North Dakota Water Resources Research Institute
Annual Technical Report
FY 2006

Introduction

This report describes the activities of the North Dakota Water Resources Research Institute (NDWRRI) during the period of March 1, 2006 to February 28, 2007.

The ND WRRI is one of the 54 institutes known collectedly as the National Institutes for Water Resources (NIWR). The NDWRRI was founded in 1965, by authority of Congress (Water Resources Research Acts of 1964, 1972, 1984, and 1990), and is administrated through the United States Geological Survey. Section 104 of the Water Resources Research Act requires the NDWRRI to apply its Federal allotment funds to:

1. Plan, conduct or otherwise arrange for competent research that fosters: (A) the entry of new research scientists into the water resources field, (B) training and education of future water resources scientists, engineers, and technicians; (C) the preliminary exploration of new ideas that address water problems or expand understanding of water and water-related phenomena; and (D) the dissemination of research results to water managers and public.

2. Cooperate closely with other college and universities in the state that have demonstrated the capability for research, information dissemination and graduate training, in order to develop a statewide program designed to resolve State and regional water and related land problems.

3. Cooperate closely with other institutes and other organizations in the region to increase the effectiveness of the Institute and for the purpose of promoting regional cooperation.

This year, NDWRRI once again allocated its 104(B) resources to fund the Graduate Fellowship research projects. The institute also continued its efforts to enhance communications between the State and Federal agency personnel and university faculty and students. Three proposals were submitted by the faculty for the National Competitive 104(G) grant program. One of them was funded. NDWRRI also worked closely with the Environmental and Conservation Sciences program of North Dakota State University (NDSU), Energy and Environmental Research Center at University of North Dakota (UND), and the International Water Institute, Fargo, ND on water related research issues and collaboration. NDWRRI co-sponsored an international conference on water, March 13-15, 2007 with the International Water Institute, Fargo, North Dakota. The Director organized and chaired two sessions creating a forum for presentation of the WRRI Fellowship research to the public. Several WRRI Fellows and their advisors participated.

Program Management

The Institute continued the same administrative mechanism with a director managing the institute program with the help of a State Advisory Committee. Dr. G. Padmanabhan, Professor of Civil Engineering, is the director. Linda Charlton, a NDSU employee, has been working part-time for the Institute to assist the director with Institute finances, communications and information transfer. The State Advisory Committee consists of three members representing the three principal water agencies in North Dakota: State Water Commission, State Department of Health, and the USGS North Dakota District. In addition, the Institute also has a Technical Advisory Committee consisting of faculty from two universities, North Dakota State University and University of North Dakota.

State Appropriation

The State Water Commission continued again this year its support to the 2006 2007 federal 104(B) funding for the Graduate Research Fellowship program of NDWRRI. This is third year the SWC provided 15% match in support of the Fellowship program.

University Support

North Dakota State University and the University of North Dakota administrations consider the NDWRRI activities important and are supportive of its efforts.

Institute Location

The Institute continues to operate from the Administrative Building of the College of Engineering and Architecture of North Dakota State University in Fargo, North Dakota, The director may be reached at:

ND Water Resources Research Institute North Dakota State University Department of Civil Engineering Fargo, ND 58105 Phone: (701) 231-7043 Fax: (701) 231-6185 E-mail: G.Padmanabhan@ndsu.edu

State Advisory Committee

The State Advisory Committee provided guidance on water resources research priorities in the State and region, and participated in the review and evaluation of research proposals and projects. The current committee members are:

Gregg Wiche, District Chief, U.S. Geological Survey, Water Resources Division, Bismarck, North Dakota

William Schuh, Water Appropriation Division, North Dakota State Water Commission, Bismarck North Dakota

Mike T. Sauer, Environmental Health Section, North Dakota Department of Health, Bismarck, North Dakota

The committee members are senior officials in the three agencies in North Dakota responsible for much of the water resources research done outside of NDSU and UND in North Dakota.

Research Program

ANNUAL BASE GRANT (104-B)

In the past several years NDWRRI has offered competitive fellowships to NDSU and UND graduate students for research on water resources topics under a Graduate Research Fellowship (GRF) program effectively using the modest amount of the 104(B) annual base grant. The program meets the requirements of Section 104 of the Water Resources Research Act of 1984. The fellowship program encourages entry of young university faculty and new research scientists into the water resources field; provides training and education to future water resource scientists and engineers; promotes exploration of new ideas that address water problems or expand understanding of water quantity, quality and related phenomena; and engages university faculty in collaborative research programs seeking supports from entities concerned with water problems.

This year, the NDWRRI continued the GRF program and applied bulk of the federal allotment to it. The GRF program is administrated and monitored by the director. Applications are invited from the graduate students and their advisors of the two research universities of the State, NDSU and UND. A rigorous review by the State Advisory Committee and other water professionals in the state determines the awards. Active participation of the academic advisors of the students in meeting matching requirement and seeking co-funding from local, state and other sources is another positive aspect of the program. Periodical review of the progress of the students in meeting the fellowship expectations is ensured by seeking reports from the students and by encouraging them to make presentations in local and regional technical seminars and conferences.

Guidelines for the 2006-2007 Graduate Research Fellowship competition were posted on the Institute website in September 2005, and the competition was announced in the faculty news publications of the two university campuses in October. The following is the request for application that was published on the UND and NDSU campus newsletters, and distributed by e-mail lists:

ND WRRI Calls for Applications for Graduate Research Fellowships

The ND Water Resources Research Institute announces its 2006 Graduate Research Fellowship program. NDSU and UND Graduate students who are conducting or planning research in water resources areas may apply for fellowships varying from three summer months to a full year in duration. Stipends may range from $800-$1,400 per month. The fellowship funds must be applied between March 2006 and February 2007.

Projects proposed for fellowship support should relate to water resources research issues in the state or region. Regional, state, or local collaborations or co-funding will strengthen an application. Fellowships have a matching requirement of two non-federal dollars to one federal dollar. Applicants should have a plan of study filed and should have a thesis research topic chosen at the time of applying. Applications need to be prepared in consultation with advisors. Advisors of the applicant should co-sign the applications.

Applications are due in the office of the director by 5:00 p.m., November 18, 2005. The proposals will be reviewed by a panel of faculty and state water resources research professionals. Announcement of awards will be made by early January.

Consult the ND WRRI website, http://www.ndsu.edu/wrri, for background on the program, and guidelines for preparation of applications. Applicants and advisors who are new to the program are urged to contact ND WRRI Director, G. Padmanabhan, at 231-7043, or G.Padmanabhan@ndsu.nodak.edu.

Send your applications to Dr. G. Padmanabhan, Director, ND Water Resources Research Institute, North Dakota State University, CIE 201E, Department of Civil Engineering and Construction, P.O. Box 5285, Fargo, ND 58105.

NDWRRI GRADUATE RESEARCH FELLOWSHIPS

Twelve fellowships were awarded in FY2006. The titles of the fellowship projects awarded are given below and details are provided for each project under separate project sections. Six of the Fellowships are renewals, three M.S. and three Ph.D. The renewals are Ali Tackett, Kendall Goltz, William Lenarz, Dan McEwen, Michael Newbrey, and Tedros Tesfay.

2006-07 Fellows and their projects:

Graduate Student Project Title Advisor Ali Tackett, M.S. Biological Sciences, NDSU Molecular phylogeography of Etheostoma nigrum (Rafinesque) in the upper Midwest. Dr. Craig Stockwell, Assistant Professor, Biological Sciences, NDSU Ara Anderson*, M.S. Biological Sciences, NDSU The life history of Hexagenia limbata (Serville) (Ephemeroptera:Ephemeridae) in North Dakota and Minnesota streams Malcolm Butler, Professor of Zoology Department of Biological Sciences, NDSU Christopher Hill, M.S. Civil Engineering, NDSU Using Entrapped Cell Systems for Treating Supernatant from Anaerobic Digester of the Moorhead Wastewater Treatment Plant Dr. Eakalak Khan, Assistant Professor, Civil Engineering, NDSU Dan McEwen, Ph.D Biological Sciences, NDSU Stoichiometry and the transfer of mercury from benthic macroinvertebrates into game fish Malcolm Butler, Professor of Zoology Department of Biological Sciences, NDSU Kendall Goltz, M.S. Natural Resources Management, NDSU The Impact of Wetlands and Wetland Easements on North Dakota Land Values Dr. Jay Leitch, Professor of Agricultural Economics, NDSU Mary Schuh, M.S. Soil Science, NDSU Farm-scale reconnaissance of estrogens in subsurface waters Dr. Frank Casey, Assistant Professor of Soil Physics, NDSU Michael Newbrey, Ph.D Biology, NDSU Comparative Study of fossil and extant fish growth: Including analyses of Mean annual temperature in the geologic record Dr. Allan Ashworth, Professor, Geology, NDSU Ryan Klapperich, M.S. Geology , UND Analysis of Associated Bedrock-Aquifer System Sediments: Origins of Electron Donor-Rich Aquifers in Eastern North Dakota Dr. Scott Korom, Professor, Geology and Geological Engineering, UND Tedros Tesfay, Ph.D. Geology, UND Modeling Groundwater Dentrification by Ferrous Iron using PHREEQC Dr. Scott Korom, Professor, Geology and Geological Engineering, UND Wei Zheng, Ph.D. Biological Sciences, NDSU Classification of Macroinvertebrate Communities across Red River Drainage Basin Malcolm Butler, Professor of Zoology Department of Biological Sciences, NDSU William Lenarz, M.S. Geology, UND Effect of flow path processes on the geochemistry and quality of water discharged along the seepage face at Pigeon Point, Sheyenne delta aquifer, Ransom County, North Dakota Dr. Phil Gerla, Geology and Geological Engineering, UND Yuhui Jin, Ph.D. Chemistry, UND Rapid and Sensitive Determination of Bacteria in Water Using Nanoparticles Julia Zhao, Assistant Professor, Department of Chemistry, UND

* Ara Anderson had to leave the graduate program due to unavoidable circumstances before much progress was made and duly informed us so. The amount was re-awarded to another deserving NDSU graduate student of Dr. Malcolm Butler: Name: Melissa Konsti Major: MS Student in Zoology Title: Top-down and bottom-up effects on the abundance of periphyton in shallow lakes

NATIONAL COMPETITIVE PROGRAM (104-G)

The Director, G. Padmanabhan, again this year, encouraged several faculty members from NDSU and UND to submit good proposals for the NIWR-USGS National Competitive Grant program (104-G). Three proposals (one from NDSU and two from UND) were submitted through the ND Institute. The proposal Collaborative Research on In Situ Denitrification and Glyphosate Transformation in Ground Water: NAWQA Eastern Iowa Basins Study Unit of Dr. Scott Korom, UND was funded for three years for a total of $91,988.

Evolution of Fish Growth and its Response to Climate Change

Basic Information

Title:	Evolution of Fish Growth and its Response to Climate Change
Project Number:	2003ND25B
Start Date:	3/1/2003
End Date:	2/28/2007
Funding Source:	104B
Congressional District:	1
Research Category:	Climate and Hydrologic Processes
Focus Category:	Climatological Processes, Wetlands, None
Descriptors:	
Principal Investigators:	Allan Ashworth, Allan Ashworth

Publication

EVOLUTION OF FISH GROWTH AND ITS RESPONSE TO CLIMATE CHANGE

DESCRIPTION OF THE REGIONAL WATER PROBLEM

It is important to consider the implications of climatic change on surface water resources in light of potential consequences of global warming. Fossils can be used to examine the effects of climate change on fish because of the long-term nature of the data. North Dakota boasts some of the best long-term data sets in the form of a fossil record to measure the effects of climatic warming on fish populations. This dissertation research will provide insight for fishery biologists and wetland ecologists concerning the long-term response of contemporary fish growth and population trends in North Dakota given potential climatic changes.

LITERATURE SUMMARY AND PRIOR WORK

Aspects of population dynamics have rarely been studied from ancient ecosystems. However, fossils can provide information about growth, mortality, and numerous other ecological processes that have been examined in contemporary systems (Ricker 1975). In a relatively recent study, which detailed the ageing of *Joffrichthys triangulpterus* scales and the subsequent modeling of their growth, provided insight into the population dynamics of an extinct osteoglossid fish (Newbrey and Bozek 2003). However, there are no contemporary species within the genus *Joffrichthys* to use as a reference for a comparative growth analysis and the data only represent one population. More recently, I have conducted research with an extinct form of pike and compared its growth to living forms of *Esox* (i.e., northern pike and muskellunge). The procedure has since been repeated for yellow perch and the hiodontids (goldeyes) in the fossil record. The research details a correlation between growth and mean annual temperature and has the potential to elucidate the effects of climate change on fish growth.

Climatological processes strongly influence growth rates of fish and as a result climatic warming may have a long-term impacts on fish populations. Growth can vary depending on aquatic and ambient mean annual temperatures (Gillooly et al. 2002). Air temperature strongly influences surface water temperature (Livingstone and Lotter 1998) and therefore fish growth. Temperature has a strong influence on metabolic rate (Q_{10} relationship) and growth of ectothermic taxa; a temperature increase of 10°C increases the metabolic rate two to three fold and reaction rates increase 100-200% (Cossins and Bowler 1987). A quantification of the relationship between growth of certain fishes and temperature will provide a greater understanding of the effects of climatic warming on fish.

My dissertation research focuses on a comparative growth of several groups of fish. Specifically, I will contrast growth of fish in the fossil record to that of living fish. The analyses are important for fishery biologists and ecologists in North Dakota who are interested in the implications of climatic change on surface water resources and fish. For example, I am studying a fossil glacial lake site in North Dakota that has produced fossil specimens of contemporary fish species. The environment of the fossil lake changed from a cool wet climate with tamarack, black spruce, birch and aspen to a contemporary prairie-pothole region. The change occurred over a period of about a thousand years thus giving us insight into ecological processes that are affected by current climate changes.

Numerous studies have modeled growth of individual populations of yellow perch, members of Hiodontidae (goldeyes), and the pikes (i.e., Scott and Crossman 1973; Becker 1983; etc.) and in all publications combined, from a variety of latitudes and ambient mean annual temperatures providing a spectrum of growth curves to analyze. The combined growth curves will provide a correlation between growth and mean annual temperature and thus provide an index to estimate the effect of climate change on fish growth. My dissertation research will provide insight to fishery biologists and surface water

ecologists about the effects of temperature change on contemporary fish populations in North Dakota and other areas.

SCOPE AND OBJECTIVES

The overall objective of my dissertation research is to describe changes in fossil fish growth by comparing fossil specimens to extant fish populations and to relate the changes in growth in the fossil record from a variety of fossil fish sites to climatic change using unrelated paleoclimate data. For example, some site-specific objectives include: 1.) to conduct an excavation at the Seibold site near Jamestown, ND to collect fossil yellow perch; 2.) to describe growth of fossil yellow perch; 3.) to compile and analyze contemporary yellow perch growth data in order to contrast growth in relation to the fossils; and 4.) to analyze contemporary yellow perch growth in relation to ambient mean annual temperature in order to understand the effects of climate change on fish.

These objectives will be repeated for numerous other localities, represented in museum collections, and containing the following taxa: the mooneyes and goldeyes of *Hiodon*; and the members of the pikes, *Esox*. These genera are represented in the present North American and North Dakota fish assemblages, in the fossil record from the Cretaceous to the present, and in museum collections.

METHODS, PROCEDURES, AND FACILITIES

Numerous publications contain the information needed to locate specimens (e.g., Grande 1984; Grande and Bemis 1998; Grande 1999; Li et al. 1997; etc.) suitable for data. Typically, hard structures (i.e., scales, otoliths, cleithra, fin rays, spines, and vertebrae) from captured fish are aged and growth is calculated using the relation between growth of these structures and fish total length (Carlander 1969). Total length is the distance from the anterior-most tip of the head to the vertical plane of the posterior caudal fin tips. For this study, fossil scales and vertebrae will be aged in the lab and from museum collections by counting annulus marks or light and dark pairs of bands on bones. Fishery biologists use Von Bertalanffy growth curves (Von Bertalanffy 1938) to assess growth rates. The curve is fit to the total lengths in this study for each age class:

$$TL_t = L_\infty[1\text{-}e^{-K(t-t_0)}]$$

where: TL_t = Total length (cm) at t (age in years); L_∞ = maximum total length; K = the Brody growth coefficient; t = time (i.e., age in years); t_0 = time at age zero (time at theoretical zero length).

Fish growth data was taken from the published literature to contrast extant growth curves. Site specific mean annual temperature (MAT) data rounded to the nearest 0.1°C will be taken from the WorldClimate© web site and when possible checked for accuracy with Northern Oceanic and Atmospheric Administration (NOAA) data. Ambient MAT will be used in a linear regression analysis and regressed to the natural log transformed total lengths (cm) of fish to obtain a correlation between MAT and growth:

$$\mathrm{Ln}\left(TL_{\mathrm{Age\,t}}\right) = m \times \sqrt{\mathrm{MAT}_E + 10°\mathrm{C}} + b$$

where: $\mathrm{Ln}(TL_{\mathrm{Age\,t}})$ = natural log transformed total length (TL cm) at (t) years of age; m = slope parameter of linear regression; MAT_E = mean annual temperature (MAT) at sites of extant (E) populations; b = intercept parameter of linear regression. Furthermore, ultimate total lengths, growth coefficients, and longevities can be used in place of total length at a given age and the relationships to MAT can also be used to quantify the effects of climate change on aspects of life history.

ANTICIPATED RESULTS AND BENEFITS

Trends in growth characteristics through time will provide insight into the evolutionary trends of fish, while the geographic patterns (i.e., movement) of closely related taxa in the geologic record will provide a better understanding of the response of fish to climate change. Fish respond to climate through variable growth, however, growth rates are only plastic to the extent of genetic variability. A changing climate will force fish to geographically relocate to maintain thermal constancy as in other poikilotherms.

The effects of climatic warming on contemporary fish species can be better understood with an investigation of extant fish growth in relation to temperature; and additional insights will be gained from an examination of the responses of fish in the geologic record during a changing climate. Quantification of growth characteristics in relation to MAT of several living taxa will provide a new and/or more comprehensive understanding of fish growth in relation to their environment. Given the potential for global warming and the questions surrounding the response of fishes, an understanding of fish growth in relation to temperature, and the correlations between fossil fish, evolution, and climatic warming in the geologic record will provide insight into the effects of climatic warming on contemporary fish species. Additionally, fishery biologists will be able to reference the mathematical relationships produced from this research and determine if management strategies (i.e., slot sizes, bag limits, etc.) need to be modified to maintain a healthy fishery. Potential growth rates are often used among other variables to determine biomass yield in a fishery, which is important to consider if harvest rates are too high. For example, a fisheries biologist in North Dakota could use this research to compare the growth and size of fish predicted for the fishery at a particular MAT. The relationship between growth and temperature could offer a new method to determine if management strategies need to be modified.

The results of this study will provide a better understanding of evolution of natural processes to biologists studying the pothole region in North Dakota. New climatic information from this study will be of interest to researchers studying changes in temperature and wet/dry cycles in North Dakota. The results of this study will be published in a series of publications.

PROGRESS OF RESEARCH

As an overview, I have nearly completed my data collection but I have recently found two new collections to visit next summer. In all, I have examined thousands of specimens and aged hundreds of fish fossils. I have 1) correlated contemporary yellow perch (*Perca flavescens*), muskellunge (*Esox masquinongy*), northern pike (*Esox lucius*), chain pickerel (*Esox niger*), goldeye (*Hiodon alosoides*), growth to temperature, 2) showed that climatic events such as that at the Cretaceous / Tertiary boundary and the Early Eocene Thermal Maximum are coincident with evolutionary events, 3) showed the effects of climate change on fossil fish colonization, biogeography, and population trends of fishes.

The results have been presented at eight scientific meetings from 2003 and 2005 (i.e., International Union for Quaternary Research, Society of Vertebrate Paleontology, Northern Plains Biological Symposium, North Dakota Wildlife Society, Dinosaur Park Symposium, Mesozoic Fishes 4). Two papers have been published to date: A paper detailing the effects of climate change on fish colonization and population trends, Canadian Journal of Fisheries and Aquatic Sciences 61:1807-1816 and a techniques paper, “Recognition of annular growth on centra of Teleostei with application to Hiodontidae of the Cretaceous Dinosaur Park Formation” published in the Dinosaur Park Symposium (Newbrey and Wilson 2005). Some example results: centra from Cretaceous (70 million years old) hiodontids and are significantly smaller than those of 50 million year ago. Those centra in the Cretaceous and Eocene are both significantly smaller than those from ND today. As the climate has been cooling since the Cretaceous, hiodontids have been getting significantly larger, a pattern also seen in the pike family (Newbrey et al. 2005: extended abstract). The comparison of *Esox* and esocoid growth patterns

was presented at the last international Mesozoic Fishes Meeting in Madrid, Spain, August, 2005. A 64+ page manuscript is in preparation for submission to the symposium proceedings as part of a prestigious set of articles that come our every four years.

Furthermore, major climatic shifts in the geologic record have been correlated in my data with evolutionary shifts in the hiodontids and the pikes, which both inhabit North Dakota today. Life spans have been getting longer for both groups, and growth rates have been changing. The interesting correlation is that more ancestral, living members of these groups show similar significant trends and characteristics in growth, lifespan, total length at age four and other ages, and ultimate total length with regard to temperature across their ranges today. These significant relationships provide numerical models with which to quantify the effects of climate change on fish. The data suggests that as the climate warms fish will mature sooner at smaller sizes thereby suggesting effects on population dynamics, predator-prey relationships, and ultimately our fishery stocks!

An oral presentation was given to Society of Vertebrate Paleontology Annual Meeting (an international organization): "Newbrey, M.G., A.C. Ashworth, and M.V.H. Wilson. 2004. Geographic trends in North American Freshwater Fishes from the Cretaceous to the Pliocene." and the Northern Plains Biological Symposium in 2005. This research addresses the trends in fish movement across latitude and through time, which is my dissertation research objective 2. The results are being used to interpret and corroborate the results from my other dissertation objectives concerning evolutionary shifts in growth patterns. I have constructed the largest freshwater fossil fish database to date, which is nearly double the size of previous fish reviews published in 1981 and 1986 and consists of nearly 400 taxonomic entries. Preliminary results are very interesting and suggest that fish dispersal is tracking with climate. The age – latitude relationships for 54 taxa within 37 families of freshwater fishes were examined from over 150 fossil localities from the Late Cretaceous to the Pliocene (~100 to 2 mya) in North America. To examine the long-term effects of climate, we compared the changes in latitudinal data with changes in paleotemperatures based on the oxygen isotope analyses of benthic foraminifera. A regression analysis indicates the paleolatitude distribution of fishes is negatively correlated with paleotemperature. This relationship suggests that fish populations are shifting in response to changing thermal conditions, which may help explain many of the patterns in long-term fish dispersal. WRRI was acknowledged in both presentations and will be in the subsequent manuscript. The manuscript will be submitted to the journal Nature.

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Modeling Groundwater Denitrification by Ferrous Iron Using PHREEQC

Basic Information

Title:	Modeling Groundwater Denitrification by Ferrous Iron Using PHREEQC
Project Number:	2003ND27B
Start Date:	3/1/2003
End Date:	2/28/2007
Funding Source:	104B
Congressional District:	At large
Research Category:	Water Quality
Focus Category:	Groundwater, Models, Nitrate Contamination
Descriptors:	
Principal Investigators:	Scott Korom

Publication

1. Tesfay, T. and S. F. KOROM,2006, "The relative roles of electron donors in aquifer denitrification reactions: insights from geochemical modeling", 40th annual meeting, North-Central section, the Geological Society of America, University of Akron, Akron, Ohio, April 20-21.
2. Korom, S., 2007, "Denitrification in the Red River Basin and Beyond: How Aquifer Sediments Influence Water Quality", Third International Water Conference, International Water Institute, Grand Forks, March 13-15.
3. Tesfay, Tedros and Scott Korom, 2006,"Modeling Groundwater Denitrification by Ferrous Iron Using PHREEQC", Technical Report No: ND 06-03, North Dakota Water Resources Research Institute, Fargo, North Dakota.
4. Tesfay,Tedros, 2006,"Modeling Groundwater Denitrification by Ferrous Iron using PHREEQC", Ph.D Dissertation, Department of Geology and Geological Engineering, University of North Dakota, Grand Forks,North Dakota.

MODELING GROUNDWATER DENITRIFICATION

BY FERROUS IRON WITH PHREEQC

DESCRIPTION OF THE PROBLEM

Studies made by members of the University of North Dakota (UND) denitrification research team show that organic carbon and sulfides are active electron donors in North Dakota and Minnesota aquifers (Korom et al. 2005). However, the role of Fe(II) was overlooked because the geochemical evidence for ferrous iron is more difficult to decipher as Fe(III) precipitates out from the aqueous solution. Thus far, little was known about the significance of solid phase ferrous iron. My research complements the previous works by investigating the two inseparable issues, abundance of biologically available ferrous iron and its role in the denitrification processes. Geochemical modeling, PHREEQC, is employed to gain insight into the *in situ* denitrification processes that take place via all possible electron donors.

LITERATURE REVIEW AND PRIOR WORK

UND denitrification team's efforts, including the latest geochemical modeling work (Skubinna, 2004), have shown evidently the role of pyrite (FeS_2) in reducing nitrates (Skubinna, 2004; Schlag, 1999). The remaining nitrate sinks were entirely attributed to organic carbon with the assumption that inorganic carbon has been produced but latter lost from solution via precipitation of Ca-Mg-CO_3. The bases of these arguments are the decline of Ca^{2+} and Mg^{2+} in the N-ISM, but not in C-ISM, as well as XRD measurement of precipitates collected from sampling bottles (Schlag, 1999). However, this accounts for only a small portion of the remaining nitrate. For example in the Elk Valley aquifer about 7 % (first tracer test) of the denitrification can be explained by the precipitation of magnesian calcite (Schlag, 1999; Skubinna, 2004). The rest of the nitrate sink was explained by a similar argument but with the assumption that enough Ca^{2+} and Mg^{2+} have been desorbed from mineral surfaces and latter on co-precipitated with C(+4) from solution (Korom et al. 2005). Nevertheless, the amount of cations that can be released from solid surfaces into the solution is limited. Commonly, the laboratory measured cation exchange capacity (CEC) and that of the CEC value used in geochemical modeling are significantly different (personal communication with Parkhurst; Skubinna, 2004). Barton and Karathanasis (1997) discovered, from the study of eight morphologically and physicochemically different pair of intact and disturbed soils that routine CEC measurements overestimates ion-exchange processes by about 49.1 %. The above assumption was probably the main reason for the ignorance of the possible role of reduced metals, such as Fe(II), in the previously studied aquifers of our region (Schlag, 1999). PHREEQC, based on the database and Gaines-Thomas convention, provides important information once the exchanger value and solution are defined in the input file (Parkhurst and Appelo, 1999). For practical modeling proposes, therefore, the exchanger (X-), presented in PHREEQC (mol/l), is determined by trial and error (Skubinna, 2004; personal communication with Parkhurst). Numerous runs are performed using different values for the exchanger (X-) until a good match is achieved between the modeled and the actual concentrations of cations in the ISM. That means the sample collected first after tracer injection is compared closely with the pre-injection cation composition of the native water to estimate the amount of Ca^{2+} and Mg^{2+} (meq/l) desorbed from the mineral surfaces into solution. If all these cations are thought to be co-precipitated with bicarbonate, then the maximum amount of bicarbonate that could be produced by organic-carbon-

supported-denitrification can be determined. For example, using the X^- value of 3.5 mmole determined by Skubinna (2004) the maximum Ca^{2+} and Mg^{2+} that can be exchanged for K^+ are about 0.501 mmol/l. This in turn can boost the role of organic carbon by only 17 % (for the Time = 589 days with a net nitrate amount of 2.42 mmol/l). This confirms that there should be another possible electron donor, presumably Fe(II).

SCOPE AND OBJECTIVES

Mixing of oxidized (nitrate polluted) water and reduced waters at depth trigger important multiphase aquifer hydrogeochemical reactions (Appelo and Postma, 1996). These reactions can be reproduced by injecting oxidants like nitrate along with a conservative anion that has similar physicochemical properties to that of the oxidant. Some of the common aquifer geochemical reactions are ion exchange, reversible reactions (dissolution and/or precipitation of dominant minerals), and redox reactions (Tesoriero et al., 2000). The latter reaction is naturally slow but when microbially catalyzed it has enormous environmental significance. The disequilibrium in the redox state between the two mixing waters instigates environmentally important reactions that change the fate of redox sensitive contaminants such as NO_3^- (Kehew, 2001).

METHODS, PROCEDURES AND FACILITIES

Aquifer denitrification reactions are complex natural processes that require consideration of the role of bacteria along with the thermodynamic and kinetic principles (Appelo and Postma, 1996). In a classic sense, complete equilibrium of any given system is achieved when it occupies a specific region of space without any spontaneous tendency for a change (Bethke, 1996). In a more practical sense equilibrium to groundwater environments is assumed when the rate of geochemical reaction is greater than that of the flow of groundwater (Postma et al. 1991, and references therein). However, in most natural geochemical environments equilibrium cannot be developed fully among all interacting multi-phases and the inference of local or partial equilibrium makes more sense.

For local equilibrium, a portion of the system develops equilibrium but as the fluid traverses the aquifer, it encounters fresh minerals, and the reaction progresses in discrete time steps (Bethke, 1996). A partial equilibrium geochemical modeling that included ion exchange, reversible reaction and redox reactions is used in this project (Figure 1). These modeling scenarios produced a series of potential recovered water qualities and the last in the sequence expected to reflect the target solution for that particular sampling date.

ANTICIPATED RESULTS

Solutions of four sampling dates from each site were selected for modeling proposes and each solution was allowed to pass through sequential reaction steps, and then finally compared with the target solution. After the net nitrate was determined, for each time step, the role of each electron donor was investigated starting with pyrite. Next, the maximum amount of organic carbon that can be calculated back from the directly measured and concealed inorganic carbon is considered. The concealed amount of Ca^{2+} and Mg^{2+} represents not only that in solution but also the fraction that may have been released from the sorbent and latter coprecipitated together with inorganic carbon. Finally, the remaining net nitrate unaccounted by these two major electron donors is attributed to Fe(II).

Table 1. Relative roles of the three common electron donors in natural denitrification reactions of North Dakota and Minnesota Aquifers.

Research Site	Electron Donors	OC %	FeS_2 %	Fe(II) %
Akeley (MN)	Range/Average in %	46 – 60/51.2	3.0 – 14/7.47	27 – 50/41.3
Perham-M (MN)	Range/Average in %	1.0 – 21/9.31	59 – 83/71.3	7.0 – 40/19.4
Perham-W (MN)	Range/Average in %	19 – 32/25.9	0.0 - 1.0/0.44	68 – 80/73.6
Luverne (MN)	Range/Average in %	28 – 36/32.9	25 – 41/30.5	32 – 39/36.5
Robinson (ND)	Range/Average in %	0.0 – 23/7.81	1.0 - 5.0/2.31	75 – 99/89.9
Karlsruhe-S (ND)	Range/Average in %	23 – 27/25.1	14 – 28/21.4	46 – 63/53.5
Larimore-2TT (ND)	Range/Average in %	19 – 30/24.7	22 – 48/37.7	27 – 48/37.5

PROGRESS TO DATE

All aqueous analytical data, mineralogy and chemistry of sediments and geochemical modeling works are evidently showing the proportional role of all electron donors (Figure 2) and Fe(II) supported denitrification has a significant role as a natural remediation tool. For example, the diagrams of Akeley (MN) research site demonstrate that CEC and reversible reactions are responsible for the geochemical evolution observed in the N-ISM and that redox reactions are not important for the C-ISM (See figures in Appendix). Unlike the C-ISM, the nitrate chamber is expected to duplicate the redox disequilibrium observed in natural aquifers. Then, the initial solution was forced to react with the three electron donors, based on the methodology explained earlier. That means the net nitrate was reduced by the proportional role of the three major electron donors. The role of each electron donor varies for the solutions of the modeled sampling dates, however, the following ranges were deduced from the "REDOX REACTION" modeling exercise: - OC 46-60 %, FeS_2 3-14 % and Fe(II) 27-50 % for Akeley (MN) (Figure 2).

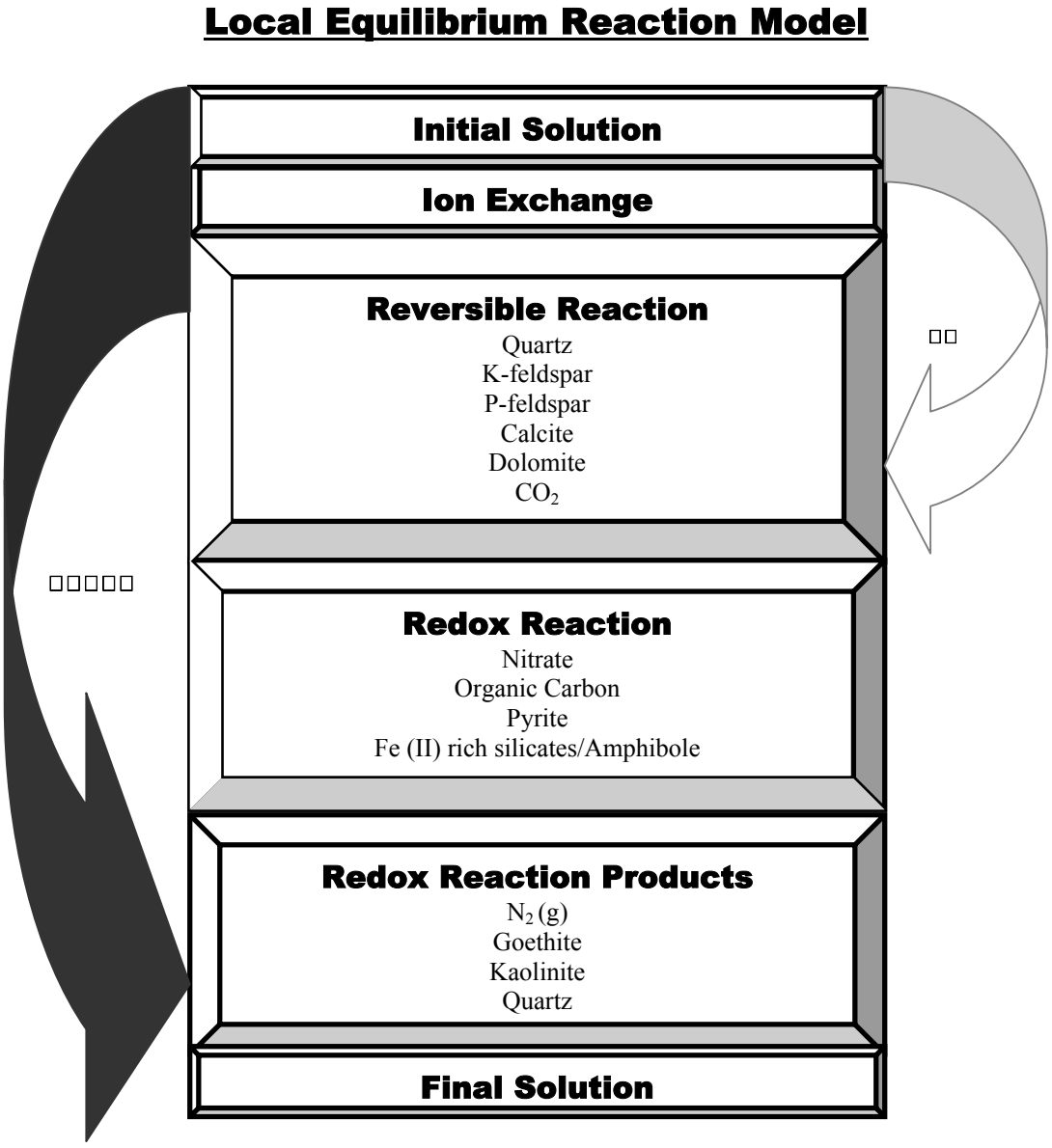


Figure 1. Partial equilibrium modeling conceptual representation; note that control chamber (C-ISM) and nitrate chamber (N-ISM) acquire different steps in the modeling sequence.

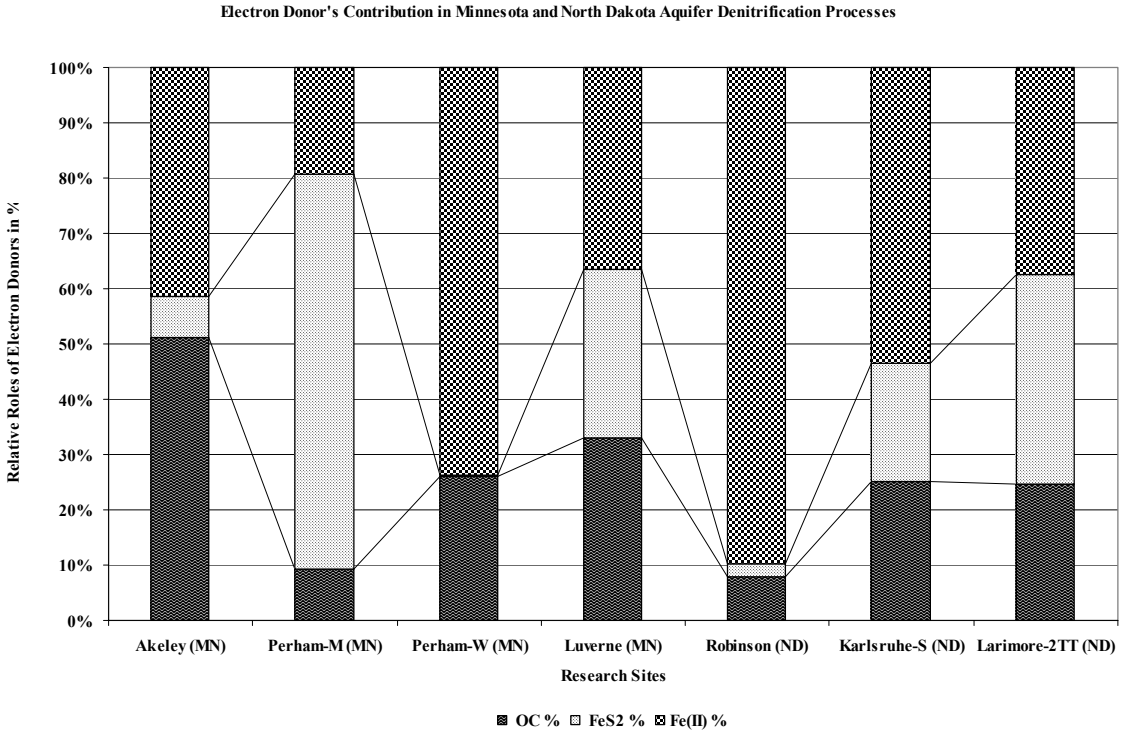
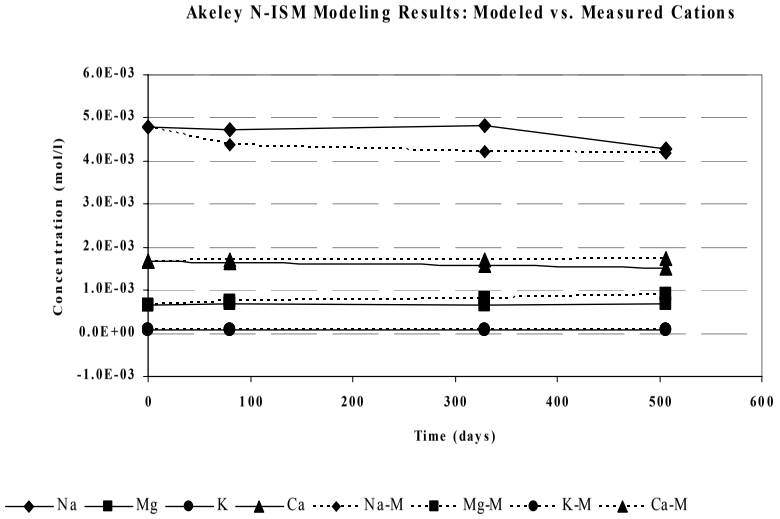
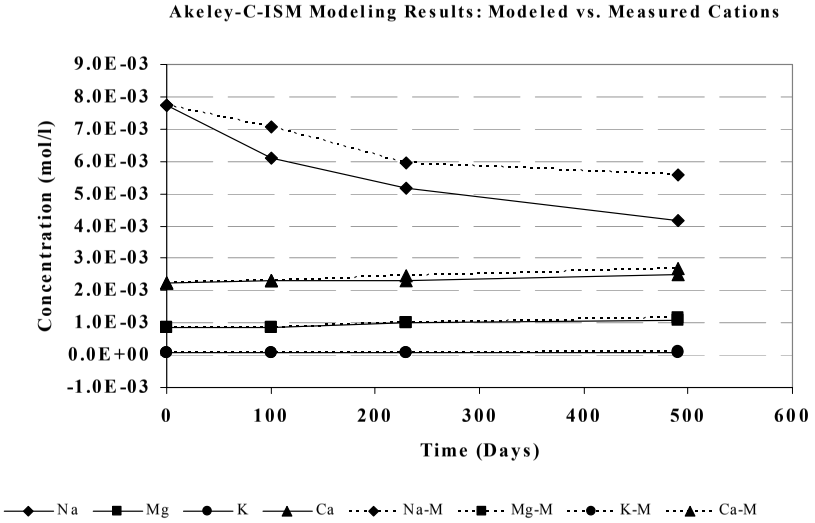
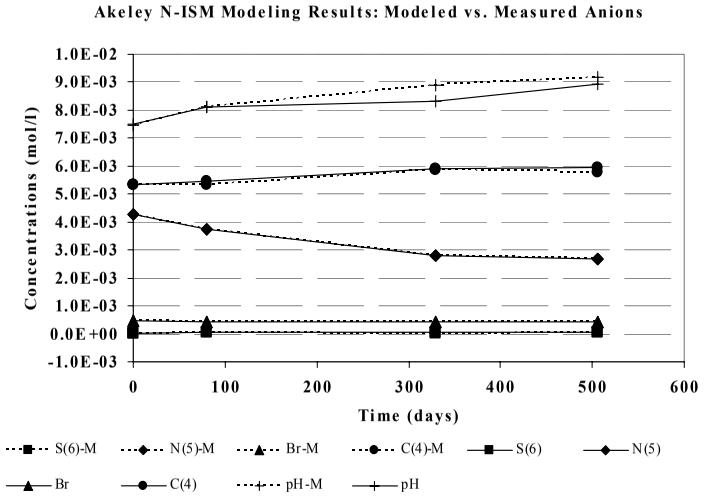


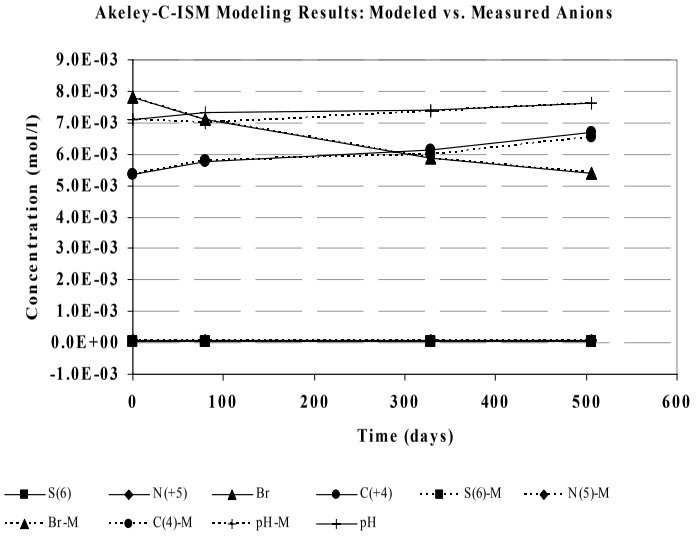
Figure 2. Average contribution of each electron donor in the natural denitrification reactions of North Dakota and Minnesota aquifers, as computed via advanced geochemical modeling, PHREEQC, employing the concept of partial geochemical modeling.

Appendix. Additional data comparing the modeled ("M" with dashed lines) vs. measured ions at the Akeley site.









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Stoichiometry and the Transfer of Mercury from Benthic Microinvertebrates into Game Fish

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STOICHIOMETRY AND THE TRANSFER OF MERCURY FROM BENTHIC MACROINVERTEBRATES INTO GAME FISH

REGIONAL WATER PROBLEM

Mercury (Hg) damages the central nervous system, altering the way that nerves conduct electrical impulses and divide, leading to lowered cognitive and metal functioning or in especially acute circumstances cerebral palsy, mental retardation or death (NRC 2000). Its effects are especially harmful to fetuses or infants during development of the nervous system. Hg emissions have continued to increase since the industrial revolution, entering aquatic food chains via atmospheric precipitation where they suspend in the water column and are uptaken by phytoplankton or settle to the bottom where they become available to bacteria (Swain *et al.* 1992). Phytoplankton, with acquired Hg, is either ingested by zooplankton or sinks to the substrate as detritus. In turn, detritus and bacteria provide a food source to macroinvertebrate benthic organisms (i.e., benthos) and bioaccumulation occurs up through trophic levels eventually to game fish that are consumed by humans (Swain *et al.* 1992, Bodaly *et al.* 1993, Fitzgerald *et al.* 1998). As a result, the EPA and various state agencies issue advisories for fish consumption where impairment occurs. The North Dakota Department of Health currently has consumption advisories listed for Devils Lake, Red River, Lake Oahe/Missouri River, Lake Sakakawea, and other waterbodies (NDDH 2003).

LITERATURE REVIEW

Most Hg in fish tissue comes through their diet, and since benthic invertebrates are important to the diets of most fish species, they likely transfer a high proportion of Hg from the environment (Ryder and Kerr 1978, Harris and Snodgrass 1993, Hall *et al.* 1997). Vander Zanden and Vadeboncoeur (2002) investigated the contribution of zoobenthic organisms to common fish species inhabiting north-temperate lakes. They combined diet data from 470 fish populations (15 species) and stable isotope data from another 90 populations (11 species) and found that on average, across all species considered, zoobenthos were a direct contributor to 50% of fish diet and indirectly provided another 15% as food for zoobenthos-supported fishes, showing that on average greater than 65% of fish diets rely on zoobenthos. Blumenshine *et al.* (1997) found that for fish across a taxonomic spectrum, feeding ranged from 18-90% on benthos with a mean reliance of 55%, indicating benthos were more important than planktonic resources. In a community of six piscivorous species, for all combined, the three most important prey species were yellow perch (*Perca flavescens*), amphipods, and dipterans (Liao *et al.* 2002). Even if benthos are not a primary food source for piscivores or planktivores, they are stabilizing; once a primary food source is gone, fish will feed on benthos (Blumenshine *et al.* 1997). Not all benthos store Hg in the same amounts with the result that some taxa will have higher concentrations relative to others (Tremblay and Lucotte 1997, Wong *et al.* 1997, Hall *et al.* 1998). Benthos concentrate Hg differentially based on size, functional feeding group, and other physiological processes (Feltmate and Williams 1991, Parkman and Meili 1993). As such benthos community structure likely affects the availability of Hg to fishes.

The kinds of benthos and fish in lakes as well as subsequent Hg accumulation rates are likely determined by nutrient limitations according resource competition theory and ecological stoichiometric (i.e., mass balance) principles (Tilman 1982). Resource competition theory, from which ecological stoichiometry is built, predicts that, in a community where two taxa, with differing body stoichiometries, are in competition, the taxon whose stoichiometry is most similar to the resource will prevail (Tilman 1980, Tilman 1981, Tilman 1982). Ecological stoichiometry predicts nutrient limitation, more than energy, determines the reproductive and competitive success of species (Reiners 1986, Sterner 1990, Elser *et al.* 1996, Sterner and Elser 2002). The direction of energy and nutrients is mostly unidirectional from benthos to pelagia, where fish act as an important vector of that transfer through ingestion of energy and nutrients in one habitat and excretion in others (Vanni 1995, Schindler *et al.* 1996, Sarvala *et al.* 1999, Jeppesen *et al.* 2000, Schindler and Scheuerell 2002).

In both terrestrial and aquatic systems, the essential stoichiometric currency is ratios of carbon:nitrogen:phosphorus; carbon because it is such a ubiquitous element in organisms, and phosphorus and nitrogen because these elements are most likely to be limiting. Carbon, nitrogen, and phosphorus are the prime constituents of major biomolecules (i.e., lipids, nucleic acids, proteins). Carbon, although an important element in organisms, is generally not limiting because of its abundance; nitrogen can be limiting, but typically its supply meets the relative needs of organisms. Phosphorus, however, is often scarce relative to needs and is thus limiting, even though it is typically needed in a much lower absolute amount relative to nitrogen or carbon. Most production in lakes is limited by phosphorus (Elser *et al.* 1990).

Relevant data for hypothesis testing of stoichiometric theory include nutrient content of consumers, understanding of homeostatic control of those nutrients in the consumer, and nutrient content of consumer resources (Sterner and George 2000). Stoichiometric data are beginning to be generated for common freshwater fish, and are sufficiently constant that published results should give a good estimate for species across environmental gradients (Davis and Boyd 1978, Sterner and George 2000, Tanner *et al.* 2000). Frost *et al.* (2003) made a first effort at stoichiometric analysis of benthic invertebrates, comparing across 9 orders from different lake environments. They found that nutrient ratios for insect orders did not differ substantially among lakes with differential nutrient supply regimes, suggesting similar homeostatic control of nutrient composition as previously found in zooplankton (Andersen and Hessen 1991). N:P ratios from lowest to highest were Amphipoda, Ephemeroptera, Trichoptera, Anisoptera, Diptera, Hemiptera, Zygoptera, Hirudinea, and Coleoptera.

Principles of mass balance apply to Hg as much as any other element in an ecosystem. As such, stoichiometry drives the dynamics of Hg accumulation from benthos to fish. For example, in a phosphorus limited system, for a fish species to maximize growth it will either have to eat high quality food (e.g., low C:P) or a higher amount of low quality food (e.g., high C:P). Suppose that to maximize growth a fish species needs to consume a given amount of phosphorus per year. In such a case, it would have to consume less high quality food compared to the low quality food to sustain maximum growth. Furthermore, consider a scenario where the low quality food has a low P:Hg relative to the high quality food. In this extreme case, Hg would accumulate to high concentrations quickly in fish. These dynamics would affect fish species differently relative to their requirement for phosphorus. These sorts of relationships can be determined with the appropriate data using coupled bioenergetic and mass balance models (Reiners 1986, Kraft 1992).

Benthic macroinvertebrate communities have historically served as good indicators of a variety of environmental conditions (Rosenberg and Resh 1993). The most stable benthos populations occur in habitats whose physical location protects organisms from the effects of near surface perturbations (Brinkhurst 1974). This research will assess the role of using benthos not only for an indicator of nutrient status of a lake, but of the susceptibility of a lake system to Hg accumulation.

SCOPE AND OBJECTIVES

I propose continuation of a research program addressing taxonomy, stoichiometry, and benthic-pelagic coupling in the context of benthic contribution to Hg accumulation to fisheries in Minnesota and North Dakota lakes along a trophic gradient from oligotrophic to eutrophic. The objectives of this study are as follows:

1) Characterize the benthic community of lakes under a variety of nutrient regimes
2) Quantify the biomass of the constituent members of the benthic communities in those lakes
3) Quantify carbon, nitrogen, phosphorus, and mercury ratios for those communities as a whole
4) Model how those ratios impact rates of mercury accumulation and concentrations in piscivorous game fish likely to inhabit those lakes (e.g., lake trout, walleye, smallmouth bass, northern pike, etc.)
5) Use appropriate regression models to test whether there is a relationship between Hg and P (as a limiting nutrient) in the benthos
6) Provide applicable information for managers of mercury-susceptible systems

METHODS

Twelve lakes will be sampled for benthic invertebrates including 3 deep, oligotrophic trout-lakes in northeast Minnesota, 3 shallow, eutrophic lakes from North Dakota and 6 mesotrophic lakes in Minnesota and North Dakota. Each lake will be sampled using a stratified sampling method, with 20 grabs per lake comprised of 60% of sampling effort at <1 m and the remaining effort split between two strata based on depth. Sampling sites will be randomly chosen using a grid on bathymetric maps, and sites will be located in the field with a GPS and depth finder. All grabs from a lake will be pooled and organisms will be separated to order or family. Samples will be dried for 24 h and dry mass will be recorded for each taxonomic group alone. All organisms will be homogenized and ground and subsamples will be analyzed for carbon, nitrogen, phosphorus, and mercury. These data will be used in a bioenergetics model to predict mercury concentration levels in game fish species, using literature-derived values for parameters not measured in the field.

DELIVERABLES

I will provide a framework by which lakes can be assessed for susceptibility to high Hg concentrations in fish. A stoichiometric underpinning can provide managers with a relatively easy method of assessing fish susceptibility to Hg by measuring a subsample of benthos for C:N:P. Manuscripts will be prepared for the bioenergetics models produced in terms of the relationship between nutrient limitation and Hg accumulation.

PROGRESS TO DATE

I have developed our benthic sampling and fauna sorting technique over the summers of 2004-05 in a related project. Computer software has been obtained for bioenergetics modeling, and I have been successful in developing mock models with fictitious data that represent those that will be empirically derived. I am in the process of determining phosphorus concentrations of invertebrates and have contacted laboratories that provide carbon, nitrogen, and mercury analysis services. Presently, I am conducting an in-depth literature review on stoichiometric theory as well as the environmental toxicology of mercury accumulation in aquatic systems.

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The Impact of Wetlands and Wetland Easements on North Dakota Land Values

Basic Information

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End Date:	2/28/2007
Funding Source:	104B
Congressional District:	1
Research Category:	Not Applicable
Focus Category:	Wetlands, Economics, Management and Planning
Descriptors:	
Principal Investigators:	Jay Andrew Leitch, Jay Andrew Leitch

Publication

THE IMPACT OF WETLANDS AND WETLAND EASEMENTS ON NORTH DAKOTA LAND VALUES

DESCRIPTION OF THE STATE/REGIONAL WATER PROBLEM BEING INVESTIGATED

Wetlands are ubiquitous across the Prairie Pothole region of North and South Dakota and Minnesota and numerous federal, state, and non-governmental agencies are actively involved in purchasing wetland conservation easements from private landowners. Common examples are the Small Wetland Acquisition Program of the United States Fish and Wildlife Service (USFWS), the Wetlands Reserve Program (WRP), and the Natural Resource Conservation Service (NRCS). To ensure that such programs are effective in encouraging landowners to place wetlands under easement while minimizing the expenditure of public funds, it is necessary that the impact of both wetlands and wetland easements on land values be fully understood.

KEY LITERATURE AND RESEARCH

My advisor (S. Shultz) along with a colleague at the University of Minnesota (S. Taff) recently completed a preliminary (test phase) of this research in three counties in southeastern North Dakota. The results published in the article: '**Implicit Prices of Wetland Easements in Areas of Production Agriculture'** in the journal Land Economics, 80 (4), 2004, are summarized in the following abstract:

Impacts of Fish and Wildlife Service wetland easements on agricultural land values in North Dakota were estimated by regressing sale process on physical and institutional characteristics of sold parcels. While easements on temporary wetlands did not influence prices, each additional acre of permanent wetland under easement decreased average prices by $321 (-79%). Because non-eased permanent wetlands were shown to reduce land prices by $161/ac., we can estimate the implicit price of a wetland easement per se to be 160$/acre – 6% below historical easement payment levels in the study area. Alternative model specifications further demonstrate the importance of separating eased and non-eased wetlands and of accounting for their hydrologic condition.

SCOPE AND OBJECTIVES OF THE PROPOSED RESEARCH

I have expanded Dr. Shultz's previous research to include all 39 North Dakota counties west of the Missouri River, since these counties contain almost all of easements statewide. I am currently quantifying the impact of temporary and semi-permanent wetlands and wetland easements on land values within these counties during the 2000-2004 time period. Since there has recently been an increasing trend in the sale of land for outdoor recreation purposes, I have begun to study the impact of land parcels sold for hunting/recreation purposes on the value of surrounding agricultural land sales because

this was not done in Dr. Shultz's earlier research. All 53 counties will be considered in this portion of the project.

METHODS AND PROCEDURES

1) Data have been collected associated with all nonconfidential agricultural land sales across all 53 counties comprising the State of North Dakota. More than 200 of these sales have been identified as potential (buyer out of state or resident of larger North Dakota city) recreational land sales. The SSURGO digital soils database has been used to quantify soil productivity within the sold parcels. Data have been collected for the years from 2000 to 2004. Collected sales data include a legal description of the property, sale price, date, and buyer/seller name. Three types of sales have been excluded: 1) all sales that are less than 40 acres in size. 2) arms-length transactions (between family members), and 3) sales which include buildings and other non-land assets.

2) The boundaries of all sale tracts have been digitized into a GIS database by identifying and selecting field boundaries in the common land unit (CLU) database of the NRCS based on legal descriptions of the sale tract. In cases where CLU boundaries do not directly correspond to sales, particular quarter section polygons of the public land survey (PLS) GIS coverage will be selected and digitized.

3) Land uses (acres of cropland versus pastureland) within individual sales tracts have been estimated through spatial overlays of the 2003 or 2005 CDL coverages. In addition, land uses specifically adjacent to wetlands and easements have been quantified using GIS buffering techniques.

4) The relative productivity of the sale tracts has been represented by spring wheat yields (bushels per acre) for cropland, and range productivity (pounds of forage per acre) for pastureland. These soil productivity measures have been calculated through spatial overlays of tracts and the SSURGO soils database, and by weighting SSURGO yields based on acreage within all unique SSURGO soil polygons within tracts.

5) The acreage of temporary and semi-permanent wetlands within sale tracts has been quantified through spatial overlays of the NWI Basin (Reynolds et al., 1996). Temporary wetlands hold water in the early spring but are usually dry by mid-summer. Semi-permanent wetlands hold water throughout the growing season during most years.

6) Estimation of conservation easement acreage within sale tracts has been accomplished by overlaying USFWS (SWAP) easement boundaries with sale tract and NWI wetland coverages.

7) The hydrologic (wetness) condition of all wetlands and easements has been determined through GIS-based spatial overlays of water classifications of the NASS-CDL, and visual inspections of overlaid NAIP color aerial photography. Overlays were specific to the year of sale and imagery. The goal has been to confirm the water coverage assumed with NWI classifications which was necessary because the surface water acreage of wetlands

can vary dramatically due to changing precipitation conditions across the state. These results will be compared to those obtained by Royle (Royle et. al., 2002) which were determined by statistical methods.

8) The following hedonic (price-attribute) multiple regression model will be estimated:

$$P_L = f(Z_s, Z_p, Z_w, Z_f),$$

where the dependent variable P_L is the sale price ($/acre); Z_s are sale characteristics including size and possible locational details; Z_p represents relative productivity measures of crop and pasture land; Z_w represents wetland characteristics (NWI classifications with water coverage estimates, as well as land uses immediately surrounding wetlands); and Z_f represents wetland easement restrictions.

9) Alternative model specifications will be evaluated including varying the Basin classification categories of NWI wetlands, utilizing alternative buffer sizes to account for land uses adjacent to wetlands, and by using alternative functional form (linear, log-linear, and semi-log relationships).

10) Marginal implicit prices will be calculated for each of the explanatory variables. These measure the expected change in the average sale price per acre associated with a 1-unit change in the quantity of an explanatory variable while holding all other explanatory variables constant at their mean levels. Finally, the marginal implicit prices of both wetlands and wetland easements for agricultural and recreational land sales will be mapped across all 53 counties comprising the State of North Dakota.

ANTICIPATED RESULTS (DELIVERABLES) & BENEFITS

A Departmental Report for lay audiences and a journal article for academics will be prepared that contain estimates of the impact of different types of wetlands and wetland easement on both agricultural and hunting/recreation land values across all 53 counties of North Dakota. Presentations summarizing research results will also be made to local and national groups (farmer groups, state and national agencies, and academic conferences). These estimates are expected to be used by the USFWS, the USDA-NRCS, rural appraisers, land owners, and land buyers/sellers to evaluate fair market prices for wetland easements, and land purchases with and without different types of wetlands.

PROGRESS TO DATE

4,332 agricultural land sales from the years 2000 to 2004 have been collected from county courthouse public records in all 53 North Dakota counties. 775 of the 4,332 total land sales were found to contain wetland easements. The boundaries of all sales tracts have been digitized into a GIS database by identifying and selecting field boundaries in the common land unit (CLU) database of the NRCS based on the legal descriptions of the database. Land uses (acres of cropland vs. pastureland) have been calculated through spatial overlay of the year 2003 or 2004 NASS CDL coverages. The relative productivity

of the sale parcels is represented by spring wheat yields for cropland, and pounds of forage per acre for pastureland. These soil productivity measures were calculated through spatial overlays of tracts and the SSURGO soils database. The type of wetlands found within the sale tracts has been quantified using spatial overlays of the NWI Basin. Conservation easement acres within sale tracts were determined by overlaying USFWS easement boundaries with the sale tract and NWI wetland coverages. The hydrologic (wetness) condition of all wetlands and easements has been determined using spatial overlays of water classifications of the NASS CDL, and visual inspections of overlaid NAIP color aerial photography.

In addition, 210 land purchasers that reside either in one of the larger North Dakota cities or outside the state were identified as potential recreational land buyers. Survey information about the nature of the sale was obtained from 152 of the 210.

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Royle, J. Andrew, Mark D. Koneff, and Ron E Reynolds. 2002. "Spatial Modeling of Wetland Condition in the U.S. Prairie Pothole Region". *Biometrics* 58, 270-279.

Shultz, S. and S. Taff. 2004. "Implicit prices of wetland easements in areas of production agriculture". *Land Economics* 80(4):501-512.

Assessing the Effectiveness of Local Water Institutions in Water Management

Basic Information

Title:	Assessing the Effectiveness of Local Water Institutions in Water Management
Project Number:	2005ND86G
Start Date:	9/1/2005
End Date:	8/30/2008
Funding Source:	104G
Congressional District:	North Dakota
Research Category:	Social Sciences
Focus Category:	Law, Institutions, and Policy, Economics, Water Quality
Descriptors:	None
Principal Investigators:	Robert Hearne

Publication

1. Hearne R., 2006, "Water Quality Monitoring Among Local Agencies in the Red River Basin", 2006 National Water Quality Monitoring Conference. San José CA. May 9.
2. Kritsky, C. C. and Robert Hearne, 2007, "Characteristics of Effective Local Water Management Organizations in the Red River Basin", Third International Water Conference, International Water Institute, Grand Forks, March 13-15.
3. Hearne R., 2006, "Criteria and Indicators for Effective Water Management Institutions", Red River Basin Conference Winnipeg, Manitoba, January 12.
4. Hearne R., 2006, "Interbasin Transfer to Reduce Flooding: The Case of Devils Lake, North Dakota", University Council on Water Resources Conference. Santa Fe, NM. July 18.

ASSESSING THE EFFECTIVENESS OF L LOCAWATER INSTITUTIONS IN WATER MANAGEMENT

Project Description

There are a variety of formal and informal local institutions that are involved with water resources management in rural areas. These governmental and non-governmental institutions have different objectives, different legal statuses, and different affiliations with state and local governments. Research is needed to assess the roles and effectiveness of local water institutions. As new initiatives to improve water quality are being proposed, it is important to assess the capacity of existing institutions to meet new and evolving needs. The objective of this research is to improve local management of water resources by providing policy makers and agencies with an improved understanding of the characteristics of successful local institutions. This research will focus on the Red River of the North basin in Minnesota and North Dakota although some assessment of Manitoba institutions will also be included. The basin is fairly homogeneous in terms of land use and geographic features, but features three completely different sets of water law, which makes it an excellent case study of institutions. The overall objective of this research is to strengthen local water management institutions so that they may better meet evolving local and basin wide needs, especially the maintenance of water quality. Specific objectives of the research include: 1) Develop a set of objective and subjective criteria and indicators to evaluate local water management institutions; 2) Provide a review of the different governmental and nongovernmental institutions in the basin, classify their goals, activities and chartered purposes, and identify overlaps and functions that are not being addressed; 3) Identify and evaluate the characteristics of local water institutions that have a demonstrated capability to meet local goals and wider goals of the greater river basin; 4) Assess the use of: scientific and technical information; extension education and training programs; and other support provided by governmental and non-governmental agencies; 5) Analyze institutions and agencies likely behavior in a decision-making situation and further develop decision-making support tools; 6) Identify the characteristics of institutions that successfully evolve to meet new challenges; 7) Analyze preferences of a sample of residents and stakeholders toward watershed management issues and the types of institutions that they trust; and 8) Disseminate results to various forums including local workshops and scientific journals. Objective and subjective criteria and indicators for local water institutions will be refined for local circumstances by interviewing and surveying assorted State and Federal agencies who work on water management issues. A survey of local water institutions will be used to: identify goals, activities, and accomplishments; assess their understanding and use of technical information and extension training; and provide an understanding of how these institutions have evolved to meet changing needs. This survey will be supported by another survey of local leaders, county commissioners, and mayors. The Legal-Institutional Analysis Model will be used to assess negotiation strategies. A number of choice experiments and a stated preference technique that can estimate the non-market value for environmental goods and services, will be used to analyze residents and leaders preferences towards water management programs and institutional frameworks.

Benefits of the Research

By identifying the attributes of local institutions that effectively achieve their own goals and/or further goals of water quality maintenance, this research will:1) ascertain whether existing institutional frameworks should be adapted to meet evolving needs or new institutions should be developed to address emerging issues such as water quality monitoring and enforcement; 2) support local institutions by identifying key characteristics that facilitate effectiveness; 3) assess the benefits and costs associated with having water resource institutions defined along county lines as opposed to watershed lines; 4) support the development of extension and education programs that strengthen local institutions by specifically addressing key characteristics of effectiveness; and 5) help policy makers in the design strategies to monitor and enforce nonpoint source pollution abatement initiatives. Based upon this research and subsequent reviews and comments, recommendations will be made to political leaders and lawmakers, agency officials, and local stakeholders.

This project will collaborate with the North Dakota State University's (NDSU) M.S. programs in Agribusiness and Applied Economics, and Natural Resources Management and is expected to support at least two M.S. students. It should produce at least two M.S. theses, a number of extension reports, at least two peer-reviewed scientific journal articles, and at least one workshop to present results to regional and local water management leaders.

Planned First and Second-Year Goals

During the first year period of September 2005 – August 2006 planned goals were to:

1) Develop a set of objective and subjective criteria and indicators to evaluate the effectiveness of local water management institutions;
2) Review the different governmental and nongovernmental institutions in the basin, classify their current goals and activities as well as their chartered purposes, and identify overlaps as well as functions that are not being addressed; and
3) Identify and evaluate the characteristics of local water institutions that have a demonstrated capability to meet local goals and wider goals of the greater river basin, including water quality monitoring and participation in the establishing TMDLs.

During the period of September 2006- August 2007 planned goals included:

4) Assess the use of: 1) scientific and technical information provided by USGS and other agencies; 2) extension education and training programs; and 3) other support provided by governmental and non-governmental agencies and organizations;
5) Analyze institutions' and agencies' likely behavior in a decision-making situation, such as watershed and basin planning, or conflict resolution and further develop decision-making support tools;
6) Identify the characteristics of institutions that successfully evolve to meet new challenges; and

7) Analyze preferences of a sample of residents and stakeholders toward watershed management issues and the types of institutions they trust.

Progress towards Goals

A review of water management organizations and institutions has been conducted.

A set of criteria and indicators for effective public water management organizations was developed and used to develop a survey instrument in 2006. A survey of organization managers and board members was conducted in December 2006 – January 2007. Survey data is currently being analyzed. This analysis will: i) identify and evaluate the characteristics of local water institutions that have a demonstrated capability to meet local goals and wider goals of the greater river basin; ii) assess the use of scientific and technical information, extension education and training programs, and other support provided by governmental and non-governmental agencies and organizations; and iii) identify the characteristics of institutions that successfully evolve to meet new challenges. A MS thesis from this work will be presented and defended in June or July 2007.

An analysis of institutions' and agencies' likely behavior in a decision-making situation has not been conducted. This objective was to have been completed with the support of USGS personnel from Ft. Collins Research center. However, given that grant funds can not be used for USGS travel, we have not been able to complete this task. Currently there are no plans to complete this activity.

A second survey of informed stakeholders will be conducted in late 2007. Currently the population to be sampled is being identified and initial consultations to support the development of the survey instrument are being conducted.

A further analysis of measures of "effectiveness" is being initiated. The results of this analysis should be used with the data on organizational activities and attributes.

Farm-Scale Reconnaissance of Estrogens in Subsurface Waters

Basic Information

Title:	Farm-Scale Reconnaissance of Estrogens in Subsurface Waters
Project Number:	2006ND100B
Start Date:	3/1/2006
End Date:	2/28/2007
Funding Source:	104B
Congressional District:	1
Research Category:	Ground-water Flow and Transport
Focus Category:	Groundwater, Non Point Pollution, Water Quality
Descriptors:	None
Principal Investigators:	Francis Xavier McKeon Casey

Publication

1. Casey, F., Mary Schuh, G. L. Larsen, Heldur Hakk and Zhaosheng Fan, 2007, "Fate of Manure-borne, Land-Applied Hormones", Third International Water Conference, International Water Institute, Grand Forks, March 13-15.

FARM-SCALE RECONNAISSANCE OF ESTROGENS IN SUBSURFACE WATERS

DESCRIPTION OF STATE OR REGIONAL WATER PROBLEM BEING INVESTIGATED

Endocrine disrupting chemicals (EDCs) are present in animal manures applied to the soil. There is increasing concern on both state and regional levels that EDCs may be leaching to ground water, and that it may be posing a threat to human health through drinking water. Because of this, the fate and transport of EDCs is an important problem of both state and regional concern.

KEY LITERATURE AND PRIOR WORK

Kavlock et al. (1996) define an EDC as, "an exogenous agent that interferes with the production, release, transport, metabolism, binding, action, or elimination of natural hormones responsible for the maintenance of homeostasis and the regulation of developmental processes in the body." According to Kolpin et al. (2002), natural and synthetic hormones are EDCs that are of particular concern because of their potency and widespread detection. An apparent increase in physiological and reproductive disorders in animals such as fish, turtles, shellfish, gastropods, and mammals has raised concern over the persistence of EDCs in the environment. The effects of these chemicals on human beings remain unknown, but there is concern that evidence of the chemicals' toxicity with animals could be an early indication of dangers that EDCs could present to humans. Disquieting trends, such as increased breast cancer in women and decreased fertility in men have already been linked, in some studies, to EDCs (e.g., Sharpe and Skakkebaek, 1993; David and Bradlow, 1995).

The agricultural use of animal waste as fertilizer is one means of environmental exposure to hormones that has raised concern, because of the intensification of livestock production and crop production near areas of widespread urbanization. Soto et al. (2004) have shown that runoff from concentrated feedlot operations can enter surface waters and result in hormone concentrations that could adversely affect aquatic health. A study by Panter et al. (2000) has attributed the phenomena of vitellogenin production in male fish (*Pimephales promelas*) to increased estrogen levels. Their study showed that vitellogenin production was induced in male fathead minnows that were exposed to 17ß-estradiol for 21 d at concentrations as low as 30 ng/L.

Dr. Francis Casey is my advisor and the project leader of research focusing on estradiol and testosterone and their metabolites at the North Dakota State University. He has conducted past experiments related to the fate and transport of EDCs, and since 2000 he has lead a team of interdisciplinary scientists in a study on the fate and transport of reproductive hormones in the soil. Work by Casey et al. (2003, 2004, 2004) has already been completed to characterize and quantify 17ß-estradiol and testosterone and their metabolites in the soil. Laboratory studies have demonstrated the transformation of 17ß-estradiol and testosterone in natural sediments into various metabolites; however, details of the transformation pathways are still undetermined in the natural environment. It has been found that these hormones degrade readily and are strongly bound to the soil; nonetheless, they are consistently detected in the environment at low concentrations.

The following are related research papers:

Casey, F.X.M., G.L. Larsen, H. Hakk, and J. Simunek. 2003. Fate and Transport of 17ß-Estradiol in Soil-Water Systems. Environ. Sci. Technol. 37(11):2400-2409.

Casey, F.X.M., G.L. Larsen, H.Hakk, and J. Simunek. 2004. Fate and Transport of Testosterone in Agriculturally Significant Soils. Environ. Sci. Tecnol. 38(3):790-798.

Casey, F.X.M., J. Lee, and J. Simunek. 2005. Sorption, Mobility, and Transformation of Estrogenic Hormones in Natural Soil. J. Environ. Qual. 34:1372-1379.

David, D.L., and H.L. Bradlow. 1995. Can environmental estrogens cause breast cancer. Sci. Am. 273:166-172.

Kavlock, R.J. 1991. Overview of endocrine disruptor research activity in the United States. Chemosphere. 39:1227-1236.

Kolpin, D.W., E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, and H.T. Buxton. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance. Environ. Sci. Technol. 36:1202-1211.

Panter, G.H., R.S. Thompson, and J.P. Sumpter. 2000. Intermittent exposure of fish to estradiol. Environ. Sci. Technol. 34:2756-2760.

Sharpe, R.M., and N.E. Skakkebaek. 1993. Are oestrogens involved in falling sperm counts and disorders of the mail reproductive tract? Lancet 341:1392-1395.

Sonnenschein, C., and A.M. Soto. 1998. An updated review of environmental estrogen and androgen mimics and antagonists. J. Steroid Biochem. Molec. Biol. 65:143-150.

Soto A.M., J.M. Calabro, N.V. Prechtl, A.Y. Yau, E.F. Orlando, A. Daxenberger, A.S. Kolok, L.J. Guillette, B. le Bizec, I.G. Lange, and C. Sonnenschein. 2004. Androgenic and estrogenic activity in water bodies receiving cattle feedlot effluent in eastern Nebraska, USA. Environ. Health Perspec. 112:346-352.

Thompson, M.L. 2005. Occurrence, Persistence, and Pathways of 17ß-Estradiol in Agricultural Soils: A Field Study. M.S. thesis North Dakota State Univ., Fargo.

SCOPE AND OBJECTIVES OF THE PROPOSED RESEARCH

Lysimeters located on a swine (*Sus scrofa*) farm in North Dakota have been installed to monitor the amount of 17ß-estradiol leached through soil, and monitoring wells have also been installed to monitor the surficial aquifer. Based on known application rates in manure and laboratory-determined degradation rates, it was anticipated that most of the hormones would be metabolized or bound to the top 10 cm of soil. Instead, 2003 data (unpublished) show substantial

amounts of 17ß-estradiol leached through the soil and detected in the shallow aquifer. 17ß-Estradiol was even found in the control plot, where no manure was applied, which provided evidence for the antecedent existence of this hormone in the soil or that it was leached from somewhere else.

This project proposes to identify the causes of the unexpectedly high 17ß-estradiol detections. First, the farmer injects his swine waste lagoon material into the field soil at this research location. The soils in this area are aquic and have redoximorphic features (i.e. faint mottling) with 16 cm of the soil surface, which suggests large fluctuations in the water table. Moreover, these soils are derived from water deposited sand, which decreases the sorption of 17ß-estradiol (Casey et al., 2003). We propose the following hypotheses for causes: (1) There exists high levels of background 17ß-estradiol from the lagoon material the farmer injected in the field; (2) High water-table conditions cause a cool and anaerobic environment in which 17ß-estradiol persists; and (3) The ground water that contains the 17ß-estradiol is transporting exogenous hormones into the lysimeters in soil horizon.

METHODS, PROCEDURES, AND FACILITIES

The research site is a swine farm in North Dakota, where previous and ongoing field studies have and are being conducted by NDSU, concerning the fate and transport of hormones in soil. The farm is an excellent location for the study, because hormone data from lysimeters in four test plots are already available, and six wells are already in place for measurement of water levels and ground-water hormone concentrations.

Both water levels and ground water hormone concentrations will be measured. In addition stratified soil samples will be collected from the surface to the water table, and used to quantify the distribution of the 17ß-estradiol with depth. These findings will be combined with historic lysimeter and well data to examine the spatial and temporal variability of the 17ß-estradiol present in the soil and to determine the causes of its high persistence.

ANTICIPATED RESULTS AND BENEFITS FROM THE PROPOSED STUDY

It is anticipated that anaerobic conditions will be found in the soil, preventing the degradation of 17ß-estradiol and its metabolites. We also expect to find a correlation between the concentration of 17ß-estradiol in ground water and the height of the water table, and the concentrations of the hormones detected by the lysimeters.

It is hoped that this project will provide valuable information on the behavior of 17ß-estradiol and its metabolites, allowing an assessment of the effectiveness of natural restorative processes in the environment. The proposed ground-water research, combined with data obtained by Casey et al. (2005) on the sorption and mobility of 17ß-estradiol in soil will help to define the interaction of soil and ground water with respect to the retention and transport of 17ß-estradiol. Knowledge of these retention and transport processes may be useful in the development of remediation strategies. Furthermore, since 17ß-estradiol is a prototype for other EDCs, results will contribute to a general understanding of EDCs and other possible organic contaminants.

The proposed project will also benefit society and the environment by serving as an educational tool. Dr. Casey has disseminated his group's findings to the public through several presentations and publications. Past experimental work has been integrated into education

through cooperation with local K-12 students and teachers. A strong effort has been made to incorporate and train individuals from the community and to reach out to underrepresented individuals. Members of the NDSU Soil Sciences department have been involved with school and community programs such as "Extending Your Horizons," which encourages young women in the fields of math and science by fostering awareness about career opportunities related to these studies. They have also participated in "Sunday Academy," an NDSU-Tribal School collaborative project designed to encourage American Indian students in North Dakota to pursue careers in science, math, and engineering. I would like to participate in programs such as these. I believe they would help promote an awareness of the environmental issues that I will be researching. They will also help facilitate interest in the sciences and technology amongst K-12 students and others who, otherwise, might not be given the opportunity to learn from such information.

PROGRESS TO DATE

The environmental phenomena to be studied have been identified and we have formulated specific research objectives and hypotheses. I have begun a literature review of the characteristics, properties, and environmental interactions of the chemicals to be studied. Previous local research on the hormones to be researched has been reviewed. I have also begun a literature review on field and laboratory methods for accomplishing the described experimental objectives and will next formulate them into a plan for sampling and analysis.

Analysis of Associated Bedrock-Aquifer System Sediments: Origins of Electron donor-rich Aquifers in Eastern North Dakota

Basic Information

Title:	Analysis of Associated Bedrock-Aquifer System Sediments: Origins of Electron donor-rich Aquifers in Eastern North Dakota
Project Number:	2006ND101B
Start Date:	3/1/2006
End Date:	2/28/2007
Funding Source:	104B
Congressional District:	At large
Research Category:	Ground-water Flow and Transport
Focus Category:	Groundwater, Hydrogeochemistry, Geochemical Processes
Descriptors:	None
Principal Investigators:	Scott Korom

Publication

ANALYSIS OF ASSOCIATED BEDROCK-AQUIFER SYSTEM SEDIMENTS: ORIGINS OF ELECTRON DONOR-RICH AQUIFERS IN EASTERN NORTH DAKOTA

Project Background

Nitrate pollution has long been recognized as the most prevalent form of groundwater pollution. The only effective process to remediate nitrate contaminants is denitrification, typically through natural attenuation. This process reduces nitrate (NO_3^-) to harmless nitrogen gas. The process occurs naturally, requiring only an oxygen-limited environment, the presence of nitrogen digesting bacteria, and the availability of electron donors. The three most common electron donors are organic carbon, sulfide (typically as pyrite, FeS_2), and ferrous iron minerals. Research has also shown that the controlling factor in this reaction has typically been the availability of suitable electron donors within the aquifer sediments.

The long-term goal of this research is to develop an aquifer nitrate vulnerability index based on the supply of electron donors in the aquifer sediments. This project contributes toward that goal by considering the abundance of electron donors in the likely parent materials comprising the aquifer sediments. Based on the experience of previous UND denitrification research and literature reports, shale units are more likely to have large supplies of electron donors than other regional rock types. Shale is relatively soft and easily weathered and comprises much of the bedrock in eastern North Dakota. Therefore, shale units are the most likely contributors to sediments in nearby aquifers. My hypothesis is that regional bedrock units rich in electron donors should correlate to nearby aquifer sediments that are potentially rich in electron donors. The practical utility of this work is that an initial list of aquifers with potentially high denitrification capacities in North Dakota may be made using geological investigations. Later, more extensive and more expensive geochemical analyses would be necessary to confirm the denitrification capacity of specific aquifers and specific aquifer sites. However, much of the initial work could be done with data already available and in conjunction with future drilling performed by the North Dakota State Water Commission (NDSWC).

Progress

Geochemical analysis and literature review are ongoing. The bedrock samples were collected this previous summer in conjunction with the State Water Commission. They were collected in a general east to west transect across Stustman, Foster, and Steele counties. Two to three samples were collected when the shale was penetrated, representing an upper and lower, and occasionally mid-level sample. Samples from

Kidder, Benson, Nelson, and Cass Counties have also been provided from other drilling projects. In total there are 39 samples representing 21 different sites and seven lithologic units. XRD analysis has been preformed on many of the samples and shows that the shale units are rich in quartz, clay minerals, and occasionally calcite, as was expected. Pyrite, micas, and iron bearing minerals have also been detected. XRD has also verified that the lithologic units are generally uniform in space and depth, meaning a single sample can be considered representative. Work has also begun to analyze organic carbon content in the samples. There are not enough data to date to be conclusive. Analysis of other electron donors, sulfide and iron, will begin in earnest.

Significance:

This research will investigate the link between the denitrification capacities of eastern North Dakota aquifers with the electron donor composition of the surrounding bedrock. If such a link is possible, a qualitative index (low, medium, and high) of aquifer denitrification capacity based on the probable source of the parent material will be developed. This index could then be used to focus, in a cost-effective fashion, more extensive and expensive geochemical analysis on specific aquifers or zones in specific aquifers.

Molecular Phylogeography of Etheostoma nigrum (Rafinesque) in the upper Midwest

Basic Information

Title:	Molecular Phylogeography of Etheostoma nigrum (Rafinesque) in the upper Midwest
Project Number:	2006ND120B
Start Date:	3/1/2006
End Date:	2/28/2007
Funding Source:	104B
Congressional District:	1
Research Category:	Biological Sciences
Focus Category:	Ecology, None, None
Descriptors:	None
Principal Investigators:	Craig Stockwell

Publication

MOLECULAR PHYLOGEOGRAPHY OF *ETHEOSTOMA NIGRUM* (RAFINESQUE) IN THE UPPER MIDWEST

DESCRIPTION OF CRITICAL STATE OR WATER PROBLEM TO BE INVESTIGATED

The geologic history and abundant potential study sites of the upper Midwest provide a unique opportunity for the assessment of spatial genetic diversity. The Johnny Darter, *Etheostoma nigrum* (Rafinesque), with its large range and abundant populations, is an excellent species to study to answer phylogeographic questions about North Dakota and Minnesota. I will examine the genetic diversity of *E. nigrum* by using microsatellite PCR primers designed initially for other species of *Etheostoma* and recently optimized for *E. nigrum*. This information will provide not only the inferred gene flow among the darters but will also provide a baseline against which to evaluate gene flow for other fish species located in the same water bodies. For instance, many game fish are stocked and transferred within and among watersheds with no genetic monitoring. By studying a benthic fish with a small home range, it will be possible to uncover the phylogeographic structure among the various watersheds of the upper Midwest. In turn, this information can be used by managers for conserving genetic diversity within and among watersheds.

SCOPE AND OBJECTIVES

With the advent of molecular techniques, the field of ecology has become dramatically dynamic. It is now possible to use these new techniques to answer a wide variety of ecological questions. I am particularly interested in using molecular genetic markers to address these questions. My time with the Stockwell Lab at NDSU will help me develop the skills I need to become a better scientist and learn the techniques that are the future of ecological work. It is with these goals in mind that I have began to examine the genetic relationships among *Etheostoma nigrum* (Rafinesque), Johnny Darter, populations in the North Dakota and Minnesota.

Many of the watersheds of the northern Midwest have been isolated since the end of the Pleistocene and offer great opportunity for assessing the recent (evolutionarily speaking) genetic divergence and gene flow among fishes in the upper Midwest (Underhill 1958). *Etheostoma nigrum* is a small, benthic fish in the perch family. It is commonly found in the lakes and streams of North Dakota and Minnesota. *E. nigrum*, found from the Hudson Bay to southern Mississippi and Colorado to the Atlantic coast, is one of the most abundant and wide-ranging species of *Etheostoma* genus (Eddy and Underhill 1978 and Kuehne 1983). Prior observations have noted significant morphological differences among these fish in the lakes and streams of the upper Midwest (Dr. Jim Grier, personal communication). However, despite its abundance and range, little work has been conducted to better understand this species. *E. nigrum* is a non-migratory fish with movements confined only locally before and after the spawning season (NatureServe 2005 and Winn 1958). In addition, because of their benthic habits, the fish are unlikely to be transferred via an angler's bait-bucket. Due to these two facts, fine-scale structure is

likely to exist among populations of *E. nigrum*. It is because of these reasons that I have chosen to use *E. nigrum* as a model species to study genetic diversity and gene flow among the lentic and lotic systems of North Dakota and Minnesota.

Polymerase chain reaction (PCR)-based analysis of microsatellites is one of the most popular techniques for determining the genetic diversity of established populations (Avise 2004). Microsatellites are a type of co-dominant DNA marker inherited in a Mendelian fashion (DeWoody and Avise 2000). That is, an individual fish will have one allele from the female parent and another from the male parent. Each population is likely to contain alleles that are unique to the population. Thus, using PCR to amplify microsatellites, it will be possible to examine the genetic uniqueness of each *E. nigrum* population and what, if any, gene flow is occurring among populations.

The first step in performing a microsatellite analysis involves creating the PCR primers. Once primers are designed, it is then possible to screen large numbers of individuals (Avise 2004). This work has already been accomplished for two species of *Etheostoma*. Published literature provides nine different PCR microsatellite primers for *E. virgatum* and *E. olmstedi*, the latter belonging to the same subgenus as *E. nigrum* (Porter et al. 2002 and DeWoody et al. 2000). Once optimized for *E. nigrum,* these primers can be used to PCR-amplify DNA that can then be used with an automated fragment analysis program to determine the genetic diversity and gene flow within and among populations of *Etheostoma nigrum* in the major watersheds of North Dakota and Minnesota. Similar success has been achieved in the Stockwell Lab with congeners of *Cyprinodon tularosa*, a threatened fish of New Mexico (Jones et al. 1998, Stockwell et al. 1998, and Iyengar et al. 2004).

METHODS, PROCEDURES, AND FACILITIES

During ice-free months, fish will be collected from sites chosen among the streams and lakes of North Dakota and Minnesota. Sampling sites will be located within the watersheds of the upper Missouri, Red River of the North, and upper Mississippi Rivers. Ten study sites will be identified in North Dakota and Minnesota. Each site will measure approximately 300 meters in length. Sites will be chosen based on the habitat preferences of *Etheostoma nigrum.* Multiple populations will be sampled within various river systems, possibly the Pipestem, Turtle, and Ottertail Rivers, to evaluate fine scale genetic structure. Future plans include sampling the James, Wild Rice, and upper Missouri Rivers and Devil's Lake in North Dakota. In Minnesota, samples will be taken from hydrologically isolated and interconnected lakes in the Lakes Region (western MN) as well as the Buffalo and Otter Tail Rivers. Fish will be collected with seines or snorkeling equipment. Captured fish will be sequestered in a living-well to first anesthetize and then sacrifice the organisms. Fish will be collected under collecting permits issued to Dr. Craig Stockwell by the states of North Dakota and Minnesota.

Once received in the lab, a fin clip will be taken from each of the voucher specimens. The fin clips can then be used in the lab for DNA extraction. The DNA will then be amplified using PCR and each of the nine optimized primers. Each primer flanks a different

microsatellite repeat. The PCR amplified samples, now representing nine loci for each fish, will then be ran on a Beckman Coulter CEQ8000 automated DNA sequencer using an automated fragment analysis program. The alleles of each individual will be scored and compare to others form its own population and geographically distant populations.

Data will be analyzed using Genetic Data Analysis (GDA). Each population will be evaluated for herterozygosities (expected and observed) allelic diversity, percent loci polymorphism, and Hardy-Weinberg equilibrium. I will also evaluate genetic structure within and among populations with Wright's F-statistics (Weir and Cockerham 1984). A hierarchical analysis will be conducted treating populations, rivers, and drainages as three distinct levels in the hierarchy. This will allow me to partition variance among populations within rivers, rivers and lakes within drainages, and between drainages.

ANTICIPATED RESULTS AND BENEFITS

The genetic evaluation of *E. nigrum* populations may have management implications. As stated above in the research description, most of the fish populations in the upper Midwest have been isolated since the end of the Pleistocene. However, many of the streams and lakes are hydrologically connected. As a result, managers often transfer and stock game fish from one water body to another with little to no regard for the genetic structure of the systems. This practice is based upon the idea that gene flow will occur in systems that are hydrologically connected; but in actuality, gene flow is largely influenced by the migratory habits of individual species. Species with small home ranges may have little gene flow between closely located populations. Over time, these populations develop a unique genetic identity, often adapting to local conditions. When fish transfers are planned without consideration of this diversity, populations become genetically homogenous. This results in a loss of genetic variation among populations and perhaps even outbreeding depression (Leberg 1992). This is especially important if populations are locally adapted. Understanding the current diversity and gene flow of *E. nigrum* in the watersheds of North Dakota and Minnesota will aide in the establishment of management and conservation units as well as help managers plan for the transfer and stocking of fishes. *E. nigrum* are particularly useful for evaluating phylogeographic structure because they are non-migratory and also unlikely to be accidentally transferred due to their use of benthic habitats.

PROGRESS TO DATE

Preliminary work with *Etheostoma nigrum* and the published primers is promising. All nine primers amplify DNA in all *E. nigrum* individuals tested. To date, the primers are assumed to amplify microsatellites. This is due to the fact that the allele sizes from *E. nigrum* are similar to the alleles in the primer-specific congeners. Future plans including sequencing the nine loci amplified to determine if the amplified samples are, in fact, microsatellites. However, for the purpose of *E. nigrum* phylogeography, a primer that amplifies diagnostic fragments can be used to determine genetic diversity and gene flow among the Mississippi River, Red River of the North, and Missouri River watersheds.

As stated earlier, 408 fish have been collected and are currently stored in the Stockwell Lab. The sample locations include the Pipestem River, Beaver Creek, Turtle River, and Forest River in North Dakota. In Minnesota, fish have been collected from Felton Creek, Hay Creek, Fishhook River, Shell River, Mississippi River, Lake Christina, and Lake Ida (a hydrologically closed system). Research is progressing as scheduled. In addition to sequencing the *E. nigrum* microsatellites, future work will include the completion of DNA extraction from collected fish, amplification of the 9 microsatellites in all 408 individuals, automated fragment analysis, and genetic data analysis.

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Classification of Macroinvertebrate Communities across Red River Drainage Basin

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CLASSIFICATION OF MACROINVERTEBRATE COMMUNITIES ACROSS RED RIVER DRAINAGE BASIN

REGIONAL WATER PROBLEM

Changes in environmental conditions in running waters lead to changes in benthic macroinvertebrate communities. Therefore, many indicators of stream or river pollution focus on macroinvertebrates. Species richness, evenness, and abundance tend to exhibit great correlation with in-stream habitat conditions. If relationships between benthic invertebrates and their habitats are deterministic, it should be possible to predict macroinvertebrate distribution. The distribution of invertebrates in any river is likely to depend on multiple habitat features. Multivariate relationships between invertebrates and habitats are associative and do not indicate causes and effects, so these relationships should be tested with specific field experiments. Also, variables found to be important descriptors of invertebrate composition may be location specific. A more complete understanding of relationships between habitat structure and community variables is needed to identify ecological patterns, and to devise improved strategies for maximizing diversity, abundance and production of commercially and ecologically important species. Appropriate spatial and temporal scales of measurements are equally required for determining cause-and-effect relationships between habitat structure and community variables.

There is increasing recognition of the need for biological information on the Red River of the North. Recently (Nov. 2-3, 2005) the International Water Institute (IWI), in collaboration with the International Red River Board (IRRB), and the International Joint Commission (IJC), sponsored a workshop to develop approaches for assessing and monitoring the health of this important aquatic ecosystem. Following two days of discussion, there was consensus that too little biological information currently exists for certain goals of the workshop to be met. Specifically, hopes of identifying targeted biological communities for monitoring, and of establishing common sampling methods, were premature, as so little biological research has been conducted on the main stem and the lower tributaries of the Red River. A recent report (Laidlaw 2004) by the Energy & Environmental Research Center (EERC) at UND serves as a starting point for development of appriopriate biological monitoring tools for the Red River Basin. I propose research to help fill this information need, by inventorying macroinvertebrate communities in major habitats of the main-stem Red River, and assessing suitable sampling techniques for monitoring these communities. Such information will make a valuable contribution toward the development of a comprehensive research proposal for the Red River main stem that is the IJC's charge to the Aquatic Ecosystem Committee of the IRRB (Fritz 2005).

LITERATURE REVIEW

The structure and composition of benthic macroinvertebrate communities in freshwater ecosystems are very often used for the biological assessment of water quality and to evaluate the impact of chemical and other pollutants (Wiens & Rosenberg, 1984; Hellawell, 1986; Mance, 1987; Rosenberg & Resh, 1993).

Stream community structure changes with variation of in-stream habitat (Gorman & Karr, 1978; Huryn & Wallace, 1987). Macroinvertebrate abundance and taxonomic richness are generally highest in stream environments where stones, woody debris, plants and other forms of habitat structure are abundant (Downes et al, 2000; Matthaei et al, 2000; Voelz & McArthur, 2000). Species richness, evenness, and abundance tend to exhibit great correlations with instream habitat (Simley et al, 2005).

Habitat structure is defined as physical objects in an environment that provide habitat (e.g., rocks, trees, shells of sessile animals). Habitat structure promotes greater biomass, abundance and diversity of organisms by enhancing the abundance and variety of resources and creating multiple substratum types and variable

hydrodynamic regimes (Bertness & Leonard, 1997; Lenihan, 1999; Downes et al, 2000; Syms & Jones, 2000). For example, increased colonizable surface area and microhabitat diversity created by habitat structure reduces the intensity of competition for living space (Downes et al, 2000; Ellner et al, 2001). Habitat structure also reduces the frequency of localized species extinctions due to chance by providing refuges from predation and physical disturbance, like wave force and strong currents (Bertuness & Leonard, 1997; Lipcius et al, 1998). Additionally, habitat structure indirectly benefits organisms by increasing the abundance and diversity of food resources by increasing particulate matter abundance (Fournier & Loreau, 1999; Siler et al, 2001).

That habitat structure generally has positive effects on biological communities is well established, but a more complete understanding of relationships between habitat structure and community variables is needed to identify ecological patterns and devise improved strategies for predicting macroinvertebrate distribution, maximizing diversity, abundance and production of commercially and ecologically important species (Lipcius et al,1998; Lenihan, 1999). Identification and consistent use of biologically meaningful measures of habitat structure are considered critical in attaining these goals (Commito & Rusignuolo, 2000; Downes et al, 2000). Appropriate spatial and temporal scales of measurements are also required for elucidating cause-and-effect relationships between habitat structure and community variables (Downes et al, 1998; Attrill et al, 2000).

However, sometimes large spatial and temporal variations in community structure are observed in both impaired and unimpaired sites. Variation in distribution and abundance of benthic organisms may be caused by differences among sites in flow-rate (Newbury, 1984), stream size and distance to the source (Minshall, Petersen & Nimz, 1985), substrate (Minshall & Minshall, 1977), vegetation (Vincent, 1983), and temperature and stream discharge (Bournard et al, 1987; Boulton & Lake, 1992). Seasonal variability of such factors at a site (e.g. Wade et al, 1989) is one of the prominent causes of temporal variation in the community. The phenology of species within a community will also alter the observed composition of the community throughout the year. The degree to which phenology affects the observed taxonomic richness, diversity or calculated biotic indices of a community depends on the species involved (Rosillon, 1987). In the context of bioassessment and monitoring, temporal variation may influence judgement as to whether or not a site is degraded (Linke et al, 1999). It is clear that the time scale of sampling, particularly when it is carried out over more than one season, can significantly affect results of a bioassessment. A predictive model incorporating time would have higher predictive power and result in a more sensitive bioassessment. A closer look at the nature of structural changes within the community between the seasons might give evidence of whether or not human impact caused the site to fail.

The distributions of many macroinvertebrates vary both spatially and temporally, such that collecting representative samples that are representative of the natural community structure is extremely difficult or impossible (Resh & Jackson, 1993; Merritt et al, 1996). The method chosen should collect representative and relatively comprehensive samples of the benthic community (Resh, 1995). The data used for biological assessment should be acquired by a variety of sampling techniques, which have been developed and modified over time (Flannagan & Rosenberg, 1982; Mackay et al, 1984; Rosenberg & Resh, 1993; Merritt et al, 1996).

SCOPE AND OBJECTIVES

The objectives of this research are to 1). Inventory the macroinvertebrate communities of different habitat conditions in the main stem Red River 2). Explore the temporal changes of these benthic communities through the open water season and 3). Compare the results of outcomes provided by different sampling techniques. These objectives will allow me to evaluate potential relations between aquatic invertebrate communities and environmental conditions.

METHODS

Sites will be randomly selected in three reaches of the main stem Red River: above Fargo (Oxbow), within Fargo (Oak Grove), and below Fargo (Harwood). Sampling periods will be chosen from May to September, during which different sampling techniques will be applied to each site according to habitat: main-channel using a Ponar sampler, channel slopes using a core sampler, and sweep net to collect from snags. If possible, drift nets will be deployed during the night time, and retrieved them the next day. Site variables will be measured on each sampling occasion using meters, water samplers, direct observations or visual estimations. Water quality samples will be taken back and analyzed by chemistry laboratory. Macrobenthic invertebrate samples will be preserved in 70% ethanol and separated from river sediments using a surcrose-floatation procedure (Anderson 1959). Organisms will be identified to the lowest practicable level by a stereoscope and with appropriate taxonomic keys. The identification results will be compared and complied with published and known records.

My advisor, Dr Malcolm.Butler will provide field facilities for all field data collection (i.e., equipment storage, sampling process). Lab analysis of samples will be conducted at NDSU.

DELIVERABLES

The macroinvertebrate faunas of large, rivers throughout the world have been poorly studied by comparison to the vast literature on smaller, upland streams. There has been a handful of studies on large rivers around the world, including rivers in Australia (Schulze & Walker, 1997; Sheldon & Walker, 1997, 1998), Austria (Humpesch & Elliott, 1990), France (Cogerino et al., 1995; Bournaud et al., 1996, 1998), North America (Wells & Denmas, 1979), Spain (Muñoz & Prat, 1994), and Russia (Zhadin & Gerd, 1970; Mordukai-Boltovskoi, 1979). Very little research has been done to benthic marcroinvetebrates in the Red River. The anticipated results of this study will comprise my Ph.D. dissertation, and will be presented at conferences, and submitted for publication to peer-reviewed journals. Ultimately, this work will be available for use in developing large scale international monitoring assessment of Red River, and advance our understanding of aquatic ecology to the benefit of biomonitoring and water resources in North Dakota and Minnesota.

PROGRESS TO DATE

Literature reviewing has been partly finished. Solicitations have been made to the North Dakota Department of Health (NDDH). A list of macroinvertebrates taxa collected from the Red River in a recent study is available from the Energy & Environmental Research Center (EERC). We have inquired and confirmed the ability of the North Dakota State University (NDSU) Chemistry Department to aid in analysis of water parameters. We have taken a preliminary survey on the Red River in September 2005. We have attended the International Water Institute (IWI) Red River of the North Assessment Workshop on November 2~3, 2005.

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Effect of flow path processes on the geochemistry and quality of water discharged along the seepage face at Pigeon Point, Sheyenne delta aquifer, Ransom County, North Dakota

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1. Lenarz, William and Philip J. Gerla, 2007, "Effect of Land Cover and Pattern on the Quality of Groundwater Discharged from Springs at The Nature Conservancy's Pigeon Point Preserve, Southeastern North Dakota", Third International Water Conference, International Water Institute, Grand Forks, March 13-15.
2. Gerla, Philip J. and William Lenarz, 2007, "Effect of Land Cover and Pattern on the Quality of Groundwater Discharged from Springs and Seeps at Pigeon Point, Southeastern North Dakota", Technical Report ND07-01, North Dakota Water Resources Research Institute, North Dakota State University, Fargo, North Dakota.

EFFECT OF FLOW PATH PROCESSES ON THE GEOCHEMISTRY AND QUALITY OF WATER DISCHARGED ALONG THE SEEPAGE FACE AT PIGEON POINT, SHEYENNE DELTA AQUIFER, RANSOM COUNTY, NORTH DAKOTA

DESCRIPTION OF THE CRITICAL STATE OR REGIONAL WATER PROBLEM TO BE INVESTIGATED

The proposed focuses on the relationship between land use, infiltration processes, and the quality of water discharged along the expansive seepage face at Pigeon Point, an area of springs and fens in the Sheyenne delta aquifer. Interestingly, the lowest seeps, suggesting longest pathlines, have the least mineralized water (350 uS/cm) while springs and seeps at higher elevation reveal increasing mineralization (500 uS/cm). Results of the work will address the following questions:

• The Sheyenne delta aquifer constitutes an important source of high quality groundwater in southeast North Dakota and may provide water for Fargo in the future. To what extent doesgeomorphology and land cover influence infiltration, recharge, and groundwater quality in the Sheyenne delta aquifer?

• What are the vadose and phreatic processes that result in changes to hydrogeochemistry of water along a flow path?

• Why do shorter groundwater pathlines that discharge at the Pigeon Point seepage face produce more mineralized water? How is the greater mineralization reflected in relative concentrations of cations and anions?

• How might future land use changes influence the quality of groundwater discharged in the Pigeon Point nature preserve? How can other parts of the Sheyenne delta aquifer be managed to best protect groundwater quality?

SCOPE AND OBJECTIVES OF THE PROPOSED RESEARCH

The goal of this research project is to determine changes in groundwater quality along aquifer pathlines that discharge at the Pigeon Point seepage face, Ransom County, North Dakota. The objectives are:

(1) characterize the infiltration characteristics of soils in the recharge/capture zone,
(2) document the changes in the composition of infiltrating water as a function of depth, soil type/geomorphology, and land cover, and
(3) explain the reason for spatial variability of hydrogeochemistry across the site.

The proposed study area covers about six square kilometers within the recharge/capture zone of Pigeon Point, which lies along the south side of the Sheyenne River where it

transects the Sheyenne delta aquifer (Figure). The landscape and surficial geology of this area was developed by glacial deposition during the Wisconsinan glacial period, especially due to the formation, deposition, and eventual draining of glacial Lake Agassiz. During the period of time that glacial Lake Agassiz was at the Herman and Campbell levels, approximately 11,000 to 13,000 years ago, the Sheyenne Delta formed in eastern Ransom County. The delta likely resulted from large load deposition as the Sheyenne River emptied into glacial Lake Agassiz. Well-sorted sand dunes comprise the southern portion of the Pigeon Point site, with fluvial-lacustrine fine sands lying northward, which are eroded and exposed along the Sheyenne River.Till underlies most of the site at a depth ranging from 20 to 30 meters. Within the Pigeon Point area there is an extensive seepage face comprised of springs and fens. Eight major springs at the seepage face coalesce to form four small, perennial streams that flow into the Sheyenne River. Fens, or groundwater-fed wetlands, occur along nearly the entire seepage zone. These wetlands slope northward and host several rare and unusual boreal plant species, which do not occur elsewhere in North Dakota or at any locations farther south than Pigeon Point.

METHODS, PROCEDURES, AND FACILITIES

At the site there are two wells in place that can be used for sampling groundwater. Six shallow
wells and eleven soil-water samplers in three nests across the study area have been installed along a single selected pathline. One of the nests was placed in the dunes toward the southern border of the study area, while the other two nests were placed in the pasture and restored prairie down-gradient near the seepage face and the Sheyenne River. The placement of the three nests allows for synoptic collection of the soil water and groundwater within spring and seep capture zone. Either The Nature Conservancy or the U.S. Forest Service, Sheyenne National Grassland, owns the area encompassed by the study site. There is land under private ownership bordering the study site, but this study can be conducted without accessing these areas.

Within each of the three nests, soil-water samplers were installed at different depths depending on the water table depth and the thickness of the root zone. Because of large infiltration and recharge rates in the dunes, the water table tends to be very shallow (< 1.5 m) in small intra-dune basins. Samplers were placed at the following depths: 0.7, 1.0, 1.5 m. In all cases, samplers were placed beneath the densest root zone to help assure collection of deeply infiltrating water. The ceramic cup of the samplers was packed with 200-mesh silica flour and when water samples are collected, the sampler is evacuated at approximately -0.4 MPa (-60 psi) and sampled 6 to 12 hours later.

Three-foot, 1-¼ inch stainless steel well points with galvanized steel casing were driven about one-two meters below the water table for sampling groundwater. In addition to the samples that are collected from the three nests, each of the two wells already in place supplement the samples collected from each nest. To complete the water sample collection, samples from each of the six largest springs along the seepage face are also collected for analysis.

During the course of the research project, there will be three sampling periods. Each of these sampling periods will take place during a different season to determine if groundwater quality and chemical transport vary across/through the study area. The seasonal samples will also illustrate how dissolved constituents in groundwater change during different times of the year, and how concentrations vary throughout the year. The first round took place in October. The second round of sampling will take place in April to sample water influenced by the spring thaw and winter run-off. The final sampling will take place in mid to late summer when drier conditions are likely, providing a different view of concentrations and oxidation/reduction. This sampling period will ideally take place after significant precipitation in order to study infiltration and chemical transport during a wet pulse following the growing season.

During each sampling period, standard operating procedures for the collection and preservation of groundwater samples for chemical analysis will be followed, as set forth by the North Dakota State Department of Health (field sampling protocol report published in 1995). These methods will be supplemented with the following ASTM standard guides:

D4696-92(2000) Pore-Liquid Sampling from the Vadose Zone
D5903-96(2001) Planning and Preparing for a Groundwater Sampling Event
D6517-00 Field Preservation of Ground-Water Samples
D6564-00 Field Filtration of Ground-Water Samples
D6634-01 Purging and Sampling Devices for Ground-Water Monitoring Wells

While in the field, samples are measured for temperature, pH, conductivity, and dissolved oxygen. After collection, the samples are transported to the Environmental Analytical Research Laboratory (EARL) on the University of North Dakota campus in Grand Forks for further analysis.

The analytical work done in the EARL lab comprises a large portion of the project time and expense. Using the equipment in the lab, and knowledge already obtained in the operation of this analytical equipment, a large expense has been saved by personally analyzing all samples instead of having them sent out for analysis. The three main analytical methods used will are flame atomic absorption spectrometry (FAAS), total organic carbon analysis (TOC), and ion chromatography (IC). The flame atomic absorption spectrometer is used to analyze samples for major cations, including calcium, magnesium, and sodium; the ion chromatograph is used to analyze for these major anions: chloride, sulfate, and nitrate, while the TOC carbon analyzer is used to analyze samples for both total organic carbon and inorganic carbon.

Oxidation-reduction conditions likely control the variability in groundwater composition. The upper parts of the seepage face shows discharge of groundwater with elevated Fe concentration, suggesting reducing conditions. Pathline analysis strongly suggests that recharge of these waters takes place within nearby wetlands and soils with a well-developed, organic-rich A-horizon. Lower springs, which may be more oxidizing, receive water from dunes. Because of disequilibrium and multiple redox couples, redox probes will likely have little value (e.g. Lindberg and Runnells, 1984). Instead, we propose to

track qualitatively redox conditions at the sample site by analyzing directly pumped and filtered groundwater for DO, nitrate, iron, and manganese using a portable spectrophotometer. Results should be sufficient to use pE-pH diagrams to show the spatial variability of redox.

In conjunction with soil water and groundwater sampling and analysis, work to better understand the physical conditions of infiltration and recharge will be completed during the project. At and near the instrumentation sites, matric potential, moisture content, and soil permeability will be estimated using transducer tensiometers, portable time-domain reflectometry surveys, and a disk infiltrometer. Significant differences in hydrology between different land cover, slope, and aspect are anticipated. These data will be used to create basic numerical models of infiltration and recharge using the USDA Salinity Laboratory's HYDRUS-2D code. Equipment and software for this work is available from UND and The Nature Conservancy.

Interpretation:

When the water samples from each of the sampling periods have been analyzed in the laboratory, the results will be interpreted in relationship to the infiltration, recharge, and groundwater flow processes. The analytical results will reveal the chemical mass and redox changes as water flow through the vadose zone and along the flow path to the springs. By examining the results from each of the three sampling periods, we will be able to determine the spatial and temporal control on groundwater ionic and redoxchemical signature. Through these interpretations, we will better understand the hydrogeochemical processes that lead to the water quality in the springs and the aquifer as a whole.

ANTICIPATED RESULTS AND BENEFITS FROM THE PROPOSED STUDY

Through the analysis of the water samples taken from across the site at the different times of the year, it will be possible to develop a conceptual model of the hydrogeochemistry that leads to the groundwater quality in the springs and seepage face, and for the aquifer as a whole. The benefit of this will be a better understanding of physical and geochemical processes operative in this portion of the Sheyenne delta aquifer, and how these may influence the water quality within both the Sheyenne River and the Sheyenne delta aquifer. In addition, the apparent reversed pattern of elevation and dissolved solids found in the seepage face will be explained. This type of situation may benefit understanding of the environment surrounding seepage faces and fen-producing environments. The study can benefit downstream cities, such as Fargo, as they continue to search for options to enhance the municipal supply of high quality water. Understanding the relationship of land cover and land use to hydrogeochemistry will be crucial in balancing the fresh water needs of society, agriculture, and the natural habitat in the unique Sheyenne delta ecosystem. In addition to these results, the work will lead to the completion of a master’s degree at the University of North Dakota.

PROGRESS TO DATE

This project officially started during the spring 2005 semester. Research began with a literature review of relevant articles to the study environment, types of sampling procedures and analyses, and chemical redox processes. Throughout the spring 2005 semester, the approach to the field work was continually refined until the current layout was selected. It was finally determined that three instrument nests would be necessary to successfully sample the water moving along a pathline through the Sheyenne delta aquifer. Supplies were purchased and collected during the months of May, June, and July. During a period of three weeks in August all of the sampling and monitoring instruments were installed in compliance with the research outline. Continued site development was continued during the month of September to ensure that a successful sample collection would take place in October. During the month of September there was also sediment collection at each of the three instrument nests. Using a hand auger, sediment samples were collected from the ground surface to the top of the water table. The sediment samples that were collected will be used to determine overall grain size changes across the study site. This information will be useful when studying the infiltration rate of surface water to the aquifer across the study site. On October thirteenth the first sampling took place. Over the two weeks following the sample collection, the water analyses took place in the EARL lab on the UND campus. The water analyses of the first water samples have been completed.

An initial observation of results from the water analyses clearly illustrates a general trend of reducing environments moving from south to north towards the Sheyenne River. Beginning at the instrument site situated in the dunes on the southern most portion of the study site and moving north along the pathline through sites two and three, the following observations can be made:

- The level of dissolved oxygen decreases from site one to site three.
- Manganese levels increase steadily from site one to site three.
- Iron is virtually undetectable at sites one and two, but appears at site three.
- Chloride and calcium both increase steadily from site one to site three.
- Potassium and sodium rise only slightly from site one to site three.
- The level of total carbon and inorganic carbon nearly double from site one to site three
- The level of total carbon and inorganic carbon is significantly different between the spring samples collected at higher elevations (Springs 1-4) compared to the spring samples collected at a lower elevation (Spring 5-6).

Based on the observations made above, it is clear that there is a more reduced environment closer to the springs and the Sheyenne River. This initial sample collected and interpretation supports the idea that the water flowing from the springs at the higher elevation is indeed coming from a more reduced environment. In addition, the more reduced water samples collected at the higher elevation springs lends support to the hypothesis that the recharge area for the higher springs is closer to the springs themselves, while the recharge area for the lower springs (more oxidized) is likely coming from further away in the dunes. Additional sample collection in April and July

will provide additional results to further support the results from the first sample collection.

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Collaborative Research on In Situ Denitrification and Glyphosate Transformation in Ground Water: NAWQA Eastern Iowa Basins Study Unit

Basic Information

Title:	Collaborative Research on In Situ Denitrification and Glyphosate Transformation in Ground Water: NAWQA Eastern Iowa Basins Study Unit
Project Number:	2006ND126G
Start Date:	8/1/2006
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Congressional District:	1
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Focus Category:	Hydrogeochemistry, Agriculture, Nitrate Contamination
Descriptors:	None
Principal Investigators:	Scott F Korom, Paul D. Capel

Publication

COLLABORATIVE RESEARCH ON IN SITU DENITRIFICATION AND GLYPHOSATE TRANSFORMATION IN GROUND WATER: NAWQA EASTERN IOWA BASINS STUDY UNIT

RESEARCH PROBLEM

Contamination of ground water by nitrate and pesticides is widespread in some areas of the country and can threaten drinking water supplies. It is well known that the most important removal mechanism of nitrate and most pesticides from ground water is biodegradation, but the in situ transformation rates are largely unknown. In this study, two 180-L stainless steel chambers forming in situ mesocosms (ISMs) of aquifer sediments will be installed below the water table at the NAWQA agricultural chemicals study sites in the glaciated part of Iowa. This work will extend the use of this technique to examine denitrification in an area characterized by high dissolved iron concentrations and to measure the transformation rate of the extensively-used herbicide, glyphosate.

OBJECTIVES

The objectives for the research are:

1. Measure the denitrification and glyphosate transformation rates in the two ISMs.
2. Determine whether the denitrification is better fit by zero-order or first-order reaction rates.
3. Determine what donors are contributing electrons for the denitrification and their relative amounts.
4. Incorporate the results of the two ISMs into the existing databank of nine other ISM sites in glacial outwash aquifers in the Upper Midwest.
5. Update the available data of the apparent isotopic enrichment factor for 15N in nitrate versus denitrification rate among of ISM sites.
6. Update the nitrate vulnerability index and extrapolate the findings to similar, unmonitored agricultural and environmental settings.

Aquifer sediment samples will be collected from the Iowa site and analyzed for grain-size distributions, mineralogy, and major e- donors (organic carbon, sulfide, and ferrous iron) to determine optimum locations for installation of the ISMs, provide insights on the types and heterogeneity of e- donors at the site, and provide the e- donor supply data at the Iowa site that can be compared to previous ISM studies in the Upper Midwest. After the ISM chambers are installed, they will be purged and then amended with nitrate and bromide, which serves as a tracer for nitrate. Any loss of nitrate beyond that explained by dilution of the bromide tracer is attributed to denitrification. The ISMs will be sampled over time (months) and the decreases in nitrate concentrations will be used to calculate rates of denitrification. Modeling of the evolution of the geochemistry in the ISMs will provide insights into what donors contributed electrons to the denitrification and their relative amounts. The field experiment will be repeated a second time; however, in

addition to nitrate and bromide, glyphosate will be added. Denitrification information from the second test will provide insights into the variability of the results from the first test. More importantly, the attenuation and transformation of glyphosate (with the dominate metabolite, AMPA) will be studied in both the presence and absence of nitrate to determine the fate of glyphosate in oxidizing and reducing conditions. The results of this study will provide site-specific transformation rates for nitrate and glyphosate and extend the aquifer nitrate vulnerability index that was developed in earlier studies. This information is vital for the development of tools and quantitative methods to characterize the transport and fate of agricultural chemicals in the Eastern Iowa Basins Study Unit, the Upper Midwest, and beyond.

PROGRESS

The starting date for the grant was August 1, 2006. Since that time, two trips have been taken to the Iowa field site to collect core samples from the site. Using a Geoprobe and working with USGS personnel from the Iowa City office, we collected 51 aquifer samples from 10 borings each up to 24 ft. deep. Samples or subsamples from all 51 of the cores went to the USGS in Iowa City and 43 cores subsamples went to The University of North Dakota (UND). Those going to UND are currently being analyzed for grain-size distributions, organic carbon contents, moist colors, and mineralogy, by x-ray diffraction (XRD). So far, grain sizes, colors, and organic carbon analyses have been done on about 30% of the samples and XRD has been done on about 10% of the samples. No more sediment samples will need to be collected. Based on core logs, preliminary sites have been chosen as possible locations for the ISMs.

In addition, I have studied several papers related to the project objectives and I have requested estimates from several companies for stainless steel to build the ISMs. The following stainless steel components have been ordered or obtained:

- ¾-in. and 2-in. riser pipes
- ¾-in. and 2-in. couplers
- ¾-in. nipples
- 3/8-in. top plates
- 3 sizes of screen
- 2 x 5-ft long, 16-in. diameter schedule 20 pipe sections

One Masters student working towards his M.S. in Environmental Engineering started on the project on January 1, 2007, as a half-time research assistant. . He recently went to the Iowa site with the PI to collect sediment cores and he continues to work on the physical, chemical, and mineralogical analysis of the cores.

The project is on schedule based on the timeline given in the project proposal.

Top-down and Bottom-up Effects on the Abundance of Periphyton in Shallow Lakes

Basic Information

Title:	Top-down and Bottom-up Effects on the Abundance of Periphyton in Shallow Lakes
Project Number:	2006ND136B
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Focus Category:	Surface Water, Water Quality, Nutrients
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Principal Investigators:	Malcolm George Butler

Publication

1. Konsti, M. K., K.D. Zimmer, B.R. Herwig , M.A. Hanson, J.A. Younk, and M.G. Butler, 2007, "Effects of Macrophytes, Nutrients, and Fish on Periphyton Abundance in Shallow Lakes", Third International Water Conference, International Water Institute, Grand Forks, March 13-15.

Top-down and Bottom-up Effects on the Abundance of Periphyton in Shallow Lakes

Regional Water Problem

Shallow lakes are the most common lake type in North America, yet our ecological understanding of these systems is poor relative to deeper "sport fish" lakes. Throughout the Prairie Pothole Region (PPR), landscape alterations have directly and indirectly altered the character and quality of regional waterbodies. The ecological value of these shallow aquatic ecosystems decreases as conditions favor a turbid, phytoplankton-dominated condition with low abundance and diversity of invertebrates and submerged aquatic plants (Scheffer 1998). Waterbodies in the turbid state are considerably less valuable to migrating waterfowl than clearwater, plant-dominated systems. Much evidence points to nutrients as a cause of high periphyton biomass, just as utrient loading enhances planktonic algae. Fish presence in a shallow basin ay also favor increased algae, both planktonic and periphytic. Periphyton is detrimental to acrophytes, and ultimately may contribute to a basin shifting from the clear-water state to the ess valuable turbid state. We need to better understand what controls periphyton, and the role it ay play in shifts from the clear-water state to the turbid-water state in shallow lakes within the PPR.

Literature Review

Shallow lakes can exist in two alternative stable states: a clear-water state dominated by macrophytes and a turbid-water state dominated by algae. Macrophytes play an important role in maintaining water clarity by tying up available nutrients and reducing the amount of sediment resuspension. They also protect invertebrates from predation, host periphyton communities, serve as spawning habitat and shelter for small fish, and provide habitat and food for waterfowl. Many previous studies have implicated increased phytoplankton in concurrent loss of macrophytes, favoring the view that phytoplankton induce shifts to the turbid state. Recently, more attention has been given to the role periphyton, specifically epiphyton, or algae growing attached to dead or living aquatic plants. The littoral zone of shallow lakes is typically extensive, and plants can often be found growing on up to 100 percent of the lake bottom.

The amount of periphyton growing on macrophytes is directly affected by grazing invertebrates (Allen 1971, Brönmark 1989, Hann 1991, Rosemond et al. 1993, McCollum et al. 1998, Hann et al. 2001), availability of nutrients (Cattaneo and Kalff 1980, Fairchild et al. 1985) and light (Wetzel 1983, Goldsborough and Brown 1991), as well as indirectly by the presence of fish (Brönmark 1989, Walker et al. 1998, Marklund et al. 2002) through a cascading effect within the food web. Therefore, periphyton biomass may be controlled from the “top down” by a fish-grazer interaction, and/or from the “bottom-up” by nutrient and light availability. Each lake is unique, differing in the presence, types, and abundance of fish, grazing invertebrates, periphyton, and macrophytes, the available nutrients, and the clarity of the water. Few researchers have observed the complete fish-invertebrate-periphyton-macrophyte trophic cascade

(Jones and Sayer 2003), as most studies have focused on just one of the top-down or the bottomup interactions.

Predatory fish directly reduce the abundance of grazing invertebrates (Walker et al. 1998, Karjalainen et al. 1999, Marklund et al. 2002, Ruetz et al. 2004) and indirectly affect algal biomass through changes in the abundance and behavior of invertebrate grazers (Power 1990, McIntosh and Townsend 1996). When fish are present and/or abundant, periphyton biomass will be higher, and when fish are absent periphyton will be suppressed by higher invertebrate grazing pressure.

Grazing invertebrates have been reported to be negatively correlated with periphyton biomass (Hillebrand 2002, Jones et al. 2002). Controlled experiments have utilized enclosures and exclosures to manipulate the presence or absence of invertebrates interacting with the plants and algae (Ruetz et al. 2004). Cattaneo (1983) conducted a similar experiment and found that periphyton biomass decreased significantly with the presence of oligochaetes and chironomids. Gastropods can also greatly suppress periphyton abundance, particularly the larger (>200μm^3), loosely attached cells (Martin et al. 1992).

Nutrient availability in a wetland can have important influences on the biomass of periphyton. Some studies have reported that an increase in available nutrients correlates with an increase in periphyton biomass (Cattaneo and Kalff 1980, Fairchild et al. 1985, Carrick and Lowe 1988), while others have found no such correlation (Jones et al. 2002, Jones and Sayer 2003). An increase in nutrients also tends to increase the turbidity of a shallow lake (Scheffer et al. 1993). This increase in turbidity causes a decrease in light penetration. When light is limited, periphyton biomass is limited as well (Goldsborough and Brown 1991). In shallow, clear lakes, where light can penetrate deeply into the water column, periphyton production can make a substantial contribution to total production by the primary trophic level (Wetzel 1983).

This study is designed to sample the abundance and composition of species involved in the fish-invertebrate-periphyton-macrophyte trophic cascade, along with other variables (nutrients, turbidity, and phytoplankton) to identify factors influencing periphyton biomass in shallow prairie lakes. Most controlled experiments have involved enclosures and exclosures, placed in a single body of water, to manipulate the presence or absence of fish and/or invertebrates interacting with the plants and algae. These experimental studies have also used from one to only a few species per trophic level (i.e. fish, invertebrates, etc.) and very few studies have evaluated more than two trophic levels. Observational studies typically involve around ten lakes, which limits the amount of variation within treatments as well as the number of study lakes in either the clear- or turbid-water state. Grazing and nutrient effects on periphyton biomass in streams have been widely studied, but little is known about periphyton levels in shallow lakes, or the influence various trophic levels can have on macrophyte abundance and water clarity. We hypothesize that grazing invertebrates will have stronger impacts on periphyton abundance, relative to available nutrients, but that nutrients will still play a role in determining periphyton abundance. We are sampling 75 lakes in two different study areas in Minnesota to test this littoral cascade hypothesis.

Scope and objectives

This study will provide better understanding of interactions contolling to periphyton within shallow lake ecosystems, by accomplishing these objectives:

1. Sample the epiphyton, invertebrate, fish, macrophyte, phytoplankton, and nutrient/light interactions within 75 shallow lakes in Minnesota.
2. Sample shallow lakes of varying water clarity (clear- vs turbid-water state) and nutrient input (LO & HI impact landscapes).
3. Test various combinations of explanatory variables in models to determine if periphyton is controlled from the top-down, from the bottom-up, or by a combination of both.
4. Determine which variables best predict periphyton biomass (nutrients, light, fish, invertebrates).
5. Determine how periphyton biomass and its predictor variables differ between study landscapes, among lakes within each landscape, and with depth in each lake.

Methods

Two areas in western Minnesota have been chosen for study. The northern area is located in eastern Polk County, while the southern area spans the region from southern Grant County to the northern edge of Stevens County. Each study area comprises approximately 560 km^2. Study sites were selected by randomly choosing from groups of candidate lakes conforming to criteria identified as part of a broader study. While compiling candidate lakes, surface area, depth, distance to roads, adjacent upland cover and other criteria were taken into account. A total of 75 wetlands were chosen using this method, roughly half within the northern study area and remaining sites in the southern study area. All sites selected are semipermanent or permanent (type IV or V) with regard to duration of flooding (Stewart and Kantrud 1971). Periphyton, invertebrate, macrophyte, and fish abundance, plus nutrient and light availability were sampled throughout the 2005 and 2006 summers.

Periphyton biomass (Chl *a*) is determined by deploying artificial substrates for five weeks. Sampling devices were set out in mid-June and collected in late-July each year (average time in water is 5 weeks). These devices consisted of a polyester braided rope (¼" thick, 1½ m long) with a brick anchor attached to one end and a float on the other, with three vinyl microscope slides attached using zip-ties at individual depths along the rope (10, 50, and 90cm from surface). The total height of the sampling device was approximately 1.5m. Using artificial substrata instead of collecting living plants permits a uniform surface type, area, and orientation, and therefore less variation in the sampling of periphyton biomass. Some species of plants may be able to alter the periphyton community, so by using an artificial substrate the species of macrophytes present will not be a factor. Cattaneo and Kalff (1979) concluded that periphyton production did not differ between natural and artificial plants. Substrates were placed vertically in the water column at a depth of ~1.5 m. Since periphyton and grazer biomass varies with depth (James et al. 2000), deploying substrates at specific depths controlled for this influence. Three devices were deployed in each lake, near the same locations

where invertebrates were sampled. Upon collection, each sample was removed from the lake with care to limit disturbance to the periphyton, placed in a container with tap water, and stored in a dark cooler until be processed in the lab within 12 hours.

Periphyton biomass will be estimated from chlorophyll *a* analyses (APHA 1989). Periphyton is scraped off slides into a dish with a razor blade, and a sub-sample was filtered onto a glass fiber filter (Whatman GF/C). Filters were frozen until processing in the lab. Each sample is allowed to steep for 24 hours in a separate tube with 90% acetone. Fluorometry is used for determining chlorophyll *a*, since it is more sensitive than spectrophotometry and thus requires fewer samples. Fluorescence is measured at 430 nm and 663 nm, and calibration factors are derived to convert fluorometric readings to concentrations of chlorophyll *a*.

Zooplankton were sampled twice per year, once in early-June, and again in Late-July, by collecting two replicate vertical column samples (Swanson 1978a) at six open water locations in each wetland. Estimates will be made of density, biomass, and taxon richness of zooplankters. Relative abundance of free-swimming invertebrates was obtained with submerged activity traps (ATs) (Swanson 1978b, Murkin et al. 1983, Ross and Murkin 1989). Six ATs were deployed at the interface of open water and emergent macrophytes, and left in each wetland for 24 hours. Abundance (counts of dominant forms) and taxon richness of macroinvertebrates will be determined, paying special attention to identifying taxa considered to be grazers/scrappers.

Density and trends in abundance of submerged macrophytes were assessed using a modified technique of Jessen and Lound (1962) and Deppe and Lathrop (1992). In each wetland, submersed macrophytes were sampled at 20 stations in early August each year. Four transects were established perpendicular to the longest axis of the lake, with 5 stations established along each transect. Therefore, sampling stations were apportioned among 3 depth strata (open water, transition, nearshore). Two samples were collected from each station using a weighted plant rake, with frequency of occurrence calculated for each plant species and all taxa combined. The first sample was weighed to determine the relative abundance (mass) of macrophytes overall. Metaphyton (e.g. *Cladophora* spp.) and macroalgae (e.g. *Chara* spp.) were assessed along with vascular plant species during these surveys.

Three surface water samples were taken along the middle of each wetland during early- June, and again in late-July each year. These samples were stored on ice and transported immediately to the Minnesota Department of Agriculture chemistry lab (St. Paul, MN) for analysis of chlorophyll *a*, total and Kjeldahl nitrogen, and total phosphorus. Turbidity and specific conductance were measured in the field with a portable nephelometer and conductivity meter, respectively. Phytoplankton biomass were estimated from chlorophyll *a* (Strickland and Parsons 1972). Collection of samples for chlorophyll *a* simultaneously with measurement of turbidity allows assessment of the contribution of phytoplankton to turbidity, and ultimately to light attenuation. A secchi disk was also used to determine water clarity, by sampling the middle of each lake once in early-June, and again in late-July each year.

All fish sampling was conducted during late-July each year. Three mini-fyke nets (9.5 mm bar mesh with 4 hoops, 2 throats, 7.62 mm lead, and a 0.69 X 0.99 rectangular frame opening into the trap) were set overnight in each lake. One experimental gill net (76.2 m multifilament net with 19, 25, 32, 38, and 51-mm bar meshes) was set along the

deepest depth contour available in wetlands less than 2-m deep or parallel to shore along a 2-m contour in wetlands with sufficient depth. This protocol has been shown to be effective in sampling fish assemblages in small lakes from other regions (Tonn and Magnuson 1982, Rahel 1984, Jackson and Harvey 1989, Robinson and Tonn 1989). This should enable us to capture both small- and large-bodied fish, and species from all the major trophic guilds (e.g. planktivores, benthivores, and piscivores) potentially present in the study wetlands. All species of fish sampled were counted and returned alive to the wetlands if possible. Voucher specimens were retained for laboratory identification when field identification could not be made.

Multivariate techniques will be used to interpret the interactions between biotic and abiotic variables. Specifically, two types of gradient analysis, principle components analysis (PCA) or correspondence analysis (CA) will be used (ter Braak 1995). These techniques are able to identify and summarize major patterns in the data, and by considering all species and sites at once, they permit detection of patterns that univariate techniques are unable to identify. Such patterns include which species vary the most among sites, which species have positive/negative associations, which species are most abundant in specific sites, as well as which sites are most similar/dissimilar in terms of species abundance, and how much individual sites differ in community composition. Abundance and composition of fish, invertebrates, and macrophytes may be correlated with the various variables, such as nutrients, light, and periphyton biomass.

Finally an information-theoretic (IT) approach, a type of model selection, will be used to determine which variables are related to periphyton biomass. Models will be selected *a priori* to ensure inclusion of appropriate variables, and comprised of various combinations of the variables. This approach will then be used to select the model best supported by the data, and dismiss others that are less plausible. Model selection considers both problems associated with overfitting the data (using too many parameters) and underfitting the data (using an insufficient number of parameters).

Deliverables

This study will improve understanding of the fish-invertebrate-periphyton macrophyte cascade, including direct impacts of nutrients and light on periphyton biomass, and the indirect impact of periphyton on macrophyte abundance. An understanding of which variables most influence periphyton abundance can help managers maintain these shallow lakes in the Clearwater state. Manuscripts will be prepared and submitted describing these findings.

Progress to date

Field data has already been collected for the full two years of the study. We sampled fish, macrophytes, nutrients, phytoplankton, periphyton, and invertebrates. Many invertebrate samples still need to be processed, but other lab work is well underway. I am currently in the early stages of analyzing data and writing manuscripts.

Financial Information

The research described in this proposal is a collaborative effort involving the University of St. Thomas (St. Paul, MN), North Dakota State University (Fargo, ND), and research scientists from both the Fisheries and Wildlife sections of the Minnesota Department of Natural Resources (MN DNR) (Bemidji, MN). The MN DNR has supported this project by covering many costs associated with travel, equipment purchase, and data collection. Funding from the NDWRRI will be used for graduate stipend, student assistance with invertebrate samples, lab supplies, and travel to meet with cooperators in the MN DNR about data analysis and publications.

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Rapid and Sensitive Determination of Bacteria in Water Using Nanoparticles

Basic Information

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End Date:	2/28/2007
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Descriptors:	None
Principal Investigators:	Julia Xiaojun Zhao

Publication

1. Jin, Y., X. Zhao, M. Wu, 2006, "Determination of bacteria using luminescent nanoparticles", Pittcon 2006, Orlando, FL.
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3. Jin, Y., Jenna Parisien, M. Wu, and Julia Zhao. 2007, "Sensitive Determination of Bacterial Cells Using Fluorescent Nanoparticles". Third International Water Conference, International Water Institute, Grand Forks, March 13-15.

RAPID AND SENSITIVE DETERMINATION OF BACTERIA IN WATER USING NANOPARTICLES

SIGNIFICANCE OF THE PROPOSED RESEARCH TO THE STATE OF NORTH DAKOTA

Sensitive and Rapid Detection of Bacteria in Drinking Water

Bacteria can grow or re-grow in distribution systems of drinking water. In fact, potable water is a major source of some bacteria colonization,[7,8] for example *L. pneumophila* and *E coli.* etc. The *L. pneumophila* in potable water can replicate rapidly and increase in virulence.[9] The British Communicable Disease Surveillance Center reported that 19 of 20 hospital outbreaks of Legionnaires' Disease in the United Kingdom from 1980 to 1992 were attributed to the *Legionella*-contaminated potable water.[10] Given the low infectious dose of pathogenic bacteria, the presence of even a single bacterium in potable water may pose a serious health risk. Therefore, sensitive and rapid detection of bacteria in water is critical.

However, the current definitive method for the detection of bacteria is the culture of the organism, which requires about 24 hours for bacterial growth.[11] The method is too slow to meet the public need. The PCR-based method can detect bacteria within six hours; but the method requires pre-enrichment of the target bacteria. The proposed method will be able to specifically identify target pathogenic bacteria at a single bacterium level within 30 minutes in water samples. The method will be accurate, rapid and sensitive to meet the public need.

Application of Nanotechnology in North Dakota

Nanotechnology has been a rapidly developing area in recent years and is becoming a hot topic worldwide. However, it is an area that is underdeveloped in North Dakota. In the University of North Dakota, Dr. Zhao is the first faculty member working in the nanotechnology area. Dr. Zhao's group is the only nanotechnology research group at UND. There is a great need to develop emerging nanotechnology in North Dakota to advance the economic and educational development of the state. Four students in Dr. Zhao's group will participate in the proposed project: a postdoctoral research associate, a Ph.D. student, an American Indian undergraduate student, and a high school student. The proposal will undoubtedly benefit the development of North Dakota, both in terms of economy and education.

BACKGROUND OF THE APPLICANT'S RESEARCH GROUP

The proposed work will build upon Dr. Julia Zhao's previous successes in synthesizing fluorescent nanoparticles and identifying bacteria. In these fields, Dr. Zhao holds three patents, has authored three book chapters, 25 conference presentations and 41 journal articles. Her previous work on bacteria determination using nanotechnology has been published in ***Proc. Natl. Acad. Sci. USA (PNAS).***[12] This paper was among the top 100 articles accessed in 2004 in ***PNAS***. This work was also featured in a ***Nature*** research highlight section on October 2004.[12] So far, Dr. Zhao has developed several methods for the synthesis of different types of silica nanoparticles.[13-

[19] in 2005, Dr. Zhao received four research grants for development of functionalized silica nanoparticles. One of these grants, the Society of Analytical Chemists of Pittsburgh Start Award, is highly competitive and is only offered to **one national awardee each year**. Currently, Dr. Zhao's group has four Ph.D. students, one postdoctoral research associate, two undergraduate students, and one high school student working on five research projects in bionanotechnology.

The co-advisor, Dr. Min Wu in the Department of Biochemistry and Molecular Biology in the School of Medicine and Health Sciences at UND, has abundant experience in the field of bacteriology. His earlier significant work on genetically engineered macrophages as cell therapy to increase immune function against bacteria was published in ***Proc. Natl. Acad. Sci. USA*** [20] and received media coverage including ***Nat. Rev. Immun.***[20] He has also worked on cell signaling and host-pathogen interaction with bacterial infection (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) for many years.[21-23]. Dr. Wu has also worked on mechanisms of DNA damage/repair and cell death in lung cells, indicated by his significant work published in high impact journals such as ***Cell Death and Differentiation, Breast Cancer Research*** etc.[24,25] Dr. Wu's recent work on new chemical conjugates containing omega-3 fatty acid for cancer therapy has attracted a broad media attention including BBC and Fox59 Channel (June 2005).

Dr. Zhao and Dr. Wu have have successfully co-advised the applicant on a project of the study of toxicity of the luminescent nanomaterials to living systems; [2,5,6] In this project, Dr. Zhao will advise the applicant on the development of nanoparticles and the techniques of conjugation of the antibodies to the nanoparticles. Dr. Wu will advise the applicant on bacteria culture and determination. So far, they have obtained some promising results.

PROJECT DESCRIPTION AND OBJECTIVES

The objective of this proposal is to develop a rapid and ultrasensitive method for the specific identification and quantitative determination of pathogenic bacteria in water. The major feature of the proposed method is the employment of fluorescent nanoparticles as target bacteria identifiers that could emit strong fluorescent signals. The method will consist of three major steps. First, the nanoparticles will react with a specific antibody to form a conjugate of nanoparticle-antibody (NP-Ab). Second, based on the antibody-antigen reaction, the NP-Ab conjugates will identify target bacteria cells from a sample by attaching the NP-Ab to the bacteria surface antigen. Third, target bacteria will be qualitatively and quantitatively determined by measuring the fluorescence intensity and wavelength (Figure 1).

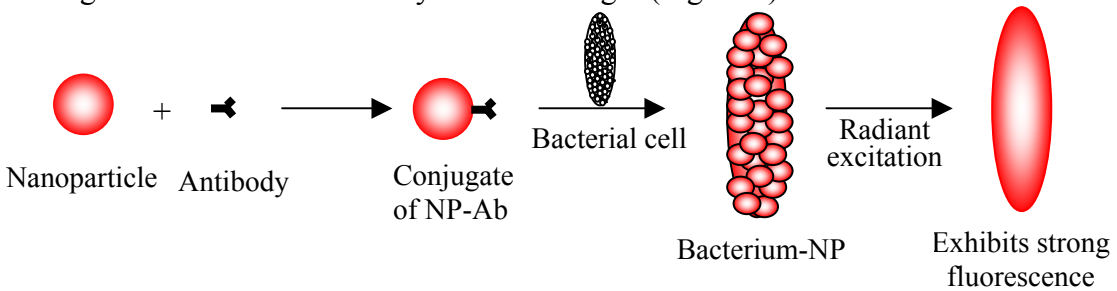

Figure 1 Schematic diagram of determination of bacteria using fluorescent nanoparticles

The method will have three major advantages.

(A) High sensitivity. The nanoparticles will be synthesized using organic dye molecules. Thousands of dye molecules will be encapsulated within a single nanoparticle. Traditional dye labeling method for the determination of bacteria only links one dye molecule to an antibody, which reacts with a single antigen. The proposed method links one nanoparticle, rather than one dye molecule, to each antibody. The advantage is that a single nanoparticle contains thousands of dye molecules, resulting in a highly amplified fluorescent signal. Furthermore, because a bacterial surface contains a number of antigens, a single bacteria cell can be linked with multiple highly fluorescent nanoparticles, making the determination of single bacteria possible.

(B) High specificity and accuracy. The high specificity and accuracy will come from two factors. (1) The identification of the target bacteria will be based on the antibody-antigen reaction. The selected antibody will only specifically recognize a target bacterial antigen. (2) Due to the strong fluorescent signals provided by nanoparticles, the signal difference between target and non-target cells is significant even in the presence of only a single target bacterium. Thus, the false positive reading will be reduced dramatically.

(C) Rapid determination. The size of the nanoparticles is adjustable in the range of 10 nm to 60 nm in diameter. The small size makes nanoparticles highly mobile, which enables them to easily reach target bacteria in a matrix. According to our previous study, the assay will take less than 30 minutes to complete the sample preparation and the determination of bacteria. Compared to the traditional bacteria detection method, plate counting, which takes about 24 hours, the proposed assay will significantly reduce the bacteria determination time.

This proposal will focus on following two specific goals.

Goal 1: Development of Quantum Dot-like Highly Fluorescent Nanoparticles

Two quantum dot-like fluorescent nanoparticles have been developed in Dr. Zhao's group (see preliminary results below). In this proposal, more such nanoparticles will be developed. The nanoparticles will emit fluorescent signals at different wavelengths when a single excitation is used. In this aspect, the nanoparticles will have a similar property as quantum dots. On the other hand, the nanoparticles will contain a large number of dye molecules. Therefore, the fluorescence intensities will be much stronger than those of quantum dots. The principle of the synthesis of the nanoparticles will be based on the fluorescence resonance energy transfer.

Goal 2: Specific, Rapid and Sensitive Identification of Target Bacteria

The proposal will focus on the method development. Targets can be any interested bacteria. Since the specific identification of target bacteria is based on antibody-antigen reaction, as long as a specific antibody to target bacteria is available, the bacteria would be identified. Primarily, *Legionella pneumophila*, *E coli.,* and *Klepsiella pneumoniae* will be our target bacteria. The reason of selecting *Legionella pneumophila* (*L. pneumophila*) as one of the targets is that *L. pneumophila* can grow easily in potable water. Meanwhile, it is among the top three microbial causes of community-acquired pneumonia. The research has demonstrated that *L. pneumophila* causes more severe disease than most common bacterial pathogens associated with community-acquired pneumonia. However, accurate diagnosis of the disease has been a significant challenge since sensitive determination of *L. pneumophila* is difficult. The principle discovered in this study could be extended to the rapid and sensitive determination of various bacteria.

RESEARCH PLAN, EXPECTED RESULTS AND FACILITIES

Development of Quantum Dot-like Fluorescent Nanoparticles

Quantum dot (QD) is a novel nanomaterial that has attracted researchers' great interest due to its unique advantage, which is simultaneously giving different emission wavelengths based on varying sizes when a single excitation source is used.[26] However, quantum dots are restricted by their low quantum yield that results in limited signals when they are used as luminescent labeling materials. Meanwhile, most of QDs are toxic to living systems which limits their applications in biological and medical fields. The designed nanoparticles will possess the advantage of quantum dots and overcome their disadvantages. Due to very high quantum yields of the organic dye molecules used, the nanoparticles will emit much stronger fluorescence signals than QDs. Meanwhile, the silica based fluorescent nanoparticles are not toxic to living systems according to current research results.[2] This nanoparticle is expected to be of great interest as a novel labeling nanomaterial for simultaneous analysis of multiple biomolecules, especially for labeling cells.

Principle of the method: The key idea of this design is to use energy transfer of fluorescence molecules to produce various dye-doped nanoparticles with different colors using a single excitation source. For instance, as shown in Figure 2, the emission wavelength of fluorescence molecule A is near the absorbance wavelength of fluorescence molecule B. As molecule A's excitation light is applied to a solution containing molecule A only, molecule A will emit at 514 nm. Subsequently, molecule B is added to the molecule A solution and thus form a mixture of A and B (with a proper ratio of A to B), the emission energy of molecule A is absorbed by molecule B. So, the emission of molecule B is observed and molecule A is quenched.

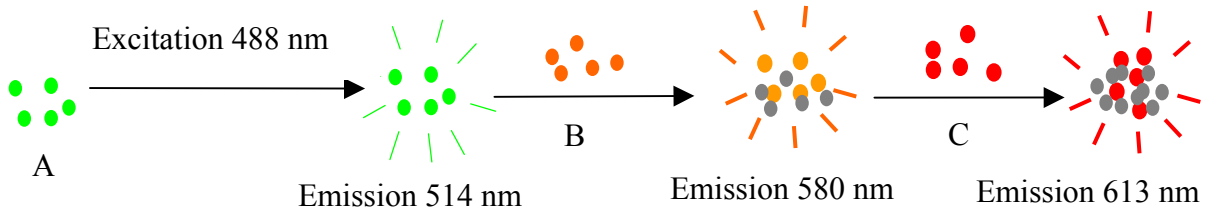

Figure 2 Multiple fluorescence signals using a single excitation source based on fluorescence resonance energy transfer

Furthermore, molecule C is added to the mixture of A and B, the emission energy of molecule B will be absorbed by molecule C. Then, the mixture will emit C's spectrum and A and B are all quenched. Therefore, different mixtures will simultaneously emit different colors using a single excitation source. By doping a mixture of dyes into a silica matrix, quantum dots-like nanoparticles will be produced.

Synthesis approach: The reverse microemulsion method will be used for synthesis of dye-doped silica nanoparticles. Silica matrix can be formed by polymerizing tetraethylorthosilicate in the microemulsion. Several dye molecules will be chosen, such as fluorescein, tetramethylrhodamine, and Alexa Fluor dyes. The desgined nanoparticles are expected to emit strong fluorescent signals at different wavelengths.

SPECIFIC AND RAPID IDENTIFICATION OF TARGET BACTERIA

Conjugation of the antibody onto nanoparticles. The research plan will be described using *L. pneumophila.* The other target bacteria will be determined using the same method. Currently**,** more than 14 serogroups of *L. pneumophila* have been identified. Among them, serogroup 1 accounts for more than 80 percent of the reported cases of legionellosis caused by *L. pneumophila*. One phenotypic difference between avirulent and virulent *L. pneumophila* is the presence of flagella; isogeneic avirulent strains obtained by passage lose their flagella. A surface antigen of *L. pneumophila* serogroup 1 that is recognized by one particular monoclonal antibody is associated with virulence. The monoclonal antibody is commercially available. The antibody will be immobilized onto the nanoparticle surface. The conjugated conditions will be optimized, including ratio of the nanoparticle to antibody, reaction temperature, reaction time, and pH.

Specific identification of *L. pneumophila* from a mixture of bacteria. The NP-Ab conjugates will be used to identify target bacteria from a mixture of bacteria. Based on a specific antibody-antigen reaction, the conjugates will only react with the antigen of the serogroup 1 of *L. pneumophila*. Thus, the target bacteria will be labeled with certain colored nanoparticles. A slightly physical adsorption of NP-Ab to the non-target cells may occur. The effective washing using PBS buffer will minimize this problem.

Quantitative determination of target bacteria. To accurately determine the amount of target bacteria, three types of instruments to measure fluorescence will be used. The first one is a spectrofluorometer to determine the fluorescence intensity of bacterial cells in solution. The fluorescence intensity will be proportional to the amount of target bacteria in the solution. The fluorescence intensities of unknown samples will be measured, and consequently, the amounts of target bacteria will be determined based on the calibration curve. The second instrument is a plate reader fluorometer. The instrument can perform high throughput determination of multiple samples. Currently, 384-well plate and 96-well plate can be used in our plate reader fluorometer. Thus, up to 384 samples can be analyzed simultaneously. The third type of instrument will be fluorescence microscopes. Each bacterial cell will show a fluorescence spot on the image. By counting fluorescence spots on the image, the number of the target bacteria can be obtained.

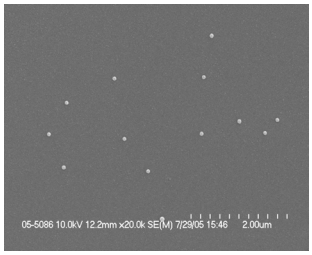

Figure 3 SEM image of the nanoparticles

Facilities. Olympus Fluorescence Microscope, JY HOBRIBA Fluorolog-3 Spectrofluorometer, Fluorescence Microplate Reader. Siemens P-4 single crystal X-ray diffractometer, FT-IR spectrometers, ICP emission spectrometers. Scanning Electron Microscope, Transmission Electron Microscope, Confocal Fluorescence Microscope. EACI horizontal/vertical laminar flow hood, NuAir CO_2 incubator, and IEC clinical centrifuge required for performing tissue culture.

PRELIMINARY RESULTS

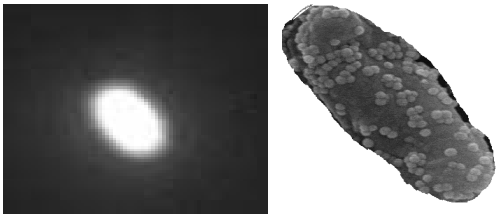

Figure 4 Fluorescence image and SEM image of a *Salmonella* cell conjugated with fluorescent nanoparticles

Synthesis of Multiple Colored Fluorescent Nanoparticles. Most recently, several multiple colored fluorescent silica nanoparticles have been developed in Dr. Zhao's group. These nanoparticles emit fluorescence at different wavelengths with high intensities. The fluorescence intensity of one nanoparticle is about 10,000 times higher than one dye molecule. The size of the nanoparticles is very uniform with a diameter of 50 nm (Figure 3, scanning electron microscopy (SEM) image).

Immobilization of Antibodies onto the Nanoparticle Surface. The specific antibodies were immobilized onto the nanoparticle surface for identification of target cells. The immobilization of antibodies was based on the reaction of amine groups on the antibody with carboxyl groups on the nanoparticles. Before the immobilization, the surfaces of the nanoparticles were activated using 5 mL of 100 mg/mL of EDC (1-ethyl-3-3(3-dimethylamino-propyl) carbodimide hydrochloride) and 5 mL of 100 mg/mL of NHS (N-hydroxy-succinimide) in an MES (morpholineethane-sulfonic acid) buffer (pH 6.8), for 25 minutes at room temperature with continuous stirring. Water-washed nanoparticles were dispersed in 10 mL of 0.1 M PBS (pH 7.3). To covalently immobilize monoclonal antibodies onto the nanoparticle surface, 5 mL of 0.1 mg/mL nanoparticles were reacted with 2 mL of 5 μg/mL antibody for 2-4 hours at room temperature with continuous stirring, followed by washing with a PBS buffer. Dr. Zhao has previously detected *Salmonella* using the nanoparticles (Figure 4).

LITERATURE CITED

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Using Entrapped Cell Systems for Treating Supernatant from Anaerobic Digester of the Moorhead Wastewater Treatment Plant

Basic Information

Title:	Using Entrapped Cell Systems for Treating Supernatant from Anaerobic Digester of the Moorhead Wastewater Treatment Plant
Project Number:	2006ND99B
Start Date:	3/1/2006
End Date:	2/28/2007
Funding Source:	104B
Congressional District:	1
Research Category:	Water Quality
Focus Category:	Treatment, Surface Water, Water Quality
Descriptors:	None
Principal Investigators:	Khan Eakalak

Publication

1. Hill, Christopher and Eakalak Khan, 2007, "Application of Entrapped Cell Systems for Treatment of Anaerobic Sludge Digester Supernatant", Third International Water Conference, International Water Institute, Grand Forks, March 13-15.

Using Entrapped Cell Systems for Treating Supernatant from Anaerobic Digester of the Moorhead Wastewater Treatment Plant

Project Description

The scope of this research consists of studying the use of entrapped cells to treat supernatant from Moorhead Wastewater Treatment Plant sludge digesters. There are a number of benefits to side stream treatment of this supernatant: Accommodation of lower flows; prevention of shock to the mainstream biological process; and prevention of the bypass of ammonia to the river. Cell entrapment is a process which has been applied to domestic wastewater for the removal of both organic carbon and nitrogen. This is the first study to apply the cell entrapment process to highly concentrated wastewater. The main focus of this research is on the removal of nitrogen from Moorhead Wastewater Treatment Plant supernatant. Typically nitrogen is removed through a series of biological processes, nitrification followed by denitrification. Entrapped cell systems can follow this conventional configuration or nitrification/denitrification can be performed in a single reactor. The oxygen diffusion limitation of the entrapment matrix creates an environment in which both nitrifying and denitrifying bacteria can coexist. Both conventional two-step and simultaneous nitrogen removal are investigated in this research.

Progress of Work

In addition to the comprehensive literature review which began before and will continue throughout this fellowship, a substantial amount of time and energy has been spent in the Environmental Engineering Laboratory, North Dakota State University and at the Moorhead Wastewater Treatment Plant. The research progress thus far is briefly described below.

The characteristics of the supernatant are of importance. Therefore, a statistical analysis was performed, using the SAS Enterprise Guide, on data obtained from the plant. The results are presented in Table 1. The data used were based on the total population of samples collected and tested between January 1, 2005 and September 19, 2005.

Table 1 Statistical Analysis Results

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Summary Statistics Results

The MEANS Procedure

Variable	Label	Mean	Std Dev	Minimum	Maximum	N	Lower 95% CL for Mean	Upper 95% CL for Mean
TS	TS	0.2319048	0.0247476	0.2100000	0.3300000	63	0.2256722	0.2381374
TVS	TVS	55.8555556	3.1936822	50.0000000	61.0000000	63	55.0512371	56.6598740
COD	COD	2426.73	324.3843403	1624.00	3400.00	63	2345.04	2508.43
TBOD5	TBOD5	49.3555556	18.4147187	27.6000000	87.6000000	63	44.7178687	53.9932424
TSS	TSS	464.7619048	100.2393449	260.0000000	940.0000000	63	439.5169527	490.0068569
NH3-N	NH3-N	2055.75	301.1676721	1535.00	2348.00	63	1979.90	2131.59

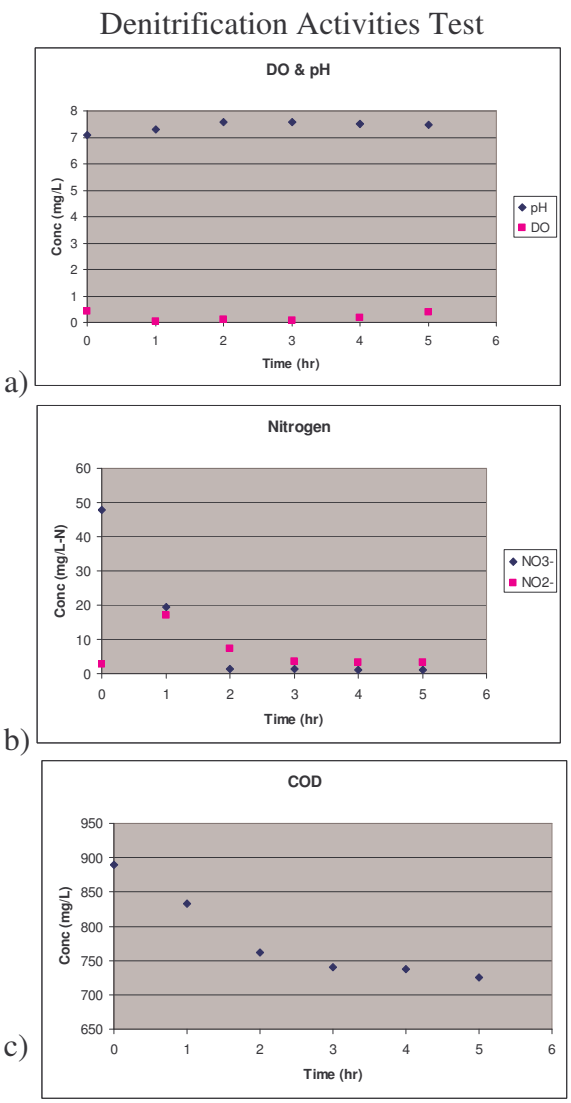
Note: TS, are in %; TVS, are in % of TS; COD, are in mg/L; TBOD5, are in mg/L; TSS, are in mg/L; NH3-N, are in mg/L as nitrogen

Nitrifying and denitrifying bacteria were cultivated initially using synthetic wastewater until sufficient biomass was obtained, approximately 3 months. After testing the activity of the bacteria (data not shown), they were then entrapped both separately and combined (1.5 nitrifier mass:1 denitrifier mass) into a cellulose triacetate matrix. The activity of the nitrifying bacteria after entrapment was virtually zero and suggests that the procedures for entrapping nitrifying bacteria in cellulose triacetate may substantially reduce their activity. It was decided that calcium alginate matrix should be explored in further entrapment experiments.

Once again, nitrifying and denitrifying bacteria were cultivated until sufficient biomass was obtained, approximately 3 months. For the denitrifying bacteria, synthetic wastewater was used as before but for the nitrifying bacteria the supernatant from Moorhead Wastewater Treatment Plant was used. After testing the activity of the bacteria, they were entrapped, both separately and combined, into a calcium alginate matrix. The pre-entrapment activity test results are shown in Figures 1 and 2.

Both activity tests illustrate that the bacteria are performing as expected. It should be noted that the nitrifying culture appears to be composed of mainly ammonia oxidizing bacteria because there is an accumulation of nitrite. This is often seen in high concentrated ammonia wastewater due to the fact that ammonia levels are toxic to nitrite oxidizing bacteria.

Denitrifying bacteria require organic carbon as electron donors to remove nitrogen from wastewater. It can be seen from the statistical analysis (Table 1) that there is a significant amount of chemical oxygen demand (COD) in the supernatant. However, based on corresponding biochemical oxygen demand (BOD), which was very low, most of COD was biorecalcitrant. “Hard” COD is the description typically given to the COD found in sludge digester supernatant. “Hard” referring to the difficulty microorganisms have in consuming this organic carbon. The source of organic carbon is currently being investigating for the denitrifying bacteria in an entrapped cell system. A study of the co-entrapped systems for nitrogen removal with and without the addition of methanol as a carbon source is the latest experiment. Due to the fact that the experiment is not complete the results will be reported at a later date.



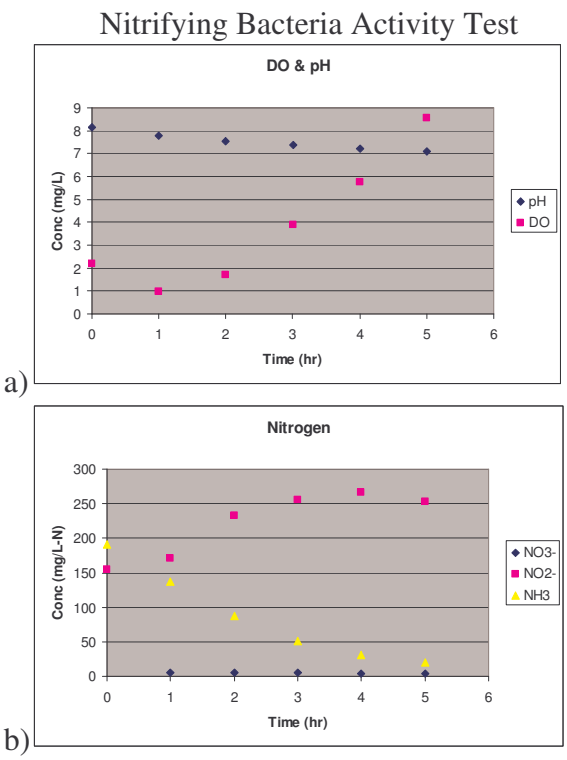


Figure 2 Nitrification Activity Test a) pH & DO b) Nitrogen

Figure 1
Denitrificaiton Activity Test a) pH & DO b) Nitrogen c) COD

Work Remaining

The work remaining for this fellowship includes the completion of the activity and carbon utilization kinetics. Two experiments for the activity and carbon utilization kinetics have been complete. It is estimated that two or three more experiment are require to obtain solid data for analysis. After each experiment, a representative sample of entrapped cells is collected and stored. These samples are to be analyzed using fluorescence-antibody labeling, detecting the spatial location of nitrifying and denitrifying bacteria and possible shedding light on the interaction between the bacteria in the entrapped cell system. After or during the fluorescence experiments, the reactors will be setup and operated in a CSTR configuration and data collected. The data shall be analysis to determine to most feasible configuration and a paper will be submitted to report the findings.

Information Transfer Program

INFORMATION DISSEMINATION

Information dissemination is done through an annual newsletter initiated in 1992, a website initiated in 1999, and presentations and publications by grant and fellowship recipients. The institute's website address is http://www.ndsu.edu/wrri. The newsletter is usually issued in the month of December of each year. Past newsletters can be accessed through the institute web site. Technical reports of Fellowship projects authored by the Fellows and advisers are also placed on the institute web site.

NDWRRI continued its sponsorship of the Biotic Resources Seminar Series at North Dakota State University.

ND WRRI and Civil Engineering Department of North Dakota State University cosponsored a seminar on Dam-Break Flood in Natural Channels by Dr. Arup K. Sarma, Associate Professor of Civil Engineering, Indian Institute of Technology (IIT)-Guwahati, India. Dr. Sarma has worked on dam-break flood wave propagation modeling of some of the Himalayan rivers in the state of Assam located in the northeast part of India. Dr. Sarma was here in the USA to participate in the Environmental and Water Resources Institute conference of the American Society of Civil Engineers in Omaha, Nebraska.

NDWRRI co-sponsored the Third International Water Conference titled "Research Education in an International Watershed: Implications for Decision Making" in Grand Forks, North Dakota, in March 13-15, 2007. The conference organized by the International Water Institute brought administrators, researchers, professionals and educators to Grand Forks, North Dakota to discuss water resources, flood control and water quality management issues related to the Red River of the North. The WRRI Director worked with the IWI in the planning and organization of the conference. He developed and chaired two sessions in the conference which provided a forum for presentation of the WRRI Fellowship research to the public. Several WRRI Fellows and their advisors participated. Ten past and present WRRI Fellows and three advisers made presentations at the conference. The Institute affiliate faculty chaired three session in all:

Concurrent Session 4C: Water Quality in Rivers and Streams Moderated by G. Padmanabhan, Director, ND Water Resources Research Institute, North Dakota State University, Fargo, ND.

. Upper Souris River TMDL Background Study. Wei Lin, Civil Engineering and Environmental and Conservation Sciences, Bernhardt Saini-Eidukat, Geosciences and Environmental and Conservation Sciences, Joseph Super, Environmental and Conservation Sciences, North Dakota State University, Fargo, ND, and Michael Ell, Environmental Administrator, North Dakota Department of Health, Bismarck, ND.

Simulation of Constituent Transport in the Red River of the North Basin, North Dakota and Minnesota, During Unsteady-Flow Conditions, 1977 and 2003-04. R.A. Nustad, Environmental Engineer, USGS, North Dakota Water Science Center, Grand Forks, ND, and J.D. Bales, Hydrologist, USGS, North Carolina Water Science Center, Raleigh, NC

Application of Entrapped Cell Systems for Treatment of Anaerobic Sludge Digester Supernatant. Christopher Hill and Eakalak Khan, Associate Professor, Department of Civil Engineering and Construction, North Dakota State University, Fargo, ND.

Concurrent Session 6A: Soils, Bacteria and Wastewater Treatment. Moderated by G. Padmanabhan, Director, ND Water Resources Research Institute, North Dakota State University, Fargo, ND.

. The Fate of Manure-borne, Land-Applied Hormones. Francis Casey, Associate Professor, Mary Schuh, graduate student, Dept. of Soil Science, Gerald L. Larsen, Research Chemist/Research Leader, Heldur Hakk, Research Chemist, Bioscience Research Lab, USDA-ARS, Fargo, ND, Zhaosheng Fan, graduate student, Dept. of Soil Science, North Dakota State University, Fargo, ND.

. Denitrification in the Red River Basin and Beyond: How Aquifer Sediments Influence Water Quality. Scott F. Korom, Associate Professor, Department of Geology and Geological Engineering, University of North Dakota, Grand Forks, ND.

Sensitive Determinatrion of Bacterial Cells Using Fluorescent Nanoparticles. Yuhui Jin, graduate student, Dept. of Chemistry, Jenna Parisien, student, Dept of Chemistry, Min Wu, Assistant Professor, Dept. of Biochemistry, School of Medicine and Health, and Julia Xiaojun Zhao, Associate Professor, Dept. of Chemistry, University of North Dakota, Grand Forks, ND.

Concurrent Session 6C: Flood, Droughts and Water Management. Moderated by Phil Gerla, Professor, Department of Geology and Geological Engineering, University of North Dakota, Grand Forks, ND.

Characteristics of Effective Local Water Management Organizations in the Red River Basin. Craig C. Kritsky, graduate student and Robert R. Hearn, Assistant Professor, Department of Agribusiness and applied Economics, North Dakota State University, Fargo, ND

The Red River Management Consortium. Daniel J. Stepan, Senior Research Manager, Energy & Environment Research Center, University of North Dakota, Grand Forks, ND.

The presentations by the Fellows and Institute affiliate faculty can be viewed at the International Water Institute's web site:

http://www.internationalwaterinstitute.org/2007proceed.htm

Research results of NDWRRI Graduate Research Fellows were published and presented in various other conferences also.

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 NCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	0	0	0	0	0
Masters	7	3	0	0	10
Ph.D.	5	1	0	0	6
Post-Doc.	0	0	0	0	0
Total	12	4	0	0	16

Notable Awards and Achievements

Publications from Prior Projects

1. 2002ND9B ("Evaluation of walleye to suppress fathead minnow populations in Type IV & V wetlands.") - Conference Proceedings - Potthof, A. J., K.D. Zimmer, B.R. Herwig, M.G. Butler, M.A. Hanson, J.R. Reed, B.G. Parsons, M.C. Ward, D.W. Willis, 2007, Effects of Piscivore Introduction on Prairie Wetland Ecosystems: Trophic Interactions Can Alter Ecosystem Structure. Third International Water Conference, International Water Institute, Grand Forks, March 13-15.
2. 2003ND28B ("A Study of Microbial Regrowth Potential of Water in Fargo, North Dakota and Moorhead, Minnesota") - Water Resources Research Institute Reports - Museus, Trent A. and Eakalak Khan, 2006, "A Study of Microbial Regrowth Potential of Water in Fargo, North Dakota and Moorhead, Minnesota",Technical Report No: ND 06-01, North Dakota water Resources Research Institute, Fargo, North Dakota.
3. 2005ND75B ("Evaluating and Validating the Index of Plant Community Integrity for the Assessment of Temporary, Seasonal, and Semi-permanent Wetlands in the Prairie Pothole Region") - Conference Proceedings - Hargiss, C.L.M., E.S. DeKeyser, D. Kirby,2006, "Development and Evaluation of an Index of Plant Community Integrity for Assessing Wetland Plant Communities", 2006 Annual Society of Range Management Meeting, Vancouver, BC.
4. 2005ND75B ("Evaluating and Validating the Index of Plant Community Integrity for the Assessment of Temporary, Seasonal, and Semi-permanent Wetlands in the Prairie Pothole Region") - Water Resources Research Institute Reports - Hargiss, Christina L.,Edward S. DeKeyser, and Donald R. Kirby, 2006,"Evaluation of an Index of Plant Community Integrity for Assessing Wetland Plant Communities in the Prairie Pothole Region", Technical Report No: ND 06-02, North Dakota Water Resources research Institute, Fargo, North Dakota.
5. 2004ND49B ("Analysis and Model Simulation of Stormwater Runoff -- A Study of Land Use and System Design on Discharge Flow Rates and Water Quality") - Dissertations - Gautam, Brajesh., 2007,"Analysis and Model Simulation of Stormwater Runoff in the Red River of the North: A Study of Land-Use on Discharge and Water Quality",MS Thesis, Department of Civil Engineering, North

dakota State University, Fargo, North Dakota.