, estuarine, marine, and anthropogenic sources (Hoefs, 1980; Peters *et al.*, 1978; Sweeney *et al.*, 1978; Kaplan, 1983; Mariotti *et al.*, 1984). These differences are measured by a magnetic sector mass spectrometer as ratios of the heavier isotope ( $^{15}\text{N}$ ) to the lighter isotope ( $^{14}\text{N}$ ). This sample ratio is then compared to the isotopic ratio of an international standard (atmospheric nitrogen in the case of  $\delta^{15}\text{N}$ ) and the difference expressed as a delta value ( $\delta$ ) in per mil units (parts per thousand of the  $^{15}\text{N}/^{14}\text{N}$  ratio). Positive delta values indicate more of the heavy isotope is present than in the international standard, while negative delta values indicate that more of the light isotope is present (Hoefs, 1980). The natural distribution of light stable isotopes (H,C,O,N,S) is not uniform. Because of slightly different chemical behavior of the heavy and light isotopes related to mass and energy contents, biogeochemical and physical systems will concentrate isotopes in different abundances that are directly related to the dominant chemical pathways (Fritz and Fontes, 1980, 1986). Light stable isotopes (H, C, N, O, S) occur in relatively great abundance, and the isotopic composition of naturally occurring compounds can be determined with an analytical precision that is much greater than natural variations. The application of stable isotope geochemistry to environmental and biogeochemical investigations has therefore become a standard and very powerful tool.

For example, the differences between the carbon isotopic values of terrestrial and marine plants are known to result from the type of photosynthetic pathway used to fix inorganic carbon and the isotopic composition of the carbon pool available to the plants (Deines, 1980). The nitrogen isotopic composition of plants is similarly determined by the nitrogen nutrient pool available to the plant. Fixation of atmospheric  $\text{N}_2$  into fertilizers results in fertilizer isotopic compositions that range from -4.1 to +1.9 (Black and Waring, 1977), while the final isotopic ratio can vary according to the amounts of  $\text{NH}_4$  and  $\text{NO}_3$  nitrogen present in the fertilizer (Freyer and Aly, 1975). Nitrogen isotopes in soils exhibit variations according to soil type (Cheng *et al.*, 1964). Naturally occurring soil nitrogen can have a wide range of values. Non-hydrolyzable soil nitrogen (-1 to +4 per mil) is similar to atmospheric nitrogen, while hydrolyzable soil nitrogen that is exported from soil systems is usually isotopically more positive. Soil  $\text{NO}_3$  nitrogen isotopic values are typically in the range of +4 to +8 per mil (Heaton, 1986). Nitrogen in excreted waste is mainly in the form of urea which is hydrolyzed to ammonia and converted to nitrate with resulting nitrogen isotopic values that are in the +10 to +20 per mil range (Kreitler 1975, 1979). The degree of nitrate  $^{15}\text{N}$  enrichment in sewage is controlled by ammonia volatilization, and this material almost always has nitrate nitrogen isotopic values higher than +10 per mil (Heaton, 1986).

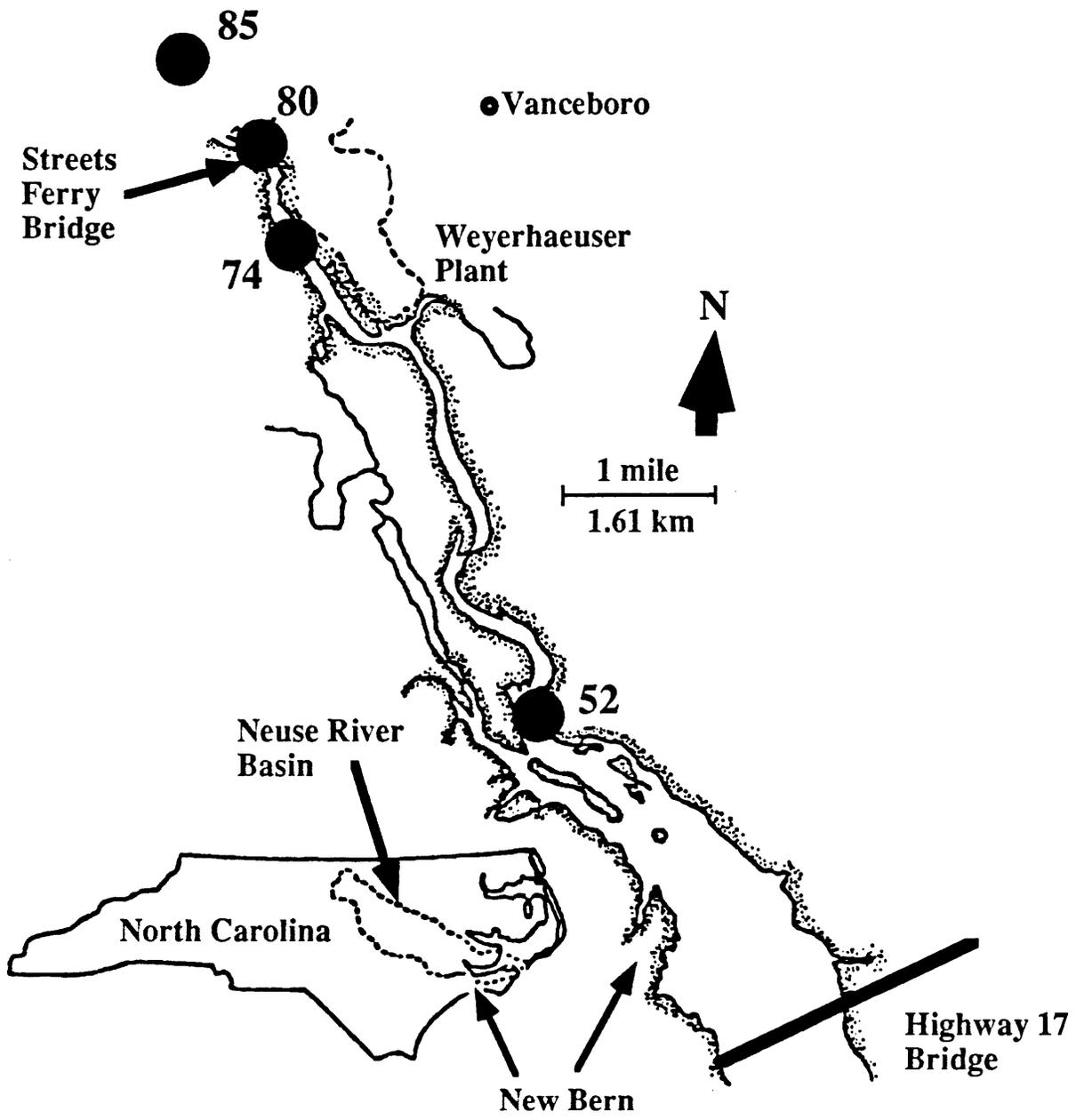
Therefore, anthropogenic and fertilizer nitrate can be distinguished isotopically, even after the fertilizer nitrate has been processed by the soil microbial cycle.

Once exported to aquatic systems, the nitrogen isotopic composition of organic matter can be altered by aquatic biogeochemical processes (Macko and Estep, 1984). California rivers that drain agricultural areas have anomalously enriched particulate nitrogen isotopic values when compared to rivers that drain unaltered forest basins (Peters et al., 1978). This is most likely the result of excess nutrient input coupled with nutrient recycling (Heaton, 1986). Changes in the isotopic composition of particulate nitrogen and ammonia are controlled primarily by detritus input, *in situ* production, nutrient uptake and nutrient regeneration, i.e. by normal biogeochemical cycling. Large changes in the isotopic composition of inorganic nitrate most commonly can occur by nitrification (Mariotti et al., 1981) or denitrification (Cline and Kaplan, 1975).

In this investigation, the isotopic composition of nitrate was monitored over a two year period in the lower Neuse River at Streets Ferry Bridge, a location just upstream from New Bern, N.C (Figure 2). Nitrate is by far the chief form of inorganic nitrogen in the Neuse River system and accounts for over 90% of the inorganic nitrogen pool (Paerl, 1987). The nitrate isotopic composition at this point in the watershed will monitor all the source inputs to the river system over the sampling period. The objective of this study was to characterize the nitrate source inputs and to determine if these inputs changed on a seasonal basis. Nutrient levels, concentrations of chlorophyll *a*, temperature, pH, and water flow rate and direction were also monitored to clarify temporal changes in physio-chemical and biological conditions of the Neuse River Basin during the study period. The sampling site was located above the estuarine portion of the Neuse River (Figure 2, Site #80). Dissolved nitrate samples were analyzed from point and non-point sources to the Neuse River Basin, as well as the dominant fertilizer types used in the area (obtained from the State Agricultural Division, Fertilizer Department). Throughout 1986 drought conditions persisted, while 1987 was a year with a more typical annual hydrological cycle. This fortuitous difference in the two sampling years allowed comparisons to be made between drought and non-drought conditions.

## METHODS

Surface water samples were obtained on a monthly basis (winter) and bi-weekly basis (summer) from the Streets Ferry Bridge sampling site (#80, Figure 2) above New Bern, N.C. over a 24 month sampling period from March 1986 to March 1988. The concentrations of soluble inorganic nitrogen ( $\text{NH}_4$  &  $\text{NO}_3$ ) phosphorus, and chlorophyll *a* were measured according to the methods presented in Paerl (1983), as were pH, temperature, and water flow direction for each sampling period. Samples for isotopic analysis were obtained by filtering 3 to 10 liters of river surface



**Figure 2.** Sampling sites in the lower Neuse. Site #80 is the site that is the focus of this study and where the nitrate samples were collected for isotopic analysis.

water through pre-combusted (550°C for 4 hours) GFF glass fiber filters. The filters and the filtrate were immediately frozen and maintained at -20°C until isotopic analysis. The analytical procedure for nitrogen isotopic analysis of dissolved nitrogen species ( $\text{NH}_4$  &  $\text{NO}_3$ ) has traditionally been completed by complex wet chemical digestions and LiOBr liberation of molecular  $\text{N}_2$  (Ross and Martin, 1970). Although this methodology is time consuming and dangerous (use of a volatile Br liquid), the main disadvantage of this technique is that it is very difficult to get a sufficient number of dissolved nitrogen isotopic analyses to do a detailed time series study. We have developed an ion sieve adsorption/combustion technique along the general outline of Cifuentes et al. (1988) for this analytical procedure, which is described below. This ion sieve technique is an important advancement because it is more efficient, allows smaller sized samples to be analyzed, has lower analytical blanks, and avoids the use of toxic Br reagents.

Dissolved nitrogen isotopic analyses were completed on the filtered river water samples after the samples were thawed at room temperature. Approximately 1-3 liters of the river water was acidified to below a pH of 3.00 with 2-5 ml of 12N HCL. The sample was then reduced to 125 ml by roto-evaporation (Heaton and Collett, 1985). The 125 ml sample was then transferred to a distillation system, and basified to a pH of greater than 11.0 with 3-6 ml of 40% NaOH. The sample was then brought up to a 300 ml volume by dilution with nitrogen free de-ionized/redistilled water. Ammonia was then steam distilled out of the sample with approximately 200 ml of water and collected in a trap containing 15 ml of 0.0025 N HCL with W-85 zeolite ion sieve (Union Carbide) which adsorbed the ammonium after HCL protonation. The zeolite was cleaned for 2 hours at 200°C and stored in a vacuum oven at 50°C prior to  $\text{NH}_4^+$  loading. The  $\text{NH}_4^+$  loaded zeolite was then filtered onto pre-combusted GFF (glass fiber) filters and combusted by a double quartz ampoule method to yield  $\text{N}_2$  (Showers, unpublished lab manual). The remaining river water sample was cooled and the nitrate was reduced to ammonia with 1 gram of Devarda's alloy. The Devarda's alloy was ground to a 100 mesh size under an argon atmosphere and cleaned at 400°C for 8 hours before nitrate reduction. The sample was mixed for 30 minutes to reduce all nitrate to ammonia, and then steam distillation, ion sieve adsorption, concentration and combustion was complete as described above.

The purified  $\text{N}_2$  gas was analyzed in a Finnigan MAT 251 Ratio Mass Spectrometer (RMS) in the NCSU Stable Isotope Lab. Purified gas samples, sealed *in vacuo* in the 6 mm borosilicate breakseals were injected into the RMS inlet system via a Cajon "cracker" system (DesMarais and Hayes, 1976). Molecular nitrogen samples were desorbed off the 5A mole sieve at 40°C to avoid any isotopic fractionation (Table 1). Data are reported as per mil deviations from atmospheric nitrogen (Mariotti, 1983). The  $\text{N}_2$  RMS machine gas standard was an argon-free  $\text{N}_2$  tank gas that was calibrated against atmospheric nitrogen prepared by cupric oxide reduction (Mariotti, 1983). The nitrogen Q-factor (mass 40 - argon interference in atmospheric nitrogen gas standards; Mariotti 1984a) was determined to be negligible for the NCSU 251 RMS at atmospheric argon

**TABLE 1**  
MOLE SIEVE DESORPTION TEMPERATURE  $\delta^{15}\text{N-N}_2$

<u>Temperature <math>\text{N}_2</math> Desorbed (<math>^{\circ}\text{C}</math>)</u>	<u>Difference from Tank Gas Std (per mil - <math>\delta^{15}\text{N}</math>)</u>
-7.1	-.596
+0.4	-.443
+12.7	-.285
+30	-.079
+40	-.009
+50	+.054
+60	+.126
+70	+.170
+90	+.250
+120	+.369
+150	+.511
+200	+.698

**TABLE 2**  
Q FACTOR ANALYSIS OF ARGON INTERFERENCE IN ATMOSPHERIC  $\text{N}_2$  STD

$Q = \frac{m/z\ 40}{m/z\ 28}$	$\Delta\ \delta^{15}\text{N}\ (\text{‰})$
-----	-----
1.43	-.012
1.48	+.009
1.52	-.012
1.70	0.000
1.92	-.023
1.96	-.004
1.97	-.022
1.99	-.024
2.223 - Atmospheric Nitrogen Ave.	n=9
2.4	+.022
2.46	+.015
2.46	-.016

concentrations (Table 2). Comparison of the ion sieve  $\text{NH}_4^+$  reduction technique to the traditional LiOBr technique (Ross and Martin, 1970) showed the W-85 ion sieve technique to be comparable. The W-85 ion sieve technique is therefore preferred because it avoids the use of dangerous bromide reagents and difficult wet chemical digestions. Comparison of the W-85 ion sieve technique to standard combustion showed no isotopic difference (Table 3). Blanks were run for each combustion batch. Using the W-85 ion sieve adsorption/combustion technique, the nitrate blank was  $0.6\ \mu\text{M}$  with an isotopic composition of  $-0.6$  per mil. Blank corrections were made after Hayes (1983).

**TABLE 3**  
**ANALYSIS OF NO<sub>3</sub> AND NH<sub>4</sub> STANDARDS BY COMBUSTION AND**  
**ION SIEVE / NH<sub>4</sub> REDUCTION METHODS**

<u>METHOD</u>	$\delta^{15}\text{N-NO}_3$	$\delta^{15}\text{N-NH}_4$
NO <sub>3</sub> -REDUCTION	3.49 ‰ (n=12; ± 0.36 ‰)	
NO <sub>3</sub> COMBUSTION	3.89 ‰ (n=6; ± 0.20 ‰)	
NH <sub>4</sub> -REDUCTION		-1.87 ‰ (n=12; ± 0.97 ‰)
NH <sub>4</sub> -COMBUSTION		-1.68 ‰ (n=14; ± 0.12 ‰)

## RESULTS

All the physical/chemical data collected during this study are summarized in Appendix 1. Discharge and flow rate and direction varied greatly over the two year sampling period. The 1986 period was a drought year with little winter/spring runoff, and discharge never exceeded 4000 cfs at the USGS gauging station at Kinston NC (Figure 3). The 1987 hydrograph was more typical with high winter/spring runoff peaking at 14000 cfs. Discharge then rapidly dropped through the May/June period to low flow summer conditions with discharge under 1000 cfs throughout the rest of the year. The USGS hydrographic data from Contentnea Creek, which drains predominately rural areas, shows a similar biannual pattern with the 1987 discharge peak at about 5000 cfs (Figure 4). Flow direction and rate in the lower Neuse (Figure 5) are similar to the discharge hydrograph with low flow occurring throughout the 1986 period and in late summer 1987. Conductivity data (Figure 6) suggest that river flow may have reversed in the lower Neuse during the late 1986 summer months and in the fall at the end of the 1986 drought period. Conductivity data during the rest of the sampling period suggest that while estuarine influences may be seen at Site 52 during lower flow conditions, Site 80 is dominated by riverine processes during all periods except at the end of the 1986 drought period.

Winter water temperatures at the Streets Ferry Bridge sampling site were below 10°C. Water temperature rose to late July - August maxima of approximately 30°C each year (Figure 7). The pH values remained slightly below 7.0 throughout the sampling period, with 1987 average values slightly lower than those in 1986 (Figure 8). Chlorophyll *a* concentrations varied seasonally, with maximum levels occurring during the spring/summer bloom periods. The 1986 spring "bloom" period showed chlorophyll *a* levels of 40 µg/l during drought conditions. The 1987 spring/summer bloom period showed high chlorophyll *a* levels exceeding 80 µg/l (Figure 9). Nitrate concentrations varied from 40 to 120 µM/l (560 to 1680 NO<sub>3</sub>-N µg/l) without a clearly distinguishable annual pattern. Nitrate concentrations showed increased variability during the 1986

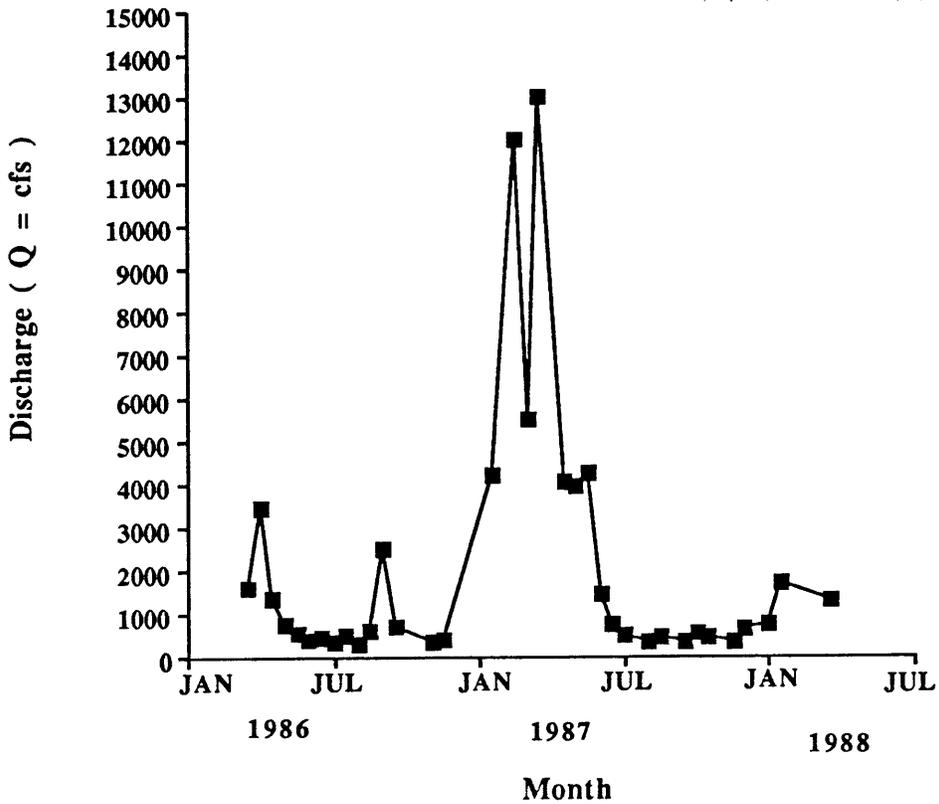


FIGURE 3. Discharge rate at Kinston, N.C. (from the USGS)

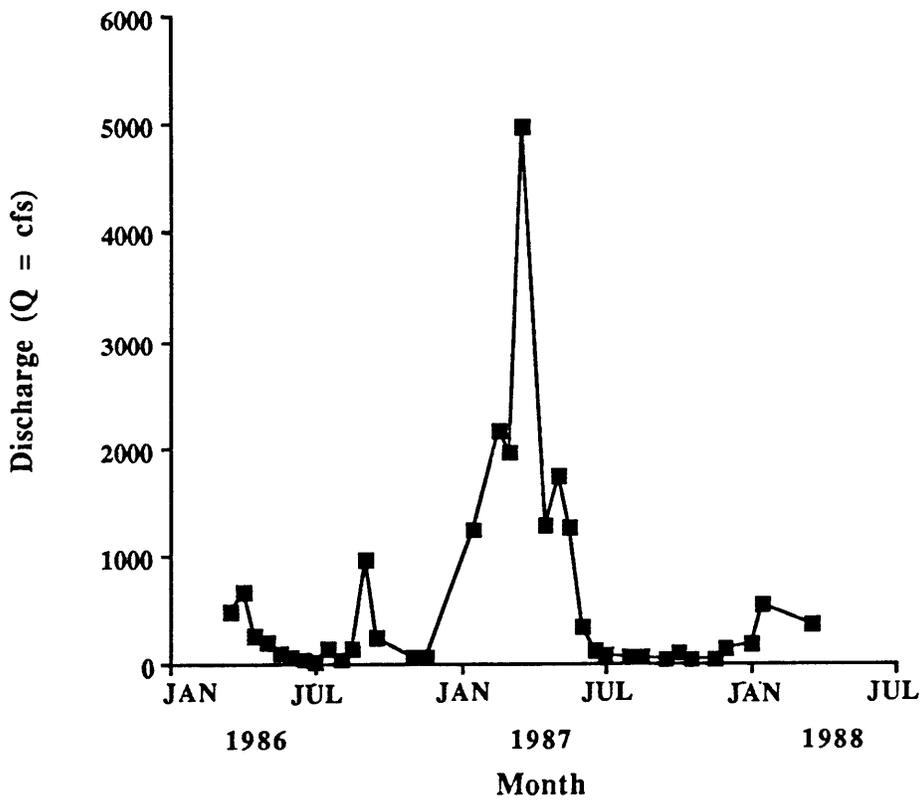


FIGURE 4. Discharge rate in Contentnea Creek, N.C. (from the USGS)

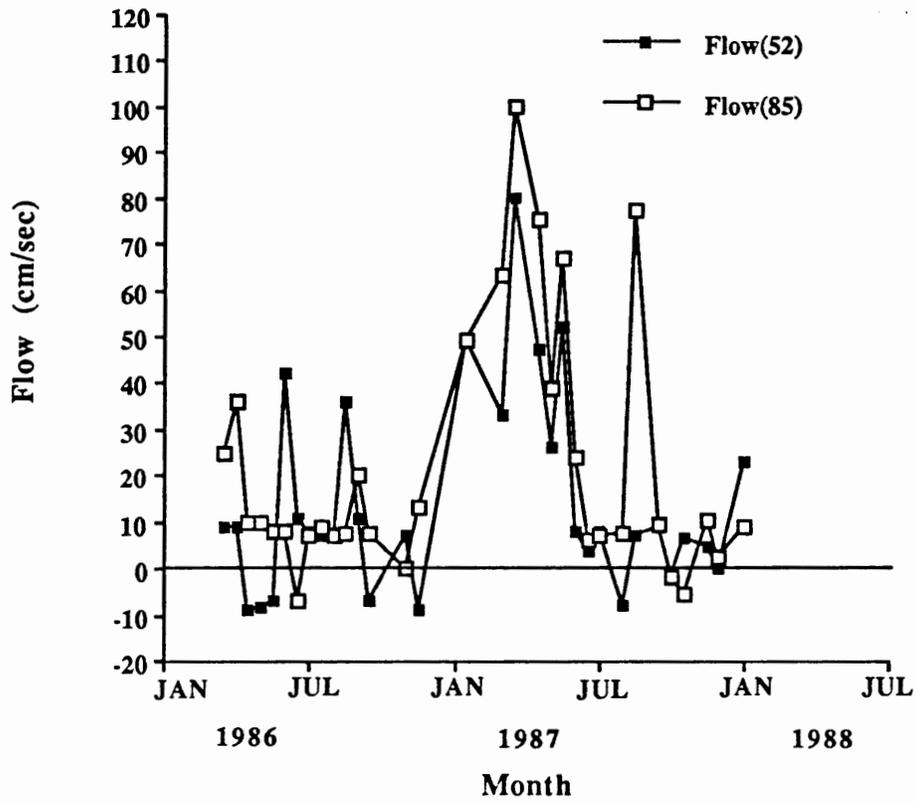


FIGURE 5. Flow direction in the lower Neuse River measured at Site #80

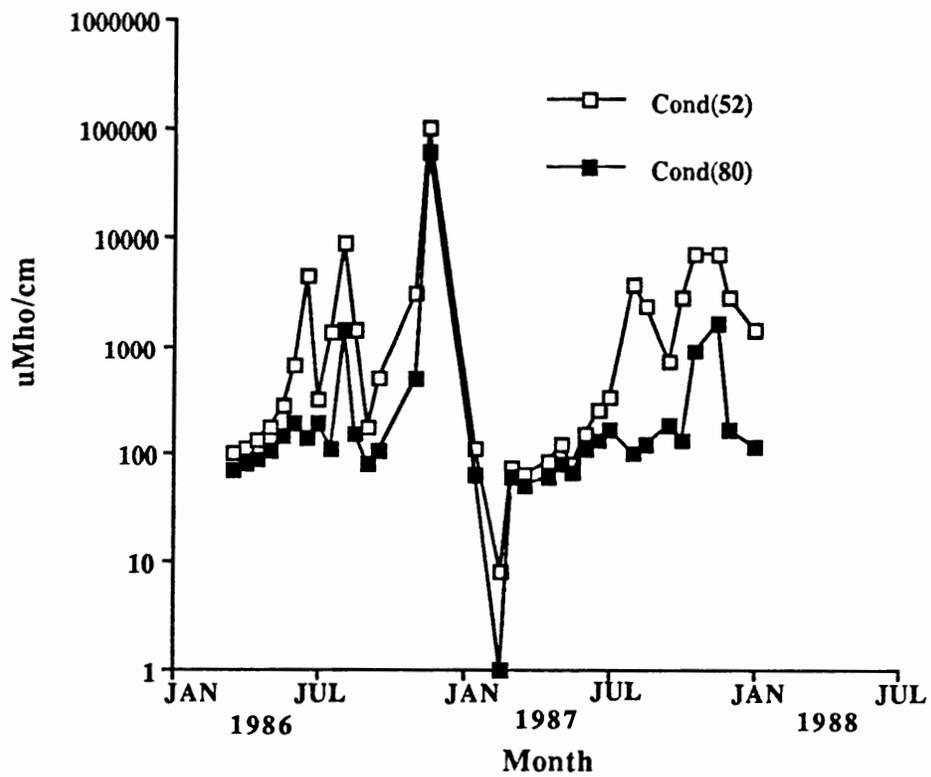


Figure 6. Conductivity in the lower Neuse River measured at Site #80.

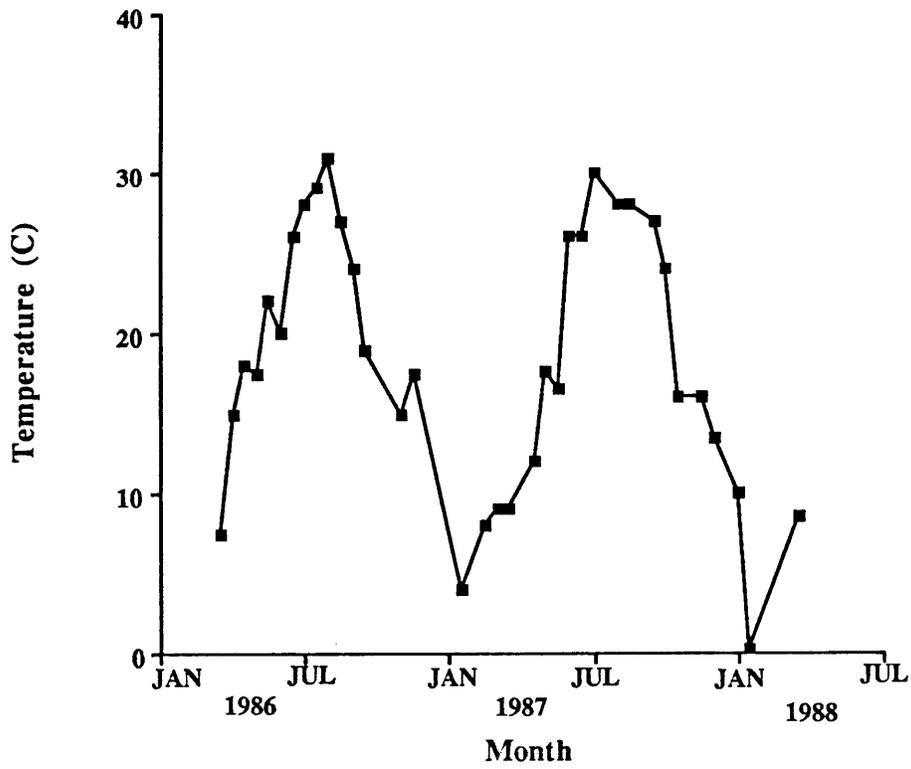


FIGURE 7. Temperature at Site #80 during the sampling period.

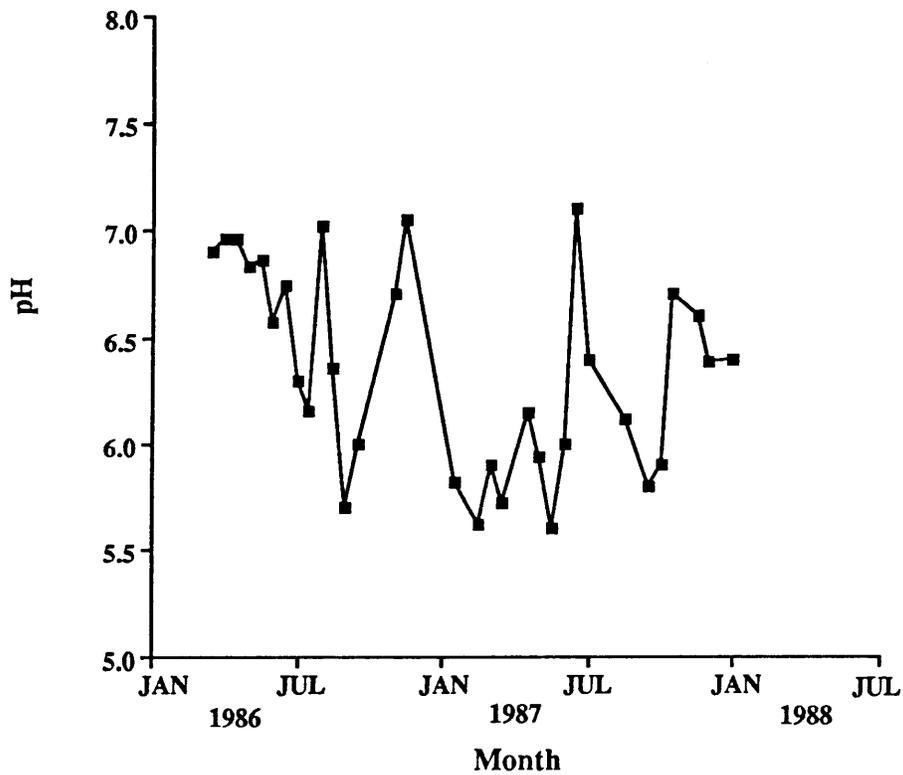


Figure 8. Surface water pH at Site #80 during the sampling period.

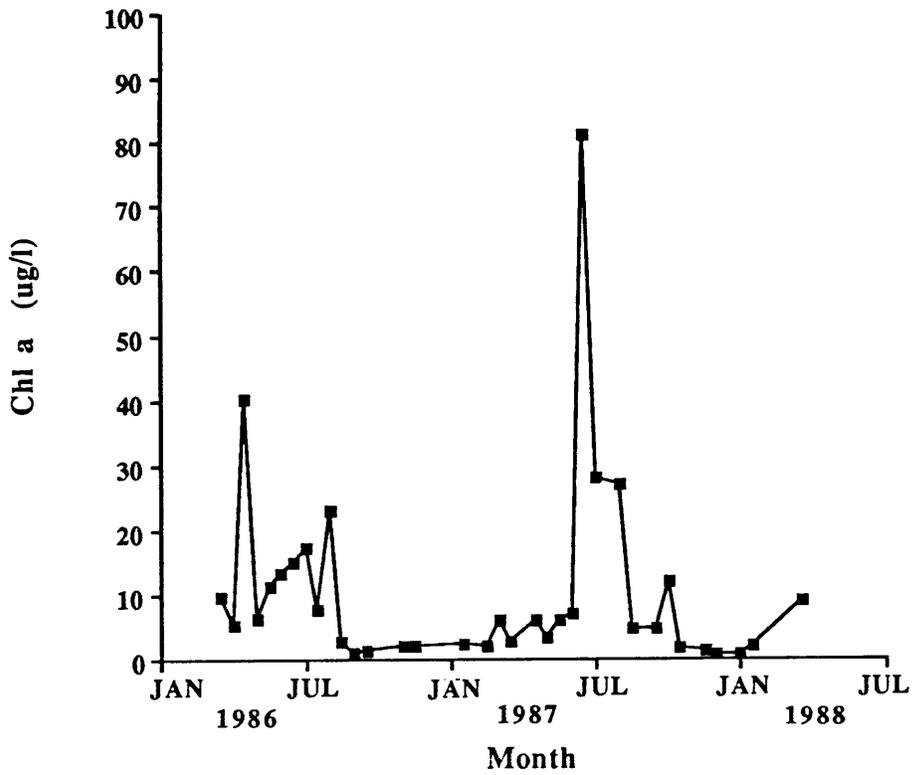


FIGURE 9. Chlorophyll a concentrations at Site 80

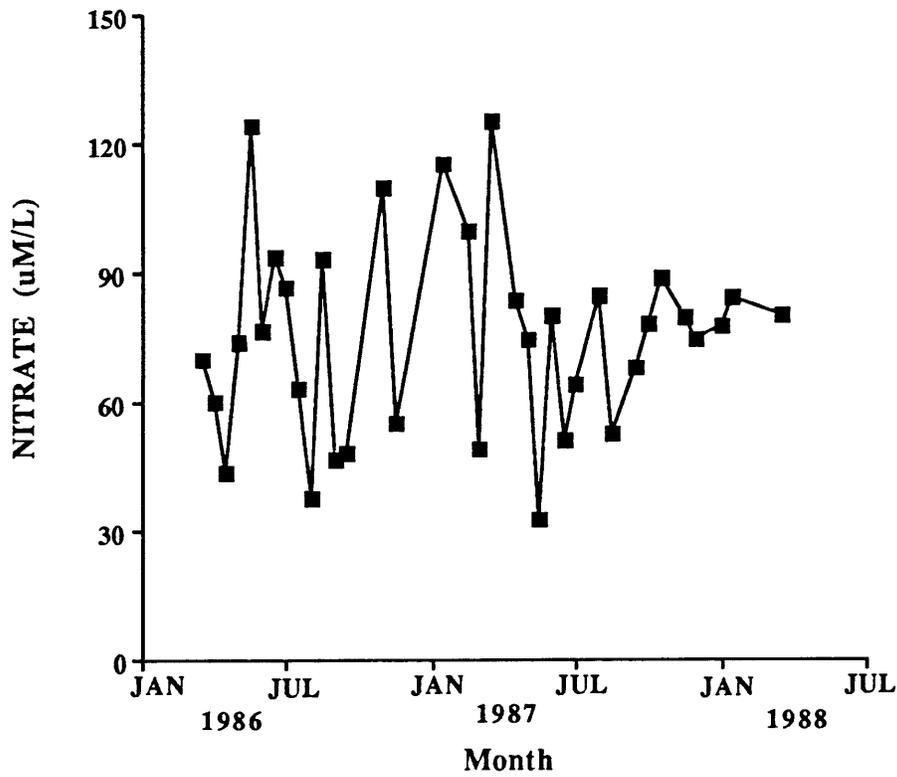


FIGURE 10. Surface water nitrate concentration at Site #80

sampling period (Figure 10). Ammonium concentrations were consistently low (0-16  $\mu\text{M/l}$  or 0-224  $\text{NH}_4\text{-N}$   $\mu\text{g/l}$ ) with winter periods showing a slight increase in concentrations over summer periods (Figure 11). Phosphorus concentrations varied seasonally with higher concentration levels in the summer months (9-15  $\mu\text{M/l}$  or 279-465  $\text{PO}_4\text{-P}$   $\mu\text{g/l}$ ) and lower concentrations occurring in the winter months (3-6  $\mu\text{M/l}$  or 93-186  $\text{PO}_4\text{-P}$   $\mu\text{g/l}$ , Figure 12).

To compare the nitrate isotopic values of the river surface water collected above New Bern to potential point and non-point sources, dissolved nitrate samples were collected in April/May 1987 from Creeping Swamp and Beaver Dam Creek agricultural drainage areas on the Coastal Plain (Jacobs and Gilliam, 1983), and in January and May, 1987 from the Raleigh and Durham MSTPs. In addition, 7 different types of dominant fertilizers used in North Carolina were obtained from the State Agricultural Division, Fertilizer Department and analyzed isotopically for comparison (Figure 13, Table 4). Urea,  $\text{NO}_3^-$ , and  $\text{NH}_4^+$  fertilizers used in North Carolina have particulate nitrogen isotopic values that range from -1 to +2 per mil. The nitrogen isotopic composition of nitrate in surface waters draining from agricultural areas was below +9 per mil, while the  $\delta^{15}\text{N-NO}_3$  from

**TABLE 4**  
 **$\delta^{15}\text{N}$  ISOTOPIC VALUES OF POINT AND NON-POINT SOURCES IN THE NEUSE RIVER WATERSHED**

<u>NITROGEN SOURCE</u>	<u><math>\delta^{15}\text{N-PON}</math></u>	<u><math>\delta^{15}\text{N-NO}_3</math></u>	<u><math>\delta^{15}\text{N-NH}_4</math></u>
<b>FERTILIZER</b>			
( $\text{NH}_4$ ) <sub>2</sub> SO <sub>4</sub> /KNO <sub>3</sub>	+1.27 ‰ (4)		
UREA-DAP#1	+0.79 ‰ (2)		
UREA-DAP #2	-0.33 ‰ (3)		
UREA-DAP#3	+1.33 ‰ (2)		
<b>POINT SOURCE</b>			
RALEIGH MSTP (1-28-87)	+6.21 ‰	+12.24 ‰	+3.45 ‰
RALEIGH MSTP (4-28-87)	+5.77 ‰	+11.73 ‰	+6.75 ‰
DURHAM MSTP (4-28-87)	-0.02 ‰	+11.08 ‰	+6.86 ‰
<b>NON-POINT SOURCE</b>			
BEAVER CREEK (4-21-87)	+9.99 ‰	+6.33 ‰	+13.26 ‰
BEAVER CREEK (5-13-87)	+9.83 ‰	+6.81 ‰	+16.86 ‰
CREEPING SWAMP (5-13-87)	+14.22 ‰	+9.00 ‰	+13.98 ‰

Raleigh and Durham MSTP discharge was consistently at or above +11 per mil. These point and non-point source isotopic results from the Neuse River Basin are similar to the published values of studies that characterize nitrate sources in other areas (Heaton, 1986), and suggest that nitrogen isotopes can be used to differentiate point and non-point inputs to the Neuse River system.

The isotopic composition of nitrate in surface waters collected above New Bern shows a annual pattern (Figure 14). Nitrogen isotopic values in the +4 to +9 per mil range occur during the winter months, while isotopic values above +9 per mil occur during the rest of the year. When these

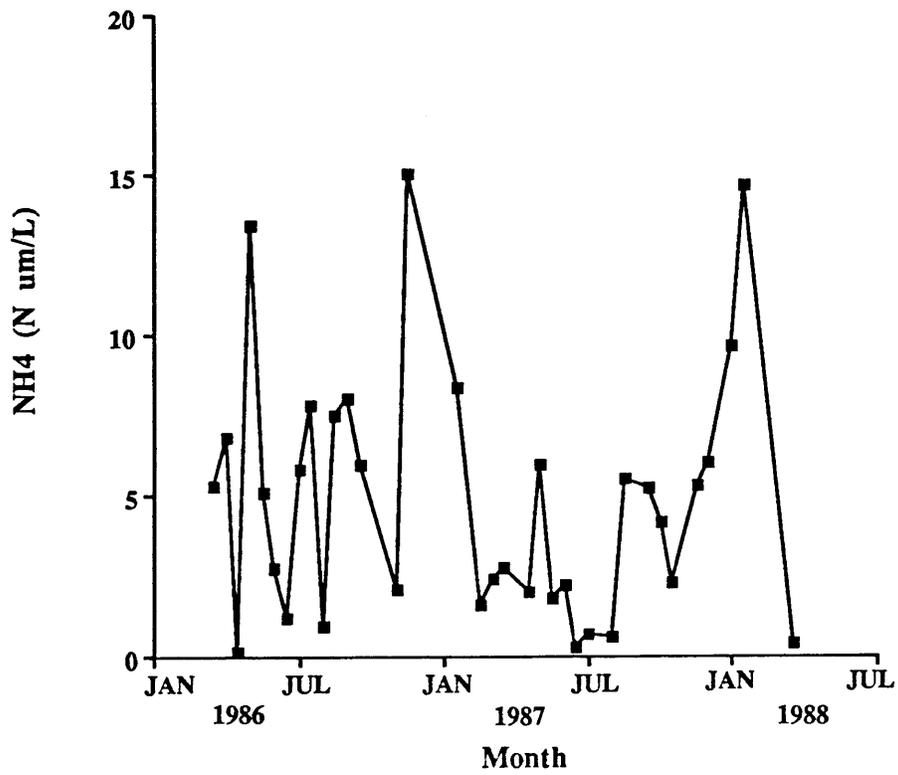


FIGURE 11. Ammonium concentration at Site #80

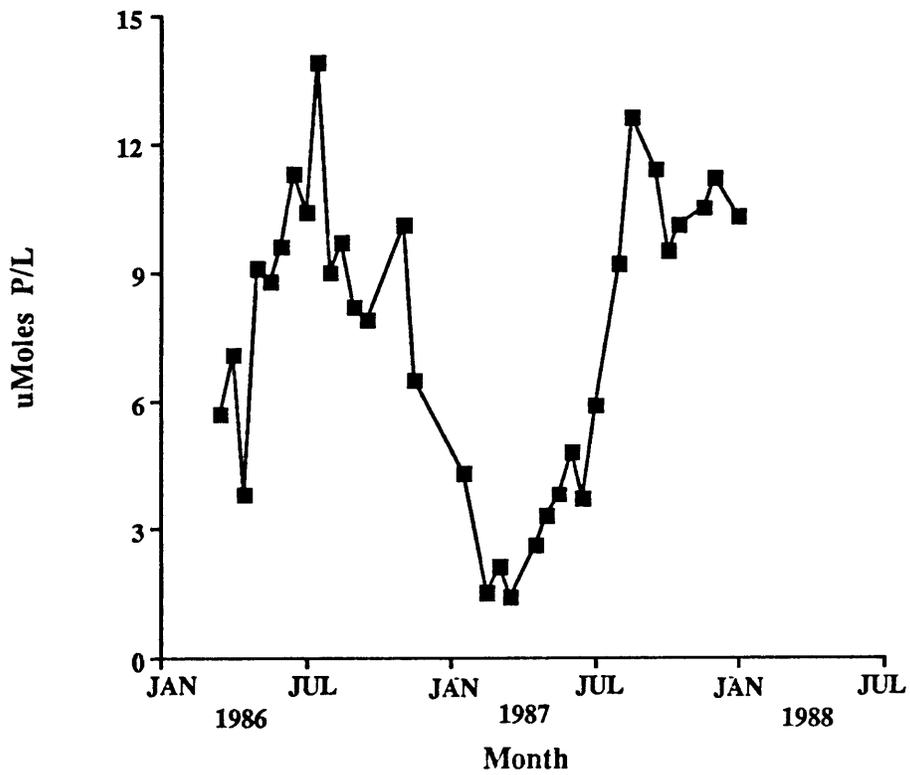


FIGURE 12. Phosphorus concentration at Site #80

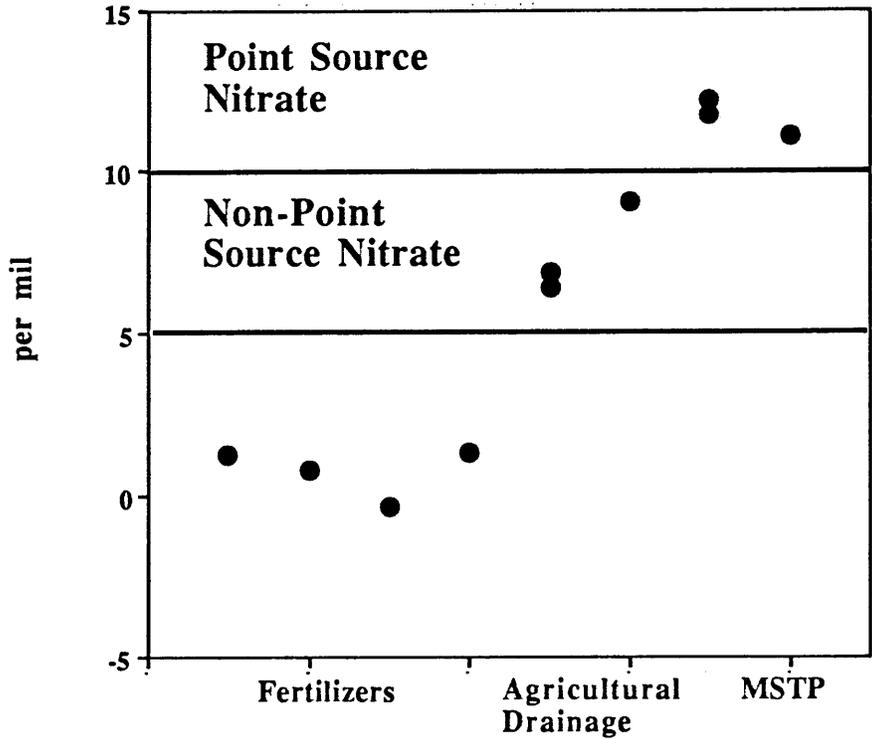


Figure 13. Nitrogen isotopic composition of nitrate sources to the Neuse River Basin

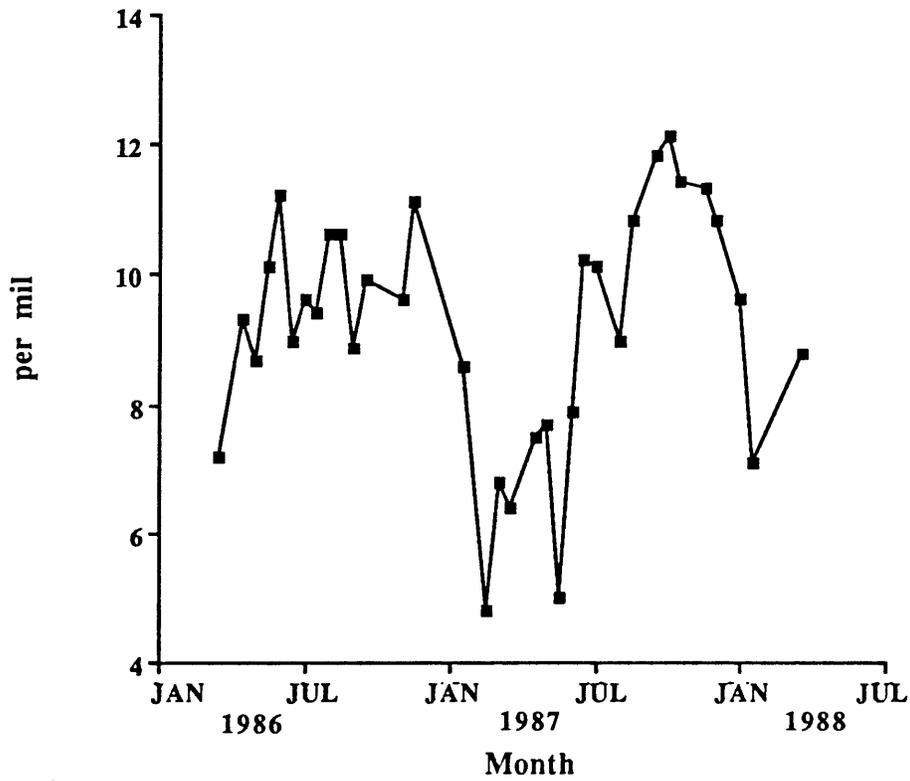


FIGURE 14. Nitrogen isotopic composition of nitrate at Site #80

values are plotted against the discharge rate measured at the USGS gauging station at Kinston (Figure 15), it is apparent that the lower isotopic values of nitrate that fall in the non-point source range occur during periods of high river discharge. During periods of low river discharge the isotopic composition of riverine nitrate falls into the point source isotopic range.

## DISCUSSION

### Temporal Separation of Nitrate Loading

The fact that the nitrogen isotopic composition of riverine nitrate is temporally correlated to discharge rate in the Neuse River system can be explained by the flushing of non-point agricultural nitrogen into the river system during periods of rainfall and subsequent runoff (Jacobs and Gilliam, 1985). During the wet winter months when rainfall is high, the non-point source nitrogen largely from agricultural areas dominates the riverine nitrate reservoir. Point source nitrate dominates during late summer and drought low flow conditions when no runoff is present. The relationship of the annual nitrate nitrogen isotopic signal to discharge suggests that the nitrogen loading in the Neuse River basin exhibits a distinct temporal separation of sources throughout the year. To understand the relationship of the isotopic character of fertilizer nitrogen to high discharge riverine nitrate, an understanding of pathways of non-point source nitrate to surface and ground water systems is required.

In the Neuse River watershed, fertilizer application is concentrated during the late winter and early spring (Humenik et al., 1983). After application, fertilizer nitrogen is taken up by plants which isotopically resemble the available source nitrogen or the isotopic composition of the fertilizer. The plant nitrogen then becomes involved in the soil microbial cycle, which mineralizes soil organics through several intermediate steps into hydrolyzable and non-hydrolyzable soil nitrate (Delwiche and Steyn; 1970, Miyake and Wada; 1971, Freyer and Aly, 1974; Mariotti et al., 1980). Hydrolyzable soil nitrate isotopic values generally range from +4 to +9 (Heaton 1986). Forest soils may have values in the lower portion of the range, while cultivation tends to increase the concentration and isotopic composition of hydrolyzable soil nitrate (Mariotti, 1984b; Heaton, 1986). Nitrate exported from agricultural areas will therefore isotopically resemble the soil nitrate reservoir, not the isotopic composition of the fertilizer. Over-fertilization can account for excessive release of nitrate from soils (Heaton, 1986). During wet rainy periods, soil nitrate is readily transported into middle and lower coastal plain streams, and instream losses of nitrate are not sufficient to counter heavy loading from non-point sources (Jacobs and Gilliam, 1985).

The nitrogen isotopic composition of surface water nitrate documents that non-point source soil nitrate dominates the riverine nitrate pool after wet periods flush the soil nitrates into the river. It is well documented that atmospheric precipitation collects a variety of gaseous, organic and inorganic

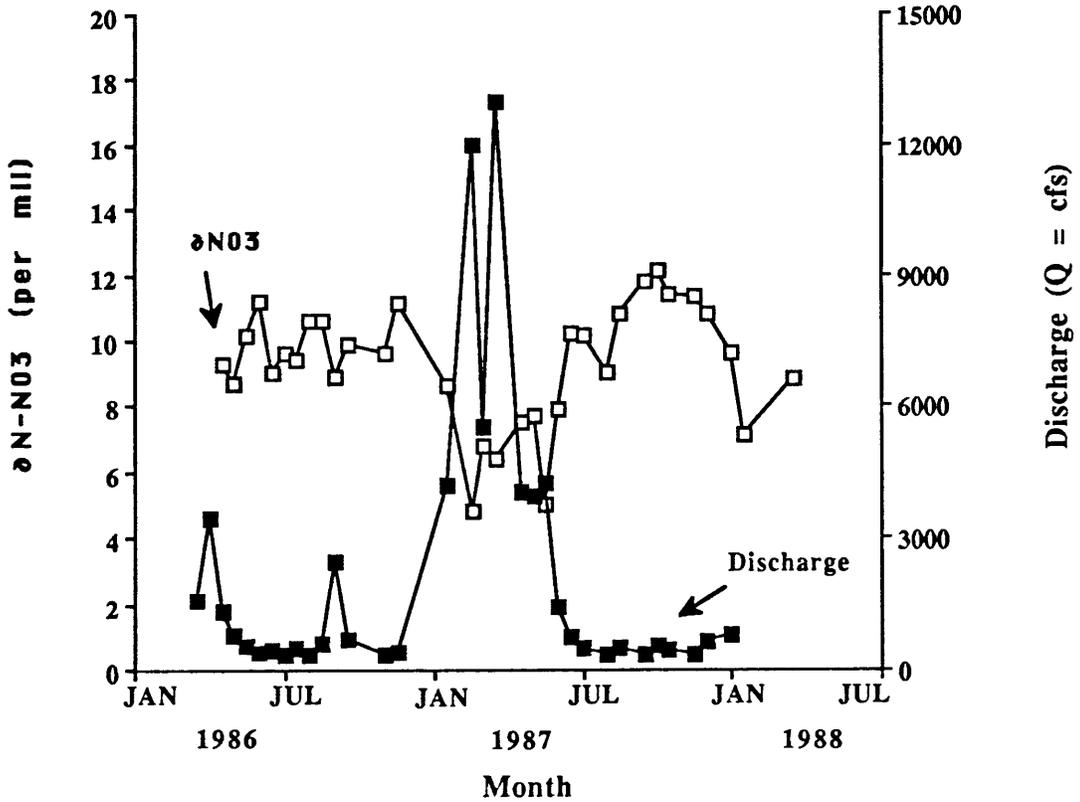


Figure 15. The isotopic composition of nitrate plotted versus discharge rate at Site #80.

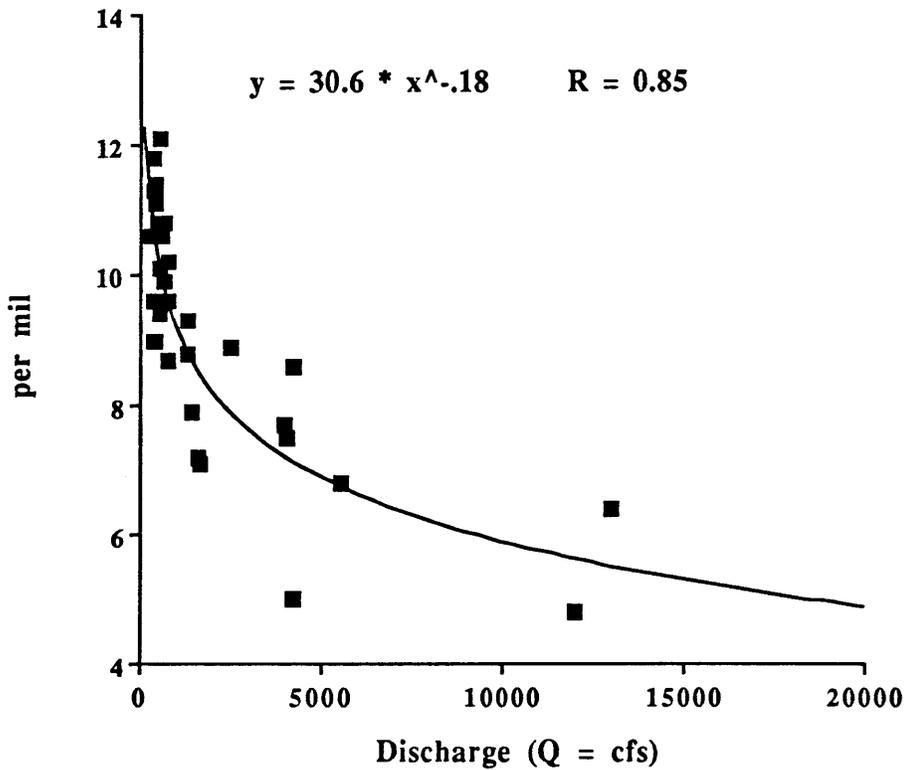


FIGURE 16. Nitrogen isotopic composition of nitrate vs discharge rate in the lower Neuse has a significant exponential relationship.

species of nitrogen during runoff and percolation, and that downward drainage tends to contain nitrate as the only chemically important species (Heaton, 1986). During the drought year of 1986, the isotopic composition of riverine nitrate did not decrease, presumably because low rainfall during this period did not flush non-point source soil nitrate into the river system. Nitrate is by far the dominant dissolved nitrogen species in the riverine nitrogen pool, which can be readily assimilated by a wide range of phytoplankton species (Paerl, 1987). During low flow summer conditions, point source nitrate dominates the river nitrogen pool. It is therefore very likely that point source nitrogen is utilized by nuisance algae during the summer blooms when low flow stagnant summer conditions combined with the physical conditions (light and temperature) permit such blooms to proliferate and persist. Why then do blooms occur in some years and not in other years? The answer to this important question may be found in the unusual mixing relationship between point and non-point nitrate sources as the river transits to low flow conditions.

### **Mixing Relationships of Nitrogen from Different Sources**

Nitrogen isotopes have been used to trace the discharge of sewage into the sea and identify the source of suspended detritus in estuaries and nearshore waters (Mariotti et al., 1984; Peters et al., 1978; and Sweeney et al., 1978). Suspended organic matter in unpolluted rivers have a nitrogen isotopic composition that ranges from 0 to +3 per mil, while oceanic biota commonly synthesize nitrogen from oceanic nitrate which has elevated isotopic compositions of +5 to +10 because of the fractionation associated with oceanic denitrification. These studies describe the mixing of terrigenous detritus with marine phytoplankton as a simple linear mixing of two fixed endpoints. In groundwaters studies, animal or sewage waste nitrate is differentiated from fertilizer nitrate on the basis of nitrogen isotopic signatures (Kreitler 1975, 1979). Because of the localized and highly concentrated nature of most point sources, the mixing relationship is also usually linear (Heaton, 1986). A plot of the Neuse River surface nitrate isotopic composition versus Q (discharge rate) shows an exponential relationship with a significant correlation factor of 85% (Figure 16). If a simple two end member mixing of non-point and point nitrate were controlling the nitrate nitrogen isotopic composition in the surface waters of the Neuse, as has been described for groundwater and nearshore marine systems, then a linear relationship between Q and  $\delta^{15}\text{N-NO}_3$  would be expected. The fact that there is an exponential relationship between the isotopic composition of nitrate and discharge suggests that there is an additional reservoir through which the nitrate must pass which causes a residence time delay between the mixing of the point and non-point reservoirs (Zuber, 1986). The surface water nitrate isotopic trends measured at the Streets Ferry Bridge sampling site integrates the inputs and mixing processes that occur over the entire watershed.

## Identification of the Additional Mixing Reservoir and Impact on Bloom Potential

Two potential additional mixing reservoirs can be mentioned here that are known to play a significant role in the Neuse River watershed discharge dynamics - groundwater and wetlands. Determination of the respective contributions of rainwater, groundwater and soil water to flood discharge in surface water systems has been estimated by physio-chemical means (conductivity, temperature), radioisotopes (tritium), and stable oxygen isotopes. Stable oxygen isotope tracers and isotope mass balance equations have shown in several watersheds that the initial discharge is predominately surface runoff, but that the dominant source of discharge is pre-storm groundwater that can make up to 90% of the total flood (Fontes, 1980). The concentration of pre-storm groundwater in the total flood and the isotopic composition of groundwater need to be evaluated and compared to surface waters in the Neuse River basin. Groundwaters in North Carolina have not accumulated high levels of  $\text{NO}_3$ , because of high rainfall levels that result in rapid flushing of nitrate in the saturated surface aquifer to surface water systems (USGS 1987). Large amounts of non-point source nitrate could be transferred to the Neuse River after fertilizer application without significantly increasing the nitrate concentration in river waters due to the dilution effect of this rapid flushing.

The flooding and draining of extensive wetlands could also produce the observed exponential relationship. Agricultural development in the lowland areas of eastern North Carolina required improved drainage systems. Drainage waters collected from a network of canals are discharged into natural streams or sounds, which increases peak runoff rates and N & P nutrient loading (Gilliam & Skaggs, 1986). The forest species which inhabit these wetlands are adapted to prolonged hydroperiods (Hook, 1984) and are highly efficient at assimilating inorganic nutrients. These wetlands are used as pollution buffer areas for lowland agricultural drainage water (Chescheir *et al.*, 1987). There are also more than 250 sites in North Carolina that discharge municipal effluent to wetlands (Kuenzler *et al.*, 1982). Kuenzler *et al.* (1982) suggest that unchannelized wetlands are a sink for nitrate as a result of extensive denitrification, while channelized wetlands lack extensive denitrification, enhance nitrate loading and do not act as a nitrate sink. Denitrification has a large isotopic fractionation (Delwiche and Steyn, 1970) which should be readily identified by isotopic characterization of different wetlands nitrate isotopic composition.

The isotopic composition of nitrate suggests that the influence of non-point source nitrate can extend into the critical spring period when the character of the biological species succession is determined during the initial spring bloom (Paerl, 1988). Non-point source nitrate continues to "leak" into the Neuse River system as the river transits to low flow conditions, possibly as wetlands drain or as the groundwater tables fall to the late summer levels. The extent and duration of this non-point source influence during the initial spring bloom period would therefore be

determined by the shape of the annual hydrograph. This exponentially decreasing non-point influence would be at a maximum during years with wet winters and dry summers. This is the type of annual hydrographic pattern which seems to support the development of intense nuisance blooms during the late summer low discharge periods (Paerl, 1987, 1988). An understanding of this exponential mixing process is crucial to the development of regulation strategies for nitrate inputs to the Neuse River watershed. Future work should address the mechanism of the exponential mixing process and the relationship of river discharge, the timing of the initial spring bloom period and nitrate non-point source loading modulation by this exponential mixing process.

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APPENDIX 1 DATA

DATE	PO <sub>4</sub> μM/L	NH <sub>4</sub> μM/L	NO <sub>3</sub> μM/L	δ <sup>15</sup> N per mil	Chl a μM/L	Temp °C	Q @ Kinston ft <sup>3</sup> /sec	Q @ Hookerton ft <sup>3</sup> /sec	pH	Flow cm/sec	Flow cm/sec	Cond μMho/cm	Cond μMho/cm
YEAR 1986													
March 5	5.7	5.29	69.9	7.2	9.6	7.5	1600	483	6.9	9	25	100	68
March 19	7.1	6.8	60.2		5.2	15	3460	658	6.96	9	36	110	80
April 3	3.8	0.14	43.6	9.3	40.1	18	1330	263	6.96	-9	10	130	85
April 15	9.1	13.39	73.9	8.7	6.2	17.5	773	195	6.83	-8.5	10	170	105
May 1	8.8	5.07	124.1	10.1	11.2	22	539	103	6.86	-7	8	270	140
May 15	9.6	2.71	76.5	11.2	13.3	20	402	65	6.57	42	8	650	190
June 10	11.3	1.21	93.7	9	15.1	26	439	42	6.74	11	-7	4500	135
June 24	10.4	5.78	86.4	9.6	17.2	28	364	24	6.3	8	7	310	190
July 8	13.9	7.78	62.9	9.4	7.8	29	515	139	6.16	7	9	1350	110
July 21	9	0.92	37.57	10.6	23	31	321	42	7.02	7.5	7	9000	1400
August 7	9.7	7.5	93.1	10.6	2.6	27	581	142	6.36	36	7.5	1400	148
August 21	8.2	8	46.5	8.9	1.1	24	2480	962	5.7	11	20	170	80
September 11	7.9	5.9	48.1	9.9	1.3	19	680	241	6	-7	7.5	500	101
October 23	10.1	2.06	109.6	9.6	2	15	343	50	6.7	7	0	3100	500
November 10	6.5	15	54.8	11.1	2	17.5	391	54	7.05	-9	13	100000	60000
YEAR 1987													
January 6	4.3	8.3	115	8.6	2.3	4	4210	1320	5.82	49	49	110	61
February 3	1.5	1.58	99.5	4.8	2.1	8	12000	2150	5.62			8	0
February 26	2.1	2.38	48.8	6.8	6	9	5510	1960	5.9	33	63	70	60
March 5	1.4	2.72	124.9	6.4	2.8	9	13000	7020	5.72	80	100	62	50
April 6	2.6	2.01	83.7	7.5	6	12	4060	1280	6.15	47	75	82	60
April 17	3.3	5.9	74.4	7.7	3.3	17.6	3950	1740	5.94	26	39	120	80
May 6	3.8	1.81	32.7	5	6.1	16.5	4230	1250	5.6	52	67	80	65
May 28	4.8	2.18	80.1	7.9	7	26	1430	477	6	8	24	150	110

DATE	PO <sub>4</sub> μM/L	NH <sub>4</sub> μM/L	NO <sub>3</sub> μM/L	δ <sup>15</sup> N per mil	Chl a μM/L	Temp °C	Q @ Kinston ft <sup>3</sup> /sec	Q @ Hookerton ft <sup>3</sup> /sec	pH	Flow cm/sec	Flow cm/sec	Cond μMho/cm	Cond μMho/cm
June 9	3.7	0.24	50.8	10.2	81	26	744	124	7.1	4	6	250	130
June 30	5.9	0.69	64.1	10.1	28	30	506	87	6.4	7.8	7.2	330	160
July 23	9.2	0.63	84.5	9	27	28	363	51		-7.8	7.5	3700	100
August 6	12.6	5.45	52.4	10.8	4.8	28	472	56	6.12	7.2	76.9	2320	120
September 1	11.4	5.23	68	11.8	4.5	27	359	37	5.8	8.8	9.5	700	175
September 28	9.5	4.14	78.2	12.1	12.1	24	555	95	5.9	-1.2	-1.9	2800	132
October 14	10.1	2.28	88.5	11.4	1.66	16	433	48	6.7	6.6	-5.3	7000	880
November 6	10.5	5.28	79.5	11.3	1.27	16	338	48	6.6	4.6	10.5	6900	1600
November 30	11.2	6.03	74.4	10.8	0.68	13.5	626	138	6.39	0	2.2	2850	165
December 17	10.3	9.63	77.5	9.6	0.78	10	770	179	6.4	22.7	9	1400	115
YEAR 1988													
January 12		14.59	84.2	7.1	1.95	0.2	1690	531					
March 2		0.39	80	8.8	9.02	8.5	1290	355					