

**Pennsylvania Water Resources Research Center
Annual Technical Report
FY 2014**

Introduction

The Pennsylvania Water Resources Research Center (PA-WRRC), founded in 1964, is authorized by Congress as one of the nation's 54 water resources research institutes comprising the National Institutes of Water Resources (NIWR). In keeping with the Land Grant mission of the host Universities, each institute serves a tripartite mission of University research, education, and outreach in advancing pressing problems in water quality and quantity. The program is administered by the U.S. Department of the Interior through the U.S. Geological Survey, in a unique Federal-State-University partnership. The PA-WRRC is located on the University Park campus at the Pennsylvania State University. There, PAWRRC resides within the Penn State Institutes of Energy and the Environment (PSIEE). The PA-WRRC continues to receive significant support contributions from PSIEE, which funds the Director's time spent in water center administration and provides additional staff support for administrative, accounting, communications, and research functions.

The PA-WRRC receives USGS 104B federal base funding that is distributed via a small grants competition to researchers at academic institutions across Pennsylvania. A request for proposals for this competition was broadly disseminated. Given the level of funding available in FY14, we were able to make 6 awards, intended to be one-year small grants allowing faculty to conduct research and outreach on problems important to Pennsylvania. None of the federal funding was used to pay overhead costs, and PA-WRRC matched every dollar of its base appropriation with at least two dollars from non-federal sources.

Twenty-two students (8 undergrads, 4 masters, 9 doctoral, and 1 postdoc) were supported or partially supported as part of the PA-WRRC 104B projects during FY14, from multiple academic institutions across Pennsylvania.

Research Program Introduction

Research Projects.

Four projects supported during FY14 were research-oriented, addressing unanswered questions in water resources and seeking solutions to water challenges.

PI Kyle Bibby from the University of Pittsburgh worked to develop treatment options for Marcellus Shale wastewater through cultivation of algae. Results demonstrated that algae growth in produced water (i.e., wastewater from hydraulic fracturing) is limited for practical applications; however, removal of certain problematic pollutants of produced waters such as calcium, strontium, manganese and radium was observed either during algae cultivation or by biosorption.

PI Brady Porter from Duquesne University studied the range expansion and population structure of five Pennsylvania State threatened fish species. The investigators developed new environmental DNA and molecular genetic techniques to trace aquatic species. Results showed that the threatened spotted darter may not be expanding its range as effectively as other imperiled darters despite improving water quality over the past several decades.

PI Jay Stauffer from Penn State University studied the effects of the introduction of nonnative fish species in the Great Lakes on aquatic resources. Results show that the abundance of the non-native gobies had increased since they were first documented in Pennsylvania's waters of Lake Erie in 1996. In all of the cases studied, the number of native benthic fishes captured in the trawls decreased as the numbers of Round Gobies increased; highlighting that the spread of non-native species into the Great Lakes creates concerns for aquatic life and water quality.

PI Huichun Zhang from Temple University worked to develop quantitative models for the removal of emerging contaminants by cation exchange resins. Results showed that polymeric cation exchange resins can effectively remove cationic organic contaminants from contaminated water and the developed predictive model can accurately estimate sorption capacity of a diverse range of cationic contaminants at different pH.

Long-term Responses of Stream Chemistry to Changes in Atmospheric Deposition in Mid-Appalachian Forests of Pennsylvania

Basic Information

Title:	Long-term Responses of Stream Chemistry to Changes in Atmospheric Deposition in Mid-Appalachian Forests of Pennsylvania
Project Number:	2009PA120S
USGS Grant Number:	G09AP00118
Sponsoring Agency:	None
Start Date:	7/1/2009
End Date:	6/30/2014
Funding Source:	Supplemental
Congressional District:	5
Research Category:	Climate and Hydrologic Processes
Focus Category:	None, None, None
Descriptors:	
Principal Investigators:	Elizabeth W. Boyer

Publications

1. Boyer, E.W., J.W. Grimm, K.S. Horner, J.S. Lynch, and M.A. Borden (2010). Atmospheric Deposition in Pennsylvania: Spatial and Temporal Variations 2009. Report prepared for the Pennsylvania Department of Environmental Protection by the Pennsylvania Water Resources Research Center, 255 p.
2. Boyer, E.W., J.W. Grimm, K.S. Horner, and M.A. Borden (2010). Atmospheric Mercury Deposition in Pennsylvania in 2009. Report prepared for the Pennsylvania Department of Environmental Protection by the Pennsylvania Water Resources Research Center, 65 p.
3. Boyer, E.W., J.W. Grimm, K.S. Horner, J.S. Lynch, and M.A. Borden (2010). Atmospheric Deposition in Pennsylvania: Spatial and Temporal Variations 2009. Report prepared for the Pennsylvania Department of Environmental Protection by the Pennsylvania Water Resources Research Center, 255 p.
4. Boyer, E.W., J.W. Grimm, K.S. Horner, and M.A. Borden (2010). Atmospheric Mercury Deposition in Pennsylvania in 2009. Report prepared for the Pennsylvania Department of Environmental Protection by the Pennsylvania Water Resources Research Center, 65 p.
5. Boyer, E.W., J.W. Grimm, K.S. Horner, J.S. Lynch, and M.A. Borden (2010). Atmospheric Deposition in Pennsylvania: Spatial and Temporal Variations 2009. Report prepared for the Pennsylvania Department of Environmental Protection by the Pennsylvania Water Resources Research Center, 255 p.
6. Boyer, E.W., J.W. Grimm, K.S. Horner, and M.A. Borden (2010). Atmospheric Mercury Deposition in Pennsylvania in 2009. Report prepared for the Pennsylvania Department of Environmental Protection by the Pennsylvania Water Resources Research Center, 65 p.

7. Boyer, E.W., J.W. Grimm, K.S. Horner, J.S. Lynch, and M.A. Borden (2010). Atmospheric Deposition in Pennsylvania: Spatial and Temporal Variations 2009. Report prepared for the Pennsylvania Department of Environmental Protection by the Pennsylvania Water Resources Research Center, 255 p.
8. Boyer, E.W., J.W. Grimm, K.S. Horner, and M.A. Borden (2010). Atmospheric Mercury Deposition in Pennsylvania in 2009. Report prepared for the Pennsylvania Department of Environmental Protection by the Pennsylvania Water Resources Research Center, 65 p.

USGS 104S PROJECT UPDATE FY2014

PROJECT TITLE & PRINCIPAL INVESTIGATOR

Long-term Responses of Stream Chemistry to Changes in Atmospheric Deposition in Mid-Appalachian Forests of Pennsylvania

Elizabeth W. Boyer, Department of Ecosystem Science & Management, Pennsylvania State University

PRINCIPAL FINDINGS AND SIGNIFICANCE

Acidic atmospheric deposition has impacted forests and waterways of the northeastern United States; largely attributed to emissions of compounds to the atmosphere stemming from fossil fuel combustion. As forested ecosystems of the eastern USA continue to adjust to dynamic changes in atmospheric deposition, long term monitoring (LTM) is critical in order to understand effects on water quality. This project continues long-term observations within 5 acid-sensitive forested watersheds (known as the LTM watersheds) within the Mid-Appalachian region of Pennsylvania.

Acidic atmospheric deposition can have serious effects on aquatic ecosystems. For example, acidified waters can impair the ability of fish gills to extract oxygen from water and change the mobility of certain trace metals (e.g., aluminum, cadmium, manganese, iron, arsenic, mercury), which in turn can place fish and other species sensitive to these metals at risk. Major negative impacts of acid deposition on forested watersheds that have been documented in various watersheds in the northeastern USA include: 1) Declines in pH of stream water which can lead to decreased biodiversity especially in fishes and macro-invertebrates; 2) Increases in dissolved inorganic aluminum concentrations in stream water which can lead to mortality of aquatic life; and 3) Leaching of base cations from soils which decrease the ability of a watershed to buffer itself from incoming acids.

Due to the Clean Air Act Amendments of 1990 and other policies, rates of acidic atmospheric deposition have greatly decreased in the eastern United States in recent decades. Questions remain about whether acid-sensitive forested watersheds are able to recover from the legacy of acid deposition, and the length of time that it will take for recovery to occur.

The susceptibility of a water body to acidification depends on the ability of the water and catchment soils to neutralize the acid deposition it receives. The best measure of this ability is acid neutralizing capacity (ANC), which characterizes the amount of dissolved compounds that will counteract acidity. Every body of water has a measurable ANC, which depends largely on the surrounding catchment's physical characteristics, such as geology, soils, and size. The ANC of a body of water reflects the relative proportions of positive and negative ions entering the water from sources such as atmospheric inputs and the soil and bedrock surrounding and underlying the water body. The higher the ANC, the more acid a water body can neutralize and the less susceptible it is to acidification. Considering long term results over the past three decades in the five study catchments, gradual decreases in stream sulfate

and increases in ANC levels in streams have been noted, largely in response to the reductions in emissions associated with the Clean Air Act Amendments of 1990.

In recent years, we have completed synoptic sampling of stream segments throughout three forested, headwater catchments under summer base-flow conditions, and have completed the data analysis in FY14. While all three watersheds have been subject to heavy loadings of acid rain in recent decades, two of the watersheds are poorly buffered and thus acid-sensitive (LTM watersheds), while one of the watersheds is well buffered and thus acid-tolerant (non LTM watershed). Results for the two LTM acid sensitive watersheds indicate that some chemical recovery (i.e. increased pH, decreased aluminum concentrations) has occurred in the streams. However, the data show that these watersheds are still chronically acidified in terms of the ANC of the stream waters, which has not decreased. This response is associated with the fact that base cations and strong acid anions dissolved in streamflow both decreased over time, and thus ANC did not improve. The two acid-sensitive LTM streams thus still show high risk of episodic acidification, and biological recovery has not yet occurred. Results show that the acid tolerant watershed is able to support acid sensitive aquatic life and high water quality even though it is subjected to similar depositional loads as more sensitive watersheds. The well buffered watershed sampled in this study is at little risk of becoming acidified at any point throughout the year.

During FY14, we also continued to measure the basic stream chemistry and stream flow with monthly, base flow sampling in the 5 acid-sensitive LTM watersheds of Pennsylvania (considered to be the “LTM” or long term monitoring streams), to further establish a record of change. The project is ongoing and is not yet complete.

STUDENTS & POSTDOCS SUPPORTED

Brendan Reed, MS Student in Forest Resources; Department of Ecosystem Science and Management; Pennsylvania State University.

PUBLICATIONS

Reed, Brendan. Water Quality of Forest Streams in Pennsylvania Impacted by Acidic Atmospheric Deposition. Master's Thesis, Pennsylvania State University.

PRESENTATIONS

Boyer E, J Grimm, K Eklof, L Iavorivska, P Drohan, J Bennett, and C Grant. Effects of climatic variability and land use on atmospheric deposition, with implications for water quality in forested catchments of the Northeastern United States. UCOWR/NIWR/CUAHSI Conference on Water Systems, Science, and Society Under Global Change. Medford, MA, June 2014.

Reed BC and EW Boyer. Water Quality of Three Forest Streams in Pennsylvania Impacted by Atmospheric Deposition. UCOWR/NIWR/CUAHSI Conference on Water Systems, Science, and Society Under Global Change. Medford, MA, June 2014.

(Invited) Boyer EW. Water Quality in Catchments of the Northeastern USA: Toward Understanding Impacts of Atmospheric Deposition and Climatic Variability. Department of Earth Sciences Seminar Series, Uppsala University, December 3, 2014.

DeWalle D, EW Boyer, and AR Buda. Relationships Between Long-term Atmospheric Wet Deposition and Stream Chemistry in Mid-Appalachian Forest Catchments. American Geophysical Union, San Francisco, CA, December 2014.

PHOTO(S) OF PROJECT



This forested, headwater stream in the mid-Appalachian region of Pennsylvania is poorly buffered and acid sensitive. In response to decreases in acidic atmospheric deposition, this stream has shown partial chemical, yet not biological recovery to date.

Developing A Treatment Option for Marcellus Shale Wastewater Through Cultivation of the Algae Dunaliella salina (year 2)

Basic Information

Title:	Developing A Treatment Option for Marcellus Shale Wastewater Through Cultivation of the Algae Dunaliella salina (year 2)
Project Number:	2013PA208B
Start Date:	4/15/2014
End Date:	2/28/2015
Funding Source:	104B
Congressional District:	14
Research Category:	Engineering
Focus Category:	Wastewater, Water Use, None
Descriptors:	None
Principal Investigators:	Kyle Bibby

Publications

There are no publications.

FINAL PROJECT REPORT

Pennsylvania Water Resources Research Center

PROJECT TITLE: Developing A Treatment Option for Marcellus Shale Wastewater through Cultivation of the Algae *Dunaliella salina*

PRINCIPAL INVESTIGATOR: Kyle Bibby, Assistant Professor, University of Pittsburgh, Department of Civil and Environmental Engineering, bibbykj@pitt.edu, 412-624-9207

PROBLEM and RESEARCH OBJECTIVES

Disposal of produced water from hydraulic fracturing is challenged with high salt and radionuclides concentration. New regulations limit the acceptance of produced water in municipal wastewater treatment plants¹. Current disposal approach is deep well injection; however, some regions (e.g. Pennsylvania) do not have sufficient capacity for this method¹. Moreover, recently induced seismicity has been associated with deep well injection². Our objective is to use halophilic microalgae *Dunaliella salina* to remove salt and radionuclide content through extracellular polymeric substances (EPS) produced by the algae during cultivation and biosorption to enable produced water reuse in future hydraulic fracturing operations or reduce treatment requirements for surface disposal.

METHODOLOGY

***D. salina* growth and salt removal experiments**

D. salina growth was determined with optical density (OD) measurement (at 680 nm via Genesis UV-Vis, Model G10S Spectrophotometer) and cell counting (Hemocytometer). Results of the experiments revealed that optical density measurement is a better method for the determination of *D. salina* growth compared to cell counting since in stress conditions (high salt concentrations and lack of nutrients), *D. salina* cells tend to aggregate and make the cells impossible to count individually under microscope.

Experiments were conducted in 50,000 mg/L NaCl media and synthetic produced water (50,000 mg/L NaCl+CaCl₂ media) (Figure 1) as well as real produced water. A control group was provided with growth media (2X Erdschreiber's media, UTEX Culture Collection, TX). All experiments performed with biological triplicates. In order to maintain optimal growth conditions, pH was adjusted to 7.5-8 with NaOH in real produced water, once every three days.

Conductivity of the samples was measured with a conductivity meter (Accumet AP75, Fisher Sci.) before and after the synthetic produced water experiments in order to determine salt uptake performance of extracellular polymeric substances (EPS) produced during the growth of *D. salina*. Similarly, in the real produced water experiments, atomic absorption spectrophotometer (AAS, Perkin Elmer 1000) was used to analyze the chemical content of the produced water before and after experiment to determine any removal of dissolved salts and metals in the samples after they were exposed to algae growth.

Evaluation of radium removal

Biosorption experiments

Radium removal with fresh and autoclaved *D. salina* biomass was investigated for the effect of pH, TDS, biomass, calcium, and initial radium concentration. Algae biomass was harvested at late exponential phase (optical density of 8 at 680 nm) and centrifuged in 1.5 mL centrifuge tubes at 4°C, 10 000 rpm for 15 min. Algae pellets were washed with DI water three times. Harvested biomass was autoclaved for 15 minutes and stored at 4°C to be used in respective experiments. Dry weight of harvested biomass was measured and 0.04 g/L dry weight was used in experiments unless otherwise stated. Experiments were conducted in 50 mL volume and polypropylene falcon tubes were used to minimize radium uptake on the container surface. RaCl₂ (Ra-226 source) was obtained from Pennsylvania State University and a stock solution of 1155±14.5% was prepared and measured with a gamma spectrometer with a high-purity germanium detector (Canberra BE 202). A radium concentration of 3000 pCi/L was used in the experiments. After the radium addition, samples were shaken at 180 rpm for 5 minutes and centrifuged at 10 000 rpm for 5 minutes. pH of the samples noted as 5±0.3 after the addition of RaCl₂. Contact time was determined as 6 hours. Biomass was added and samples were shaken at 150 rpm for 6 hours. Controls were provided with no biomass addition. Radium uptake was calculated by:

$$q = \frac{(C_0 - C_s) * V}{M}$$

where; C₀ = Control concentration at the end of contact time (6 hours),

C_s = Sample at the end of contact time (6 hours),

V = Sampling volume,

M = Dry biomass weight corresponding to sampling volume.

Radium concentration of the real produced water samples was measured with gamma spectrometry and liquid scintillation method together with barium sulfate precipitation was used in all the remaining experiments. Experiments were conducted with biological triplicates and technical replicates in liquid scintillation method. Biological replicates were conducted for measurements with gamma spectrometry.

BaSO₄ precipitation method and liquid scintillation chromatography (LSC)

A sample volume of 10 mL was put in 1.5 mL micro-centrifuge tubes and centrifuged at 10 000 rpm, 4°C for 7 minutes. Supernatant was withdrawn carefully into 15 mL polypropylene falcon tubes. Samples were then poured into 50 mL Teflon beakers (Chemglass Life Sciences). 1.82 mL of 20 mM BaCl₂·2H₂O (2746 mg/L) solution was added to have 5 mg barium in the sample. 20 mL of 1 M H₂SO₄ was added to the mixture and heated at 50°C for an hour on a heater table to allow BaSO₄-Ra precipitate formation. 0.45 µm mixed cellulose ester membrane filter paper (MF-Millipore, HAWG) was used to collect the BaSO₄-Ra precipitate. 2 mL of 0.25 M EDTA (pH

adjusted to 9 with HCl) was used to transfer the precipitate from filter to 20 mL glass vials (Econo glass, Perkin Elmer). Taps of the glass vials were then loosely screwed and vials were heated at 50°C until the solution becomes clear. After the solution cooled down, 14 mL of Ultima Gold universal LSC cocktail added and samples were counted by LSC for 60 min in the specific energy range (170–230 keV) to select for dominant radium-226 count.

Gamma spectrometry measurements

A high resolution Apex Gamma spectrometry system (Ortec) with a high-purity germanium (HPGe) detector (Canberra BE 202) was used to quantify the radionuclide activities. Following biosorption experiments, samples were evaporated into dryness in oven at 120°C. Residuals were then transferred into 47 mm diameter petri dishes (Millipore) and sealed with gas seal tape. The thickness of all samples in petri dish was controlled to less than 1 mm (2 gram dry weight) to minimize the impact of geometry on the accuracy of gamma-spectrometry measurement. Ra-226 activity was directly quantified by measuring gamma ray emission at 186 KeV peak.

PRINCIPAL FINDINGS AND SIGNIFICANCE

D. salina growth in simulated and real produced water

During algae cultivation experiments decreased algae growth was observed in 50,000 mg/L NaCl medium and synthetic produced water (50,000 mg/L TDS NaCl + CaCl₂) compared to control conditions (Figure 1). This result could suggest possible inhibitory effect of calcium on *D. salina*. Moreover, cultivation in real produced water did not demonstrate promising algae growth for practical purposes (Figure 2).

Synthetic produced water experiments showed that there is no decrease in the conductivity of the samples after algae cultivation. However, in real produced water experiments, AAS analysis revealed that there is up to 15, 27 and 33% removal of calcium, strontium and manganese, respectively. Results shown in Figure 3 were averaged from replicated experiments. Although iron removal appeared to be significant, initial concentration of iron in the produced water was very low (<1 ppm).

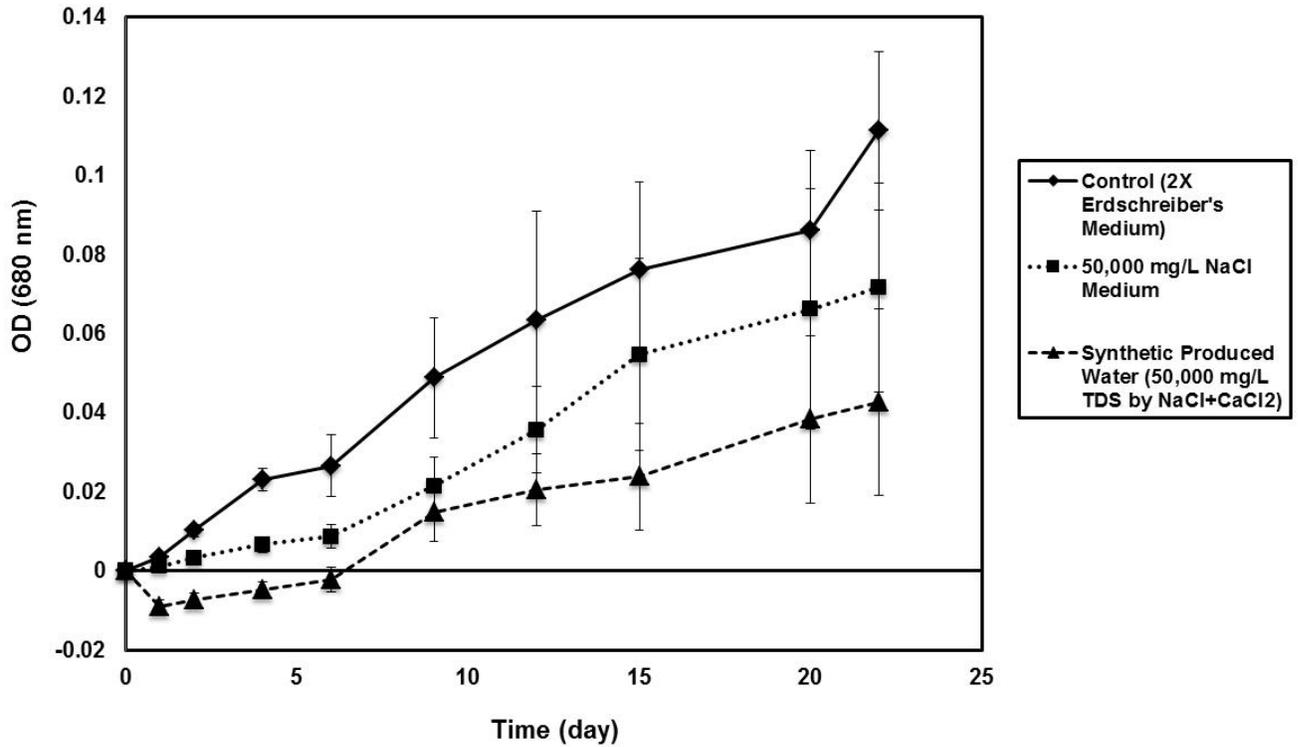


Figure 1. Comparative *D. salina* growth curves represented as optical density (680 nm) as a function of time

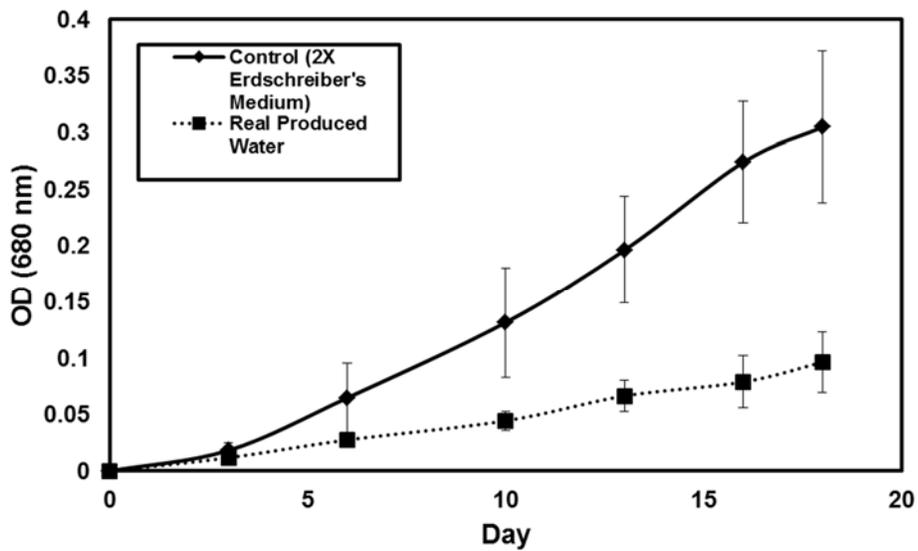


Figure 2. *D. Salina* growth with control and real produced water, (pH adjusted)

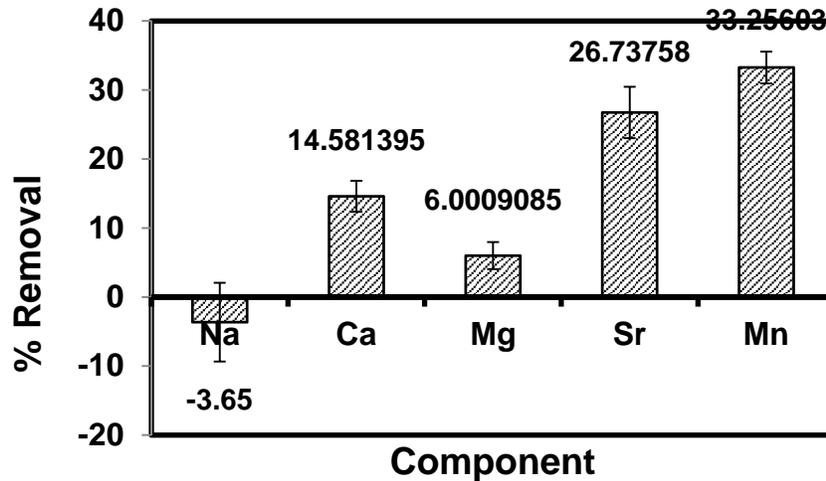


Figure 3. Removal of components in real produced water after *D. salina* growth

Radium biosorption experiments

Experiments were conducted with fresh and autoclaved algae biomass demonstrated higher radium uptake with autoclaved biomass than with fresh biomass; thus, autoclaved biomass results are included for evaluation.

Radium biosorption experiments with *D. salina* biomass were conducted in deionized water to determine the effect of pH, TDS, calcium (as a competing ion), initial radium and biomass concentration. pH experiments showed that radium uptake on the walls of the polypropylene container increases with increasing pH, as previously shown by previous work³ conducted with glass and polyethylene containers, reaching to a maximum at pH 9. Figure 5 shows radium concentration detected in controls. Radium concentration at pH 9 is 1180 pCi/L although initial radium concentration was 3000 pCi/L. Thus a slightly acidic pH, pH= 5 was selected for future experiments. Following the addition of RaCl₂ in deionized water samples, a pH value of 5±0.3 was achieved.

Simulated produced water experiment, where effect of TDS concentration was evaluated showed decreasing radium uptake with increasing salt concentration. Effect of TDS experiment showed 14,466 pCi/g_{biomass} radium uptake at 50,000 mg/L TDS concentration (Figure 6); however, experiments in real produced water with a TDS concentration of 54,915 mg/L showed only 3935 pCi/g_{biomass} radium uptake (Table 1). These results together with calcium ion effect experiment with fresh biomass (Figure 9) suggest probable common ion effect in real produced water that competes with radium for available biosorption sites. Similarly, real produced water experiments also showed a decreasing percent radium removal as TDS of the sample increases (Table 1). Radium uptake increased in the real samples with increasing salt concentration. This is probably due to increasing initial radium concentration in the samples, which is similar to the effect seen in Figure 7. No radium uptake detected in Sample 1.

Figure 8 shows radium uptake as a function of increasing biomass concentration. As expected, radium uptake decreases and percent removal increases as biomass concentration increases in the samples.

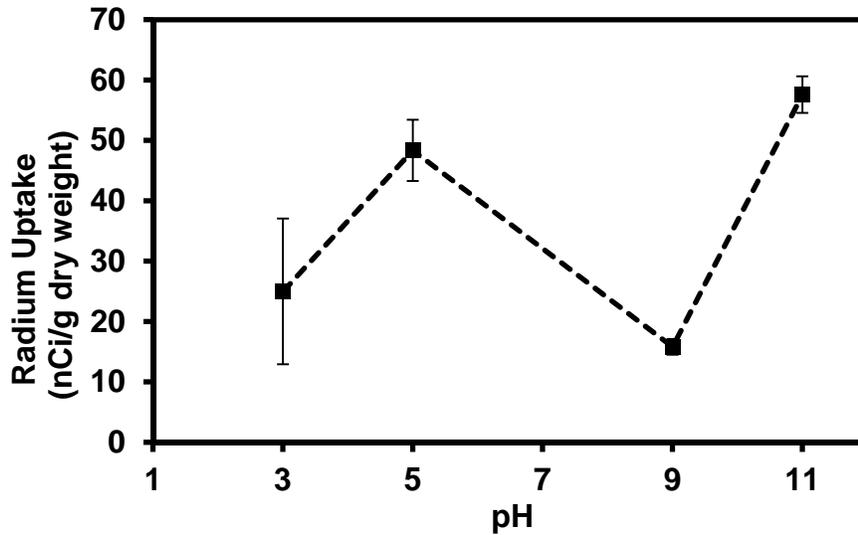


Figure 4. pH effect on radium biosorption

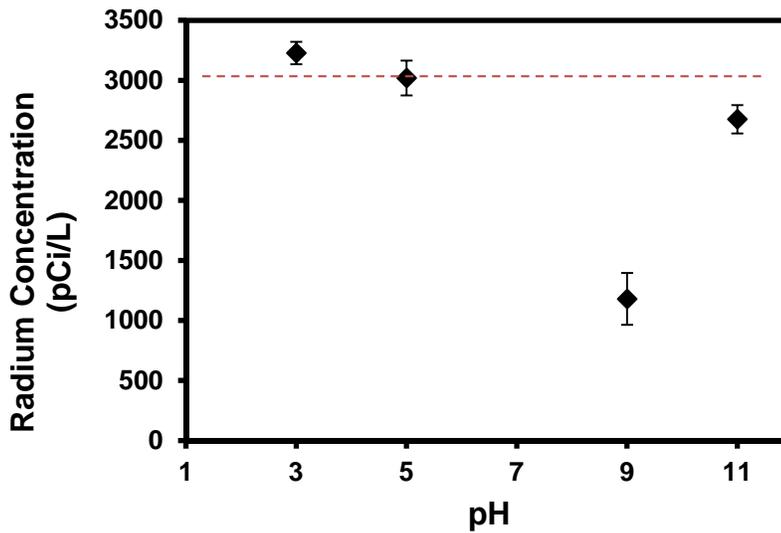


Figure 5. Radium concentration in control conditions with changing solution pH (Initial radium concentration is constant = 3000 pCi/L)

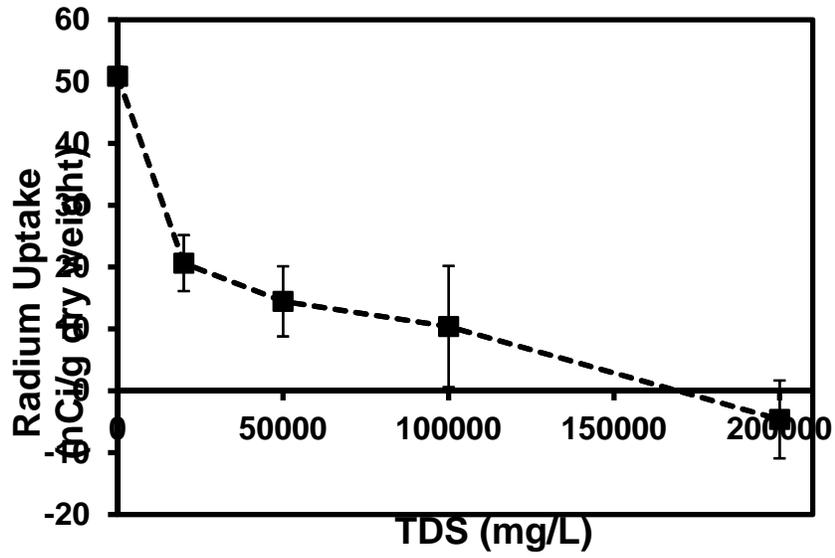


Figure 6. Effect of salt concentration on radium biosorption

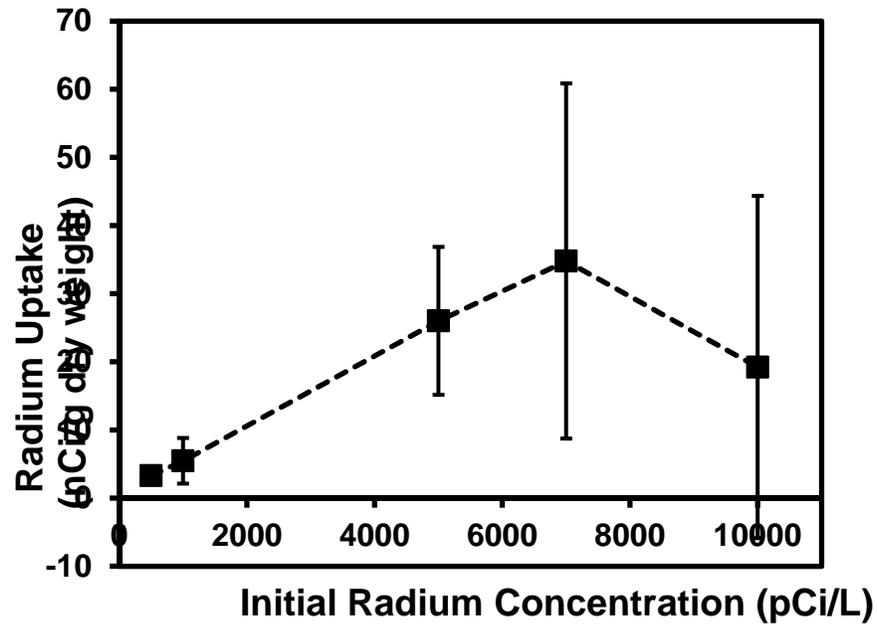


Figure 7. Radium uptake as a function of initial radium concentration

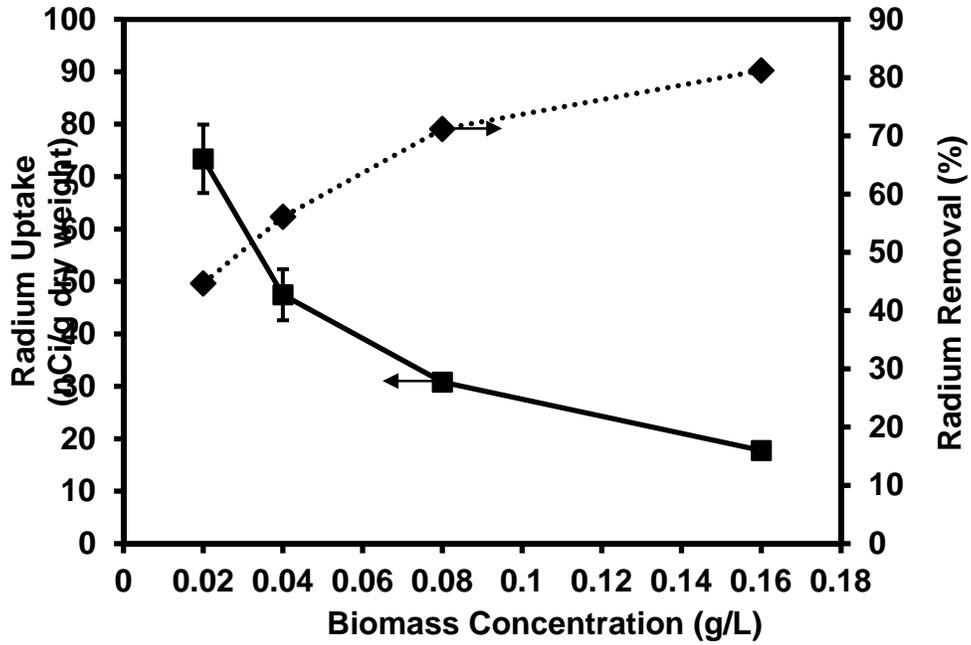


Figure 8. Radium uptake as a function of biomass concentration

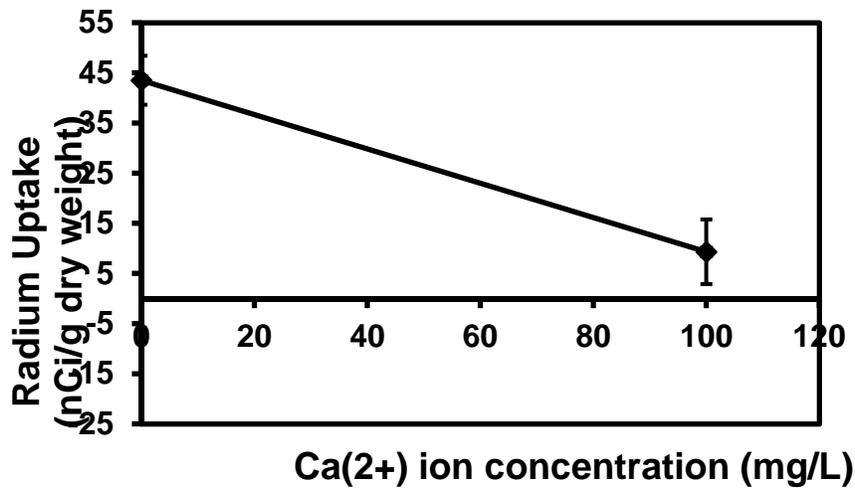


Figure 9. Competing ion effect of calcium on radium biosorption

Table 1. Radium biosorption in real produced water

Sample	S1 (Day 1)	S2 (Day 5)	S3 (Day 15)
TDS (mg/L)	17785	54915	94005
Cl (mg/L)	6852	29653	52640
Ba (mg/L)	70	1405	2687
Sr (mg/L)	46	651	1127
Ca (mg/L)	349	3938	6292
Mg (mg/L)	39	381	630
pH	7.4	7.4	7.5
Ra-226 (pCi/L)	874±215	1430±340	3330±595
Ra-226 Uptake (pCi/g dry weight)	n.d.	3934.55	5764.15
Ra-226 Removal (%)	-	9.63	6.67

**n.d. Uptake not detected*

STUDENTS & POSTDOCS SUPPORTED

Name: Benay Akyon (Primary student on project)

Major: Environmental Engineering

Degree: Graduate Student, Ph.D.

Name: Elyse Stachler

Major: Environmental Engineering

Degree: Graduate Student, Ph.D.

Name: Arthur Moncrieffe

Major: Environmental Engineering

Degree: Graduate Student, MS

PUBLICATIONS

A peer-reviewed journal article is expected to be submitted by the end of August, 2015.

Tentative title: Radium Biosorption in Saline Wastewater by the Halophilic Algae *Dunaliella salina*. Benay Akyon, Kyle Bibby. To be submitted August, 2015.

INFORMATION TRANSFER ACTIVITIES

Poster presentation at the ASCE Shale Energy Engineering Conference student poster session with the title of “*Biosorption mechanisms of Dunaliella salina as a treatment technology for high salinity wastewaters*”.

Poster presented at Pitt SSOE CEE Graduate Student Research Day.

AWARDS & ACHEIVEMENTS

Benay Akyon won the runner-up at the ASCE Shale Energy Engineering Conference student poster competition with the title of “*Biosorption mechanisms of Dunaliella salina as a treatment technology for high salinity wastewaters*”

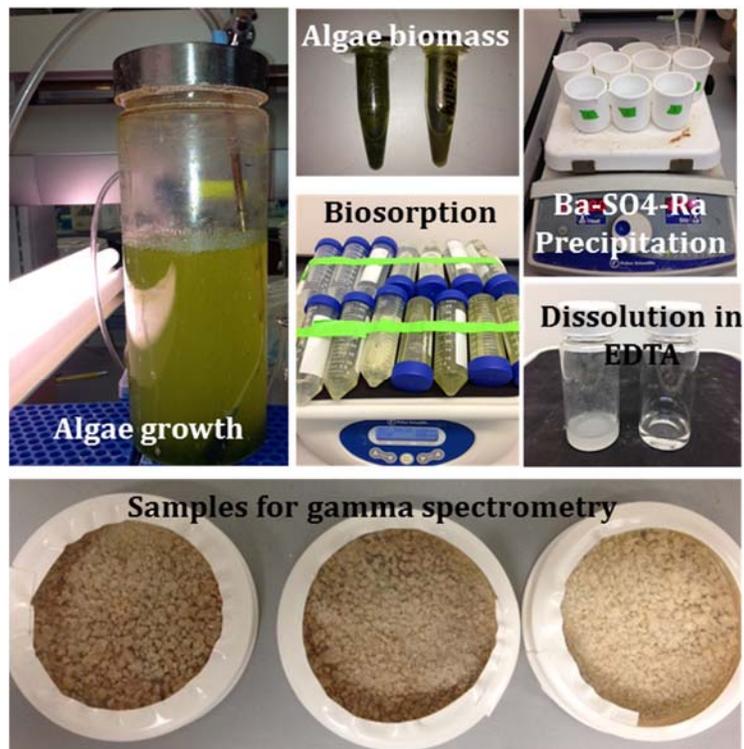
REFERENCES:

1. Lutz, B. D.; Lewis, A. N.; Doyle, M. W., Generation, transport, and disposal of wastewater associated with Marcellus Shale gas development. *Water Resources Research* 2013, 49, (2), 647-656.
2. Keranen, K.; Weingarten, M.; Abers, G.; Bekins, B.; Ge, S., Sharp increase in central Oklahoma seismicity since 2008 induced by massive wastewater injection. *Science* 2014, 345, (6195), 448-451.
3. Tsezos, M.; Keller, D., Adsorption of radium-226 by biological origin absorbents. *Biotechnology and bioengineering* 1983, 25, (1), 201-215.

PHOTOS OF PROJECT



Experimental set-up for the *D. salina* growth experiments



Experimental scheme for radium biosorption experiments

Range Expansion and Genetic Population Structure of Five Pennsylvania State Threatened Fish Species using Environmental DNA (eDNA) and Molecular Genetic Techniques (year 2)

Basic Information

Title:	Range Expansion and Genetic Population Structure of Five Pennsylvania State Threatened Fish Species using Environmental DNA (eDNA) and Molecular Genetic Techniques (year 2)
Project Number:	2013PA209B
Start Date:	4/15/2014
End Date:	2/28/2015
Funding Source:	104B
Congressional District:	14
Research Category:	Biological Sciences
Focus Category:	Conservation, Methods, Management and Planning
Descriptors:	None
Principal Investigators:	Brady Porter

Publications

There are no publications.

FINAL PROJECT REPORT REQUEST

Pennsylvania Water Resources Research Center

PROJECT TITLE & PRINCIPAL INVESTIGATOR

Range expansion and genetic population structure of five Pennsylvania State threatened fish species using environmental DNA (eDNA) and molecular genetic techniques.

Brady A. Porter, Associate Professor of Biological Sciences, Duquesne University, porterb@duq.edu, 600 Forbes Ave., Pittsburgh, PA 15282, phone: (412) 396-5786.

PROBLEM and RESEARCH OBJECTIVES

The upper Ohio, lower Allegheny, and lower Monongahela Rivers are classified as recovering systems, so there is need to accurately monitor and document recovery of the fish fauna and track state threatened and imperiled species. Unfortunately, traditional sampling methods for large riverine habitats can be inefficient when sampling for imperiled species that are low in abundance. The current Pennsylvania State mandated surveying method for benthic fish in large river environments is electrified-benthic trawling which is costly, labor intensive, potentially dangerous to the operators, and often harms the fish by abrasion from the sediment and debris entering the trawl. This is particularly problematic when sampling for imperiled species that are already low in abundance. Another drawback to trawling is that it doesn't survey pelagic species.

Environmental DNA (eDNA) sampling has the potential to be a versatile, rapid and less invasive technique to detect the presence of imperiled aquatic species without the limitations of traditional sampling methods. eDNA sampling for fish fauna has advantages over traditional sampling methods because 1) aquatic environments keep DNA suspended in the water column and therefore, rare species can be detected without ever being seen 2) fish don't need to be removed from their environment to take a sample, and 3) the eDNA detection technique has been shown to work using water samples from all types of habitats (e.g. ponds, wetlands, rivers, and streams) with the ability to detect many types of organisms – fish, frogs, salamanders, mammals, crustaceans, and insects [1-4]. Ultimately, these advantages result in further benefits that include accurate target species identification, enhanced species detection sensitivity, and an overall reduction in sampling costs [5] that will prove beneficial to all agencies responsible for monitoring aquatic environments (e.g. PA. Fish & Boat Commission, PA DEP, environmental consultants and the scientific community).

The objectives of this study were to 1) develop and validate an aquatic eDNA assay for imperiled darter species detection, and 2) use a combination of traditional surveying techniques to monitor range expansion and genetic population structure of five species of darters in western Pennsylvania (PA) that have rankings ranging from state threatened to critically imperiled (three in the subgenus *Nothonotus*: bluebreast darter - *Etheostoma camurum*, spotted darter - *Etheostoma maculatum*, Tippecanoe darter - *Etheostoma tippecanoe*, and two in the genus *Percina*: gilt darter - *Percina evides* and river darter - *Percina shumardi*).

Our first specific aim was to develop cutting-edge eDNA methods to detect the presence of the imperiled target species from water samples as they expand their geographic range in the upper Ohio River system. eDNA can be defined as DNA that is extracted from environmental samples (e.g. water, soil, sediment) and is isolated before physical/visual detection of the target species [6]. The predominant sources of fish eDNA are derived from their urine, feces, and sloughing epidermal cells [1]. eDNA sampling has the potential to be a rapid and less invasive technique to detect the presence of state imperiled darter species and potentially for a variety of additional elusive aquatic species.

Surveys conducted since 2003 have revealed evidence of range expansion in both *Percina* and *Nothonotus* darters [7-11]. Other studies have shown that some of the threatened species were in close downstream proximity to L/D installations [7,12,13]. This information helped us formulate our next working hypothesis and second specific aim that range expansion of the five imperiled species is linked to critical habitat that has been created by the turbulence on the downstream side of L/D installations. Historically, these installations have been perceived as converting lotic ecosystems into a series of deep, continuous pools or lentic habitat. However, it appears as though sufficient turbulence has created habitat that the target species may be utilizing [8,14]. Their small populations and disjunct distributions are most likely due to habitat fragmentation from previous water quality degradation and the navigational lock and dam system [7,15-19]. We used electrified-benthic trawling (2 minute sampling effort/trawl) downstream of ten L/D installations on the Ohio, Beaver, Allegheny, and Monongahela Rivers and backpack electrofishing (in nine adjacent tributaries) to collect data for aim 2 that were used to 1) validate eDNA findings 2) identify critical habitat within the large river environment, and 3) identify tributaries that are inhabited by the target species and potentially used for spawning.

Darter populations in the upper Ohio River system are at their eastern most range and show disjunct distributions. They appear to exist within their fragmented habitat as metapopulations (i.e. many large and small spatially isolated subpopulations). With our third aim (still in progress) we will use microsatellite DNA analysis on confirmed samples to determine the genetic population structure and metapopulation dynamics of the imperiled species to prioritize conservation management efforts. We hypothesize that metapopulation models can be developed to identify source populations, determine levels and direction of gene flow, and track recent colonization events (new sink populations), in correlation to the locations of locks and dams to make more informative conservation management decisions. These models have recently been used in viability analysis for conservation management to describe colonization and extinction rates for individuals in fragmented habitat. Our target species' landscape genetics will be modeled using more appropriate and spatially realistic occupancy models such as the logistic regression model (LRM) and the incidence function model (IFM) [20]. Individuals will be genotyped using multi-locus microsatellite (nuclear, DNA repeat motifs) analyses. Ultimately, understanding metapopulation structure and dynamics will facilitate management strategies that prioritize conservation efforts toward genetically diverse source populations compared to smaller, genetically depauperate, and ephemeral sink populations.

METHODOLOGY

eDNA

Water Sampling

L/D Tail-water Sampling: A transect 50 to 150 m below the L/D installation was established as the starting point for seven evenly spaced trawls. At the head of each trawl, 2.0 L of water was collected from the bottom of the river using a sterilized Van Dorn bottle for a total of 14.0 L per site. Water samples were stored on ice in sterilized coolers and filtered at the lab within 24-h. Between L/D installation surveys, all equipment was decontaminated with a solution of 20% bleach and rinsed with deionized water.

Tributary Sampling: Just below the first riffle upstream from the confluence of the tributary and the river, six sub-surface grab samples were collected using sterile 1.0 L plastic bottles (total 6.0 L per site). All samples were taken prior to any personnel or equipment entering the water. All samples were stored on ice and returned to the lab for filtering within 24-h of collection.

Sample Processing, Filtering, and DNA Extraction

eDNA water samples were processed at Duquesne University's pre-PCR forensics laboratory inside of a UV sterilized biological hood within a dedicated pre-PCR room. The water samples (2.0 L per filter) were vacuum filtered using 0.7 μm sterile glass-fiber filters. Turbid samples (> 8.0 NTU) were filtered by stacking a 1.5 μm glass-fiber filter on top off the 0.7 μm filter to decrease pore clogging. Filters were placed into new storage bags and stored at -80 °C until extraction.

The two most commonly used eDNA extraction kits (as determined by a literature review - MoBio PowerWater DNA Isolation Kit and Qiagen DNeasy Blood and Tissue kit) were tested to compare total eDNA extraction efficiency by using three replicate samples for each kit and from two L/D installations. Both manufacturers' extraction protocols were modified to handle the logistics of processing glass micro-fiber filters. In this report, only data from Allegheny River L/D #6 will be discussed because three of the five target species (*E. camurum*, *E. tippecanoe*, and *P. evides*) were documented with electrified-benthic trawling at this location. Because of elevated turbidity levels the day of sampling, each 2.0 L water sample was filtered with a 1.5 μm filter stacked on top of a 0.7 μm filter to prevent filter clogging. Therefore, three water samples were processed with each kit with the 1.5 and 0.7 μm filters extracted separately. Extracted DNA was quantified with a Qubit Fluorometer (2.0) using a double stranded DNA broad range assay kit. Samples were prepared per manufacturers' specifications and read in triplicate. DNA concentrations were averaged and standard deviations calculated. Once robust polymerase chain reaction (PCR) primers are selected, both kits will be screened to determine which most efficiently removes PCR inhibitors.

Gene Selection, Primers, and Polymerase Chain Reaction (PCR)

The mitochondrial NADH Dehydrogenase Subunit 2 (*ND2*) gene was initially selected as the target gene for eDNA detection for several reasons. First, mitochondrial genes are multi-copy, which should increase chances of amplification. Next, the entire mitochondrial genome across nine species of darters was analyzed for variability, and it was determined that *ND2* was the most variable [21]. Lastly, primers (both sequencing and PCR) were developed and optimized by Ritchea [21] and Howell [9] which we anticipated would streamline our primer selection and

amplification process. GenBank deposited sequences of *ND2* for the target species were aligned using DNASTAR/Lasergene - MegAlign software (v 8.1.3) and analyzed for variable sites to develop multi-plex PCR primers. Alignment verified that darters from the sub-genus *Nothonotus* (bluebreast, tippecanoe, and spotted darters) and darters from the genus *Percina* were to be developed for multi-plex PCR primers respectively. Because eDNA in water samples is likely to be fragmented and degraded, amplicon sizes were designed to be within 150-200 base pairs (bp). We hypothesized that target species detection utilizing fragment analysis of PCR product would be more sensitive than traditional visual, agarose-gel based assays. Therefore, to reduce stochastic PCR bias and to detect multiple species in one PCR reaction, primer sets were manually (i.e. visually using sequence alignments) designed with conserved reverse primers and species-specific forward primers uniquely labeled with fluorescent dyes. Each 20.0 μL PCR reaction contained: 50 ng template DNA, 1x Phusion HF buffer, 400 μM dNTPs, 0.5 μM each primer, and 0.2 μL Phusion DNA Polymerase. PCR was performed on a BioRad C1000 Touch thermocycler under the following conditions: 1) 98 $^{\circ}\text{C}$, 0:30, 2) 98 $^{\circ}\text{C}$, 0:10, 3) 72 $^{\circ}\text{C}$, 0:30, 4) 72 $^{\circ}\text{C}$, 0:15, 5) repeat (steps 2-4) 34x, 6) 72 $^{\circ}\text{C}$, 10:00. eDNA PCR products were first visually screened on a 2% agarose gel containing 1% ethidium bromide. For fragment analysis, 2.0 μL of PCR product was mixed with 8.0 μL of LIZ 500 size standard/formamide (0.25 μL LIZ + 9.25 μL formamide per reaction) and then analyzed with an ABI 3130 genetic analyzer. Species detection was determined by the presence of a species-specific fragment size and fluorescent color.

Fragment Analysis Detection Sensitivity vs. Agarose Gel Visualization

Before we could confidently apply fragment analysis detection methods to eDNA field samples, individual and multi-plex PCR reactions were performed using genomic DNA extracted from *Nothonotus* darter fin clips. DNA was quantified with a Qubit dsDNA broad range assay quantification kit. DNA stocks (20 ng/ μL) were made for each species. A series of serial dilutions was made that reduced DNA concentrations by 50% in each dilution down to the lowest concentration of 39.6 pg/ μL (= 0.0396 ng/ μL). Using the serial dilutions, PCR was run with each species and their respective primer set, and one multi-plex PCR with all three *Nothonotus* species (1 μL of DNA/rxn).

L/D and Tributary Fish Sampling

Ten L/D installations on four river systems (Beaver River, Ohio River, Allegheny River, and Monongahela River) were sampled using a PSU (Penn State University) trawl, otherwise known as an electrified-benthic trawl. A transect 50 to 150 m below the L/D installation was established as the starting point for seven evenly spaced trawls. Each trawl consisted of two minutes of sampling effort. All fish species were identified and enumerated. Caudal fin clips were collected from target species and preserved in 100% ethanol for microsatellite genotyping analysis.

Tributaries within 1.0 km downstream of L/D installations were selected for sampling if the watershed size was $\geq 5.0 \text{ mi}^2$. This size standard was derived from previous sampling which documented that local tributaries within that size range are large enough to support darter populations, specifically, *Etheostoma* darters in the subgenus, *Nothonotus* [9]. Each tributary was electrofished for 100 m starting at the first riffle upstream of the confluence with the main river.

Genetic Population Structure Analysis and Metapopulation Dynamics

DNA was extracted from fin clips following standard phenol:chloroform extraction methods [22]. Twenty-two microsatellite loci previously developed in *Etheostoma scotti* [23] and *Etheostoma caeruleum* [24] were screened for PCR amplification in the bluebreast darter (*E. camurum*). Loci were screened by optimizing 12.0 μ L *Taq* polymerase PCR reactions with: 24-36 ng template DNA, varying $MgCl_2$ concentrations, and optimizing annealing temperatures. Loci exhibiting amplification were selected for further testing to confirm polymorphism. Loci polymorphism is currently being assessed using bluebreast darter DNA from local individuals and individuals previously sampled from Big Darby Creek in Ohio. The Pennsylvania and Ohio individuals will be genotyped using an ABI 3130 Genetic Analyzer and scored using GENESCAN software (Applied Biosystems Inc.) [23,25]. Genotyping data will be used to determine population structure with the Bayesian analysis software STRUCTURE, which uses multi-locus allelic frequency data to determine the probability of an individual's assignment to one population versus another. Loci that can properly distinguish our local bluebreast darters from the Ohio population will be selected and all bluebreast darters will be analyzed for various measurements of genetic diversity, gene flow, and divergence between populations using the software ARLEQUIN.

PRINCIPAL FINDINGS AND SIGNIFICANCE

eDNA Method Development

After eDNA water samples were collected and filtered, we tested several major components of eDNA method development before the processing of field samples could begin: 1) develop PCR protocols and confirm that the manually designed, species-specific PCR primers would not cross-amplify non-target species, 2) test the detection limits of fragment analysis to accurately detect PCR amplification using DNA from confirmed specimens versus visual detection with agarose gels, and 3) compare the DNA extraction efficiency of the two most commonly used eDNA extraction kits.

PCR Development and Testing

Initial screening of primer sets for target species within the genus *Percina* were not species specific and would need redesigned. *Nothonotus* primers were developed for multi-plex PCR. Initial screenings showed variable results for specificity. Therefore, we spent considerable time attempting to optimize the Phusion polymerase PCR and thermocycle conditions, and we even tested a *Taq* polymerase PCR assay to achieve species-specificity. Attempts were made to amplify eDNA from several field samples, but weak positive control PCR reactions further indicated that the primers were problematic. After exhausting all possibilities, we determined that new primers needed to be designed. New primers were designed by using the publically available software, Primer-BLAST (<http://www.ncbi.nlm.nih.gov/tools/primer-blast>) [26]. Primer-BLAST has the ability to design and screen species-specific primers against DNA sequences within the GenBank database or with custom DNA sequence databases to screen for specificity while following the thermodynamics of successful primer design. Because darters are the second most diverse clade of fish in North American [27] and readily hybridize [27,28], we

extended primer design to target species-specific primers within three mitochondrial genes (*ND2*, *COI*, and *Cytb*). The most recently published list of all species of Percidae documented within the Ohio River watershed of Pennsylvania [29] was referenced to build customized DNA sequence databases for all three genes using a combination of GenBank sequences and DNA sequences derived from Sanger sequencing local specimens. Customized primer design parameters were entered into Primer-BLAST with the customized databases used to check for species-specificity. The two highest ranking primer sets were selected for each target species and three positive control species. Therefore, a total of sixteen primer sets were designed and are currently being assessed.

Previous work by Thomsen [1,30] demonstrated that generalized fish mini-barcoding primers could be used to detect multiple fish species utilizing next generation sequencing (NGS) of aquatic eDNA. The high throughput capacity of NGS has the ability to overcome PCR primer bias and the potential to provide community level data on the entire fish community. Therefore, eDNA extracted from eight of our sample locations was PCR amplified using generalized mini-barcode *Cytb* primers published by Thomsen [30]. Samples were prepared for sequencing on the Illumina Mi-Seq platform. Sequencing was just completed and data is currently being processed.

Detection Limits of Fragment Analysis Using Genomic DNA

Individual PCR product visualized using 2% agarose gel electrophoresis showed that no product was visible below 625 pg DNA/reaction, Figure 1. Because agarose gel electrophoresis has limited resolution to detect closely sized amplicons, visualization of a multi-plex PCR reaction shows up as one band (data not shown). In contrast, using fragment analysis visualization of the fluorescently tagged amplicons (and closely sized amplicons) using Peak Scanner software, we confirmed that all three species could be detected in one multi-plex PCR reaction, Figure 2. Fragment analysis of the individual PCR reactions was variable (Peak Scanner images not shown):

E. tippecanoe PCR product was visible down to the lowest concentration even though product was not discernable on the agarose gel (Figure 1)

E. camurum PCR product was not visible below 1,250 pg/ μ L regardless of detection method

E. maculatum PCR product was detectable at all concentrations, but the negative control indicated contamination

The serial dilution assay supports the earlier conclusion that new primers needed developed. Under laboratory conditions, PCR amplification should be very robust and working efficiently in order to increase the chances of species detection from environmental samples. eDNA from water samples will likely possess very small quantities of target DNA in the presence of PCR inhibitors. Using funding from an additional source, aquarium experiments to determine eDNA detection levels using common darter species were conducted and will be processed once the new set of primers are screened for specificity.

Final conclusions from the fragment analysis experiments:

Fragment analysis detection of species-specific amplicons was more sensitive (species-dependent) than agarose gel visualization

Fragment analysis over comes the limitations of using agarose gel visualization for multi-plex PCR of closely sized, species-specific amplicons

Species-specific amplicons from multi-plex PCR were successfully detected with the ability to differentiate targets that are 1) only one base pair different in size and 2) utilizes unique fluorescent tags as a secondary measure for positive species-specific detection (Figure 2) Fluorophore strength varies - Emac-6FAM > Ecam-VIC > Etip-NED which confirms that the rarest species should be labeled with 6-FAM, but forward primer ratios between species need to be optimized to have similar detection efficiency

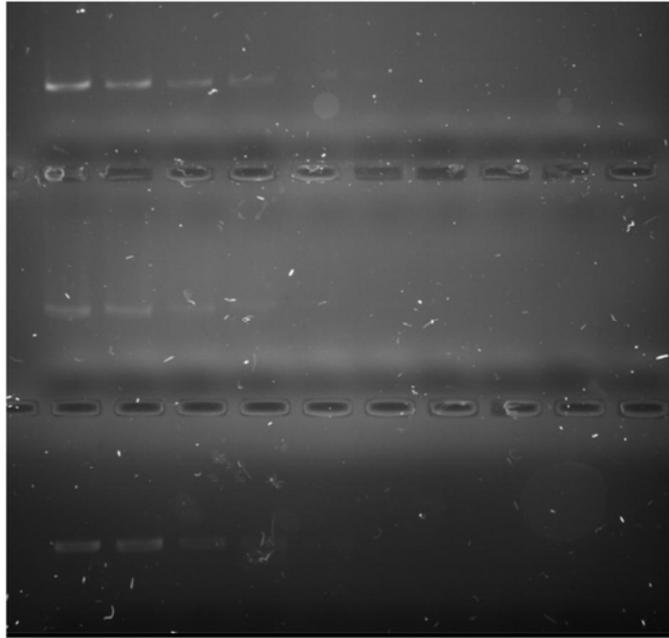


Figure 1. Agarose gel visualization of PCR product from individual PCR reactions in *Nothonotus darters*.

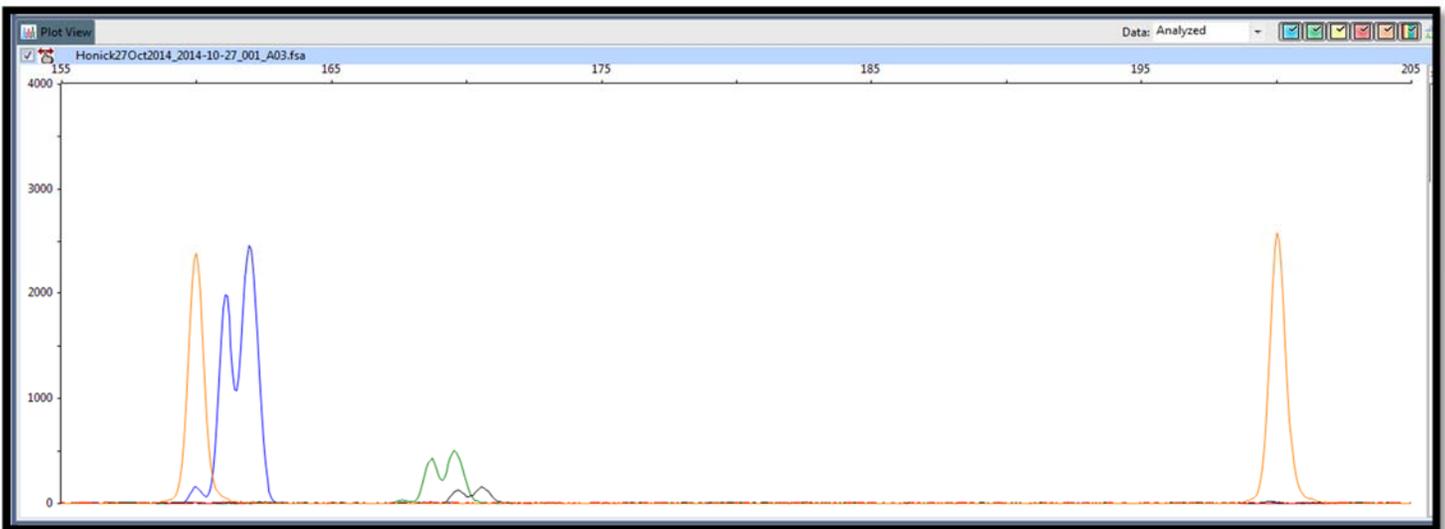
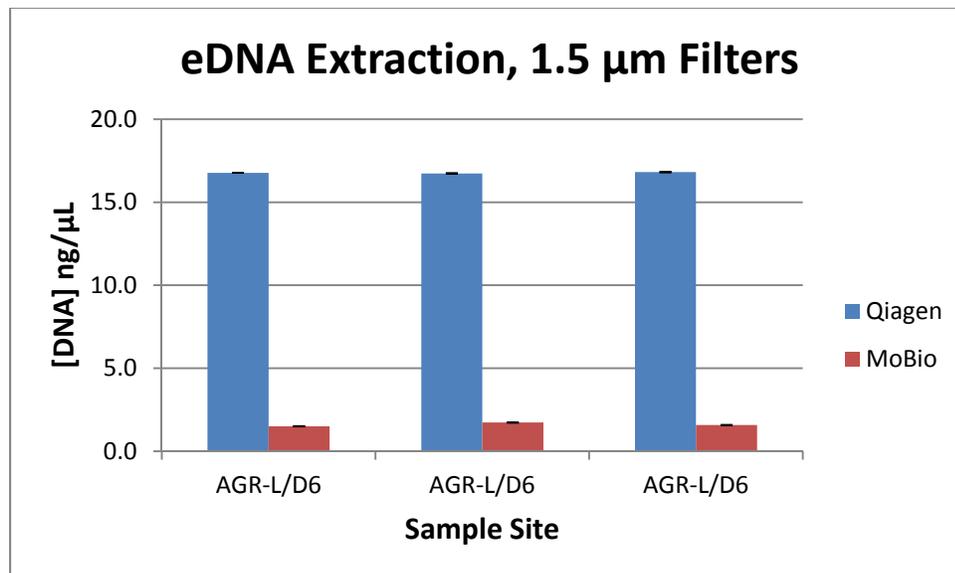


Figure 2. Fragment analysis electropherogram from Peak Scanner (v 2.0) illustrating simultaneous detection of all three species under multi-plex conditions: blue peak = spotted darter, green peak = bluebreast darter, black peak = Tippecanoe darter, orange peaks = LIZ 500 size standards

eDNA Extraction Kit Comparison

To increase the effectiveness of eDNA detection from water samples, DNA extraction methods should be optimized to provide maximum DNA yields. Therefore, the two most commonly cited eDNA extraction kits for processing aquatic eDNA samples were tested: MoBio PowerWater DNA Isolation and Qiagen Dneasy Blood and Tissue kit. Samples taken from below Allegheny River L/D #6 were chosen for the test because three of the five target species (*E. camurum*, *E. tippecanoe*, and *P. evides*) were documented at the location with electrified-benthic trawling. Regardless of filter pore size, the Qiagen kit extracted more DNA per 2.0 L of water than MoBio, Figure 3. As a general trend the 1.5 μm filters captured more DNA, Figure 3. However, the final elutions from each kit were visibly different with Qiagen extractions having a slight amber color indicating the potential presence of PCR inhibitors (e.g. melanin, or tannins) while the MoBio extractions appeared very clear. Once new primer sets have been selected, the extraction kits will be tested to see which most effectively reduces PCR inhibition.



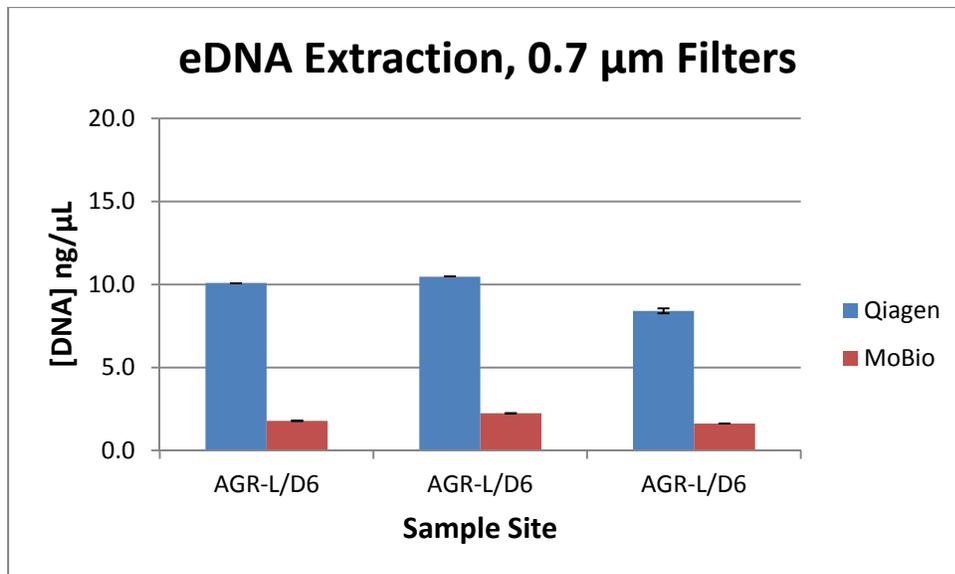


Figure 3. DNA extraction kit comparison showing the amount of total DNA extracted from glass-microfiber filters (error bars = standard deviation)

L/D and Tributary Fish Sampling

Sampling below ten L/D installations with electrified-benthic trawling to survey for big-river darters, and backpack electrofishing of nine adjacent tributaries, we documented 22 and 30 species of fish, respectively (data not shown). Summary of target species abundance indicates that *E. camurum* was the most abundant species in both the tributary and river sampling while *E. maculatum* was the least abundant with only one individual documented below L/D #3 on the Allegheny River, Table 1. Surveys conducted since 2003 have revealed evidence of range expansion in both *Percina* and *Nothonotus* darters [7-11]. Freedman [10] utilized Missouri benthic trawling in the upper Ohio River from Pittsburgh downstream to the Pennsylvania State border (survey completed in 2007) and documented the river darter, *Percina shumardi*, for the first time in Pennsylvania. In addition, they collected five spotted darters, *E. maculatum*, (below Dashields L/D) citing this as evidence for range expansion. In contrast, Argent and Kimmel [11] assessed benthic fish community structure both above and below fourteen L/D installations on the Allegheny and Monongahela Rivers, but did not document the presence of the spotted darter. Benthic trawling surveys in the Dashields and Montgomery pools of the Ohio River by Argent in 2011-2012, again, did not document any spotted darters (unpublished data). Most recently, Argent and Kimmel [31] surveyed 203 km of the Allegheny River (from 2007 – 2011), which included 122 km of the navigable section starting at Pittsburgh, PA upstream to L/D #9, and then 81 km upstream of L/D #9 to the confluence with French Creek. Utilizing a Missouri trawl within the navigable section and with sample sites spaced at approximately 1.6 km apart (a total of 6 min trawling effort/site), Argent and Kimmel [31] were unable to document the presence of any spotted darters. In the free-flowing section, the shallow depths of the river caused them to switch to backpack electrofishing 50-100 m sections spaced approximately 1.6 km apart. Regardless of the change in habitat and the free-flowing section's connectivity to the established spotted darter populations of French Creek and the headwaters of the upper Allegheny River [19], Argent and Kimmel [31] only documented four individuals within 81 km.

The conclusions from our fish surveys below ten L/D installations and nine adjacent tributaries are:

- We were unable to document the spotted darter in the Ohio River in the tail waters below the Montgomery or the Emsworth L/D, and we only found one spotted darter below Allegheny L/D #3. This suggests that the spotted darter may not be expanding its range as efficiently as our other target species and that they still occur in small, disjunct populations.
- Our data combined with the findings of Argent and Kimmel [11,31] suggests that classifying spotted darters as in a state of range expansion may be premature and warrants further investigation.
- Our data supports the concept of range expansion of the river darter up into the lower reaches of the Allegheny and Monongahela Rivers.
- The gilt darter, *Percina evides*, appears to be expanding its range down the Allegheny River and into the upper Ohio River. Previous surveys by Koryak [8], Argent & Kimmel [31], and Freedman [10] only document the gilt darter as low as pool #5 on the Allegheny River. We documented the gilt darter below L/D # 3, 4, & 5 on the Allegheny River, and nine individuals below the Emsworth backchannel L/D on the Ohio River.
- The bluebreast and Tippecanoe darters were the most abundant target species in both tributary and big-river surveys indicating that they are effectively expanding their ranges throughout the lower Allegheny and upper Ohio Rivers.

Table 1. Summary of target species abundance from tributary electrofishing and electrified-benthic trawling.

Tributaries Sampled						
Site	Tributary To:	Abundance				
		<i>E. camurum</i>	<i>E. tippecanoe</i>	<i>E. maculatum</i>	<i>P. evides</i>	<i>P. shumardi</i>
Bull Cr.	Allegheny R.	15	1	—	—	—
Chartiers Run	Allegheny R.	—	—	—	—	—
Deer Cr.	Allegheny R.	23	1	—	—	—
Kiskiminetas R., 1	Allegheny R.	10	25	—	—	—
Kiskiminetas R., 2	Allegheny R.	7	6	—	—	—
Taylor Run	Allegheny R.	—	—	—	—	—
Flaugherty Run	Ohio R.	—	—	—	—	—
Little Sewickley Cr.	Ohio R.	13	—	—	—	—
Montour Run	Ohio R.	2	—	—	—	—
Moon Run	Ohio R.	1	—	—	—	—
Electrified Benthic Trawling						
Site	Lock & Dam	Abundance				
		<i>E. camurum</i>	<i>E. tippecanoe</i>	<i>E. maculatum</i>	<i>P. evides</i>	<i>P. shumardi</i>
Allegheny River	L/D #2	4	2	—	—	1
Allegheny River	L/D #3	37	37	1	7	—
Allegheny River	L/D #4	56	41	—	20	—
Allegheny River	L/D #5	67	13	—	10	—
Allegheny River	L/D #6	23	43	—	15	—
Beaver River	Dam #1	3	—	—	—	—
Monongahela River	L/D #2	2	2	—	—	1
Monongahela River	L/D #3	—	—	—	—	—
Ohio River	Emsworth Emsworth	—	—	—	—	—
Ohio River	BC*	3	16	—	9	1
Ohio River	Montgomery	—	2	—	—	19

* Back Channel

STUDENTS & POSTDOCS SUPPORTED (

Anthony Honick, Biology, Ph.D. candidate, Duquesne University
Emily Shemanski, Biology, undergraduate, Duquesne University
Taylor Cutteridge, Biology, undergraduate, Duquesne University

PUBLICATIONS

Honick, Anthony; Brian Zimmerman, Mike Koryak and Brady Porter, *Range expansion of Nothonotus darters within the upper Ohio River watershed. In preparation.*

INFORMATION TRANSFER ACTIVITIES

Duquesne University Graduate Student Research Symposium, November 7, 2014: *Surveying Five Pennsylvania State Threatened Darters Using Traditional and Environmental DNA (eDNA) Techniques.* Anthony S. Honick and Dr. Brady Porter, poster #37

Honick, A.S. and Porter, B.A. *The design and implementation of environmental DNA (eDNA) techniques for threatened and endangered darters of the upper Ohio River watershed.* The American Fisheries Society, Pennsylvania Chapter – Spring Technical Meeting, April 25, 2014

AWARDS & ACHEIVEMENTS

Bayer Graduate Research Fellowship – \$11,837.50 (Fall Semester, 2014)

As a result of the active funding and the resulting research progress that has been achieved by the Ph.D. candidate, Anthony Honick.

PHOTO OF PROJECT



Identifying and enumerating fish caught below Allegheny River L/D #3 - the only location where the imperiled.

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Developing Quantitative Models for the Removal of Quaternary Ammonium Ions as Representative Cationic Emerging Contaminants by Cation Exchange Resins (year 2)

Basic Information

Title:	Developing Quantitative Models for the Removal of Quaternary Ammonium Ions as Representative Cationic Emerging Contaminants by Cation Exchange Resins (year 2)
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Principal Investigators:	Huichun Judy Zhang

Publications

There are no publications.

FINAL PROJECT REPORT

Pennsylvania Water Resources Research Center

PROJECT TITLE & PRINCIPAL INVESTIGATOR

Title: Developing Quantitative Models for the Removal of Quaternary Ammonium Ions as Representative Cationic Emerging Contaminants by Cation Exchange Resins

Principal Investigator: Huichun (Judy) Zhang, Ph. D. Department of Civil & Environmental Engineering, Temple University.

ONE-SENTENCE SOUND BYTE STYLE DESCRIPTION OF THE PROJECT OR FINDINGS

This research showed that polymeric cation exchange resins can effectively remove cationic organic contaminants from contaminated water and the developed predictive model can accurately estimate sorption capacity of a diverse range of cationic contaminants at different pH.

PROBLEM and RESEARCH OBJECTIVES

Many organic compounds are ionic in the aquatic environment. Aromatic amines such as substituted anilines, extensively used as pesticides, dyestuffs, and pharmaceuticals, can exist in aqueous solution as either cationic species under solution pH less than their pKa values or neutral species at pH greater than their pKa¹⁻⁴. Other industrially significant compounds like quaternary ammoniums, commonly found in effluents from disinfectant, surfactant, fabric softener, antistatic agent, and wood preservation industries, exist in the cationic form in wastewater effluents^{5, 6}. The toxic effects of cationic organic contaminants (COCs) on human health and the environment require their efficient removal from contaminated water^{2, 5-7}. Although many treatment methods for water and wastewater effluents containing organic compounds have been developed, including photocatalytic oxidation⁸, electrolysis⁹, adsorption⁷, oxidation¹⁰, biodegradation^{11, 12}, and membrane¹³, there are only limited attempts for COCs removal from aqueous environments.

Ion exchange resins that consist of polymeric matrices and different functional groups can effectively remove ionic organic compounds (IOCs)¹⁴⁻¹⁶ by exchanging with ionic solutes in solution. For cation exchange resins (CXR), the functional groups are mostly sulfonate or carboxylate groups^{17, 18}. Although activated carbon has been structurally modified for removing ionic compounds, it has shown lower selectivity and sorption capacity (about 100 times lower) than ion exchange resins in the removal of IOCs¹⁹. In addition, regeneration of the exhausted resin is attainable at ambient temperature with low attrition rates as opposed to activated carbon whose thermal regeneration process is costly and energy intensive with high attrition rates^{20, 21}.

Although sorption of IOCs by ion exchange resins is mainly a result of ion-exchange processes, it is also controlled by factors including hydrophobicity and types of functional groups of the solute, size and nature of the resin functional groups, dielectric constant of the solvent, and moisture content and matrix of the resin²²⁻²⁶. Several studies have shown that interactions between the nonpolar moieties (NPM) of an organic ion and those of the resin matrix determine the selectivity of the ion toward the resin^{23, 24, 27}. For example, selectivity of polystyrene anion

exchange resins for alkanesulfonic anions increased with increasing chain length of the anions²³; higher selectivity was observed for larger aliphatic quaternary ammonium cations toward polystyrenesulfonic CXRs^{28, 29}; and selectivity increased with increasing number of aromatic rings in the solutes due to the possibility of π - π interactions between the aromatic compounds and the resin²⁷. In addition, sorption reflects differences in interactions forces between solute-sorbent and solute-solvent, thus solvent-associated interactions (e.g. water-solute, water-sorbent, water-water interactions) have a profound effect on selectivity of sorption. For instance, sorption of ions with higher hydration energies or by resins with higher moisture contents is more difficult because expelling water molecules from the organic ions or from the resin matrix is more energy demanding²⁴⁻²⁶.

Poly-parameter linear free energy relationships (pp-LFERs) have been widely used for predicting partition behavior of neutral compounds from water to organic solvents^{30, 31} or sorption of neutral compounds by various sorbents^{32, 33} (eq 1). In the pp-LFERs method all possible interactions between a solute and a sorbent are considered in a general equation developed by Abraham³⁴⁻³⁷:

$$SP = eE + sS + aA + bB + vV + c \quad (1)$$

where SP represents a solvation property that is related to solute and solvent properties in a linear free energy relationship, and independent variables or descriptors E, S, A, B, and V account for the properties of the solute. E is the excess molar refraction accounting for nonspecific interactions due to London dispersive forces and Debye forces; V is McGown's characteristic molecular volume that accounts for cavitation energy and some additional nonspecific interactions; S is a combined dipolarity/polarizability descriptor; A and B are the overall solute hydrogen bond acidity and basicity; and c is a constant used to capture all other additional forces and might be entropy related³⁰. The regression coefficients e , s , a , b , v , and c are determined by multiple linear regression analysis and reflect differences in the interaction forces between the solvent and the sorbent³⁸.

Interactions that are responsible for sorption of organic ions by ion exchange should resins include the same nonspecific and specific (e.g. H-bonding, π - π) interactions for neutral solutes^{24, 39}. In addition, electrostatic interactions is an important part of the specific interactions^{40, 41}. To take into account any additional interactions in partition of an ion between two solvents, Abraham's group introduced new descriptors J^+ and J^- for cationic and anionic solutes respectively, where J^- is zero for cations and J^+ is zero for anions. Also, both descriptors are zero for neutral solutes⁴²⁻⁴⁴:

$$SP = eE + sS + aA + bB + vV + j^+J^+ + j^-J^- + c \quad (2)$$

Like other regression coefficients in eq 1, j^+ and j^- represent differences in the additional interaction forces between the two solvents.

Our recent work has successfully used the pp-LFERs approach to examine sorption of neutral organic solutes onto three non-ionic resins⁴⁵ or organic anions and neutral solutes onto three anion exchange resins⁴⁶. Despite these, only a very limited number of studies have reported the uptake of organic cations by CXRs^{28, 39} with little variation in the structures of the cations, understanding of molecular interactions remains phenomenological, and there is no *quantitative* model available to determine either sorption capacity or the level of electrostatic and non-electrostatic interactions between cations and CXRs. Toward this end, we investigated sorption mechanisms of 12 cationic and 8 neutral organic compounds over a wide range of aqueous

concentrations on two CXRs (MN500 and Amberlite 200). Using the isotherm data and the pp-LFERs approach (eq 2), we interpreted the sorption mechanisms and developed predictive models to calculate sorption capacity and selectivity of a diverse range of organic compounds on the studied resins under changing pH. Solvent-associated interactions were then removed using ideal gas phase conversion to separate the effect of solvent-associated interactions from that of solute-sorbent interactions⁴⁵. In this method, aqueous sorption was converted to sorption by a water-wet sorbent from the ideal gas phase where there are no intermolecular interactions^{45, 47, 48}. The aqueous sorption isotherms were then reconstructed to analyze differences in the solute-sorbent interactions which were obscured in the original aqueous sorption.

METHODOLOGY

Materials.

Two strong CXRs, MN500 and Amberlite 200, and one weak CXR, Amberlite IRC-76, were provided by Purolite® and Dow® (U.S.) respectively (properties in Table S2 and analytical methods in Texts S6-8). Both strong CXRs are polystyrenic, MN500 is microporous while Amberlite 200 is porous; Amberlite IRC-76 is polyacrylic. Prior to use, the resins were sequentially washed by HCl, NaOH, NaCl and methanol (details in Text S4). Six aliphatic and three aromatic quaternary ammoniums, four substituted phenols, four substituted anilines, imidazole, and nitrobenzene (NB) were purchased from Fisher Scientific. Analytical grade chemicals were used as purchased to prepare solutions with nanopure deionized water. The properties of solutes and their abbreviations are listed in Table 1.

Adsorption Experiments.

Batch experiments for adsorption isotherms were carried out in amber glass bottles with Teflon-lined screw caps at ambient temperature (22±1°C). For each isotherm at least 17 experimental data points were collected. The concentration of NaCl was 20 mM in all samples to keep ionic strength constant, and the aqueous concentration of all ionic solutes was between 0.003 and 4 mM. For neutral solutes, the equilibrium concentration range was selected based on the compound solubility. Trial experiments were conducted to determine solid to solution ratios to achieve 25%-75% removal of the solutes. The pH was adjusted using 0.2 M NaOH and HCl to keep the solutes in the desired charged form. The amber bottles containing resin and the target compound solution were shaken under 175 rpm for 48 hours. Preliminary kinetic experiments showed that all sorption reached equilibrium within a few hours and there was no significant removal after about 10 hours. After reaching equilibrium, NB, anilines, and phenols were analyzed using high-performance liquid chromatography (HPLC-Agilent) and the quaternary ammonium cations and imidazole were analyzed using a total organic carbon analyzer (TOC-Shimadzu) (details in Text S5). Sorption of the target compounds was determined from the differences between the initial and final concentrations.

Table 1. Physical-Chemical Properties of the Selected Organic Compounds.

Compound	logK _{GW} ^a	Hydration Energy ^c (KJ/mol)	Solute Descriptors ^d					
			E	S	A	B	V ^g	J ⁺
Aniline	-4.03	-5.09	0.96	0.96	0.26	0.41	0.82	0
4-Nitroaniline (4-NA)	-7.12	-22.72	1.24	1.83	0.51	0.34	0.99	0
2-Nitroaniline (2-NA)	-5.13	-11.36	1.18	1.37	0.30	0.36	0.99	0
4-Chloroaniline (4-CA)	-4.35	-6.91	0.91	1.36	0.36	0.25	0.96	0
Phenol	-4.59	-8.28	0.81	0.89	0.60	0.30	0.78	0
4-Nitrophenol (4-NP)	-7.66	-25.80	1.07	1.72	0.70	0.22	0.95	0
2-Nitrophenol (2-NP)	-3.1	0.22	1.02	1.05	0.05	0.37	0.95	0
4-Chlorophenol (4-CP)	-5.05	-10.91	0.92	1.24	0.55	0.22	0.90	0
2,6-Dichlorophenol (2,6-DCP)	-3.96	-4.96	0.90	0.90	0.38	0.24	1.02	0
4-Methylphenol (4-MP)	-4.15	-5.77	0.82	0.87	0.57	0.31	0.92	0
Nitrobenzene (NB)	-3.12	0.10	0.87	1.11	0.00	0.28	0.89	0
Protonated aniline (Aniline ⁺)	-14.5 ^b	-64.82	0.81	1.62	1.93	0	0.84	0.62
Protonated N,N-dimethylaniline (DMA ⁺)	-14.2 ^b	-63.11	0.81	1.85	1.84	0	1.12	0.81
Protonated Imidazole (Imidazole ⁺)	-16.5 ^b	-76.23	0.56	2.5	1.61	0	0.56	1.05
Tetramethylammonium (NMe ₄ ⁺)	-9.76	-37.80	-0.20	1.48	0.82	0	0.81	1.24
Tetraethylammonium (NEt ₄ ⁺)	-9.27	-35.00	-0.52	1.81	0.57	0	1.41	1.52
Tetrapropylammonium (NPr ₄ ⁺)	-8.67 ^b	-32.17	-0.07	1.72	0.49	0	1.92	1.64
Tetrabutylammonium (NBu ₄ ⁺)	-8.16 ^b	-29.36	-0.10	1.90	0.40	0	2.48	1.65
(2-Chloroethyl)trimethylammonium (CETM ⁺)	-14.4 ^b	-64.25	0.11 ^e	1.83 ^f	0.85 ^f	0	1.06	1.80 ^f
(2-Bromoethyl)trimethylammonium (BETM ⁺)	-15.4 ^b	-69.95	0.29 ^e	1.86 ^f	0.82 ^f	0	1.11	2.00 ^f
Phenyltrimethylammonium (PTM ⁺)	-16.5 ^b	-76.23	0.67 ^e	2.07 ^f	0.82 ^f	0	1.26	2.00 ^f
Benzyltriethylammonium (BTM ⁺)	-15.8 ^b	-72.23	0.53 ^e	2.40 ^f	0.57 ^f	0	1.82	2.20 ^f
Vinylbenzyl trimethylammonium (VBTM ⁺)	-17.8 ^b	-83.64	0.89 ^e	2.17 ^f	0.87 ^f	0	1.64	2.15 ^f

^aK_{GW} is the dimensionless Henry's law constant⁴¹; ^bestimated based on the modified bond contribution method⁴⁴; ^cestimated based on $\Delta G_{G-w,i} = -RT \ln K_{GW} + RT \ln V_w/V_G$ where V_w and V_G are the molar volume of water and the gas phase, respectively⁴⁵; ^dAbraham descriptors^{31,42}; ^eestimated from refractive index⁴²; ^festimated based on the group contribution method (Table S3)⁴⁹; ^gMcGowan's characteristic molecular volume⁴⁹.

Estimating Abraham Descriptors. Estimating descriptors for solutes that do not have reported descriptor values is important for using pp-LFERs. Descriptor V is the McGowan characteristic volume and was estimated based on the volumes of all atoms and the number of bonds in the molecular structure^{35,49}. Descriptor E can be estimated from refractive index⁴². Cations typically have negligible H-bond basicity, so we fixed their B values at 0. Two methods were used for estimating descriptors A, S, and J⁺ for quaternary ammoniums: the modified group contribution method⁴⁹, and the method based on the reported pp-LFERs between compound's partition coefficients (lnP) and solute descriptors³¹. In the first method, the properties of each compound arise from the contributions of all functional groups in the structure. Thus, solute descriptors can be estimated as the sum of descriptor values of all functional groups. Tetramethylammonium (NMe₄⁺) was used as the basic structure and its reported descriptor values (Table 1) were modified based on the contributions of all additional functional groups in each solute structure. Detailed calculations are in Table S3.

In the pp-LFERs approach, the descriptors of a solute can be estimated based on the reported regression coefficients (*c*, *e*, *s*, *a*, *b*, *v*, and *j*⁺) for multiple solvent-water systems and its respective experimental lnP values in these systems. First, the experimental lnP values were estimated based on the ion transfer energy ($\Delta G_{tr}^o = -RT \ln P$) from SPARC (<http://www.archemcalc.com/sparc.html>), an online chemical properties calculator. Second, the lnP values of the solute in the solvent-water systems were calculated based on the known

regression coefficients for each solvent using eq 2 and hypothetical descriptor values. Then, mathematical procedures such as Solver® in Microsoft Excel were used to calculate the unknown descriptors by minimizing differences (i.e. mean weighted square errors MWSE) between the experimental $\ln P$ values and the calculated ones³⁵. Note that it is necessary to have solvents with different physical chemical properties, and the number of solvent-water systems should be greater than the number of unknown descriptors. The descriptors for DMA⁺ and imidazole⁺ were estimated based on the descriptors for the corresponding neutral solutes. Abraham and Acree³¹ have developed a set of equations to predict descriptors for protonated amines based on the descriptors for neutral amines.

Estimating Henry's Law Constants ($\log K_{GW}$) for Cationic Solutes. Henry's law constants are required for the gas phase conversion analysis. Two approaches were examined for accuracy in estimating $\log K_{GW}$ values. The first approach is based on $\Delta G_{G-W} = -RT \ln K_{GW}$, where ΔG_{G-W} were calculated in SPARC or Gaussian in WebMO (<http://www.webmo.net/demo/index.html>), a web-based interface for computational chemistry programs. In comparison with the reported Henry's law constants, neither programs estimated ΔG_{G-W} accurately (data now shown). The second approach is the modified bond contribution method⁵⁰ where $\log K_{GW}$ can be estimated from $\log K_{GW}$ of a structurally-related compound by adding or subtracting contributions of all additional bond values in the molecular structure (see one example in Table S4). The obtained $\log K_{GW}$ values were then slightly adjusted ($\pm < 0.55$ log units which is similar to the reported error for experimentally determined $\ln P$ ³¹) to improve precision of the predictive model (root mean square error values or RMSE) after the gas-phase conversion.

PRINCIPAL FINDINGS AND SIGNIFICANCE

pH effect on sorption capacities of resins. The pH effect on the sorption of neutral aromatic and quaternary ammonium compounds was studied to examine the contribution of electrostatic versus non-electrostatic interaction to sorption capacity. The sorption capacity of quaternary ammoniums on two strong cation exchange resins, MN500 and Amberlite200, is pH-independent. The quaternary cations are permanently charged, indicating that electrostatic interactions are pH independent. Sorption of nitrobenzene (NB) on the cation exchange resins is also pH independent but is significantly lower than that of the cations, this is because neutral NB does not have any electrostatic interactions with the ion exchange resins. The pH effect was then investigated for aniline ($pK_a = 4.67$) as a compound that undergoes proton transfer reaction under changing pH conditions. Aniline is protonated at $pH < pK_a$ and neutral at $pH > pK_a$. Sorption of aniline is strongly pH-dependent which significantly decreased when the ratio of protonated aniline to neutral aniline decreased, showing that the protonated aniline had higher sorption capacity than neutral aniline. We attributed this to the fact that protonated aniline undergoes ion exchange reactions (electrostatic) in addition to hydrophobic (nonelectrostatic) interactions. Also, sorption of aniline slightly decreased when pH was lower than 3, due to competition with H^+ for the sorption sites.

Aqueous Sorption Isotherms. In general, MN500 exhibits higher sorption capacity than Amberlite 200, because MN500 has a high proportion of micropores (more than 48% of pore volume) which enhanced sorption energy due to superposition of force fields of the opposite walls⁵¹. Although Amberlite 200 has a lower moisture content than MN500, it has a significantly lower surface area (42 versus 370 m^2/g), thus, there are less sites available for solute sorption. Sorption affinity of organic compounds on MN500 and Amberlite 200 follows the order of: aromatic cations > aliphatic cations > neutral aromatic solutes. The higher affinity of the cationic compounds indicates that the primary

sorption process is ion exchange. The interactions between the nonpolar moieties of the ions and of the resins and solvent-associated interactions are two important factors that influence on the resin selectivity. The higher sorption affinities of the aromatic cations can be attributed to the strong non-electrostatic interactions resulting from $\pi - \pi$ interactions between the aromatic rings of the resin matrix and the aromatic cations. While the neutral aromatic compounds have the ability of forming $\pi - \pi$ interactions, the lack of electrostatic interactions resulted in their much lower removal by the cation exchange resins.

The sorption behavior of two neutral compounds (phenol and NB) on ionic and nonionic resins was studied in order to investigate the effect of resin structure. The neutral resins showed much higher sorption capacities (at least 5 times higher) than the ionic resins with sorption capacity following the order of: MN200 > XAD-4 > XAD-7 > MN500 > IRA-96 > IRA-910. This behavior can be mainly contributed to the higher surface areas of the neutral resins and the highly hydrophilic nature of the ionic resins (due to the presence of functional groups). Sorption of the neutral compounds on MN200 exhibits the highest sorption capacity among the neutral resins due to its high surface area and microporous structure with high sorption potential⁴⁵. Comparing with XAD-7, XAD-4 has a larger pore volume (1.25 versus 1.12 cm³/g) and a higher proportion of micropores (12.4% > 2.3%). Moreover, XAD-7 has the highest moisture content (67%) due to its polar acrylic structure, while the moisture content of XAD-4 and MN200 is 56% and 58%. Lower sorption affinity of IRA-96 and IRA-910, two anion exchange resins, can be related to their higher moisture contents than the cation exchange resin MN500. IRA-910 and IRA-96 have higher ion exchange capacities (5.33 and 6.19 meq/g-dry respectively) than MN500 and Amberlite200 (1.07 and 2.43 meq/g-dry respectively), which can result in more hydrophilic structures.

Multiple linear correlations between the natural logarithm of selectivity and Abraham's solute descriptors was conducted at different sorbed concentrations to examine the contribution of different interactions to the overall selectivity of the cations. We found that selectivity was mainly promoted by dipolar/polarizability effects; induced dipole (E), electrostatic (J⁺), and H-accepting interactions (A) had some positive effects on the selectivity. H-bond basicity (B) had a large positive effect which applies to the neutral compounds only since B is almost zero for all cations. After the gas phase conversion, the contribution of solvent-associated interactions to the Gibbs free energy change has been eliminated, and consequently the contribution of solute-sorbent interactions can be obtained. Unlike the aqueous phase, electrostatic interaction has the most dominant effect on the sorption of the cationic compounds from the gas phase.

Development of Predictive Model. A predictive model was developed based on the above obtained regression coefficients at various sorbed concentrations and was then used to estimate aqueous equilibrium concentration at any given Q_e . Details of the model development are included in our recently accepted paper in Environmental Science & Technology.⁵² **Figures 1** illustrates the correlation of the estimated equilibrium concentrations using the predictive model with the experimental values. The good correlation results indicate that the predictive model is able to accurately predict the sorption behavior of a diverse range of cationic and neutral solutes. Overall these findings will provide guidance to resin selection processes in a given treatment scenario, and will also help polymer industry synthesizing resins with desired physical-chemical properties.

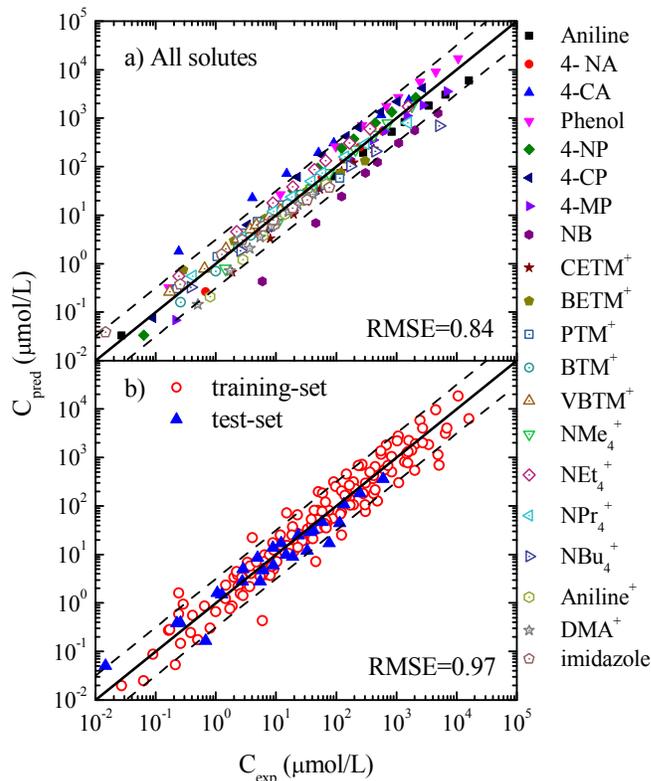


Figure 1. Aqueous equilibrium concentrations calculated based on the predictive model versus the experimental values for a) a training-sets including 12 cations and 8 neutral aromatic solutes with 169 data points and b) a training-set of 17 solutes with 153 data points and 3 test set solutes (4-NA, PTM⁺, and imidazole) with 27 data points for MN500. The solid lines show a perfect prediction, where the predicted value for C_e exactly equals the experimental value; the dashed lines are 0.5 log units above or below the solid lines.

Conclusions

Overall, our results show that CXRs can be effective in the removal of COCs from contaminated water. Understanding the sorption mechanism is fundamental for selecting and synthesizing appropriate resin for a given contaminant. The sorption of organic solutes by CXRs is mainly affected by resin properties like moisture content, resin matrix, and surface area as well as solute structure. The selective removal of ionic organic contaminants by CXRs through ion exchange processes is promoted by non-electrostatic interactions between the non-polar moieties of the solutes and the resin matrix. pp-LFERs enable us to identify the predominant molecular interactions between solute and resin and to develop predictive models for estimating the sorption capacity of a target contaminant on a given resin at any environmentally relevant pH. Further addition of compounds with diverse structures to the training set will expand the ability of the predictive models to handle a large number of solutes. The gas-phase conversion is a useful approach for eliminating solute-solvent effects which obscure solute-sorbent interactions in the aqueous phase.

Significance

To the best of our knowledge, this study is the first to systematically examine the fundamental mechanisms of COCs removal by CXRs. The contributions of *solute-resin* and *solvent-associated* interactions to the overall adsorption energy have been separately quantified, which

offer deeper insights into adsorption mechanisms. Predictive models for the first time were developed to predict the adsorption capacities of a number of COCs by two representative CXRs. In addition, the developed models and approaches will lead to a mechanistic understanding of the adsorption processes of other commonly used sorbents. A fundamental understanding of the adsorption mechanisms and development of predictive models will contribute to a major advance in the development of resins as a new water treatment technology targeting COCs removal. Application of the developed models and approaches to other sorbents will also take the understanding of adsorption processes in general to a new level that is not accessible using current approaches.

The occurrence of COCs in our water systems and in the environment is among the greatest environmental challenges facing the State and the Country. Meeting this challenge with conventional treatment technologies alone is difficult and costly. Development of a new water treatment process using ion-exchange resins will directly protect human health from drinking water contamination and prevent environmental contamination from wastewater discharge. This work has also provided environmental engineers and water utilities with the criteria to select the type and quantity of CXRs for a given treatment scenario. This knowledge, together with findings for anion exchange resins, will also enable chemical companies to design more suitable resins for separation and purification purposes.

STUDENTS & POSTDOCS SUPPORTED

Nastaran Jadbabaei, Civil & Environmental engineering, Temple University, Ph.D. Candidate
Shubo Wang, Civil & Environmental engineering, Temple University, Master student
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Quiana M Waters, Civil & Environmental engineering, Temple University, undergraduate student

PUBLICATIONS (follow style formats on the next page)

Jadbabaei, N. and **Zhang, H.** 2014 “Sorption Mechanisms and Predictive Model for the Removal of Cationic Organic Contaminants by Cation Exchange Resins”. *Environ. Sci. Technol.* 48, 14572–14581.

Wang, S. and **Zhang, H.**; 2015 “Predictive Modeling of Bi-Solute Adsorption by Polymeric Resins based on Adsorbed Solution Theories (ASTs)”, *Environ. Sci. Technol.* In preparation.

Wang, Shubo, 2015 Predictive Modeling of Bi-Solute Adsorption by Polymeric Resins based on Adsorbed Solution Theories (ASTs), “MS Dissertation”, Department of Civil and Environmental Engineering, College of Engineering, Temple University, Philadelphia, PA, 70 pages.

Zhang, H. and Jadbabaei, N. 2014, Developing Quantitative Models for the Removal of Quaternary Ammonium Ions as Representative Cationic Emerging Contaminants by Cation Exchange Resins, Final Report Submitted to Pennsylvania Sea Grant, Temple University, Philadelphia, PA, 11 pages.

Zhang, H., Jadbabaei, N., 2015, Developing Quantitative Models for the Removal of Quaternary Ammonium Ions as Representative Cationic Emerging Contaminants by Cation Exchange Resins, Final Report Submitted to Pennsylvania Water Resource Research Center, Temple University, Philadelphia, PA, 13 pages.

INFORMATION TRANSFER ACTIVITIES

Jadbabaei, N.; **Zhang, H.**; “Revisit sorption mechanisms of organic compounds on cation exchange resins”, 246th ACS National Meeting, Division of Environmental Chemistry, Indianapolis, IN, USA, Sept. 8 – 12, 2013.

Zhang, H. “Emerging Contaminants: Oxidation by Binary Metal Oxide Mixtures and Removal by Polymeric Sorbents”, Department of Civil Engineering, HeFei University of Technology, Anhui, China, July 26, 2013.

Zhang, H. “Emerging Contaminants: Oxidation by Binary Metal Oxide Mixtures and Removal by Polymeric Sorbents”, College of Environmental Science and Engineering, Shanghai JiaoTong University, Shanghai, China, June 28, 2013.

Jadbabaei, N.; **Zhang, H.**; “Revisit sorption mechanisms of organic compounds on cation exchange resins”, American Water Resources Association, Philadelphia Metropolitan Area Section (AWRA-PMAS) on “Constructed Wetlands for On-site Wastewater Treatment”, Philadelphia, PA, Feb 20, 2014.

Zhang, H. “Understanding and Modeling Removal of Neutral and Ionic Organic Contaminants by Polymeric Resins”, US–China Drinking Water Treatment Symposium, Penn State Harrisburg, Harrisburg, PA, October 4, 2014.

Wang, S. and **Zhang, H.**; “Predictive Modeling of Bi-Solute Adsorption by Polymeric Resins based on Adsorbed Solution Theories (ASTs)”, 250th ACS National Meeting, Division of Environmental Chemistry, Boston, MA, USA, Aug. 16 – 20, 2015.

AWARDS & ACHIEVEMENTS (of PIs, students, or staff on the project)

Jadbabaei, N. American Chemical Society, The Division of Environmental Chemistry, “**Certificate of Merit**” awarded for the oral presentation “Revisit sorption mechanisms of organic compounds on cation exchange resins” at the 246th ACS National Meeting

Jadbabaei, N. Student Poster Scholarship Award of the 2013/2014 year, American Water Resources Association, Philadelphia Metropolitan Area Section (AWRA-PMAS)

PHOTOS OF PROJECT

Please include 2 graphics or photos with captions, if possible. These may be used in our annual report, web page, and/or brochure, and may be used by the National Institutes of Water Resources.

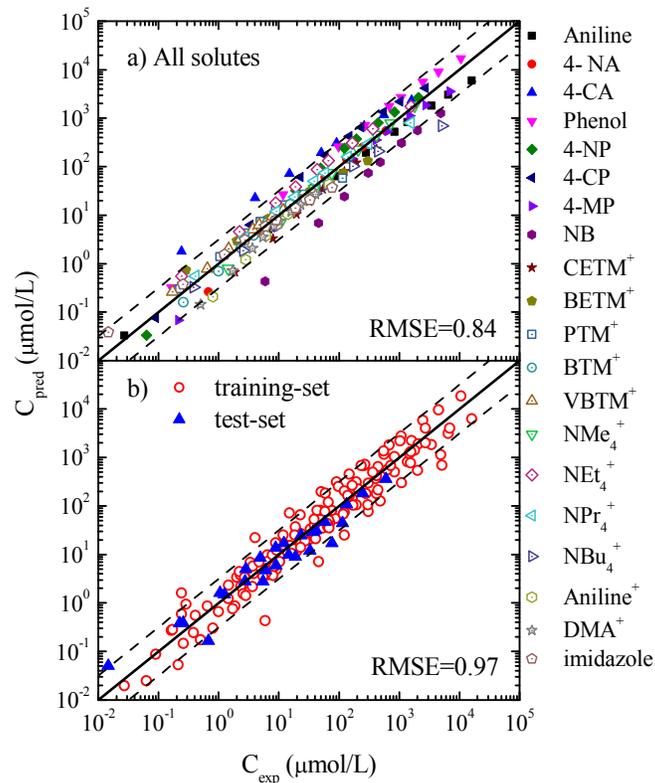


Figure. Aqueous equilibrium concentrations calculated based on the predictive model versus the experimental values for a) a training-sets including 12 cations and 8 neutral aromatic solutes with 169 data points and b) a training-set of 17 solutes with 153 data points and 3 test set solutes (4-NA, PTM⁺, and imidazole) with 27 data points for MN500. The solid lines show a perfect prediction, where the predicted value for C_e exactly equals the experimental value; the dashed lines are 0.5 log units above or below the solid lines.⁵²



Amber bottle reactors containing a target contaminant and one studied resin

Citations

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Evaluation of Round Goby and Tubenose Goby Habitat Disturbance and Impacts to Native Fishes (year 2)

Basic Information

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FINAL PROJECT REPORT
Pennsylvania Water Resources Research Center

PROJECT TITLE & PRINCIPAL INVESTIGATOR

Evaluation of Round Goby and Tubenose Goby Habitat Disturbance and Impacts to Native Fishes – Phase II

Jay Stauffer, Department of Ecosystem Science and Management, Pennsylvania State University

PROBLEM and RESEARCH OBJECTIVES

The purpose of this study is to report on the decline of benthic fishes in the Pennsylvania portion of Lake Erie, to determine if there was a shift in diet of the native fishes when Round Gobies were present in selected Pennsylvania tributaries of Lake Erie, to document the presence of the Tubenose Goby in Pennsylvania, and to compare growth rates of Round Goby from the lake and those from the streams in artificial streams, when fed only macroinvertebrates.

METHODOLOGY

Annual assessment by the Pennsylvania Fish and Boat Commission Lake Erie Research Unit (LERU) includes offshore (> 12 meters) bottom trawling from the RV Perca during the summer and fall. This effort provides an abundance index of forage fish, young-of-the-year yellow perch, and to some extent adult fish inhabiting hypolimnetic waters. Historically, catches were represented as catch per tow-hour. In 1998, the trawl gear was calibrated using SCANMAR, providing accurate measurements of trawl dimensions while the gear was being fished. The trawl gear (otter boards, cables, 4-seam Marinovich trawl with 12.8 m head rope lined with a 0.64 cm inner mesh bag) used by the LERU has been the standard since 1985. Distance trawled is calculated by recording starting and ending points from an onboard GPS. Area trawled was calculated by multiplying distance trawled by the wing-spread of the trawl; thus, we were able to estimate the number of fish per hectare trawled.

We sampled Round Gobies in littoral habitats using electrofishing, hook and line, and beach seines. Electrofishing was largely ineffective, because Round Gobies either escaped to rock cover before experiencing galvanotaxis, or sank to the bottom in and among rock cover following galvanonarcosis. Hook and line sampling was most effective in areas too deep to seine or where large rocks prevented seining. Seines were effective over sand and small gravel and in shallow littoral habitats.

All Round Gobies collected were anesthetized using MS222, preserved in 10% formalin and transported to the laboratory for data collection. In the laboratory, we measured standard lengths (nearest mm) using an electronic dial calipers and weights (nearest 0.1g) using an electronic balance. Gender was determined by examining external genitalia (MacInnis and Corkum 2000). Both saggital otoliths were removed from 147 gobies (88 males and 59 females) and allowed to dry for several days prior to examining for age. Whole otoliths were immersed in oil and viewed under a dissecting microscope at 10-24x magnification. Two researchers independently estimated age from each otolith. If researchers disagreed on an age estimate for a fish, then the otolith was re-examined by both researchers simultaneously, and a consensus age was estimated. If the researchers could not reach a consensus on age, the fish was eliminated

from subsequent analyses. We reached a consensus age for all 59 females; ages of 3 of the 88 males examined were not included in analyses because of lack of consensus.

We described population-size structure using a length-frequency histogram. We analyzed growth by creating length-at-age plots and then fitting von Bertalanffy growth functions. We tested for differences in parameters of the von Bertalanffy growth function using Rao's asymptotic chi-squared test (Rao 1973) as described in Galucci and Quinn (1979). We performed separate analyses for each gender and habitat type (pelagic versus littoral) in which gobies were collected to determine if the growth function varied by gender or habitat type.

Invasion of tributaries

Fishes were collected in Twentymile Creek from the mouth of the stream to the waterfall (Site 1 - N 42 15'38.71 W 79 46'49.33) and just below the old railroad bridge (Site 2 - N 42 15'29.48 W 79 46'39.68) with two 1.5 m x 3.05 m x 3.2 mm seines to determine the fish composition and species diversity in areas where gobies were present and absent. Additionally, we electrofished 100 meters (25-m below the sites, 50-m sites, and 25-m above the sites) of the stream reach. All fishes collected were identified to species and released with the exception of voucher specimens for each species. Percent composition, population estimates, and Brillouins diversity indices were calculated for each site.

Nine plots were constructed in Elk Creek near the mouth (42° 1'10.65"N, 80°22'17.09"W) in August 2012 from non-treated plywood with 2 x 4 m inside diameter that was filled with substrate. These plots were placed as a 3 x 3 design in the stream and filled with three different types of substrate (2b gravel (small substrate), #1 gravel (medium substrate) and rip-wrap (large substrate). Sampling took place once a month from June until September 2012 and included a twenty-four hour survey. Every 8 hours, three of the nine plots were shocked, one from each substrate type (small, medium and large). A seine was positioned downstream to collect the fishes. Fishes were anesthetized in MS222, fixed in formalin and preserved in ethanol for later identification. Water velocity was recorded before the first drift sample collection and after the last drift sample collection. Drift nets were placed downstream of each plot for fifteen minutes, every three hours, to collect macroinvertebrates that drift in the water column. These nets were then examined and aquatic insects were preserved in ethanol for later identification. Macroinvertebrates residing in the substrate were collected using D-frame kick-nets, at each plot, at the end of the twenty-four hour sampling period. The substrate was preserved in ethanol and examined under dissection scopes to search for macroinvertebrates.

Stomachs were removed from all benthic fishes collected and the contents identified to the lowest possible taxon (family or genus). Macroinvertebrates collected were also enumerated and identified to the lowest possible taxon. Stomach contents of all benthic fishes were examined to detect trophic partitioning, which occurred among species. We compared these data to the results of the macroinvertebrate collections and calculated electivity indices. Macroinvertebrates and fishes were collected upstream of the waterfalls (42° 0'24.90"N, 80°21'13.89"W) in Elk Creek in August as described above.

Artificial streams were constructed and placed in the Tom Ridge Center. Two streams were populated with Round Gobies from the lake and two streams with Round Gobies from the tributaries. Fourteen individuals were released into each of the 4 streams, keeping like specimens together, stream and lake. Two artificial streams of opposite flow housed stream gobies and two artificial streams housed the round gobies. Specimen lengths were recorded prior to entry into the streams.

Weekly additions of micro and macro invertebrates collected by four separate 30-second kick net collections from streams in the Erie drainage. Additional samples were collected for identification of species present during sampling. Collections were populated into each of the 4 artificial streams at one week intervals for a period of 4 weeks. Invertebrate identification was completed by hand sorting .

PRINCIPAL FINDINGS AND SIGNIFICANCE

Relative abundance of Round Gobies in Lake Erie

The abundance of Round Goby had increased since they were first documented in Pennsylvania's waters of Lake Erie in 1996. By 1998, Round Gobies became the dominant component of assessment trawls. Goby abundance peaked in 2001 when, on average, over 2,500 gobies were caught per hectare trawled. Goby abundance declined in 2002 to less than 1,000 gobies per hectare (Fig. 1). In all cases, the number of native benthic fishes captured in the trawls decreased as the numbers of Round Gobies increased (Fig. 1).

Year-class strength and abundance of fishes fluctuate naturally; thus, we recorded the presence/absence of the benthic species in the trawl samples (Fig. 2). With the exception of the Trout-perch, *Percopsis omiscomaycus*, all other benthic species had a drastic decrease or were absent from all the trawls in the years subsequent to the population explosion of the Round Goby.

Population structure of Round Gobies

The length-weight relationship for gobies was similar to that reported by others and is similar to that reported for Round Gobies in their native habitat (Fig. 3) (Charlebois et al. 1997, MacInnis and Corkum 2000, Phillips 2002). Males and females had similar length-weight relationships and neither slopes nor intercepts were significantly different based on overlapping 95% confidence intervals.

Male gobies lived longer than females. All fish age 4 and older were males, and only 2 females reached age 3. The oldest goby was a 6-yr old, 154 mm standard-length male. Male gobies in Pennsylvania waters of Lake Erie were larger than females (Fig. 4). The longest male goby was 165 mm, whereas the largest female goby was 110 mm. There was no major difference in size of females captured in pelagic habitats versus females captured in littoral habitats, but there was a difference for males. All males greater than 119 mm were captured in littoral habitat, most among the large boulders close to shore.

Sample size of females was too small to compare von Bertalanffy analyses for pelagic and littoral areas, so we pooled females and described the growth parameters with a single equation (Fig. 5). Littoral males reach their asymptotic length (L_{∞}) faster than pelagic males. There was no detectable difference in theoretical maximum length between pelagic and littoral males.

Collections with the electric trawl in Presque Isle Bay resulted in the capture of Tubenose Gobies, *Proterorhinus semilunaris*, which was the first record of this species in Pennsylvania; thus the study was expanded to examine the diet of this newly discovered invasive species. Round Goby, Tubenose Goby, and the endangered Iowa Darter, *Etheostoma exile* all consumed Zebra Mussels, but these mussels comprised almost the entire diet of the two goby species (Table 1). *Etheostoma exile* fed primarily on freshwater shrimp species.

Invasion of tributaries and Presque Isle Bay

More individual fishes were captured by electrofishing above the waterfalls on Twentymile Creek, Site 2, than below the waterfalls, Site 1 (Table 2). Eight species were found

at both sites, which were not found at the other site. One of these was the Round Goby. Other species collected at Site 1, but not at site 2 included one specimen of *Lepisosteus osseus* and one *Notropis atherinoides*. Round Gobies comprised 45% of the total fishes at the lower site. Rainbow Darters comprised the second largest species composition, 35% in the bottom site and the fourth largest species composition at the top site. In total, Rainbow Darters constituted the greatest percentage of benthic species composition at both sites, when Round Gobies were excluded from Site 1. Brillouin's diversity index at site 1 and 2 were 1.39 and 2.11, respectively.

The kicknet samples downstream in Elk Creek yielded primarily flies (Chironomidae, Tipulidae), mayflies (Baetidae, Caenidae), hellgrimmites (Corydalidae), beetles (Elmidae) and caddisflies (Hydropsychidae) (Table 3). The Rainbow Darters in this stream reach consumed mayflies followed by caddisflies and flies (Table 3). The Round Goby consumed primarily mayflies (Table 3). The kicknet samples upstream in Elk Creek (Table 4) yielded planaria (Tricladida), flies (Chironomidae, Simuliidae), stoneflies (Perlidae), mayflies (Baetidae), beetles (Psephenidae), caddisflies (Hydropsychidae, Polycentropodidae), and amphipods (Gammaridae). The Rainbow Darters in this reach consumed primarily mayflies and flies (Table 4).

Analysis of the trend data depicted a significant difference in the growth rates of the individuals from differing environments. Those that originated in stream habitats experienced a larger average (5.6mm; .713g) size increase during the 4-week trial than those from the lake (2.6mm; <.01g). With a P-value of <0.05 in comparison with residual weight of sampled species, this indicates significant differences in the study groups.

STUDENTS & POSTDOCS SUPPORTED

Sara Mueller, MS student, Department of Ecosystem Science and Management, Penn State University

PHOTO OF PROJECT



Table 1. Stomach contents of *Etheostoma exile*, *Neogobius melanostomus*, and *Proterorhinus marmoratus*, from Presque Isle Bay.

Presque Isle Bay					
September 2011					
<i>E. exile</i> stomach content		<i>N. melanostomus</i> stomach content		<i>P. marmoratus</i> stomach content	
n = 13		n = 26		n = 5	
Taxon	Average Percentage	Taxon	Average Percentage	Taxon	Average Percentage
Zebra Mussel	3.8	Zebra Mussel	67	Zebra Mussel	27
Mussel Species	0	Mussel Species	2.9	Mussel Species	0
Freshwater Shrimp	30.4	Freshwater Shrimp	0	Freshwater Shrimp	0
Insect Species	0.4	Insect Species	0.8	Insect Species	0
Fish egg	0	Fish egg	0	Fish egg	1

Table 2. Fishes collected downstream (Station 1) and upstream (Station 2) of the waterfall on Twentymile Creek, 22 June, 2003.

Species	Station 1	Station 2
<i>Lepisosteus osseus</i>	1	0
<i>Oncorhynchus mykiss</i>	4	15
<i>Salmo trutta</i>	0	1
<i>Campostoma anomalum</i>	17	53
<i>Cyprinella spiloptera</i>	1	0
<i>Luxilus cornutus</i>	1	0
<i>Luxilus chrysocephalus</i>	0	22
<i>Nocomis micropogon</i>	4	124
<i>Notropis atherinoides</i>	1	0
<i>Pimephales notatus</i>	0	1
<i>Rhinichthys atratulus</i>	2	59
<i>Rhinichthys cataractae</i>	0	26
<i>Semotilus atromaculatus</i>	0	4
<i>Catostomus commersoni</i>	0	1
<i>Hypentelium nigricans</i>	1	0
<i>Ameiurus nebulosus</i>	0	2
<i>Noturus flavus</i>	3	0
<i>Ambloplites rupestris</i>	1	1
<i>Lepomis gibosus</i>	2	2
<i>Lepomis macrochirus</i>	2	1
<i>Micropterus dolomieu</i>	7	0
<i>Etheostoma caeruleum</i>	88	48
<i>Etheostoma flabellare</i>	2	6
<i>Percina caprodes</i>	0	4
<i>Cottus bairdi</i>	0	11
<i>Neogobius melanostomus</i>	113	0
Total	250	388
Brillouin's Diversity Index	1.39	2.11

Table 3. Macroinvertebrates June 2012 and stomach contents of fishes with electivity index.

	<i>Macroinvertebrates</i>		<i>Fish Stomach Content</i>			
			<i>Macroinvert ebrates In Stomach (%)</i>		<i>Macroinvert ebrates In Stomach (%)</i>	
	<i>Driftnet</i>	<i>Kicknet</i>	<i>E. caeruleum</i>	<i>Electi vity Index</i>	<i>N. melanstomu s</i>	<i>Electi vity Index</i>
<i>Taxa</i>	<i>% Environment</i>	<i>% Environ ment</i>	<i>n = 3</i>		<i>n = 15</i>	
<i>Pronoterus</i>	0.94	--	--	--	--	--
<i>Berosus</i>	--	0.73	--	--	--	--
<i>Dubiraphia</i>	--	0.08	--	--	--	--
<i>Stenelmis</i>	--	0.82	--	--	--	--
<i>Coleoptera</i>	0.94	--	--	--	--	--
<i>Chironomidae</i>	13.21	12.11	50.00	0.61	25.00	0.35
<i>Dineutus</i>	--	0.04	--	--	--	--
<i>Caenis</i>	6.6	21.9	--	--	--	--
<i>Callibaetis</i>	1.89	--	--	--	--	--
<i>Centroptilum</i>	0.94	--	--	--	--	--
<i>Baetidae</i>	0.94	--	--	--	--	--
<i>Stenonema</i>	--	0.24	--	--	--	--
<i>Tricorythodes</i>	57.55	62.32	--	--	75.00	0.09
<i>Ameletus</i>	0.94	--	--	--	--	--
<i>Ephemeroptera</i>	4.72	--	50.00	--	--	--
<i>Physidae</i>	--	0.04	--	--	--	--
<i>Hirudinea</i>	--	0.04	--	--	--	--
<i>Gastropoda</i>	4.72	--	--	--	--	--
<i>Caecidotea</i>	--	0.16	--	--	--	--
<i>Argia</i>	--	1.43	--	--	--	--
<i>Cordulgaster</i>	--	0.04	--	--	--	--
<i>Hydropsyche</i>	2.83	--	--	--	--	--
<i>Agraylea</i>	0.94	--	--	--	--	--
<i>Hydroptila</i>	0.94	--	--	--	--	--
<i>Paleagapetus</i>	0.94	--	--	--	--	--
<i>Cheumatopsych e</i>	--	0.04	--	--	--	--
<i>Tricoptera</i>	0.94		--	--	--	--

Table 4. Macroinvertebrates from kick net samples and stomachs from Troutperchs in Elk Creek above first waterfall.

Kicknet Sample			<i>E. caeruleum</i> stomach content (n= 17)	Electivity Index
Order	Family	Percentage		
Tricladida	N/A	11.72	0	-1.0
Trichoptera	Hydropsychidae	8.45	14	-0.3
	Polycentropodidae	15.80		
Coleoptera	Psephenidae	7.08	0	-1.0
	Elmidae larvae	1.63		
	Elmidae adult	0.27		
Ephemeroptera	Baetidae	15.53	58.5	0.6
Plecoptera	Perlidae	0.54	1.1	0.3
Diptera	Chironomidae	37.60	17.6	-0.4
	Simulidae	0.27		
Amphipoda	Gammaridae	0.27	0	-1.0
Oligochaeta	N/A	0.82	0	-1.0
Megaloptera	N/A	0.00	3.1	N/A
Odonata	Coenagrionidae	0.00	6.2	N/A

Emerging Contaminants in Vernal Ponds Across a Human Impact Gradient

Basic Information

Title:	Emerging Contaminants in Vernal Ponds Across a Human Impact Gradient
Project Number:	2014PA214B
Start Date:	4/15/2014
End Date:	2/28/2015
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Congressional District:	5
Research Category:	Water Quality
Focus Category:	Water Quality, Non Point Pollution, None
Descriptors:	None
Principal Investigators:	Heather Gall

Publications

There are no publications.

FINAL PROJECT REPORT
Pennsylvania Water Resources Research Center

PROJECT TITLE & PRINCIPAL INVESTIGATOR(S)

Emerging Contaminants in Vernal Ponds Across a Human Impact Gradient

PI: Heather E. Gall, Penn State, Department of Agricultural and Biological Engineering

Co-PI: Tracy Langkilde, Penn State, Department of Biology

Co-PI: Elizabeth Boyer, Penn State, Department of Ecosystem Science and Management

Co-PI: David Miller, Penn State, Department of Ecosystem Science and Management

PROBLEM and RESEARCH OBJECTIVES

Humans use a large variety of chemicals in their everyday lives, including over-the-counter medications, prescription drugs, and personal care products, which become part of the wastewater stream. Wastewater treatment plants were not designed to remove these chemicals, and therefore these products and their metabolites remain in the effluent. In addition to human sources of ECs, veterinary pharmaceuticals and hormones are given to livestock raised in animal feeding operations to increase growth and reduce disease. The land-application of animal and human wastes to agricultural fields as a nutrient source inadvertently introduces ECs into the environment; however, the processes governing their fate and transport are not well understood.

Many ECs are known or suspected to cause endocrine disruption, particularly in sensitive aquatic organisms. Hormones can cause endocrine disruption in fish at concentrations as low as 1 ng/L (Leet et al. 2011). Triclosan, a common antimicrobial, affects tadpole hindlimb development at concentrations as low as 0.15 µg/L (Veldhoen et al. 2006). The effects of pesticides have been widely studied and are known to adversely impact frogs and other amphibians, with the effects becoming magnified in the presence of multiple contaminants (e.g., Egea-Serrano et al. 2012). Although the presence of ECs in surface water is widespread (Kolpin et al. 2002) and some laboratory studies have been conducted to understand the impact of controlled exposure of amphibians to ECs, no data exist for the occurrence of ECs in natural vernal ponds - temporary water bodies that are critical amphibian breeding sites - that are known to be highly susceptible to contaminant accumulation.

Amphibian populations are declining globally, largely due to human activities (Collins 2013, Wake and Vredenburg 2008), with water quality degradation due to pollution thought to be threatening more than 20% of amphibians worldwide (IUCN 2004). Quantifying the impacts of environmental contaminants on amphibian populations remains a challenge, given complex interactions among multiple stressors and changing global environmental conditions. Degraded water quality due to pollution from anthropogenic activities is widespread and the presence of multiple contaminants in water bodies is ubiquitous, affecting amphibian breeding habitat. Dose-response experiments have had a crucial role in understanding contaminant impacts on amphibians (e.g., Hayes et al. 2002). However, predicting impacts on amphibian populations in the wild requires understanding the potential of amphibians to adapt via selection and plasticity to increased contaminant levels. Amphibians are known to display strong local adaptation to some contaminants, such as de-icing agents (Brady 2012, Hopkins et al. 2013, Sanzo and Hecnar 2006) and pesticides (Cothran et al. 2013). These findings suggest that it is possible for amphibians to evolve tolerance to higher levels of environmental contaminants. Understanding this is critically important for prioritizing conservation efforts. Although many studies have

attempted to quantify the impacts of a single contaminant on amphibians and other aquatic organisms, it is unlikely that these contaminants exist as the sole stressor outside of controlled laboratory studies. Furthermore, there is significant evidence that stressors behave synergistically, causing multiplicative effects beyond what could be predicted by summing up the impacts of each stressor individually (Relyea and Mills 2001, Sih et al. 2004, Relyea 2009). Therefore, studies that focus on a single stressor offer limited insight into the actual impacts experienced by amphibians in degraded habitats. Relyea and Diecks (2008) emphasized the importance of conducting mesocosm studies that more accurately simulate amphibian exposure to ecologically-relevant combinations of contaminants and stressors in nature.

This project sought to advance our understanding of the fate and transport of ECs and the potential ecological impacts that these contaminants may have on native vernal pond amphibians. Our specific objectives were to: (i) link the occurrence of ECs in vernal ponds with land use across a human impact gradient; (ii) quantify the hydroperiod of these ponds to inform EC fate and transport; and (iii) determine if wood frogs are affected by “cocktails” of contaminants.

METHODOLOGY

Objective #1: Link the occurrence of ECs in vernal ponds with land use across a human impact gradient

Ten ponds were selected in Centre County, Pennsylvania based on two main criteria: (i) known, or likely to be, used as breeding habitat by vernal pond amphibians; and (ii) had drainage areas that spanned a human impact gradient, ranging from 100% forested to 100% agricultural land use. Six of the ponds are located on Penn State University Property (5 at Penn State’s Living Filter, which is irrigated with treated wastewater effluent, and 1 at the Rock Springs Agronomy Research Farm, which receives dairy manure applications) and four of the ponds were located in the Scotia Barrens State Game Lands. Drainage areas for each of the vernal ponds were delineated in ArcGIS (ArcMap 10.1) using contour maps with 0.61 m (2 ft) intervals available through PASDA (Pennsylvania Spatial Data Access). The most recent land cover maps available (2001) were downloaded from the United States Geological Survey (USGS) National Map Viewer and Download Platform. ArcGIS was used to calculate land use percentages in each drainage area.

Water samples were to be collected from each pond during two sampling events: (i) in the fall after post-harvest manure applications; and (ii) in the early spring after pesticide and fertilizer applications. Fall samples were collected in October 2014. Spring samples are scheduled to be collected in May 2015. The timing of the sample collection is also driven by rainfall, such that samples coincide with the first major rainfall event following application dates. Samples are analyzed according to methods approved by the Environmental Protection Agency for: 7 hormones of human and livestock origin, 25 pesticides and their metabolites, nutrients (nitrogen, phosphorus, and carbon), and pH.

Objective #2: Quantify the hydroperiod of the vernal ponds

Autonomous sensors measure water level in each pond to quantify variability within the hydroperiod, allowing us to monitor water input (surface runoff) events that are likely to transport contaminants into these ponds, and to monitor pond drying. The hydroperiod dynamics are important for the wood frog, as their survival is contingent upon them metamorphosing prior to the ponds drying in the summer months when evapotranspiration is high. The sensors collect

water level data every 15 minutes, which are downloaded by the graduate and/or undergraduate students onto a rugged field laptop.

Objective #3: Assess the impacts of “contaminant cocktails” on wood frog growth and survival

Wood frogs are site-fidelic, such that they almost always return to the vernal pond from which they were born, allowing them to adapt to local environmental conditions, including the presence of contaminants (Brady 2012, Hopkins et al. 2013, Sanzo and Hecnar 2006). We used a split-clutch experimental design to compare the growth and survival of tadpoles from the same egg mass (i.e., clutch) raised in contaminated and uncontaminated sites. Two sites at the Living Filter were considered contaminated, as they are surrounded by agricultural fields and receive treated wastewater effluent irrigation. Two sites at the Scotia Barrens State Game Lands were selected as the uncontaminated sites. Four egg masses from each of Scotia Barrens ponds were collected within 72 hours of being laid. Each egg mass was placed in its own plastic container next to two ponds from which the masses were collected until the eggs hatched (approximately 2 weeks). Water samples were collected to test for possible BPA leaching during the time the eggs were in the containers. Within a week after hatching, 60 tadpoles from each egg mass were collected for translocation. The tadpoles were translocated such that 30 tadpoles from each egg mass were deployed in a different uncontaminated pond and 30 were deployed in a contaminated pond at the Living Filter (see Figures 1 and 2 below). Each set of 30 tadpoles was placed in a cylindrical mesh enclosure (see photos in section below).

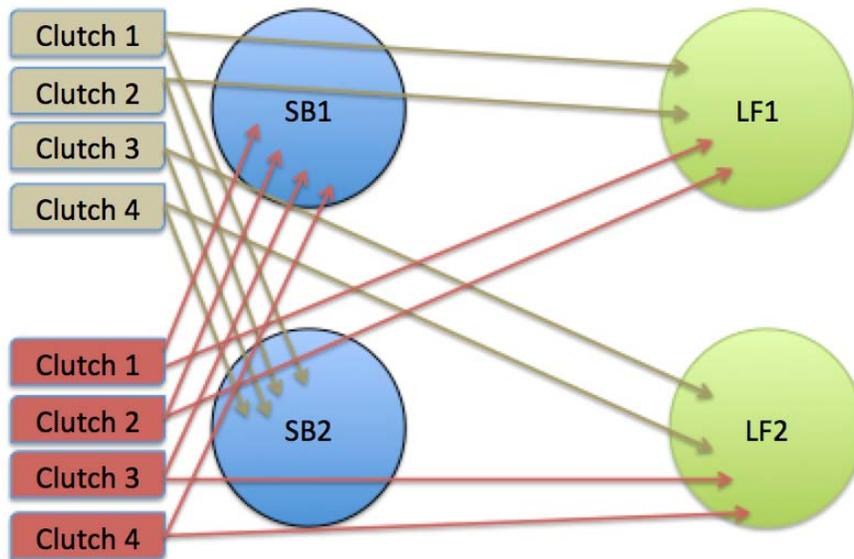


Figure 1. Tadpole Translocation Schematic.

Note that SB1 and SB2 are the two uncontaminated sites at the Scotia Barrens State Game Lands and LF1 and LF2 are the two contaminated sites at Penn State’s Living Filter.

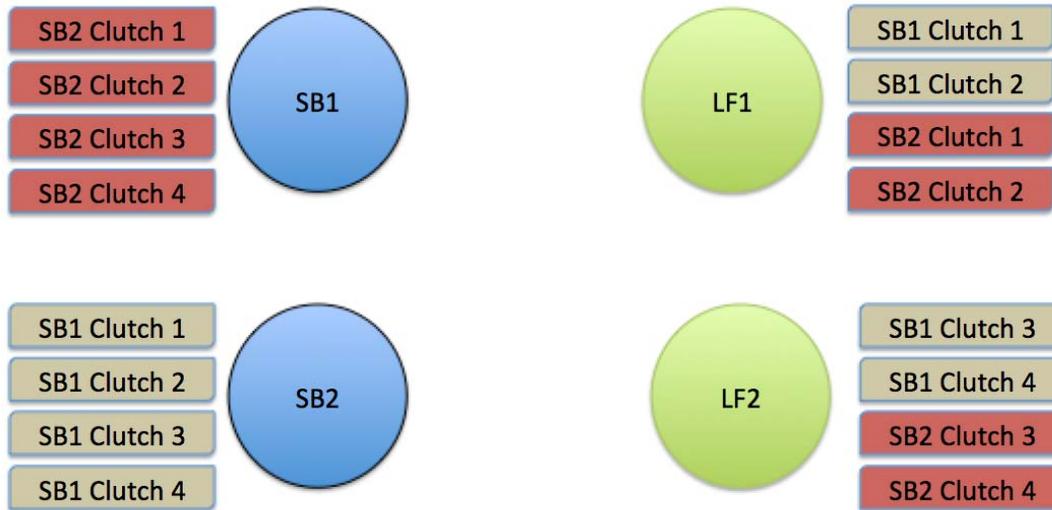


Figure 2. Final Locations of Translocated Tadpoles.

PRINCIPAL FINDINGS AND SIGNIFICANCE

The project is ongoing, and therefore only preliminary results and their expected significance are provided here.

Objective #1: Water samples were collected at 6 of the 10 ponds in October 2014. Four of the ponds in the Scotia Barrens State Game Lands were dry in the fall, and therefore could not be sampled. The data collected at the 5 Living Filter (LF 1-5) sites and 1 Rock Springs (RS) site are given below:

Site ID	Coordinates	pH	NH ₃ -N [mg/L]	NO ₃ +NO ₂ [mg-N/L]	TKN [mg/L]	TP [mg/L]	DOC [mg/L]	Caffeine [ng/L]	Estrogens [ng/L]
LF1	40.83147, -77.89389	6.59	ND*	ND	5.51	0.74	13.4	51	ND
LF2	40.83481, -77.90292	7.08	0.15	1.96	3.33	1.14	8.27	340	ND
LF3	40.830528, -77.86533	6.81	0.26	3.38	2.99	1.01	7.6	910	ND
LF4	40.83265, -77.8907	6.85	ND	1.82	2.26	0.66	8.98	41	ND
LF5	40.83137, -77.89924	7.16	0.22	1.84	3.18	1.5	7.14	130	ND
RS	40.70026, -77.96532	7.45	ND	ND	1.52	ND	6.86	2.4	ND

TKN = Total Kjeldahl Nitrogen, TP = Total Phosphorus, DOC = Dissolved Organic Carbon
 ND = Not detected

Currently, no data on the presence of emerging contaminants in vernal ponds exists. Therefore, the results of the water quality samples collected across the human impact gradient will be the first data set of its kind. Although the sampling was limited to two times, the results provide a snapshot in time across space of the presence of multiple contaminants in these sensitive and understudied ecosystems. The data will be leveraged as preliminary results to apply for larger, external funds. Although no estrogen concentrations were detected in the October 2014 sampling event, an earlier round of samples collected in October 2013 did have hits for estrogens at one of the Living Filter sites. Therefore, the timing of effluent irrigation and our sampling may affect whether or not concentrations are detected, given the fast half-lives of these compounds.

Additionally, the results can be used to inform the design of more controlled mesocosm-scale laboratory studies, as the data provide information about the environmentally relevant concentrations of multiple water quality parameters.

Objective #2: Water level sensors were deployed at all ten sites from September 2014 through mid-November 2014 and removed over the winter months. They were re-deployed in mid-March 2015 and will remain in the field until late Fall 2015 before they are removed prior to freezing conditions this coming winter. The water level data can provide insight into how effluent irrigation influences the natural variability in water level and the duration of the hydroperiod compared to the less impacted sites in Scotia Barrens. For example, it appears that the effluent irrigation extends the hydroperiod of the Living Filter ponds, as the four sites that were dry in fall 2014 were the sites in Scotia Barrens, whereas the remainder of the sites had water. Figure 3 below shows how rainfall and effluent irrigation impacted the water level at four of the Living Filter sites in Fall 2014. Each event caused significant increases in water level in each pond.

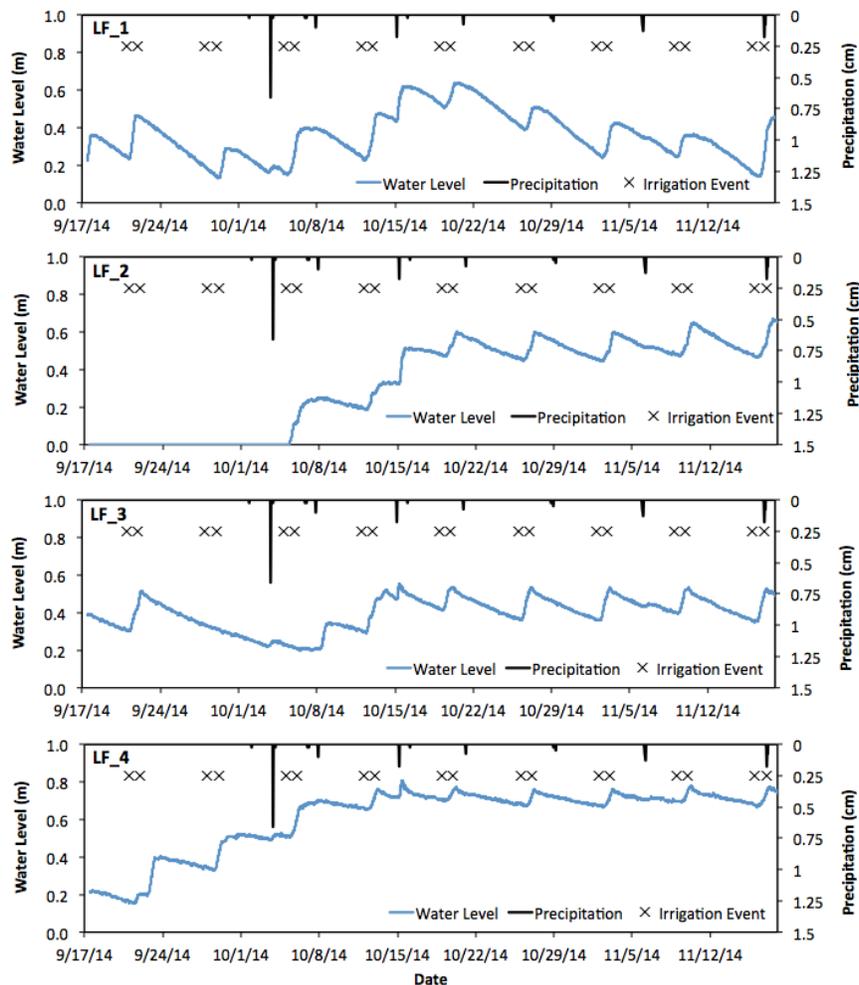


Figure 3. Time series data of hyetographs and water levels at LF sites 1-4. The timing of irrigation events are shown as “X” across the top of the figure. Each effluent irrigation event is ~5 cm over a 12-hour time period.

Objective #3: The results of the tadpole translocation experiment will not be known until later this summer, as the experiment is ongoing until the tadpoles reach metamorphosis. Currently,

survival in each enclosure has been < 50%, with some enclosures having approximately 7 tadpoles remaining, others having only 2 or 3, and a few having no tadpoles remaining. In one instance, loss of tadpoles was due to escaping from the enclosure. In other cases, predators managed to get into the enclosures. Given that tadpoles at the impacted sites have survived in some of the enclosures, we do not believe that any of the deaths have been water quality related. We will be documenting when the tadpoles in each enclosure at each site begin to develop hind limbs. So far, metamorphosis has not yet begun. Previous research has indicated that tadpoles under stress may have accelerated metamorphosis, becoming frogs that are smaller compared to sites that have fewer stressors (predators, water quality, rapidly declining water levels, etc.). Therefore, the timing of metamorphosis will be an important indicator as to whether or not the impacted (Living Filter) ponds contain more stressors compared to the less-impacted (Scotia Barrens) ponds. When possible, we will make comparisons between tadpoles that came from the same egg clutch. We will leverage this information as preliminary data and apply for external funding to conduct more field studies and to design more controlled laboratory studies. This study is the first of its kind, so we expect the data to appeal to funding agencies such as NSF (Evolutionary Biology program).

It is noteworthy to mention that the Penn State Office of Physical Plant, which operates the Living Filter, is extremely supportive of the work our group has done at the Living Filter ponds. They are interested in the data we are collecting and have worked with us to operate the irrigation laterals impacting our sites on a consistent weekly schedule. They have also provided additional funds to support additional water quality samples from the ponds at which the tadpoles have been deployed.

STUDENTS & POSTDOCS SUPPORTED

Post-Doctoral Researchers:

Lindsey Swierk, Ecology, PhD, Penn State

Malcolm Taylor, Agricultural and Biological Engineering, PhD, Penn State

Graduate Students:

Bradley Carlson, Ecology, PhD, Penn State

Aminul Chowdhury, Agricultural and Biological Engineering, PhD, Penn State

Odette Mina, Agricultural and Biological Engineering, PhD, Penn State

Undergraduate Students:

Alexandra Everhart, Ecosystem Resources Management, Penn State

Marc Gluberman, Biological Engineering, Penn State

Mariana Gonzalez, General Engineering, Penn State

Kelley Harrington, Civil Engineering, Penn State

Daniel Schultz, Biological Engineering, Penn State

PUBLICATIONS

Mina, Odette, Heather Gall, Bradley Carlson, Tracy Langkilde, 2014, A preliminary assessment of endocrine disrupting compounds in vernal ponds in Central PA, in Proceedings of the American Society of Agricultural and Biological Engineers, ASABE, Montreal, Canada, Paper No. 1910944.

INFORMATION TRANSFER ACTIVITIES

Oral Presentations:

Mina, O., H. Gall, B. Carlson, and T. Langkilde. 2014. A preliminary assessment of endocrine disrupting compounds in vernal ponds in Central Pennsylvania. *2014 American Society of Agricultural and Biological Engineers and Canadian Society for Bioengineering Annual International Meeting*. Montréal, Québec, Canada, July 15.

Gonzalez, M., O. Mina, and H. Gall. 2014. Assessing the impact of land use on water quality in vernal pools. *Penn State College of Engineering Research Initiative Research Symposium*, Penn State, University Park, PA, July 2.

Poster Presentations:

Gall, H., O. Mina, and A. Chowdhury. 2015. Fate, Transport, and Impacts of Human and Animal Sources of Emerging Contaminants. *Penn State Institutes of Energy and the Environment Bar Charts & Barbecue: Annual Picnic & Poster Symposium*, Penn State, University Park, PA, May 1.

Harrington, K., O. Mina, and H. Gall. 2015. Assessing the impacts of wastewater irrigation on water levels in vernal pools. *Penn State Undergraduate Research Symposium*, Penn State, University Park, PA, April 8.

Gall, H., O. Mina, B. Carlson, and T. Langkilde. 2014. Assessing the presence of endocrine disrupting compounds in vernal pools. *Science of Environmental Toxicology and Chemistry North America Focused Topic Meeting: Endocrine Disruption*. Research Triangle Park, NC, Feb. 5.

Mina, O., H. Gall, B. Carlson, and T.L. Langkilde. 2014. Assessing the presence of endocrine disrupting compounds in vernal pools. *Penn State Institutes of Energy and the Environment Bar Charts & Barbecue: Annual Picnic & Poster Symposium*, Penn State, University Park, PA, April 25.

Mina, O., H. Gall, B. Carlson, and T.L. Langkilde. 2014. Assessing the presence of endocrine disrupting compounds in vernal pools. *Environmental Chemistry and Microbiology Student Symposium*, Penn State, University Park, PA, March 28.

PHOTOS OF PROJECT



Field technician, Jeremy Harper (left), and PhD student, Aminul Chowdhury (right), setting up containers in which the wood frog eggs will hatch at one of the control sites in the Scotia Barrens State Game Lands.



Wood frog egg mass resting on screening to hatch at a vernal pond in the Scotia Barrens State Game Lands. Tadpoles will be translocated to another control site and a contaminated site within a week of hatching.



Field technician, Eric Teitsworth, deploying newly hatched tadpoles in enclosures at a vernal pond in Penn State's Living Filter. Wastewater effluent irrigation can be seen in the far right. A real-time monitoring station (right) collects water level and various water quality parameters continuously.

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Information Transfer Program Introduction

Outreach Projects.

Two projects supported during FY14 were oriented toward information transfer, making research-based information available to user communities.

PI Bryan Swistock from Penn State University and a group of extension educators measured water quality in springs from across the state of Pennsylvania, which are popular sources of drinking water with the public. Results showed that roadside springs are routinely used by over 10% of Pennsylvania residents but often contain unsafe levels of bacteria, protozoans or other pollutants. Penn State Extension created and disseminated educational resources to increase awareness about the risks of drinking water from untreated roadside springs.

PI Beth Boyer from Penn State University and PA-WRRC graduate student interns worked to deliver educational and outreach programs. PA-WRRC co-sponsored numerous conferences and academic seminar series. PA-WRRC facilitated outreach at community events, in local elementary schools.

Drinking Water Testing and Education for Roadside Springs in Pennsylvania

Basic Information

Title:	Drinking Water Testing and Education for Roadside Springs in Pennsylvania
Project Number:	2014PA212B
Start Date:	4/15/2014
End Date:	2/28/2015
Funding Source:	104B
Congressional District:	5
Research Category:	Ground-water Flow and Transport
Focus Category:	Water Quality, Education, Groundwater
Descriptors:	None
Principal Investigators:	Bryan Reed Swistock

Publications

There are no publications.

FINAL PROJECT REPORT
Pennsylvania Water Resources Research Center

PROJECT TITLE & PRINCIPAL INVESTIGATORS

Drinking Water Testing and Education for Roadside Springs in Pennsylvania

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PROBLEM and RESEARCH OBJECTIVES

Roadside springs are pervasive across many parts of Pennsylvania. Residents using both public and private drinking water supplies seek out these springs to avoid the use of aesthetically inferior home or public water supplies. A 1990 report by the Pennsylvania General Assembly suggested an inventory and testing of roadside springs along with educational efforts to assist in curbing risks associated with consumption of drinking water from these unprotected water supplies. However, little research or additional information has been gathered since that report.

A 2013 pilot study of 35 roadside springs located in 19 counties across Pennsylvania by the Penn State Water Resources Extension team found that over 90% fail at least one health-based drinking water standard but this study was based on just one sample from each spring. More data on the use of roadside springs along with more detailed water quality data are needed to better quantify the health risks associated with these water supplies and target educational efforts for roadside spring users.

The overall goal of this project was to reduce the public health risk from the use of untreated roadside springs as a drinking water source. The objectives of the project were:

- 1) Determine the approximate use of roadside springs from attendees at various Penn State Extension water resources programs.
- 2) Obtain additional information about the water quality and temporal variability of water quality from a set of heavily-used roadside springs across Pennsylvania and
- 3) Create educational resources to increase awareness about the risks of drinking water from untreated roadside springs.

METHODOLOGY

The initial testing in 2013 identified 12 of 35 roadside springs that represented an especially large risk due to the presence of *E. coli* bacteria. From these 12 springs, ten springs were selected for additional testing (Figure 1). Locations and other characteristics of each spring are shown in Table 1.

Each spring was visited by project personnel quarterly (June, September, December and March) to collect water quality samples. All samples were returned within 24 hours of collection to the Penn State Agricultural Analytical Laboratory and analyzed for pH, NO₃-N, aluminum, arsenic, barium, calcium, copper, lead, iron, manganese, sulfate, magnesium, chloride, total coliform bacteria, *E. coli* bacteria, alkalinity, corrosivity index, total dissolved solids, total suspended solids, and hardness. During each seasonal sample collection visit, project personnel also documented the flow and use of each roadside spring.

During the Fall 2014 and Spring 2015 sample periods, additional samples were collected from eight springs for analysis of *Giardia* and *Cryptosporidium* oocysts. Eight springs were included in this testing due to cost limitations. Samples were collected into 10 liter cubitainers and shipped overnight on ice to the Analytical Services laboratory in Williston, Vermont. *Giardia* and *Cryptosporidium* were quantified in each sample using EPA Method 1623.1

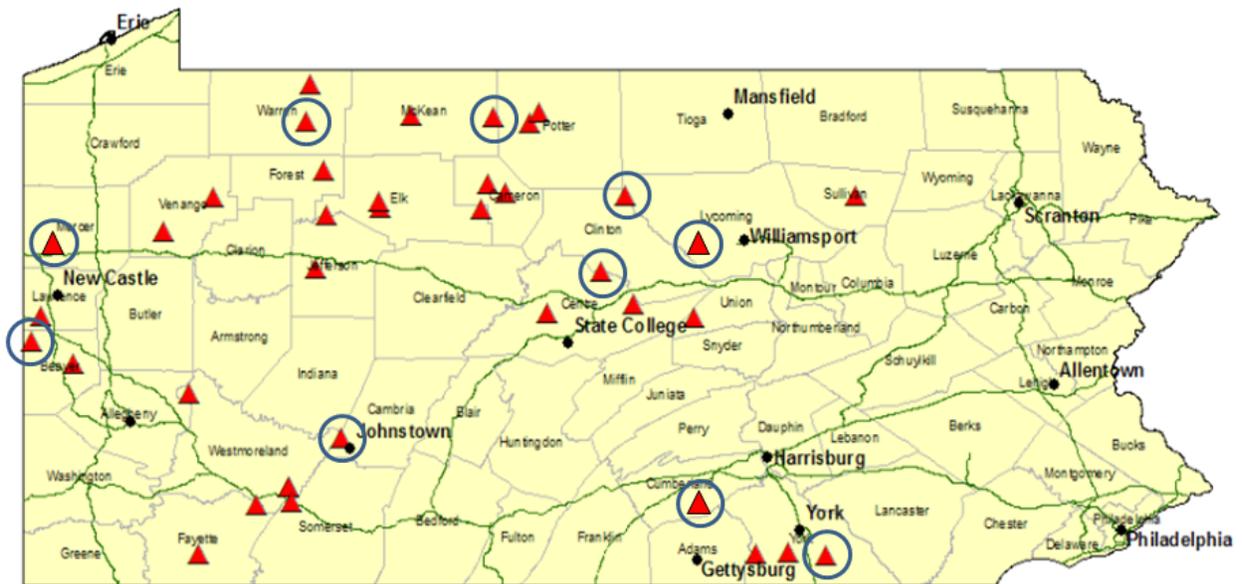


Figure 1. Location of the 10 roadside springs tested in 2014-15 (circled triangles) in comparison to the 35 springs tested in the 2013 pilot study (red triangles).

Table 1. Location and flow characteristics (gallons per minute) for each roadside spring visited during 2014-15.

Spring name	GPS Location	County	Minimum Flow (gpm)	Maximum Flow (gpm)
Lycoming	41.44149, -77.577726	Lycoming	0.41	1.69
Orviston	41.107285, -77.697819	Centre	1.54	27.5
Route 6	41.792680, -78.224632	McKean	0.15	5
Rippling Run	39.850927, -76.597183	York	2.3	10
Ridge Road	40.801122, -80.47606	Beaver	2.0	10
Chapman Dam	41.771313, -79.136143	Warren	6.6	13.3
Heffley Spring	40.375233, -78.966547	Cambria	3.0	39.0
Laurel Forge	40.034661, -77.272190	Cumberland	1.5	14.8
Waterville	41.22679, -77.32473	Lycoming	2.97	5.26
West Middlesex	41.165823, -80.443087	Mercer	2.0	8.5

Roadside spring use was estimated through informal surveys of attendees at various Penn State Extension Water Resources workshops across the state. These workshops focused on rural drinking water, pond management, stormwater, pesticide certification, irrigation management, groundwater education, or other water topics. Attendees were asked two questions about their use of roadside springs related to their frequency of use and reasons for using roadside springs. Most responses were collected informally using TurningPoint response cards incorporated into PowerPoint presentations.

PRINCIPAL FINDINGS AND SIGNIFICANCE

Use of Roadside Springs

A total of 1,035 were surveyed about their use of roadside springs at 55 educational programs across the state. Responses were distributed across the state including northcentral (312), northeast (173), northwest (41), southcentral (287), southeast (156), and southwest (66).

Overall, 30% (310) of respondents indicated that they had consumed water from a roadside spring at least once while 69% (722) had never consumed roadside spring water. The remaining 1% (6) gave no response. Of the 30% who had consumed roadside spring water, 18% collected water infrequently (12% every few years, 6% annually), while 12% collected spring water regularly (4% every few months, 5% monthly and 3% weekly).

When asked about their primary reasons for consuming roadside spring water, the most common responses were the better taste of the water and the idea of “natural” unpolluted and untreated water (Figure 2). Other less common reasons included camp/seasonal use, convenience or replacement for bad home water. The most common “other” uses for roadside spring water were water for household or garden plants, water for aquariums, and drinking water while hiking or biking.

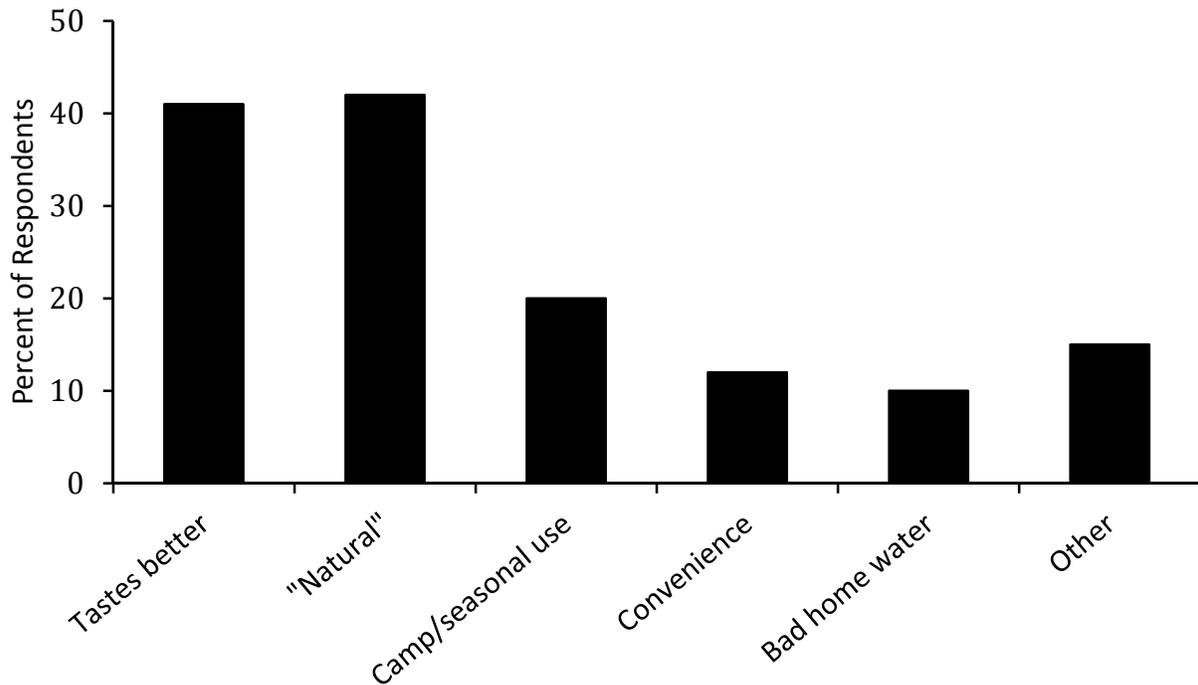


Figure 2. Reasons for use of roadside spring water given by 1,034 respondents.

Roadside Spring Water Quality

Water quality summaries for each water test parameter are shown in Table 2 for the ten roadside springs. Also shown in Table 2 are the percent of the ten springs which failed drinking water standards at least once for each parameter and the percent of all of the roadside spring samples that failed drinking water standards for each parameter.

Coliform bacteria were the most prevalent drinking water problem found in the springs, occurring above the drinking water standard in all of the samples from each of the ten springs (range 3 to 2,420 colonies per 100 mL). *E. coli* bacteria was also a common problem occurring in 80% of the springs at least once and in 43% of all of the samples collected from the springs. Other health related pollutants were absent from spring water but occasional aesthetic issues were noted including low pH and corrosive water or high levels of total dissolved solids (TDS), sulfate, iron, or aluminum.

Table 2. Water quality summary statistics for the ten roadside springs. All results are in mg/L except bacteria (colonies per 100 mL), pH, and corrosivity (LSI index). All results for arsenic and copper were below detection limits.

Parameter	Mean	Minimum	Maximum	% of springs failing	% of all samples failing
Health-Related Pollutants					
Coliform bacteria	192	3	2,420	100%	100%
<i>E. coli</i> bacteria	11	0	165	80%	43%
Barium	0.02	0.009	0.031	0%	0%
Lead	0.001	<0.001	0.004	0%	0%
Nitrate-N	0.89	<0.50	4.36	0%	0%
Aesthetic Pollutants					
pH	7.02	5.84	8.03	30%	18%
Total Dissolved Solids	126	<20	842	10%	8%
Chloride	11.3	<5	62.5	0%	0%
Sulfate	41.9	<10	390	10%	8%
Iron	0.09	<0.05	0.57	10%	10%
Manganese	0.003	<0.005	0.01	0%	0%
Corrosivity (LSI)	-1.93	-4.72	1.19	80%	80%
Aluminum	0.06	<0.005	0.398	20%	5%
Alkalinity	47.7	3.1	253	N/A	N/A
Suspended Solids	1.71	<1	8.0	N/A	N/A
Hardness	89.1	5.9	654	N/A	N/A

Results from the two samples (Fall 2014 and Spring 2015) collected from eight the springs for protozoan testing are shown in Table 3. Seven of the eight springs (88%) contained *Giardia* or *Cryptosporidium* oocysts during one or both of the sample times but the concentrations of oocysts per liter were generally low (below 10 oocyst per liter). Coliform and *E. coli* bacteria concentrations during the March 2015 protozoan testing are also shown in Table 3. Coliform bacteria were present in all samples and were generally higher in springs that contained protozoan oocysts. Conversely, *E. coli* bacteria were absent from several springs that contained protozoan oocysts making it a surprisingly poor indicator of the presence or absence of protozoans. Several springs that had protozoans during both sample time periods did not have *E. coli* bacteria and the one spring that did not have protozoans during either sample period tested positive for *E. coli* bacteria.

Table 3. Giardia and Cryptosporidium occurrence (oocysts per L) in samples collected from eight roadside springs in Fall 2014 and Spring 2015 in comparison to bacteria concentrations.

Spring	September 2014		March 2015			
	Giardia	Cryptosporidium	Giardia	Cryptosporidium	Total Coliform bacteria (#/100 mL)	<i>E. coli</i> (#/100 mL)
Lycoming	5.28	5.01	0.09	0	62	0
Orviston	0	0	0	0	59	5
McKean	0	0	6.49	2.23	>201	0
Rippling Run	5.7	6.36	0	0	>201	0
Ridge Road	0	0	6.29	4.26	>201	8
Warren	4.07	4.71	6.75	6.93	50	0
Heffley	6.32	6.05	0	0	32	2
Laurel Forge	0	0	6.82	5.57	>201	0

Table 4 provides the mean flow and water quality concentrations across the four seasonal samples collected at each spring. Percentage after mean shows the percent of springs failing the drinking water standard during that season.

Water flows were highest in the summer and spring and lowest in the fall. Most spring flows were between 1 and 10 gallons per minute. Water quality concentrations were consistent across seasons and agree with the overall annual results presented in Table 2. Aesthetic water quality (metals, pH, chloride, etc.) were consistent between seasons. Concentrations of total coliform bacteria were most variable between seasons (Figure 2). Higher bacteria concentrations were generally observed in spring and summer, presumably due to wetter and warmer conditions promoting surface water infiltration into roadside springs. Counts of coliform bacteria in roadside springs were highest in the early summer of 2014 then declined through fall and winter before beginning to increase again in spring 2015.

Table 4. Mean concentrations (percent of springs failing drinking water standards) in each season. All results are in mg/L except bacteria (colonies per 100 mL), pH, and corrosivity (LSI index). All results for arsenic and copper were below detection limits.

Parameter	Summer 2014	Fall 2014	Winter 2014	Spring 2015
Flow (gallons per minute)	9.5	3.4	5.3	10.4
Health-Related Pollutants				
Coliform bacteria	402 (100%)	169 (100%)	87 (100%)	110 (100%)
<i>E. coli</i> bacteria	20 (50%)	19 (50%)	1 (30%)	5 (40%)
Barium	0.018	0.018	0.016	0.017
Lead	0.002	0.001	0.001	0.001
Nitrate-N	0.91	0.77	0.93	0.95
Aesthetic Pollutants				
pH	6.85 (20%)	7.12 (10%)	6.99 (30%)	7.14 (10%)
TDS	117 (10%)	144 (10%)	125 (10%)	118 (10%)
Chloride	11.4	12.2	10.8	10.8
Sulfate	39.7 (10%)	48.1 (10%)	44.8 (10%)	35.3 (10%)
Iron	0.10 (10%)	0.07 (10%)	0.08 (10%)	0.10 (10%)
Manganese	0.003	0.003	0.003	0.003
Corrosivity	-2.2 (80%)	-1.74 (80%)	-1.97 (80%)	-1.82 (80%)
Aluminum	0.05	0.03	0.04	0.10 (20%)
Alkalinity	43	54	50	44
TSS	2	1	2.1	1.7
Hardness	83	102	94	76

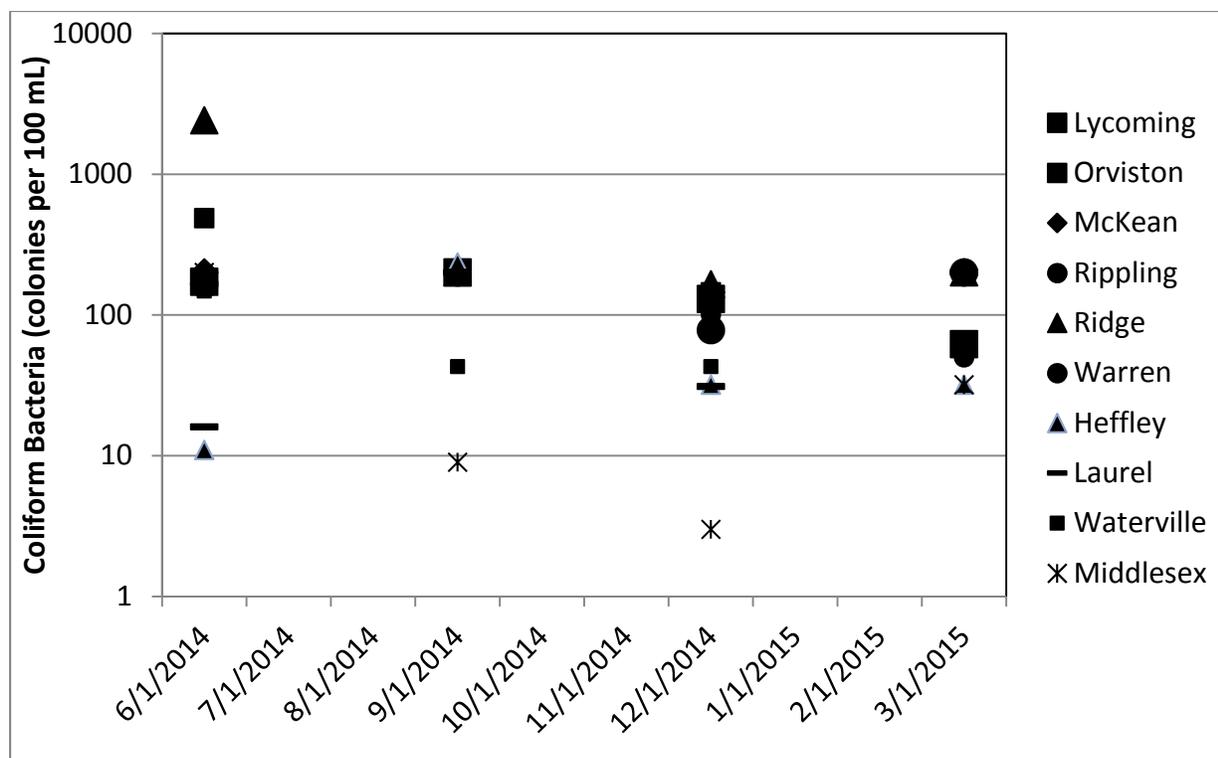


Figure 2. Concentrations of total coliform bacteria in each roadside spring throughout the year (note log scale on vertical axis).

Results from this study show that roadside springs are a routine source of drinking water for about 10% of the rural Pennsylvania population with another 20% indicating that they use roadside springs occasionally for drinking water.

The seasonal, detailed testing of ten roadside springs in this study found that all springs fail at least one safe drinking water standard including the presence of *Giardia* and/or *Cryptosporidium* oocysts. Water quality in roadside springs was generally stable throughout the year although bacteria levels tended to be higher during warmer and wetter conditions (spring and summer). Overall, results from this study suggest that the consumption of untreated roadside spring water represents a significant public health risk warranting future education and outreach efforts.

STUDENTS & POSTDOCS SUPPORTED - NONE

PUBLICATIONS

- Swistock, Bryan, James Clark, Susan Boser, Dana Rizzo, Diane Oleson, Amy Galford, Mark Madden, and Gary Micsky, 2015 (submitted), Issues associated with the use of untreated roadside springs as a source of drinking water, *Journal of Contemporary Water Research and Education*
- Swistock, Bryan, 2015 (in press). *Roadside Springs*, Penn State Extension fact sheet, 4 pp. <http://extension.psu.edu/natural-resources/water>

INFORMATION TRANSFER ACTIVITIES

The project investigators presented results from this study at a variety of Penn State Extension water resources workshops and events across the state including safe drinking water clinics, pond management workshops, irrigation programs, Ag Progress Days, Farm Show and pesticide certification courses. Detailed of educational efforts include:

- Overall, roadside spring education was a component in 56 different programs which were presented to 1,619 residents. Of these attendees 887 (55%) completed an on-site paper or TurningPoint evaluation. A total of 845 (95%) learned new information and 486 (55%) indicated that they had learned a great deal of new information. 690 (78%) were planning on taking some action as a result of hearing the presentation. Of particular importance was the presentation provided for 18 township supervisors at the 2014 Pennsylvania State Association of Township Supervisors meeting in Hershey, PA. Townships own and maintain the right-of-way where many roadside springs are located across the state.
- A statewide news release was prepared and disseminated in the *Watershed Winds* online newsletter in Spring 2014 and again in Spring 2015.
- An article entitled *Should you drinking from roadside springs?* was published in the Penn State *Ag Science* Magazine in Spring 2014 (page 8).
- A statewide webinar was presented 86 attendees on *A Study of Roadside Springs in Pennsylvania*. The recorded webinar and supporting materials can be viewed at: <http://extension.psu.edu/natural-resources/water/webinar-series/past-webinars/a-study-of-roadside-springs-in-pennsylvania>
- A poster presentation entitled *A Study of Roadside Springs of Pennsylvania* was presented to 210 attendees at the May 2014 Pennsylvania Groundwater Symposium.
- A paper was presented on *Issues Associated with the Use of Untreated Roadside Springs as a Source of Drinking Water* at the 2015 Pennsylvania Groundwater Symposium. A poster on the same work will be presented at the 2015 UCOWR conference in Las Vegas, NV.
- Several water resources Extension Educators have also published news releases in their local communities sharing research results and extension information regarding this roadside spring project.

PHOTOS OF PROJECT



Diane Oleson explains results from the roadside spring project at the Pennsylvania Groundwater Symposium in May 2014.



Jim Clark collects a sample from a roadside spring in northcentral Pennsylvania.



Jim Clark (Water Resources Extension Educator, McKean County) presents results from the roadside spring project to over 150 attendees at the 2015 Pennsylvania Groundwater Symposium.

Pennsylvania Water Resources Research Center Outreach Program

Basic Information

Title:	Pennsylvania Water Resources Research Center Outreach Program
Project Number:	2014PA213B
Start Date:	4/15/2014
End Date:	2/28/2015
Funding Source:	104B
Congressional District:	5
Research Category:	Not Applicable
Focus Category:	Education, Conservation, Water Use
Descriptors:	None
Principal Investigators:	Elizabeth W. Boyer

Publications

There are no publications.

FINAL PROJECT REPORT
Pennsylvania Water Resources Research Center

PROJECT TITLE & PRINCIPAL INVESTIGATOR(S)

Pennsylvania Water Resources Research Center Education & Outreach Program

Elizabeth W. Boyer, Department of Ecosystem Science and Management, Penn State University

PROBLEM and RESEARCH OBJECTIVES

Concerns over water resources have been growing in Pennsylvania in recent years, in response to severe droughts and floods, a growing population, increasing demands for water, and the need to understand how changes in land use, climate, and energy extraction (e.g., natural gas) affect water quantity and quality. The Pennsylvania Water Resources Research Center will co-sponsor conferences that promote dialog of important water resources issues in Pennsylvania and beyond. Further, we will offer educational outreach programs targeted to multiple audiences.

METHODOLOGY

Objective 1. Co-sponsor conferences of broad interest to water resources issues in Pennsylvania.

The Pennsylvania Water Resources Research Center (PA-WRRC) helped to plan, co-sponsor, and participate several conference events in FY2014. Goals of these activities are to:

- *Discuss water issues of importance to Pennsylvania.* We aim to bring together diverse audiences to discuss water issues of regional importance. We will encourage dialog and discussion among stakeholders, involving academia, state and local agencies, utilities, non-governmental organizations, consultants, and more. This will foster collaboration and cooperation toward articulating water challenges, common goals, and potential solutions. Further, we aim to discuss the role of research in advancing understanding of important water resources issues in Pennsylvania. This will encourage synthesis of data, information, and results.
- *Enable networking.* We recognize that networking among researchers, educators, and stakeholders is one key to both professional growth and to impactful science. The conference will provide mechanisms for professional networking for all participants through formal activities (e.g., introductions, panel discussions and breakout groups, and the oral/poster presentation sessions), and informal activities (ample time for discussions to unfold during breaks, meals, and social activities).
- *Mentoring of early career academic researchers.* We aim to facilitate strong participation of early career researchers (including assistant professors, post-doctoral associates, and graduate students), inviting them to participate in the Conferences, and providing awards to offset their travel and registration costs. Further, there will be a suite of awards for best presentations by graduate students, and we have planned a special graduate program session

to facilitate sharing of ideas and networking. This will help to spark further research collaborations.

Objective 2. Provide 10 “let’s talk about water” outreach programs to local events and local schools, engaging with the community on water issues.

PA-WRRC engaged in water education and outreach by offering science, technology, and math (STEM) education programs focused on water resources, tailored to appropriate age audiences. Our graduate interns and undergraduate interns, themselves involved in STEM fields of research, will provide water educational outreach programs -- attending community events, visiting some local classrooms in the public schools, and having groups of pre-K students visit our water labs on the Penn State Campus.

Importantly, we recognize that, in addition to being competent researchers, STEM graduate students must be able to communicate science and research to a variety of audiences. Graduate and undergraduate students will gain facility with explaining science to people of many ages. The graduate students also inspire transformation in the K-12 formal and informal learning environments and stimulate interest in science and engineering among students and teachers. Further, our outreach activities will engage PA-WRRC with the community, providing opportunities to discuss water issues with a variety of stakeholders and providing visibility of our organization.

PRINCIPAL FINDINGS AND SIGNIFICANCE

Objective 1. Co-sponsor conferences of broad interest to water resources issues in Pennsylvania.

In FY2014, the Pennsylvania Water Resources Research Center co-sponsored the following conferences:

- 2014 Pennsylvania Groundwater Symposium: *Building Groundwater Knowledge Through Monitoring*, held in State College, PA, May 7, 2014. PA-WRRC and PSIEE affiliated researchers Lara Fowler and Paul Knight were among the keynote speakers for the conference. PA-WRRC provided support for 3 graduate students to attend the conference.
- 2014 NIWR/UCOWR/CUAHSI Conference: *Water Systems, Science, and Society Under Global Change* held in Medford, Massachusetts, June 18-20, 2014. PA-WRRC director Elizabeth Boyer gave a talk at the conference and served on the conference organizing committee. PA-WRRC provided support for 5 graduate students to attend the conference as well as 1 water resources extension associate (James Clark)

- 2014 Mid-Atlantic Regional Water Conference: *The Future of Mid-Atlantic Water Supply and Infrastructure*, held in Shepherdstown, West Virginia, September 23-24, 2014. PA-WRRC Director Beth Boyer participated in plenary session discussion panel and served on the conference planning committee. PA-WRRC provided support for 4 graduate students to attend the conference.

Objective 2. Provide “let’s talk about water” outreach programs, engaging a wide community of stakeholders in water issues.

PA-WRRC and PSIEE co-sponsored the following high-profile academic seminar series in FY14, related to water resources on the campus of Penn State University, toward discussing how research is advancing water resources issues:

- Marine Science and Technology Earth Talks (co-sponsored with primary leadership from the PSU Earth and Environmental Systems Institute).
- Nutrient Wicket Problems (co-sponsored with primary leadership from the PSU Center for Nutrient Solutions).
- National Ground Water Association Darcy Lecturer Dorte Wildenschild. She gave a talk entitled “Opportunities and Limitations for Porous Media Characterization and Process Quantification Using X-ray Tomography” on October 1, 2014 (PA-WRRC was the primary sponsor).

During FY2014, PA-WRRC engaged with stakeholders in many outreach events

- Provided support and leadership for the Penn State student chapter of the American Water Resources Association, engaging about 40 undergraduate and graduate students at Penn State in educational and outreach activities.
- PA-WRRC graduate interns Sarah Tzilkowski and Lidiia Iavorivska worked in the Phillipsburg, PA and Bellefonte, PA elementary schools to offer 10 water education activities for 2nd grade science classes – focusing on the water cycle, watersheds, and water quality. These programs were offered in cooperation with colleagues from Penn State’s Center for Science and the Schools, facilitating existing partnerships with K-12 schools and classroom teachers, and meeting science standards from the state of PA.
- PA-WRRC disseminated information about our research, education, and outreach programs at “CrickFest” in Coburn, PA promoting water conservation and education, and at “Penn State Ag Progress Days” in Rock Springs, PA. Both of these events draw thousands of community members and community leaders who are interested in water resources issues.
- PA-WRRC supported a summer internship for graduate student Amber Stilwell of Mercyhurst University for an internship in aquatic ecology and water quality based at the Tom Ridge Environmental Center in Erie. This is supported by USGS PA water science center and PAWRRC.

PA-WRRC Director Elizabeth Boyer provided leadership for a review of water sciences at Penn State during FY14, via a University wide Water Task Force Committee. PA-WRRC led 3 open town hall meetings to discuss water research, education, outreach, and opportunities at Penn State, and solicited wide participation among water-interested faculty and researchers from across the Penn State campuses. The committee assessed Penn State’s water-related activities

and developed a strategic plan for enhancing water research, education, and outreach. PA-WRRC and PSIEE arranged for an ad-hoc External Advisory Committee met at Penn State on August 5th 2014, to review the Water Task Force strategic plan report, and to help us consider how best to: 1) Coordinate a wide array of water programs and interests across the University; 2) Prioritize future investments toward advancing water sciences at Penn State; and 3) Raise the profile of Penn State water sciences nationally and internationally. The reviewers included Larry Band (Chair), Greg Characklis, Wendy Graham, and Deb Swackhamer. They submitted a useful report to Penn State with ideas on the above, which was distributed to Administration as well as water faculty and staff. Penn State is actively engaged in implementing recommendations from the Water Task Force Report.

PA-WRRC Director Elizabeth Boyer worked with Pennsylvania Sea Grant, supporting their application to become a Sea Grant College. Boyer participated in the PA Sea Grant external evaluation by NOAA in October 2014 in Erie, PA.

PA-WRRC Director Elizabeth Boyer and PSIEE affiliate Henry Lin initiated discussion toward developing a dual-title graduate degree in Water at Penn State. Students admitted to a primary graduate program may also have the option of enrolling in a secondary area of study to receive a dual-title degree. PA-WRRC facilitated an initial open discussion for faculty from across the campus who were interested in participating, toward developing this educational program. Ideas and outcomes were discussed and compiled from this town-hall style meeting. One key outcome of this program will be better-facilitating interdisciplinary water education and research.

STUDENTS & POSTDOCS SUPPORTED

Lidiiia Iavorivska, PhD student, Department of Ecosystem Science and Management, Penn State University

Sarah Tzilkowski, PhD student, Department of Ecosystem Science and Management, Penn State University

PUBLICATIONS (follow style formats on the next page)

Williams MR, AR Buda, HA Elliott, J Hamlett, EW Boyer, and JP Schmidt. (2014). Groundwater Flow Path Dynamics and Nitrogen Transport Potential in the Riparian Zone of an Agricultural Headwater Catchment. *Journal of Hydrology*. DOI:10.1016/j.jhydrol.2014.02.033.

PRESENTATIONS

In FY14, Principal Investigator Boyer and collaborators presented many talks about water resources research to a wide variety of audiences. These included:

- Britson AJ, DH Wardrop, **EW Boyer**, and PJ Drohan. Differences in water chemistry, soil chemistry, and plant litter quality in high and low disturbance wetlands. Joint Aquatic Sciences Meeting, Portland, OR, May 2014. *Student award for best poster.*
- Buda AR, SS Tzilkowski, LC Kibet, RB Bryant, **EW Boyer**, AL Allen, PJ Kleinman, and EB May. Terrestrial sources of urea to water in a mixed land use watershed: exploring the roles of current and past nitrogen management. Joint Aquatic Sciences Meeting, Portland, OR, May 2014.
- Boyer E**, J Grimm, K Eklof, L Iavorivska, P Drohan, J Bennett, and C Grant. Effects of climatic variability and land use on atmospheric deposition, with implications for water quality in forested catchments of the Northeastern United States. UCOWR/NIWR/CUAHSI Conference on Water Systems, Science, and Society Under Global Change. Medford, MA, June 2014.
- Clark J, B Swistock, **E Boyer**, M Madden, and D Rizzo. Natural gas well drilling and rural drinking water supplies. UCOWR/NIWR/CUAHSI Conference on Water Systems, Science, and Society Under Global Change. Medford, MA, June 2014.
- Iavorivska L, **E Boyer**, J Grimm, J Fuentes. Concentration and composition of organic matter in precipitation of Pennsylvania as affected by climatic factors. UCOWR/NIWR/CUAHSI Conference on Water Systems, Science, and Society Under Global Change. Medford, MA, June 2014.
- Lawler D, **E Boyer**, and P Drohan. Effects of Atmospheric Mercury Deposition on Isolated Wetland Environments. UCOWR/NIWR/CUAHSI Conference on Water Systems, Science, and Society Under Global Change. Medford, MA, June 2014.
- Reed BC and **EW Boyer**. Water Quality of Three Forest Streams in Pennsylvania Impacted by Atmospheric Deposition. UCOWR/NIWR/CUAHSI Conference on Water Systems, Science, and Society Under Global Change. Medford, MA, June 2014.
- (Invited, Plenary) EW Boyer**, R Alexander, J Needoba, and R Smith. Lateral transfers of carbon from terrestrial watersheds to the oceans: Rivers and groundwater. Coastal Carbon Synthesis Community Workshop, Woods Hole Oceanographic Institution, Falmouth, MA, August 2014.
- Eklöf, K., **Boyer, EW.**, Drohan, P. and Iavorivska, L. Will refilling a northern Appalachian impoundment cause extensive methylation? International Union of Forest Research Organizations World congress, 5-11 October 2014, Salt Lake City, UT, USA.
- Eklöf, K., Schelker, J., Sørensen, J., Åkerblom, S., Kraus, A., Meili, M., Weyhenmeyer, GA., von Brömssen, C., Laudon, H., **Boyer, EW.**, and Bishop, K. Effects of forestry on mercury runoff in catchment-scale studies in Sweden. International Union of Forest Research Organizations World congress, 5-11 October 2014, Salt Lake City, UT, USA.
- Swistock B, **E Boyer**, J Clark, M Madden, and D Rizzo. A comparison of water well quality before and after nearby fracking of gas wells in Pennsylvania. National Ground Water Association Workshop on Groundwater Quality and Unconventional Gas Development: Is There a Connection? Pittsburgh, PA, November 2014.
- (Invited) Boyer EW.** Water Quality in Catchments of the Northeastern USA: Toward Understanding Impacts of Atmospheric Deposition and Climatic Variability. Department of Earth Sciences Seminar Series, Uppsala University, December 3, 2014.
- (Invited) Boyer EW**, PJ Drohan, D Lawler, J Grimm, C Grant, KJ Eklof, J Bennett, and MD Naber. Anthropogenic Mercury Accumulation in Watersheds of the Northern Appalachian Mountains. American Geophysical Union, San Francisco, CA, December 2014.

- (Invited) Boyer EW** and L Fowler. Social Justice and Water Issues in the 21st Century. American Geophysical Union, San Francisco, CA, December 2014.
- DeWalle D, **EW Boyer**, and AR Buda. Relationships Between Long-term Atmospheric Wet Deposition and Stream Chemistry in Mid-Appalachian Forest Catchments. American Geophysical Union, San Francisco, CA, December 2014.
- Iavorivska L, **EW Boyer**, J Grimm, and JD Fuentes. Atmospheric Deposition of Organic Carbon in Pennsylvania as Affected by Climatic Factors. American Geophysical Union, San Francisco, CA, December 2014. *AGU student travel grant award.*
- Eklöf, K., **Boyer, EW.**, Drohan, P., Iavorivska, L., Harper, J., Brown, M., Fink, C., Gogno, J., and Reed, B. *Mercury methylation trends pre and post refilling in a Northern Appalachian impoundment.* AGU Fall Meeting, 15-19 December, San Francisco, CA, USA.
- (Invited) Boyer EW.** Atmospheric Mercury Deposition and Accumulation in Mid-Appalachian Catchments. Cornell University, Biogeochemistry and Environmental Science and Sustainability Seminar Series, February 27, 2015.

USGS Summer Intern Program

None.

Student Support					
Category	Section 104 Base Grant	Section 104 NCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	8	0	0	0	8
Masters	4	0	0	0	4
Ph.D.	9	0	0	0	9
Post-Doc.	1	0	0	0	1
Total	22	0	0	0	22

Notable Awards and Achievements