

# Comprehensive trend analysis of nutrients and related variables in a large eutrophic estuary: A decadal study of anthropogenic and climatic influences

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## *Abstract*

We used a decadal data set, with weekly to biweekly sampling in April–October and monthly sampling in November–March, to characterize climatic (hurricane-level storms, a sustained 3-yr drought) and anthropogenic influences on N and P concentrations and loadings to a large eutrophic, poorly flushed estuary, the Neuse Estuary of the Albemarle–Pamlico Estuarine System. Mass volume transport data were obtained with cross-estuary transect flow measurements taken near the entrance to the estuary. Although trends were minimally influenced by hurricanes, analyses were significantly affected by the sustained drought near the end of the study. As examples, decreasing trends in total N (TN), total P (TP), and bottom-water dissolved oxygen concentrations, and in TN loadings were significant considering all data, but these trends were not significant when the sustained drought was excluded from analysis. In addition, the trend in TN loading was especially sensitive to the initial sampling period.  $\text{NH}_4^+$  concentrations dramatically increased (overall by ~500%) as a persistent trend regardless of attempts to control for climatic events. An increasing trend in  $\text{NH}_4^+$  also was documented in an adjacent, rapidly flushed Coastal Plain estuary, the Cape Fear. The  $\text{NH}_4^+$  data suggest a regional-scale effect of high inputs from inadequately controlled, increasing nonpoint sources. The fragility of TN loading trends, the striking increase in  $\text{NH}_4^+$  concentrations, and the lack of management emphasis on controlling nonpoint sources such as “new” industrialized swine production collectively do not support recent reports of achievement of a 30% reduction in TN loading to the Neuse. Nonpoint sources remain a critical target for reduction to alleviate the negative effects of cultural eutrophication in this system, as in many estuaries throughout the world.

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We dedicate this study to Robert G. Wetzel, in tribute to his integrity and his many seminal contributions that have significantly advanced aquatic science.

... anthropogenic impacts on water quality are strong and the need for characterizing ambient conditions and temporal trends in these conditions is correspondingly urgent.

—Jassby et al. (1997)

More than two thirds of coastal rivers and bays in the United States are moderately to severely degraded from cultural eutrophication, exacerbated by poor flushing and significant human population growth in coastal areas (Bricker et al. 1999; National Research Council [NRC] 2000). Despite this general recognition, there are as yet few success stories in achieving significant reductions, especially of non-point nutrient sources, to estuaries and coastal embayments (NRC 2000).

Cloern (2001) described three phases in the evolving conceptualization of the overall effects of cultural eutrophication in aquatic ecosystems: Phase I historically emphasized changing nutrient inputs, phytoplankton response, and bottom-water oxygen depletion; Phase II, described as the contemporary phase, has emphasized system-specific attributes that modulate a complex suite of direct and indirect responses to enrichment, leading to large differences among freshwaters and estuarine-coastal systems in their sensitivity to eutrophication; and Phase III, for the early 21st century, was projected to address several questions about effects of system-specific attributes on ecosystem response to eutrophication, nutrient enrichment interactions with other stressors, and response linkages. As an additional question proposed for guiding scientific research in Phase III, Cloern (2001) asked how deeper scientific understanding of coastal eutrophication can be used to improve ecosystem restoration or rehabilitation. In this paper, we describe a decadal study that falls within Cloern's (2001) Phase I, with some limited Phase II insights. Yet this study illustrates the present status of progress in understanding cultural eutrophication: that for most estuarine-coastal aquatic ecosystems, sufficient long-term data are only beginning to become available to assess trends in nutrient concentrations and loadings; that there is still only limited understanding of climatic influences on these trends; and that the trends themselves are a "moving target," changing significantly depending on the period used as time zero ( $T_{\text{initial}}$ ). Accordingly, Jassby et al. (1997) described apparent Phase I research from Cloern (2001), the quantitative characterization of trends in anthropogenic effects on water quality, as an ongoing, urgent need.

Here, we describe a decadal effort to address that need in a major U.S. estuary toward addressing the question of how improved understanding of trends in nutrient concentrations and loadings in this system can be used to improve efforts to restore it. The eutrophic Albemarle-Pamlico Estuarine Ecosystem of North Carolina is the second largest estuary and the largest lagoonal estuary on the U.S. mainland (Steel 1991). The Outer Banks enclose this estuary except for several narrow inlets connecting it with the Atlantic Ocean. This characteristic enhances the Albemarle-Pamlico's function as a significant fish nursery ground; the broad, shallow (depth generally 2–4 m), sheltered system provides fully half of the total nursery area for commercially important species along the entire U.S. Atlantic Coast (Steel 1991). Its enclosed waters are wind mixed, with little tidal effect (Reed et al. 2004),

and poorly flushed, with mean residence times in major tributaries estimated at 50–100 d over an annual cycle (Christian et al. 1991; Steel 1991).

These features make the Albemarle-Pamlico especially sensitive to anthropogenic nutrient loading. Winds easily re-suspend nutrients that were deposited in the sediments, and poor flushing tends to retain nutrients in these waters (Christian et al. 1991; Giffin and Corbett 2003). Water quality in many of its tributaries is degraded by excessive turbidity and nutrient loading from human activities (Steel 1991), and the estuary is considered one of the most eutrophic in the southeastern United States (Bricker et al. 1999). Moreover, the Albemarle-Pamlico is hurricane prone: Over the past ~100 yr, more severe storms have struck the U.S. Atlantic Coast than any other region on Earth, with North Carolina second only to Florida in the number of major storms that have made landfall (U.S. Weather Service 2005). These major storms have caused flooding of concentrated (confined) swine feed operations (CAFOs), which have occurred in high density since the late 1980s in the watershed near the upper estuary (Burkholder et al. 1997). Moderate to high precipitation has also led to bypassing of raw sewage from municipal wastewater treatment plants (WWTPs); flooding of WWTPs in the aftermath of Hurricanes Fran (1996) and Floyd (1999) led to sustained treatment bypasses and the release of large amounts of raw sewage for weeks following the storms (Bales 2003). Although such major storms can cause acute short-term adverse effects on estuarine systems, they can also function beneficially in system flushing and renewal (Bales 2003; Burkholder et al. 2004).

Numerous publications have described environmental conditions and trends in the Albemarle-Pamlico at the end of the 20th century, especially in one of its major tributaries and largest nutrient contributor (McMahon and Woodside 1997), the Neuse Estuary (Qian et al. 2000; North Carolina Department of Environment and Natural Resources [NC DENR, formerly the Department of Environment, Health, and Natural Resources, DEHNR] 2001; Stow et al. 2001). However, in estimating nutrient loadings to the Neuse Estuary, researchers often were forced to rely on concentration and flow data from locations 13–70 km upstream from the entrance to the estuary, with differences in basin drainage area ranging from 11% to 65% (Epperley and Ross 1986; Bales and Robbins 1999). Extrapolative nutrient loading models were developed in some cases to account for these differences, but their accuracy was reduced by subsequent installation of dams, roads, or other flow modifiers. Similar approaches commonly have been used in other estuaries (e.g., Jaworski 1981; Boynton et al. 1995). In addition, models generally have had to rely on a synthesis of data on nutrient concentration from multiple sources, with variations imposed by differences in sampling, analytical methodologies, and uneven temporal and spatial distributions. These factors have impeded adequate assessment of trends in nutrient concentrations and loadings and effectiveness of management strategies designed to reduce eutrophication. For the Neuse, nutrient loading estimates derived from these concentration and flow data have been used to evaluate the success of management strategies to reduce oxygen deficits, algal blooms, and massive fish kills through targeted reduction

of a total maximum daily load for nitrogen (NC DENR 1999; Reckhow 2003; Wool et al. 2003).

In this study, we used a network of long-term monitoring stations and a newly developed flow model to assess trends in nutrient concentrations and loadings and response variables in the Neuse Estuary. Our study differs from previous efforts in the following features: Other recent studies date from 1998 to 2000, and most were based on monthly data (one data point per month). Our study adds ~5 additional years of new information and used monthly averages from a higher frequency data set during April–October (two to four data points per month; one data point per month in November–March). Previous studies (e.g., Qian et al. 2000; Stow et al. 2001; Borsuk et al. 2003) were also based on midchannel data only, taken from the highest flushing area, which could have underestimated nutrient concentrations and loadings. Our study captured conditions in side channels, where much of the nutrient loading and algal blooms occur (Glasgow and Burkholder 2000). The previous studies used flow data ~13–75 km upstream from the estuary, or attempted to extrapolate downstream to the estuary from those data. In contrast, the flow model used in this study was based on empirical acoustic Doppler current profiling data collected near the oligohaline edge of the estuary. The data used for this model encompassed conditions ranging from low flow (during a sustained 3-yr drought) to very high flow (1999, post–Hurricanes Dennis, Floyd, and Irene). Finally, other recent studies (e.g., Stow et al. 2001; Stow and Borsuk 2003; Borsuk et al. 2004) used flow-adjusted concentrations, which ideally can help to account for flow variability and provide a more accurate estimate of monthly concentrations in riparian systems (U.S. Geological Survey [USGS] 2001). In the Neuse system, however, flow-adjusted models have explained only ~20–55% of the variance in nitrogen concentrations at selected stations along the river, with lower correlations at stations near the estuary (Stow and Borsuk 2003). Our flow model, developed for this study with empirically collected flow data near the estuary, was used in combination with concentration data that were measured over the decadal period at the same locations. As an additional novel contribution, we examined climatic (hurricane-level storms and a sustained drought) influences on eutrophication in this estuary, augmented by a series of autoregression models and an overview of some major nutrient sources in the Neuse watershed.

Our analysis indicates decreasing trends in total N (TN) and total P (TP) concentrations and loadings and in bottom-water dissolved oxygen (DO) concentrations in the estuary over the past decade, but these trends can primarily be explained by climatic events (a sustained 3-yr drought near the end of the study) rather than management actions because nutrient reductions from improved management practices in the Neuse watershed over the past decade have been offset by increases in human and swine populations and related factors. In contrast, we report a striking increase in  $\text{NH}_4^+$  concentrations. We present evidence for a similar increase in  $\text{NH}_4^+$  concentrations in another major Coastal Plain river basin. Analysis of the  $\text{NH}_4^+$  concentration data, reflecting “new N” as well as regenerative inputs, indicates that despite certain nutrient sources targeted for reduction in present

management plans, eutrophication is progressing in this estuary from addition of inorganic N ( $\text{N}_i$ ) by inadequately controlled nonpoint nutrient sources.

## Study area

The Neuse River and Estuary flow ~320 km through the Piedmont and Coastal Plain to Pamlico Sound (Fig. 1). The upstream boundary of the study area (area drained,  $1.37 \times 10^4 \text{ km}^2$ , or about 85% of the basin) was 13 km downstream from New Bern (population 23,000; NC DENR 2002), in the vicinity of the entrance to the oligohaline estuary under average flow conditions (Bowen and Hieronymus 2003; Fig. 1). At New Bern, the Neuse abruptly widens and changes from a flowing river to a wind-mixed estuary that eventually flows into Pamlico Sound (Fig. 1). Approximately 50% of the Neuse waters in the Coastal Plain are contributed by subsurface flow during low-flow conditions (Spruill 1997; Weaver 1998).

The watershed contains ~16,900 km of stream length, with ~66 km<sup>2</sup> of run-of-river impoundments and  $1.5 \times 10^3 \text{ km}^2$  of estuaries (NC DENR 2002). The system drains  $\sim 1.62 \times 10^4 \text{ km}^2$ , with natural and cultivated forests (38%), agriculture (29%), and wetlands (14%) dominating the watershed (U.S. Environmental Protection Agency [EPA] 1999; U.S. Department of Agriculture [USDA] 2001). Urban areas, which compose ~13% of the Neuse watershed, continue to rapidly expand and diversify (USDA 2001). The estuarine study area is mesohaline and has typical estuarine flow dynamics, with outflow at the surface and inflow at the bottom along with a predominant south shore surface outflow (Reed et al. 2004). Statistically significant cross-estuary and along-estuary differences occur in the mesohaline estuary in both halocline structure and nutrient concentrations (Reed et al. 2004). The broad and shallow nature of this system results in complex hydrodynamics, including wind-induced upwelling of high-salinity bottom water.

At present, the Neuse watershed contains  $\sim 1.35 \times 10^6$  people (about 16% of the state's population; NC DENR 2002), with 54% of the basin population located in ~10% of the land area. Forest and cultivated croplands decreased  $\sim 7.28 \times 10^3 \text{ km}^2$  over the past decade, giving way to land development (USDA 2001; Line et al. 2002; National Agricultural Statistics Service 2004). The watershed also contains  $\sim 1.75 \times 10^6$  swine ( $\sim 3.5 \times 10^6$  swine produced per year, or 18% of the state's annual total, mostly in concentrated animal feed operations or CAFOs), contributing an equivalent in sewage of about 7 million people (American Society of Agricultural Engineers 1998), and  $\sim 4.8 \times 10^7$  poultry produced per year (USDA 2001; Table 1). CAFOs are a recent feature on the landscape, mostly added since the late 1980s (reviewed in Burkholder et al. 1997), with  $\sim 1.9 \times 10^7$  swine produced in North Carolina per year (NC DENR 2002). Thus, CAFOs represent a relatively new nonpoint source of mammal wastes (Table 1). Most CAFOs in North Carolina, including the Neuse watershed, are in the eastern Coastal Plain near the estuaries on former wetlands (Burkholder et al. 1997). The water table lies about 1 m, on average, from the surface soils; moreover, much of the land

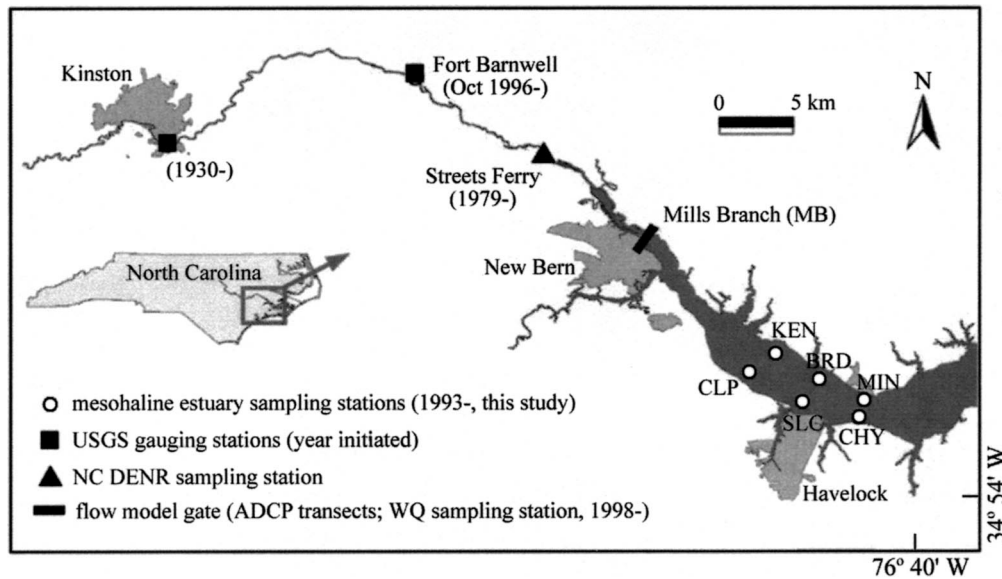


Fig. 1. The Neuse River and Estuary, showing USGS flow stations and the Mills Branch (MB) gate (generally the oligohaline edge of the estuary) for the flow model. The six main estuarine sampling sites are also shown, each  $\sim 750$  m out from shore: Carolina Pines (CLP), Kennel Beach (KEN), the Neuse near the Beard Creek confluence (BRD), the Neuse near the Slocum Creek confluence (SLC), Minnesott Beach (MIN), and Cherry Point (CHY). Municipal boundaries (New Bern, Havelock, Minnesott) are indicated in grey. Note that prevailing winds tend to mix the waters especially toward the north shore, with the south shore relatively more protected (Reed et al. 2004).

previously had been ditched or tile-drained to channel runoff to receiving rivers and estuaries (Steel 1991). CAFOs release high quantities of  $\text{NH}_3$  and other nutrient forms into the atmosphere and adjacent surface and subsurface waters (e.g., Burkholder et al. 1997; Mallin 2000; Walker et al. 2000).

The Neuse contributes the largest percentage of N and P to the Albemarle–Pamlico Sound, accounting for almost

35% of the N and 50% of the P loadings, although it represents only  $\sim 20\%$  of the total contributing drainage area (Spruill et al. 1998; USDA 2001; North Carolina Center for Geographic Information and Analysis 2002). The state's "nutrient-sensitive waters" (NSW) classification was applied to the eutrophic Neuse River and Estuary in the late 1980s because the system sustains major algal blooms, ox-

Table 1. Estimated N and P contributions (average per year) of point and selected nonpoint sources to the Neuse watershed.

Source*	No.	N ( $\text{kg yr}^{-1}$ )	P ( $\text{kg yr}^{-1}$ )
Major WWTPs (discharge)	157	$1.1 \times 10^5$	$4.8 \times 10^5$
Other major point sources (discharge)	21	$8.8 \times 10^4$	$3.7 \times 10^5$
Synthetic fertilizers	$2.9 \times 10^8$ kg	$2.9 \times 10^7$	$1.3 \times 10^6$
Swine CAFOs ( $3.5 \times 10^6$ animals produced)			
Active	460	$2.0 \times 10^7$	$4.6 \times 10^6$
Inactive	75	+?	+?
Broiler chickens (CAFOs, other; $3.79 \times 10^7$ animals produced)	4	$6.4 \times 10^5$	$2.0 \times 10^5$
Turkeys ( $1.04 \times 10^7$ produced)	—	$4.1 \times 10^6$	$1.1 \times 10^6$
Cattle ( $8.5 \times 10^5$ animals produced)	7	$3.6 \times 10^6$	$9.0 \times 10^5$

\* Major point sources are defined as discharging  $\geq 3.7 \times 10^6$  L  $\text{d}^{-1}$  (1.0 million gallons per day); estimates exclude sludge and bypasses. Swine CAFOs are defined as containing  $\geq 250$  animals as standing stock (NC DENR 2004). Broiler chicken and turkey CAFOs are defined as having  $\geq 30,000$  birds as standing stock; the four in operation account for a small portion of the standing crop of  $\sim 4.83 \times 10^7$  birds produced  $\text{yr}^{-1}$  in the Neuse watershed. Cattle operations are defined as containing  $\geq 1,000$  confined beef or 700 mature dairy cattle (liquid manure systems requiring National Pollution Discharge Elimination System general permits) or 100 confined cattle (liquid manure systems requiring a state general nondischarge permit). The above estimates exclude materials leached from dead animals (1–8% of production in swine) and leachate from inactive swine CAFOs. Synthetic fertilizer quantities pertain to multinutrient and single-nutrient fertilizers shipped within the watershed annually per fiscal year. Livestock, poultry, and fertilizer data are from Powers and Van Horn (1998) and NC DACS (2002).

xygen deficits, and fish kills during most annual cycles (NC DENR 1997a). Through 1997, P was the nutrient targeted for reduction in the Neuse system. Focus shifted in 1998 to development of a total maximum daily load (TMDL; NC DENR 1999) for total nitrogen. Run-of-river impoundments in the upper Neuse, as well as lower freshwater segments, have supported late summer cyanobacterial blooms (e.g., *Microcystis aeruginosa* Kützing; Christian et al. 1986), and cryptic cyanobacteria have also been abundant in the estuary (Fensin 1997). Freshwater blooms, limited by light availability and P inputs (e.g., Christian et al. 1986; Cuker et al. 1990), give way to estuarine blooms of dinoflagellates and other flagellates (Mallin 1994; Pinckney et al. 1997; Burkholder et al. 1999).

The mesohaline estuary supports massive dinoflagellate blooms, especially during high-precipitation winter–spring seasons (Mallin 1994; Springer et al. 2004). Mesohaline phytoplankton productivity has been positively correlated with delivery of nitrate from upper freshwater segments (Mallin et al. 1993). Colimitation of both N and P has been demonstrated for the estuarine plankton during spring (Rudek et al. 1991), indicative of high nutrient loading (e.g., Fisher et al. 1992), with N limitation invoked for the remainder of the annual cycle (Rudek et al. 1991). Other researchers, however, have reported that the N:P concentration ratio might have substantially increased and have suggested that the estuary could be sustaining a shift toward P limitation extending longer than the spring season (Qian et al. 2000). The relative isolation of the Neuse Estuary from ocean inputs and the extended flushing rates promote non-conservative mixing and substantial recycling of these nutrients (Christian et al. 1991).

During this decadal study, human population in the Neuse watershed increased by 16% (North Carolina State Data Center 2004), impervious surface area from urbanization doubled (USGS 1980; EPA 1999), and use of synthetic fertilizers decreased by ~20% (NC Department of Agriculture and Consumer Services 2002). The swine population increased ~285% since 1990. At the same time, nutrient reductions occurred through improved effluent treatment in some municipal WWTPs, and an agreement was also forged by cropland agriculturalists to plan to apply ~40% less fertilizer (Center for Agricultural Partnerships 2002).

## Materials and methods

*Sampling design and measurements*—The estuarine water quality study extended from May 1993 to June 2003. Six sites in the mesohaline Neuse Estuary were sampled (Fig. 1): Carolina Pines (CLP; Global Positioning System [GPS] coordinates 34.9693N, 76.9125W; referred to as site 38 or FLN in Glasgow and Burkholder 2000), Kennel Beach (KEN; GPS coordinates 35.0171N, 76.9068W), Beard Creek (BRD; GPS coordinates 34.9981N, 76.8709W; referred to as site 36 in Glasgow and Burkholder 2000), Slocum Creek (SLC; GPS coordinates 34.9568N, 76.8851W; referred to as RAD in Glasgow and Burkholder 2000), Cherry Point (CHY; GPS coordinates 34.9474N, 76.8159W), and Minnesott Beach (MIN; GPS coordinates 34.9638N, 76.8124W).

It should be noted that station SLC was also the discharge area of a major point source to the Neuse Estuary, the WWTP of the U.S. Marine Corps Air Station, Cherry Point. This WWTP began discharge in 1994 ( $7.6 \times 10^6$  L d<sup>-1</sup>; maximum capacity  $1.33 \times 10^7$  L d<sup>-1</sup>) and was permitted at  $\leq 30$  mg NO<sub>3</sub><sup>-</sup> L<sup>-1</sup> and  $\leq 2$  mg TP L<sup>-1</sup> (NC DENR–DWQ permitting records). The six stations were sampled weekly throughout 1993–1998. From 1999 to 2003 they were sampled biweekly from April through October, and monthly from November through March. Infrequent departures from this schedule resulted from field instrumentation malfunctions or from severe storms that prevented sampling. A seventh station, Mills Branch (MB), near the oligohaline edge of the estuary under average flow conditions (Bowen and Hieronymus 2003), was added for the last 4.5 yr of the study (January 1999–June 2003; Fig. 1).

Sampling and analytical procedures recognized by state and federal regulatory agencies (40CFR Part 136; American Public Health Association [APHA] et al. 1992; EPA 1993) were used together with sanctioned modifications as EPA variances for analysis of N and P species. Certification with DWQ and EPA involved external audit of analytical procedures, data and quality assurance measures, and participation in blind performance evaluation studies. Certain physical and chemical variables were determined in situ with a Hydrolab H<sub>2</sub>O multiprobe and transmitter with SVR4-DL datalogger and included depth profiles (0.5-m intervals) for temperature, salinity, pH, and DO. The Hydrolab datasonde was calibrated daily during use periods. Photosynthetically active radiation was recorded with a LiCor datalogger and 4 $\pi$  spherical sensor with light attenuation coefficients calculated following Wetzel and Likens (2001). Transparency was also assessed from Secchi depth data (Wetzel and Likens 2001). An integrated water column sampler (modified from Cuker et al. 1990) was used to collect samples from the upper and lower water column (0.0–1.5 m, and 1.5 m to just above the bottom sediments at 2.5–3.5 m, respectively, in deeper sites). The water column sampler was thoroughly rinsed with site water before sampling. Samples were maintained in darkness on ice for transport to the laboratory and were then refrigerated or frozen as appropriate until analysis (EPA 1993).

In addition to the routine monitoring regime described above, event-driven sampling efforts were undertaken (1998–2003) to track phytoplankton blooms (chlorophyll *a* [Chl *a*] > 40  $\mu$ g L<sup>-1</sup>, the state standard for acceptable water quality; NC DEHNR 1996a) throughout the study area from Mills Branch (MB) to MIN–CHY (Fig. 1). Bloom events were detected on the basis of observations by field personnel during routine monitoring or through detection by our remote monitoring network (from 2000 on; Glasgow et al. 2004) of instrumented platforms at the six sites on the basis of pH and DO profiles. Subsamples were preserved with acidic Lugol's solution (Wetzel and Likens 2001) in the field and held at 4°C until analysis for bloom-forming taxa (light microscopy for nontoxic dinoflagellates forming winter blooms).

Samples for analysis of suspended solids (SS) were maintained at  $\leq 4^\circ\text{C}$ , filtered within 48 h, and measured gravimetrically (method 2540D, APHA et al. 1992). Chl *a* sam-

ples were filtered under low vacuum (Whatman GF/C filters, 55–69 kPa) and low light ( $20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) within 12 h of collection and were stored frozen ( $-20^\circ\text{C}$ ) with desiccant in darkness until analysis within 2 months. Chl *a* was extracted in 90% basic acetone (EPA 1997a; Wetzel and Likens 2001), and fluorescence was determined with a Turner 10-AU fluorometer.

Nutrients were analyzed with a Technicon (Traacs 800) or Lachat Instruments (Quickchem 8000) autoanalyzer. Variances from the EPA and the NC DENR–DWQ were obtained to enable use of procedures for nutrient sample storage and analysis (substitution of freezing at  $-20^\circ\text{C}$  for acidification; 2-month limit) that accommodated low-level analysis of estuarine matrices (EPA 1992, 1997b). Water samples for TP analysis were frozen at  $-20^\circ\text{C}$  until analysis with a variance of EPA method 365.1 (EPA 1992, 1993; practical quantitation limit [APHA et al. 1992]  $10 \mu\text{g L}^{-1}$ ).  $\text{NH}_4^+$  samples were preserved immediately with phenol in the field (Glasgow and Burkholder 2000) and were held in darkness on ice during transport. Analysis followed a variance of EPA method 350.1 (EPA 1993; practical quantitation limit  $12 \mu\text{g NH}_4^+ \text{L}^{-1}$ ). Samples for  $\text{NO}_3^- + \text{NO}_2^-$  analysis (hereafter referred to as nitrate) were frozen and analyzed within 2 months with a variance of EPA method 353.4 (EPA 1992; practical quantitation limit  $6 \mu\text{g NO}_3^- \text{L}^{-1}$ ). Samples for total Kjeldahl N analysis (TKN = free  $\text{NH}_3 + \text{N}_o$  [organic N]) were assayed as in Glasgow and Burkholder (2000) with a state-certified method and a detection limit of  $1,000 \mu\text{g L}^{-1}$  from May 1993–April 1994; for the remainder of the study, we used a modification of EPA method 351.2 (EPA 1993; samples held at  $-20^\circ\text{C}$  and not preserved with sulfuric acid; practical quantitation limit  $140 \mu\text{g N L}^{-1}$ ). Values for TN (as TKN +  $\text{NO}_3^-$ ) and TP were used to calculate TN:TP ratios (June 1994–June 2003; molar basis).

*Mass water transport*—A boat-mounted acoustic Doppler current profiler (ADCP; Workhorse Rio Grande model, 1,200 kHz direct reading; RD Instruments) was used to obtain discharge measurements from cross-estuary transects taken at Mills Branch, New Bern, North Carolina (Fig. 1). As mentioned, this site is located in the vicinity of the estuary's oligohaline edge under average flow conditions (Bowen and Hieronymus 2003). ADCP with bottom-tracking technology can more accurately estimate river discharge into estuarine systems (as opposed to measurements extrapolated from a single point-determined or fixed-location gauging station at substantial distance upstream), by effectively separating out bidirectional (upriver and downriver) estuarine influences (reviewed in Glasgow and Burkholder 2000; Reed et al. 2004). Such estuarine influences can include increased local winds (directed upstream and downstream) or precipitation events (Reed et al. 2004). The separation of bidirectional estuarine influences made it possible to exclude upstream movement in calculating net downstream volume of flow.

The ADCP was configured to give short-term velocity errors of  $2.6 \text{ cm s}^{-1}$ . The error value of this standard configuration is  $\sim 1.6$ -fold greater than the theoretical standard deviation of a perfect ADCP system (Therault 1986). The ADCP was attached to a boom mounted to the bow of a small boat (8-m-long customized Albemarle boat with cen-

tral console). Data were taken from shore to shore at Mills Branch at a boat velocity of 4–5 knots  $\text{h}^{-1}$ . The positioning antenna for the differential GPS (Ashtech BR2G) was mounted on a raised standard that was attached to the ADCP boom, allowing maximum accuracy in positioning the boat for transect replication. The ADCP data included 28 transects with  $\geq 95\%$  downstream flow ( $N = 28$  dates; cross-estuary transects taken in January 1999–August 2001; 53 cross-estuary transects generated 1,737 ADCP ensembles). If more than one transect on a given date met the 95% downstream flow criterion, the data were averaged (e.g., 12 transects on 3 March 2001, 9 transects on 4 April 2001). The number of ensembles in each transect was dependent on boat speed, water depth, ADCP configuration, and other parameters.

The estimates for mass water transport into the estuary on the basis of these data were compared with estimated values from another model that has been used historically in the Neuse. That model relies on an equation that was developed by Weyerhaeuser (Thut 1980) before installation of a major dam on the Neuse in the upper watershed (Falls of the Neuse, which impounded 35 km of the river; NC DEHNR 1993; NC DENR 2002), which extrapolates discharge measured by a fixed-point USGS station at Kinston, North Carolina ( $\sim 70$  km upstream from Mills Branch). On the basis of the results of a linear regression between the measured ADCP and the estimated extrapolated Weyerhaeuser values, the Weyerhaeuser equation tended to underestimate flow during lower flow conditions and to overestimate during higher flow conditions.

The ADCP data were used to develop an updated, segmented linear regression model to predict discharge at Mills Branch, North Carolina, from measured flow (USGS fixed-point station) at Kinston, North Carolina. These ADCP data encompassed conditions ranging from low flow (drought years) to very high flow (1999, post-Hurricanes Dennis, Floyd, and Irene). Flow rates at Kinston in the data set used to develop the prediction equations ranged from  $13$  to  $750 \text{ m}^3 \text{ s}^{-1}$ , compared with a range of  $7$  to  $1,014 \text{ m}^3 \text{ s}^{-1}$  for the complete decadal data set. Predicted values for the larger data set involved extrapolation of higher flow data only from 6 d post-Hurricane Fran (0.15% of the data) during the 10-yr study. Overall, the new model should be more accurate than those based on the older straight line model. Mills Branch was used as the model gate because it is located farther downstream near the oligohaline edge of the estuary and was more inclusive of upstream hydrologic contributions than USGS gauging stations that historically have been used for regulatory-based assessments of nutrient loadings to the Neuse Estuary (e.g., DiPiero et al. 1994; NC DENR 1999; Qian et al. 2000; Fig. 1). It also included the flow from a major groundwater discharge area located in the vicinity of New Bern and the oligohaline edge of the estuary, important because subsurface flow contributes  $\sim 50\%$  of the total Neuse flow during low-flow conditions (Spruill 1997; Weaver 1998). In addition, the location for the new gate includes input from where the Castle Hayne aquifer is exposed to the land surface and comes into direct contact with the Neuse River (Winner and Coble 1996; Weaver 1998). The Castle Hayne aquifer was determined to be the source of a large

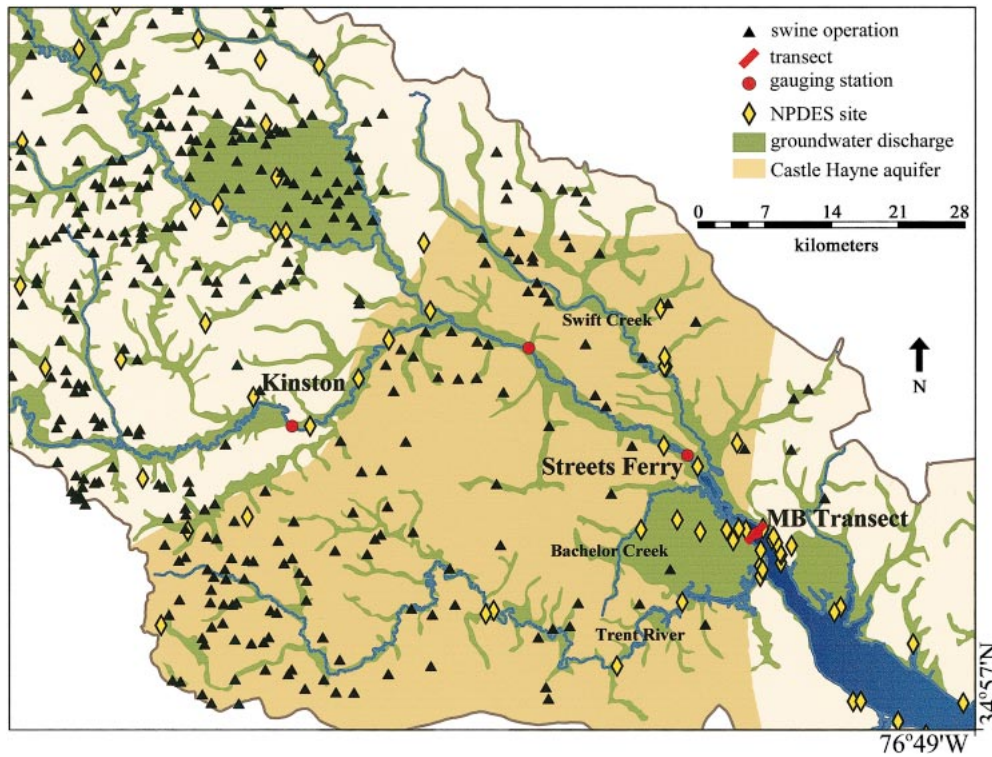


Fig. 2. Map showing hydrologic inputs to the Neuse River and Estuary, including major groundwater discharge areas and the Castle Hayne aquifer, in the Mills Branch (MB) model gate area near the oligohaline edge of the Neuse Estuary under average flow conditions. Map also shows the close proximity of swine CAFOs and National Pollution Discharge Elimination System sites.

increase in discharge between Kinston and the area near the confluence with Swift Creek under low-flow conditions (Weaver 1998; Fig. 2).

The fitted segmented regression model was developed using nonlinear least squares to estimate the coefficients for the low- and high-flow segments and the unknown change-point (Qian and Richardson 1997). The resulting model ( $r^2 = 0.91$ ), with an estimated change-point at Kinston of  $155 \text{ m}^3 \text{ s}^{-1}$ , is described by Eqn. 1 and 2.

$$\text{Kin} < 155 \text{ m}^3 \text{ s}^{-1}:$$

$$\text{MB} = 192.117 + 0.154846 \times \text{Kin} \quad (1)$$

$$\text{Kin} \geq 155 \text{ m}^3 \text{ s}^{-1}:$$

$$\text{MB} = -27.364053 + 1.570529 \times \text{Kin} \quad (2)$$

Plots comparing the measured USGS flow values at Kinston, the measured ADCP values at Mills Branch, and the model-predicted values at Mills Branch by date are shown in Fig. 3. Daily flow estimates at Mills Branch were then generated over the study period by applying the segmented linear regression model to the long-term flow data set available from the USGS gauging station at Kinston.

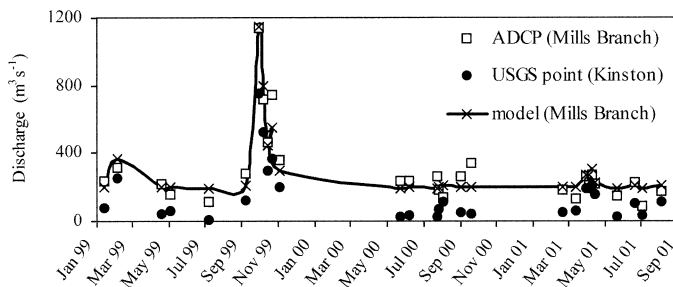


Fig. 3. Comparison of discharge from the fixed-point USGS station at Kinston (closed circles) and empirical measurements at Mills Branch (open squares) obtained with cross-river–transect ADCP, by date. The flows predicted from the segmented linear regression model developed from ADCP data are also shown (solid line). Model-predicted points are connected for visual clarity, with no intent to portray as continuous data.

**Nutrient loadings**—The modeled daily flow at MB was subsequently coupled with nutrient concentration data to estimate TP and TN daily loadings. Mills Branch was a desirable location for the loading gate to the estuary, where the incorporation of upstream contributions to the estuary is most comprehensive with limited estuarine influence. Routine nutrient sampling at this site was not initiated until 1999, and this limitation necessitated use of one additional nutrient data source and development of modeled transfer functions to construct a 10-yr data set for the calculation of a loading trend at Mills Branch. Therefore, monthly midchannel nutrient data from the state environmental agency at a Streets Ferry station, ~10 km upstream from Mills Branch, were used to supplement our data collected at Mills Branch. His-

torically, the state data set was coupled with direct flow measurements from upstream USGS gauging stations, or with flow models based on these stations, to estimate nutrient loadings (e.g., Thut 1980; NC DENR 1999; Stow et al. 2001).

It was determined from PROC UNIVARIATE *t*-tests (SAS Institute 1999) that TP concentrations from these two locations (Mills Branch and Streets Ferry) collected over the same period (January 1999–December 2001) were not statistically different. Thus, the January 1993–1998 Streets Ferry data and the 1999–2003 Mills Branch data were pooled to estimate the approximate decadal trend in TP loading (January 1993–June 2003). However, the TN data from the two locations collected during the 1999–2001 period were statistically different ( $p < 0.05$ ). Therefore, differences between TN concentrations from Streets Ferry and Mills Branch during 1999–2001 were modeled with PROC ARIMA (SAS Institute 1999) to produce a transfer function (Brocklebank and Dickey 2003) that was then used to backcast TN concentrations at Mills Branch for January 1993–December 1998. Specifically, we calculated the percent difference ( $P$ ) between the two sites,  $P = (X - Y)/X$ , where  $X$  represents the observed concentration at Streets Ferry and  $Y$  represents the concentration at Mills Branch. We determined  $P$  from the data (available in 1999–2001) on  $X$  and  $Y$  and used the relationship  $Y = (1 - P)X$  to impute a value of  $Y$  from the observed  $X$  when  $Y$  was missing. Because  $P$  might not be a constant function over time, we fitted a model to  $P$  over the period when  $X$  and  $Y$  data were available. We then reversed the time order of those data so that a “forecast” would actually move backward in time from the first observed available  $P$ . We refer to these forecasts from the reverse-ordered data as “backcasts.” After considering several models, we selected the estimated general least squares model for the  $P$  data to account for autocorrelation, with a period 12 sine wave plus ARIMA errors. In this way, the direct measurements available from Mills Branch from January 1999 to June 2003 were then coupled with the backcasted data for use with the flow estimates to estimate TN loadings and the associated trend analysis. It should be noted that we found no significant trend in flow over the decadal study, and very low correlation between flow and TN concentration (Mills Branch station:  $r^2 = 0.07$  for all years;  $r^2 = 0.17$ , yearly mean) and did not use flow-adjusted concentrations to estimate nutrient loadings because flow-adjusted concentration models have explained only ~35–40% of the variance in nitrogen concentrations in the lower Neuse near the estuary (Stow and Borsuk 2003).

*Data management and statistical analyses*—Data were analyzed with Statistical Analysis System software (SAS—PC SAS version 8.01; SAS Institute 1999). The data matrix contained >135,000 observations on physical, chemical, and biological variables from >350 sampling periods, including more than ~4,030 records on nutrient concentrations.

Time series models for the nutrient data were created with PROC ARIMA, a parametric autoregressive, integrated, moving average modeling technique that assumes normality in distribution and allows strong statistical evaluation of seasonal and linear trends (SAS Institute 1999). Analyses were

completed with and without extreme values, which did not significantly influence the trends in the data. Various models have been used in previous work to assess nutrient trends in the Neuse and other estuaries, such as nonparametric models (e.g., Berryman et al. 1988; Reckhow et al. 1992; Qian et al. 2000) or dynamic regressions (e.g., Lamon et al. 2004), which allow the magnitudes of the seasonality terms and the trend slopes to change over time. The modeling approach used here combined the parametric PROC ARIMA with some nonparametric features. PROC ARIMA was used rather than seasonal trend decomposition (STL) using loess (locally weighted regression; Cleveland et al. 1990) because loess is a regression technique that uses local smoothing and does not take into account the beginning and end of the time series. Seasonal Kendall Tau would divide the data series into 12 segments (months); the data would then be analyzed by shorter segments for trends, which can produce errors and results that have less power. ARIMA, in contrast, has the capability of analyzing forward and backward (i.e., as a moving average; Brocklebank and Dickey 2003).

PROC ARIMA was performed on monthly averages to ensure equal spacing of the data and to preserve the environmental variability observed in nutrient concentrations, which tend to exemplify extreme value distributions. A natural log transformation was then performed on all nutrient data. This gave a more symmetric error distribution and reduced the magnitude of any large errors. A further advantage to the use of transformed data is that percent changes become additive changes on the logarithmic scale, making the data more amenable to statistical evaluation, in that most statistical models are additive in nature. The model-building process consisted of choosing from several model components in addition to the linear trend term to measure long-term secular movement. They included a sine and cosine of period 12 months, which would deliver an exact sine wave of period 12 and harmonic sine and cosine pairs (Brocklebank and Dickey 2003). For example, the first harmonic had period 6 months and went through two cycles per year; the next went through three cycles. The result was a period-12 function that allowed for flexibility in the sine wave, while imposing regular behavior (i.e., a deterministic monotone trend and a repeating seasonal pattern).

The autoregressive and moving average coefficients  $p$  and  $q$ , respectively, were obtained by analyzing the normal, partial, and inverse autocorrelation functions. The  $p$  and  $q$  terms were included so that the model used data from the previous month and the same month from the previous year. The final model was selected by minimizing the Akaike information criterion (AIC) and checking that the residuals were random, or white noise. This method ensured that each parameter was individually tailored to select the model with the best fit. Among the models considered were some with seasonal autoregressive terms, thus allowing local departures from the linear trend and seasonal pattern. Autoregressive terms were added at lags 1 and 12 in a seasonal multiplicative model (Brocklebank and Dickey 2003). The lag 1 term considered the previous month's deviation from that regular pattern and adjusted the forecast up or down accordingly. The lag 12 term made a similar adjustment on the basis of the deviation observed for the same month in the last year. In this way,

Table 2. Comparison of trend results from ARIMA (parametric) and seasonal Kendall Tau (nonparametric) models. Bold indicates significance.

Nutrient	Years	Trend <i>p</i> value	Trend value	AIC†	Change in units	Unit	Model
ARIMA							
TP	93–03	<b>0.036**</b>	−0.0022	40.1	−26	μg	$p=1$ ; time cos cos2 sin sin2
TN	94–03	<b>0.027**</b>	−0.0028	−125.1	−272	μg	$p=(1)(12)$ ; time cos cos2 sin sin2
NH <sub>4</sub> <sup>+</sup>	93–03	<b>&lt;0.0001***</b>	0.0149	240.8	49	μg	$q=1$ ; time cos cos2 sin sin2
NO <sub>3</sub> <sup>−</sup> +NO <sub>2</sub> <sup>−</sup>	93–03	0.403	0.068	380.8	23	μg	$p=(1)(12)$ ; time cos cos2 sin sin2
TKN	93–03	0.102	−0.0019	−107.5	−179	μg	$p=1$ $q=1$ ; time cos cos2 sin sin2
SS	93–03	<b>0.011**</b>	−0.0023	65.6	−2	mg	$p=1$ $q=1$ ; time cos cos2 sin sin2
SRP	93–03	0.491	−0.0020	203.0	−5	μg	$p=(1)(12)$ ; time cos cos2 sin sin2
Chl <i>a</i>	93–03	0.533	0.0014	195.7	3	μg	$p=1$ ; time cos sin
TN:TP‡	93–03	0.513	0.0013	43.8	3	—	$p=1$ $q=1$ ; time cos cos2 sin sin2
TP load	93–03	0.220	−0.0016	12.9	−1.5×10 <sup>4</sup>	kg	$p=1$ $q=1$ ; time cos cos2 sin sin2
TP load	93–99	0.963	0.0001	31.0	704	kg	$q=2$ ; time cos sin
TN load	93–03	<b>0.017**</b>	−0.0026	−12.2	−2.5×10 <sup>5</sup>	kg	$p=1$ ; time cos cos2 sin sin2
TN load	93–99	0.313	−0.0019	3.1	−1.3×10 <sup>5</sup>	kg	$p=1$ ; time cos sin
		<i>p</i> value	Tau statistic	Slope statistic			
Seasonal Kendall Tau							
TP	93–03	<b>0.099*</b>	−0.2437	−2.4	−25	μg	
TN	94–03	<b>0.027**</b>	−0.4559	−35.8	−325	μg	
NH <sub>4</sub> <sup>+</sup>	93–03	<b>0.004***</b>	0.5361	4.1	42	μg	
NO <sub>3</sub> <sup>−</sup> +NO <sub>2</sub> <sup>−</sup>	93–03	0.424	0.0741	0.1	1	μg	
TKN	93–03	<b>0.020**</b>	−0.4524	−26.9	−272	μg	
SS	93–03	<b>&lt;0.0001***</b>	−0.2943	−0.2	−2	mg	
SRP	93–03	0.236	−0.1228	−0.4	−4	μg	
Chl <i>a</i>	93–03	0.454	0.0565	0.2	2	μg	
TN:TP‡	93–03	0.564	−0.0873	−0.2	−2	—	
TP load	93–03	0.268	−0.1333	−1,181	−1.2×10 <sup>4</sup>	kg	
TP load	93–99	0.763	−0.0397	−421	−2,946	kg	
TN load	93–03	<b>0.012**</b>	−0.3926	−30,461	−3.2×10 <sup>5</sup>	kg	
TN load	93–99	0.194	−0.1905	−29,298	−2.1×10 <sup>5</sup>	kg	

† Akaike information criterion.

‡ Molar ratio.

\*  $p \leq 0.1$ \*\*  $p \leq 0.05$ .\*\*\*  $p \leq 0.005$ .

the trend and seasonal influence become more flexible. The resulting model created with the ARIMA method represented a linear combination of the past values of a given nutrient, past errors (also called shocks or innovations), and current and past values of other time series (SAS Institute 1999).

The parametric statistical modeling approach used here included some nonparametric features, which provided a higher level of power with the parametric method than could have been achieved with a nonparametric test alone. The models were evaluated to ensure that they were reasonable (including examination of AIC, chi-square residuals for autocorrelation, etc.; see Table 2 for the model and AIC associated with each model formation for the principal set of decadal data). Although parametric models are more powerful if the assumptions are correct, assumption failure can cause problems in some aspects of inference (Brocklebank and Dickey 2003). Nonparametric models (e.g., Qian et al. 2000) guard against failure of some assumptions at the expense of sacrificing some power to detect effects when they are actually there. In recognition of these trade-offs, we also

analyzed the primary data set with the seasonal Kendall Tau modeling approach (Reckhow et al. 1992) for comparison. On the basis of the preferred methodology in the literature and because the seasonal Kendall Tau assigns ranks, we used the median statistic when analyzing the monthly concentration data for each parameter with this approach.

Trend analysis was performed for each of the following parameters in the Neuse data set—concentrations of SS, TP, SRP, TN, NO<sub>3</sub><sup>−</sup>+NO<sub>2</sub><sup>−</sup>, TKN, and NH<sub>4</sub><sup>+</sup>—with the use of monthly means (ARIMA) calculated from the data and with TN and TP loadings summed by month from the daily loadings. The concentration trends were determined from the mesohaline estuary stations; the loading trends were predicted for the Mills Branch site with the NC DENR data from the Streets Ferry station to augment our Mills Branch data. TN loading trends for 1993–2003 were compared with trends from June 1994 to 2003, considering the previously mentioned methodological differences among data sources. Trend analyses were also conducted for the TN:TP molar ratios (June 1994–June 2003,) and Chl *a*. (1) Evaluation of

the entire data set (May 1993–June 2003), which included 10 tropical cyclones, was compared with (2) analysis of this data set, excluding the most intensive hurricane seasons in 1996 and 1999; (3) analysis of the 1993–1999 data to assess effects of an  $\sim 3$ -yr sustained drought (2000–2002); and (4) analysis of the 1993–1999 data set, excluding the 1996 and 1999 hurricanes. (5) Other alterations included separating the decadal data set into upper and lower depth bins. In addition, we examined the influence of the starting point ( $T_{\text{initial}}$ ) on trend analyses for TP concentrations and loadings, TN concentrations and loadings, DO concentrations (depth  $\geq 3.0$  m), and Chl *a* by running the analyses with each of the first 6 months as  $T_{\text{initial}}$ .

All statistical analyses were performed at an  $\alpha = 0.05$  level of significance; significance at  $\alpha \leq 0.10$  was also reported. The null hypothesis of zero slope over time was tested. If the model(s) indicated a significant linear trend, the null hypothesis was rejected, and the trend parameter was used to calculate beginning and end values of the trend and to calculate percent change over the series. All models were run on the log-transformed data and then transformed back to the original units for graphical display. Although the trend analysis plots showing concentrations (i.e., untransformed units) from ARIMA appear linear, they actually were curvilinear; depending on the range of data, this was difficult to visually discern. Transformation bias (i.e., from transforming back to the original scale from the log scale; Spruegel 1983) was not accounted for because such effort would have introduced an unknown error term into the equation; it would have been difficult to add or subtract an unknown quantity of error and evaluate whether the resulting trend was in the correct direction.

Finally, flow was examined as a potential covariate to determine whether it was necessary to adjust for the influence of flow on concentration (i.e., to check whether an observed trend in a response variable might have simply been due to increased or decreased flow). There were no significant trends in either the Kinston flow or the predicted flow at Mills Branch over the study. The flat trend line in flow, considered together with the low correlation of flow-adjusted concentrations at downstream sites (reported in Stow and Borsuk 2003), did not support use of flow-adjusted concentrations (USGS 2001).

## Results

**Climatic conditions**—During this study, the Neuse Estuary was affected by 10 tropical cyclones with high flooding and a sustained 3-yr drought (2000–2002; Fig. 4). Eastern North Carolina was under drought conditions throughout most of summer 1993, followed by precipitation from Hurricane Emily (category 3), which passed near the Outer Banks in late August. Precipitation in 1994 was close to the long-term average (on the basis of the 100-yr annual mean for North Carolina; Southeast Regional Climate Center 2004), followed by a high-precipitation year in late winter through midsummer 1995, setting a 100-yr record for the Piedmont in the upper Neuse watershed (State Climate Office of North Carolina 2004). A second high-precipitation

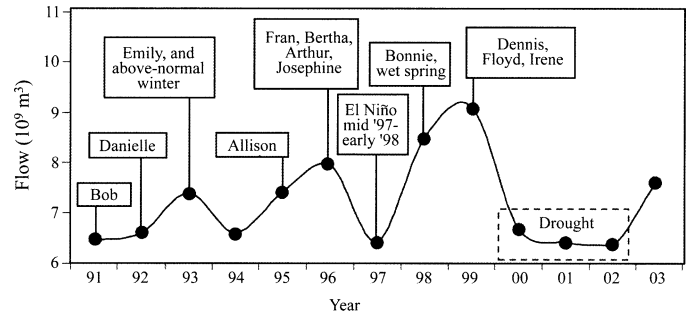


Fig. 4. Annual volume of flow to the Neuse Estuary at Mills Branch during the 1990s to 2003, estimated from daily USGS flow data at Kinston by the segmented linear regression model (names indicate tropical storms). Note that mass water transport at Mills Branch was 15% lower per year during 2000–2002 than during all other years of the study because of a sustained 3-yr drought.

year, 1996, included intense storms, especially Hurricanes Bertha (level 3; early July) and Fran (level 3; early September). Fran was the most severe of the hurricanes in 1993–1996, in part because its path followed the length of the Neuse watershed (Bales and Childress 1996; Bales 2003). Although spring 1998 was a wet period (highest precipitation period on record since 1930 at the Kinston USGS station and double the mean seasonal flow), annual precipitation totals in 1997–1998 approximated the long-term mean. A highly active hurricane season characterized 1999, including Hurricanes Dennis (which struck North Carolina twice), Floyd, and Irene (Burkholder et al. 2004). Of these, Floyd caused a 500-yr flood in some areas of the Neuse basin (Bales 2003).

In marked contrast, the sustained drought affected the study area during 2000–2002 (State Climate Office of North Carolina 2003). This long-term drought was evaluated as the worst sustained by North Carolina in more than 100 yr, and much of the state was declared a federal drought disaster area for agriculture. The precipitation total for 2000–2002 was the fourth lowest on record, and the mid and upper Neuse watershed accrued rainfall deficits equivalent to more than a year's average precipitation (Southeast Regional Climate Center 2004). This sustained drought was followed by an above-average precipitation year in 2003 (State Climate Office of North Carolina 2003), especially for the first 6 months included in this study.

**General environment**—Site-specific and interannual variability in salinity and halocline depth occurred in this ecosystem (Reed et al. 2004). Depending on seasonal precipitation patterns, surface salinities (means  $\pm 1$  SE) varied from  $4.1 \pm 2.3$  to  $12.1 \pm 5.3$  (grand mean, surface waters  $9.3 \pm 0.1$ ) and ranged from 0.0 to 26.8. These variable ranges encompassed conditions reported in other studies of this system (e.g., Pinckney et al. 1997; Glasgow and Burkholder 2000). Analysis of the light attenuation values indicated substantial turbidity in this estuary. High SS values were recorded in the lower water column during winter–spring 1995–1996 ( $25\text{--}30$  mg L<sup>-1</sup>, twofold higher than maxima during other years), and reflected both sediments from runoff

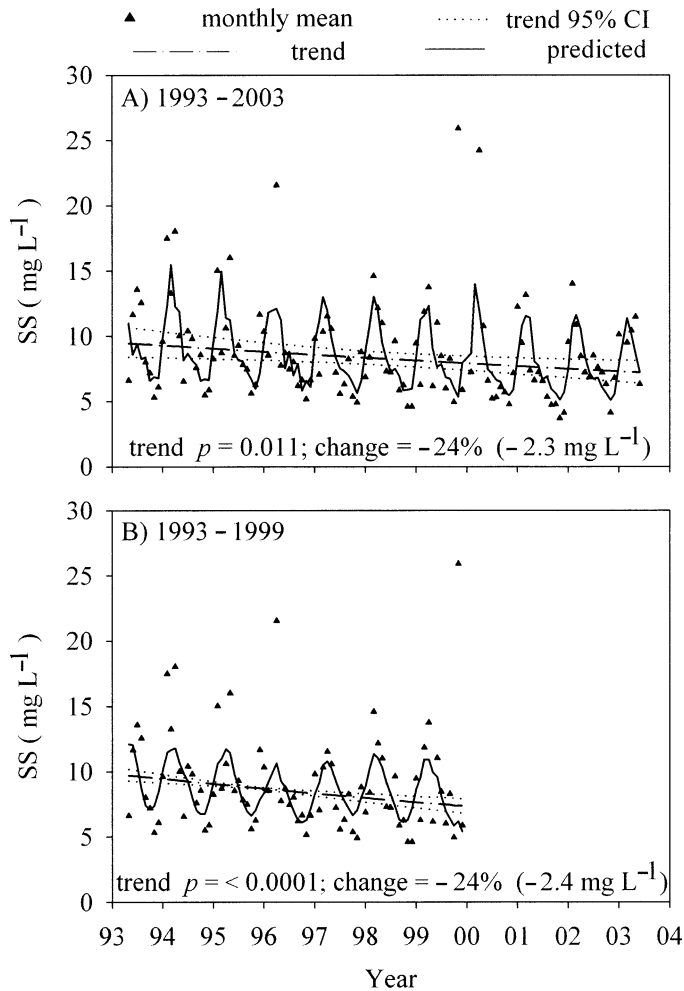


Fig. 5. Suspended solids concentrations in the Neuse Estuary, considering the six mesohaline stations collectively (A) over the decadal study (1993–2003;  $n = 121$  months; 4,015 samples) and (B) excluding the period post-1999 with the sustained drought ( $n = 79$  months; 2,664 samples). Linear trend lines (dashed) were calculated by least squares means for all sites. Also shown are the 95% confidence limits (dotted lines), predicted (model-fitted) values (solid line),  $p$  value (probability associated with the linear slope), percent change in SS, and corresponding change in SS concentration (in parentheses).

and a massive nontoxic dinoflagellate bloom. High SS was also measured after major storms (e.g., up to  $22 \text{ mg L}^{-1}$  post-Hurricane Fran vs.  $\sim 5.5 \pm 1.0 \text{ mg L}^{-1}$  pre-Fran). Overall, SS concentrations decreased by 24% over the study (1993–2003,  $p = 0.01$ ) or predrought years 1993–1999 ( $p < 0.0001$ ; Fig. 5).

**Dissolved oxygen**—During warmer months in all years, hypoxia/anoxia occurred in the lower third of the water column at all six mesohaline sites, in violation of the state standard for fish health ( $\geq 5 \text{ mg DO L}^{-1}$ ; NC DEHNR 1996a; Fig. 6). Density stratification with formation of salt wedges exacerbated bottom-water hypoxia. Runoff associated with high precipitation in late winter and early spring, with contributed organic loadings, likely led to the bottom-water ox-

xygen sags that were sometimes observed early in the growing season (mid-March through early April). Hypoxia/anoxia in more than the lower third of the water column occurred infrequently in localized areas during most years. Exceptions occurred during July 1995, several days in early October 1995, and in localized areas for short periods following hurricanes. During late summer 1996, Hurricane Fran caused loading of millions of liters of raw sewage, swine industry wastes, and swamp waters into the Neuse (NC DEHNR 1996b), resulting in complete water column anoxia and fish kills in upstream freshwater segments, but not in the mesohaline estuary (Burkholder et al. 1999). In contrast, the higher flood volume and dilution of Hurricanes Dennis, Floyd, and Irene resulted mostly in short-term bottom-water hypoxia; no major fish kills were reported (Burkholder et al. 2004).

Trend analysis indicated that for all mesohaline sites considered collectively, water column DO decreased (total water column by 9%,  $p = 0.070$ ; depth  $\geq 0.5 \text{ m}$  by  $-11\%$ ,  $p = 0.047$ ) and bottom-water DO significantly decreased (depth  $\geq 3 \text{ m}$  by  $-19\%$ ;  $p = 0.040$ ) over the decadal study (Table 3; Fig. 6A–C). However, there was no significant change in DO when post-1999 years, including the sustained drought, were excluded from the analysis (1993–1999,  $p = 0.372$ – $0.501$ ; Fig. 6D–F). For the six sites considered separately ( $N = 121$  dates with  $\geq 2,170$  records per site), overall water column DO concentrations significantly decreased at SLC (by 15%,  $p = 0.032$ ), KEN (by 10%, borderline significance at  $p = 0.056$ ), and CLP (by 14%,  $p = 0.020$ ).

**Phytoplankton**—Phytoplankton biomass as Chl  $a$  generally was  $15$ – $25 \mu\text{g L}^{-1}$  over the decadal study (grand mean  $20.3 \pm 0.3 \mu\text{g L}^{-1}$ ), indicative of eutrophic conditions (Mallin 1994; Bricker et al. 1999; Fig. 7). In the mesohaline estuary, trend analysis indicated no significant change in Chl  $a$  over the decadal study, with or without the 1996 and 1999 hurricane seasons, except at KEN (61% increase from 1993 to 2003,  $p = 0.038$ ,  $n = 120$ ; 693 samples), where several dense winter–early spring blooms occurred. During the last 4 yr of the study, including the sustained drought, analysis of the Chl  $a$  data showed a significant increasing trend (75% increase from 2000 to 2003;  $p = 0.0001$ ,  $n = 40$ ; 1,348 samples), indicating enhancement of algal blooms during sustained drought conditions, with high Chl  $a$  especially in winter 2001–2002.

Winter blooms of dinoflagellates and cryptophytes were documented during 5 of the 10 yr (Figs. 8, 9). Dominant taxa included the dinoflagellates *Heterocapsa rotundata*, *Heterocapsa triquetra*, and toxigenic *Prorocentrum minimum*. Lower densities of cryptophytes [*Chroomonas marina* (Büttner) Butcher and *Ochromonas minima* Thronsen] were often associated with dinoflagellate-dominated blooms. During the first winter of the study, a major bloom consisting mostly of *P. minimum* occurred in the mesohaline estuary, with Chl  $a$  concentrations of  $>100 \mu\text{g L}^{-1}$  for 2 months. Maximal Chl  $a$  levels were sustained from January through April ( $150$ – $180 \mu\text{g L}^{-1}$ ), with  $\leq 300 \mu\text{g L}^{-1}$  in March. They occurred during high-precipitation winters and have been correlated with TN inputs (e.g., Mallin 1994). Similar blooms occurred in the subsequent two winters: winter

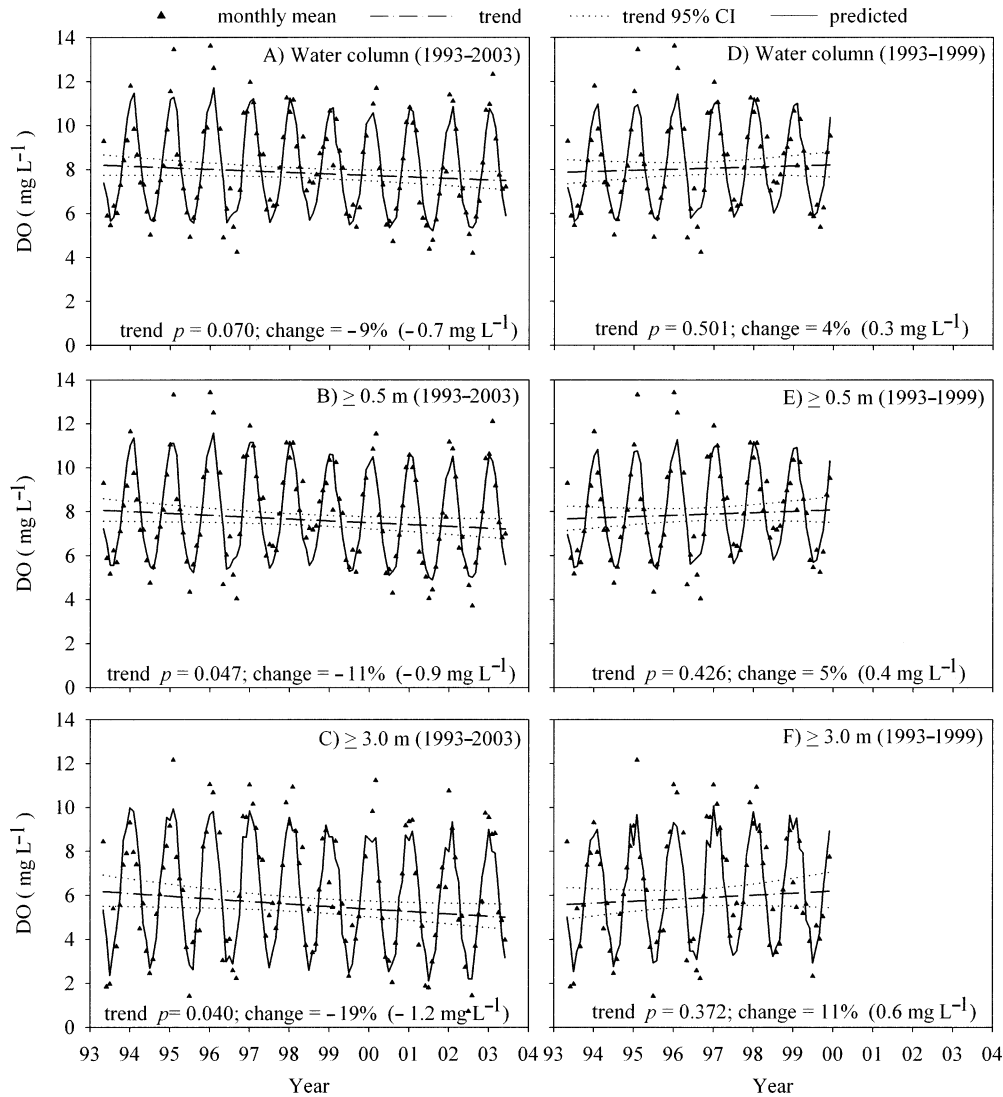


Fig. 6. Dissolved oxygen concentrations in the Neuse Estuary over the decadal study considering the six mesohaline stations collectively, and plotted separately for depth  $\geq 0.5$  m and depth  $\geq 3.0$  m. Linear trend lines (dashed) were calculated by least squares means for all sites. Also shown are the 95% confidence limits (dotted lines), predicted (model-fitted) values (solid line),  $p$  value (probability associated with the linear slope), percent change in DO, and corresponding change (in parentheses) in concentration (A–C) over the decadal study (1993–2003) as (A) total water column ( $n = 121$  months; 13,192 samples), (B) depth  $\geq 0.5$  m ( $n = 121$  months; 11,171 samples), (C) depth  $\geq 3.0$  m ( $n = 121$  months; 2,106 samples); (D–F) excluding the period post-1999 with the sustained drought (1993–1999) as (D) total water column ( $n = 79$  months; 7,585 samples), (E) depth  $\geq 0.5$  m ( $n = 79$  months; 6,547 samples), and (F) depth  $\geq 3.0$  m ( $n = 79$  months; 1,322 samples).

1998–1999 (three-species equal mix of *P. minimum*, *H. triquetra*, and *H. rotundata*) and winter 2001–2002 (Fig. 8). Beyond the monthly winter sampling regime, additional sampling documented Chl *a* concentrations as high as  $800 \mu\text{g L}^{-1}$  (28 March 2002) in bloom patches. Blooms of dinoflagellates did not form in winters following the highest intensity hurricane seasons, probably reflecting washout and burial of cysts during these major storms. Summer blooms of toxigenic dinoflagellates (*Pfiesteria* spp.; Burkholder et al. 1999, 2005; Brownie et al. 2002) were common in years with few or no tropical cyclones (1991–1995); smaller

blooms occurred after the 1996 hurricane season in 1997–1998, and no toxic *Pfiesteria* activity was documented during or after hurricane year 1999 (Burkholder et al. 2004).

**Nutrient concentrations**—TP and TN concentrations (grand mean for the mesohaline estuary sites collectively,  $120 \pm 1 \mu\text{g TP L}^{-1}$ , range 10–690  $\mu\text{g TP L}^{-1}$ ;  $890 \pm 5 \mu\text{g TN L}^{-1}$ , range 70–5,295  $\mu\text{g TN L}^{-1}$ ) indicated eutrophic conditions, as expected (Figs. 9, 10A). Throughout the study, TN<sub>i</sub> and TP increased during periods of higher precipitation, as reported in other work on the Neuse (e.g., Christian et al.

Table 3. Depth-dependent trends in dissolved oxygen (DO) concentrations in the mesohaline Neuse Estuary study area. Data were log transformed over a period of 121 months. Bold indicates significance.

Depth (m)	Total samples	Trend <i>p</i> value	Change over series (%)	Change over series (mg DO L <sup>-1</sup> )
All depths	13,192	<b>0.070*</b>	-9	-0.70
≥0.5	11,171	<b>0.047**</b>	-11	-0.85
≥1.0	9,319	0.163	-12	-0.90
≥1.5	7,388	<b>0.090*</b>	-13	-0.95
≥2.0	5,568	0.144	-13	-0.88
≥2.5	3,775	0.184	-13	-0.86
≥3.0	2,106	<b>0.040**</b>	-19	-1.17

\*  $p \leq 0.1$ .

\*\*  $p \leq 0.05$ .

1991; Rudek et al. 1991; Mallin et al. 1993). During warmer months,  $\text{NH}_4^+$  significantly increased in the bottom waters compared with colder months (Student's *t*-test: total water column,  $p = 0.0002$ ; lower water column,  $p < 0.0001$ ; data not shown), likely associated with decomposition and regeneration processes (Glibert 1988) and with salt wedge and hypoxic conditions in the enriched reducing environment of the lower water column (Boyer et al. 1994).

Trend models indicated that TP concentrations decreased over the study, with or without inclusion the 1996 and 1999 hurricane seasons (1993–2003 + hurricane data, 23% decrease,  $p = 0.036$ ; Table 4; Fig. 9A), especially in the lower water column (-29%,  $p = 0.001$ ). The trend was also significant when the two most severe hurricane periods and the post-1999 years with the sustained drought were excluded from analysis (1993–1999;  $p = 0.0046$ ; Table 4). However, the trend was not significant when the analysis excluded only the post-1999 data ( $p = 0.201$ ; Table 4; Fig. 9B). Total N concentrations significantly decreased over the decadal period, considering the mesohaline stations collectively (1993–2003 or 1994–2003;  $p = 0.069$  and  $0.027$ , respectively), but when post-1999 years were excluded, there was no significant trend (e.g., Tables 4, 5; Fig. 10A,D). With the parametric approach, there also were no significant trends in TKN concentrations over the 10-yr study, with or without consideration of hurricane periods or the sustained drought. Analyses with and without periods with Hurricanes Fran and Floyd generally yielded similar N trends. Among N species,  $\text{NO}_3^-$  composed  $30 \pm 1\%$  (grand mean  $\pm 1$  SE) of the total  $\text{N}_i$  concentration available throughout the water column in the mesohaline estuary, but only  $\sim 5\%$  of the TN. In association with precipitation events,  $\text{NO}_3^-$  increased by  $\sim 120\%$  (maximum concentrations at  $\sim 500 \mu\text{g L}^{-1}$ ) throughout the study area. However, the  $\text{NO}_3^-$  trends for 1993–2003 and 1993–1999 were not significant ( $\pm$ hurricane periods,  $\pm$ the sustained drought;  $p > 0.10$ ; Fig. 10B,E). Although trend analyses for most parameters were sensitive to the beginning period used (Table 6), the majority of the analyses that used different months as  $T_{\text{initial}}$  supported the trends described above. The trend analysis for TN was most sensitive to  $T_{\text{initial}}$  (Table 6). Nevertheless, 50% (67%, if the trend with  $T_{\text{initial}}$

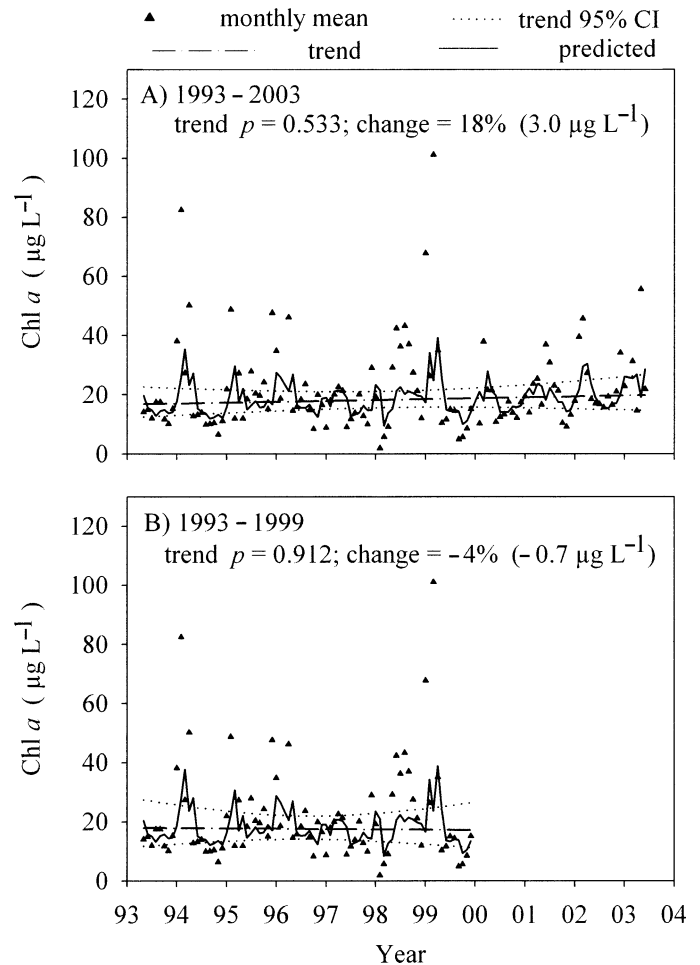


Fig. 7. Phytoplankton Chl *a* concentrations in the Neuse estuary considering the six mesohaline stations collectively (A) over the decadal study ( $n = 121$  months; 3,993 samples), and (B) excluding the period post-1999 with the sustained drought ( $n = 79$  months; 2,645 samples). Linear trend lines (dashed) were calculated by least squares means for all sites. Also shown are the 95% confidence limits (dotted lines), predicted (model-fitted) values (solid line), *p* value (probability associated with the linear slope), percent change in Chl *a*, and corresponding change (in parentheses) in concentration.

= October 1994 is considered significant at  $p = 0.059$ ) of the analyses with each month from June to November 1994 as  $T_{\text{initial}}$  yielded significant negative trends in TN concentrations (Table 6).

Ammonium generally was  $< 40 \mu\text{g L}^{-1}$  during low-precipitation periods in the first half of the study, increasing to  $\sim 250 \mu\text{g L}^{-1}$  in the lower water column during early summer precipitation events, then to  $250 \mu\text{g L}^{-1}$  and  $> 600 \mu\text{g L}^{-1}$  in the upper and lower water columns, respectively, following high-precipitation periods in summer. There was a striking increase in  $\text{NH}_4^+$  concentrations over the decadal study (1993–2003; Table 4; Fig. 10C). Thus, overall, coincident with the decline in TN concentrations over that period,  $\text{NH}_4^+$  concentrations increased significantly in the mesohaline estuary (503% increase from 1993 to 2003,  $p < 0.0001$ ). The increasing trend was also significant when the

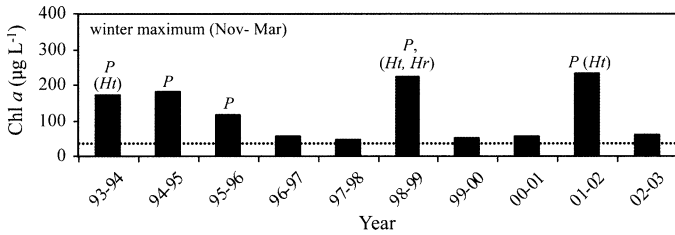


Fig. 8. Chl *a* maxima during winter blooms from the routine monthly sampling regime, indicating dominant taxa as *P* (*Prorocentrum minimum*), *Ht* (*Heterocapsa triquetra*), and *Hr* (*Heterocapsa rotundata*). These taxa made up more than 90% of the total phytoplankton cells. Corresponding cell densities were as follows: 1993–1994,  $6.10 \times 10^4$  *P. minimum* cells  $\text{ml}^{-1}$  and  $0.9 \times 10^3$  *H. triquetra* cells  $\text{ml}^{-1}$ ; 1994–1995,  $1.02 \times 10^5$  *P. minimum* cells  $\text{ml}^{-1}$ ; 1995–1996,  $1.90 \times 10^4$  *P. minimum* cells  $\text{ml}^{-1}$ ; 1998–1999,  $1.15 \times 10^4$  cells  $\text{ml}^{-1}$  each of *P. minimum*, *H. triquetra*, and *H. rotundata*; and 2001–2002,  $3.15 \times 10^4$  *P. minimum* cells  $\text{ml}^{-1}$  and  $1.90 \times 10^4$  *H. triquetra* cells  $\text{ml}^{-1}$ . The dashed line represents North Carolina's Chl *a* standard for acceptable water quality ( $<40 \mu\text{g L}^{-1}$ ; NC DENR 1996a).

post-1999 years with the sustained drought were excluded from the analysis (increase of 188% from 1993 to 1999,  $p = 0.015$ ; Table 4; Fig. 10F).

Molar TN:TP ratios were similar among sites and were generally  $<30$  (grand mean from June 1994 to June 2003,  $21.3 \pm 0.2$ ,  $N = 108$  dates; 3,547 records), indicating a transition between potential N and potential P limitation (Geider and La Roche 2002). TN:TP ratios were lowest in the summer season (mean  $16.6 \pm 0.2$ ). Higher TN:TP ratios were observed in spring (March–April; seasons statistically determined as in Glasgow and Burkholder 2000), with maxima typically at 36 to 48 (mean  $25.0 \pm 0.3$ ). Winter TN:TP ratios were also high (mean  $31.1 \pm 0.7$ ). In addition, there were significant decreasing trends for TN:TP ratios in the mesohaline estuary (1994–2003; overall, 22% decrease,  $p = 0.019$ ). The data support an increased potential importance of phosphorus in controlling phytoplankton production, as reported in other studies (Rudek et al. 1991, down-estuary; Qian et al. 2000). However, there was no significant trend in TN:TP molar ratios when the post-1999 years with the sustained drought were excluded from analysis, with or without the hurricane periods ( $p > 0.3$ – $0.9$ ). Moreover, during much of the year, elevated N and P indicated enriched conditions for both nutrients in supporting phytoplankton growth (Hecky and Kilham 1988).

Generally, results compared well between the parametric and nonparametric approaches, with the exception of TKN (Table 2). Although the ARIMA detected no significant trend in TKN over the decadal data set, the seasonal Kendall Tau detected a significant decreasing trend ( $p = 0.020$ ). Changes in concentration units and  $p$  values for the other parameters were similar, except that the trend for TP had a lower level of significance, whereas the trend for SS had a higher level of significance with the seasonal Kendall Tau. The overall conclusions of decreasing or increasing trends were the same.

**Nutrient loadings**—Over the decadal study, loadings to the oligohaline estuary were estimated at  $9.7 \times 10^5$  kg TP

$\text{yr}^{-1}$  and  $9.6 \times 10^6$  kg TN  $\text{yr}^{-1}$ . There was no significant trend in TP loading from 1993 to 2003, with or without the 1996 and 1999 hurricane seasons or the sustained drought post-1999 (Table 4; Fig. 11A,D). There was a decreasing trend in TN loading (by 28% from 1993 to 2003,  $p = 0.017$ ) with or without the 1996 and 1999 hurricane seasons (Table 4; Fig. 11B), but TN loading did not change significantly when the sustained drought was excluded from the analysis ( $p = 0.313$ ; Table 4; Fig. 11E). A comparison of loading trends between the ARIMA and seasonal Kendall Tau approaches yielded similar  $p$  values and conclusions (Table 2). The TN loading trend was highly sensitive to the beginning period used (Tables 4–6; Fig. 11C,F); analysis from June 1994 to June 2003 yielded no significant trend ( $p = 0.127$ ).

## Discussion

Assessment of the effects of cultural eutrophication on aquatic ecosystems fundamentally requires consideration of temporal scale and sources of variation for reliable evaluation of changes in TN and other nutrient inputs over time (Phase I of Cloern's [2001] evolving conceptual model of coastal eutrophication problems). The detailed, decadal data set from this study contributes new information about climatic effects on trends in nutrient concentrations and loadings to a major eutrophic estuary, with implications about shifts in various nutrient sources, progress in management strategies to control them, and future efforts needed to reduce eutrophication. Our statistical approach used a moving average and incorporated harmonics and covariance terms designed to address seasonal aspects of the data set, as well as the potential influences of autocorrelation. Nevertheless, interpretations are complicated, as in other studies (e.g., Gallegos et al. 1992; Jassby 1998), by the sensitivity of trend analysis to the disproportionate influence of points at the beginning and end of the evaluation period and the potential variation from one period to the next, which can be large in shallow, wind-driven systems such as the Neuse Estuary.

It has been estimated that pristine temperate zone ecosystems in North America and Europe export 75–230 kg N  $\text{km}^{-2} \text{yr}^{-1}$  (median 133 kg N  $\text{km}^{-2} \text{yr}^{-1}$ ; NRC 2000), but human activity is estimated to have increased the N flux (e.g., by  $>4$ -fold in the Mississippi River, 8-fold in rivers of the northeastern United States, and 11-fold in rivers draining to the North Sea; Howarth et al. 2002). Over the past decade, the Neuse had moderate nutrient loadings ( $9.7 \times 10^5$  kg TP  $\text{yr}^{-1}$  and  $9.6 \times 10^6$  kg TN  $\text{yr}^{-1}$ , or, averaged over the watershed from the headwaters to New Bern, 86 kg P  $\text{km}^{-2} \text{yr}^{-1}$  and 850 kg N  $\text{km}^{-2} \text{yr}^{-1}$ ) compared with other systems. As examples, the average N flux per area for waters flowing into the North Atlantic Ocean (Howarth et al. 1996) was estimated at 486 kg N  $\text{km}^{-2} \text{yr}^{-1}$ , with high fluxes along the coasts of northwestern Europe (1,300–1,450 kg N  $\text{km}^{-2} \text{yr}^{-1}$ ), the northeastern United States (1,070 kg N  $\text{km}^{-2} \text{yr}^{-1}$ ), and the Chesapeake Bay (840–1,022 kg N  $\text{km}^{-2} \text{yr}^{-1}$ ). A flux of  $\sim 42$  kg P  $\text{km}^{-2} \text{yr}^{-1}$  was estimated for the main stem upper Chesapeake Bay (Magnien et al. 1992), and 4.5 kg P  $\text{km}^{-2} \text{yr}^{-1}$  for relatively pristine rivers in northern Canada (Howarth et al. 1996). The sensitivity of the poorly flushed

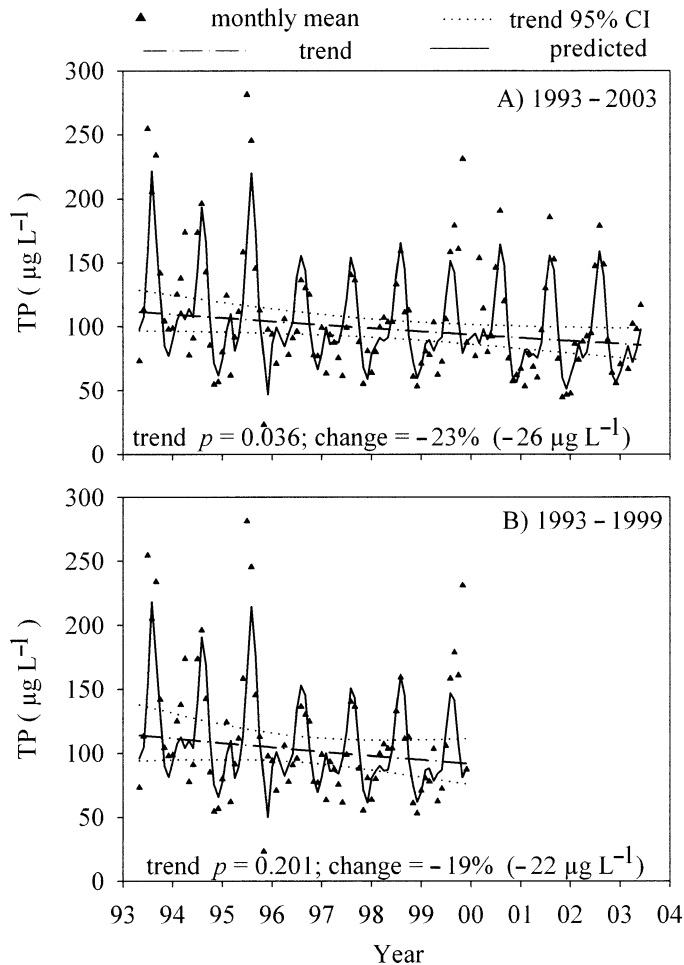


Fig. 9. Total phosphorus concentrations in the Neuse Estuary considering the six mesohaline stations collectively (A) over the decadal study ( $n = 121$  months; 4,006 samples) and (B) excluding the period post-1999 with the sustained drought. Linear trend lines (dashed) were calculated by least squares means for all sites. Also shown are the 95% confidence limits (dotted lines), predicted (model-fitted) values (solid line),  $p$  value (probability associated with the linear slope), percent change in TP, and corresponding change in concentration (in parentheses).

Neuse to only moderate nutrient loading is manifested by the high phytoplankton biomass, extensive bottom-water hypoxia, and massive fish kills that continue to characterize this system, as is true of many other estuaries despite ongoing efforts to reduce nutrient concentrations and loads (NRC 2000).

Climatic changes (droughts, El Niños, hurricanes, wet seasons) have been shown to strongly influence estuarine ecosystems, alone or with interacting anthropogenic effects (e.g., Valiela et al. 1996; Verity 2002). In this study, hurricane-level storms, represented by the two most severe events during the decadal period, had little effect on the outcome of trend analyses for the parameters examined. Others have also reported that, in shallow estuaries frequently disturbed by hurricanes, there can be relatively rapid recovery in water quality and have suggested that estuaries, like stream eco-

systems (Yount and Niemi 1990), can be resilient to such storms (Constanza et al. 1993; Burkholder et al. 2004). In contrast, the sustained 3-yr drought near the end of the study significantly affected the trend analyses.

*Nutrient concentrations*—Although nutrient loading estimates are important for management considerations (e.g., TMDLs imposed by the federal Clean Water Act; NRC 2001), ambient nutrient concentrations are a key variable supporting algal assemblage shifts, excessive algal production, and associated adverse effects of eutrophication (Hecky and Kilham 1988). The strength of the data set contributed by this decadal study lies especially in its extensive nutrient concentration measurements.

Concentrations of TN and TP significantly decreased in the Neuse Estuary, but the decreases were drought related. These findings extend temporally beyond Glasgow and Burkholder (2000) and Qian et al. (2000), who had earlier found that pronounced decreases in P concentrations detected in the upper Neuse River because of the P detergent ban (1988; NC DEHNR 1991) persisted downstream in the estuary. In contrast, nitrate concentrations did not significantly change overall (1993–2003), with or without inclusion of the sustained drought in the analysis, although nitrate had significantly increased over the first half of the study (Glasgow and Burkholder 2000). As a major new finding,  $\text{NH}_4^+$  concentrations dramatically increased in the mesohaline estuary over the 10-yr period, whether the data were analyzed with or without inclusion of major hurricane seasons or the sustained 3-yr drought that occurred near the end of the study. Analysis of other experimental data has suggested that  $\text{NH}_4^+$  is the major nutrient that supports the winter phytoplankton blooms, which contribute the highest algal production in this system (e.g., Fan et al. 2003). Two questions are merited: Is the Neuse unique in this striking ammonium increase? What major sources might be involved?

On the basis of the source data and proximity, we suspected inputs from swine CAFOs as a major  $\text{NH}_4^+$  source. North Carolina's Coastal Plain contains the highest density of swine CAFOs in the United States, known to contribute high ammonia in both leachate and aerosols from effluent spraying and land application waste management practices (Walker et al. 2000). In the North Carolina Coastal Plain,  $\text{NH}_4^+$  is a major component of wet N atmospheric flux and has been increasing over the past two decades (see National Atmospheric Deposition Program data in NC DENR 2001). Walker et al. (2000) estimated that ammonia accounts for ~42% of the N emissions in North Carolina, and suggested that a significant fraction of the N deposited to North Carolina coastal and estuarine waters could be in the form of  $\text{NH}_x$ . They reported that atmospheric emissions in eastern North Carolina from swine CAFOs ( $p < 0.01$ ), fertilizers ( $p < 0.10$ ), and turkey/broiler operations ( $p < 0.05$ ) were significantly higher in 1990–1997 than in 1982–1989; only emissions from non-broiler chickens decreased during 1990–1997 ( $p < 0.01$ ). Furthermore, the increase in average swine emissions accounted for ~84% of the increase in average annual emissions from all sources between the two periods analyzed by Walker et al. (2000). Atmospheric  $\text{NH}_3$  is deposited in surface waters within a ~100-km radius from

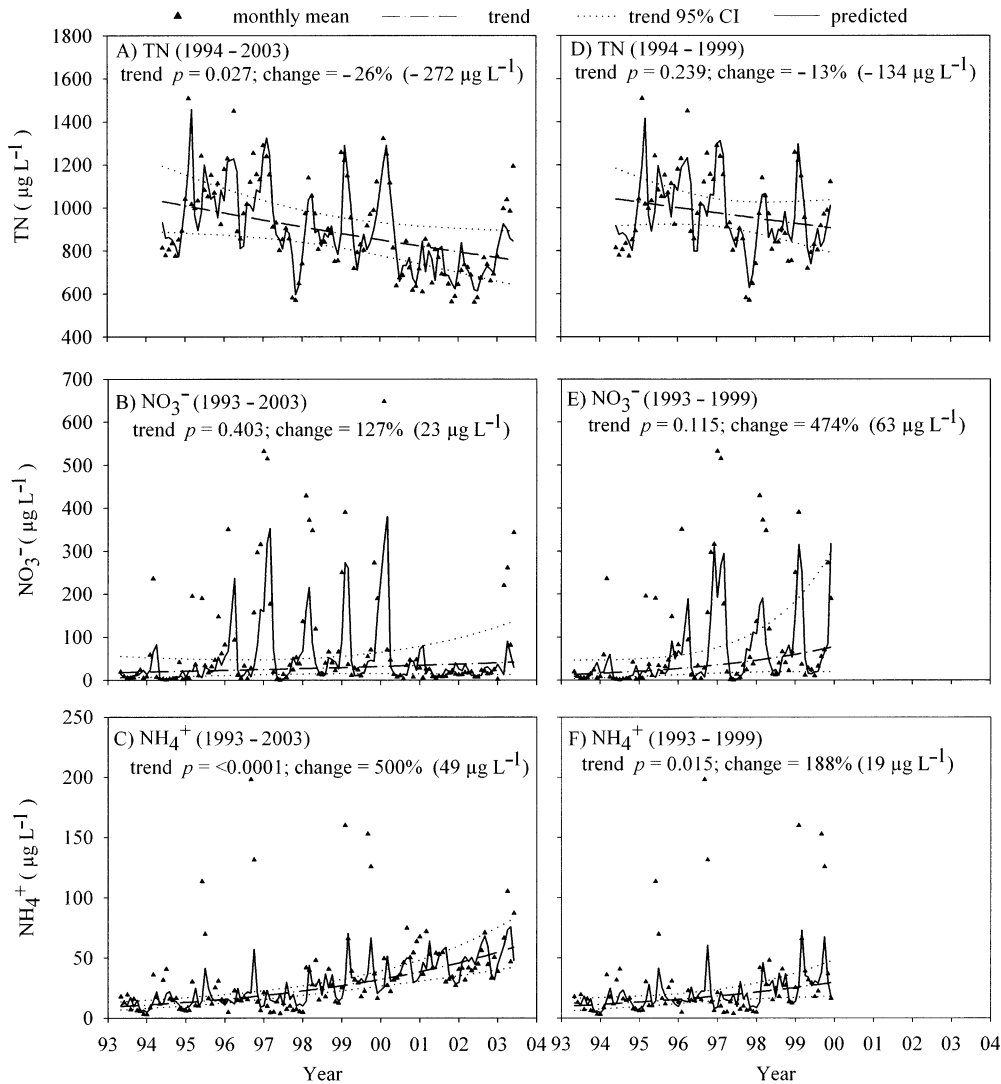


Fig. 10. Nitrogen concentrations in the Neuse Estuary considering the six mesohaline stations collectively (A–C) over the decadal study as (A) TN ( $n = 108$  months; 3,567 samples), (B)  $\text{NO}_3^-$  ( $n = 121$  months; 3,982 samples), and (C)  $\text{NH}_4^+$  ( $n = 121$  months; 3,987 samples) and (D–F) excluding the period post-1999 with the sustained drought as (D) TN ( $n = 66$  months; 2,220 samples), (E)  $\text{NO}_3^-$  ( $n = 79$  months; 2,629 samples), and (F)  $\text{NH}_4^+$  ( $n = 79$  months; 2,650 samples). Note that trends for  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations were based on data from 1993 on, whereas the trend for TN concentrations was based on data from June 1994 on because of methodological differences in data collection prior to June 1994. Linear trend lines (dashed) were calculated by least squares means for all sites. Also shown are the 95% confidence limits (dotted lines), predicted (model-fitted) values (solid line),  $p$  value (probability associated with the linear slope), percent change in the N species, and corresponding change in concentration (in parentheses).

the emission source, and both wet and dry deposition likely are important (Asman and Van Jaarsveld 1992; Aneja et al. 2003). Data from two National Atmospheric Deposition Program stations in eastern North Carolina estimate wet deposition of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  at  $8.8\text{--}13.7 \times 10^{-2} \text{ kg N km}^{-2} \text{ yr}^{-1}$ , although  $\sim 80\%$  of the N was in the oxidized form (EPA 2000b). Dry deposition of particulate  $\text{NO}_3^-$  and  $\text{NH}_4^+$  and gaseous  $\text{HNO}_3$  has been estimated at  $1.5\text{--}3.2 \times 10^{-2} \text{ kg N km}^{-2} \text{ yr}^{-1}$  (EPA 2000a).

We compared our results with data collected for an adjacent river, the Cape Fear, with the use of comparable meth-

ods over a similar period (Fig. 12). This system drains the largest watershed in North Carolina, with 27% of the State's human population and 50% of the swine population ( $\sim 5 \times 10^6$  swine), the latter mostly in the Northeast Cape Fear and Black River tributaries, which lie within the Coastal Plain (Mallin 2000). Ammonium data were examined from four representative Cape Fear stations (Fig. 12). Station Navassa (NAV; GPS coordinates 34.2594N, 77.9877W) is in the main stem Cape Fear River  $\sim 7$  km upstream from its confluence with the Northeast Cape Fear River. This station is oligohaline and mostly influenced by freshwater discharge from

Table 4. Trend analyses in nutrient concentrations and loadings and Chl *a* concentrations, depending on the data source and initial period used, from PROC ARIMA analysis (1993–2003 or 1993–1999, excluding the sustained 3-yr drought). Bold indicates significance.

Period	[TP]	TP load	[TN]	TN load	[NH <sub>4</sub> <sup>+</sup> ]	[Chl <i>a</i> ]
993–2003 (all; +hurricanes, sustained drought)						
<i>p</i> value	<b>0.036**</b>	0.220	<b>0.069*</b>	<b>0.017**</b>	<b>&lt;0.0001***</b>	0.533
% change	–23%	–18%	–21%	–28%	+503%	+18%
<i>n</i> (months)	121	124	120	124	121	121
No. of samples	4,006	3,803	3,948	3,818	3,987	3,993
95% CI	–23.5% to –23.2%	–18.0% to –17.6%	–22.1% to –20.7%	–27.9% to –27.6%	+501% to +506%	+17.3% to +18.2%
1993–2003, excluding 1996, 1999 hurricane periods						
<i>p</i> value	<b>0.005***</b>	0.197	0.280	<b>0.028**</b>	<b>&lt;0.0001***</b>	0.437
% change	–26%	–17%	–16%	–28%	+509%	+21%
<i>n</i> (months)	121	124	120	124	121	121
No. of samples	3,901	3,621	3,842	3,621	3,879	3,886
95% CI	–25.7% to –25.4%	–17.7% to –17.1%	–18.2% to –14.3%	–28.7% to –27.3%	+498% to +520%	20.6% to 22.0%
1993–1999 (+hurricanes, –sustained drought)						
<i>p</i> value	0.201	0.963	0.922	0.313	<b>0.015**</b>	0.912
% change	–19%	+1%	–1%	–15%	+188%	–4.1%
<i>n</i> (months)	79	83	78	83	79	79
No. of samples	2,657	2,556	2,601	2,556	2,650	2,645
95% CI	–19.4% to –19.4%	+0.58% to +1.17%	–1.2% to –1.0%	–14.9% to –14.6%	+188% to +188%	–4.3% to –3.9%
1993–1999 (–hurricanes, –sustained drought)						
<i>p</i> value	<b>0.0046***</b>	0.471	0.838	0.134	<b>0.061*</b>	0.704
% change	–32%	–12%	–2%	–23%	+171%	+15%
<i>n</i> (months)	79	83	78	83	79	79
No. of samples	2,552	2,374	2,495	2,374	2,542	2,538
95% CI	–31.2% to –31.9%	–13.2% to –10.7%	–2.7% to –1.8%	–25.2% to –21.5%	+154% to +188%	+13.0% to +16.5%

\*  $p \leq 0.10$ .\*\*  $p \leq 0.05$ .\*\*\*  $p \leq 0.005$ .

the Piedmont, as well as the Coastal Plain-derived Black River about 23 km upstream. Station Sarecta (SAR; GPS coordinates 34.9797N, 77.8625W) is located in the Northeast Cape Fear River. There are ~340 swine CAFOs within a 20-km radius of this station (Mallin and Cahoon 2003). The third station, Sta. NCF117 (GPS coordinates 34.3634N,

77.8968W) is 110 km downstream from Sarecta and ~40 km upstream from the city of Wilmington in a tidally influenced, freshwater segment of the Northeast Cape Fear River at its confluence with the main-stem Cape Fear. The fourth station, Channel Marker 61 (M61; GPS coordinates 34.1938N, 77.9573W), is in the upper Cape Fear Estuary

Table 5. Trend analyses in TN concentrations and loadings from June 1994 to 2003 or June 1994 to 1999 data, excluding the sustained 3-yr drought. The data from 1993 were excluded here for comparison to Table 4. Bold indicates significance.

Parameter	1994–2003 (all data)	1994–2003 (–1996, 1999 hurricane periods)	1994–1999 (+hurricanes, –3-yr drought)	1994–1999 (–1996, 1999 hurricanes, –3-yr drought)
TN concentration				
<i>p</i> value	<b>0.027*</b>	<b>0.013*</b>	0.239	0.213
% change	–26%	–27%	–13%	–14%
<i>n</i> (months)	108	108	66	66
No. of samples	3,567	3,461	2,220	2,114
95% CI	–27.6% to –25.2%	–28.2% to –26.6%	–13.5% to –12.4%	–14.3% to –12.6%
TN loading				
<i>p</i> value	0.127	0.311	0.738	0.826
% change	–20%	–14%	–5%	–4%
<i>n</i> (months)	107	107	66	66
No. of samples	3,287	3,105	2,040	1,858
95% CI	–20.5% to –20.0%	–14.2% to –13.4%	–5.0% to –4.7%	–6.0% to –1.8%

\*  $p \leq 0.05$ .

Table 6. Sensitivity of trend analysis to the beginning conditions, showing variability from consideration of each of the first six months as  $T_{\text{initial}}$  [TP concentration, DO, and Chl *a*, 116–121 months (May–Oct 93–03); TN concentration, 119–124 months (Jun–Nov 94–03); TN and TP loads, 103–108 months (Jan–May 93–03). Bold indicates significance.

Trend statistics	[TP]	TP load	[TN]	TN load	[DO]	[Chl <i>a</i> ]
Avg. <i>p</i> value	<b>0.035**</b>	0.439	<b>0.076*</b>	<b>0.049**</b>	<b>0.059*</b>	0.641
Avg. SD	0.013	0.204	0.065	0.028	0.020	0.073
Avg. change	–24%	–12%	–24%	–25%	–19%	13%
Avg. SD	1.56	4.84	4.02	2.78	1.61	2.96
<i>p</i> value (range)	0.018 to 0.056	0.220 to 0.771	0.008 to 0.164	0.017 to 0.083	0.031 to 0.085	0.533 to 0.728
Trend (range)	–26.4% to –21.9%	–17.8% to –4.5%	–30.6% to –19.7%	–28.1% to –21.7%	–21.1% to –16.1%	+9.8% to +17.8%

\*  $p \leq 0.10$ .

\*\*  $p \leq 0.05$ .

(mean salinity of 6 for 2002–2003) near the Port of Wilmington, ~8 km downstream from Sta. NCF117. Samples at these stations were collected monthly on the outgoing tide for ~8 yr, from June 1995 (NAV, NCF116, M61) or February 1996 (SAR) through June 2003.

In the lower Cape Fear during 1996–2003, significant increasing trends in  $\text{NH}_4^+$  concentrations were documented at all four stations (Fig. 12), whereas significant changes in TN and nitrate concentrations were not discerned. The largest increases in  $\text{NH}_4^+$  occurred at Stas. NCF117 (315% increase,  $p < 0.0001$ ,  $n = 96$ ) and SAR (148% increase,  $p = 0.028$ ) on the Northeast Cape Fear with a high density of CAFOs in its watershed; the lowest increase occurred at Sta. NAV (64% increase,  $p = 0.025$ ), on the main-stem Cape Fear below the Black River and above the Northeast Cape Fear tributary. The increases, although substantial, have been lower than in the Neuse, likely because the Cape Fear is tidal with high flushing (Mallin et al. 2000), whereas the Neuse waters have a longer residence time and are wind mixed with little tidal influence (Reed et al. 2004).

Analysis of these data, considered together with our data for the Neuse, indicates that significant increases in  $\text{NH}_4^+$  concentrations over the past decade may characterize estuaries draining watersheds and airsheds with high densities of swine and poultry CAFOs, via runoff and leachate into subsurface flow (see review in Burkholder et al. 1997) and atmospheric wet and dry deposition (Walker et al. 2000; Whittall et al. 2002). The TN load to the Albemarle–Pamlico watershed from atmospheric deposition and subsequent dry or wet fallout has been estimated at ~38%, among the highest known for U.S. watersheds (EPA 2000a). The contribution of atmospheric  $\text{NH}_3$  and P deposition by CAFO animal confinement buildings, lagoons, and sprayfields to nutrient budgets in natural systems has not been fully characterized, but >80% of total swine  $\text{NH}_3$  emissions in the southeastern United States originate in North Carolina (EPA 1998; Aneja et al. 2003), and there was a 30% increase in airborne  $\text{NH}_3$  during the 1990s, 47% and 25% of which was contributed by swine and poultry industrialized animal operations, respectively (NC DENR 1997b, 2002). N and P loading from direct runoff would be more substantial when CAFOs were flooded and in direct contact with surface waters (Mallin 2000; Mallin et al. 2000) or following major effluent spills from ruptured CAFO lagoons (nutrients as well as rich organics, fecal coliforms, and microbial pathogens; Burkholder et al. 1997). CAFOs would be expected to contribute sig-

nificant loadings of  $\text{NH}_4^+$ , as well, to other waterways in the Americas and Europe, where this industry has expanded (Webb and Archer 1994; USDA 2001). The  $\text{NH}_4^+$  in the North Carolina estuaries would also have been contributed from other external sources, such as WWTPs and land-applied sludge, stack emissions, and septic systems with inadequate nitrification. Internal  $\text{NH}_4^+$  loading from regeneration, partial denitrification, and resuspension (Glibert 1988; Koike and Sørensen 1988), and increased rates of bacterial decomposition of  $\text{N}_0$  from wetland waters could be important as well.

Partitioning the relative contribution of the different components that compose  $\text{NH}_4^+$  loading is a challenge because of the many potential input sources. Atmospheric wet and dry deposition (Walker et al. 2000; Whittall et al. 2002), groundwater (Harned et al. 1995), estuarine sediments (Giffin and Corbett 2003), and partial denitrification (Glibert 1988) are all potential sources that must be addressed in future research to determine the source contributions of the striking increases in  $\text{NH}_4^+$  concentration that were documented in both the Neuse and Cape Fear systems, and to further evaluate the overall importance of  $\text{NH}_4^+$  in driving phytoplankton production and bloom formation (Fan et al. 2003).

*Nutrient loadings*—Trend analysis of TP loading was relatively insensitive to  $T_{\text{initial}}$  in this study, and no significant change was detected with or without consideration of the sustained 3-yr drought. Thus, although potential P sources have continued to increase in the watershed, there is as yet no apparent signal for increased P loading to the estuary. These findings differ from earlier trend analyses, which had suggested a significant decreasing trend in TP loading (Glasgow and Burkholder 2000; Qian et al. 2000). Although P is relatively insoluble, it can be moved by precipitation into adjacent waters from P-saturated soils or from manures applied to soils (NRC 2000). Phosphorus accumulation in soils used for disposal of swine CAFO wastes in the Neuse watershed was already appreciable in 1993, when three counties (~20% of the land) within the watershed had sufficient P from CAFO wastes to exceed the P requirements of all non-legume crops and forages (Barker and Zublena 1995). The lack of a significant decreasing trend in TP loading over the past decade suggests that benefits earlier derived from the P detergent ban in the late 1980s (NC DEHNR 1991) and other improvements (e.g. biological nutrient removal in major

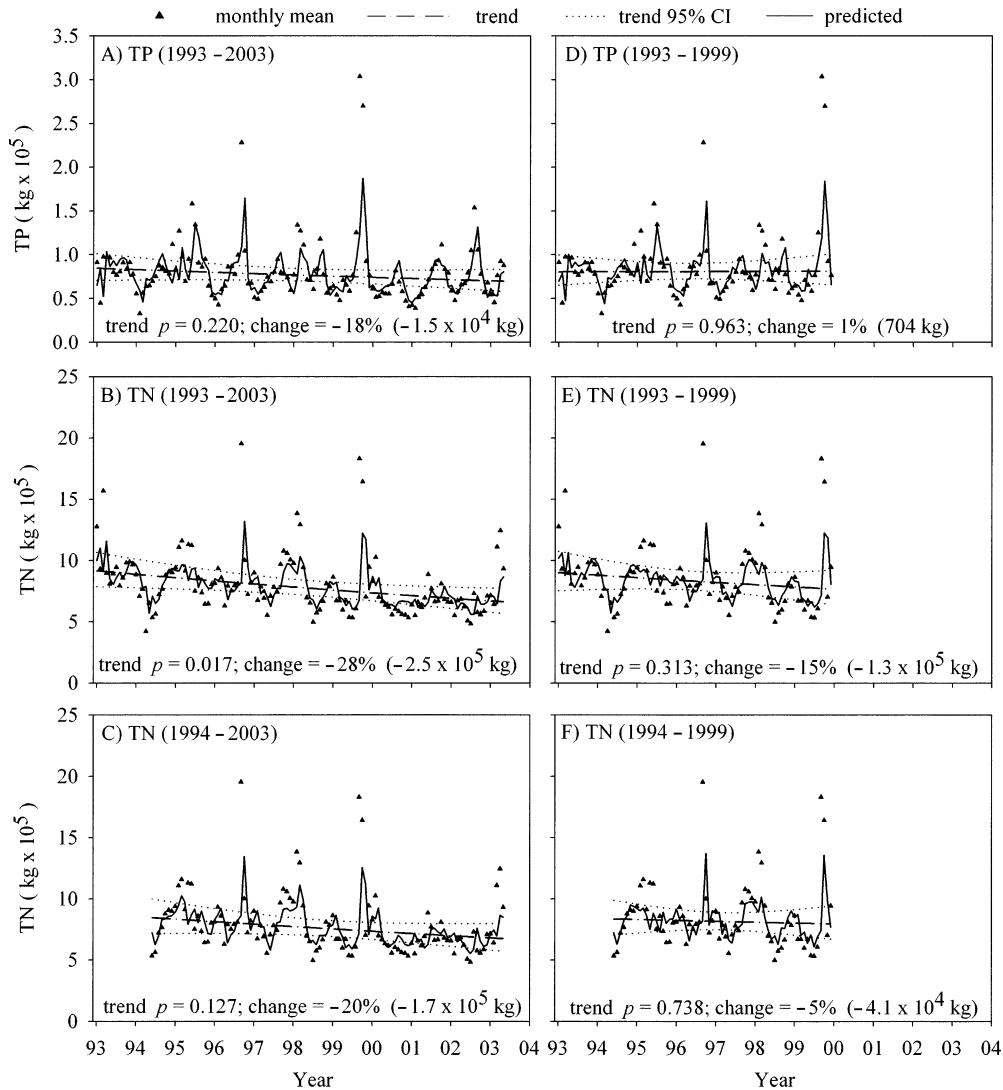


Fig. 11. Total phosphorus and total nitrogen loadings considering the six mesohaline stations collectively (A–C) including the sustained 3-yr drought as (A) TP loading (1993–2003;  $n = 124$  months; 3,803 samples), (B) TN loading (1993–2003;  $n = 124$  months; 3,818 samples), (C) TN loading from 1994 to 2003 ( $n = 107$  months; 3,287 samples), and (D–F) excluding the period post-1999 with the sustained drought as (D) TP loading (1993–1999;  $n = 83$  months; 2,556 samples), (E) TN loading (1993–1999;  $n = 83$  months; 2,556 samples), and (F) TN loading (1994–2003;  $n = 66$  months; 2,040 samples). Linear trend lines (dashed) were calculated by least squares means for all sites. Also shown are the 95% confidence limits (dotted lines), predicted (model-fitted) values (solid line),  $p$  value (probability associated with the linear slope), percent change in TP or TN loading, and corresponding change in concentration (in parentheses).

WWTPs) have gradually been offset by increasing P inputs. Thus, P could become increasingly important in eutrophication of the Neuse, as has been hypothesized for other estuaries (e.g., Fisher et al. 1992; Hagy et al. 2004) under increasing watershed development. The overall decrease (22%,  $p = 0.019$ ) in TN:TP concentration ratios in the Neuse Estuary (also reported by Qian et al. 2000) lend additional support for increased importance of P.

As mentioned, TN trend analyses can be especially sensitive to  $T_{\text{initial}}$ , given the broad range and variability in concentrations measured from one date to the next. We found a significant decreasing trend (28%) in TN loading to the

Neuse Estuary only when 1993 and the sustained 3-yr drought near the end of the study were included in the analysis. Our findings for the Neuse Estuary differ from those of several other studies (e.g., Stow et al. 2001; Stow and Borsuk 2003). Some long-term analyses have indicated that TN loads to the Neuse Estuary have increased by at least 30% over recent decades (Stanley 1988; Dodd et al. 1993). In contrast, other analyses through 1998–2000 for the upper watershed indicate that TN levels have been decreasing or stationary since 1995 (Qian et al. 2000; NC DENR 2001). However, the lower river and estuary historically have sustained high nutrient loading (Steel 1991), and in the lower

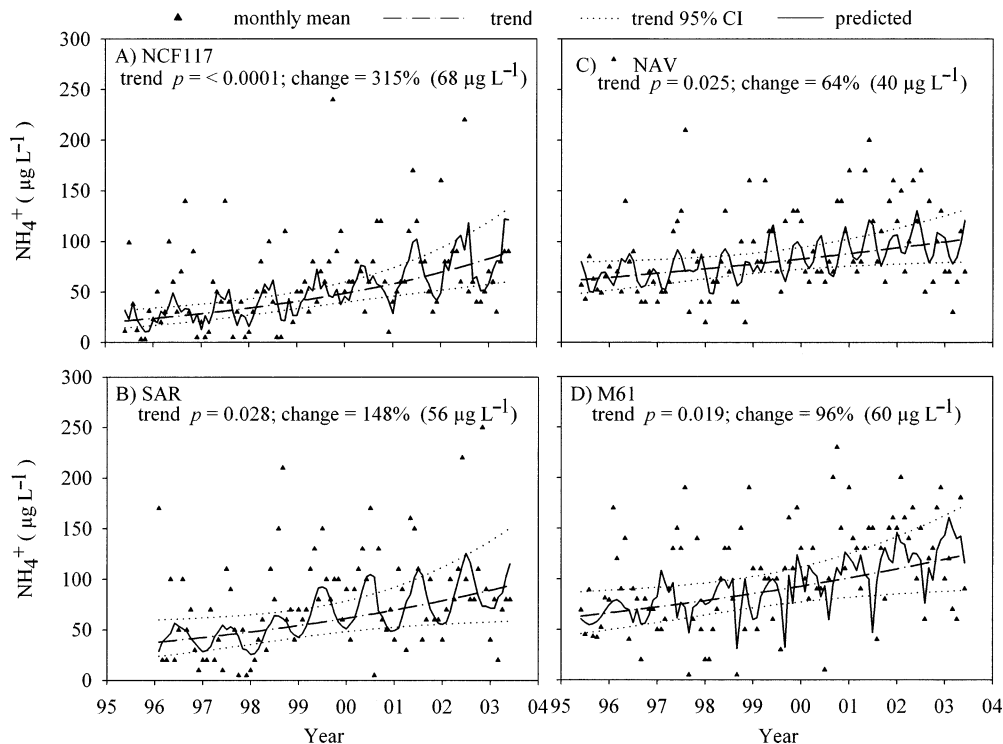


Fig. 12. Ammonium concentrations at four stations in the Cape Fear Estuary from monthly data as (A) Sta. NCF 117 (June 1995–June 2003;  $n = 96$  months; 97 samples), an oligohaline segment of the Northeast Cape Fear River at its confluence with the main stem Cape Fear; (B) Sta. Sarecta (SAR, February 1996–June 2003;  $n = 88$  months; 89 samples), an oligohaline segment in the Northeast Cape Fear; (C) Sta. Navassa (NAV, June 1995–June 2003;  $n = 96$  months; 97 samples), in the mainstem Cape Fear River  $\sim 7$  km upstream from its confluence with the Northeast Cape Fear River; and (D) Channel Marker 61 (M61, June 1995–June 2003;  $n = 96$  months; 97 samples), in the upper Cape Fear Estuary  $\sim 8$  km downstream from NCF 117. Linear trend lines (dashed) were calculated by least squares means for all sites. Also shown are the 95% confidence limits (dotted lines), predicted (model-fitted) values (solid line),  $p$  value (probability associated with the linear slope), percent change in DO, and corresponding change in concentration (in parentheses).

Neuse watershed, a major nonpoint nutrient source, swine CAFOs, sustained exponential growth from the late 1980s through the mid-1990s.

The differences between our estimates and others' partly reflect reliance among those studies on concentration data collected with diverse methods and different sampling locations and frequencies over different periods. Different modeling approaches have also been used (e.g., Stow et al. 2003; Wool et al. 2003). Some researchers, as mentioned, have attempted to control for climatic events through the use of flow-adjusted concentrations (e.g., Stow et al. 2001; Stow and Borsuk 2003). This approach showed a decline in flow-adjusted TN concentrations at Kinston and Fort Barnwell from 1995 to 2000, after  $\sim 15$  yr of relative stability (1979–1995; Stow and Borsuk 2003), but as mentioned, the model could explain only  $\sim 35$ – $40\%$  of the variability in TN concentrations at the station nearest the estuary and closest to our study area (Fort Barnwell; Stow and Borsuk 2003). Researchers have applied different methods, as well, for estimating volume of flow to the estuary. Ours is the first study based on flow data obtained in close proximity to the Neuse Estuary, collected by ADCP to directly measure inflow and outflow across the estuary. In related research, ADCP was

used to demonstrate a varied flow field in both the horizontal and vertical extent of the estuary, with meteorological-dependent bidirectional discharges and resulting shear layers (Reed et al. 2004). Although valuable information was gained with single transect data, long-term flow patterns could not be discerned, but long-term flow patterns were distinguishable from composite ADCP visualizations. The persistent circulation patterns over time are important in explaining variations in observed lateral differences in flow or site-specific differences in biological phenomena. Areas of increased or decreased water mass retention (eddies, null zones, areas of low or high flow) were identified with a composite method of processing multiple ADCP transect data sets, which can be used to plan an improved monitoring scheme for ongoing efforts. The improved accuracy in estimating these bidirectional flows will also be an important consideration in refining nutrient loadings and residence times.

*Eutrophication of the Neuse: The science–policy interface*—Cloern (2001) suggested that scientists' evolving conceptual model of eutrophication needs to consider how improved understanding of trends in nutrient concentrations

and loadings in estuaries can be used to improve efforts to restore aquatic ecosystems. The following points are included toward addressing that goal.

Management strategies for water quality protection in many U.S. estuaries during the 1970s to mid-1990s focused mostly on P control of point source discharges (e.g., NC DEHNR 1991; NRC 2000). Because these efforts previously targeted P reductions without concomitant N reductions, the magnitude of algal blooms in the Neuse, as well as other lower rivers and estuaries, has probably increased. In the Chesapeake Bay, experiments by Fisher et al. (1992) showed that P reductions caused decreased growth of freshwater algae so that  $N_i$  uptake was reduced during downstream transport (e.g., of highly mobile nitrate), and more  $N_i$  thus was available to support estuarine blooms. The premise that increased P limitation in upstream freshwater segments promotes increased N concentrations and phytoplankton production downstream in estuaries was supported by analysis of a long-term data set in the Chesapeake Bay (Harding and Perry 1997). In the Neuse, Mallin et al. (1993) found a significant correlation between upper watershed river flow and estuarine nitrate loading and phytoplankton productivity following a 2-week lag period, indicating that nitrate from freshwater segments can travel long distances and stimulate estuarine phytoplankton. Comanagement of N and P is expensive and sometimes uses conflicting best-management practices (BMPs; Clausen et al. 1989; NRC 2000); therefore, in many locations, N controls are only beginning to be imposed (NRC 2000). As for the Neuse, data available for many estuaries under increasing cultural eutrophication point to the need for N and P comanagement to reduce the adverse effects of eutrophication and help restore water quality. It is also important to note that attempts to quantitatively update nutrient inventories for U.S. watersheds will be hampered, as in this study, because of changes in required agricultural reporting in 2001 and thereafter. Adequate monitoring and reporting of anthropogenic point and nonpoint sources continues to be a critical need for resource managers and scientists in assessing eutrophication effects and the success of restoration efforts (NRC 2000).

As exemplified by the Neuse Estuary, the importance of controlling agricultural, urban, and other nonpoint nutrient sources to reduce eutrophication to aquatic ecosystems, although scientifically established, continues to be overlooked or underemphasized because of economic and political constraints (e.g., Chesapeake Bay Program 1997; NRC 2000). Even in well-funded efforts to reduce nutrient loading and hypoxia, targeted goals generally have not been realized because of increased nonpoint loading. In the late 1980s, for example, resource managers targeted a 40% reduction in controllable nitrogen sources to the Chesapeake Bay (Chesapeake Bay Program 1997), but <20% reduction in TN loading was achieved over an ~15-yr period despite major decreases in point source loadings, increased buffer areas, and other imposed management strategies (Chesapeake Bay Program 2005). Thus, Hagy et al. (2004) recommended that a significant decrease in the area affected by anoxia in the Chesapeake Bay would (still) require 40% reduction in TN loading.

The TMDL developed for the Neuse targeted a 30% re-

duction in TN loading by all sources to the estuary, to be accomplished during the 5-yr period from 1998 to 2002 (NC DENR 1997a). The fragility of TN loading trends, as shown in this study; the striking increase in  $NH_4^+$  concentrations; and the lack of management emphasis on achieving major reductions in most nonpoint sources collectively do not support reports to the general citizenry of achievement of a 30% reduction in TN loading to the Neuse by anthropogenic sources over the past 5 yr. Aside from improved treatment of municipal sewage point sources, cropland farmers located mostly in the upper and mid watershed initiated a program in 1998 to decrease their N fertilizer application by 30%, and additional BMPs for cropland agriculture were installed from 1996 to 2003 as well (Neuse Basin Oversight Committee 2004). A noticeable decrease in TN concentrations within the estuary from this program might not be detected, however, until a lag period of a decade or more. Although the Neuse Basin Oversight Committee (2004, p. 10) reported that "for the basin as a whole, the Neuse agricultural community in 2003 has achieved a 42% nitrogen reduction," the statement pertains to cropland farmers and refers to N fertilizer use, rather than in-stream N levels. Efforts to reduce N loading actually have not been widely adopted by other major sources of agricultural loading, such as industrialized animal agriculture. Moreover, contributions of certain nonpoint sources, such as leachate from septic tanks (Simpson 2004) and land application of sludge from human wastes (Rockefeller 2002), are poorly known but could also be substantial with increased pressures from coastal development.

Stationary or increasing trends in nutrients, as in the Neuse, despite concerted efforts by resource managers to reduce nutrient inputs, likely reflect expanding nonpoint sources that continue to be inadequately addressed, mainly through voluntary BMPs (Steel 1991; Burkholder et al. 1997). Thus, it is not surprising that declining TN and TP trends in the Neuse Estuary can be explained primarily by climatic events, as nutrient reductions from improved management practices in the Neuse watershed over the past decade have been offset by increases in human and animal populations (especially swine) and related factors. Given ongoing, increased development of watersheds drained by estuaries in many regions of the world (Vitousek et al. 1997), goals for nutrient reduction that do not account for increased coastal and basin development should be adjusted upward at 5–10-yr intervals, and interpretations from long-term ( $\geq 10$  yr) data sets, on the basis of consistent methodologies with adequate detection limits, must recognize the importance of climatic events that can drive trend analyses. Indirect methods (e.g., transfer function and ARIMA procedures) will continue to be necessary to estimate anthropogenic- and event-driven climatic changes in estuaries increasingly affected by watershed development and cultural eutrophication. With further long-term observations, greater confidence in loading estimates will be realized, which in turn will strengthen the reliability of predictive models for eutrophication effects and system recovery.

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