

**Water Resources Research Institute of the
University of North Carolina
Annual Technical Report
FY 2008**

Introduction

During the Fiscal Year 2008 reporting period, the Water Resources Research Institute of The University of North Carolina supported research from 6 universities for 37 related projects. Research priorities, as directed by the WRRRI (Water Resources Research Institute) Advisory Committee included the following: A. Water Availability, Use and Climate Interaction 1. Water use: Research is needed to improve processes for collecting data and estimating use of water in North Carolina. 2. Effectiveness of conservation measures: Research is needed to improve estimates of the effectiveness of conservation measures for urban water use during droughts. 3. Climate forecast and water availability during droughts: Incorporate the climate forecast to improve the ability to manage reservoirs. Determine frequency, duration, and recovery from periods of low precipitation.

B. Drinking Water Quality 1. Contaminant screening: Evaluate current and new techniques to screen drinking water regulated contaminants and other contaminants of special health concern at water intakes and upstream sampling locations. 2. Compliance with drinking water standards: Evaluate how water suppliers can most effectively comply with regulations for disinfection by-products 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 and monitor lead, copper and other metals in household plumbing. 5. Water supply security: Determine effective approaches/strategies to protect water supplies from the source to the end user.

C. Nutrients and Water Quality 1. Chlorophyll *a* standard: Determine effectiveness of the 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.

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. Determine which practices are the most effective for addressing coastal stormwater discharges. Estimate the monetary benefit of the BMPs and improvements. 2. Low Impact Development (LID): Determine which LID techniques work best in North Carolina to minimize development impacts to watersheds.

E. Monitoring and Assessment of Special Management Programs Determine the dynamics of fecal coliform, sediment and nutrients in urban streams and stormwater detention facilities in the Jordan Lake Reservoir watershed, Neuse River Basin, Tar-Pamlico River Basin, and other areas that are subject to TMDLs.

F. Agricultural Impacts 1. Research is needed to determine water quality impacts (nutrients and fecal coliform) of special classes of agricultural operations such as land application of animal waste, pasture operations, municipal biosolids, and organic farming. 2. Determine whether there is a nitrogen reduction benefit in partial lagoon covers and re-use methane systems. Determine the effect of spray operations on nitrogen runoff.

G. Aquatic Ecosystem Functions and Instream Flow Needs There are a variety of ecosystem function issues that need to be researched and evaluated to improve water quality and habitat protection: 1. Effectiveness of riparian buffers in restoration, especially in the mountain region. 2. Effective approaches to aquatic ecosystem restoration 3. Strategies and effectiveness of aquatic weed control 4. Instream flow and wetland functional assessment approaches 5. Degradation of trout streams 6. Protection of rare and endangered aquatic species in North Carolina streams.

H. Waste Management 1. Septic systems: Evaluate the contributions of septic tanks to groundwater and nearby streams. 2. 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.

I. Water Quality Monitoring 1. Mercury: Research is needed to better understand sources and transport mechanisms for mercury in water, sediment, and tissue. A statewide assessment of conditions that favor methyl mercury is needed. 2. 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 nutrient interactions. 3. Economic and feasibility assessment of water quality and remote environmental monitoring programs 4. Biological stressor studies in impaired waters

J. 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.

Research Program Introduction

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

Priority water research needs for the FY 2008 program were developed in close consultation with the Institutes' Advisory Committee. Following their annual meeting, a statement of priority research needs was developed. The proposal solicitation, as in the past, is sent to all presidents and relevant department heads of senior colleges and universities in North Carolina as well as historically black colleges, to apprise them of the opportunity to submit proposals. The call for proposals is also sent to an email distribution list of approximately 180 university faculty across North Carolina. The proposals received are sent to the Technical Committee and to external peer reviewers to determine the relevancy, need for the proposed research and relative strength and weaknesses. The Technical Committee meets to review all comments made by reviewers and make recommendations regarding proposal funding. Criteria that will be used to judge proposals are: · the scientific quality of the proposed work, · the need for this research in North Carolina, · how closely the project relates to the WRI 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, · the cost of the proposed work, opportunities for application in teaching..

Protecting Receiving Waters: Removal of Biochemically Active Compounds from Wastewater by Sequential Photochemical and Biological Oxidation Processes

Basic Information

Title:	Protecting Receiving Waters: Removal of Biochemically Active Compounds from Wastewater by Sequential Photochemical and Biological Oxidation Processes
Project Number:	2007NC70B
Start Date:	3/1/2007
End Date:	8/31/2008
Funding Source:	104B
Congressional District:	2
Research Category:	Biological Sciences
Focus Category:	Treatment, Acid Deposition, Water Quality
Descriptors:	
Principal Investigators:	Detlef Knappe

Publication

1. Baeza, A.C., 2008, Removal of pharmaceutical and endocrine disrupting chemicals by sequential photochemical and biological oxidation processes. Ph.D. Dissertation, Department of Civil, Construction, and Environmental Engineering, NC State University, Raleigh, NC, 200 pages.
2. Baeza, C. and D.R.U. Knappe, 2009, Characterization of oxidation products formed in UV/H₂O₂ processes: Biochemical activity and biodegradation potential, In Proc. of AWWA Research Symposium Emerging Organic Contaminants, American Water Works Association, Denver, CO, 6 pages.

Title: Protecting Receiving Waters: Removal of Biochemically Active Compounds from Wastewater by Sequential Photochemical and Biological Oxidation Processes

Statement of the Critical Regional and State Water Problem

The presence of biochemically active compounds (BACs) such as endocrine disrupting chemicals (EDCs), antimicrobial compounds, and other pharmaceutically active compounds (PhACs) in the aquatic environment is an issue of great importance. For example, the presence of EDCs may cause intersexuality in fish, and the presence of antimicrobial compounds may lead to the evolution of antibiotic-resistant bacteria. The Washington Post [1] recently reported that a USGS study found that at least 80% of male smallmouth bass caught in Virginia and Maryland tributaries of the Potomac River grew eggs. In addition, 54% of male largemouth bass caught in the Potomac River near the Blue Plains wastewater treatment plant (WWTP) of Washington D.C. showed signs of feminization and 23% were intersex.

While the specific cause of the feminization of male fish in the Potomac watershed has not yet been identified, other studies have linked incidents of intersexuality to the presence of EDCs that enter streams through WWTP discharges [2, 3, 4]. BACs are poorly removed during conventional wastewater treatment [5, 6, 7], and WWTP discharges are therefore an important source, through which BACs are introduced into the environment. Recent studies have shown that BACs are now ubiquitous in surface waters throughout the United States [8].

One can argue that similarities exist between the Potomac watershed and the Neuse River watershed in North Carolina. For example, rainfall, water usage, and population growth patterns are somewhat similar. Furthermore, the Potomac flows into the ecologically sensitive Chesapeake Bay, which serves as a breeding and rearing ground for many species of fish in the Atlantic Ocean. In North Carolina, the Neuse flows into the similarly sensitive Pamlico Sound, and its watershed includes rapidly expanding urban areas (Durham, Cary, Raleigh). Conventionally treated wastewater from these municipalities is discharged into the Neuse or its tributaries. In addition, some water reuse projects have been implemented or are in the planning stage (irrigation, stream augmentation), which may also affect the water quality of the Neuse and its tributaries. Advanced wastewater treatment strategies are therefore needed that effectively eliminate the discharge of BACs into the environment.

Statement of Benefits

The goal of the proposed research is to develop an advanced wastewater treatment strategy that provides a barrier against the release of BACs into North Carolina surface waters and that yields readily biodegradable oxidation intermediates. Benefits of the proposed research include not only improved habitat for aquatic life, but also improved water quality for drinking water treatment plants that rely on surface water sources impacted by upstream WWTP discharges. The initial step of the proposed treatment strategy relies on the UV/H₂O₂ process to remove BACs via photolysis and hydroxyl radical oxidation. While this treatment option is expected to be effective for parent compound removal, little is known to date about the biochemical activity and environmental persistence of oxidation intermediates.

While *in vitro* biological activity assays such as the Yeast Estrogen Screen (YES) and Minimum Inhibitory Concentration (MIC) assay have been used to assess oxidative removal of estrogenic and antimicrobial activity, respectively, the results from such tests do not always concur with those obtained by *in vivo* tests. This point was illustrated in a UV/H₂O₂ treatment study [9] that showed that removal rates of estrogenic activity were more rapid when assessed by

the YES assay than when assessed by an *in vivo* vitellogenin assay involving Japanese medaka fish. The results of the *in vivo* test suggested that some photooxidation intermediates exhibited estrogenic activity. Furthermore, little is known about the environmental persistence of such photooxidation intermediates. As a result, the proposed research will investigate what photooxidation conditions are required to produce BAC intermediates that are readily biodegradable. The biodegradation of photooxidation intermediates can be accomplished by microorganisms in (1) receiving streams (conventional WWTP discharge scenario or stream augmentation scenario), (2) biological filters that follow the photooxidation step, or (3) soils (e.g., water reuse scenarios involving irrigation or soil aquifer treatment). For the current proposal, scenario 1 will be simulated in Task 3. Scenarios 2 and 3 could be tested if follow-up funding can be obtained.

Nature, Scope, and Objectives of Research

Hypothesis: The hypothesis that will be tested in this research is that UV/H₂O₂ oxidation effectively removes BACs and their associated biological activity in WWTP effluent matrices. In addition, we will test the hypothesis that BAC oxidation products are readily biodegradable by microorganisms in NC surface waters. The validity of the above hypotheses will be tested with six BACs: the antimicrobial compounds sulfamethazine (SMZ), sulfadiazine (SDZ), and trimethoprim (TMP), the EDCs bisphenol-A (BPA) and 17- α -ethinyl estradiol (EE2), and the analgesic compound diclofenac. Figure 1 depicts the chemical structures of the six contaminant probes as well as the position of the ¹⁴C label (where applicable).

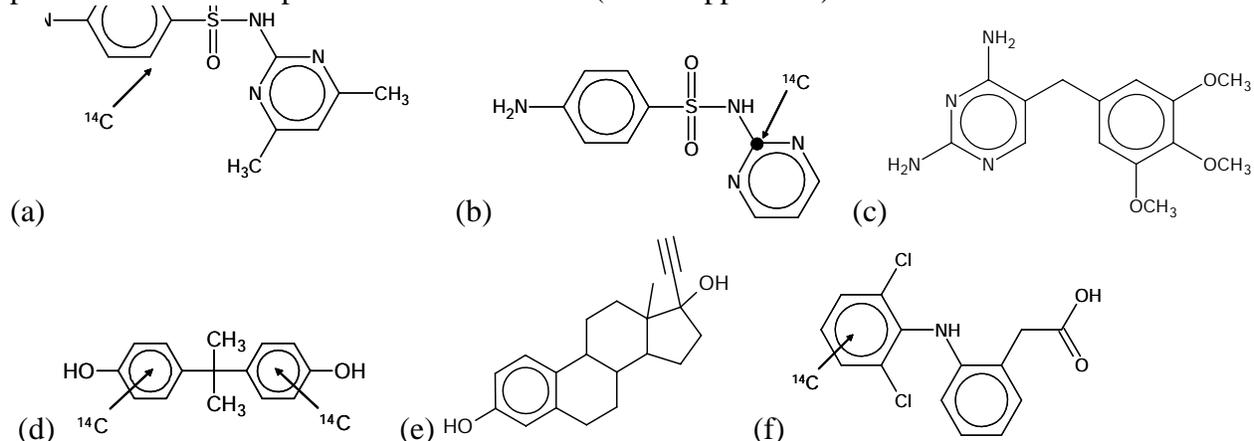


Figure 1: Chemical structures of (a) ¹⁴C-sulfamethazine, (b) ¹⁴C-sulfadiazine, (c) trimethoprim, (d) ¹⁴C-bisphenol-A, (e) 17- α -ethinyl estradiol, and (f) ¹⁴C-diclofenac.

These compounds were chosen for several reasons. First, many of these compounds are poorly degraded in WWTPs, and as a result have been detected in surface waters throughout the United States. Second, our group has experience with analytical methods for these compounds as well as with *in vitro* biological activity assays including the Yeast Estrogen Screen (YES) and Minimum Inhibitory Concentration (MIC) assays for detection of estrogenic and antimicrobial activities. Third, four of the selected BACs (SMZ, SDZ, bisphenol-A and diclofenac) are available in ¹⁴C-labeled form, which will allow us to trace the biological mineralization of photooxidation intermediates *at environmentally relevant concentrations*. Finally, SMZ and SDZ were specifically chosen because of the location of the ¹⁴C label – tests with SMZ will

allow us to test mineralization of the aniline moiety of sulfonamides while tests with SDZ will allow us to test the mineralization of the diazine moiety that is common in many sulfonamides.

Objectives: The principal objective of the proposed research is to quantify the effectiveness of combining UV/H₂O₂ and biological oxidation processes for the mineralization of six BACs (the antimicrobial compounds sulfamethazine, sulfadiazine, trimethoprim, the EDCs bisphenol-A and 17- α ethinyl estradiol, and the analgesic diclofenac) that commonly occur in conventionally treated wastewater. Specific objectives are (1) to evaluate the effects of the WWTP effluent matrix on photolysis and photooxidation rates of six BACs (**Task 1**), (2) to quantify the mineralization potential of ¹⁴C-labeled BAC oxidation products as a function of UV/H₂O₂ oxidation conditions (**Task 2**), and (3) to measure biodegradation rates of ¹⁴C-labeled BAC oxidation products by microorganisms in NC surface waters at different dilution rates (**Task 3**).

As part of task 1 will also quantify (1) effects of the WWTP matrix on removal rates of biological activity by YES and MIC assays and (2) effects of UV/H₂O₂ treatment conditions (UV fluence, H₂O₂ concentration) on effluent organic matter characteristic such as biochemical oxygen demand (BOD₅), assimilable organic carbon (AOC) concentration, dissolved organic carbon (DOC) concentration, and UV absorbance at 254 nm (UV₂₅₄).

Timeline. Figure 2 summarizes the timeline for the 3 research tasks, which are described in more detail below. Progress reports will be submitted on a quarterly basis, and I final report will be submitted by 2/29/08.

Task	2007								2008				
	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	
Task 1													
Task 2													
Task 3													

Figure 2: Timeline of project tasks.

Methods, Procedures, and Facilities

Task 1: Characterization of the photochemical oxidation process

Objectives: (1) Quantify and compare photolysis and UV/H₂O₂ photooxidation rates of six BACs (Figure 1) in ultrapure water and a WWTP effluent, (2) quantify removal rates of biological activity (estrogenic and antimicrobial activity), and (3) quantify changes in effluent organic matter (EfOM) characteristics as a result of UV/H₂O₂ treatment.

Approach: Photolysis and UV/H₂O₂ oxidation rates of BACs will be quantified with a quasi-collimated beam (QCB) apparatus, a schematic of which is shown in Figure 3. The purpose of the QCB apparatus is to ensure that the UV rays reaching the sample are exactly perpendicular, which allows for accurate measurement of UV energy at the surface of the sample. Therefore, we can accurately measure the UV fluence delivered to the sample. The QCB is equipped with a low pressure (LP) UV lamp, and LP UV fluences (doses) in the range of 0 to 1000 mJ cm⁻² will be tested. Initial experiments will be conducted to quantify photolysis rates (no H₂O₂ added) of the six BACs shown in Figure 1. Experiments will be conducted in ultrapure water (UPW) and in WWTP effluent from the North Cary Water Reclamation facility (Cary, NC). These experiments will show whether the WWTP effluent matrix decreases (lower transmittance of UV light) or

enhances (through sensitized photoprocesses that lead to the formation of reactive species in the WWTP effluent) BAC removal rates.

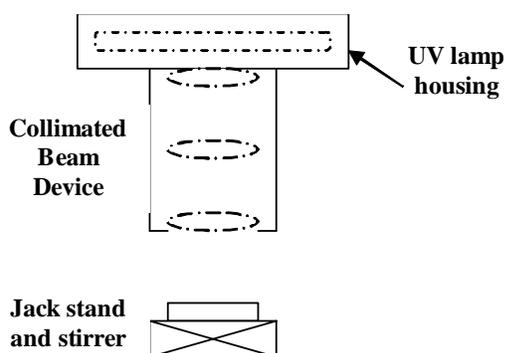


Figure 3: Schematic of basic “Quasi-Collimated Beam” UV Reactor

To quantify BAC oxidation rates in the UV/H₂O₂ process, additional QCB experiments will be conducted with the same UV fluence values used in photolysis experiments and with H₂O₂ concentrations of 2, 6, and 10 mg L⁻¹. Initial UV/H₂O₂ experiments will be conducted with the hydroxyl radical probe *para*-chlorobenzoic acid (*p*-CBA) to measure OH radical concentrations in UPW and in WWTP effluent [10, 11]. Once the OH radical concentration has been measured for each H₂O₂ concentration and water, removal rates of the 6 BACs will be predicted from photolysis rates (determined in the initial phase of this task), the measured OH radical concentration from *p*-CBA experiments, and second-order OH radical rate constants ($k_{OH,C}$) reported in the literature (Table 1). The method to be utilized for this prediction is outlined in detail in [11]. The predicted BAC removal rates will be verified with QCB experiments conducted in UPW and WWTP effluent spiked with individual BACs and with a mixture of all six BACs shown in Figure 1.

Table 1: Second-order rate constants for the reaction of OH radicals with selected BACs

Compound	$k_{OH,C}$ (M ⁻¹ s ⁻¹)	References
Sulfamethazine	5x10 ⁹ , 4.5x10 ⁹	12, 10
Sulfadiazine	3.7x10 ⁹	12
Trimethoprim	6.9x10 ⁹	13
Bisphenol-A	1.02x10 ¹⁰	14
17- α -Ethinyl estradiol	9.8x10 ⁹ , 1.08x10 ¹⁰	14, 15
Diclofenac	7.5x10 ⁹	15

BACs will be separated and quantified using HPLC methods already developed in our lab [10, 14].

Additional UV/H₂O₂ experiments will be conducted to measure removal rates of biological activity. The removal of estrogenic activity will be tested with BPA and EE2, spiked into WWTP effluent at environmentally relevant concentrations, and will be quantified with the YES assay [16]. For SMZ, SDZ, and TMP, the removal rate of antimicrobial activity will be measured with the MIC assay as outlined by [10].

Changes in EfOM characteristics resulting from UV/H₂O₂ treatment will be quantified by comparing BOD₅ levels, AOC concentrations, DOC concentrations, and UV₂₅₄ values of the WWTP effluent prior to and following UV/H₂O₂ treatment. Data from earlier experiments will be used to select UV/H₂O₂ treatment conditions that lead to 90 and 99% removal of the most difficult to oxidize BAC. BOD, DOC, and UV₂₅₄ will be measured using Standard Methods, and AOC will be measured using a new flow-cytometric method [17] that is and currently being implemented in our laboratory. Because of the quantities of water necessary to perform the

EfOM characterization tests, a 3-L recycle system equipped with an annular UV reactor containing a LP UV lamp (Trojan) will be used to conduct UV/H₂O₂ oxidation experiments for this portion of task 1. This reactor has been used previously for kinetic studies in our lab [10].

Task 2: Characterization of the mineralization potential of ¹⁴C-labeled BAC photo-oxidation products

Objectives: (1) Using ¹⁴C-labeled SMZ, SDZ, BPA, and diclofenac, quantify the mineralization potential of photooxidation intermediates in batch biological processes, and (2) determine UV/H₂O₂ treatment conditions for optimizing mineralization potential.

Approach: To achieve Task 2 objectives, batch reactors will be used to determine the mineralization potential of SMZ, SDZ, BPA, and diclofenac photooxidation intermediates. Batch tests permit screening of a wide range of photooxidation conditions and will be utilized to find optimal treatment conditions (UV fluence and H₂O₂ concentration) that maximize the conversion of BACs into readily biodegradable intermediates. In Task 2 experiments, WWTP effluent will be spiked with ¹⁴C-labeled SMZ, SDZ, BPA, or diclofenac at environmentally relevant concentrations (~1 µg/L), and treated with a range of UV fluences (0-1000 mJ cm⁻²) and H₂O₂ concentrations (0, 2, 6, and 10 mg L⁻¹). Upon photochemical oxidation, samples will be inoculated with a consortium of bacteria harvested from the Neuse River just upstream of the Raleigh WWTP. This consortium will be isolated and maintained as described by [17] and will permit sample inoculation with a known concentration of cells. The cell concentration of the sample will be determined by flow cytometry following staining with SYBRGreen [17]. SMZ and SDZ were chosen for this task because of the location of the ¹⁴C label within their structure (Figure 1). By comparing mineralization rates of these two species, we can obtain information about the relative mineralization rates of photooxidation products derived from the aniline ring (SMZ) and the diazine ring (SDZ). Additionally, diclofenac is very recalcitrant in conventional wastewater treatment [18] and will thus be an excellent challenge BAC for testing the effects of UV/H₂O₂ treatment on enhancing mineralization potential.

Reactors for biological oxidation experiments were initially developed in our laboratory to test the bioavailability of sorbed organic contaminants (Figure 4, [19]). The reactors are equipped with a NaOH trap that will capture ¹⁴CO₂ produced from the mineralization of ¹⁴C-labeled photooxidation intermediates. A second vial containing H₂O₂ will serve as a source of oxygen to maintain aerobic conditions.

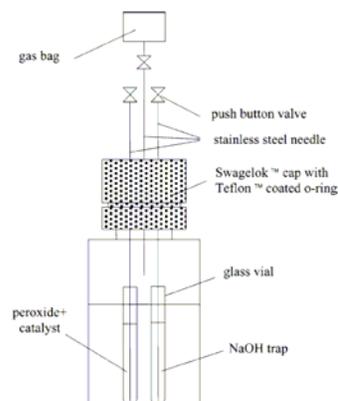
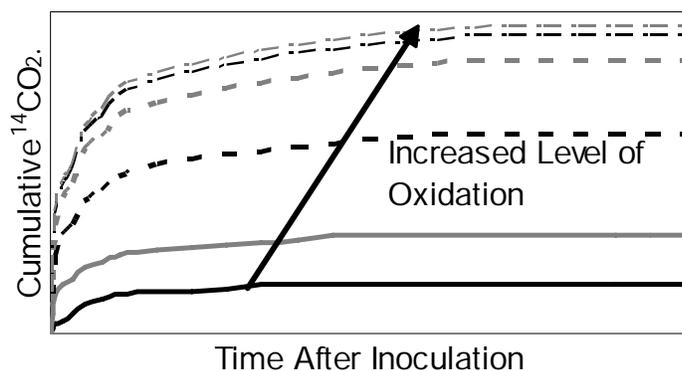


Figure 4: Batch reactor for biological oxidation experiments [19].

Contents of the NaOH trap will be periodically removed for analysis by liquid scintillation counting to determine the rate of ¹⁴CO₂ production, and replaced with fresh 2N NaOH solution. Batch tests will be terminated when ¹⁴CO₂ production is no longer measurable. At the completion of batch tests, ¹⁴C in the aqueous phase (both suspended and dissolved) will be measured to obtain a ¹⁴C mass balance. Results from experiments conducted without photooxidation pretreatment will serve as a baseline for assessing the effectiveness of

photooxidation processes for enhancing biological mineralization. Control experiments, in which aerobic biological activity will be shut down via sodium azide addition, will be conducted in parallel with biotic experiments. All biological oxidation experiments will be conducted in triplicate.

Figure 5 displays a possible outcome of the biological oxidation experiments. Utilizing the same initial hydrogen peroxide concentration and varying applied UV fluence, Figure 5 depicts the expected cumulative formation of $^{14}\text{CO}_2$ as a function of time after inoculation of the photooxidized WWTP effluent. Increased UV fluence translates into a greater degree of BAC degradation and likely into the formation of more biodegradable products. The data of interest will be the initial rates of $^{14}\text{CO}_2$ formation and the mineralization potential, which is the maximum percentage of parent compound ^{14}C mineralized (visually represented as the plateau of Figure 5). The goal of Task 2 experiments is to identify the point of diminishing returns at which



more severe UV/H₂O₂ oxidation conditions do not lead to enhanced mineralization rates and do not increase the mineralization potential during the biological oxidation step

Figure 5: Potential outcomes from the Photooxidation and biodegradation experiments.

Task 3: Determination of mineralization rates of photooxidation products in receiving waters

Objective: Using one ^{14}C -labeled BAC, quantify the mineralization rate of photooxidation intermediates in batch biological processes designed to simulate the discharge of treated wastewater into a receiving stream.

Approach: Task 3 experiments are designed to simulate the discharge of photooxidized wastewater into receiving streams. The principal goal of the experiment is to quantify the mineralization rate of ^{14}C -labeled photooxidation intermediates in two NC surface waters at three dilution rates during warm and cold weather conditions. Task 3 experiments will be conducted with one ^{14}C -labeled BAC that proved to be the most challenging to remove in Task 1 and Task 2 experiments. In Task 3 experiments, WWTP effluent samples, spiked with the ^{14}C -labeled BAC, will first be treated with the optimal UV/H₂O₂ condition for maximizing mineralization potential as determined in Task 2. Upon photooxidation, samples will be mixed at levels of 10%, 50%, and 90% (v/v) with two NC surface waters: (1) Neuse River water (collected just upstream of the Raleigh WWTP) and (2) Pigeon House Branch water (collected at a proposed stream augmentation site). Photooxidized waters will be mixed with NC surface waters in the batch reactor to simulate variability in dilution rates that might occur when treated wastewater is discharged into small or large streams during wet, average, or drought periods. Furthermore, Task 3 experiments will be conducted with receiving water samples that will be collected during summer and winter months to investigate seasonal variations in mineralization rates of

photooxidation intermediates. The water temperature of each stream will be recorded at the time of sampling, and the temperature conditions will be replicated during the biological oxidation experiments in our laboratory. Biological oxidation experiments will be initiated within one day of stream water sample collection.

Samples from the NaOH trap of the batch reactor (Figure 4) will be collected and analyzed as in task 2, and the experiment will be terminated when $^{14}\text{CO}_2$ production is no longer measurable. At the completion of batch tests, ^{14}C in the aqueous phase (both suspended and dissolved) will be measured to obtain a ^{14}C mass balance. Results from experiments conducted without photooxidation pretreatment will serve as a baseline for assessing the effectiveness of photooxidation processes for enhancing biological mineralization in receiving waters. Control experiments, in which aerobic biological activity will be shut down via sodium azide addition, will be conducted in parallel with biotic experiments. All tests will be conducted in triplicate.

Results from these experiments will yield a first approximation of the expected environmental half-lives of BAC photooxidation intermediates. This information will be useful to estimate the degree of mineralization that can be accomplished by stream microorganisms between a WWTP discharge location and a downstream water treatment plant intake. Also of interest will be an estimate of the expected mineralization that would occur in the Neuse River between a WWTP discharge and Pamlico Sound.

Facilities

Equipment required to conduct the proposed research (collimated beam apparatus, annular UV reactor, HPLC, liquid scintillation counter, incubators) is available in the Environmental Engineering research laboratory at NC State University or is available for our use on the NC State campus (flow cytometer).

Related Research

Since the first documented example of a widespread sexual disruption in wild fish resulting from exposure to ambient levels of BACs in British waters was reported in 1998 [20], many studies have detected BACs in surface waters throughout the world [e.g., 8, 21, 22]. One important source of BACs is the discharge pipe of municipal WWTPs, which do not effectively remove a number of BACs before discharge into receiving waters [23, 24].

The advanced oxidation technology proposed for use in this study is the UV/H₂O₂ process. In this process, hydrogen peroxide molecules absorb UV energy and break down into hydroxyl radicals ($\cdot\text{OH}$). The hydroxyl radical is highly reactive, oxidizing many BACs with rate constants on the order of $10^9 - 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ [e.g. 13-15]. To date, most studies evaluating the effectiveness of the UV/H₂O₂ process for BAC removal and removal of associated biochemical activity have focused on drinking water treatment conditions and thus evaluated process performance in relatively clean water matrices ranging from pure lab water to stream and lake waters [e.g. 10, 13, 16]. These studies have shown that although UV/H₂O₂ oxidation is negatively impacted by the presence of background organic matter and high alkalinity, the process is still capable of oxidizing BACs and removing associated biological activity at reasonable operating conditions. For example, estrogenic activity associated with EE2 was almost completely removed using LP UV and $5 \text{ mg L}^{-1} \text{ H}_2\text{O}_2$ with 200 mJ cm^{-2} applied UV fluence while $400\text{-}500 \text{ mJ cm}^{-2}$ of applied fluence were required to achieve similar removals in two surface waters (Figure 6a) [14]. While UV disinfection typically occurs at fluence values of less than 100 mJ cm^{-2} , a full scale UV/H₂O₂ oxidation process at a Dutch drinking water

treatment plant designed for pesticide removal utilizes UV fluence values on the order of ~ 600 mJ cm^{-2} [25].

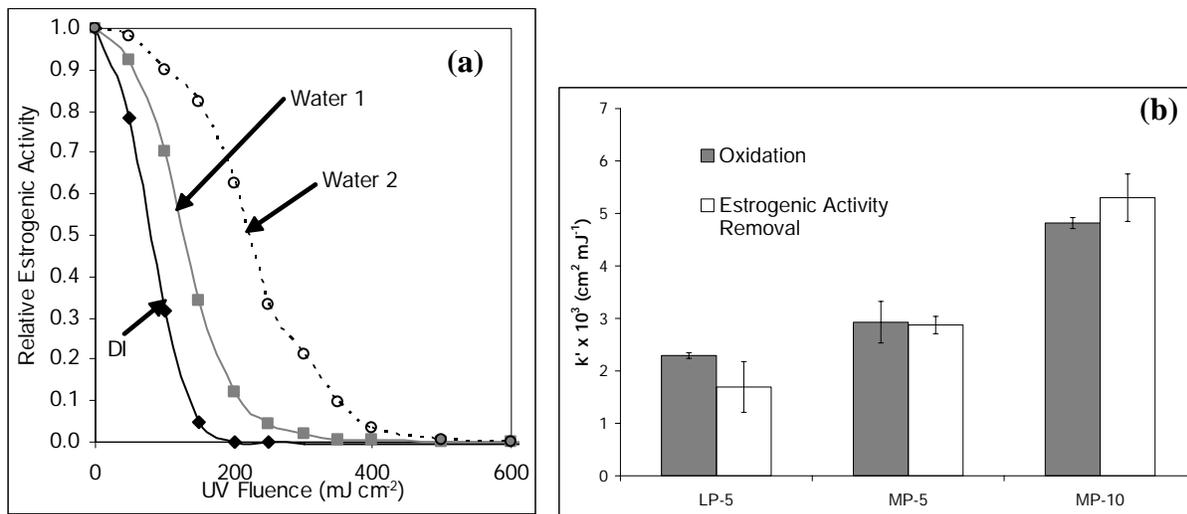


Figure 6: (a) UV/H₂O₂ removal of estrogenic activity in DI water and 2 natural waters and (b) comparison of EE2 oxidation and estrogenic activity removal rates with low pressure (LP) and medium pressure (MP) UV/H₂O₂ processes; value after LP or MP signifies H₂O₂ concentration in mg/L (from [14])

In some instances (e.g., EE2 (Figure 6b) [14] and sulfamethoxazole and SMZ [10]) removal rates of the parent BAC and the associated biochemical activity are identical; however, this lock-step behavior does not always occur. In [9], removal of estrogenic activity as measured with the *in vitro* YES assay was slower than oxidative removal of BPA. When an *in vivo* assay measuring vitellogenin induction in male fish was utilized to detect removal of estrogenic activity associated with BPA, the disparity was more pronounced. Additionally, an increase in larval mortality was observed at certain UV/H₂O₂ treatment conditions. The paper reiterates that although UV/H₂O₂ is effective in oxidizing BACs, the byproducts may still be harmful.

Water quality can be improved with UV/H₂O₂ treatment if we can show that BAC oxidation products are readily biodegradable at environmentally relevant concentrations. Oxidation processes yield intermediates that are more biodegradable than the parent compounds, and this concept is being exploited in the treatment of industrial wastewaters. By 2001, more than 100 literature examples were available indicating the plausibility and utility of sequential chemical and biological oxidation processes for the treatment of wastewaters containing recalcitrant pollutants such as effluents from textile mills, paper mills, tannery, and olive mills, pesticide wastewaters, etc. [26]. However, little is known to date about the fate of oxidation intermediates of organic compounds that are present at trace levels in municipal wastewaters. The proposed work will fill this gap, examining how photooxidized BAC intermediates are biodegraded by natural consortia of microorganisms.

Training Potential

It is anticipated that **one PhD student** (Carolina Baeza) will be trained in the field of Environmental Engineering by participating in this project. This student has been supported on an NSF Graduate Research Fellowship and is now on an NWRI fellowship that will continue to

provide partial support throughout the course of the proposed project. In addition, **one visiting scholar** (Erik Rosenfeldt) will contribute to this project. The PI has worked and continues to involve one to two undergraduate research assistants in ongoing research project. It is anticipated, that **one undergraduate student** will be trained by the proposed research project.

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Curriculum Vitae

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Education

Ph.D. Environmental Engineering	University of Illinois, Urbana, IL	January 1996
M.S. Environmental Engineering	University of Illinois, Urbana, IL	May 1991
B.S. Civil Engineering	University of Illinois, Urbana, IL	May 1989

Professional Experience

2002 – present Associate Professor, Dept. of Civil, Construction, and Environmental Engineering, NC State University

2005 (7/1-12/31) Visiting Scholar, EAWAG, Swiss Federal Institute of Technology (ETH) Zürich, Switzerland

1996 - 2002 Assistant Professor, Department of Civil Engineering, NC State University

Research Interests

Trace organic contaminants, adsorption processes, advanced oxidation processes, coagulation, effects of algae on water quality and treatment processes, natural organic matter, bioavailability, fate of organic contaminants in landfills.

Teaching Interests

Physical/chemical fundamentals of environmental engineering processes, water treatment plant design, unit operations and water chemistry laboratories.

Recent Publications (Water-Treatment-Related)

1. Baeza, A.C. and D.R.U. Knappe. "Removal of Sulfonamides and Associated Antimicrobial Activity by UV Photolysis and UV/H₂O₂ Processes." In *Proc. 2006 AWWA Annual Conference and Exhibition*, San Antonio, TX, June 11-15, 2006.
2. Knappe, D.R.U. "Chapter 9 - Surface Chemistry Effects in Activated Carbon Adsorption of Industrial Pollutants." In *Interface Science in Drinking Water Treatment – Theory and Applications*, Newcombe, G. and Dixon, D. (Eds.), Academic Press: Oxford, UK, 2006.
3. Quinlivan, P.A.; L. Li; and D.R.U. Knappe. "Effects of Activated Carbon Characteristics on the Simultaneous Adsorption of Aqueous Organic Micropollutants and Natural Organic Matter." *Water Research*, 39(8): 1663-1673, 2005.
4. Li, L.; P.A. Quinlivan; and D.R.U. Knappe. "Predicting Adsorption Isotherms for Aqueous Organic Micropollutants from Activated Carbon and Pollutant Properties." *Environmental Science and Technology*, 39(9): 3393-3400, 2005.
5. Knappe, D.R.U. and A.A. Rossner Campos. "Effectiveness of High-Silica Zeolites for the Adsorption of Methyl Tertiary-Butyl Ether from Natural Water." *Water Science and Technology: Water Supply*, 5(5): 83-91, 2005.

6. Badruzzaman, M.; P. Westerhoff; and D.R.U. Knappe. "Intraparticle Diffusion and Adsorption of Arsenate onto Granular Ferric Hydroxide (GFH)." *Water Research*, 38(18): 4002-4012, 2004.
7. Knappe, D.R.U.; L. Li; P.A. Quinlivan; and T.B. Wagner. *Effects of Activated Carbon Characteristics on Organic Contaminant Removal*. Awwa Research Foundation: Denver, Colorado, 2003.
8. Li, L.; P.A. Quinlivan; and D.R.U. Knappe. "Effects of Activated Carbon Surface Chemistry and Pore Structure on the Adsorption of Organic Contaminants from Aqueous Solution." *Carbon*, 40(12): 2085-2100, 2002.
9. Newcombe, G.; J. Morrison; C. Hepplewhite; and D.R.U. Knappe. "Simultaneous Adsorption of MIB and NOM onto Activated Carbon: II. Competitive Effects." *Carbon*, 40(12): 2147-2156, 2002.

Selected Current Research Projects (Water-Treatment-Related)

1. Removal of antimicrobial compounds and their associated biochemical activity by UV/H₂O₂ processes. Student support through NSF Graduate Fellowship and NWRI Fellowship.
2. Removal of 2-methylisoborneol and geosmin with high-silica zeolites and zeolite-enhanced ozonation. PI: Knappe, D.R.U. Agency: AwwaRF (\$150,000; 1/1/06 – 12/31/07).
3. Evaluation of Computational Fluid Dynamics (CFD) for Modeling UV-Initiated Advanced Oxidation Processes. PI: Ducoste, J.J., Co-PI Knappe, D.R.U. Agency: AwwaRF (\$150,000; 1/1/06 – 12/31/07).
4. Predicting Single-Solute Adsorption Isotherms for Non-Regulated Contaminants from Fundamental Adsorbent and Adsorbate Properties, PI, U.S. EPA (\$65,000; 8/16/03 – 9/30/06).

Professional Activities, Honors, and Awards

Professional Society Memberships:

American Society of Civil Engineers, American Water Works Association, International Water Association, American Chemical Society, American Geophysical Union, Association of Environmental Engineering and Science Professors.

Registration:

EIT, Illinois

Honors and Awards:

- 2006 AWWA Water Science and Research Division Best Poster Award for poster entitled "Removal of Sulfonamides and Associated Antimicrobial Activity by UV Photolysis and UV/H₂O₂ Processes"
- 2003 Bill Horn Kimley-Horn Faculty Award for excellence in graduate and undergraduate teaching and other accomplishments, NC State University
- 2001 AWWA Water Science and Research Division Best Paper Award
- 2000 Young Civil Engineer Achievement Award, University of Illinois.
- Thesis advisor of David Briley whose MS thesis entitled "Optimization of Coagulation Conditions for the Removal of Algae in Conventional Water Treatment" won 2nd place in the 1999 AEESP/Montgomery Watson Master's Thesis Award competition.

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

Basic Information

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

Publication

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-Forsell 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).

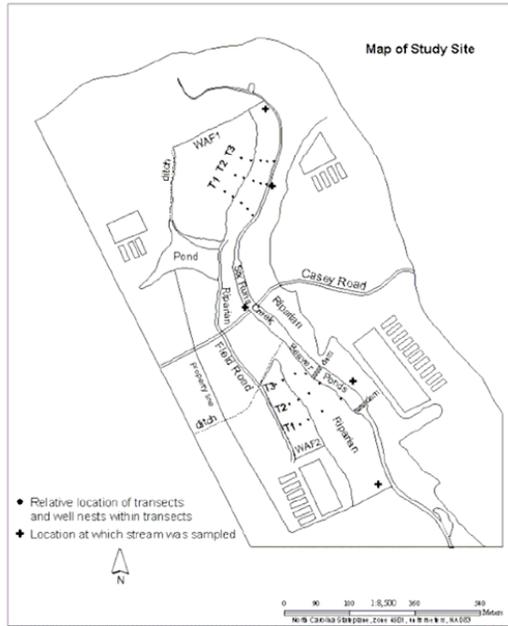


Figure 1. Map of study site. Figure found in Israel et al., 2005

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*), streptomycin/spectinomycin (*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	AATGCCCAGTCGGCAGCG			
<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.

Antibiotics	Concentrations (µg/ml)	No. of Variables
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).

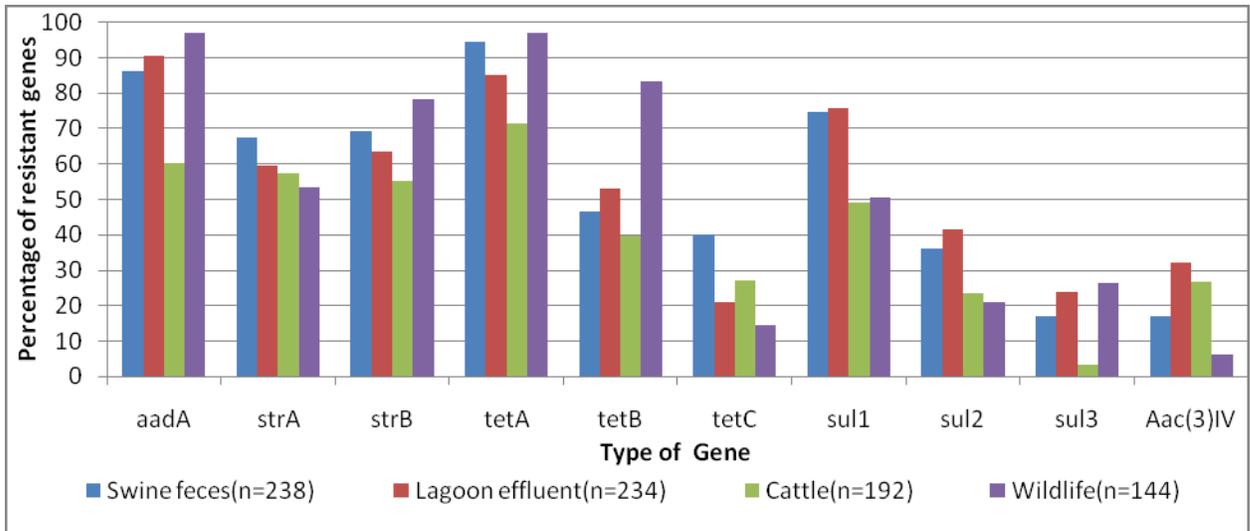


Figure 2: Distribution of antibiotic resistant gene types in known sources.

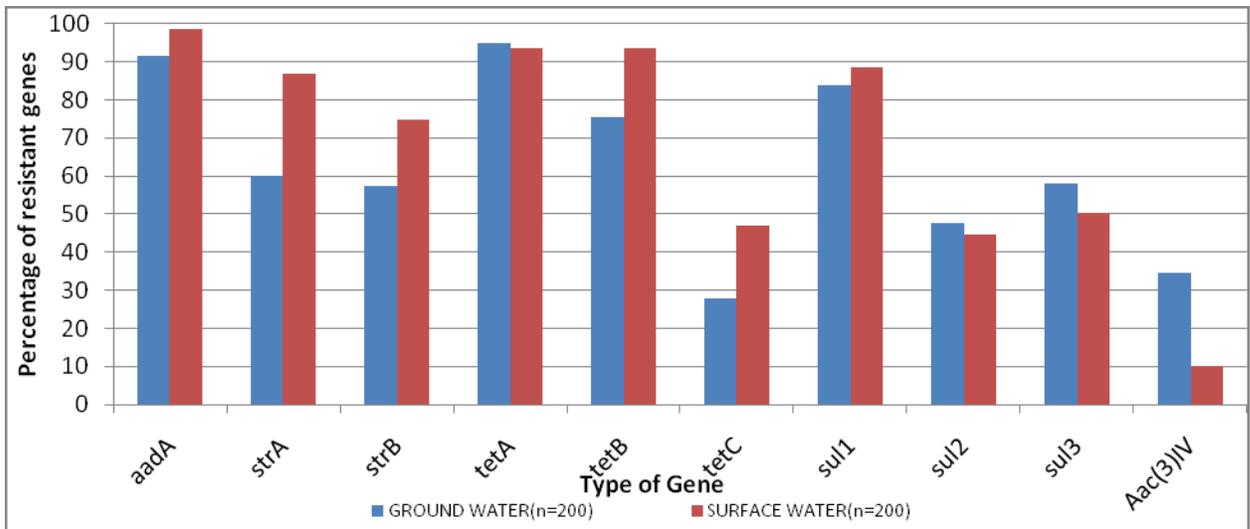


Figure 3: Distribution of antibiotic resistant gene types in environmental sources.

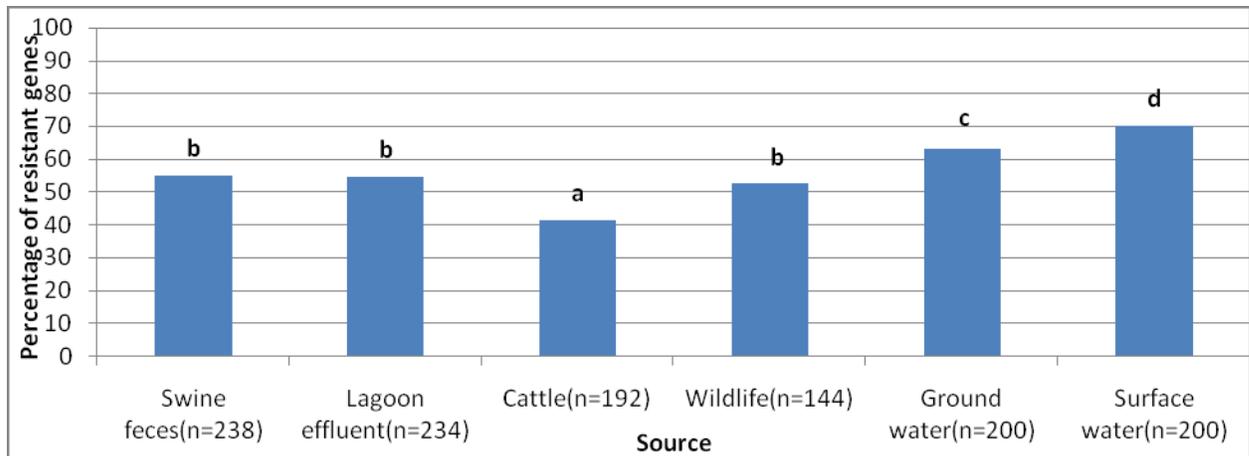


Figure 4: Distribution of antibiotic resistant genes among the sources ($P < 0.05$).

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.

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

Basic Information

Title:	Development of an Analytical Method for Taste and Odor Compounds and Application to NC Drinking Water Sources and Finished Waters
Project Number:	2008NC107B
Start Date:	3/1/2008
End Date:	2/28/2009
Funding Source:	104B
Congressional District:	2
Research Category:	Water Quality
Focus Category:	Surface Water, Treatment, Water Quality
Descriptors:	
Principal Investigators:	Detlef Knappe

Publication

Title

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

Problem

Many drinking water utilities face treatment challenges related to the presence of taste and odor (T&O) compounds in their water source. During a taste and odor episode, a sensitive instrumental method that can quickly and reliably identify and quantify common T&O compounds will greatly aid the selection of effective treatment strategies. Many compounds have odor threshold concentrations (OTCs) in the low ng L⁻¹ range and some even in the sub-ng L⁻¹ range (e.g. geosmin has an OTC of 4 ng L⁻¹ and 2,4,6-trichloroanisole and 2,4,6-tribromoanisole have OTCs of 0.03 ng L⁻¹).

Research Objectives

One objective of the proposed study is to extend a GC-MS/MS method for MIB and geosmin, which we are currently using in our laboratory, to a suite of about ~20 additional compounds that are commonly associated with T&O problems in drinking water. The analytical method includes a headspace solid-phase microextraction (SPME) step to concentrate analytes and has a detection limit of 1 ng/L for MIB and geosmin. The proposed GC-MS/MS method will feature components of previously developed methods (e.g. Scilimenti and Krasner 2003, Young and Suffet 1999), but it is expected to be more sensitive and thus capable of detecting a larger number of T&O compounds because of the added MS/MS component.

A second objective is to apply the analytical method developed in Task 1 to (1) document the identity and concentration ranges of T&O compounds in NC drinking water sources and (2) evaluate the effectiveness of full-scale treatment practices for the removal of taste-and-odor compounds in NC drinking water treatment plants. Distribution system samples will be analyzed as well to identify whether biological processes in the distribution system lead to the production of haloanisoles. Samples will be collected on a bi-weekly basis over an 8-month period at two NC municipalities.

A timeline for the proposed research is shown in Figure 1. Method development (Task 1) will involve a continuing PhD student who developed the GC-MS/MS method for MIB and geosmin in our laboratory. It is anticipated that the PhD student's familiarity with the GC-MS/MS instrument and with MS/MS method development will allow us to quickly expand our existing method to incorporate ~20 additional T&O compounds. In Task 2, we will develop a sampling plan and protocol with two NC municipalities. In Task 3, samples from the two project participants will be collected and analyzed on a bi-weekly basis (first two weeks in each month will be dedicated to utility 1 and second two weeks of each month to utility 2).

Methodology

Task 1: Development of GC-MS/MS Method

Objective: Develop an analytical method, with which common T&O compounds can be identified and quantified to concentrations below their odor threshold concentrations (OTCs).

The analytical method requires effective preconcentration, separation and detection of the T&O compounds. In the preconcentration step, solid phase microextraction (SPME) of T&O compounds from the headspace of a water sample using a 1-cm divinylbenzene/ carboxen/ polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber is carried out at 65°C for 30 minutes. Then the compounds are separated by gas chromatography using a DB5-ms column (30m x 0.25 mm, 0.25 µm film thickness) and the following temperature program: 35°C for 23 minutes, raise temperature to 139°C at a rate of 4 °C/min, raise

temperature to 301°C at a rate of 27°C/min, hold at 301°C for 5 minutes. Finally an ion trap mass spectrometer operating in the positive chemical ionization MS/MS mode is used to identify and quantify the compounds. The instrument used for analysis is a Varian 3800 GC equipped with a CombiPal SPME autosampler and Varian Saturn 2200 tandem mass spectrometer (MS/MS).

Task 2: Develop a sampling plan and protocol with two NC municipalities

Objectives: (1) Identify sampling locations with utility participants. (2) Develop sampling protocol.

Approach: Sampling locations will be identified in collaboration with personnel from two utility participants. The goal is to collect samples such that we can document T&O compound occurrence in the source water and T&O compound fate/generation during treatment and distribution. For example, source water samples may be collected at different depths at the intake structure and at locations at which bloom development is common. In addition, samples will be collected across the treatment train (terminal reservoir/presettling basin, (pre)ozonation, chlorinated filters, clearwell) and at different water ages in the distribution system (including at least one known stagnation point).

Based on results obtained in Task 1, a written sampling protocol will be developed and transferred to personnel at utility participants. The sampling protocol will be based on Standard Methods and will most likely require utility participants to collect duplicate samples in 40-mL EPA vials (headspace-free). Vials will be placed in a cooler and shipped on ice by overnight carrier to the Environmental Engineering Research Lab at NC State University, where samples will immediately be prepared for analysis. To enhance T&O compound stability during sample shipping, sample pH may need to be adjusted to ~pH 4.5 (Khiari et al. 1999).

Task 3: Identification and Quantification of T&O compounds in NC drinking water sources and finished waters

Objectives: (1) Apply the developed GC-MS/MS method to identify and quantify T&O compounds in samples collected from two utility participants. (2) Evaluate the effectiveness of full-scale treatment practices for the removal of taste-and-odor compounds at utility participants. (3) Identify and quantify T&O compounds that may be produced during disinfection or through biological processes in the distribution system

Approach: Samples from two utility participants will be analyzed on a bi-weekly basis for 8 months (see Figure 1 for schedule). The analytical method developed in Task 1 will be used for sample analysis. From the collected data, utilities will learn which T&O compounds are in their source water, which treatment processes are effective for the removal of individual T&O compounds, and which T&O compounds are generated during treatment via the addition of oxidants/disinfectants and during distribution (e.g. biological methylation of halophenols).

Using the data collected in Task 3, we will put together a matrix that will provide utilities with information regarding the effectiveness of treatment processes for the removal of individual T&O compounds. If the proposed study shows that some T&O compounds occur in NC water sources that do not correspond well to common treatment options, a follow-up study could be conducted to identify treatment solutions that can lower the concentration of that compound to a concentration that is below its OTC.

Principal Findings

To date, the following steps in the method development have been completed: (1) compound selection, (2) identification of retention times, (3) identification of parent ion mass, and (4) optimization of MS/MS conditions that maximize production of the quantitation ion. To ensure effective identification of the parent ion masses and retention times of individual T&O compounds, analyses were initially conducted in single-stage MS mode. This was done by SPME analysis of a 10-mL sample containing 2 g of NaCl and 1 µg L⁻¹, 10 µg L⁻¹ or 50 µg L⁻¹ of an individual T&O compound. The total ion chromatogram (TIC, capturing all ions

in the 40-650 m/z range) was used to identify both the retention time and parent ion mass. Next, MS/MS conditions were optimized by varying the resonant excitation amplitude to determine the voltage that most effectively fragmented the parent ion into the desired quantitation ion. Table 1 summarizes parent and quantitation ion masses as well as the retention times that were observed for each compound. The results in Table 1 illustrate that the method is currently able to resolve all compounds except 2,4,6-trichloroanisole and *trans, trans*-2,4-decadienal. Two GC analyses may therefore be necessary to resolve all of the T&O compounds shown in Table 1.

Table 1. Masses of parent and quantitation ions and retention times for 18 taste and odor compounds.

Compound	Molecular weight (g/mol)	Parent ion mass (m/z)	Quantitation ion mass (m/z)	Retention time (minutes)
dimethyl disulfide	94.2	95	80.7	4.911-4.999
<i>cis</i> -4-heptenal	112.17	95	66.8	13.353-13.410
1-heptanal (heptaldehyde)	114.19	96.7	68.7	14.090-14.200
dimethyl trisulfide	126.26	127	92.7	22.380-22.562
6-methyl-5-hepten-2-one	126.2	108.8	66.8	26.740-26.863
<i>trans, trans</i> -2,4-heptadienal	110.15	111	92.8	29.660-30.015
2-isopropyl-3-methoxypyrazine	152.19	153	120.8	34.386-34.473
<i>trans</i> -2, <i>cis</i> -6-nonadienal	138.2	121	92.8	37.483-37.570
2-isobutyl-3-methoxypyrazine	166.22	167	124.8	38.598-38.659
2-methylisoborneol	168.3	151	94.8	38.774-38.846
β -cyclocitral	152.23	153	108.8	40.368-40.425
2,3-benzopyrrole (indole)	117.15	118	90.8	43.409-43.701
2,4,6-trichloroanisole	211.5	211	176,196	44.184-44.217
<i>trans, trans</i> -2,4-decadienal	152.23	153	134.8	44.185-44.350
2,3,6-trichloroanisole	211.5	211	176,196	45.614-45.647
geosmin	182.3	165	108.8	46.978-47.010
β -ionone	192.3	193	174.8	49.225-49.290
2,4,6-tribromoanisole	344.83	345	265.8	50.916-50.980

To complete the method development portion, MS/MS conditions will be optimized for two additional T&O compounds (1-penten-3-one and *cis*-3-hexenyl acetate). Furthermore, several deuterium-labeled compounds will be selected as surrogate standards to assess T&O compound recovery by SPME. Standard curves will then be developed and method detection limits identified. Once method development is completed, we will start working with NC utilities to determine the identity and concentration of taste and odor compounds in their water sources and in their finished waters.

Significance

A highly sensitive analytical method will be developed that will permit the identification and quantification of many common T&O compounds in NC drinking water sources and finished waters. To date, little is known about which compounds cause T&O problems in NC drinking waters, and water treatment professionals typically have to adjust treatment processes on a trial and error basis to improve the T&O quality of the water. Knowledge about which compounds are responsible for T&O problems would allow utilities to tailor

their treatment approaches to the compound(s) involved in a particular T&O episode such that the desired finished water quality is obtained in an effective and economical manner.

Apart from learning which T&O compounds are present in NC source waters, results of this research will also evaluate the fate and potential generation of T&O compounds across full-scale water treatment processes and in drinking water distribution systems. Thus, utilities will not only gain insights into which treatment strategies effectively control T&O problems but also into which T&O compounds may be produced during oxidation/disinfection and/or through biological processes in the distribution system.

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

Basic Information

Title:	Development of the Semi-Empirical Ammonia Deposition and Emission (SEADE) Model for Application to North Carolina Coastal Watersheds
Project Number:	2008NC108B
Start Date:	3/1/2008
End Date:	2/28/2010
Funding Source:	104B
Congressional District:	2
Research Category:	Water Quality
Focus Category:	Acid Deposition, Models, Nutrients
Descriptors:	
Principal Investigators:	Wayne Robarge, Robert Austin, John Walker

Publication

Title

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

Problem

Globally, domestic animals are the largest source (22 Tg N yr^{-1} , $1 \text{ Tg} = 10^{12} \text{ g}$) of atmospheric NH_3 , comprising approximately 40% of natural and anthropogenic emissions combined, while synthetic fertilizers and agricultural crops together contribute an additional $12.6 \text{ Tg NH}_3\text{-N yr}^{-1}$ (23% of total emissions) (Bouwman et al., 1997). Within and downwind of agricultural regions, NH_x 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_3 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_3 emissions, the concentration of NH_4^+ 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_x to eastern North Carolina ecosystems, the magnitude of NH_3 dry deposition remains unknown. Measurements of ambient 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). In a recent study at a swine facility in eastern North Carolina, Walker et al. (2007) estimated that approximately 10% of NH_3 emissions from the barns/lagoon complex were deposited within 500m downwind. Dry deposition rates $> 50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ were predicted for distances $< 100 \text{ m}$ from the emissions complex, which included a forested riparian area. At 500 m from the source, dry NH_3 deposition was $16 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, which was approximately 3.5X wet deposition of NH_4^+ . These results suggests that dry NH_3 deposition exceeds the critical nitrogen load (Kuylenstierna et al., 1998) for ecosystems in the vicinity of animal production facilities. The contribution of NH_3 dry deposition to total N deposition in North Carolina coastal watersheds, however, remains unknown. Without information on the magnitude and spatial variability of NH_3 deposition in mixed agricultural regions (i.e., crop and animal production), assessments of ecosystem health and the potential for nitrogen saturation/eutrophication are incomplete.

Research Objectives

Results from this study will include:

- 1) annual watershed scale estimates of NH_3 dry deposition for the Cape Fear and Neuse River basins;
- 2) seasonal estimates of NH_3 concentration and net air-surface exchange fluxes at 100 m horizontal resolution within the model domain;
- 3) summary of net NH_3 fluxes by primary land use type and across seasons;
- 4) atmospheric nitrogen deposition budget for the Cape Fear and Neuse River basins;
- 5) evaluation of SEADE model version 1 with respect to ambient concentration predictions;
- 6) recommendations for continued model development and future research.

Here we propose a new model for estimating 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. This approach will produce an initial watershed scale estimate of NH₃ dry deposition which takes into account the bi-directional nature of NH₃ 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.

Methodology

In this research we present a new method for predicting ambient NH₃ concentrations and bi-directional air-surface exchange at field to watershed scales in areas where animal production intensity is high. The method proposed includes the development of three main systems: 1) a facility scale NH₃ emissions inventory; 2) a model for predicting atmospheric NH₃ concentrations; 3) and a model for predicting NH₃ net air-surface exchange rates. The model is described in detail in the following sections.

Emissions

Ammonia emissions are calculated for individual animal production facilities included in the North Carolina Department of Environment and Natural Resources, Division of Water Quality 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, and 30,000 or more confined poultry using a liquid waste management system. Annual emission rates are calculated for each facility based on number and type of animals as described below. Annual emissions are then scaled by an average monthly temperature to partition the emissions into a monthly inventory. The results of Walker et al., 2004 show that the NH₃ 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 NH₃ animal⁻¹ yr⁻¹; Asman, 1992), sows (16.43 kg NH₃ animal⁻¹ yr⁻¹; van der Hoek, 1998), and fattening (market) hogs (6.39 kg NH₃ animal⁻¹ yr⁻¹; 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 NH₃ yr⁻¹); 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 NH₃ animal⁻¹ yr⁻¹) and BEF_{MH} is the basic emission factor for market hogs (kg NH₃ animal⁻¹ yr⁻¹). 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.

To account for seasonal variability, annual emissions are monthly allocated by scaling with ambient temperature (Walker et al., 2007). 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 NH₃ emission rate (kg) for month i (E_i), where a_o is the average monthly emission rate in kg NH₃ (annual emission/12). Coefficients α and β 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 (°C)

Atmospheric Concentrations

Atmospheric NH₃ concentrations will be calculated as a function of distance from the nearest animal production facility. Distance algorithms were developed from weekly integrated concentrations measured along horizontal gradients from 10 to 700m downwind of a 5000 animal swine facility (Walker et al., 2007). In this study, a non-linear model of the form

$$NH_3 = aX^{-0.75} \quad (5)$$

is used to predict the ground level NH₃ concentration ($\mu\text{g m}^{-3}$) at each point in the model domain as a function of distance (X in units of m) from each source, 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.

Coefficient a in equation (5) varies by month as a result of seasonality in emissions. The results of Walker et al. (2007) were used to derive the following relationship between the coefficient A and monthly emissions ($R^2 = 0.82$)

$$a_j = 29.0E_j + 35.1 \quad (6)$$

where E_j is monthly emission in units of kg NH₃ at facility j . The intercept was not statistically significant at $p = 0.01$ and is therefore set to zero.

Air-Surface Exchange Fluxes

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 NH₃ (Deerhake et al., 2005). For NH₃, a model framework that recognizes the bidirectional nature of the exchange process is required. In this study, NH₃ 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 NH₃ flux (F_T) to the net emission potential of the canopy (i.e., foliage and soil or water), or surface concentration (χ_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 (χ_o), defined by Nemitz et al. (2001) as

$$\begin{aligned} \chi_c = & [\chi_a (R_a R_b)^{-1} + \chi_s \{(R_a R_s)^{-1} + (R_b R_s)^{-1} + (R_g R_s)^{-1}\} + \chi_g (R_b R_g)^{-1}] \\ & \times \{(R_a R_b)^{-1} + (R_a R_s)^{-1} + (R_a R_w)^{-1} + (R_b R_g)^{-1} + (R_b R_s)^{-1} \\ & + (R_b R_w)^{-1} + (R_g R_s)^{-1} + (R_g R_w)^{-1}\}^{-1} \end{aligned} \quad (7)$$

where χ_a is the atmospheric 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 χ_s is the stomatal compensation point. NH_3 exchange with the ground is described by the soil compensation point (χ_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}).

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

$$\chi(z_0) = \frac{\chi_a R_a^{-1} + \chi_g R_g^{-1} + \chi_c R_b^{-1}}{R_a^{-1} + R_b^{-1} + R_g^{-1}} \quad (8)$$

The net flux may then be calculated as

$$F_t = -\frac{\chi_a - \chi(z_0)}{R_a} \quad (9)$$

The aerodynamic resistance is calculated as a function of the standard deviation of wind direction (σ_θ), and wind speed (u) according to Hicks et al. (1987) assuming that the atmosphere is considered unstable when global radiation (G) exceeds 100 W m^{-2} (Meyers et al., 1998). The boundary-layer resistance is calculated according to Dyer 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 NH_3 transfer (R_s) is assumed equal to that of water vapor (H_2O) corrected for differences in molecular diffusivity. Stomatal resistance to H_2O 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 H_2O transfer, and the applicability of H_2O resistance schemes to other gases, are well established, much less is known about the cuticular resistance (R_w) of NH_3 . Previous studies have shown that cuticular adsorption of 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 NH_3 concentration in air as the cuticle becomes saturated. This has important implications for the work presented here, as very high concentrations of 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 mixed moorland vegetation. 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 to 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 NH_3 , leaves may act as a source or sink depending on the ratio of the ambient concentration to the stomatal compensation point (χ_s) (Farquhar et al., 1980; Husted and Schjoerring, 1995). χ_s is a function of temperature and the apoplastic concentrations of NH_4^+ and 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 NH_3 and NH_4^+ in the soil pore space and fresh/salt water solution establishes a soil/water compensation point, referred to here as χ_g (Dawson, 1977; Nemitz, 2001) with a corresponding specification of Γ_g . For example, fertilized soils, which have a large value of Γ_g , will be a net source of NH_3 in the model, while forest soils or open water, which conversely have small

values of Γ_g , may oscillate between source and sink throughout the year. Thus, the model takes into account bidirectional 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, the most appropriate values have been selected from peer reviewed literature (e.g., references above; Walker et al., 2006; Walker et al., 2007).

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

Model Implementation

Geographic Information System (GIS) software and custom built components are used exclusively in the atmospheric concentration part of the SEADE model. The GIS is used to create, maintain and store the emissions inventory and in the analysis and visualization of model outputs. The custom components compute concentration fields and include ArcInfo scripts and C code executed in batch mode. Because of computational concerns and the possible 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 used in the atmospheric concentration model. These scripts manage the spatial boundaries of the model domain and assure 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 maximize the efficiency of the atmospheric concentration model.

The SEADE modeling system is implemented by first calculating facility scale emissions, followed by calculation of ambient NH_3 concentrations, followed by calculation of net air-surface exchange fluxes. As described above, a concentration is predicted at each point within the model domain for each facility. The maximum value is retained for each prediction point and the farm and distance recorded. Next, the concentration field is converted to raster form and the air-surface exchange rate is calculated at each cell by applying land use parameters, meteorological data, and concentrations following the framework described above.

In calculating 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.

Output from the proposed analysis will include: 1) annual watershed scale estimates of NH_3 dry deposition for the Cape Fear and Neuse River basins in the form of maps and tables; 2) seasonal estimates of NH_3 concentration and net air-surface exchange fluxes at 100 m horizontal resolution within the model domain in the form of maps and tables; 3) summary of net NH_3 fluxes by primary land use type and across seasons.

Model Evaluation

The emissions inventory will be evaluated by aggregating facility scale emissions up to the county scale and comparing with estimates generated directly from county-level activity data reported by the North Carolina Department of Agriculture and Consumer Services (NCDACS). Because county-level statistics from NCDACS are not developed from permitting requirements, this approach provides an independent evaluation of the NCDQA facility scale database. The physical location of facilities as reported in the NCDWQ database will be cross-checked against georeferenced color photos from the National Agriculture Imagery Program (NAIP). Large farms with multiple animal waste lagoons will be split based on adjacent barn size and number, and facilities with erroneous spatial locations will be corrected using the areal imagery. Where facility and lagoon location is questionable, further clarification will be requested from the NCDWQ.

Performance of the atmospheric concentration model will be evaluated by comparing predictions against measurements from the Carolina Ammonia Monitoring Network (CAMNet), which is operated by investigators Walker and Robarge. CAMNet consists of 24 monitoring sites within the Cape Fear and

Neuse River basins at which weekly integrated NH_3 concentrations are measured at ground level using the ALPHA passive sampler (Tang, 2001). Sites are located at varying distances (100 m to 6 km) from different types and sizes of animal production facilities. Two sites are considered “clean” background sites not directly influenced by emissions from animal facilities.

Potential total uncertainty in predicted air-surface exchange fluxes will be assessed using a sensitivity analysis similar to Cooter and Schwede, 2001. Monte Carlo simulations will be used to examine the sensitivity of flux predictions to uncertainty in individual model inputs. Estimates of measurement precision and accuracy will be used for meteorological inputs and corresponding resistance parameterizations. For parameters such as R_w , I_s^- and I_s^+ , inputs will be varied within the range of reported values. This model evaluation approach will: 1) generate an estimate of potential uncertainty in concentration and flux predictions; 2) identify the most influential model inputs; and, consequently; 3) produce a priority list for future experimental work.

Atmospheric Nitrogen Budget

The final component of the project is to evaluate the importance of NH_3 dry deposition within the context of the complete atmospheric nitrogen deposition budget (Sparks et al., 2007). To do this we will compare annual and seasonal watershed scale estimates of NH_3 dry deposition to the other primary atmospheric nitrogen components, which include: wet deposited NO_3^- , NH_4^+ , and dissolved organic nitrogen; dry deposited NO_3^- and NH_4^+ aerosol, and dry deposited oxidized forms of nitrogen (NOy) including HNO_3 . Data sources include the National Atmospheric Deposition Program/National Trends Network (NADP, 2007) and Clean Air Status and Trends Network (CASTNet, 2007). Estimates of the contribution of organic nitrogen species will be based on the work of Sparks et al. (2007) and Whittall and Paerl (2001).

Principal Findings

To date, work on the collection, validation and parameterization of the facility scale NH_3 emissions inventory is complete. The model for predicting atmospheric NH_3 concentrations is finished and undergoing calibration with newly acquired data from the Carolina Ammonia Monitoring Network (CAMNet) stations (Figure 2). Preliminary results show expected Spatiotemporal patterns of highest concentrations during summer and highest deposition rates in the immediate vicinity of sources (Figure 1). The bi-directional modeling approach captures differences in net exchange rates between systems of high (fertilized crops) and low (forest) nitrogen status.

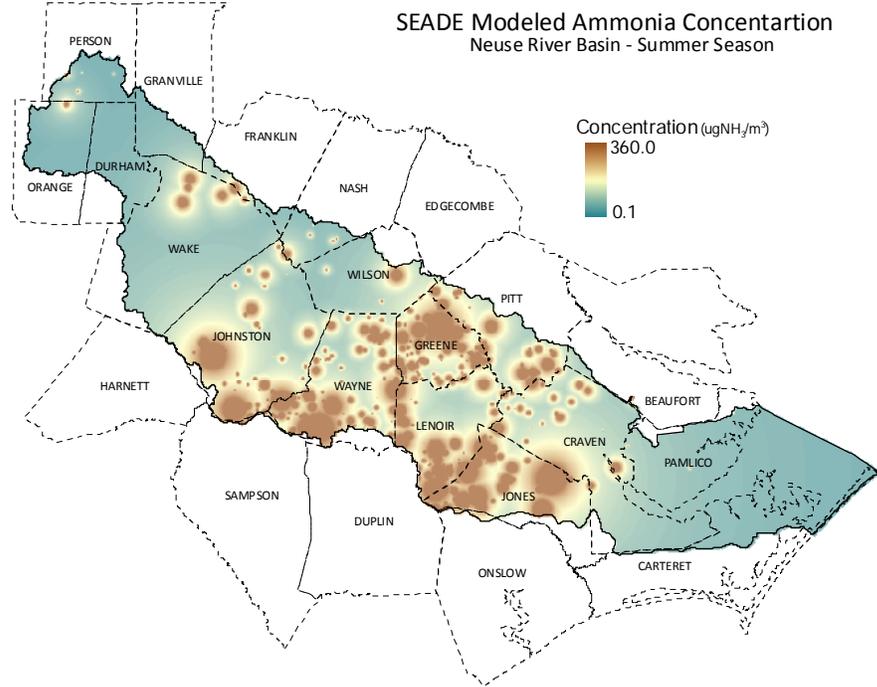


Figure 1: SEADE predicted ammonia concentration in the Neuse River Basin during the summer season

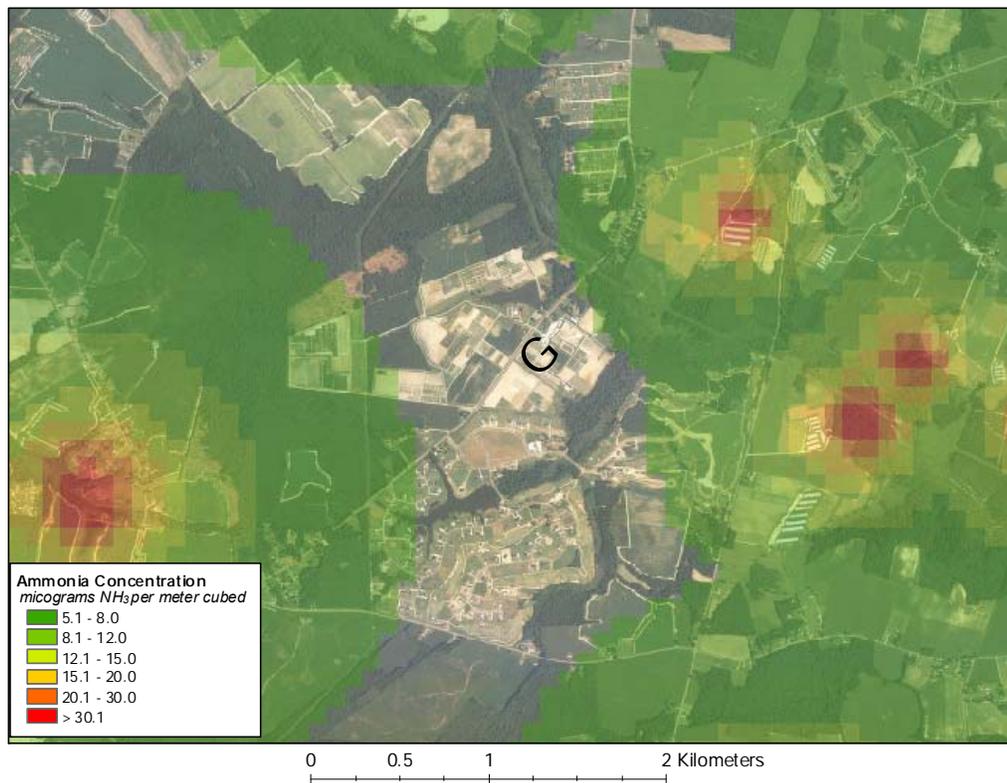


Figure 2: Predicted Ammonia Concentration surrounding Clinton research station for the summer season. The 'X' marks the location of 1 of 24 passive sampler locations used to validate the model output.

Significance

While significant progress has been made in determining wet deposition inputs of NH_x to eastern North Carolina ecosystems, the magnitude of NH₃ dry deposition remains unknown. Measurements of ambient NH₃ 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. (2007) estimated that approximately 10% of NH₃ emissions from the barns/lagoon complex were deposited within 500m downwind. Dry deposition rates > 50 kg N ha⁻¹ yr⁻¹ were predicted for distances < 100 m from the emissions complex, which included a forested riparian area. At 500 m from the source, dry NH₃ deposition was 16 kg N ha⁻¹ yr⁻¹, which was approximately 3.5X wet deposition of NH₄⁺. These results suggests that dry NH₃ deposition exceeds the critical nitrogen load (Kuylenstierna et al., 1998) for ecosystems in the vicinity of animal production facilities. The contribution of NH₃ dry deposition to total N deposition in North Carolina coastal watersheds, however, remains unknown. Without information on the magnitude and spatial variability of NH₃ deposition in mixed agricultural regions (i.e., crop and animal production), assessments of ecosystem health and the potential for nitrogen saturation/eutrophication are incomplete.

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

Basic Information

Title:	Improved Drought Management Strategies for the Triangle Area Utilizing Climate Information based Probabilistic Streamflow Forecasts
Project Number:	2008NC113B
Start Date:	3/1/2008
End Date:	12/31/2009
Funding Source:	104B
Congressional District:	2
Research Category:	Climate and Hydrologic Processes
Focus Category:	Drought, Hydrology, Water Supply
Descriptors:	
Principal Investigators:	Sankarasubramanian Arumugam

Publication

1. 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.
2. Golembesky, K., A. Sankarasubramanian, and N. Devineni (2009), 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, 135(3).

Title

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

Problem

Despite the relative abundance of water in North Carolina (NC), increasing demand at major metropolises make the local and regional water supply systems vulnerable to even moderate drought conditions. Given that most of the existing water supply systems serve multiple purposes, operating these systems to meet the increased water demand often causes frequent shortages resulting in restrictions, even though the supply may be exhibiting its natural variability. For instance, in the Triangle Area in NC, the demand has grown by about 20%-62% from 1995-20002 resulting in three severe droughts/shortages (summers of 2002, 2005 and 2007)⁴ in the past five years. Unless closely monitored using various sector-specific criteria, the impacts of droughts are progressive, persistent (at longer timescales >1 month) and pervasive over a larger area. Uncertainty in seasonal climate, competition among water uses (e.g., water supply versus water quality), and standard operating policies (e.g., fixed rule curves) for allocation pose significant challenges with drought management plans being retroactive or based on impact assessments (monitoring). ***We propose to address these issues in Triangle Area water management by investigating the utility of various proactive water conservation measures that could be invoked contingent on the climate information based streamflow forecasts.***

Multi-year drought during 1998-2002 caused severe hardship and economic losses across most of NC². Several local and state-wide water supply systems experienced record shortages and many communities operated under mandatory water conservation plan during 2001-2003. The triangle area, having close to 10-12% of the population of NC, is currently reeling under exceptional drought with most of the systems reporting mandatory water restrictions. The two major water supply systems in the area, Falls Lake and Jordan Lake, are multipurpose (primarily water supply, water quality and flood control) and the debate is currently on how to control the releases from these systems without significantly affecting the ecological and downstream flow conditions. Despite mandatory water restrictions in the region, record high temperature led to increased water consumption in the region. With 65% probability of having La Nino conditions in the tropical Pacific during the fall and winter of 2007, the situation is quite alarming with the possibility of below normal rainfall conditions during the winter. Given that most of the cities have invested in alternate water management plans such as waste water reuse, it is imperative that proactive conservation measures needs to be triggered before the onset of drought, which could improve system resilience and reduce its vulnerability during critical periods of drought.

Research Objectives

A strategy to improve water allocation in the Neuse basin is proposed by developing a seamless integration climate-information based streamflow forecasts into water systems planning (3 months to 6 months) and operation. The proposed research will develop long-lead probabilistic streamflow forecasts in the Neuse basin contingent on both local land-surface and exogenous climatic conditions. Retrospective streamflow forecasts will be combined with a reservoir management model to understand the utility of streamflow forecasts in operating the Falls Dam. With the decadal variability in the tropical Atlantic Sea Surface Temperature (above-normal conditions) resulting in more hurricanes, it is imperative to develop a prognostic approach for water management in the Neuse basin given the basin accounts for 22% of state's population. Such an approach based on climate information could help water managers to prepare well in advance to reduce the impacts resulting from hydroclimatic extremes.

Methodology

Probabilistic Streamflow Forecasts Development: First, an assessment of different AGCMs' ability to predict both winter (January-March) and summer (June-September) precipitation over the Neuse basin will be investigated. This will be analyzed online at IRI data library (<http://iridl.ldeo.columbia.edu/>). Based on that, the best AGCM will be selected for developing the streamflow forecasting model. Three different approaches can be adopted when developing climate-information based streamflow forecasts: (a) Couple AGCM outputs with a Regional Spectral Model (RSM) whose outputs are combined with a large-scale watershed model¹⁶(b) Statistically downscale AGCM precipitation to streamflow at a particular point of interest^{18,19} (c) Develop a low dimensional statistical model that predicts the streamflow based on dominant climate predictors that influence the streamflow/rainfall potential over the basin¹⁷. Given only one year for this study, we will pursue approaches (b) and (c) to develop climate-information based streamflow forecasts. Coupling of AGCM with RSM and a watershed model will be pursued as future research activities. To pursue approach (b), we will develop different statistical downscaling methodologies to predict streamflow based on the selected AGCM's precipitation grids^{18,19}. To develop a low-dimensional statistical model, detailed diagnostic analyses will be first carried out to identify the dominant predictors that influence the streamflow potential of the Neuse basin. The study will exploit the NC state climatological office's database (<http://www.nc-climate.ncsu.edu/cronos/>) and various other databases including climate Diagnostic Center (<http://www.cdc.noaa.gov/PublicData/>) to perform diagnostic analyses for predictor identification. We will employ state-of-the-art multivariate techniques including independent component analyses (ICA)²⁰ to develop predictors that are independent to each other. Once the predictor set is developed, we will develop retrospective probabilistic streamflow forecasts for the Falls Lake using different statistical approaches including parametric and nonparametric regression techniques. Figure 6 shows an example of a probabilistic streamflow forecasts developed for a reservoir system in Ceara, North East Brazil.

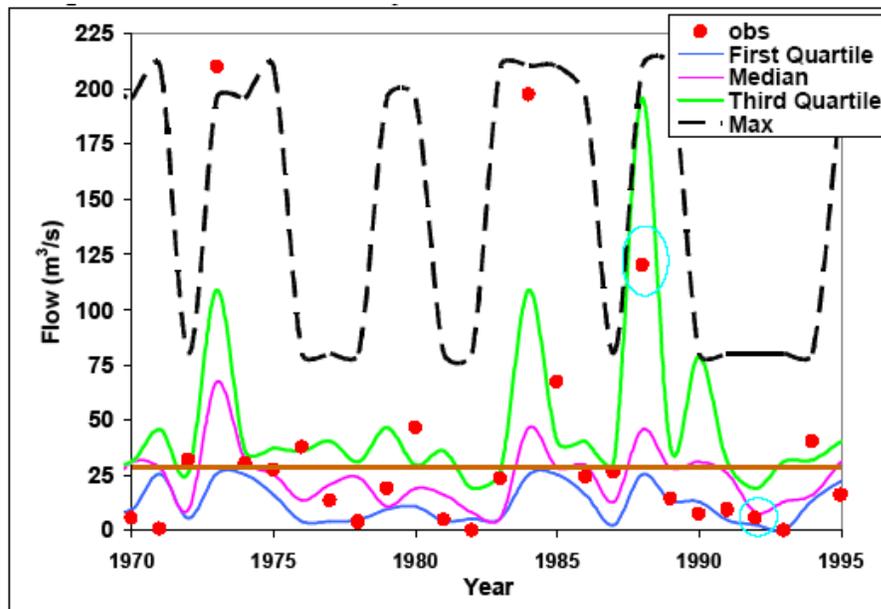


Figure 6: Retrospective, Leave one-out cross-validated 7-months ahead streamflow forecast ensembles for the Oros reservoir, Ceara, NE Brazil¹⁷. The correlation between the observed streamflow and the predicted median streamflow is 0.7 over the period 1949-1996. Predictors employed for this purpose include Nino3.4 and Atlantic Dipole¹⁷. Note the ensembles shift according to the nature of flows for the two circled years in comparison to the long-term average (solid brown line). More than 60% of the mass is above the long-term average for the above

normal inflow year in 1988, whereas in a below normal year in 1992, more than 80% of the mass is below the long-term average.

Reservoir Analyses: To develop a customized reservoir management model for Falls Lake, the study will employ MORAPS, which has been tested on many basins for climate forecasts application. Figure 7 shows a snapshot of MORAPS for representing a multi-reservoir system in the Greater Horn of Africa¹⁴. MORAPS incorporates novel features with the ability to run both retrospective analyses and to perform adaptive analyses of reservoir systems for real-time decision-making. Downscaled streamflow forecasts based on AGCM precipitation can also be used as an input to the model. MORAPS also incorporates a novel contract structure^{11,15} with an ability to perform the analyses under both optimization and simulation modes. Using retrospective streamflow forecasts developed from the study, we will employ MORAPS to identify optimal operating policies for the Falls Dam for reducing downstream flood damages as well as meeting both water quality and water quantity targets.

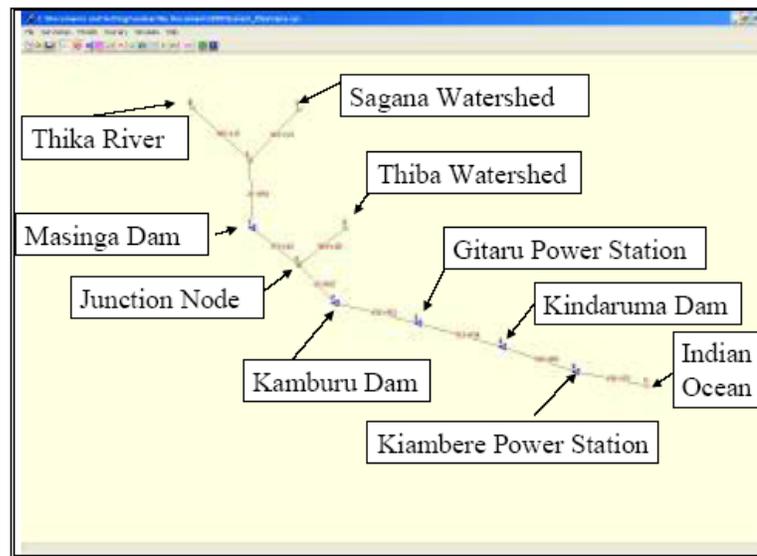


Figure 7: A snap shot of the Tana River 7-Forks Reservoir Management Model in the Greater Horn of Africa shown within MORAPS (Authors: Sankarasubramanian Arumugam, PI of this proposal and Upmanu Lall; copyrighted to IRI, Columbia University).

Dissemination: An important goal of this research is to develop a prognostic approach to improve water management in the Neuse basin, which can help local/state water managers prepare well in advance for mitigation of the impacts resulting from hydroclimatic extremes. In this regard, we would like to invite NC DENR (Division of Water Resources), USACE (Wilmington District) and NC Drought Monitoring Council as external advisory board. In collaboration with them, we plan to perform the retrospective analyses of Falls Lake management contingent on probabilistic streamflow forecasts. A web portal will also be developed as part of the project that will update the long-lead streamflow forecasts for the Neuse basin on a monthly basis. We envisage that this effort for Neuse basin will motivate other basins in NC to incorporate to follow a prognostic, climate-information driven approach towards water management.

Relevance to Other Neuse Basin Initiative and Scope for Future Research

Given the diversity of hydrologic and geographic settings as well as water management issues related to the ongoing developments in the basin, Neuse has been a target basin towards monitoring, understanding and modeling of hydrological processes from both national and state

agencies. Neuse which is located in the Albemarle-Pamlico drainage basin has been identified as one of the 20 selected basins for National Water Quality Assessment Program from the USGS³³. Consortium of Universities for the Advancement of Hydrologic Science (CUAHSI), a collection of Universities for advancing hydrologic science, has identified Neuse as a target basin for setting up hydrologic observatory³⁴. NC state DENR has developed a detailed water management plans to protect water quality and quantity issues in the basin given 22% of state's population live in the Neuse basin^{35,36,37}. Several non-profit organizations, NC Water Resources Research Institute and Neuse River Foundation support many basin wide initiatives^{38,39,40}. Research findings from this proposal will support the above programs in quantifying the uncertainty related seasonal streamflow potential as well as in providing prediction outlooks on floods and droughts. Findings from this research will also support future research initiatives of the PI that includes understanding the linkages between climate variability and ground water availability, utility of climate forecasts in reservoir/lake water quality management to guide the TMDL process and in understanding the importance of policy instruments in forecast applications in water management.

Principal Findings

Three specific objectives were proposed and the progress and the work needs to be completed under each objectives are detailed below:

Objective 1: Develop climate information based streamflow forecasts for the winter (January-February-March, JFM) and summer season (July-August-September, JAS) for the Jordan Lake

Streamflow forecasts using climate information has been developed for both systems (Falls Lake and Lake Jordan). The developed forecasts are obtained by multimodel combination with individual models' forecasts being obtained from statistical downscaling of GCM forecasts and from low-dimensional statistical models having SSTs from tropical Pacific and Atlantic oceans as the predictors. We have also developed a unique multimodel combination algorithm that evaluates the performance of the individual models contingent on the predictor state. This work has been published in Water Resources Research.

(b) Develop a Triangle Area Water Management Model (TAWMM) that explicitly considers the water contracts and downstream releases from both Falls Lake and Lake Jordan.

Two separate reservoir models have been developed and the potential utility of transferring water from Lake Jordan to Falls Lake has been assessed using the forecasts developed for the winter and summer seasons. The developed reservoir models have been validated in simulating the observed storages based on the observed inflows and the initial reservoir levels recorded at the beginning of the season. The utility of climate forecasts in improving Falls Lake management has been published in the Journal of Water Resources Planning and Management.

(c) Combine the retrospective analyses of Jordan Lake with the retrospective analyses of Falls Lake to develop a combined water management strategies for the Triangle region.

Both reservoir models were combined with the streamflow forecasts developed for each basin and a detailed retrospective analysis for transferring the water from Lake Jordan and Falls Lake has been explored. The ability to transfer water has been assessed based on the probability of meeting the target reservoir storage in both reservoirs. For instance, if the probability of meeting the target storage is higher in Lake Jordan, then different scenarios of water transfer from Lake Jordan to Falls Lake is developed such that the probability of meeting the end of season target storage in Lake Jordan does not go below its climatological probability. Our findings show that climate forecasts have great potential in aiding water transfer from Cape Fear River basin to the Neuse basin. It is important to note that the probability of meeting the target storage is based on the water supply storage alone, which implies that the proposed water transfer mechanisms does not affect downstream releases from both systems.

Significance

Benefits/Information from this project will support other ongoing activities in the Neuse including Neuse river basin planning program (supported by DENR), National Water Quality Assessment Program (supported by USGS) and NC Drought Monitoring (supported by Division of water resources, DENR) in coordination with the state's climate office. Analyses from this research will also promote identification of alternate river basin management plans during critical drought conditions including conjunctive use, instream flow maintenance and estuaries management. Informative web portal will be developed that summarizes the hydroclimatic predictability of the Neuse basin as well as updates of streamflow potential for the seasons ahead. Potential implications and its relevance to several ongoing researches in the Neuse basin will include quantitative representation of uncertainty in streamflows to support TMDL process, development of seasonal water management plans considering conjunctive use for the coastal zone and prediction outlooks for floods and droughts. We envisage that this effort for Neuse basin will motivate other basins in NC to incorporate to follow a prognostic, climate-information driven approach towards water management.

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Information Transfer Program Introduction

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 WRI seminars and the Annual Conference. WRI continues to be a sponsor of continuing education credits by the NC Board of Examiners of Engineers and Surveyors. This allows WRI to offer Professional Development Hours (PDHs) and contact hours for attendance at WRI seminars, the Annual Conference, and other workshops WRI sponsors.

NC WRI Information Technology Transfer

Basic Information

Title:	NC WRI Information Technology Transfer
Project Number:	2008NC114B
Start Date:	3/1/2008
End Date:	2/28/2009
Funding Source:	104B
Congressional District:	2
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	
Principal Investigators:	Kelly A Porter

Publication

Information Transfer Program

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

The WRRI Information Transfer Program includes the WRRI Annual Conference, which the institute has done since 1998. The 11th Annual Conference was held on October 8-9, 2008 and was titled "Drought Management and Water Conservation," held at the NCSU McKimmon Center in Raleigh, NC. It is the state's premier water research conference. Many questions about North Carolina's water resources will be addressed through research presented by university and corporate researchers, students, local, state, and federal government agency representatives, and environmental professionals. The Institute's goal is to provide a forum for attendees to become informed and educated on the most current research addressing water resource issues in North Carolina, as well as network and discuss water-related issues with other attendees. More than 250 people attended WRRI's 2008 Annual Conference on October 8 and 9 to hear scientists, economists, engineers, and policy specialists from universities; local, state and federal agencies; and private consulting firms share their latest work. Participants chose from 76 technical presentations covering the full spectrum of water-related issues. Drought management and water conservation was the focus of the 2008 Annual Conference plenary session and luncheon. On October 8th, the conference opened with our Keynote Speaker **Dr. Matthew C. Larsen**, Associate Director for Water at the United States Geological Survey (USGS). His presentation is titled *Water for America: Information for Water Management in the 21st Century*. Dr. Larsen has programmatic responsibility for all water-related research and activities at the USGS including flooding, water quality, drought, climate change and water availability. Our luncheon speaker, **Mr. John N. Morris**, served as director of the NCDENR Division of Water Resources for 28 years, and spoke on: *Given North Carolina's Continuing Growth and the Potential for Recurring Droughts, How Can We Assure Our Future Water Supply?* Over the 1.5 day conference over 60 oral presentations were delivered in the concurrent sessions. The first day closed with a poster and exhibit reception. The Annual Conference continued the morning of October 9th with more water-related technical presentations.

The WRRI Information Transfer Program includes workshops supported by the NC Department of Environment and Natural Resources (DENR), Land Quality Section along with the NC Sedimentation Control Commission (SCC). Workshops held during this period include:

- (1) Two Spring Erosion and Sediment Control Planning and Design Workshop, March 11-12, 2008 in Wilmington, NC, and April 9-10, 2008 in Hickory, NC;
- (2) One Fall Planning and Design Erosion and Sedimentation Control Workshops, February 25-26, 2009 in Raleigh, NC; and
- (3) Erosion and Sedimentation Control Local Programs Training Workshop, January 27-28, 2009 in Southern Pines, NC.

Another way WRI provides Information Transfer is through the North Carolina Water Resources Association (NCWRA) Luncheon and Forums:

- (1) April 7, 2008: "Greening Development to Protect Watersheds: Can Urban Land Use Planning Make a Difference?" Dr. Philip Berke, Professor of City and Regional Planning, Director Center for Sustainable Community Design, Institute for the Environment, UNC, Chapel Hill;
- (2) December 8, 2008: "It Came From Another Continent: Managing Aquatic Invasive Plants," William Frazier, Water Quality Lab and Pretreatment Manager, City of High Point Public Services Dept. and; Robert Richardson, Assistant Professor Aquatic and Non-cropland Weed Mgmt Crop Science Dept. NCSU; and,
- (3) February 16, 2009: "The Grass Is Always Greener Under the Water Sprinkler: The Role of Water Reuse in an Integrated Water Resource Management Approach," Don Safrit, Technical Specialist, McKim & Creed, P.A.

In addition, two seminars were sponsored by Progress Energy to provide Information Transfer:

- (1) April 1, 2008: Water Allocation Law in Southeastern States – "The Southeastern Water World: Where are We, How Did We Get Here & Where Are We Going?" Dr. James E. Kundell, Professor Emeritus, University of Georgia Carl Vinson Institute of Government' and "Water Management Law in North Carolina, 2008 – Present and Prospective," Dr. Milton S. Heath, Jr., Professor of Public Law and Government, UNC Chapel Hill School of Government.
- (2) November 14, 2008: "Comparing Price and Non-price Approaches to Urban Water Conservation," Dr. Sheila M. Olmstead, Associate Professor of Environmental Economics, Yale School of Forestry and Environmental Studies.

WRI maintains six electronic mail lists (listservs):

- (1) Water-Research list - 205 subscribers – inform water researchers from NC universities about calls for papers, grants, upcoming conferences, student internships, etc.;
- (2) WRI-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.

WRI maintains its own website (<http://www.ncsu.edu/wri>). The website provides on-line access to the WRI-News, the WRI technical reports and their summaries, water research seminars, and information on WRI-sponsored workshops, conferences, and seminars. During this fiscal year, WRI continues to make the reports available on the WRI website.

WRI administers the NC Urban Water Consortium (UWC) and meets with its' 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, WRI 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. Currently there are 11 member cities/special districts in North Carolina that met on the following dates: March 20, 2008 – Charlotte; June 30, 2008 – Winston-Salem; September 30, 2008 – Greensboro; and December 10, 2008 – Durham.

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 18, 2008 – High Point; June 12, 2008 – Greensboro; September 25, 2008 – Raleigh; and December 11, 2008 - Charlotte.

WRRR Research Reports

Basic Information

Title:	WRRR Research Reports
Project Number:	2008NC144B
Start Date:	3/1/2008
End Date:	2/28/2009
Funding Source:	104B
Congressional District:	2
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	
Principal Investigators:	Kelly A Porter

Publication

New WRRRI Research Reports – During the year, the Institute published the following reports for distribution to users throughout the state and nation. Reports are now distributed through the WRRRI website. The documents are uploaded into the library repository and available for downloading or printing.

WRRRI-384 – Identifying the Origins and Attachment Behavior of Non-Point Source Microbial Contaminants, by G. Characklis, University of North Carolina at Chapel Hill.

WRRRI-386 – Water Quality Impacts of NC's Largest Wetlands Mitigation Bank, by E. Bernhardt, Duke University.

WRRRI-388 - Neuse River Estuary Fish Monitoring Project: Phase II Monitoring, by Catherine McClellan, Lisa Eby, and Larry Crowder, Duke University Marine Laboratory.

WRRRI-389 – Multi-level Financial Analysis of Residential Water and Wastewater Rates and Rate-Setting Practices in North Carolina, by J. Hughes, University of North Carolina at Chapel Hill.

WRRRI-SR-26 – Designing Hydrologic Observatories: A Paper Prototype of the Neuse Watershed, by K. Reckhow, Duke University.

WRRRI-SR-27 – Determining Inspection and Maintenance Requirements and Cost for Structural BMPs in North Carolina, by B. Hunt, NC State University.

USGS Summer Intern Program

None.

Student Support					
Category	Section 104 Base Grant	Section 104 NCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	7	0	0	0	7
Masters	2	0	1	0	3
Ph.D.	4	0	1	0	5
Post-Doc.	1	0	0	0	1
Total	14	0	2	0	16

Notable Awards and Achievements

Publications from Prior Years

1. 2007NC75B ("Stable Isotope Tracers to Quantify Impervious Area Effects on Baseflow to Coastal Plain Streams") - Conference Proceedings - O Driscoll, M.A. and Allen, T. 2008. Urbanization and the Hydrologic Response to Storm Events. NC Coastal Hazards: The Implications of Severe Storms and Sea Level Rise on Coastal North Carolina. UNC- Coastal Studies Institute Workshop. Jockey s Ridge State Park, May 28, 2008.
2. 2007NC75B ("Stable Isotope Tracers to Quantify Impervious Area Effects on Baseflow to Coastal Plain Streams") - Conference Proceedings - DeLoatch, J.P., O Driscoll, M.A., Brinson, M.M., and Hardison, E.C. 2008. Urban Land-Use Effects on Stream-Groundwater Interactions along Inner Coastal Plain Streams, NC. Geological Society of America National Meeting, Houston, TX (October 2008).
3. 2007NC75B ("Stable Isotope Tracers to Quantify Impervious Area Effects on Baseflow to Coastal Plain Streams") - Conference Proceedings - O Driscoll, M.A., Hardison, E.C., DeLoatch, J.P., and Brinson, M.M. 2008. Indicators of Stream and Riparian Zone Condition In Urbanizing Inner Coastal Plain Watersheds, North Carolina. Geological Society of America National Meeting, Houston, TX (October 2008).
4. 2007NC75B ("Stable Isotope Tracers to Quantify Impervious Area Effects on Baseflow to Coastal Plain Streams") - Conference Proceedings - Harnsberger, D., O Driscoll, M., and Chadwick, J. 2008. The effects of urban land use on water table elevations and nitrate processing in groundwater discharging to Coastal-Plain streams of the Greenville Area. 2nd Annual Research & Creative Achievement Week, March 31-April 4, 2008. East Carolina University, Greenville, NC.
5. 2007NC75B ("Stable Isotope Tracers to Quantify Impervious Area Effects on Baseflow to Coastal Plain Streams") - Conference Proceedings - Harnsberger, D., O Driscoll, M., and Chadwick, J. 2008. The effects of urban land use on riparian water table levels and groundwater nutrient processing along low-order Coastal Plain tributaries, Greenville, NC. Riparian ecosystems and buffers: Working at the water s edge. American Water Resources Association Conference, Virginia Beach, VA. June 30-July 2, 2008.
6. 2007NC75B ("Stable Isotope Tracers to Quantify Impervious Area Effects on Baseflow to Coastal Plain Streams") - Conference Proceedings - Harnsberger, D., O Driscoll, M., and Chadwick, J. 2008. The effects of urban land use on water table and nitrate levels in groundwater discharging to low-order Coastal Plain streams. Geological Society of America SE Section Meeting, Charlotte, NC. April 10, 2008.
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