Indiana Water Resources Research Center
Annual Technical Report
FY 2013

Introduction

Overview: This report covers the activities of the Indiana Water Resources Research Center (IWRRC) for the period March 1, 2013 to February 28, 2014 and was written by Ronald F. Turco, Director of the center. The report is provided to meet requirements and obligations under the 104 (B) of the USGS water centers program. The objectives of the fiscal year 2013 program of the IWRRC have been: (1) to continue to engage the water community in the State of Indiana as related to water research and education; (2) to chair the dedicated water community at Purdue University–the Purdue Water Community (http://www.purdue.edu/dp/water/about.php); (3) foster a research programs that encompass water issues related to: contaminants that is primarily focused on the Wabash River and in support of the projects aid in the development of grant submission for major efforts; (4) continue to support an outreach program related to water and water quality (in particular rural water protection/safety) and (5) to strengthen interactions with State regulatory agencies and Federal Agencies via active participation in a series of well water protection education programs, meetings with USGS, and Indiana Department of Natural Resources. In the last year we have supported externally reviewed 104(B) projects, maintained a functional website (www.iwrrc.org) been involved in the development, submission and management of number of grant proposals including a record number of 104g submissions. In terms of web resources we have maintained the digital library of most of our back issues of water center reports and made them available via the Purdue University Library at “IWRRC Technical Reports”(http://docs.lib.purdue.edu/watertech/). This year for the first time we made raw data from our river sampling program available to the public (https://purr.purdue.edu/search/?terms=publications:turco\ ). This site includes three years of water data from 5 sites and data from Purdue Water Quality Field Station.

The IWRRC and Wabash River Enhancement Corporation (WRRC) have maintained a strong relationship over the past year. We are now in the third year of our 319 project entitled "Region of the Great Bend of the Wabash River Implementation project” where IWRRC is active with WREC in helping to evaluate projects for potential cost-share under the implementation effort and we engaged in number of new project startups. The IWRRC-WREC collaboration led to project entitled “Deer Creek-Sugar Creek Watershed Management Plan and Implementation Program.” Work on this effort has been almost completed. The relation with WREC led to the development of student grant effort that was submitted to the Ford College Community Challenge (C3) community grant supports student-led, community-based projects around the theme “Building Sustainable Communities.” The effort has engaged a number of undergraduate and graduate students in three local water quality projects. The students have completed service projects at: Oakland High School, Purdue University, and Cary Home. The IWRRC has been active with the Purdue University water community (PWC) and have facilitated a number of campus wide meetings to engage this group. We have been primarily interested in developing grant applications. International, we have continued to work with Purdue’s office of global engineering on a number of water projects with efforts in China, India and Mexico. We have worked with the Purdue University Ecopartnership (http://www.purdue.edu/discoverypark/ecopartnership/) and have recently initiated efforts with a possible project in Colombia. Our goal has been the development of effective partnerships leading to real improvements in water quality. For this reporting period, we continue the strategic outreach alliance with the Purdue Pesticide Program office for the development of document and educational materials on methods to prevent water contamination. By leveraging our funds with the Purdue Pesticide Program office’s core efforts we are using the opportunity to include the IWRRC in many of their programs. Our efforts have established a constant and vital outreach effort that is associated with prevention rather than remediation of environmental problems. In the future we are increasing our support of the PPP office.

Project 01: Program Administration and State Coordination

The administrative portion of the project has been used to support the management of the IWRRC's research projects and to facilitate the development of other research projects. We have also stepped up our efforts to coordinate campus level interactions (helping to create the Purdue Water Community) with state and federal

agencies. All of these efforts have the ultimate goal of improving the quality of water resources in the State of Indiana. We have used a limited amount of money on the administrative portion but it has allowed the IWRRC director some means to invest time in the efforts to integrate with state and federal agencies. Most of IWRRC funds are used for projects and the director's time is contributed to the project. The IWRRC director has worked with state and federal environmental agencies, the governments of Indiana's cities and counties and key citizen groups on water education and water resources planning activities. In this way, the results from the research projects can be transferred to interested individuals in the state. The IWRRC director will participate in important national and international meetings related to water and environmental protection.

Projects Areas

1. Work with community projects has continued including working with the Wabash River Enhancement Corporation (WREC) on a Volunteer Water Quality Monitoring project to allow opportunities for volunteer monitors to assess water quality conditions throughout the watershed. WREC its partners conducted a fall and spring Wabash River Sampling Blitz in 2013. Connecting volunteers to the Wabash River: During each spring and fall event, approximately 175 volunteers mobilize to collect water samples from 258 stream sites within the Region of the Great Bend of the Wabash River and Wildcat Creek watersheds. The Wabash Sampling Blitz is dedicated to helping watershed residents learn more about the quality of the Wabash River. This portion of the Blitz provides information to the community through various media outlets, public information discussions, the WREC Wire (e-newsletter), the Wabash Sampling Blitz website and via partner newsletters and programs. During the previous 6 events (going back 3 years), volunteer groups sampled three to four stream sites collecting field measurements for temperature and transparency, using test strips to analyze pH and nitrate at a minimum, and filling sample bottles for laboratory analysis of E. coli, nitrate+nitrite, orthophosphorus, and total organic carbon. Sample results were mapped by subwatershed drainage and posted to www.wabashriver.net as soon as possible following the event. In total 600 unique volunteers participated in the sampling blitzes.

2. We have also continued working along with the Wabash River Enhancement Corporation, our partners, and education and outreach committees to provide numerous opportunities for watershed stakeholders to learn about the Wabash River and the Region of the Great Bend of the Wabash River watershed; facilitated education-based events; and coordinated programs to recognize the opportunities and commitments made by businesses and individuals throughout the watershed. Public meetings, the Clear-Blue-Green business certification program, field days, workshops, and the Wabash Sampling Blitz are just some of the activities used to educate our stakeholders.

3. In conjunction with the Tippecanoe County soil and water conservation district office, we have been developing a new theme area related to the use of cover crops to reduce N leaching and runoff as well as to improve soil health. This links to the use of infield anaerobic bioreactors as a means of reducing soluble N that can be discharged to surface water.

4. The Wabash River runs some 764 km (475 mi), is situated across five 8-digit Hydrologic units (HUC), crosses 19 counties and at its full distance stretches from the Ohio border in the Northeast corner of the state to the Southwest corner where it combines with the Ohio River below Mount Vernon on its way to the Mississippi River. Our goal is simple: develop a coordinated research and management agenda for work on the Wabash River. The long-term goal of the effort is to help re-establish the Wabash River as a healthy water body that provides quality recreation and economic value to the state. In December we hosted a one-day meeting in December entitled: The Wabash River Workshop at The Campus Center @ IUPUI. Our keynote was given by Dr. Bill Richardson, Research Aquatic Ecologist, U.S. Geological Survey and was titled: Interactions among geomorphology, nutrients and foodweb productivity of the Upper Mississippi River: a case study with speculation. This was followed by 10 other presentations with over 50 water scientist from the around the state were in attendance. A second meeting is in the planning stages for Fall 2014.

5. Dr. Turco has been an active participant in educational programing related to well water and aquifer health. He given 6 Ground water talks to various groups at six different locations in the state of Indiana.

Grant Applications Submitted thorough/with IWRRC:

a. (Funded and ongoing) USDA-CIG $165,000 Using cover crops to improve soil health and moisture retention. b. (Funded and ongoing) IDEM-319 $240,000 Region of the Great Bend of the Wabash River Implementation Project with L. Prokopy, S. Peel and R. Goforth.

c. (Funded and ongoing) USDA-CAP: $2,875,642 Sustainable Production and Distribution of Bioenergy for the Central USA with J. Volenec, S. Brouder, others

d. (Funded and ongoing) IDEM-319 $132,000 Deer Creek-Sugar Creek Watershed Management Plan and Implementation Program. Project with S. Peel and R. Goforth.

e. (Funded and ongoing) SUNGRANTS: Optimization of biomass productivity and environmental sustainability for cellulosic feedstocks: Land capability and life cycle analysis. $875,000 S.M. Brouder, PI, R.F. Turco, J.J. Volenec, D.R. Smith and G. Ejeta CoPIs

f. (Closed) USDA NRI, Managed Ecosystems. Ecological services of agro-biofuels: productivity, soil C storage, and air and water quality. $399,999. Submitted Dec. 2007. S.M. Brouder, PI, R.F. Turco, J.J. Volenec, D.R. Smith and G. Ejeta CoPIs. g. (Closed) ALCOA 95,000 Bauxite Residue in Soil. R. Turco.

h. (Closed) IDEM-319 Development and Demonstration of Outcomes-Based Evaluation Framework for the Indiana Nonpoint Source Program. Developed with Jane Frankenberger, and Linda Prokopy.

i. (Closed) IDEM 319: Wabash River: Lafayette-West Lafayette Reach of the Wabash River Watershed Management Plan. Submitted in conjunction with the Wabash River Enchantment Corporation $700,000. L. Prokopy, L. Bowling, K. Wilson and R. Turco. Bridging grant approved for one year of additional support.

j. (Closed) USDA Conservation Effects Assessment Program. $660,000. Watershed-Scale Evaluation of BMP Effectiveness and Acceptability: Eagle Creek Watershed, Indiana. Developed with Jane Frankenberg, Lenore Tedesco, Jerry Shively, Linda Prokopy. This was an outgrowth of an effort submitted last year to EPA but note funded: Creating sustainable drinking water supplies for Central Indiana: Innovations to achieve reductions in watershed and reservoir nutrient levels

External Board of Advisors Membership: Dr. Jack Wittman, Ph.D., INTERA, Bloomington IN Dr. Bill Guertal Director, USGS Indiana Water Science Center, Indianapolis IN Mr. Jeff Martin, USGS Indiana Water Science Center, Indianapolis IN

Faculty Advisory Committee: Dr. Linda Lee, Professor and Director of ESE Dr. Indrajeet Chaubey, Agriculture and Biological Engineering Dr. Larry Nies, Civil and Environmental Engineering Dr. Inez Hua, Civil and Environmental Engineering

Research Program Introduction

None.

Transport and retention of atrazine, metolachlor, carbaryl, and chlorothalonil in agricultural streams

Basic Information

Title:	Transport and retention of atrazine, metolachlor, carbaryl, and chlorothalonil in agricultural streams
Project Number:	2012IN333B
Start Date:	3/1/2012
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	IN-006
Research Category:	Water Quality
Focus Category:	Agriculture, Ecology, Hydrology
Descriptors:	
Principal Investigators:	Melody J. Bernot, Daniel Elias

Publications

1. Elias, D, and MJ Bernot. 201X. In preparation. Abundance and transport of atrazine, metolachlor, carbaryl, and chlorothalonil in central Indiana streams. Microbial Ecology.
2. Elias, D, MJ Bernot. 2013. Abundance and transport of atrazine, metolachlor, carbaryl, and chlorothalonil in central Indiana streams. 18th BSU Student Symposium. Ball State University, Muncie, IN. March
3. Elias, D, MJ Bernot. 2013. Effects of atrazine, metolachlor, carbaryl and chlorothalonil on stream sediment nutrient dynamics. Seminar Series ID 705, Environmental Sciences Program. Ball State University. Muncie, IN. March
4. Elias, D, MJ Bernot. 2012. Abundance and transport of atrazine, metolachlor, carbaryl, and chlorothalonil in central Indiana streams. Ohio Valley Society of Environmental Toxicology and Chemistry.Oxford , OH. Septembe
5. Elias, D, MJ Bernot. 2012. Abundance and transport of atrazine, metolachlor, carbaryl, and chlorothalonil in central Indiana streams.Indiana Water Resources Association. Mitchell, IN. June
6. Elias, D, MJ Bernot. 2012. Abundance and transport of atrazine, metolachlor, carbaryl, and chlorothalonil in central Indiana streams. Society for Freshwater Science. Louisville, KY. May.
7. Elias, D, MJ Bernot. 2012. Abundance and transport of atrazine, metolachlor, carbaryl, and chlorothalonil in central Indiana streams. Indiana Academy of Science. Indianapolis, IN. March.
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12. Elias, D, MJ Bernot. 2012. Abundance and transport of atrazine, metolachlor, carbaryl, and chlorothalonil in central Indiana streams. Society for Freshwater Science. Louisville, KY. May.
13. Elias, D, MJ Bernot. 2012. Abundance and transport of atrazine, metolachlor, carbaryl, and chlorothalonil in central Indiana streams. Indiana Academy of Science. Indianapolis, IN. March.
14. Jarvis, AL. The effects of the psychiatric drug carbamazepine on freshwater invertebrate communities and ecosystem dynamics. MS Thesis. Ball State University, May 2014.
15. Bernot, MJ, AL Jarvis, RJ Bernot. 2014. Aquatic Neuroses: The effects of the pharmaceutical carbamazepine on aquatic ecosystem dynamics. Joint Aquatic Sciences Meeting, Portland, OR. May.
16. Lee, JH, MJ Bernot. 2014. The toxicity and effects of carbamazepine on Yellow Perch (Perca flavescens) growth and behavior. Ball State University Student Symposium. March.
17. Abell, NS, MJ Bernot. 2014. The influence of stimulants on the freshwater snail, Physa acuta. Ball State University Student Symposium. March.

IWRRC 2012 Project Report

Title: *Transport and retention of atrazine, metolachlor, carbaryl, and chlorothalonil in agricultural streams*

Project Type: Research

Start Date: 3/01/2012 **End Date:** 2/28/2013

Congressional District: IN-006

Focus categories: AG, ECL, HYDROL, NPP, ST, SW, TS, WQL

Keywords: agricultural contaminants, nonpoint pollution, streams, toxicology

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Abstract / Summary

Agricultural pesticides have the potential to affect receiving aquatic ecosystems. However, few studies have provided direct measures of pesticide movement within freshwaters. Atrazine, metolachlor, carbaryl and chlorothalonil have high usage rates throughout the Wabash River watershed and are potentially toxic to algae, aquatic plants, invertebrates, fish, and humans. Thus, a more comprehensive understanding of their transport and fate in freshwaters is needed to effectively manage these compounds. Research provided novel and direct measures of contaminant transport and retention in central Indiana streams. Specifically, *in situ* enrichment experiments of atrazine, metolachlor, carbaryl and chlorothalonil were conducted to directly quantify dissolved and sediment movement of these agricultural contaminants. Research was guided by one primary question with related hypotheses: What factors affect transport and retention of agricultural pesticides in lotic ecosystems? Enrichment experiments were conducted in July and October of 2011 to coincide with summer and fall stream conditions. In July, nitrate uptake length was longer relative to atrazine, carbaryl and chlorothalonil. In contrast, for the month of October, atrazine and metolachlor uptake lengths were longer relative to nitrate. Changes in nutrient uptake in response to atrazine, metolachlor, carbaryl and chlorothalonil were observed for ammonium with increasing concentration of metolachlor. Also, there was a decrease in remineralization of nitrate and decrease in phosphate uptake with increasing concentration of chlorothalonil. This research enhanced our understanding of atrazine, metolachlor, carbaryl and chlorothalonil transport and retention in freshwaters and provided data necessary for effective management of these agricultural chemicals and freshwater resources.

Problem:

This study will be conducted in the Upper White River Watershed, a tributary to the Wabash River and **one of the most degraded watersheds in the country** (USEPA 2002). This watershed is ideal for studying the influence of agricultural activity on aquatic ecosystems as the watershed is >70% agricultural land. Since the Clean Water Act was passed by Congress in 1972, environmental managers and policy makers have made substantial headway in identifying and reducing point source discharge in the nation's waterways (USEPA 2002). Yet, >40% of these water bodies remain impaired, often because of nonpoint source pollution (Carpenter et al. 1998, USEPA 2002). Nonpoint source pollution from agricultural activity poses a particular problem for water quality management because the responsibility is spread among entire populations, complicating source identification and reduction. Further, nonpoint pollution often degrades freshwater integrity to a greater extent than point pollution (Atasoy et al. 2006). Agricultural pollutants include nutrients, sediments, and trace organic compounds. Of these contaminants, trace organic compounds are the least understood. Agriculturally derived trace organics can be divided into functional categories including herbicides, insecticides, and fungicides. These categories represent dozens of trace organic compounds though their potential environmental effects are likely distinctly different due to distinct chemical properties. The frequency of use, detection in freshwater, and

potential toxicity varies with individual compounds (Murray et al. 2010). In the Wabash River watershed, two herbicides (atrazine and metolachlor), one insecticide (carbaryl) and one fungicide (chlorothalonil), have both high usage rates and high frequency of detection in receiving waters. Further, these compounds have peak concentrations that may adversely affect the aquatic community (Munn et al. 2006) and human health (Margni et al. 2003; Yang et al. 2006; Ochoa-Acuna et al. 2009a), as well as potential sub-lethal effects on fecundity, growth, behavior and metabolic processes (Fairchild et al. 1996; Kashian et al. 2002) of aquatic organisms at expected environmental concentrations (Hashimoto et al. 1981; Ma et al. 2006; Larson et al. 1999; Myers et al. 2000). ***A more comprehensive understanding of how agriculturally derived trace organic compounds are transported and retained in aquatic ecosystems is needed to mitigate potential impacts on freshwater integrity including impaired function.*** Without more comprehensive data to guide management programs, we may incorrectly assume that these programs are adequate for maintaining or improving water resources. The proposed research will address these concerns by **directly** quantifying trace organic movement in streams. These data are essential for managers to make informed decisions about current and future management of water resources.

Research Objectives:

Research was guided by one primary question: **What factors affect transport and retention of agricultural pesticides in lotic ecosystems?** Research incorporated *in situ* experimental enrichments of agricultural contaminants to directly quantify movement in the ecosystem. Several research hypotheses guided data analyses:

H1: Transport of pesticides will be greater with increased discharge.

H2: Transport of pesticides will decrease with organic matter availability in conjunction with increased sorption and microbial activity.

H3: Dissolved chlorothalonil transport will be lower relative to atrazine, metolachlor, and carbaryl due to higher sediment sorption.

Methodology:

Enrichment experiments were conducted July 22nd, 2011 and October 18th, 2011. Experiments were conducted on a 1 km reach of a 2nd order tributary to Jakes Creek which is located on a Ball State University owned field station and has a history of agricultural activity in the surrounding sub-watershed (Figure 1). The tributary is located in the greater Wabash River watershed. During each enrichment experiment, sampling sites were marked every 50 m along the length of the reach with one site located upstream the enrichment to serve as a reference point. Commercial grade pesticides were used to prepare an enrichment solution and include Atrazine 4L, Me-too-lachlor, Sevin and Daconil. In addition, the enrichment solution included bromide as a conservative trace and nitrate (as KNO_3) to foster comparison. The enrichment solution was made in a 25 mL carboy which was dripped into the experimental reach for ~2h (until equilibrium

was achieved) at ~0.016 L/min. Atrazine, metolachlor, carbaryl and chlorothalonil concentrations achieved via the enrichment were an order of magnitude below toxicity for any aquatic organisms but representative of concentrations measured previously in aquatic ecosystems (1.0, 0.6, 0.05 and 0.005 µg/L, respectively).

Once bromide equilibrium was achieved at the most downstream station, water and sediment sampling were conducted at each station along the reach. For dissolved pesticide analyses, two filtered water samples (1000 ml) were collected at each sampling site using a 60 mm syringe connected to a glass fiber filter (0.7 mm pore size) and placed into amber baked glass bottles. These samples were delivered within 12h to the Indiana State Department of Health (ISDH) Environmental Laboratories, Indianapolis for analyses. Atrazine, metolachlor and chlorothalonil were analyzed following method 525.2. These pesticides were extracted from water samples by passing 1 liter of sample water through a disk containing a solid matrix with a chemically bonded C18 organic phase (liquid-solid extraction, LSE). The compounds were eluted from the LSE disk with small quantities of ethyl acetate followed by Methylene Chloride. The sample components were separated, identified, and measured by a gas chromatography/mass spectrometry (GC/MS) system. Carbaryl analysis followed method 531.1. This pesticide was injected into a reverse phase HPLC column. Separation of carbaryl was achieved using gradient elution chromatography. After elution, carbaryl was hydrolyzed with 0.05N sodium hydroxide at 100°C. The methyl amine formed during hydrolysis was reacted with o-phthalaldehyde (OPA) and thiofluor to form a highly fluorescent derivative which was detected by a fluorescence detector. Separate filtered water samples (200 mL) were also collected at each sampling site for analysis of nutrients (nitrate, phosphate, ammonium) and bromide via ion chromatography (DIONEX 3000) and carbon via combustion (Schimadzu TOC-VS) at Ball State University following standard procedures. For sediment pesticide analyses, ~50 g homogenized sediment (top 5 – 10 cm) was collected into glass containers (150 ml) and also delivered to ISDH for analyses following extraction. For analysis of organic matter in sediments, sediment sub-samples were dried in a Model 30 GC laboratory oven for 48 h followed by combustion in a Barnstead Thermolyne FB 1400 muffle furnace and weight. Physicochemical parameters were also measured at each sampling site using a Hydrolab minisonde for oxygen, pH, temperature, turbidity, and conductivity and a Watson-Marlow flow meter to estimate discharge at each sampling site with measurements of depth, width and velocity.

Data collected in conjunction enrichment experiments were used to calculate water and sediment-bound transport metrics (uptake length, velocity, rate, retention, export) and compared among compound type and season using analysis of variance (ANOVA).

Results

Enrichment experiments were conducted in July and October (2011) in a 3rd order stream (Jakes Creek, Muncie, Indiana). Ten sites were marked 50 m apart for a total reach length of 500 m. A 25 L carboy was prepared with atrazine, metolachlor, carbaryl, chlorothalonil, and nitrate, at concentrations of 2.0, 1.0., 0.01, 0.05 µg/L, and 2 mg/l, respectively, and added to the stream reach at 0.016 l/min using a peristaltic geo-pump

for 120 min (sampling at 100 min).Water and sediment samples were collected and stream physicochemical parameters were measured using a Hydrolab® and Watson-Marlow flow meter. Enrichment experiments in July quantified longer uptake rates for nitrate (312 m) and chlorothalonil (222 m) relative to carbaryl (102 m) and atrazine (30 m) (Figure 1). In October, uptake lengths for metolachlor (188 m) and atrazine (98 m) were longer, relative to nitrate (44m). Shorter uptake length for nitrate in October relative to July, suggests lower concentration of dissolved nitrate, and greater retention of nitrate relative to pesticides.

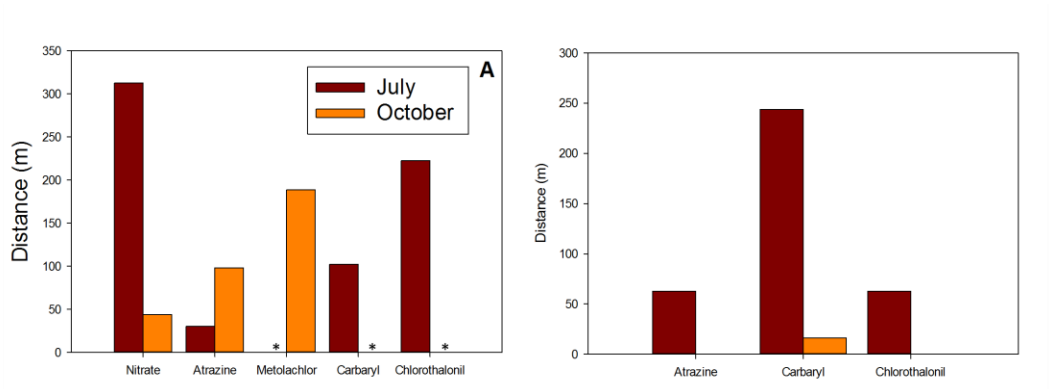

Figure 1. Uptake length of (A) dissolved pesticides; and, (B) sediment bound pesticides for atrazine, metolachlor, carbaryl, and chlorothalonil, relative to nitrate, in July and October enrichment experiments. * = Data not available.

For the sediment microbial community experiment there was little variation of ammonium, nitrate, and phosphate uptake within pesticides (Table 1). Also, there was a preferential uptake of ammonium over nitrate and a net remineralization of phosphate (Table 1). Uptake rate, loss of ammonium from the water column to the benthos, was negative correlated ($P < 0.05$) to metolachlor and was nonlinearly associated (polynomial cubic response) under increasing concentrations of this pesticide (Fig. 2). On the other hand, NH_4-N loss was not significantly correlated with increasing concentrations of atrazine, carbaryl or chlorothalonil. Nitrate uptake rate was positively correlated ($P < 0.05$) with a negative uptake (remineralization, organic forms to inorganic forms of nutrient) in response to increasing concentrations of chlorothalonil (Fig. 3). Nitrate dynamics were associated with a polynomial cubic response to chlorothalonil. In contrast, nitrate uptake of the sediment microbial community exposed to atrazine, metolachlor or carbaryl were not significantly correlated. In response to exposure to chlorothalonil, sediment microbial community phosphate uptake rate was negatively correlated with a linear response, $P < 0.0001$ (Fig. 4). No other pesticides were correlated with phosphate loss.

Table 1. Mean and range of nutrients uptake rates (mg/gdm/d)

Pesticides	Ammonium	Nitrate	Phosphate
Atrazine	0.76 *(0.50 - 0.92)*	0.001 *(0 - 0.002)*	-0.002 *(-0.004 - 0.003)*
Metolachlor	0.87 *(0.40 - 1.18)*	0.001 *(0.0002 - 0.002)*	-0.001 *(- 0.003 - 0.002)*
Carbaryl	0.67 *(0.30 - 1.07)*	0.03 *(0.02 - 0.03)*	-0.01 *(-0.02 - 0.003)*
Chlorothalonil	0.67 *(0.34 - 1.01)*	0.001 *(-0.001 - 0.003)*	-0.03 *(-0.05 - 0.003)*

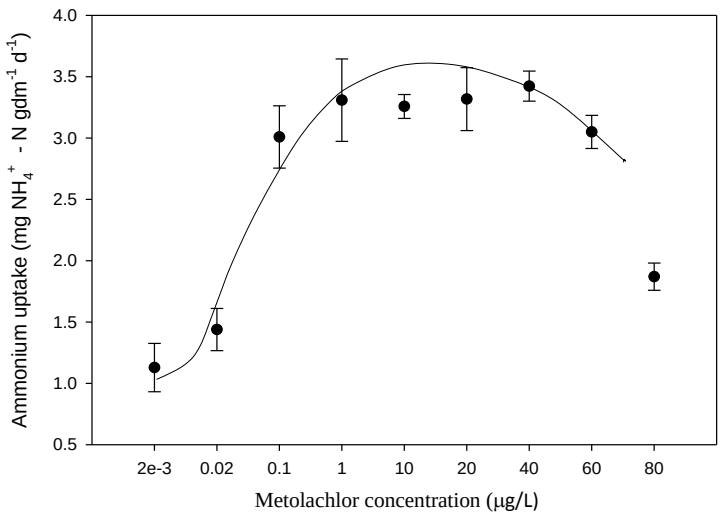

Figure 2: Mean Ammonium uptake rate after 24 h incubation (4 replicates, 10 treatments, N = 40) in response to increasing concentrations of metolachlor. Bars: SE.

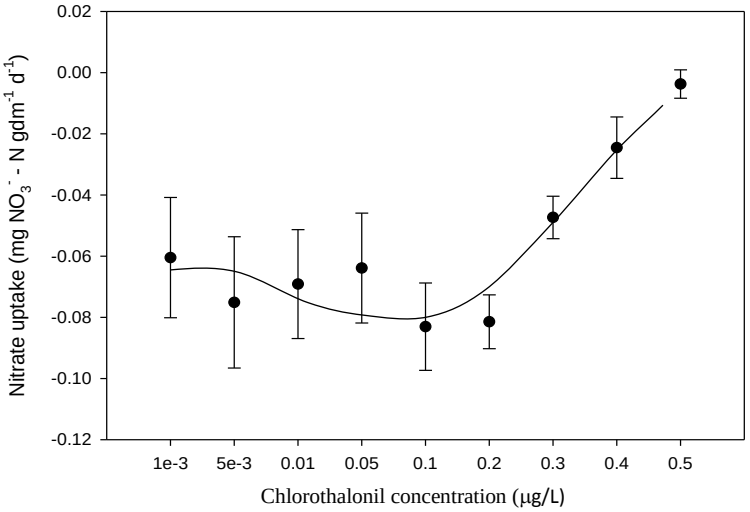

Figure 3: Mean nitrate uptake rate after 24 h incubation (4 replicates, 10 treatments, N = 40) in response to increasing concentration of chlorothalonil. Bars: SE.

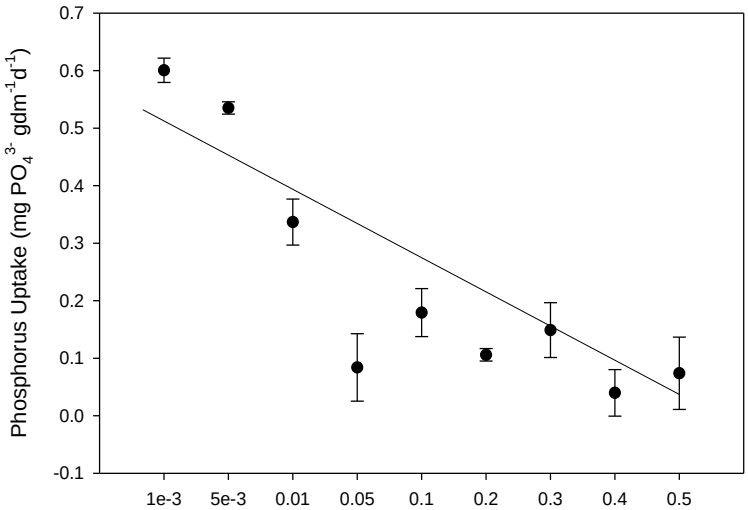

Figure 4: Mean phosphorus uptake rate after 24 h incubation (4 replicates, 10 treatments, N = 40) in response to increasing concentrations of chlorothalonil. Bars: SE.

Major Conclusions and Significance

These data suggest spatial and temporal variation in both abundance of pesticides as well as transport and retention within the ecosystem. Atrazine and metolachlor were detected in sediments suggesting accumulation from previous spraying seasons. Atrazine and metolachlor dissolved concentrations were within range of sub lethal effects on aquatic organisms. Atrazine had a shorter uptake length relative to nitrate, which could indicate a nitrate saturated stream and/or fast assimilation of the pesticide.

The different effects of atrazine, metolachlor, carbaryl and chlorothalonil on nitrogen and phosphorus uptake by sediment microbial communities are mainly influenced by chemical characteristics of pesticides (modes of action, Kow) and species present. Increasing availability of NH4-N and/or phosphorus (nutrient loss) could potentially affect stream export, reduce water quality and increase eutrophication. Remineralization of NO3-N in response to increasing concentrations of chlorothalonil could indicate energy demanding detoxification processes that require the oxidation of organic substrates. The equations generated in this research can be used to complement models of nitrogen/phosphorus availability in streams and to determine the potential changes in nutrient dynamics in the increasing presence of pesticides in lotic ecosystems.

Publications

<u>Presentations</u>

Elias, D, MJ Bernot. 2013. Abundance and transport of atrazine, metolachlor, carbaryl, and chlorothalonil in central Indiana streams. 18th BSU Student Symposium. Ball State University, Muncie, IN. March

Elias, D, MJ Bernot. 2013. Effects of atrazine, metolachlor, carbaryl and chlorothalonil on stream sediment nutrient dynamics. Seminar Series ID 705, Environmental Sciences Program. Ball State University. Muncie, IN. March

Elias, D, MJ Bernot. 2012. Abundance and transport of atrazine, metolachlor, carbaryl, and chlorothalonil in central Indiana streams. Ohio Valley Society of Environmental Toxicology and Chemistry.Oxford , OH. September

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Elias, D, MJ Bernot. 2012. Abundance and transport of atrazine, metolachlor, carbaryl, and chlorothalonil in central Indiana streams. Indiana Academy of Science. Indianapolis, IN. March.

Publications

Elias, D, MJ Bernot. In preparation. Abundance and transport of atrazine, metolachlor, carbaryl, and chlorothalonil in central Indiana streams. Microbial Ecology.

Grant Submissions:

Elias, D. Transport of atrazine, metolachlor, carbaryl and chlorothalonil in freshwater ecosystems. Sigma Xi Grants in Aid of Research. Total Request: $1000.

Elias, D. Synergistic and antagonistic effects of atrazine, metolachlor, carbaryl and chlorothalonil on freshwater organisms. Ball State University ASPIRE. Total Request: $500. 2012-2013.

Elias, D. Frequency and abundance of atrazine, metolachlor, carbaryl and chlorothalonil in central Indiana freshwaters and sediment. Indiana Academy of Sciences. Total Request: $3,000.

Elias, D. Environmental fate of atrazine, metolachlor, carbaryl and chlorothalonil in central Indiana freshwaters. Environmental Protection Agency. Total Request: $42,000/annually.

Students

A total of 5 graduate students and 2 undergraduate students were involved with this project.

Monitoring and Modeling of Hydrodynamic Processes at the Wabash-Tippecanoe Confluence

Basic Information

Title:	Monitoring and Modeling of Hydrodynamic Processes at the Wabash-Tippecanoe Confluence
Project Number:	2012IN334B
Start Date:	3/1/2012
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	IN-004
Research Category:	Climate and Hydrologic Processes
Focus Category:	Floods, Geomorphological Processes, Models
Descriptors:	None
Principal Investigators:	Venkatesh Merwade, Indrajeet Chaubey, Reuben R Goforth

Publications

1. None to report
2. None to report

Title: Monitoring and modeling of hydrodynamic processes at the Wabash-Tippecanoe Confluence

Abstract / Summary:

Flooding is the major natural disaster affecting the state of Indiana. With growing concerns related to climate and land use changes, there is a need to develop sustainable approaches towards floodplain management. The objective of this project is to set-up an instrumentation network at the confluence of Wabash and Tippecanoe rivers to monitor the hydrodynamics of high flows including velocity, stage, groundwater level and sediments. It is expected that such monitoring will create data that will enable to develop better understanding of the behavior of Wabash River for different flow conditions. This understanding will in-turn will enable addressing other environmental and ecological issues related to flooding along Indiana rivers.

Problem:

This project addresses the critical problem of flooding and its impact on other natural systems. While the past approach in handling high floods is through hard (or structural) measures (e.g., levees), there is a growing consensus that a better and more sustainable way is to propose and implement soft (or non structural) measures (sometimes in conjunction with structural measures) that will involve the usage of temporary floodplain storage to alter the flood hydrograph. Proposing and implementing soft measures involves understating the floodplain hydrodynamics and its interaction with other natural processes related to floodplain storage, ecology and geomorphology. The work proposed in this project is the first step towards accomplishing the goal of sustainable flood management through monitoring and modeling of floodplain hydrodynamics.

Research Objectives:

The objectives of this project are:

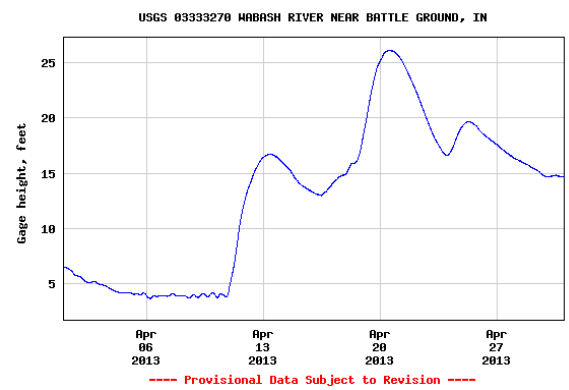
1. Install the hydrodynamic instrumentation network including acoustic dopplers and pressure transducers along the Wabash-Tippcanoe Confluence.
2. Real-time publication of data through United States Geological Survey (USGS) and WaterHUB (www.water-hub.org).
3. Develop a hydrodynamic model that uses the collected data to simulate the behavior of Wabash and Tippecanoe rivers at high flow conditions.

Methodology:

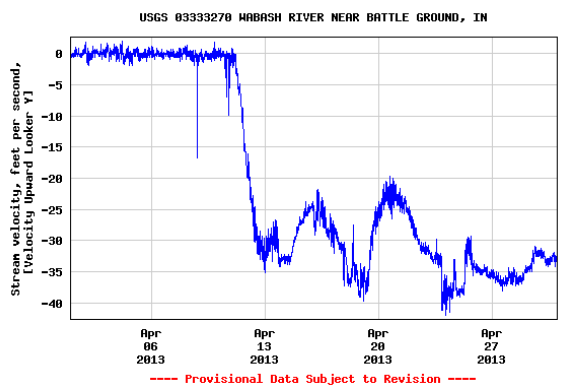
The methodology involved setting-up and installing the instruments in co-operation with the Indiana Water Science Center. This included a reconnaissance survey to determine the location of sites, getting appropriate permits, preparing the sites, and installing the instruments. The instruments were installed in November of 2012, and the data are available online since the beginning of 2013. A graduate student is now working to set-up a 1D HEC-RAS hydraulic model for calibration using the data available from the field.

Results:

The instruments are working as expected, and were able to capture the recent April 2013 flood with stage and velocity data as shown below. These data will be very useful in calibrating the model and testing some hypotheses related to river-floodplain interaction during high flow events.



Stage data from April 1 – 30 for Wabash River near Battle Near Battle Ground



Velocity data* from April 1 – 30 for Wabash River near Battle Near Battle Ground.

*Due to the orientation of the instrument, the velocity values are recorded as negative.

Major Conclusions and Significance

The instrumentation network is able to provide unprecedented hydrodynamic data hydrodynamic data that were not available for Wabash River. The results from this project will enable to: (i) investigate the role of velocity as a surrogate flood early warning signal by evaluating stage and velocity data; (ii) quantify floodplain storage for different types of flood hydrographs during different flow conditions and seasons; (iii) understand flood hydrodynamics at Wabash-Tippecanoe river confluence; (iv) determine the role of backwater flows in describing flood hydrographs and peak attenuation at downstream locations; (v) observe the effect of river hydrodynamics on fish habitat usage and community structure; and (vi) quantify the effect of river hydrodynamics on ecosystem services.

Publications: Nothing to report. The website with data is currently only accessible to Indiana Water Science Center and the PI.

Grant Submissions: Nothing to report.

Students: Nikhil Sangwan, MS student in Civil Engineering, was involved in helping with the instrumentation set-up. Currently, Siddharth Saksena, MS student in Civil Engineering, is setting-up the HEC-RAS model for the Wabash-Tippecanoe confluence.

Identifying Opportunities for Soil and Water Conservation With Indiana s County Surveyors and Drainage Boards

Basic Information

Title:	Identifying Opportunities for Soil and Water Conservation With Indiana s County Surveyors and Drainage Boards
Project Number:	2012IN335B
Start Date:	3/1/2012
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	Entire State
Research Category:	Social Sciences
Focus Category:	Agriculture, Conservation, Hydrology
Descriptors:	None
Principal Investigators:	Linda Prokopy, Nathan Mullendore

Publications

1. Mullendore, Nathan and Linda S. Prokopy. 2013. Surveying the Surveyors: Exploring Awareness, Attitudes, and Behavior among Indiana's Ditch Managers. 34th Annual IWRA Spring Symposium.
2. Mullendore, Nathan and Linda S. Prokopy. 2013. Surveying the Surveyors: Exploring Awareness, Attitudes, and Behavior among Indiana's Ditch Managers. 34th Annual IWRA Spring Symposium.

Identifying Opportunities for Soil and Water Conservation With Indiana's County Surveyors and Drainage Boards

Focus Categories: AG, COV, HYDROL, LIP, M&P, NPP, WQL, WQN
Keywords: Drainage Water Management, Drainage Policy, Channelized Streams, Water Quality, Nonpoint Source Pollution, Diffusion of Innovation
Principal Investigator: Linda Stalker Prokopy, Ph.D.

May 2013 – Progress Update

Abstract/Summary
Indiana's water quality and quantity problems have been widely recognized and documented. Since more than 70% of the state's total area consists of agricultural lands, a significant portion of conservation effort has focused on farmers and the management practices they can voluntarily adopt to protect soil and water resources. Comparatively less attention has been given to the structure and maintenance of the waterways themselves. Indiana contains tens of thousands of miles of "regulated drains" that are controlled by county surveyors and county drainage boards. These lower order streams represent biotic communities that comprise the headwaters of the state's many rivers and creeks. Traditional management, however, reduces these waterways to their most basic function as conveyances, ignoring their role in the ecosystem as hosts for biotic and abiotic processes that actively regulate the fate and transport of nutrients and farm chemicals. Novel techniques and practices such as two-stage ditches, controlled drainage structures, in-ground bioreactors, and constructed in-stream wetlands represent promising alternatives to traditional management approaches. Despite extensive development and research, many of these tools remain underutilized. Drainage water management inherently involves agreements and cooperation between multiple stakeholders with different missions and goals, a factor that contributes to slow diffusion of innovation. This project seeks to better understand the primary decision makers behind drainage water management. By exploring the attitudes, knowledge, and motivations of Indiana's county surveyors and drainage board members, insights will be gained into how these groups function and make management decisions, helping to foster new collaborations and earlier adoption of conservation technology.

Problem
Indiana's rivers and streams exhibit a variety of water quality problems, many of which are attributable to erosion and runoff from agricultural lands. Anthropogenic nutrient loading, chemical contamination, and sedimentation affect aquatic systems throughout the state, causing cumulative problems downstream in local places like the Eagle Creek Reservoir and as

far away as the Gulf of Mexico. In many ways, however, Indiana's water resource issues are as much a problem of *quantity* as they are of *quality*. Private landowners and other land managers have significantly modified drainage pathways to achieve agricultural production goals, protect private property, and prevent localized flooding. These actions have increased the magnitude of peak flows and the frequency of major flood events, exacerbating the water quality problems caused by land management practices.

Indiana's 92 county surveyors and 100+ drainage boards collectively oversee tens of thousands of miles of "regulated drains", which are open ditches or tile drains with precise legal and geographic definitions. While certain drain types and maintenance regimes are undeniably part of water resource problems, improvements can just as easily make them part of the solution. Techniques and practices such as two-stage ditches, controlled drainage structures, in-ground bioreactors, and constructed in-stream wetlands represent promising alternatives to traditional management approaches. This project seeks to better understand the primary decision makers behind drainage water management. By exploring the attitudes, knowledge, and motivations of Indiana's county surveyors and drainage board members, insights will be gained into how these groups function and make management decisions, helping to foster new collaborations and earlier adoption of conservation technology.

Research Objectives

- Survey county surveyors and drainage board members in Indiana to understand their awareness of and attitudes toward water quality issues and innovative drainage practices
- Analyze data and prepare one extension publication and one peer-reviewed journal article discussing results and recommendations
- Disseminate findings to conservation partners in Indiana at professional meetings and conferences

Methodology

The survey instruments employed in this research project will be distributed to all of Indiana's county surveyors by email. Reminder emails and phone calls will be used to achieve an adequate response rate. Mail surveys will be distributed to drainage board members throughout the state using a five-wave approach (Dillman 2000). Data will be analyzed using SPSS.

Progress to Date

In the early phases of this project, we reviewed the limited literature regarding the social dimensions of ditch management. We also reviewed results from previous informal surveys of

Indiana surveyors. The knowledge gained from these experiences was combined with feedback from other conservation agencies to design our survey instrument. We have worked with the Indiana County Surveyors Association, The Nature Conservancy, and other Purdue University researchers to revise the survey, with plans to distribute it in spring 2013.

Several opportunities to utilize the results arose during the design process. We will be meeting with The Nature Conservancy in June to discuss how our social dimensions data can be used to improve two-stage ditch outreach. We will also incorporate our results into an ongoing project with the Environmental Defense Fund that focuses on holistic watershed planning, including innovative in-stream filtration practices.

Publications

Mullendore, Nathan and Linda S. Prokopy. Surveying the Surveyors: Exploring Awareness, Attitudes, and Behavior among Indiana's Ditch Managers. Abstract selected for presentation at the 34th Annual IWRA Spring Symposium, June 20, 2013.

Grant Submissions

None

Students

A total of six undergraduate and graduate students have participated in this project:

Brian MacGowan (Graduate)
Patrick Freeland (Graduate)
Rebecca Perry-Hill (Graduate)
Amber Mase (Graduate)
Ziang Cheng (Undergraduate)
Liz Monell (Undergraduate)

Niche breadth variation of fish assemblages for the entire Wabash River

Basic Information

Title:	Niche breadth variation of fish assemblages for the entire Wabash River
Project Number:	2012IN341B
Start Date:	3/1/2012
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	IN-006
Research Category:	Biological Sciences
Focus Category:	Ecology, Geomorphological Processes, Hydrogeochemistry
Descriptors:	None
Principal Investigators:	Mark Pyron

Publications

1. None to report
2. None to report

General Report Format

Report Format

Project Id:
Title: Niche breadth variation of fish assemblages for the entire Wabash River
Project Type: Research
Start Date: 3/01/2012 **End Date:** 2/28/2013
Congressional District: Indiana 6th Congressional District
Focus Categories: ECL, GEOMOR, HYDGEO, HYDROL
Keywords: Fish assemblages, geomorphology, hydrology, substrates
Principal Investigators: Mark Pyron

Abstract / Summary Understanding the species- and assemblage-habitat relationships of large river fishes has been historically limited by the large size and spatial complexity of river ecosystems. I have successfully used a Geographic Information System (GIS) database constructed with spatially explicit physical data as well as information on occurrences of individual taxa to detect habitat use patterns for complete fish assemblages at smaller scales. I tested for fish assemblage variation in the Wabash River from the upper reaches to the confluence with the Ohio River. Niche breadth and niche position were calculated using environmental variables (bathymetry, riverbed sediment composition, woody debris presence, and flow velocity) assembled in a spatially explicit GIS framework.

Problem: The stream fishes of Indiana provide the state many positive returns including benefits to anglers, for ecosystem services, and as a portion of a natural heritage. Although there are several local extinctions, the majority of fish species that were here historically are still present. However, the current fish assemblages of Indiana have higher occurrences of species that are tolerant to human disturbances (Jacquemin & Pyron 2011). There is a need to further our understanding of natural fish assemblages and how they respond to habitat changes.

The Wabash River includes the longest free-flowing stretch in the eastern U.S. and as such, is a unique system for study. Fish assemblages in the Wabash River vary spatially and temporally, and this variation appears to be caused in part by hydrologic variation. Fish assemblages change predictably from upstream to downstream (Vannote et al. 1980), with changes in river gradient and hydrology that produce increasingly smaller substrate particle sizes with decreasing gradient (Pyron & Lauer 2004). Temporal changes in fish assemblages vary with spatial scale. At local site scales, annual flood event frequency controls the substrate composition (Pyron et al. 2010) and local fish assemblage structure varies with substrate composition (Mueller & Pyron 2010). At a 230-km river distance spatial scale, the fish assemblage underwent gradual, predictable change during a 25-year period (Pyron et al. 2006). During this 25-year period, hydrologic alterations have increased (Pyron & Neumann 2008), and these alterations likely had negative impacts on stream assemblages.

The associations between stream fish species abundance, assemblage structure, and available habitat have been studied using two primary approaches (Jackson et al. 2001). The first approach relies on small-scale studies of microhabitat use or habitat partitioning and is often based on detailed measures of local habitat variation combined with subsequent observations of individual fish uses of the quantified habitats (e.g., Grossman & Freeman 1987). While useful for describing fish species distributions and assemblage structure at the scale of small stream reaches, Jackson et al. (2001) concluded that the accumulation of such detailed information would be overwhelming for describing species- and assemblage-habitat relationships at larger spatial scales. The second approach uses large-scale studies to test for assemblage patterns that are correlated with environmental variables. Such large-scale studies examine the effects of environmental variables on fish assemblage structure at watershed or larger scales (Hoeinghaus et al. 2007). While both approaches have merits, neither is sufficient to effectively determine how fish species are distributed relative to spatially explicit habitat availability in large rivers, which are intermediate between the smaller streams studied in the first approach

and the watershed scale focused on in the second approach. Consequently, our understanding of the ecology of large river taxa is relatively limited.

Understanding the species- and assemblage-habitat relationships of large river fishes has been historically limited by the large size and spatial complexity of river ecosystems. However, we have used a Geographic Information System (GIS) database constructed with spatially explicit physical data and information on occurrences of individual taxa to detect habitat use patterns for species (Pyron et al. 2011). Our pilot study successfully demonstrated the potential for testing hypotheses associated with environmental factors that have not been amenable with other analytical approaches. This current proposal is to apply the same method for collecting and analyzing fish species data for the complete Wabash River length (700 km) to identify niche breadth for all fishes, and variation in fish assemblage structure for an entire river. Niche breadth and niche position are calculated using environmental variables (bathymetry, riverbed sediment composition, woody debris presence, and flow velocity) assembled in a spatially explicit GIS framework (Knouft et al. 2011). We will use three resolutions to identify variation in niche breadth at different scales.

Methodology:

FIELD COLLECTIONS/LAB PROCESSING

I collected environmental data for the entire Wabash River length and fish samples for four 5-km reaches. I collected channel bathymetry data using sonar-based depth measurements (Pyron et al. 2011) along the Wabash River from Lafayette, IN to the confluence with the Ohio River. Sediment composition data were collected at the 5-km collection reaches using a pole method (Mueller & Pyron 2010). At each location where the pole is probed into the sediment, a water velocity observation at 60 % of depth was collected, and a GPS waypoint recorded on a GPS unit. Woody debris locations throughout the reach were recorded as presence from sonar information.

I used a boat electrofisher to sample fishes along 5-km transects at four locations to represent variation in geomorphology (Figure 1). The boat is outfitted with a electrofishing apparatus and associated booms and electrodes, and is operated at a frequency of 60 Hz and 8 AMPS. I sampled in a back-and-forth pattern from shore to shore. Electrofishing was conducted in a downstream direction with one crew member on the bow identifying stunned fish (spotter), one crew member piloting the boat, one crew member (recorder) recording species identified by the spotter and entering waypoints on a Trimble GeoXH handheld Global Positioning System (GPS), which provides locality data at sub-30 cm accuracy. Fishes stunned with the boat electrofisher were not captured; rather, spotters, selected based on their familiarity with Wabash River fish species, identified stunned fishes as they surface. This allowed efficient sampling for multiple reaches during a few days while at the same time maintaining species-level identification for most sampled fishes. The GPS unit was used to record waypoints representing the positions (latitude and longitude) of each fish or group of fishes identified by the spotters as the boat was piloted in a downstream direction at < 4 km / h. For each waypoint entered, the recorder noted all species identified by the spotters for that waypoint. Thus, waypoints provide spatially explicit data describing the locations in the river where one or more individual fishes were observed so that these data can be incorporated into a GIS for calculation of species-habitat associations.

ANALYSES

Habitat data from sidescan sonar images were converted to GIS point layers. These data were used with an inverse distance weighting interpolation method in ArcGIS (version 9.3) to estimate values of bathymetry, sediment, and water velocity of the area that was not surveyed within the stream channel of the study reach (Dinehart & Burau 2005, Knouft et al. 2011). Resulting GIS datasets from the interpolation routine are a set of raster files containing continuous bathymetry, sediment, and water velocity data. Because habitat data often co-vary, I reduced the GIS environmental data using Principal Components Analysis (PCA). Habitat use by each species in each sampling period was calculated from mean PC scores for occupied locations (Knouft et al. 2011). The degree of specialization (niche breadth) and marginality (niche position) of the habitat occupied by a species can be quantified by comparing the mean and variance occupied by a species to the mean and variance of the global range of available habitats. Niche breadth is quantified as the standard deviation of the global habitat divided by the standard deviation of the species habitat, and measures the relative range of habitat in which a population occurs (Hirzel et al. 2002). Species with low scores (high niche breadth) are habitat generalists that occupy habitats with relatively high variance compared to the global habitat variance, while species with high scores (low niche breadth) are habitat specialists that occupy narrow ranges of habitat with a low variance compared to the global habitat variance (Doledec et al. 2000). Niche position is the deviation of the species habitat from the global habitat mean and is quantified as the absolute difference between the global habitat mean and the species habitat mean, divided by 1.96 standard deviations to remove bias associated with variance in the global distribution (Hirzel et al. 2002), allowing comparisons across sites or sampling periods. Niche position scores can range from 0 to 1, with a score of 0 indicating a species mean at the global mean, while a score of 1 indicates a species that occupies habitats relatively far from the global mean.

Niche specialization and niche position estimates were generated for each species using global mean and standard deviation values calculated from each GIS-based PC dataset and the mean and standard deviation measures of species habitat use (Knouft et al. 2011). Population density (individuals/collection site) for each species was calculated as the total number of individuals collected divided by the total number of collection sites. Multivariate analysis (Canonical Correspondence Analysis) was used to test hypotheses for the relationship between niche measures and population density: 1) niche specialization is negatively correlated with population density among species; 2) niche position is negatively correlated with population density among species; and, 3) a model incorporating measures of niche specialization and niche position to predict variation in population density.

Preliminary Results. We captured 3,520 individuals in 51 species of fishes at 1,713 GPS coordinates, in the four 5-km reaches (Table 1). We used a Canonical Correspondence Analysis (CCA) to examine patterns in species abundances with environmental variables. The CCA resulted in two significant axes ($P < 0.01$) based on randomizations of eigenvalues and species-environment correlations (Figure 2). Latitude and longitude locations, substrate size, mean water depth, and presence of woody debris provided explanation of fish assemblage variation in the CCA.

My laboratory is currently analyzing these data for a final report that will be submitted before Sep 2013.

Figure 1. Locations of four 5-km reaches on the Wabash River.

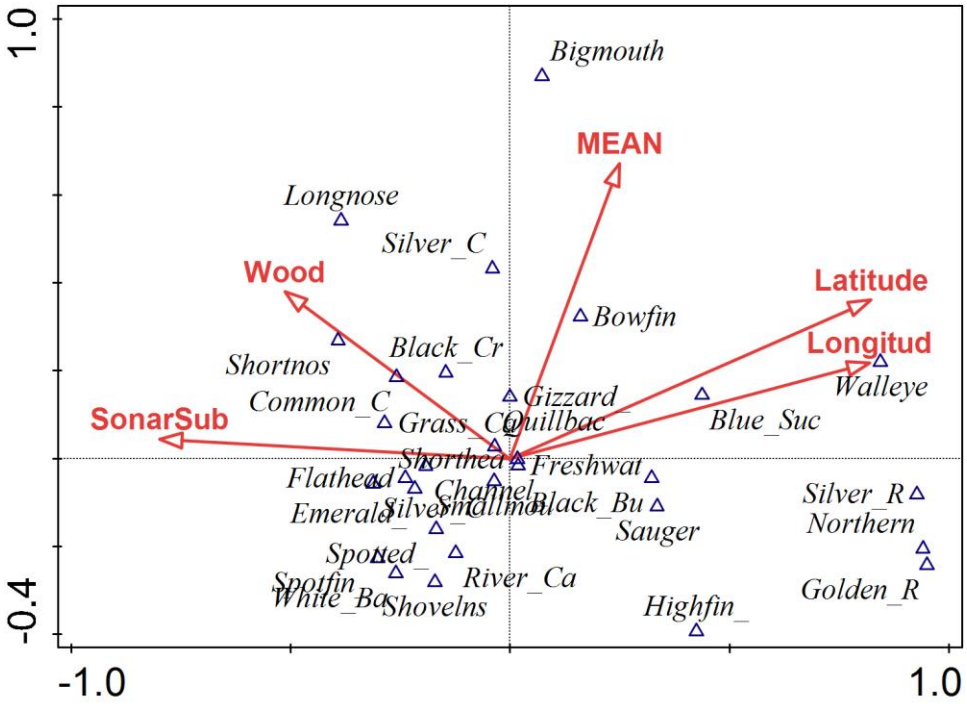


Figure 2. First two axes of a Canonical Correspondence Analysis for fish and environmental variables at four 5-km reaches of the Wabash River. Vectors represent significant environmental predictors of fish abundance.

Major Conclusions and Significance. Our GIS analyses resulted in the ability to predict Wabash River fish assemblage variation using several environmental variables. The results demonstrated that river location was the strongest variable explaining variation in fish assemblages. Fish assemblages are expected to change predictably from upstream to downstream (Vannote et al. 1980), with changes in river gradient and hydrology that produce increasingly smaller substrate particle sizes with decreasing gradient (Pyron & Lauer 2004). The downstream locations had larger substrates, increased woody debris, and similar mean depth than upstream sites.

Fish assemblages change temporally with human effects and resulting in local or regional extinctions (Matthews 1998). The Wabash River is not a pristine ecosystem and has multiple current anthropogenic impacts, including altered hydrologic regime, agricultural effects, and urbanization (Pyron et al. 2006). The altered hydrology likely contributes strongly to variation in substrate composition in the river. The altered hydrology of Wabash River includes increased minimum flows, decreased maximum flows, and increased fall rates that are a result of agricultural management of water drainage, and dam releases from upstream reservoirs. Local effects of increased fall rates are bank erosion, especially in locations lacking riparian vegetation. Sloughing of banks that are composed of fine sediments results in increased silt substrates and wider, shallow channel form. Potential improvements to the hydrology and stability of the Wabash River include modification of dam releases to more closely mimic natural precipitation events

(Richter & Thomas 2007), and reducing the rapid drainage of water in agricultural subwatersheds, allowing water to reach groundwater tables.

References

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Jacquemin, S. & Pyron, M. 2011. Fishes of Indiana streams: current and historic assemblage structure. Hydrobiologia 665:39-50.

Knouft, J.H., Caruso, N.M., Dupre, P.J., Anderson, K.A., Trumbo, D.R. & Puccinelli, J. 2011. Using fine-scale GIS data to assess the relationship between intra-annual environmental niche variability and population density in a local stream fish assemblage. Methods in Ecology and Evolution 2:303-311.

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Pyron, M., Lauer, T.E. & Gammon, J.R. 2006. Stability of the Wabash River fish assemblages from 1974 to 1998. Freshwater Biology 51: 1789-1797.

Pyron, M. & Neumann, K. 2008. Hydrologic alterations in the Wabash River watershed. River Research and Applications 24:1-10.

Richter, B.D. & Thomas, G.A. 2007. Restoring environmental flows by modifying dam operations. Ecology and Society 12:12 online URL: http:www.ecologyandsociety.org/vol12/iss1/art12/

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R. & Cushing, C.E. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences 37:130-137.

Publications I expect to submit a manuscript in fall 2014 using these current data. My lab group has published the following manuscripts from IWRRC funded research:

Pritchett, J. and M. Pyron. 2012. Fish assemblages respond to habitat and hydrology in the Wabash River, Indiana. River Research and Applications 28:1501-1509.

Pyron, M., R. Goforth, J. Beugly, S. Morlock and M. Kim. 2011. A GIS approach for explanation of fish assemblage structure in a large river. River Systems 19:239-247.

Pritchett, J. and M. Pyron. 2011. Predicting fish assemblages from substrate variation in a turbid river: grab samples compared with pole probing. North American Journal of Fisheries Management 31:574-579.

Grant Submissions: na

Students The project included two graduate students (Luke Etchison, Julie Backus) and one undergraduate student (Adam Garza).

Connectivity & Functioning of Wetlandscapes: A Complex Network Modeling Analysis

Basic Information

Title:	Connectivity & Functioning of Wetlandscapes: A Complex Network Modeling Analysis
Project Number:	2012IN342B
Start Date:	3/28/2012
End Date:	2/1/2014
Funding Source:	104B
Congressional District:	IN-004
Research Category:	Climate and Hydrologic Processes
Focus Category:	Wetlands, None, None
Descriptors:	None
Principal Investigators:	Suresh Rao

Publications

1. Logan, L. et al. 2013. Freshwater Wetlands: Balancing Food and Water Security with Resilience of Ecological and Social Systems. In: CLIM Book Chapter (in press).
2. Logan, L. et al. 2013. Freshwater Wetlands: Balancing Food and Water Security with Resilience of Ecological and Social Systems. In: CLIM Book Chapter (in press).

IWRRC Project Update (May 31, 2013)

P.S.C. Rao

School of Civil Engineering & Agronomy Department

Purdue University

Abstract / Summary

Wetlands occupy a small fraction of the land area, and yet constitute an important element of landscapes by providing multiple, essential ecological services. Wetlands play a vital role as landscape elements, providing ecological, biogeochemical, and hydrological benefits as well as socioeconomic, food, and freshwater security. Large-scale alterations to landscapes and the hydrologic cycle to meet demands of growing increasingly affluent populations have left wetlands impaired. Typically viewed as single units, we conceptualize wetlands as hydrologically and ecologically connected self-organized, dynamic, complex networks. Wetland networks and their many functions are linked to produce persistent self-emergent patterns and landscape resilience. Through this viewpoint, wetland restoration and recreation efforts can move toward optimization strategies that preserve landscape resilience while promoting socioeconomic, food, and freshwater water security

Hydrologic temporal dynamics of wetlands, as governed by hydro-climatic forcing (i.e., rainfall patterns; ET losses) and anthropogenic modifications to land use and management (e.g., artificial surface and subsurface drainage networks; urbanization; croplands) have profound impacts on the spatial structure, hydrologic and ecological connectivity, and ecological functioning of inland wetlands. This project focuses on various approaches to modeling the spatial and temporal dynamics of wetlands, as an approach to exploring the patterns evolving at multiple scales as a result of anthropogenic and hydro-climatic variability.

Problem:

Wetlands play diverse roles in providing multiple ecosystem services (e.g., as hydrologic and biogeochemical buffers and filters; as ecological habitats for aquatic vegetation and animals). Wholesale loss of wetlands because of land use change and clime change is a major problem across the globe. In addition, environmental heterogeneity (e.g., spatial and temporal variability in rainfall and ET loss patterns) introduces another element of variability in how the structure and functioning of wetlands evolves in diverse landscapes. The resilience of aquatic species that thrive in wetlands and the spatial/temporal patterns that evolve are then dependent to a large extent on the hydrologic variability within a given wetland and its connectivity to all other wetlands distributed across the landscape of interest.

Research Objectives:

The primary goal of this project is to develop new modeling techniques to understand eco-hydrological spatial and temporal dynamics of wetlands. Wetlands are conceived as water bodies of different sizes and shapes, and spatially organized

in particular patterns across the wetlandscapes. Temporal dynamics of the spatial distribution and eco-hydrologic connectivity of these wetlands is of specific interest. Various stochastic modeling approaches and network analysis tools are being used to understand the role of hydro-climatic forcing, landscape topography, pedologic and vegetation features.

Methodology:

Research approaches used include the following:

1. Stochastic analytical and numerical modeling approaches for modeling water balance in wetlands
2. Critical analysis and synthesis of published data on spatial distribution and organization of wetlands in the U.S.
3. Preliminary analysis of wetlands in a region to explore vulnerability of ecological networks with the each network serving as a node.
4. Preliminary network analysis of species dispersal in a wetland ecological network.
5. Review and critical assessment of published papers related to wetlands structure and functions
6. Develop research collaborations with colleagues at Purdue and universities in other locations

Results:

1. A parsimonious stochastic numerical model was developed (Matlab codes) to describe temporal dynamics of water depth and storage in geographically and hydrologically isolated wetlands. Major drivers of hydrologic variability in wetlands have been now identified through model simulations [in collaboration with Prof. Gianluca Botter at the University of Padua, Italy].
2. This model was extended to consider geographically isolated but hydrologically connected (via shallow groundwater) wetlands distributed across a landscape [with Dr. Jeryang Park, former PhD student; now Asst. Prof. at Hongik University, Seoul, South Korea]
3. Analysis of frequency distribution of wetland sizes in U.S. 50 states, and spatial analysis in selected regions (e.g., North Dakota; North Carolina; etc.) [with Dr. Heather Gall, post-doc at Purdue Univ., Jeryang Park & Elin Karlsson, PhD student, Purdue Univ.]
4. Preliminary analysis of wetlands as aquatic habitat nodes in an ecological network has been completed, and the vulnerability to fragmentation with removal (loss) of key nodes has been evaluated. In addition, a new stochastic modeling framework is being developed to examine the dynamics of species dispersal along a wetland ecological network [with Dr. Jeryang Park; Samueil Hassan, Ph.D. student; Prof. Satish Ukkusuri, Purdue University].
5. The role of wetlands as water bodies, spatially organized across a wetlandscape, on water and energy exchange at a regional scale is being evaluated [with Elin Karlsson; and Prof. Dev Niyogi, Purdue Univ.]

6. Work has been initiated [with Prof. Nancy Emery, Purdue University] to examine the correlations between hydrologic variability within vernal pool landscapes (in California) and the evolution and persistence of vegetation patterns.
7. Helped co-found Global Wetlands Ecohydrology Network (GWEN), an international consortium of scientists engaged in the study of wetlands as ecological habitats governed primarily of hydrologic variability [with Prof. Gia Destouni, Stockholm University].

Major Conclusions and Significance:

Size frequency distribution of wetlands, like that of lakes, follows a truncated power-law for data derived for all 50 U.S. states. Size truncation depends on the landscape changes imposed by urbanization or conversion to croplands, and of course on the topographic features of the landscapes. Hydrologic dynamics within a single wetland or multiple wetlands across a landscape are dominants controlled by the rainfall patterns (frequency and depth variations); wetland geometry (i.e., bathymetry); and degree of hydrologic connectivity with the landscape (e.g., groundwater). The model we developed can be used to forecast such hydrologic variability in wetlands, and then examine how the dynamics of multiple wetland functions are moderated by hydrologic conditions.

Wetlands serves as habitat nodes in ecological networks, and loss of nodes can lead to habitat fragmentation and impairment of aquatic species dispersal. Preliminary work completed to date has identified several approaches, based on network analysis, as being useful for this purpose. Hydrologic variability within a single wetland, and across several wetlands in a landscape, also controls the emergence of the spatial and temporal patterns of aquatic vegetation. Initial work, combining field observations with model simulations, has been initiated to understand the link between hydrologic temporal variability and distribution and persistence of vegetation in landscapes containing vernal pools.

Publications:

Logan, L. et al. 2013. Freshwater Wetlands: Balancing Food and Water Security with Resilience of Ecological and Social Systems. In: CLIM Book Chapter (in press).

Grant Submissions:

- NESS Doctoral Fellowship Application (submitted by Elin Karlsson)
- NSF Pre-proposal submitted (invited for full proposal); with Dr. Nancy Emery, Purdue University

Students & Post-Docs

- Elin Karlsson; current PhD student, ESE IGP; co-supervised by Drs. Suresh Rao & Dev Niyogi

- Jeryang Park; completed PhD (Civil Eng.), December 2012; Currently Asst Prof., Civil Engineering Dept., Hongik University, Seoul, South Korea
- Heather Gall; Post-doc (School of Civil Eng.,), Purdue Univ.; Asst Prof., Ag & Biol Eng Dept, Penn State Univ (Effective July 1, 2013)
- Bryan Otten, Junior, EEE, Purdue Univ. (COE SURF student)

Attached-Growth Lagoons for Wastewater Nitrification in Small Communities

Basic Information

Title:	Attached-Growth Lagoons for Wastewater Nitrification in Small Communities
Project Number:	2013IN356B
Start Date:	3/1/2013
End Date:	2/28/2015
Funding Source:	104B
Congressional District:	IN-004
Research Category:	Water Quality
Focus Category:	Nutrients, Treatment, Water Quality
Descriptors:	
Principal Investigators:	Ernest R. Blatchley, Ronald F. Turco

Publications

There are no publications.

ATTACHED-GROWTH SYSTEM FOR NITRIFICATION AT LOW TEMPERATURE

Zhe Sun[1] and Ernest R. Blatchley III[1,2]
[1]Lyles School of Civil Engineering, Purdue University
[2]Division of Environmental & Ecological Engineering, Purdue University

Abstract: Lagoon systems are commonly used in small communities for domestic wastewater treatment. These systems are simple and economical to operate, and are often appropriate for use in areas where land costs are low. Lagoons are effective for removal of conventional pollutants under warm-weather conditions; however, biochemical nitrification is hindered in suspended-growth lagoons during periods of extended cold weather. Attached-growth nitrification systems, as compared to suspended-growth systems, have been reported to yield improved biochemical nitrification under cold-weather conditions. In this work we investigated a commercially-available biochemical nitrification reactor (i.e., BOBBER) that includes surfaces to promote development of attached-growth nitrifying communities. The BOBBER system has been applied to an aerobic lagoon system at Wingate, IN. Results of monitoring of this system have indicated improvements in oxidation of ammonia-N, especially during winter months.

To examine the behavior of this system in a more controlled setting, two laboratory-scale mini-BOBBER systems were installed in a temperature-controlled room. The systems were treated with an ammonium chloride feed solution, and allowed to approach steady-state conditions at nominally 20°C, 15°C, 10°C, and 5°C. Concentrations of nitrate and nitrite, as well as pH and other process variables were measured daily during these experiments. These results were analyzed to quantify ammonia-N removal rates. Similar calculations were applied to the data from the full-scale lagoon system at Wingate.

Ammonia-N removal in the laboratory-scale lagoon system always exceeded 95%, even when at temperatures of 4.6°C. However, the full-scale lagoon system was not as effective, with ammonia-N removal of approximately 74% at the same temperature. Possible explanations for these differences in behavior include: competition from heterotrophs, relatively low influent ammonia-loading into Wingate second lagoon, and a relatively small population of nitrifying bacteria at Wingate.

These findings suggest that it is possible to maintain effective biochemical nitrification in attached-growth systems even under cold-weather conditions. Satisfactory performance of these systems will depend on hydraulic design and N loading rates of the systems.

Problem and Research Objectives: Attached-growth systems appear to be less sensitive to cold weather conditions than suspended-growth systems. Field implementation of a commercial system (BOBBER) designed to promote development of an attached-growth nitrifying community at Wingate, IN has shown promise, in that nitrification was improved at during winter conditions. As such, these systems may represent a viable alternative for ammonia-N control in small wastewater treatment facilities. Therefore, to improve our understanding of attached-growth nitrification in lagoon systems, a laboratory-scale (mini-BOBBER) lagoon system was installed in the Environmental Engineering Laboratories at Purdue University.

The objectives of this study were to:

i. Compare nitrification dynamics at low temperature in a real lagoon system using full-scale BOBBER reactors with a laboratory-scale lagoon system using mini-BOBBER reactors.
ii. Quantify nitrification dynamics under controlled conditions, involving pure ammonia-N feed without organic carbon substrates.
iii. Investigate the relationships between ammonia-N removal rate and ammonia-N concentration in attached-growth systems at different temperatures.

Methodology: A series of mini-BOBBER experiments was conducted. The mini-BOBBER systems were designed and operated to simulate the nitrification process at the Wingate lagoon system. Both types of media used in these experiments were suspended at Wingate second lagoon for 2 weeks to establish the nitrifier attached communities.

To mimic attached-growth nitrification at Wingate, two mini-BOBBER systems provided by Bradley Environmental were installed in a temperature controlled room. The reactors were operated under the identical conditions, in terms of reactor volume, temperature, influent composition, influent flow rate and discharge rate, aeration rate, etc. BOBBER system 1 was operated with Kaldnes K1 media with a specific surface area of 300 m^2/m^3 while the BOBBER system 2 was operated with RK media with a specific attach area of 750 m^2/m^3. The two BOBBER reactors were operated for 101 days with 4 temperature stages (nominally 20°C, 15°C, 10°C, and 5°C). For each temperature stage, the performance of the system was allowed to approach a steady-state condition. Figure 1 is a schematic illustration of the setup of the mini BOBBER experiments.

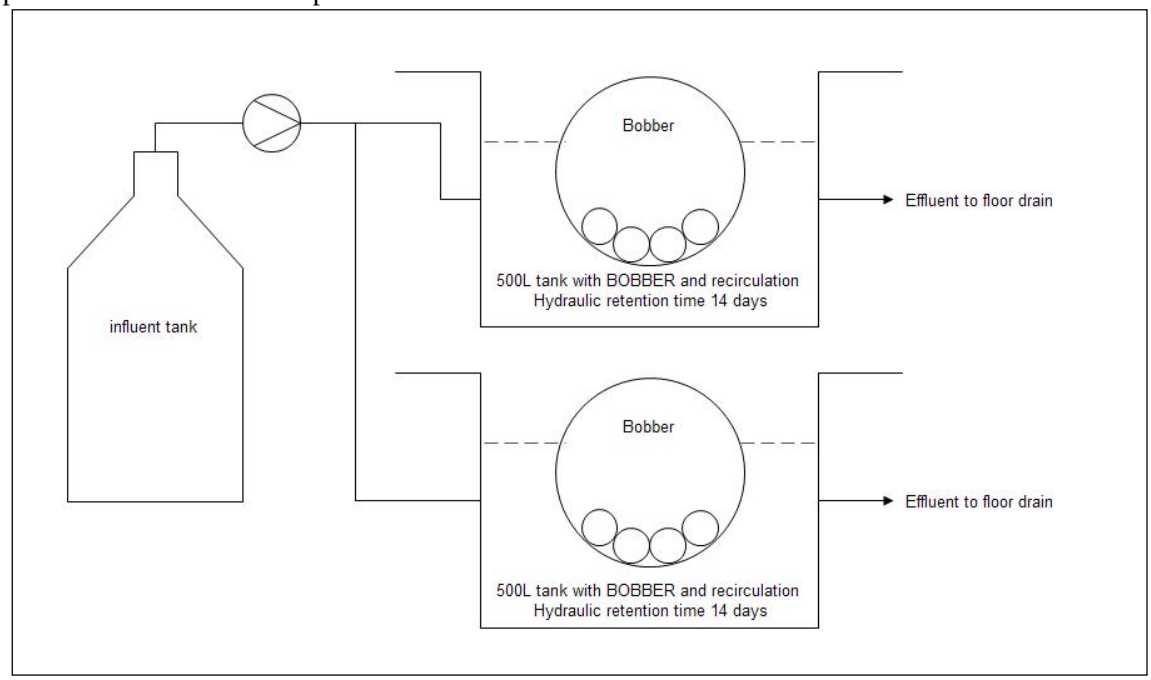

Figure 1. Schematic of mini BOBBER experiments.

Synthetic wastewater (SWW) from the influent tank was pumped into two tanks that included mini-BOBBER reactors (See Figure 2 (a)) at a fixed flow rate (24.5 mL/min). This flow rate was chosen to provide a hydraulic retention time of 14 days, which is representative of the operation of the Wingate lagoon system. In order to keep influent and discharge flow rate the same, the treated water was designed to overflow through a PVC pipe (See Figure 2a) to a floor drain.

The mini-BOBBER reactor is pictured in Figure 2b. There were two air supplies: air supply #1 provided aeration and drew water into the circulating part from the inlet at the bottom; air supply #2 promoted circulation of water and media in the mini-BOBBER reactor. Both air supplies promoted O_2 transfer, which was needed to satisfy O_2 that was expressed by biochemical oxidation of influent ammonia-N. Water was designed to spray out from outlet on the top of mini-BOBBER reactor.

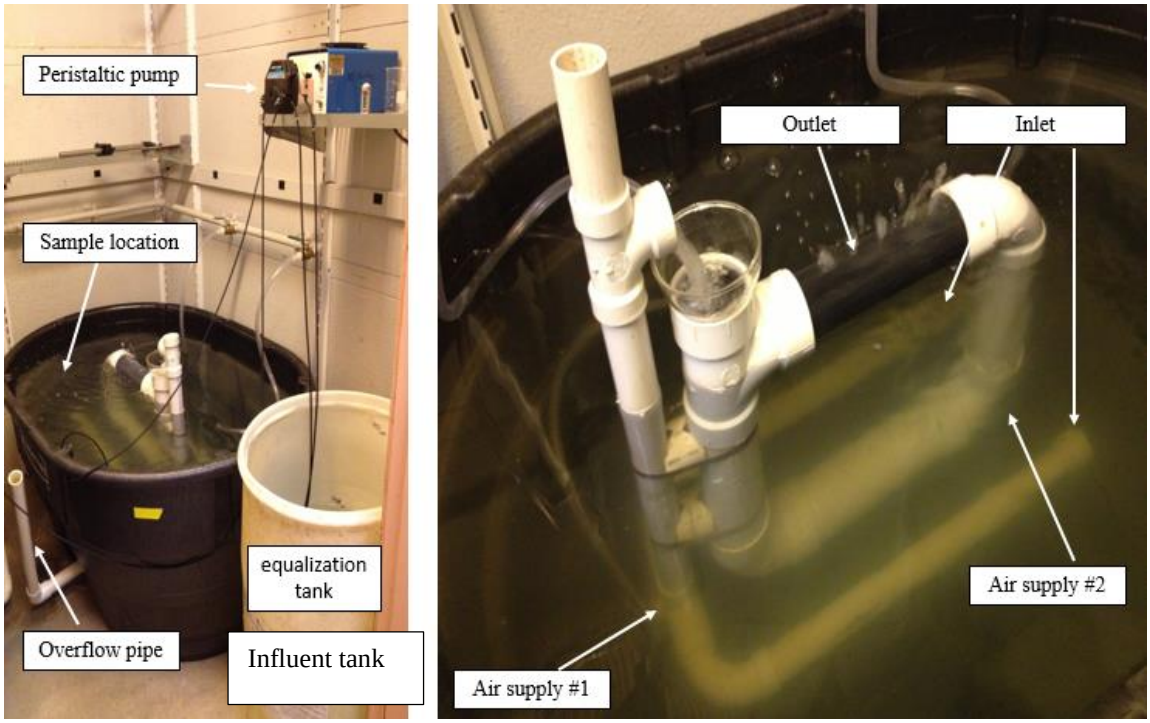

Figure 2. Images of mini-BOBBER experiment design (a) Left, image of lab-scale lagoon system; (b) Right, image of mini-BOBBER reactor.

Experiments with the mini-BOBBER systems were conducted as follows:

1. Each tank was filled with 500 L synthetic wastewater (SWW, composition defined below) and placed in a temperature-controlled room at an initial temperature of 20°C.
2. Both types of media were inoculated with a mixed culture of bacteria from the Wingate secondary lagoon, and subsequently the inoculated media (3.78 L of media per tank) were placed in the two mini-BOBBER reactors.
3. The influent tank was filled with SWW (Roughly 70 L per day) for a whole day before service, and the peristaltic pump was set at the flow rate of 24.5 mL/min. Water samples in influent tank and both mini-BOBBER tanks were taken periodically to measure pH, temperature, DO, ammonia-N, nitrite-N, and nitrate-N.
4. When the system had been operated under steady-state at 20°C for 2 weeks, the temperature was decreased to 15°C. Then, following similar steps, the system temperature was decreased to 10°C and 5°C, sequentially.
5. The system was operated at 5°C for 46 days.

SWW was used for all experiments in this study. SWW containing roughly 40 mg/L ammonia-N was prepared by dissolving ammonia chloride in dechlorinated tap water. This ammonia-N loading was determined based on the average influent ammonia-N concentration at Wingate lagoon. No organic carbon was included in the SWW. The composition of Purdue tap water, as reported by the Purdue water utility for some metals and other inorganic constituents, is included in Table 1.

Table 1. Composition of Purdue Tap Water with respect to common inorganic constituents.

pH	Alkalinity (mg/L as $CaCO_3$)	Calcium hardness (mg/L as $CaCO_3$)	Magnesium hardness (mg/L as $CaCO_3$)	Fe (mg/L)	Mn (mg/L)	Cu (mg/L)
7	320	300	120	0.49	0.154	0.57

According to the Consumer Confidence Report provided by Purdue University, West Lafayette Campus Water Works, most nutrients required for nitrifiers growth are included in Purdue tap water. Furthermore, tap water was dechlorinated by sodium bisulfite prior to use to minimize the influence of chlorine on microorganisms. During dechlorination, sodium bisulfite was added at a concentration of 10 mg/L to tap water to create a stock solution. Sodium bisulfite was added in slight stoichiometric excess of residual chlorine concentration to ensure complete dechlorination. Free chlorine and total chlorine were measured by the DPD/KI Colorimetric Method, as defined by *Standard Methods for Examination of Water and Wastewater*.

Samples were collected for measurements of pH, DO, temperature, ammonia-N, nitrite-N, and nitrate-N. pH was measured by an Accumet model 50 pH/ion/conductivity meter (Fisher Scientific) connected to a ROSS Ultra combination pH electrode (ORION). DO and temperature were measured by a YSI 550A DO meter. Ammonia-N was measured by an Accumet model 50 pH/ion/conductivity meter (Fisher Scientific) connected to a 95-12 ammonia electrode (ORIONNitrite-N and nitrate-N were measured by an Ion Chromatography (DIONEX) using an IonPac AS19 4*25 mm Analytical column (RFIC).

Principle Findings: The mini-BOBBER experiments were conducted over a period of 101 days, during which time the performance of each system was monitored using daily measurements of influent and effluent quality. Operating parameters including pH, DO, and temperature were measured daily, and remained stable during the entire experiment. Only the results for ammonia-N removal and the fates of nitrogen are reported here. Additional details of the results of these experiments will be presented in a manuscript that is to be submitted for publication.

Figure 3 illustrates time-course measurements of ammonia-N concentration for the mini-BOBBER experiments. Ammonia-N concentration showed a rapid decline during the first 10 days of the experiment. Then ammonia-N concentration reached steady-state roughly at first temperature stage. Comparatively small changes of ammonia-N concentration were observed during the three subsequent temperature stages (See Figure 3).

The rapid ammonia-N concentration decrease during the first 10 days of the experiment was probably due to a rapid increase of the concentration of nitrifiers, which was promoted by a high initial ammonia-N concentration (roughly 45 mg/L as ammonia-N). This period was also characterized by a rapid decrease of DO; at the end of this period, and for the remainder of the experiment, DO concentrations were maintained at or near equilibrium values for the temperatures of the experiment (data not shown). Because the system appeared to be undergoing acclimation in the first 10 days of the experiment, average ammonia-N concentration in the effluent was calculated without data from the first 10 days.

The average ammonia-N concentrations in both mini-BOBBER tanks during the four stages of the experiment are listed in Table 2. As temperature decreased, the average ammonia-N concentration increased slightly. However, effluent ammonia-N concentration was consistently well below the permit limitation values that are imposed on most municipal WWTPs, which are typically in the range of 3-6 mg/L (as N).

Table 2. Average ammonia-N concentration in both mini BOBBER tanks.

	Ammonia-N (mg/L)	
Temperature (°C)	Kaldnes K1 media	RK media
19.7	0.76	0.81
14.3	0.80	0.77
9.6	0.91	0.98
4.6	1.50	1.43

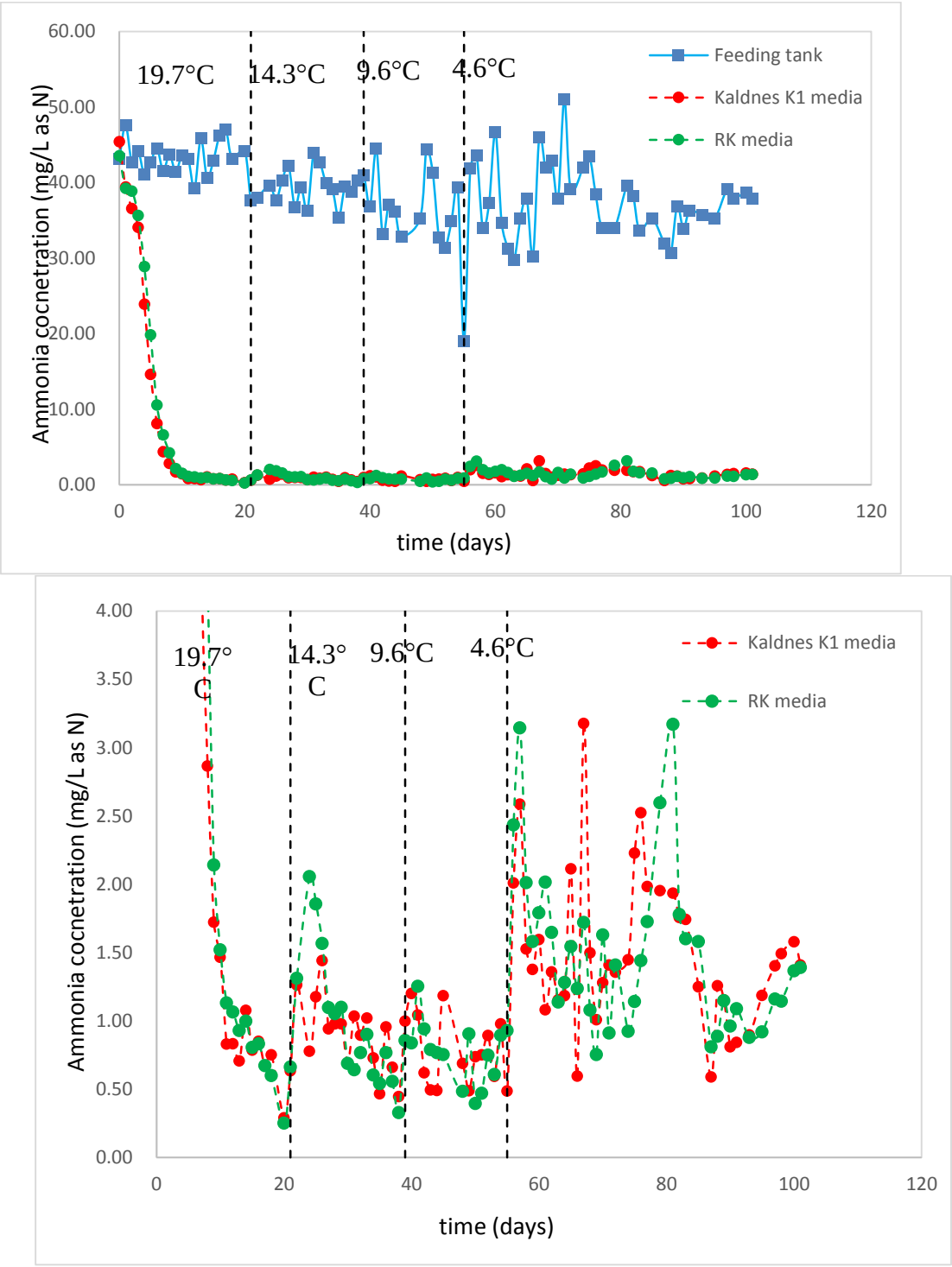

Figure 3. Time-course measurements of Ammonia-N from mini-BOBBER experiments. Also included are daily ammonia-N concentrations of both mini-BOBBER systems corresponding to average water temperature in each stage of the experiment. Upper panel gives the general ammonia-N concentration in both feeding tank and reactor, while the lower panel gives a more detailed ammonia-N concentration in the reactor with small vertical scale.

Time-course measurements of Nitrite-N and nitrate-N for the mini BOBBER experiments are illustrated in Figure 4. The nitrite-N and nitrate-N concentrations in the reactor with Kaldnes K1 media achieved equilibrium faster than the reactor with RK media. The possible reason for this is the different media type used. Since Kaldnes K1 media is smaller in size, the mixing for Kaldnes K1 media could be more efficient, which could lead to faster consumption of ammonia-N.

However, after the initial ammonia-N loadings in both reactors were depleted, nitrite-N and nitrate-N in both reactors achieved similar steady-state concentration. Additionally, nearly all nitrite-N in both reactors was converted to nitrate-N. Ammonia-N appeared to be a growth-limiting factor and the surface area for both types of media were not fully utilized. Therefore, in spite of the difference in specific surface area for both types of media, the steady-state nitrite-N and nitrate-N concentrations in both reactors were similar.

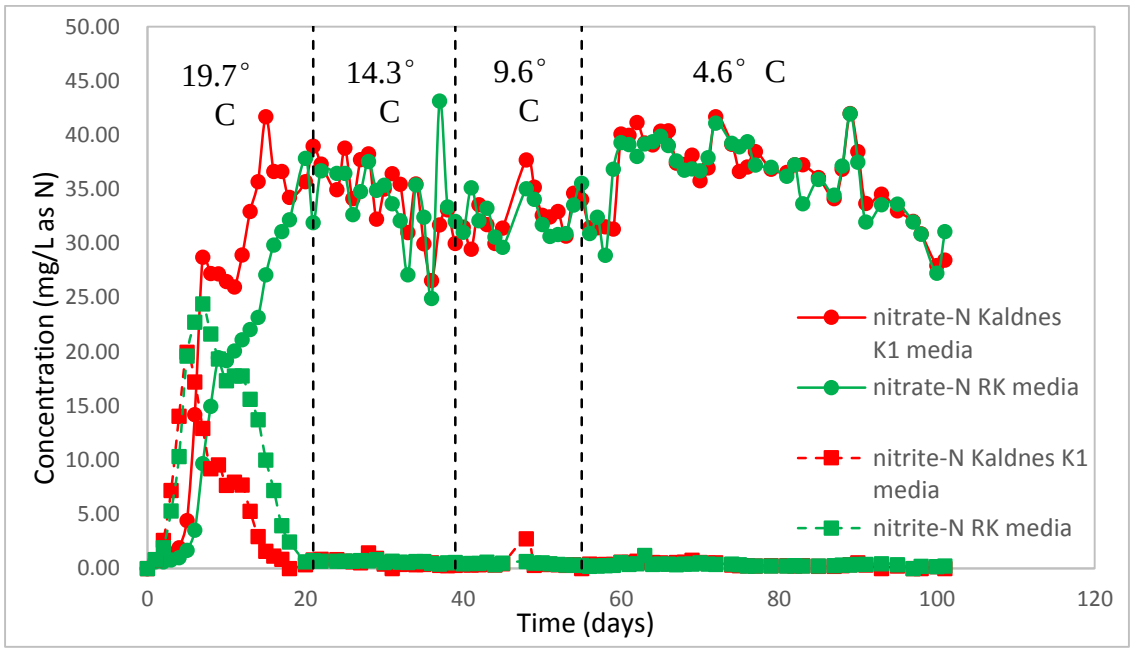

Figure 4. Nitrite-N and nitrate-N data of mini BOBBER experiments. Also included are daily nitrite-N and nitrate-N concentrations of both mini BOBBER systems corresponding to average water temperature in each stage of the experiment.

Nitrogen balances were conducted for both mini-BOBBER reactors based on the assumptions that all nitrogen in the system existed as ammonia-N, nitrite-N, and nitrate-N. Therefore, the nitrogen balance calculations were conducted under the assumption that no molecular nitrogen (N_2) or other forms of nitrogen (*e.g.*, N_2O) were present in the system. No measurements of N_2 or N_2O were included in this research. Therefore, formation of N_2 or N_2O in the liquid phase and escape of these compounds from the liquid phase to the gas phase were not accounted for in this model. Based on these assumptions, a nitrogen balance was developed, as shown in Eq. (1):

$$C_{total,N}=C_{ammonia,N}+C_{nitirite,N}+C_{nitrate,N} \quad (1)$$

Figure 5 illustrates the time-course behavior of $C_{total,N}$ from these nitrogen balance calculations for both reactors. The total nitrogen for both systems was dominated by nitrate-N for the majority of the period of these experiments, exclusive of the induction period (first 10-15 days), even after the temperature was reduced to 4.6°C. This indicates that the mini-BOBBER reactors with both types of media were able to convert most ammonia-N to nitrate-N at this ammonia-N loading, even under low temperature conditions.

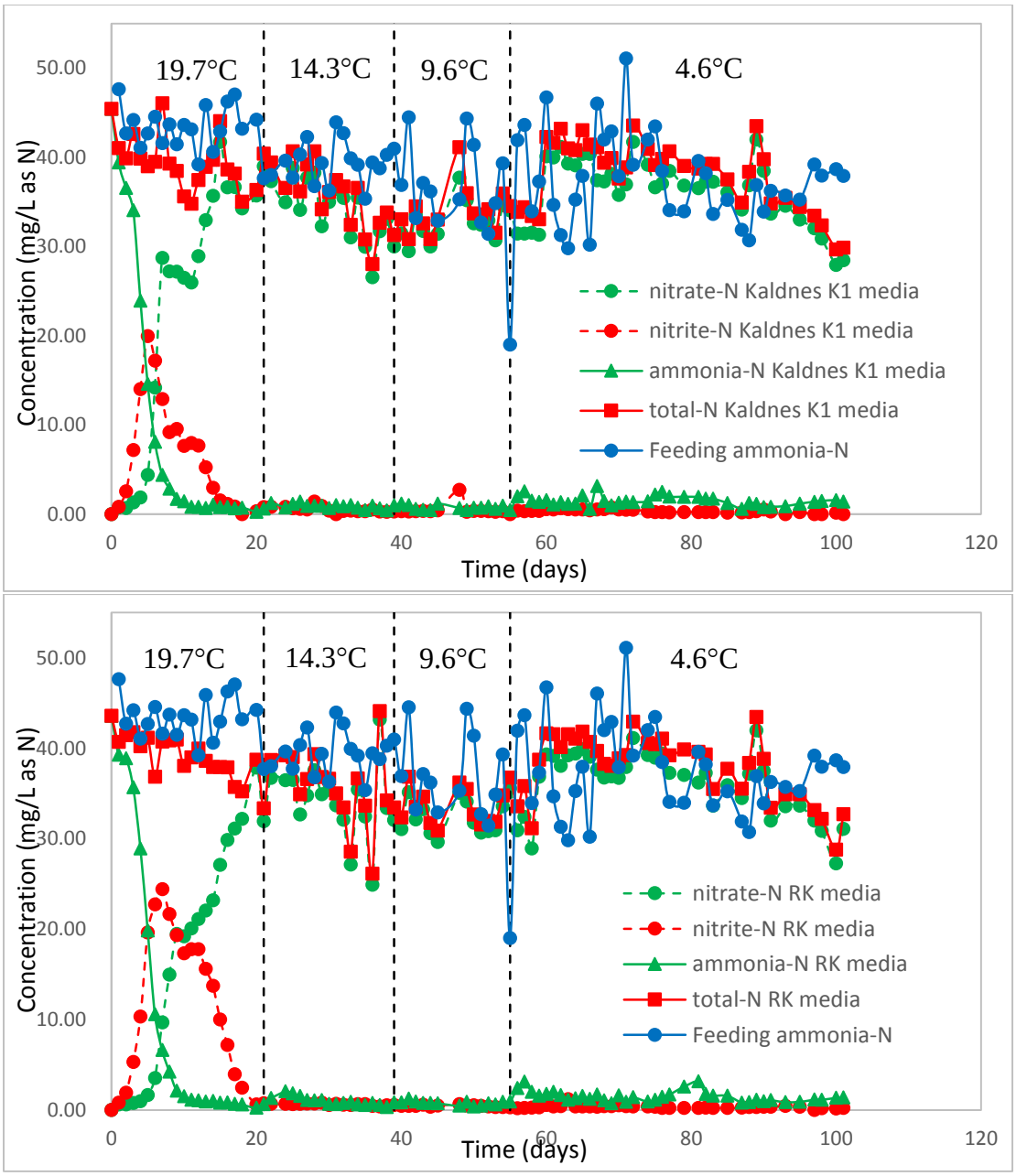

Figure 5. Nitrogen balance: (a) upper panel, nitrogen balance of the system using Kaldnes K1 media; (b) lower panel, nitrogen balance of the system using RK media.

Calculations of ammonia removal rate were conducted by application of the principles of mass balance. The reactor systems were each assumed to be well-mixed and only the media surfaces were counted in the calculation (*i.e.*, the walls of the tanks were not included in these calculations).

Figure 6 illustrates the specific ammonia-N removal rate as a function of time for both reactor systems over the course of this experiment. Table 3 provides a summary of the mean and standard deviation of the specific ammonia-N removal rate for both systems in all four phases of the experiment. Collectively, these summaries indicate that the two systems achieved similar ammonia-N removal rates after the initial period of acclimation. Temperature did not appear to alter the rate of ammonia-N removal substantially under this operating condition.

Table 3. Average specific ammonia-N removal rate at different temperatures.

	Specific ammonia-N removal rate (g/day/m^2)	
Temperature	Kaldnes K1 media	RK media
19.7 C	1.41+0.23	0.59+0.13
14.3 C	1.14+0.17	0.46+0.07
9.6 C	1.07+0.20	0.43+0.06
4.6 C	1.07+0.32	0.42+0.12
Media surface area (m^2)	1.14	2.85

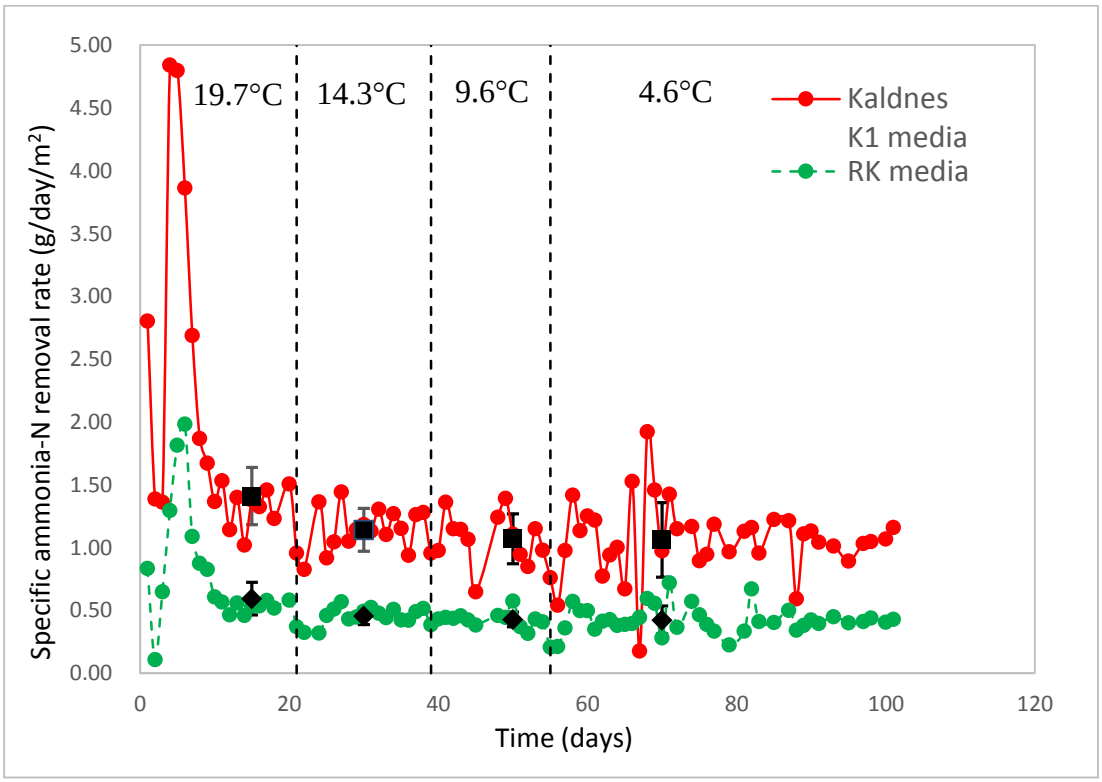

Figure 6. Specific ammonia-N removal rate of mini BOBBER experiments. Also included the average specific ammonia-N removal rate during each temperature stage.

In the mini-BOBBER experiments, efficient ammonia-N removal was achieved at temperatures from 4.6°C to 19.6°C. The observed ammonia-N removal was over 95%, even at 4.6°C. However, ammonia-N removal at the Wingate second lagoon where the BOBBERs were installed was less efficient (approximately 71%). The average specific ammonia-N removal rate (at temperature 5°C) at the second lagoon of the Wingate system was 0.66 g/day·m^2. For comparison, the average specific ammonia-N removal rate (at temperature 5°C) observed from the mini-BOBBER experiments was 1.07 g/day·m^2.

Possible reasons for the discrepancy between observations from mini BOBBER experiments and Wingate second lagoon include the following:

- The mini-BOBBER system was provided with an influent that contained ammonia-N as the only source of reduced-N, with no organic carbon. The presence of organic carbon will promote development of a heterotrophic community, which will compete with the nitrifying community. The presence of organic carbon and heterotrophs in real lagoon systems could hinder nitrification.
- The influent ammonia-N concentration into the second lagoon at Wingate was roughly 20 mg/L as N in cold-weather periods (During warm periods, the influent ammonia-N concentration into the second lagoon was round 2 mg/L as N). However, the feed solution concentration used in the mini-BOBBER experiments was roughly 40 mg/L as ammonia-N. A lower ammonia-N loading could contribute to the lower ammonia-N removal rate at Wingate. For example, if the ammonia loading is lower than the nitrification rate, the nitrifiers will consume most of the ammonia-N, and the effluent ammonia-N will be low.
- Nitrifiers in the mini-BOBBER experiments were fed a relatively stable ammonia-N loading. However, the ammonia-N loading at Wingate second lagoon was less stable. During warm periods, almost all ammonia-N was oxidized in lagoon 1 and the influent ammonia-N concentration into the second lagoon was typically 2 mg/L or less (ammonia-N loading for the mini-BOBBER experiments was roughly 40 mg/L). So the accumulation of nitrifiers at Wingate was presumably slower and the nitrifying bacteria population stayed at relatively low level, as compared to the mini-BOBBER system. When temperature decreased, the ammonia-N loading in the Wingate second lagoon increased, possibly because there were not enough nitrifiers to deal with the large ammonia-N loading. As a result, the ammonia-N removal rate decreased.
- The average bulk ammonia-N concentration in Wingate second lagoon (at temperature 5°C level) was 11.1 mg/L, while the average bulk ammonia-N concentration in mini BOBBER system (at temperature 5°C level) was approximately 1.5 mg/L. According to the relatively low ammonia-N removal rate with a higher bulk ammonia-N concentration observed at Wingate second lagoon, the main cause of this should be lack of nitrifying bacteria.

Significance: Attached-growth nitrification with mini-BOBBER reactors was demonstrated to achieve efficient ammonia-N removal (>95%) over a wide range of temperatures (4.6°C to 19.7°C). According to monitoring data from the Wingate lagoon system, the ammonia-N removal rate was increased greatly after the installation of the BOBBER reactors. However, the improvement still needs to be validated under conditions of extended cold temperature.

Attached-growth nitrification was observed to be less temperature sensitive than suspended-growth nitrification, which supports the application of attached-growth nitrification during cold periods. Additionally, the maximum ammonia-N removal rate under 4.7°C and 22.6°C were measured with SSW. However, these results were observed with simple ammonia-N loading, the performance with real wastewater still needs to be tested.

Attached-growth nitrification (such as BOBBER reactors) has great potential to deal with ammonia-N under low temperature range. This could be applied in many small communities with winter nitrification problems at relatively low cost (compared to mechanical wastewater treatment plant). Additionally, pre-attached BOBBER reactors could be added to new constructed wastewater treatment systems, the pre-attached BOBBER reactors are easy to transport and install, and they are able to perform nitrification immediately after installation.

Publications: A manuscript is being prepared for possible publication in the refereed literature. Another manuscript has been submitted for consideration as a podium presentation at a regional conference.

Grant Submissions: No additional proposals have been submitted. However, we are working with Bradley Environmental to examine possible sources of additional funding to extend this work.

Students: Funds from this grant were used to support Mr. Zhe Sun, who used this work as the basis of his M.S. research. Mr. Sun successfully defended his thesis in Spring 2014.

Multi-Year Assessment of Water Use and Reuse in the Wabash River Watershed

Basic Information

Title:	Multi-Year Assessment of Water Use and Reuse in the Wabash River Watershed
Project Number:	2013IN358B
Start Date:	3/1/2013
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	IN-004
Research Category:	Engineering
Focus Category:	Water Use, Management and Planning, Surface Water
Descriptors:	
Principal Investigators:	Loring F Nies, Chad Jafvert

Publications

1. Indiana Association for Floodplain and Stormwater Management, Inc September 11-13, 2013 --- Pokagon State Park “Assessment of Water Use and Reuse in the Wabash River Watershed”
2. Wabash River Workshop IWRRC and Purdue Water Community December 13, 2013 “Assessment of Water Use and Reuse in the Wabash River Watershed”

Assessment of Water Use and Reuse in the Wabash River Watershed

Principal Investigator: Dr. Larry Nies, Civil Engineering Purdue University

Abstract / Summary
The management of water resources is a critical function to sustain food production, manufacturing, energy production, potable water production, recreation, and landscape beneficial uses, including maintaining species diversity. Every sector of the economy is dependent upon access to water. Vast amounts of data are collected by numerous agencies without coordination, integration or organization of the many heterogeneous data sets such that sophisticated high performance data analysis and resource management is not possible. The untapped potential for increased economic development and improved environmental stewardship achievable by application of modern information management tools is enormous. Because these data are archived in discrete heterogeneous databases maintained by State and federal agencies the capability to holistically manage critical water resources at watershed scales (e.g. the Wabash River) does not exist. By assessing current and past water use and reuse in the Wabash River Watershed, we will be able to identify how much, and for what purposes, the water resources of the basin are being used, and if there have been any temporal changes or emerging patterns over the past 5 years. We will identify characteristics of current databases and determine how to enhance access to currently available data to support watershed scale management, and recommend how to modify the acquisition and storage of water data to adapt to future information needs. Recently, the NRC concluded that an analysis of the current status of indirect (i.e., unplanned) water reuse is a critical need for understanding the role of water reuse in meeting fresh water demands. The implications in terms of water resources planning, human health, and freshwater ecosystems conservation are significant. We propose to complete a multi-year indirect water reuse analysis for the Wabash River Watershed by documenting both water discharges and water extractions within the watershed geographically and by specific use categories.

Problem and Research Objectives:
Massive amounts of water quality and water resource data are collected and archived continuously by federal and environmental regulatory agencies on all 50 States. The management of water resources is a vital and critical function necessary to sustain food production, manufacturing, energy production, potable water production, recreation, and landscape beneficial uses, including maintaining species diversity. Every sector of the economy is dependent upon access to water. Although vast amounts of data are collected by numerous agencies there is no coordination, integration or organization of the many heterogeneous data sets such that sophisticated high performance data analysis and resource management can occur. The untapped potential for increased economic development and improved environmental stewardship that could be achieved from the application of modern information management tools is enormous. Because these data are archived in discrete heterogeneous databases maintained by State and federal agencies, each with limited scope and mission, the capability to holistically manage critical water resources at watershed scales (e.g. the Wabash River) does not exist. For example it is exceedingly difficult to estimate the amount of *de facto* water reuse that occurs within the Wabash River basin. In the context of population growth, economic development, climate change and environmental stewardship, the increasing demand for freshwater makes it necessary to find innovative ways to manage our water resources in a sustainable way. The Wabash River Watershed has been historically a water rich area. Due to land use changes, river flows have actually increased over the preceding decades and water availability has not been considered a major issue. However, severe droughts such as the one

experienced during the summer of 2012 highlight the need for understanding how the water is being used and reused throughout the watershed. Understanding use patterns by the critical non-agricultural human components and activities, and the implications that these uses have on other systems, including agriculture and natural systems, is crucial information required for effective planning and management of water resources. Moreover, future conditions such as anticipated increasing demand for freshwater resources concurrent with reductions in surface and ground water availability will require preparation to consider new scenarios. We should be able to use currently available data to inform a review of current policies on water use and reuse, and support decision making about new strategies for water resource management. In order to successfully achieve these goals, it is important to have accessible information and management tools that provide relevant information for different stakeholders in water resource management decision making.

Methodology:

The extent of water reuse is measured by calculating the fraction of used water in a surface water resource. It is equal to the ratio of all wastewater discharges above a specific point in the stream to the stream flow at that specific point. The volume of wastewater discharged into a watershed is determined by adding the reported discharge values contained in the U.S. EPA National Pollutant Discharge Elimination System (NPDES) databases on the reported monthly basis. All types (i.e., classifications) of facilities located within the watershed boundaries are considered (U.S. EPA 2010). The U.S. Geological Survey (USGS) National Water Information System (NWIS) database provides historical data on stream flow (NWIS, 2012), and is used as a reference for calculating the relationship between NPDES discharges and stream flow at specific locations and time points in the watershed. For a complete water balance, state agencies' withdrawals databases and USGS water consumption estimates are integrated to determine the volumes of water extracted within the watershed and the purposes of those extractions. The water reuse calculation and water balance will be performed on all 24 8-digit Hydrological Unit Code (HUC) watersheds within the larger watershed. This will further allow integrated analysis at each successively larger HUC levels and at the entire Wabash River watershed level.

Part of this research project consists of developing organizational/relational data structures for the disparate data within the state and federal agencies databases, and to link the resulting merged data set harmoniously with existing visualization and environmental management tools. Available and accessible software, such as Oracle database software, Microsoft Excel and ArcGIS, will be used to develop a set of management tools that include data sets, summary tables, and informative maps. Trends in data from month to month and between months over the 6 year period will be evaluated with statistical software to note any outliner values and/or potential drivers to statistical disparities, such as wet vs. dry year variations, or any reasons for increased demand, such as new energy production systems coming online.

Principle Findings

During the low flow months, humans extracted, used and pumped through a NPDES permitted facilities on average the equivalent of the entire flow of the river. This is equivalent to emptying the entire flow of the Wabash River on a daily basis and refilling it at its confluence with the Ohio River continuously for a four month period.

Significance
Documentation of the "human water cycle" at larger watershed scales provides relevant information to different stakeholders who influence water resource management decision-making. Considering the magnitude of human influence on low flow conditions, prospective well-meaning water recycling projects to reuse wastewater for such purposes as crop or landscape irrigation should carefully consider the effects this could have on the current watershed hydrology and downstream human and ecosystem needs.

Publications

Presentations
Indiana Association for Floodplain and Stormwater Management, Inc
September 11-13, 2013 --- Pokagon State Park
"Assessment of Water Use and Reuse in the Wabash River Watershed"

Wabash River Workshop
IWRRC and Purdue Water Community
December 13, 2013
"Assessment of Water Use and Reuse in the Wabash River Watershed"

Manuscript
Wiener, M.J., C. T. Jafvert, and L. F. Nies. 2014. Water Use and Reuse in the Wabash River Watershed. Environmental Science and Technology. In Review.

Grant Submissions: None yet.

Students
One PhD student was involved with this project; M. Julia Wiener.

The Role of Silver Nano Particles on Microbial Activities in Natural Surface Water: Effects of Aqueous Chemistry

Basic Information

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Publication

1. non Yet

The Role of Silver Nano Particles on Microbial Activities in Natural Surface Water: Effects of Aqueous Chemistry

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Abstract
Silver nano particles (NPs) are the largest and fastest growing category of nanotechnology-based medicines and consumer products. After the products are consumed, washed, or discarded, silver NPs can be released into the environment, especially to a natural water body like the Wabash River. Silver is reported to be highly toxic to some aquatic organisms and as an antibiotic may also damage or alter the most abundant and vulnerable beneficial microbial communities in the environment, such as *Gordonia* sp. The Wabash River flows through 19 counties and receives large amounts of treated sewage or untreated storm sewage from many cities in Indiana. Silver can enter the river water either as nanoparticles, nanoparticle aggregates, or soluble ions. It is reported that the toxicity of silver nanoparticles is primarily related to the released Ag^+ as the metal silver is oxidized by oxygen and then dissolved. However, considering the complex chemical background of natural surface water, the silver NPs can have complicated processes such as dispersion vs. aggregation, dissolution vs. precipitation, oxidation vs. reduction, and complexation with the background chemicals such as chloride, surfactants, and dissolved natural organic matters. The experimental results show that the average particle size and dispersivity of silver NPs play an important role in the toxicity of silver NPs. Aggregation is signification for silver NPs in DI water. However, the presence of 3 mM SDS or 8 mg/L Suwannee River NOM reduced the aggregation of silver NPs apparently. Overall, the degree of aggregation increased in the order of 3 mM SDS < 8 mg/L NOM < DI water < 10 mM NaCl < 10 mM $NaNO_3$. When the silver NPs had an elevated concentration from 7.3 to 29.2 mg/L in DI water, the log inactivation of the *Gordonia* sp. increased from 0.16±0.04 to 0.45±0.13. However, the log inactivation rate increased to 1.40±0.26 and 0.87±0.12 in the presence of 3 mM SDS or 8 mg/L NOM, respectively with 29.2 mg/L silver NPs. On the other hand, 10 mM NaCl reduced the log inactivation rate to 0.07±0.07 due to causing moderate aggregation of silver NPs and forming likely less toxic silver chloride species.

Problem and Research Objectives
Nanomaterials have been widely introduced into our daily life as medicines and consumer products to improve our health and quality of life. After consumption, these nanomaterials are released into the environment, especially to natural water bodies, such as the Wabash River. However, their environmental consequences are largely unknown. Among various commonly used nanomaterials, silver nano particles (NPs) are of the most concern because of their toxicity. Annual global demand for silver is about 1,040.6 million ounces in 2011 [1]. The metal is used in a vast array of industrial and consumer products. The trend of increasing productions of silver-containing products is expected to continue. Monovalent ionic silver (Ag^+) has broad-spectrum antimicrobial properties. It is used in silver(I) sulfadiazine, the preferred antimicrobial agent for the treatment of serious burns [2]. The increase of bacterial resistance against other antibiotics like penicillin enlarges the usage of silver-based pharmaceutical compounds [3]. In addition, silver NPs are increasingly used in clothing, cosmetics, food storage containers, electronics, and optical devices as an anti-microbial agent [4, 5]. Silver NPs are readily to be released from these products to the environment, especially through washing processes [6].

The Wabash River is an important water resource and ecological system supporting both population growth and the economic development of Indiana. It drains approximately 1,438 square miles through the heartland of Indiana [7]. The Wabash River flows through 19 counties and receives large amounts of treated sewage or untreated storm sewage from many cities

including Bluffton, Logansport, Veedersburg, Lafayette, West Lafayette, and Vincennes in Indiana [7]. Certainly, silver NPs are of a big concern along with other emerging environmental contaminants, such as pharmaceutical compounds.

Silver is reported to be highly toxic to some aquatic organisms [8]. And as an antibiotic, it may also damage or alter beneficial microbial communities in the environment [9, 10]. For the *Gordonia* sp., a suborder of *Corynebacterineae*, possess the ability to degrade xenobiotics and industrial pollutants. Many of the isolates of *Gordonia* have been derived from industrially influenced habitats and wastewater treatment systems [11]. As a result, the *Gordonia* sp. can be regarded as one of the beneficial microorganisms in wastewater plants and natural surface water bodies. Our previous study indicated that the sessile form of the *Gordonia* sp. are more resistant to benzene than the planktonic form [12]. Silver can enter the river water either as nanoparticles, nanoparticle aggregates, or soluble ions [6]. The toxicity of silver nanoparticles is primarily related to the released monovalent ionic silver (Ag^+) as the metal silver is oxidized by oxygen and then dissolved [5, 13], a process that is facilitated at low pH [9]. However, considering the complex chemical background of natural surface waters, the silver NPs can have complicated processes such as dispersion vs. aggregation, dissolution vs. precipitation, oxidation vs. reduction, and complexation with the background chemicals such as chloride, surfactants, and dissolved natural organic matter (NOM). Obviously these processes will affect the forms of silver, and thus its antimicrobial effects. Currently there have been large gaps in knowledge about the aqueous chemistry on the toxicity of silver NPs. Understanding the behaviors of silver NPs at different solution conditions would help to model and predict their toxicity to microorganisms, the most abundant and vulnerable part in the aqueous ecological system.

Three main objectives of this project are listed below.

i) Synthesize and characterize silver NPs, including the particle size and the morphology.
ii) Systematic investigation of the solution chemistry on the release of Ag^+ and the silver morphology. The solution chemistry includes chloride, anionic surfactant, and NOM.
iii) Evaluate the microbial growth rate and activities in a synthetic solution with silver NPs. The *Gordonia* sp., in planktonic form will be tested against silver. The chemical effects observed in objective (ii) will be tested respectively by measuring the inactivation rate of the microorganisms.

Methodology

Synthesis of silver NPs. Nanoparticles of silver were prepared by the method of reduction of the complex cation $[Ag(NH_3)_2]^+$ with D-maltose [3, 14]. The initial concentration of the reaction components were 1 mM and 10 mM for $AgNO_3$ and D-maltose, respectively. The concentration of the used ammonia was 5 mM. Sodium hydroxide solution was added to the reaction system to adjust the value of pH at about 11.5, as well as to achieve a reaction time of several minutes.

Silver NPs' morphology and size measurement. The produced silver particle diameter was about 40~90 nm (see Figure 1). The size distribution and the morphology of the silver NPs were examined by transmission electron microscopy (TEM) (Tecnai G2T20, FEI), dynamic light scattering (DLS) (measuring the hydrodynamic diameter) (Zetasizer Nano ZS, Malvern), and UV-Vis absorbance (Lambda 25, PerkinElmer). The presence of different amounts of anionic surfactant (sodium dodecyl sulfate), chloride, and Suwannee natural organic matter were tested

with respect to the solubility of silver and the morphology of the particles, respectively. Atomic absorption spectroscopy (AAnalyst 200, PerkinElmer) was used to analyze total silver concentration following acidification by 10% (v/v) HNO_3.

Inactivation of the Gordonia sp. with silver NPs. Stock cultures of the *Gordonia* sp. (ATCC# BAA-558) were grown in yeast extract glucose (10 g of dextrose and 10 g of yeast extract per liter of distilled water) at 30 °C and 200 rpm for about 3 days until stationary phase by monitoring the growth by optical density measurement (OD_{600}) at specific time intervals. The microbial concentration was around 10^8 CFU/mL [12]. Evaluation of silver NPs on microbial growth and activities in planktonic form was performed by the following steps.

i) Purify the grown *Gordonia* sp. by centrifugation in a 50 ml Falcon tube at 5000 rpm for 5 min. After centrifugation, remove the supernatant by decanting, resuspend the cells in 25 ml DI water by vortexing completely and centrifuge again as above. Repeat centrifugation for four times totally. Finally, resuspend the purified cells in 25 ml DI water and transfer the cells to a sterile 125 ml flask with 25 ml sterile DI water for a total volume of 50 ml; mix. The target concentration of the purified microbe is 10^5-10^6 CFU/mL.
ii) Mix varied chemicals, such as silver NPs, chloride, Suwannee River natural organic matter, or sodium dodecyl sulphate (SDS) without the purified cells in small sterile test tubes, sonicate the chemical solution mixture for 5 min in an ultrasonic bath. Then vortex the purified cell suspension prepared in Step 3 for 20 s and transfer 1.0 mL to the chemical solution. The final volume of each sample is 3.5 mL (2.5 mL chemical solution + 1.0 mL purified cell suspension). Vortex each sample for 20 s.
iii) After mixing according to the table, put the solution mixtures on a shaker at room temperature shaking for 6 hours.
iv) Do bacterial plate counting at the end of Step iii). Plate 10^{-4}, 10^{-5}, 10^{-6}, and10^{-7} in triplicate.

The dosed silver concentration ranged from 7.3 to 29.2 mg/L. The pH level was between 5.7 and 6.8 for all of the test solutions. The chemical effects were investigated for their effects on the dispersivity and toxicity of silver NPs. In the meantime, control tests of the same chemical solution but without silver NPs were performed to calculate the log inactivation rate, which is $-\log(N/N_0)$, where N is the number/mL of survived microorganisms with silver NPs, N_0 is the number/mL of survived microorganisms of the same chemical solution but without silver NPs.

Sample analyses. All of the sample analyses followed the standard methods [15]. The analyses included total silver concentration by atomic absorption spectroscopy, the size and the morphology of silver NPs by TEM , DLS, and UV-Vis absorbance. The inactivation of *Gordonia* sp. was quantified by heterotrophic plate counts, which the growth medium was composed of 10 g of dextrose, 10 g of yeast extract and 15 g of agar powder per liter of distilled water. The medium was autoclaved at 121 °C with 20 min cycle.

Principle Findings

Characterization of synthesized silver NPs

Figure 1 shows the TEM images of the synthesized silver NPs. From the figure, the silver NPs have globular shape generally. The diameter of the silver NPs was in the range of 40~90 nm. Figure 2 shows the UV-Vis absorbance of the silver NPs, which has the peak of absorbance at 453 nm.

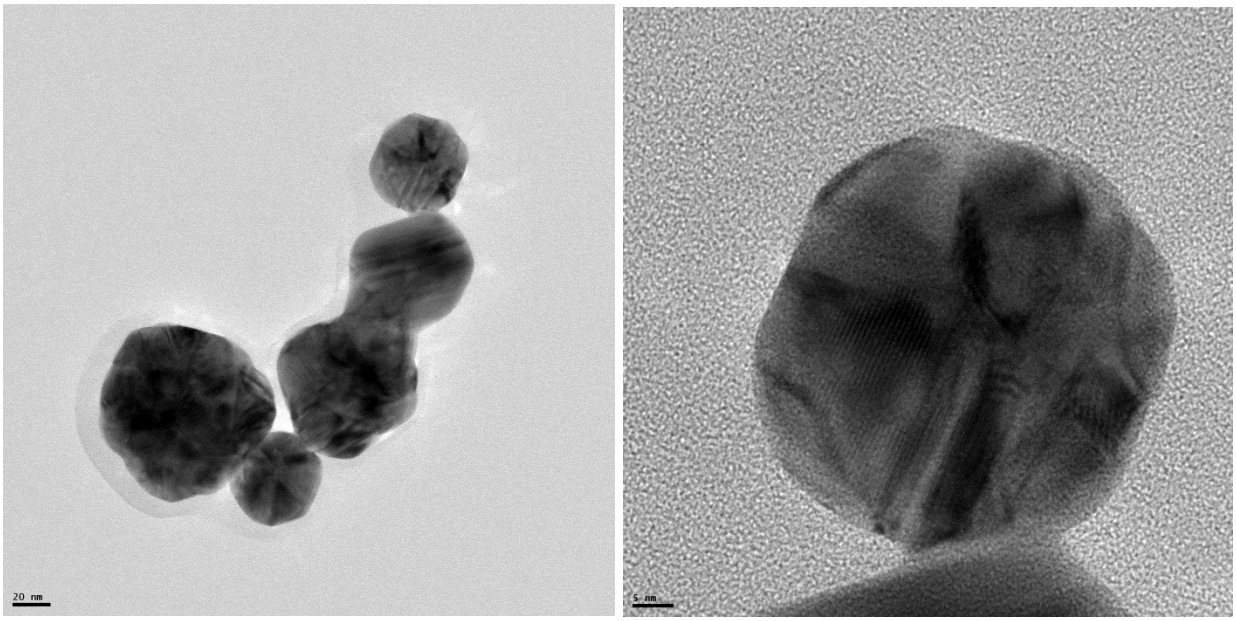

Figure 1. TEM images of the synthesized silver NPs. Copper grids were used in the TEM examination. (The scale bar is 20 nm in the left image and 5 nm in the right image)

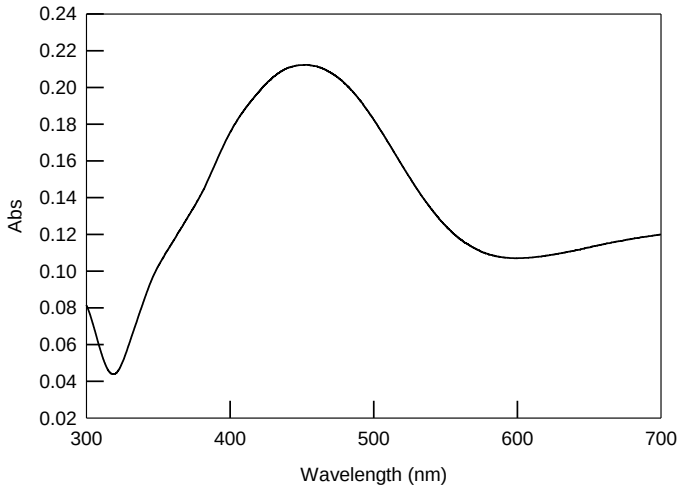

Figure 2. UV-Vis absorbance of the synthesized silver NPs.

Solution chemistry on aggregation of silver NPs

Figure 3 shows the average diameter of the silver NPs in different aqueous solutions at the beginning and after 90 minutes. In DI water, silver NPs aggregated from 154.9±109.4 nm to 295.7±126.5 nm after 90 minutes. Based on the TEM results shown in Figure 1, individual silver NPs ranged from about 40 to 90 nm. As a result, aggregation is signification for silver NPs. However, the presence of 3 mM SDS or 8 mg/L NOM reduced the aggregation of silver NPs apparently, which yielded an average diameter of 161.3±92.2 and 174.3±95.3 nm, respectively

after 90 minutes. In contrast, 10 mM $NaNO_3$ induced the severest aggregation, which had 857.9±245.1 nm after the same period. It is likely that $NaNO_3$ caused double layer compression or charge screening effect of silver NPs, thus enhanced aggregation. Different from $NaNO_3$, NaCl can form silver chloride species with silver NPs. More detailed discussions are presented later. Overall, the degree of aggregation increased in the order of 3 mM SDS < 8 mg/L NOM < DI water < 10 mM NaCl < 10 mM $NaNO_3$.

The average particle size and dispersivity of silver NPs play an important role in determining the toxicity of silver NPs [3, 5, 16]. It was reported that Ag^+ is the dominant species for the antimicrobial effect of silver NPs [13]. Smaller particle size and better dispersivity of silver NPs implies more Ag^+ release and better contact with microorganisms, thus a stronger bactericidal effect is expected. An oxide layer of Ag_2O formed on metallic silver NPs' surface when in contact with dissolved oxygen in water during the synthesis and purification processes [17]:

$$2Ag^0 + \frac{1}{2}O_2 = Ag_2O \qquad (1).$$

The oxidized layer dissolves to release silver ions as follows [17]:

$$Ag_2O(s) + H_2O = 2Ag^+ + 2OH^- \qquad (2).$$

At neutral pH range, the measured zeta potential of silver NPs was around -65 mV [18]. Despite charge repulsion among silver NPs, aggregation still happened as evidenced in Figure 1.

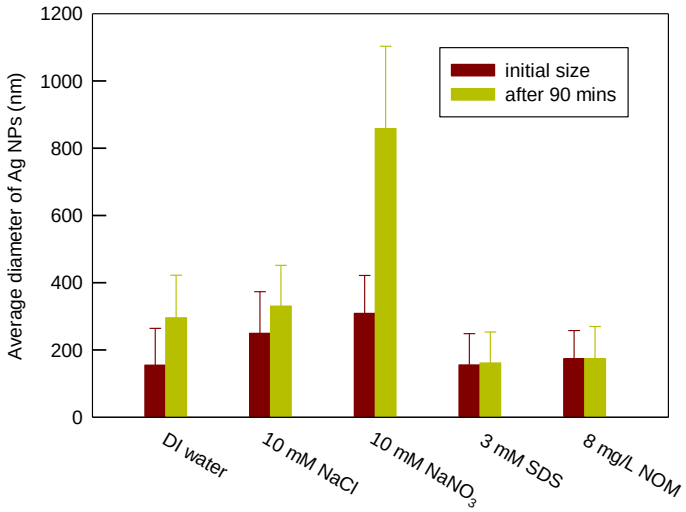

Figure 3. Sizes of the silver NPs in different aqueous solutions at the beginning and after 90 minutes.

Inactivation of Gordonia sp. by silver NPs

Figure 4 shows that *Gordonia* sp. were grown in yeast extract glucose medium at 30 °C and 200 rpm for about 50 hours until stationary phase by monitoring the growth by optical density measurement at 600 nm (OD_{600}) at different time intervals. After that, the *Gordonia* culture was purified by centrifuge with DI water for four times before being mixed with different chemical solutions with or without silver NPs.

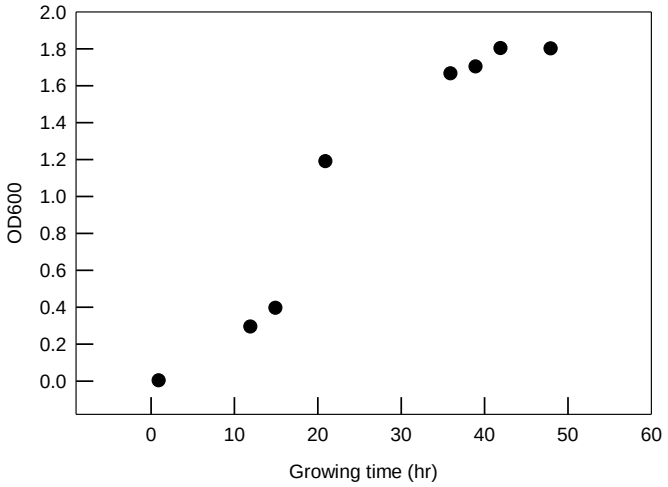

Figure 4. The growing curve of *Gordonia* sp. before the tests of inactivation by silver NPs.

Figure 5 shows that a greater log inactivation of the *Gordonia* sp. occurred at a higher dosage of silver NPs in DI water. More specifically, the log inactivation increased from 0.16±0.04 to 0.45±0.13 when the silver NPs increased from 7.3 to 29.2 mg/L.

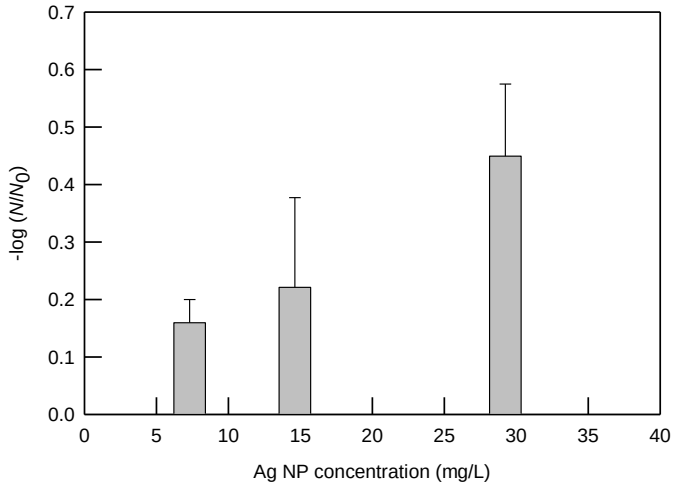

Figure 5. Log inactivation of the *Gordonia* sp. at different silver NPs in DI water.

The presence of SDS enhanced the bactericidal effect of silver NPs as shown in Figure 6. Higher log inactivation rates of *Gordonia* sp. were observed at greater concentrations of SDS. The log inactivation increased from 0.41±0.48 to 1.40±0.26 when the concentration of SDS increased from 1 to 3 mM. Note here the SDS concentration is much less than the critical micelle concentration, which was reported as 7 mM in water [19]. SDS is an anionic surfactant, which can increase steric repulsion among silver NPs [20, 21]. As a result, Figure 3 shows small particle size and well dispersed silver NPs in 3 mM SDS solution, which improved bactericidal effect of silver NPs.

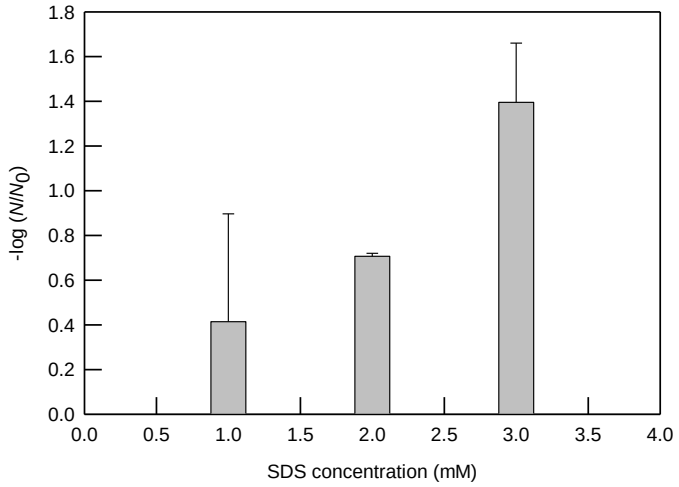

Figure 6. Effect of sodium dodecyl sulfate (SDS) on the log inactivation of the *Gordonia* sp. by 29.2 mg/L silver NPs.

Similar to SDS, NOM exhibits the character of anionic surfactant [22]. It facilitates the dispersion and steric repulsion among silver NPs, as shown in Figure 3. Consequently, like SDS, greater log inactivation of *Gordonia* sp. was found at higher NOM concentrations, i.e. the log inactivation increased from 0.07±0.27 at 2 mg/L NOM to 0.87±0.12 at 8 mg/L NOM. Similar finding was report by Morones et al. [23].

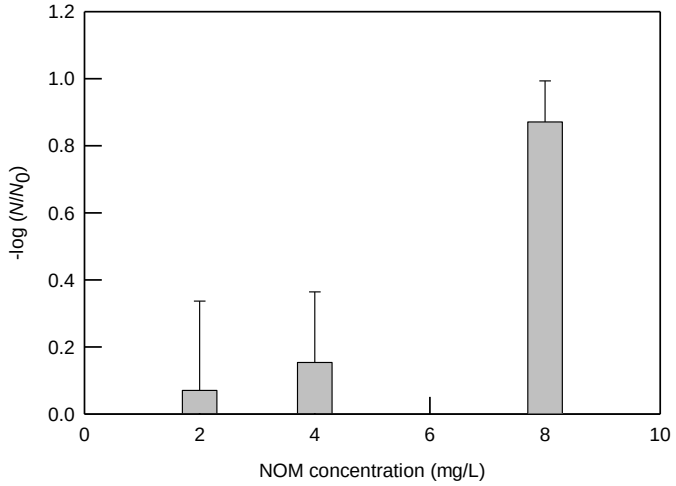

Figure 7. Effect of Suwannee River natural organic matter (NOM) on the log inactivation of the *Gordonia* sp. by 29.2 mg/L silver NPs.

However, the presence of chloride decreased the toxicity of silver NPs. As illustrated in Figure 8, at 10 mM NaCl the log inactivation rate decreased to 0.07±0.07 from 1.06±0.01 at 3 mM NaCl. Figure 3 shows moderate aggregation of silver NPs in the presence of 10 mM NaCl, which may reduce the toxicity of silver NPs. However, chloride may react with Ag^+ to form AgCl(aq), $AgCl_2^-$, and $AgCl_3^{2-}$. At 10 mM NaCl, AgCl(aq) and $AgCl_2^-$ are found at roughly equivalent

concentrations, with lesser amounts of Ag^+ and $AgCl_3^{2-}$ [10]. Consequently, chloride not only caused moderate aggregation of silver NPs, but also reduced the amount of Ag^+, the dominant species for bactericidal effect. Although the bactericidal effect at 3 and 5 mM NaCl is greater than DI water with 29.2 mg/L silver, the control tests were performed in different NaCl solutions or DI water, respectively; thus N_0 is different.

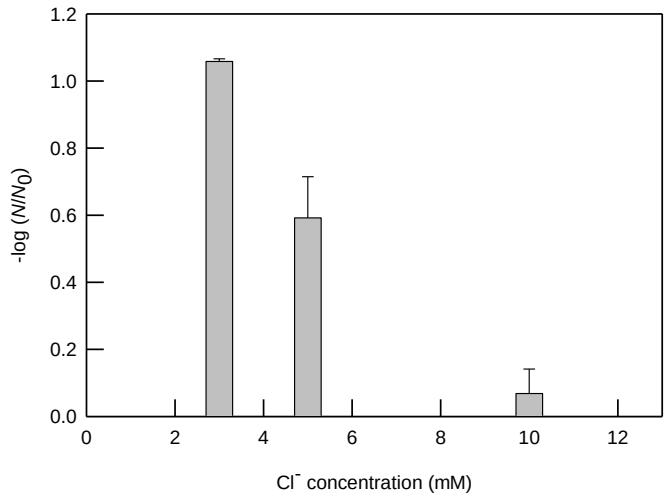

Figure 8. Effect of chloride on the log inactivation of the *Gordonia* sp. by 29.2 mg/L silver NPs.

Major Conclusions and Significance

Silver NPs are increasingly used in medicine and consumer products because of silver's broad spectrum antimicrobial property. This project investigated the effects of solution chemistry on the bactericidal efficiency of silver NPs on *Gordonia* sp., a group of beneficial bacteria in natural water bodies and wastewater treatment. The average particle size and dispersivity of silver NPs play an important role in the toxicity of silver NPs. The experimental results indicate that aggregation is signification for silver NPs in DI water. However, the presence of 3 mM SDS or 8 mg/L NOM reduced the aggregation of silver NPs apparently. Overall, the degree of aggregation increased in the order of 3 mM SDS < 8 mg/L NOM < DI water < 10 mM NaCl < 10 mM $NaNO_3$. The log inactivation of the *Gordonia* sp. increased from 0.16±0.04 to 0.45±0.13 when the dosage of silver NPs increased from 7.3 to 29.2 mg/L in DI water. However, the log inactivation rate rose to 1.40±0.26 and 0.87±0.12 in the presence of 3 mM SDS or 8 mg/L NOM, respectively with 29.2 mg/L silver NPs. In contrast, 10 mM NaCl reduced the log inactivation rate to 0.07±0.07, because NaCl caused moderate aggregation of silver NPs and formed likely less toxic silver chloride species.

Publications

A journal article is being prepared based on the research results from this study. In addition, some of the results from this project will be featured in an oral presentation in 2014 World Environmental & Water Resources Congress in Portland, Oregon.

Grant Submissions

A grant application is in preparation.

Students

The project employed two undergraduate students Francis Yorio and Louisa Orr to conduct the research activities proposed in this study. They were systematically trained with fundamental research skills from theoretical, experimental, and analytical aspects. In addition, this project was featured in CE 365 Introduction to Environmental Engineering for 15 undergraduate students.

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Abundance, Transport, and Retention of Pharmaceuticals and Personal Care Products (PPCPs) in Central Indiana Streams

Basic Information

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Descriptors:	None
Principal Investigators:	Melody J. Bernot

Publication

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Abundance, transport and retention of pharmaceuticals and personal care products (PPCPs) in central Indiana streams

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Abstract / Summary

Persistence of pharmaceuticals and personal care products (PPCPs) in the environment is an emerging concern due to potential adverse effects on aquatic ecosystems and transport to human drinking water sources. We quantified both human and veterinary PPCPs throughout freshwaters of central Indiana underscoring the ubiquity of these contaminants. Research combined descriptive sampling of PPCP abundance with innovative enrichment studies to directly quantify PPCP transport and retention in freshwater. Additionally, we conducted *in vitro* and *in situ* experimental assessments of PPCP influence on aquatic organisms. Research was guided by two objectives: 1) Develop predictive models of PPCP abundance in lotic ecosystems; and, 2) Quantify variation in PPCP retention and transport via descriptive sampling and experimental techniques. We sampled 19 3rd order streams in central Indiana during baseflow conditions. Dissolved pharmaceutical concentrations were measured at each site in addition to stream physiochemical characteristics and organic matter biomass. Caffeine (24-135 ng/L), carbamazepine (1-88 ng/L), cotinine (4-34 ng/L), naproxen (5-15 ng/L), and paraxanthine (49-62 ng/L) concentrations varied among sites. Pearson correlations indicated only stream temperature, width, and autotrophic abundance were correlated with pharmaceutical concentrations. Structural equation modeling identified causal relationships between stream variables and pharmaceutical concentrations. Significant pathways included filamentous algal biomass and sediment percent organic matter effects on carbamazepine as well as nutrient and discharge effects. Additionally, significant effects of macrophyte biomass on caffeine were identified. These data and analyses highlight multiple interacting controls on pharmaceutical abundance in freshwater ecosystems. Experimental assessments identified sub-lethal effects of carbamazepine on both macroinvertebrates and yellow perch. Ephemeroptera had longer molt cycles when exposed to environmentally-relevant concentrations of carbamazepine in addition to a higher prevalence of abnormal behaviors. Yellow perch had lower growth rates when exposed to environmentally-relevant concentrations of carbamazepine in addition to reduced response to stimulus. At the ecosystem scale, carbamazepine was found to alter species diversity, organic matter composition, and nutrient concentrations through effects on specific organisms.

Problem and Research Objectives:

This study was conducted in the Upper White River (UWR) watershed, a tributary to the Wabash River and one of the most degraded watersheds in the country (USEPA 2002). The UWR watershed in central, Indiana encompasses 2,718 mi^2 across 16 counties and provided an excellent system to study PPCPs due to varied land use and PPCP sources. Since the Clean Water Act was passed by Congress in 1972, environmental managers and policy makers have made substantial headway in identifying and reducing point source discharge in the nation's waterways. Yet, >40% of these water bodies remain impaired, often because of nonpoint source pollution which often degrades freshwater integrity to a greater extent than point pollution. PPCPs can enter freshwaters via multiple sources including disposal of surplus drugs, human excretion into sewage, and runoff associated with therapeutic treatment of livestock. These sources can be routed to freshwater via

urban sewage transport, ineffective or leaking septic tanks and landfills, or overland runoff. There have been numerous studies quantifying measurable concentrations of PPCPs and their metabolites in surface and groundwaters. Higher concentrations of PPCPs in water are generally associated with wastewater treatment effluent, but concentrations vary with secondary waste treatment procedures. Further, although effluent serves as a source of PPCP pollutants, streams across the US not influenced by wastewater can also have measureable concentrations of waste contaminants from residential, industrial, and agricultural sources. Once PPCPs enter into freshwater, they may be transformed, retained, or exported downstream. PPCP compounds can be transformed via biodegradation or photodegradation; retained via assimilation or sediment sorption, or if the compound is mobile and recalcitrant, exported downstream unaltered. All of these pathways may influence transport downstream to drinking water sources. Previous studies have consistently demonstrated the prevalence of PPCP compounds. However, current knowledge does not include an understanding of the spatiotemporal patterns of PPCP abundance in freshwater. Further, environmental transport and fate of these compounds have not been quantified to assess regulatory need. Because PPCPs are designed to have a physiological effect, it is likely they may influence organisms even at trace concentrations.

The overall objective of the study was to quantify spatial and temporal variability of abundance, transport, and retention of PPCPs in streams of the Upper White River (UWR) watershed of central Indiana. Research combined descriptive sampling with experimental assessments to directly quantify PPCP transport and retention in lotic ecosystems. Research was guided by two primary objectives:

1. Develop predictive models of PPCP abundance in lotic ecosystems.
2. Quantify variation in PPCP transport and retention using short-term enrichment experiments.

In addition, *in vitro* and *in situ* experiments were conducted to quantify the effect of PPCPs on aquatic organism populations, communities, and ecosystems.

Methodology:

*Descriptive Measurements: S*tream sites in the UWR watershed were selected for sampling and represented a range of urban and agricultural activity. At each site and sampling event, sediment and dissolved PPCP concentrations were measured in addition to multiple physiochemical parameters and characterization of the sub-watershed. Water and sediment samples were analyzed using high performance liquid chromatography with tandem quadruple mass spectrometric detection in collaboration with the Indiana State Department of Health (ISDH) chemical laboratories (Indianapolis). Analytes measured in each sample included 1,7-dimethylxanthine, acetaminophen, caffeine, carbamazepine, cotinine, DEET, gemfibrozil, ibuprofen, lincomycin, naproxen, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfathiazole, triclocarban, triclosan, trimethoprim, and tylosin. Field blank samples and matrix samples were also collected at each site and sampling event to ensure data quality.

Additional filtered water samples (250 mL) were collected at each sampling event and site for chemical analysis at Ball State University including anions (NO_3, Br, Cl, PO_4, SO_4) and cations (NH_4, Li, K, Mg) using a Dionex 3000 Ion Chromatograph and dissolved organic carbon concentrations using a Schimadzu TOC-VC carbon analyzer. These chemical analyses aided identification of PPCP sources (e.g., manure, tile drainage, wastewater). Additional sediment samples (100 cm^3) were also collected and analyzed for organic matter and elemental content to inform potential for PPCP sorption as well as serve as an indicator of microbial activity and potential biodegradation of PPCPs. Physical parameters were also measured at each sampling event including flow, depth, width, oxygen, pH, temperature, specific conductivity and light to enable assessments of abiotic factors influencing PPCP abundance (e.g. photodegradation, dilution).

PPCP Enrichment Experiments: We enriched a an experimental stream reach on a Ball State University Field Station (Cooper-Skinner Farm) with select PPCP compounds (acetaminophen, carbamazepine, DEET, triclosan) to quantify transport and retention. Enrichment protocols followed standard nutrient enrichment protocols and consisted of dripping (~0.14 L/min) a concentrated solution of the target compound (contained in a 20 L carboy) and potassium bromide (conservative tracer) into the stream using a peristaltic pump until equilibrium was achieved (~2 hrs depending on discharge). Enrichments increased PPCP concentration to a maximum environmentally-relevant concentration within the reach (150 ng/L acetaminophen; 10 ng/L carbamazepine; 400 ng/L DEET; 10 ng/L triclosan). These enrichment concentrations were two orders of magnitude lower than lethal concentrations to any organism and high enough that fine resolution in concentration changes could be detected. Once equilibrium was achieved, downstream sites were sampled over ~30 min, moving from downstream to upstream, to quantify loss of the target compound over the reach followed by termination of the addition. Water and sediment chemistry was also measured in addition to physiochemical characteristics as described above.

Statistical Analyses: Descriptive data were analyzed using both Bonferroni-corrected Pearson correlation statistics and multiple regression analyses. Independent variables (e.g., discharge, nutrient concentration, physiochemical characteristics) correlated with PPCP concentrations were used to develop predictive multiple regression models identifying factors controlling PPCP abundance. Multiple regression models were developed for all compounds with >60% detection frequency. Correlation and regression analyses informed development of predictive models using structural equation modeling. Structural equation modeling (SEM) was used to test *a priori* hypothesized causal pathways influencing pharmaceutical abundance and persistence in the environment. Data were modeled using SPSS statistical software equipped with AMOS17.0 (Amos v. 17.0 SPSS, Chicago, Illinois, USA). Data collected in conjunction with enrichment experiments were used to calculate PPCP transport metrics (uptake length, velocity, rate, retention, export) and compared among compound types using analysis of variance (ANOVA). Additionally, correlation and regression statistics were used to identify factors controlling retention and transport. All correlation, regression and ANOVA

statistical analyses were performed using the SAS statistical program (SAS Institute, Inc.; System for Windows V8).

Table 1. Summary of physical, chemical and biological characteristics of streams sampled (N = 19) and pharmaceutical concentrations.

Physical Characterisitics	*Mean*	*SD*	Chemical Characteristics	*Mean*	*SD*	Biological Characteristics	*Mean*	*SD*	Pharmaceutical Abundance	*Mean*	*SD*
Light (% open)	26.3	19.9	Cl (mg/L)	47.6	20.5	SPOM (g/L)	0.018	0.016	Caffeine (ng/L)	37.8	29.5
pH	8.2	0.2	NO_3-N (mg/L)	3.4	4.0	CBOM (g/m^2)	6.5	9.2	Carbamazepine (ng/L)	20.1	25.2
Temperature ©	25.9	2.6	NH_4-N (mg/L)	0.1	0.1	MAC (g/m^2)	3.8	10.3	Cotinine (ng/L)	10.8	8.7
DO (mg/L)	6.5	1.4	DIN (mg/L)	3.5	4.0	FG (g/m^2)	46.2	64.7	Naproxen (ng/L)	5.6	2.3
TDS (g/L)	83.0	19.5	PO_4-P (mg/L)	0.4	0.3	FBOM (g/m^2)	294.6	344.4	Paraxanthine (ng/L	51.2	3.3
Wetted Width (m)	20.3	12.1	SO_4 (mg/L)	83.9	58.6	FBOM (% organic)	1.5	0.9			
Flow (m/s)	0.1	0.1	TC (mg/L)	96.8	23.3	EP (g/m^2)	3.7	6.0			
Depth (m)	0.38	0.23				Total Autrophic Biomass (g/m^2)	53.7	61.6			
Discharge (L/s)	1056	1410				Total Heterotrophic Biomass (g/m^2)	301.0	342.6			

Principle Findings:

Caffeine (24-135 ng/L), carbamazepine (1-88 ng/L), cotinine (4-34 ng/L), naproxen (5-15 ng/L), and paraxanthine (49-62 ng/L) concentrations varied among sites (Table 1). Correlation analyses identified multiple physical, chemical and biological stream characteristics related to pharmaceutical concentrations. Carbamazepine was positively correlated with nitrate (NO_3) and dissolved organic nitrogen (DIN) concentration ($p < 0.01$; Figure 1) as well as total dissolved carbon and temperature ($p < 0.05$) suggesting wastewater sources of PPCPs. Caffeine was positively correlated with macrophyte biomass as well as coarse benthic organic matter abundance and stream temperature ($p < 0.05$). Paraxanthine and naproxen were also positively correlated with macrophyte biomass ($p < 0.01$). Cotinine concentrations were not correlated with any measured variable ($p > 0.05$; data not shown).

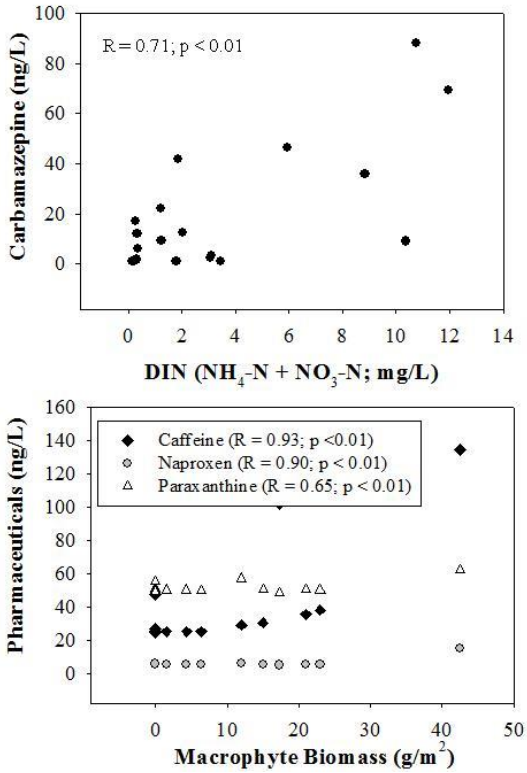

Figure 1. Correlations between dissolved inorganic nitrogen and carbamazepine (top panel) as well as macrophyte biomass and caffeine, naproxen and paraxanthine.

Multiple regression analyses explained > 95% of the variation in caffeine, cotinine, and naproxen concentrations but only 74% of the variation in carbamazepine and 58% of paraxanthine variation. Macrophyte biomass and DIN were the most consistent predictors of pharmaceutical concentrations across compounds though other physical and chemical characteristic were also explanatory including light, oxygen, flow, fine benthic organic

matter, depth, and phosphate. Structural equation modeling (SEM) identified significant causal relationships between stream variables and total pharmaceutical concentration (sum of all measured compounds) with a significant fit of the covariance matrix (Figure 2). Significant pathways included flow and depth effects on discharge; discharge effects on ammonium; ammonium effects on total pharmaceutical concentrations; organic matter content on ammonium; and total dissolved solids on total pharmaceutical concentrations. Discharge and organic matter content of fine benthic material, though necessary for model fit, were not significant pathways explaining total pharmaceutical concentrations. Interestingly, light availability did not influence total pharmaceutical concentrations or autotrophic production (as biomass). Total autotrophic biomass yielded a better model fit than biomass of individual autotrophic compartments (macrophytes, epilithon, filamentous green algae.

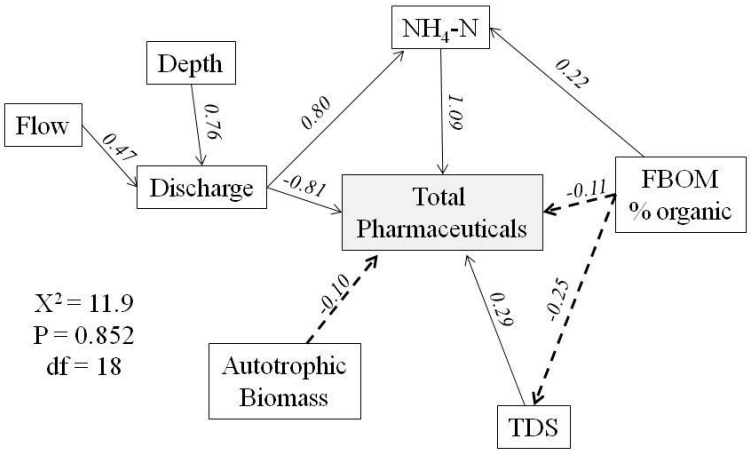

Figure 2. Structural equation model to describe controls on total dissolved pharmaceutical concentrations (sum of all pharmaceuticals measured) in central Indiana streams. Boxes are variables in the model. Numbers are standardized path coefficients. Error variance was calculated for all variables (not shown). Solid lines indicate significant paths in the model. Dashed lines denote hypothesized pathways not significant in the model but necessary for model fit. Model fit statistics noted.

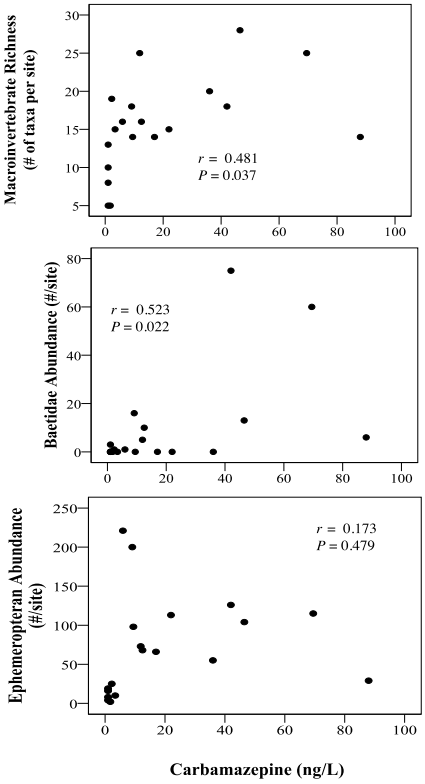

Figure 3. Bivariate correlations of carbamazepine (CBZ; ng/L), macroinvertebrate richness (# of taxa per site), and Baetidae and Ephemeroptera abundance (# of individuals per site). N = 19.

Because carbamazepine was a dominant pharmaceutical measured in our descriptive study, we focused on carbamazepine for some additional analyses to understand potential organismal and ecosystem effects of this specific compound. Macroinvertebrate richness across study streams (N = 19) was positively correlated with increasing concentrations of carbamazepine (Figure 3). Using structural equation modeling (SEM) we inferred that carbamazepine influenced macroinvertebrate richness through indirect pathways linked to Baetidae abundance (data not shown). Baetidae abundance influenced ephemertopteran abundance and FBOM percent organic matter, both of which altered macroinvertebrate richness.

To better understand the effect carbamazepine has on life history characteristics of aquatic organisms and consumer-resource interactions, we assessed the influence of carbamazepine on the development, growth and behavior of mayfly nymphs (Stenonema sp.) and the alterations in food consumer-resource interactions between Stenonema and algae (Chaetophora) in laboratory microcosms. Carbamazepine influenced the development and behavior of Stenonema nymphs and the body dimensions of adult individuals (Figure 4). However, it appears that carbamazepine does not influence consumer-resource interactions at concentrations found in surface waters. The pharmaceutical carbamazepine may influence the behavior, growth and development of mayflies, which could have significant consequences at the population, community and ecosystem level.

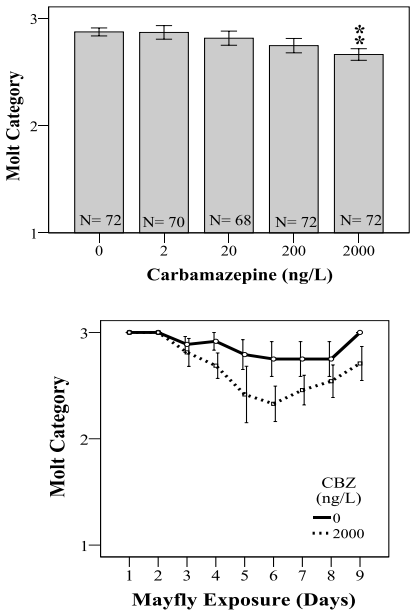

Figure 4. Molt category for mayfly nymphs exposed to carbamazepine (CBZ) treatments and molt category of mayfly nymphs over exposure (9 d) for control and 2000 ng/L CBZ treatment. Each data point represents the mean molt category for all *Stenonema* individuals in each treatment ± 1 SE. Categories ranged from 1-3, 1 being the newest molt and 3 the oldest. ** $P < 0.01$

Additionally, we conducted a 31 d *in situ* mesocosm experiment to quantify effects of environmentally-relevant concentrations of carbamazepine on invertebrate communities and ecosystem processes. Mesocosms were populated with four gastropod taxa (Elimia, Physa, Lymnaea and Helisoma), zooplankton, filamentous algae and phytoplankton. Invertebrate diversity increased in the presence of carbamazepine. Additionally, carbamazepine altered the biomass of Helisoma and Elimia, induced a decline in Daphnia pulex abundance and shifted the zooplankton community towards copepod dominance. Lastly, carbamazepine decreased decomposition and altered primary production and dissolved nutrient concentrations.

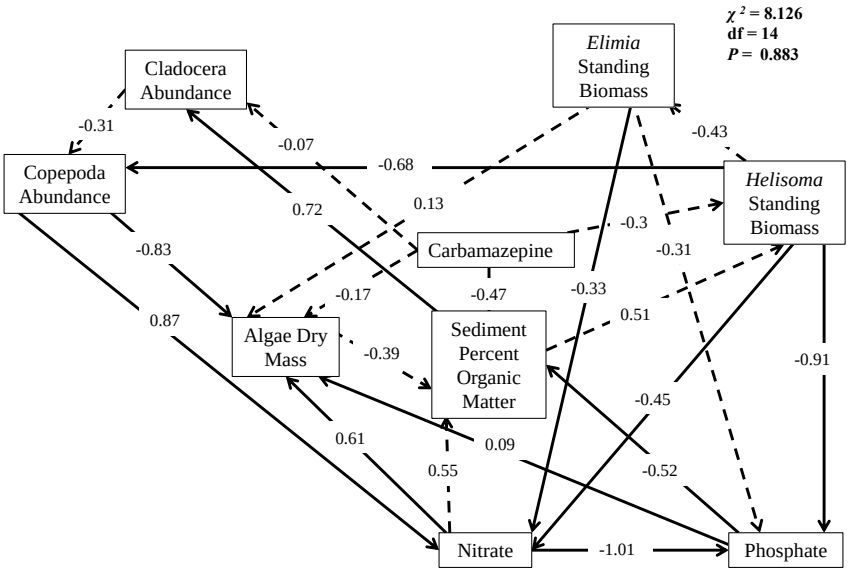

Figure 5. Structural equation model describing the relationship between carbamazepine and the freshwater ecosystem. The model (χ^2 = 8.126, df = 14, P = 0.883) accounts for a substantial portion of the variability in the abiotic components of the ecosystem (r > 0.50). Numbers are standardized path coefficients. Solid lines indicate significant paths in the model (P < 0.05). Dashed lines are non-significant hypothesized pathways (P > 0.05).

Changes in the invertebrate community identified using structural equation modeling were through direct (i.e., exposure to carbamazepine) and indirect pathways (i.e., changes in food resource availability; Figure 5). These data show the psychiatric drug carbamazepine may alter freshwater community structure and ecosystem dynamics and could have profound effects on natural systems.

Laboratory chronic toxicity tests were also conducted to assess carbamazepine effects on yellow perch. Yellow perch growth (as increases in length and weight were negatively affected by carbamazepine concentrations. Additionally, yellow perch exposed to carbamazepine were less likely to respond to stimuli.

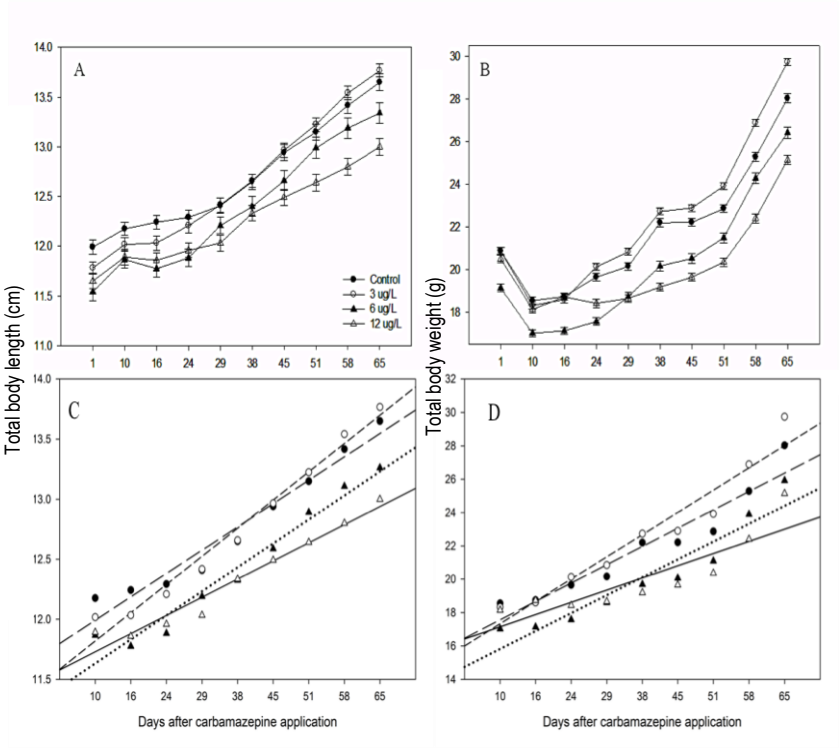

Figure 6. Change of total body length (A) and total body weight (B) of yellow perch after carbamazepine exposure. Symbols are mean +/- SE of three replicates per treatment. Slope difference of total body length (C) and body weight (D) among treatment groups with regression.

Significance

Overall, pharmaceutical concentrations detected in central Indiana streams were within the range previously documented in freshwaters throughout the United States though carbamazepine concentrations were ~5x higher than previous studies in central Indiana.
Consistent with previous studies in central Indiana, ammonium concentrations and stream discharge were not significant variables in correlation and regression analyses. However, structural equation models identified discharge and ammonium as important variables to model fit. These data characterized one time point (summer baseflow) when conditions likely yield lower pharmaceutical

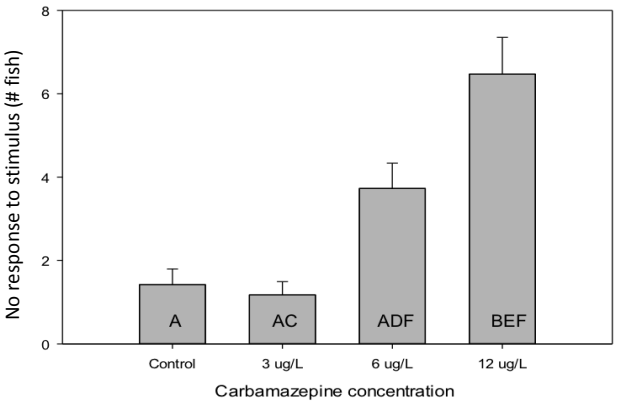

Figure 7. Carbamazepine effect on yellow perch behavior as response (movement) to stimulus after chronic exposure. Significant differences in stimulus response was measured at higher concentrations of carbamazepine ($p < 0.001$).

concentrations due to high potential for photo- and biodegradation as well as reduced input. This, coupled with higher autotrophic biomass in summer relative to other times of the year, suggests mechanisms controlling pharmaceutical abundance are temporally variable. Concentrations of pharmaceuticals measured in this study can influence aquatic organism reproduction and behavior. We found carbamazepine may alter the macroinvertebrate community structure of freshwater streams in central Indiana, which could potentially lead to alterations in resource availability (i.e., presence and use of FBOM) and predator-prey interactions (i.e., altered functional feeding groups present). *In vitro* experiments also demonstrated that environmentally relevant concentrations of carbamazepine could have chronic effects on Stenonema and Chaetophora. Further, an *in situ* mesocosm experiment demonstrate how environmentally relevant concentrations of carbamazepine alter the communities and processes of freshwater ecosystems.

Publications

Jarvis AL, MJ Bernot, RJ Bernot. *In review*. Relationships between the psychiatric drug carbamazepine and freshwater macroinvertebrate community structure. *Ecological Applications*. Submitted May 2014.

Jarvis, AL, MJ Bernot, RJ Bernot. *In review*. The effects of the pharmaceuticals carbamazepine on life history characteristics of flat-headed mayflies (Heptageniidae) and aquatic resource interactions. *Ecotoxicology*. Submitted April 2014.

Jarvis, AL, MJ Bernot, RJ Bernot. *In review*. The effects of the psychiatric drug carbamazepine on freshwater invertebrate communities and ecosystem dynamics: An *in situ* mesocosm experiment. *Ecological Applications*. Submitted May 2014.

Bernot, MJ, J Justice. *In press*. Survey of Personal Care Products (PCPs) in the United States. S. Díaz Cruz, D. Barceló (eds.). The Handbook of Environmental Chemistry: Personal Care Products in the Aquatic Environment. Springer.

Lee, JH, MJ Bernot. *In preparation*. The toxicity and effects of carbamazepine on Yellow Perch (*Perca flavescens*) growth and behavior. *Ecotoxicology*. To be submitted August 2014.

Abell, NS, MJ Bernot. *In preparation*. The influence of stimulants on the freshwater snail, *Physa acuta*. *Ecotoxicology*. To be submitted August 2014.

Bernot, MJ, RJ Bernot, LM Caffo, CL Crismore, D Elias, P Flores, J Justice, JH Lee, HL Madinger, and R Osborne. *In preparation*. Relationships between physiochemical characteristics, community structure and pharmaceutical concentrations in central Indiana streams. *Science of the Total Environment*. To be submitted August 2014.

Presentations

Bernot, MJ, AL Jarvis, RJ Bernot. 2014. Aquatic Neuroses: The effects of the pharmaceutical carbamazepine on aquatic ecosystem dynamics. Joint Aquatic Sciences Meeting, Portland, OR. May.

Lee, JH, MJ Bernot. 2014. The toxicity and effects of carbamazepine on Yellow Perch (*Perca flavescens*) growth and behavior. Ball State University Student Symposium. March.

Abell, NS, MJ Bernot. 2014. The influence of stimulants on the freshwater snail, *Physa acuta*. Ball State University Student Symposium. March.

Lee, JH, MJ Bernot. 2013. Invited Seminar. Transfer of 17a-ethynylestradiol and carbamazepine in a food chain established by invasive species: Toxicity, pharmacokinetics and bioaccumulation. Taylor University, Department of Chemistry. October 2013.

Bernot, RJ, MJ Bernot, LM Caffo, CL Crismore, D Elias, P Flores, J Justice, JH Lee, HL Madinger, R Osborne. 2013. Parasites as indicators of drug contamination: relationships among parasites, gastropods and pharmaceuticals in Midwestern streams. American Society for Parasitology. Québec City, Québec.

Jarvis, A, MJ Bernot, RJ Bernot. 2013. The effects of the pharmaceutical carbamazepine on aquatic macroinvertebrate community structure. Indiana Water Resources Association, Muncie. June.

Bernot, MJ, RJ Bernot, LM Caffo, CL Crismore, D Elias, P Flores, J Justice, JH Lee, HL Madinger, R Osborne. 2013. Relationships between physiochemical characteristics, community structure and pharmaceutical concentrations in central Indiana streams. Society for Freshwater Science, Jacksonville, FL. May.

Jarvis, A, MJ Bernot, RJ Bernot. 2013. The effects of the pharmaceutical carbamazepine on aquatic macroinvertebrate community structure. Society for Freshwater Science, Jacksonville, FL. May.

Lee, JH, MJ Bernot. 2013. Transfer of 17a-ethynylestradiol and carbamazepine in a food chain established by invasive species: Toxicity, pharmacokinetics and bioaccumulation. Ball State University Student Symposium. March.

Jarvis, A, MJ Bernot, RJ Bernot. 2013. The effects of the pharmaceutical carbamazepine on aquatic macroinvertebrate community structure. Ball State University Student Symposium. March.

Jarvis, AL, MJ Bernot, RJ Bernot. 2013. The effects of the pharmaceutical carbamazepine on aquatic macroinvertebrate community structure. Indiana Academy of Science, Indianapolis. March.

Drosos, I, MJ Bernot. 2013. Factors influencing anion loss and retention in sediment. Ball State University Student Symposium. March.

Thesis

Jarvis, AL. The effects of the psychiatric drug carbamazepine on freshwater invertebrate communities and ecosystem dynamics. MS Thesis. Ball State University, May 2014.

Grant Submissions

Lee, JH. *Funded*. 2014-2015. Assessing acute and chronic toxicity of carbamazepine and 17α-ethynylestradiol on yellow perch (*Perca flavescens*). Indiana Academy of Science Senior Research Grant. Total Award: $2,914.

Lee, JH. *Funded*. 2014-2015. Assessing acute and chronic toxicity of carbamazepine and 17α-ethynylestradiol on yellow perch (*Perca flavescens*) BSU ASPiRE Graduate Research Program. Total Award: $500.

Drosos, I. *Funded*. 2014-2015. Carbamazepine loss and retention in sediments. Ball State University ASPiRE Undergraduate Student Research. Total Award: $300.

Becker, JC, MJ Bernot, TE Lauer. 2014-2015. *Funded.* RiverPACE: A Nationwide Riverine Pharmaceuticals Assessment, Collection, and Education Project. Discovery Group. Total Award: $19,972.

Bernot, MJ, RJ Bernot, J Becker, K Epp. *In review*. A systems-based approach to quantify abundance, fate and effects of pharmaceuticals and personal care products (PPCPs) in lotic ecosystems. Environmental Protection Agency (EPA-G2014-STAR-E1): Systems-Based Research for Evaluating Ecological Impacts of Manufactured Chemicals. Total Request: 799,523. Submitted March 2014.

Bernot, MJ, RJ Bernot. Not Funded. Preliminary Proposal: RUI: Predicting abundance, transport and effects of PPCPs in lotic ecosystems in a changing world. National Science Foundation Division of Environmental Biology. Submitted January 2014.

Bernot, MJ. Not funded. Water Environment Research Foundation Unsolicited Research Program. Freshwater Pharmaceutical and Personal Care Product Risk Management. Submitted August 2013.

Bernot, MJ. Not funded. Occurrence of pharmaceuticals in freshwater: Quantifying human exposure to trace contaminants. National Institute of Health. Submitted October 2013.

Students

A total of 3 graduate students and 4 undergraduate students were involved with project activities.

Graduate Students (Ball State University): Amanda Jarvis, MS student; Jee Hwan Lee, PhD student; Courtney Crismore, MS student
Undergraduate Students (Ball State University, BS Biology students): Natalie Abell, Isis Drosos, Susannah Dragash, Nicole Woodall

Estimating Capture Probabilities of Common Stream Fish in the Eastern Corn Belt Plain

Basic Information

Title:	Estimating Capture Probabilities of Common Stream Fish in the Eastern Corn Belt Plain
Project Number:	2013IN361B
Start Date:	3/1/2013
End Date:	2/28/2015
Funding Source:	104B
Congressional District:	IN-006
Research Category:	Biological Sciences
Focus Category:	Conservation, Methods, Models
Descriptors:	
Principal Investigators:	Thomas E Lauer, Jason C Doll

Publication

1. None yet

ESTIMATING CAPTURE PROBABILITIES OF COMMON STREAM FISH IN THE EASTERN CORN BELT PLAIN

Prepared By:
Jason C. Doll, Graduate Student
Thomas E. Lauer, Ph.D., Professor and Director
Aquatic Biology and Fisheries Center
Department of Biology
Ball State University
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May, 2014

Abstract:

Imperfect detection of fish can lead to biased estimates of abundance and assemblage level descriptors such as the Index of Biotic Integrity. Accentuating this bias are two items. First the schooling behavior of fish confounds the efficacy of traditional models in estimating abundance and capture probability. Second, we know habitat influences fish distribution and catchability. Although multi-pass depletion sampling of fish can be used to estimate some of the bias, assuming fish behave independently, quantifying both schooling and habitat bias effects is required to fully understand fish abundance. Thus, our objective in this study is to determine how imperfect detection of common stream fishes is influenced by schooling behavior and a suite (14) of physicochemical variables. To meet this objective, we conducted multi-pass depletion surveys at 16 randomly selected sites in the Eastern Corn Belt Plain ecoregion in Indiana. A total of 1,193 fish representing 65 species were sampled during the project. We will use these data and extend an existing model to estimate abundance and capture probability of schooling and non-schooling species. These estimated capture probabilities can then be applied to standard electrofishing surveys to improve the accuracy of abundance estimates and can be applied to biological monitoring tools to create multimetric indices that are more sensitive to perturbation.

Problem and Research Objectives:

Stream health can be assessed using multimetric indices, such as the Index of Biotic Integrity (IBI), and multivariate analysis (ter Braak 1986; Karr 1991). The IBI has been used to assess the health of Indiana's streams in the Eastern Corn Belt Plain for over a decade (Simon & Dufour 1997). This multimetric index and other similar ones were patterned after Karr (1981) and have been widely used and accepted. Unfortunately, the sensitivity in detecting shifts in fish assemblages using an IBI is restricted to major perturbations (Trebitz et al. 2003). Improvements in describing stream health and improving sensitivity are possible using other methods, such as multivariate analysis (Thomas & Hall 2006), but typically require different, or more extensive data. One such factor limiting sensitivity in the IBI is our ability to obtain accurate estimates of fish abundance as capture probability is influenced by environmental variables. By refining something this basic with respect to capture (e.g., reduce variability), we will advance our ability to detail stream health, regardless of the multimetric method employed in the analysis.

Obtaining accurate estimates of fish abundance in streams are problematic. Standard sampling methods for an IBI (Simon & Dufour 1997) typically consists of single pass electrofishing catch per unit effort as an index of abundance. This method assumes the probability of capturing a fish is 1 (i.e., perfect capture probability), capture probability is spatially constant, and equal among species. These assumptions are not ecologically reasonable and often violated, thereby increasing bias, adding noise to the dataset and limiting our ability to detect change. Variability in capture probabilities can also be exaggerated due to fluctuations in physicochemical factors. For example, differences in conductivity and turbidity across disparate waters could influence capture efficacy, limiting our ability to describe whether differences in the fish assemblage exist. The need to evaluate factors influencing capture probabilities is real and must be resolved to improve our understanding of the fish community. Ultimately, this study will lead to better fisheries management decisions and evaluation of water quality through biological interpretation not just in the Eastern Corn Belt Plain, but elsewhere.

Our objective is twofold: 1. Determine capture probabilities of common stream fishes in wadeable streams of the Eastern Corn Belt Plain while accounting for schooling behavior, and 2. determine how variation in physicochemical parameters among the streams may influence capture probabilities. To meet these objectives we will conduct multi-pass depletion surveys (both fish and physicochemical parameters) at a minimum of 25 randomly selected sites and use the data to model factors influencing capture probabilities of fishes. We will also extend the models of Royle (2004) for non-schooling species and Martin et al. (2011) for schooling species to account for covariates of detection. We hypothesize that the physicochemical parameters being measured will significantly affect capture probabilities of fishes in wadeable streams of the Eastern Corn Belt Plain. We also hypothesize that not accounting for schooling behavior will bias capture probabilities and abundance estimates of schooling species.

Methodology:

Sixteen sites were sampled between June 19 and August 8, 2013. Each site was closed off with a block net placed at the upstream and downstream ends to restrict fish movement during the survey. Three passes with a tote-barge DC electrofishing unit were conducted in an upstream pattern. At the end of each pass, fish were counted and held in large tanks until the survey was completed. Species that required visual examination under a dissecting microscope were preserved in formalin on site and transported to Ball State University. Methodology has been approved by the Ball State University Animal Care and Use Committee (IACUC Protocol #:317058-1).

Fourteen physicochemical parameters were collected at each site to evaluate their influence on capture probabilities (conductivity, turbidity, stream width, water temperature, average depth, stream gradient, habitat type (pool, riffle, run, glide), and substrate type (fines, gravel, cobble, boulder). Conductivity, water temperature, and turbidity were measured with a Hydrolab DS5 probe with a Hach Trimble Recon field computer. Stream width and sampling distance was measured with a laser range finder. Average depth was measured by taking depth measurements at seven equidistant points along a transect. Transects were spaced every 10 m. Stream gradient will be measured using USGS topographic maps. Habitat was further assessed at each site using the Qualitative Habitat Evaluation Index (Rankin 1989).

Principle Findings:

A total of 1,193 fish representing 65 species were sampled during the project. Species diversity at individual sites ranged from 18 to 37 species. The most diverse sites were Wildcat Creek (37 species), Whitewater River (32 species), and Nolan's Fork (29 species). The relationship between the physicochemical parameters and QHEI metrics on detectability will be analyzed in the coming months.

Significance:

This project will determine capture probabilities of common stream fish sampled throughout the Eastern Corn Belt Plain. The influence of fish schooling behavior and commonly measured physicochemical variables will be incorporated into the model to provide more

accurate estimates of abundance. By accounting for these confounding variables, sensitivity in secondary data summaries (e.g., multimetric indices) will increase the ability to detect perturbations. The results will not only provide a methodology to improve our interpretation of water quality using multimetric indices, but also benefit managers and researchers nationwide in obtaining the best possible estimates of species abundance.

Publications:

The project is ongoing and the data are still being analyzed. We anticipate one presentation at the 2015 Midwest Fish and Wildlife conference in Indianapolis and one manuscript submitted for publication.

Grant Submissions: None

Students:

Four graduate students conducted all field work.

One graduate student and two undergraduate students identified fish in the lab.

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Linking Cover Crops to Improved Stream Water Quality via Field-Scale Soil Sampling, in Shatto Ditch Watershed, IN

Basic Information

Title:	Linking Cover Crops to Improved Stream Water Quality via Field-Scale Soil Sampling, in Shatto Ditch Watershed, IN
Project Number:	2013IN362B
Start Date:	3/1/2013
End Date:	2/28/2015
Funding Source:	104B
Congressional District:	IN-001, IN-002
Research Category:	Biological Sciences
Focus Category:	Nitrate Contamination, Non Point Pollution, Surface Water
Descriptors:	
Principal Investigators:	Jennifer L Tank, Sheila F Christopher

Publication

1. None yet

ESTIMATING CAPTURE PROBABILITIES OF COMMON STREAM FISH IN THE EASTERN CORN BELT PLAIN

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Abstract:

Imperfect detection of fish can lead to biased estimates of abundance and assemblage level descriptors such as the Index of Biotic Integrity. Accentuating this bias are two items. First the schooling behavior of fish confounds the efficacy of traditional models in estimating abundance and capture probability. Second, we know habitat influences fish distribution and catchability. Although multi-pass depletion sampling of fish can be used to estimate some of the bias, assuming fish behave independently, quantifying both schooling and habitat bias effects is required to fully understand fish abundance. Thus, our objective in this study is to determine how imperfect detection of common stream fishes is influenced by schooling behavior and a suite (14) of physicochemical variables. To meet this objective, we conducted multi-pass depletion surveys at 16 randomly selected sites in the Eastern Corn Belt Plain ecoregion in Indiana. A total of 1,193 fish representing 65 species were sampled during the project. We will use these data and extend an existing model to estimate abundance and capture probability of schooling and non-schooling species. These estimated capture probabilities can then be applied to standard electrofishing surveys to improve the accuracy of abundance estimates and can be applied to biological monitoring tools to create multimetric indices that are more sensitive to perturbation.

Problem and Research Objectives:

Stream health can be assessed using multimetric indices, such as the Index of Biotic Integrity (IBI), and multivariate analysis (ter Braak 1986; Karr 1991). The IBI has been used to assess the health of Indiana's streams in the Eastern Corn Belt Plain for over a decade (Simon & Dufour 1997). This multimetric index and other similar ones were patterned after Karr (1981) and have been widely used and accepted. Unfortunately, the sensitivity in detecting shifts in fish assemblages using an IBI is restricted to major perturbations (Trebitz et al. 2003). Improvements in describing stream health and improving sensitivity are possible using other methods, such as multivariate analysis (Thomas & Hall 2006), but typically require different, or more extensive data. One such factor limiting sensitivity in the IBI is our ability to obtain accurate estimates of fish abundance as capture probability is influenced by environmental variables. By refining something this basic with respect to capture (e.g., reduce variability), we will advance our ability to detail stream health, regardless of the multimetric method employed in the analysis.

Obtaining accurate estimates of fish abundance in streams are problematic. Standard sampling methods for an IBI (Simon & Dufour 1997) typically consists of single pass electrofishing catch per unit effort as an index of abundance. This method assumes the probability of capturing a fish is 1 (i.e., perfect capture probability), capture probability is spatially constant, and equal among species. These assumptions are not ecologically reasonable and often violated, thereby increasing bias, adding noise to the dataset and limiting our ability to detect change. Variability in capture probabilities can also be exaggerated due to fluctuations in physicochemical factors. For example, differences in conductivity and turbidity across disparate waters could influence capture efficacy, limiting our ability to describe whether differences in the fish assemblage exist. The need to evaluate factors influencing capture probabilities is real and must be resolved to improve our understanding of the fish community. Ultimately, this study will lead to better fisheries management decisions and evaluation of water quality through biological interpretation not just in the Eastern Corn Belt Plain, but elsewhere.

Our objective is twofold: 1. Determine capture probabilities of common stream fishes in wadeable streams of the Eastern Corn Belt Plain while accounting for schooling behavior, and 2. determine how variation in physicochemical parameters among the streams may influence capture probabilities. To meet these objectives we will conduct multi-pass depletion surveys (both fish and physicochemical parameters) at a minimum of 25 randomly selected sites and use the data to model factors influencing capture probabilities of fishes. We will also extend the models of Royle (2004) for non-schooling species and Martin et al. (2011) for schooling species to account for covariates of detection. We hypothesize that the physicochemical parameters being measured will significantly affect capture probabilities of fishes in wadeable streams of the Eastern Corn Belt Plain. We also hypothesize that not accounting for schooling behavior will bias capture probabilities and abundance estimates of schooling species.

Methodology:

Sixteen sites were sampled between June 19 and August 8, 2013. Each site was closed off with a block net placed at the upstream and downstream ends to restrict fish movement during the survey. Three passes with a tote-barge DC electrofishing unit were conducted in an upstream pattern. At the end of each pass, fish were counted and held in large tanks until the survey was completed. Species that required visual examination under a dissecting microscope were preserved in formalin on site and transported to Ball State University. Methodology has been approved by the Ball State University Animal Care and Use Committee (IACUC Protocol #:317058-1).

Fourteen physicochemical parameters were collected at each site to evaluate their influence on capture probabilities (conductivity, turbidity, stream width, water temperature, average depth, stream gradient, habitat type (pool, riffle, run, glide), and substrate type (fines, gravel, cobble, boulder). Conductivity, water temperature, and turbidity were measured with a Hydrolab DS5 probe with a Hach Trimble Recon field computer. Stream width and sampling distance was measured with a laser range finder. Average depth was measured by taking depth measurements at seven equidistant points along a transect. Transects were spaced every 10 m. Stream gradient will be measured using USGS topographic maps. Habitat was further assessed at each site using the Qualitative Habitat Evaluation Index (Rankin 1989).

Principle Findings:

A total of 1,193 fish representing 65 species were sampled during the project. Species diversity at individual sites ranged from 18 to 37 species. The most diverse sites were Wildcat Creek (37 species), Whitewater River (32 species), and Nolan's Fork (29 species). The relationship between the physicochemical parameters and QHEI metrics on detectability will be analyzed in the coming months.

Significance:

This project will determine capture probabilities of common stream fish sampled throughout the Eastern Corn Belt Plain. The influence of fish schooling behavior and commonly measured physicochemical variables will be incorporated into the model to provide more

accurate estimates of abundance. By accounting for these confounding variables, sensitivity in secondary data summaries (e.g., multimetric indices) will increase the ability to detect perturbations. The results will not only provide a methodology to improve our interpretation of water quality using multimetric indices, but also benefit managers and researchers nationwide in obtaining the best possible estimates of species abundance.

Publications:

The project is ongoing and the data are still being analyzed. We anticipate one presentation at the 2015 Midwest Fish and Wildlife conference in Indianapolis and one manuscript submitted for publication.

Grant Submissions: None

Students:

Four graduate students conducted all field work.

One graduate student and two undergraduate students identified fish in the lab.

References Cited:

Karr, J.R. 1981. Assessment of biotic integrity using fish communities. Fisheries 6:21-27.

Martin, J., J.A. Royle, D.I. Mackenzie, H.H. Edwards, M. Kery, & B. Gardner. 2011. Accounting for non-independent detection when estimating abundance of organisms with a Bayesian approach. Methods in Ecology and Evolution 2:595-601.

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Royle, J.A. 2004. N-mixture models for estimating population size from spatially replicated counts. Biometrics 60:108-115.

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Ter Braak, C.J.F. 1986. Canonical correspondence analysis: A new eigenvector technique for multivariate direct gradient analysis. Ecology 67:1167-1179.

Thomas, J.F., & T.J. Hall. 2006. A comparison of three methods of evaluating aquatic community impairment in streams. Journal of Freshwater Ecology 21(1):53-63.

Trebitz, A.S., B.H. Hill, & F.H. McCormick. 2003. Sensitivity of indices of biotic integrity to simulated fish assemblage changes. Environmental Management 32(4):499-415.

Information Transfer Program Introduction

None.

Pollution Prevention Strategies for Farmers and Commercial Input Applicator Businesses

Basic Information

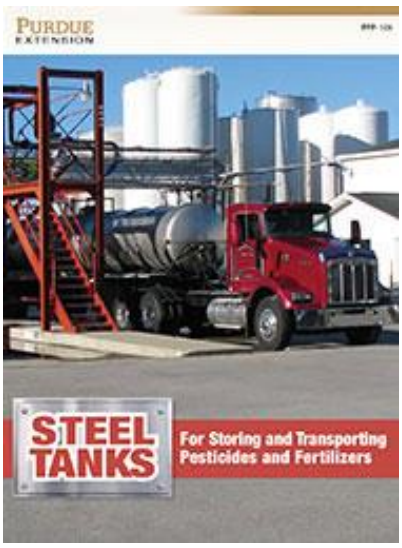
Title:	Pollution Prevention Strategies for Farmers and Commercial Input Applicator Businesses
Project Number:	2013IN363B
Start Date:	3/1/2013
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	IN-004
Research Category:	Water Quality
Focus Category:	Economics, Climatological Processes, Groundwater
Descriptors:	None
Principal Investigators:	Fred Whitford

Publications

There are no publications.

Outreach Project: Steel Tanks for Storing and Transporting Pesticides and Fertilizers

PI: Dr. Fred Whitford, Purdue University



Publication Name and Authors: Whitford, F., D. Nowaskie, B. Peas, D. Sink, E. Fuller, G. Bunn, C. Kominski, T. Cobbs, G. Blasé, and A. Schmerge. *Steel Tanks for Storing and Transporting Pesticides and Fertilizers* (PPP-105). 2013.

Project Accomplishments:

a. Publication. 72 pages, full color. Printed 7,500 copies.

b. Publication Accessibility on Website:
https://ag.purdue.edu/extension/ppp/Documents/PPP-105.pdfc.

c. Purdue University News Release:
http://www.purdue.edu/newsroom/releases/2013/Q4/purdue-extension-publication-helps-identify-proper-pesticide,-fertilizer-tanks.htmld.

d. Examples of newsletter, magazine, and trade publications using information:

•http://farmindustrynews.com/farm-equipment/new-publication-purdue-identifying-proper-pesticide-and-fertilizer-tanks
•http://editiondigital.net/article/Purdue+Extension+Publication+Helps+Identify+Proper+Pesticide,+Fertilizer+Tanks/1627212/0/article.html
•http://www.highbeam.com/doc/1G1-354587168.html

e. Hard Copy Distribution: All pesticide extension coordinators in the United States, all Indiana county extension agriculture and natural resources educators, and all licensed pesticide application businesses in Indiana. Hard copies distributed at extension programs where the senior author presented on poly tanks.

f. PowerPoint Distribution: Agencies, private businesses and associations were furnished the PowerPoint that the senior author uses at programs. In this way, others were teaching their employees or members on steel tanks.

g. Presentations:

- Storage and application tanks: poly, fiber, mild or stainless steel. Indiana Fertilizer Advisory Board. West Lafayette, Indiana.
- Storage and application tanks: steel, fiber, or poly? University of Missouri Crop Injury and Diagnostic Clinic. Columbia, Missouri.
- Poly, Fiberglass, and Steel Tanks: The Pros and Cons of Each Tank Type. Seaside, Oregon.

USGS Summer Intern Program

None.

Notable Awards and Achievements