Indiana Water Resources Research Center Annual Technical Report FY 2017

Introduction

The Indiana Water Resources Research Center (IWRRC) is administered by Purdue University through the leadership of Director, Dr. Linda S. Prokopy, Professor in the Department of Forestry & Natural Resources and Managing Director, Laura A. Esman. Our objectives included (1) support research opportunities that address water issues within Indiana through the 104(B) small grants program, (2) increase visibility and awareness of the Center throughout the state, and (3) explore the opportunity to directly train young scientists through the US Geological Survey (USGS) Student Internship Program.

Research Program Introduction

104(B) Small Grants Program

The majority of the base funding received by the IWRRC is used to support a small grants program to fund research opportunities that address water issues within Indiana. All proposals submitted under the FY2017 Request for Proposals were externally reviewed by subject area experts within Indiana and outside of the state. Five proposals were funded including: • Communicating the State of Indiana Water Resources. PIs: Dr. Keith Cherkauer and Dr. Laura Bowling, Purdue University • Effects of land use type on abundance and type of microplastic pollution – a contaminant of emerging concern in Indiana rivers. PI: Dr. Gary Lamberti, University of Notre Dame • Effects of viruses on the development of harmful algal blooms. PI: Dr. Zhi (George) Zhou, Purdue University • Examining Anthropogenic Impacts on the Wabash River System. PIs: Dr. Jeffery Stone and Dr. Jennifer Latimer, Indiana State University • WaterWorks: A game to teach water systems thinking. PIs: Dr. Shahzeen Attari, Indiana University

In addition, four projects from FY2016 remained active during this reporting period: • Floodplain restoration and nutrient retention in the Wabash River Basin. PIs: Dr. Sara McMillan and Dr. Venkatesh Merwade, Purdue University • Nutrient removal efficiency of a combined surface/subsurface flow wetland system. PI: Dr. Pierre-Andre Jacinthe, Indiana University Purdue University Indianapolis • Pilot investigation of variable contaminant loads in fish as a result of foraging and habitat specialization. PIs: Dr. Tomas Hook and Timothy Malinich, Purdue University • Water and nutrient recovery from aquaculture effluents through vegetable production. PIs: Dr. Hye-Ji Kim, Dr. Paul Brown, Bob Rode, Dr. Cary Mitchell, Purdue University

One 104(G) project was active during this reporting period: • Can there ever be enough? Analysis of the adoption, penetration and effectiveness of urban stormwater best management practices. PIs: Dr. Laura C. Bowling and Dr. Linda S. Prokopy, Purdue University

We developed the 2018 RFP for the 104(B) program in consultation with other Water Resource Research Centers in the region. Water Centers within the Mississippi River Basin agreed to include a statement in the 2018 RFP indicating a focus area was funding complimentary research on Harmful Algal Blooms. We continued the expanded funding opportunity within the 104(B) small grants program allowing applications for a larger grant that included a collaborative, interdisciplinary team, integrating social and physical dimensions. We also continued requesting external peer review of applications that included collecting comments to return to grant applicants.

Increase Visibility and Awareness of the IWRRC

One of our goals is to increase awareness of the IWRRC throughout the state by increasing our visibility and organizing our communication with academic institutions throughout the state. We maintain the IWRRC website (iwrrc.org) that includes funding opportunities, such as 104(B) and 104(G) grant programs, projects funded through IWRRC, a list of upcoming relevant conferences and meetings, and our partners. We continue to develop the website and hope to include publications and reports and eventually information on the State of Indiana water resources. In addition, we maintain a listserv of statewide academic researchers working on water related issues. The listserv is being used to disseminate water related information and funding opportunities. We have also included the option to join this listserv through the website.

IWRRC has an Advisory Council of key water stakeholders throughout the state, including representatives from federal and state agencies and academic institutions. We held one meeting of the Advisory Council and communicated with them through email throughout the year.

We have maintained our connection with the Indiana Water Resources Association (IWRA), which has a long history of being a main convener of water researchers within Indiana. The IWRA holds an annual symposium that is well attended by state and federal agencies, conservation partners, and academics. IWRA is also a huge supporter of student participation through presentations and posters. IWWRC co-sponsored the June 2017 Symposium and was represented in four research presentations and three student posters. Dr. Linda Prokopy, Director, announced the small grants program grantees and introduced the development of the State of Indiana Water Resources website (FY16 funded project). In addition, the Director and Managing Director were able to attend the IWRA annual membership business meeting and will continue to co-sponsor the IWRA annual Symposium.

February 2018, the Director attended the annual National Institutes for Water Resources (NIWR) meeting in Washington DC. This meeting provided valuable exposure to other Center Directors to learn the structure of other centers, how they fund research, and the various water issues.

IWRRC continued its webinar series in September 2017 by co-hosting a webinar with Michigan State University's Institute of Water Research. Drs. Linda Prokopy (Purdue University) and Francis Eanes (Bates College) presented Agriculture, water & conservation in the Saginaw Bay Watershed: Results from a social science evaluation of the agricultural and conservation community, sharing the results of surveys and interviews conducted with agricultural producers, crop advisors, and conservation professionals in the Saginaw Bay watershed.

Throughout the year, the Director has participated in conferences and on committees, increasing the visibility of the IWRRC, including: • Participated on the planning committee for the annual conference of the Soil and Water Conservation Society. She also presented on numerous projects that integrate social sciences into the management of water resources. • Served on Steering Committee for the Indiana Climate Change Impacts Assessment. • Served on the author team for USDA's Climate Indicators for Agriculture report. • Served on Steering Committee for FFAR Convening Event on Innovation Pathways to Sustainability • Served on FFAR Advisory Council on Forging Innovation Pathways to Sustainability

USGS Student Internship Program

We continue to investigate the opportunity to hire an intern through this program.

Grant Applications Submitted Through/With IWRRC Researchers associated with the IWRRC and/or funded through the 104(B) small grants program have been able to use project outcomes to explore other funding opportunities. In addition, the Director was involved in the submission of numerous grants during the year that benefitted the IWRRC. Below is a list of grant applications submitted based on work with the IWRRC: • (Funded) Conservation Technology Information Center. $107,279. Conduct Producer Forums to Improve Outreach and Communication • (Funded) IDEM-319. $724,994 St. Mary's Watershed Initiative. • (Funded) Notre Dame Undergraduate Glynn Family Honors Program Summer Research Grant. $4,500. Physiological Effects of Microplastic Ingestion on Yellow Perch (Perca flavescens). • (Funded) NSF-CBET. $312,066 Nitrogen and Phosphorus Dynamics in Restored Floodplains of Intensively Managed Watersheds • (Funded) Purdue University, College of Agriculture, AgSEED Program. Healthy Food, Healthy Environment: Developing best management practices for Hydroponic and Bioponic crop production systems in Indiana • (Funded) The Nature Conservancy. $257,902. Non-Operator Landowners and Soil Health • (Funded) The Nature Conservancy. $86,418. Saginaw Bay Evaluation, Year 2.

• (Funded) The Nature Conservancy. $61,250. Social Indicators Assessment in the Upper White River Watershed. • (Funded) USDA-NIFA. $462,498. Does Crop Insurance Inhibit Climate Change Irrigation-Technology Adoption • (Funded) USDA NRCS/Starkey Farm Partnership. $268,866. Edge of Field Monitoring Program.

Current IWRRC Leadership Director: Dr. Linda S. Prokopy, Department of Forestry and Natural Resources, Purdue University

Managing Director: Laura A. Esman, Department of Forestry and Natural Resources, Purdue University

Advisory Council: Jeff Frey, US Geological Survey Dr. Mark Pyron, Ball State University Jill Reinhart, Natural Resources Conservation Service MaryLou Renshaw, IN Department of Environmental Management Dr. Todd Royer, Indiana University Jordan Seger, IN State Department of Agriculture Dr. Ron Turco, Purdue University

Can there ever be enough? Analysis of the adoption, penetration and effectiveness of urban stormwater best management practices

Basic Information

Title:	Can there ever be enough? Analysis of the adoption, penetration and effectiveness of urban stormwater best management practices
Project Number:	2014IN377G
USGS Grant Number:	
Start Date:	9/1/2014
End Date:	8/31/2017
Funding Source:	104G
Congressional District:	IN-4
Research Category:	Water Quality
Focus Categories:	Water Quality, Management and Planning, Non Point Pollution
Descriptors:	None
Principal Investigators:	Laura C. Bowling, Linda Stalker Prokopy

Publications

1. Gao, Y., N. Babin, A.J. Turner, C.R. Hoffa, S. Peel, and L.S. Prokopy, 2016. Understanding urban-suburban adoption and maintenance of rain barrels, Landscape and Urban Planning http://www.sciencedirect.com/science/article/pii/S0169204616300330
2. Gao, Y., N. Babin, A.J. Turner, C.R. Hoffa, S. Peel, and L.S. Prokopy, 2016. Understanding urban-suburban adoption and maintenance of rain barrels, Landscape and Urban Planning http://www.sciencedirect.com/science/article/pii/S0169204616300330
3. Bowling, L., Turco, R., Hoover, F., Peel, S. (2016). Region of the Great Bend of the Wabash River Water Quality Summary Report. Wabash River Enhancement Corporation. Available at:http://static1.1.sqspcdn.com/static/f/319113/27408873/1484172558920/IDEM+Water+Quality+Summary_F
4. Gao, Y., Church, S. P., & Prokopy, L. S. (2016). 2016 Great Bend of the Wabash River Watershed Urban Resident Survey Executive Summary Report. West Lafayette: Purdue University.
5. Gao, Y., N. Babin, A.J. Turner, C.R. Hoffa, S. Peel, and L.S. Prokopy, 2016. Understanding urban-suburban adoption and maintenance of rain barrels, Landscape and Urban Planning http://www.sciencedirect.com/science/article/pii/S0169204616300330
6. Bowling, L., Turco, R., Hoover, F., Peel, S. (2016). Region of the Great Bend of the Wabash River Water Quality Summary Report. Wabash River Enhancement Corporation. Available at:http://static1.1.sqspcdn.com/static/f/319113/27408873/1484172558920/IDEM+Water+Quality+Summary_F
7. Gao, Y., Church, S. P., & Prokopy, L. S. (2016). 2016 Great Bend of the Wabash River Watershed Urban Resident Survey Executive Summary Report. West Lafayette: Purdue University.

Indiana Water Resources Research Center Project Report

Report 2017 Program Report

Project Id: 2014IN377G

Title: Can there ever be enough? Analysis of the adoption, penetration and effectiveness of urban stormwater best management practices

Project Type: Research

Period: March 1, 2017 – February 28, 2018

Congressional District: IN-004

Focus Categories: Water Quality, Management and Planning, Non Point Pollution

Keywords: urban stormwater BMPs, social indicators, water quality

Principal Investigators:

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Abstract / Summary: While agricultural systems have utilized Best Management Practices to reduce pollution for a number of years, work on urban stormwater management is lacking. The West Lafayette-Lafayette communities spanning the banks of the Wabash River in north central Indiana have a combined population of over 215,000 people. Like many similar sized communities across the country, the region is struggling to deal with increasing stormwater impacts on water quality. Stormwater conservation practices, such as rain gardens, rain barrels and permeable pavement offer the potential of decreasing stormwater volumes and reducing water quality impacts, but their utilization is generally lower than their agricultural counterparts. The goal of this proposed work is to improve water quality planning and implementation through recommendations to improve the overall adoption, penetration and permanence of urban stormwater BMPs. Our research approach blends statistical analysis with social science techniques to determine 1) how many BMPs do we need? and 2) how can we get them in the watershed?

Problem: Stormwater management, including the infrastructure for water conveyance, drainage and treatment, is an increasing water problem for communities of all sizes. This project is addressing the need to improve and enhance the nation's water supply through evaluation of what limits adoption of urban stormwater conservation practices. Urban streams are among those with the lowest water quality in the country. Improvements to stormwater conveyance and treatment infrastructure alone cannot resolve the problem.

Stormwater conservation practices, such as rain gardens, rain barrels and permeable pavement offer the potential of decreasing stormwater volumes and reducing water quality impacts, but their utilization is generally lower than their agricultural counterparts. Poor penetration is attributed to several reasons, including more numerous landowners with less property, a limited number of cost incentive programs and fewer formal public education programs than found in the agricultural community. Secondly, there is little demonstrated ability to show watershed-scale water quality improvement due to BMP implementation in urban environments, which is a function of both the needed intensity of BMP implementation to enact a desired change, as well as the statistical design of a monitoring program that can detect the expected rates of change.

Research Objectives:

This project is addressing the knowledge gap regarding the watershed-scale effectiveness of urban stormwater BMPS, starting with how many BMPs it takes to show statistically significant water quality improvement and extending to the willingness of landowners to adopt. Our specific project objectives include:

- Evaluate the level of adoption and intensity and duration of sampling needed to demonstrate statistically significant change;
- Assess the factors influencing practice adoption, penetration and permanence; and
- Develop watershed management planning strategies for achieving urban water quality goals.

During this project period, specific efforts were directed at evaluating the level of adoption needed to demonstrate statistically significant change through watershed modeling (Objective 1) and assessing the factors influencing adoption and permanence of rain barrels and rain gardens (Objective 2).

Methodology:

Objective 1:

The LTHIA-LID 2.1 model (Liu et al., 2015) was used to evaluate the level of BMP adoption for meaningful load reduction that was defined by the local stakeholders and determined by statistical analysis. Seven scenarios were simulated by implementing six different types of BMPs to all suitable areas. The seventh scenario simulated water quality improvement with all practices combined. For cost analysis, the median value of local data from the Wabash River Enhancement Corporation (WREC) database were used, together with the results of the model simulation.

Objective 2:

Mail social indicator surveys were conducted by the Natural Resources and Social Science lab at Purdue University in 2016. One thousand surveys were mailed to urban residents in Tippecanoe County, Indiana. The address lists were purchased from Survey Sampling International. The Dillman Tailored Design Method was used to contact residents on the address list up to five times (Advance letter, 1st mailing of paper survey, reminder postcard, in-person drop-off and pick-up for single family homes and 2nd mailing of survey to apartment dwellers, 3rd mailing of a paper survey with a reminder postcard). The 2016 response rate was 31.4% (% not including bad addresses. n=255, excluding duplicated or invalid responses).

Results:

Objective 1:

Implementation of BMPs reduced simulated annual runoff between 0.2 to 26%. The smallest reduction resulted from implementing green roofs because the area suitable for green roofs is very small compared to the area suitable for other practices. The maximum simulated load reductions were 14%, 17%, 29%, and 14% for Nitrate, TP, TSS and E. coli, respectively. Pollutant load reductions were not proportional to the cost of implementation and porous pavement was the most cost effective practice for load reduction.

Objective 2:

People had positive perceptions towards rain gardens and rain barrels, in terms of their benefits in reducing ponding, managing stormwater in a cost-effective way, and improving water quality. In specific, people recognized the features of rain gardens in increasing the feeling of nature and improving the appearance of the surrounding.

However, according to our analysis on people with different experience of these practices, people who have already adopted the practice are in favor of rain gardens, while people who have not yet adopted are still concerned about maintenance issues, risk of bugs and insects, skills required, and time required.

Major Conclusions and Significance

Objective 1:

Based on LTHIA model simulations, the achievable load reduction for TSS exceeds both the target reduction determined by stakeholders as part of the existing watershed management plan, as well as the level of change that is necessary foir statistical significance, with 21 years or more of sampling. That means water quality targets can be achieved with BMPs alone. However, it will take a very long sampling time and even with maximum BMP implementations, achieving statistically significant reductions in TP and E. coli is not possible. Therefore, for urban streams, it is not feasible to tie measures of success of watershed management planning to statistically significant reductions in load of these quantities.

Objective 2:

The concerns for maintenance, risk of bugs and insects, skills and time required, are not the actual concerns for people who have already adopted the practice, this provides implication for outreach organizations to deliver clearer information for potential users of urban stormwater management practices and to help address these concerns so that more people will implement the practices. This result will help to promote the adoption and diffusion of urban stormwater management practices among residential communities and have a positive impact on local water quality.

Publications:

Presentations:

1. Gao, Y. and L. Prokopy, 2017, Resident perspective on stormwater conservation practices in Indiana, Oral presentation at the 2017 Association for Environmental Studies and Sciences (AESS) 9th Annual Conference, June 22nd, 2017, Tucson, AZ
2. Rahman, S., Hoover, F-A, and L. Bowling, 2017, Can There Ever Be Enough to Impact Water Quality? Evaluating BMPs in Elliot Ditch, Indiana Using the LTHIA-LID Model. Oral presentation delivered at the American Geophysical Union annual conference, December 11-15, 2017, New Orleans, Louisiana.
3. Rahman, S., Hoover, F-A, and L. Bowling, 2017. Can There Ever Be Enough? Implementing BMPs in Elliot Ditch, Indiana Using the LTHIA-LID Model and a Power Analysis. 38th Annual Indiana Water Resources Association Symposium, June 28-30, 2017, Marshall, Indiana.

Paper:

Gao, Y., S. Church, S. Peel and L. Prokopy, 2017, Public perception towards river and water conservation practices: opportunities for implementing urban stormwater management practices, Journal of Environmental Management, submitted and under minor revision.

Grant Submissions: None

Students:

PhD students:

1. Yuling Gao, PhD Candidate, Department of Forestry and Natural Resources
2. Fushcia-Ann Hoover, PhD (completed August 2017), Department of Agricultural and Biological Engineering
3. Md. Sanoar Rahman, PhD Candidate, Department of Agricultural and Biological Engineering

Current Reduction Status Regional Tracker (CrsTracker)

Basic Information

Title:	Current Reduction Status Regional Tracker (CrsTracker)
Project Number:	2015IN401S
USGS Grant Number:	
Sponsoring Agency:	U.S. Army Corps of Engineers
Start Date:	6/1/2017
End Date:	5/31/2018
Funding Source:	104S
Congressional District:	
Research Category:	Not Applicable
Focus Categories:	None, None, None
Descriptors:	None
Principal Investigators:	Bernard Engel

Publications

1. None
2. None
3. None
4. The tool is published as a web page available for the public and stakeholders to use at this link: http://lthia.agriculture.purdue.edu/crs/ .

Indiana Water Resources Research Center Project Report

Report 2017

USGS Special Projects

Title: Current Reduction Status Regional Tracker (CrsTracker)
Project Type: Research

Period: March 1, 2017 – February 28, 2018

Congressional District: (Great Lakes Watershed in parts of MN, WI, MI, IL, IN, OH)

Focus Categories: Water Quality and Nutrients

Keywords: STEPL, BMP, Phosphorous, Nitrogen

Principal Investigators:

Bernie Engel, Head and Professor;

Yaoze Liu, Postdoctoral Research Scientist;

Larry Theller, Geographic Systems Analyst;

Purdue University Department of Agricultural and Biological Engineering

Abstract / Summary

The objective of this project is to create a web-based tool, CrsTracker, to allow users to evaluate the impacts of four best management practices (BMPs) which may be installed, or proposed for installation, in multi-scale watersheds across the Great Lakes region. The goal of the impact analysis is to gain an understanding of the cumulative effects of these varied BMPs in the region. The CrsTracker ranking tool is designed to tally total BMP acres and use a form of the EPA watershed spreadsheet management model, STEPL (Spreadsheet Tool for Estimating Pollutant Loads), to estimate impact (cumulative load reduction) of these specific classes of BMPs in reducing nitrogen, phosphorous, and sediment flowing into the Great Lakes.

The CrsTracker tool [http://lthia.agriculture.purdue.edu/crs/] is designed to feed data into the Purdue ABE implementation of the STEPL model engine, providing a tabulation or "current standings" output which users can either view or download to use as prepopulated parameters for their own detailed scenario modeling.

Problem:

There is a lack of an overall mechanism to estimate the pollution reduction produced by the many varied BMPs which have been installed or that are considered for installation by various NGO and governmental units around the Great Lakes since GLRI began. For example, the Corps of Engineers could greatly benefit from a single catalog where various BMPs, either proposed or constructed, can be entered and tracked.

Research Objectives:

The ultimate goal of this tool is to provide information to allow the presentation of a "reduction status current standing" for each watershed, without requiring a dedicated analysis run by a specific user. The design would support tracking BMPs installed by particular groups to allow "crowd-sourcing" or addition of BMPs by the various groups and agencies responsible for them.

To facilitate the process, several new BMPs were added to the STEPL engine, which produces the pollution reduction estimates. Understanding the pollution reduction value of these new practices requires significant research, which was done as part of this proposal.

The project team worked with Corps of Engineers personnel to create the list of BMPs included in the STEPL tool. The BMPs added to this implementation of STEPL include:

1) the conversion of farmland to habitat with some wetlands
2) the restoration of degraded wetlands
3) enhancement of degraded wetlands
4) restoration of riparian habitat

For the new BMPs, pollutant reduction coefficients were developed for each practice based on scientific literature and expert opinion.

Methodology:

The research team adapted core technologies which are implemented in a current Purdue online model: Purdue STEPL Web. The CrsTracker tool uses the analysis module from STEPL to summarize the impacts of the proposed or installed BMPs. Data flows between the browser and the software engine using state-of-the-art JSON (JavaScript) programming. This design, repurposing current software systems, allowed a rapid prototyping and development sequence for the tool. The design includes a public facing map-based interface. Stakeholders are able to monitor the current state of impacts by clicking on the map. To upload BMP implementation data, stakeholders are able to go to the map to draw a new BMP.

The Purdue STEPL Web model is a web-enabled version of the STEPL spreadsheet tool. The original spreadsheet was created by EPA as a set of Excel macros and enhanced several times. This version has several adaptations by Purdue researchers, such as connection to an online database of precipitation, and enhancements to optimize selection of BMPs to attain water quality goals. STEPL uses two simple approaches to calculate nutrient and sediment loads from different land uses: USLE and SCS Curve Number (CN) method. As does the original, it calculates annual load reductions resulting from various BMPs. The algorithm uses precipitation, landuse, and soil data to compute surface runoff volume, nutrient loads, and sediment delivery. The nutrients include nitrogen and phosphorous. The version of Purdue STEPL which is executed by CRS Tracker needs only a polygon and a county FIPS code as input from the user. The STEPL engine uses the L-THIA service to delineate a watershed if needed (two of four BMPS need this) for the BMP polygon, then extracts NLCD 2011 landuse, and SSURGO (2015) soil for the polygon. The FIPS code is resolved to state and county name. The ratio of the watershed to the actual BMP area is computed, and used in appropriate BMPs. Landuse is converted from NLCD categories to STEPL's simpler categories. Precipitation is generated by CLIGEN 5 precipitation simulator using a county-based .par file (built on observed data through 2014), then USLE factors are pulled from an EPA database based on the county name provided. Landuse categories resolved from NLCD categories supported by this STEPL engine include cropland, pastureland, forest, and urban. The original STEPL categories of user-defined, feedlot, septic systems and numbers of animals are not contained in NLCD landuse and thus not implemented in this context. The conversion from existing landuse category to wetland BMP does not include any additional standard STEPL BMPS (e.g. strip-cropping, porous pavement) as they are not applicable in this project.

Management practices influence the computation through application of nutrient reduction efficiencies per BMP per applicable area for the list of supported practices. The list of current BMPs include 4 practices of interest to the Corps of Engineers as described above.

Results: Details of the literature review and analysis

BMP-1. The conversion of farmland to habitat with some wetlands.
The definition of this BMP is that a specified area changes from farmland to a combined habitat (native grass, prairie grass, shrubs, very little tree area), with various amounts of the area draining to wetlands.

The percent reduction values of TN, TP, BOD, and TSS for this practice were estimated based on a total area (A1) to change, an area of wetland (A2, calculated from "percent of the total area drains to wetlands" and the assumption that the area of wetland was 4 percent of drainage area (USEPA, 1999)). Most of the area (area left, A1-A2) was changed to habitats (curve numbers were changed to that of habitat, which was treated as forest land use), and various amounts of habitat area drained to wetlands. The drainage area of wetlands was assumed to be 0%, 5%, 10%, 15%, and 25% of the total area, respectively. The median values of percent reductions of TN, TP, BOD, and TSS for this practice were used. Based on the following tables (Table 1 and Table 2), conversion of farmland to habitat with some wetlands can reduce TN, TP, BOD, and TSS by 94%, 95%, 99%, and 99%.

Table 1. Major inputs used to simulate conversion of farmland to habitat with some wetlands

	Curve Number				
	HSG A	HSG B	HSG C	HSG D	
Farmland	67	78	85	89	Same as curve numbers of cropland in STEPL web
Habitat	39	60	73	79	Same as curve numbers of forest in STEPL web
	Pollutant reduction (%)				
	TN	TP	BOD	TSS	
Farmland	N/A	N/A	N/A	N/A	
Wetland	37	35	86	55	Same as the restoration of degraded wetlands in this file
Farmland	area (acre)	1000			
	Soil Group	B			
CLIGEN	Waterloo, IN				
USLE parameters	**R**	**K**	**LS**	**C**	**P**
Cropland	140	0.3	0.11	0.2	1
Pastureland	140	0.3	0.11	0.04	1
Forest	140	0.3	0.11	0.003	1
user defined	140	0.3	0.11	0	1

Table 2. Simulation results of the conversion of farmland to habitat with some wetlands

		pollutants load	percent reductions (%)
Original Loads-Farmland	TN (lb)	4867	/
	TP(lb)	568	/
	BOD (lb)	3137	/
	Sediment (US ton)	246	/
Habitat with some wetlands			

wetlands (% of whole area)		pollutants load	Percent reductions (%)
0%	TN (lb)	275	94
	TP(lb)	29	95
	BOD (lb)	44	99
	Sediment (US ton)	4	98
5%	TN (lb)	274	94
	TP(lb)	29	95
	BOD (lb)	42	99
	Sediment (US ton)	4	99
10%	TN (lb)	273	94
	TP(lb)	29	95
	BOD (lb)	41	99
	Sediment (US ton)	3	99
15%	TN (lb)	272	94
	TP(lb)	29	95
	BOD (lb)	39	99
	Sediment (US ton)	3	99
20%	TN (lb)	271	94
	TP(lb)	28	95
	BOD (lb)	37	99
	Sediment (US ton)	3	99
25%	TN (lb)	270	94
	TP(lb)	28	95
	BOD (lb)	36	99
	Sediment (US ton)	3	99

BMP-2. The restoration of degraded wetlands.
This BMP is defined as returning a degraded wetland and its functions to a pre-existing condition or as close to that condition as is possible (IWWR, 2003; NRCS, 2010b).

We assume that degraded wetlands in current condition have no impact in reducing pollutant loads. In modeling, then, they currently have the impact of a body of water (no pollution production or reduction) until they are selected to be “restored” at which time a pollution reduction coefficient is imposed. We assume that the restored wetlands have the same performance as new constructed wetlands. Only pollutant concentration reduction of wetland is considered, runoff volume reduction is not considered. Reduction values of TP and TSS were from the International Stormwater BMP Database (http://www.bmpdatabase.org/). TN and BOD reduction values were from literature as shown in Table 3. The reductions of TN, TP, BOD, and TSS for restoration of degraded wetlands were 37%, 35%, 86%, and 55%, respectively.

Table 3. TN and BOD reductions for restoration of degraded wetlands

TN reduction (%)	References	BOD reduction (%)	References
37	Kovacic et al. (2000)	66	Maine et al. (2009)
36.8	Zhang et al. (2010)	85.5	Zhang et al. (2010)
47.5	Vymazal (2007)	93.5	Wießner et al. (2005)
29	Panswad and Chavalparit (1997)	65	Maine et al. (2007)
56	Metcalf and Eddy Inc (1991)	81	Hayes et al. (1987)
88.5	Nyakang'o and van Bruggen (1999)	90	Metcalf and Eddy Inc (1991)
85	Koottatep and Polprasert (1997)	88.5	Rivera et al. (1997)
44	Hammer and Knight (1994)	97	Laber et al. (1999)
16	Birch et al. (2004)	65	Billore et al. (1999)
24	NPRPD (2007)	98	Nyakang'o and van Bruggen (1999)
22	Maine et al. (2006)	77	Maine et al. (2006)
37	Median of above	86	Median of above

BMP-2. Constructed wetlands.
Constructed wetlands are artificial ecosystems with hydrophytic vegetation for water treatment (NRCS, 2010c).

Only pollutant concentration reduction of wetland is considered, runoff volume reduction is not considered. Reduction values of TP and TSS were from the International Stormwater BMP Database (http://www.bmpdatabase.org/). TN and BOD reduction values were from literature as shown in Table 3. The reductions of TN, TP, BOD, and TSS for construction of new wetlands were 37%, 35%, 86%, and 55%, respectively, which are the same as those used above in "restoration of degraded wetlands."

BMP-3. Enhancement of degraded wetlands.
Enhancement of degraded wetlands is defined as increasing one or more of the functions performed by an existing wetland beyond original condition of the wetland. There is often an accompanying decrease in other functions (IWWR, 2003; NRCS, 2010a).

We assume that degraded wetlands in their current condition have no impacts in reducing pollutant loads. We assume degraded wetlands are enhanced with increased TN, TP, BOD, and sediment retention by increasing water storage volume per drainage area. Efficiencies for enhancement of degraded wetlands in reducing pollutants are greater than those for restoration of degraded wetlands. In modeling, this means a higher reduction coefficient for pollutants is used to represent restoration of degraded wetlands.

Based on the performance of free water surface wetlands, and Equations 2.1, 6.2, and 6.42 from Kadlec and Wallace (2008), the percent reductions of TN and TP from 50th to 90th percentiles were calculated. The results were adjusted proportionally to the newly built wetland performance using the 50th percentile values. The 75th percentiles values were used as the efficiencies of enhancement of degraded wetlands. Calculation results are shown in Table 4 and Table 5. Due to lack of data, BOD and TSS reductions were assumed to be the same as that of newly built wetlands. Efficiencies of enhancement of degraded wetlands in reducing TN, TP, BOD, and TSS were 57%, 54%, 86%, and 55%, respectively.

$$q = \frac{Q}{A} \quad (2.1)$$

where

q = hydraulic loading rate (HLR), m/d

A = wetland area (wetted land area), m^2

Q = water flow rate, m^3/d

$$\frac{C_o - C^*}{C_i - C^*} = \frac{1}{(1 + k/Pq)^P} \quad (6.2)$$

where

C_o = outlet concentration, mg/L

C_i = inlet concentration, mg/L

C^* = background concentration, mg/L

k = modified first order areal constant, m/d

P = apparent number of TIS

q = hydraulic loading rate, m/d

$$J = kC \quad (6.42)$$

where

C = concentration, g/m^3

J = removal per unit area, or load removed, $g/m^2 \cdot d$

k = rate coefficient, m/d

Table 4. TN efficiency of enhancement of degraded wetlands

Percentile	Load Removed (g/m^2*yr)	Rate Coefficient (m/yr)	Reduction (%)	Proportionally Adjusted (%)
0.5	129	12.6	44.40	37
0.6	214	17.1	54.04	45
0.7	375	24.2	64.90	54
0.8	550	29.6	71.11	59
0.9	1973	39.2	81.33	68

Table 5. TP efficiency of enhancement of degraded wetlands

Percentile	Load Removed (g/m^2*yr)	Rate Coefficient (m/yr)	Reduction (%)	Proportionally Adjusted (%)
0.5	6	10	41.72	35
0.6	11.3	13.1	49.94	42
0.7	26.6	16.7	57.72	48
0.8	45.6	25	70.45	59
0.9	92	60	90.99	76

BMP-4. Restoration of riparian habitat.
Published work on restoration of riparian habitat includes two types of restoration, horticultural restoration and process restoration (Griggs, 2009). 'Horticultural restoration' refers to a high level of site management and external human inputs that include site preparation (land-leveling, disking), planting of nursery-grown trees and shrubs in predesigned patterns, irrigation, and chemical weed-control. 'Process restoration' strives to reestablish stream or river processes onto the site, which attempts to restore a site by working with existing stream or river processes.

This project explores 'process restoration', such as breaking a ditch to make the form of flood plains different. The drainage area is assumed to be big (at least 10^6 times the restored area based on Table 7 and Table 8) relative to the restored area. Reduction per unit area per year ($g/m^2/yr$) was used to represent this BMP. Based on Table 6, restoration of riparian habitat can reduce TN, TP, BOD, and TSS by 7.4, 1.98, 0, and 710 $g/m^2/yr$, respectively.

Table 6. Performance of restoration of riparian habitat

	TN ($g/m^2/yr$)	TP ($g/m^2/yr$)	TSS ($g/m^2/yr$)
Noe and Hupp (2005)	3.5 - 13.4	0.22- 4.13	
Craft and Casey (2000)	1.4	0.12- 0.75	120–1300
Yarbro (1983)	--	0.17	
Brinson et al. (1980)	7.3	0.54	
Brown (1978)	--	3.2	
Mitsch et al. (1979)	--	3.6	
Johnston et al. (1984)	12.8	2.6	2000
Kleiss (1996)			600–800
Kuenzler et al. (1980)		0.10–0.28	39–305
Noe and Hupp (2009)	2.2-12.6	0.21-3.48	303-4600
summary	1.4-13.4	0.1-4.13	
Median	7.4	1.98	710

Table 7. Land use distributions in drainage area

	Percent (%)
Urban	10
Cropland	50
Pastureland	20
Forest	20
Soil Group	B

Table 8. Reductions for various sizes of drainage areas

	Pollutant reduced	Pollutants from Drainage area					
	by 1 acre restored area	1 acre	10 acre	50 acre	100 acre	1000 acre	1000000 acre
TN (kg)	29.9	1.7	16.9	84.3	168.6	1686.2	1686233.8
TP (kg)	8.0	0.2	2.3	11.4	22.8	228.3	228316.6
BOD (kg)	0.0	1.3	12.9	64.3	128.5	1285.0	1285035.6
TSS (kg)	2873.3	241.1	2411.0	12054.8	24109.6	241095.7	241095650.1

Percent reductions (%)							
		1 acre	10 acre	50 acre	100 acre	1000 acre	1000000 acre
TN	/	100	100	36	18	2	0.002
TP	/	100	100	70	35	4	0.004
BOD	/	0	0	0	0	0	0.000
TSS	/	100	100	24	12	1	0.001

Major Conclusions and Significance:

The main conclusion of this project is that it is possible and reasonable to use nutrient load reduction factors to represent the environmental impact of various wetland BMPs used to convert farmland to various wetland types.

Summary of the resulting BMP characteristics and treatments:

BMP	**Process and Treatment Area**	**Annual Total Nitrogen Reduction**	**Annual Total Phosphorous Reduction**	**Annual Total Suspended Sediment Reduction**
Conversion of farmland to habitat combined with wetland habitat	Draw polygon to be converted. Program will calculate pre-existing load of the drawn area based on landuse and soil. By definition the wetland is at least 4 % of the property area, the rest is habitat.	94%	95%	99% .
Restoration of Degraded Wetlands	Draw polygon for wetland. Program will delineate this for watershed. Calculate pre-existing load of the watershed based on landuse and soil. The functional treatment area is 25x the BMP area or less. The proportion of excess watershed to functional BMP area is used to scale the full load before reduction factor is applied to the STEPL load to see the BMP reduced load.	37%	35%	55%
Enhancement of Degraded Wetlands	Draw polygon for wetland. Program will delineate this for watershed. Calculate pre-existing load of the watershed based on landuse and soil. The functional treatment area is 25x the BMP area or less. The proportion of excess watershed to functional BMP area is used to scale the full load before reduction factor is applied to the STEPL load to see the BMP reduced load.	57%	54%	55%
Restoration of Riparian Wetland Habitat	Draw polygon of restoration area, river provides large input load. Ignore pre-existing load and landuse. Program will calculate area of restoration (drawn area) and calculate a reduction based on area and large load. By definition the user feels that the watershed of the stream is more than 1000 x the BMP area.	66 pounds **per restored acre**	18 pounds **per restored acre**	6300 pounds **per restored acre**

Implementation of BMP 1:
The software calculates the load of the drawn area, based on the original non-wetland landuse. The BMP will apply **percent reductions** to the EMC table of TN, TP, BOD, and TSS by 94%, 95%, 99%, and 99%, respectively.

Implementation of BMP-2:
The drainage area of the wetland is required. The drainage area has multiple land uses and possibly multiple soils. A new L-THIA editor tool was built based on the NRCS pond delineator that has users trace the wetland, and then the drainage area into the wetland is delineated and tabulated.

For "restoration" or "construction" each landuse has preliminary nutrient load multiplied by the reductions of TN, TP, BOD, and TSS as 37%, 35%, 86%, and 55%, respectively (basically applied to all EMC of the drainage area.).

Implementation of BMP-3:
Efficiencies of enhancement of degraded wetlands in reducing TN, TP, BOD, and TSS are 57%, 54%, 86%, and 55%, respectively with reductions applied to the EMC of all the landuses within the drainage area.

Example of drainage area method to use in CRSTracker tool to reduce loads under BMP 2 and BMP 3.

Logical Process.

Step 1 draw BMP area "A". Store as A in acres.
Step 2 calculate effective area "F". Compute 25 * A and store as F.
Step 3 delineate area A. Store watershed area "W" as BMPwshed.
Step 4 compute BMPxf which is the proportion of watershed that is untreated.
BMPxf = (1- ((W-F)/W)).
Step 5 compute full load for entire watershed W in STEPL. = "L"
Step 6 compute treatable effective load, "TEL" by reducing full load by ratio of untreated watershed to full watershed.
TEL = (L* BMPxf)
Step 7 compute reduced load (RL) from treatable effective Load. E.G. for BMP 3 (enhanced, this is 57% reduction in N)
RL = TEL * (1-.57)   This is reduced load in pounds or tons.
Step 8 compute reduction R in load by BMP. R = (L – RL) = reduction in pounds or tons.

Nitrogen Reduction Example.

BMP = 60 acres.
Effective area = F = 60 * 25 = 1500 acres
W (watershed) = 3200 Acres.
BMPxf = (1 – (3200 – 1500) / 3200) or = (.53125) The watershed has 53 % too large an area, can only treat 47% .
The soil is "D" and the landuse is cropland (central Michigan). The STEPL load is 21,000 lbs. of N.
The treatable load or TEL = 47% of the STEPL load L.
21,000 * .47 = 9870 lbs. N.

BMP 3 has .57% reduction in N as defined from literature. So we reduce treatable load by removing 57% of TEL.
9870 * (1 - .57) = 4244 lbs. N left remaining after reduction as new reduced load "RL".
Total reduction in lbs., is (TEL – RL) or 9870 – 4244 = 5626 lbs. reduced by BMP at Rate = 5626 lbs. / 60 acres = 93.8 lbs. / acre N reduction rate.

Phosphorous Reduction Example.

Same physical example area, P reduction is 54% from literature.
BMPxf = .53125. (effective BMP area is 47% of watershed)
STEPL P Load is 4719.
Treatable P load (47%) is 47 * 4719 = 2217.9 lbs.
Reduction is 54% of that treatable load.
New load is (1 - .54) 2217.9 = 1020 lbs.
Reduction in pounds = 2217 – 1020 = 1197 lbs.
Reduction rate = 1197 lbs. / 60 acres = 20 lbs. per acre.

Implementation of BMP-4:

The tool calculates the area of the just the constructed wetland and applies the reduction constant, which reduces the near-infinite incoming riverine load of TN, TP, BOD, and TSS by 7.4, 1.98, 0, and 710 $g/m^2/yr$, respectively. This is equivalent to 66 lbs, 17.7 lbs, 0 lbs, and 6334 lbs, respectively, reduction per constructed acre.

Publications:

The tool is published as a web page available for the public and stakeholders to use at this link: http://lthia.agriculture.purdue.edu/crs/ .

Scholarly articles will follow in the future.

Grant Submissions: none yet

Students: postdoctoral associate Yaoze Liu

Floodplain restoration and nutrient retention in the Wabash River Basin

Basic Information

Title:	Floodplain restoration and nutrient retention in the Wabash River Basin
Project Number:	2016IN392B
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End Date:	2/28/2018
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Congressional District:	IN-004
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Descriptors:	None
Principal Investigators:	Sara Kristen McMillan, Venkatesh Merwade

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1. None
2. Saksena, S., Merwade, V., and Singhofen, P.J. Evaluating the role of subsurface processes in explaining river-floodplain hydrodynamics: Moving beyond traditional floodplain mapping. Advances in Water Resources. In review.
3. McMillan, S.K., A. Johnson, G. Noe, S. Saksena, S. Dey, V. Merwade. Characterizing drivers of nitrogen and phosphorus flux in restored riverine floodplains. Restoration Ecology. In preparation.

Indiana Water Resources Research Center Project Report

Report 2017 Program Report Format

Project Id: 2016IN392B

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Focus Categories: Floods, Hydrology, Nutrients, Water Quality

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Abstract / Summary

Excess nitrogen and phosphorus in receiving waters is associated with high nutrient inputs in watersheds that are intensively managed for agriculture. The majority of export occurs during high flows that mobilize dissolved nitrogen and phosphorus as well as sediment and associated pollutants. In undeveloped watersheds, floodplains intercept this flood pulse creating optimal conditions for sedimentation, increasing contact between nutrient-rich waters and biologically active soils, and filtering dissolved nutrients and other pollutants from groundwater. Reducing conditions that develop during inundation promote nitrogen removal via denitrification but can cause release of phosphorus from sorption sites that are maintained under oxic conditions. Quantifying the relative contributions of these opposing processes on water quality is critically needed for successful restoration design and implementation.

Through an integrated modeling and monitoring approach we are identifying the functional relationships that exist between sources and sinks of nutrients (e.g., denitrification, phosphorus release) and hydrodynamic and biogeochemical drivers using the Wabash-Tippecanoe River confluence as a model system. We have developed a 2-dimensional hydrodynamic model of the system and have begun seasonal measurement of nitrogen and phosphorus transformations in floodplain sediments. Preliminary modeling results highlight the potential for water storage in the floodplains that flood multiple days each year on average. While soil characteristics varied laterally with increased carbon and more bioavailable carbon nearest the river, these patterns were not observed in denitrification. Instead, denitrification rates were similar across the floodplain and higher in the spring ($p=0.0066$) than in the winter likely reflecting temperature control of biological activity. Future efforts are focused on continued seasonal measurement of nutrient biogeochemistry, development of hydrodynamic metrics that quantify river-floodplain connectivity, and integration of nutrient loading, retention and hydrologic connectivity through a statistical modeling approach. This will allow us to scale results temporally and spatially to estimate the net impact of floodplain processes on nutrient retention across the Wabash-Tippecanoe River confluence and the Wabash River Basin. Collectively, this will build the foundation for an integrated and interdisciplinary analysis of the complex environmental controls on nutrient retention in river-floodplain ecosystems.

Problem

Floodplains are responsive to flood pulsing and hydrologic connectivity with the channel (Junk et al., 1989; Tockner et al., 1999). Although floodplains occupy a small fraction of the total landscape, they retain a disproportionate amount of nutrients and sediment (Mayer et al., 2007; Noe and Hupp, 2009). During high flow events, riverine water overtops the channel banks and is hydrologically connected to the surrounding floodplain. The flowpath widens causing velocities to slow and retention times to increase, both of which are critical to sediment and nutrient trapping. Restoration of these functions through breaching of levees to re-establish natural flood pulsing is a management strategy that shows great promise. In fact, nearly 30,000 acres of riverine floodplains have been restored in the Wabash River Basin by the NRCS. A literature review of nutrient retention in floodplains of large rivers suggests that as much as 600 lb/acre may be retained annually (Dee et al., 2014). However, the primary controls on N and P transformations in floodplains remain poorly understood during flooding events in the complex, tile-drained landscape of the Midwestern U.S.

Spatial variation in sedimentation rates in floodplains is controlled by multiple variables including river discharge, channel morphology, duration of inundation, and lateral proximity to the stream sediment source (Steiger and Gurnell, 2002). Similarly, rates of nutrient transformation depend on lateral and longitudinal gradients in floodplains, with higher rates found in areas with greater organic matter, nutrient content and wetness (Noe et al., 2013). Flood duration is often linked to increased sedimentation and nutrient trapping (Noe and Hupp, 2005). However, much is still unknown about how fine-scale (e.g., topography, vegetation, groundwater flow) and large-scale (e.g., channel morphology, elevation) impact flow velocities. We expect those areas that are inundated more frequently to deposit greater amounts of sediment and associated nutrients. High water tables in these areas will also contribute to reducing conditions in the subsurface that enhance rates of nitrate removal via denitrification. However, these same flooded conditions can cause release of attached phosphorus. Additionally, during periods of intense flooding, solutes and sediment previously deposited on in these near-stream floodplains can be resuspended and transported downstream. Coupling these processes with a detailed hydrodynamic model to determine the extent and duration of flooding will allow us to link these processes at a larger scale and provide information to water quality managers regarding restoration of floodplains in the Wabash River Basin and throughout the agricultural Midwest.

Research Objectives

Our long-term goal is to develop a robust predictive tool to quantify river-floodplain connectivity and its impact on sediment and nutrient retention using the Wabash-Tippecanoe River confluence as a model system. This project is the start of that effort to by quantifying the extent and duration of inundation following storm-driven water and nutrient pulses with the biogeochemical processes that either retain or remove N and P. Specifically, we are (1) quantifying flooding hydrodynamics (e.g., frequency, extent and velocity) using a modeling approach, (2) measuring key ecosystem processes driving loading, transformation, and retention of N and P in floodplain sediments seasonally, and (3) scaling these results temporally and spatially to estimate the net impact of floodplain processes on N and P retention at the Wabash-Tippecanoe River confluence.

Methodology:

The Wabash-Tippecanoe River confluence at Prophetstown State Park includes restored prairies and floodplain wetlands on the western side of the Tippecanoe and Wabash Rivers. These floodplains were formerly used for agricultural production. Both sites become inundated multiple times each year on average and have relatively shallow subsurface aquifers. At each floodplain site, we established a network of 15 transects from river edge to the hillslope to capture variability in floodplain connectivity and soil type (Figure 1). Sedimentation markers have been placed at each tile to monitor deposition of alluvial sediments during inundation.

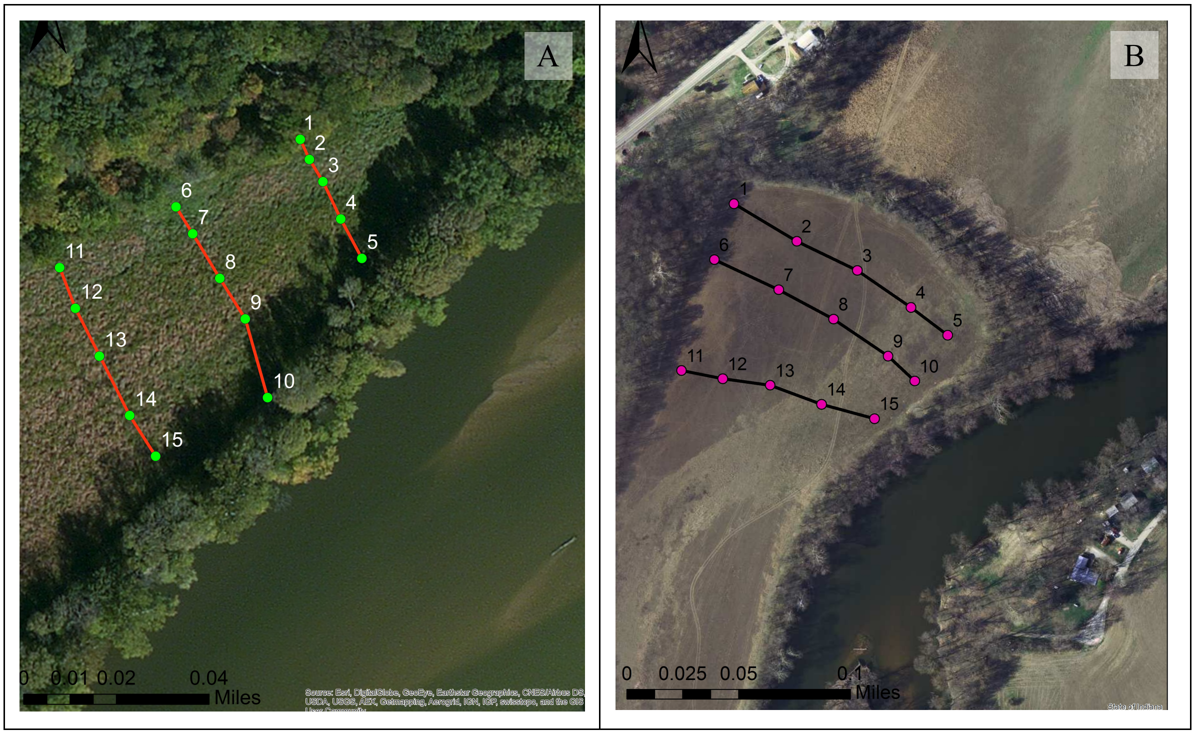


Figure 1: Floodplain transects at (A) Wabash River and (B) Tippecanoe River.

Through previous funding from INWRRC, USGS and Purdue, we developed a 1D steady-state HEC-RAS model of the Wabash-Tippecanoe confluence. This model is based on high-resolution bathymetry data for the river reaches and LIDAR data for the floodplain. We have used these data along with publicly available stream gauge and land use data to develop a flood prediction model using the Integrated Channel and Pond Routing (ICPR) model. This model is unique in that it is able to simulate surface and subsurface flow dynamics. While the ICPR model is being developed, we are using the ID HEC-RAS model to quantify spatial flooding extents based on expected storm return intervals.

We are currently measuring nutrient transformations seasonally at 9 locations in each of the floodplain sites (i.e., river edge (R) = 5,10,15; mid-floodplain (M) = 3,8,13; hill slope edge (HS) = 1,6,11). We collected intact sediment cores to a depth of 20 cm to measure net N and P flux in the laboratory under inundated conditions. Nutrient data are currently being analyzed and will be reported in the final report later this year. To quantify denitrification, we are using two methods: (1) acetylene block bottle assays which allow rapid measurement at multiple locations and is a method that has been used extensively over the past 30 years (Groffman et al. 2006) and (2) sacrificial bottle assays using membrane inlet mass spectrometry (MIMS) which provides a more precise estimate of in situ denitrification and respiration (Reisinger et al. 2016). Because the MIMS method is new and has not been applied in floodplain sediments, we are using both approaches to compare results and validate the new approach. We had unforeseen method development issues which limited our ability to make these measurements during the fall. As a result, we have a full set of both measurements in January 2017 (winter) and Spring 2017 (spring).

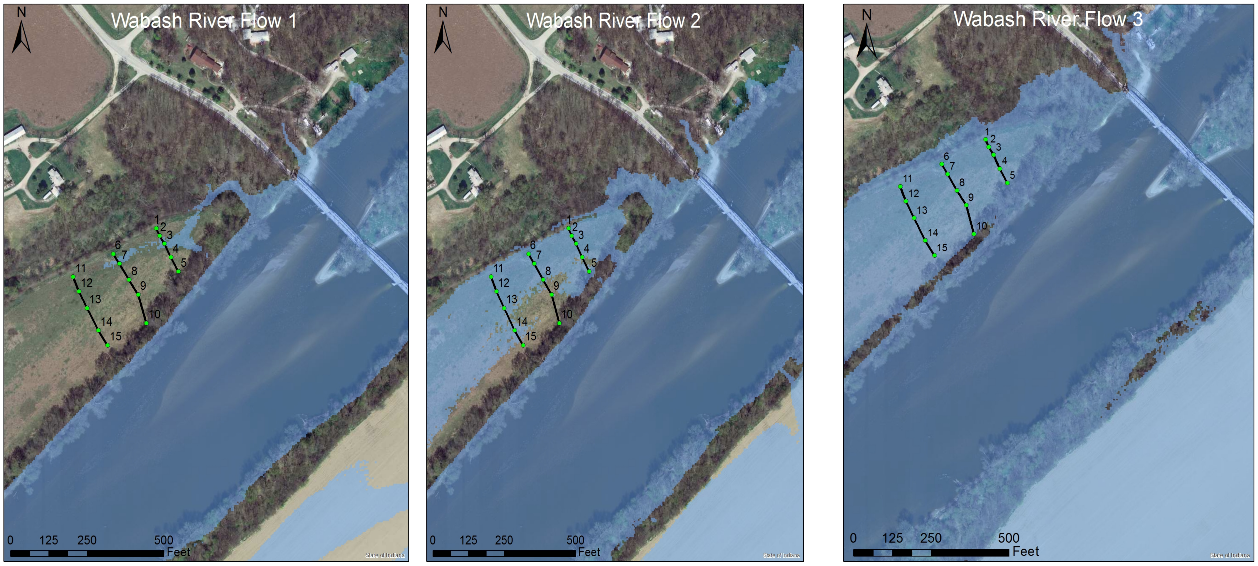
We are currently using the ICPR model to quantify the magnitude and direction of water velocities within the river and the floodplain, which is critical to understanding the potential for settling and resuspension of particles and attached pollutants. We will synthesize hydrodynamic modeling results into a series of metrics that quantify river-floodplain connectivity, such as time inundated, proportion of floodplain area inundated, variance in temporal and spatial connectivity, and surface water velocity at each floodplain biogeochemistry measurement location. We will then develop a time series of connectivity (e.g., water depth and velocity) across each of the floodplain sites, focusing initially on the transect locations. This will allow us to relate flooding hydrodynamics with the effects of topography, vegetation and sub-surface flows, but more importantly with fluxes of N, P and sediment. We recognize that 1 year of flux data is insufficient to develop a robust statistical model of nutrient loading and retention and have applied for additional funding to continue. However, we will use our data and values from the literature to develop an initial model that we can refine as more measurements are made.

Results

Hydrodynamics:

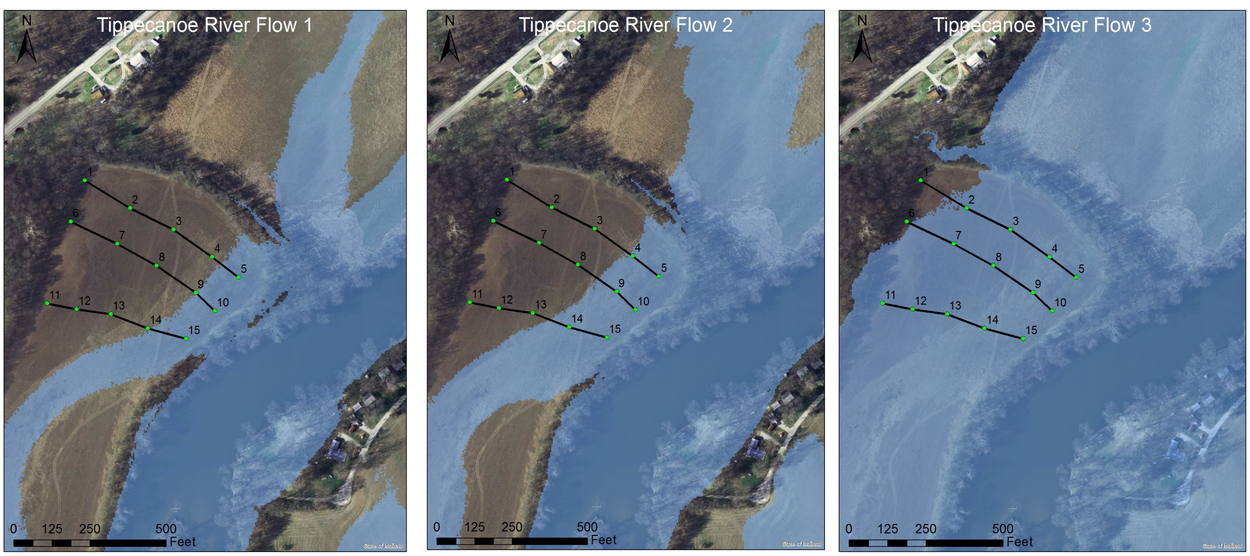
We analyzed flow records to produce a frequency distribution of daily flows throughout the year. These percentiles were calculated using the daily flows and show that for an average of 10% of the year (36 days) at least part of the floodplain is inundated for both locations and that nearly the entire floodplain is inundated 2% (7 days) of the year (Figure 2). Of note, is the relic channel on the Tippecanoe River that experiences more frequent and longer duration flooding.

Wabash River



Inundation at 90th percentile of daily flow | Inundation at 95th percentile of daily flow | Inundation at 98th percentile of daily flow

Tippecanoe River



Inundation at 90th percentile of daily flow | Inundation at 95th percentile of daily flow | Inundation at 99.8th percentile of daily flow

Figure 2. Flood extent and inundation at different flow percentiles at monitoring stations at Wabash and Tippecanoe sites.

Preliminary results from the IPCR model highlight the improvement in flood prediction accuracy using integrated models and high-resolution bathymetric data (Figure 3). Compared to results from the 1D model, these allow for finer scale resolution of stage dynamics. Fine topographic changes have been shown to be important controls on nutrient biogeochemistry (Wolf et al. 2013) and we plan to use both models to develop the most widely applicable and representative statistical model linking connectivity metrics with nutrient retention.

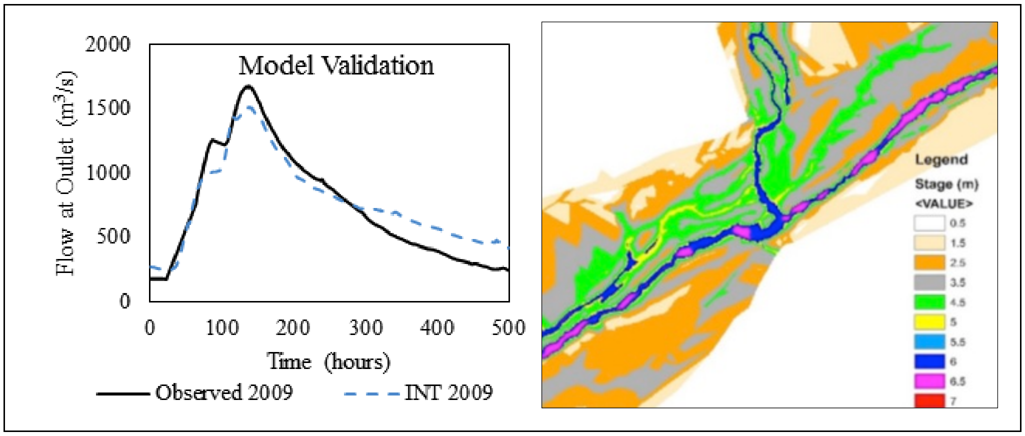


Figure 3: ICPR model validation and fine-scale resolution of water depth

Nutrient retention:

Preliminary data show distinct differences in soil characteristics laterally and between the sites. We collected sediment samples at all 15 locations at each site (Figure 1). These data revealed significant differences in C:N ratios which is an indicator of carbon quality (Figure 4). We observed more bioavailable carbon at the Tippecanoe River compared to the Wabash River (C:N 12.8 ± 0.95 versus 16.1 ± 0.82, $p<0.0001$). Additionally, C:N ratios increased laterally with the sites closest to the river having lower ratios. Comparison of total organic matter content (%) also showed significantly higher values at sites that are more frequently flooded near the river edge (R=10.02 ± 2.46, M=7.31 ± 0.80, HS = 7.43 ± 0.91; p=0.0005). Soil properties are an aggregate of multiple factors at the local and watershed scale, including flood frequency, vegetation, land use history and other sediment sources from the watershed. We

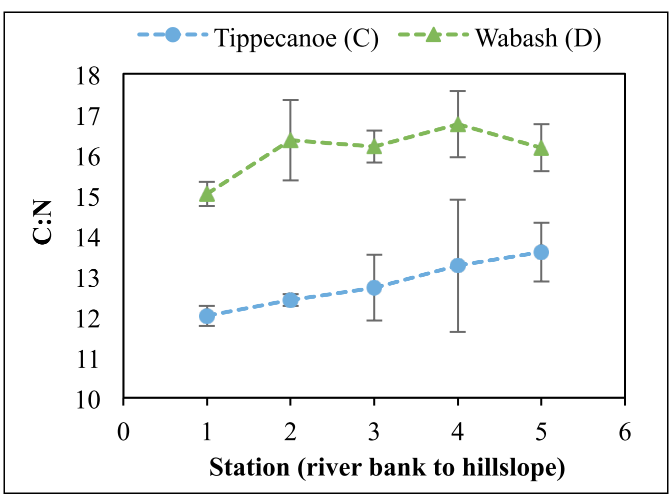


Figure 4: Carbon:nitrogen ratios at restored floodplains sites on Tippecanoe and Wabash Rivers. Error bars are one standard deviation, n=3 per station.

hypothesized that these differences would also affect trapping and processing of riverine nutrients and sediment. We did not observe similar patterns based on landscape position in denitrification rates determined using the acetylene block method (Figure 5). Rates were more variable and higher in the spring (p=0.0066) than in the winter likely reflecting temperature control of biological activity. In 24-hour intact flux core experiments, we observed that the site was generally a sink for nitrate with correlates well with increased rates of denitrification under flooded conditions (Figure 6). As soils become more anoxic, soil respiration proceeds from using oxygen as the primary electron acceptor to nitrate thereby increasing rates under prolonged inundation. We also observed significant correlations with denitrification and the total organic carbon present in the soil and mean days flooded suggesting that denitrification at these sites may be limited by carbon availability and redox conditions (Figure 7). Both sites were generally a source of ammonium as organic matter mineralization contributes to ammonification and release of ammonium (Figure 6). Phosphorus flux was generally low and highly variable during the 24-hour incubation (Figure 6). Future work is focused on extending the duration time and further differentiating the sorption capacity of these soils for P.

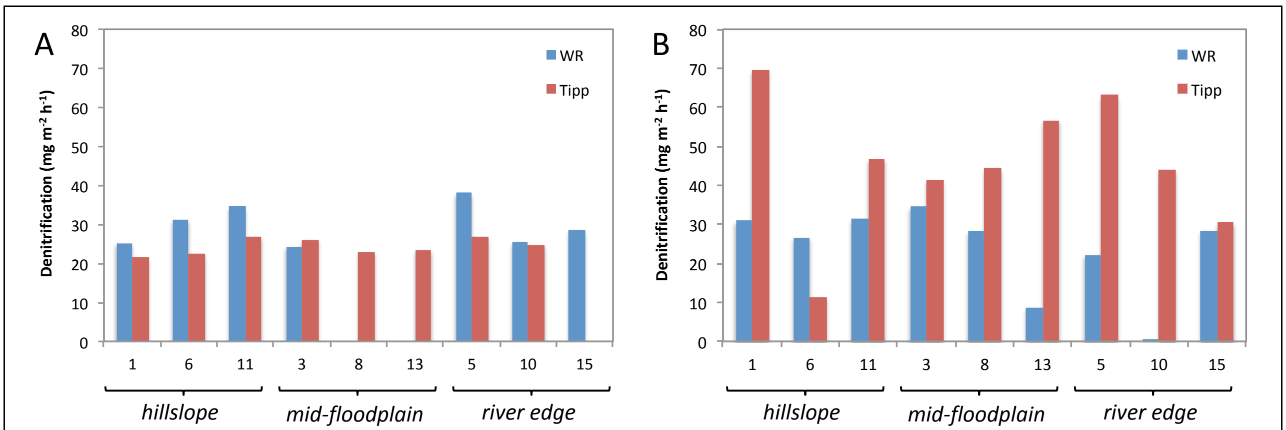


Figure 5: Denitrification rates as measured using acetelyne block bottle assays in the (A) winter and (B) spring of 2017 in the WR (Wabash River) and Tipp (Tippecanoe River) floodplains.

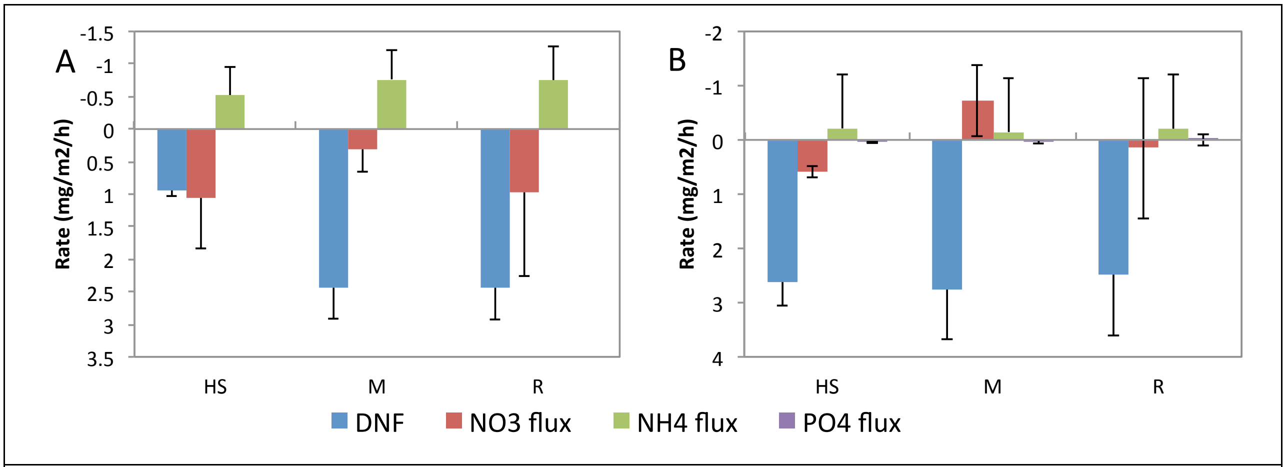


Figure 6: Nutrient flux and denitrification (DNF) rates in the Winter 2017 at (A) Tippecanoe River and (B) Wabash River sites respectively. No significant differences were observed among the locations (n=3 for each transect; HS = hillslope, M = mid-floodplain; R = river edge).

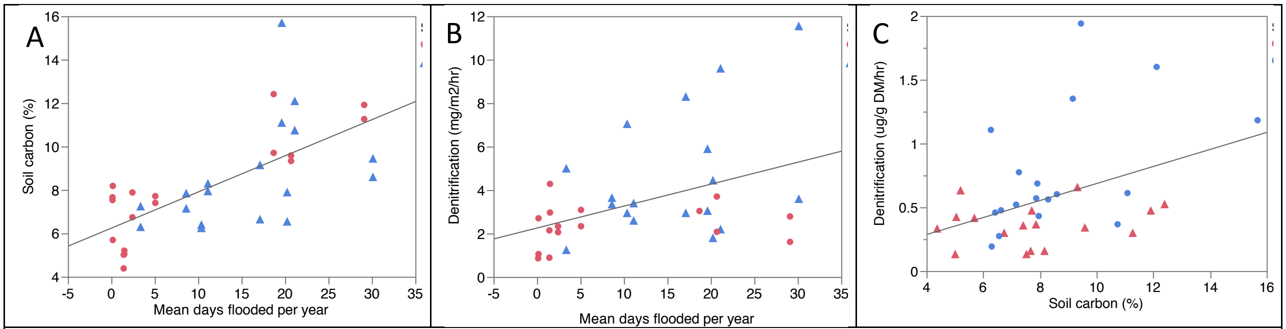


Figure 7: (A). Soil carbon (r=0.677, p<0.0001) and (B) denitrification (r=0.416, p=0.013) as a function of mean days flooded each year. (C) Relationship between denitrification and soil carbon (r=0.399, p=0.018). Wabash River sites are in blue and Tippecanoe River sites are in red.

Future plans

We anticipate completing a manuscript on the field and modeling activities included in this report with a target submission date of August 2018. Results of this study formed the basis for a successful grant submission to the National Science Foundation and will allow us to explore the controls on denitrification but also phosphorus dynamics in these restored floodplains.

We have developed a cooperative relationship with Indiana Department of Natural Resources (IDNR) and the Prophetstown Park Manager (Jason Getz) in particular. This has allowed us to better coordinate field activities with their prairie management but also to better frame our questions that would be helpful for managers. To this end, we established two additional sites for the NSF project including a working farm in the Wabash River floodplain and a wetland mitigation project on the Prophetstown Park property. All of these sites are historically significant sites with early Native American settlements so we have been also working closely with the Indiana Department of Cultural Resources within IDNR to ensure that sampling activities do not disturb any known archeological sites.

Major Conclusions and Significance

Impacts of this research will help influence restoration activities in Indiana as the state invests in practices to reduce nutrient loads from agricultural lands. Phosphorus export from Indiana's portion of the Lake Erie Basin contributed to the harmful algal blooms in Lake Erie that left more than 400,000 people in Toledo without drinking water in 2014 (Smith et al., 2015), and N and P from Indiana agricultural land contributes to hypoxia in the Gulf of Mexico (Rabalais, 2001). To address these concerns, Indiana has developed a Nutrient Reduction Strategy (ISDA, 2016) to reduce nutrient runoff by encouraging voluntary, incentive-based conservation practices. Great potential exists for floodplain restoration to significantly reduce nutrient loads and programs such as the Wetland Reserve Enhancement Partnership provide a mechanism for the National Resource Conservation Service to leverage resources to initiate restoration of high priority wetlands to improve water quality and wildlife habitat. However, characterization and prediction of the environmental factors driving successful restoration is needed to find optimal locations that achieve the greatest impact per dollar invested. This work will provide a needed synthesis of the impacts of floodplain reconnection on the net retention of nitrogen and phosphorus.

As floodplains intercept high riverine discharges, retention time increases and opportunities for biological and physical processes occur. However, reducing conditions created during flooding can have contrasting results, including retention of sediment and particulate bound phosphorus, removal of soluble nitrate via denitrification, and release of soluble reactive phosphorus. Our results show that denitrification is indeed enhanced in these systems and changes as expected with seasonality.

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Publications

Saksena, S., Merwade, V., and Singhofen, P.J. Evaluating the role of subsurface processes in explaining river-floodplain hydrodynamics: Moving beyond traditional floodplain mapping. Advances in Water Resources. *In review.*

McMillan, S.K., A. Johnson, G. Noe, S. Saksena, S. Dey, V. Merwade. Characterizing drivers of nitrogen and phosphorus flux in restored riverine floodplains. Restoration Ecology. *In preparation.*

Presentations

McMillan, S.K., C. Alford, A. Johnson, G. Noe, S. Saksena, S. Dey, V. Merwade. Effects of floodplain restoration on nitrogen and phosphorus dynamics in agricultural watersheds. 12th International Symposium on Biogeochemistry of Wetlands. Coral Springs, FL. April 2018.

McMillan, S.K. Floodplain restoration from headwaters to rivers. Kent State University, Department of Biology Seminar, September 22, 2017.

McMillan, S.K. Ecosystem restoration: Does restoring structure lead to function? Curriculum for the Environment and Ecology Seminar, University of North Carolina, Chapel Hill, NC. April 2017.

McMillan, S.K., V. Merwade, G. Noe. Nitrogen and phosphorus flux in restored riverine floodplains in agricultural watersheds. Indiana Water Resources Association Annual Meeting. Marshall, IN. June 2017

McMillan, S.K., V. Merwade, G. Noe. Nitrogen and phosphorus flux in restored riverine floodplains in intensively managed watersheds. Society of Wetland Sciences Annual Meeting. San Juan, PR. June 2017

Saksena, S., and Merwade, V. Integrated Modeling of Surface-Subsurface Processes to Understand River- Floodplain Hydrodynamics in the Upper Wabash River Basin. World Environmental and Water Resources Congress 2017, Sacramento, CA, pp. 60–68.

Saksena, S., V. Merwade and P. Singhofen. Evaluating the impact of temporal and spatial scale variability on floodplain storage in the Upper Wabash River basin, 2016 AWRA Spring Specialty Conference on GIS and Water Resources, Sacramento, CA, July 2016.

Saksena, S. and V. Merwade. Evaluating the impact of spatial and temporal rainfall variability on river-floodplain hydrodynamics, Abstract H24B-05 presented at the 2016 Fall Meeting, AGU, San Francisco, CA Dec. 2016.

Grant Submissions

This project has allowed us to collect data and further refine our research questions. We submitted proposals to the USGS and the USDA, both of which were not funded but we received positive feedback. We also submitted a more theoretical approach to the NSF-CBET in October 2016, which was funded for 3 years at $312,066. We also have one student (Danielle Winter) awarded an NSF Graduate Research Fellowship and she will focus her PhD research on this project. The NSF-CBET funding allowed us to recruit another highly motivated student (Shannon Donahue) for this biogeochemistry component in McMillan's lab and fund the PhD of current student (Sayan Dey) in Merwade's lab on the hydrodynamics component.

Students

Through this project, we have supported learning and discovery of 2 undergraduate students (Caitlin Nelligan, ABE and Evan Pesut, ABE), and supplemented funding for 1 MS student (Celena Alford, ABE) and 1 PhD student (Siddarth Saksena, CE).

Pilot investigation of variable contaminant loads in fish as a result of foraging and habitat specialization

Basic Information

Title:	Pilot investigation of variable contaminant loads in fish as a result of foraging and habitat specialization
Project Number:	2016IN393B
Start Date:	3/1/2016
End Date:	2/28/2018
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Congressional District:	IN-004
Research Category:	Not Applicable
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Descriptors:	None
Principal Investigators:	Tomas Hook, Timothy Malinich

Publications

1. None
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Indiana Water Resources Research Center Project Report

Report 2017 Program Report Format

Project Id: 2016IN393B

Title: Pilot investigation of variable contaminant loads in fish as a result of foraging and habitat specialization

Project Type: Research

Period: Final report through February 28, 2018

Congressional District: IN-004

Focus Categories: Ecology, Toxic Substances

Keywords: Yellow Perch, Black Crappie, Mercury, Population Trophic Structuring

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Abstract / Summary

Contaminants such as mercury are typically quantified as measures of central tendency/averaged across all sampled fish of the same species within the same body of water. Many contaminants accumulate in fish from prey items consumed in their environment. Various studies suggest that individuals within a population do not all exhibit the same feeding and habitat residence patterns, and therefore may have varying risks of contamination, e.g., from mercury. If popular sportfish such as yellow perch, *Perca flavescens*, or black crappie, *Pomoxis nigromaculatus*, exhibit diet plasticity and specialize on pelagic or benthic prey, then some groups of fish within populations may have elevated contaminant loads and pose a greater risk to consumers. We hypothesized that diet plasticity and specialization in fish populations could lead to a bi-modal distribution of contaminant loads. We sampled yellow perch and black crappie from 5 different lakes in northern Indiana and evaluated the trophic ecology of fish using a combination of stable isotopes ($\delta^{13}C$, $\delta^{15}N$), and morphology. Finally, we examined the relationships between mercury loads of individual fish and their diet/habitat specialization. Yellow perch in most of our lakes exhibited broader trophic niches compared to black crappie. Across both species we found lake specific differences in isotopes, morphology and mercury content. Within lakes, we found that individual mercury concentrations were not distributed parametrically. Further, stepwise regression tests to identify good predictors of mercury content found that in addition to fish length, mercury within fish were often associated $\delta^{15}N$ and $\delta^{13}C$ isotope ratios and several morphological axes which may indicate specialization for particular habitats or food items.

Problem:

Mercury is a persistent contaminant problem in Indiana's waterways (Management, 2014). Mercury contamination has the potential to directly impact Indiana residents by accumulation in body tissues through consumption of fish species such as catfish, yellow perch and black crappie. Accumulation of mercury through diet can increase risk of several debilitating diseases, brain damage and kidney damage. Understanding how a population of fish within a lake may have varying contaminant risks can help us understand the risk to Indiana's recreational angling community.

Research Objectives:

Use stable isotopes ($\delta^{13}C$ & $\delta^{15}N$) and morphology to characterize the trophic complexity of yellow perch and black crappie among northern Indiana lakes.

- Determine if trophic positions vary among populations in yellow perch and black crappie.
- Using morphological landmarks, characterize the morphology of yellow perch and black crappie and identify any signs of specialization such as body shapes identified with pelagic (fusiform bodies) or benthic habitats (deeper bodies).

Examine the intra-population mercury content of fish populations

- Examine intra-population variation in total mercury concentrations of individual yellow perch and black crappie.
- Identify any trophic measures, in addition to total fish length and sex, that may act as predictors of mercury concentrations within fish.

Methodology:

Fish and Lakes

Black crappie and yellow perch were collected in 5 Northern Indiana glacial lakes (Backwater, Jimmerson, Skinner, Sylvan, and Wawasee: see Figure 1) by the Indiana Department of Natural Resources as a part of annual surveys in March-June 2016. The lakes varied from one another across multiple biotic and abiotic metrics (Table 1). Fish were collected by trap net and preserved in a -20°C freezer. In the laboratory, fish were digitally imaged on a concave board using a Panasonic DMC-TS5 camera with a ruler for identifying scale. In addition, each fish was sexed, measured for total length (to 1 mm), mass (to 0.1 g), and had 1-3 g of dorsal muscle tissue removed from just below the anterior end of the dorsal fin to measure stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and mercury analyses (total methylmercury). Size and sex can have influences (Bastos et al., 2016) on stable isotopes and mercury therefore a subset of fish from the five lakes were selected to approximately equally represent each sex, and fall within a total length range of 200-250mm (see Table 2 for breakdown by lake and fish type). In total, we selected 96 yellow perch and 141 black crappie for our study.

In August of 2016, invertebrates from 4 of the represented lakes (Jimmerson, Shipshewana, Skinner, Sylvan, and Wawasee) were collected from 2 replicate samples of pelagic and benthic resources in the nearshore and offshore regions of each lake. Pelagic samples from these lakes were collected by a horizontal tow of a 50μm zooplankton net. Benthic samples were collected by petite ponar (15mm x 15 mm) grabs. Pelagic samples were found to be full of filamentous algae and zooplankton and were unable to be separated in the lab, therefore we measured these together to represent a general pelagic seston signal in our stable isotope analysis. Benthic samples were sorted manually in the lab, removing commonly found invertebrates (i.e. chironomids, amphipods, and gastropods) consumed by black crappie and yellow perch. Chironomids were collected in all lakes sampled except Jimmerson and were used to measure baseline benthic isotope signals, see *Stable Isotope* methods below, in each lake. For Jimmerson Lake, amphipods and gastropods were used to establish a baseline signal.

Stable Isotopes

Dorsal muscle tissues for $\delta^{13}C$ and $\delta^{15}N$ isotope ratio analysis were stored in a -20°C freezer prior to preparation. Samples were dried (60 °C), ground, and sent to the Cornell Isotope Laboratory for analysis, where they were analyzed using a ThermoFinnigan Delta Plus mass spectrometer and NC2500 elemental analyzer. Carbon isotope ratios were mathematically adjusted to account for lipids (Post et al., 2007). Lakes will often vary in their isotopic baselines, therefore to examine relationships among lakes we adjusted isotope ratios using isotopic baselines (i.e. Post 2002). This adjustment allows for the comparison of trophic positions of fish from each lake. Baseline samples were prepared in the same manner as the fish tissue and sent to the Cornell Stable Isotope Lab for analysis. Following baseline corrections, we compared the trophic positions of yellow perch and crappie among lakes, including total length of fish as a covariate, with a permutated multivariate analysis of variance (perMANOVA) using the R function ADONIS within package vegan (Oksanen et al., 2016). Following this analysis we conducted separate univariate ANOVA and Tukey post-hoc tests of nitrogen trophic positions and carbon trophic positions to determine how lakes differed from one another. Additional analyses involving stable isotopes, described below, were conducted for each lake separately and did not require baseline corrections for $\delta^{13}C$ and $\delta^{15}N$.

Morphometrics

Morphometric analysis of crappie and perch were conducted, separately, using similar methodologies. Landmark points were used to measure morphology, using eighteen points for yellow perch and 13 points for black crappie (Figure 2). Landmarks were assigned using the program TpsDig (Rohlf, 2005). Fish shapes were normalized via the procrustes procedure in the program R (R Core Team, 2014) using the function GeoMorph (Adams et al., 2018). This method adjusts images for differences due to size, scale, and position through rescaling, translation and rotation of images. Some variation in shape was attributable to differences in allometry, and this variation was accounted for by the following procedure. Procrustes points were tested within a perMANOVA along with the centroid sizes of individuals. Centroid size is the average distance of each landmark point to the image center and is closely related to fish size (Klingenberg, 2016). The residuals from this perMANOVA were extracted and used for all subsequent analyses (See Parsons et al. 2016 for similar methods).

The general morphological differences of fish, of the same species, among lakes were first compared using a perMANOVA. Following this a principle components analysis (PCA) was used to examine how shapes varied among lakes (i.e., one PCA for data across all lakes. This analysis collapsed our landmark data into a series of PC axes describing the major variation in the landmark morphology. The top 3 axes, generally covered a cumulative 75% of the variation, and were used along with stable isotopes in regression models to predict mercury content (Described below).

Mercury

Total mercury (Hg) content was measured using a thermal decomposition (gold) amalgamation atomic absorption spectrophotometer direct mercury analyzer (DMA-80, Milestone Inc.; For similar methods see Cladis et al. 2014). Fish samples were run in duplicate, along with a standard (Tort-3) and mercury was calculated as a wet weight (μg/kg). Following the first run of mercury processing, we calculated the coefficient of variation (CV) of each sample by dividing the standard deviation of the sample by the mean and multiplying by 100. Samples with CV over 10% were run a second time in duplicate. After this rerun of samples, the mean mercury values of every individual were recalculated with the addition of the re-run of high CV samples. These means were used for mercury values in all further analyses. Mercury values represent the total mercury content within the muscle tissue and may consist of both inorganic and organic (methyl-mercury) mercury. However, previous studies have shown that the vast majority of mercury within muscle tissue consists of methyl-mercury due to its ability to accumulate in tissue (Boening, 2000).

Mercury distributions within fish populations was first examined using the Shapiro-Wilks test for normality, a common assumption for contaminants in populations. The mercury content was further tested across lakes for yellow perch and black crappie using a MANOVA. Baseline mercury levels among lakes may vary depending on current and historic mercury deposition, as well as lake size, water clarity, and primary production. We also considered relationships between mercury and the trophic positions of fish as well individual sex and size. These analyses were conducted by nesting individuals by lake and testing mercury content of trophic position and sex, while including length as a covariate. Finally, we conducted a series of Pearson correlations, to examine potential relationships of mercury content with the total length of fish, $\delta^{13}C$ and $\delta^{15}N$ isotope ratios. These correlations were repeated separately for each lake since the baseline values of both isotope ratios and mercury levels may vary among lakes.

Finally, we examined the strength of different individual characteristics (e.g. sex, length, isotope ratios, morphology etc.) as predictors of mercury content. Toward this goal we conducted a forwards and backwards stepwise linear regression (SLR) for each species within each lake. Specifically we used total mercury as a dependent variable and the following independent variables: size and sex of fish, isotope values ($\delta^{13}C$ and $\delta^{15}N$), and the first 3 principal component scores describing morphology. Models were compared using Akaike Information Criterion (AIC). The top models for each lake are listed in Table 4. All analyses were conducted using R (R Core Team, 2014).

Results

In all of our analyses we found a strong lake effect on mercury concentrations and stable isotope ratios. This effect was expected and baseline values were collected for isotope corrections to help account for this. Limitations in sampling opportunities prevented us from collecting baseline samples across different seasons, which may impact the interpretation of isotope results below. Further, several analyses in morphology and mercury analysis were limited in their ability to discern patterns due to uneven sample sizes.

A comparison of isotope baselines among lakes shows that each lake varied in its baseline $\delta^{13}C$ and $\delta^{15}N$ ratios and similar patterns are reflected in the fish from each lake (see Figure. 3 for isotope ratio values and invertebrate baseline values). Baseline values among lakes appear to vary across all lakes; however seasonal differences between isotopes examined in the fish and those examined in invertebrates likely impact the effectiveness of baseline corrections. This is particularly evident in cases where benthic invertebrates such as chironomids appear more 'pelagic', more negative, in carbon signals than isotopes measured from mixed zooplankton samples. Fish trophic positions calculated from baselines suggest different trophic patterns among lakes (YEP R^2=0.231, p=0.0001;BLC R^2=0.671, p=0.0001); after accounting for the differences in fish total length (YEP R^2=0.577, p=0.0001; BLC R^2=0.671, p=0.0001). Interactions between the two variables were also significant for perch but not for crappie (YEP R^2=0.026, p=0.001; BLC R^2=0.001, p=0.863). In yellow perch, post-hoc ANOVA and Tukey tests found the differences were attributed to both carbon trophic positions and nitrogen trophic positions in lakes Wawasee and Sylvan. In black crappie, differences among lakes were driven primarily by nitrogen trophic positions, particularly from Lake Jimmerson and by carbon trophic positions in lakes Jimmerson and Sylvan.

Morphology of yellow perch and black crappie provided some support of specialization of fish among and within lakes. Yellow perch were tested for morphological differences related to lake and sex specific differences (Lake R^2=0.06 p=0.002, Sex R^2= 0.03 p=0.031). Black crappie also demonstrated differences between lakes and sex (Lake R^2=0.13 p=0.001, R^2=0.02 Sex p=0.001), and in comparison to perch, lake effects explained more shape variation for crappie. The ratio of male to female perch was skewed toward one sex or another in all perch lakes except Lake Wawasee, which may have influenced the explanatory contribution of sex within the perMANOVA. Distributions of morphology along the first PC axes often appeared to relate to the orientation of head and tail structures, orienting more dorsally (toward the surface) or ventrally (toward the benthos).

Mercury distributions, tested by Shapiro-Wilks tests, within fish populations were not always normally distributed. In yellow perch populations, lakes Jimmerson and Skinner both exhibited non-normal distributions. Crappie populations also exhibited non-normal distributions within lakes Jimmerson, Skinner, and Wawasee. Differences in mercury content were observed among lakes in each species of fish but not among different sexes (Figure 4). An ANCOVA, using length as a covariate, confirmed predictions for mercury variation among lakes in perch ($p<0.0001$) and crappie ($p<0.0001$). In both cases, length was also an important predictor of mercury content (perch $p=0.007$, crappie $p<0.0001$). The nested ANCOVA examining sex specific mercury concentrations found no significant differences between the sexes in either black crappie ($p=0.1$) or yellow perch ($p=0.15$). In both species, length continued to be a good predictor of mercury content. In perch, this analysis may have been hindered by the bias in sex ratios in all but Lake Wawasee.

Correlation analyses conducted separately on each lake, between Hg, length and trophic positions (Table 3) demonstrated a great deal of variety in Hg responses to trophic measures (see Figure 5 for a visual of total Hg in fish and their isotope ratios). Interestingly, nitrogen isotopes had mixed responses to mercury content, usually with weak correlations in either positive or negative directions. In general both species exhibited positive total mercury correlation with enrichment in $\delta^{13}C$. In almost every case, length was positively correlated with mercury.

Stepwise linear regressions were conducted separately by lake, due to the strong differences among lakes in isotope ratios and Hg, and similar patterns emerged among lakes. All initial models for each species in each lake were tested using the same potential explanatory variables (see Table 4 for model breakdown). Length of fish is always a top predictor of Hg content in fish. Nitrogen isotopes, a measure of trophic level, were also consistently a top predictor for most fish in many of the lakes. For black crappie in lakes Sylvan and Skinner, $\delta^{13}C$ isotope ratios were also among the best predictors for Hg. Sex of the fish was an important predictor of mercury content in Lake Wawasee black crappie and Backwater Lake yellow perch. However, uneven sex ratios may have contributed to the perch result. In both fish, in several lakes, morphology axes were significant predictors of Hg content. In Backwater Lake for black crappie, all three PCA axes were included in the final model.

Major Conclusions and Significance

The work completed by this study is a preliminary exploration of complex intra-population trophic patterns and total mercury contamination in yellow perch and black crappie. Our measured trophic indicators (morphology and stable isotopes) varied among and within lakes. We observed that yellow perch have broad variation in trophic indicators, in comparison to black crappie, suggesting individual yellow perch may utilize different trophic pathways. These pathways may impact the average level and variation of total Hg contamination within perch populations. Further, although black crappie on average displayed a narrower range of trophic indicators, some patterns observed in our study were not expected and could suggest differences in trophic pathways and mercury contamination between lakes. Additional exploration of the ideas developed through this study could serve to help identify fish populations with varying risk of contaminants, potentially identifying methods to lower mercury risk to recreational anglers consuming sportfish in Indiana.

Within our study, we identified that patterns of Hg contamination varied among lakes, and that within lakes Hg did not generally follow parametric distributions. This suggests that a more robust sampling of fish populations may be required to capture the true distributions of mercury contaminants within Indiana lakes. Total Hg values calculated from only a handful of representative fish, which may or may not be a targeted species for consumption, may underestimate the risk of Hg consumption for recreational anglers from a particular lake.

We attempted to connect total Hg to trophic measures, but found mixed and often loose connections between Hg and either isotope ratios or morphology. Our trophic analyses may have been limited by the lack of baseline signals for mercury (Lavoie et al., 2013) and the lack of seasonal baselines for isotope ratios. It is possible that future studies may find stronger connections with more intense sampling of fish and invertebrate diet items. Interestingly, we found correlation of Hg with $\delta^{15}N$ were occasionally negative in direction. Nitrogen isotope ratios are linked closely with trophic position (Post, 2002) wherein higher trophic consumers generally consume larger prey. Higher trophic consumers are also expected to experience greater bioaccumulation and previous studies have observed trends between Hg and $\delta^{15}N$ (Bank et al., 2007). Despite the findings in $\delta^{15}N$, larger fish in our study did have positive associations with total Hg, similar to other fish research (Farkas et al., 2003). Follow up correlations indicate that nitrogen and fish total length were positively associated with one another. The negative associations with $\delta^{15}N$ may be further evidence that links between total Hg and trophic patterns may not be clear cut within our sampled lakes and fish may be feeding in different trophic pathways with different risks to Hg accumulation. Since we were not able to gather ideal prey baselines, future studies should consider sampling multiple prey items within lakes and utilizing mixed models to determine how different prey may be contributing to diets.

Finally, it may also be important to investigate the influence of seasonal trends on total mercury and stable isotopes in fish within these small temperate lakes. In small temperate lakes, seasonal changes in production (Watras et al., 1995) and invertebrate prey (Abrantes et al., 2006; Tallberg et al., 1999) could in turn alter the rates of mercury accumulation of fish. For instance it was found that common bream, *Abramis brama*, exhibit seasonal changes in mercury loads and that much of the seasonal variation may be attributable to differences in fish condition (Farkas et al., 2003). Seasonal differences could be due to variable production, precipitation, dissolved organic carbon and even the changing colors of lakes (Mierle and Ingram, 1991). In addition to seasonal variation, fish may also have interannual variation in mercury contamination (Greenfield et al., 2005). It is also established that different sexes in some fish (Bastos et al., 2016; Madenjian et al., 2015) may vary in total Hg due to different metabolic rates which may change with season and across years. If mercury accumulation varies seasonally in northern Indiana lakes, this could have an important effect on appropriate angling periods, catch limits and consumption advisories for recreational fish species.

Publications

Indiana Water Resources Research Conference, 2017; Marshall, Indiana; Poster presentation; *Investigation of variable contaminant loads in fish as a result of foraging and habitat specialization*

Ecological Society of America's Annual Conference (Abstract Accepted) 2018, New Orleans, LA; Oral Presentation; *Investigation of variable contaminant loads in fish as a result of foraging and habitat specialization*

Students Undergraduate-1

PhD-1

PostDoc-1

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Tables

Table 1. Physical characteristics of lakes.

Lake	Mean Lake Depth (m)	Surface Area (ha)	Lake Perimeter (km)	Chlorophyll a (mg/m^3)	Temperature (at 1.5 m)	Dissolved Oxygen (at 1.5 m)	Total Catch Area (km^2)	Lake SDI
Back Water	-	56.66	9.05	18.327	24.4	3.45	107.2	3.38
Jimmerson	17.07	114.53	22.72	1.56	26.01	8.14	105.39	5.11
Skinner	9.75	50.59	4.36	26.29	26.3	10.22	36.14	1.76
Sylvan	10	254.96	21.69	17.65	26.6	7.47	-	3.83
Wawasee	23.46	1059.5	46.05	1.43	27.2	7.61	67.9	3.70

Table 2. Yellow perch and black crappie, sample size (in parentheses) of each sex, mean & standard deviation of total length (mm) for each Indiana Lake examined.

	Yellow Perch		Black Crappie	
Lake	Male	Female	Male	Female
Back Water	195±0 (1)	194.9±13.0 (25)	206.2±27.2(9)	226.4±16.9(8)
Jimmerson	(0)	(0)	247.9± 14.6(11)	257.3±17.7 (21)
Skinner	(0)	(0)	156.4±18.1 (19)	155.5±16.1 (11)
Sylvan	238.4±19.9 (37)	250±0 (1)	250.5±24.3 (13)	240.1±15.1 (13)
Wawasee	199.2±28.4 (12)	194.1±26.6 (20)	225.5±28.6 (17)	235.7±7.6 (19)

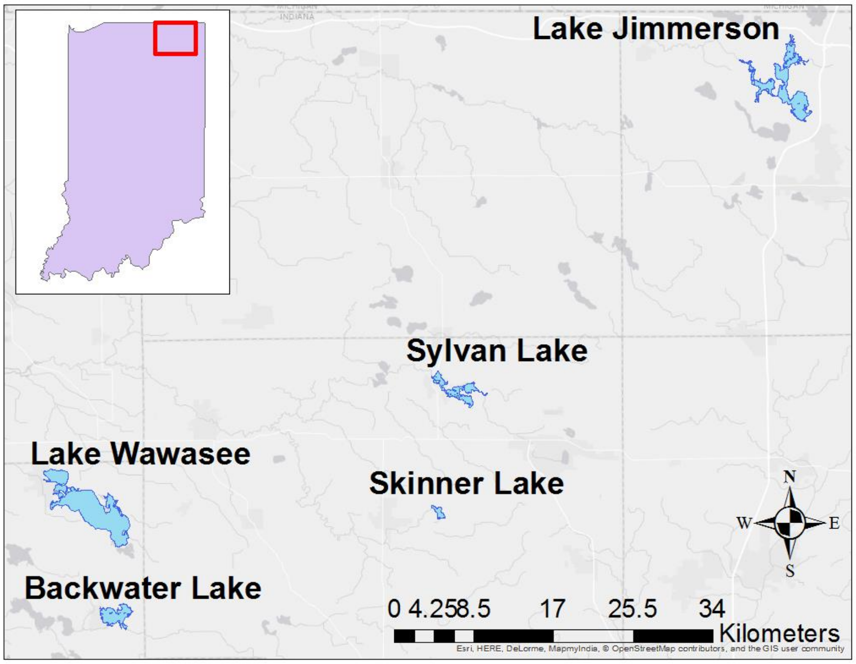
Table 3. Summary table of Pearson correlations of fish length and isotope ratios with mercury. Correlations were examined in each species for the combination of all lakes, and for each separate lake. Number in parenthesis is the total number of fish for yellow perch and black crappie (YEP|BLC).

		Yellow Perch	Black Crappie
All Lakes (96|141)	Length	-0.17	0.47
	Carbon	0.70	-0.24
	Nitrogen	-0.40	-0.72
Back Water (26|17)	Length	0.29	0.78
	Carbon	0.16	0.68
	Nitrogen	0.05	0.82
Jimmerson (0|32)	Length	NA	0.52
	Carbon	NA	0.19
	Nitrogen	NA	0.22
Skinner (0|30)	Length	NA	0.65
	Carbon	NA	-0.56
	Nitrogen	NA	-0.64
Sylvan (38|26)	Length	0.64	0.59
	Carbon	-0.12	0.56
	Nitrogen	0.02	-0.34
Wawasee (32|36)	Length	0.53	0.46
	Carbon	0.38	0.03
	Nitrogen	-0.11	-0.15

Table 4. Initial and final models from the forward-backward stepwise regression for each lake, and species. Final model AIC values are provided for each lake. Carbon values are corrected for lipids. The first three morphological principle component axes are included in each model and generally accounted for 75% of the morphological variation.

Fish Species	Lake	Model	Variables	AIC
Yellow Perch	Back Water	Initial Model	$^{13}C+^{15}N+L_{tot}+Sex+PC1+PC2+PC3$	
		Final Model	$^{15}N+L_{tot}+Sex+PC3$	151.4
	Sylvan	Initial Model	$^{13}C+^{15}N+L_{tot}+Sex+PC1+PC2+PC3$	
		Final Model	$^{15}N+L_{tot}$	170.9
	Wawasee	Initial Model	$^{13}C+^{15}N+L_{tot}+Sex+PC1+PC2+PC3$	
		Final Model	$^{15}N+L_{tot}$	236.5
Black Crappie	Back Water	Initial Model	$^{13}C+^{15}N+L_{tot}+Sex+PC1+PC2+PC3$	
		Final Model	$^{15}N+PC1+PC2+PC3$	87.0
	Jimmerson	Initial Model	$^{13}C+^{15}N+L_{tot}+Sex+PC1+PC2+PC3$	
		Final Model	$^{15}N+L_{tot}+PC1$	269.3
	Skinner	Initial Model	$^{13}C+^{15}N+L_{tot}+Sex+PC1+PC2+PC3$	
		Final Model	$^{13}C+L_{tot}$	128.0
	Sylvan	Initial Model	$^{13}C+^{15}N+L_{tot}+Sex+PC1+PC2+PC3$	
		Final Model	$^{13}C+^{15}N+L_{tot}+PC1$	112.5
	Wawasee	Initial Model	$^{13}C+^{15}N+L_{tot}+Sex+PC1+PC2+PC3$	
		Final Model	$^{15}N+L_{tot}+Sex$	206.7

Figure 1. Map of Indiana lakes examined for isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and total mercury content.



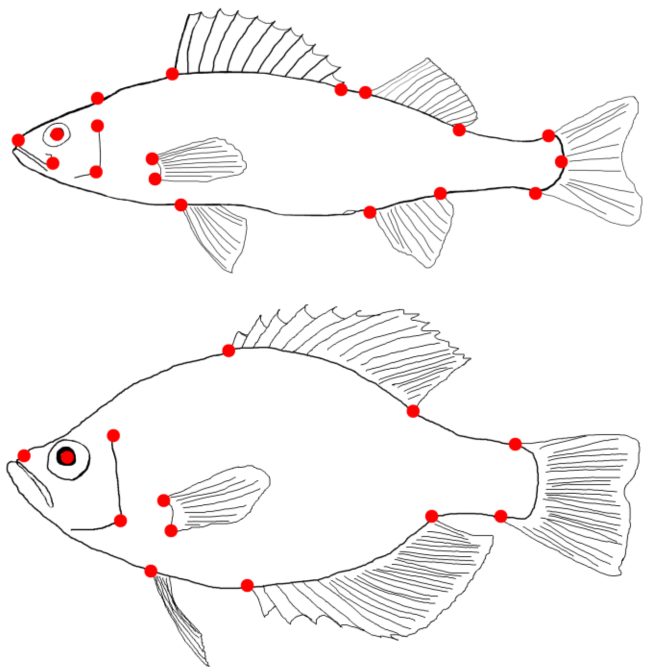


Figure 2. Landmark points on yellow perch (top) and black crappie (bottom) used for morphometric analysis.

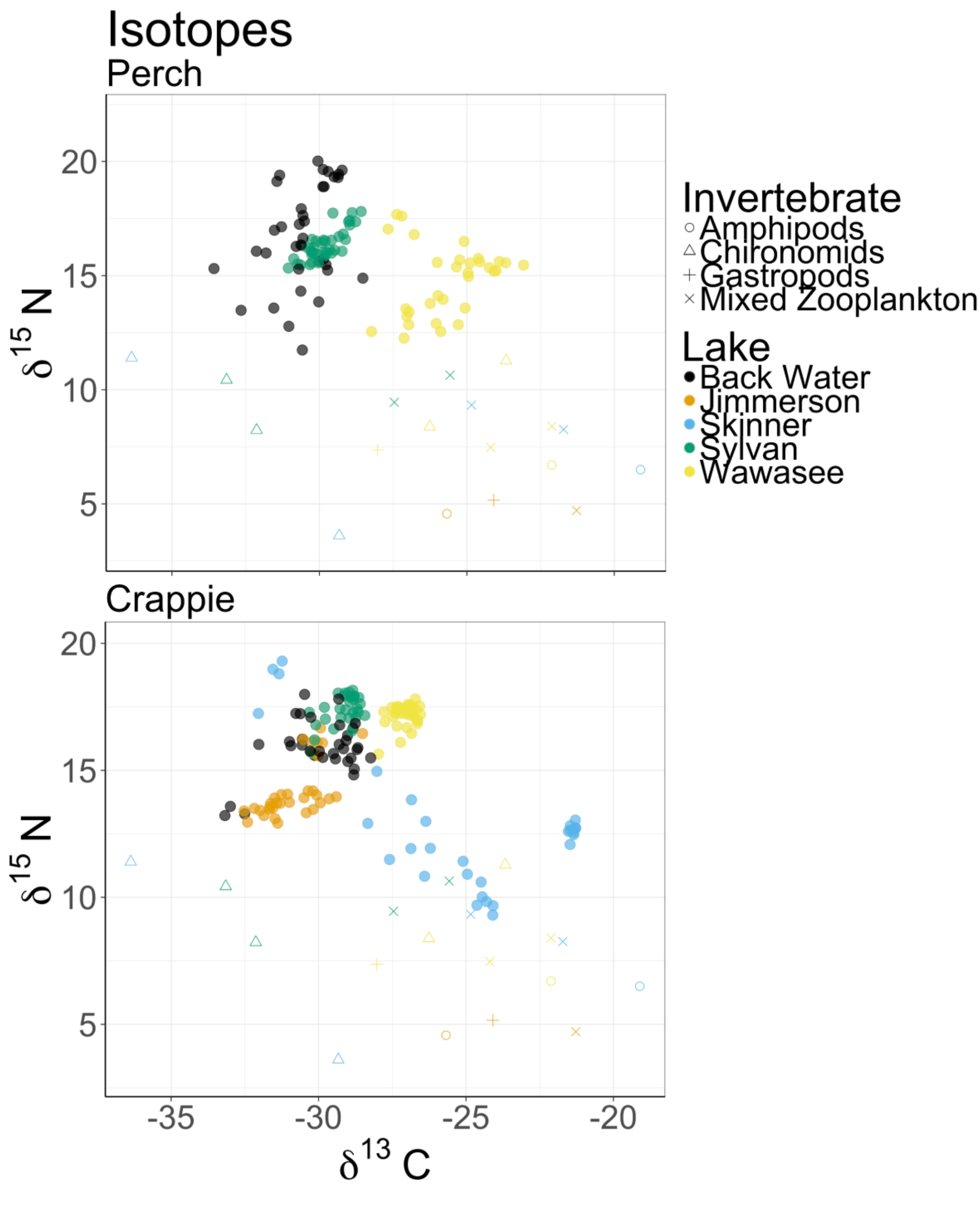


Figure 3. Isotope ratios for yellow perch (top) and black crappie (bottom). Carbon ratios represent lipid corrected carbon ratios.

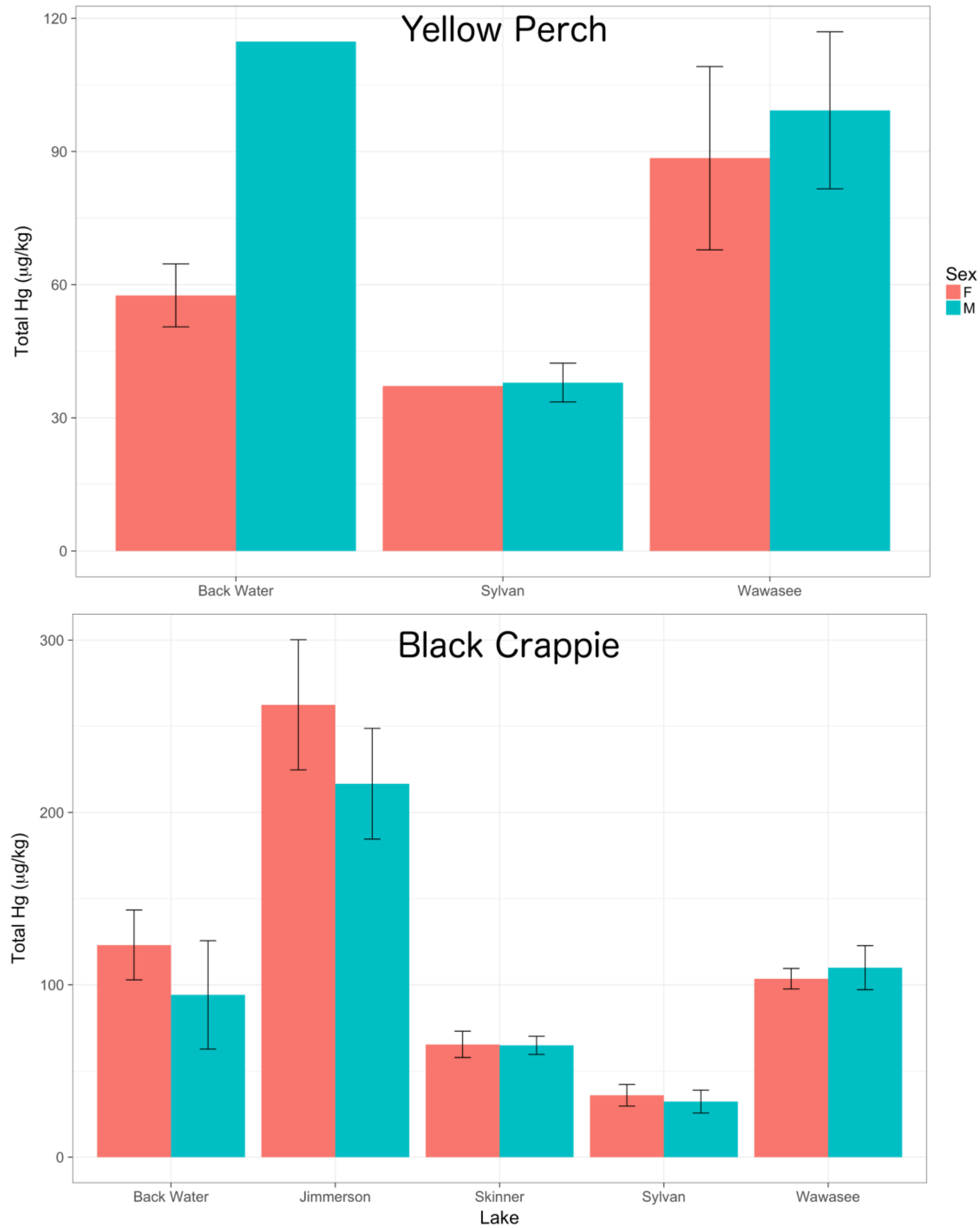


Figure 4. Mean with 95% confidence interval of total mercury for male (blue) and female (red) fish in yellow perch (top) and black crappie (bottom) populations. Note, male yellow perch in Backwater and female yellow perch in Sylvan are missing error bars since only a single representative of this sex was collected in 2016.

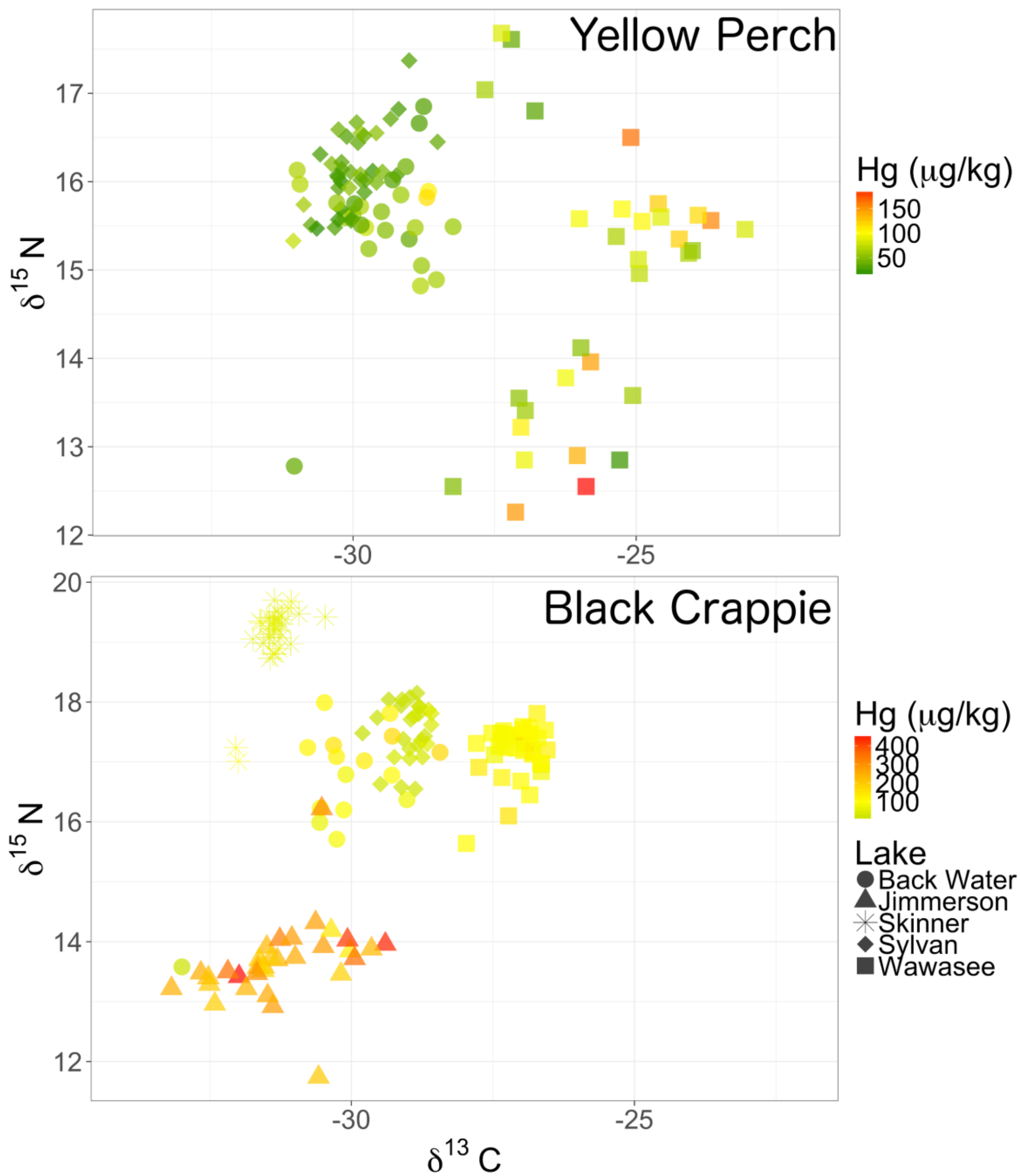


Figure 5. Isotope ratios for yellow perch (top) and black crappie (bottom) where individual fish have been color coded to represent total Hg levels measured in the fish[1]. Carbon isotope ratios represent lipid corrected values.

[1]Total mercury colors have been set to represent species separately.

Water and nutrient recovery from aquaculture effluents through vegetable production

Basic Information

Title:	Water and nutrient recovery from aquaculture effluents through vegetable production
Project Number:	2016IN394B
Start Date:	3/1/2017
End Date:	12/31/2018
Funding Source:	104B
Congressional District:	IN-004
Research Category:	Not Applicable
Focus Categories:	Agriculture, Conservation, Irrigation
Descriptors:	None
Principal Investigators:	Hye-Ji Kim, Paul Brown, Cary Mitchell, Robert Rode

Publications

1. Yang, T. and H.J. Kim. 2016. Effect of Plant Species on Waste Water Recycle in Aquaponics. American Society for Horticultural Science, Atlanta, GA. August 7-11, 2016 (poster presentation).
2. Yang, T. and H.J. Kim. 2016. Comparison of Tomato Production between Aquaponics and Hydroponics. American Society for Horticultural Science, Atlanta, GA. August 7-11, 2016 (poster presentation).
3. Yang, T. and H.J. Kim. 2016. Effect of plant species on nitrogen and phosphorus recovery from aquaculture effluents. HLA Research Retreat. Four Points. May 9, 2016 (poster presentation).
4. Yang, T. and H.J. Kim. 2016. Conservation of aquaculture wastewater and nutrients through vegetable crop production. Agroenviron 2016: 10th International Symposium on Agriculture and the Environment. May 23-27, 2016 (poster presentation).
5. Wongkiew, S., B.N. Popp, H.J. Kim, and S.K. Khanal. 201X. Fate of nitrogen in floating-raft aquaponic systems using natural abundance nitrogen isotope. International Biodeterioration & Biodegradation (Under review)
6. Kim. H.J. and Yang, T. 2017. tentative title: N and P use efficiency of an aquaponics system (in preparation)
7. Yang, T. and H.J. Kim. 201X. tentative title: Comparison of aquaponics and hydroponics on the production of tomato, basil and lettuce (in preparation)

Indiana Water Resources Research Center Project Report

Report 2017 Program Report Format

Project Id: 2016IN394B

Title: Water and nutrient recovery from aquaculture effluents through vegetable production

Project Type: Research

Period: March 1, 2017 – February 28, 2018

Congressional District: IN-004

Focus Categories: AG, COV, IG, NC, NU, WW, WS, WU

Keywords: Nitrogen, Phosphorus, Eutrophication, Aquaponics, Wastewater, Water Reuse, Sustainability, Food Security

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Abstract / Summary

As an integrated food production system that links aquaculture with hydroponic crop production in a recirculating ecosystem, aquaponics holds great promise for helping to ameliorate multiple global challenges through efficient use of resources for crop production. We found that N and P use efficiency of aquaponics is higher than that of hydroponics, but the efficiency of both systems is still low due to high nutrient inputs and high N loss through denitrification. We are working on optimizing the system by modifying multiple parameters including crop species, fish species, water flow rate, nutrient management (fish feed), and physical and chemical parameters of recirculating water to optimize the system. This research will significantly contribute to the enhancement of system efficiency and reduction of environmental wastes.

Problem:

Aquaculture systems generate considerable amounts of wastewater containing compounds such as nitrogen (N), phosphorus (P), and suspended solids (Cripps et al, 2000), and therefore, the large-scale application of aquaculture is restricted by land and water utilization as well as by environmental concerns (Islam, 2005). Aquaponics is an integrated system that links aquaculture with hydroponic production in a recirculating ecosystem by converting aquaculture wastewater into plant nutrients (Tyson et al., 2011). Aquaponic systems recycle more than 98% of their water (Al-Hafedh et al., 2008) and dramatically reduce discharge of nutrient-rich wastewater to the environment, with greater potential for profitability by simultaneously producing two cash crops. Since this is a soilless culture, many of the disease issues associated with conventional soil-based farming are not a concern. Although it is in its infancy, aquaponic food production has enormous potential to be the most efficient and space-saving method of farming, and to become the next green revolution to provide sustainable solutions for the nexus across water, energy, land and food.

The addition of feed is the main source of nutrient input and indirect production of pollutants in aquaculture-generated wastewaters. Approximately about 25% of the nitrogen input is converted into fish biomass, and over 70% is excreted into the aqueous phase in the form of total ammonia nitrogen (TAN; NH_3 and NH_4^+) (Hargreaves, 1998). Nitrifying bacteria in aquaponic systems oxidize ammonium (NH_4^+) to nitrite (NO_2^-) and further convert it into nitrate (NO_3^-). Plants uptake NO_3^- and NH_4^+ to produce biomass as nitrogen is required in the highest amounts by plants, and NO_3^- is typically the preferred form of nitrogen (Marschner, 2012). Since fish feed is one of the most significant costs in operating an aquaponic system (Broughton and Quagrainie, 2013) and the release of nutrient-rich wastewater effluents can lead to eutrophication and other environmental concerns, *it is essential to effectively remove N and P from aquaculture wastewater effluents through crop production and to improve nitrogen and phosphorus use efficiency within the system.* Most plants have the capability of absorbing and assimilating both nitrate and ammonium ions; but the physiological responses to these ions can vary greatly among plant species (Kraiser et al., 2011). Although the effects of nitrogen form on plant growth have been demonstrated in other production systems, information on *nutrient flows through the continuum of feed, nutrients in aquaculture wastewater, and plant yield in aquaponic systems is lacking.* Different plant species display different shoot and root morphological and

Fig. 1. *Aquaponics systems in the Purdue HORT greenhouse complex.*

physiological characteristics, and therefore, some vegetables with higher growth rate of edible parts, and/or enhanced root characteristics may more effectively recover N and P from wastewater effluents for biomass production, while significantly reducing the release of these elements into the environment.

Research Objectives:

The ***specific objectives*** of the proposed research are to:

Objective 1: Investigate N and P removal and recovery from fish wastewater as affected by vegetable production.
Objective 2: Evaluate N and P use efficiency of vegetable crops from wastewater effluents.
Objective 3: Optimize mass balance of N and P in aquaponics through proper combinations of aquatic animals and plant species.

Methodology

Studies have been conducted since 2015 with aquaponics systems established in the Purdue greenhouse complex (Fig. 1). Tilapia (*Oreochromis niloticus*) at a stocking density of 20-25 kg/m^3 were fed commercial fish-feed (41% protein and 1.1% P) recommended for tilapia-based aquaponics systems at about 1% of their weight daily. The pH, dissolved oxygen (DO), and electrical conductivity (EC; total soluble salts (TSS)) of aqueous phase were monitored on a regular basis.

Objective 1: Three model crops, lettuce, basil, and tomato, were chosen in this study for their differences in edible parts as well as morphological and physiological functions. The crops were separately grown in tilapia-based aquaponics and compared with the ones in hydroponics controls. Water quality parameters were monitored as stated above, and plant growth parameters (plant fresh weight, height, leaf number and length, and fresh weight) were recorded weekly. At harvest, the growth parameters were measured again, and plant tissues were separately harvested and processed for nutrient analysis. Water was sampled throughout production period for nutrient analysis using an ion chromatography.

Objective 2: At the end of the production cycle, plant parts were separately harvested, and fresh and dry weights were determined. The initial and final fresh weights of fish was also determined. Total N in samples of fish, plants, fish-feed, and suspended solids (microbial biomass) was determined by LECO TruSpec C/N analyzer (LECO Corp., St. Joseph, MI). Samples of fish, plants, fish-feed, and suspended solids (microbial biomass) were dried, processed, and their total P content was determined by persulfate digestion procedure (Hach Permachem Reagent, HACH, Loveland, CO). N and P use efficiency was calculated based on the ratio of nutrient uptake (nutrient contents in fish and plant tissue) and nutrient input (fish feed).

Objective 3: Vegetable crops and fish species will be grown in aquaponics systems at the selected stocking density. Comparative analysis of productivity potentials will be conducted to compare the type of vegetables, type of aquatic animals, and/or type of feed. The yield and quality of vegetables, water consumption, and other relevant process variables will be measured.

Results

Objective 1&2:

We found that the EC continuously increases in the effluents, with significant accumulation of soluble salts including nitrates (NO_3^-) and phosphates (PO_4^{3-}) (Fig. 3). Variation for N and P acquisition of plant species has been observed (Yang and Kim, 2016), as shown by changes in differential recovery of nutrients from aquaculture effluents associated with their rapid biomass production (Table 1).

Although closed-loop systems are known to be more efficient in water and nutrient use than open-loop or conventional field production systems, our studies demonstrate the current N and P levels used recirculating hydroponic and aquaponic systems are not sustainable, and generate significant amount of environmental wastes (Fig. 4.). We also found that plants have different ability to recover N and P from the wastewaters, with the highest degree of recovery in tomato, and the lowest degree in lettuce, and that plant biomass production, growth rate, and harvest method are associated with N and P utilization capacity to effectively sequester these pollutants from wastewaters, resulting in variations in N- and P-use efficiencies of an aquaponics system (Table 1). Our research provided evidence that N is more effectively used in aquaponics compared to hydroponics, but the efficiency of both systems is still low due to high nutrient inputs and high N loss through denitrification (Fig. 4). In an aquaponics environment where P diffusion is not an issue, major contributors for resource recovery are higher biomass production rate, and a larger sink (shoot) for extra N and P assimilation. It should be noted that despite the continued recovery of N and P through biomass production of plant species, nitrate accumulated over time in aquaponics, while phosphate gradually reached and maintained averagely at 10 mg/L. While this concentration is considered very low in commmercial hydroponic system, PI proved that this is sufficient for crop production.

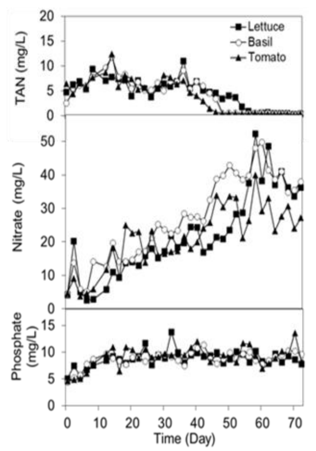

Fig. 3. *Variation of N and P concentrations in an aquaponic system as influenced by plant species.*

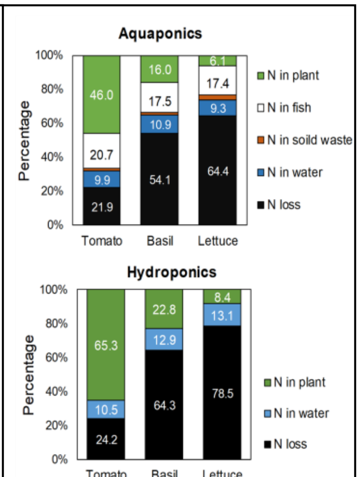

Fig. 4. *N budgets of the major crops grown in aquaponics and hydroponics.*

Objective 3: We found that higher water flow rate (3000 L/day) in tilapia-based aquaponics creates better initial water quality (lower pH, lower NH_4^+ and NO_2^- concentrations, lower water temperature) for crop growth. Medium-flow-rate is suitable for fast-growing crops without decreasing yield, while high-flow-rate is

Table 1. *Comparisons of aquaponics and hydroponics in yield and N- and P-use efficiency.*

Plant crop	Production system	Yield (g FW) / 3 months			N use efficiency (%)		P use efficiency (%)	
		Marketable Part	Unmarketable Parts		Plant	Plant + fish	Plant	Plant + fish
Tomato	Aquaponics	152 a	868 b	512 a	13.5	18.6	30.5	42.01
	Hydroponics	115 a	1661 a	338 a	14	14.0	46.1	46.1
Basil	Aquaponics	233 b	–	124 b	2.5	7.0	5.6	15.9
	Hydroponics	287 a	–	170 a	4.3	4.3	14.0	14.0
Lettuce	Aquaponics	121 b	–	24 b	1.3	5.8	2.9	13.0
	Hydroponics	197 a	–	31 a	3.0	3.0	9.7	9.7

suitable for slow-growing crops. Flow rate does not have any effect on the growth performance of medium-growing crops.

We have received new sets of tilapia (warm-water fish) and trout (cold-water fish) and currently performing research trials in combination with warm-season crops and cool-season crops. With that, it is expected that the research project will be completed by the end of this year.

Major Conclusions and Significance

- N and P use efficiency was higher in aquaponics compared to hydroponics, but the efficiency of both systems is still low due to high nutrient inputs and high N loss through denitrification. We will modify physical and chemical parameters to optimize the system, which will significantly enhance the system efficiency and reduce environmental wastes.
- Aquaponics system can be more energy-efficient and yield higher productivity by setting it at a proper flow rate depending on crop species.

Publications

Papers

1. Wongkiew, S., B.N. Popp, **H.J. Kim**, and S.K. Khanal. 2018. Fate of nitrogen in floating-raft aquaponic systems using natural abundance nitrogen isotope (submitted to International Biodeterioration & Biodegradation)
2. Quagrainie, K.K., R.M.V.Flores[*†], **H.J. Kim**, and V. McClain[†]. 2017. Economic analysis of aquaponics and hydroponics production in the U.S. Midwest. Journal of Applied Aquaculture 30 (1):1-14.
3. Yang, T[†]. and **H.J. Kim*.** 2018. Comparison of aquaponics and hydroponics on the production of tomato, basil and lettuce. (will be submitted in August)
4. Yang, T[†]. and **H.J. Kim*.** 2018. tentative title: N and P removal and recovery in an aquaponics system. (will be submitted in October)

Abstracts

1. **Kim, H.J.** and T. Yang[†]. 2017. Nutrient use efficiency in aquaponics and hydroponics. HortScience 52(9):S232.
2. Yang, T[†]. and **H.J. Kim.** 2017. Growth and Yield of Vegetables Grown in Aquaponics in Comparison to Hydroponics. HortScience 52(9):S202.
3. Yang, T[†]. and **H.J. Kim.** 2017. Growth and productivity of vegetable crops as affected by nutrient management practices in an aquaponics system. HortScience 52(9):S344.
4. **Kim, H.J.** and T. Yang. 2017. Effects of plant species on nitrogen and phosphorus removal in aquaponic system. Aquaculture America 2017 Abstracts, p. XX. San Antonio TX.
5. **Kim, H.J.** and T. Yang. 2017. Growth and yield comparison of lettuce, basil, and tomato grown in aquaponics and hydroponics systems. Aquaculture America 2017 Abstracts, p. XX. San Antonio TX.

Presentations

1. •**Kim, H.J.** 2017. Aquaponics basics and promoting health tourism in Okinawa. University of the Ryukus, Okinawa, Japan. December 19, 2017 (open only to selected audience)

2. •**Kim, H.J.** 2017. Enhancing resource efficiency for crop productivity and quality. Department of Horticulture, South China Agricultural University. December 27, 2017. (invited seminar speaker, n=40)
3. •**Kim, H.J.** 2017. Smart Farming: Enhancing resource efficiency for crop productivity and quality. Department of Horticulture and Landscape Architecture 2017 Fall Seminar Series, Purdue University, West Lafayette, IN. October 27, 2017. (invited seminar speaker, n=40)
4. •**Kim, H.J.** and T. Yang†. 2017. Nutrient use efficiency in aquaponics and hydroponics. American Society for Horticultural Science, Waikoloa, HI. September 19-22, 2017 (oral presentation).
5. •Yang, T†. and **H.J. Kim.** 2017. Growth and Yield of Vegetables Grown in Aquaponics in Comparison to Hydroponics. American Society for Horticultural Science, Waikoloa, HI. September 19-22, 2017 (oral presentation).
6. •Yang, T†. and **H.J. Kim.** 2017. Growth and productivity of vegetable crops as affected by nutrient management practices in an aquaponics system. American Society for Horticultural Science, Waikoloa, HI. September 19-22, 2017 (poster presentation).
7. •**Kim, H.J.** and T. Yang. 2017. Effects of plant species on nitrogen and phosphorus removal in aquaponic system. Aquaculture America 2017 Abstracts, p. XX. San Antonio TX. February 21, 2107(oral presentation).
8. •**Kim, H.J.** and T. Yang. 2017. Growth and yield comparison of vegetables grown in aquaponics and hydroponics systems. Aquaculture America 2017. San Antonio TX. February 21, 2107(oral presentation).

Grant Submissions

External Grants Submitted

- **Kim, H.J. (PI),** Z. Zhou, and V. Aggarwal (6/12018-5/30/2021) WERF, WRF: Nutrient recovery for energy-efficient food production in aquaponics systems. Amount requested: $330,000. Submitted to National Science Foundation. (not funded)
- Brown, P. and **H.J. Kim (Co-PI).** (1/1/2018-12/31/2020). Water efficient food production systems for Egypt and U.S. Amount requested: $ 199,753.11. Submitted to the U.S.-Egypt Science and Technology Joint Fund. (not funded)

Internal Grant

- **Kim, H.J. (PI),** A.Deering, and K. Quagrainie (5/1/2018-4/30/2019) Healthy Food, Healthy Environment: Developing best management practices for Hydroponic and Bioponic crop production systems in Indiana, Purdue University, College of Agriculture, AgSEED Program (funded)

Students

Undergraduate: 7

Masters: 0

PhD student: 2

PostDoc:0

Visiting scholar: 2

Nutrient removal efficiency of a combined surface/subsurface flow wetland system

Basic Information

Title:	Nutrient removal efficiency of a combined surface/subsurface flow wetland system
Project Number:	2016IN396B
Start Date:	3/1/2016
End Date:	2/28/2018
Funding Source:	104B
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Focus Categories:	Wetlands, Nutrients, Water Quality
Descriptors:	None
Principal Investigators:	Pierre-Andre Jacinthe

Publication

1. Elias D., Wang L., Jacinthe P.A. 2018. A meta-analysis of pesticide loss in runoff under conventional tillage and no-till management. Environmental Monitoring and Assessment 190: 79. https://doi.org/10.1007/s10661-017-6441-1.

Indiana Water Resources Research Center Project Report

Report 2017 Program Report Format

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Title: Nutrient removal efficiency of a combined surface/subsurface flow wetland system

Project Type: Research

Period: March 1, 2017 – February 28, 2018

Congressional District: IN-007

Focus Categories: Wetlands, Nutrients, Water Quality

Keywords: constructed wetlands, edge of field, nitrate, SRP, greenhouse gas

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Abstract / Summary

Constructed wetlands located downslope from agricultural fields can intercept nutrients-laden runoff and tile waters, thereby contributing to water quality improvement in agricultural watersheds. However, wet soil conditions can lead to enhanced emission of greenhouse gases (GHG), and that could offset the water quality benefits of constructed wetlands. Thus, there is a need to determine conditions under which optimum nutrient removal and minimum GHG emission can be achieved.

Treatment wetlands are constructed using different design concepts, including free water surface (FWS), horizontal subsurface flow (HSSF), and vertical subsurface flow (VSSF) wetlands. At the present, it is unclear how design parameters could affect wetland systems performance. To address this question, a qualitative analysis was conducted using data from peer-reviewed articles stemming from studies conducted in natural and constructed wetlands. We collected peer-reviewed studies, conducted in both natural and constructed wetlands, that reported nutrient removal and GHG emission (CO_2, CH_4, N_2O).

Results showed a clear influence of the dominant vegetation community on both nutrient retention and GHG emission from wetlands. Compared to other macrophytes, we observed higher NH_4 and lower P removal in wetlands dominated by *Canna indica*. Emission of CO_2 was 1.5-fold higher in vegetated than in non-vegetated wetlands. Under low-water table conditions, wetlands acted as CH_4 sinks, but emitted N_2O at rates sometimes 100-fold higher than under high-water table, suggesting complete denitrification (reduction of N_2O to N_2) under water-saturated conditions. Nutrient retention and GHG emission varied with wetland type as determined by water flow path. In general, N and P removal was higher in FWS wetlands than in other systems. N removal was also higher in horizontal subsurface flow (HSSF) than in vertical subsurface flow (VSSF) wetlands. A similar trend was observed regarding CH_4 emission (HSSF>VSSF) but the opposite was noted for N_2O emission (HSSF<VSSF). FSW had higher CH_4 and N_2O emission than the other types of constructed wetlands (either VSSF or HSSF) and natural wetlands. CO_2 emission was highest in natural wetlands, followed by VSSF, HSSF, and FSW.

Information gathered in this synthesis should inform the design of constructed wetlands to optimize nutrient removal efficiency and address air quality concerns. Specifically, our results suggest that: (i) when seeding constructed wetlands, plant species can be selected to optimize nutrient removal and minimize GHG emission, and (ii) the FWS wetlands might be the most cost-effective option as they provide similar environmental benefits as the more complex and expensive subsurface flow systems.

Problem:

Agricultural intensification has led to increased export of nitrogen (N) and phosphorus (P) from agricultural fields into streams, rivers, and lakes resulting in impairment of surface waters. In the Mississippi Basin, the export of nutrients from tile-drained US Midwest croplands is blamed water quality degradation observed both within and outside the region. The US Environmental Protection Agency (EPA) has established a maximum concentration limit of 10 mg N L^{-1} for nitrate (NO_3^-) in drinking water. This limit is often exceeded in rivers and streams, especially after rainfall events following fertilizer application to crop fields. In Indiana, ~47% of streams assessed in 2016 were considered impaired (IDEM, 2016) due to elevated nutrients concentration (total phosphorus > 0.3 mg P L^{-1}; nitrate >10 mg NO_3-N L^{-1}), although other factors (e.g. pesticides, low dissolved oxygen, high E. coli load) have also contributed to that designation. These nutrient-enriched aquatic ecosystems often experience algal blooms with the subsequent decomposition of algal biomass resulting in hypoxia (oxygen-depleted waters; < 2 mg O_2 L^{-1}) and fish kills. Further, the Mississippi and the Atchafalaya river systems contribute an estimated 91% of total N delivered to the Gulf of Mexico (Rabalais et al., 2002). Therefore, the Corn Belt (Ohio, Indiana, Illinois, Iowa) has been identified as the main contributor to the expanding (doubling in size in 10 years) hypoxic zone or dead zone in the Gulf of Mexico (Dale et al., 2010).

Because of its climate and generally flat landscape, nearly half of all croplands in the Midwest is equipped with subsurface tile drainage that allows for rapid removal of excess moisture and timely implementation of farming operations. It is well recognized that tile drainage installation accelerates the flux of water and nutrients from croplands. The interception of agricultural runoff and tile-drainage discharge in wetlands (natural or constructed) strategically located downslope of cultivated fields could help reduce nutrient export and mitigate these water quality challenges. A constructed wetland is a setting where plant nutrients and other agricultural pollutants can be retained through physico-chemical reactions, used by emergent vegetation and transformed by soil microbes. Wetlands are transitional lands between terrestrial and aquatic systems, and can be connected to either surface water sources or a shallow groundwater system. Natural wetlands have well-documented ecological functions including wildlife habitats, groundwater recharge, water retention and flood control, sediment stabilization, carbon sequestration and nutrients removal (Woodward and Yong-Suhk, 2001). Constructed wetlands have received attention as a cost-effective approach to deal with diffuse pollution in the environment. They are designed to reproduce the biogeochemical processes occurring in natural wetlands and create optimum conditions for the biotransformation of pollutants into harmless products. Numerous studies have examined the effectiveness of constructed wetlands to retain and transform nutrients (e.g. Kovacic et al., 2000; Poe et al., 2003). Nitrogen and phosphorus removal rates can be variable but, in some cases, rates as high as 98% and 96% have been reported (Hunt and Poach, 2001; Newman et al., 1999; Schaafsma et al., 1999). Nutrient loading, water temperature, flow regime and wetland age are key controlling factors of that variability.

Constructed wetlands engineered systems designed to reproduce biogeochemical conditions and processes observed in natural wetlands, and create controlled environments for successful treatment of nutrient-laden agricultural runoff. Constructed wetlands reported in the literature are based on various design concepts, but the free water surface (FWS), the horizontal subsurface flow (HSSF) and the vertical subsurface flow (VSSF) wetland are the most common types. The FWS typically consists of a basin that may or may not support an emergent vegetation community, and can be either a stagnant or a flow-through system. In contrast to the FWS system in which water is open to the atmosphere, the subsurface flow systems (Fig. 1) consist of porous media (sand, gravel, soil, bark mulch…) through which water flows underground either vertically (VSSF) or horizontally (HSSF).

While the water-quality protection benefits of wetlands (both natural and constructed) are widely reported, there is also a paucity of data regarding their impact on air quality, specifically emission from wetlands of the greenhouse gases (GHG) carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4). These trace gases play important roles in the chemistry and energy balance of the earth's atmosphere. In addition, N_2O and CH_4 participate in stratospheric ozone depletion. Atmospheric concentration of these gases has steadily increased during the last 150 years (IPCC, 2013). Water saturation leads to O_2 exclusion from soil pore space and development of suboxic conditions in wetland soils. These conditions could result in enhanced production of N_2O (via denitrification) and CH_4 (via methanogenesis). Enhanced transfer of GHG from wetlands into the atmosphere is a legitimate concern since such emissions could offset the water quality benefits provided by these ecosystems. Therefore, information regarding GHG dynamics is an important component of our understanding of wetland ecosystems. Thus, there is a need to determine the environmental conditions and design options that result in efficient nutrient removal and at the same time minimize GHG emission from wetlands.

Research Objectives:

Most past studies have focused on wetlands efficiency as measured by nutrient removal. While some studies have examined the intensity of GHG exchange with the atmosphere, there has been to our knowledge no previous attempt to concurrently evaluate the impact of wetland design on nutrient removal and GHG emission from constructed wetlands. Here, we use a meta-analysis approach to address this information gap. The objective of this study was to identify the conditions for optimal nutrient removal and mitigation of GHG emissions in natural and constructed wetlands.

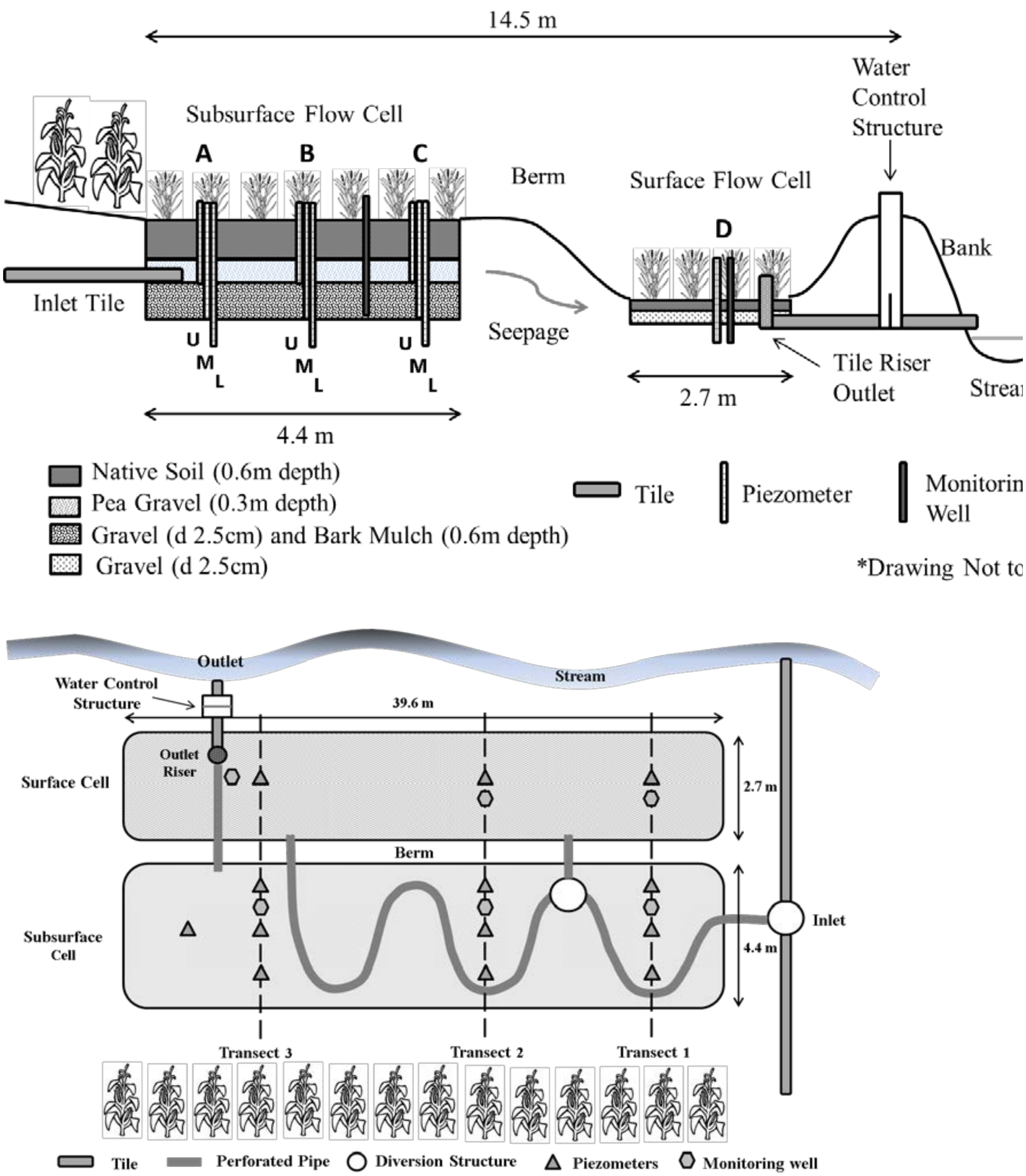

Fig. 1. Cross-sectional view (top) of a constructed wetland with two compartments – an horizontal subsurface flow (HSSF) and the free surface water flow (FSW) compartment. The top diagram shows the different layers of through which water flows in the HSSF. The bottom diagram is a top-down view of the wetland showing water flow directions in the HSSF compartment (water transported through serpentine-like perforated tube) and in the FSW compartment. Location of piezometers and monitoring wells is also indicated. This wetland is constructed downslope from an actively-managed agricultural field at Starkey Farms (Brownsburg, IN).

Methodology

A literature survey of peer-reviewed journal articles about natural and constructed wetlands, nutrient removal, and GHG emissions (CO_2, CH_4, N_2O) was conducted using the Web of Science and Google Scholar databases. Peer-reviewed articles published in English from 1977 to 2017 were collected (N = 100). Studies that did not provide results on nitrate, nitrite, ammonium, ammonia, total nitrogen, total phosphorus, phosphate retention or greenhouse gases emissions were excluded, narrowing the number of available studies to 40 (Table 1). Data collected focused on the factors and conditions associated with nutrient removal as well as GHG emission rates. Wetland conditions included type of wetland (e.g. FSW, subsurface flow), dominant emergent vegetation (e.g. *Typha angustifolia, Zizania latifolia*), and environmental conditions (i.e. temperature, organic matter, water table). Below is a synthesis of our analysis of the data collected.

Results

Results (Fig. 2) showed that nutrient removal was generally higher in vegetated than in non-vegetated wetlands (e.g. NO_3: 14% vs. 11%; Total P: 50% vs. 33%). Nutrient retention was also influenced by the dominant vegetation occurring in a wetland, with *Canna indica* and *Stenotapharum secundatum* exhibiting higher ammonia removal (10%) than *Typha latifolia* (6.8%) and *Phragmites australis* (9.1%) but lower total P removal (*C. indica*, 4%; *S. secundatum*, 8%) than *T. latifolia* (12%) and *P. australis* (16%). Greenhouse gases emission was positively affected by presence and type of plants. On average, GHG emission was 1.6 times higher in vegetated than in non-vegetated wetlands (Fig 3).

Environmental and hydrological conditions influenced nutrient retention and GHG emission from wetlands. Specifically, total N removal increased with increasing organic matter content. In wetlands with high water table, we observed significantly higher rates of CH_4 emission (28 times) but lower rates of N_2O emission (126 times) than in wetlands with low water table. Further, in accordance with general trends in water temperatures, emission rates of CH_4, N_2O and C_2O were consistently higher in summer relative to spring, fall, and winter.

Wetland type, as determined by design and water flow path, influenced nutrient retention. Horizontal subsurface flow wetlands (HSSF) had higher total N removal than vertical subsurface flow wetlands (VSSF). In contrast, VSSF had higher total P removal than HSSF (Fig. 2). Free surface water wetlands (FSW) had higher total N removal than subsurface flow (HSSF and VSSF) wetlands and natural (Fig. 2). GHG emission was also influenced by wetland type. We observed that FSW had higher CH_4, and N_2O emissions than subsurface flow (VSSF, HSSF) and natural wetlands (Fig.3). Among the subsurface flow wetlands, N_2O emission was generally higher in VSSF than in HSSF systems. A contrasting was observed with regard to CH_4 with emission rates generally higher in HSSF relative to VSSF wetlands. Emission of CO_2 was highest in natural wetlands, followed by VSSF, HSSF, and FSW.

Major Conclusions and Significance

- Although nutrients in agricultural runoff can be successfully removed in constructed wetlands, GHG emission cannot be ignored. The intensity of GHG emission was found to vary with the dominant vegetation community. Although much remains to be learned about the coupling of vegetation community structure and GHG production/transport in wetlands, this finding suggests that it might be possible to select different plant species combinations to optimize nutrient removal and minimize GHG emission from constructed wetlands.

- Selection of wetlands design can have a definitive impact on system performance as measured by high nutrient removal and low GHG emission. More efficient N and P removal was achieved with the FWS wetlands (Fig. 2) than with either of the subsurface flow systems (HSSF and VSSF). Although GHG emission was generally greater in the FWS wetlands than in the other types, difference was minimal and likely not significant (Fig. 3). Taken together, these results suggest that both water quality and air quality objectives can be best achieved with the FWS design. This observation is significant, and specifically means that the FWS design can provide similar environmental benefits as the more complex and expensive subsurface flow systems. These results are certainly intriguing, and therefore warrants further site-specific investigation.

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Table 1. Peer-reviewed research articles included in the meta-analysis. Abbreviation: GHG = greenhouse gas.

Reference	Type of wetland	Location	Measurement
Barbera et al. 2014	constructed	Italy	GHG
Boyt et al. 1977	natural	Florida, US	Nutrients
Brix et al. 2001	natural	Denmark, Hungary, Czech Republic	GHG
Bubier and Moore 1994	natural	Minnesota, US	GHG
Calheiros et al. 2007	constructed	Portugal	Nutrients
Sim et al. 2008	constructed	Malaysia	Nutrients
Chiemchaisiri et al. 2009	constructed	Thailand	GHG
Christense et al. 2003	natural	Iceland, Siberia	GHG
Clair et al. 2002	natural	Canada	GHG, nutrient
Van der Gon 1995	natural	Phillipines	GHG
Gersberg et al. 1983	constructed	California, US	Nutrients
Gottschall et al. 2007	constructed	Canada	Nutrients
Hu et al. 2010	natural	China	Nutrients
Inamori et al. 2007	constructed	Japan	GHG
Jahangir et al. 2016	constructed	Estonia, Finland, Norway	GHG
Jordan et al. 2003	restored	Maryland, US	Nutrients
Lee et al. 2009	constructed	multiple locations, review	Nutrients
Liikanen et al. 2006	constructed	Finland	GHG
Liu et al. 2009	constructed	Japan	GHG
Maltais-Landry et al. 2009	constructed	Canada	GHG
Mander et al. 2014	constructed	multiple locations, review	GHG
Maucieri et al. 2017	constructed	multiple location, review	GHG
Morse et al. 2012	natural	North Carolina, US	GHG
Picard et al. 2005	natural	Ohio, US	Nutrients
Schrier-Ujil et al. 2011	natural	Netherlands	GHG
Sha et al. 2011	natural	Ohio, US	GHG
Silvan et al. 2004	constructed	Finland	Nutrients
Sovik and Klove 2007	constructed	Norway	GHG
Sovik et al. 2006	constructed	Norway	GHG
Strom et al. 2007	constructed	Sweden	GHG
Strom et al. 2003	natural	Sweden	GHG
Tai et al. 2002	constructed	China	GHG
Tang et al. 2009	constructed	China	Nutrients
Tao 2015	constructed	New York, US	GHG
Teiter and Mander 2005	constructed	Estonia	GHG
Vymazal 2007	constructed	NA	Nutrients
Wang et al. 2008a	constructed	Japan	GHG
Wang et al. 2008b	constructed	Japan	GHG
Wu et al. 2017	constructed	China	GHG
Lin et al 2002	constructed	Taiwan	Nutrients

Fig. 2. Mean nutrient removal (and standard deviation) expressed as percentage of nutrient input for different types of wetlands. Total N is the sum of total Kjeldahl nitrogen (ammonia, organic and reduced nitrogen) and nitrate. Total P is the sum of soluble reactive and organic P. Abbreviations: FWS = free water surface wetland; HSSF = horizontal subsurface flow wetland; VSSF = vertical subsurface flow wetland.

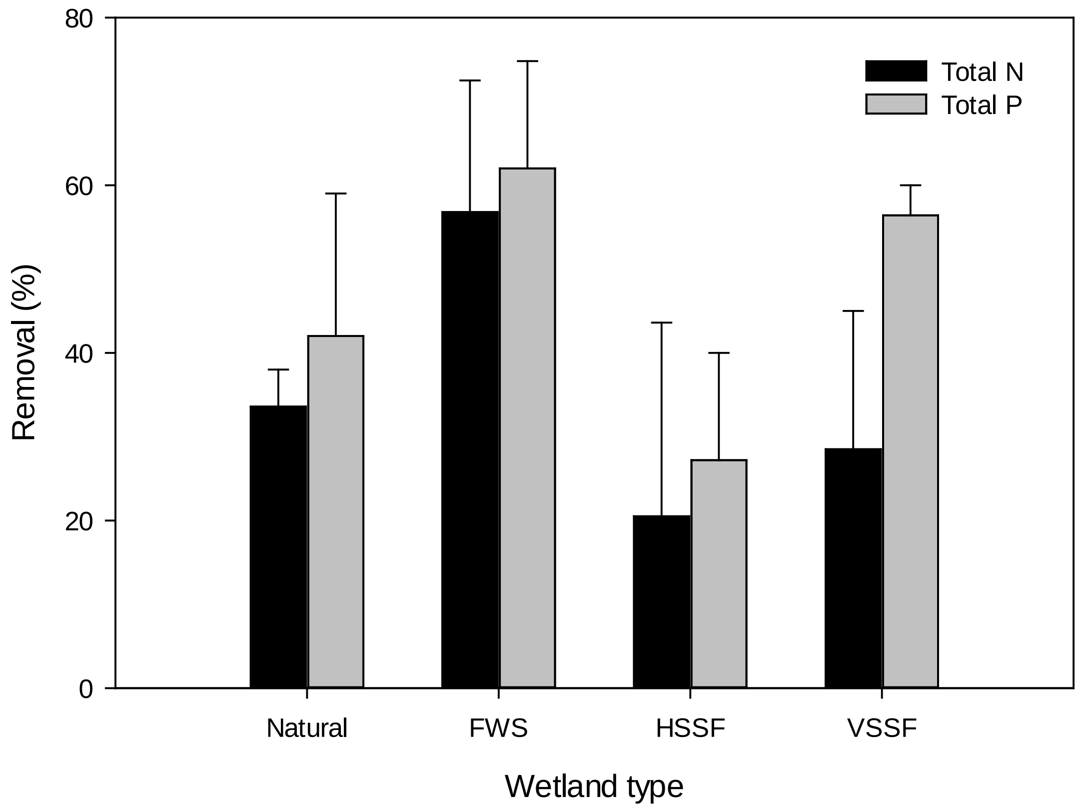
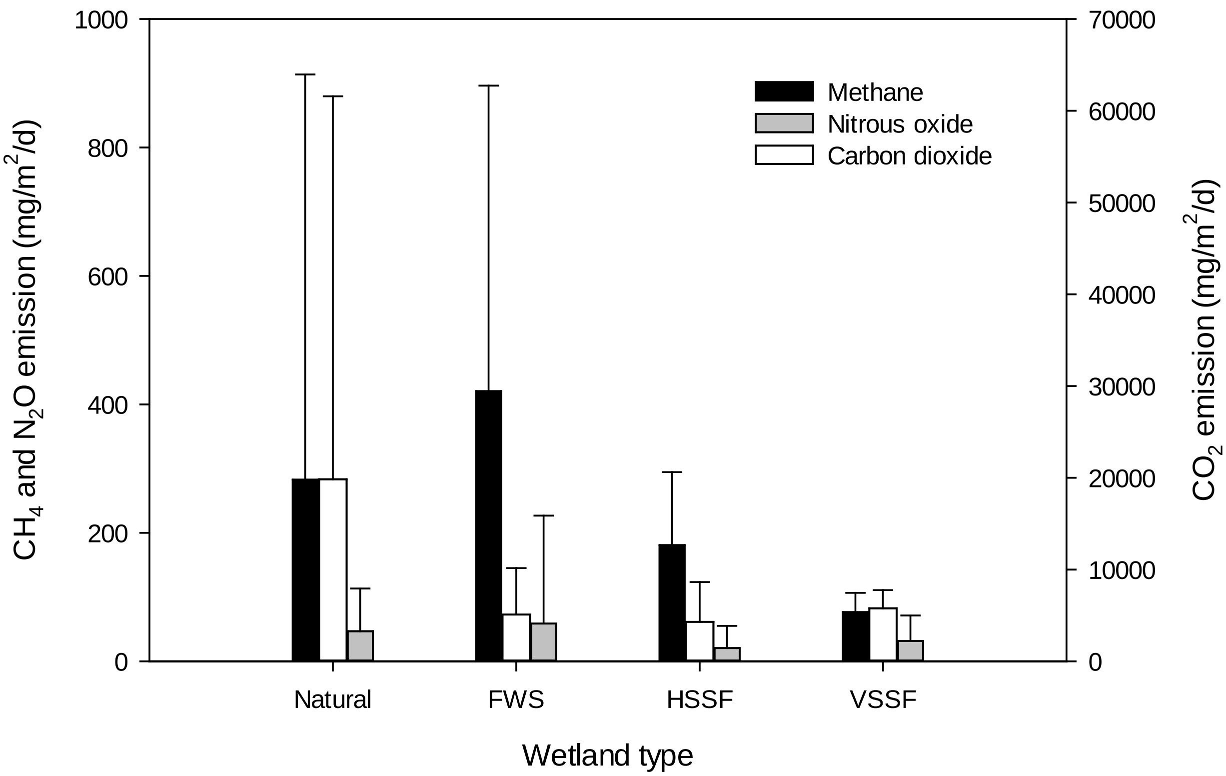


Fig. 3. Mean (standard deviation) emission of greenhouse gas (GHG) for different types of wetlands of methane. Gas fluxes are reported in units: CO_2-C mg/m^2/d, CH_4-C mg/m^2/d and N_2O-N mg/m^2/d. Abbreviations: FWS = free water surface wetland; HSSF = horizontal subsurface flow wetland; VSSF = vertical subsurface flow wetland.



Publication
Elias D., Wang L., Jacinthe P.A. 2018. A meta-analysis of pesticide loss in runoff under conventional tillage and no-till management. Environmental Monitoring and Assessment 190: 79. https://doi.org/10.1007/s10661-017-6441-1.

Presentation
Career Success Tour at Starkey Farms, October 20, 2016: On-site presentation/description of constructed wetland for high school students attending the "Future Farmers of America (FFA)" convention in Indianapolis. Attendance: 70.

Grant Submission:
Edge of Field Monitoring Program. USDA NRCS/ Starkey Farms Partnership. $268,866 (project funded through 2020 by NRCS; extension with USGS under consideration).

Postdoctoral researcher
- Daniel Elias

Students
The following students have contributed to the project:
- Graduate student (1): Danielle Follette
- Undergraduate students (3): Amanda Evans, Jonathan Jacinthe, Ashley Gunther

Effects of Land use Type on Abundance and Type of Microplastic Pollution- A Contaminant of Emerging Concern in Indiana Rivers

Basic Information

Title:	Effects of Land use Type on Abundance and Type of Microplastic Pollution- A Contaminant of Emerging Concern in Indiana Rivers
Project Number:	2017IN402B
Start Date:	3/1/2017
End Date:	2/28/2019
Funding Source:	104B
Congressional District:	IN-002
Research Category:	Water Quality
Focus Categories:	Wastewater, Water Quality, Ecology
Descriptors:	None
Principal Investigators:	Gary A. Lamberti

Publications

1. Hartlage, M.S., W.M. Conard, K.E. O'Reilly, and G.A. Lamberti. May 2018. Microplastic Distribution Above and Below Large Dams in Indiana, USA. Poster Presentation at Notre Dame College of Science Joint Annual Meeting, Notre Dame, Indiana.
2. Hartlage, M.S., W.M. Conard, K.E. O'Reilly, and G.A. Lamberti. May 2018. Microplastic Distribution Above and Below Large Dams in Indiana, USA. Poster Presentation at Annual Meeting of the Society for Freshwater Science, Detroit, Michigan.
3. O'Reilly, K.E., W. Conard, and G.A. Lamberti. October 2017. Microplastics in Aquatic Ecosystems: Tiny Plastics, Big Problem? Youtube Video. Available at: https://www.youtube.com/watch?v=n_O42Cs9sbg&index=8&list=PLuk3dGfI10k1g98NkBHHNEhDg_mAKdYiw

Indiana Water Resources Research Center Project Report

2017 Progress Report

Project ID: 2017IN402B

Title: Effects of land use type on abundance and type of microplastic pollution – a contaminant of emerging concern in Indiana rivers

Project Type: Research

Period: March 1, 2017 – February 28, 2018 (NCE to February 28, 2019)

Congressional District: IN-2

Focus Categories: Wastewaster (WW), Water Quality (WQL), Ecology (ECL), Surface Water (SW)

Keywords: Microplastics, Land Use, Ecotoxicology, Pollution, Rivers

Principal Investigators:

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Abstract / Summary:

Water quality degradation resulting from human activities represents a threat to environmental and human health. Contaminants of emerging concern, including microplastics (plastic particles <5 mm in size), are understudied in flowing waters of the Midwestern USA including in Indiana. Microplastics enter rivers and streams through a variety of anthropogenic pathways (e.g., wastewater effluent, breakdown of larger plastic debris, atmospheric deposition) and can negatively impact aquatic organisms through both direct consumption with food and indirect contamination from sorbed toxins. Due to their small size, removal of microplastics once introduced to aquatic ecosystems is impractical and thus source-tracking and input reduction are essential. We quantified the concentration and types (e.g., microbeads, fibers, fragments) of microplastics in rivers of 9 Indiana watersheds and 31 separate sites representing a gradient of land use (i.e., agricultural, urban, or forested). We hypothesized that, compared to forested systems, watersheds dominated by agricultural or urban land use would have higher concentrations but different forms of microplastics due to human activities. We also predicted that concentrations would increase with distance downstream. Preliminary results suggest that microplastic distribution is similar across land-use types and with longitudinal distance, with fibers being the dominant form present. Identifying the sources and types of microplastics in Indiana waters will provide valuable information for our state and is critical for the development of management actions for this emerging contaminant.

Problem:

The extent and level of microplastic pollution, an emerging contaminant, in Indiana waterways is unknown despite the potential for negative impacts on ecological and human systems.

Research Objectives:

The objectives of this study are to: (1) provide Indiana managers, researchers, and decision-makers with a first-ever baseline measurement of the concentration and types of microplastics in major Indiana waterways and (2) determine if microplastic concentration or type vary with land use and longitudinal position in the watershed. Ultimately, this project will help guide future monitoring and remediation of microplastic pollution.

Methodology:

Methods consist of four main steps: (1) site selection, (2) field sampling, (3) laboratory sample processing, and (4) data analysis and dissemination. As of February 2018, we have completed steps 1 and 2 and have completed a portion of step 3. We anticipate the completion of step 3 by October 2018 and step 4 by January 2019.

1. We **selected sites** for field sampling and obtained permission to access sites as necessary. Using GIS analysis, we selected three watersheds that well represented each of three land-use categories (forested, urban, agricultural). To do so, we used ArcGIS along with two datasets, the National Land Cover Database (NLCD) and watershed boundary data

(USGS). Within each of the watersheds identified, we chose to sample at three sample sites. The first sample site was always at the lower confluence of the watershed to effectively sample the entire watershed. The second sample site was in the middle of the watershed. The third sample site was in a headwater stream or tributary. Sample sites were carefully chosen to not be immediately downstream of cities, wastewater treatment plants, or dams.

2. **Field sampling** was conducted in October 2017 during a 10-day continuous period and without any rain events in that watershed four days prior to sampling. Physical and chemical water quality parameters (water depth, dissolved oxygen, temperature, conductivity, and pH) were collected *in situ* at each site using a water quality multiprobe. We collected two types of microplastic samples: (1) bulk water samples and (2) sediment samples. Bulk water samples (n=3) were collected by filling a 2-L glass bottle approximately 0.3 m below the water surface (McCormick et al. 2014, Masura et al. 2015). Sediment samples (n=3) were collected by taking a sediment core (10 cm depth, 3 cm diameter) stored in glass mason jars. Sediment samples were collected at 21 of 31 sites since it was unsafe to collect sediment sample at some locations (e.g., steep banks, deep water). (Note: In the time since our initial proposal was written, net samples have been used less often for microplastics sampling since studies have found that bulk water samples are just as accurate, require less sampling effort, and can be readily compared among sites.) Furthermore, we have substituted sediment samples in our sampling protocol in place of net sampling.

3. We are actively **processing samples** in the laboratory following methods outlined by the National Oceanic and Atmospheric Administration (Masura et al. 2015). Our lab was taught this protocol in the laboratory of Dr. Timothy Hoellein at Loyola University Chicago, where microplastics research is ongoing. We first run samples through a set of increasingly smaller-pore sieves to remove large non-plastic materials such as organic matter and large rocks. Samples from 300-µm and 4.75-mm sieves are then isolated into clean, covered glass jars and dried in an oven at 60 °C for 72 hours. A wet peroxide oxidation (WPO) is then used to separate microplastics from any organic material. Briefly, hydrogen peroxide is used in the presence of an iron (II) catalyst to digest organic material but not plastics. Following the WPO, a saturated sodium chloride solution is added to the sample, which is placed in a funnel hanging vertically from a ring stand. The sample settles for at least 2 hours. The now density-separated sample is filtered under vacuum onto multiple glass microfiber filters (GF/F) to visualize microplastics. The filters are dried at 60 °C for 24 hours and then viewed under a stereomicroscope where all microplastics are counted and categorized (i.e., pellet, foam, fiber, or fragment). Categorization and counts of each filter are performed twice by independent observers such that numbers agree within 5%. If 5% agreement is not achieved, a third independent evaluation of the sample is performed. Particles per cubic meter (p/m^3) and percentages of microplastic types will be calculated for each bulk and sediment sample. Procedural control samples are inserted at all stages of this project to account for potential contamination from atmospheric deposition, contaminated laboratory water, or personal clothing. To date, we have processed bulk water samples and anticipate sediment sample processing to be completed by October 2018.

4. **Data analysis** will be performed on the counts and categories of the microplastics. We will use multivariate ordination techniques including principal components analysis (PCA) and non-metric multidimensional scaling (NMDS) to evaluate landscape and water quality variables that influence microplastic concentrations and composition. Results from this research will then be used to develop a spatial model (e.g., IN heat map) describing microplastic distribution across land uses, which will be disseminated to Indiana user groups.

Results:

Since we have not yet completed sample processing, results are forthcoming. We anticipate having results compiled for the bulk water samples by June 2018 and sediment sample results compiled by October 2018. These mileposts are consistent with the one-year no-cost extension granted to this project because of the delay in receiving funding.

Major Conclusions and Significance:

As results are forthcoming, we can only speculate on conclusions and result significance. The results of this research will provide Indiana managers, researchers, and decision-makers with a first-ever baseline measurement of the concentration and types of microplastics in major Indiana waterways. Results will shed light on whether land use influences the concentration and types of microplastics observed as well as the location of potential 'hot spots' of microplastic pollution within the state of Indiana. As warranted, state management agencies can then target particular watersheds for microplastics monitoring and reduction. This research also has public health implications because contaminants on the surface of microplastics may be transferred up the food web (and potentially to humans via fish consumption) or degrade municipal water quality. While recent legislation has banned the use of plastic microbeads in cosmetics (Microbead-Free Waters Act of 2015, H.R. 1321), microplastics will continue to exist in our waterways due to their multiple sources, persistence, and sorptive features (Free et al. 2014).

Upon project completion, we will utilize multiple means to disseminate results to the scientific community, resource managers, and general public. We will present our findings at the Indiana Water Resources Association Annual Symposium in 2019 and at one additional conference, such as the annual meeting of the Society for Freshwater Research. Results will be disseminated through peer-reviewed journal papers and public outreach. At least one peer-reviewed journal manuscript will be produced focusing on the distribution and types of microplastics found in Indiana watersheds that vary in land use type.

Publications:

Presentations

Conard, W.M., K.E. O'Reilly, M.S. Hartlage, and G.A. Lamberti. May 2018. Land Use Effects on Microplastic Pollution in Indiana Rivers. Oral presentation at Annual Meeting of the Society for Freshwater Science, Detroit, Michigan.

Conard, W.M., K.E. O'Reilly, M.S. Hartlage, and G.A. Lamberti. May 2018. Microplastics in Midwestern waters: An emerging contaminant. Oral presentation for GLOBES Graduate Symposium, South Bend, Indiana.

Hartlage, M.S., W.M. Conard, K.E. O'Reilly, and G.A. Lamberti. May 2018. Microplastic Distribution Above and Below Large Dams in Indiana, USA. Poster Presentation at Notre Dame College of Science Joint Annual Meeting, Notre Dame, Indiana.

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O'Reilly, K.E., W. Conard, and G.A. Lamberti. October 2017. Microplastics in Aquatic Ecosystems: Tiny Plastics, Big Problem? Youtube Video. Available at: https://www.youtube.com/watch?v=n_O42Cs9sbg&index=8&list=PLuk3dGfI10k1g98NkBHHNEhDg_mAKdYiw

Grant Submissions:

Matthews, Laura. Physiological Effects of Microplastic Ingestion on Yellow Perch (*Perca flavescens)*. Notre Dame Undergraduate Glynn Family Honors Program Summer Research Grant. May – July 2018. $4,500. Funded.

Students Engaged:

High School: 1
Undergraduate: 3
Ph.D.: 2

Literature Cited:

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WaterWorks: A Game to Teach Water Systems Thinking

Basic Information

Title:	WaterWorks: A Game to Teach Water Systems Thinking
Project Number:	2017IN403B
Start Date:	3/1/2017
End Date:	2/28/2019
Funding Source:	104B
Congressional District:	IN-009
Research Category:	Water Quality
Focus Categories:	Conservation, Education, Management and Planning
Descriptors:	None
Principal Investigators:	Shahzeen Attari, Mike Sellers

Publications

1. Attari, S. Z., Poinsatte-Jones, K., & Hinton, K. (2017). Tapping perceptions of water systems. Judgment and Decision Making, 12(3), 314-327.
2. Game Website (temporary host): http://water.studiocypher.com/

Indiana Water Resources Research Center Project Report

Report 2017 Program Report Format

Project Id: 2017IN403B

Title: WaterWorks: A game to teach water systems thinking

Project Type: Research

Period: March 1, 2017 – February 28, 2018

Congressional District: IN-9

Focus Categories: COV, EDU, M&P, WU

Keywords: water game, gamification, systems thinking, perceptions, education, drinking water, system dynamics

Principal Investigators:

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Abstract / Summary Public understanding of the water system is vital in dealing with the myriad of water challenges facing the world today. A lack of deep systems understanding of how water needs to be treated to be delivered to the home and what happens to the water once it leaves the home can pose severe sustainability and adaptation challenges. Neglecting natural water resources can lead to environmental, socio-political, and economic concerns. Prior research conducted by our lab (Attari *et al.*, 2017) had Indiana University student participants (N = 578) *draw* how water reaches the tap in an average home in the U.S. and is then returned to the natural environment. We also conducted an expert elicitation (N = 15) to create a simplified correct diagram to code each student drawing. Results showed major gaps in understanding, where 29% of the student participants did not draw a water treatment plant, 64% did not draw a wastewater treatment plant, and 1 in 5 participants depicted untreated wastewater returning to the natural environment. For the majority of non-environmental students, the water system stops at the home. These gaps reveal a critical area for public environmental education efforts. Using the funds from our grant, we created an interactive online video game to teach players about the water system. Our game, called WaterWorks, simulates a localized region where the player is responsible for building and maintaining a water system. The final goal of this project is to test whether game play can improve systems understanding and how players understand the risks associated with water quality, quantity, and infrastructure.

Problem: Given the many misperceptions people have about how our water system works, our aim was to identify a fun and interactive method to teach people about the water system. In the future we also aim to assess learning using built in survey questions.

Research Objectives: Our research objectives were to create an online game that was playable and then test whether game play led to better systems thinking and water use.

Methodology: We worked with Studio Cypher, a game development company in Bloomington Indiana to create the online Game.

Results A rough version of the game can be found here: http://water.studiocypher.com/ A click through explanation of how our water system works can be found here: http://water.studiocypher.com/about.html. The developers are finishing the game currently and we should make it freely accessible when completed.

Major Conclusions and Significance Given that the water game is now built, my collaborator Mike Sellers and I will use it in our courses as an educational tool and debug the game as we go. We will then identify ways in which playing the game helps players understand the water system better by using pre- and post-surveys. This last research portion will be paid for by my startup funds.

Presentations in 2017

- University of Illinois at Urbana-Champaign (Department of Agricultural and Consumer Economics)
- Michigan State University (Plenary, Fate of the Earth: Climate-Food-Energy-Water Nexus)
- Midwest Undergraduate Cognitive Science Conference (Keynote)
- University of California, Merced (School of Natural Sciences)
- University of California, Berkeley (Institute of Personality and Social Research)
- Center for Advanced Study in the Behavioral Sciences, Stanford University (Board of Directors)

Publications

Paper: Attari, S. Z., Poinsatte-Jones, K., & Hinton, K. (2017). Tapping perceptions of water systems. *Judgment and Decision Making, 12*(3), 314-327.
http://journal.sjdm.org/17/17124/jdm17124.pdf
Game Website (temporary host): http://water.studiocypher.com/

Grant Submissions: None at this time.

Students Undergraduate student: Carrisa Knox – who created all the images and animations of the game. This Fall she will be a new PhD student at University of Michigan studying systems design – an area very related to the water systems game project.

Examining Anthropogenic Impacts on the Wabash River System

Basic Information

Title:	Examining Anthropogenic Impacts on the Wabash River System
Project Number:	2017IN404B
Start Date:	3/1/2017
End Date:	2/28/2019
Funding Source:	104B
Congressional District:	IN-008
Research Category:	Water Quality
Focus Categories:	Surface Water, Nutrients, Sediments
Descriptors:	None
Principal Investigators:	Jeffrey Stone, Jennifer C Latimer

Publication

1. O'Neil E, Stone JR, 2017, Marrying GIS and diatom analyses: reconstructing the history of water quality of the Wabash River, 24th North American Diatom Symposium, Poster Presentation.

Indiana Water Resources Research Center Project Report

Report 2017 Program Report Format

Project Id: 2017IN404B

Title: Examining Anthropogenic Impacts on the Wabash River System

Project Type: Research

Period: March 1, 2017 – February 28, 2018

Congressional District: IN-8

Focus Categories: Surface Water (SW), Nutrients (NU), Sediments (SED), Water Quality (WQL)

Keywords: Diatoms, Biogeochemistry, Pollution, Nutrients, Invasive Species

Principal Investigators:

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Abstract / Summary: The Wabash River in the regions around Terre Haute are characterized by strong seasonal variability in nutrient flux and diatom communities, driven primarily by regional flooding. Nutrient influxes from run-off in agricultural areas result in nitrate and phosphate concentrations that are particularly high in the spring and summer months, respectively. Sites upstream of Terre Haute are characterized by elevated levels of ammonium during the winter months, potentially related to differences in farming practices. Diatom community patterns at the six locations we studied were also strongly seasonal in nature. Diatom abundances were lowest during the summer months. Diatom productivity within the Wabash River near Terre Haute was typically more strongly associated with nitrate concentrations than ammonium or phosphates. Heavy metal contamination was not observed from water samples collected during the study period.

Problem: Because it drains a vast area of agricultural landscapes, the Wabash River Valley currently comprises some of the highest nutrient yields to the Mississippi River waterways. Of the more than 800 watersheds in the US that contribute nutrients to the Gulf of Mexico, multiple watersheds within the Wabash River Valley have been identified among the most substantial sources for nutrient pollution. While aquatic eutrophication is commonly viewed as a nuisance because it leads to low water clarity, low water quality, unpleasant odors, and an unfavorable shift in the color of the water, eutrophication has many more serious implications. Excessive nutrients commonly lead to harmful algal blooms that may create toxic water environments and alter aquatic community compositions.

Research Objectives: 1) Expand on analyses of the anthropogenic impacts upon water conditions in the Wabash River Valley. 2) Quantify the nature their impact on the aquatic environment. 3) Provide a baseline for seasonal variations that may occur in the Wabash River associated with water and nutrient fluxes and determine other man-made impacts. 4) Characterize the introduction of invasive species and anthropogenic impacts through sediment core analyses in stream-adjacent lakes.

Methodology: Each week, for approximately 1.5 years, a set of sites along the Wabash River are monitored for changes in a series of biological and aqueous geochemical components, including: nitrogen, phosphorus, diatoms, and trace metals. Geochemical monitoring includes the use of a multiprobe sonde and water samples collected for laboratory analyses. Seasonal trends and longer-term patterns are used to explore research objectives 1 through 3 listed above. Six sites were located to meet these objectives. One site well upstream from Terre Haute, one site near the confluence of Otter Creek, several sites along the length of Terre Haute, and one site farther downstream of Terre Haute. Long-term records of anthropogenic impacts and introduction of invasive diatom species are to be analyzed from lake sediment cores collected from lakes near the Wabash River that are inundated during seasonal flooding events.

Results: The Wabash River in the regions around Terre Haute are characterized by strong seasonal variability in nutrient flux and diatom communities. Generally speaking, flooding during the spring and summer months typically leads to a high concentration of nitrates and phosphates in the river; most likely this is a result of influx from run-off in agricultural areas. Phosphate concentrations are particularly high in the summer months and moderately high in the fall months at all sites, except for those that are far removed from agricultural areas. Sites located upstream of Terre Haute are unique in that they occasionally express high concentrations of ammonium during the winter months, which may indicate introduction from Otter Creek and the surrounding agricultural areas, possibly resulting from a difference in agricultural practices, such as late autumn application of fertilizers and an absence of cover crops. Heavy metal contamination was not observed from water samples collected during the study period. Diatom community patterns are also strongly seasonal in nature and tend to follow the patterns established by nutrient concentrations. Diatom abundances were lowest during the summer months, which phosphate concentrations were highest; this may be a response to low light penetration or higher production by other algal groups. Because of delayed funding, sediment cores from river-adjacent lakes have not yet been collected, but are expected to be analyzed this summer as part of the Summer Undergraduate Research Experience program at Indiana State.

Major Conclusions and Significance: Senescence and decomposition of organic material from algal blooms also removes oxygen from the water. In streams, this is commonly referred to as oxygen sag. Oxygen sag within stream systems and negatively impacting the environmental quality, and changing the stream community to less desirable organisms. Lowered water quality has particularly strong impacts on the aquatic insect and fish communities. Excessive nutrients concentrations entering the Gulf of Mexico have been repeatedly linked to hypoxic events, which have substantially degraded the fishing, shrimp, and clam industries in the Gulf Coast.

Publications: To date, this work has resulted in three presentations, including one regional scientific conference, one national scientific conference, and one institutional seminar presentation. We anticipate these results will also be presented at the Annual Meeting for the Geological Society of America (Indianapolis, Fall 2018) and a MS thesis (anticipated defense in Fall 2018).

O'Neil E, Stone JR, Latimer J, 2018, Analyzing the water quality of the Wabash River using diatom species assemblages and geochemistry, Indiana Academy of Sciences Annual Meeting, Indianapolis, Oral Presentation.

O’Neil E, Stone JR, Latimer JC, 2017, Analyzing the water quality of the Wabash River using diatoms, macroinvertebrates, and geochemistry. USGS Brown Bag Speaker Series, Indianapolis, IN.

O’Neil E, Stone JR, 2017, Marrying GIS and diatom analyses: reconstructing the history of water quality of the Wabash River, 24th North American Diatom Symposium, Poster Presentation.

Grant Submissions: None (yet).

Students: This research has directly supported one MS thesis student (Ellen O’Neil), and indirectly supported two undergraduate student researchers (Jordyn Loveall, Hillary Johnson) at Indiana State University. Undergraduate researchers have contributed to the project through laboratory and field assistance, but have also been funded (so far) primarily through internal resources at Indiana State. Additional direct support for continued undergraduate research is expected for this coming summer.

Effects of Viruses on the Development of Harmful Algal Blooms

Basic Information

Title:	Effects of Viruses on the Development of Harmful Algal Blooms
Project Number:	2017IN405B
Start Date:	3/1/2017
End Date:	2/28/2019
Funding Source:	104B
Congressional District:	IN-004
Research Category:	Water Quality
Focus Categories:	Nitrate Contamination, Non Point Pollution, Nutrients
Descriptors:	None
Principal Investigators:	Zhi George Zhou

Publications

There are no publications.

Indiana Water Resources Research Center Project Report

Report 2017 Program Report Format

Project Id: 2017IN405B

Title: Effects of viruses on the development of harmful algal blooms

Project Type: Research

Period: March 1, 2017 – February 28, 2018

Congressional District: IN-4

Focus Categories: NC, NPP, NU, SW, WQL

Keywords: Harmful algal blooms, viruses, nutrients

Principal Investigators:

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Abstract / Summary

Harmful algal blooms (HAB) are overgrowth of algae that could foul up surface waters, consume oxygen in the water, and produce harmful toxins that are harmful to humans and animals. HABs have been reported as a major environmental problem in all 50 states of the United States. Many studies have been done on the factors that can affect the development of HABs, such as sunlight, temperature, low turbulence, and nutrient sources, but only limited studies have been down to evaluate the effects of viruses on the development and decay of HABs, even though that viruses are the most abundant biological entity in aquatic systems and some studies suggest that viruses are driving the life-and-death dynamics of algal blooms. The overall goal of this project was to evaluate the effects of viruses on the development of HABs. In this study, Chlorovirus PBCV-1 was isolated from natural environment and its invasion to algae was observed by transmission electron microscopy. In addition, qPCR primers were designed to quantify Chlorovirus PBCV-1 in total microbial community and validated with the traditional plaque assay method. Experiments are being conducted to evaluate the effects of nitrogen and phosphorus on viral of algae. The results will improve fundamental understanding of effects of viruses on microbial structure and functions of algae and contribute to the development of control strategies for HABs, which is still one of the most costly and challenging environmental problems in the world.

Problem

In natural environments, algal growth result in harmful algal blooms (HABs) and foul up surface waters with massive biomass, cause anoxia in the water, and release toxins into ecosystems that are harmful to humans and animals (Anderson et al. 2002). HABs widely occur in the United States, and have been reported as a major environmental problem in all 50 states (US Environmental Protection Agency 2016). Algae are commonly found in Indiana lakes and streams (Indiana Department of Management 2016). The recent HAB outbreak in neighboring Ohio in 2015 has generated great public health concern for residents in Indiana. To address the concern of HABs, the Indiana Department of Environmental Management (IDEM) and three other state agencies are monitoring blue-green algae, a.k.a. cyanobacteria, in lakes at in Indiana (Indiana Department of

Management 2016). High cyanobacteria counts (> 100,000 cells/ml as defined by the World Health Organization) have been reported in over 11 lakes in Indiana, such as Brookville Lake, Raccoon Lake, and Mississinewa Lake (Indiana Department of Management 2016), which indicates an urgent need for further studies HABs.

Many factors have been identified to affect the behavior of algal growth and decay during HABs, such as sunlight, temperature, low turbulence, and nutrient sources, and phosphorus and nitrogen (Anderson et al. 2002). Most existing studies on HABs focus on environmental factors for the development of HABs. For example, the dominant factors controlling HABs by *A. fundyense* in Nauset were identified as water temperature and residence time of cells (Ralston et al. 2015). Nutrients have been found to be important factors on HAB formation and several HAB species were identified in bloom concentrations adjacent a site discharging phosphate-processing effluent into an estuary within Tampa Bay (Garrett et al. 2011). Another study indicated anthropogenic nutrients promoting HABs is site-specific and hydrodynamic dependent (Davidson et al. 2014).

However, an important, yet less investigated, process is algal decay. HABs may be removed by fungi, bacteria, and ciliates. One previous study indicated that freshwater algal cultures were suppressed by a fungus strain *Trichaptum abietinum* within 48 hours of co-incubation due to its preying ability on the algal cells (Jia et al. 2010). Another study indicated that an algicidal bacterium *Deinococcus sp.* can induce growth inhibition on HAB species *A. tamarense* (Li et al. 2015). Four HAB species were inhibited by a marine bacterium due to its supernatant stress (Hou et al. 2016). Predator-induced changes in phytoplankton movements resulted in a reduction in encounter rate and a 3-fold increase in net algal population growth rate (Harvey and Menden-Deuer 2012).

However, only limited studies have been done to evaluate algal decay by viruses, although viruses are the most abundant biological entity in aquatic systems (Gachon et al. 2010). One study on lytic viruses (PgV) and their infections on algal species *Phaeocystis globosa* indicated that viral infection plays an important role in *P. globosa* dynamics and the diversity of both host and virus community (Baudoux and Brussaard 2005). Another study on the brown tide showed that viruses may be a major source of mortality for brown tide blooms in regional coastal bays of New Jersey and New York (Gastrich et al. 2004). Other studies have shown that viruses can control host algal populations and play an important role in structuring microbial communities (Fuhrman 1999, Gachon et al. 2010, Van Etten and Dunigan 2012). Recently, a study combined satellite and *in situ* data on the life cycle of a mesoscale (~10-100km) bloom in the North Atlantic and found that high levels of specific viruses infected coccolithophore cells, indicating that viruses are driving the life-and-death dynamics of algal blooms, even when all other factors stays essentially the same (Lehahn et al. 2014). However, studies on viral infections on algal cells are still limited and therefore there remains a <u>*critical need for research*</u> to evaluate viruses on the decay of algae.

Research Objectives

The overall goal is to evaluate the effects of viruses on the development and decay of algae. The specific research objectives are to:

1) develop qPCR primers to quickly and accurately quantify virus abundance;
2) evaluate the growth and decay of algal cells under the exposure of various nutrient loading; and
3) elucidate the mechanisms of virus infection on the decay of algal cells.

Methodology

Microalgal cultures and growth conditions.

Fresh water microalgal strain *Chlorella sp.* (ATCC 50258) was used as a representative algal strain in this study. Stock culture was cultivated in ATCC 847 growth medium at 25 ℃. ATCC medium 847 contains 1.0 g Proteose Peptone, 250 mg $NaNO_3$, 25 mg $CaCl_2$, 75 mg $MgSO_4$, 75 mg K_2HPO_4, 175 mg KH_2PO_4, 25 mg NaCl, and a drop of 1.0% $FeCl_3$ solution in 1.0 L distilled water. The growth of microalgae was determined by a hemocytometer (HAUSSER SCIENTIFIC) and a fluorescence microscope (Nikon Eclipse Ni with Plan Fluor x40 objective). LIVE/DEAD® BacLight™ Bacterial Viability Kits were used to stain live/dead cells after viral infection. The excitation/emission wavelengths for these dyes are about 480/500 nm for SYTO 9 stain (live) and 490/635 nm for propidium iodide (dead).

Algal virus isolation and identification.

Water sample with *Chlorovirus* was collected from a lake in Celery Bog Nature Area, West Lafayette, IN. The collected water sample was filtered through a syringe filter with 0.2 µm PTFE membrane (Thermo Scientific) to remove non-viral particles. Then *Chloroviruses* in the filtrate were isolated by the modified plaque assay method (Van Etten et al. 1983). A mixture of 100 µL diluted filtrate (approximately 50 PFU per 100 uL), 300 µL *Chlorella* cells (2×10^8 to 4×10^8 cells/mL), 100 µL erythromycin stock (1000 mg/L) and 5 mL of soft-agar medium was poured onto a 1.5% agar plate. Agar medium (1.5%) and soft-agar medium (0.75%) were prepared by adding 15 g and 7.5 g agar per liter ATCC 847 medium for plaque assay. After a week's incubation at 25 ℃, viral plaques formed on agar plate were counted. *Chlorovirus* from a single plaque on the algal lawn was isolated and amplified in host culture for future use. To maintain algal viruses, 300 µL filtrate was added to 100 mL host microalgae culture (3×10^7 to 7×10^7 cells/mL). After three days' incubation, new virus stock was prepared by filtering the viral rich suspension through a 0.2 µm syringe filter. The viral rich filtrates were stored at 4℃ for future use.

The type of *Chlorovirus* from single plaque was identified by polymerase chain reaction (PCR). A freeze-thaw pretreatment procedure consisting of three cycles of heating at 95 °C for 2 mins and freezing to solid was used to extract DNA. Four algal virus-specific primers listed in **Table 1** (Rusanova 2010) were examined to identify the isolated *Chlorovirus*. Each 50 µL PCR reaction contains 2 µL of the pretreated sample, 10 µM virus specific PCR primers, DreamTaq DNA Polymerase, and 10X DreamTaq green buffer. Negative controls were using water to substitute the sample. All PCR reactions were performed in a Biorad 1000-Series Thermal Cycler with denaturation at 95°C for 2 min, followed by 39 cycles of heating at 95°C for 30s, annealing at primer-specific temperatures (**Table 1**) for 45s and extension at 72°C for 1 min. At the end of cycling, all PCR reactions were subjected to a final extension step at 72°C for 30 min. After PCR, 8 µL of the reaction product were loaded into 1.5% Bio-rad Certified™ agarose gel stained with SYBR safe stain and subject to electrophoresis in a Bio-Rad electrophoresis cell. The electrophoresis results were obtained by Bio-Rad Gel Doc™ XR+ with Image Lab™ software.

Table 1. PCR primers used in this study

Primer name	Forward primer	Reverse primer	Annealing temperature (C)	Amplicon size (bp)
CVMS	AAGAAGGGAGC ATACTTCACGC	CAAAATGTAAGG GTAATAGATCTTC	50	645
PBCVS	CTTATCGCAGCT CTCGATTTTG	GTTCGGTGCTCGG AAATCCTTC	44	600
ATCVS	AAGAAAGGTGCC TACTTTGAAC	AGGTCGTTCGGA GCTTTGTACT	48	610
CHLV	CCWATCGCACG WCTMGATTTTG	ATCTCVCCBGCV ARCCACTT	52	560+

Infected and uninfected algal cells were observed under a Tecnai T12 transmission electron microscope (TEM). Samples were prepared by Purdue Electron Microscopy Facility modified from the method described by (Greiner et al. 2009). Target algae suspensions were centrifuged to obtain an algal pellet and were fixed with a cacodylate-buffered (pH6.8) 2% glutaraldehyde and 2% formaldehyde solution. After washing in buffer, samples were post-fixed in the same buffer with 2% OsO4. The samples were dehydrated in a series of graded acetone and then embedded in Embed 812 Resin. At last diamond knives were used to acquire ultrathin sections. The ultrathin sections were stained with uranyl acetate and lead citrate for TEM observation.

Development of new qPCR primers to quickly and accurately quantify virus.

Traditionally, plaque assay has been used as a standard method to determine virus abundance through the quantification of plaque-forming units (PFU) on agar plates filled with host cells (Van Etten et al. 1983). However, plaque assay is time-consuming and it usually takes few days before results can be collected. Conversely, quantitative real-time PCR (qPCR) can overcome these disadvantages by quickly and quantitatively measuring virus gene copy number in a few hours and directly detecting DNA without cultivation biases. In this study, qPCR primers were developed to quantify algal virus and validated with results from plaque assay.

The genome of *Chlorovirus* PBCV-1 was obtained on NCBI and protein coding gene sequence of A185R was used to design primers in this study. PrimerQuest Tool from

Integrated DNA Technologies was used to generate qPCR primers and the five highest scoring pairs of primers on the candidate list were purchased and applied in this study (Table 2).

Table 2. Newly designed qPCR primers for PBCV-1

Primer name	Forward primer	Reverse primer
185R 1	ACTACGCAATTCCTGACGATAAG	GAGAGCTGCGATAGGTGTAAAG
185R 2	GGGTGCGTACTTTACACCTATC	CACCAGAGTTTCTGGACTCATATT
185R 3	GAACGACTTGGAGGTGAGTT	CAGAACGCGATGGAATGTTTAG
185R 4	CAACTTCCGTGGACAACAAATC	GTTGCACCTTCGTACTTACCT
185R 5	ATTGAGACTGGACTGGGAAAG	TTCGCGAGATCGTCCAATAG

The new qPCR approach was validated with plague assay. Virus lysate was prepared by inoculating 50 mL algae culture at exponential stage (3×10^7 to 7×10^7 cells/mL) in a 20 µL virus stock. After three days of incubation, 50 mL lysate was serially diluted to 10^{-7} in PBS buffer. The dilutions of 10^{-6} and 10^{-7} were subject to plaque assay as previously described (Van Etten et al. 1983). The original lysate and dilutions of 10^{-1} to 10^{-5} were treated by freeze-thaw pretreatment before analyzed with qPCR. Each 20 µL qPCR reaction contained 1 µL of the pretreated sample, 10 µL Biorad supermix, 1 µL qPCR primers, and 7 µL water. Negative controls were conducted with distilled instead of DNA sample. All qPCR reactions were performed in a Bio-Rad CFX96^{TM} Real-Time System began with denaturation at 95°C for 3 min, followed by 39 cycles of heating at 95°C for 5s, annealing and extension at 60 ℃ for 30s. Standard curves for qPCR were generated from a set of eight-fold serially diluted standards (ranging from 4.06×10^9 to 4.06×10^2 gene copies per µL).

Evaluate the decay of algal cells under the exposure of various nutrient loading.

To test the decay of algal cells under various nutrient levels, modified BG-11 growth medium was prepared by adding 37.5 mg $MgSO_4 \cdot 7H_2O$, 18 mg $CaCl_2 \cdot 2H_2O$, 1 mL EDTA ferric solution and 1 mL micronutrient solution to 1 L water. EDTA ferric solution was prepared with 0.3 g ferric citrate and 0.05 g EDTA in 1 L water. Micronutrient solution was made by adding 2.86 g H_3BO_3, 0.222 g $ZnSO_4 \cdot 7H_2O$, 0.035 g $CoCl_2 \cdot 6H_2O$, 0.055 g $CuCl_2 \cdot 2H_2O$ and 0.23 g $Na_2MoO_4 \cdot 2H_2O$ to 1 L water. Growth medium were prepared with various nutrient levels (Table 3) with PBS buffer and 1 M sodium nitrate stock. The selection of low and medium nutrient level were based on oligotrophic and eutrophic conditions as previously described (Ma et al. 2015, Smith et al. 1999). High nutrient level followed nutrient levels in the original BG-11 growth medium to provide sufficient nutrient supply.

Table 3. Nutrient levels selected

Modified BG-11 medium	Low (mg/L)	Medium (mg/L)	High (mg/L)
Nitrogen	0.35	14	41
Phosphorus	0.05	1	53

Algae suspension at exponential stage (approximately 1×10^7 cells/mL) was collected and centrifuged, and the pellets were washed three times by 0.85% NaCl solution before resuspended in the nine modified BG-11 medium with various nutrient levels (3×3), respectively. Then all samples were inoculated with virus stock (multiplicity of infection 0.1) and started incubating at 25 ℃. Two samples were collected periodically from each vial for qPCR and live/dead staining experiments under a fluorescence microscope.

Results

Growth of Chlorella and PBCV-1 algal virus

Growth of *Chlorella sp.* was observed by inoculating 100 µL of culture in 100 mL ATCC Medium 847 and incubating at 25 ℃ with a 14 hour light/10 hour dark cycle. As shown in Figure 1, *Chlorella* grew rapidly during the first 10 days and increased from 2×10^4 cells/mL to 2×10^7 cells/mL after approximately two weeks of incubation. Based on these results, two weeks of incubation time was used for the following experiments to cover the exponential growth phase.

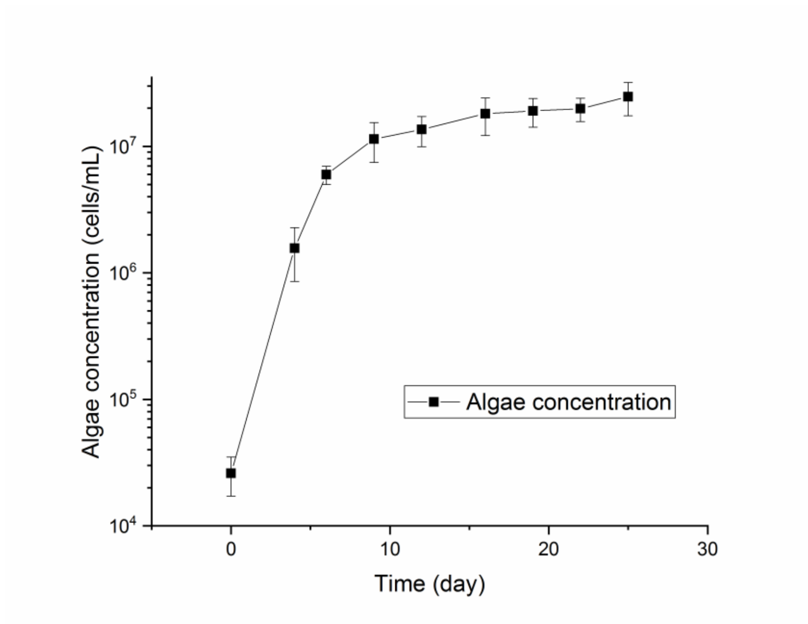

Figure 1. Growth behavior of *Chlorella sp.*

Chlorovirus was isolated successfully (

Figure ***2***). Virus from one single plaque on the lawn was harvested and examined by PCR with primers targeting various types of *Chloroviruses*. According to the gel electrophoresis result (

Figure ***2***), the only band appeared at 600 to 700 bp was targeting *Paramecium bursaria Chlorella virus 1* (PBCV-1), which is consistent with our expectation.

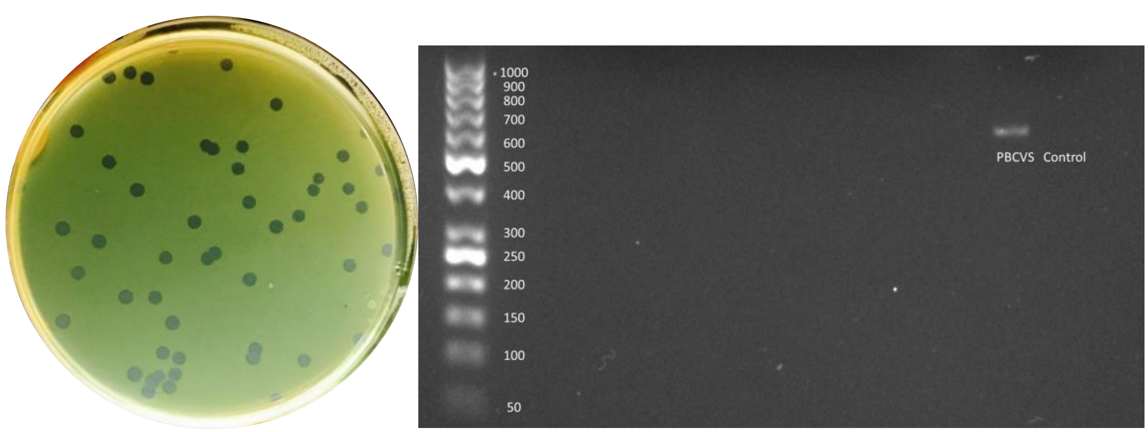

Figure 2. Pictures of plaque assay (left) and gel electrophoresis result (right)

TEM analysis of PBCV-1 infection of algal cells

The results of TEM analysis showed both a health algae cell and an infected algae cell (
Figure 3*), which clearly showed that Chlorella was attacked by PBCV-1 viruses—particles with hexagonal structures in*

Figure *3* (b).

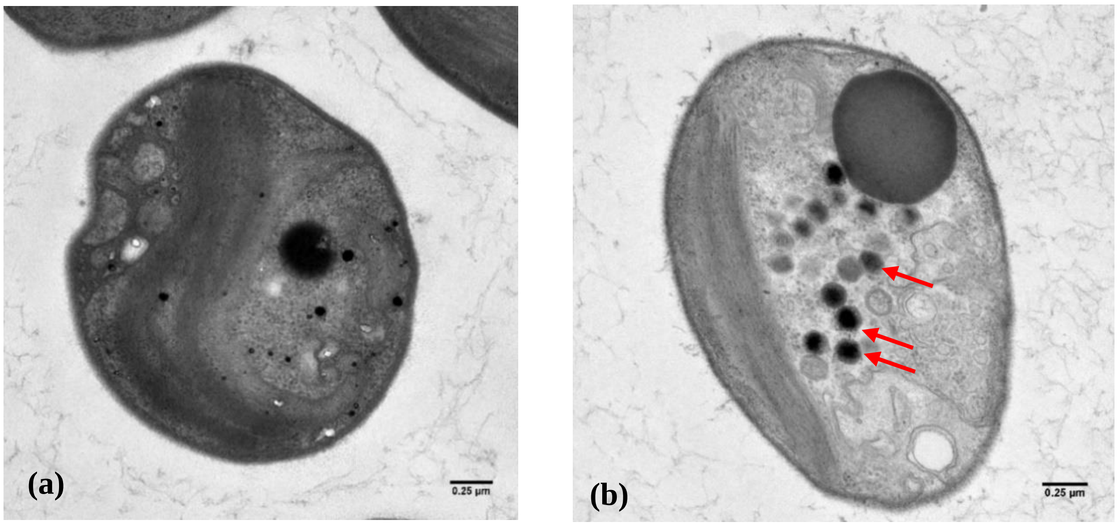

Figure 3. TEM pictures of uninfected (a) and infected (b) *Chlorella sp.* cells

Development of new qPCR primers to quantify PBCV-1 virus

The newly designed qPCR primers were tested and compared with plaque assay results. Primer specificity and standard of target gene was tested before applied to samples. Cycle threshold (Ct) values of negative controls for A185R1 primer ranged from 29.72 to 30.15 and Ct values of 4×10^9 target molecules ranged from 7.32 to 7.46. Standard curve of target gene was made from 4×10^9gene copies to 4×10^2gene copies (**Figure *4*** a). The single peak in melting curve of A185R1 primer (**Figure *4*** b) indicated that the binding between this primer and target gene was specific in the standard. Only A185R1 primer has been tested so far and other designed primers will be tested in the future.

Subsequently, qPCR was used to directly quantify viruses. The original virus concentration was determined as $4.85\times10^9\pm1.35\times10^9$ PFU/mL by plaque assay. According to qPCR results (Figure 4 c), samples above 4.85×10^5 PFU/mL were successfully detected. Binding between primer A185R1 and target gene was also tested to be specific in real samples (Figure 4 d).

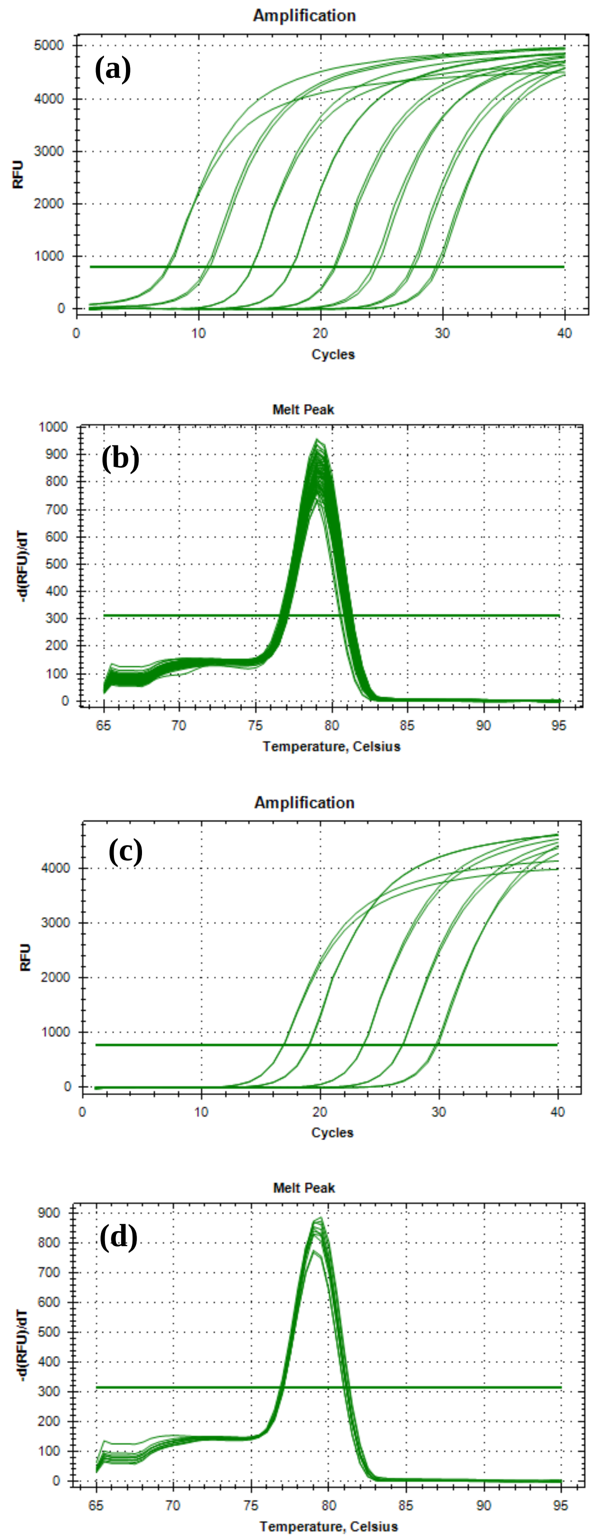

Figure 4. Real-time PCR result of A185R 1 primer (a) standard curve, (b) melting curve of standard, (c) heat/cold treated samples with various virus abundance (d) melting curve of samples

The results of qPCR and plaque assay were compared and agreed well (Figure 5). A single-factor ANOVA test was also performed and the resulting p value of 0.247 indicated that there was no significant difference between plaque assay and qPCR. The results showed the qPCR approach with our newly developed primers accurately quantified virus concentration without the long waiting time in a conventional plaque assay.

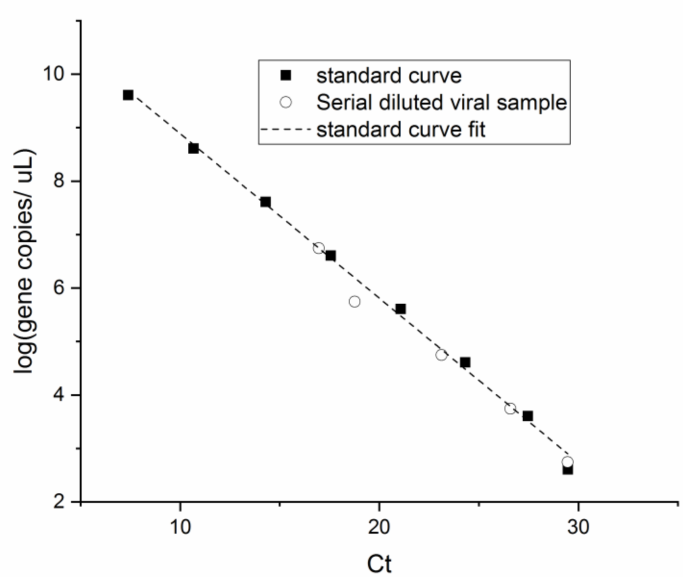

Figure 5. Quantification of PBCV-1 virus by qPCR method

Evaluate the decay of algal cells under the exposure of various nutrient loading.

The preliminary results of the effects of nutrient levels on viral infection of algal cells are shown in Figure 6 and Figure 7. No significant differences were observed among the current data. The results might be explained by the relative high multiplicity of infection (0.068) applied in this set of experiments. Lower multiplicity of infection (approximately 10^{-6}) will be used in future experiments to evaluate decay of algal cells under the exposure of various nutrient loading

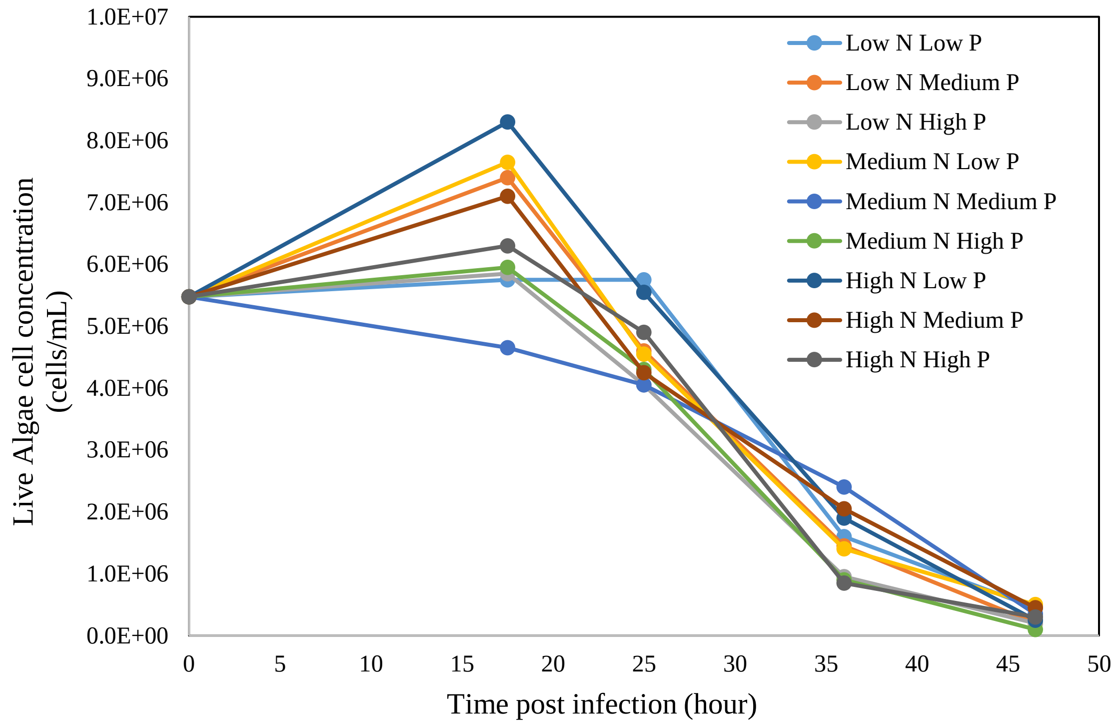

Figure 6. Live cells post infection under various nutrient levels

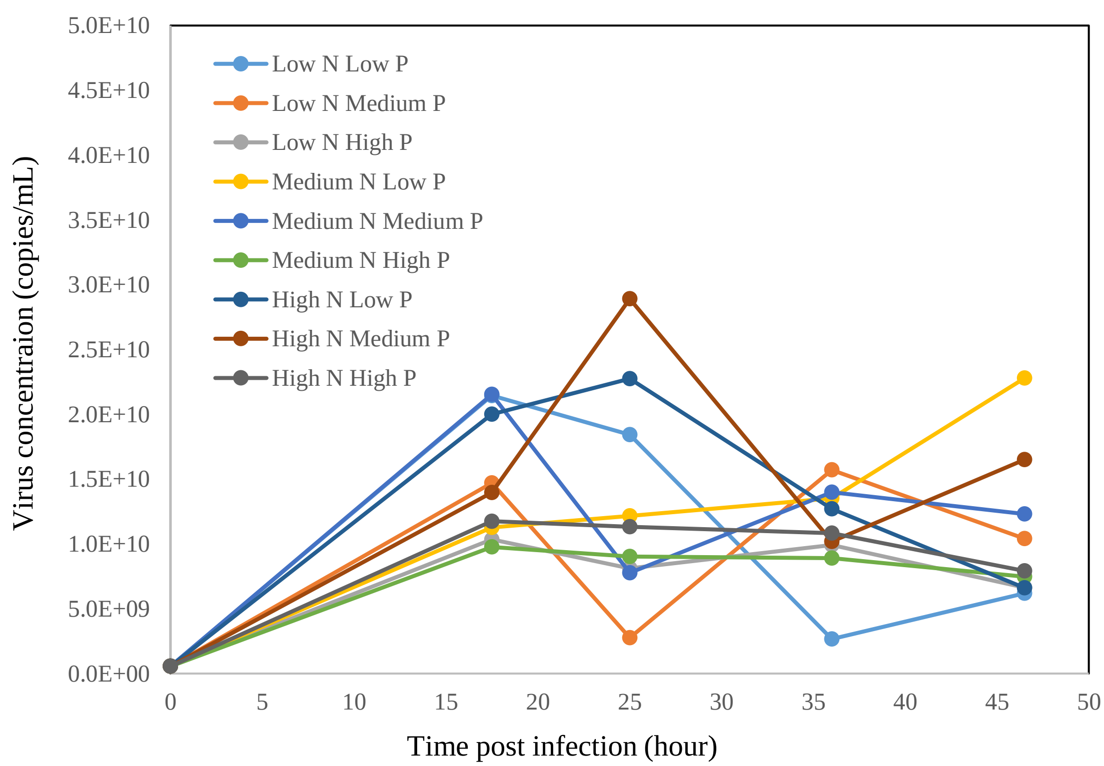

Figure 7. Virus concentration post infection under various nutrient levels

Major Conclusions and Significance

We have isolated an algal virus PBCV-1 that specifically infects an algal species *Chlorella*. The results of algal infection were observed in a TEM analysis. We have successfully developed new qPCR primers to quantify PBCV-1 virus and validated it with plaque assay. We are currently evaluating the growth and decay of algal cells under the exposure of various nutrient loading and will elucidate the mechanisms of virus infection on the decay of algal cells.

The results will improve fundamental understanding of effects of viruses on microbial structure and functions of algae and contribute to the development of control strategies for HABs, which is still one of the most costly and challenging environmental problems in the world.

Publications

We plan to submit an abstract to the 39th Annual Indiana Water Resources Association Symposium by May 18, 2018.

Additional results will be used to prepare a manuscript for a peer-reviewed journal when the project is finished next year.

Grant Submissions

We have not submitted a grant based on the current results yet as this is still an on-going study.

Students

One Ph.D. student at School of Civil Engineering has been supported by this grant.

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Communicating the State of Indiana Water Resources

Basic Information

Title:	Communicating the State of Indiana Water Resources
Project Number:	2017IN406B
Start Date:	3/1/2017
End Date:	2/28/2019
Funding Source:	104B
Congressional District:	IN-004
Research Category:	Water Quality
Focus Categories:	Water Quantity, Water Supply, Water Use
Descriptors:	None
Principal Investigators:	Keith Aric Cherkauer, Laura C. Bowling

Publications

There are no publications.

Indiana Water Resources Research Center Project Report

Report 2017 Program Report Format

Project Id: 2017IN406B

Title: Communicating the State of Indiana Water Resources

Project Type: Research

Period: March 1, 2017 – February 28, 2018

Congressional District: IN-4

Focus Categories: WQN, WS, WU

Keywords: Water resources, groundwater supply, surface water supply

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Abstract / Summary

Water resources are sources of water that are of sufficient quality to meet human needs, when and where they are needed. Therefore, they reflect both water supply – the useable sources of surface and groundwater, as well as demand, where and when water is being extracted for a purpose. Long-term water scarcity results when these two are out of balance, such that the human demand for water represents the majority of renewable supply. Sustainable use of water resources therefore requires the balanced allocation of renewable natural resources to people, farms and ecosystems. Balanced allocation in turn requires that we understand the nature of the available resources, including the mean, seasonal variability and extreme conditions. The purpose of the project is to create an up-to-date easily accessible web portal that geospatially displays metrics to convey the state of both Indiana ground and surface waters. The code written for this project retrieves data from the USGS online water database, completes automated data quality checking, calculates metrics for the current state (end of last water year) and long-term trends for Indiana water resources, and outputs this analysis. All analysis is conducted for the last 30 water years up to and including the most recent water year. The current state of Indiana water resources is assigned based on a ranking of how the current groundwater and surface water metrics compare to previous water years. Long-term trends are also based on the last 30 water years of surface and groundwater data. Results from this analysis are summarized in 4 sets of metrics corresponding to the current state of groundwater, current state of surface water, groundwater trends, and surface water trends. These are imported into an online ArcGIS platform to geospatially represent the location and status of water resources within Indiana using interactive webmaps. These webmaps are combined with snapshots of groundwater, and surface water levels, and the end of water year drought monitoring state can be found on the State of Indiana Water Resources Website.

Problem:

Water resources are sources of water that are of sufficient quality to meet human needs, when and where they are needed. Therefore, they reflect both water supply – the useable sources of surface and groundwater, as well as demand, where and when is water being extracted and for what purpose. Long-term water scarcity results when these two are out of balance, such that the human demand for water represents the majority of renewable supply. Sustainable use of water resources therefore requires the balanced allocation of renewable natural resources between natural and anthropogenic activities. Balanced allocation in turn requires that we understand the nature of the available resources, including the mean, seasonal variability and extreme conditions. The problem is, that although many federal (e.g. USGS, NOAA, USCOE) and state agencies (IDNR, IDEM) have their own publicly-available databases of water quantity, there is no one portal to obtain an overall summary of water availability for the entire state of Indiana. Each agency has its own website and methods of displaying this information, their own data formats, and their own methods for computing summary statistics. There is a need for tools to integrate this disparate data into a single web-interface to summarize and inform the public on the current state of Indiana's water resources, and use the data to compute statewide metrics on water availability.

Research Objectives:

- Develop tools to extract water resource data for specific time periods (such as groundwater, river and reservoir water storage values at the end of the water year) from various state and national agencies including the NRCS, IDEM, IDNR, USGS, and the Corps of Engineers, and to integrate those disparate data types into a geodatabase for further analysis.
- Review and calculate water availability metrics that provide insight into the state of water resources and allow one to rank them statistically to put the current state of water resources into perspective using historical observations.
- Create maps of water storage and availability metrics and how conditions for the previous water year rank relative to the historical record.
- Develop informational materials that will be integrated into the web site to help explain the data and metrics being presented.

Methodology:

Data: The data displayed on the preliminary website and used for analysis was acquired from the United States Geological Survey (USGS) database. The data used for the groundwater analysis was daily mean groundwater levels given in feet below the land surface. The data used for the surface water analysis was daily mean flow rate given in cubic feet per second. Data regarding the groundwater and surface water sites including latitude, longitude, elevation, aquifer (groundwater), and hydrologic unit code (HUC) or drainage area (surface water) was also retrieved from the USGS. Only data from the previous 30 water years was used for analysis for both groundwater and surface water. This cutoff was chosen in order to create a consistent length of time across sites to produce an equal comparison. Groundwater wells and surface water sites were excluded if they did not contain at least 300 daily mean values for at least 24 of the 30 years in question. Any data points with incomplete dates, dates that did not include the day, month, and year of acquisition, were also removed. A few of the groundwater sites provided water level as a height above a vertical datum, and these values were converted to depth below surface based on the provided land surface elevation above the specified datum given by USGS for each of those sites. Additionally, any groundwater sites that provided only a daily maximum and minimum, the average was assumed to be an appropriate approximation of the mean daily water level due to the minimal variation in groundwater levels.

Current State: The parameters chosen to represent the current state of Indiana ground water were annual mean, 1-day maximum, 1-day minimum (groundwater), 7-day minimum (surface water), and range [1-day maximum – 1-day minimum] (groundwater) as well as the value on September 30th (the last day of the water year). One-day maximum and 1(7)-day minimum flow were chosen to represent the periods of high and low water levels respectively. Range was chosen to represent the rate and degree of recharge of the groundwater over the coarse of the year, and the September 30th value was chosen to be the ‘current state’ of groundwater resources in Indiana as it is the last recording for the water year. These metrics were then ranked using the Hazen ranking system in order to compare the most recent water year (2017) to the previous 29 water years. The Hazen rankings vary between 0 and 1, not including the bounds of 0 or 1. Values closer to 1 are indicative of a wet year when evaluating mean annual levels or a greater range (recharge) when evaluating the groundwater range metric.

Trends: The parameters chosen to be representative of long-term trends in Indiana water were annual mean, 1-day maximum, 1-day minimum (groundwater), 7-day minimum (surface water), and range (groundwater). Trends were evaluated for each of these using the Mann-Kendall test for statistical significance. The Mann-Kendall test was chosen as it is a non-parametric, rank based test that is distribution free and not significantly affected by the presence of outliers. For this study, a confidence level of 90% was used as a cutoff in determining if a ‘significant’ trend was present. The magnitude of the trends was determined using the Thiel-Sen slope estimation. This approximation is based on the median value of the slopes and is therefore resistant to outliers as well.

Results

Program Development: Programs have been developed that automatically retrieve data from the USGS online water database based on the most recently ended water year. The previous 30 years of data for Indiana groundwater levels and surface water flowrates are used. Several data quality checks are performed in order to ensure validity and uniformity within the dataset. This includes the data restrictions to ensure at least 300 daily values are present for each water year used in analysis and at least 80% of the water years for each site contain adequate data. Next, the current state metrics are calculated for each of the two (groundwater and surface water) datasets for sites that contain data for at least 24 years including the most recent water year. The long-term trends are also calculated for the two datasets for sites that contain 24 years of data though no restriction was implemented requiring the most recent water year to contain data to include sites in the trend analysis. Finally, these calculated metrics are outputted in 4 ASCII style tables (Groundwater Current State, Surface Water Current State, Groundwater trends, and Surface Water Trends) that are formatted with site information (latitude, longitude, etc.) in order to be imported to an ArcGIS platform.

Website: Tables created in the previous section were then used to create four webmaps using the ArcGIS online platform. These are interactive webmaps with layers displaying each of the metrics used to show the state of Indiana waters. These maps also contain site information such as elevation, HUC, and aquifer. The website also contains snapshots of water levels for the most recent water years across the state. There were 7 groundwater, 7 streamflow sites, and 5 reservoir sites selected to show the trend of water levels throughout the most recent water year. These snapshots contain graphs created using the graphing tools on the USGS website. Each graph contains the water level over the course of the current water year overlaid with the long-term average for the period of record for the site. Accompanying each of these snapshot pages is a short summary of the observed trends for the current year. Additionally, there is a page displaying drought conditions across the state on the last record date of the most recent water year, which is retrieved from the National Drought Mitigation Center. All of the snapshots and drought monitoring images and graphs are acquired automatically using the coding. Background information such as the abstract above and a more detailed methodology section are also include in the website as well as links to the data sources.

Major Conclusions and Significance

The results of this work are an up-to-date web portal for obtaining information, data and insight into the current state of surface and groundwater resources in Indiana that can be used by land managers, agency personnel and legislators to inform short term and long-term water management decisions.

Publications

None

Grant Submissions:

None

Students

1 – Masters Student – Department of Agricultural and Biological Engineering

Information Transfer Program Introduction

None.

USGS Summer Intern Program

None.

Student Support					
Category	**Section 104 Base Grant**	**Section 104 NCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	19	0	0	0	19
Masters	4	0	0	0	4
Ph.D.	7	3	0	0	10
Post-Doc.	3	0	0	0	3
Total	33	3	0	0	36

Notable Awards and Achievements