Arkansas Water Resources Center Annual Technical Report FY2015

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Arkansas Water Resources Center Annual Technical Report FY2015

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Abstract

This publication serves as the annual report to the U.S. Geological Survey regarding the 104B program projects and activities of the Arkansas Water Resources Center (AWRC) for FY2015. This document provides summary information for each of the projects funded through the 104B base grant. This year, the AWRC funded 3 faculty research proposals and 6 student centered proposals with faculty advisors. Faculty projects include: 1) "REWARD: Rice Evapotranspiration and Water Use in the Arkansas Delta", Benjamin Runkle, University of Arkansas, Department of Biological and Agricultural Engineering; 2) "Runoff Water Quality from Managed Grassland Amended with a Mixed Coal Combustion Byproduct", David Miller, University of Arkansas, Department of Crop, Soil and Environmental Sciences (CSES); and 3) "Characterization of Phosphorus Stores in Soils and Sediments and the Potential for Phosphorus Release to Water, Related to Land Use and Landscape Position within a Watershed", Andrew Sharpley, University of Arkansas, Department of CSES. Student projects with a faculty advisor that were funded include: 1) "Optical Water Quality Dynamics During Receding Flow in Five Northwest Arkansas Recreation Rivers", Thad Scott and Amie West, University of Arkansas, Department of CSES and Environmental Dynamics, respectively; 2) "Continuation of analysis for host-specific viruses in water samples collected from select 303(d) listed streams in the Illinois River Watershed", Kristen Gibson and Jay Jackson, University of Arkansas, Department of Food Science; 3) "Creating an Annual Hydrologic Dataset in Forested Ozark Streams", Michelle Evans-White and Allyn Fuell, University of Arkansas, Department of Biological Sciences; 4) "Relationship Between Nutrients, Macrograzers Abundance (Central Stonerollers and Crayfish), and Algae in Ozark Streams", Michelle Evans-White and Kayla Sayre, University of Arkansas, Department of Biological Sciences; 5) "Elucidation of a Novel Reaction Pathway for N-Nitrosamine Formation", Julian Fairey and David Meints, University of Arkansas, Department of Civil Engineering; and 6) "Does Environmental Context Mediate Stream Biological Response to Anthropogenic Impacts?", Sally Entrekin and Lucy Baker, University of Central Arkansas, Department of Biological Sciences.

This publication also covers research projects from FY2014 where project extensions were granted, thus warranting updated final project reports. In this publication is also a summary of the Arkansas Water Resources Center's administration and information transfer programs, student involvement, notable awards and achievements, and publications of previous 104B projects.

Keywords: Arkansas Water Resources Center, 104B Program Funding, Information Transfer, Water Quality

Report Introduction

The Arkansas Water Resources Center (AWRC or Center) is part of the network of 54 water institutes established by the Water Resources Research Act of 1964 and is located at the University of Arkansas in Fayetteville. Since its formation, the AWRC in cooperation with the US Geological Survey and the National Institutes for Water Resources has focused on helping local, state and federal agencies understand, manage and protect water resources within Arkansas.

The Center has contributed substantially to the State's understanding of its water resources through scientific research and volunteer monitoring efforts. Additionally, the training of students – the future generations of scientists and engineers – is a top priority for the Center. The AWRC directs its research funding priorities toward providing local, state and federal agencies with scientific data necessary to make informed decisions that enhance their ability to protect and manage water resources throughout the State. AWRC helps to fund and coordinate research to ensure good water quality and adequate quantity to meet the needs of Arkansas today and into the future.

Another priority mission of the Center is the transfer of water resources information to stakeholders within Arkansas and around the country. The AWRC holds an annual water conference to address current water issues and solutions. The Center also publishes numerous types of publications including technical reports, peer-reviewed journal articles, and monthly electronic water newsletters. The use of social media has allowed the Center to reach more people, with a growing number of interested individuals from state agencies, water organizations, and the greater public.

The AWRC continues to enhance its activities to successfully implement its core missions – to generate competent research, train future water scientists and engineers, and actively disseminate information to water stakeholders throughout Arkansas. This report details these activities of the Center during the past project year (March 1, 2015-February 29, 2016).

Research Management Introduction

Since its formation, the Arkansas Water Resources Center (AWRC or Center) has focused on helping local, state and federal agencies manage and protect Arkansas' water resources. The Center has contributed substantially to the State's understanding of its water resources through scientific research and volunteer monitoring efforts. Additionally, the training of students – the future generations of scientists and engineers – is a top priority for the Center.

Scientific Research

Each year, several researchers across the state submit proposal applications for research grants from the AWRC through the USGS 104B program. The AWRC directs its research funding priorities toward providing local, state and federal agencies with scientific data necessary to make informed decisions that enhance their ability to protect and manage water resources throughout the State. During this past year, the AWRC research program successfully promoted the dissemination and application of research results to stakeholders through publications, conferences and workshops. The research program also emphasized the training of future scientists and engineers who are focused on water resources and watershed management, and supported undergraduate, Masters and Ph.D. levels. The "seed" grants provided to research faculty through this program have led to the development of larger research proposals submitted to other funding agencies and also have provided research opportunities to new faculty and more senior faculty investigating new areas in water resources.

When soliciting research proposals for funding through the USGS 104B program, the Center emphasized the following objectives:

- Arrange for applied research that addresses water supply and water quality problems
- Train the next generation of water scientists and engineers
- Support early career faculty in water research and preliminary data
- Support faculty changing focus or addressing emerging water issues
- Transfer research results to stakeholders and the public
- Publish 104B funded research in peer-reviewed scientific literature
- Cooperate with other colleges, universities and organizations in Arkansas to create a coordinated statewide effort to address state and regional water problems.

Center projects generally focus on topics concerned with the quality and quantity of surface water and ground water, especially regarding non-point source pollution, land use and climate change, agriculture in the delta region, and sensitive ecosystems. To formulate a research program relevant to current water issues in Arkansas, the Center worked closely with state and federal agencies and academic institutions. The following water research topics are currently important to Arkansas:

- Physical, chemical and biological characteristics of streams, reservoirs and aquifers, and how these influence nutrient transport and water quality
- The trends in water quality over time and how things change in response to watershed management
- Source water protection related to drinking water quality and availability
- Non-point source impacts on water quality
- Point-source impacts on water quality, especially from effluent discharges

- Contaminant transport through streams and other aquatic ecosystems
- Water quality and availability, especially in eastern Arkansas
- The biological response to nutrient gradients in lentic and lotic ecosystems
- Development of mechanisms for improving the quality and quantity of water supplies

Each of the proposals selected for funding this past year addressed one or more of the priority research topics and or objectives of the Center. The Center also encourages research proposals that support the USGS national water mission in one of its broad areas, including:

- Increase knowledge of water quality and quantity
- Improve understanding of water availability
- Evaluate how climate, hydrology and landscape changes influence water resources
- Create and deliver decision-making tools that support water management
- Improve the country's response to water-related emergencies

The AWRC has a Technical Advisory Committee (TAC) composed of representatives of state and federal water resources agencies, academia, industry and private groups. A subset of the TAC reviewed and ranked proposals submitted to the AWRC - USGS 104B Program for funding, which helped ensure that funds addressed a variety of current and regional water resource issues.

In FY2015, the AWRC, with the guidance of the TAC, funded 3 faculty research proposals totaling $60,000 and 6 student research proposals with a faculty advisor totaling $28,000. Faculty projects that were funded include: 1) "REWARD: Rice Evapotranspiration and Water Use in the Arkansas Delta", Benjamin Runkle, University of Arkansas, Department of Biological and Agricultural Engineering; 2) "Runoff Water Quality from Managed Grassland Amended with a Mixed Coal Combustion Byproduct", David Miller, University of Arkansas, Department of Crop, Soil and Environmental Sciences (CSES); and 3) "Characterization of Phosphorus Stores in Soils and Sediments and the Potential for Phosphorus Release to Water, Related to Land Use and Landscape Position within a Watershed", Andrew Sharpley, University of Arkansas, Department of CSES. Student projects with a faculty advisor that were funded include: 1) "Optical Water Quality Dynamics During Receding Flow in Five Northwest Arkansas Recreation Rivers", Thad Scott and Amie West, University of Arkansas, Department of CSES and Environmental Dynamics, respectively; 2) "Continuation of analysis for host-specific viruses in water samples collected from select 303(d) listed streams in the Illinois River Watershed", Kristen Gibson and Jay Jackson, University of Arkansas, Department of Food Science; 3) "Creating an Annual Hydrologic Dataset in Forested Ozark Streams", Michelle Evans-White and Allyn Fuell, University of Arkansas, Department of Biological Sciences; 4) "Relationship Between Nutrients, Macrograzers Abundance (Central Stonerollers and Crayfish), and Algae in Ozark Streams", Michelle Evans-White and Kayla Sayre, University of Arkansas, Department of Biological Sciences; 5) "Elucidation of a Novel Reaction Pathway for N-Nitrosamine Formation", Julian Fairey and David Meints, University of Arkansas, Department of Civil Engineering; and 6) "Does Environmental Context Mediate Stream Biological Response to Anthropogenic Impacts?", Sally Entrekin and Lucy Baker, University of Central Arkansas, Department of Biological Sciences.

Once these scientists were funded, the Center coordinated and administered the grants, allowing the researchers to concentrate on providing a quality project. Support was provided to researchers in the form of accounting, reporting and water sample analysis (through the AWRC Water Quality Laboratory).

Volunteer Water Quality Monitoring

The Center supported and worked closely with Ozarks Water Watch, a non-profit water resources organization. AWRC provided guidance and support to StreamSmart, a program of Ozarks Water Watch. AWRC personnel conducted a formal training workshop related to sample collection and site assessment to volunteers. The Center also supported this program by funding the laboratory analysis of water samples collected by volunteer citizen scientists. AWRC supported another program of Ozarks Water Watch called Beaver LakeSmart by participating on the advisory board and providing guidance to the program director.

Student Training

In addition to funding research proposals that emphasized the training of students, the Center provided several training opportunities directly. This direct student support included:

- The AWRC participated in the Research Experience for Undergraduates (REU) program by advising students through the scientific research process.
- The AWRC helped train undergraduate students by mentoring them through their freshman engineering research projects at the University of Arkansas.
- The Center supported paid student work where the student gained experience in the water quality laboratory and in data organization and analysis.
- The AWRC continued with its second annual paid summer internship for high school students. The student intern was expertly trained in geographical information systems (GIS) and successfully completed several GIS products associated with a variety of Center-related research projects.

During this past year, 23 students and postdoctoral researchers were trained through participation in research projects and through the AWRC directly.

Project Title: Is persistence of plasmids in antibiotic resistant E. coli isolated from stream water impacted by integrons and conjugation or mobilization genes?
Project Number: 2014AR350B
Start Date: 3/1/2014
End Date: 2/29/2016
Funding Source: 104B
Congressional District: AR-003
Research Category: Biological Sciences
Focus Category: Ecology, Water Quality, Wastewater
Principal Investigator: Mary Cathleen Savin

Publications and Presentations:

Suhartono, and M. C. Savin, 2014, Occurrence of class 1 integron and mobilization genes associated with plasmid mediated-trimethoprim/sulfamethoxazole-resistant bacteria isolated from wastewater treatment plant effluent and stream water in Northwest Arkansas at 3rd Annual Student Water Conference (SWC), Stillwater, OK.

Suhartono, and M. C. Savin, 2014, Plasmid-mediated class 1 integron and mobilizations genes are prevalent in antibiotic resistant effluent and stream bacteria at Gamma Sigma Delta Student Research Competition, Fayetteville, AR.

Suhartono, and M. C. Savin, 2015, Influence of selected integrase and/or mobilization genes on the persistence of trimethoprim and sulfamethoxazole resistant Escherichia coli, at Arkansas Water Resources Center Annual Watershed and Research Conference, Fayetteville, AR.

Suhartono, and M. C. Savin, 2015, Persistence of plasmids in antibiotic resistant stream water Escherichia coli harboring integron, conjugation, and/or mobilization genes at The Water Microbiology Conference, Chapel Hill, NC.

Suhartono, S., M. C. Savin, and E. E. Gbur, 2016, Genetic redundancy and persistence of plasmid-mediated trimethoprim/sulfamethoxazole resistant environmental Escherichia coli, Water Research, Submitted.

Suhartono, S., M. C. Savin, and E. E. Gbur, 2016, Transmissible plasmids and integrons shift Escherichia coli population towards larger multiple drug resistance numbers, Environmental Science and Pollution Research, Submitted.

Suhartono, and M.C. Savin, May 2016, Dissemination and Persistence of Plasmid Located Antibiotic Resistant Genes Associated with Integrase and Mobilization Genes in Wastewater Treatment Plant Effluent and Stream Water Bacteria, "PhD Dissertation (expected)," Cell & Molecular Biology, Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR.

Suhartono, S., & Savin, M. (2016). Conjugative transmission of antibiotic-resistance from stream water Escherichia coli as related to number of sulfamethoxazole but not class 1 and 2 integrase genes. Mobile Genetic Elements, 6(6), e1256851.

Suhartono, S., M. C. Savin, and E.E. Gbur. 2016. Genetic redundancy and persistence of plasmid-mediated trimethoprim/sulfamethoxazole resistant effluent and stream water Escherichia coli. Water Research. 103:197-204. http://dx.doi.org/10.1016/j.watres.2016.07.035.

Project Title: Is persistence of plasmids in antibiotic resistant *E. coli* isolated from stream water impacted by integrons, conjugation or mobilization genes?

Project Team: Mary C. Savin, Department of Crop, Soil, and Environmental Sciences (CSES), Cell & Molecular Biology (CEMB), University of Arkansas, Fayetteville, AR 72701
Suhartono, Cell & Molecular Biology (CEMB), Department of Crop, Soil, and Environmental Sciences (CSES), University of Arkansas, Fayetteville, AR 72701

Executive Summary:

Persistence of antibiotic resistant bacteria may be facilitated by the presence of conjugation and mobilization (*mob*) and integron (*intI*) genes associated with bacterial plasmids. Plasmids extracted from 139 antibiotic resistant *E. coli* isolated from treated effluent and receiving stream water were used to detect and characterize *mob* genes and class 1 and 2 integrase genes using PCR amplifications. Plasmid persistence was determined using mesocosm incubations for a total of 76 *E. coli* in which the antibiotic resistant determinants for trimethoprim and sulfamethoxazole were confirmed. *E. coli* were grown in the presence of sub-inhibitory concentrations of trimethoprim or sulfamethoxazole, and the density of bacteria (log CFU/mL) was determined during an 11-day experiment. This investigation confirmed the occurrence of class 1 and 2 integrons and indicated the positive relationship of the presence of the integron with the increasing number of phenotypic multiple antibiotic resistances (MAR). The mob_{F12} gene was most frequently detected, and the co-occurrence of two or three *mob* genes, which often was mob_{F12} in combination with either mob_{P51} or mob_{Qu}, resulted in a higher proportion of increased MAR in the resistant *E. coli* population. Over an 11-day experiment, isolates persisted at concentrations greater than log 8.99 CFU mL^{-1} in the presence of sub-inhibitory sulfamethoxazole regardless of integron and mobilization gene designation. In the presence of sub-inhibitory trimethoprim, isolates harboring plasmids $mob^{+}intI^{+}$ were less persistent compared to isolates without either or with a gene from either group individually. Overall, resistance in plasmids remained relatively stable during the experiment.

Introduction:

Antibiotic resistant bacteria (ARB) are a major public problem, with concern increasing about their persistence in the environment. Despite different disinfection protocols in different WWTPs and reductions in culturable *Escherichia coli, E. coli* and broad-host-range (BHR) plasmids (Akiyama et al., 2010) and antibiotic resistance genes (ARG) (MacLeod and Savin, 2013) remain in discharged WWTP effluents, which lead to inputs of corresponding plasmids into receiving streams. Persistence in stream water may be facilitated by the presence of *mob* genes and integrons associated with bacterial plasmids. The research objectives were to determine the presence of integrase and mobilization genes and the relationship with multiple antibiotic resistance (MAR) number in antibiotic resistance bacteria, and to determine the influence of those genes towards the persistence of antibiotic resistant *E. coli* plasmids that were originally isolated from treated wastewater effluent and receiving stream water.

Methods:

Previous investigations recovered a number of *E. coli* possessing ARG (Akiyama and Savin, 2010) and plasmids (Akiyama et al., 2010) from one site upstream (20 m upstream, M1), wastewater treatment plant (WWTP) effluent discharge (ME), and two sites downstream (640 m (M2) and 2000 m (M3)) of the pipe discharging water from the Fayetteville, Arkansas WWTP into Mud Creek. Plasmid extractions from antibiotic resistant *E. coli* were carried out using the Wizard® Plus SV Minipreps DNA Purification System (Promega, Madison, WI, USA) according to the manufacturer's instruction. Plasmids were then used as templates to detect and characterize *mob* and *intI* genes and confirm the presence of sulfamethoxazole and trimethoprim resistance genes.

Genes related to resistance to sulfamethoxazole (*sul1, sul2,* and *sul3* gene), trimethoprim (*dfrA1, dfrA8, dfrA12, dfrA14, dfrA17*, and *dfrB3* gene), integrons (*intI1* and *int2*), and mobilization (*mobP11, mobP14, mobP51, mobF11, mobF12, mobQ11,* and *mobQu*) were determined using PCR amplifications (Pei et al., 2009; Šeputienė et al., 2010; Mazel et al., 2000; Alvarado et al., 2012). All PCR amplifications were performed in 20 µL reactions containing 1× PCR buffer, 2.5 mM $MgCl_2$, 200 µM dNTPs, 400 ng/µL bovine serum albumin (Merck KGaA, Darmstadt, Germany), 0.5 µM of each primer, 1 µL of template DNA, and 0.5 U of GoTaq DNA polymerase (Promega, Madison, WI, USA). DEPC-treated water (EMD Millipore, Darmstadt, Germany) was used as no template control (NTC) run in parallel with samples. The PCR reactions were carried out using a PTC-200 thermocycler (MJ Research, Waltham, MA) under conditions as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, annealing (55.9°C for *sul1* and *sul2;* 60.8°C for s*ul3*; 46°C for *dfrA1*; 56.3°C for *dfrA8;* 52°C for *dfrA12;* 44°C for *dfrA14*; 44°C for *dfrA17*; 56°C for *dfrB3*; 62°C for *IntI1*; 50°C for *Int2*; 60°C for *mobp11;* 50°C for *mobp14*; 58°C for *mobp52;* 53°C for *mobf11*; 55°C for *mobf12*; 50°C for *mobq12*; 64°C for *mobqu*) for 30 s, and 72°C for 60 s, with a final extension at 72°C for 8 min. PCR products were analyzed on 1.5 % (w/v) agarose gels with ethidium bromide at 100V for 50 min in Tris-borate-EDTA (TBE) buffer and documented using Kodak EDAS 290 gel documentation and analysis system (Eastman Kodak Co., Rochester, NY) to assess bands of the expected size. Additional confirmation of the PCR products was performed through DNA sequencing (Eurofin Genomics, Kansas City, Kansas, USA).

The influence of plasmid-mediated mob and *intI* genes on persistence of *E. coli* isolates over time was tested in 500-mL sterile Erlenmeyer flasks containing 200 mL synthetic wastewater made from components as described by McKinney (1962) supplied with antibiotics (either 0.19 µg L^{-1} trimethoprim or 0.5 µg L^{-1} sulfamethoxazole). A total of 76 Isolates resistant to both sulfamethoxazole (80 µg mL^{-1}) and trimethoprim (4 µg mL^{-1}) were placed into one of four groups according to *intI* and *mob* gene presence/absence combinations: group I ($mob^{+}intI^{+}$), group II ($mob^{-}intI^{+}$), group III ($mob^{+}intI^{-}$), or group IV ($mob^{-}intI^{-}$). The flasks were maintained at 23 °C for 11 days, with 3 mL removed from each flask after 1, 3, 5, 7, 9 and 11 days of incubation. The colony forming unit (CFU) number on day 1, 7, 9, and 11 was determined using plate count assay on selective tryptic soy agar media supplemented with either sulfamethoxazole (80 mg L^{-1}) or trimethoprim (4 mg L^{-1}).

A statistical analysis was performed to evaluate the effects of occurrence of *mob* and *intI* genes towards the multiple antibiotic resistance (MAR) number with 95% confidence intervals using GLIMMIX procedure on SAS 9.4 (Cary, North Carolina, USA). The data were analyzed based on a multinomial logit model with a cumulative logit link function and the results were back-transformed to the proportion scale for presentation of the results. Following the preliminary overall test for treatment effects, contrasts were used to compare individual pairs of treatments ($P \leq 0.05$) on the cumulative logit scale. Additionally, an analysis of variance (ANOVA) was performed to evaluate the effects of mobilization and/or integron presence or absence, days of incubation, and the combination of gene presence/absence and time on bacterial concentration in the presence of each antibiotic. When appropriate, means were separated by Fisher's least significant difference (LSD) at $\alpha = 0.05$. Analysis was performed using GLM procedure with 95% confidence intervals on SAS 9.4 (Cary, NC).

Results:

There was a statistically significant difference ($P = 0.0014$) in *mob* gene distribution among plasmids of isolates across MAR number (Figure 1a). A total of 65 (46%) isolates conferred transmissible

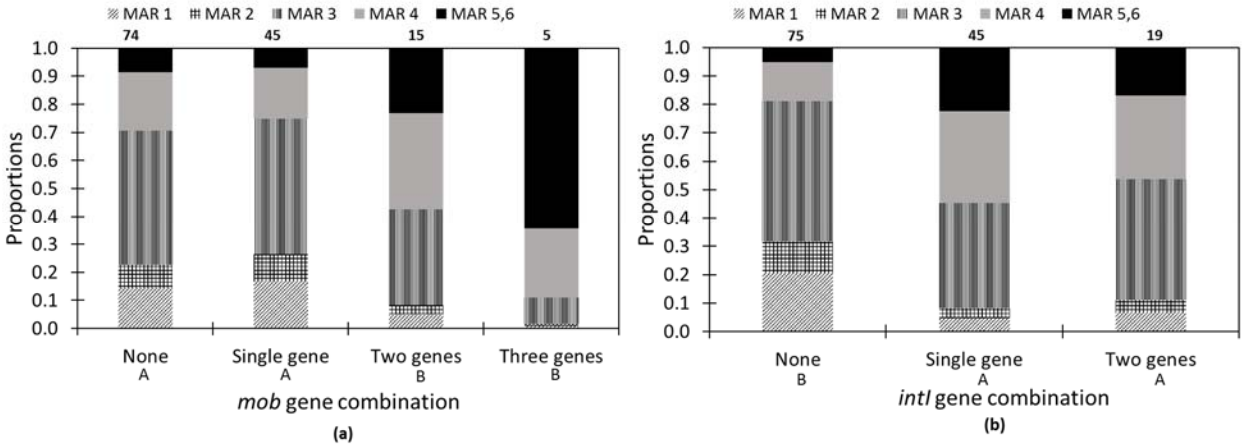

Figure 1. Relationship of estimated proportion of (a) *mob* genes (P = 0.0014) and (b) *intI* genes (P < 0.0001) among multiple antibiotic resistant *E. coli* isolated from Mud Creek in Fayetteville, Arkansas (N = 139). Designations with the same letter at the bottom of gene combinations are not significantly different using an overall test for equality of distributions on the cumulative logit scale contrasts procedure ($P \leq 0.05$). Numbers at the top represent the sum of occurrences by column.

plasmids indicated by the presence of *mob* genes. A mob gene, mob_{F12}, was most prevalently detected on plasmids from 54 (39%) of total 139 isolates or 83% of the total transmissible plasmids. The co-occurrence of two or three *mob* genes, which often was mob_{F12} in combination with either mob_{P51} or mob_{Qu} resulted in a higher proportion of increased MAR in the resistant *E. coli* population. The *mobP11*, *mobP14*, and *mobQ11* genes were not detected. Similar to the *mob* genes, there was also a significant difference (P < 0.0001) in distribution of *intI* genes among plasmids of isolates across MAR number (Figure 1b). The occurrence of integrons, particularly class 1 integrons, alone or in combination, shifted the distribution proportion of *E. coli* isolates such that more of the population possessed larger MAR numbers (MAR 3 to MAR 5 or 6) (Figure 1b).

Sulfamethoxazole and trimethoprim resistance genes were confirmed in plasmids of 76 isolates. In terms of persistence, there was significant effect of incubation time (P = 0.0062) on bacterial concentration when grown in the presence of sub-inhibitory concentration of sulfamethoxazole regardless the mob-integron designation (data not shown). Despite its significant decrease on day 11, isolates persisted during incubation such that concentrations remained at almost 1 billion CFU per mL. In the presence of trimethoprim, there was a significant interaction of integrase by mobilization gene presence or absence with incubation time (P = 0.0365) affecting bacterial growth (Table 1). Bacterial concentration harboring plasmids with both integron and mobilization genes decreasing over time; however, after 11 days, bacterial concentrations in all treatments remained over 1 billion CFU per mL. Isolates harboring plasmids absent in either or both integron and mobilization genes did not significantly decrease in concentration during the experiment.

Conclusions:

Having two or more *mob,* or one or two *intI*, contributed to significantly increasing the proportion of the *E. coli* population exhibiting larger multiple phenotypic antibiotic resistances. In the presence of sub-inhibitory concentrations of sulfamethoxazole, isolates persisted regardless of integron and mobilization gene designation, whereas in the presence of trimethoprim, isolates harboring plasmids with both integron and mobilization genes decreased in concentration during an 11-day experiment. However, there was little significant differentiation in persistence among the four groups designating presence and absence of integron and mobilization genes. Overall, these findings indicate that treated effluent

Table 1. Means of cell density (log CFU mL^{-1}) grown on trimethoprim based on presence or absence of integron and mobilization (mob) genes, and time of incubation (N = 76).

Time of incubation (day)	Integron	Mob Absent	Mob Present
1	Absent	9.32a	9.18bcd
	Present	9.15cdef	9.26ab
7	Absent	9.23abcd	9.25abc
	Present	9.24abcd	9.15def
9	Absent	9.16cde	9.14def
	Present	9.19bcd	9.08ef
11	Absent	9.19bcd	9.17bcde
	Present	9.25abc	9.06f

*Means followed by a similar letter are not significantly different ($P < 0.05$).

containing multiple antibiotic resistant bacteria may be an important source of integrase and mobilization genes. Resistant plasmid persistence appear to have potential for stability in the environment. Sulfamethoxazole- trimethoprim resistant bacteria may have a high degree of genetic redundancy and diversity conferring resistance to each antibiotic which may lead to persistence of the bacteria in the stream environment, although the role integrase and mobilization genes towards persistence is unclear.

References:

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Šeputienė, V., Povilonis, J., Ružauskas, M., Pavilonis, A. and Sužiedėlienė, E. 2010. Prevalence of Trimethoprim Resistance Genes in *Escherichia coli* Isolates of Human and Animal Origin in Lithuania. Journal of Medical Microbiology, 59, 315-322.

Project Title:	Improved ensemble forecast model for drought conditions in Arkansas using residual re-sampling method
Project Number:	2014AR352B
Start Date:	3/1/2014
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	001
Research Category:	Climate and hydrologic processes
Focus Category:	Drought, climatalogical processes, hydrology
Principal Investigator:	Yeonsang Hwang

Publications and Presentations:

Egan, H. and Y. Hwang, 2015, Arkansas Drought Variability at Create @ STATE: A Symposium of Research, Scholarship and Creativity, Jonesboro, AR.

Egan, H., H. Tyler,M. Land, 2015, Arkansas Climate Diversity, Arkansas Water Resources Center Annual Water Conference, Fayetteville, AR.

Tyler, H., M. Land and Y. Hwang, 2015, Arkansas Climate: With or Without Uniform? at Create @ STATE: A Symposium of Research, Scholarship and Creativity, Jonesboro, AR.

Project Title: Improved ensemble forecast model for drought conditions in Arkansas using residual re-sampling method

Project Team: Yeonsang Hwang, College of Engineering, Arkansas State University

Executive Summary:

Successful prediction of drought stages in Arkansas is essential for sustainable use of water resources in Arkansas. Stochastic ensemble forecast model utilizes flexible historical residual resampling technique to provide a short term monthly (1 to 3-month lead) forecast of drought condition in climate divisions in Arkansas. The short-term monthly simulation showed varying forecast skills in different lead-time and years. Long-term forecast capability was tested by performing seasonal predictions based on random re-sampling technique. Further analysis of the climate variability in the state is needed to improve the forecast skills of this model.

Introduction:

Drought is a part of natural variability while the impact on natural resources and industry due to drought events can be mitigated with proper planning and preparation (Steinmann 2006). As the cost of drought during the three-year period between 1987 and 1989 was estimated to be 39 billion dollars combining energy, agriculture, water losses, etc. in the US, increasing water use for agriculture activities, power generation, and municipal growth has added concerns to water resources sustainability in the state of Arkansas. Liu et.al. (2013) also examined the past drought and presented the drought scenarios.

The most recent updates from the IPCC highlights that the contrast in precipitation between wet and dry seasons will increase amid non-uniform changes in the global water cycle in response to the predicted global warming in the 21th century (IPCC 2013). IPCC's draft report also states that regional scale prediction is still problematic, and would create additional uncertainty in hydro-climate conditions in Arkansas. Historical data does show noticeable seasonal and annual climate variability in precipitation and temperature in the state (SPPI, 2008). Considering this uncertainty, any prediction of hydro-climate variables is challenging but very important in water management and planning in the region.

Through this project, numerical models were tested for monthly forecast of drought stages in climate divisions (9 regions by NOAA) with short-term prediction (up to 3-month lead). Long-term dry/wet condition projection were also tested. We anticipate this tool to be utilized to improve local, regional, and state water management plans in the future.

Methods:

This forecast idea is based on a flexible statistical framework that utilizes residuals in local regression fits. Conventional ensemble forecast techniques take advantage of historical climatological and hydrologic data, but those techniques are limited in terms of the forecast performances in two ways. First, historical data only allows developed models predict within the variability of existing data (normally instrumental measurements), and therefore all generated forecast becomes very sensitive to the length and quality of collected time series. Sequential index method (repeating annual historical data to generate future prediction) is a good example, and forecast products are limited by the observed variability. Secondly, the model won't generate possible extreme conditions not existing in historical data. Given the uncertainty in the model, historical data, and future changes in climate variability, it becomes more important to utilize natural variability and forecast extreme conditions.

Residual resampling technique focuses on the natural statistical properties in the historical data and utilizes the residuals in rather simple mathematical models such as linear regression. Calculated residuals of regression models are collected (re-sampled) and distributed to generate ensemble forecasts. While residual calculation is easily done over the entire time series, different strategies can be applied to resample the residuals.

Similar residual resampling techniques have been applied to streamflow forecasts (e.g., Prairie et al. 2006), and the study of auto-regressive features in drought indices have been utilized in the past. Popular drought indices such as PDSI (Palmer Drought Severity Index) and SPI (Standardized Precipitation Index) have been examined and shown as auto-regressive processes in earlier researches (e.g., Guttman 1998). However, previous research has been focused on deterministic forecast techniques until Carbone and Dow (2005) examined the possibility of ensemble forecast for drought indices using a historical random sampling technique in South Carolina.

A series of experimental application of this approach at a different spatial scales was tested in South Carolina (Hwang and Carbone 2009) and later in Arkansas (Martinez 2012, Yan and Hwang 2014). Although the latest model successfully performed three-month lead drought stage forecasts in Arkansas' 9 climate divisions, this forecast model showed limitations due to the built-in autoregression process. For example, change of drought condition due to large rainfall in September over the eastern side of the state wasn't captured in the interquartile range of the forecasted values. In this project, baseline residual sampling technique will be applied to the 9 climate divisions in Arkansas to verify the advantages and disadvantages of this technique. All drought and climate information are compiled from NOAA NCDC (National Climate Data Center) historical archives. For statistical analyses and forecast model development, open source statistics package R is utilized. Among other geostatistics libraries pre-developed and available through R communities, locfit by Loader (1997) provides basic data-driven analysis using non-parametric polynomial approach. This approach is known to be good for non-linear historical data.

Results:

Monthly PDSI forecast model with 1 to 3-month lead-time is used to produce 1000 ensemble members per month using historical data set from NOAA NCDC. All predictions are calculated from the partial time series up to the current month to perform hind-cast to correctly evaluate the forecast skills. Different from the local (at climate stations) forecast tested by Hwang and Carbone (2009), regional (for climate divisions) drought forecast model was applied without weather forecast based residual resampling strategy. In this previous work, all selected residuals were tagged by pre-determined categories based on the monthly temperature and precipitation of the year with respect to climate normal of the station. This allowed the residuals to be more effectively selected. For example, with above normal temperature and precipitation as the forecasted monthly weather condition of the target month, residuals from the years with similar condition were more frequently used than other years.

In this work, short-term forecast for monthly historical PDSI utilizes random residual resampling process. This means that the residuals were randomly sampled from the constructed regression model for the entire historical time series. Also, all forecasts are produced with a seed value generated from the linear local correlation model. This correlation model relates the drought conditions of current and target months depending on the forecast lead time. All correlation models are built without current year's data for fair performance evaluation. Figure 1 shows differences in forecast skills for 1 to 3 month forecast lead time and sample forecast on 2010 in the central Arkansas region (Climate Division 5). It is clear that 1-month forecast shows better confidence (better capture of PDSI in boxplots) than 3-month forecasts (longer whiskers and off-box data). However, the 3-month forecast still captures observed values quite well in many months. Our results show different forecast quality through the years in the time series due to the nature of random sampling approach. This model shows the forecast skills ranging between 0.4 and 0.1 in KSS (Kuiper Skill Score, Wilks 2011) throughout all 9 Climate Divisions in Arkansas.

Rank Histogram (Wilks 2011) is one of the popular graphical measures to examine the quality of ensemble climate forecast models. In pre-defined bins evenly divided in the full range of forecast ensembles, location of observed values are tallied. For example, in the ensemble forecasts ranging between -1 to 4, observed value of -0.95 will add a count in the first bin (with the bin width of 0.1) to the far left side of the chart. Flat diagram implies an ideal ensemble forecast that captures natural events with good variation on both above and below observation all times. Inverse U-shaped chart implies rather 'accurate' forecasts that captures the natural events close to the median forecasts values frequently.

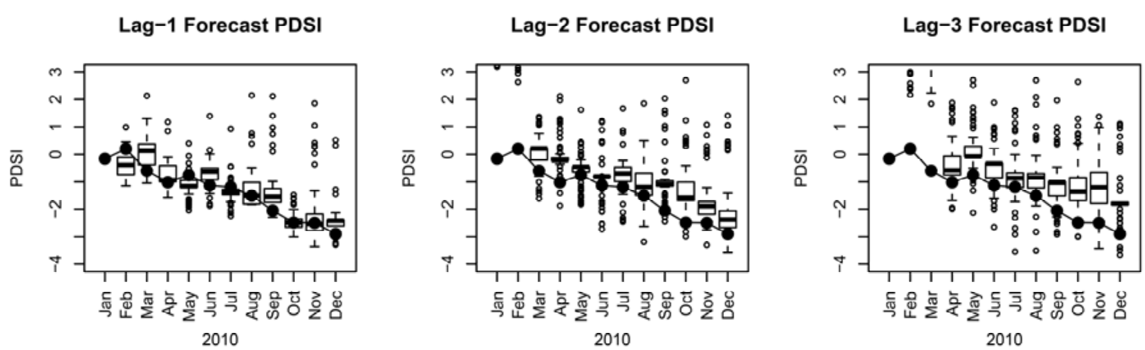

Figure 1. Example PDSI forecast in Arkansas Climate Division 5 (central Arkansas) using historical residual resampling technique. Lag-1, 2, and 3 represents the forecast lead of 1, 2, and 3 months, respectively. For example, Lag-2 forecasts are predicted by utilizing all data available 2 months before the target month. Box plots show the ensemble range, and solid circles show observed (actual) monthly values. The length of the boxes indicates the interquartile range of all generated ensemble indices, and the whiskers show the 5th and 95th percentile range.

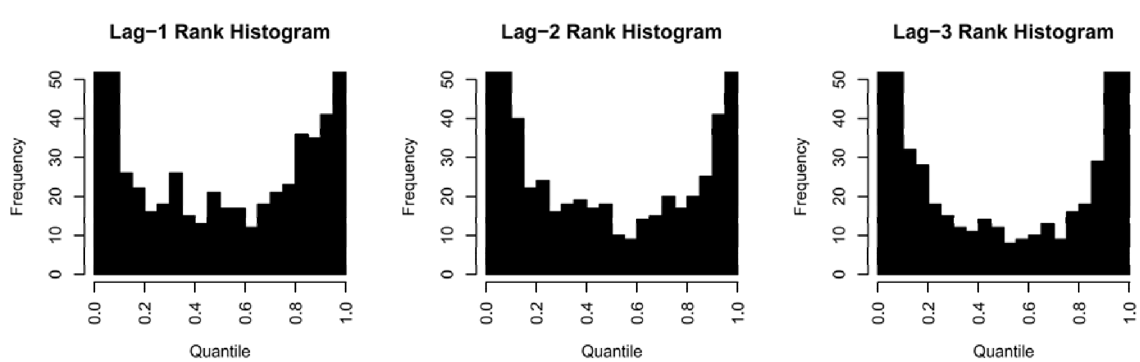

Figure 2. Example forecast skill measure (Rank Histogram) of PDSI forecast in Arkansas Climate Division 5. Locations of observed data are tallied in generated ensemble range.

However, this can also be achieved by generating ensemble forecasts with very large variation, which leads to inefficient products for actual design and planning. For our models, U-shaped histograms were obtained and shown in Figure 2. This possibly implies 1) forecast ensemble range is too narrow to perfectly re-generate natural variability in the record, or 2) overall forecast skill is too low to capture the observation within ensemble's interquartile ranges. Given the idea that our 1-month lead forecast records as high as 0.4 in KSS, the former explains the results better. This ensemble range can easily be adjusted based on users' (i.e., decision makers) willingness to take a different level of uncertainty in the forecast outputs.

Short-term forecast was also tested without looking at the monthly drought correlation between the current and target months. This strategy would be useful and effective when the model output is more expected to generate extreme events such as severe drought beyond historical monthly trend or abrupt change in drought conditions both in dry-to-wet and wet-to-dry conditions. In order to achieve this goal, seed forecast values are generated randomly on the target months first. Similar to the first model, collected residuals are randomly added to the seed forecast to generated ensemble forecast (1000 members). For all climate divisions, ensemble forecast showed much bigger range than the first model. This was clearly shown in wider interquartile range in boxplots similar to Figure 1. However, rank histogram still was U-shaped, which implies that the forecast needs further improvements. KSS calculated for this model was significantly lower (-0.05 ~ 0.3) than the first model, too. This is mainly because of the poor median forecast given from random selection.

Long-term PDSI projection was performed through random sampling over the entire state. Given the large uncertainty in this index averaged over large spatial scale, only seasonal projection was generated with 3-month moving window. For example, forecast in season 'J' utilized the average monthly PDSI from December, January, and February. Also, season 'F' projection was based on January, February, and March. Figure 3 shows the overall performance of this seasonal projection. Future model application will focus on the improvement in seasonal drought projection skill by incorporating climate scenarios such as CO_2 emission scenario or fixed rate temperature increase due to possible climate change in the future.

Conclusions and Recommendations:

The ensemble forecast model used in this work is based on two different residual random sampling strategies and additional application in seasonal scale drought projection;

1. Historical residual resampling model provides simple but valuable platform to be modified and applied to drought prediction.
2. As expected, resampling technique is capable of producing useful forecast skill for moderate progression of drought.
3. Ensemble technique captures uncertainties in the climate system for moderate progression of drought.
4. Limitations do exist in this simple method when drought condition changes beyond seasonal trend in the record in Arkansas. Rank Histogram clearly reflects this.
5. Climate division level climate statistics must be analyzed in conjunction with climate forecast products in the area for further model improvement. This will allow better median forecast quality and will make overall ensemble ranges properly positioned.

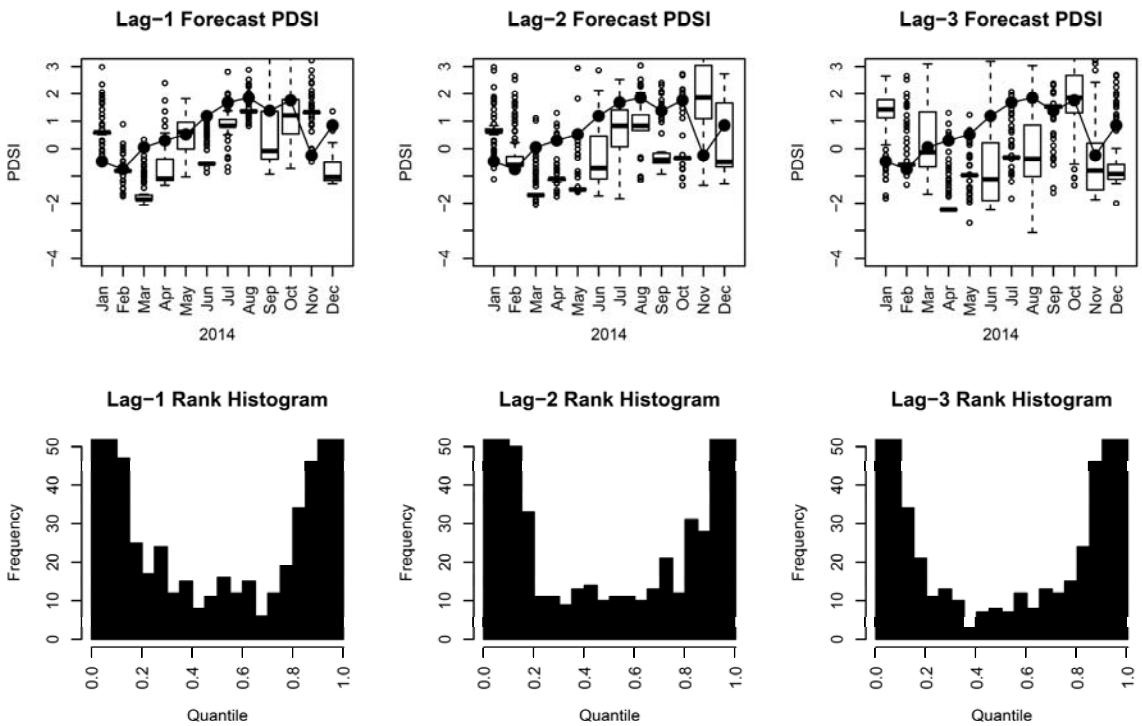

Figure 3. Example seasonal forecast and it's skill measure (Rank Histogram) of PDSI forecast in Arkansas. For comprehensive testing and analysis, seasonal forecasts are presented using 3-month moving window (e.g., January forecast is for DJF, and February is for JFM, etc).

Further study on climate variability in Arkansas will be essential to improve the quality of drought prediction. This includes the study of climate teleconnections, seasonal correlations, variability of key climate variables, etc.

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Project Title:	Hydrogeology and Biogeochemical Evolution of groundwater in Big Creek and Buffalo River Basins and Implications for Concentrated Animal-Feeding Operations
Project Number:	2014AR355B
Start Date:	3/1/2014
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	003
Research Category:	Water quality
Focus Category:	Hydrogeochemistry, groundwater, agriculture
Principal Investigator:	Phil D. Hays

Publications and Presentations:

Roland II, V.L. and P.D. Hays, 2016 (expected). The role of organic matter in the fate and transport of antibiotic resistance, metals, and nutrients in the karst of Northwest Arkansas. "MS Thesis", Environmental Dynamics Program, University of Arkansas, Fayetteville, AR.

Roland, V, 2014, Hydrogeologic and water quality investigation of Big Creek in the Buffalo River Watershed near a major concentrated-animal feeding operation at National Association of Black Geosciences, Washington State University Tri-Cities, WA.

Roland, V, 2014, What’s up with the Buffalo River: Rolling out the Science at Public Town Hall Meeting, Fayetteville, AR.

Project Title: Biogeochemical Evolution of groundwater in Big Creek and Buffalo River Basins and Implications for Concentrated Animal-Feeding Operations

Project Team: Victor L. Roland II, Environmental Dynamics, University of Arkansas-Fayetteville
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Executive Summary:

Concentrated animal feeding operations (CAFOs) can be a source of organic matter, nutrients and bacteria to waterways. This study aims to assess the response of bacteria to increases in dissolved organic carbon (DOC) concentrations and the role of bacteria and increases in DOC in dissolved nitrogen and phosphorus assimilation. Water samples were collected at several sites throughout the study area in July 2014 and January 2015 and analyzed for nutrients and bacteria counts. Laboratory microcosm experiments were conducted using water collected from a spring in Mt. Judea, AR. Mason jars were filled with spring water and gravel sized rocks collected at the spring. The spring water was amended with phosphate, $\delta^{15}N$ –labeled nitrate, and $\delta^{13}C$-labeled acetate. The microcosms were sampled at weekly intervals through the first 3 weeks of the experiment and one final sampling at 13 weeks. Samples were analyzed for total nitrogen, dissolved oxygen, $\delta^{15}N$-NO_3, $\delta^{18}O$-NO_3, and $\delta^{13}C$ -DIC.

Introduction:

CAFOs are sources of organic matter, nutrients, bacteria, and other products that can potentially influence water quality (Wantanabe et al., 2010; Ko et al., 2008; Jarvie et al., 2013; Varnel & Brahana, 2003). The impact of increasing labile organic matter can lead to major shifts in microbial ecology, biogeochemical processes, and potentially degraded water-quality. Organic matter has been linked with the transport of endocrine disrupting compounds (Yamamoto et al., 2003), and metals (Seiler and Berendonk et al., 2012). This study is part of a larger study aimed to assess the role of organic matter in the transport and fate of antibiotics and antibiotic resistance in karst groundwater. Karst springs are particularly vulnerable because of preferential pathways that connect groundwater and surface water, which allows rapid transport of contaminants. This study will assess the role of carbon and nutrient cycling in the development of biomass in epikarst springs. The objectives of the project were; (1) to model changes in microbial metabolic activity based on DOC concentration using laboratory microcosm studies, (2) to model the effect of DOC concentration on nitrate attenuation, (3) to quantify changes in biomass production under elevated DOC and nutrient conditions.

Methods:

Water samples were collected in July 2014 and January 2015. Sampling site locations are shown in Figure 1. Big Creek upstream is located 3.0 miles upstream of the CAFO and the Big Creek downstream sampling location is located 4 miles downstream of the CAFO. The Buffalo River upstream site is located 0.1 miles upstream of the confluence with Big Creek and the downstream site on the Buffalo River is located 0.25 miles downstream of the confluence. Dye Spring is an epikarst spring discharging groundwater from a perched limestone aquifer approximately 2 miles south of the CAFO. Land cover in the recharge area of the spring consists of agricultural pastures and forested areas. Temperature, pH,

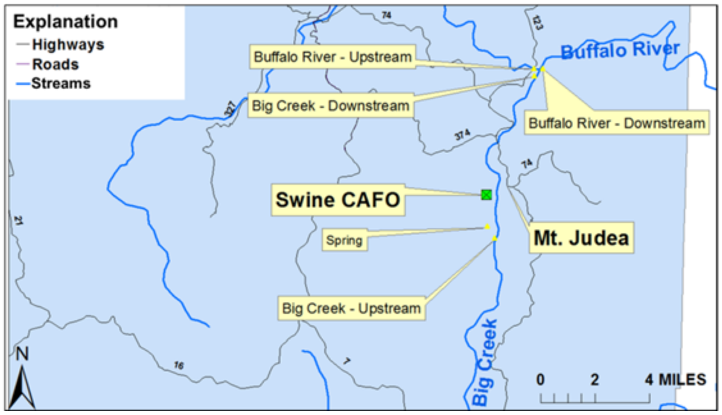

Figure 1. Map of sampling locations in Mt. Judea, AR.

specific conductivity, and dissolved oxygen measurements were taken at the time of sampling. Water quality samples were collected in Nalgene or Teflon sample bottles. Samples analyzed for total nitrogen and total phosphorus were filtered and acidified using 0.2 % sulfuric acid. All samples were stored on ice during transit to the laboratory. Samples were stored at 4 °C before analysis. Total Phosphorus and total nitrogen were simultaneously analyzed using alkaline persulfate digestion (APHA, 4500-Pj). Sulfate analysis was conducted using barium sulfate turbidimetric method (USEPA 375.4). The method for the analysis of ammonia was conducted using the salicylate-hypochlorite method adapted from Reardon and others (1966). Biological water quality samples were collected in Teflon sample bottles and transported to the laboratory. The heterotrophic plate count method was modified to determine the concentration of live heterotrophic bacteria cells in water samples (APHA, 9215). Biological water samples were shaken before 10 µL aliquots were used to inoculate a 10% strength Trypticase Soy Agar media. Samples were allowed to incubate at 35°C for 48 hours.

Laboratory microcosm

Laboratory microcosms were conducted in a dark environment at 13 °C for 13 weeks to simulate conditions in epikarst. Autoclaved gravel was added to 1.0 L mason jars and the jars were filled with spring water and amended with organic carbon, nitrate, and phosphate. Three dissolved organic concentrations were used in the experiments; 1.0 mg/L, 10.0 mg/L, and 100 mg/L. Acetate was chosen as an organic carbon source because it is easily metabolized by bacteria. The microcosms also received three different nutrient treatments; nitrogen (KNO_3), phosphate ($NaPO_4$), and nitrogen and phosphate at 0.1 mg/L, 1.0 mg/L and 10 mg/L. Nutrient concentration ranges were determined based on historical phosphorus and nitrogen observations at the spring. Labeled δ^{15}N-nitrate ($K^{15}NO_3$) and labeled- δ^{13}C -acetate ($^{13}C_2H_3NaO_2$) were used to enrich the isotopic compositions of nitrate and dissolved organic carbon in the microcosms to 1000‰, respectively. The microcosms were sampled at weeks 1 – 3 and at week 13. Phosphoric acid conversion of DIC to CO_2 was used to measure δ^{13}C –DIC. Conversion of available nitrate to N_2O gas by the bacteria *Pseudomonad aereofaciens* was used to measure δ^{15}N-nitrate. Nitrate isotope values were measured relative to ambient air, and δ^{13}C –DIC were reported versus the Vienna Pee Dee Belemnite (VPDB) standard. Dissolved oxygen was also measured using the Winkler titration method. Biofilm biomass was collected from the microcosms by scrubbing with a stiff plastic bristle brush in 150 mL of Millipore water. The samples were centrifuged, and the supernatant was decanted from the samples before freeze drying. The dry mass of the biofilm biomass was then recorded.

Results:

Field pH ranged from 6.38 to 9.64 standard pH units for all sampling sites, Table 1. The mean pH of surface water was 8.23 ±0.92 standard pH units and ranged from 7.32 to 9.64. The mean pH of groundwater discharging from Dye Spring was 7.07 ±1.14 and had pH measurements ranging from 6.38 to 8.38. Mean water temperature during summer sampling was 23.89 ±3.4°C and 17.73 ±0.25°C for surface and groundwater, respectively. Surface water and groundwater mean temperatures in the winter were 8.3 ± 1.35°C and 13 ± 2.45°C, respectively. Mean specific conductance of the surface water samples was 207.9 ± 46°C; in groundwater mean specific conductance was 413 ± 19.7°C. Dye Spring had the least dissolved oxygen; however, water at all sites was aerobic.

Biological water quality is presented in Figure 2. Mean heterotrophic bacteria counts were greatest upstream on Big Creek ($p<0.001$). Dye Spring had the second greatest concentration of heterotrophic bacteria, 334 ± 73 cfu/10µL. There was no statistically significant difference in heterotrophic bacteria concentrations at upstream and downstream sites on the Buffalo River.

Water chemistry was similar at all sites. Total nitrate and phosphate concentrations in the Buffalo River and Big Creek were less than 1 mg/L during all sampling events, with exception given to the downstream site on Buffalo River July 14, 2014, Table 1. The concentration of total phosphorus downstream on the Buffalo River 1.77 mg/L was unusually high for phosphate concentrations in the

Table 1. Field parameters (measured at time of sampling), and measured water quality parameters. NA – constituent not measured, Total Phosphorus and total nitrogen MDL<0.02, Ammonia-nitrogen MDL<0.002 mg/L.

Sampling Location	Sampling Date	Temp. (°C)	pH	Specific Conductance (µS/cm)	DO (mg/L)	Total Nitrogen (mg/L)	Total Phosphorus (mg/L)	NH_3-N (mg/L)	SO_4 (mg/L)
Buffalo River (Upstream)	7/17/2014	25.9	7.32	217.3	7.00	0.18	<0.02	0.01	12.1
	1/30/2015	8.5	7.92	213	NA	0.21	0.02	NA	NA
Buffalo River (Downstream)	7/14/2014	26.6	7.44	225	8.01	0.22	1.77	<0.002	12
	1/30/2015	7.1	8.59	216.4	NA	0.22	<0.02	NA	NA
Big Creek (Upstream)	7/17/2014	19	7.51	149.7	9.89	0.16	0.14	<0.002	11.9
	1/30/2015	10.2	9.64	131.5	NA	0.07	0.02	NA	NA
Big Creek (Downstream)	7/14/2014	24.02	7.9	273.3	8.43	0.23	<0.02	0.01	12.8
	1/30/2015	7.7	9.49	236.8	NA	0.23	0.02	NA	NA
Dye Spring	7/17/2014	17.55	6.45	407.1	6.65	2.52	0.04	0.05	11.7
	8/12/2014	17.9	6.38	435	6.38	NA	<0.02	NA	NA
	1/30/2015	13	8.38	397	NA	3.24	0.02	NA	NA

Buffalo River. The typical range of total phosphorus concentrations in the Buffalo River at baseflow from 1991 – 2001 was 0.004 mg/L and 0.040 mg/L (White et al., 2004). Additional sampling will be necessary to determine the validity of this measurement. Dye Spring had significantly greater total nitrogen values when compared to other sampling sites (p=0.02). Total nitrogen concentrations during sampling events at Dye Spring were 2.5 mg/L and 3.24 mg/L in summer and winter, respectively. The chemical and biological composition of the spring water is controlled by a thick soil layer covering the recharge area of the spring. Water infiltrates through the soil, but is altered chemically and biologically before discharging at the spring. In the Buffalo River and Big Creek, lower bacteria and nutrient concentrations were observed and are examples of dilution effects. Big Creek originates from seeps upstream and gains flow moving downstream. Upstream the dilution effect is minimal when compared to the downstream site because of additional flow gained from groundwater and other surface-water features.

Laboratory Microcosms

Data from laboratory microcosm experiments show little change in the isotopic composition of δ^{13}C-DIC in microcosm treatments with 1.0 mg/L and 10 mg/L, DOC Figure 3a. In the first three weeks of

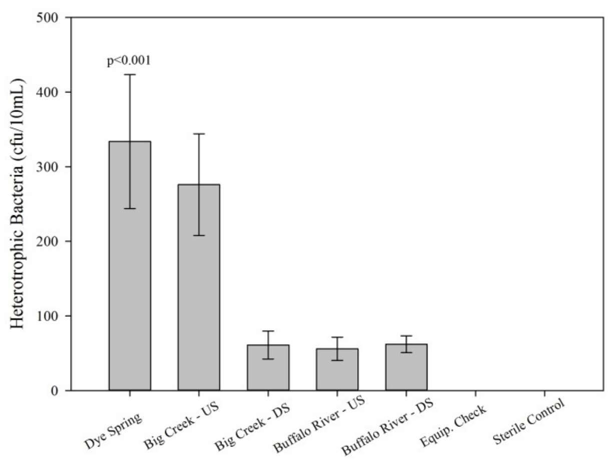

Figure 3. Heterotrophic Bacteria Concentration in biological water quality samples, shaded bar represents mean concentration, and error bars represent the ± standard deviation

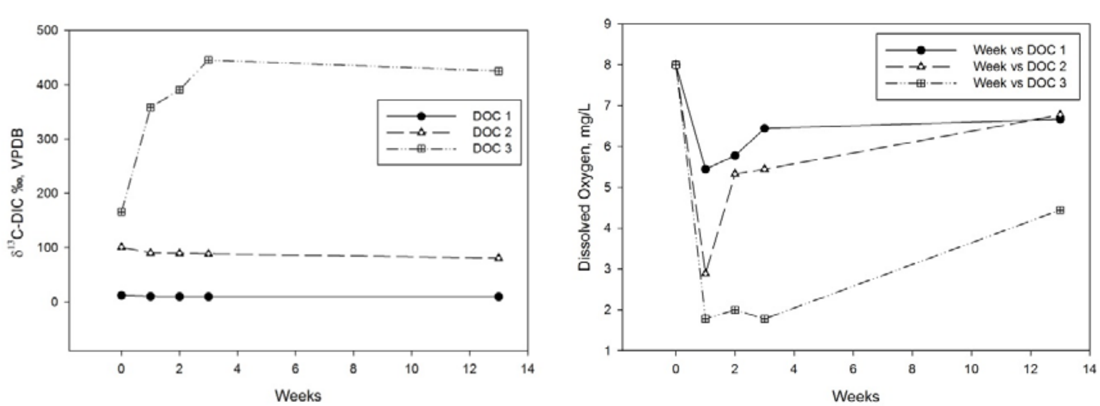

Figure 2. Overview of δ13C-DIC isotopic composition (left), and dissolved oxygen in microcosm samples (right); DOC amendments are represented as follows: DOC1 represents 1.0 mg/L DOC, DOC2 represents 10 mg/L DOC, and DOC3 represents 100 mg/L DOC.

sampling 1.0 mg/L and 10.0 mg/L, DOC treatments became isotopically lighter before plateauing for the remainder of the experiment. The $\delta^{13}C$-DIC compositions of microcosms treated with 100 mg/L, DOC displayed a different trend, becoming isotopically heavier in the first three weeks. After the third sampling period, $\delta^{13}C$-DIC changed little before the final sampling period at week 13. Increasing $\delta^{13}C$-DIC values is an indication of microbial transformation of the isotopically heavy ^{13}C-DOC added to the microcosm at the beginning of the experiment. Decreasing and or unchanged $\delta^{13}C$-DIC values would indicate exhaustion of ^{13}C-DOC, gas exchange with the environment outside of the microcosms, or no quantifiable significant transformation of DOC to DIC. Dissolved oxygen decreased in the initial three weeks of sampling when the biological oxygen demand was greatest and increased as oxygen in the headspace of the microcosms equilibrated with the remaining water and as microbial activity decreased over time Figure 3b. Figure 4 shows $\delta^{15}N$-NO_3 and $\delta^{18}O$-NO_3 compositions of water samples collected from laboratory microcosms. This data provides information on the attenuation of nitrate under varying concentrations of DOC, nitrate, and phosphate. The line drawn represents a slope of 0.5, which provides good isotopic indication of denitrification activity occurring in the microcosms. Denitrification activity was detected in microcosms containing more DOC, indicating the limiting nature of low concentrations of DOC on denitrification over the impact of nitrate concentration. This occurs in large part because DOC drives respiration and biological oxygen demand. Denitrification is an anaerobic to micro-aerobic process, and without sufficient amounts of DOC, dissolved oxygen concentrations remain too great to observe significant denitrification activity. Secondly, denitrification is enzymatically coupled with DOC oxidation; therefore ideal conditions for denitrification must have a sustained DOC source and anaerobic conditions. Biomass production in the microcosms did not vary significantly across the various treatments with respect to DOC concentration or nutrient concentration ($p=0.4$). The mean quantity of biomass collected from DOC1 microcosms was 19.36 ± 6.34 mg, from DOC2 microcosms 21.86 ± 1.41 mg, and from DOC3 microcosms 13.40 ± 2.45 mg Figure 5. Therefore, while DOC had a significant impact on DIC production and isotopic composition, DO concentration, and denitrification the total biomass produced was not significantly impacted.

Conclusions:

Water-quality in the Buffalo River, Big Creek, and Dye spring has been consistent over the course of the study. The early conclusion that may be drawn from the laboratory studies is that the presence of organic matter in karst systems can cause changes microbial activity based on concentration. More available organic matter provided indication of nitrate removal and increased biomass production. Organic matter limits denitrification and respiration, affecting microbial productivity and the evolution of

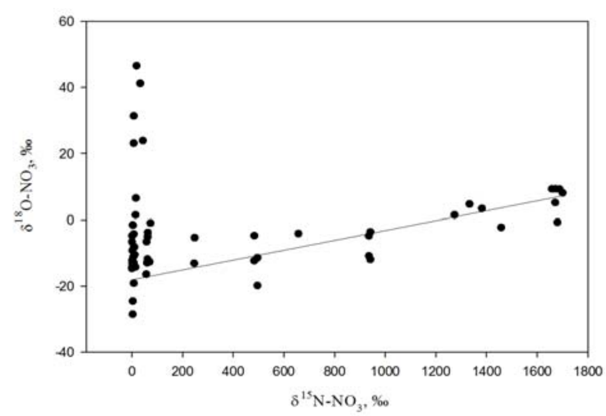

Figure 4. Overview of $\delta^{15}N$-NO_3 and $\delta^{18}O$-NO_3 isotopic composition in microcosm water samples, line is drawn at a slope of 0.5; data points on or near line indicate denitrification activity.

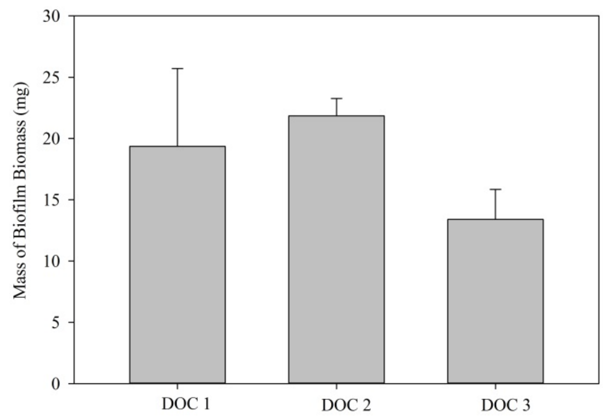

Figure 5. Mean biofilm biomass collected from laboratory microcosms. DOC 1 represents 1.0 mg/L DOC, DOC 2 represents 10.0 mg/L DOC, and DOC 3 represents 100.0 mg/L DOC. Columns represent mean values and error bars represent standard deviation of samples.

water chemistry. The broader significance of the findings of this study implicates organic matter as a key indicator of the assimilatory capacity of karst groundwater environments, specifically in the case of microbial contributions. When labile organic matter is available at higher concentrations, the influence of microbial processes on water quality are greater than the opposing conditions when organic matter is more recalcitrant and sparse. Groundwater management strategies can be improved by increased monitoring of the flux of organic matter at the surface and subsequently as DOC into groundwater flowpaths. The findings of this study will be a part of the PI's dissertation and future publications.

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Project Title: Lower Cutoff Creek Monitoring
Project Number: 2014AR359B
Start Date: 3/1/2014
End Date: 2/29/2016
Funding Source: 104B
Congressional District: 004
Research Category: Water quality
Focus Category: Sediments, water quality, non point pollution
Principal Investigator: Kelly J. Bryant

Publications and Presentations:

Bryant, K. and H. Liechty, 2014, Water quality monitoring in Bayou Bartholomew at Delta States Farm Management (SERA-35), Tillar, AR.

Project Title: Lower Cutoff Creek Monitoring
Project Team: Kelly Bryant, School of Agriculture, University of Arkansas at Monticello
Hal Liechty, School of Forest Resources, University of Arkansas at Monticello

Executive Summary:

Water samples were collected once per week over a 10 month period at four locations along Lower Cutoff Creek and 4 locations along Upper Cutoff Creek. Total Suspended Solids (TSS) was measured for each sample. Results indicated that these two creeks had relatively low levels of TSS throughout the study period. There was no significant difference in TSS between the two creeks. The data did not identify any "hot spots" on either creek that would assist in locating point source pollution of TSS. The road crossings at each sample site had no measurable impact on the TSS in either of the creeks. Continued monitoring of these two creeks at site four is warranted to better understand these two sub-watersheds and their contribution to silt loads and turbidity on Bayou Bartholomew.

Introduction:

Bayou Bartholomew is one of ten priority watersheds identified in the 2011-2016 Nonpoint Source Pollution Management Plan published by the Arkansas Natural Resources Commission. The plan identifies silt loads and turbidity as a key element causing degradation to the streams in the watershed. The need for additional water quality data in this HUC 8 watershed is great.

SWAT model simulations performed by Saraswat, Leh, Pai and Daniels divide the Bayou Bartholomew watershed into 44 sub-watersheds. The modeling was designed to identify sub-watersheds where mitigation efforts should be focused first. Lower Cutoff Creek is one of those areas in regard to sediment. The SWAT model, however, was only calibrated and validated at the larger watershed scale. Little to no data was available on the HUC 12 levels, especially for Lower Cutoff Creek. It is worth noting that while the SWAT model predicts Lower Cutoff Creek to be high in sediment concentration, the sub-watershed is flanked by four sub-watersheds that are modeled to have only half the sediment concentration percentile.

This study seeks to identify portions of Lower Cutoff creek where sediment concentrations are the greatest, ultimately leading to identification of sediment sources and offering solutions. If no "hot spots" are found, or if specific sources in "hot spots" cannot be identified, then a more general approach to cleaning the sub-water shed would be in order such as wide spread BMP adoption or a mitigation bank. In addition, this study collects water samples along Upper Cutoff Creek for comparison. This will provide one year of observations as to the relative sediment concentration between the two adjoining sub-watersheds.

Methods:

Seven locations along Lower Cutoff Creek and seven locations along Upper Cutoff Creek were selected for water sampling sites, and these included sites upstream and downstream from high-water bridges. Water samples were collected weekly from April 2014 to January 2015, and these collections occurred during both base- and storm-flow conditions. Weeks when water was present and flowing at all locations, samples were collected at each location. Weeks when water was not flowing at a location, no sample was collected at that location. At six of the bridge locations a sample was collected upstream as well as downstream of the bridge in an effort to measure the impact of the bridge on sediment levels in the creek.

All water samples were delivered to the water quality lab at the UAM School of Forest Resources. Total suspended solids were analyzed for each sample and the data recorded. In all cases the variable being measured in this study is total suspended solids (TSS).

In addition, the watersheds associated with each creek were delineated and information on area, land use and stream length was determined for each watershed upstream from each sampling location.

Results:

Summary statistics for TSS measurements collected over the study period are displayed in Table 1. Sediment concentrations in Lower Cutoff Creek were not different than those in Upper Cutoff Creek, with average concentrations of 13 mg/L and ranging from 1 to 80 mg/L throughout the study period. This result is interesting and surprising since the SWAT model ranked Lower Cutoff Creek as an area with high sediment loads and Upper Cutoff Creek to have relatively low sediment loads. Bridges, which have been implicated in increased sediment transport, didn't cause an increase in sediment concentrations downstream compared to upstream.

The downstream TSS readings for Lower Cutoff Creek by sample site are displayed in a Box-and-Whisker plot (Figure 1). The box contains 50% of the observations at each location. Readings ranged from less than one to thirty mg/l except for three samples that were greater than 30 but less than 50. In general, site 4 had greater TSS readings than the other sites. Sites three and four are downstream of sites one and two.

The downstream TSS readings for Upper Cutoff Creek by sample site are also displayed in a Box-and-Whisker plot (Figure 2). Readings ranged from less than one to thirty mg/l. Site two always had readings between 6.8 and 12 except on three occasions. In general, site 3 had greater TSS readings than the other sites. Sites 3 and four had larger boxes than sites 1 and 2 indicating a wider dispersion of observations.

Statistical tests were run on the data sets depicted in Figures 1 and 2 including an ANOVA with Bartlett's test for equal variances and Tukey's Multiple Comparison Test. The means at each location were not significantly different on Lower Cutoff Creek, while the means on Upper Cutoff were significantly different at $P < 0.05$ (Table 2). The Tukey test shows that location 1 is significantly different from location 3 on Upper Cutoff Creek.

The Lower Cutoff Creek watershed encompasses 51,665 acres while the Upper Cutoff Creek

Table 1. Summary statistics of weekly total suspended solids measurements for all observations on Lower Cutoff Creek and Upper Cutoff Creek by creek and by stream direction; April 2014 to January 2015.

	Lower Cutoff Creek*	Upper Cutoff Creek*	Upstream **	Downstream**
	(mg/l)	(mg/l)	(mg/l)	(mg/l)
Maximum	48.00	77.60	77.60	48.00
Minimum	0.40	0.40	0.40	0.40
Mean	12.29	13.00	13.21	11.96
Std. Deviation	9.68	10.65	12.24	8.66
C.V.	0.79	0.82	0.93	0.72

* includes upstream and downstream samples. **Includes both Upper and Lower Cutoff Creeks.

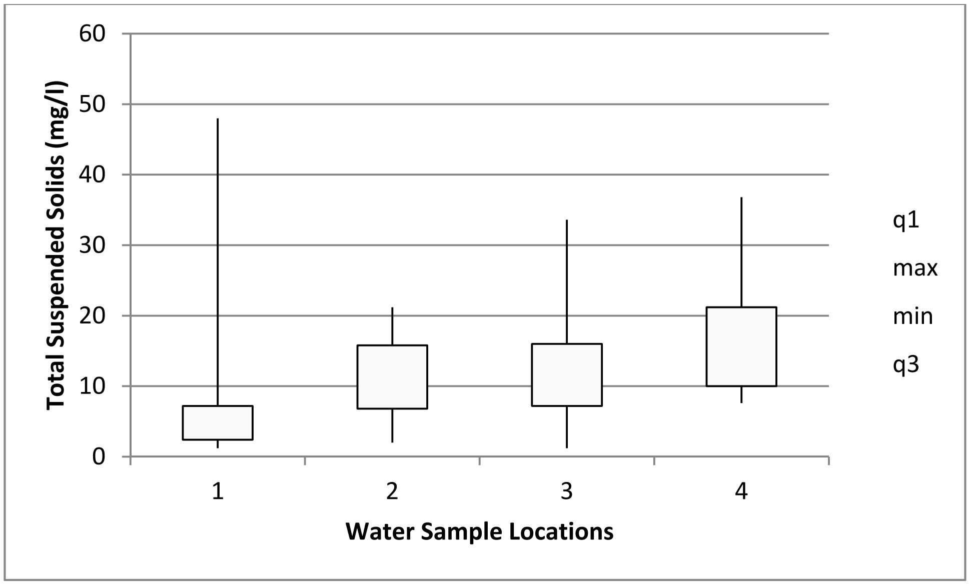


Figure 1. Total suspended solids measured downstream of the road crossing at four sites on Lower Cutoff Creek; April 2014 to January 2015.

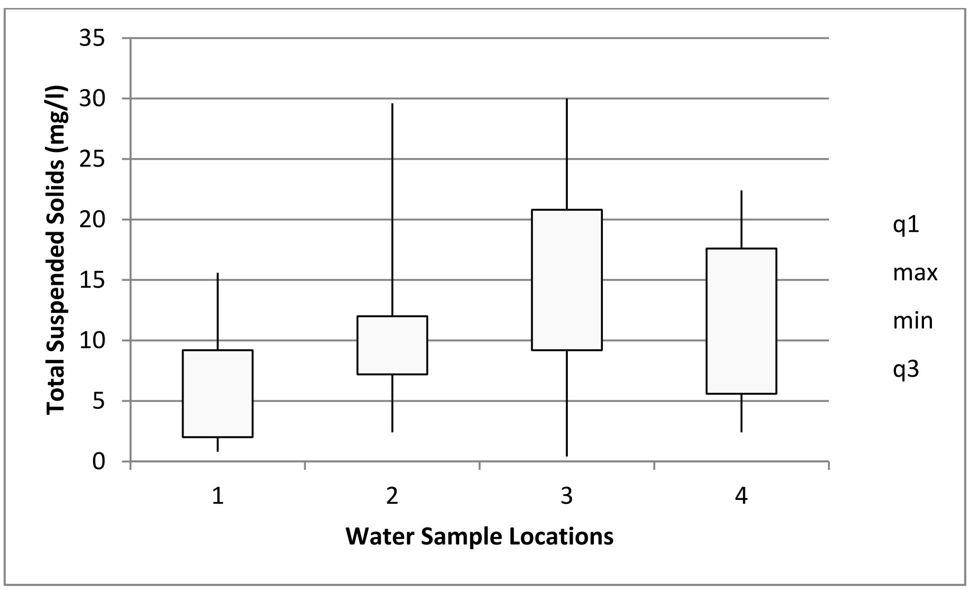


Figure 2. Total suspended solids measured downstream of the road crossing at four sites on Upper Cutoff Creek; April 2014 to January 2015.

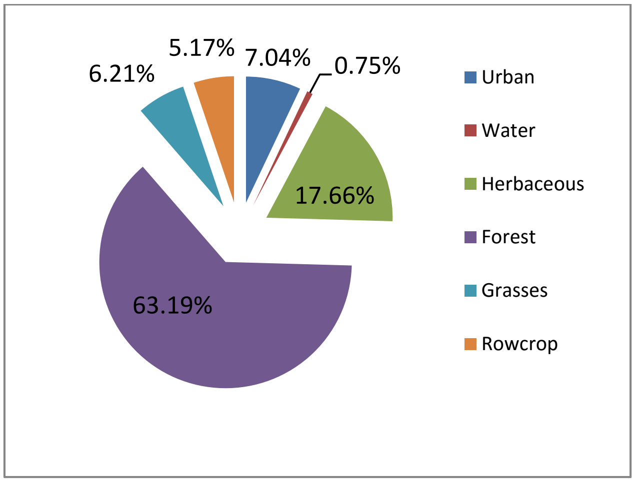
watershed is comprised of 60,404 acres. Land use for the two sub-watersheds is displayed in Figure 3. Both watersheds are well over 50% forest. Lower Cutoff Creek has a larger urban component and a larger row crop component than Upper Cutoff Creek.

Conclusions:

This study shows that sediment concentrations were not different between Lower and Upper Cutoff Creeks, which varied in priority rankings based on the watershed model. This underscores the potential limitations of using model estimations to identify priority sub-watersheds as target areas for the

Table 2. ANOVA test results for Lower and Upper Cutoff Creeks.

	Lower Cutoff Creek	Upper Cutoff Creek
P value	0.255	0.0205
P value summary	ns	*
Are means signif. different? (P < 0.05)	No	Yes
Number of groups	4	4
F	1.402	3.559
R squared	0.08723	0.1731



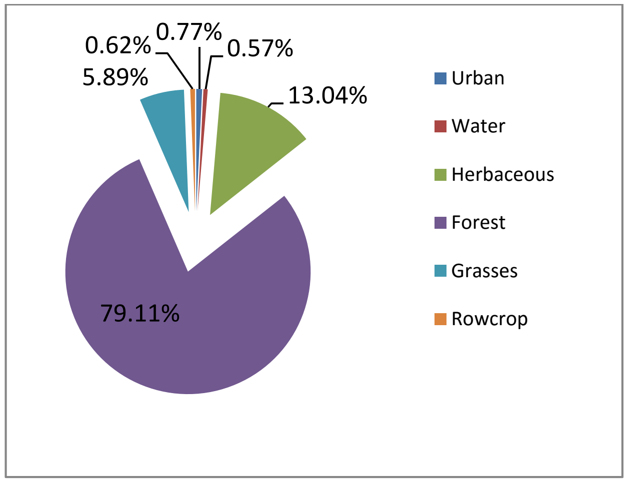


Figure 3. Land use in the Lower Cutoff Creek sub-watershed (left) and Upper Cutoff Creek sub-watershed (right).

implementation of best management practices. However, there are differences in land use and hydrology between the two sub-watersheds that could influence sediment concentrations at a range of flow conditions. Water-quality monitoring can provide important information to water resource managers about how watershed characteristics influence the water quality of streams and rivers.

The U.S. Geological Survey measures suspended sediment concentrations along Bayou Bartholomew at locations including; 1) near Meroney, AR; 2) at Garrett Bridge; and 3) near McGehee, AR. Readings for these locations are published on the internet for various dates including May 28, 2014; July 22, 2014; November 13, 2014; December 19, 2014; and January 4, 2015. Of the eleven observations at these locations during the study period of this project, the nine observations in 2014 ranged from 12 mg/l to 60 mg/l which is consistent with our findings. The January 4, 2015 samples collected near Meroney and at Garret Bridge had suspended sediment concentrations of 108 mg/l and 133 mg/l respectively. This study collected one final water sample on January 8, 2015 of 15.2 mg/l. The level of sediment observed along Upper and Lower Cutoff Creeks in 2014 is consistent with that observed by the U.S.G.S. along Bayou Bartholomew.

Project Title:	Characterization of phosphorus stores in soils and sediments and the potential for phosphorus release to water, related to land use and landscape position within a watershed
Project Number:	2015AR364B
Start Date:	3/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	003
Research Category:	Water quality
Focus Category:	Nutrients, surface water, agriculture
Principal Investigator:	Andrew Sharpley

Publications and Presentations:

Dodd, R.J., A.N. Sharpley, 2015, The impact of legacy P and conservation practices, at Alberta Agriculture and Rural Developments Nutrient Management Team, invited webinar.

Dodd, R.J., A.N. Sharpley, and H.P. Jarvie, 2015, Phosphorus sinks to phosphorus sources: The impact of legacy P and conservation practices, in Soil Science Society of America Annual Meeting, Minneapolis, MN.

Dodd, R.J. and A.N. Sharpley, 2015, Recognizing the role of soil organic phosphorus in soil fertility and water quality, Resources, Conservation and Recycling, 105: 282-293.

Dodd, R.J. and A.N. Sharpley, 2016, Conservation practice effectiveness and adoption: Unintended consequences and implications for sustainable phosphorus management, Nutrient Cycling in Agroecosystems 104: 373-392.

Project Title: Characterization of phosphorus stores in soils and sediments and the potential for phosphorus release to water, related to land use and landscape position within a watershed.

Project Team: Rosalind Dodd, Dept. of Crop, Soil & Environmental Science, University of Arkansas
Andrew Sharpley, Dept. of Crop, Soil & Environmental Science, University of Arkansas
Casey Dunigan, Washington County Soil and Water Conservation District.

Executive Summary:

Phosphorus (P) concentrations within the Illinois River can impair water quality and are of regional concern. Agricultural soils are commonly considered to be a major contributor of P to this watershed. However, P can accumulate within various positions in the landscape including riparian areas, stream banks and stream sediments and provide an additional legacy P source. This research aimed to determine the potential for P release from these different landscape positions from three different land uses: farm, urban parkland and a natural forest. Soil samples were taken from six landscape positions: field soils, the edge of the riparian area, the middle of the riparian area, the stream bank, the edge of the stream bed and the stream bed across the three land uses. Sequential fractionation was used to determine the speciation of P within these soils and water extractable P was determined to provide an indication of the potential for P release. Results suggested that the farm field soils posed the largest risk of P loss to runoff. However, there was no significant difference in soil P in the stream sediments across the different land uses indicating that agricultural use did not lead to P accumulating in the sediments. The results also highlighted the potential for release of organic P forms which are largely overlooked and recommends that soil and water sampling strategies should monitor both inorganic and organic P forms. The speciation of soil P varied across the landscape positions. Importantly, the stream sediments were found to be dominated by the reductive soluble P forms. This indicates that a change in redox state could potentially mobilize a large store of P and care must be taken to ensure these sediments do not become anoxic.

Introduction:

Phosphorus (P) is widely accepted as a key factor contributing to the eutrophication of many streams, rivers and lakes (Carpenter et al. 1998). The P content of streams is often related to the surrounding land use, for example in northwest Arkansas the amount of dissolved reactive P (DRP) in streams was shown to increase with increasing proportion of pasture and urban development within a watershed (Haggard et al. 2003; 2007). The long-term application of poultry litter in excess of the crop nutrient requirements has led to a buildup of P within these pasture soils (Slaton et al. 2004) and there is growing concern that these soils will pose a chronic risk of P loss to surface waters and lead to large lag times between mitigation efforts to decrease P loss and observed improvements in water quality (Meals et al. 2010).

The accumulation of P within the landscape and the subsequent remobilization has been termed as a legacy P effect (Kleinman et al. 2011) and relates not only to P stored within agricultural field soils but stores of P which have accumulated in riparian areas, streambanks and bed sediments (Sharpley et al. 2013). Much of the deposition, sorption and remobilization processes occur in transition zones between the edge of field and the watercourse e.g., riparian buffer zones (Javie et al. 2013). While we know hotspots of P accumulation exist within a watershed, the location, potential impact and residence time of these stores is poorly understood (Jarvie et al. (2013). Furthermore, the speciation of the stores of soil/sediment P and how these vary related to land use and landscape position is unclear and much of the past research has focused solely on the inorganic P forms. Organic P can represent a significant proportion of the total soil P, especially in manured pasture soils and there is growing interest in the contribution of organic P forms to plant production (Nash et al. 2014) and the potential release to water (Darch et al. 2014).

Based in Goose Creek, a tributary of the Illinois River, this work aims to identify the location and magnitude of legacy P stores within a watershed and to determine their potential impact on P loss to water in order to improve long-term water quality at a watershed scale. The following specific objectives were assessed:

1. To determine the forms of P within the soils and riparian, streambank and stream bed sediments within Goose Creek.
2. To determine which areas of the watershed are acting as a source or sink of P to Goose Creek.
3. To determine the impact of land use and riparian management on the P speciation and release potential within these zones.

Methods:

Site identification

Working with Arkansas Association of Conservation District (AACD) personnel, 5 agricultural sampling sites were identified, 4 of which are on the main stem and 1 on Owl Creek. In addition samples were taken from Creekside Park and Mt. Kessler to provide urban and forest comparison sites. For sampling locations see Figure 1.

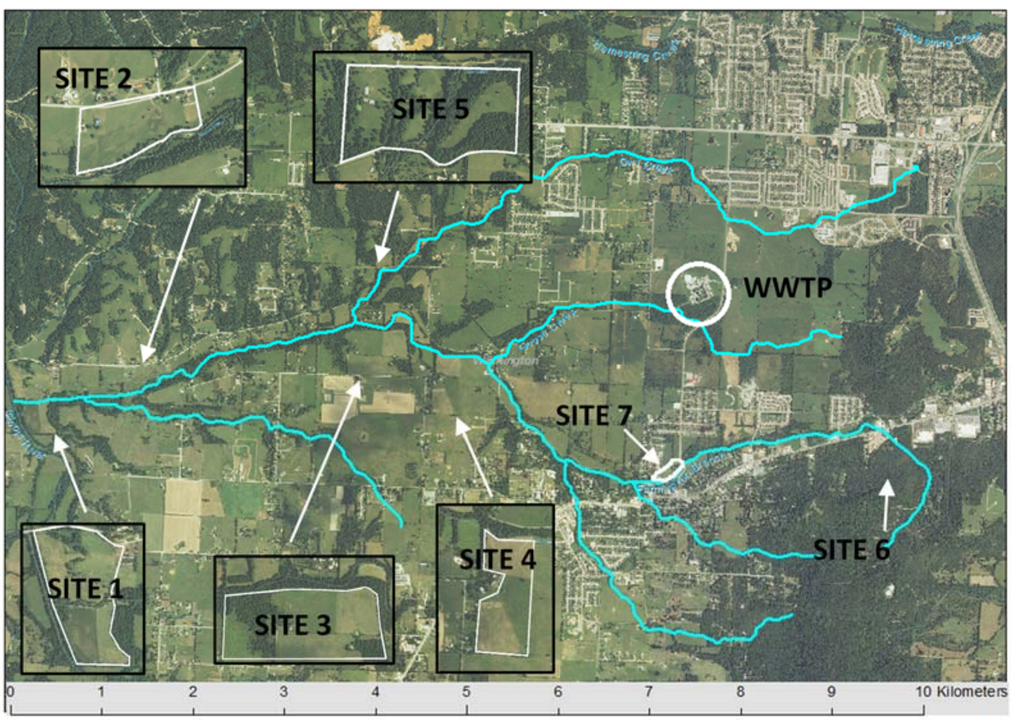


Figure 1: Map showing sampling sites on Goose Creek. Sites 1 – 4 are agricultural, site 6 is forest headwater site, and site 7 is an urban park. WTTP marks the wastewater treatment plant.

Sampling

At each site three transects were laid crossing through the field/park/forest, the riparian zone and into the stream bed. Soil samples were taken to a depth of 4", using a slide hammer, from three locations in the field, park or forest and bulked to give one sample and from one location each at the edge of the riparian zone and in the middle of the riparian zone. A sample of the top 0-4" of soil from the exposed face of the stream bank was collected using a hand trowel. A sample of sediment from the edge of the stream bed to a depth of 4" was taken with a spade. Finally samples of the stream bed sediment were taken from three locations across the stream channel and bulked to give one sample. To take the stream bed samples a bottomless bucket was sunk into the stream bed to interrupt the stream flow. Samples were then taken from inside the bucket with a spade to a depth of 4" to minimize the loss of fine sediment. This sampling strategy yielded six samples per transect from different parts of the landscape, denoted as a zone: (1) field, park or forest,(2) riparian edge, (3) riparian middle, (4) stream bank, (5) stream edge and (6) stream bed. Additionally at each site, where the stream was flowing, a grab sample of the stream water was collected.

All samples were collected over two consecutive days during August 2016 under base-flow conditions. The samples were returned to the lab the same day. Water samples were filtered < 0.45 µm and submitted to the Arkansas Water Quality Laboratory for analysis for dissolved reactive P (DRP) and total dissolved P (TDP) following potassium persulfate digestion.

Soil and sediment analysis

Soil samples were air dried and sieved to < 2mm. The stream edge and bed samples were wet sieved < 2mm prior to air drying. Soil pH was measured in water (1:2 soil-to-solution ratio), samples from the 3 transects from each site were bulked and particle size determined from each zone via gravimetric analysis (Gee and Bauder, 1986).

All P analysis were carried out in accordance with the SERA 17 Methods of P Analysis Handbook (Pierzynski, 2000). Mehlich-3 extractable P (M3-P), Al and Fe were determined and the degree of P sorption saturation (DPPS) calculated. Water extractable P (WEP) was extracted in a 1:10 soil-to-solution ratio and the inorganic WEP (WEP_i) content analyzed via the molybdate blue method. Additionally the extract was digested with potassium persulfate prior to colorimetric analysis to determine the total WEP (WEP_t). The organic WEP (WEP_o) was then inferred as the difference between WEP_i and WEP_t.

Phosphorus speciation was determined using the sequential fractionation scheme of Zhang and Kovar (2000). This yielded five fractions, NH_4Cl-P, NH_4F-P, NaOH-P, citrate bicarbonate dithionate-P (CBD-P) and H_2SO_4-P which correspond to the following P species respectively: soluble or loosely bound P, Al bound P, Fe bound P, reductant soluble P and calcium bound P. The P content of each fraction was determined colorimetrically to determine inorganic P and following potassium persulfate digestion to determine total P with organic P inferred as the difference. All fractions were added together to determine total soil P (TP) and the inorganic or organic fractions were added to determine total inorganic or organic P (P_i and P_o)

Statistical analysis

The study design consisted of seven field sites with soil samples taken from three transects at each site. The transects were considered to be replicates and results are presented as a mean of these transects. All data was inspected for normality and a log10 transformation was performed on all WEP and M3-P results prior to statistical analysis. A one-way analysis of variance was carried out by site and by zone and specific differences between sites or between zones were determined using a Tukey multiple comparisons test at the $p < 0.05$ level of significance.

Results:

Water extractable P

Water extractable P (WEP) can be used to give an indication of the potential for P release from soils and sediment to water. In the agricultural soils total WEP and WEP_i showed a general decrease along the transect from the field soils to the stream bed sediment. In contrast, there was no difference in total WEP or WEP_i across the zones in the park or forest soils (Fig. 2). Similarly in the park and forest soils these sediments showed higher concentrations of M3-P than the park and forest soils.

In addition to this general tread there were significant differences in TWEP and WEP_i across the different farm sites. Sites 1, 2 and 5 all showed significant differences between the field soils and the corresponding soils taken from the forest and park sites ($p < 0.05$) (Table 1). Additionally, these sites while WEP was elevated in the field soils the concentration was significantly lower in the stream edge and stream bed sediments and sites 1 and 5 showed no significant difference between park and forest sediments in these zones. This indicates that while manure application and agricultural land use increases the potential for P loss from the field soils this does not increase the soluble fraction within the stored stream bed at these sites.

However, field soils of farm sites 3 and 4 showed significantly and appreciably lower concentrations of TWEP and WEP_i which were not significantly different to the other landscape zones or to the park or forest land uses ($p < 0.05$). This was despite the soils sites 3 and 4 having similar M3-P concentrations to the other farm sites (Table 1). Interestingly site 3 showed significantly higher WEPi concentrations than the stream edge sediments from the park site despite having similar M3-P concentrations in the soil samples ($p<0.05$). This highlights the variation among sites, potentially as a

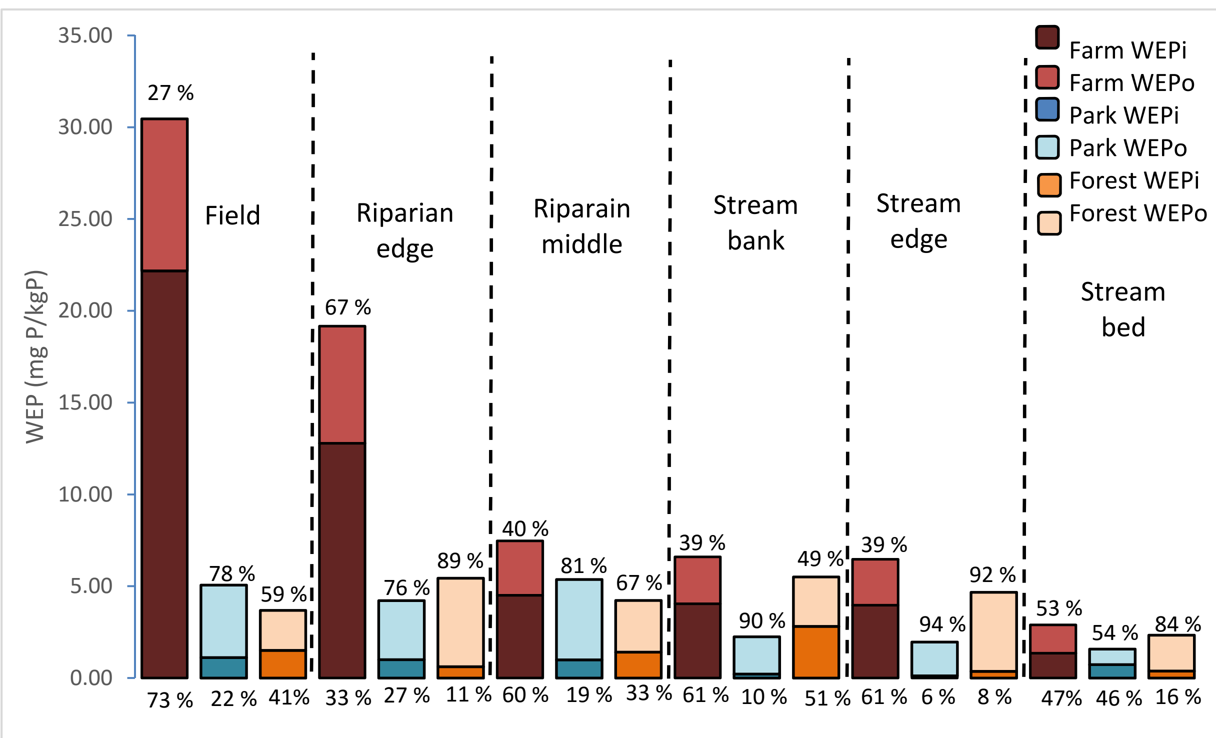


Figure 2: Water extractable P concertation across land use and landscape zones. Figures show the % of total WEP in the inorganic (top) and organic (bottom) fraction respectively.

result of differences in soil properties.

In contrast to WEP_i there were no significant differences in WEP_o between the different land uses (Fig 2., Table 1). However as a proportion of TWEP, the inorganic fraction was dominant in all the samples, except the stream bed for the all the farm sites while WEP_o was the dominant fraction in almost all of the

Table 1: Mean total (TWEP), inorganic (WEPi) and organic (WEPo) water extractable P concentrations and Mehlich (III)-P (M3-P) concentrations for each site and landscape zone. Capital and lower case letters denote significant differences between sites and zones respectively from a Tukey's multiple comparisons analysis of variance at the p <0.05 level of signficance.

Site	**Land use**	**Zone**	**TWEP**	**WEPi**	**WEPo**	**M3-P**
1	Farm	Field	33.71A^{a}	25.27A^{a}	8.44A^{a}	280.3
2	Farm	Field	67.88A^{a}	55.43A^{a}	12.44A^{a}	172.2
3	Farm	Field	4.96C^{ab}	2.95B^{a}	2.01A^{a}	175.9
4	Farm	Field	8.32BC^{a}	4.52B^{a}	3.80A^{a}	78.9
5	Farm	Field	37.42A^{a}	22.70A^{a}	14.72A^{a}	34.0
6	Forest	Field	3.68C^{a}	1.51B^{a}	2.18A^{a}	47.4
7	Park	Field	5.06BC^{a}	1.11B^{a}	3.95A^{a}	52.1
1	Farm	Riparian edge	22.98A^{a}	17.14A^{a}	5.83A^{a}	123.8
2	Farm	Riparian edge	27.71A^{ab}	23.20A^{ab}	4.51A^{ab}	91.1
3	Farm	Riparian edge	11.67AB^{a}	3.58BCD^{a}	8.08A^{a}	54.2
4	Farm	Riparian edge	13.60AB^{a}	7.70ABC^{a}	5.90A^{a}	19.8
5	Farm	Riparian edge	19.89A^{ab}	12.3AB^{a}	7.57A^{a}	25.3
6	Forest	Riparian edge	5.43B^{a}	0.61D^{a}	4.82A^{a}	22.4
7	Park	Riparian edge	4.22B^{ab}	1.00CD^{a}	3.22A^{a}	28.5
1	Farm	Riparain middle	10.95A^{ab}	7.40A^{ab}	3.55A^{ab}	75.5
2	Farm	Riparain middle	4.50A^{c}	1.81A^{bc}	2.69A^{ab}	72.4
3	Farm	Riparain middle	5.30A^{ab}	2.77A^{a}	2.53A^{ab}	37.5
4	Farm	Riparain middle	12.03A^{a}	7.83A^{b}	4.20A^{a}	29.1
5	Farm	Riparain middle	4.58A^{bc}	2.72A^{a}	1.86A^{a}	14.7
6	Forest	Riparain middle	4.23A^{a}	1.41A^{a}	2.82A^{a}	16.8
7	Park	Riparain middle	5.37A^{a}	0.99A^{a}	4.37A^{a}	27.1
1	Farm	Stream bank	10.43A^{ab}	7.57A^{ab}	2.86A^{ab}	68.7
2	Farm	Stream bank	9.20AB^{bc}	5.13A^{abc}	4.07A^{ab}	62.8
3	Farm	Stream bank	2.06B^{b}	0.34BC^{a}	1.72A^{b}	77.8
4	Farm	Stream bank	6.05AB^{a}	4.07A^{ab}	1.98A^{a}	180.5
5	Farm	Stream bank	5.25AB^{abc}	3.12AB^{b}	2.13A^{a}	235.6
6	Forest	Stream bank	5.51AB^{a}	2.81ABC^{a}	2.70A^{a}	253.2
7	Park	Stream bank	2.24AB^{ab}	0.21C^{ab}	2.03A^{ab}	223.4
1	Farm	Stream edge	2.33AB^{b}	0.98AB^{b}	1.35A^{b}	15.9
2	Farm	Stream edge	3.66AB^{c}	1.66AB^{bc}	2.00A^{a}	96.2
3	Farm	Stream edge	6.17A^{ab}	2.58A^{a}	3.59A^{ab}	190.3
4	Farm	Stream edge	3.16AB^{a}	0.83AB^{b}	2.33A^{a}	180.2
5	Farm	Stream edge	2.41AB^{c}	0.86AB^{b}	1.55A^{a}	151.1
6	Forest	Stream edge	4.67AB^{a}	0.37B^{a}	4.30A^{a}	115.4
7	Park	Stream edge	1.96$^{B\ bc}$	0.12B^{b}	1.84A^{ab}	112.7
1	Farm	Stream bed	2.58A^{b}	1.12AB^{b}	1.46A^{a}	142.8
2	Farm	Stream bed	2.43A^{c}	1.18A^{c}	1.25A^{a}	203.5
3	Farm	Stream bed	4.50A^{ab}	2.31A^{a}	2.19A^{ab}	177.0
4	Farm	Stream bed	2.56A^{a}	0.74AB^{b}	1.81A^{a}	89.7
5	Farm	Stream bed	2.41A^{c}	1.41AB^{b}	1.01A^{a}	32.3
6	Forest	Stream bed	2.33A^{a}	0.38B^{a}	1.95A^{a}	39.6
7	Park	Stream bed	1.58A^{c}	0.73B^{a}	0.85A^{b}	47.3

forest and park samples. This suggests that P release from agricultural soils is largely in the most available orthophosphate form while organic P can be a significant portion of P loss from non-agricultural soils.

P fractionation

Total soil P (TP) concentrations were higher in the farm samples compared to park or forest samples for the field and riparian edge zones only. Stream edge and stream bed sediments of the park samples had higher TP concentrations than the farm sites despite lower WEP, indicating storage of P in less mobile forms (Fig. 3). In contrast to WEP, proportions of P_o and P_i were similar in the soil samples and P_o dominated TP in the stream sediments. The soil samples from the forest sites were dominated by P_o, likely reflecting the increased organic matter inputs at this site. However, the stream sediments were also dominated by P_i indicating that at all sites P_o fractions were more mobile. This is in agreement with many field trials showing greater mobility of dissolved organic P forms (e.g. Leytem et al. 2002).

Soil P fractionation showed that NaOH-$P_{i,}$ which corresponds to Fe-bound P was the dominant inorganic fraction in the farm and forest soils across the majority of landscape positions. This is in accordance with the large volume of studies documenting P fractionation of manures agricultural soils (Negassa and Leiweber, 2009). However in the park soils CBD-P_i, corresponding to reductant soluble P was the largest fraction (Table 2). For the organic fraction NaOH-P_o, which corresponds to that bound within organic matter, was dominant across all land uses in the field and riparian soils but CDB-P_o, corresponding to reductive soluble P_o, dominated in the farm and park sediment samples. The high concentrations of both Pi and Po in the reductive soluble form highlights the potential for P release following a change in redox conditions. This will be particularly significant in the stream sediment samples.

In-stream water quality

Water samples were taken from sites where the stream was flowing at the time of sampling. Due to only one sample being taken it was not possible to carry out statistical analysis but TDP concentrations

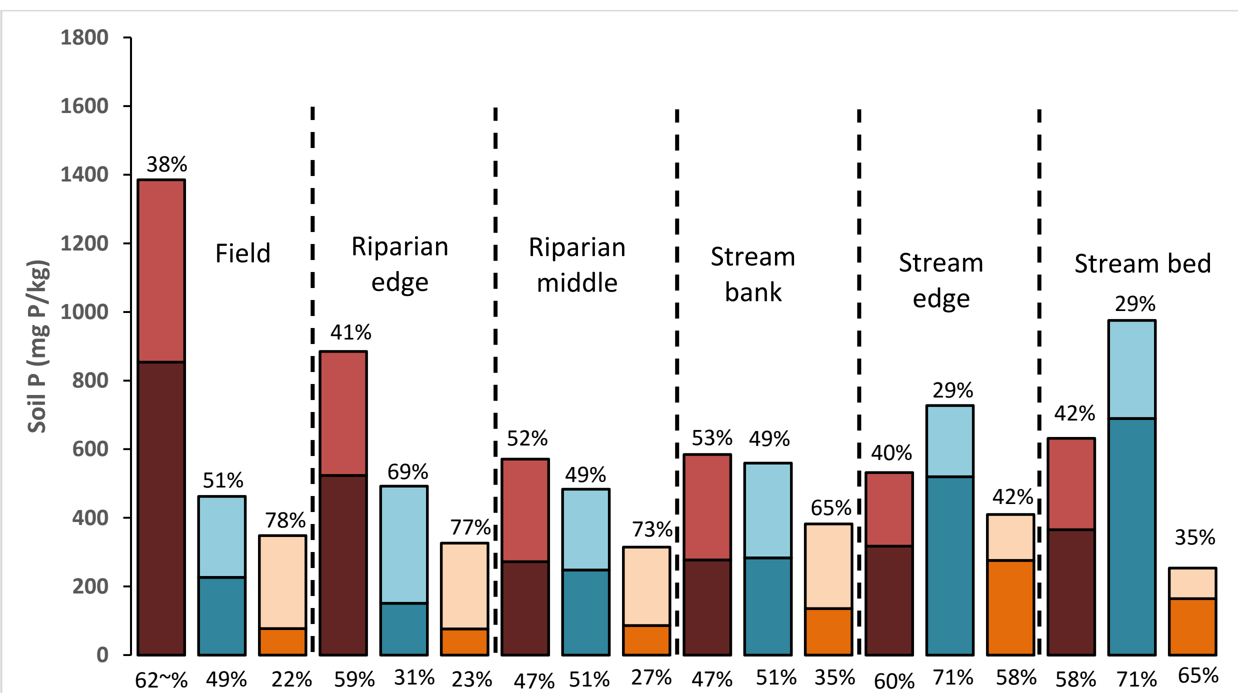


Figure 3: Total soil P, total inorganic P and total organic P across land use and landscape zones. Figures show the % of total WEP in the inorganic (top) and organic (bottom) fraction respectively.

Table 2: Soil P fractionation results showing mean concentrations of inorganic and organic P in the various fractions across land use and landscape zones. Results in bold highlight the dominant forms.

Land use	**Zone**	**NH4Cl-Pi**	**NH4Cl-Po**	**NH4F-Pi**	**NH4F-Po**	**NaOH-Pi**	**NaOH-Po**	**CBD_Pi**	**CBD-Po**	**H2SO4-Pi**	**H2SO4-Po**	Total [illegible]
Farm	Field	85.3	21.5	185.7	85.6	**369.3**	**252.5**	124.4	145.7	88.7	26.8	8[illegible]
Park	Park	17.6	13.9	20.3	15.0	50.3	**122.5**	**85.0**	59.2	53.1	25.4	2[illegible]
Forest	Forest	5.4	15.7	5.6	23.7	**29.1**	**185.3**	26.0	39.0	11.3	7.3	[illegible]
Farm	Riparian edge	33.3	8.1	112.4	27.0	**233.8**	**169.8**	100.1	142.9	43.8	13.8	5[illegible]
Park	Riparian edge	12.2	7.9	9.8	17.7	47.5	**166.2**	**62.3**	120.2	19.0	29.3	1[illegible]
Forest	Riparian edge	4.8	17.5	3.6	21.4	**31.3**	**169.5**	24.0	36.0	12.5	5.7	[illegible]
Farm	Riparian middle	14.5	5.4	42.8	19.5	**106.4**	**133.2**	78.5	121.2	30.0	19.8	2[illegible]
Park	Riparian middle	14.4	7.1	8.9	10.4	66.2	**121.2**	**78.3**	65.0	80.1	32.0	2[illegible]
Forest	Riparian middle	3.2	9.1	3.5	13.8	**40.0**	**153.2**	25.7	30.2	13.5	22.4	[illegible]
Farm	Stream bank	9.1	3.4	38.0	11.2	**99.7**	124.1	89.8	**155.4**	40.6	13.4	2[illegible]
Park	Stream bank	7.8	4.4	8.2	7.7	61.8	91.3	**73.0**	**146.2**	132.5	26.5	2[illegible]
Forest	Stream bank	2.6	5.5	3.6	9.4	41.7	**110.8**	**66.0**	109.8	21.7	11.2	1[illegible]
Farm	Stream edge	6.6	3.1	31.2	7.9	**128.0**	83.7	89.6	**108.5**	61.9	11.5	3[illegible]
Park	Stream edge	4.8	1.7	12.2	3.9	**126.0**	47.0	118.0	**123.7**	258.7	31.3	5[illegible]
Forest	Stream edge	6.4	2.7	6.8	5.5	**76.5**	**84.5**	69.3	32.3	116.9	9.0	2[illegible]
Farm	Stream bed	7.0	1.5	26.1	6.0	**164.9**	73.6	84.0	**166.3**	83.5	18.6	3[illegible]
Park	Stream bed	10.7	2.8	12.8	49.5	145.2	51.0	**153.0**	**122.0**	367.8	60.5	6[illegible]
Forest	Stream bed	1.8	1.3	3.9	3.6	11.9	**46.6**	**72.7**	18.2	74.4	19.3	1[illegible]

were largely similar across the 4 farm sites sampled and the park site despite differences in WEP (Fig. 4). However, while DRP dominated the TDP concentration in the farm sites this fraction was much lower in the park sites where DOP made up 81 % of the TDP. This reflects the differences in WEP fractionation found across these sites and highlights the importance of considering DOP forms for water quality.

Conclusions and Recommendations:

Soil P concentrations in the agricultural soils of Goose Creek were elevated compared to forest and urban park soils. This was also the case for WEP concentrations, which were very high in the field soils of three of the five farm sites. As WEP provides an indication of the potential for P release to water this suggest that these soils will be contributing a significant amount of P to runoff. However, there was a large reduction in all soil P forms and WEP along the transect and there was no significant difference in the stream sediments between the different land uses indicating that the long-term agricultural management at these sites has not lead the enrichment of P the streambed sediments compared to the natural forested headwaters.

Interestingly, the field soils at two of the five agricultural sites showed much lower WEP concentrations, which were not significantly different to that from the park or forest soils. This did not reflect differences in M3-P concentrations and may be a reflection of soil properties. Additionally, the stream bank samples across all sites had very high M3-P concentrations but low WEP. This indicates that M3-P is not a good indicator of the potential risk for P loss questioning the validity of its use for nutrient management. The results also indicated that the organic P fraction can contribute to total WEP especially in the forest and park sites. This was also reflected in the stream water data where DRP in the park site sample was very low and the DOP fraction was large and there was little difference in total dissolved P between the farm and park sites. While DRP is the most readily available form for algal uptake, DOP can be utilized via enzyme hydrolysis (Whitton et al. 1991) and can negatively impact water quality. Hence, we would recommend that both soil and water sampling strategies should consider both inorganic and organic P fractions.

Phosphorus fractionation showed differences in the dominant P fractions across the different land use and landscape types. Importantly, the stream sediments tended to be dominated by the reductive

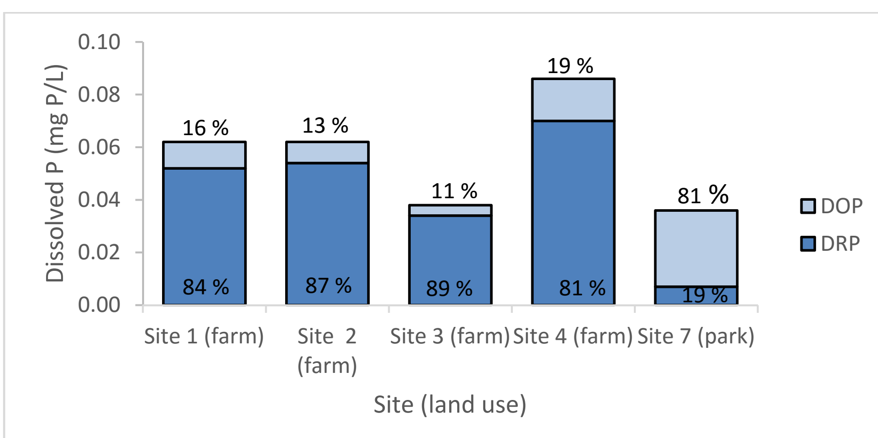


Figure 4: Total dissolved P concentration in stream samples at the time of soil and sediment sampling. Figures show the % of total WEP in the inorganic (top) and organic (bottom) fraction respectively.

soluble P form. This has implications for water quality as a change in redox state could mobilize this large store of P, therefore it is important to ensure that these sediments do not become anoxic.

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Project Title: REWARD: Rice evapotranspiration and water use in the Arkansas delta
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Funding Source: 104B
Congressional District: 003
Research Category: Climate and hydrologic processes
Focus Category: Irrigation, water quality, water use
Principal Investigator: Benjamin R.K. Runkle

Publications and Presentations:

Runkle BRK, K. Suvočarev, S.F. Smith, M. Reba, 2016, Alternate Wetting and Drying as an effective management practice to reduce methane in Arkansas rice production, in 36th Meeting of the Rice Technical Working Group, Galveston, TX.

Management Practice to Reduce Methane in Arkansas Rice Production, in AGU Fall Meeting, San Francisco, CA.

Suvocarev K, M. Reba, B.R.K. Runkle, 2015, Using the Surface Renewal Technique to Estimate CO2 Exchange from a Rice Field to the Atmosphere, in AGU Fall Meeting, San Francisco, CA.

Suvočarev K, M. Reba, B.R.K. Runkle, 2016, Surface Renewal: An alternative to the Eddy Covariance method?, in invited talk at the University of Nebraska Lincoln.

Reavis, C., B.R.K. Runkle, May 2017 (expected), Evapotranspiration in Mid-South Rice Fields, MS Thesis, Department of Biological and Agricultural Engineering, University of Arkansas, Fayetteville, AR.

Project Title: REWARD: Rice Evapotranspiration and Water use in the Arkansas Delta

Project Team: Benjamin R. K. Runkle, Department of Biological & Agricultural Engineering, University of Arkansas, Fayetteville
Support from Michele Reba, USDA-ARS Delta Water Resources Unit, Jonesboro, Arkansas; Kosana Suvočarev, Colby Reavis, and S. Faye Smith, University of Arkansas.

Executive Summary:

Rice agriculture uses 35% of Arkansas's irrigation water and contributes to the unsustainable depletion of the state's water resources. New rice irrigation methods introduced to reduce field methane emissions are also known to reduce overall water use, but their influences on field evapotranspiration (ET) are unclear. The main method under development is known as Alternate Wetting and Drying (AWD), which floods the soil and then allows a strategic dry down; such cycles can occur 4-5 times per growing season. In this study we measure ET from a pair of adjacent production-scale fields under conventional and AWD irrigation. We measure with the micrometeorological eddy covariance technique and generate gap-filled seasonal estimates using a moving-window statistical approach. To our surprise the AWD field generated slightly higher ET fluxes than the neighboring conventionally flooded field (603 mm and 584 mm, respectively). The AWD field also sustained greater plant heights, leaf area indices, and harvest yields, so we suspect that the greater plant biomass and root activity generated higher transpiration rates even when the field surface was not flooded. This experiment is still underway, as the field irrigation strategies will be swapped for the 2016 growing season and additional modeling of the sites' full water balances are under development. The implications of the initial findings are (1) potential reductions in evaporation are balanced by increases in transpiration, (2) there may be increased "green water use efficiency" with AWD irrigation, and (3) a full water balance that includes infiltration, percolation, and irrigation on- and off-flows must be conducted to clarify water savings. If the water savings can be validated in pilot studies in different Mid-South regions, AWD could be implemented on a larger scale as a regular practice.

Introduction:

Rice agriculture uses 35% of Arkansas's irrigation water and contributes to the unsustainable depletion of the state's water resources (Reba et al., 2013; ANRC, 2014). A variety of new irrigation methods have been proposed to reduce water use, including alternate wetting and drying (AWD), which floods the soil and then allows a strategic dry down before reflooding to save water, reduce the risk of the straighthead disability on rice, and decrease field methane production. This method reduces greenhouse gas emissions by more than 70% (including from methane, which is produced under water-saturated conditions and is 20-30 times more potent as a greenhouse gas than CO_2) (Rogers et al., 2013; Linquist et al., 2015). In other settings (e.g., India), AWD is known to reduce overall irrigation applications by 30-50% (Sudhir-Yadav et al., 2011) so it should have the effect of preserving ground- and surface-water resources and related pumping and application costs.

The driving hypothesis of the research is that the AWD-field's ET is significantly less than the conventionally irrigated field. A proportionally greater fraction of the AWD-field's ET is expected to be in the form of transpiration rather than evaporation as there are fewer periods where the surface is flooded. We focused the project on carefully quantifying the ET flux that is most closely associated with plant water use and yield production. This term is also likely to be the largest consumptive portion of the water balance and has direct relevance to the field's energy balance, carbon balance, and greenhouse gas production. We measure and compare seasonal ET from two fields under AWD and conventional management strategies using the quasi-continuous eddy covariance approach, as described in the methods.

Methods:

Site description

Two privately farmed, adjacent rice fields (34° 35' 8.58" N, 91° 44' 51.07" W) located just outside of Humnoke, Arkansas, were used for this research. Each field is approximately 350 m wide from north to south and 750 m long from east to west (i.e., 26 ha). One field was managed with continuous flooding (CF) during the rice growing season and the other with AWD management practice, facilitating a direct comparison of the two types of systems with minimal spatial separation. Both sites have been zero-graded and thus have approximately 0% slopes. Although only about 12.3% of total rice in Arkansas is grown on zero-graded land, this practice is growing due to the potential to save water in the fields (Hardke, 2015), to serve as a carbon-offset credit option (ACR, 2014) and to receive credit in the Natural Resources Conservation Service's Environmental Quality Incentives Program (EQIP). The sites are not tilled and are flooded for two months in winter for duck habitat and hunting. The dominant soil mapping unit in this area is a poorly-drained Perry silty clay. The fields were drill-seed planted April 7 (AWD) and April 8 (CF), given an irrigation flush on May 3 (CF) and May 4 (AWD), and given a permanent flood on May 16 (CF) and May 18 (AWD). The AWD field dried on June 5 and received 3 more dry periods through the summer.

Approach

A combination of different measurement and modeling methods are employed to constrain the ET flux at different temporal scales ranging from hourly to seasonal. The primary environmental drivers of ET, such as wind speed, radiation, and plant canopy cover, were collected for later use in a process-based model to enable better predictions of ET. Water table height was measured at both fields using Ceramic Capacitive Pressure Level Transmitters (Keller USA) as piezometers in shallow dip-wells.

This proposal is situated within a larger research project aimed to measure year-round land-atmosphere fluxes of energy, water vapor, CO_2 and CH_4 from a side-by-side pair of rice fields. The fluxes were measured using the micrometeorological eddy covariance technique (Baldocchi et al., 1988). For these measurements, we installed a 3D sonic anemometer (CSAT3, Campbell Scientific, Inc, USA), an open-path CO_2/H_2O infared gas analyzer (LI-7500A, LI-COR Inc., Lincoln, NE, USA), and an open-path CH_4 analyzer using wavelength modulation spectroscopy (LI-7700). The instruments were installed on towers at each field, at 2 m above the soil surface (AWD field) and 2.2 m (CF field). Sensor data was recorded at 20 Hz and through an Analyzer Interface Unit (LI-7550) with a LI-COR SMARTflux™ automated processing system. Each tower, equipped with eddy covariance sensors and other low frequency biometeorological sensors, was located at the north end of its field, approximately in the center by east and west. The dominant southern winds enabled a data collection footprint over each targeted fields. The high-frequency data collected from the eddy covariance system was processed and quality controlled using EddyPro software (v. 6.1, LI-COR Inc., Lincoln, NE, USA) to compute half-hourly estimates of CH4, CO_2, ET, and sensible heat flux from each field. Typical eddy covariance corrections were also applied within this software. The ET fluxes were gap-filled using a standard moving-window lookup table approach that correlates flux magnitudes to common meteorological variables (Reichstein et al., 2005; Reddyproc online tool).

Supplemental measurements of plant stature were determined through plant height, leaf area index (LAI), and harvest yield. The plant height was measured at ten arbitrarily chosen locations per field per measurement period. The LAI was measured with the LAI-2200 (LI-COR), a non-destructive plant canopy analyzer operating via canopy light interception and radiative transfer modeling. This measurement was performed at five arbitrarily chosen locations per field per measurement period with at least 10 m from the field edge to avoid potential distorting effects of horizontal penetration of light into the canopy. A GPS-enabled John Deere GreenStar 3 2630 Harvest Monitor was attached to the harvesting combine and recorded location-based wet and dry harvest weights from both fields, with measurements spaced approximately 2 m apart.

Results:

The project successfully measured the evapotranspiration flux (presented as latent heat, Figure 1) using the eddy covariance method. Due to wind direction requirements, instrument reliability, and measurement quality checking, 27% and 30% of the half-hour measurements were used for the Conventional and AWD fields, respectively. There was greater data coverage in the key growing season period from June 1 to July 17. The gap-filling model, across the entire data period, predicts observed LE fluxes with root mean square error of 39.8 W m^{-2} and coefficient of determination (r^2) of 0.94.

The key finding is that there is slightly greater evapotranspiration from the AWD field than the Conventional field during the 2015 growing season. This difference – from 1477 to 1431 MJ m^{-2}; or 603 mm to 584 mm – is slight but consistent with plant conditions (detailed below) that seem to enhance growth at the AWD field. This response may be due to the strong ability of rice roots to pull water from the soil matrix and from the relatively short length of each dry down period (approximately 11 days).

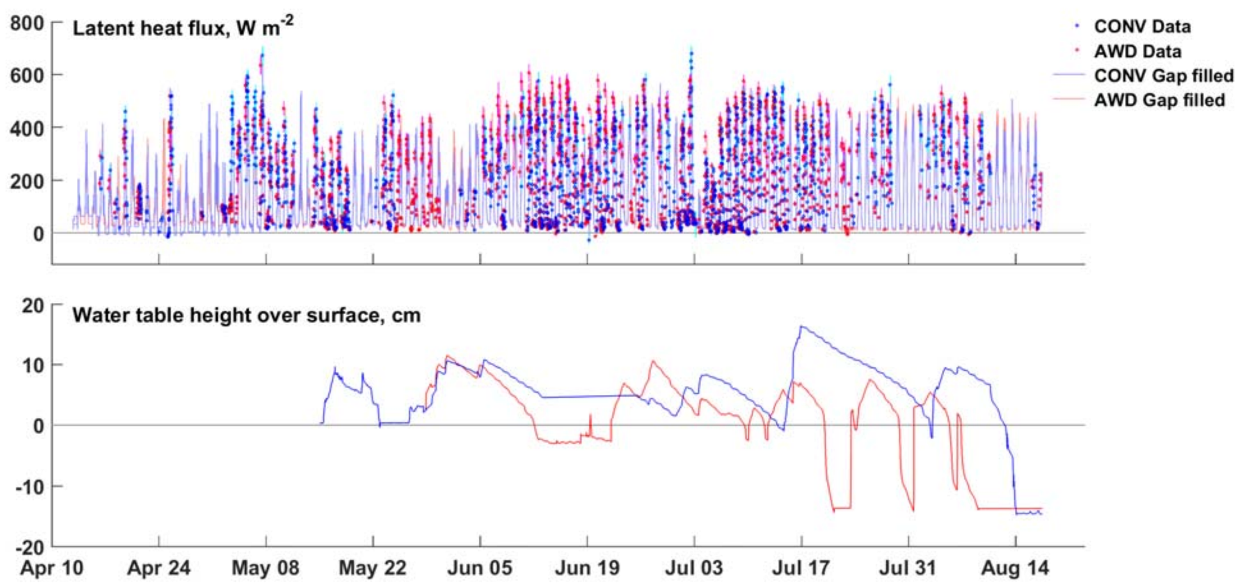

Figure 1: Preliminary data from the REWARD 104B project. The evapotranspiration flux, presented continuously (gap-filled; solid lines) and cumulatively (dashed lines) in latent energy terms, in two comparison rice fields from the 2015 growing season in Humnoke, AR. Fluxes are measured by eddy covariance and gap-filled using an automated moving window, semi-empirical look-up method based on flux responses to meteorological conditions (Reichstein et al., 2005). Contrary to the hypothesis in the REWARD project, the AWD field generates slightly higher ET than the conventionally-flooded field (Cumulative LE flux was 1477 MJ m^{-2} from the AWD field and 1431 MJ m^{-2} from the Conventional Field). The AWD field dried down from June 11-23 and four more times afterward. Water table height measurements were installed only in mid-May though most of the previous period was characterized by a subsurface water table.

The plants grown under AWD conditions were taller and had higher LAI by the end of the season (Figure 2). The AWD field was consistently either more full or equal to the conventional field at each measurement point. LAI follows the characteristic shape with a late-season decline in canopy thickness after grain filling. The greater plant heights and LAI in the AWD field may have contributed to the slightly higher harvests from the AWD field as well (Figure 3). The likely implication is that the AWD field had greater transpiration as a proportion of total ET than the conventionally flooded field.

Conclusions and Recommendations:

The shift from conventional flooding to AWD irrigation will change the regional water balance, inducing alterations in field rates of evaporation, transpiration, infiltration, and runoff. Local measurements of these terms will help in managing water demand and irrigation scheduling as well as constrain estimates of groundwater recharge, the regional meteorological energy balance, and downstream water quality. Uncertainty in the field application and water use of rice is explicitly noted in the Arkansas Water Plan as a challenge for adequately predicting state water supplies. The project findings help to reduce uncertainty in the evapotranspiration from rice fields and will have significant and practical effects in the state's water management.

This research provokes several intriguing questions for follow-up investigations. The first effect was similar rates of ET due to the preponderance of transpiration in the vertical water budget. This result will be modeled and investigated in further detail in an ongoing USGS project. Future plans also include switching the field treatments so that the AWD field will receive conventional flooding and vice versa, to account for potential changes in drainage or soil moisture wicking between the fields. If the water savings can be validated in pilot studies in different Mid-South regions, AWD could be implemented on a larger scale as a regular practice.

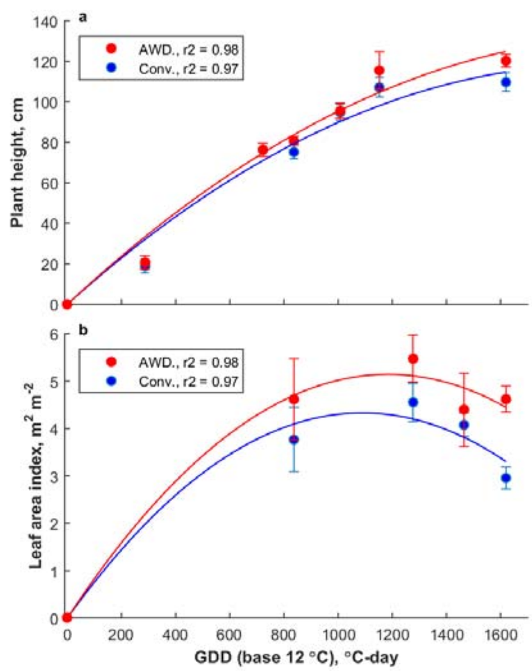

Figure 2: (a) Plant height and (b) leaf area index presented as responses to growth degree days (GDD). The final meausrement point is 12 August 2015. GDD is measured cumulatively from 8 April 2015. Error bars indicate standard deviations from the mean over 10 (height) and 5 (LAI) measurements. A quadratic curve, forced through the origin, is shown for convenience (the coefficient of determination – r^2 value – for this relationship is provided in legend).

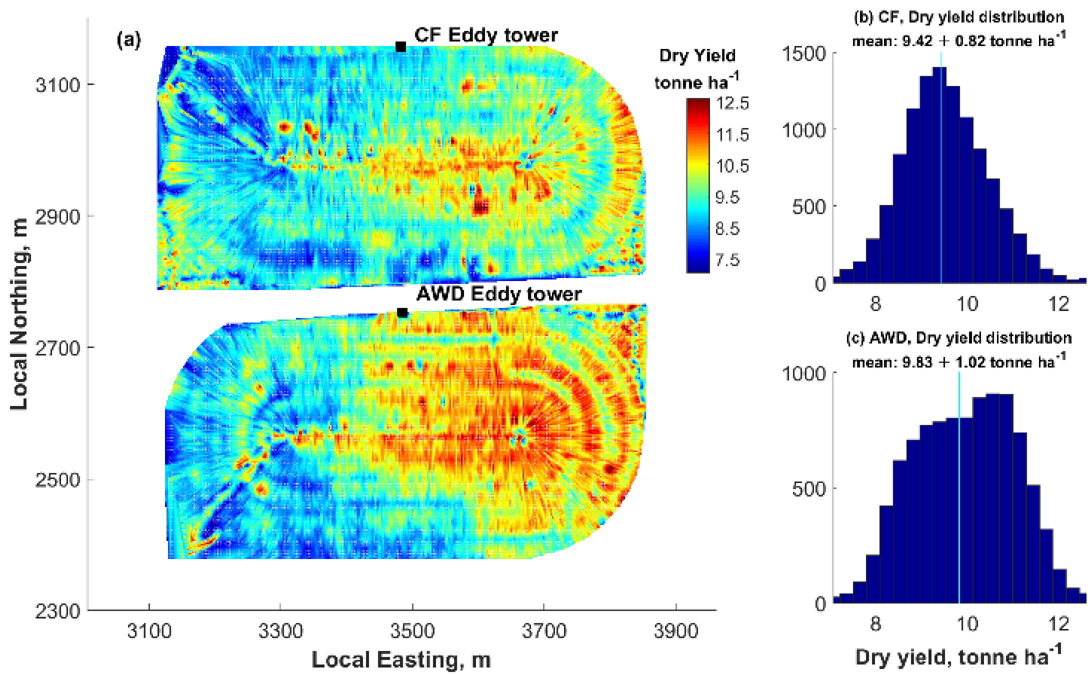

Figure 3: Study sites and harvest dry yield map. The locations of the eddy covariance towers are marked on the conventionally flooded (CF) and alternate wetting and drying (AWD) fields. The fields are separated by a canal and two levees. The towers are located at (CF: 34° 35'19.82 N, 91° 45'06.00" W; AWD: 34° 35'06.71 N, 91° 45'06.10" W). (a) The yield maps are interpolated from points taken approximately 2 m apart, measured during harvest via automated and GPS-enabled yield monitor. (b) and (c) Histograms of the CF and AWD field yields, respectively, are presented. The fields are each approximately 27 ha in size.

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Focus Category: Sediments, nutrients, recreation
Principal Investigator: J. Thad Scott

Publications and Presentations:

West, A. O., J. M. Nolan, J. T. Scott, 2015, Physical and Chemical predictors of human perceptions of water quality in southwestern Ozark rivers, in Joint Assembly Meeting, Montreal, Canada.

West, A. O., J. M. Nolan, J. T. Scott, 2016, Human Perceptions of rivers of the southwestern Ozarks and links to optical water quality, in Oklahoma Clean Lakes and Watersheds Association, Stillwater, OK.

West, A.O., J.M. Nolan, and J.T. Scott, 2015, Physical and Chemical predictors of human perceptions of water quality in southwestern Ozark rivers, in Arkansas Water Resources Center Annual Meeting, Fayetteville, AR.

West, A.O., J.M. Nolan, J.T. Scott, 2015, Physical and Chemical predictors of human perceptions of water quality in southwestern Ozark rivers, in Oklahoma Clean Lakes and Watersheds Association, Stillwater, OK.

West, A.O. and J.T. Scott, 2016, Black disk visibility, turbidity, and total suspended solids: a comparative evaluation, Limnology and Oceanography Methods, conditionally accepted.

West, A.O. and J.T. Scott, May 2016, Optical water quality and human perceptions of rivers, PhD Dissertation, Environmental Dynamics, University of Arkansas, Fayetteville, AR, 160 pp.

West, A.O., J.M. Nolan, and J.T. Scott, 2015, Optical water quality and human perceptions: a synthesis, WIREs Water, 3(2), 167–180, doi:10.1002/wat2.1127.

West, A.O., J.M. Nolan, and J.T. Scott, 2016, Optical water quality and human perceptions of rivers: an ethnohydrology study, Ecosystem Health and Sustainability, in review.

Project Title: Optical water quality dynamics during receding flow in five Northwest Arkansas recreational rivers

Project Team: Amie O. West, Environmental Dynamics Program, University of Arkansas
J. Thad Scott, Crop, Soil, and Environmental Science, University of Arkansas

Executive Summary:

Understanding optical water quality and particulate matter dynamics in recreational rivers is integral in shaping management strategies that maintain ecosystem health, perceived value and appeal, and regional economic significance in a changing environment. Suspended sediment strongly governs optical water quality and is ecologically, as well as aesthetically significant. Increased sedimentation is among the most widespread concerns in rivers throughout the world and a dominant portion of sediment transport occurs in response to increased flow. Thus, it is important to characterize particulate matter concentrations in rivers under changing flow conditions. This study sought to describe optical water quality and particulate concentration dynamics as flow recedes after precipitation events in five ecologically and recreationally significant rivers of the southwestern Ozarks. We found that relationships between particulate concentrations and hydrograph variables were dependent upon catchment characteristics and discrete events were highly variable. We determined optical water quality measures to be strongly correlated to particulate matter concentrations, and may be well suited for describing variability in the absence of more intensive monitoring programs.

Introduction:

Increased sedimentation is among the most widespread pollutant concerns in US rivers, and is the primary cause of impairment in Arkansas rivers and streams (US EPA 2008). Suspended sediment in rivers is greatly influenced by land use within the watershed, and can transport adsorbed pollutants downstream (Dodds and Whiles 2010). Settling of suspended solids can affect benthic organisms and may alter the structure and productivity of the biotic community (Ryan 1991). The exact relationship of suspended sediment concentration with discharge can vary based on sediment availability, precipitation intensity, distance of sediment source, seasonality (Williams 1989), shear strength and sediment cohesiveness (Ji 2008), and catchment soil type (Sander et al. 2011). Accurately characterizing variability in sediment concentrations and transport in individual rivers often requires costly, time-consuming, and intensive, long-term monitoring, and is arguably impractical in many cases.

Optical water quality (OWQ) is defined as the suitability of water for its role in the environment as governed by its composition and the geometric structure of the light field (Tyler 1978, Kirk 1988). Because it involves the behavior of light in both the visible and photosynthetically available part of the electromagnetic spectrum, OWQ is relevant to water resources management (Julian et al. 2013). OWQ can affect water temperature, fish predation, predator evasion, photosynthesis, and many other biogeochemical reactions (Wetzel 1975, Kirk 2011). Suspended particulate matter is often the dominant influence on OWQ in rivers [*Davies-Colley and Close*, 1990; *Davies-Colley and Smith*, 2001; *Julian et al.*, 2008]. Inorganic and organic particulates influence OWQ differently based on size, shape, and composition (Davies-Colley et al. 1993, Gippel 1995), and inorganic clay particles can carry substantial amounts of adsorbed organic matter (Brown and Matthews 2006). Although light availability is a fundamental factor in river ecology, few studies exist that characterize US rivers in terms of OWQ (Julian et al. 2008).

We undertook this study seeking to characterize variability in suspended particulate matter and OWQ as flow recedes after precipitation events in five ecologically and recreationally significant rivers of the southwestern Ozarks in Arkansas, US (Table 1).

We also sought to investigate how the organic proportion of particulate matter is related to specific aspects of the hydrograph. Our measured water quality (WQ) variables were chosen to allow us to test the following hypotheses (Figure 1): (1) precise relationships between particulate matter concentrations and discharge will be event specific because particulate matter concentrations in rivers are sensitive to many environmental influences; (2) the organic proportion of suspended sediment will increase with time after the event peak because of diminishing carrying capacity for heavier inorganic sediments and more favorable conditions for sestonic organisms; And (3) measurements of horizontal black disk visibility and particulate matter concentrations will respond similarly to events, and may serve to generally describe the dynamics as flow recedes in rivers, in the absence of more intensive water quality measurements, because OWQ is strongly governed by scattering by suspended particulates.

Table 1. Catchment characteristics of study rivers; land use from *US Geological Survey* [2011], and Level III ecoregion from *Woods et al.*, [2004]; BM, Boston Mountains; OH, Ozark Highlands

	Gage #	Area (km^2)	Urban (%)	Forest (%)	Agriculture (%)	Ecoregion
Buffalo R.	07056000	2147	3.2	83.2	11.2	BM, OH
Illinois R.	07195430	1489	16.4	28.5	52.9	OH
Kings R.	07050500	1365	4.1	67.5	25.8	OH
Mulberry R.	07252000	966	3.2	90.7	4.7	BM
War Eagle Cr.	07049000	681	4.6	58.0	35.1	OH

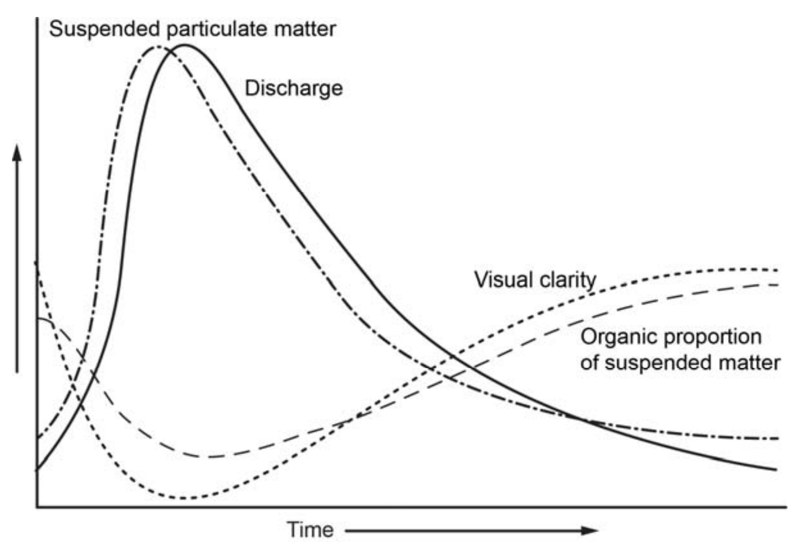

Figure 1. Hypothesized model of relationships of suspended particulate matter and optical water quality as flow recedes in individual hydrograph events

Methods:

This study took place between March and October in 2014 and 2015. Site visits were initiated by precipitation events and occurred at intervals of approximately once every 1 to 3 days as flow receded. For each sampling event, we calculated the average of three consecutive in-situ black disk visibility measurements (Davies-Colley 1988) (BDV). We collected grab samples, transported them on ice, and stored them at ~4° C at the lab at the University of Arkansas, where they were processed within 48 hours. We filtered up to 1L of water through Whatman GF/F 47 mm glass fiber filters for analysis of total suspended solids concentration (TSS) (APHA 2005). We filtered samples through Whatman GF/F 25 mm glass fiber filters for analysis of suspended chlorophyll *a* (Schl) and particulate nitrogen (PN) concentrations. We used a Turner Designs Model 7200 Trilogy™ flourometer fitted with an absorbance module to measure Schl by the acid digestion method following overnight extraction with acetone (APHA 2005). We used a Thermo Scientific™ Flash 2000 Organic Elemental Analyzer to measure PN (APHA 2005).

All data were $\log_{10}$ transformed to account for the tendency for log-normal distributions in water quality data (Hirsch et al. 1991), with the exception of proportion data, which were logit transformed as recommended by Warton and Hui [2011]. All statistical tests were performed on the transformed data with a critical alpha level of 0.05. We tested each WQ variable for equality of variance among the rivers with Levene's test and performed an omnibus one-way analysis of variance (ANOVA) to determine whether differences in means of measured parameters existed among the rivers. Upon detection of significant differences in those parameters with equal variance among rivers, we performed multiple comparisons using Tukey's honestly significant difference (HSD) test in the "stats" package in R (R Core Team 2015), which automatically adjusts for unequal sample sizes. Parameters for which we determine unequal variance among rivers, we used the Games-Howell method to test pairwise differences because it is less sensitive to variance inequalities (Games and Howell 1976). We manually identified the peak of each flow event and calculated the length of time after the hydrograph peak (TAP) for each sample. We critically analyzed relationships between particulate concentrations and Q/TAP using Pearson's correlation coefficient and ordinary least squares regression (OLS). We further examined differences in regression relationships among select well-represented events to compare event-specific dynamics within rivers using OLS and analysis of covariance (ANCOVA). We assumed the inorganic contribution to PN was negligible [following *Beusen et al.*, 2005] because PN is greatly dominated by proteins, amino acids, and nucleic acids (Meybeck 1982, Dodds and Whiles 2010), and is well correlated to particulate organic carbon at TSS concentrations of our study (Ittekkot and Zhang 1989). Therefore, we examined OLS regressions of the ratio of PN to TSS versus TAP to investigate changes in the relative organic content of TSS as flow receded.

Results:

Summary statistics for each measured variable are shown in Table 2. Correlation and ANCOVA results are summarized in Table 3. Analyses of variance indicated significant differences ($p < 0.05$) among rivers in means for every measured WQ variable (Figure 2). Hydrographs and relationships between particulate matter concentrations and discharge for each river are presented in Figures 3-7.

The ratio of PN to TSS was significantly and positively correlated with TAP in BUF, ILL, and KIN, indicating that suspended particulates were more dominated by organic matter with as flow receded. However, even though reduced velocity and increased clarity as flow receded may have offered more favorable conditions for sestonic algae proliferation, we observed declining concentrations of Schl as flow receded. We suggest sloughing of periphyton from upstream during high flow obscured our ability to observe whether an increase in sestonic primary productivity contributed to PN:TSS. Regressions in MUL and WAR indicated slopes were not significantly different from zero (Figure 8), suggesting no relationship between organic proportions of suspended particulate matter with TAP. ANCOVA of the relationship of PN:TSS with TAP only resulted in significant interaction effects among the two events in

Table 2. Geometric mean and (multiplicative standard deviation) for measured water quality variables

	Black disk visibility (m)	Total suspended solids (mg/L)	Suspended chlorophyll-a (μg/L)	Particulate nitrogen (mg/L)
Buffalo R.	1.01 (2.22)	7.79 (2.75)	1.14 (1.94)	0.09 (1.91)
Illinois R.	0.42 (1.78)	33.48 (2.51)	2.70 (2.84)	0.22 (2.04)
Kings R.	0.90 (2.09)	9.99 (3.85)	1.43 (2.31)	0.11 (2.50)
Mulberry R.	0.71 (1.63)	10.81 (1.72)	0.46 (1.96)	0.09 (1.54)
War Eagle Cr.	0.61 (2.15)	15.78 (2.73)	1.38 (1.84)	0.13 (1.81)

Table 3. Correlation coefficients for relationships among measured variables and discharge (Q; m^3/s) and time after event peak (TAP; d); text in bold indicates regression slope was significantly different from zero ($p < 0.05$); asterisk indicates ANCOVA returned significant interactions among discrete hydrograph events

	BDV (m)	TSS (mg/L)	Schl (μg/L)	PN (mg/L)	PN:TSS
Buffalo R.					
Q	-0.02*	0.16	0.01	0.04*	-0.30
TAP	**0.72***	**-0.77**	-0.22	**-0.79***	**0.57**
Illinois R.					
Q	**-0.84**	**0.89**	**0.68***	**0.87**	**-0.68**
TAP	**0.64**	**-0.69***	**-0.69**	**-0.72**	**0.49**
Kings R.					
Q	**-0.80**	**0.80***	**0.66**	**0.67***	**-0.80***
TAP	**0.72***	**-0.78***	**-0.72**	**-0.69***	**0.79**
Mulberry R.					
Q	**-0.51**	**0.48**	-0.37	0.09	**-0.68***
TAP	**0.80**	**-0.70**	**-0.72**	**-0.90**	-0.06*
War Eagle Cr.					
Q	**-0.81***	**0.81**	0.19	**0.66**	**-0.65**
TAP	**0.53**	**-0.51**	**-0.43**	**-0.57**	0.14

MUL, suggesting the slope of this relationship is not event-specific in the other four rivers. The slopes of PN:TSS *versus* TAP in MUL and WAR were not significantly different than zero and demonstrated substantial scatter.

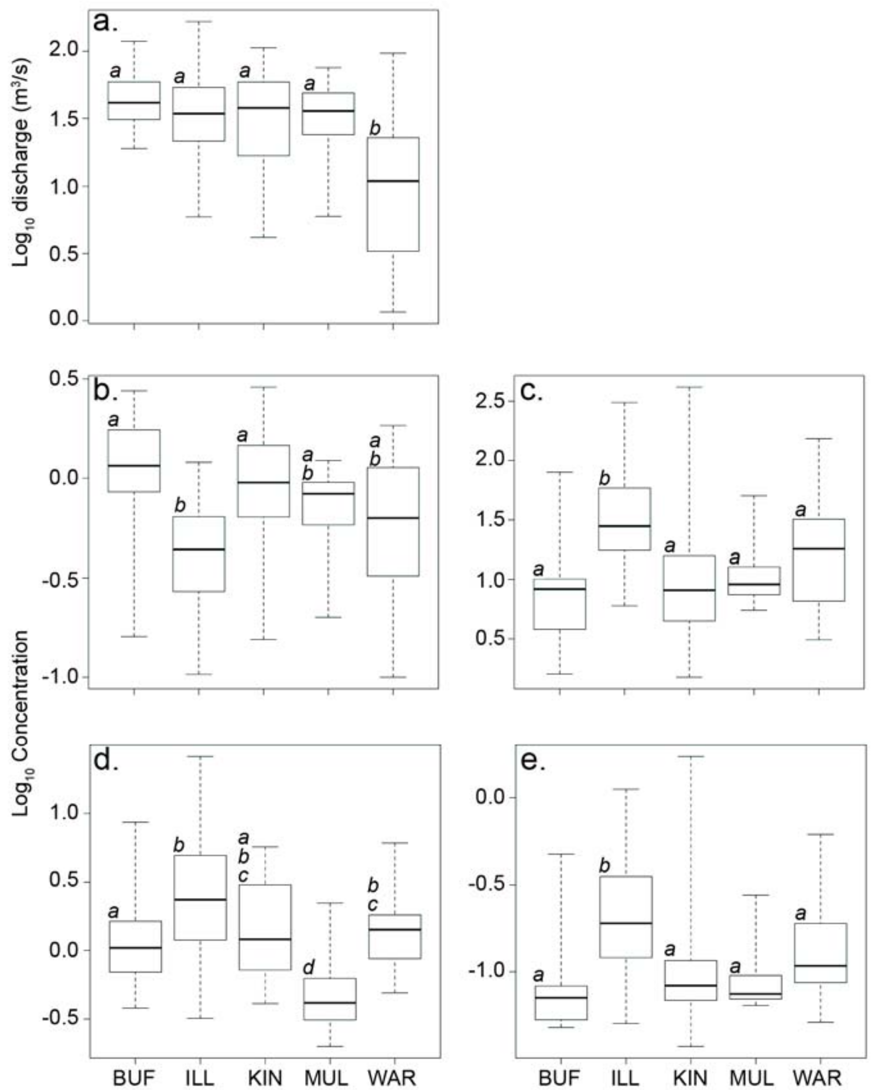

Figure 2. Boxplots of $\log_{10}$ transformed variables; a) discharge (m^3/s); b) black disk visibility (m); c) total suspended solids (mg/L), d) suspended chlorophyll *a* (µg/L); e) particulate nitrogen (mg/L); letter above boxed indicate statistical differences in pairwise comparisons ($p < 0.05$)

Water quality variables in BUF and MUL were either weakly or not significantly correlated with discharge over the complete study period (Table 3). In discrete events, the intuitive relationship of increased particulate matter with increased discharge was much more evident in BUF. Interaction effects in BUF indicated the magnitude of response in BDV and PN were dependent upon the specific hydrograph event. However, the lack of significant interactions among discrete events in MUL (Figure 7) may be because WQ measures were generally less variable than in BUF. While both rivers are dominated by forested land, the difference between BUF and MUL was likely related to other catchment characteristics. The MUL watershed exists fully within the Boston Mountains ecoregion, and the steeper gradient underlain by sandstone means event flow is likely more dominated by overland flow, with little groundwater-surface water interaction (Adamski et al. 1995). Whereas, while the headwaters of BUF are in the Boston Mountains ecoregion, the Ozark Highland ecoregion dominates the BUF watershed, and the karst geology promotes substantial groundwater contributions to flow (Adamski et al. 1995), effectively diluting the storm response.

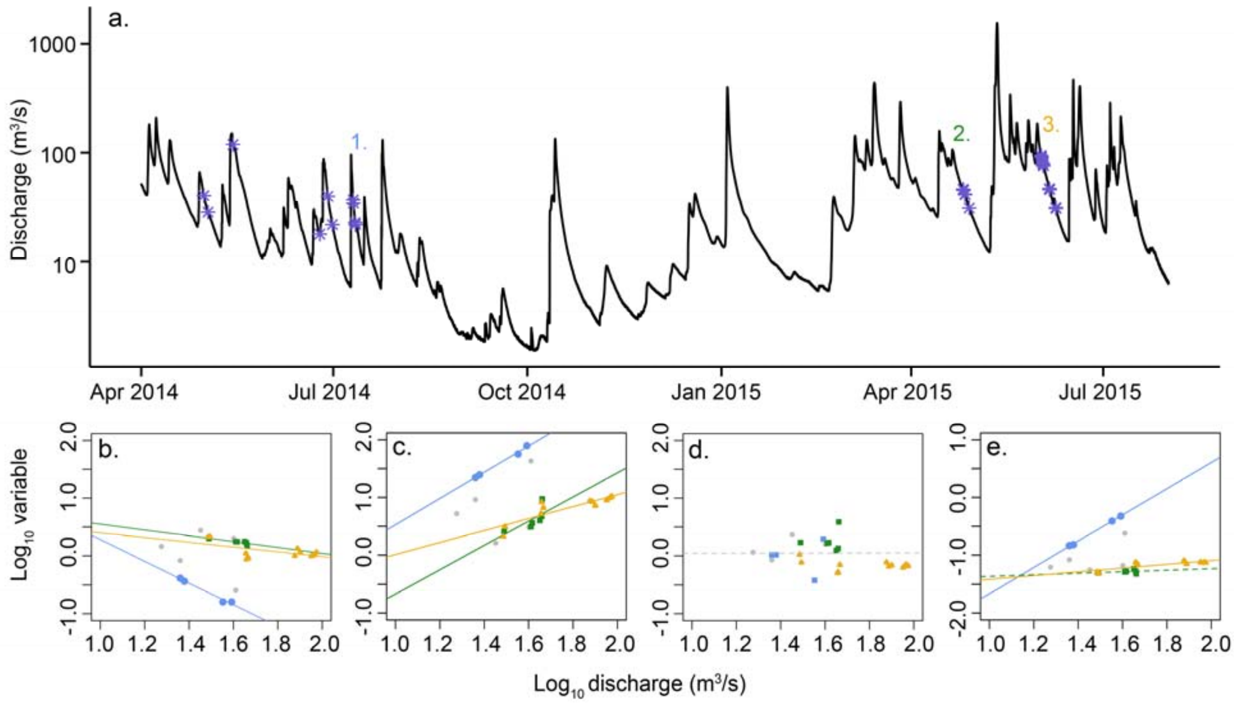

Figure 3. Buffalo River hydrograph (a) and ordinary least squares regression results of $\log_{10}$ transformed water quality variables *versus* $\log_{10}$ transformed discharge (m^3/s); b) black disk visibility (m); c) total suspended solids (mg/L); d) suspended chlorophyll-*a* (mg/L); e) particulate nitrogen (mg/L); multiple lines indicate significant interaction effects (ANCOVA); dashed lines indicate the regression slope was not significantly different from zero.

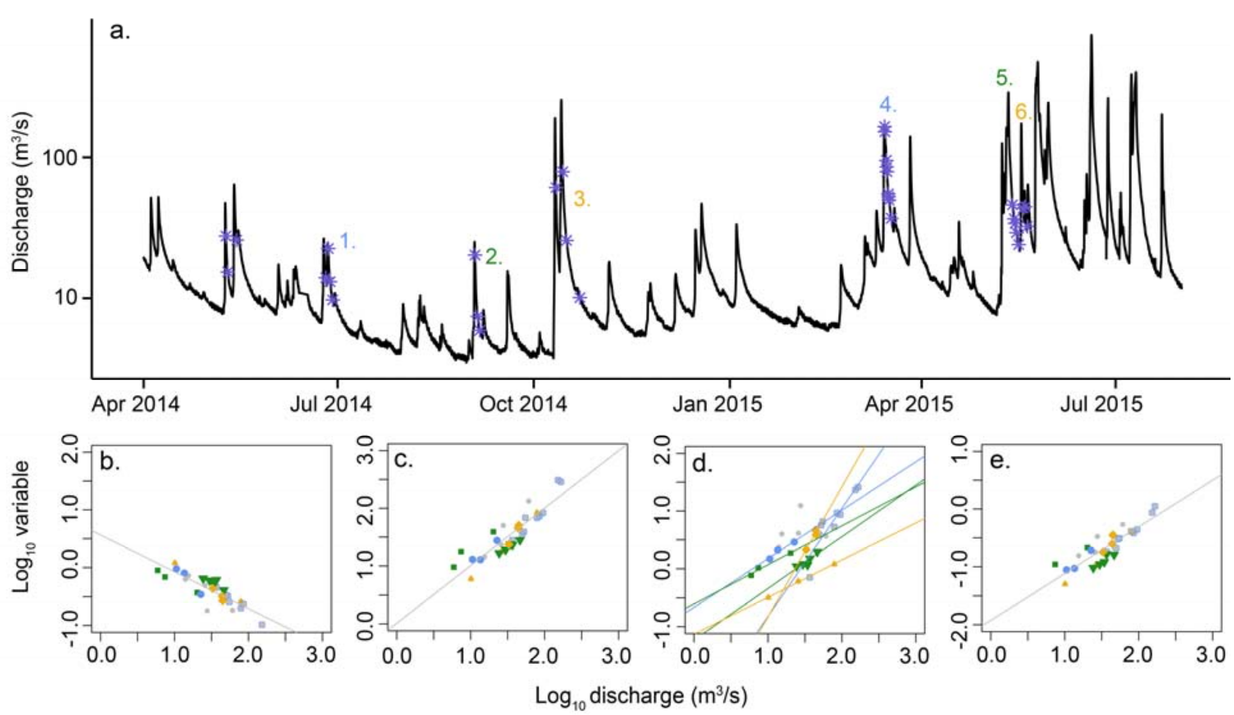

Figure 4. Illinois River hydrograph (a) and ordinary least squares regression results of $\log_{10}$ transformed water quality variables *versus* $\log_{10}$ transformed discharge (m^3/s); b) black disk visibility (m); c) total suspended solids (mg/L); d) suspended chlorophyll-*a* (mg/L); e) particulate nitrogen (mg/L); multiple lines indicate significant interaction effects (ANCOVA); dashed lines indicate the regression slope was not significantly different from zero.

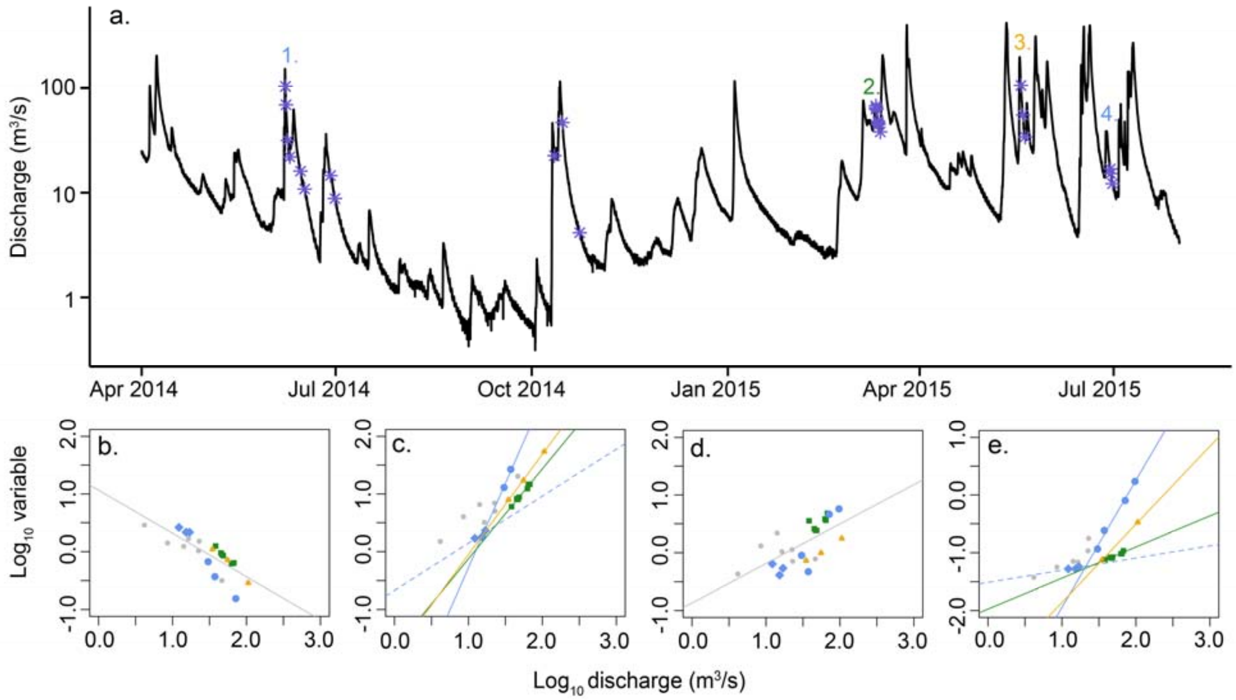

Figure 5. Kings River hydrograph (a) and ordinary least squares regression results of $\log_{10}$ transformed water quality variables *versus* $\log_{10}$ transformed discharge (m^3/s); b) black disk visibility (m); c) total suspended solids (mg/L); d) suspended chlorophyll-*a* (mg/L); e) particulate nitrogen (mg/L); multiple lines indicate significant interaction effects (ANCOVA); dashed lines indicate the regression slope was not significantly different from zero.

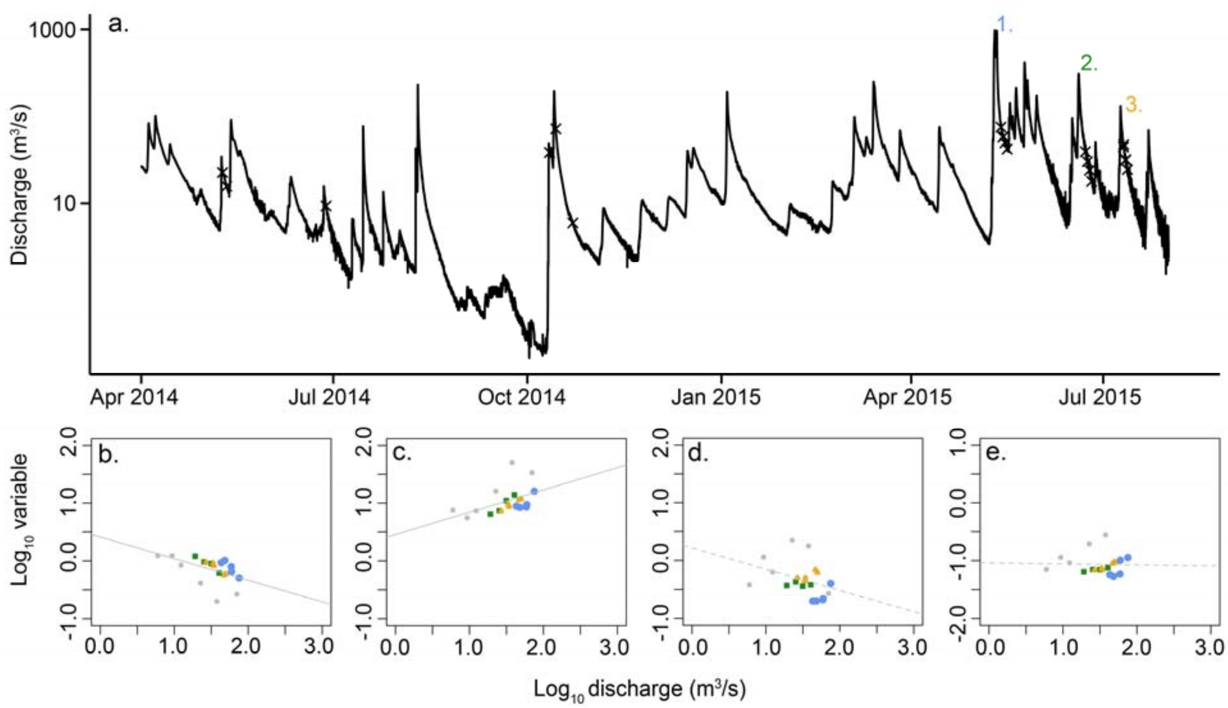

Figure 6. Mulberry River hydrograph (a) and ordinary least squares regression results of $\log_{10}$ transformed water quality variables *versus* $\log_{10}$ transformed discharge (m^3/s); b) black disk visibility (m); c) total suspended solids (mg/L); d) suspended chlorophyll-*a* (mg/L); e) particulate nitrogen (mg/L); dashed lines indicate the regression slope was not significantly different from zero.

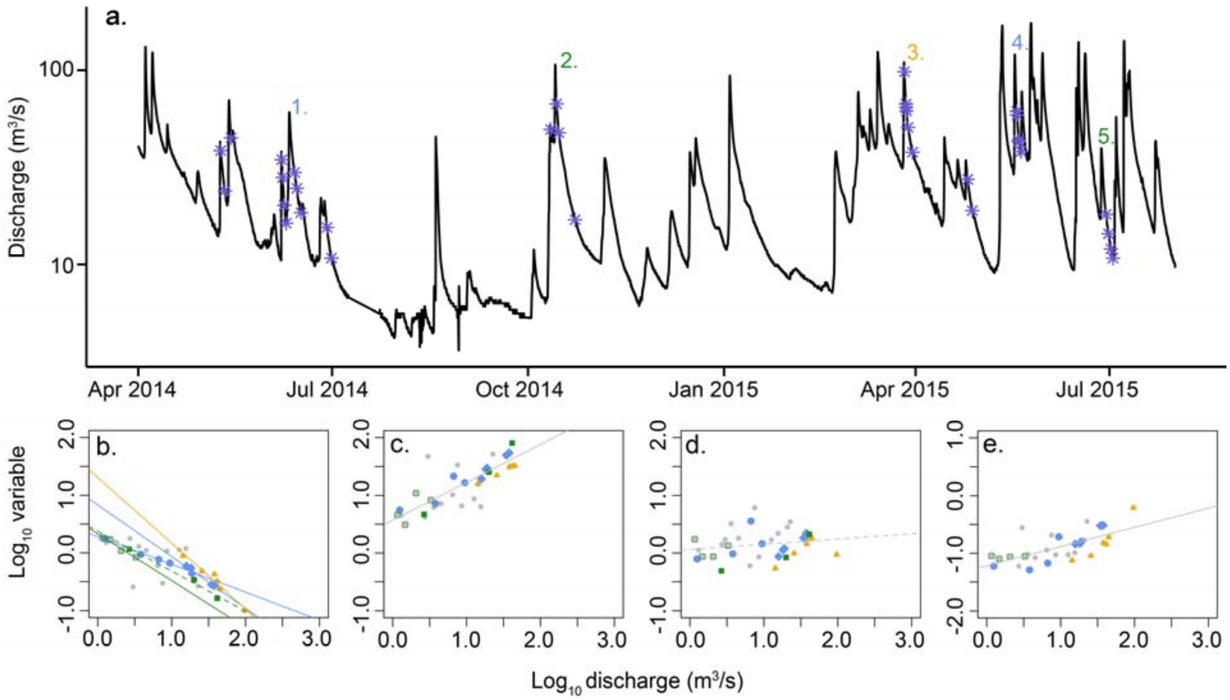

Figure 7. War Eagle Creek hydrograph (a) and ordinary least squares regression results of $\log_{10}$ transformed water quality variables *versus* $\log_{10}$ transformed discharge (m^3/s); b) black disk visibility (m); c) total suspended solids (mg/L); d) suspended chlorophyll-*a* (mg/L); e) particulate nitrogen (mg/L); multiple lines indicate significant interaction effects (ANCOVA); dashed lines indicate the regression slope was not significantly different from zero.

Alternately, in ILL, KIN, and WAR, discharge was relatively strongly correlated with WQ variables, except Schl in WAR. Interaction effects among well-sampled events were more common in KIN (Figure 5) than in ILL and WAR. It appeared that variability in event magnitudes and antecedent conditions of discrete events in ILL and WAR did not influence relationships between particulate concentrations and discharge as strongly as they did in KIN. Agricultural and urban land use are dominant contributors to excessive nutrient concentrations in surface waters (Carpenter et al. 1998), and agriculture is a principle source of sediment pollution in the US (Waters 1995). We suspect our observations in ILL are likely a result of a drainage area in nearly 70% agricultural and urban land use, with point and nonpoint source nutrient pollution (Green and Haggard 2001). WAR has the second greatest agricultural land use (35%) of the rivers in our study, and the second highest geometric mean concentrations of TSS, Schl, and PN (Figure 2; Table 2). It is reasonable to suggest our results in ILL and WAR are characteristics of their watersheds, as they are less prone to natural temperance provided by the forested landscape, i.e., sediment storage and release thresholds (Walling 1999) and riparian nutrient uptake (Peterjohn and Correll 1984). Our observations in these five rivers suggest as the watershed is more influenced by agricultural practices in the Ozarks, particulate concentrations may be more tightly coupled to event discharge. The event-specific relationships we hypothesized were generally only observed in BUF and KIN.

Measurements of OWQ can be an effective, affordable method for characterizing sediment concentrations in rivers (Davies-Colley et al. 2014). BDV may be a viable surrogate for TSS when developed with localized models [*Ballantine et al.*, 2014]. Our results suggest that OWQ measurements may be valuable in characterizing receding flow dynamics in the absence of resources supporting more precise chemical and physical characterization. However, because we did not observe patterns of event-specific control in BDV relationships analogous to those of particulate matter concentrations (as assessed by ANCOVA), BDV may not demonstrate similar sensitivities as concentration measurements as flow recedes in Ozark rivers. Though perhaps not generally a strong control, colored dissolved organic

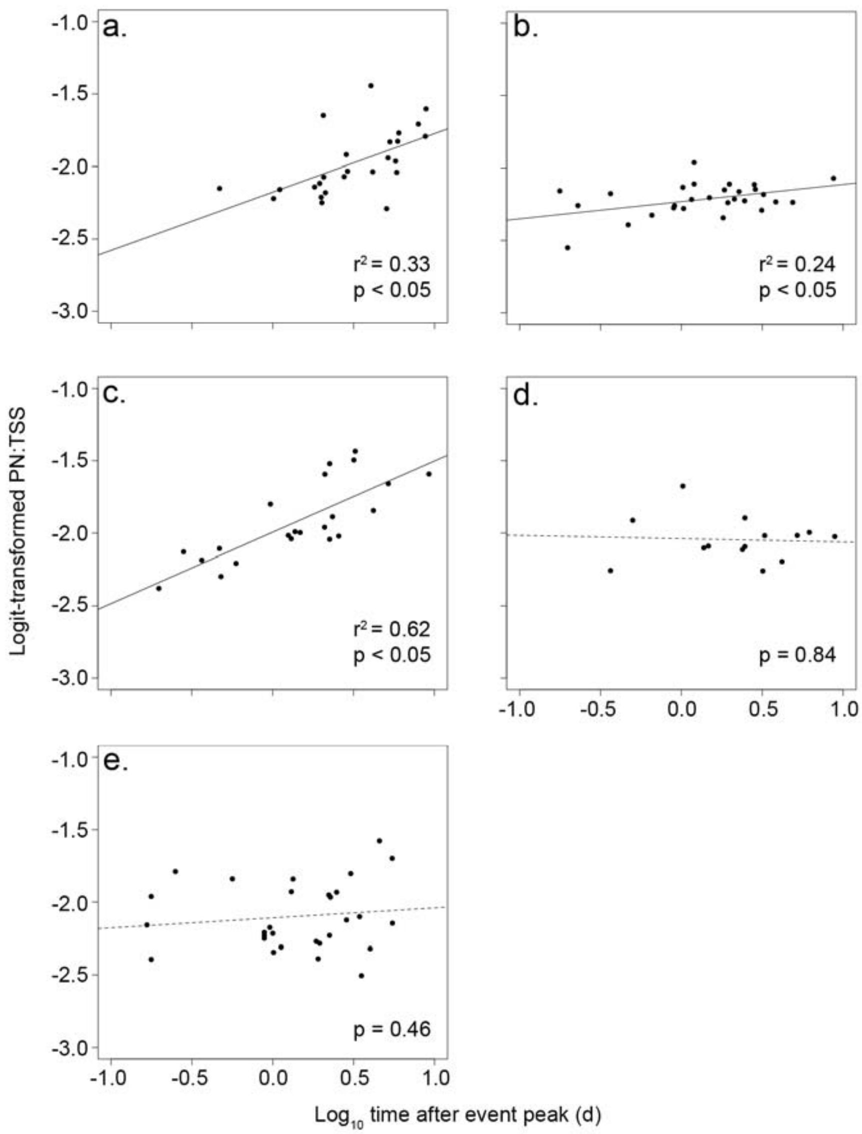

Figure 8. Ordinary least squares regression results for logit transformed PN:TSS versus $\log_{10}$ transformed time after hydrograph peak, dashed line indicates regression slope was not significant different than zero; a) Buffalo River; b) Illinois River; c) Kings River; d) Mulberry River; e) War Eagle Creek.

matter can influence BDV, especially following precipitation events (Julian et al. 2008). Characteristics of dissolved organic matter in the Ozarks can also vary with land use (Brisco and Ziegler 2004). We propose, when general characteristics of particulate matter concentrations as flow recedes in rivers of the Ozarks are sufficient, BDV can be an inexpensive and adequate tool. Nevertheless, more research is needed to determine sensitivities of simple optical methods to particle size distributions, organic proportions, and dissolved components in rivers before considering them for detailed characterizations.

Conclusions and Recommendations:

This study helps to describe variability in OWQ in five recreational rivers of the Ozarks in Arkansas. Outdoor recreation in Arkansas generates approximately $10 billion in consumer spending each year (Outdoor Industry Association 2012). Visitors to the Buffalo National River alone spent over $56 million in 2014 (National Park Service 2015). OWQ is particularly relevant in human perceptions of water quality (Smith et al. 1995, House and Fordham 1997, West et al. 2015) and judgments of suitability for recreation (Egan et al. 2009, Smith et al. 2015). Given the popularity of kayaking and canoeing in the Ozarks, many recreationalists will be likely to experience the rivers during periods of increased flow, thus offering a social application for the increased frequency of water quality assessment that may be facilitated using OWQ methods. We acknowledge, however, that recreational visitation also occurs during lower flow conditions, especially in summer. Our study was limited to approximately the first eight days after peak flow. Future work could extend this time period to characterize sediment concentration and OWQ dynamics as event flow shifts to base flow conditions in recreational rivers of the Ozarks.

A better understanding of particulate dynamics and their influence on OWQ may be valuable to water resources management in recreational rivers of the US. Our study showed that particulate matter concentrations in rivers in the southwestern Ozarks are temporally variable, and precise relationships with the hydrograph can differ based upon catchment characteristics, and among specific events within the same catchment. This study also demonstrated the relatively weak relationship of particulate matter concentrations with discharge in less-disturbed rivers, and more predictable relationships in agricultural watersheds. Because OWQ measurements can be useful for characterizing general particulate matter dynamics, we suggest they be considered for more frequent monitoring in scenic and ecologically sensitive rivers as climate and land use changes continue to take effect in the region.

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Project Title:	Continuation of analysis for host-specific viruses in water samples collected from select 303(d) listed streams in the Illinois River Watershed
Project Number:	2015AR368B
Start Date:	3/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	003
Research Category:	Water quality
Focus Category:	Non point pollution, surface water, water quality
Principal Investigator:	Kristen E. Gibson

Publications and Presentations:

Gibson, K.E., J.M. Jackson, S.L. Lampman, J.B. Carter, T.J. Moore, and G. Almeida, 2015, Use of Coliphage and Enteric Viruses for Fecal Source Tracking in Impaired Streams in the Illinois River Watershed, in International Symposium on Waterborne Pathogens, Savannah, GA.

Project Title: Continuation of Analysis for Host-Specific Viruses in Water Samples Collected from Select 303(d) Listed Streams in the Illinois River Watershed

Project Team: Kristen Gibson, Department of Food Science, University of Arkansas

Executive Summary:

In Northwest Arkansas, several streams within the Illinois River Watershed (IRW) have been placed on the 303(d) list for impaired waterbodies. In 2012, there were 13 streams—including 5 reaches of the Illinois River—on the 303(d) list for the IRW, and of these, 8 (62%) were due to elevated *Escherichia coli* levels. Moreover, the source of fecal contamination is listed as unknown for all but one stream. The objectives of our first study were to: 1) collect and process water samples from 303 (d) listed streams within the IRW and 2) determine likely dominant sources of fecal contamination over multiple seasons including "off-seasons" (e.g., when recreational activity is minimal). From May 2013 to April 2014, 462 samples were collected – approximately 20 samples from each sampling site (n = 23). Each sample was analyzed for *E. coli*. In addition, male-specific, coliphage (FRNA and FDNA) were analyzed by USEPA Method 1602 followed by isolation of individual plaques (up to 15 from each sample) and PCR to determine FDNA or FRNA as well as genogroup (G). For detection of additional markers of fecal contamination (i.e. host specific enteric viruses), polyethylene glycol (PEG 8000) precipitation was performed on 200 ml portions of samples (n = 38) determined to have elevated levels of coliphage (i.e. > 50 PFU). During the eleven-month sampling period, most streams had *E. coli* levels exceeding the 126 MPN/100ml cut-off; however, levels seemed to be lower from November 2013 to March 2014. Data also indicate a lack of correlation between levels of *E. coli* and coliphage (r^2 = 0.279). A large library of coliphage (n = 2,164) was archived for which a subset were analyzed and typed in order to glean more information about potential fecal source. During the second part of the study, 1,334 coliphage plaque isolates have been analyzed by PCR and reverse transcription (RT) PCR to determine FDNA or FRNA status—1,276 and 58, respectively. The FRNA isolates belong primarily to GI (n = 39) followed by GIII (n = 9), GII (n = 4), and GIV (n = 2) with GI FRNA associated primarily with animals. Analysis of 38 samples by PCR and RT-PCR for presence of host-specific and pathogenic enteric viruses revealed the following: human adenovirus (n = 38), human polyomavirus (n = 7), bovine enterovirus (n = 4), and porcine sapovirus (n = 0). Overall, this study generated much needed information on the levels of *E. coli* and coliphage in impaired waterbodies due to fecal contamination in the IRW.

Introduction:

In Northwest Arkansas, several streams within the Illinois River Watershed (IRW) have been placed on the 303(d) list for impaired water bodies. In 2012, there were 13 streams—including 5 reaches of the Illinois River—on the 303(d) list for the IRW, and of these, 8 (62%) were due to elevated *Escherichia coli* levels. Moreover, the source of fecal contamination is listed as unknown for all but one stream. Current standard methods for the evaluation of microbial water quality involve the use of generic bacterial indicators such as enterococci, fecal coliforms, and *E. coli*. However, these indicator bacteria do not provide enough information to determine the source of the fecal contamination or the actual risk to public health. In order to help prevent these streams from remaining on the 303(d) list, identification of the primary origins/sources of fecal pollution is needed.

In 2013, the AWRC 104b Program funded our study titled "Fecal Source Characterization in Select 303(d) listed Streams in the Illinois River Watershed with Elevated Levels of *Escherichia coli*". The objectives of the proposed study were to: 1) collect and process water samples from 303(d) listed streams within the IRW and 2) determine likely dominant sources of fecal contamination over multiple seasons including "off-seasons" (e.g., when recreational activity is minimal). Male-specific, ssRNA coliphage viruses (FRNA) and host-specific enteric viruses were the primary microbial targets for determination of likely fecal contamination. We generated a large library of coliphage (n = 2,164) of which a subset were analyzed and typed in order to glean more information about potential fecal source. Analysis of a subset of samples by PCR and RT-PCR for presence of host-specific and pathogenic enteric viruses was also proposed. Therefore, the primary purpose of this project was to complete the analysis of the coliphage isolates as well as analyze the samples with elevated levels of coliphage for the presence of host-specific viruses.

Methods:

Analysis of coliphage. For selection of FRNA and FDNA coliphage, *E. coli* strain C3000 host was utilized. Following quantification by the single agar overlay (SAL) procedure, individual plaques (up to 15 from each sample) were isolated using a sterile micropipette tip, resuspended in 500 µl of SM buffer, and stored at -80°C until analysis. For nucleic acid extraction, coliphage plaque suspensions (up to 6 for each sample) were incubated at 94°C for 3 min. Following extraction, the samples were analyzed by conventional PCR using FDNA specific primers. Those samples that were negative for FDNA were then analyzed by reverse transcription PCR (RT-PCR) using FRNA specific primers (Table 1). Once confirmed FRNA, the samples were analyzed to determine the specific FRNA genogroup as described by Friedman et al. (2011).

Analysis of host-specific markers. For detection of additional markers of fecal contamination, polyethylene glycol (PEG 8000) precipitation was performed on 200 ml of samples (n = 38) determined to have elevated levels of coliphage (i.e. > 50 plaque forming units). The resulting pellet was resuspended in disodium phosphate and total nucleic acid (RNA and DNA) extraction was performed as describe in Lambertini et al. (2008). The extracted nucleic acid was analyzed by real time PCR for the presence of human polyomaviruses, bovine enteroviruses as well as porcine and human adenoviruses (Table 1).

Table 1. Target microorganisms for determination of likely fecal source by PCR and RT-PCR.

Target microorganism	Primary Origin	Reference Method
male-specific ssRNA coliphage GI and GIV	animal	Friedman *et al*. (2011)
male-specific ssRNA coliphage GII and GIII	human	
human polyomavirus JC and BK	human	McQuaig *et al.* (2009)
human adenovirus	human	Jothikumar *et al.* (2005)
bovine enterovirus	bovine	Jiménez-Clavero *et al.* (2005)
porcine adenovirus	porcine	Wolf *et al.* (2010)

Statistical Analysis. All statistical analyses were performed using JMP® Pro 12.0 on $\log_{10}$ transformed values of *E. coli* and total coliphage concentrations. Thus far, the relationship between *E. coli* and coliphage concentrations has only been determined and analyzed. Future analyses will include nonparametric tests to compare the proportion of each FRNA genogroup and $\log_{10}$ quantities of *E. coli* in stream water samples under various physical water quality conditions (Ogorzaly et al., 2009) as well as paired *t* tests to compare the $\log_{10}$ geometric means of the density data grouped by land use impact, if known. A chi-square or Fisher exact test will be used to evaluate potential significance between frequencies of coliphage and other target microorganism detection and proportions of FRNA genogroups among land use categories. Additional analyses may include examination of bivariate associations with sample data as described in Cole et al. (2003). Briefly, “0” will be entered when FRNA or other target microorganisms are below the detection limit while “1” will be entered for presence of microorganisms. Based on the statistical tests described above, the strength of association of the probable fecal source and presence of target microorganisms will be determined.

Results:

The results for levels of *E. coli* and coliphage at each sampling location were reported previously. Briefly, during the eleven-month sampling period, most streams had *E. coli* levels exceeding the 126 MPN/100ml cut-off; however, levels seemed to be lower from November 2013 to March 2014. Based on bivariate analysis and a linear fit model, the relationship—predictive value—between *E. coli* and total coliphage concentrations is relatively weak (r^2 = 0.379) although the relationship is statistically significant ($p < 0.0001$) meaning significantly different from a R-squared value of zero.

Of the 462 samples collected—20 from each sampling site—2,154 coliphage were archived for analysis. Out of 2,154 coliphage, 1,334 were analyzed to determine whether FDNA or FRNA coliphage resulting in 1,276 (95.5%) and 58 (4.5%), respectively. Overall, 18 sampling sites had at least one positive for FRNA; however, 60% of the FRNA positive samples were from Clear Creek, Muddy Fork, and Little Osage Creek. Moreover, 71% of FRNA coliphage were obtained from 3 sampling dates corresponding to 3.6 to 8.9 cm rain events within 0 to 4 days preceding sample collection. Last, FRNA genogroup typing indicated a higher prevalence of animal-associated fecal pollution (71%) as opposed to human-associated (29%). With respect to host-specific viruses, analysis of 38 samples by real time PCR and RT-PCR for presence of host-specific and pathogenic enteric viruses revealed the following: human adenovirus (n = 38), human polyomavirus (n = 7), bovine enterovirus (n = 4), porcine adenovirus (n = 0).

Conclusions and Recommendations:

Based on the data for coliphage, there are a few observations that can be made. First, there is a possible association between precipitation events and the presence of FRNA coliphage in receiving waters. This potential association may be due to run-off from urban areas as well as agricultural areas as 71% of FRNA were in animal-associated genogroups. However, most of the FRNA coliphage were detected in Clear Creek, Muddy Fork, and Little Osage Creek with the former known to be impacted by urban run-off and the latter two could be impacted more so by municipal discharge. More research is needed to understand these potential associations. It is important to note that previous research has demonstrated that the four FRNA genogroups "trend" toward specific fecal sources as indicated in Table 1 (Cole et al., 2003); however, animal associated genogroups (GI and GIV) do not distinguish between wildlife and livestock which is a limitation of this source tracking tool.

Second, FDNA dominated the coliphage population in the samples. One reason for this is possibly due to the selection of *E. coli* C3000 host for detection of coliphage which is used primarily for somatic coliphage as opposed to male-specific, FRNA coliphage. There is an *E. coli* host that is used for detection of FRNA specifically, but we had decided not to use this host for various reasons. The other reason for the dominance of FDNA in the coliphage population could be that FDNA has been shown to comprise a high proportion of male-specific coliphage population in municipal wastewater and discharge, bovine and swine wastes (Cole et al., 2003) as well as environmental waters (Ravva et al., 2015). There may be opportunity to evaluate the predictive value of FDNA coliphage when paired with land use data.

Last, human adenoviruses were present in all samples (n = 38) that had elevated levels of coliphage (> 50 plaque forming units). This is interesting since human adenoviruses have been proposed as a virus indicator due to their ubiquity in human-associated wastewaters; however, it is still surprising that 100% of these samples were positive which warrants further investigation.

Overall, these data provide much needed information on the levels of coliphage in impaired waterbodies due to fecal contamination in the IRW. The dominance of FDNA coliphage could be indicative of a greater human influence in the watershed compared to animals if land use data is also considered during more in depth analysis of the data. Conversely, animal-associated FRNA coliphage were more prevalent within the 58 FRNA coliphage identified. In addition, based on these data, there is a possibility that human adenoviruses could serve as a valuable indicator of human fecal pollution in watersheds; however, more research is needed to confirm this association.

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Project Title:	Creating an annual hydroecological dataset in forested Ozark streams
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D.J. Hall, A.K. Dodd, and M.A. Evans-White, 2015, Physicochemical characteristics of streams in two flow regimes of the Ozark Highlands and Boston Mountains, Arkansas, USA, in Arkansas Water Resources Center Annual Conference, Fayetteville, AR.

Dodd, A.K. and M.A. Evans-White, 2019 (expected), Flow regime influences on whole stream metabolism and greenhouse gas fluxes in the Ozark Highlands and Boston Mountains Arkansas, USA, PhD Dissertation, Department of Biological Sciences, University of Arkansas, Fayetteville, AR.

Project Title: Creating an Annual Hydroecological Dataset in Forested Ozark Streams

Project Team: Michelle Evans-White, Department of Biological Sciences, University of Arkansas
Allyn Dodd, Department of Biological Sciences, University of Arkansas

Executive Summary:

Assessing and predicting ecological alteration is an important management strategy as streams continue to be impacted by the conversion of forested land to agricultural and urban areas. Relating environmental factors such as flow regime with ecological processes provide a decision-making tool to support water management. I sought to assess ecosystem metabolism in two dominant natural flow regimes, groundwater flashy and runoff flashy flow types, in minimally-impacted (>85% forested catchment area) Ozark streams to characterize connections between biological activity and hydrology. Study streams consisted of three groundwater flashy streams and three runoff flashy streams. I collected dissolved oxygen and temperature data every fifteen minutes with YSI DS5X multiparameter sondes via the single-station method from April 2015 to March 2016 to determine annual rates of gross primary production (GPP), ecosystem respiration (ER), and net ecosystem metabolism (NEM). Reaeration coefficients for metabolism estimates were calculated via surface renewal models for each stream. Discharge, total phosphorus and total nitrogen were measured monthly throughout the study. Annual gross primary production, ecosystem respiration, and net ecosystem metabolism were similar between flow regimes ($p= 0.27$; $p= 0.45$; $p=0.72$), but exhibited a high degree of variation in all three metrics over the study period. Though not statistically significant, groundwater flashy streams exhibited greater gross primary production as well as more negative ecosystem respiration, which may indicate that groundwater systems are slightly more productive than runoff systems. Net ecosystem metabolism ranged from -394 to 174 mg O_2 m^{-2} y^{-1} across streams, but values in runoff flashy streams varied more widely than groundwater streams. Discharge was similar between flow regimes ($p= 0.55$), as were total nitrogen concentrations ($p=0.13$). Gross primary production and ecosystem respiration tended to be higher in groundwater flashy streams, likely due to stable base flows during dry summer periods as well as less turbid water. Net ecosystem metabolism was negative in both flow regimes, but both autotrophic and heterotrophic streams were present in both flow classes, highlighting that flow class characteristics and land cover may not be the most important predictors of differences in ecosystem production in these systems. Rather, these data indicate a “mosaic” of carbon dynamics across northern Arkansas. Further work to discern flow regime differences based on hydrologic characteristics measured in the field are needed to confirm flow class model predictions, and to what degree intermittency may influence variability in ecosystem metabolism rates.

Introduction

The Arkansas Natural Resources Council is in the process of updating the state water plan. The goal of the plan is to provide a framework for the long-term sustainable use for the health, well-being, environmental, and economic benefit of Arkansas (ANRC 2014). The plan was conceived with little data on flow-ecology relationships that can provide more accurate estimates of the water resources needed to maintain the biological integrity and ecosystem function of state waters. Future state water plans will benefit from studies examining how hydrology and landscape changes influence Arkansas stream biota and ecosystem processes.

Assessing and predicting ecological alteration is an important management strategy as streams continue to be impacted by the conversion of forested land to agricultural and urban areas. Anthropogenic land use alters physical characteristics of streams as well as ecosystem function (Allan 2004, Poff et al. 2006). Relating environmental factors such as flow regime with ecological processes provide a decision-making tool to support water management (Poff et al. 2010). Several natural flow regime categories exist for streams within the Ozark forested biome (Leasure et al. 2016*;* Fig.1) that may result in variation in ecosystem function within this biome. It is necessary to examine the extent of variation in ecosystem function explained by flow classification within reference forested streams before assessing the effects of land use change on these systems. Therefore, I propose to examine flow-ecosystem function relationships within two predominant flow classes (runoff flashy and groundwater flashy) in the Ozark forested biome streams that can be used in future projects as a basis to compare stream function in altered landscapes within these flow regimes.

Whole-stream metabolism is a measure of primary production and ecosystem respiration that serves as an interface between water quality and ecosystem characteristics such as carbon availability, nutrient uptake rates, and trophic structure (Dodds 2007). Metabolism is driven by a suite of factors, such as light and nutrients, which can be influenced by changes in the landscape (Bernot et al. 2010). The indirect and direct susceptibility of metabolism to land use change makes it a good metric for assessing impacts at the ecosystem level. Additionally, daily metabolism can be vary temporally due to changes in light levels, organic matter inputs, algal biomass, and hydrology (Roberts et al. 2007). Annual metabolism integrates this variability and estimates are greatly dependent upon the frequency of daily measurements; less frequent measurements can result in erroneous annual metabolism budgets for a given stream (Roberts et al. 2007). The large dependence of these annual budgets on flow timing and amounts suggests that they will differ significantly across differing natural flow regimes within the same biome. While others have examined daily metabolism in Ozark streams, these studies were short in duration, likely missing patterns or variation in metabolism that would be useful in characterizing natural Ozark forested stream function.

The objective of this study was to assess ecosystem metabolism under two dominant natural flow regimes in Ozark forested streams. Stream metabolism was calculated from measures of primary production and ecosystem respiration from which inferences regarding overall ecosystem carbon and nutrient dynamics may be made. Annual gross primary production was expected to be higher in streams exhibiting groundwater flashy flow regimes, as groundwater streams never completely dry. The other dominant flow regime in Northwest Arkansas, runoff flashy, dries several days to weeks of the year, leading to the demise of the algal community in areas of no flow. Thus, annual gross primary production was predicted to be lower in runoff flashy streams given that the algal community dried and required recolonization. I predicted that both stream types would be net heterotrophic, with ecosystem respiration outpacing primary production, given that all streams in the proposed study were forested and thus received annual subsidies of leaf litter every autumn.

Methods:

This study took place in six minimally-impacted ($\geq$ 85% forested area in the catchment) streams in Northwest Arkansas. Three streams per flow type were selected from groundwater flashy and runoff flashy flow regimes. These two natural flow regimes were spatially clustered within the Ozark Highlands and Boston Mountains ecoregions, respectively. Four streams were located upstream of USGS gaging stations. Discharge was measured monthly using the mid-section method.

Dissolved oxygen and temperature were measured every 15 minutes by Hydrolab DS5X multiparameter sondes (Hach Company, Loveland, CO) from April 2015 to February 2016 via the single-station method. Stream metabolism was calculated based on diel changes in dissolved oxygen and temperature measurements according to Bott (2006). Reaeration coefficients were calculated via the surface renewal model method. Preliminary corrections for groundwater contributions to reaches receiving appreciable inputs were made according to Hall and Tank (2005) by measuring dissolved oxygen in water at discernible upwellings as well as discharge down the reach to determine springwater gains and losses from springs to the sonde. We measured total nitrogen by automated cadmium reduction on a Lachat Quikchem 8500 (Hach Company, Loveland, Colorado). Total phosphorus was measured using the ascorbic acid method. (APHA 2005)

T-tests were utilized to determine differences in nutrients, discharge, primary production, respiration, and metabolism between flow classes. Regression analysis was employed to examine relationships between discharge and net ecosystem metabolism.

Results:

Groundwater streams and one runoff stream, Murray Creek, did not dry for any time throughout the study period. However, two streams modeled as runoff flashy streams dried for over one month, from September 24th to November 6th, 2015.

Discharge measured across all streams ranged from 0.4 to 1.48 m^3/s, with groundwater streams exhibiting an average discharge of 0.88 (+/- 0.32) m^3/s and runoff streams exhibiting an average discharge of 0.66 (+/- 0.10) m^3/s. Discharge was similar between flow regimes ($p=0.55$).

Across all streams, total gross primary production (GPP) over the duration of the study ranged from 134 to 530 g O_2 m^{-2} y^{-1}. GPP did not differ between flow regimes ($p= 0.27$). Mean GPP in groundwater streams was 344 (+/- 95) g O_2 m^{-2} y^{-1}, while mean GPP in runoff streams was 203 (+/- 55) g O_2 m^{-2} y^{-1}. Ecosystem respiration (ER) varied from -54 to -912 g O_2 m^{-2} y^{-1}, and was also similar between both flow regimes ($p=0.45$). Mean respiration was -464 (+/- 224) g O_2 m^{-2} y^{-1} in groundwater streams and -238 (+/- 145) g O_2 m^{-2} y^{-1} in runoff streams. Three streams exhibited positive net ecosystem metabolism, indicating an autotrophic system, while three streams yielded negative, or heterotrophic, metabolism (Table 1); however, these were not demarcated by flow class. Streams exhibited a high degree of within-class variation- groundwater systems exhibited total net ecosystem metabolism from -378 to 29 g O_2 m^{-2} y^{-1},

Table 1. Summary of sites and parameters measured over the course of the study, from April 2015 to late February 2016

		GPP	**ER**	**NEM**	**Discharge**	**TP**	**TN**
Flow Class	**Site**	g O_2 m^{-2} y^{-1}	g O_2 m^{-2} y^{-1}	g O_2 m^{-2} y^{-1}	m^3/s	µg/L	mg/L
Runoff	Big Piney	130	-524	-394	0.49	3.28	0.04
Runoff	Little Piney	310	-136	174	0.85	2.69	0.05
Runoff	Murray	169	-55	114	0.65	5.52	0.12
Groundwater	Sylamore	247	-259	-12	1.48	6.36	0.23
Groundwater	Roasting Ear	534	-912	-378	0.76	10.00	0.45
Groundwater	Spring	250	-221	29	0.4	12.45	1.15

with an average of -120 (+/- 129) g O_2 m^{-2} y^{-1}. Runoff streams yielded a range of metabolism from -394 to 174 g O_2 m^{-2} y^{-1}, averaging -35 (+/- 180) g O_2 m^{-2} y^{-1} (Figures 1, 2). Net ecosystem metabolism was not related to discharge across streams ($p=0.70$, $R^2=0.04$) (Figure 3).

Primary production, respiration, and metabolism exhibited similar seasonal trends across flow regimes throughout the study timeline. Gross primary production peaked in late summer (August to September), declined following abscission, and began to increase once more in January. Ecosystem respiration was highest in the months following abscission (October to December), but remained low and stable the rest of the year in runoff streams while groundwater streams tended to exhibit greater and more variable ecosystem respiration throughout the year. Over the year, metabolism in runoff flashy systems was more variable than groundwater streams.

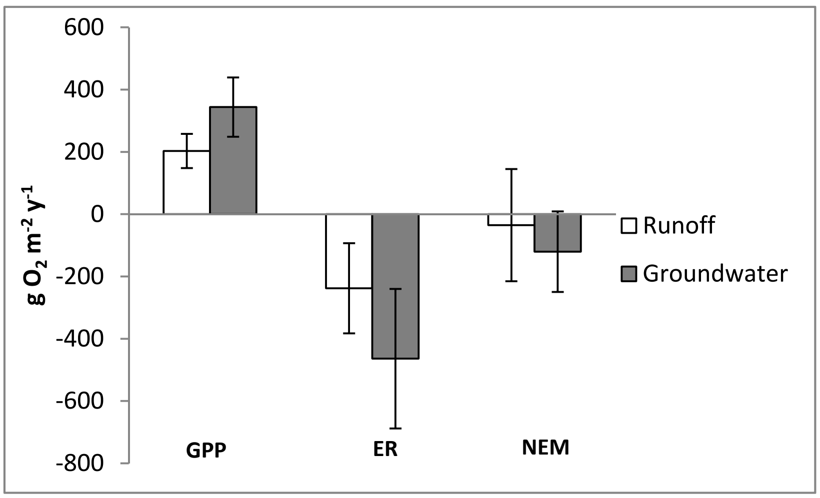

Figure 1. Gross primary production (GPP), ecosystem respiration (ER), and net ecosystem metabolism (NEM) in runoff (white boxes) versus groundwater (gray boxes) flashy streams.

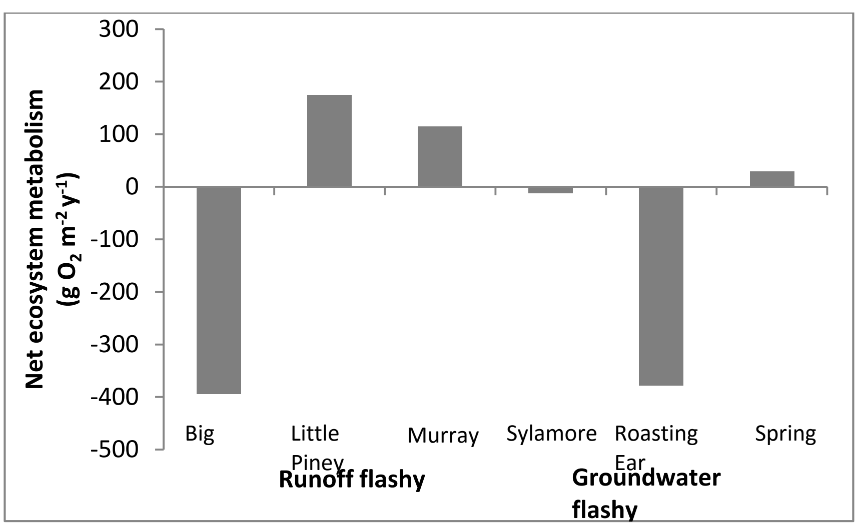

Figure 2. Net ecosystem metabolism across study sites

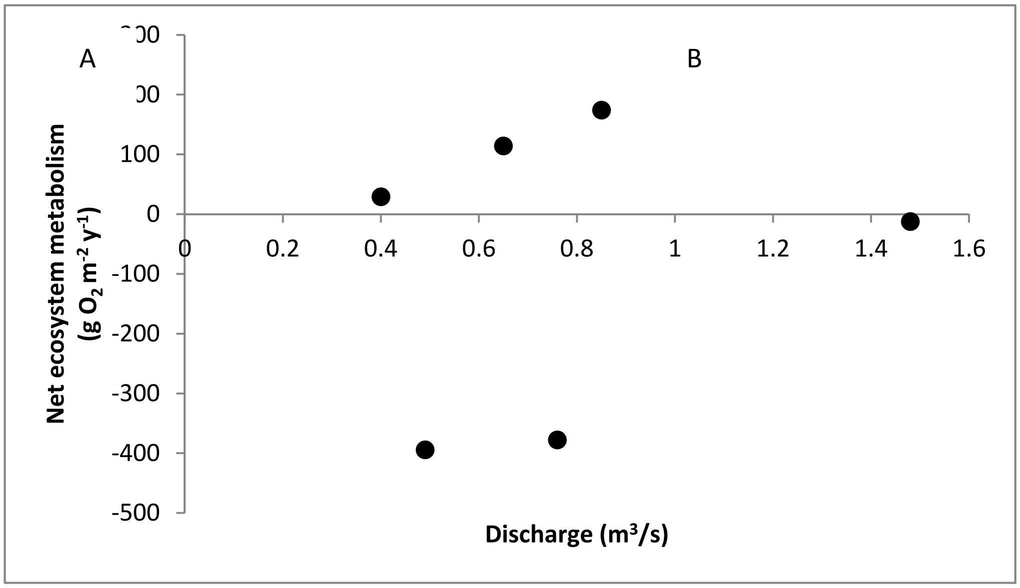

Figure 3. Average monthly net ecosystem metabolism across sites was not related to average monthly discharge ($p=0.70$, $R^2=0.04$).

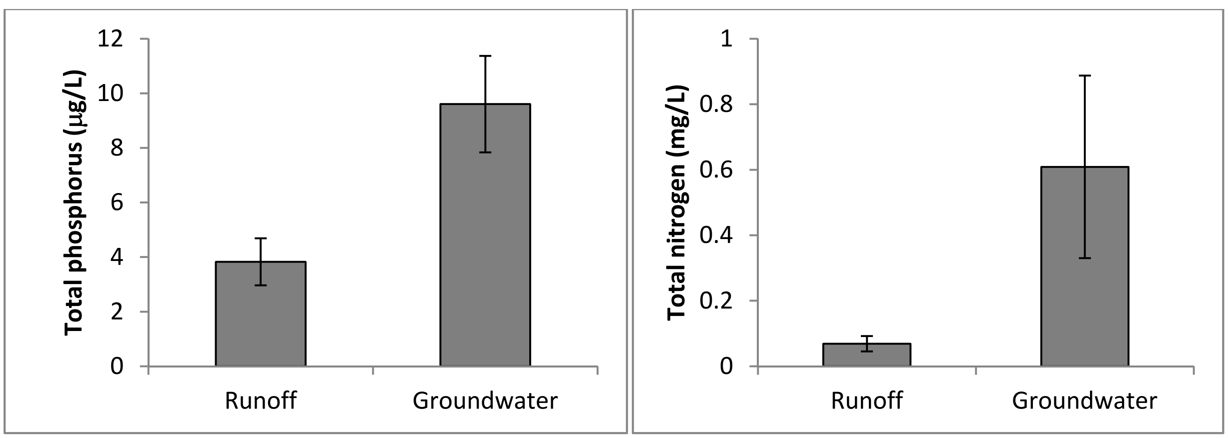

Figure 4. A) Mean total phosphorus (+/- 1 SE) and B) mean total nitrogen (+/- 1 SE) in runoff vs. groundwater flashy streams.

Total phosphorus was higher in groundwater streams ($p= 0.04$), though phosphorus levels were low across sites, averaging 9.6 (+/- 1.77) µg/L in groundwater flashy streams and 3.8 (+/- 0.86) µg/L in runoff flashy streams. Overall, total phosphorus levels fell between 2.7 and 12.4 µg/L (Figure 4A). Total nitrogen was similar between flow classes ($p= 0.13$) ranging from 0.04 to 1.2 mg/L across systems. Groundwater flashy streams revealed total nitrogen concentrations of 0.61 (+/- 0.1) mg/L while runoff flashy streams yielded 0.07 (+/- 0.01) mg/L (Figure 4B).

Conclusions and Recommendations:

Two streams that fell under the runoff flashy classification according to model predictions dried for three weeks longer than model criteria for that flow class. This indicates that these streams may actually fall under the intermittent runoff flow class, but given that my observations were made over only one year and flow class analyses utilized a long period of record (at least 50 years for reference gages used in the model), more work is needed to equivocally reject the original classification of these two streams. Further, both streams are dominated by runoff sources, which still allows for analysis of differences based on dominant flow sources. It is important to note, however, that more field measurements are needed to confirm model classifications, especially in headwaters streams, where the resolution of data used in flow regime classification was low.

While not statistically significant, gross primary production as well as ecosystem respiration tended to be greater in groundwater streams, which may be due to stable flows that sustain algal biomass during periods of little to no rainfall. Additionally, groundwater streams appear to allow more light to pass through; I observed a greenish tint in runoff flashy streams that makes the stream water nearly opaque in some areas (mainly pools). This is likely a byproduct of the karst inherent to the Boston Mountains ecoregion and not anthropogenic sediment pollution upstream, but is an important consideration given that this phenomenon tends to lower primary production in these systems. It is worth noting that differences in gross primary production and ecosystem respiration may indeed exist between groundwater flashy and runoff flashy regimes, but were obscured by low sample size (N=6) and study duration. It is possible that potential differences between flow classes may exist over interannual time scales, and that the full scope of variation in primary production and respiration inherent in each flow regime was not captured by my sample size.

I observed similar patterns in timing of peak primary production and ecosystem respiration throughout the study, though the magnitude and exact timing of maxima, minima, and variation in these metrics exhibited flow class- specific trends. This may be an artifact of ecoregion differences in the timing of abscission and leaf out. Future work will include repeated measures statistics to further explore potential temporal trends and differences in primary production, ecosystem respiration, and net metabolism.

Runoff flashy streams exhibited greater variation in metabolism rates over the course of the study, though data for both flow types highlight the high degree of variation inherent in ecosystem function across classifications. Proximal and/or distal factors not directly attributable to flow regime or immediately adjacent land cover are likely driving metabolism rates, perhaps in ways that are more site-specific than flow class or ecoregion-specific alone. Importantly, streams of the Ozark Highlands and Boston Mountains are inherently distinct; impacts to one system, even within the same flow regime and land cover category, may have vastly different effects on downstream habitats and biota.

Interestingly, nutrient levels were higher in groundwater flashy streams than runoff flashy streams. Both flow classes included streams that had been subject to past agriculture, but groundwater systems may be especially susceptible to higher concentrations of legacy nutrients, though nutrient levels were low across all sites.

I can provide no "rules of thumb" for managing these streams based on net ecosystem metabolism within the context of flow regime, as Northern Arkansas streams represent a mosaic of carbon uptake and transport. However, this work provides a reference for future evaluation of ecosystem function within a flow regime framework, which is important for establishing regional and national environmental flow standards. These efforts are also helpful for comparing urban and agricultural systems to forested streams to ascertain human alteration of stream function and water quality. Importantly, this work reveals that level of intermittency rather than water source may be an important factor governing the amount of inherent variation in ecosystem metabolism across systems in an ecoregion.

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Project Title: Relationship between nutrients, macrograzers abundance (Central Stonerollers and Crayfish), and algae on Ozark Streams

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Executive Summary:

Stream anthropogenic nutrient enrichment can cause instream and downstream problems of excess algal growth, which can constrain the recreational use of streams and reduce stream biodiversity (Millennium Ecosystem Assessment 2005, Evans-White et al. 2013). Elevated nutrients in streams can increase algal growth and community composition promoting taxa that are a concern for public health (Dodds and Welch 2000). A dose- or stressor-response relationship between nutrient levels and stream benthic algae is being developed by Arkansas Department of Environmental Quality (ADEQ) in accordance with Arkansas' Regulation No. 2 narrative, but the study will not measure grazer activity; grazers can be important determinants of stream benthic algal biomass and production. Specifically, some of the variation in the relationship between nutrients and benthic algae may be explained by grazer activity (Stevenson et al. 2012). Intense grazing pressure by benthic algivores, such as stonerollers and crayfish, may decrease the slope of the relationship between nutrients and algae, thus dampening the magnitude of the effect of nutrient enrichment in streams. Our objective was to examine how large-bodied abundant grazers in Ozark streams may modify the dose-response relationship between nutrients and algal biomass in Ozark Highland streams; these data can be considered when the state is developing their numeric nutrient standards. Stonerollers and crayfish were collected by backpack electrofishing at fifteen sites in the Ozark Highlands ecoregions of Oklahoma and Arkansas. Spatial repeated-counts sampling was conducted on each stream segment. Biomass of stonerollers was estimated using length to dry mass relationships from all sites, and site-specific biomass was estimated. Crayfish species and numbers were recorded. Linear regression was used to examine stoneroller, crayfish, and nutrient effects on algal biomass measured in a separate study during the same season at each site. Linear regression of residuals of chlorophyll a to nutrients against macrograzer estimates were not statistically significant. However, accurate stoneroller abundances are difficult to obtain due to limitations of current methodology. Manipulative experiments excluding grazers may be more effective at estimating large-bodied grazer effects in these study systems. Further considerations should be given to development of novel abundance sampling methods such as prepositioned areal electrofisher which may allow for increased precision of sampling by decreasing sampling biased introduced by fishes moving out of sampling area.

Introduction:

Nutrient pollution to streams can cause instream and downstream problems of excess algal growth, which can constrain the recreational use of streams and reduce stream biodiversity (Millennium Ecosystem Assessment 2005, Evans-White et al. 2013). In the Ozark Highlands, stream nutrient concentrations can be directly related to the land use practices, such as agriculture, poultry farming, and cattle farming (USGS 2007, Stevenson et al. 2012) within the watershed that contribute non-point nutrients and to urban point sources, such as sewage treatment plants (Haggard 2010, White et al. 2014). Elevated nutrients in streams can increase algal growth and shift the algal community composition towards taxa that are a concern for public health or reduce the recreational value of the water body (Dodds and Welch 2000). Algae can often be limited by N, P, or sometimes both (Dodds et al. 2002). Local studies have suggested that nutrients can be a determining factor of algal biomass in Ozark Highland streams and have suggested that algal growth is limited by N in Ozark streams (Power et al. 1988, Lohman et al. 1991, Lohman and Jones 1999). Therefore, increasing concentration of N, P, or both may result in increased algal biomass and eutrophication (Lohman et al. 1992, Lohman and Jones 1999, Dodds and Welch 2000, Dodds et al. 2002). The US Environmental Protection Agency (USEPA) requires US states and tribal nations to develop freshwater numeric nutrient criteria for nitrogen (measured as Total Nitrogen) and phosphorus (measured as Total Phosphorus); Arkansas is currently gathering data to develop these criteria.

In 2000, the USEPA provided possible national nutrient criteria standards for 13 Aggregate Ecoregions (Arkansas belonging to IX, X, IX) divided into smaller level III Nutrient Ecoregions. These were based off of 75th percentile nutrient concentration distributions for each region that may not account for finer spatial-scale regional variations (Haggard et al. 2013), which could result in numeric criteria that are perceived as too conservative or not conservative enough. Additionally, these standards rely solely on statically methodology and do not consider biological data. Therefore, many states have begun the task of gathering additional data to aid in the development of regional nutrient criteria standards based on scientific methods that can include assessment of algal biomass (USEPA 2013). A dose- or stressor-response relationship between nutrient levels and stream benthic algae is being developed by Arkansas Department of Environmental Quality (ADEQ) in accordance with Arkansas' Regulation No. 2 narrative. Relationships between nutrient concentrations and algae in this region can be variable (Stevenson et al. 2012, Haggard 2010) because other factors in addition to nutrient concentrations can affect benthic algal concentrations. Specifically, some of the variation in the relationship between nutrients and benthic algae may be explained by grazer activity (Stevenson et al. 2012).

Intense grazing pressure by benthic algivores (i.e., grazers) may decrease the slope of the relationship between nutrients and algae, thus dampening the magnitude of the effect of nutrient enrichment in streams. Algal grazing by stonerollers (*Campostoma spp.*) and crayfish (*Orconectes spp.*) can be imp- ortant determining factors on algal biomass in Ozarks streams. High stoneroller densities can elicit grazing pressures that affect algal biomass and community composition (Power et al.1988) and can substantially decrease algal biomass in high nutrient streams (Steward 1987). Crayfish are important grazers in Ozark streams and are important components of energy flow within streams (Whitledge and Rabeni 1997, Flinders and Magoulick 2007), and reported to consume much or more of the detrital and algal materials then other benthic macroinvertebrates. Stable isotope studies have provided evidence suggesting that crayfish diet may be more dependent on algae than the stoneroller diet and crayfish and stoneroller experimental manipulations have suggested that each grazer can reduce stream benthic algal biomass at natural densities (Evans-White et al. 2001), which emphasizes the importance of studying grazer pressure by both grazer types. Our objective is to examine how grazers may modify the dose-response relationship between nutrients and algal biomass in Ozark Highland streams. This dose-response relationship can be considered when the state is developing their nutrient standards.

Hypotheses

Stoneroller and crayfish abundance will explain the variation in regression models predicting algal biomass from nutrient concentrations. Nutrients will have a positive effect on algal biomass. Streams with greater crayfish and stoneroller abundances will have lower than expected algal biomass based on the estimated regression line with nutrients.

Methods:

Site Description

Fifteen sites were sampled in the Ozark Highlands Nutrient Ecoregion of Eastern Oklahoma and Northwest Arkansas, which is held within aggregate Ecoregion XI. Five of the sites were located in the Eucha-Spavinaw Watershed and ten where located in the Illinois River Watershed. Land use data is known Arkansas streams, but is not as well documented in Oklahoma streams. (Table 1). Sites were selected along a phosphorus gradient with concentrations of Total Phosphorus (TP) ranging from 0.08-0.16 mg/l and Nitrite-Nitrate (NO_2NO_3-N) ranging from 0.15-8.3 mg/l. Sites were scouted prior to sampling to assess sizes that would allow for backpack electrofishing sampling, sites chosen were small to medium sized streams with an average width ranging from 4-18 m, average depth ranging from 0.11-.282 m, and average velocity ranging from 0.08-0.42 m/s.

Collection

Sampling was conducted from 5-29 August 2015. The experimental unit being the stream segment (n=15) with three spatially-distinct riffles. Spatially-replicated count sampling was done whereby a minimum of three riffles were sampled at each stream segment and five quadrates (5 m^2) were sampled within each riffle. A modified-quantitative kick-net and backpack electrofishing (Smith-Root LR-24) method was used to sample grazer populations (Flinders and Magoulick 2005, Magoulick and Lynch 2015). Specifically, the methods were modified to increase the area sampled to an area of five meters-square. A three-person crew composed of one person equipped with a backpack electrofishing unit and two kickers, started five meters upstream of a seine (3mm mesh) held five meters in width by two people perpendicular to flow. The electrofishing crew slowly moved downstream to the seine while dislodging the substrate and actively electrifying the water which allowed fish and crayfish to be dislodged and

Table 1: List of all study sites within Ozark Highlands level III Nutrient Ecoregion. Five streams were sampled from the Ecuha-Spavinaw abbreviated Eucha, and ten found in the Illinois River watershed. Land use data is well known for Arkansas, but not for Oklahoma. Site ID will be used throughout the report as appreciation in all tables and figures that follow. Land use data provided by University of Arkansas' Center for Advanced Spatial Technologies (2006).

Stream	Site ID	State	Watershed	Latitude	Longitude	Land Use
Illinois River	ILLI1	AR	Illinois	35.953990	-94.249406	61% Forest, 28% Pasture, 7% Herbacous
Evansville Creek	EVAN1	OK	Illinois	35.877400	-94.570586	--
Spring Creek	SPRG3	OK	Illinois	36.148334	-95.154753	--
Saline	SALI1	OK	Eucha	36.281539	-95.093206	--
Little Saline	LSAL1	OK	Eucha	36.284553	-95.088672	--
Spavinaw Creek	SPAV1	AR	Eucha	36.384845	-94.480992	46% Pasture, 47% Forest, 3% Urban
Barren Fork	BARR2	OK	Illinois	35.919056	-94.619319	--
Flint, Gentry	FLIN1	AR	Illinois	36.239731	-94.500696	53% Pasture, 35%Forest, 7% Urban
Beaty Creek	BEAT1	OK	Eucha	36.354951	-94.776667	--
Goose Creek	GOOS1	AR	Illinois	36.056029	-94.291228	56% Pasture, 26% Forest, 12% Urban
Osage Creek	OSAG2	AR	Illinois	36.221997	-94.290074	43% Urban, 36% Pasture, 13% Forest
Ballard Creek	BALL1	OK	Illinois	36.061371	-94.573153	--
Osage Creek	OSAG1	AR	Illinois	36.265925	-94.237772	43% Urban, 36% Pasture, 13% Forest
Flint Creek	FLIN3	OK	Illinois	36.214540	-94.665494	--
Spring Creek	SPAR1	AR	Eucha	36.243673	-94.239325	43% Urban, 36% Pasture, 13% Forest

coherence into the downstream seine. Greater lengths were covered in streams where the width was less than five meters to standardize the area sampled. All stonerollers and crayfish were collected from the seine and put into separate five gallon buckets after each electrofishing pass. Raw count and standard length of stonerollers as well as species and carapace length of crayfish were recorded. Substrate, flow, depth, and width were taken at each quadrate while habitat length and electrofishing seconds were recorded at each riffle. Chlorophyll *a,* ash free dry mass, and nutrient measurements were taken within two weeks of the sampling time frame by a separate study group.

Calculating Biomass

A subset of stonerollers were retained and used to estimate length-mass relationships to determine total population biomass (Evans-White et al. 2001). Specifically stonerollers from four sites were used for the length-mass relationship (Illi1, Ball1, Beat1, and Sprg3), which represented low, middle, and high phosphorus concentrations along the gradient (total N=246). Stonerollers were dried at 48°C for a minimum of 72 hours. Once removed from oven, fish were put into desiccator for minimum of 1 hour. Fish were then weighted to the nearest 0.1mg. Length-mass relationship between natural log transformed dried weight and standard length were then calculated and used to estimate total biomass of stonerollers per sample reach.

Calculating Nutrient-Macrograzer Relationship

Multiple linear regression was used to examine stoneroller, crayfish, and nutrient effects on algal biomass. First nutrients, TP, NO_2NO_3-N, where regressed against the natural log of chlorophyll *a* and the residuals were computed. The stoneroller biomass and crayfish counts were then regressed against the residuals from the nutrient-chlorophyll *a* relationship.

Results:

Count data of stoneroller and crayfish was collected at all 15 sites (Table 2) and stoneroller counts were compared to other local studies (Table 3). Biomass relationship between length and dry mass of stonerollers was significant ($y=3.11x-12.8$, $R^2=0.80$, $p<0.001$). Nutrient and algae regression revealed a medium and significant correlation (Figure 1). Regression between residuals of nutrients to stoneroller biomass and crayfish count had low correlation coefficients and were not statistically significant (Figure 2, Figure 3).

Conclusions and Recommendations:

Linear regression showed no statistically-significant relationship between corrected residuals and stoneroller biomass or crayfish counts (Figures 3 and 4). Although sites were sampled along a gradient, our data did not account for the variation in algae along this gradient. Stonerollers are common in the Ozarks and inhabit streams in high quantities. They feed in large schools ranging in size from 200-500 individuals when left undisturbed (Matthews et al. 1987). However, stonerollers are described as active swimmers who face the current while consuming algae (Matthews 1998) which can help justify quick and robust swimming habits (Scott and Magoulick 2006). This along with their schooling behaviors often make them hard to sample quantitatively and can result stochastic sampling counts (Table 3). Since numerous studies have quantified the large scale to which stoneroller consume algae (Power and Matthews 1983, Steward 1987, Power et al. 1985, 1988, Power 1990, Evans-White et al. 2001) more effective sampling methods need to be implemented to understand the abundances of stonerollers in streams.

Table 2: Count and relative abundance of stonerollers and crayfish captures in the Ozark Highlands Ecoregion of Oklahoma and Arkansas.

Site ID	Counts	Count/m^2	Counts	Count/m^2
	Campostoma sp.		***Orconectes sp.***	
ILLI1	145	0.39	192	0.51
EVAN1	152	0.41	285	0.76
SPRG3	82	0.22	121	0.32
SALI1	26	0.07	81	0.22
LSAL1	51	0.14	376	1.00
SPAV1	78	0.21	236	0.63
BARR2	168	0.45	170	0.45
FLIN1	12	0.03	99	0.26
BEAT1	205	0.55	383	1.02
GOOS1	190	0.51	235	0.63
OSAG2	65	0.17	163	0.43
BALL1	166	0.44	226	0.60
OSAG1	120	0.32	370	0.99
FLIN3	35	0.09	59	0.16
SPAR1	210	0.56	281	0.75

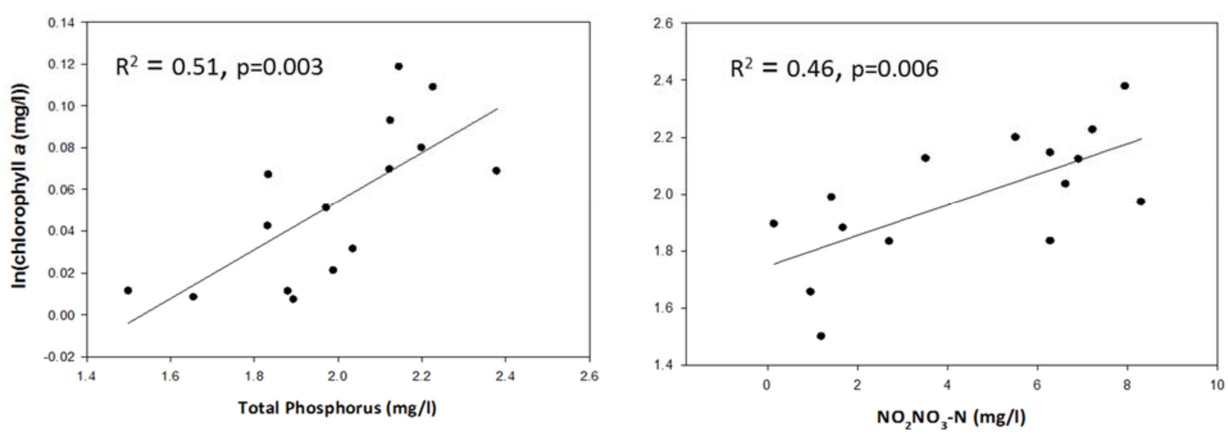

Figure 1: The relationship between natural log transformed chlorophyll *a* (mg/l) on Total Phosphorus (TP) and Nitrate-Nitrite (NO2NO3-N). All measurement were taken within two weeks of macrograzer sampling by a separate study group. Both significantly correlated.

Other methods for quantifying stonerollers may be more proficient such as using three pass electrofishing, barge electrofishing, or prepositioned electrofishing method. Three pass electrofishing method is often used in fish studies, but requires streams with smaller widths. In this method block-nets are set up at the up-stream and down-stream portions of the sample reach in order to prohibit the stonerollers from escaping the sampling area which prohibits large numbers of fish from escaping (Peterson et al. 2004). This method allows for high detection of fish species, however, it was not possible on most of the streams we sampled because the widths surpassed block-net size ranges. In situations

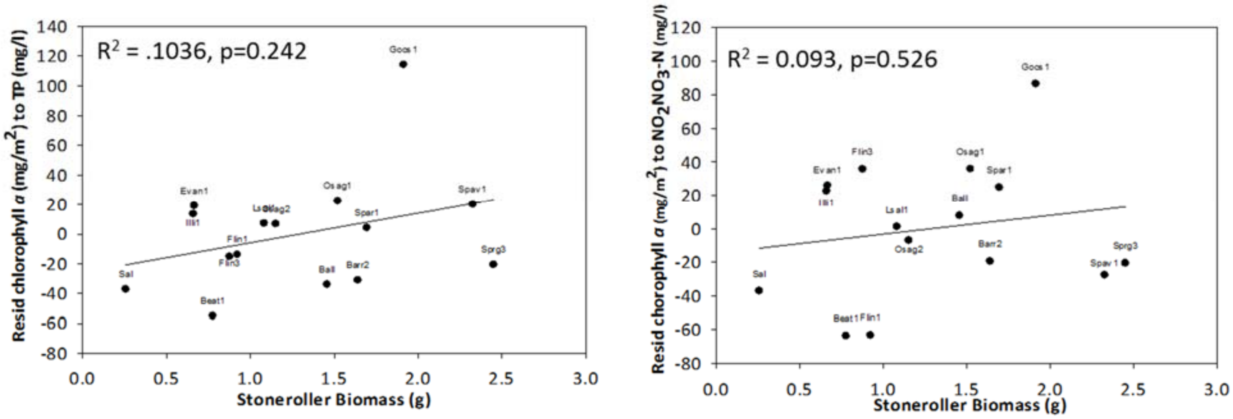

Figure 2: The graph shows the residuals of chlorophyll *a* on Total Phosphorus (TP) and Nitrate-Nitrite (NO2NO3-N) regressed against stoneroller biomass. The residuals are a measure of variation in chlorophyll *a* not explained by nutrient concentrations. The regression between these residuals and stoneroller biomass (g) was not significant with very low R^2 values.

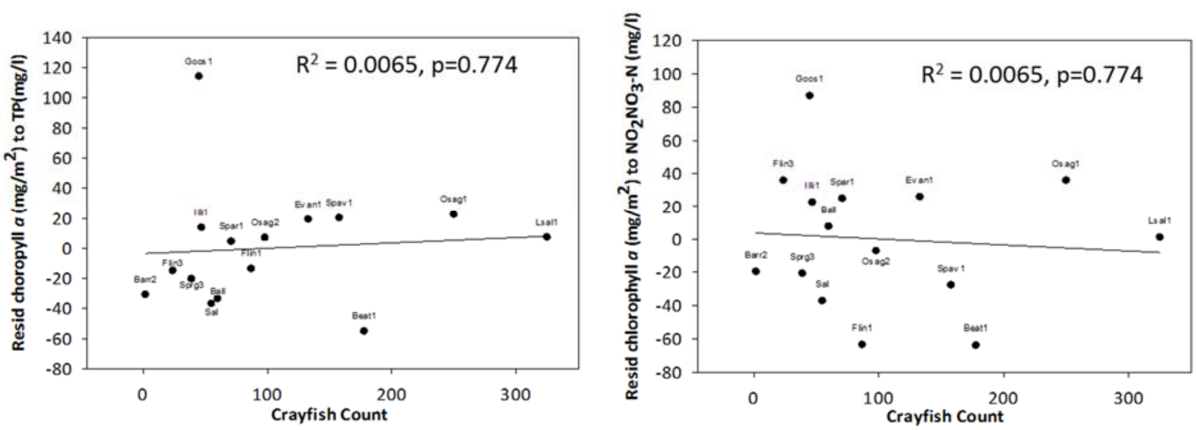

Figure 3: The graph shows the residuals of chlorophyll *a* on Total Phosphorus (TP) and Nitrate-Nitrite (NO2NO3-N) regressed against crayfish count or relative abundance. The residuals are a measure of variation in chlorophyll *a* not explained by nutrient concentrations. The regression between these residuals and stoneroller biomass (g) was not significant with very low R^2 value.

where the stream is too wide for standard backpack electrofishing, consideration can be given toward barge electrofishing with robust block net set-up (Meador and McIntyre 2003). Weaver et al. recently proposed a method for quantifying fish using a prepositioned areal electrofisher (2014). This device is a quadrate which allows electrical flow and is powered by a generator. This prepositioned electrofisher would be placed in the stream, fish would be allowed to recolonize the area, and then generator would be turned on allowing for less biased and more precision in estimations of fish populations (Weaver et al. 2014). Future consideration should be given to other methods of sampling stoneroller abundance in streams of middle order such as the streams sampled in our study. In addition, it is important to understand how effective these procedures are both before and after sampling since spatial and temporal variability in fish communities can affect population estimates (Meador and McIntyre 2003).

Table 3: Literature and agency search of previous fish surveys. Raw stoneroller counts (not corrected from area) were extrapolated from the studies. All data correspond to aggregate ecoregion XI, within Ozark Highlands level III Nutrient Ecoregion with is consistent with the our study area. Method described by Dauwalter and Edmund where greater areas were collected in streams with higher mean standard widths. Method described by Ross et al. where at least five seine hauls were collected from each site during the years of 1972-1981. Arkansas Department of Environmental Quality (ADEQ) follows the methods described by EPA Rabid Bioassessment Protocol (Barbour et al. 1999).

River	Counts	Method	Source
Big Creek, AR	610 ‖ 721	Electrofish	Dauwalter & Edmund 2003 ‖ ADEQ 1999-2001
Brush Creek, AR	237 ‖ 199	Electrofish	Dauwalter & Edmund 2003 ‖ ADEQ 1999-2001
Clear Creek, AR	383 ‖ 271	Electrofish	Dauwalter & Edmund 2003 ‖ ADEQ 1999-2001
Diles Creek, AR	154 ‖ 232	Electrofish	Dauwalter & Edmund 2003 ‖ ADEQ 1999-2001
N. Big Creek, AR	1082 ‖ 102-707	Electrofish	Dauwalter & Edmund 2003 ‖ ADEQ 1999-2001
Long Creek, AR	421 ‖ 573	Electrofish	Dauwalter & Edmund 2003 ‖ ADEQ 1999-2001
Mill Creek, AR	423 ‖ 933	Electrofish	Dauwalter & Edmund 2003 ‖ ADEQ 1999-2001
Mud Creek, AR	390 ‖ 728	Electrofish	Dauwalter & Edmund 2003 ‖ ADEQ 1999-2001
N. Sylamore Creek, AR	53 ‖ 198	Electrofish	Dauwalter & Edmund 2003 ‖ ADEQ 1999-2001
Piney Creek, AR	53-1082 ‖ 201	Seine ‖ Electrofish	Ross et al. 1986 ‖ ADEQ 1999-2001
Dry Creek, AR	119	Electrofish	Dauwalter and Edmund 2003
Greasy Creek, AR	257	Electrofish	Dauwalter and Edmund 2003
Hampton Creek, AR	343	Electrofish	Dauwalter and Edmund 2003
Tuttle Bend, AR	598	Electrofish	Dauwalter and Edmund 2003
Upshaw Creek	864	Electrofish	Dauwalter and Edmund 2003
Osage CreeL, AR	256-713	Electrofish	ADEQ 1999-2001
Spavinaw, AR	89-253	Electrofish	ADEQ 1999-2001
Spring Creek, AR	220-1417	Electrofish	ADEQ 1999-2001
Flint Creek, AR	535	Electrofish	ADEQ 1999-2001
Sager Creek, AR	356	Electrofish	ADEQ 1999-2001

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Principal Investigator:	Julian Fairey

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D.M. Meints II, W. Zhang, and J.L. Fairey, 2014, Assessing sources of total N-nitrosamines in drinking water systems, in America Chemical Society (ACS) National Meeting, San Francisco, CA.

Project Title: Elucidation of a novel reaction pathway for *N*-nitrosamine formation

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Executive Summary:

In this study, a chemiluminescence-based total *N*-nitrosamine (TONO) assay was adapted to include a solid-phase extraction (SPE) step to assess the role of a biologically derived chemical as an *N*-nitrosamine precursor. Specifically, the role of hydroxylamine – a key nitrification intermediate – was assessed as a function of five sample treatments related to the TONO assay (*Untreated*, *$HgCl_2$* only, sulfanilamide [*SAA*] only, *$HgCl_2$ + SAA*, and *HCl*) in terms of TONO (measured in the aqueous phase), SPE-TONO (measured in methanol) and NDMA by GC-FID. A series of batch reactor experiments were performed with various combinations of 3.52 mM hydroxylamine, 35.2 mM dimethylamine (a known NDMA precursor) and 3.52 mM monochloramine. However, several analytical interferences were discovered, associated with excess hydroxylamine in the samples, which obscured results from the TONO assay, GC-FID (for NDMA), and ion chromatography (for nitrite). In the aqueous phase with dimethylamine present, hydroxylamine was catalyzed by (1) *$HgCl_2$* to nitrite and NDMA and (2) *$HgCl_2$ + SAA* to NDMA only, as any nitrite formed was removed by SAA. In the methanol phase, hydroxylamine and dimethylamine were catalyzed to NDMA on the activated carbon in the SPE cartridges. However, these experiments revealed a previously unconsidered NDMA formation pathway, in which hydroxylamine is catalyzed to peroxynitrite ($ONOO^-$) in the presence of dissolved oxygen and subsequently reacts with dimethylamine to form NDMA. Recommendations are provided to guide the design of *N*-nitrosamine formation pathway experiments.

Introduction:

Nitrification episodes are prevalent in chloraminated drinking water distribution systems (CDWDSs) (Kirmeyer et al., 1995) and may exacerbate *N*-nitrosamine formation through the production of hydroxylamine, a key intermediate. In the nitrification process, biological ammonia oxidation to nitrite occurs in two steps: (1) the ammonia monooxygenase enzyme catalyzes ammonia oxidation to hydroxylamine (NH_2OH) and (2) the hydroxylamine oxidoreductase enzyme catalyzes hydroxylamine oxidation to nitrite (Kim and Gadd, 2008). Hydroxylamine is known to react with dimethylamine, $(CH_3)_2NH$, to form unsymmetrical dimethylhydrazine (UDMH) (Yang et al., 2009), which in turn can react with dissolved oxygen to form NDMA (Lunn and Sansone, 1994). Hydroxylamine has been implicated in the formation of NDMA during ozonation (Zhang et al., 2014), so it is reasonable that if biological ammonia oxidation occurs during chloramination, the hydroxylamine produced may react with monochloramine (Wahman et al., 2014) to form peroxynitrite ($ONOO^-$), if dissolved oxygen is present. Peroxynitrite is a known nitrosating agent (Uppu et al., 2000), but its role in *N*-nitrosamine formation under nitrification conditions in chloramine systems remains unknown.

In this study, a total *N*-nitrosamine (TONO) assay, developed by Mitch and colleagues (Kulshrestha et al., 2010) was adapted to include a solid-phase extraction (SPE) step, which is faster and simpler than a continuous liquid-liquid extraction, but may suffer from various analytical interferences. In particular, *N*-nitrosamines are known to form in SPE cartridges by catalysis reactions on the surfaces of the activated carbon (Padhye et al., 2011). Abiotic experiments were completed to assess the role of hydroxylamine in NDMA formation, as related to nitrification in CDWDSs. These results unexpectedly revealed the presence of multiple interference pathways associated with the SPE-TONO assay that could be used to guide methodological improvements and help explore alternative NDMA formation pathways.

Methods:

Solid Phase Extraction and Quenching Agents. For TONO measurements, 500 mL samples were concentrated by SPE and eluted to an organic solvent. The SPE columns were conditioned by sequential rinsing with solvent and water as follows: 3 mL of solvent followed by aspiration (repeated once), 3 mL of solvent and left wet (repeated once), and 3 mL of Milli-Q water and left wet (repeated four times). To load the sample onto the SPE columns, a sample delivery system was used to draw each 500 mL sample through a single column into a waste container at a flow rate of approximately 5 mL min^{-1}. This was followed by an aspiration period of 10 minutes of atmospheric air at full vacuum. To elute the *N*-nitrosamines from the SPE columns, 12 mL of solvent was passed through each column drop-wise and collected in a centrifuge tube. The eluted extract was passed through a wetted drying column (rinsed with 6 mL of solvent), which consisted of 6 g sodium sulfate encapsulated by glass fiber frits in a 6 mL glass SPE column and followed by 3 mL of organic solvent in an attempt to purge the drying column of any remaining *N*-nitrosamines. The sample extracts were then concentrated from ~15 mL to precisely 1 mL in a 37°C water bath using an evaporator with a gentle stream of lab-grade nitrogen. The 1 mL sample extracts were transferred to individual 2 mL amber glass vials sealed with PTFE lined screw caps and stored at -20°C.

As described by Kulshrestha et al. (2010), interferences caused by *S*-nitrosothiols and nitrite can produce false-positive signals in the chemiluminescence detector and thus need to be quenched in the sample extracts. *S*-nitrosothiols were quenched from the 1 mL sample extracts with 100 µL of the mercuric chloride solution (20 g L^{-1} in Milli-Q water) and allowed to sit in the dark for 30 minutes. Next, nitrite was quenched with 100 µL of the sulfanilamide solution (50 g L^{-1} in 1 N HCl) and allowed to sit in the dark for 15 min. Interfering compounds were quenched and *N*-nitrosamines quantified within 2 weeks of sample extraction.

Total N-nitrosamine Analysis. Total *N*-nitrosamines were quantified in the purified sample extracts using a chemiluminescence NO detector (Eco Physics CLD 88sp), as detailed by Mitch and Dai (2012). Output signals from the chemiluminescence detector were discretized at 0.2 second intervals and captured using a MS Excel macro. These data were then imported into MATLAB R2012a to calculate the area under each sample peak using a summation and baseline subtraction formula. Each sample peak area was then compared to that of the standard curve preceding its respective injection to determine the concentration as NDMA based on the volume of the injection and the initial volume of the sample processed by SPE, if applicable.

Hydroxylamine Experiments. The impact of hydroxylamine on TONO and NDMA formation were assessed in batch reactors at room temperature (20-22°C) with combinations of hydroxylamine (3.52 mM), monochloramine (3.52 mM), and dimethylamine (35.2 mM). Each batch reactor consisted of an amber glass bottle filled with 400 mL of 10 mM sodium borate (prepared in Milli-Q water) and purged with O_2 for 10 minutes to achieve ~40 mg L^{-1} dissolved oxygen (DO). Each reagent addition was followed by an allotted time prior to other amendments, as follows: sodium borate (10 minutes), hydroxylamine (5 minutes), monochloramine (5 minutes), dimethylamine (5 minutes), and the combined sample (60 minutes). Various combinations of the TONO sample treatments were investigated to assess potential interferences on aqueous phase aliquots and methanol extracts following SPE. Regardless of the sample phase (i.e., aqueous or methanol), the TONO standard curve was prepared by direct injections of NDMA into methanol, as described previously. The following five treatments were assessed: (1) untreated (i.e., no sample treatment), (2) sulfanilamide only (i.e., samples dosed with 100 ⍰L of 50 g L^{-1} sulfanilamide in 1 N HCl and held in the dark for 15 minutes), (3) mercuric chloride only (i.e., samples dosed with 100 ⍰L of 50 g L^{-1} mercuric chloride and held in the dark for 30 minutes), (4) mercuric chloride and sulfanilamide, and (5) HCl only. Aqueous phase samples were measured by the TONO assay following the five sample treatments by direct injection into the reaction chamber. Between 404-412 mL of each aqueous phase sample was processed by SPE and eluted into 10 mL of methanol, but was not further concentrated using the nitrogen gas blowdown step to avoid further volatile losses. These samples were subjected to the five sample treatments followed by the TONO assay and GC-FID.

Results:

TONO and NDMA formation were assessed in batch reactors containing combinations of hydroxylamine (3.52 mM), dimethylamine (35.2 mM), and monochloramine (3.52 mM). *N*-nitrosamines were measured in triplicate in (1) aqueous phase aliquots taken prior to SPE (TONO Aqueous, Table 3) and (2) methanol following SPE (TONO Solvent and NDMA by GC-FID, Table 1). Aqueous TONO data for the batch reactors with hydroxylamine only (Table 1) showed a comparatively large TONO response in the $HgCl_2$-treated aqueous phase sample (57,249 μg L^{-1} as NDMA), which was subsequently removed by treatment with SAA. The corresponding data in Table 2 indicate a high concentration of nitrite in this sample (18,356 μg L^{-1} as N), presumably from mercury-aided catalysis of hydroxylamine reacting with oxygen (Wahman et al., 2014). This result demonstrates the need to use SAA when applying the TONO assay to waters that do not contain nitrite, such as those with hydroxylamine that could produce an interference signal by $HgCl_2$ catalyzing nitrite formation. An additional observation from the hydroxylamine only experiments (Table 1) is the apparent production of nitrite during IC analysis from residual hydroxylamine in the sample. $HgCl_2$ treatment presumably removed any remaining hydroxylamine in the sample by catalyzing hydroxylamine's reaction with oxygen, producing nitrite as a product. Also, treatment with SAA (by itself or with $HgCl_2$) should result in complete nitrite removal and the associated TONO response. Therefore, residual hydroxylamine is only expected in the *Untreated* and *SAA*-only treated samples, and nitrite is only expected in the *Untreated* and *$HgCl_2$*-only treated samples, producing an associated TONO response. While the *$HgCl_2$ + SAA* treated sample had an expected non-detectable nitrite (Table 2) and minimal TONO response (<10 μg L^{-1} as NDMA, Table 1), the *SAA*-only treated sample had a measurable nitrite concentration (1,522 μg L^{-1} as N) with a minimal TONO response (<19 μg L^{-1} as NDMA), suggesting formation of nitrite during IC analysis. Taken together, these results indicate that hydroxylamine present in the *SAA*-only treated sample was converted to nitrite during IC analysis.

For the batch reactors with hydroxylamine and dimethylamine, Tukey's tests were done to compare the triplicate means between treatments. For NDMA and TONO in methanol, there were no statistically significant differences between sample treatments, indicating potentially interfering compounds (e.g., nitrite) were not present in the methanol following SPE or created by the treatment (e.g., $HgCl_2$). For TONO in the aqueous phase, the comparatively high TONO response in the *$HgCl_2$*-treated sample (62,134 μg L^{-1} as NDMA) was attributed to nitrite (15,455 μg L^{-1} as N, Table 2) and NDMA, presumably from mercury catalyzing the reaction of the residual hydroxylamine in the presence of dissolved oxygen. As in the hydroxylamine only experiments, residual hydroxylamine may have resulted in nitrite production during IC analysis as the *SAA*-only treatment had a nitrite concentration (1,350 μg L^{-1} as N) without a correspondingly large TONO response (77 μg L^{-1} as NDMA). In contrast, the *$HgCl_2$ + SAA* treatment showed an undetectable nitrite concentration and a large TONO response (15,834 μg L^{-1} as NDMA). This suggests that hydroxylamine and dimethylamine reacted to form UDMH, which subsequently reacted with dissolved oxygen catalyzed by mercury to form NDMA. In sum, two interferences were apparent in the aqueous phase batch reactors with hydroxylamine and dimethylamine: (1) nitrite and NDMA interferences produced by $HgCl_2$ treatment and (2) an NDMA interference produced by *$HgCl_2$ + SAA* treatment.

Table 1. Total *N*-nitrosamines and *N*-nitrosodimethylamine formed from reactions with 3.52 mM hydroxylamine (NH_2OH), 3.52 mM monochloramine (NH_2Cl), and 35.2 mM dimethylamine ($(CH_3)_2NH$) for various sample treatments

Reagents	Treatment	Total *N*-nitrosamine Assay (TONO)		NDMA	Nitrite Equivalent TONO [a] $\mu g\ L^{-1}$ as NDMA
		$\mu g\ L^{-1}$ as NDMA Average ± 95% confidence interval			
		Aqueous [b]	Solvent [c]	Solvent [c]	
NH_2OH + $(CH_3)_2NH$ + NH_2Cl	Untreated	3,230 ± 534	527 ± 55	420 ± 23	NA
	SAA	133 ± 22	332 ± 97	200 ± 55	NA
	$HgCl_2$	6,243 ± 209	521 ± 48	489 ± 49	7,561
	$HgCl_2$ + SAA	765 ± 218	326 ± 112	393 ± 234	NA
	HCl	141 ± 18	407 ± 17	323 ± 100	NA
NH_2OH + $(CH_3)_2NH$	Untreated	1,358 ± 1,237	13,087 ± 1,298	9,028 ± 771	13,835
	SAA	77 ± 48	10,623 ± 3,721	9,558 ± 1,039	9,009
	$HgCl_2$	62,134 ± 4,187	12,145 ± 892	8,935 ± 1,015	54,534
	$HgCl_2$ + SAA	15,834 ± 2,866	11,882 ± 1,149	7,930 ± 1,487	NA
	HCl	100 ± 108	12,222 ± 957	6,283 ± 909	9,330
NH_2OH	Untreated	118	NM	NM	25,578
	SAA	< 19	NM	NM	9,330
	$HgCl_2$	57,249	NM	NM	70,622
	$HgCl_2$ + SAA	< 10	NM	NM	NA
$(CH_3)_2NH$	Untreated	125	NM	NM	NA
	SAA	49	NM	NM	NA
	$HgCl_2$	326	NM	NM	NA
	$HgCl_2$ + SAA	102	NM	NM	NA

[a] Theoretical response from nitrite in TONO assay based on 1:1 molar yield and 100% efficiency
[b] Sample processed in aqueous phase without solid-phase extraction
[c] Sample concentrated by solid-phase extraction and eluted into methanol; values corrected for the estimated NDMA extraction efficiency (70%, see text)
HCl – treated with 100 µL of 1 N HCl
$HgCl_2$ – treated with 100 µL of 20 g L^{-1} mercuric chloride and held in the dark 30 minutes
$HgCl_2$ + SAA – treated with mercuric chloride followed by sulfanilamide
NA – not applicable
ND – not detected
NM – not measured
SAA – treated with 100 µL of 50 g L^{-1} sulfanilamide in 1 N HCl and held in the dark 15 minutes

For the batch reactors with hydroxylamine, dimethylamine, and monochloramine, Tukey's tests were done to compare the triplicate means between treatments. For NDMA and the TONO samples in methanol (Table 3), there were no statistically significant differences between sample treatments, indicating potentially interfering compounds (e.g., nitrite) were not present in the methanol following SPE. In contrast, for the aqueous phase TONO samples, statistically significant differences were found between the *Untreated* sample and the other treatments, which was attributed to the formation and quenching of nitrite in the presence of hydroxylamine by $HgCl_2$ and SAA, respectively (Table 2). Interestingly, HCl treatment resulted in a comparatively low TONO response in the aqueous phase (141 ± 18 µg L^{-1} as NDMA), suggesting hydroxylamine in its acidic form (NH_3OH^+, $pK_a \approx 6$) does not react with dimethylamine to form UDMH. This result is in agreement with Zhang et al. (2014) that found the reaction between hydroxylamine and dimethylamine to form UDMH was pH dependent.

Comparing the batch reactors containing hydroxylamine, dimethylamine, and monochloramine with those containing hydroxylamine and dimethylamine indicated that in the (1) aqueous phase, hydroxylamine reacted in the $HgCl_2$-treatment, and (2) solvent phase, hydroxylamine reacted with the activated carbon in the SPE cartridges. NDMA formed at over one order of magnitude greater in the batch reactors with hydroxylamine and dimethylamine compared to those with monochloramine (Table

Table 2. Inorganic nitrogen formed from reactions with 3.52 mM hydroxylamine (NH_2OH), 3.52 mM monochloramine (NH_2Cl), and 35.2 mM dimethylamine ($(CH_3)_2NH$) for various sample treatments

		Aqueous phase concentrations, µg L^{-1} as N Average ± 95% confidence interval		
Reagents	**Treatment**	**Nitrite**	**Nitrate**	**Ammonium**
NH_2OH + $(CH_3)_2NH$ + NH_2Cl	Untreated	1,126 ± 210	1,644 ± 1,441	46,267 ± 1,931
	SAA	ND	2,667 ± 168	49,262 ± 9,516
	$HgCl_2$	1,806 ± 211	2,048 ± 53	7,200 *
	$HgCl_2$ + SAA	ND	2,033 ± 179	40,122 ± 232
	HCl	ND	2,620 ± 77	41,542 ± 2,075
NH_2OH + $(CH_3)_2NH$	Untreated	2,689 ± 130	512 ± 431	4,983 ± 483
	SAA	1,350 ± 143	4,593 ± 691	ND
	$HgCl_2$	15,455 ± 224	ND	5,300 *
	$HgCl_2$ + SAA	ND	ND	ND
	HCl	1,421 ± 293	5,015 ± 271	ND
NH_2OH	Untreated	2,709	5,783	3,021
	SAA	1,522	5,128	ND
	$HgCl_2$	18,356	497	3,486
	$HgCl_2$ + SAA	ND	ND	ND
$(CH_3)_2NH$	Untreated	BDL	BDL	3,873
	SAA	ND	ND	ND
	$HgCl_2$	ND	ND	2,634
	$HgCl_2$ + SAA	ND	ND	ND

BDL – below detection limit
HCl – treated with 100 µL of 1 N HCl
$HgCl_2$ – treated with 100 µL of 20 g L^{-1} mercuric chloride and held in the dark 30 minutes
$HgCl_2$ + SAA – treated with mercuric chloride followed by sulfanilamide
ND – not detected
SAA – treated with 100 µL of 50 g L^{-1} sulfanilamide in 1 N HCl and held in the dark 15 minutes
* One of three samples had detectable concentrations
Method detection limits for nitrite, nitrate, and ammonium were respectively 304-, 226- and 775 µg L^{-1} as N

3). This suggests that excess hydroxylamine was present in the batch reactors without monochloramine and reacted with dimethylamine and dissolved oxygen in the SPE cartridges to form NDMA.

Padhye et al. (2011) showed that N-nitrosamines formed from secondary amines by nitrogen fixation on activated carbon. Additionally, the solvent TONO and NDMA results support the assertion that any nitrite present or formed does not elute from the SPE process, as both were insensitive to treatment type (i.e., *SAA* only or *$HgCl_2$ + SAA* should have quenched nitrite and reduced the TONO and NDMA, but Tukey's tests for the solvent phase showed no difference amongst any treatments). Therefore, following SPE, hydroxylamine and monochloramine are not present and only NDMA and dimethylamine remained.

For the batch reactors with hydroxylamine and dimethylamine, the comparatively low *Untreated* aqueous TONO (1,358 µg L^{-1} as NDMA) further supports the assertion that NDMA formation was catalyzed by the activated carbon in the SPE cartridges. Logically, the majority of this aqueous TONO signal was associated with nitrite (2,689 µg L^{-1} as N, Table 2) that subsequently reacted with *$HgCl_2$ + SAA* to form N-nitrosamines (15,834 µg L^{-1} as NDMA, Table 1, but nitrite was not detected, Table 2). In this case, an alternative NDMA formation mechanism is also plausible, one that does not involve UDMH. Here, hydroxylamine is catalyzed to peroxynitrite ($ONOO^-$) in the presence of dissolved oxygen by $HgCl_2$(Anderson, 1964), and $ONOO^-$ subsequently reacts with dimethylamine to form NDMA.(Masuda et al., 2000)

Conclusions and Recommendations:

In summary, the presence of hydroxylamine presents two problems in assessing total *N*-nitrosamine formation: (1) in the aqueous phase, hydroxylamine is catalyzed by $HgCl_2$ to nitrite and NDMA, and (2) in the solvent phase, hydroxylamine reacts with dimethylamine and is catalyzed to NDMA on the surfaces of the activated carbon in the SPE cartridges. Additional experiments should be done to assess the role of hydroxylamine in *N*-nitrosamine formation at lower molar ratios and longer reaction times to ensure no unreacted hydroxylamine is present in the batch reactors prior to measurement of *N*-nitrosamines by TONO and GC-FID and anions by IC. Further, batch experiments with UDMH will help elucidate other potential NDMA reaction pathways, similar to the one proposed involving peroxynitrite. Further examination of extraction techniques and quenching agents are necessary to eliminate method-derived interferences from the TONO assay and GC-FID measurement of NDMA.

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Baker, L., and S. Entrekin, 2015, Does environmental context mediate stream biological response to anthropogenic stressors?, in UCA College of Natural Sciences and Mathematics, Conway, AR.

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Baker, L., M.A. Evans-White, and S. Entrekin, 2015, Does environmental context mediate anthropogenic stressors?, in Natural Areas Association, Little Rock, AR.

extent of stream biological degradation from human activities?, in Society of Environmental Toxicology and Chemistry Special Session, Denton, TX.

Baker, L., M.A. Evans-White, and S. Entrekin, 2016, How do environmental context and human activities in a stream basin interact to alter macroinvertebrate community structure?, in American Fisheries Society Arkansas State meeting, Fairfield, AR.

Baker, L., M.A. Evans-White, S. Entrekin, 2016, Stream basin physical characteristics and human activities interact to influence aquatic macroinvertebrate responses, in Society of Freshwater Science, Sacramento, CA.

Baker, L., S. Entrekin, and M.A. Evans-White, 2015, Does environmental context mediate stream biological response to anthropogenic stressors?, in American Fisheries Society State meeting, Benton, AR.

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Verkamp, H., L. Baker, M.A. Evans-White, S. Entrekin, 2016, in Society of Environmental Toxicology and Chemistry Special Session, Denton, TX.

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Project Title: Does environmental context mediate stream biological response to anthropogenic impacts?

Project Team: Lucy Baker, Biology Department, University of Central Arkansas
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Executive Summary:

Unconventional natural gas (UNG) requires land-clearing for infrastructure, water withdrawal, and chemicals for hydraulic fracturing that could alter water quality. The degree that UNG development alters nearby stream quality may also depend on stream basin natural characteristics such as slope and soil type. We adapted a multi-metric model that ranks sensitivity and exposure in Fayetteville Shale headwater stream basins. Basin vulnerability is a combination of sensitivity and exposure used to compute relative risk of biological degradation. We predicted macroinvertebrate communities in basins with UNG and pasture would experience greater compositional change across a vulnerability gradient than basins without UNG. We sampled macroinvertebrates in 40 basins over a gradient of vulnerability in streams with UNG and pasture and with pasture only. Macroinvertebrate diversity and percent Ephemeroptera, Plecoptera and Trichoptera (%EPT) declined linearly as vulnerability increased. Erosive soils appeared to be driving this relationship, which may indicate an interaction between soil erodibility and human activities to alter instream habitat. Conversely, macroinvertebrate density and biomass increased and then decreased in an apparent threshold across the same vulnerability gradient. Human disturbances on a landscape are typically associated with increased nutrient inputs that would be expected to support larger organisms but biomass and density declined at a vulnerability score of 260. This decline may be evidence of sub-lethal effects caused by chemical contamination or habitat degradation. Vulnerability explained more variation in macroinvertebrate communities than sensitivity or exposure alone, suggesting an interaction between the landscape natural characteristics and human disturbances. Sensitivity variables, soil erodibility and slope, drove the differences in macroinvertebrate community composition across a vulnerability gradient. In contrast to our hypothesis, all macroinvertebrate metrics responded similarly in basins with and without UNG. Our results suggest that UNG activities alter landscapes and habitat similar to other land uses, mainly pasture. However, the apparent threshold shown in macroinvertebrate biomass and density may be a result of cumulative human activities. As land alteration continues in the Fayetteville Shale, our predictive model could be used to identify basins that are more or less susceptible to degradation and subsequent differences in communities as a tool to protect ecological integrity.

Introduction:

As of 2014, 9,259 kilometers of Arkansas streams were listed as impaired by metals, nutrients, pathogens, or other water quality metric violations, while harboring 183 state-listed species of greatest conservation need (SGCN) (ADEQ, 2014). Unconventional natural gas (UNG) and agriculture as pasture are common human disturbances in the Fayetteville Shale located in north-central Arkansas. UNG and pasture require land development, road construction, and freshwater that could increase sediment, nutrients, and pollutants in streams (Entrekin et al. 2011, Peirre et al. 2015, Poff et al. 1997). Land conversion decreases biodiversity in ecosystems (Turner 2015). A decrease in biodiversity can reduce the resiliency of ecosystems to disturbances and leave biological communities more vulnerable to degradation (Naeem, 2006). Approximately 500 new shale gas wells per year are predicted through

2025 in the Fayetteville Shale (Arkansas Water Plan) and production is estimated to decline in 2030 (Browning et al. 2014). As humans continue to alter landscapes, it is important to identify cumulative human effects to stream biological communities to improve river network water quality and conserve and restore watersheds with the greatest existing biological integrity.

Climate, geology, and topography determine basin natural characteristics that influence stream structure, function, and biological communities. However, anthropogenic disturbances have disrupted these predictable relationships by conversion of natural landscapes, hydrology and natural channel alterations, and climate change acceleration (Dodds et al. 2015). Basin natural characteristics (i.e. slope, soil type) may interact with human stressors to affect the extent of disturbance to stream biota. For example, basins with steeper slopes could experience more runoff and erosion. Basin sensitivity was therefore a combination of environmental characteristics that make a basin more or less resistance or resilient to degradation following disturbance (McCluney et al. 2014) (Appendix A). Basin exposure was landscape-level anthropogenic activities that threaten freshwater systems (Paukert et al. 2010) (Appendix B). Basin vulnerability to disturbance was quantified as the basin sensitivity multiplied with the exposure (Entrekin et al. 2015). A greater vulnerability score indicates a basin more likely degraded or with greater potential for degradation with additional stressors. Basin sensitivity and exposure indices have been developed for analysis of stressor-exposure impacts on streams within basins (Paukert et al. 2010, Matteson and Angermeier 2007, Vorosmarty et al. 2010). However, few empirical studies have tested these models against biological change (i.e. macroinvertebrate communities) in basins with varying natural characteristics and regional stressors (Clapcott et al. 2014).

Our objective was to quantify the macroinvertebrate change along a gradient of vulnerability in basins with UNG and pasture and basins with pasture only. We analyzed macroinvertebrate communities because they have features that reflect landscape-stream connections (Baxter et al. 2005). Macroinvertebrate community metrics can be broken into two categories: compositional and aggregate (Micheli et al. 1999). Compositional metrics include relative abundance and diversity that reflect habitat quality and heterogeneity necessary to support organisms with different physiological traits. We predicted that compositional metrics like percent sensitive taxa (% EPT) and diversity will decrease as vulnerability increases because of greater and more intense disturbances that reduce habitat quality (Figure 1). Aggregate macroinvertebrate metrics include total biomass, and density that reflect resource availability. We predicted that aggregate metrics like total biomass or collector-gatherer density would increase as vulnerability increased because of more nutrient inputs from human activities (Figure 1). We also predicted that basins with UNG and pasture would have a greater extent of community change because of more intense and recent landscape disturbances.

Methods:

Vulnerability Model (Entrekin et al. 2015)

Landscape natural characteristics that influence stream resistance and resilience were identified using literature and available data, then classified as sensitivity variables (Appendix A). We computed sensitivity variables for 140 headwater stream basins in the Fayetteville Shale, AR. Sensitivity variables in each basin were ranked based on a calculated quartile of the cumulative distribution of all 140 basins, where ≤25% were 1, ≤50% were 2, ≤75% were 3 and ≥100% were 4. Precipitation, permeability, and wetland ranks were inversed because basins with less precipitation, less wetlands, and a lower permeability rate were considered more susceptible to biological degradation. Ranks were summed for an overall basin sensitivity score. Common human activities in the Fayetteville shale were identified as exposure variables and categorized similarly to sensitivity scores (Appendix B). Exposure and sensitivity scores were multiplied to generate a vulnerability score (sensitivity x exposure), which described a basin's risk of biological degradation.

Study sites

We sampled 40 streams across north-central Arkansas with basins ranging from 5.9 km^2- 84.5 km^2 (Figure 2). Basins were primarily forested or pasture and 18 basins were exposed to UNG (Appendix C and D). Basins were selected to achieve a gradient of vulnerability and either with UNG and pasture or without UNG. UNG well densities in basins with UNG ranged from 0.04 wells/km^2 – 2.90 wells/km^2

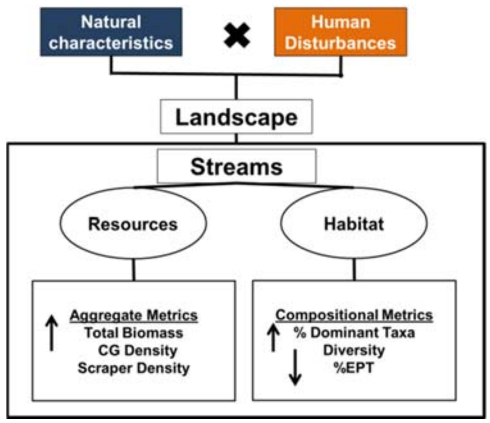

Figure 1: Macroinvertebrate communities reflect altered landscape-stream connections.

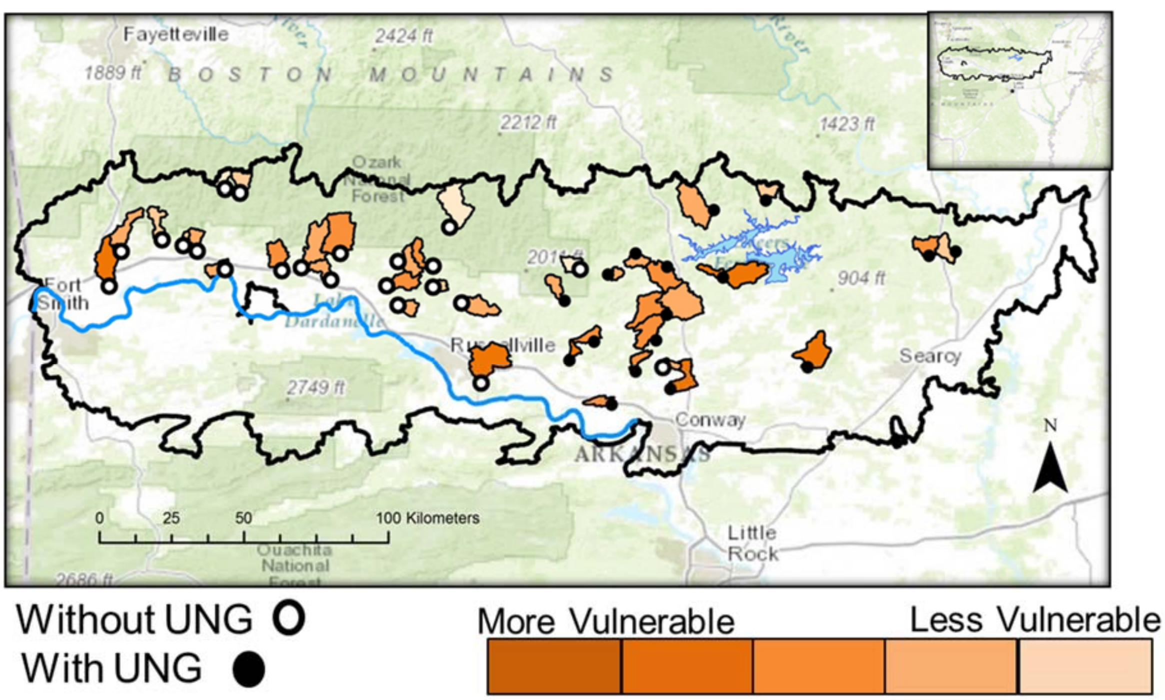

Figure 2: Macroinvertebrates were sampled in 40 headwater streams across a gradient of vulnerability. Twenty-two streams were not exposed to UNG but had a gradient of pasture and 18 streams were had a gradient of UNG with pasture

according to 2015 data from Arkansas Gas and Oil Commission (Appendix D). Basins without UNG had a gradient of pasture and basins with UNG had variable pasture.

Field Sampling

Macroinvertebrates were sampled in 40 streams across a gradient of vulnerability and in basins with UNG and pasture or without UNG. Macroinvertebrates were sampled in streams one time quantitatively using a Hess sampler with a 250*u*m mesh size from May - June 2015. Two consecutive riffles were sampled (3 samples from each riffle). We recorded substrate composition and percent fine sediments at each stream. Macroinvertebrates were stored in 70% ethanol and transported to the laboratory where they were sorted into greater than 1 mm and less than 1 mm size classes. Macroinvertebrates were identified to genera in most cases (Merritt et al. 2008). Habitat variables and water quality metrics such as depth, discharge, pH, conductivity, dissolved oxygen, and substrate composition were recorded at the time of macroinvertebrate sampling. Substrate, canopy cover, riparian zone stability, and embeddedness were estimated over the sampled reach using methods from the Safe Harbor Agreement and Candidate Conservation Agreement with Assurances Habitat Assessment (Earlywine 2014).

Statistical Methods

Macroinvertebrate community compositional and aggregate metrics were compared across a gradient of vulnerability in basins with a gradient of UNG and variable pasture or without UNG and a gradient of pasture using an analysis of covariance (ANCOVA). The covariate was vulnerability and the factors were basins with UNG and pasture or without UNG. If slopes were heterogeneous between sites with and without UNG, separate regression lines were fit. We compared slopes from regressions between exposure, sensitivity, exposure variables, and sensitivity variables that explained variation among vulnerability scores.

Results:

Compositional metrics

All compositional variables responded as we predicted across a vulnerability gradient; however, unlike we predicted, basins with and without UNG responded similarly (Figure 3A, B, and C, Table 1). As stream vulnerability increased the percent dominant taxa increased and ranged from 21% - 85% (Figure 3A). Non-tanypodinae, a tolerant fly larvae, and *Leucrocuta*, a sensitive mayfly, primarily dominated macroinvertebrate communities. As stream vulnerability increased Shannon's diversity decreased linearly regardless of UNG presence (Figure 3B, Table 1). Shannon's diversity typically ranges from 1.5 - 3.5, representing high biodiversity in natural systems (MacDonald 2003). Diversity in sampled streams ranged from 0.5 – 3.0. Percent EPT ranged from 2% - 73%, and were dominated by a combination of sensitive and tolerant mayflies, sensitive stoneflies, and tolerant caddisflies (Figure 3C, Appendix E). Neither exposure nor sensitivity alone explained as much variation as vulnerability in any compositional metrics. However, sensitivity variables, mainly kfactor, slope, and precipitation explained significant variation among all compositional metrics. Exposure variable, percent crop explained variation in diversity, despite little crop in any study basins (Mattson and Angermeir 2007). Percent EPT decreased across an exposure gradient primarily from greater pasture. Percent dominant taxa were not significantly associated with any exposure variables.

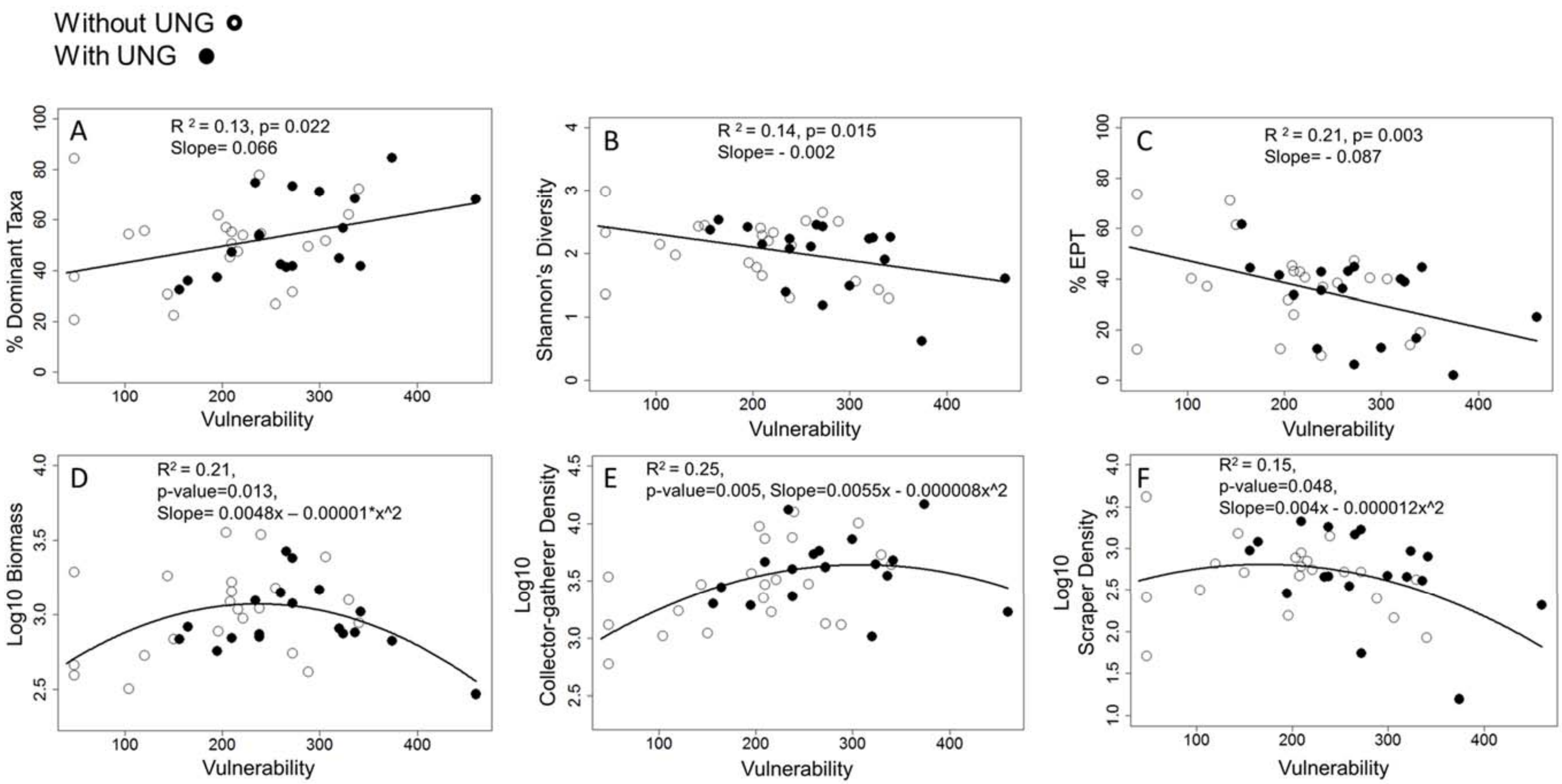

Figure 2: Compositional metrics were analyzed across a gradient of vulnerability in sites with and without UNG. (A) Percent dominant taxa in streams responded similarly to vulnerability in basins with UNG and pasture and pasture alone. As vulnerability increased, the percent dominant taxa or unevenness in the community increased. (B) Shannon's diversity in streams responded similarly to vulnerability despite the presence of UNG. However, vulnerability did explain a significant amount of variation in stream diversity. (C) Percent EPT taxa significantly decreased as vulnerability increased regardless of UNG activity. (D) Total biomass responded similarly in streams with UNG and pasture and pasture alone. As vulnerability increased, total biomass increased to an apparent threshold at a vulnerability score of 260. (E) Collector-gatherer density responded similarly in basins with UNG and pasture and with pasture alone. Collector-gatherer density increased as vulnerability increased until an apparent threshold at about 300. (F) Scraper density responded similarly in basins with UNG and pasture and pasture alone. As vulnerability increased, scraper density increased very little until a vulnerability score of 260 where density decreased.

Aggregate Metrics

All aggregate metrics increased to a point and then decreased at a vulnerability score of approximately 260 (Figure 3D, E, and F). There were no differences in responses between sites with pasture and UNG and pasture only (Table 1). Neither sensitivity nor exposure explained significant variation in total biomass; however, vulnerability did (Figure 3D). Sites with a score of ≤ 260 were dominated by more sensitive organisms and sites with a vulnerability score > 260 had communities dominated by more tolerant organisms (Appendix F). Both sensitivity and exposure significantly predicted variation in collector-gatherer density; however, exposure appears to be driving the apparent threshold (R^2= 0.12, p=0.027, R^2= 0.14, p=0.018, respectively). Scraper density, predominantly two mayflies, increased little and then decreased at vulnerability score of about 260 (Figure 3F, Appendix F). Neither sensitivity nor exposure explained a significant amount of variation in scraper density across streams. Sensitivity variables, soil erodibility, percent wetland, slope, and precipitation explained some variation in scraper density. Exposure variables crop and pasture were the main contributors to the vulnerability/ aggregate metrics relationships.

Conclusions and Recommendations:

Streams in the Fayetteville Shale harbor 48 aquatic species of greatest conservation need (USFWS, 2015). Our vulnerability model predicted % dominant taxa, macroinvertebrate diversity, % EPT taxa, total biomass, collector-gatherer density, and scraper density. Vulnerability described more variation in compositional and aggregate metrics than sensitivity or exposure alone, suggesting an interaction between the landscape natural characteristics and human disturbances. Compositional metrics and aggregate metrics were associated with both exposure and sensitivity variables highlighting the importance of multi-metric models in understanding the relationship between landscapes and streams and to describe the potential for biological degradation (Paukert et al. 2011, Truchy et al. 2015).

Table 1: Analysis of Covariance was used to test the heterogeneity of slopes in sites with UNG and pasture and pasture alone. If sites were homogenous, exposure types were combined and a regression line was fit. Biomass and functional feeding group densities were fit with a polynomial regression because of an apparent threshold effect.

Variable	Factor	F	df	P-value	Exposure Type	Slope	P-value	R^2
% Dominant Taxa	Vulnerability	2.5	3,36	0.017	Combined	0.07	0.02	0.13
	With/Without UNG	0.46		0.646				
	Interaction	N.A.						
Diversity	Vulnerability	-2.62	3,36	0.013	Combined	-0.002	0.015	0.14
	With/Without UNG	-0.66		0.514				
	Interaction	N.A.						
% EPT	Vulnerability	-2.85	3,36	0.007	Combined	-0.087	0.003	0.21
	With/Without UNG	-0.2		0.843				
	Interaction	N.A.						
Total Biomass	Vulnerability	0.33	3,36	0.746	Combined	0.0048x-0.00001x^2	0.013	0.21
	With/Without UNG	0.62		0.536				
	Interaction	N.A.						
Collector-Gatherer Density	Vulnerability	1.91	3,36	0.065	Combined	0.0055x – 0.000008x^2	0.005	0.25
	With/Without UNG	-0.39		0.698				
	Interaction	N.A.						
Scraper Density	Vulnerability	-2.18	3,36	0.036	Combined	0.004x – 0.000012x^2	0.048	0.15
	With/Without UNG	-1.22		0.23				
	Interaction	N.A.						

We found that there were no significant differences in macroinvertebrate community metrics between basins exposed to UNG and pasture and basins exposed to a gradient of pasture only, meaning that UNG activity is stressing landscapes similarly to other human activities. However, we have observed an apparent threshold (~260), where cumulative human disturbances may be having sub-lethal effects and inhibiting biomass accrual. We have also identified natural characteristics that appear to be most important in a basin's resistance and resilience to disturbance in the Arkansas River Valley region, including kfactor, slope, precipitation, and percent wetland. We saw two functionally different responses between compositional metrics (linear) and aggregate metrics (parabolic) that may reflect the extent and type of degradation.

Compositional metrics decreased linearly with vulnerability, suggesting that more vulnerable basins experience greater stream habitat degradation (Figure 1). We found that soil erodibility, slope, and precipitation explained the most variation in compositional metrics. Slope explained significant amounts of variation but the relationship was opposite of what we expected. We predicted that steeper slopes would make a basin more sensitive to degradation because of flashier hydrology and less time for water and contaminants to infiltrate. However, diversity, and % EPT taxa increased with greater slopes and percent dominant taxa decreased with greater slopes. This may be due to an increase in habitat heterogeneity caused by varying substrate deposition and flow patterns (Statzner and Higler 1986). We also found a strong positive correlation between percent forest and slope in sampled basins (r=0.82).

Macroinvertebrate aggregate metrics increased across a vulnerability gradient and then declined, suggesting that there is a point where landscape disturbance negates the positive effects of increased nutrients on biomass and density (Figure 1). We found that total biomass, collector-gatherer density, and scraper density increased across a vulnerability gradient; however, these metrics began to decrease when vulnerability reached 260. Aggregate metrics were primarily driven by sensitivity variables, specifically percent wetlands, slope, and kfactor. However, sensitivity variable/ aggregate metric relationships were linear and did not appear to be the sole cause of the threshold effect. The sharp decrease in aggregate metrics may be caused by an increase in chemical contamination or organic pollution that inhibits biomass accrual (Woodcok and Huryn 2006, Entrekin et al. 2011). Increased salt concentrations in streams may have sub-lethal effects and inhibit organismal growth (Tyree et al. 2016).

Diversity of a community is determined first at a regional scale, where dispersal barriers and evolutionary events influence the organisms found within a region. Macroinvertebrate alpha diversity in our basins was determined at the local scale and represent species that were able to persist through a particular disturbance regime (Ricklefs 2004, Naeem 2006). Diversity decreased as vulnerability increased, suggesting that our vulnerability model may represent a gradient of disturbance intensity. Human activities and natural characteristics quantified as exposure and sensitivity can be described as disturbances to stream because they represent an event or events that have a frequency, intensity, and severity outside of predictable range (Resh et al., 1988). Aggregate metrics in our study exhibit functional responses that support the intermediate disturbance hypothesis, where greater vulnerability or disturbance increases stream productivity to a point when taxa loss occurs (Connell 1978).

Our results suggest that UNG extraction alone does not cause greater habitat or resource alterations that would create a different functional response in macroinvertebrate communities than other human disturbances (i.e. pasture). Surprisingly, percent crop and pasture were the only exposure variable that explained significant variation in macroinvertebrate metrics. UNG production has slowed in the Fayetteville Shale since 2012 from about 30 average rig counts to about 7 average rig counts (Arkansas Oil and Gas Commission). Less UNG development may reflect a decrease in associated UNG activities, like traffic, land clearing, and potential chemical spills. However, UNG extraction added to preexisting human activities could be playing an important role in the aggregate metrics threshold response.

Recommendations

We have identified stream basins that have natural landscape characteristics and anthropogenic stressors that make them vulnerable to biological degradation. We recommend our model be used to help USGS and resource managers decide where new development should be avoided to maintain ecological integrity (Appendix G). Basins that have a suite of less sensitive landscape natural characteristics may respond better to restoration projects. For example, a stream draining a basin with a low kfactor, steep slopes, and surrounding wetlands would be more suitable for restoration or conservation than a basin with a high kfactor, low slopes, and few surrounding wetlands. Finally, we recommend that there be more investigation on the sub-lethal effects of cumulative human disturbances. The apparent threshold responses of aggregate metrics suggest that cumulative human disturbances and more sensitive natural characteristics are interacting to alter a predictable resource/biomass response.

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Appendix A: Landscape natural characteristics that describe the susceptibility of a stream basin to degradation from human disturbances were identified using literature and available data. Natural characteristics were classified as sensitivity variables.

Sensitivity Variables	**Description**	**Data Source**
Average slope (degree)	A larger slope increases basin sensitivity because water moves over landscapes faster and carries nutrients and contaminants	Slope raster calculated from the 100 m DEM in ArcGIS.
Soil erodibility factor (k factor)	K factor is a measure of erosive capability of a soil; therefore, the higher the k factor the more sensitive a basin.	STATSGO soils data for the Conterminous United States; http://water.usgs.gov/GIS/metadata/usgswrd/31XML/ussoils.xml
Drainage density of NHDplus flowlines (km/km^2)	More streams per area in a basin increase sensitivity because of a higher probability of contamination reaching a flow path.	NHDplus data; http://www.horizonsystems.com/nhdplus/
% Wetlands (NLCD class 90 &95)-Inversed	Greater percent of wetlands increase sensitivity because greater connectivity to streams and greater undeveloped land.	2006 NLCD datasets; http://www.mrlc.gov/nlcd06_data.php
Precipitation (mm)-Inversed	More precipitation makes a basin less sensitive because the ecosystem was not stressed by lack of water.	PRISM 30 year normal; http://www.prism.oregonstate.edu/normals/
Soil Permeability (inches/hour)-Inversed	The lower the average permeability rate the more sensitive a basin is to degradation because of greater runoff.	STATSGO soils data for the Conterminous United States; http://water.usgs.gov/GIS/metadata/usgswrd/31XML/ussoils.xml

Appendix B: Human disturbances that threaten aquatic biological communities in the Fayetteville Shale. Disturbance variables are classified as exposure variables.

Exposure Variable	**Description of associated disturbance**	**Data Source**
Well density (vertical wells)	Disturbances caused by roads, well pad, and pipelines.	Arkansas Oil and Gas Commission (ftp://www.aogc2.state.ar.us/GIS_Files/)
Well density (nonvertical wells)	Disturbance caused by roads, well pads, water withdrawal, contaminates, and pipelines	Arkansas Oil and Gas Commission (ftp://www.aogc2.state.ar.us/GIS_Files/)
Dam Density (#/km^2)	Flow and nutrient restrictions	NID dataset: http://nid.usace.army.mil/cm_apex/f?p=838:12
Mine Density (#/km^2)	Disturbance from construction and roads	USGS Mineral Resources Database: http://mrdata.usgs.gov/mrds/
Road density (km/km^2)	Contaminants from vehicles and increased impervious surfaces	TIGER 2010 Streets; http://datagateway.nrcs.usda.gov/GDGOrder.aspx?order=QuickState
% Impervious surfaces	Decreased water infiltration causing flashier systems and increased nutrients	2006 NLCD datasets; http://www.mrlc.gov/nlcd06_data.php
% Pasture	Habitat alteration, increased nutrients and sediments	2006 Arkansas Land Use dataset http://gis.arkansas.gov/?product=land-use-land-cover-fall-2006-raster
% Row crop	Habitat alteration, increased nutrients and sediments	2006 Arkansas Land Use dataset http://gis.arkansas.gov/?product=land-use-land-cover-fall-2006-raster

Appendix C: Sensitivity variables calculated for each sampled basin.

HUC12	Stream Name	Permeability	Precipitation	Stream density	% Wetland	Slope	Kfactor
80203010301	Bayou Des Arc	1.41	1280.11	2.00	0.01	4.35	0.27
111102030102	Beardy Branch	1.72	1298.39	1.70	0.00	2.60	0.29
110100140204	Butler Creek	1.72	1316.33	1.52	0.19	5.67	0.27
111102020806	Cedar Creek (Big River)	1.01	1275.95	2.28	0.00	3.58	0.28
111102050106	Cedar Creek (Cove Creek)	1.26	1284.29	1.82	0.05	5.30	0.32
110100140601	Choctaw Creek	1.72	1316.33	1.64	0.01	6.09	0.27
111102010904	Cravens	1.72	1329.66	1.68	0.00	5.68	0.27
111102050202	Creben Creek	1.24	1267.14	3.01	0.06	5.15	0.34
110100130401	Departee Creek	1.40	1252.88	1.11	0.00	4.74	0.25
111102020202	Dirty Creek	1.41	1299.88	1.34	0.03	6.06	0.32
111102030101	Driver Creek	1.61	1302.79	1.32	0.00	8.76	0.24
111102020204	EF Horsehead	1.29	1307.99	1.73	0.17	7.97	0.30
111102010704	Fane Creek	1.61	1395.44	1.45	0.00	16.59	0.24
111102030303	Galla Creek	1.20	1308.75	1.74	0.07	4.06	0.32
111102030504	Gap Creek	1.46	1257.32	1.26	0.11	5.43	0.32
111102010905	Gar Creek	1.34	1270.32	1.49	0.00	4.37	0.33
111102020705	Granny	1.50	1276.37	1.73	0.00	6.69	0.30
111102050203	Greenbrier Creek	1.38	1262.30	1.94	1.10	3.01	0.32
110100140506	Hill Creek	1.40	1298.41	1.63	0.00	9.11	0.25
111102050104	Hogan	1.17	1298.78	2.11	0.03	5.03	0.32
111102020802	Indian Creek	1.61	1371.43	1.39	0.00	16.57	0.24
111102050107	Jacks Fork	1.14	1262.30	1.03	3.81	3.87	0.32
111102010504	Little Froggy Bayou	1.83	1271.98	1.48	0.02	3.60	0.33
111102010805	Little Mulberry	2.11	1346.74	1.40	0.06	7.58	0.27
111102020303	Little Spadra	1.31	1272.41	1.59	0.14	5.26	0.33
111102010802	Maxie Creek	0.99	1329.66	1.24	0.00	5.93	0.29
111102021001	McCoy Creek	1.38	1280.09	1.60	0.01	5.91	0.28
111102020804	Mill Creek	1.53	1286.03	1.59	0.03	8.07	0.26
111102010801	Mill Mulberry	1.50	1379.33	1.61	0.00	11.31	0.27
111102010703	Mountain Creek	1.61	1394.66	1.37	0.00	15.60	0.24
111102050101	North Fork Cadron	1.72	1298.84	1.87	0.01	5.04	0.27
111102050105	Pine Mountain	1.38	1303.32	1.62	0.08	4.03	0.30
111102030204	Pool Hollow	1.34	1265.97	2.10	12.69	4.26	0.31
111102030203	Prairie Creek	1.36	1274.13	1.95	0.12	2.42	0.29
111102030105	Rock Creek	1.44	1295.27	1.66	0.00	7.32	0.30
111102020704	Slover Creek	1.50	1276.49	1.63	0.00	6.11	0.30
111102020301	Spadra Creek	1.45	1339.40	1.64	0.03	11.74	0.30
110100140901	Tenmile Creek	1.50	1253.48	1.19	0.02	3.04	0.25
110100140403	Weaver Creek	1.40	1302.61	1.65	0.06	8.91	0.25
111102020805	Wilson Creek	1.44	1262.39	1.24	0.00	8.18	0.29

Appendix D: Exposure variables for sampled basins

HUC12	Stream	% Urban	% Crop	% Pasture	UNG Density	Mine Density	Dam Density	Vertical Well Density	Road Density	Impervious Surfaces
80203010301	Bayou Des Arc	1.09	0.00	55.32	1.04	0.00	0.11	0.00	4.31	0.32
111102030102	Beardy Branch	3.18	0.00	44.56	2.40	0.00	0.40	0.00	6.48	0.64
110100140204	Butler Creek	1.59	0.00	24.31	3.00	0.00	0.21	0.00	8.13	0.14
111102020806	Cedar Creek (Big River)	2.08	0.00	38.90	0.00	0.00	0	0.00	5.64	0.36
111102050106	Cedar Creek (Cove Creek)	1.73	0.00	35.04	2.11	0.00	0.25	0.00	5.96	0.08
110100140601	Choctaw Creek	2.37	0.00	28.58	2.93	0.00	0.24	0.00	7.59	0.33
111102010904	Cravens	0.68	0.00	35.44	0.00	0.00	0	0.00	3.25	0.02
111102050202	Creben Creek	3.38	0.00	27.68	0.25	0.00	0.18	0.04	3.72	0.14
110100130401	Departee Creek	4.20	0.00	38.86	0.41	1.00	0.03	0.00	7.18	0.70
111102020202	Dirty Creek	2.00	0.01	41.69	0.00	0.00	0	0.57	8.72	3.41
111102030101	Driver Creek	0.00	0.00	0.45	0.00	0.00	0	0.12	4.64	0.04
111102020204	EF Horsehead	1.74	0.00	31.29	0.00	0.00	0	0.00	3.25	0.02
111102010704	Fane Creek	0.00	0.00	0.44	0.00	0.00	0	0.04	3.72	0.14
111102030303	Galla Creek	11.74	0.01	38.04	0.00	0.00	0	0.85	6.21	0.06
111102030504	Gap Creek	23.67	0.01	29.13	0.06	0.00	0	0.05	4.78	0.34
111102010905	Gar Creek	23.63	0.00	35.67	0.00	0.00	0	1.43	9.53	2.12
111102020705	Granny	1.46	0.00	18.92	0.00	0.00	0	0.48	8.92	0.51
111102050203	Greenbrier Creek	25.15	0.00	34.25	0.19	0.00	0	0.83	6.40	0.54
110100140506	Hill Creek	0.88	0.00	31.10	0.04	0.00	0	0.33	6.19	0.26
111102050104	Hogan	2.68	0.00	34.12	2.40	0.00	0.19	0.55	18.98	0.19
111102020802	Indian Creek	0.44	0.00	0.48	0.00	0.00	0	0.96	4.16	0.13
111102050107	Jacks Fork	4.39	0.00	51.01	0.00	1.00	0	0.17	3.35	0.21
111102010504	Little Froggy Bayou	26.63	0.00	47.34	0.00	0.00	0	0.00	2.47	0.04
111102010805	Little Mulberry	2.84	0.00	33.60	0.00	0.00	0	0.60	6.41	0.22
111102020303	Little Spadra	5.09	0.00	46.31	0.00	0.00	0	0.58	3.97	0.04
111102010802	Maxie Creek	0.19	0.00	31.90	0.00	0.00	0	0.80	4.70	0.08
111102021001	McCoy Creek	3.05	0.00	36.95	0.00	0.00	0	1.06	4.60	0.29
111102020804	Mill Creek	0.44	0.00	10.98	0.00	0.00	0	0.00	11.59	0.18
111102010801	Mill Mulberry	0.56	0.00	5.55	0.00	0.00	0	0.00	5.71	0.09
111102010703	Mountain Creek	0.66	0.00	2.24	0.00	0.00	0	0.00	8.46	0.22
111102050101	North Fork Cadron	1.47	0.00	31.51	2.90	2.00	0.35	0.00	13.08	0.18
111102050105	Pine Mountain	4.93	0.00	49.96	2.59	2.00	0.26	0.05	4.21	1.51
111102030204	Pool Hollow	2.85	0.00	40.15	1.29	0.00	0.18	0.06	27.39	1.67
111102030203	Prairie Creek	1.74	0.00	64.81	2.08	1.00	0.15	0.00	4.20	0.21
111102030105	Rock Creek	0.10	0.00	3.90	0.14	0.00	0	0.05	8.91	0.28
111102020704	Slover Creek	1.10	0.00	34.46	0.00	0.00	0	0.00	5.57	0.50
111102020301	Spadra Creek	0.83	0.00	16.33	0.00	0.00	0	0.00	6.87	0.28
110100140901	Tenmile Creek	4.75	0.00	50.45	0.48	1.00	0.27	0.00	5.55	0.12
110100140403	Weaver Creek	1.81	0.00	21.26	0.49	0.00	0.05	0.06	7.76	0.45
111102020805	Wilson Creek	0.87	0.00	26.53	0.00	2.00	0	0.05	9.23	1.21

Appendix E: Density and biomass for each taxa found, sorted by functional feeding group

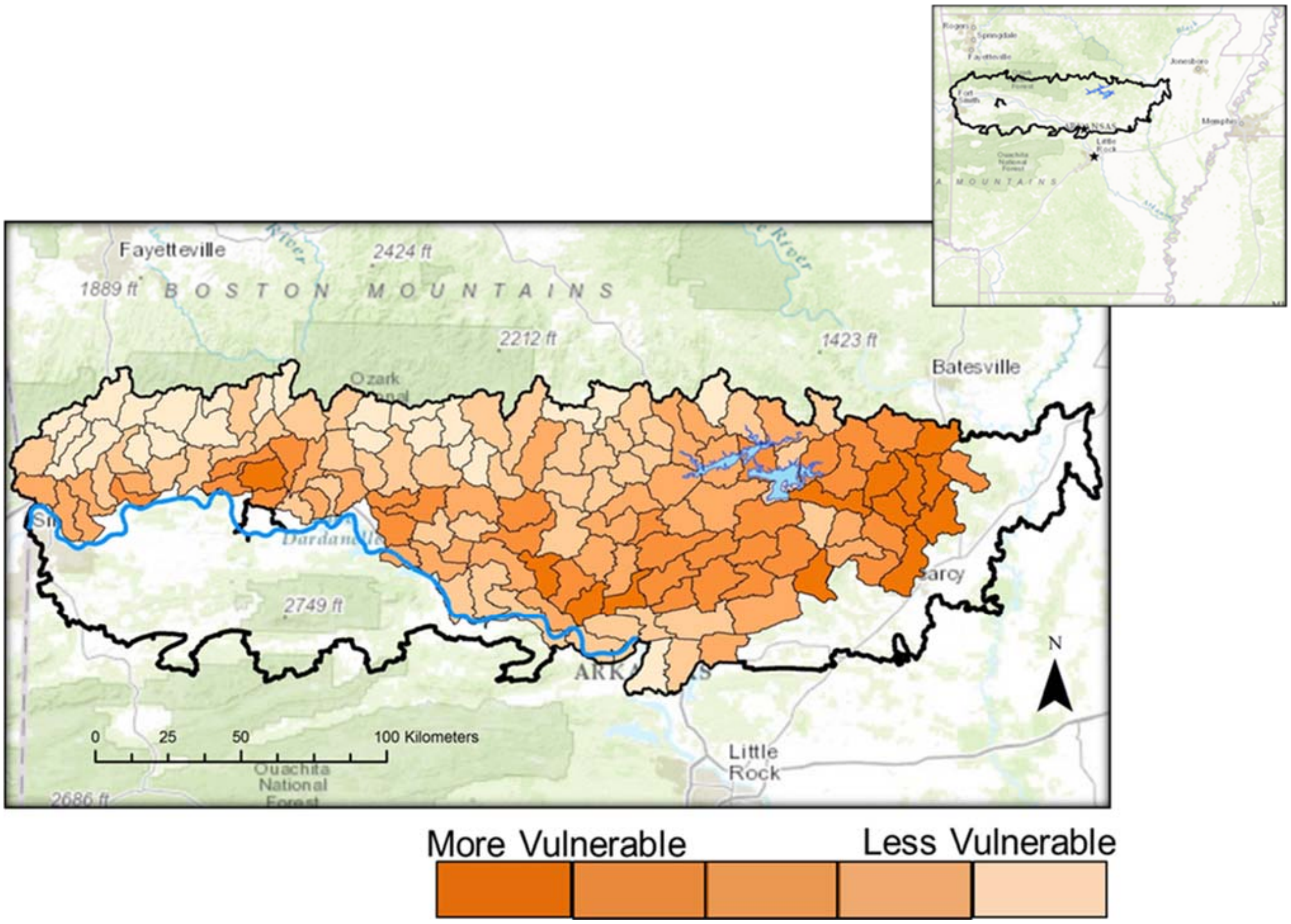
FFG	Order	Family	Genus	Density	Biomass
Collector-gatherer	Coleoptera	Elmidae	Ancyronyx	11.76	5.75
			Dubiraphia	100.00	99.44
			Dubiraphia (A)	94.12	87.94
			Elmidae	188.24	1.38
			Optioservus	43.79	19.48
			Optioservus (A)	11.76	22.85
			Ordobrevia	120.22	113.15
			Ordobrevia (A)	14.12	17.94
			Stenelmis	313.90	144.90
	Diptera	Chironomidae	Non-Tanypodinae	3564.55	126.71
		Ephydridae	Ephydridae	53.70	1.74
	Ephemeroptera	Baetidae	Acentrella	223.53	113.37
			Baetidae	41.18	10.86
			Baetis	351.50	52.25
			Plauditus	94.12	44.69
		Caenidae	Caenis	194.60	23.21
		Ephemerellidae	Ephemerella	11.76	4.55
			Ephemerellidae	11.76	4.92
		Ephemerellidae	Eurylophella	82.35	27.04
			Serratella	146.78	70.40
		Heptageniidae	Rhithrogena	108.24	210.03
			Stenacron	106.95	87.87
		Leptophlebiidae	Leptophlebia	154.95	36.46
			Leptophlebiidae	137.32	6.00
			Paraleptophlebia	157.25	52.49
		Tricorythidae	Tricorythodes	111.98	15.07
	Plecoptera	Chloroperlidae	Alloperla	14.71	6.23
	Trichoptera	Leptoceridae	Leptoceridae	69.68	0.67
			Mystacides	11.76	0.37
			Setodes	11.76	0.37
			Triaenodes	66.05	1.01
		Psychomyiidae	Psychomyia	11.76	16.50
Collector-filterer	Diptera	Simuliidae	Prosimulium	418.75	16.04
		Simuliidae	Simulium	26.14	3.21
	Ephemeroptera	Isonychiidae	Isonychia	93.67	100.17
	Trichoptera	Brachycentridae	Brachycentrus	23.53	76.69
		Hydropsychidae	Cheumatopsyche	589.85	93.93
			Hydropsyche	28.05	198.49
		Philopotamidae	Chimarra	90.18	16.02
			Wormaldia	23.53	36.73
Scraper	Coleoptera	Curculionidae	Curculionidae (A)	11.76	4.45
		Elmidae	Stenelmis (A)	43.92	63.90
		Lampyridae	Lampyridae	11.76	15.98
		Psephenidae	Psephenus	68.74	48.12
		Scirtidae	Scirtidae	11.76	0.41
	Ephemeroptera	Baetidae	Heterocloeon	358.16	23.37
		Heptageniidae	Heptageniidae	127.06	23.43
			Leucrocuta	464.65	128.91
			Stenonema/Maccaffertium	160.58	66.00
	Hemiptera	Corixidae	Corixidae	11.76	2.03
	Lepidoptera	Crambidae	Crambidae	53.54	1.91
	Trichoptera	Glossosomatidae	Agapetus	97.35	10.38
		Helicopsychidae	Helicopsyche	39.01	36.03

		Hydroptilidae	Hydroptila	231.79	19.54
		Phryganeidae	Phryganeidae	11.76	3.11
Predator	Coleoptera	Dytiscidae	Agabus	11.76	59.97
			Dytiscidae	19.61	8.27
			Hygrotus	11.76	10.18
			Laccophilus	11.76	0.41
			Oreodytes	24.62	4.61
		Gyrinidae	Dineutus	21.67	114.08
			Gyretes	11.76	5.97
			Gyrinidae	11.76	0.05
		Hydrochidae	Hydrochus	11.76	0.97
		Hydrophilidae	Berosus	11.76	2.72
	Diptera	Ceratopogonidae		44.52	7.26
		Chironomidae	Tanypodinae	298.30	23.46
		Empididae	Empididae	25.55	0.31
			Hemerodromiinae	30.00	2.65
		Tabanidae	Chrysops	14.71	85.25
			Silvius	15.69	170.71
		Tipulidae	Hexatoma	37.51	105.25
			Pilaria	21.24	7.77
	Megaloptera	Corydalidae	Corydalus	13.57	1299.01
			Nigronia	21.05	20.51
		Sialidae	Sialis	85.78	5.03
	Odonata	Aeshnidae	Aeshna	11.76	3.42
			Aeshnidae	24.51	28.00
		Calopterygidae	Calopterygidae	11.76	215.99
		Coenagrionidae	Amphiagrion	17.99	41.44
			Argia	22.22	119.98
		Corduliidae	Corduliidae	11.76	142.85
		Gomphidae	Arigomphus	15.69	86.79
			Gomphidae	11.76	104.76
			Stylogomphus	26.24	9.98
			Stylurus	11.76	1.15
	Plecoptera	Chloroperlidae	Chloroperlidae	11.76	0.08
			Haploperla	194.36	125.01
		Perlidae	Acroneuria	23.53	30.21
			Agnetina	20.17	89.67
			Hansonoperla	83.82	13.14
			Neoperla	101.68	92.36
			Perlesta	77.20	53.74
			Perlidae	64.71	7.66
			Perlinella	14.12	27.27
		Perlodidae	Isoperla	40.00	58.59
	Trichoptera	Polycentropodidae	Polycentropus	44.39	23.23
Shredder	Diptera	Tipulidae	Tipula	30.10	1081.06
			Tipulidae	117.65	1.56
	Plecoptera	Capniidae	Allocapnia	279.89	57.71
			Capniidae	17.65	5.96
		Leuctridae	Paraleuctra	82.35	23.80
		Nemouridae	Amphinemura	13.45	9.21
			Nemouridae	11.76	14.14
		Taeniopterygidae	Taeniopteryx	47.06	0.34
	Trichoptera	Lepidostomatidae	Lepidostoma	11.76	41.18
		Limnephilidae	Hydatophylax	15.13	355.68
			Limnephilidae	11.76	0.36

Appendix F: Macroinvertebrates density and biomass from 40 streams in Arkansas.

Latitude	Longitude	HUC12	Stream Name	Density (# organisms m^{-2})	Biomass (mg $m^{-2)}$
-92.025701	35.2498	80203010301	Bayou Des Arc	6492.81	1042.85
-92.645702	35.485183	111102030102	Beardy Branch	16084.31	1249.82
-92.559717	35.537984	110100140204	Butler Creek	3501.57	742.05
-93.298226	35.407453	111102020806	Cedar Creek (Big River)	12417.65	1652.91
-92.496839	35.318065	111102050106	Cedar Creek (Cove Creek)	9072.55	2669.54
-92.462544	35.50462	110100140601	Choctaw Creek	7081.70	2398.05
-93.922742	35.545037	111102010904	Cravens Creek	2858.82	1083.35
-92.560013	35.238779	111102050202	Creben Creek	9227.45	1466.11
-91.565672	35.543021	110100130401	Departee Creek	5260.78	826.66
-93.659802	35.495409	111102020202	Dirty Creek	17186.27	2451.72
-92.73289	35.499182	111102030101	Driver Creek	1456.21	392.14
-93.599332	35.503273	111102020204	East Fork Horsehead	17788.24	3447.72
-93.83645	35.701662	111102010704	Fane Creek	8525.49	1931.71
-93.040002	35.209904	111102030303	Galla Creek	5637.91	878.11
-92.634528	35.154717	111102030504	Gap Creek	5323.53	1188.13
-93.836243	35.498286	111102010905	Gar Creek	9135.29	1102.69
-93.298079	35.518212	111102020705	Granny Creek	4455.82	1427.60
-92.451923	35.194621	111102050203	Greenbrier Creek	17531.37	666.85
-92.155868	35.672478	110100140506	Hill Creek	3695.42	683.11
-92.46436	35.382667	111102050104	Hogan's Creek	6323.53	744.03
-93.138069	35.605472	111102020802	Indian Creek	1407.84	458.29
-92.475542	35.249278	111102050107	Jacks Fork	4364.71	774.16
-94.196414	35.455981	111102020303	Little Froggy Bayou	6368.63	1259.47
-94.159299	35.544052	111102010504	Little Mulberry	3521.57	1225.43
-93.507553	35.471989	111102010805	Little Spadra	15325.49	3581.61
-93.966732	35.558363	111102010802	Maxie Creek	2699.35	534.02
-93.100479	35.41357	111102021001	McCoy Creek	4403.92	946.50
-93.189774	35.507045	111102020804	Mill Creek	2062.09	553.37
-94.032149	35.573469	111102010801	Mill Creek (Mulberry River)	1529.41	319.85
-93.789667	35.691643	111102010703	Mountain Creek	7907.84	1817.37
-92.288264	35.477223	111102050101	North Fork Cadron	4654.90	762.84
-92.463344	35.38267	111102050105	Pine Mountain	8320.26	695.33
-92.766245	35.266721	111102030204	Pool Hollow	2298.04	295.64
-92.689253	35.314939	111102030203	Prairie Creek	2041.18	804.58
-92.781321	35.418116	111102030105	Rock Creek	2771.90	571.51
-93.333775	35.456683	111102020704	Slover Creek	4195.42	1501.40
-93.478487	35.53922	111102020301	Spadra Creek	1988.24	414.11
-91.648085	35.532217	110100140901	Tenmile Creek	6805.88	1409.98
-92.317331	35.647752	110100140403	Weaver Creek	6517.65	706.48
-93.189132	35.453684	111102020805	Wilson Creek	1856.32	687.48

Appendix G: We calculated vulnerability scores for Hydrologic Unit Code 12 (HUC12) in the Fayetteville Shale region and in the Arkansas River Valley.



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Principal Investigator:	David M. Miller

Publications and Presentations:

Burgess-Conforti, J., 2015, Liming characteristics of a Class-C fly ash and a high calcium dry flue gas desulfurization by-product, in Agronomy Society of America, Minneapolis, MN.

Burgess-Conforti, J. and D.M. Miller, 2016 (expected), Liming characteristics of a Dry Flue-Gas-Desulfurization By-product and its Effect on Runoff Water Quality, MS Theis, Department of Crop, Soils, and

Project Title: Runoff Water Quality from Managed Grassland Amended with a Mixed Coal Combustion Byproduct

Project Team: Jason R. Burgess-Conforti, Department of Crop, Soils, and Environmental Science, University of Arkansas
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Executive Summary:

Millions of megagrams of coal combustion byproducts (CCBs) are produced annually in the United States. Certain CCBs have physical and chemical characteristics that provide potential for use as a soil amendment. The objective of this experiment was to examine the effect of land application of a dry flue gas desulfurization (DFGD) by-product on runoff water quality. Dry FGD by-product was applied to a managed grassland in May of 2015 and trace element concentrations in runoff water were measured following each runoff-producing event for a 2-mo period. There were no significant differences in cumulative runoff volume or cumulative loading of As, Be, Cd, Co, Cr, Cs, Cu, Ni, Pb, Rb V, and U between the amended and unamended plots. Cumulative loading of Se was significantly higher in amended plots compared to the unamended control. Additional research is needed to fully understand the environmental impacts of land applying coal combustion by-products, but our results suggest that runoff water quality from DFGD-amended grassland is very similar to that from unamended grassland.

Introduction:

The 1990 Clean Air Act Amendments mandated a reduction in SO_2 emissions from coal-fired power plants, resulting in installation of flue gas scrubbers and the production of flue gas desulfurization (FGD) by-products. Flue gas desulfurization by-products are produced when a calcitic sorbent is injected into flue gases to trap and remove SO_2. Dry FGD emission control systems often combine FGD by-products and coal fly ash (siliceous particulate matter produced when coal is burned) together resulting in a by-product with characteristics different from those of fly ash alone or wet FGD by-products. Dry FGD by-products are often a mixture of fly ash, unreacted sorbent, calcium sulfite ($CaSO_3$• 0.5 H_2O), and calcium sulfate ($CaSO_4$• $2H_2O$) (Kost et al., 2005). Dry FGD by-products are typically alkaline and may potentially be beneficially reused as a soil amendment to raise soil pH. Dry FGD by-products also contain plant essential nutrients such as Ca, S, K, Mg, P, B and Zn and can be used as a soil amendment for increasing soil nutrient concentrations.

In 2008, only 8.3% of DFGD by-products were beneficially reused, which left 1.5 million megagrams to be disposed of in surface impoundments and landfills (ACAA, 2008). Coal combustion by-products contained in landfills and surface impoundment pose significant environmental contamination risks. If it were to be shown that DFGD by-products can be utilized as a soil amendment without adversely affecting the environment, more DFGD by-products might be utilized beneficially. The purpose of this experiment was to monitor the effect of land application of a DFGD by-product to a managed grassland on runoff quality over a 2-mo period.

Materials and Methods:

Six plots, 6-m long by 1.5-m wide, were located at the University of Arkansas Agricultural Research and Extension Center in Fayetteville, Arkansas on a 5% west-to-east slope. The research plots were located in an area mapped as a Captina silt loam (fine-silty, siliceous, active, mesic Typic Fragiudult; Table 1). Aluminum gutters were positioned on the down-slope edge of each plot to direct runoff into subsurface collection bottles which were covered with acrylic sheets to prevent direct precipitation from contaminating runoff samples. The six experimental plots were arranged in a randomized block design with three replications of two treatments (i.e., amended and unamended) to evaluate the effect of DFGD land application on runoff water quantity and quality.

The DFGD used was collected from the John W. Turk Power Plant in Hempstead County, Arkansas by a dry scrubber using an Alstom Novel Integrated Desulfurization design. Chemical characteristics of the DFGD by-product are presented in Table 2. Dry flue gas desulfurization byproduct treatments in this study included two application rates imposed once as a single application. Dry FGD byproduct was applied at a rate equivalent to 9 (amended) and 0 (unamended) Mg DFGD ha^{-1}. Dry flue gas desulfurization byproduct was evenly applied to plots on May 18, 2015.

Following DFGD application, runoff water was collected from each plot after every runoff-producing precipitation event from May 18, 2015 until July 9, 2015. Total runoff volume captured by the collection system was measured for each plot following each runoff-producing precipitation event. The first 15 mL of runoff from each plot was used to determine EC and pH immediately following collection of runoff samples and was then immediately discarded. Runoff pH was measured using a pH electrode (Orion Triode, No. 91-79 ORP) and EC was measured using a conductivity cell (VWR symphony, No. 11388-382). Any remaining runoff subsample was then filtered through a 1.6-µm glass microfiber filter (Whatman GFA-1820-110; Whatman International Ltd., Maidston, England) and then vacuum filtered through a 0.45-µm Metricel membrane filter (GN-6; Pall Life Sciences Corporation, Ann Arbor, MI). Following filtration, runoff samples were acidified by adding one drop of 36% (w/w) HCl per 10 mL of filtrate. Acidified aliquots were used to determine elemental concentrations of As, Be, B, Cd, Co, Cr, Cu, Mo, Ni, Pb, Rb, Se, Sr, and V by ICP-MS. Mercury concentrations were determined by ALS Environmental, Inc. (Tucson, AZ) in accordance with EPA method 7470a using a manual cold-vapor technique.

Results:

Fourteen precipitation events occurred during the study period and mean runoff volumes are presented in Table 3.The cumulative load of Se was significantly higher ($P < 0.05$) in the plots that received DFGD by-product than the control for the first two months following application (Table 4). Cumulative loads of other trace elements analyzed were not significantly different between the treated and control plots. Although not significantly different, cumulative runoff, loads As, Be, Cd, Co, Cr, Cs, Cu, Ni, Pb, V, and U were numerically higher in the treated plots compared to the unamended control. The high variation between replicates in both the control and amended plots resulted in cumulative loads that were numerically different but not statistically different. Cumulative loads of some elements such as Cs and Pb from plots that received the DFGD by-product were nearly twice those from the unamended control but due to deviation, neither were significant at α=0.05.

Mobility of selenium in the environment is controlled by soil pH and redox potential (Eh). In oxic alkaline soils, the highly mobile SeO_4^{2-} (selenate) is the predominant form of Se (Mayland et al., 1991).

Table 1. Chemical characteristics of a Captina silt loam prior to application of a dry flue gas desulfurization by-product

			Mehlich 3 extractable nutrients (mg kg^{-1})										
Soil Series	pH	EC µmhos cm^{-1}	P	K	Ca	Mg	S	Na	Fe	Mn	Zn	Cu	B
Captina	6.708	60.6	30.6	45.8	828.9	30.6	6.2	10.7	167.8	71.4	5.2	0.5	0.1

Table 2. Chemical characteristics of a dry flue gas desulfurization by-product originating from the John W. Turk Power Plant in Hempstead County, Arkansas.

Element	mg kg^{-1}
As	9.1
Be	2.16
B	336.36
Cd	0.61
Co	14.21
Cr	39.05
Cs	0.38
Cu	106.35
Hg	0.81
Ni	26.03
Pb	7.68
Rb	6.35
Se	15.77
V	127.07
U	2.73
EC (2:1)	4.02 ms cm^{-1}
pH	10.7

Table 3. Mean runoff volumes of a managed grassland that received a dry flue gas desulfurization by-product and an unamended control collected from 5/20/15 to 7/9/2015.

	Volume of Runoff Collected (mL)	
Date	Control	Treatment
5/20/2015	0†	169.33
5/25/2015	260	491.67
5/27/2015	60	195.00
6/1/2015	255	1341.00
6/14/2015	315	223.33
6/15/2015	495	450.00
6/16/2015	83.33	90.00
6/17/2015	522.5	645.00
6/26/2015	215.5	400.33
7/2/2015	310.33	546.00
7/3/2015	31	365.00
7/7/2015	414.67	911.67
7/8/2015	102.67	273.00
7/9/2015	127.5	0†

† Plots that received no runoff during the precipitation event

Table 4. Cumulative runoff, mean electrical conductivity (EC), pH, and cumulative trace element loads for a 2-month period (May-June 2015) from a managed grassland that received a one-time application of a dry flue gas desulfurization by-product. Means with the same letter within a row are not significantly different at α=0.05.

	Treatment	
Parameter	Control	Treatment
Cumulative Runoff (L)	2.8a	5.5a
Mean EC (μs cm^{-1})	215.8a	168.3a
Mean pH	6.34a	6.24a
As†	7.81a	11.81a
Be	0.34a	0.51a
Be	299.48a	477.64a
Cd	1.84a	2.51a
Co	4.28a	5.32a
Cr	2.98a	5.04a
Cs	1.25a	3.21a
Cu	65.84a	109.53a
Hg‡	BDL	BDL
Ni	9.17a	14.64a
Pb	4.65a	8.31a
Rb	32.42a	32.85a
Se	21.21a	37.62b
V	42.8a	85.57a
U	0.48a	1.46a

†Cumulative loads of trace elements are in g
‡Hg concentrations were below detectable limits (BDL)

Mobility of Se in the environment can be further enhanced in soils with high phosphate concentrations. Phosphate out competes Se for soil colloid adsorption sites. Due to continuous application of poultry litter in northwest Arkansas, soils often have high phosphorous concentrations which may have increased the susceptibility of Se to runoff.

Conclusions and Recommendations:

Although there were no significant difference between the amended and unamended plots in cumulative loadings of As, Be, Cd, Co, Cr, Cs, Cu, Ni, Pb, V, and U, these elements may have been taken up by plants, accumulated in the soil, or leached through the soil profile. The U.S. Geologic Survey should continue to research alternative methods of CCB disposal that may be more sustainable than the current disposal methods which can pose significant risk for environmental contamination. Beneficial reuse of CCBs as a soil amendment can only be accomplished if it can be shown that land application will not result in contamination of natural resources. The experiment described in this report is ongoing and will be completed in May of 2016. Complete statistical analyses of runoff water quality, plant uptake, and soil accumulation of trace elements and heavy metals will be performed upon completion of data collection.

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Principal Investigator: Brian E. Haggard

Publications and Presentations:

Scott, E.E. and B.E. Haggard, 2015, E. coli numbers in streams are related to land cover in the stream buffer zone, in University of Arkansas GIS Day, Fayetteville, AR.

Scott, J.T. and B.E. Haggard, 2015, Implementing effects-based water quality criteria for eutrophication in reservoirs: linking standard development and assessment methodology, Journal of Environmental Quality, 44:1503-1512.

Johnston, R., H.N. Sandefur, P. Bandekar, M. Matlock, B. Haggard and G. Thoma, 2015, Predicting changes in yield and water use in the production of corn under climate change scenarios, Ecological Engineering, 82: 555-565.

Johnson, T., L. Edgar, B. Haggard, and K. Rucker, 2015, Student Perceptions of the [State] Water Resources Center, Water Resources and Water Issues, Natural Sciences Education, 44:136-142.

McCarty, J.A., B.E. Haggard, M.D. Matlock, N. Pai, and D. Saraswat, 2016, Post-model validation of a deterministic water model using measured data, Transactions ASABE, 59(2):497-508.

McCarty, J.A., and B.E. Haggard, 2016, Can we manage nonpoint source pollution using nutrient concentrations during seasonal baseflow?, Agricultural and Environmental Letters, 1:160015.

Scott, E.E., Z.P. Simpson, and B.E. Haggard, 2016, Constituent loads and trends in the Upper Illinois River Watershed and Upper White River Basin, Arkansas Water Resources Center, Fayetteville, AR, MSC377, 89 pp.

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Simpson, Z.P., E.E. Scott and B.E. Haggard, 2015, Constituent load estimation in the Lower Ouachita-Smackover Watershed, Arkansas Water Resources Center, Fayetteville, AR, MSC373, 11 pp.

Scott, J.T. and B.E. Haggard, 2015, Evaluating the assessment methodology for the chlorophyll-a and secchi transparency criteria at Beaver Lake, Arkansas, Arkansas Water Resources Center, Fayetteville, AR, MSC372, 24 pp.

Scott, E.E. and B.E. Haggard, 2015, [Data Report-Water Quality for] Water Quality Monitoring for Selected Priority Watersheds in Arkansas, Upper Saline, Poteau and Strawberry Rivers, Arkansas Water Resources Center, Fayetteville, AR, DR-WQ-MSC369.

Scott, E.E. and B.E. Haggard, 2016, [Data Report-Water Quality for] Water Quality Monitoring and Constituent Load Estimation in the Upper Illinois River Watershed, 2009, Arkansas Water Resources Center, Fayetteville, AR, DR-WQ-MSC363.

Scott, E.E. and B.E. Haggard, 2016, [Data Report-Water Quality for] Water Quality Monitoring and Constituent Load Estimation in the Upper Illinois River Watershed, 2009, Arkansas Water Resources Center, Fayetteville, AR, DR-WQ-MSC363.

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Scott, E.E., 2015, Water News, January Newsletter, Arkansas Water Resources Center.

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Project Title: Information Transfer Program
Project Team: Brian E. Haggard, University of Arkansas, Arkansas Water Resources Center, Department of Biological and Agricultural Engineering
Erin E. Scott, Arkansas Water Resources Center

Introduction:

An important component of the Arkansas Water Resources Center's (AWRC) mission is the transfer of water resources information to the user community within Arkansas and the region. This community of users includes researchers, resource planners and managers, environmental consultants, environmental advocacy entities, lawyers, and the general public. The transfer of information was accomplished through the following 7 avenues:

1. Hold and sponsor annual water resources conference during the project year.
2. Prepare and disseminate monthly email newsletters that address water related activities and news throughout Arkansas and regionally.
3. Publish technical reports and data reports on water research and water quality monitoring projects.
4. Maintain the AWRC website as a primary portal for accessing technical reports, notices, newsletters, conference registration, AWRC Water Quality Laboratory information, and AWRC library materials.
5. Update the AWRC Facebook and Twitter page throughout the year.
6. Expand the Center's reach to inform the public by means of University-wide electronic news (e.g. "Newswire") and possibly news through a national platform.
7. Center-related research published in peer-reviewed journals, presentations at scientific conferences and meetings, and support of students seeking graduate degrees. This includes 104B funded research as well as other Center-related research.

The dissemination of water resources information through the 7 primary avenues listed above reaches a broad audience throughout Arkansas and neighboring states.

Annual Water Conference:

Over 150 people attended the annual water conference held in July 2015. Attendees included stakeholders from municipalities, state agencies, research institutions, non-profit groups, environmental consulting firms, and the general public from throughout Arkansas and the region. Topics included:

- Animal manure and the land-water interface
- Agricultural water management in the delta
- Urban stormwater management
- Emerging research by students funded through the USGS 104B program.

In conjunction with the annual conference, the Center hosted a stormwater inspector certification course titled "BMP Design, Application and Inspection for Construction Sites". This course was taught by a CMS4S certified instructor for Stormwater Inspector certification. Over 80 people attended and were certified through course, including construction site managers and workers, designers, developers, inspectors, and other interested individuals.

Electronic Newsletters:

The AWRC distributed monthly electronic newsletters to several hundred people from local and state agencies, municipalities, academia, non-profit organizations, consulting firms, students, and many other stakeholders. Electronic newsletters continue to be a valuable means of distributing important information related to water resources. The Center published news articles on current research being done throughout the State, especially projects funded through the USGS 104B program, recent activities of the Center, the USGS, and other organizations, funding opportunities, and other timely water-related news.

The AWRC populates a section of the newsletter for "Upcoming Events" to highlight not only Center-related events and activities, but also those of other local or national organizations such as ADEQ, ANRC, Beaver Watershed Alliance, Illinois River Watershed Partnership, and the US EPA. AWRC also updates a "Jobs" section each month aimed to provide recent graduates or early career people some guidance and examples of current job openings related to water science and engineering.

Publications:

AWRC published 7 technical reports and 8 water-data reports on the Center's website during this past project year (March 2015-February 2016). These technical reports included the USGS annual report, the USGS annual summary, water research and monitoring reports from projects funded by state or local water organizations, as well as reports by scientists not related to the Center in an effort to make available important information in addition to or in lieu of peer-reviewed articles. Water-data reports are published on AWRCs website and provide easy access to years-worth of Center-related water quality monitoring data associated with the data collected for the technical reports. These data reports are available to the public and can be accessed as neatly-organized Microsoft Excel data files.

Website:

The AWRC website is the primary portal for stakeholders to access important and useful water resources information. During this past year, Center-staff have worked to improve the usability of the website and the availability of water resources information. The website serves as a platform to provide:

- Immediate electronic availability of almost all AWRC publications
- A warehouse of raw data provided as water-data reports associated with research and monitoring projects
- Information about submitting a water sample to the AWRC Water Quality Laboratory
- Information on upcoming conferences and funding opportunities, especially USGS 104B and 104G grants, and other events.

Maintenance of the AWRC website is a critical component of the AWRC's information transfer program.

Social Media:

The AWRC continues to expand its presence on social media. During this past year, staff utilized Facebook and twitter to disseminate information about the activities of the Center including funding opportunities, conference materials, and research findings. Social media also has been a great way to network and share ideas and stories among water stakeholders and organizations. The Center shares posts from other water or water-related organizations about current news or upcoming events. During this past project year, the Center began posting the monthly electronic newsletters on Facebook and started utilizing the "boost post" function. This has resulted in posts reaching over 5,000 people, with increased user engagement. The use of Hootsuite enabled our twitter activity to at least mirror our Facebook posts.

Other News Outlets:

The AWRC began reaching out to communications staff at the University of Arkansas, University Relations Department, to increase the Center's reach and inform the greater public through additional news outlets. Specifically, AWRC worked with University Relations to run a story on a local water-research project that the Center had recently published. This news article was distributed via email to over 25,000 faculty, staff and students at the University of Arkansas, and also available on a national news platform accessed by communications professionals around the country. The story was picked up by a contributing editor for the American Society of Civil Engineers and published in that organization's national magazine.

Publications, Presentations and Degrees:

When soliciting research proposals through the USGS 104B program, AWRC emphasizes several objectives, including the future publication of research results in peer-reviewed scientific literature. During this past year, 12 publications have been submitted or accepted into peer-reviewed scientific journals. These publications are listed within each project report or in the section for publications from previous project years.

AWRC also emphasizes the presentation of research results at local, national and international meetings and conferences, and the support of graduate research assistants. During this past year, 31 oral and poster presentations were given by student and faculty researchers at conferences around the country. Additionally, 9 graduate students either successfully completed their graduate studies and have published their thesis or dissertation, or are expected to graduate in coming years.

Conclusions:

One of the primary missions of the AWRC is the transfer of information to water resources stakeholders. Through the use of an annual water conference, electronic newsletters, publication of reports, maintenance of the website, engagement through social media, and utilization of additional news outlets, AWRC continues to reach a broad audience throughout Arkansas and even the Nation. The Center has helped to ensure that water resources managers have the information necessary to help guide important management decisions.

Student Support FY2015					
Category	**Section 104 Base Grant**	**Section 104 NCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	10	0	0	0	10
Masters	7	0	0	0	7
PhD	4	0	0	0	4
Post-doc	1	0	0	0	1
Total	22	0	0	0	22

Notable Awards and Achievements

Lucy Baker was awarded third place for the student oral presentations at the Natural Areas Association Conference held in Little Rock, AR. This award recognized outstanding student presentations that occurred over the two day conference. Lucy competed with approximately 30 students.

Publications from Prior Years

1. 2011AR313B ("Continued Investigation of Land Use and Best Management Practices on the Strawberry River Watershed") - Articles in Refereed Scientific Journals - Brueggen-Boman, T.R., S.Choi, and J.L. Bouldin, 2015, Response of Water Quality Indicators to the Implementation of Best Management Practices in the Upper Strawberry River Watershed, Arkansas, Southeastern Naturalist Journal, 14(4): 697-713.
2. 2014AR349B ("Assessing total nitrosamine formation and speciation in drinking water systems") - Articles in Refereed Scientific Journals - Do, T.D., J.R. Chimka, and J.L. Fairey, Improved (and Singular) Disinfectant Protocol for Indirectly Assessing Organic Precursor Concentrations of Trihalomethanes and Dihaloacetonitriles, Environmental Science and Technology, 49: 9858-9865.
3. 2014AR354B ("Economics of Multiple Water-Saving Technologies across the Arkansas Delta Region") - Articles in Refereed Scientific Journals - Kovacs, K., M. Mattia, G. West, 2015, Landscape irrigation management for maintaining an aquifer and economic returns, Journal of Environmental Management, 160: 271-282.
4. 2014AR354B ("Economics of Multiple Water-Saving Technologies across the Arkansas Delta Region") - Conference Proceedings - Kovacs, K., 2015, Regional Irrigation Management with Conjunctive Surface and Groundwater Use, Agricultural and Applied Economics Association Annual Meeting, San Francisco, CA.
5. 2014AR354B ("Economics of Multiple Water-Saving Technologies across the Arkansas Delta Region") - Conference Proceedings - West, G. and K. Kovacs, 2015, Spatial irrigation management to sustain groundwater and economic returns, SERA35: Delta Region Farm Management and Agricultural Policy Working Group, Vicksburg, MS.