Water Resources Research Institute of the University of North Carolina Annual Technical Report FY 2005

Introduction

During the Fiscal Year 2005 reporting period, the Water Resources Research Institute of The University of North Carolina supported research from five universities for seven related projects. Research priorities, as directed by the WRRI (Water Resources Research Institute) Advisory Committee included the following: emerging contaminants (EDCs & PPCPs), drinking water protection, water supply/drought issues, erosion and sediment control/stormwater, nutrients and water quality, agriculture issues, water/wastewater financing and funding, economic, legal and regulatory issues, river basin management, infrastructure issues, on-site wastewater management, and monitoring and data analysis. The research projects reported herein provide relevant data within the WRRI priority constraints.

The information transfer program continued to focus on disseminating results of sponsored research and providing information on emerging water issues, regulations, and problems. Results of research are desseminated by publication of technical completion reports, summaries in the WRRI newsletter, publication of summaries on the WRRI website, and presentations by investigators at WRRI seminars and the Annual Conference. WRRI continues to be a sponsor of continuing education credits by the NC Board of Examiners of Engineers and Surveyors and the NC Board of Landscape Architects. This allows WRRI to offer Professional Development Hours (PDHs) and contact hours for attendance at WRRI seminars and the Annual Conference.

Research Program

The Water Resources Research Institute of The University of North Carolina is responsible for fostering and developing a research training and information dissemination program responsive to the water problems of the State and region. To develop its programs, the Institute maintains an aggressive effort to interact and communicate with federal, state, and local water managers. The close contact with water managers is a basis for determining the ever-changing water research priorities.

Priority water research needs for the FY 2005 program were developed in close consultation with the Institutes' Advisory Committee. Following their annual meeting, a statement of priority research needs was developed. The proposal solicitation, as in the past, is sent to all presidents and relevant department heads of senior colleges and universities in North Carolina as well as historically black colleges, to apprise them of the opportunity to submit proposals. The call for proposals is also sent to an email distribution list of about 180 university faculty across North Carolina. The proposals received are sent to the Technical Committee and to external peer reviewers to determine the relevancy, need for the proposed research and relative strength and weaknesses. The Technical Committee meets to review all comments made by reviewers and make recommendations regarding proposal funding. Factors considered in the review of proposals are: (1) scientific quality of the proposed work; (2) need for the results of the research in North

Carolina and the region; (3) the probability that useful results can be obtained in one-year; and (4) the potential for the continued support from other funding sources.

Use of Indicators to Distinguish Between Point and Non-Point Sources of Chemical Contamination in North Carolina Streams

Basic Information

Title:	Use of Indicators to Distinguish Between Point and Non-Point Sources of Chemical Contamination in North Carolina Streams
Project Number:	2005NC44B
Start Date:	3/1/2005
End Date:	2/28/2006
Funding Source:	104B
Congressional District:	4
Research Category:	Water Quality
Focus Category:	Agriculture, Non Point Pollution, Waste Water
Descriptors:	chemical monitoring, endocrine active substances, organic compounds, wastewater treatment
Principal Investigators:	Howard Weinberg

Publication

Title

Use of Indicators to Distinguish Between Point and Non-point Sources of Chemical Contamination in NC Streams (70213)

Problem and Research Objectives

Effluent discharged into receiving streams from wastewater treatment plants has to meet National Pollutant Discharge Elimination System permit levels on a variety of parameters that are designed to protect the stream's ecology and aquatic life from deleterious effects and to ensure that the natural flora and fauna can remediate the residual chemicals and micro-organisms prior to subsequent usage. No such equivalent protection of the natural environment is afforded through the operations of the large number of on-site wastewater treatment systems that serve almost half of the North Carolina population. When these systems fail, their untreated effluents can run off the ground surface and reach surface waters compromising downstream ecosystems and perhaps even the downstream drinking water treatment plant intakes. The management of nonpoint source contamination wasn't designed to account for the presence of chemicals with far different properties to those mimicking natural compounds and the presence of pharmaceutically and endocrine active chemicals with biological functions in environmental waters is testament to the ineffectiveness of current contaminant control. Drugs used for human and animal therapy and endocrine-disrupting compounds are introduced into agricultural systems via land application of recycled wastewater and accumulated biosolids as well as through direct usage of pesticides. The widespread domestic use of many of these compounds also ensures that they will be present in septic systems and in landfills. Leakage and runoff from any of these systems will contribute significant loading into receiving waters and contribute to impairment. It is unknown what percentage of accumulations of these compounds derive from point and non-point sources but from extrapolation of what is known about nonpoint pollution from regulated compounds, the contribution from the nonpoint sources is likely to be very significant.

As this research aims to provide a strategy for subsequent application to a monitoring program, the monitoring aspect of this project will be limited to a preliminary demonstration of capability. There is strong evidence that widely used anthropogenic chemicals, some with pharmaceutically or endocrinally active properties, are present in our streams an reservoirs feeding drinking water treatment plants. Among those frequently cited in the literature are synthetic hormones, steroids, pesticides, surfactants, antibiotics, antidepressants, and caffeine. Whereas, the future use of biochemical markers may be capable of identifying a specific activity in waters, such markers cannot differentiate between their sources. What is needed first is a strategy to make this differentiation possible. We propose, therefore, to use our strengths as analytical and environmental chemists to identify chemical markers of NPS contamination and then select two to three on-site wastewater systems and farms using biosolids for land application and wastewater for irrigation where we will be able to track these markers together with two to three chemicals representative of PhACs and EASs. Finally, we will sample up- and downstream of the points of identified runoff from the nonpoint sources to demonstrate a causative link with elevated levels of these compounds in the sampled waters. By identifying widely used chemicals that are attenuated during conventional wastewater treatment, we aim to show that the presence of these compounds in receiving streams is due to nonpoint sources of contamination and subsequently use these compounds as indicators of such contamination. This proposal seeks preliminary answers to the following questions regarding point and nonpoint source contamination of receiving streams and groundwater by pharmaceutically active- and endocrine disrupting chemicals:

1. How can we best evaluate the most appropriate surrogates for a vast array or anthropogenic chemicals that characterize the presence of wastewaters discharged from municipal treatment plants in receiving stream?
2. Which representative chemical measurement would be a stronger indicator of contamination of streams from nonpoint sources?
3. What correlations can be drawn between the indicator measure and the concentrations of targeted emerging chemical contaminants of concern?
4. Can the identification of these chemicals be traced to a specific source or group of sources where control of release might be a better approach to protecting water quality?

Almost all the impaired 303(d) streams in the Neuse and Cape Fear river basins have biological impacts from urban runoff or agriculture and would provide a sound basis for the demonstration of the concept of differentiation between sources of contamination. The list of impaired waters in the Cape Fear and Neuse River basins (NC DENR, 2003) will initially guide the selection of sample collection sites in high priority rivers and streams with sample points upstream of the point of NPS discharge collected as a control. With the use of the developed NPS chemical indicators, a limited sampling strategy will be developed that will confirm the presence of the point source indictors in representative wastewater plant effluents and of the nonpoint source indicators in runoff and leach fields at stream locations that will not be impacted by upstream wastewater treatment plant discharges. We will target those water bodies that are suspected of impairment from urban and agricultural runoff and which are currently being targeted by the PI on another study that is evaluating the use of ubiquitous environmental organisms as a marker for changes in antibiotic resistance profiles of bacteria found in riparian sediments up and downstream of a municipal wastewater treatment plant. With input from our colleagues at the North Carolina division of the U.S. Geological Survey (Gloria Ferrell), the Division of Environmental Health (DEH) NPS Section at DENR (Barbara Hartley-Grimes) and the DWQ-Basinwide and Estuary Planning (Jay Sauber) we will design an initial screening of two rivers or creeks from each basin collecting samples upstream, at the nonpoint discharge into the stream, and then downstream. Grab samples will be collected from each of the predetermined locations in the sequence in which they geographically occur moving downstream from the control. In summary, then, the objectives of this study are to identify chemical indicators that distinguish between point and nonpoint sources of contamination and then to survey for their presence along with targeted PhACs and EASs in the Neuse and Cape Fear River Basins at sample collection sites known to be upstream of point source discharges and downstream of such discharges.

Methodology

The following components are being accomplished for successful completion of the stated objectives:

A. Generate a focused list of target chemicals based on usage in localized populations. Prepare methods.
B. Testing of wastewater plant influents and effluents to demonstrate attenuation of chemicals during treatment
C. Testing of agricultural runoff and leach fields from septic tank discharges to identify the presence of the same chemicals in A.
D. Winter sampling season, sample collection and analysis
E. Spring/summer sampling season, sample collection and analysis
F. Data interpretation and compilation of report presenting conclusions and determination if the issue needs further study

Establishing the Indicators
We carried out a comprehensive review of occurrence data in surface waters and from wastewater treatment plant effluent studies with the objective of selecting representative chemicals including pharmaceutically active compounds (PhACs) and endocrine active substances (EASs) that are unique to the practices generating nonpoint sources of contamination and yet are remediated during wastewater treatment. While there are several microbiological approaches that attempt to distinguish between point and nonpoint sources (e.g. Parveen et al, 1997; Wiggins et al, 1999) none are a "magic bullet" and all depend on the collection and analysis of a large and statistically valid number of isolates which would add significantly to the proposed budget yet provide a great deal of uncertainty in the outcome. In year one of this study we investigated chemicals associated with domestic use (for septic systems) that appear to be highly remediated during conventional wastewater treatment and, therefore, would not be present in a receiving stream prior to the nonpoint source addition.

Herberer et a1 (2002) found 99.9% removal of caffeine from conventional wastewater treatment yet it was detected in over 70% of streams tested by Kolpin et a1 (2002). Caffeine is unlikely to sorb to soil (Seiler et al. 1999) but is degraded aerobically by microbes. Drewes, et al. (2003) found that caffeine was not detected in tertiary treatment or in groundwater under soil irrigated with wastes. Caffeine does not sorb to any major extent on particles (Peeler 2004) suggesting that there is likelihood that it will be found in the aqueous phase of septic tank effluent. Pederson et a1 (2005) found that caffeine was not detectable above the method detection limit in runoff from agricultural fields irrigated with treated effluent. At levels around 40 to 140 mg in an 8 oz drink of tea or coffee and from 9-46 mgl in a12 oz soft drink (Wilson 1999) there are expected to be high levels of caffeine in the wastewater from domestic on-site treatment making caffeine a suitable choice as an indicator of surface water runoff from septic leachate. From the information regarding removal efficiency in conventional wastewater treatment

Triclosan is an antibacterial agent with widespread occurrence in consumer products. This compound is prevalent in domestic waste since it is a component of cosmetics, such as toothpaste, shampoo, and soap that are commonly washed down the drain. Several studies have shown a range of removal rates for triclosan from wastewater treatment plants. Bester et a1 (2003) reported approximately 30% of triclosan sorbed to activated sludge and that only about 5% is easily removed from biosolids. Key removal of triclosan occurs during secondary treatment which utilizes microbial aerobic and anaerobic degradation. This treatment process also removes particulate organic matter which triclosan may be sorbed to. Sabaliurius (2003) compared activated sludge and trickling filter treatments and found over 90% removal for both.

That study found that triclosan was affected by sorption and ionization. However, Singer et al. (2002) found 79% biodegradation removal during wastewater treatment, 15% into sludge and 6% remaining in the effluent at an average concentration of 42 ng1L. Additionally, triclosan has been found to undergo photolysis degradation once in natural surface waters with ranges of 0.06-0.33 ngl Lkr (Bester et al. 2003). Once in surface waters, there is some degradation of triclosan due to photolysis. Sabaliurius, et a1 (2003) found instream removal ranged from 0.06-0.33 ngl Lkr with a half life of 2.1-3.3 hours. Morrall et al. (2004) found that the settling of suspended solids within the river played a large role in the natural removal. This study found that overall, 19% of triclosan was lost due to sorption into suspended solids and settling within the river.

Using these removal rates, we are able to take typical flow rates for an identified wastewater treatment plant and demonstrate the likelihood of triclosan being present downstream of discharge. These calculations are shown in Table 1 and suggest a plant effluent discharge concentration of triclosan at about 15 ng/L that it will decrease to levels below the detection limit of 10 ng/L once released into the receiving surface water stream.

To compliment these findings, caffeine has been detected at 137 mg/L and triclosan at 6mg/L in the aqueous phase of septic tanks (Wren 2001).

Possible indicators from wastewater treatment plants (WWTPs) are stable chemicals produced during final disinfection and although many conventional plants are switching from chlorine to ultra-violet for this process, many rural plants still depend on chlorine. Haloacetic acids (HAAs) are one such subgroup of byproducts produced during chlorination of wastewaters provided the wastewater is well nitrified before the addition of chlorine.

Table 1. Calculations for Levels of Triclosan in Plant Effluent Discharges

Workable Model			
Based on ML/day influent estimated of OWASA			
Concentration influent (ng/L)	385.32		
removal rate WWTP	0.96		
amount removed (ng/L)	369.90		
Co = concentration in effluent (ng/L)	15.41		
Q wwtp	155.40		
Qriver	194.00		
Cro=Co*Qwwtp/Qwwtp+Qr (ng/ft3)	8.08		
Instream removal rate ng/L/ hr	0.06	0.21	0.33
Instream removal rate ng/ft3/s	0.00	0.00	0.00
Cro/removal rate = time (s)	17137.44	4896.41	3115.90
Cro/removal rate = time (hr)	285.62	81.61	51.93
Stream mean velocity (ft/s)	0.54		
distance removal(ft) = removal time * velocity	9254.22	2644.06	1682.58
Based on population served by OWASA			
Concentration influent (ng/L)	351.92		
removal rate WWTP	0.96		
amount removed (ng/L)	337.85		
Co = concentration in effluent (ng/L)	14.08		
Q wwtp	155.40		
Qriver	194.00		
Cro=Co*Qwwtp/Qwwtp+Qr (ng/ft3)	7.38		
Instream removal rate ng/L/ hr	0.06	0.21	0.33
Instream removal rate ng/ft3/s	0.00	0.00	0.00
Cro/removal rate = time (s)	15652.22	4472.06	2845.86
Cro/removal rate = time (hr)	260.87	74.53	47.43
Stream mean velocity (ft/s)	0.54		
distance removal(ft) = removal time * velocity	8452.20	2414.91	1536.76

Caffeine and Triclosan

During year one of this project we have evolved sensitive methods for the analysis of these two indicators in the complex matrix of septic wastewater and subsequent discharges which are now described. The septic effluent samples are centrifuged at 2500 rpm for 15 minutes. The supernatant is then passed through a 0.45µm filter. Surface water is also filtered through at 0.45 pm filter. 100 mL of sample are processed using 3mL Oasis HLB cartridges (Waters Corporation, Milford, MA). The cartridges are first rinsed with 3mL hexane, 3mL ethyl acetate, 3rnL methanol, and 5mL 10mM pH 7 phosphate buffer at a rate of 15rUmin. The sample is then passed through at a rate of 15mL/minute. After the sample, the cartridges are washed with 5mL 10mM pH 7 phosphate buffer and allowed to dry under vacuum for 30 minutes and dry under nitrogen gas for 15 minutes. Cartridges are then washed with 2mL of hexane and then eluted with 3mL of 3:7 methyl tert-butyl ether (MtBE):acetonitrile. Silica gel cartridges are washed with 10 mL 3:7 MtBE:acetonitrile. The extract from the HLB cartridges is passed through the cartridge and this eluent is caught in a test tube. The sample is allowed to dry under vacuum and the cartridge subsequently eluted with 3 mL 3:7 MtBE:acetonitrile, which is caught in the same test tube. The extracts are blown to dryness and then reconstituted in 200µL acetonitrile. These samples are then derivatized by adding 50µL of silylating agent (BSTFA) and 50µL of pyridine spiked with hexachlorobenzene as internal standard. This mixture is heated for 35 minutes at 65 C. After derivatization, the extracts are analyzed on a gas chromatograph with mass spectrometrtic detection (GC-MS). Figures 1 and 2 are sample spectra of the caffeine and triclosan standards.

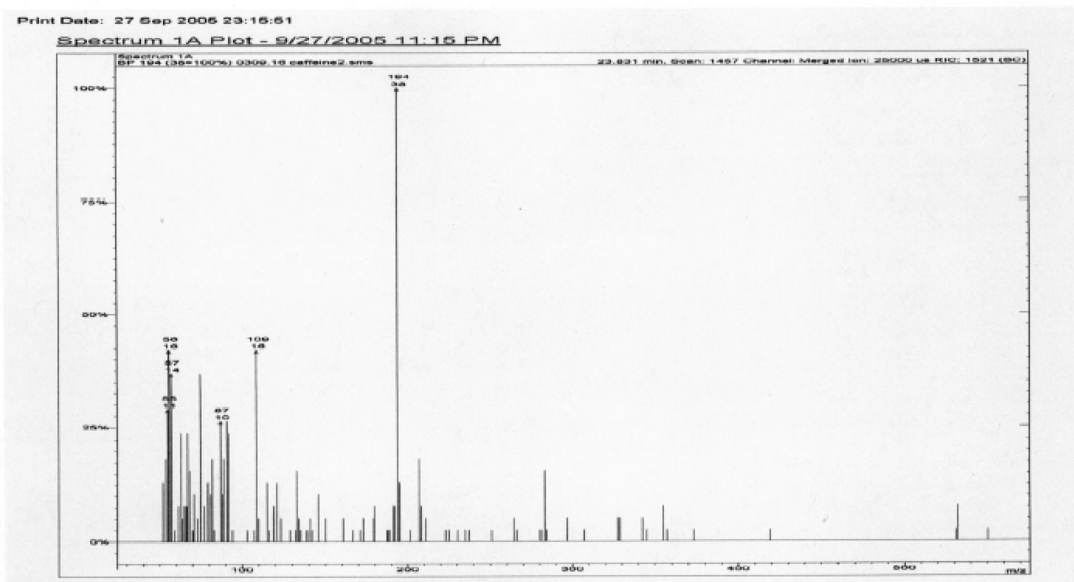

Figure 1. Spectra of caffeine standard prepared at 0.5mgL in acetonitrile.

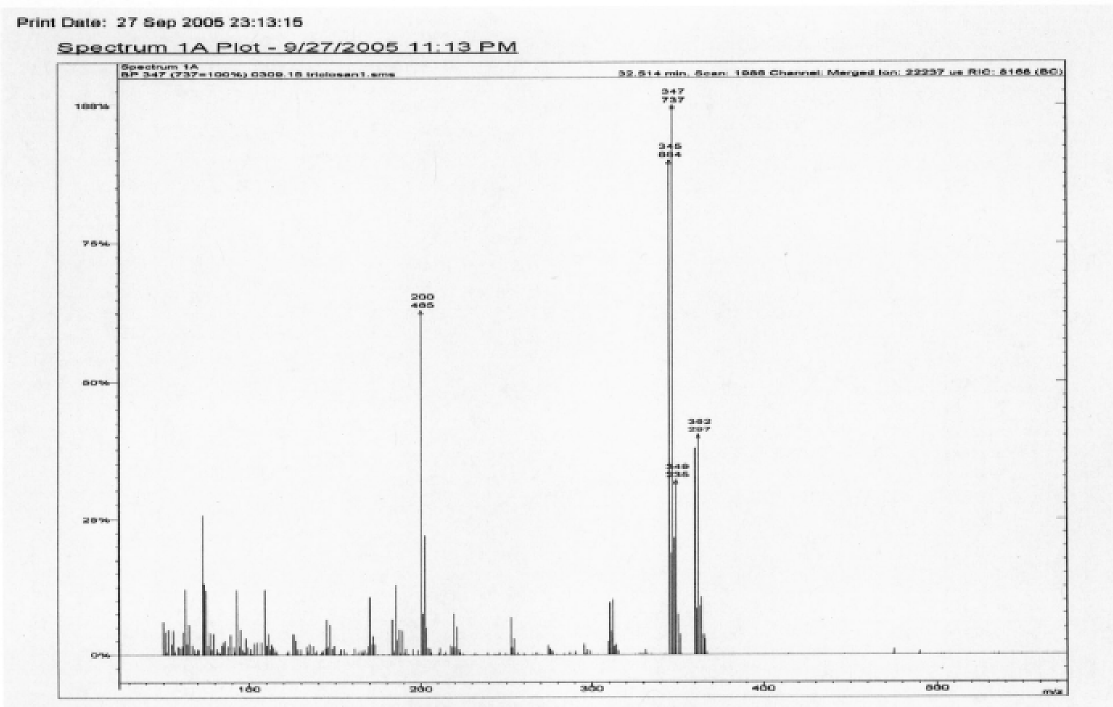

Figure 2. Spectra of triclosan standard prepared at 0.5mgL in acetonitrile.

HAA Analysis
We are in the process of trying several new methods to detect lower concentrations of HAAs in surface water. So far, the most successful method involves blowing down sample extracts after derivatization with diazomethane. From an initial test with methyl esters of the HAAs, there was about 50% recovery. Although this recovery is not high, the chromatograms were much cleaner than other concentration methods. In a recent experiment with surface water samples blown down, the average recovery for dichloroacetic acid (DCAA) was 57% and for trichloroacetic acid (TCAA) was 41%. Early application of this method has found levels of DCAA and TCAA at 2 to 5 µg/L in the receiving waters after discharge of dechlorinated effluent from a conventional wastewater treatment plant. Using the concentration technique, we expect to lower detection limits from 1 g/L which is the established limit using existing U.S. EPA methods to around 50 ng/L allowing for tracking the discharge as it moves downstream.

Principal Findings

In the first year of this project we have undertaken an extensive investigation of the literature to identify chemical tracers that can distinguish between point and nonpoint sources. This has required evaluating the chemical and physical properties of a variety of relatively new chemicals that are now found in wastewater effluents and determining which are more effectively removed by conventional wastewater treatment compared to on-site treatments. As a result of this review we have selected two commonly used household chemicals which are likely to be found at high levels in the liquid phase of septic pump tanks while being effectively removed during municipal wastewater treatment. Caffeine is present in a wide variety of beverages as well as in some medications and triclosan is an antibiotic that is present in almost all household cleaning materials. Both of these chemicals were shown to meet these specified criteria and to persist in septic tank effluents. As potential indicators of nonpoint sources of surface water contamination their levels were often shown to be below detection limits a few hundred feet downstream of wastewater treatment plant discharges while persisting in runoff from failed septic systems. In terms of identifying tracers of municipal plant discharges, we are targeting two sets of chemicals; those produced during the final chlorination stages of conventional wastewater treatment which would not normally be present in the effluent of septic systems.

In the second year of this study, we will examine chemical tracers of surface water contamination whose source is in land applied biosolids.

Significance

It is anticipated that the results of the proposed study will generate the tools that can then be used in a future studies to effectively determine the contribution of emerging chemicals from both point and nonpoint sources of contamination into the State's watersheds. Clearly, the costs associated with any required changes in management practices or contamination cleanup will be much less if this issue can be addressed without much further delay. Without the proposed marker study, we will remain unable to correlate watershed impairment with NPS contaminants and monitoring of streams will prove inconclusive on the sources of contamination.

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Estuarine sediment beds as a reservoir for human pathogens: monitoring transport of populations of enterococci and Vibrio in the Neuse River Estuary

Basic Information

Title:	Estuarine sediment beds as a reservoir for human pathogens: monitoring transport of populations of enterococci and Vibrio in the Neuse River Estuary
Project Number:	2005NC45B
Start Date:	3/1/2005
End Date:	2/28/2006
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Congressional District:	3
Research Category:	Water Quality
Focus Category:	Sediments, Ecology, Surface Water
Descriptors:	suspended sediments, water quality monitoring, bacteria, river beds
Principal Investigators:	Rachel T Noble, Stephen Fries

Publication

1. Hsieh, J.L., J.S. Fries, and R.T. Noble, in press. Vibrio and phytoplankton dynamics during the summer of 2004 in a eutrophying estuary. Ecol. Applications.
2. Fries, J.S., R.T. Noble, H.W. Paerl, H.W., and G.W. Characklis, submitted. Particle suspensions in the Neuse River Estuary: Identifying contributions of resuspension, runoff, and phytoplankton. Estuaries.
3. Hsieh, J.L., J.S. Fries, and R.T. Noble, in prep. Dynamics of Vibrio spp. in the Neuse River Estuary, NC: Improvements for predictive models. Environ. Microbiol.
4. Fries, J.S., R.T. Noble, G.M. Kelly, and J.L. Hsieh. submitted. Storage of Vibrio sp. in estuarine sediments following storm events: possible population shifts and future human health threat? EOS.
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8. Fries, J.S., R.T. Noble, H.W. Paerl, and G.W. Characklis, 2005, "Turbidity and Particle Suspensions in the Neuse River Estuary: Identifying Contributions of Resuspension, Runoff, and Phytoplankton," in Estuarine Research Federation Meeting, Norfolk, VA.
9. Fries, J.S., G. Kelly, and R.T. Noble, 2006. "Dynamics of enterococci and Vibrio sp. concentrations associated with estuarine disturbance during passage of a coastal hurricane" in the 9th Annual Conference of the Water Resources Research Institute of the UNC: Preparedness for Natural and Manmade Disasters, Raleigh, NC.

Title

Estuarine Sediment Beds as a Reservoir for Human Pathogens: Monitoring Transport of Populations of Enterococci and *Vibrio* sp. in the Neuse River Estuary (70214)

Problem and Research Objectives

Estuaries are buffer zones between rivers and oceans, where many anthropogenic inputs are absorbed or modified. Several mechanisms could provide a sink for this material, including dispersal within the estuary, burial, wind-induced flushing, or storage in the sediment. While this range of mechanisms exhibits unique influences in each estuary depending on hydrographic forcing, the duration that pathogenic organisms remain viable and pose risk to public health critically depends on their deposition, lifestyle in the sediment, and resuspension. Microorganisms contained within anthropogenic inputs have the potential to persist, or even proliferate, in estuaries.

The Neuse River Estuary (NRE) is an ecologically and economically-important tributary of North Carolina's Albemarle-Pamlico Sound system. It is a shallow, bar-built estuary with minimal tidal influence and flows dominated by wind and river inflow. This estuary is experiencing a decline in water quality as a response to urban (approximately 1.5 million people) and agricultural growth in its watershed. The watershed also includes coastal communities that depend heavily on fishing, aquaculture, and tourism to sustain the economy as well as inland communities where farming and other businesses play an important role. We chose the NRE as a prime location for a study quantifying the potential for sediments to serve as reservoirs and a significant source of bacterial populations during resuspension events. The NRE typifies shallow, Atlantic and Gulf coastal plain estuarine ecosystems physically, chemically, and biologically. It is also an estuary that is experiencing rapid urbanization and agricultural development in its watershed, including confined animal feeding operations (CAFOs), which has led to the current increase in nutrient loading and subsequent eutrophication. It is a tractable estuarine ecosystem (~60 km along its axis) that is presently intensively monitored for water quality and habitat characteristics. It is the subject of several hydrologic, biogeochemical, and water quality modeling efforts that are germane to this project's research goals (e.g. Bowen and Hieronymus 2003). Finally, it is representative of many other river basins in the U.S., thus making findings in the NRE widely applicable.

The NRE has been widely studied in terms of water quality and monitored for a span of over 20 years thanks largely to the efforts of Dr. Hans Paerl through multiple projects such as NRE Modeling and Monitoring (MODMON) project (http://www.marine.unc.edu/neuse/modmon). This research has largely included study of chemical and nutrient inputs that affect phytoplankton dynamics and eutrophication and modeling of physical parameters and trophic structure. In more recent years there has been a growing emphasis on the study of additional microbial contaminants.

Most of North Carolina's key production of swine, chickens, and turkeys is located in the coastal plain region, including the Neuse River watershed. The swine population alone has increased from less than a million head in 1998 to over 12 million in 2002 and there are now more than 3 times as many swine as humans in the state's coastal watersheds (NC Dept of Environmental and Natural Resources). This industry generates about 10 times as much waste as the human population, with most of the waste released to the environment untreated. In addition, there are approximately 40 million turkeys and chickens raised in the state's coastal watersheds annually. A potentially serious problem is the increase in viral and bacterial pathogens that are transported from these upstream areas of the NRE downstream toward the coast. Bacterial and viral pathogens in hog waste are of major concern because many of these microbes are able to infect humans. Current treatment processes used in animal production are inadequate to prevent the release of potential human pathogens to the environment, which occurs by way of contaminated overland flow from spray irrigation and through groundwater. Other common sources of fecal contamination to the NRE are sewage treatment plant effluents, on-site wastewater treatment and manure spraying systems, agricultural animal manure runoff, sanitary wastes from boats, wildlife feces, and stormwater and other non-point source runoff.

Studies of water contamination in the NRE and other systems have noted increased fecal indicator concentrations in surface water during times of increased rainfall (Patz 1998; Ashford 1998; Mager 1996; Fries et al. in press), suggesting runoff as an important source of pollution. Recreational water quality standards rely on enumeration of indicator bacteria as proxies for the potential presence of fecal contamination. Studies have suggested that enterococci are an indicator of fecal contamination for several reasons: in recreational bathing waters, enterococcus concentrations correlate closely with the incidence of gastrointestinal illness (Dufour and Ballentine 1986) and fecal enterococci are resistant to chemical and physical stress and therefore persist in the marine environment (Kuhn et al. 2000; Frahm et al. 1998). Of the facultative anaerobic organisms common in human fecal flora, enterococci have been found in almost all subjects with a mean level of $8x10^8$ per gram feces (Klessen et al. 2000).

Sediment studies also benefit our understanding of the dynamics of bacterial populations not typically associated with contamination from anthropogenic sources. *Vibrio* species are naturally-occurring, heterotrophic bacteria ubiquitous in estuaries and coastal systems worldwide, including North Carolina. Some *Vibrios* have been shown to be human and animal pathogens (Center for Disease Control: http://www.cdc.gov/ncidod/dbmd/diseaseinfo/). Among the most notable pathogenic species, *Vibrio cholerae* is the organism responsible for one of the world's recurring epidemics, cholera. *Vibrio vulnificus* and *Vibrio parahaemolyticus* are more commonly known as a cause of food borne illness, which occurs primarily through the consumption of contaminated shellfish. *V. vulnificus* can also cause skin infections when open wounds are exposed to seawater containing these bacteria (Strom and Paranjpye 2000). *Vibrio vulnificus* is of particular concern as it can develop into serious and life threatening infections, particularly in immunocompromised individuals. There are several hundred cases of *Vibrio* infections each year in the United States, including an average of 16 cases annually in NC from 2000 to 2005 (NC Dept of Health and Human Services:

http://www.epi.state.nc.us/epi/) and five fatalities from infections in New Orleans following Hurricane Katrina (CDC 2005).

The ecology of *Vibrios* has been studied in estuaries throughout the world. Each *Vibrio* species has specific temperature and salinity requirements. In general, if the salinity requirements are met, the number of *Vibrio*s correlates strongly with temperature and increases in warmer months (Motes 1998). It has been shown that *Vibrio cholerae* demonstrate particle attachment to organic particles (Huo 1996). Other recent research suggests that the presence of particles may influence *Vibrio* populations. Louis et al. (2003) have shown that Susquehanna River inflow contributes to the variability in the occurrence of *V. cholerae* in Chesapeake Bay. This may be due to salinity changes or salinity may be an indicator of other changes in the system. Pfeffer (2003) found that *V. vulnificus* in the Neuse had positive correlations with temperature and turbidity, as well as other environmental parameters. To date, work on the sediment reservoirs of *Vibrio* have been directed at the roles of particle attachment providing a transport mechanism for sediment-water exchange (Randa et al. 2004). Our observations in the NRE support the possibility that a significant portion of *Vibrio* (~30%) are attached and this fraction is higher when environmental conditions (salinity) are unfavorable for growth or particulate loads are high (Hsieh et al. in revision).

Fecal bacteria have shown an affinity for fine particle attachment, providing advantages in survivability and increasing deposition to sediments. Bacterial mortality due to predation or environmental exposure is reduced for enterococci attached to particles (Davies and Bavor 2000; Jin et al. 2004). The ability of these organisms to survive longer than coliforms in sediments supports the use of enterococci as a fairly stable and conservative indicator of contamination (Craig et al. 2004). While particle attachment provides longer periods of viability, the time in suspension is reduced as particles settle to the bed. These particles have the potential to contaminate shellfish and create 'hot-spots' of sediment contamination (De Luca-Abbott et al. 2000). The sedimentation of new inputs of fecal contamination reinforces the importance of making accurate predictions of inputs due to resuspension of settled bacteria. Studies of bacterial survivability and exchange with coastal sediments benefit the design and execution of models predicting transport of pathogens (Steets and Holden 2003).

The NRE provides an optimal environment to assess the importance of sediment-derived populations of indicator and pathogenic bacterial species such as enterococci and *Vibrio* sp., respectively. There are significant levels of both in the NRE (Fries et al. in press; Hsieh et al. in revision) and in Pamlico Sound (unpublished data), and the range of salinity and temperature in the estuary is optimal for populations of both groups to remain viable in the water column for long periods of time (days to months), permitting transport and resuspension to occur. The connections between sediment and microbial transport may simply be due to particle attachment. Previous observations in NRE bottom water has demonstrated that approximately 30-40% of cells in both bacterial groups attach to particles that settle faster than 1.2 mm per hour (Fries et al. in press). This portion of the total population is subject to the movements of the particles or sediment.

Monitoring of microbial contamination of inland waters and estuaries usually focuses on the impacts of rainfall as the presumed major source. This study sought to quantify the potential for sediments to represent a significant source of bacterial populations during resuspension events. These events could be driven by wind without rain or by wind coincident with rainfall (storms) in shallow systems. The Neuse River Estuary is such a system and is the target of intense water quality monitoring for nutrient and bacterial dynamics.

Methodology

Study Site

The site for this work was in the NRE in eastern North Carolina. Results from ongoing extensive water quality monitoring has established the importance of the section of the river near the city of New Bern as a transitional region between water columns dominated by runoff and resuspension and those dominated by phytoplankton (Fries et al. submitted). Understanding the interactions between particles and bacterial populations is a critical link in microbial transport models which need sedimentation terms for losses from the water column (Fries et al. in press). Following deposition, future resuspension events may represent a source of bacteria to the water column not often included in models or management frameworks.

Several periods of wind and rain were experienced at the study site during the summer of 2005 (Figure 2). These events strongly influenced the salinity in bottom waters, the vertical stratification, and the resuspension of sediments. Of particular note, Hurricane Ophelia passed the study site on September 14, with the highest winds (> 40 mph) and a total of 4.5 inches of rainfall. The approach of this storm subjected the area to 16 days of winds from the northeast (National Weather Service at New Bern Airport: http://www.erh.noaa.gov/mhx/f6.html). Given the alignment of this wind direction with the long axis of Pamlico Sound and the lower NRE, increases in salinity from a surge into the upper NRE was expected.

TACO Design

To capture the dynamics of resuspension, this study pursued a sampling strategy beyond the limitations of boat-based, fair-weather sampling often used for detection of microbial contamination. The Time-series Aquatic Contamination Observer (TACO) was designed and built as a research platform for acquisition of water samples *in situ* (Figure 3). Sampling was programmed on a regular schedule and based on instrument triggers using an ISCO 6712 sampler. A total of twenty four, 1 liter samples could be collected. For this study, a turbidity sonde (FTS-12) was used to trigger samples based on 10 minute averages of turbidity (NTU). Temperature and turbidity variance were also logged on the ISCO logger at 10 minute intervals.

Water Quality Measures

In addition to temperature and turbidity from the sonde connected to the sampler, water quality measures were also obtained from the samples themselves. Salinity was measured using a refractometer. Total suspended solids (TSS), particulate organic carbon (POC), and the ratio of POC to particulate nitrogen (CN) were measured using duplicate 50 ml samples on 0.7 m glass fiber filters using a Carlo Erba NA1500. Particle size distribution was measured using a Beckman Coulter Multisizer III. From the distribution, the integrated fine particle volume (FPV) of particles from 3 to 60 m in diameter was calculated. Complementary measurements of several water quality parameters for bottom water were also available from a United States Geological Survey monitoring station located 50 feet from the TACO (data available at http://waterdata.usgs.gov/nc/nwis/uv/?site_no=02092162).

Microbiological Measures

Enterococci were quantified using Enterolert™ media with Quanti-tray/2000® (IDEXX Laboratories) incubated at 41 °C for 24 hours. The most probable number (MPN) for each sample was calculated based on aggregate numbers of large and small positive wells (Hurley and Roscoe 1983). The method employed for *Vibrio* analysis was a hybrid dilution series based on our previous knowledge of concentrations as a function of salinity. Samples were filtered through 47 mm nitrocellulose (0.4 µm pores) filters, and then plated on Thiosulfate Citrate Bile Sucrose (TCBS) agar. Plates were incubated for 24 hours at 37 °C, after which time colonies were counted and recorded per dilution. Multiple sample volumes (factor of 5 between plates) were prepared with the central volume targeting 30 colonies on a plate, based on the salinity model published by Hsieh et al. (in revision). To maintain a minimum of 5 ml solution per filter, smaller volumes were diluted with sterile phosphate buffered solution (PBS). Only colonies that were green or yellow and exhibited relief from the surface of the filter were counted, and reported in colony forming units (CFU).

A common problem with microbiological analyses of water samples is the decay of concentrations in bottles stored *in situ* until recovery. Tests were conducted in the field using samples stored in the sampler during each deployment. When samples were recovered, duplicate samples were taken: one was taken back to the lab for analysis and the other left in the sampler until the next recovery (2-10 days). Following analysis of the stored sample, a decay rate was computed for each bacterial group and some particle measures (Table 1). Decay rates were only computed for samples that remained above the level of detection for the respective methods and dilutions. To complement this data, laboratory time series were also conducted using the same bottles stored at room temperature in the dark (similar to field conditions). These results were comparable to the field results and included in the average decay rates used. Given the large potential influence of this decay, samples stored longer than four days were excluded from analyses and not presented as part of this study. Decay of particle measures were neglected as they showed little decay through time.

Table 1. Decay measurements (expressed as d^{-1}) based on field and laboratory incubations

Days		TSS	POC	Enterococci	*Vibrio*
2.0	LAB	-0.07	-0.09	-0.49	-0.39
4.0	LAB	-0.06	-0.02	-0.31	-0.36
4.9	TACO	0.07	0.06	-0.75	-0.36
6.3	TACO	0.03	0.02	-0.22	-0.47
6.8	TACO	0.04	-0.11	BLD	-0.12
6.9	TACO	0.01	-0.03	BLD	-0.33
7.0	TACO	-0.01	0.00	BLD	-0.51
7.0	TACO	0.00	-0.05	-0.36	-0.11
7.0	LAB	-0.02	NM	BLD	BLD
8.0	TACO	-0.01	-0.10	BLD	-0.11
8.0	LAB	-0.05	NM	-0.36	BLD
9.0	TACO	0.00	-0.11	BLD	-0.50
	TACO avg	0.02 ± 0.01	-0.04 ± 0.02	-0.44 ± 0.16	-0.31 ± 0.06
	LAB avg	-0.05 ± 0.01	-0.06 ± 0.03	-0.38 ± 0.05	-0.38 ± 0.01
	BOTH avg	0.00 ± 0.01	-0.04 ± 0.02	-0.41 ± 0.08	-0.33 ± 0.05

NM = not measured for this test
BLD = one or both time points were below level of detection for this test

Sediment Coring

Sediment sampling in the NRE occurred on seven occasions between July and October, 2005. Sediment cores were taken to a depth of six to 24 cm using a metal corer five cm in diameter. Cores were carefully transported back to the lab. Once in the lab, overlying water was withdrawn with a pipette and a portion was collected and analyzed as a water sample (procedures above) to check for accidental resuspension during transport and handling. The core was extruded and three sub-cores (1.54 cm^2) were collected with cut syringe barrels. The top 1 cm of sediment was sectioned from sub-cores and used for various analyses. Samples for total sediment CN using a Carlo Erba NA1500 were stored in a -20^{o}C freezer prior to analysis. Samples for microbiological analyses were resuspended in 25 ml sterile phosphate buffer solution (PBS). Sediment suspensions were analyzed as water samples for both IDEXX (1:100 dilution) and TCBS filter plating (0.1 and 0.5 ml diluted in PBS). MPN or CFU were expressed per gram of total surface sediment. Samples spent 30 to 90 minutes in suspension prior to being used in these analyses and fine sediment in both trays and filters was evenly distributed.

Resuspended sediment was used for grain size analyses as well. Grain size analyses of particles (>63 µm) were done using a series of sieves (63µm, 125 µm, 250 µm, 500 µm, and 1000 µm). Sieved sub-samples were dried over night at 55-60 °C and weighed. The fine fraction (<63 µm) was resolved using the particle size distribution (as described above). Particle counts were made from 1:400 dilution of sample (50 µl of sample in 20 ml diluent).

Principal Findings

This study has made great progress in addressing a set of research questions targeting improved understanding of sediment-mediated transport of bacteria in the Neuse River Estuary:

- ***Do viable populations of enterococci and Vibrio exist in the sediment? Are they in a culturable state?*** At the study site (near New Bern, NC), significant populations of metabolically active organisms have been found in surficial sediments. The dynamics of these populations are particularly interesting. For enterococci (fecal indicator), a stable population was found in the sediments, representing a potential source for water column contamination during resuspension. Meteorological forcing altered the concentrations of *Vibrio* (genus of native bacteria that could include potentially pathogenic species) throughout the summer and fall. The advection and deposition of *Vibrio* at the study site, where significant concentrations are rare, represents a model for relocation of bacterial populations due to storms that has public health implications for other estuaries.

- ***Does sediment resuspension contribute significant amounts of enterococci and Vibrio to the water column through resuspension?*** Results show a clear linkage between turbidity and bacterial concentrations. Events where both increase are difficult to study without the *in situ* sampler and demonstrate the importance of time scales shorter than routine monitoring intervals (weeks). Results also reinforce the potential for wind (without rain) events to generate contamination not included in most event-response sampling for management and protection of public health.

- ***How does the particle loading that can occur after major precipitation events affect Vibrio populations?*** While *Vibrio* clearly depended on events, secondary fluxes (e.g., dissolved organic matter, nutrients) may exert some control on populations following rain and wind events. Observations at the study site deviated from our previous monitoring of *Vibrio* concentrations, as a function of environmental conditions. It appears that the combination of high salinity and resuspension at this site provided optimal conditions for *Vibrio* growth beyond established models. Following Hurricane Ophelia, the combination of high suspension (increased surface area for attachment) and low salinity due to runoff inputs (attachment induced due to stress) may be the mechanism responsible for the observed increase in sediment concentrations.

The results of this study represent one component of the role of resuspension in estuarine water quality by uniquely combining observations of indicator and pathogenic microorganisms and particulate matter in the NRE. Given the size of the NRE and the many beneficial uses of its water to the communities around it, this project has the potential to demonstrate the importance of resuspension to water quality. For enterococci, a key indicator of fecal contamination, we have documented high concentrations of the bacteria not only in the water column after storms, but also in sediment cores. Similar results have been found for *Vibrio*. Our results demonstrate the importance of suspended material as a contributing factor to water quality measurements taken after heavy wind events. Fecal indicators (enterococci) and native bacteria (*Vibrio*) are not the only bacteria of public health interest that are likely to be found in the NRE. Other potential, more dangerous pathogens of concern include *Salmonella*, *Campylobacter*, *Yersinia*, *Listeria*, and possibly a range of enteric viral pathogens (Mark Sobsey, pers. comm.). It is clear that understanding the particle attachment characteristics of both bacterial pathogens and indicators is vital for successful management of our estuarine waters.

Significance

- The results of this study reinforce the importance of high-frequency sampling and coring of surface sediments to complement water quality monitoring, especially for efforts targeting bacterial groups with potential human health impacts.

- Through the support gained from this project, we have also been able to successfully design and build an autonomous sampling platform that permitted *in situ* sampling during times that boat use was impossible (heavy storms) and over important time scales (hours to days). The TACO sampling platform has already gained notoriety in the small field of estuarine sediment dynamics, and several other research groups have contacted us for a blueprint. In particular, researchers working in near-shore coastal environments that are heavily impacted by stormwater runoff are interested in building similar systems to characterize plume formation and dispersion. This study represents an important part of ongoing research demonstrating the importance of resuspension over short time scales in the NRE.

- This project highlights the advances in our understanding of microbial transport attainable using *in situ*, adaptive sampling. Researchers that monitor aquatic environments are rapidly embracing the ability to observe systems on scales that are appropriate to both the processes and resources used in these environments. With respect to time, these scales are usually shorter than the frequency of monitoring measurements conducted using boats and personnel. While this study was a significant step forward in monitoring the Neuse River Estuary, the need remains to continue and expand *in situ* sampling.

- Future monitoring efforts should also incorporate sediment sampling into the regularly scheduled and event-response efforts. The interaction between bacteria, suspensions, and weather are not sufficiently understood for effective prediction of concentrations and protection of the public. Without any measure of the resident bacteria in sediments, events (e.g., wind without rain) that cause significant resuspension may go unpredicted and possibly undetected. Based on our measurements, there is a high likelihood that this is occurring for enterococci in the upper Neuse.

- There are many potentially useful measurements and advanced techniques not utilized during this project due to funding and/or time limitations. Elements that were not sampled but will be included in future TACO deployments are nutrients and dissolved organic matter concentrations. We have hypothesized in this study that the period of elevated *Vibrio* during September was due to the confluence of high mixing and salinity at the study site. These concentrations deviate significantly from models based on the whole estuary and may represent a bacterial response to a flux of dissolved resources from the sediment that, along with salt, provide unique conditions for growth beyond observations in similar salinity downstream.

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Characterization of Surface Water/Ground Water Interactions along the Tar River using Ground Penetrating Radar

Basic Information

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Publication

Title

Characterization of Surface Water/Ground Water Interactions Along the Tar River Using Ground Penetrating Radar (70215)

Problem and Research Objectives

Groundwater inputs are important to streams for their influence on stream hydrology and ecology (Hayashi and Rosenberry 2002). Spatial variability of groundwater inputs to streams is common due to aquifer heterogeneity, slope, and variability in land cover. Groundwater withdrawals may also affect groundwater inputs to streams by pirating water from them (O'Driscoll 2004, Lautier 2001). The degree to which a stream interacts with the underlying ground water system is important for a variety of scientific, practical, and legal reasons, such as wellhead protection (Nnadi and Sharek 1999), bank filtration (Sheets et al. 2002), stream ecology (Brunke and Gonser 1997), and non-point source pollution from adjacent lands (Hill et al. 1998).

In the past, various methods have been used to study surface water-ground water interactions in diverse hydrogeological settings and at various scales (Edwards 1998, Harvey and Wagner 2000, and Woessner 2000). Common techniques include seepage runs (Zelwegger et al. 1989), seepage meters (Lee 1977, Isiorho and Meyer 1999), remote sensing (Atwell et al. 1971), radioactive and stable isotope tracers (Hoehn and Santschi 1987), water chemistry (Katz et al. 1997), dye tracers (Bencala et al. 1984, Triska et al. 1993), piezometery (Lee and Cherry 1978, Geist et al. 1998), biological investigations (Stanford and Ward 1993), numerical models (Nield et al. 1994), and water temperature (Silliman et al. 1995).

Stream channel sediment hydraulic properties are typically heterogeneous (Jones and Mullholland 2002). Numerous piezometers are required to adequately characterize hydraulic properties of an active river channel. Piezometer installation and monitoring in active river channels is difficult and expensive. In the Coastal Plain of North Carolina, it is difficult to maintain river channel piezometer installations for long periods because of flooding due to tropical storms and hurricanes. Practical techniques are needed to characterize the geological framework of the active river channel that controls the river's relationship with the ground water system.

Recently, ground penetrating radar has emerged as a tool to characterize complex heterogeneities in paleochannels and floodplain settings (Naegeli et al. 1996 and Beres et al. 1999). GPR has been used in river channels to characterize the sediments adjacent to gravel-bed river channels (Naegeli et al. 1996). In this study, they dug a trench to ground-truth the GPR profiles. In the Rhine valley of northeastern Switzerland GPR surveys were conducted in step mode to characterize the glaciofluvial sediment framework. The GPR data was ground-truthed against outcrop photographs (Beres et al.1999). These studies have shown that groundpenetrating radar has the potential to improve our understanding of the sedimentary framework of active river channels, and how rivers and groundwater systems interact.

An improved understanding of river-groundwater interactions along coastal plain rivers is important because water chemistry within these river systems is strongly influenced by the connections and fluxes between river and groundwater systems (Spruill 2004). These interactions are strongly controlled by the near-channel stratigraphic framework and the surficial aquifer. The surficial aquifer that extends across the Coastal Plain of North Carolina ranges from 1-68 meters thick (Lautier 2001). It consists of fine grained sand, silt, clay, and shell materials typically of Holocene to Pleistocene in age. The complex stratigraphy of floodplain settings, active channel sediments, the surficial aquifer, and other shallow aquifers influence the direction and magnitude of ground water flows and associated nutrients to rivers along the Coastal Plain. The nature of river-groundwater interactions along coastal plain rivers is not well known. We used a two-fold approach to study groundwater interactions with the Tar River: using physical hydrograph separations and field hydrogeophysical approaches to look at long-term and spatial variations of groundwater inputs to the Tar River and the geological controls on these inputs. Our study objective was to characterize river-groundwater interactions along the Tar River.

Methodology

The distribution of floodplain and river channel sediments adjacent to coastal plain rivers is complex and requires numerous sediment cores to characterize, yet is very important to understanding river-ground water interactions and contaminant transport. Eighteen piezometers and 39 meters of split spoon cores and hand auger samples were used to characterize the subsurface and groundwater inputs along a 22 kilometer stretch of the Tar River, eastern North Carolina, USA. Additionally, 2-D and 3-D GPR data were collected using a GSSI SIR-2000 system with a 200 MHz antenna, to define the shallow stratigraphic framework. The ultimate goal was to use GPR to assess the hydraulic characteristics of floodplain and channel deposits.

Hydrograph separations and discharge analysis

Daily discharge data was obtained from the U.S. Geological Survey stream gage at Tarboro, NC (USGS Gage 02083500 Latitude 35°53'40", Longitude 77°31'59", Drainage Area - 2,183 miles^2 or 5653 km^2). This record spans the period of 1931-2002 and was used to determine long-term variations in baseflow contributions to the Tar River. In addition, the daily discharge data from the U.S. Geological Survey stream gage at Tarboro, NC was statistically analyzed for the period from 1931-2002 to determine long-term trends in discharge and discharge variability over time. U.S. Geological Survey stream flow records from Tarboro and Greenville were used to quantify seasonal downstream increases in stream flow over the period of record (1997-2005).
The Greenville gage has a record from 1997-present (USGS Gage 02084000 Latitude 35°37'00", Longitude 77°22'22", Drainage Area-2,660 miles^2 or 6890 km^2). To determine large-scale ground water inputs to the Tar River, differences in baseflow were compared between Tarboro and Greenville.

Mechanical hydrograph separation was performed on the discharge data using a hydrograph analysis model (W.H.A.T.- Web-based Hydrograph Analysis Tool) developed by Lim et al. 2005. The local minimum method was chosen to separate the stream hydrograph into baseflow and stormflow components. This method analyzes each daily measurement of streamflow. A discharge point is considered the local minimum if it is the lowest discharge in one half the interval minus 1 day (0.5(2N-1) before and after the date being considered (Sloto and Crouse 1996). The baseflow values for each day between local minimums are estimated by linear interpolations, the lowest points on the hydrograph are connected by straight lines, anything above this line is considered stormflow and anything below is considered baseflow. The line for the entire data series of daily streamflow from 1931-2003 was estimated using the model and the associated stormflow and baseflow components were estimated.

Geophysical surveys

Ground penetrating radar (Geophysical Survey Systems Inc., Subsurface Interface Radar System-2000 with 200 MHz antenna) was used to characterize heterogeneity in the underlying active river channel sediments. The stratigraphy beneath the river bottom was imaged to depths up to approximately 5 meters. GPR transect data was collected in continuous mode, and at higher spatial resolution at targeted sites to characterize subsurface stratigraphy along 18 segments of the 22 km study reach. To reference GPR data we used sediment logs and hydraulic conductivity information from borings within and adjacent to the river channel. The GPR antenna was floated in a rubber raft and data were collected in continuous mode (Figure 12). Navigation was acquired using a Trimble GPS, and differentially corrected position data were linked to the GPR data by waypoint and scan number. Twenty-one surveys included cross-sections perpendicular to the river channel.

GPR data was processed using Radan v.7 (copyright Geophysical Survey Systems, Inc.). Raw data were filtered to remove background noise and gain was adjusted to bring out horizons and other reflectors. Processed GPR data was then uploaded into Canvas v.8, where color interpretations, scale and direction were added.

Sediment Sampling and Ground Water/Surface Water Monitoring

Eighteen piezometers and 39 m of split spoon cores and hand auger samples were used to characterize the subsurface near the Tar River. Split-spoon samples of floodplain and active channel sediments were obtained during piezometer installation to reference GPR transects. Slug tests were performed at all piezometers to characterize the hydraulic properties of the surrounding sediments. The Bouwer and Rice Slug Test Method was used (Fetter 1994). Water level changes during slug tests in each piezometer were recorded using Hobo water level recorders.

Piezometers were used to characterize the interaction of ground water and surface waters of the study reach consisting of a 22 km stretch along the Tar River, Pitt County, North Carolina, USA. We selected five locations for ground water and surface water monitoring, as indicated in Figure 6. At four of the five locations along the Tar River nested piezometers were installed adjacent to the river at shallow and deeper depths of 4 and 7m (respectively) below the channel sediment-water interface. These installations were performed with a hollow-stem auger drill rig and sediment cores were collected using a split-spoon sampler. The fifth location at US Route 264 was not instrumented with nested piezometers , but was instrumented with channel piezometers. Channel piezometers were installed at all sites within the river channel. Channel piezometers had screens that were 0.76 m long and the bottom of the screens were typically installed approximately 1.83 m below the sediment-water interface. Piezometers were typically secured to large trees located along the stream banks (Figure 13). Casing elevations for all piezometers were surveyed using a laser theodolite.

Hydraulic conductivity was estimated for 18 piezometers. River-ground water head gradients were measured in each piezometer every two weeks since September 2005. In addition, on the south side of the river the surface water and ground water levels and temperatures were recorded with HOBO pressure transducers at 30 minute intervals and downloaded monthly using a laptop computer. Measured hydraulic conductivity values were used to calculate ground water flux to and from the river channel (Darcy's Law), using head gradients based on those measured in the river.

Water temperature recorders and pressure transducers were installed in stream channel piezometers adjacent to the river. Surface water temperature and stream stage were also recorded at these locations. Ground water temperature and water level measurements were recorded at all five sites to quantify temporal variations in ground water flux to the river channel. Water temperature and hydraulic head data were downloaded on a monthly basis.

Principal Findings

Long-term (1931-2003) baseflow analysis for the Tar River at Tarboro indicated that baseflow is the major component of river discharge along the Tar (60% on average). There have been slight changes in baseflow discharge along the Tar over this time period. In general, baseflow has become more variable over time, with an increase in the occurrence of high and low flow events. These changes may be due to changes in climate and/or land-use over time.

Urbanization, stormwater runoff, wastewater discharge, water supply withdrawals, and interbasin transfers may all affect the frequency, timing, and magnitude of baseflow discharge over time in the Tar River basin. In addition, subtle changes in climate that have occurred over the last 50 years may also influence baseflow discharge. Work done by Boyles (2000) indicates that the climate in North Carolina has been slowly changing since the 1950s with a common pattern of increased rainfall during fall and winter and decreased rainfall in summer months. This change in rainfall patterns may explain trends in Tar River baseflow since increased precipitation during high baseflow periods in the winter could cause the occurrence of high baseflows to increase, whereas lesser rainfall in summer months could result in a decrease in occurrence of low baseflows. If this rainfall trend continues the summer low baseflows along the Tar may be susceptible to further decreases in the future.

Seasonal variations in baseflow are common along the Tar and the extreme low baseflows are typical occurrences in the summer months due to increased solar radiation, warmer temperatures, increased evaporation, and plant uptake of surface water and ground water. During summer months the Tar River is vulnerable to low baseflows that are related to recent weather patterns and this time period is likely to be the most sensitive to future climate change in the region. A comparison of annual baseflow and rainfall grouped by season (dormant vs. growing) showed dormant season rainfall to be most important to annual groundwater recharge and baseflow generation within the Tar Basin. The amount of dormant season rainfall that occurs annually has a greater influence on the baseflow discharge to the Tar than rainfall during the growing season. If rainfall amounts change in the region as a result of climate change, the modifications of rainfall distribution throughout the year will be important to determining the effects on baseflow to this and other coastal plain rivers. Typically, the greatest variability in baseflow occurred during the months of September and October, due to hurricane effects on baseflow. Baseflow magnitudes can be extremely low or high during these months depending on recent storm activity. If the frequency and magnitude of hurricane and tropical storm landfalls change in the future this will have an effect on baseflow discharge to the Tar, particularly during the fall.

Baseflow inputs along the Tar typically increase downstream from Tarboro to Greenville. However, there are several time periods where baseflow decreases downstream, indicating channel losses or large amounts of evapotranspiration between the gauges. During our study, stream losses were also indicated in several piezometers along the Tar. For the period of April 1997-Feb 2006 the average baseflow increase downstream was 199 ft^3/s or a 20% increase relative to baseflow at Tarboro. This translates to groundwater inputs of approximately 9 ft^3/s/mile. Seasonally there is significant variation in baseflow increases downstream ranging from 4 ft^3/s/mile during summer to 17.5 ft^3/s/mile during winter.

Variations in river-groundwater interactions were noticeable with time and distance along the Tar and were observed in channel and nested piezometers. The river was typically gaining groundwater, however several instances of losing segments were observed. Hydraulic conductivity variations were large between sites, with the range of hydraulic conductivity measured in piezometers of $10^{-2.03}$-$10^{-7.03}$ cm/s, with a median value of $10^{-3.38}$ cm/s, representative of sandy channel sediments. A pattern was evident in the hydraulic conductivity data, the channel sediments on the north side of the river typically had greater hydraulic conductivity values when compared to those on the south side of the river. Sediment cores and GPR data indicate that there are differences in sediment type that are related to the channel asymmetry commonly observed along the Tar. Generally the south side of the river has steep banks, and is underlain by Pliocene to Cretaceous sediments that often contain marine or estuarine clays that tend to have low hydraulic conductivities. On the north side of the river the floodplain is extensive, the topography is gentle, and the underlying sediments tend to be sandy deposits that are likely reworked alluvial sediments. These sediments tend to be more permeable, hence groundwater inputs on the north side of the river tend to be greater than those on the south side of the river. Clay sediments on the south side of the river may also cause groundwater inputs to occur as springs or seeps which were not inventoried in this study. The general presence of sandy sediments along the north side of the river is one reason for the high concentration of sand and gravel pits on the north side of the river when compared to the south side.

Cross-sections of the river channel were typically asymmetrical, with the steeper banks almost always located on the southern side of the river. The channel asymmetry that occurs along the Tar is noticeable for the entire study reach and this pattern is also common along other Coastal Plain Rivers in Virginia, North Carolina, and South Carolina, indicating that these differences in hydraulic conductivity and the groundwater fluxes may also occur at a regional scale. Several studies have indicated that this floodplain asymmetry may be related to uplift in the region, causing rivers to incise to the south and preserving reworked fluvial deposits to the north (Sexton 1999 and Soller 1988).

Another pattern related to channel asymmetry was observed in the groundwater specific conductance data. Typically the specific conductance of groundwater underlying the Tar varied depending on what side of the river it was sampled along. This is likely related to differences in residence time and groundwater flowpaths adjacent to and underlying the river. Greater hydraulic conductivity sediments were found to typically have lower groundwater specific conductance values. This relationship between hydraulic conductivity and specific conductance in channel groundwaters may be useful in future studies to quantify river groundwater interactions and channel hydraulics of this and other coastal plain rivers. Ground penetrating radar was found to be a useful tool in determining the bathymetry of the river channel and the nature of the sediments underlying the river channel. The stratigraphy beneath the river bottom was imaged to depths up to approximately 4 to 5 meters using GPR transect data collected in continuous mode. Data collected along the Tar River indicated that GPR appears to be well-suited to characterize the variability of active channel sediment properties along and perpendicular to the river channel at depths of several meters below the channel.

Two notable limitations to the use of GPR in these coastal plain systems exist, first the signal is attenuated in clay sediments so the GPR data may only indicate the depth to the first clay layer. Second, as salinity increases in coastal plain rivers towards the coast, the GPR signal becomes attenuated in the water column.

The hydrograph separation analysis indicated that baseflow (groundwater) comprises 60% of the Tar River streamflow over time. Hydrograph separations and discharge analysis revealed that baseflow contributions to the Tar River have changed since the 1930s. The magnitude and variability of baseflow feeding the Tar River have changed slightly, daily mean baseflow has decreased by 49 cubic feet per second (cfs) (1.34 m^3/s) and daily minimum baseflows have dropped 33 cfs (0.93 m^3/s). The variability of baseflow within a given year as measured by the coefficient of variation has increased by 8% when comparing data before 1971 and after 1971.

Ground water head data indicated that the shallow water table aquifer had a high degree of complexity on a local scale. Sediment samples and slug tests conducted in stream-channel piezometers indicated that the geology between the north and south sides of the river varied significantly, with a direct effect on the movement of ground water through the river channel.

Ground water flux into and out of the channel varied between the north and south sides of the river by as much as four orders of magnitude. The differences appear to be related to stratigraphic differences between the north and south sides of the river. GPR transects successfully located key hydrogeologic elements such as clay layers (confining beds), sand lenses, and active channel bedforms, which had a direct impact on the movement of ground water. GPR is a useful tool for the characterization of subsurface sediments underlying river channels and can provide information on the interactions between the shallow water table aquifer and surface waters along coastal plain rivers.

Future work will include various field tasks to improve the understanding of the relationship between GPR transects and sediment hydraulic properties. A sediment sampling program is being developed to obtain deeper sediment samples underneath the river channel (drill /vibracore ~ 5-10m depth) to develop an improved understanding of GPR profiles and their relationships with groundwater inputs. Future groundwater monitoring at the sites will help to develop relationships between groundwater flux and specific conductance of ground water along the Tar and we will seek to monitor specific conductance during storm events to determine how groundwater fluxes vary during runoff episodes. In addition more hydraulic conductivity data will be collected along the river in temporary wells to better determine the spatial variability of hydraulic conductivity in the river channel sediments and their relationships to groundwater flux and ground penetrating radar data.

Significance

Future work will include various field tasks to improve the understanding of the relationship between GPR transects and sediment hydraulic properties. A sediment sampling program is being developed to obtain deeper sediment samples underneath the river channel (drill /vibracore ~ 5-10m depth) to develop an improved understanding of GPR profiles and their relationships with groundwater inputs. Future groundwater monitoring at the sites will help to develop relationships between groundwater flux and specific conductance of ground water along the Tar and we will seek to monitor specific conductance during storm events to determine how groundwater fluxes vary during runoff episodes. In addition more hydraulic conductivity data will be collected along the river in temporary wells to better determine the spatial variability of hydraulic conductivity in the river channel sediments and their relationships to groundwater flux and ground penetrating radar data.

Ground penetrating radar surveys should be run along all major coastal plain rivers in North Carolina and correlated with the geology. These data would help indicate locations where the rivers are in connection with important aquifers or are separated by aquicludes. A map of these features would be very useful in determining areas where groundwater management may affect rivers or vice versa.

Ground penetrating radar surveys should be run along piedmont and mountain rivers in North Carolina. Future work should evaluate the effectiveness of ground penetrating radar as a subsurface investigation tool in these settings.

In this study groundwater fluxes were typically several orders of magnitude larger on the north side of the river when compared to the south side. Hydraulic characteristics of sediment along the Tar River were dependent on the side of the river they were measured along. The river is in contact with Pliocene or older marine or estuarine sediments that tend to have clays and silts on the south side of the river. On the north side, the river is frequently in contact with reworked fluvial sediments which tend to be better sorted and coarser, typically fine to coarse sands. From observations made by other researchers this pattern is quite common along other coastal plain rivers in Virginia, North Carolina, South Carolina and Georgia. If similar behavior exists in other coastal plain rivers it is likely that the effects of land-use will vary based on the side of the river. Contaminants from septic systems, leaking underground storage tanks, and other anthropogenic sources on the north side of the river will be more likely to migrate to the river when compared to similar land-use on the south side of the river. Future work should address the variability in contaminant transport due to floodplain asymmetry along coastal plain rivers.

The degree of asymmetry of the Tar floodplain is notable. The Tar has been migrating to the south for at least thousands of years. The incision of the river and the presence of terraces to the north has allowed for the preservation of Holocene and Pleistocene sediments on terraces to the north. These sediments may hold important information with regards to past climate, hurricane occurrence, and flood frequency along the Tar. With new age dating technologies, such as optically stimulated luminescence (OSL), dating of these terraces and the various sediments underlying them may help unravel the past climate of the region.

Measurement of hydraulic conductivity in channel sediments is necessary to determine the hydraulic properties of river channels and their interactions with groundwater systems. However, this requires installing numerous piezometers or wells throughout a river basin which can be very labor intensive and expensive. Based on our hydraulic conductivity data obtained from channel piezometers along the Tar and their relationship with specific conductance data obtained from the same piezometers it may be possible to develop a relationship between hydraulic conductivity and specific conductance of groundwater as a means to estimate hydraulic conductivity in the channel. Future work will aim to evaluate the effectiveness of this approach.

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A Comparison of Drought Tolerance in Common Herbaceous Wetland Macrophytes as Indicated by Plant Growth, Water Status, and Oxidative Stress

Basic Information

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Title

A Comparison of Drought Tolerance in Common Herbaceous Wetland Macrophytes as Indicated by Plant Growth, Water Status, and Oxidative Stress (70216)

Problem and Research Objectives

Part 1: Drought Susceptibility in Emergent Wetland Angiosperms: a Comparison of Water Deficit Growth in Five Herbaceous Perennials

Wetland ecosystems by nature are integrally tied to hydrology (Collins and Battaglia 2001; De Steven and Toner 2004). Consequently, mechanisms that alter prevailing hydrologic conditions, including mechanical and climateological, may sharply influence wetland function and value. Whereas water fluctuations involving episodic inundations and desiccation can facilitate increased plant diversity (Bush et al. 1998; Olson 2004; Mulhouse et al. 2005), extended periods of submersion or drought may foster decreased plant diversity, including the possible promotion of monotypic stands of invasive aquatic species (Pezeshki et al. 1998; Galatowitsch et al. 2000; Bonilla-Warford and Zedler 2002; Kercher and Zedler 2004). Williams and Hudak (2005) suggested that drought and low water availability are among the greatest threats to constructed wetlands in north-central Texas, and that herbaceous species (e.g., *Sagittaria latifolia*, and *Eleocharis quadrangulata*) are particularly vulnerable to water deficits. Similarly, Holland et al. (1995) reported the loss of several urban wetlands in Oregon due to drought-associated water deficits, and that drier-end wetlands (e.g., seasonally flooded) were often more susceptible to human disturbances. Moreover, droughts beyond recoverable time periods for established macrophytes, could eliminate valuable habitat necessary for both migratory waterfowl and indigenous wildlife (Sorenson et al. 1998; Williams and Hudak 2005).

Despite selective environmental pressures that would seemingly promote tolerance to both flooding and exposure, many wetland plants are unable to withstand even short periods of water scarcity (Steudler and Touchette 2003; Mulhouse et al. 2005; Williams and Hudak 2005). This is a fundamental concern, as wetlands are considered to be among the most threatened habitats globally (Gopal and Junk 2000). In a paper published in 2002, Jacobs et al. noted our lack of knowledge and the need for further studies on how extreme water table fluctuations influence wetland vegetation. While, there is growing interest in defining drought-associated changes in natural vegetation, driven primarily by climate change models predicting increases in drought severity and duration in the mid-latitudes, much of this work has been focused on grasslands and forests (e.g., Mangan et al. 2004; Breshears et al. 2005), and comparatively fewer studies have exclusively considered wetland systems (Sorenson et al. 1998; Dawson et al. 2003). Therefore, the purpose of this study was to evaluate growth responses of five herbaceous wetland species (monocots *Carex alata*, *Juncus effusus*, and *Peltandra virginica*, and dicots *Saururus cernuus*, and *Justicia americana*) to simulated drought conditions (up to 6 wks with 1-in-25 yr precipitation low and water withdrawal). Emergent herbaceous plants were selected in favor of woody vegetation because of their ease of manipulation under controlled greenhouse conditions and due to their relative vulnerability to low water supply (Williams and Hudak 2005). For this study, it was anticipated that the herbaceous wetland plants would demonstrate varying degrees of drought tolerance, ranging from rapid vegetative die-off to minimal adverse effects. Thereby, the results of this study could be useful in providing a framework to gauge relative tolerance to drought among different wetland plant species. As a secondary application, this study could also provide valuable insight into proper plant selection for wetland restoration/ creation in areas with sporadic water availability (including stormwater retention wetlands).

Freshwater wetlands often exist as transitional areas between terrestrial uplands and deep open water. Thus they are fundamentally sensitive to changes in hydrology. Some of the more dramatic changes in wetland water supply occur during extensive droughts, where both precipitation and soil water table markedly decline. While it is generally understood that herbaceous wetland macrophytes are more sensitive to decreased water availability than wetland trees, the degree of susceptibility among wetland herbs remains relatively unexplored. Therefore, the purpose of this study was to evaluate plant growth responses of five herbaceous wetland species (monocots *Carex alata*, *Juncus effusus*, and *Peltandra virginica*, and dicots *Saururus cernuus*, and *Justicia americana*) to simulated drought conditions (up to 6 wks in a 1-in-25 yr precipitation low with receding soil water tables).

Part 2: Drought Tolerance Versus Drought Avoidance: A Comparison of Plant-Water Relations in Herbaceous Wetland Plants Subjected to Water Withdrawal and Repletion

Water deficits can result in significant declines in overall plant productivity and with increasing water scarcity can promote high rates of plant mortality. While a number of studies have addressed physiological acclimations to low water availability on agriculturally important species, comparatively fewer studies have considered wetland vegetation. This discrepancy is not surprising when considering aquatic plants, by nature, are constrained to environments characteristically dominated by excessive water. Nevertheless, wetlands represent an intermediate between terrestrial and open-water systems, and are thus fundamentally sensitive to changes in hydrology. Seemingly slight changes in water level may result in substantial adjustments in both species richness and productivity (Mitch and Gosselink 1986; Amlin and Rood 2002). Furthermore, when considering wetland hydroperiods, some systems (e.g., intermittently exposed, and seasonally flooded wetlands) can sustain extended periods of flooding and exposure (Cowardin et al. 1979; Wilcox 2002). In extreme cases, seasonally flooded wetlands will undergo enormous fluctuations in water availability as soil water tables oscillate from 50 cm aboveground to 100 cm belowground over a period of weeks (Mitsch et al. 1979). Furthermore, the degree of water deprivation can worsen during episodes of unseasonably low precipitation or drought; influencing both the degree of water deficit and extending the period of which these aquatic plants must tolerate water stressed conditions.

Drought, as defined as the absence of appreciable precipitation over an extended period of time allowing for the depletion of soil moisture with a concomitant injury to plants

(Kramer 1983), can influence natural plant distributions and productivity. Adjustments by plants to drought may involve avoidance and/or tolerance. Drought avoidance includes responses such as increased stomatal and cuticular resistance, changes in leaf area and anatomy, and changes in leaf orientation (Morgan 1984; Jones and Corlett 1992; Zlatev 2005). Whereas, drought tolerance involves maintaining adequate cell turgor, while preventing disruptions in cellular metabolism (Munns 1988; Savé et al. 1993). Tolerance has been attributed to at least two mechanisms - osmotic adjustment (involving inorganic ions, carbohydrates, and organic acids), and changes in cellular/ tissue elasticity (i.e., bulk elastic modulus; ϵ).

Studies suggest that plant metabolic processes are more responsive to turgor and cell volume conditions rather than fluctuations in water potential (Jones and Corlett 1992; Zlatev 2005). While dehydration of cells during water deficits can result in lower osmotic potential by confining existing solutes into smaller volumes, true osmotic adjustment necessitates the accumulation and buildup of these ions or compounds in excess of pre-stress conditions (Bray 1993). Drought-induced changes in tissue elasticity can also modify the relationship between turgor pressure and cell volume contributing further to drought tolerance (Blake et al. 1991; Saito and Terashima 2004).

Understanding the basis of plant water relations in emergent wetland herbs may provide some insight into the capacity of wetland species to maintain metabolic activity during extended periods of drought. Furthermore, any species that is capable of modifying their water relations during periods of low water availability may have a competitive advantage over plants incapable of altering water status. Therefore, the purpose of this study was to evaluate the effects of water stress on plant water relations in five herbaceous wetland species (monocots *Carex alata* Torr., *Juncus effusus* L., *Peltandra virginica* L., and dicots *Saururus cernuus* L., *Justicia americana* L. Vahl.). The ability of these plants to adjust to simulated drought conditions was evaluated using pressure-volume isotherms, which provided insights into leaf osmotic adjustment, tissue elasticity, plant water potential, and turgor pressure. Furthermore, plant productivity and water use efficiencies (WUE) were also evaluated to gain additional insights into wetland plant responses associated with short periods of water deficit and repletion.

Methodology

Part 1: Drought Susceptibility in Emergent Wetland Angiosperms: a Comparison of Water Deficit Growth in Five Herbaceous Perennials

Growth Conditions

Five herbaceous obligate wetland plants (*Carex alata* Torr., *Juncus effusus* L., *Peltandra virginica* L., *Saururus cernuus* L., and *Justicia americana* L. Vahl.) were grown under controlled greenhouse conditions in 20 L microcosms containing natural lake water (~ 25 cm) and sediments (~20 cm) at plant densities comparable to those observed in natural field populations (60-100 shoots m^{-2}). Prior to the initiation of water deficits, the plants were allowed to acclimate within the microcosms for 4 weeks to ensure plant establishment as indicated by active growth and productivity. Drought conditions were initiated by removing surface water, over a 1-wk period, until the water level reached the upper portion of the substratum. During this period drought-treated plants (n=5 for each treatment) received watering of foliage and sediment comparable to a 1-in-25 year low precipitation rate and periodicity (1.0 cm water at 7-day intervals; based on summer precipitation data from the central Piedmont region of North Carolina over a 75 yr period). Control microcosms (n=5) were watered at mean summer precipitation rates and periodicity (2.9 cm water at 7-day intervals), while maintaining water levels at 25 cm above the substratum. Drought treated microcosms were exposed to 2-, 4-, or 6-wks of simulated drought, followed by a 2- to 6-wk recovery period consisting of pre-drought conditions (i.e., mean summer precipitation rates and periodicity, and 25 cm of standing water). Throughout the study, temperature (daily maximum and minimum) and relative humidity (RH; daily maximum and minimum) were monitored using a temperature and humidity data logger (Hobo H8 logger, Onset Computer Corporation, Bourne, MA).

Growth and Productivity Measurements

Growth measurements (n = 5) were recorded at weekly intervals and included phytomass (above- and belowground tissue), leaf area (LA), relative growth rate (RGR), and unit leaf rate (ULR; also termed net assimilation rate). Phytomass was determined by carefully removing plants from microcosms, as facilitated by the soft sediments which minimized loss of belowground tissues. The plants were sorted between aboveground- (stems and leaves) and belowground tissues (roots and rhizomes), and dried (70°C) to constant weight. LAs were measured using scanned digital images (7100 USB scanner, Visioneer Inc., Pleasanton, CA) of individual leaves against a white background (as described in Ferris et al. 2001, with modifications described in O'Neal et al. 2002). The images were then imported into an image processing and analysis program, Scion Image (Scion Corporation, Frederick, MD), for leaf area calculations. RGRs were calculated based on the production of dry matter at weekly intervals as defined by the following equation:

$$RGR = \frac{\ln W_{x+1} - \ln W_x}{t_{x+1} - t_x}$$

where W is the weight of dry matter reported for consecutive collection periods (x and x+1), and t represents the time interval (in wks) between collections. While RGRs are considered a fundamental measure of plant productivity and are important in the comparisons of plant performance between species and/or treatment effects, it is limited in its ability to evaluate causal factors that shape plant productivity (Beadle 1985). Differences in productivity are often associated with the plant's assimilation capacity as reflected in leaf area. Therefore, ULR measurements are often considered in growth analyses because they encompass both dry-mass production and assimilation capacity. ULR is defined as the increase in plant dry-mass over time per unit of assimilatory material, and was calculated in this study as follows:

$$(W_{x+1} - W_x)\ (\ln LA_{x+1} - \ln LA_x)$$

$$ULR = \frac{}{(LA_{x+1} - LA_x)\ (t_{x+1} - t_x)}$$

where LA (as described above) and W were measured at consecutive sample periods (Beadle 1985; Hunt 1990).

Data Analysis
Along with other measurements, mean percent differences from controls for growth parameters were presented for comparisons. These values were calculated as follows:

$$\%\ \text{Difference} = \left(1 - \frac{\text{treatment}}{\text{control}}\right) *100$$

and allowed for general evaluations between treatments and their respective controls. These values were not analyzed statistically, and were used to merely represent the magnitude of change when significant differences were observed.

A repeated measure ANOVAs (general linear model [GLM] procedure) were performed using SAS statistical software (SAS Institute Inc., Cary, NC) on RGR, ULR, phytomass, leaf area, and aboveground/belowground tissue ratios. Pre-planned comparisons were performed for each sample collection using LS-means to evaluate treatment responses at each time interval. All comparisons were considered statistically significant when p-values were less than 0.05.

Part 2: Drought Tolerance Versus Drought Avoidance: A Comparison of Plant-Water Relations in Herbaceous Wetland Plants Subjected to Water Withdrawal and Repletion

Growth Conditions
Five herbaceous obligate wetland plants (*Carex alata* Torr.*, Juncus effusus* L., *Peltandra virginica* L., *Saururus cernuus* L., and *Justicia americana* L. Vahl.) were grown under controlled greenhouse conditions in 20 L microcosms containing natural lake water (~ 25 cm) and sediments (~20 cm) at plant densities comparable to those observed in natural field populations (60-100 shoots m^{-2}). Prior to the initiation of water deficits, the plants were allowed to acclimate within the microcosms for 4 weeks to ensure plant establishment as indicated by active growth and productivity. Drought conditions were initiated by removing surface water, over a 1-wk period, until the water level reached the upper portion of the substratum. During this period drought-treated plants (n=5 for each treatment) received watering of foliage and sediment comparable to a 1-in-25 year low precipitation rate and periodicity (1.0 cm water at 7-day intervals; based on summer precipitation data from the central Piedmont region of North Carolina over a 75 yr period). Control microcosms (n=5) were watered at mean summer rates and periodicity (2.9 cm water at 7-day intervals), while maintaining water levels at 25 cm above the substratum. Drought treated microcosms were exposed to 2 wks of simulated drought, whereas drought-recovered microcosms consisted of 2 wks of simulated drought

followed by a 2 wks return to pre-drought conditions (i.e., mean summer precipitation rates and periodicity, and 25 cm of standing water).

Plant-Water Status

Leaf relative water content (θ) was evaluated according to Joly (1985) using the following equation:

$$\theta = \frac{(W_f - W_d)}{(W_t - W_d)}$$

where W_f was the fresh weight recorded during collection, W_t was the turgid weight, and W_d was the oven dry weight (70°C, until constant weight). Pre-dawn Ψ_{xylem} were determined by using a Scholander pressure chamber (Model 1000, PMS Instrument Co., Albany, Oregon, USA) on young fully extended leaves enclosed within foil bags for 2 hrs.

A Scholander pressure chamber was used to determine Ψ_{leaf} on leaves from each species to compare water status of control and drought recovered plants. Water deficits were established by exposing leaves to transpirational water loss on a laboratory bench. Turner et al. (1984) favored this approached, over elevated pressurization, because it minimized the possibility of Ψ disequilibria between apoplastic and symplastic tissues. Pressure-volume isotherms were constructed by plotting the reciprocal of Ψ_{leaf} against θ. First order regression analyses were performed on the linear portion of the curve. This line is equivalent to leaf Ψ_{π}, and can be used to determine θ at turgor loss point (θ^{tlp}), the osmotic potential at full saturation (Ψ_{π}^{sat}), osmotic potential at turgor loss point ($\Psi\pi^{tlp}$), and symplastic volume of the total water content (θ_{sym}). Bulk elastic modulus (ϵ) was obtained from the initial part of the curve, following Ψ_{π} correction, as described in the following equation:

$$\epsilon = \frac{d\ \Psi_p}{d\ \theta}\ \theta_{sym}$$

where changes in tugor potential (Ψ_p) were compared against changes in θ, and relative symplastic water content (Koide et al. 1989).

Data collected from pressure-volume isotherms were also used to generate Höfler diagrams for control and drought accumulated plants. The values derived for each point within the diagram represents the mean value among replicates with comparable water content. Because θ for these replicates were within rages, the best polynomial fit represents a population estimate for the mean value within that range of water content. Consequently, data represented by Höfler diagrams may not precisely match the data derived from P-V analysis on each individual plant.

Transpiration and Water Use Efficiency

A gravimetric technique was used to estimate transpiration on whole plants according to Slavík (1974) and Kramer (1983). This involved careful removal of plants from the microcosm and enclosing the sediment/ root complex in polyethylene bags to prevent water evaporation from the soil. The soft sediment used during this experiment facilitated the plants removal with negligible damage to root structure. The plants were maintained within a greenhouse under environmental conditions comparable to plants within the microcosms (i.e., 10% neutral density shading of ambient light; 26 ± 2°C; 45.4 ± 3.2 % RH). Transpiration measurements were integrated over the course of the day with measurements reported at 60 min intervals. Drought treated plants were restricted to sediments collected from the 2-week drought treatment with no additional water added, whereas control and drought-recovered plants were placed in saturated soils with standing water to replicate typical water supply.

Water use efficiency (WUE) was estimated according to Kramer (1983; as modified by Gaiser et al. 2004, and Gao et al. 2004), defined as the amount of water used (via transpiration) per unit dry matter produced.

$$\text{WUE} = \frac{\text{Water used in transpiration}}{\text{Dry mater produced}}$$

Dry matter productivities were estimated as the mean increase in total biomass (both aboveground and belowground tissues) reported during the week of transpiration measurements (n = 5 for each microcosm).

Growth and Productivity

Growth measurements included both absolute growth (i.e., the amount of dry matter produced plant^{-1} day^{-1}), and relative growth (i.e., the amount of dry matter produced gram^{-1} [of plant] day^{-1}). In this case plants were collected at weekly intervals (n=5) and the increase in total dry mass (above- and belowground biomass collectively) were used to estimate plant productivity/ growth. Survival measurements were estimated as the percent change in living plants over the two week drought treatment.

Data Analysis

Data for controls and drought treated plants were compared using a non-parametric Kruskal-Wallis one-way analysis of variance (ANOVA), followed by a Tukey multiple comparison test for post-hoc evaluations. All comparisons were considered significant at a p-value of less than 0.05.

<u>Prinicipal Findings</u>

Report 1: Drought Susceptibility in Emergent Wetland Angiosperms: a Comparison of Water Deficit Growth in Five Herbaceous Perennials

Of the five species studied, three (*J. americana*, *S. cernuus*, and *J. effusus*) had no survivors after six weeks of simulated drought. *J. americana*, appeared to be the most sensitive to water deprivation with a 67% decrease in plant phytomass and an 85% decrease in leaf area with only two weeks of drought, and complete mortality after three weeks. While *P. virginica* also had significant decreases in biomass, leaf area, relative growth rate (RGR) and unit leaf rate (ULR), in as little as two weeks of drought, no noticeable decreases in survival were observed. In contrast, when *J. effusus* experienced between 2- and 4-weeks of water deprivation, there were significant increases in RGR, ULR, phytomass, leaf area, and shoot: root ratios. *S. cernuus* and *C. alata* remained relatively unaffected following four weeks of drought; however by the fifth week, there were significant declines in leaf area for both species. In general, this study provides experimental evidence on how herbaceous macrophytes grow under drought conditions. This basic understanding is fundamental if we are to develop better working models on how wetlands will respond to changing environmental conditions that lead to decreased water supply.

In conclusion, our current understanding on how emergent wetland perennials respond to water deficits is incomplete. This study provides experimental evidence on how plant growth and productivity can be altered during drought events. In general, plants in this study were severely impacted (*J. americana*), moderately impacted (*S. cernuus*, and *P. virginica*), largely unaffected (*C. alata*), or benefited (*J. effusus*) by a combination of decreased precipitation and water table drawdown that simulated a 1-in-25 year drought.

Table 2. Summary of growth responses observed in five species of herbaceous wetland plants to 2-, 4-, and 6-wks of drought. Parameters include relative growth rate (RGR), unit leaf rate (ULR), total plant phytomass (Biomass), leaf area, shoot: root ratio (S:R ratio), and percent survival. Numerical values represent the mean maximum percent difference observed from controlled (emergent) conditions, and the values in parentheses represent the week when the greatest difference was observed. The plus or minus signs indicate an increase/ positive response or a decrease/ negative response. Survival is based on the number of individuals remaining as a percent of the initial (for weeks 4 and 10). NC was used to indicate no significant change from the control (repeated measures ANOVA).

						% Survival	
Species	RGR	ULR	Biomass	Leaf Area	S:R Ratio	Week-4	Week-10
Justicia americana							
2-wk	NC	NC	$-67^{(7)}$	$-85^{(7)}$	NC	75 ± 15	87 ± 7
4-wk[5]	NC	NC	$-46^{(5)}$	$-19^{(5)}$	$+83^{(5)}$	61 ± 22	0 ± 0
6-wk[5]	NC	NC	$-48^{(5)}$	$-35^{(5)}$	NC	54 ± 8	0 ± 0
Saururus cernuus							
2-wk	NC	NC	NC	NC	$+369^{(7)}$	84 ± 19	80 ± 18
4-wk	NC	NC	NC	NC	$+351^{(5)}$	83 ± 24	86 ± 15
6-wk[7]	NC	NC	$-75^{(7)}$	$-32^{(7)}$	NC	32 ± 8	0 ± 0
Peltandra virginica							
2-wk	$-231^{(4)}$	$-235^{(4)}$	$-53^{(10)}$	$-64^{(5)}$	NC	91 ± 7	103 ± 10
4-wk	$-395^{(6)}$	$-394^{(6)}$	$-64^{(8)}$	$+44^{(3)}/-77^{(8)}$	NC	90 ± 7	98 ± 4
6-wk	$-226^{(6)}$	$-356^{(7)}$	$-84^{(9)}$	$-82^{(9)}$	NC	93 ± 13	94 ± 9
Juncus effusus							
2-wk	$+1062^{(4)}$	$+1579^{(4)}$	$+306^{(10)}$	$+180^{(4)}$	NC	86 ± 18	92 ± 9
4-wk	$+692^{(4)}$	$+842^{(4)}$	$+198^{(10)}$	$+136^{(10)}$	NC	115 ± 30	96 ±24
6-wk[8]	$+655^{(4)}$	$+925^{(4)}$	NC	$+175^{(4)}$	$+209^{(8)}$	49 ± 4	0 ± 0
Carex alata							
2-wk	NC	NC	NC	NC	NC	113 ± 9	108 ± 13
4-wk	NC	NC	NC	NC	NC	126 ± 22	122 ± 25
6-wk	NC	NC	NC	$-47^{(10)}$	NC	98 ± 3	79 ± 4

Part 2: Drought Tolerance Versus Drought Avoidance: A Comparison of Plant-Water Relations in Herbaceous Wetland Plants Subjected to Water Withdrawal and Repletion

In this study, simulated drought resulted in significant decreases in xylem water potential (Ψ_{xylem}) for all five species, suggesting that these plants were physiologically affected by water deficit. Four of the five species showed outward signs of drought avoidance, including significant reductions in transpiration (*C. alata*, *P. virginica*, *J. americana*, and *S. cernuus*) and modifications of leaf area (*P. virginica* and *J. americana*). Interestingly, while adjustments in transpiration were observed for most plants during the dry period, no significant changes in water use efficiencies (WUE) were detected until after water repletion. That is, only two species (*C. alata* and *P. virginica*) had enhanced WUE as water availability returned to normal. Drought conditions also promoted drought tolerance responses in all five species, as indicated by a change in bulk modulus of elasticity (ϵ; all species) and decreased osmotic potential (Ψ_π; *P. virginica*). Taken as a whole, this study reveals two contrasting drought tolerance strategies in wetland herbs. While four of the species alter ϵ to generate declines in Ψ, *P. virginica* favored decreases in osmotic potentials (as indicated by decreases in Ψ_π at full saturation and at turger loss point).

Table 1. Plant and soil parameters collected following two weeks of simulated drought. Data includes relative water content (θ), xylem water potential (Ψ_{xylem}; MPa), aboveground biomass, belowground biomass, and percent survival. Letters represent the statistical relationship between each treatment when differences were detected (the same letter on different values are considered statistically similar). Data is presented as means ± 1 S.E. (n = 5).

Species (treatment)	θ (%)	$-\Psi_{xylem}$	Aboveground (g)	Belowground (g)	Survival (%)
Monocots					
Carex alata					
Control	91.7 ± 3.7	0.24 ± 0.07	0.03 ± 0.003	0.007 ± 0.001	108.3 ± 8.3
Drought	84.5 ± 7.3	0.76 ± 0.18*	0.04 ± 0.009	0.004 ± 0.004	125.5 ± 21.7
Recovered	91.1 ± 2.4	0.51 ± 0.07*	0.03 ± 0.004	0.004 ± 0.003	113.3 ± 9.4
Juncus effusus					
Control	91.2 ± 3.3	0.66 ± 0.08	0.14 ± 0.01	0.10 ± 0.02	119.4 ± 19.0
Drought	70.6 ± 3.5*	0.94 ± 0.14*	0.19 ± 0.05	0.09 ± 0.01	114.6 ± 29.9
Recovered	89.9 ± 4.8	0.51 ± 0.12	0.21 ± 0.07	0.09 ± 0.02	86.1 ± 17.8
Peltandra virginica					
Control	87.9 ± 1.0	0.03 ± 0.01	0.91 ± 0.21	1.05 ± 0.30	87.3 ± 3.6
Drought	84.3 ± 3.4	0.12 ± 0.03*	0.37 ± 0.07*	1.81 ± 0.32	90.0 ± 7.1
Recovered	84.1 ± 3.1	0.59 ± 0.04*	0.62 ± 0.12	1.04 ± 0.23	90.7 ± 7.1
Dicots					
Justicia americana					
Control	95.9 ± 0.5	0.32 ± 0.04	0.66 ± 0.06	1.17 ± 0.21	92.6 ± 7.28
Drought	74.8 ± 1.9*	0.87 ± 0.25*	0.34 ± 0.05*	1.01 ± 0.19	61.3 ± 22.0
Recovered	90.1 ± 5.1	1.12 ± 0.11*	0.16 ± 0.06*	0.32 ± 0.11*	74.9 ± 15.0
Saururus cernuus					
Control	96.9 ± 2.4	0.34 ± 0.05	0.24 ± 0.05	0.37 ± 0.12	102.6 ± 9.8
Drought	88.2 ± 1.1*	0.96 ± 0.16*	0.20 ± 0.03	0.14 ± 0.05	82.9 ± 23.5
Recovered	92.9 ± 2.0	0.89 ± 0.16*	0.21 ± 0.03	0.17 ± 0.04	83.9 ± 19.2

Table 2. Plant water status parameters derived from pressure-volume isotherms on control and drought-recovered plants. Parameters include water fraction at turgor loss point (θ_{tlp}; %), symplastic water fraction (θ_{sym}; %), osmotic potential at full saturation ($-\Psi_{\pi}^{sat}$; MPa), osmotic potential at the turgor loss point ($-\Psi_{\pi}^{tlp}$; MPa), and bulk modulus of elasticity (ε; MPa). Statistical differences from the control are indicated by asterisks ($p < 0.05$; one-way ANOVA). Data is presented as means ± 1 S.E. ($n = 8$).

Species (treatment)	θ_{tlp}	θ_{sym}	$-\Psi_{\pi}^{sat}$	$-\Psi_{\pi}^{tlp}$	ε
Monocots					
Carex alata					
Control	0.79 ± 0.01	0.54 ± 0.03	1.11 ± 0.06	1.26 ± 0.09	1.69 ± 0.36
Recovered	0.77 ± 0.04	0.50 ± 0.04	0.37 ± 0.03*	0.67 ± 0.09*	0.56 ± 0.06*
Juncus effusus					
Control	0.84 ± 0.03	0.52 ± 0.05	0.33 ± 0.03	0.52 ± 0.04	0.71 ± 0.10
Recovered	0.86 ± 0.03	0.61 ± 0.05	0.36 ± 0.05	0.54 ± 0.08	1.64 ± 0.26*
Peltandra virginica					
Control	0.92 ± 0.01	0.82 ± 0.01	0.52 ± 0.02	0.84 ± 0.02	4.26 ± 0.88
Recovered	0.85 ± 0.03*	0.61 ± 0.05*	0.86 ± 0.08*	1.38 ± 0.11*	1.95 ± 0.47*
Dicots					
Justicia americana					
Control	0.85 ± 0.01	0.32 ± 0.06	1.15 ± 0.08	1.43 ± 0.10	1.91 ± 0.24
Recovered	0.92 ± 0.02*	0.77 ± 0.04*	0.69 ± 0.03*	1.11 ± 0.06*	4.18 ± 0.62*
Saururus cernuus					
Control	0.90 ± 0.01	0.38 ± 0.05	1.38 ± 0.15	1.58 ± 0.17	3.60 ± 0.45
Recovered	0.93 ± 0.01*	0.71 ± 0.01*	0.76 ± 0.01*	1.03 ± 0.06*	8.49 ± 0.99*

Significance

Part 1: Drought Susceptibility in Emergent Wetland Angiosperms: a Comparison of Water Deficit Growth in Five Herbaceous Perennials

As our basic understanding of how different wetland macrophytes respond to changing soil water tables increases with future studies (including both controlled greenhouse experiments and field investigations) we should be able to develop more generalized patterns necessary for predicting plant-drought interactions. This basic understanding is fundamental if we are to develop better working models on how wetlands will respond to changing environmental conditions that lead to decreased water supply - including mechanical disturbances associated with human activities and projected decreases in water availability due to climate change.

Part 2: Drought Tolerance Versus Drought Avoidance: A Comparison of Plant-Water Relations in Herbaceous Wetland Plants Subjected to Water Withdrawal and Repletion

In contrast, the former case of increased cell wall rigidity in drought stressed plants would allow for decreases in both turgor and water potential with only a small decrease in plant water content. Furthermore, a change in cell wall elasticity requires far less energy than the metabolically driven alternative of increasing organic compatible solutes (Lo Gullo et al. 1986). Consequently, Corcuera et al. (2002) suggested that plants growing in soils low in both water and nutrient content would favor the lower energy process of cell wall modification. While all species studied in this investigation can be found in organically rich soils typical of many freshwater wetland habitats, *J. americana*, *J. effusus*, and *S. cernuus* are also commonly found in nutrient poor clay soils typical of central Piedmont reservoirs of North Carolina (Touchette et al. 2000).

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Is There a Relationship Between Phosphorus and Fecal Microbes in Aquatic Sediments?

Basic Information

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7. Knowles, John E., Lawrence B. Cahoon, and Steven M.K. Gill. 2006. Sediment-associated phosphorus in drainage features in southeastern North Carolina. Journal of the North Carolina Academy of Science 122:39-48.

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9. Toothman, Byron. 2006. "Effects of Phosphorus and Labile Organic Carbon on Sediment-Associated Fecal Indicator Bacteria" M.S. thesis, Program in Marine Science, UNC Wilmington, Wilmington, NC, 54 pp.
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Title

Is There a Relationship Between Phosphorus and Fecal Microbes in Aquatic Sediments? (70209)

Problem and Research Objectives

Microbial contamination of surface waters, particularly by fecal material, is one of the most challenging problems facing environmental managers, necessitating closures of large areas of otherwise economically valuable waters to shellfishing and recreational uses. North Carolina currently has over 300,000 acres of estuarine waters closed to shellfishing (N.C. Shellfish Sanitation Section records), and 40,000 acres conditionally closed to shellfishing following rain events (Mallin et al. 2001a). Selected recreational areas receive periodic bathing advisories owing to microbial contamination (N.C. Shellfish Sanitation Section records). The potential threats to human health represented by these closures add to the economic damage they actually cause. Remediation of this contamination is clearly important for economic and public health reasons (Maiolo and Tschetter 1981).

Research indicates that P is the key limiting factor for bacterial populations in many aquatic ecosystems, including lakes (Currie 1990; Morris and Lewis 1992), the coastal ocean (Bjorkman and Karl 1994; Cotner et al. 2000), and salt marshes (Sundareshawar et al. 2003). Kirchman (1994) found that heterotrophic bacteria are responsible for a large portion of inorganic P uptake in lakes, much more than for ammonia uptake. Mallin et al. (2001b, 2002a, 2004) demonstrated P stimulation of ATP, heterotrophic bacteria, and BOD in two blackwater streams and two blackwater rivers in the NC coastal plain. Nitrogen inputs directly stimulated the autotrophs (phytoplankton), but not the heterotrophs. A recent study by an M.S. student in the Marine Science program at UNC Wilmington examined the effects of sediment P levels on fecal coliforms in relatively undeveloped estuarine watersheds and in experimental microcosms (Rowland 2002). At field sites, increases in persulfate-oxidizable P levels ("persulfate-P") correlated with increasing sediment fecal coliform levels. Manipulations of persulfate-P levels in experimental microcosms across the same range of concentrations observed in the field studies yielded similarly significant correlations with fecal coliform populations (Fig. 1). These results suggested that P may limit fecal microbial populations in shallow aquatic sediments, at least when sediment P levels are otherwise low.

Phosphorus pollution of surface waters is widespread (Correll 1998), but nitrogen pollution has received more attention owing to problems with algae blooms (Bricker et al. 1999). Sources of P pollution include domestic and wild animal wastes, human wastes, and fertilizers. Mechanisms of P loading to surface waters include both permitted and improper point discharges of wastes and non-point runoff. Our previous research has shown strong positive correlations between total suspended solids and P in wet detention ponds (Mallin et al. 2002b). Research has shown that human uses of fertilizers and animal wastes have elevated soil levels of P (Cahoon and Ensign 2004) and increased discharges from land to coastal waters (Liu et al. 1997; Daniel et al. 1998; Sharpley and

Tunney 2000). Cahoon (2002) showed that fertilizer use in residential neighborhoods was linked to elevated sediment P concentrations in the Bradley Creek watershed, where elevated sediment fecal coliform levels also occurred.

Phosphorus (P) pollution is well known to contribute to eutrophication problems in North Carolina's waters. A phosphate detergent ban in 1989 (N.C. D.E.M. 1991) and other measures to limit point and non-point source discharges of pollutants have implicitly recognized the potential for phosphorus to cause water quality problems. Recent increases in soil P levels in much of eastern North Carolina, caused by application of animal manures and use of commercial fertilizers, have resulted in a stronger emphasis on agricultural P management, evidenced by North Carolina's promulgation of a "Phosphorus Loss Assessment Tool" (PLAT; Osmond et al. 2005). However, the rationale for these management measures has been control of algal blooms. Only recently has the importance of P limitation of bacteria in aquatic ecosystems been recognized.

We originally hypothesized that phosphorus concentrations limit the concentrations of fecal indicator microbes in aquatic sediments, and proposed to use both observational and experimental approaches to test this hypothesis. Therefore, we identified two main and several corollary objectives:

1. Determine the relationship between sediment phosphorus levels and sediment concentrations of fecal indicator bacteria (fecal coliforms, streptococci, and enterococci) in several different watersheds in eastern NC:

- Sample in a wide range of habitats to provide a sufficient range of parameter values to detect a relationship if one exists.
- Describe the concentrations and variability in fecal indicator concentrations.
- Determine the relationship between sediment fecal indicator concentrations and P levels.
- Define the components or fractions of "sediment P" using different extraction and measurement techniques.
- Determine the relationships, if any, between fecal indicator concentrations and other potentially relevant parameters.

2. Determine experimentally the response of fecal microbial concentrations in sediments to manipulated variations in phosphorus and organic substrate availability.

An event we could not foresee when we wrote our original proposal, in the form of a major sewage spill and subsequent runoff event, provided an opportunity to evaluate the response of populations of sediment fecal indicator bacteria to sudden fecal contamination uncoupled from storm water runoff. On Friday, July 1, 2005 the middle branch of Hewletts Creek at Pine Grove Road was subjected to a raw sewage spill of 3,000,000 gallons (~11,360 m^3). This occurred when a 60 cm force main coupling repair burst apart. This line carried sewage from Wrightsville Beach to a pump station on Bradley Creek, then to a pump station (#34) on Hewletts Creek (near the breach) then on to the Wilmington South Side Wastewater Treatment plant on River Road (the plant discharge is near Channel Marker 54 in the Cape Fear River Estuary). This line had been built in the mid 1980s using EPA funds. A citizen called the City at approximately 6:20 AM with a complaint; city workers were on site at 7:10 AM and found an obvious major leak. The workers turned the pump off but sewage continued to flow into the creek. During the course of the day they dug down 2-3 m to find the problem, finally finishing a temporary repair at 10:30 PM. The workers estimated that the spill had begun about 5:00 AM; thus the sewage spill occurred over a near-18 hour period (Hugh Caldwell, City of Wilmington, personal communication). Some waste flowed into the creek or nearby swamp forest, and some flowed into the nearest storm drains, which drain directly into Hewletts Creek. Both the North Carolina Division of Water Quality (DWQ) and the N.C. Shellfish Sanitation Section were alerted that morning, and as a result the N.C. Division of Marine Fisheries closed the creek and a large section of the Intracoastal Waterway (ICW) to shellfishing, and Shellfish Sanitation closed that area to swimming. This section of the ICW encompassed the area between the Wrightsville Beach Bridge and ICW Channel Marker 141 near Peden Point, including all tributaries between.

We requested and received an extension of our funded research project to take advantage of the opportunity this spill event presented to study fecal contamination of sediments, adding another objective to our original set:

3. Determine the response of sediment populations of fecal indicator bacteria to human sewage spills and runoff events.

Methodology

This study examined the distribution and concentration of fecal indicator bacteria in sediments in diverse aquatic habitats in southeastern North Carolina, and examined their relationship to different measures of sediment phosphorus, hypothesized to limit aquatic bacteria, and other parameters, using both observational and experimental approaches.

Sampling Locations: Sampling was conducted at several sets of locations in southeastern North Carolina. One set of locations was distributed through portions of Duplin and Pender counties, and included several sites sampled on a monthly basis since 1995 by the Lower Cape Fear River Program (LCFRP), directed by co-PI Mallin (www.uncw.edu/cmsr/aquaticecology/lcfrp/) in addition to 48 sites selected as representative of natural and man-made drainage features. These were fresh water sites in mostly rural locations and were sampled once or twice for this project. Another set of sampling locations included 11 public and private boat launching ramps and five nearby shoreline locations for comparison, where people come into direct contact with water and sediments, throughout New Hanover County, the most urbanized of North Carolina's 20 coastal counties. These sites were sampled several times on a monthly basis for this project. Another set of locations sampled for this project included a large number of stream and pond sites in central New Hanover County, sampled once or twice for this project. Most of these stream locations were also sampled on a monthly basis by the City of Wilmington Watersheds/New Hanover County Tidal Creeks Program (www.uncw.edu/cmsr/aquaticecology/TidalCreeks/), also directed by co-PI Mallin, but a number of detention ponds were included for the purposes of this project. A set of sites in the Bradley Creek watershed, including several also sampled monthly by the New Hanover County Tidal Creeks Program, was sampled intensively (monthly since January, 2003) as part of previous research projects and for this project. Finally, we sampled in the Hewletts Creek watershed in response to a major sewage spill in the middle branch of that creek in July, 2005 and again in September, 2005.

Fecal Indicator Bacteria: Sediment samples for analyses of sediment fecal microbial concentrations were collected in triplicate using sterile 2.3 cm diameter coring tubes. Sediment fecal coliforms, fecal streptococci, and fecal enterococci were analyzed using a suspension, dilution, and filtration procedure. Individual cores were suspended with stirring for two minutes using a magnetic stirrer in one liter of phosphate buffer solution (0.25M KH_2PO_4, pH adjusted to 7.2 with 0.1N NaOH). Triplicate aliquots (usually 10 ml or one ml) were withdrawn from stirred suspensions using sterile volumetric pipets and immediately filtered onto sterile membrane filters (0.45 µm pore size, 47 mm diam), after which standard incubation and plate count techniques were used for each group of fecal indicator bacteria. Triplicate filters for determination of fecal coliform bacteria were incubated for 24 hours at 44.5 ^{o}C in a Fisher Isotemp digital water bath on mFC agar (Fisher/Difco), following method 9222D (APHA 2001). Similarly, triplicate filters for determination of fecal streptococci were incubated for 48 hours at 35 ^{o}C in a Fisher Isotemp digital water bath on mEnterococcus agar (Fisher/Difco), following method 9230C.3.b (APHA 2001). Finally, triplicate filters for determination of fecal enterococcus bacteria were incubated for 48 hours at 41 ^{o}C in a Fisher Isotemp digital water bath on mE agar (Fisher/Difco), following method 9230C.3.a (APHA 2001). Bacterial colonies satisfying the respective criteria for each method were counted after incubations using an Olympus SZ-III stereo-microscope. Colony counts were expressed as Colony-Forming Units (CFU)/100 ml of suspension and calculated as CFU g^{-1} and CFU cm^{-2} of cored sediment.

Sediment Parameters: Sediment samples for analyses of chemical parameters were taken in triplicate by the same coring methods as for bacterial analyses. Triplicate cores for the respective sets of analyses were selected randomly from each set of six cores taken at each sampling location and time. Sediment cores destined for chemical analyses were iced immediately, frozen initially at -20 ^{o}C, then stored at -85 ^{o}C for 24 hours prior to lyophilization using a Virtis Benchtop 3.3 Vacu-Freeze lyophilizer. Lyophilized sediment samples were homogenized and stored in sealed containers at room temperature prior to sub-sampling for chemical analyses.

Sediment P analyses were conducted by two methods. Valderrama's (1981) boric acid-persulfate digestion method employs boric acid-persulfate digestion of a pre-weighed, dry sediment sample by autoclaving, followed by analysis of orthophosphate using the standard molybdenum-blue method (Parsons et al. 1984). Reagent blanks were prepared for each batch of samples, and blank values subtracted from sample values. Standardization with sodium glycero-phosphate yielded approximately 98% recovery. [Sediment P] was expressed as *u*g P (g sediment)$^{-1}$ and as *u*g P cm^{-2}. This method was used for samples from the LCFRP and Bradley Creek sites for consistency with the longer sediment P data base from those collections, dating back to 2002, and for comparison with the results of Rowland (2002), whose findings prompted this study. We think the persulfate-P method more accurately extracts and represents bio-available P than more vigorous digestion techniques commonly used for total sediment-P analyses, i.e., perchloric acid-nitric acid digestions (Strickland and Parsons 1972; Cahoon et al. 1990).

We also adapted the modified SEDEX sequential sediment P extraction technique of Anderson and Delaney (2000) to estimate the different fractions of P in sediments. Lengthy experimentation with the specific technique published by Anderson and Delaney (2000) identified several problems with its chemistry for our sample matrices, however. This led us to employ a combination of the standard Mehlich III soil testing technique (Mehlich, 1984) and modified SEDEX techniques to distinguish and quantify sediment P fractions. A four-part sequential extraction and measurement procedure was used to partition soil P into fractions. Step one was extraction of 2.0 g dried, homogenized sediment by shaking for five min with 20 ml of the Mehlich III reagent (0.2N acetic acid, 0.25N ammonium nitrate, 0.013N nitric acid, 0.015N ammonium fluoride, 0.001M EDTA). Following centrifugation, five ml of supernatant was analyzed directly for soluble reactive phosphorus (SRP) by the molybdenum-blue spectrophotometric technique (Parsons et al. 1984). This fraction was designated M3-SRP. Another nine ml sub-sample of the extract was digested by autoclaving with one ml boric acid - potassium persulfate reagent (Valderrama, 1981) to convert organic phosphates to ortho-phosphate, and then analyzed for SRP as above to yield Mehlich-III extractable total phosphorus, designated M3-TP. Mehlich III-extractable organic phosphorus, calculated by subtraction of M3-SRP from M3-TP, was designated M3-OP. Standardization of the molybdenum-blue analysis was performed with each batch of samples using 3.0 *u*M KH_2PO_4. Step two was based on the modified SEDEX procedure for analyses of authigenic P and particulate organic P, respectively (Anderson and Delaney 2000). The solid residue from the Mehlich III extraction was digested in 13 ml 0.01 N H_2SO_4 for 16 hr with stirring, after which 10 ml of the supernatant was used for molybdenum-blue spectrophotometry, as above, and the calculated results designated as authigenic P. Finally, the remaining residue was treated with one ml of 50% $MgNO_3$, dried, combusted for two hr at 550^oC, digested in 13 ml 0.01 N H_2SO_4 for 24 hr, then analyzed by molybdenum-blue spectrophotometry and designated as particulate organic phosphorus (POP). Soil P fractions were calculated as *u*g P g sediment^{-1}. The average of three replicate values from each sample site was calculated along with standard deviation for each P fraction. This method was employed for samples collected at boat ramp sites and drainage feature sites in Duplin and Pender counties.

The carbohydrate content of sediment samples was analyzed by the phenol-sulfuric acid method of Underwood et al. (1995). Approximately five mg of lyophilized, homogenized sediment was suspended in 1.0 ml of distilled water, to which 0.5 ml of 5% aqueous phenol solution and 2.5 ml of conc sulfuric acid were added while stirring vigorously. Resulting absorbance was measured at 485 nm on a Milton-Roy Spectronic 401 spectrophotometer in a one cm cuvet against a reagent blank. Standard curves were established each time the assay was performed using a dilution series of dextrose

($C_6H_{12}O_6$) and carbohydrate contents were expressed as μg C (g sediment)$^{-1}$. The average of three replicate values from each sample site was calculated along with standard deviation.

Water Quality Parameters: Water column data were collected as part of the City of Wilmington Watersheds/New Hanover County Tidal Creeks Program, with supplemental funding from the City of Wilmington to collect and analyze post-sewage spill samples in Hewletts Creek. Water column field parameters (water temperature, pH, dissolved oxygen, turbidity, and conductivity) were measured at each site using a YSI 6920 Multi-parameter Water Quality Probe (sonde) linked to a YSI 610 display unit and calibrated prior to each sampling event. At other locations and times water temperature, conductivity, salinity, and dissolved oxygen data were collected using a hand-held YSI 85 Multi-Parameter water quality meter, calibrated prior to each use. Nitrate+nitrite (= 'nitrate') and orthophosphate were measured in triplicate following flitration using standard wet chemistry techniques (Parsons et al. 1984). Samples for total nitrogen (TN) and total phosphorus (TP) were collected in duplicate on site as well and were digested using a persulfate/boric acid agent following Valderrama (1981). Nitrate, orthophosphate, TN and TP were analyzed at the UNCW Center for Marine Science Nutrient Laboratory by a Bran & Luebbe AutoAnalyzer 3. Samples for ammonium were collected in duplicate, field-preserved with phenol, stored on ice, and analyzed in the laboratory according to Parsons et al. (1984). Fecal coliform samples were collected and determined using the mFC method (9222D, APHA 2001). Chlorophyll *a* samples were analyzed in triplicate for filtered samples extracted in acetone according to Welschmeyer (1994) and US EPA (1997, using a Turner AU-10 fluorometer calibrated with chlorophyll *a* (Sigma). Rainfall data for southeastern North Carolina were obtained from USWS rain gaging station at the Wilmington International Airport, and were reported as cumulative totals for each 24-hr period beginning at 0700 hrs local time.

Experimental Protocols: Sediment cores were collected as above at sampling locations in the Bradley Creek watershed and in several other locations for experimental determinations of responses of fecal indicator bacteria to added P and organic carbon. A randomly selected triplicate set of sediment cores was analyzed for initial concentrations of fecal coliform and fecal enterococcus bacteria as above. Four treatments were used in a 2 x 2 design, executed in triplicate: three sediment cores were incubated with one liter of incubation medium (0.4% NaCl buffered to pH 8.0 with sodium borate) +100 μg P/liter (as KH_2PO_4), three sediment cores were incubated with one liter of incubation medium + 1000 μg dextrose C/liter, three sediment cores were incubated with one liter of incubation medium + 100 μg P/liter + 1000 μg dextrose C/liter, and three sediment cores were incubated with one liter of incubation medium only. Incubations lasted 24 hours at 37^{o}C, after which sediment cores were processed for analyses of fecal coliform and fecal enterococcus bacteria as above.

Hewletts Creek Sewage Spills: Regulators from the N.C. D.W.Q. sampled the area on Saturday, July 2, then again on July 4 and 6. Researchers from the UNCW Aquatic Ecology Laboratory sampled the water column in the area on July 3, 5, 7, 15, 21, and on August 8. Researchers from the UNCW Department of Biology and Marine Biology collected sediment samples for fecal coliform bacteria and enterococci on July 6, 8, 11, 13, 15, 18, 20, 22, 26, 29, August 2 and August 11. Stations sampled included MB-PGR (spill site), SB-PGR (south branch at Pine Grove Rd.), NB-GLR (north branch at Greenville Loop Rd.), HC-M (creek mouth), HC-3 (at a dock on the north shore of the creek), HC-NWB (the northwest branch of the creek between HC-3 and the tributary stations), the Masonboro Channel on the ICW south of the creek mouth, and the Shinn Creek channel on the ICW north of the creek mouth. Stations sampled for sediment bacteria included MB-PGR, SB-PGR, NB-GLR and DOCK, a control site located near the junction of Hewletts Creek and the ICW. A second sewage spill occurred in the Hewletts Creek watershed on Sept. 15, 2005, when a force main burst near Shipyard Blvd., apparently owing to flooding caused by Hurricane Ophelia's rains, discharging approximately 750,000 gallons (~2840 m^3) of sewage, much of which flowed through storm drains into the middle branch of Hewletts Creek. Sampling efforts similar to the previous scheme were conducted by UNCW researchers on Sept. 19 and 23.

Statistical Analyses: Data sets were examined for normality using the Shapiro-Wilk test. Non-normal data sets were transformed as appropriate; bacteria concentration data were typically log-transformed (Log[counts+1]). When transformation could not yield a normal distribution, non-parametric statistical tests were used to analyze original data. The effects of sediment P, carbohydrate, and other variables on bacterial concentrations were analyzed by linear regression. Correlations between bacterial indicators of fecal contamination were determined using Pearson's product-moment correlation. Comparisons of bacterial or sediment phosphorus concentrations among sites or treatments were performed using Analysis of Variance for normal data sets. All statistical analyses were performed on SAS Institute's JMP version 4.0, except as noted.

Soil P fraction concentrations (M3-SRP, authigenic P and total P) from drainage feature locations were compared within 11 paired sites using one-way analysis of variance. The relationship between Mehlich-III P (SRP, OP and total) and authigenic P concentrations for all sites was examined using Pearson's product-moment correlation analysis. One-way analysis of variance and Tukey's HSD *a posteriori* tests were used to compare mean sample soil P concentrations among sites categorized by different adjacent land covers, after confirming that distributions within each category and class of soil P were "normally" distributed using the Shapiro-Wilk test of goodness of fit to a "normal" distribution. Log transformations were used to address issues of non-normality, particularly skewness.

Results of experimental incubations employing a 2 x 2 design were tested for normality using the Shapiro Wilk test, and found to be non-normal. Results of experimental incubations employing a 2 x 2 design were log-transformed and analyzed by two-way ANOVA (Sokal and Rohlf, 1995); pooled experimental results were analyzed by a Kruskal-Wallis test.

Principal Findings

Populations of fecal indicator bacteria and the pathogens whose presence they indicate are widely distributed and frequently very abundant in shallow aquatic sediments. Measures of fecal indicator bacteria in the overlying water column are far more frequently conducted, however. Water quality regulations are currently based on water column concentrations of indicator bacteria, even though sediments represent a significant reservoir and may support survival and growth of these contaminants. This study examined the distribution and concentration of fecal indicator bacteria in sediments from a diverse set of aquatic habitats in southeastern North Carolina, examined their relationship to different measures of sediment phosphorus, a macronutrient hypothesized to limit aquatic bacteria, as well as other parameters, using a combination of observational and experimental approaches. Finally, major sewage spills in one tidal creek watershed provided an opportunity to test ideas about sediment-associated fecal indicator bacteria and the factors supporting them.

The concentrations of fecal indicator bacteria (fecal coliforms, fecal streptococcus, and fecal enterococcus) were measured in sediments from a diverse set of aquatic habitats in southeastern North Carolina, including 76 sites in fresh water drainages in the Lower Cape Fear River basin and in fresh water and estuarine watersheds, at boat ramps and adjacent beaches, and in detention ponds in New Hanover County. Concentrations of fecal coliform, fecal streptococcus, and fecal enterococcus bacteria varied from 0 up to 10^3-10^4 Colony-Forming Units (CFU) /cm^2 of sediment. Concentrations of these indicator bacteria types were significantly correlated with each other. Our study is the first of which we are aware quantifying fecal enterococcus in aquatic sediments. Our results show that levels of this indicator are relatively higher than those of fecal coliforms, and the correlation between the two indicators gives confidence that enterococcus concentrations are similarly useful in indicating the presence of actual pathogens. Average concentrations of fecal coliform and fecal enterococcus bacteria, if totally suspended in a 1-m deep water column, were frequently higher than relevant regulatory standards for human body contact (200 CFU/100 ml for coliforms, 33 CFU/100 ml for enterococcus) and significantly higher than the standard for shellfishing waters (14 CFU/100 ml for coliforms). Thus, the concentrations of fecal indicator bacteria were indicative of problematic levels of contamination at many times and places in southeastern North Carolina, including situations in which exposure of the public to potentially harmful levels of these contaminants is likely.

It was originally hypothesized, based on published literature and previous studies in our laboratories, that concentrations of the macronutrient, phosphorus (P), in sediment matrices might limit survival and growth of sediment populations of fecal indicator bacteria. When sediment P as persulfate-labile P was measured and compared to simultaneous measures of sediment-associated fecal coliforms and enterococcus, no significant relationships were found. Concentrations of persulfate-P in sediments frequently exceeded the relatively low levels previously found to limit bacterial survival and growth. When sediment P was extracted and fractionated using a combination of techniques, including the standard Mehlich-III soil test extraction technique as well as acid-extraction and combustion techniques, comparisons of fecal coliforms and enterococcus concentrations measured simultaneously with the P fractions similarly revealed no usefully significant relationships. Thus it was concluded that levels of sediment P generally exceeded those that limit fecal indicator bacteria populations in aquatic sediments in this region.

Authigenic P was the dominant portion of sediment P in most samples, and was positively correlated with M3-SRP values, which suggested an interaction of anthropogenically added P with natural or added soil minerals, especially calcium, which may be added to soils as lime or land plaster (gypsum). This fraction is not part of the soil test P evaluated and reported by the N.C. Division of Agronomy. The general predominance of authigenic P in these sediment samples indicated that conventional soil test procedures fail to measure most of the P in this landscape much of the time. If the correlation between M3-SRP and authigenic P reflects a chemical transformation of M3-SRP to authigenic P by reaction with soil minerals, then actual export of added P from agricultural fields might also be underestimated by use of conventional soil test P measures.

Pair-wise regressions of fecal indicator bacteria concentrations against other parameters from intensive sampling in the Bradley Creek watershed in New Hanover County revealed effects of salinity and temperature on fecal coliform concentrations in sediments, but multiple regression analysis showed an effect of rainfall within the previous 24 hours. The latter result indicated that storm water runoff was an important mechanism driving recruitment of fecal indicator bacteria to aquatic sediments. The significant negative relationship between salinity and fecal coliform concentrations in sediments also indicated a relationship between runoff and bacterial recruitment to sediments.

Experimental evaluations of the responses of fecal indicator bacteria to combinations of added phosphorus and organic substrate revealed that fecal bacteria did respond positively to added P when background P levels were low; however, field sites where sediment P levels were that low were largely restricted to forested areas. Urbanization of forested areas, or transformation to agriculture will likely cause increased sediment P levels that would remove P limitation of fecal bacterial growth. Experimental organic carbon additions more frequently and more generally stimulated growth of these bacteria populations. Preliminary evidence indicates that storm water runoff may also be elevated in bio-available organic carbon, so this may be an additional factor to be considered in evaluating the effects of storm water runoff on bacterial contamination in surface waters and sediments.

Major sewage spills in the Hewletts Creek watershed in the summer of 2005 provided an additional opportunity to study recruitment and survival of sediment-associated fecal indicator bacteria. Results of an intensive monitoring effort after the first major spill, on July 1, 2005, showed that the concentrations of fecal indicator bacteria attenuated faster in the water column than in sediments, and that bacteria in the sediments survived at higher relative levels longer than in the water column. The sudden introduction of large amounts of fecal contamination to the watershed and subsequent large increases in sediment concentrations supported the statistically significant relationship between runoff events and sediment concentrations described above. Sediment bacteria information was used to justify continuing closure of the Hewletts Creek basin to swimming, and supports the argument that sediment contamination by fecal bacteria after such spills should be considered by public health officials and regulations.

Significance

Our research, along with our studies of the July 1 sewage spill demonstrated that following a major pollution incident where human or animal waste is involved, sampling the water column for fecal bacteria is not sufficient to obtain a complete picture of the system in terms of human health issues. Large quantities of the polluting bacteria settle to the sediments and remain viable for weeks to months, and are subject to resuspension in the water column after a mixing event. Fecal bacteria on or in the sediments are largely protected from UV radiation, a principal means of death or deactivation in the water column. Also, the sediments contain carbon, nitrogen, and phosphorus, key nutrient for survival and growth. We recommend that regulatory authorities devise sampling and assessment plans for pollution incidents that consider sediment-associated fecal bacteria. Furthermore, sediment sampling of fecal indicators should be included in normal monitoring programs by regulatory agencies, as these sediment-associated fecal microbes represent a readily available source of pollution to the overlying water column.

During the sediment phosphorus sampling portion of this project it became evident that standard laboratory procedures for sediment P analysis underestimate an important component of sediment P, authigenic P. The general predominance of authigenic P in these sediment samples indicates that conventional soil test procedures fail to measure most of the P in this landscape much of the time. If the correlation between M3-SRP and authigenic P reflects a chemical transformation of M3-SRP to authigenic P by reaction with soil minerals, then actual export of added P from agricultural fields might also be underestimated by use of conventional soil test P measures. We recommend that regulatory agencies and researchers revise soil P tests so that they will account for all major fractions of P in soils.

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Arsenic and Heavy Metal Leaching Potential from Broiler Litter Stockpiled on Bare Soil

Basic Information

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Descriptors:	poultry, copper, mananese, zinc, nitrate, phosphate
Principal Investigators:	Sanjay B. Shah, Garry L. Grabow, David H. Hardy, Dean Hesterberg, Rodney L Huffman, James Parsons

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Title

Arsenic and Heavy Metal Leaching Potential from Broiler Litter Stockpiled on Bare Soil (70208)

Problem and Research Objectives

North Carolina ranks second behind Minnesota in turkey production, producing 39 million birds in 2004. After hatching, turkey poults are separated by sex and raised in brooder houses for 6-8 weeks. Thereafter, the birds are transferred to grow-out houses where the hens are kept for 13-14 weeks and the toms for 18-20 weeks. Typically, each batch of turkey poults is raised on fresh litter and after the brooding, the litter is deep-stacked to kill pathogens and then used in the grow-out houses. After each grow-out, the crust containing much of the excreta and feed is removed and may be replaced by fresh litter. Depending on the grower's management practices and the integrator's requirements, the litter in the grow-out house may be cleaned out annually or less often. Most litter (also broiler litter) is used as a nutrient source for crop production. If crop and environmental conditions are favorable, the poultry litter may be land-applied; otherwise, it is stockpiled until conditions are suitable for land application. In North Carolina, poultry (both turkey and broiler) litter is generally stockpiled over the winter months and then applied to corn ground in spring. While some producers apply litter to hayfields during summer, in the absence of disposal alternatives, others may stockpile litter for application to winter wheat in fall and spring.

Poultry litter contains many nutrients that while useful as plant nutrients, can cause water quality problems if they contaminate ground or surface waters. Constituents in turkey litter that are known to degrade water quality are nitrate-nitrogen (NO_3^--N), ammoniacal-N (NH_4^+-N+NH_3-N), phosphorus (P), pathogens, heavy metals [e.g., copper (Cu), manganese (Mn), and zinc (Zn)], and metalloids [arsenic (As)]. Organic As formulations, mostly as roxarsone (~28.5% As; 3-nitro-4-hydroxyphenylarsonic acid), are used for controlling coccidial intestinal parasites, blackhead disease in turkey, and as a growth promotant; they are also fed to broilers and swine. Between 1995 and 2000, >96% of feed mills blended roxarsone in concentrations ≥11 mg/kg-feed in broiler grower and starter feed with ~80% mills using 37-50 mg/kg (Chapman and Johnson 2000). Since roxarsone is largely excreted, microbes in litter and soil can mineralize roxarsone into more toxic and mobile species, such as, arsenate (As(V)) and arsenite (As(III)). Based on multiple sources, Sims and Wolf (1994) reported that poultry litter contained up to 77 mg-As/kg-dry weight (dw). Wershaw et al. (1999) estimated that 1,000 Mg of roxarsone and its degradation products were introduced into the environment annually through land-application of poultry litter.

Experts suggest that stockpiling poultry litter under cover (shed or tarpaulin) can reduce nutrient losses through runoff, leaching, or volatilization. Currently, there are no regulations on covering litter stockpiles in North Carolina; anecdotal evidence suggests that 70-95% of litter stockpiles are formed on bare ground with no cover (T. Cutts, NRCS, pers. com. 2005). In uncovered stockpiles, litter constituents may be transformed into more mobile and toxic species due to favorable litter [e.g., high dissolved organic carbon (C) and microbial concentrations] and environmental (e.g., high temperature and moisture content) conditions. Such soluble constituents of litter stockpiled on bare soil may be transported by rainfall into the soil beneath the stockpile. Since As in poultry litter is mostly in the water soluble form (Jackson and Miller 1999), there is potential for As to leach into the soil. The Coastal Plain of North Carolina supports a large poultry industry, has coarse-textured soils, high and fluctuating water tables (causing redox effects), and heavy rainfall (>1,270 mm, SERCC, 2003). Hence there is concern in this region that As in poultry litter may contaminate shallow groundwater. Shallow groundwater in the Coastal Plain is usually within 6 m of the soil surface and much higher during the wetter months and may also be used as drinking water. Arsenic is a carcinogen that can also cause gastrointestinal, neurological, dermal, hematological, cardiovascular, peripheral vascular, and immune system effects; additionally, it may also be a potent endocrine disruptor (Momplaisir et al. 2001). Given that most chronic As poisoning cases are due to inorganic As in drinking well water, the EPA has established a maximum contaminant level (MCL) of 0.01 mg/L for As in drinking water.

However, other pollutants such as NO_3^--N, Cu, Mn, and Zn in poultry litter that can also impact drinking water quality. On a wet basis, turkey litter contains 2.75% total Kjeldahl-N (TKN) (NCCE 2005) which raises concerns about leaching of N species (NO_3^--N and ammoniacal-N) into the soil. There is also concern about Cu, Mn, and Zn contaminations since concentrations of Cu, Mn, and Zn in poultry litter are reported as high as 1,003, 667, and 669 mg/kg (Sims and Wolf 1994). While NO_3^--N can cause potentially fatal methemoglobinemia in infants, high Cu levels can cause gastrointestinal effects in the short term and kidney and liver damage in the longer term. Hence, the EPA established MCLs for Cu (1.3 mg/L) and NO_3^--N (10 mg/L) as part of the primary drinking water standards. In addition to having low to moderate mammalian toxicity (McBride 1994), Mn and Zn impart undesirable test, color or odor to drinking water. The EPA has established secondary standards for Mn (0.05 mg/L) and Zn (5 mg/L).

While there is considerable research on leaching of litter constituents from land-applied litter, no literature on leaching of As and heavy metals from stockpiled poultry litter could be located. Further, no published studies on the impact of stockpiling litter on the transformation of As species that could impact mobility and toxicity of these species are available. Hence, the overall goal of the proposed research was to evaluate the potential of constituents in turkey litter stockpiled on bare soil to contaminate groundwater. The specific research objectives were:

1. to evaluate transformations of As, N, and P species and changes in total C concentrations within the stockpiled turkey litter;
2. to monitor movement of As species, NO_3^--N, ammoniacal-N, phosphate, Cu, Mn, and Zn beneath and outside the litter stockpile footprint; and
3. to correlate Mehlich 3 soil test P, Cu, Mn, and Zn concentrations with total concentrations.

Methodology

This study was used to evaluate the transformation of As and other turkey litter constituent species during stockpiling and monitor movement of As and other species from stockpiles into the soil below. During an 11-month study, four turkey litter stockpiles were monitored during the summer and four more during winter. Turkey litter samples collected prior to and at the end of stockpiling were analyzed for various constituents. Soil samples were collected down to 610 mm depth both beneath stockpiles and beyond the stockpile footprints. Temperature and moisture conditions inside the stockpiles as well as in the soil below the stockpiles were favorable for biochemical transformation and movement of litter constituents. Arsenate was the dominant water soluble (WSE) As specie recovered in the litter at the end of stockpiling and some WSE-As was lost from the stockpiles. While roxarsone was completely degraded in the winter stockpiles, summer stockpiling increased roxarsone concentrations. Stockpiling resulted in highly mobile constituents (e.g., nitrate) impacting the entire sampled depth while less mobile species (e.g., WSE-As) mainly impacted the top 305-mm depth though there was some leaching into the deeper layer. The predominant WSE-As species in the soil was As(V) and high WSE-phosphorus levels in the soil could increase WSE-As leaching due to competitive sorption-desorption.

Principal Findings

Since arsenic (As) is a carcinogen and can also cause other diseases, the EPA has established a maximum contaminant level of 0.01 mg/L for As in drinking water. However, As is fed to turkey (also broiler and swine) mostly as roxarsone for controlling coccidial intestinal parasites, blackhead disease in turkey, and as a growth promotant. Since roxarsone is largely excreted, microbes in litter and soil can mineralize roxarsone into more toxic and mobile species, such as, arsenate (As(V)) and arsenite (As(III)). When litter is removed from the turkey house, it may be land applied immediately or stockpiled to be applied later. When litter is stockpiled on bare ground without cover, as is common practice in North Carolina, warm and moist conditions inside the stockpile combined with rainfall can result in transformation of litter constituents, including As into more mobile and toxic species that may then leach into the soil. Over time, depending on soil properties and water table depth, As from turkey litter could contaminate the ground water. Turkey litter also contains many other constituents such as nitrate-nitrogen (NO_3^--N), ammonium-N (NH_4^+-N), phosphorus (P), and heavy metals that while useful as plant nutrients, can cause water quality problems if they contaminate ground or surface waters.

While researchers have evaluated the mobility of litter constituents in land-applied litter, no literature on mobility of As and other constituents from stockpiled poultry litter is available. The objectives of this study were (i) to evaluate transformation of As and other constituent species during stockpiling; (ii) to monitor movement of As and other species beneath the stockpile; and (iii) to correlate Mehlich 3 P and heavy metal concentrations with total concentrations. Four turkey litter stockpiles (~4.1 Mg ea.) were established in Orangeburg loamy sand soil in Clinton, NC, during May to November 2004 for 161 d (summer stockpiles). Two stockpiles received simulated rainfall (under rainfall deficit conditions). Two stockpiles were instrumented for measuring temperature and moisture content. Turkey litter samples were collected prior to and at the end of stockpiling. Soil samples were collected down to 610 mm depth both beneath stockpiles (center, half diameter, and diameter) as well as beyond the stockpile footprints. After removing the summer stockpiles and leaving the site bare for 13 d, four winter stockpiles (November 2004 to April 2005) were established for 162 d. The winter stockpiles were treated the same as the summer stockpiles but were not instrumented. The study ran from May 2004 to April 2005. Twenty-four turkey litter samples and 96 soil samples were analyzed for moisture content, pH, electrical conductivity (EC), total carbon (C), dissolved organic C (DOC), total N, NH_4^+-N, NO_3^--N, total P, water soluble extract (WSE)-P, total As, WSE-As, total copper (Cu), total zinc (Zn), and total manganese (Mn). Solid phase and water soluble As speciation were also performed on the turkey litter and soil samples. Some important conclusions are summarized below.

- High temperature (>20°C higher than ambient temperature) and moist conditions in the stockpiles during summer stockpiling provided favorable conditions for biochemical activity in the stockpiles.
- Increase in total concentrations of most species (except C, N, and As) during stockpiling were attributed to large losses of C (only winter) and N.
- During summer stockpiling, pH and concentrations of water soluble constituents (except NO_3^--N) were higher in the core due to leaching from the skin (0-150 mm) into the core. However, during winter stockpiling, concentrations of all water soluble constituents decreased in the core as well, possibly, due to leaching into the soil below.
- Reduced WSE-As concentrations in both the skin and core in the winter stockpiles indicated that WSE-As leached into the soil below.
- Roxarsone completely degraded during winter stockpiling. However, roxarsone concentrations were higher at the end of summer stockpiling (vs. the initial samples) due to reasons that were unclear.
- Both solid-phase and water-soluble phase As speciation indicated that As(V) was the dominant As specie in the litter at the end of stockpiling (both summer and winter).
- Application of simulated rainfall to the stockpiles generally did not increase leaching losses (vs. natural rainfall only) probably due to the small depths of application (48.8 mm in summer and 32.0 mm in winter) and generally deficit rainfall conditions.
- Soil beneath the stockpile stayed warmer and wetter than the soil outside the stockpile footprints indicating that constituents leached into the soil from the stockpiles would have more favorable conditions for biochemical transformations and movement.
- Beneath the stockpiles, generally, the highest concentrations of most litter constituents in the soil were at the half diameter followed by the center. Greatest changes in pH were observed at the center and greater NO_3^--N leaching occurred at the diameter location.
- Highly mobile constituents (e.g., NO_3^--N) and pH impacted the entire 610 mm depth of soil; less mobile species (e.g., WSE-As and WSE-P) impacted mainly the top 305-mm depth though there was some evidence of leaching into the deeper layer.

- Concentrations of highly reactive and mobile species (e.g., NO_3^--N) were generally lower throughout the soil profile at the end of the study than after summer stockpiling probably due to transformation within and leaching below the sampling depth. However, concentration of less reactive species (e.g., WSE-As) were higher in the soil at the end of the study.
- Solid phase As speciation analyses indicated that As in the soil was mainly in the As(V) form, consistent with the findings in the turkey litter after stockpiling.
- Total soil As concentrations beneath stockpiles were always <5 mg/kg, comparable to background soil As concentrations in the 305-610 mm depth. However, stockpiling resulted in elevated WSE-As concentrations throughout the sampling depth even though no background WSE-As had been detected.
- High WSE-P concentrations in the litter and the underlying soil can induce arsenate leaching by competitive sorption-desorption. Arsenic leaching would be of greater concern in poultry litter containing higher As concentrations. Sims and Wolf (1994) reported total As concentrations up to 77 mg/kg while the highest concentration in this study was <16 mg/kg.
- There was evidence of Cu leaching down into the soil profile from the stockpiles.
- Based on analyses in the top 76 mm of Orangeburg loamy sand soil and based on coefficient of determination (r^2), Mehlich 3 analysis provided the best index of total concentration estimate for Zn, followed by P, Cu, and finally, Mn.

<u>Significance</u>

1. Poultry producers should be persuaded not to stockpile litter in the open on bare ground. Ideally, litter should be stored in a covered shed with a concrete floor; however, a less expensive solution would be to store the litter on a tarpaulin and cover it with a tarpaulin as well. A proper storage system will not only reduce the potential for groundwater contamination but also runoff and volatilization losses of litter constituents.
2. There is need to evaluate movement of litter pollutants through the soil on poultry farms where litter is stockpiled on bare ground by monitoring soil, groundwater and well water samples. Monitoring of farms should be prioritized based on arsenic content in feed or litter, duration of stockpiling on bare soil, soil texture, water table depth, and drinking water source (humans or animals).
3. Currently there are no regulations governing poultry litter stockpiling in North Carolina. Neighboring states such as Georgia and South Carolina have litter stockpiling regulations. The NC Department of Environment and Natural Resources may need to investigate the need for such regulations to safeguard water quality.

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Integration of High Resolution Imagery in Cost-effective Assessment of Land Use Practices Influencing Erosion and Sediment Yield

Basic Information

Title:	Integration of High Resolution Imagery in Cost-effective Assessment of Land Use Practices Influencing Erosion and Sediment Yield
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Principal Investigators:	Siamak Khorram, Stacy Nelson

Publication

1. Hester, D.B., H.I. Cakir, S.A.C. Nelson, and S. Khorram, (submitted in June 2006). Per-pixel Classification of QuickBird Hyperspatial Satellite Imagery for Urban Land Cover Mapping. Photogrammetric Engineering and Remote Sensing.
2. Hester, B., expected completion: 2006. Urban Land Cover and Watershed Monitoring Using QuickBird Hyperspatial Satellite Imagery. Ph.D. dissertation, Dept. of Forestry and Environmental Resources, College of Natural Resources, North Carolina State University, Raleigh, NC.
3. Khorram, S., S.A.C. Nelson, H.I. Cakir, D.B. Hester, 2005. Integration of high resolution imagery in cost-effective assessment of land use practices influencing erosion and sediment yield - Phase I. Submitted to WRRI: CEO Technical Report 221, 35 pp., Center for Earth Observation, North Carolina State University.
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9. Hester, B., S. Khorram, S.A.C. Nelson, and H.I. Cakir, 2005. Application of High resolution Satellite Imagery to Land Cover Study in a Rapidly Urbanizing Watershed in the North Carolina State University 2005 College of Natural Resources Future of Forests and Natural Resources Distinguished Lecture: Graduate Student Poster Session. Raleigh, NC.
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Title

Integration of High Resolution Imagery in Cost-effective Assessment of Land Use Practices Influencing Erosion and Sediment Yield (70207)

Problem and Research Objectives

National concerns have increasingly focused on the degradation of this nation's water quality and associated resources. As the Nation's population increases, so does society's ability to continuously alter the landscape leading to amplified surface loadings from storm and watershed overflow, increased suspended sediments in runoff, and agricultural and industrial drainage problems. Monitoring land use has become critically important as best management controls must now directly contend with the need for additional agricultural, industrial and urban growth and the desire to protect water quality. Improving degraded watersheds and streams require accurate and current land use and land cover (LU/LC) data. The Center for Earth Observation's previous study showed that, state-of-the-art, IKONOS imagery provides an effective means of obtaining LU/LC data within an urban watershed. IKONOS is particularly effective at delineating impervious surfaces prevalent in urban areas. However, IKONOS classifications based on single date imagery have some limitations with regard to other LU/LC classes. The delineation of bare and disturbed soils proved problematic. Bare and disturbed soils are a small part of the total proportion of the area within a watershed but these areas play a critical role in water quality and sediment load. Bare and disturbed soils are nearly identical spectrally and are often misclassified with fallow agriculture. Additionally, grass and open space which are commonly a significant part of an urban/suburban watershed, are often confused with agriculture. Due to the seasonal nature of agriculture, using multi-date imagery could be effective in distinguishing agriculture from the grass/open space class and from the bare/disturbed soil class.

Quantifying accurate LU/LC change within a watershed is an important component of monitoring watershed quality. During our previous study, IKONOS proved to be an effective means to quantify land use composition within an urban watershed. However, our initial analysis of LU/LC only provided a snapshot of the watershed land use composition at a single point in time. To completely understand the impact land use has on water quality, it is also important to accurately assess the type and position of changes occurring within the watershed. This can be accomplished through change detection using remotely sensed imagery. Currently, most LU/LC change detection studies have used lower resolution imagery. However, lower resolution imagery may be incapable of accurately detecting small-scale or mixed LU/LC classes that fall below the resolution of the imagery but still may be significant contributors of upland sediment load.

The objectives of this research was to develop a classification procedure for high-resolution satellite data that would provide a cost-effective method for identifying and quantifying LU/LC categories that have the potential for high erosion and sediment contribution within an urbanized watershed.

Methodology

The study area, originally the Hominy Creek watershed, North Carolina, was revised, with WRRI approval, to include highly active and continuously changing watersheds in Northeast Raleigh, North Carolina. The level and scale of change occurring in the North Raleigh area would provide greater indications of the water quality, impervious surface areas, high-growth indices, and their linkages produced through the techniques being developed for this study. The North Raleigh study area contains primarily urban and suburban land uses interspersed with large forested clusters. The study area totals 71.5 km^2 and is located just northeast of the capital city of Raleigh, and has been sited as one of the areas of highest growth from 2000 to 2004.

The general approach for this study consisted of four steps: (1) pre-processing of the data; (2) evaluation of different data fusion, classification and post processing techniques; (3) final classification procedure based on the results from evaluation process; and (4) accuracy assessment. The pre-processing step involved accurate geometric registration of the images, atmospheric corrections, etc. The evaluation process consisted of applying the three different data processing tools (data fusion, classification method, and post processing filter) to assess their effect on the classified maps. Any differences in map accuracy should be largely explained by the effect of these tools. Consequently, the third step was the determination of the final classification procedure based on the input from the evaluation process. In the final step, accuracy assessment of the final thematic map was performed.

It is important to distinguish the terms “classification method” and “classification procedure.” Classification method describes the choice of a specific algorithm to assign raw image values to pre-determined land use/land cover (LU/LC) types. On the other hand, classification procedure is a much broader term involving pre-processing of the images, selection of the classification algorithm, post-processing, and the accuracy assessment.

Principal Findings

Six distinct LU/LC categories having the greatest impact on water quality were determine from our classification analysis; Deciduous Trees, Evergreen Trees, Herbaceous Vegetation, Bare/Disturbed Soil, Water, Impervious. Image classification performed using a supervised classification procedure and a 3x3 majority filter algorithm, yielding an overall classification accuracy of 80.29 percent (Kappa 0.73). The users’ accuracy for the impervious surface category was 94 percent (Kappa 0.89). The results suggest the classification accuracy and level of detail produced from high-resolution imagery, using the procedures detailed in this study, can be used to accurately identify and quantify levels of LU/LC which influence water quality within highly urbanized watersheds, particularly impervious surfaces.

The high accuracy of the final thematic maps produced from Quickbird imagery (61 cm at the highest spatial resolution) in this study is of considerable note. This degree of overall classification accuracy is often very difficult to achieve in highly mixed LU/LC landscapes, as well as in highly urbanized landscapes. A parallel CEO classification study, incorporating the identical north Raleigh watershed study area, using 30-meter resolution Landsat Enhanced Thematic Mapper (ETM) data, produced a similar level of accuracy but not at the level of detail produced by this study, as shown in Figure 1. Consequently, for the same study area, the amount of impervious surface estimation was identified as approximately 32 percent in the Landsat study. The Quickbird based classification suggested that only 22 percent of the study area was actually impervious.

This over-estimation of impervious surfaces with Landsat data is not a limitation of accuracy but rather a limitation of details. In the parallel CEO study mentioned above, the impervious surfaces were assimilated by three urban classes (low intensity, medium intensity, and high intensity urban) based on the level of imperviousness within each 30 by 30 meter pixel. In such studies, the impervious surface amount for a given area (such as a watershed) is estimated using an imperviousness level assigned to each urban class. This impervious level is given as part of the definition of these classes and is represented as a simple percentage (i.e., a pixel is considered to be low intensity urban if 20 to 40 percent of the pixel is impervious). A crude transformation using these percentages is thus widely used to determine percent imperviousness. For example, in EPA's "rapid watershed planning handbook", 60 percent of high intensity residential, 40 percent of low intensity residential, and 90% of commercia1/industria1 areas are considered impervious (Caraco et. al. 1998). These types of generalizations are inherently subject to overestimations or under-estimations of impervious surface for a given area. In contrast, using high resolution data, impervious surfaces can be directly mapped, thus bypassing the limited and indirect means that are usually necessary. By developing and comparing image classification techniques, this study produced very high total-area, overall classification accuracies in the North Raleigh watershed study area. The use of a supervised classification procedure, along with a 3x3 majority filter algorithm, achieved the highest levels of overall classification accuracy (84 percent, Kappa 0.74).

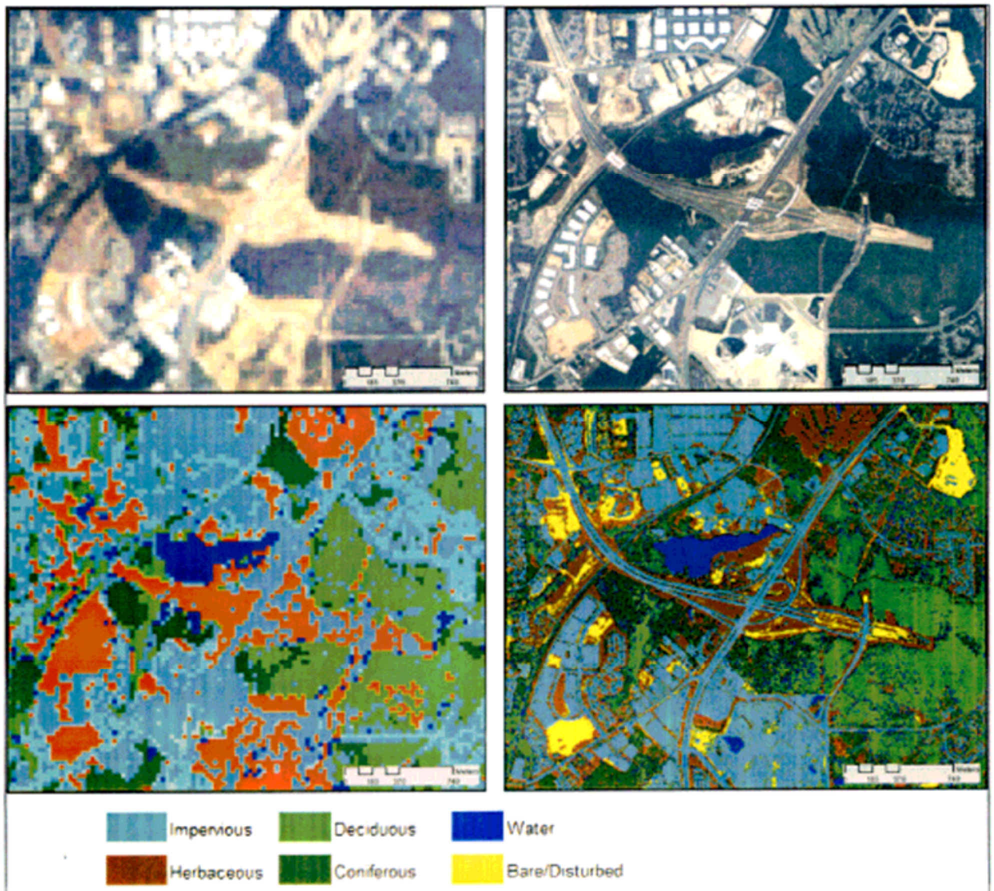

Figure 1 Comparison of land cover classifications based on Landsat ETM+ and Quickbird imagery. On the left is a Landsat ETM+ image and, below it, a classification based on that image. On the right, the same area is represented by Quickbird data with a classification based on it displayed below. The two classifications both have an overall accuracy better than 80%.

The Cakir-Khorrarn (CK)-data fusion technique developed by NCSU's CEO (patent pending) provided surprising and impressive improvements in spectral classifications of the study area using high-resolution Quickbird data. The CK-data fusion method has proven to be superior to extracting improved classification information in comparison to existing commercial techniques (Khorram et al. 2003). Current commercial "pan sharpening" techniques do not retain the spectral integrity of the multispectral data. Thus these techniques result in a fused image that is capable of only limited use for LU/LC classification procedures. The CK-fusion technique results in only minimal spectral distortion in the original data characteristics. This enables users to incorporate enhanced analyses on the fused images (i.e., more detailed classifications). This study represents a first attempt at applying this technique to high-resolution Quickbird data.

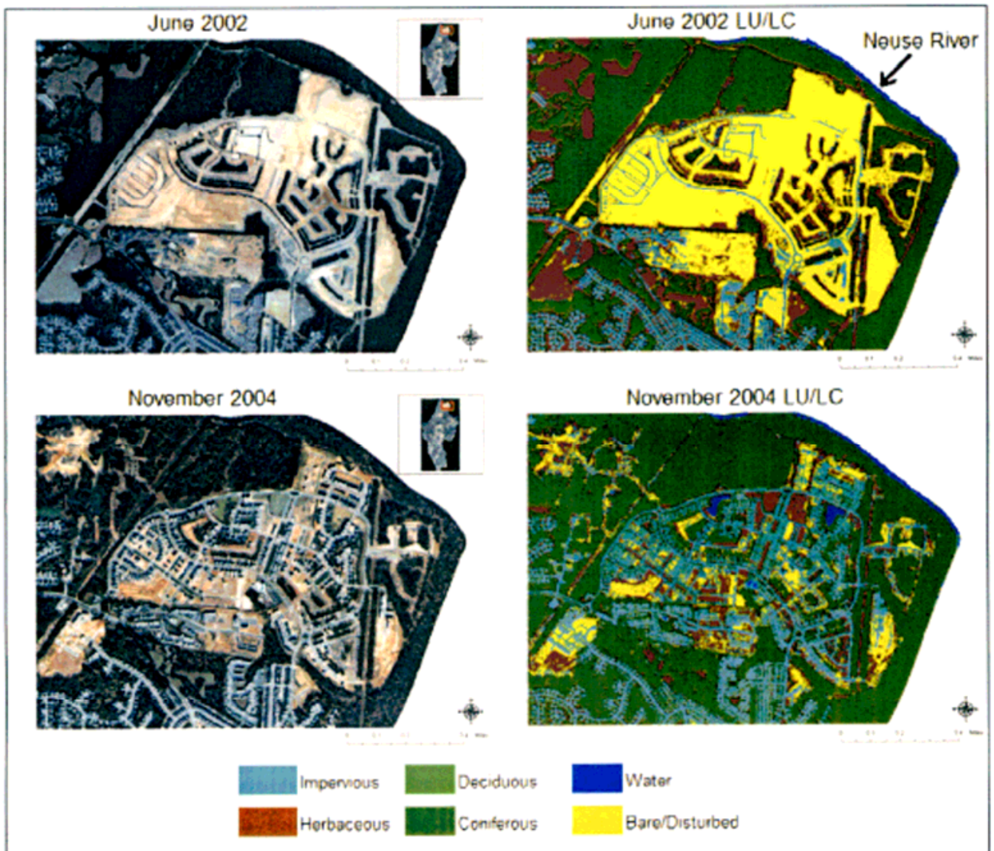

Figure 2 A portion of the study area that has experienced dramatic land cover change between 2002 (top) and 2004 (bottom).

This study concluded with an initial analysis of the feasibility of developing high-resolution change detection using Quickbird data. This preliminary change detection within the North Raleigh study area, using summer 2002 and winter 2004 imagery, confirmed that this area is undergoing dynamic levels of LU/LC changes within very short time intervals and can be monitored using Quickbird high-resolution data. This level of rapid growth has been successfully captured in the preliminary change detection analysis illustrated in Figure 2.

Significance

The identification and quantification of current LU/LC categories within a watershed, as performed in this study, represents a very important component in understanding landscape contributions to downstream erosion and sediment yield. However, it is also critically important to understand the changes in these LU/LC categories that are taking place in the watershed. Changes in LU/LC can provide an indication of the stability and sensitivity of LU/LC categories and the ability for water quality modelers to incorporate these changes into their estimation of future water quality impairment capacities.

The availability of high-resolution satellite imagery already promises resource managers a cost-effective means of securing up-to-date land cover information.

References

Caraco, D. R. Claytor, P. Hinkle, H. Kwon, T.Schueler, C.Swann, S. Vysotsky, and J. Zielinski. 1998. *Rapid Watershed Planning Handbook: A Comprehensive Guide for Managing Urbanizing Watersheds.* Prepared by Center For Watershed Protection, Ellicott City, MD, for U.S. Environmental Protection Agency, Office of Wetlands, Oceans, and Watersheds, and Region V.

Khorram, S. H.I. Cakir, and D.F. Stallings. 2003. Comparison of remotely sensed data from different sensors with different spatial and spectral resolutions to detect and characterize riparian stream buffer zones. EPA Spectral Sensing of Vegetation Conference, Las Vegas, Nevada.

Information Transfer Program

In addition to activities related to specific research projects, WRRI maintains a strong information transfer program by cooperating with various state agencies and professional organizations to sponsor workshops and other events and by seeking grants for relevant activities. During the current fiscal year, WRRI continued to be designated by the N.C. Board of Examiners for Engineers and Surveyors as an Approved Sponsor of Continuing Professional Competency activities for Professional Engineers and Surveyors licensed by the State of North Carolina. In addition, WRRI is an approved sponsor for the N.C. Board of Landscape Architects to offer contact hours. This allows WRRI to offer Professional Development Hours to engineers and surveyors for attending our water resources research seminars and our Annual Conference.

WRRIs Information Transfer Program includes workshops supported by the NC Department of Environment and Natural Resources (DENR), Land Quality Section along with the NC Sedimentation Control Commission (SCC). Workshops held during this period include: (1) Two Advanced Erosion and Sediment Control Planning and Design Workshop, 3/2-3/2005, Wrightsville Beach, NC and 4/28-29/2005, Asheville, NC; (2) WRRI Preconference Symposium: Low Impact Development Approaches for Sustainable Water Management, 4/4/2005, Raleigh, NC; (3) WRRI Annual Conference: Managing Water Quality & Quanatity: Integrating Science, Technoloy & Policy, 4/5/05, Raleigh, NC; (4) Two Basic Planning and Design Erosion and Sedimentation Control Workshops, 10/26-27/05, Hickory, NC, 12/14-15/05 Wilmington, NC; and (5) Erosion and Sedimentation Control Local Programs Training Workshop, 1/31-2/1/06, Southern Pines, NC.

WRRI maintains six electronic mail lists (listserves): (1)Water Research list - 180 subscribers - inform water researchers from NC universities about calls for papers, grants, upcoming conferences, student internships, etc.; (2)WRRI-News list - 700 subscribers - informs researchers, local governments, municipalities, interest groups etc. abouts calls for papers, grants, upcoming conferences and events, etc.; (3)NCWRA-info list - 150 subscribers - provides information of NCWRA sponsored events; (4)Sediments list - 200 subscribers - sends out SEDIMENTS newsletter and information on erosion and sediment control regulations; (5)& (6) Urban Water Contortium(UWC) and UWC-Stormwater list - for Urban Water Consortium member communications.

WRRI maintains its own website (http://www.ncsu.edu/wrri). The website provides on-line access to the WRRI-News, the WRRI technical report summaries, water research seminars, and information on WRRI-sponsored workshops, conferences, and seminars.

Another way WRRI provides Information Transfer is through the NC Water Resources Association Luncheon and Forums: (1) September 12, 2005, Raleigh, NC, "New Approaches for Measuring Pathogens and Indicators in Environmental Waters" Rachel T. Noble, PhD, Assistant Professor, University of North Carolina at Chapel Hill, Institute of Marine Sciences; (2) December 5, 2005, Raleigh, NC "If You Build It, Will They Come? Evaluating Cumulative and Secondary Environmental Impacts and Local Growth Options" J. Scott Lane, AICP, Director of Planning, The Louis Berger Group, Inc. Shari Bryant, Piedmont Region Coordinator, Habitat Conservation Program, N.C. Wildlife Resources Commission; and (3) February 13, 2006, Raleigh, NC, "Hurricane Effects: Following the Path of Rita" Beth Wrege, Hydrogeologist, U.S. Geological Survey.

WRRI Research Seminar Series

Basic Information

Title:	WRRI Research Seminar Series
Project Number:	2005NC67B
Start Date:	3/1/2005
End Date:	2/28/2006
Funding Source:	104B
Congressional District:	2
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	
Principal Investigators:	David Moreau, Kelly Porter

Publication

Organized and sponsored the following seminars by investigators working under WRRI grants:

Dr. Deanna Osmond
NC State University
“Effectiveness of Agricultural Best Management Practices”
Tuesday, March 15, 2005, 3:00 pm
1132 Jordan Hall, NC State University

Dr. Siamak Khorram
NC State University
“Current Trends in Land Use/Land Cover Mapping, Change Detection, and Monitoring from Remotely Sensed Data”
Tuesday, April 12, 2005, 3:00 pm
1132 Jordan Hall, NC State University

Dr. Phil Singer
UNC Chapel Hill
"Estimated Compliance with the Proposed Stage 2 Disinfection By-Products Rule for Eleven Water Utilities in North Carolina"
Wednesday, September 28, 2005, 3:00 pm
1132 Jordan Hall, NC State University

Dr. Sankar Arumugam
NC State University
"Improved Water Allocation Using Climate Information Based Streamflow Forecasts: Decision Analyses and Possibilities"
Wednesday, October 5, 2005, 3:00 pm
1132 Jordan Hall, NC State University

Dr. Francis de los Reyes
NC State University
"Improving Dewatering of Wastewater Biosolids Using Innovative Approaches"
Tuesday, October 18, 2005, 3:00 pm
1132 Jordan Hall, NC State University

Dr. Sanjay Shah
NC State University
"Pollutant Leaching Potential from Turkey Litter Stockpiled on Bare Soil"
Tuesday, November 15, 2005, 3:00 pm
1132 Jordan Hall, NC State University

Dr. Craig Allan
UNC Charlotte

"Hydrological and Biogeochemical Investigations of Riparian Buffers in the Piedmont and Blue Ridge Regions of North Carolina"
Tuesday, November 29, 2005, 3:00 pm
1132 Jordan Hall, NC State University

Dr. Carmelo Tomas
UNC Wilmington
"Evidence for Nutrient Regulation of Harmful Algal Blooms in Wilson Bay, New River, NC"
Wednesday, November 30, 2005, 3:00 pm
1132 Jordan Hall, NC State University

Dr. Greg Characklis
UNC-Chapel Hill
"Microbial Partitioning and its Impact on Stormwater Management"
Tuesday, January 17, 2006, 3:00 pm
1132 Jordan Hall, NC State University

Dr. Larry Cahoon
UNC Wilmington
"Is There a Relationship between Phosphorus and Fecal Microbes in Aquatic Sediments?"
Tuesday, February 7, 2006, 3:00 pm
1132 Jordan Hall, NC State University

The WRRI Institute NEWS

Basic Information

Title:	The WRRI Institute NEWS
Project Number:	2005NC68B
Start Date:	3/1/2005
End Date:	2/28/2006
Funding Source:	104B
Congressional District:	2
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	
Principal Investigators:	Kelly Porter

Publication

Published the *WRRI News* three times during the reporting period. The WRRI News is an 8-page newsletter that covers a wide range of water-related topics from current federal and state legislation and regulatory activities to new research findings, water-related workshops and conferences, and reviews of water-related publications. The WRRI News is sent to nearly 4,300 federal and state agencies, university personnel, multi-county planning regions, city and local officials, environmental groups, consultants, businesses and individuals.

New WRRI Research Reports

Basic Information

Title:	New WRRI Research Reports
Project Number:	2005NC69B
Start Date:	3/1/2005
End Date:	2/28/2006
Funding Source:	104B
Congressional District:	2
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	
Principal Investigators:	David Moreau

Publication

New WRRI Research Reports – A demand for Institute reports continues. During the year, the Institute published the following reports for distribution to users throughout the state and nation. In addition, new Journal Article Series (JA) in which journal articles are submitted to peer reviewed journals in lieu of institute final reports are listed. .

WRRI-343-C – Calibration and Verification of a Two-Dimensional, Laterally Averaged Mechanistic Model of the Neuse River Estuary.

WRRI-350 – Environmental Analysis of Swine Waste Management Technologies Using the Life-Cycle Method.

WRRI-351-A – Nitrate Flux from Ground to Surface Waters Adjacent to the Neuse River Waste Water Treatment Plant.

WRRI-352 – A Systematic Evaluation of Polyacrylamide for Sediment and Turbidity Control.

WRRI-353 – Effect of Management Practices on Denitrification in Soils Fertilized with Liquid Swine Waste.

WRRI-354 – Nitrogen Cycling Dynamics in Agricultural Fields Fertilized with Liquid Swine Waste–Microbial Nitrification and Denitrification.

WRRI-JA-11 – Artificial Neural Networks for Forecasting Watershed Runoff and Stream Flows.

WRRI-JA-12 – Lithology and Mineral Chemistry of the Castle Hayne Limestone – An Important Coastal Plain Aquifer in North Carolina.

WRRI-JA-13 – Membrane Treatment of Secondary Effluent for Subsequent Use.

WRRI-JA-14 – Influence of Temporal Variations in Water Chemistry on the Pb Isotopic Composition of Rainbow Trout (Oncorhynchus mykiss).

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 NCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	5	0	2	0	7
Masters	8	0	0	0	8
Ph.D.	1	0	0	0	1
Post-Doc.	3	0	0	0	3
Total	17	0	2	0	19

Notable Awards and Achievements

Publications from Prior Projects

1. 2002NC3B ("A Systematic Evaluation of Polyacrylamide for Sediment and Turbidity Control") - Conference Proceedings - Mitasova, H., Bernstein, D., Harmon, R.s., Hofierka, J., and McLaughlin, R., 2005, Monitoring and modeling natural and anthropogenic terrain change: Spatial analysis and simulations of impact on landscape processes, in Proceedings of the International GIS Planet conference, Estoril, Portugal, CDROM.
2. 2002NC3B ("A Systematic Evaluation of Polyacrylamide for Sediment and Turbidity Control") - Conference Proceedings - Mitasova, H., Thaxton, C., Hofierka, J., McLaughlin, R., Moore, Al, and Mitas, L., 2005, Path sampling method for modeling overland water flow, sediment transport and short term terrain evolution in Open Source GIS, in, Elsevier, Proceedings of the XVth International Conference on Computational Methods in Water Resources (CMWR XV), Chapel Hill, NC, pp. 1479-1490.
3. 2002NC3B ("A Systematic Evaluation of Polyacrylamide for Sediment and Turbidity Control") - Articles in Refereed Scientific Journals - Mitasova, H., Mitas, L. Ratti, C., Ishii, H. Alonso, J., and Harmon, R.S. (In Press), Real-time Human Interaction with Landscape Models Using a Tangible Geospatial Modeling Environment, IEEE Computer Graphics & Applications, Special Issue - GeoVisualization.