Iowa State Water Resources Research Institute
Annual Technical Report
FY 2002

Introduction

Research Program

Modeling, GIS, and Technology Transfer in Support of TMDL Development and Implementation in Iowa

Basic Information

Title:	Modeling, GIS, and Technology Transfer in Support of TMDL Development and Implementation in Iowa
Project Number:	2002IA1B
Start Date:	3/1/2001
End Date:	2/28/2003
Funding Source:	104B
Congressional District:	Iowa 3rd
Research Category:	Not Applicable
Focus Category:	Water Quality, Models, Law, Institutions, and Policy
Descriptors:	TMDL, water quality, GIS, modeling, environmental policy
Principal Investigators:	Udoyara Sunday Tim, James L Baker, Ramesh Kanwar

Publication

1. Tim, U.S., 2003. Biophysical modeling for environmental policy decisions: Assessing model creditability and scientific integrity (in preparation).
2. Tim, U.S., 2003. TMDL development and implementation: A review of state water quality programs and TMDL approaches. Technical Report # TMDL-01/0003. Department of Agricultural & Biosystems Engineering, Iowa State University, Ames, Iowa 50011.

Problem and Research Objectives

The federal Clean Water Act (CWA) employs a variety of interrelated management and policy instruments to regulate environmental pollution and protect water quality. These instruments include the National Pollutant Discharge Elimination System or NPDES (33 US C § 1342, CWA section 402), which represents CWA's primary mechanism for achieving and enforcing water quality standards. However, Congress recognized that technology-based effluent controls (generally grouped under point source controls) alone may not be sufficient to enforce applicable water quality standards. Thus, Congress enacted section 303 (d) of the CWA that involves a complex statutory scheme which requires states (herein referred to the collection of states, territories, and authorized tribes) to identify waters where point source controls are insufficient to maintain and improve the water quality standard. The water quality management approach established by Congress forces the states to assess their waters, establish water quality standards based on designated and beneficial uses, prioritize water quality improvement need, and establish total maximum daily loads or TMDLs for such impaired waters and pollutants.

Whereas the CWA requires states to identify waters not meeting quality standards and to develop plans for cleaning them up, the TMDL program, as defined under section 303(d), is designed to determine the maximum amount of pollutant load that a waterbody can absorb and still meet the established quality standard. The program then apportions that maximum load among the various pollution sources (nonpoint and point) in order to facilitate their control. Both section 303(d) and the TMDL program identified a three-step process for moving beyond effluent based controls to more technologically based standards. First, states must establish a list of impaired waters (or water quality limited segments), and this list must identify and priority-rank the waters where point source controls alone would be insufficient to achieve desirable quality standards. Next, for each waterbody identified in the 303(d) list, each state must establish a TMDL or load capacity assessment consistent with the priority ranking in the 303(d) list. Finally, the TMDLs are to be incorporated into, and implemented pursuant to, the state's water quality management plans established under section 303(e) and defined under section 305(b) of CWA. The states are to submit the 303(d) list and the respective calculations for EPA approval. In turn, EPA is required to review the list and TMDLs and either approve or disapprove them as appropriate. If, for example, the 303(d) list and the TMDL implementation plan meet EPA approval, they are then incorporated into the state's water quality management plans. Disapproval, on the other hand, mandates the identification and/or establishment of the TMDL by EPA. Furthermore, if a state fails to establish an acceptable 303(d) list or TMDL, the EPA is also required to intervene and perform the state's duties.

Defined as "the sum of the individual waste load allocations for point sources and load allocations from nonpoint sources and natural backgrounds" (40 CFR 130.2) , the TMDL program forms the basis for developing best management practices (BMPs) for water quality control and plays a key role in stakeholder involvement in watershed management. All stages in the TMDL development process require sound science and the ability to translate complex water quality data into coherent, concise packages so that agencies and stakeholders can understand the issues and evaluate alternative management options. The basis for TMDL development rests on a wide range of factors, including source water assessments, expected ability to meet the TMDL limits, terrestrial and aquatic ecosystem monitoring and modeling, in addition to resource economics. To assist the states, EPA has provided a compendium of tools and models to aid in the TMDL development and implementation plan. However, the reliability of these models for general applications remains questionable. There is still a considerable gap between models developed for the prediction of point and nonpoint source pollution and those that can be used to support TMDL analyses and load allocation. Consequently, many in the production agriculture and natural resource management communities have expressed concerns over the lack of science behind the TMDL modeling and planning process. For example, the former Secretary of Agriculture, Mr. Dan Glickman and many others have expressed concern over the TMDL program. In particular, Mr. Glickman stated: "*the USDA is concerned about the science being used in assessing and attributing the effects of nonpoint source pollution. These models have a high degree of uncertainty and there are gaps in the data regarding what is natural background pollution versus what is caused by human activity.*" Given these uncertainties, there is a critical need for an objective evaluation of existing ecological models and analytical tools that are used in the TMDL development. There is equally a need for a concise demonstration of how these models can be used to establish quantitative measures of the relationship between pollutant sources and water quality impacts. Equally critical is the need to suggest possible areas of improvement of these models for a more proactive and adaptive implementation of the TMDL program by stakeholders.

The problems faced by states in developing TMDLs vary widely across water quality issues and problems. Efficient and equitable development of TMDL requires a sound scientific and technical base and appropriate tools not currently available. Successful water quality management in the United States has always depended on applying good science and on the efficacy of modeling techniques. This research was established to provide enhanced decision support for TMDL analysis by: (1) critically evaluating existing terrestrial and aquatic models and TMDL planning tools to insure that they are based on sound science and are used in a sound manner; (2) providing tools for estimating waste loads associated with biological pollutants under different watershed conditions and management practices; (3) developing objective criteria for choosing among models, data sources, and implementation plans based on the priorities of all stakeholders; and (4) demonstrating the use of integrated models for assessing the potential ecological benefits of TMDL implementation at the watershed scale. The specific project objectives are:

1. To undertake a science-based evaluation of existing models for their use in TMDL development and implementation and suggest areas for future refinements.

2. To integrate algorithms developed for waterborne pathogens (specifically bacteria) into the SWAT biophysical model to facilitate use in development of nutrients and microbial TMDLs in tiled drained watersheds in Iowa.

3. To assess the potential ecological and water quality benefits of TMDL implementation in an agriculturally dominated watershed in Iowa to serve as a case study.

The overarching goal of the project is to enhance the effectiveness of watershed water quality management efforts and improve the scientific basis and computer models for TMDL development and implementation. The products from this research should increase the likelihood of acceptance of the TMDL process by regulators and stakeholders and help assure that the entire TMDL development process meets the desired water quality goals.

Methodology

Objective 1: To undertake a science-based evaluation of existing models for their use in TMDL development and implementation and suggest areas for future refinements.

Several recent reports have identified many inconsistencies in the methodologies used by states in their TMDL program and the lack of sound scientific principles in the choice of models for prediction pollutant loads. In a report titled “Water Quality—Inconsistent State Approaches Complicate Nation’s Efforts to Identify Its Most Polluted Waters” the General Accounting Office (GAO) concluded that “states have developed varied approaches to setting water quality standards, monitoring water quality, and assessing water quality data to make listing determinations” and recommended that the EPA “provide additional guidance to the states on carrying out the key functions….that influence how states identify the waters for their section 303(d) lists” (GAO, 2000). Another report by the National Research Council (NRC) examined the scientific basis of the TMDL program and recommended development of mathematical models that can more effectively link environmental stressors to biological responses of ecosystems (NRC, 2001). Yet another report by the EPA’s Federal Advisory Committee (FACA) recommended that EPA’s top priorities for science and model development should include improving monitoring and modeling capabilities and providing technical assistance to the states (EPA 2002). These reports and other similar studies point to the need for a more rigorous, unbiased review of the scientific basis of the TMDL program, particularly the methodologies and models used in characterizing pollution sources and pollutant loads.

As a major component of this research project, our goal was to conduct a comprehensive yet critical review of existing water quality models that are used in the TMDL program and to identify their strengths and weaknesses. Some of the issues addressed in the research included the following: What are the scientific principles behind the existing terrestrial and aquatic ecosystem models? Is the philosophical basis of these models in concert with current scientific understanding of biotic and abiotic processes? Does the model address the environmental attributes that are required in the TMDL program? Are the existing water quality models supported by data routinely collected from monitoring programs? What are the physical, biological, and chemical processes incorporated into these models?

Indeed, the past decades have witnessed an increased interest in the use of ecological models in a wide variety of applications. With this explosive growth in model development have come increased concerns about the models' suitability and reliability in policy situations. Users of ecosystem models have begun to ask very specific questions such as: How can we tell if a model of a highly complex ecosystem is a good model for the existing conditions? Is the use of a simple model instead of a complex model justified? How can we judge the relative merits (strengths and limitations) of different models? What is the "best available" ecological model, given the nature of the landscape and waterscape? How do we judge the reliability of the predictions that models provide? Modelers and stakeholders alike are interested in identifying terrestrial and aquatic ecosystem models that are optimal for applications in the TMDL program.

In evaluating and identifying a suitable terrestrial and aquatic ecosystem model for TMDL development, particularly the prediction of pollutant loads, many factors must be considered. In addition to those questions identified earlier, other issues prevail. For example: What is the appropriate scale of resolution—temporal and spatial? What are the various uncertainties associated with the data and the model? How can these model uncertainties be quantified and applied to the estimation of total maximum pollutant load? This research focused on addressing some of these issues by creating a model evaluation protocol with the goal of identifying "best available" models for use in TMDL development under varying landscape conditions and management regimes.

The steps used in the model evaluation process include assembling, through comprehensive review of the literature (e.g., reports, proceedings, and compendia), candidate models that are available for predicting pollutant fate and transport in terrestrial and aquatic environments. Other sources of information on models include Web-based digital libraries and resource agency publications. Through these sources, about 250 different terrestrial models and 60 aquatic ecosystem models having different spatial and temporal resolution and philosophical principles were identified. From this list about 130 terrestrial and 35 aquatic ecosystem models were selected. At this stage of model evaluation, a model was removed from further consideration if it failed to meet prescribed qualitative benchmarks (e.g., technical support, ease of use, documentation, availability, etc.).

The second phase of model evaluation involved assembling basic information on each model and developing a set of evaluation criteria and matrix. The evaluation criteria involved 30 different philosophical, scientific, and technical aspects upon which to judge the merits of a model. Examples include: modeled biophysical processes, spatial and temporal scale, model documentation, availability of model source code, modifiability of model source code, level of technical support, the availability of documentation, ease of use, and data requirements. For each criterion, an evaluation matrix that consisted of ratings (ranging from 0 to 5) was used to derive a cumulative score for a model. A model whose score exceeded a specified cumulative value was identified for a third-level, more rigorous and critical review. This third level of model evaluation focused on model applicability to the TMDL program, ease of model refinements, and many other software engineering issues. Evaluation criteria considered under this level of model review include: Does the model provide reliable simulation of the water quality constituents required in the TMDL program? Do the modeling components allow for simulation of watershed with mixed land use? How does the model handle low flows or storm events? Is the model robust and scientifically defensible? Is the model implementation commensurate with available resources and technical expertise? How explicit are the modeling and parameter uncertainties treated in the model? Through this process 10 terrestrial models and 7 aquatic ecosystem models were identified as "optimal" models for the TMDL program.

The fourth and final level of model evaluation involved examination of the critical components of each of the "optimal" models for their applicability to the estimation of daily pollutant loads. We also examined the potential for refinement of each model to address some of the limitations identified in the model review. Since the majority of the TMDLs require estimation of pollutant loads from nonpoint sources, the models selected were those that are watershed-based or can be integrated with standard graphical interface with tools such as the geographic information systems (GIS). The models that we recommended were those that can easily be linked, either loosely or closely, with an aquatic ecosystem (or receiving water) model to provide a more comprehensive, integrated tool for pollutant load estimation in receiving waterbodies. Details of the evaluation process including the evaluation criteria and matrix can be obtained from the PIs.

Objective 2: To integrate algorithms developed for waterborne pathogens (specifically bacteria) into the SWAT biophysical model to facilitate use in development of nutrients and microbial TMDLs in tiled drained watersheds in Iowa.

Comprehensive models that not only predict the fate and transport of toxic substances in agro-ecosystems are needed for the implementation of the TMDL program. Newer, faster computers have made possible the development of sophisticated and highly complex models that predict the movement of nutrients and pesticides in fields and watersheds. However, very few of these models have incorporated functional components that predict microbial fate and transport in terrestrial environment. Furthermore, for many of the existing models that estimate bacterial transport, very simplified functional relationships are used. For example, the movement of microbial pathogens has been simulated assuming standard biophysical relationships developed for non-conservative pollutants. The modeling of microbial fate and transport in urban and rural landscape require significantly different approaches, as well as process-based functional relationships, from those used for conventional pollutants such as nutrients and pesticides.

In this research, we developed a process-based component functional model for incorporation into the Soil and Water Assessment Tool or SWAT model (Arnold et al., 1998) to provide a more comprehensive and robust analytical tool for developing TMDLs for watersheds impaired by both chemical and biological pollutants. Constitutive equations that govern the fate and transport of microorganisms (bacteria) in soil and water were developed based on governing abiotic and biotic processes in agricultural landscapes.

The SWAT model is the latest of the family of field-scale and watershed-scale models developed by researchers at the Agricultural Research Services of the U.S. Department of Agriculture. In particular, SWAT was developed as a replacement to the SWRRBWQ model designed to evaluate hydrology and water quality of agricultural fields under different management practices and the ROTO model that allows simulation of subsurface hydrology. The SWAT model predicts the effects of agricultural management, climate, reservoir management, groundwater withdrawals, and water transfer on hydrology, sediment transport, and chemical yields on large agricultural watersheds. In terms of spatial scale, SWAT can be used to analyze watersheds and catchments of up to 100 square miles by subdividing the landscape into homogeneous land units. Temporally, simulations with the SWAT model can be performed on an event basis or continuously on a daily basis for up to 100 years.

A unique feature of the SWAT model that differentiates it from the SWRRBWQ model is subdivision of the watershed or land area into subunits or subwatersheds and the further division of these subunits into smaller homogeneous areas or hydrological response units (HRUs) according to spatial variability of soil, topography, and land cover. The SWAT model is designed to preserve the spatially-distributed parameters of the entire watershed as well as the homogeneity of the subwatersheds. From the biogeochemical cycling perspective, the components of the SWAT model include: hydrology, which estimates water budget and incorporates components such as weather, surface runoff, return flow, percolation, evapo-transpiration, transmission losses, ponds and reservoir storage, crop growth, irrigation water transfer, groundwater flow, and channel routing; sediment yields including erosion from agricultural land management; soil temperature; climate; and agricultural chemical transport, which predicts the fate, cycling, and transport of nitrogen, phosphorus, and pesticides in soil and water. The primary inputs required by the model include weather (e.g., daily precipitation, daily maximum and minimum temperatures, solar radiation, and relative humidity), soils, topography (e.g., land elevation), vegetation cover, and agricultural land management practices. With these inputs, SWAT simulates standard water quality parameters such as total nitrogen concentration, peak flow, runoff volume, and sediment yield.

Objective 3: To assess the potential ecological and water quality benefits of TMDL implementation in an agriculturally dominated watershed in Iowa to serve as a case study.

Under this objective, our primary goal was to demonstrate the application of an ecological model as a tool for the TMDL program through a simplified case study. Because sufficient data was not available to check the microbial fate and transport equations assembled under Objective 3, we decided to examine the performance of the SWAT model in predicting nutrient load in a relatively large agricultural watershed. The modeling experiences obtained from this case study mirror similar experiences of state resource managers. Thus, our observations matched those of other researchers and resource planners and identified the limitations in many of the widely used watershed water quality models including SWAT. Below is a description of the research methods and results.

SWAT Modeling. As described previously, SWAT is a biophysical, semi-distributed, continuous, daily time step model designed to simulate water yield, sediment delivery, and nutrient and pesticide loading from large, ungauged watersheds. The model uses datasets typically available from government agencies. It is capable of predicting the relative impact of agricultural management and land use over long time periods. The SWAT model is also equipped with a pre- and post-processing interface that is built upon the ArcView GIS interface system.

The GIS interface of SWAT is set up as an extension of ArcView®. This configuration gives the interface the flexibility to use special features available in other ArcView® extension packages. The ArcView SWAT version of the model allows geo-referenced data to be pre-processed for entry into the model. After model simulation, the GIS component post-processes the model output and displays the data as graphics, charts or tables. The key processes, which impact water quality, are discussed below.

Hydrology. The water balance is the basic hydrodynamic component of the model. The water balance equation used is:

$$SW_t = SW_0 + \sum(R_{day} - Q_{surf} - E_a - w_{seep} - Q_{gw})$$

where SW_t is the final soil water content (mm water), SW_0 is the initial soil water content (mm water), R_{day} is the amount of precipitation for the day (mm water), Q_{surf} is the amount of surface runoff for the day (mm water), E_a is the amount of evapo-transpiration for the day (mm water), w_{seep} is the amount of water entering the vadose zone from the soil profile for the day (mm water), and Q_{gw} is the amount of return flow for the day (mm water). Because SWAT uses a daily time step, the water balance is calculated every day of the simulation period. The predicted water yield from a given land area is important because it determines the concentration of pollutants being removed from the land area. The major component of water yield is surface runoff. The quantity of surface runoff impacts the amount of soil and chemicals transported to a receiving waterbody.

Sediment Yield. The predicted soil erosion rate and sediment yield is calculated for each hydrologic response unit (HRU) with the Modified Universal Soil Loss Equation (MUSLE) (Williams, 1975). This equation uses surface runoff volume and peak rate to predict erosion rate and sediment delivery from small watersheds. MUSLE is derived from the Universal Soil Loss Equation (USLE) developed by Wischmeier and Smith (1978). The MUSLE equation adapted for use in the model is:

$$Sed = 11.8 \cdot (Q_{surf} \cdot q_{peak} \cdot area_{hru})^{0.56} \cdot K_{USLE} \cdot C_{USLE} \cdot P_{USLE} \cdot LS_{USLE}$$

where *Sed* is the sediment yield (metric tons), 11.8 is a unit conversion constant, Q_{surf} is the surface runoff volume (mm water/ha), q_{peak} is the peak runoff rate (m^3/s), $area_{hru}$ is the area of the hydrologic unit area (HRU) in hectares, K_{USLE} is the USLE soil erodibility factor, C_{USLE} is the USLE cropping and management factor, P_{USLE} is the USLE conservation support practices factor, and LS_{USLE} is the USLE slope length and steepness factor. The Q_{surf} and q_{peak} are calculated every day precipitation occurs. If surface runoff occurs, then sediment yield is calculated for that day. Because crop growth affects Q_{surf} and q_{peak}, C_{USLE} is also updated daily to reflect changes in the plant growth and land cover.

Crop Growth. Crop growth is simulated in SWAT by using the modeling approach used in the Erosion Productivity Impact Calculator (EPIC) model (Williams et al., 1983). The EPIC model allows for the variation in growth for different plant species and variation due to climate and growth conditions.

Nutrients. Nitrogen (N) and phosphorus (P) management and movement are simulated in SWAT using the modeling approaches in the GLEAMS model (Leonard et al., 1987). Thus SWAT simulates the movement and transformations of nitrogen between two mineral (ammonium and nitrate) and three organic (active, stable and fresh) soil nitrogen pools. Monitoring three mineral (labile in solution, labile on soil surface and fixed in soil) and three organic pools (active, stable and fresh) of soil phosphorus simulates soil phosphorus movement and transformation.

SWAT Modeling Database. The modeling database consisted of those elements that represent the agricultural landscape and the spatially varying characteristics of land use, land cover, and climate. The landscape terrain was represented by the digital elevation model or DEM, a graphical representation of the land slope steepness and aspect (direction). The DEM is prepared as a 30-meter grid polygon format. Each “cell” of this 30-meter by 30-meter grid is given a single elevation value. This GIS coverage (Figure 1) determines watershed and sub-basin (subwatershed) boundaries and can be used to derive hydrologic parameters including land slope, aspect, and flow accumulation. The DEM is available through the Iowa Department of Natural Resources Geological Services Bureau (IDNR-GSB).

The digitized streams are line representations of accumulated perennial water flow over the soil surface. This coverage is important for the routing (i.e. movement and transformation) of runoff and pollutants originating in the watershed. The stream coverage was created by the hydrologic modeling component of SWAT utilizing the DEM.

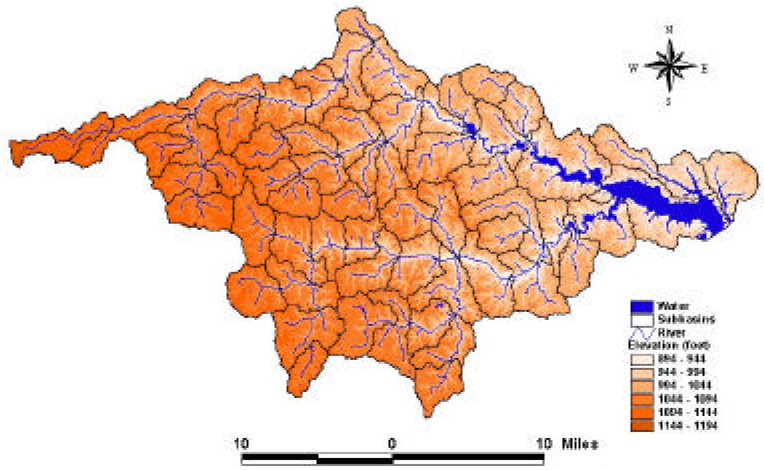

Figure 1. Digitized Elevation Model of the watershed

Sub-basin outlets are geo-referenced points on a stream or river. Outlets may occur in series on larger streams such that the outlet of one sub-basin contributes channel flow to a downstream sub-basin. A sub-basin is the land area contributing surface runoff to the sub-basin outlet. The sub-basin file was created in-house following Natural Resources Conservation Service (NRCS) and USGS criteria for developing 14-digit Hydrologic Units.

The land use/land cover information for the study area was prepared as a 30-meter grid polygon format. Each “cell” of this 30-meter by 30-meter grid is designated a single land cover type. This coverage (see Figure 2) is used to define the plant growth characteristics SWAT will use to simulate the area. This coverage is part of the USGS National Land Cover Dataset using 1992 Landsat Thematic Mapper imagery and supplemental data (USGS, 2000).

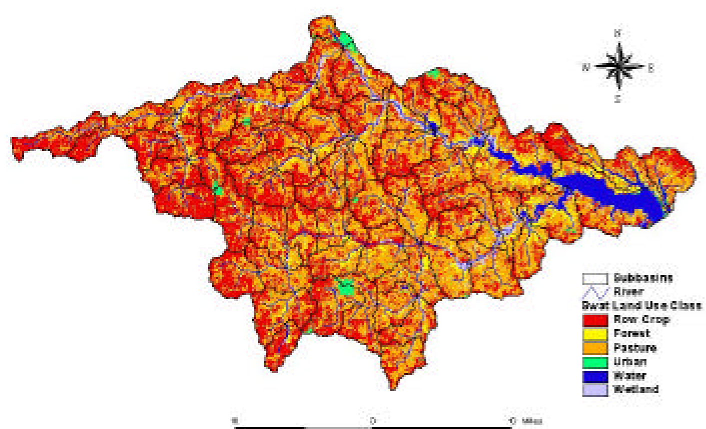

Figure 2. Land use, land cover coverage for SWAT modeling

Soils data and the spatial distribution of soil properties within the study area were also prepared as a 30-meter grid polygon format. Each “cell” of this 30-meter by 30-meter grid is designated a single soil type. This coverage is used to define the soil chemical and physical properties SWAT will use to simulate the area. The township digital soil coverage of Appanoose, Clark, Decatur, Lucas, Monroe, and Wayne Counties and the Iowa Soil Properties and Interpretations Database (ISPAID) are the original sources of the information for the soils coverage (see Figure 3). The Iowa soils data was linked to the SWAT soils database by use of the SCS Soils 5 column of ISPAID and the S5ID number from the soilsia.dbf in SWAT.

Figure 3. Soils digital information used in the SWAT modeling

Three types of files are maintained to simulate weather. These files are the measured daily maximum and minimum temperature file, the measured daily precipitation file, and weather generator input file. The SWAT model comes complete with a climate generation model and the monthly average parameters for more than 1100 weather stations throughout the contiguous United States. For this project, measured daily maximum and minimum temperature and precipitation data from four long-term recording stations close to the watershed were obtained from local sources. The monthly data for these recording stations were obtained from the Iowa State University Agronomy Department Agricultural Meteorology website at: http://www.agron.iastate.edu/climodat/. The weather stations are located near the towns of Centerville, Chariton, Corydon and Osceola (see Figure 4). SWAT simulates the weather by sub-basin. If data from multiple weather stations is available, the distance from the centroid of each sub-basin to each weather station is calculated. The sub-basins are then assigned to the closest weather station for their respective climate data.

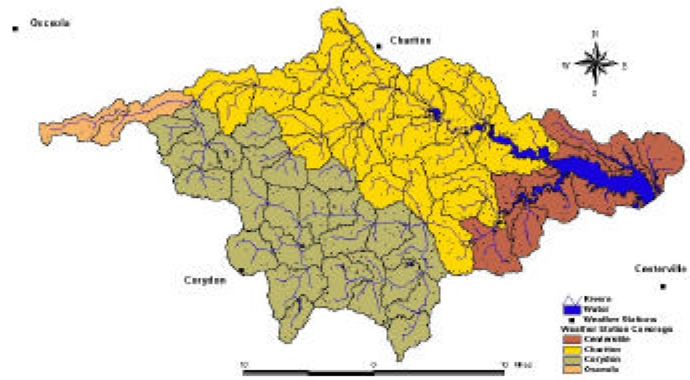

Figure 4. Spatial location of the weather stations used in the modeling

Nutrient management database used in the SWAT model contains 54 commonly available chemical fertilizers, organic fertilizers, and animal manures. Table 1 lists the chemical and physical properties of fertilizers needed by the model for anhydrous ammonia (82-0-0), diammonium phosphate (18-46-0), and urea (45-0-0) fertilizer. The definitions of the fertilizer characteristics were obtained from the SWAT User's Manual.

Table 1. Nutrient inputs used in the SWAT modeling case study

Fertilizer Name	FMINN	FMINP	FORGN	FORGP	FNH3N
Anhydrous Ammonia	0.82000	0.00000	0.00000	0.00000	1.00000
Urea	0.45000	0.00000	0.00000	0.00000	1.00000
Diammonium Phosphate	0.18000	0.20200	0.00000	0.00000	0.00000
FMINN	Fraction of mineral N (NO3 and NH4) in fertilizer (kg min-N/kg fertilizer)				
FNH3N	Fraction of mineral N in fertilizer applied as ammonia (kg NH3-N/kg min-N)				

Implementing SWAT to Rathbun Lake Watershed. Because SWAT is a semi-distributed model, it can simulate discrete, small homogeneous areas within a sub-basin. However, to effectively use this small-scale capability, one must know the assumptions made within the model and the limitations imposed due to the variability of each of the inputs and the resolution of the spatial databases. The amount of detail required of the model will be determined, in part, by selected project objectives. One objective of this project was to demonstrate the predictive capability of the SWAT model and its relevance to the TMDL program. One approach was to rank watershed areas (i.e., the 61 sub-basins in the watershed) according to their relative environmental impact.

Delineating Hydrologic Response Units. Hydrologic Response Units or HRUs are the unique combinations of land use and soil that occur within an individual sub-basin. The SWAT model allows the user to select how an HRU is defined (see Figure 5). One option is to select the predominant land use and predominant soil for each sub-basin. This would then be a single HRU for each sub-basin. The second option is to select multiple HRUs by moving adjustable threshold scale bars for land use and soil that define the threshold criteria. To develop a multiple HRU option, the threshold for land use was first selected. The sliding threshold scale bar ranges from 1% to the maximum percent of any land use in any sub-basin in the watershed. For example, if 10% threshold for land use was selected, this means that within each sub-basin, only those land uses that have at least 10% areal coverage in the sub-basin will be used to define HRUs. Land uses comprising less than 10% areal coverage within the sub-basin will not be simulated. The land area where these minor land uses exist will be distributed back to the remaining land uses in relative proportion to the initial extent of these land uses within the sub-basin. This last step is done so that all of the land areas within a sub-basin have an assigned HRU.

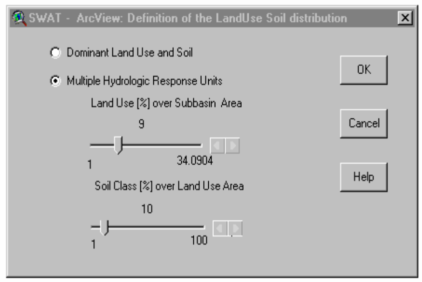

Figure 5. Screen layout for delineating HRU

The same procedure can be applied to delineating HRUs on the basis of the soil criterion. However, when selecting the soils threshold level, the threshold applies to the areal extent of the soils within a specific land use within a sub-basin. The scale bar for soils ranges from 1% to the maximum extent of any soil within any land use within any sub-basin. The scale bars of the land use and soils operate independently of each other (see Figure 5). Therefore, one can, for example, select 10% land use threshold and 20% soil threshold.

The multiple HRU option was selected for this project. The threshold limits set for creating HRUs was 9% land use and 10% soils. This resulted in creating and simulating 513 HRUs within the 1427 km^2 watershed for the baseline scenario. These thresholds were selected for this project based upon details of the land use and soil coverages. Table 2 summarizes how the multiple HRU land use threshold impact selection of HRUs and the respective land uses compared to the original data in the GIS database.

Table 2. Impact of threshold choice on HRU delineation and watershed land use simulated.

Land use	GIS coverage (ha)	1% SWAT threshold (ha)	9% SWAT threshold (ha)
Forest (mixed, deciduous)	13,536	13,574 (100%)	10,505 (78%)
Urban (residential, quarries commercial, urban grass, barren rock)	3,010	2,856 (95%)	538 (19%)
Wetland (wooded, herbaceous)	6,798	6,798 (100%)	1,752 (26%)
Water	5,455	5,113 (94%)	4,424 (81%)

The multiple HRU option determines the number of unique land use and soil combinations simulated and, therefore, the amount of detail to be simulated.

Model Calibration and Validation. There is a widely accepted axiom in watershed hydrologic modeling that if the water balance is estimated accurately, then other processes and parameters that utilize these estimates will also be accurately predicted. Thus, the model reliability assessment was focused on establishing the trustworthiness of the SWAT hydrologic modeling component. In the model reliability assessment, the water yield prediction from the SWAT model was compared to measured stream flow from USGS stream gage #06903400 on the Chariton River near the town of Chariton. The basis of comparison was yearly average stream flow from 1966 to 1986. The SWAT model was calibrated by adjusting selected input parameters that yield predictions of water flow within acceptable values of the observed flow. The t-statistic was calculated as follows:

$$t_{calculated} = \frac{\bar{x} - \bar{y}}{s/\sqrt{n}}$$

where $\bar{x}$ = the average of the predicted stream flow values, $\bar{y}$ = the average of the observed stream flow values, s is the standard deviation of the predicted stream flow values, and n is the number of observations (years). The t-statistic calculated was $|0.617|$. The tabular t-statistic at 0.05 probability and 20 degrees of freedom is 1.725. Based upon these t-statistic values, the null hypothesis cannot be rejected; that is, there is no difference between the observed and predicted stream flow. Figure 6 shows the correspondence between the observed and predicted average annual stream flow at the indicated gage station. It is noted that the years 1973 and 1982 appear as outliers to the rest of the data. Both years exceeded long-term average precipitation by 50% and 43%, respectively. In general, the model predictions appear to be within reasonable and acceptable range of uncertainty.

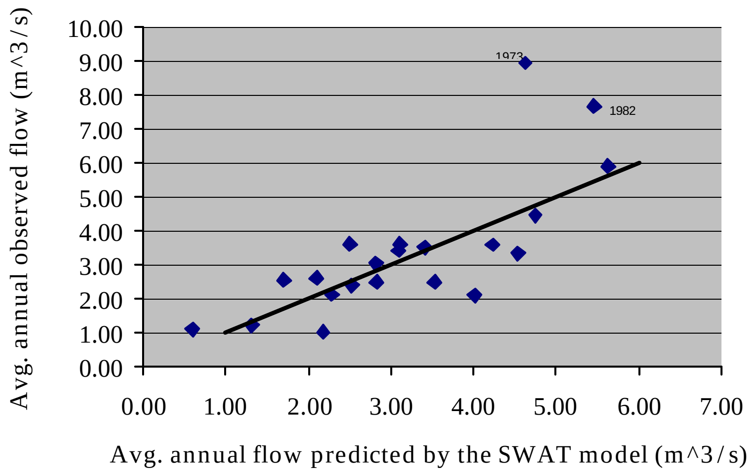

Figure 6. Relationship between SWAT predicted and field observed water yields.

Other standard performance measures were used to explain the reliability of the SWAT model prediction and measured values of the water yield. Table 3 summarizes the values of the performance measures of model reliability compared to the ideal values.

Table 3. Comparison of model performance using standard measures of reliability.

Performance measure	Ideal value	Calculated value (1966-1986 data)	Calculated value (1987-1999 data)
Maximum Error (ME)	0	4.32	4.22
Root Mean Square Error (RMSE)	0	38	40
Modeling Efficiency (EF)	1	0.56	0.59
Coefficient of Determination (CD)	1	2.19	3.03
Coefficient of Residual Mass (CRM)	0	0.05	0.17

Principal Findings and Significance

Simulation Setup. The initial conditions included setting fraction of soil water field capacity in the basin file to 0.6 and all other adjustments were made during the calibration process. The simulation period for all the output maps discussed below was from 1990 to 1999. This time frame was selected because the model GIS land use coverage most closely approximates the current watershed land use. The revised crop, pesticide, fertilizer, and weather databases discussed earlier were used. Model output is presented as average annual output for the ten-year period.

Typical Simulation Results. The results of the SWAT model demonstration component of the research are presented as a series of tables and maps produced from the SWAT model simulated output. The SWAT model was developed as a tool for understanding the processes occurring in watersheds and for documenting the relative changes that can be expected by manipulating the model inputs. Figure 7 identifies the sub-basin numbers, while Table 4 provides the sub-basin ranking of six output parameters discussed for the current land use conditions.

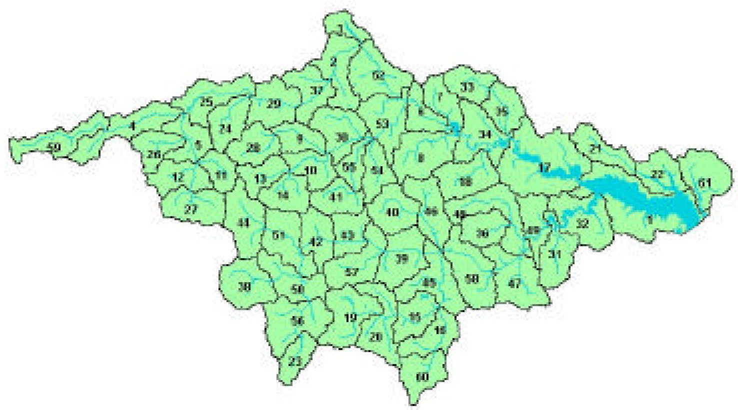

Figure 7. Sub-basin identification and numbering.

Table 4. Selected SWAT-generated model output under current land use and land management condition.

(Sorted by output columns, maximum to minimum values)													
SUB*	WYLD** mm/yr	SUB	SYLD+ Mg/ha/yr	SUB	ORGN++ kg N/ha/yr	SUB	SEDP# kg P/ha/yr	SUB	NSURQ@ kg N/ha/yr	SUB	SOLP% kg P/ha/yr
4	250	38	0.242	9	50	9	9	23	7.8	37	0.6
59	233	33	0.195	37	40	21	8	26	7.5	2	0.6
37	225	18	0.184	24	40	37	8	38	7.5	53	0.6
2	224	48	0.179	38	39	4	8	27	6.7	30	0.6
53	222	56	0.165	4	39	38	8	49	6.5	25	0.6
25	222	40	0.162	30	36	24	8	42	6.5	52	0.6
29	222	46	0.147	21	36	59	7	53	6.4	6	0.6
49	218	9	0.146	2	34	14	7	2	6.3	29	0.6
52	218	4	0.123	35	33	41	7	20	6.3	49	0.6
32	211	37	0.116	29	33	26	7	25	6.2	4	0.5
31	206	30	0.106	41	33	2	7	43	6.1	40	0.5
27	206	17	0.102	33	33	33	7	37	6.1	46	0.5
9	206	42	0.097	59	32	30	7	31	6.0	9	0.5
17	206	19	0.092	14	32	27	7	5	6.0	35	0.5
6	205	36	0.092	52	32	23	7	56	5.9	18	0.5
30	204	51	0.090	53	31	44	6	50	5.8	31	0.5
18	203	50	0.087	25	31	25	6	60	5.7	58	0.5
46	203	31	0.087	8	31	29	6	29	5.7	15	0.5
40	201	47	0.086	18	31	28	6	4	5.6	26	0.5
24	197	32	0.084	36	30	56	6	11	5.6	8	0.5
48	196	23	0.084	26	30	52	6	30	5.5	24	0.5
26	195	24	0.083	40	30	18	6	52	5.5	33	0.5
3	193	25	0.082	7	29	35	6	40	5.4	48	0.5
22	193	58	0.082	48	29	5	6	12	5.3	34	0.5
38	192	8	0.082	13	28	40	6	46	5.3	59	0.5
33	187	49	0.079	10	28	13	6	51	5.3	27	0.5
23	187	39	0.078	28	28	12	6	47	5.3	42	0.5
8	187	52	0.078	44	28	8	6	18	5.2	17	0.5
35	187	29	0.077	5	27	53	6	32	5.2	47	0.5
58	185	44	0.073	27	27	7	6	15	5.1	7	0.5
42	184	53	0.072	56	26	36	6	6	5.1	50	0.5
34	184	2	0.071	23	25	10	5	57	5.0	43	0.4
36	181	21	0.070	12	25	48	5	9	4.9	38	0.4
21	179	20	0.068	50	25	19	5	58	4.9	36	0.4
5	178	57	0.066	16	24	50	5	35	4.8	5	0.4
7	177	45	0.066	42	24	42	5	19	4.8	12	0.4
47	177	27	0.063	34	24	51	5	48	4.8	32	0.4
15	176	14	0.063	51	24	54	5	59	4.7	23	0.4
61	175	41	0.062	54	24	16	5	17	4.7	51	0.4
43	173	59	0.059	22	24	20	5	28	4.7	21	0.4
12	173	26	0.059	46	23	11	5	8	4.6	56	0.4
51	172	43	0.057	55	23	22	5	39	4.5	16	0.4
19	166	35	0.057	17	22	55	4	34	4.5	3	0.4

Table 4 (continued)

SUB*	WYLD** mm/yr	SUB	SYLD+ Mg/ha/yr	SUB	ORGN++ kg N/ha/yr	SUB	SEDP# kg P/ha/yr	SUB	NSURQ@ kg N/ha/yr	SUB	SOLP% kg P/ha/yr
41	166	16	0.051	19	21	34	4	16	4.5	41	0.4
16	165	1	0.042	43	21	46	4	33	4.4	39	0.4
1	159	60	0.042	31	21	60	4	24	4.4	20	0.4
56	156	34	0.040	11	21	43	4	36	4.4	54	0.4
10	154	5	0.037	49	21	17	4	45	4.3	19	0.4
50	152	28	0.036	20	20	57	4	21	4.2	10	0.4
39	150	61	0.033	57	20	31	4	7	4.1	55	0.4
54	147	13	0.032	39	20	39	4	14	4.1	60	0.4
55	145	12	0.029	47	19	49	4	13	3.8	11	0.3
11	142	10	0.028	60	19	45	4	22	3.6	22	0.3
20	140	7	0.028	45	18	47	4	44	3.6	45	0.3
45	132	22	0.026	58	17	3	4	41	3.5	13	0.3
14	131	15	0.025	32	17	32	3	3	3.5	57	0.3
60	127	54	0.024	6	16	6	3	1	3.4	14	0.3
57	126	11	0.024	3	15	58	3	10	2.9	28	0.3
44	123	6	0.018	61	14	15	3	54	2.8	61	0.3
28	122	55	0.015	15	14	61	2	55	2.8	1	0.3
13	117	3	0.008	1	8	1	2	61	2.5	44	0.3

* Sub-basin number

** Water yield

+ Sediment yield

++ Organic nitrogen yield attached to the sediment

\# Phosphorus yield attached to the sediment

@ Soluble nitrogen yield

% Soluble phosphorus yield

Water Yield. Water yield is the amount of water that eventually flows in the stream and exits the watershed outlet. The water originates from precipitation falling on the watershed or is added to the system through irrigation and is partitioned into several pathways. The three pathways contributing to water yield are: surface runoff, lateral flow of water through the soil profile to the stream, and stream recharge from the shallow aquifer. Surface runoff is the dominant pathway contributing to water yield. Therefore, factors that increase surface runoff will increase water yield. Table 5 shows the effects that soil type and land use have on simulated water yield. Water yield increases as percent imperviousness of land use increases (e.g., Forest WYLD < Row Crop WYLD < Urban WYLD). Water yield also tends to increase with decreasing soil water infiltration (e.g., soil hydrologic group B WYLD< soil hydrologic group C WYLD< soil hydrologic group D WYLD). Definitions for the soil hydrologic groups can be found in the SWAT User's Manual. Figure 8 illustrates the water yield from the 61 sub-basins for the current land use and land management practices.

Table 5. SWAT model simulated water yield by soil type and current land use categories.

Soil	Hyd Grp[1]	Landuse[2]					
		AGRL	FRSD	PAST	URMD	WATR	WETL
				--mm/yr--			
IA004	B	169	136	121			105
IA031	B			136			
IA033	B		134				
IA044	B						99
IA065	B	135	112	81			
KS111	B	178		117	169		
KS146	B	167	89	101	159		
KS175	B	211			190		
MO003	B					0	77
MO007	B	158		87			
IA040	C	273		216	256		
IA043	C				228		
IA053	C		178				
MO009	C			166	222		
MO011	C			187			
MO012	C		182				187
MO018	C	248	181	198			169
MO023	D		293	208			
MO031	D	238	240	203	280		

[1]Soil Hydrologic Group

[2]Landuse Categories for HRUs: AGRL = Agricultural Land, FRSD = Forest, PAST = Pasture, URMD = Urban Land, WATR = Water, and WETL = Wetland

Sediment Yield. Sediment yield is the amount of soil eroded from the sub-basin and delivered to the stream reach. SWAT uses the MUSLE equation to estimate this amount of sediment produced. Sediment deposition in streams and water bodies clogs the drainage network, destroys vital habitat for fish and other invertebrates, and reduces storage capacity and water depth in lakes and reservoirs. Sediment in the water column causes turbidity and reduces light penetration. In addition, sediment is an important parameter for water quality because other potential pollutants are bound to the sediment. Therefore, as the quantity of sediment increases, the potential for other pollutants to be present increases. Table 6 shows the effect soil type and land use has on sediment yield. Agricultural land (row crop) is the dominant source of upland sediment per hectare. Sediment yield tends to increase as water infiltration decreases (e.g., soil hydrologic group B SYLD< soil hydrologic group C SYLD< soil hydrologic group D SYLD).

Nutrient Loading. Nitrogen (N) and phosphorus (P) were the primary nutrients simulated in the study, in part because of concerns for eutrophication and the recently emerging concerns about hypoxia in the Gulf of Mexico. Both of these nutrients are present as sediment-bound (adsorbed) and as solutes in water. The nutrients dissolved in water will reach Lake Rathbun much more readily than the sediment-bound nutrients. Table 7 shows the simulated impacts of soil type and land use on sediment-bound (adsorbed) P yield. The adsorbed P is predominantly from agricultural (row crop) land

and is directly related to the quantity of sediment yield. Table 8 shows the effect soil type and land use have on soluble phosphorus yield. Soluble phosphorus tends to increase as infiltration rate decreases (e.g., soil hydrologic group B SOLP < soil hydrologic group C SOLP< soil hydrologic group D SOLP). Pasture land use also had the highest soluble phosphorus yield. Figure 9 illustrates the soluble phosphorus yield from each sub-basin for the current land use and land management.

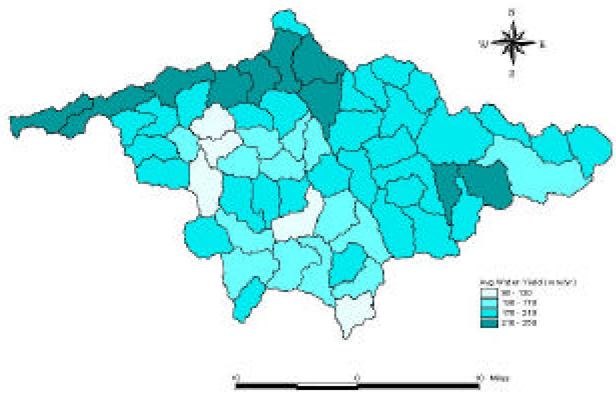

Figure 8. SWAT model predicted average water yield by sub-basin

Table 6. SWAT model simulated sediment yield by soil type and current land use categories.

Soil	Hyd Grp[1]	Landuse[2]					
		AGRL	FRSD	PAST	URMD	WATR	WETL
		--Mg/ha/yr--					
IA004	B	0.039	0.000	0.001			0.001
IA031	B			0.001			
IA033	B		0.000				
IA044	B						0.001
IA065	B	0.029	0.000	0.001			
KS111	B	0.095		0.003	0.000		
KS146	B	0.051	0.000	0.001	0.000		
KS175	B	0.064			0.000		
MO003	B					0.000	0.001
MO007	B	0.056		0.000			
IA040	C	0.153		0.012	0.000		
IA043	C				0.000		
IA053	C		0.000				
MO009	C			0.001	0.000		
MO011	C			0.000			
MO012	C		0.001				0.008
MO018	C	0.056	0.002	0.005			0.002
MO023	D		0.003	0.002			
MO031	D	0.239	0.002	0.010	0.000		

[1]Soil Hydrologic Group

[2]Landuse Categories for HRUs: AGRL = Agricultural Land, FRSD = Forest,
PAST = Pasture, URMD = Urban Land, WATR = Water, and WETL = Wetland

Table 7. SWAT model simulated sediment-P yield by soil type and current land use categories.

Soil	Hyd Grp[1]	Landuse[2]					
		AGRL	FRSD	PAST	URMD	WATR	WETL
		--kg/ha/yr--					
IA004	B	30.9	0.7	0.4			3.6
IA031	B			0.5			
IA033	B		0.4				
IA044	B						4.2
IA065	B	21.9	0.6	0.4			
KS111	B	49.6		1.8	1.4		
KS146	B	47.9	0.7	0.7	1.3		
KS175	B	36.1			1.4		
MO003	B					0.0	5.5
MO007	B	41.5		0.4			
IA040	C	60.6		4.0	1.4		
IA043	C				1.4		
IA053	C		1.6				
MO009	C			1.0	1.4		
MO011	C			1.1			
MO012	C		2.8				7.8
MO018	C	26.4	1.8	1.6			4.2
MO023	D		5.6	2.4			
MO031	D	47.5	4.1	4.0	1.2		

[1]Soil Hydrologic Group

[2]Landuse Categories for HRUs: AGRL = Agricultural Land, FRSD = Forest, PAST = Pasture, URMD = Urban Land, WATR = Water, and WETL = Wetland

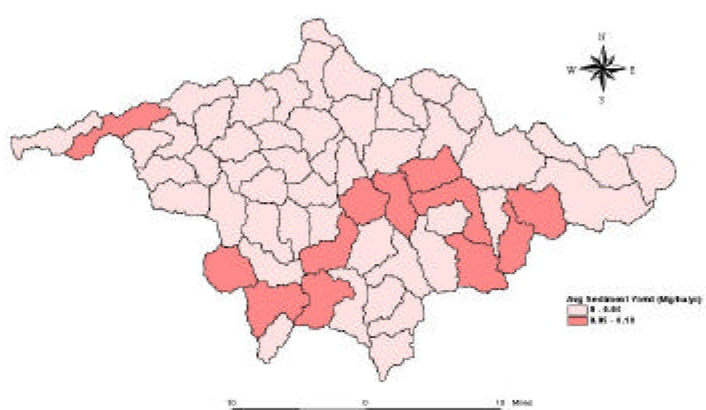

Figure 9. SWAT model predicted soluble-P load by sub-basin.

Table 8. SWAT model simulated dissolved-P by soil type and current land use categories.

Soil	Hyd Grp[1]	Landuse[2]					
		AGRL	FRSD	PAST	URMD	WATR	WETL
		--kg P/ha/yr--					
IA004	B	0.122	0.063	0.451			0.258
IA031	B			0.511			
IA033	B		0.059				
IA044	B						0.189
IA065	B	0.088	0.040	0.260			
KS111	B	0.132		0.374	0.104		
KS146	B	0.120	0.047	0.368	0.091		
KS175	B	0.149			0.120		
MO003	B					0.000	0.295
MO007	B	0.114		0.333			
IA040	C	0.218		0.789	0.102		
IA043	C				0.124		
IA053	C		0.102				
MO009	C			0.620	0.115		
MO011	C			0.674			
MO012	C		0.129				0.561
MO018	C	0.176	0.113	0.763			0.387
MO023	D		0.207	0.813			
MO031	D	0.177	0.170	0.790	0.056		

[1]Soil Hydrologic Group

[2]Landuse Categories for HRUs: AGRL = Agricultural Land, FRSD = Forest, PAST = Pasture, URMD = Urban Land, WATR = Water, and WETL = Wetland

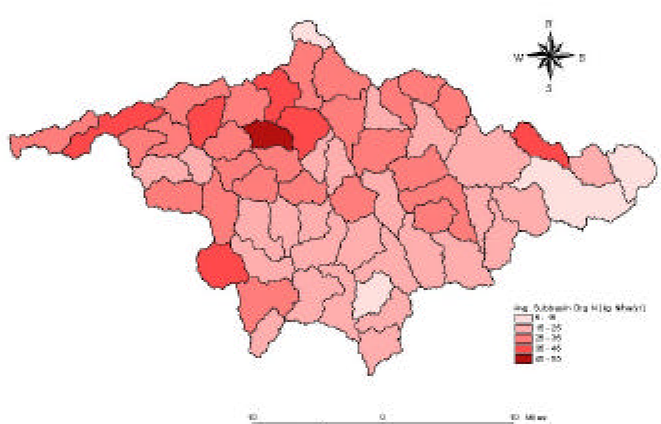

Figure 10. SWAT model predicted sediment-bound N by sub-basin.

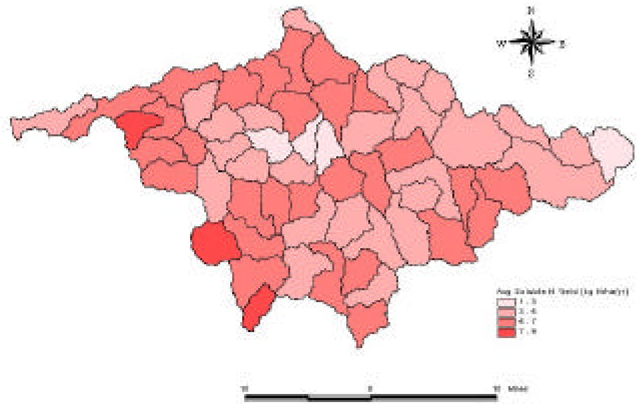

Figure 11. SWAT model predicted soluble N by sub-basin.

The model simulated loads of N and, in the adsorbed or sediment-bound phase, followed similar trends as adsorbed P and a function of soil type, land use, and land management. The source of adsorbed N to channels is predominantly from agricultural (row crop) land use and is directly related to the quantity of sediment yield. Figures 10 and 11 show the spatial distribution of adsorbed-phase and dissolved-phase N loads from each sub-basin. The effect of soil type and land use on soluble N is similar to that of soluble P. Soluble N tends to increase as infiltration rate decreases. Pasture land use also has the highest soluble N load. Overall, the results of the SWAT model application show the capability of the model in the development of nutrient TMDLs. However, during the model application, a number of shortcomings in model performance were observed. There is need to refine the SWAT model to enhance usability and to allow the specification of events with shorter durations. If provided with the requisite high quality data, the SWAT model has potential to produce reasonably accurate and acceptable estimates of pollutant loads nested to establish the assimilative capacity of a waterbody.

Conclusions

The Clean Water Act (CWA), and its many amendments, has been viewed as one of the most successful environmental regulations in terms of achieving statutory goals and has gained widespread support by interest groups and the general American public. However, during the past decade, many have questioned whether actions intended to achieve "swimmable and fishable waters" are worth the implementation costs and have called on federal agencies to conduct cost-benefit assessments of the program before widespread implementation. These criticisms have originated from many sectors of society, including industry that has opposed the imposition of stringent and potentially costly requirements on effluent discharge standards. Criticism has also come from agribusiness groups and farmers who contend that federal regulations are a costly intrusion on private land use and land management decisions. States and local jurisdictions have voiced concerns about the CWA, fearing that it imposes new unfunded mandates in the midst of tight capital and human resources. Environmental groups, on the other hand, believe that more stringent regulation and fine-tuning is not only needed to strengthen the CWA but also to address the remaining environmental degradation problems from human activities. All these concerns are legitimate, given EPA's projected $4.3 billion annual costs of TMDL implementation, the estimated 20,000 waterbodies across the U.S. that are not meeting water quality standards, and the fact that as many as 40,000 TMDLs will have to be developed by the states.

As described by the EPA, TMDL relates to the amount of pollutants a waterbody can accept on a daily basis without violating its designated/beneficial use, referred to as "assimilative capacity" or "load capacity." The process of developing a TMDL can be categorized into five basic steps: (1) identification of pollutants of concern; (2) estimation of the waterbody's loading capacity or assimilation capacity for those pollutants; (3) estimation of the pollutant loading from all sources—point and nonpoint—to the waterbody; (4) determination of the total allowable pollutant load to the waterbody; and (5) allocation of pollution loading to each source, including a margin of safety. Each of these steps requires sound scientific principles, particularly the use of existing water quality monitoring data to estimate daily load and wasteload from known and diffuse sources. It requires the use of mathematical models to establish cause-and-effect relationships between human activities and environmental responses (in the form of water quality impacts). Indeed, process-oriented mathematical models offer cost-effective alternatives to large-scale, long-term field monitoring programs that document potential benefits of watershed and waterbody restoration strategies and can be used to measure the efficacy of different land management strategies. However, the potential for substantial costs and adverse environmental and human health impacts from the improper use of a mathematical model must be recognized.

This research established a science-based approach for evaluating, reviewing, and selecting mathematical models, including those that are being used or can be used by states to support their TMDL development and implementation program. The research developed a set of qualitative criteria for model selection and identified "candidate" or "optimal" terrestrial and aquatic ecosystems for use in determining pollutant load and waste-load. The models selected were deemed to have the capacity to support the estimation and allocation of TMLD for common chemical pollutants and sediments. Some of the models identified as optimal were also deemed suitable candidates for refinements to enhance their ability to simulate fate and transport of all forms of pollutants—chemical and biological. One of the models selected as optimal was modified and applied to a relatively large agricultural watershed to serve as a case study. Specifically, the SWAT model was modified to incorporate a functional component for predicting pathogen fate and transport in agricultural landscapes, thereby providing a new modeling system for predicting impact of physical, chemical and biological processes in terrestrial ecosystems. From the experiences obtained in this research, we can conclude that in spite of the many deficiencies of mathematical models of hydrology and water quality, they continue to possess unmistakably proven capability and are invaluable analytical tools and decision-support systems in environmental management and natural resource planning.

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Predicting sorption, mobility, accumulation, and degradation potential of antibiotics in Iowa’s soil/water environment

Basic Information

Title:	Predicting sorption, mobility, accumulation, and degradation potential of antibiotics in Iowa’s soil/water environment
Project Number:	2002IA4B
Start Date:	3/1/2001
End Date:	2/28/2003
Funding Source:	104B
Congressional District:	Iowa 3rd
Research Category:	None
Focus Category:	Agriculture, Non Point Pollution, Toxic Substances
Descriptors:	None
Principal Investigators:	Steven Fales

Publication

Research Problem

Approximately 31.6 million pounds of antibiotics are used in the production of poultry (10.6 million pounds), hogs (10.3 million pounds), and cattle (3.7 million pounds) in the United States each year (Mellon et al., 2001). Over three fourths of these antibiotics (24.6 million pounds) are given to healthy animals in low doses to promote growth (Levy, 1997). Most of the antibiotics given to farm animals are not metabolized in the body, rather they are excreted in the active form (Lee et al., 2000). The fate of antibiotics introduced into soil and aquatic environments with manure and other animal wastes is largely unknown. However, there is much concern that the presence and persistence of low levels of antibiotics in soil and aquatic environments could encourage the buildup of existing antibiotic-resistant bacterial populations and promote the development of new populations (Henry, 2000).

In Iowa, Earthen Waste Storage Structures (lagoons) are widely used for temporary storage of liquid animal wastes with the intent of protecting surface and ground water from contamination and allowing farmers to use the wastes in a timely fashion. Liquid animal wastes are generally spread on agricultural soils both as a means of disposal of the wastes and as a nutrient source for crop production. The Iowa Dept. of Public Health (1998) found relatively high concentrations of chlortetracycline (11 to 540 µg/L) and erythromycin (10 to 275 µg/L) in such liquid animal wastes. The report also indicated that many of the 18 *E. coli* isolates, all three Salmonella species, and an isolate of Enterococcus demonstrated resistance to one or more of the antibiotics.

The antibiotics most commonly added to livestock feed as growth promoters (1 to 100 mg per head per day) are chlortetracycline (Aureomycin), oxytetracycline (Terramycin) and macrolide (erythromycin) (Sewell, 1993; FAC, 1998; Herman et al., 1995). The fate of these compounds in Iowa soils depends on sorption and desorption of the antibiotics on soils, leaching, and the rates of chemical, photochemical, and microbial decomposition of the antibiotics. The basic hypothesis of the study is that the fate (sorption/desorption, leaching, and decomposition) of antibiotics in soil environments is strongly influenced by the chemical reactions between the antibiotics and soil constituents.

Specific Objectives

1. Characterize three common Iowa soils and isolate and characterize reactive soil components (clay-humic complexes, clay minerals and humic materials) from these soils.

2. Quantify sorption of tetracycline and chlorotetracycline on the soils and soil components.

3. Determine the effects of staturating cation (Ca vs K) and ionic strength (I=0.05 and I=0.005) on sorption of tetracycline and chlorotetracycline on the soils and soil components .

4. Quantify the influence of sorption on tetracycline and chlorotetracycline degradation rates.

5. Quantify mobility of tetracycline and chlorotetracycline in soil columns.

Methodology

Soil samples, surface (0-15 cm) and subsurface (≥ 15 cm), were collected from three sites representing three different soil series and a range of soil physical and chemical properties. Both the studied soils and the general sampling locations had been previously characterized (McBride et al., 1987). Based on interviews with the landowners or operators, specific sampling sites that had never received manure applications were selected. The soils were characterized using standard analytical procedures to determine pH in $CaCl_2$, pH in KCl, pH in water, organic C, organic H, organic N, % sand, % coarse silt, % fine silt, % clay and extractable cations (Ca, Mg, Na, and K).

Soil components were physically and chemically separated from the soils and prepared for the sorption and degradation studies. Clay-humic complexes were isolated from the soils by sedimentation (<2 µm e.s.d.). Portions of the clay-humic complexes were K- or Ca-saturated by washing in 1M KCl or 0.5 M $CaCl_2$ and then dialyzed against distilled water and freeze dried. Other portions of the clay-humic complexes were treated with 30% H_2O_2 for removal of the humic materials before being K- and Ca- saturated, dialyzed against distilled water and freeze-dried. Humic materials were separated from the three soils by hydrolyzing Na-saturated samples in 0.1 M NaOH under and N_2 purge. After the hydrolysis the humic materials were separated by centrifugation, neutralized to pH 7, K- or Ca-saturated, dialyzed and freeze-dried.

A batch equilibration technique has been designed and is being used to measure sorption of tetracycline and chlortetracycline on the various soils and prepared soil components. HPLC is being used to quantify tetracycline and chlortetracycline in the supernatant solutions and sorption is determined by difference. Variables being tested include soil components (clay-humic complexes, clay minerals, and humic substances), saturating cation (K vs Ca), and ionic strength (I=0.05 and I=0.005). Previous research has demonstrated the importance of pH, hence in this study pH is being carefully controlled at 6.5. The data will be used to prepare four point sorption isotherms with three replications for each point.

After the sorption studies are complete, tetracycline will be incubated under both sterile and non-sterile conditions in aqueous controls and with soil components exhibiting both high and low sorption. Tetracycline will also be incubated under both sterile and non-sterile conditions with the soil exhibiting the highest sorption capacity. Degradation kinetics will be quantified for these systems by extracting tetracycline from the samples at various times during the incubations and quantifying parent and degradation products by HPLC.

The final stage of the research will be a column leaching study. Intact soil columns treated with tetracycline and chlortetracycline will be leached with high and low ionic strength solutions with different ratios of K and Ca. The ionic strength and the K:Ca ratios of the leaching solutions will be selected to both encourage and discourage colloid mobility. Leachate will be analyzed by HPLC.

Principal Findings and Significance

The project is not yet far enough along to report findings and significance with respect to tetracycline sorption, degradation and mobility in Iowa soils. Prior to Dr. Evangelou's death in March 2002, soil samples had been collected and initial analysis of the samples provided basic soil characterization data. Since March of 2002, the project has been reorganized and refocused. Specifically, we have conducted a literature review, refined the hypotheses being tested, developed new specific objectives, and designed three major sets of experiments focused on testing those hypotheses. From the literature review, it was apparent that the effects of pH on sorption and degradation of tetracyclines in soil environments have been carefully studied. However, little information was available distinguishing whether tetracylclines are dominantly sorbed on soil clays or soil humic materials and the effects of saturating cation and ionic strength on sorption, degradation, and mobility of tetracyclines in soils. Therefore, the focus of the project has been targeted on filling these knowledge gaps.

The soils sampled for this study are listed in Table 1. Clay content of the sampled soils ranged from 19.2 % in the Nicollet surface sample to 34.6% in the Clarinda subsoil sample. Organic C content ranged from 0.44% for the Fayette subsoil sample to 1.65 for the Fayette surface soil sample. Total exchangeable cations ranged from 13.6 $cmol_c\ kg^{-1}$ for the Nicollet surface soil to 19.7 $cmol_c\ kg^{-1}$ for the Fayette subsoil. The pH values in KCl ranged from 4.5 in the Clarinda subsoil sample to 6.5 in the Fayette surface soil sample. In general, the properties of the sampled soils are sufficiently diverse to allow a reasonable assessment of the influence of soil properties and soil components on the fate of antibiotics.

Table 1: Soil sampled for the study.

Sample Site	**Soil Series**	**Classification**
Tama Co.	Fayette	Fine-silty mixed superactive mesic, Typic Hapludalfs
Boone Co.	Nicollet	Fine-loamy mixed superactive mesic, Aquic Hapludolls
Clarke Co.	Clarinda	Fine smectitic mesic, Vertic Argiaquolls

Preliminary chemical characterization of tetracycline, chlortetracycline, and oxytetracycline were performed. UV-vis absorbance spectra of the antibiotics dissolved in water, and various concentrations of KCl, $CaCl_2$, $MgCl_2$, and $AlCl_3$ were obtained using a UV-vis spectrophotometer (Varian Instruments, Cary 50 Bio model, Walnut Creek, CA, USA). Calibration curves for quantifying concentrations of the various antibiotics dissolved in water were developed for two wavelengths, near 270 nm (W1) and 370 nm (W2). Solubility of the oxytetracycline in water was measured by determining the concentration where the absorbance-concentration relationship deviates from Beer's-law.

Potentiometric titrations indicate two and possibly three pKa's for the tetracyclines. The solubility of oxytetracycline was found to be approximately 300 mg L^{-1}. UV-VIS spectroscopy revealed two prominent absorption maxima near 280 and 360 nm for oxytetracycline and tetracycline and two prominent sorption maxima near 280 and 370 nm for chlortetracycline. Absorption spectra for all three tetracyclines were only slightly affected by background $CaCl_2$ (0 to 50 meq L^{-1}) and $MgCl_2$ (0 to 40 meq L^{-1}) concentrations. By contrast, the presence of as little as 2 meq L^{-1} $AlCl_3$ substantially altered the absorbance spectra for all three tetracyclines. The cause of change in the absorbance spectra in the presence of $AlCl_3$ is not clear, but may indicate either a pH effect or the formation Al-tetracycline complexes. More work is needed to resolve the cause of this effect. The results demonstrate that tetracycline, chlortetracycline, and oxytetracycline concentrations in water and both $CaCl_2$ and $MgCl_2$ solutions can be quantified by UV-VIS spectroscopy with linear response for the 0 to 20 mg L^{-1} concentration range. The presence of Al in aqueous solution, however, may cause problems with spectrometric analysis.

Major accomplishments during the last year include the physical separation and chemical preparation of cation saturated soil components (clay-humic complexes, clay minerals, and humic materials) from the studied soils and the development and testing of an HPLC method for quantification of tetracyclines. Considerable effort was expended developing the HPLC technique. The tetracyclines are not well behaved in HPLC because they have three ionizable moieties and are zwitterions over a large pH range. Several published HPLC methods performed poorly on our HPLC system, and considerable refinement of one of those methods was necessary to obtain high quality analytical data.

Personnel

The history of this project is complex. During most of the first year, preliminary work on the project was done by an undergraduate student, Kim Grosenheider, under the supervision of Dr. Evangelou. In December 2001, a graduate student, Jutta Pils, and a postdoctoral associate, Dr. Leticia S. Sonon, were assigned to work on separate aspects of the project. Since Dr. Evangelou's death on March 24, 2002, Dr. Sonon has been assigned to another project. Dr. Steven Fales, Agronomy Department Chair, assumed the role of principal investigator for the project. Work on this project is currently being conducted by Ms. Jutta Pils, a Ph.D. graduate student in Agronomy, under the supervision of Dr. David Laird, USDA, ARS, NSTL and Collaborative Professor in Agronomy. Ms. Pils has completed all course work and passed her preliminary examination in February 2003.

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Effects of grazing management on sediment and phosphorus losses in pastures

Basic Information

Title:	Effects of grazing management on sediment and phosphorus losses in pastures
Project Number:	2002IA7B
Start Date:	3/1/2001
End Date:	2/28/2003
Funding Source:	104B
Congressional District:	Iowa 3rd
Research Category:	Not Applicable
Focus Category:	Nutrients, Surface Water, Solute Transport
Descriptors:	
Principal Investigators:	James Richard Russell, John L. Kovar, Steven K. Mickelson, Wendy J. Powers, Richard C Schultz

Publication

Problem and Research Objectives

The amounts of sediment and phosphorus in water runoff from agricultural lands are of concern because of the potential for siltation and eutrophication brought on by high phosphorus concentrations of surface waters. Currently, there is limited information about the total sediment and phosphorus loads in runoff coming from pastureland in the Midwest. Because vegetation limits soil disruption caused by the impact of raindrops and forage roots hold soil particles, forages harvested at an appropriate height through suitable grazing management should maintain water infiltration and minimize sediment and phosphorus losses in water runoff from pastures. The objectives of this experiment were to quantify the amounts of sediment and phosphorus in the runoff from pasturelands managed by different systems, develop tools to monitor and control sediment and phosphorus loss from pastures, and develop best management practices for producers to control sediment and phosphorus losses while optimizing productivity of pastures.

Methodology

Pasture Management. Three blocks of approximately 2.75 hectares were located on hills with slopes up to 15° in a smooth bromegrass (*Bromus inermis*) pasture at the Iowa State University Rhodes Research and Demonstration Farm near Rhodes, Iowa. Each block was subdivided into five 0.4-hectare paddocks, with a 6-meter wide lane at the top for cattle movement and a 10-meter wide buffer area at the bottom. Prior to the initiation of grazing in 2001, soil samples were collected to depths of 0 to 5 cm and 5 to 10 cm to determine soil P and K levels. Diammonium phosphate was applied in the spring of 2001 so that all pastures were at least at an optimum level (11 - 15 ppm P) of phosphorus. Soils in all paddocks contained an optimum level (81 - 120 ppm K) or greater of potassium; therefore, no additional potassium was applied. In both years urea was applied at a rate of 200 kg/ha before the start of grazing in the spring and 115 kg/ha at the initiation of the forage stockpiling period, in August, to all pastures. Sandbags were placed around the perimeter of the pastures and between each paddock to prevent contamination from runoff by natural rainfall events from outside the experimental area and between neighboring paddocks.

Grazing treatments were randomly assigned to each of the 5 paddocks in each plot. Treatments included an ungrazed control (U), summer hay harvest with winter stockpiled grazing to a residual sward height of 5 cm (HS), continuous stocking to a residual sward height of 5 cm (5C), rotational stocking to a residual sward height of 5 cm (5R), and rotational stocking to a residual sward height of 10 cm (10R). Grazing was initiated on May 29, 2001, and May 7, 2002, with 3 mature Angus cows in each grazed paddock.

In the continuous stocking system, cattle were removed from the paddocks after the sward height decreased to 5 cm. Paddocks were allowed a rest period of 7 to 10 days to limit regrowth, thereby simulating continuous stocking. In the rotational stocking systems, cattle were removed from the paddocks after the sward height decreased to 5 or 10 cm. Paddocks were allowed rest periods of 35 days to allow plant regrowth in rotational stocking. Forage sward heights were measured with a raising plate meter (4.8 kg/m^2) twice weekly during the grazing seasons. During the 2001 grazing season, mean total grazing days were 491, 360, and 274 cow-days/ha, and during 2002 grazing season, mean total grazing days were 400, 316, and 257 cow-days/ha for the 5C, 5R, and 10R stocking systems, respectively.

First-cutting hay was harvested from the HS treatment in June of 2001 and 2002, yielding 1079 and 1471 kg of forage dry matter per acre, respectively. Regrowth from these paddocks was clipped in early August of each year to initiate forage stockpiling, but the yield of clipped forage was inadequate to bale. Each paddock in the HS system was stocked in mid-November of each year and grazed to a residual sward height of 5 cm, allowing grazing for 47 and 59 cow-days/ha in 2001 and 2002, respectively.

Rainfall Simulations. To determine sediment and phosphorus loss in water runoff, rainfall simulations were conducted 4 times per year for 2 years. Year 1 simulations were conducted in the late spring, mid-summer, and autumn of 2001, and early spring of 2002. Year 2 simulations were conducted in late spring, mid-summer, and autumn of 2002 and early spring 2003. Six simulation sites were selected within each paddock—3 within a low slope range (1° to 7°) and three in a high slope range (7° to 15°). Six simulation sites were selected within the buffer zone below each paddock. Three of these sites were at the base of the paddock and 3 were 10 meters within the buffer strip. Rainfall simulation locations were identified with GPS so that the same locations could be used during each sampling period. Rainfall simulators were 0.5 x 1.0 meter and assembled so that the uphill side of the simulator was 1 meter high. Each rainfall simulation ran for 1.5 hours at a precipitation rate of 7.1 cm/hour. The water source used was rural water that had been filtered through an additional 0.45 µm filter, to remove particulate matter. During simulations, the amount of rainfall and runoff was measured at 10-minute intervals, and a sample of runoff was collected and added to a composite sample that was used to determine total sediment, total phosphorus, and soluble phosphorus. Surface roughness was measured with a 41-pin meter with a length of 2 meters; ground cover was determined by the percentage of pins on the pin meter striking plant material. During simulations, soil samples were taken adjacent to each site at depths of 0–5 cm and 5–12 cm for determination of Bray-1 phosphorus and soil moisture. Penetration resistance was measured at 3.5-cm intervals to a depth of 35 cm using a Bush Recording Penetrometer; readings from the 0 to 10 cm depth, 10 to 20 cm depth, and 20 to 35 cm depth were averaged for statistical analysis. Sward height was measured using a rising plate meter (4.8 kg/m^2), and a forage sample was clipped from a 0.25-m^2 area adjacent to the rainfall simulation site to determine the mass of forage dry matter.

Principal Findings and Significance

Grazing Effects in Paddocks. The proportion of rainfall lost as runoff was less ($P<0.05$) in the U paddocks than in all other treatments during both years 1 and 2. In year 1, the proportion of rainfall lost as runoff was greater ($P<0.05$) in the late spring (36%) than in mid-summer (11.8%), autumn (13.1%), or early spring (7.1%) across all treatments. Similarly, in year 2, the proportion of rainfall lost as runoff in late spring (19.4 %) was greater ($P<0.05$) than the mid-summer (7.5%), autumn (11.8%), and early spring (12.6%) periods.

There were no differences in mean concentrations of sediment in runoff between stocking treatments in either year. Mean total P concentrations in the runoff were greater in paddocks with the 5C and 5R treatments than other treatments in both years ($P<0.05$). Mean sediment and total P concentrations did not differ between months in year 1, but soluble P concentrations were greater ($P<0.05$) in the late spring than the other sampling periods. In year 2, mean sediment and total P concentrations in runoff did not differ between sampling periods. However, soluble P concentration in runoff was less in the early spring than it was in other sampling periods.

Erosion of sediment was not different between treatments in year 1. In year 2, the 5C paddocks contributed greater amounts of erosion ($P<0.05$) than the other treatments. The greatest amount of erosion occurred in the late spring period across all treatments in both years ($P<0.05$). Of the pasture physical characteristics measured sediment loss was most highly correlated with percent surface cover ($Y=889.2 - 19.95\ x + 0.108\ x^2$, $r^2=0.3362$), where Y is the sediment loss in kg/ha/simulation and x is the percentage of ground covered with plant material.

Losses of total P were greater ($P<0.05$) from paddocks with the 5C and 5R treatments than U paddocks in year 1, while the HS and 10R treatments were intermediate and not significantly different from any of the other treatments (Table 1). Total P losses were greater ($P<0.05$) from 5C treatment then from all other treatments in year 2. Losses of soluble P were lower ($P<0.05$) from U paddocks than from other treatments in year 1. In year 2 the 5R treatment had greater ($P<0.05$) soluble P losses than U, with the HS, 5C, and 10R intermediate to, and not significantly different from, either the U or 5R treatments. In years 1 and 2, 89% and 76% of the total P in the runoff was in the form of soluble P.

Table 1. Annual sediment, total P and soluble P in runoff.

	Sediment, (kg/ha)		**Total P, (kg P/ha)**		**Soluble P, (kg P/ha)**	
	Year 1[b]	Year 2	Year 1	Year 2	Year 1	Year 2
U[a]	11.4	4.8[c]	0.06[c]	0.03[c]	0.04[c]	0.02[c]
HS	34.5	17.8[c]	0.23[c,d]	0.10[c]	0.19[d]	0.04[c,d]
5C	61.2	118.2[d]	0.41[d]	0.40[d]	0.29[e]	0.13[c,d]
5R	61.9	30.5[c]	0.41[d]	0.21[c]	0.35[e]	0.17[d]
10R	46.2	17.8[c]	0.26[c,d]	0.09[c]	0.20[d,e]	0.04[c,d]

[a] U = Ungrazed, HS = Summer Hay Harvest/Winter Stockpile Grazing, 5C = 5cm Continuous Grazing, 5R = 5cm Rotational Grazing, 10R = 10cm Rotational Grazing.
[b] Different letter within the same column denotes a difference, ($P<0.05$).

High slope areas had a greater percentage of rainfall lost as runoff than low slope areas in both years (21.2 vs. 14.6% in year 1 and 16.0 vs. 9.8% in year 2) across all treatments and months ($P<0.05$). There was no effect of slope on sediment or total P and soluble P concentrations or total and soluble P losses in runoff for either year. Sediment loss from high slope areas was greater ($P<0.05$) than from low slope areas in year 1 (14.7 vs. 7.3 kg/ha) across all treatments. There was no significant effect of slope on sediment loss in year 2.

In both years, sward heights of the grazed paddocks were greatest in the early summer period ($P<0.05$). By later sampling periods, the paddocks had been sufficiently grazed to reach their prescribed forage sward height.

Soil moisture in the upper 5 cm was greater in year 1 than in year 2 ($P<0.05$), 23.3% and 20.5%, respectively. Soil moisture was greater in the U paddocks (24.6% and 22.1% for year 1 and 2, respectively) than in all other paddocks ($P<0.05$) in both years, with no difference in soil moisture between the other treatments (23.1%, 23.8%, 23.0%, and 21.8% in year 1 and 19.6%, 20.8%, 20.9%, and 20.0% in year 2 for 5C, 5R, 10R, and HS, respectively). In both years soil moisture followed the same trend with soil moisture high in the late spring, lowest in the mid-summer, intermediate in the autumn, and high in early spring ($P<0.05$). Soil moisture was 27.5%, 16.2%, 22.9%, and 26.6% in year 1 and 24.2%, 13.6%, 21.9%, and 23.8% in year 2 for late spring, mid-summer, autumn, and early spring, respectively.

Mean penetration resistance in the 0 to 10 cm depth for the four sampling periods in year 1 was lowest for the U treatment (20.5 kg-force), intermediate for the HS (23.5 kg-force) treatment, and greatest in the summer grazing treatments (25.0, 26.1, and 26.2 kg-force for the 5C, 5R, and 10R, respectively; $P<0.05$) but did not differ between summer grazing treatments. In year 2, mean penetration resistance in the 0 to 10 cm depth the U paddocks were lower ($P<0.05$) (24.8 kg-force) than all other treatments. However, there were no differences in penetration resistance between paddocks with different forage utilization systems (30.6, 33.9, 33.5, and 34.3 kg-force for the HS, 5C, 5R and 10R treatments, respectively). Mean penetration resistance in the 10 to 20 cm depth was unaffected by treatment in either year, averaging 27.2 and 34.7 kg-force across all treatments for year 1 and 2, respectively. Similarly, mean penetration resistance in the 20 to 35 cm depth was unaffected by treatment in either year averaging 28.6 and 39.5 kg-force for year 1 and 2, respectively.

Averaged across months, surface cover in ungrazed paddocks was greater ($P<0.05$) than paddocks in which forage was harvested either as hay or grazed. In both years, surface cover in the 5C paddocks was lower than paddocks with other treatments ($P<0.05$). Mean surface covers were 99.0%, 93.5%, 82.8%, 89.9%, and 93.3% in year 1 and 99.1%, 95.5%, 89.1%, 91.4%, and 94.1% in year 2 for the U, HS, 5C, 5R, and 10R treatments, respectively.

Surface roughness did not differ by treatment or time in either year. Soil Bray-1 P concentrations in the upper 5 cm were 20 to 25 ppm at the initiation of the experiment and did not differ between treatment or sampling period in either year. However, total P losses during rainfall simulations were greater from simulation sites that had greater soil Bray-1 P concentrations.

Buffer Effects. Mean sediment concentrations in the runoff were not affected by simulation location or month in either year. However, mean total P and soluble P concentrations in run-off were greater (P<0.05) in the paddocks than at the base of the paddock or 10-meter in the buffer in both years. Over the two years, mean concentrations of total and soluble P from paddocks were 49.5% and 47.4% greater, respectively, (P<0.05) than the mean values within the buffers. This result indicates that grazing will increase the amount of P that is available for transport within a pasture, but it rapidly becomes immobile again in ungrazed buffer areas.

In year 1, the proportion of rainfall lost as runoff was greater (P<0.05) in the paddocks than at the paddock base and at 10 meters within the buffer. In year 2, the proportion of rainfall lost as runoff was greater (P<0.05) in the paddocks and at the paddock base than at 10 meters within the buffer. In year 1, percent runoff was 17.1%, 11.7%, and 8.6% and in year 2 percent runoff was 12.8%, 12.8%, and 7.5% from the paddock, base of the paddock, and 10 m within the buffer, respectively. These differences can partially be attributed to the differences in soil slope, soil texture, and forage composition that exist between locations.

In year 1, there was no difference in sediment loss between the paddock and the two locations within the buffer. In year 2, sediment loss was greatest from the paddock, lowest from 10m within the buffer, and intermediate at the base of the buffer (Table 2). As a result of differences in rainfall infiltration and the total phosphorus concentration of runoff, total phosphorus flows from the paddocks were 3.5 and 7.0 times greater (P<0.05) than those from the paddock base and in the buffer in year 1 and 2.0 and 4.0 times greater (P<0.05) than those from the paddock base and in the buffer in year 2. Amounts of soluble P in the runoff were 3.0 and 24 times greater in the buffer and at the base of the paddock than in the paddock in year 1.

Table 2. Sediment, Total P, and Soluble P losses within the paddocks, at the base of the paddock, and 10m within the buffer.

	Sediment, (kg/ha)		Total P, (kg P/ha)		Soluble P, (kg P/ha)	
	Year 1[b]	Year 2	Year 1	Year 2	Year 1	Year 2
Paddock[a]	44.0	38.4[a]	0.28[a]	0.16[a]	0.24[a]	0.08
Base	27.6	21.2[a,b]	0.08[b]	0.08[b]	0.08[b]	0.03
Buffer	20.4	10.4[b]	0.04[b]	0.04[b]	0.01[b]	0.02

[a] Paddock = Average across all paddocks, Base = At the paddock-buffer interface, within the buffer, Buffer = Within the buffer, 10m down slope from the paddocks
[b] Different letter within the same column denotes a difference, (P<0.05).

Across treatments, mean forage sward heights in paddocks, at the paddock base, and 10 meters in the buffer strip were 9.4, 17.3, and 18.1 cm in year 1 and 12.2, 22.9, and 24.6 cm in year 2 (P<0.05).

Penetration resistance in the upper 10 cm of soil was greater in the paddocks than at either the paddock base or 10 meters in the buffer strip ($P<0.05$) for all sampling periods except late spring of year 1. In both years at all locations and depths, penetration resistance was low during the late spring, increased to a maximum in mid-summer, decreased to an intermediate level by autumn, and had returned to late spring levels by early spring. These differences not only represent treatment effects but are also influenced by soil moisture and texture differences between location and sampling periods.

Major Findings. Of the physical measurements, the proportion of ground cover was most highly related to sediment loss. Results imply that sediment and phosphorus losses in pasture runoff may be reduced by managing rotational stocking to maintain adequate sward height and/or using vegetative buffer strips along pasture streams. Such management practices are particularly important in pastures on soils with high Bray-1 P concentrations.

Time line of research and outreach activities for the March 2002 to February 2003 time period

- April 2002 - Early spring rainfall simulations
- April/May 2002 - Processed runoff samples from April rainfall simulations
- May 2002 - Grazing initiated on pastures, Hay cut
- June 2002 - Late spring rainfall simulations, Processed runoff samples from June rainfall simulations
- July 2002 - Presented preliminary research results at the Rhodes Research and Demonstration Farm Field Day, Presented first years data at the American Forage and Grassland Conference meetings (Title "Effects of grazing management on sediment and phosphorus runoff"), Mid-summer rainfall simulations
- July/August 2002 - Processed runoff samples from July rainfall simulations
- October 2002 - Summer grazing period terminated, Fall rainfall simulations
- October/November 2002 - Processed runoff samples from October rainfall simulations
- November 2002 - Stockpile forage grazing period
- January 2003 - Results from 2001 and 2002 summarized for *2003 ISU Beef Research Report*, titles: "Effects of Grazing Management on Pasture Production and Phosphorous Content of Forage (A Progress Report)" and "Effects of Grazing Management on Sediment and Phosphorous Losses in Runoff (A Progress Report)." Copies available online at http://www.iowabeefcenter.org.

Evaluating the effectiveness of restored wetlands for reducing nutrient losses from agricultural watersheds

Basic Information

Title:	Evaluating the effectiveness of restored wetlands for reducing nutrient losses from agricultural watersheds
Project Number:	2002IA9B
Start Date:	3/1/2001
End Date:	2/28/2003
Funding Source:	104B
Congressional District:	Iowa 3rd
Research Category:	Not Applicable
Focus Category:	Wetlands, Nutrients, None
Descriptors:	
Principal Investigators:	Arnold G Van der Valk, William G. Crumpton

Publication

Nontechnical Summary

This study examined the effectiveness of recent wetland restorations and land use conversions for reducing nutrients in agricultural runoff in the Iowa Great Lakes watershed. It had two major research objectives: (1) to monitor nutrient concentrations in the inputs and outputs of restored wetlands to see how effective they are as nutrient sinks, and (2) to monitor nutrient concentrations in the outflow of subwatersheds differing in the extent of wetland restoration and set-aside acreage to determine if these differences have significantly reduced the levels of nutrients in subwatershed outflows. A review of available data on the 278 restored wetlands indicates that runoff from, at most, about 20% of the upland areas in the Iowa Great Lakes watershed passes through restored wetlands. In addition, the wetland restorations are located primarily in areas that are no longer cultivated, and, consequently, most of the wetlands do not receive significant agricultural runoff. Where they do receive agricultural drainage, the restored wetlands were effective sinks for total nitrogen (TN), but their effectiveness as sinks for total phosphorous (TP) is less clear. For subwatersheds, restoring wetlands and taking uplands out of crop production reduces the concentrations of total nitrogen in their outflows significantly, but effects on total phosphorus are unclear.

Project Goals and Objectives

Water quality data from the Iowa Great Lakes indicates that concentrations of nutrients in these lakes have not declined as a result of the restoration of hundreds of wetlands in the watershed. Why restoring wetlands has not lowered nutrient concentrations in the Iowa Great Lakes is the overarching goal of this study. This study investigated two possible reasons why restored wetlands may not be effective nutrient sinks: (1) Restored wetlands may not yet have the nutrient removal capacity of natural wetlands; and (2) the restored wetlands in the watershed may not intercept sufficient nutrient runoff to significantly impact overall nutrient inputs into the lakes.

The four specific objectives of the study were:

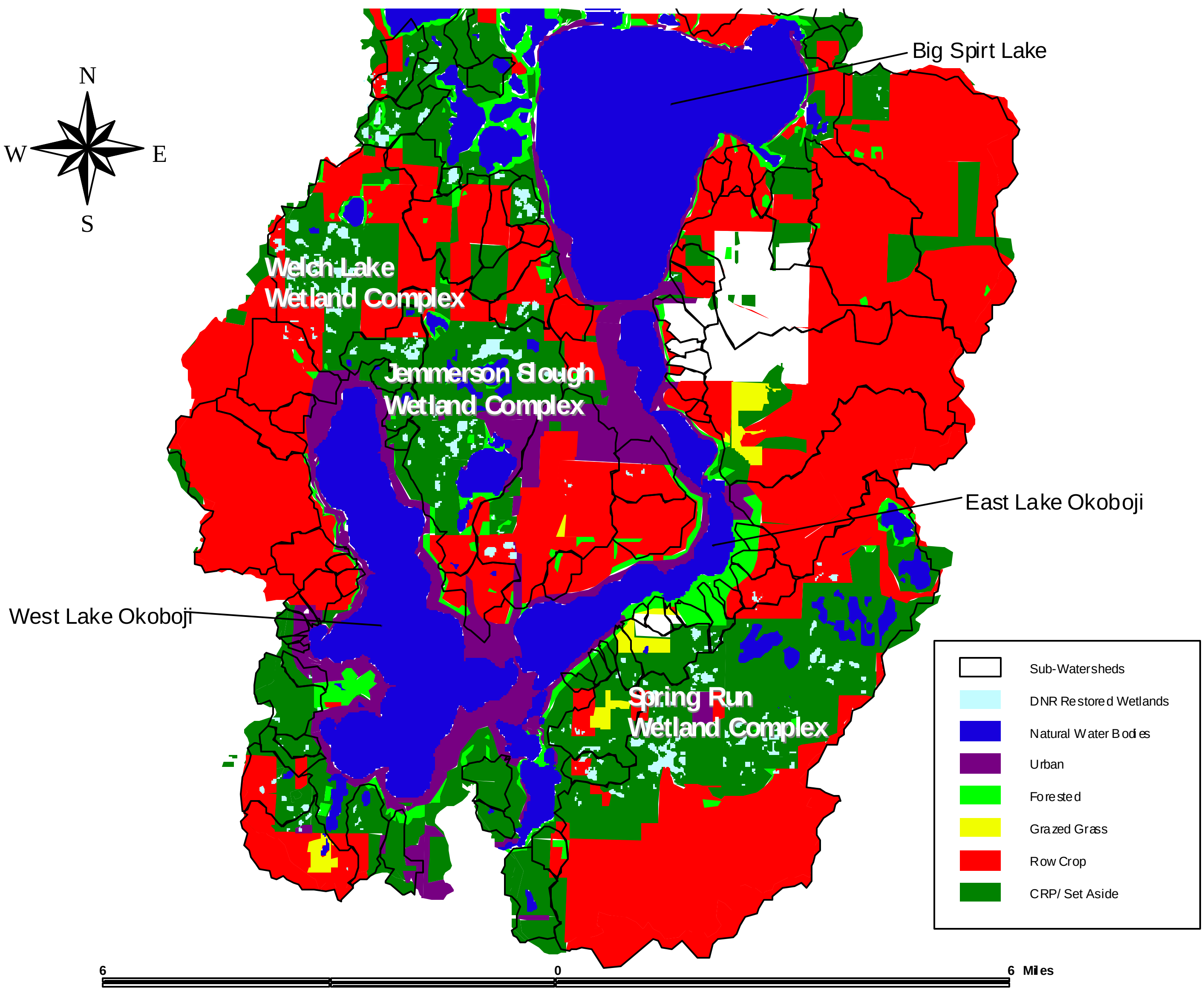
(1) To determine the number, location, and size of the restored wetlands in the Iowa Great lakes watershed.
(2) To determine the composition, abundance, and distribution of the vegetation and biomass of living and dead vegetation in selected restored wetlands.
(3) To estimate nutrient removal capacity of selected restored wetlands by measuring nutrient input and output concentrations.
(4) To measure the nutrient losses from subwatersheds primarily in row crops with and without restored wetlands.

Results

(1) Restored Wetland Inventory. All available data on restored wetlands in the Iowa Great Lakes watershed were obtained from the Dickinson County offices of the Iowa Department of Natural Resources and the Natural Resources and Conservation Service of the USDA. Information available about these restored wetlands was highly variable and often very limited. Digitized land-use and topographic maps of the watershed were used to collect data on the location, area, and catchment size of each restored wetland (Figure 1).

By the end of the summer of 2002, there were 278 restored wetlands in the Iowa Great lakes watershed. For the most part, these restored wetlands were found in clusters or complexes on large tracts of land managed by the Iowa Department of Natural Resources. In these areas, the uplands have mostly been taken out of row crops and converted to some type of perennial grassland. The total area of these 278 restored wetlands is only 360 ha (888 acres) or 1.2% of the upland area of the Iowa Great Lakes watershed. The total area of the potential catchments of these restored wetlands is about 6,429 ha (15,873 acres) or 21.5% of the upland area of the Iowa Great Lakes watershed. This represents the maximum area of the potential catchments of these 278 wetlands and was derived from an analysis of terrain models. The actual catchments undoubtedly have a smaller area. In short, most of the restored wetlands in the watershed are small (ca. 1.3 ha or 3.19 acres) and they are located primarily in a small number of publicly owned areas that are no longer in row crops. Consequently, most of these wetlands do not intercept significant amounts of agricultural runoff.

Figure 1. Map of the Iowa Great Lakes Region with land use practices, delineated subwatersheds, and restored wetlands shown.



(2) Vegetation of Restored Wetlands. To investigate whether the state of development of restored wetlands affects their capacity as nutrient sinks, five restored wetlands were selected for monitoring and detailed sampling of their vegetation. Finding suitable wetlands whose nutrient inputs and outputs could be monitored proved difficult. An analysis of available records for restored wetlands eliminated the majority of them from consideration. Most of the wetlands received little or no agricultural runoff and lacked well-defined inputs and outputs that could be sampled. Of the 278 sites examined only about 30 were identified in preliminary screening as potential study sites. Of these 30 wetlands, the five that could be most reliably sampled were selected based on site evaluations. The selected wetlands ranged in size from 0.313 ha (0.773 acres) to 3.59 ha (8.865 acres), and their catchments ranged from 14.6 ha (36 acres) to 114.5 ha (283 acres).

In the summer of 2001, sampling of the vegetation and standing crop of the five selected restored wetlands was initiated. Each wetland was divided into a series of parallel zones and each zone was sampled using a randomly located transect in the zone. Samples were collected in quadrats placed at random intervals along these transects. The cover of each species in each 1m x 1 m quadrat was recorded and then all aboveground vegetation clipped and bagged. All standing crop samples were oven dried and weighed. Tables 1 and 2 summarize the vegetation data for each of these five wetlands. In general, their vegetation was similar and dominated by a small number of common wetland species. The vegetation of four of the five wetlands was dominated by *Phalaris arundicnacea* (reed canary grass) and *Typha glauca* (cattail). The vegetation of the fifth wetland (wetland 8), which was a dammed up stream and deeper than the others, was dominated by submerged aquatics, *Potamogeton* spp. (pondweeds). Other common species were *Scirpus fluviatilis* (great river bulrush) and *Scirpus validus* (soft-stem bulrush). Although there were submerged aquatic and emergent zones in these wetlands, they were not as dense or species rich as those found around comparable extant prairie potholes in NW Iowa.

The mean standing crop or biomass in restored wetlands ranged from 40 to 735 g/m^2 and averaged 430 g/m^2 (Table 1). This is considerably lower than standing crops found in natural wetlands in northern Iowa, ca. 600 to 1,000 g/m^2. The standing dead component of the vegetation was again dominated by *Phalaris*, *Typha* , and *Scirpus* species. The standing dead or necromass ranged from 23 to 393 g/m^2 and averaged 260 g/m^2 (Table 2).

(3) Restored Wetland Nutrient Inputs and Outputs. In the five wetlands whose vegetation was sampled, nutrient concentrations of inputs and outputs were estimated weekly using grab samples in 2001 and 2002. All of the water samples collected were analyzed for total nitrogen (TN) and total phosphorous (TN) using standard methods.

Table 3 summarizes the annual input and output concentrations of TN and TP. The overall mean input concentration of TN for all five wetlands over both years was 19.0 mg/l while the mean annual output concentration was 2.93 mg/l. This is a mean reduction in TN concentrations of about 85%. However, wetland catchments were too small and flows were too low and variable to estimate mass loading to the wetlands or mass reductions by the wetlands during the study period. Only 2 of the 5 wetlands received flow for more than a few weeks after sampling was initiated in 2001, and there was little flow entering any of the wetlands during 2002 due to drought conditions. Two of the wetlands had no outflow at all during 2002. Although it is clear that all five wetlands reduced TN significantly over both years, it is not clear whether they significantly affected TP. The overall mean total phosphorus concentration in the inputs of these five restored wetlands was 0.189 mg/l and the overall mean concentration in the outputs was 0.108 mg/l. However, inflow and outflow TP concentrations were too variable to draw any conclusions regarding reductions.

There is no correlation between nutrient reduction and either living or dead biomass. For example, wetland 8, whose total biomass was only 40 g/m^2, and wetland 16, whose biomass was 735 g/m^2, in 2001 had TN reduction of 83% and 87%, respectively. Nothing in our data suggests that the nutrient removal capacity of restored wetlands is limited because they do not yet have comparable vegetation or biomass to that of the extant wetlands in the region.

Table 1. Percent frequency of the most common species found in restored wetlands and their mean total biomass.

Species	Wetland 1	7	8	12	16
			Submerged Species		
Ceratophyllum demsersum	53%	30%	0%	7%	8%
Lemna minor	7%	11%	0%	33%	56%
Lemna trisulca	13%	21%	0%	21%	0%
Myriophylum spicatum	40%	14%	0%	42%	2%
Potamogeton spp.	33%	49%	83%	18%	25%
			Emergent Species		
Alisma plantago-aquatica	0%	4%	0%	4%	0%
Eleocharis palustris	0%	8%	0%	2%	0%
Leersia orryzoides	13%	1%	3%	2%	0%
Phalaris arundinacea	60%	6%	20%	40%	81%
Sagittaria latifolia	0%	4%	3%	1%	0%
Scirpus fluviatilis	33%	14%	0%	7%	2%
Scirpus validus	40%	30%	3%	1%	19%
Sparganium eurycarpum	7%	0%	15%	1%	2%
Typha glauca	20%	93%	0%	33%	65%
			Other Species		
Asclepias incarnata	7%	1%	0%	0%	4%
Carex spp.	7%	0%	3%	0%	2%
Cirsium arvense	7%	25%	8%	0%	2%
Mentha arvensis	13%	3%	0%	0%	0%
Polygonum spp.	0%	34%	0%	1%	2%
Minor species	0%	0%	0%	2%	0%
Number of Quadrats	15	71	40	84	48
Mean biomass (g/m^2)	452	412	272	40	735
Basin Area (acres)	0.77	4.70	1.87	8.33	3.66

Table 2. Percent frequency of standing dead species and their mean total necromass.

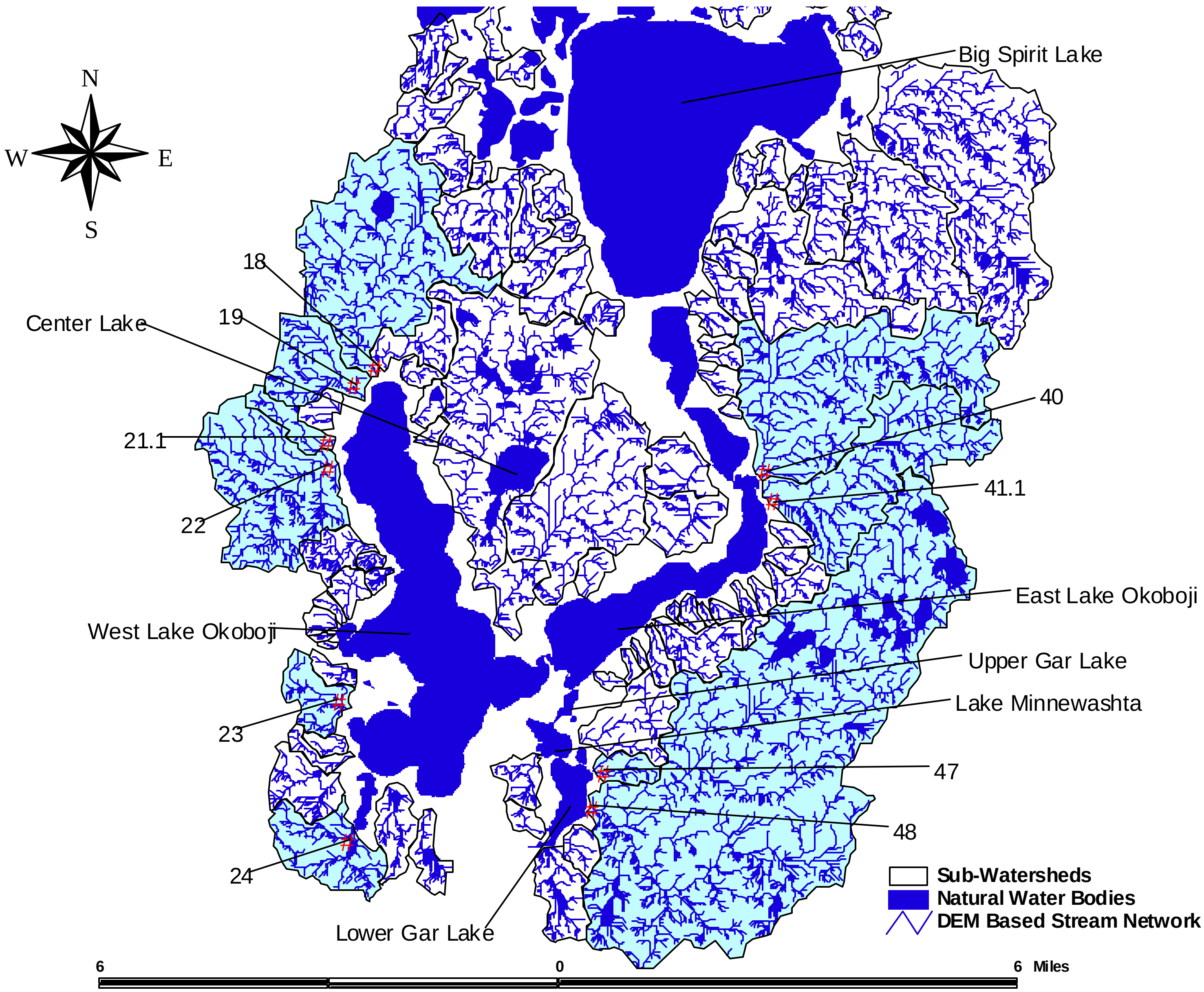
Species	Wetland 1	7	8	12	16
			Submerged Species		
Ceratophyllum demsersum	0%	1%	0%	0%	0%
Lemna minor	0%	0%	0%	0%	2%
Myriophylum spicatum	0%	0%	0%	1%	0%
Potamogeton spp.	0%	0%	0%	1%	0%
			Emergent Species		
Alisma plantago-aquatica	0%	0%	0%	1%	0%
Eleocharis palustris	0%	3%	0%	0%	0%
Leersia orryzoides	7%	0%	0%	1%	0%
Phalaris arundinacea	13%	0%	5%	32%	75%
Scirpus fluviatilis	13%	7%	0%	1%	2%
Scirpus validus	13%	8%	0%	0%	0%
Sparganium eurycarpum	7%	0%	0%	2%	2%
Typha glauca	20%	66%	0%	21%	44%
			Other Species		
Asclepias incarnata	0%	1%	0%	0%	2%
Carex spp.	7%	0%	0%	0%	0%
Cirsium arvense	0%	11%	0%	0%	0%
Mentha arvensis	0%	1%	0%	0%	0%
Polygonum spp.	0%	3%	0%	0%	0%
Minor species	0%	1%	0%	0%	0%
Number of Quadrats	15	71	40	84	48
Mean necromass (g/m^2)	114	511	23	168	393

Table 3. Annual mean concentrations (mg/l) of total nitrogen (TN) and total phosphorus (TP) in the inflows and outflows from five restored wetlands in Dickinson County, Iowa.

	Total Nitrogen (TN)		Total Phosphorus (TP)	
Sample Location	2001	2002	2001	2002
Wetland 1				
1_IN_E	20.88	19.45	0.091	0.06
1_IN_S	20.4	No Flow	0.118	No Flow
1_OUT	6.53	7.65	0.063	0.052
Wetland 7				
7_IN	22.66	No Flow	0.089	No Flow
7_OUT	2.08	No Flow	0.054	No Flow
Wetland 8				
8_IN	17.5	26.2	0.506	0.165
8-OUT	2.94	5.24	0.145	0.178
Wetland 12				
12_IN	7.49	8.15	0.236	0.303
12_OUT	0.65	0.83	0.211	0.083
Wetland 16				
16_IN	14.96	13.197	0.236	0.086
16_OUT	2.02	1.4	0.117	0.07

(4) Subwatershed Nutrient Outputs. In 2000, 2001, and 2002, grab samples were collected at the outflows from 10 selected subwatersheds (Figure 2) differing in predominant land use and in extent of restored wetlands (Table 4). All of the water samples were analyzed for total nitrogen and total phosphorous. There were five subwatersheds that were mostly cropland (19, 21.1, 22, 40, and 41), 2 intermediate subwatersheds with extensive wetland restoration and with less than 50% cropland (18, 48), and two subwatersheds (23, 47) nearly entirely in restored wetlands and set-aside programs. The remaining subwatershed was 69% cropland transitioning to pasture with a pastured wetland (24).

Figure 2. Map of Iowa Great Lakes region with 10 selected subwatersheds and sampling locations shown.



Nitrate concentrations were closely related to subwatershed land use, being highest in subwatersheds with predominantly cropland and falling to near detection limits in subwatersheds with extensive wetland restoration and set-aside. Nitrate comprised the major fraction of TN in subwatersheds with much cropland and the pattern of TN concentrations was similar to that of nitrate. As in the case of nitrate, TN concentrations were closely related to subwatershed land use, with highest concentrations in subwatersheds dominated by cropland and lowest concentrations in subwatersheds with little or no cropland. The mean annual concentration of TN in outflows from the five subwatersheds mostly in row crops ranged from 5.26 to 19.0 mg/l (Table 4). For the 2 subwatersheds with greatest extent of restored wetlands and the least amount of land in cultivation, mean annual TN concentrations in outflows ranged were 1.0 and 1.6 mg/L. The two intermediate subwatersheds had mean annual TN concentrations of 4.34 and 6.31 mg/L. A comparison of long-term patterns in TN concentrations (Figure 3) illustrates the separation in TN concentrations among subwatersheds with extensive cropland (40), versus intermediate amounts of cropland (48), versus no significant cropland (47). In general, concentrations of TN were lower in outflows from subwatersheds with extensive wetland restoration and conversion of cropland to set-aside. However, the relative contribution of wetland restoration and set-aside programs is obscured by the correlation of these land use changes. Subwatersheds with extensive wetland restoration tended to have extensive cropland conversion.

TP concentrations displayed more short-term variability than TN concentrations and were less clearly related to subwatershed land use. A comparison of long-term patterns in TP concentrations (Figure 4) illustrates considerable overlap in TP concentrations across subwatersheds with land use ranging from extensive cropland (40), through intermediate amounts of cropland (48), to no significant cropland (47). Mean annual TP concentrations (Table 4) in the subwatersheds predominantly in cropland ranged from 0.069 to 0.168 mg/L, while in subwatersheds with the least cropland and the greatest extent of wetland restoration and set-aside mean annual TP concentrations were 0.086 and 0.106 mg/L. The two intermediate subwatersheds had mean annual TP concentrations of 0.109 and 0.180 mg/L. Subwatersheds with extensive set-aside and restored wetlands do not consistently have lower TP concentrations in their outflows than those predominantly in row crops and without restored wetlands.

Nutrient concentrations in weekly grab samples may not reflect the true, flow-weighted average concentrations in subwatershed outflows, and this is especially likely in the case of TP. Weekly grab samples fail to capture major flow events, during which much of the TP load to the lakes is probably transported, and patterns in TP concentrations cannot be assumed to reflect patterns in P mass export from subwatersheds or P mass loading to the lakes. The pattern of flow events is likely to be a primary determinant of nutrient loading to the lakes. This can be illustrated by comparing the long-term patterns in nutrient loading crudely estimated from TN and TP concentrations and relative water yield for the region based on stream flow measurements (Figure 5 and 6). Patterns in TN and TP concentrations (Figures 3 and 4) do not reflect patterns in TN and TP mass transport (Figures 5 and 6). Better estimates of nutrient loading require continuous flow measurements and automated sampling of subwatershed outflows to estimated flow-weighted concentrations and mass transport. These data are needed in order to calibrate watershed scale models of nutrient loading and develop a targeted approach to wetland siting for nutrient reduction in the IGL watershed. In the summer of 2003, selected IGL subwatersheds were instrumented with automated samplers with continuous flow monitoring to address this need.

Although there is significant variation in the concentration of TN in outflows from subwatershed to subwatershed, as expected, restored wetlands and land set-aside programs are effective in reducing nitrate losses from subwatersheds. For total phosphorus, thought to be the major nutrient responsible for algal blooms in most lakes, the outcome is less clear. The results from the subwatershed studies parallel those from the restored wetland studies. In both cases, TN concentrations are reduced consistently, but TP varies much more, both spatially and temporally.

Table 4. Mean annual concentration (mg/l) of total nitrogen (TN) and total phosphorus (TP) from selected subwatersheds of the Iowa Great Lakes. Subwatersheds arranged by land use.

Watershed			Total Nitrogen (TN)				Total Phosphorus (TP)			
	Acres	% Row Crop	2000	2001	2002	Mean	2000	2001	2002	Mean
Predominately Row Crop										
E3(40)	2980	85	5.92	7.54	8.86	7.44	0.11	0.166	0.095	0.124
E4(41)	1946	95	9.16	10.72	14	11.29	0.101	0.189	0.089	0.126
W14(19)	660	88	3.25	5.51	7.01	5.26	0.092	0.137	0.088	0.106
W13(22)	1760	93	5.16	10.91	12.53	9.53	0.14	0.275	0.089	0.168
W14(21.1)	236	100	17.34	19.15	20.52	19	0.076	0.063	0.068	0.069
Mixed Row Crop and Grassland										
G3(48)	9359	38	4.69	3.45	4.87	4.34	0.09	0.146	0.091	0.109
W2(18)	2702	45	5.78	7.08	6.08	6.31	0.169	0.212	0.16	0.18
Predominately ungrazed grassland										
G6(47)	185	0	1.28	1.02	0.69	1	0.099	0.125	0.095	0.106
W10(23)	371	0	No flow	2.88	1.76	1.56	No flow	0.165	0.094	0.086
Other										
W9(24)	743	69	No flow	1.54	3.38	1.64	No flow	0.286	0.439	0.242

Figure 3. Comparison of measured concentrations of Total N for 2000 to 2002 in streams draining selected watersheds in the Iowa Great Lakes Region with different land use.

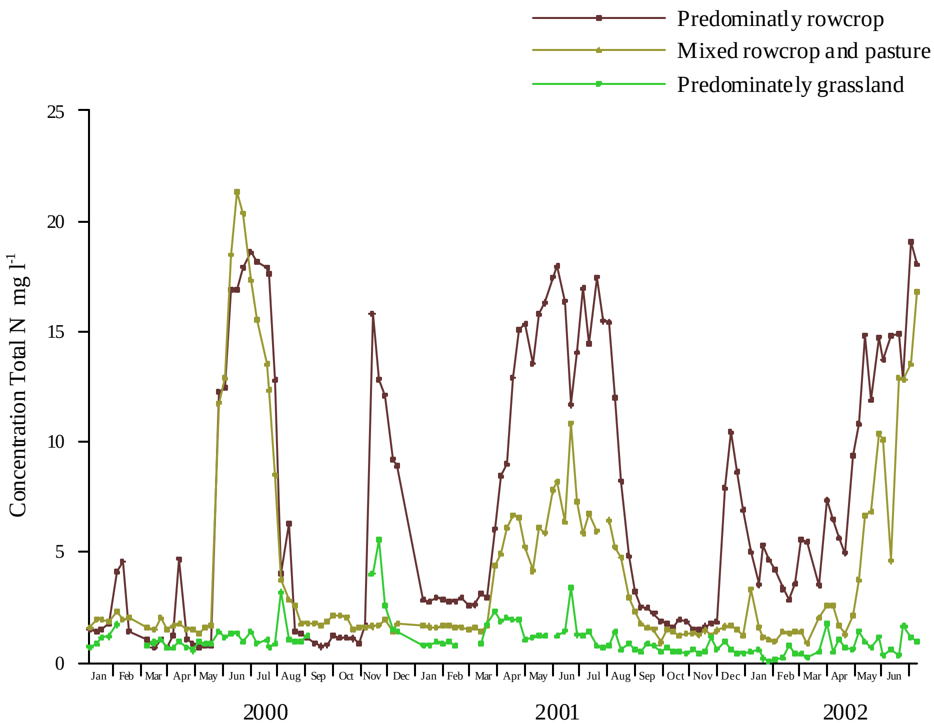


Figure 4. Comparison of measured concentrations of Total P for 2000 to 2002 in streams draining selected watersheds in the Iowa Great Lakes Region with different land use.

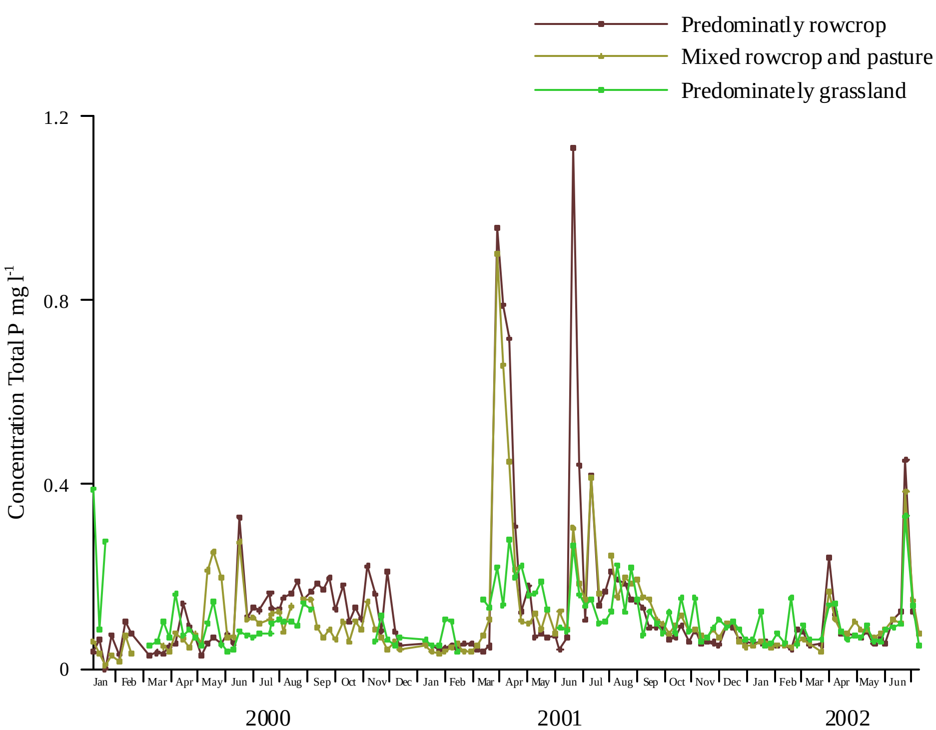


Figure 5. Estimated loading rates of Total N for 2000 to 2002 in streams draining selected watersheds in the Iowa Great Lakes Region with different land use.

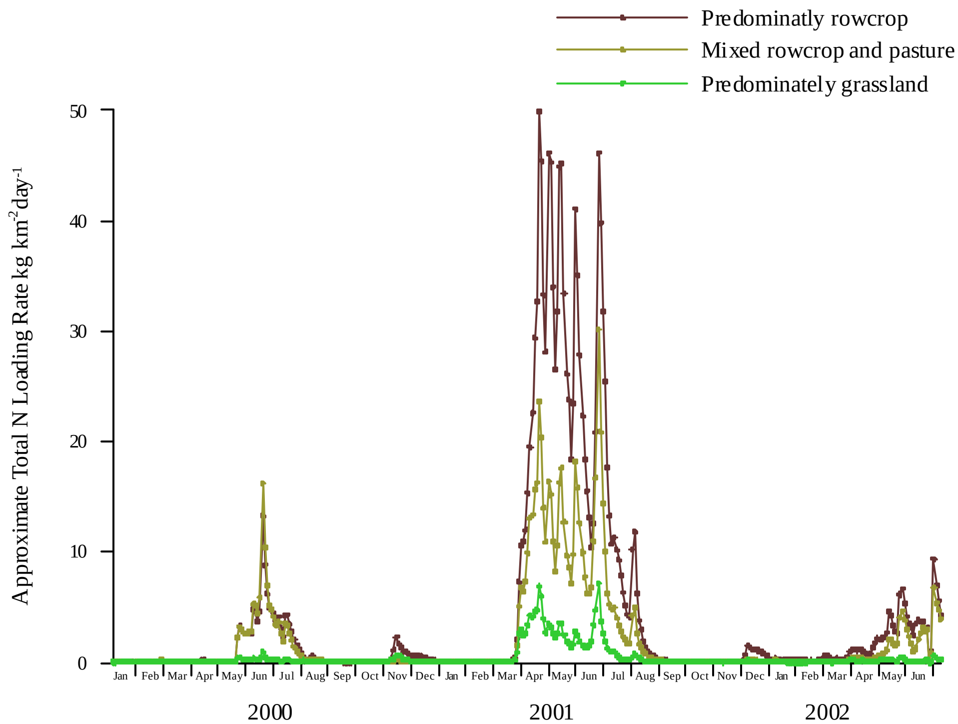
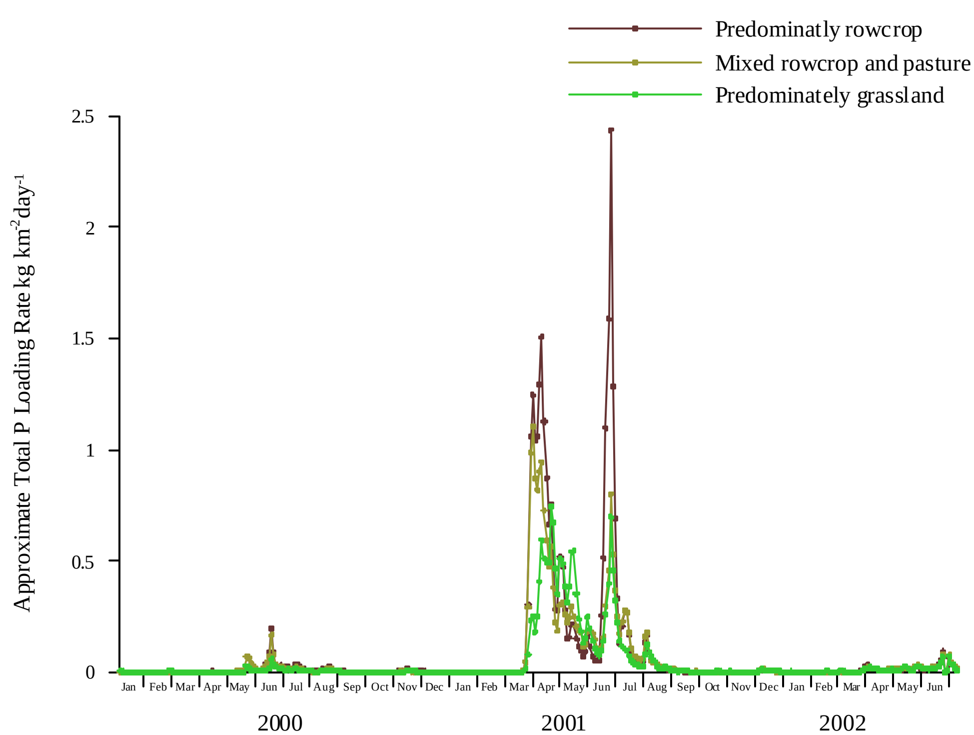


Figure 6. Estimated loading rates of Total P for 2000 to 2002 in streams draining selected watersheds in the Iowa Great Lakes Region with different land use.



Conclusions

Although nearly 280 wetlands have been restored in the Iowa Great Lakes watershed, at best, these wetlands intercept runoff from about 20% of the uplands in the watershed. For TN, this suggests that nitrogen inputs may have been reduced by about 15%. For TP, they would be reduced less than 10%. Consequently, it is not surprising that nutrient concentration in the Iowa Great Lakes has not begun to decline. When all sources of nutrients into these lakes are considered (dry fallout, wet fallout, internal loadings, urban runoff, etc.), the effects of wetland and upland restorations on nutrient levels in the lakes are still too small to be detectable.

Publications and Presentations

All of the research is ongoing and no publications have resulted yet. One thesis should be completed in the next year. A presentation on some of the preliminary results was made at the annual Midwest Limnology Conference. This study was featured in the Leopold Letters newsletter (Vol. 13, No.4, Winter 2001). It was also discussed in an interview on WOI Radio's noontime show. Currently, a graduate student, Brandon Dittman, is using data collected on the characteristics of restored wetlands for a creative component as part of his MS degree in Water Resources.

Fate and transfer of antibiotic-resistance genes excreted by farm animals

Basic Information

Title:	Fate and transfer of antibiotic-resistance genes excreted by farm animals
Project Number:	2002IA10B
Start Date:	3/1/2002
End Date:	2/28/2003
Funding Source:	104B
Congressional District:	Iowa 1st
Research Category:	Not Applicable
Focus Category:	Agriculture, Groundwater, Non Point Pollution
Descriptors:	Genetic drift, bacteria, contaminant transport, natural attenuation, and public health
Principal Investigators:	Pedro J Alvarez

Publication

Problem and Research Objectives

Antibiotic resistance is a public health concern of great urgency due to a growing inefficacy of antimicrobial agents to treat infectious diseases. This is mainly due to the propagation of antibiotic resistance genes among bacteria, which is exacerbated by the potential overuse of antimicrobials in humans and the intensive use of antibiotics in animal agriculture for non-therapeutic purposes such as growth promotion and disease prevention (Mellon et al., 2001). Recent studies have found that antibiotic resistance genes occur in bacteria in the environment as a direct result of animal agriculture (e.g., swine production facilities), and that soil and groundwater in the vicinity of such facilities may be *potential sources of antibiotic resistance in the food chain* (Chee-Sanford et al., 2001). However, such genes have not yet been considered as environmental pollutants, and little is known about the fate and transport of antibiotic resistance genes when released to the environment as a result of direct runoff, groundwater infiltration from lagoons, or manure spreading activities. Critical knowledge gaps include the rate and extent of gene propagation (including bacterial migration and inter-specific gene transfer from enteric to soil bacteria) and the effect of environmental factors such as soil characteristics and water chemistry on the persistence of antibiotic resistance. Learning about these issues is important to assess the impact of antibiotic resistance genes on public and environmental health and to determine the need for regulatory action in states where animal agriculture is common.

This study addresses the effect of antibiotic exposure (e.g. tetracycline (TC)) on indigenous soil microorganisms in simulated runoff infiltration conditions. Emphasis was placed on addressing the following issues: (1) persistence of TC in a flow through column, (2) effect of the presence of an antibiotic on the microbial community (populations) within a flow through column, and (3) genetic characterization of the microorganisms isolated from the columns exposed to the antibiotic.

The main goals of the ongoing research are to:

<u>Short Term</u>

1. Characterize the fate and transport of TC in soil.
2. Determine the effect of sustained TC exposure on the development of TC-resistant strains.

<u>Long Term</u>

1. Monitor the development and relative abundance of TC-resistant strains.
2. Genotypic characterization of resistant strains.
3. Model the resistance gene transfer.

Methodology

Flow-through Columns. Two cylindrical, 30-cm-long, flow-through glass columns (Kontes Glass Company, Vineland, NJ) were modified with six sample ports located at 2, 5, 9, 14, 19, and 24 cm from the bottom inlet of the column. Inlet and outlet three-way valves were placed at the respective locations. The columns were secured in a vertical position and tightly packed with soil (University of Iowa Softball Field). The columns were wrapped in aluminum foil to minimize algal growth and possible antibiotic photodegradation.

Two-L reservoir bottles were equipped with 3-hole caps (Kontes Glass Company, Vineland, NJ) and wrapped in aluminum foil. Masterflex Neoprene® tubing (Cole-Parmer Co.) and a Masterflex peristaltic pump (Cole-Parmer Co.) were used for the delivery of the feed solution. The pump flow rate was adjusted as to achieve a column flow rate range between 3.0 and 4.0 mL/hr. The flow rate for the control column (TC -) was approximately 3.4 mL/hr and 3.6 mL/hr for the TC-enriched column. Bromide tracer studies were conducted on both columns prior to addition of the feed solutions.

The feed solution for both columns consisted of synthetic ground water (von Gunten and Zorbist, 1993) as nutrient source and sodium acetate as a carbon source (10 mg/L). In addition, one feed solution was amended with tetracycline-hydrochloride (T3383, Sigma Co.) at 10-50 mg/L.

Concentration Profiles. The concentrations of acetate and tetracycline were monitored along the column length (inlet, outlet, and sample ports) approximately every two months since column initiation. Standard curves for both chemicals were prepared monthly to ensure measurement accuracy. Acetate concentrations were measured via an anion chromatograph equipped with an auto-sampler apparatus (Alltech 570), an IonPAc AS14 column (Dionex), and a conductivity detector (Dionex). Tetracycline content was analyzed via a manual injection HPLC pump (Alltech 426) equipped with a HPLC column (Supelco, Discovery C8, 59353-U) and a variable wavelength detector (Dionex). Detection conditions were as follows: 680mL 0.1 Ammonium Oxalate, 270mL Dimethylformamide, 50 mL 0.2M Dibasic Ammonium Phosphate (pH 7.6), at 1 mL/min, 20 µL, Isocratic, Ambient, UV at 280 nm. The elution time for tetracycline was approximately 3 min.

Microbial Enumeration. Initially, agar plate counts for the enumeration of microbial populations were performed. The effluent from both columns was collected and 100 µL were streaked onto the R2A agar plates, with the intent to quantify the total heterotrophic populations. R2A plates enriched with tetracycline (50 mg/L) were also streaked with the column effluent in order to quantify the antibiotic-resistant microorganisms. Several attempts with this method yielded variable and irreproducible results.

A modified MPN 96-well plate technique was adapted for microbial enumeration of the column effluent. Growth media containing succinate and resazurin solution was used for the enumeration of the total heterotrophic population, and tryptic soy broth enriched with tetracycline (50 mg/L) was used for the antibiotic-resistant microorganisms. This quantification was based on visual scoring of the color change (blue to red for resazurin) and growth-induced TSB-turbidity development and subsequent statistical analysis.

Genetic Analysis. Effluent from the tetracycline exposed column was used for the isolation of antibiotic-resistant strains. Tetracycline enriched R2A agar plates were streaked with the effluent and incubated at 30°C for periods of 2-5 days, depending on the growth rates (appearance of colonies). Individual colonies were restreaked onto TC-enriched R2A agar plates, incubated, isolated, and restreaked a second time in order to ensure strain "purity."

Bacterial DNA was extracted with kits according to manufacturers' protocols (Qiagen). A Mastercycler® thermocycler device (Eppendorf) was purchased for the Polymerase Chain Reaction (PCR) gene detection techniques. PCR amplification was performed on the extracted DNA according to the protocols provided in the reaction kits (PanVera). The typical (50 μL) reaction mixture consisted of 0.25 μL DNA polymerase (Ex Taq), 5.0 μL 10X buffer, 4.0 μL dNTP mix (2.5 mM), 5.0 μL DNA template, 4.0 μL primers (forward and reverse), and 27.75 μL H_2O. The amplification was performed as previously described by Aminov, et.al. (2001). Briefly, the cycle steps were: (1) an initial denaturation at 94°C (5 min) followed by 25 cycles of 94°C (30s), (2) annealing at 30s and 30s extension (72°C), and (3) extension at 72°C (7 min). The annealing temperatures and the sequences for each primer are shown in Table 1.

DNA primers were constructed for the following tet-determinants coding for Ribosomal Protection Proteins (RPP): TetB(P), Tet(M), Tet(O), Tet(Q), Tet(S), Tet(T), Tet(W), and OtrA. PCR products were analyzed by electrophoresis on a 1.2% (wt/vol) agarose gel containing ethidium bromide. The expected sizes of the amplification products were, 168bp for TetW, 169bp for TetB(P), Tet(Q), Tet(S), Tet(T), 171bp for Tet(M), Tet(O), and 212 bp for OtrA. 16S ribosomal DNA (rDNA) analysis of the isolated strains was also conducted.

Table 1: PCR Primers targeting RPP classes (Aminov et al., 2001)

Primer	Class Targeted	Annealing T (°C)	Sequence
TetB/P-FW TetB/P-RV	Tet B P	46	AAAACTTATTATATTATAGTC TGGAGTATCAATAATATTCAC
TetM-FW TetM-RV	Tet M	55	ACAGAAAGCTTATTATATAAC TGGCGTGTCTATGATGTTCAC
TetO-FW TetO-RV	Tet O	60	ACGGARAGTTTATTGTATACC TGGCGTATCTATAATGTTGAC
OTR-FW OTR-RV	Otr A	66	GGCATYCTGGCCCACGT CCCGGGGTGTCGTASAGG
TetQ-FW TetQ-FW	Tet Q	63	AGAATCTGCTGTTTGCCAGTG CGGAGTGTCAATGATATTGCA
TetS-FW TetS-FW	Tet S	50	GAAAGCTTACTATACAGTAGC AGGAGTATCTACAATATTTAC
TetT-FW TetT-FW	Tet T	46	AAGGTTTATTATATAAAAGTG AGGTGTATCTATGATATTTAC
TetW-FW TetW-FW	Tet W	64	GAGAGCCTGCTATATGCCAGC GGGCGTATCCACAATGTTAAC

FW= forward, RV= reverse

Principal Findings and Significance

Initially, tetracycline concentrations were monitored in the influent and effluent of the exposed column. Approximately 97% of the initial tetracycline was degraded within the column length (Figure 1).

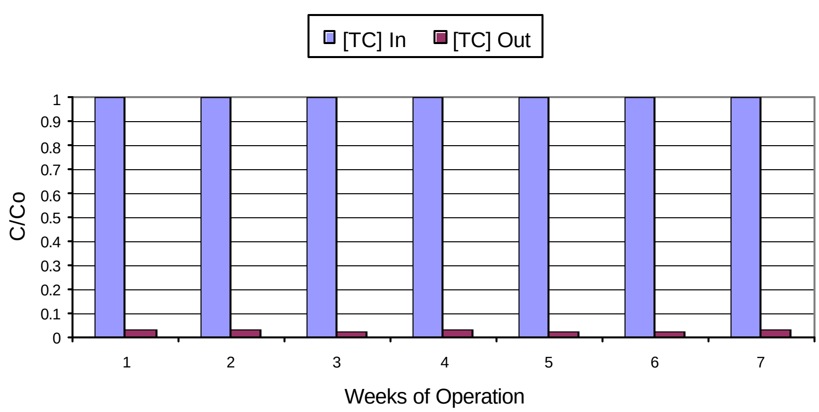


Figure 1: Inlet and outlet standardized antibiotic concentrations
for TC-enriched column (operation start date: 8/9/2002)

Tetracycline and acetate concentration profiles along the length of the columns were monitored in order to determine the antibiotic degradation behavior within the column and monitor microbial utilization of the carbon source. Three column-length profiles have been performed since column initiation. A representative profile is shown in Figure 2. All obtained profiles exhibited similar acetate and tetracycline behaviors within the columns.

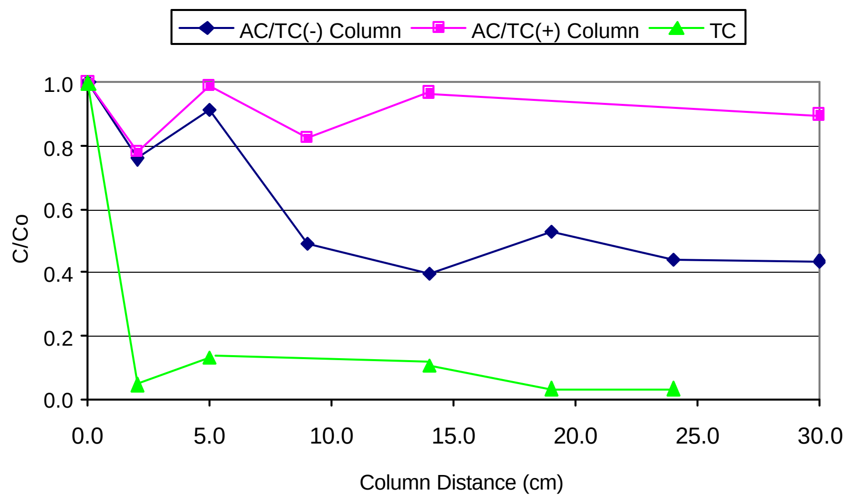


Figure 2: Tetracycline and acetate concentration profiles (12/2002)

The degradation of tetracycline appears to take place within the first 2.5 cm of the column and presumably occurred upon initial contact with the column soil (no sampling port was available at this location). Batch studies conducted to understand the behavior of tetracycline when in contact with soil are described later in the report. Trace amounts of the antibiotic were present throughout the length of the column and at the outlet sampling port.

Assuming that acetate consumption is indicative of microbial activity, acetate profiles for the two columns suggest decreased microbial concentrations within the TC-enriched column. The presence of approximately 80-90% of the original acetate added to the TC-enriched column (Fig.2) suggests a very low level of acetate consumption by the microorganisms present in the column, which, in turn, can be assumed as an overall lower number of microbes than in the non-enriched column, where the acetate concentrations appear to be oscillate between 40-60% of the feed concentration. The removal of acetate in the non-enriched column may also be assumed as a process limited by the dissolved oxygen (DO) concentration.

MPN microbial counts corroborated the acetate profile data with a lower total heterotrophic population in the TC-enriched column. The antibiotic-resistant populations in both columns were also significantly smaller than the respective total heterotrophic populations, but there seemed to be no statistical difference between the resistant counts of the two columns (Figure 3). However, the percentage of resistant microorganisms increased in the TC-enriched column from approximately 4% to 35%.

Again, this was apparently due to the decrease of the total heterotrophic population in the enriched column, rather than to the development of antibiotic resistance in the indigenous strains.

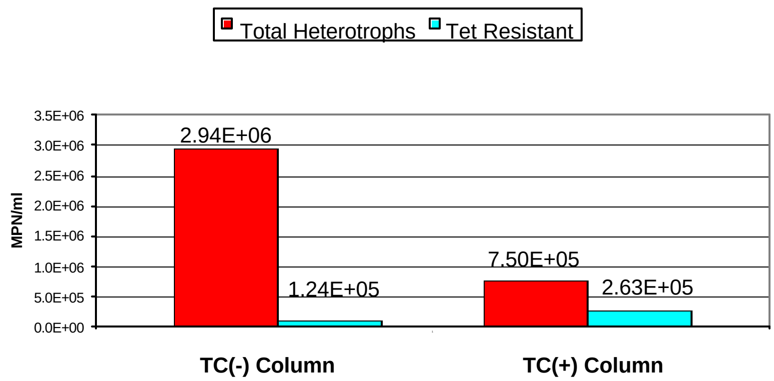


Figure 3: MPN microbial enumeration

Tetracycline has been reported to be highly unstable in light conditions due to photodegradation. Batch experiments were performed to assess the stability of an aqueous tetracycline solution under light and dark conditions. Two 100-mL solutions were prepared with one beaker completely covered in aluminum foil to simulate dark conditions and with the second beaker exposed to environment light. Surprisingly, no statistical differences were observed for the degradation rates of the two solutions, with approximately 85% of the original tetracycline remaining after 400 hours (Figure 4).

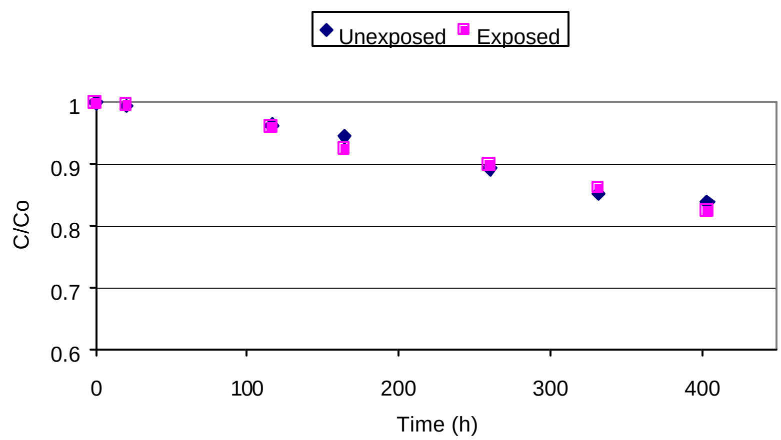


Figure 4: Tetracycline degradation under light (exposed) and dark (unexposed) conditions

To investigate the behavior of tetracycline when in contact with soil environments, batch studies were performed with the same soil source that was used for the column packing. 250-mL amber-glass reaction bottles were filled with 100-mL of distilled water and 10 grams of soil. The solution was mixed thoroughly by vigorous shaking and the initial pH was measured (pH ≈ 7). A 100 mg/L tetracycline solution was prepared, and the pH was also measured (pH ≈ 4). Fifty mL of the TC solution were added to the soil mixture and shaken immediately. The pH and the tetracycline concentrations of the resulting solution were measured within 1 minute of the TC addition. Approximately 96% of the initial antibiotic was removed upon contact with the soil, which concurs with the tetracycline degradation behavior observed in the tet-enriched column. Along with the disappearance of the antibiotic, a rise in pH of the solution is observed, suggesting some form of *alkaline hydrolysis* as the mechanism of tetracycline degradation (Figure 5).

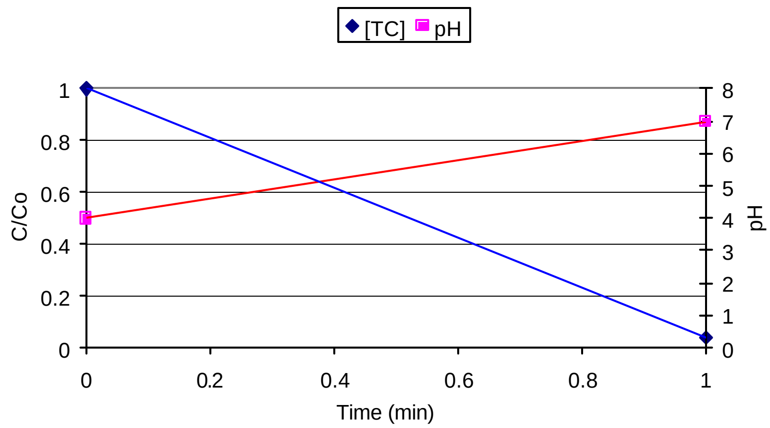


Figure 5: Batch degradation of TC and pH behavior

Microbial strains (courtesy of Dr. Svetlana Kocherginskaya, UIUC) carrying the tet-determinants of interest were grown and the plasmids containing these determinants were extracted. PCR amplification of the template DNA yielded products approximately 160-220 bp., which is in agreement with previously published results (Aminov et al., 2001). The positive PCR controls are shown in Figure 6 with standard DNA ladder samples flanking the amplified products. The remaining determinants (TetB(P), TetW, TetT) have been successfully amplified but are not shown.

Isolation, DNA extraction, and PCR amplification steps were followed with the TC-resistant strains harvested from the TC-enriched column, but, to date, no RPP-tet-determinant has been detected in any of these strains. Since the presence of extracted DNA was confirmed by gel electrophoresis, it may be assumed that the isolated TC-resistant strains do not carry the determinants we had targeted. We are currently screening for the genes coding the Ribosomal Protection Protein (RPP), which represent a portion of the total TC-resistance determinants. Genes responsible for the efflux pump mechanism for antibiotic excretion present another important resistance mechanism available to the microorganisms. Thus, it is very likely that the isolated TC-resistant strains contained one of the efflux pump genes, explaining their ability to grow on antibiotic medium without carrying the RPP determinants we had targeted.

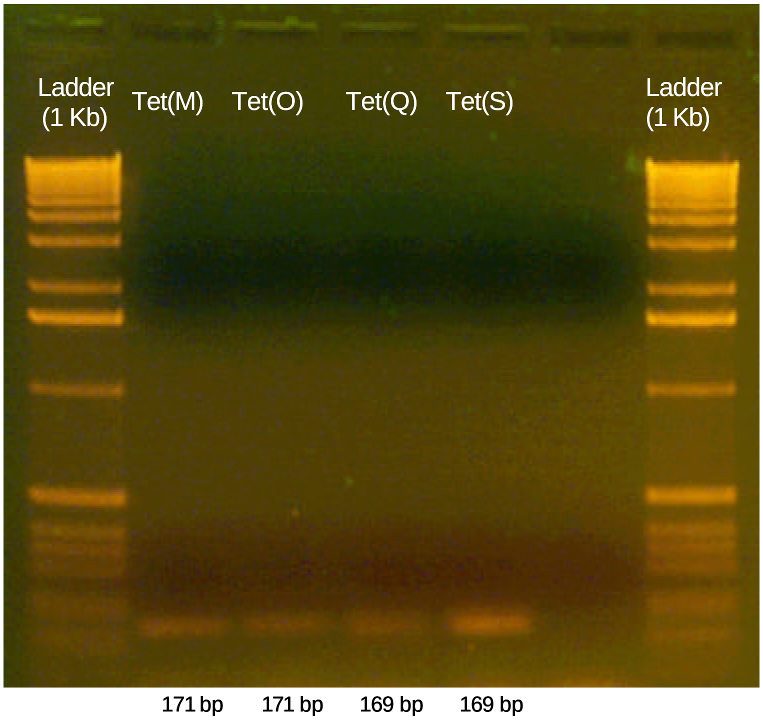


Figure 6: Tet-resistant strains, positive controls
(amplicon sizes 169-171 bp.)

Future Research Directions

Currently, antibiotic resistance could be considered to be an environmental pollution problem with gene vectors as the target contaminants. However, little is known about the fate and transfer of such gene vectors when released to the environment during direct runoff, groundwater infiltration from lagoons, or manure spreading activities (Figure 7). Critical knowledge gaps include the rate and extent of gene propagation (including bacterial migration and inter-specific gene transfer from enteric to soil bacteria) and the effect of environmental factors and sustained antibiotic exposure on the persistence of antibiotic resistance.

Accordingly, one possibility for future research would be the expansion of tet-resistance determinants targeted. This would include the current screening for the RPP genes as well as the efflux pump determinants (TetA-TetE, TetG, TetH, TetK, TetL, TetA(P), OtrB). Upon successful genetic detection and analysis of the tet-resistant strains, the spatial distribution of the resistance genes within the flow-through columns will be addressed in detail. This approach will not only provide a more complete idea of the type of resistance genes present in the columns, but it will also facilitate the mathematical modeling of the dynamics of propagation of these genes, which is an objective of this research project. Determining the fate, transport, transfer, and decay kinetics of gene vectors will thus help formulate more accurate mathematical models to support regulatory and management decisions (e.g., to set total maximum daily loads [TMDLs]).

The effect of antibiotic dosing will also be addressed in the near future. The on/off impact of antibiotic application will be investigated in order to elucidate the effect of such administration on the microbial communities, first, by means of microbial enumeration, and, second, by genetic analysis. The shifts in tet-resistant population will be monitored during and after antibiotic application with a predicted decrease in the amounts of resistant microbes after the withdrawal of the antibiotic from the column feed solutions. Upon this removal, the time necessary for the community to return to the "original" (pre-antibiotic) state will be monitored in order to ascertain the effect of antibiotic dosing as experienced in animal agricultural applications (also periodic).

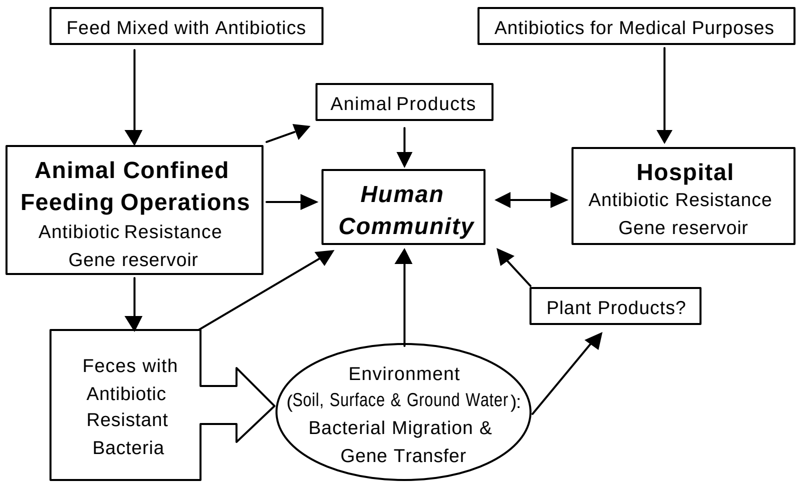


Figure 7. Potential Exposure Pathways for Antibiotic Resistance Genes

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Relationship of Nitroso Compound Formation Potential to Drinking Source Water Quality and Organic Nitrogen Precursor Source Characteristics

Basic Information

Title:	Relationship of Nitroso Compound Formation Potential to Drinking Source Water Quality and Organic Nitrogen Precursor Source Characteristics
Project Number:	2002IA16G
Start Date:	9/1/2002
End Date:	8/31/2004
Funding Source:	104G
Congressional District:	first
Research Category:	None
Focus Category:	Water Use, Toxic Substances, Agriculture
Descriptors:	None
Principal Investigators:	Richard Louis Valentine

Publication

13. Title: Relationship of Nitroso Compound Formation Potential (NCFP) to Drinking Source Water Quality and Organic Nitrogen Precursor Source Characteristics

14. Statement of Critical Regional or State Water Problem:

Recent research indicates that certain disinfection practices may result in the formation of significant amounts of N-nitrosodimethylamine (NDMA), and quite likely other nitroso compounds in drinking water. These compounds are believed formed when chlorine is added to water containing ammonia, and certain organic nitrogen compounds ("precursors"). Measurements in several drinking water distribution systems suggest that unprotected sources receiving point and non-point waste discharges are particularly susceptible to their formation, especially when chloramination is practiced.

The formation of NDMA and possibly other nitroso compounds in drinking water is an emerging concern because they are generally carcinogenic, mutagenic, and teratogenic (O'Neill et al., 1984). For example, the nitrosamine, N-nitrosodimethylamine, NDMA ($(CH_3)_2NNO$) is a particularly potent carcinogen. Risk assessments from California's Office of Environmental Health Hazard Assessment (OEHHA) and US EPA identify lifetime de minimis (i.e., 10^{-6}) risk levels of cancer from NDMA exposures as 0.002 ppb (2 ng/L) and 0.0007 ppb, respectively. In February of 2002 the California Department of Health Services established an interim action level of 0.01 ppb (10 ng/L) in drinking water.

Many drinking water sources in the Midwest and other parts of the country are unprotected receiving point and non-point waste discharges. Municipal and industrial waste discharges, and those associated with agricultural practices, are potentially important sources of the organic nitrogen precursors required for the formation of nitroso compounds. These waters are correspondingly expected to be susceptible to nitroso compound formation from chlorination and especially chloramination. This may limit the use of some water sources for drinking water or restrict treatment options that otherwise have desirable characteristics. Initial observations indicate that some consumers are being exposed to undesirable levels of NDMA. Organic nitrogen is therefore not a simple benign pollutant typically associated with nutrients as generally thought..

A need exists for an improved understanding of the nature and extent of this potential problem. Work is especially needed that relates nitroso compound formation potential to source water quality and origin of organic nitrogen precursors, watershed uses, and to biogeochemical processes that could influence the quantity and types of nitroso compounds potentially produced.

15. Statement of Results or Benefits:

This project will obtain information on 1) quantities and selected types of nitroso compounds that could be formed in drinking source waters, 2) the relationship of their formation potential to source water quality and organic nitrogen precursor source characteristics, and 3) the influence of several biogeochemical processes that may influence their formation.

The anticipated benefits from this study are several fold. First, the results of the proposed study will provide a link between ambient water quality and its use for public supplies. For example, source water quality my limit the suitability of some commonly used disinfection practices such as chloramination. This is particularly important given that chloramination is being widely adopted as the preferred method of disinfection in distribution systems of utilities that cannot meet newly established EPA rules on the formation of halogenated DBPs when free chlorination is practiced. Information gained in this study will also be important in making decisions about the need for comprehensive occurrence and risk assessments, and if warranted, the need to minimize the occurrence.

Identifying specific factors making waters susceptible to nitroso compound formation will aid in assessing the potential extent of the problem and point to mitigation strategies. For example, these might involve organic nitrogen control either through treatment modifications or source control, careful selection of drinking source waters and well locations, and modification of waste and agricultural waste management practices. Clearly, consideration of the potential for nitroso compound formation should be one aspect of any discussion relating drinking water quality to point and non-point pollution, including that from to agriculture and concentrated animal feeding operations.

16. Nature, Scope, and Objectives

Nature and Scope. Nitroso compounds are a class that includes numerous carcinogens, mutagens, and tetraogens. Approximately 300 of these compounds have been tested, and 90% of them have been found to be carcinogenic in a wide variety of experimental animals (Magee, 1982; O'Neill, 1984). Until recently it was believed that the occurrence of nitroso compounds in drinking water and wastewater was due to contamination of the source water. Recent laboratory and field studies, however, show that N-nitrosodimethylamine (NDMA), a particularly potent carcinogen, can be produced as a consequence of drinking water and wastewater disinfection (California DHS, 2002; Najim and Trussell, 2001; Choi and Valentine, 2002; Mitch and Sedlak, 2002). Specifically NDMA is produced by reactions of chlorine in water containing both ammonia, and certain organic nitrogen compounds. It is therefore a newly recognized "disinfectant by-product" (DPB). Formation of other types of nitroso compounds by this mechanism is suspected because of structural and reactivity similarities. Furthermore the occurrence potential appears significant in particular, in unprotected drinking source waters receiving a variety of waste discharges. These are likely the source abundant organic nitrogen precursors and ammonia.

It is hypothesized that water supplies receiving municipal and industrial waste discharges and in particular, agriculture related wastes are particularly susceptible to nitroso compound formation particularly if chloramination disinfection is practiced. It is also proposed that NDMA be but one representative of a new class of disinfectant by-products, the nitroso compounds, many of which are of a health, concern. The quantities and types in drinking source water will depend then on the nature of the inputs of organic nitrogen (both quantities and origin) as well as to processes that can affect the specific types and concentrations of precursor compounds.

There will likely be a genesis of nitroso compound formation potential dependent on such factors as biodegradation and photolysis. Biological processes in particular will be important as it is well established that soluble microbial products include significant amounts of organic nitrogen which differs in characteristics from material consumed for growth and energy requirements (Parkin and McCarty, 1981). These products include many substances that could act as nitroso compound precursors. Alternatively, biotransformation may reduce the NCFP if the characteristic precursors are highly degradable. Passage of the water through an aquifer will also influence nitroso compound formation potential through biological and possibly geochemical processes. Many organic nitrogen compounds also exhibit high light absorption characteristics and hence high potential for direct photolysis and transformation to other compounds.

Objectives. Based upon the ascertained research needs, the following specific objectives of this research study have been formulated with respect to the relationship of source water quality and nitroso compound formation potential (NCFP) as a newly recognized disinfectant by-product:

1. Characterize the nature of the NCFP (types and quantities), in a variety of "susceptible" surface and groundwater drinking source waters, and examine the relationship of NCFP to source water quality and land usage.
2. Characterize the NCFP of a variety of organic nitrogen sources (municipal effluents, CAFO lagoons etc).
3. Conduct microcosm studies to evaluate the influence of natural transformation processes (photolysis, biological transformation) on the NCFP.

Time-line. This study will be conducted over a period of 2 years starting September 1, 2002 (Table 1). We will initially focus on developing analytical methods for several different nitrosamine compounds and their precursors during the first 4-5 months. Once we have established analytical methods and reliable method detection limits, we will be able to the measure nitroso compound formation potential in various water samples as discussed in the next section. Source water sampling will be coordinated with ongoing USGS activity. We anticipate collecting these samples and measuring NCFP immediately upon their receipt on a monthly basis over about a 12-16 month period. Studies involving sources of organic nitrogen precursors will not depend on the USGS scheduling. It is anticipated that this work, along with microcosm studies can be conducted during the second year in parallel with the source water studies (months 12-21). The last three months will be used to focus on data interpretation, and thesis and journal paper preparation.

Table 1. Time-line of Project Tasks

	2002				2003												2004							
Task Description	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A
Project start-up/MS purchase	-	-																						
Initial methods development																								
NDMA			-	-																				
Nitroso alkylamines-others			-	-	-																			
Organic nitrogen precursors						-	-	-																
Source water NCFP determinations					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Organic nitrogen precursor/waste NCFP													-	-	-	-	-	-	-	-	-			
Microcosm studies																								
Aging/biotransformation													-	-	-	-	-	-	-	-	-			
Photolysis															-	-	-	-						
Thesis, paper preparation																					-	-	-	-

17. Methods, Procedures, and Facilities:

NCFP determination. Three standardized tests will be used measure NCFP. One will measure the formation potential from reaction of preformed monochloramine (NCFP-M), the second will measure the formation potential from free chlorine (NCFP-FC), and the last will measure the formation potential from reaction of nitrite (NCFP-N). It is anticipated that the formation potential from reaction of monochloramine will yield the most nitroso compounds. The NCFP-FC test will be conducted since ambient levels of ammonia can be quite large in some samples leading to formation of monochloramine. The NCFP-N test will be conducted to measure direct nitrosation. It is anticipated that this will yield comparatively small amounts of nitroso compounds. However, several organic nitrogen precursors, such as Thiram (Graham et al, 1995), are known to react rapidly with nitrite, and therefore reaction of nitrite should be evaluated. In general the importance of nitrite would also be minimal, as most drinking sources do not contain very much nitrite although partial nitrification in some well water, and in distribution systems sometimes is observed.

All samples will be adjusted to pH 8.3 by using acid or base addition. In the NCFP-M test, preformed monochloramine will be prepared at an ammonia to chlorine molar ratio of 2:1. An aliquot will be added to the stirred samples at a concentration of 4 mg/L as chlorine (0.05 mM). In the NCFP-FC test, hypochlorous acid (free chlorine) will be added at 4 mg/L as chlorine, and in the NCFP-N test, sodium nitrite will be added at 2 mg-N/L. Solutions will be incubated in the dark at 20 ^{o}C for 24 hours and 72 hours then nitroso compounds will be quantified. Relatively concentrated wastes (precursor sources) may have to be diluted with purified water before measurements can be made. Initial concentrations of nitroso compounds will be measured in the source waters as part of the control to account for background levels (Nikaido et al. 1977).

NDMA will be measured in all samples. In addition, a select number of other nitroso compounds will be determined. Table 2 is a list of candidate nitroso compounds and their organic nitrogen precursors that will be considered. They all are of health concern and are likely to be of environmental importance since the precursors are relatively common constituents of wastewater and river/lake water (Sachter et al, 1997; Nikaido et al, 1977). Studies will initially focus on the formation of several alkyl nitrosoamines because it is believed that they will react via the proposed pathway, and because we do not anticipate any analytical difficulty due to their similarity with NDMA. We will consider adding additional compounds when we determine our analytical capabilities with regards to them.

Table 2. List of candidate nitroso compounds and their organic nitrogen precursors

Nitroso compound	Organic Nitrogen Precursor
N-Nitrosodimethylamine	Dimethylamine
N-Nitrosodiethylamine	Diethylamine
N-Nitrosoethylmethylamine	Ethylmethylamine
N-Nitrosodi-n-butylamine	Di-n-butylamine
N-Nitrosodi-n-propylamine	Di-n-propylamine
N-Nitrosodibutlylamine	Dibutylamine
N-Nitrosodiphenylamine	Diphenylamine
N-Nitrosopiperidine	Piperidine
N-Nitrosomorpholine	Morpholine
N-Nitrosopiperazine	Piperazine
N-Nitrosopyrrolidine	Pyrrolidine
N-Nitroso-N-ethylurea	Ethylurea
N-Nitroso-N-methylurea	Methylurea

Source water studies. A variety of ongoing USGS studies will allow them to provide samples for determination of the nitroso compound formation potential as well as information on water quality (nitrogen, phosphorus, dissolved organic carbon, suspended solids) and land usage. In particular, the USGS has generated a large amount of information to be used in this study as a guide in selection of surface sampling sites and for the interpretation of results. A recent report by Becher et al (2001) summarizes surface water quality from 1996 to 1998 at 11 intensively monitored locations in four watersheds. In addition, it provides information on land usage. Differences in water quality are attributed largely to agricultural practices which it is hypothesized, will be reflected in differences in nitroso compound formation potential.

Surface water continues to be assessed by monthly collection of samples from small streams such as the Wapsipinicon River near Tripoli, to large rivers such as the Mississippi River at Clinton, IA. Although most land is used for row-crop agriculture in Iowa, the effect of concentrated animal facilities (CAFO's) on stream quality is being investigated by the USGS by sampling the South Fork of the Iowa River. Point source inputs to streams are being evaluated by collection of samples in and near municipal sewage outfalls to evaluate the occurrence of organic waste-water contaminants. We anticipate obtaining samples at approximately 11 surface sampling locations on a monthly basis.

A special activity will involve measuring the NCFP along the course of a river. The Iowa River will probably be used for this with sampling at approximately 10-15 locations, including the influent and effluent to the Coralville reservoir. This will result in information on the impact of a large reservoir, and multiple inputs of point and non-point wastes, as well as on the fate of organic nitrogen compounds and NCFP.

As part of ongoing USGS projects, samples are collected from municipal wells to assess the ambient quality of water for public supply. Municipal wells are completed in variety of different aquifers that range from shallow vulnerable alluvial to deep protected bedrock aquifers. Water-quality of shallow alluvial aquifers will be evaluated by collection of samples from monitoring wells at approximately 10 locations over a 12 month period. Samples will be simultaneously obtained from nearby surface source waters for comparison.

Samples will also be obtained at two municipal utilities practicing chloramination to compare the NCFP test results with what is actually being produced in their distribution systems after treatment. One site will be Cedar Rapids, which obtains water from shallow alluvial wells on the Cedar River, and where measurements in the distribution show significant formation of NDMA. Another will utilize surface water from the Mississippi River such as Burlington, Iowa.

Precursor sources. Samples will be obtained from several municipal wastewater treatment facilities practicing different treatment methods. These will include conventional activated sludge, trickling filter, lagoons, and extended aeration activated sludge. Samples will also be obtained samples from several lagoons used to treat animal wastes at CAFO sites (with the assistance of Dr. Melvin Stewart at Iowa State University). The idea is to directly determine the contribution of these materials to the NCFP and to provide material for microcosm studies. Ideally these will come from locations potentially impacting source waters used in this study.

Microcosm studies. Microcosm studies will examine nitroso compound formation potential genesis as a function of water age in several (6-10) source water samples and river water diluted wastes. Each microcosm will be a four-liter sample, stored in the dark at 20 ^{o}C. We will age these one week to two months. It is hypothesized that biological activity will influence the extent and distribution of nitroso compound formation. Aging may favor the ultimate formation of NDMA while more complicated nitroso compounds may predominate in less aged water. Conversely, biodegradation of precursors compound may cause a reduction in formation potential. Some samples will also exposed to simulated sunlight using a Suntest solar simulator to examine the influence of sun on NCFP. Only a limited number of studies will be conducted with source water due to equipment limitations.

Facilities and analytical systems. The USGS will provide extensive information on water quality (organic nitrogen, TOC, inorganic nitrogen) of the samples provided to Iowa. This information is obtained as part of their ongoing studies using their own facilities and standard protocols. Additional analytical work will be conducted in the Environmental Engineering and Science Laboratories (EESL) at the University of Iowa. Most of the functions of the EESL take place in the facility (4800 ft^2, opened Summer 2001) located in the Seamans Center for the Engineering Arts and Sciences. Some projects are supported at the division of the EESL located at the University Water Treatment Plant - a 2,400 ft^2 facility designed for both research needs and teaching purposes. The laboratory is fully equipped with a wide selection of modern analytical equipment. It is managed by a full-time laboratory director (Dr. Craig Just) who is responsibile for student training, equipment maintenance, methods development, and most importantly, laboratory QA/QC.

It is anticipated that the general methodology developed for the analysis of NDMA at the University of Iowa will be extended for the analysis of a variety of specific nitroso compounds (Taguchi et al., 1994). In this approach the nitroso compound will be determined by an isotope dilution gas chromatography/mass spectrometer (GC/MS) method. Prior to extraction, all 1 L samples are dosed with the deuterated nitroso compound as an internal standard if available. Otherwise deuterated NDMA will be used as an internal standard. Isotopically labeled nitroso compounds in methanol other than NDMA, seem unfortunately, to be available only for a few precursor candidates. To the 1 L sample is added 200 mg of carbonaceous adsorbent (Ambersorb 572, Aldrich) and then the sample is shaken for 1 hour at 200 rpm. The Ambersorb beads are vacuum filtered onto a glass fiber filter, and dried in air for 30 minutes. Beads are transferred to a 2-mL amber vial where beads are soaked with 0.5 mL of methylene chloride for 20 minutes before analysis. A 95 μL aliquot of methylene chloride extract is injected into GC/MS (Varian) equipped with Large Volume Injector (Optic2). The nitroso compound will be quantified based on the mass detection of the characteristic molecular ion. The MDL at the 99% confidence level for NDMA is expected to be approximately 2.0 ng/L.

Specific organic nitrogen precursors will be determined by GC-MS methodology (Sachter et al, 1997) or by HPLC. Total organic nitrogen will be measured using the Kjeldahl method (APHA, 1993). Nitrate and nitrite will be determined by ion chromatography using a Dionex IC, and total organic carbon by Shimadzu TOC 4000 analyzer (APHA, 1993).

18. Related Research:

Environmentally oriented NDMA occurrence and formation studies have been generally empirical in nature and have focussed primarily on determining if, not how, nitrosamines may be formed in water. All have assumed that nitrite is a required reactant. Lab based studies usually involve its addition along with an appropriate organic nitrogen containing precursor. This is based upon the widely held belief that the primary formation mechanism involves the classical acid catalyzed nitrosation. This reaction is however, also biologically catalyzed and likely will account for any background levels of nitroso compounds in the untreated waters (Ayanaba and Alexander, 1974; Nikaido et al, 1977).

The recent concern about the occurrence of NDMA originated when it was found in highly purified wastewaters intended for recycle and reuse. It was found at alarming levels, sometimes exceeding 1000 ng/L. Its formation as a consequence of some treatment step was suspected because it was absent in the plant effluent. Subsequent observations found it in some treated drinking waters while absent in the influent streams (California Department of Health Services, 2002). Based upon suspected linkage to disinfection practices, Najim and Trussell (2001) showed formation of over 16 ng/L in one chloraminated drinking water and approximately 400 ng/L in a filtered and chlorinated tertiary waste water. They hypothesized that NDMA formation was somehow related to the common practice of chlorination or chloramination (formation of chloramines by reaction of chlorine and ammonia).

Recently Choi and Valentine (2002) and Mitch and Sedlak (2002) independently proposed a novel mechanism to describe NDMA formation in chlorinated and chloraminated water containing DMA as a model precursor. Choi and Valentine (2002) also developed a kinetic reaction model (Figure1) of use in making predictions about NDMA formation. The proposed mechanism is based largely on studies of hydrazine formation (a rocket fuel) and its oxidation (Lunn et al, 1991; Castegnaro et al, 1986; Cahn and Powell, 1954). The key reactions include the formation of monochloramine from the initially added HOCl (Reaction 1), the reaction of chlorine with DMA to form dimethylchloramine, DMCA (Reaction 2), and the slow transfer of active chlorine from monochloramine to DMA to form more DMCA (Reaction 3). Formation of NDMA is initiated by the formation of 1,1-dimethylhydrazine (UDMH) intermediate from the reaction of DMA with monochloramine (Reaction 4), followed by the oxidation of UDMH by monochloramine to NDMA (Reaction 5). Figures 2 and 3 show a close correspondence between measured NDMA concentrations and that predicted by the comprehensive model in chlorinated and chloraminated water containing DMA.

It should be noted that addition of nitrite to DMA containing solutions yielded very little NDMA. This shows as expected, that classical nitrosation is not likely an important formation mechanism involving simple organic amines at drinking water conditions unless some unrecognized catalytic process is occurring. Fast reactions of nitrite with some "unusual" precursors such as Thiram (Graham et al., 1995), a pesticide cannot be ruled out however. The general importance of this possibility needs to be evaluated in control studies along with formation by the direct chlorine reaction route.

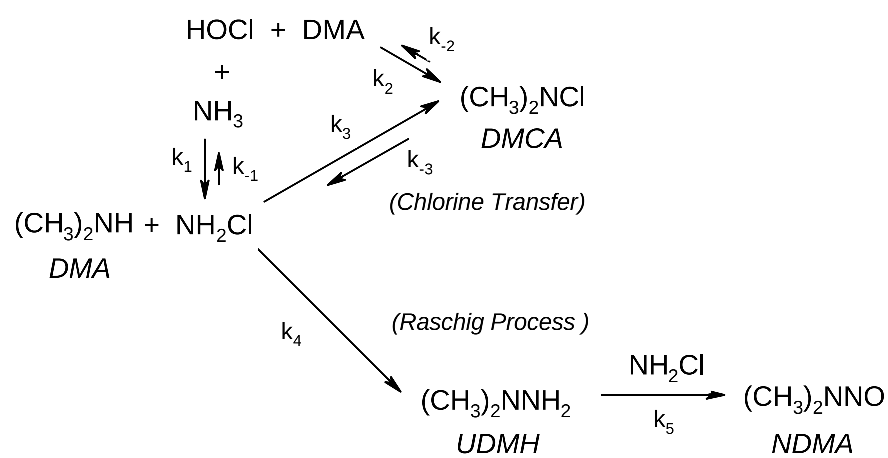

Figure 1. Mechanism of NDMA formation in chlorinated waters containing DMA and ammonia.

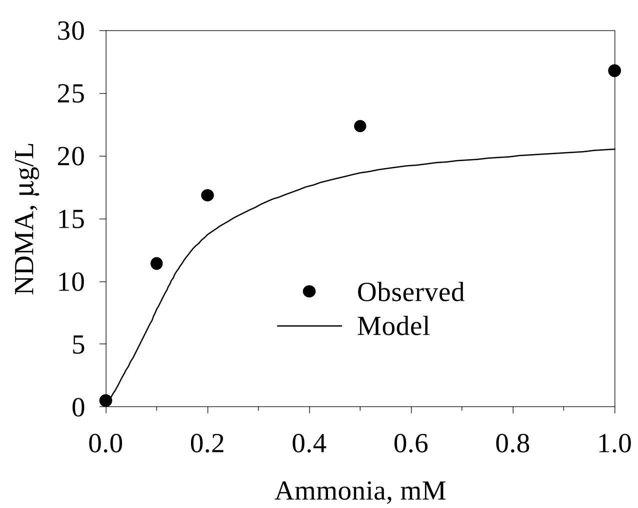

Figure 2. NDMA formation after 24 hours contact. Addition of 0.1 mM HOCl to a mixture of 0.1 mM DMA and ammonia. pH 7.0 ± 0.1; 4 mM bicarbonate buffer. Ionic strength was adjusted to 0.05 M with NaCl. Temperature 25^{o}C.

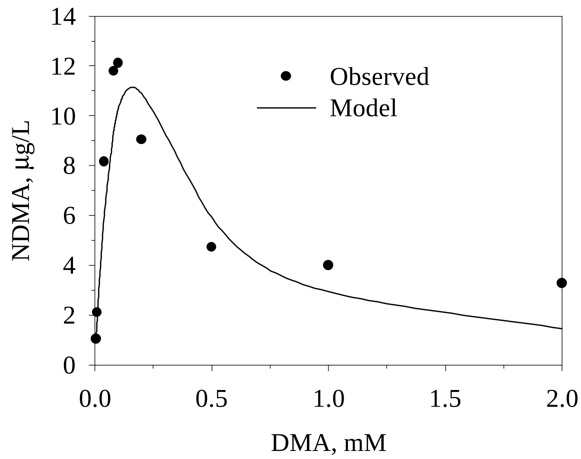

Figure 3. NDMA formation after 24 hours as a function of DMA concentration. Preformed monochloramine concentration was fixed at 0.1 mM. The pH was adjusted to 7.0 ± 0.1 using 1 mM bicarbonate buffer. Temperature 25 ^{o}C.

The rationale for the applicability of this mechanism to the formation of some other types of nitroso compounds is based upon precursor structural similarities (Rowe and Audreith, 1956). For example diethylamine and dibutylamine should yield formation of N-nitrosodiethylamine and N-nitrosodimethylamine by the same mechanism. This however, has not been demonstrated either in the field or in the lab. In addition, no studies have been conducted examining nitroso compound formation by this mechanism in waters thought susceptible as described in this proposal. Studies on the genesis of NCFP and the influence of natural attenuation processes are a logical extension of recent work.

The PI is currently participating in an American Water Works Association Research Foundation (AWWARF) sponsored project surveying NDMA occurrence in treated drinking water in the US and Canada. An important finding is that the highest NDMA formation appears to occur in chloraminated waters obtained from unprotected and "susceptible" sources (unpublished data). For example, concentrations increased from <1 ng/L in the raw water to 6 ng/L in the effluent, and then to approximately 20 ng/L in the distribution system of the City of Cedar Rapids, Iowa. The source is a shallow alluvial well on the Cedar River.

The proposed project will build upon this work and studies recently supported by an ISWRRI seed grant that ends in May 2001. The proposed study does not duplicate any work in these other projects, which were instrumental in developing the hypotheses in this proposal.

19. Training Potential:

This project will support two doctoral graduate students or one doctoral and one master's student at the 1/2 time level for two years. These students will work both with Professor Valentine as well as help in sampling under the direction of Stephen J. Kalkhoff (see section 20). In addition, one undergraduate student from our undergraduate environmental engineering program or from chemistry will be hired for each of the two summers. We anticipate having work-study funds or funds from the Research Experience for Undergraduates-REU program to augment this.

20. Statement of Government Involvement:

The PI will work very closely with Stephen J. Kalkhoff, Chief, Eastern Iowa Basins, National Water-Quality Assessment Program (NAWQA) study unit. He will participate in planning, sampling, and in collaboration on resulting papers. Working together with the two students, he will facilitate the integration of this project with several ongoing USGS studies.

First, the U.S. Geological Survey will provide historical data, ongoing water-quality monitoring data, and ancillary information as part of the collaborative efforts in this project. Data collected as part of the Eastern Iowa Basins (EIWA) study unit of the National Water-Quality Assessment Program (NAWQA) and other historical data indicate that organic compounds including nitrogen containing compounds vary seasonally. Dissolved organic nitrogen concentrations generally are greatest during snowmelt and rain events in early spring when runoff from the land surface is occurring. As intuitively expected, greater concentrations of organic compounds occur in streams draining watersheds with large numbers of concentrated animal feeding facilities. Because of the NAWQA's program needs to relate water quality to natural and human factors that effect it, the U.S. Geological Survey has also compiled a variety of ancillary data, land use, hydrology, etc for the sampling sites that would be available to help understand the nitroso formation potential of water resources in Iowa. This information will provide guidance in the selection of source water sampling locations and times.

The U.S. Geological Survey District is also involved with several projects to investigate water-quality conditions in Iowa and is collecting data from a variety of environments for which a determination of nitroso compound formation potential and precursors would be value. For example, samples are collected from municipal wells to assess the ambient quality of water for public supply, surface water is assessed by monthly sampling of several streams and rivers, and point source inputs to streams are being evaluated by collection of samples in and near municipal sewage outfalls to evaluate the occurrence of organic waste-water contaminants. This extensive sampling effort will allow the U.S. Geological survey to both provide analytical data on organic nitrogen concentrations and provide samples for determination of the nitroso compound formation potential.

21. Information Transfer Plan

The primary form of information transfer will be through peer-reviewed publications in nationally recognized journals, and presentations at national and regional meetings of several organizations (e.g. AWWA, WEF). Subject matter would be relationship of water quality to nitroso formation potential, sources of nitroso compounds precursors, influence of aquifer passage on the NCFP, correlation of land use practices with NCFP, and the NCFP of various wastes.

The intended audience will be 1) water treatment and supply professionals, 2) state and federal government agencies involved with water quality (e.g. Iowa DNR, EPA, USGS), and 3) members of the agricultural community who must deal with ag waste issues. Dr. Melvin Stewart, Department of Agriculture and Biosystems at Iowa State University has agreed to facilitate potential presentations sponsored by our extension service.

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An Integrated Immunological-GIS Approach for Bio-monitoring of Ecological Impacts of Swine Manure Pollutants in Streams

Basic Information

Title:	An Integrated Immunological-GIS Approach for Bio-monitoring of Ecological Impacts of Swine Manure Pollutants in Streams
Project Number:	2002IA25G
Start Date:	9/15/2002
End Date:	9/15/2005
Funding Source:	104G
Congressional District:	Iowa’s 3rd district
Research Category:	None
Focus Category:	Non Point Pollution, Water Quality, Methods
Descriptors:	None
Principal Investigators:	James A. Roth, Dusan Palic, Bruce Willard Menzel, Clay Lynn Pierce

Publication

1. Title

An Integrated Immunological-GIS Approach for Bio-monitoring of Ecological Impacts of Swine Manure Pollutants in Streams

2. Statement of critical regional or State water problem

Thirty years after enactment of the Clean Water Act, 40% of our nation's rivers, lakes, and coastal waters are still considered unfit for fishing, swimming, drinking or aquatic life. The U.S. EPA identified agricultural operations as the primary cause of non-point source pollution in the nation's impaired rivers and lakes. At least 10 % of the nation's impaired river miles are affected by pollution from livestock operations (3.). In portions of the Midwest, confinement livestock operations are a particular problem in this regard. Spills and dumping of fecal material occurred over one thousand times at feedlots in the ten Midwest U.S. states between 1995 and 1998. Over the past two decades, swine production in the U.S. has increasingly shifted to a large-scale, corporate model. Today, two percent of the hog operations in the U.S. produce over 46% of the total hog population (26.). In Iowa, the largest swine production state, about 21 million tons of manure are produced annually, chiefly at large-scale confinement operations (pers. comm., Dr. Jeffery Lorimor, ISU Department of Agricultural and Biosystems Engineering). Commonly, the manure from such operations is held in earthen lagoons for anaerobic decomposition prior to application as a fertilizer to crop fields. As older-style lagoons age, their failure and leakage is a growing problem. Improper application of the fertilizer and its runoff from cropland also result in manure delivery to local waters. Because of its volume, composition, and handling methods, therefore, swine fecal contamination is a serious threat to environmental quality in regional waterways and especially in Iowa (1.). Local citizens are becoming increasingly intolerant of the environmental cost of confinement livestock production (36.)

Cases of massive deaths of aquatic organisms, often referred to as fish kills, are an extreme manifestation of the ecological impact of fecal contamination. Typically, they result from high concentrations of toxic ammonia contained in the manure or from depletion of dissolved oxygen in the water caused by decomposition of the pollutant. From 1995 to 1998, over 13 million fish were killed in more than two hundred documented manure spills in the Midwest (35.). In the past 20 years, fecal pollution was the single most important cause of 495 documented fish kills in Iowa, accounting for over one-quarter of the diagnosed cases, and hog manure was the primary pollutant (2.). Although cases of acute toxicity of manure pollution capture the headlines, there are other impacts that may be of equal, or perhaps greater, long-term ecological importance. For example, fecal pollutants contain nutrients, especially nitrogen and phosphorus compounds, microorganisms and other materials that upset ecological processes and impair water quality for human uses (13.). Additionally, exposure to sub lethal pollutant concentrations can interfere with normal life processes of wildlife such as feeding, reproduction, defense and disease resistance. This can result in gradual declines and even extirpations of animal populations and communities. Such chronic effects of manure pollution are poorly known, because of the difficulty of measuring them and placing them in ecological context. Moreover, low-level delivery of fecal pollutants can portend larger catastrophic inputs, for example, when a gradually leaking storage lagoon eventually bursts or an erosive, manure-fertilized crop field receives heavy rainfall.

State and federal agencies engaged in reducing non-point source water pollution are interested in obtaining new technologies for identifying, measuring and anticipating pollution occurrence. Clearly, development of tools that could integrate biological and environmental information to produce site-specific predictive models for guiding pollution-prevention management practices is highly desirable. Benefits of such tools and management practices would accrue locally and more broadly throughout interstate drainage basins. The highly publicized Gulf of Mexico hypoxia situation is an example of the geographically widespread impacts of agricultural (and other) pollution in the Mississippi River basin. The proposed research would develop a novel tool that integrates molecular biological and ecological approaches to quantitatively evaluate environmental impacts of swine manure pollutants. Although the technique will be developed with specific reference to Midwestern waters, it will be more broadly applicable, both geographically and with reference to other forms of pollution that engender immune responses in animals. Thus, we believe that the technique has potential to be widely adopted by state and federal environmental management agencies.

3. Statement of results or benefits

The expected results include 1) testing, evaluation and application of techniques that quantitatively measure fish immune response to swine manure exposure, 2) development of predictive models for estimating site-specific fecal pollution potential in Midwestern warmwater stream systems based on local landscape features and farming practices, and 3) a marriage of the two techniques as a new and cost-effective pollution biomonitoring methodology. Several benefits will result from the research. First, the work will extend knowledge in basic immunobiology science. Knowledge of the fish immune system is very limited. The assays that will be tested in the project were developed for homeothermic animals - mammals and birds. Adaptation of their use for fish will contribute to knowledge of fish immune reactions. This will be valuable information, not only in the context of pollution control, but also relative to fish health. Aquaculture, or fish farming, is the fastest growing segment of American agriculture, and fish health-related issues are a major factor inhibiting its continuing development. Second, the refined immunological assay methods will provide a relatively simple and fast tool for detecting ecological impacts of low-level and long-term manure pollution. Third, applications of the technique will permit identification of livestock operations and croplands that potentially pose threats to aquatic ecological integrity from manure seepage and runoff. Fourth, the research will contribute to development of the USGS IRIS and Aquatic Gap projects that are underway at Iowa State University and partially funded by the Iowa Department of Natural Resources (see below).

4. Nature, scope and objectives of the project, including a timeline of activities

Nature: The proposed research reflects the need for integrated, multidisciplinary approaches to deal with complex environmental issues. It will combine physiological laboratory techniques, computer modeling of agricultural landscapes and non-point source pollution pathways, and field-based ecological analyses to create a new and integrated approach for evaluating impacts of livestock fecal contamination on Midwestern streams.

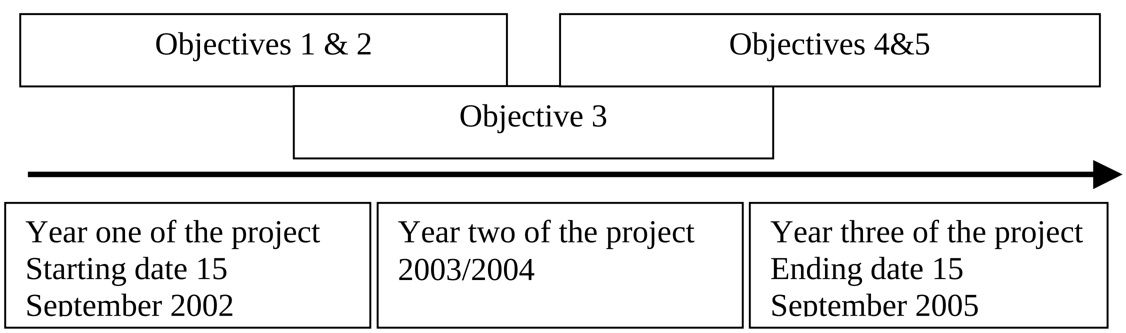
Scope: The research relates to two major priorities of the NIWR National Competitive Grants Program.

A) It will complement work by the USGS related to non-point source pollution, contributing to

development of integrated watershed decision support tools for assessing organics and microorganisms transport and fate, along with their effects on aquatic systems.
B) It promises development of a new water quality sensor technology that will be based on integrated methodologies and will provide results that are readily accessible through the Internet.

<u>Objectives:</u> This research is predicated on the hypothesis that low levels of swine liquid manure slurry and anaerobic lagoon liquid released to open water cause changes in immunological response in fish and increase fish susceptibility to infection.
The initial objectives, therefore, are 1) to evaluate this hypothesis through a series of laboratory immunological assays applied to the test organism, the fathead minnow (*Pimephales promelas*) and 2) to identify one or more assays for use as a bio-monitoring technique to detect ecological impact of manure pollution in nature. A subsequent task involves use of digital environmental databases that are maintained and managed by the USGS BRD Iowa Cooperative Fish and Wildlife Research Unit at Iowa State University. The objective is 3) to characterize a number of Iowa watersheds and stream systems according to their potential susceptibility to hog manure pollution and to use this information to design a water quality and fish sampling regime. Finally, water and fish collected at selected stream sites will be analyzed through a battery of chemical and immunological procedures with the objectives 4) to quantitatively measure ecological impact of manure pollution on the streams, and 5) to evaluate the utility of this approach as a biomonitoring tool for environmental protection agencies.

Suggested timeline:



5. Methods, procedures and facilities
The fathead minnow is a native Iowa species, abundant and ubiquitous in small streams (14.). Thus, it is a good choice as a representative of fish communities exposed to low level concentrations of swine manure pollutants released into Iowa waters. Moreover, it is commonly used as a standard bioassay organism in toxicological analyses, so there is substantial knowledge on its tolerance to a wide array of environmental physical conditions and pollutants (15.). Additionally, colonies can be easily established and maintained in the laboratory. Fathead minnows used for the experiment will be raised in a controlled environment, without previous exposure to swine manure.

The bacteriological composition and characteristics of swine manure slurry and lagoon liquid are generally known, a number of bacterial varieties being present (16.). During manure storage and manipulation, some bacteria die and degrade. Consequently, it is expected that a number of chemical varieties of released bacterial lipopolysaccharides (LPS) are present in both manure forms, reflective of different bacterial sources. Since thorough bacteriological analyses of the manure and water are time consuming and expensive, the LPS component of the bacterial cell wall will be used as a measure of bacterial variety and concentration. Furthermore, since LPS is considered an activator and/or promoter of phagocytic cell activity (17.), the amount of LPS in manure samples can serve as a measure of immunologically active substances present (20). The immune response will be determined by activity of phagocytic cells, through several forms of measurement. Evidence from limited research involving fish suggests that several respiratory burst activity (RBA) assays are useful for determining phagocytic function in fish, and the procedure also seems to hold promise as a bio-indicator for fish health (6.). Additionally, histological examinations of melano–macrophage centers (MMC) in liver, spleen, kidneys, intestines, skin and gills will be used to measure effects of long term exposure to manure (19.).

Geographic Information Systems (GIS) technologies provide a tool to enter, store, manipulate and integrate geo-referenced data on, for example, landscape features, water quality, and aquatic organisms (30.). The project will apply GIS technology and landscape modeling to calculate possible swine pollutant flow path patterns in Iowa watersheds having large hog confinements and in those where liquid manure fertilizer is applied on crop fields. Using this approach, we will estimate temporal and spatial distribution of manure loads and concentrations that reach receiving waters. This will provide the basis for a field sampling regime to determine actual conditions of water quality and fish communities at stream sites selected to represent a range of calculated manure pollutant loadings. Water quality data on fecal coliform bacteria, phosphorus compounds and ammonia, among others, will be evidence concerning actual loading of manure material. Ecological impact of the pollution will be evaluated by the developed immunological assays performed on wild-caught fathead minnows. Statistical comparisons will be made between the calculated and measured evidence for the pollutant to determine the accuracy and reliability of the immunological approach in actual practice. As a further check on the procedure, Index of Biotic Integrity (IBI) values will be calculated based on wild fish community parameters. The IBI is a commonly used bioindicator of stream environmental quality. It serves as a summary measure of biotic community response to pollution and other forms of habitat degradation. It is being used routinely for long-term environmental monitoring programs in Iowa and other Midwestern states. This design, therefore, will allow comparisons between this established coarse-scale environmental indicator and the experimental fine-scale immunological indicator.

The project will involve the following stages and procedures.
Stage 1 – Determine the lethal concentration for 50% of the experimental population of fish exposed to swine liquid manure slurry and swine anaerobic lagoon liquid. This procedure is necessary in order to calculate the range of concentrations that will be applied in the actual experiment, since the experimental fish should not experience any mortality during long-term toxicity tests. For this, different concentrations of the test substances, measured as LPS, will be used in static water aquaria containing 10 to 15 fish each, following standard procedural guidelines of the U.S. EPA OPPTS 885.4200 (23.). To determine LPS concentrations, the

Limulus amoebocyte lysate assay will be used (20.). In brief, five different concentrations of test substance will be used in each of two aquaria, together with a positive (concentration that causes 100% mortality) and negative (no active substance) control. Mortalities will be measured twice a day (morning/evening) and then calculated as LC 50 for 96 hours. Once LC 50 is determined, the range of sub lethal concentrations will be calculated and used in long term toxicity testing.

Stage 2 – Develop and standardize procedures for isolating phagocytic cell populations from fish tissue samples (21.) and use them for the battery of assays: iodination, ingestion and RBA (18.). Iodination assays measure the antimicrobial activity in phagocytic cells based on activity of the myeloperoxidase – hydrogen peroxide – halide ion system. Ingestion assays measure actual phagocytosis using labeled particles submitted to phagocytes. The RBA assays will include three different procedures. The procedure that shows best results with fish cells will be selected for further study: 1) *Superoxide-dismutase (SOD) inhibitable reduction of cytochrome c.* Briefly, upon appropriate stimulation, phagocytes produce superoxide anion that is converted to hydrogen peroxide in the presence of SOD. Peroxide then oxidizes p-hydroxy-phenylacetate (PHPA) in the presence of horseradish peroxidase to a fluorescent product $PHPA_2$ (18.). 2) *Oxidation of 2'7'dichloro-dihydro- fluorescine (H_2DCF).* This is based on a combination of esterase and peroxidase activity towards different reactive oxygen species produced during oxidative burst (17.). 3) *Luminol enhanced chemiluminescence.* In this assay , the phagocytic activity is measured using a set of biochemical reactions, mainly myeloperoxidase activity, again based on superoxide anion release and induced measurable chemiluminiscence (18.) The experimental fish will also undergo histological examination of MMC forming centers by standard histological methods (19.). MMC are aggregates of phagocytic cells in the sites of infection. If the stimulus for their gathering lasts long enough, melanin production by the cells increases in order to prevent accumulation of free radical byproducts of phagocyte activity. These centers are visually identified by their dark melanin-based color and are often used as a non-specific sign of chronic infection (19.).

Stage 3 – Apply assays developed in Stage 2 to fish exposed to different sub lethal concentrations of swine liquid manure slurry and anaerobic lagoon liquid calculated in Stage 1. Exposure of the fish will follow standard U.S. EPA procedures (23.). In short, a range of five different sub lethal concentrations of substance will be used in two aquaria. Each aquarium will have 150 fish at the beginning, and starting after two days of exposure, 5-10 fish will be sacrificed weekly and subjected to examination.

Stage 4 – Submit fish to a challenge test using *Aeromonas hydrophila* as the pathogen and determine relative percent survival (RPS). The challenge test is a standard procedure for evaluating immunomodulation effects of different substances (23., 24.). It consists of two steps. The first is to vaccinate the experimental animals with a commercially available vaccine. The second step is to challenge vaccinated and non-vaccinated groups with the pathogen and then measure RPS. In this study, the challenge test will be performed on groups of fish that were either previously exposed to sub lethal concentrations of manure or not so exposed. All groups will be assayed for phagocyte cell function during the challenge period.

Stage 5 – Statistical evaluation of data using appropriate methodology for finding the differences between groups of test organisms exposed to different conditions and toxicants. Possible approaches include analysis of variance, contingency-table analysis, and two types of multivariate procedures – principal components analysis and logistic regression (25.).

Stage 6 – GIS-mediated flow path analysis of manure delivery to waterways (30.). This stage will rely heavily on cooperation with the USGS Iowa Cooperative Fish and Wildlife Research Unit (ICFWRU) and its Iowa River and Stream Information System. IRIS is a digital database for integrating physical, chemical and biological information into a comprehensive, statewide information system for interior Iowa rivers. It is linked with the Iowa Aquatic Gap program that is also managed by the ICFWRU and funded by USGS BRD. Using information in these databases, a number of Iowa watersheds will be selected that represent a range of likely hog manure influences on local waters. For each watershed, we will determine the drainage-basin morphometry and the manure production or application rate. The 500 m resolution digital elevation model (DEM) will be utilized in several steps involving delineation of the stream network, identification of stream sites for fish and water sample collection, determination of the drainage area at each site, calculation of landscape parameters that characterize this drainage area and estimation of watershed-averaged manure deposition rate. We will use ArcView software for advanced GIS geospatial analysis using raster and vector data supported by ArcGIS desktop extensions for application to the USGS Hydrologic Modeling System, HEC-HMS. In order to use this software we will formulate statistical models capable of representing the spatio-temporal variability in surface waters and build a model for predicting concentrations of manure loads under different hydrologic conditions and for different agricultural chemical application and deposition rates. We expect to utilize the Agricultural Nonpoint Source Pollution Model (AGNPS) (38.) and perhaps the Feedlot Evaluation Model (37.) as the bases for developing the new models.

Stage 7 – Field testing of methods. Samples of water and fish communities will be collected using standard techniques (34.) at stream sites determined from Stage 6 analysis. Cursory evaluation of general health status of the catch will be done, and most fish will be preserved for later laboratory inspection and enumeration, but a subsample of fathead minnows will be taken alive and assayed for immune function using methods developed in Stage 2.

Stage 8 – Integration of field data in the IRIS system, comparative analysis of the data obtained with Fish Index of Biotic Integrity (IBI) and fish innate immune system changes, and testing of the spatial/temporal prediction model for estimate of manure impact on selected sites (31.).

Facilities that will be used are the fish culture and maintenance facility in the ISU Department of Animal Ecology, immunology laboratory in the ISU Department of Veterinary Microbiology and Preventive Medicine, and the ISU Geographical Information Systems Facility.

6. Related research

The innate immune system is the collection of defense mechanisms that protect the organism against microbial infection, with no need for prior exposure to the microbe. The link between low-level environmental contamination and activation of the fish IIS has been suspected for some time (4., 5.). The IIS has several attractive features for application to bio-monitoring. First, it promptly reacts on changes caused by interaction between the animal and its environment. Second, changes of innate immunity can influence susceptibility to disease and provide another measurement tool (6.). Third, innate immunity appears to be evolutionarily conservative (7.), so that the response towards a given pollutant by one species (of fish, for example) may reflect similar responses among other species in the local community. Therefore, techniques for measuring the IIS response potentially can be standardized and used to predict entire aquatic community responses. Finally, sampling of the different components of the IIS can provide a set of inter-related parameters that can be used to discern pattern formation in the response towards a specific pollutant (6.). A cellular component in the IIS is the phagocytic cell, a part of the immune system that actively ingests foreign particles encountered in the body or on mucosal surfaces (such as the gills of fish). An activated phagocyte releases a spectrum of different chemical compounds that can be measured in order to monitor the immune response (8.).

Fish species and communities are commonly used as bioindicators of aquatic environmental conditions (9.) for several reasons. Fishes are often abundant and are generally easy to capture. Their biology, ecology and long-term population responses to pollution are well known, and they have cultural values that Society appreciates. Use of fishes as biomarkers of innate immunity changes is based on several additional considerations. First, impairment of innate immunity is more pronounced in fish than, for example, in mammals (10.). Second, changes in fish innate immunity may be appropriate for evaluating overall condition of the aquatic environment (11.). Third, long-term bio-monitoring results can be used for evaluating potential impacts on human and ecosystem health, because the route for the toxicant often leads to humans through aquatic systems (12., 13.).

7. Training potential

A. Ph. D. graduate student – 1. Dusan Palic, DVM, will use this project as the basis of his Ph.D. dissertation in Immunobiology and Fisheries Biology. During this research he will have an opportunity to continue his scientific education through combining medical expertise with ecosystem approaches to solving large scale problems at higher levels of complexity.
B. Undergraduate students – 10. The students will be recruited from the ISU Department of Animal Ecology and other biology programs. They will be employed as hourly assistants during the academic year and in summer. Their duties will include maintaining the stock of experimental minnows, assisting with the immunological assays, collecting fish and water samples from stream sites, and performing water quality tests.

8. Statement of government involvement

Dr Clay Pierce, Assistant Leader (Fisheries) of the Iowa Cooperative Fish & Wildlife Research Unit, Biological Resources Division - U.S. Geological Survey is Co-Principal Investigator of this project. Dr Pierce has expertise in fish and stream ecology and experience with Geographical Information Systems. His responsibilities as federal collaborator in the project include assistance on proposal preparation, literature review and consulting data sources from federal project, active involvement in choosing field sites for the research using projections from path flow analysis models and evaluating field data analysis in hydroecological context. Also, Dr Pierce will take active part in coordinating implementation of collected data in IRIS information system, presentation of the results through IRIS and the World Wide Web, and adviser role for the graduate student.

9. Information transfer plan

Information derived from the project will be transmitted to scientists, environmental managers, and the general public. Scientific audiences will be reached initially through presentations at scientific conferences. Examples include annual meetings of the Iowa Academy of Science (state level), the Midwest Fish and Wildlife Conference (regional level) and the American Fisheries Society (national level). Ultimately, articles will be published in scientific research journals such as the *Journal of Environmental Management, Fish and Shellfish Immunology,* and *Ecological Toxicology.* The research will be a registered project with the Iowa Agriculture and Home Economics Experiment Station and the Iowa Cooperative Fish and Wildlife Research Unit at Iowa State University. As such, annual and final reports will be provided for the USDA CRIS reporting system and the Unit's own annual report. In this way, project results will be available to the national and international scientific community. Our field work, especially, will involve coordination with local natural resources managers of the Iowa Department of Natural Resources, the USDA Natural Resources Conservation Service, and the USGS state office. Through these interactions, we will advise the management community about the project and its results. If the integrated biomonitoring approach is ultimately deemed feasible, workshops will be held for staffs of these agencies to introduce them to the procedures and encourage their adoption for management applications. The general public and the private mass communication sector will be informed about the project in several ways: through established public information channels of ISU Extension and the ISU Colleges of Agriculture and Veterinary Medicine, and by the IRIS WWW page:
(http://madagascar.gis.iastate.edu/iris/viewer.htm).

10. Investigator's qualifications

Two page resumes for investigators are included as separate documents at the end of this proposal.

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Information Transfer Program

USGS Summer Intern Program

Student Support

None

Notable Awards and Achievements

Publications from Prior Projects