

**Water Resources Research Institute of the  
University of North Carolina  
Annual Technical Report  
FY 2009**

# Introduction

During 2009-2010 (Fiscal Year 2009), the Water Resources Research Institute (WRI) 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.

Research priorities continue to be identified by the WRI Advisory Committee, composed of representatives of several federal and state agencies, local governments, industries, and non-governmental environmental organizations (NGOs). A technical review committee is also convened on an annual basis to advise WRI staff on the scientific merit of research proposals submitted for funding. Full-time faculty members from all North Carolina institutions of higher education are eligible to receive grants from WRI.

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 disseminated by publication of technical completion reports, summaries in the WRI newsletter, publication of summaries on the WRI website, and presentations by investigators at the WRI Annual Conference. WRI 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 WRI to offer Professional Development Hours (PDHs) and contact hours for attendance at the WRI Annual Conference and other workshops and seminars that WRI sponsors.

## Research Program Introduction

During 2009-2010 (Fiscal Year 2009), WRRI continued its regular program of fostering research, training, and information transfer responsive to water issues of the state and region. Results from Institute-supported research efforts are expected to assist local, municipal, state, regional and federal agencies improve their decision-making in the management and stewardship of their water resources. To help it chart and sponsor a research program responsive to the water resource issues and opportunities in North Carolina, WRRI interacts closely with the N.C. Department of Environment and Natural Resources, other agencies, water and power utilities, and an array of research and outreach programs within the UNC system and at private higher educational institutions across North Carolina. A research advisory committee provides input, guidance, and review of the Institute's research priorities on an annual basis. This committee is composed of representatives of several federal and state agencies, local governments, industries, and non-governmental environmental organizations (NGOs). A technical review committee is also convened on an annual basis to advise WRRI staff on the scientific merit of research proposals submitted for funding. Full-time faculty members from all North Carolina institutions of higher education are eligible to receive grants from WRRI.

Each year, in a day-long session, members of this committee bring to the table present and future needs they see for water research. Via a facilitated formal process, an extended list of research needs and/or new emphases is developed. The results of this process are shared with prospective investigators as part of WRRI's annual call-for-proposals. Proposals that address the annual priorities and meet peer review and other criteria receive preferential consideration for funding. Research priorities, as determined via the above process, are incorporated into our Section 104 Objectives on an annual basis. The proposal solicitation, as in the past, is sent to relevant contacts 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 approximately 200 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. Criteria that was be used to judge proposals include: the scientific quality of the proposed work; the need for this research in North Carolina; how closely the project relates to the WRRI mission; the usefulness of the results for managing water resources in North Carolina; the probability that useful results can be obtained in one year; the potential for continued support from other funding sources; and the cost of the proposed work, opportunities for application in teaching.

The research priorities for FY 2009-2010 are listed as:

A. Water Availability, Use and Climate Interaction 1. Water supply and demand: Investigate potential effects of climate change on both supply and demand for water. 2. Effectiveness of conservation measures, including measures that improve the long-term efficiency of water use and measures that reduce demand during droughts 3. Climate forecasts to improve water management during droughts 4. Water Reuse Evaluate quality of gray water and harvested rainwater that has potential for reuse. Evaluate the cost and other factors affecting alternative approaches to reuse.

B. Drinking Water Quality 1. Contaminant screening: Evaluate current and new techniques to screen drinking water regulated contaminants and other contaminants of special concern upstream of water intakes and downstream of wastewater discharges. 2. Compliance with drinking water standards: Evaluate how water suppliers can most effectively comply with regulations for disinfection by-products (including nitrogenous DBPs) and other difficult-to-comply parameters. 3. Groundwater quality and safety: Evaluate criteria for groundwater standards, groundwater aquifer storage and recovery systems; groundwater safety for individual homes and small public systems with special attention to arsenic and radon. 4. Evaluate factors affecting lead,

## Research Program Introduction

copper and other metals in household plumbing.

C. Water Quality and Nutrient Monitoring, Management and Impacts 1. Chlorophyll *a* standard: Evaluate alternatives to the use of chlorophyll *a* standard as a water quality indicator. 2. Nutrient balance in watersheds: Determine how various watershed nutrient sources and sinks respond to changes over time. Determine the expected time lags in changes to surface and ground water nutrient loads. Determine the hydrological influences on nutrient cycling. Evaluate the different approaches/strategies to water monitoring and the costs involved. 3. Improved effectiveness of water quality monitoring across North Carolina: More effective monitoring is needed for permitting, modeling use support, and assessment of trends. Determine frequency of sampling in relation to environmental indicators, and fate and transport of important pollutants. 4. Economic and feasibility assessment of water quality and remote environmental monitoring programs 5. Agricultural impacts Determine water quality impacts (nutrients, fecal coliform, pharmaceuticals, etc.) of special classes of agricultural operations such as land application of animal waste, pasture operations, municipal biosolids, and organic farming. Determine effect of spray operations on runoff of nutrients.

D. Urbanization Impacts on Water Quality 1. Stormwater Management: Evaluate how well stormwater best management practices (BMPs) and management strategies protect downstream water quantity and quality at the watershed scale. Evaluate performance of practices for addressing coastal stormwater discharges. Evaluate the barriers to stormwater retrofits and off-stream management for water quality. 2. Low Impact Development (LID): Determine which LID techniques work best in North Carolina to minimize development impacts to watersheds.

E. Aquatic Ecosystem Functions and Instream Flow Needs 1. Effective approaches to aquatic ecosystem restoration 2. Instream flow and wetland functional assessment approaches 3. Protection of rare and endangered aquatic species in North Carolina streams.

F. Waste Management 1. Biosolids disposal: Determine fate and transport of nutrients and pathogens from agricultural uses of biosolids. Determine alternative uses and markets for biosolids generated from wastewater. 2. Investigate factors leading to sanitary sewer overflows, including disposal of fats, oils, and greases.

G. Groundwater 1. Shallow groundwater of the Coastal Plain: Determine the relationship between the Coastal Plain's surficial aquifers, surface water, and the first confined aquifer. Determine the relationship between the transfer of contaminants and waste discharges from land and surface waters to surficial aquifers and vice versa. 2. Naturally occurring chemicals: Compile latitude and longitude coordinates and well and water depth of areas that have naturally occurring chemicals in soil that affect groundwater. Working with USGS datasets would be useful.

# Antibiotic Resistance and Water Quality: Land Application of Swine Lagoon Effluent as a Potential Source of Antibiotic Resistant Genes in Surface Water

## Basic Information

<b>Title:</b>	Antibiotic Resistance and Water Quality: Land Application of Swine Lagoon Effluent as a Potential Source of Antibiotic Resistant Genes in Surface Water
<b>Project Number:</b>	2007NC76B
<b>Start Date:</b>	3/1/2007
<b>End Date:</b>	2/28/2010
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	2
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Water Quality, Agriculture, None
<b>Descriptors:</b>	swine waste, bacteria, irrigation, lagoons
<b>Principal Investigators:</b>	Alexandria Graves, Daniel Israel

## Publication

1. Liwimbi, L.C. 2009. Antibiotic Resistance and Water Quality: Land Application of Swine Lagoon Effluent as a Potential Source of Antibiotic Resistant Genes in Surface Water., MS Thesis, Dept. of Soil Science, NC State University, Raleigh, NC.

## Title

Antibiotic Resistance and Water Quality: Land Application of Swine Lagoon Effluent as a Potential Source of Antibiotic Resistant Genes in Surface Water

## Problem

The use of antibiotics in animals is suspected to be a major route of the transference of antibiotic resistant bacteria to humans, even when different antibiotics are used in animals than in people. Mathematical models have been used to evaluate the medical impacts of simultaneously using the same antibiotic in food animals and human medicine. Analysis from the mathematical models demonstrates that animal antibiotic use may hasten the appearance of antibiotic resistance and decrease the efficacy of antibiotic used in humans. A number of reports have specifically linked antibiotic use in livestock with the spread of antibiotic resistant pathogenic bacterial to humans. North Carolina is the home of our Nation's second largest swine industry. Most of this swine production is restricted to a small geographical area in southeastern North Carolina. This high concentration of swine production may increase the risk of antibiotic resistant bacteria from swine operations reaching the nearby surface waters. If antibiotic resistance and the presence of antibiotic resistant genes are occurring at an elevated level in swine waste, then it logically follows that antibiotic resistant genes found in bacteria are potentially discharged during land application of swine lagoon effluent and have the potential to reach nearby surface waters. The goal of this study is to evaluate the association of antibiotic resistance genes found in *E.coli* isolated from swine with the actual phenotypic expression of the resistance. Additionally to develop an antibiotic resistance database for *E. coli* isolates from a commercial swine facility and assess its efficacy for tracking movement of bacteria from swine confinement houses to surface waters. The appearance of swine-manure derived bacteria in shallow groundwater near the stream or in the stream would document the need for improved mitigation strategies. To establish that swine manure-derived bacteria are discharged to surface waters, source tracking methods will be used.

The predominant manure management choice for swine is the lagoon system. Anaerobic lagoons are widely used in temperate climates in the United States for the treatment of swine manure. They are simple to manage and very effective in reducing organic matter and nutrients when properly designed and operated (Bicudo et al, 1999). Anaerobic lagoons store, treat and minimally dilute the waste from concentrated animal feeding operations (CAFO). Lagoons, however, were not designed to control pathogens, despite the fact that swine manure contains as high as a billion protozoa, fungi and bacteria per gram.

Previous studies showed that pathogens can persist in swine lagoon liquid and sludge, in manure piles, and in waste litter (Plym-Forsshell 1995; Radtke and Gist 1989). Pathogens are more likely to persist in liquid or moist waste, and in sludge or lagoon treatments, which do not heat manure to a high enough temperature to kill pathogens (Kudva et al. 1998). Hog manure may contain pathogens like *Cryptosporidium* and *Salmonella*, which can cause diarrhea in normal healthy adults, but can be fatal in children, the elderly and other groups at risk. (Sobsey et al, 1999). Raw hog waste applied to crops can contain 100 to 10,000 times the number of pathogens that is allowed in treated human waste (Sobsey et al, 1999). However, since raw hog waste is rarely if ever applied to crop land in North Carolina, the level of human pathogens in effluent from treatment lagoons applied to crops is likely to be lower than that reported for raw manure (Sobsey, et al. 1999) Nevertheless, since pathogens move easily through air and water, there is potential for transmission from swine operations to humans.

## Research Objectives

The goal of this study is to evaluate the association of antibiotic resistance genes found in *E.coli* isolated from swine with the actual phenotypic expression of the resistance. Additionally to develop an antibiotic

resistance database for *E. coli* isolates from a commercial swine facility and assess its efficacy for tracking movement of bacteria from swine confinement houses to surface waters.

1. Determine the relationship between presence of antibiotic resistance genes for tetracycline, sulfonamides, streptomycin and apramycin resistant genes found in *E. coli* strains from swine manure, lagoon effluent and nearby ground and surface waters with the actual phenotypic expression of the resistance.
2. Develop a database of antibiotic resistance patterns for *E. coli* isolated from swine manure, cattle manure, wildlife manure, human and pets.
3. Evaluate the usefulness of this database for assessing movement (or dispersal) of *E. coli* from a confined swine operation to a nearby stream.

The goal of this study is to identify and quantify *E.coli* isolates with antibiotic resistant genes in raw swine manure, lagoon effluent from a commercial swine facility and in nearby ground and surface waters. The appearance of swine-manure derived bacteria in shallow groundwater near the stream or in the stream would document the need for improved mitigation strategies. To establish that swine manure-derived bacteria are discharged to surface waters, source tracking methods will be used.

### Methodology

The Soil Science Department has well equipped laboratories for molecular and microbiological analysis of manure, water/wastewater, and soil. Dr. Graves's laboratory is equipped with a Mastercycler ep *realplex* real-time thermal cycling system, eppendorf thermocycler for conventional PCR, agarose gel electrophoresis units, gel documentation systems, membrane –filter manifolds, centrifuges, water baths, incubators, refrigerators, -20°C and -80°C freezers. The lab also houses PC computers with internet access.

**Study Site:** The study site (Figure 1) is a commercial swine farm with a standing herd of 4400 finishing animals, located in a 275 ha watershed along the upper reach of Six Runs Creek, which flows in a southerly direction in eastern Sampson County, NC. The study site is approximately 18 km north of Clinton, NC. The study site has two waste application fields. The stream adjacent to waste application field 1 flows in a channel, but the segment adjacent to waste application field 2 is impounded by two beaver dams and forms an elongated pond. Below the lower beaver dam the stream flows in a channel as it exits the producer's property. Four swine operations with 23 swine houses are located in this watershed. Fields receiving swine-lagoon effluent (approximately 40 ha) and cropped with coastal bermuda grass managed for hay or as grazed pastures are situated on both sides of the creek. A forested riparian buffer of variable width (41 to 87m) is located between the waste application fields and the creek. Three transects of piezometers (wells) have been installed in each of two waste application fields and the adjacent forested riparian system on the west side of Six Runs Creek for sampling of shallow ground water. Each transect has four or five well nests positioned on the side slope of the field, at the field edge, in the riparian zone, and at the stream edge. In the waste application fields, wells within a nest have been placed 1 m apart and screened at three different depths: near top of water table, and at two greater depths below the water table (Israel et al., 2005).

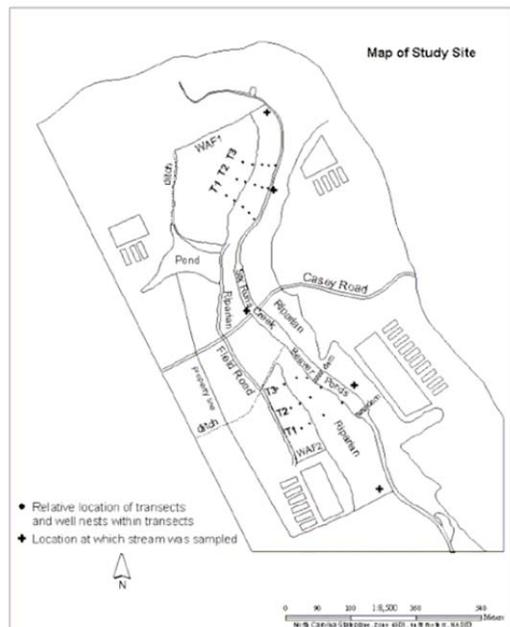


Figure 1. Map of study site. Figure found in Israel et al., 2005

**Procedures for Objective 1:** A combined total of 300 *E. coli* isolates from swine houses, lagoons, ground and surface waters will be evaluated for antibiotic resistance genes and phenotypic expression of antibiotic resistances. Shallow groundwater will be sampled from wells in the sprayfield and at the stream edge and the stream will be sampled upstream, adjacent to and down stream of the swine operation. Swine manure and lagoon effluent samples will be serially diluted (surface water and groundwater samples will not be diluted) and filtered on membrane filters. Filters will be transferred to plates and incubated at 44.5°C. After 24 h single colonies will be picked and transferred to liquid media and incubated at 37°C. After 24 h an aliquot of each culture will be taken for PCR analysis and another aliquot will be transferred to micro-well plates for the antibiotic resistance evaluations.

*PCR detection of resistance genes.* Bacterial lysates will be used as templates for the PCR reactions. Lysates will be prepared by resuspending a loopful of bacteria from a fresh overnight culture on a blood agar plate will be resuspended in 500µl of water, homogenized and heated at 95°C for 15 min. After cooling to room temperature, suspensions will be centrifuged for 3 min at maximum speed in a microcentrifuge. A 1-µl volume of the supernatant will be used as a template for each 25-µl PCR mixture. The primers and protocols for major resistance genes for tetracycline (*tetA*, *tetB*, and *tetC*), sulfonamides (*sul1*, *sul2*, and *sul3*), streptomycinspectinomycin (*strA/strB*, *aadA*), and apramycin [*aac(3)IV*] are described in Table 1. All polymerase chain reactions will be completed with the following temperature cycling: 1 cycle of 4 min at 95°C; 35 cycles, each consisting of 1 min at 95°C, 1 min at annealing temperature given in Table 1 followed by 1 min at 72°C; and 1 cycle of 7 min at 72°C. SYBR Green I (Applied Biosystems) will be used to detect the amplified product. The product will be run through gel electrophoresis to confirm fragment location.

Table 1. Single PCR conditions and control strains

Gene	Primer name	Primer sequence	Anneal (°C)	Fragment size (bp)	Positive control
<i>aadA</i>	4Fa	GTGGATGGCGGCCTGAAGCC	68	525	AMR-002d
	4Ra	AATGCCAGTCGGCAGCG			
<i>strA</i>	2Fa	CCTGGTGATAACGGCAATTC	55	546	AMR-009d
	2Ra	CCAATCGCAGATAGAAGGC			
<i>strB</i>	3Fa	ATCGTCAAGGGATTGAAACC	55	509	AMR-009d
	3Ra	GGATCGTAGAACATATTGGC			
<i>tetA</i>	TetA-Lb	GGCGGTCTTCTTCATCATGC	64	502	RO8d
	TetA-Rb	CGGCAGGCAGAGCAAGTAGA			
<i>tetB</i>	TetB-Lb	CATTAATAGGCGCATCGCTG	64	930	PB#11d
	TetB-Rb	TGAAGGTCATCGATAGCAGG			
<i>tetC</i>	TetC-Lb	GCTGTAGGCATAGGCTTGGT	64	888	PB#02d
	TetC-Rb	GCCGGAAGCGAGAAGAATCA			
<i>sul1</i>	Sul1-Lb	GTGACGGTGTTCGGCATTCT	68	779	AMR-130d
	Sul1-Rb	TCCGAGAAGGTGATTGCGCT			
<i>sul2</i>	Sul2-Lb	CGGCATCGTCAACATAACCT	66	721	AMR-130d
	Sul2-Rb	TGTGCGGATGAAGTCAGCTC			
<i>sul3</i>	Sul3-Fc	GAGCAAGATTTTTGGAATCG	51	880	RL0044c
	Sul3-Rc	CATCTGCAGCTAACCTAGGGCTTTGGA			
<i>aac(3)IV</i>	Aac4-Ld	TGCTGGTCCACAGCTCCTTC	59	653	AMR-075d
	Aac4-Rd	CGGATGCAGGAAGATCAA			

a Reference: Boerlin et al., 2005; b Reference: Lanz et al., 2003; c Reference: Perreten and Boerlin, 2003; d Reference: Boerlin et al., 2005.

*Antibiotic resistance analysis of isolates.* Various antibiotic concentrations will be used to determine antibiotic resistance patterns in target microorganisms (Table 2). The antibiotics/concentrations were selected based on previous success from other ARA studies and their common use in human and veterinary practice (Mathew et al., 1999). Each of the thirty-eight antibiotic/concentrations is added separately to flasks of autoclaved and cooled Trypticase Soy Agar (TSA, BBL) from stock antibiotic solutions to achieve the desired concentration, and then poured into sterile 15x100mm petri dishes. Control plates (no antibiotics) are included with each set. Isolates are transferred from the microwell plate using a stainless steel 48-prong replica plater (Sigma). The replicator is flame-sterilized (95% ethanol) after inoculation of each TSA plate. The inoculant is allowed to soak into the agar and the plates are then incubated for 48 hours at 37°C. Resistance to an antibiotic is determined by comparing each isolate to the growth of that isolate on the control plate. A one (1) is recorded if that isolate grew (a round colony, mostly filled) and a zero (0) is recorded for no growth. This is repeated for each isolate on each of the 30 antibiotic plates.

This information will allow correlation of occurrence of antibiotic resistance genes carried by

isolates with the expression of antibiotic resistances encoded by these genes. This will allow an assessment of the level of expression of antibiotic resistance genes in the *E. coli* population.

Table 2. Antibiotics and concentrations used in ARA.

<b>Antibiotics</b>	<b>Concentrations (µg/ml)</b>	<b>No. of Variables</b>
Erythromycin	60, 70, 90, and 100	4
Neomycin	2.5, 5.0, and 10	3
Oxytetracycline	8, 16, 32, 64, and 128	5
Streptomycin	8, 16, 32, 64, and 128	5
Tetracycline	8, 16, 32, 64, and 128	5
Cephalothin	16, 32, 64, and 128	4
Apramycin	16, 32, and 64	3
Sulfamethoxazole	64, 128, 256, and 512	4
Trimethoprim- Sulfamethoxazole	8, 16, 32, 64, and 128	5
Control	No antibiotic	2
Total		40

**Procedures for Objective 2.** Strains of *E. coli* will be isolated from known fecal waste samples to develop a known source library. No more than 10 isolates will be taken from a given sample of each manure source to build a database of 1000-1200 isolates. The known source categories will be composed of swine, cattle, wildlife and pets. Over 300 *E. coli* isolates from swine and 300 *E. coli* isolates from cattle have already been collected for database development. Antibiotic resistance analysis on 1000-1200 known isolates will be performed as described under Objective 1.

*Statistical Analysis for ARA:* Variables for the analyses include the number of antibiotics used and the degree of pooling of sources. Each analysis produces a classification set for every known source isolate. The correct classification rates are calculated using one set of antibiotic resistance patterns (ARPs) both to establish the classification rule and as test subjects (Harwood et al., 2000). The number of isolates from a given source that are placed in the correct source category by discriminant analysis is termed the rate of correct classification. The average rate of correct classification (ARCC) for the database is obtained by averaging the correct classification percentages for all sources (Harwood et al., 2000). The holdout method of cross validation, in which isolates from known sources are randomly removed from the data set and treated as test subjects, will be used as a more rigorous test of the predictive power of the databases (Harwood et al., 2000). To determine whether the known database are large enough or has ample representation, artificial clustering will be used. Artificial clustering involves randomly assigning equal numbers of isolates from each source and applying discriminant analysis to determine the random ARCC. Our database will contain 4 source types, swine, cattle, wildlife and pets. The random ARCC should be approximately 25% for each source. Thus, any percent significantly greater than the 25% ARCC indicates that the known source database is not representative. If the ARCC for a source segment of the database is found not to be representative, isolates will be added until the problem is corrected. By doing so, assures that the database will serve as a good point of reference for identifying unknown source isolates collected from Six Runs Creek. The development and validation of this database will allow determination of the source of unknown *E. coli* isolates obtained from the Six Runs Creek.

**Procedures for Objective 3.** Water samples will be collected from a total of 5 stream sampling sites, once a month for nine months. Sampling sites will consist of upstream (above waste application field 1, see Figure 1) and downstream sites in relation to the swine facility. The sampling regime is designed to capture possible seasonal variation in host sources contributing bacterial loading to Six Runs Creek. Ground water samples will be collected from the wells of transect two at each waste application fields (figure 1). Sampling from these sites will occur once every other month for nine months.

Isolation of *E. coli* will be performed by membrane filtration of a known volume of a water sample passed through a membrane filter that is then placed on media that is selective for the target microorganism. After incubation for 24 hr in a 44.5°C water bath, colonies will be transferred to 96-microwell plates containing 0.2 ml colilert broth specific for the target microorganism, and incubated for 24 h at 37°C. Twenty-four isolates from each water sample will be evaluated using antibiotic resistance analysis to determine its source. Antibiotic resistance analysis will be performed as described under objective 1. Isolates identified as swine will be evaluated for the presence antibiotic resistant genes using procedures described in objective 1.

### Principal Findings

Microbial source tracking by means of antibiotic resistance analysis (ARA) and polymerase chain reaction (PCR) have been performed on *E. coli* recovered from groundwater and surface water. Out of a total of 192 groundwater well samples only 7 wells had *E. coli* counts greater than 250 cfu/100ml, representing 3.6% of the samples. Of the 3.6% of groundwater samples that had elevated levels of *E. coli*, MST indicated that both lagoon effluent and wildlife (bird, deer, and unknown wildlife sample) were the major contributors of this contamination.

Surface water samples had *E. coli* counts that were consistently higher than the recreational standard of 250 cfu/ 100 ml. Elevated surface water counts were not surprising as beavers had taken residence in the stream and built a dam. We recently made contact with a professional wildlife trapper and were able to collect feces from beaver, nutria and raccoon; ARA and PCR profiles from these three sources will be added to the database and all the data collected from the groundwater and surface water will be re-evaluated against the more comprehensive database.

To date, a total of 1,208 *E. coli* isolates from swine feces, lagoon effluent, cattle, wildlife and nearby ground and surface waters (n=238, 234, 192, 144, 200 and 200, respectively) have been evaluated for phenotypic expression of resistance to various concentrations of the following antibiotics: erythromycin, neomycin, oxytetracycline, streptomycin, tetracycline, cephalothin, apramycin, trimethoprim, and rifampicin. All the isolates displayed multiple antibiotic resistances. These isolates have also been evaluated for antibiotic resistance genes. Genotypic evaluation indicated the presence of *aadA*, *strA*, *strB*, *tetA*, *tetB*, *tetC*, *sul1*, *sul2*, *sul3*, and *aac(3)IV* ARGs in all the sources of isolates.

- Swine feces and lagoon effluent isolates: Had high levels (*aad*, *strA*, *strB* *tetA* and *sul1*); intermediate (*tetB* and *tetC*); low (*sul2*, *sul3* and *Aac(3)IV*) (Figure 2).
- Cattle isolates: Had high levels (*aadA* and *tetA*); intermediate (*strA*, *strB*, *tetB* and *sul1*); while wildlife isolates had high levels (*aadA*, *strB*, *tetA* and *tetB*); intermediate (*strA* and *sul1*). Both sources had low levels of *tetC*, *sul2*, *sul3* and *Aac(3)IV* genes (Figure 2).
- Ground and surface water isolates: Had high levels (*aadA*, *strA*, *tetA*, *tetB* and *sul1*); intermediate (*strB*, *sul2* and *sul3*) and low (*Aac(3)IV*). Both *strB* and *tetC* genes increased in surface water (Figure 3).
- ARGs in isolates from swine feces, lagoon effluent and wildlife sources were not significantly different (Figure 4).
- ARGs in isolates from both ground and surface water were significantly greater than all the known sources (P<0.05) (Figure 4).

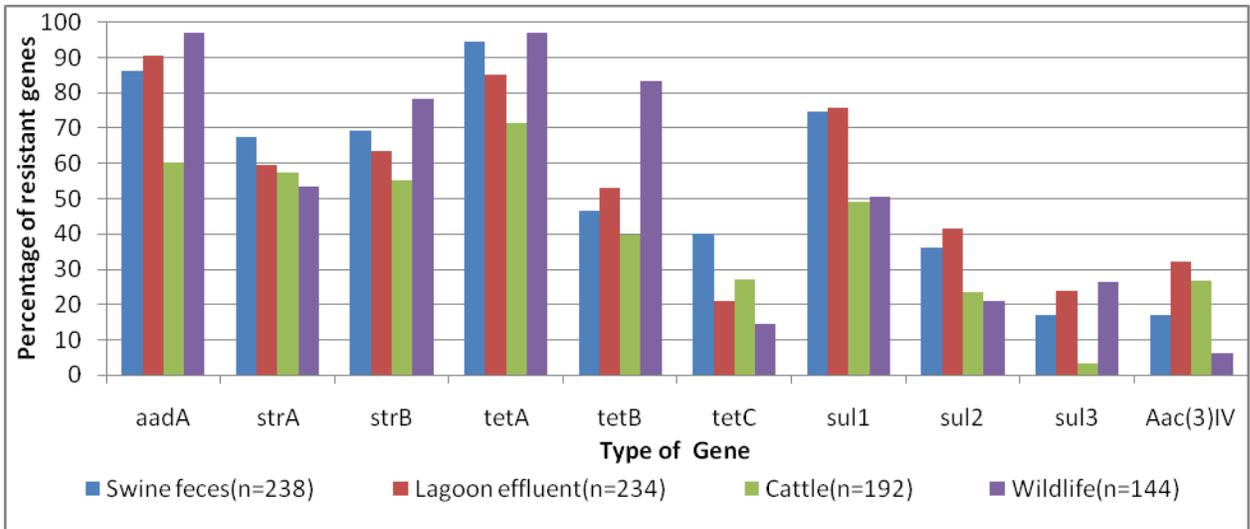


Figure 2: Distribution of antibiotic resistant gene types in known sources.

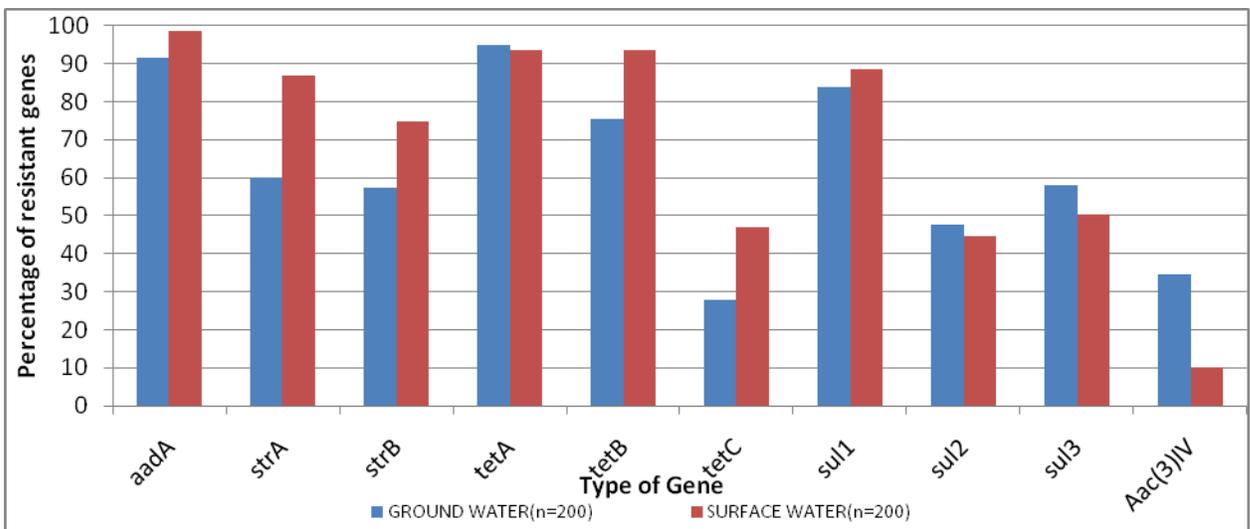


Figure 3: Distribution of antibiotic resistant gene types in environmental sources.

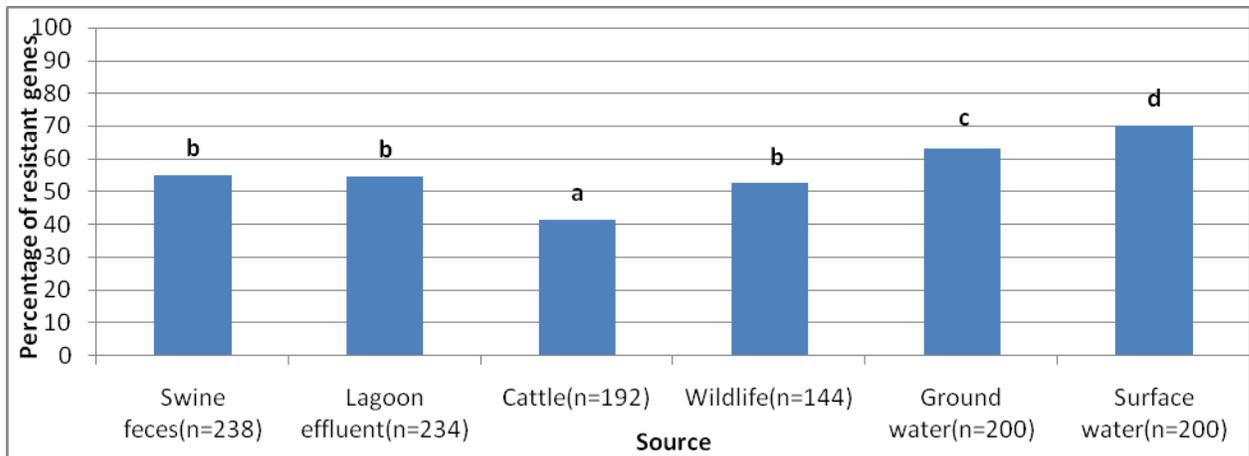


Figure 4: Distribution of antibiotic resistant genes among the sources (P<0.05).

### Significance

Microbial resistance to antibiotics is spreading fast; incidence of vancomycin resistance has increased from less than 1% to 17% within a span of 10 years (Pfaller et al., 1998). This study is intended to evaluate the association of antibiotic resistance genes found in *E.coli* isolated from swine with the actual phenotypic expression of the resistance. Additionally to develop an antibiotic resistance database for *E. coli* isolates from a commercial swine facility and assess its efficacy for tracking movement of bacteria from swine confinement houses to surface waters. Quantitative polymerase chain reaction will provide robust, sensitive and highly discriminant data. The results of this research will provide important information regarding the role of land application of lagoon effluent in spreading of bacteria with antibiotic resistance genes to surface waters. Early diagnosis of the problem will allow for the development of improved technologies and mitigation strategies.

This work can be used to track sources responsible for fecal pollution in the environment and also to make decisions based on scientific evidence to establish if waste management systems are working properly. The study can also provide timely answers to questions about antibiotic resistance. Swine production is not the major source of fecal pollution in the creek but multiple sources are responsible. This might also be the case for other similar locations dominated by swine. However, considerations should be made for the role of wildlife in transporting *E. coli* from lagoons to the streams, such as birds, turtles, etc. They were surprised that some resistant genes were more pronounced in wildlife than the livestock where antibiotics are mostly used.

### References

Aminov, R. I., N. Garrigues-Jeanjean, and R. I. Mackie. 2001. Molecular ecology of tetracycline resistance: development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins. *Appl. Environ. Microbiol.* 67:22-32.

Ananthaswamy, Anil. Spreading problem: Superbug genes are getting into soil and water-will humans be next? 2001. *New Scientist Magazine*.

Bates, J. 1997. Epidemiology of vancomycin-resistant enterococci in the community and the relevance of farm animals to human infection. *J. Hosp. Infect.* 37: 89-101.

Bicudo, J. R., L. M. Safley, and P. W. Westerman. 1999. Nutrient content and sludge volumes in single-cell recycle anaerobic swine lagoons in North Carolina. *Trans. ASAE* 42(4): 1087-1093.

Bischoff, K. M., D. G. White, M. E. Hume, T. L. Poole, and D. J. Nisbet. 2005. The chloramphenicol resistance gene *cmIA* is disseminated on transferable plasmids that confer multiple-drug resistance in swine *Escherichia coli*. *FEMS Microbiol. Lett.* 243:285-291.

Boerlin, Patrick, Rebecca Travis, Carlton L. Gyles, Richard Reid-Smith, Nicol Janecko Heather Lim, Vivian Nicholson, Scott A. McEwen, Robert Friendship, and Marie Archambault. 2005. Antimicrobial Resistance and Virulence Genes of *Escherichia coli* Isolates from Swine in Ontario. 71(11):6753-6761.

Bryan, A., N. Shapir, and M. J. Sadowsky. 2004. Frequency and distribution of tetracycline resistance genes in genetically diverse, nonselected, and nonclinical *Escherichia coli* strains isolated from diverse human and animal sources. *Appl. Environ. Microbiol.* 70:2503-2507.

Centers for Disease Control and Prevention (CDC). 1998. Report to the State of Iowa Department of Public Health on the Investigation of the Chemical and Microbial Constituents of Ground and Surface Water Proximal to Large-Scale Swine Operations by E.R. Campagnolo and C.S. Rubin, National Center for Environmental Health, Center for Disease Control and Prevention, Atlanta, GA.

Diaz Granados, C. A., and J. A. Jernigan. 2005. Impact of vancomycin resistance on mortality among patients with neutropenia and enterococcal bloodstream infection. *J. Infect. Diseases* 191 (4):588-595.

Donoho, A. L. 1984. Biochemical studies on the fate of monensin in animals and in the environment. *J. Anim. Sci.* 58:1528-1539.

Elmund, G. K., S. M. Morrison, D. W. Grant, and M. P. Nevins. 1971. Role of excreted chlortetracycline in modifying the decomposition process in feedlot waste. *Bull. Environ. Contam. Toxicol.* 6:129-135.

Feinman, S. E., and J. C. Matheson. 1978. Draft environmental impact statement: subtherapeutic antibacterial agents in animal feeds. Food and Drug Administration Department of Health, Education and Welfare Report, p. 372. Food and Drug Administration, Washington, D.C.

Flanagan, D. A., L. G. Gregory, J. P. Carter, A. Karakas-Sen, D. J. Richardson, and S. Spiro. 1999. Detection of genes for periplasmic nitrate reductase in nitrate respiring bacteria and in community DNA. *FEMS Microbiol Lett.* 177:263-270.

Gavalchin, J., and S. E. Katz. 1994. The persistence of fecal-borne antibiotics in soil. *J. Assoc. Off. Anal. Chem. Int.* 77:481-485.

Goni-Urriza, M., M. Capdepuy, C. Arpin, N. Raymond, P. Caumette, and C. Quentin. 2000. Impact of an urban effluent on antibiotic resistance of riverine *Enterobacteriaceae* and *Aeromonas* spp. *Appl. Environ. Microbiol.* 66:125-132.

Graves, A. K., C. Hagedorn, A. Teetor, M. Mahal, A. M. Booth, and R. B. Reneau, Jr. 2002. Antibiotic resistance profiles to determine sources of fecal contamination in a rural Virginia watershed. *J. Environ. Qual.* 31:1300-1308.

Guerra, B., E. Junker, A. Schroeter, B. Malorny, S. Lehman, and R. Helmuth. 2003. Phenotypic and genotypic characterization of Antimicrobial resistance in German *Escherichia*

- coli* isolates from cattle, swine, and poultry. J. Antimicrob. Chemother. 52:489-492.
- Haapapuro, E. R., N. D. Barnard, and M. Simon. 1997. Review animal waste used as livestock feed: dangers to human health. Prev. Med. 26:599-602
- Hagedorn, C., S. L. Robinson, J. R. Filtz, S. M. Grubbs, T. A. Angier, and R. B. Reneau. 1999. Determining sources of fecal pollution in a rural Virginia watershed with antibiotic resistance patterns in fecal streptococci. Appl. Environ. Microbiol. 65:5522-5531.
- Harwood, V. J., J. Whitlock, and V. Withington. 2000. Classification of antibiotic resistance patterns of indicator bacteria by discriminant analysis: Use in predicting the source of fecal contamination in subtropical waters. Appl. Environ. Microbiol. 66:3698-3704.
- Israel, D.W., W. J. Showers, M. Fountain and J. Fountain. 2005. Nitrate Movement in Shallow Ground Water from Swine-Lagoon-Effluent Spray Fields Managed under Current Application Regulations. J. Environ. Qual. 34:1828-1842.
- Krapac, I. G., W. S. Dey, C. A. Smyth, and W. R. Roy. 1998. Impacts of bacteria, metals, and nutrients on groundwater at two hog confinement facilities, p. 29-50. *In* Proceedings of the National Ground Water Association Animal Feeding Operations and Groundwater: Issues, Impacts, and Solutions A Conference for the Future. National Groundwater Association, St. Louis, Mo.
- Krapac, I. G., W. S. Dey, W. R. Roy, B. G. Jellerichs, and C. Smyth. 2000. Groundwater quality near livestock manure pits, p. 710-718. *In* 8th International Symposium on Animal, Agricultural and Food Processing Wastes. American Society for Agricultural Engineering, Des Moines, Iowa.
- Kudva, Indira T., Kathryn Blanch, and Carolyn J. Hovde. 1998. Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. Applied and Environmental Microbiology. 64 (9): 3166-3174.
- Lanz, R., P. Kuhnert, and P. Boerlin. 2003. Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland. Vet. Microbiol. 91:73-84.
- McAllister, T. A., L. J. Yanke, G. D. Inglis and M. E. Olson. 2001. Is antibiotic use in dairy cattle causing antibiotic resistance? Advances in Dairy Technology. 13:229-247.
- Mathew, A. G., W. G. Upchurch and S. E. Chattin. 1998. Incidence of antibiotic resistance in fecal *Escherichia coli* isolated from commercial swine farms. J. Anim. Sci. 76:429-434.
- Maynard, C., J. M. Fairbrother, S. Bekal, F. Sanschagrin, R. C. Levesque, R. Brousseau, L. Masson, and J. Harel. 2003. Antimicrobial resistance genes in enterotoxigenic *Escherichia coli* O149:K91 isolates obtained over a 23-year period from pigs. Antimicrob. Agents Chemother. 47:3214-3221.
- Mazel, D. 2004. Integrons and the origin of antibiotic resistance gene cassettes. ASM News 70(11):520.
- Odland, Brant A., Meredith E. Erwin, and Ronald N. Jones. 2000. Quality Control Guidelines for Disk Diffusion and Broth Microdilution Antimicrobial Susceptibility Tests with Seven Drugs for Veterinary Applications. J. Clin. Microbiol. 38 (1):453-455.
- Perreten, V., and P. Boerlin. 2003. A new sulfonamide resistance gene (*sul3*) in *Escherichia*

*coli* is widely spread in the pig population of Switzerland. *Antimicrob. Agents Chemother.* 47:1169-1172.

Pfaller, Michael A., Ronald N. Jones, Gary V. Doern, Kari Kugler, and The Sentry

Participants Group. 1998. Bacterial Pathogens Isolated from Patients with Bloodstream Infection: Frequencies of Occurrence and Antimicrobial Susceptibility Patterns from the SENTRY Antimicrobial Surveillance Program (United States and Canada, 1997). *Antimicrob. Agents Chemother.* 42(7):1762-1770.

Plym-Forshell, L. 1995. Survival of *Salmonellas* and *Ascaris Suum* Eggs in a thermophilic biogas plant. *Acta Vet Scand.* 36(1): 79-85.

Radtke, TM and Gist, GL. 1989. Wastewater sludge disposal — antibiotic resistant bacteria may pose health hazard. *J Environ Health* 52 2, pp. 102–105.

Smith, D. L., Harris, A. D. Johnsom, J. A. Silbergeld, E. K., and J. G. Morris, Jr. 2002. Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. *Proc. Natl. Acad. Sci.* 99(9):6434-6439.

Sobsey, M., V.R.Hill and D. Cole. 1999. Public Health Impact of Intensive Livestock Operations: Human Communicable Disease Concerns. Presented at *First Annual Public Health Symposium: Public Health Impact of Intensive Livestock Operations*, July 15–16, 1999, at North Carolina State University, Raleigh, NC. Sponsored by the North Carolina Department of Health and Human Services.

Teuber, M. 2001. Veterinary use and antibiotic resistance. *Curr. Opin. Microbiol.* 4:493-499.

# Development of an Analytical Method for Taste and Odor Compounds and Application to NC Drinking Water Sources and Finished Waters

## Basic Information

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There are no publications.

## **Title**

Development of an analytical method for taste and odor compounds and application to NC drinking water sources and finished waters

## **Problem and Research Objectives**

The presence of taste and odor (T&O) compounds in drinking water is an important issue for many utilities. In general the public perception is that the aesthetic quality of water is a good indicator of how safe the water is to drink. Thus water utilities meeting primary drinking water standards will not be able to guarantee consumer satisfaction unless their water is also taste and odor free (McGuire, 1995). Considerable financial resources are spent by the water industry to prevent and control T&O problems. For example, a T&O survey of about 800 US and Canadian water utilities showed that water utilities on an average spent 4.5% of their total budget to control T&O problems (Suffet et al., 1996).

The causes of T&O problems have been largely attributed to microbial byproducts, disinfectants and disinfection byproducts, and distribution system materials (AwwaRF and Lyonnaise des Eaux, 1995; Whelton and Dietrich, 2004). Most T&O compounds implicated in consumer complaints have been microbial metabolites formed in surface waters (Suffet et al., 1999; Peter et al., 2009). Actinomycetes, cyanobacteria, algae and even fungi have been associated with the occurrence of T&O compounds (Gerber, 1979; Watson, 2003; Zaitlin and Watson, 2006). T&O problems commonly occur when 'culture-like conditions' (a 2- to 5- week period with little or no rain, average flows less than 6500 cfs and water temperatures 17°C or greater) exist in reservoirs (Raschke et al., 1975). Such conditions may occur with increasing frequency in North Carolina, which experienced extreme drought conditions in 2002, 2007 and 2008 in >30% of the state (ncwater.org). Apart from the water source, T&O compounds may also form during treatment and distribution. Therefore, it is important for water utilities to screen their source and finished waters as well as distribution system samples for T&O compounds to determine the identity and origin of these compounds.

The principal objective of this study was to develop an analytical method that can be used by drinking water utilities to simultaneously detect and quantify 19 compounds commonly associated with T&O problems in drinking water. To assure sensitivity and specificity, gas chromatography tandem mass spectrometry (GC-MS/MS) was used. Sample preconcentration was achieved by head space solid phase microextraction (SPME) which has emerged as an ideal preconcentration technique with such advantages as small sample volume, solvent-free extraction, and adaptability to automation. Additional objectives of this study were to develop standard curves and to identify the limit of quantitation (LOQ) for each compound. The final objective was to apply the developed method to identify and quantify T&O compounds in several North Carolina water samples including a drinking water source, tap water, and water from lakes that were experiencing algae/cyanobacteria blooms.

## **Methodology**

To meet the objectives of this study, the following tasks were completed:

- 1) Validate suitable conditions for effective headspace SPME preconcentration and separation of the 19 T&O compounds on the GC-column.
- 2) Determine optimal MS/MS system settings.
- 3) Develop calibration curves for the 19 T&O compounds.
- 4) Determine the limit of detection (LOD) and limit of quantitation (LOQ) for each T&O compound.
- 5) Test the developed method with different water samples from NC: algal bloom samples, drinking water and distribution system samples were analyzed using the method and calibration curves developed.

Table 3.1 Names, properties, and sources of compounds used in this study.

Compound Name	CAS Number	Purity (%)	Stock solution concentration in methanol ( $\mu\text{g/ml}$ )	Density of neat material ( $\text{g/cm}^3$ )	Vendor
dimethyl disulfide	624-92-0	99.9	1000		Supelco
hexanal-d12		99.5 atom%D		0.890	CDN Isotopes
1-hexanal	66-25-1	98		0.814	Aldrich
cis-4-heptenal	6728-31-0	$\geq 98$		0.850	SAFC
1-heptanal (heptaldehyde)	111-71-7	$\geq 95$		0.818	Fluka
dimethyl trisulfide	3658-80-8	99.9	1000		Supelco
cis-3-hexenyl acetate	3681-71-8	$\geq 98$		0.897	SAFC
trans, trans-2,4-heptadienal	4313-03-5	90		0.881	Aldrich
2-isopropyl-3-methoxypyrazine	25773-40-4	99.9	100		Supelco
trans-2, cis-6-nonadienal	557-48-2	95		0.866	Aldrich
2-isobutyl-3-methoxypyrazine	24683-00-9	99.9	100		Supelco
methylisoborneol-d3	135441-89-3	>99 atom%D			CDN Isotopes
2-methylisoborneol	2371-42-8	99.9	100		Supelco
$\beta$ -cyclocitral	432-25-7	$\geq 90$		0.943	SAFC
2,3-benzopyrrole (indole)	120-72-9	99.9	2000		Supelco
trans, trans-2,4-decadienal	25152-84-5	85		0.857	Aldrich
2,4,6-trichloroanisole	87-40-1	99.1	100		Supelco
2,3,6-trichloroanisole	50375-10-5	99.9			Sigma-Aldrich
geosmin	19700-21-1	99.8	100		Supelco
$\beta$ -ionone	79-77-6	96		0.945	Aldrich
2,4,6-tribromoanisole	607-99-8	99			Aldrich
sodium chloride	7647-14-5	Certified ACS			Fisher Scientific
methanol(LC-MS chromasolv)	67-56-1	99.9			Fluka
acetone( GC resolv)	67-64-1	99.5			Fisher Scientific

Table 3.1 summarizes information about the T&O compounds and other chemicals used in this study. Apart from their name, CAS number, purity, and source, Table 3.1 lists the stock standard solution concentration if the compound was purchased in methanol or the density of the neat compound if the pure substance was purchased.

#### *Preconcentration*

Solid phase microextraction (SPME) of T&O compounds from the headspace of a water sample was carried out at 65°C for 30 minutes using 1-cm DVB/CAR/PDMS SPME fiber. To ten mL of sample, about 2.5 grams of sodium chloride was added in 20-mL glass vials. Salt addition significantly decreases the solubility of the compounds in water and along with stirring has been shown to accelerate the mass-transfer into the headspace of the vial (Bao et al., 1999). The salt-sample mixture was placed in the autosampler tray of the Combi PALTM that was programmed to perform the following sequential steps: (1) preheat sample in the agitator at 300 rpm for 30 minutes at 65°C to ensure that the sample is at 65°C before extraction begins, (2) insert SPME fiber through the septum and adsorb T&O compounds from the headspace for 30 minutes in the agitator (at a fiber depth of 22 mm from the bottom of the vial and the agitator set at 250 rpm), and (3) desorb at 250°C for 5 min into the GC injector (0.8-mm glass liner) that operated initially in the splitless mode. The injector was switched to the split mode with a split ratio of 20:1 after 3.00 minutes of extraction. Every 30 seconds during the 30-minute extraction, the agitator rotation was stopped for 2 seconds. The carrier gas was helium at a flow rate of 1.5 mL/min. The Combi PALTM settings and the GC-injector settings are summarized in Table 3.3.

Table 3.3 SPME Analysis conditions and GC-injector settings

Combi PAL™ settings	
Incubation Temperature	65°C
Agitator On time	30 seconds
Agitator Off time	2 seconds
Vial Penetration	22 mm
Extraction time	30 minutes
Injection penetration	54 mm
Desorption time	5 minutes
Middle Injector Type 1177	
Oven temperature	250 °C
Split ratio initial	On at 20:1
Split ratio at 0.01 minutes	Off
Split ratio at 3.00 minutes	On at 20:1
Column flow	1.5 mL/min

#### *Separation*

Separation of T&O compounds was achieved with a FactorFour VF-5ms column with the following temperature program: 35°C for 23 minutes, ramp to 139°C at a rate of 4 °C/min, ramp to 301°C at a rate of 27°C/min, and hold at 301°C for 5 minutes. Many T&O studies have utilized the same or similar columns and the temperature program was previously shown to be effective for a method developed for 12 fishy, swampy, and grassy odor compounds (Scimienti and Krasner, 2003). The optimal method run time was determined to be seventy minutes and twenty seconds by the Varian Software.

#### *Detection/Quantification*

Compound identification was done by verifying the spectral masses and retention times obtained in previous studies (Young and Suffet, 1999; Scimienti and Krasner, 2003; and application notes from Varian Inc.). Compound specific mass spectrometer settings were determined with the help of the automated methods development (AMD) feature of the Varian Software (MS Workstation ver 6.9). The AMD determines the optimum voltage for resonant excitation while injecting the parent ion to produce the quantitation ion in the ion trap of the MS.

#### *Preparation of stock standard and intermediate standards*

The stock standard solutions purchased in methanol were kept at -17°C and were used prior to the listed expiration date. For compounds purchased in neat form, stock standard solutions were prepared by dissolving a certain volume in  $\mu\text{L}$  ( $2 / \text{density of the neat compound}$ ) in 2 mL of methanol to

obtain a 1000 mg/L stock standard solution. E.g. to prepare 1000 mg/L of 1-hexanal stock,  $2 / 0.814 = 2.5 \mu\text{L}$  of the neat compound was added to 2 mL methanol. Intermediate standards were prepared from stock standard solutions and stored at  $-17^{\circ}\text{C}$  for up to two months. Table 3.4 summarizes the stock standard solution concentrations for each compound and provides details on the preparation of intermediate standards and calibration stock mixtures.

Table 3.4 Concentrations of stock standard solutions and procedures used to obtain intermediate standards and calibration standard mixtures.

Compound Name	Stock standard solution concentration in methanol (mg/L)	Volume of stock standard solution added to acetone to prepare 2mL of intermediate standard ( $\mu\text{L}$ )	Volume of stock/intermediate standards to make calibration mixtures ( $\mu\text{L}$ )
<b>Calibration Mixture 1 (Group 1)</b>		Intermediate Standards (5mg/L each)	Calibration stock mixture containing 125 $\mu\text{g/L}$ of each compound in 2mL acetone
2-isopropyl-3-methoxypyrazine	100	100	50
2-isobutyl-3-methoxypyrazine	100	100	50
2-methylisoborneol	100	100	50
$\beta$ -cyclocitral	1000	10	50
2,4,6-trichloroanisole	100	100	50
2,3,6-trichloroanisole	1308	7.6	50
geosmin	100	100	50
$\beta$ -ionone	1000	10	50
2,4,6-tribromoanisole	100	100	50
<b>Calibration Mixture 2 (Group 2)</b>		No intermediate standards. Calibration stock mixture prepared directly from stock standard solutions	Calibration stock mixture containing 12.5 mg/L of each compound in 2mL acetone
dimethyl disulfide	1000		25
1-hexanal	1000		25
cis-4-heptenal	1000		25
1-heptanal (heptaldehyde)	1000		25
dimethyl trisulfide	1000		25
cis-3-hexenyl acetate	1000		25
trans, trans-2,4-heptadienal	1000		25
trans-2, cis-6-nonadienal	1000		25
2,3-benzopyrrole (indole)	2000		12.5
trans, trans-2,4-decadienal	1000		25
<b>Internal Standards</b>			Second intermediate standard of 100 $\mu\text{g/L}$ in 2mL acetone for MIB-d3
methyl-d3-isoborneol	1120	8.9	40
hexanal-d12	2657	3.8	

#### Calibration mixtures and standards

Based on instrument responses of preliminary sample analyses, compounds were grouped into a high and a low sensitivity bin. Group 1 compounds exhibited high sensitivity while Group 2 compounds exhibited lower sensitivities. Accordingly, two calibration mixtures were prepared as follows: A high-concentration calibration mixture (12.5 mg/L) for Group 2 compounds and a low-concentration calibration mixture (125  $\mu\text{g/L}$ ) for Group 1 compounds. The calibration curves were obtained with seven aqueous calibration standards. Serial dilutions of the calibration mixtures (12.5 mg/L and 125  $\mu\text{g/L}$ ) yielded intermediate solutions from which the seven calibration standards were prepared (Table 3.5). MIB-d3 and hexanal-d12 were the internal standards for the Group 1 and Group 2 compounds, respectively (Table 3.4). The internal standards were spiked at 10 ng/L (MIB-d3) and 500 ng/L (hexanal-d12) in every calibration standard.

Table 3.5 Calibration concentrations, concentration ranges of the two groups of chemicals and volume spiked in 10 mL of deionized water.

Chemical mix/Internal Standard	Group 1 Compounds			Group 2 Compounds			Internal Standards					
	Concentration to be achieved in 10mL sample (ng/L)	Concentration of calibration mixture and serially diluted mixtures (µg/L)	Volume added to 10mL sample (µL)	Concentration to be achieved in 10mL sample (ng/L)	Concentration of calibration mixture and serially diluted mixtures (mg/L)	Volume added to 10mL sample (µL)	Concentration to be achieved in 10mL sample (ng/L)		Concentration of calibration stock mix		Volume added to 10mL sample (µL)	
Calibration Points							MIB-d3	Hex-d12	MIB-d3 (µg/L)	Hex-d12 (mg/L)	MIB-d3	Hex-d12
1	0.1	1.25	0.8	10	0.125	0.8	10	500	100	5	1	1
2	0.5	1.25	4.0	50	0.125	4.0	10	500	100	5	1	1
3	1	12.5	0.8	100	1.25	0.8	10	500	100	5	1	1
4	5	12.5	4.0	500	1.25	4.0	10	500	100	5	1	1
5	10	62.5	1.6	1000	6.25	1.6	10	500	100	5	1	1
6	25	62.5	4.0	2500	6.25	4.0	10	500	100	5	1	1
7	50	125	4.0	4000	12.5	3.2	10	500	100	5	1	1

#### *Isotope dilution method*

Quantification of the compounds was accomplished using an isotope dilution procedure. The concept behind the isotope dilution method is that an isotopically labeled analog of the target analyte will have a similar extraction recovery, ionization response and retention time as the compound under study. It has been shown to account for extraction inefficiency and loss of analytes because of intersample variability in many studies and was also shown to be effective across different sample matrices (Stanford and Weinberg, 2007). Deuterium labeled MIB (MIB-d) and hexanal (hexanal-d12) were the two internal standards in this study. Their retention times nearly overlapped with those of their respective unlabelled counterparts, and the same MS segments were used to capture both the labeled and unlabelled parent ions in this method.

#### **Principal Findings**

- A GC-MS/MS method was developed with which 19 common T&O compounds can be analyzed in a single analysis.
- The calibration curves developed had high coefficients of determination ( $r^2 > 0.950$ ). The calibration data of both Group 1 and Group 2 compounds were best described with a power law.
- Limits of quantitation (LOQs) were approximately 1 ng/L for Group 1 compounds and 100 ng/L for Group 2 compounds. For 12 of the 17 compounds with known odor threshold concentrations (OTCs), the LOQs were at or below the OTC. Consequently, the developed analytical method is capable of detecting developing T&O problems for 12 common T&O compounds before consumers can detect objectionable tastes and odors in their water. Results from the developed analytical method therefore allow utilities to develop source water management and treatment strategies in a more proactive manner.

- To test the developed analytical method, algal/cyanobacterial bloom samples from three North Carolina ponds/lakes were analyzed. Of the compounds targeted in this study all compounds except 2,4,6-tribromoanisole were detected in the bloom samples. Geosmin,  $\beta$ -ionone and *trans*-2,*cis*-6-nonadienal were present at concentrations that greatly exceeded their respective OTCs. Effective geosmin and *trans*-2,*cis*-6-nonadienal removal during drinking water treatment can be accomplished by activated carbon adsorption and/or ozonation processes. Chlorination alone is also effective for *trans*-2,*cis*-6-nonadienal removal. The removal of  $\beta$ -ionone can be accomplished by ozonation, and the effectiveness of other treatment processes for  $\beta$ -ionone is not known to date.
- A comparison of results obtained for non-filtered and 0.45- $\mu$ m filtered samples suggested that many T&O compounds in the bloom samples were present as intracellular compounds. Conventional treatment (coagulation, flocculation, sedimentation) should be effective for the removal of intracellular compounds, and utilities should be careful with the application of preoxidation processes that may lead to the release of intracellular compounds that can otherwise be removed by conventional treatment.
- For Raleigh raw and tap water sample there was no significant difference in the measured concentrations of  $\beta$ -cyclocitral and geosmin, suggesting no significant effect of both the treatment and distribution system on these compounds. Geosmin was present at a concentration that was near its OTC while the  $\beta$ -cyclocitral concentration was well below its OTC.

In the developed analytical method, deuterated MIB (MIB-d3) and hexanal (hexanal-d12) were used as the internal standards to obtain the response factors. Future work should consider including additional stable isotope analogs of the targeted T&O compounds. The use of additional internal standards that more closely match compound classes that were not well represented by the current two internal standards may improve recoveries and enhance accuracy and precision for such compounds as dimethyl disulfide and dimethyl trisulfide or the dienals.

Furthermore, in the IT-MS/MS optimization procedure, only the CID amplitude was experimentally optimized. The optimization of other instrumental parameters such as the parent ion isolation window, CID time, broadband amplitude, CID bandwidth, modulation range, filament current and ion trap temperature should therefore be considered in future work.

Finally, the use of different GC columns (e.g. columns designed for polar compounds) and a 2-cm DVB/CAR/PDMS SPME fiber should be evaluated in future work to see whether one or both can lower the LOQs and MDLs of those compounds, for which current analytical limits are above their OTCs.

### **Significance**

An analytical method was developed to detect and quantify 19 compounds commonly associated with taste and odor (T&O) problems in drinking water. The method can be used by utilities during T&O episodes to quickly and reliably detect T&O compounds and determine their concentrations. Knowledge about the identity and concentration of T&O compounds will greatly aid utilities in the selection of appropriate treatment strategies.

Head space solid phase microextraction (SPME) was used to concentrate T&O compounds, and gas chromatography (GC) followed by tandem mass spectrometry (MS/MS) was used to separate, detect, and quantify the T&O compounds. For 12 of the 17 targeted T&O compounds with known odor threshold concentrations (OTCs), limits of quantitation were below the OTC. This result suggests that the developed method is capable of detecting developing T&O problems for these 12 compounds and allow utilities to implement treatment strategies before consumers can detect objectionable tastes and odors in their water. The developed method was tested by analyzing three water samples from North Carolina ponds and lakes that experienced algae/cyanobacteria blooms. In addition, Raleigh source and tap water samples were analyzed.

Of the 19 targeted T&O compounds, all but 2,4,6 tribromoanisole were detected in the collected samples. In the bloom samples, geosmin,  $\beta$ -ionone, and trans-2,cis-6-nonadienal most frequently occurred at concentrations that exceeded their OTCs, sometimes by a factor of >100. A comparison of results for non-filtered and filtered (0.45  $\mu$ m membrane) samples suggests that many T&O compounds were predominantly present inside algae/cyanobacteria cells. Only geosmin and  $\beta$ -cyclocitral were present at measurable levels in Raleigh source and tap water. Concentrations in the raw and tap water samples were similar and at levels that were at or below the OTCs of geosmin and  $\beta$ -cyclocitral.

## References

- American Public Health Association. 2005. *Standard Methods for the Examination of Water and Wastewater*, 21st edition, various pages.
- Arthur, C. and Pawlischyn, J. 1990. "Solid phase microextraction with thermal desorption using fused silica optical fibers." *Anal. Chem.* 62(19):2145.
- AwwaRF and Lyonnaise des Eaux. 1995. *Advances in Taste-and-Odor Treatment and Control*. I.H. Suffet, J. Mallevalle, and E. Kawczynski, eds. American Water Works Association Research Foundation, Denver, CO.
- AwwaRF and Lyonnaise des Eaux. 1987. *Identification and Treatment of Tastes and Odors in Drinking Water*. J. Mallevalle and I.H. Suffet, eds. American Water Works Association Research Foundation, Denver, CO.
- Bjurman, J., Kristensson, J. 1992. "Production of volatile metabolites by the soft rot fungus *Chaetomium globosum* on building materials and defined media." *Microbios* 72(290):47.
- Boerjesson, T., Stoellman, U.M., Schnuerer, J.L. 1993. "Off-odorous compounds produced by molds on oatmeal agar: Identification and relation to other growth characteristics." *J. Agric. Food Chem.* 41(11):2104.
- Bruchet, A. 1999. "Solved and unsolved cases of taste and odor episodes in the files of inspector Cluzeau." *Water Science and Technology* 40(6):15.
- Bruchet, A. and Duguet, J. 2004. "Role of oxidants and disinfectants on the removal, masking and generation of tastes and odours." *Water Science and Technology* 49(9):297.
- Buttery, R.G and Ling, L.C. 1973. "Earthy aroma of Potatoes." *J. Agric. Food. Chem* 21(4):745.
- Buttery, R.G, Guadagni, D.G, Ling, L.C. 1978 . "Volatile aroma components of cooked artichoke." *J. of Agric Food Chem.* 26 (4):791.
- Buttery, R.G, Guadagni, D.G, Ling, L.C, Seifer R.M. and Lipton W. 1976. "Additional volatile components of cabbage, broccoli, and cauliflower." *J. Agric. Food Chem.* 24(4):829.
- Cancho, B., Ventura F., Galceran M. 1999 . "Behavior of halogenated disinfection by-products in the water treatment plant of barcelona, spain." *Bull. Environ. Contam. Toxicol.* 63(5):610.
- Collins, R.P. and Kalnins, K. 1965 . "Volatile constituents produced by the alga *Synura-petersenii* - II. alcohols, esters and acids." *Air Water. Pollut.* 9(7-8):501.
- Cook, D. and Newcombe, G. 2004. "Can we predict the removal of MIB and geosmin with PAC by using water quality parameters?" *Water, science & technology: water supply* 4(4):221.
- Cotsaris, E., Bruchet, A., Mallevalle J., Bursill D.B. 1995. "The identification of odorous metabolites produced from algal monocultures." *Water Science and Technology* 31(11):251.
- Delahunty, C.M., Eyres, G., Dufour, J.P. 2006. "Gas chromatography-olfactometry." *Journal of Separation Science* 29(14):2107.
- Diaz, A, Fabrellas, C, Ventura, F. 2005. "Determination of the odor threshold concentrations of chlorobrominated anisoles in water." *J. Agric. Food Chem.* 53(2):383.
- Dietrich, A.M., Hoehn, R.C., Dufresne, L.C., Buffin, L.W., Rashash, D.M.C. and Parker, B.C. 1995. "Oxidation of odorous and nonodorous algal metabolites by permanganate, chlorine, and chlorine dioxide." *Water Sci. Tech.* 31(11): 223-228.
- Fabrellas, C., Cardenoso, R., Devesa, R., Flores, J., Matia, L. 2004. "Taste and odor profiles (offflavors) in the drinking waters of the Barcelona area (1996-2000)." *Water Science and Technology* 49(9):129.
- Gerber, N.N. 1979. "Volatile Substances from Actinomycetes: Their Role in the Odor Pollution of

Water." Crit. Rev. Microbiol. 7 (3):191.

Gerber, N.N. 1969. "A volatile metabolite of actinomycetes, 2-methylisoborneol." J. Antibiot. 22(10):508.

Gerber, N.N. 1968. "Geosmin from microorganisms is trans- 1, 10-dimethyl-trans-9-decalol." Tetrahedron Lett. (25):2971.

Gerber, N.N. and Lechevalier, H.A. 1965. "Geosmin, an earthy-smelling substance isolated from actinomycetes." Appl. Microbiol. 13(6):935.

Gordon, D. T. and Morgan, M. E. 1972. "Principal Volatile Compounds in Feed Flavored Milk." J. Dairy Sci. 55(7):905.

Guadagni, D.G and Buttery, R.G. 1978. "Odor threshold Of 2,3,6-trichloroanisole in water." J. Food Sci. 43(4):1346.

Henatsch, J. J. and Jüttner, F. 1983. "Volatile odorous excretion products of different strains of *Synechococcus* (cyanobacteria)." Water Science and Technology 15(6-7):259.

Jüttner, F. 1995. "Physiology and biochemistry of odorous compounds from freshwater cyanobacteria and algae." Water Science and Technology 31(11):69.

Jüttner, F. 1984. "Characterization of *Microcystis* strains by alkyl sulfides and beta cyclocitral." Zeitschrift Für Naturforschung, C, A Journal of Biosciences 39(9-10):867.

Jüttner, F. 1984. "Dynamics of the Volatile Organic Substances Associated with Cyanobacteria and Algae in a Eutrophic Shallow Lake." Appl. Environ. Microbiol. 47(4):814.

Jüttner, F. 1983. "Volatile odorous excretion products of algae and their occurrence in the natural aquatic environment." Water Science and Technology 15(6-7):247.

Jüttner, F. 1981. "Detection of Lipid Degradation Products in the Water of a Reservoir During a Bloom of *Synura uvella*." Appl. Environ. Microbiol. 41(1):100.

Jüttner, F. 1976. "Beta-Cyclocitral and alkanes in *microcystis* (Cyanophyceae)." Zeitschrift Für Naturforschung, C, A Journal of Biosciences 31(9-10):491.

Jüttner, F. and Watson S.B. 2007. "Biochemical and ecological control of geosmin and 2-methylisoborneol in source waters." Appl. Environ. Microbiol. 73(14):4395.

Jüttner, F. and Hahne, B. 1981. "Volatile excretion products of *Poteroochromonas malhamensis*. Identification and formation." Zeitschrift Für Pflanzenphysiologie 103(5):403.

Jüttner, F. and Muller, H. 1979. "Excretion of octadiene and octatrienes by a freshwater diatom." Naturwissenschaften 66(7):363.

Jüttner, F., Höflacher, B. and Wurster, K. 1986. "Seasonal analysis of volatile organic biogenic substances (Vobs) in fresh-water phytoplankton populations dominated by *Dinobryon*, *Microcystis* and *Aphanizomenon*." J. Phyco. 22(2):169.

Khiari D., Barrett S.E., Suffet I. 1997. "Sensory GC analysis of decaying vegetation and septic odors." American Water Works Association. Journal 89(4):150.

Khiari, D., Suffet, I.H., Barrett, S.E. 1995. "Extraction and identification of chemicals causing grassy odors in flavor profile analysis (FPA) reference standards." Water Science and Technology 31(11):93-8.

Khiari D, Suffet I.H., Barrett S.E. 1995. "The determination of compounds causing fishy/swampy odors in drinking water supplies." Water Science and Technology 31(11):105-12.

Khiari, D., Brenner, L., Burlingame, G.A. and Suffet, I.H. 1992. "Sensory gas chromatography for evaluation of taste and odor events in drinking water." Water Science and Technology 25(2):97.

Khiari D., Bruchet A., Gittelmann T., Matia L., Barrett S.E. and Suffet I.H. 1999. "Distribution generated taste-and-odor phenomena." Water Science and Technology 40(6):129.

Khiari, D, Young, C.C, Amah, G., Ye, Q., Atasi K., Huddleston, J., Suffet, I.H. 1999. "Factors affecting the stability and behavior of grassy odors in drinking water." Water Science and Technology 40(6):287-92.

Kikuchi, T., Mimura, T., Moriwaki, Y., Ando, M., Negoro, K. 1974. "Metabolites of a diatom, *Synedra rumpens* Kutz, isolated from water in Lake Biwa. identification of odorous compounds, n-hexanal and n-heptanal, and analysis of fatty acids." Chem. Pharm. Bull 22(4):945.

Krasner, S., Hwang, C.J. and McGuire, M.J. 1983. "A standard method for quantification of earthy-musty odorants in water, sediments and algal cultures." Water Science and Technology 15(6-7):127.

Malleret L and Bruchet A. 2002. "A taste and odor episode caused by 2,4,6-tribromoanisole." American Water Works Association Journal 94(7):84.

Malleret L., Bruchet A., Hennion M. 2001. "Picogram determination of "earthy-musty" odorous

compounds in water using modified closed loop stripping analysis and large volume injection GC/MS." *Analytical Chemistry* 73(7):1485.

March, R.E. 1997. "An introduction to quadrupole ion trap mass spectrometry." *Journal of Mass Spectrometry* 32(4):351.

MacDougall, D., Amore, F.J., Cox, G.V., Estes, F.L. 1980. "Guidelines for data acquisition and data quality evaluation in environmental chemistry"; ACS Committee on Environmental Improvement. *Anal. Chem.* 52(14):2249.

McGuire, M. 1999. "Advances in treatment processes to solve off-flavor problems in drinking water." *Water Science and Technology* 40(6):153.

Miller III, A., Scanlan, R. A., Lee J. S. and Libbey L. M. 1973. "Identification of the Volatile Compounds Produced in Sterile Fish Muscle (*Sebastes melanops*) by *Pseudomonas fragi*." *Appl. Microbiol.* 25(6):952.

Murray, K.E., and Whitfield, F.B. 1975. "The occurrence of 3-alkyl-2-methoxypyrazines in raw vegetables." *J. Sci. Food. Agric.* 26(7):973.

Pan, L. and Pawliszyn, J. 1997. "Derivatization/solid-phase microextraction: New approach to polar analytes." *Anal Chem* 69(2):196.

Peter, A. and von Gunten, U. 2009. "Taste and odour problems generated in distribution systems: A case study on the formation of 2,4,6-trichloroanisole." *Aqua* 58(6):386.

Peter, A. and Von Gunten, U. 2007. "Oxidation kinetics of selected taste and odor compounds during ozonation of drinking water." *Environ. Sci. Tech.* 41(2):626.

Peter, A., Koster O, Schildknecht A, von Gunten U. 2009. "Occurrence of dissolved and particlebound taste and odor compounds in swiss lake waters." *Water Res* 43(8):2191.

Pirbazari, M., Borow, H.S. Craig, S., Ravindran, V. and McGuire, M.J. 1992. "Physical chemical characterization of five earthy-musty-smelling compounds." *Water Science and Technology* 25(2):81.

Raschke, R.L., Carroll, B. and Tebo, L.B. 1975. "The relationship between substrate content, water quality, actinomycetes and musty odours in the broad river basin." *J. Appl. Ecol.* 12(2):535-60.

Rashash, D.M.C., Dietrich, A.M., Hoehn R.C. 1997. "FPA of selected odorous compounds." *American Water Works Association. Journal* 89(4):131.

Rashash, D.M.C., Dietrich, A.M., Hoehn, R.C. and Parker, B.C. 1995. "The influence of growth conditions on odor-compound production by two chrysophytes and two cyanobacteria." *Water Science and Technology* 31(11):165.

Rashash, D.M.C.; R.C. Hoehn; A.M. Dietrich; T.J. Grizzard; and B.C. Parker. 1996. *Identification and Control of Odorous Algal Metabolites*. American Water Works Association Research Foundation, Denver, CO.

Rigal, S. and Danjou, J. 1999. "Tastes and odors in drinking water distribution systems related to the use of synthetic materials." *Water Science and Technology* 40(6):203.

Schierberle, P., Ofner, S., Grosch, W. 1990. "Evaluation of Potent Odorants in Cucumbers (*Cucumis sativus*) and Muskmelons (*Cucumis melo*) by Aroma Extract Dilution Analysis." *J. Food Sci.* 55(1):193.

Scimienti, M.J. and S.W. Krasner. 2003. "The development of a solid-Phase microextraction technique for determining fishy, swampy, or grassy odors in drinking water." In Proc. 2003 AWWA WQTC, Philadelphia, PA.

Skoog, D.A., and Leary, J.J. 1992. *Principles of Instrumental Analysis*, fourth edition, Saunders college Publishing, various pages.

Suffet, I.H., Schweitzer, L., Khiari, D. 2004. "Olfactory and chemical analysis of taste and odor episodes in drinking water supplies." *Rev. in Env. Sci. and Biotech.* 3(1):3.

Suffet, I.H., Khiari, D., Bruchet, A. 1999. "The drinking water taste and odor wheel for the millennium: Beyond geosmin and 2-methylisoborneol." *Water Science and Technology* 40(6):1

Suffet, I. H., Corado, A., Chou, D., McGuire, M. Butterworth, S. 1996. "AWWA Taste and Odor Survey." *Journal AWWA* 88(4): 168.

Tabachek, J. and Yurkowski M. 1976. "Isolation and identification of blue-green algae producing muddy odor metabolites, geosmin, and 2-methylisoborneol, in saline lakes in Manitoba." *Journal of the Fisheries Research Board of Canada* 33(1):25.

Tombouliau, P., Schweitzer, L., Mullin, K., Wilson, J. and Khiari D. 2004. "Materials used in drinking water distribution systems: contribution to taste-and-odor." *Water Science and Technology* 49(9):219.

U.S. Environmental Protection Agency, EPA method 40 CFR- Part 136 (Appendix B).2003.

Van Gemert, L.J. and Nettenbreijer, A.H. 1977. *Compilation of Odour Threshold Values in Air and Water*. National Institute of Water Supply, Voorburg, The Netherlands.

Vial, J. and Jardy, A. 1999. "Experimental Comparison of the different approaches to estimate LOD and LOQ of an HPLC method." *Anal. Chem.* 71(14):2672.

Watson, S. 2004. "Aquatic taste and odor: A primary signal of drinking-water integrity." *Journal of Toxicology and Environmental Health. Part A* 67(20-22):1779.

Watson, S. 2003. "Cyanobacterial and eukaryotic algal odour compounds: Signals or by-products? A review of their biological activity." *Phycologia* 42(4):332.

Watson, S., Satchwill, T., Dixon, E., McCauley, E. 2001. "Under-ice blooms and source-water odour in a nutrient-poor reservoir: Biological, ecological and applied perspectives." *Freshwat Biol.* 46(11):1553.

Watson, S., Brownlee, B., Satchwill, T. and McCauley E. 1999. "The use of solid phase microextraction (SPME) to monitor for major organoleptic compounds produced by chrysophytes in surface waters." *Water Science and Technology* 40(6):251.

Wilkins, K.. 1996. "Volatile metabolites from actinomycetes." *Chemosphere* 32(7):1427.

Wisconsin Department of Natural Resources. 1996. *Analytical Detection Limit Guidance*.

Yano, H., Nakahara, M. and Ito, H. 1988. "Water blooms of *Uroglena americana* and the identification of odorous compounds." *Water Science and Technology* 20(8-9):75.

Young, W.F.; H. Horth; R. Crane; T. Ogden; and M. Arnott. 1996. "Taste and odour threshold concentrations of potential potable water contaminants." *Water Res.* 30(2): 331-340.

Young, C.C. and I.H. Suffet. 1999. "Development of a standard method-analysis of compounds causing tastes and odors in drinking water." *Water Sci. Tech.* 40(6): 279-285.

Zaitlin, B., Watson, S., Ridal, J., Satchwill, T., Parkinson, D. 2003. "Actinomycetes in lake ontario: Habitats and geosmin and MIB production." *American Water Works Association Journal* 95(2):113.

Zaitlin, B. and Watson, S.B. 2006. "Actinomycetes in relation to taste and odour in drinking water: Myths, tenets and truths." *Water Res.* 40(9):1741-53.

# Development of the Semi-Empirical Ammonia Deposition and Emission (SEADE) Model for Application to North Carolina Coastal Watersheds

## Basic Information

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<b>Principal Investigators:</b>	Wayne Robarge, Robert E Austin, John Walker

## Publications

There are no publications.

## **Title**

Development of the semi-empirical ammonia deposition and emission (SEADE) model for application to North Carolina watersheds

## **Problem and Research Objectives**

Globally, domestic animals are the largest source (22 Tg N yr<sup>-1</sup>, 1 Tg = 10<sup>12</sup> g) of atmospheric NH<sub>3</sub>, comprising approximately 40% of natural and anthropogenic emissions combined, while synthetic fertilizers and agricultural crops together contribute an additional 12.6 Tg NH<sub>3</sub>-N y<sup>-1</sup> (23% of total emissions) (Bouwman et al., 1997). Within and downwind of agricultural regions, NH<sub>x</sub> therefore represents a significant fraction of atmospherically derived N entering terrestrial and aquatic systems (Whitall and Paerl, 2001). In natural systems where N is the limiting nutrient, atmospherically derived reactive N may have beneficial effects on productivity, including increased photosynthesis (Sievering et al., 2000) and accumulation of inorganic soil N (Padgett et al., 1999). Recent studies also indicate that enhanced N deposition may increase the carbon storage capacity of temperate forests (Sievering, 1999). When N input exceeds system requirements, however, environmental stresses such as soil acidification (Roelofs et al., 1985), forest decline (Nihlgard, 1985), and eutrophication of surface waters (Paerl, 1995; Paerl and Whitall, 1999) may occur. During the 1990's, the Coastal Plain region of North Carolina experienced a significant increase in agricultural NH<sub>3</sub> emissions, owing primarily to growth in swine and poultry populations (Walker et al., 2000a), which are most concentrated in the Cape Fear and Neuse River basins. Beginning with the increase in NH<sub>3</sub> emissions, the concentration of NH<sub>4</sub><sup>+</sup> in precipitation also increased significantly in this part of the state (Walker et al., 2000a; Walker et al., 2000b; Paerl and Whitall, 1999). Whitall and Paerl (2001) estimate that atmospheric wet deposition contributes 20 to 40% of biologically available "new" nitrogen entering North Carolina coastal waters. Furthermore, Paerl and Whitall (1999) report that high atmospheric nitrogen deposition rates are generally coincident with regions experiencing harmful algal bloom expansion, a symptom of eutrophication.

While significant progress has been made in determining wet deposition inputs of NH<sub>x</sub> to eastern North Carolina ecosystems, the magnitude of NH<sub>3</sub> dry deposition remains unknown. Measurements of ambient NH<sub>3</sub> concentrations across eastern North Carolina suggest the potential for high dry deposition rates in areas densely populated by animal production facilities (Robarge et al., 2002; Walker et al., 2004). In a recent study at a swine facility in eastern North Carolina, Walker et al. (2008) estimated that approximately 10% of NH<sub>3</sub> emissions from the barns/lagoon complex were deposited within 500m downwind. Dry deposition rates > 50 kg N ha<sup>-1</sup> yr<sup>-1</sup> were predicted for distances < 100 m from the emissions complex, which included a forested riparian area. At 500 m from the source, dry NH<sub>3</sub> deposition was 16 kg N ha<sup>-1</sup> yr<sup>-1</sup>, which was approximately 3.5X wet deposition of NH<sub>4</sub><sup>+</sup>. These results suggests that dry NH<sub>3</sub> deposition exceeds the critical nitrogen load (Kuylenstierna et al., 1998) for ecosystems in the vicinity of animal production facilities. The contribution of NH<sub>3</sub> dry deposition to total N deposition in North Carolina coastal watersheds, however, remains unknown. Without information on the magnitude and spatial variability of NH<sub>3</sub> deposition in mixed agricultural regions (i.e., crop and animal production), assessments of ecosystem health and the potential for nitrogen saturation/eutrophication are incomplete.

This study presents a new model for estimating NH<sub>3</sub> dry deposition at field to watershed scales in areas of intensive animal production, taking the Neuse and Cape Fear River basins as an initial case study. This approach will produce an initial watershed scale estimate of NH<sub>3</sub> dry deposition which takes into account the bi-directional nature of NH<sub>3</sub> air-surface exchange with vegetation, soils, and water and resolves spatial features, such as concentration and deposition gradients around individual animal facilities that are currently missed by larger regional air quality models.

The specific objectives of this study are to:

1. provide annual watershed scale estimates of NH<sub>3</sub> dry deposition for the Cape Fear and Neuse River basins;

2. estimate seasonal NH<sub>3</sub> concentration and net air-surface exchange fluxes at 100 m horizontal resolution within the model domain;
3. summarize the net NH<sub>3</sub> fluxes by primary land use type, counties, and across seasons;
4. establish an atmospheric nitrogen deposition budget for the Cape Fear and Neuse River basins;
5. evaluate the SEADE model with respect to ambient concentration predictions;
6. provide recommendations for continued model development and future research.

## **Methodology**

The SEADE model consists of three components: 1) a facility-scale NH<sub>3</sub> emission inventory; 2) a spatial model for predicting atmospheric NH<sub>3</sub> concentrations; and 3) a model for predicting net NH<sub>3</sub> air-surface transfer rates. The model is described in detail in the following sections.

### *Emission Inventory*

Ammonia emissions are calculated for individual animal production facilities included in the North Carolina Department of Environment and Natural Resources, Division of Water Quality (NCDWQ) Registration Database. Operations include those deemed permitted under 15ANCAC2H.0217 Rule for Waste Not Discharged to Surface Waters. Animal operations are registered under this rule if the facility serves 250 or more swine, 100 or more confined cattle, 75 or more horses, 1000 or more sheep, or 30,000 or more confined poultry and are using a liquid waste management system. An annual emission rate is calculated for each facility based on the number and type of animals as described below. Annual emissions are scaled by an average monthly temperature to partition the emissions into a monthly inventory. The results of Walker et al., 2004 show that the NH<sub>3</sub> emissions budget for the Neuse and Cape Fear River basins is dominated by swine (60% of total) followed in importance by turkeys (10%), chickens (9%), cows (8%), and fertilizer (6.5%).

To account for differences in per-swine emissions resulting from feed formulations used in phase feeding programs, emissions are calculated for each of the 19 facility types contained in the DWQ database using a combination of emission factors for boar stud/gilts (11.0 kg NH<sub>3</sub> animal<sup>-1</sup> yr<sup>-1</sup>; Asman, 1992), sows (16.43 kg NH<sub>3</sub> animal<sup>-1</sup> yr<sup>-1</sup>; van der Hoek, 1998), and fattening (market) hogs (6.39 kg NH<sub>3</sub> animal<sup>-1</sup> yr<sup>-1</sup>; van der Hoek, 1998). Where a combination of sows and fattening pigs are present, the following calculation is used:

$$ER_T = (DC_T \times CF \times FOS \times BEF_S) + (DC_T \times CF \times FMH \times BEF_{MH}) \quad (1)$$

where  $ER_T$  is the facility scale annual emission rate (kg NH<sub>3</sub> yr<sup>-1</sup>);  $DC_T$  is facility design capacity in number of animals,  $CF$  is a correction factor for actual percent capacity, assumed to be 0.95,  $FOS$  is the fraction of sows present (0.1), and  $FMH$  is the fraction of market hogs present (0.9);  $BEF_S$  is the basic emission factor for sows (kg NH<sub>3</sub> animal<sup>-1</sup> yr<sup>-1</sup>) and  $BEF_{MH}$  is the basic emission factor for market hogs (kg NH<sub>3</sub> animal<sup>-1</sup> yr<sup>-1</sup>). The fraction of sows and market hogs is calculated from the most recent North Carolina market statistics (NCDACS, 2007).

When only one animal type is present at a facility, the annual emission rate reduces to

$$ER_T = DC_T \times CF \times BEF_T \quad (2)$$

where  $BEF_T$  is the basic emission factor for animal type  $T$ . Basic emission factors for the remaining animal categories, including cattle (e.g., milk, beef), horses, sheep, goats, and poultry (e.g., pullets, layers, broilers, and turkeys) are taken from Asman, 1992.

Although very few poultry facilities are contained in the CAFO's database, a yearly emission estimate for poultry was needed for model calibration. In 10 out of 24 sites the closest facility to a CAMNet site was a poultry house as determined from aerial imagery. From the imagery we are able to determine the number of barns contained by the facility, however, due to the lack of permit data we were unable to determine the type of house (e.g. layer, pullet, turkey, or broiler). As such, an average yearly emission of  $0.4 \text{ kg NH}_3 \text{ bird}^{-1} \text{ yr}^{-1}$  was used to best estimate the emissions from these facilities.

To account for seasonal variability, annual emissions are monthly allocated by scaling with ambient temperature (Walker et al., 2008). A linear regression model of the form

$$E_i = a_o - \alpha \cos\left(\frac{2\pi i}{12}\right) - \beta \sin\left(\frac{2\pi i}{12}\right) \quad (3)$$

is used to estimate the facility scale  $\text{NH}_3$  emission rate (kg) for month  $i$  ( $E_i$ ), where  $a_o$  is the average monthly emission rate in  $\text{kg NH}_3$  (annual emission/12). Coefficients  $\alpha$  and  $\beta$  are given by

$$\alpha = -8.9\left(\frac{a_o}{T}\right) \text{ and } \beta = -5.6\left(\frac{a_o}{T}\right) \quad (4)$$

where  $T$  is the average annual temperature ( $^{\circ}\text{C}$ )

#### *Atmospheric Ammonia Concentrations*

*Models.* Atmospheric  $\text{NH}_3$  concentrations are calculated as a function of distance from the nearest animal production facility. Three different distance-decay models, following the form of a shifted power law ( $y = ax^k + \varepsilon$ ), were compared for their ability to predict ground level  $\text{NH}_3$  air concentrations. The main advantage of the shifted power-law formulation is its accuracy over a broader range of distance than that of logarithmic law formulation (Zou et al., 2006). The models were compared to analyze the effects of both the exponential decay constant  $k$  and the initial emissions coefficient  $a$ .

Model I was developed from weekly integrated concentrations measured along horizontal gradients from 10 to 700m downwind of a 5000 animal swine facility (Walker et al., 2008) and takes the form

$$\text{NH}_3 = a_j X^{0.75} \quad (5)$$

Coefficient  $a$  in equation (5) varies by month as a result of the seasonality in emissions. The results of Walker et al. (2008) were used to derive the following relationship between the coefficient  $a$  and monthly emissions ( $R^2 = 0.82$ )

$$a_j = 0.29E_i + 35.1 \quad (6)$$

where  $E_j$  is monthly emission in units of kg  $\text{NH}_3$  at facility  $j$ . The intercept was not statistically significant at  $p = 0.01$  and is therefore set to zero.

Model II varies  $a$  by month using the same procedure as Model I but varies the decay exponent  $k$  by season. Model III uses a fixed seasonal coefficient  $a$  and applies the same seasonally derived decay exponent  $k$  as used in Model II. As described below, Models II and III were developed from the CAMNet database (see section 2.1.2.2).

Model validation measures were derived both externally (Model I) and internally (Models II and III). An external model validation was performed on Model I, developed from an independent dataset (Walker et al., 2008) to CAMNet observations. Models II and III were developed and validated (internally) using only the CAMNet data in a split-sample approach. In general, an external model validation provides a more robust evaluation of model performance, however, it is considered reasonable to use internal model validation to calculate internal measures of validation such as the prediction error (Hastie et al, 2001). For Models II and III, the monitoring sites were divided into two sets of data, two-thirds of the sites (16 stations) for calibration and one-third of the sites (8 stations) for model validation. Because the concentration model is highly dependent on the distance to the nearest animal facility it was important to subset the data so that the calibration data contained sites with a full range of distances to the closest animal facility. To ensure that the model remained robust in regards to the distance to the nearest animal facility yet unbiased in the selection of calibration sites, the CAMNet sites were ordered by their distance to the nearest facility and then broken into 8 sets of 3 stations. A random number generator was used to pick one station out of each set of 3 that would be used for validation, the other two used for model calibration (Appendix Table X).

Model performance was evaluated using the regression coefficient, mean bias (MB), normalized mean bias (NMB), root mean square error (RMSE), and normalized mean error (NME) according to the following equations:

$$\begin{aligned} \text{MB} &= \frac{1}{N} \sum_1^N (C_m - C_o), \\ \text{NMB} &= \frac{\sum_1^N (C_m - C_o)}{\sum_1^N C_o} 100\%, \\ \text{RMSE} &= \sqrt{\frac{1}{N} \sum_1^N (C_m - C_o)^2}, \\ \text{NME} &= \frac{\sum_1^N |C_m - C_o|}{\sum_1^N C_o} 100\%, \end{aligned} \tag{7a - 7d}$$

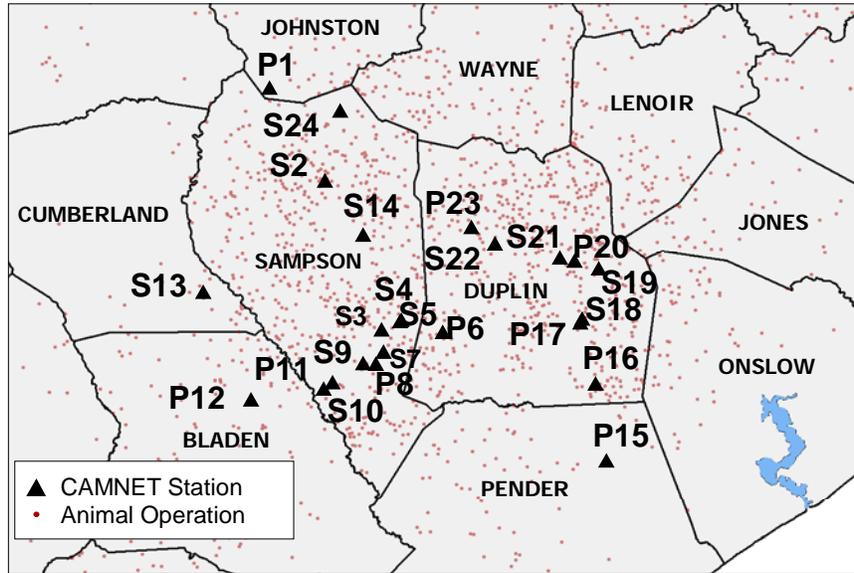
where  $C_m$  and  $C_o$  represent model and observed concentrations, respectively.

#### *Observations*

The Carolina Ammonia Monitoring Network (CAMNet), which is operated by investigators Walker and Robarge, consists of 24 monitoring sites within the Cape Fear and Neuse River basins at which weekly integrated  $\text{NH}_3$  concentrations are measured at ground level using passive air samplers (Figure 1).

Samplers were positioned to measure NH<sub>3</sub> concentrations in the vicinity of different types of animal facilities and at a range of distances from 100 to 4500 meters away. The sites were positioned in random directions from the nearest facility and in various landscape settings. Of the 24 sites, 10 sites were located closest to poultry operations, 14 closest to swine. The size of the facilities ranged from a 12 barn poultry facility to a 27,000 feeder-to-finish swine operation.

Figure 1: Location of CAMNET monitoring stations used to calibrate the monthly emission factor for ground level ammonia concentration.



Ground level NH<sub>3</sub> air concentrations were measured with the ALPHA passive sampler (Tang et al., 2001). The sampler consists of a 6 mm long, 21 mm inner diameter FEP Teflon tube. One end contains a 5µm PTFE membrane, through which NH<sub>3</sub> gas diffuses and is adsorbed onto an acid-coated collection filter located at the other end of the diffusion path. The membrane prohibits particle collection and thus the NH<sub>3</sub> concentration is not biased high by collection of ammonium aerosol. The membrane also forms a quasi-laminar layer of air adjacent to its outer surface which serves to establish a turbulence-free diffusion path between the membrane and the collection filter, thus avoiding “wind-shortening” of the diffusion path. Within CAMNet, samplers were deployed in replicate (2) for 1 to 2 weeks at each measurement location (Figure 1) in an open bottom rain shelter fixed at a height of 1.5 m above ground.

The ambient concentration of NH<sub>3</sub> determined by the passive sampler depends on the mass of NH<sub>3</sub> adsorbed by the collection filter, exposure duration, and diffusion coefficient. The mass of NH<sub>3</sub> adsorbed by the collection filter is calculated by:

$$Q = (c_e - c_b)v \quad (8)$$

where  $v$  is the volume of the extract (mL),  $c_e$  is the filter extract concentration of NH<sub>4</sub><sup>+</sup> (µg mL<sup>-1</sup>), and  $c_b$  is the extract concentration of an unexposed travel blank (µg mL<sup>-1</sup>). Each batch (weekly or biweekly) of samples is bracketed by two sets of  $N = 6$  travel blanks, which account for contamination during transport to and from the field site. In this case,  $c_b$  represents the median of these two sets of travel blanks.

The concentration of NH<sub>3</sub> in air is then calculated as:

$$[NH_3] = Q/V \quad (9)$$

where  $V$  is the effective volume of air sampled (L). The theoretical volume of air sampled is:

$$V = DA t/L \quad (10)$$

where  $D$  is the temperature dependent diffusion coefficient of NH<sub>3</sub> in air (Massman, 1998),  $A$  is the tube cross sectional area (mm<sup>2</sup>),  $t$  is the time of exposure, and  $L$  is the length of the diffusion tube (mm).

Collection filters (25 mm, Swiftlab, UK) were coated with a solution of 5% (w/w) phosphorous acid in methanol. Exposed collection filters were extracted in 2.5 mL deionized water and stored at 4 °C prior to analysis by ion chromatograph (Dionex model DX-120, Dionex, Sunnyvale, CA). Laboratory and field (travel blanks) were analyzed along with field-exposed samples. Six laboratory blanks were processed for each batch (defined by weekly or biweekly sampler deployment period) of field exposed samples and represent extracts from samplers that are prepared in the laboratory using standard procedures then extracted immediately and stored at 4 °C until analysis. Six field blanks were processed for each batch of field exposed samples and represent extracts from unexposed samplers that are transported to and from the field sites with field exposed samplers. The travel blank characterizes all sources of contamination and variability related to laboratory procedures and transport to and from the field site.

Previous studies with passive devices have shown that the effective sampling rate ( $S$ )

$$S = DA/L = V/t \quad (11)$$

is lower than the theoretical value due to additional resistance to diffusion by the PTFE membrane at the sampler entrance (Tang et al., 2001). The sampling rate must therefore be characterized and calibrated over the range of sampling conditions (i.e., NH<sub>3</sub> concentration, duration of exposure, etc.) either by field comparison to an independent reference method (Tang et al., 2009) or exposure to a known concentration of NH<sub>3</sub> under controlled conditions. By quantifying the mass ( $Q$ ) of NH<sub>3</sub> collected by the sampler at a particular air concentration  $[NH_3]$  and exposure period ( $t$ ), equations (9) and (11) can be solved for the effective sampling volume ( $V$ ) and uptake rate ( $S$ ), respectively. Finally, by monitoring temperature during exposure, an adjustment to the diffusion coefficient ( $D$ ) may be derived using equation (10).

Similar to the approach of Walker et al. (2008), we characterized the uptake rate ( $S$ ) by exposing the ALPHA sampler to a range of NH<sub>3</sub> concentrations (1 to 100 µg m<sup>-3</sup>) in a well-mixed Teflon-lined 61cm(L)×30.5cm(W)×19cm(H) acrylic chamber over exposure periods ranging from 1 – 7 days. Ammonia concentrations were generated by mixing NH<sub>3</sub> (10 ppm ± 5% or 100 ppm ± 5%; Airgas; Durham, NC) with clean air via calibrated mass flow controllers or critical orifice. The concentration of NH<sub>3</sub> in air entering and exiting the chamber was monitored using a dual-cell Pranalytica photoacoustic NH<sub>3</sub> detector (Nitrolux Model 200; Pranalytica, Inc.; Santa Monica, CA). Our experiments yield a median value ( $N = 40$ ) of  $S = 0.00356 \pm 0.00015 \text{ m}^{-3} \text{ hr}^{-1}$ , which agrees closely with the value of  $0.00324 \text{ m}^{-3} \text{ hr}^{-1}$  obtained by Tang et al. (2009) via field comparison to the DELTA denuder system (Sutton et al., 2001). The primary sources of uncertainty in our calculation are accuracy of the reported NH<sub>3</sub> cylinder concentration (±5%) and the precision of the passive sampler during the exposure experiments (8%), yielding a total

uncertainty  $\approx 9.5\%$ , which is on the same order as the difference (9.4%) between our estimate of S and that of Tang et al. (2009).

Detection limit, or minimum detectable concentration ( $L_D$ ), was calculated as a function of the standard deviation of field blanks ( $\sigma_o$ ) as outlined by Currie (1999)

$$L_D = 2t_{1-\alpha, \nu} \sigma_o \quad (12)$$

where  $t$  is the Student's t-statistic with  $\nu$  degrees of freedom and a 5% probability of accepting the alternative hypothesis "analyte present" when it is false ( $\alpha = 0.05$ ). Equation (12) assumes constant variance between  $L = 0$  and  $L = L_D$  and a 5% probability of accepting the null hypothesis "analyte absent" when it is false ( $\beta = 0.05$ ). Because the batchwise variance of the travel blank is not constant over time,  $L_D$  is determined for each batch of  $N = 6$  travel blanks. Method precision was determined as the median absolute relative difference between collocated duplicate samples.

Accuracy of the ALPHA sampler was assessed by comparison to phosphorous acid coated glass (URG Corp., Chapel Hill, NC) annular denuders (U.S. EPA, 1997; Perrino and Gherardi, 1999). Denuders were operated for 24 hours at a mass flow controlled (URG Corp., Chapel Hill, NC) air sampling rate of 10 Lpm. Denuders were extracted with 10 mL deionized water and analyzed for  $\text{NH}_4^+$  by ion chromatography (Dionex DX-120; Dionex, Sunnyvale, CA). ALPHA/denuder comparisons took place at Duke Forest, near Chapel Hill, NC, and the Clinton Horticultural Crops Research Station, near Clinton, NC (Site 14 [S14], Fig. 1), from January to November, 2009. The Duke Forest site is a suburban site characterized by relatively low  $\text{NH}_3$  concentrations (Sparks, et al., 2008) while the Clinton site is in an area of widespread animal and crop production and therefore experiences much higher  $\text{NH}_3$  concentrations (Robarge et al., 2002). For this comparison, the mean and median daily denuder  $\text{NH}_3$  concentrations during a weekly period containing  $N = 7$  observations (i.e., denuders were not operated in replicate) was compared to the mean and median of  $N = 4$  replicate ALPHA weekly measurements. Denuder precision during previous studies was  $< 10\%$  expressed as coefficient of variation of replicates (Robarge et al., 2002; Bash et al., 2010).

Agreement between the two methods was assessed by calculating the median absolute relative percent difference between average concentrations and by reduced major axis regression. Ayers (2001) demonstrated in an analysis of air quality data that RMA was superior to ordinary least squares regression, which tends to underestimate the slope parameter and overestimate the intercept when both X and Y variables contain error. Regression analysis comparing weekly mean and median ALPHA and denuder samples ( $N = 72$  observations) showed good agreement between the two methods. Slopes ( $\pm$  standard error) were  $0.90 \pm 0.027$  and  $0.99 \pm 0.034$  for mean and median weekly concentrations, respectively, and corresponding  $R^2$  values were 0.94 and 0.92. Intercepts of 0.02 and 0.01 for mean and median concentrations, respectively, were not statistically significant ( $P > 0.1$ ). The median absolute relative difference between average ALPHA and denuder concentrations was 0.29%, with highest values observed at concentrations below  $0.25 \mu\text{g NH}_3 \text{ m}^{-3}$ . At such low concentrations, the ALPHA concentration becomes increasingly sensitive to the blank correction, a problem that would be reduced to some degree for longer exposures (i.e., increasing ratio of exposed vs. blank concentration in sample extract).

The median LOD ( $48.0 \mu\text{g NH}_4^+ \text{-N L}^{-1}$ ,  $N = 48$ ) is equivalent to 0.24 and  $0.12 (\mu\text{g NH}_3 \text{ m}^{-3})$  in air for 1 and 2 week exposures, respectively, at  $25^\circ\text{C}$ . Sampler precision, defined as the median absolute relative percent difference (RPD) between duplicate samples, was 6.9% ( $N = 1081$ ). RPD tends to increase with

decreasing concentration below  $\approx 1 \mu\text{g NH}_3 \text{ m}^{-3}$ , reaching a median value of 30% for the 20 observations surrounding (10 above/10 below) the weekly detection limit of  $0.24 \mu\text{g NH}_3 \text{ m}^{-3}$ .

#### *Ammonia Air-Surface Exchange Model*

Ammonia may be either emitted from or deposited to vegetation, soil, and water, depending on the “compensation point” of the underlying surface. For this reason, the traditional approach of calculating deposition fluxes by applying a deposition velocity to the atmospheric concentration, which is appropriate for gases with a zero surface concentration such as sulfur dioxide and nitric acid (Fowler and Unsworth, 1979; Hicks et al., 1987), is not appropriate for  $\text{NH}_3$  (Deerhake et al., 2005). For  $\text{NH}_3$ , a model framework that recognizes the bidirectional nature of the exchange process is required. In this study,  $\text{NH}_3$  air-surface exchange (flux) is calculated using the two-layer canopy compensation point model developed by Nemitz et al. (2001), in which the competing processes of emission and deposition within the foliage-soil (or water) system are taken into account by relating the net canopy-scale  $\text{NH}_3$  flux ( $F_t$ ) to the net emission potential of the canopy (i.e., foliage and soil or water), or surface concentration ( $\chi_o$ ). The system of equations describing the net canopy flux ( $F_t$ ), as well as component fluxes [i.e., stomatal ( $F_s$ ), cuticular ( $F_w$ ), and ground ( $F_g$ )], is given by Nemitz et al. (2001).

Total and component fluxes are dependent on the canopy compensation point ( $\chi_c$ ), defined by Nemitz et al. (2001) as

$$\begin{aligned} \chi_c = & [\chi_a (R_a R_b)^{-1} + \chi_s \{ (R_a R_s)^{-1} + (R_b R_s)^{-1} + (R_g R_s)^{-1} \} + \chi_g (R_b R_g)^{-1}] \\ & \times \{ (R_a R_b)^{-1} + (R_a R_s)^{-1} + (R_a R_w)^{-1} + (R_b R_g)^{-1} + (R_b R_s)^{-1} \\ & + (R_b R_w)^{-1} + (R_g R_s)^{-1} + (R_g R_w)^{-1} \}^{-1} \end{aligned} \quad (13)$$

where  $\chi_a$  is the atmospheric  $\text{NH}_3$  concentration,  $R_a$  is the atmospheric aerodynamic resistance,  $R_b$  is the atmospheric boundary layer resistance,  $R_s$  is the leaf stomatal resistance,  $R_w$  is the leaf cuticular resistance, and  $\chi_s$  is the stomatal compensation point.  $\text{NH}_3$  exchange with the ground is described by the soil compensation point ( $\chi_g$ ) and the ground resistance ( $R_g = R_{ac} + R_{bg}$ ), which is determined by the in-canopy aerodynamic ( $R_{ac}$ ) and ground boundary-layer resistances ( $R_{bg}$ ). In the case of exchange over open water, the ground resistance reduce to zero.

Upon determination of  $\chi_c$ , the surface concentration [ $\chi(z_0)$ ] may be calculated according to

$$\chi(z_0) = \frac{\chi_a R_a^{-1} + \chi_g R_g^{-1} + \chi_c R_b^{-1}}{R_a^{-1} + R_b^{-1} + R_g^{-1}} \quad (14)$$

The net flux may then be calculated as

$$F_t = - \frac{\chi_a - \chi(z_0)}{R_a} \quad (15)$$

The aerodynamic resistance is calculated as a function of the standard deviation of wind direction ( $\sigma_\theta$ ), and wind speed ( $u$ ) according to Hicks et al. (1987) assuming that the atmosphere is considered unstable when global radiation ( $G$ ) exceeds  $100 \text{ W m}^{-2}$  (Meyers et al., 1998). The boundary-layer resistance is calculated according to Duyzer et al. (1992). Following Hicks et al. (1987), friction velocity ( $u_*$ ) is calculated from the near-neutral approximation as a function of  $R_a$  and  $u$ .

The bulk stomatal resistance to  $\text{NH}_3$  transfer ( $R_s$ ) is assumed equal to that of water vapor ( $\text{H}_2\text{O}$ ) corrected for differences in molecular diffusivity. Stomatal resistance to  $\text{H}_2\text{O}$  is calculated as a function of  $G$ , air temperature ( $T$ ), and the vegetation specific minimum resistance ( $R_{smin}$ ) according to the standard parameterization of Wesely (1989). While the factors controlling stomatal resistance to  $\text{H}_2\text{O}$  transfer, and the applicability of  $\text{H}_2\text{O}$  resistance schemes to other gases, are well established, much less is known about the cuticular resistance ( $R_w$ ) of  $\text{NH}_3$ . Previous studies have shown that cuticular adsorption of  $\text{NH}_3$  depends on the chemical characteristics of the cuticle and surface water layers (Flechard et al., 1999). Studies [(Fowler et al. (1998), Jones et al. (2007))] have also shown an increase in  $R_w$  with increasing  $\text{NH}_3$  concentration in air as the cuticle becomes saturated. This has important implications for the work presented here, as very high concentrations of  $\text{NH}_3$  are expected near animal production facilities. In this case, we use the concentration-dependent  $R_w$  parameterization of Jones et al. (2007) for *C. vulgaris*. While it is recognized that this parameterization may not be generally applicable, specific parameterizations for vegetation types across eastern North Carolina have not yet been developed.

The in-canopy aerodynamic (turbulent) resistance ( $R_{ac}$ ) is calculated according Zhang et al. (2003) as a function of  $u_*$ , single-sided leaf area index (LAI), and the vegetation specific minimum in-canopy aerodynamic resistance ( $R_{acmin}$ ). The additional boundary layer resistance ( $R_{bg}$ ) at the ground is calculated according to Schuepp (1977) and applied similarly to Nemitz et al. (2000). The sum of  $R_{ac}$  and  $R_{bg}$  establishes the total ground resistance ( $R_g$ )

In the case of  $\text{NH}_3$ , leaves may act as a source or sink depending on the ratio of the ambient concentration to the stomatal compensation point ( $\chi_s$ ) (Farquhar et al., 1980; Husted and Schjoerring, 1995).  $\chi_s$  is a function of temperature and the apoplastic concentrations of  $\text{NH}_4^+$  and  $\text{H}^+$  (Nemitz et al., 2000). The leaf emission potential  $\Gamma_s = \text{NH}_4^+/\text{H}^+$  has been shown to vary widely as a function of plant and soil nitrogen status (Schjoerring et al., 1998; Sutton et al. 1997; Flechard and Fowler, 1998). Similar to the leaf apoplast solution, the equilibrium between gaseous  $\text{NH}_3$  and  $\text{NH}_4^+$  in the soil pore space and fresh/salt water solution establishes a soil/water compensation point, referred to here as  $\chi_g$  (Dawson, 1977; Nemitz, 2001) with a corresponding specification of  $\Gamma_g$ . For example, fertilized soils, which have a large value of  $\Gamma_g$ , will be a net source of  $\text{NH}_3$  in the model, while forest soils or open water, which conversely have small values of  $\Gamma_g$ , may oscillate between source and sink throughout the year. Thus, the model takes into account bidirectional  $\text{NH}_3$  exchange with vegetation as well as soil and fresh/salt water. Since direct measurements of apoplast and water/soil solution chemistry are not yet available for all of the surface types within the model domain, appropriate values have been selected from peer reviewed literature (e.g., references above; Walker et al., 2006; Walker et al., 2008; Cooter et al., 2010). Gamma values for individual land use categories are held constant throughout the year.

Air-surface exchange fluxes are calculated by land use according to the 2001 National Land Cover Data imagery classification, which contains 21 land use classes at 30m horizontal resolution (NLCD, 2001). Land use specific inputs include  $\Gamma_s$ ,  $\Gamma_g$ , LAI,  $R_{acmin}$ , and  $R_{smin}$  (Appendix A, Table 12). The model is driven with hourly meteorological data from the nearest NC AGNet station (NCSCO, 2007), including wind speed, standard deviation of wind direction, air temperature, and soil temperature.

## MODEL IMPLEMENTATION

Geographic Information System (GIS) software and custom built components are used to calculate and analyze the atmospheric concentration field within the SEADE model. The GIS is used to create, maintain and store the emissions inventory and in the analysis and visualization of model outputs. Custom algorithms compute concentration fields and include ArcInfo scripts and C code executed in batch mode. Because of computational concerns and a potential future need for supercomputer compatibility, the concentration field model is developed with support for cross-platform compilation.

The model domain is controlled by scripts that use both the CAFO's database and a predefined lattice distance. Export functions within the scripts create the input files required by the atmospheric concentration model. These scripts manage the spatial boundaries and verify that the output resolution and facility locations are based on the select coverage area. When a non-rectangular model domain is specified, lattice points that fall outside the coverage area are removed in order to reduce the computational time of the atmospheric concentration model.

The SEADE modeling system is implemented by first calculating facility scale emissions, followed by calculation of ambient  $\text{NH}_3$  concentrations, followed by calculation of net air-surface exchange fluxes. As described above, a concentration is predicted at each point within the 100m by 100m lattice, producing a distribution of  $N = Z$  concentration estimates where  $Z$  equals the total number of animal production facilities within the model domain. To produce smooth concentration fields, the maximum concentration at each point is retained and the source farm and distance are recorded. Next, the concentration field is converted to a raster data model and the air-surface exchange rate is calculated at each cell by applying land use parameters, meteorological data, and concentrations following the framework described in the *Ammonia Air-Surface Exchange Model*.

To calculate air-surface exchange rates, hourly meteorological data for the period of interest are reduced to an average diurnal profile. The diurnal profile of meteorological parameters is then used to generate a diurnal profile of air-surface exchange at each prediction point. Hourly estimates are then aggregated to produce a representative daily flux at each model prediction point, which is then scaled up to a seasonal value.

## Principal Findings

Globally, domestic animals are the largest source (22 Tg N yr<sup>-1</sup>, 1 Tg = 10<sup>12</sup> g) of atmospheric  $\text{NH}_3$ , comprising approximately 40% of natural and anthropogenic emissions combined, while synthetic fertilizers and agricultural crops together contribute an additional 12.6 Tg  $\text{NH}_3\text{-N}$  y<sup>-1</sup> (23% of total emissions) (Bouwman et al., 1997). Within and downwind of agricultural regions,  $\text{NH}_x$  therefore represents a significant fraction of atmospherically derived N entering terrestrial and aquatic systems (Whitall and Paerl, 2001). While significant progress has been made in determining wet deposition inputs of  $\text{NH}_x$  to eastern North Carolina ecosystems, the magnitude of  $\text{NH}_3$  dry deposition remains unknown. Measurements of ambient  $\text{NH}_3$  concentrations across eastern North Carolina suggest the potential for high dry deposition rates in areas densely populated by animal production facilities (Robarge et al., 2002; Walker et al., 2004).

This study presents a new model for estimating  $\text{NH}_3$  dry deposition at field to watershed scales in areas of intensive animal production, taking the Neuse and Cape Fear River basins as an initial case study. The SEADE model consists of three components: 1) a facility-scale  $\text{NH}_3$  emission inventory; 2) a spatial model for predicting atmospheric  $\text{NH}_3$  concentrations; and 3) a model for predicting net  $\text{NH}_3$  air-surface transfer rates. This approach produces an initial watershed scale estimate of  $\text{NH}_3$  dry deposition which accounts for the bi-directional nature of  $\text{NH}_3$  air-surface exchange with vegetation, soils, and water and resolves

spatial features, such as concentration and deposition gradients around individual animal facilities that are missed by larger regional air quality models.

The facility-scale NH<sub>3</sub> emission inventory was developed from the North Carolina Department of Environment and Natural Resources, Division of Water Quality (NCDWQ) Registration Database. To validate facility locations, the animal operations were overlaid with the Farm Services Agency (FSA) 2006 National Aerial Imagery Program (NAIP) imagery and manually rectified within a Geographic Information System (GIS). Comparison between the CAFO-based emissions inventory and the independently collected statistics from the North Carolina Department of Agriculture Statistics (NCDAS) service provides an estimate of total basin-wide NH<sub>3</sub> swine emission within 6 percent of the SEADE emissions inventory.

Atmospheric NH<sub>3</sub> concentrations are calculated as a function of distance from the nearest animal production facility. Three different distance-decay models, following the form of a shifted power law (  $y = ax^k + \epsilon$  ), were compared for their ability to predict ground level NH<sub>3</sub> air concentrations. Model I was developed from weekly integrated concentrations measured along horizontal gradients from 10 to 700m downwind of a 5000 animal swine facility (Walker et al., 2008), Model II varied  $a$  by month using the same procedure as Model I but varied the decay exponent  $k$  by season. Model III used a fixed seasonal coefficient  $a$  and applied the same seasonally derived decay exponent  $k$  as used in Model II. Model performance was evaluated using the regression coefficient, mean bias (MB), normalized mean bias (NMB), root mean square error (RMSE), and normalized mean error (NME). For Model I, regression plots revealed strong correlation between model and observations and followed a pattern of model overestimation at low concentrations and underestimation at high concentrations. In particular, mean bias ranged from -1.49  $\mu\text{g NH}_3 \text{ m}^{-3}$  in the winter to 2.1  $\mu\text{g NH}_3 \text{ m}^{-3}$  in the summer, indicating that the model tended to overestimate during warm months and underestimate during cold months.

In this study, NH<sub>3</sub> air-surface exchange (flux) is calculated using the two-layer canopy compensation point model developed by Nemitz et al. (2001), in which the competing processes of emission and deposition within the foliage-soil (or water) system are taken into account by relating the net canopy-scale NH<sub>3</sub> flux ( $F_T$ ) to the net emission potential of the canopy (i.e., foliage and soil or water), or surface concentration ( $\chi_o$ ). The air-surface exchange fluxes are calculated by land use according to the 2001 National Land Cover Data imagery classification, which contains 21 land use classes at 30m horizontal resolution (NLCD, 2001). The model is driven with hourly meteorological data from the nearest NC State Climate Office AGNet station (NCSCO, 2007).

For the year, the air-surface exchange model predicted a net dry deposition totaling 20.6 million kg NH<sub>3</sub>, 6.9 million kg within the Neuse and 13.6 million kg within the Cape Fear. In the Neuse, deposition averaged 1.2, 0.9, 1.2, and 0.6 kg ha<sup>-1</sup> for the spring, summer, fall, and winter respectively. In the Cape Fear, deposition averaged 1.3, 1.3, 1.4, and 0.6 kg ha<sup>-1</sup> respectively. On an annual scale, NH<sub>3</sub> is deposited to low nitrogen systems (forests, wetlands) and emitted from high nitrogen (fertilized) systems (cultivated crops, pasture).

Total deposition to both river basins was 20.6 million kg NH<sub>3</sub> on an annual scale, which represents 36% of total emissions calculated from the SEADE emissions inventory and 27% of total emissions calculated from the NCDAS database. Thus, the majority of NH<sub>3</sub> emitted within the two river basins is either wet deposited or transported out of the river basins.

## **Significance**

Based on this study, it is concluded that a semi-empirical model that spatially predicts NH<sub>3</sub> emission and deposition is capable of capturing dry NH<sub>3</sub> at field-to-watershed scales in areas of intensive animal production and on a seasonal basis. With a further refinement of the emissions inventory, increased sampling regime, and additional improvements in the compensation point for different land cover classes, this model offers great promise in linking the complex, field-scale mechanistic processes with the large, regional-scale deposition models.

In this analysis we have identified three priority areas for improving air concentration and flux estimates which will be the focus of Phase II of the development of the SEADE model, which are described below.

1. Further refinement of the emissions inventory would enable more accurate prediction of NH<sub>3</sub> air concentrations. Specifically, we will attempt to add poultry facilities to the emissions inventory by working with county extension offices to identify the location, types, and sizes of poultry facilities. A secondary objective is to develop a more refined protocol for splitting large swine facilities into multiple emission complexes, which will reduce the influence of a small number of very large facilities.
2. The model presented here is limited in its ability to predict ground level air concentrations due to a relatively small number of measurement sites available for development and evaluation. Additional concentration measurements were taken at 25 different sites in 2008 – 2009. The next version of the model will employ these additional data for development and testing of the air concentration model. We will also examine the usefulness of including the Lizzie dataset, from which Model I was developed, in the construction and validation of Models II and III.
3. Additional testing will be performed to optimize the implementation of the air concentration model. As described above the model produces a distribution of air concentrations at each model grid point consisting of N observations equal to the number of animal facilities within the model domain. Currently only the maximum concentration is retained, assuming that the nearest animal facility dominates the seasonal average concentration. Forthcoming analyses will examine other approaches, such as a summed concentration weighted by distance to the corresponding facility.
4. In addition to the semi-empirical models presented here, future analyses will compare concentration predictions from traditional Gaussian and Lagrangian dispersion models.

## **References**

- Asman, W. A. H., 1992. National Institute of Public Health and Environmental Protection.
- Ayers, G. P., 2002. *Atmos. Environ.*, 35, 2432-2425.
- Bilthoven, the Netherlands 1992.
- Bouwman, A.F., *et al.*, 1997. *Global Biogeochemical Cycles*, 11, 561-587.
- Burkholder, J.M., *et al.*, 2005. *Water Resources Research Institute*.
- Burkholder *et al.*, 2005. *Limnol. Oceanogr.* 51, 463 - 487.
- CASTNet, 2010. Clean Air Status and Trends Network. <http://www.epa.gov/castnet/>
- Cooter, E.J., and Schwede, D.B., 2000. *J. Geophys. Res.* 105, 6695 – 6707.
- Cooter, E.J., *et al.*, 2010. *Atmos. Environ.*, in press.
- Currie, L., 1999. *Analytica Chimica Acta*, 391, 105 – 126.
- Dawson, G.A., 1977. *J. Geophys. Res.* 82, 3125 – 3133.
- Deerhake *et al.*, 2006. Proceedings of the Workshop on Agricultural Air Quality: State of the Science. 250 – 258.
- Duyzer, J. H., *et al.*, 1992. *Environ. Poll.* 75, 3 – 13.

- Farquhar, G.D., *et al.*, 1980. *Plant Physiol.* 66, 710 – 714.
- Flechard, C.R., Fowler, D., 1998. *Quart. J. Roy. Met. Soc.* 124, 759 – 791.
- Flechard, C.R., *et al.*, 1999. *Quart. J. R. Meteorol. Soc.* 125, 2611 – 2641.
- Fowler, D., Unsworth, M. H., 1979. *Q. J. R. Meteorol. Soc.* 105, 767 – 783.
- Hastie T., R. Tibshirani and J. Friedman, The elements of statistical learning: data mining, inference and prediction. , Springer-Verlag, New York (2001) 14 .
- Hicks, B. B., *et al.*, 1987. *Water Air Soil Pollut.* 36, 311 – 330.
- Husted, S., Schjoerring, J. K., 1995. *Plant Physiol.* 109, 1453 – 1460.
- Jones, M.R., *et al.*, 2007. *Atmos. Environ.*, 41, 2049 – 2060.
- Lin, M., *et al.*, 2009. *Atmos. Chem. Phys. Discuss.*, 9, 17157-17181.
- Massman, W. J., 1998. *Atmos. Environ.*, 32, 1111 – 1127.
- Meyers, T.P., *et al.*, 1998. *J. Geophys. Res.* 103, 22645 - 22661.
- Nemitz, E., *et al.*, 2000. *Agric. For. Meteorol.* 105, 405 – 425.
- Nemitz, E., *et al.*, 2001. *Quart. J. Roy. Met. Soc.* 127, 815 – 833.
- NADP, 2010. National Atmospheric Deposition Program. <http://nadp.sws.uiuc.edu/>
- NCDWQ, 2007. North Carolina Division of Water Quality
- NCDACS, 2007. North Carolina Department of Agriculture and Consumer Services
- NCSCO, 2007. North Carolina State Climate Office. <http://www.nc-climate.ncsu.edu/>
- NLCD, 2001. National Land Cover Data. <http://www.mrlc.gov/>
- Nihlgard, B., 1985. *Ambio*, 14, 2-8.
- Padgett, P., *et al.*, 1999. *Atmos. Environ.*, 33, 769-781.
- Paerl, H.W., 1995. *Ophelia*, 41, 237-259.
- Paerl, H.W., 1997. *Limnol. Oceanogr.* 42, 1154 – 1165.
- Paerl, H.W., Whitall, D.R., 1999. *Ambio*, 28, 307-311.
- Perrino, C., *et al.*, 2001. *Atmos. Environ.*, 35, 331-341.
- Qian *et al.*, 2000. *Environ. Sci. Technol.* 34, 4474 – 4482.
- Robarge, W.P., *et al.*, 2002. *Atmos. Environ.*, 36, 1661-1674.
- Roelofs, J.G.M., *et al.*, 1985. *Plant and Soil*, 84, 45-56.
- Schjoerring, J. K., *et al.*, 1998. *Atmos. Environ.* 32, 491 – 498.
- Schuepp, P.H., 1977. *Bound.-Lay. Meteorol.* 12, 171 – 186.
- Sievering, H., 1999. *Nature*, 400, 629-630.
- Sievering, H., *et al.*, 2000. *Glob. Biogeochem. Cyc.* 14, 1153-1160.
- Sparks *et al.*, 2008. *Glob. Ch. Biol.*, doi: 10.1111/j.1365-2486.2007.01526.x.
- Sutton, M.A., *et al.*, 1997. Gaseous Exchange with Grassland Systems. Wallingford, Oxon, UK, 131 – 139.
- Sutton, M.A., *et al.*, 2001 *Water, Air, and Soil Pollution: Focus* 1, 145–156.
- Tang, Y.S., *et al.*, 2001. *Sci. World* 1, 513 – 529.
- Tang, Y.S., *et al.*, 2009. Center for Ecology and Hydrology, Edinburgh.
- U. S. Environmental Protection Agency. Compendium Method IO-42: Determination of Reactive Acidic and Basic Gases and Strong Acidity of Fine-Particles (<2.5 µm). EPA/625/R-96010a; U.S. EPA: Cincinnati, OH, 1997.
- van der Hoek, K. W., 1998. *Atmos. Environ.*, 32, 315-316.
- Walker, J.T., *et al.*, 2008. *Atmos. Environ.*, 42, 3407 – 3418.
- Walker, J.T., *et al.*, 2006. *Agric. For. Meteorol.* 138, 54 - 68.
- Walker, J.T., *et al.*, 2000a. *Environ. Sci. Technol.*, 34, 3527-3534.
- Walker, J.T., *et al.*, 2000b. *Atmos. Environ.*, 34, 3407-3418.
- Weseley, M. L., 1989. *Atmos. Environ.* 23, 1293 – 1304.
- Whitall, D.R., Paerl, H.W., 2001. *J. Env. Qual.*, 30, 1508-1515.
- Yamartino, R.J. 1984. *Journal of Climate and Applied Meteorology* 23: 1362–1366.
- Zhang, L., *et al.*, 2003. *Atmos. Chem. Phys.* 3, 2067 – 2082.
- Zou *et al.*, 2006 X. Zou, Z. Shen, T. Yuan, S. Yin, J. Cai, L. Chen and W. Wang, *Atmospheric Environment* **40** (2006), pp. 8068–8073.

# Improved Drought Management Strategies for the Triangle Area Utilizing Climate Information based Probabilistic Streamflow Forecasts

## Basic Information

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## Publications

1. Devineni, N., and A. Sankarasubramanian. 2010, Predictability of U.S. Winter Precipitation: Role of ENSO state in Developing Multimodel Combinations, in press, Monthly Weather Review.
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3. Sankarasubramanian, A., N. Devineni, and S. Ghosh, Multi-model Ensembling of Probabilistic Streamflow Forecasts: Role of Predictor State, Space in Skill Evaluation, Institute of Statistics Mimeo Series, 2595, NC State University, 2006.
4. Arumugam, S. and N. Devineni, Predictability of U.S. Winter Precipitation: Role of ENSO state in Developing Multimodel Combinations, American Geophysical Union Fall Conference, San Francisco, December 10-14, 2007.
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6. Arumugam, S., N. Devineni, and S. Ghosh, Multi-model Ensembling based on Predictor State Space: Seasonal Streamflow Forecasts and Causal Relations, American Geophysical Union Conference, San Francisco, December 11-15, 2006.
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8. Golembesky, K., S. Arumugam and N. Devineni, Improved Management of Falls Lake Reservoir during the Summer Season Using Climate Information Based Monthly Streamflow Forecasts: Role of Restrictions in Water Supply and Water Quality Management, WRI Annual Conference, Raleigh, NC, March 27-28, 2007.

9. Devineni, N. and S. Arumugam, Improved Drought Management of Falls Lake Reservoir: Role of Multimodel Streamflow Forecasts in Setting up Restrictions, USA-Korea Conference, Raleigh, NC, July 16-19,2009.

## **Title**

Improved water management strategies for the Neuse Basin utilizing climate-information based probabilistic streamflow forecasts

(Utilizing three-month ahead multimodel streamflow forecasts for improving the management of Falls Lake)

## **Problem and Research Objectives**

The multi-year drought during 1998-2002 caused severe hardship and economic losses across most of North Carolina (NC) (Weaver, 2005). Several local and state-wide water supply systems experienced record shortages and many communities operated under mandatory water restrictions from 2001-2003 (Weaver, 2005). A similar situation existed during the 2005 drought and is present during the current 2007 drought throughout the state (<http://www.ncdrought.org/>). Economic losses in NC for the year 2002 were estimated to be \$398 million for agriculture and \$15-\$20 million for municipalities (Weaver, 2005). Unless closely monitored using various sector-specific indicators, the impacts of droughts are progressive, persistent and pervasive over a large area. Thus, updating drought management plans not only requires monitoring but also needs to include prognostic information about the streamflow potential in the upcoming seasons to support proactive management measures such as restrictions and hedging. In this study, we combine three-month ahead climate information based multimodel streamflow forecasts with a reservoir management model that can take ensembles of reservoir inflows to invoke prescribed levels of restriction for water supply.

Droughts experienced by regional water supply systems often result from reduced streamflow/precipitation potential which could occur due to varying exogenous climatic conditions such as tropical sea surface temperature (SST) (Ropelewski and Halpert, 1987; Piechota and Dracup, 1996; Barlow et al. 2001). As water supply systems can experience shortages in supply owing to its (inflows) natural variability, resulting deficits are exacerbated by increased demand resulting from urbanization and population growth in the region (Lyon et al. 2005; Vorosmarty et al. 2000). For instance, in the Triangle Area in NC, the demand has grown by about 20%-62% from 1995-2000 (Weaver, 2005) resulting in three severe droughts (summers of 2002, 2005 and 2007) in the past five years. Given that most of the water supply systems are multipurpose, operating these systems to meet the increased demand under reduced streamflow availability could be very challenging.

The main intent of this study is to apply climate information based streamflow forecasts from three models - parametric regression, semi-parametric resampling and multimodel (obtained by combining the former two models) – for setting up restrictions on water supply releases from the Falls Lake reservoir in NC. By performing retrospective reservoir analyses, we basically compare the forecasted end of the season target storage probabilities with the climatological probabilities to set up restrictions on water supply releases.

## Methodology

### **FALLS LAKE SYSTEM: MANAGEMENT MODEL DEVELOPMENT**

Falls Lake is a man-made reservoir in the upper Neuse River, NC (Figure 1) operated by the US Army Corps of Engineers (USACE), since December 1983, to serve five purposes: (1) Flood control, (2) Water supply, (3) Water quality, (4) Recreation, and (5) Fish and Wildlife. The lake is long and narrow in shape and extends 28 miles up the Neuse River. Three rivers—the Eno, Flat and Little Rivers— provide the majority of inflows. As a water supply reservoir, Falls Lake provides Raleigh, by contract, with up to 100 million gallons of water a day (MGD). Due to the population growth in the City of Raleigh and in the suburbs served by Falls Lake over the last decade, storage conditions in Falls Lake have been increasingly stressed recently resulting in three severe droughts (2002, 2005, and 2007) which occurred over the last five years. Current drought management and monitoring activities are coordinated by the NC Drought Management Advisory Council (NCDMAC) in coordination with various state and federal agencies in NC.

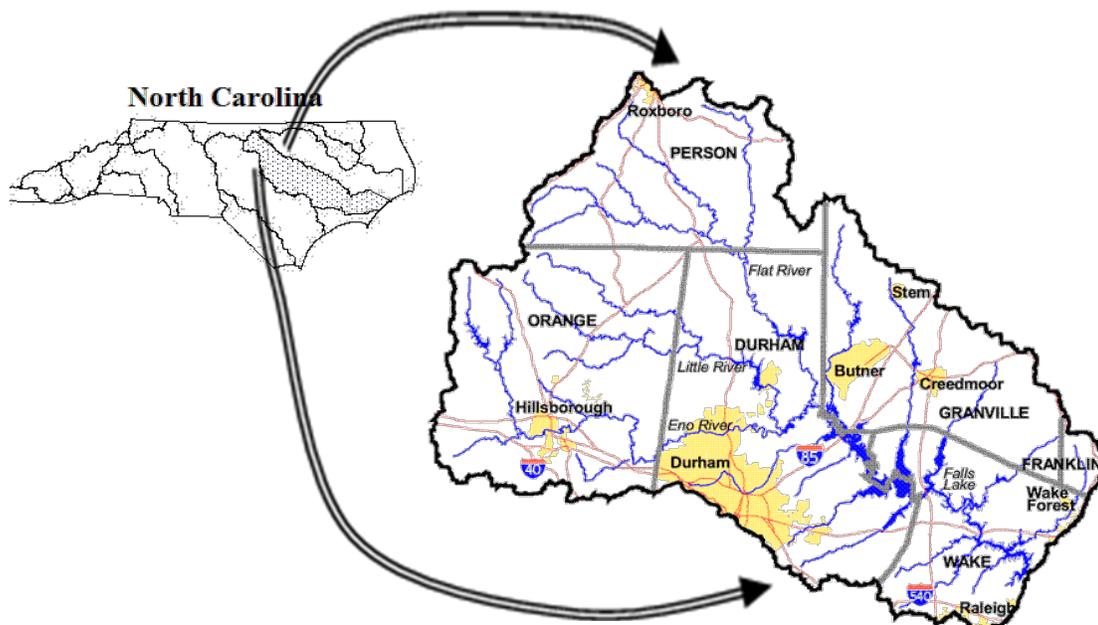


Figure 1: Location of Neuse River basin and Falls Lake Reservoir in the upper Neuse river basin.

### **Data and Operational Constraints**

For operational purposes, reservoir storages of Falls Lake are divided into various pools: (a) Flood control pool (controlled storage, 251.5 ft -264.8 ft and uncontrolled storage, 264.8 ft -289.2 ft) (b) Conservation pool (251.5 ft -236.5 ft) with two separate storage accounts for water quality and water supply (c) Sediment Dead Storage (236.5 ft -200 ft). All elevations (in ft) are based on North America Vertical Datum of 1927 (NAVD27). Both water supply and water quality releases are met based on the storages in conservation pool by devoting 39% of the conservation pool storage volume to water supply and the remaining 61% to water quality purposes.

The USACE uses 251.5 ft (131, 395 acre-feet) as the operational rule curve or the target pool level, which is obtained based on the average monthly flows recorded at Falls Lake [\[http://epec.saw.usace.army.mil\Falls\\_WC\\_Plan.pdf\]](http://epec.saw.usace.army.mil\Falls_WC_Plan.pdf). Thus, the USACE tries to ensure the reservoir level at operational rule curve in the beginning (July 1) and end (September 30) of the summer season. During wet summer years (e.g., 1996 and 1999), the above-normal inflows forces the reservoir level above 251.5 ft posing operational constraints on flood control and recreation. Under such situations, the USACE releases additional water to maintain the operational rule curve to reduce the downstream flood risk.

Normal outflows for protecting downstream water quality in the Neuse River are 254 cubic feet per second (cfs). However, reservoir outflows during below-normal storage conditions could be reduced to 100 cfs (April to October) and 60 cfs (November to March) after consultation with all stakeholders. Additional information such as monthly releases, stage-storage and stage-water spread area relationships was obtained from USACE to develop the Falls Lake simulation model, which is described in detail in the following section.

### Falls Lake Reservoir Model Formulation

Given the seasonal (T-month lead) ensemble inflow forecasts  $q_j^k$  and initial reservoir storage,  $S_0^*$ , at the beginning of the allocation period (for this analyses, July 1<sup>st</sup>) with  $j=1,2,\dots, N$  denoting the forecast years ( $N$  = total number of years of retrospective forecasts) and  $k = 1,2,\dots, K$  is the index representing a particular member out of 'K' ensembles, the Falls Lake simulation model determines the seasonal releases  $R_1$  and  $R_2$  representing water supply and water quality allocations respectively with a specified reliability of  $(1-p_{f1})$  and  $(1-p_{f2})$ , where  $p_f$  - implies failure probability. In addition, the water allocation model incorporates an end of the season target storage,  $S_T^*$  (T- denoting the forecast lead time in months) that is associated with a failure probability  $p_s$ . For instance, in the case of Falls Lake  $S_T^*$  corresponds to the storage of the reservoir at 251.5 ft operational rule curve. The simulation model could also estimate the probabilistic constraints (in equations (7) and (8)), reliability of supply for each use  $((1-p_{f1})$  and  $(1-p_{f1}))$  and  $p_s$  given the specified demand  $R_1^*$  and  $R_2^*$ , for each use along with  $S_T^*$  and  $S_0^*$ . Using the basic continuity equation, we update the seasonal storage equations for each ensemble member 'k' for the forecasting year 'j'.

$$S_{T,j}^k = S_{0,j}^* + q_j^k - E_j^k - (R_{1,j} + R_{2,j}) \quad \dots (1)$$

where seasonal storage equations are constrained so that the storage is between the minimum and maximum possible storage,  $S_{min}$  and  $S_{max}$  respectively.

$$S_T^k = \min(S_T^k, S_{max}), S_T = \max(S_T^k, S_{min}) \quad \dots (2)$$

In the event, the end of season storage falling below the minimum possible storage,  $S_{min}$ , we encounter deficits,  $SD_j^k$ , which needs to be distributed among the users as restrictions.

$$SD_j^k = (S_{min} - S_{T,j}^k) \mid S_{T,j}^k < S_{min} \quad \dots (3)$$

$$SD_j^k = \sum_{i=1}^2 w_{i,j}^k; w_{i,j} = \alpha_i R_{i,j} \quad \dots (4)$$

The restrictions,  $w_i$ , for each user could be specified exogenously as a fraction,  $\alpha_i$ , of the target release,  $R_i$ . The restriction fraction,  $\alpha_i$ , could also be allowed to vary depending on the restriction level.

Evaporation,  $E_j^k$  is computed as a function of average storage during the season using the water spread area and storage information of the reservoir.

$$E_j^k = \psi_j \delta_1 ((S_0^* + S_T^k) / 2)^{\delta_2} \quad \dots (5)$$

where  $\psi_j$  is the seasonal evaporation rate,  $\delta_1$  and  $\delta_2$  are coefficients describing the area-storage relationship. In this study, we employed spline interpolation for obtaining the water spread area corresponding to the average season storage computed for each ensemble. It is important to note that the evaporation is evaluated implicitly for each streamflow member in the ensemble. The estimated average lake evaporation rate ( $\psi_j$ ) is = 0.995 ft/season (after adjusting with the pan coefficient of 0.7) for the summer, which is obtained from the monthly pan evaporation recorded at Chapel Hill, NC.

The objective is to determine  $R_i$ , such that the releases for  $i^{\text{th}}$  use is bound by the minimum and maximum demand for the season.

$$R_{i,\min} \leq R_i \leq R_{i,\max} \quad \dots(6)$$

Similarly, we also enforce the probability of having the end of the season storage,  $S_T$ , less than the target storage,  $S_T^*$  to be small represented by its failure probability (Prob),  $p_s$ , using equation (7).

$$\text{Prob}(S_T \leq S_T^*) \leq p_s \quad \dots(7)$$

To ensure the obtained release,  $R_i$ , is met with high reliability,  $(1-p_{fi})$ , we include

$$\text{Prob}(w_i \leq w_i^*) \leq p_{fi} \quad \dots(8)$$

where  $w_i^*$ , specified by the user, denotes the maximum restriction that could be enforced for each user as part of restrictions. This constraint basically accounts the uncertainty in releases. Thus, the obtained seasonal release may be between the desired bounds  $R_{i,\min}$  and  $R_{i,\max}$ , but the specified release  $R_i$  has a small probability,  $p_{fi}$ , of facing restrictions being lesser than  $w_i^*$ . The restriction  $w_i$  is calculated for each ensemble member 'k' using the restriction fraction,  $\alpha_i$ , based on equation (4). In this study, we basically specify  $w_i^* = 0$  for all the analyses.

Looking across all the traces in the ensemble, the model computes the following probabilities to evaluate equations (7) and (8):

- (1)  $\text{Prob}(w_i \leq w_i^*)$  is estimated from the number of traces in which  $(w_i \leq w_i^*)$  / total number of traces, N. This includes the calculation of the failure to meet the two specified demands, water supply and water quality.
- (2)  $\text{Prob}(S_T < S_T^*)$  is obtained from the number of traces in which  $S_T < S_T^*$  / total number of traces, N

We consider N=500 ensembles that represent the average seasonal streamflow during the summer (July, August and September, JAS). In this study, instead of obtaining  $R_1$  and  $R_2$  for the specified constraints, we specify the water supply release (in Figure 2),  $R_{1,j}^*$ , and water quality release,  $R_{2,j}^*$ , (with the average flow being equal to 254 cfs or 100 cfs) to estimate the probabilistic constraints in equations (7) and (8). The above probabilities are then computed across the ensembles to evaluate the above-listed constraints. Though the model is presented in a simulation framework, it could be extended into an optimization-simulation model by including compensations under restrictions along with a detailed contract structure (Arumugam et al. 2003).

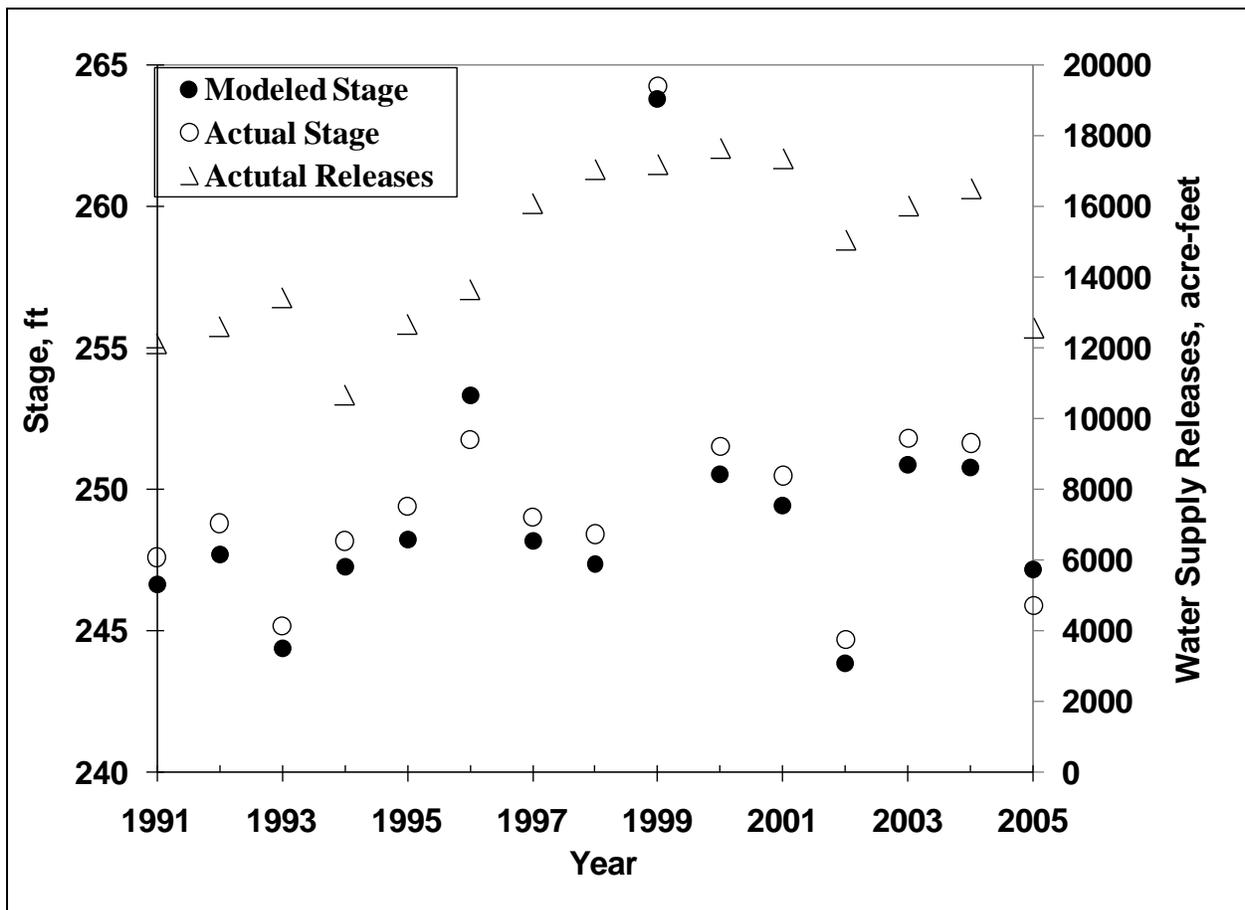


Figure 2: Comparison of modeled stages with the observed stages in September for the period 1991-2005. Figure also shows the reported water supply releases during JAS from Falls Lake. Modeled stages are obtained upon simulating the model with observed flows, releases and by forcing the model with the initial storage recorded each year on July 1.

### Reservoir Model Verification

Prior to performing the retrospective reservoir analyses using the streamflow forecasts, model verification was performed from 1991-2005 by comparing the reservoir model's ability to predict the observed end of September storages. The model simulations were performed by forcing the model with the observed flows during JAS and initial storages in July to determine the end of the September storages by allocating the reported releases for water quality and water supply. This verification provides a check on the mass balance of the reservoir model as well as in its ability to model the conservation storage pool into two separate accounts (*i.e.*, water supply and water quality storages). Figure 2 shows the observed and model predicted stages at the end September—the end of the season stage. The observed and modeled storages obtained from the reservoir model were converted into stages using the available stage-storage relationship for Falls Lake. Figure 2 clearly shows that the developed model is quite reasonable in predicting the observed September storages upon simulation with observed flows and reported releases. This gives us the confidence in employing the simulation model presented here for further analyses that utilize the seasonal streamflow forecasts from three models for invoking restrictions.

## SEASONAL STREAMFLOW FORECASTS FOR FALLS LAKE

This section briefly describes the development of streamflow forecasts for Falls Lake during the summer season. For additional details on the streamflow forecasting model, predictor identification and the skill of cross-validated forecasts, see the forecasting paper (Devineni et al. 2008) and the technical report (Sankarasubramanian et al. 2006) (available online: <http://www.stat.ncsu.edu/library/papers/mimeo2595.pdf>). Seasonal streamflow forecasts were developed for the summer season based on April, May and June (AMJ) climatic information, denoted by anomalous SST conditions in the tropical Pacific, tropical North Atlantic and over the North Carolina coast.

Predictor identification using Spearman rank correlation was performed on the International Research Institute for Climate and Society (IRI) data library between the global SSTs (<http://iridl.ldeo.columbia.edu/SOURCES/KAPLAN/EXTENDED/v2/ssta/>) and the seasonal streamflows. Grid points of SSTs (black rectangles) in Figure 3a that have significant correlation with the predictand were considered as predictors in developing the forecasts. The correlations shown in Figure 3a are for 78 years of flows. Thus, if the absolute value of correlation is greater than 0.22, then we expect the correlation between the predictor and predictand to be statistically significant (at 95% confidence level). Since the SST grid points were spatially correlated, principal component analysis was performed and the first two principal components (explained 73% of total variance exhibited in SSTs) were retained for model development. We also considered spring season (April-June) streamflow and the previous month streamflow (June alone) as surrogate predictors to incorporate land surface conditions such as soil moisture. But, the correlations between the previous month/seasonal flows and the summer flows are statistically not significant.

We consider two non-linear models, parametric regression (with the predictand being cube-root of the flows) and semi-parametric resampling models [Souza and Lall, 2003], in developing multimodel forecasts. Since the skewness of the recorded summer flows is 1.9, we applied cube-root transformation for developing the parametric regression model. With regard to individual model selection, one can even consider land surface model in developing streamflow forecasts. Studies have considered objective criterion along with stepwise regression to select the best combination of nonlinear models in developing multimodel forecasts (Regonda et al. 2006). In this study, the resulting seasonal streamflow forecasts from parametric regression and semi-parametric resampling models were then combined using a multimodel combination algorithm to develop improved seasonal streamflow forecasts [Devineni et al. 2008, Sankarasubramanian et al. 2006].

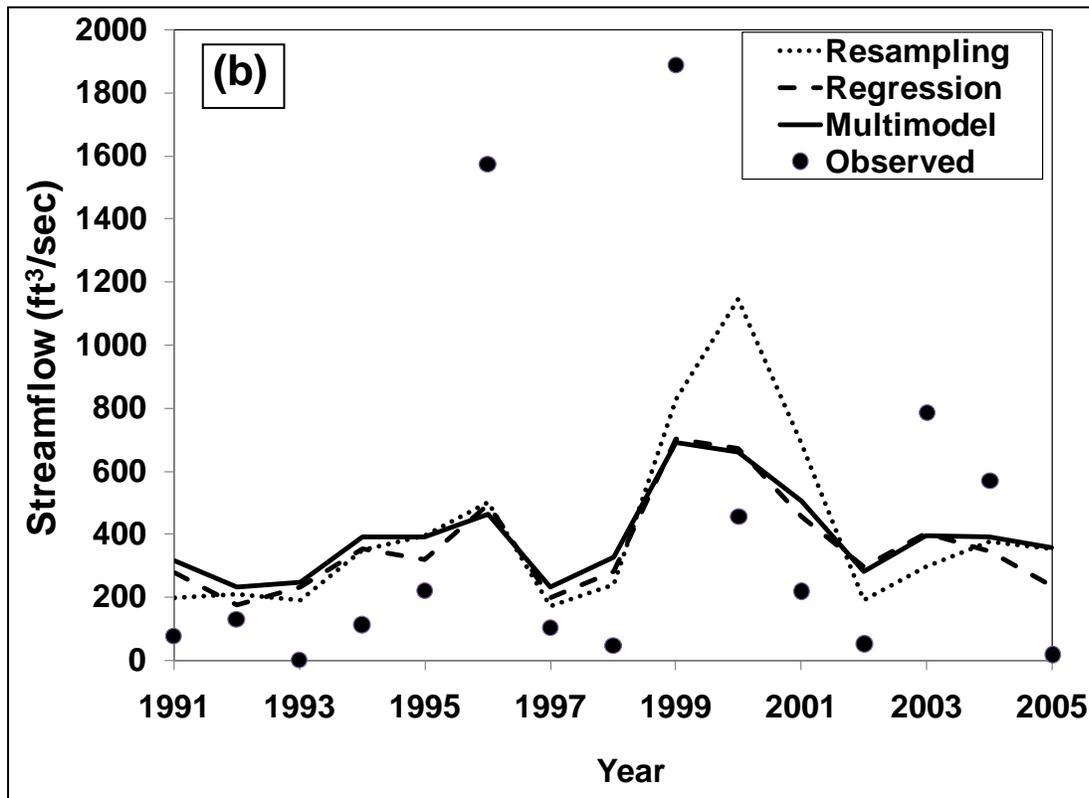
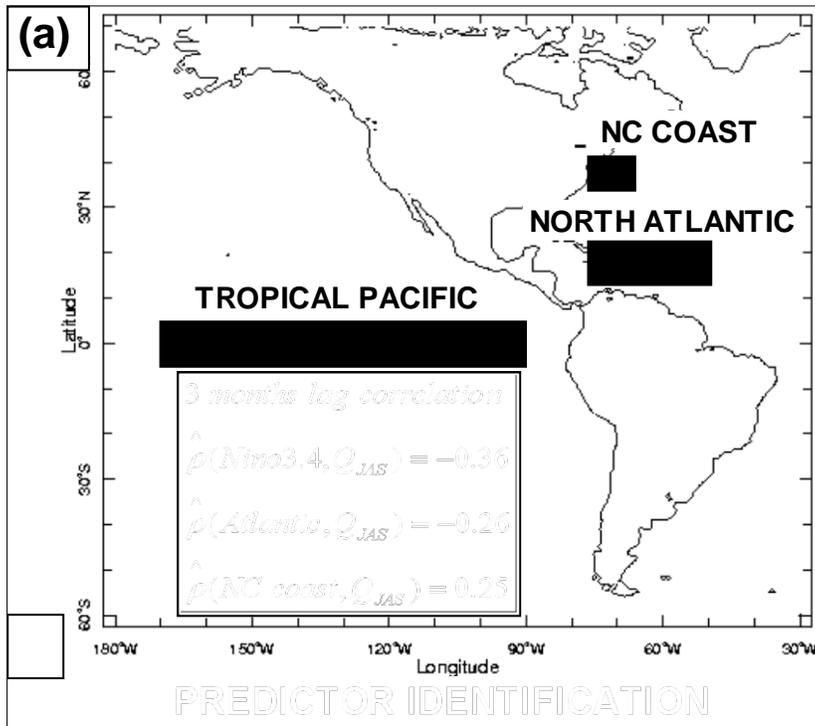


Figure 3: Leave-one out Cross-validated seasonal (JAS) streamflow forecasts for the Falls Lake from three forecasting models (Figure 3b -Regression, Resampling and Multimodel) along with the with the employed predictors (Figure 3a). Principal components of the three SST regions (shown as rectangles in Figure 3a) were performed and the dominant two components were employed for developing the streamflow forecasts represented in the form of ensembles.

In this study, we employed seasonal streamflow forecasts from three models – regression, resampling and multimodel – for improving the drought management of Falls Lake. The adaptive forecasts for the period 1976-2005 were developed by training the model using the observed flows and predictors available from 1928-1975. The correlations between the observed flows and the ensemble mean of the seasonal streamflow forecasts are 0.44, 0.49 and 0.51 for resampling, regression and multimodel respectively, which are statistically significant for the 30 years of validation. Figure 3b shows the adaptive forecasts from the three models for the period 1991-2005. The forecasts (in Figure 3b) are shown as conditional mean, which is obtained from the 500 ensembles of the conditional distribution of streamflows developed for each year. Representing the conditional distribution with large ensembles will only lead to better estimates of probability constraints (equations (7) and (8)) without improving the skill of the probabilistic forecasts. For instance, with regard to parametric regression model, the actual information content in the forecasts is purely determined by its conditional mean and conditional variance. We also consider the null forecast, the climatological ensembles, whose ensembles were developed by simple bootstrapping of JAS flows. This approach is reasonable, since there is no year-to-year correlation between summer flows at Falls Lake. These streamflow forecasts and the initial storages observed on July 1 were provided as input to the reservoir management model to estimate the reliabilities of meeting the water supply releases (in Figure 2) and minimum water quality releases as well as to estimate the probability of end of September storage being below the target storage (corresponding to target stage 251.5 ft) ( $\text{Prob}(S_T < S_T^*)$ ).

### **Principal Findings**

A reservoir simulation model that uses ensembles of streamflow forecasts is presented and applied for allocating water during the summer season (JAS) from the Falls Lake Reservoir in the Neuse River basin, NC. Given the initial storage at the beginning of the season and ensembles of seasonal streamflow forecasts, the simulation model can estimate the reliability of the specified target releases and the end of the season target storage probability.

The customized simulation model for Falls Lake was analyzed using JAS seasonal streamflow forecasts from three models: *parametric regression*, *semi-parametric resampling* and *multimodel forecasts* (obtained from the former two models). The performance of these three models in estimating probability of end of the season storage being lesser than the target storage was evaluated by comparing with the estimates of probability of end of the season storage being lesser than the target storage from climatological ensembles to predict below-normal storage conditions, which could help in invoking restrictions for improving storage conditions at the end of the summer season.

Analyses of Falls Lake using the simulation model without constraining the end of season target storage showed 100% reliability of meeting target releases, implying the entire seasonal demand could be met purely based on initial storage. This invalidated the utility of streamflow forecasts available for the summer season. However, by constraining the system to meet the end of the season target storage, we show clearly that the estimates of probability of end of the season storage being lesser than the target storage from forecasts are higher than the climatology estimates during below-normal summer inflow

years and vice versa during above-normal inflow years, thereby indicating the utility of forecasts in invoking restrictions. By invoking restrictions during JAS based on the predicted estimates of probability of end of the season storage being lesser than the target storage, the study shows that, by validating with JAS observed flows, increased storage conditions result in September. Among the three streamflow forecasting models, multimodel streamflow forecasts seem to better predict the change in streamflow potential, thus resulting in reduced false alarms and missed targets in predicting below-normal storage conditions at the end of September. Thus, applying multimodel forecasts would reduce uncertainty from individual models which could lead to better decisions and also could improve public confidence in utilizing seasonal streamflow forecasts for water management application.

### **Significance**

In developing seasonal water allocation policies, initial storages may ensure 100% reliability of supplying target releases for the intended uses, thereby limiting the utility of climate forecasts. But, ensuring the end of the season target storage (or the operational rule curve) will be met with high probability could offer additional insights for invoking the appropriate level of restrictions during below-normal inflow years. Further, as the water demand increase over the service area (due to urbanization and population growth), the initial storage may no longer ensure 100% reliability which will necessitate the application of climate forecasts for invoking restrictions. During above-normal inflow years, since the forecasts based probability of end of the season storage being lesser than the target storage will be lower than its climatological probability, forecasts based allocation would avoid unnecessary restrictions if the initial storage is lower than the operational rule curve. On the other hand, if the initial storage is higher than the operational rule curve, then additional release could be considered to reduce the downstream flood risk such that the forecasts based estimates of probability of end of the season storage being lesser than the target storage is equal to its climatological probability.

The retrospective analysis presented in this study could also be utilized to determine the appropriate beginning of the season storage under future increased demand scenarios. Using climatological ensembles, we can estimate the increased beginning of the season storage,  $S_0$ , which needs to ensure the current climatological probability of end of the season storage being lesser than the target storage will remain unchanged even under future release scenarios. Similarly, the proposed formulation also could be utilized to develop rule curves that change according to the inflow potential. The study shows that by restricting reservoir releases during below-normal years, the probability of end of the season storage being lesser than the target storage could be increased. To develop rule curves for this scenario, we can specify the end of the season target storage based on the target storage and obtain previous month target storages that will ensure the restricted releases during the season. It is also important that these rule curves need to be updated regularly based on the updated climate information, which is important towards better prediction of intra-seasonal variability in streamflows.

The main advantage in utilizing multimodel forecasts is in reducing model uncertainty by constituting ensembles from multiple models. In our multimodel combination scheme, higher weight is given to the individual model that performs well under similar predictor conditions. For instance, if an individual model performs better during El Nino conditions, then higher number of ensembles is drawn from that particular model under similar predictor conditions. By combining individual models with climatology, we reduce the overconfidence in individual model forecasts to develop multimodel forecasts that has reduced false alarms and missed targets. This study clearly shows that employing such multimodel forecasts for season-ahead water allocation provides a more reliable way to develop appropriate management strategies such as invoking (or not invoking) restrictions during below-normal (above-normal) years. Our future studies will focus on better management of water supply systems under increased demand

potential without resorting to capacity expansion and investments on new systems by considering alternate water uses (e.g. reclaimed water) and trading.

## **References**

- Arumugam, S., A. Sharma, and U.Lall, Water Allocation for multiple uses based on probabilistic reservoir inflow forecasts, Proceedings, IAHS, Soppore, 2003.
- Barnston, A. G., S. J. Mason, L. Goddard, D. G. DeWitt, and S. E. Zebiak (2003), Multimodel ensembling in seasonal climate forecasting at IRI, *Bulletin of the American Meteorological Society*, 84, 1783-+.
- Barlow, M., S. Nigam, and E. H. Berbery (2001), ENSO, Pacific decadal variability, and US summertime precipitation, drought, and stream flow, *Journal of Climate*, 14, 2105-2128.
- Dettinger, M.D. and H.F. Diaz (2000), Global characteristics of stream flow seasonality and variability, *Journal of Hydrometeorology*, 1 (4): 289-310.
- Devineni, D., A.Sankarasubramanian, and S. Ghosh (2008), Multi model Ensembling of Streamflow Forecasts: Role of Predictor State in Developing Optimal Combinations, Accepted in *Water Resources Research*.
- Doblas-Reyes, F. J., M. Deque, and J. P. Pielke (2000), Multi-model spread and probabilistic seasonal forecasts in PROVOST, *Quarterly Journal of the Royal Meteorological Society*, 126, 2069-2087.
- Georgakakos, A.P., H.M.Yao, M.G.Mullusky, and K.P.Georgakakos (1998), Impacts of climate variability on the operational forecast and management of the upper Des Moines River basin, *Water Resources Research*, 34 (4), 799-821.
- Guetter, A. K., and K. P. Georgakakos (1996), Are the El Niño and La Niña predictors of the Iowa River seasonal flow? *J. Appl. Meteor.*, 35, 690-705.
- Hamlet, A.H., D. Huppert and D.P. Lettenmaier (2002), Economic value of long-lead streamflow forecasts for Columbia River hydropower, *J. Water Resour. Planning and Management* 128, 91-101.
- Hamlet, A.F., and D.P.Lettenmaier (1999), Columbia River streamflow forecasting based on ENSO and PDO climate signals, *Journal of Water Resources Planning and Management*, 125 (6), 333-341.
- Hartmann, H. C., T. C. Pagano, S. Sorooshian, and R. Bales (2002), Confidence builders - Evaluating seasonal climate forecasts from user perspectives, *Bulletin of the American Meteorological Society*, 83, 683-+.
- Kyriakidis, P.C., N.L.Miller and J.Kim (2001), Uncertainty propagation of regional climate model precipitation forecasts to hydrologic impact assessment, *Journal of Hydrometeorology*, 2 (2): 140-160.
- Leung, L.R., A.F.Hamlet, D.P. Lettenmaier and A.Kumar (1999), Simulations of the ENSO hydroclimate signals in the Pacific Northwest Columbia River basin, *Bulletin of the American Meteorological Society*, 80 (11): 2313-2329.
- Lyon, B., N. Christie-Blick, and Y. Gluzberg (2005), Water shortages, development, and drought in Rockland County, New York, *Journal of the American Water Resources Association*, 41, 1457-1469.
- Maurer, E. P., and D. P. Lettenmaier (2004), Potential effects of long-lead hydrologic predictability on Missouri River main-stem reservoirs, *Journal of Climate*, 17, 174-186.
- Pagano, T. C., H. C. Hartmann, and S. Sorooshian (2001), Using climate forecasts for water management: Arizona and the 1997-1998 El Niño, *Journal of the American Water Resources Association*, 37, 1139-1153.
- Piechota, T.C., F.H.S.Chiew, J.A.Dracup and T.A.McMahon (2001), Development of exceedance probability streamflow forecast, *Journal of Hydrologic Engineering*, 6 (1), 20-28.

Piechota, T.C., and J.A. Dracup (1996), Drought and regional hydrologic variation in the United States: Associations with the El Nino Southern Oscillation. *Water Resources Research*, 32(5):1359-1373.

Regonda, S. K., B. Rajagopalan, M. Clark, and E. Zagona (2006), A multimodel ensemble forecast framework: Application to spring seasonal flows in the Gunnison River Basin, *Water Resour. Res.*, 42, W09404, doi:10.1029/2005WR004653.

Ropelewski, C.F., and M.S. Halpert (1987), Global and Regional Scale Precipitation Patterns Associated with the El-Nino Southern Oscillation. *Monthly Weather Review*, 115(8):1606-1626.

Sankarasubramanian, A., N.Devineni, and S. Ghosh (2006), Multi model Ensembling based predictor state space: Seasonal forecasts and climate relation, *Institute of Statistics Miniseries*, North Carolina State University, Raleigh.

Sankarasubramanian, A., U. Lall, and S. Espuneva (2008), Role of Retrospective Forecasts of GCMs Forced with Persisted SST anomalies in Operational Streamflow Forecasts Development, *Journal of Hydrometeorology*, 9(2), 212-227.

Schaake, J., and L.Larson (1998), Ensemble Streamflow Prediction (ESP): Progress and Research needs, Special Symposium on Hydrology, Am.Meteorol., Soc., Boston, Mass, J19-J24, 1998.

Sicard, E., R. Sabatier, H. Niel, and E. Cadier (2002), A new approach in space-time analysis of multivariate hydrological data: Application to Brazil's Nordeste region rainfall, *Water Resources Research*, 38, (12): Art. No. 1319.

Souza, F., and U. Lall (2003), Seasonal to interannual ensemble streamflow forecasts for Ceara, Brazil: Applications of a multivariate, semiparametric algorithm. *Water Resources Research*, 39(11): p. Article No. 1307.

Steinemann, A. C. (2006), Using climate forecasts for drought management, *Journal of Applied Meteorology and Climatology*, 45, 1353-1361.

Vorosmarty, C. J., P. Green, J. Salisbury, and R. B. Lammers (2000), Global water resources: Vulnerability from climate change acid population growth, *Science*, 289, 284-288.

Weaver, C.J., *The Drought of 1998-2002 in North Carolina-Precipitation and Hydrologic Conditions*, in *USGS Scientific Investigations Report*. 2005.

Yao, H., and A. Georgakakos (2001), Assessment of Folsom Lake response to historical and potential future climate scenarios, 2- reservoir management, *J. Hydrology*, 249, 176-196, 2001.

# Protecting Receiving Waters: Removal of Biochemically Active Compounds from Wastewater by Ozonation and Activated Carbon Adsorption Processes

## Basic Information

<b>Title:</b>	Protecting Receiving Waters: Removal of Biochemically Active Compounds from Wastewater by Ozonation and Activated Carbon Adsorption Processes
<b>Project Number:</b>	2009NC120B
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<b>End Date:</b>	12/31/2010
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	02
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Treatment, Acid Deposition, Water Quality
<b>Descriptors:</b>	Endocrine disrupting chemicals, Pharmaceutically active compounds, adsorption, biodegradation, oxidation, wastewater treatment, water reuse
<b>Principal Investigators:</b>	Detlef Knappe, Francis de los Reyes

## Publications

There are no publications.

## **Title**

Protecting Receiving Waters: Removal of Biochemically Active Compounds from Wastewater by Ozonation and Activated Carbon Adsorption Processes

## **Problem and Research Objectives**

The presence of biochemically active compounds (BACs) such as endocrine disrupting chemicals (EDCs), antimicrobial compounds, and other pharmaceutically active compounds in the aquatic environment is an issue of great importance. For example, the presence of EDCs may cause intersexuality and gender bending in fish, and the presence of antimicrobial compounds may lead to the evolution of antibiotic-resistant bacteria.

A recently completed study in our laboratory showed that UV/H<sub>2</sub>O<sub>2</sub> oxidation is an effective, but costly (and energy-intensive) process for the removal of BACs from wastewater. Compared to UV/H<sub>2</sub>O<sub>2</sub>, ozonation and powdered activated carbon (PAC) adsorption processes are expected to be more cost-effective and energy-efficient (Joss et al. 2008). However, strategies for the effective incorporation of PAC adsorption or ozonation processes into NC WWTPs need to be developed. For example, little is known about the optimal addition point for PAC in WWTPs. Similarly, little information is available about the ozone demand exerted by typical NC wastewaters.

The objectives of this research are (1) to measure oxidation kinetics of six model BACs during ozonation of NC wastewater matrices and, with the aid of a mathematical model, predict ozone doses required to achieve BAC oxidation levels of 90 and 99% for wide range of BACs and (2) to identify suitable powdered activated carbon (PAC) types and effective PAC addition points in wastewater treatment plants and determine PAC doses that yield BAC removals of 90 and 99%.

## **Methodology**

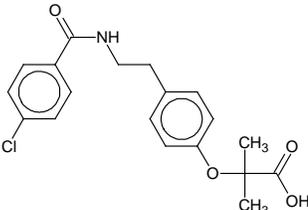
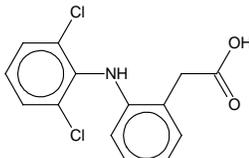
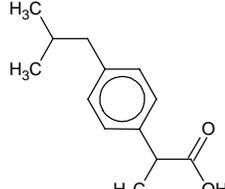
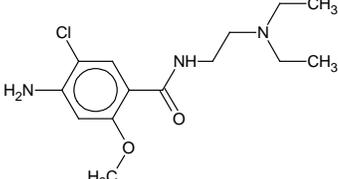
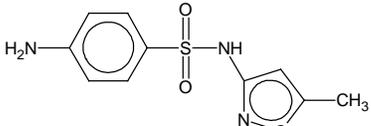
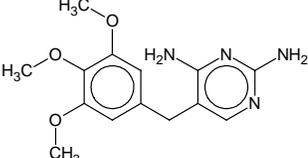
### **Pharmaceuticals**

Table 1 shows the six pharmaceuticals that were selected for this study. The selected compounds are commonly found in effluent-impacted water bodies and are expected to respond differently to oxidative and adsorptive treatment technologies.

Table 2 summarizes physical-chemical characteristics of the selected pharmaceuticals, including the octanol-water partition coefficient of the neutral form of each compound (logP) and the octanol-water partition coefficient at pH 7 (logD). The pK<sub>a</sub> values illustrate that BZF, DCF, IBP, and SMX are predominantly present in their anionic form at neutral pH. In contrast, the cationic form of MCP dominates at pH 7. For TMP, cationic and neutral forms coexist in almost equal proportions at neutral pH.

SMX, BZF, IBP, and DCF stock solutions were prepared daily in phosphate buffered ultrapure (DI) water (pH 7); TMP stock solutions were prepared in acidified DI water (pH 4) to enhance solubility; and MCP stock solutions were prepared in DI water. All stock solutions were prepared at concentrations of 2.0 mg/L, and stock solutions were filtered through a 0.22- $\mu$ m PTFE membrane before use. The targeted initial pharmaceutical concentration was ~100  $\mu$ g/L (<0.5  $\mu$ M). This concentration is sufficiently low that the determined removal percentages are expected to match those obtained at concentrations more commonly encountered in the wastewater.

**Table 1. Model pharmaceuticals**

Name	Abbreviation	Compound Class	Molecular Structure
bezafibrate	BZF	lipid regulator	
diclofenac	DCF	non-steroidal anti-inflammatory	
ibuprofen	IBP	analgesic	
metoclopramide	MCP	antiemetic	
sulfamethoxazole	SMX	antibiotic	
trimethoprim	TMP	antibiotic	

**Table 2. Properties of selected pharmaceuticals**

Chemical	Molecular Weight (g/mol)	pK <sub>a</sub>		logP	logD (pH 7)
		Acid [0/-]	Base [+0]		
BZF	361.82	3.3 <sup>*</sup>		3.46	0.03
DCF	296.15	4.2 <sup>+</sup>		4.06	1.28
IBP	206.28	4.9 <sup>+</sup>		3.72	1.16
MCP	299.8		9.3 <sup>+</sup>	2.22	-0.31
SMX	253.28	5.8 <sup>*</sup>		0.89	-0.27
TMP	290.32		7.1 <sup>+</sup>	0.79	0.38

Predicted with Advanced Chemistry Development (ACD/Labs) Software v. 8.14 (as listed in SciFinder Scholar)

<sup>+</sup> Experimentally determined values as listed in EPI Suite v. 4.0 database

### Water

Wastewater treatment plant effluent (WWTPE) collected from the North Cary Water Reclamation Facility is used. Upon collection, WWTPE was stored in 55-gal stainless steel drums at 4°C. Water was used as collected. The dissolved organic carbon (DOC) concentration of the WWTPE was 7.5 mg/L and the pH was 7.9. For reference, experiments were also conducted in two North Carolina drinking water sources: (1) 50/50 blend of Cane Creek Reservoir and University Lake waters (source of OWASA plant in Carrboro, NC) and (2) Cape Fear river water (source for Wilmington, NC). DOC concentrations of OWASA and Cape Fear river waters were 5.1 and 6.9 mg/L, respectively, and pH values were 7.2 and 7.0, respectively.

### Powdered Activated Carbons

Four PACs prepared from different base materials and with different activation methods were studied (Table 1). Three PACs (NuChar, Hydrodarco B, and WPH) were used in their as-received form. In addition, we prepared a superfine version of the WPH PAC, which we termed S-WPH. The mean diameter of S-WPH was ~0.3 µm while those of the as-received PACs were in the range of 17-25 µm.

**Table 3. Characteristics of PACs.**

PAC Name	Manufacturer	Material	Activation Method
NuChar	MeadWestvaco	Wood	Chemical
Hydrodarco B	American Norit	Lignite Coal	Thermal
WPH	Calgon Carbon Corporation	Anthracite Coal	Thermal
S-WPH	Custom-made	Anthracite Coal	Thermal

### Batch tests to determine the adsorption kinetics of pharmaceuticals on PAC

Batch kinetic tests were performed with the four PACs shown in Table 3. Pharmaceuticals were spiked at an initial concentration of ~100 µg/L into WWTPE. Experiments were conducted in 32-oz. amber glass bottles, and solutions were mixed with a PTFE-coated magnetic stir bar. Experiments were conducted at

ambient water pH unless otherwise specified. After taking samples to determine the initial pharmaceutical concentration, the desired amount of PAC was added under continuous mixing. Samples for pharmaceuticals analysis were taken after PAC contact times of 2, 5, 10, 15, 30, 60 and 120 minutes. Additional samples were taken after a contact time of 2 weeks. Solution pH was measured at the beginning and end of each kinetic test (Orion pH meter 420 A, Fisher Scientific, Pittsburgh, PA).

#### **Batch tests to determine ozonation and hydroxyl radical oxidation kinetics**

Batch tests are being conducted to determine the effectiveness of wastewater ozonation on pharmaceutical removal. Initial tests were conducted to determine the rate of ozone decay in WWTP. Ozone was spiked at O<sub>3</sub>/TOC ratios of 0.25, 0.5, 0.75, and 1.0 mg O<sub>3</sub>/mg DOC. Remaining ozone concentrations were measured by sampling reactor contents with a syringe containing 25 µL of a 10 mM cinnamic acid solution. The purpose of the cinnamic acid is to quench the remaining ozone in the sample. In the process, cinnamic acid is stoichiometrically converted to benzaldehyde, which is quantified by HPLC analysis. The remaining ozone concentration is then calculated from the amount of benzaldehyde formed.

Similar tests are being conducted to measure the rate of para-chlorobenzoic acid (p-CBA) conversion. The compound p-CBA is essentially non-reactive towards molecular ozone but is readily oxidized by hydroxyl radicals. From these data, we will be able to predict the rate at which the oxidation of BACs occurs due to molecular ozone and due to the presence of hydroxyl radicals.

#### **Analytical methods**

Concentrations of the six pharmaceuticals were determined by a high-performance liquid chromatography (HPLC, Breeze, Waters Corporation, Milford, MA) system equipped with a dual-wavelength UV detector. Pharmaceuticals were separated on a C18 column (2.6 µm, 4.6 × 100 mm, Kinetex C18 100A, Phenomenex, Torrance, CA). Prior to analysis, samples were filtered through a 0.22-µm PTFE membrane. Concentrations in bench-scale tests were sufficiently high that samples could be analyzed by direct injection, i.e. without sample preconcentration.

A gradient method was used for the analysis of SMX, TMP and MCP. Eluent A consisted of 50% v/v acetonitrile and 50% v/v 25 mM ammonium acetate buffer (pH 5) and eluent B of 10% v/v acetonitrile and 90% v/v 25 mM ammonium acetate buffer (pH 5). Each analysis started with 100% eluent B. From 1 to 9 minutes, the eluent was ramped linearly from 100% eluent B to 75% eluent B and 25% eluent A. Isocratic methods were used for BZF, DCF, and IBP. For BZF, the mobile phase was 25% v/v acetonitrile and 75% v/v 25 mM ammonium acetate buffer (pH 5). For DCF and IBP, the mobile phase was 37% v/v acetonitrile and 63% v/v 25 mM ammonium acetate buffer (pH 5). The mobile phase flow rate was 1 mL/min for all analyses. The detector wavelength was set at 266 nm for SMX, 238 nm for TMP, 274 nm for MCP, 240 nm for BZF, 282 nm for DCF, and 222 nm for IBP.

TOC and DOC were measured with a total organic carbon analyzer (Model TOC-5000A, Shimadzu Scientific, Columbia, MD). UV<sub>254</sub> absorbance was measured with a UV/vis spectrophotometer.

Solution pH was measured with a calibrated pH meter (Orion pH meter 420 A, Fisher Scientific, Pittsburgh, PA).

#### **Principal Findings**

Figures 1 and 2 compare uptake rates of six pharmaceuticals for PAC doses of 10 and 20 mg/L, respectively. Results were obtained with NuChar PAC in OWASA water. The data in Figures 1 and 2 show that MCP and TMP are the most adsorbable compounds; BZF and DCF are in the intermediate range; and IBP and SMX are two least adsorbable compounds.

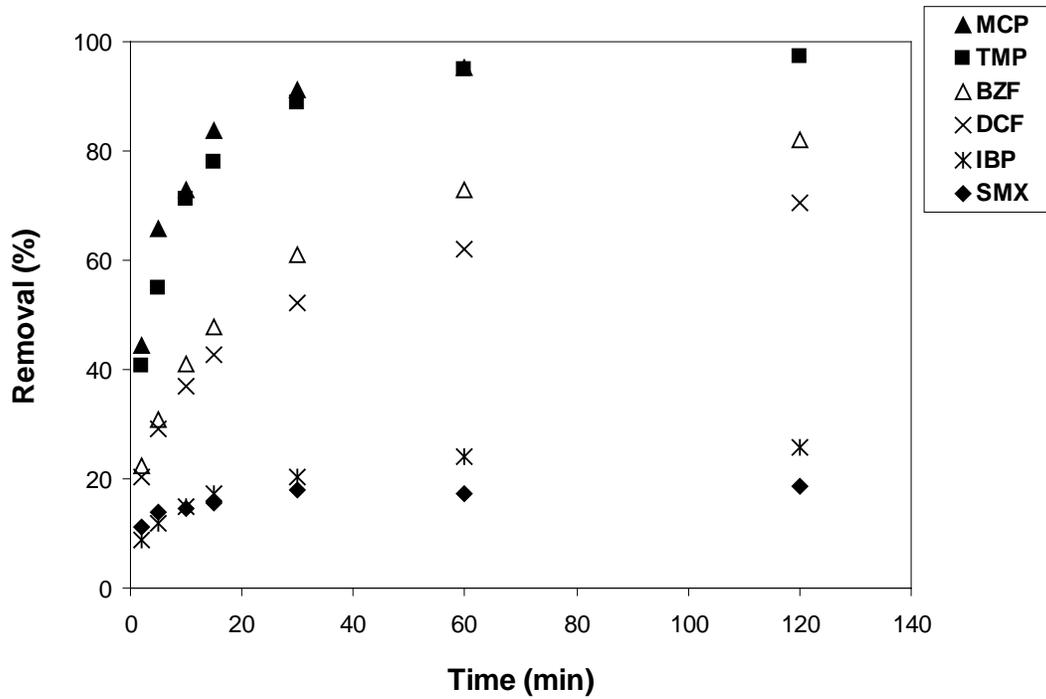


Figure 1. Adsorption uptake kinetics for six pharmaceuticals. PAC type: NuChar, PAC dose: 10 mg/L, pH: 7.2. Water: OWASA.

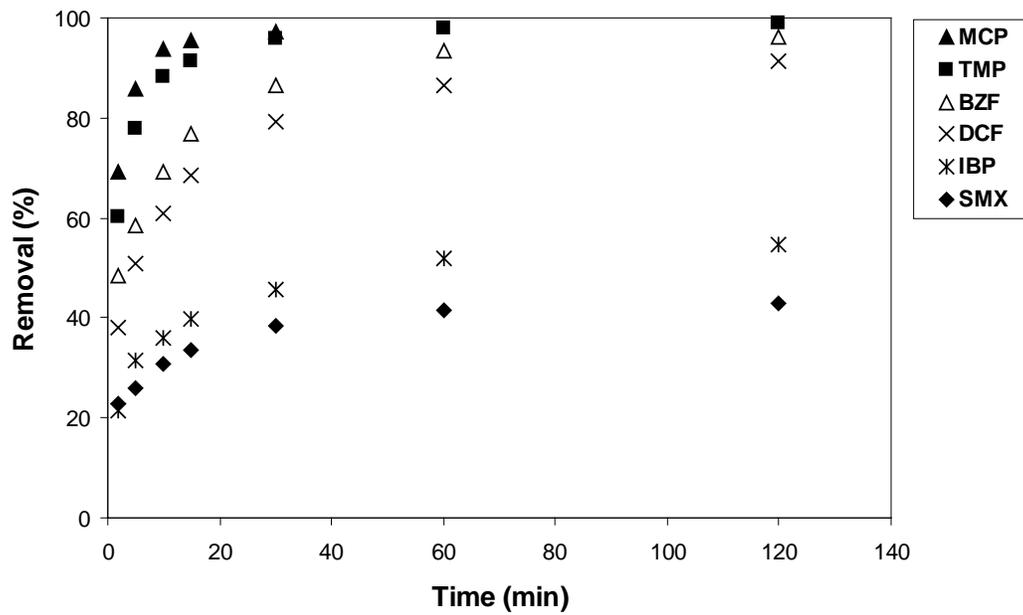


Figure 2. Adsorption uptake kinetics for six pharmaceuticals. PAC type: NuChar, PAC dose: 20 mg/L, pH: 7.2. Water: OWASA.

Additional experiments were conducted with TMP and SMX to compare the effects of the background water matrix on the effectiveness of PAC for BAC removal. The following background water matrices were tested: (1) OWASA water, (2) Cape Fear river water, and (3) Cary WWTP. Results for SMX and TMP are shown in Figures 3 and 4, respectively. The results show that the removal of SMX and TMP was similar in the two drinking water sources, but lower in the WWTP. The effects of the WWTP matrix were especially pronounced for SMX because the WWTP exhibited both a higher DOC concentration and a higher pH. SMX is a weak organic acid that transitions from the neutral to the anionic form as pH increases (Figure 5a). As a result, the adsorbability of SMX decreases with increasing solution pH. The effects of the WWTP matrix on TMP removal were less pronounced. TMP is a weak organic base that transitions from the cationic to the neutral form as pH increases (Figure 5b). As a result, its adsorbability increases with increasing pH. Even though the pH of the WWTP was higher than that of the two drinking water sources, TMP removal was lower in the WWTP. Thus, the WWTP contained organic matter that competed more strongly with the trace organic contaminants than the organic matter in the two drinking water sources.

### **Significance**

The goal of the proposed research is to evaluate two advanced wastewater treatment strategies (ozonation and activated carbon adsorption) that, when applied individually, are expected to provide (cost-)effective barriers against the release of BACs into North Carolina surface waters. Benefits of the proposed research include new information for NC utilities on the effectiveness and cost of advanced wastewater treatment processes for BAC removal. Incorporation of advanced treatment processes into NC WWTPs would lead to improved habitat for aquatic life and improved water quality for drinking water treatment plants that rely on surface water sources impacted by upstream WWTP discharges.

# Seasonal Streamflow Forecasts for the Hydrologic Unit Code (HUC-8) Basins in North Carolina utilizing Multimodel Climate Forecasts

## Basic Information

<b>Title:</b>	Seasonal Streamflow Forecasts for the Hydrologic Unit Code (HUC-8) Basins in North Carolina utilizing Multimodel Climate Forecasts
<b>Project Number:</b>	2009NC122B
<b>Start Date:</b>	3/1/2009
<b>End Date:</b>	2/28/2011
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	2
<b>Research Category:</b>	Climate and Hydrologic Processes
<b>Focus Category:</b>	Drought, Hydrology, Water Supply
<b>Descriptors:</b>	Hydroclimatology, Hydrological Forecasting, Climate, Multimodel combination, climate forecasts
<b>Principal Investigators:</b>	Sankarasubramanian Arumugam, Ryan Boyles

## Publications

There are no publications.

## Title

Seasonal Streamflow Forecasts for the Hydrologic Unit Code (HUC-8) Basins in North Carolina utilizing Multimodel Climate Forecasts

## Problem and Research Objectives

Despite the relative abundance of water in North Carolina (NC)<sup>11</sup>, increasing demand at major metropolitan areas make the local and regional water supply systems vulnerable to even moderate drought conditions<sup>12</sup>. For instance, in the Triangle Area in NC, the demand has grown by about 20%-62% from 1995-2000 resulting in three severe droughts/shortages (summers of 2002, 2005 and 2007) in the past five years. Reservoir systems in humid regions are typically designed as within-year storage systems with an intent to capture only the seasonal variability in streamflow, but do not carryover the deficit/surplus from year to year (e.g., reservoirs in the western US are designed for over-year). Thus, the low storage (typically expressed as % of annual streamflow volume) and increasing urban demand necessitate the importance of utilizing climate-information based streamflow forecasts to develop strategies for seasonal and multiseason water management. Unfortunately, the climate (precipitation and temperature) forecasts from General Circulation Models (GCMs) that are issued by the National Center for Environmental Prediction (NCEP) are available at large spatial scales (2.5°×2.5°) and do not provide streamflow forecasts.

Though rainfall in NC has been shown to have significant predictability to various climatic signals such as El-Nino Southern Oscillation and Atlantic Dipole<sup>13,14</sup> limited effort has been undertaken in developing seasonal streamflow forecasts<sup>15,16</sup>. It has been shown that the associations between the precipitation and large-scale climatic signals vary to a greater degree within the NC<sup>18</sup>. For instance, if El-Nino conditions exist in the tropical Pacific during the winter, coastal watersheds in NC experience increased precipitation, whereas the mountain watersheds in NC experience decreased precipitation<sup>18</sup>. One possibility for the difference in potential is due to orographic precipitation in the mountainous watersheds. To address this issue, it is important to downscale the large-scale precipitation from GCMs to local variability using either statistical or dynamical downscaling. Given the State water supply plan encourages development of local water supply plans<sup>19</sup>, it is important to develop seasonal streamflow forecasts customized at the hydrologic unit code level. In this study, **the investigators will downscale the large-scale climate forecasts issued by various national agencies and centers to develop seasonal streamflow forecasts at the 8-Digit Hydrologic Unit Code (HUC-8) basins to support various ongoing drought management activities in NC.**

Four specific objectives are encompassed in the proposed study:

**Objective 1:** Assemble precipitation forecasts from multiple General Circulation Models (GCMs) and Regional Climate Models (RCMs) available from various research and national agencies and analyze their ability to predict seasonal streamflow variability in HUC-8 basins.

**Objective 2:** Statistically downscale the precipitation forecasts available from GCMs and RCMs and optimally combine them to develop seasonal streamflow forecasts for the HUC-8 basins.

**Objective 3:** Update the streamflow forecasts every month during winter and summer seasons using the updated climate forecasts issued during the season from national centers.

**Objective 4:** Disseminate both retrospective and real-time streamflow forecasts developed for the winter and summer seasons through the NC CRONOS database.

The primary goal of this research is to develop seasonal streamflow forecasts and update them every month during the season over HUC-8 basins in NC. For this purpose, we plan to combine retrospective and real-time climate forecasts issued using multiple GCMs and downscale the multimodel climate forecasts to develop seasonal streamflow forecasts.

Uncertainties in the climate forecasts could arise either in projecting the boundary conditions, Sea Surface Temperature (SST), or from the atmospheric conditions. To reduce this uncertainty, predictions

from climate models are represented as ensembles or as scenarios of precipitation and temperature. Apart from these two sources of uncertainties, the most important uncertainty is the model uncertainty, which basically arises in the selection of Atmospheric GCMs. Recent studies have shown that combining climate forecasts from multiple atmospheric GCMs improve the seasonal climate forecasts<sup>2,17</sup>. In this study, we propose to combine precipitation forecasts from multiple GCMs using a new multi-combination technique developed in the project supported by NC WRRI. The developed multimodel combination algorithm basically evaluates the skill of GCMs contingent on the most influential predictor state<sup>13,17</sup>. Our efforts in utilizing the above algorithm in developing streamflow forecasts for the Falls Lake<sup>13</sup> during the summer season have suggested appropriate policies for invoking restrictions for water supply releases and to meet the end of the season storage during drought years<sup>16</sup>.

## **Methodology**

### ***Objective 1: Predictability of Seasonal Streamflow Using Climate Forecasts***

For Objective 1, we will obtain retrospective precipitation forecasts from various GCMs and RCMs available from NCEP, International Research Institute for Climate and Society (IRI) and other research institutes for the summer and winter seasons. Utilizing the streamflow and precipitation available from the NC CRONOS database for the HUC-8 basins, we will assess the skill of the precipitation forecasts in predicting the observed streamflow/precipitation during winter and summer seasons. The investigators will utilize various skill measures ranging from simple correlation to rigorous rank probability skill score for evaluating the skill of probabilistic forecasts. Given that regional climate models incorporate local topography and land-surface atmospheric interactions better, their ability to predict streamflow for basins receiving orographic rainfall (particularly for basins in the western NC) will be greatly enhanced. We will assess the skill of climate forecasts from GCMs and RegCM3 in predicting the streamflow variability in HUC-8 basins during the winter and summer seasons.

### ***Objective 2: Statistical Downscaling and Multimodel Combination***

This section details the statistical downscaling and multimodel combination methodologies employed in developing streamflow forecasts over the HUC-8 basins. Given that the climate forecasts from General Circulation Models (GCM) are available at large spatial scales ( $2.5^{\circ} \times 2.5^{\circ}$ ), three different approaches can be adopted to develop climate-information based streamflow forecasts: (a) couple GCM outputs with a Regional Climate Model (RCM) whose outputs could be combined with a large-scale watershed model<sup>1</sup> (b) statistically downscale GCM-predicted precipitation, which could be given as an input into a watershed model<sup>20</sup> and (c) develop a low dimensional statistical model that predicts the streamflow based on dominant climate predictors that influence the streamflow/rainfall potential over the basin<sup>13</sup>.

**Statistical Downscaling:** Under Objective 2, we will statistically downscale precipitation forecasts from RCMs (available at 20 Km X 20 Km) and GCMs to streamflow available at HUC-8 basins. For basins that are dominated by rainfall-runoff events, statistical downscaling of GCM/RCM precipitation forecasts is a better option (instead of using watershed models which requires downscaling to precipitation), since streamflow is a spatial integrator of precipitation<sup>20</sup>.

Various techniques have been reported for statistical downscaling that includes simple regression (model output statistics) models<sup>20</sup>, generalized linear regression models, nonparametric approaches<sup>3</sup>, and non-homogeneous hidden Markov models<sup>21</sup>. We will explore these downscaling methods for developing streamflow forecasts over the HUC basins.

**Multimodel Combination:** Using the streamflow forecasts downscaled from various GCMs and RCMs, we will investigate methodologies to combine them optimally to develop multimodel streamflow forecasts. Recent studies have shown that multi-model climate forecasts have better skill than single model forecasts<sup>2,20</sup>. The commonly employed strategy for developing multimodel ensembles is to give higher weights for a model that has better predictability (e.g., mean square error) or optimize the weights for each model in such a way that the developed multimodel ensembles will have improved predictability. In this study, we will employ a new approach<sup>13</sup> for developing multi-model ensembles by analyzing the model's predictability from the predictor state space, SST conditions. Under this approach, if the model

performs very poorly during La Nina conditions, then it will receive lesser weight during those conditions. Similarly, if all the models perform poorly during neutral ENSO conditions, then higher weights could be given for climatology.

### **Multimodel Combination Algorithm Conditioned on the Predictor State:**

Let us denote the streamflow forecasts available after downscaling as  $Q_{i,t}^m$ , where  $m=1,2,..,M$  denotes the downscaled forecasts from 'M' different models,  $i = 1,2, ..N_m$  represents ensembles of the streamflows with ' $N_m$ ' denoting the total number of ensembles under each model and  $t= 1, 2...T$  denotes years for which the seasonal forecasts,  $Q_{i,t}^m$ , available over a given HUC basin. We assume that the models have a common underlying predictor vector,  $\mathbf{X}_t$  (e.g., ENSO state), which influences the conditional distribution of climatic attributes that are represented using ensembles. Figure 1 provides a flow chart indicating the steps in implementing the proposed multi-model ensembling conditioned on the predictor state. Even if the models do not have a common predictor particularly in the context of GCM forecasts, one could use the leading principal component of the underlying boundary conditions (for instance SSTs) as the common predictor across all the models.

Developing multi-model ensembles based on optimal combination requires the observed climatic variable  $Y_t$  which could be used to get the skill of the probabilistic forecasts based on Rank Probability Score (RPS) to obtain weights  $w_t^m$ . Thus, the weights for each model will be obtained in a leave-one out cross validation by leaving out the observation in a particular year and using the rest of the years to estimate the weights. It is important to note that RPS is evaluated each year using the ensembles representing the conditional distribution. Let us denote the RPS of the probabilistic forecasts,  $Q_{i,t}^m$ , for each time step as  $RPS_t^m$ . RPS provides the total error between the cumulative distribution of categorical forecasts in comparison to the cumulative distribution of observed variable, which is assumed to be zero below the observed category and one beyond the observed category. Rank Probability Skill Score (RPSS) compares the skill of the RPS of the forecasts with the RPS of climatology. RPSS is similar to correlation with a value 1 indicating good skill and value lesser than zero indicating a poor forecast. Details on computation of RPS and RPSS are given in the investigators' paper<sup>13</sup>.

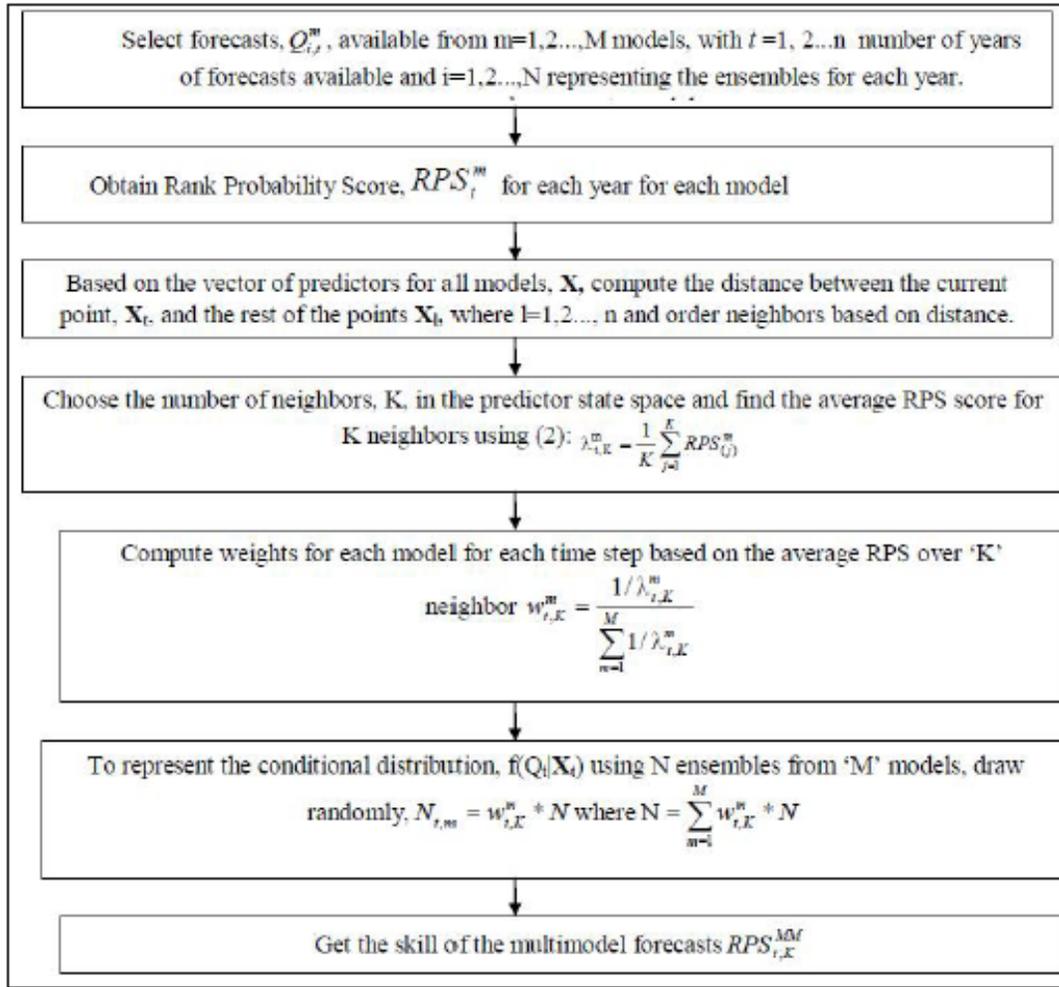


Figure 1: Multimodel combination algorithm proposed for combining downscaled streamflow forecasts (Adapted from Devineni et al., 2008<sup>13</sup>).

Our approach to assess the skill of the model is to look at its ability to predict under similar climatic conditions (denoted by the SSTs), which could be identified by choosing a distance metric that computes the distance between the current predictor state,  $\mathbf{X}_t$  and the historical predictor vector  $\mathbf{X}$ . One could use simple Euclidean distance or a more generalized distance measure such as Mahalanobis distance metric, which is more useful if the predictors' exhibit correlation among them. Compute the distances  $d_{it}$  between the current conditioning state  $\mathbf{X}_t$  and the historical predictor vector  $\mathbf{X}_l$  as

$$\mathbf{d}_{it} = \sqrt{(\mathbf{X}_t - \mathbf{X}_l)^T \hat{\Sigma}^{-1} (\mathbf{X}_t - \mathbf{X}_l)} \quad \dots (1)$$

where  $\hat{\Sigma}$  denotes a consistent estimate of the variance-covariance matrix of the historical predictor vector  $\mathbf{X}$ . Using the distance vector  $\mathbf{d}$ , identify the ordered set of nearest neighbor indices by  $\mathbf{J}$ . Thus,  $j^{\text{th}}$  element in the distance vector metric provides the  $j^{\text{th}}$  closest  $\mathbf{X}_l$  to the future state  $\mathbf{X}_t$ . Using this information, we assess the performance of each model in the predictor state space as,

$$\lambda_{t,K}^m = \frac{1}{K} \sum_{j=1}^K RPS_{(j)}^m \quad \dots (2)$$

where  $RPS_{(j)}$  denotes the skill of the downscaled streamflow forecasts for the year that represents the  $j^{\text{th}}$  closest condition (obtained from  $\mathcal{J}$ ) to the future condition  $\mathbf{X}_t$ . In other words,  $\lambda_{t,K}^m$  summarizes the average skill of the downscaled streamflow forecasts from model 'm', by choosing 'K' years that resemble very similar to the current condition,  $\mathbf{X}_t$ .

Using  $\lambda_{t,K}^m$  obtained for each model at each time step, we obtain the weights for multimodel combination so that models with better performance during a particular climatic conditions needs to be represented with more number of ensembles in comparison to a model with lower predictability under those conditions. It is important to note that RPS is a measure of error in predicting the probabilities and it is evaluated based on the entire ensembles. Next, we define the weights as,

$$w_{t,K}^m = \frac{1/\lambda_{t,K}^m}{\sum_{m=1}^M 1/\lambda_{t,K}^m} \quad \dots (3)$$

If  $\lambda_{t,K}^m$  is zero for few models  $l \leq M$ , then the weights  $w_{t,K}^m$  are distributed equally between the models for which  $\lambda_{t,K}^m$  is non-zero with the rest of models weights being equal to zero.

The multi-model climate change projections for each time step could be developed by drawing  $w_{t,K}^m * N_m$  ensembles from each model to constitute the multi-model ensembles. Thus, one has to specify the number of neighbors 'K' to implement this approach. It is also important to note that choosing fewer K does not imply that the multimodel forecasts are developed using the observed predictand and predictors based in the identified K similar conditions. In fact, the streamflow forecasts  $Q_{i,t}^m$ , are forecasts developed based on the observed values of the predictand and predictor over a particular training period. Thus, we use the weights,  $w_{t,K}^m$ , only to draw the ensembles from  $Q_{i,t}^m$ . The simplest approach for selecting number of neighbors is to find a fixed 'K' that provides improved skill under multimodel streamflow forecasts over the calibration period. The above algorithm was also employed has in improving winter precipitation over the US by combining precipitation forecasts from seven different GCMs<sup>17</sup>. The performance of multimodel ensembles will also be compared with individual model's skill using various verification measures such as average RPS, average RPSS and correlation during both winter and summer seasons. For HUC basins that receive streamflow from controlled releases, we will combine precipitation forecasts from GCMs to issue multimodel precipitation forecasts.

### **Objective 3: Updated Streamflow Potential during the Season**

It has been shown that updating the streamflow forecasts during the season improves the season forecasts and reduces the error in predicting the intraseasonal variability in streamflows<sup>20</sup>. As part of this work, we also plan to update the seasonal streamflow forecasts during both winter and summer seasons to quantify the updated streamflow potential during the seasons. The methodology to develop updated streamflow forecasts is the same as that of developing streamflow forecasts at the beginning of the season (Objective 2), but we will utilize the climate forecasts updated every month to develop multimodel streamflow forecasts for the remaining months in the season.

### **Objective 4: Disseminate Experimental Streamflow Forecasts with NC CRONOS server**

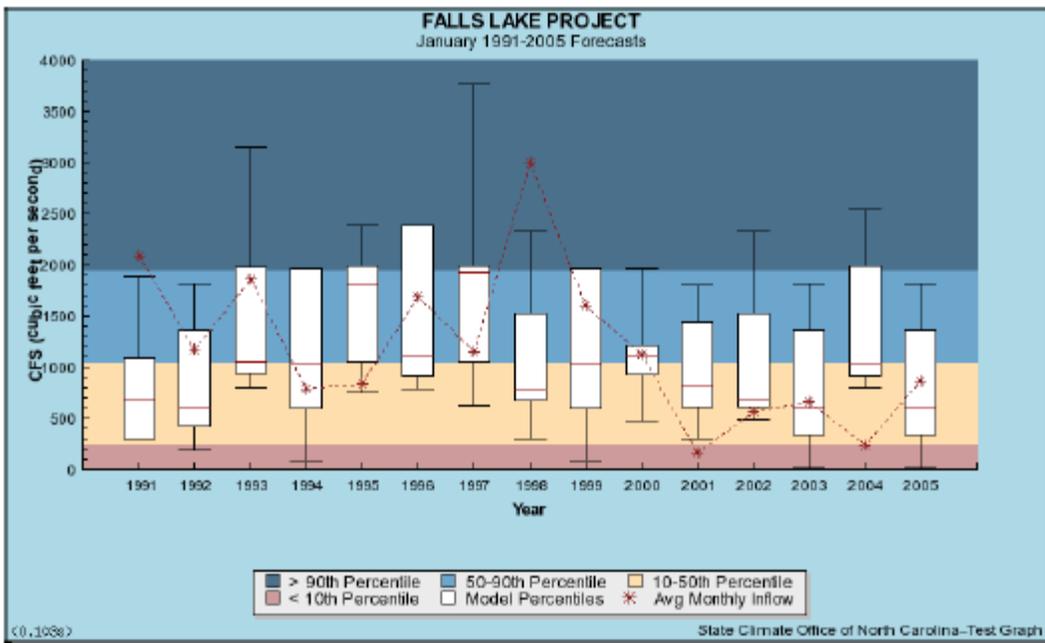
Regarding Objective 4, the proposal team will disseminate the developed forecasts through NC CRONOS database. CRONOS is the real-time and historical environmental database available through the State Climate Office's web site that provides both climate and hydrological observations for NC and

surrounding states. Data collected in this archive include quality-controlled observations from most state and federal monitoring networks, including NOAA, USDA, USGS, NC DENR, and the State Climate Office. Data from many of these networks are updated hourly, and historical observations for some locations are available for 100+ years. The water resource data archived on CRONOS is also mirrored at the DENR Division of Water Resources for their planning and monitoring application. These data and the products produced by them provide the basis for routine climate monitoring, especially during times of drought. On an experimental basis, we will provide real-time seasonal streamflow forecasts for the 2009 summer season and 2010 winter season. Both retrospective and real-time streamflow forecasts will be made available as tercile categories and as ensembles of streamflows which could be utilized for reservoir management. Educational modules and tutorials explaining tercile categories and skill measures will be made available at NC CRONOS website so that the public/agencies are aware of the basis behind the research product. Frequently asked questions from the public on forecast products will also be archived.

### **Principal Findings**

*Disseminate both retrospective and real-time streamflow forecasts developed for the winter and summer seasons through the NC CRONOS database.*

A nice web portal that can both upload and disseminate the streamflow forecasts from the NC-CRONOS database has been developed (<http://hatteras.meas.ncsu.edu/ajmcnama/>). The portal can display both retrospective forecasts and individual year forecasts along with detailed info on the forecast skill summary. A sample figure from the web page is displayed here.



Under individual year forecasts, the user has been provided with the option to download the forecast files under various file formats. Under retrospective forecasts, the skill of the forecasts are summarized using simple correlation, relative root mean square error, mean squared skill score and rank probability skill score. Details regarding the skill measures have also been provided in the website.

We plan to continue on this work in the summer by archiving the retrospective forecasts from multiple models as well as disseminating the forecasts for various basins in NC. One undergraduate student will be working in the summer to develop the forecasts. The other student, who worked on the project last summer, will complete the portal development so that it could be used to disseminate the real-time streamflow forecasts.

## **Significance**

One of the main goals of this research is to develop the capacity and the tools for realtime streamflow forecasting utilizing climate information in NC. The State Climate Office of NC is the nodal agency in disseminating the climate information throughout NC. By archiving the retrospective forecasts and disseminating the real-time climate information based streamflow forecasts for NC basins, we anticipate increased awareness in utilizing climate information for water sector decision making. All the developed modules and downscaling tools will be transferred to the State Climate Office of NC, so that real-time forecasting could be continued on a regular basis. Developed tools and modules will also be available in the state climate office website for public consumption. PIs will also submit new proposal to NOAA's Climate Prediction Program for Americas to continue this effort on a regular basis. The proposed research will also synergize with other ongoing research at NCSU funded by National Science Foundation on utilizing multi-time scale streamflow forecasts for improving water management as well as with other activities in the SCO-NC on climate information dissemination for the Southeast region.

## **References**

1. Wood, A.W., Kumar, A. and Lettenmaier, D.P., A retrospective assessment of National Centers for Environmental Prediction climate model-based ensemble hydrologic forecasting in the western United States, *Journal of Geophysical Research-Atmospheres* 110 (D4): Art. No. D04105, 2005.
2. Rajagopalan, B., U. Lall, and S. E. Zebiak, 2002: Categorical climate forecasts through regularization and optimal combination of multiple GCM ensembles, *Monthly Weather Review.*, 130, 1792–1811.
3. Gangopadhyay, S., M. Clark, and B. Rajagopalan (2005), Statistical downscaling using Knearest neighbors, *Water Resources Research*, 41, W02024, 1-23, 2005.
4. <http://www.srh.noaa.gov/alr/>
5. Shaake, J., and Larson, L., Ensemble Streamflow Prediction (ESP): Progress and Research needs, Special Symposium on Hydrology, Am.Meteorol., Soc., Boston, Mass, J19-J24, 1998.
6. Trenberth, K. E., C. J. Guillemot, Physical Processes involved in the 1988 Drought and 1993 Floods in North America. *Journal of Climate*, 9, 6, 1288–1298, 1996.
7. Cayan, D. R., K. T. Redmond, and L. G. Riddle, ENSO and Hydrologic Extremes in the Western United States. *J. Climate*, 12, 2881-2893, 1999.
8. Dettinger, M.D. and H.F. Diaz, Global characteristics of stream flow seasonality and variability, *Journal of Hydrometeorology*, 1 (4): 289-310, 2000.
9. Guetter, A. K., and K. P. Georgakakos, 1996: Are the El Niño and La Niña predictors of the Iowa River seasonal flow? *J. Appl. Meteor.*, 35, 690–705.
10. Piechota, T. C., and J. A. Dracup, 1996: Drought and regional hydrologic variation in the United States: Associations with the El Niño-Southern Oscillation. *Water Resour. Res.*, 32, 1359–1374.
11. Moreau, D.H., North Carolina's Abundant Water Resources: Supply, Use and Imbalances, WRRRI News letter, 355, May–June 2006.
12. Weaver, C.J., The Drought of 1998–2002 in North Carolina—Precipitation and Hydrologic Conditions, USGS Scientific Investigations Report, 2005.
13. Devineni, N., A. Sankarasubramanian, and S. Ghosh (2008), Multi-model Ensembling of Probabilistic Streamflow Forecasts: Role of Predictor State Space in Skill Evaluation, *Water*

*Resources Research*, 44, W09404, doi:10.1029/2006WR005855.

14. Roswintiarti, O., D.S.Niyogi, and S.Raman., Teleconnections between the tropical Pacific sea surface temperature anomalies and North Carolina precipitation anomalies during El Niño events, *Geophysical Research Letters*, 25, 4201-42, 1998.

15. Jamie R. Rhoads, Dev dutta S. Niyogi, and Sethu Raman, Mesoscale analysis of severe weather and ENSO interactions in North Carolina., *Geophysical Research Letters*, Vol. 27, pp 2269-2272, 2000.

16. Golembesky, K.,A. Sankarasubramanian, and N. Devineni, Improved Management of Falls Lake Reservoir during the Summer Season using Climate Information based Monthly Streamflow Forecasts: Role of Restrictions in Water supply and Water Quality Management, *Journal of Water Resources Planning and Management*, in press, 2008.

17. Devineni, N., and A. Sankarasubramanian (2008), Predictability of U.S. Winter Precipitation: Role of ENSO state in Developing Multimodel Combinations, Submitted to Monthly Weather Review.

18. <http://www.nc-climate.ncsu.edu/climate/enso.html>

19. [http://ncwater.org/Water\\_Supply\\_Planning/NC\\_Water\\_Supply\\_Plan/](http://ncwater.org/Water_Supply_Planning/NC_Water_Supply_Plan/)

20. Sankarasubramanian, A., U. Lall, and S. Espunova, Role of Retrospective Forecasts of GCMs Forced with Persisted SST anomalies in Operational Streamflow Forecasts Development, *Journal of Hydrometeorology*, 9(2), 212-227, 2008.

21. Robertson AW, Kirshner S, Smyth P. (2004), Downscaling of daily rainfall occurrence over northeast Brazil using a hidden Markov model. *Journal of Climate*, 17(22):4407-4424.

# Using PCR-based Methods to Assess Microbial Contamination from Swine CAFOs in Surface and Groundwaters

## Basic Information

<b>Title:</b>	Using PCR-based Methods to Assess Microbial Contamination from Swine CAFOs in Surface and Groundwaters
<b>Project Number:</b>	2009NC123B
<b>Start Date:</b>	3/1/2009
<b>End Date:</b>	2/28/2011
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	7
<b>Research Category:</b>	Ground-water Flow and Transport
<b>Focus Category:</b>	Groundwater, Surface Water, Water Quality
<b>Descriptors:</b>	Swine manure, CAFO, Microbial source tracking, Quantitative PCR, Bacteroides
<b>Principal Investigators:</b>	Bongkeun Song, Lawrence B. Cahoon, Michael A. Mallin

## Publication

1. Arfken, A., M.I. Haltom, M.A. Mallin and B Song, 2010, Molecular Detection and Quantification of Swine Fecal Contamination in the Northeast Cape Fear River and Black River, Water Resources Research Institute Annual Conference, Raleigh, NC, March 30-31, 2010.

## Title

Using PCR-based methods to assess microbial contamination from swine CAFOs in surface and groundwaters

## Problem and Research Objectives

Large scale industrialized swine production is rapidly increasing in the United States. In North Carolina, swine inventory rose from 2.7 million in 1990 to over 9 million head in 1997 (Mallin 2000). North Carolina's 2300 concentrated animal feeding operations (CAFOs: Figure 1.A.) generate large quantities



B



Figure 1. Map of hog farms in Eastern North Carolina River Basin (A) and an example of hog lagoon operation system (B).

(annually >15 million metric tons statewide) of pig manure slurry (PMS), a mixture of pig feces and urine that is pumped or drained from massive hog houses into outdoor holding ponds called lagoons (Figure 1.B.). This material is a highly concentrated source of nitrogen, phosphorus, and fecal bacteria, viruses and protozoans, some of which are pathogenic (Mallin and Cahoon 2003). When the lagoon reaches a certain level the liquid supernatant is sprayed onto surrounding fields, where some nutrients are absorbed by soils or crops, and some of the microbes are killed by UV radiation or protozoan predation. These spray fields are subject to hurricanes and other major storms that spread fecal contamination and nutrient pollution to nearby and downstream water bodies (Mallin 2000). Additionally, normal rainfall and stormwater runoff, as well as subsurface

movement of microbes, causes microbial contamination of watersheds that leads to the spread of pathogenic microorganisms, which have become one of the major public health concerns (Cho and Kim, 2000; Krapac et al., 2002).

Pollution from CAFOs during and after large storms has been well documented in North Carolina (Mallin 2000 and references within). Chronic runoff and subsurface movement from sprayfields also occurs that contributes to water quality degradation in North Carolina rivers, estuaries, and groundwater supplies, but no definitive investigation has been conducted to identify swine manure contamination as a source of chronic microbial pollution in watersheds. Thus, the investigators will examine the levels of swine manure contamination in waterways of hog farm areas by detecting and quantifying the 16S rRNA genes of hog specific *Bacteroides-Prevotella*. This project will provide the first assessment of microbial water quality impacts traceable specifically to swine waste operations in NC watersheds.

The goal of this research is to determine the water quality and health-related impacts of swine manure waste in the streams, rivers and groundwaters of North Carolina. Our specific objectives are:

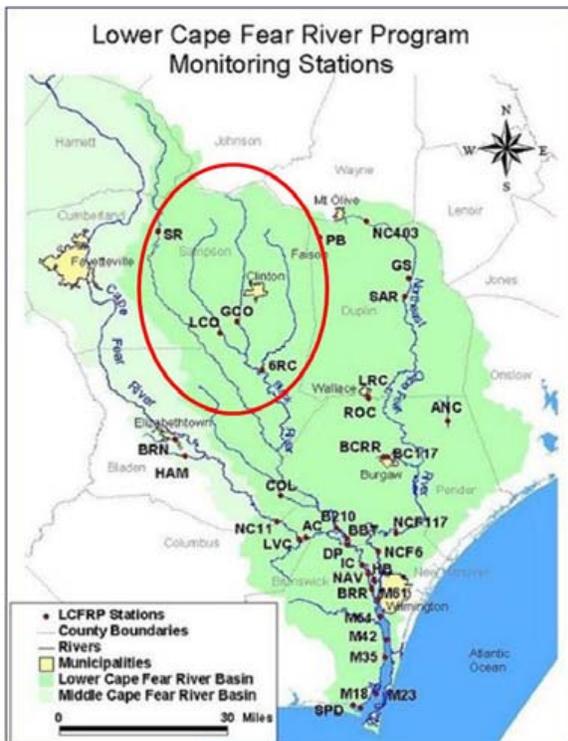
- 1) To enumerate fecal indicator bacteria from water samples using conventional methods.

- 2) To detect swine manure contamination in water samples using PCR-based methods specific for the 16S rRNA genes of *Bacteroides-Prevotella* living in swine.
- 3) To quantify the levels of swine manure contamination in water using Q-PCR of the hog specific *Bacteroides-Prevotella* 16S rRNA genes.
- 4) To monitor spatial and temporal variation of contamination levels in different locations.
- 5) To measure water quality parameters such as ammonia, nitrate, total nitrogen, orthophosphate, and total phosphorous, etc., and correlate concentrations of these parameters with swine manure contamination in surface and groundwater of CAFO-impacted watersheds.

## Methodology

### 1. Sampling sites

Bimonthly sampling will be conducted in the lower Cape Fear River watershed, which contains the largest concentration of swine CAFOs in North Carolina (approximately 5,000,000 head (Cahoon et al., 1999)). The investigators will have the benefit of cost sharing with the Lower Cape Fear River Program, which conducts a regular monthly, State-certified sampling program at 35 locations within including streams, rivers, and the estuary.



Sites to be sample will include streams in CAFO-rich areas [Six Runs Creek (6RC), Great Coharie Creek (GCO), Little Coharie Creek (LCC), Goshen Swamp (GS), the upper Northeast Cape Fear River at Sarecta (SAR)], non-swine areas below point source dischargers as “other pollution” controls such as sites below industrial and municipal discharges (Panther Branch (PB), lower Burgaw Creek(BC117)), and pristine stream sites (Colly Creek(COL)). Liquid samples of suspended PMS will be also collected from hog lagoon systems near the sampling stations to compare the *Bacteroides-Prevotella* communities. In addition, at least 10 groundwater samples in Sampson County will be examined to determine swine manure contamination in potential drinking water sources. In collaboration with community groups, Dr. Steve Wing of the Department of Epidemiology at UNC-Chapel Hill will provide water samples from drinking wells in communities where he has been engaged in epidemiological studies for many years (see attached support letter). Such samples will be collected using strict protocols and chain-of-custody procedures.

### 2. Enumeration of Coliform bacteria

Samples from surface and groundwater will be analyzed for fecal coliform bacteria concentrations using standard methods (membrane filtration method (mFC). The water samples (100 ml and 10 ml duplicates each) will be filtered through a sterile 0.45µm-pore-size mixed cellulose ester filter, which will be placed on m-FC medium broth at 44.5°C for 24 hr. The blue colonies on the filters will be counted as fecal coliforms (Method 9222-D, APHA 1995). *Enterococcus* bacteria will be enumerated as well using Method 9230-C.

### 3. Detection and quantification of swine manure contamination.

For molecular analysis, one liter of water samples will be filtered through 0.2 µm-poresize membrane filters. The filters will be stored at -80 °C until analyzed. The investigators will extract DNA from the filters using a Power Soil DNA extraction kit (Mo Bio laboratories) and conduct two different molecular

applications to detect and quantify the 16S rRNA genes of the pig-specific *Bacteroides-Prevotella* group as indicators for PMS contamination in the watershed.

**T-RFLP analysis:** T-RFLP is a molecular technique to examine microbial community structures by comparing DNA fingerprint profiles. The profiles are generated by digesting a PCR-amplified product of a target gene using one or more restriction enzymes. The PCR amplification is conducted with a fluorescent labeled primer and a non-labeled primer, which generates a mixture of amplicon with a fluorescent label at one end. The 16S rRNA genes of the *Bacteroides-Prevotella* group will be amplified with the primers Bac32F and Bac708R, which are specific for the detection of the general *Bacteroides-Prevotella* group (Bernhard & Field, 2006a,b). The amplicons will be purified and digested with one of the selected restriction enzymes. The different sizes of fragments will be separated through an ABI2100 gene analyzer (Applied Biosystems), which will provide the profiles of fragments based on fragment lengths. The investigators will look for the T-RFs with 149, 238 and 240 bps, which are the specific T-RFs corresponding to the pig-specific *Prevotella* group. The investigators found initial evidence of PMS contamination in the Black River using this T-RFLP analysis. The samples obtained from 6RC and B210 sites had T-RFs with sizes of 149, 238 and 240 bp, which showed the presence of pig-specific *Prevotella* group in the Black river water. However, the station SAR located in the Northeast Cape Fear River was negative for the PMS contamination based on the TRFLP analysis. **Thus, the investigators can rapidly monitor and detect the PMS contamination in a large number of samples during the bimonthly sampling efforts.**

**Quantitative PCR assays:** Q-PCR is a PCR method to detect and quantify the increase of PCR products using fluorescent dye and a real-time PCR machine (Heid et al., 1996). Two different methods (SYBR Green and Taqman) can be used to determine the increase of PCR products. In the SYBR Green assay, PCR product formation is quantitatively monitored by determining the increase in fluorescence after binding a fluorescent DNA stain (SYBR Green) to the amplicon (Higuchi et al., 1991). SYBR Green Q-PCR requires a set of gene-specific primers, which will be used to amplify a 100-150 bp fragment of the gene. SYBR Green binds all double-stranded DNA molecules, therefore real-time PCR amplification that is monitored with SYBR Green must be optimized so that the primer-dimer formation and non-specific amplification do not occur. Because the melting curve of a product is dependent on its GC content, length, and sequence, specific amplification can be distinguished from non-specific amplification by examining the melting curve of the PCR products (Ririe et al., 1997). Specific PCR primers for pig-*Bacteroides-Prevotella* were previously developed and used to detect PMS contamination in freshwater rivers (Okabe et al., 2007). The investigators tested the primers with the samples used for T-RFLP analysis above. The investigators detected the presence of pig-specific *Bacteroides-Prevotella* group at Six Runs Creek only (draining a watershed with 153 CAFOs) located in Sampson County, which showed the high specificity of the primers detecting the pig-*Bacteroides-Prevotella* 16S rRNA genes. SYBR Green Q-PCR was developed to quantify the pig-*Bacteroides-Prevotella* 16S RNA genes in water samples (Okabe et al., 2007; Okabet & Shimazu, 2007). The investigators will optimize the QPCR protocol and conduct the quantification assays with the water samples collected from the sites described above. The investigators will also examine seasonal variation of the PMS contamination with samples collected bimonthly over a year. The Q-PCR assays will provide the copy number of the pig-*Bacteroides-Prevotella* 16S rRNA genes in each sample. The investigators will normalize the copy number per total concentration of DNA to compare the levels of PMS contamination at different locations. **Based on the quantification of the pig-*Bacteroides-Prevotella* 16S rRNA genes, the investigators will estimate the levels of PMS contamination in surface water and groundwater around hog lagoon areas as well as downstream of the rivers in the Cape Fear River watershed.**

#### 4. Measurement of water quality

The strongest signals of swine CAFO pollution, in addition to fecal microbes, are provided by elevated nutrient levels. Samples will be analyzed using EPA and APHA State-certified techniques for ammonia, nitrate, TN, orthophosphate, and TP. For many of the surface sites the investigators will have the benefit of cost-sharing by the Lower Cape Fear River Program to reduce chemical analyses costs. For surface water samples the investigators will also have physical water quality data including water temperature, pH, dissolved oxygen, conductivity and turbidity. Data from sampling locations in the lower Cape Fear River watershed dating back to 1995 can be viewed at: [www.uncwil.edu/cmsr/aquaticceology/LCFRP/](http://www.uncwil.edu/cmsr/aquaticceology/LCFRP/).

Data analyses will include evaluation of quantitative measures of each nutrient metric as well as nutrient ratios; fecal wastes typically have lower N:P ratios than unimpacted environmental samples.

#### *5. Correlation between swine manure contamination and water quality in NC watersheds*

Once the investigators have obtained the nutrient and physical water quality data, fecal coliform counts, and data on detection and quantification of pig-specific *Bacteroides-Prevotella* 16S rRNA genes from each sample, the investigators will conduct correlation analyses, linear regression and principal component analysis (PCA) to determine statistical relationships among swine manure microbial contamination metrics and water quality parameters at watershed scales. Depending on the levels of correlation among the water quality parameters, the investigators might be able to suggest which specific parameter or parameter combinations can be used as a proxy of PMS contamination in watersheds. The investigators will also examine the correlation between the levels of nutrients and the copy numbers of the 16S rRNA genes, which will determine if nutrients are originated from the hog lagoon systems.

#### **Update:**

In order to monitor hog-specific fecal contamination in the Cape Fear River watershed, the investigators are employing two molecular techniques: T-RFLP (Terminal Restriction Fragment Length Polymorphism) and qPCR (Quantitative Polymerase Chain Reaction) of 16S rRNA genes in *Bacteroides-Prevotella*. T-RFLP is a DNA fingerprinting method, which is used to compare the structural similarities of *Bacteroides-Prevotella* communities in hog lagoon wastes and water samples collected from 9 stations in two watersheds; the Black River and the Northeast Cape Fear River. The qPCR technique is used to quantify the levels of hog manure contamination in each water sample based on the copy numbers of hog specific *Bacteroides-Prevotella* 16S rRNA genes. In addition, fecal coliform bacteria counts and nutrient analyses have been conducted along with the molecular analysis.

#### **Principal Findings**

The investigators conducted bimonthly sampling from 5 sites (LCO, GCO, 6RC, B210 and COL) in the Black River watershed and 4 locations (PB, GS, SAR and BC117) in the Northeast Cape Fear River watershed. The LCO, GCO, 6RC, PB, GS and SAR sites are considered as potential contamination sites since many swine CAFOs are concentrated near the sampling stations (Table 1). Station B210 is located in the Black River proper well downstream of hog farm concentrated areas, but integrates inputs from several tributaries rich in swine CAFOs. Some sites, including BC117 and PB contain point-source municipal or industrial wastewater discharges that frequently impact water quality at our sampling sites. The COL site is a pristine control due to its location neither upstream nor downstream from major swine CAFOs and industrial or municipal areas. In addition, waste samples from several hog lagoons in both watersheds were collected as controls for swine manure contamination and to test molecular analysis of hog specific *Bacteroides-Prevotella* 16S rDNA.

Based on T-RFLP and qPCR analyses, temporal variation of hog lagoon contamination in both watersheds was observed in 2009. The highest level of hog lagoon contamination was detected in the PB site on May while the second highest was at 6RC on September, and SAR on July based on qPCR assay (Figure 2). PB is the northernmost monitoring site of the Northeast Cape Fear River watershed (containing an industrial waste water discharge as well as 13 swine CAFOs, one located close to our sampling station), and 6RC and SAR are non-point source areas located downstream of highly concentrated hog farm areas (Table 1). The abundance of hog specific *Bacteroides-Prevotella* 16S rDNA was generally higher in Sites LCO, GCO, 6RC, PB, GS and SAR compared to B210, located in a fifth-order river where there is considerable dilution (Figure 2). Interestingly, the BC117 site on Burgaw Creek, which feeds into the Northeast Cape Fear River had substantial levels of hog lagoon contamination, even though it is heavily influenced by the Town of Burgaw Wastewater Treatment Plant discharge and has only four CAFOs in the watershed (Table 1). T-RFLP analysis showed similar community structures of *Bacteroides-Prevotella* groups in lagoon waste contaminated sites. The community structure of PB in May

shared high similarities with those examined from LCO, GCO, 6RC, GS, SAR and BC117. There was no detection of hog lagoon contamination at the pristine control COL site (Figure 2).

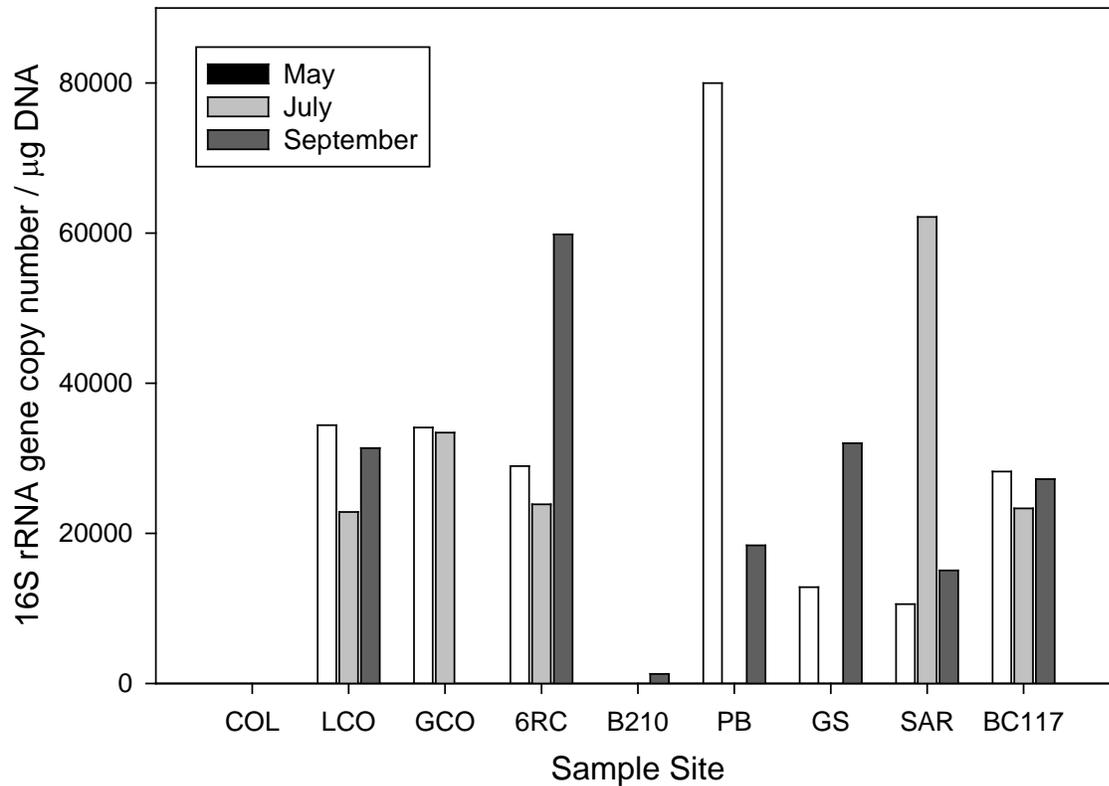
The point source areas, PB and BC117, were the stations most frequently exceeding the North Carolina fecal coliform water contact standard (Table 1), sometimes quite substantially. Although heavily influenced by point source discharges, these sites nonetheless experienced fecal contamination from swine CAFOs, each on at least two occasions when their counts exceeded the standard. One non-point area, SAR in the upper Northeast Cape Fear River, exceeded the standard on three of six occasions, and showed swine fecal source contamination on the two sampling occasions when the largest fecal coliform counts occurred, May and September. The remaining stations either exceeded the standard once or not at all (Table 1), and several (LCO, GCO, 6RC and GS) showed frequent swine fecal influence.

Table 1. Fecal coliform bacteria counts (CFU/100 mL) at test sites in the Black and northeast Cape Fear River watersheds, 2009; also, the approximate number of swine CAFOs in the watershed is presented. **Bolded** means the contact standard of 200 CFU/100 mL was exceeded.

Sites	Month					# of CAFOs
	March	May	July	September	November	
Black River watershed						
LCO	<b>257</b>	46	10	19	55	63
GCO	17	37	55	46	136	95
6RC	<b>1,140</b>	46	19	127	109	153
COL	19	86	46	na	109	6
Northeast Cape Fear watershed						
B210	28	10	118	64	172	300+
SAR	64	<b>450</b>	91	<b>728</b>	<b>310</b>	na
GS	19	195	<b>1,546</b>	118	46	119
PB	46	<b>546</b>	<b>290</b>	<b>3,700</b>	<b>580</b>	13
BC117	37	<b>10,000</b>	<b>530</b>	<b>1,546</b>	<b>410</b>	4

na – not available

Figure 2. Quantitative PCR of hog specific *Bacteroides-Prevotella* 16S rDNA in water samples collected from 9 stations in May, July and September of 2009. Abundance of gene copy number is normalized by  $\mu\text{g}$  of total DNA extracted from filters.



### **Significance**

Frequent and widespread contamination by waterborne pathogens in coastal and inland water resources is a major aspect of pollution in North Carolina. Both human and animal fecal pollution contribute to waterborne diseases. Point and non-point sources of contamination are typically monitored based on cultivation and enumeration of fecal indicator bacteria (*Escherichia coli*, fecal coliform bacteria, fecal enterococci). However, the origin of fecal contamination cannot be identified using these count methods, which hinders proper assessment and remediation of bacterial contamination in multiple-use watersheds. Alternative monitoring methods have been developed by detecting and enumerating fecal anaerobes such as *Bacteroides*, *Bifidobacterium* and *Clostridium* (see the review: Savichtcheva and Okabe, 2006).

These new methods are based on molecular techniques with the use of polymerase chain reaction (PCR) to evaluate the presence and absence of the indicator bacteria with host-specific PCR primers. In addition, real-time PCR has been applied to enumerate the number of target organisms in water samples without cultivation efforts. Thus, molecular methods have been used to detect and quantify the levels of fecal contamination from different host sources, such as human, cow and pig.

Swine manure stored in lagoons or spread on crop fields can be a source of fecal contamination and significant water quality degradation in adjacent watersheds. Estimating the scales of swine manure contamination in streams, rivers and groundwater will allow

development of proper and effective efforts to manage and remediate water quality in NC. Thus, this proposed research will generate the following benefits:

- 1) Defining levels of swine manure contamination in surface and groundwater in areas near or downstream of swine CAFOs.
- 2) Providing potential health risk assessment by quantifying microbial contamination.
- 3) Estimating spatial and temporal scales of contamination related to CAFO operations.
- 4) Identifying improper management of swine waste operation systems in hog farms.
- 5) Evaluating effectiveness of current swine waste Best Management Practices (BMPs).
- 6) Providing science-based advice for waste management and remediation to enhance water quality of NC watersheds.

## **References**

- APHA (1998) *Standard methods for the examination of water and wastewater*. 20th Ed. American Public Health Association, Washington, D.C.
- Bernhard AE, Field KG (2000a) Identification of nonpoint sources of fecal pollution in coastal waters by using host specific 16S ribosomal DNA genetic markers from fecal anaerobes. *Appl Environ Microbiol* 66: 1587–1594.
- Bernhard AE, Field KG (2000b) A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides Prevotella* genes encoding 16S rRNA. *Appl Environ Microbiol* 66: 4571–4574.
- Boehm AB, Fuhrman JA, Mrse RD, Grant SB (2003) Tiered approach for identification of a human fecal pollution source at a recreational beach: case study at Avalon Bay, Catalina Island, California. *Environ Sci Technol* 37: 673–680.
- Burkholder JM, Mallin MA, Glasgow HB Jr, Larsen LM, McIver MR, Shank GC, Deamer-Melia N, Briley DS, Springer J, Touchette BW, Hannon EK (1997) Impacts to a coastal river and estuary from rupture of a swine waste holding lagoon. *J Environ Qual* 26: 1451–1466.
- Cahoon LB, Mikucki JA, Mallin MA (1999) Nitrogen and phosphorus imports to the Cape Fear and Neuse River basins to support intensive livestock production. *Env Sci Tech* 33:410-415
- Cho JC, Kim SJ (2000) Increase in bacterial community diversity in subsurface aquifers receiving livestock wastewater input. *Appl Environ Microbiol* 66: 956-965.
- Cotta MA, Whitehead TR, Zeltwanger RL (2003) Isolation, characterization and comparison of bacteria from swine faeces and manure storage pits. *Environ Microbiol* 5:737-745.
- Crane SR, Moore JA, Grismer ME, Miner JR (1983) Bacterial pollution from agricultural sources: A review. *Trans Amer Soc Agricul Engin* 26: 858–872.
- Daly K, Stewart CS, Flint HJ, Shirazi-Beechey SP (2001) Bacterial diversity within the equine large intestine as revealed by molecular analysis of cloned 16S rRNA genes. *FEMS Microbiol Ecol* 38:141–151.
- Davies CM, Long JAH, Donald M, Ashbolt NJ (1995) Survival of fecal microorganisms in marine and freshwater sediments. *Appl Environ Microbiol* 61: 1888–1896.
- Desmarais TR, Solo-Gabriele HM, Palmer CJ (2002) Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. *Appl Environ Microbiol* 68:1165–1172.
- Dick LK, Bernhard AE, Brodeur TJ, Santo Domingo JW, Simpson JM, Walters SP, Field KG (2005a) Host distributions of uncultivated fecal *Bacteroides* bacteria reveal genetic markers for fecal source identification. *Appl Environ Microbiol* 71: 3184–3191.
- Dick LK, Simonich MT, Field KG (2005b) Microplate subtractive hybridization to enrich for *Bacteroidales* genetic markers for fecal source identification. *Appl Environ Microbiol* 71: 3179–3183.
- Edwards DR, Daniel TC (1992). Environmental impacts of on-farm poultry waste disposal—a review. *Bioresource Tech* 41: 9–33.
- Evans RO, Westerman PW, Overcash MR (1984) Subsurface drainage water quality from land application of swine lagoon effluent. *Trans Ameri Soc Agricul Engin* 27: 473–480.
- Field KG, Bernhard AE, Brodeur TJ (2003) Molecular approaches to microbiological monitoring: fecal source detection. *Environ Monit Assess* 81: 313–326.

Gilliam JW, Huffman RL, Daniels RB, Buffington DE, Morey AE, Leclerc SA (1996) Contamination of surficial aquifers with nitrogen applied to agricultural land. Report No. 306. Water Resources Research Institute of the University of North Carolina, Raleigh, N.C.

Heid CA, Stevens J, Livak KJ, Williams PM (1996) Real time quantitative PCR. *Genome Methods* 6: 986–994.

Higuchi R, Dollinger G, Walsh PS, Gelfand DH (1991) Simultaneous amplification and detection of specific DNA sequences. *BioTechnol* 10: 413.

Hold GL, Pryde SE, Russell VJ, Furrie E, Flint HJ (2002) Assessment of microbial diversity in human colonic samples by 16S rDNA sequence analysis. *FEMS Microbiol Ecol* 39:33–39

Huffman RL, Westerman PW (1995) Estimated seepage losses from established swine waste lagoons in the lower coastal plain of North Carolina. *Trans Ameri Soc Agricul Engin* 38: 449–453.

Iannotti EL, Fischer JR, Sievers DM (1982) Characterization of bacteria from swine manure digester. *Appl Environ Microbiol* 43:136-143.

Krapac IG, Dey WS, Roy WR, Smyth CA, Stormont E, Sargent SL, Steele JD (2002) Impacts of swine manure pits on groundwater quality. *Environ Pollut* 120: 475-492.

Leser TD, Amenuvor JZ, Jensen TK, Lindecrona RH, Boye M, Moller K (2002) Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. *Appl Environ Microbiol* 68: 673–690.

Mallin MA (2000) Impacts of industrial animal production on rivers and estuaries. *Ameri Sci* 88: 2-13.

Mallin MA, Cahoon LB (2003) Industrialized animal production – a major source of nutrient and microbial pollution to aquatic ecosystems. *Popul Environ* 24: 369-385.

Mallin MA, Burkholder JM, McIver MR, Shank GC, Glasgow HB Jr, Touchette BW, Springer J (1997) Comparative effects of poultry and swine waste lagoon spills on the quality of receiving stream waters. *J Environ Qual* 26: 1622–1631.

Matsuki T, Watanabe K, Fujimoto J, Miyamoto Y, Takada T, Matsumoto K, Oyaizu H, Tanaka R (2002) Development of 16S rRNA-gene-targeted group-specific primers for the detection and identification of predominant bacteria in human feces. *Appl Environ Microbiol* 68: 5445–5451.

Mawdsley JL, Bardgett RD, Merry RJ, Pain BF, Theodorou MK (1995) Pathogens in livestock waste, their potential for movement through soil and environmental pollution. *Appl Soil Ecol* 2: 1–15.

Meays CL, Broersma K, Nordin R, Mazumder A (2004) Source tracking fecal bacteria in water: a critical review of current methods. *J Environ Manag* 73: 71-79.

Okabe S, Okayama N, Savichtcheva O, Ito T (2007) Quantification of host-specific *Bacteroides-Prevotella* 16S rRNA genetic markers for assessment of fecal pollution in freshwater. *Appl Microbiol Biotechnol* 74: 890-901.

Okabe S, Shimazu Y (2007) Persistence of host-specific *Bacteroides-Prevotella* 16S rRNA genetic markers in environmental waters: effects of temperature and salinity. *Appl Microbiol Biotechnol* 76: 935-944.

Peu P, Brugere H, Pourcher A-M, Kerouredan M, Godon J-J, Delgenes J-P, Dabert P (2006) Dynamics of a pig slurry microbial community during anaerobic storage and management. *Appl Environ Microbiol* 72: 3578-3585.

Ririe KM, Rasmussen RP, Wittwer CT (1997) Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. *Anal Bioch* 245:154-160.

Santoro AE, Boehm AB (2007). Frequent occurrence of the human-specific *Bacteroides* fecal marker at an open coast marine beach: relationship to waves, tides and traditional indicators. *Environ Microbiol* 9: 2038-2049.

Savichtcheva O, Okabe S (2006) Alternative indicators of fecal pollution: relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. *Water Res* 40: 2463-2476.

Scott TM, Rose JB, Jenkins TM, Farrah SR, Lukasiak J (2002) Microbial source tracking: Current methodology and future directions. *Appl Environ Microbiol* 68:5796–5803.

Seurinck S, Deforidit T, Verstraete W, Siciliano SD (2004) Detection and quantification of the human-specific HF183 *Bacteroides* 16S rRNA genetic marker with real-time PCR for

assessment of human faecal pollution in freshwater. *Environ Microbiol* 7: 249–259

Shanks OC, Atikovic E, Blackwood D, Lu J, Noble RT, Santo Domingo J, Seifring S, Sivaganesan M, Haugland RA (2008) Quantitative PCR for detection and enumeration of genetic markers of bovine fecal pollution. *Appl Environ Microbiol* 74: 745–752.

Simpson JM, Santo Domingo JW, Reasoner DJ (2002) Microbial source tracking: state of the science. *Environ Sci Technol* 24: 279–5288.

Snell-Castro R, Godon J-J, Delgenès J-P, Dabert P (2005) Characterisation of the microbial diversity in a pig manure storage pit using small subunit rDNA sequence analysis. *FEMS Microbiol Ecol* 52:229-242.

Solo-Gabriele HM, Wolfert MA, Desmarais TR, Palmer CJ (2000) Sources of Escherichia coli in a coastal subtropical environment. *Appl Environ Microbiol* 66: 230–237.

Stone KC, Hunt PG, Coffey SW, Matheny TA (1995) Water quality status of a USDA water quality demonstration project in the Eastern Coastal Plain. *J Soil Water Conser* 50: 567–571.

Tajtakova M, Semanova Z, Tomkova Z, Szokeova E, Majoroa J, Radikova Z, Sebokova E, Klimes I, Langer P (2006) Increased thyroid volume and frequency of thyroid disorders signs in schoolchildren from nitrate polluted area. *Chemosphere* 62: 559–564.

Ward MH, deKok TM, Levallois P, Brender J, Gulis G, Nolan BT, VanDerslice J (2005) Workgroup report: drinking-water nitrate and health—recent findings and research needs. *Environ Health Perspect* 113:1607–1614.

Westerman PW, King LD, Burns JC, Cummings GA, Overcash MR (1987) Swine manure and lagoon effluent applied to a temperate forage mixture: II. Rainfall runoff and soil chemical properties. *J Environ Qual* 16: 106–112.

Whitehead TR, Cotta MA (2001) Characterisation and comparison of microbial populations in swine faeces and manure storage pits by 16S rDNA gene sequence analyses. *Anaerobe* 7:181-187.

## **Information Transfer Program Introduction**

The Water Resources Research Institute (WRI) is heavily geared to providing water resources information to the water professional. These professionals are typically with federal, state and local government, industry, agriculture, consulting groups, and environmental groups, or are private entrepreneurs. The main forms of information transfer are through an Institute internet site, bi-monthly newsletter, conferences, seminars, forums, workshops and research publications.

WRI 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 WRI to offer Professional Development Hours (PDHs) and contact hours for attendance at the WRI Annual Conference and other workshops, seminars and forums that WRI sponsors.

# NC WRI Information Technology Transfer

## Basic Information

<b>Title:</b>	NC WRI Information Technology Transfer
<b>Project Number:</b>	2009NC126B
<b>Start Date:</b>	3/1/2009
<b>End Date:</b>	2/28/2010
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	2
<b>Research Category:</b>	Not Applicable
<b>Focus Category:</b>	None, None, None
<b>Descriptors:</b>	
<b>Principal Investigators:</b>	Kelly A Porter

## Publications

There are no publications.

## Information Transfer Program

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

## WRRRI Annual Conference

The WRRRI Information Transfer Program includes the WRRRI Annual Conference, which the institute has done since 1998. It is the state's premier water research conference where research is presented by university and corporate researchers, students, local, state, and federal government agency representatives, and environmental professionals. After trying something new by having the WRRRI Annual Conference in the fall of 2008 (October 8-9, 2008), it was decided to move the Annual Conference back to the spring of the year which means for this reporting cycle, an Annual Conference did not take place. The next annual conference took place on March 30-31, 2010, which will be reported on in the 2010 Annual Technical Report.

## Newsletter

Published the *WRRRI News* five times during the reporting period. The WRRRI 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 WRRRI News is now sent electronically to 850 federal and state agencies, university personnel, multi-county planning regions, city and local officials, environmental groups, consultants, businesses and individuals. It is also posted on the WRRRI website: <http://www.ncsu.edu/wrri/newsletter/>

## Internet Services

The Water Resources Research Institute of The University of North Carolina maintains an internet site: <http://www.ncsu.edu/wrri>. The purposes of the internet site are:

- to provide wider dissemination of research results, such as WRRRI research reports (<http://www.ncsu.edu/wrri/reports/index.html>), and information produced for our newsletter ( <http://www.ncsu.edu/wrri/newsletter/>),
- to provide researchers quick and easy access to proposal and project related materials (proposal guidelines and forms, guidelines for authors, etc), and
- to provide links to other water-related organizations that might be of use to the research and management communities in North Carolina.
- to provide timely information on workshops, conferences, seminars and other educational opportunities, and presentations from WRRRI sponsored events:
  - WRRRI Annual Conference Programs: <http://www.ncsu.edu/wrri/conference/past.html>
  - Erosion and Sedimentation Control Planning and Design Workshops: [http://www.ncsu.edu/wrri/events/esc\\_workshops/pastescworkshops.html](http://www.ncsu.edu/wrri/events/esc_workshops/pastescworkshops.html)
  - Local Programs: <http://www.ncsu.edu/wrri/events/localprograms/index.html>
  - North Carolina Water Resources Association Forums: <http://www.ncsu.edu/wrri/events/ncwra/pastforums.html>
  - Progress Energy Water Resources Seminars: <http://www.ncsu.edu/wrri/events/progressenergyseminars/pastforums.html>

### **WRRI Electronic Lists**

WRRI maintains the following electronic mail lists (listservs) for information transfer purposes:

- (1) Water-Research list - 205 subscribers – inform water researchers from NC universities about calls for papers, grants, upcoming conferences, student internships, etc.;
- (2) WRRI-News list - 825 subscribers - informs researchers, local governments, municipalities, interest groups etc. about calls for papers, grants, upcoming conferences and events, etc.;
- (3) NCWRA-info list - 240 subscribers - provides information of the North Carolina Water Resources Association sponsored events;
- (4) Urban Water Consortium (UWC) for Urban Water Consortium member communications;
- (5) and UWC-Stormwater Group list for the UWC Stormwater Group member communications.

### **Audio-Visual Productions**

The Water Resources Research Institute designed a tabletop exhibit to display at conferences, workshops, and other events in which we participate. The exhibit is a 7' x 4', 4-panel display bearing our name and logo along with photos and descriptions of several current research projects. This display highlights our current research and publications at our annual conference and at other events.

Most presentations given at workshops and seminars sponsored by WRRI are uploaded to the WRRI website for public viewing (see under WRRI Internet Services). Investigators or their graduate students have presented or will present oral or poster presentations at a WRRI Annual Conference. Many of these presentations are available from past WRRI Annual Conferences at: <http://www.ncsu.edu/wrri/conference/past.html>

### **NC Urban Water Consortium**

WRRI administers the NC Urban Water Consortium (UWC) and meets with the members quarterly. The consortium was established in 1985 by the Institute, in cooperation with several of North Carolina's larger cities to provide a program of research and development and technology transfer on water problems that urban areas share. Through this partnership, WRRI and the State of North Carolina help individual facilities and regions solve problems related to local environmental or regulatory circumstances. Participants support the program through annual dues and enhancement funds and guide the program through representation on an advisory board, selection of research topics, participation in design of requests for proposals, and review of proposals. In July 2009, the UWC gained a new member of the Town of Cary Department of Public Works and Utilities. There are 12 member cities/special districts in North Carolina that met on the following dates: March 11, 2009 – Burlington; June 17, 2009 – Wilmington; September 23, 2009 – Chapel Hill; and December 11, 2009 – Raleigh.

In 1998, several members of the NC UWC partnership formed a special group to sponsor research and technology transfer on issues related to urban stormwater and management. The Urban Water Consortium (UWC) Stormwater Group is administered by WRRI. Participants support the program through annual dues and enhancement funds. They guide the program through representation on an advisory board, selection of research topics, participation in the design of requests for proposals and review of proposals. Currently there are eight members that met on the following dates: March 12, 2009 – Durham; June 11, 2009 – Fayetteville; September 30, 2009 – Wilmington; and December 16, 2009 – Winston-Salem.

### **Other WRRI Sponsored Workshops, Forums and Seminars**

The WRRI Information Transfer Program includes workshops supported by the NC Department of Environment and Natural Resources (DENR), Division of Land Resources Land Quality Section along with the NC Sedimentation Control Commission (SCC). Workshops held during this period include ([http://www.ncsu.edu/wrri/events/esc\\_workshops/pastescworkshops.html](http://www.ncsu.edu/wrri/events/esc_workshops/pastescworkshops.html)):

- (1) One Spring Erosion and Sediment Control Planning and Design Workshop, February 25-26, 2009 in Raleigh, NC
- (2) One Fall Planning and Design Erosion and Sedimentation Control Workshop, November 4-5, 2009 in Hickory, NC
- (3) Erosion and Sedimentation Control Local Programs Training Workshop, February 3-4, 2010 in Atlantic Beach, NC.

WRI formed a new partnership with NC DENR Division of Water Quality (DWQ) to begin the NC DWQ Stormwater Programs Workshops (<http://www.ncsu.edu/wri/events/dwq/>):

- (1) One DWQ Stormwater Programs Workshop, April 30, 2009 in Raleigh, NC
- (2) One DWQ Stormwater Programs Workshop (with a focus on Coastal Stormwater rules and programs), October 21, 2009 in Wilmington, NC

Another way WRI provides Information Transfer is through the North Carolina Water Resources Association (NCWRA) Luncheon and Forums (<http://www.ncsu.edu/wri/events/ncwra/>):

- (1) April 6, 2009: "Measuring the value of water to competing users during times of scarcity"; Dr. Laura Taylor, Director, Center for Environmental and Resource Economics Policy (CEnREP), and Professor of Ag. and Resource Economics, NC State University
- (2) September 11, 2009: "Renewable and Sustainable Options for Charlotte-Mecklenburg Utilities Wastewater Treatment Plants"; Ms. Jacqueline Jarrell, Environmental Management Division Superintendent, Charlotte-Mecklenburg Utilities Department
- (3) December 7, 2009: "Recent and Current Projects and Initiatives in Hazard Risk Management for North Carolina Water Resources"; Hope Morgan, GISP, GIS Manager, Office of Geospatial & Technology Management, NC Dept of Emergency Management and Jack Moyer, National Water Security and Preparedness Lead URS Corporation;
- (4) February 22, 2010: "Trends in the Development of Israel's Water Sector"; Oded Fixler, Deputy Director General for Engineering in the Israeli Water and Sewage Authority.

In addition, two seminars were sponsored by Progress Energy at NCSU's Jane S. McKimmon Center to provide Information Transfer

(<http://www.ncsu.edu/wri/events/progressenergyseminars/pastforums.html>)

- (1) April 16, 2009: "Utility Management Challenges for the 21st Century: Managing in an Era of Uncertainty", by Mr. Chuck Clarke, Chief Executive Officer of Cascade Water Alliance, Washington
- (2) November 20, 2009: "CI-FLOW: A prototype total water system for forecasting inland and coastal flooding in Eastern North Carolina"; by Kevin Kelleher, Deputy Director, National Severe Storms Laboratory (NSSL), and Rick Luettich, Ph.D., Professor and Director, UNC Institute of Marine Sciences

<b>Student Support</b>					
<b>Category</b>	<b>Section 104 Base Grant</b>	<b>Section 104 NCGP Award</b>	<b>NIWR-USGS Internship</b>	<b>Supplemental Awards</b>	<b>Total</b>
<b>Undergraduate</b>	5	0	0	0	5
<b>Masters</b>	5	0	2	0	7
<b>Ph.D.</b>	2	0	1	0	3
<b>Post-Doc.</b>	0	0	0	0	0
<b>Total</b>	12	0	3	0	15

## Notable Awards and Achievements

WRRI Under New Leadership In August 2009, N.C. State University announced that Dr. Michael Voiland, executive director of the N.C. Sea Grant Program, will also serve as permanent director of WRRI, overseeing the institute's statewide research and technology transfer programs. Voiland had been acting director of WRRI since April. In September 2009, WRRI announced that Dr. David Genereux, professor in the NCSU Department of Marine, Earth and Atmospheric Sciences, had been named to a one-quarter-time position as WRRI's associate director for research. His duties include working with the WRRI Advisory Committee to establish research priorities, managing the annual request for proposals (including proposal review and award decisions), advising prospective investigators, reviewing final reports, and advancing technology transfer.

From research project funded titled "Improved water management strategies for the Neuse Basin utilizing climate-information based probabilistic streamflow forecasts" by Dr. Sankar Arumugam, Civil and Environmental Engineering, NC State University; Naresh Devineni, PhD student, working with Dr. Sankar Arumugam in Civil and Environmental Engineering at NC State University, received an outstanding student paper award for his presentation "Multimodel Ensembles of Streamflow Forecasts: Role of Predictor State in Developing Optimal Combinations" at the 2007 Fall AGU (American Geophysical Union) conference in San Francisco.

Khorrarn, Siamak, Stacy A.C. Nelson, Halil Cakir, and Barry Hester. Center for Earth Observation, NC State University. Integration of High-Resolution Satellite Imagery in Cost-Effective Assessment of Land Use Practices Influencing Erosion and Sediment Yield. Report 378 of the Water Resources Research Institute of The University of North Carolina. (<http://www.lib.ncsu.edu/resolver/1840.4/3589>) (USGS Grant Agreement Number 01HQGR0096) A journal article on similar work by the investigators (Cakir, H. I., Khorrarn, S., and Nelson, S.A.C. 2006. Correspondence Analysis Approach for Detecting Land Use Land Cover Changes. *Remote Sensing of Environment*. 102(3-4): 306-317.) was ranked #9 and #8 by ScienceDirect in the Environmental Science Remote Sensing of Environment sub-category as one of the Top 25 Hottest Articles over two consecutive 3-month rating periods; April-June 2006 and July-September 2006.

Title: Exploring mercury transport mechanisms in aquatic systems: A statewide assessment of factors affecting methylmercury contamination of food webs and fish Research funded by US Geological Survey conducted by Dr. Derek Aday, Biology Dept, NC State University

Description and Significant Impacts: Mercury contamination of aquatic systems has received much attention recently because of potential health effects on humans and wildlife. Although the factors affecting mercury deposition, its conversion to biologically active methylmercury, and its bioaccumulation in aquatic systems have been identified, equivocal results for particular species and systems have hampered policy making. Our study addresses this problem through a comprehensive, statewide synthesis of the available data on fish mercury contamination in North Carolina and the environmental factors associated with methylmercury formation and transport through aquatic food webs. Using data collected by the North Carolina Department of Environment and Natural Resources, the U.S. Environmental Protection Agency, and others, we examined the relationships between a suite of biotic and abiotic factors and tissue mercury concentrations in fish from North Carolina water bodies. Multivariate tests were conducted to create predictive models relating environmental variables to mercury levels in fish, and Akaike's information criterion was used to examine the relative strengths of the candidate models. The best model in our analyses ( $R^2=0.81$ ) included species, fish trophic status, ecoregion, and pH. Other important drivers of mercury accumulation were land use patterns (the percentage of the subbasin that is agricultural) and site type (swamps versus lakes, rivers, and bays).

Year 2 In the second year of the study, mercury dynamics in relation to mercury point-source proximity was investigated. Most mercury contamination in aquatic biota originates from coal-fired power plants. They

quantified the relative importance of mercury point-source deposition and proximity to coal-fired power plants on mercury and selenium accumulation in two species of fish. Lower tissue mercury and higher tissue selenium concentrations were found in fish collected near coal-fired power plants. As well, mercury accumulation in fish were driven more by biotic and waterbody characteristics than atmospheric deposition, whereas selenium accumulation in fish was driven by atmospheric deposition near power plants. The researchers were also able to improve upon their predictive model, by accounting for the error introduced to the model through selenium mitigated reductions in fish tissue mercury near coal-fired power plants.

Although previous investigations have indicated similar individual relationships, their study is unique in that they examined the relative importance of a large number of biotic and abiotic variables across a range of environments, ecosystems, and species.

Research Highlighted in various news articles: New model predicts hot spots for mercury in fish Eureka Alert: [http://www.eurekaalert.org/pub\\_releases/2008-12/ncsu-nmp120108.php](http://www.eurekaalert.org/pub_releases/2008-12/ncsu-nmp120108.php)

New model predicts hot spots for mercury in fish Mercury levels in fish are prompting widespread consumption advisories and uncertainty among consumers over which species are safe to eat. Now researchers at North Carolina State University have developed a model that will help scientists and regulators around the country predict which areas are likely to have fish with high mercury levels a breakthrough that should help officials address public uncertainty by developing health advisories for specific water bodies and fish species.

The NC State researchers have created a statistical model that can incorporate data on the variety of factors that influence mercury levels in fish tissue . such as the pH of the water and the size and species of the fish to identify those aquatic ecosystems that are likely to have fish with high mercury levels. "We want to be predictive," says NC State's Dr. Derek Aday, "rather than reacting to events after they've happened." Aday, an assistant professor of biology at NC State who is part of the research team, says the model can be used "to develop specific health advisories for water bodies and species rather than sweeping advisories." Current advisories tend to restrict consumption of certain species for an entire state or region out of concern that mercury levels in the fish could adversely affect human health.

While the NC State effort has so far focused on North Carolina, Aday says, "The goal is to create a template that could be used in systems throughout the country. Specific variables may change, but the approach would be the same." In fact, Aday says, "we've identified a suite of variables that we believe will be consistent in driving mercury dynamics across many aquatic systems." The new model is a synthesis of a number of smaller statistical models that allows researchers to examine the combination of factors that can drive contaminants in aquatic systems.

In order to collect data for use in the new model, the researchers synthesized water quality, fish tissue mercury and environmental data that had been collected by North Carolina agencies since 1990. That database was then used to construct the statistical model.

Medical News Today: <http://www.medicalnewstoday.com/articles/131469.php> Physorg:  
<http://www.physorg.com/news147354867.html> Bio-medicine:  
<http://www.bio-medicine.org/biology-news-1/New-model-predicts-hotspots- for-mercury-in-fish-6113-1/>  
News at NCSU: <http://news.ncsu.edu/releases/nc-state-develops-model-to-predict-hotspots-for-mercury-levels-in-fish/>

## **Publications from Prior Years**

1. 2001NC1461B ("Role of Sediment Processes in Regulating Water Quality in the Cape Fear River") - Articles in Refereed Scientific Journals - Sundareshwar, P.V., C.J. Richardson, R.A. Gleason, P.J. Pellechai, and S. Honomichl, 2009, Nature versus nurture: Functional Assessment of Restoration Effects on Wetland Services using Nuclear Magnetic Resonance Spectroscopy, Geophysical Research Letters, Vol, 36, L03402.