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Dynamics of NH_4^+ and NO_3^- Uptake in the Water Column of the Neuse River Estuary, North Carolina

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ABSTRACT: In an attempt to more fully understand the dissolved inorganic nitrogen dynamics of the Neuse River estuary, $^{15}NH_4^+$ and $^{15}NO_3^-$ uptake rates were measured and daily depth-integrated rates calculated for seven stations distributed along the salinity gradient. Measurements were made at 2–3-wk intervals from March 1985 to February 1989. Significant dark NH_4^+ uptake occurred and varied both spatially and seasonally, accounting for as much as 95% of light uptake with the median being 33%. Apparent NH_4^+ uptake ranged from 0.001 μmol N l^{-1} h^{-1} to 4.2 μmol N l^{-1} h^{-1}, with highest rates occurring during late summer–fall in the oligohaline estuary. Apparent NH_4^+ uptake was significantly related to NH_4^+ concentration ($p < 0.01$); however, the regression explained <3% of the variation. Daily-integrated NH_4^+ uptake ranged from 0.1 mmol N m^{-2} d^{-1} to 133 mmol N m^{-2} d^{-1} and followed the trend of apparent uptake. Annual NH_4^+ uptake of the estuary was significantly lower in 1988 than for any other year. Dark uptake of NO_3^- was only 14% of maximum light uptake. Apparent NO_3^- uptake rates ranged from 0.001 μmol N l^{-1} h^{-1} to 1.84 μmol N l^{-1} h^{-1} with highest rates occurring in the oligohaline estuary. Apparent NO_3^- uptake was significantly related to NO_3^- concentration ($p < 0.01$); however, the regression explained <5% of the variation. In general, NO_3^- uptake was only 20% of total dissolved inorganic nitrogen (DIN) uptake. Daily-integrated NO_3^- uptake ranged from 0.1 mmol N m^{-2} d^{-1} to 53 mmol N m^{-2} d^{-1} and followed similar patterns of apparent uptake. Annual NH_4^+ uptake was 11.39 mol N m^{-2} yr^{-1}, 10.28 mol N m^{-2} yr^{-1}, 10.93 mol N m^{-2} yr^{-1}, and 7.38 mol N m^{-2} yr^{-1}, with the 4-yr mean being 10.0. Annual NO_3^- uptake was 3.12 mol N m^{-2} yr^{-1}, 3.40 mol N m^{-2} yr^{-1}, 1.96 mol N m^{-2} yr^{-1}, and 1.84 mol N m^{-2} yr^{-1}, with the 4-yr mean being 2.6. The total annual DIN uptake was more than twice published estimates of phytoplankton DIN demand, indicating that there is an important heterotrophic component of DIN uptake occurring in the water column. The extrapolation of nitrogen demand from primary productivity results in serious underestimates of estuarine nitrogen demand for the Neuse River estuary and may be true for other estuaries as well.

Introduction

Estuaries are continuously enriched with a wide array of chemicals from the watershed, including the plant growth-stimulating nutrients nitrogen (N) and phosphorus (P). Many estuaries successfully incorporate and attenuate large and/or highly variable pulses of nutrient inputs by a combination of processes, including assimilation into standing stock of primary producers, recycling via grazing, and bacterial decomposition, sedimentation, and export. Elevated concentrations of dissolved inorganic nitrogen (DIN) can result in increased phytoplankton production and biomass, which may in turn be followed by increased populations of consumer animals. This trophic transfer contributes to the value of estuaries for human purposes (Neilson and Cronin 1981).

Excessive nutrient enrichment can be detrimental to estuaries as has been demonstrated in a number of instances around the nation. High nutrient levels may lead to blue-green algal blooms in the fresher portions of estuaries and to dinoflagellate blooms in the more saline stretches. We chose to concentrate our research emphasis on N rather than P because many studies (e.g., Goldman et al. 1973; Nixon 1981; Boynton et al. 1982; Nixon and

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Pilson 1983) have confirmed the conclusion of Ryther and Dunstan (1971) that N, not P, is "the critical limiting factor to algal growth and eutrophication in coastal marine waters." For North Carolina estuaries, N limitation has been demonstrated in several past studies; the early evidence was reviewed by Williams (1973) and additional support was provided by Thayer (1974) for Cape Lookout, North Carolina. Both the Pamlico and Chowan estuaries appear to be N-limited during the summer (Kuenzler et al. 1979; Stanley and Hobbie 1981). Finally, several studies in the Neuse River estuary also support the contention of N limitation (Fisher et al. 1982; Matson et al. 1983; Paerl 1983; Mallin et al. 1991).

This study of the Neuse River estuary was designed to elucidate the effects of DIN loading from the watershed and river on nuisance blooms of the cyanobacterium *Microcystis aeruginosa* occurring in the lower third of the river above the freshwater–seawater interface (FSI). We were also interested in addressing the effects of variable DIN loading on primary productivity and nitrogen uptake by phytoplankton throughout the estuary. Our data on primary productivity have been published elsewhere (Boyer et al. 1993) and a synthesis paper concerning N loading and N recycling is in preparation. This paper is concerned with describing spatial, seasonal, and interannual patterns of DIN uptake in the Neuse River estuary.

Unlike the many estimates of daily-integrated primary productivity (P_d in g C m^{-2} d^{-1}), there is only one study to date that measured and integrated DIN uptake along the salinity gradient of an estuary over annual cycles (Pennock 1987). The method of measuring N uptake is different than that of P_d but the depth integration process is identical. Uptake of DIN (typically NH_4^+ and NO_3^-) is measured by the incorporation of ^{15}N-labeled salts into the filterable particulate fraction (Neess et al. 1962; Dugdale and Goering 1967). The specific uptake rate (ν in h^{-1}) adjusted for the concentration of particulate nitrogen (PN) in the sample is given as apparent uptake or transport (ρ in μmol N l^{-1} h^{-1}). As with photosynthesis, the conversion of ρ to the more usable value, daily-integrated DIN uptake (V_d in mmol N m^{-2} d^{-1}), requires knowledge of the specific relationship between ρ and irradiance (I), the water column light extinction coefficient (k), and the daily light regime. Estimates of annual DIN uptake (V_y, in mol N m^{-2} yr^{-1}) require that V_d be extrapolated daily and summed over the year. An estimate of V_y for an entire estuary also requires extrapolation among sampling stations along the salinity gradient. Therefore, it is important that the sampling resolution for elucidating both spatial and temporal variation of an ecological process be relevant to the scale of final analysis.

An alternative to direct measurement of DIN uptake is the concept nitrogen demand (Harrison and Hobbie 1974; Nixon and Pilson 1983; Degobbis et al. 1986). Nitrogen demand is defined as the phytoplankton N requirement needed to balance primary productivity. It is extrapolated from CO_2 uptake experiments by using the Redfield ratio, C:N = 6.6 (mol : mol). One problem with using this approach concerns the variable diel nature of C vs N uptake rates. In the Delaware Bay, Pennock (1987) found that the average short-term C:NH_4^+ uptake ratio was 34. When integrated over depth and with the inclusion of dark uptake, the C:NH_4^+ uptake ratio decreased to 8.5. Analysis of nitrogen dynamics in the Chowan River (Stanley and Hobbie 1977) showed that short-term C:NH_4^+ uptake ratio varied from 60 early in the morning to <1 at night. However, when integrated over depth for the course of the day, the C:NH_4^+ uptake ratio ranged from 0.7 to 6.9. Miyazaki et al. (1987) described the delay that occurs between C and N uptake during daylight hours as being related to the physiological necessity of the phytoplankton to build up a carbon reserve before actively assimilating NH_4^+ and NO_3^-. It is questionable whether phytoplankton balance their C:N ratio over the course of a day. Miyazaki and Ichimura (1986) showed that while the C:N of PN ranged from 5.9 to 7.8, the C:N uptake ratio of NH_4^+ ranged from 4.8 to 12.9.

It has also become clear that phytoplankton are not the only organisms that assimilate DIN in aquatic systems. In parallel CO_2 and NH_4^+ uptake experiments, Laws et al. (1985) found that NH_4^+ uptake rates could not be explained by phytoplankton C fixation alone and concluded that heterotrophic bacteria must have been responsible for the difference. Probyn and Painting (1985) found that 50% of the NH_4^+ uptake at two of their five stations in the Antarctic was accounted for by the <1 μm fraction. Wheeler and Kirchman (1986) showed that 78% of the NH_4^+ uptake in coastal waters off Georgia was due to prokaryotes. Using another approach, Findlay et al. (1991) measured bacterial production in the Hudson River and found that it was four times higher than algal primary production. Bacteria meet their N demands by assimilating either organic or inorganic N. Since bacteria have a lower C:N than phytoplankton (2.3–8.3, Linley and Newell 1984) and because their contribution to DIN uptake can be quite large, it is highly likely that previous assessments of total nitrogen uptake for estuaries and coastal waters have been seriously underestimated.

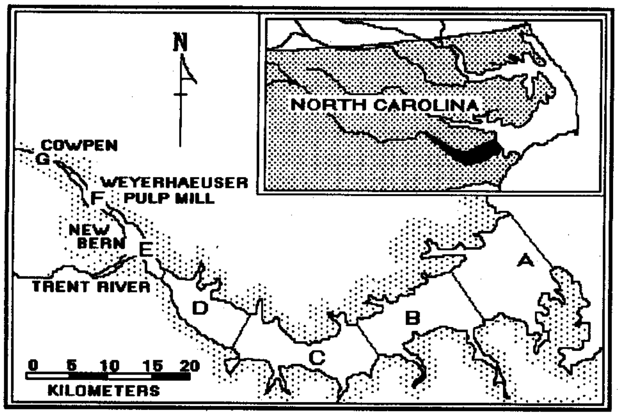

Fig. 1. Location and delimited areas of sampling stations in the Neuse River estuary. Inset shows the Neuse River drainage basin in relation to the Albemarle-Pamlico Sound system.

Materials and Methods

Study Site

The Neuse River estuary (Fig. 1) covers ~400 km^2 and drains ~16,000 km^2 of land, the majority of which is either agricultural or forested (Stanley 1988). Almost 79% of the total nitrogen loading and 55% of the total phosphorus loading results from nonpoint sources (Stanley 1988). The remainder comes from 16 municipal and industrial point sources, most of which are sewage treatment plants. However, the largest single discharge is from a Weyerhauser paper pulp mill at the head of the estuary above New Bern (Fig. 1). Physical factors and nutrient distributions in the Neuse River estuary have been described elsewhere (Christian et al. 1991).

The Neuse River flow regime is generally characterized by highest flows in winter–spring and lowest flows in the summer–fall, with periodic pulses occurring as a result of storms (Christian et al. 1991). During winter–spring 1987, three flooding events occurred in the Neuse River that had long-term effects on physical conditions and biological processes throughout the river-dominated ecosystem. Salinity decreased within the estuary for the duration of the flood, but the flood also had an effect on salinity that lasted through the summer and fall of 1987. The highest estuarine salinities measured for the 4 yr studied occurred in 1988 (Christian et al. 1991).

Mean salinities at stations A, B, and C were not significantly different from each other ($p < 0.05$; Boyer et al. 1993) but were significantly higher than stations D and E, which in turn were significantly higher than stations F and G (Table 1). For purposes of discussion we define these zones as the mesohaline estuary, oligohaline estuary, and river, respectively.

TABLE 1. Physical characteristics of sampling stations along the Neuse River estuary. Light extinction coefficient is represented by k.

Station	Km from Mouth	Mean Depth (m)	Area (km^2)	Median k (m^{-1})	Median Salinity (‰)
A	2.4	4.32	121.7	1.21	13.1
B	9.4	3.57	100.8	1.19	11.8
C	24.5	2.85	88.9	1.58	9.7
D	41.4	2.53	61.4	2.21	6.3
E	52.3	2.26	17.3	2.95	2.8
F	63.0	2.64	3.0	3.50	0.3
G	76.3	2.20	0.6	2.39	0.0

Sample Collection and Analysis

Water column data and water samples were collected at 2–3-wk intervals from seven stations distributed throughout the Neuse River estuary for the 4-yr period beginning March 1985 and ending February 1989 (Fig. 1). At each station, dissolved oxygen (mg l^{-1}), temperature (°C), and salinity (‰) were measured at 0.5-m depth intervals through the water column using a YSI Model 57 DO meter and a YSI Model 33 S-C-T meter, respectively. Measurements of photosynthetically active radiation (PAR in μE m^{-2} s^{-1}) were taken every 0.2 m through the water column using a LI-COR 192SA flat sensor (1985–1987) or a 193SB spherical sensor (from 1987) for later calculation of light extinction coefficient (k). Photic depth was defined as the depth of the water column to 1% of surface irradiance ($Z_p = -\ln(0.01)/k$). Total insolation (I_o in watts m^{-2}) was continuously monitored at East Carolina University using a roof-mounted Eppley pyranometer and then converted to μE m^{-2} s^{-1} before use.

Water samples were collected approximately 0.2 m below the surface in polyethylene bottles and kept at or below ambient temperature in the dark. Samples were transported to the laboratory and processed within 7 h after collection. Filtered water (Whatman 934-AH) was stored frozen or refrigerated for later analysis of ammonium (NH_4^+), nitrate plus nitrite (NO_3^-), filterable reactive phosphorus (FRP), and dissolved Kjeldahl nitrogen (DKN). Analyses followed United States Environmental Protection Agency (1979) and American Public Health Association (1985) procedures, with digestions according to Jones and Bradshaw (1989). Preliminary studies showed there was little nitrate in samples; therefore we assumed the nitrate plus nitrite pool to be primarily NO_3^-. Material on filters was frozen for later analysis of chlorophyll *a* (Chl *a* in μg l^{-1}), particulate nitrogen (PN), and particulate phosphorus (PP) following procedures in the aforementioned references. NO_3^-, FRP, DKN, PN, and PP were analyzed with an Orion Scientific Instruments Corporation segmented flow analyzer; other analyses were done manually.

DIN uptake rates were measured using the stable isotope ^{15}N as a tracer (Neess et al. 1962; Dugdale and Goering 1967; Stanley and Hobbie 1981). Duplicate water samples were spiked with 99% $^{15}NH_4Cl$ or $K^{15}NO_3$ (MSD Isotopes, Cambridge, Massachusetts) at concentrations approximating 10–50% of ambient concentrations and incubated at ambient temperatures under either light (393 $\mu E\ m^{-2}\ s^{-1}$) or dark conditions. Light was supplied by eight cool-white (GE-F40CW) fluorescent lights alternated with eight wide-spectrum (GE-F40PL/AQ) lights. Subsamples were filtered through precombusted Whatman GF/C glass-fiber filters at initial and twice more for periods up to 6 h. Filters were dried at 60°C before being converted to N_2 gas by a micro-Dumas procedure (Fiedler and Proksch 1975). Briefly, PN values were used to determine the portion of the filter needed for analysis. Samples were added to precombusted glass tubes along with precombusted CuO and CaO, evacuated, sealed, and combusted at 450°C overnight. Gas samples were analyzed for $\%^{15}N$ enrichment using a NOI-5 Statron optical emission spectrophotometer (Beckman, DDR). Sample $\%^{15}N$ values were corrected by comparison to a range of $^{15}NH_4Cl$ standards.

At approximately 3-mo intervals, samples from stations A, D, and G were incubated for $^{15}NH_4^+$ and $^{15}NO_3^-$ uptake under a series of neutral density screens representing a range of six light intensities (0 $\mu E\ m^{-2}\ s^{-1}$, 4 $\mu E\ m^{-2}\ s^{-1}$, 16 $\mu E\ m^{-2}\ s^{-1}$, 74 $\mu E\ m^{-2}\ s^{-1}$, 156 $\mu E\ m^{-2}\ s^{-1}$, and 393 $\mu E\ m^{-2}\ s^{-1}$). These "light-series" data were used to generate K_I, the light half-saturation constant ($\mu E\ m^{-2}\ s^{-1}$) and ρ_{max}, the maximum apparent uptake rate under saturating light conditions ($\mu mol\ N\ l^{-1}\ h^{-1}$) using the Michaelis-Menten equation described below.

DATA ANALYSIS

Apparent uptake rate or transport was calculated using a modified Eq. 4 from Collos (1987):

$$\rho = \nu \times PN = \frac{(^{15}N_t - {}^{15}N_o) \times PN}{(^{15}N_p - {}^{15}N_o) \times t}$$

where ρ = apparent uptake rate ($\mu mol\ N\ l^{-1}\ h^{-1}$), ν = specific rate (h^{-1}), $^{15}N_t$ = final percent ^{15}N enrichment (as atom % excess) in the particulate fraction, $^{15}N_o$ = initial percent ^{15}N enrichment in particulate fraction, and $^{15}N_p$ = initial percent ^{15}N enrichment of dissolved pool.

Results are reported as ρ because ν is affected by amount of detrital N in the sample and by the uptake of unlabeled N (Dugdale and Goering 1967; MacIsaac and Dugdale 1972; Kanda et al. 1985; Dugdale and Wilkerson 1986; Collos 1987). Apparent NH_4^+ uptake was not corrected for isotope dilution from remineralization but incubations were kept under 6 h to minimize this effect.

Apparent uptake vs irradiance curves for light-series incubations were examined and both the maximum uptake rate (ρ_{max}) and the light half-saturation constant (K_I) were estimated using least squares regression with modified Haynes-Woolf transformation of the Michaelis-Menten equation (Lehninger 1975). The routine experiments resulted in a separate ρ_{max} for all seven stations. We assigned the K_I values to those other stations in their respective salinity group for that specific date. Additionally, K_I was extrapolated between light series experiments for each routine measure of N uptake based on one light level.

We calculated daily depth-integrated uptake (V_d) by using a discrete depth-interval integration technique similar to that of Fee (1973) but using a modified Michaelis-Menten equation set in spreadsheet form using Lotus 1-2-3:

$$V_i = \frac{(\rho_{max} - \rho_d) \times (I_o e^{-kz}) \times 200}{K_I + (I_o e^{-kz})} + \rho_d$$

where V_i = hourly uptake per depth interval, ρ_d = dark apparent uptake rate, and z = water column depth interval (0.2 m, which accounts for the 200 in the equation). The V_i for each depth interval were summed over depth to get hourly-integrated uptake (V_h, in mmol N $m^{-2}\ h^{-1}$) for the whole water column. The calculation was repeated for each hour of the day, and V_h were summed over time to get V_d (mol N $m^{-2}\ d^{-1}$). This procedure was repeated for each of the seven stations for all of the 82 sampling dates.

Annual integrated uptake (V_y in mol N $m^{-2}\ yr^{-1}$) of the estuary was calculated by first multiplying V_d for each station and date by the area of its corresponding estuary segment (Fig. 1) to arrive at mol N $segment^{-1}\ d^{-1}$. These values were then used to calculate Mmol N $segment^{-1}\ yr^{-1}$ (Mmol = 10^6 mol) by extrapolation among sampling dates. Summing the annual N uptake values for the seven segments gave annual N uptake for the estuary, which was then divided by the total benthic area of the estuary to give V_y. This integration procedure was repeated for both NH_4^+ and NO_3^-. Values of V_y for both NH_4^+ and NO_3^- were summed to arrive at annual DIN uptake for the estuary.

Data were normalized by the natural log transformation. Least squares regression analyses were performed on transformed data and coefficients of determination (r^2) were calculated using SYSTAT (Wilkinson 1988). The median was chosen as the nonparametric statistic of comparison because

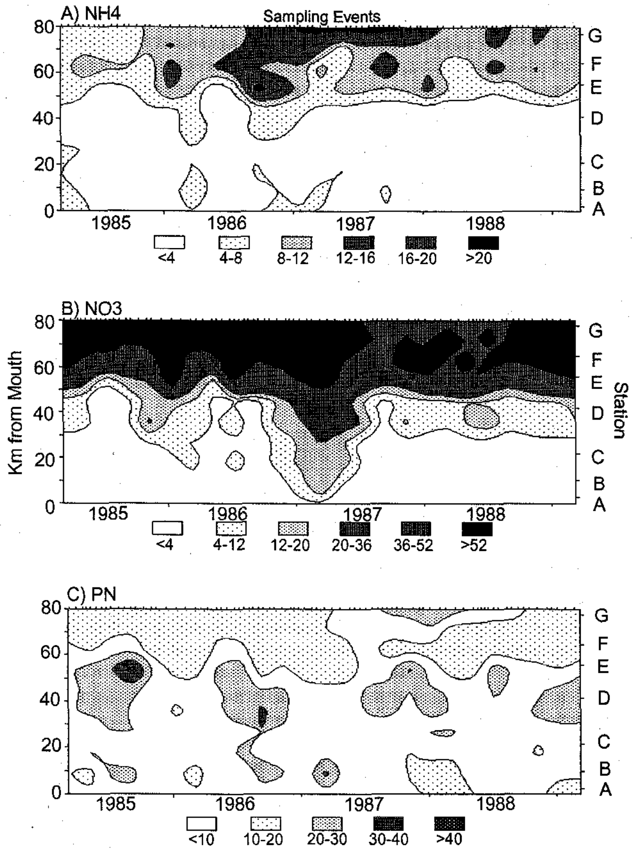

Fig. 2. Isopleths of nitrogen concentrations (μM) as in the Neuse River estuary during the 1985–1989 sampling period. Sampling events are shown as tick marks on the top axis. (A) NH_4^+, (B) NO_3^-, (C) particulate nitrogen.

most of the data were skewed to the right (see Christian et al. 1991 for a more thorough discussion). The 95% confidence interval of the median as a box-and-whisker plot (McGill et al. 1978; Wilkinson 1988) was used to visually determine whether differences in variables among stations, seasons, and years were statistically significant ($p < 0.05$). This graphical analysis is a rapid and powerful method of comparing grouped data. We also ran ANOVA on the same data as verification of box-and-whisker results.

The space-time contour plots of data along the salinity gradient of the estuary were generated using the Kriging function and TOPO module of Surfer (Golden Software). Grids were set up in proportion to both sampling sites and events to eliminate artifacts. A known dataset was used to verify the accuracy of the cubic splined contour lines produced using this method. Plots were converted to bitmap files by screen capture in Windows (Microsoft), shaded using Paintbrush (Microsoft), and labeled in Freelance (Lotus).

Results and Discussion

CHEMICAL CHARACTERISTICS

Ammonium concentrations for the 4 yr measured were generally highest in the river (often >8 μM), decreased in the oligohaline zone to <4 μM, then often rose again at the mouth (Fig. 2A). Concentrations of NH_4^+ in the summer were significantly lower ($p < 0.05$) than in any other season. The 1987 flood had little effect on NH_4^+ concentrations. We know that NH_4^+ is a minor component of total nitrogen loading in winter when runoff is high (Christian et al. 1989); therefore, we would not necessarily expect NH_4^+ concentrations to increase with river flow. The low NH_4^+ concentrations in the mesohaline zone during 1988 were due to intrusion of high-salinity, low-nutrient water from Pamlico Sound. There were no significant interannual differences in median NH_4^+ concentrations at each station for the 4 yr studied.

The most dramatic effect of the winter–spring 1987 flood was on the distribution of NO_3^- within the estuary (Fig. 2B). Most of the nitrogen input to the Neuse River comes in the form of NO_3^- as runoff from the watershed during winter (Christian et al. 1989). Nitrate concentrations in the mesohaline estuary were usually well below 4 μM during the winter and undetectable (<0.1 μM) during summer due to a combination of dilution by Pamlico Sound water, denitrification, and incorporation into plankton biomass (Christian et al. 1991). Because of the high river flows, NO_3^- concentrations of up to 25 μM were found as far down-estuary as station B, only 9.4 km from the mouth. Median NO_3^- concentrations at all stations for 1987 were significantly higher ($p < 0.05$) than any other year studied.

PN also displayed both a spatial and seasonal signal in the Neuse River estuary (Fig. 2C) with highest PN concentrations occurring during late summer–early fall in the oligohaline estuary below New Bern (station E, 52 km). PN concentrations followed a similar distribution pattern as that of Chl *a* (Boyer et al. 1993) and were significantly related ($r^2 = 0.419$, $p < 0.001$); therefore, it is not unreasonable to assume that much of the PN was due to phytoplankton biomass. If we assume that the molar ratio C:Chl *a* = 65 (Harris 1986) and a Redfield C:N = 6.6, then N:Chl *a* should be 9.8. Using this value in a regression analysis showed that algal N accounted for two-thirds of PN. A bloom of the dinoflagellate *Heterocapsa triqueta* occurred in the lower estuary in winter–spring 1987 (Christian et al. 1991; Boyer et al. 1993). This was clearly seen as a peak in PN in Fig. 2C. There were no significant differences ($p < 0.05$) in median station PN levels among years.

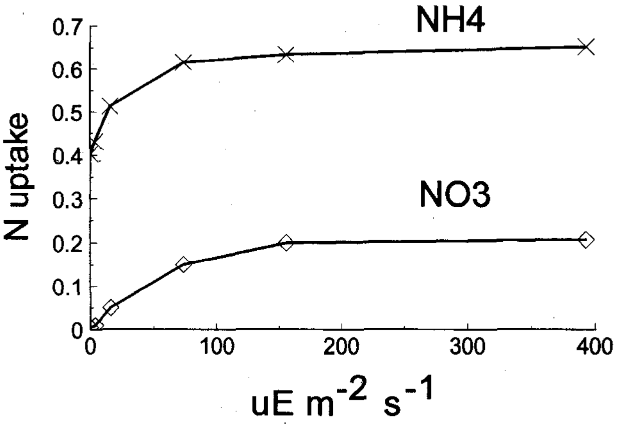

Fig. 3. Typical apparent DIN uptake (ρ in μmol N l^{-1} h^{-1}) vs irradiance (I) curves for NH_4^+ (×) and NO_3^- (◇). The high dark uptake of NH_4^+ is evident.

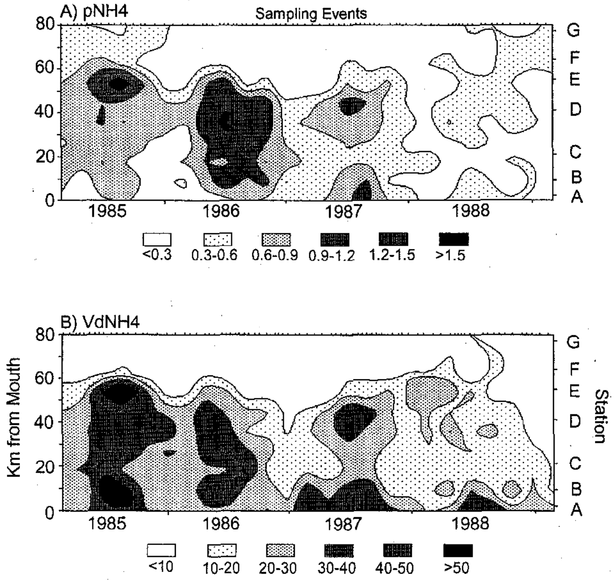

Fig. 4. (A) Isopleths of apparent NH_4^+ uptake rates (ρ in μmol N l^{-1} h^{-1}) along the Neuse River estuary during 1985–1989 sampling period. (B) Isopleths of daily integrated NH_4^+ uptake rates (V_d in mmol N m^{-2} d^{-1}) for the Neuse River estuary during 1985–1989 sampling period.

AMMONIUM UPTAKE

All light-series incubations resulted in saturation of ρ at or below 393 μE m^{-2} s^{-1}. No cases of photoinhibition were observed; therefore, we assumed that all other incubations performed at this light level would be equal to ρ_{max}. The light half-saturation constant, K_I, for NH_4^+ uptake ranged from 10 μE m^{-2} s^{-1} to 150 μE m^{-2} s^{-1}, with the median being 37. There were no significant differences in K_I among stations; however, the range in K_I for stations D and G was much greater than for station A. Values of K_I increased linearly with water temperature for all stations ($p < 0.001$), with the upriver station G possessing the best fit ($r^2 = 0.51$), compared to station A ($r^2 = 0.30$) and station D ($r^2 = 0.17$).

In the Neuse, NH_4^+ uptake was not as light-dependent as CO_2 uptake (Boyer et al. 1993). Most of the light-series incubations exhibited a typical light saturation curve but did not pass through the origin (Fig. 3). Instead, the curves were positively shifted on the y-axis with a substantial amount of uptake occurring in the dark. At times, this dark or background uptake of NH_4^+ (ρ_d) accounted for as much as 95% of ρ_{max}, with the median being 33%. No significant differences in median ρ_d:ρ_{max} were seen between the estuary (A–E) and the river or among seasons, but there were significant differences among years ($p < 0.05$). Median ρ_d:ρ_{max} in 1988 was 0.47, significantly higher than the other years ($p < 0.01$); however, the actual magnitude of dark uptake was unchanged. It was the light-dependent component of NH_4^+ uptake that was lower in 1988. The ρ_d:ρ_{max} ratio of NH_4^+ uptake was not related to temperature, salinity, DIN, PN, or any other measured parameter.

The distribution of ρ_{max} (Fig. 4A) was similar to that of PN (Fig. 2C). Since PN is used in the equation to calculate ρ_{max} and because much of the PN is associated with phytoplankton biomass, the regression of ρ_{max} with PN was highly significant ($r^2 = 0.69$, $p < 0.001$). The range in ρ_{max} was 0.001 μmol N l^{-1} h^{-1} to 4.2 μmol N l^{-1} h^{-1}. Highest ρ_{max} occurred in summer–fall in the oligohaline zone around stations D and E, decreased down estuary, and then increased at the mouth, except in summer 1986 when high rates were evenly distributed throughout the estuary. For the 4 yr measured, median ρ_{max} values were significantly higher ($p < 0.05$) in the estuary than the river (Fig. 5A). In addition, there was a general decline in ρ_{max} over time (Fig. 5B), with measured values for 1988 being significantly lower ($p < 0.05$) than any of the other years, possibly as a result of oligotrophic Pamlico Sound water intrusion. Apparent NH_4^+ uptake was significantly related to NH_4^+ concentration ($p < 0.01$); however the regression explained <3% of the variance.

Daily-integrated NH_4^+ uptake rates, V_d-NH_4^+, varied three orders of magnitude from 0.1 mmol N m^{-2} d^{-1} to 133 mmol N m^{-2} d^{-1} (Fig. 4B). As was the case for the distributions of PN and ρ_{max}, highest V_d-NH_4^+ occurred during late summer–fall in the oligohaline zone, decreased down estuary, and then increased at the mouth. The median V_d-NH_4^+ for all stations in 1988 was significantly lower than for other years ($p < 0.05$).

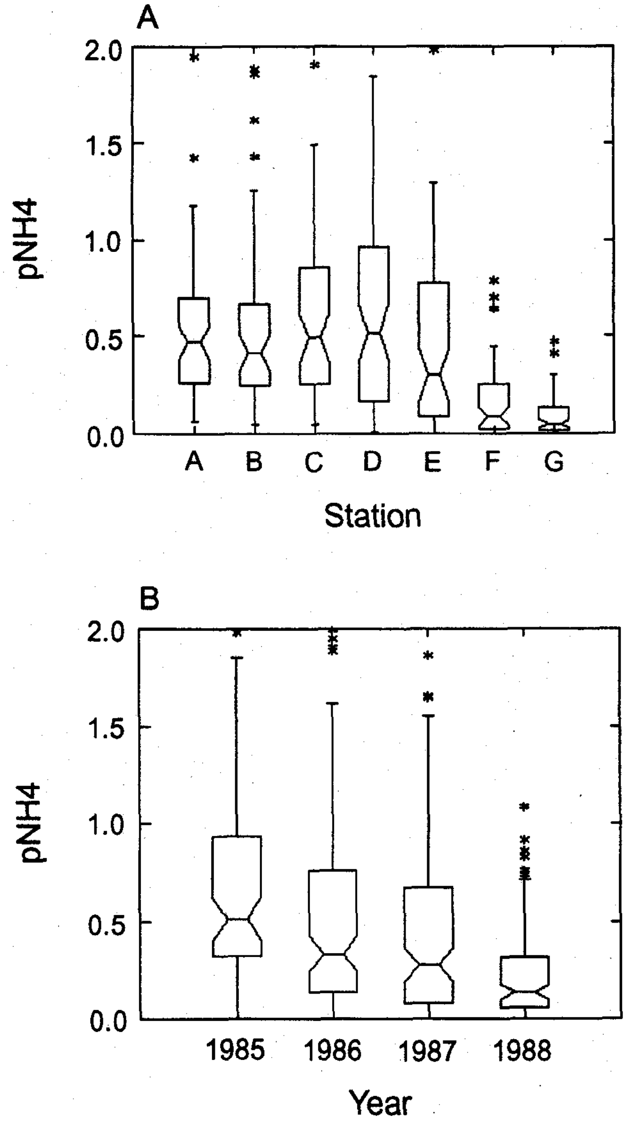

Fig. 5. (A) Box-and-whisker plot of apparent NH_4^+ uptake rates (ρ in μmol N l^{-1} h^{-1}) by station. The median is indicated by the horizontal line within the box. The notches represent the 95% confidence interval around the median. The upper and lower ends of the box are the quartiles while the ends of the vertical line represent the data range. Outliers, as defined by SYSTAT, are shown as asterisks (*). If the notches of separate boxes do not overlap horizontally, their medians are significantly different ($p < 0.05$). (B) Box-and-whisker plot of apparent NH_4^+ uptake rates (μmol N l^{-1} h^{-1}) by year showing significantly lower uptake for 1988.

NITRATE UPTAKE

As with NH_4^+, all NO_3^- uptake experiments achieved saturation at or below 393 μE m^{-2} s^{-1}. No photoinhibition of NO_3^- uptake was observed at this irradiance level. The K_I values for NO_3^- uptake ranged from 9.5 μE m^{-2} s^{-1} to 279 μE m^{-2} s^{-1}, with the median being 48.9. Unlike NH_4^+, no significant differences in K_I were found among stations. No significant relationship between K_I and temperature was observed, but highest K_I values at all stations were found in late summer–early fall.

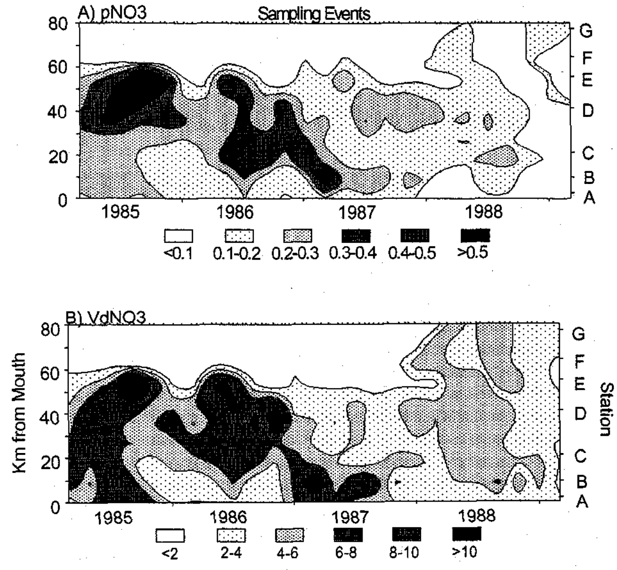

Fig. 6. (A) Isopleths of apparent NO_3^- uptake rates (ρ in μmol N l^{-1} h^{-1}) along the Neuse River estuary during the 1985–1989 sampling period. (B) Isopleths of daily integrated NO_3^- uptake rates (V_d in mmol N m^{-2} d^{-1}) along the Neuse River estuary during the 1985–1989 sampling period.

Median ρ_d:ρ_{max} increased from 0.14 in the mesohaline zone to 0.25 in the river and may have been related to the presence of high ambient NO_3^- concentrations. No seasonal differences in ρ_d:ρ_{max} were observed, but as with NH_4^+, the ρ_d:ρ_{max} of NO_3^- uptake was significantly higher during 1988 due to low light-dependent uptake rates.

Maximum apparent NO_3^- uptake rates ranged from 0.001 μmol N l^{-1} h^{-1} to 1.84 μmol N l^{-1} h^{-1} (Fig. 6A). Highest ρ_{max} measurements occurred in the oligohaline estuary at station D and decreased toward the mouth except during the flood when a peak of NO_3^- uptake activity occurred near the mouth due to the dinoflagellate bloom previously mentioned. Patterns in NO_3^- uptake showed marked interannual variability. For 1985, ρ_{max} values in the river were some of the highest measured in this study, while almost all NO_3^- uptake rates in 1988 were <0.2 μmol N l^{-1} h^{-1}, significantly lower ($p < 0.05$) than the other years.

Comparison between patterns of NO_3^- concentrations and NO_3^- uptake revealed the influence of both phytoplankton production and NO_3^- concentration on the potential of NO_3^- uptake. The relationship between ρ_{max} and ambient NO_3^- concentration was significant ($p < 0.01$) but explained <5% of the variance. In many cases, low ρ_{max} oc-

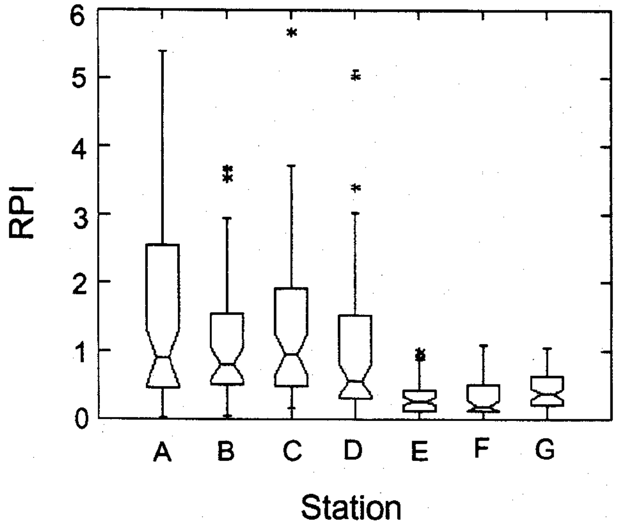

Fig. 7. Box-and-whisker plot of relative preference index (RPI) of NO_3^- showing the difference between the estuary and river.

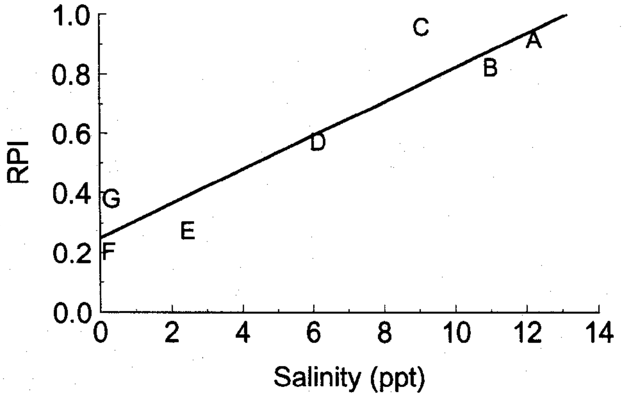

Fig. 8. Relationship between median relative preference index (RPI) of NO_3^- and salinity (‰).

curred when NO_3^- levels were highest, as in the river where neither NO_3^- uptake nor primary productivity were limited by NO_3^- concentration (Boyer et al. 1993). In the oligohaline estuary, NO_3^- uptake rates were high and corresponded with rapidly declining NO_3^- concentrations. In the mesohaline estuary, NO_3^- levels were lowest because of both biological uptake and physical mixing (Christian et al. 1991). It is possible that NO_3^- uptake rates may have been limited by NO_3^- concentrations; however this may also have been due to the increased availability of NH_4^+ in the region.

Daily integrated NO_3^- uptake rates, V_d-NO_3^-, varied 2.5 orders of magnitude from 0.1 mmol N m^{-2} d^{-1} to 53 mmol N m^{-2} d^{-1} (Fig. 6B). As with ρ_{max}, V_d-NO_3^- was highest during late summer–fall in the oligohaline estuary except during the spring 1987 dinoflagellate bloom at the mouth when high NO_3^- levels were present. Patterns in V_d-NO_3^- showed marked interannual variability. In 1985–1986, V_d-NO_3^- values in the river were some of the highest measured in this study, while virtually all NO_3^- uptake rates in 1988 were <4 mmol N m^{-2} d^{-1}. Although ρ_{max} for 1988 was significantly lower than other years ($p < 0.05$), there was no significant difference in V_d-NO_3^- among years.

In the Neuse River estuary, the ρ_d:ρ_{max} of NO_3^- uptake was half that of NH_4^+ uptake (0.16 vs 0.33). This was typical for all estuarine stations and may have been the result of preferential uptake of NH_4^+ by phytoplankton and bacteria. The only exceptions occurred in the riverine portion where the ρ_d:ρ_{max} of NO_3^- uptake increased to 0.25 due to the presence of high NO_3^- concentrations.

Median ρ_{max}-NO_3^- was 31% of ρ_{max}-NH_4^+. By excluding station G, where NO_3^- concentrations sometimes reached >100 μM, ρ_{max}-NO_3^- dropped to 28% of ρ_{max}-NH_4^+. Another way to examine the relationship between apparent NH_4^+ and NO_3^- uptake is to use the relative preference index (RPI) for a nitrogen source (McCarthy et al. 1977), where

$$\mathrm{RPI} = \frac{(\rho NO_3^-/\rho DIN)}{(NO_3^-/DIN)}.$$

An RPI < 1 indicates a preference for other DIN (such as NH_4^+) while an RPI > 1 means that the plankton preferred to use NO_3^-. Figure 7 shows

TABLE 2. Amount of DIN uptake (Mmol N yr^{-1}) for each station and the Neuse River estuary by year (where Mmol = 10^6 mol).

	NH_4^+					NO_3^-				
Station	1985	1986	1987	1988	Mean (±SE)	1985	1986	1987	1988	Mean (±SE)
A	1,440.0	1,226.5	1,911.9	1,299.1	1,469.4 (17.6)	450.2	381.6	317.5	174.4	330.9 (10.8)
B	1,100.3	1,245.4	861.3	860.4	1,016.8 (12.8)	261.0	347.0	119.8	242.3	242.5 (9.7)
C	1,100.2	1,080.3	862.3	466.1	877.2 (17.2)	250.7	317.5	148.2	155.5	218.0 (9.0)
D	668.9	428.1	572.3	230.9	475.0 (13.8)	214.1	243.7	160.9	122.1	185.2 (7.4)
E	169.2	64.7	89.1	47.9	92.7 (7.3)	51.2	44.9	20.0	25.9	35.5 (3.8)
F	5.8	3.6	4.6	1.7	3.9 (1.3)	0.9	1.6	5.0	2.5	2.5 (1.3)
G	0.6	0.5	0.6	0.2	0.5 (0.4)	0.2	0.9	1.3	0.3	0.7 (0.7)
Estuary	4,485.1	4,049.0	4,302.1	2,906.5	3,935.7 (26.6)	1,228.2	1,337.1	772.7	723.0	1,015.2 (17.6)

TABLE 3. Annual DIN uptake on an areal basis (mol N m^{-2} yr^{-1}) for each station and for the total Neuse River estuary.

	NH_4^+					NO_3^-				
Station	1985	1986	1987	1988	Mean (±SE)	1985	1986	1987	1988	Mean (±SE)
A	11.8	10.1	15.7	10.7	12.1 (1.6)	3.7	3.1	2.6	1.4	2.7 (0.1)
B	10.9	12.4	8.5	8.5	10.1 (1.4)	2.6	3.4	1.2	2.4	2.4 (1.0)
C	12.4	12.1	9.7	5.2	9.8 (1.8)	2.8	3.6	1.7	1.8	2.5 (0.9)
D	10.9	7.0	9.3	3.8	7.8 (1.8)	3.5	4.0	2.6	2.0	3.0 (0.9)
E	9.8	3.8	5.1	2.8	5.4 (1.8)	2.9	2.6	1.2	1.5	2.0 (0.9)
F	1.9	1.2	1.5	0.6	1.3 (0.7)	0.3	0.5	1.6	0.8	0.8 (0.8)
G	1.1	0.9	1.1	0.4	0.9 (0.6)	0.4	1.6	2.3	0.5	1.2 (1.0)
Estuary	11.4	10.3	10.9	7.4	10.0 (1.5)	3.1	3.4	2.0	1.9	2.6 (0.9)

the median RPI for each station as a function of salinity. There are two points that should be addressed from these data. First, median RPI values for all stations were <1 denoting an overall preference for NH_4^+ as a nitrogen source throughout the estuary. Second, the range in RPI clearly shows that there were many instances when NO_3^- was the preferred N source at the estuarine stations. It is also interesting to note that the stations which had highest NO_3^- concentrations showed lowest RPI values. Phytoplankton at stations E–G, with median NO_3^- levels >30 μM, still preferred to use NH_4^+ as the main nitrogen source. It is also clear that the RPI is related to salinity in the Neuse (Fig. 8). Median RPI declined linearly with decreasing salinity from estuary to river possibly as a consequence of change in phytoplankton community structure (Pennock 1987).

ANNUAL DIN UPTAKE

Annual estimates of NH_4^+ and NO_3^- mass uptake (Mmol N $segment^{-1}$ yr^{-1}) of the entire estuary are shown in Table 2. Between 81% and 90% of the mass NH_4^+ uptake occurred in the mesohaline zone, 9% and 19% in the oligohaline estuary, and <1% in the river. This was largely because of the respective areas of each segment (Table 1). A similar trend was seen for mass NO_3^- uptake, where 75–79% of the total occurred in the mesohaline zone, 20–23% in the oligohaline estuary, and <1% in the river.

V_y-NH_4^+ for the Neuse River estuary was 11.39 mol N m^{-2} yr^{-1}, 10.28 mol N m^{-2} yr^{-1}, 10.93 mol N m^{-2} yr^{-1}, and 7.39 mol N m^{-2} yr^{-1}, with the 4-yr mean ± SE being 10.0 ± 1.3 mol N m^{-2} yr^{-1}. V_y-NO_3^- for the Neuse River estuary was 3.12 mol N m^{-2} yr^{-1}, 3.40 mol N m^{-2} yr^{-1}, 1.96 mol N m^{-2} yr^{-1}, and 1.84 mol N m^{-2} yr^{-1}, with the 4-yr mean being 2.6 ± 0.9 mol N m^{-2} yr^{-1}. Annual NO_3^- uptake accounted for only 20.5% of the total DIN uptake of the estuary. Annual DIN uptake of the estuary was 14.51 mol N m^{-2} yr^{-1}, 13.68 mol N m^{-2} yr^{-1}, 12.89 mol N m^{-2} yr^{-1}, and 9.22 mol N m^{-2} yr^{-1}, with the 4-yr mean being 12.6 ± 1.5 mol N m^{-2} yr^{-1}.

Annual primary productivity for the Neuse River estuary for the 4 yr measured was 455.6 g C m^{-2} yr^{-1} (Boyer et al. 1993). The phytoplankton nitrogen requirement as calculated using the Redfield ratio was only 5.7 mol N m^{-2} yr^{-1}. Therefore, the mean DIN uptake of the Neuse River estuary based on direct measurements is more than twice the nitrogen demand extrapolated from primary productivity measurements. We believe that much of this discrepancy is due to dark or background DIN uptake by the nonphotosynthetic plankton fraction (e.g., bacteria) (Wheeler and Kirchman 1986; Harrison and Wood 1988). If the C:N of bacteria is assumed to be 5 (Findlay et al. 1991) and all N requirements are met by DIN, then annual bacterial productivity (B_y in g C m^{-2} yr^{-1}) can be estimated from the difference between measured and extrapolated DIN demand. For the 4 yr studied, B_y was 410.5 g C m^{-2} yr^{-1}, an amount almost equal to P_y. This may be an underestimate as organic N also contributes to bacterial production; however, it is clear that the use of the Redfield ratio to estimate nitrogen demand of estuaries can result in serious underestimates because the heterotrophic component is not included in that total.

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