Maryland Water Resources Research Center
Annual Technical Report
FY 2015

Introduction

During Funding Year 2015, the Maryland Water Resources Research Center (MWRRC) supported a variety of research and outreach activities that address the diversity of water issues in the State and the Chesapeake Bay Region. The Center's outreach/information transfer event recognized the International Year of Soils, in recognition of the critical role that soils play in the water cycle. Two new research projects spanned the rural-urban spectrum in Maryland: investigating emerging contaminants in poultry litter, and quantifying the performance of building- and site-scale stormwater management technology. A research seed project helped to launch a study of bioremediation using duckweed. Four graduate students received summer fellowships: investigating the ecological effects of agricultural safeners (widely considered to be inert ingredients in herbicides); assessing the ability of riparian forest buffer systems to reduce nutrient inflow to streams; advancing the art of modeling small-scale stormwater control measures; and quantifying patterns and trends in nutrient and sediment delivery to the Chesapeake Bay. Three research projects begun in previous years were completed during this period, with new contributions to our understanding of road salt in groundwater, invasive and native wetland plants, and a non-lethal approach to monitoring for intersex in fish.

Research Program Introduction

With 104B funding, the Maryland Water Resources Research Center supported two research projects, one seed grant, and four graduate student summer fellowships in Funding Year 2015.

- The Fate of Emerging Contaminants in Poultry Litter Digestion (2015MD324B); PIs: Stephanie Lansing, Stephanie Yarwood, Lance Yonkos (Environmental Science & Technology, University of Maryland, College Park)
- Water balance of a green roof integrated with a constructed wetland and rain garden for urban water management (2015MD326B); PI: David Tilley (Environmental Science & Technology, University of Maryland, College Park)
- Potential use of duckweed (Lemna minor) in bioremediation of rare earth element-containing effluents (2015MD327B); PIs: Johan Schijf (Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science), Ed Landa (Environmental Science & Technology, University of Maryland, College Park)
- Retrospective Analysis of Nutrient and Sediment Loadings and Trends in the Chesapeake Bay Watershed (Graduate Fellowship) (2015MD329B); Qian Zhang (PhD student, Geography & Environmental Engineering, Johns Hopkins University, Advisor: W.P. Ball)
- *Chironomus riparius:* A Tool for Studying Ecological Effects of "Inert" Safeners (Graduate Fellowship) (2015MD330B); Kasey Bolyard (M.S. student, Biological Sciences, Towson University; Advisor: S. Gresens)
- Assessing hydrogeomorphological constraints on water quality functions of forested riparian buffers in Western Maryland (Graduate Fellowship) (2015MD331B); Stephanie Siemek (Ph.D. student, Appalachian Laboratory, University of Maryland Center for Environmental Science; Advisor: K. Eshleman)
- Storm Water Runoff and Water Quality Modeling in Urban Maryland (Graduate Fellowship) (2015MD332B); Jing Wang (M.S. student, Civil & Environmental Engineering, University of Maryland, College Park; Advisor: B. Forman)

Three 104(b) projects funded in previous years were completed during this reporting period:

- Tracing the rates of road salt runoff movement from impervious surface to stream and the effects on soil and aquifer geochemistry (2014MD313B, continuation of 2013MD306B); PIs: Joel Moore and Steven Lev (Physics, Astronomy & Geosciences, Towson University).
- Wetland restoration: Experimental effects of soil carbon:nitrogen ratio on growth of invasive and native Phragmites australis (common reed) (2014MD314B); PI: Andrew Baldwin (Environmental Science & Technology, University of Maryland College Park).
- Validation of non-lethal laparoscopic technique for detection of intersex in regional black bass populations (2014MD315B, continuation of 2013MD305B); PI: Lance Yonkos (Environmental Science & Technology, University of Maryland College Park).

A University of Maryland project selected for support under the 104(G) program in 2014 was active during this performance period:

- Environmental Concentrations and Exposure Effects of Environmental Gestagens on a Sentinel Teleost Fish (2014MD321G); PI: Edward Orlando (Animal & Avian Sciences, University of Maryland, College Park).

One coordination grant was active during this performance period:

- Impact of the '308 Reports' on Water Resources Planning and Development in the U.S. and Implications of these Results for the Future (G16AP00019); PIs: Gerald Galloway, Jeffrey Brideau, Kaye Brubaker (University of Maryland, College Park); Agency: U.S. Army Corps of Engineers, Institute for Water Resources.

Progress Report for Coordination Grant G16AP00019

Note: This project was not listed among the MWRRC projects on NIWR.net; therefore, a brief progress report is included here.

Report by Jeffrey Brideau, Ph.D.
June 30, 2016

Below, is a brief summary of the progress made on the project *Impact of the "308 Reports" on Water Resources Planning and Development in the United States and Implications of these Results for the Future*, funded by the National Institutes of Water Resources (NIWR) through a grant administered by the United States Geological Survey (Grant/Cooperative Agreement Number G16AP00019).

In January 1927, the U.S. Congress, speaking through the Rivers and Harbors Act (PL 70-560), instructed the U.S. Army Corps of Engineers (USACE) to prepare a nationwide series of river surveys to determine the feasibility of developing hydroelectric power in combination with navigation, irrigation, and flood control measures. Collectively known as the "308 Program" – named after House Document 308, 69th Congress, 1st Session, which defined the surveys' scope and intent – this program marked the first national, basin-wide, multipurpose water resources planning program in the United States.

The stated objective of this research project is:

"to explore the linkages between the production of large-scale water resources planning studies by a federal agency, and the material development of water resources at the federal, state, and local levels. Through the analysis of the set of reports, participant motivations, as well as contemporary economic and political events, key lessons will be sought with applications for the development of 21st century water resources planning in the United States."

To achieve this end, the co-PI – Dr. Jeffrey Brideau – spent the first few months of the project (October 2015 – February 2016) developing a specific research agenda, identifying and reviewing the relevant secondary literature, and constructing a series of research benchmarks and proposed projected outputs.

One of the central efforts made during this early phase was the identification of archival repositories and specific record groups for the collection of data related to three specific case studies. In March 2016, Dr. Brideau traveled to Augusta, Athens, and Atlanta, Georgia on the first of several planned research trips. In Georgia, he accumulated over 5,500 images of documentary materials related to construction and reception of the 308 report on the Savannah River. Dr. Brideau spent much of the following month at the National Archives and Records Administration (NARA) in College Park, collecting the corresponding national-level records (RG 77 – Records of the Office of Chief of Engineers), and accumulated another 5,000 research images in the process.

This abundance of research material is currently under review, and Dr. Brideau has begun to develop the first of the proposed case studies. To this end, in June 2016, Dr. Brideau presented a draft paper at the University Council on Water Resources (UCOWR)/NIWR annual conference in Pensacola, FL. His presentation outlined the 308 program’s historical significance and suggested avenues to evaluate its continued relevance as well as the lessons it offers to present-day water resources managers. The paper incorporated the Savannah River case study and the feedback received will be used to revise and redraft a paper to be submitted for academic publication in Autumn 2016.

Another notable outcome from the early months of this project was an expansion of the research agenda and activities to be conducted under the auspices of the associated grant. This new work included: the collection and analysis of unanticipated sources of research material located in regional and local archival repositories; as well as a refocused and expanded set of project objectives and research questions based on a more thorough understanding of the relevant literature, the availability of data, and a series of conversations between the co-PI and the Institute for Water Resources (IWR). Additionally, Dr. Brideau has been asked to design and implement an IWR oral history project to capture the histories of water resources planning and development as well as institutional dynamics at IWR. To facilitate the development of this oral history project, the co-PI collaborated with the Army Corps of Engineers’ Office of History and the Army’s Center for Military History, hopefully forging lasting institutional relationships moving forward.

Additional information on the project’s status, ongoing research endeavors, citations, or anticipated publications and other outputs can be provided by contacting the co-PI at: jbrideau@umd.edu

Tracing the rates of road salt runoff movement from impervious surface to stream and the effects on soil and aquifer geochemistry

Basic Information

Title:	Tracing the rates of road salt runoff movement from impervious surface to stream and the effects on soil and aquifer geochemistry
Project Number:	2014MD313B
Start Date:	6/1/2014
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	2
Research Category:	Water Quality
Focus Category:	Hydrogeochemistry, Geochemical Processes, Water Quality
Descriptors:	None
Principal Investigators:	Joel Moore, Joel Moore

Publications

There are no publications.

Completion Report

Tracing the rates of road salt runoff movement from impervious surfaces to stream and the effects on soil and aquifer geochemistry

2014-MD-313B

March 1, 2013 – February 29, 2016

1) Narrative summary

Problem and Research Objectives

Runoff from impervious surface that contains dissolved road salt (NaCl) clearly affects the chemistry of soils, groundwater, and streams. However, effects on soils, groundwater, and streams typically have been studied in isolation from each other. Also, the effects of stormwater management basins on road salt movement from impervious surfaces to streams have not been investigated. The research funded by this grant has the goal of addressing the following questions about road salt movement from impervious surfaces to streams in a system with stormwater management basins. The study site is located in Owings Mills, MD located in a suburban part of Baltimore County, MD.

a) What are the rates of Na and Cl movement from impervious surface to stream?
b) Where and how along the flow paths from road to stream are Na and Cl retained?
c) What is the effect of Na retention on soil and shallow aquifer chemistry, particularly on pH and the concentrations of important plant macronutrients like calcium (Ca), magnesium (Mg), and potassium (K) in the cation exchange complex?

Methods

The methods employed include collection of

- Physical data such as stream discharge and water table levels
- Collection of water and soil/aquifer matrix samples at the field site through June 2016
- Chemical analysis of those samples in the Urban Environmental Biogeochemistry Laboratory at Towson University including
  - Concentration of cations and anions in water via ion chromatography
  - Composition of the cation exchange complex via $BaCl_2$ extraction and then ion chromatography
  - Soil pH via 0.1M $CaCl_2$ suspension and pH probe
- Deployment of pressure and conductivity sensors to continuously monitor water level and the total dissolved content
- In collaboration with colleagues from Johns Hopkins, we did an electrical resistivity survey in April 2016 to image the salt plumes in the floodplain aquifer. We will do a second survey in late summer or early fall 2016.

Principal Findings

Stream discharge and water table levels increased from fall into winter as trees shed their leaves and evapotranspiration rates decreased. Na^+ and Cl^- were loaded into groundwater via stormwater management basins (SMBs) and then moved via surface and groundwater flow into first- and second-order streams. In groundwater below the SMBs, Cl^- concentrations increased rapidly at the beginning of each winter and approached or exceeded seawater concentrations (Fig. 1). Through early spring, Cl^- and Na^+ concentrations decreased slowly.

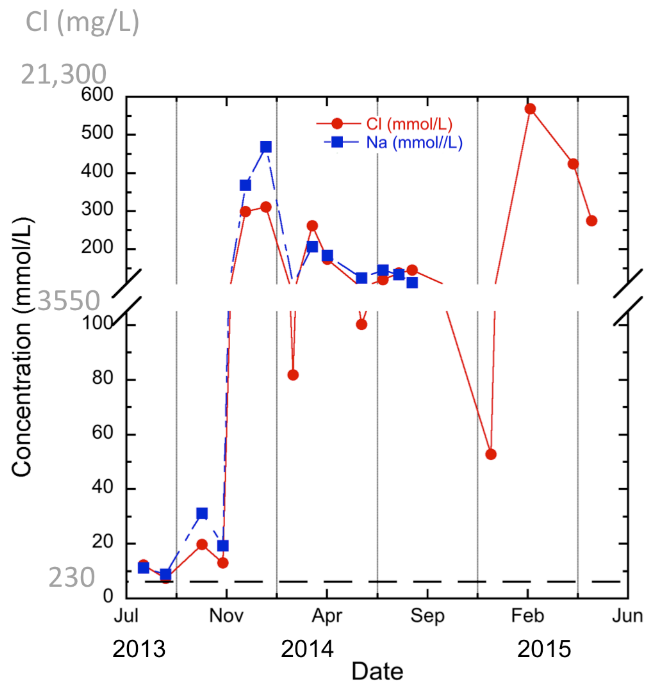

Fig. 1: Calcium, chloride, and sodium concentrations in groundwater from July 2013 to June 2015. The groundwater was collected from under a stormwater management basin that collects runoff from a ~200 unit townhome community along with secondary and tertiary roads. Sodium and chloride concentrations were approached or exceeded concentration of seawater concentrations (535 mmol Cl^- L^{-1}) and were ~2100 times higher than concentrations measured in groundwater collected from a forested watershed ~15 km to the northeast.

Cl^- and Na^+ concentrations increased from 100s of mg L^{-1} in fall to 1000s of mg L^{-1} in winter and then declined into early spring was seen in shallow groundwater (~1 m) downgradient of the SMBs. In deeper groundwater (~2.5 m), Cl^- and Na^+ concentrations remained relatively stable across the seasons at several hundreds of mg L^{-1}.

The movement of high Na^+ water through groundwater has increased the pH and Na^+

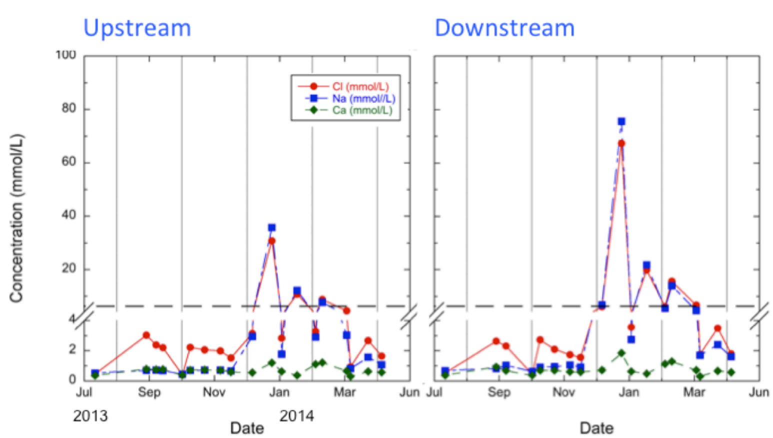

Fig. 2: Calcium, chloride, and sodium concentrations from July 2013 to June 2014 from locations upstream and downstream where water from two stormwater management basins enters the stream via surface and groundwater. Cl^- and Na^+ concentrations are 125 times higher than in a reference watershed ~15 km to the northeast with similar bedrock and a similar size.

concentrations in soils and aquifer matrix downgradient of the SMBs. Compared to unaffected soils, soil pH increased from a value of ~4.5 to pH 6–7 and the Na^+ fraction of the cation exchange complex increased from 0 to 20–40%. Measured Na^+ fractions of the cation exchange complex in road salt–affected soils matched well with predictions from the PHREEQC geochemical model. Na^+ replaced H^+, Mg^{2+}, and, to a lesser extent, Ca^{2+} in the cation exchange complex.

Significance

Results from this study increase understanding of how road salt affects the chemistry of soils/aquifer matrices, groundwater, and streams. Understanding groundwater and stream chemistry across seasons will provide information that will be useful in predictive models of road salt impacts in the future. Understanding of the effects of high Na^+ water on soil and aquifer matrix chemistry provides information that is important for assessment of the longer term impact of road salt. In addition to presentations at professional meetings, this work has been presented to and discussed at several venues with environmental scientists and regulators from the Maryland Department of Environment, Maryland Department of Natural Resources, and US Geological Survey.

2) Publication citations for March 1, 2015 onward

Articles in Refereed Scientific Journals

Snodgrass, Joel; Joel Moore; Steven M. Lev; Ryan E. Casey; David Ownby; Robert Flora; Grant Izzo**,** 2016 *in review*, Influence of Modern Stormwater Management Practices on Transport of Road Salt to Surface Waters , Environmental Science and Technology

Abstracts/Conference Proceedings (Undergraduate co-author)*

Gregory Woodward*; and Joel Moore**,** 2015, Road salt (NaCl) runoff increases soil pH and significantly alters cation exchange chemistry in soils and aquifers, *2015 Annual Geological Society of America Meeting,* Baltimore, MD.

Joel Moore, 2016, Stormwater management basins as a source of year-round salt loading to groundwater and streams, *Road salt usage and environmental impacts workshop* sponsored by Maryland Water Monitoring Council, Patuxent National Wildlife Visitor's Center, MD.

3) Students supported

MWRRC and matching funds have supported 2 undergraduate students and 1 masters student.

4) Notable achievements and awards

The work supported by this grant has been well received and I gave the following talks related to this research:

Research seminars

Sept. 2015: *Use of a rural-urban watershed gradient to investigate processes that control urban stream geochemistry*. Department of Geosciences, University of Delaware

Nov. 2015: *How does urbanization alter stream chemistry?* Department of Earth Sciences, Dickinson College

Talks to local and regional governmental groups

Jan. 2016: *Road salt*. Maryland Department of Environment, Baltimore, Maryland

June 2016: *Road salt and water quality*. Salt Management Workshop, Metropolitan Washingon Council of Governments, Washington, DC.

Wetland restoration: Experimental effects of soil carbon:nitrogen ratio on growth of invasive and native Phragmites australis (common reed)

Basic Information

Title:	Wetland restoration: Experimental effects of soil carbon:nitrogen ratio on growth of invasive and native Phragmites australis (common reed)
Project Number:	2014MD314B
Start Date:	3/1/2014
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	5th and 8th Congressional District of Maryland
Research Category:	Biological Sciences
Focus Category:	Wetlands, Nutrients, Invasive Species
Descriptors:	None
Principal Investigators:	Andrew Baldwin, Stephanie Ann Yarwood

Publications

1. Baldwin, Andrew H., Amr E. Keshta, Martina Gonzalez Mateu, Diane E. Leason, and Stephanie A. Yarwood, 2015. Carbon cycling, invasives, and restoration: an overview of current research on the tidal Patuxent. Patuxent River Conference, Jefferson-Patterson Park and Museum, St. Leonard, Maryland (Poster presentation).
2. Baldwin, Andrew H., Amr E. Keshta, Martina Gonzalez Mateu, Diane E. Leason, and Stephanie A. Yarwood, 2015. Carbon cycling, invasives, and restoration: an overview of current research on the tidal Patuxent. Poster presentation. Patuxent River Conference, Jefferson-Patterson Park and Museum, St. Leonard, Maryland, June 2015. (http://www.paxcon.org/uploads/5/7/6/6/5766937/pax_2015_baldwin_poster.pdf)

Completion Report

Wetland restoration: Experimental effects of soil carbon:nitrogen ratio on growth of invasive and native *Phragmites australis* (common reed)

Prepared for: Maryland Water Resources Research Center

Project Number: 2014MD314B

Project duration: March 1, 2014 - February 28, 2016

Principal Investigator:
Andrew H. Baldwin
Department of Environmental Science and Technology
University of Maryland, College Park, MD 20742
baldwin@umd.edu 301-405-7855

Co-PI: Stephanie A. Yarwood

Prepared by: A.H. Baldwin, S.A. Yarwood, and M. Gonzalez Mateu (graduate student)

Submitted: July 13, 2016

Project Goals, Methods, and Research Accomplishments

Objectives

Objective 1: Assess aboveground and belowground growth, ecophysiology, and competitive performance of native and invasive *Phragmites* in mixtures and monocultures, planted in soil from either restored or natural wetland and subjected to increased, decreased, or unaltered soil C:N ratio (manipulated by addition of wood shavings or urea nitrogen).

Objective 2. In the monoculture plantings used in Objective 1, quantify AM fungi colonization of roots of native and invasive *Phragmites* and relate AM fungi abundance to plant responses to C:N ratio treatments in soils from restored and natural wetlands.

Objective 3: In a separate experiment, determine N and C mineralization rates in soils from restored and natural wetlands subjected to increased, decreased, or unaltered soil C:N ratio.

Methods

We conducted Objective 3 first because it was a lab study that only required collecting soil from the field and could be accomplished in a short period of time. Soils were sampled in October of 2014 from two freshwater tidal wetlands. Soils were amended with urea, glucose, or sawdust, corresponding to a low C:N (urea), a high C:N with a labile carbon source (glucose) and a high C:N with a recalcitrant C source (sawdust). To determine Potential Nitrogen Mineralization (PMN), the amended soils were incubated under anaerobic conditions in glass serum bottles at 40C for 10 days, and soil extracts analyzed for NH4-N. To determine emissions of carbon dioxide and methane, amended soils were incubated at 35C for 25 days and gas samples analyzed on a gas chromatograph.

Objectives 1 and 2 were originally planned only as greenhouse experiments, but we decided to conduct the Objective 1 study as a field experiment as well as in the greenhouse instead because: 1. We had unforeseen micronutrient limitation in the greenhouse that needed to be solved before greenhouse experiments could be conducted; 2. We were encouraged by results from Objective 3 that field treatments would be effective, and 3. Field studies, while harder to maintain environmental control, are more realistic than greenhouse studies. (The micronutrient problem has since been addressed, and found to be reduced iron limitation, in a separate experiment conducted by a group of undergraduate Capstone students in Environmental Science and Technology, ENST). Early in 2015 we collected additional rhizomes, propagated them in the greenhouse (Figure 1), and planted them in the field at Jug Bay Wetlands Sanctuary, in a tidal freshwater marsh where invasive *Phragmites* had been sprayed with herbicide during the fall of 2014. A boardwalk was constructed to reduce disturbance to the

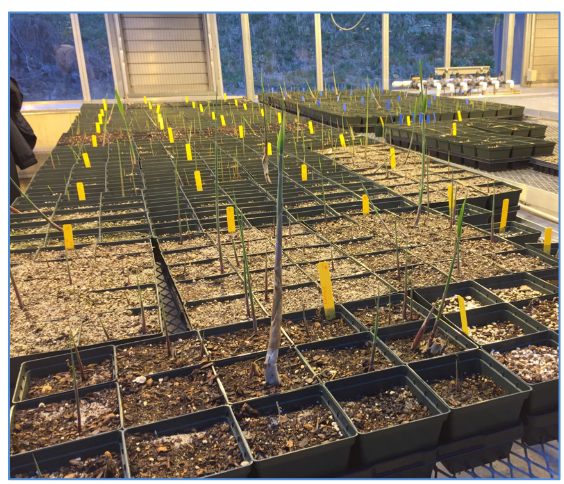

Figure 1. Native (yellow tags) and invasive (blue tags) *Phragmites* shoots emerging from rhizomes collected on the Patuxent river. Plants were grown to abou 1 m height and planted in the field C:N ratio experiment.

marsh substrate (Figures 2 and 3). Plants of native and invasive *Phragmites* were planted in monoculture and mixture, and treatments to alter soil C:N ratio were added (Figure 4). The treatments were: urea (22g), wood shavings (1.25kg), both, or no treatment to 1-m^2 plots.

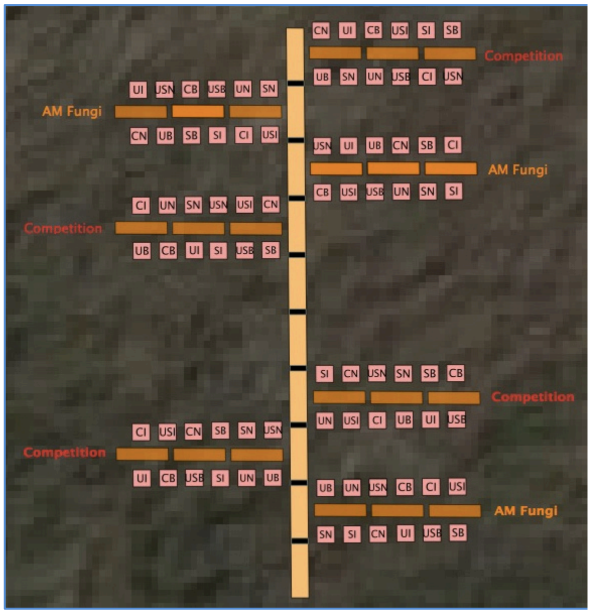

Figure 2. Jug Bay site showing the experimental design. Boardwalks (tan and brown) reduce the disturbances during sampling. Each treatment is assigned in each block and can be identified by the following letter: U=urea, S=sawdust, C=control, N=Native, I=Invasive, B=Both native and invasive.

Figure 3. Experimental site at Jug Bay Wetland Sanctuary.

Figure 4. Application of carbon treatment to experimental plot (pine wood shavings, 1.5 kg/m^2).

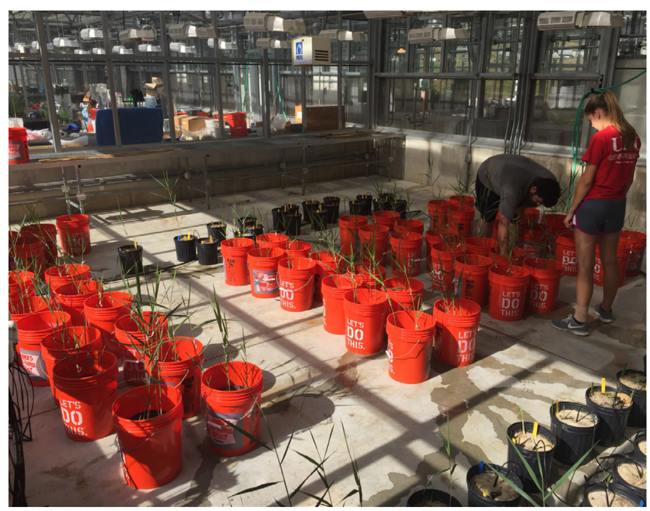

Figure 5. Greenhouse experimental units for studying effects of carbon and nitrogen additions on competitive outcomes between native and invasive *Phragmites*.

For the Objective 1 field experiment, treatments and monitoring will continue through the 2016 growing season. Repeated measurements include stem height, stem basal diameter, number of stems per plot, hydrology, and soil characteristics. When the experiment concludes, we will harvest the above and belowground biomass and determine leaf area. Then we will use that information to explore the net balance of native and invasive *Phragmites* interaction, using an index of relative interaction intensity.

In the early spring of 2016, we collected additional rhizomes and set up a greenhouse experiment (Figure 5). For this experiment we planted native and invasive *Phragmites* in mixture and monoculture in pots within treatment tanks (buckets). A low-nutrient supplement of Osmocote slow-release fertilizer was applied to all pots, and then pots were exposed to a 2x2 factorial arrangment of urea nitrogen addition and carbon (wood shavings) as was done in the field. To encourage mycorrhizal colonizion, an inoculum of soil from wetlands where native and invasive *Phragmites* were confirmed to have fungal infection was added to each pot before planting. We will terminate this experiment in August 2016.

For Objective 2, we refined and tested the methods for AM fungi colonization on native and invasive in *Phragmites* roots collected from the Choptank and Patuxent subestuaries. We are sampling every two weeks during the 2016 growing season on the Choptank River along a salinity gradient to determine the stabililty of endophytic fungi populations. At the end of the the Patuxent field and greenhouse studies (Objective 1 experiments), colonization and genetic identification of AM fungi and DSE will be performed on harvested roots of plants subjected to the different carbon and nitrogen addition treatments.

Research accomplishments

In the laboratory incubation study (Objective 3), Potential Nitrogen Mineralization was affected by the increased carbon amendments only in the natural wetland soil. Both the glucose and sawdust treatments effectively decreased nitrogen mineralization compared to the control soil (Figure 6). Application of urea significantly increased mineralization for both natural and restored sites compared to the control and the other amendments as expected. The carbon addition treatments significantly increased carbon dioxide production in both types of wetland soils, while the urea treatment had no significant effect on CO2 production compared to controls (Figure 7).

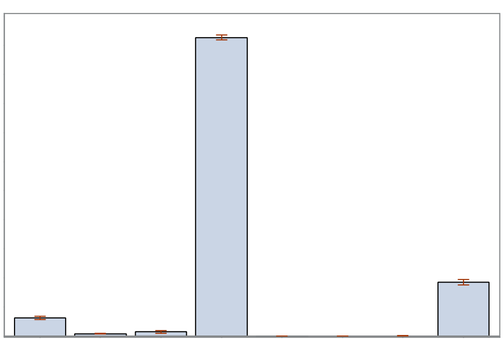

Figure 6. Potential nitrogen mineralization after 10 days of anaerobic incubation. Means and standard error bars are shown, and different letters indicate statistical significance between treatment means (P<0.001). P=Patuxent Wetland Park, W=Wooton's Landing, C=Control, G=Glucose, SD=Sawdust, U=Urea.

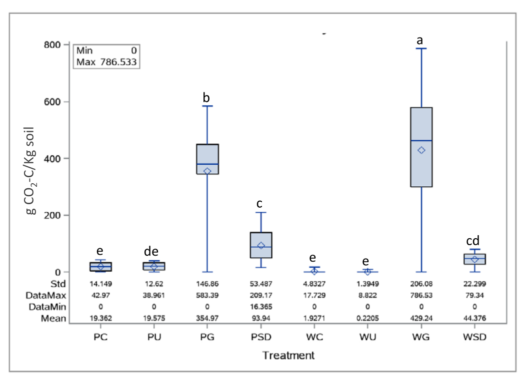

Figure 7. Boxplot showing total carbon dioxide production (gCO2-C/Kg of soil per day) after 25 days of anaerobic incubation. Different letters indicate statistical significance (P<0.001). P=Patuxent Wetland Park, W=Wooton's Landing, C=Control, G=Glucose, SD=Sawdust, U=Urea.

As mentioned previously, we encountered micronutrient limitation of native *Phragmites* plants grown in the greenhouse. The primary symptom was leaf chlorosis (yellowing) and ultimately poor growth. To identify the limiting nutrients, the PIs worked with a group of students in the ENST undergraduate Capstone course to develop and carry our a two-semester greenhouse experiment. Two possible limiting minerals, magnesium (Mg2+) and reduced iron (Fe2+), were applied to native *Phragmites* in a factorial combination of Mg and Fe (all of which received Osmocote slow-release fertilizer), plus an additional treatment that received no minerals or fertilizer. A number of measuresments were made, but the results are evident in the photograph in Figure 8: reduced iron was the primary cause of chlorosis (in fertilized plants) and strongly limiting growth.

Figure 8. Native *Phragmites* grown under different supplements of reduced iron, magnesium, and fertilizer. The experiment was carried out by undergraduate ENST Capstone students.

For Objective 2, we found that AM fungi colonized both native and invasive *Phragmites* (Figure 9A; only native *Phragmites* shown). We also found dark septate endophytes (DSE), which were not expected (Figure 9B). The function of DSE is unknown but they may play a role in stress tolerance or nutrient uptake. We successfully grew about about a dozen isolates of DSE on agar plates (Figure 10) in preparation for genetic sequencing and found that DSE colonization was greater at higher than at lower salinity, suggesting a role in stress tolerance (Figure 11).

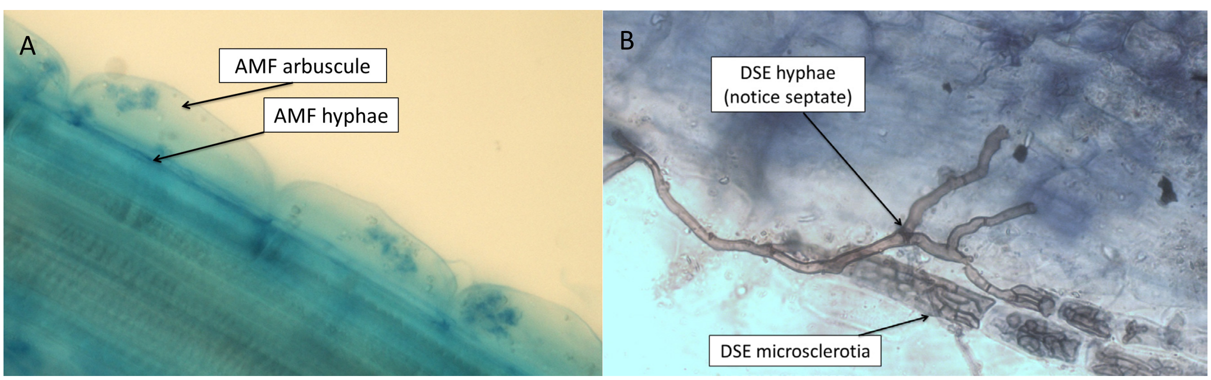

Figure 9. Arbuscular-Mycorrhizal Fungi (AM Fungi, A) and Dark Septate Endophytes (DSE, B) that have colonized roots of native *Phragmites*. Source: Martina Gonzalez Mateu.

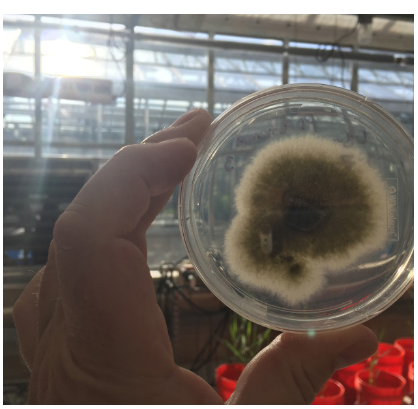

Figure 10. DSE isolated from *Phragmites* roots and grown on agar plates in preparation for genetic identification.

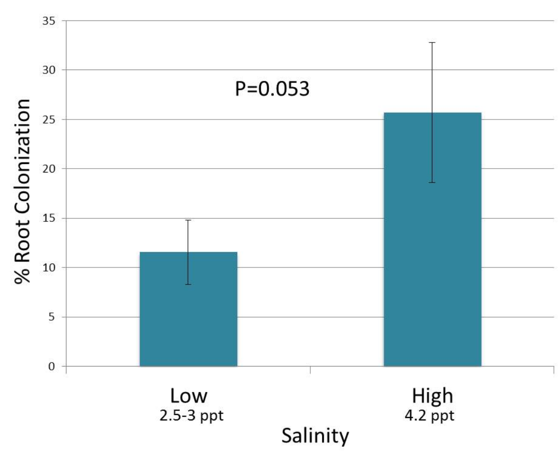

Figure 11. Colonization of *Phragmites* by DSE at different salinities along the Choptank River subestuary.

The most important and novel finding of the Objective 1 field experient so far is that, at some sites where invasive *Phragmites* was killed using herbicide, planted native *Phragmites* can be successfully established that will outperform invasive Phragmites, even under different nutrient availabilities (Figure 12). Urea addition favored native *Phragmites* in both monoculture (Figure 12A) and mixture (Figure 12B) plantings. This was surprising because we had hypothesized that invasive *Phragmites* would produce greater biomass and competitively dominate under nitrogen-enriched conditions. We had also hypothesized that native *Phragmites* would competively dominate in mixture plots subjected to carbon addition (which should immobilize nitrogen), and this was observed in our plots (pet bedding treatment, Figure 12 B). However, this result may not be due to our treatments given the surprising finding that native *Phragmites* dominated across all treatments in competition (mixture) plots (Figure 12B). Additional monitoring during 2016 and the final harvest will provide additional data that will aid in interpretation of these results.

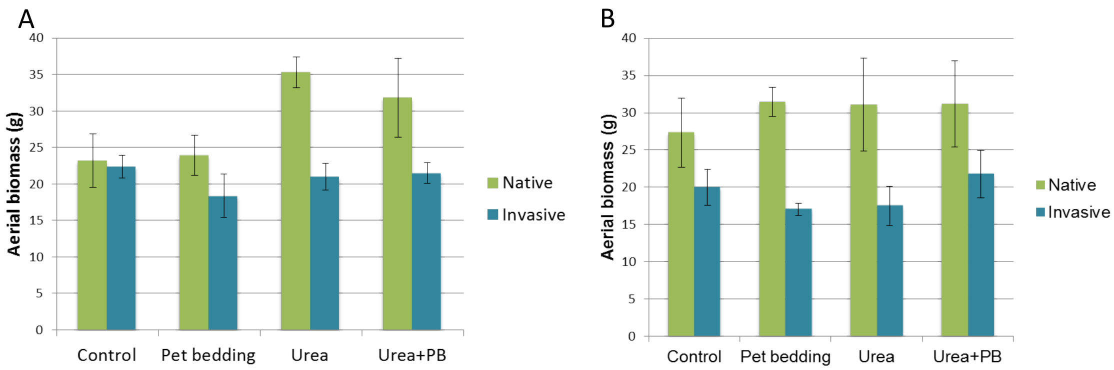

Figure 12. Estimated aerial biomass of native and invasive *Phragmites* in field plots in monoculture (A) and mixture (B) subjected to carbon addition (pet bedding wood shavings), urea nitrogen, both (Urea+PB), or no additions (Control).

Publications and Presentations

Baldwin, Andrew H., Amr E. Keshta, Martina Gonzalez Mateu, Diane E. Leason, and Stephanie A. Yarwood, 2015. Carbon cycling, invasives, and restoration: an overview of current research on the tidal Patuxent. Poster presentation. Patuxent River Conference, Jefferson-Patterson Park and Museum, St. Leonard, Maryland.

Leason, D., M. Gonzalez, and A. Keshta. *Phragmites* on the Patuxent, 2016. (Gonzalez portion: 1. Can carbon additions improve the competitive ability of native *Phragmites*? 2. Root endophytes in Phragmites roots: Identification and functional role). Oral presentation, Patuxent River Conference, Smithsonian Environmental Research Center, Edgewater, Maryland.

Students supported

Undergraduate

The project partially supported a group of 7 undergraduate students in the two-semester ENST Capstone course series, who developed and carried the micronutrient limitation experiment on native *Phragmites*, under the mentorship of the PIs. Additionally, 8 undergraduate students working in the PIs' labs have participated in the project and discussed the project during presentations given by the graduate student during lab group meetings.

M.S.

The project provided the primary research funding for Martina Gonzalez Mateu, an international M.S. student in the MEES program co-advised by the PIs. At least 2 other M.S. students from the PIs' labs have also participated in the research or heard presentations on the project.

Ph.D.

No Ph.D. students were directly supported by the project, although at least 2 Ph.D students in the PIs' labs have participated in the research or contributed to the project during presentations at lab group meetings.

Validation of non-lethal laparoscopic technique for detection of intersex in regional black bass populations

Basic Information

Title:	Validation of non-lethal laparoscopic technique for detection of intersex in regional black bass populations
Project Number:	2014MD315B
Start Date:	7/1/2014
End Date:	6/30/2015
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Publications

1. Yonkos, Lance; Christine Kim; Angela Leasca; Winston Liu; Shivani Patel; Laura Poulsen; Shefali Shah; Taylor Throwe Renuka Tripu, 2014. "Poultry Litter-Induced Intersex in Regional Largemouth Bass Populations," Chesapeake and Potomac Regional Chapter of the Society of Environmental Toxicology and Chemistry Annual Meeting. Grasonville, MD. April 28, 2014 (poster presentation).
2. MacLeod, Alexander; Lance Yonkos, 2014, "Validation of a Laparoscopic Method for Collecting Testis from Largemouth Bass (Micropterus salmoides) for Non-Lethal Detection of Intersex (Testicular Oocytes)," Chesapeake and Potomac Regional Chapter of the Society of Environmental Toxicology and Chemistry Annual Meeting. Grasonville, MD. April 28, 2014 (poster Presentation).
3. Yonkos, Lance; Christine Kim; Angela Leasca; Winston Liu; Shivani Patel; Laura Poulsen; Shefali Shah; Taylor Throwe Renuka Tripu, 2014, "Testicular oocytes and vitellogenin induction in largemouth bass (Micropterus salmoides) exposed to 17 -estradiol and poultry litter at different ages," Society of Environmental Toxicology and Chemistry 35th Annual Meeting. Vancouver, BC. November, 2014 (poster presentation).
4. MacLeod, Alexander; Lance Yonkos, 2014, "Validation of a Laparoscopic Method for Collecting Testis from Largemouth Bass (Micropterus salmoides) for Non-Lethal Detection of Intersex (Testicular Oocytes)," Society of Environmental Toxicology and Chemistry 35th Annual Meeting. Vancouver, BC. November, 2014 (poster presentation).
5. MacLeod, Alexander; Lance Yonkos, 2015. Validation of a Laparoscopic Method for Collecting Testis from Largemouth Bass (Micropterus salmoides) for Non-Lethal Detection of Intersex (Testicular Oocytes), Association of Mid-Atlantic Aquatic Biologists, Cacapon, WV, March 25, 2015 (poster presentation).
6. Kim, Christine; Angela Leasca; Winston Liu; Shivani Patel; Laura Poulsen; Shefali Shah; Taylor Throwe Renuka Tripu; Lance Yonkos, 2014, "Poultry Litter-Induced Intersex in Regional Largemouth Bass Populations," University of Maryland College Park, 2014 Maryland Day, College Park, MD, April 26, 2014 (poster presentation).
7. Kim, Christine; Angela Leasca; Winston Liu; Shivani Patel; Laura Poulsen; Shefali Shah; Taylor Throwe Renuka Tripu; Lance Yonkos, 2014, "Poultry Litter-Induced Intersex in Regional

Largemouth Bass Populations," University of Maryland College Park – Undergraduate Research Day, College Park, MD, April 30, 2014 (poster presentation).

8. Yonkos, Lance; Christine Kim; Angela Leasca; Winston Liu; Shivani Patel; Laura Poulsen; Shefali Shah; Taylor Throwe Renuka Tripu, 2014. "Poultry Litter-Induced Intersex in Regional Largemouth Bass Populations," Chesapeake and Potomac Regional Chapter of the Society of Environmental Toxicology and Chemistry Annual Meeting. Grasonville, MD. April 28, 2014 (poster presentation).
9. MacLeod, Alexander; Lance Yonkos, 2014, "Validation of a Laparoscopic Method for Collecting Testis from Largemouth Bass (Micropterus salmoides) for Non-Lethal Detection of Intersex (Testicular Oocytes)," Chesapeake and Potomac Regional Chapter of the Society of Environmental Toxicology and Chemistry Annual Meeting. Grasonville, MD. April 28, 2014 (poster Presentation).
10. Yonkos, Lance; Christine Kim; Angela Leasca; Winston Liu; Shivani Patel; Laura Poulsen; Shefali Shah; Taylor Throwe Renuka Tripu, 2014, "Testicular oocytes and vitellogenin induction in largemouth bass (Micropterus salmoides) exposed to 17 -estradiol and poultry litter at different ages," Society of Environmental Toxicology and Chemistry 35th Annual Meeting. Vancouver, BC. November, 2014 (poster presentation).
11. MacLeod, Alexander; Lance Yonkos, 2014, "Validation of a Laparoscopic Method for Collecting Testis from Largemouth Bass (Micropterus salmoides) for Non-Lethal Detection of Intersex (Testicular Oocytes)," Society of Environmental Toxicology and Chemistry 35th Annual Meeting. Vancouver, BC. November, 2014 (poster presentation).
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13. Kim, Christine; Angela Leasca; Winston Liu; Shivani Patel; Laura Poulsen; Shefali Shah; Taylor Throwe Renuka Tripu; Lance Yonkos, 2014, "Poultry Litter-Induced Intersex in Regional Largemouth Bass Populations," University of Maryland College Park, 2014 Maryland Day, College Park, MD, April 26, 2014 (poster presentation).
14. Kim, Christine; Angela Leasca; Winston Liu; Shivani Patel; Laura Poulsen; Shefali Shah; Taylor Throwe Renuka Tripu; Lance Yonkos, 2014, "Poultry Litter-Induced Intersex in Regional Largemouth Bass Populations," University of Maryland College Park – Undergraduate Research Day, College Park, MD, April 30, 2014 (poster presentation).

NIWR FINAL REPORT

PROJECT ID: MWRRC PROJECT # 2013-MD-305B

TITLE: VALIDATION OF NON-LETHAL LAPAROSCOPIC TECHNIQUE FOR DETECTION OF INTERSEX IN REGIONAL BLACK BASS POPULATIONS

DATE: July 8, 2016

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1. SUMMARY

Regional Water Problem

Intersex in the form of testicular oocytes (TO) has been reported with high prevalence in regional smallmouth bass *(Micropterus dolomieu)* and largemouth bass *(Micropterus salmoides)* populations [Blazer et al 2007; 2011; Iwanowicz et al 2009; Yonkos et al 2014]. Prevalence in males from Potomac, Shenandoah, Susquehanna, and several Eastern Shore Watersheds often exceeds 80%. The presence of TO is generally accepted as evidence of exposure to endocrine disrupting compounds (EDCs) and suggestive of reproductive compromise and population level effects [Hinck et al 2009]. This raises concerns for the aquatic food web and for the quality of regional drinking water resources. Potential sources of estrogenic contaminants are numerous (e.g., WWTP effluent, agricultural and urban runoff, etc), but, as yet, no causal link to any particular source or contaminant has been identified that adequately explains the spatial heterogeneity of TO occurrence and severity [Blazer et al 2012]. Continued surveillance of resident fish populations is necessary to determine areas of particular concern and to employ a weight-of-evidence framework for identifying culprit contaminants/activities.

Black bass (largemouth and smallmouth) are climax predators within regional freshwater aquatic food webs. Because they appear uniquely sensitive to EDCs, they are the logical choice as sentinel species for monitoring contaminant effects. However, current methods for identification of TO in require field collection and sacrifice of fish for histological identification of oocytes within testis of male specimens. Generally a minimum of ten male fish is considered necessary to accurately assess TO prevalence and severity at a particular site. Because black bass are only minimally sexually dimorphic (and only during the spawning season), sampling efforts also involve collection and sacrifice of numerous females before achieving the requisite number of male fish. Where bass populations are already imperiled through over-exploitation, habitat modification, contaminant-impacts, and/or competition from invasive species, this level of experimental collection necessarily places an additional impact on population number. Thus, populations in regions where analysis of fish is most warranted are among those least able to accommodate this additional pressure. Employing a non-lethal means of determining TO prevalence will serve to alleviate this pressure while still allowing field assessment of endocrine disruption within impacted populations. Further, targeted regions can be re-sampled over multiple seasons or years to observe temporal shifts including recovery or further decline without undue influence to fish populations.

Project Objectives

Fish populations in many Chesapeake tributaries are sufficiently threatened that any additional burden of research-related collection is difficult to justify. This makes development of an effective non-lethal monitoring technique paramount. To that end, we examined the efficacy of endoscopy via the genital pore (laparoscopy) as a non-lethal, minimally-invasive sampling technique for collection of largemouth bass *(Micropterus salmoides)* gonadal tissue for the detection of testicular oocytes (TO). Laparoscopic insertion into the body cavity allows testis tissue collection via biopsy without penetration through the body wall. The method has been demonstrated previously to be effective for non-lethal gender identification in largemouth bass by allowing internal visualization and biopsy of gonadal tissue with high survival, rapid healing, and minimal infection [Matsche 2013]. Our research expands on previous efforts by collecting multiple testis biopsies (n=5) equidistant along a single testis lobe with the purpose of quantifying TO prevalence and severity. This was addressed in four non-sequential phases: *(1) Model Development:* determination of testis biopsy number and size necessary to quantitatively establish TO prevalence and severity; *(2) Recovery and Healing:* determination of post-operative survival and healing following multiple-biopsy tissue collection; *(3) Spawning Capability:* determination of post-operative spawning capability; and *(4) Field Validation:* comparison of non-lethal laparoscopic tissue collection and conventional tissue dissection for efficacy of detecting TO in native fish collected from regional waters with a history of high intersex prevalence.

Hypotheses

H_1: Laparoscopic testicular tissue collection from mature male largemouth bass will heal rapidly and result in minimal mortality

H_2: Laparoscopic testicular tissue collection will not interfere with natural spawning of mature male largemouth bass

H_{3a}: Laparoscopic testicular tissue collection from mature male largemouth bass will allow non-lethal detection of testicular oocyte prevalence comparable to conventional methods that require sacrifice and dissection of specimens

H_{3b}: Laparoscopic testicular tissue collection from mature male largemouth bass will allow non-lethal quantification of testicular oocyte severity comparable to conventional methods that require sacrifice and dissection of specimens

Methods

The research project uses a non-lethal laparoscopic technique (pioneered by fish pathologist Mark Matsche, MD DNR, Cooperative Oxford Laboratory, Oxford, MD) to collect testis biopsy samples prior to routine collection of tissue via dissection. Comparison of efficacy of TO detection by laparoscopic tissue collection versus routine sacrifice and dissection was undertaken to determine the appropriateness of the method for field assessment of TO prevalence and severity. Post-laparoscopic survival, healing and spawning ability were also investigated to estimate the actual level of impact the procedure would have if employed on native bass populations. Efforts toward satisfaction of the several research objectives occurred concurrently with fish from various sources satisfying multiple purposes. For example, hatchery-reared and field-collected largemouth bass were used variously to ensure sufficient numbers of specimens for method comparison, model development, and/or post-laparoscopic survival, healing, and spawning assessment. Field sampling was done in coordination with on-going MD DNR, USGS, and US FWS population assessment efforts allowing use of already targeted-collections to minimize additional sampling impacts. Processing of biopsied and dissected tissues was by routine histological processing and necessarily required sacrifice of research fish. Briefly, tissue samples were dehydrated in alcohol, embedded in paraffin, sectioned at 6 µm, and stained with hematoxylin and eosin [Luna, 1992].

Laparoscopy – Refinement of non-lethal testis tissue collection

Methods for testis tissue collection have been adapted from Matsche [2013] to allow collection of multiple testis biopsies. Briefly, individual largemouth bass were anesthetized with tricaine methanesulfonate (Finquel®, Argent Laboratories, Redmond, WA) and placed dorsal side down between angled plastic positioning blocks with procedures initiated immediately after movement ceases. The urogenital opening was gently dilated using a blunt obturator with surgical lubricant (Surgilube®) followed by insertion of the examination sheath through the urogenital opening to the urinary bladder [Matsche 2013]. The exposed end of the examination sheath was then angled 30° towards the posterior of the fish (to direct the tip toward the head) and 30° toward the left body wall. The obturator was then removed and replaced with the laparoscope and biopsy forceps which were used to perforate the urinary bladder and access the body cavity. To assist with laparoscopic viewing the coelom was inflated using a low-pressure air supply connected to a stopcock on the examination sheath. Guided by the laparoscope, the examination sheath was advanced into the coelom caudally along the left dorsal region of the body wall until the gonad was encountered and gender determined. If determined to be female, procedures were discontinued and the fish was revived (or sacrificed) as necessary. If determined to be male, the five biopsies were collected along an approximately equidistant transect from the left gonad and placed in 10% neutral buffered formalin. The examination sheath remained in body cavity with only the flexible forceps removed during all five biopsies. If survival was to be assessed, a passive integrated transponder (PIT) was inserted into the body cavity prior to removal of the examination sheath. If comparison with testis cross-sections was required, fish were sacrificed (via decapitation) and testis removed via routine dissection.

Phase I – Biopsy Model Development and Validation

The primary goal of this project was to determine whether biopsies could detect TO with the same precision as existing methods using cross-sections from sacrificed fish and whether a severity index could be constructed to facilitate future research. The first step involved determining the instrument size and number of biopsies necessary to provide sufficient tissue for quantitative analysis of TO prevalence and severity. This was necessary to ascertain the utility and limitations of the laparoscopic technique as a non-lethal means of quantifying TO. The approach required taking multiple biopsies at discrete intervals along the testis using instruments of several sizes with cross-sections taken subsequently from regions between biopsies. Briefly, five biopsies were taken along the left testis lobe using forceps meant for small fish (1.7 mm diameter) and five biopsies were taken along the right testis lobe using forceps meant for larger fish (3.3 mm diameter). Routine histological processing of all biopsies and cross-sections allowed comparison of the methods for detection and enumeration of TO. For each specimen, at least one histological section from each of the five tissue segments and ten biopsies was examined via light microscopy for TO presence and other pathology. Establishment of TO prevalence involved observation of one or more discernible oocytes within preserved testis cross-sections and/or biopsies from an individual specimens. All tissue samples of adequate quality were examined for the presence of oocytes under low and moderate magnification (4x and 10x objectives, respectively) with confirmation of potential oocytes determined under high magnification (40x objective). Determination of TO severity conformed as closely as possible to the ranking system described by Blazer et al [2007] for smallmouth bass (*Micropterus dolomieu*). Briefly, five cross-sections taken equidistant along the testis lobe were examined by light microscopy using a 10x objective (approx. 4 mm^2 of tissue) and scored for severity as follows: *Focal Distribution* (Score 1): single oocyte within a microscopic field; *Diffuse Distribution* (Score 2): more than one oocyte in a field of view without physical association with neighboring oocytes; *Cluster Distribution* (Score 3): more than one but less than five closely associated oocytes; *Zonal Distribution* (Score 4): more than five closely associated oocytes or numerous clusters in a field of view. Ranks for each section were averaged to determine a severity rank for the individual specimen. This system is currently being modified in an attempt to provide equivalent results for biopsy collected tissues. Issues that remain to be resolve include: (1) whether biopsies can consistently collect tissues from appropriate regions of the testis and (2) how many biopsy sections are necessary to provide quantitatively similar results to conventional methods.

Phase II – Survival Study

In order be deemed effective post-laparoscopy survival must be high with minimal long-term compromises to fish health. Maintaining batches of fish after the laparoscopic procedure allowed for monitoring of infection and assessing post-operative healing and survival. Fish were anesthetized and, as described above, approximately five biopsies were taken equidistant along the length of the left testis. Post-operative survival was investigated initially as part of a dedicated "survival" study (March 2014) using hatchery-reared fish (SmartFish Farms, Auburn, KY). Additional survival data was accrued on fish used in satisfaction of other project objectives. In the dedicated survival study a subset of fish was sacrificed immediately after the procedure (e.g., controls), another cohort was sacrificed at one week to investigate incidence of infection or other pathology, and the remainder were sacrificed at one month to investigate healing and establish long term survival. On sacrifice testis tissue was also collected for comparison of biopsy vs routine dissection. This served to make more robust the sample size for method validation and model development.

Phase III – Reproduction Study

The third phase of the project was intended to determine if biopsy collection of the testis adversely affects reproductive capability of male fish. It is important to determine whether fish remain competent to spawn following laparoscopic biopsy of testis. Releasing a wild-caught mature male largemouth bass rendered sterile by the laparoscopic procedure could potentially be more damaging to a population than actually sacrificing the fish on collection. He will continue to occupy a territory, consume forage species and attract females to spawn but will not fertilize eggs so will contributing no progeny.

We attempted to complete the reproduction study by field collecting largemouth bass and using laparoscopy to definitively establish gender. Females received no additional treatment while males had the standard five biopsies collected from the left testis. Fish were then segregated by gender and held for a prescribed recovery interval (approximately 14 days) before being paired in spawning raceways at the Manning Hatchery (Cedarville, MD) and monitored for spawning activity. Unfortunately, highly variable weather conditions during the spring of 2014 made the largemouth bass spawning season unpredictable. Paired bass at the Manning Hatchery (both those treated via laparoscopy and control fish) failed to spawn. Because bass spawn only once per year we were unable to pursue this project phase any further. We were able to use the male fish from this portion of the project to further investigate survival and healing and to make more robust the sample size of fish used in comparing biopsy examination to conventional dissection.

Phase IV – Field Validation Study

The final phase of the study involved field validation of laparoscopic testis sampling as an effective tool for identifying TO prevalence and severity. To achieve this endpoint largemouth bass were collected regionally via boat electrofishing from the Potomac and Anacostia Rivers during the spring and summer 2014; largemouth and smallmouth bass was collected in conjunction with USGS efforts in Vermont during summer 2014; and largemouth bass collections were performed during spring 2015 in collaboration with University of Georgia researchers in a lake in Georgia with a history of high TO prevalence. In each system females were released once identified to minimize the number of fish removed from the population. An effort was made to collect testis via laparoscopic biopsy from a minimum of 10 mature male fish for assessment of TO prevalence and severity. An electronarcosis system was used rather than MS222 to avoid the need for quarantine. This allowed immediate release of females. Males were treated as described previously (i.e., after collection of biopsies fish were sacrificed, testis was removed and cross-sectioned, and all tissues were processed for histological examination). In all, approximately 100 field collected male bass were examined for this study. To increase the sample size (i.e., number of specimens available for comparison of TO detection via biopsy and cross-section), preserved testis tissue was sampled via biopsy from approximately 100 male smallmouth bass previously collected and examined by USGS personnel. Tissues were collected “blind” from fish populations known to have high TO prevalence.

Results

Determination of the ability of testis biopsies to detect TO requires a multitude of fish with the intersex condition, preferably demonstrating a range of severities. During initial collection efforts we encountered few fish with TO. This limited the ability to make a meaningful comparison between the two methods. Subsequent live (Vermont, June 2014; Georgia, Mar 2015) and post-mortem (USGS, Apr 2015) tissue collections dramatically increased the number of specimens with TO.

Tissue collection, processing, and histological analysis of slides was completed in late 2015 and early 2016. Overall results indicate that biopsy collection via laparoscopy was able to detect TO prevalence with approximately 80% of the efficacy of conventional cross-sections from sacrificed fish. There was, however, significant variability between sampled populations with biopsy collection actually exceeding cross-section sampling in at least one instance. While laparoscopic tissue collection shows promise for detecting testicular oocytes, several limitations became apparent as a result of the current study. When TO severity is high, laparoscopic detection compares favorably to traditional lethal detection methods. Prevalence in similarly detected by the two methods and estimates of severity based on enumeration of encountered TO are of similar magnitude. However, when severity is low (i.e., where TO occur rarely within mature testis) laparoscopic tissue collection was not found to match traditional methods for TO prevalence detection. This disparity is a reflection of the comparative amount of tissue observed by each method and the region of testis sampled. Transverse sections provide substantial area for observation, the larger the diameter of the testis the more tissue that is observed. Likewise, transverse sections ensure encountering all regions of the testis including central "hilus" regions where TO have been found to occur most frequently. Because biopsies are of a fixed size, area of observation does not change with size of testis. Biopsies are also not certain to include the hilus region. To address the in-equality in area between biopsy and transverse sections, we estimated that 5 step sections per biopsy would provide an equivalent "unit of observation" to one transverse section. This proved to be an under-estimation, especially for sample populations comprised of numerous large individuals. Overall, our 5 step-section units averaged only 60% of average transverse sections. In consequence, where TO were rare (i.e., where severity was low) detection was less frequent because less tissue was observed. Poor TO detection at low severity may not be a particularly significant limitation. Generally, reproductive effects in fish are not believed to occur unless TO is very severe. Therefore, populations of fish with low TO severity would not generally be considered in need of increased management activity or of interest for further investigation.

Survival — Results from the dedicated "survival" study indicate post-surgery survival to 28 days is high (approx. 90%) as is healing based on integrity of the urinary bladder (≥ 90% of surviving fish). Survival of laparoscoped fish employed in satisfaction of other project *Phases* (e.g., spawning capability) indicate that this is a reasonable estimate of survival. Several caveats to be explored include the implications of season and/or water temperature on survival and health of fish following laparoscopic tissue collection. For example, fish with large parasite loads, like those collected from the Anacostia River, arrive with compromised immune function suggesting a greater likelihood of post-operative infection and subsequent mortality.

Spawning Capability — Eight pairs of largemouth bass were maintained in spawning raceways at the Manning Hatchery, Cedarville, MD. These fish were collected regionally (mostly from the Potomac River) over approximately the month of April 2014. Unfortunately, unusually cold and variable spring weather impeded normal spawning of largemouth bass in the region, both in natural surface waters and in the hatchery ponds. While male fish in raceways demonstrated typical spawning behaviors (e.g., nest preparation and coaxing of females), females released no eggs so males released no sperm. Without the natural release of eggs to induce the subsequent release of milt, post-operative spawning capability could not be assessed. As largemouth bass spawning is seasonal and highly temperature dependent, we were unable to address this research phase during the period of project

Application to Other Species

At the request of MD DNR, US FWS, and USGS fisheries biologist, the laparoscopic method has been adapted for use with several other fish species of interest:

- First is the smallmouth bass which has been found in the Potomac and Shenandoah Rivers to have a high incidence of TO. This species has also suffered population declines in these regions as a result of fish kills over the past decade. While no causal link has been established, the possibility exists that TO induction and premature mortality are related to a common contaminant exposure occurring at some earlier life stage. Laparoscopic tissue collection will allow non-lethal investigation of TO prevalence in those regions where smallmouth bass populations are at particular risk. As this species is very similar to the largemouth bass, only minimal adaptation of the method is required. Also, since smallmouth bass generally occupy a distinct range from that of largemouth bass, this expands the inland surface water systems that can be investigated for TO using this method.

- The northern snakehead *(Channa argus)* is a large fast-growing predatory fish species native to China but now invasive and established in many Chesapeake Bay tributary rivers. The species competes effectively with largemouth and smallmouth bass but grows faster so adds an additional stressor to systems where it is present. Currently very little is known about the reproductive biology of the snakehead including spawning frequency and other fecundity criteria. Even knowledge of whether females are single or multiple spawners is lacking. For this reason the laparoscopic method is being adapted to non-lethally investigate gonad development in female northern snakehead. The intent is to track gonad development in individual female fish over protracted periods to determine seasons of maturation and establish whether fish are single or multiple spawners. This information is considered paramount when devising management strategies. As the northern snakehead differs morphologically in significant ways from the largemouth bass, modification of the laparoscopic method will be more substantial and may require different instruments.

Significance

Results suggest laparoscopic tissue collection has promise as a non-lethal field sampling strategy. Since mortality is generally low the method appears especially well suited for use in regions where fish populations are already compromised. The method also shows significant adaptability for application to other species of interest, broadening the scope of potential studies. This procedure could provide fishery managers and scientists with an additional tool for monitoring fish populations for intersex both temporally (by repetitive sampling of individual fish) and spatially (by including compromised regions without causing undue impact). Field studies could be designed to correlate observed effects (i.e., TO prevalence and severity) with particular land use practices (e.g., non-point source agricultural runoff, WWTP effluent discharges) in an attempt to identify culprit contaminants and devise management strategies. There also exist the potential to use the method to non-lethally sample other tissues (e.g., liver) for contaminant analysis.

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2. PUBLICATIONS ASSOCIATED WITH THE RESEARCH PROJECT

All work associated with this project has been completed. Two manuscripts are planned for publication describing methods developed and results generated during completion of the project. The first is under revision by project collaborators and will be submitted by the end of July 2016. The second will be submitted for journal publication by the end of September, 2016

MacLeod AH, V Blazer, M Matsche, and LT Yonkos. 2016. A novel non-lethal laparoscopic approach to detect intersex in largemouth bass *(Micropterus salmoides)* and smallmouth bass *(Micropterus dolomieu)*. Planned for submission to *Environmental Toxicology and Chemistry*, July 2016.

MacLeod A and L Yonkos. 2016. Implication of tissue collection techniques on assessments of testicular oocyte prevalence and severity in black bass. Planned for submission to *Environmental Toxicology and Chemistry*, September 2016.

There have been numerous poster and platform presentations including eleven abstracts accepted at regional and national professional society meetings and four public presentations.

Accepted abstracts at professional scientific meetings

MacLeod AH and LT Yonkos. 2016. The Uncertainties Associated with Detection and Quantification of Intersex (Testicular Oocytes) in Fish. Society of Environmental Toxicology and Chemistry 35th Annual Meeting. Orlando FL. November, 2016 (poster presentation; abstract accepted)

MacLeod AH and LT Yonkos. 2016. Complications and Considerations of Assessing Intersex in Wild Fish Populations. Chesapeake and Potomac Regional Chapter of the Society of Environmental Toxicology and Chemistry Annual Meeting. Columbia, MD. April 18, 2016 (platform presentation).

MacLeod AH. Complications and Considerations of Assessing Intersex in Wild Fish Populations, 5th Young Environmental Scientists Meeting of the Society of Environmental Toxicology and Chemistry, Gainesville, FL. February 28th – March 2nd, 2016 (platform presentation).

MacLeod AH and LT Yonkos. 2016. Validation of a Laparoscopic Method for Collecting Testis from Largemouth Bass *(Micropterus salmoides)* for Non-Lethal Detection of Intersex (Testicular Oocytes). 21st Annual Maryland Water Monitoring Council Conference, November 13th , 2016 (platform presentation).

MacLeod AH and LT Yonkos. 2015. Non-lethal Detection of Intersex (Testicular Oocytes) from Largemouth Bass *(Micropterus salmoides)* Using Laparoscopy. Society of Environmental Toxicology and Chemistry 35th Annual Meeting. Vancouver, BC. November, 2015 (platform presentation; abstract accepted)

MacLeod AH and LT Yonkos. 2015. Validation of a Laparoscopic Method for Collecting Testis from Largemouth Bass *(Micropterus salmoides)* for Non-Lethal Detection of Intersex (Testicular Oocytes). Chesapeake and Potomac Regional Chapter of the Society of Environmental Toxicology and Chemistry Annual Meeting. Columbia, MD. April 24, 2015 (platform presentation).

MacLeod AH and LT Yonkos. 2015. Validation of a Laparoscopic Method for Collecting Testis from Largemouth Bass *(Micropterus salmoides)* for Non-Lethal Detection of Intersex (Testicular Oocytes). Association of Mid-Atlantic Aquatic Biologists. Cacapon, WV. March 25, 2015 (poster presentation).

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MacLeod AH and LT Yonkos. 2014. Validation of a Laparoscopic Method for Collecting Testis from Largemouth Bass (*Micropterus salmoides*) for Non-Lethal Detection of Intersex (Testicular Oocytes). Chesapeake and Potomac Regional Chapter of the Society of Environmental Toxicology and Chemistry Annual Meeting. Grasonville, MD. April 28, 2014 (poster presentation).

Yonkos L, C Kim, A Leasca, W Liu, S Patel, L Poulsen, S Shah, T Throwe and R Tripu. 2014. Poultry Litter-Induced Intersex in Regional Largemouth Bass Populations. Chesapeake and Potomac Regional Chapter of the Society of Environmental Toxicology and Chemistry Annual Meeting. Grasonville, MD. April 28, 2014 (poster presentation).

Public presentations:

MacLeod AH and LT Yonkos. 2015. Non-lethal Detection of Intersex (Testicular Oocytes) from Largemouth Bass *(Micropterus salmoides)* Using Laparoscopy. MS Exit Seminar - Department of Environmental Science and Technology Seminar Series. April 15, 2015.

MacLeod AH and LT Yonkos. 2014. Validation of a Laparoscopic Method for Collecting Testis from Largemouth Bass (*Micropterus salmoides*) for Non-Lethal Detection of Intersex (Testicular Oocytes). University of Maryland - Environmental Science and Technology Department Annual Graduate Student Research Day. College Park, MD, May 13, 2014.

Kim C, A Leasca, W Liu, S Patel, L Poulsen, S Shah, T Throwe and R Tripu, and L Yonkos. 2014. Poultry Litter-Induced Intersex in Regional Largemouth Bass Populations. University of Maryland College Park – Undergraduate Research Day. College Park, MD, April 30, 2014.

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University of Maryland College Park. 2014 Maryland Day. College Park, MD, April 26, 2014.

3. STUDENTS SUPPORTED BY RESEARCH PROJECT

One master's student in the University of Maryland - Department of Environmental Science and Technology, Alex MacLeod, focused the majority of his thesis research on validation of laparoscopic tissue collection as a viable means of non-lethal testicular oocyte detection and quantification. By adapting techniques and equipment to provide quality results, he optimized the method for rapid and reproducible biopsy collection on bass testis from fish of various ages/sizes. He also adapted the method for use on other species including the invasive northern snakehead *(Channa argus)*. Additionally, he worked with regional partners to demonstrate the utility of laparoscopy as a sound approach for field monitoring of fish condition. Included were fisheries biologists and environmental toxicologists from MD DNR, USGS Leetown Science Center, US FWS Chesapeake Bay Field Office, the University of Georgia, and elsewhere.

A Gemstone Undergraduate Research Team comprised of eight (8) undergraduate students from various University of Maryland academic departments was been supported in part by this MWRRC-funded project. They investigated poultry litter-induced intersex in largemouth bass by exposing batches of fish at various ages to aqueous poultry litter mixtures before growing to maturity for assessment of testis pathology. While they did not actually use the laparoscopic procedure on their test fish, they were trained on the method and assisted Alex MacLeod in his research efforts.

4. NOTABLE ACHIEVEMENTS AND AWARDS

Alex MacLeod was awarded a $5,000 Dean's Fellowship from the College of Agriculture and Natural Resources largely in recognition of the quality of his laparoscopy validation research project. He presented preliminary results of the project at the Society of Environmental Toxicology and Chemistry (SETAC) national (poster; Nov 2014) and regional (platform; Apr 2015) meetings, and presented final results of the research at the 2015 SETAC national meeting in November 2015. Alex completed his thesis in May, will submit two manuscript for publication in coming months and will continue for a PhD in the ENST graduate program beginning in the fall.

Environmental Concentrations and Exposure Effects of Environmental Gestagens on a Sentinel Teleost Fish

Basic Information

Title:	Environmental Concentrations and Exposure Effects of Environmental Gestagens on a Sentinel Teleost Fish
Project Number:	2014MD321G
USGS Grant Number:	G14AS00014
Start Date:	9/1/2014
End Date:	8/31/2017
Funding Source:	104G
Congressional District:	MD-005
Research Category:	Water Quality
Focus Category:	Water Quality, Toxic Substances, Wastewater
Descriptors:	None
Principal Investigators:	Edward F Orlando, Michael T Meyer, Patrick Phillips

Publications

There are no publications.

Department of the Interior, United States Geological Survey, National Institutes for Water Resources

PROGRESS REPORT

September 1, 2014 – February 28, 2015

1. **Title of Project and Grant ID:** Environmental Concentrations and Exposure Effects of Environmental Gestagens on a Sentinel Teleost Fish (2014MD321G)
2. **Principal Investigator and Organization:** Edward F. Orlando, University of Maryland College Park
3. **Date of Report:** June 12, 2015
4. **Overview of Progress.**

   In the first six months of our study, we have completed (a) setting up the fish exposure system, (b) testing the exposure system and capability of our lab group by doing an ethanol exposure, (c) executing our first gestagen exposure experiment, and working out gestagen analysis methods. We have interesting data from both the ethanol solvent exposure and the first gestagen exposure experiments. We are off to a strong start, and I am pleased with our progress.

5. **Project Hypothesis, Objectives, and Progress Update.**

   A. *Summarize the hypothesis and objectives of your project.*

   We hypothesize that gestagen exposure will negatively affect the reproductive health of fathead minnows and that progesterone and at least some of the progestins will be measured in ng/L (biologically active concentrations) in environmental samples from the Southeast Stream Quality Analysis (SESQA) study.

   Objective 1: We will assess the exposure effects of gestagens on fathead minnow reproductive health using a combination of behavioral and physiological endpoints to determine the most effective gestagen and the most sensitive endpoints.

   Objective 2: We will augment an existing 41 hormone method, which already includes progesterone and one progestin norethindrone, developed by Dr. Mike Meyer (Co-PI), with three additional progestins that are not in the method but that are required for this study: levonorgestrel, gestodene, and drospireone.

   Objective 3: We will determine the concentration of gestagens in water, POCIS, and sediment samples from the SESQA study sites. Results from this objective will provide the first comprehensive examination of the occurrence of environmental gestagens in stream systems in the United States. We will also quantify concentration of gestagens in the lab exposure studies of Objectives 1 and 4.

   Objective 4: We will integrate the findings from Objective 1-3 to determine environmentally relevant exposure concentrations for individual gestagens and also test one gestagen mixture to verify and compliment the results of the earlier objectives, and to initiate the identification of biomarkers of gestagen exposure, which currently are not known.

   B. *Which objectives have you completed to date?*

   We have substantially begun Objectives 1 and 2 (please see preliminary results below). Results and monthly progress is discussed in a group conference call on the 2nd Tuesday of each month with Drs. Michael Meyer (co-PI), Dana Kolpin (USGS, Lawrence, KS), and David Alvarez (USGS, Columbia, MO).

C. What work is planned during the next 6-12 months?

We will complete the first and second objectives and make substantive progress on the third objective. We will present preliminary results at the North American Conference of Comparative Endocrinology, Ottawa Canada, June 21-25, 2015. We will have our first paper in review from the research supported by this grant by the end of first year and will be working on a second manuscript. Our first face-to-face group meeting (same individuals as in 5B. above) will occur at the Society of Environmental Toxicology and Chemistry annual meeting, Salt Lake City, UT, November 1-5, 2015.

6. Is there anything the DOI/USGS/NIWR needs to be notified of relevant to the study's progress?

Progress is on target or greater than our projected schedule.

7. Preliminary results.

Dr. Mike Meyer has initial results suggesting that he will have no difficulty measuring the gestagens in this project at the required ng/L concentrations. He is currently working on maximizing sensitivity of the assays and validating those methods.

In the Orlando Lab's first experiment, we have tested the effects of different concentrations of ethanol (0, 0.001, 0.0001, and 0.00001% EtOH) on the reproductive behavior and fecundity of fathead minnows. We find no published papers that have systematically studied the effects of ethanol on these ecologically critical endpoints in fish. This was very surprising to us. We chose our ethanol concentrations based on the literature and made sure we were at or below the concentrations used by others. We found a negative impact on numbers of eggs produced by fish at the two highest concentrations with no difference seen in the 0.00001% EtOH and water only control.

In our second experiment, we exposed fish to water only, water plus 0.0000095% EtOH, 10 ng/L, and 100 ng/L gestodene (in 0.0000095% EtOH). Gestodene is a synthetic gestagen or progestin that is used in oral contraceptives. In previous research by my lab, we found that gestodene did not activate the *nuclear progesterone receptor* but was a strong activator of the *nuclear androgen receptor* of fathead minnows (doi: 10.1021/es501428u). In the current study, gestodene exposure of reproductive adult fathead minnows was associated with a dramatic decrease in egg production (fecundity) and masculinization of secondary sex characteristics (male phenotype including nuptial tubercles and finspot, induced in female fish). Preliminary analysis suggests that behavior of both females and males is altered and may be contributing to the decrease in spawned eggs. *The importance of these data is that decreased egg production and altered reproductive behavior have strong links to effects on population (recruitment to the next generation) and, by extension, aquatic ecosystem health.*

Funding from the DOI/USGS/NIWR is enabling the training of one PhD student and approximately six undergraduate research assistants. Two of these undergraduates are considering research careers in part because of this experience.

The Fate of Emerging Contaminants in Poultry Litter Digestion

Basic Information

Title:	The Fate of Emerging Contaminants in Poultry Litter Digestion
Project Number:	2015MD324B
Start Date:	3/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	MD-005
Research Category:	Water Quality
Focus Category:	Treatment, Water Quality, Agriculture
Descriptors:	None
Principal Investigators:	Stephanie Lansing, Stephanie Ann Yarwood, Lance Yonkos

Publications

There are no publications.

Final Report for
Fate of Emerging Contaminants in Poultry Litter Digestion
2015MD324B

Reporting period: March 1, 2015-February 29, 2016
Project duration: March 1, 2015-February 29, 2016

Prepared for:
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Submitted April 29, 2016

BACKGROUND INFORMATION

Regional Water Pollution Problem and Project Solution

The Delmarva Peninsula is considered one of the top poultry producing regions in the United States. Three Bay counties – Sussex County, DE; Lancaster County, PA; and Wicomico County, MD – are among the top 20 poultry producing counties in the nation (USEPA, 2010). The region generates 600 million birds and 1.6 billion lbs of poultry litter annually, which consists of bedding, feathers, manure and feed (Fisher et al., 2005). Best management practices for poultry wastes include land application of litter, which is rich in nitrogen and phosphorus. However, agriculture is the largest single source of nutrient loading in the Chesapeake Bay (USEPA, 2010). Excessive land application of poultry waste has impacted water quality in surface and ground waters through the region. Surface run-off from manure-amended fields has resulted in harmful algal blooms, widespread anoxia, increased microbial pathogens and declines in submerged aquatic vegetation. Most previous research has focused on documenting nutrient and pathogen pollution from poultry wastes, but recent attention has focused on non-traditional poultry litter-associated contaminants (PLACs), as their effects on surface waters are relatively unknown (Fisher et al., 2005; Burkholder et al., 2006). PLACs in poultry litter include veterinary antibiotics, naturally-occurring steroid hormones, trace metals, and pesticide residues (Bolan et al., 2010). Previous studies have demonstrated the potential for PLACs to be transported into ground and surface waters from litter amended fields (Dutta et al., 2012; Chee-Sanford et al., 2009), and the potential risks to human and environmental health. Burkholder et al. (2006) published the findings of a workshop on Environmental Hazards Impacts of Concentrated Animal Feeing Operations (CAFOs) and concluded that there is a need to document and promote best management practices that minimize input of antibiotics from CAFOs into fresh water.

In 2010, over 29 million pounds of antimicrobials were sold for livestock use in the U.S., which is estimated to be 3 to 4 times the amount of antibiotics used by humans (USFDA, 2011). However, estimating antimicrobial use in poultry is difficult. The USDA reports at least 50 active antimicrobial compounds have been approved for use in poultry (USFDA, 2009). Mellon et al. (2001) estimated that nearly 40% of all antimicrobials used for non-therapeutic purposes in livestock were administered to poultry. Many of these compounds are identical to those administered to humans in clinical settings, including tetracyclines, macrolides, and fluoroquinolones (USFDA, 2004). In poultry litter, antibiotics are present in the unused feed and in the manure as both the parent compound and secondary metabolites. When applied to fields, manure breaks down and antibiotics within the manure matrix can be transported into waterways, taken up by plants and higher organisms, and select for antibiotic resistance in the environment (Martinez, 2009; Kumar et al., 2005; Chee-Sanford et al. 2009). Recent attention has also focused on the environmental effects of sub-lethal concentrations of antibiotics, which can promote the proliferation of resistant microbes and often fall below the detection limits of traditional analytical methods (Andersson and Hughes, 2014). Future research should focus on methods that detect the bioavailability of antibiotics to organisms in the environment.

Poultry litter also contains naturally synthesized hormones excreted in the manure (Zhao et al., 2008), including estrogens, androgens and progestogens. While data are limited on hormone excretion of poultry, a recent analysis estimated that poultry litter application to Maryland farmland contributes 35.27 lbs/yr of progesterone and 24.3 lbs/yr of estrone to the environment (Bevacqua et al., 2011). These endocrine-disrupting compounds and their metabolites can enter aquatic environments through runoff from cropland fertilized with animal manure. While hormones are typically present in surface waters at low concentrations, these compounds remain biologically active even at low levels (ppb) (Burkholder et al., 2006). Recent research has shown that the reproductive biology, physiology, and fitness of fish and other aquatic organisms may be adversely affected by exposure to these low concentrations of steroid hormones (Zhao et al., 2008). Future research on the occurrence, fate and transport of naturally-occurring hormones in poultry litter is necessary to understand the potential impact on Maryland ecosystems.

Until recently, research into emerging contaminants in the environment has focused on optimizing traditional analytical methods to detect low concentrations of these compounds in complex matrices, including soil and manure. However, analytical methods have several disadvantages for assessing chemicals in the environment. First, they are unable to determine toxicity to living organisms or whether the chemical is present in a form that is harmful to living cells. Secondly, analytical methods do not fully account for possible synergistic or antagonistic effects on living organisms by other chemicals present in complex matrices (Costanzo et al., 1998). Specifically, quantitation of estrogens and antibiotics by liquid chromatography (LC) methods requires significant sample clean-up and chemical extraction, to the point that sample media to be quantitated differs significantly from media to which fish are actually exposed. Likewise, LC methods only quantitate target analytes without regard for other constituents that may contribute estrogenicity or antibiotic resistance, such as heavy metals (Georgescu et al., 2011; Berg et al., 2005). The relative contribution of various estrogenic and antibiotic constituents may be difficult to determine, especially where more potent contributors, such as Estradiol (E2) are at or below analytical detection limits.

Consequently, there is a trend in toxicological research towards using living organisms to screen for contaminants and advance understanding of the effects of these compounds in the environment. Antibiotics at low concentrations stimulate or depress bacterial gene expression at the transcriptional level. These changes can be detected as luminescence effects on bacterial promoter-reporter constructs with a *lux*-promoter (Goh et al., 2002). Lux-based yeast assays have successfully been used as a rapid means of assessing water samples for estrogenic activity (Sanseverino et al., 2009). Applied to manure samples, bioluminescent assays offer the advantages of reflecting the amount of the contaminant available to organisms when the manure is released into the environment through land application or run-off. For hormones, biological assays using bioluminescent yeast estrogen screen (BLYES) can be used measure total estrogenicity (Sanseverino et al., 2009). Furthermore, these biological assays could provide insight into the matrix effect of manures on the transformation of antibiotics and hormones.

Anaerobic digestion (AD) of animal manures is used to produce on-farm renewable energy in the form of methane-enriched biogas, while reducing greenhouse gases, fecal pathogens, organic pollutants and odor. While the mechanisms of biogas production are well understood, the fate and transport of antibiotics and hormones during AD have only recently been explored. Previous studies have indicated variable degradation of antibiotics in dairy and swine manures in AD systems. Arikan et al. (2006) reported a 59% decrease of tetracycline in dairy manure after a 64-day AD retention time. Varel et al. (2012) demonstrated a correlation between AD temperature and total reduction of chlortetracycline and monensin in dairy and swine manure. Further research is needed to understand whether antibiotic degradation is a result of biological activity in the reactor or abiotic factors in the digestion process, such as time and temp. Nevertheless, results from AD of other animal manures at mesophilic temp. (35-37°C) have indicated a reduction in several antibiotic concentrations, including those used in poultry.

The fate of naturally-occurring hormones during poultry litter digestion has not been explored. A prior study by Hakk et al. (2005) reported degradation of biologically active 17β-estradiol in heated composting of poultry litter. Paterakis et al. (2012) reported > 50% removal of steroid estrogens in the anaerobic digestion of primary and mixed sewage sludge, although the exact mechanism was undetermined. Microbial breakdown appears to be a key route for the degradation of hormones, which should be facilitated by the multi-faceted microbial-driven AD process (Zhao et al., 2008).

Since the anaerobic digestion environment has both abiotic and biotic conditions that should assist in the degradation of antibiotics and naturally-occurring steroid hormones in poultry litter, it has the potential to reduce these contaminants in the waste and, subsequently, the environment. Additionally, AD can reduce nutrients and pathogens in livestock wastewater (Lansing and Martin, 2006; Lansing et al., 2008) and generate biogas that can be integrated into the farm's power grid (Giesy et al., 2005). AD technology has potential as a sustainable waste management system for Maryland poultry farmers that can improve regional water quality and provide an economic benefit to regional businesses.

OBJECTIVES

Anaerobic digestion is a waste-to-energy process that reduces GHG emissions, provides on-farm energy and still allows for application of nutrients in manure onto agricultural fields. The application of anaerobic treatment to poultry litter on a commercial scale has gained interest in the region. Current research at the University of Maryland is investigating methods of removing nitrogen and phosphorus from digested litter to address water quality concerns from nutrient pollution. However, the fate of antibiotics and estrogens in poultry litter digesters had not been explored. The long-term goal of project is to provide a manure treatment option to poultry farmers that would have the benefits of producing energy, reducing pollutants, and providing a high quality fertilizer that can be relocated off-farm, if necessary, and applied more appropriately to reduce the contaminant load to the environment. In order to achieve this goal, it is essential to evaluate antibiotics in poultry litter and the effects of digestion on these compounds. This project explored the fate of antibiotics and hormones in poultry litter through AD and the potential for AD to reduce these contaminants in the manure prior to field application. Furthermore, this study was aimed to evaluate biological assay methods for assessing the biological availability of antibiotics and estrogens to organisms, which is the primary concern for their presence in the environment. The specific objectives of the project were to: 1) evaluate concentrations of antibiotics and hormones in poultry litter pre and post-digestion using standard analytical methods (LC-MS/MS); 2) evaluate the biological effect of these concentrations on microorganisms using biological assays; and 3) compare the results of the measured concentrations with the determined biological effect.

RESULTS

Poultry Litter Anaerobic Digester

The poultry litter digester was designed, constructed and loaded with inoculum from a dairy manure anaerobic digester located at the USDA Beltsville Agricultural Research Center on October 1, 2014 (Figure 1). Chipped poultry litter was obtained from a commercial poultry farm on the Eastern Shore of Maryland. Poultry litter loading into the digester began on October 2014. The litter loading regime started at five days per week and was subsequently decreased to the current loading rate of three times per week.

Figure 1: The 850 L poultry litter digester located at USDA Beltsville Agricultural Research Center.

Total biogas produced, percent methane (CH_4), total solids (TS), volatile solids (VS), pH, and temperature were measured at least once weekly, with total Kjeldhal nitrogen (TKN) and ammonia nitrogen content measured biweekly. Chemical oxygen demand (COD), soluble chemical oxygen demand (sCOD), and FOS/TAC ratio (a measure of the ratio between volatile fatty acids to alkalinity) were measured monthly. Methane and H_2S concentrations in biogas were measured using a Biogas 5000 meter (LANDTEC, Colton, CA). The TS and VS were measured by heating to constant mass, first at 105 ºC, and then at 550 ºC (APHA 2005), while COD and sCOD (samples for sCOD acidified to pH ≤2 and filtered through 0.45 um filter) were analyzed using the HACH® (HACH, Loveland, CO) adapted digestion method (Jirka et al., 1975). Ammonia nitrogen and TKN were analyzed from samples acidified to pH≤2 and filtered through 0.45 um filter using Lachat (HACH, Loveland, CO) (QuikChem Method 10-107-06-2-O for NH_3-N and QuikChem Method 13-107-06-2-D for TKN). FOS/TAC analysis was conducted by quantifying the amount of 0.1 N acid samples required to bring down the pH of filtered samples to 5 and from 5 to 4.3.

Between August 2015 and March 2016, the poultry litter digester produced an average 101 L CH_4/kg VS (Figure 2), with the methane content in the biogas averaging 56% and hydrogen sulfide concentration averaging above 0.79%.

The pH of the digester content (effluent) remained at the optimal level (Table 1). Total solids, VS, and COD destruction were found to be low for anaerobic

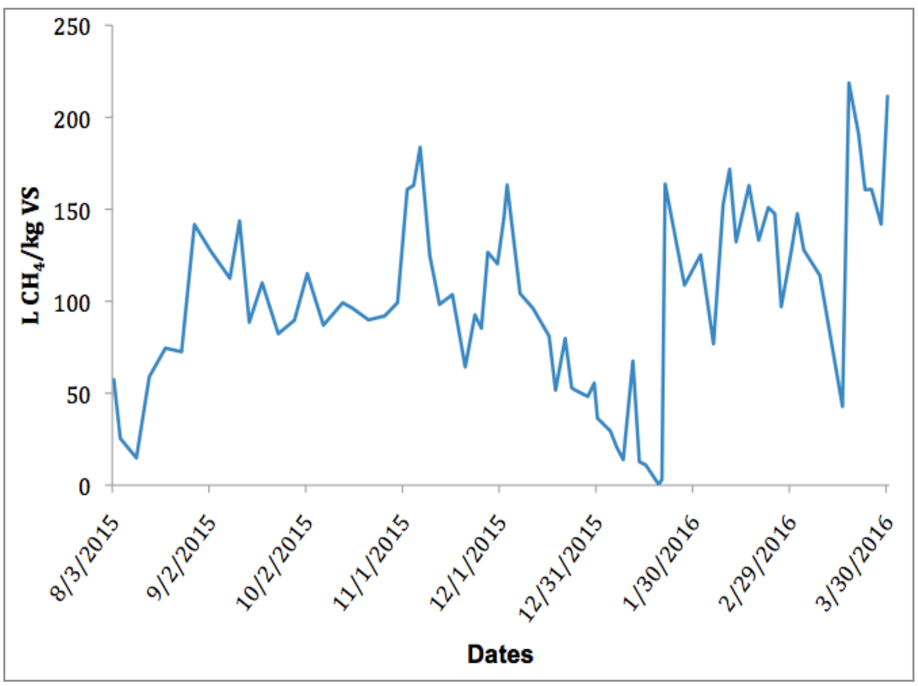


Figure 2: Methane production by poultry digester operating at USDA Beltsville Agricultural Research Center (August 2015 – March 2016).

digestion at 20, 25, and 33%, respectively. This is likely due to the high concentration of recalcitrant wood chips present within the poultry litter. Indeed, only 22% of the total COD could be attributed to soluble substrates that can be easily accessed by microorganisms.

The average effluent FOS/TAC ratio (0.48) was found to be higher than the optimal ratio of 0.2 (Table 1). This was due to a few months (i.e. November and December 2015), where the value rose to more than 1. The ratio, however, decreased between January and March 2016 to the optimal level of approximately 0.2. As expected, there was only a slight decrease in the TKN values between the influent and the effluent (13%), as nutrients are largely preserved in the digestion process. Ammonium content within the digestion process increased (by 39%), as ammonium is released when organic matter within the poultry litter is degraded.

Table 1. Summary of poultry litter digestate analysis (January 2015 – March 2016)

	Influent	**Effluent**
pH	7.34 ± 0.13	7.34 ± 0.03
Total solids (g/kg)	62.5 ± 4.4	49.8 ± 4.0
Volatile solids (g/kg)	42.8 ± 3.1	34.3 ± 3.1
COD (g/L)	40.1 ± 5.3	27.0 ± 4.1
sCOD (g/L)	9.16 ± 0.99	5.44 ± 0.63
FOS/TAC ratio	1.25 ± 0.35	0.48 ± 0.20
TKN (mg/L)*	1810 ± 136	1580 ± 35
NH_3-N (mg/L)*	368 ± 26	512 ± 30

*Values up to September 2015, with latest data still under analysis.

Estrogen Analysis

Sample extraction: Preparation of anaerobic digester influent and effluent samples for quantification of estrogen analytes (LC-MS/MS) and estrogenicity (BLYES) was complicated by substantial matrix interference resulting in higher than expected method detection limits (MDL). Typically 1.0 L aqueous environmental samples would be filtered (0.7 µm GFF) before extraction to Oasis® HLB (hydrophilic / lipophilic balanced) SPE cartridges. Collected analytes would be eluted from cartridges with methanol, taken to dryness and reconstituted to 1.0 mL, producing a 1000x concentration of target analytes and yielding a BLYES assay MDL of approximately 0.1 ng EEQ/L and LC-MS/MS MDL of approximately 0.6 ng/L. Because the anaerobic digester influent and effluent samples were so concentrated, 50 mL conical bottom tubes of media were centrifuged and only 20 mL aliquots of supernatant were filtered before extraction. This resulted in a BLYES MDL of approximately 5.0 ng EEQ/L (acceptable for preliminary analysis) and LC-MS/MS detection MDL of 30 ng E2/L (unacceptably high even for preliminary analysis). Modification of extraction methods, including ultra-centrifugation and subsequent lyophilization of aqueous and solids fractions, is underway to allow targeted analyte recovery and effectively reducing MDL for LC-MS/MS analysis.

Estrogenicity: Despite limitations in sample preparation, estrogenicity > 5.0 ng/L was detectible in all anaerobic digester influent (n = 4) and effluent (n = 4) samples analyzed (Figure 3). Concentrations averaged 13 ± 5.4 ng/L and 14 ± 2.9 ng/L for influent and effluent samples, respectively. These results suggest anaerobic digestion did not produce a meaningful reduction in estrogenicity within the aqueous sample fraction. This finding is preliminary and requires additional investigation before confirmation. Analyses were only conducted on aqueous fractions of samples. Therefore, it is possible that total estrogenicity was reduced, but that additional

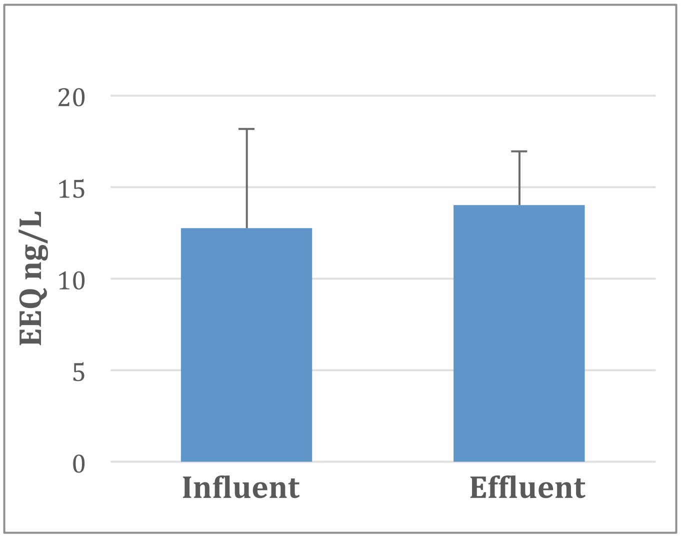


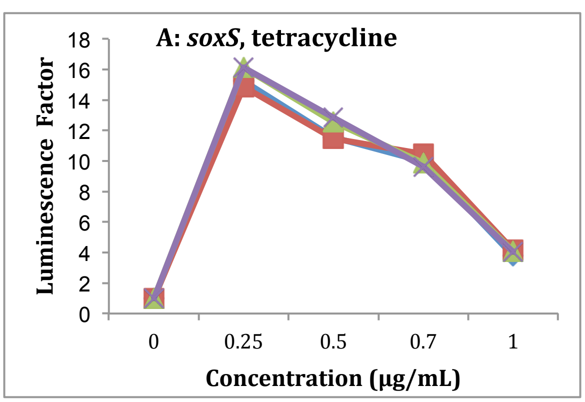
Figure 3: Mean estrogenicity, as measured by BLYES assay, in AD influent and effluent samples (error bars are standard deviation).

estrogenic constituents partitioned into the aqueous phase as solids were eliminated during anaerobic digestion. Analysis of estrogens in source poultry litter and aqueous and solid compartments of multiple influent and effluent samples would allow calculation of an "estrogen budget" and determination of changes related to anaerobic digestion.

Antibiotics Analysis

Reporter Strains: A component of this project was the development of a method to determine the biological effect of antibiotics found in pre- and post-digested poultry litter (influent and effluent). Five bacterial reporter strains (two *E. coli* strains with *lux* plasmids containing *soxS* and *micF* promoters (called *soxS* and *micF* from henceforth), and three *Salmonella* strains with *lux* plasmids containing *ilvC*, *ilvLG*, and *tsr* promoters (termed as *ilvC, ilvLG, and tsr* from henceforth)), as referenced in Tsui et al. (2004) and Melamed et al. (2012), were used to evaluate the biological effect of five different pure antibiotics before using them for the influent and effluent samples. To our knowledge, these reporter strains have yet to be used for analyzing wastewater. Both *E. coli* strains were tested for their bioluminescence response to tetracycline, oxytetracycline, and sulfadimethoxine, while the three *Salmonella* strains were tested for response to lincomycin and erythromycin. Four different concentrations that were approximately the range used within the referenced literature were used to test the effect of antibiotic concentration on the bioluminescence of these reporter strains.

Four wavelengths (395, 470, 527, and 570 nm) and five time points (2, 4, 6, 8, and 10 hours) were used in the test. Responses of the bacterial reporter strains were normalized to the bioluminescence measured for the corresponding bacteria without antibiotics (termed as luminescence factor), and the luminescence factor graphed against the pure antibiotics concentration to create calibration curves. The wavelength did not appear to have major impact on the luminescence factor (Figure 4).



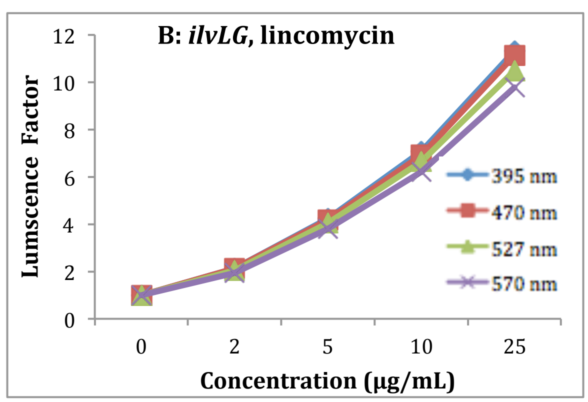
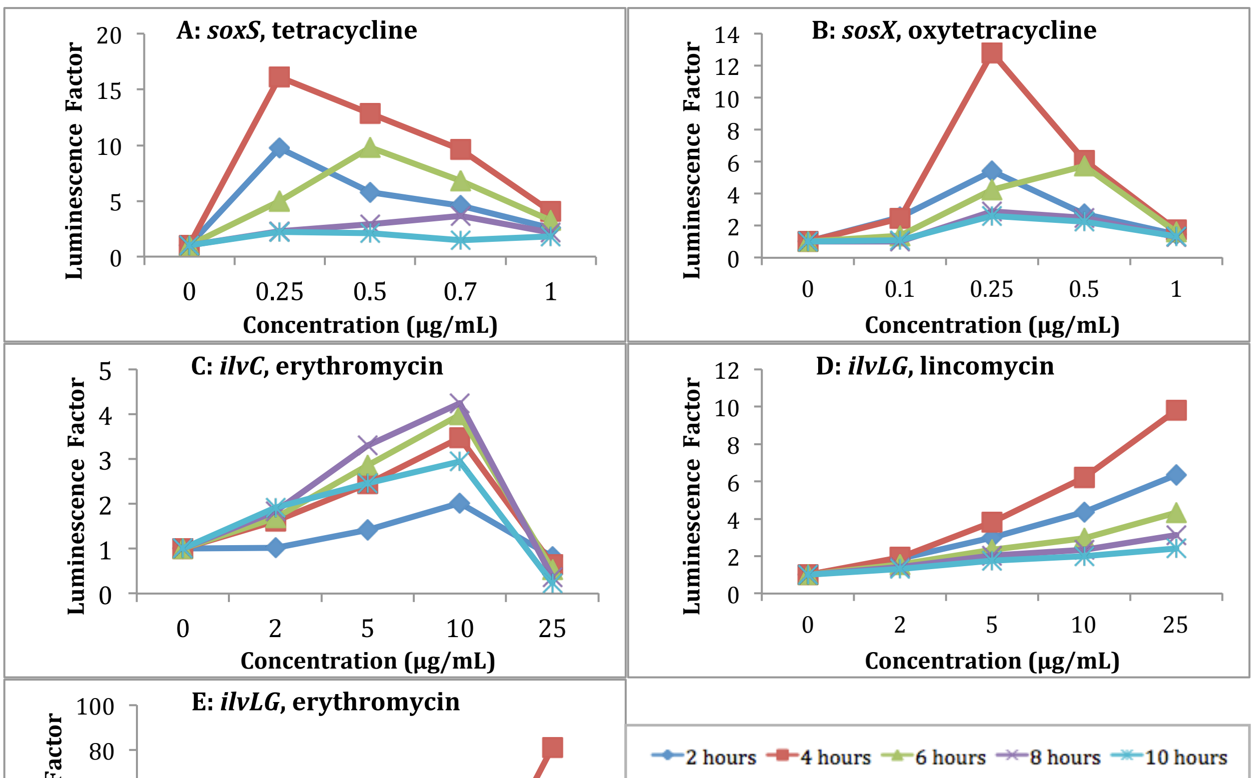


Figure 4: Two examples of the luminescence factor versus antibiotics concentration curve demonstrating the lack of difference between the four wavelengths tested. Curves were *soxS* response to tetracycline (A) and *ilvLG* response to lincomycin.

Four hours was observed to be an ideal time point, since the luminescence factor was highest at this time point and/or the trend for luminescence factor versus antibiotic concentration could clearly be observed (Figure 5). A reporter strain was considered to be suitable if its luminescence factor could follow a trend of an initial increase, a peak, and followed by a decrease, as the concentrations of antibiotics increased (See Figure 5A, time point 4 hours for an example); bioluminescence would increase as concentration of antibiotics increased, but would decrease due to inhibition. Of the five reporter strains, the *soxS*, *ilvC*, and *ilvLG* were deemed to be suitable candidates for quantifying tetracycline (*soxS*), oxytetracyline (*soxS*), lincomycin (*ilvLG*), and erythromycin (*ilvC* and *ilvLG*). The *micF* strain was not found to be suitable for measuring all three antibiotics that it was tested for since the luminescence factor remained at approximately one unit or decreased below one. In addition, the *tsr* strain was not used further since its luminescence factor for similar antibiotics was lower than those observed for the *ilvC* and *ilvLG* strains (luminescence factor of approximately one to two in *tsr*, versus, one to 80 in the latter two). Strain *ilvC* was only suitable for measurement of erythromycin but not lincomycin, since its luminescence factor for the latter antibiotic remained at approximately one despite changes in the antibiotic concentration.



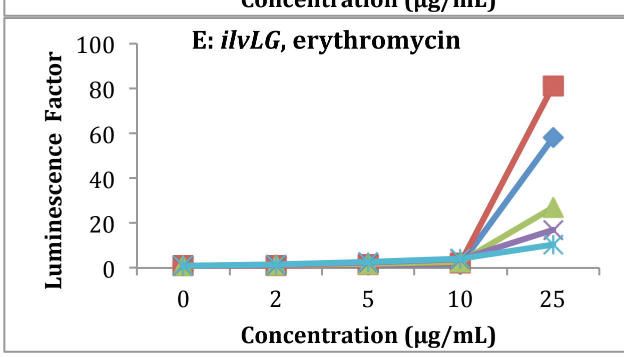
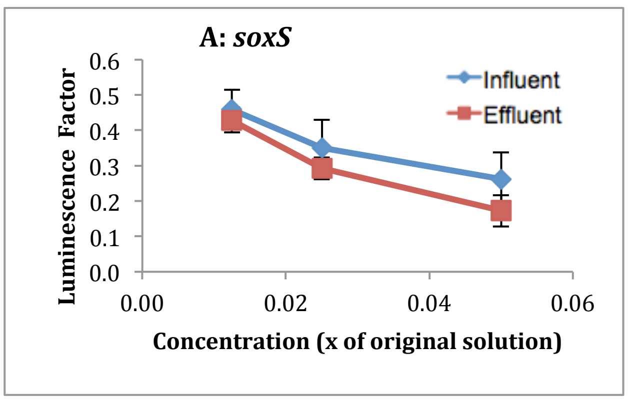
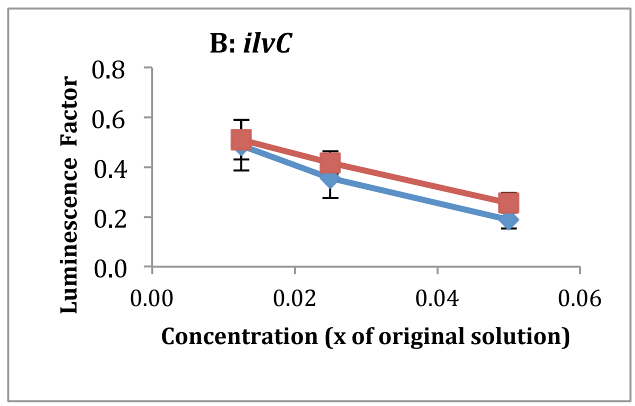


Figure 5: Calibration curves showing luminescence at five different time points for *soxS* strain to pure tetracycline (A), *soxS* strain to pure oxytetracycline (B), *ilvC* strain to pure erythromycin (C), and *ilvLG* strain to pure lincomycin (D) and pure erythromycin (E).

Antibiotic Quantification of poultry litter digester influent and effluent: Modification of extraction methods is underway to allow targeted analyte recovery for the LC-MS/MS analysis. The three reporter strains (*soxS*, *ilvC*, and *ilvLG*) were used to quantify the biological effect of the antibiotics within the influent (n=4) and effluent (n=4) of the digester. The aqueous portion the influent and effluent samples obtained from centrifugation were sterile-filtered using a 0.2 µm filter. Samples were diluted 20, 40, and 80 times before use in the analysis, resulting in final concentrations of 0.0125x, 0.0250x, and 0.050x.

The results showed that regardless of the dilution factor, the addition of influent or effluent samples to *soxS* and *ilvC* resulted in luminescence factor of less than one, indicating that the addition of samples to these two strains caused inhibition (Figures 6A and 6B). In contrast, the addition of influent or effluent samples to the *ilvLG* strain resulted in increased luminescence, albeit slight (luminescence factor ranging from 1.1 to 1.6) (Figure 6C). The decreasing luminescence factor with more concentrated samples could be due to two reasons: 1) the antibiotics within these samples are at the higher end or higher than the values of the calibration graphs, resulting in inhibitory effects; and/or 2) there are other compounds, such as ammonia or volatile fatty acids, within the influent and effluent aqueous matrix that could have inhibited the growth of the reporter strains.





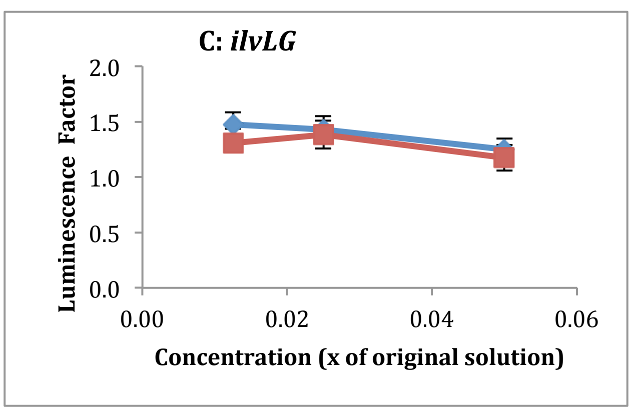
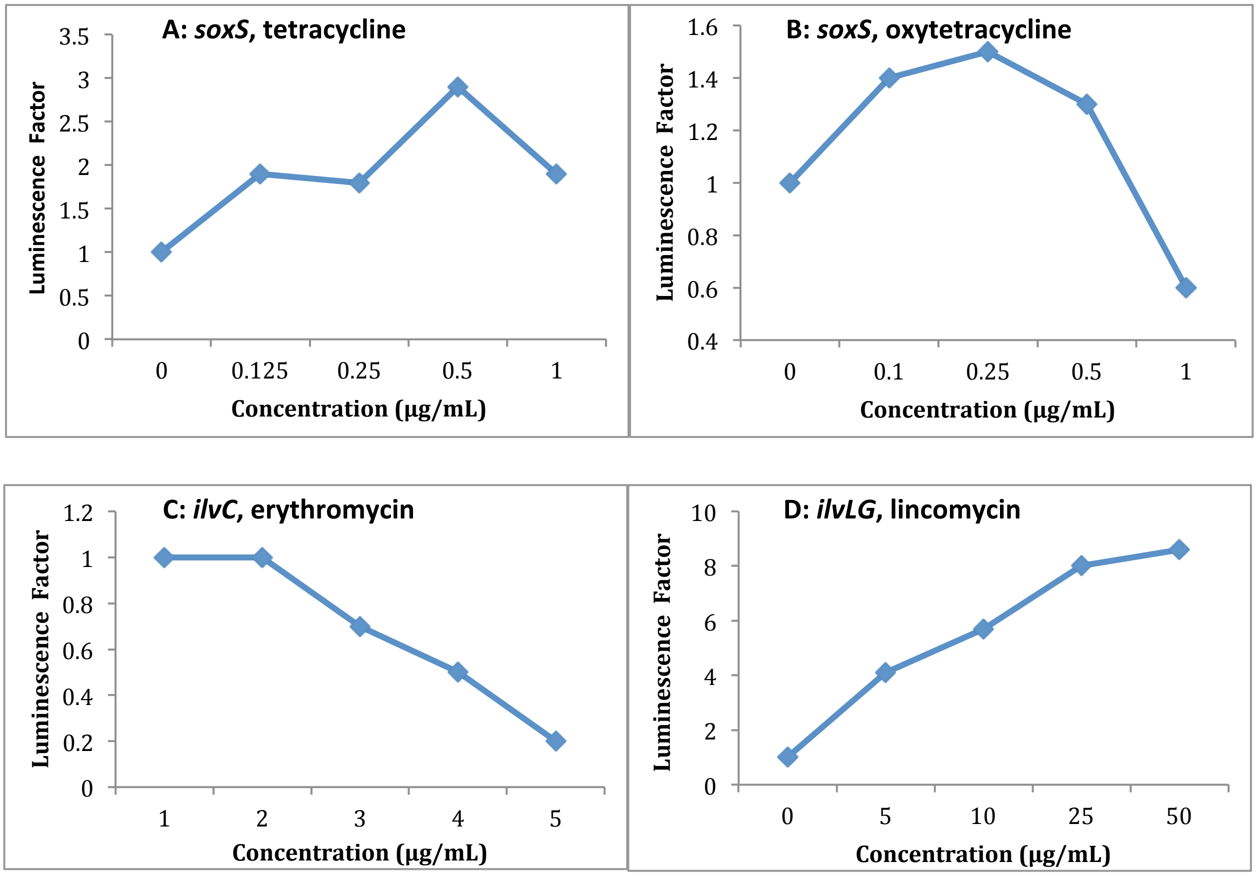


Figure 6: Luminescence responses of *soxS* (A), *ilvC* (B), and *ilvLG* (C) to aqueous portion of influent and effluent samples that were diluted 20, 40, and 80 times (error bars are standard deviation).

Additionally, it should also be mentioned that some of the reporter strains were not specific enough to distinguish one antibiotic from the other. For instance, while luminescence factor for *ilvLG* in the presence of influent and effluent samples were higher than one unit, it is not clear from the results if the luminescence is due to the presence of erythromycin or lincomycin.

Finally, the calibration curve from the earlier reporter strains analysis was not reproducible in this test that included the influent and effluent samples. For instance, while the luminescence factor for *ilvLG* strain ranged from 1 to 81 units for erythromycin during the reporter strain testing phase, the luminescence factor observed in the subsequent test only ranged from 1 to 23 (Figure 7 in contrast to Figure 5 at time point 4 hours).



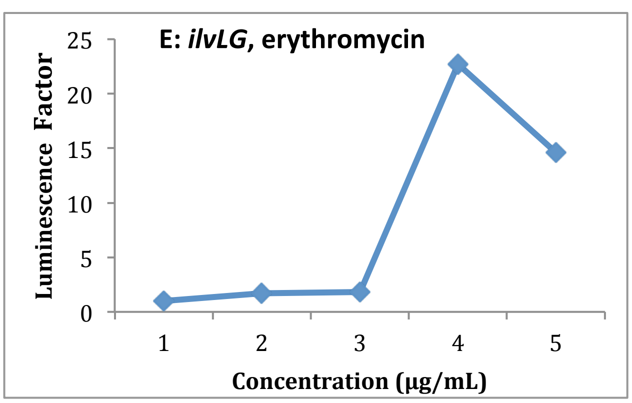


Figure 7: Calibration curves showing luminescence responses during testing with influent and effluent samples for *soxS* strain to pure tetracycline (A), *soxS* strain to pure oxytetracycline (B), *ilvC* strain to pure erythromycin (C), and *ilvLG* strain to pure lincomycin (D) and pure erythromycin (E).

Therefore, the following further investigations will be conducted:

1. Obtain antibiotics level from the LC-MS/MS to give an approximate range for the type and level of antibiotics present. While these samples were sent away for analysis several

months ago, the results have not been received due to the need to find an alternative extraction method. While the reporter strains by itself could not be used to distinctively identify a particular type of antibiotic, its results complemented with the LC-MS/MS data could give a more holistic picture for the type, quantity, and biological effect of antibiotics present within the poultry litter.

2. Analyze samples that have undergone extraction and elution through the Oasis® HLB (hydrophilic / lipophilic balanced) SPE cartridges. This could remove many of the unknown inhibitory compounds, as well as assist in concentrating the antibiotics present within the samples.

3. Determine the reproducibility of the calibration curves for the different reporter strains.

CONCLUSIONS

This investigation was conducted to quantify the fate and effect of antibiotics and naturally-occurring hormones in poultry litter pre- and post-anaerobic digestion to advance understanding of the behavior of these contaminants in AD systems. Preliminary results from the BLYES assay suggested that the anaerobic digestion did not produce a meaningful reduction in estrogenicity within the aqueous sample fraction. This finding is preliminary and requires additional investigation before confirmation. Further investigation into the estrogen quantity in aqueous and solid compartments of multiple influent and effluent samples to allow calculation of an "estrogen budget" and determine changes related to anaerobic digestion is needed. Sample preparation for the LC-MS/MS proved to be complicated, with a MDL that was unacceptably high. Modification of extraction methods, including ultra-centrifugation and subsequent lyophilization of aqueous and solids fractions, is underway. Finally, bacterial reporter strains were used to evaluate the biological effects of antibiotics within the influent and effluent samples. While three of reporter strains were able to function properly with pure antibiotics, issues with reproducibility of calibration curves, bacterial inhibition by sample, and non-specificity were encountered. Further research is being conducted to address these issues.

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Water balance of a green roof integrated with a constructed wetland and rain garden for urban water management

Basic Information

Title:	Water balance of a green roof integrated with a constructed wetland and rain garden for urban water management
Project Number:	2015MD326B
Start Date:	4/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	5
Research Category:	Engineering
Focus Category:	Hydrology, Ecology, Water Quantity
Descriptors:	None
Principal Investigators:	David R Tilley

Publication

1. Thompson, R., D. Tilley, 2015. Retention and thermal performance of a thin sloped green roof on a sustainable home. Cities Alive: 13th Annual Green Roof and Wall Meeting, Brooklyn, NY, October 2015.

Report to NIWR/MWRRC
2015MD326B

Water balance of a green roof integrated with a constructed wetland and rain garden for urban water management

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1. Narrative Summary

Water is a defining feature of America's landscape, heritage and prosperity, therefore, managing this critical resource is increasingly important to the well-being of human society and the environment. Polluted stormwater runoff is one of the most important threats to water quality globally. Quick conveyance of stormwater adversely affects stream hydrology, and can degrade geomorphology of aquatic systems via channelization and erosion [1, 2]. Furthermore, road runoff contains sediments, pesticides, metals such as copper, cadmium, zinc, as well as organic pollutants such as polycyclic aromatic hydrocarbons (PAH) [3]. When transported to receiving waters, many chemical contaminants are toxic to aquatic organisms and plants. Water quality is also degraded as many urban areas utilize combined sewers to convey household sewage and stormwater runoff to wastewater facilities. When runoff exceeds system capacity, combined sewer overflows (CSOs) occur, discharging polluted runoff and sewage to water bodies [4].

Green stormwater infrastructure (GSI) is increasingly being recognized as a sustainable approach to stormwater management. GSI comprises a set of technologies designed to mimic the hydrology and filtration capacity of undeveloped landscapes [1]. Green infrastructure technologies, like green roofs, do so by making use of the natural abilities and functions of ecosystems (i.e., soil, plants, bacteria) to restore water-energy balance to urban ecosystems through reducing or diverting excess stormwater [5]. This is due to their ability to infiltrate, intercept and return water to the atmosphere via evapotranspiration [2].

Despite several studies showing an intimate connection between the energy-water balances of a green roof, few studies have simultaneously evaluated stormwater retention and thermal performance of green roofs in residential settings. Furthermore, residential application of green roofs is projected to increase because many homes possess sloped roofs that have weight load restrictions. Thus many new green roofs could likely be sloped, extensive, light-weight and shallow.

This study aimed to simultaneously research the retention of a 10^{o} sloped, 2.5 inch green roof located on a sustainable home in Rockville, Maryland (USA). The effects of storm characteristics (size, intensity and frequency) and soil moisture on stormwater retention were studied. Furthermore, this study researched the effect of soil water content on evapotranspiration rates, which has strong implications to green roof retention because ET reduces antecedent soil moisture, which likely reduces stormwater runoff.

2. Methods

System Overview

Three types of green infrastructure were integrated into the design of *WaterShed*, the 2011 winning sustainable solar house built for the U.S. Department of Energy Solar Decathlon competition by the University of Maryland (Fig. 1). It was acquired by one of Maryland's regional energy companies, Pepco Holdings, Inc., and is now permanently housed in Rockville, MD at the Pepco WaterShed Sustainability Center. The green roof served as the main study site. The LiveRoof Lite modular green roof (6.35 cm deep, 10^{o} sloped, 28.9 m^2) sits on a highly insulated roofing system (R-value=50) with a white membrane. Substrate composition is 84.5% engineered shale, 4.5% sand, and 11% compost. Sedum species composition include: *Sedum album*, *S. spurium*. *S. reflexum*, *S. spurium*, and *S. sexangula*re. To evaluate retention and thermal performance, an Integrated Monitoring System (Campbell Scientific Data Loggers and instrumentation) was set up to quantify water, energy and weather parameters every 15 minutes.

Figure 1: Integrated green infrastructure the Pepco WaterShed Sustainability Center in Rockville, MD (2014, Photo Courtesy of S. Tjaden).

Retention and Evapotranspiration

To quantify water retention across multiple rain events between July 2014 and March 2015, the Soil Depletion Method was applied. A total of 107 rain events were identified. Rain events were defined as the period between the time precipitation began and ended. Independent rain events were defined by a separation of at least 6 hours [7, 12].

Figure 2: Volumetric water content sensors within the green roof were used in the Soil Depletion Method to calculate retention and evapotranspiration.

The Soil Depletion Method used volumetric water content (VWC) sensors (Campbell Scientific CS655 Water Content Reflectometer) within the green roof (Fig. 2) to determine changes in substrate storage between data recordings (15 min) ($\pm\Delta S = S_{t15}-S_{t0}$). Where, $+\Delta S$ signifies retention, and $-\Delta S$ is water loss due to substrate drainage or ET. During a rain event, total retention was calculated as the sum of +ΔS. Over prolonged periods of time, where there is no runoff or drainage, ET equals –ΔS. Since ET is small during rain events (<4mm/day), it was estimated from average ET between rain events. Total ET per day was calculated as the sum of –ΔS. Since ET is attributed to the change in water status, the soil depletion method could only be confidently applied during warmer months (July-Sept). During colder months, plant cover and ET diminishes. Thus, any water loss over prolonged periods of time could be due to substrate drainage or runoff.

VWC sensors calculate the dielectric permittivity from signal attenuation analyses through its probes. Finally, it applies the Topp equation to estimate volumetric water content from dielectric permittivity.

Regression analysis was used to determine the effects of VWC and storm characteristics on retention. Event size (mm), was classified as total precipitation during a rain event. Storm frequency, or time between events (days), was the time between the end one of one event and the beginning of the next. Storm intensity (mm/min) was defined as total precipitation over the length of the storm. Pre-event substrate water content was defined as the average water content 1 hour prior to the beginning of an event.

3. Principal Findings and Significance

Compared to previous studies on extensive green roofs, where up to 60% mean retention was observed, the shallow, sloped green roof at WaterShed had a mean retention of 27 % (±11) for rain events occurring from July to November. Average percent retention was generally lower and more variable in December through March (13 % ±16.), with many rain events retaining no water. Low percent retention is likely attributed to the thin depth of the media and the 10^{o} roof slope. Previous findings have shown that during the warm season, green roofs are characterized by higher ET, enabling retention capacity to regenerate quickly [2]. Furthermore, there were several rain events that exhibited no retention during the cold season. On several occasions it was observed that the green roof substrate was frozen, which likely severely diminished retention.

Overall, event size was the single biggest predictor of retention. In both seasons, larger storm events produced more absolute retention ($p<0.0001$) (Fig. 5A). However, in July-November larger storm events produced less retention as a percent of precipitation ($p<0.05$) (Fig. 5B). This agrees with previous findings, where the effect of event size, VWC and vegetation on stormwater retention was studied on an un-irrigated extensive green roof system in Central Texas. Event size explained 55.4% of the retention rate in trays with substrate only and 70.6% of the variation observed in vegetated trays [6]. Likewise, a smaller percentage of total rainfall was retained with

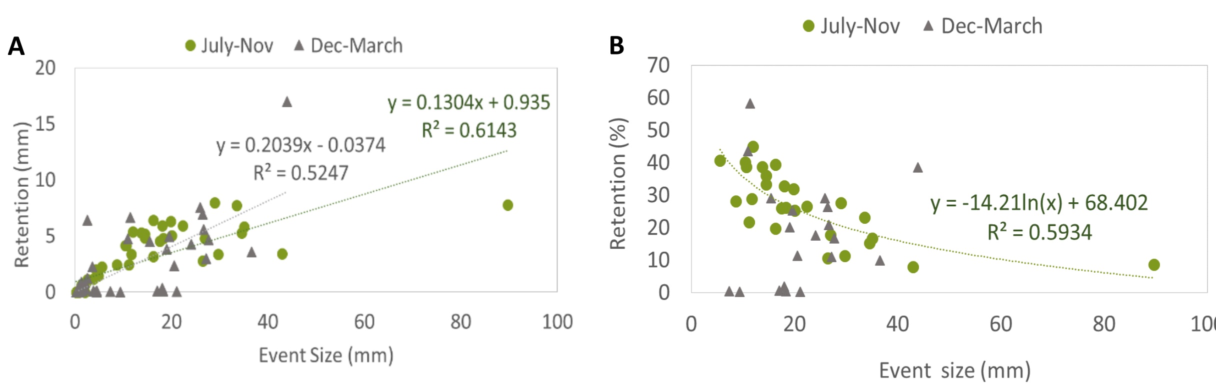

Figure 5: (**A**) Larger storm events produced more absolute retention ($p<0.0001$), (**B**) but less as a percent of precipitation in July-November ($p<0.05$).

increasing event size [6]. Lower correlation between event size and retention, as well as no significant relationship between event size and percent retention in in December-March could be due to freezing temperatures or senesced vegetation. Poor ET and frozen substrate would severely limit retention capacity.

Low antecedent VWC and longer time between events improved retention in July-November ($p<0.05$) (Fig. 6A, 6B). Time between event was strongly tied to substrate VWC, as greater time between events is imperative to substrate drying out, improving a green roofs ability to retain subsequent water [6]. Furthermore, longer time between events would likely allow for more plant water uptake via ET. No significant relationship between antecedent water content, time between events and retention was observed during the months of December through March, likely due the several storm events where the substrate may have been frozen and no retention ensued.

Evapotranspiration rates during the months of July through September were greater when VWC was high ($p<0.0001$) (Fig. 6C), indicating plants play an intimate role in reducing water content between storms for improved retention. Other studies have shown green roof ET improves with increasing soil moisture and plant type. Substrate water content affected ET differentially in *S. kamtschaticum* species (R^2=0.93, $p<0.0001$) compared to *S. album* (R^2=0.80, $p<0.0001$) [7]. Findings indicate that on thin extensive green roofs, which are low in water content (<0.15 m^3/m^3), and tend to dry out quickly, soil moisture may be the limiting factor in ET, instead of climatic and crop factors. Due to limitations with the Soil Depletion Method, evapotranspiration could only be determined July-September It was assumed after this time, ET rates are extremely low. Thus, with low ET in the cold season, it is likely that more time is needed between storms to regenerate retention capacity, or thaw out frozen substrates.

There was no correlation between storm intensity and retention during both seasons, indicating rapid regeneration of retention capacity regardless of event rate (Fig. 6D). However,

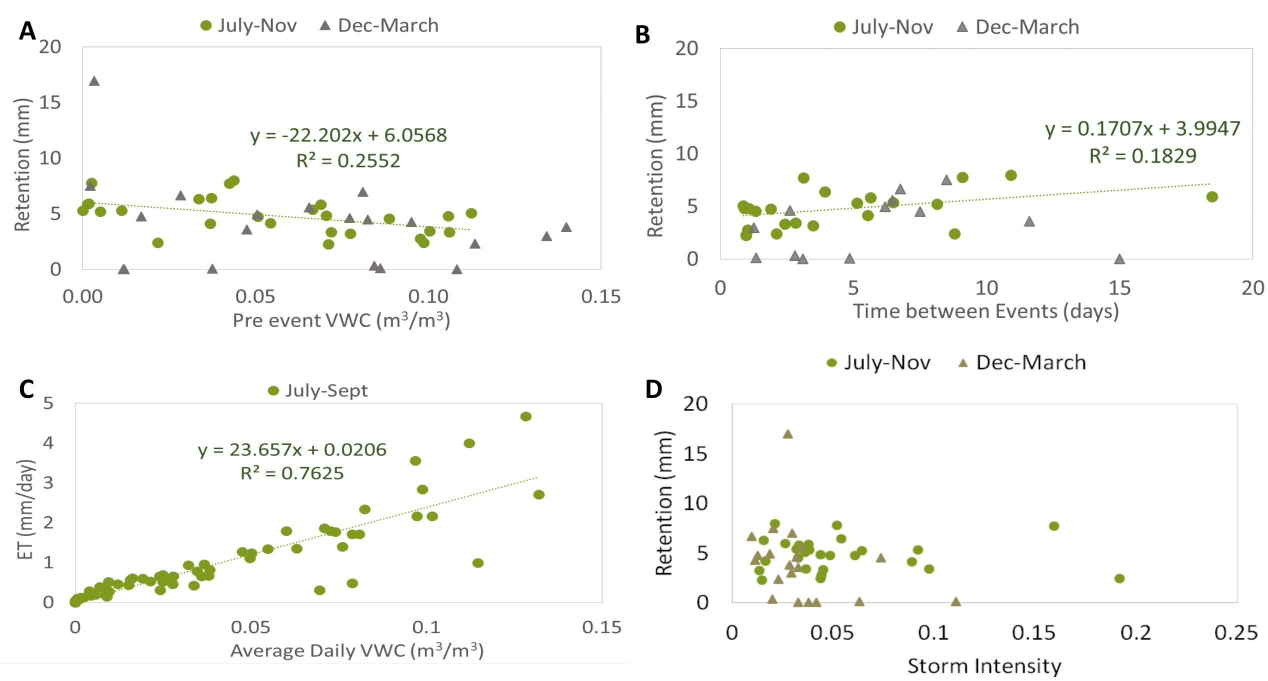

Figure 6: (**A-B**) Low antecedent water content and greater time between events improved retention in July-November ($p<0.05$). (**C**) Evapotranspiration rates were greater when soil water content was high ($p<0.0001$). (**D**) No correlation between storm intensity and retention was observed.

this trend may simply be attributed to moderately intense storms falling during this season (< 0.30 mm/min). Quantitative analysis of runoff characteristics on extensive green roofs have shown a high water retention capacity to rainfall of less than 0.33 mm/min. As rainfall intensity increases beyond 0.33 mm/min, water-retention capacity decreased [8].

Many infiltration capacity studies show reduced crop canopy makes ground surfaces prone to the harsh impact of intense storms, resulting in soil compaction, erosion and reduced infiltration capacity [9]. Typically when rainfall intensity exceeds infiltration capacity of soils, runoff ensues. Therefore, an inverse relationship between storm intensity and retention due to the finite retention capacity of a green roof was expected [10].

Overall, the correlations between season, VWC and storm characteristics indicate a need for more hydrological studies on thin, sloped green roofs. Particularly since greater storm size, intensity and frequency are expected under climate change scenarios. Furthermore, the characteristics indicate that there may be potential trade-offs for irrigating green roofs, as greater soil moisture may increase ET, yet limit retention if time between events is low.

Publication citations

Thompson, R., D. Tilley, 2015. Retention and thermal performance of a thin sloped green roof on a sustainable home. Cities Alive: 13th Annual Green Roof and Wall Meeting, Brooklyn, NY

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Number of students supported

Ph.D. students supported = 1

Notable Achievements

Rhea Thompson and David Tilley organized a Green Infrastructure Stormwater Workshop in partnership with Maryland Sea Grant to be convened at the Pepco Watershed Sustainability center in Rockville, MD on June 9th, 2016.

Potential use of duckweed (Lemna minor) in bioremediation of rare earth element-containing effluents

Basic Information

Title:	Potential use of duckweed (Lemna minor) in bioremediation of rare earth element-containing effluents
Project Number:	2015MD327B
Start Date:	4/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	District 5
Research Category:	Water Quality
Focus Category:	Geochemical Processes, Treatment, Wastewater
Descriptors:	None
Principal Investigators:	Johan Schijf, Edward R Landa

Publications

There are no publications.

Research Report on 2015MD327B "Potential use of duckweed (*Lemna minor*) in bioremediation of rare earth element-containing effluents".
Reporting Period 03/01/2015 – 02/29/2016
Principal Investigator: Johan Schijf, UMCES/Chesapeake Biological Laboratory; Co-PI: Edward R. Landa, University of Maryland College Park (ENST).

1. Narrative summary

Problem and research objectives

Yttrium and the rare earth elements (YREEs) are a globally scarce mineral resource that is in increasing demand for expanding technologies, such as the color screens of cell phones and other handheld devices. Since China controlled about 95% of the world YREE market a few years ago, there has been an effort to revitalize US YREE production through enhanced mining and refining. YREE extraction from ore is a low-yield and chemically intensive process that has a significant potential for generating contaminated liquid and solid waste streams. Little is known about the human and environmental toxicity of the YREEs, but several studies indicate that it may be substantial. In this project we investigated the possibility of using duckweed (*Lemna minor*) as a suitable target for the biomonitoring and/or bioremediation of YREE-rich industrial effluents. Duckweed is a small aquatic vascular plant that occurs ubiquitously in stagnant freshwater systems (ponds and slow streams) and is known to have a pronounced tendency to adsorb a wide range of transition metals. It is hardy and grows rapidly by vegetative propagation. As it floats on the water surface it quickly adsorbs metals directly from solution, whereupon it can be easily harvested and processed for YREE recovery or safe disposal, if necessary after drying or ashing to reduce volume. In this project we grew duckweed in culture and conducted two experiments to examine its ability to adsorb YREEs under natural conditions, with special emphasis on uptake capacity and kinetics.

Methods

A continuous culture of duckweed (*Lemna minor*) was established with starting material from the Carolina Biological Supply Co. in four 9-Gal. fish tanks (**Fig. 1**). Duckweed was grown in three of the four tanks on Hoagland's F/2 medium that was renewed once or twice per week. The fourth tank was used for rotation during cleaning, whereby the plants were transferred with a plastic skimmer spoon. The tanks were equipped with growth lights on a timer set to an 16 h/8 h night/day cycle. No aeration or temperature control was required (**Fig. 2**). The medium was freshly prepared in Milli-Q water from basal salt mixture (Caisson Labs). Once the culture filled three tanks, excess material was regularly discarded to prevent overcrowding and keep the plants healthy. The culture was sustained for 5 months until it was infected by a fungus and collapsed. The contents of a single tank were harvested twice during this period for sorption experiments.

Two sorption experiments were performed in wide-mouth jars with magnetic stirring, placed in a fish tank with the same light conditions as the culture (**Fig. 3**). Each jar contained one liter of tapwater spiked with ~100 ppb each of Y and the 15 REEs. The spike solution was made up in Milli-Q water from individual YREE-chloride salts and minimally acidified, so that its addition to the experimental solution would change conditions as little as possible. At t = 0, about 5-10 g

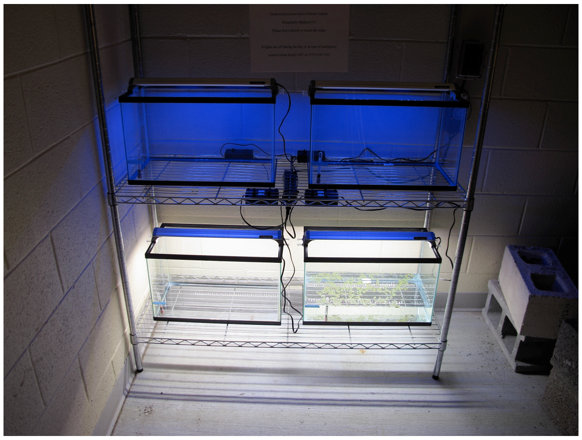

Fig. 1 Rack with rotating duckweed cultures maintained in the L. Eugene Cronin complex at CBL. Three of the four tanks were occupied at any time. Top two tanks are shown with 'night' lighting.

Fig. 2 One of the culture tanks. Duckweed eventually covered the entire surface and was maintained in Hoagland's F/2 medium, renewed biweekly. No aeration or temperature control were required.

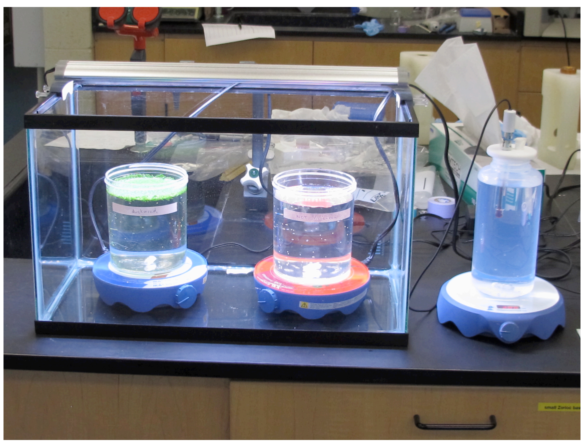

Fig. 3 Experimental setup for YREE sorption study.

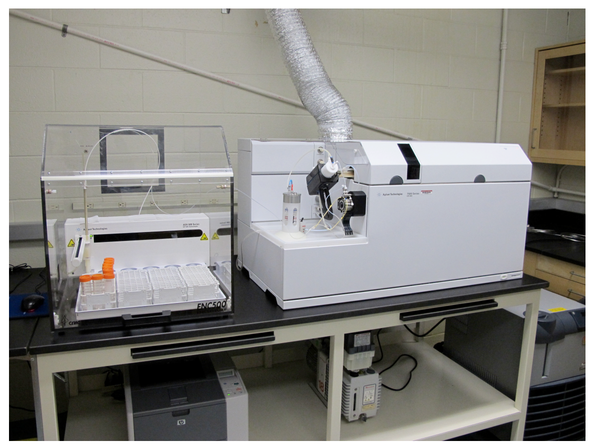

Fig. 4 ICP-MS in the laboratory of PI Johan Schijf.

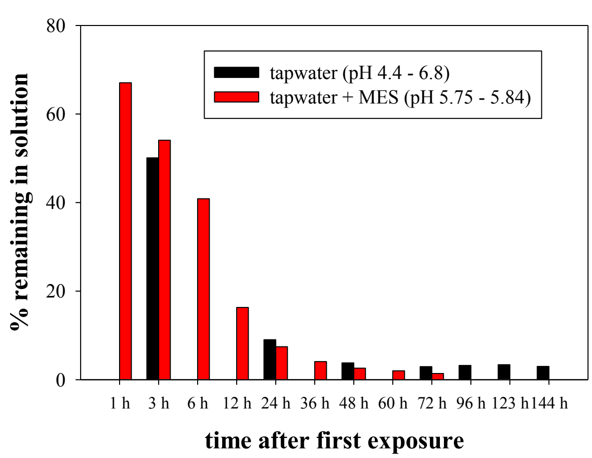

Fig. 5 Erbium sorption on duckweed in tapwater, with free-drifting pH and buffered with MES (pH 6).

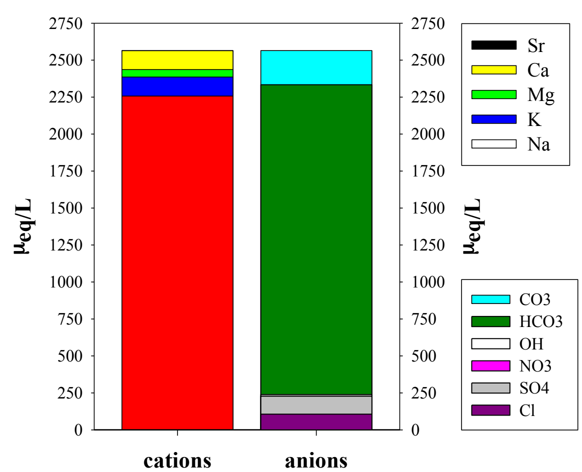

Fig. 6 Major ion composition of CBL tapwater. Alk ~ 2300 μeq/L at pH ~ 9 based on charge balance.

(wet weight) of duckweed from the culture was added to one of the jars whereas the other was used as a negative control. At fixed times, 5-mL 0.2-μm cartridge-filtered samples were collected in polypropylene centrifuge tubes and acidified with HNO_3. Solution pH was measured in the control jar at each time point with a glass electrode, continuously calibrated against a pH 3.00 standard (**Fig. 3**) and in the sample jar before addition and after final removal of the duckweed. YREE concentrations in the filtered samples were measured on an Agilent ICP-MS against an external calibration line, using standard procedures (**Fig. 4**). YREE concentrations in duckweed were measured in the same manner, after drying for several days in an oven at 95°C to determine dry weights and subsequent microwave digestion in a mixture of HNO_3 and H_2O_2. Major cation (Na, K, Mg, Ca) and anions (chloride and sulfate) in tapwater were measured, respectively, by Perkin-Elmer ICP-AES in the CBL Nutrient Analytical Services Laboratory and by Dionex ion chromatography in the laboratory of Dr. Laura Lapham. Carbonate alkalinity was estimated from these analyses based on the measured pH and charge balance.

Principal findings

Duckweed was found to be easy to culture, even for non-experts such as ourselves. After several weeks of growth the plants formed a lush, cohesive mat that was apparently held together by filamentous green algae. For the purpose of these experiments, the duckweed can therefore not be considered a mono-culture and at least some of the YREE sorption may have to be attributed to the algal cells. However, this situation probably more closely resembles what is found in natural systems. The cultured duckweed was found to contain negligible amounts of YREEs, mostly the light REEs (La-Nd), which is consistent with a minor presence of clay particles.

Both sorption experiments indicate that YREEs are quickly adsorbed by duckweed from tapwater at subneutral to circumneutral pH (4-6), at levels of 100 ppb for individual elements. An example is given for erbium (Er) in **Fig. 5**, but all YREEs essentially are adsorbed to the same extent. About 50% of the initial dissolved concentrations was taken up by the duckweed within 3 hours and >90% within 24 hours. In the first experiment, which was continued for 144 hours until the plants showed signs of deterioration, final YREE adsorption reached levels of 96-98%. To ensure that sufficient light and oxygen reached the duckweed, the experiments were conducted in open vessels and weighing of the solution before and after the experiments indicated that evaporation amounted to about 20 mL per day. Minimal YREE removal was observed in the negative control (no duckweed), where some YREEs were recovered in an acid leach of the vessel upon completion of the experiment, which was attributed to non-specific adsorption since the pH was too low for YREE hydroxide precipitation. When evaporation, wall adsorption, and YREEs remaining in the solution were accounted for, a mass balance calculation and ICP-MS analysis of the plant tissue showed that most of the YREEs removed from the solution were indeed adsorbed by the duckweed. A missing fraction of the initial YREE concentrations, corresponding to about 20% of the duckweed material, was most likely due to loss of tissue during solution sampling and final harvest.

In the first sorption experiment, pH was allowed to drift freely in order to keep conditions as natural as possible. However, the pH of CBL tapwater was found to be about 9, probably due to addition of sodium hydroxide during water treatment, to facilitate chlorination. Analysis of the tapwater by ICP-AES, initially performed to determine background Ca concentrations, confirmed the overwhelming presence of Na, as well as a carbonate alkalinity similar to that of seawater, as calculated from the pH and charge balance (**Fig. 6**). The experimental solutions were therefore acidified to pH ~ 4.5 to eliminate carbonate which might interfere with YREE sorption through formation of stable complexes. The negative control stayed at this pH, yet the sample solution drifted up to pH ~ 7, complicating the interpretation of the results. The experiment was therefore repeated in the presence of MES, a non-coordinating buffer that is not toxic to plants. In the second sorption experiment, the pH remained at pH 5.8±0.1 in both jars. This apparently had no effect on the sorption process (**Fig. 5**). However, besides the losses discussed above, another fraction of YREEs was missing in this experiment, centered on the middle REEs (Sm-Eu). This fraction could not be accounted for, but was most likely adsorbed on the centrifuge tubes after collection of the filtered samples. It suggests that the amount of acid added to stabilize the samples was insufficient to overcome the MES buffering. This is an issue that will have to be addressed in future experiments.

Significance

Our experiments indicate that duckweed rapidly takes up dissolved YREEs from freshwater under natural conditions (room temperature and pH ~ 6), at levels much higher than background concentrations in rivers and lakes, removing about 50% of the initial load within 3 h and >90% within 24 h, after which adsorption continues as long as the culture stays healthy. Our results do not indicate whether the YREE uptake is due to active transport through the cell membrane or to passive, pH-dependent cation exchange on the cell surface. The exact mechanism has repercussions for the reversibility of the uptake process and for competition from other metals, neither of which was address in our preliminary experiments. Two additional experiments were planned but, since the first sorption experiment had to be repeated, these could not be conducted before the duckweed culture collapsed. The first was designed to study YREE sorption in the presence of variable levels of Ca, which is an abundant and physiologically important metal that binds to the same functional groups on the cell surface that are likely to bind the YREEs. If YREE uptake involves cation exchange, the effect of Ca is expected to be minor, but it may be substantial if YREEs are transported into the cell by Ca-specific ion channels. The second experiment was designed to study YREE sorption in water from a local wetland that is rich in natural organic ligands such as humic acids. These would form YREE complexes in solution that could significantly lower their uptake under more natural conditions. Our preliminary results are encouraging and these and other corollary experiments may yet be done if additional funding can be procured and a new duckweed culture established.

2. No publications have resulted from this project to date.

3. Ms. Shannon M. Burns, an undergraduate student at the University of Georgia, assisted with this project while she was an REU student at CBL during the summer of 2015, working on unrelated research. The UMCES REU program is funded by NSF (OCE-1262374) and administered by the Maryland Sea Grant Program.

4. No notable achievements or awards resulted from this work.

Retrospective Analysis of Nutrient and Sediment Loadings and Trends in the Chesapeake Bay Watershed (Graduate Fellowship)

Basic Information

Title:	Retrospective Analysis of Nutrient and Sediment Loadings and Trends in the Chesapeake Bay Watershed (Graduate Fellowship)
Project Number:	2015MD329B
Start Date:	6/1/2015
End Date:	8/30/2015
Funding Source:	104B
Congressional District:	MD 7
Research Category:	Water Quality
Focus Category:	Non Point Pollution, Water Quality, Methods
Descriptors:	None
Principal Investigators:	Kaye Lorraine Brubaker, William Ball

Publications

1. Zhang, Q. and W.P. Ball. 2015. Concentration-Discharge Relationships for Nutrients and Sediment in Major Tributaries to Chesapeake Bay: Typical Patterns and Non-Stationarity, poster presentation at Maryland Water Monitoring Council (MWMC) Annual Conference, Linthicum Heights, MD, November 13, 2015.
2. Zhang, Q., R.M. Hirsch, and W.P. Ball. 2016. Long-Term Changes in Sediment and Nutrient Delivery from Conowingo Dam to Chesapeake Bay: Effects of Reservoir Sedimentation, Enviromental Science & Technology 50(4), 1877-1886, DOI 10.1021/acs.est.5b04073

MARYLAND WATER RESOURCE RESEARCH CENTER

Summer Graduate Fellowship 2015 Final report

Retrospective Analysis of Nutrient and Sediment Loadings and Trends in the Chesapeake Bay Watershed

(MWRRC Grant Number: 2015MD329B)

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Background and Objectives

Toward controlling hypoxia in Chesapeake Bay, management programs have focused for decades on reducing nitrogen (N), phosphorus (P), and suspended sediment (SS) loads from the Bay watershed (Shenk and Linker, 2013). To assess the reduction progress and refine control strategies, managers need to better understand the nature and causes of historical changes in N, P, and SS loads at different areas of the watershed (**Figure 1**).Toward that end, we need to better understand the historical trends in riverine fluxes, changes in watershed sources, and the relationships between the two aspects.

In many river monitoring programs, water-quality constituent concentrations have been sampled only a few times every month. Therefore, it is often necessary to estimate concentration and loading for days without sampling. In this regard, Hirsch *et al.* (2010) have developed a method called "Weighted Regressions on Time, Discharge, and Season (WRTDS)". For each day of estimation, WRTDS re-evaluates the dependencies of concentration on time, discharge, and season based on data most relevant to the estimation day (Hirsch *et al.*, 2010; Hirsch and De Cicco, 2015). Consequently, WRTDS can better represent the varying seasonal and flow-related patterns and are more resistant to flux-estimation bias than prior methods (Moyer *et al.*, 2012; Hirsch, 2014). This work aims to further improve the method by adding explanatory variables on antecedent wetness condition (Macrae *et al.*, 2010; Murphy *et al.*, 2014).

Riverine nutrient concentrations have been observed to exhibit long-range dependence (LRD), since watershed can store, transport, and mix solutes over a diverse range of spatial and temporal scales (Kirchner and Neal, 2013). Because LRD processes can induce trend-like behaviors, statistically significant trends may be falsely declared more frequently than expected. Currently, there is still lack of understanding on LRD estimation approaches for river water-quality data, which are typically irregular and thus cannot be examined by traditional methods.

In the above context, our main objectives are: (**A**) to apply WRTDS to evaluate long-term trends of riverine loadings from the Bay watershed and identify the linkages to source inputs, (**B**) to improve WRTDS using additional variables that account for antecedent conditions, and (**C**) to evaluate methods for LRD estimation in irregular time series. For Objective A, we have conducted three specific analyses: (**A1**) nutrient and sediment export from the Susquehanna River Basin, (**A2**) changes in net deposition in the "nearly-full" Conowingo Reservoir, and (**A3**) non-stationarity in concentration-discharge relationships in Chesapeake's major tributaries.

This work has been supported by a combination of funds from the Maryland Water Resources Research Center, the U.S. Geological Survey, and the Maryland Sea Grant. The MWRRC Fellowship has made it possible to continue and complete my work toward the research objectives.

Research Methods

(A1) Export of Nitrogen, Phosphorus, and Sediment from the Susquehanna River Basin (SRB): Analysis of Decadal-scale Trends and Sub-basin Mass Balances

We have conducted a comprehensive evaluation of (1) temporal trends of nutrient and sediment loads at seven long-term sites and (2) spatial variations of nutrient and sediment budgets of major sub-basins in the SRB, the Chesapeake Bay's largest tributary (**Figure 2**). We focused on SS, TP, TN, dissolved P (DP), particulate P (PP), dissolved N (DN), and particulate N (PN). For each constituent at each site, we have run WRTDS (Hirsch and De Cicco, 2015) to produce the daily true-condition and flow-normalized loading estimates. For trend analysis, we have focused on the synthesis of flow-normalized estimates. We have also compiled and analyzed watershed source input data from the Chesapeake Community Modeling Program (Shenk and Linker, 2013), which include atmospheric deposition, fertilizer, manure, and point sources. In addition, we have used the true-condition estimates to calculate the relative flux contributions by Susquehanna's sub-basins and examined the effects of streamflow and land use on constituent export.

(A2) Long-Term Changes in Sediment and Nutrient Delivery from Conowingo Dam to Chesapeake Bay: Effects of Reservoir Sedimentation

We have evaluated the concentration and loading histories from sites above and below the Conowingo Reservoir for the period between 1986 and 2013 to provide new insights on sediment and nutrient processing within the reservoir. These sites included Conowingo, Marietta, and Conestoga (**Figure 3**). Specifically, we performed three types of analyses on SS, TP, and TN with increasing use of statistical modeling: (1) identification of temporal changes in concentration-discharge relationships at sites above and below the reservoir using observed data, (2) evaluation of net deposition trend in the reservoir system using mass-balance analysis on loadings estimated by standard WRTDS models, and (3) analysis of changes of reservoir function by better accommodating streamflow variability through the development of three different historical stationary models of the concentration relation to discharge and season. In all three analyses, special attention has been given to the identification of discharge intervals associated with major changes in reservoir performance.

(A3) Characterization of Non-Stationary Concentration-Discharge (C-Q) Relationships in Chesapeake Bay Tributaries

We have analyzed fifteen long-term sites on nine major non-tidal tributaries to Chesapeake Bay to better understand the nature and change of C-Q relationships for nutrient and sediment across the Bay watershed. Specifically, we fitted non-parametric LOWESS (locally weighted scatterplot smoothing) curves to the paired C-Q data to classify C-Q relationships (*i.e.*, dilution, chemostasis, and mobilization) for SS, TP, and TN at each site. To examine temporal changes, we constructed the *C-Q* scatterplots for two non-overlapping decadal periods, *i.e.*, 1985-1995 and 2005-2015. In addition, we applied WRTDS to illustrate changes in C-Q relationship the regression surfaces and also compared the changes in relative contributions of low-, mid-, and high-flow classes between the two decadal periods.

(B) Improvement of Riverine Flux Estimation with Flow Anomalies

We have focused on variables that can be directly derived from daily flow discharge data, including flow anomalies (Vecchia *et al.*, 2009), average discounted flow (Wang *et al.*, 2011), base-flow index (Lyne and Hollick, 1979), and flow gradient. For each constituent of interest, namely, chloride (Cl), nitrate-plus-nitrite (NO_x), total Kjeldahl nitrogen (TKN), soluble reactive phosphorus (SRP), TP, and SS, we have collected the nearly-daily water-quality data for several tributaries to Lake Erie and Ohio River (National Center for Water Quality Research, 2015). To mimic the roughly monthly sampling resolution for many traditional monitoring programs, we performed Monte Carlo sub-sampling of the original concentration data to produce five concentration subsets. With each subset, we have run the original and modified WRTDS models (13 in total) to estimate daily concentrations and fluxes. These estimates were then compared with the observed concentrations and fluxes to calculate model residuals. Nash-Sutcliffe efficiency was quantified for each model to examine their performances.

(C) Evaluation of Methods for Estimating Long-Range Dependence (LRD) in Irregular Water Quality Time Series

We have evaluated and compared several approaches for estimating LRD using Monte Carlo simulation. First, we simulated 30 replicates of regular time series for a wide range of prescribed LRD values, ranging from “white noise” to “Brownian noise”. These regular series each have a length of 9125, equivalent to 25 years of daily samples. We then converted the regular series to irregular series using gap intervals that were simulated with negative binomial distributions, based on distribution parameters representative of real water-quality data. For the simulated gappy data, we have applied several types of methods to estimated LRD strengths, including interpolation methods, spectral analysis, and wavelet analysis. Finally, the estimated LRDs were compared with the prescribed (“true”) LRD to quantify the performance of each method.

Results

(A1) Export of Nitrogen, Phosphorus, and Sediment from the Susquehanna River Basin (SRB): Analysis of Decadal-scale Trends and Sub-basin Mass Balances

Nutrient and sediment loadings have generally declined at all sites except below Conowingo Reservoir in the last three decades (**Figure 4**), indicating watershed-wide progress after decades of management controls. Period-of-record declines in riverine yield are generally smaller than those in source input, suggesting the possibility of continuing contribution from legacy surface and sub-surface stores. Net contributions from all six sub-basins upstream of Conowingo Reservoir have followed similar patterns for both streamflow (Q) and constituent loads (L). Strong linear log[L]~log[Q] relationships confirm that streamflow is a principal factor controlling rates of constituent export and that the constituent exports have not been supply-limited. In addition, long-term median yields of N, P, and SS all correlate positively with the area fraction of agricultural or urban lands but negatively with that of forested land, and these patterns were observed under all flow classes.

(A2) Long-Term Changes in Sediment and Nutrient Delivery from Conowingo Dam to Chesapeake Bay: Effects of Reservoir Sedimentation

Monitoring data show that SS and TP concentrations at the reservoir inlet have declined under most discharges in recent decades, but without corresponding declines at the outlet, implying recently diminished reservoir trapping. Second, best estimates of mass balance suggest decreasing net deposition of SS and TP in recent decades over a wide range of discharges (**Figure 5**), with cumulative mass generally dominated by the 75^{th}~99.5^{th} percentile of daily Conowingo discharges. Finally, results from stationary models also support the conclusion of diminished trapping of SS and TP under a range of discharges that includes those well below the literature-reported scour threshold. Overall, these findings suggest that decreased net deposition of SS and TP has occurred at sub-scour levels of discharge.

(A3) Characterization of Non-Stationary Concentration-Discharge (C-Q) Relationships in Chesapeake Bay Tributaries

Long-term C-Q data across the Chesapeake Bay's tributary watersheds reveal dominant chemostasis and mobilization effects for dissolved and particulate constituents, respectively (**Figure 6**). Separation of the monitoring data into non-overlapping decadal periods revealed clear non-stationarity in C-Q relationships for many of the selected site-constituent combinations. Such temporal changes reflected changes in dominant watershed sources and are consistent with literature. In addition, WRTDS has provided a powerful tool for validating the LOWESS derived C-Q relationships and for providing additional insights. These findings highlight the potential pitfalls of assuming stationary C-Q relationships when estimating constituent concentrations and fluxes or analyzing their temporal trends.

(B) Improvement of Riverine Flux Estimation with Flow Anomalies

Results show that performances of the original and modified WRTDS models with respect to flux estimation vary with site and with constituent (**Figure 7**). In many cases, all modified models have performed similarly with or slightly better than the original WRTDS model. Significant improvements were achieved for some constituents, including Cl with the short-term flow anomaly model, NO_x with the long-term flow anomaly model, and SS with the average discounted flow model. These findings confirms the value of incorporating antecedent conditions in flux estimation. None of the proposed terms, however, was useful for improving the estimation of TKN or SRP.

(C) Evaluation of Methods for Estimating Long-Range Dependence (LRD) in Irregular Water Quality Time Series

Results suggest that none of the existing methods fully account for the effects of data irregularity on LRD estimation (**Figure 8**). First, the results illustrate the danger of using interpolation for gap-filling when examining auto-correlation, as the interpolation methods consistently under-estimate or over-estimate LRDs under a wide range of LRDs and gap distributions. Second, the long-established Lomb-Scargle spectral method also consistently under-estimates LRD. Its modified form, using the 5% lowest frequencies for spectral slope estimation, has small bias but very poor precision. Third, a wavelet-based method, coupled with an aliasing filter, generally has the smallest bias and root-mean-squared error among all methods for a wide range of conditions of LRDs and gap distributions. The aliasing method, however, does not account for data irregularity, thus introducing some bias. Overall, the wavelet method is recommended for estimating LRD in irregular time series until improved methods are developed.

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Figures

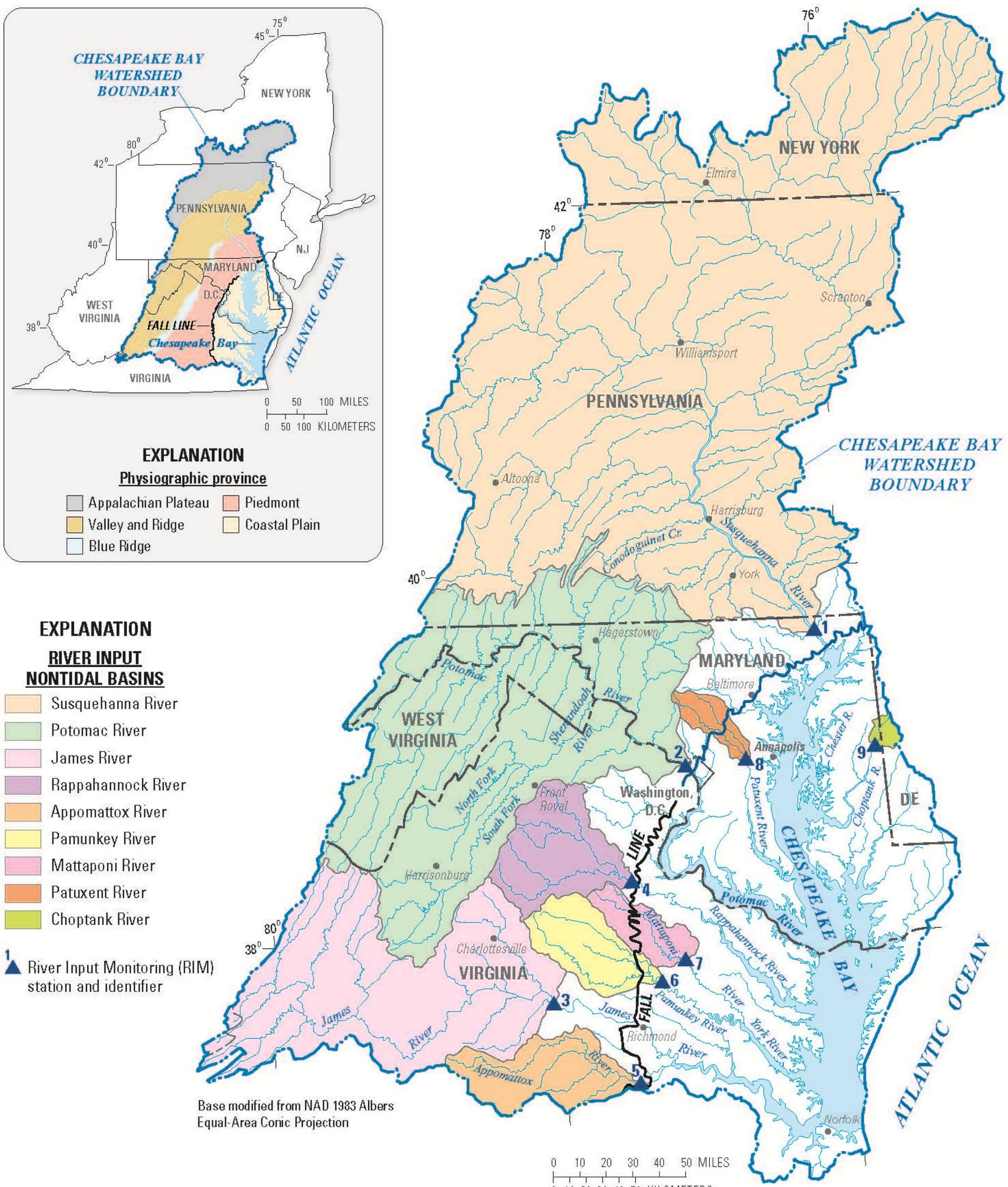

Figure 1. Chesapeake Bay watershed and monitoring sites at the fall-line of the nine major tributaries. This figure was reproduced from Moyer *et al.* (2012) with permission. Inset shows the major physiographic provinces.

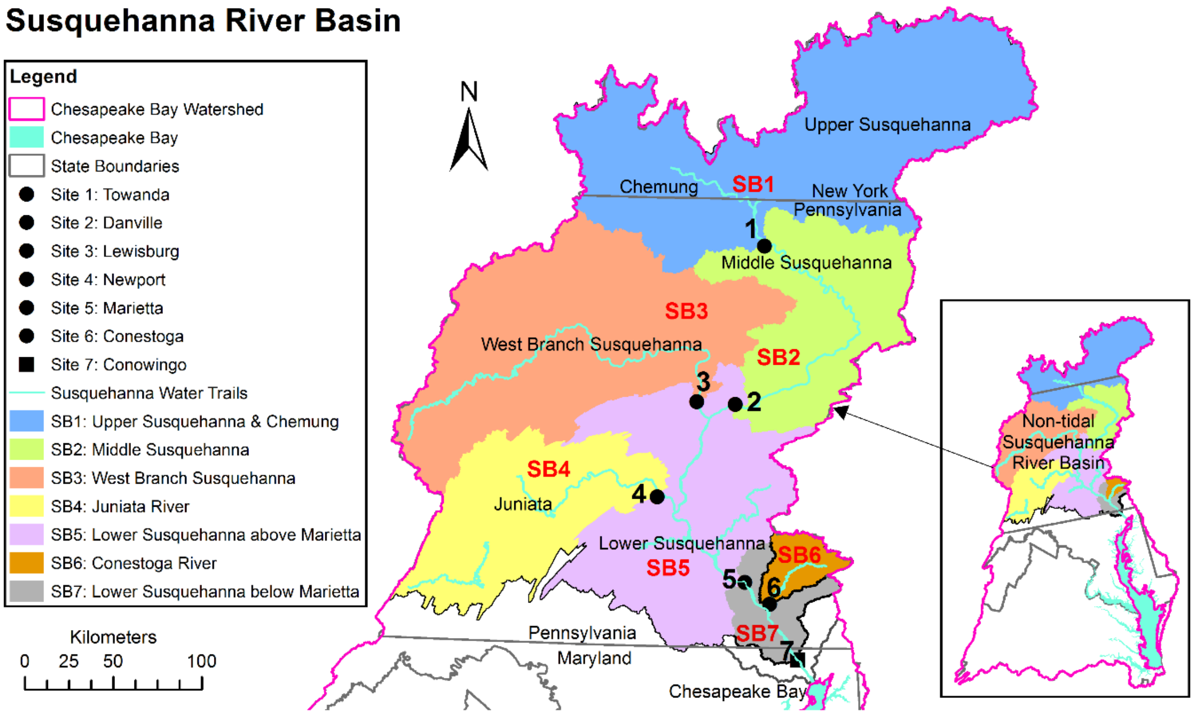

Figure 2. Map of Susquehanna River Basin (SRB), showing the seven sub-basins (SB1-SB7) as formed by seven monitoring sites (No. 1-7). Conowingo (#7) is the non-tidal SRB's outlet. Inset shows the SRB's location in Chesapeake Bay Watershed.

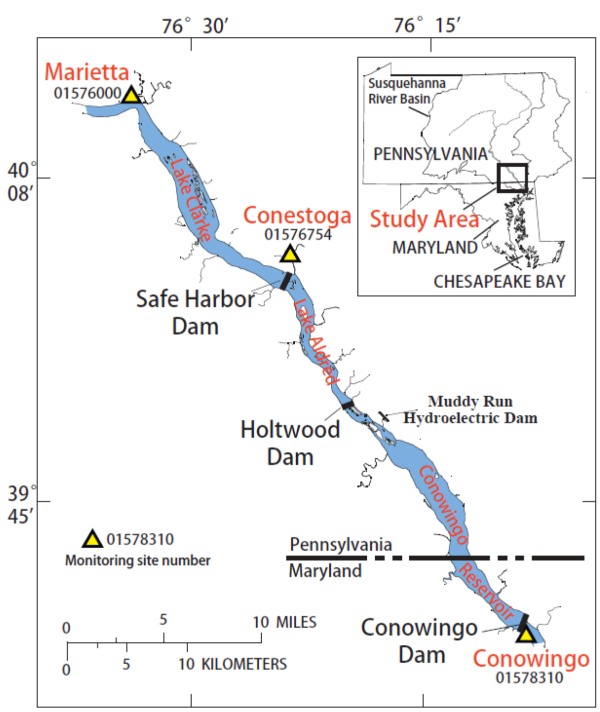

Figure 3. Map of the Lower Susquehanna River Reservoir System consisting of Lake Clarke, Lake Aldred, and Conowingo Reservoir. Yellow triangles indicate the three monitoring sites: Conowingo, Marietta, and Conestoga.

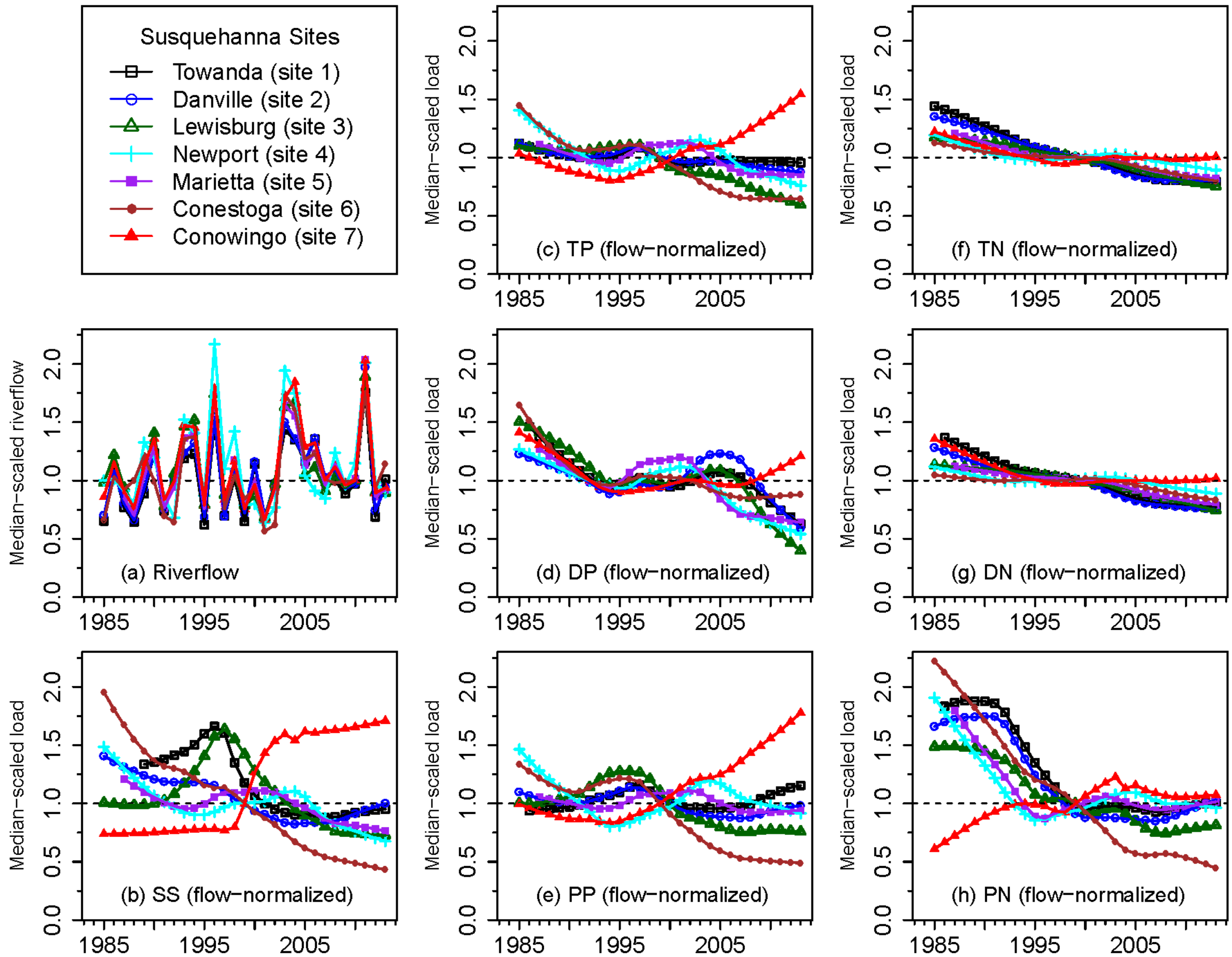

Figure 4. Annual streamflow discharge (a) and annual *flow-normalized* loading trends of SS (b), TP (c), DP (d), PP (e), TN (f), DN (g), and PN (h) at the Susquehanna sites. Note that all values have been scaled by respective long-term annual medians.

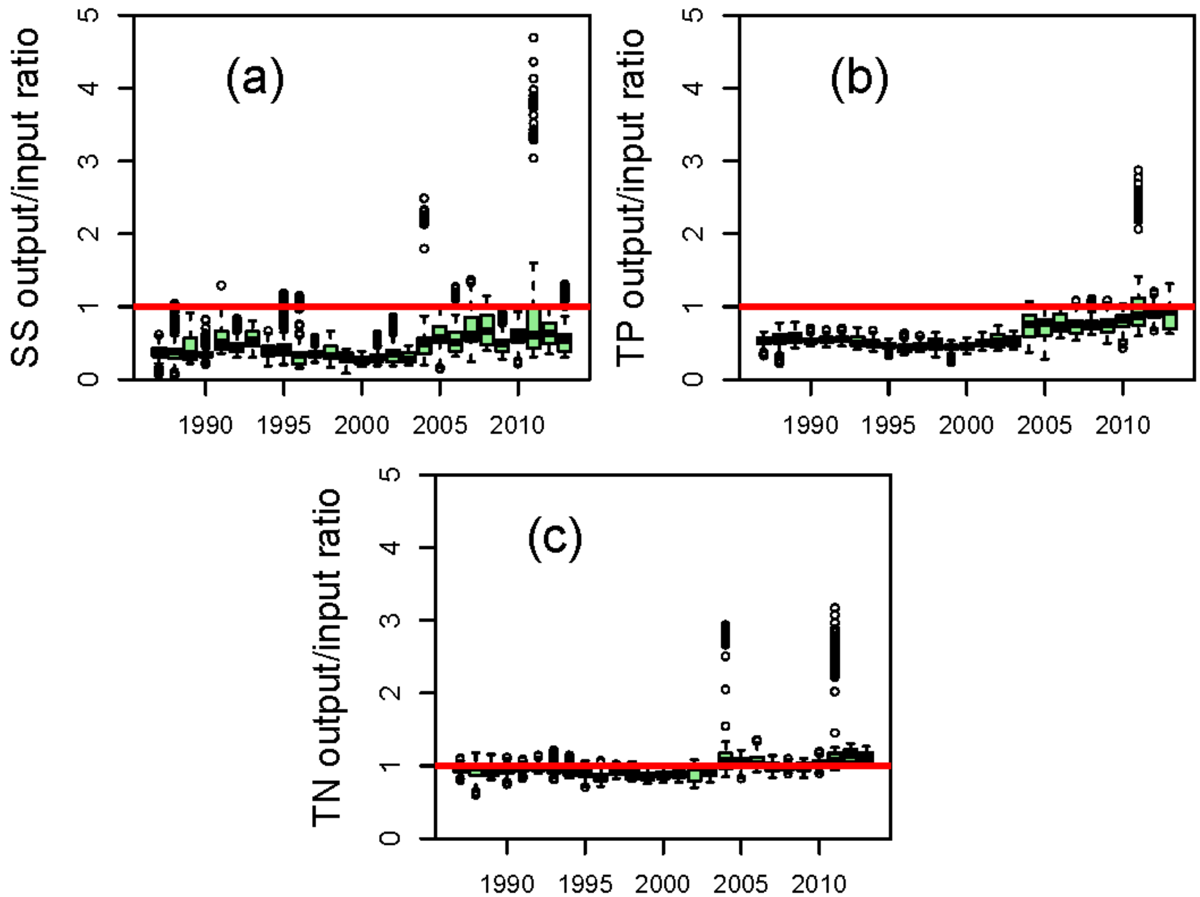

Figure 5. Annual boxplots of estimated output/input ratios based on 35-day moving averages of input and output for (a) suspended sediment (SS), (b) total phosphorus (TP), and (c) total nitrogen (TN) based on loading estimates from the standard WRTDS models. (Note that ratios less than 1.0 indicate net deposition.)

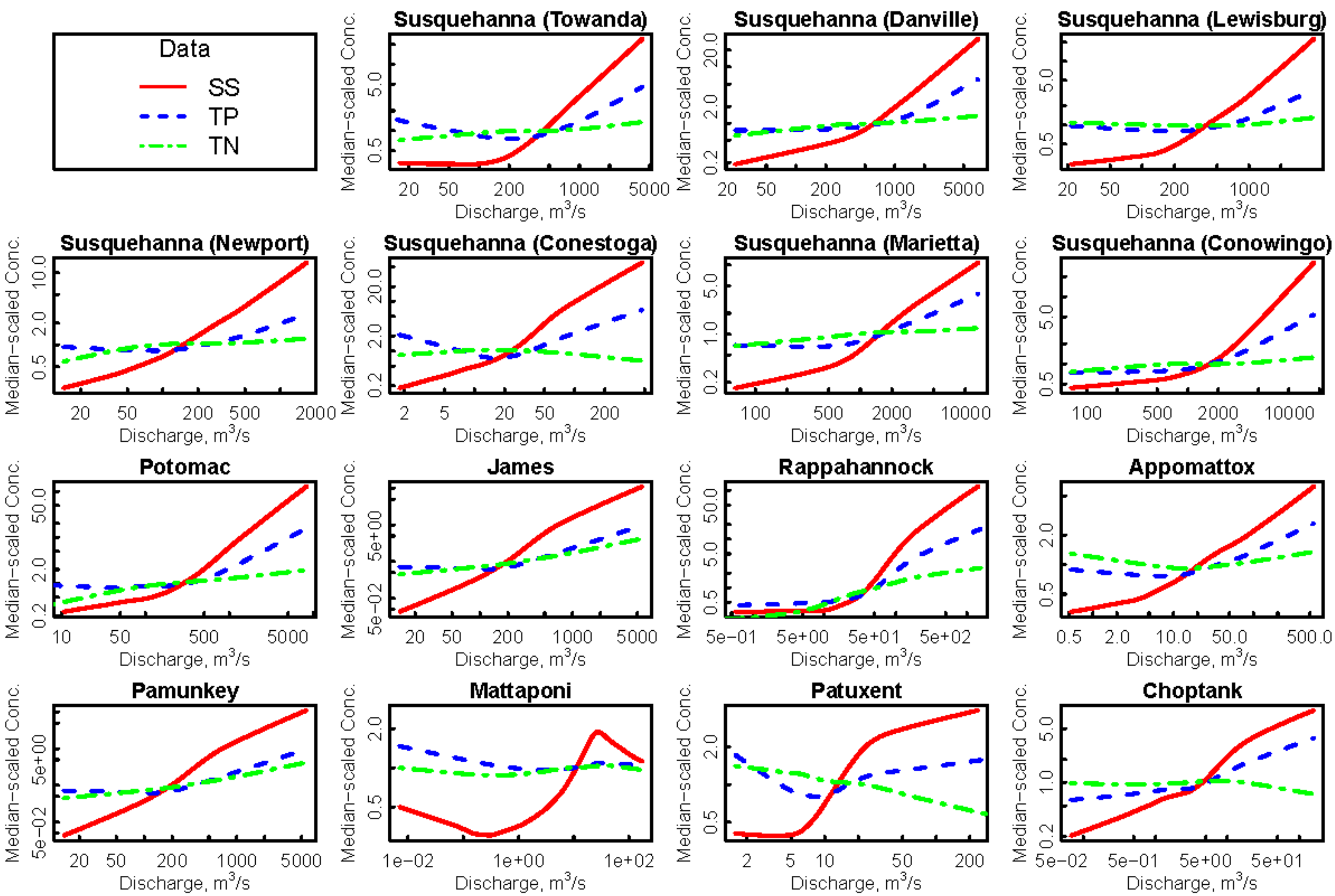

Figure 6. Concentration-discharge relationships for suspended sediment (SS), total phosphorus (TP), and total nitrogen (TN) data at the 15 Chesapeake sites. Median-scaled concentrations are presented to aid comparison across site and species. Smooth lines are fitted non-parametric LOWESS curves. Note that both axes are on logarithmic scale. Data points are omitted for visual clarity.

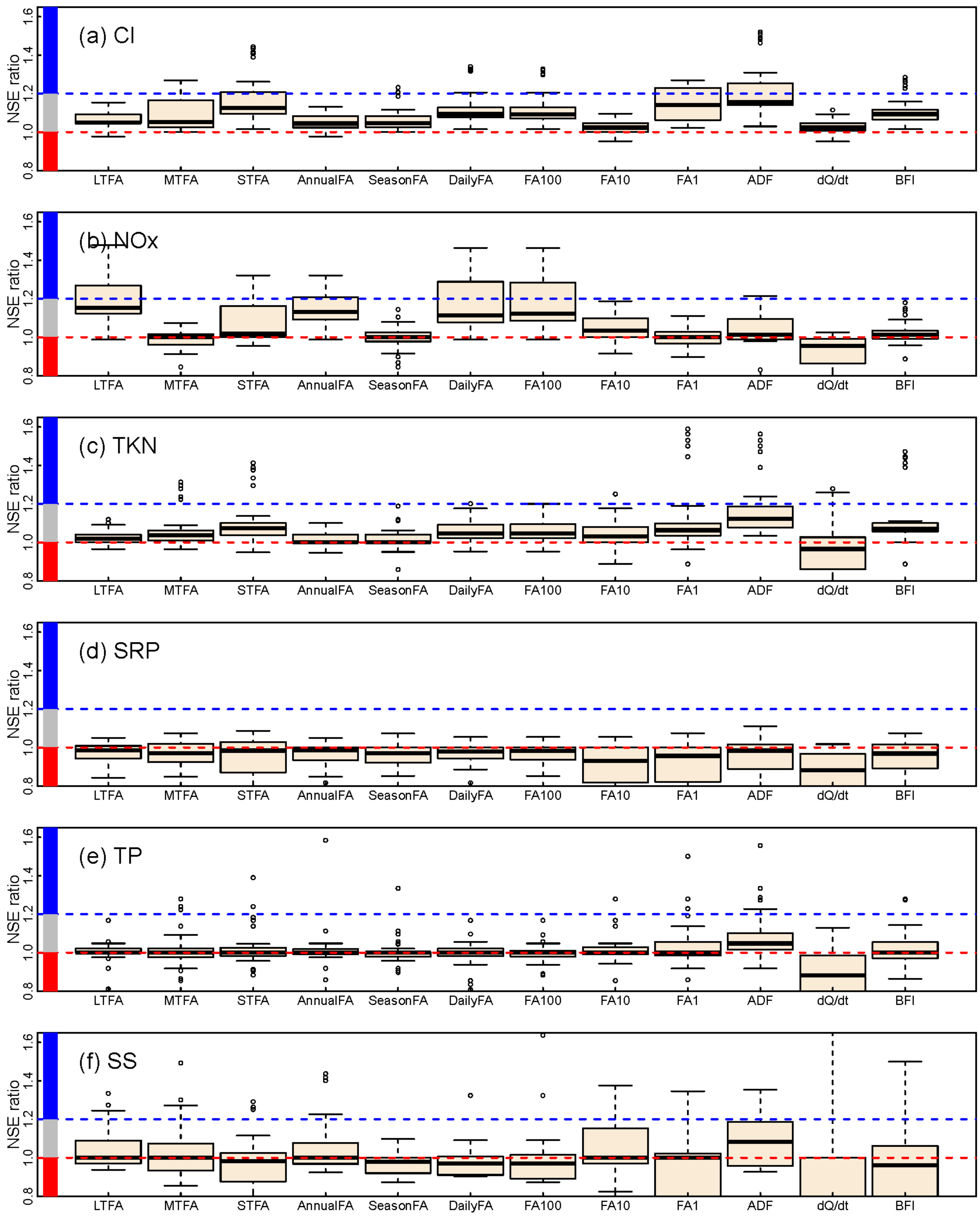

Figure 7. Boxplots of ratio between Nash-Sutcliffe efficiency (NSE) of each modified model and NSE of the original WRTDS model for the seven sites for six constituents: (a) Cl, (b) NOx, (c) TKN, (d) SRP, (e) TP, and (f) SS. The ratios are divided to three regions: (1) major improvement (ratio > 1.2; indicated by blue bars), (2) minor improvement (1 < ratio < 1.2; grey bars), and (3) inferior performance (ratio < 1.0; red bars).

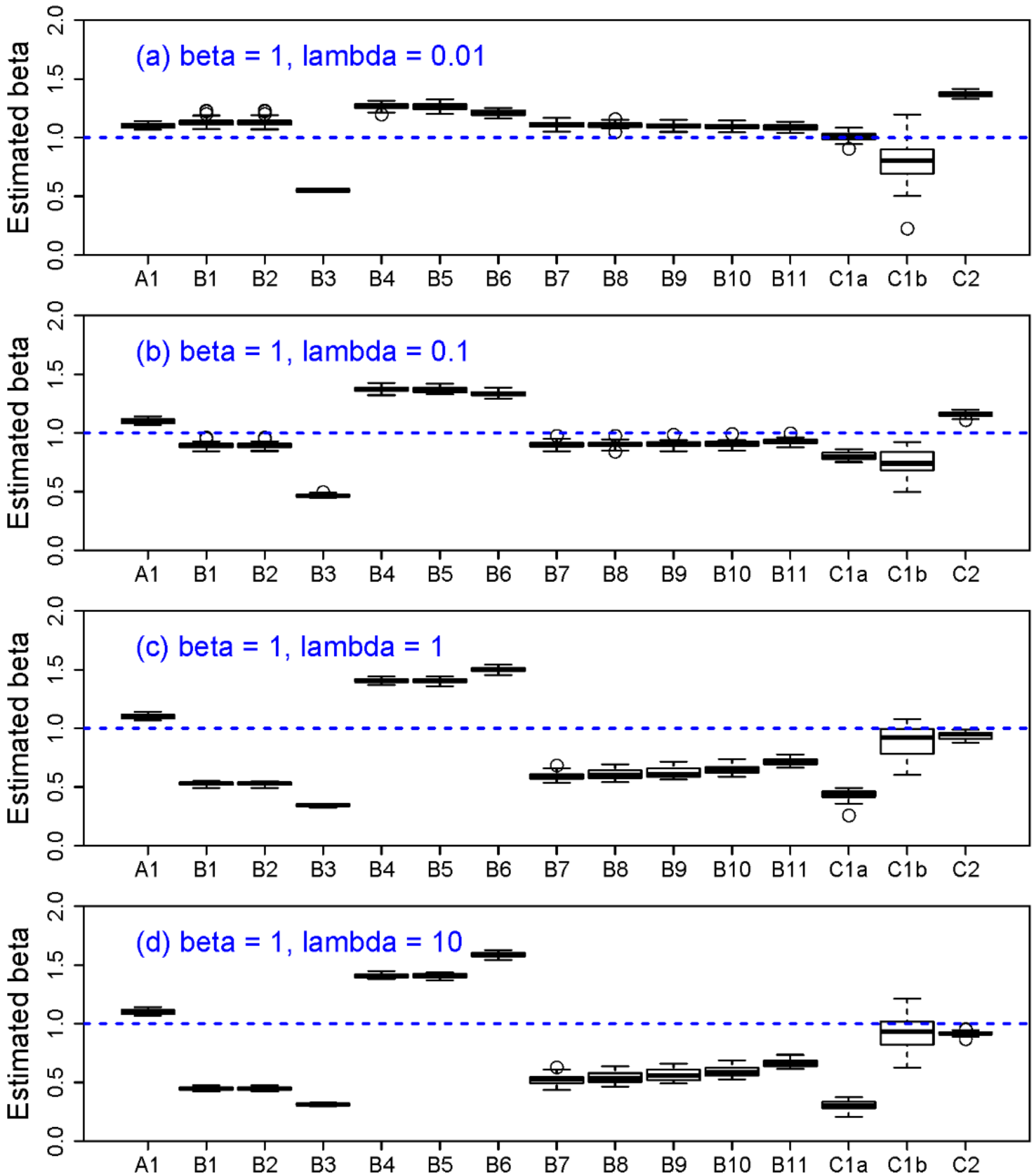

Figure 8. Comparison of bias in estimated LRD strengths in irregular data that are simulated with β = 1 (30 replicates), series length of 9125, and gap intervals simulated with (a) NB (λ=0.01, μ=1), (b) NB (λ=0.1, μ=1), (c) NB (λ=1, μ=1), and (d) NB (λ=10, μ=1).

List of Publications and Conference Presentations

<u>Journal Manuscript(s) under Review</u>

Zhang, Q., R.M. Hirsch, and W.P. Ball. 2015. *Long-Term Changes in Sediment and Nutrient Delivery from Conowingo Dam to Chesapeake Bay: Effects of Reservoir Sedimentation*, **Environmental Science & Technology**, under review.

Zhang, Q., W.P. Ball., and D.L. Moyer. 2015. *Decadal-scale Trends and Sub-basin Mass Balances for Nitrogen, Phosphorus, and Sediment in the Susquehanna River Basin, USA*, **Science of the Total Environment**, under review.

<u>Journal Manuscript(s) in Preparation</u>

Zhang, Q. and W.P. Ball. 2015. *Characterization of Non-Stationary Concentration-Discharge Relationships in Chesapeake Bay Tributaries*, manuscript in preparation.

Zhang, Q. and W.P. Ball. 2015. *Improvement of Riverine Flux Estimation with Flow Anomalies*, manuscript in preparation.

Zhang, Q. and C.J. Harman. 2015. *Evaluation of Methods for Estimating Long-Range Dependence in Irregular Water Quality Time Series*, manuscript in preparation.

<u>Conference Presentation(s)</u>

Zhang, Q. and W.P. Ball. 2015. *Non-stationary Concentration-Discharge Relationships for Nitrogen, Phosphorus, and Sediment for Nine Major Tributaries of the Chesapeake Bay*, oral presentation at American Geophysical Union (AGU) Fall Meeting, San Francisco, CA, December 14-18, 2015.

W.P. Ball, Q. Zhang, and R.M. Hirsch. 2015. *Effects of Reservoir Sedimentation on Sediment and Nutrient Removal in the Lower Susquehanna River Reservoir: An Input-Output Analysis Based on Long-Term Monitoring*, oral presentation at American Geophysical Union (AGU) Fall Meeting, San Francisco, CA, December 14-18, 2015.

Zhang, Q. and W.P. Ball. 2015. *Concentration-Discharge Relationships for Nutrients and Sediment in Major Tributaries to Chesapeake Bay: Typical Patterns and Non-Stationarity*, poster presentation at Maryland Water Monitoring Council (MWMC) Annual Conference, Linthicum Heights, MD, November 13, 2015.

Zhang, Q. W.P. Ball., and D.L. Moyer. 2015. *Long-Term Export of Nitrogen, Phosphorus, and Sediment in the Susquehanna River Basin: Analysis of Decadal-Scale Trends and Sub-Basin Mass Balances*, oral presentation at Geological Society of America (GSA) Annual Meeting, Baltimore, MD, November 1-4, 2015.

Chironomus riparius: A Tool for Studying Ecological Effects of “Inert” Safeners (Graduate Fellowship)

Basic Information

Title:	Chironomus riparius: A Tool for Studying Ecological Effects of “Inert” Safeners (Graduate Fellowship)
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Principal Investigators:	Kaye Lorraine Brubaker, Susan Gresens

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1. Bolyard, Kasey; Susan Gresens, John Sivey, Chris Salice, 2015, Chironomus riparius: A tool for studying the ecological effects of “inert” safeners, in SETAC North America 36th Annual Meeting Meeting Program, SETAC, Salt Lake City, Utah, p. 57.

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Chironomus riparius: A Tool for Studying Ecological Effects of "Inert" Safeners
(Graduate Fellowship)
2015MD330B

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Background and Objectives

"Safeners," chemicals that protect crops from herbicidal injury when mixed into formulation with active herbicides, have emerged over the past few decades.[1,2] The dichloroacetamide class of safeners, which includes benoxacor, dichlormid, furilazole, and AD67, protect corn crops from the thiocarbamate and chloroacetamide classes of herbicides[2,3] (Figure 1). Dichloroacetamide field application rates are estimated at over 2 million kg/year in the United States.[4] S-metolachlor, a major chloroacetamide commonly paired with benoxacor or dichlormid, had an estimated annual usage in the United States of at least 16 million kg in 2012.[4] Nevertheless, the environmental distribution and fate of safeners is little studied.[5] Considered "inert" ingredients in herbicide formulations[2], safeners are registered differently than herbicide active ingredients in the United States.[2]

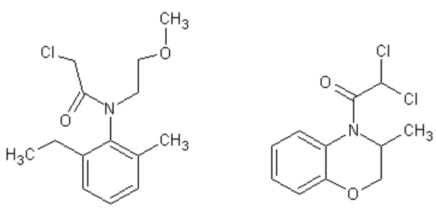

Figure 1. Molecular structures of the chloroacetamide herbicide metolachlor (left) and its typical safener, benoxacor (right).

Although fate and effects data are required for inert ingredients when being registered in the United States, there is a large knowledge gap regarding ecological effects studies, for example, chronic sediment toxicity studies, in regards to the dichloroacetamide safeners.[4] Given the extent of the use of safeners in the U.S. and elsewhere, this lack of ecotoxicity data represents a significant uncertainty in assessing the ecological risks of agrochemicals.[4] Within anaerobic reaction chambers under laboratory conditions, it has been demonstrated that dichloracetamide safeners can undergo reductive dechlorination, transforming into products that more closely represent their active herbicidal counterparts (Figure 2).[5]

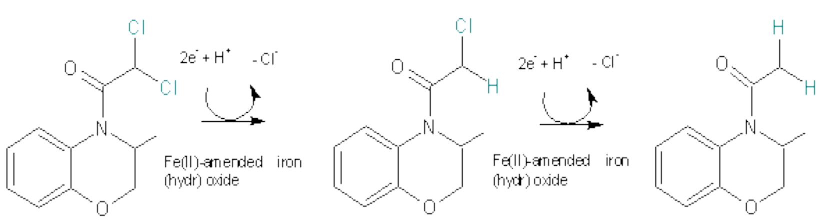

Figure 2. Reductive dehalogenation of benoxacor into its transformation products, monochlorobenoxacor and deschlorobenoxacor, in the presence of Fe(II)-amended iron (hydr) oxide, adapted from reference 4

The intermediate product, which contains one geminal chlorine, could be important for ecotoxicity testing, since this chemical could be more biologically active in non-target organisms than the original.[5]

Research Methods

A major goal of this project is to determine the possible toxicity of four compounds, S-metolachlor, benoxacor, monochlorinated benoxacor (a transformation product), and a mixture (S-metolachlor + benoxacor), to larval *C. riparius*, within a chronic, 28-day sediment toxicity study. For our experiments, we used a field-collected (Mt. Washington, MD), iron-rich soil, and amended appropriately with DI water, sieving the particles to 2 mm, creating an aquatic sediment-like substrate. We also added 30% organic matter by dry sediment weight in the form of alpha-cellulose powder (Sigma-Aldrich), to encourage the progression of anaerobic conditions within our spiked-sediment.

We ran three range-finding studies, a high set (ranging from 0.01 mg/kg-100 mg/kg) of benoxacor, and two low sets (ranging from 0.1 µg/kg-1000 µg/kg) of benoxacor and S-metolachlor, before choosing definitive concentrations for the *C. riparius* microcosms. Range-finding studies use a smaller number of replicates (1-2 per treatment) to give the researcher a preliminary idea of which chemical concentrations will likely elicit effects of interest in the study organism. Our first definitive block resulted in an extremely similar, non-monotonic dose-response curve for emergence ratios within the benoxacor treatment group as was seen in the high-concentration range-finder we ran for this chemical.

Our definitive toxicity experiments used sediment spiked following OECD 218 protocols[6], by spiking small batches of sediment for each chemical at five nominal concentrations: 0.01, 0.1, 1, 10, and 100 mg/kg. This was done similarly for the three individual chemicals. For the mixture (S-metolachlor + benoxacor) the sediment was spiked with equal parts of each compound, achieving nominal concentrations of 0.02, 0.2, 2, 20, and 200 mg/kg. 1 to 3 day-old (first-instar) larvae were initiated into each experimental replicate in equal age proportions (4 one-day-olds, 12 two-day-olds, and 4 three-day-olds, for a total of 20 individuals per replicate. Biological observations were made once daily until either an organism was found dead (larvae or pupae) or the first adult organism emerged, whichever occurred first. At this point, observations began to be made twice daily.

Because of the size of the study, it is being conducted in two replicate blocks of treatments: block 1 has been completed, and the second block of the experiment is to be completed in December. Each block contained two replicates per chemical treatment and concentration, as well as two replicates for a solvent control group, and two more replicates for a negative control group. The complete dataset will have four replicates for each chemical and concentration.

A second part of our research was documenting the spike recovery of benoxacor within smaller models of our microcosms, sans *C. riparius*. Within two aqueous-only chambers, 3.1 mg of benoxacor was spiked into 150 mL of DI water (chamber 1) or 150 mL of a premade culture medium (modified Elendt M7 medium, chamber 2) achieving an 80 uM concentration. In two other chambers, 3.1 mg of benoxacor was either spiked onto sand (chamber 3) or the Mt. Washington sediment (chamber 4) achieving a 100 mg/kg concentration by dry weight. Both of these chambers had 150 mL of M7 medium overlying their respective bottom substrates. Aqueous samples were taken from all four chambers periodically throughout 28 days, using a plastic 3 mL plastic syringe, and these samples were filtered using 0.2 µm nylon filers. The chambers with bottom-substrate were also sampled periodically from within the substrate. Samples were collected using a 3 mL plastic syringe, then shaken and stored in 5 mL of methanol, effectively removing all recovered benoxacor from the substrate.

Results

The first block of our *C. riparius* experiment revealed some obvious trends. A lethal effect of monochlorinated benoxacor, S-metolachlor, and the mixture is discernible at the highest treatment levels (100 mg/kg, 200 mg/kg for the mixture). A non-monotonic dose-response is suggested by the emergence ratio means of the treatments with benoxacor and monochlorinated benoxacor. But, with only two replicates to-date, the form of the responses is not clear. Regression analysis will be completed on biological variables (emergence ratio, average individual body weight) once the second block of the experiment is completed. Time-to-event analysis for emergence times will also be examined. There is evidence that the form of the *C. riparius* responses that we are measuring may vary between the mixture and the individual chemicals (Figure 3).

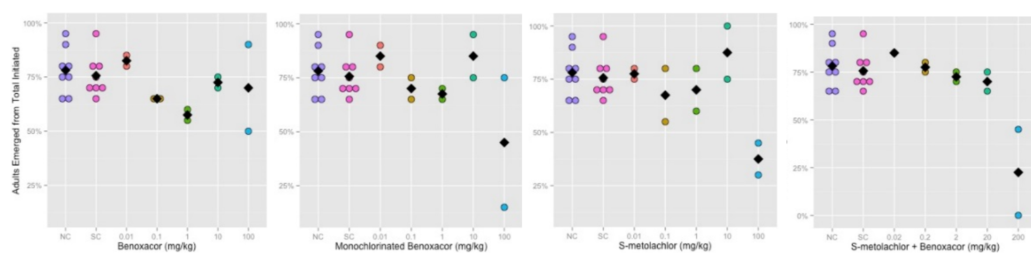

Figure 3. Dot plots depicting the spreads and means (black dot) of emergence ratios for each chemical and experimental concentration. The entire control group for this first experimental block is included (and therefore the same) on each of the four graphs.

Spike recovery of benoxacor remained constant over time in the aqueous-only microcosms (Figure 4, left). Benoxacor reached an equilibration point in the water column of the spiked-sand microcosm (right). However, a loss of the compound was evident in aqueous samples from the spiked-sediment microcosm (right), suggesting transformation of benoxacor occurred. Since these chambers (which didn't contain *C. riparius*) were proportionately the same as the biological microcosms used in the toxicity experiments, it is inferred that the same magnitude and rate of benoxacor transformation within the sediment chamber occurred in the toxicity microcosms.

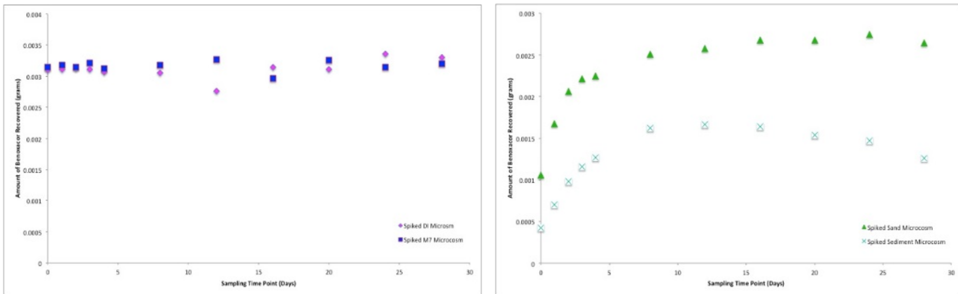

Figure 4. Graphs depicting benoxacor recovered in aqueous-only microcosms (left) and substrate-containing microcosms (right).

Experimental results to date show that benoxacor exhibits similar toxic effects relative to its partnered herbicide S-metolachlor. Benoxacor does not appear to have been evaluated previously within any chronic aquatic or sediment peer-reviewed studies. Although our chemicals in question do not seem to elicit a severely toxic response in *C. riparius*, a moderate effect may be occurring. The shape and degree of the response to these chemicals is also interesting, and we anticipate the second block experiment and subsequent analysis being completed. The more sensitive, sub-lethal variables, such as body weight and time-to-emergence, may prove to be important affected end-points within our chronic exposure system.

The information from the spike-recovery experiment suggests that the choice of materials used in our experiments (such as the type of sediment) can affect the transformation of chemicals (i.e. benoxacor to monochlorinated benoxacor) and thus their relative toxicities. If we had used a standard formulated, kaolin-based sediment, this benoxacor dechlorination should not have occurred. Spike recovery data suggests that the non-monotonic response in *C. riparius* to benoxacor may in part be due to its transformation to monochlorinated benoxacor. The two chemicals elicited very similar dose-response curves within our chronic exposure experiment. Barring any significant toxic response in our organism or not, this work will achieve novel insight to the behavior of safening chemicals in model benthic systems, as well as to the chronic effects of these chemicals and their mixtures in benthic organisms.

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Publication

Bolyard, Kasey; Susan Gresens, John Sivey, Chris Salice, 2015, *Chironomus riparius*: A tool for studying the ecological effects of “inert” safeners, in SETAC North America 36th Annual Meeting Meeting Program, SETAC, Salt Lake City, Utah, p. 57

Assessing hydrogeomorphological constraints on water quality functions of forested riparian buffers in Western Maryland (Graduate Fellowship)

Basic Information

Title:	Assessing hydrogeomorphological constraints on water quality functions of forested riparian buffers in Western Maryland (Graduate Fellowship)
Project Number:	2015MD331B
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Research Category:	Water Quality
Focus Category:	Water Quality, Nutrients, Geomorphological Processes
Descriptors:	None
Principal Investigators:	Kaye Lorraine Brubaker, Keith N. Eshleman

Publications

There are no publications.

Stephanie Siemek
University of Maryland Center for Environmental Science (UMCES)
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Assessing hydrogeomorphology constraints on water quality functions of forested riparian buffers in Western Maryland

MWRRC Grant# 2015MD331B

Advisor: Dr. Keith Eshleman
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Background and Objectives

Riparian forest buffer systems (RFBS) are considered to be a best management practice (BMP) in reducing nutrient runoff into the Chesapeake Bay, especially from agricultural fields (CBW, 2012). Although very few data exists supporting riparian buffers at a larger watershed scale (McCarty et al, 2008), the Chesapeake Bay Program (CBP) is currently working towards restoring 900 miles of stream banks and shorelines with forest buffers in order to restore the Bay by 2025 (CBW, 2012). Therefore, more research is needed to ensure riparian buffers are a best management strategy in each of the geological provinces of the Bay's watershed. Most experimental studies of the efficacy of nutrient removal by RFBS's have been conducted in the Coastal Plain (Bradburn et al., 2010; Lowrance et al., 1997; Sweeney et al., 2002). The relatively few studies of RFBS's elsewhere have found a relatively wide range of performance across the various physiographic provinces. Previous research has suggested that the hydrogeomorphological setting of RFBS's and associated hydrologic flowpath (i.e., nature of the surface water-groundwater interaction) may determine the functionality of buffers, as shallow flow paths are optimal for function because they allow for water interception within the rhizosphere (Lowrance, 1997). The overall goal of the research conducted as part of this MWRRC summer fellowship was to assess the extent to which RFBS's are reducing nutrient runoff from a representative Ridge and Valley watershed in western Maryland. The specific project objectives were to:

- Characterize the temporal and spatial variability in water quality along the mainstem of a stream (Murleys Branch) draining a mixed land use watershed in the Ridge and Valley (R&V) province of western Maryland using a repeating synoptic survey design;
- Identify the primary factors governing the variations in water quality from the headwaters of the watershed to the outlet;
- Quantify the extent of interaction between surface water and groundwater within two representative reaches of the mainstem (one reach with an RFBS and a control reach lacking an RFBS)

Summer Activities/Research Methods

The field study was conducted in the Murleys Branch watershed (area = 31.1 km^2) near Flintstone, Maryland (Fig. 1). Land use in the basin is a mix of forest, agriculture, and rural residential. Some of the landowners participate in the Conservation Reserve Enhancement Program (CREP) where farmers are given incentives through the government to plant trees in riparian zones to reduce erosion and nutrient pollution into streams. Like many other watershed systems in the R&V physiographic province, streamflow in the mainstem (and perhaps in some individual tributaries) is influenced by flows from two springs (one in the southern region known as Murleys Branch Spring and the other in the northern region known as Warm Spring). A portion of the discharge from Flintstone Creek upwells into Murleys Branch near Warm Spring. Flintstone Creek is a river part of a neighboring watershed separated by a mountain, but has been verified by locals that it flows underneath the ground, surfaces, and then merges into Murleys Branch. These hydrologic features demonstrate the complexity of R&V hydrology and underline the challenges associated with understanding water quality and its relationship with land-use and conservation activities at the basin scale.

Land-use was determined using ArcMap 10.2.2 and imagery acquired from Department of Geographical Sciences (DGS) at University of Maryland (UMD) using Light Detection and Ranging (LiDAR). From this data, land-use was defined and calculated (e.g. percent of canopy and agriculture); planted riparian buffers were identified and verified using documentation from the Department of Natural Resources (DNR). Watersheds (and local contributing areas) were delineated using ArcHydro or TauDEM routines. Synoptic stream surveys (32 stations) were conducted on a bi-monthly basis to characterize the spatial and temporal variations in water quality along the mainstem of Murleys Branch during the study. Water samples were analyzed for specific conductance, pH, acid neutralizing capacity (ANC), total dissolved nitrogen (TDN), total phosphorus (TP), orthophosphate- P (PO_4-P), ammonia (NH_3-N), nitrite (NO_2-N), nitrate (NO_3-N), chloride (Cl), bromide (Br), sulfate (SO_4), and dissolved organic carbon (DOC) using established analytical methods.

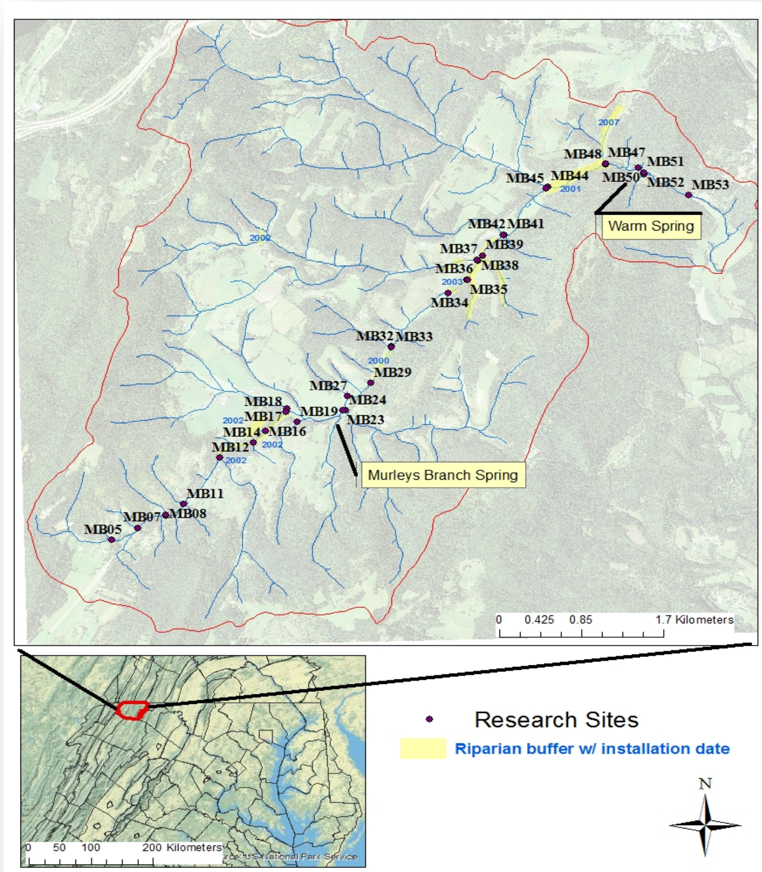

Figure 1 Map of Murleys Branch watershed in Flintstone, Maryland. Points in upper right map indicate study sites along the mainstem and yellow polygons represent riparian buffers. Streamwater flows from south to north.

An intensive field study of surface and groundwater interactions within two representative reaches of the mainstem (Fig. 2) was conducted to assess the potential influence of planted RFBS's on water quality in Murleys Branch. The upstream reach contains an RFBS that was planted as part of the CREP in 2000, while the lower reach does not contain a planted RFBS. Upstream and downstream nodes of both reaches were instrumented using established USGS gaging methods, including a staff gage and continuously recording pressure transducer to monitor gage height (15 minute interval; Fig. 3). The four stations are:

- MB29 is the upstream station located near the southern end of the riparian buffer
- MB32 is the downstream station located at the northern end of the riparian buffer (the local contributing area associated with this reach is 33.75 hectares based on terrain analysis of a 30m DEM)
- MB33 is the upstream station for the control reach
- MB34 is the downstream station for the control reach (the local contributing area associated with this reach is 63.87 hectares)

After installing the stream gaging equipment, the following field methods were employed throughout the study:

- Hobo loggers were deployed at each of the four stations to provide continuous in situ measures of specific conductance and water temperature (Fig. 4);
- Grab water samples were collected bi-weekly for nutrient analysis (same analyses were performed as the bimonthly samples with the addition of total suspended solids (TSS);
- Streamflow was measured bi-weekly using the velocity area method to develop a rating curve for each station;
- Runoff hydrographs for the study period were created for each station using the continuous gage height records and the rating curves; and
- Net lateral inflow hydrographs (normalized by the local contributing area of each reach) were determined from the differences in upstream and downstream discharge.

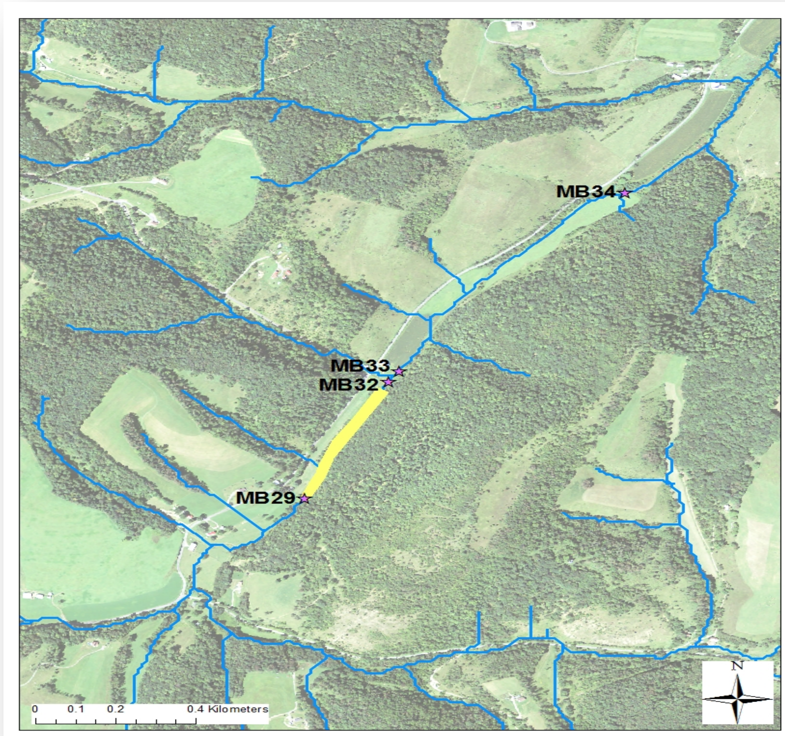

Figure 2. Map showing the locations of the two intensively studied stream reaches, gaging stations (purple stars), planted buffer (yellow polygon), and local contributing areas.

Figure 3. Research sites MB29 (top left), MB32 (top right), MB33 (bottom left), and MB34 (bottom right). Pictures show staff gages within the streams at each of the research stations.

Figure 4. Housing units for Hobo loggers were built and employed to protect instruments from damage.

Quality control procedures were carefully followed both in the lab and in the field in order to reduce sample contamination. Water samples were kept on ice in a cooler until they could be transported back to the lab and filtered within 24 hours of collection using a 0.45 µm membrane filter. Once filtered, they were aliquoted into proper containers, cations and DOC aliquots were preserved with nitric acid and phosphoric acid, respectively, and samples were stored in a specific temperature environment (4°C or -20°C depending on storage standards) until lab analysis was completed.

Results/Lessons Learned/ Next steps

As this was my first research experience in field hydrology, I obtained valuable experience in 1) fabricating and installing stream monitoring equipment; 2) periodically downloading water data to a laptop computer; 3) measuring discharge using the velocity area method and constructing rating curves; 4) analyzing gage height and discharge hydrographs using HOBOware: 5) filtering water samples in the lab and analyzing them for ANC, specific conductance, and nutrient concentrations.

Results from the synoptic survey showed spatial and temporal variations with the constituents tested (Fig. 5 & 6) with no obvious trends with all RFBS locations. NO3-N concentrations generally declined from upstream to downstream and NH3-N, PO4-P, and TDP varied with some concentrations explained by land-use. For instance, tributary MB17, which runs along a cattle farm, had high nutrient concentrations and reach MB29-MB32, which has a RFBS, showed a detectable reduction in all four nutrient concentrations. However, nutrient reductions can occur for multiple reasons, including watershed management (e.g. RFBS), in-stream biological activity, or dilution from less contaminated, surfacing springs. Therefore, all these possibilities must be taken into account before confirming RFBS are responsible for nutrient reductions. RFBS adjacent to reach MB29-MB32 does not protect against any upland agricultural runoff. Corn is grown adjacent to reach MB33-MB34; however, nutrient concentrations were not much higher in this reach. The sudden drop in nutrients in MB29-MB32 indicates some type of dilution effect, rather than a nutrient mitigation effect from the RFBS. I couldn't get permission to install the groundwater wells for the intensive study, but such data could have helped better discern the nutrient removal functions of the RFBS.

Constituents tested that may give some implication to flow patterns based on the water's chemistry, included SO_4, Cl, ANC, and conductivity. SO_4 and Cl are both very high in MB32 sample, where high SO4 can possibly be due to chemical weathering of bedrock (Mayer et al., 2010) and Cl concentration may be a legacy effect from winter road salt. ANC and conductivity both decline along the mainstem, but at MB32 dramatically increase. Variations in the stream's chemistry may result from deep, ground water interacting with bedrock, surfacing, and then merging into the stream.

During the intensive study, the hydrology of reaches MB29-MB32 and MB33-MB34 were further investigated. An intense storm occurred late June, knocking down two staff gages; therefore, data up to that event was omitted. Reach MB29-MB32 had a higher mean lateral flow than reach MB33-MB34, which was negative (Fig. 7A & B). Mean runoff from MB29 (Fig. 7C) catchment had a small contribution to streamwater compared to the lateral flow. Town Creek discharge, provided by USGS, is shown to compare trends (Fig. 7D), which had similar results as MB29. Results indicate MB33-MB34 is either a losing reach or MB29-MB32 has an additional source of water discharge.

Data collected from the conductivity and temperature HOBO loggers showed a diurnal and seasonal variation as expected. Next steps may include setting loggers at multiple depths to measure streambed temperature profile that will signify if the streams are losing or gaining reaches (Constantz, 2007). More flow measurements and analysis between nutrient data and land-use/soil-type within the watershed will also be performed.

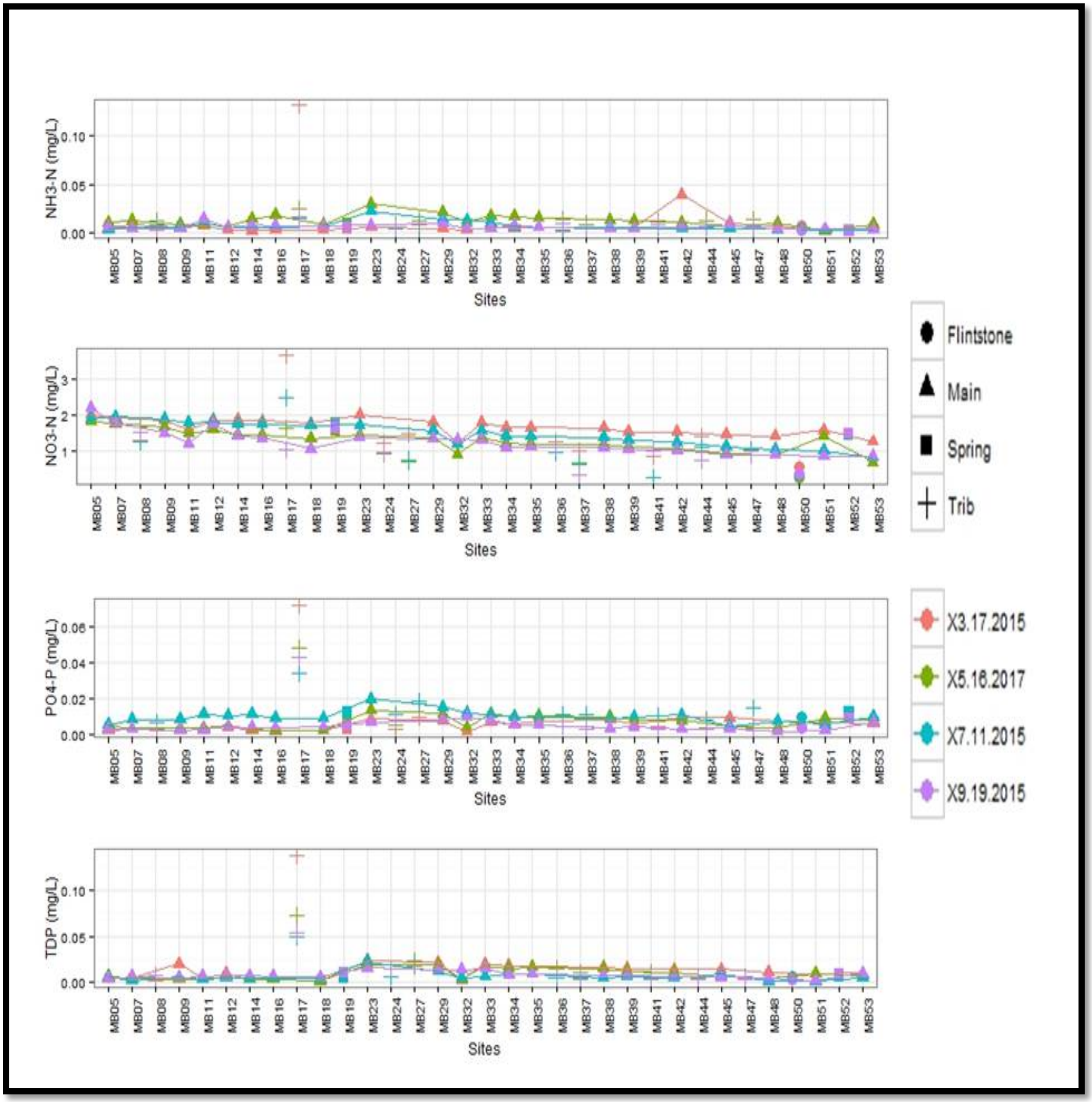

Figure 5 Spatial and temporal variations of NH3, NO3, PO4-P, and TDP within the mainstem. MB05 is the most southern region within the watershed, where Murleys Branch flows northward. NO3 decreases as the stream flows further downstream. All four nutrients peak at MB17, which is a tributary that runs along farmland before discharging into Murleys Branch. MB29-MB32 shows a decrease in all four nutrients, possibly from dilution from a spring or a functional RFBS. Other sites must be further investigated to understand nutrient concentrations.

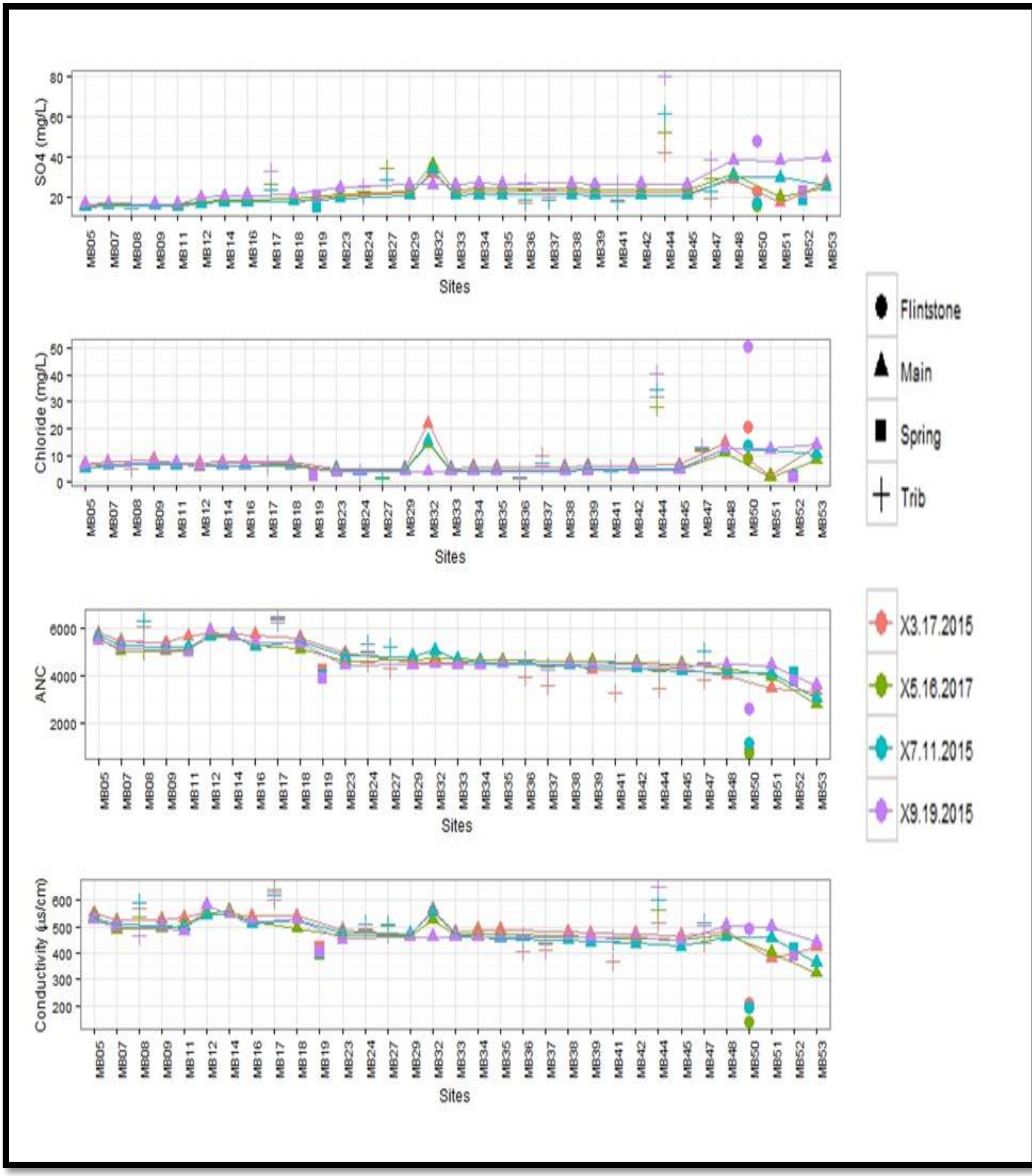

Figure 6 Spatial and temporal variations of SO4, Cl, ANC, and conductivity in Murleys Branch watershed. Each constituent shows a sudden peak at MB29-MB32, possibly from the discharge of water that has reacted with deep bedrock. Results will be investigated further to determine relationship with soil type, geology, and/or land-use.

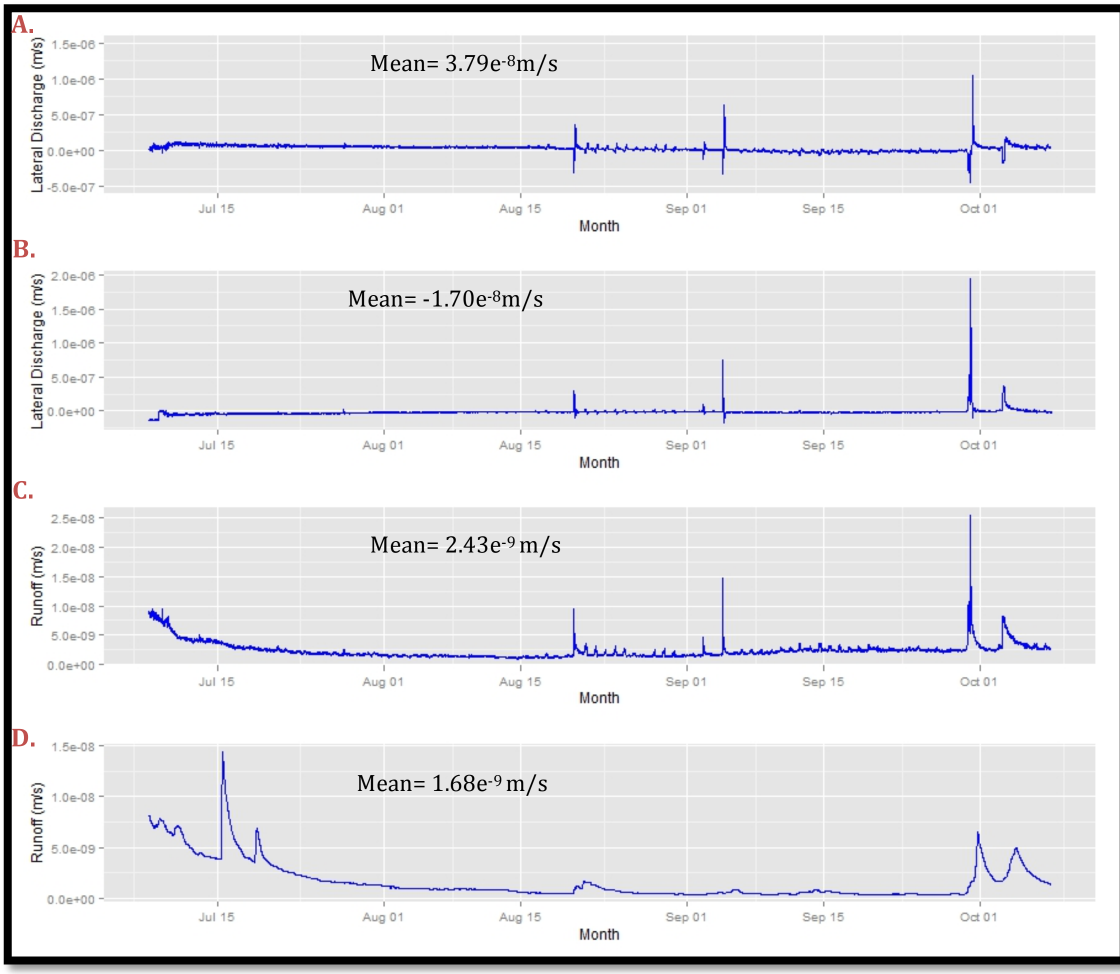

Figure 7 Top two graphs (A.&B.) show lateral inflow for reach 1 (MB29_32) and reach 2 (MB33_34), respectively. Third graph (C.) shows MB29 runoff normalized by contributing area of drainage basin. Forth graph (D.) shows Town Creek from a nearby USGS gaging site for comparison. Both show similar trends with peaks from rain events.

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Storm Water Runoff and Water Quality Modeling in Urban Maryland (Graduate Fellowship)

Basic Information

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Principal Investigators:	Kaye Lorraine Brubaker, Barton Forman

Publication

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Name: Jing Wang

Civil and Environmental Engineering

University of Maryland, College Park

Advisor: Dr. Barton Forman, baforman@umd.edu

Storm Water Runoff and Water Quality Modeling in Urban Maryland

Number: 2015MD332B

Background and Objective:

Urbanization significantly affects storm water runoff through the creation of new impervious surfaces such as highways, parking lots, and rooftops. Such changes can adversely impact the downstream receiving water bodies in terms of physical, chemical, and biological conditions. In order to mitigate the effects of urbanization on downstream water bodies, stormwater control measures (SCMs) have been widely used (e.g., infiltration basins, bioswales). A suite of observations from an infiltration basin installed adjacent to a highway in urban Maryland was used to evaluate stormwater runoff attenuation and pollutant removal rates at the well-instrumented SCM study site. In this study, the Storm Water Management Model (SWMM) was used to simulate the performance of the SCM.

The purpose of this study is to apply and calibrate SWMM using observed runoff flows and pollutant concentration measurements collected from an existing infiltration basin installed adjacent to Highway 175 in Columbia, Howard County, Maryland. The infiltration basin is designed to treat the runoff from a small portion of the highway (Natarajan 2012). The main objectives of the research are:

1. To setup the SWMM model for use in the study watershed.
2. To automatically calibrate and validate the SWMM model for the MD 175 infiltration basin. The calibrated model should be able to reproduce the past hydrological and water quality-related features of the study area.
3. To explore model sensitivities related to parameters, which can be used to improve model calibration via a reduction in the parameter space.

An automatic calibration routine will be developed for SWMM model calibration and validation, which can improve calibration efficiency and can eventually be used by greater SWMM modeling community.

Research Methods:

Study Domain

The study site is located along MD 175 East in Columbia, Howard County, Maryland. It is a small drainage area (2.9 ha) that includes an existing infiltration basin.

Based on topography delineation, the total area of the study site is 2.9 ha of which 33% is impervious. The weighted curve number for infiltration is 75. The drainage area consists of impervious highway surfaces and grassy areas directly connected to the infiltration basin. Runoff from the entire drainage area is concentrated into the grassy area and then flows into the infiltration basin. The infiltration basin has one inflow and one outflow point via installation of calibrated weirs.

Model Setup

In this study, both water quantity and water quality variables were of interest. Therefore, the study area was modeled in SWMM for both runoff and pollutant loads.

Based on the hydrologic behavior of the study area, four "subcatchments" were established in SWMM model that included two highways and two grassy areas. Two channels and one culvert were established in SWMM to reproduce the flow routing for the study domain.

Based on previous field observation, the infiltration basin in the study area was ponded with water through the entire study period. Therefore, it is reasonable to treat the infiltration basin as a wet pond, which can be represented as a node “storage unit” in SWMM.

Meteorological Boundary Conditions

The hourly precipitation was obtained from the NOAA National Climate Data Center’s Quality Controlled Local Climatological Data (QCLCD). Normals daily station USW00093721, closest to the study site, was selected for this research.

Measurement Datasets

The runoff and water quality data collected from 2009 and 2012 by Dr. Allen Davis’ research group were used for the model calibration and verification. The runoff flows at the inlet and outlet of the infiltration basin were recorded continuously at a 2-minute interval for 103 different storm events (Natarajan 2012). Meanwhile, water quality samples were collected at the inlet and the outlet of the infiltration basin during a subset of these rainfall events.

Auto-Calibration Procedure

Calibration begins by generating at least m+1 sets of model parameters automatically where m = number of parameters being identified. In this study, the boundaries (feasible ranges) of input parameters were defined based on peer-reviewed literature. The distribution of the input parameters was assumed to be uniform (non-informative) and no serial correlation between these characteristic. Therefore, the parameters can be calculated within feasible boundaries as follows:

$$X_{i,j} = L_i + r_{i,j}(U_i - L_i) \qquad (3.3.1-1)$$

where $X_{i,j}$ is the value of the ith parameter in the jth replicate; U_i is upper bound of the ith parameter; L_i is lower bound of the i th parameter; $r_{i,j}$ is random variable that has a uniform distribution on the continuous range [0, 1]; i is ith parameter; and j is j th replicate for a set of size J (Barco et al. 2008).

<u>Sensitivity analysis for SWMM parameters</u>

SWMM parameters exhibit different sensitivity when modeling different watersheds (Tsihrintzis and Hamid 1997; Barco et al. 2008). Therefore, a sensitivity analysis was conducted to improve calibration efficiency and reduce the overall parameter space in this study.

In the study, the parameters related to subcatchment (width, surface slope, Manning's N for impervious/pervious area, depression depth on impervious/pervious area, percent of impervious area with no depression storage, runoff curve number, and drying day) were calculated by weighting proportionally to their drainage areas.

<u>Model Calibration (with Validation)</u>

In this study, inflows and outflows were simulated at 2-minute interval simultaneously considering all selected storm events (Shinma and Reis 2014).

The objective functions for water quantity were calculated by weighting the inflow and outflows,

$$\begin{cases} NSE = \alpha \cdot NSE_{inflow} + \beta \cdot NSE_{outflow} \\ Rel.B = \alpha \cdot Rel.B_{inflow} + \beta \cdot Rel.B_{outflow} \\ R = \alpha \cdot R_{inflow} + \beta \cdot R_{outflow} \end{cases} \quad (3.3.3.-1)$$

in which $NSE, Rel.B, and\ R$ are Nash-Sutcliffe coefficient, relative bias and correlation coefficient for the model, respectively; $NSE_{inflow}, Rel.B_{inflow}, and\ R_{inflow}$ are for model inflows; $NSE_{outflow}, Rel.B_{outflow}, and\ R_{outflow}$ are for model outflows; α is weighted factor for inflows ($\alpha = 0.8$); and β is weighted factor for outflows ($\beta = 0.2$). Because we have more confidence for inflow measurements rather than outflow measurements.

The objective functions for water quality were relative bias, root mean square error, and correlation coefficient. In this study, only the inflow water quality was calibrated due to the lack of outflow water quality data.

Results:

Model Setup

The delineation of study area was based on the hydrologic behavior. There are four "subcatchments", two channels and one culvert established in SWMM model (Figure 1).

Subcatchments "highway_1" (0.7405 ha) and "highway_2" (0.4443 ha) represent disconnected impervious highways. Subcatchments "grass_right" (0.8145 ha) and "grass_left" (0.1481 ha) represent two grassy areas in the study area.

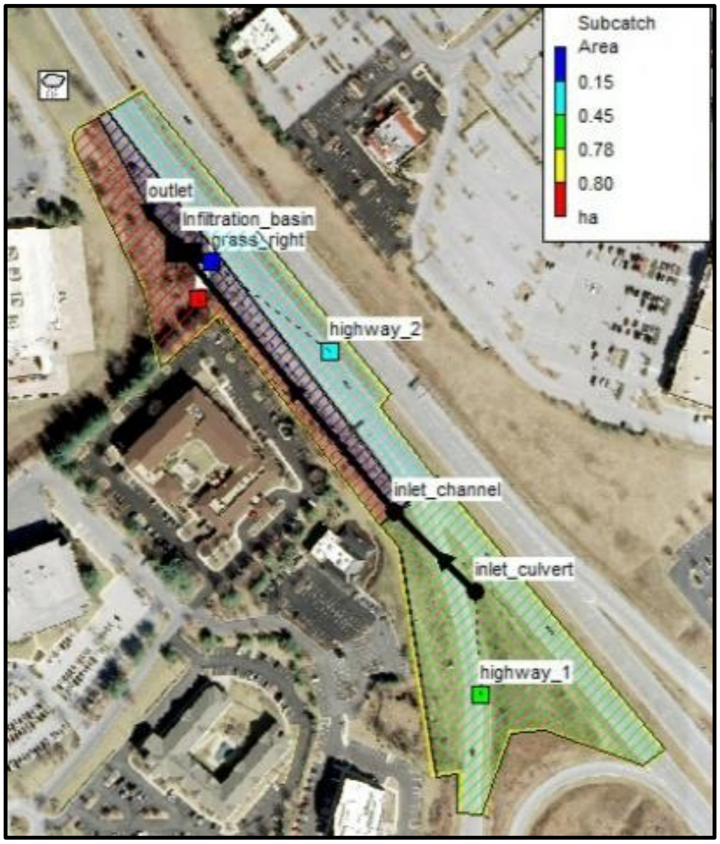

Figure 1. Modeled study site in SWMM

Parameter Sensitivity Analysis for Inflows

Sensitivity analysis was carried out to narrow down the parameters space for model inflow calibration. The relative sensitivities of inflow peak discharge, peak time, and integrated inflow to parameters were analyzed.

The results narrowed the inflow parameter dimensionality from 38 to 29. Critical parameters selected for model inflow calibration included sucatchment width, slope, Manning's N for impervious area, depression storage for impervious/pervious area, runoff curve number, Percent of impervious area with no depression storage and inlet channel roughness.

Model Calibration and Validation Results for Flows

The model calibration period was from August, 2009 to April, 2011, while the validation period was from April, 2011 to August, 2012. There were total 36 storm events during calibration period. And during validation period, there were total 36 storm events. The calibration and validation results for flows are listed in Table 1. Figure 2 and Figure 3 shows the comparison of hydrographs between SWMM and field-observations for water flows in calibration and validation periods.

Table 1. Statistics of Model Flows.

Hydrology Parameters	Calibration Period 2009/08-2011/04	Validation Period 2011/04-2012/08
NSE [-]	0.4303	0.1552
R [-]	0.6853	0.4041
Relative Bias [%]	−8.38%	−39.60%

The NSE in both the calibration and validation periods were greater than 0, which indicated the flow discharges simulated by SWMM model was better than the mean of observed discharge. For calibration period, the values indicated a relative good model performance. As expected, statistics in the validation period were worse than those obtained in the calibration period.

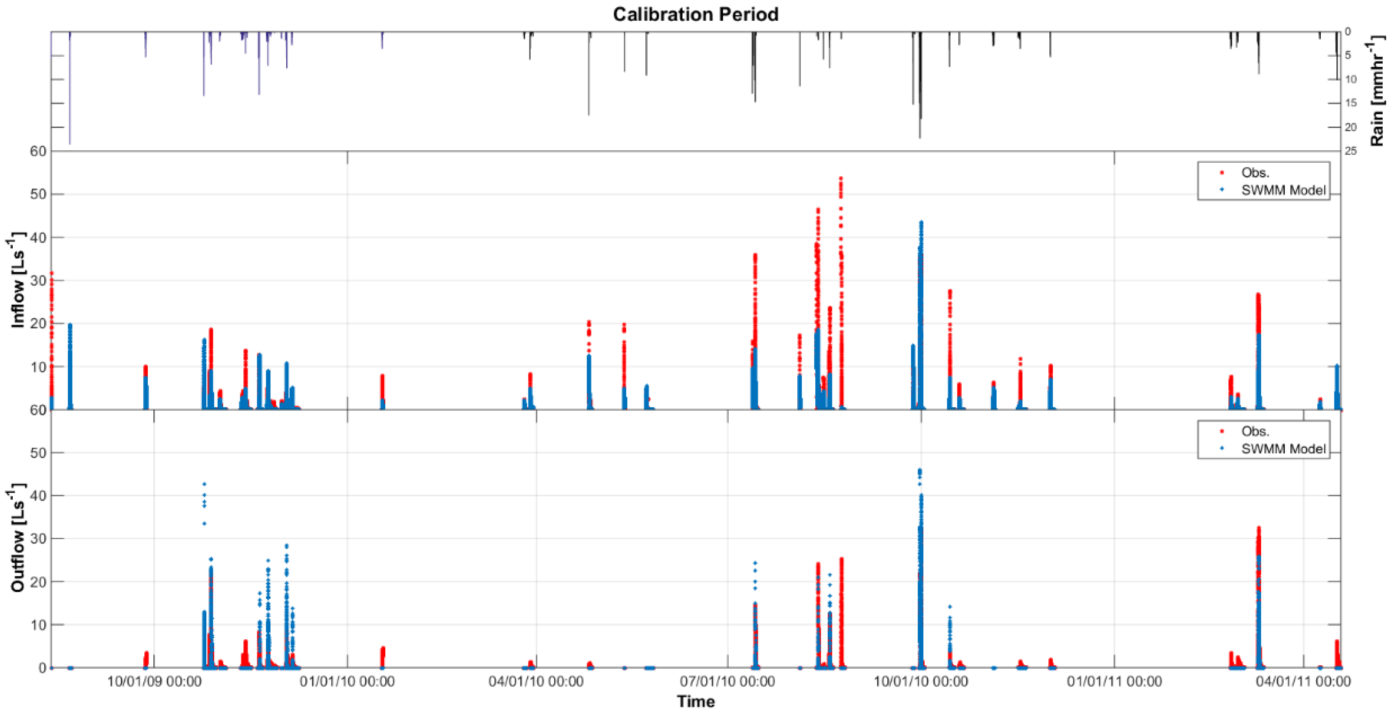

Figure 2. Inflow and Outflow in Field-observation and SWMM (Calibration).

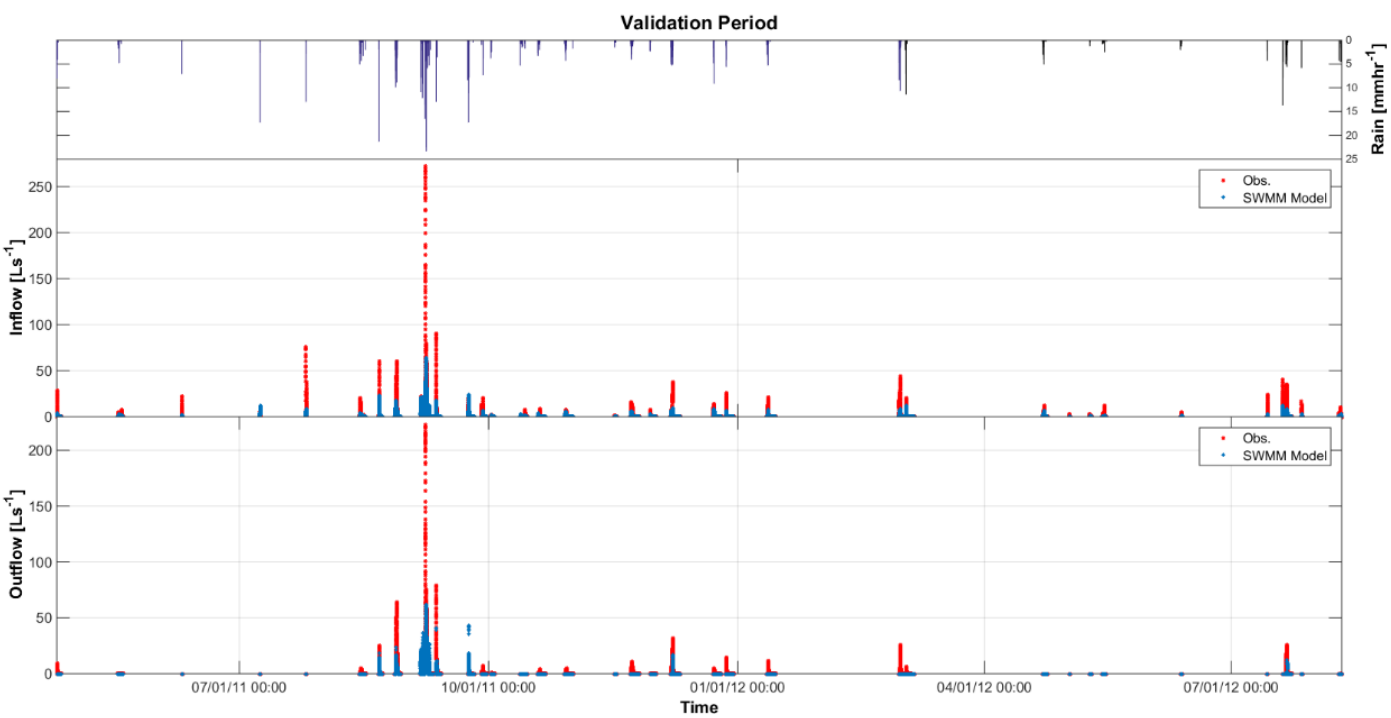

Figure 3. Inflow and Outflow in Field-observation and SWMM (Validation).

Model Calibration and Validation Results for Water Quality

The model calibration period for water quality was from August, 2009 to December, 2010, while the validation period was from February, 2011 to May, 2012. There were total 13 storm events during calibration period. And during validation period, there were total 13 storm events. In this study, only inflow total suspended solids (TSS) concentration was simulated due to the lack of outflow water quality data.

The statistics for model water pollutant TSS including model root mean square error (RMSE) and correlation coefficient (R). The model calibration and validation results are listed in Table 2.

Table 2. Statistics of Model Inflow Water Quality.

Water Quality Parameters	Calibration 2009/08-2010/10	Validation 2011/02-2012/05
R [-]	0.3530	0.2314
RMSE [mg/L]	169.2	137.5
Relative Bias [%]	-1196%	5772%

The statistics for inflow quality (TSS) were poor for both calibration and validation. The results reflect that simulation of urban runoff quality is very difficult. Very large uncertainties arise both in the representation of the physical, chemical and biological processes and in the acquisition of data and parameters for model algorithm (Gironas et al. 2009).

Future Work:

One of the most important advantages of model is to predict the future. Coupled with future climate change projections, the calibrated model is able to predict the performance of the infiltration basin and the hydrologic behavior of the urban site.

Reference:

Barco, J., K. M. Wong, and M. K. Stenstrom. 2008. Automatic calibration of the US EPA SWMM model for a large urban catchment. *Journal of Hydraulic Engineering*.

Gironas, J., L. A. Roesner, J. Davis, and National Risk Management Research Laboratory (U.S.). 2009. Storm water management model applications manual. Cincinnati, OH: National Risk Management Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, 1 online resource (179 p.).

Natarajan, P. 2012. Evaluation of transitional performance of a stormwater infiltration basin managing highway runoff.

Shinma, T. A., and L. R. Reis. 2014. Incorporating multi-event and multi-site data in the calibration of SWMM. *Procedia Engineering* 70:75-84.

Tsihrintzis, V. A., and R. Hamid. 1997. Modeling and management of urban stormwater runoff quality: a review. *Water Resources Management* 11 (2):136-164.

The list of publications:

Jing Wang, 2015, "Storm Water Runoff and Water Qulatiy Modeling in Urban Maryland", MS Dissertation, Civil and Environmental Engineering, University of Maryalnd, College Park, MD, P-ii.

Information Transfer Program Introduction

The Center's major Information Transfer effort (beyond publications and presentations by the PIs themselves) is the annual Maryland Water Symposium. This year's Symposium was the 13th such event.

Maryland Water Issues - Educational Event

Basic Information

Title:	Maryland Water Issues - Educational Event
Project Number:	2014MD320B
Start Date:	3/1/2014
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	MD-001
Research Category:	Not Applicable
Focus Category:	Education, None, None
Descriptors:	None
Principal Investigators:	Kaye Lorraine Brubaker

Publications

There are no publications.

2015 Maryland Water Education Event: Closure

Due to schedule and budget constraints, the proposed event was not held.

Maryland Water Symposium 2015

Basic Information

Title:	Maryland Water Symposium 2015
Project Number:	2015MD328B
Start Date:	3/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	MD 5
Research Category:	Not Applicable
Focus Category:	Education, None, None
Descriptors:	None
Principal Investigators:	Kaye Lorraine Brubaker

Publications

There are no publications.

Maryland Water Resources Symposium 2015

The Maryland Water Resources Research Center hosted "Celebrating Soil," the Maryland Water 2015 Symposium on Dec. 7, 2015. This was the 13th symposium hosted by the Center.

This year's symposium departed from the one-day series of speakers that has become our tradition. It consisted of an informal evening poster session with light refreshments, followed by a screening of the film, "Symphony of the Soil" (Deborah Koons Garcia, http://www.symphonyofthesoil.com/)

Contributed posters included:

- Spectral Studies of Dissolved Organic Matter from Anaerobically Digested Biosolids with and without Thermal Hydrolysis Pretreatment
- Do Chemically Contaminated Subaqueous Soils Present a Challenge for Classification?
- Influence of Different Wastewater Solids Treatment Methods on Concentrations of Triclosan, Triclocarban, and their Degradation Products
- Identifying Problematic Hydric Soils Derived from Red Parent Materials in the United States
- Organic Matter Decomposition in Restored and Natural Wetlands
- Predicting nitrogen leaching losses from intensifying agriculture in sub-Saharan Africa
- Quantifying Contaminants in Bear Creek Sediment: Validation of Novel Analytical Techniques and Implications for Risk Assessment
- Investigating the construction of a permeable reactive barrier and the soil microbial profile for the remediation of a trichloroethylene plume at a Superfund site
- Eighth International Acid Sulfate Soil Conference at University of Maryland, July 17-23, 2016

Participants also contributed educational and artistic items on the theme of soils.

About 50 participants registered. Most were from the University of Maryland community (faculty, research staff, graduate and undergraduate students), although state and local agencies and consulting firms were represented.

2015
International
Year of Soils

(http://www.fao.org/soils-2015/en/)

USGS Summer Intern Program

None.

Student Support					
Category	Section 104 Base Grant	Section 104 NCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	17	6	0	0	23
Masters	6	0	0	0	6
Ph.D.	3	1	0	0	4
Post-Doc.	1	0	0	1	2
Total	27	7	0	1	35

Notable Awards and Achievements

Publications from Prior Years

1. 2013MD308B ("The role of streams in nitrogen fluxes from watersheds in the Choptank Basin (Graduate Fellowship)") - Articles in Refereed Scientific Journals - Gardner, J.R., T.R. Fisher, T.E. Jordan, K.L. Knee, 2016, Balancing watershed nitrogen budgets: accounting for biogenic gases in streams, Biogeochemistry 127 (2-3), 231-253, DOI 10.1007/s10533-015-0177-1
2. 2012MD286B ("Forest N retention in the Chesapeake Bay watershed: A role in water quality restoration? (Graduate Fellowship)") - Articles in Refereed Scientific Journals - Sabo, R.D., D.M. Nelson, K.N. Eshleman, 2016, Episodic, seasonal, and annual export of atmospheric and microbial nitrate from a temperate forest, Geophysical Research Letters, 43(2), 683-691, DOI 10.1002/2015gl066758
3. 2004MD68B ("Using Bioaugmentation to Improve the Biodegradation of Chlorinated Compounds in Wetlands -- Summer Fellowship") - Articles in Refereed Scientific Journals - Schiffmacher, E.N., J.G. Becker, M.M. Lorah, M.A. Voytek, 2016, The effects of co-contaminants and native wetland sediments on the activity and dominant transformation mechanisms of a 1,1,2,2-tetrachloroethane (TeCA)-degrading enrichment culture, Chemosphere, 147, 239-247, DOI 10.1016/j.chemosphere.2015.12.033
4. 2014MD317B ("Impacts of Stormwater Management on Greenhouse Gas Fluxes from Urban Streams (Graduate Fellowship)") - Articles in Refereed Scientific Journals - Smith, R. M., and S.S. Kaushal, 2015, Carbon cycle of an urban watershed: exports, sources, and metabolism, Biogeochemistry, 126(1-2), 173-195.
5. 2011MD247B ("Environmental Suitability of Fly Ash Use in Highway Structural Fills (Graduate Fellowship)") - Articles in Refereed Scientific Journals - Komonweeraket, K., B. Cetin, A. Aydilek, C.H. Benson, and T.B. Edil, 2015, Geochemical Analysis of Leached Elements from Fly Ash Stabilized Soils, Journal of Geotechnical and Geoenvironmental Engineering, 141(5).
6. 2011MD247B ("Environmental Suitability of Fly Ash Use in Highway Structural Fills (Graduate Fellowship)") - Articles in Refereed Scientific Journals - Komonweeraket, K., B. Cetin, A.H. Aydilek, C.H. Benson, and T.B. Edil, 2015, Effects of pH on the leaching mechanisms of elements from fly ash mixed soils, Fuel, 140, 788-802.
7. 2010MD209B ("Leaching of Heavy Metals from High Carbon Fly Ash--Stabilized Soils in Highway Embankments") - Articles in Refereed Scientific Journals - Cetin, B., A.H. Aydilek, and L. Li, 2014, Trace Metal Leaching from Embankment Soils Amended with High-Carbon Fly Ash, Journal of Geotechnical and Geoenvironmental Engineering, 140(1), 1-13.