

**New Jersey Water Resources Research Institute  
Annual Technical Report  
FY 2009**

# Introduction

The New Jersey Water Resources Research Institute (NJWRRI) supports a diverse program of research projects and information transfer activities. 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 supporting graduate students with developing their thesis research. The funding is intended to initiate novel and important research efforts by both faculty and students, and thus emphasizes 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.

Research projects span a wide range of topics in water resources. In the first faculty project, Bini sought to study the effects of wastewater treatment plant (WTP) effluents on the composition and resistance of microbial communities in surface waters and sediments. This could help determine if antibiotics contribute to the emergence of infectious diseases by affecting the development of reservoirs of antibiotic resistance genes within natural microbial communities. In the second faculty project, Miskewitz tested a new method to measure sediment oxygen demand (SOD). The new method has the ability to incorporate the effects of stream velocity, with reduced impacts on the benthic layer and reduced measurement times. The final faculty project was going to determine and improve the efficacy of using chlorophyll, the most abundant natural pigment, as the next generation photocatalyst for water decontamination. However, this project ended early when the PI moved to a university outside of New Jersey.

Graduate students have similarly carried out an impressive range of research. Babson and his advisor sought to characterize the biochemical pathways of anaerobic degradation processes in waste and wastewater management systems, allowing the systems to recover fuel products and mitigate associated wastewater streams. Bugel and his advisor investigated the link between aryl hydrocarbon receptor agonists and reproductive effects in killifish (*Fundulus heteroclitus*). Results suggest that long-term exposure to exogenous contaminants may lead to short-lived biomarker responses due to biochemical adaptation. Oka and her advisor analyzed groundwater samples from a manufacturing gas plant impacted site for the presence of metabolic and molecular biomarkers of anaerobic hydrocarbon degradation. This is one of the first studies in New Jersey that has combined the use of metabolic biomarkers along with catabolite gene *bssA* to evaluate the in situ anaerobic bioremediation potential of a hydrocarbon impacted site.

Additionally, Joshua Galster and Kirk Barrett of Montclair State University received funding from the NIWR/USGS National Competitive Grant Program for a study of the relationship between urbanization and stream baseflow. This three-year grant will study streams and watersheds in ten East Coast states. Initial findings of this research are reported.

The goal of our information transfer program is to bring timely information about critical issues in water resource sciences to the public, and to promote the importance of research in solving water resource problems. The information transfer program continues to focus on producing issues of the newsletter that provide a comprehensive overview of a particular water resource topic, as well as one issue a year that highlights water research occurring in New Jersey. The program continues to develop the NJWRRI website ([www.njwrri.rutgers.edu](http://www.njwrri.rutgers.edu)) into a comprehensive portal for water information for the state. We also collaborate with other organizations in sponsoring and producing conferences.

## **Research Program Introduction**

The New Jersey Water Resources Research Institute has had a policy, yearly re-affirmed by the Advisory Council, of using the research dollars to promote new and novel directions of research. To this end, three projects directed by research faculty at institutions of higher learning around the state were selected, and three grants-in-aid were awarded to graduate students who are beginning their research. In both cases, 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 resource issues of importance to the state.

In addition, this fiscal year a project from New Jersey received funding through the NIWR/USGS National Competitive Grant Program. The initial findings of that research are reported here.

# Antibiotic pollution of aquatic habitats and impact on the development of environmental pools of resistance in natural microbial communities

## Basic Information

<b>Title:</b>	Antibiotic pollution of aquatic habitats and impact on the development of environmental pools of resistance in natural microbial communities
<b>Project Number:</b>	2009NJ188B
<b>Start Date:</b>	3/1/2009
<b>End Date:</b>	2/28/2010
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	6
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Water Quality, Groundwater, Non Point Pollution
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Elisabetta Bini

## Publications

1. Honarbakhsh M., D. Sannino, A. Gomes, and E. Bini. 2010. Occurrence of antibiotic resistant bacteria and resistance genes in wastewater effluents and surface waters. 110th General Meeting, American Society for Microbiology, San Diego, May 23-27, 2010. (poster presentation)
2. Honarbakhsh M., D. Sannino, A. Gomes, and E. Bini. 2010. Occurrence of antibiotic resistant bacteria and resistance genes in wastewater effluents and surface waters. Meeting in Miniature of the Theobald Smith Society, NJ Branch of the ASM. New Brunswick, NJ, April 29th, 2010. (poster presentation)
3. Honarbakhsh M., D. Sannino, A. Gomes, and E. Bini. 2010. Occurrence of antibiotic resistant bacteria and resistance genes in wastewater effluents and surface waters. 4th Annual Mini-Symposium on "Microbiology at Rutgers University: Cultivating Traditions, Current Strength, and Future Frontiers". New Brunswick, NJ, February 1-2, 2010. (poster presentation)

## Problem and Research Objective

This project aims to study the effects of wastewater treatment plant (WTP) effluents on the composition and resistance of microbial communities in surface waters and sediments. The hypothesis being tested is that antibiotics contribute to the emergence of infectious diseases by affecting the development of reservoirs of antibiotic resistance genes within natural microbial communities. Affected environments are expected to be those exposed to detectable concentrations of antibiotics, such as in the immediate surroundings of wastewater treatment plants. To prove a correlation between antibacterial agents' pollution and development of resistance in natural ecosystems, the following aims will be pursued:

***Aim #1. Determination of antibiotics in wastewater effluents and sediments.*** The concentrations of a variety of antibiotics will be measured at different sites in the area surrounding WTPs. Water and sediment samples will be tested. Occurrence of specific antibiotics will be compared to the data obtained from corresponding sampling sites in objectives #2 and #3 to evaluate if there is a correlation between the sets of values.

***Aim #2. Testing cultured isolates for antibiotic resistance.*** Environmental isolates from the area surrounding WTPs will be tested for their resistance to different classes of antibiotics. Isolates will be identified by 16S rRNA gene sequencing. Presence of genes conferring resistance to specific antibiotics will be verified by PCR. These data will provide the linkage between microbial species and resistance genes, and will provide evidence whether or not natural communities have developed antibiotic resistance.

***Aim #3. Community structure and metagenomic analysis of antibiotic resistance determinants.*** The analysis will be conducted applying TRFLP and quantitative real-time PCR techniques to total DNA extracted from waters and sediments. This will be used to assess the composition of affected microbial communities and the extent and distribution of resistance genes. The analysis will also quantify the proportion of individual resistance genes in such samples, regardless of the culturability of the species present.

## Methodology

***Study area and sample collection.*** The Somerset Raritan Valley Sewerage Authority, established in 1958, is a regional wastewater treatment plant located in Somerset County, NJ. After treatment, the wastewater is discharged to a channel which eventually merges with the Raritan River. Samples were collected from the final effluents of the plant, indicated with a P in the figure, and from two locations downstream of the sewage discharge, at N 40° 33.287' W 74° 34.069' and at N 40°33.160' W 74° 33.797', indicated with RI and RII, respectively. The distance between the RI and RII is approximately 500 m (Fig.1).



**Figure 1. Arrows indicate the sites where samples were collected**

Water samples were concentrated by filtration and plated on Muller Hinton agar. Individual strains were purified by repeated streak transfers on the same medium.

*Assessment of antibiotic presence in wastewater effluents and sediments.* Water samples were concentrated by lyophilization samples and analyzed by HPLC-MS-MS using the Core Research facilities of the Biotechnology Center for presence of antibiotics. Antibiotic standards were prepared in a range of concentrations and analyzed by HPLC for an assessment of their retention times and optimization of the conditions of separation and detection. Peaks corresponding to each antibiotic were directed to a mass spectrometer for further analysis. Standard curves for each antibiotic were constructed using the MS peak area as a function of the amount of antibiotic. These standard curves will be used in to quantify the concentration of antibiotics present in the water samples collected at the locations described in Fig.1.

*Characterization of antibiotic resistance in culturable isolates.* MIC (antibiotic minimal inhibitory concentration) for each antibiotic was determined on Muller Hinton Broth, in microwell plates. Serial dilutions, from 200 to 15,000 ug/ml, of each of the six antibiotics were tested. Cultures of each strain were incubated overnight at 37°C and growth was assessed by OD measurement at 600nm.

*Extraction of metagenomic DNA.* Immediately after collection, 500 ml of each composite sample were filtered on nitrocellulose membranes and DNA was isolated by phenol extraction. The DNA was further purified on a Cs chloride gradient.

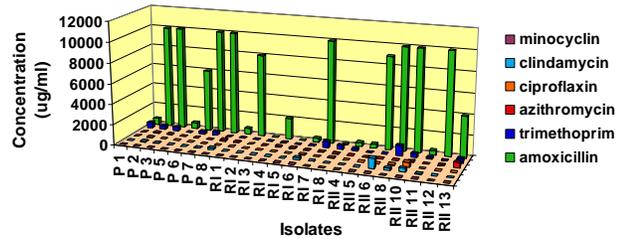
*TRFLP (terminal restriction fragment length polymorphism) analysis.* Genes corresponding to the 16S rRNA were PCR amplified from the environmental DNA using a fluorescent dye-labeled forward primer, subjected to restriction digestion, and the resulting fragments were separated and visualized on a sequencing platform. The same technique will be applied to the analysis of the diversity of antibiotic resistance genes.

*Construction of 16S rRNA gene libraries.* 16S rRNA genes were amplified from the metagenomic DNA using the universal primers Bact-8F (5'-AGAGTTTGATCCTGGCTC AG) and Univ-1517R (5'-ACGGCTACCTTGTTACGACTT). PCR amplicons were cloned into Topo vectors (Invitrogen), transformed into *E. coli* and individual colonies were screened by RFLP to identify unique clones for sequencing. Using the same method, we will also construct libraries of antibiotic resistance genes.

## Principal Findings and Significance

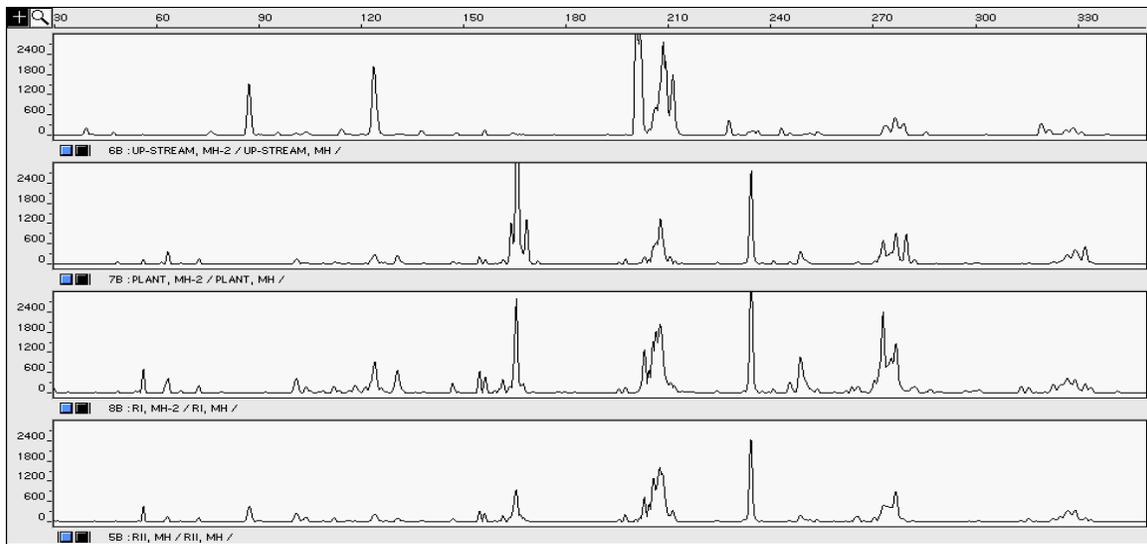
*Culture-dependent methods.* The prevalence of antibiotic resistance in strains isolated from plant effluents (P) and downstream from the wastewater treatment plant (RI and RII) was investigated. The sensitivity of pure isolates to different antibiotics was first assessed using the disc-diffusion method on Muller Hinton Agar. The following antibiotics were tested: amoxicillin, azithromycin, clindamycin, ciprofloxacin, minocyclin, and trimethoprim. All isolates resulted resistant to amoxicillin, and many showed azithromycin and trimethoprim resistance. To a lesser extent, resistance to clindamycin, ciprofloxacin and minocyclin was also observed for some isolates. The analysis of further isolates collected upstream from the discharge site, and from pristine environments (Pine Barrens), is currently in progress.

*Characterization of antibiotic resistance in culturable isolates.* Most isolates resulted resistant to elevated concentrations of amoxicillin, while some of the bacteria, regardless of the site of sampling, displayed resistance to low concentrations of all of the other antibiotics. Resistance to multiple antibiotics was observed in most isolates (Fig. 2).



**Figure 2. Minimal inhibitory concentration of selected antibiotics**

*Microbial biodiversity of the four sites.* TRFLP (terminal restriction fragment length polymorphism) is a culture independent technique which was applied to study the complexity of the microbial community based on variation in the 16S rRNA genes in all four samples. To generate fluorescently-labeled terminal restriction fragments, amplification of the 16S rRNA gene was carried out by using a fluorescent dye-labeled forward primer. The amplicons were subjected to restriction enzyme digestion and then separated and detected on a sequencing platform. Different peaks correspond to 16S rRNA genes with different digestion patterns, and each TRFLP profile provides a fingerprint of the microbial community under analysis. The comparison of the four samples indicates that some peaks are common to all samples, although their intensity may vary, and some are unique. In particular, the composition of the “Upstream” community appears most dissimilar from the sites P, RI and RII (Fig. 3).



**Figure 3. Preliminary TRFLP results show that the intensity of specific peaks shared among the four sites varies from site to site.**

*Conclusions.* We investigated the occurrence of antibiotic resistance in bacterial isolates from the final effluents of the Somerset Raritan Valley Sewage Authority in New Jersey, and from two locations downstream of the sewage discharge. Bacteria belonging to different genera were identified using culture-dependent techniques and 16S rRNA gene sequencing. Isolates from the wastewater effluent included various *Bacillus*, *Enterobacter*, *Acinetobacter*, and *Staphylococcus* species. *Enterobacter* and *Staphylococcus* species were not present in samples collected from the river downstream of the plant, which was characterized by the presence of *Brevibacterium*, *Chryseobacterium*, *Aeromonas*, and *Delftia* strains. The antibiotic susceptibility phenotypes were determined by the disc-diffusion method for six of the most frequently prescribed antibiotics: amoxicillin, azithromycin, clindamycin, ciprofloxacin, minocyclin, and trimethoprim. All the isolates resulted resistant to elevated concentrations of amoxicillin, while some of the bacteria, regardless of the site of sampling, displayed resistance to low concentrations of all of the other antibiotics. Resistance to multiple antibiotics was observed in most isolates. Results suggest that resistance to specific antibiotics might be due to the lateral transfer of resistance genes from wastewater effluents to freshwater bacteria.

## A modified photosynthesis process for water purification

### Basic Information

<b>Title:</b>	A modified photosynthesis process for water purification
<b>Project Number:</b>	2009NJ191B
<b>Start Date:</b>	3/1/2009
<b>End Date:</b>	7/1/2009
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	6
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Water Quality, Treatment, Methods
<b>Descriptors:</b>	
<b>Principal Investigators:</b>	Shaurya Prakash

### Publications

There are no publications.

## Project Summary:

Photocatalysis provides a new pathway for developing better, more efficient and cost-effective methods for water decontamination. There is growing research in finding new materials and methods for developing visible light photocatalysts for potential use of solar energy for water decontamination applications. One such material, provided to us by nature, is chlorophyll. In the absence of quenchers such as carotenoids, visible light interaction with chlorophyll leads to generation of singlet oxygen which is a highly reactive oxygen species. This reactive oxygen species can interact with organic matter to cause oxidation and eventually decontaminate water. The goal of this research effort was to determine and improve the efficacy of using chlorophyll, the most abundant natural pigment, as the next generation photocatalyst for water decontamination. An experimental approach was to be used to identify critical parameters required for optimization of chlorophyll use. Furthermore, a model nanofluidic device was to be built to overcome challenges related to short lifetime and subsequent diffusion length of singlet oxygen.

The project was terminated early (in 3 months after the start date) due to the PI moving from Rutgers University to The Ohio State University. Only initial fabrication experiments were conducted as NJWRRI funds were not transferred to continue research in Ohio.

During the brief duration of the project, the undergraduate student began fabrication of the originally proposed nanofluidic device and was making progress towards developing a robust and repeatable methodology for device fabrication.

## Development of a profile SOD measurement technique

### Basic Information

<b>Title:</b>	Development of a profile SOD measurement technique
<b>Project Number:</b>	2009NJ193B
<b>Start Date:</b>	3/1/2009
<b>End Date:</b>	2/28/2010
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	6
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Methods, Water Quality, Hydrology
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Robert J. Miskewitz

### Publication

1. Miskewitz, R., Francisco, K. and Uchrin, C. (2010) Comparison of a novel profile method to standard chamber methods for measurement of Sediment Oxygen Demand, Journal of Environmental Science and Health, Vol. 45 No. 7.

***Problem/Objective:***

Sediment oxygen demand (SOD) is the sum of the dissolved oxygen removed from the water column by the respiration of organisms living in the sediment and the oxidation of reduced chemicals found in the sediment. The SOD in a stream can vary based on sediment age, surface area, depth of deposits, temperature, and chemical and biological characteristics. SOD is often considered an indicator of the health of the system because sediment populations remain relatively stable while the overlying water can be transient.

The most common method of SOD measurement involves enclosing a known volume of water in a chamber over a known area of the riverbed surface, then measuring the change in dissolved oxygen concentration over a period of time. The rate of oxygen depletion in the water is then used to calculate the SOD. However, one of the most important environmental factors that will affect the SOD is the flow rate of water over the sediment/water interface, which cannot be simulated in an SOD chamber. The flow conditions also represent one of the largest sources of uncertainty in SOD measurements. It has been observed that SOD increases proportionally to increasing stream velocity, especially at low velocities. This dependence on flow is thought to be related to mixing in the near surface boundary layer; however it has also been shown that even small changes in the stream velocity impact the benthic respiration as well as the chemical oxygen demand in sediments.

The SOD measurement methodology tested in this study is based upon a characterization of the flow in the near sediment boundary layer and the transport of dissolved oxygen down a concentration gradient. The main advantage that it has over chamber methods is the ability to measure the SOD flux as a function of the flow. In addition, the measurement time is reduced from two hours to ten minutes. To test this methodology, side by side comparison of the chamber and profile methods were conducted.

***Methodology:***

SOD was measured during three days at two locations in July 2009. Deployments were conducted for a period of approximately six hours each. During this time at least two chamber measurements were attempted and the profile was sampled continuously. The deployment locations were the Millstone River in Hillsborough, NJ and the Lawrence Brook in Milltown, NJ. These locations were chosen because they had the following conditions: the water depth was at least 0.8 meters and the center channel was regular and had a relatively flat bottom. During the deployments, water temperature was measured by the profile system. These measurements were then used to correct the SOD.

**Chamber Method:** The chamber was placed on the bottom of a stream and secured into the sediments with care so as not to disturb the bottom material in the study area. It was important to obtain a tight seal around the bottom of the chamber to ensure that dissolved oxygen is not transported into or out of the system volume. An In-Situ Inc. Rugged Dissolved Oxygen (RDO) Pro optical dissolved oxygen probe was installed with an airtight seal at the top of the chamber. The system was then left in place for a period of 40 to 130 minutes, during which the dissolved oxygen in the chamber was measured each minute. The change in dissolved oxygen in the chamber is due to SOD and water column respiration. The impacts of the water column respiration were removed using a dark bottle procedure. SOD was calculated using the following expression:

$$SOD = \frac{\Delta C_{DO} V}{\Delta t A} \quad (1)$$

Where  $V$  is the volume of the chamber ( $0.029 \text{ m}^3$ ), and  $A$  is the surface area of the exposed sediment ( $0.101 \text{ m}^2$ ). The dissolved oxygen concentration was plotted against time. The slope of the plotted data ( $\Delta C_{DO}/\Delta t$ ) is the rate of oxygen uptake by the sediment corrected for water column respiration.

**Profile Method:** The profile method is based upon transport through the logarithmic boundary layer that develops when fluid flows over a flat plate. The method takes advantage of the fact that the flux,  $q_{SOD}$ , and the gradient of dissolved oxygen,  $\frac{dC}{dz}$ , are proportional in the boundary layer. The constant of proportionality is a diffusion coefficient. If the stream flow is laminar, the coefficient is the molecular diffusion coefficient for oxygen in water, but in order to properly represent a realistic stream flow, a turbulent diffusion coefficient must be used. In this case, the turbulent diffusion coefficient is referred to as the vertical eddy diffusivity,  $\varepsilon_z$ .

$$q_{SOD} = \varepsilon_z \frac{dC}{dz} \quad (2)$$

The eddy diffusivity is not a function of dissolved oxygen transport parameters, but rather the turbulence present in the flow itself. The eddy diffusivity can then be calculated via Equation 3. This expression calculates the vertical eddy diffusivity as a function of the friction velocity,  $u^*$ , elevation above the bed,  $z$ , and the depth,  $d$ , of the water.

$$\varepsilon_z = \kappa u^* z \left(1 - \frac{z}{d}\right) \quad (3)$$

The value of  $\kappa$  is the von Karmen constant which has a value of 0.4.

Measurements of the gradient of dissolved oxygen concentration above the sediment surface and the vertical eddy diffusivity were collected. The gradient of dissolved oxygen concentrations was measured via three RDO Pro optical dissolved oxygen probes installed on a rack at 10 cm, 20.8 cm and 31.6 cm above the sediment. The dissolved

oxygen gradient was measured at a rate of 1 Hz and averaged over a one minute period. The SOD flux was then calculated for length of the deployment.

In order to calculate the vertical eddy diffusivity, the friction velocity,  $u^*$ , must be measured. The friction velocity is determined by taking the square root of the covariance of the turbulent fluctuations in the vertical and horizontal velocities,  $u^* = \sqrt{u'w'}$ . These measurements were collected using a Sontek Acoustic Doppler Velicometer (ADV) which measured the velocity of the water at a point 4 cm above the sediment surface at a resolution 10 Hz. Using these measurements, 10 minute average  $u^*$  values were calculated. The  $u^*$  values were then used to calculate the eddy diffusivity. Since the dissolved oxygen gradient measurements were calculated using three probes 10.8 cm apart, the average eddy diffusivity was calculated by integrating equation 2 between the probes and dividing by the interval. In this way the average flux was calculated between the probes.

$$q_{SOD} = \varepsilon_z \frac{\Delta C}{\Delta z} \quad (4)$$

The system was built into a rack structure that held the dissolved oxygen probes and the ADV (Figure 1.). The structure was built to resemble a sawhorse. The dissolved oxygen probes were oriented perpendicular to the current and located 5 cm away from the sample volume of the ADV. The legs were located far enough from the sensors to avoid any disturbance to the sediment and the flow.

### ***Results and Discussion:***

Chamber and profile SOD measurements were collected on July 21, 2009 in the Millstone River in Hillsborough, NJ and on July 22 and 28, 2009 in the Lawrence Brook in Milltown, NJ. The two systems were installed at least 2 meters apart laterally across the river from each other. Measurements were collected generally between 9:30 to 14:00 on each day. Measurements collected while personnel were in the stream to move the chamber were discarded. A total of seven chamber measurements were collected over three days; these include one in the Millstone River and six in the Lawrence Brook. Meanwhile a total of 50 profile measurements were collected, including eight in the Millstone River. Chamber measurements in the Lawrence Brook varied from 2.7 to 8.9  $\text{g/m}^2/\text{day}$  and had a mean of 5.0  $\text{g/m}^2/\text{day}$ . Concurrent profile measurements varied from 1.3 to 13.5  $\text{g/m}^2/\text{day}$  and had a mean of 7.16  $\text{g/m}^2/\text{day}$ . In the Millstone River the chamber measurement was 4.6  $\text{g/m}^2/\text{day}$  while the profile measurements varied from 0.5 to 2.2  $\text{g/m}^2/\text{day}$  with a mean of 1.32  $\text{g/m}^2/\text{day}$ . The measurements made via both profile and chamber methods were consistent with previous published values for similar streams (Table 1.).

**Table 1. SOD Measurements**

		SOD Measurements (mg/m <sup>2</sup> /day)
Millstone River	7/21/2009 (chamber)	4.6
	7/21/2009 (profile)	0.5 – 2.2
Lawrence Brook	7/22/2009 (chamber)	3.6 – 9.5
	7/22/2009 (profile)	1.3 – 10.1
Lawrence Brook	7/28/2009 (chamber)	6.7 – 13.0
	7/28/2009 (profile)	4.7 – 13.5
Passaic River NJ (freshwater) <sup>1</sup>	1983	0 - 2.43
Dead River, NJ <sup>2</sup>	2005	5.11 - 17.34
Black Creek, SC <sup>3</sup>	1980	0.8 - 9.34

Note: All measurements were corrected to 20° C.

<sup>1</sup> Giga and Uchrin, 1990.

<sup>2</sup> Otubu et al., 2006.

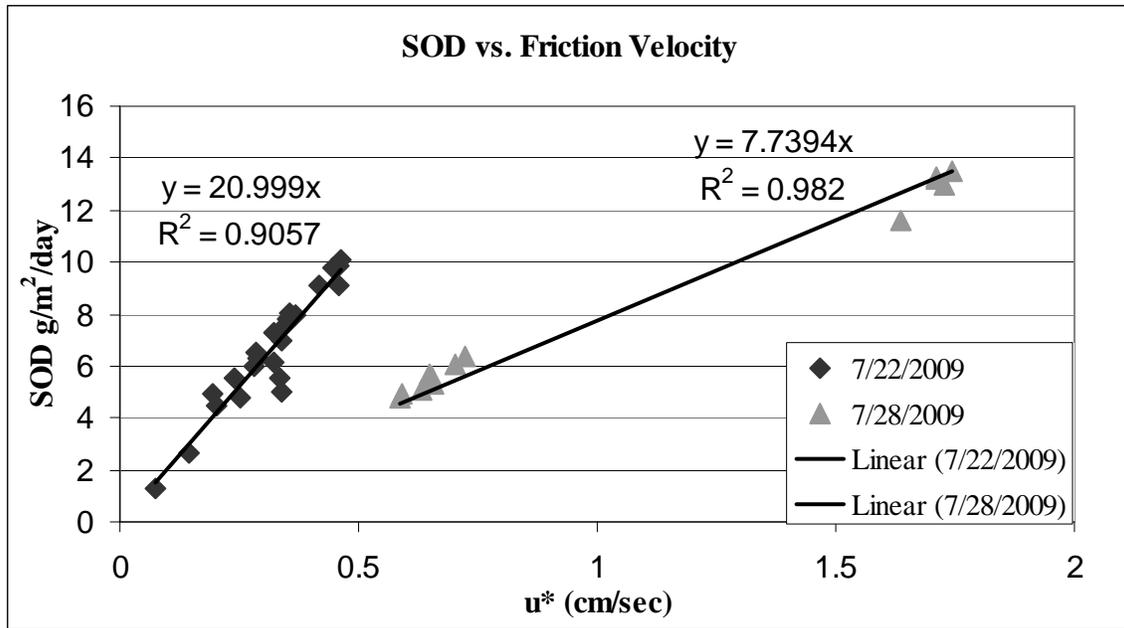
<sup>3</sup> Mancini et al. 1986.

The two rivers are different in character and size. The Millstone River drains an area of 608 km<sup>2</sup> and its bed can be characterized as sandy. Installing the chamber was difficult due to the fact that the sandy bottom resulted in leaks. The chamber was pushed into the sediment to create a seal. Some disruption of the sediments may have occurred in the effort to secure a good seal along the bottom of the chamber and resulted in exposure of buried sediments which may have impacted the SOD measurements. The Lawrence Brook, by contrast, drains a much smaller area of 96 km<sup>2</sup> that is comprised of more suburban and urban areas. The bottom sediments in the Lawrence Brook are silty and contain a high amount of organic matter, and it was much easier to obtain a good seal for the chamber.

It is apparent that while the SOD measurements made via the profile method are very close to those made with the chamber method, significant differences arose. Those differences presumably occurred from the impacts of velocity and the fact that there

was a large storm in between the two sampling events that may have altered the stream bed enough to impact the SOD.

It has been observed that SOD increases proportionally to increasing stream velocity. This is often a confounding variable in chamber studies. A well developed flow regime that can simulate the existing stream conditions is practically impossible in a chamber. During the two days that the profile and chamber systems were deployed in the Lawrence Brook stream conditions varied greatly, especially on 7/28/09. There was a storm the previous day and the river flow was elevated during the first half of the deployment (9AM -11 AM), returning to a more normal condition within a few hours (12 Noon). As a result, measurements collected on both of these days exhibited a fairly wide range of flow conditions. Flow conditions were compared to SOD using the friction velocity,  $u^*$ , because by definition, it is constant over the height of the boundary layer and therefore yields a far more consistent parameter than the measured velocity. It is also a direct measure of mixing in the water column. A linear relationship of  $u^*$  on SOD is clearly seen on both of the two days of measurement in the Lawrence Brook (Figure 1.).



**Figure 1.** SOD measurements compared to friction velocity,  $u^*$ .

Development of a model of SOD as a function of the friction velocity in river would enable more accurate prediction of the dissolved oxygen in the system. Murphy and Hicks (1986) state that for modeling purposes, simulation of bottom velocities as near as possible to field conditions should be the goal of any SOD experiment. The profile

method enables not only measurement of SOD at field conditions but could also enable a SOD -  $u^*$  rating curve to be developed.

It is also clear that the magnitude of SOD can be impacted by disturbance of the surface of the benthic layer. The instances of disturbed sediments in this study resulted from increased suspension of sediment during elevated runoff events and from placement of the chamber for SOD measurement. On July 23, 2009, during the week between the two sampling events, there was a rainfall of 1.73 inches and the flow in the Lawrence Brook was elevated. This storm event disturbed the sediment surface thus changing the magnitude of SOD with respect to the mixing in the boundary layer. After the storm event, the friction velocity required for SOD of a similar magnitude a week prior was significantly higher. This result agrees the idea that during the storm larger particles such as sand were suspended due to elevated flows and settled out as the flow rate decreased. These larger particles are commonly associated with lower SOD and thus greater velocities were required for elevated SOD.

Measurements made with the chamber were also apparently impacted by disturbance of the sediments, especially during chamber placement. One reason for this is that the topmost layer of undisturbed sediments is assumed to be mostly oxidized and results in a relatively smaller SOD on the overlying water column. Once disturbed, the organic-rich sediments that are underlying the topmost sediments are exposed to the oxygen rich water and SOD increases. On July 28, 2009, one of the fluxes measured in the chamber was higher than expected. This was presumably due to the act of placing the chamber, which exposed the benthic sediments underlying the newly deposited surface.

### ***Conclusions:***

This study sought to develop a methodology for measuring SOD that could incorporate the effects of stream velocity. Currently, the vast majority of SOD measurements are taken via the chamber method of Murphy and Hicks. During this study, SOD chamber measurements were collected alongside dissolved oxygen profile SOD measurements. The profile measurements were found to be in relative agreement with the chamber measurements, as well as previously documented measurements in previous studies. The strength of the profile method is that it takes into account the dependence of SOD on streamflow. In addition, much higher temporal resolution can be attained with SOD measurements, on the order of ten minutes rather than two hours using the chamber method. Another important advantage of the new method is that there is less impact to the benthic layer when taking the measurement, thereby yielding a result that is closer to the natural SOD present in the stream. The main potential drawback of the new method is the expense and expertise required to use the ADV.

# Innovative Research and Development for Environmental Protection and Sustainable Waste and Wastewater Management System Design

## Basic Information

<b>Title:</b>	Innovative Research and Development for Environmental Protection and Sustainable Waste and Wastewater Management System Design
<b>Project Number:</b>	2009NJ194B
<b>Start Date:</b>	3/1/2009
<b>End Date:</b>	2/28/2010
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	6
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Wastewater, Groundwater, Water Quality
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	David Babson, Donna E. Fennell

## Publication

1. Babson, David, and Donna Fennell. 2009. System-wide Energy and Carbon Balances for a Sustainable Waste-to-Energy Infrastructure. Sun Grant Initiative Energy Conference, Washington DC.

## Project Summary

Research Priority Topic II values “novel approaches to water resource problems and water science,” and the proposed research falls into this priority topic.

The continued utilization of fossil-fuel energy sources is transferring carbon from the geosphere to the atmosphere and hydrosphere at an unsustainable rate, and is causing global warming and ocean acidification [1-3]. This fact is fundamental to the current debate about energy policy, and has fused energy and environmental policy together [4]. Recovering energy from biogas generated from degrading organic substrates (biomass) is attractive because there are numerous sources of cheap biomass to use, including municipal solid waste (MSW) [5-6]. The ability to characterize and understand the significant biochemical pathways involved in anaerobic degradation processes will allow optimized systems to effectively recover valuable fuel products from degrading waste while minimizing and simultaneously treating associated wastewater streams [7]. The achievement of energy security, while simultaneously protecting the environment and waterways, is of paramount concern in New Jersey.

New Jersey has 21 waste-to-energy facilities associated with existing landfills, and its development of an energy master plan [8] has changed the paradigm for future waste management system design. New systems will seek to further minimize environmental contamination and wastewater accumulation while maximizing energy recovery. Trade-offs between available energy recovery and leachate (wastewater) treatment exist, and optimized systems would focus on efficiency enhancements with respect to both.

Energy recovery from landfill biogas has mainly been viewed as a byproduct of solid waste and wastewater disposal for many years and has not been optimized [9-10]. The prospect of quantifying the specific system energy inputs and outputs using an energy balance, and analyzing how future systems could be designed to minimize energy inputs and maximize outputs, is new [11]. Placing a greater focus on system energy sustainability cannot, however, diminish the need to require minimal impact from the waste and wastewater management sector. The accumulation of nitrogen rich contaminated wastewater is a specific concern. Thus, nitrogen species (ammonia) accumulation associated with waste degradation should be minimized as a system driver and must be part of the considered processes for sustainable design [12].

In traditional anaerobic digestion, organic matter is converted to methane gas (a biofuel), and unwanted byproducts, such as ammonia, are liberated as the organic material degrades. If recovered, ammonia can be catalytically converted to generate hydrogen - an additional biofuel.

A theoretical design scheme for an integrated system to carry out anaerobic digestion, ammonia separation and hydrogen recovery has been established to determine system energy requirements and biofuel (methane and hydrogen) outputs. Energy demands such as heating, fluid pumping, reactor mixing, and ammonia cracking were characterized, and

compared to the potential biofuel outputs over a range of possible feedstock carbon to nitrogen (C:N) ratios.

Material and energy flows for the hypothetical system are a function of the input flows, which can be varied to estimate a system-wide energy balance for a range of possible input values and material compositions. The model was constructed in a Microsoft Office Excel spreadsheet (Microsoft Corporation, Redmond, WA), and input variables and flow stream dependence were propagated throughout the model system. The model consists of a separate worksheet for each of the three parts of the integrated system, but dependent flows on any one worksheet correspond to and are linked to appropriate flows or energy values in another.

The modeled outputs were a function of the assumed inputs which were held constant while the feedstock C:N ratio was varied (Table 1).

**Table 1** - Variable input values for analysis of C:N ratio impact on system energy accumulation

Variable Description	Input Value
C:N Ratio	Variable (3 to 136, (g C/g N))
Dry-Solids Mass Flow	1,000 kg TS/hr
Fraction of Feed Stock Degraded	0.80
Moisture Content (wt.% water)	90.0%
Aqueous TAN Loading (in Stream 2)	100 mg NH <sub>4</sub> <sup>+</sup> -N/L
Percent Recycle of Stream 8	65.0%
Ambient-Digester Temperature Difference	40.0 K
Internal Efficiency	0.35

The total recoverable energy decreased as the feedstock C:N ratio decreased because less carbon was available for methane production. However, the overall system energy balance was positive. This indicated that the integrated system generates more potential energy in the form of methane and hydrogen than it consumed over a broad range of C:N ratios.

For feedstock C:N ratios below 21 (g C/g N), ammonia stripping became the greatest system energy demand, and consistently increased as a fraction of the total system energy demand as the nitrogen loading increased (C:N ration decreased). Several process energy integration cases were assessed. With no process energy integration, the system required between 23% and 34% of the total energy generated, but under optimized conditions this could be reduced to between 8% and 17%. Although the recoverable energy decreased as the feedstock C:N ratio decreased, normalizing total integrated energy potential (hydrogen plus methane) to the stoichiometric methane potential for a given C:N ratio indicated that the integrated process generated more energy than anaerobic digestion alone.

Decreasing the C:N ratio of the feedstock also improved the effluent biogas quality by increasing the methane fraction by as much as 29% (above 80% CH<sub>4</sub>). Finally, the model identified significant process tradeoffs to be optimized, such as the recycle flux and minimum liquid set point. The model also provided a basis and justification for further research of such processes.

This research establishes the wastewater contaminant, ammonia, as an additional biofuel to be recovered from anaerobic systems. It also begins to justify simultaneous methane and ammonia recovery from integrated systems that recover valuable fuel products from degrading biomass while minimizing and treating associated aqueous discharge streams.

## References

1. Cox, P.M., et al., *Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model*. Nature, 2000. **408**(6809): p. 184-187.
2. Hoegh-Guldberg, O., et al., *Coral Reefs Under Rapid Climate Change and Ocean Acidification*. Science, 2007. **318**(5857): p. 1737-1742.
3. Bates, B.C., et al., *Climate Change and Water*. 2008, Intergovernmental Panel on Climate Change: Geneva. p. 210.
4. Hochschild, D. and P. Shah, *On Our Energy Future*, in *San Francisco Chronicle*. 2007: San Francisco. p. E-5.
5. Consonni, S., M. Giugliano, and M. Grosso, *Alternative strategies for energy recovery from municipal solid waste: Part A: Mass and energy balances*. Waste Management Special Issue - VARIREI 2003, 2005. **25**(2): p. 123-135.
6. Consonni, S., M. Giugliano, and M. Grosso, *Alternative strategies for energy recovery from municipal solid waste: Part B: Emission and cost estimates*. Waste Management Special Issue - VARIREI 2003, 2005. **25**(2): p. 137-148.
7. He, R., et al., *Biological degradation of MSW in a methanogenic reactor using treated leachate recirculation*. Process Biochemistry, 2005. **40**(12): p. 3660-3666.
8. BPU, *Section 2: Goals, Objectives and Performance Measures*, in *State of New Jersey Energy Master Plan (Draft 10/13/2006)*. 2006, New Jersey Board of Public Utilities State of New Jersey Energy Master Plan: Trenton, NJ. p. 1-6.
9. Reinhart, D.R. and T.G. Townsend, *Landfill Bioreactor Design and Operation*. 1998, Boca Raton, FL (USA): CRC Press LLC. 189.
10. Thompson, S. and S. Tanapat, *Waste Management Options for Greenhouse Gas Reduction: Modelling Methane Generation for Different Waste Management Options*. Environmental Informatics Archives, 2004. **2**: p. 242-251.
11. Babson, D., et al. *Development of a Dynamic Energy Balance to Assess Operating Efficiency of the Burlington County Bioreactor Landfill in New Jersey (USA)*. in *Sardinia 2007: Eleventh International Waste Management and Landfill Symposium*. 2007. Sardinia, Italy: CISA, Environmental Sanitary Engineering Centre.
12. El-Haggag, D.S.M., *Sustainability of Municipal Solid Waste Management Sustainable Industrial Design and Waste Management*. 2007, Academic Press: Oxford. p. 149-196.

in vivo fish assays to study the anti-estrogenic action of polycyclic aromatic hydrocarbons and to evaluate endocrine activity

# Development of two in vivo fish assays to study the anti-estrogenic action of polycyclic aromatic hydrocarbons and to evaluate endocrine activity in NJ wastewater effluents

## Basic Information

<b>Title:</b>	Development of two in vivo fish assays to study the anti-estrogenic action of polycyclic aromatic hydrocarbons and to evaluate endocrine activity in NJ wastewater effluents
<b>Project Number:</b>	2009NJ198B
<b>Start Date:</b>	3/1/2009
<b>End Date:</b>	2/28/2010
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	6
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Water Quality, Wastewater, Toxic Substances
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Sean Bugel, Keith R Cooper

## Publications

1. Bugel, S.M., White, L.A., Cooper, K.R., 2010. Impaired reproductive health of killifish (*Fundulus heteroclitus*) inhabiting Newark Bay, NJ, a chronically contaminated estuary. *Aquat. Toxicol.* 96 (3): 182-193.
2. Bugel, S.M., 2009. An integrated biomarker approach for assessing exposure and effects of endocrine disruptors and other contaminants in killifish (*Fundulus heteroclitus*) from the New York–New Jersey Harbor Estuary. M.S. Thesis Dissertation. Department of Environmental Sciences, Rutgers University, New Brunswick, NJ. 100 pages.

## **Project Summary**

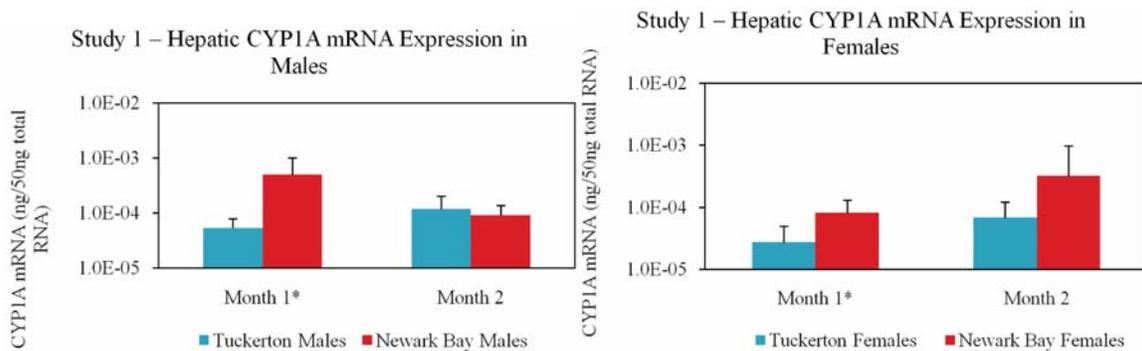
The proposed research sought to investigate the link between exposure to aryl hydrocarbon receptor (AhR) agonists and reproductive effects in killifish (*Fundulus heteroclitus*). Early studies from our lab reported finding evidence for endocrine disruption in both male and female killifish inhabiting Newark Bay, NJ (Bugel, 2009; Bugel *et al.*, 2010). These findings included decreased gonadal weights and altered gonadal development in both genders, decreased egg-yolk precursor protein expression (vitellogenin) in the female, altered steroid signaling expression (gonadal aromatase) in the female, and a potential relationship between activity of the aryl hydrocarbon pathway and vitellogenin expression. As a result, we sought to further investigate the contaminant related effects on reproductive processes in Newark Bay, and explore the relationship between aryl hydrocarbon exposure and these newly discovered reproductive impacts.

Aryl hydrocarbon receptor agonists (i.e. dioxins, furans, polycyclic aromatic hydrocarbons) are found throughout the NY-NJ Harbor Estuary and are among the highest concentrations ever reported. These contaminants are thought to be impacting fish populations by interfering with normal endocrine signaling. Subsequent experiments have explored contaminant impacts on the biochemical level of reproductive pathways and the potential relationship between AhR agonist exposure and endocrine disruption. Early results indicate that exposure of naïve male killifish to the Newark Bay environment can elicit an estrogenic response evidenced by induction of the vitellogenin pathway, a female egg-yolk protein. This biomarker response was attenuated with increased exposure time, and may explain why previous studies have found no evidence for exposure of estrogenic contaminants to aquatic species in this ecosystem, despite the presence of known estrogenic contaminants. These findings indicate that conventional exposure biomarker studies may lead to premature conclusions that are based on biomarker responses that give false negatives. In a separate study, when killifish from both Newark Bay and the reference population were injected with 17 $\beta$ -estradiol, both genders from Newark Bay exhibited vitellogenin induction, although to a much lesser degree compared to induction in the reference population. This shifted dose-response curve for vitellogenin induction indicates a biochemical impact on the estrogen signaling pathways within Newark Bay and a decreased responsiveness to estrogen. The doses at which the Newark Bay killifish have a significantly lower protein induction are environmentally and physiologically relevant. Therefore, a decreased responsiveness in males may complicate using vitellogenin as a conventional biomarker in this population and may help explain why the reproductive impacts in the female that have been previously reported. Additional laboratory studies are being performed to explore exactly how the aryl hydrocarbon pathway interacts with the estrogen pathway to elicit these effects.

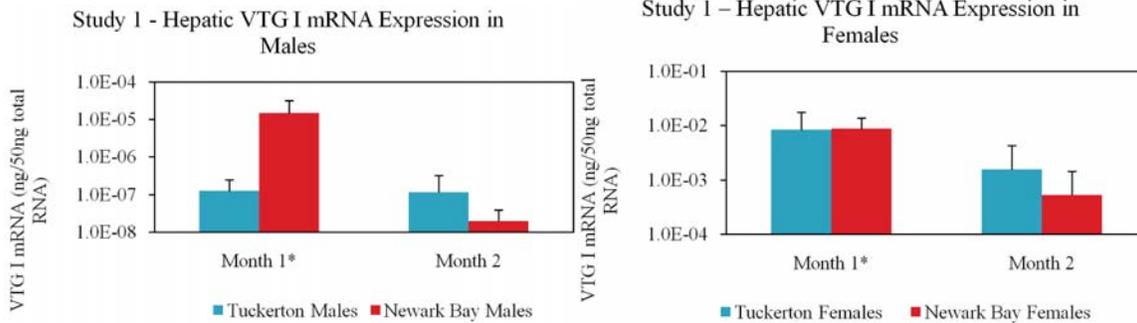
*Study 1 – Naïve killifish exposure to the Newark Bay Environment and biomarker responses*

This study explored the biomarker responses of a naïve population (previously un-exposed to contaminants) transplanted from Tuckerton, NJ, a relatively pristine ecosystem, into Newark Bay, an ecosystem highly contaminated with a complex mixture of chemicals (dioxins, furans, polyaromatic hydrocarbons, pesticides, metals, PCBs, pharmaceuticals).

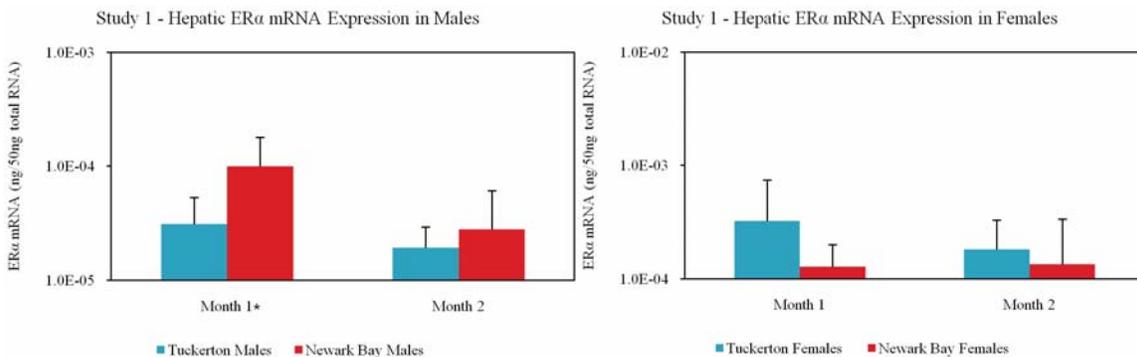
Tuckerton killifish were placed in several galvanized steel cages at both Tuckerton and Newark Bay locations, from May 24, 2009, to July 21, 2009, at a density of 15 males and 15 females per cage. Sub-samples of fish were collected from the cages at one month (June 22) and two months (July 21) exposure time and analyzed by qPCR for various biomarker responses (hepatic cytochrome P450 1A, vitellogenin and estrogen receptor alpha mRNA expression). These biomarkers were chosen as surrogate measures of exposure to aryl hydrocarbon receptor agonists, estrogenic compounds, and impacts on the estrogen receptor alpha pathway, respectively.



**Figure 1.** Cytochrome P4501A expression is a biomarker for exposure to aryl hydrocarbons that induce the AhR pathway. *Left Figure:* Newark Bay males were significantly induced at one month exposure time. By two months exposure the male CYP1A expression returned to reference levels. *Right figure:* Newark Bay females were also significantly induced at one month exposure and were not significantly different from the reference population at two months. The insignificant findings at the second month were contrary to what is normally expected with increased exposure and represents an attenuated response that is believed to be an adaptation for living in a toxic environment with chronic up-regulation of a metabolic enzyme. \*Significant at  $p < 0.05$  using Student's t-test. N = 4-6.



**Figure 2.** Vitellogenin is an egg-yolk precursor protein normally expressed only in adult females. This protein is produced by the liver and shuttled via the blood to developing follicles in the ovary. Follicles take up vitellogenin and cleave the protein into several dozen proteins during the maturation of the egg. Vitellogenin is driven by the estrogen receptor, and expression in males is indicative of exposure to exogenous contaminants that bind to the estrogen receptor. *Left Figure:* In males, there was a significant 111-fold increase in hepatic vitellogenin mRNA expression at one month. This biomarker response was attenuated and had returned to reference levels by the end of the second month. These results indicate that estrogenic contaminants are present at Newark Bay and can elicit a biological effect. *Right Figure:* No effect on female expression of vitellogenin was observed. \*Significant at  $p < 0.05$  using Student's t-test.  $N = 4-6$ .



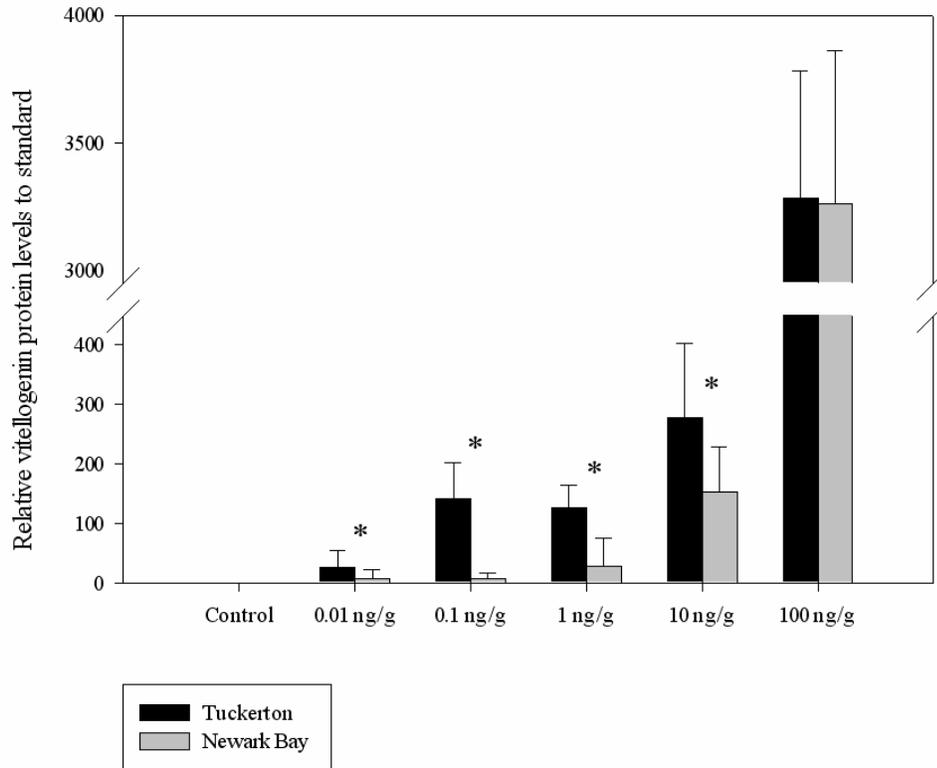
**Figure 3.** The estrogen receptor alpha ( $ER\alpha$ ) is generally accepted as the isoform responsible for vitellogenin induction, and has previously been shown to be effected by exposure to AhR agonists.  $ER\alpha$  levels were measured to help explain the down-regulation (attenuation) of vitellogenin in males at two months exposure (Figure 2). *Left Figure:* Male expression of  $ER\alpha$  was significantly higher at one month, and had decreased to reference levels by the second month. *Right Figure:* No significant effect on  $ER\alpha$  was measured in females. \*Significant at  $p < 0.05$  using Student's t-test.  $N = 4-6$ .

*Study 2 – Vitellogenin induction by graded doses of 17 $\beta$ -estradiol (E2) in a contaminant impacted population*

Previous studies have demonstrated no vitellogenin induction in the Newark Bay male killifish population and decreased expression in females (Bugel, 2009; Bugel *et al.*, 2010). However, Study 1 (above) demonstrated that Newark Bay killifish were exposed to estrogenic contaminants at concentrations that elicited biological effects. We therefore hypothesized that Newark Bay killifish have a decreased sensitivity to 17 $\beta$ -estradiol (E2) and would therefore exhibit a shifted dose-response curve.

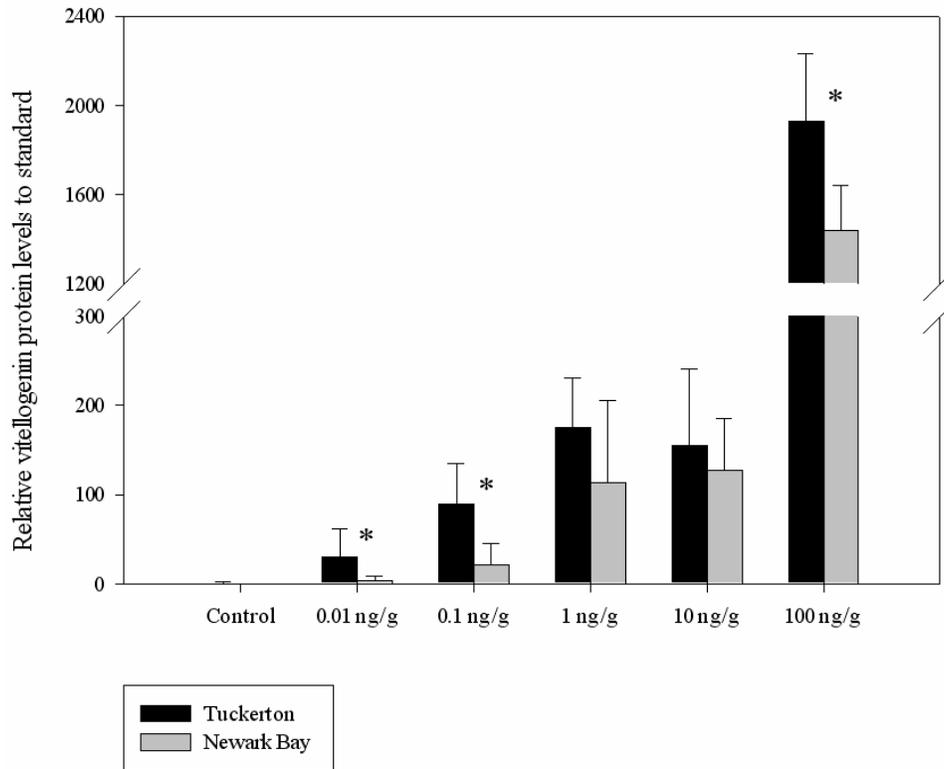
Killifish were collected from Tuckerton (reference population) and Newark Bay, NJ, in October, 2009, a period of the year when fish are reproductively inactive. Fish were acclimated to laboratory conditions for one week. Fish were injected intraperitoneally with graded doses of E2 at concentrations of 100, 10, 1, 0.1, and 0.01 ng E2/g body weight using 10  $\mu$ L of corn oil/g body weight. Fish were sacrificed after four days and tissues were collected (blood plasma and liver). Circulating levels of vitellogenin protein were analyzed by immunoblotting and hepatic estrogen receptor alpha expression was analyzed by qPCR.

## Study 2 - Male Vitellogenin Dose-Response



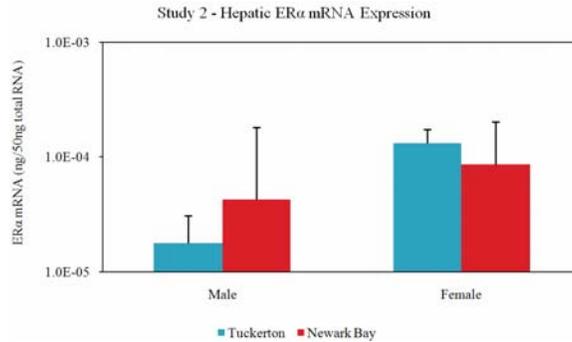
**Figure 4.** Dose-response of 17β-estradiol (E2) induction of vitellogenin protein in males. Newark Bay males had a shifted dose-response curve evidenced by significantly lower levels of vitellogenin produced at lower concentrations of E2. Induction in the Newark Bay males was similar to the reference population only at the highest dose (100 ng E2/g body weight). This concentration range is comparable to the estrogen activity of environmentally relevant concentrations of estrogenic contaminants. Therefore, interpretation of vitellogenin biomarker responses may be complicated by an unknown anti-estrogenic mechanism in this population. \*Significant at  $p < 0.05$  using Student's t-test. N = 4-6.

## Study 2 - Female Vitellogenin Dose-Response



**Figure 5.** Dose-response of  $17\beta$ -estradiol (E2) induction of vitellogenin protein in females. Newark Bay females also had a shifted dose-response relationship, although vitellogenin responses were much more variable. This is likely due to endogenous levels of estradiol within the fish, despite this experiment being conducted out of the breeding season. At the highest dose, Newark Bay had significantly less induction, indicating that Newark Bay females have a lower capacity for vitellogenin production. This data supports that Newark Bay females have a decreased hormonal response that results in lower levels of protein than what is normal. This decreased responsiveness is thought to contribute to the reproductive impacts previously reported by Bugel *et al.* (2010).

\*Significant at  $p < 0.05$  using Student's t-test. N = 4-6.



**Figure 6.** Hepatic estrogen receptor alpha (ER $\alpha$ ) mRNA levels were evaluated as a surrogate measure of the estrogen hormone receptor. We hypothesized that Newark Bay killifish would have significantly lower levels of ER $\alpha$  which would explain the shifted ER-VTG dose response curves in Figures 2 and 3. Hepatic expression was not significantly different between males or between females. \*Significant at  $p < 0.05$  using Student's t-test. N = 4-6.

## Project Conclusions

There has been limited evidence suggesting that the Newark Bay ecosystem contains estrogenic contaminants that elicit biological effects on aquatic species. This may be due to altered biochemical pathways in indigenous fish populations which have led to down-regulation of pathways that are under chemical stress and, therefore, a decreased sensitivity to induction. However, exposing a naïve population to the Newark Bay environment has proven a valuable tool. Naïve killifish caged at Tuckerton have significantly higher vitellogenin expression levels (111-fold) after one month, indicating exposure to estrogenic contaminants. This biological response is attenuated over time and returned to reference levels after two months. This supports the hypothesis that biomarker responses can be short-lived due to biochemical adaptation and therefore negative findings may not be conclusive. Furthermore, indigenous killifish from Newark Bay had a decreased sensitivity to vitellogenin induction by 17 $\beta$ -estradiol, suggesting an anti-estrogenic effect in this population. This may be due to cross-talk of the AhR pathway, or by chronic stimulation by exogenous contaminants that have hormonal properties. These possibilities will be explored in future studies based on the results presented here.

## References

Bugel, S.M., White, L.A., Cooper, K.R., 2010. Impaired reproductive health of killifish (*Fundulus heteroclitus*) inhabiting Newark Bay, NJ, a chronically contaminated estuary. *Aquat. Toxicol.* 96 (3): 182-193.

Bugel, S.M., 2009. An integrated biomarker approach for assessing exposure and effects of endocrine disruptors and other contaminants in killifish (*Fundulus heteroclitus*) from the New York–New Jersey Harbor Estuary. M.S. Thesis Dissertation. Department of Environmental Sciences, Rutgers University, New Brunswick, NJ. 1-100.

# Application of molecular and metabolic biomarkers of anaerobic hydrocarbon degradation as evidence for natural attenuation in New Jersey groundwater samples

## Basic Information

<b>Title:</b>	Application of molecular and metabolic biomarkers of anaerobic hydrocarbon degradation as evidence for natural attenuation in New Jersey groundwater samples
<b>Project Number:</b>	2009NJ199B
<b>Start Date:</b>	3/1/2009
<b>End Date:</b>	2/28/2010
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	6
<b>Research Category:</b>	Water Quality
<b>Focus Category:</b>	Groundwater, Toxic Substances, Water Quality
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Amita Oka, Lily Young

## Publications

1. Oka, Amita; Craig Phelps; Xiangyang Zhu; Diane Saber; and Lily Young, 2009, Evidence for Anaerobic Biodegradation of Hydrocarbons in the Subsurface Environment. Abstract presented at Goldschmidt 2009, Davos, Switzerland. *Geochimica et Cosmochimica Acta*, 73(13), Suppl. 1, A968.
2. Oka, Amita; Craig Phelps; Xiangyang Zhu; Diane Saber; and Lily Young, 2009, Metabolic Biomarkers and Biomolecular Signatures Provide Evidence for Natural Attenuation of Hydrocarbons in Anoxic Groundwater, 109th General Meeting, American Society for Microbiology, American Society for Microbiology, Washington, DC, 157.
3. Oka, Amita R., 2009, Anaerobic Benzene Degradation in Culture and Hydrocarbon Degradation in the Subsurface Environment, Ph.D. Dissertation, Dept. of Environmental Sciences, Rutgers, the State University of New Jersey, New Brunswick, New Jersey, 164 pages.

## Project summary

### Research problem and research objectives

Groundwater is a valuable source of fresh water and is one of the most important sources of drinking water in the U.S. Groundwater can be impacted by organic contaminants due to naturally occurring sources or due to human intervention, rendering the water resources unsuitable for use. Although many physical and chemical methods of remediation can be applied for site clean up, these can be resource intensive, and alternative bioremediation strategies are required.

In situ bioremediation makes use of the capability of naturally occurring microorganisms to degrade organic contaminants and may be a cost effective option of remediation for certain sites. Often the impacted sites turn anoxic due to rapid growth of aerobic bacteria, and it is therefore essential to understand anaerobic biodegradation processes. Anaerobic biodegradation of hydrocarbons has been studied under controlled conditions in several laboratory studies, and the outcomes of this research can be applied to field studies.

The objective of this study was to analyze groundwater samples from a manufacturing gas plant impacted site, and evaluate them for the presence of two types of biomarkers of anaerobic hydrocarbon degradation; (i) specific metabolites of anaerobic naphthalene and 2-methyl naphthalene degradation (metabolic biomarkers), and (ii) functional gene *bssA* coding for the alpha subunit of benzyl succinate synthase, a crucial enzyme in anaerobic hydrocarbon degradation (molecular biomarker). Such a study is unique because it involves study of metabolic biomarkers of anaerobic degradation of polycyclic aromatic hydrocarbons (PAHs), along with the molecular biomarker (*bssA* gene) in the New Jersey groundwater samples. Such a combined analysis has not been reported before from New Jersey. This study can be used to verify anaerobic biodegradation of PAHs at the study site, and its results can be applied for design of other field studies and evaluation of remediation strategies at other impacted sites around the state.

### Methodology

Detailed methods and materials can be found in Oka (4).

**Groundwater Sampling:** Six monitoring wells (MW) were selected at a manufactured gas plant (MGP) impacted site, from which groundwater was collected for this study (see Figure 1). Samples for analysis of DNA were filtered with 0.22  $\mu\text{m}$  filters and filters were frozen for further analysis. Samples for analysis of metabolic intermediates were not filtered, but were acidified in the field and stored at 4°C.

**Extraction and analysis of metabolic intermediates:** Solvent extracts of water samples were analyzed using a GC-MS. Metabolic intermediates were identified by a comparison of the retention time and the mass spectra of the samples to the standards analyzed similarly, or by comparison with published mass spectra. Abundance of the base peaks was used for quantification of metabolic intermediates.

**DNA extraction end-point PCR analysis:** DNA was extracted by using a commercially available kit. End-point PCR analysis of the 16S rRNA gene and *bssA* gene was performed on each DNA extract. Eubacteria specific 16S rRNA gene primers (5) were used to ensure the presence of eubacteria in the water samples. The *bssA* gene PCR primers and the program were as described by Winderl et al. (8).

**qPCR analysis:** The qPCR analysis with SYBR green assay was performed on all DNA extracts for quantification of the target genes in the samples. For 16S rRNA gene and *bssA* gene analysis, primers developed by Suzuki et al. (7) and Beller et al. (2, 3) were used, respectively. Two pairs of primers were used for *bssA* gene analysis; one was based on toluene-degrading denitrifying bacteria (defined as *bssA*-Denitrif qPCR) (2), and the other was based on sulfate-reducing/methanogenic toluene-degrading bacteria (defined as *bssA*-Sulf-Meth qPCR) (3). A modification of the qPCR protocols by Beller et al. (2, 3) was used for 16S rRNA gene and *bssA* gene analysis.

Calibration curves for *bssA*-Denitrif qPCR and 16S rRNA gene qPCR were prepared by using dilutions of *Thauera aromatica* T1 genomic DNA, while the calibration curve for *bssA*-Sulf-Meth qPCR was obtained by using dilutions of *Desulfobacterium cetonicum* DSM7627 (*Desulfosarcina cetonica* DSM7267) genomic DNA. The number of gene copies in the genomic DNA extracts was calculated by using the equation:

$$\text{Gene copies } \mu\text{L}^{-1} = (\mu\text{g DNA } \mu\text{L}^{-1} / \text{bp genome}^{-1})(\text{bp } \mu\text{g}^{-1} \text{ DNA}) (\text{genes genome}^{-1})$$

For qPCR analysis we assumed that: (i) the size of the genomic DNA of T1, and *D. cetonicum*, each was 4.6 Mbp. (ii) the genomic DNA of T1 had only one 16S rRNA gene and 1 *bssA* gene, and genomic DNA of *D. cetonicum* has only 1 *bssA* gene. (iii) the calibration curves were representative of the other bacteria in the environment. It was also assumed that both *bssA* gene primers sets used in this study for qPCR analysis would be able to detect *bssA* genes in the environmental samples.

## Principal findings

**Groundwater was impacted with monoaromatic and polycyclic aromatic hydrocarbons:** Contaminants detected in the water samples are given in Table 1. The highest concentration of benzene, toluene, ethylbenzene and xylenes (BTEX), and naphthalene was present in MW-40, which is within the source area.

**Reducing conditions had developed at the impacted wells:** Characteristics of the water samples, like dissolved oxygen (D.O.) and oxidation-reduction potential (ORP) (Figure 1), indicate that the wells within the plume area had depleted levels of dissolved oxygen and had developed reducing conditions. Therefore, microbial activity in the impacted wells is expected to be occurring mainly under anaerobic conditions.

**Metabolic intermediates specific to anaerobic microbial degradation were detected in the groundwater:** Four different metabolic intermediates of anaerobic PAH degradation were detected in the groundwater samples: 2-naphthoic acid (2-NA),

tetrahydro-2-naphthoic acid (TH-2-NA), hexahydro-2-naphthoic acid (HH-2-NA), and methyl-naphthoic acid (MNA), and at least one of these metabolites were detected in each well. Distribution of the metabolites detected in the water samples is shown in Figure 2. Metabolites TH-2-NA and HH-2-NA can be formed only under anaerobic conditions (1, 6, 10). Therefore their presence in the groundwater specifically indicates anaerobic microbial degradation of substrates.

Detection of anaerobic hydrocarbon degradation metabolites in high abundance in MW-24 indicates that this well contains a microbial community that is enriched for anaerobic PAH degradation. Presence of such easily metabolized metabolic intermediates indicates ongoing anaerobic microbial degradation processes, and not accumulation of metabolites over time.

**Detectable bacterial community was present in all samples:** 16S rRNA gene PCR product was detected in all samples with end-point PCR analysis (Figure 3A) indicating that all samples contained detectable eubacterial DNA.

**Bacteria capable of anaerobic degradation of hydrocarbons were present in the impacted wells:** Detection of analogues of *bssA* genes in the impacted wells (Figure 3B) clearly indicates that bacteria capable of anaerobic hydrocarbon degradation are present in the groundwater at these wells.

A comparison of the end-point PCR results of 16S rRNA gene and the *bssA* gene indicate that although detectable bacterial community was present in all the monitoring wells, hydrocarbon-degrading bacteria were enriched particularly within the plume (MW-24, 29 and 40), likely due to the presence of the hydrocarbon contaminants.

**Eubacteria were more abundant within the impacted wells:** As shown in Figure 4, the highest density of 16S rRNA genes was detectable in MW-29, an impacted well. Similar 16S rRNA genes abundance was detected in two other impacted wells, MW-40 and MW-24.

The abundance of 16S rRNA genes in all the impacted wells was 1-2 orders of magnitude higher than in the non-impacted wells. These results indicate enrichment of bacterial population specifically in the impacted wells likely due to prolonged exposure to organic contaminants.

**Relatively high abundance of hydrocarbon degrading bacteria was detected in MW-24:** *BssA* gene products could be quantified only in MW-24. Our results indicate that MW-24 contains a relatively high abundance of the analogues of bacteria containing *bssA* genes detectable by methods used in this study.

Although *bssA* gene analogues were detected in all impacted wells by end-point PCR analysis (Figure 3), the detection limit of the qPCR assay and/or its limited coverage of *bssA* gene diversity in the environment were likely responsible for lack of *bssA* gene detection by qPCR at MW-29 and MW-40.

## Significance

This study has established a relationship between distribution of metabolic intermediates, catabolite gene *bssA* and hydrocarbon contaminants present in the impacted groundwater in New Jersey. From the results, it can be seen that the impacted monitoring wells have a relatively high abundance of metabolic and molecular biomarkers, indicating microbial activity. In addition, based on the results of this study an area enriched for anaerobic hydrocarbon degradation (MW-24) at the impacted site could also be identified, and the results of independent analysis of the biomarkers are in agreement. These multiple lines of evidence provide conclusive proof that the impacted site has a potential for natural attenuation.

This site was studied by our laboratory before, and metabolic intermediates of anaerobic PAH and monoaromatic hydrocarbon degradation were detected, but molecular biomarkers were not available then (9). To our knowledge, this is one of the first studies in New Jersey that has combined the use of metabolic biomarkers along with catabolite gene *bssA* to evaluate the in situ anaerobic bioremediation potential of a hydrocarbon impacted site. This study also differs from a previously reported similar study (3) as it applies a combination of biomarkers of anaerobic hydrocarbon degradation, including gene *bssA*, to evaluate bioremediation potential of a site that was historically contaminated and is not manipulated by addition of contaminants.

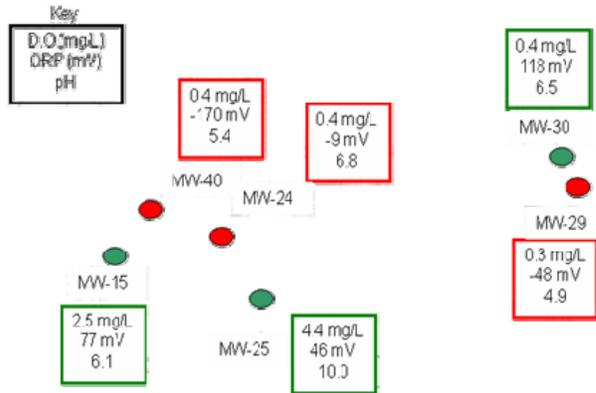
Data collected in this study is valuable as it can be used to establish the robustness of biomarkers in field conditions and can be applied to evaluate in situ bioremediation as an alternative strategy for site clean up at this and other sites.

## Tables and Figures

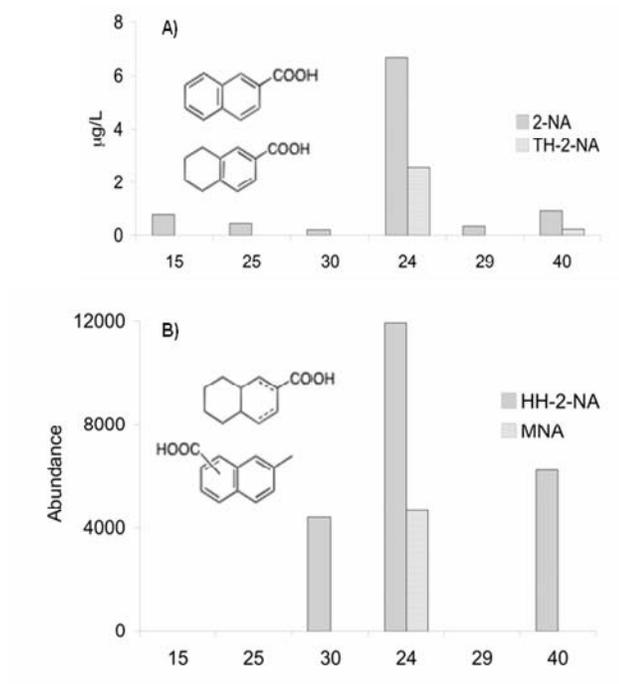
	Monitoring wells					
	Outside the plume			Inside the plume		
	15	25	30	24	29	40
BTEX	-	-	-	-	121.9	1724
2-Methylnaphthalene	-	-	-	-	-	-
Naphthalene	-	-	-	0.5	-	19
Phenanthrene	-	-	0.02	0.05	-	0.05

**Table 1.** Concentration of organic contaminants in the samples ( $\mu\text{g/L}$ ).

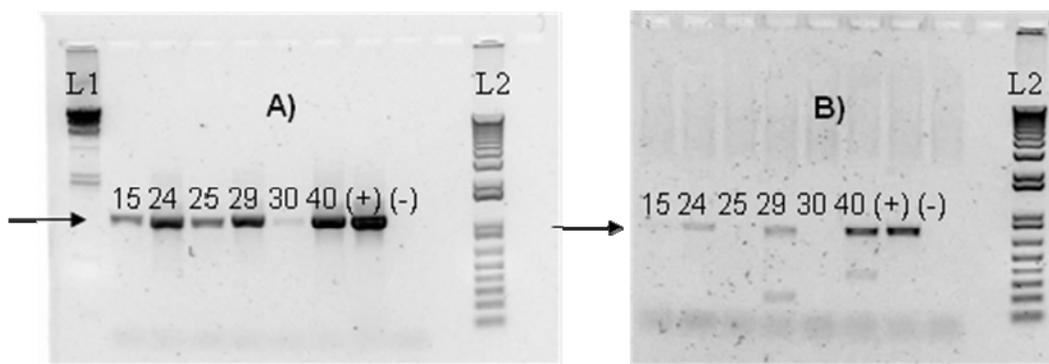
-; not detected



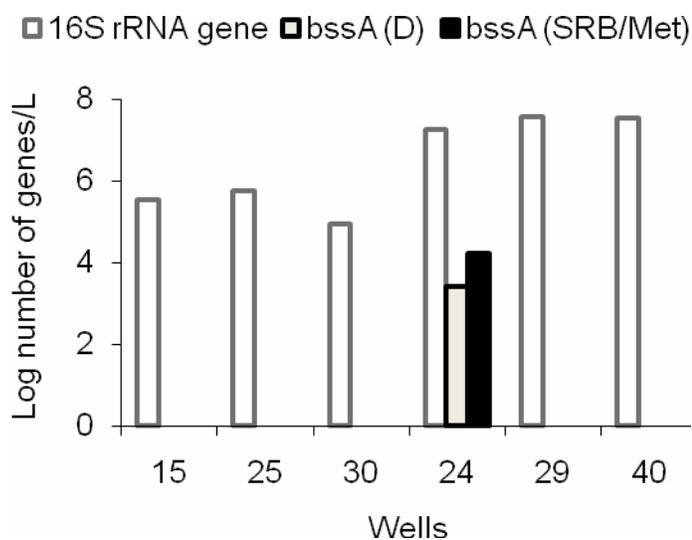
**Figure 1.** Location of the monitoring wells at the MGP site in Glassboro, NJ is shown in the map. Wells within the plume (MW-24, 29 and 40) are shown in red while wells outside the plume (MW-15, 25 and 30) are shown green. The scale at the bottom of the map reads: 1.5 cm is 250 ft. Dissolve oxygen (D.O.), oxidation reduction potential (ORP), and pH at each well are given.



**Figure 2.** The concentration of 2-NA and TH-2-NA (A) and the abundance of HH-2-NA and MNA (B) in the groundwater samples. TH-2 NA was also detected in MW-15, 25, and 29, but was below our quantification limit.



**Figure 3.** Electrophoresis using 1% agarose gel with *Bacteria* specific 16S rRNA gene PCR A) and *bssA* gene PCR products B). L1:  $\lambda$  HindIII, L2: Kb+ ladder. Arrows indicate position of expected size of the PCR product and numbers stand for monitoring wells. *Thauera aromatica* T1 Genomic DNA was used as template for positive control (+) and sterile water was used for negative control (-).



**Figure 4.** Abundance of eubacterial 16S rRNA genes and *bssA* genes in toluene-degrading denitrifying bacteria (*bssA*-Denitrif) or toluene-degrading sulfate-reducing/methanogenic (*bssA*-Sulf-Meth) in the groundwater samples estimated using qPCR analysis.

## References

1. **Annweiler, E., A. Materna, M. Safinowski, A. Kappler, H. H. Richnow, W. Michaelis, and R. U. Meckenstock.** 2000. Anaerobic degradation of 2-methylnaphthalene by a sulfate-reducing enrichment culture. *Appl. Environ. Microbiol.* **66**:5329-33.
2. **Beller, H. R., S. R. Kane, T. C. Legler, and P. J. Alvarez.** 2002. A real-time polymerase chain reaction method for monitoring anaerobic, hydrocarbon-degrading bacteria based on a catabolic gene. *Environ. Sci. Technol.* **36**:3977-84.
3. **Beller, H. R., S. R. Kane, T. C. Legler, J. R. McKelvie, B. S. Lollar, F. Pearson, L. Balsler, and D. M. Mackay.** 2008. Comparative assessments of benzene, toluene, and xylene natural attenuation by quantitative polymerase chain reaction analysis of a catabolic gene, signature metabolites, and compound-specific isotope analysis. *Environ. Sci. Technol.* **42**:6065-72.
4. **Oka, A. R.** 2009. Anaerobic Benzene Degradation in Culture and Hydrocarbon Degradation in the Subsurface Environment. Rutgers, the State University of New Jersey, New Brunswick.
5. **Oka, A. R., C. D. Phelps, L. M. McGuinness, A. Mumford, L. Y. Young, and L. J. Kerkhof.** 2008. Identification of critical members in a sulfidogenic benzene-degrading consortium by DNA stable isotope probing. *Appl. Environ. Microbiol.* **74**:6476-80.
6. **Sullivan, E. R., X. Zhang, C. Phelps, and L. Y. Young.** 2001. Anaerobic mineralization of stable-isotope-labeled 2-methylnaphthalene. *Appl. Environ. Microbiol.* **67**:4353-7.
7. **Suzuki, M. T., L. T. Taylor, and E. F. DeLong.** 2000. Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5'-nuclease assays. *Appl. Environ. Microbiol.* **66**:4605-14.
8. **Winderl, C., S. Schaefer, and T. Lueders.** 2007. Detection of anaerobic toluene and hydrocarbon degraders in contaminated aquifers using benzylsuccinate synthase (*bssA*) genes as a functional marker. *Environ. Microbiol.* **9**:1035-1046.
9. **Young, L. Y., and C. D. Phelps.** 2005. Metabolic biomarkers for monitoring *in situ* anaerobic hydrocarbon degradation. *Environmental Health Perspect.* **113**:62-67.
10. **Zhang, X., E. R. Sullivan, and L. Y. Young.** 2000. Evidence for aromatic ring reduction in the biodegradation pathway of carboxylated naphthalene by a sulfate reducing consortium. *Biodegradation* **11**:117-24.

Does urbanization decrease baseflow? A historical, empirical analysis in the coastal states of Eastern United States

## Does urbanization decrease baseflow? A historical, empirical analysis in the coastal states of Eastern United States

### Basic Information

<b>Title:</b>	Does urbanization decrease baseflow? A historical, empirical analysis in the coastal states of Eastern United States
<b>Project Number:</b>	2009NJ210G
<b>Start Date:</b>	9/1/2009
<b>End Date:</b>	8/31/2011
<b>Funding Source:</b>	104G
<b>Congressional District:</b>	NJ 8th
<b>Research Category:</b>	Climate and Hydrologic Processes
<b>Focus Category:</b>	Surface Water, Water Quantity, Management and Planning
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Joshua C. Galster, Kirk Barrett

### Publications

There are no publications.

Does urbanization decrease baseflow? A historical, empirical analysis in the coastal states of Eastern United States

## **Problem and Research Objectives**

Approximately half of the U.S. population depends on surface water (rivers and reservoirs) for their drinking water. During dry weather, rivers and reservoirs are fed by baseflow. Many of these areas are urbanizing, in some cases rapidly. Theoretically, urbanization will cause a decrease in baseflow, which means urbanization is a threat to water availability for about half the population. Reduced baseflow can also negatively affect stream biota. The problem is that it is not clear if (and to what degree) this theoretical linkage between urbanization and decreased baseflow is actually experienced in the real world; there are several processes associated with urbanization that could confound the theoretical relationship. This project will help resolve this relationship by conducting a large spatial and temporal scale, empirically-based investigation into the urbanization-baseflow relationship. The project will determine if (or how likely) there really is a relationship between urbanization and decreased baseflow in a real watershed.

This project will provide the most complete assessment about whether/how urbanization actually affects baseflow. Water supply managers and land development regulators can make use of this information to help better understand the effects of land development and manage it accordingly, especially in rural and water supply watersheds. The project results should be useful in assessing the threat posed by urbanization to dry-weather water availability and stream ecology.

Annual baseflow is an important metric for minimal stream flows and is a critical value for water resource managers, wildlife interests and groundwater connectivity. One potentially significant but currently under-studied impact on baseflow is urbanization. We propose to analyze historic United States Geological Survey (USGS) gage data to determine trends in baseflow over time. Baseflow data would be collected for the states of New Jersey, Pennsylvania, Delaware, Maryland, and Virginia. We will use different timeframes and baseflow metrics for their utility in identifying trends. We propose to use four metrics of annual baseflow: 1) baseflow per unit drainage area (BF); 2) ratio of BF to precipitation (BF/P); 3) BF as a fraction of total flow (BF/TF); and, 4) the annual minimum daily average flow per unit drainage area (AMDAF). Trends will be identified using the non-parametric Mann Kendall statistical test and the Sen slope estimator.

## **Methodology**

We are empirically investigating the relationship between urbanization and stream baseflow by examining the stream discharge records maintained by the United States Geological Survey in 10 states: NY below the Adirondacks, CT, NJ, PA, DE, MD, VA, NC, SC, and GA. We will select gages that have continuous records for at least 25 years and have had substantial changes in the amount of impervious surfaces within the watersheds. As a control, we will also analyze trends in 10 gaged watersheds per state that showed near constant imperviousness. We will separate baseflow from other stream flows using a digital filtering method and aggregate daily baseflow to create an annual baseflow time series for each gage. We will then compute time series of three baseflow statistics: 1) annual baseflow per unit drainage area, 2) ratio of baseflow to precipitation and 3) baseflow fraction of total flow. We will have both undergraduate and graduate students assisting us with these tasks.

## **Principal Findings and Significance**

Initial results from 31 unregulated streams across the four physiographic provinces of New Jersey (with streamflow records ranging from 25 to 95 years) are presented. The percentage of streams with significant (95% confidence) increasing trends vs. decreasing trends was 13% vs. 6% for BF, 16% vs. 23% for BF/P, and 13% vs. 13% for BF/TF. The sizeable differences among metrics indicate the need to carefully choose the metric when analyzing for trends in baseflow. The metrics were examined in series of 10-year blocks to compare trends in partial records to those in full records. Stream that showed long-term trends were likely to have one or more 10- year period(s) in which there was no trend. This finding underscores the need for long-term records if trends are to be detected.

Our aim is to expand on these initial results by including other states with similar physiographic regions (e.g., Ridge and Valley, Piedmont, Coastal Plain, etc.) and also undergoing urbanization that places pressure on the local water resources. Students have been active in collecting and analyzing the USGS gages that meet the criteria above. Currently over 600 gages in the ten states have been determined to meet the criteria above and will be analyzed for baseflow and precipitation trends. This work will continue the summer of 2010 and during the academic year of 2010-2011, and will be coordinated by a graduate student at Montclair State.

## **Information Transfer Program Introduction**

The information transfer program serves an important purpose to the state's water resource community. The goal is to bring timely information about critical issues in water resource sciences to the public, and to promote the importance of research in solving water resource problems. The program accomplishes this goal through a variety of means. One focus is on producing a newsletter that provide a comprehensive overview of current water resource issues. The program continues to develop the NJWRRI website ([www.njwrri.rutgers.edu](http://www.njwrri.rutgers.edu)) into a comprehensive portal for water information for the state. We also collaborate with other organizations in sponsoring and producing conferences.

# Information Transfer Program

## Basic Information

<b>Title:</b>	Information Transfer Program
<b>Project Number:</b>	2009NJ200B
<b>Start Date:</b>	3/1/2009
<b>End Date:</b>	2/28/2010
<b>Funding Source:</b>	104B
<b>Congressional District:</b>	6
<b>Research Category:</b>	Not Applicable
<b>Focus Category:</b>	None, None, None
<b>Descriptors:</b>	None
<b>Principal Investigators:</b>	Christopher Obropta, Diana Morgan

## Publications

There are no publications.

## Information Transfer Program

The information transfer program has emphasized development of the website and e-based communications with stakeholder groups. It has also focused on the production of substantive newsletters addressing specific water resource issues as an effective way to communicate information to the public.

Although no issues of the newsletter were produced during 2009, one issue of the newsletter is about to be released and a second is in progress. The first issue focuses on research and extension programs occurring in the Raritan River basin. Our annual research update issue is currently in production. It is intended to showcase NJWRRI-funded research and also to illustrate the importance of research in solving water-related problems. Each issue of the newsletter is approximately eight pages. It is primarily distributed via our e-mail lists to approximately 2,000 people throughout the state, and as paper copies to all members of the New Jersey legislature and Congressional delegation.

Our website ([www.njwrri.rutgers.edu/](http://www.njwrri.rutgers.edu/)) has been continually updated with information on water resource events and information in New Jersey, the U.S. and around the world. The home page and 'events' pages are regularly updated to highlight upcoming events, publications and other water-related news. The website is our primary means of information transfer to the water community and the public, and we will continue to update and improve its functionality with new pages and greater content.

We continue to expand and use targeted, group-specific e-mail lists to bring relevant information to specific audiences. Targeted lists include a list of scientists/principal investigators, water resource managers, non-governmental organizations and people affiliated with NGOs, and policy-makers. The lists are continuously updated and expanded, and are used to keep these groups informed of events, conferences, publications, and funding opportunities. These lists enable us to initiate and maintain frequent contact with stakeholder groups. We believe these lists are an excellent method of keeping the water-related public aware of NJWRRI, as well as informed about water-related news and information.

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. As a member of the council, we co-sponsored the 2009 New Jersey Water Monitoring and Education Summit on November 18-19, 2009.

NJWRRI is a platinum-level co-sponsor of the 4th Passaic River Symposium to be held June 22, 2010 at Montclair State University. We are providing scholarships for students and non-profits to attend the symposium, and NJWRRI-funded research projects will be presented. We are also planning to co-sponsor a summit on nutrient management that would present the current understanding of nutrient fate and transport from suburban and urban landscapes. The audience and participants will be a group of scientists, environmental agency representatives, industry leaders, policy makers, and other stakeholders from New Jersey.

# USGS Summer Intern Program

None.

<b>Student Support</b>					
<b>Category</b>	<b>Section 104 Base Grant</b>	<b>Section 104 NCGP Award</b>	<b>NIWR-USGS Internship</b>	<b>Supplemental Awards</b>	<b>Total</b>
<b>Undergraduate</b>	2	4	0	0	6
<b>Masters</b>	0	0	0	0	0
<b>Ph.D.</b>	4	0	0	0	4
<b>Post-Doc.</b>	0	0	0	0	0
<b>Total</b>	6	4	0	0	10

## **Notable Awards and Achievements**

The research outcomes of project 2009NJ199B, "Application of molecular and metabolic biomarkers of anaerobic hydrocarbon degradation as evidence for natural attenuation in New Jersey groundwater samples," were shared with Gas Technology Institute (GTI), IL. These data were used for an internal study by GTI, which evaluated parallel lines of evidence for verification of natural attenuation at manufactured gas plant impacted sites.

The Philadelphia Water Department has requested a trial of the methodology developed by project 2009NJ193B, "Development of a profile SOD measurement technique." Preliminary field work will be conducted in June 2010.

David Babson, PI on project 2009NJ194B, "Innovative Research and Development for Environmental Protection and Sustainable Waste and Wastewater Management System Design," is invited to present his research at the Rutgers - NSF IGERT International Symposium on Biofuels and Bioenergy in June 2010.

## Publications from Prior Years

1. 2003NJ39B ("Validation of the PMF (Preprocessor to MODFLOW for Fractured Media) Package") - Articles in Refereed Scientific Journals - Mun, Y., and C.G. Uchirin, 2004. "Development and Application of a MODFLOW Preprocessor Using Percolation Theory for Fractured Media," J. Amer. Wat. Resources Assoc., 40:229-239.
2. 2003NJ39B ("Validation of the PMF (Preprocessor to MODFLOW for Fractured Media) Package") - Dissertations - Mun, Yuri. 2004. Development of the PFM Package, a MODFLOW Preprocessor to Simulate Ground Water Flow and Contaminant Transport in Fractured Media Using Percolation Theory, PhD Dissertation, Dept. of Environmental Science, Rutgers University, New Brunswick, NJ. 242 pages.
3. 2003NJ42B ("Microbial respiration of arsenic and selenium") - Articles in Refereed Scientific Journals - Narasingarao, P. and M.M. Häggblom. 2007. *Pelobacter seleniigenes* sp. nov., a selenate-respiring bacterium and reclassification of *Malonomonas rubra* as *Pelobacter ruber* comb. nov. *International Journal of Systematic and Evolutionary Microbiology*. 57: 1937-1942.
4. 2003NJ42B ("Microbial respiration of arsenic and selenium") - Articles in Refereed Scientific Journals - Rauschenbach, I, P. Narasingarao P and M.M. Häggblom. 2010. *Desulfurispirillum indicum* sp. nov., a selenate and selenite respiring bacterium isolated from an estuarine canal in southern India. *Int J Syst Evol Microbiol*. 2010 Apr 16. DOI 10.1099/ijs.0.022392-0 [Epub ahead of print]
5. 2003NJ42B ("Microbial respiration of arsenic and selenium") - Articles in Refereed Scientific Journals - Han, S., P. Narasingarao, A. Obraztsova, J. Gieskes, A.C. Hartmann, B.M. Tebo, E.E. Allen, and D.D. Deheyn. 2010. Mercury Speciation in Marine Sediments under Sulfate-Limited Conditions. *Environ. Sci and Technol*. Publication Date (Web): April 29, 2010. DOI: 10.1021/es903112m.
6. 2003NJ44B ("Seed Dispersal Dynamics in a Restored Salt Marsh: Implications for Restoration Success") - Dissertations - Hicks, P. L. 2004. Seed Dispersal Dynamics and Vegetation Community Development in a Restored Brackish Marsh: Implications For Restoration and Management. MS Thesis. Dept of Ecology and Evolution, Rutgers University, New Brunswick, NJ. 125 pp.
7. 2003NJ44B ("Seed Dispersal Dynamics in a Restored Salt Marsh: Implications for Restoration Success") - Other Publications - Hicks, P. L. and J. M. Hartman. 2004. Seed Dispersal Dynamics and Plant Community Development in a Restoration Urban Salt Marsh. Society of Wetland Scientist Conference, Seattle, WA. July 18-23, 2004.
8. 2003NJ47B ("Potential Nitrogen Saturation in Urban Wetlands") - Dissertations - Stander, Emilie, 2007, The effects of urban hydrology and elevated atmospheric deposition on nitrate retention and loss in urban wetlands, "Ph.D. Dissertation," Graduate Program in Ecology & Evolution, Graduate School – New Brunswick, Rutgers University, New Brunswick, NJ, 206 pp.
9. 2002NJ16G ("Wetlands in urban regions: connections among wetland structure, wetland function and regional water quality") - Dissertations - Stander, Emilie, 2007, The effects of urban hydrology and elevated atmospheric deposition on nitrate retention and loss in urban wetlands, "Ph.D. Dissertation," Graduate Program in Ecology & Evolution, Graduate School – New Brunswick, Rutgers University, New Brunswick, NJ, 206 pp.
10. 2002NJ16G ("Wetlands in urban regions: connections among wetland structure, wetland function and regional water quality") - Articles in Refereed Scientific Journals - Stander, E. K. and J. G. Ehrenfeld. 2009. Rapid assessment of urban wetlands: functional assessment model development and evaluation. *Wetlands* 29(1): 261-276.
11. 2002NJ16G ("Wetlands in urban regions: connections among wetland structure, wetland function and regional water quality") - Articles in Refereed Scientific Journals - Stander, E. K. and J. G. Ehrenfeld. 2009. Rapid assessment of urban wetlands: do hydrogeomorphic classification and reference criteria work? *Environmental Management*. 43(4): 725-742.

12. 2004NJ68B ("Dechlorination of Polychlorinated Dibenzo-p-Dioxins and Dibenzofurans by Dehalorespiring Bacterial Cultures ") - Dissertations - Lui, Fang. 2007. Microbial Dechlorination of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans: Pathways, Kinetics and Environmental Implications. Ph.D. Dissertation. Department of Environmental Sciences. Rutgers, The State University of New Jersey, New Brunswick, NJ, 188 pp.
13. 2004NJ72B ("Seed Dispersal Dynamics in Restored and Intact Salt Marshes: Implications for Restoration Success") - Dissertations - Hicks, P. L. 2004. Seed Dispersal Dynamics and Vegetation Community Development in a Restored Brackish Marsh: Implications For Restoration and Management. MS Thesis. Dept of Ecology and Evolution, Rutgers University, New Brunswick, NJ. 125 pp.
14. 2004NJ72B ("Seed Dispersal Dynamics in Restored and Intact Salt Marshes: Implications for Restoration Success") - Other Publications - Hicks, P. L. and J. M. Hartman. 2004. Seed Dispersal Dynamics and Plant Community Development in a Restoration Urban Salt Marsh. Society of Wetland Scientist Conference, Seattle, WA. July 18-23, 2004.
15. 2004NJ74B ("High resolution geophysical imaging as a novel method for noninvasive characterization of contaminated wetlands: application to Kearny Marsh ") - Conference Proceedings - Mansoor, N., Slater, L., Trubacco, T. and Baz, M., 2003, Assessing and monitoring groundwater contamination from landfill leachate in Kearny Marsh using high-resolution geophysics, The Meadowlands Symposium: A Scientific Symposium on the Hackensack Meadowlands, October 9-10, 2003. Lyndhurst, NJ, Abstract.
16. 2004NJ74B ("High resolution geophysical imaging as a novel method for noninvasive characterization of contaminated wetlands: application to Kearny Marsh ") - Articles in Refereed Scientific Journals - Mansoor, N. and L. Slater. 2007. Aquatic electrical resistivity imaging of shallow-water wetlands. *Geophysics*. 72, 5, F211-221.
17. 2004NJ75B ("Fate of Brominated Flame Retardants in New Jersey Wastewater Treatment Facilities") - Dissertations - Lui, Fang. 2007. Microbial Dechlorination of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans: Pathways, Kinetics and Environmental Implications. Ph.D. Dissertation. Department of Environmental Sciences. Rutgers, The State University of New Jersey, New Brunswick, NJ, 188 pp.
18. 2005NJ84B ("The Influence of Urbanization on Watershed Nitrogen Cycling Watersheds") - Other Publications - Rosenzweig, B. and P. Jaffe. 2009. Continuous Monitoring of Nitrogen Retention in an Urban Stormwater Detention Pond. American Geophysical Union Fall Meeting, San Francisco, CA. December 11-15, 2009. *Eos Trans. AGU*, 90(52), Fall Meet. Suppl., Abstract B11A-0468. (Poster presentation)
19. 2005NJ86B ("Microbial degradation of MTBE in anaerobic environments") - Other Publications - Youngster, L. K. G., T. K. G. Youngster, L. J. Kerkhof, and M. M. Häggblom. 2007. Analysis of low-biomass, anaerobic, MTBE-degrading bacterial communities 107th General Meeting of the American Society for Microbiology, Toronto, Canada, May 21-25, 2007.
20. 2005NJ86B ("Microbial degradation of MTBE in anaerobic environments") - Book Chapters - Häggblom, M. M., L. K. G. Youngster, P. Somsamak, and H. H. Richnow. 2007. Anaerobic Biodegradation of Methyl tert-Butyl Ether (MTBE) and Related Fuel Oxygenates. In Allen I. Lasking, Sima Sariaslani, and Geoffrey M. Gadd (ed), *Advances in Applied Microbiology*,. Amsterdam, Elsevier. 62:1-20.
21. 2005NJ86B ("Microbial degradation of MTBE in anaerobic environments") - Articles in Refereed Scientific Journals - Youngster, L. K. G., P. Somsamak, and M. M. Häggblom. 2008. Effects of co-substrates and inhibitors on the anaerobic O-demethylation of methyl tert-butyl ether (MTBE). *Appl. Microbiol. Biotechnol.* 80:1113-1120.
22. 2005NJ86B ("Microbial degradation of MTBE in anaerobic environments") - Other Publications - Youngster, L. K. G., L. J. Kerkhof, and M. M. Häggblom. 2009. Community characterization of anaerobic methyl tert-butyl ether (MTBE) degrading enrichment cultures. 109th General Meeting of the American Society for Microbiology, Philadelphia, PA. May 17-21, 2009.

23. 2005NJ86B ("Microbial degradation of MTBE in anaerobic environments") - Articles in Refereed Scientific Journals - Youngster, L. K. G., L. J. Kerkhof, and M. M. Haggblom. Community characterization of anaerobic methyl tert-butyl ether (MTBE)-degrading enrichment cultures. *FEMS Microbio. Ecol.* 72 (2): 279-288. Published online Jan. 22, 2010, DOI: 10.1111/j.1574-6941.2010.00841.x
24. 2005NJ86B ("Microbial degradation of MTBE in anaerobic environments") - Dissertations - Youngster, L.K.G., 2009, Microbial degradation of the fuel oxygenate Methyl Tert-Butyl Ether (MTBE). Ph.D. Dissertation, Graduate Program in Microbiology and Molecular Genetics, Rutgers, The State University of New Jersey, New Brunswick, NJ, 132 pages.
25. 2005NJ89B ("Impacts of Organic Matter Heterogeneity on Desorption and Availability of Sediment-bound PCBs") - Dissertations - Sharma, Samriti, 2007, Impact of soil organic matter heterogeneity and soil aggregation on the sorption of herbicides by soils, Ph.D. Dissertation, Department of Environmental Sciences, Rutgers University, New Brunswick, NJ, 170 pages.
26. 2005NJ91B ("RESISTANCE OF FRACTURED ROCK DECHLORINATING BACTERIA TO PRESSURE FROM HEAVY METALS") - Other Publications - Son, E.-K.; K.Y. Lee; and D.E. Fennell. 2008. Characterization of a novel Dehalococcoides enrichment culture dehalorespiring chlorinated ethenes. 108th General Meeting of the American Society for Microbiology. June 1-5, Boston, MA. (poster presentation)
27. 2005NJ91B ("RESISTANCE OF FRACTURED ROCK DECHLORINATING BACTERIA TO PRESSURE FROM HEAVY METALS") - Articles in Refereed Scientific Journals - Krumins, V.; Park, J.-W.; Son, E.-K.; Rodenburg, L.A.; Kerkhof, L.J.; Haggblom, M.M.; and Fennell, D.E. 2008. Sustained PCB dechlorination enhancement in Anacostia River sediment. *Water Research*, 43, 4549 – 4558.
28. 2005NJ91B ("RESISTANCE OF FRACTURED ROCK DECHLORINATING BACTERIA TO PRESSURE FROM HEAVY METALS") - Dissertations - Son, E.-K. 2009. Characterization of bacterial processes in the subsurface and the atmosphere. Ph.D. Dissertation. Dept. of Environmental Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ, 181 pp.
29. 2005NJ129B ("Undergraduate Research Internships") - Articles in Refereed Scientific Journals - Moffett, Donna and John Hasse. 2006. Looking for the Relationship Between Sprawl and Water Quality: A Case Study of Gloucester County, NJ. *Middle States Geographer*, 39:26-33.
30. 2006NJ100B ("Nitrate removal in urban wetlands: examining the roles of vegetation, soils, and hydrology in the creation of hot spots and hot moments of denitrification") - Other Publications - Palta, Monica M. and Joan G. Ehrenfeld. 2008. "Linking form to function: Using soil properties to predict nitrogen removal across an urban riparian landscape." The American Water Resources Association Summer Specialty Conference: Riparian Ecosystems and Buffers, Virginia Beach, Virginia.
31. 2006NJ100B ("Nitrate removal in urban wetlands: examining the roles of vegetation, soils, and hydrology in the creation of hot spots and hot moments of denitrification") - Other Publications - Palta, Monica M. and Joan G. Ehrenfeld. 2009. "Denitrification function in brownfield soils: Using texture to predict nitrate removal across an urban riparian landscape." The Society for Wetland Scientists Annual Conference, Madison, Wisconsin.
32. 2006NJ101B ("Female Hormones in Surface Water of Central/Northern New Jersey: Impacts of Combined Sewer Overflows versus Treated Wastewater Discharge") - Dissertations - Sharma, Samriti, 2007, Impact of soil organic matter heterogeneity and soil aggregation on the sorption of herbicides by soils, Ph.D. Dissertation, Department of Environmental Sciences, Rutgers University, New Brunswick, NJ, 170 pages.
33. 2006NJ102B ("Cranberry Agriculture as Wildlife Habitat in the Pine Barrens Wetland Ecosystem") - Other Publications - Wen, Ai. 2008. Species composition in the seedbank of abandoned cranberry farms: From a restoration perspective. ESA 2008 Annual Meeting. PS 67-151, Milwaukee, Wisconsin. Poster presentation.

34. 2006NJ102B ("Cranberry Agriculture as Wildlife Habitat in the Pine Barrens Wetland Ecosystem") - Other Publications - Wen, Ai. 2009. Effects of agriculture's legacy on wetland succession and its implication on restoration. SWS 2009 Annual Meeting. Madison, WI. Oral presentation.
35. 2006NJ118B ("Integrated Assessment of Economic and Water Quality Impacts of Agricultural Best Management Practices in Upper Cohansey River Watershed") - Articles in Refereed Scientific Journals - Qiu, Z. 2009. Identifying Critical Source Areas in Watersheds for Conservation Buffer panning and Riparian Restoration. *Environmental Management* 44(5):968-980.
36. 2006NJ118B ("Integrated Assessment of Economic and Water Quality Impacts of Agricultural Best Management Practices in Upper Cohansey River Watershed") - Articles in Refereed Scientific Journals - Qiu, Z., C. Hall and K. Hale. 2009. Evaluation of cost-effectiveness of conservation buffer placement strategies in a River Basin. *Journal of Soil and Water Conservation* 64(5): 293-302.
37. 2007NJ132B ("Development of Microscale Membrane Extraction for trace Monitoring of Pesticides and other Emerging Pollutants in Water") - Articles in Refereed Scientific Journals - Hylton, Kamilah, Manuvesh Sangwan, and Somenath Mitra, 2009, Microscale membrane extraction of diverse antibiotics from water, *Analytica Chimica Acta*, 653 (1), 116-120.
38. 2007NJ139B ("Phosphate and Thermal Stabilization of Dredged Sediments for Reuse as Construction Material") - Articles in Refereed Scientific Journals - Ndiba, P. K.; Axe, L., 2009, Sequential Extraction of Phosphate and Thermal Treated New York/New Jersey Harbor Dredged Sediments, *Environmental Engineering Science*, 26 (12), 1755-1764.
39. 2007NJ139B ("Phosphate and Thermal Stabilization of Dredged Sediments for Reuse as Construction Material") - Articles in Refereed Scientific Journals - Ndiba, P. K.; Axe, L., 2010, Risk assessment of metal leaching into groundwater from phosphate and thermal treated sediments, *Journal of Environmental Engineering*, 136 (4), 427-434.
40. 2007NJ141B ("Restored oyster reef habitat use by the American Eel (*Anquilla rostrata*) in the Lower Delaware Bay") - Articles in Refereed Scientific Journals - Taylor, J. and D Bushek. 2008. Intertidal oyster reefs can persist and function in a temperate North American Atlantic estuary. *Mar. Ecol. Prog. Ser.*, 361: 301-306.
41. 2007NJ145B ("A Quantitative Approach to Linking Temporal Variations of Groundwater Level with Nitrogen Cycling in New Jersey Wetlands") - Other Publications - Yarlequé, C., D. Giménez, A. Posadas, E. Stander, J. Ehrenfeld and R. Quiroz. 2008. Groundwater Level Dynamics across Temporal Scales for Urban and Rural Wetlands: A Wavelet-Multifractal Model. ASA-CSSA-SSSA 2006 International Annual Meetings, October 5-9, Houston, TX.
42. 2007NJ145B ("A Quantitative Approach to Linking Temporal Variations of Groundwater Level with Nitrogen Cycling in New Jersey Wetlands") - Other Publications - Yarlequé, C., D. Giménez, A. Posadas, E. Stander, J. Ehrenfeld and R. Quiroz. 2008. Groundwater Level Dynamics across Temporal Scales for Urban and Rural Wetlands: A Wavelet-Multifractal Model. International Workshop on Urban Wetland Ecology and Restoration, December 12-19, Xiamen, China.
43. 2008NJ162B ("Micro Scale Solid Phase Extraction using Carbon Nanotubes for Rapid Detection of Organic Pollutants in Water Resources") - Articles in Refereed Scientific Journals - Sae-Khow, Ornthida; Somenath, Mitra, 2009, Carbon nanotubes as the Sorbent for Integrating  $\mu$ -SPE within the Needle of a Syringe, *J. Chromatogr. A*, 1216, 2270-2274.
44. 2008NJ162B ("Micro Scale Solid Phase Extraction using Carbon Nanotubes for Rapid Detection of Organic Pollutants in Water Resources") - Dissertations - Sae-Khow, Ornthida, 2010, Microextraction Techniques using Carbon Nanotubes as the Media, Ph.D. Dissertation, Department Chemistry and Environmental Science, New Jersey Institute of Technology, Newark, NJ, 104 pages.
45. 2008NJ154B ("Volatilization of PCBs from the Tappan Zee region of the Hudson River") - Other Publications - Sandy, A. L., L.A. Rodenburg, and R.J. Miskewitz, 2009, Direct Measurement of Air/Water Exchange Mass Transfer Coefficients of PCBs, Platform presentation SETAC 30th Annual Meeting in North America, November 19-23, 2009, New Orleans, LA.
46. 2008NJ161B ("Microbial Mobilization of Arsenic and Selenium Oxyanions in Subsurface Aquifers") - Other Publications - Rauschenbach I, Narasingarao P, Haggblom MM (2009) Selenium oxyanion

- reduction: a ubiquitous process in the environment. Abstract Q-210, American Society for Microbiology 109th General Meeting, Philadelphia, PA, May 17-21, 2009.
47. 2008NJ164B ("Identifying the source of excess fine-grained sediments in New Jersey rivers using radionuclides") - Conference Proceedings - Galster, Josh, Kirk Barrett, Huan Feng, Jared Lopes, Nicole Bujalski, 2009, Using 210Pb and 137Cs to identify the bank vs. soil contributions to excess fine-grained sediments in urban and rural New Jersey river channels, in Geological Society of America Abstracts with Programs, Denver, Colorado, v. 41 p. 577.
  48. 2008NJ164B ("Identifying the source of excess fine-grained sediments in New Jersey rivers using radionuclides") - Other Publications - Bujalski, Nicole, Jared Lopes, Josh Galster, Kirk Barrett, Huan Feng, 2009, Using radionuclides to investigate New Jersey rivers for sources of excess fine grained sediment, Montclair State University Third Annual Student Research Symposium, Montclair, New Jersey.
  49. 2008NJ164B ("Identifying the source of excess fine-grained sediments in New Jersey rivers using radionuclides") - Conference Proceedings - Galster, Josh, Kirk Barrett, Huan Feng, Jared Lopes, Nicole Bujalski, 2009, Using atmospherically-deposited radionuclides to identifying the source of excess fine-grained sediments in two New Jersey watersheds, in Association of American Geographers 2009 Annual Meeting Abstract Volume, Las Vegas, Nevada.
  50. 2008NJ164B ("Identifying the source of excess fine-grained sediments in New Jersey rivers using radionuclides") - Conference Proceedings - Galster, Josh, Kirk Barrett, Huan Feng, Nicole Bujalski, Jared Lopes, 2008, Identifying the source of fine-grained sediment using radionuclides, in Geological Association of New Jersey Annual Meeting Abstract Volume, Montclair, New Jersey, v. 25.
  51. 2008NJ164B ("Identifying the source of excess fine-grained sediments in New Jersey rivers using radionuclides") - Conference Proceedings - Galster, Josh, Kirk Barrett, Huan Feng, Nicole Bujalski, Jared Lopes, 2008, Characterizing the source of fine-grained sediments in New Jersey rivers using radionuclides, in Geological Society of America Abstracts with Programs, Denver, Colorado, v. 40, p. 194.
  52. 2008NJ164B ("Identifying the source of excess fine-grained sediments in New Jersey rivers using radionuclides") - Conference Proceedings - Galster, Josh, Kirk Barrett, Huan Feng, Jared Lopes, Nicole Bujalski, 2008, Identifying the source of excess fine-grained sediments in New Jersey rivers using radionuclides, in NEGSA Abstracts with Programs, Denver, Colorado, v. 40.