New Jersey Water Resources Research Institute
Annual Technical Report
FY 2015

Introduction

The New Jersey Water Resources Research Institute (NJWRRI) supports a diverse program of research projects. With oversight from the Advisory Council, which sets the Institute's Research Priorities, the available funds are divided between supporting faculty with 'seed' projects or new research initiatives and funding graduate students to develop their thesis research. The funding is intended to initiate novel and important research efforts by both faculty and students, thus emphasizing new research ideas that do not have other sources of funding. We hope to support the acquisition of data that will enable further grant submission efforts and in the case of students, lead to research careers focused on cutting-edge research topics in water sciences.

Three faculty initiated projects were awarded and eight grants-in-aid were awarded in FY2015 to graduate students who are beginning their research. We expect that the research is exploratory and is not supported by other grants. The intent is that these projects will lead to successful proposals to other agencies for further support. The larger goal of the research component of the Institute's program is to promote the development of scientists who are focused on water resources issues of importance to the state. Updates on three graduate student projects and one faculty project from FY2014 are included as well as an update on one graduate student project from FY2013.

In FY2015, the NJWRRI continued to emphasize the development and upkeep of the website and e-based communications with stakeholder groups. We also continue to participate in the New Jersey Water Monitoring Council, a statewide body representing both governmental and non-governmental organizations involved in water quality monitoring. Furthermore, in FY2015, the NJWRRI co-sponsored a conference “Fixing Flooding: One Community at a Time, Innovative Solutions using Green Infrastructure” on February 26, 2016 at the Middlesex County Fire Academy in Sayreville, New Jersey with the Rutgers Cooperative Extension Water Resources Program. The purpose of the conference was to educate stakeholders and engage them in addressing the impacts of stormwater runoff from impervious cover. The workshop acknowledged on-going efforts by the Sustainable Raritan River Collaborative and showcased their work as well as the work completed as part of the National Fish and Wildlife Foundation (NFWF) project “Incorporating Green Infrastructure Resiliency in the Raritan River Basin (NJ)” to date. The conference provided attendees an opportunity to network and develop new partnerships so they can better achieve the goal of a sustainable Raritan River Basin. The presentations and more information about the conference can be found at http://www.water.rutgers.edu/GreenInfrastructureConference.html.

Research Program Introduction

None.

Assessing the role of wetland flora as a sink for nutrients in subsurface gravel wetlands in New Jersey

Basic Information

Title:	Assessing the role of wetland flora as a sink for nutrients in subsurface gravel wetlands in New Jersey
Project Number:	2013NJ341B
Start Date:	3/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	NJ-004
Research Category:	Water Quality
Focus Category:	Nutrients, Non Point Pollution, Wetlands
Descriptors:	
Principal Investigators:	Kyle Seiverd, Louise Wootton

Publications

There are no publications.

NJWRRI FY2015 Annual Report Information

Project Title: Assessing the role of wetland flora as a sink for nutrients in subsurface gravel wetlands in New Jersey

Subaward agreement no. 4943

(1) **PI information**: List all PIs, addresses, email addresses, phone numbers

Subaward PI:

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(2) **Numbers of Students Supported**:

Undergraduates: 0

Masters' students: 1

Ph. D. students: 0

Postdoctoral assocs:0

(3) **Project Summary**:

Few of the studies to date on the role of plants in nutrient sequestration have been carried out in the field, and in my literature review I found few that were carried out in New Jersey. Thus it is imperative to assess the potential of various plant species commonly used in storm water basin plantings for removing nutrients from runoff in order to guide best management practices (BMP) in future basin designs. In addition, plant community composition is influenced by water level, amounts of leaf litter and soil fertility (Weiher and Keddy 1995). Thus, it might be expected that different plants will outcompete others in different wetlands. Determining which species are most successful in establishing in the different wetlands can help managers to select planting palettes that will be effective for the type of wetland in question.

The expected outcomes of this project were to provide data on the short-term effects of stormwater basin design on the competitive outcomes between plant species, as well as on their biomasses, and nutrient content. In addition, once our analyses are completed we will provide data on seasonal changes in plant nutrient sequestration. Together, these data can be used to guide choices of plants for use in storm water basin plantings as well as to inform decision making about optimal harvest times for maximizing nutrient removals from systems such as these.

Methodology - give a general summary of procedures and methods actually implemented

Four subsurface gravel wetlands, each of a varying design were constructed at Georgian Court University (Table 1). Eight wetland plant species were monitored during the growing season to assess establishment success and survival. Six replicates of each monitored species were harvested at the start, middle and end of the growing season and separated into above ground and below ground biomass samples. Above ground samples were sorted by color, with green samples being at least 50% green, the remaining was classified as brown biomass. Roots were washed in tubs using water and patted dry using paper towel (Figure 1). Samples were massed and placed in a drying oven for a minimum of 72 hours before being massed again to assess wet and dry biomass.

Table 1: Subsurface gravel wetlands constructed at Georgian Court University.

Wetland	Design
University of New Hampshire Design (UNH)	Original replicate of wetland designed by UNH
Advanced Bioretention Systems (ABS)	vertical, rather than horizontal water flow with mostly sand soil composition.
Simplified UNH Design #1 (S-UNH-1)	UNH design modified by excavated to a depth of 3'and providing an additional foot of gravel
Simplified UNH Design #2 (S-UNH-2)	UNH design modified by removal of baffles and simplified plumbing

Figure 1: Washing of roots for below ground biomass of *Spartina alternifolia.*

Dried green above ground biomass for each species was ground using a blender. Approximately 0.2 grams of each replicate was pooled by species and placed in a sample tube. These plants are being analyzed for nutrient content at the beginning, mid and end of growing season. Nutrient analyses are being completed at the Rutgers Soil and Plant Analysis Laboratory with results expected by the end of May 2016. Nutrient content will then be compared within and between subsurface gravel wetlands using repeated measures ANOVAs.

Principal Findings and Significance –

Preliminary findings of above ground dry green biomass were that *Juncus effusus* had the greatest amount of success across all four wetland designs. Throughout the growing season, *J. effusus* showed no decrease (Figure 2) in above ground dry green biomass. The dry root biomass of *J. effusus* also increased in all four wetland designs. The greatest increase of dry root biomass was in the UNH with an average increase of 867% (Figure 3).

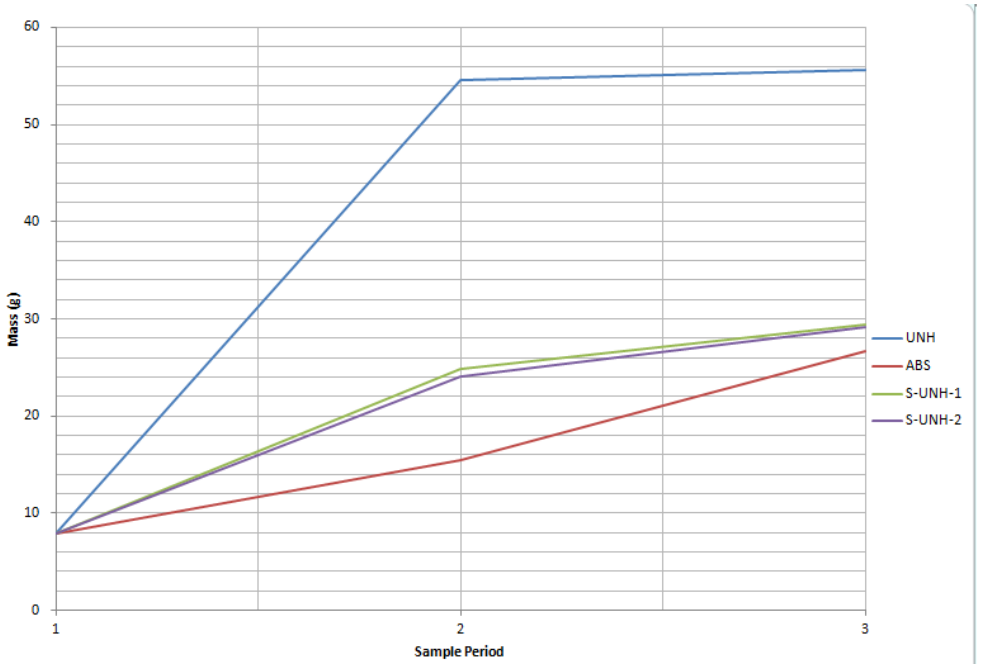

Figure 2: *J. effusus* average green biomass in four wetlands throughout the 2015 growing season.

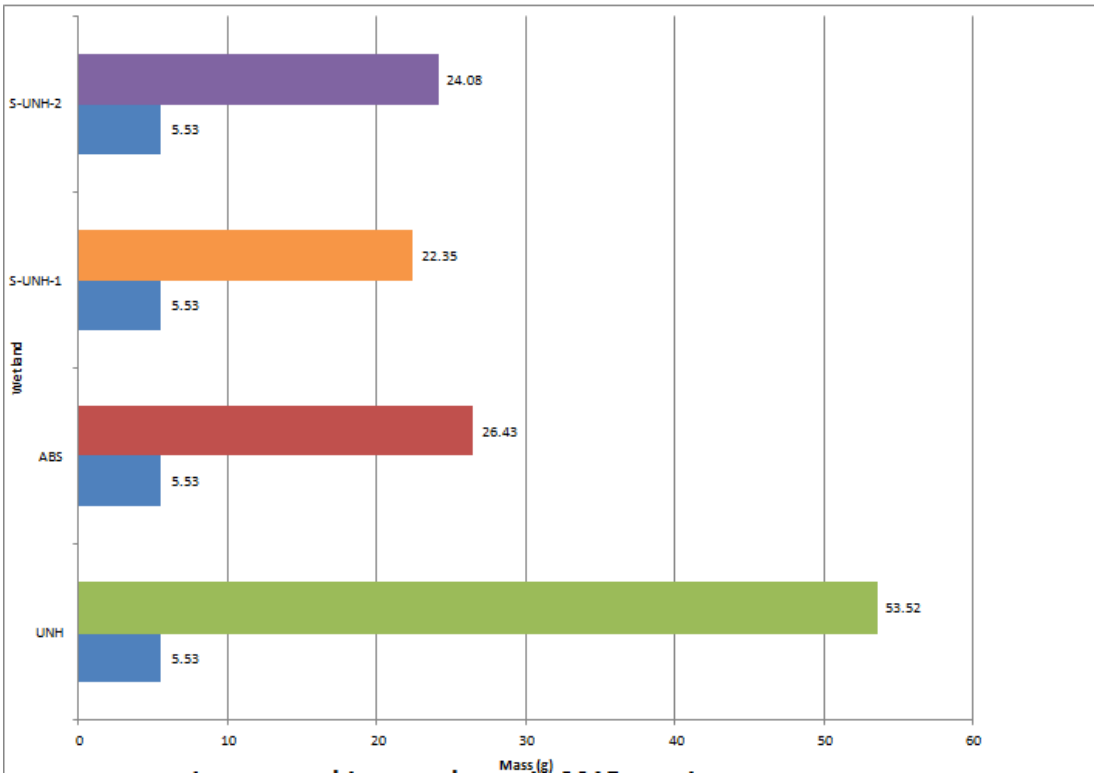

Figure 3: *Juncus effusus* dry root biomass before (blue) and after the 2015 growing season in 4 subsurface gravel wetlands at Georgian Court University.

Spartina alternifolia showed the least amount of success across all four wetland designs. A decrease in above ground green biomass was found across all wetlands (Figure 4). Dry root biomass of *S. alternifolia* also decreased across all four wetlands (Figure 5).

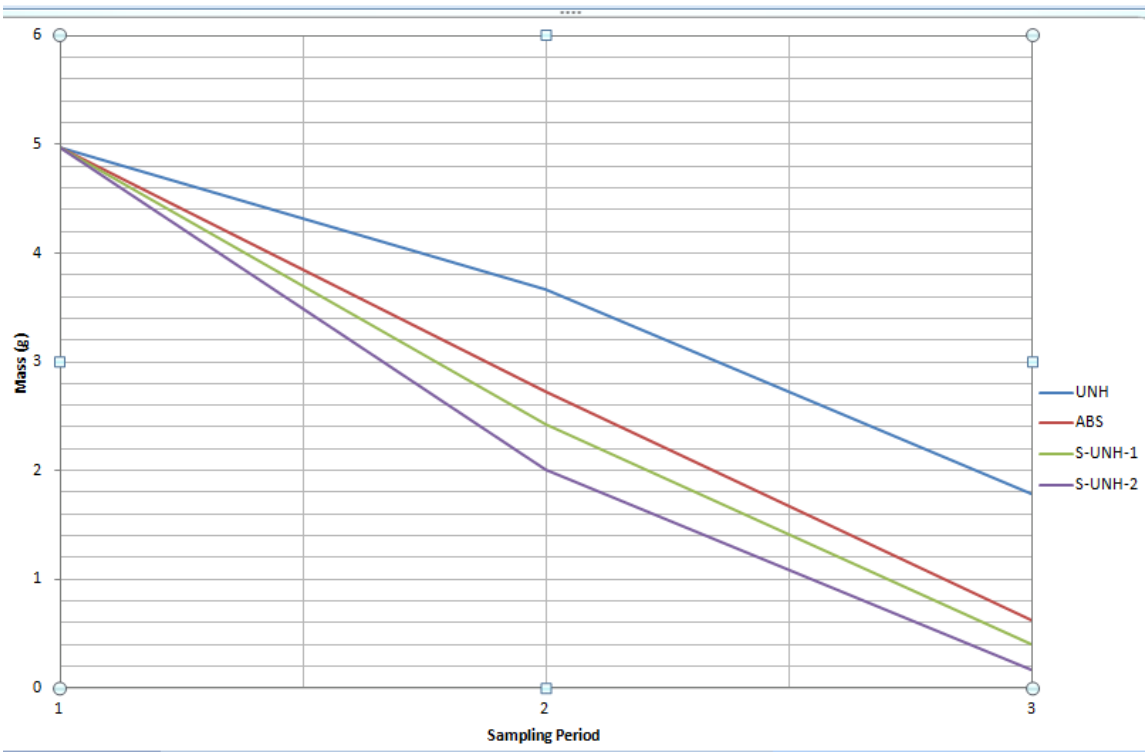

Figure 4: *Spartina alternifolia* dry green biomass in four wetlands throughout the 2015 growing season at Georgian Court University.

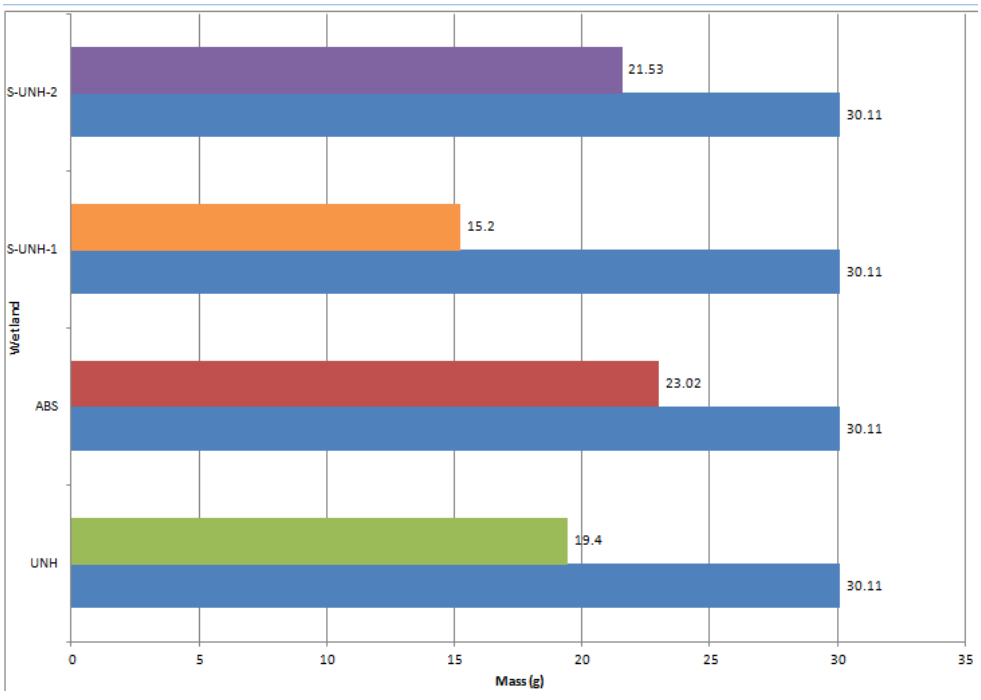

Figure 5: *Spartina alternifolia* dry root biomass before (blue) and after the 2015 growing season in 4 subsurface gravel wetlands.

Of the four wetland designs, the original University of New Hampshire subsurface gravel wetland showed the greatest proliferation of total above ground green biomass (Figure 6). Green dried biomass showed a peak during the midseason harvest (August), with a slight decline at the end of season harvest (October). Pooled dry root biomass (Figure 9) aligned with above ground green biomass, with UNH having the greatest increase compared to the other wetland designs. The ABS had the least pooled biomass increase, showing a decline over the entire growing season.

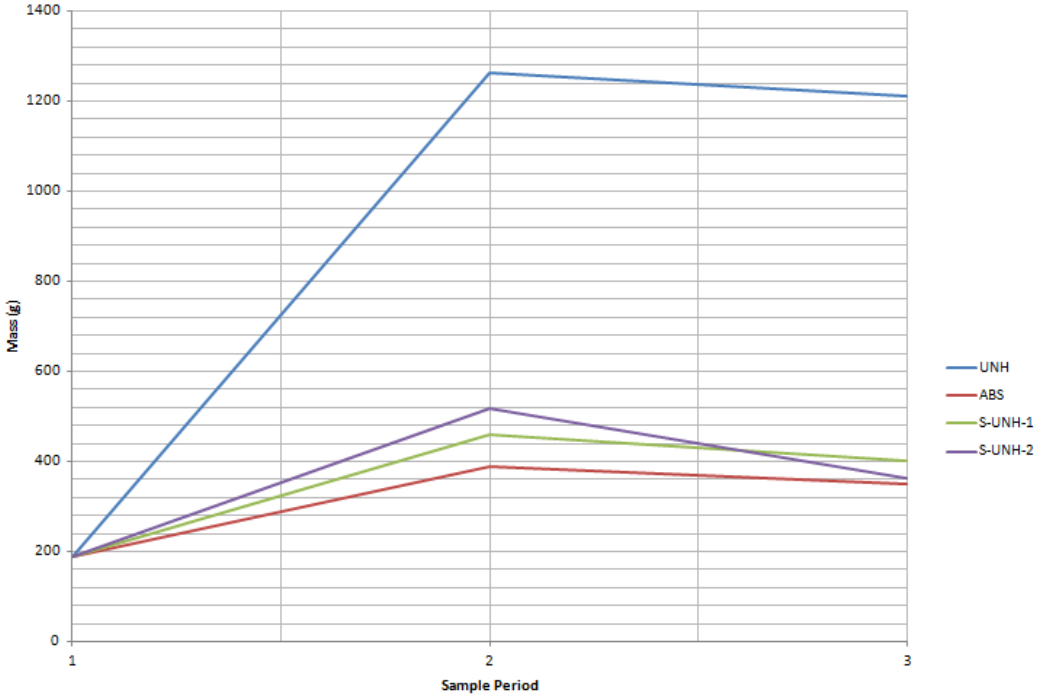

Figure 6: Pooled dry green biomass between wetlands in 2015 growing season.

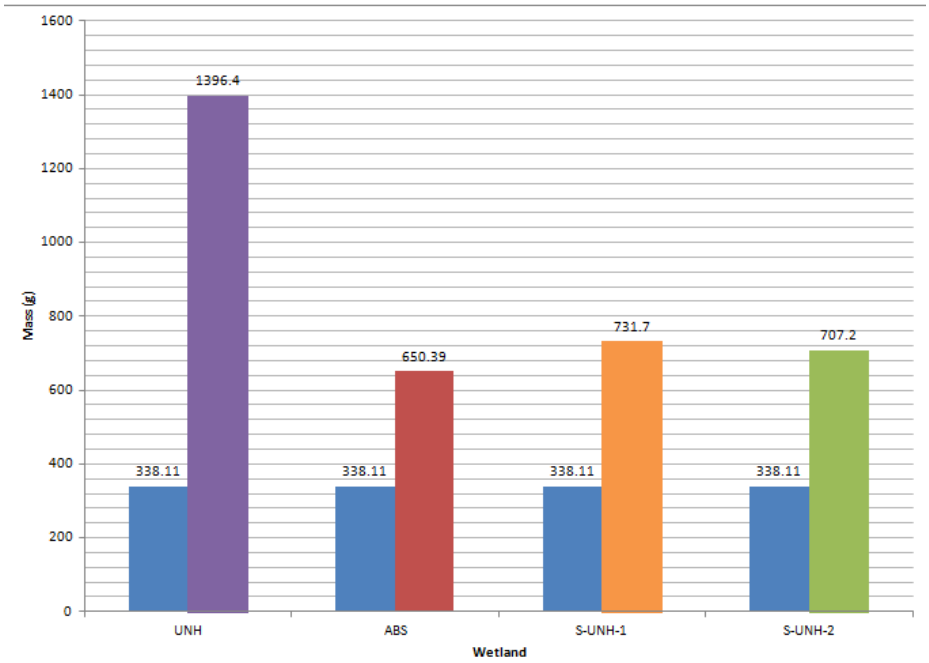

Figure 7: Pooled dry root biomass between wetlands at the start (blue) and end (colored) of the 2015 growing season.

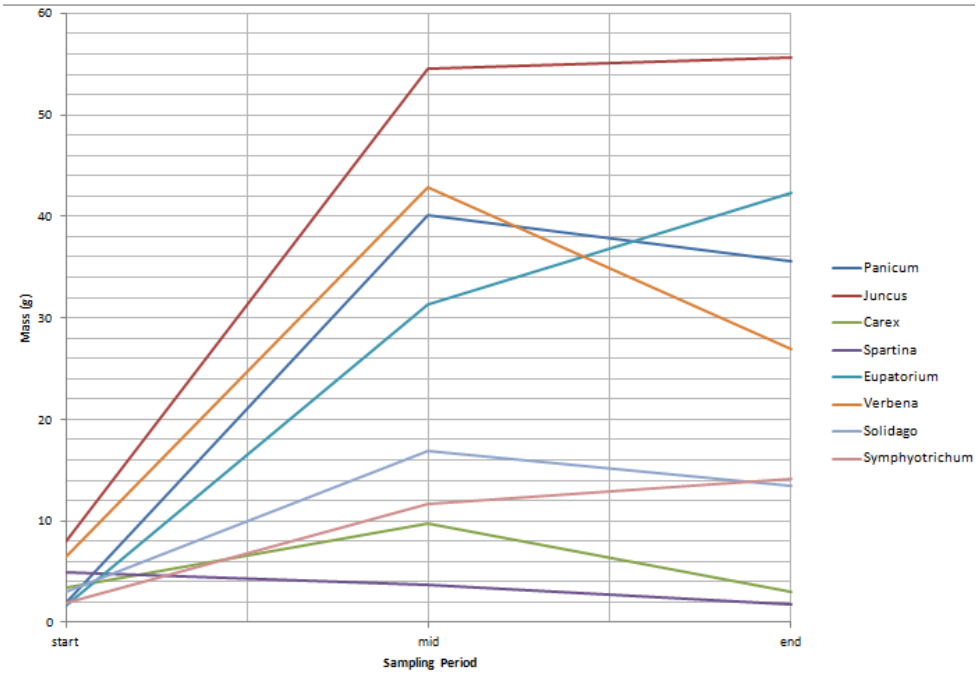

Figure 8: Average above ground green biomass of 8 species in UNH during the 2015 growing season.

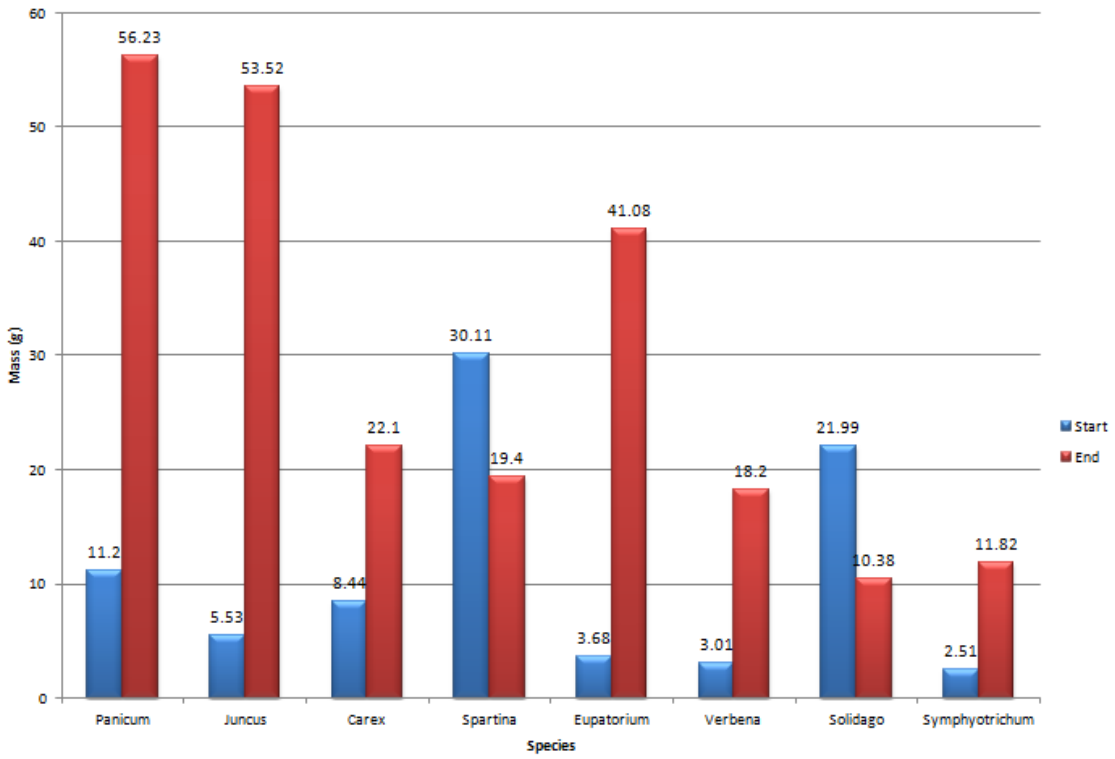

Figure 9: Average root biomass change of 8 species in the UNH during the 2015 growing season.

A majority of species flourished in UNH. Six of the eight species monitored showed increases in above ground green and root biomass (Figures 8 and 9). *Spartina alternifolia* decreased in above and below ground biomass. *Solidago sempervirens* also showed a decline in root biomass, however above ground green biomass increased at midseason then declined at the end of season. This decrease could be attributed to herbivory (Figure 10).

Figure 10: Herbivory of *Solidago sempervirens* in ABS during 2015 growing season.

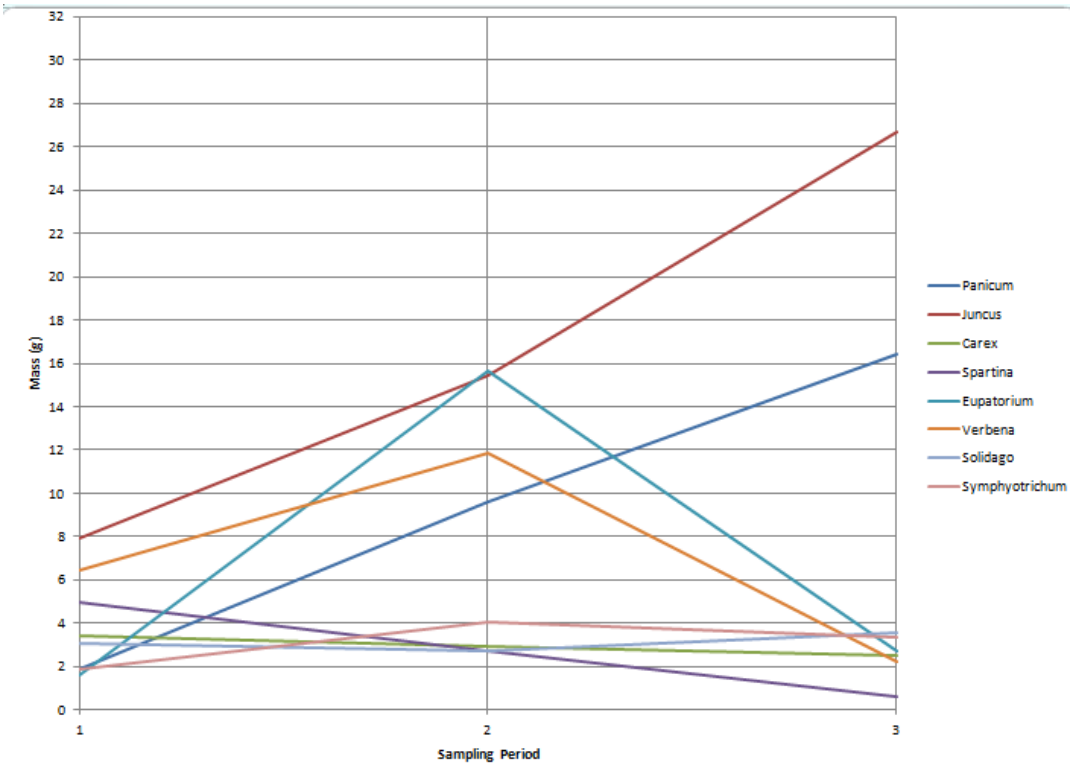

Figure 11: Average above ground green biomass of 8 species in ABS during the 2015 growing season.

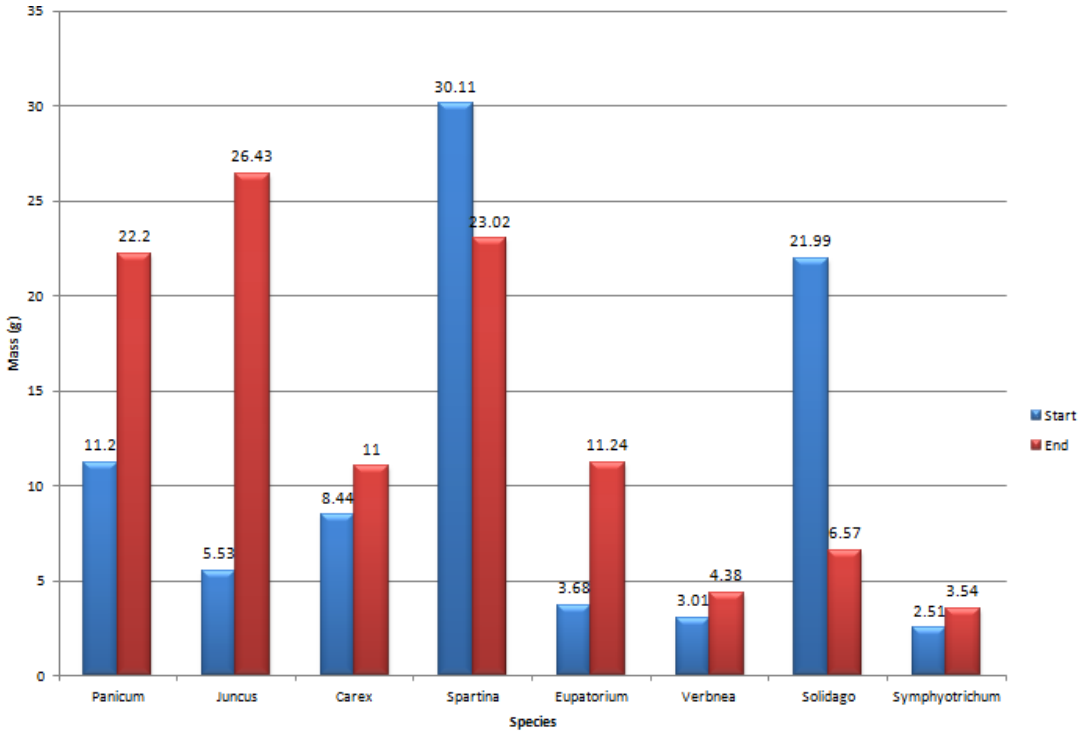

Figure 12: Average root biomass change of 8 species in ABS during the 2015 growing season.

ABS had the least amount of above ground growth across all eight species. *Juncus effusus* and *Panicum virgatum* had the greatest success in ABS (Figures 11 and 12). Both species increased in above ground green and root biomass. Although most species had gains in root biomass, the same increases were not seen above ground. *Eupatorium perfoliatum* had an increase in root biomass and its green biomass peaked midseason, however most of the above biomass had died by the end of the season.

S-UNH-1 and S-UNH-2 showed similar degrees of success for above ground biomass. *Juncus effusus* and *Panicum virgatum* increased in above ground green biomass throughout the growing season (Figures 13 and 14). *Spartina alternifolia* had the least success S-UNH-1 and S-UNH-2. It had a decrease in root biomass and aboveground green biomass (Figures 13,14,15,16).

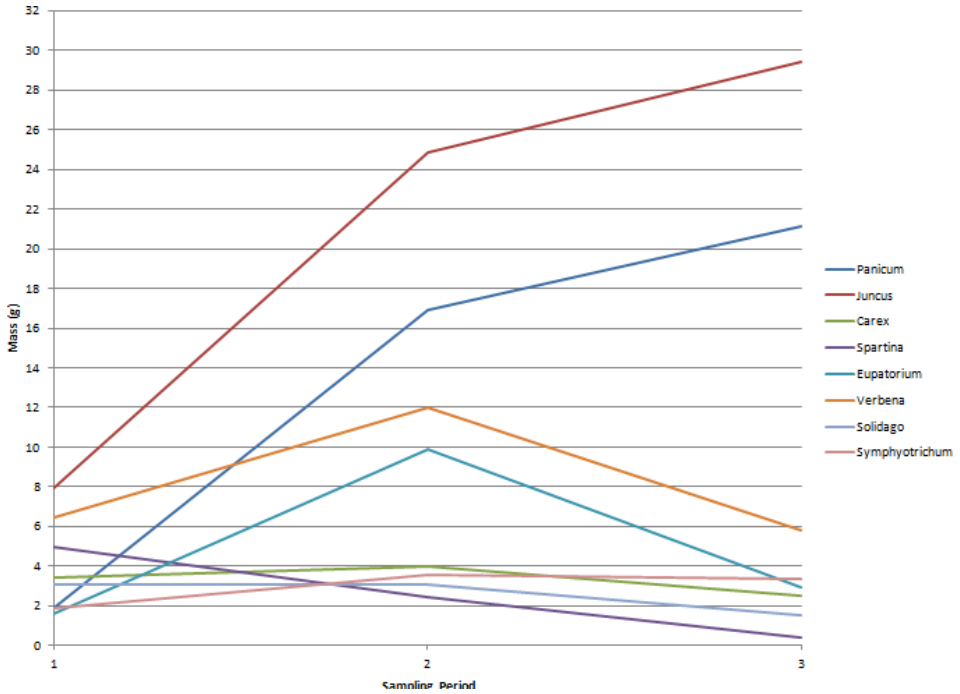

Figure 13: Average above ground green biomass of 8 species in S-UNH-1 during 2015 growing season.

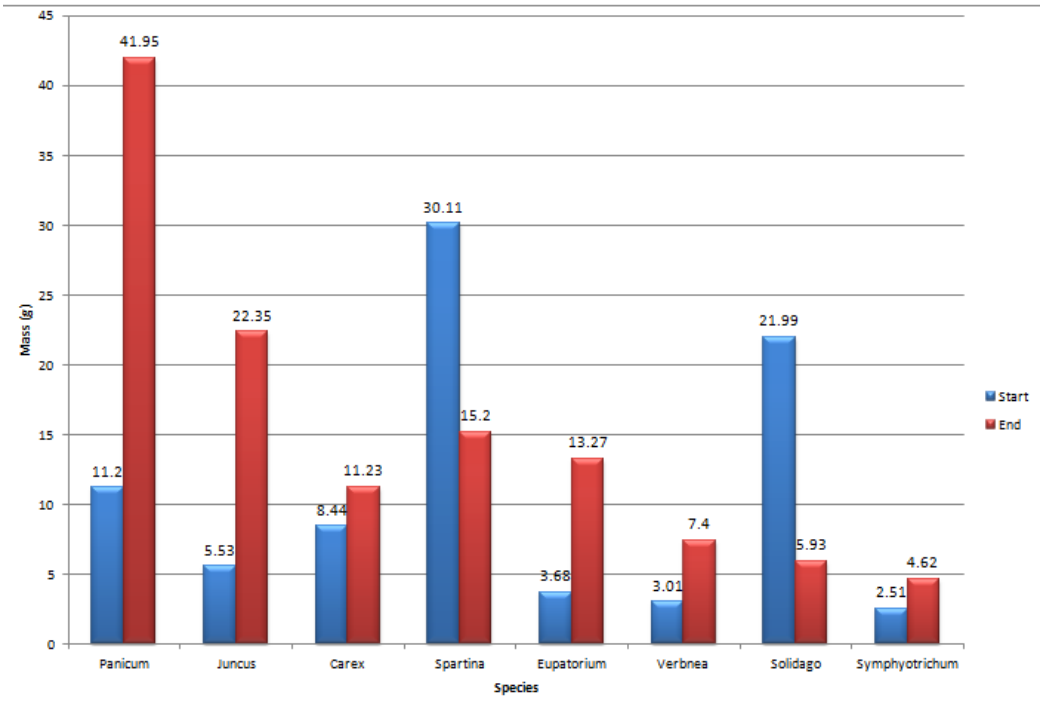

Figure 14: Average root biomass change of 8 species in S-UNH-1 during 2015 growing season.

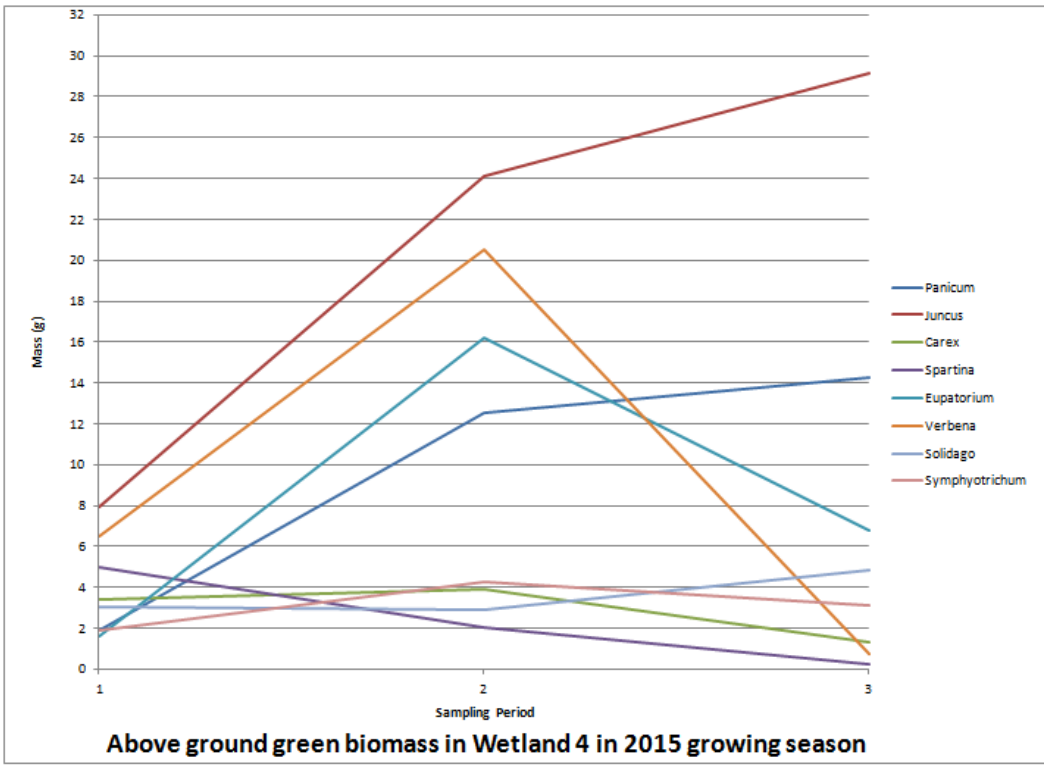

Figure 15: Average above ground green biomass of 8 species in S-UNH-2 during 2015 growing season.

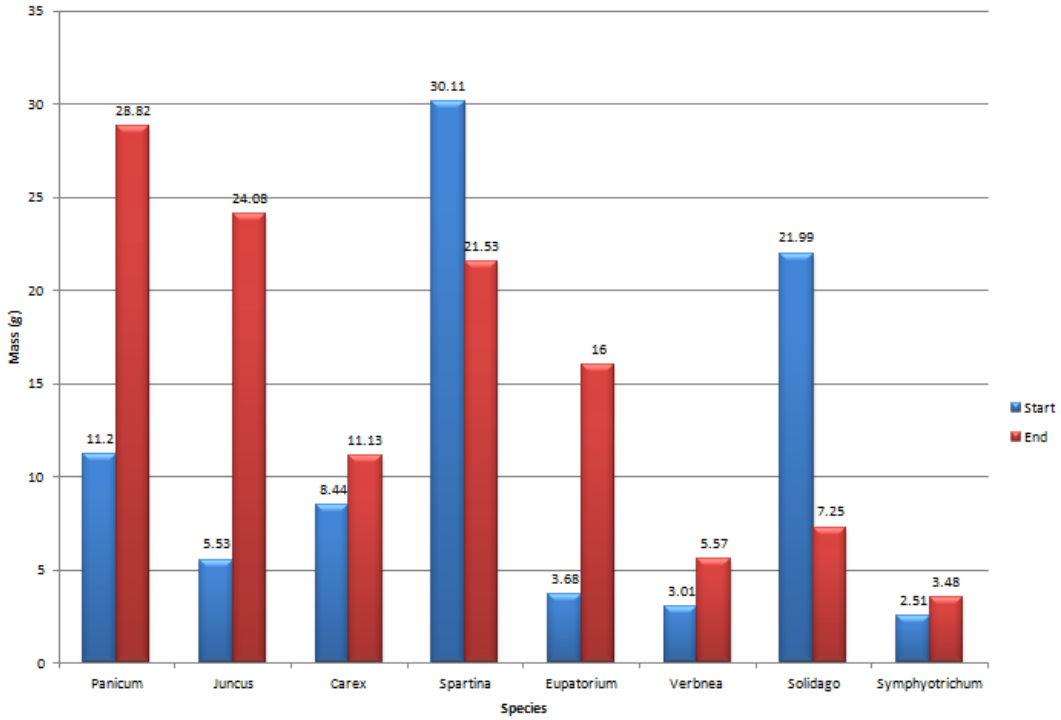

Figure 16: Average root biomass change of 8 species in S-UNH-2 during the 2015 growing season.

Discussion

Based on these data, when deciding on a subsurface gravel wetland design, UNH will result in the greatest increase in biomass during the first year of wetland establishment. ABS had the smallest increases in plant biomass and plant survivorship. Herbivores, such as white-tailed deer (Figure 17) and groundhogs will impact plant success and establishment. Although some herbivory occurred across all four wetlands, it was relatively minimal (plants were selected based on their perceived resistance to deer browsing) and there was still an overall gain in above ground green biomass.

Figure 17: White-tailed deer hoof prints in ABS at Georgian Court University.

A majority of the species monitored had a peak above ground green biomass midseason (August). This suggests best management practice for these types of wetland would include a midseason harvest for optimal nutrient removal from the system. Mowing the wetlands midseason and removing the cut plant materials will prevent nutrients being released by decomposition to be returned to the water flowing out of these systems, improving water quality.

Of the eight species monitored, *Juncus effusus* and *Panicum virgatum* had the greatest success in establishment. Both increased in biomass regardless of wetland design and had minimal to no herbivory. *Spartina alternifolia* had the least success, declining in above and below ground biomass across all four wetland designs. There was little to no herbivory of *S. alternifolia*, suggesting that the lack of success could be the result of incompatibility for growth in these types of wetland.

When designing a gravel wetland, the University of New Hampshire Subsurface Gravel Wetland resulted in the greatest plant species success. Of the eight species monitored, *Juncus effusus* and

Panicum virgatum had the greatest rate of establishment and growth. Assuming nutrient content of materials is similar (results pending analysis), midseason harvest by mowing and removal of plant material would be optimal for reducing nutrients released by decomposition.

(4) **Publications or Presentations**:

None to report at this time

Modeling hydrologic and stream temperature response to land-use and climate change in developed and developing watersheds: a comprehensive analysis

Basic Information

Title:	Modeling hydrologic and stream temperature response to land-use and climate change in developed and developing watersheds: a comprehensive analysis
Project Number:	2014NJ350B
Start Date:	3/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	NJ-001
Research Category:	Not Applicable
Focus Category:	Models, Water Supply, Management and Planning
Descriptors:	None
Principal Investigators:	Joseph A Daraio

Publications

1. Bechtold, A., M. McCarthy, C. Spurgin, J. Tucci (undergraduate students) and J.A. Daraio, 2015, Climate Change Impacts on Stream Flow in Two New Jersey Watersheds,17th Annual Rowan University Science, Technology, Engineering, & Mathematics (STEM) Student Research Symposium, April 24, 2015, Glassboro, NJ (Poster)
2. Daraio, J.A., 2014, A Comparative Analysis of Hydrologic Response to Climate Change in Developed and Undeveloped Watersheds on the New Jersey Coastal Plain, AGU Fall Meeting, December 15 19, 2014, San Francisco, CA (Poster)
3. Under review Daraio, J.A. “Potential Climate Change Impacts on Stream Flow and Groundwater Storage in Two Watersheds on the New Jersey Coastal Plain” Journal of Hydrologic Engineering

Modeling hydrologic and stream temperature response to land-use and climate change in developed and developing watersheds: a comparative analysis.

1 PI Information

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2 Students Supported

Undergraduates: 3
Masters' students: 1

3 Project Summary

3.1 Problem and Research Objectives

The complexity of processes that determine the hydrologic response of a watershed makes it difficult to predict the potential impacts of changes in land-use and climate without a high degree of uncertainty (Chen et al., 2011). Our understanding and ability to predict runoff response of watersheds to land-use and climate change, has been growing and is an area of active research (Hay, Markstrom, et al., 2011; Ogden et al., 2011; Roderick and Farquhar, 2011; Tong et al., 2011; Viger, Hay, Markstrom, et al., 2011). While the processes controlling runoff response to land-use change and urbanization are relatively well understood and have been much documented (WEF and ASCE, 2012), each watershed is unique. The basic runoff generating processes may be the same or similar, however the interactions between rainfall, infiltration, overland flow, subsurface flow, groundwater flow, and stream flow are likely to differ between watersheds and even within sub-basins of a watershed, e.g. within urbanized sub-basins of a larger watershed. Climate change can affect basin hydrologic response through changes in precipitation that affect runoff timing, volume, and intensity, but few generalizations can be made (Daraio and Bales, 2014).

There is a need for place-based research that can illuminate both general hydrologic understanding and provide insight to the response of local watersheds and potential economic and

infrastructure impacts. The main aspect of the proposed work is to develop rainfall-runoff models in two watersheds in New Jersey in order to assess and understand through a comparative approach how each watershed responds. These models will be used for issues that are particularly important for New Jersey. For example, rising sea level due to climate change could impact coastal infrastructure for over 6 million people in New Jersey (Williamson et al, 2008), and there is little known about how hydrologic impacts may interact with seal level rise, e.g. to affect both coastal and inland flooding. The impacts of Hurricane Sandy highlight the vulnerability of New Jerseys coastal infrastructure. Additionally, it is not known if and how sea level rise may impact freshwater ecosystems further inland.

Objectives The objectives of the proposed project are 1. to develop, calibrate, and validate rainfall-runoff models for two watersheds in New Jersey with urban, developing, and undeveloped areas, 2. to use these models to assess the potential impacts of climate change through 2100 on hydrologic response in these basins under current land-use conditions, and 3. to put in place a network of stream temperature sensors in these watersheds for collection of field data to be used for analysis and the development of stream temperature models.

3.2 Methodology

3.2.1 Site description

Hydrologic models were developed for two watersheds that lie within the Outer Coastal Plain of New Jersey, the upper Maurice River and the Batsto River (Figure 1). The NJ coastal plain is relatively flat, it ranges in elevation from 119 m to sea level, with an unconsolidated to semi-consolidated surficial geology. This area is dominated by the Pinelands, which runs from Ocean county, just south of Monmouth county to Cumberland county just north of the Delaware Bay, includes most of Burlington and Atlantic counties, and parts of Camden, Gloucester, and Cape May counties (Figure 1). The area primarily consists of sandy, acidic soil underlaid by the Kirkwood-Cohansey Aquifer. These coarse sands are porous in nature, which allows for rapid infiltration. The upper Maurice River watershed includes part of the western border of the Pinelands. The river becomes an estuary downstream of Union Lake, in Millville, NJ. The Batsto River watershed is located entirely within the Pinelands area, and the majority is within the Pinelands National Reserve. The Batsto watershed is dominated by forest and wetlands, and the upper Maurice watershed is mixed urban, forest, and agriculture (Table 1).

The hydrologic regime for each river is similar with the highest mean flows in the early spring and lowest mean flows at the end of summer (Figure 2). Mid- to late summer into early autumn is the time when the highest flows occur (due to the occurence of hurricanes and tropical storms), and also the greatest variability of flows occurs during this period. Therefore, the units of analysis, or seasons, represented in this study were chosen based on the hydrology of these systems. The period from early July to the beginning of October (end of the water year) was chosen as the summer in order to include the periods with greatest flow variation and highest peak flows (Figure 2). Flows in this period are most important for design of infrastructure in particular. The spring was chosen to capture the beginning of the period of declining mean/median flows to the start of the summer. Autumn was chosen from the end of summer to the end of the calendar year, and winter was defined from the start of the calendar year to the start of spring. All analysis was done on a seasonal basis using these definitions.

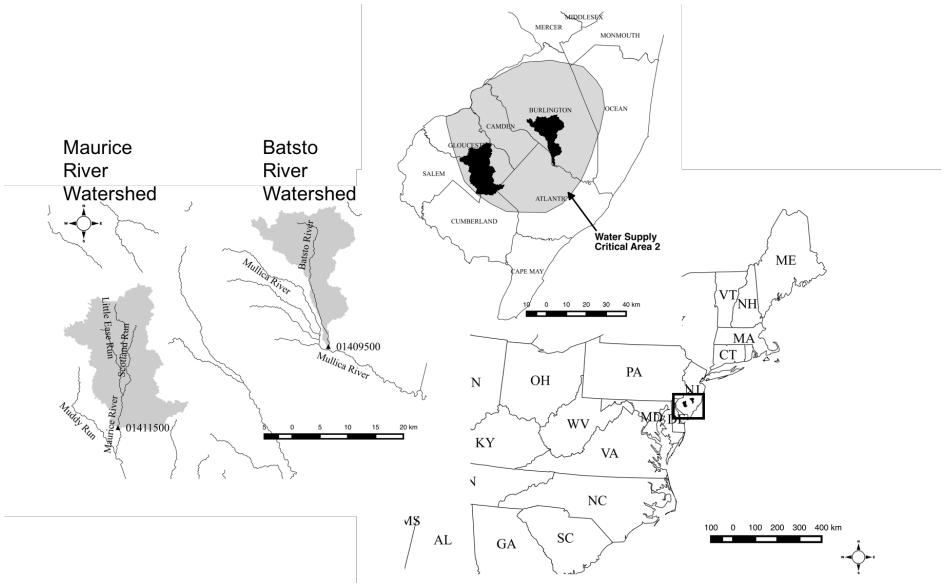

Figure 1: Locations of the Upper Maurice River watershed (290 km^2) and the Batsto River watershed (180 km^2). Outlets of each watershed was set at USGS stream gage locations (triangles). The gage at Norma, NJ was used for the Maurice River (USGS site ID 01411500), and the gage at Batsto, NJ was used for the Batsto River (site ID 01409500). Approximate area of Water Supply Critical Area 2 (adapted from Charles, Nawyn, et al. 2011)

3.2.2 Watershed modelling

The Precipitation-Runoff Modeling System (PRMS), a distributed hydrologic model (Leavesly, Markstrom, et al., 2006), was used to simulate the hydrologic response of the watersheds under selected climate change scenarios. Questions have been raised as to whether a model calibrated with historical data will provide good results for future hydrologic projections since downscaling relatationships may not be valid for future climate (Willems et al., 2012). Discussion of this issue is beyond the scope of this paper, however PRMS has been widely tested and used for climate change impact assessment (Dams et al., 2015; Daraio and Bales, 2014; Hay, Markstrom, et al., 2011; LaFontaine et al., 2015; Mendoza et al., 2015; Mizukami et al., 2016; Viger, Hay, Markstrom, et al., 2011). PRMS models with 116 hydrologic response units (HRUs) and 236

Table 1: Land use as a percentage of total basin area for the upper Maurice and Batsto River watersheds.

Land Use	**Batsto**	**Maurice**
Agriculture	7.4%	22.1%
Barren Land	0.1%	0.9%
Forest	60.0%	30.2%
Urban	5.6%	28.2%
Water	1.77%	1.2%
Wetlands	25.2%	17.5%

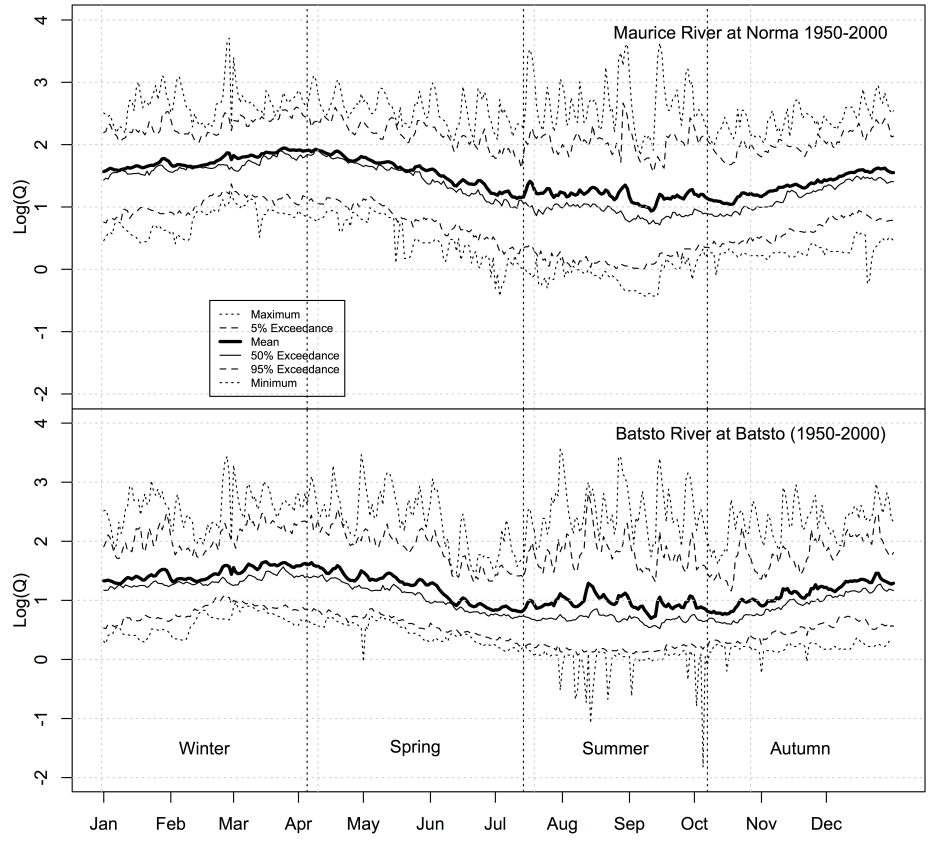

Figure 2: Maximum, minimum, mean, and median stream flow Q (m^3s^{-1}), and 5% and 95% exceedance levels for each day of the year for the upper Maurice River (top) and Batsto River (bottom) for water years 1950–2000.

HRUs were developed for the upper Maurice River and Batsto River basins, respectively. The depression storage option (Viger, Hay, Jones, et al., 2010) was used for PRMS in both basins. The Muskingum method for streamflow routing was used. The model was calibrated step-wise following (Hay and Umemoto, 2007; Hay, Leavesly, et al., 2006) using Luca (Let us calibrate). Solar radiation was calibrated first, followed by potential evapotranspiration (PET), streamflow volume, and finally stream flow timing. The calibration period for the model was water years 1990–1996, and the model was evaluated for water years 1997–2003. Stream flow (volume and timing) was calibrated to mean daily flow and mean monthly flow, weighted equally.

Data Daily maximum and minimum air temperature and daily precipitation were derived using observed 12 km^2 gridded data (Maurer et al., 2002) and observed potential evapotranspiration (PET) was estimated based on 1 km^2 gridded solar radiation data from Thornton et al., (2014), available from the USGS Geo Data Portal. The R package EcoHydRology (Fuka et al., 2014) was used to calculate PET from solar radiation using the Preistly-Taylor equation. *A priori* parameterization of PRMS was done using the GIS weasel (Viger, 2008) from the data bin provided from the USGS (Roland Viger). The data bin includes land use, cover, and soils

(STATSGO) coverages from 2000 (see Leavesly, Hay, et al. 2003).

Model calibration and performance Model performance was evaluated using the R package hydroGOF (Zambrano-Bigiarini, 2014). Simulated hydrographs in both basins are more spikey than observed data (Figures 3 and 4), especially for smaller events, which indicates simulations have too much runoff or not enough surface storage for some of the smaller events. However both models were able to be fit well over the seven year calibration period. Nash-Suttcliffe efficiencies were between 0.5 and 0.6 for the Batsto model for both mean daily and mean monthly simulations, and other metrics of model performance indicate a relatively good fit of the model (index of agreement $d > 0.8$, Kling-Gupta efficiencies ≈ 0.7, and high volumetric efficiencies, VE). The fit model for the Batsto showed biases of -1.3% for both mean daily and mean monthly flow. Model fit for mean daily flow for the Maurice River was adequate, and the fit was better for mean monthly flow (NSE = 0.73, $d = 0.92$, KGE = 0.77). Bias for mean daily flow was -0.7% and for mean monthly flow was -0.8% for the Maurice. Model fit for seasonal flows, representing inter-annual variation, was good in all seasons for both models (Figure 3). Seasons are defined differently in the hydroGOF package then they are in this study, however the seasonal performance provides and indication of how well the models represented interannual variation.

Evaluation of the models over water years 1997 to 2003 indicated that both models performed well for mean daily and mean monthly flows (Figures 3 and 4). The Maurice model performed consistently across all seasons. The Batsto model performed well in the winter but had a consistent positive bias in the summer and autumn (45% and 35% respectively), and simulations followed observed flow patterns well. Model performance was deemed acceptable for use in the analysis of climate change factors performed here. However, using a coupled surface/groundwater model will likely improve model performance because the properties of these watersheds, with porous sandy soils, are such that groundwater flow is a significant part of the hydrology of the systems.

3.2.3 Climate change projections

Dynamically downscaled Bias Corrected Constructed Analogs (BCCA) V2 daily climate projections from the CMIP5 multi-model ensemble (USBR, 2013; Taylor et al., 2012) were used to derive meteorological data (precipitation, maximum and minimum air temperature) to drive PRMS models. The CMIP5 General Circulation Models (GCMs) used in this analysis are listed in Table 2. Shapefiles of both basins with delineated HRUs were uploaded to the USGS Geo Data Portal, and the area-weighted grid statistics algorithm was used to obtain daily mean precipitation, and maximum and minimum air temperature for each HRU. Data for the historical period of 1970–2000 and future projections for the period 2020–2100 were downloaded for each CMIP5 model in Table 2.

A total of 154 climate projections were used for simulations in each basin. Selection of CMIP5 climate projections was based on data availability from the USGS Geo Data Portal. PRMS simulations for the historical period (water years 1971–2000) provided baseline data from which which climate change measures were calculated. PRMS output of daily mean stream flow, daily mean ground water storage, daily total precipitation were used from historical simulations to provide a baseline mean value for each variable, except precipitation. For precipitation, mean

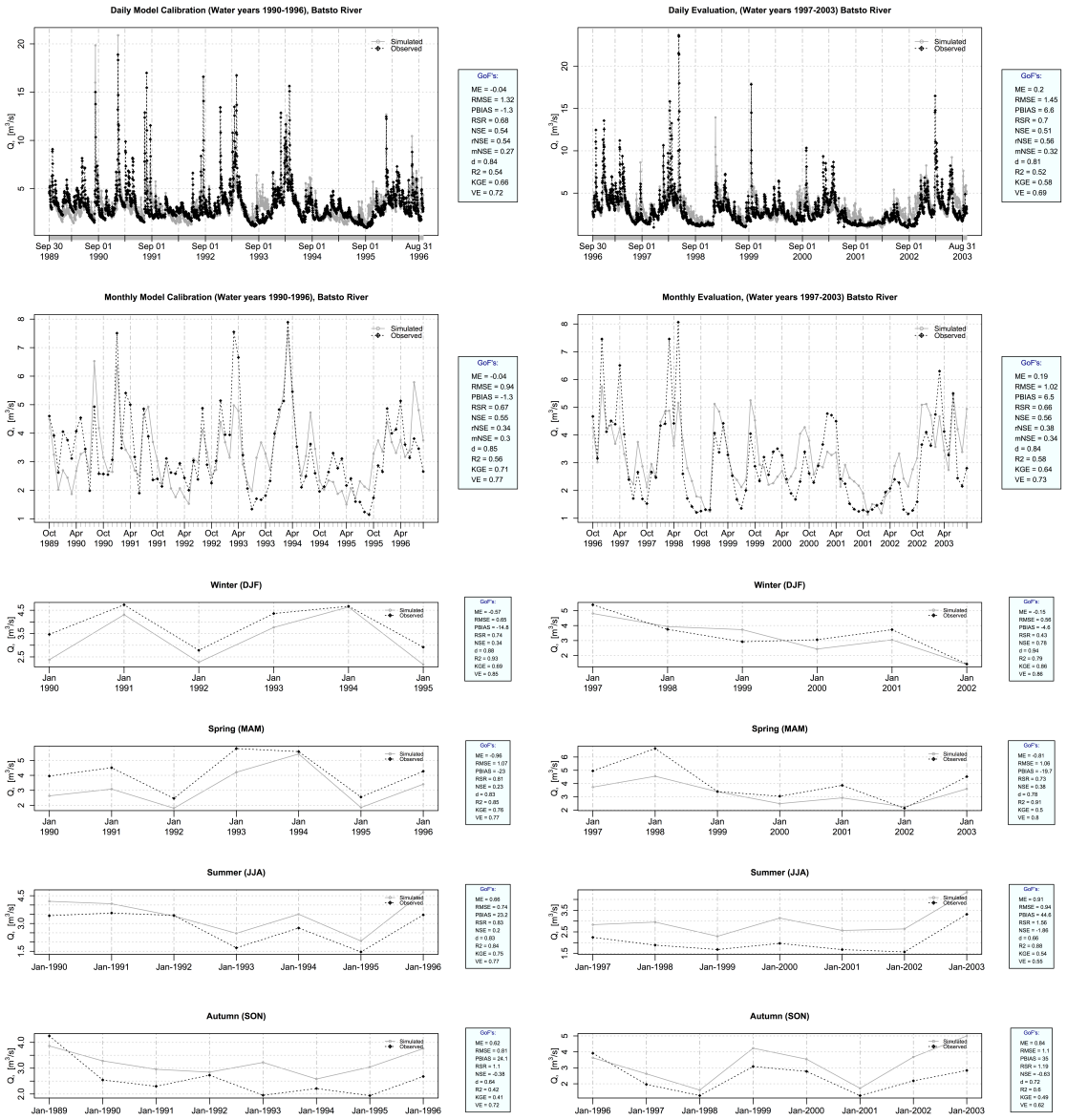

Figure 3: Calibration and evaluation of simulations for the Batsto River for daily mean flow, monthly mean flow and, seasonal mean flow. Goodness-of-Fit (GoFs) are shown on the right of each hydrograph. ME = mean error; RMSE = root mean squared error; PBIAS = Percent bias ; RSR = ratio of RMSE to the standard deviation of observed data; NSE = Nash-Sutcliffe efficiency, rNSE = relative Nash-Sutcliffe efficiency; mNSE = modified Nash-Sutcliffe efficiency; d = index of agreement; R2 = coefficient of determination (R^2); KGE = Kling-Gupta efficiency; and VE = volumetric efficiency.

total precipitation was calculated as a baseline. For GCMs with more than 1 historical run, the baseline value was calculated from all historical runs combined.

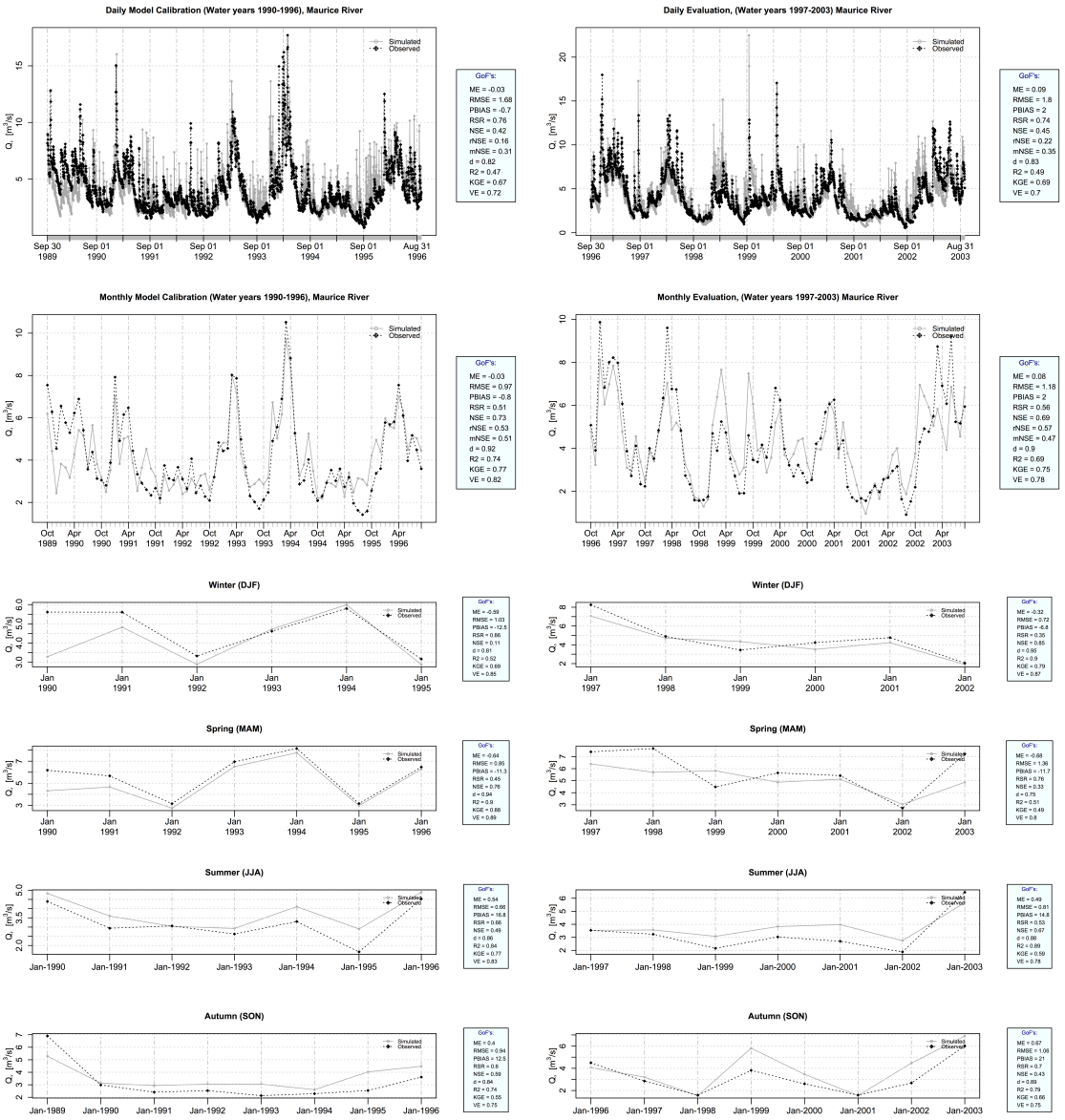

Figure 4: Calibration and evaluation of simulations for the Maurice River for daily mean flow, monthly mean flow and, seasonal mean flow.. Goodness-of-Fit (GoFs) are shown on the right of each hydrograph. ME = mean error; RMSE = root mean squared error; PBIAS = Percent bias ; RSR = ratio of RMSE to the standard deviation of observed data; NSE = Nash-Sutcliffe efficiency, rNSE = relative Nash-Sutcliffe efficiency; mNSE = modified Nash-Sutcliffe efficiency; d = index of agreement; R2 = coefficient of determination (R^2); KGE = Kling-Gupta efficiency; and VE = volumetric efficiency.

Seasonal anomalies Analysis was done on a seasonal basis given the consistency of model performance at the seasonal time scale as described in Section 3.2.2, with seasons defined by as above (see Figure 2). Seasonal anomalies for stream flow, storage, and total precipitation were calculated using historical run(s) for each CMIP5 GCM under each climate change scenario.

Table 2: List of models from the CMIP5 multi-model ensemble used in this paper.

			Scenario, number of runs	
Modeling Center (or Group)	**Institute ID**	**Model Name**	**Historical**	**RCP 2.6, 4.5, 8.5**
Commonwealth Scientific and Industrial Research Organization (CSIRO) and Bureau of Meteorology (BOM), Australia	CSIRO-BOM	ACCESS1.0	1	0, 1, 1
Beijing Climate Center, China Meteorological Administration	BCC	BCC-CSM1.1	1	1, 1, 1
College of Global Change and Earth System Science Beijing Normal University	GCESS	BNU-ESM	1	0, 1, 1
Canadian Centre for Climate Modelling and Analysis	CCCMA	CanESM2	5	5, 5, 5
National Center for Atmospheric Research	NCAR	CCSM4	2	2, 2, 2
Community Earth System Model Contributors	NSF-DOE-NCAR	CESM1(BGC)	1	0, 1, 1
Centre National de Recherches Météorologiques/Centre Européen de Recherche et Formation Avancée en Calcul Scientifique	CNRM-CERFACS	CNRM-CM5	1	0, 1, 1
Commonwealth Scientific and Industrial Research Organization in collaboration with Queensland Climate Change Centre of Excellence	CSIRO-QCCCE	CSIRO-Mk3.6.0	10	10, 10, 10
Institute for Numerical Mathematics	INM	INM-CM4	1	0, 1, 1
Institut Pierre-Simon Laplace	IPSL	IPSL-CM5A-LR	4	3, 4, 4
		IPSL-CM5A-MR	1	1, 1, 1
Japan Agency for Marine-Earth Science and Technology, Atmosphere and Ocean Research Institute (The University of Tokyo), and National Institute for Environmental Studies	MIROC	MIROC-ESM	1	1, 1, 1
		MIROC-ESM-CHEM	1	1, 1, 1
Atmosphere and Ocean Research Institute (The University of Tokyo), National Institute for Environmental Studies, and Japan Agency for Marine-Earth Science and Technology	MIROC	MIROC5	3	3, 3, 3
Max-Planck-Institut für Meteorologie (Max Planck Institute for Meteorology)	MPI-M	MPI-ESM-MR	3	1, 3, 1
		MPI-ESM-LR	3	3, 3, 3
Meteorological Research Institute	MRI	MRI-CGCM3	1	1, 1, 1
Norwegian Climate Centre	NCC	NorESM1-M	1	1, 1, 1
		Total simulations:	41	33, 41, 39

Seasonal stream flow anomalies were calculated using the following.

$$\hat{Q}_{n_{sy,a_i}} = \overline{Q}_{n_{sy,f_i}} - \overline{Q}_{n_{s,b_i}} \tag{1}$$

where $\hat{Q}_{n_{sy,a_i}}$ ($\mathrm{m^3 s^{-1}}$) is the seasonal stream flow anomaly, a, for the season, s, daily mean stream flow for year y for GCM i, $\overline{Q}_{n_{sy,f_i}}$ is the seasonal daily mean stream flow for the year y for run f of GCM i, and $\overline{Q}_{n_{s,b_i}}$ is the baseline, b, mean seasonal daily mean stream flow for all runs of GCM i over the period 1971–2000. Anomalies for each season were grouped by bin, $n = 4$, within ranges at probability levels = 0.05, 0.50, and 0.95, where flows in the range of

prob($Q \leq 0.05$) were exceeded 95% of the time, flows with prob($Q \geq 0.95$) were exceeded 5% of the time, with other flow ranges for prob($0.05 < Q \leq 0.5$), and prob($0.5 < Q < 0.95$). Anomalies were also calcuated with all probability levels pooled, i.e with $n = 1$ (all flows).

Similarly, for the seasonal storage anamoly,

$$\hat{S}_{n_{s_y,a_i}} = \overline{S}_{n_{s_y,f_i}} - \overline{S}_{n_{s,b_i}} \tag{2}$$

Where $\hat{S}_{n_{s_y,a_i}}$ is the seasonal storage anomaly (mm) for the seasonal daily mean storage for year y for GCM i, $\overline{S}_{n_{s_y,f_i}}$ is the seasonal daily mean storage for the year y for run f of GCM i, and $\overline{S}_{n_{s,b_i}}$ is the baseline, b, mean seasonal daily mean storage for all runs of GCM i over the period 1971–2000, with n as above.

Seasonal anomalies for precipitation were calculated using the mean seasonal total precipitation as follows.

$$\hat{P}_{s_y,a_i} = \sum P_{s_y,f_i} - \overline{\sum P}_{s,b_i} \tag{3}$$

Where $\hat{P}_{s_y,a_i}$ is the seasonal precipitation anomaly, a, (mm) for the seasonal (s) total precipitation for year y for GCM i, $\sum P_{s_y,f_i}$ is the seasonal total precipitation for the year y for run f of GCM i, and $\overline{\sum P}_{s,b_i}$ is the baseline, b, mean seasonal total precipitation for all runs of GCM i over the period 1971–2000. Precipitation anomalies were not calculated for separate bins.

In order to allow for comparisons across both basins, and to compare the relative response of stream flow, storage, and precipitation both within and across basins, anomalies were normalized to the mean seasonal values for each variable. Therefore

$$\widehat{Q}_{n_{s_y,a_i}} = \frac{\hat{Q}_{n_{s_y,a_i}}}{\overline{Q}_{n_{s,b_i}}} \tag{4}$$

$$\widehat{S}_{n_{s_y,a_i}} = \frac{\hat{S}_{n_{s_y,a_i}}}{\overline{S}_{n_{s,b_i}}} \tag{5}$$

$$\widehat{P}_{s_y,a_i} = \frac{\hat{P}_{s_y,a_i}}{\overline{\sum P}_{s,b_i}} \tag{6}$$

are the normalized seasonal stream flow, storage, and precipitation anomalies, respectively.

Projections for stream flow, storage, and precipitation were estimated for the periods for water years 2051–2065 and 2084–2099. Anomalies were calculated for each GCM giving a total of 41 simulations from 18 GCMs for historical runs, 33 simulations from 13 GCMs under the RCP 2.6 scenario, 41 simulations from 18 GCMs under the RCP 4.5 scenario, and 39 simulations from 18 GCMs under the RCP 8.5 scenario (Table 2). Each simulation resulted in the calculation of 4 seasonal anomalies for each year of simulation. Sample sizes ranged from 30 to 300 for a season's anomalies for historical runs, and from 14 to 140 for a season's anomalies for each time period for future projections.

Evaluation of the performance of simulations using downscaled bias corrected climate projections was done for the period 1971–2000. Goodness-of-fit measures used for calibration and evaluation of the model cannot be used for evaluation using climate projections because

GCMs can only replicate climate averages, not daily flows. Evaluation was done using Mann-Whitney-Wilcoxon tests for flows grouped by exceedance, or probability levels. Mann-Whitney-Wilcoxon tests provide estimates for the difference in means (observed $-$ simulated) for each probability level.

3.3 Principal Findings and Significance

Overall performance of PRMS simulations using meteorological data from the CMIP5 multi-model ensemble was good. On average over the historic period, CMIP5 driven simulations overestimated flows by 0.13 m^3s^{-1} (3.9%) for the Batsto River and underestimated flows by 0.18 m^3s^{-1} (3.9%) for the Maurice River (Tables 3 and 4). The GCM driven simulations performed better at low flows than at high flows, and these results are consistent with the performance of PRMS in both basins over the calibration and evaluation periods.

3.3.1 Precipitation

Precipitation anomalies showed normal distributions under each basin under RCP 2.6 with slightly increasing precipitation in spring and summer to 2065 and 2099 (Figure 5). Precipitation change was also similar in both basins under RCP 4.5 and 8.5 in most seasons except for winter and autumn where precipitation anomalies indicated no change in the Batsto basin and decreases in the Maurice basin. Simulations indicated mean decreases of 6.6% and 5.6% in winter and autumn, respectively, under RCP 4.5, and of 12% and 10% under RCP 8.5 in the Maurice basin. A relatively sharp gradient of increasing mean annual precipitation occurs over the Maurice basin from south to north, and the Batsto basin has a slight gradient of increasing precipitation from outlet to headwaters (Figure 6). It is likely that climage change impacts on precipation may differ between these basins despite their proximity. Additionally, there was greater variation in precipitation under all RCP scenarios that increased over the periods ending in 2065 and 2099. The increase in variance was greatest at the end of the century in the summer in both basins for all GCMs.

3.3.2 Stream flow and basin storage

For all flows combined, stream flow anomalies in the Batsto River seemed to follow the same pattern as did precipitation anomalies in the basin (Figure 7b). Stream flow anomalies in the Maurice River, however, did not follow the same patterns as precipitation anomalies in all seasons (Figure 7a). While the mean precipitation showed a decrease in autumn in the Maurice basin, mean stream flow anomalies were near 0. Additionally, spring precipitation showed an increase in Maurice basin, but again, mean stream flow anomalies indicated no change. Groundwater storage anomalies in both basins followed the same pattern as stream flow anomalies (Figure 7c, d), as was expected for these basins given the strong connections between stream flow and groundwater. There was significantly greater variation ($p < 0.05$) in stream flow anomalies in Batsto River than in the Maurice River in the winter under all scenarios (F-test).

Anomalies for stream flow and ground water storage for flows at different exceedance levels provide a more detailed picture of the hydrologic responses of these basins (Figure 8). Stream flow and groundwater storage anomalies did not change much from the period ending 2065 to the

Table 3: Difference in mean stream flow (m^3s^{-1}) for stream flow projections using downscaled bias-corrected GCM simulations to drive the rainfall-runoff model for the Batsto River for the historic period 1971–2000. Difference in the mean was calculated using the Wilcox test in the statistical package R. Results are shown for all flows combined, and for flows in the range for each probability level. Positive values indicate that observed flows were greater than simulated flows. * represent statistically significant difference in means at $p < 0.05$. Mean difference for all GCMs was calculated using only mean flows that were significantly different.

			Probability level			
	Run	**All**	$\mathbf{p \leq 0.05}$	$\mathbf{0.05 < p \leq 0.5}$	$\mathbf{0.5 < p < 0.95}$	$\mathbf{p \geq 0.95}$
ACCESS1-0_historical_r1i1p1	1	-0.090*	-0.250*	-0.301*	0.246*	3.65*
bcc-csm1-1_historical_r1i1p	1	-0.097*	-0.130*	-0.245*	0.142*	3.04*
BNU-ESM_historical_r1i1p1	1	-0.197*	-0.339*	-0.372*	0.098*	2.64*
CanESM2_historical_r1i1p1	1	-0.210*	-0.216*	-0.333*	-0.016	2.59*
	2	-0.161*	-0.249*	-0.295*	0.062*	2.82*
	3	-0.232*	-0.265*	-0.374*	-0.001	2.56*
	4	-0.106*	-0.175*	-0.247*	0.131*	2.55*
	5	-0.153*	-0.189*	-0.266*	0.025	2.84*
CCSM4_historical_r1i1p1	1	-0.015	-0.286*	-0.239*	0.356*	3.75*
	2	-0.211*	-0.383*	-0.396*	0.099*	2.98*
CESM1-BGC_historical_r1i1p1	1	-0.180*	-0.387*	-0.361*	0.135*	2.86*
CNRM-CM5_historical_r1i1p1	1	-0.139*	-0.384*	-0.354*	0.219*	3.49*
CSIRO-Mk3-6-0_historical_r10i1p1	1	-0.165*	-0.401*	-0.373*	0.178*	3.65*
	2	-0.082*	-0.312*	-0.278*	0.249*	3.45*
	3	-0.100*	-0.243*	-0.239*	0.138*	2.97*
	4	-0.119*	-0.295*	-0.292*	0.174*	3.11*
	5	-0.146*	-0.253*	-0.289*	0.089*	2.93*
	6	-0.100*	-0.197*	-0.302*	0.225*	3.40*
	7	-0.108*	-0.288*	-0.266*	0.164*	3.03*
	8	-0.098*	-0.339*	-0.276*	0.205*	3.42*
	9	-0.172*	-0.321*	-0.372*	0.159*	2.99*
	10	-0.120*	-0.348*	-0.323*	0.224*	3.11*
inmcm4_historical_r1i1p1	1	-0.133*	-0.262*	-0.279*	0.120*	2.67*
IPSL-CM5A-LR_historical_r1i1p1	1	-0.103*	-0.336*	-0.312*	0.243*	3.52*
	2	-0.185*	-0.415*	-0.366*	0.128*	3.22*
	3	-0.114*	-0.322*	-0.337*	0.248*	3.75*
	4	-0.132*	-0.286*	-0.319*	0.168*	3.52*
IPSL-CM5A-MR_historical_r1i1p1	1	-0.226*	-0.451*	-0.401*	0.075*	3.17*
MIROC-ESM-CHEM_historical_r1i1p1	1	-0.081*	-0.406*	-0.294*	0.294*	3.34*
MIROC-ESM_historical_r1i1p1	1	-0.173*	-0.334*	-0.358*	0.130*	3.29*
MIROC5_historical_r1i1p1	1	-0.124*	-0.317*	-0.303*	0.182*	2.98*
	2	-0.035*	-0.304*	-0.254*	0.338*	3.32*
	3	-0.176*	-0.386*	-0.383*	0.164*	3.41*
MPI-ESM-LR_historical_r1i1p1	1	-0.041*	-0.219*	-0.220*	0.262*	3.23*
	2	-0.064*	-0.276*	-0.227*	0.222*	3.04*
	3	-0.058*	-0.274*	-0.236*	0.243*	3.49*
MPI-ESM-MR_historical_r1i1p1	1	-0.137*	-0.346*	-0.332*	0.191*	3.29*
	2	-0.238*	-0.453*	-0.412*	0.069*	2.90*
	3	-0.126*	-0.339*	-0.303*	0.183*	2.94*
MRI-CGCM3_historical_r1i1p1	1	-0.054*	-0.357*	-0.316*	0.370*	4.07*
R_NorESM1-M_historical_r1i1p1	1	-0.150*	-0.384*	-0.322*	0.156*	2.86*
Mean difference, m^3s^{-1}		-0.131 (-3.9%)	-0.334 (-27%)	-0.312 (-15%)	0.176 (4.3%)	3.167 (30%)
Mean flow, m^3s^{-1}(observed)		3.34	1.24	2.06	4.04	10.6

period ending 2099 under all climate change scenarios. Both basins showed an increase in flows and storage at low flows (95% exceedance) along with a high degree of variability across model

Table 4: Difference in mean stream flow (m^3s^{-1}) for stream flow projections using downscaled bias-corrected GCM simulations to drive the rainfall-runoff model for the Maurice River for the historic period 1971–2000. Difference in the mean was calculated using the Wilcox test in the statistical package R. Positive values indicate that observed flows were greater than simulated flows. * represent statistically significant difference in means at $p < 0.05$. Mean difference for all GCMs was calculated using only mean flows that were significantly different.

			Probability level			
	Run	**All**	$\mathbf{p \leq 0.05}$	$\mathbf{0.05 < p \leq 0.5}$	$\mathbf{0.5 < p < 0.95}$	$\mathbf{p \geq 0.95}$
ACCESS1-0_historical_r1i1p1	1	0.225*	-0.130*	-0.188*	0.948*	3.60*
bcc-csm1-1_historical_r1i1p	1	0.020	-0.067*	-0.247*	0.494*	2.43*
BNU-ESM_historical_r1i1p1	1	0.091*	-0.298*	-0.251*	0.737*	2.62*
CanESM2_historical_r1i1p1	1	0.120*	-0.128*	-0.158*	0.640*	2.51*
	2	0.131*	-0.164*	-0.159*	0.678*	2.55*
	3	0.031	-0.194*	-0.249*	0.550*	2.25*
	4	0.203*	-0.140*	-0.093*	0.771*	2.73*
	5	0.078*	-0.114*	-0.162*	0.518*	2.47*
CCSM4_historical_r1i1p1	1	0.343*	-0.246*	-0.081*	1.135*	3.83*
		0.098*	-0.318*	-0.281*	0.809*	2.90*
CESM1-BGC_historical_r1i1p1	1	0.116*	-0.350*	-0.222*	0.778*	2.68*
CNRM-CM5_historical_r1i1p1	1	0.142*	-0.314*	-0.242*	0.850*	3.28*
CSIRO-Mk3-6-0_historical_r10i1p1	1	0.185*	-0.232*	-0.195*	0.882*	3.38*
	2	0.288*	-0.184*	-0.111*	1.030*	3.37*
	3	0.219*	-0.183*	-0.099*	0.822*	3.04*
	4	0.224*	-0.187*	-0.114*	0.863*	3.01*
	5	0.240*	-0.132*	-0.071*	0.836*	2.79*
	6	0.219*	-0.150*	-0.152*	0.895*	3.29*
	7	0.248*	-0.132*	-0.087*	0.880*	2.97*
	8	0.230*	-0.220*	-0.136*	0.910*	3.30*
	9	0.182*	-0.232*	-0.193*	0.882*	3.00*
	10	0.237*	-0.197*	-0.166*	0.978*	3.24*
inmcm4_historical_r1i1p1	1	0.182*	-0.149*	-0.166*	0.827*	2.89*
IPSL-CM5A-LR_historical_r1i1p1	1	0.168*	-0.290*	-0.209*	0.876*	3.18*
	2	0.136*	-0.413*	-0.215*	0.818*	3.20*
	3	0.175*	-0.236*	-0.194*	0.853*	3.33*
	4	0.197*	-0.209*	-0.170*	0.875*	3.16*
IPSL-CM5A-MR_historical_r1i1p1	1	0.064*	-0.430*	-0.279*	0.728*	2.99*
MIROC-ESM-CHEM_historical_r1i1p1	1	0.238*	-0.344*	-0.154*	1.000*	3.11*
MIROC-ESM_historical_r1i1p1	1	0.104*	-0.253*	-0.241*	0.725*	3.32*
MIROC5_historical_r1i1p1	1	0.174*	-0.313*	-0.178*	0.855*	2.94*
	2	0.306*	-0.220*	-0.103*	1.076*	3.32*
	3	0.112*	-0.295*	-0.259*	0.808*	2.90*
MPI-ESM-LR_historical_r1i1p1	1	0.274*	-0.151*	-0.049*	0.901*	2.84*
	2	0.252*	-0.203*	-0.046*	0.845*	2.78*
	3	0.260*	-0.208*	-0.060*	0.880*	3.10*
MPI-ESM-MR_historical_r1i1p1	1	0.139*	-0.242*	-0.205*	0.780*	2.97*
	2	0.020	-0.419*	-0.325*	0.678*	2.76*
	3	0.152*	-0.253*	-0.173*	0.774*	2.82*
MRI-CGCM3_historical_r1i1p1	1	0.302*	-0.335*	-0.147*	1.141*	3.76*
R_NorESM1-M_historical_r1i1p1	1	0.163*	-0.315*	-0.169*	0.812*	2.84*
Mean difference, m^3s^{-1}		0.178 (3.9%)	-0.195 (-13%)	-0.183 (-6.7%)	0.840 (14%)	3.14 (25%)
Mean flow, m^3s^{-1} (observed)		4.61	1.47	2.76	5.93	12.5

simulations. Anomalies in both basins for high flows (5% exceedance) indicate a decrease with climate change. However, given that the model underestimates high flows consistently, this result is questionable.

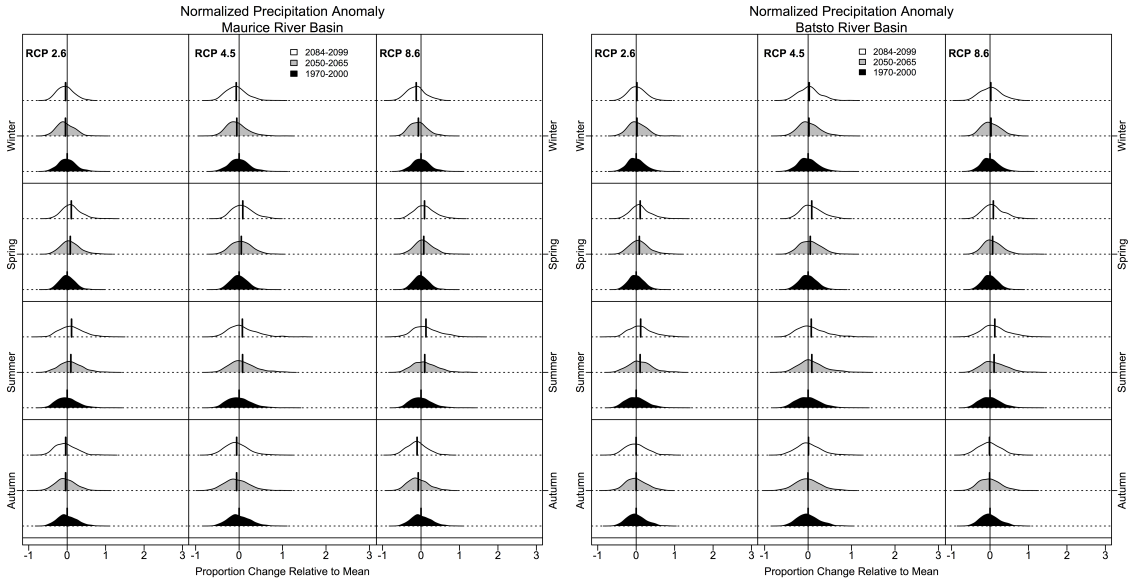

Figure 5: Distribution of seasonal total precipitation anomalies normalized to the seasonal mean total precipitation simulated from base models from GCMs for the period 1971-2000 for the Maurice River basin (right) and the Batsto River basin. Solid bar shows the location of the mean value for each distribution. The vertical line is at 0 indicating no change from the mean of the base models.

The most notable differences between the response of each basin was between the groundwater storage anomalies (Figure 8c, d). Anomalies for groundwater storage in the Batsto basin showed less variation and more consistent trends over the seasons for most scenarios and probability levels (Table 5). At flows between 5% and 50% exceedance levels, under RCP 4.5 and 8.5 simulations indicate a decrease in ground water storage in winter and spring, no change in summer and an increase in autumn by 2065 in the Maurice basin. Simulations indicated little change in this pattern through 2099. By contrast, for the Batsto basin, simulations indicated an increase in ground water storage for this range of flows by 2065 in all seasons except winter. This pattern persisted in the Batsto for spring, summer and autumn, with an increase in storage in winter by 2099. For flows between 50% and 95% exceedance levels, simulations indicated a similar decrease in groundwater storage in the Maurice basin. Whereas storage anomalies in the Batsto basin show little change through 2099, except in winter when there could be a slight decrease in storage.

3.3.3 Discussion

Anomalies for precipitation were consistent with overall performance of the CMIP5 multimodel ensemble, which has indicated overall increased winter precipitation on the east coast of North America and an increase in annual precipitation 5-10% for the northeast United States (Maloney et al., 2014). The CMIP5 multimodel ensemble also underpredicted winter precipitation on average with a mean bias of -3.16% with a range of 14.36 to - 28.15%, and underpredicted the annual runnoff to precipitation ratio (Sheffield, Barrett, et al., 2013). PRMS simulations using climate change projections from the CMIP5 multimodel ensemble showed an increase in

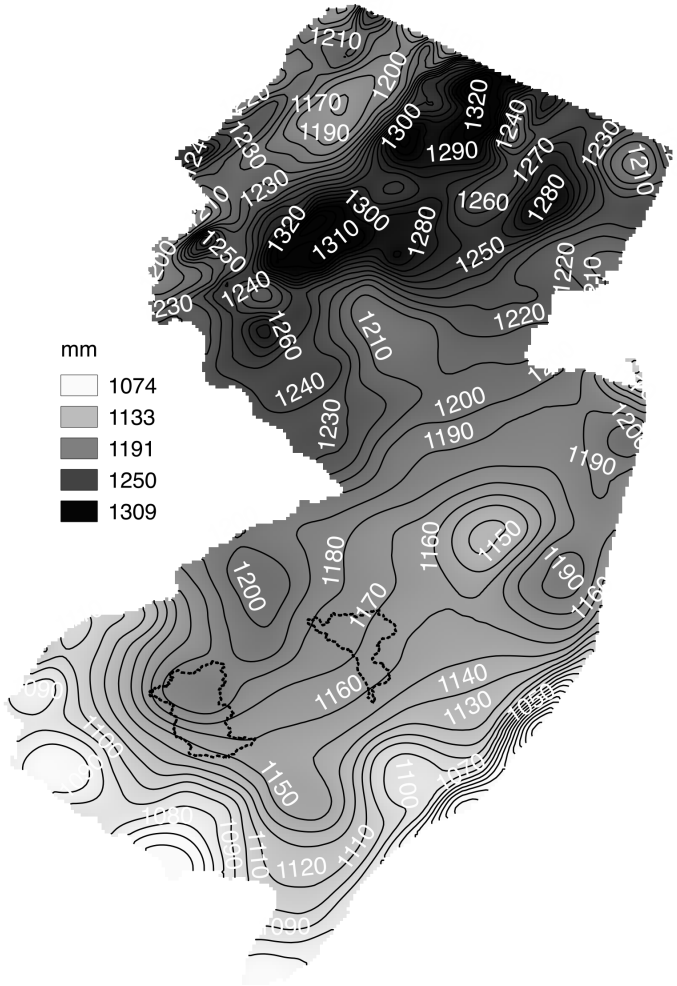

Figure 6: Distribution of mean annual precipitation for New Jersey. Data obtained from PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu, created 4 Feb 2004.

precipitation overall in both basins, but showed a slight decrease and no change of winter precipitation in the Maurice and Batsto basins, respectively.

Variance for precipitation anomalies was greatest at the end of the century in the summer in both basins for all GCMs and is most likely due to model uncertainty for the CMIP5 multimodel ensembles. It is expected that uncertainty will increase for projections that extend over longer time periods. However since this increase in uncertainty was not consistent across all seasons, it is likely that there will be greater variance in precipitation during the summer months in both basins. Water supply management strategies in both basins will likely face similar challenges due to increased variation in precipitation with climate change, though decisions may differ for other considerations, such as the need for aquatic habitat conservation for endangered species in the Batsto.

The Maurice and Batsto basins are separated by less than 40 km, and have similar

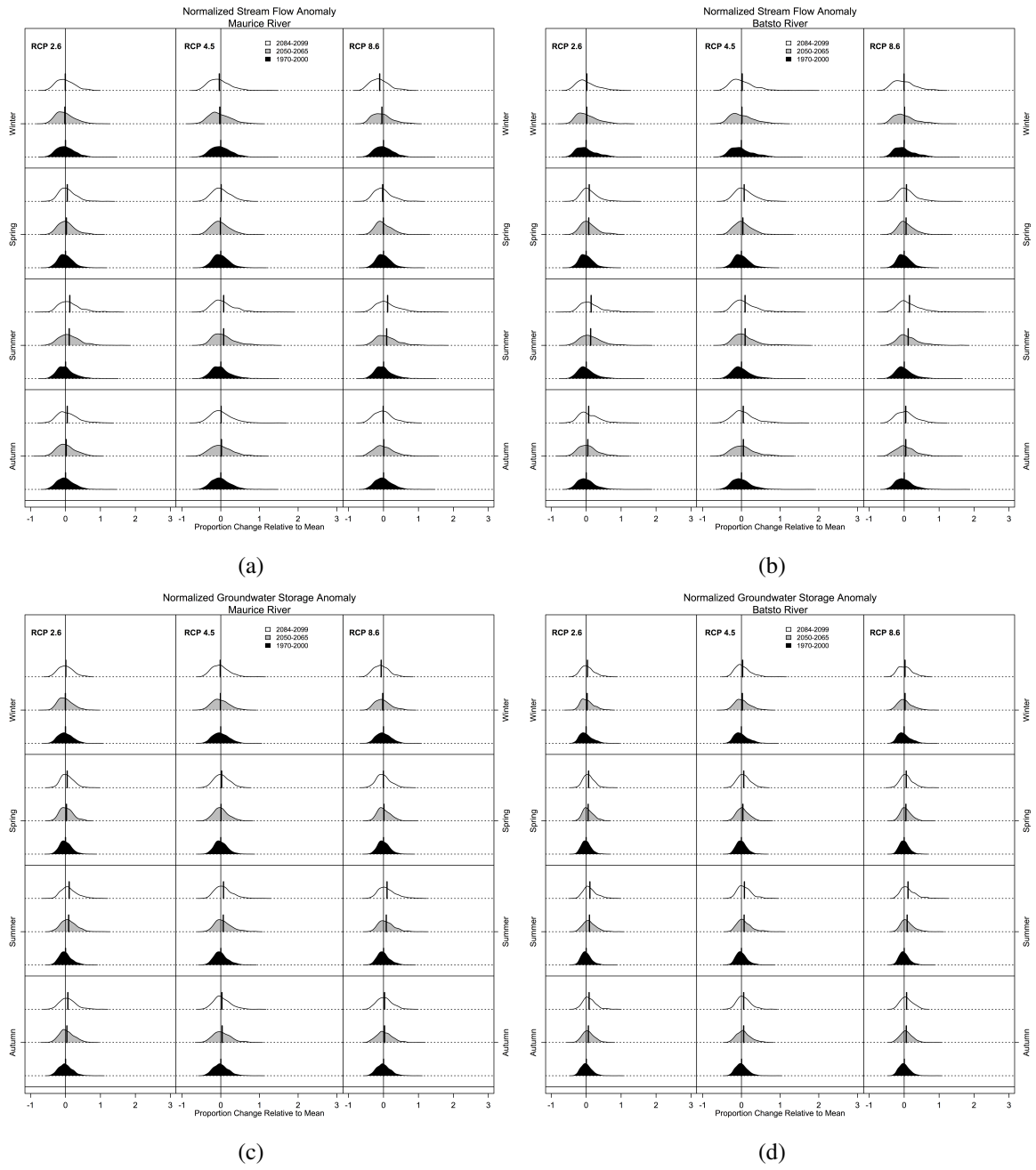

Figure 7: Distribution of seasonal flow anomalies normalized to the seasonal mean flows for the Maurice River (a) and the Batsto River (b), and of seasonal storage anomalies normalized to the seasonal mean storage for the Maurice River (c) and the Batsto River (d) simulated from base models from GCMs for the period 1971-2000. Solid bar shows the location of the mean value for each distribution. The vertical line is at 0 indicating no change from the mean of the base models.

topography and elevation, yet precipitation anomalies indicated some differences in patterns of change in each basin. Despite its small size, New Jersey has five distinct climatic zones, and precipitation has historically shown a zonal pattern from the coastal plain of southern NJ to the

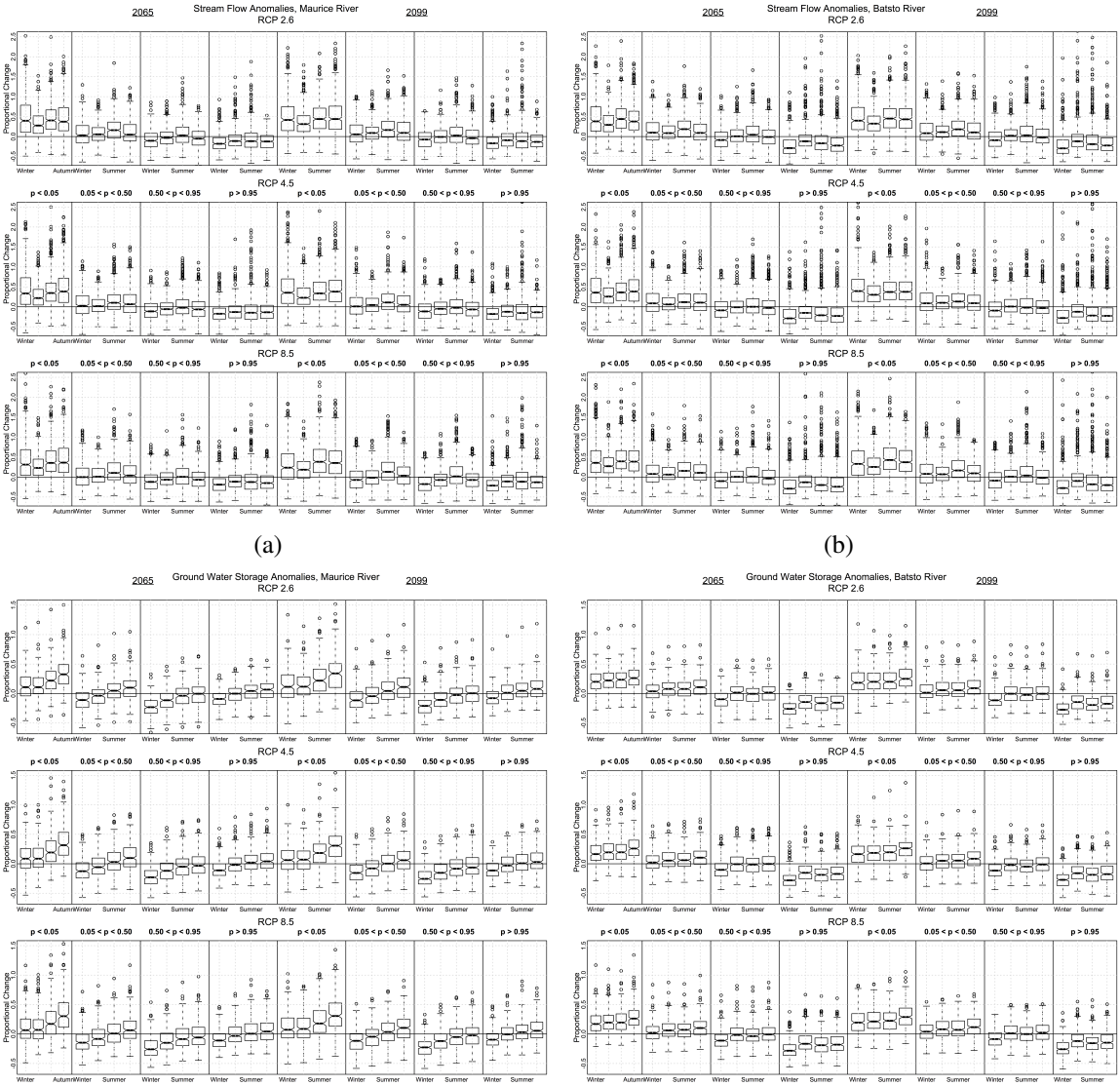

Figure 8: Box plots of seasonal flow anomalies for the Maurice River (a) and the Batsto River (b) and groundwater storage anomalies for the Maurice River (c) and the Batsto River (d) for flows in the range of each probability level. Anomalies were normalized to the mean flows in each bin for each season simulated from base models from GCMs for the period 1971-2000. Solid bar shows the location of the mean value for each distribution. The vertical line is at 0 indicating no change from the mean of the base models. If notches in boxplots do not overlap 0, or other notched areas of other boxplots then this provides strong evidence the there is a difference in the medians (R Core Team, 2015).

highlands in the north. Observed patterns of precipitation along with the results obtained here suggests that climate change impacts on precipitation may vary over relatively small spatial scales. Stream flow response in the Batsto and Maurice basins tended to vary with precipitation, which is consistent with the behavior of watersheds in general in the conterminous United States (McCabe and Wolock, 2011). How much this relationship may change in the future in the

Table 5: Estimate of the ratio of variances (F-test) for groundwater storage anomalies in the Maurice basin to those in the Batsto basin. Values > 1 indicate greater variance for anomalies in the Maurice basin. * indicates a significant difference in variance at the $p < 0.05$ level.

	Scenario	$\mathbf{p \leq 0.05}$	$\mathbf{0.05 < p \leq 0.5}$	$\mathbf{0.5 < p < 0.95}$	$\mathbf{p \geq 0.95}$
			Winter		
2065					
	RCP 2.6	1.55*	1.31	1.11	0.98
	RCP 4.5	1.60*	1.26	1.09	1.08
	RCP 8.5	1.77*	1.44*	1.21	1.11
2099					
	RCP 2.6	1.61*	1.40*	1.14	1.13
	RCP 4.5	1.63*	1.33*	1.14	1.03
	RCP 8.5	1.57*	1.32*	1.12	1.00
			Spring		
2065					
	RCP 2.6	1.53*	1.40*	1.16	0.92
	RCP 4.5	1.56*	1.40*	1.20	1.01
	RCP 8.5	1.76*	1.54*	1.31*	1.03
2099					
	RCP 2.6	1.64*	1.46*	1.22	1.00
	RCP 4.5	1.57*	1.42*	1.26	1.01
	RCP 8.5	1.53*	1.39*	1.21	0.93
			Summer		
2065					
	RCP 2.6	1.68*	1.61*	1.40*	1.23
	RCP 4.5	1.85*	1.60*	1.42*	1.23
	RCP 8.5	2.14*	1.82*	1.58*	1.34*
2099					
	RCP 2.6	1.96*	1.72*	1.45*	1.27
	RCP 4.5	1.75*	1.58*	1.51*	1.29*
	RCP 8.5	1.81*	1.62*	1.43*	1.20
			Autumn		
2065					
	RCP 2.6	1.83*	1.68*	1.39*	1.15
	RCP 4.5	2.02*	1.69*	1.41*	1.24
	RCP 8.5	2.33*	1.90*	1.58*	1.22
2099					
	RCP 2.6	2.04*	1.81*	1.44*	1.31
	RCP 4.5	2.05*	1.71*	1.50*	1.15
	RCP 8.5	1.97*	1.70*	1.44*	1.16

Maurice and Batsto basin, e.g. due to land use change, is not clear. However, since the response of stream flow in the basins for the most part followed precipitation patterns, the implications for management of water supply and design of infrastructure is likely to be different despite their proximity, contrary to the above.

Overall, PRMS simulations using the CMIP5 multimodel ensemble significantly

underpredicted high flows in both basins. In general, GCMs are not skillful in simulating extreme events at the subgrid scale (Neelin, 2011), and the CMIP5 multimodel ensemble has been shown to underpredict tropical cyclone frequency in the Atlantic (Sheffield, Camargo, et al., 2013). This can help explain some of the underprediction of high flows in summer and autumn since southern NJ is often impacted by tropical systems. However the trend in simulations for Maurice and Batsto basins indicated underprediction at flows greater than median flows and overprediction at flows less than median flows. The consistency of these errors allows conclusions to be drawn about relative changes in stream flow with climate change, but it is not possible to quantify absolute changes.

In the Pinelands region, groundwater recharge tends to be lower in the growing season, most likely because of increased evapotranspiration (Walker et al., 2011). Depth to the water table varies seasonally, and much of this variation is likely due to local soil permeability and the makeup of the aquifer, and there are strong interactions between subsurface flows and wetlands, lakes and streams (Walker et al., 2011). Much of the Maurice basin contains land that can be classified as Pinelands, and the entire Batsto basin is in the Pinelands region. The porous sandy soils of the region result in both basins having closely linked groundwater and surface water connections. Annual base flow for the Maurice River was from 74 to 92% of total flow over the period 1933–1986 (Lacombe and Rosman, 1995), and an estimated 45% of annual precipitation became discharge at the USGS stream gage at Norma from 1933–1986. The remainder would either be lost to ET or stored in the basin as surface or groundwater. While similar data for the Batsto River were not available, since the Batsto basin is located entirely within the Pinelands, it is likely to have greater connection to the subsurface. Storage was weakly correlated with precipitation, but it is related to precipitation through stream flow.

Simulations indicate an increase in stream flow in both basins consistent with observed trends in stream flow in this region over the past 110 years (Yang et al., 2014). Both basins showed an increase in flows and storage at low flows ($< 5\%$ exceedance) along with a high degree of variability across model simulations at low flows in particular. Simulation results and climate projections in general indicate an increase in summer convective precipitation in eastern North America with more rainfall falling in fewer events (Guinard et al., 2014). It is also likely that there will be fewer small events. How this will impact watersheds with different characteristics may not be the same. In these basins with porous substrates, it seems that more rainfall will lead to greater stream flow and groundwater storage even if it occurs in fewer events, which is consistent with evidence found by Liu, (2011) that higher average rainfall per event will tend to increase groundwater recharge and vegetation growth. It is an open question as to whether this type of rainfall pattern will lead to greater vegetation growth in the Pinelands. Its unique aspects are due to the low water holding capacity of the sandy soils, so it is more likely that the Pinelands watersheds will show primarily increased groundwater recharge.

Unsurprisingly, the groundwater storage anomalies estimated by pooling all flows indicated similar changes in storage in both basins. Simulations for both basins showed either no change or a slight increase in storage for all emission scenarios, with one exception. However, some differences in the response of groundwater storage to climate change between the basins was indicated at different exceedance levels. In particular, variability in groundwater storage anomalies were greater in the Maurice basin than in the Batsto. The Batsto basin has more uniform land use consisting of around 88% undeveloped land within the Pinelands, and is therefore more likely to show less variation in the connection of surface and ground waters. The

Maurice basin, on the other hand, has approximately 45% forest and wetlands and almost 30% urban land-use. The large amount of impervious area is likely to have a significant impact on groundwater recharge by increasing runoff and decreasing recharge (Shuster et al., 2005). The Batsto basin also showed less variation in groundwater storage anomalies by season, which is consistent with a more uniform land use in the basin with significantly less impervious area.

Simulated groundwater storage anomalies may raise some potential implications with regard to water supply. In the Maurice basin storage anomalies were greater in summer and autumn than in winter and spring, and anomalies indicated a more consistent trend in the Batsto. It seems that storage in the Batsto basin may be more balanced throughout the year than in the Maurice with increased storage during low flow periods. Land-use has a strong impact on how climate change impacts hydrology (Daraio and Bales, 2014), and it seems most likely that the different responses simulated in these basins can in part be attributed the different land-use patterns within these basins.

A limitation of the models developed here was that the models did not explicitly include water withdrawal from the basins. However, the models were developed from observed stream flow data that implicitly included withdrawal, since withdrawal was occurring within the basins over the period of record. The assumption inherent in the calibration and evaluation of these models was that withdrawal rates remained constant at recent levels. Therefore projections include the implicit assumption of no increased pressure due to population growth in addition to the explicit assumption of no land-use change. Groundwater withdrawals are known to have an impact on stream flow in these basins. For instance, simulations by Charles and Nicholson, (2012) indicated that base flow reduction in the Pinelands due to groundwater withdrawal can approach up to 30 to 50 percent, and cause a decline of as much as 10 percent of total wetland area.

The Maurice basin is very likely to see significant more growth through the 21st century than the Batsto basin, and the potential exists that groundwater storage will decrease more than simulations here suggest. If this is the case, then future development and water management within the Maurice basin will face different challenges than those that may be faced in the Batsto. In particular, it will be important for new storm water infrastructure and upgrades to existing infrastructure to maximize retention and recharge of groundwater. In order to better quantify these potential changes and set design standards for the Maurice basin, there is a need to combine an existing MODFLOW model (Cauller and Carleton, 2006) with PRMS developed here. This will provide the means to more fully analyze the potential impacts of climate change, land-use change, and potential increased in withdrawal demand on groundwater storage and water supply in the Maurice and Batsto basins.

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4 Publications and Presentations

Under review

Daraio, J.A. "Potential Climate Change Impacts on Stream Flow and Groundwater Storage in Two Watersheds on the New Jersey Coastal Plain" *Journal of Hydrologic Engineering*

Poster Presentions:

Bechtold AL, McCarthy ME, Spurgin CM, Tucci JM, and Daraio JA 2015. "Climate Change Impacts on Stream Flow in Two New Jersey Watersheds." 18^{th} Annual Rowan University Science, Technology, Engineering, & Mathematics (STEM) Student Research Symposium, Glassboro, NJ, April 24, 2015

Daraio, J.A. 2014. "A Comparative Analysis of Hydrologic Response to Climate Change in Developed and Undeveloped Watersheds on the New Jersey Coastal Plain." AGU Fall Meeting, San Francisco, CA, December 15–19, 2014.

Developing nano-activated carbon based technology for groundwater remediation

Basic Information

Title:	Developing nano-activated carbon based technology for groundwater remediation
Project Number:	2014NJ352B
Start Date:	3/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	NJ-006
Research Category:	Not Applicable
Focus Category:	Groundwater, Treatment, Toxic Substances
Descriptors:	None
Principal Investigators:	Chengyu Chen, Weilin Huang

Publications

There are no publications.

NJWRRI FY2014 Annual Report

Developing carbon nanoparticles (CNPs) based technology for groundwater remediation

Chengyu Chen

(1) PI information:

Dr. Weilin Huang (thesis advisor)

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(2) Numbers of Students Supported:

1 Ph.D. student: Chengyu Chen

Rutgers, The State University of New Jersey, Graduate School, Department of Environmental Sciences, 14 College Farm Road, New Brunswick, New Jersey 08901; Email: chengyuc@scarletmail.rutgers.edu; Telephone: (732) 789-3077; Amount of funding: $5,000.

(3) Project Summary:

a. Problem and Research Objectives

The proposed research program addresses groundwater pollution and remediation, one of pressing environmental issues in the state of New Jersey. New Jersey has the most superfund sites in the US [1], many of which have volatile organic compound (VOCs), e.g., 4-chlorophenol (4CP) and aniline, as major pollutants in their groundwater systems [2]. Conventional technologies such as pump and treat are not cost effective, and *in situ* remediation technologies such as bioremediation and permeable reactive barriers (PRBs) have disadvantages including the risk of toxic intermediates and adverse impacts on secondary water quality objectives [3]. More recent developments proposed to inject nanoscale zero-valent iron (nZVI) or nano carbon tube (CNT) materials to groundwater for remediation of chlorinated solvents. However, these nanomaterials have limited success due to their poor transport properties. Although nZVI has been reported to have high reactivity [4], it agglomerates rapidly into larger micron-sized particles [5] in water, substantially reducing the efficiency of degradation process [6]. Study demonstrated that nZVI had limited mobility (traveling for a few meters at most from the injection well) and had longevity up to a few months [7]. Similarly, because CNT has lengths up to micrometers [8] and settles quickly in water, it does not have good transport properties for penetrating through aquifer materials or dispersing well in groundwater systems. Meanwhile, CNT does not exhibit high adsorption capacities towards chlorinated solvents [9].

To address these problems, the objectives of this research are to study four novel types of carbon nanoparticles (CNPs), which include nano-sized activated carbons and nano-sized biochars. The CNPs are hypothesized to have stronger adsorption capacities for chlorinated organic compounds in water, and more importantly, have specific properties to remain stable against natural sedimentation and particle aggregation in natural groundwater conditions. Such properties are indicative that the CNPs will remain suspended and dispersed well over extended time period

in natural groundwater conditions, so that they can travel long distance in aquifer to adsorb typical volatile organic compounds.

Systematic experiments have been conducted for characterizing the physicochemical properties of the CNPs, quantifying their adsorption capacities for two model VOCs contaminant in groundwater – 4CP and aniline, and studying the stability properties of CNPs in terms of the sedimentation in quiescent purified water and the CNPs aggregation process in quiescent natural groundwater conditions under different ionic strength and pH conditions. Results so far have shown these CNPs to be very promising for remediating groundwater contaminated with VOCs via an *in situ* injection method.

b. Methodology

i. Sample Characterization

Four types of commercial carbon nanoparticles (CNPs) produced from different raw materials were purchased and named as CNP1, CNP2, CNP3, and CNP4, where CNP1 and CNP2 were nano-sized biochar, and CNP3 and CNP4 were nano-sized activated carbon. A powdered activated carbon (PAC) and a multi-walled carbon nanotube (CNT) material were also used in this study for comparison. All carbonaceous materials were used as received. They were measured for their total N_2-BET specific surface area (SSA), total pore volume, average pore radius, and distribution of the surface area within the pores using a nitrogen gas adsorption technique. Transmission electron microscope (TEM) and Scanning electron microscope (SEM) were employed to examine their sizes and morphologies. Elemental analysis (EA) and X-ray photoelectron spectroscopy (XPS) were conducted to quantify their elemental compositions. Fourier transform infrared spectroscopy (FTIR) spectra were obtained for characterizing the functional groups. Dynamic light scattering (DLS) measurements were performed to determine their size distribution.

ii. Adsorption Experiment

Aqueous adsorption of 4-chlorophenol (4CP) and aniline on the four types of CNPs, PAC, and CNT was investigated in this study. Adsorption isotherms were measured at room temperature (25ºC) and pH 6 using a batch system. Each batch reactor contained 50mL of 4CP or aniline solutions with various initial concentrations. A constant mass of a sorbent was added to achieve around 50% reductions in the initial solute concentration upon equilibrium. Duplicate samples were prepared for each batch system. Reactors were mixed by tumbling top to bottom at 150 rpm. After attainment of adsorption equilibrium, mixtures of solution and sorbent were taken at from the reactors and were filtered through 0.22 μm. The filtrates were analyzed for the aqueous 4CP or aniline concentrations using a high performance liquid chromatography (HPLC) equipped with a diode array UV detector. Control experiment was run to access the loss of solute to reactor components. Both the Freundlich isotherm equation and the Langmuir isotherm equation were employed to fit the adsorption isotherm data obtained from the batch experiments.

Adsorption kinetics experiments were also performed using the batch method. For each type of the sorbents, duplicates of aqueous solution of 4CP or aniline and a fixed mass of sorbent were added to 500mL screw cap flasks. The reactors were mixed completely by tumbling top to bottom at 150rpm and 25ºC. Samples of the solution-sorbent mixtures were taken at different time intervals, and were filtered and analyzed using the same methods as above.

iii. Electrophoretic Mobility Measurements

To investigate the stability of the CNP particles in natural groundwater system against sedimentation and particle aggregation, the electrophoretic mobility (EPM) of the four types of CNPs was measured using electrophoretic light scattering at different solution chemistry. The EPM of the CNPs were converted to zeta potentials (ζ) with the software built-in the instrument. The EPM were measured for CNPs at different solution chemistry resembling natural groundwater conditions, using sodium chloride or calcium chloride at different ionic strength or pH conditions. All measurements were conducted at 20 ºC.

iv. Sedimentation Experiments

Four types of CNPs, PAC, and CNT were prepared in purified water to compare their sedimentation rates. Each sample after sonication was diluted with deionized water to make aqueous solution of the same concentration. 4 mL of stock solution of each sample was introduced into a clean spectrophotometer cuvette. Samples were sealed and stored in dark at room temperature for natural sedimentation. The light intensity, *I*, of the samples were measured periodically over two months by a at 675 nm wavelength. The fraction of particles remained suspending in water over time was measured as the light intensity at time t, *I(t)*, over its initial light intensity, *I(0)*. All experiment and measurements were conducted at pH 6.

v. Determination of CNPs Aggregation Process and Kinetics

To determine the aggregation processes of CNPs in different solution chemistry, time-resolved dynamic light scattering (DLS) measurements were performed for the hydrodynamic sizes of CNPs. The sonicated CNPs aqueous solutions were diluted with deionized water to make the same concentration. For each experiment, 2 mL of CNPs solution was introduced into a clean glass vial. pH of the solution was adjusted before 2 mL of prepared electrolyte solution with predetermined concentration was introduced into the vial containing the NAC suspensions to induce aggregation. The vial was briefly vortexed before being inserted into the DLS instrument and measurement was started immediately. The scattered light was detected by a photo-detector at a scattering angle of 90º. The intensity-weighted hydrodynamic radius, R_h, of the particles measured was determined through cumulant analysis. The aggregation processes of CNP at the starting 20 minutes were recorded by measuring the R_h. The samples were then sealed and left undisturbed in dark for continued aggregation. The hydrodynamic radius and the polydispersity of the CNP samples remained suspending in solution was monitored over two months to observe the stability against aggregation by the electrolytes in solution. All DLS measurements were conducted at 20 ºC.

To estimate the stability of CNPs against aggregation, the aggregation kinetics and the critical coagulation concentration (CCC) of the CNPs at different solution chemistry were determined from the aggregation data obtained above. At the initial stage of nanoparticle aggregation, the hydrodynamic radius measured by DLS, $R_h(t)$, increases linearly with time, *t*. Therefore, the initial aggregation rate constant, *k*, can be determined from the slope of the linear range of the aggregation profiles. The aggregation attachment efficiencies, α (equivalent to the inverse stability ratio, *1/W*), which range from 0 to 1, were calculated to quantify the initial aggregation kinetics of the CNPs at different electrolyte concentrations and pH conditions. The calculation was done by normalizing the slopes obtained under different electrolyte concentrations by the slope obtained under favorable aggregation conditions, which refers to fast or nonrepulsive aggregation conditions. From the determined attachment efficiency profiles, the CCC of the CNPs at different natural groundwater conditions was determined. By extrapolating through the fast and slow aggregation regimes, the

intersections of the extrapolation yield CCC [10], which were indicative of the CNPs stability against aggregation at the specific solution conditions.

c. Principal Findings and Significance

i. Sample Characterization

DLS, TEM, and SEM analysis have shown that all four types of CNPs are mainly spherical particles and some irregularly shaped ones, with diameter around 100 nm; PAC has diameter in micron range; and CNT has diameter in 100 nm while length in microns. N_2-BET SSA analysis showed that the specific surface area of CNP1, CNP2, and CNP3 are around 1000 m^2/g; while CNP4, PAC, and CNT have SSA about 300, 600, and 40 m^2/g, respectively. Such results indicated that CNP1, CNP2, and CNP3 with much higher SSA should have much higher adsorption capacity for VOCs compared to CNP4, PAC, and CNT. BET SSA analysis also showed that for all types of CNPs, more than 80% of pores are within radius of 20 Å, while PAC and CNT have much larger pores. Elemental analysis showed that the oxygen content of CNPs were about 10%, while XPS showed agreed that CNPs have oxygen content of about 6%. The difference in results between EA and XPS is because EA analyzed for the bulk sample while XPS probes the sample surface. Yet the high oxygen content from two analyses both suggested possible negative charges present on the CNP surface. The C-O and C=O stretching vibration from FTIR analysis of CNP samples also demonstrated the presence of oxygen containing functional groups. However, potentiometric titration of CNP samples is still needed to provide further evidence of the origin of CNP charge on the particle surface, which is the key to explaining the stability of CNPs in water against sedimentation and aggregation.

ii. Adsorption Experiments

So far experiments have been performed on the adsorption of 4CP and aniline by the four types of CNPs and PAC. All adsorption data fit better to the Freundlich isotherm equation than the Langmuir isotherm model, where the fitting of the data to the Freundlich isotherm model produced fitting coefficients of determination, R^2, all above 0.98, while Langmuir isotherm had R^2 below 0.98. The difference on the fitting between these two models may be due to the fact that Langmuir isotherm assumes homogeneous sorbent surface and monomolecular layer of adsorbate coverage with no interaction among adsorbed molecules, however, the activated carbon and biochar samples used in the experiments are heterogeneous materials. In general, the adsorption of 4CP was much stronger by CNPs and PAC comparing to the adsorption of aniline. This may be due to the stronger hydrophobic interactions between the carbon materials and 4CP comparing to aniline, where 4CP has a higher $\log(K_{ow})$ value of 2.39 compared to 0.90 for aniline [11]. The lower water solubility of 4CP (26 g/L) compared to aniline (34 g/L) is also consistent with such results [12]. For adsorption of both 4CP and aniline, CNP1, CNP2, and CNP3 showed only slightly higher adsorption capacity compared to PAC, but exhibited much higher adsorption capacity compared to CNP4. This correlated well with the SSA data from sample characterization.

Adsorption kinetics experiments for 4CP showed that CNP1, CNP2, CNP3, and PAC removed up to 50% of 4CP from the aqueous phase in 3 minutes, given that the adsorbent amounts added to solution were up to 80% removal of adsorbate from aqueous phase upon equilibrium. The fast adsorption rates should be due to the high SSA of these four carbon materials. However, CNP1, CNP2, and CNP3 reached equilibrium in 1 hour, while PAC did not reach equilibrium until 5 days. The higher rates of adsorption from CNP1, CNP2, and CNP3 may be due to their much smaller

average pore radius (17 Å) compared to PAC (35 Å). The smaller pores of CNPs may trap 4CP upon entry, while the larger pores of PAC may release adsorbed 4CP thus taking longer time to reach adsorption equilibrium. Compared to these four carbon materials, CNP4 adsorbed 4CP much more slowly because of its much smaller SSA. The adsorption kinetic experiments for aniline showed similar trends as 4CP as discussed, however, the overall adsorption rates of aniline were faster compared to 4CP, given up to about 70% of aniline adsorbed from aqueous phase in 3 minutes of contact with the carbon materials. This phenomenon may be explained from the size of the adsorbate given other conditions the same. Aniline with a smaller molecular weight (93 g/mol) may enter the carbon pores more quickly than 4CP with molecular weight of 128 g/mol, even though the equilibrium adsorption capacity of aniline was lower than 4CP as discussed previously.

The major findings in adsorption experiments so far have shown that CNP1, CNP2, and CNP3, in comparison with PAC and CNP4, have high adsorption capacity for VOCs such as 4CP and aniline, and can remove these contaminants from water in minutes upon contact. Such promising carbonaceous materials can be applied in groundwater remediation of VOCs or act as emergent treatment agents for organic chemical spills.

iii. Stability of CNPs against Sedimentation and Aggregation in Water

The measured EPM values were converted to Zeta Potential (ζ) for the CNPs in purified water. CNP1, CNP2, and CNP3 had Zeta Potential of about -50 mV, while CNP4 had -30 mV. Such negative values indicate that the surface of the CNPs is very negatively charged, which agree with the high oxygen content from EA and XPS analysis. With the negatively charged surface, CNPs should be very stable against sedimentation and aggregation in water because of the strong repulsive electrostatic force existing between each carbon nanoparticle.

We have compared the natural sedimentation rates among the CNPs to PAC, CNT, ZVI, and fullerene nanoparticles (C_{60}) in quiescent purified water. Results showed that CNT and ZVI settled almost completely from water after one hour; PAC and C_{60} settled after 4 days and 20 days, respectively; however, all four types of CNPs had about half of the particles remained suspending in water after 2 months. The hydrodynamic radius, R_h, of CNPs samples after 2 months measured by DLS remained about 50 nm as the original size, indicating no significant aggregation occurred. Therefore, CNPs are very stable in quiescent purified water against settling and aggregation compared to other commonly particles used for groundwater remediation.

The stability of CNPs against aggregation was further examined from the carbon nanoparticles aggregation under different sodium chloride or calcium chloride concentrations. The concentrations of NaCl used in the experiments to induce CNP aggregation ranged from 0 to 600 mM, and $CaCl_2$ ranged from 0 to 200mM at pH 6. For all CNPs, the Zeta Potentials became less negative as electrolyte concentrations increased. As discussed before, the carbon nanoparticles with high negatively charged surface experience strong repulsive electrostatic forces at low electrolyte concentration. However, as salt concentration increases, the negative charges on the CNP surface are screened out and attractive van der Waals forces dominate between the nanoparticles [10]. Therefore, Zeta Potential became less negative and particle aggregation started. Such effects of $CaCl_2$ were much more significant than NaCl, due to the fact that divalent ions are much more effective coagulants than monovalent ions.

The aggregation processes can be observed from the aggregation profiles of CNPs measuring particle R_h with time. Consistent with the change in Zeta Potentials, higher electrolyte concentrations induced faster aggregation rates. However, as electrolyte concentrations increased to certain value, the aggregation rates stopped increasing. By converting the slopes of the

aggregation profiles at different electrolyte concentrations, the attachment efficiencies, α, between 0 and 1 were calculated. By extrapolating through the fast and slow aggregation regimes, the intersections of the extrapolation yield critical coagulation concentrations (CCC) of each CNP at the presence of NaCl and $CaCl_2$. For CNP1, CNP2, and CNP3, the CCC of NaCl were 120 mM, and of $CaCl_2$ were 5 mM. For CNP4, the CCC of NaCl was 70 mM, and of $CaCl_2$ was 3 mM, which is in agreement with the Schultz-Hardy Rule [13]. Because typical natural groundwater has sodium concentrations less than 9 mM, and calcium concentrations less than 2.5 mM [14], CNPs should remain stable against aggregation in groundwater conditions.

Continued observation on the R_h and polydispersity of the aggregated CNPs in previous electrolyte solutions over 1 month has further proven the stability of CNPs against aggregation in natural groundwater conditions. Results showed that in the presence of NaCl concentration less than 15 mM, R_h of CNPs remained within 100 nm over 1 month; and in the presence of $CaCl_2$ concentration less than 1 mM, R_h of CNPs remained within 200 nm over 1 month. The polydispersity index increased with time of aggregation. It suggested that the aggregation process increased the size distribution of CNPs, creating carbon particles with more widely distributed sizes as aggregation proceeded.

The pH effect on the Zeta Potential and aggregation of CNPs was also examined. For all CNPs, their Zeta Potentials become increasingly negative at higher pH conditions. This may be due to the fact that the surface functional groups on the CNPs become deprotonated as pH increases. The aggregation profiles of CNPs in NaCl and $CaCl_2$ at different pH conditions reviewed consistent results with the change of Zeta Potentials. Lower pH enhanced CNPs aggregation rates, while higher pH created more negatively charged surface thus suppressed the aggregation process. Nevertheless, because natural groundwater typically has pH within 6.5 to 8.5, CNPs should remain stable against aggregation as the results obtained at pH 6.

In conclusion, in quiescent or slowly flowing natural groundwater system where the ionic strength and pH are under typical conditions, the novel CNPs suspension may remain relatively stable as nanoparticles without aggregation over months. This may be very different from other agents or nanoparticles such as CNT and ZVI which are commonly used for groundwater remediation. This high resistance to aggregation properties may enable CNPs to remain as nanoparticles when they travel long distance in groundwater systems to reach the contaminated zone. In addition, the CNPs used this study have high adsorption capacity and fast adsorption rate for organic pollutants such as 4CP and aniline. With these two unique properties, it is likely that the carbon nanoparticles examined in this study could be used as strong and reactive particles for effectively remediating groundwater systems polluted with organic chemicals.

(4) Publications or Presentations:

Oral presentation of the findings supported by this funding was given as a seminar on 11 November, 2015 at Rm 223, Department of Environmental Sciences, Rutgers University, 14 College Farm Road, New Brunswick, NJ 08901. The presentation was entitled "Colloidal Stability of Activated Carbon Nanoparticles (CNPs) in Aquatic Environments."

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Parasite biomass and nutrient stoichiometry in a stream ecosystem

Basic Information

Title:	Parasite biomass and nutrient stoichiometry in a stream ecosystem
Project Number:	2014NJ355B
Start Date:	3/1/2015
End Date:	2/29/2016
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Congressional District:	NJ-006
Research Category:	Not Applicable
Focus Category:	Ecology, Nutrients, Surface Water
Descriptors:	None
Principal Investigators:	Rachel Paseka, Michael V. K. Sukdeo

Publications

1. Paseka, Rachel, 2015, Deviation from homeostasis and elemental imbalance in host-parasite interactions, Theo Murphy International Scientific Meeting - Elements, genomes and ecosystems: cascading nitrogen and phosphorus impacts across levels of biological organisation, Kavli Royal Society International Centre, Buckinghamshire, UK (Poster)
2. Paseka, Rachel, 2015, Deviation from homeostasis and elemental imbalance in host-parasite interactions, Conference on Biological Stoichiometry, Trent University, Peterborough, Ontario, Canada (Poster)
3. Paseka, Rachel, 2015, Elemental imbalance in host-parasite interactions, Rutgers Ecology and Evolution Student Seminar, New Brunswick, NJ (Presentation)
4. Paseka, Rachel and Michael Sukhdeo, 2014, Parasite biomass in streams of the New Jersey Pine Barrens, American Society for Parasitologists, New Orleans, LA (Presentation)
5. Paseka, Rachel (IN PROGRESS). “Ecological Stoichiometry of Parasitism”, Ph.D. Dissertation, Ecology and Evolution Graduate Program, Rutgers University, New Brunswick, NJ.
6. Paseka, Rachel. 2016. "Low parasite biomass in oligotrophic streams differs from previous estimates in diverse communities." Under review at Freshwater Science.

NJWRRI FY2014-Extension Annual Report

(1) **PI Information**

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(2) **Numbers of Students Supported**:

Undergraduates: 0
Master's students: 0
Ph.D. students: 1
Postdoctoral assocs.: 0

(3) **Notable Achievements**

I completed the preliminary proposal defense required by my graduate program in 2015, and I continue to receive support from the NSF Graduate Research Fellowship Program.

(4) **Project Summary**:

The following is a summary of a dissertation chapter that I completed this year. The full manuscript is currently in review at *Freshwater Science.* Funding from NJWRRI supported purchases of much of the sampling equipment and lab supplies that I used for this project, and I continue to use these items for my ongoing dissertation projects.

"Low parasite biomass in oligotrophic streams differs from previous estimates in diverse communities."

Problem and Research Objectives

Parasites are diverse and ubiquitous in nature, but their cryptic presence commonly leads to their omission from studies on the organization of communities and ecosystems. Parasites are increasingly recognized as an important functional group, but there are many unanswered questions about the ecosystem-level consequences of parasitism (Ostfeld et al. 2008, Hatcher et al. 2012, Preston et al. 2016). The distribution of biomass among taxa reflects the flow of energy and matter through ecosystems, and comparing the relative biomass of parasitic and free-living organisms is one approach to approximate the functional importance of parasitism at the ecosystem scale. The first report of ecosystem-level biomass distribution that included parasites was remarkable, with parasites accounting for large amounts of total consumer biomass in estuaries on the Pacific coast of North America. In these ecosystems, parasite biomass density exceeded the standing stock biomass of some free-living consumer groups, including the birds that serve as top predators (Kuris et al. 2008). Though this study did not address the mechanistic roles that parasites might play in these estuarine ecosystems, the report of high parasite biomass density suggested that parasites may mediate substantial components of ecosystem functioning.

Comparative biomass studies across ecosystems very rarely include parasites, so it is unknown whether high parasite biomass is common or specific to the type of system studied by Kuris et al. (2008). Subsequent studies of California ponds (Preston et al. 2013) and New Zealand lakes (Lagrue and Poulin 2015a) reported parasite biomass densities roughly one order of magnitude lower than what was estimated in the Pacific estuaries, but still exceeding the biomass of some free-living groups. In addition to differences in the overall density of parasite biomass among systems, these studies reveal substantial variation in parasite biomass distribution among various taxa and life cycle stages. As these data continue to accumulate, they will provide opportunities to test hypotheses concerning relationships among ecosystem characteristics, constraints on parasite density, and the functional importance of parasitism at the ecosystem scale.

I conducted standardized, seasonal sampling of fish and macroinvertebrates to measure the standing stock biomass of all consumers in streams of the New Jersey Pine Barrens. I dissected all free-living individuals collected to quantify the abundance and biomass of their macroparasites. The conversion of these data into standing stock biomass density estimates for stream ecosystems allowed comparisons among taxa, streams, and seasons. I also compared these results to those from several other aquatic ecosystems and evaluated the potential influence of sampling methodology on apparent differences among ecosystems.

Methodology

I sampled two streams within the Mullica River basin of the New Jersey Pine Barrens from August 2013 to July 2014. The streams are small and have low flow rates, with a sandy substrate and high levels of allochthonous inputs. The two streams are 4.8 miles from one another at the points sampled but differ in water quality due to differences in surrounding land use. Skit Branch (GPS coordinates 39.767215 N, -74.676949 W) predominantly drains protected pine forest within Wharton State Forest, and it is characterized by low levels of pH, conductivity, and dissolved nutrients. Muskingum Brook (39.817195 N, -74.737765 W) lies outside of the preserved area and drains a mixture of agricultural, developed, and forested land. Relative to Skit Branch, Muskingum Brook is higher in both pH and dissolved nutrients (Morgan and Good 1988, Zampella et al. 2001).

The sampling area at each stream was a 100 m reach, split into 5 20 m transects. To minimize disturbance of adjacent transects, I sampled downstream transects before moving upstream. I sampled macroinvertebrates every 3 months by taking 1 Hess sample (0.1 m^2) in a haphazardly chosen area of the streambed and 1 D-net sample (0.3 m^2) along the stream bank at each transect (Hauer and Resh 2006). I sampled fish monthly by seining continuously in each transect for 10 minutes (1/8" mesh seine), collecting every fish caught during this time. This sampling scheme led to the collection of a total of 40 macroinvertebrate samples (10 per season) and 60 fish samples (15 per season) for each stream. All samples were frozen on the day of collection and thawed at a later date for processing.

Macroinvertebrate samples were rinsed through a 500 μm sieve to remove fine benthic matter, and entire samples were scanned visually to remove all macroinvertebrates from stream detritus. I identified each free-living organism to species (fish) or family (macroinvertebrates) and then dissected all individuals to remove, identify, and count macroparasites present in all tissues. This study includes all metazoan macroparasites detected in the samples; bacterial, fungal, and protozoan parasites were not quantified. Monogenean parasites infecting the gills of several fish species were not counted because detachment during sample freezing prevented reliable quantification of this group.

To measure fish biomass, I removed all parasites and intestinal contents, dried remaining tissues at 60° C for a minimum of 3 days, and weighed each fish individually. For free-living invertebrate biomass, I used published length-mass regressions (Benke et al. 1999, Méthot et al. 2012) to estimate the dry mass of each individual from its measured length. I measured parasite biomass by pooling parasites onto pre-weighed, fiberglass filters (Whatman GF/F), drying at 60° C, and weighing with a microbalance (± 0.0001 mg). I calculated the mean individual mass of each parasite species and stage, then used this mean value for stream biomass calculations.

Using the dry mass values for fish, macroinvertebrates, and parasites collected at each stream, transect, and season, I converted these values to consumer biomass density (mg dry mass/m^2 of stream). Some key assumptions were necessary for this conversion. First, using an estimate of 5% fish capture rate and seasonal measurements of stream transect area, I estimated the total dry mass of fish per stream transect for each season. I applied this same transformation to parasite stages infecting fish. For macroinvertebrates and their parasites, I calculated biomass density as the quotient of dry mass collected in each sample and sampler area. Finally, I used bootstrapping (Manly 2007) to estimate the means and confidence limits of parasite contribution to total consumer biomass for each stream and season.

To compare the results of this study with those of 3 other aquatic systems where parasite biomass density has been measured, I compiled data on the mean biomass density of parasites and the total percentage of consumer biomass made up of parasites from prior studies (Kuris et al. 2008, Preston et al. 2013, Lagrue and Poulin 2015b).

Principal Findings

The dataset produced from this sampling scheme contains temporal and spatial abundances of all free-living organisms >500 μm and their parasites (Table 1). Overall, I collected 13 fish species, 46 macroinvertebrate families (insects, crustaceans, and molluscs), and 9 macroparasite species.

	Muskingum Brook		**Skit Branch**	
	Number of taxa	**Number of individuals**	**Number of taxa**	**Number of individuals**
Insects	35	6,091	33	2,987
Fish	10	358	8	192
Crustaceans	2	40	1	7
Molluscs	3	348	1	6
Parasites	9	2,362	7	1,621

Table 1. Summary of free-living and parasitic consumer taxa collected from two streams in the New Jersey Pine Barrens. For fish and parasites, number of taxa represents species. For insects, crustaceans, and molluscs, number of taxa represents families.

All major groups of free-living consumers collected significantly surpassed parasites in total biomass density (Fig. 1). The mean biomass density of parasites significantly differed between streams: 0.16441 mg/m^2 (± 0.027195 SEM) in Muskingum Brook and 0.08572 mg/m^2 (± 0.01878 SEM) in Skit Branch. The overall percentages of consumer dry mass made up of parasites were not significantly different between streams, 0.00733% (95% CI=0.00462-0.01112) at Muskingum Brook and 0.00643% (95% CI=0.00312-0.0117) at Skit Branch. All consumer groups fluctuated slightly in biomass density among seasons, but the total percentage of consumer biomass composed of parasites did not show large seasonal variation (Fig. 2).

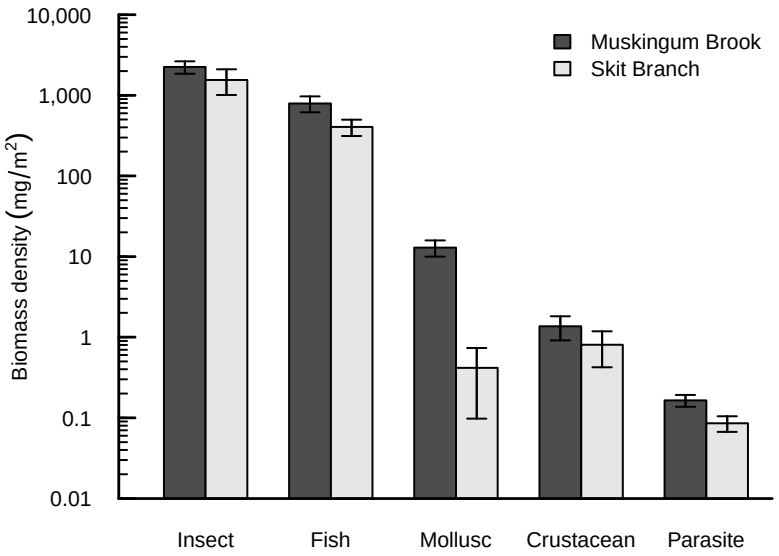

Figure 1. Mean biomass density (mg/m^2) ± SEM of free-living and parasitic consumers in two Pine Barrens streams.

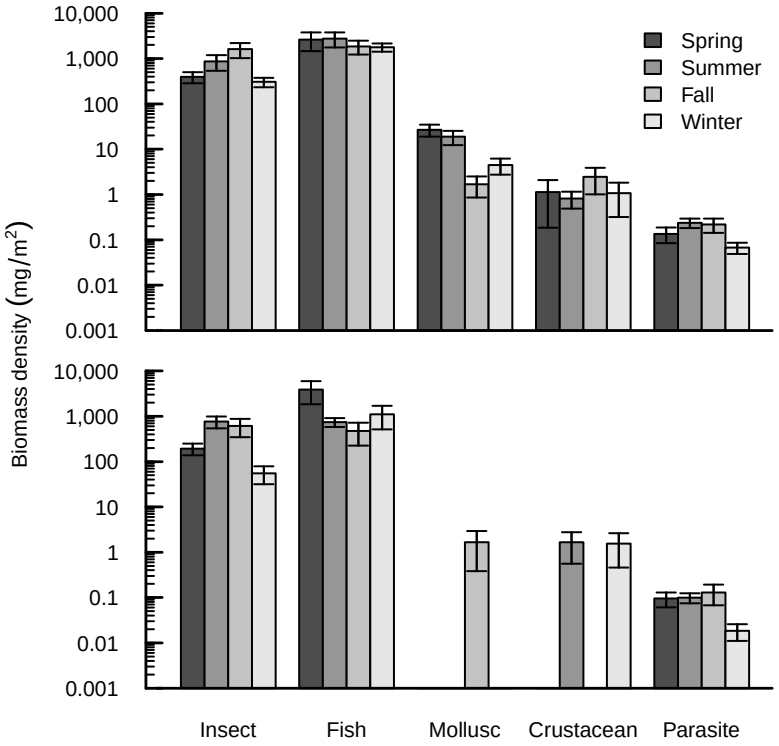

Figure 2. Mean biomass density (mg/m^2) ± SEM of free-living and parasitic consumers in Muskingum Brook (top) and Skit Branch (bottom), split by season.

The distribution of parasite biomass among taxa varied considerably between streams (Fig. 3). All four major groups of macroparasites (trematodes, cestodes, acanthocephalans, and nematodes) contributed substantially to parasite biomass density at Muskingum Brook. Nematodes comprised the majority of parasite biomass at Skit Branch, and cestodes were entirely absent there.

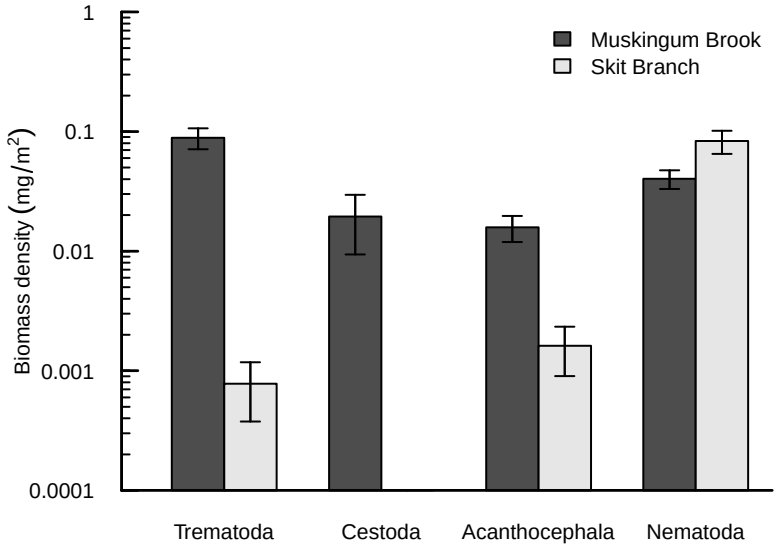

Figure 3. Mean biomass density (mg/m^2) ± SEM for four major parasite taxa in two Pine Barrens streams.

Free-living organisms harboring at least one macroparasite species made up a large portion of biomass in both streams (Fig. 4). Parasitized individuals made up 29.234% (95% CI=14.275-50.791) of free-living consumer biomass at Muskingum Brook and 21.476% (95% CI=8.793-43.366) of free-living biomass at Skit Branch. The mass of parasitized fish exceeded that of unparasitized fish in both Muskingum Brook (82.474% fish biomass parasitized) and Skit Branch (71.08% fish biomass parasitized). All other consumer groups (insects, molluscs, and crustaceans) were lower in unparasitized biomass than in parasitized biomass at Muskingum Brook, and parasitized individuals of some groups were not collected at Skit Branch.

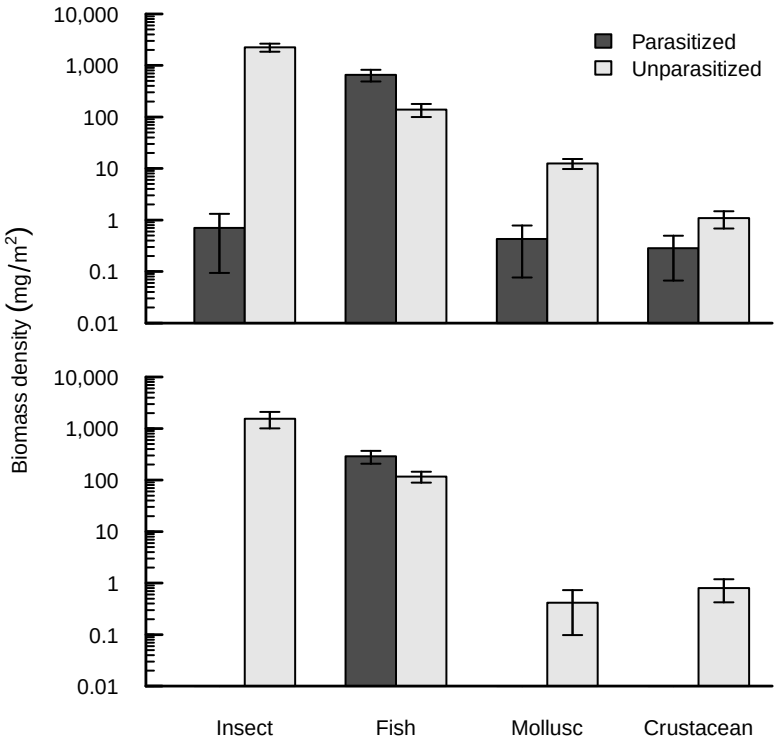

Figure 4. Mean biomass density (mg/m^2) ± SEM of free-living consumers divided by infection status for Muskingum Brook (top) and Skit Branch (bottom).

Parasite biomass density varies substantially within and among study systems, and the values reported here for Pine Barrens streams are lower than those for any other system studied (Fig. 5). Differences in sampling and reporting methods likely contribute to some of the observed differences in biomass density among systems, as two datasets report wet mass (Kuris et al. 2008, Lagrue and Poulin 2015a) and two report dry mass (Preston et al. 2013, present study). Considering the percentage of gross consumer biomass that is actually comprised of parasites (percent parasite biomass) allows a cross-system comparison less influenced by methodology. Both parasite biomass density and percent parasite biomass show that the Pine Barrens streams studied here have the lowest parasite biomass values for any system studied. The Pacific estuaries that were the original focus of this type of research surpass other ecosystem types in both measures of parasite biomass.

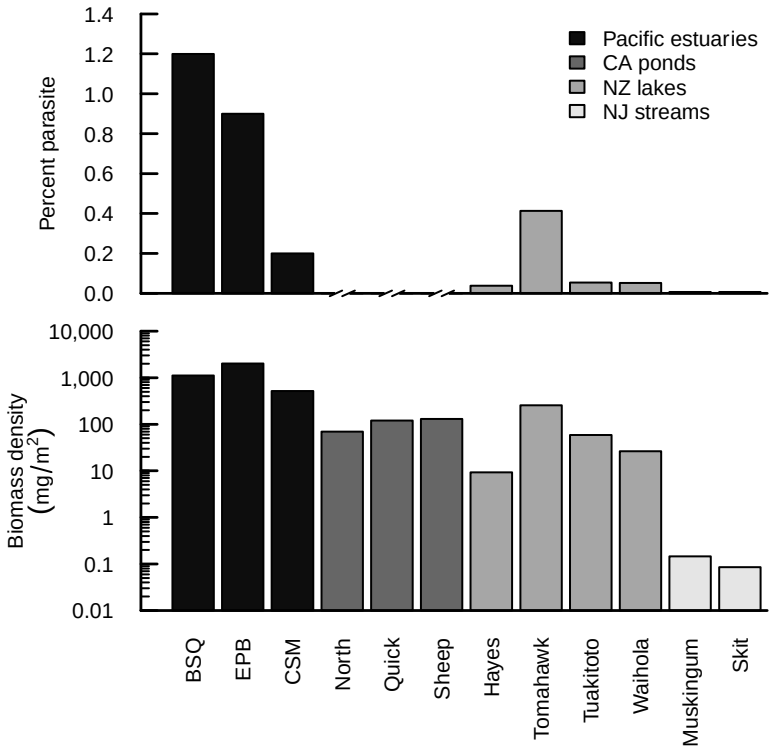

Figure 5. Overall percentage of consumer biomass comprised of parasites (top) and mean biomass density of parasites (bottom) in the 4 aquatic ecosystems for which comparative biomass studies have been conducted.

Significance

Comparative studies that estimate the densities of parasitic and free-living organisms at the ecosystem-scale are useful for trying to understand the ecological function of parasitism (Kuris et al. 2008, Preston et al. 2013), as well as for testing general ecological theories (Hechinger et al. 2011, Lagrue et al. 2015). However, caution is necessary in the generalization of results from past studies, and in making comparisons among ecosystems. Previous estimates of very high parasite biomass density in some aquatic ecosystems are not universal. Variation in parasite biomass density within and among ecosystem types suggests that ecosystem properties may lead to differences in available energy for parasites, but the patterns and mechanisms are not yet known. Even at low densities, there are numerous direct and indirect mechanisms by which parasites may mediate ecosystem processes such as productivity, decomposition, and nutrient cycling

(Hatcher et al. 2012, Preston et al. 2016). Additional empirical data involving a greater diversity of parasite taxa, ecosystem types, and ecological processes must be coupled with the development of a theoretical framework to understand the importance of parasitism at the ecosystem scale.

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(4) **Publications or Presentations**:

Paseka, Rachel (IN PROGRESS). "Ecological Stoichiometry of Parasitism", Ph.D. Dissertation, Ecology and Evolution Graduate Program, Rutgers University, New Brunswick, NJ.

Paseka, Rachel. 2016. "Low parasite biomass in oligotrophic streams differs from previous estimates in diverse communities." Under review at *Freshwater Science*.

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Development of a new, effective and low-cost adsorption material to enhance Low Impact Development (LID) techniques for prevention of urban stormwater pollution in New Jersey

Basic Information

Title:	Development of a new, effective and low-cost adsorption material to enhance Low Impact Development (LID) techniques for prevention of urban stormwater pollution in New Jersey
Project Number:	2014NJ357B
Start Date:	3/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	NJ-008
Research Category:	Not Applicable
Focus Category:	Water Quality, Treatment, Methods
Descriptors:	None
Principal Investigators:	Hanieh Soleimanifar, Yang Deng

Publications

1. Soleimanifar, Hanieh, Y. Deng, D. Sarkar, 2014, Water Treatment Residual (WTR)-Coated Mulches for Mitigation of Toxic Metals and Nutrient in Polluted Urban Stormwater, New England Graduate Student Water Symposium, University of Massachusetts, Amherst, MA (Poster)
2. Soleimanifar, Hanieh; Yang Deng, Laying Wu, Dibyendu Sarkar, 2016, Water treatment residual (WTR)-coated wood mulch for alleviation of toxic metals and phosphorus from polluted urban stormwater runoff, Chemosphere, Vol. 154, 289-292.
3. Soleimanifar, Hanieh; Yang Deng, Laying Wu, Dibyendu Sarkar, 2016, Water treatment residual (WTR)-coated wood mulch for alleviation of toxic metals and phosphorus from polluted urban stormwater runoff, 101ST Annual New Jersey Water Environment Association (NJWEA) Conference, Atlantic City.
4. Soleimanifar, Hanieh; Yang, Deng, 2014, Water Treatment Residuals-Coated Mulches for Mitigation of Urban Stormwater Pollutants - Batch and Column Studies, New England Graduate Student Water Symposium, UMass Amherst. (Oral Presentation)

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(2) **Numbers of Students Supported**:

Undergraduates: 0
Masters' students: 0
Ph. D. students: 1
Postdoctoral associate: 0

(3) **Any Notable Achievements** (Awards, Recognition, etc.), or direct application of the research by Management Agencies, Nonprofits/NGOs, etc.

N/A

(4) **Project Summary**:

Problem and Research Objectives

Urban runoff, as a serious non-point source, is becoming a major pollution issue in urbanized areas such as New Jersey that is the most populous state (Duke et al., 1998). The most frequently found pollutants in urban stormwater include toxic metals (e.g. Cu, Zn and Pb), nutrients (N and P), and hydrocarbon (e.g. gasoline). Bioretention basin is a leading low-impact development (LID) technique that utilizes soil retention to mitigate stormwater pollutants, and allow for stormwater infiltration at a small scale in developed areas (Hsieh and Davis, 2005). A typical bioretention basin consists of sand and overlying vegetation and mulch. Although a bioretention basin can, to some degrees, reduce certain pollutants present in urban runoff, its application is restricted in the following three aspects. First, heavy metals are accumulated at the top soil so that frequent replacement of soil is required. Otherwise, certain metals in soil will finally exceed the EPA regulated levels (Weiss et al., 2008). Second, the reported nutrient P removal efficiencies of bioretention basins are highly unstable, and even the P release into infiltrated stormwater is observed (Dietz and Clausen, 2005; Dietz and Clausen, 2006; Wu et al., 1996). Third, hydrocarbon (e.g. petroleum) may significantly degrade the quality of soil in bioretention basins. Therefore, it is an urgent demand in New Jersey and many other states to develop appropriate technologies that can substantially enhance the performance of bioretention basins for urban stromwater management.

Water treatment residuals (WTR) are coagulation byproducts of traditional water treatment. Alum (Al) or iron (Fe) - WTRs have been reported to be produced over 2 million tons annually in USA (AWWARF, 1997). This waste is usually disposed of within landfills. However, this option is being challenged by limited landfill space and prohibitive disposal costs. Over the past decades, WTR has been demonstrated to remove toxic metals (e.g. Pb), metalloids (e.g. As), phosphorus, and certain organic wastes from water. However, direct application of WTR as filter media is not practically feasible, because WTR blocks are readily formed to prevent water infiltration. This

study will develop new mulch by coating recycled WTR on it. Benefits are pronged: 1) to reduce the pollutants entering soil in bioretention basins; and 2) to extend the lifetime of bioretention basins. Using different mulches for removal of common stormwater pollutants were attempted (Jang et al., 2005; Mahfoz, 2008). However, it is novel to reuse WTR (an industrial waste) as a new mulch coating is novel in urban stormwater management. And the research proposed here has not been supported by any other grants.

Specific objectives and hypotheses

The long term goal of this study is to develop effective, low-cost, and sustainable LID techniques to address stormwater pollution in an urbanized environment. The primary objective of this study is to evaluate the potential of WTR-coated mulch to strength the performance of bioretention basins in removal of typical urban runoff pollutants. The central hypothesis is that Al-WTR and/or Fe-WTR can effectively and irreversibly adsorb heavy metals, phosphorous and hydrocarbons in urban runoff. To test the hypothesis, the following specific objectives are pursued:

Objective I: to properly prepare and characterize different types of WTR-coated mulches;

Objective II: to determine the optimal modified mulch type and operating conditions;

Objective III: to evaluate leaching potential of undesirable chemicals from exhausted WTR-coated mulches under rainfall and landfilling conditions.

Methodology

Research methods, experimental design and expected results. Two coatings (i.e. Fe-WTR and Al-WTR) and two mulches (i.e. original wood mulch and scrap rubber mulch) were used to synthesize four different mulch types, including Fe-WTR-coated wood and tire mulches, and Al-WTR-coated wood and tire mulches. These materials were characterized with different analytical techniques. Thereafter, bench-scale batch tests were conducted with synthetic urban stormwater to obtain key kinetic data. Two types of stormwater pollutants were analyzed, including metals (Pb, Cu, and Zn) and phosphorous. Finally, column tests were carried out to test the performance of these WTR-coated mulches under a continuous flow condition. The optimal modified mulch type was selected in terms of the treatment performance. In addition, Synthetic Precipitation Leaching Procedure (SPLP) and Toxicity Characteristic Leaching Procedure (TCLP) tests examined whether certain chemicals of concern may leach out from the modified mulches under different scenarios.

Materials Fe-based and Al-based WTRs were provided from Passaic valley water commission and Bridgewater Water Treatment Plant respectively. The WTR was completely mixed, dried by air, and then sieved by 2-mm sieve (Deng et al., 2011). Wood mulch was purchased from HomeDepot, and original scrap rubber mulch was supplied from New Jersey RubbeRecycle Inc. The mulches were rinsed with deionized water a few times, and then air dried (Jang et al., 2005). Synthetic urban runoff was prepared with the composition as shown in the following table.

Table 1 Chemical composition of synthetic polluted urban runoff

Parameter	Sources	Concentration(mg/L)

pH	$NaOH / HNO_3$	6.9
Cu	$Cu(NO_3)_2.2.5H_2O$	0.1
Zn	$Zn(NO_3)_2.6H_2O$	0.6
Pb	$Pb(NO_3)2$	0.1
P	Na_2HPO_4	3 (as P)
Motor oil	-	20
Total dissolved solids	$CaCl_2$	120
PIPES	$C_8H_{18}N_2O_6S_2$	10mM

Preparation of WTR-coating mulch

Wood and rubber mulches (1cm x 1cm) were selected, rinsed, and air dried. Mulch glue, an environmentally friendly binder, was used to completely attach the Fe-WTR and Al-WTR powders on the mulch surfaces. Raw wood and rubber mulches were used in the control group. To properly coat wood and rubber mulches, 1:3 and 1:6 WTRs to mulch weight ratios were required, respectively. Less than 1% of coated WTRs came off in the process of rinsing. Considering the weight of glue, the final weight percentage of WTRs was 15-20% of the coated wood mulch and 8-10% of the coated rubber mulch.

Experimental design

Preparation of WTRs-coated mulch:

Characterization of WTR-coated mulch. Different analytical techniques were used to characterize the Fe-WTR-coated wood mulch, including: 1) electron microscopy (SEM) – (Energy Dispersive Spectroscopy (EDS) (Hitachi S-3400N) – morphology and elemental composition of WTR coating. 2) CHNS analysis will be employed to measure total C, N and S contents in the WTR coatings.

Batch tests. Batch tests were carried out in flasks with 1L synthetic runoff on a rotary shaker. Reaction temperature was controlled at 25^{o}C. For each type of WTR-coated mulch, kinetics tests was first conducted to determine key kinetics data in removal of metals (Pb, Cu, and Zn) and phosphorous.

Column tests. Mini columns (3'' diameter and 15cm height) were loaded with 5 cm height of four different coated and raw mulches. The synthetic urban runoff was continuously pumped into the columns and filtrate was periodically collected for analysis. Tests proceeded until 50 bed volumes of solution passed through the column. Pumping for 9 hours with a 21 ml/min flowrate satisfied the 50 bed volume goal.

SPLP and TCLP tests. The spent WTR-coated mulches in column tests were collected for Synthetic Precipitation Leaching Procedure (SPLP) (EPA SW-846 Method 1312) and Toxicity Characteristic Leaching Procedure (TCLP) (EPA SW-846 Method 1311) tests, respectively. SPLP is designed to simulate material left in-situ exposed to rainfall and evaluate the leaching potential of the modified mulches caused by rainfall. TCLP is designed to evaluate mobility of hazardous wastes in simulated landfilling conditions.

Analytical method Heavy metals (i.e., Cu, Zn, and Pb) were quantified using an inductively coupled plasma-mass spectroscopy (ICP-MS) (Thermo, X Series II, XO 472). Phosphorous was measured with HACH tests kits using a UV/Vis spectrophotometer (HACH DR5000).

Principal Findings and Significance

Objective I: to properly prepare and characterize different types of WTR-coated mulches (to be continued)

1) *Characterization of WTRs:* Table 2 summarizes the data from acid digestion and CHNS analysis. Total iron percentage was high in Fe-WTRs compared to total aluminum while this was vice versa for Al-WTRs. Carbon percentage was higher for Fe-WTRs. Nitrogen percentage was similarly low for both types.

Table 2 Percentage of total iron, aluminum, carbon and nitrogen in Al-WTRs and Fe-WTRs

Sorbent	Fe%	Al%	C%	N%
Al-WTRs	0.8	5.6	5.74	1.35
Fe-WTRs	11.3	0. 5	11.49	1.55

2) *Characterization of WTR-coated mulch:* Figures 1-6 show SEM images for raw and coated mulches. Raw uncoated wood mulch has a fibrous surface texture while raw rubber mulch SEM images showed a polymeric texture. All coated mulches showed a rough surface texture with particle size of millimeter order while raw mulches had a particle size in the order of µm. SEM and EDS analysis (figure 7-12) showed that WTRs coating was primarily amorphous materials consisting of Fe, Al, Si, O and K. EDS images of coated mulches show Aluminum and iron peaks related to Al-WTRs and Fe-WTRs respectively.

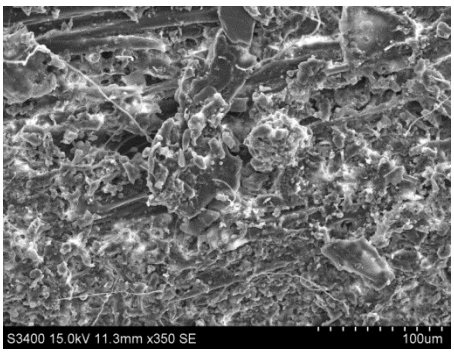


Figure 1 SEM image- Wood mulch

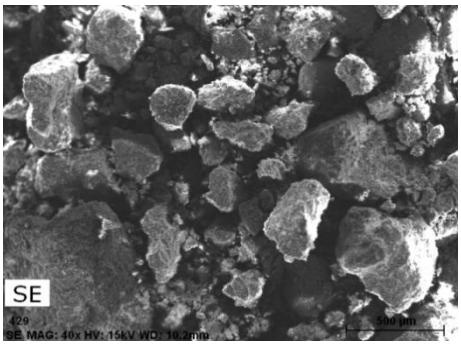


Figure 2 SEM image- Al-WTRs coated wood mulch

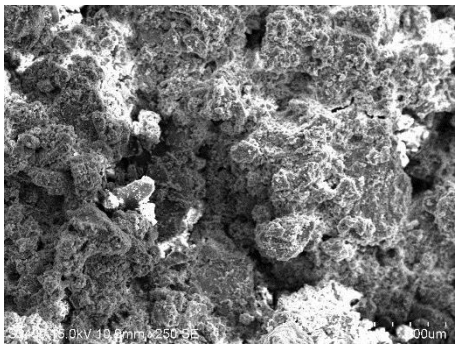


Figure 3 SEM image- Fe-WTRs coated wood mulch

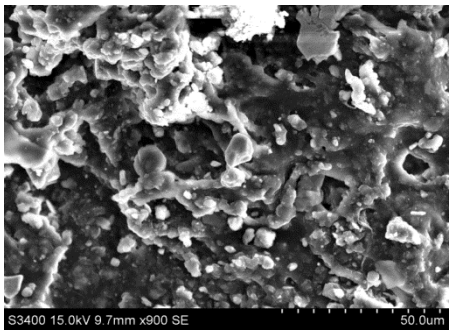


Figure 4 SEM image- Rubber mulch

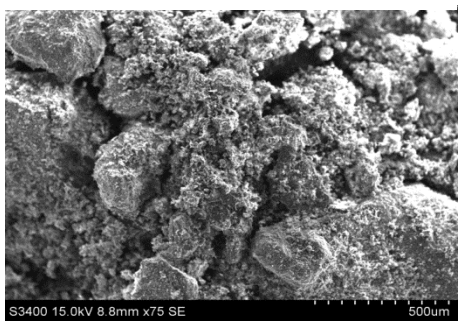


Figure 5 SEM image- Al-WTRs coated rubber mulch

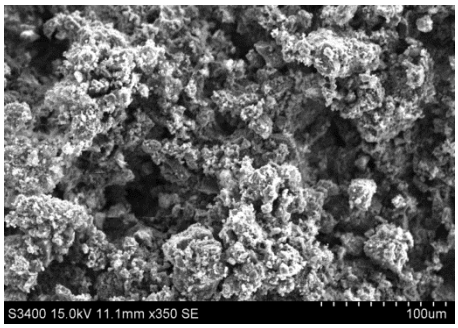


Figure 6 SEM image- Fe-WTRs coated rubber mulch

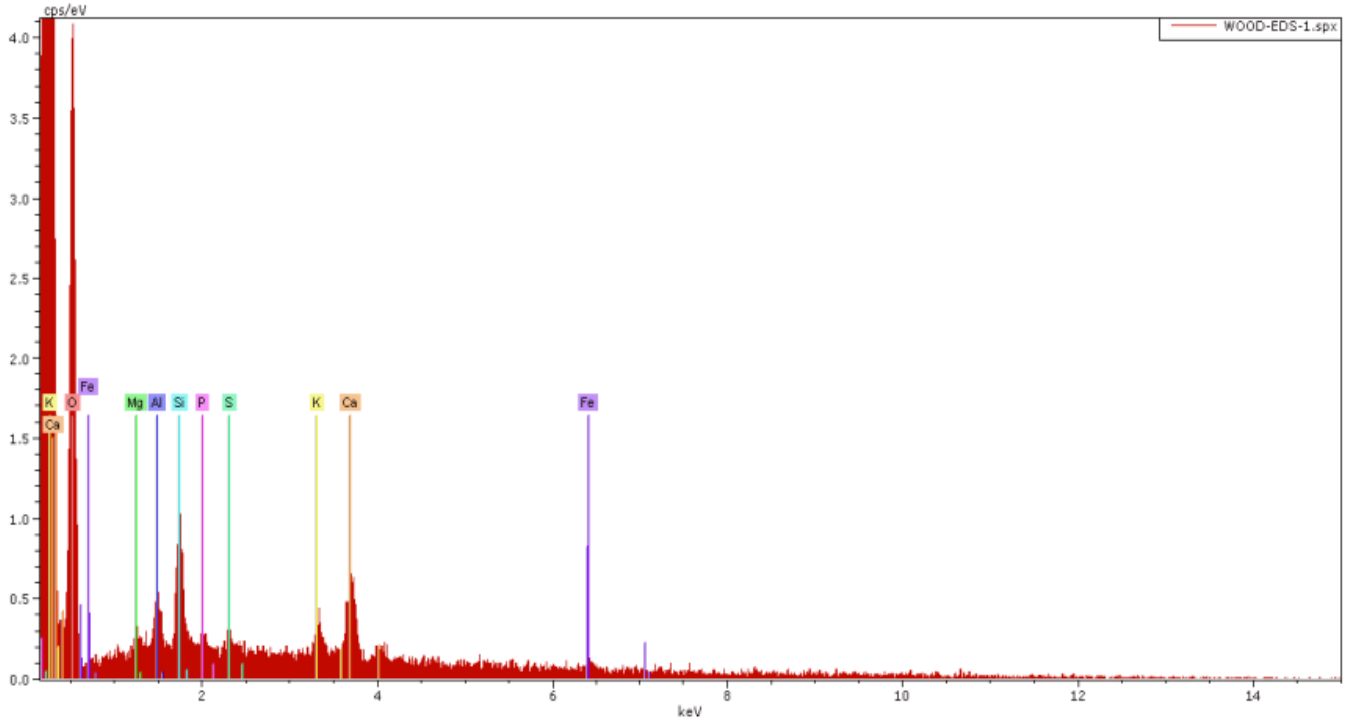


Figure 7 EDS image- Wood mulch

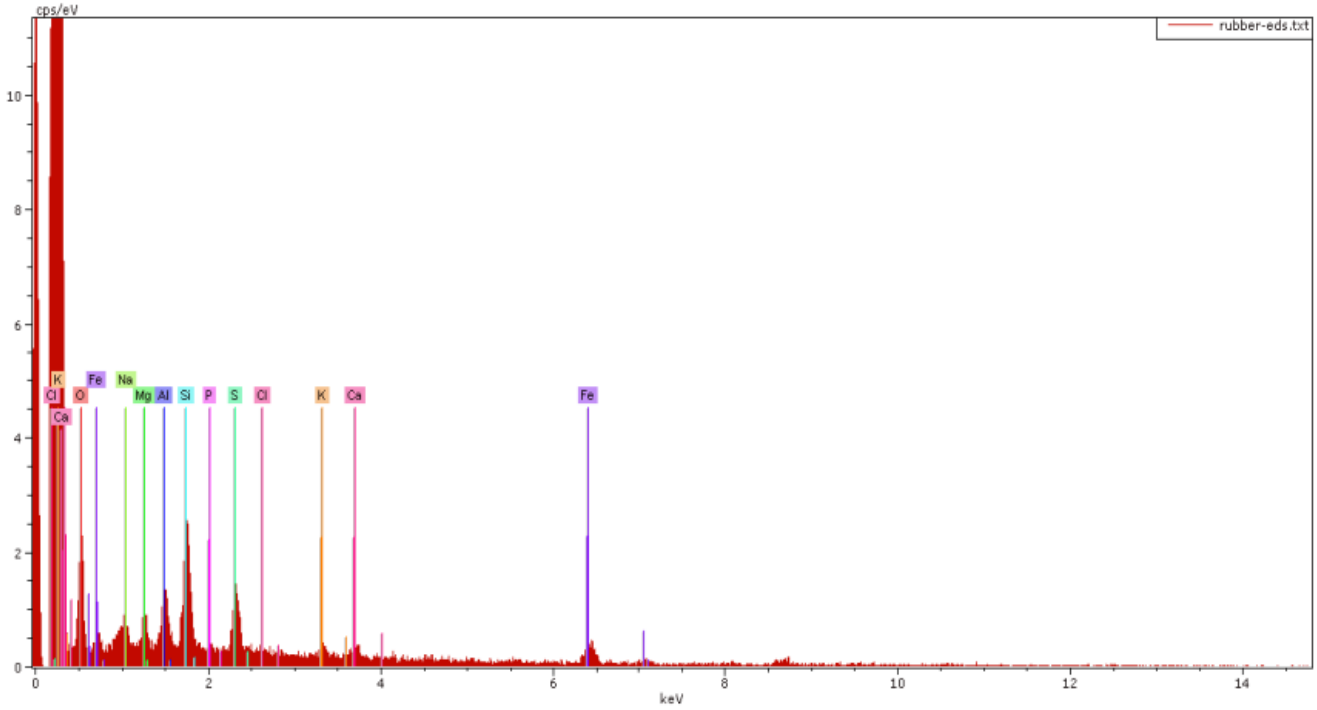


Figure 8 EDS image- Rubber mulch

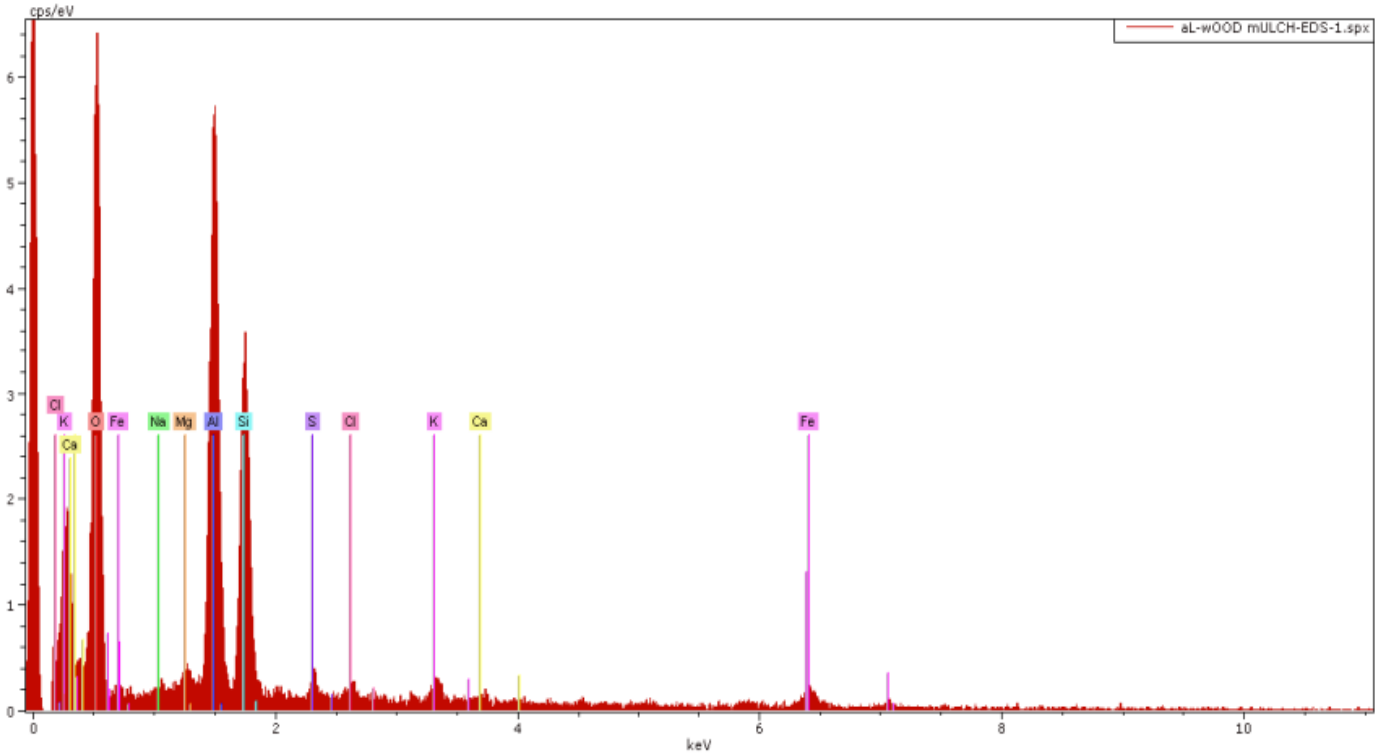


Figure 9 EDS images- Al-WTRs Coated Wood Mulch

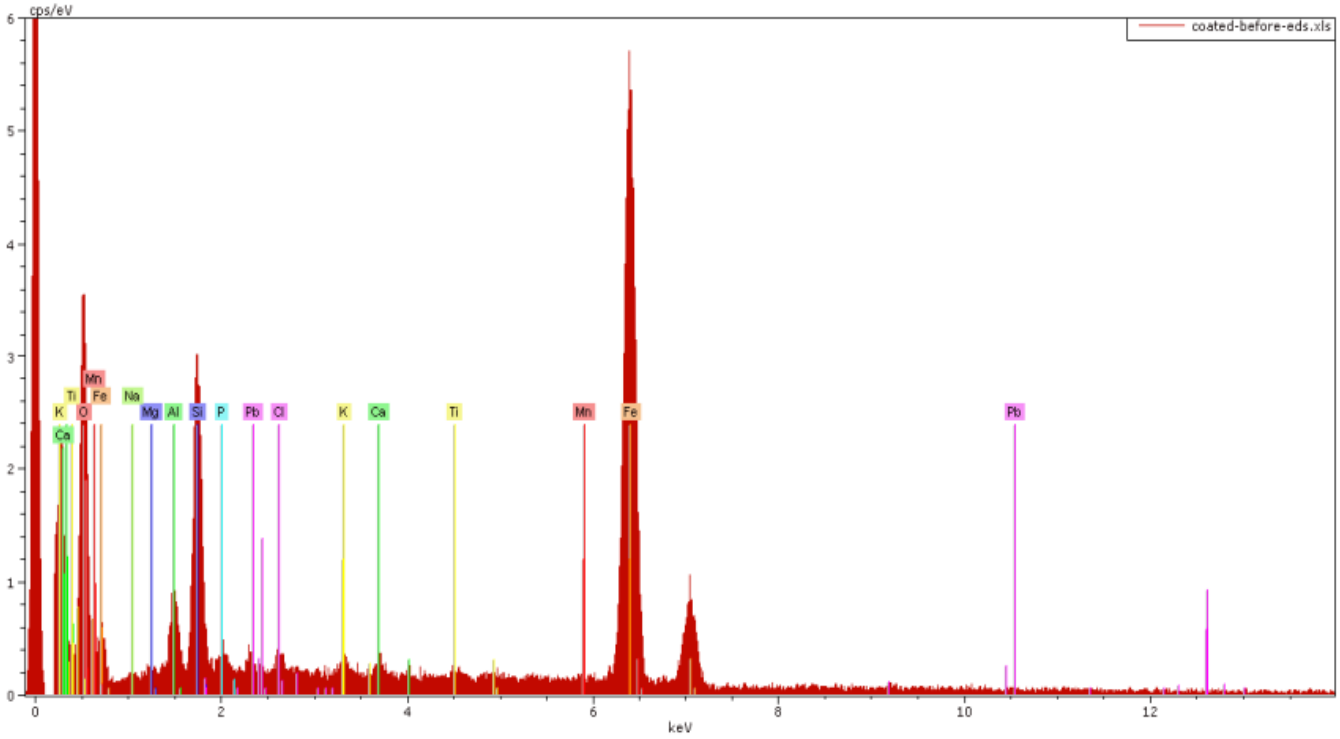


Figure 10 EDS image- Fe-WTRs Coated Wood Mulch

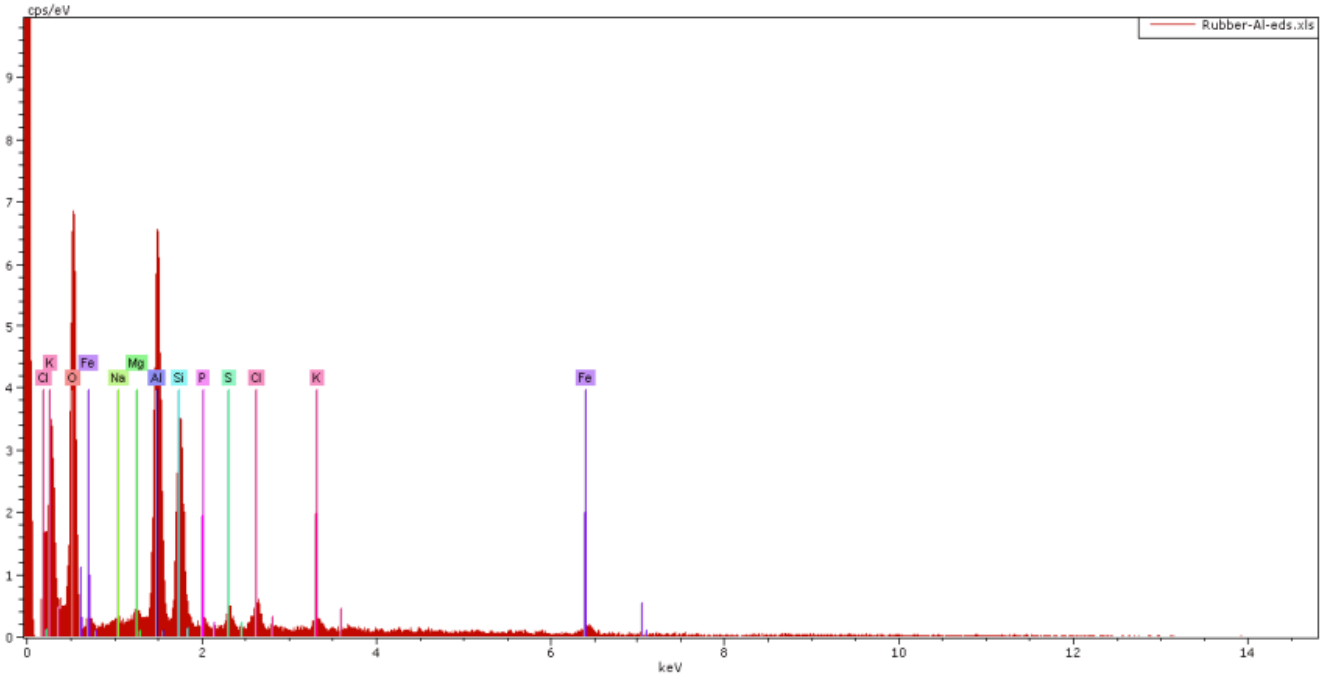


Figure 11 EDS image- Al-WTRs coated rubber mulch

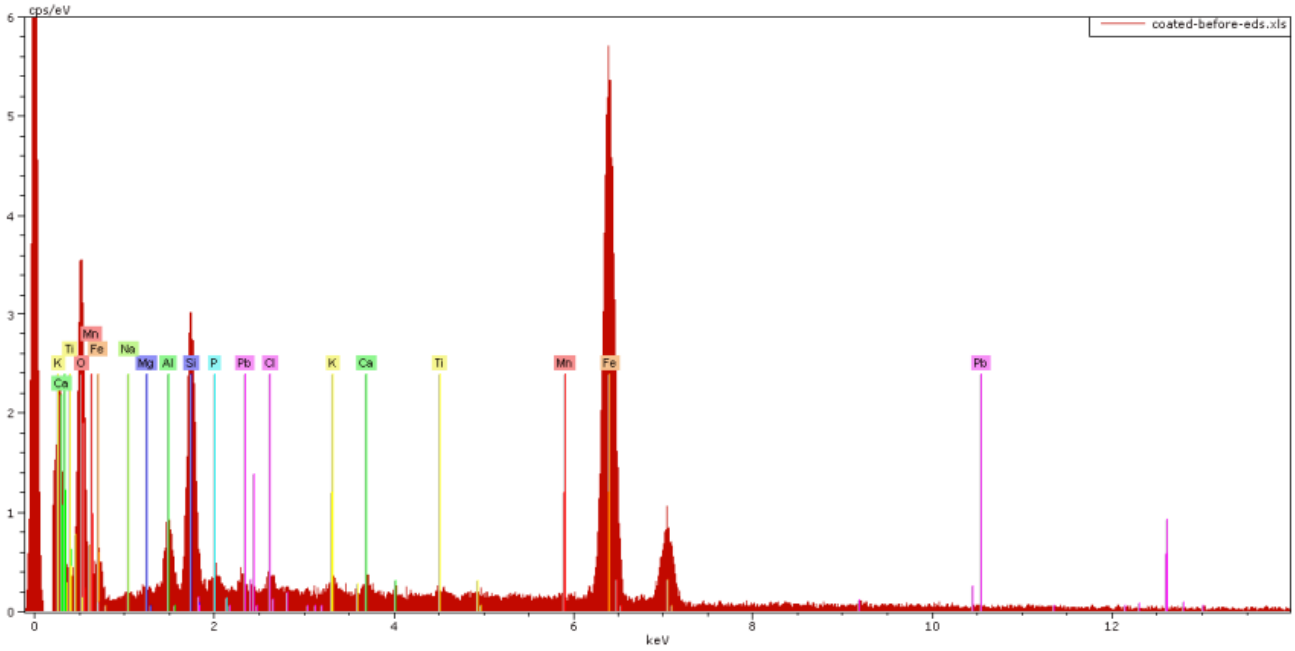


Figure 12 EDS image- Fe-WTRs coated rubber mulch

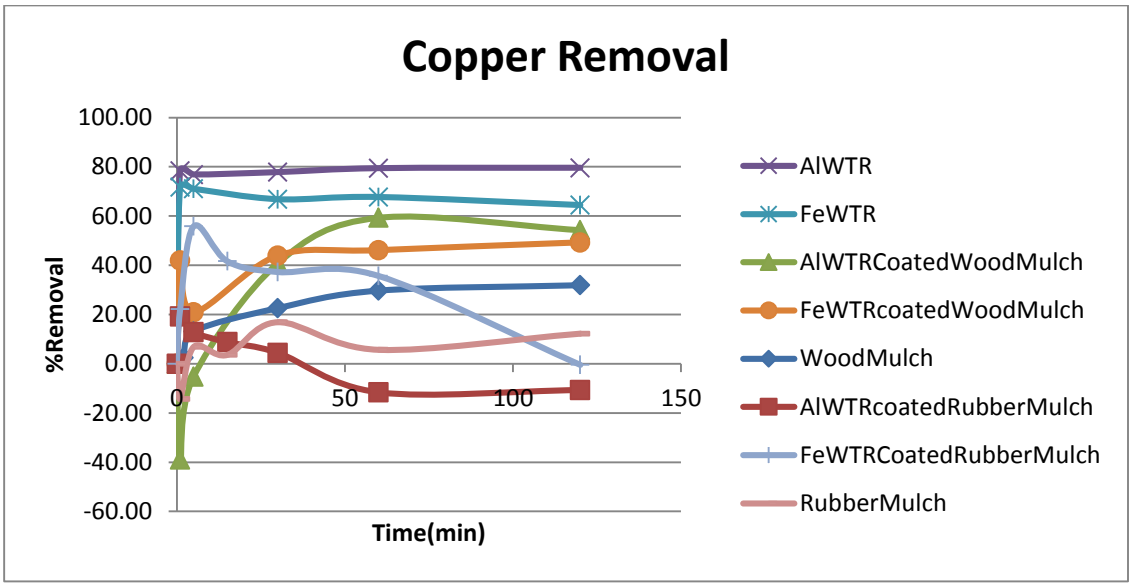
Objective II: to determine the optimal modified mulch type and operating conditions (100% completed)

Kinetic Tests

10 g/L Fe or Al-WTR were used in each test. To properly coat wood and rubber mulches, 1:3 and 1:6 WTR to mulch weight ratios were required, respectively.

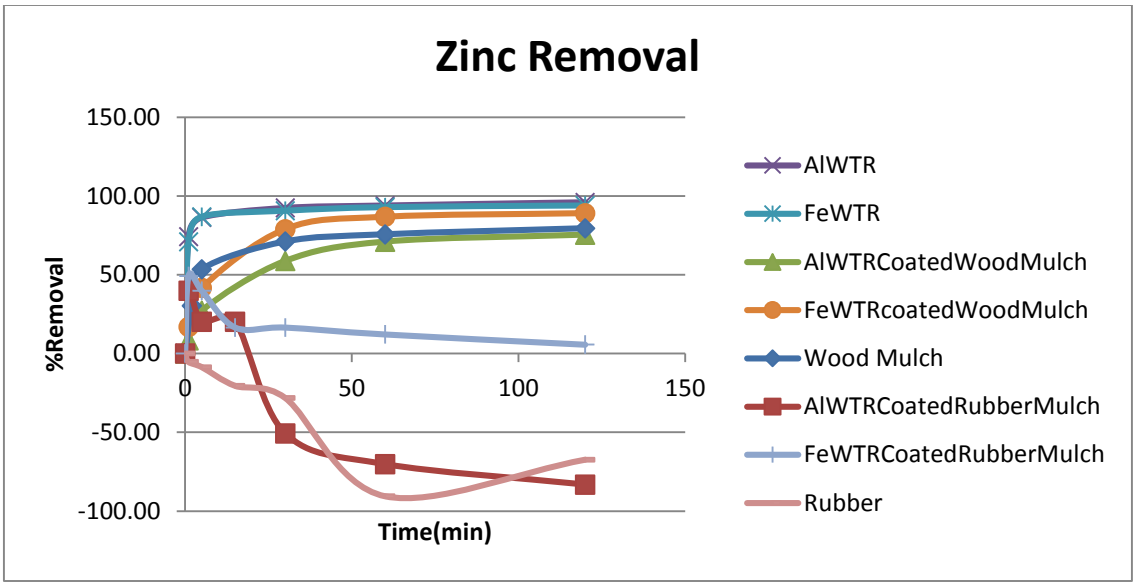
Copper:

Eight different sorbents were tested. WTR only was better than WTR-coated mulch in terms of Cu removal because part of the active sites on WTRs were occupied by the mulch. Among 4 different coated mulches, Al-WTR coated wood mulch showed the highest and the most stable sorption capacity. Fe-WTR Rubber mulch removed copper immediately, but slightly released Cu with time. Of interest, Al-WTR coated tire rubber mulch released Cu.



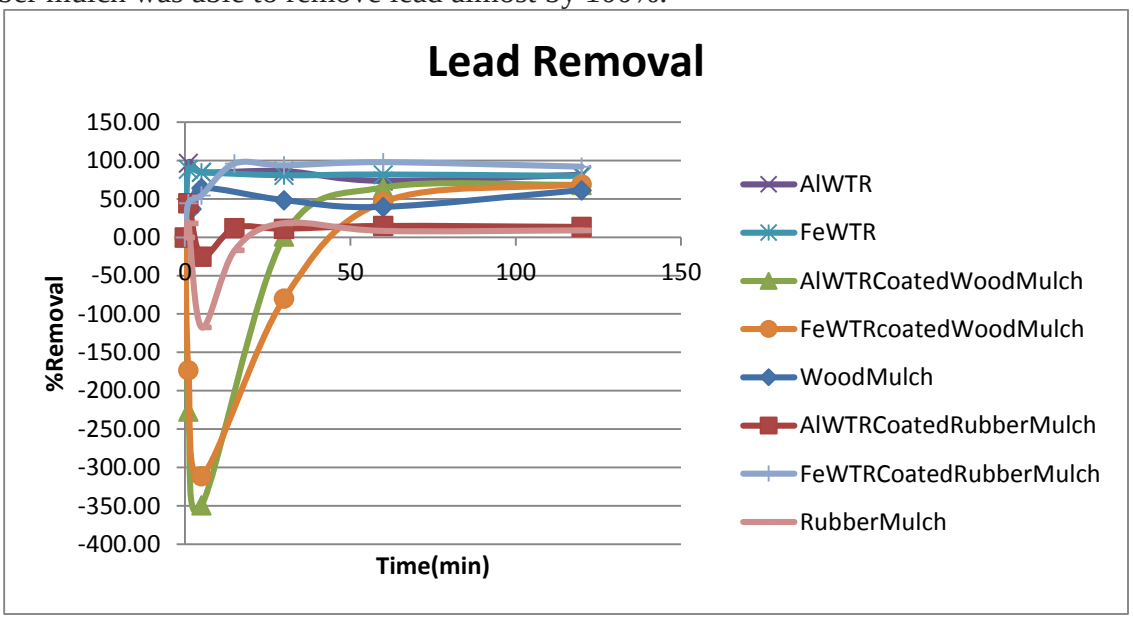
Zinc:

Similar to copper, zinc was also released from rubber. The removal process by Al or Fe-WTR coated rubber mulch was instant but the release of zinc occurred later. Fe-WTR coated wood mulch exhibited a better capacity for zinc.



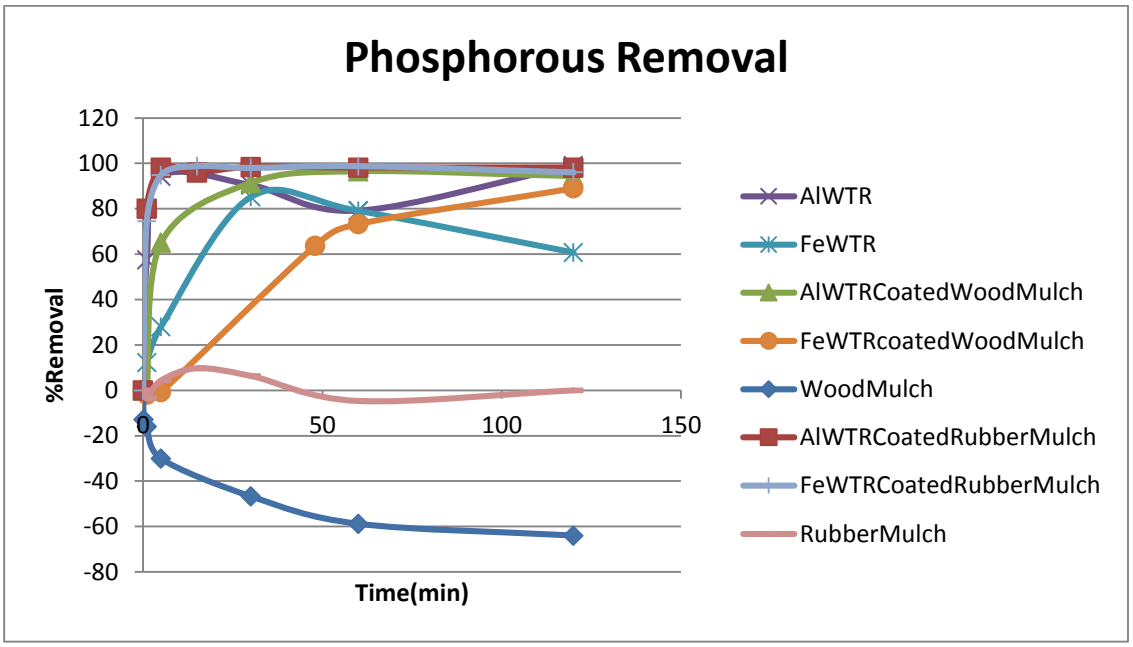
Lead:

Wood alone, Fe WTR coated wood mulch and Al WTR coated mulch appeared to release lead at the beginning but then the coated wood mulch was able to remove lead up to 70%. Fe-WTR coated Rubber mulch was able to remove lead almost by 100%.



Phosphorous:

Wood and tire mulches only could not remove P. Even the former contributed P to the synthetic runoff. Al and Fe WTR coated rubber mulches both rapidly removed almost 98% of the phosphorous. Al-WTR coated wood mulch adsorption of P was relatively slow. Within 5 and 30 min., 65% and 90% of the phosphorous were reduced, respectively. Although the coated rubber mulches were effective for removal of Pb and P, they might release unwanted copper and zinc. Therefore, the following column tests were conducted to compare Al-WTR coated wood mulch and Fe-WTR coated wood mulch.



Kinetics Parameters:

Based on the data obtained, we determined key kinetics parameters including reaction orders and rate constants for four different sorbents.

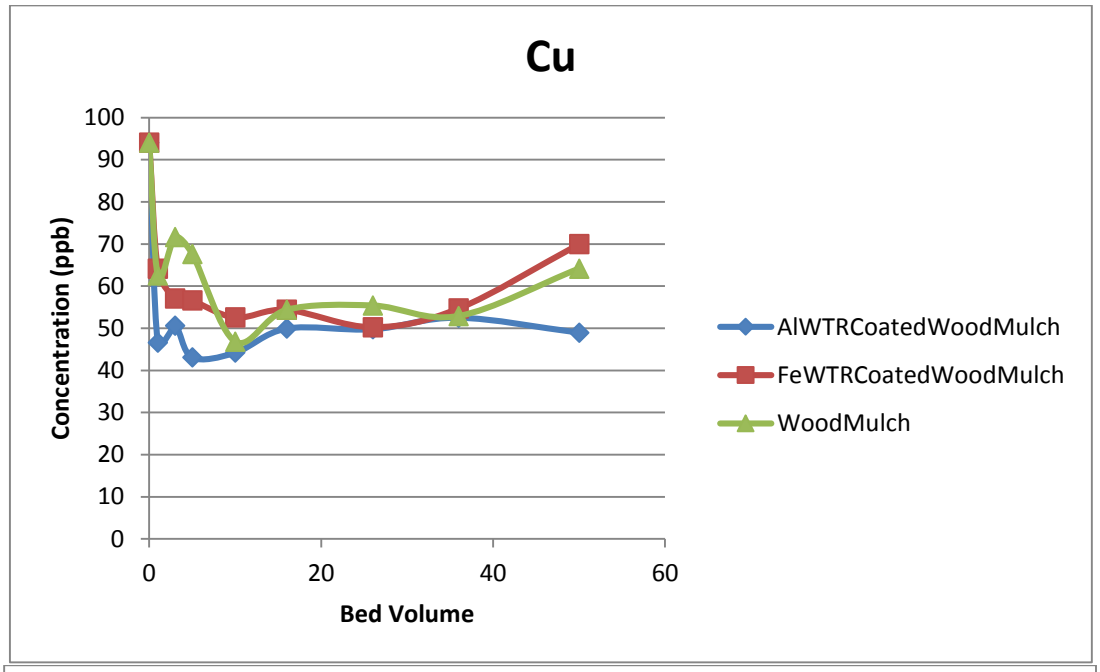
Sorbent-Ion	0- order		1st order		2nd order	
	k	R^2	k	R^2	k	R^2
AlWTRCoatedWoodMulch-Cu	0.26	0.62	0.01	0.68	0.0003	0.70
AlWTRCoatedWoodMulch-Zn	4.52	0.72	0.01	0.82	0.00003	0.91
AlWTRCoatedWoodMulch-Pb	0.19	0.41	0.02	0.65	0.003	0.84
AlWTRCoatedWoodMulch-P	0.02	0.49	0.02	0.62	0.08	0.54
FeWTRCoatedWoodMulch-Cu	0.13	0.39	0.004	0.45	0.0001	0.50
FeWTRCoatedWoodMulch-Zn	5.35	0.63	0.02	0.78	0.00008	0.91
FeWTRCoatedWoodMulch-Pb	0.08	0.45	0.02	0.71	0.006	0.89
FeWTRCoatedWoodMulch-P	0.02	0.89	0.02	0.99	0.03	0.96
AlWTRCoatedRubberMulch-Cu	-0.002	0.59	-0.00004	0.56	-0.12	0.62
AlWTRCoatedRubberMulch-Zn	-0.008	0.66	-0.000007	0.56	-10.47	0.74
AlWTRCoatedRubberMulch-Pb	0.0015	0.01	0.0002	0.0008	-0.0001	0.0031
AlWTRCoatedRubberMulch-P	0.01	0.16	0.02	0.28	0.10	0.31
FeWTRCoatedRubberMulch-Cu	-0.11	0.15	-0.003	0.15	-0.00006	0.14
FeWTRCoatedRubberMulch-Zn	-1.72	0.22	-0.003	0.22	-0.000004	0.22
FeWTRCoatedRubberMulch-Pb	0.03	0.34	0.02	0.30	0.02	0.15
FeWTRCoatedRubberMulch-P	0.009	0.17	0.02	0.17	0.07	0.05

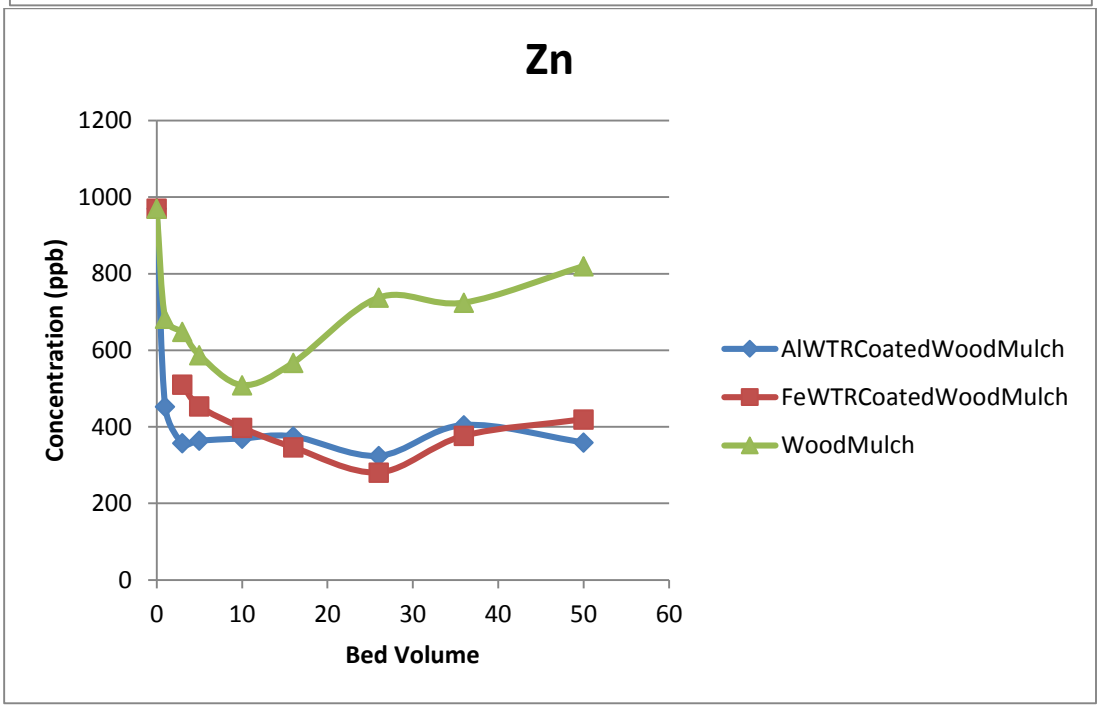
The removal of heavy metals by the coated wood mulch was better described by second-order model while the removal of phosphorous by the coated wood mulch showed mostly to be a first

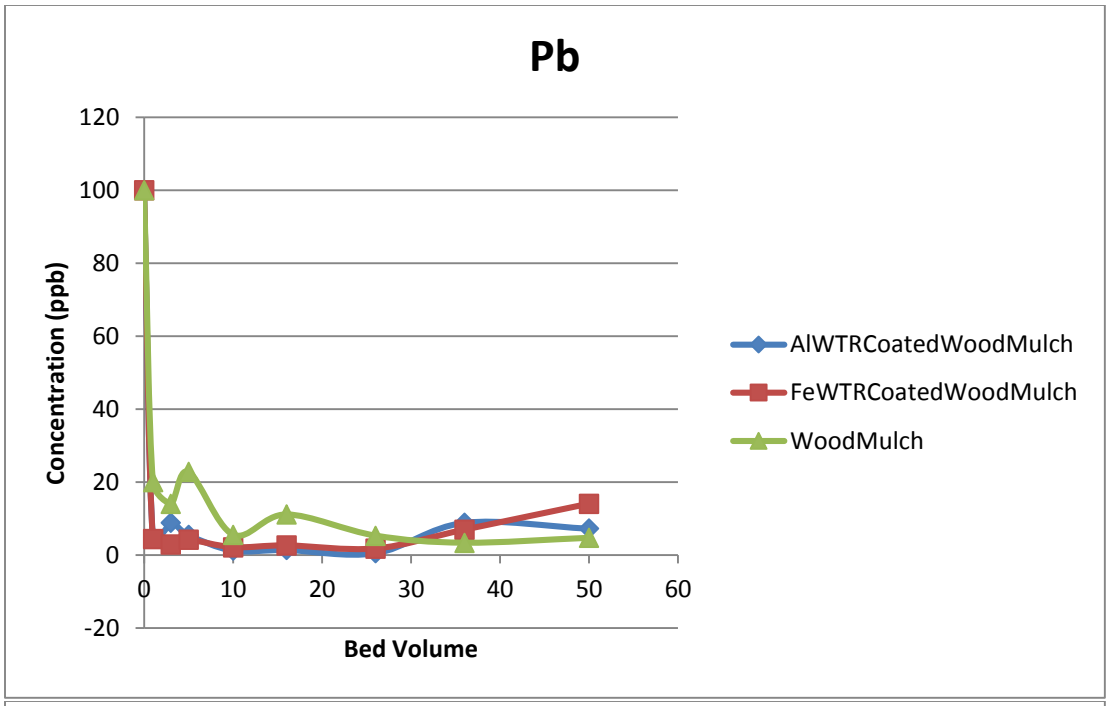
order reaction. Examining three different kinetic order models in most cases did not give us a good model for the removal of heavy metals and phosphorous by the coated rubber mulch.

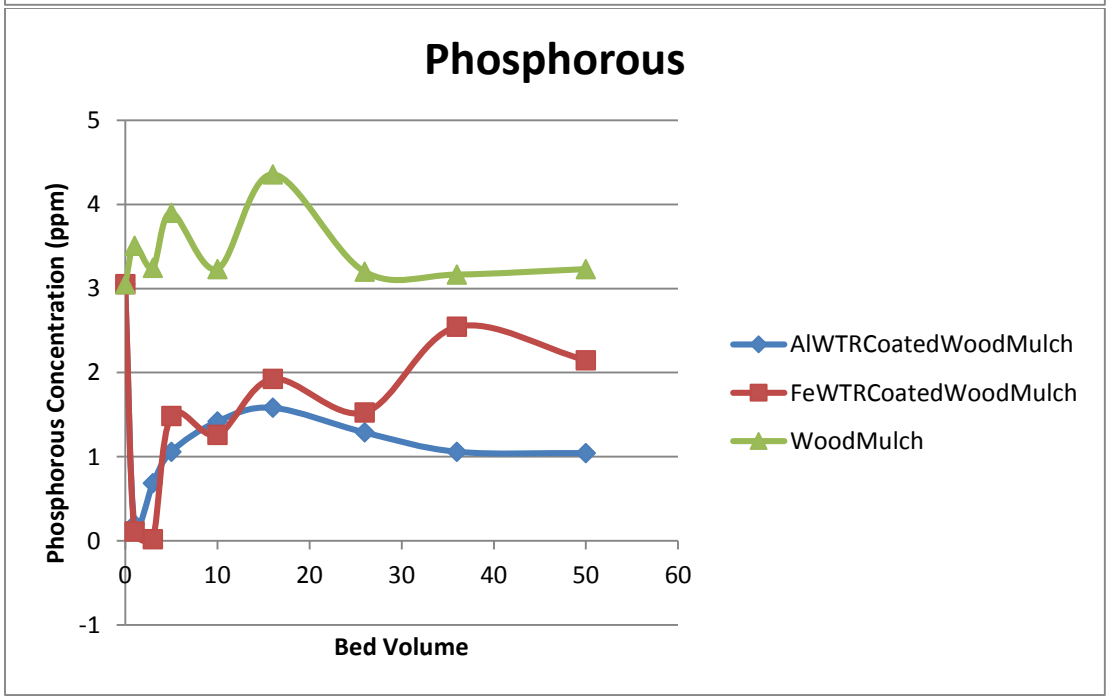
Column Tests:

Column tests including three sorbents (wood mulch alone, Al-WTR coated mulch and Fe-WTR coated Mulch) were performed. Results showed that Al-WTR coated wood mulch was the best candidate.









Objective III: to evaluate leaching potential of undesirable chemicals from exhausted WTR-coated mulches under rainfall and landfilling conditions (100% completed).

We examined Al and Fe WTR coated wood mulches before and after tests under landfill and rainfall situation and compared the amounts of released chemicals of concern with the standards. Data are shown below. Results suggest that the release of undesired chemicals under landfilling and rainfall conditions is not a concern.

	Concentration (ppb)							
	Cr	As	Se	Ag	Cd	Ba	Hg	Pb
TCLP-AlWTRcoatedWoodMulch-BeforeTest	47.29	2.977	-2.057	0.03	2.408	3603	4.287	7.513
TCLP-FeWTRcoatedWoodMulch-BeforeTest	30.15	2.537	0.552	0.055	2.052	2292	2.705	-0.024
TCLP-AlWTRcoatedWoodMulch-AfterTest	38.59	2.257	-1.86	0.065	3.025	8565	3.138	4.735
TCLP-FeWTRcoatedWoodMulch-AfterTest	5.356	0.948	-0.082	0.35	0.722	1223	2.404	1.545
SPLP-AlWTRcoatedWoodMulch-BeforeTest	29.33	1.972	-1.236	0.022	3.295	10230	2.015	2.043
SPLP-FeWTRcoatedWoodMulch-BeforeTest	14.16	1.97	1.402	0.118	2.711	2502	1.818	0.504
SPLP-AlWTRcoatedWoodMulch-AfterTest	3.918	0.867	-0.174	0.263	0.803	1766	1.861	0.593
SPLP-FeWTRcoatedWoodMulch-AfterTest	18.83	2.28	-0.307	0.05	3.184	4651	1.541	1.039
TCLP Limit	5000	5000	1000	5000	1000	100000	200	5000

(4) **Publications or Presentations**:
List any publications, presentations, published abstracts reporting the WRRI-supported work. Oral or poster presentations at conferences should be specified as such.

1. Articles in Refereed Scientific Journals
   Soleimanifar, Hanieh; Yang Deng, Laying Wu, Dibyendu Sarkar, 2016, Water treatment residual (WTR)-coated wood mulch for alleviation of toxic metals and phosphorus from polluted urban stormwater runoff, Chemosphere, Vol. 154, 289-292.
2. Poster Presentation
   Soleimanifar, Hanieh; Yang Deng, Laying Wu, Dibyendu Sarkar, 2016, Water treatment residual (WTR)-coated wood mulch for alleviation of toxic metals and phosphorus from polluted urban stormwater runoff, 101ST Annual New Jersey Water Environment Association (NJWEA) Conference, Atlantic City.

3. Oral Presentation
   Soleimanifar, Hanieh; Yang, Deng, 2014, Water Treatment Residuals-Coated Mulches for Mitigation of Urban Stormwater Pollutants - Batch and Column Studies, New England Graduate Student Water Symposium, UMass Amherst.

4. Poster Presentation

Soleimanifar, Hanieh; Yang, Deng; Dibyendu, Sarkar , 2014, Water Treatment Residual (WTR)-Coated Mulches for Mitigation of Toxic Metals and Nutrient in Polluted Urban Stormwater, New England Graduate Student Water Symposium, UMass Amherst.

Assessing the partitioning and treatment of microbial agents during wet weather flow

Basic Information

Title:	Assessing the partitioning and treatment of microbial agents during wet weather flow
Project Number:	2015NJ361B
Start Date:	3/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	NJ-006
Research Category:	Water Quality
Focus Category:	Treatment, Water Quality, Models
Descriptors:	None
Principal Investigators:	Nicole Fahrenfeld, Nicole Fahrenfeld

Publications

1. Eramo, A., Delos Reyes, H.D., and Fahrenfeld, N. (accepted, Platform). Disinfection of microbial agents in combined sewer overflows using the green disinfectant peracetic acid. Water Environment Federation Technical Exposition and Conference (WEFTEC). New Orleans, LA. September 2016.
2. Eramo, A., Delos Reyes, H.D., and Fahrenfeld, N. (alternate Platform). Assessing the partitioning and treatment of microbial agents from combined sewer effluent during wet weather. 101st Annual New Jersey Water Environment Association Conference and Exhibition. Atlantic City, NJ. May 19, 2016
3. Fahrenfeld, N., Eramo, A., Delos Reyes, H., Yam, M. (Invited panelist). Controlling pathogens in wet weather flows. Parasites and Pathogens: Ecological and Medical Impacts of Global Climate Change. Hofstra University. Hempstead, NY. October 16, 2015.
4. Delos Reyes, H.D., Eramo, A., and Fahrenfeld, N. (Poster). Antibiotic resistance genes in wet weather flow: Impact of combined sewer overflows on New Jersey water quality. 101st Annual New Jersey Water Environment Association Conference and Exhibition. Atlantic City, NJ. May 18, 2016.
5. Eramo, A., Delos Reyes, H.D., and Fahrenfeld, N. (Poster). Flux and treatment of antibiotic resistance genes in CSO. 101st Annual New Jersey Water Environment Association Conference and Exhibition. Atlantic City, NJ. May 18, 2016.
6. Oduro, R., Eramo, A., Fahrenfeld, N. (Poster). Microbial pollution in wet weather flow. Rutgers Aresty Research Symposium. April 27, 2016.
7. Delos Reyes, H., Eramo, A., Fahrenfeld, N. (Poster). Antibiotic resistance genes in wet weather flow: Impact of combined sewer overflows on New Jersey water quality. Rutgers Douglass Summer Research. October 2, 2015.
8. Yam, M., Eramo, A., Fahrenfeld, N. (Poster). Antibiotic resistant genes (ARG) in wastewater and urban surface water. Rutgers Aresty Summer Research Symposium. August 7, 2015.

PLEASE FOLLOW THIS FORMAT:

(1) **PI information**: Nicole Fahrenfeld, nfahrenf@rutgers.edu, 848-445-8416

(2) **Numbers of Students Supported**:
Undergraduates: 3
Masters' students:
Ph. D. students: 1
Postdoctoral Associates:

(3) **Any Notable Achievements**
Alessia Eramo was awarded Kenneth S. Stoller Award by NJ WEA 2016
Hannah Delos Reyes awarded scholarship by NJ WEA 2016
Reba Oduro awarded "Best STEM Poster" at Aresty Research Symposium, Rutgers University
Hannah Delos Reyes awarded poster award at NJ WEA 101st Annual Meeting. May 18, 2016.

(4) **Project Summary**:

Problem and Research Objectives – Combined sewer overflows (CSOs) degrade water quality in New Jersey through the addition of pathogens and antibiotic resistant bacteria in CSO effluent. Incidental ingestion of CSO-plagued Passaic River water presents a high risk of gastrointestinal disease (Donovan *et al.*, 2008). Likewise, high levels of antibiotic resistant bacteria in the Hudson River were associated with CSOs (Young *et al.*, 2013). Thus, solutions are needed to inactivate a broad suite of pathogens and reduce the risk of the spread of antibiotic resistance during wet weather flow events. Upgrading sewer systems in New Jersey is an $8 billion problem. Due to the high cost of upgrading sewer systems, end-of-pipe treatment options are desirable.

Major challenges for achieving end-of-pipe treatment are the (1) variable nature of the CSO effluent and (2) uncertainties associated with wet weather treatment technologies. The aims of this work are to increase our knowledge of the flux of microbial agents during wet weather flow and to evaluate the ability of peracetic acid (PAA) disinfection to reduce the risk of antibiotic resistance in CSO effluent. Application of next generation sequencing technology and methods proven for defining the partitioning of microbial agents on "settleable" particles versus suspended is proposed to provide key data on the flux of microbial agents for improving the design of treatment and modeling of CSOs. (2) We will test the effectiveness of the 'green disinfectant' PAA on reducing the load of viable antibiotic resistant bacteria and antibiotic resistance genes (ARG) in CSO effluent. A viability-based qPCR method will be applied to differentiate between ARG in viable cells and those present in nonviable cells or as extracellular DNA. Understanding this partitioning is critical for predicting the fate of antibiotic resistance gene carrying bacteria in the environment and will improve our knowledge of the effectiveness of peracetic acid as a CSO disinfectant. Thus, this research will result in critically needed data for a pressing water quality issue in New Jersey.

Methodology –

Sampling: Aqueous samples were collected for three storm events at the end of one CSO pipe (Fig. 1). Samples were collected every fifteen to thirty minutes, based on the forecasted duration of the storm, and stored on ice until processing in the laboratory. Conductivity and pH, were measured in the field with a multimeter. CSO effluent fractions were composited following collection to provide two to five representative samples from across the storm hydrograph. Aliquots of the composited samples were analyzed for total suspended solids (TSS), using standard methods. Field blanks consisting of autoclaved deionized water were prepared and analyzed for QA/QC.

To separate particle-associated ("settleable") and suspended microbial agents, methods used by Krometis et al. (2007) were adopted. Aliquots of the total and suspended CSO effluent fractions were filter concentrated and stored at -20°C until molecular analysis. To determine the loading of ARG across the unit hydrograph, qPCR was performed for select ARG (*sul*1 (Pei *et al.*, 2006), *tet*(G) (Aminov *et al.*, 2002), bla-TEM) and total bacterial population (16S rRNA), using SybrGreen chemistry.

Disinfection: Simulated CSO effluent (wastewater influent + DI water) was used to evaluate the effectiveness of peracetic acid disinfection on reducing the viable and nonviable ARG loads in effluent. Samples were aliquoted in triplicate and dosed with peracetic acid (0, 5, and 20 mg/L) during agitation. Subsamples will be removed at 10, 20, and 60 minutes and the disinfection reaction in these samples will be quenched with sodium thiosulfate (100-mg/L) and catalase (50-mg/L). Next, half of the samples were treated with propidium monoazide (PMA) prior to DNA extraction. The other half did not receive PMA treatment and were preserved (-20°C) until DNA extraction. PMA treatment allows for the differentiation of DNA from viable and nonviable sources. For PMA treatment, methods described by Nocker et al. were adapted (Nocker *et al.*, 2007, Nocker *et al.*, 2010, Fittipaldi *et al.*, 2012).

Statistical analyses: A Shapiro test was performed on log-normalized data to determine if data were normal. A paired Student's t-test was applied to test for differences in attached and suspended log-normalized *sul*1 gene copy numbers across storms. A Wilcox rank sum test was applied to test for differences in 16S rRNA gene copy numbers in the attached versus suspended fractions. ANOVA followed by a post-hoc Tukey Honestly Significant Difference test was performed on log-normalized *sul*1 gene copy numbers to determine to test for differences between storms.

Principal Findings and Significance -

Wet weather sampling:

Sampling during the course of three wet weather events was performed to determine the proportion of ARG attached versus suspended and the intra- and inter-storm variability in this partitioning (Fig. 2). Attached *sul*1 gene copies were greater than *sul*1 gene copies observed in the suspended fraction across the wet weather events ($p=5.2\times10^{-5}$). No significant difference was observed in 16S rRNA gene copy numbers between the attached and suspended concentrations across the wet weather events ($p=0.23$). Intra-storm variability was observed for Storm 1 and 3. For example, attached *sul*1 concentration increased 5.4-log and suspended *sul*1 concentrations increased 4.6-log between the first and last sampling point for Storm 3. However, the intra-storm variability did follow the pattern expected (high at beginning of storm, low in the

middle, and higher at the end) based on previous culture based studies of indicator organisms in CSO (Passerat *et al.*, 2011), likely due to the fact samples for this study were taken in the surface water outside of a CSO outfall as opposed to other studies where samples were taken within the pipe. Additionally, it is possible that the CSO immediately adjacent to the sampling location was not flowing the entire duration and/or during all storm sampling events. The outfall sampled has reported 78 discharges/year (Van Abs et al. 2014).

Comparing between the three storms, the cumulative rainfall was 2.02 inches for Storm 1, 0.32 inches for Storm 2, and was 0.58 inches for Storm 3. Results of conductivity and TSS measurements are shown in Fig. 3. For Storm 2 and increase in conductivity was observed across the storm and a decrease in conductivity was observed for Storm 3. TSS peaked for Storm 2 during the middle of the rainfall event and while TSS was greatest at the first sampling time point for Storm 3 and decreased during the rainfall event. Inter-storm variability was observed with *sul*1 gene copy numbers significantly lower in Storm 3 than Storms 1 and 2 (both $p<3\times10^{-6}$) (Fig. 4). No significant differences were observed between Storms 1 and 2 for *sul*1 gene copy numbers ($p=0.08$). Samples are currently being prepared for amplicon sequencing to determine if there are changes in the microbial community across the unit hydrograph and/or between storm events.

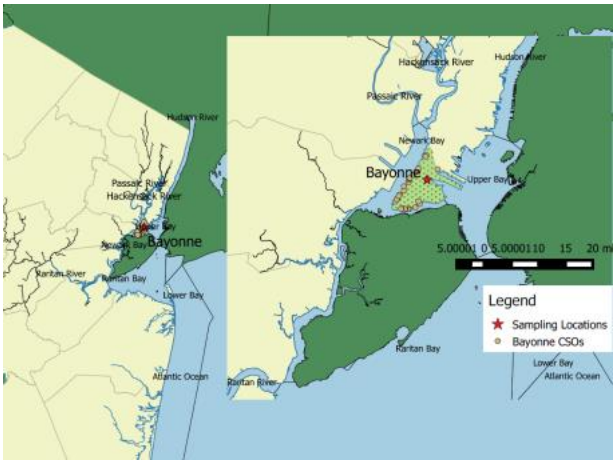


Fig.1 Map of CSO wet weather sampling location

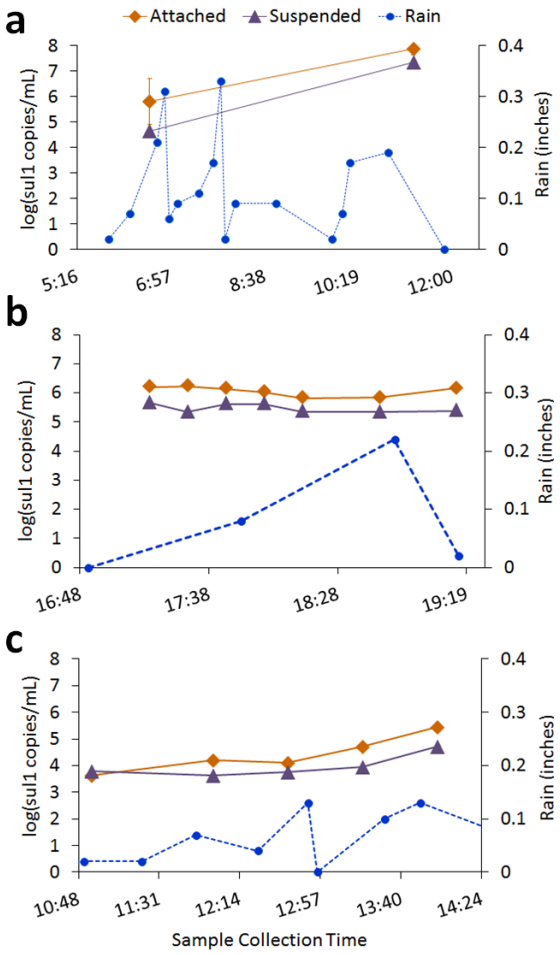


Fig. 2 *sul*1 concentrations measured by qPCR and historical rainfall data over the course of (a) storm 1 (b) storm 2 and (c) storm 3. Sample collection times for (a) and (b) represent time of grab sample. Sample collection times for (c) represent the midpoint sampling time between two consecutive composited samples. Error bars for Storm 1 represent standard deviation of replicate (N=2) samples.

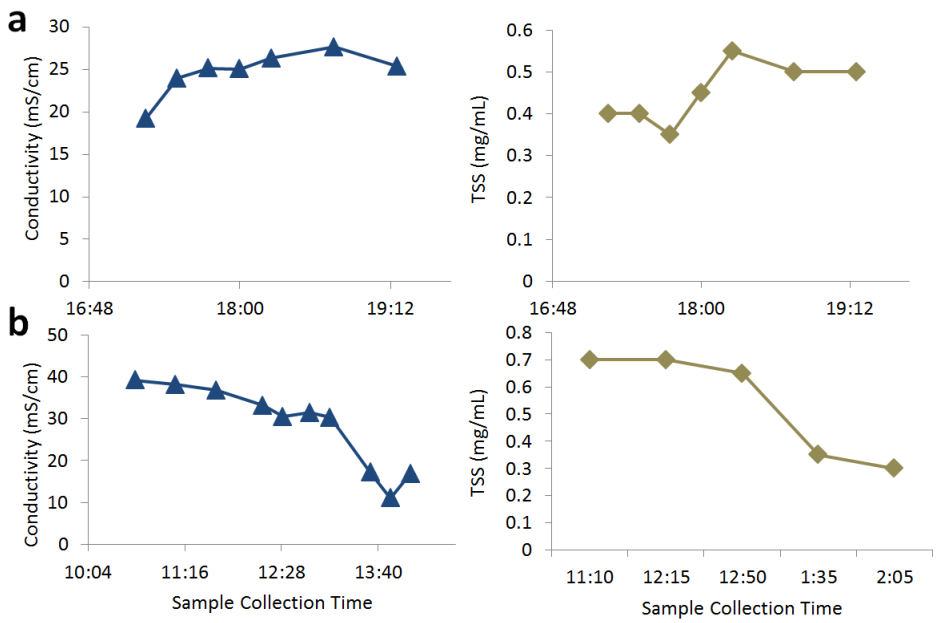


Fig. 3 Field conductivity readings and Total Suspended Solids (TSS) measured in samples collected during (a) storm 2 and (b) storm 3. Sample collection times for TSS (b) represent the midpoint sampling time between two consecutive composited samples. Other graphs represent sampling times for individual samples.

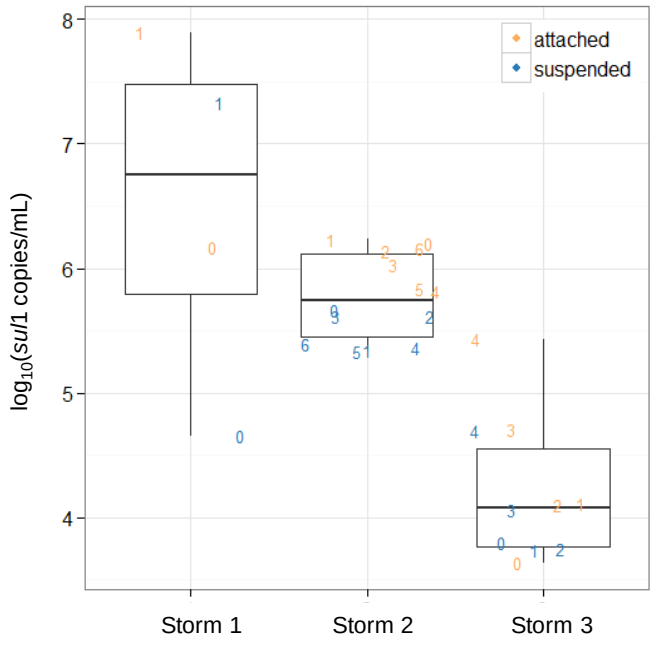


Fig. 4 Boxplot of *sul*1 concentrations by storm. Jitter overlay represents individual sampling time (e.g., 1 represents the first sample from a given storm) concentration with the color representing whether the samples were attached (orange) or suspended (blue).

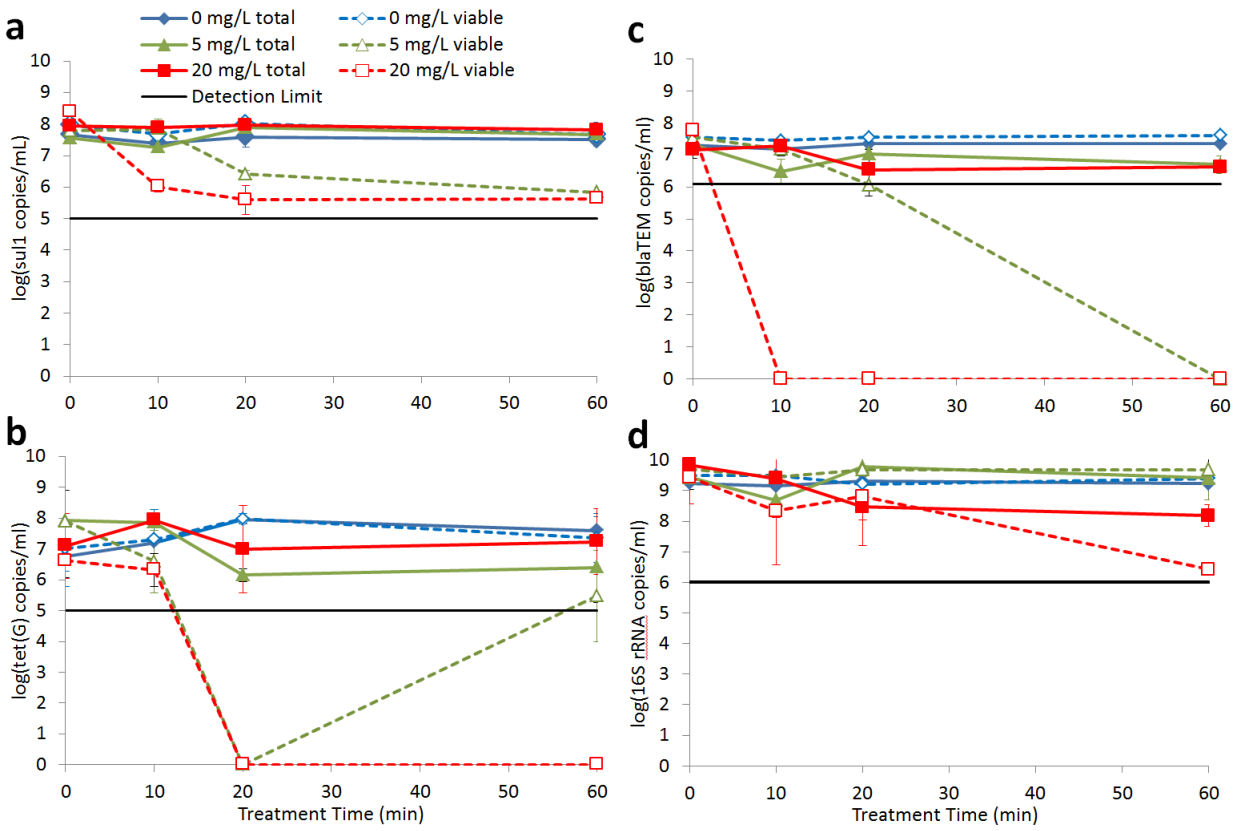


Fig 5. a. *sul*1, b. *tet*(G), c. bla-TEM, and d. 16S rRNA concentrations at 0, 10, 20 and 60 minutes of treatment with 0, 5 and 20 mg/L of PAA. Error bars represent standard deviation of triplicate samples. A viability-based qPCR assay using PMA (see text) was used to differentiate between total concentrations of ARGs and concentrations of ARGs originating only from viable cells.

Disinfection: Disinfection with PAA resulted in an average 2.7-log removal of *sul*1 in viable cells after 20 min of treatment with 20 mg/L PAA (Fig. 5a). Further reduction in the *sul*1 gene copy numbers in viable cells was not observed with increased incubation time up to 60min. Similarly, *tet*(G) was below detection after 20 min of treatment with 5 or 20 mg/L PAA. The detection limit for *tet*(G) in this study was 5-log, and an average 1.9-log removal of *tet*(G) was observed in viable cells after 20 min of treatment with 20 mg/L PAA with no change in *tet*(G) in viable cells (Fig. 5b). For bla-TEM, concentrations in viable cells fell below the detection limit after 10 minutes of treatment with 20 mg/L PAA, again, with no change in total bla-TEM concentrations. The detection limit for bla-TEM in this study was 6.1-log, representing an average 1.7-log removal of bla-TEM in viable cells after 10 min of treatment with 20 mg/L PAA (Fig. 5c). Total bacterial population was estimated using the 16S rRNA gene. Simulated CSO treated with 20 mg/L PAA, which exhibited a decreasing trend in both total and viable genes, otherwise 16S rRNA concentrations were relatively constant for other treatment levels and times (Fig. 5d). Therefore, PAA was effective at disrupting cell membranes of ARG carrying cells, but not effective at destroying ARG or 16S rRNA. This observation is consistent with research demonstrating loss of viability in antibiotic resistant bacteria at lower doses of UV than was required for ARG destruction (McKinney and Pruden, 2012). However, given that primer sets targeting short PCR inserts were used (134-247 bp) for these analyses, it is possible that part of

the ARG were destroyed but not detected with our protocols. Interestingly, the second longest primer set applied was for detecting the 16S rRNA, which had a 3.3-log removal treatment with 20mg/L PAA after 60min but no significant differences for other treatment conditions.

Significance: Understanding the partitioning of ARG is important for understanding fate in surface water and vortex separators, which are being considered for use as end-of-pipe treatment for CSO. Our results indicate that if attached ARG are considered settle-able, then treatments and fate/transport mechanisms involving settling could remove up to 7.9-log *sul*1 copies per mL of sample during wet weather flows. An average of 77.5+/-12.3% of *sul*1 gene copies were attached during the wet weather flow events. Further storm sampling at other locations should be performed to confirm these results are consistent across CSO outfalls. PAA is being evaluated as a green disinfectant for rapid treatment of CSO effluent. With respect to PAA's effect on ARG, results indicate that PAA is effective at compromising the membrane of cells carrying *sul*1, *tet*(G), and bla-TEM genes, but that destruction of these genes was not observed. These results may be used to better understand the fate of ARG during wet weather flows and to design end-of-pipe treatment systems to help prevent the spread of antibiotic resistance in the water environment. Further, these results may help in parameterization of quantitative risk assessment models given that the rate of ARG propagation can be expected to be different for ARG in viable cells (which can spread via growth and horizontal gene transfer) compared to extracellular ARG/ARG in cells with compromised membranes (which can spread via transduction).

(4) **Publications or Presentations**:

1. Articles in Refereed Scientific Journals:
In preparation

2. Book Chapter:
none

3. Dissertations:
Results will be included in A. Eramo's dissertation, anticipated graduation 2018

4. Conference Proceedings

Eramo, A., Delos Reyes, H.D., and Fahrenfeld, N. (accepted, **Platform**). Disinfection of microbial agents in combined sewer overflows using the green disinfectant peracetic acid. Water Environment Federation Technical Exposition and Conference (WEFTEC). New Orleans, LA. September 2016.

Eramo, A., Delos Reyes, H.D., and Fahrenfeld, N. (alternate **Platform**). Assessing the partitioning and treatment of microbial agents from combined sewer effluent during wet weather. 101st Annual New Jersey Water Environment Association Conference and Exhibition. Atlantic City, NJ. May 19, 2016

Fahrenfeld, N., Eramo, A., Delos Reyes, H., Yam, M. **(Invited panelist).** Controlling pathogens in wet weather flows. Parasites and Pathogens: Ecological and Medical Impacts of Global Climate Change. Hofstra University. Hempstead, NY. October 16, 2015.

Delos Reyes, H.D., Eramo, A., and Fahrenfeld, N. (**Poster**). Antibiotic resistance genes in wet weather flow: Impact of combined sewer overflows on New Jersey water quality. 101st Annual New Jersey Water Environment Association Conference and Exhibition. Atlantic City, NJ. May 18, 2016.

Eramo, A., Delos Reyes, H.D., and Fahrenfeld, N. (**Poster**). Flux and treatment of antibiotic resistance genes in CSO. 101st Annual New Jersey Water Environment Association Conference and Exhibition. Atlantic City, NJ. May 18, 2016.

Oduro, R., Eramo, A., Fahrenfeld, N. (**Poster).** Microbial pollution in wet weather flow. Rutgers Aresty Research Symposium. April 27, 2016.

Delos Reyes, H., Eramo, A., Fahrenfeld, N. (**Poster).** Antibiotic resistance genes in wet weather flow: Impact of combined sewer overflows on New Jersey water quality. Rutgers Douglass Summer Research. October 2, 2015.

Yam, M., Eramo, A., Fahrenfeld, N. (**Poster).** Antibiotic resistant genes (ARG) in wastewater and urban surface water. Rutgers Aresty Summer Research Symposium. August 7, 2015.

5. Other Publications
NA

References

Aminov RI, Chee-Sanford JC, Garrigues N, Teferedegne B, Krapac IJ, White BA & Mackie RI (2002) Development, validation, and application of PCR primers for detection of tetracycline efflux genes of Gram-negative bacteria. *Appl Environ Microbiol* **68**: 1786-1793. doi: 10.1128/aem.68.4.1786-1793.2002.

Donovan E, Unice K, Roberts JD, Harris M & Finley B (2008) Risk of gastrointestinal disease associated with exposure to pathogens in the water of the lower Passaic River. *Appl Environ Microbiol* **74**: 994-1003. doi: 10.1128/aem.00601-07.

Fittipaldi M, Nocker A & Codony F (2012) Progress in understanding preferential detection of live cells using viability dyes in combination with DNA amplification. *J Microbiol Methods* **91**: 276-289. doi: 10.1016/j.mimet.2012.08.007.

Krometis L-AH, Characklis GW, Simmons Iii OD, Dilts MJ, Likirdopulos CA & Sobsey MD (2007) Intra-storm variability in microbial partitioning and microbial loading rates. *Water Res* **41**: 506-516. doi: 10.1016/j.watres.2006.09.029.

Nocker A, Sossa-Fernandez P, Burr MD & Camper AK (2007) Use of propidium monoazide for live/dead distinction in microbial ecology. *Appl Environ Microbiol* **73**: 5111-5117. doi: 10.1128/aem.02987-06.

Nocker A, Richter-Heitmann T, Montijn R, Schuren F & Kort R (2010) Discrimination between live and dead cells in bacterial communities from environmental water samples analyzed by 454 pyrosequencing. *Int Microbiol* **13**: 59-65.

Passerat J, Ouattara NK, Mouchel J-M, Vincent R & Servais P (2011) Impact of an intense combined sewer overflow event on the microbiological water quality of the Seine River. *Water Res* **45**: 893-903. doi: 10.1016/j.watres.2010.09.024.

Pei R, Kim S-C, Carlson KH & Pruden A (2006) Effect of river landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG). *Water Res* **40**: 2427-2435. doi: 10.1016/j.watres.2006.04.017.

Young S, Juhl A & O'Mullan GD (2013) Antibiotic-resistant bacteria in the Hudson River Estuary linked to wet weather sewage contamination. *J Water Health* **11**: 297–310.

Investigation into the hydrologic benefits of soil compaction management

Basic Information

Title:	Investigation into the hydrologic benefits of soil compaction management
Project Number:	2015NJ364B
Start Date:	3/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	NJ-003
Research Category:	Water Quality
Focus Category:	Hydrology, Management and Planning, Water Quality
Descriptors:	None
Principal Investigators:	Steven Yergeau, Louise Wootton

Publications

There are no publications.

Investigation into the Hydrologic Benefits of Soil Compaction Management

1. PI Information:

a. Steven Yergeau, Environmental & Resources Management Agent
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2. Number of Students Supported:

Two undergraduate students from Georgian Court University were supported financially through this grant. The students assisted with field work and data collection during the fall of 2015.

3. Notable Achievements

None at this time to report.

4. Project Summary

Problem and Research Objectives

Landscaping and horticultural practices have resulted in hydrological alterations to lands in New Jersey. Increased compaction of soils, especially during construction, and installing and maintaining residential landscapes accounts for a portion of these alterations since compacted soils act like impervious surfaces and reduce or prevent infiltration of precipitation into the ground. Many management techniques are available to alleviate compaction, such as not using heavy machinery on wet soils, mechanically breaking up compacted soils, replacing topsoil, or planting vegetation prior to the formation of soil compaction. The goal of these best management practices (BMPs) is to restore natural hydrologic function to the local environment in a sustainable manner.

It is generally recognized that hydrology should be accounted for when planning and implementing BMPs. It is essential to provide more detail than just identifying the water source and what its movement is, and even developing a rudimentary water budget will contribute to a better understanding of site hydrology and could greatly enhance the likelihood of successful mitigation and restoration of hydrology (Morgan and Roberts 2003). The water budget is an accounting of each component of the hydrologic cycle to quantify its contribution in a particular system. A water budget is commonly calculated using a mass balance approach where inputs and outputs equal some change in water storage over time. It is useful in determining changes in overall water storage based upon changes in any individual components of the equation. A simplified water budget equation, solving for runoff (Q) from a site, is expressed as:

$$Q = P - ET - I \qquad \text{[Eq. 1]}$$

where, Q = surface water runoff; P = precipitation; ET = evapotranspiration; and I = infiltration.

The objective of this investigation is to determine the extent to which soil compaction management strategies provide improvements to local hydrology. This was accomplished through measurement of the components of the water balance, and calculation of same, for locations that are undergoing soil compaction reduction practices and comparing them to the same measurements in comparable locations not undergoing soil compaction management. The research question to be answered by this project is: what are the changes to the water balance of lands undergoing soil compaction management strategies compared to the water balance of soil systems currently altered due to development with no compaction management implementation? Specific hypotheses to be tested during the course of this project are:

- Areas that are undergoing mechanical disruption of soil compaction (Verti-Quaking) will have increased infiltration rates, higher soil storage of water, increased ET rates, and reduced runoff volumes compared to sites not implementing soil compaction management.
- Areas where BMPs are occurring will have calculated water balances similar to undisturbed, natural sites, as described in the literature.

Methodology

To determine the components of the water balance (Eq. 1), six sites were studied during the fall of 2015. Each of the three sites had a pair of plots (one plot at each site undergoing Verti-Quaking; and the other plot at each site with no Verti-Quaking, as a control). These were identified on Georgian Court University's campus and all six plots consisted of mowed turf. The six plots were marked off as 10 meter by 10 meter plots to provide enough room for the Verti-Quake machine pass over them twice. One pass was in the North-South direction and the other was in an East-West direction. This was to mimic how the device is used on other areas of campus. None of the sites underwent irrigation during the course of the study. The sites selected were in areas with known compaction and drainage problems with conditions as similar as possible for comparison. Measurements of each component of the water balance (described individually below) were taken after Verti-Quaking occurred.

Precipitation - Rainfall for Lakewood, NJ was obtained from the Community Collaborative Rain, Hail and Snow Network (CoCoRaHS) (http://www.cocorahs.org/). CoCoRaHS is a network of volunteers measuring and mapping precipitation to provide quality data for natural resource, education and research applications. There are fifteen sites located throughout Ocean County with those locations closest to the GCU campus chosen to reduce any likely error due to spatial and temporal differences normally associated with rainfall data. An Earth Networks Weather Station is currently located at GCU's campus (http://www.aws.com/wx.aspx?ID=lwdnj&RND=7.055475). This station, which has been inconsistent in the past, was used only to supplement the CoCoRaHS data when reliable, appropriate data is available and was not the primary source of precipitation values.

Evapotranspiration (ET) – ET was measured using an ETGage Model E evapotranspiration simulator (http://www.etgage.com/) mounted on a fence post and attached to a datalogger. The ETgage Model E is an automated meter for estimating ET from turf, field crops, orchards and vineyards. A covered ceramic evaporator at the top mimics the ET process of irrigated crops and turfgrass. A reservoir below the evaporator holds distilled water where the evaporator draws water from the reservoir at the same rate that plants remove water from soil. Water drawn from the reservoir must first pass through an accurately calibrated glass measuring vial, with one vial corresponding to 0.01 inch of ET. Electronics accurately sense when the vial is empty, and immediately refill it through a 3-way solenoid valve. The datalogger recorded and stored each

event as 0.01 inches, with data downloaded in the field during each weekly site visit. Only one ET Gage was set up at each pair of test plots, for a total of three gauges.

Infiltration Rate - The infiltration rate was measured using a Turf-Tec Infiltrometer (Turf-Tec International, Tallahassee, FL, USA). The infiltration test was performed by inserting the infiltrometer into the ground, filling the double-ring assembly with water, and measuring the change in water level after 5 minutes. The level of infiltration is measured on a scale (in inches) attached to the infiltrometer. The number of inches recorded during the 5 minutes is multiplied by 12 to obtain the infiltration rate in inches per hour (in/hr). The infiltration rate was measured six times in each test plot during each site visits, for a total of 36 measurements during the study.

Soil Compaction - Soil compaction was measured using a handheld static cone penetrometer (DICKEY-john Corporation, Springfield, IL, USA) and reported as the depth reached when a soil resistance of 300 pounds per square inch (psi) is reached. The distance the rod penetrates the soil is measured and recorded with deeper penetrometer depths indicating less compact soils. Twenty measurements were collected from each of the six plots for a total of 120 measurements during each week of the study. These measurements were used in conjunction with other data collected to determine if the goal of alleviating compaction is being achieved.

Measurements were taken weekly for six weeks from early October through November. The onset of cold weather impeded the ability of the investigators to get representative samples as the ground became more frozen. The measurements gathered at each location were combined in the water balance (Eq. 1) to calculate runoff (Q) and are being compared to literature-derived 'natural' water budgets to see how well the Verti-Quaking is improving local hydrology. The data are also being analyzed to compare pre- and post-management efforts. Additional data on soil texture and soil water content at all sampling locations were obtained by collecting soil samples with analyses conducted by the Rutgers University Soil Testing Laboratory.

Principal Findings and Significance

Preliminary evaluation of the soil compaction results indicate that there was no significant difference between those sites that underwent mechanical soil disruption (i.e., Verti-Quaking) and those that did not during the course of this study ($t(34) = 0.138$, $p = >0.05$). The reasons for this are still under investigation but may be due to the limited sample size. Delays to the start of sampling occurred that resulted in only several weeks in the fall were available for sampling. As a result, only six weeks of data were obtained, which included only two applications of the vertiquake to the treated areas.

Soil moisture/water content has a major impact on how well a soil will compact and there was a strong positive correlation between the soil compaction depths and soil water content (Figure 1) ($r(36) = 0.81$ [$r^2 = 0.66$], $p = 0.01$). This indicates that as a soil dries, with lower water content, the particles move closer together, increasing soil resistance. When a soil has a moisture content increase, the water decreases the resistance of the soil and the penetrometer is able to obtain a deeper measurement.

Data are still undergoing analysis and the creation of the water budgets is being evaluated. The investigators are in the process of determining additional sources of funding to enable them to sample in additional seasons, and over a few years. This will ensure that this research is reliable enough to be used to help guide programs on effectively bridging the gap between management actions and improvement to ecosystem health.

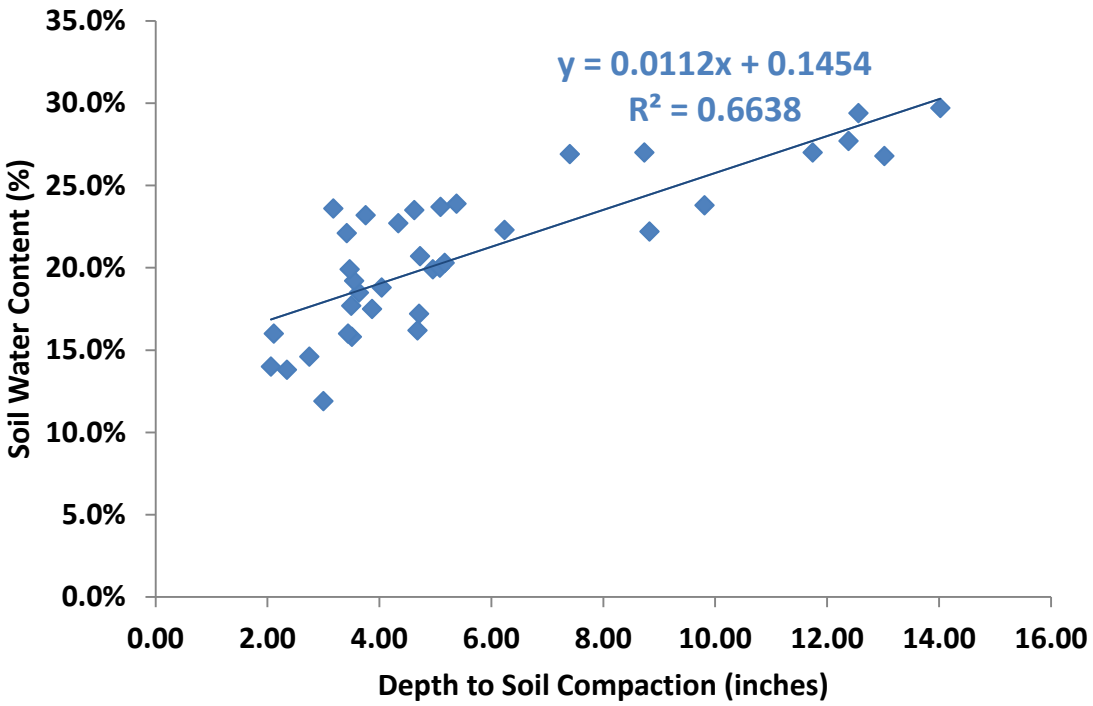

Figure 1: Plot of soil water content versus depth to soil compaction.

5. Publications or Presentations:
None at this time to report.

References

Morgan, K.L. and T.H. Roberts. 2003. Characterization of Wetland Mitigation Projects in Tennessee, USA. *Wetlands*. 23(1):65-69.

Establishing bioremediation options for dioxins and furans for the heavily contaminated sediments of the Passaic River, New Jersey

Basic Information

Title:	Establishing bioremediation options for dioxins and furans for the heavily contaminated sediments of the Passaic River, New Jersey
Project Number:	2015NJ365B
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Principal Investigators:	Haider AlMnehlawi, Donna E. Fennell

Publications

1. Almnehlawi, H.S. and Fennell, D.E.2016. Dioxin Substrate Range of Dibenzofuran-Degrading Bacteria Isolated from Contaminated Sediments A poster presented at ASM microbe conference 2016, June 16-20, 2016 Boston, MA.
2. Rao, R., Almnehlawi, H.S. and Fennell, D.E. 2016. Aerobic Biodegradation of Dibenzofuran. A poster presentation, Rutgers University Chemical Engineering undergraduate researcher participated in this research through the Douglass Residential College Introduction to Scientific Research and Project SUPER

Establishing bioremediation options for dioxins and furans for the heavily contaminated sediments of the Passaic River, New Jersey.

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Problem and Research Objectives

Human activities in the last two centuries added many hazardous pollutants to the environment. Industry, development, and urbanization negatively effect many waterways in the United States. The Passaic River is one of the water resources that has been harmed by these factors, especially the 6 to 7 miles area, that known as the Lower Passaic River (1). According to EPA, the Passaic River sediment is contaminated by dioxins (2). Dioxins have been detected in different areas over the world in various environmental compartments, soil, water, as well as in plant and animal tissues (3). Dioxins have a wide range of effects on humans, other organisms, wildlife, and natural ecosystems. They cause various types of cancer and circulation, reproductive, and respiration system diseases (4). The persistence of dioxins in the environment and their bioaccumulation in biota (5) of are great concern. It is for this reason that using food, such as fish and shellfish, from the Passaic River area has been banned. Dioxins do not have reactive groups, therefore their biodegradation is very difficult and slow, if it occurs at all (6).

Many methods are used to remove or sequester dioxins in environmental systems including ultra violet light, oxidizing chemical reagents, and capping and dredging. However, these methods are very expensive, require specialized equipment and personnel, and they may require transferring the sediments to another place for treatment (7). Using microorganisms to degrade these compounds is a good alternative method because it could be more cost effective and environmentally sustainable.

The objective of this study is to identify Passaic River bacteria that aerobically degrade lightly chlorinated dioxins. The hypothesis is: Specific native Passaic River sediment bacteria can use dioxins as a sole carbon source and transform them to less or non toxic forms

Methodology

a. Sediment collection. Samples were previously collected from Passaic River in the area of Jersey City, New Jersey (40.742445- 47.132851) by Professor John Reinfelder. These samples were used for isolation of bacterial strains. Sediments in additional areas will be recovered with the help of USEPA Region II (on-going). An Ekman grab sampler and boat were used for sediment collection. Sediment samples were transferred to the laboratory and stored in sterile glass jars at 4Cºprior to use. Environmental parameters such as temperature, pH, and salinity were measured in the water column at sampling location.

b. Media preparation. Aerobic minimal culture medium was prepared as described previously (8) with slight modification to ensure that trace metals did not precipitate. The media solution was autoclaved and the pH was adjusted to neutrality prior to use.

c. Microcosm setup. Sterile 250 ml Erlenmeyer flasks (by gravity autoclaving) were used as microcosm containers. Twenty-five ml of sterile medium was placed in the sterile flasks under a hood to keep sterile conditions, and 25mg of dibenzofuran crystals was added as a sole source of carbon. [Note that dibenzofuran and dibenzo-*p*-dioxin are closely related compounds that may be used as carbon sources by dioxin-degrading bacteria. Dibenzofuran is far less expensive and was used as carbon source for initial enrichments/isolations. Next, 1g/l of Passaic River sediment was added to each microcosm as inoculum. Microcosms were incubated at 28ºCand shaken at 180 rpm for 7-10 days. For controls, sediment was inactivated by autoclaving. Microcosm aliquots (0.25 ml) were transferred to fresh media with dibenzofuran as an enrichment process, transferring process was done every 7 days for five times . Activity was assessed by a color change after 6-7 days.

d. Isolation and purification. Minimal media agar were prepared as described in **part b** but with 15 g/l of noble agar and 50 mg/l of cyclohexamide (fungal inhibitor) added. For plating, 100 µl of diluted (10^{-1}-10^{-6}) culture was spread on the agar to get single colonies. Dibenzofuran crystals were placed directly on the lid of the plates as the carbon source. One plate was left without dibenzofuran as a control. Plates were incubated at 28Cº until colonies developed. The ability of pure cultures to degrade dibenzofuran was confirmed using the protocol described in **part c.** Isolates were stored at -80ºC in glycerol.

e. Identification of unique isolates. Polymerase Chain Reaction (PCR) with Denaturing Gradient Gel Electrophoresis (DGGE) were used to identify isolates. Briefly, DNA was extracted and amplified using bacterial 16S rRNA gene PCR primers with GC clamp as described previously (9). Individual DNA bands from the denaturing gel were excised and those bands with different migration distances—indicating potentially unique phylotypes—were sequenced to initially identify the bacteria that were isolated on dibenzofuran..PCR was done for each excised band then DNA was purified by using Exo-Sap, finally 20µl was sent to Genwize Company for sequencing. Sequencings were analyzed and blast by using Finch software program.

f. Degradation of dibenzo-*p*-dioxin and lightly chlorinated dibenzo-*p*-dioxins by isolates. Unique isolates were grown as described in **Task 1c** by inoculating the flask with a pure culture. On-going experiments are testing the isolates for degradation of dibenzo-*p*-dioxin; 2,7-

dichlorodibenzo-*p*-dioxin; 2,2-dichlorodibenzo-*p*-dioxin and 2,3,7,8 tetrachlorodibenzo-*p*-dioxin. In this case biodegradation will be tested with lower concentrations of the parent compound (as is expected in the native sediments). Those isolates that can degrade the lightly chlorinated dibenzo-*p*-dioxins will be of special interest for the river, since in situ anaerobic processes produce these more soluble forms (10). Turbidity increase, dibenzofuran crystal disappearance from the liquid media, and yellow metabolite formation indicated that these isolates used dibenzofuran as a sole carbon source.

Gas Chromatography-Mass spectroscopy analysis GC-MS

We are working on the GC.MS to achieve all the requirements of these analysis. In this analysis dioxins and furans concentrations will be measured before and after the bacterial isolates addition, Standard curve was prepared. Internal standard and surrogate standards were selected. The samples will be extracted to measure dioxins residue in each sample.

Principal Findings and Significance

a- Bacterial isolation and dibenzofuran degradation.

Pure cultures from Passaic River sediments, which can degrade dibenzofuran, were isolated. The yellow color shown in in Fig. 1 and 2 shows formation of oxidized dibenzofuran metabolites. The yellow metabolite is (2-hydroxy-4-[3'-oxo-3'H-benzofuran-2'-yliden] but-2-enoic acid (HOBB) that is produced as a yellow metabolite from dibenzofuran biodegradation.(11). Turbidity increase and dibenzofuran disappearance were observed visually and clearly after 7 days from the microcosm set up. Fig1 and 2.

Fig.1 Microcosms to assess Dibenzofuran biodegradation A: day 0, B: day 7, note: Letter (c) on the flask means control other flasks are three live replicates.

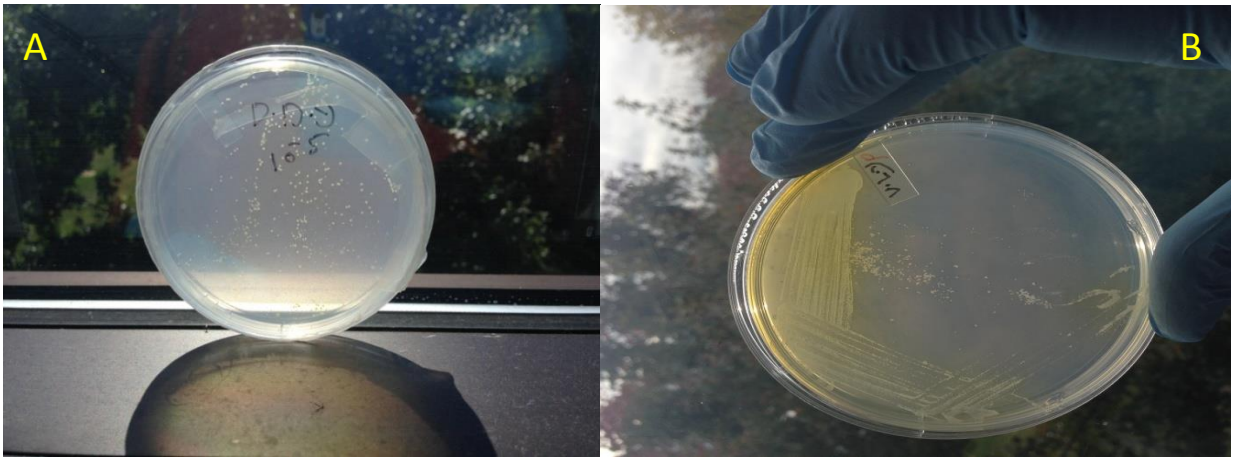

Figure (2) A: Colonies grown from 10^{-5} dilution of enriched culture, B: Isolate from a single colony of A (Pure Culture).

Identification of unique isolates.

Using PCR, DGGE gel, and sequencing of 16S rRNA genes, it was confirmed that four different species were isolated from Passaic River sediment. These isolates can degrade dibenzofuran and form yellow metabolites. Based on the sequence of the 16S rRNA gene, the isolates are most closely related to: *Paenibacillus naphthalenovorans* (98% similarity), *Achromobacter aegrifaciens*(90% similarity), *Janibacter terrae*(98% similarity), and *Acidovorax ebreus* (98% similarity). Isolates were stored in glycerol at -80C° for long term storage.

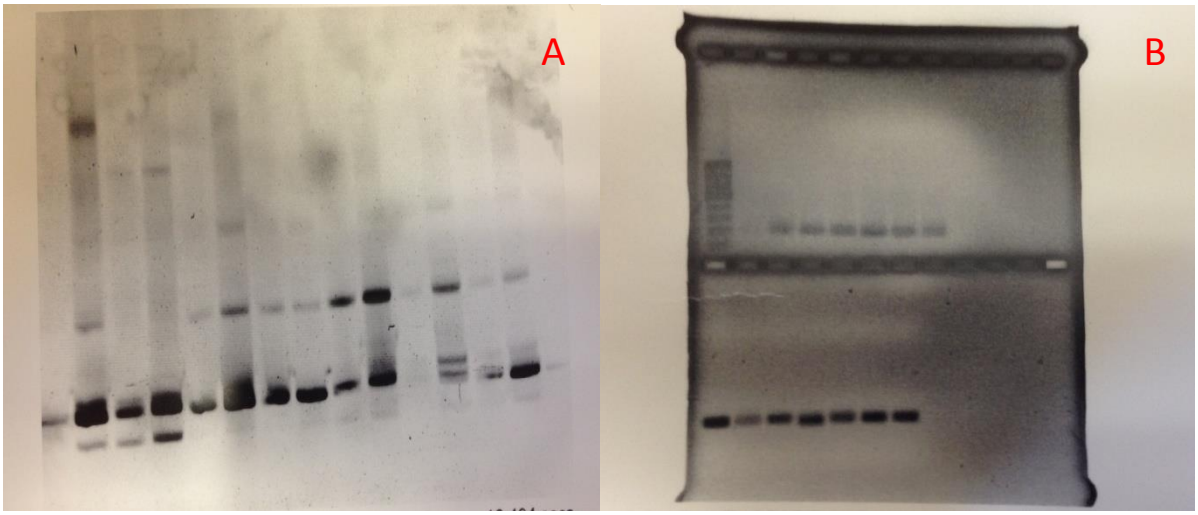

Fig.3 (A) DGGE gel shows different bands of DNA representing unique phylotypes. (B) PCR of selected bands from DGGE gel.

Degradation of dibenzo-*p*-dioxin and lightly chlorinated dibenzo-*p*-dioxins by isolates.

On-going experiments are being performed to test cultures for degradation of dibenzo-*p*-dioxin; 2, 7-dichlorodibenzo-*p*-dioxin; 2,2-dichlorodibenzo-*p*-dioxin and 2,3,7,8 tetrachlorodibenzo-*p*-dioxin. In this case, biodegradation will be tested with lower concentrations of the parent compound. Those isolates that can degrade the lightly chlorinated dibenzo-*p*-dioxins will be of special interest for the river, since in situ anaerobic processes produce these more soluble forms (10).

Publications or Presentations:
An abstract has been accepted for poster presentation in ASM microbe 2016, June 16-20, 2016 Boston, MA

Undergraduate Student Participation:

Miss Riddhima Rao, a Rutgers University Chemical Engineering undergraduate researcher participated in this research through the Douglass Residential College Introduction to Scientific Research and Project SUPER.

- **Almnehlawi, H.S. and Fennell, D.E.2016.** Dioxin Substrate Range of Dibenzofuran-Degrading Bacteria Isolated from Contaminated Sediments A poster presented at ASM microbe conference 2016, June 16-20, 2016 Boston, MA.

- **Rao, R., Almnehlawi, H.S. and Fennell, D.E. 2016.** Aerobic Biodegradation of Dibenzofuran. A poster presentation , Rutgers University Chemical Engineering undergraduate researcher participated in this research through the Douglass Residential College Introduction to Scientific Research and Project SUPER

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Bacterium isolated from dioxin-polluted environments. Systematic and Applied Microbiology, 28:165-174.

The biodegradation of pharmaceuticals in anaerobic wastewater digestate

Basic Information

Title:	The biodegradation of pharmaceuticals in anaerobic wastewater digestate
Project Number:	2015NJ366B
Start Date:	3/1/2015
End Date:	2/29/2016
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Congressional District:	NJ-006
Research Category:	Water Quality
Focus Category:	Wastewater, Treatment, Water Quality
Descriptors:	None
Principal Investigators:	Julia Campbell, Lily Young

Publications

There are no publications.

The Biodegradation of Pharmaceuticals in Anaerobic Wastewater Digestate

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Student Supported: Julia Campbell, Masters Student

Future Conference Presentation: NEMPET June 24-26, 2016. Presentation titled: Degradation of Atenolol in a Methanogenic Consortium

Abstract

Pharmaceuticals and Personal Care Products (PPCPs) are used on a daily basis by people worldwide and released into the environment by human excretion as well as improper disposal. These contaminants make their way to a wastewater treatment plant where they are incompletely removed and released into streams via wastewater effluent. While little is known about the effects of emerging PPCPs contaminants on humans, it is known that pharmaceutical compounds and their metabolites can cause adverse effects to aquatic organisms. PPCPs can also inhibit or promote processes in wastewater treatment plants such as methane production. Due to these risks and concerns, it is important to find a way to get rid of these contaminants before they reach the environment. The following research investigates anaerobic microbial degradation under methanogenic conditions as means to remove pharmaceuticals from wastewater. The effect of pharmaceuticals on methane production will also be investigated. The identification of degradation pathways and metabolites of pharmaceuticals as well as the identification of the microbial community responsible can reveal new methods to rid wastewater of pharmaceutical contaminants.

Priority Issues

PPCPs are incompletely removed from wastewater treatment plants (5,6). Wastewater treatment plants were designed to treat human waste, not the pharmaceuticals that we take and then excrete. Pharmaceuticals have been detected in streams and wastewater effluent throughout the country (6,7). Although this is a nationwide problem, in New Jersey it is of major concern because it is the most densely populated state in America. The wastewater that is produced by population is treated for fecal coliforms and BOD removal for release back into the environment. This water is the habitat of a variety of aquatic species and possibly reused for human consumption. Pharmaceuticals in the aquatic environment have been linked to the development of antibiotic resistance in bacteria and the feminization of amphibians and fish by acting as endocrine disruptors (1, 4,6, 8). Bioaccumulation in aquatic organisms is also a concern. Studies

have shown the accumulation of many types of pharmaceuticals in fishes' muscle, which is then consumed by humans (9,15). These findings are not only the beginnings to a potential public health concern, but raise concern about the health of the ecosystem and overall quality of the water.

With specific reference to wastewater treatment facilities, another issue from pharmaceutical input has arisen. Several types pharmaceuticals influence the production of methane in the wastewater treatment process (2,5,12). The production of methane is a vital to the wastewater treatment process: methanogenisis reduces the volume of waste solids by converting larger organic molecules to methane gas (17). This could then impact the efficiency of the wastewater treatment process.

With the concerns and finding stated above, it is important to find a way to remove these pharmaceuticals prior to them reaching the environment. This research investigates the impact of pharmaceuticals on microbial communities under methanogenic conditions. The aerobic microbial degradation of pharmaceuticals has been investigated to a certain extent (14). However, investigation of anaerobic microbial degradation is still limited, especially with regards to degradation under methanogenic conditions. Minimal anaerobic degradation has been shown for pharmaceuticals such as gemfibrozil, carbamazepine, and ibuprofen (3). This leaves many other questions that must be answered in order to apply this research. . The microbial community responsible and its metabolism of pharmaceuticals have yet to be identified. The identification of what pharmaceuticals that can be degrading are not fully understood either. In this research I have chosen two pharmaceuticals that do not have well established results with regards to anaerobic degradation: ibuprofen, and atenolol (fig 2). These pharmaceuticals are some of the most commonly occurring and in the highest abundances in wastewater and environmental water (6,7,18). However, little is known about their capacity to be degraded anaerobically. This research aims to elucidate anaerobic pharmaceutical degradation and its effects to further the steps to finding a solution for this contamination issue

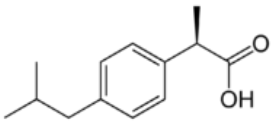

Figure 1: Structure of ibuprofen

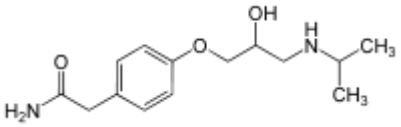

Figure 2: Structure of atenolol

Specific Objectives and Hypotheses

The microbial community in a wastewater treatment facility is exposed to a variety of contaminants including pharmaceuticals. With that being stated, I hypothesize the following with respect to the selected two compounds, ibuprofen and atenolol:

1. Exposure to each pharmaceutical compound will select for specialized sub-community capable of metabolizing the compound as a nutrient or carbon source.

2. Methane production by the methanogenic community will be impacted because of the biochemical influence of PPCPs and the microbial community shift.

Methods and Experimental Design

Anaerobic digestate was obtained from Rockland County Sewer District in New York. The treatment plant processes at 28.9 MGD at a temperature of 37°C (11). This effluent goes into the Hudson River which then feeds into the Hudson River wastershed, part of this watershed is located in New Jersey (11). This will serve as the microbial inoculum. The microbial degradation of PPCPs and production of methane gas will be determined in the following manners:

1. **Culture set up:**

   Anaerobic enrichment with 20% inoculum in methanogenic media was established in 100mL bottles with 60mL of N_2/CO_2 headspace as described in Owen et., al. Two pharmaceuticals were amended at 0.5mM: Atenolol and Ibuprofen. Separate cultures for each pharmaceutical were established with triplicate actives and duplicate sterile controls. Two background cultures without added PCPPs were established for each treatment. These cultures will be incubated in the dark at 37°C as this is the temperature that the anaerobic digester maintains.

2. **Monitoring of substrate concentration and gas production:**

   Pharmaceutical concentrations were analyzed via HPLC to determine the extent of pharmaceutical degradation. For atenolol, the mobile phase was comprised of 20% methanol, 80% Millipore water with 50 mM of anhydrous monobasic sodium phosphate, as described in Sasaki et al. For ibuprofen, the mobile phase of 70% methanol, 30% 40 mM of acetic acid was used for HPLC analysis as described by Murdoch et al. Methane production and the amount produced was determined by measuring excess headspace and utilizing the GC as described in Owen et, al.

3. **Microbial community composition:**

   Throughout incubation, the cultures were sampled to identify the microbial community as well as the changes in community structure. This was accomplished by using MO BIO Laboratories' Powersoil DNA Isolation kit. The extractions were then sent out for Illumina sequencing, this technique determines the makeup of the microbial community. This was done for both archaea and bacteria. In order to determine that the community shift was due to the influence of pharmaceuticals, DNA extraction followed by Illumina sequencing was also done on unammended (background cultures) samples at the same time point.

Results

Degradation of Pharmaceuticals

By day 98 atenolol was completely degraded (fig 3). Atenolol was transformed into an intermediate. Since the UV detector on the HPLC was able to still detect the intermediate, this means the benzene ring of atenolol is still intact and some part of the side chains are possibly being degraded. Ibuprofen was not degraded. This important to know because this means that ibuprofen cannot be removed from the wastewater treatment system via microbial processes.

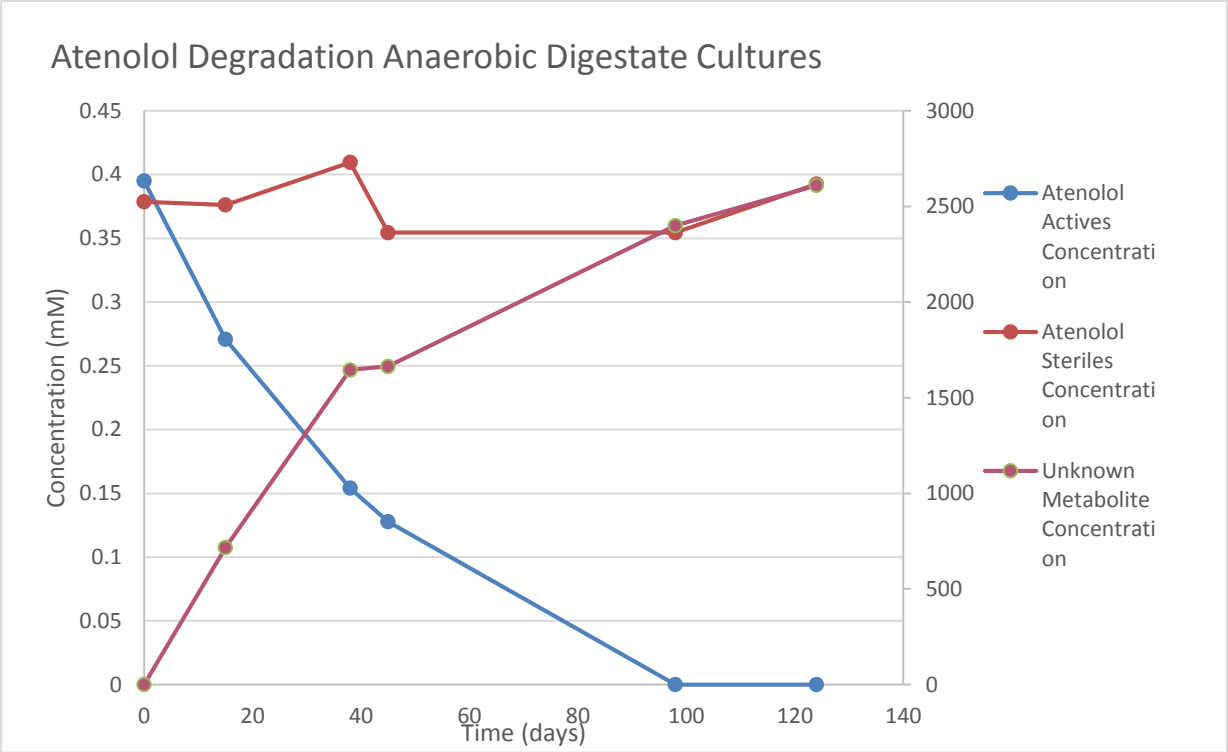

Figure 3: Degradation of atenolol

Methane Production

In both the atenolol and ibuprofen fed cultures, the addition of the pharmaceuticals enhanced the production of methane (fig 4 and fig 5). The increased production of methane shows that these pharmaceuticals do not have an inhibitory effect on methanogens, the type of archaea responsible for producing methane. Inhibition of this process would greatly hinder the effectiveness of anaerobic digestion as discussed in the priority issues section. Atenolol produced more methane than ibuprofen; this could be due to the energy it may be gaining from transformation of atenolol and therefore more end products are available for methanogenesis.

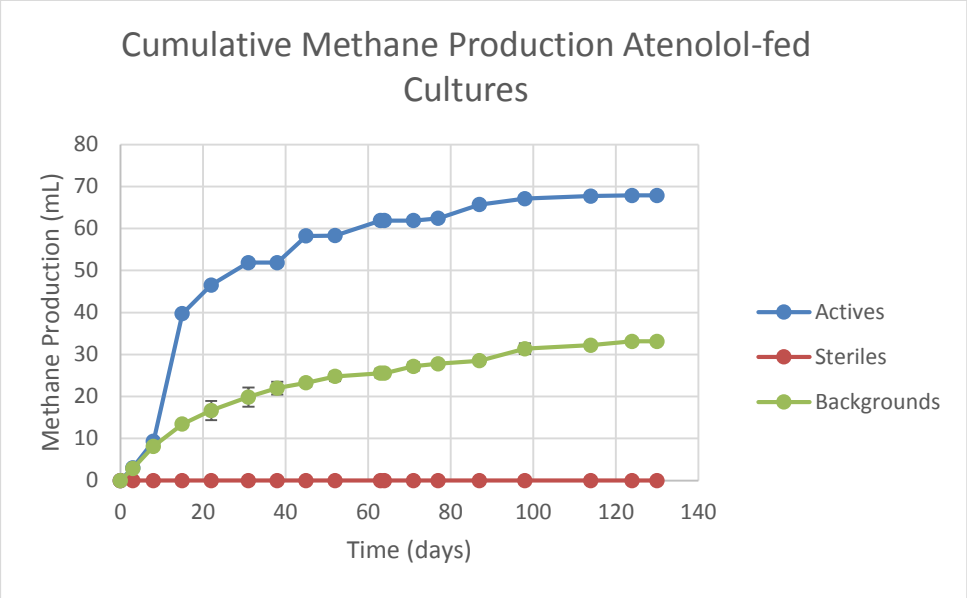

Figure 4: Methane production of atenolol-fed cultures

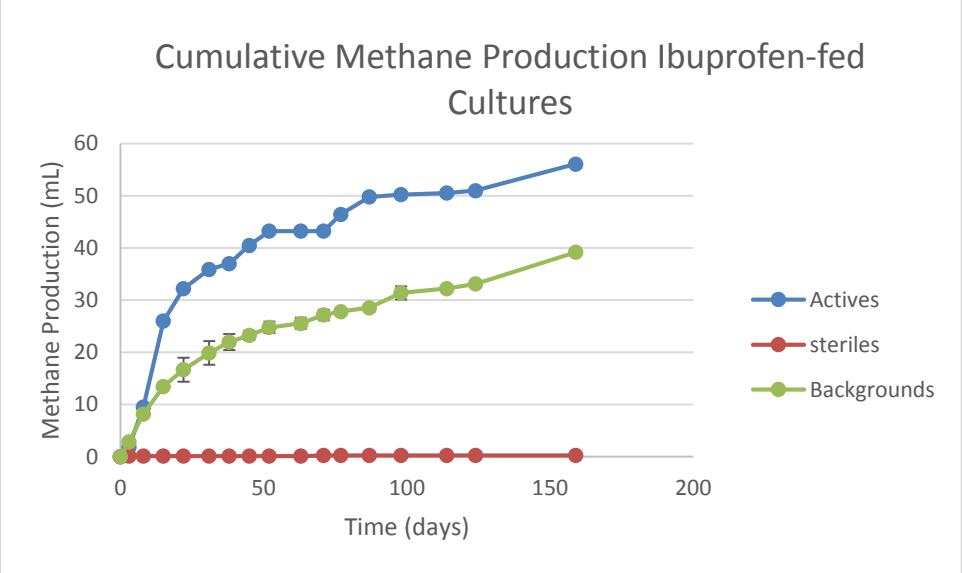

Figure 5: Methane production of ibuprofen-fed cultures

Microbial Community Composition

Illumina sequencing of the atenolol cultures was carried out after two 0.5mM feedings. This was done for bacteria, the community responsible for breaking down the compound, and archaea, the community responsible for methane production. Figure 6 shows the bacterial community

composition of an atenolol fed culture compared to an unfed background. There is little difference between the background and atenolol fed culture. The bacterial community in wastewater treatment digesters are continually exposed to a variety of compounds including pharmaceuticals (6, 7). This could explain why there is little change in the community. The archaeal community had a slight shift (fig 7). Methanobacteriales decreased slightly in the atenolol culture compared to the background. The atenolol fed culture had a slight increase in thermoplasmatales.

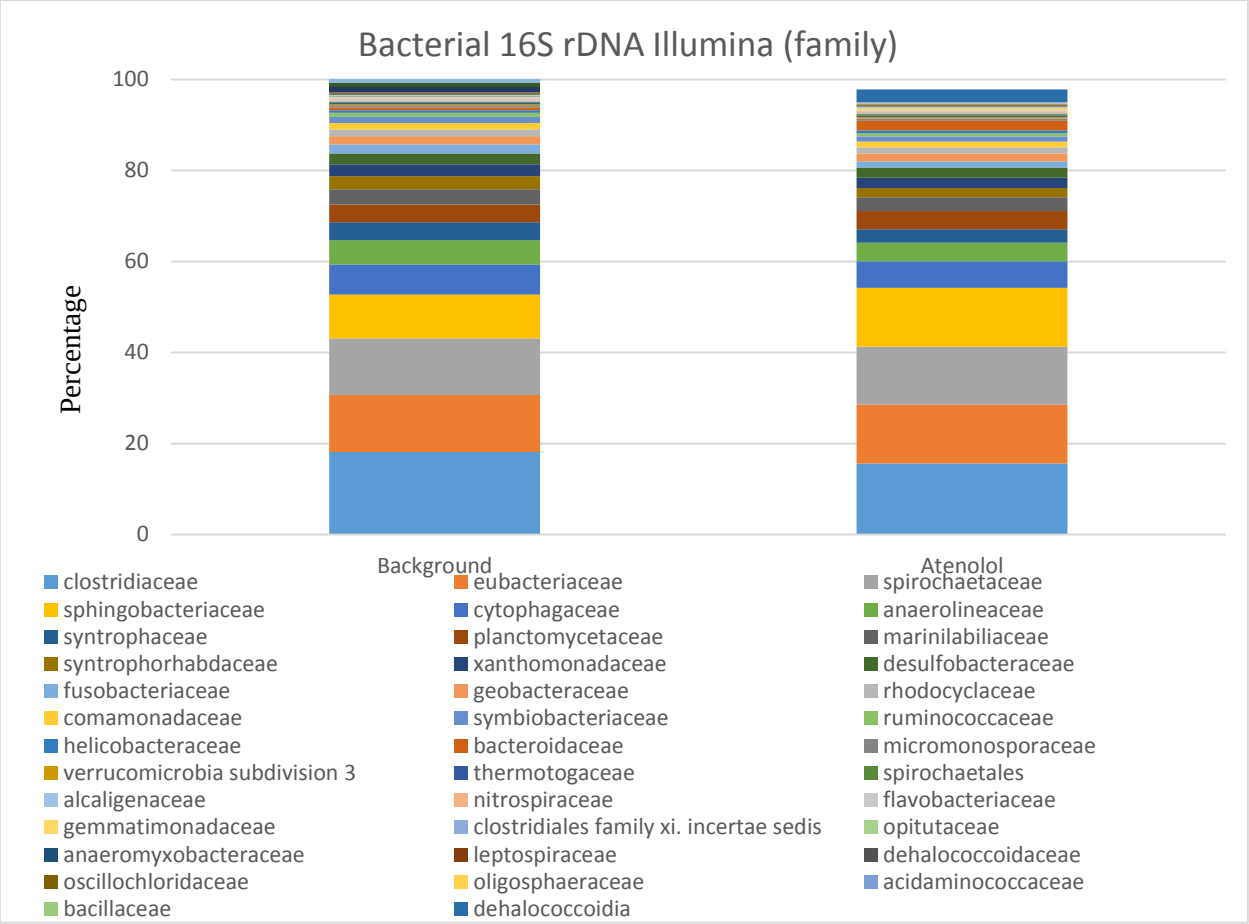

Figure 6: Bacterial community composition

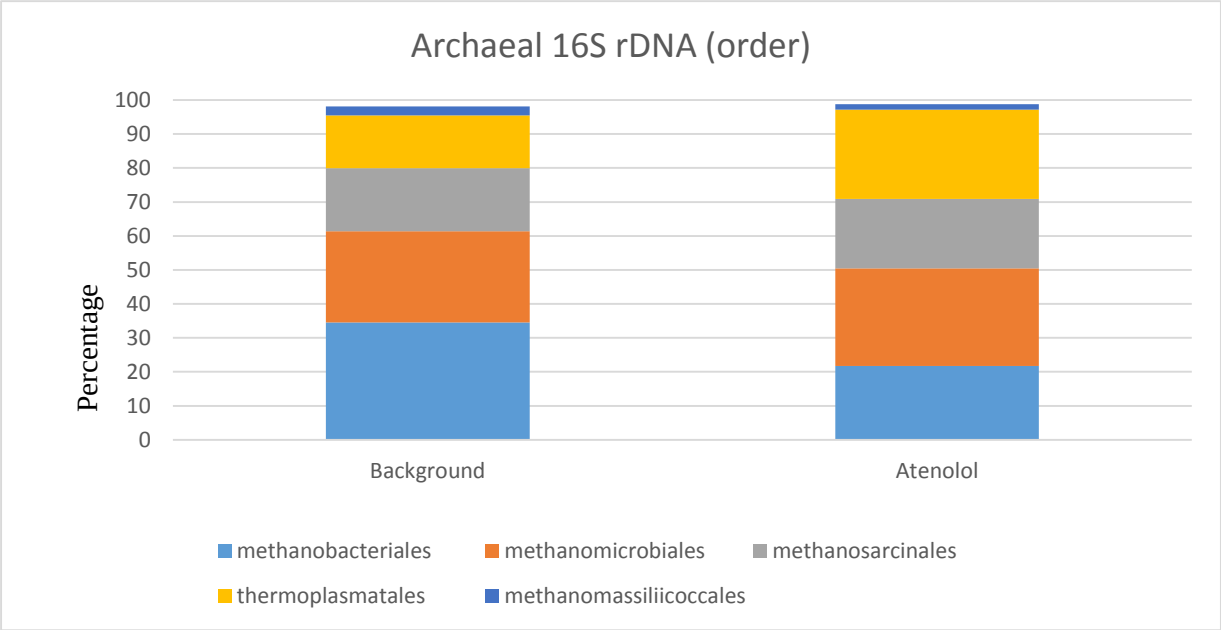

Figure 7: Archaeal community composition

Conclusions and Future Work

Atenolol was transformed into a metabolite indicating that the microbial community in an anaerobic digester can transform the pharmaceutical. Furthermore, the bacterial community before and after enrichment has little change implying that the existing community in the digester does not need to dramatically shift in order to transform this compound. Ibuprofen does not degrade showing that the anaerobic digestate community is not capable of this, or at least in this time frame.

Cultures are still being maintained. Future work includes identification of the atenolol intermediate and community analysis of the ibuprofen fed cultures.

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Assessment of seasonal variations and viability of antibiotic resistant genes from hospital point sources to surface waters of New Jersey

Basic Information

Title:	Assessment of seasonal variations and viability of antibiotic resistant genes from hospital point sources to surface waters of New Jersey
Project Number:	2015NJ367B
Start Date:	3/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	NJ-006
Research Category:	Water Quality
Focus Category:	Wastewater, Surface Water, Water Quality
Descriptors:	None
Principal Investigators:	Alessia Eramo, Nicole Fahrenfeld

Publication

1. Yam, M., Eramo, A., Fahrenfeld, N. 2015. Antibiotic resistant genes (ARG) in wastewater and urban surface water. Rutgers Aresty Summer Research Symposium. Poster.

PLEASE FOLLOW THIS FORMAT:

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(Adviser Nicole Fahrenfeld, nfahrenf@rutgers.edu, 848-445-8416)

(2) **Numbers of Students Supported**:
Undergraduates: 3
Masters' students:
Ph. D. students: 1
Postdoctoral Associates:

(3) **Any Notable Achievements**
Alessia Eramo was awarded Kenneth S. Stoller Award by NJ WEA 2016

(3) **Project Summary**:

Problem and Research Objectives –

In recent decades, the incidence of antibiotic resistance in the clinic has increased while the development of novel antibiotics has steadily decreased. Antibiotic resistance has been characterized by the Centers for Disease Control and Prevention, The White House, and the World Health Organization as one of the most serious threats to public health. Since antibiotic resistance in humans has been linked to environmental sources of antibiotic resistance genes (ARGs), it is important to characterize and limit the spread of ARGs in the environment.

Hospital effluents are a source of antibiotic resistant bacteria in municipal wastewater treatment plants (WWTPs) and surface waters throughout the world. However, regulations regarding the release of ARGs from WWTPs do not currently exist. Studies have shown fluctuations in the occurrence of different hospital infections during different periods of the year, but surprisingly little data is available about seasonal fluxes in antibiotic resistant infections. This study aims to (1) collect crucial information about seasonal variations and fate of ARGs from municipal only and hospital influenced municipal wastewaters. Samples were collected during the winter and summer seasons at two municipal WWTPs receiving hospital wastewater, at one WWTP that does not receive hospital wastewater, and from the surface water downstream of the WWTPs.

A second objective of this study is to (2) better characterize the potential for ARG proliferation in the environment. Many ARGs are located on mobile genetic elements that allow for dissemination via horizontal gene transfer mechanisms and persist in the environment even after the host bacteria dies. This study differentiates between the live and dead fraction of cells containing ARGs using the DNA cross-linking dye propidium monoazide (PMA) in a viability based qPCR assay. This is the first known study using PMA to analyze fate of ARGs in environmental samples.

Methodology –

Sampling and Chemical Analyses
Samples were collected in sterile bottles from the influent, effluent, upstream waters and receiving waters of two WWTPs serving major New Jersey hospitals (H1 and H2) and one WWTP serving an area without major medical centers (C) (Table 1). Samples were collected three times during the summer and three times during the winter. Influent and effluent grab samples from each

sampling event were collected at 8AM, 10AM and 12PM and composited in the lab. Upstream and downstream samples consisted of one grab sample per sampling event. pH, conductivity, and temperature were measured in the field with a multimeter. Total suspended solids (TSS) for all samples were measured in the lab according to standard methods.

Molecular Analyses
Total ARG quantification by filtration in influent, effluent, upstream and downstream samples. Approximately 200–600 mL of each sample were filter concentrated to collect bacteria. DNA was extracted from filters using a FastDNA® SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA). DNA was diluted to reduce inhibition and qPCR was performed to quantify the ARGs *sul*1 for resistance to sulfonamides, *tet*(G) for tetracycline resistance, and blaTEM for beta-lactamase resistance. 16S rRNA was also quantified to estimate the total bacterial population and to account for sample to sample differences in DNA extraction efficiency.

Viability based ARG quantification in effluent and downstream samples. Bacteria were harvested by centrifuging 170-ml aliquots of the composited effluent samples and downstream samples at 4,000xg for 15 min and removing the supernatant except for the last 10 mL. PMA treatment followed by DNA extraction was applied to 500-µL aliquots of each 10 mL concentrated sample. PMA treatment consisted of adding PMA to the aliquots for a final concentration of 50 µM followed by incubation in the dark for 5 minutes followed by photoactivation for 15 minutes using a PMA-Lite™ LED Photolysis Device. For quality assurance on the performance of PMA, negative controls containing only dead cells were analyzed for 20% of samples. Negative controls were heat-treated field samples at 100°C for 10 minutes followed by treatment with PMA.

PMA treated samples were used to quantify only the ARGs originating from live cells. DNA was also extracted from 500-µL aliquots not treated with PMA to quantify ARG concentration originating from nonviable cells. The parallel treatment of samples with and without PMA treatment allowed for comparison between total and live ARGs in the effluent and downstream of each WWTP. DNA was diluted 1:50 to reduce inhibition and qPCR was performed to quantify the ARGs *sul*1, tet(G), and blaTEM, along with 16S rRNA.

Principal Findings and Significance -

Filtered Samples:
Samples were filtered to compare ARG concentrations (1) at different sampling locations within a given WWTP/surface water system, (2) in summer to winter, and (3) for WWTP with and without inputs from major health care facilities. Average *sul*1 concentrations in filtered samples were highest in influent samples and lowest in upstream and downstream locations for a given sampling season and WWTP (Fig. 1). Influent concentrations of *sul*1 were approximately the same during the two seasons at all three WWTPs. Interestingly, summer *sul*1 concentrations were lower than winter concentrations in the upstream samples for sites H1, H2 and C. Concentrations were lower in summer than winter in the effluent samples for sites H2 and C and in the downstream samples for sites H1 and C. Comparing the three WWTPs sampled the control WWTP had the lowest observed *sul*1 concentrations at various sampling locations in summer. Seasonal variation was not observed in H1 effluent or in H2 downstream sampling sites. Winter results for *tet*(G) exhibited a similar pattern as *sul*1 between sampling locations with the influent exhibiting the highest

concentrations. Significant differences were not observed for *tet*(G) between H1, H2 and C WWTPs (Fig 2).

Viability Analysis:

A viability based qPCR assay was adapted to determine whether ARG were present in viable cells or extracellularly/in cells with compromised membranes. This method was applied to effluent and downstream samples to determine what proportion of the ARG were present in viable cells and to determine if this proportion shifted between the outfall at downstream sampling location. It is worth noting that the detection limit for this assay is lower than for the total ARG described in the previous section due to the fact that for these analyses cells were harvested using centrifugation. Thus, while *sul*1 was detected in all effluent and downstream samples using the filtration method, *sul*1 was detected with varying frequency in downstream and effluent samples at H1, H2 and C WWTPs using the viability assay. It was detected most frequently in the effluent of H1 (6/6 sampling events), followed by H2 (4/6 sampling events) and with least frequency at C (2/6 sampling events). For detection in the downstream samples, *sul*1 was detected most frequently in downstream of H2 (4/6 sampling events), C (3/6 sampling events), and with least frequency at H1 (1/6). The frequency of downstream detection may be a function of wastewater dilution factor and distance downstream that sampling was feasible (due to access issues due to large amounts of private property), which varies by WWTP. With the exception of the 8/31/2015 sampling at H2, *sul*1 gene copy numbers were greater in the total than the viable fraction by an average of 1.1-log units where measurable (i.e detected in both total and viable) (Fig 3).

qPCR and data analysis is currently ongoing for summer and winter samples. All samples will be run with primers quantifying *sul*1, *tet*(G), blaTEM, and 16S rRNA gene targets. Data sets are at various levels of completion (Table 2). ARG results will be evaluated and normalized to 16S rRNA data, which serves as a surrogate for the total bacterial community. Further statistical analyses are underway. In addition, other possible fate and transport factors will be evaluated such as: distance of upstream and downstream locations from respective wastewater treatment plants, ratio of hospital beds to persons in a sewershed, and approximate area of the sampled water body.

Table 1 Proximity (in miles) of upstream and downstream sampling locations to the two hospital-influenced wastewater treatment plants (H1 and H2) and the control wastewater treatment plant (C).

	Upstream	Downstream
H1	1.4	1.8
H2	2.4	1.6
C	0.75	1.1

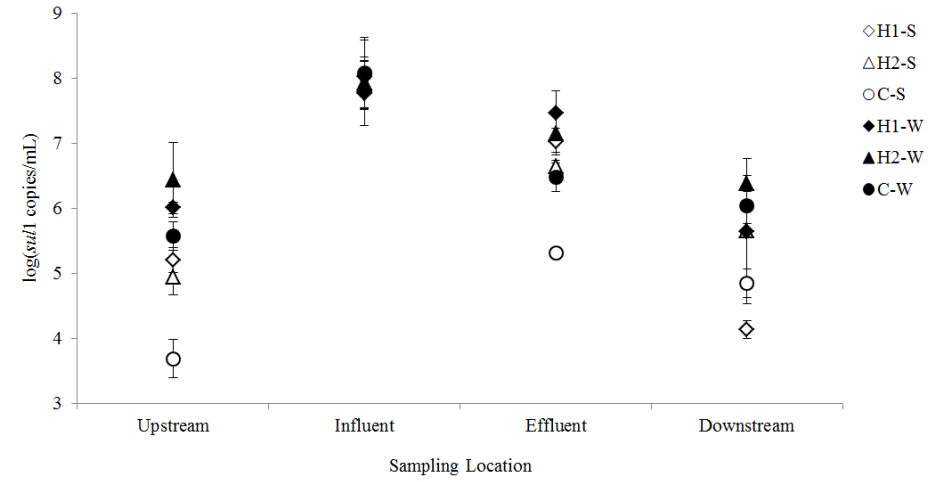

Fig. 1 Summer (S) and winter (W) upstream, influent, effluent and downstream *sul*1 concentrations measured in filtered samples (representing total *sul*1) from three wastewater treatment plants (H1, H2 and C). Error bars are the standard deviation of the three sampling events that occurred during each season.

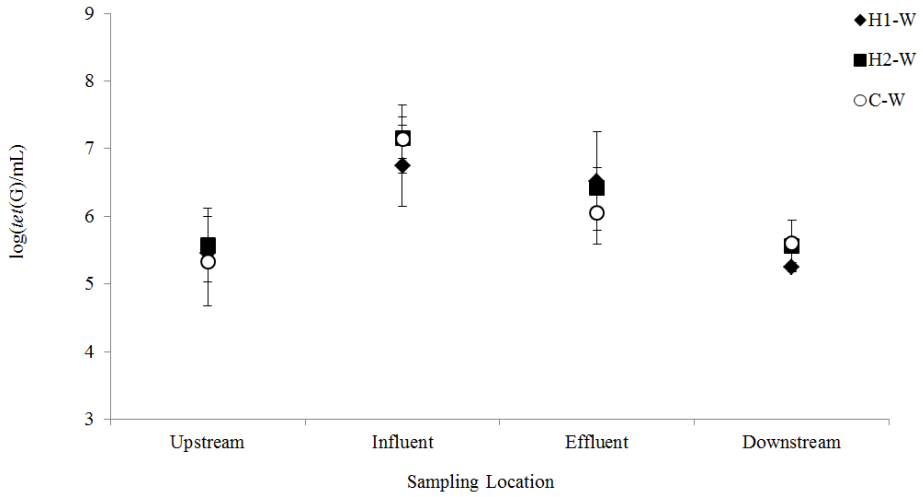

Fig. 2 Winter upstream, influent, effluent and downstream *tet*(G) concentrations measured in filtered samples from three wastewater treatment plants (H1, H2 and C). Error bars represent the standard deviation of the three sampling events that occurred during the winter season.

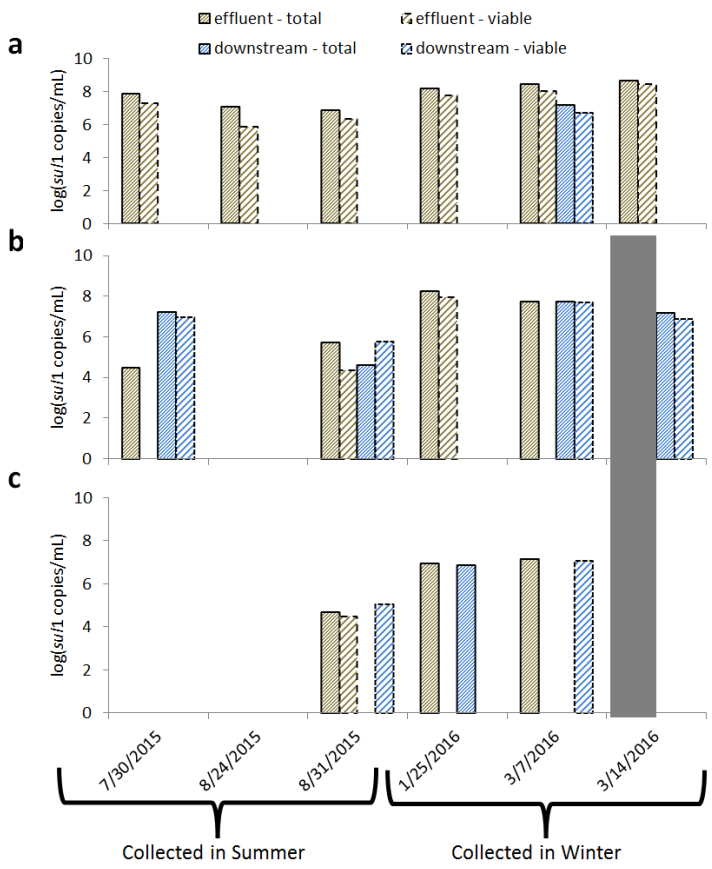

Fig. 3 Viability analysis of effluent and downstream samples collected in summer and winter from (a) H1, (b) H2, and (c) control WWTPs. Samples covered by a gray bar were not yet run at the time of analysis. *sul*1 was not detected in other samples where data is not shown.

Table 2. qPCR runs conducted to date.

		sul 1		tet(G)		bla_{TEM}		16S	
		filtered samples	live/dead samples	filtered samples	live/dead samples	filtered samples	live/dead samples	filtered samples	live/dead samples
Summer	7/30/2015	✓	✓			✓		✓	✓
	8/24/2015	✓	✓			✓		✓	✓
	8/31/2015	✓	✓					✓	✓
Winter	1/25/2016	✓	✓	✓	✓	✓	✓	✓	
	3/7/2016	✓	✓	✓		✓	✓		
	3/14/2016	✓	✓	✓		✓	✓		

Significance:

The results obtained to date demonstrated detection of various ARGs in the surface waters surrounding WWTPs in New Jersey. Average seasonal *sul*1 concentrations were higher in downstream samples than upstream samples at H2 and C in summer and at C in winter. In contrast, average downstream *sul*1 concentrations were lower than upstream samples at H1 in summer and

winter, and at H2 in winter. The results of additional genes at different times of the year may provide additional insight or trends.

This research is especially important for New Jersey because New Jersey has high de facto water reuse rates. The high ARG concentrations measured in upstream samples raise concern about ARG concentrations at drinking water collection points. The proximity of upstream sampling locations to pumping stations will be determined.

The results of this research will inform the development of treatment and policy recommendations to help combat the crises of antibiotic resistance. Final conclusions from this study will include:

- An evaluation of ARG seasonal variations in WWTP influents and effluents and receiving waters of New Jersey.
- Measurement of the ability of WWTPs to remove or inactivate ARG carrying bacteria.
- Evaluation of WWTPs' contribution to ARGs in surface waters, and if servicing a hospital population increases that contribution.
- Comparison of the live and dead fractions of antibiotic bacteria leaving WWTPs to downstream concentrations.

(4) **Publications or Presentations**:

1. Articles in Refereed Scientific Journals:
In preparation

2. Book Chapter:
none

3. Dissertations:
Results will be included in my dissertation, anticipated graduation 2018

4. Conference Proceedings

Yam, M., Eramo, A., Fahrenfeld, N. (**Poster).** Antibiotic resistant genes (ARG) in wastewater and urban surface water. Rutgers Aresty Summer Research Symposium. August 7, 2015.

5. Other Publications
NA

Perflourinated compounds (PFOS, PFOA, PFNA) exposure in laboratory zebrafish and field-caught small mouth bass produce different toxicology profiles based on morphometric, gene expression, and behavioral endpoints

Basic Information

Title:	Perflourinated compounds (PFOS, PFOA, PFNA) exposure in laboratory zebrafish and field-caught small mouth bass produce different toxicology profiles based on morphometric, gene expression, and behavioral endpoints
Project Number:	2015NJ368B
Start Date:	3/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	NJ-006
Research Category:	Water Quality
Focus Category:	Toxic Substances, Groundwater, Surface Water
Descriptors:	None
Principal Investigators:	Carrie Greenfield, Keith R Cooper

Publications

1. Greenfield CE, Cooper, KR. 2015. Morphometric and gene expression changes as a result of perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA) and perfluorooctanoic acid (PFOA) exposure to embryonic zebrafish (Danio rerio) [abstract]. Presented at: Meeting of the Hudson-Delaware Regional Society of Toxicology and Chemistry; 20-21 Apr 2015; New Brunswick, NJ
2. Greenfield CE, Cooper, KR. 2015. Locomotion, anxiety, and feeding behavior changes as a result of PFOS, PFNA and PFOA exposure to embryonic zebrafish [abstract]. Presented at: 36th Meeting of the Society of Toxicology and Chemistry; 1-5 Nov 2015; Salt Lake City, UT
3. Jantzen, Carrie; Kate Annunziato; Sean Bugel; Keith Cooper, 2016, PFOS, PFNA, and PFOA sub-lethal exposure to embryonic zebrafish have different toxicity profiles in terms of morphometrics, behavior, and gene expression, Aquatic Toxicology, (175) 160-170.

Perfluorinated Compound (PFOS, PFOA, PFNA) exposure in laboratory zebrafish produces different toxicology profiles based on morphometric, gene expression, and behavioral endpoints.

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2. Number of students supported:

Undergraduates: 2
Masters' students: 0
Ph. D. students: 3
Postdoctoral Associates: 0

3. Project Summary

Polyfluorinated compounds (PFCs) are an emerging contaminant in New Jersey watersheds (Post et al. 2013). In 2009, at least three PFCs in particular have been found in elevated concentrations in NJ public water wells (Post et al. 2013). These compounds are composed of a long carbon backbone that is fully fluorinated with either a carboxyl, alcohol, or sulfonate terminal group (Lindstrom et al. 2011). PFCs are both oil and water resistant, have a high thermal stability, are chemically inert, and can be used as surfactants. (Lindstrom et al. 2011). Due to these properties, PFCS have been used in a number of applications such as water and oil resistant coatings, aviation hydraulic fluids, fire-fighting foams, adhesives, industrial surfactants and emulsifiers (Post et al. 2013). However, these same properties also allow PFCs to be bioaccumlative and biomagnify through the food chain in the environment. Three PFCs that are commonly found in New Jersey watersheds and often studied are perfluorooctane sulfonate (PFOS), perfluorooctonate (PFOA), and perfluorononononaoic acid (PFNA)

PFC toxicity has been studied more extensively in mammals. PFOS exposure to rats and mice has been seen to cause neurotoxic effects, decreased body weight, increased liver weight and increased mortality (Lindstrom et al. 2011). PFOA exposure in rodents has led to observations of increased liver, pancreas, and testicular tumors. Additional effects include liver enlargement and changed in lipid metabolism (Cook et al. 1992). There has been limited human toxicity data on PFC exposures. PFOA has been the PFC most widely studied in humans, and correlations have been made between PFOA exposure and prostate cancer (Lindstrom et al. 2011). In studies of aquatic organisms, PFOA and PFOS have been

seen to affect growth, embryo development, metamorphosis, reproduction, and behavior (Lindstrom et al. 2011).

PFCs, in particular PFOS, have been detected in wildlife, environment, humans, and drinking water on every continent worldwide (Giesy and Kannan 2001). In 2009, at least three PFCs in particular have been found in elevated concentrations in NJ public water wells (Post et al. 2013). These include PFOA (57% of samples; up to 100 ng/L), PFOS (20% samples) and PFNA (30% samples, up to 96 ng/L)(Post et al. 2013). It has been shown that many common waste water treatment processes do not effectively remove PFCs from drinking water sources (Post et al. 2013). This indicates that not only are PFCs present in New Jersey water systems, but some are capable of bioaccumulation, biomagnification, and once in the water supply they cannot easily be removed. The combination of these factors highlights the needs for a better understanding of how these compounds function in individuals at the tissue level and determine the effects that this may have at the population level.

The specific objectives investigated in this study were to (1) determine how PFOA, PFOS, and PFNA individually and as mixtures affect gene expression of calcium modulation, tissue remodeling, and cell cycle/cell death pathways in the zebrafish model and (2) correlate the gene expression changes found after PFOA, PFOS, and PFNA exposure with behavior of zebrafish to determine effects of individual and population fitness of aquatic organisms exposed to PFCs.

Methods

The AB strain zebrafish (Zebrafish International Resource Center, Eugene, OR) were used for all experiments. Breeding stocks were bred and housed in Aquatic Habitats (Apopka, FL) recirculating systems under a 14:10 hour light: dark cycle. System water was obtained by carbon/sand filtration of municipal tap water and water quality was maintained at <0.05 ppm nitrite, <0.2 ppm ammonia, pH between 7.2 and 7.7, and water temperature between 26 and 28°C. All experiments were conducted in accordance with the zebrafish husbandry protocol and embryonic exposure protocol (#08-025) approved by the Rutgers University Animal Care and Facilities Committee.

Zebrafish embryos were exposed at 3 hours post fertilization (hpf) to PFOS, PFOA, or PFNA at concentrations of 0, 0.02 µM, 0.2 µM, or 2.0 µM (0, 20, 200, 2000 ppb) for 120 hours in a static non-renewal protocol. All compounds were dissolved in water. After this time, fish were transferred to non-treated system water and fed 2 times daily with Zeigler Larval AP50 (Aquatic Habitats, Apopka, Florida). Therefore, the only exposure was through the water from 3hpf to 120hpf (5 days), which corresponds to embryonic to yolk sac larval exposure.

The exposure followed modified OECD 212 protocol (OECD. 2011), where in addition to the endpoints of lesion presence, length, weight, and mortality as stated in the protocol, cranial facial development and gene expression were also analyzed. At 120 hpf, morphometric measurements were recorded and gene expression analyzed. Further modification to the OECD protocol was to extend the study beyond the exposure time-points which allowed for removing any chemical exposure from 120hpf to 14 dpf. At 14 dpf swim activity endpoints were collected. Each treatment compound and corresponding control group was set up as individual experiments, and the sample size was dependent on number of embryos produced from the stock breeding sets. No experiment had mortality greater than 20% of the starting sample size.

Approximately thirty individual animals from each treatment and control group were fixed in formalin and then stained for bone and cartilage following a two-color acid free Alcian Blue/ Alizarin red stain (Walker and Kimmel 2007). Photographs were taken using a Scion digital camera model CFW-1310C mounted on an Olympus SZ-PT dissecting microscope and cartilage/bone were measured using Adobe Photoshop. Endpoints examined included total body length, interoccular distance, and yolk sac size to assess larval growth, cranial facial development, and nutrient storage and usage, respectively. Measurements could be made at the nanometer level. Each experiment was independently replicated three times.

Four replicates of each treatment and control each consisting of 25 animals were exposed for 120 hours and then transferred to clean water until they were two weeks old. The swim activity was performed in 24 well plates with a single animal in each well. After 1 hour incubation under fluorescent light, the light was turned off and zebrafish recorded with an infrared filter for 30 minutes. The recordings were analyzed with Noldus Ethovision Software (Leesburg, VA) for endpoints of total distance traveled, average swim velocity, and time and frequency of swimming in the middle of the well. The total distance traveled and swimming velocity measurements are indicators of general locomotion and activity. The time and frequency in the middle of the well is a measurement of stress and anxiety (Schnorr et al. 2012). These innate behaviors have been assumed to play important roles in predator-prey interactions (Kalueff et al. 2013). Control and each treatment group had approximately N=50 fish/replicate. Each experiment was independently replicated twice.

Four replicates (N=25 fish/replicated) from each treatment and control were snap frozen in liquid nitrogen and RNA extracted using RNAzol reagent (Sigma-Aldrich, St. Louis, MO). DNA contamination was removed with the DNA-*free*™ kit (Life Technologies). Reverse transcription was performed with the High-Capacity cDNA Reverse Transcription Kit (Life Technologies, Carslbad, CA) and real-time qPCR was performed using iQ™ SYBR® Green Supermix (Bio-Rad, Hercules CA). The qPCR protocol was used: 35 cycles of: 95°C for 15 seconds and 60°C for 1 minute. The housekeeping gene used was *b-actin*, which has been determined to be unaffected by any treatments in this study. Analysis was performed using a standard curve method. The four genes examined and primer sequences are listed in Table 1. Each independent experiment was replicated 3 times.

Gene Symbol	**Gene Name**	**Primer Sequences**
slco2b1	Solute carrier organic anion transporter 2b1	F: 5'- TTG CCC TGC CTC ACT TCA TT-3' R: 5'-AGG CTG GAG TTG AGT CTG GT-3'
tfc3a	Transcription factor 3a	F: 5'-TGA GAA ACC GCA GAC CA ACT -3' R: 5'-CTT GCT GCT CCA GGT TGA GA-3'
Ihha	Indian hedgehog homolog a	F: 5'-TGA GTC CAA AGC TCA CAT CCA-3' R: 5'-AGG CTG GAA AAC AAC CAC CG-3'
Wnt5b	Wingless-type MMTV integration site family 5b	F: 5'-GCA AAG CCA TCT TTC CCT GAA-3' R: 5'-TGT ATC CCG AGC AAA AAC CTG-3'

Table 1: List of transcripts and primer sequences for gene expression analysis.

To analyze a broader selection of transcripts, messenger RNA expression was analyzed for a suite of 100 developmentally relevant transcripts using qRT-PCR methods previously described (Bugel et al. 2014). Transcripts selected for this analysis were broadly part of pathways involved in tissue remodeling,

calcium signaling, cell cycle and cell death, growth factors, angiogenesis and hypoxia (Suppl. Table 1). For this gene expression analysis, embryonic zebrafish were exposed to 2.0 uM of PFOA, PFOS or PFNA until 120 hpf. Animals were observed daily and no lesion occurrence was recorded. Four replicates (N=25 animals/replicate) were snap frozen as whole animal pool replicates at 120 hpf and analyzed. Briefly, total RNA was isolated using RNAzol® RT (Molecular Research Center, Inc., Cincinnati, OH) and complementary DNA was synthesized using the Applied Biosystems High-Capacity cDNA Reverse Transcription kit (Life Technologies, Carlsbad, CA). qRT–PCR was performed using a StepOnePlus™ Real–Time PCR System with Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA). All primers used are listed in (Bugel et al. 2014). *β-actin* was used as a housekeeping transcript for normalization, and relative expression was quantified using the ΔΔCt method (Pfaffl 2001).

All statistical analysis were performed using SigmaPlot™ (v. 11.0) and R (v. 3.2.2). Morphometric measurements were analyzed using a one-way analysis of variance (ANOVA). Swim activity was analyzed using a two way ANOVA based on treatment and 5-minute time intervals. Gene expression data was evaluated using either ANOVA, or Student's t-test when the data passed normality and variance tests. If the data was not normal, a log transformation was used, and t-test performed. After log transformation, if data cannot be normalized, a Wilcoxon test was used. Statistical significance was at a p-value $\leq$ 0.05

Results – Principle Findings and Significance

Morphometric Data

Morphometric endpoints of interoccular distance, total body length, and yolk sac area were assessed to determine if exposure to PFOS, PFOA, or PFNA affected embryonic development. For all treatment groups, all concentrations (0.02, 0.2, 2.0 µM) were sub-lethal, and there was no significant difference for the prevalence of death, embryonic abnormalities, or delayed development. Summary of all measurements for each compound can be seen in Table 2.

Total Body Length (mm)				
	Control	**0.02**	**0.2**	**2.0**
PFOS (N=26-29)	4.76±0.23	4.75±0.16	4.63±0.22*	4.67±0.23*
PFNA (N= 20-23)	4.72±0.24	4.74±0.167	4.64±0.19	4.63±0.11*
PFOA (N= 30-38)	4.79±0.12	4.83±0.11	4.83±0.22	4.68±0.13*
Interoccular (mm)				
	Control	**0.02**	**0.2**	**2.0**
PFOS (N=26-29)	0.23±0.02	0.21±0.02*	0.22±0.01*	0.22±0.02*
PFNA (N= 20-23)	0.24±0.03	0.24±0.02	0.23±0.02	0.23±0.03
PFOA (N= 30-38)	0.18± 0.05	0.19±0.04	0.19±0.03	0.20±0.04*
Yolk Sac Area (mm^2)				
	Control	**0.02**	**0.2**	**2.0**
PFOS (N=26-29)	0.45±0.06	0.48±0.08	0.47±0.04	0.43±0.06*
PFNA (N= 20-23)	0.43±0.04	0.43±0.04	0.43±0.03	0.45±0.05*
PFOA (N= 30-38)	0.48±0.06	0.50±0.08	0.49±0.04	0.55±0.08*

Table 2. Summary of morphometric endpoints measured in 5 days post fertilization (dpf) zebrafish after exposure to PFOS, PFOA, or PFNA. Values are the average ± standard deviation from the mean. An asterisk (*) indicates a statistical significant value, $p< 0.05$, one-way ANOVA compared to corresponding control.

The 5 dpf total body length measurement was used to determine if exposure to PFOS, PFOA or PFNA during the embryonic life stages had an effect on larval growth. PFOA, PFOS, and PFNA all resulted in significantly reduced body length at the 2.0 μM treatment. Additionally, PFOS exposure at 0.2 μM also significantly reduced the total body length. No significant differences were observed at lower concentrations of PFOA, PFOS, or PFNA.

Interoccular distance was used to indicate changes in craniofacial development following embryonic PFC exposure (Table 2). PFOA treatment at 2.0 μM resulted in a significant increase of interoccular distance, while PFOS at all concentrations (0.02 μM, 0.2 μM, 2.0 μM) significantly decreased interoccular distance. PFNA exposure at all doses, and PFOA exposure at the lower concentrations had no effect on this measurement.

The yolk sac is comprised of vitellogenin derived yolk-proteins, maternally supplied by the oocyte to fully support nutritional needs of the embryo/larvae prior to beginning feeding after 120 hpf. Measuring the yolk sac size is an important endpoint to determine if PFOS, PFOA, or PFNA affected the volume of the available nutrients and utilization in embryonic zebrafish. PFOS (2.0 μM) treated zebrafish had a significantly decreased yolk sac size, while PFOA (2.0 μM) and PFNA (2.0 μM) treated animals both had a significantly increased yolk sac size (Table 2).

Swim Activity

Swim activity data were collected in five minute time bins for a total of 25 minutes. Cumulative data for each measurement are listed in Table 3. PFOS (0.02 μM, 2.0 μM) PFOA (0.2 μM and 2.0 μM) and PFNA (0.2 μM) exposure caused a significant increase in the distance traveled.

Distance Traveled (mm)				
	Control	**0.02**	**0.2**	**2.0**
PFOS (N=24-35)	88.75±9.31	98.15±12.95*	95.63±10.41	108.15±11.22*
PFNA (N= 14-27)	78.40±6.87	73.78±7.89*	83.01±9.06	79.55±10.36
PFOA (N= 30-38)	93.39±9.95	104.50±12.41	97.24±10.72*	106.95±11.23*
Time in Middle of Well (seconds)				
	Control	**0.02**	**0.2**	**2.0**
PFOS (N=24-35)	166.02±22.84	229.60±41.69	209.43±32.68	169.70±31.35
PFNA (N= 14-27)	211.39±33.34	200.44±27.67	243.53±30.83*	241.92±29.68*
PFOA (N= 30-38)	208.74±37.57	195.78±29.88	195.84±30.43	193.91±37.43
Crossing Frequency (crosses/ 25 minutes)				
	Control	**0.02**	**0.2**	**2.0**
PFOS (N=24-35)	71±10	76±9*	83±13*	78±10*
PFNA (N= 14-27)	84±10	73±8	67±7	79±9
PFOA (N= 30-38)	74±9	88±13	85±12	90±12

Velocity (mm/s)				
	Control	**0.02**	**0.2**	**2.0**
PFOS (N=24-35)	0.39±0.08	0.37±0.10	0.42±0.15	0.43±0.14*
PFNA (N= 14-27)	0.42±0.14	0.36±0.08*	0.35±0.09*	0.37±0.07*
PFOA (N= 30-38)	0.42±0.10	0.43±0.10	0.41±0.09	0.45±0.14

Table 3. Summary of swim activity endpoints. Values are the average ± standard deviation from the mean. An asterisk (*) indicates a statistical significant value, $p < 0.05$, one-way ANOVA compared to corresponding control.

Swimming velocity is a measurement used to assess the average swimming speed of zebrafish for the duration of the activity assay (Table 3). Data points were obtained in five minute intervals. PFOS (0.02 µM) and PFNA (0.02, 0.2, 2.0 µM) exposure both resulted in a significant decrease in swimming velocity. In PFOA exposure (2.0 µM) swimming velocity was significantly increased.

Zebrafish can exhibit thigmotaxis (movements towards or away from a stimulus) as a stress response to new environments. The measurement of time spent swimming in the middle of the assay well and the number of times the animal swam across the well is used as an indicator of stress or anxiety (Blaser et al. 2010). PFNA exposure (0.02, 0.2, uM) significantly increased the time spent in middle of the well. PFOS and PFOA treatment had no significant effects on this endpoint. PFOS (0.02, 0.2, 2.0 µM) showed a significant increase in crossings. PFNA and PFOA did not show any significant effects on this endpoint.

Gene Expression

Targeted gene expression was analyzed for organic anion transporter 2b1 (*slco2b1*) and striated muscle development (*tfc3a*) transcripts in 120 hpf zebrafish across all concentrations (Figure 1). *Slco2b1* was significantly upregulated in PFOS (2.0 µM) and PFOA (all treatments), and significantly down-regulated in PFNA (all concentrations). *Tfc3a* expression was significantly higher in PFOS (2.0 µM) and PFOA (0.2, 2.0 µM). *Ihha* (hedgehog gene) (data not shown) showed no significant difference in expression at any PFC concentration. *Wnt5b* (calcium modulation pathway) (data not shown), had a significant increase in expression after PFOS exposure (2.0 µM, fold change +2.18).

Gene expression was performed on a battery of genes involved in tissue remodeling, cell cycle, cell death, angiogenesis, hypoxia, calcium signaling, and growth factors. Genes that were significantly different in expression after exposure to PFOS, PFOA, or PFNA are listed in Table 4 below. Of the 106 genes analyzed, *ap1s1* was the only gene that was significantly different (decreased) across all three PFCs. Of the three compounds, PFOS significantly affected the greatest number of genes (*calm3a, cdkn1a, cyp1a, flk1, tgfb1a*). PFOA and PFNA each only significantly altered one gene (*c-fos* and *tgfb1a*, respectively).

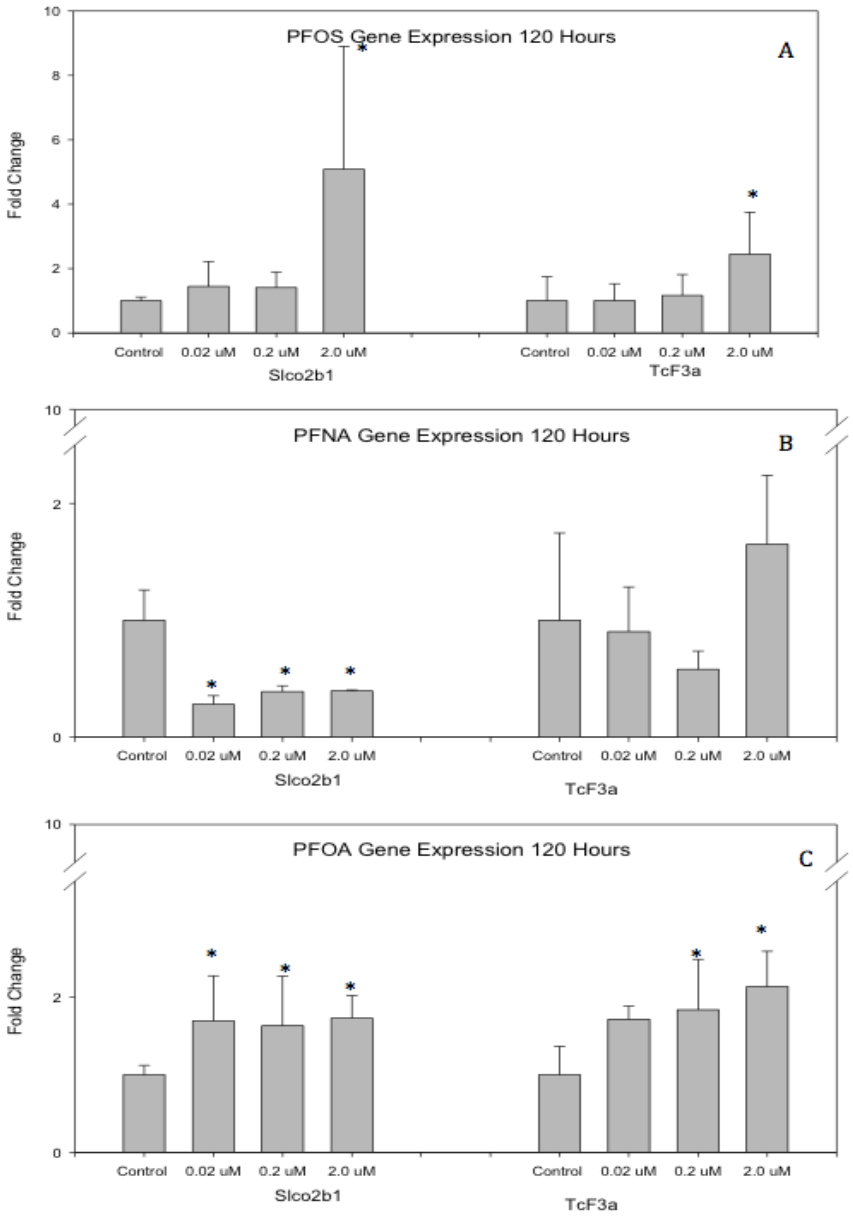

Figure 1. Embryonic zebrafish gene expression (5dpf) after exposure to [A] PFOS, [B] PFNA, and [C] PFOA. Bars represent mean fold change and standard deviation. N= 4 replicates of 25 pooled animals for each exposure group. An asterisk (*) indicates a statistical significant value, $p< 0.05$, one-way ANOVA.

Gene symbol	**Gene Name**	**Function**	**Compound**	**Fold Change ± SD**
ap1s1	Adaptor related protein complex 1, sigma subunit 1	Protein transport	PFOS PFOA PFNA	0.58 ± 0.14** 0.59 ± 0.21** 0.57 ± 0.09**

calm3a	Calmodulin 3a	Calcium ion binding	PFOS	1.17 ± 0.06*
cdkn1a	Cyclin-dependent kinase inhibitor 1A	Apoptosis, mitotic cell cycle regulation	PFOS	0.72 ± 0.13*
cyp1a	Cytochrome P450 1A	Aromatic compound metabolism	PFOS	0.60 ± 0.20*
flk1	Kinase insert domain receptor like	angiogenesis	PFOS	0.66 ± 0.17*
tgfb1a	Transforming growth factor beta 1a	Growth factor activity	PFOS PFNA	0.82 ± 0.07* 0.82 ± 0.09*
c-fos	v-fox FBJ murine osteosarcoma viral oncogene homolog Ab	Transcription factor complex	PFOA	1.63 ± 0.19*

Table 4. List of genes that were significantly increased or decreased in transcript analysis of 120 hpf zebrafish exposed to PFOS, PFOA, and PFNA (2.0 µM). An asterisk (*) indicates a statistical significant value $p < 0.05$. A double asterisk (**) indicates a statistical significant value $p < 0.01$.

Significant Findings

The toxic effects following PFOA, PFNA, and PFOS exposure to embryonic and larval zebrafish have different biomarker profiles. It is likely that both the carbon chain length and the terminal group play a role in the observed effects on morphometrics, gene expression, and behavior. A number of these changes are reported in this paper at PFC concentrations ranging from 5.0 -25.0 fold below our previously calculated LC50 values (PFOS 25 µM, PFNA 10 µM, PFOA 35 µM), and, to our knowledge, at lower concentrations than previously reported in the teleost literature.

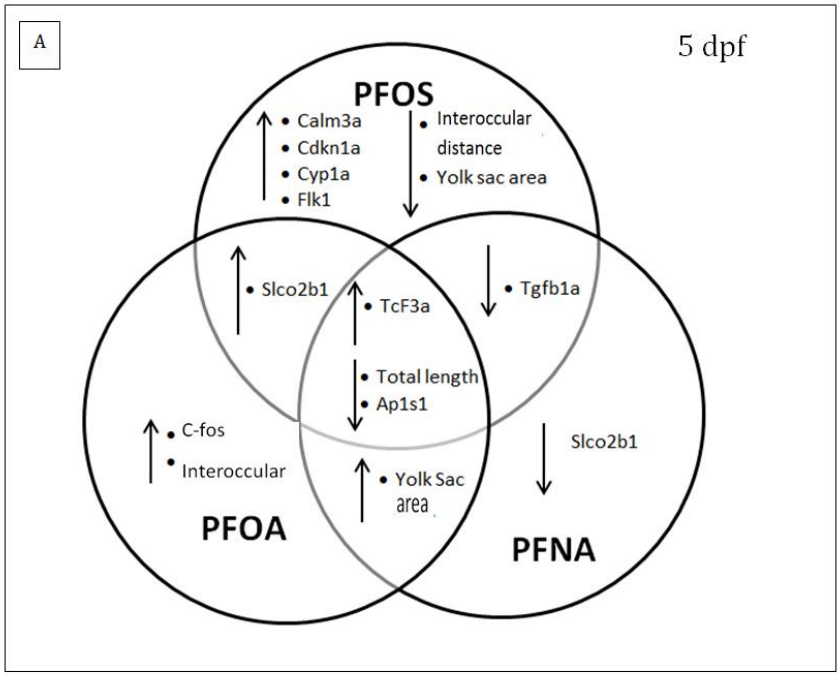

Figure 2. Venn diagram of morphometric, gene expression, and swimming activity endpoints for PFOS, PFOA and PFNA exposure at all concentrations examined for 5 days post fertilization. Up arrows (↑) indicate significant increase compared to control ($p < 0.05$). Down arrows (↓) indicate a significant decrease compared to control ($p<0.05$).

The data presented in this study support the hypothesis that sub-lethal embryonic exposure to PFOS, PFNA, or PFOA will result in different responses in regards to morphometric, behavior, and gene expression in both yolk sac fry and larval zebrafish. All three PFCs commonly resulted in a decrease in total body length, increased *tfc3a* (muscle development) expression and decreased *ap1s* (protein transport) expression at 5dpf, and hyperactive locomotor activity 14 dpf. All other endpoints measured at both life-stage time points varied between each of the PFC.

At 5 dpf, PFCs are having subcellular effects, which are being translated into morphological and behavioral effects at concentrations well below the lowest observed sub-lethal concentrations (PFOS 20 uM, PFOA 30 uM, PFNA 5 uM). PFOS was more potent than PFOA and PFNA in altering gene expression, growth, behavior and yolk sac utilization. This correlates to studies in many other organisms including daphnia, medaka, rats, and aquatic invertebrates (Cui et al. 2009; Ji et al. 2008; Li 2009). PFOS had the greatest number of significant detrimental outcomes in the endpoints studied. While PFOA exposure at 5dpf had a smaller number of significant endpoint effects, at 14dpf it had the most persistent effect on growth in the juvenile zebrafish.

Our studies have focused on the embryo to juvenile life stages, but additional studies are needed to determine what the effects of altered nutrient transport, production, and storage will be later in life in adult animals as well as in the subsequent unexposed generation.

Publications/ Presentations

Jantzen, Carrie; Kate Annunziato; Sean Bugel; Keith Cooper, 2016, PFOS, PFNA, and PFOA sub-lethal exposure to embryonic zebrafish have different toxicity profiles in terms of morphometrics, behavior, and gene expression, Aquatic Toxicology, (175) 160-170

Greenfield CE, Cooper, KR. 2015. Locomotion, anxiety, and feeding behavior changes as a result of PFOS, PFNA and PFOA exposure to embryonic zebrafish [abstract]. Presented at: 36th Meeting of the Society of Toxicology and Chemistry; 1-5 Nov 2015; Salt Lake City, UT

Greenfield CE, Cooper, KR. 2015. Morphometric and gene expression changes as a result of perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA) and perfluorooctanoic acid (PFOA) exposure to embryonic zebrafish (Danio rerio) [abstract]. Presented at: Meeting of the Hudson-Delaware Regional Society of Toxicology and Chemistry; 20-21 Apr 2015; New Brunswick, NJ

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Suspended particulate toxicity: an emerging area of concern for physiochemical exposures to fish and other aquatic organisms

Basic Information

Title:	Suspended particulate toxicity: an emerging area of concern for physiochemical exposures to fish and other aquatic organisms
Project Number:	2015NJ369B
Start Date:	3/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	NJ-006
Research Category:	Water Quality
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Descriptors:	None
Principal Investigators:	Daniel Millemann, Keith R Cooper

Publication

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(1) PI information: List all PIs, addresses, email addresses, phone numbers

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(2) Numbers of Students Supported: list the NUMBER of students in each category that were in any way supported by WRRI funding.
Undergraduates: -
Masters' students: -
Ph. D. students: 1
Postdoctoral Associates: -

(3) Any Notable Achievements (Awards, Recognition, etc.), or direct application of the research by Management Agencies, Nonprofits/NGOs, etc.

$500 Travel award from local Hudson Delaware Chapter of SETAC to participate in 5th annual YES meeting, information below.

(3) Project Summary:

Problem and Research Objectives:

Studies on the aquatic toxicity of PM have not yet been conducted and a more thorough understanding of this toxicity is necessary to address ongoing concerns in ecosystem functioning[1]. Our lab has shown that Gulf menhaden (*Brevoortia patronus*) from the northern Gulf of Mexico accumulated PAH based particulates in their heart ventricles and vasculature[2]. This accumulation occurred in a temporal pattern consistent with the Deepwater Horizon oil spill, suggesting a mechanism by which PAH derived suspended particulates are created (by atmospheric deposition or crude oil weathering) and then taken up by aquatic organisms. Our finding shows a direct correlation to the particulates and focal lesions such as necrosis due to the blocking of microcapillaries and perhaps oxidative stress resulting from desorption of reactive PAHs. This finding is supported by a study not directly related to PM; Woodhead (1981) showed that black carbon (as India ink) was absorbed through the lower gastrointestinal track of the Amazon molly (*Poecilia formosa*) and distributed to the heart ventricle and kidney [3]. This study, although not specifically examining particulate matter, determined that inert carbon was absorbed into the fish, likely by macrophages. The proposed study will demonstrate a specific exposure scenario in which toxic particulates are absorbed into a teleost species relevant to New Jersey.

Suspended PM toxicity is rarely examined in aquatic toxicology and could greatly impact the critical water resources of New Jersey. New Jersey has a history of PM issues [4] and the urbanization of coastal watersheds in addition to potential oil spills may impact aquatic organisms more than is currently understood. It has been demonstrated that atmospheric deposition of soot particulates [5] as well as the weathering of crude oil contaminants [6] are at least partly responsible for the introduction of these particulates into aquatic ecosystems. Ultimately this project will

provide evidence of PM toxicity to aquatic organisms in a way that is meaningful and applicable to water resources in New Jersey. There could be direct applications to regulate particulate loadings in the receiving waters of urbanized watersheds and as well as applications to post oil spill exposure assessments of affected wildlife. A better understanding of particulate toxicity in aquatic organisms is needed due to their formation, presence, and persistence in aquatic environments.

The goal of the proposed study is to identify the potential toxic impacts of PAH particulate matter on aquatic organisms, specifically a relevant fish species. These particulates are environmentally relevant due to their deposition on impervious surfaces as a result of anthropogenic activities as well as potential for creation from larger scale events such as oil spills, not uncommon in New Jersey.

PAH derived particulates will distribute to vital organs (kidney, heart) of the selected fish species, which will result in focal damage due to ischemia and desorption of reactive compounds from particulate surfaces.

Methodology:

All zebrafish handling methods were approved by the Rutgers IACUC guidelines. AB and Fli strain zebrafish were maintained on an Aquatic Habitats (Apopka, FL) Stand-Alone System (10% daily water change, 22–25 ◦C) with a 14:10 light:dark photoperiod. Adults were fed brine shrimp (*Artemia salina*) and TetraMin Flakes. Embryos were raised at 26◦C in embryo media.

Gavage Exposure

Two types of Fluorescebrite multifluorescent microspheres™ (0.2 µm and 1 µm in diameter; excitation maxima of 377, 517 and 588nm, and emission maxima of 479, 546 and 612nm) and acetylene black (a diesel soot surrogate) were used to assess the possible translocation of particulates across the gastrointestinal epithelium. Five to ten adult AB-strain zebrafish (approximately 24 months old) were acclimated to 10 gallon tanks for 48 hours prior to their first gavage. Zebrafish were fed throughout the course of the gavage studies with standard flake formulation in the evenings 3 hours after gavage recovery. Solutions containing 2.5% weight/volume of each of these materials were used to gavage individual adult fish with methods modified from Collymore et al (2013) [7]. Acetylene black exposure solution and the respective controls also contained 0.4% Tween 20 surfactant to assist in the suspension of the particles. Prior to gavage, fish were each anesthetized with 200 mg/L of tricaine mesylate (MS222) until they no longer responded to physical stimulus (pinching of the tail). Five µL of gavage solution was administered and fish were placed into an aerated recovery tank with fresh system water before being returned to the original holding tank. This procedure was carried out every other day for a week for a total of three oral gavages. After one additional day of depuration, fish were sacrificed for blood analysis and histopathology.

On day 6, fish were anesthetized with 200 mg/L of tricaine mesylate (MS222) to enable collection of blood. The caudal fin was removed with an industrial razor blade and a smear was created with a drop blood collected from the caudal vein. At this point, the spine was severed and a cross section of the fish was taken from behind the pectoral fin, through the anterior body cavity and used to create and impression smear of the viscera, kidney, and muscle tissue onto slides coated with poly L lysine to promote the retention of muscle and viscera. Remaining tissues were frozen (fluorescent microspheres) or fixed in 10% buffered formalin (acetylene black) for additional

analysis. Formalin fixed tissue followed typical histological processing, was sectioned at 4-5 µm, and stained with hematoxylin and eosin for light microscopy.

Plate Exposure

Two week old AB and Fli-strain zebrafish larvae were placed randomly into the first, third, and fifth rows of a clear polyethylene 48-well plate prior to exposure to the aforementioned Flourescebrite microspheres™ to test the ability of these plastic microspheres to cross gill or gastrointestinal epithelium in a static bath exposure. The zebrafish rearing solution was carefully removed from each well until 200uL remained, at which point 800 µL of exposure solution or fresh rearing solution (for controls) was added giving a total volume of 1 mL and a concentration of 50 mg/L. Row one contained the control fish, row three contained the 0.2 µm microspheres and row 5 contained the 1.0 µm microspheres. Multiple replicates were conducted without feeding to maintain water quality in the small volumes. After 24 hours, fish were transferred to clean rearing solution to rinse off any remaining microspheres adhered to the surface epithelia, sacrificed with a lethal dose of MS222 and fixed in 10% buffered formalin. Due to the loss of fluorescence over time, fish were examined within 24 hours of the conclusion of the exposure to identify the location of fluorescent microspheres with an inverted microscope fitted with a fluorescent laser light source using a Trit C filter.

Acetylene Black Feed study

Twelve AB strain zebrafish, approximately 30 days post fertilization (dpf), were acclimated in 10 gallon aquaria for 24 hours prior to being fed a 2% mass/mass mixture of acetylene black in standard fish feed to assess the impact of acetylene black under chronic conditions. Each tank was given either 40 mg of control or spiked feed once per day for 60 days prior to sacrifice with an MS222 overdose. Water changes were conducted weekly to control for ammonia and nitrate levels. Temperature was maintained between 22 and 26 degrees Celsius throughout the duration of the experiment. Sacrificed fish were measured to assess growth over the 60-day exposure period and fixed in 10% buffered formalin. Histological processing was conducted as previously described.

Pulse-Dip Exposure

Adult zebrafish and mummichog were used to determine the penetration of particulate material after an acute, high dose exposure to acetylene black. Adult AB zebrafish (approximately 2 years old) were taken from our system after being retired from active reproduction. Adult mummichog were collected from Tuckerton, New Jersey and maintained in the laboratory at 22 degrees Celsius and 20 psu. Five fish of each species were exposed for 6 hours to 50 mg/L of acetylene black in a 2 L beaker to replicate conditions similar to a stormwater pulse event with elevated concentrations of suspended solids. The control and test solutions also contained 0.02% tween to assist in the initial suspension of the acetylene black particles. After six hours, the fish were placed into 1 liter of clean water to recover from the exposure and eliminate any surface adhesion of the particles. After this recovery fish were sacrificed with a lethal dose of MS222, fixed in 10% buffered formalin, and processed for histology using methods previously described.

Data analysis

The primary endpoint in these studies was to determine the presence of particles within the vasculature of zebrafish (*Danio rerio*) using microscopy and light imaging techniques.

Fluorescebrite multifluorescent microspheres™ (0.2 μm and 1 μm in diameter; excitation maxima of 377, 517 and 588nm, and emission maxima of 479, 546 and 612nm) were detected with a TRIT C filter. Histopathology was used to assess the accumulation of acetylene black particulates into fish gastrointestinal and gill epithelium. Organ systems were analyzed for abundance and severity of lesions associated with particulate exposure as well as any other lesions of note using guidelines and methods recommended by Wolf et al (2014) [8]. Prevalence data was assessed and where appropriate Chi-Square was used to determine statistical significance.

Principal Findings and Significance:

Gavage Exposure

There were a total of 10 fish gavaged with control material and 10 fish gavaged with 2.5% acetylene black. Five fish were examined for each of the fluorescent microsphere gavage cohorts. All fish survived the repeated gavage procedure. There was no conclusive evidence of accumulation of black particulate through the gastrointestinal tract in zebrafish after gavage. There appeared to be some accumulation in the gastrointestinal epithelium as well as some pigmented sections of the parietal pericardium, however this pigmented material was not overly abundant and could not be differentiated from the natural occurrence of melanin in zebrafish tissues.

Zebrafish gavaged with fluorescent microspheres had blood smears and imprint smears prepared the day after final gavage. There was no fluorescence detected in blood collected from the caudal vein relative to controls for either of the size classes of microspheres (Figure 1 A, B, & C). Imprint smears did show abundant fluorescent in the gastrointestinal tract, but there was no detectable fluorescence in other tissues.

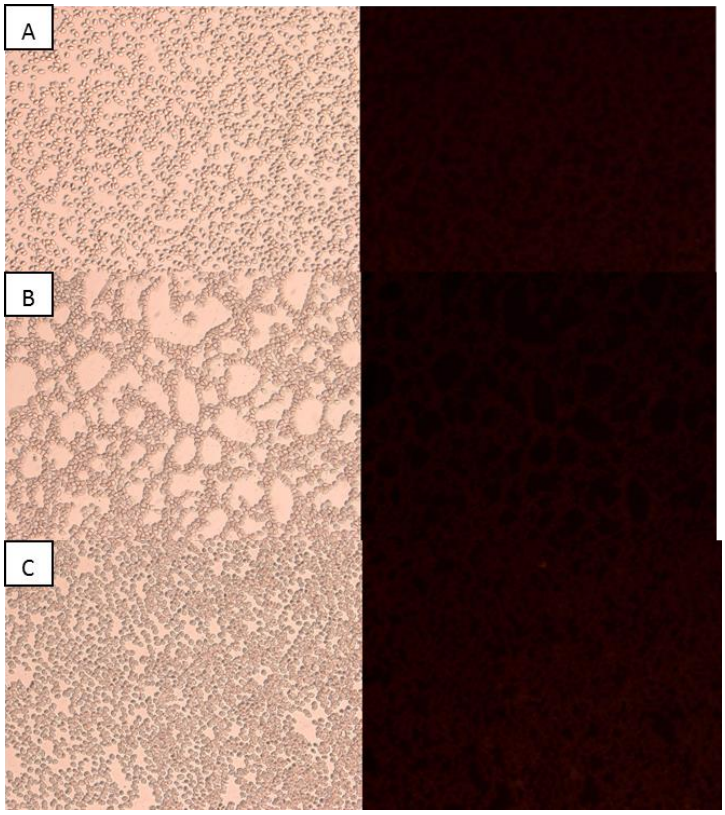

Figure 1. Comparison of blood smears of control (A), 0.2 µm (B) and 1.0 µm (C) microsphere gavaged fish. Left column is light microscopy, right column is fluorescent light with TRIT C filter.

Acetylene Black Feed study

There was 100 percent survival of fish fed a spiked acetylene black feed for 60 days. There was no significant difference in the growth of exposed fish compared to control fish based on the weight/length ratios and comparison (Figure 2). Particulates also did not appear to cross the gastrointestinal epithelium based on the histological analysis. Similar to the acetylene black gavage, areas of possible particulate accumulation could not be definitely differentiated from natural melanin deposits.

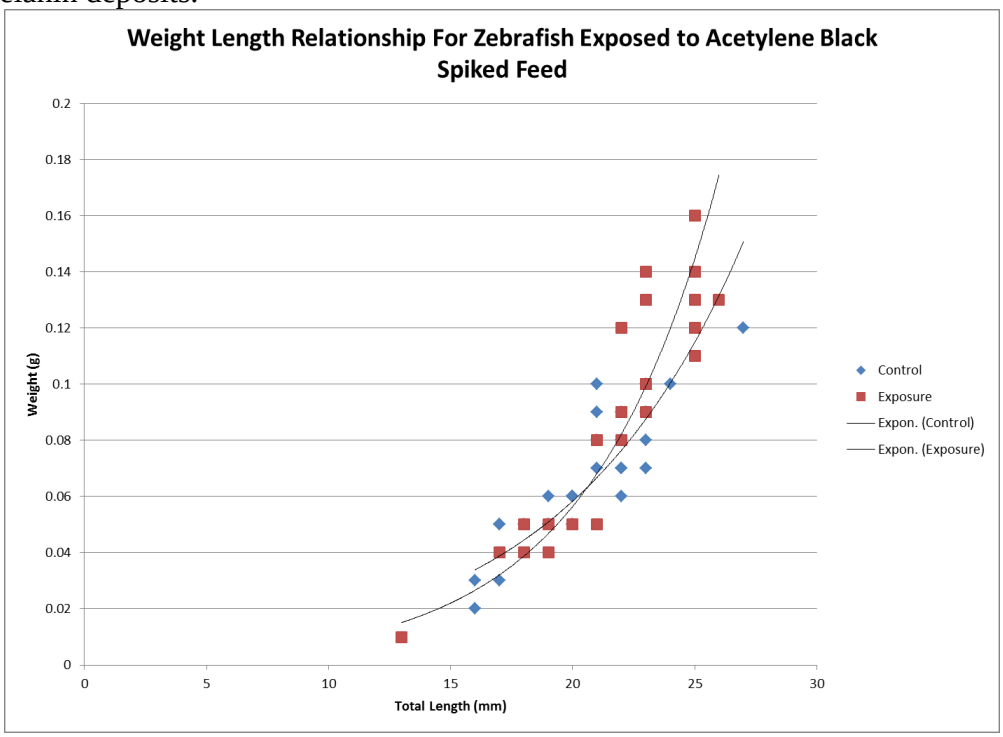

Figure 2. Comparison of the weight and length of fish exposed to acetylene black spiked feed relative to controls.

Plate Exposure

There were no differences in mortality of fourteen dpf zebrafish exposed for 24 hours to 50 mg/L of fluorescent microspheres relative to controls. Exposed larvae actively ingested and appeared to accumulate particulates across the epithelial barriers; there was no fluorescence present in controls (Figure 3 A & B). There was abundant particulate material in the gastrointestinal tract, which may dampen fluorescence in other tissue. Additional replicates are being conducted to develop methods that eliminate adhered particulate to the surface of the fish and within the gastrointestinal tract to enhance the identification of microspheres accumulating within the tissue.

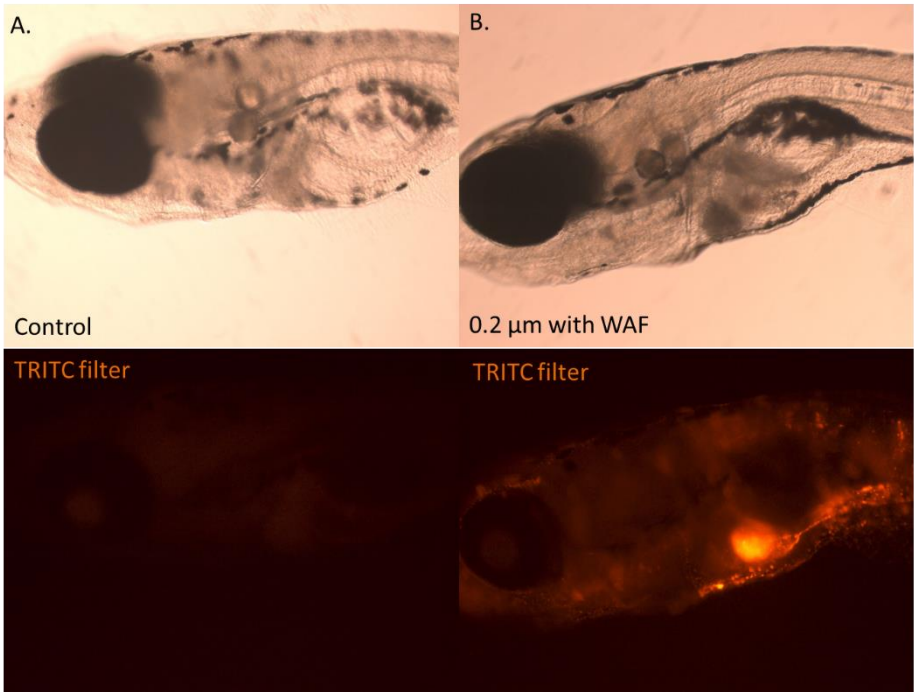

Figure 3. Fluorescent microspheres present in the gastrointestinal tract of larval zebrafish as well as in other tissues. Additional methods are being tested to eliminate dampening of accumulated particulates from the larger accumulation in the gastrointestinal tract to identify potential sinks for particulate material.

Pulse-Dip Exposure

There was 100% survival in zebrafish exposed to 50 mg/L acetylene black with 0.02 % tween, with minimal accumulation of particulate material on the external epidermis. Four out of 8 mummichog died as a result of the same exposure conditions (at 20 psu) while there was 100% survival in the controls. There was abundantly more acetylene black material associated with the external epidermis and gill tissue. There was a concentrated accumulation of black particulate beneath the operculum, on the ventral side of the gills. This collection of material appeared to be organized based on some type of removal mechanism from the gills. Histology results of the pulse-dip exposure have not been examined.

Significance/Conclusion

The initial findings of this study suggest that larval zebrafish ingest particulate material in bath exposures and this material likely enters other organ systems as well. Fluorescent microspheres are ingested and concentrate in the gastrointestinal tract, with some occurrence of fluorescence in other tissues. The lack of fluorescent particles in the blood of zebrafish gavaged with 2.5% solutions of fluorescent particles indicates that there is minimal uptake via the gastrointestinal tract, at least that results in the blood from the caudal vein. Imprint smears also support this finding; there was no fluorescent particles in tissues other than the gastrointestinal tract. In mammalian studies, uptake of fluorescent beads (and other particulate matter) appears to occur through the Peyer's Patches: lymphoid nodules associated with the lower gastrointestinal tract. Teleost species lack this lymph tissue and thus it is not surprising to find minimal evidence of particulate uptake. Particulate uptake is more likely due to gill uptake rather than crossing gastrointestinal epithelia, based on some of the previous work done in our lab showing black

particulate matter entering gill blood sinuses. Previous studies have stated that microplastics can accumulate in the liver after absorption through the gastrointestinal tract, although it remains unclear as to whether the gills play an important role in particulate uptake [9,10].

Zebrafish may not be the best model to determine particulate accumulation based on their lack of a stomach and relatively short gastrointestinal tract compared to species that are classified as herbivores and detritivores. Amazon mollies, a similar teleost species with regards to ecological niche, showed negligible relatively negligible uptake of particulate material as well based on the resemblance to melanin [3]. The results of the acetylene black bath exposure to Atlantic killifish may elucidate further species differences based on these alternative lifestyles the species tested. Natural conditions are also difficult to replicate in microcosms. Stressful conditions in urban environments contribute to secondary gill or gastrointestinal damage due to concurrent exposures. Chemicals like dispersants increase membrane permeability which would increase particulate uptake. Gill and intestinal parasites may also play a large role in the accumulation of particulates in fish vasculature based on an increased immune response or other pathology.

Suspended PM toxicity is rarely examined in aquatic toxicology and could greatly impact the critical water resources. It has been demonstrated that atmospheric deposition of soot particulates [5] as well as the weathering of crude oil contaminants [6] are at least partly responsible for the introduction of these particulates into aquatic ecosystems. Other particles, such as microplastics, have been identified and characterized in multiple surface waters. The accumulation of these particles in fish species has not been quantified and is not well understood. These studies lay a foundation to further test the potential for particulates (both particulate matter and plastics) to cross gastrointestinal or gill epithelia under a variety of exposure conditions.

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(4) Publications or Presentations:

Millemann, Daniel R.; Keith R. Cooper, 2016. Suspended particulate toxicity: An emerging area of concern for physiochemical exposures to fish and other aquatic organisms. Presented at the 5th Young Environmental Scientist Meeting 28 February–2 March 2016 Gainesville, FL, USA

Developing a green technology to remove phosphate and pharmaceuticals from wastewater effluents

Basic Information

Title:	Developing a green technology to remove phosphate and pharmaceuticals from wastewater effluents
Project Number:	2015NJ370B
Start Date:	3/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	NJ-006
Research Category:	Water Quality
Focus Category:	Treatment, Wastewater, Water Quality
Descriptors:	None
Principal Investigators:	Saumik Panja, Yang Deng

Publications

1. Panja, Saumik, Padmini Das, Rupali Datta and Dibyendu Sarkar (2015) “Preliminary Studies on Removal of Tetracycline and Ciprofloxacin from Secondary Wastewater Effluent by Vetiver Grass”, Geological Society of America Annual Conference, Baltimore, MA, November 2015.
2. Panja, Saumik, Rupali Datta, Yang Deng and Dibyendu Sarkar (2015) “Preferential Uptake between Nutrients and Pharmaceuticals by Vetiver Grass from Secondary Wastewater Matrix”, Geological Society of America Annual Conference, Denver, CO, September 2016.
3. Panja, Saumik, Rupali Datta, Yang Deng and Dibyendu Sarkar (2016) “Potential Use of Vetiver Grass to Remove Ciprofloxacin from Aquatic Media”, the 101st Annual New Jersey Water Environment Association (NJWEA) Conference – Student Poster Composition (1st place)

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Dibyendu Sarkar, Professor, Ph.D., P.G.

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(2) Numbers of Students Supported:

Undergraduates: 0
Master's students: 0
Ph.D. students: 1
Postdoctoral associates.: 0

(3) **Any notable Achievements** (Awards, Recognition, etc.), or direct application of the research by Management Agencies, Nonprofits/NGOs, etc.

The 1st Place in the 101ST Annual New Jersey Water Environment Association (NJWEA) Conference – Student Poster Composition (Presentation Title: Potential Use of Vetiver Grass to Remove Ciprofloxacin from Aquatic Media)

(4) Project Summary

Problem and Research Objectives

Degradation of water quality due to a variety of pollution has consistently been an issue of great concern. Recognition of such environmental problems inspired rigorous scientific investigations for development of novel technologies to meet with current and future water demands. Water reclamation is a sound and sustainable approach to address the water crisis issue and is highly encouraged by both USEPA and NJDEP (USEPA, 2004; NJDEP, 2005). However, currently, available reclamation methods suffer from a variety of restrictions, such as high operational costs, complex maintenance, production of undesirable treatment byproducts, and intensive labor demand. Therefore, there is an urgent need to develop innovative, viable, low cost, environmentally friendly water reuse technologies.

Traditional wastewater treatment plants (WWTPs) typically apply secondary wastewater treatment that is assoicated with an aerobic biological process, which effectively removes suspended solids and biodegradable organic matters from municipal wastewater. However, effluent organic matter (EfOMs), pathogens, nutrients, along with emerging contaminants present in the secondary effluent need to be appropriately removed before water reuse. Among the wastewater pollutants, those identified as emerging contaminants (such as pharmaceuticals) are seriously challenging our water reuse options due to their prolonged health concern and persistence in the environment, and absence of effective treatment technology. Extensive use of pharmaceutically active compounds in the public health sector and veterinary industries ultimately leads to their accumulation in water resources, so that microorganisms exposed to these pharmaceuticals become increasingly resistant (Li et al., 2010). According to the Center for Disease Control (CDC), more than 2 million U.S. people are affected by antibiotic-resistant infections every year, resulting in the death of 23,000 people (CDC report 2013). Tetracycline (TTC) and ciprofloxacin (CIP) are representatives of wastewater-driven antibiotic compounds. Karthikeyan and Meyer (2006) reported a typical TTC concentration of 48µg/L in influent wastewater streams. A significant portion of CIP is excreted in partially metabolized form from the physiological system of consumers and enters into the environment through the municipal wastewater, untreated sewage, manure stockpiles in veterinary industries, applications of manure in agricultural lands, and runoff from farms to the aquatic system (Rakshit et al., 2012).

From the viewpoint of environmental sustainability and cost-effectiveness, phytoremediation could be an effective solution to this particular problem. Vetiver grass is an ideal candidate for wastewater phytoremediation with its extensive root system, fast growth, extremophilic nature, high biomass, and extensive growth in hydroponic systems. United States Department of Agriculture (USDA) has declared this perennial grass to be a non-invasive species. Given its subtropical abundance, vetiver grass is non-native to New Jersey. However, application of vetiver grass to prevent erosion of slopes, and also to treat sewage and landfill leachate have been reported in various states, including Oklahoma, Mississippi, Florida, and Hawaii. In this study, we used the Sunshine variety of vetiver. This cultivar is incapable of producing viable seeds or spreading via rhizomes (http://plants.usda.gov/plantguide/pdf/pg_chzi.pdf). Also, we did not plant vetiver in soil but in floating pontoons in non-winter seasons as a scavenger of nutrients and pharmaceuticals. After every year (April to November), the vetiver grass will be incinerated, and new vetiver plants will be added to the floats the next season. Application of vetiver grass to polish secondary wastewater effluent is a promising solution (Ash and Truong, 2004). It can tolerate and remove a wide range of inorganic and organic pollutants such as Pb (Chen et al., 2004), Hg (Truong et al., 2000), TNT (Makris et al., 2007), and tetracycline (Datta et al., 2013). Hart et al. (2003) reported that vetiver grass can significantly reduce coliforms from a domestic wastewater effluent within 4 days of exposure. Zhao et al., (2012) demonstrated that vetiver grass can efficiently remove nutrients from secondary wastewater effluent.

Our **long-term goal** is to develop an innovative, environmentally sustainable, and economically viable wastewater reclamation technology. The primary **aim** of this project was to evaluate the performance of vetiver system (VS) in the removal of both traditional and emerging contaminants from New Jersey wastewater for the purpose of water reuse. The **central hypothesis** of this project is that nutrients and pharmaceuticals in secondary wastewater effluents can be effectively removed by VS via multiple phytoremediation mechanisms. To test the hypothesis, we investigated 3 key issues:

1) The removal efficiencies of VS for typical secondary effluent contaminants (i.e. COD, BOD_5, TN, turbidity, and pathogenic bacteria).
2) The removal capacities and kinetics of VS for both traditional (phosphate) and emerging contaminants (TTC and CIP) in secondary effluent.
3) Advantages and limitations of VS, in comparison with currently available water reuse technologies from both treatment efficiency and economic feasibility points of view.

Methodology

Secondary wastewater effluent was collected in a bulk amount from the Joint Meeting of Essex & Union Counties, situated in Elizabeth, New Jersey. The wastewater was transported in a 32-gallon bucket and stored at 4^{o}C at a cold room. The characterization of wastewater was completed within 7 days of sampling.

Full factorial tests were conducted to understand the kinetics behaviors of TTC and CIP uptake by vetiver grass. Tests were performed in PTFE bottles containing 800mL antibiotics-containing secondary wastewater effluent. The plant concentration (32 gm/L) was fixed to occupy 4% of the reaction volume. Control tests were carried out at identical conditions except that any plant is not introduced. Periodical samples were collected at designated times for analysis of TTC and CIP.

TTC and CIP in samples were measured using the method of Punamiya et al. (2013). A Finnigan surveyor plus HPLC system (Thermo Scientific) equipped with quadruple pumps coupled with a surveyor PDA plus detector (photodiode array) and a surveyor plus autosampler was used for all analysis. A hypersil gold C_{18} column (150mm × 4.6mm, 5µm) (Thermo Scientific) with a corresponding hypersil gold guard column (10mm × 4mm, 5µm) (Thermo Scientific) at room temperature was used for all separation. Mobile phase for TTC: 0.01 mol L^{-1} mixed solution of oxalic acid, acetonitrile, and methanol (150:20:20 by volume). Mobile phase for CIP: phosphoric acid: acetonitrile (78:22). The flow rate was maintained at 1.5 mL min^{-1}, with an injection volume of full loop (25µL). And the UV detector was set at 360 nm. A linear standard curve was calibrated for quantification based on the area signals. Nutrients (nitrate and phosphate) were analyzed using a Dionex Ion chromatographer. And TOC was measured using a Shimadzu TOC analyzer.

Table 1: Basic parameters and their values of secondary wastewater effluent from a wastewater treatment plant (WWTP).

Parameter	**Values**
pH	6.98-7.48
CIP (mg/L)	N/A
TTC (mg/L)	N/A
NO_3-N (mg/L)	30
TP (mg/L)	10
TOC (mg/L)	60

Principal Findings and Significance

Removal of traditional wastewater contaminants.

Vetiver grass successfully removed the traditional wastewater contaminants including nitrate (fig. 1), phosphate (fig. 2), and TOC (fig. 3) from both TTC and CIP amended wastewater matrix. Results showed that the VS was capable of removing nitrate, phosphate and TOC by 96%, 98%, and 80% respectively. With the increasing nutrient dose, the removal of antibiotics decreased. A biphasic removal was observed for the removal of nutrients and TOC, i.e. a rapid initial reduction within the first 30 days followed by a slow removal during the rest of the experimental period. In contrast, in the control experimental setup (without plants), only a slow degradation of nutrients was observed, because the only mechanism for the nutrient removal is microbial activity that was gradually developed.

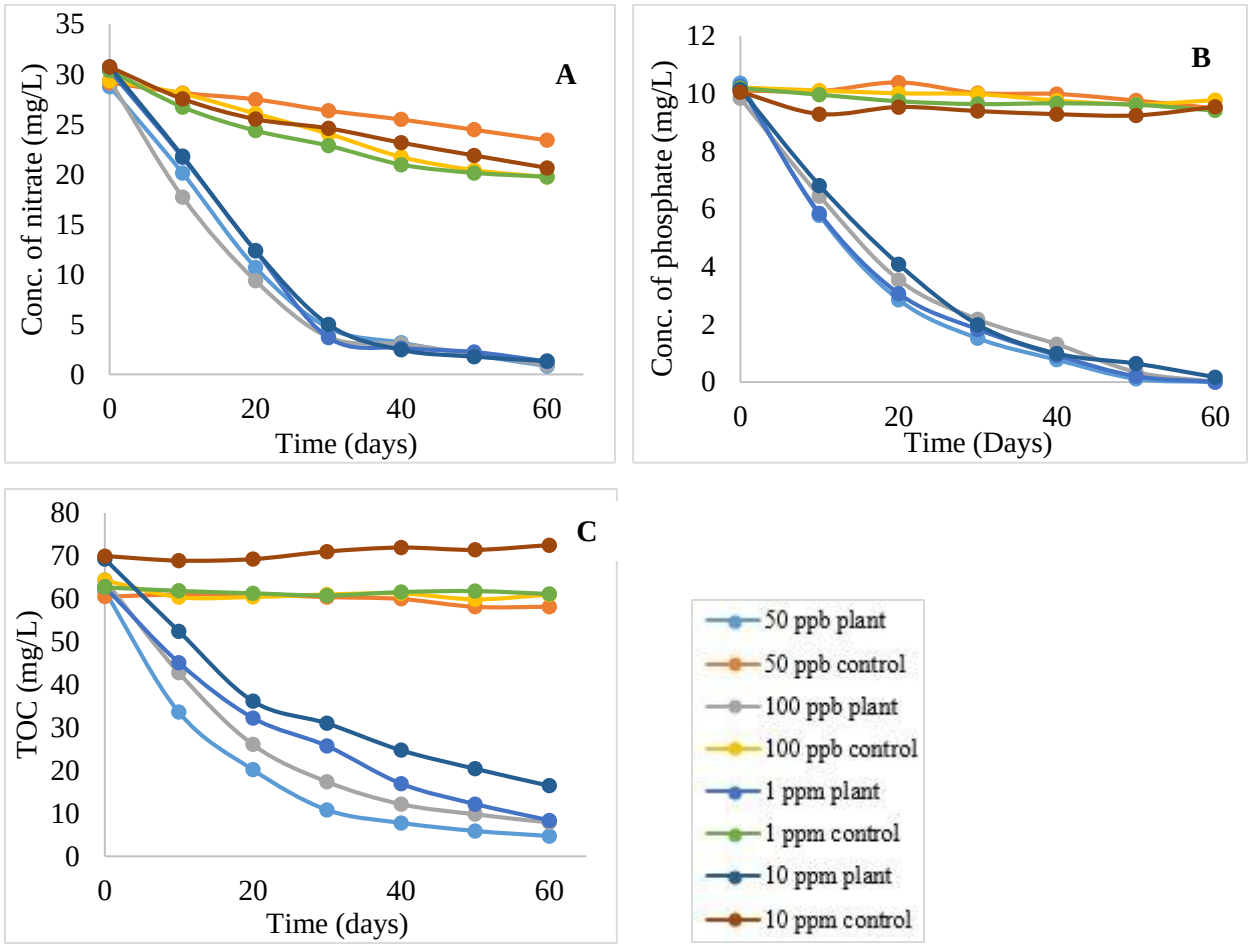

Fig. 1. Removal kinetics of target pollutants in the VS treatment and control tests: A) nitrate, B) phosphate, and C) TOC (TTC = 0.05, 0.1, 1, and 10 mg/L)

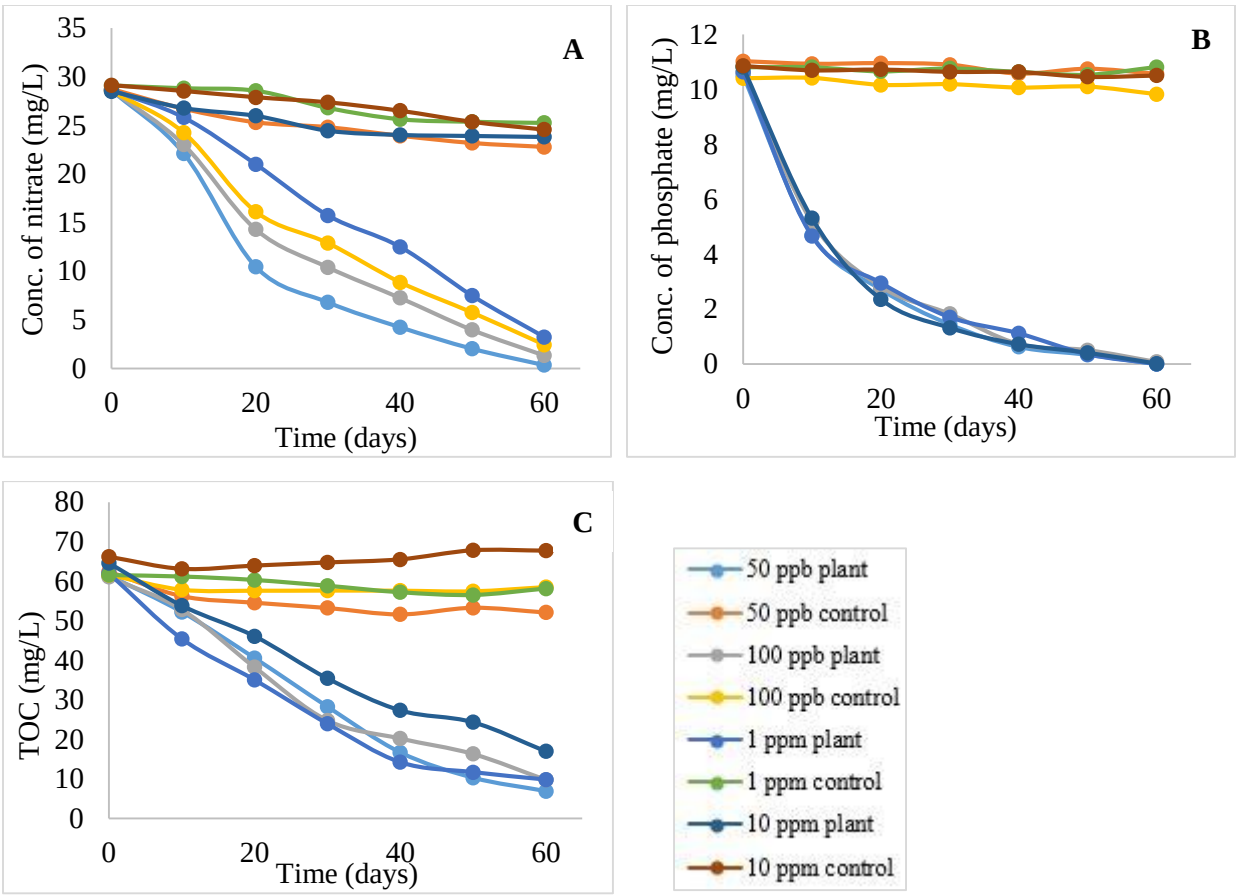

Fig.2: Removal kinetics of target pollutants in the VS treatment and control tests: A) nitrate, B) phosphate, and C) TOC (CIP = 0.05, 0.1, 1, and 10 mg/L)

Removal of representative pharmaceuticals

In our experiment, we noticed that vetiver grass remvoed TTC and CIP from secondary wastewater matrix. For TTC, a rapid removal was observed during first 5 days. At the end of the fifth day, we observed that vetiver grass successfully removed 100% TTC from 0.05, 0.1 and 1 mg/L (fig. 4,5 and 6). At an initial TTC concentration of 10 mg/L, almost all the TTC was removed within 30 days (fig. 7). The plants did not indicate any stress symptoms in response to the dosing antibiotics. The control setup (without plants) did not show any significant decrease of TTC in all of the experimental setup (0.05, 0.1, 1, 10 mg/L).

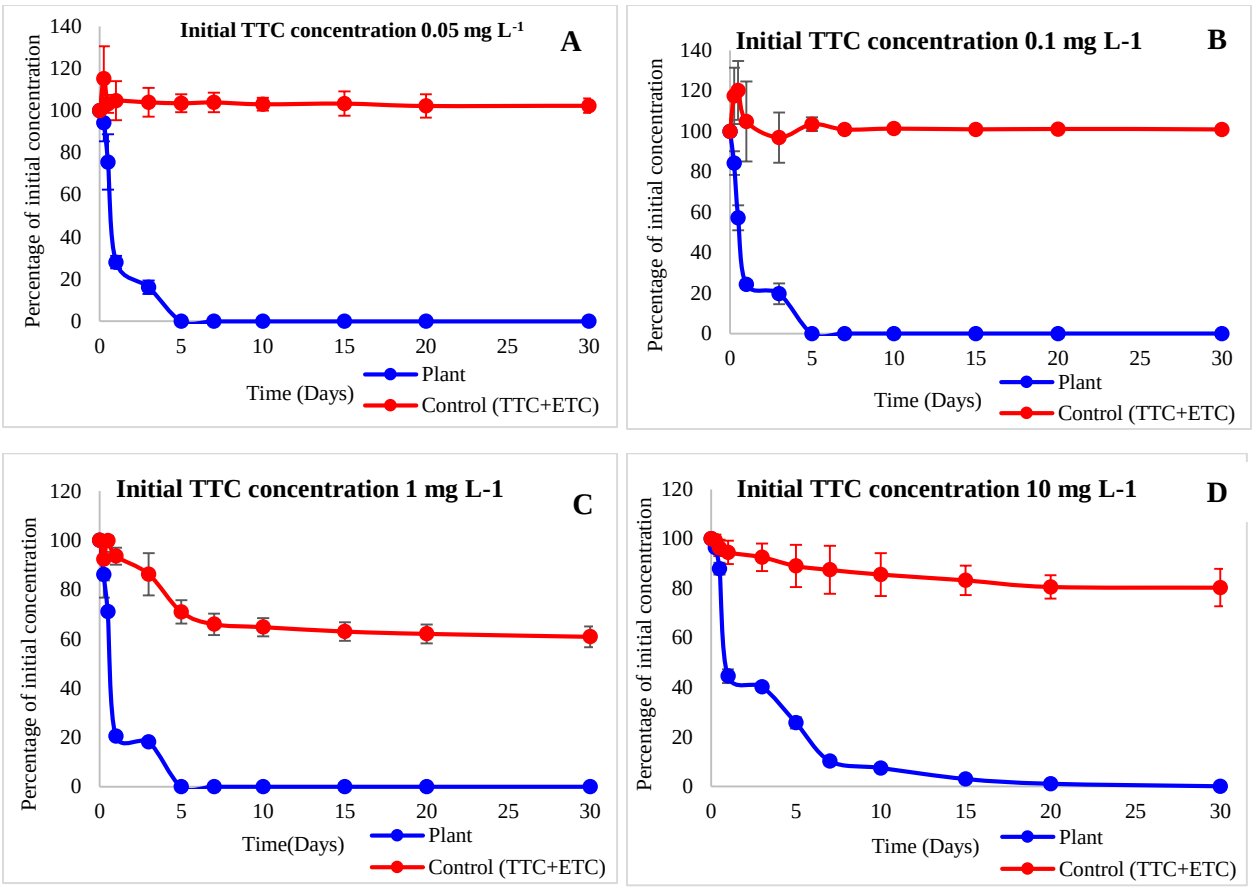

Fig.3: Removal kinetics of TTC during the VS and control treatment tests at different initial concentrations: A) 0.05 mg/L, B) 0.1 mg/L, C) 1 mg/L, and D) 10 mg/L.

For CIP, vetiver grass has also shown a similar treatment capability in a wastewater matrix, except that the CIP remediation was slower. A biphasic removal of CIP was observed, i.e. an initial rapid removal within the first 7 days followed by a slow reduction during the rest of the experimental period. Within 30 days, 100% CIP removal was achieved (fig. 11,12 and 13), except 10 mg/L CIP experimental setup at which 75% CIP was removed (fig.14). In contrast, any removal was not significantly observed in the control experimental setup.

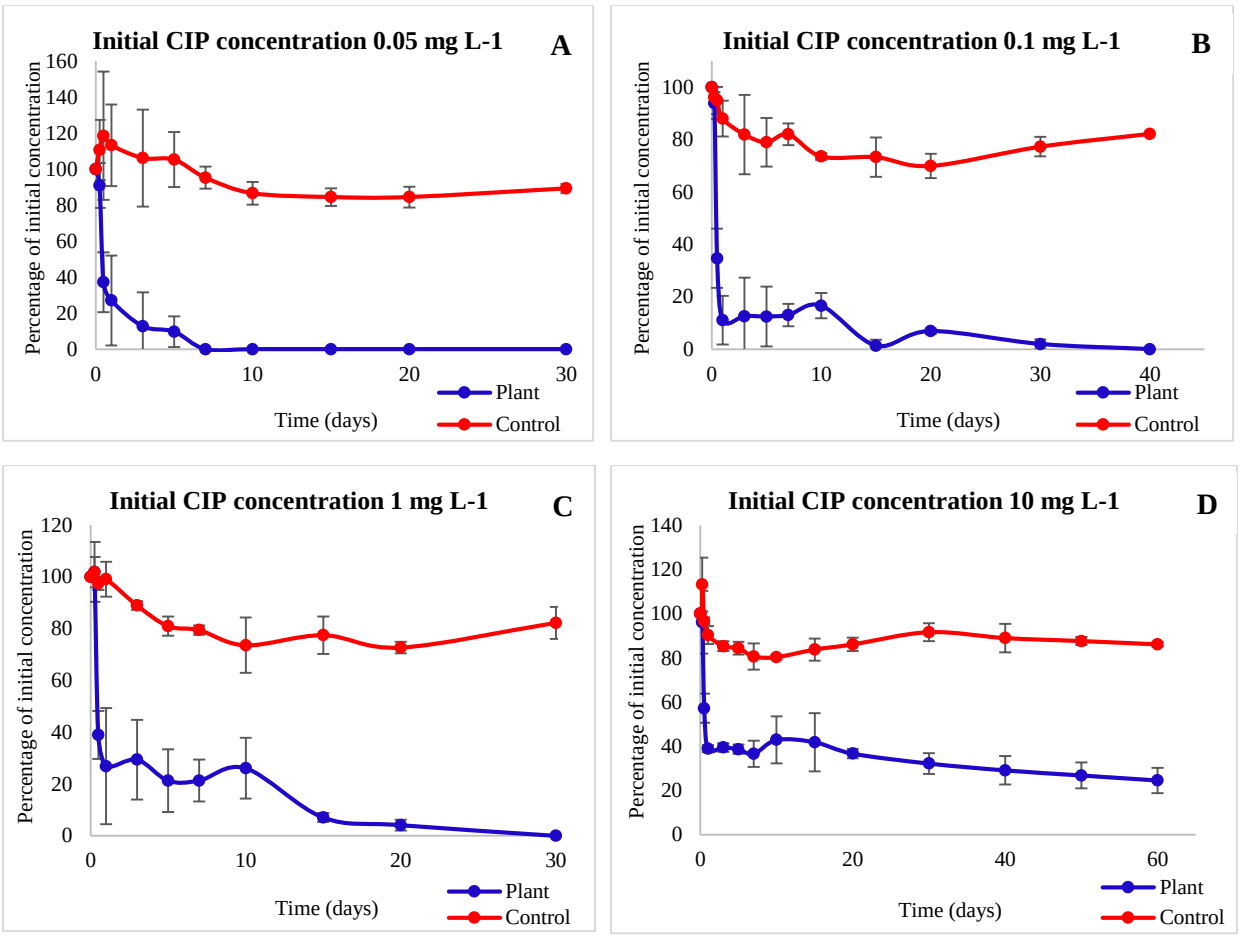

Fig.4: Removal kinetics of CIP removal at different initial CIP contrations: A) 0.05 mg/L, B) 0.1 mg/L, C) 1 mg/L, and D) 10 mg/L.

Cost analysis:

Preliminary cost analysis was made for treatment of 1000 gallons of secondary effluent as follows.

Amount of plant needed: 1% of the reaction volume

No. of plants needed = $10000 gal \times \frac{3.785\ L}{gal} \times \frac{1000\ mL}{L} \times \frac{1\ gm}{100\ mL} \times \frac{1\ plant}{40\ gm} =$ 9,462.5 ~9463 $plants$

Cost per plant = \$1

$cost_{plants}$ = $\$(9{,}463 \times 1) = \mathbf{\$9{,}463}$

Cost for pumping:

Two 92W 1000 gallons per hour pump = $cost_{pump} = 2 \times \$45.00 = \mathbf{\$90.00}$

Operating and maintenance (O&M) cost:

Cost of electricity per month

$$cost_{electricity} = 92W \times 1hr \times \frac{kWhr}{1000\,Whr} \times \frac{\$0.1}{kWhr} \times \frac{24hr}{day} \times \frac{30\,day}{month} = \mathbf{\$6.624/month}$$

Labor cost:

$$cost_{labor} = (no.of\ labors \times hourly\ wages \times \frac{hours}{day} \times no.of\ days)$$

$$(10 \times \frac{\$40}{hr} \times \frac{6hr}{day} \times 2days) = \mathbf{\$4,800}$$

Cost for reusable pontoons:

$$cost_{pontoon} = (cost\ per\ pontoon \times no.of\ pontoons)$$

$$\frac{\$60}{pontoon} \times 50\ pontoons = \mathbf{\$3,000}$$

Miscellaneous cost:

$$cost_{miscellaneous} = \mathbf{\$1,000}$$

Total one time cost:

$$(cost_{plants} + cost_{pump} + cost_{labor} + cost_{pontoon} + cost_{miscellaneous})$$

$$= (\$9{,}463 + \$90 + \$4{,}800 + \$3{,}000 + \$1{,}000)$$

$$= \$18{,}353 \sim \mathbf{\$19,000}$$

Total maintenance cost:

$$cost_{electricity} = \$6.624 \sim \mathbf{\$7}$$

(5) Publications or Presentations:

Oral presentation:
None

Poster presentation:

- Panja, Saumik, Padmini Das, Rupali Datta and Dibyendu Sarkar (2015) "Preliminary Studies on Removal of Tetracycline and Ciprofloxacin from Secondary Wastewater Effluent by Vetiver Grass", Geological Society of America Annual Conference, Baltimore, MA, November 2015.
- Panja, Saumik, Rupali Datta, Yang Deng and Dibyendu Sarkar (2015) "Preferential Uptake between Nutrients and Pharmaceuticals by Vetiver Grass from Secondary Wastewater Matrix", Geological Society of America Annual Conference, Denver, CO, September 2016.
- Panja, Saumik, Rupali Datta, Yang Deng and Dibyendu Sarkar (2016) "Potential Use of Vetiver Grass to Remove Ciprofloxacin from Aquatic Media", the 101st Annual New Jersey Water Environment Association (NJWEA) Conference – Student Poster Composition (1st place)

Effects of hard clam (Mercenaria mercenaria) grow-out operations on benthic communities in Barnegat Bay, NJ

Basic Information

Title:	Effects of hard clam (Mercenaria mercenaria) grow-out operations on benthic communities in Barnegat Bay, NJ
Project Number:	2015NJ371B
Start Date:	3/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	NJ-008
Research Category:	Water Quality
Focus Category:	Ecology, Water Quality, Surface Water
Descriptors:	None
Principal Investigators:	Rebecca Shell, Robert Prezant

Publications

1. Shell, Rebecca M. Expected presentation at the Fall 2016 meeting of the Atlantic Estuarine Research Society (AERS): “Effects of hard clam (Mercenaria mercenaria) grow-out operations on benthic communities: preliminary results from Barnegat Bay.” Community College of Baltimore County, Catonsville, MD.
2. Shell, Rebecca M. Seminar to be presented in the Winter 2017 Sustainability Seminar Series: “Effects of hard clam (Mercenaria mercenaria) grow-out operations on benthic communities: preliminary results from Barnegat Bay.” Montclair State University, Montclair NJ.

(1)

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(2) Two Masters' students were sequentially funded by this project.

(3) This funding completed data collection on this project. Analysis is ongoing – expected completion 2017.

(4) Project Summary:

Problem and Research Objectives

Infaunal bivalves, including the northern quahog (*Mercenaria mercenaria)* provide a variety of services to their immediate environment. At a basic level, the presence of infaunal clams provides habitat heterogeneity, especially as the presence of hard clams is positively correlated with seagrass cover. As a filter feeder, hard clams remove organic particulate, algae and phytoplankton and suspended sediments from the water column, filtering water at a rate of approximately 1 gallon per hour. Infaunal clams also affect sediment porosity, vertical profiles of oxygen and nutrients, and furthering the exchange of gas and waste between the sediment and the water column, driving local benthic biodiversity and overall ecosystem functioning by increasing the potential for aerobic microbial activity. It is clear that the hard clam is integral to healthy soft-sediment systems, particularly in eutrophic systems where water filtration is integral to maintaining light penetration and dissolved oxygen. It is of great concern therefore that estimated *M. mercenaria* stocks in Barnegat Bay have fallen off greatly in recent decades.

Shellfish aquaculture operations, as with terrestrial animal farming, are by necessity high-density operations. The densities maintained during clam grow-outs might be expected to detrimentally impact local communities due to locally-increased nutrient input. Conversely however, it is possible that the filtering capability of this bivalve will instead provide a net benefit to local communities from the increase in total filtering capacity. Further compounding the issue, fouling of the screen apparatus used in grow-outs can lead to increases in larval settlement and subsequent shifts in local community structure, as well as increases in overall biodiversity and biomass and changes to sediment characteristics.

The objectives of the study are to investigate changes in relative infaunal benthic invertebrate abundance and community structure caused by *Mercenaria mercenaria* grow-outs with aims to answer the following questions:

1) How do benthic invertebrate communities (as measured in terms of density, diversity, richness, dominance, evenness, and biomass) associated with grow-out enclosures change during a 12-month cycle?

2) What community shifts, if any, are caused by the intense farming of *Mercenaria*? Are the community changes due to the increases in clam abundance, or the screening equipment itself?

Methodology

No deviations from the proposal were required in 2016. My team, as planned, sampled each of the plots (screened, screened and planted, and control) in all three blocks at each of the three prescribed dates: May, August and October. As per the protocol, the screens were rolled back briefly to allow for sample collection and then immediately replaced and firmly re-secured to the benthos using curved rebar. Benthic samples (Ekman grab), sediment cores (d=15cm), plankton samples (mesh 80µm) and screen epibiota were also collected. Samples were preserved in 70% ethanol, and transported to Montclair State University for identification. The May 2016 samples are completed, with August and October at about 50% identified.

Principal Findings and Significance

The data from this project are not fully collected, and analysis is therefore not available. As expected, polychaetes and amphipods dominate the plots. Benthic molluscs are also well represented (Table 1).

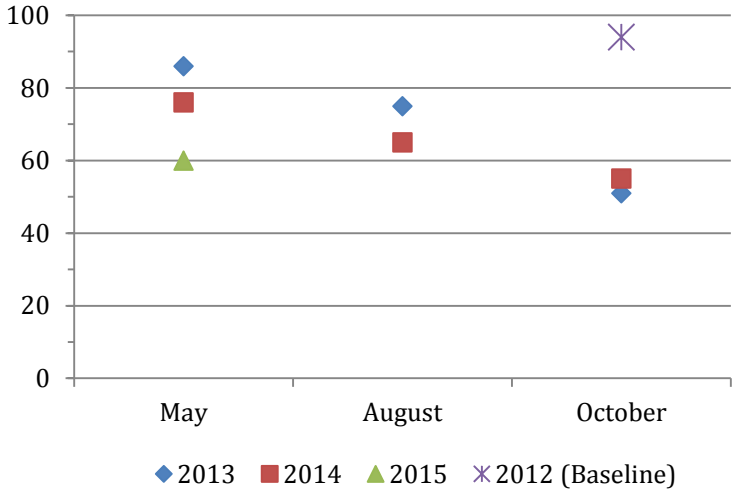
Table 1 Count of identifiable taxa by group

Mollusca		31
	(Bivalvia)	18
	(Gastropoda)	13
Polychaeta		60
Amphipoda		37
	(Caprellidae)	5
Decapoda		16
Isopoda		5
Anthozoa		3
Cumacea		2
Mysidae		1

Only 14 of these taxa have been identified on all sampling dates: four amphipods (*Ampelisca abdita, A. verrilli, Ampithoe longimana, Lysiasnopsis alba*); three molluscs (*Solemya velum, Tellina agilis, Tritia obsoleta*); six polychaetes (*Clymenella torquata, Glycera dibranchiata, Goniada* sp., *Prionospio heterobranchia, Scoloplos* sp., *Streblospio benedicti*) and *Erichsonella filiformis*, an isopod.

A few trends that have appeared could be significant. The baseline samples taken in October 2012 included 94 identifiable taxa (identified to family, many down to species where possible.) All subsequent sampling dates on the Sedge lease have shown decreased species richness as compared with the baseline samples, perhaps due to Hurricane Sandy which hit the bay directly in October 2012, just week after the plots were installed. (Remarkably, no equipment or plot set-up was lost during the storm). 2013 and 2014 saw further decreases (Figure 1). Whether this trend is limited to treatment plots or continues across controls remains to be teased out.

Figure 1. Total taxa identified over time through May 2015. (August and October 2015 are not completed.) Baseline data from October 2012 included.



Data from this project will provide information currently lacking on the practical effects of hard clam aquaculture industry on the local benthic ecosystem. Much acreage in Barnegat Bay is appropriate for clam grow-outs but is not presently being cultivated. Should the analysis, expected to be complete in 2017 and published in 2017/2018, provide evidence of overall improvement or net stability in local benthic biodiversity, we hope that policy makers will consider the ecological benefits along with the economic benefits of opening up more of this area to the industry, especially given the overall decline of local wild stocks in recent decades.

(4) **Presentations planned for Fall 2016/Winter 2017.**

Shell, Rebecca M. Expected presentation at the Fall 2016 meeting of the Atlantic Estuarine Research Society (AERS): "Effects of hard clam (*Mercenaria mercenaria*) grow-out operations on benthic communities: preliminary results from Barnegat Bay." Community College of Baltimore County, Catonsville, MD.

Shell, Rebecca M. Seminar to be presented in the Winter 2017 Sustainability Seminar Series: "Effects of hard clam (*Mercenaria mercenaria*) grow-out operations on benthic communities: preliminary results from Barnegat Bay." Montclair State University, Montclair NJ.

Development of novel membranes for reuse of brackish water and sea water desalination via membrane distillation

Basic Information

Title:	Development of novel membranes for reuse of brackish water and sea water desalination via membrane distillation
Project Number:	2015NJ372B
Start Date:	3/1/2015
End Date:	2/29/2016
Funding Source:	104B
Congressional District:	NJ-010
Research Category:	Water Quality
Focus Category:	Treatment, Water Quality, Water Use
Descriptors:	None
Principal Investigators:	Smruti Ragunath, Somenath Mitra

Publications

1. Ragunath, Smruti, Sagar Roy, and Somenath Mitra, 2016, Selective Hydrophilization of the Permeate Surface to Enhance Flux in Membrane Distillation, poster presented at 101th NJWEA Student Poster Session on May 18th, 2016 at Atlantic City, NJ.
2. Ragunath, Smruti, Sagar Roy and Somenath Mitra, 2016, Selective Hydrophilization of the Permeate Surface to Enhance Flux in Membrane Distillation, oral presentation selected for 252nd ACS National Meeting, scheduled for August 2016, at Philadelphia, PA.

1. **Title: Development of Novel Membranes for Reuse of Brackish Water and Sea Water Desalination via Membrane Distillation**

2. **Thesis Advisor Name: Somenath Mitra**

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4. **Any Notable Achievements:** Awarded 2nd place in Student Poster Competition organized by New Jersey Water Environment Association held at Atlantic City, NJ on May 18th 2016.

5. **Project Summary:**

Priority Issues

As many parts of the nation continue to suffer from water shortages, more attention needs to be paid towards conservation and recovery of clean water. The growing demand for water and depleting water reserves force us to look for engineered water sources by approaches such as water purification and desalination (1, 2). According to a recent report from United Nations, there is increasing interest in investments on water treatment methodologies due to growing demand for pure drinking water that is critical to improving public health (3).

Conventional processes for generating deionized water include Membrane Filtration, Reverse Osmosis (RO), and Multi Stage Flash Distillation (MSF). While Membrane filtration removes micro and nano particles, there is no filtration technique that can remove dissolved substances. RO requires high pressure and is good for low salinity water while MSF is energy intensive. These methods have their limitations and the development of the next generation of water purification technology is needed to provide energy efficiency, smaller instrument foot print and to generate soft water for public and industrial consumption.

Membrane distillation (MD) is a thermal evaporative process that offers advantages over traditional distillation(4, 5). It has been used in applications such as sea water desalination and food processing. The principle of separation in MD is based on the difference in volatility with vapor pressure being the driving force. Schematic diagram of a typical membrane distillation system is shown in Figure.1. Here a porous, hydrophobic membrane is used as a barrier for separating heated feed and cold permeate streams.

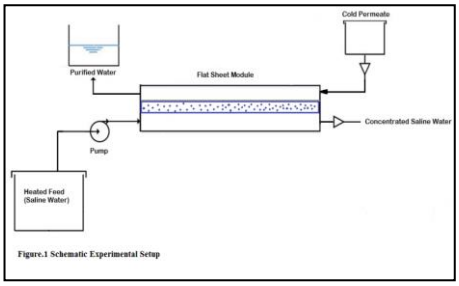

Figure.1 Schematic Experimental Setup

As a heated saline (brackish or sea) water passes through its lumen, it is partially transformed to water vapor. The hydrophobicity prevents the saline water from entering the pores. However, freed from hydrogen bonding, the water vapor passes through and is swept by a flow of cold permeate at a temperature of 15–20 º C. It is condensed on the permeate side of the membrane. MD can be operated at lower temperatures than the boiling point of the feed solutions; it requires atmospheric pressure and no vapor space. Typically, the process requires relatively low operating temperatures in the range of 60 - 90°C. This is unlike the traditional distillation processes where the operating temperatures are well above 100°C. Because of their lower energy requirement, membrane distillation is an attractive process for producing pure water.

Though membrane distillation has the potential to evolve as an energy efficient process, the key component in a membrane process is the membrane itself. The separation or purification of the desired contaminants is highly dependent on the membrane properties. Hence development of novel membrane architecture is of great importance to enhance the overall performance and efficiency of MD. Membrane properties are improved by engineering novel membranes or modification of the surface properties of existing membranes (6-8). Various membrane modification techniques have been employed for increasing the membrane performance and to generate higher flux.

Specific Objectives and Hypotheses

The research is aimed at developing a cost-effective process for brackish and sea water desalination by enhancing the performance of MD. The specific goal of this project is to develop novel membrane modification methods to create membranes with enhanced properties for generating pure water. This will be accomplished by hydrophilization of the permeate side of the membrane.

Materials and Methods

Experimental Setup

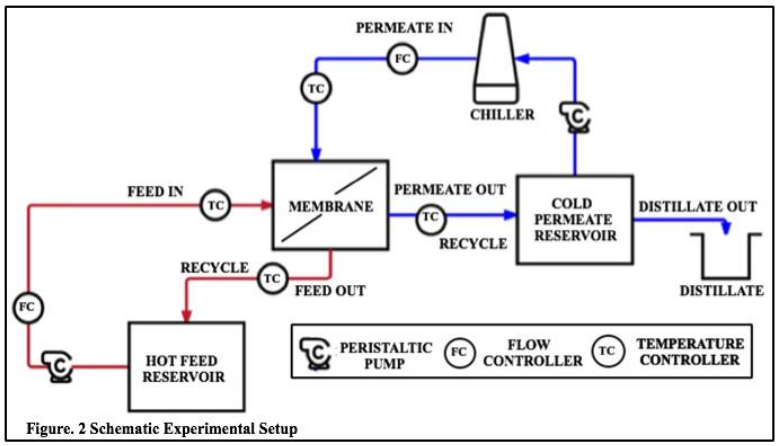

Figure. 2 Schematic Experimental Setup

MD experiments were carried out in the DCMD mode. The schematic diagram of the experimental setup is shown in Figure.2. Typical setup consists of a PTFE membrane cell having an effective membrane area of 14.5 cm^2. The membrane holder had Viton O-rings, PTFE tubing, PFA and PTFE connectors, as well as pumps for feed and permeate flow. Constant temperature water bath (Neslab Waterbath Model GP 200, NESLAB Instruments, Inc, Newington, NH, USA) was used to maintain steady feed temperature and a bench top chiller (Polyscience LS5, Cole-Parmer, USA) was used to maintain the permeate temperature around 15-20°C. Feed and permeate solutions were contacted in the membrane module in a counter current flow. Both the feed and permeate were recycled from their respective reservoirs using Master Flex Easy Load peristaltic pumps (Cole-Parmer, USA). The inlet and outlet membrane temperatures were monitored using temperature sensors (Four-channel Data logging Thermometer, RS-232, Cole-Parmer, USA). Hydrophobic Teflon membrane of 0.2 µm pore size with polypropylene support was obtained from Advantec (Toyo Roshi Kaish,Ltd, Japan).

Membrane Modification

The membrane under study was a highly hydrophobic Teflon membrane with polypropylene support. Surface modification via chemical treatment of the polypropylene backing was carried out to enhance the hydrophilicity of the permeate side. The process was initiated with treatment with chromic acid

solution which was prepared by mixing potassium dichromate ($K_2Cr_2O_7$), sulfuric acid and water in a ratio of 1:20:30 (9). After preliminary wetting in acetone, the membrane was treated with the chromic acid solution for 1 min in an oven maintained at 60^o C. The membrane was then washed with distilled water. The hydrophilization was characterized by measuring the contact angle of water droplet on membrane surface, Fourier transform infrared (FTIR) spectroscopy (Magna IR System 560, Nicolet Instrument Corporation, Wisconsin, USA) and Scanning Electron Microscope with Energy Dispersive X-ray (SEM-EDX) Spectroscopy (Leo 1530 VP, Carl Zeiss SMT AG Company, Oberkochen, Germany). Performance of the hydrophilized membrane was compared with that of the unmodified membrane by determining the flux at different flow rates, temperature and salt concentration

Results and Discussion

Membrane Characterization

The membrane was characterized using Scanning Electron Microscopy (SEM) along with Energy Dispersive X-ray (EDX) Spectroscopy. Figure. 3 (a) and (b) show EDX images of the permeate side before and after hydrophilization. The EDX analysis of permeate side of the membrane showed an increase in oxygen content from 1.5 to 7% after hydrophilization.

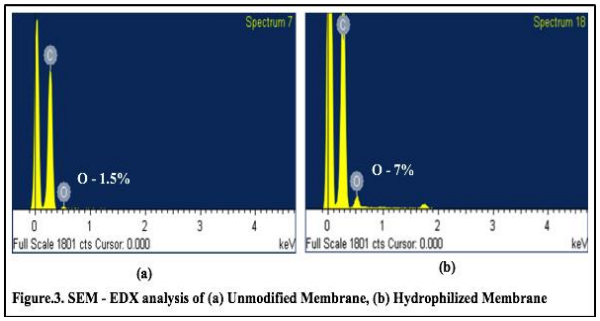

Figure.3. SEM - EDX analysis of (a) Unmodified Membrane, (b) Hydrophilized Membrane

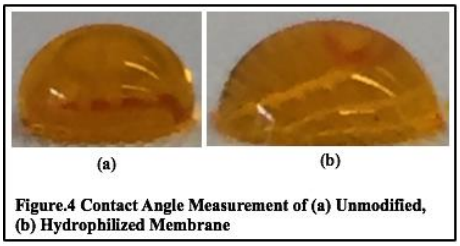

Figure.4 Contact Angle Measurement of (a) Unmodified, (b) Hydrophilized Membrane

The hydrophilicity of the modified membrane was also studied by contact angle measurements. A low contact angle on the permeate side can lead to the pore wetting of the membrane by increased surface energy (10). After hydrophilization, the contact angle was found to decrease from 94^o±2 to 73^o±2. Lowering contact angle via partial hydrophilization in the permeate side of the membrane was expected to have positive effect on the membrane performance. The photographs of the contact angle measurements performed at the permeate side of both the hydrophilized and unmodified membrane are shown in Figure. 4 (a) and (b).

Effect of Hydrophilization on Membrane Performance

Overall membrane performance was studied as a function of permeate flux attained by both hydrophilized and unmodified membranes. The effect of hydrophilization was studied as a function of temperature, flow rate and feed concentration. Figure. 5(a), presents water vapor flux as a function of temperature for both membranes. It is evident that water vapor flux increased with temperature in both of the membranes. This was attributed to the exponential increase in vapor pressure with temperature (11, 12). It was seen that the hydrophilized membranes exhibited higher water vapor flux compared to the unmodified membrane. Maximum water vapor flux of 61.4 L/m^2.hr was attained at 80°C feed temperature at a permeate flow of 200 mL/min for the hydrophilized membrane. The effect of hydrophilization of the permeate side was quite dramatic with an enhancement as high as 52% at 60° C.

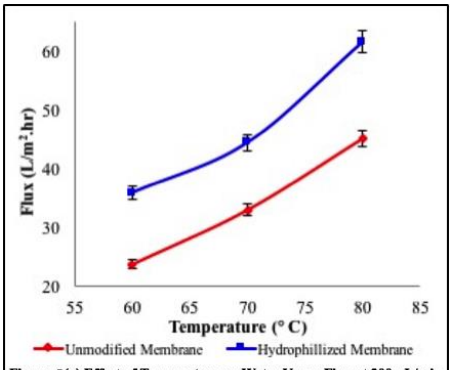

Figure. 5(a) Effect of Temperature on Water Vapor Flux at 200mL/min

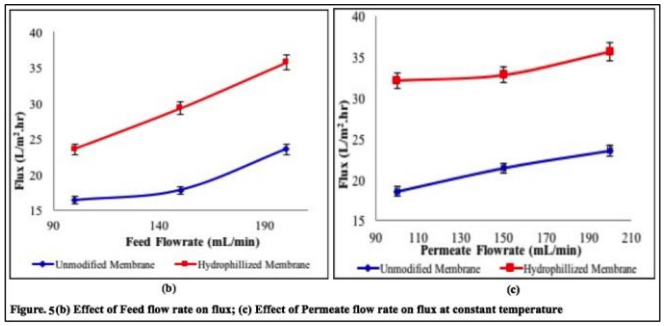

Figure. 5(b) Effect of Feed flow rate on flux; (c) Effect of Permeate flow rate on flux at constant temperature

Figure. 5 (b) shows the effect of varying feed flow rate at 60° C at a constant permeate flow rate of 200 mL/min, while Figure. 5 (c) shows the effect of varying permeate flow rate at the same temperature but at constant feed flow rate of 200 mL/min. Higher flux were observed in both cases. Nearly 73% enhancement was attained at 100 mL/min and 60°C for hydrophilized membrane at constant feed flow rates. The increase in water vapor flux for increasing flow rate was attributed to the increased turbulence and reduced boundary layer effect at elevated flow rates which helped in lowering the temperature polarization and increased the driving force for MD.

Figure. 5 (d) shows the effect of varying feed concentration on permeate flux. The high salt concentration decreased the water activity at the membrane interface. A significant boundary layer developed along the membrane interface at high feed concentrations which reduced the driving force across the membrane. This led to a decrease in permeate flux. As expected, overall water vapor flux decreased with increase in salt concentration ranging from 61.4 L/m^2.hr and 45 L/m^2.hr for pure water to 42.9 L/m^2.hr and 35.7 L/m^2.hr at 10000 ppm concentration for hydrophilized and unmodified membrane, respectively.

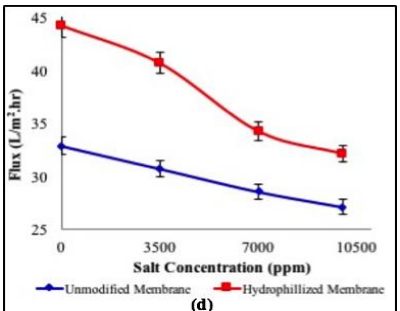

Figure.5(d). Effect of Salt Concentration on Water Vapor Flux.

Conclusion

Enhanced flux in DCMD using a hydrophilized membrane is reported. It was evident that hydrophilization was effective in rapid permeate removal thus enhancing mass transfer coefficients. The membrane distillation performance was consistently higher in case of hydrophilized membranes at all flow rates, temperature and salt concentrations. Flux enhancement reached as high as 73%.

6. Publications and Presentations:

Publication

Article titled "Selective Hydrophilization of the Permeate Surface to Enhance Flux in Membrane Distillation" by Smruti Ragunath, Sagar Roy and Somenath Mitra, is currently under review at journal of *Separation and Purification Technology*.

Presentation

(a) Poster entitled "Selective Hydrophilization of the Permeate Surface to Enhance Flux in Membrane Distillation" by Smruti Ragunath, Sagar Roy and Somenath Mitra, presented at 101th NJWEA Student Poster Session on May 18th, 2016 at Atlantic City, NJ.

(b) Oral presentation entitled "Selective Hydrophilization of the Permeate Surface to Enhance Flux in Membrane Distillation" by Smruti Ragunath, Sagar Roy and Somenath Mitra, is selected for 252ndACS National Meeting, scheduled for August 2016, at Philadelphia, PA.

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Information Transfer Program Introduction

None.

Knowledge and action on nonpoint pollution: a professional development program for educators

Basic Information

Title:	Knowledge and action on nonpoint pollution: a professional development program for educators
Project Number:	2015NJ363B
Start Date:	3/1/2015
End Date:	2/28/2016
Funding Source:	104B
Congressional District:	NJ-004
Research Category:	Water Quality
Focus Category:	Education, Non Point Pollution, Water Quality
Descriptors:	None
Principal Investigators:	Roberta Howard Hunter

Publication

1. Hunter, Roberta. 2016. Environmental PBL as Professional Development. North American Association for Environmental Education Research Symposium ,October 14, 2016, San Diego, CA

Grant Report for

Knowledge and Action on Nonpoint Pollution:

A professional development program for educators

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Numbers of Students Supported:

Undergraduates:	0
Masters' students:	0
Ph. D. students:	0
Postdoc Associates:	0

PROJECT SUMMARY

Problem and Research Objectives

Certain water resource issues lend themselves to public engagement by being accessible in both evidence and in action to remediate. Nonpoint source pollution is one such issue. While the actual physical and chemical parameters of the water quality may not be visible to most, the impacts of impaired water quality often are – excessive algal growth, high temperatures, and sedimentation can all be observed by the public, and can be addressed through both individual and collective public action. In addition, nonpoint source pollution is a near-universal issue for both urban and rural communities. The research described here piloted and examined the efficacy of a problem-based learning professional development program for formal and informal educators. Of key interest is the development of certain environmental literacy

competencies: identifying and investigating issues; using physical, ecological, and sociopolitical information to develop possible solutions; and articulating views about warranted individual and collective actions (Hollweg, Taylor, Bybee, Marconkowski, McBeth, & Zoido, 2011). The coastal lakes of Monmouth County served as a context of study for the professional development program, as they are critically impacted by nonpoint pollution. Both quantitative and qualitative methods were used to evaluate the program, which will be expanded in coming years to include classroom observations to determine the impacts on educator practice with youth.

This research addressed the *Information Transfer* priority detailed in the RFP by developing educators' understanding of the physical, ecological, and sociopolitical aspects of nonpoint pollution and their competencies in identifying individual and collective actions to be taken on the issue. Using Monmouth County's nine coastal lakes as an instructional context, educators will develop not only their own environmental literacy, but strategies to develop that of the youth with which they work. This research program is uniquely positioned for greater impact by including both formal educators (public, private, and charter teachers for grades 6-13) and informal educators (nature center, museum, and zoo/aquaria staff as well as youth development staff and volunteers) together. Even during the K-12 years, children only spend about 18.5% of their waking hours in a classroom, an amount that decreases sharply as the person grows (Feder, et al., 2009). By providing formal and informal educators the understanding and skills to address water resource issues with youth in multiple settings, we can provide life-wide learning with a great impact. While a middle or high school teacher can

work at length with 100 – 150 students per year, informal educators can work with as many as 1000 in short-term settings. The introduction of the Next Generation Science Standards (NGSS Lead States, 2013) has made it easier to connect science practice and content such as water resource understanding and management across multiple contexts.

Effective professional development for teachers is lacking in the U.S. While effective teacher professional learning is intensive, ongoing, connected to practice, and connects teachers with colleagues, most of the opportunities available to public school teachers are short term, have limited connection to teachers' actual practice, and do not support effective collaboration (Darling-Hammond, Wei, Andree, Richardson, & Orphanos, 2009). True collaborative work, rather than contrived collegiality (Hargreaves & Dawe, 1990), encompasses many of the qualities of excellence (Erickson, Brandes, Mitchell, and Mitchell, 2005). The proposed project meets these benchmarks of quality through the use of problem-based learning. Problem-based learning (PBL) is a form of collaborative learning in which learners work together to find viable solutions to a complex, ill-defined problem (Barrows, 1986; Hmelo-Silver, 2004). Though its implementation varies, PBL has four key components: problems are ill-structured and authentic, the approach is learner-centered, and teachers act as facilitators (Barrows, 2002; Hmelo-Silver & Barrows, 2006). One of the distinguishing characteristics of PBL is that the learning goals are not focused solely on content knowledge, but also problem-solving, metacognition, and self-regulated learning skills (Torp & Sage, 2002). Issues such as nonpoint source pollution which are complex and lay at the crux of science and society are an excellent match for problem-based learning.

Objectives:

1. Using Monmouth County coastal lakes as a context for a problem-based learning professional development program, prepare formal and informal educators to address nonpoint source pollution with youth in multiple contexts.
2. To create formal and informal educators who are prepared to address nonpoint source pollution and other water resource issues with at least 1000 youth in the year following completion of the program.
3. Improve the decision making competencies of environmental literacy, specifically around water resource issues, in formal and informal educators to be measured by both pre-post task-based assessment as well as analysis of online and face to face interaction through chat transcripts and session video.
4. To pilot a model hybrid problem-based learning environmental literacy professional development program integrating online and face-to-face sessions.

Methodology

Due to difficulties with recruiting for the multiple-session format originally proposed, the KAN-P program was implemented as a six-hour, one-day workshop held in conjunction with the Alliance for New Jersey Environmental Education annual conference in January 2016. The 18 attendees included 3 formal educators, 6 nonformal educators who work closely with public schools, and 9 nonformal educators from a variety of settings. During the day-long workshop, participants worked in small groups in a problem-based learning context. After an introduction to the basics of problem-based learning, groups worked collaboratively in small groups, while I

provided just-in-time instruction when necessary. Each group had a laptop available to them for research purposes, and were provided basic information sites and scenario-specific information through a LiveBinders site set up for the program (http://bit.ly/1rBSMol, access key: BayPond). They were provided with the following scenario:

> *You are a member of the planning committee for the shore town of Bayburg, NJ. Within the town is Bay Pond, a coastal lake that has been considered a treasure of the community for generations. In the past decade, Bay Pond has become polluted and is often full of algae and trash. It is no longer the picturesque spot it once was. During large storms, it sometimes floods, pouring out into the ocean, which then closes the beach because the water is not safe for swimming.*
>
> *A recent report from the DEP has identified three main issues with Bay Pond: sedimentation, eutrophication, and high microbial levels. The mayor of Bayburg has given your committee the task of creating a plan to remedy the situation and bring back the beauty of Bay Pond.*

Each group member was provided with a Bay Pond community role (i.e. Chamber of Commerce chair, café owner, environmentalist) to play during the problem-based learning work. Groups then spent several hours researching the causes of the DEP issues, and collaboratively creating solutions, which were then presented to the whole group.

Quantitative data collected was in the form of a Qualtrics survey that covered basics of non-point source pollution (sources, impacts, solutions) and problem-based learning. The Qualtrics survey was completed prior to attending the workshop and within 2 weeks after the

workshop. Qualitative data collection was through personal meaning maps completed at the beginning and end of the workshop. In addition, program evaluations were collected at the end of the workshop.

Principal Findings and Significance

Content knowledge

There were 9 participants who completed the Qualtrics survey both pre- and post-workshop. There did appear to be a ceiling effect, as participants came in to the program more knowledgeable about non-point source pollution than anticipated. For the top scoring participants, there appears to be a decrease or flat score. This is due to responses on one question "How does nitrogen waste affect aquatic health?" which became less complex on the post-test. It is apparent that the workshop positively affected knowledge for those participants who came in with less extensive knowledge about non-point source water pollution.

Table 1. Non-point source pollution content knowledge assessment

TOTAL KNOWLEDGE PRE SCORE (Out of 20)	TOTAL KNOWLEDGE POST SCORE (Out of 20)	CHANGE
18	18	0
18	15	-3
16	16	0
14	11	-3
14	16	2
13	16	3
12	15	3
9	15	6
9	13	4
MEAN 13.7	**15**	

The types of knowledge represented on the personal meaning maps changed over the course of the workshop, as well. Pre-workshop personal meaning maps focused largely on types of non-point source pollution and to a lesser extent the impacts on natural systems. In the post-workshop personal meaning maps, there was an emergence of themes of preventing and correcting non-point source pollution and the importance of community engagement in mitigation.

Problem-based learning

The Qualtrics instrument also included questions about problem-based learning to assess participants' knowledge of and attitudes towards problem-based learning. As expected, many participants had an awareness of problem-based learning, but not an understanding of it. This was changed by participation in the workshop. When asked how problem-based learning benefits learners, one respondent's answer to the question "How does problem-based learning benefit learners/students?" developed from a pre-workshop response of:

> "by allowing students to apply previous learning and research skills to find solutions to real problems"

to the following post-workshop response:

> "Helps learners to determine what they don't know, and allows for collaboration with a group to research answers to those questions. The students are more

> actively engaged in their learning, and are also learning to work together and break a problem into smaller parts."

This post-workshop answer demonstrates an awareness of the metacognitive (determining what they don't know, breaking a problem into smaller parts) and social-cognitive (engagement and collaborative learning) aspects that lend themselves to high quality learning and ultimately to learning for environmental literacy (Hollweg, et al., 2011). The connection to environmental literacy is also apparent in another participant's response to the same question post-workshop:

> "From my experience at the workshop, I realized how many different ways there are to view the same problem. We tend to identify the problem and want it solved from our own viewpoint. My experience helped me to see that your decisions effect many different people in different ways. It really helped me to learn to look at all sides. We learned both thinking strategies as well as researching the problem and finding out the facts. I think student centered learning prepares you for real life.., as opposed to lectures, assignments or exercises which really don't prepare you to be self directed."

Program evaluations

The program evaluation used was the Program Evaluation for Older Audiences from the Rutgers Department of 4-H Youth Development. This evaluation asks retrospective questions about past and current knowledge (on a scale of 1 = no knowledge to 4 = a lot of knowledge), how participants will use the information they learned, and to rate the presenter and program (1 = poor, 5 = excellent). Again, the ceiling effect is apparent, with the pre-workshop knowledge

form nonpoint source pollution fairly high (Table 2). The quality of the presenter and program were both rated very high, with means of 4.8 out of 5.

Table 2. Program evaluation knowledge self-report

	Pre-workshop mean (out of 5)	Post-workshop mean (out of 5)
Non-point source pollution	3.1	3.8
Problem-based learning	2.3	3.6

When asked how they will use the information they learned, participants said they would use it with students in schools (either their own or partner schools) (n=7), with the public, community, or their nature center (n=4), or to develop curriculum (n=4). These numbers suggest that both problem-based learning and information about problem-based learning will potentially reach a great number of learners across the state as a result of this workshop.

Publications or Presentations

Environmental PBL as Professional Development

Poster North American Association for Environmental Education Research Symposium
October 14, 2016, San Diego, CA

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USGS Summer Intern Program

None.

Student Support					
Category	**Section 104 Base Grant**	**Section 104 NCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	14	0	0	0	14
Masters	5	0	0	0	5
Ph.D.	12	0	0	0	12
Post-Doc.	0	0	0	0	0
Total	31	0	0	0	31

Notable Awards and Achievements

For 2015NJ361B: Alessia Eramo was awarded the Kenneth S. Stoller Award by NJ WEA 2016. Hannah Delos Reyes was awarded a scholarship by NJ WEA 2016. Reba Oduro was awarded “Best STEM Poster” at the Aresty Research Symposium, Rutgers University. Hannah Delos Reyes was awarded a poster award at the NJ WEA 101st Annual Meeting, May 18, 2016.

For 2015NJ367B: Alessia Eramo was awarded the Kenneth S. Stoller Award by NJ WEA 2016.

For 2015NJ369B: A $500 travel award from the local Hudson Delaware Chapter of SETAC was awarded for participating in the 5th annual Young Environmental Scientist Meeting, February 28 – March 2, 2016, Gainesville, FL.

For 2015NJ370B: A 1st Place in the 101ST Annual New Jersey Water Environment Association (NJWEA) Conference was received in the Student Poster Competition (Presentation Title: Potential Use of Vetiver Grass to Remove Ciprofloxacin from Aquatic Media).

For 2015NJ371B: A 2nd place in the Student Poster Competition organized by the New Jersey Water Environment Association held at Atlantic City, NJ on May 18th 2016 was received.