

Development of an unattended estuarine nutrient monitoring program using ferries as data-collection platforms

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Abstract

Water quality monitoring of estuarine ecosystems relies on measurement of biogeochemically important elements, including nitrogen (N), phosphorus (P), carbon (C), and silicon (Si) at a time-step relevant to the environmental conditions affecting the estuary. To improve the temporal resolution of sample collection from the Neuse River estuary, North Carolina, USA, a water sampling system was installed on a state-operated motor vehicle ferry. A gravity-filtration device consisting of a plastic cylinder (15 cm long, 4 cm wide) with a 0.7 μm pore size filter at the bottom was suspended inside the mouth of the collection bottle. Dissolved organic nitrogen (DON), orthophosphate, dissolved organic carbon (DOC), ammonium, and silica were analyzed from filtered water samples, whereas nitrate plus nitrite analysis required acidification of samples in addition to filtration. Refrigerated storage of samples for 7 days had minimal effect on nutrient concentrations. A daily sample collection scheme was employed during September and October 2005 in the Neuse River estuary. There were relatively minor changes in N, P, C, and Si nutrients during and after passage of Hurricane Ophelia in September, but high concentrations of ammonium, silica, and orthophosphate occurred at the onset of high riverine discharge during a separate storm in October. In contrast, DOC, nitrate plus nitrite, and DON reached highest values after the peak riverine discharge. This high temporal resolution sampling regimen was essential for examining estuarine response to brief meteorological and hydrologic perturbations and is applicable to many other estuaries worldwide.

Introduction

Estuarine and coastal waters worldwide are experiencing accelerated rates of nutrient loading from watershed and airshed sources (Nixon 1995, Paerl 1997, Boesch et al. 2001). Anthropogenic nutrient enrichment often causes bottom-up ecological impacts that influence estuarine primary productivity (eutrophication) and biogeochemical cycling. The concentrations of nitrogen (N), phosphorus (P), carbon (C), and silicon (Si) are fundamental parameters in the assessment of nutrient enrichment. Evaluation of trends in estuarine water quality and overall ecological condition require continuous, long-term data on these nutrient concentrations.

Historically, estuarine and coastal water sample collection has been performed by personnel on research vessels. How-

ever, the frequency of sampling is limited by the cost of vessel operations, and therefore research cruises are conducted at weekly to monthly intervals. This is particularly problematic in large, highly dynamic systems, where there is a need for high-frequency, spatially intensive sampling capable of capturing both the event scale and longer-term trends. Recently, automated water samplers have enabled more frequent sample collection, and some equipment is capable of analyzing nutrient concentrations in situ. However, current technologies for in situ analyzers are limited to only a few analytes (nitrate plus nitrite, orthophosphate, etc.) and they lack sensitivity in nutrient-depleted waters. A more effective automated sampling scheme would allow analysis of multiple water-quality parameters, resulting in a more comprehensive accounting of biogeochemical processes in these waters.

Commercial and public ferries regularly traverse estuarine and coastal ecosystems worldwide. When outfitted with automated water samplers, these ferries offer a convenient and cost-effective platform for process and water quality research (Räntäjarvi et al. 1998). Such a system has been adopted for monitoring the Neuse River Estuary (NRE) and Pamlico Sound (PS) in North Carolina, USA, part of the second largest estuarine complex in the United States (Buzzelli et al. 2003). This ferry-based monitoring program, FerryMon (www.ferrymon.org),

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allows more frequent sample collection than is available from the existing monitoring efforts underway in this estuary. The program is also expected to complement and enhance the spatial extent of a biweekly environmental assessment program, the Neuse River Modeling and Monitoring Program (ModMon, www.marine.unc.edu/neuse/modmon), underway in the NRE and PS estuaries (Luettich et al. 2000, Paerl et al. 2004). One goal of this ferry-based monitoring program is the implementation of a continuous, daily sampling regimen for the Neuse River estuary. This high-frequency data will facilitate analysis of short-term (daily to weekly) trends in N, P, C, and Si that are not currently monitored.

However, the automated sampling process requires preservation and storage of the water samples on board the ferry until they can be retrieved for analyses. The efficacy of various sample storage procedures has been examined by numerous researchers (see review by Mayer and Woo 1998). As highlighted by Gardolinski et al. (2001) in a comparison of nitrate and orthophosphate storage procedures, the effect of storage procedures depends on other chemical characteristics of the sample matrix such as calcium concentration and bacterial populations. The matrix-dependent nature of nutrient preservation is apparent from the contrasting results of other studies, as well (for example, see Thayer 1970; Avanzino and Kennedy 1993; Dore et al. 1996). Thus, the current study assessed several sample storage procedures with water from the study site on the Neuse River estuary. The preservation methods were based on those outlined in Standard Methods (APHA 1998). Additionally, a novel device for automated water filtration was developed and tested. These techniques were evaluated for their applicability in the implementation of a daily water-quality assessment program for the Neuse River estuary.

Materials and procedures

The FerryMon Project is a ferry-based water quality monitoring program operated jointly by the University of North Carolina at Chapel Hill's Institute of Marine Sciences in Morehead City, NC, and the Duke University Marine Laboratory in Beaufort, NC. As part of the collection equipment, ISCO model 6700FR refrigerated autosamplers (Teledyne Isco, Lincoln, NE, USA) were installed on 3 of the State of North Carolina Department of Transportation motor vehicle ferries that traverse the Neuse River estuary and Pamlico Sound. This study used the sampling system aboard the M/V *Lupton*, a 55-m passenger and motor vehicle ferry. The ferry operates every half-hour between Cherry Branch on the south side of the Neuse River estuary and Minnesott Beach on the north side of the estuary, a distance of ca. 1.5 km. The water intake for the ISCO was plumbed in-line with the vessel's raw-water cooling system. The vessel's cooling system continually draws in water from outside the hull at a depth of 1.5 m. Consequently, the water samples collected by the sampler come from the turbulent zone created by the vessel's 1.8 m draft, and may be a vertical integration of this portion of the water column.

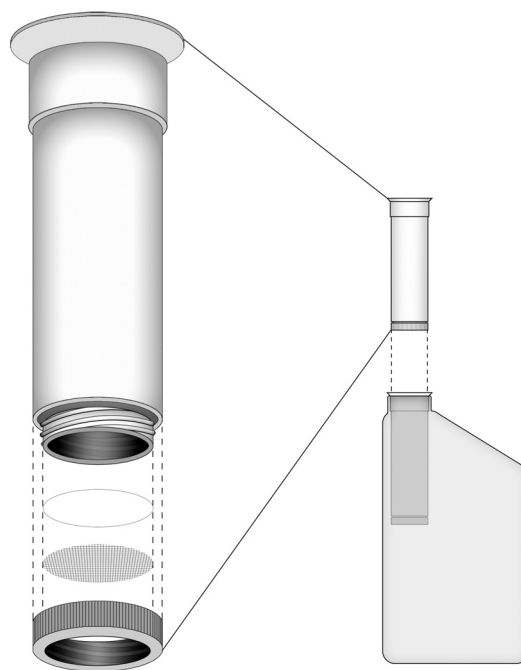


Fig. 1. Schematic of the filtration canister used for the filtration of water before acidification and refrigerated storage.

The chemical constituents of interest for this component of the FerryMon Project were ammonium (NH_4), nitrate plus nitrite ($\text{NO}_3 + \text{NO}_2$), dissolved organic nitrogen (DON), silica (SiO_2), dissolved reactive phosphorus (PO_4), and dissolved organic carbon (DOC). The refrigerated chamber within the ISCO sampler allows storage of up to twelve 2-L sample bottles at near 0°C . Standard Methods for the Examination of Water and Wastewater (APHA 1998) recommends storage of water samples for PO_4 , SiO_2 , and DOC analysis at 4°C for up to 28 days. Freezing of samples for subsequent SiO_2 analysis may result in a loss of SiO_2 . Samples for DOC analysis may be acidified to a $\text{pH} < 2.0$ with H_2SO_4 (APHA 1998). Preservation of $\text{NO}_3 + \text{NO}_2$, NH_4 , and organic nitrogen for up to 28 days can be achieved by acidification to $\text{pH} < 2.0$ with H_2SO_4 (APHA 1998).

The Neuse River estuary often has high concentrations of suspended organic (phytoplankton, zooplankton, detritus) and inorganic (silt, sand) particulate matter. Storage of water samples containing high concentrations of particulate matter (and subsequent microbial growth) may result in high variability in the subsequent nutrient analysis. Further, the effect of acidification and subsequent cell lysis on dissolved nutrient concentration is unknown. Therefore, filtration of particulate matter from the sample before acidification and storage was necessary.

To address this problem, an automated "filtration canister" was developed to reduce the concentration of particulates in the stored and preserved samples (Figure 1). The filtration canister consisted of a 15-cm length of 4 cm inner diameter PVC pipe fitted with a screw-on lid on the bottom of the tube. The center of the lid was cut out and replaced with a circular piece

of nylon mesh cloth. A combusted 47-mm Whatman GF/F glass fiber filter (0.7 μm pore size) was fitted inside the lid and was supported by the nylon mesh. The top of the tube was fitted with a collar that allowed the filtration canister to be suspended inside the mouths of the autosampler bottles (1-L polyethylene bottles, Teledyne Isco). The filtration canister held 225 mL, which drained (by gravity) through the filter. Installation of a traditional filter cartridge (available from various laboratory equipment manufacturers) was not possible due to requirements of output pressure from the sampler and the limited life span of a single filter cartridge in this turbid estuary.

Assessment of method

Evaluation of nutrient preservation techniques—A series of experiments was performed to evaluate the precision and accuracy of a combination of traditional preservation techniques (refrigeration and acidification) and the filtration procedure described above. Seven experiments were carried out over a 4-week period in which batches of water samples were collected and stored for varying periods of time (1 to 7 days). The 7 sampling events were representative of a range of estuarine chemical and biological conditions (sampling was conducted during the warmest and hence most biologically active period of the year when biota would be most likely to affect nutrient concentrations). Each experiment was conducted with water collected on separate days, and thus the initial nutrient concentrations and planktonic composition of the water differed between experiments.

On 7 occasions between 28 June 2005 and 25 July 2005, a 1.5-L sample was collected from the middle of the Neuse River estuary using the ISCO water sampler aboard the *M/V Lupton*. Individual 100-mL aliquots were transferred to separate bottles that received different preservation treatments and were subsequently stored in the refrigerated ISCO sampler for up to 7 days. The control and treatments were collected and analyzed in triplicate. The treatments included (1) no filtration, (2) no filtration with acidification, (3) filtration, and (4) filtration with acidification. The 100-mL sample passed through the filters in less than 30 min. Acidification of the samples was performed by adding 5 drops of 50% H_2SO_4 to the bottle before sample collection (previously verified to achieve a pH < 2.0 in the sample water in the bottle).

Subsamples of the filtered samples were immediately transferred to separate bottles, stored on ice during transport to the laboratory, and frozen until analysis. These subsamples were filtered and frozen on the same day they were collected. They were used as controls to compare to the batch of treatment samples stored in the refrigerated ISCO sampler for the designated period. The refrigerated chamber was kept at $\sim 0^\circ\text{C}$, which resulted in some of the samples near the rear of the chamber being frozen, whereas those nearest the front were not frozen. Based on Dore and colleagues' (1996) examination of refrigeration and freezing on concentrations of N, P, and Si, we do not anticipate that this temperature differential affected

our results. After the 1- to 7-day storage period, samples were retrieved from the *M/V Lupton* and transported to the laboratory on ice. Previously unfiltered samples were then filtered through precombusted 0.7- μm glass fiber filters. Samples were frozen until analysis.

All nitrogen, PO_4 , and SiO_2 analyses were performed using a Lachat QuikChem 8000 autoanalyzer (Lachat Instruments, Milwaukee, WI, USA). Total dissolved nitrogen was determined using in-line perchlorate digestion, and DON was determined by subtraction of $\text{NO}_3 + \text{NO}_2 + \text{NH}_4$ from the measured total dissolved nitrogen. For statistical purposes, sample concentrations below the method detection limit (MDL) were assigned concentrations of $\text{MDL} \div 3$. Dissolved organic carbon was determined on a Shimadzu 5000TOC analyzer (Shimadzu, Columbia, MD, USA) after acidification and sparging with purified air for 6 min. The effect of pH on the analysis of the nitrogen, PO_4 , and SiO_2 samples was determined by comparing a midrange calibration standard of each analyte with an acidified version of that standard. Silica was the only analyte in which the mean recovery of the nonacidified and acidified standard differed significantly ($t = 11.884$, $n = 3$, $P < 0.001$; see statistical procedures described below). The acidified SiO_2 standards were, on average, 4% lower than the nonacidified samples.

A 2-sample t test ($\alpha = 0.05$) (SPSS, Chicago, IL, USA) was used to compare the mean values of the control samples from each storage period to the treatment samples for that period. For each t test, a Levene's test for equality of variances was performed, and the results dictated the choice of the probability value used (P for equal or unequal variances).

Filtration of water samples resulted in the least number of statistically different control-versus-treatment comparisons for NH_4 , PO_4 , DON, and DOC (Figures 2, 3, and 4). Of these 4 analytes, there was only one instance (NH_4 after 5 days of storage) in which a control and treatment differed from one another. In this case, NH_4 concentration of the filtered samples after 5 days of storage were, on average, 36% higher than the control (Figure 2B) ($t = -3.579$, $df = 4$, $P = 0.023$). There was little variability in NH_4 among the replicate control samples, but greater variability in all of the treatment samples. With regard to PO_4 and DOC, even unfiltered samples stored in the refrigerated sampler for 1 to 7 days were not statistically different from their respective controls (Figures 3B and 4B). Because filtration is necessary before sample analysis, however, automated filtration is desirable because it saves the technician time during sample preparation.

Silica concentration was never significantly different between any control samples and their respective unfiltered treatments (Figure 3A). However, SiO_2 in filtered samples was 28% lower than control after 6 days of storage ($t = 3.229$, $df = 4$, $P = 0.032$) (Figure 3A).

Filtration and acidification of samples was the most effective preservation technique for $\text{NO}_3 + \text{NO}_2$. This preservation method resulted in only one statistically significant difference between the NO_3 controls and treatments: after 2 days of stor-

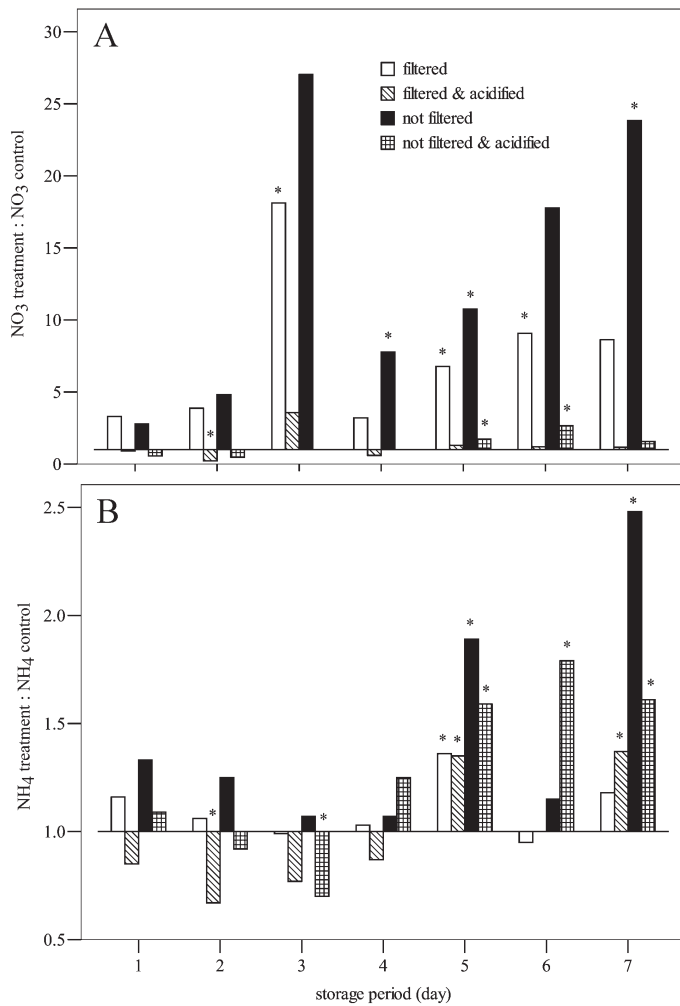


Fig. 2. Ratio of treatment:control average nutrient concentrations for NO₃ + NO₂ (A) and NH₄ (B) during varying periods of refrigerated storage. Asterisks indicate a significant difference ($P < 0.05$) between the treatment and control nutrient concentrations.

age, NO₃ was 78% lower in the filtered and acidified samples than the control ($t = 4.571$, $df = 2$, $P = 0.045$) (Figure 2A).

In summary, no single treatment was effective in preserving all analytes over the 7 days of storage. However, filtration of water from the Neuse River estuary allowed storage of PO₄, DON, and DOC for up to 7 days in the refrigerated ISCO samplers without significant deviation from the control samples. While not perfect, filtration was the most effective method of preserving samples for NH₄ analysis, and filtration plus acidification was most effective for NO₃ + NO₂ analysis. Samples for SiO₂ analysis were negatively affected by filtration in one instance.

None of the analytes exhibited temporal trends of increasing instability as a function of storage time. Therefore, whereas NH₄, NO₃ + NO₂, and SiO₂ may be negatively affected by the storage protocols identified above, these instances occurred randomly over the 7-day period. This may be in part due to the

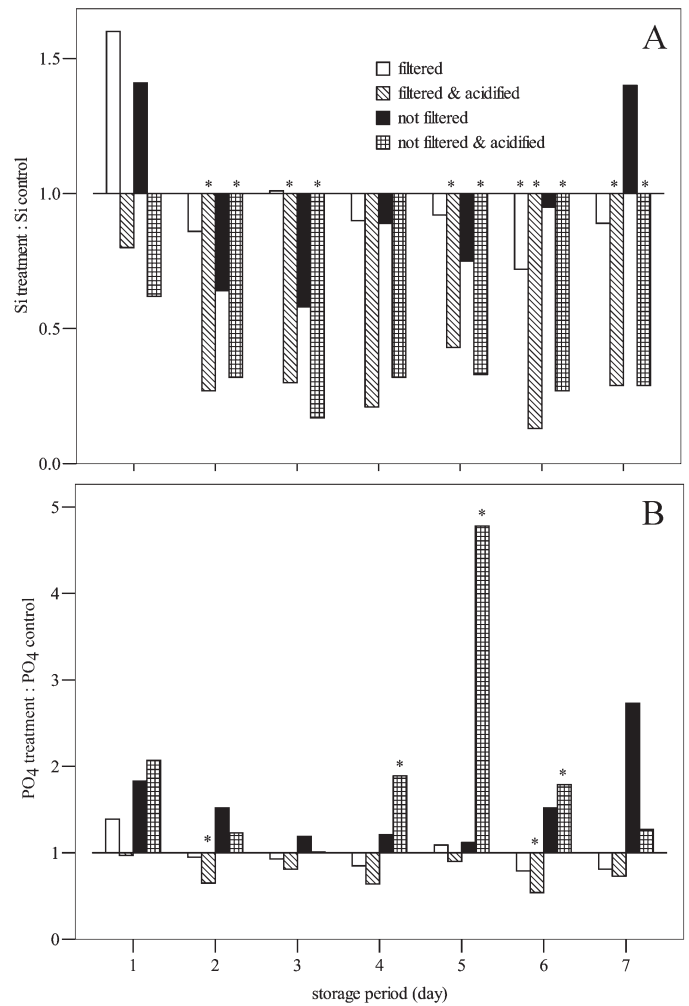


Fig. 3. Ratio of treatment:control average nutrient concentrations for Si (A) and PO₄ (B) during varying periods of refrigerated storage. Asterisks indicate a significant difference ($P < 0.05$) between the treatment and control nutrient concentrations.

fact that each of the 7 experiments was conducted with estuarine water collected on different days, and therefore the water samples were subject to different microbial populations.

Method validation—These results provide guidance on how best to implement a daily sampling regimen for N, P, C, and Si in the Neuse River estuary. A primary limitation of this method is that the ISCO sampler is capable of holding only 12 bottles of the size compatible with the filtration canister developed here. Given that this study examined the effects of storage over a 7-day period, a practical sampling strategy is limited to sampling over a 6-day period in which 2 bottles could be filled during each sampling event. These duplicate field samples could each be subjected to different treatments to best accomplish the preservation of N, P, C, and Si. For example, water collected in one bottle could be filtered for subsequent analysis of NH₄, DON, PO₄, and DOC. The second sample could be filtered and acidified for subsequent analysis

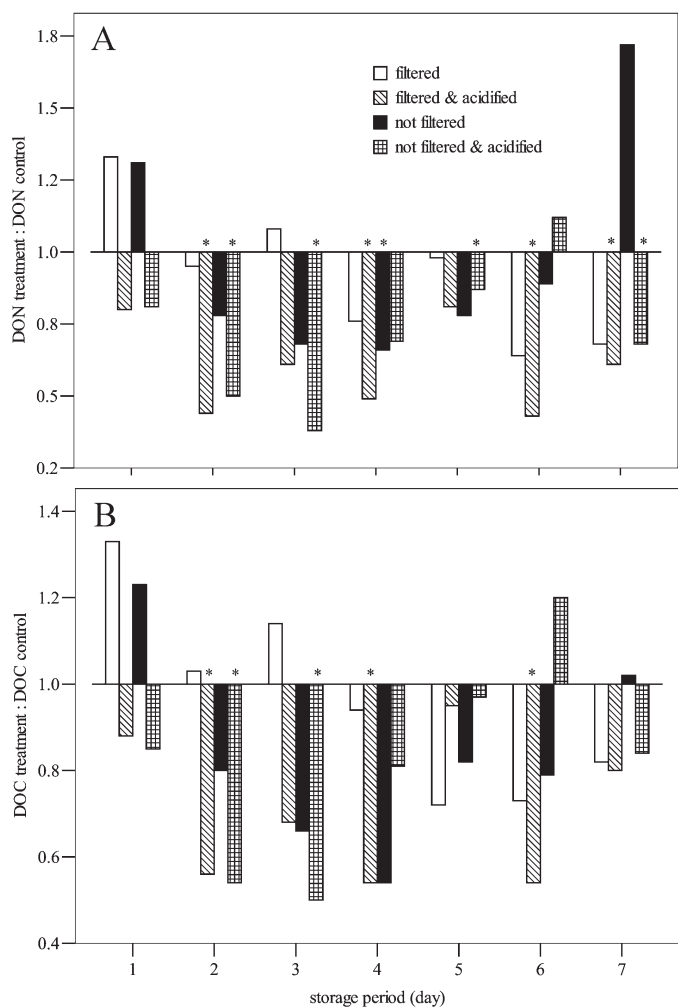


Fig. 4. Ratio of treatment:control average nutrient concentrations for DON (A) and DOC (B) during varying periods of refrigerated storage. Asterisks indicate a significant difference ($P < 0.05$) between the treatment and control nutrient concentrations.

of NO_3 . Silica could be analyzed from the filtered water sample, with the understanding that under some circumstances this may result in an underestimate of in situ conditions. Likewise, NO_3 may be underestimated in some cases, although the filtration and acidification method described here proved superior to other methods of preservation.

To validate this proposed sampling strategy, daily water samples were collected from the Neuse River estuary using the M/V *Lupton* sampling system from 8 September 2005 through 30 October 2005. Filtered samples were collected between 1200 and 1400 hours in duplicate bottles containing filter canisters, with 1 bottle acidified. Samples were retrieved from the ISCO sampler on the 7th day of storage, and bottles were washed with phosphate-free laboratory detergent, rinsed, dried, and returned to the sampler for the next daily collection. On 5 days during this period, water samples could not be collected because of technical problems with equipment or

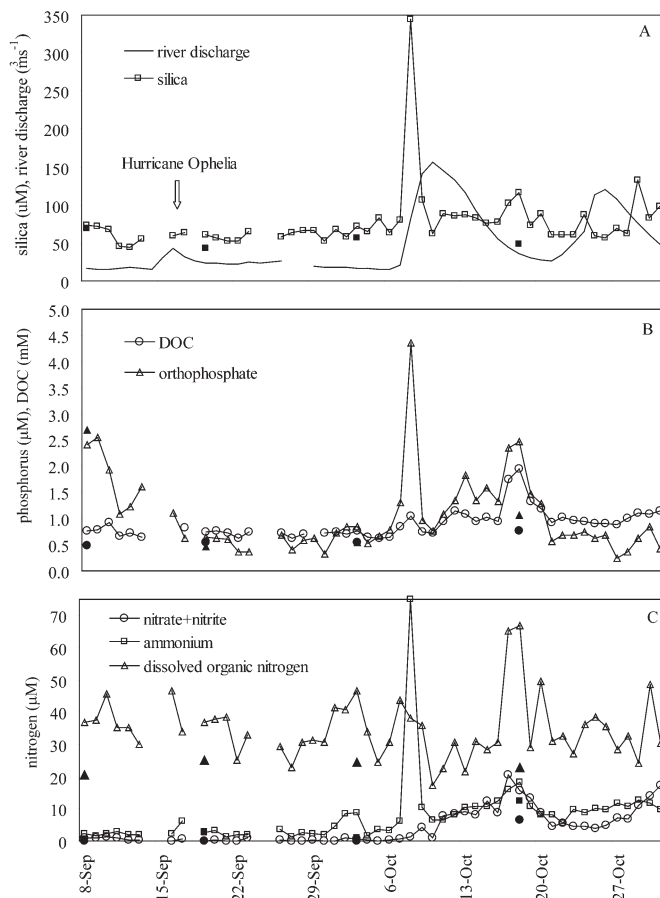


Fig. 5. Daily nutrient monitoring data from the Neuse River estuary during September-October 2005. (A) Silica concentration and Neuse River discharge (Fort Barnwell USGS gauging station 02091814), (B) dissolved organic carbon and orthophosphate concentration, and (C) inorganic and organic nitrogen concentrations. Solid symbols correspond with hollow symbols of the same shape and represent nutrient data collected and analyzed as part of the Neuse River Estuary ModMon Program.

because the ferry was not running during Hurricane Ophelia.

The data collected during this period spans a variety of meteorological and hydrologic events, including Hurricane Ophelia and a period of extensive rainfall and elevated riverine discharge (Figure 5A). Dissolved inorganic N and P concentrations were relatively low during September 2005 (< 1 and $< 2 \mu\text{M}$, respectively) (Figure 5B,C). Hurricane Ophelia affected the North Carolina coast 12 September through early 15 September, making landfall 50 km southeast of the study site on 14 September as a category 2 hurricane. Rainfall from this storm was intense (> 10 cm) along the coastal plain, but did not extend far westward and thus river inflow emanating from the Piedmont was not significantly affected (Figure 5A). Water samples were not collected on 14-15 September due to disruption of the ferry schedule by Hurricane Ophelia. Sample collection resumed on 16 September, and no nutrients were elevated above the previous week's concentration. Any effect

on nutrient concentrations by Hurricane Ophelia was thus limited to a 48-h period during which sampling was not possible.

An ephemeral spike in NH_4 , PO_4 , and SiO_2 occurred on 8 October in response to rainfall derived from an extratropical low pressure system (Figure 5A,B,C). Tropical Storm Tammy merged with this frontal system over Georgia on 6 October, and may have contributed to the precipitation delivered to the Neuse River basin as the frontal system moved northeast. Inorganic N, P, and DOC increased gradually as the flood wave passed through the estuary. This general pattern was repeated during a second storm event that peaked on 26 October, this time without an initial spike in concentration as observed on 8 October.

To demonstrate the necessity of these daily measurements of nutrient concentration in analyzing the effects of environmental perturbations on the estuarine system, Figure 5 includes nutrient concentration data collected as part of the Neuse River Estuary ModMon Project (Luettich et al. 2000, Paerl et al. 2004). The ModMon data shown are from the water sampling station corresponding with the Cherry Branch ferry crossing; data are the average of the surface and bottom nutrient concentrations. This comparison of the ModMon and FerryMon data is not intended as a comparison of the absolute concentrations of the analytes shown, since samples were collected at slightly different times of the day and reflect different integrated depths of the water column. Rather, the intention is to highlight the need for high temporal sampling resolution during discrete disturbance events such as hurricanes and river flood events. Interpretation of the effects of either of these events on the estuary would not have been possible from the biweekly sampling data. Sampling at a time-step comparable to the time-scale of meteorologic and hydrologic events (daily) is essential for proper monitoring of estuarine biogeochemistry and evaluation of the effects on estuarine condition. Increased sampling frequency during periods of accelerated phytoplankton growth may provide additional insight into short-term variability in biogeochemical condition of the estuary. However, during relatively quiescent periods in the estuary when river inflow is low and steady (such as the 2-week period over late September to early October), the biweekly sampling regimen captured the relevant range of nutrient concentrations.

The data gathered over this 2-month period revealed two important characteristics of the Neuse River estuary that would not have been apparent from existing monitoring projects. First, the data provide a nearly continuous record of biogeochemical processes during passage of a hurricane over the estuary. Previous research has shown dramatic short (i.e., days to weeks) and longer-term (months) effects of hurricanes on water quality in the Neuse River estuary (Paerl et al. 1998; Peirls et al. 2003; Paerl et al. 2006). In contrast, Hurricane Ophelia did not provide a major disruption to estuarine biogeochemistry. Whereas riverine discharge doubled during this storm, the increase was minor relative to the floods documented during previous hurricanes (Bales et al. 2000), and

therefore riverine-derived nutrient loads would not be expected to be significant. The frequency of data collection during Hurricane Ophelia supports the conclusion that effects on the Neuse River estuary were minor; in the absence of these data, however, we could only speculate on estuarine conditions between the 2 ModMon sampling events before and after the storm.

The second insight gained from these data are the time-series of chemical constituents in the estuary corresponding with the first major autumn storm to influence the Neuse River estuary beginning on 8 October and peaking 10 October. This time-series of nutrient concentrations reflects a typical pattern found in riverine storm hydrographs: an initial peak in concentration caused by the entrainment of material during elevated water level and velocity, followed immediately by a decrease in concentration resulting from dilution. Due to these rapid, daily changes in nutrient concentration, this effect would not be detectable if sampled over a weekly or biweekly basis. As a practical consequence of relying solely on the biweekly nutrient concentration data, calculation of the nutrient load (concentration \times discharge) delivered to the adjacent Pamlico Sound during the October storm event would be greatly underestimated because the transient peak in NH_4 , PO_4 , and SiO_2 would not be considered.

Discussion

Our findings point to the importance of dependability and regularity of vehicle ferries to capture relatively short-term but highly significant nutrient pulsing events, which are critical determinants of water quality conditions in the NRE. Second, a method was developed to prefilter water collected aboard the ferry, thus allowing it to be stored for up to a week without degradation of the N, P, C, and Si. We estimate that implementation of the automated daily sampling protocol described here saved > 11 h of personnel time in weekly operations.

Because this FerryMon monitoring scheme is able to sample only a single location in the estuary, it is clearly not a substitute for the less frequent, although spatially extensive, ModMon estuarine monitoring program. Identical sampling systems have been installed on 2 motor vehicle ferries operating in the adjacent Pamlico Sound (Buzzelli et al. 2003). These ferries traverse a larger area than the M/V *Lupton* described here, and thus provide a greater spatial coverage for data collection. However, the problem of sample storage space arises when considering the implementation of a continuous sampling schedule across many sites. A smaller-sized filtration canister would be required in combination with a larger number of sample bottles to extend the sampling frequency and number of locations on these other ferries.

The automated collection and filtration system developed here is applicable beyond the ferry-based sample collection scheme. It would also apply to unattended mooring or buoy-based monitoring systems. Coastal ocean and estuary observing systems would greatly benefit from such a low-cost, low-

maintenance system for collecting samples for nutrient analysis. The sample collection and storage protocols examined in this study should help guide the development of similar monitoring programs in other aquatic systems.

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