New York State Water Resources Institute Annual Technical Report FY 2015

Introduction

The Mission of the New York State Water Resources Institute (WRI) is to improve the management of water resources in New York State and the nation. As a federally and state mandated institution located at Cornell University, WRI is uniquely situated to access scientific and technical resources that are relevant to New York State's and the nation's water management needs. WRI collaborates with regional, state, and national partners to increase awareness of emerging water resources issues and to develop and assess new water management technologies and policies. WRI connects the water research and water management communities. Collaboration with New York partners is undertaken in order to: 1) Build and maintain a broad, active network of water resources researchers and managers, 2) Bring together water researchers and water resources managers to address critical water resource problems, and 3) Identify, adopt, develop and make available resources to improve information transfer on water resources management and technologies to educators, managers, policy makers, and the public.

Research Program Introduction

The NYS WRI's FY2015 competitive grants research program was conducted in partnership with the NYS Department of Environmental Conservation (DEC) Hudson River Estuary Program (HREP). The overall objective of this program is to bring innovative science to watershed planning and management. In FY2015 research was sought that fit within the context of New York State's concerns about aging public water resources infrastructure and related economic constraints on public investment. Additionally, competitive funding was directed toward projects that incorporated analysis of historic or future climate change and/or extreme weather and their impacts on communities, ecosystems, and infrastructure. The specific areas of interest for the FY2015 grants program solicitation were: 1) The current state and effectiveness of water-related infrastructure including water supply and wastewater treatment facilities; natural and "green" infrastructure; distribution networks; decentralized treatment installations; dams; culverts and bridges; constructed wetlands; etc., at providing water services regionally at reasonable cost, as well as how they compare to the natural systems they are replacing, augmenting or impacting; 2) Historic and/or future effects of climate change and extreme weather impacts on New York's communities; and climate resilience of ecosystems, infrastructure, communities, and governance institutions and/or development of strategies to increase resiliency of these systems; 3) Integration of scientific, economic, planning/governmental and/or social expertise to build comprehensive strategies for local public asset and watershed managers and stakeholders; 4) Novel outreach methods that enhance the communication and impact of science-based innovation to water resource managers, policy makers, and the public; and 5) The relationship between management in the Hudson River watershed and the Hudson estuary ecosystem, fish and wildlife.

Projects were evaluated by a panel consisting of 4 WRI staff representatives, 1 Director from another state's Water Research Institute, 1 Cornell University faculty member, 1 staff member from the NYS Department of Environmental Conservation, and 2 representatives from other NY-based academic institutions. Four research projects were initiated with 104b base funding, while another seven were initiated and funded through DEC sources that WRI leverages with its base federal grant. For FY2015, 104b-funded projects include:

1. Emerging Organic Pollutants: From College Campuses to Cayuga Lake PI: Susan Allen-GIl, Ithaca College

2. Denitrifying Bioreactors Reduction of Agricultural Nitrogen Pollution at the Watershed Scale PI: Larry Geohring, Cornell University

3. Western New York Watershed Network PI: Chris Lowry, SUNY-Buffalo

4. Population and DPS Origin of Subadult Atlantic Sturgeon in the Hudson River PI: Isaac Wirgin, New York University Medical Center

Additionally, WRI staff funded in part by the 104b program engaged in ad hoc research activities, the results of which are reported on below (authors in bold indicate WRI researchers):

1. **Sridhar Vedachalam**, Kyra T. Spotte-Smith, **Susan J. Riha**, A meta-analysis of public compliance to boil water advisories, Water Research, 94 (2016) 136-145

2. **Vedachalam. S.**, Geddes, R.R., **Riha, S.J.**, 2016, Public–Private Partnerships and Contract Choice in India's Water and Wastewater Sectors, Public Works Management & Policy, 21(1):71-96

3. McPhillips, L. and **M.T. Walter**, Hydrologic conditions drive denitrification and greenhouse gas emissions in stormwater detention basins, Ecological Engineering, 85 (2015) 67-75

4. **Vedachalam. S.**, Lewenstein, B.V., DeStefano, K.A., Polan, S.D., **Riha, S.J.**, 2015, Media discourse on ageing water infrastructure, Urban Water Journal. DOI: 10.1080/1573062X.2015.1036087

5. **Vedachalam, S.**, Vanka, V.S., **Riha, S.J.**, 2015, Reevaluating onsite wastewater systems: Expert recommendations and municipal decision-making, Water Policy, 17(6):1062-1078

6. **Rahm, B.G., Vedachalam, S.**, Bertoia, L., Mehta, D., Vanka, V.S., **Riha, S.J.**, 2015, Shale gas operator violations in the Marcellus and what they tell us about water resource risks, Energy Policy. 82:1-11

7. McPhillips, L.E., P.M. Groffman, C.L. Goodale, **M.T. Walter**, 2015, Hydrologic and biogeochemical drivers of riparian denitrification in an agricultural watershed, Water, Air, and Soil Pollution 226:169 [doi: 10.1007/s11270-015-2434-2]

Environmental Research

Basic Information

Title:	Environmental Research
Project Number:	2015NY218B
Start Date:	3/1/2015
End Date:	2/28/2016
Funding Source:	104B
Congressional District:	NY-23
Research Category:	Water Quality
Focus Category:	Water Quality, Nitrate Contamination, Wastewater
Descriptors:	None
Principal Investigators:	Susan Riha, Brian Gramlich Rahm

Publication

1. Rahm, Brian; Nicole Hill, Stephen Shaw, and Susan Riha, 2016, Nitrate Dynamics in Two Streams Impacted by Wastewater Treatment Plant Discharge: Point Sources or Sinks? Journal of the American Water Resources Association,52(3), 1–13.

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NITRATE DYNAMICS IN TWO STREAMS IMPACTED BY WASTEWATER TREATMENT PLANT DISCHARGE: POINT SOURCES OR SINKS?[1]

Brian G. Rahm, Nicole B. Hill, Stephen B. Shaw, and Susan J. Riha[2]

ABSTRACT: We examined nitrate processing in headwater stream reaches downstream of two wastewater treatment plant outfalls during low streamflow. Our objectives were to quantify nitrate mass flux before and after effluent discharge and to use field and laboratory techniques to assess the mechanism of nitrate uptake. Microcosm experiments were utilized to determine the location of nitrate processing, and molecular biomarkers were used to detect and quantify microbial denitrification. At one site, downstream nitrate mass flux was significantly (p = 0.01) lower than sum of upstream and wastewater effluent fluxes, indicating rapid stream assimilation of incoming nitrate in the vicinity of the point source. Microcosm experiments supported the theory that nitrate processing occurs in sediments. Molecular assays for denitrifcation-associated functional genes *nosZ*, *nirS*, and *nirK*, provided evidence that effluent contained enriched denitrifying communities relative to ambient stream water. Nitrate loss at the site with greater uptake was correlated with sulfate loss (p < 0.01; r^2 = 0.86), suggesting a possible link between sulfate reducing bacteria and denitrifying bacterial communities. Results suggest there is an opportunity to better understand nitrate dynamics in cases where point sources may act as point sinks under specific sets of conditions.

(KEY TERMS: nitrate; nitrogen; microbiological processes; denitrification; genetic markers; rivers/streams; total maximum daily loading; effluent wastewater discharge.)

Rahm, Brian G., Nicole B. Hill, Stephen B. Shaw, and Susan J. Riha, 2016. Nitrate Dynamics in Two Streams Impacted by Wastewater Treatment Plant Discharge: Point Sources or Sinks? *Journal of the American Water Resources Association* (JAWRA) 1-13. DOI: 10.1111/1752-1688.12410

INTRODUCTION

Anthropogenic nutrient loading in streams and rivers is a significant issue across the world and the United States (U.S.), leading in some cases to eutrophication of lakes and coastal zones, and general degradation of water quality for human use and ecosystems (Vitousek *et al.*, 1997; Caraco and Cole, 1999; Rabalais, 2002). Nitrogen inputs arise from a variety of nonpoint sources such as agricultural and urban runoff and atmospheric deposition, as well as from point sources such as industrial and municipal wastewater effluents (Johnson *et al.*, 1976; Boyer *et al.*, 2002; Driscoll *et al.*, 2003; Lofton *et al.*, 2007).

In the U.S., the Environmental Protection Agency is applying total maximum daily load (TMDL) calculations to water bodies receiving significant and detrimental nutrient inputs. TMDLs specify the maximum amount of a pollutant allowed to enter a water body

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so that the water body still meets its designated water quality standards. TMDLs also allocate portions of the total load to point and nonpoint sources, which then must be managed in accordance with a variety of regulatory, nonregulatory, and/or incentive-based guidelines. Such arrangements increase stakeholder interest in identifying nutrient inputs at point sources and identifying management solutions to reduce inputs.

Streams and rivers are important for their role in transporting nutrients from ecosystems and engineered and anthropogenic sources from one place in the landscape to another. They also play a major role in nutrient transformation processes, altering the chemical form and quantity of nutrients exported to downstream environments. For nitrogen especially, headwater streams have been identified as key locations for biogeochemical transformation processes such as mineralization of organic nitrogen, biological assimilation, and microbially mediated processes such as nitrification and denitrification (Alexander *et al.*, 2000; Peterson *et al.*, 2001; Thouin *et al.*, 2009). However, the efficiency with which small streams retain or transform nitrogen inputs can vary, and can be altered by effluent discharge from municipal wastewater treatment plants (WWTPs). While there have been some observations of high instream nitrogen removal efficiencies associated with WWTP discharge (Marti *et al.*, 2004; Ribot *et al.*, 2014), most studies have found low removal efficiencies (Haggard *et al.*, 2005; Gibson and Meyer, 2007; Lofton *et al.*, 2007), often because effluent nitrogen loads far exceeded the assimilation capacity of the water bodies. Few studies have considered systems in which the WWTP nitrogen load is only a small amount (<10%) of the total stream load, despite such systems being common. To our knowledge, even fewer studies exist focusing on the impact of WWTP effluent on stream biogeochemical transformation and associated bacterial ecology (Wakelin *et al.*, 2008; Drury *et al.*, 2013). Given the importance of instream processing of nitrogen, and the critical role played by microbial communities in river metabolic processes, more work is needed on understanding how they are linked, especially at disturbed and regulated sites such as WWTP outfalls.

There are several mechanisms by which dissolved nitrogen may be removed from streams receiving WWTP effluent, but microbial denitrification is one of the most important because nitrogen is removed from the system as a gas. Denitrification depends on a variety of factors, including the presence of an active community of microbes capable of carrying out the various metabolic steps. Assessing microbial communities as indicators of important biogeochemical processes has become more achievable through molecular biological techniques that are independent of culturing constraints (Hugenholtz *et al.*, 1998; Xu, 2006). Detection and quantification of conserved functional genes in environmental samples makes it possible to more directly link nutrient transformation with the microbial communities potentially responsible for them (Wakelin *et al.*, 2008).

Aside from the microbes themselves, denitrification rates also depend on the concentration of nitrate (NO_3^-) and organic carbon, appropriate redox conditions, and other physical factors such as temperature and pH (Lofton *et al.*, 2007; Mulholland *et al.*, 2008). Still, it is difficult to accurately predict and measure denitrification rates in river environments (Boyer *et al.*, 2006; Seitzinger *et al.*, 2006; Mulholland *et al.*, 2009). Some models used to track net nitrogen flux at watershed scales take an empirical approach, representing nitrogen processing downstream of WWTP discharges through a decay function (Boyer *et al.*, 2006; Alexander *et al.*, 2009). Models generally have difficulty parsing mechanistic drivers of nitrogen transformation, and are less useful for assessing dynamics at the site-scale. Thus, there is still a general need for more data on instantaneous measures of nitrogen transformation in streams with a focus on the underlying causes of specific processes (Boyer *et al.*, 2006).

Here, we report observations of nitrogen mass flux and uptake at two sites on two headwater streams receiving WWTP effluent in upstate New York during low flow. Our objectives were to (1) quantify NO_3^- losses in reaches impacted by WWTP discharge, (2) use laboratory microcosm studies to help assess the mechanism of that loss, and (3) use functional molecular biomarkers to detect and quantify microbial denitrification. Given our focus on headwater streams at times of low flow, we hypothesized that we would be able to observe and quantify NO_3^- processing downstream of WWTP inputs, that microbial denitrification would be a significant driver of NO_3^- uptake, and that WWTP effluent would serve to enrich the downstream denitrifying microbial community relative to upstream.

METHODS

The Method section first introduces the two study sites. The remainder of the section sequentially introduces the methods associated with each of the three primary objectives: field sampling of NO_3^- flux; microcosm studies; and evaluation of microbial biomarkers.

Study Site

Field observations were made on two streams, Fall Creek and the Owasco Inlet, both located in the Finger Lakes region of rural upstate New York (Figure 1). Fall Creek begins near Lake Como in Cayuga County and drains into Cayuga Lake in Tompkins County. The Owasco Inlet begins near the hamlet of Peruville in Tompkins County and drains into Owasco Lake in Cayuga County. Both catchments are underlain by Devonian bedrock consisting primarily of siltstones and shales, and their natural drainage systems have been impacted by Pleistocene glaciation.

On Fall Creek, the effluent pipe enters the stream just outside the Village of Freeville. Two WWTPs share this effluent pipe: an approximately 0.5 mgd (21.9 L/s) facility serving roughly 2,500 people in the Village of Dryden and a 0.125 mgd (5.48 L/s) facility serving roughly 700 people in the Village of Freeville. The Village of Freeville WWTP consists of two aerated lagoons operated in series. The Town of Dryden WWTP was upgraded between the summer 2011 and summer 2012 sampling seasons. Prior to July 2011, the Village of Dryden WWTP consisted of primary settling, a trickling filter and a rotating biological contactor. Post-2011, the WWTP consists of primary settling followed by a sequencing batch reactor. The effluent pipe from the two WWTPs is approximately 5 km long, possibly allowing the addition of infiltrated water not reflected in the discharge reported at the actual WWTPs. Because of this possibility of infiltrated water, the effluent pipe discharge at Fall Creek is estimated by using chloride (Cl^-) concentration as a conservative tracer (discussed more below).

On the Owasco Inlet, the effluent pipe enters the stream at the outfall from the Village of Groton WWTP. This is a 0.5 mgd (21.9 L/s) facility serving roughly 2,400 people, and consists of primary settling and a sequencing batch reactor. The land use upstream of the effluent pipes on both Fall Creek and Owasco Inlet is predominantly agricultural. In both cases, ambient NO_3^- concentrations are thought to be largely controlled by agricultural inputs related to feed and fertilizer, as well as atmospheric deposition (Johnson *et al.*, 2007).

Sampling was conducted during the summers of 2011, 2012, and 2014 during low flow periods such that the fractional flow contribution of WWTPs to the streams were near the annual maximum. At higher streamflows, the changes in stream nitrogen concentrations due to additions by WWTPs are small enough that they are within the margin of error of standard chemical analytical techniques. U.S. Geological Survey (USGS) stream gages are located on Fall Creek (USGS gage #04234000) approximately 13 km downstream from the WWTP discharge and on the Owasco Inlet (USGS gage #04235299) approximately 25 km downstream from the WWTP discharge. Mean annual streamflows are 5,352 and 4,332 L/s on Fall Creek and Owasco Inlet, respectively. Study reaches were identified around WWTP discharge points. We sought upstream locations that were easily accessible, and free from the influence of WWTP effluent so as to capture "ambient," or natural stream conditions. Downstream locations were chosen at which conduc-

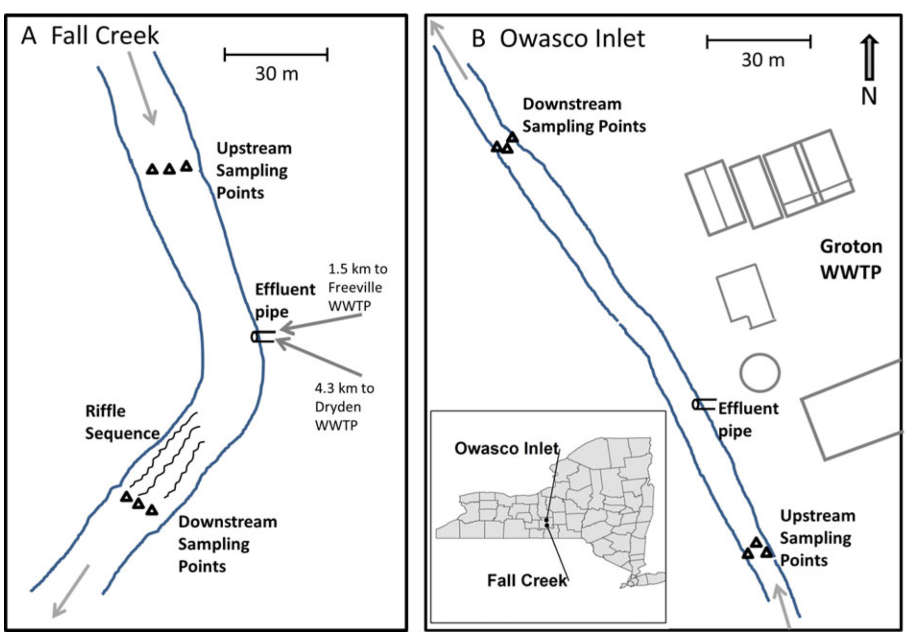

FIGURE 1. Stream Study Sites Showing (inset) the Approximate Location in Upstate New York, (A) Fall Creek, and (B) the Owasco Inlet.

tivity measurements indicated that WWTP effluent and stream water were completely mixed. At Fall Creek, the study reach consisted of a 105 m stretch, beginning at an upstream sampling location 50 m above the WWTP discharge, and ending at a downstream sampling location 55 m below the discharge. On the Owasco Inlet, the study reach began at an upstream sampling location 45 m above the WWTP discharge, and extended to a sampling location 85 m downstream.

Field Sampling and Laboratory Procedures

Field measurements were conducted so as to compare stream nitrate mass flux upstream and downstream from the point of WWTP discharge. For both the upstream and downstream location, grab samples were taken at three points across the width of the stream (Figure 1). At the effluent pipe, three samples were also taken, but there was no variation in location. All nine samples from each sampling event were chemically analyzed independently. The three concentrations at each location were then averaged to provide a measure of the mean nitrate concentration above and below the discharge pipe and from the WWTP. There were eleven sampling events on Fall Creek and five events on the Owasco Inlet. For one of the five events (on July 10, 2012) on Owasco Inlet, sampling occurred during a backwash cycle, resulting in much lower effluent flow and higher nitrate concentration.

For a subset of the sampling events, nitrite and ammonia were measured in addition to nitrate. For these events, these additional measures of nitrogen could be used to assess whether changes in nitrate were influenced by transformation to and from other forms of nitrogen. In addition to the nitrogen components, dissolved organic carbon (DOC), sulfate, and Cl^- were also sampled during some events. Cl^- loads were used to estimate discharge from the WWTP. DOC and sulfate were considered as possible controls on stream nitrogen transformation.

Water samples for ion chromatography, organic carbon and ammonium analysis were syringe filtered (nylon 0.45 μm, Cole-Parmer, Vernon Hills, Illinois) in the field and placed in polypropylene tubes (VWR, Radnor, Pennsylvania) on ice. Anions, including Cl^-, NO_3^-, NO_2^-, and SO_4^{2-} were analyzed using an ICS-2000 Ion Chromatograph (Dionex, Sunnyvale, California) with an IonPac AS-18 analytical column, 25 μL sample loop, and 21 mM KOH eluent. Analysis of NH_4^+ was performed by colorimetry at the Cornell Nutrient Analysis Laboratory. Analysis of DOC was performed on an OI Analytical (College Station, Texas) 1010 referencing a standard potassium hydrogen phthalate (KHP) solution.

Physicochemical water parameters, including dissolved oxygen, conductivity, pH, and water temperature were determined in the field using YSI (Yellow Springs, Ohio) sondes (YSI models 600 XLM and 6,920 V2) linked to a handheld, digital datalogger and display (YSI 650 MDS).

Streamflow at the sampling locations on Fall Creek and the Owasco Inlet was estimated by scaling the flow measured at the stream gage by the fraction of the watershed area above the effluent pipes (84% for Fall Creek and 18% for Owasco Inlet). Stream sampling was done during periods of sustained base flow; other studies that have evaluated the similarity of flow across scales have found that above a watershed size of approximately 8 km^2, base-flow scales closely with watershed area (Shaman *et al.*, 2004). Thus, although not a direct measurement, we assume that our estimation of streamflow is reasonably robust.

A long-term mean effluent flow for WWTP discharge at Fall Creek was available at the USEPA's Enforcement and Compliance History Online website (http://echo.epa.gov). This long-term mean was not considered representative of the actual discharge at the time of sampling, so Cl^- concentrations measured upstream (Cl_{up}), downsteam (Cl_{down}), and at the effluent pipe (Cl_{pipe}) were used in conjunction with the streamflow (Q_{stream}) to better estimate the instantaneous effluent discharge (Q_{pipe}) using a Cl^- mass balance:

$$\frac{Q_{stream}(Cl_{down} - Cl_{up})}{Cl_{pipe}} = Q_{pipe} \tag{1}$$

Chloride was considered to be a conservative tracer with no losses in the short-section of study reach. The intentional application of a Cl^- pulse is often used in the dilution gaging method of streamflow estimation (Dingman, 2002, p. 613). Effluent flow for the Owasco Inlet was provided by the WWTP operator, including duration of reactor decantation, and total volume of each batch. The Cl^- mass balance was also applied to the Owasco Inlet flow data despite greater confidence in the effluent flow reported by the WWTP. The average effluent flow estimated by the Cl^- mass balance on Owasco Inlet was not significantly different from that reported by the WWTP operator.

Nitrate flux was calculated by multiplying the nitrate concentration by the streamflow or pipe flow. The upstream flux plus the flux added by the WWTP was compared to the downstream flux. A two-tailed paired *t*-test was used to evaluate the difference between the combined upstream and WWTP nitrate flux and the downstream nitrate flux. In addition, statistical comparisons of NO_3^-, SO_4^{2-}, and DOC concentrations between upstream, downstream, and effluent samples at each field site were performed via independent *t*-test analyses. Also, to evaluate possible controls on nitrate loss, such as streamflow and ambient DOC and SO_4^{2-}, ordinary least squares regression analyses were employed. Significance was set at 5%.

Microcosm Experiments

The purpose of the microcosm experiments was to determine the denitrification potential at Fall Creek and Owasco Inlet in the water column with and without sediment present. Samples for microcosm experiments were collected from Fall Creek and Owasco Inlet during summer 2012. Unfiltered water samples were collected upstream of the WWTP pipe using pre-cleaned 2 L Nalgene HDPE bottles. Three sediment samples were collected across a lateral transect at sampling sites upstream and downstream of the pipe at both Fall Creek and Owasco Inlet by pushing a polypropylene centrifuge tube to a depth of 2 cm and sliding the cap under the opening to seal the tube. The transect sediment samples were then combined to form a composite representation of the upstream and the downstream sediment at each site. Samples were transported back to the lab on ice.

The concentration of nitrate in the upstream water samples was measured on the ion chromatograph. Sealed and pre-cleaned 125 mL wide-mouth glass jars were used for the microcosms. Sediment samples finer than 2 mm in diameter were scooped into twelve jars (six from Fall Creek and six from Owasco Inlet) with a sterilized spatula so that a thin coat completely covered the base of the jars. 100 mL of unfiltered water was then added to 24 jars so that half of the jars had sediment present and half did not. All microcosms were spiked with 10 mg/L NO_3^--N and sealed with a lid containing a septum.

Microcosm experiments were conducted over a 24-h period at 20°C. Dark conditions with no allowance for the addition of oxygen were established to ensure that observed nitrate losses could not be attributed to assimilation by autotrophs and so that heterotrophic denitrification would be the favored pathway for nitrate reduction. Final nitrate concentrations for the water column were measured after the 24-h incubation period.

A two-way analysis of variance (ANOVA) was employed to examine the effect of sampling location (Fall Creek or Owasco Inlet water) and the two microcosm treatments, with or without sediment present, on nitrate reduction over a 24-h incubation period. In addition, for each condition, a two-tailed paired *t*-test using a 5% significance level was applied to assess differences in nitrate concentrations before and after incubation.

Molecular Analyses

Molecular analyses were performed on field samples from Fall Creek and Owasco Inlet on two dates in 2012 and two in 2014. We wished to compare the relative quantity of genes (biomarkers) associated with microbial denitrifiers at upstream and downstream locations within sediment or water samples. Additional measurements of functional gene quantities were taken from WWTP effluent and discharge pipe biofilm to help explain any observed changes in downstream denitrifier biomarker quantities compared to upstream.

Water samples for molecular analyses were collected unfiltered. Sediment samples were collected in sterile 50 mL centrifuge tubes by driving the tubes into the sediment and capping them under water immediately. Biofilm samples were collected from WWTP discharge pipes by scraping wetted pipe surfaces with sterile spatulas, and depositing solids into 0.2 mL centrifuge tubes.

Microbial biomass from water samples was obtained by filtering 0.75 L stream water or effluent through a 0.22 μm Hydrophilic PVDF Durapore membrane (Sterivex-GV; Millipore, Darmstadt, Germany). Membranes were then cut into small pieces using a sterilized razor and placed into 0.2 μL centrifuge tubes for DNA extraction. For sediment and biofilm samples, DNA was extracted directly from collected solids.

DNA was extracted using the MoBio (Carlsbad, California) PowerSoil DNA Extraction kit according to manufacturer's instructions. Quantification of DNA was accomplished using the Quant-iT™ PicoGreen double-stranded DNA Assay Kit (Life Technologies, Carlsbad, California #P11496) and a reference standard curve of Lambda-phage DNA on the Tecan (Mannedorf, Switzerland) Infinite M200 Pro multimode reader with Magellan™ software.

Quantitative polymerase chain reaction (QPCR) was performed on functional genes *nosZ*, *nirS*, and *nirK* (Table 1). For *nosZ* and *nirK*, DNA from an isolate of *Pseudomonas nitroreducens* was kindly provided by Constance Roco of the Shapley lab at Cornell University for use as standard curve and positive control. For *nirS*, *Paracoccus denitrificans* was used as the standard. Triplicate QPCR amplifications of all standards, controls, and experimental samples were performed using an iCycler iQ real-time PCR detection system (Bio-Rad, Hercules, California). We used 25 μL reaction volumes containing 1X iQ SYBR Green Supermix (Bio-Rad), forward and reverse primer at a concentration of 700 nM, and approximately

TABLE 1. Quantitative PCR Gene Targets and Their Corresponding Primers.

Target	Primer ID	Primer Length	Sequence	Amplicon Size (bp)	Melting T (°C)	Reference
nirS	nirSCd3aF	20	GTSAACGTSAAGGARACSGG	425	57.1	Geets *et al.* (2007)
	nirSR3cd	19	GASTTCGGRTGSGTCTTGA		55.8	
nirK	nirK876	17	ATYGGCGGVCAYGGCGA	165	62.7	Henry *et al.* (2004)
	nirK1040	20	GCCTCGATCAGRTTRTGGTT		54.8	
nosZ	nosZ2F	23	CGCRACGGCAASAAGGTSMSSGT	267	66.5	Warneke *et al.* (2011)
	nosZ2R	21	CAKRTGCAKSGCRTGGCAGAA		60.8	

1 ng DNA. PCR conditions for *nosZ* and *nirS* were set following the protocol from Warneke *et al.* (2011). The conditions for *nirK* were 2 min at 50°C followed by 10 min at 95°C for enzyme activation, then 40 repeated cycles with 15 s at 95°C for denaturation and 1 min at 69°C for annealing, extension, and detection. Melt curve analysis was conducted after all runs to help confirm purity of amplicons.

DNA quantities were calculated using Data Analysis for Real-Time PCR (DART-PCR), a freely available Excel (Microsoft) based macro which determines threshold cycles, reaction efficiencies and relative DNA starting quantities from raw fluorescence data (Peirson *et al.*, 2003) (http://www.gene-quantification.de/download.html#dart). Values presented in this study are normalized per mass DNA extracted. This normalization allows us to compare the relative percentage of a gene pool comprised of target DNA sequences. The heterogeneous nature of the sediment, biofilm, and water samples made absolute quantification of target DNA sequences impractical and misleading.

During the DART-PCR process, differences in amplification efficiency within and between tested groups of gene targets were assessed using one-way ANOVA. Outlier samples were excluded from further analyses. For relative comparisons of denitrifier genes, paired *t*-tests and a 10% significance level were used to determine whether downstream mean biomarker quantities differed from upstream in water samples or in sediment samples at each site. Independent *t*-tests and a 10% significance level were also used to determine whether effluent mean biomarker quantities differed from upstream water samples at each site.

RESULTS

Field Sampling of Stream Nitrate

The NO_3^- mass flux was calculated at each site above and below the effluent pipe using measured concentration and flow (Table 2). One would expect the downstream flux to be nearly equivalent to the sum of the upstream flux plus the flux from the WWTP effluent. Indeed, this is what we observed at the Owasco Inlet, where there was no significant difference between the downstream flux and the sum of the upstream and effluent flux. However, at Fall Creek, the downstream flux was significantly smaller than the sum of the upstream and effluent flux ($p = 0.011$). The percent difference between the measured downstream flux and the flux expected given the sum of the upstream and effluent flux was greatest on Fall Creek during 2011 prior to the installation of treatment plant upgrades. The WWTP NO_3^- flux during the 2011 period was more than an order of magnitude higher than the flux post-2011. Thus, prior to the upgrade, effluent was contributing more than 30% of the total stream nitrate load, whereas post-upgrade effluent contributed less than 10%.

High concentrations of ammonium in either upstream or effluent flow could influence the concentration and mass flux of nitrate observed downstream, particularly through nitrification. At both sites, upstream samples contained no detectable ammonium (Table 3). Also, NH_4^+ inputs from WWTP effluent were negligible relative to nitrate. If one were to assume instream conversion of NH_4^+ to NO_3^-, NH_4^+ would have contributed less than two percent of downstream nitrate mass flux at either site, on average. A build up of nitrite downstream might suggest incomplete nitrification of ammonium. While nitrite was observed at Fall Creek (0.15 ± 0.12 mg/L NO_2^--N), there was no significant difference between observed downstream nitrite mass flux and the sum of upstream and effluent nitrite flux. Nitrite was observed at Owasco Inlet on only a single sampling date.

Significant correlations between ambient nitrate concentration and either natural streamflow or upstream DOC were not observed at either site. Upstream Fall Creek DOC concentrations ranged from 2.70 to 7.40 mg/L over nine sampling dates across the three years. Effluent DOC concentrations ranged from 4.23 to 9.68 mg/L, with four of the five highest concentrations observed during 2011. The decline in DOC after 2011 presumably corresponds to the upgrade in the WWTP. On all sampling dates

TABLE 2. Flow and Nitrate Observations. "Up" refers to the upstream location, "Down" refers to the downstream location, and "Eff" is the effluent pipe. Mass flux is calculated as the product of concentration and flow. % Diff is the percent difference between the measured downstream flux and the flux expected downstream (upstream + effluent).

	NO_3^--N (mg/L)			Q (L/s)			NO_3^--N Mass Flux (mg/s)				
Date	Up	Eff	Down	Up	Eff	Down	Up	Eff	Down	Up + Eff	% Diff
Fall Creek											
2011-07-01	1.23	7.49	0.95	1,360	105	1,465	1,673	786	1,392	2,459	−77
2011-07-08	0.91	9.59	0.66	980	33	1,013	892	319	669	1,211	−81
2011-07-15	0.96	19.5	0.92	629	27	656	604	525	604	1,128	−87
2011-08-02	0.80	8.56	0.56	533	28	561	426	241	317	668	−111
2011-09-01	0.64	3.89	0.49	701	50	751	450	194	368	644	−75
2012-06-28	1.04	1.85	0.77	636	21	657	662	39	503	702	−39
2012-07-10	0.59	1.43	0.53	418	20	438	245	28	230	273	−19
2012-07-30	0.33	0.70	0.27	758	21	779	249	15	208	264	−27
2012-08-14	0.28	1.10	0.26	418	20	438	116	21	115	138	−20
2014-08-11	0.93	1.30	0.87	859	42	901	798	55	782	853	−9
2014-09-10	1.11	1.16	0.95	669	61	730	740	71	696	811	−17
Owasco Inlet											
2012-07-10*	0.44	3.83	0.60	110	6	116	49	23	69	72	−4
2012-07-30	0.36	0.92	0.50	123	34	157	44	31	79	75	4
2012-08-14	0.33	1.53	0.75	69	40	109	22	61	82	84	−3
2014-08-11	0.82	1.21	0.83	150	55	205	123	66	170	189	−12
2014-09-10	1.11	4.80	1.69	217	49	266	240	235	449	475	−6

*On Owasco Inlet the July 10, 2012 measurement occurs during a backwash cycle of the sequencing batch reactor.

TABLE 3. Physicochemical Summary at Both Study Sites. Values for n represent sampling days on which observations were made; each observation was performed in triplicate. Data values are means ± standard deviation.

		Fall Creek		Owasco Inlet	
Parameter	Location	n	Value	n	Value
Study reach (m)			101		128
Q (L/s)	Upstream	11	749 ± 292	5	131 ± 59.1
Temperature (°C)	Upstream	6	22.2 ± 2.18	4	19.7 ± 2.90
pH	Upstream	5	8.4 ± 0.1	3	8.5 ± 0.0
Conductivity (mS/cm^3)	Upstream	6	0.431 ± 0.012	4	0.584 ± 0.050
NO_3^--N (mg/L)	Upstream	11	0.80 ± 0.31	5	0.61 ± 0.34
	Effluent	11	5.14 ± 5.80	5	2.46 ± 1.74
	Downstream	11	0.66 ± 0.26	5	0.87 ± 0.47
NH_4^+-N (mg/L)	Upstream	4	0.00 ± 0.00	4	0.00 ± 0.00
	Effluent	6	0.19 ± 0.16	4	0.17 ± 0.13
	Downstream	4	0.00 ± 0.00	4	0.02 ± 0.04
NO_2^--N (mg/L)	Upstream	9	0.15 ± 0.12	3	0.00 ± 0.00
	Effluent	9	0.78 ± 0.40	3	0.09 ± 0.16
	Downstream	9	0.19 ± 0.09	3	0.03 ± 0.04
DOC (mg/L)	Upstream	9	4.64 ± 1.57	4	5.52 ± 2.70
	Effluent	9	8.05 ± 2.18	4	4.96 ± 1.10
	Downstream	9	4.87 ± 1.58	4	5.33 ± 2.11
SO_4^--S (mg/L)	Upstream	9	5.82 ± 1.62	4	4.86 ± 0.59
	Effluent	9	13.3 ± 2.66	4	20.0 ± 8.70
	Downstream	9	5.56 ± 1.37	4	6.55 ± 1.20

DOC concentrations in effluent exceeded upstream concentrations, and mean effluent DOC was significantly higher than upstream DOC (independent, two-tailed t-test, $p = 0.002$). On Owasco Inlet, upstream DOC concentrations ranged from 2.93 to 8.92 mg/L over four sampling dates across two years. Effluent DOC concentrations ranged from 4.00 to 6.10 mg/L, and were not significantly greater than upstream values in general.

Effluent sulfate concentrations at Fall Creek and Owasco Inlet were significantly higher than upstream (independent, two-tailed t-test, $p < 0.001$ and $p = 0.046$, respectively) (Table 3). At Fall Creek, but not on the Owasco Inlet, downstream sulfate mass flux was significantly lower (p = 0.001) than the sum of upstream and effluent sulfate mass flux, suggesting removal or a transformation process. Furthermore, a regression analysis of nitrate loss and sulfate loss produced a strong positive correlation ($p < 0.001$; $r^2 = 0.86$) at Fall Creek, but not on Owasco Inlet.

Microcosms

Table 4 summarizes the average percent nitrate lost for each microcosm condition following a 24-h incubation period. Reductions in NO_3^- were observed in Fall Creek and Owasco Inlet microcosms containing sediment, whereas small increases in NO_3^- were observed in Fall Creek and Owasco Inlet microcosms that did not contain sediment. There was a significant ($p < 0.001$) reduction in nitrate when sediment was present in both Fall Creek and Owasco Inlet microcosms. Results from the two-way ANOVA showed a significant ($p < 0.001$) interaction between the water location and presence of sediment effects on the average percent nitrate reduction over 24 h. An analysis of the simple main effects showed that the effect of sediment presence on nitrate reduction at each location was significant ($p < 0.001$). In addition, with sediment present, the effect of water location (Fall Creek or Owasco Inlet) was significant ($p < 0.001$) on the level of nitrate reduction. However, there was no significant difference (p = 0.687) in the percent nitrate reduced at either site when sediment was absent. These results suggest that any denitrification that may be happening at either site is predominantly occurring in the sediment and that the denitrification potential of Fall Creek sediment was greater than Owasco Inlet sediment.

Quantitative PCR

At both Fall Creek and the Owasco Inlet, DNA corresponding to *nosZ*, *nirS*, and *nirK* were detected at all sampling locations, both in the water column and in the sediment, indicating that biomarkers for microorganisms potentially capable of denitrification were ubiquitous (Figure 2). Also at both sites, effluent water contained microbial communities with higher relative quantities of *nosZ* than ambient upstream water (p = 0.073 and 0.045 at Fall Creek and Owasco Inlet, respectively), suggesting that the WWTP might be a source of microorganisms containing this gene. At Fall Creek, effluent also contained a microbial community with higher relative quantities of *nirS* compared to upstream water (p = 0.060). For *nirK*, the only significant result was the observation at the Owasco Inlet of lower relative quantities in downstream sediments compared to upstream (p = 0.014).

TABLE 4. Average Percent Nitrate Lost Following a 24-h Incubation Period for Microcosm Experiments (n = 6 for each treatment) and Two-Tailed Paired t-Test Results.

Description	% NO_3^- Reduction after 24 h	p-Value
Fall Creek water with sediment	32.5 ± 8.18	<0.001
Fall Creek water without sediment	−3.55 ± 7.83	0.318
Owasco Inlet water with sediment	14.1 ± 3.64	<0.001
Owasco Inlet water without sediment	−1.82 ± 6.52	0.527

DISCUSSION

Nitrate Processing

We observed nitrate processing in relatively short reaches downstream of two WWTP point sources during low flow conditions when one might expect the impact of discharge on stream chemistry to be the greatest. At Fall Creek, observed downstream nitrate mass flux was significantly lower than the sum of upstream and effluent flux values, despite relatively high WWTP nitrate inputs, whereas at the Owasco Inlet there was no significant difference. Overall, the Fall Creek site appeared to have greater nitrate removal than the Owasco Inlet site. Although our methodology did not allow us to calculate nitrate uptake lengths, it is clear that uptake at Fall Creek occurred over a short distance, and that uptake length (the distance necessary for stream nitrate to return to ambient levels) was less than 55 m on all sample days. This uptake length is significantly shorter than most reported values from studies conducted on WWTP-impacted streams (Table 5). Indeed, some studies saw no significant uptake at all or even an increase in nitrate presumed to be due to nitrification of NH_4^+-rich effluents (Marti *et al.*, 2004; Haggard *et al.*, 2005).

We were interested in identifying factors that might be directly or indirectly controlling nitrogen cycling at these sites and thereby poising ambient nitrate concentrations at observed levels. A better understanding of controlling variables in ambient stream water would help us understand why nitrate uptake at Fall Creek occurs more rapidly compared to the Owasco Inlet and previously studied sites. Mechanistic controls on ambient nitrate concentrations have been proposed before. Some studies have suggested an inverse relationship between DOC con-

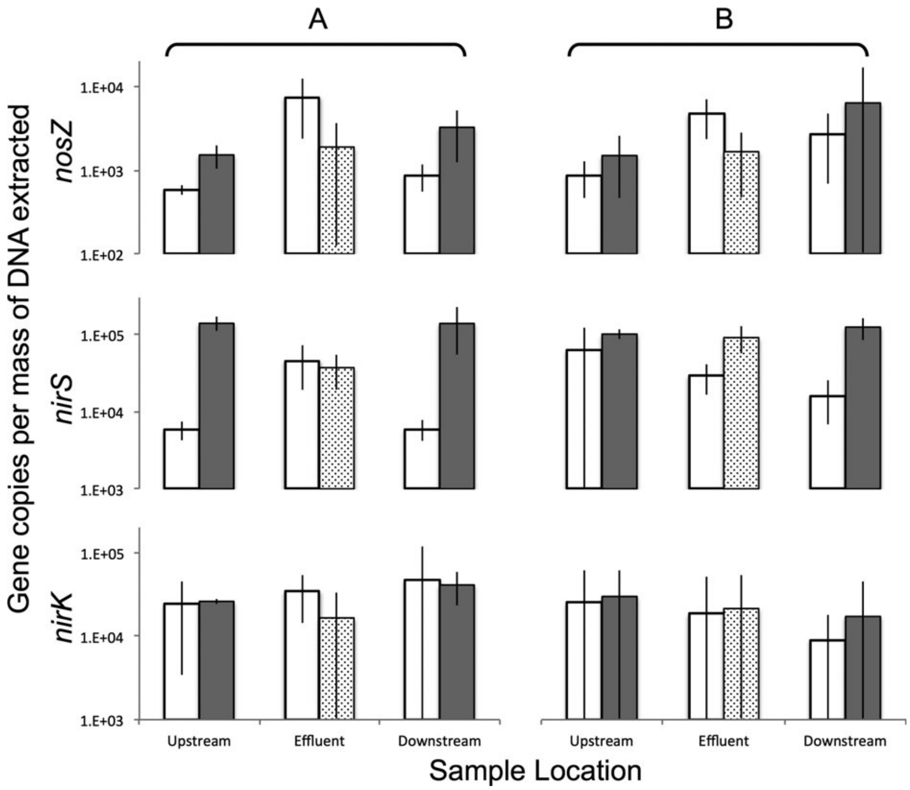

FIGURE 2. Relative Quantification of Genes *nosZ*, *nirS*, and *nirK* at (A) Fall Creek and (B) Owasco Inlet at Locations Upstream, Downstream, and within Effluent Discharge. Unshaded bars represent samples taken from filtered water; shaded bars represent sediment; dotted bars represent discharge pipe biofilm. All bars show means (n = 4 sampling days) and lines show standard deviation.

TABLE 5. Comparison of Nitrate Uptake Lengths from Selected Previous Studies (nd = not determined).

Stream	Mean Q (L/s)	Observed Downstream NO_3^--N (mg/L)	Mean Uptake Length (m)	Reference
Fall Creek, New York	749	0.66	<55	This study
Owasco Inlet, New York	178	0.87	nd	This study
Hugh White Creek, North Carolina	14	Not provided	268	Payn *et al.* (2005)
Truckee R. DR2, Nevada	3,822	0.05 (peak)	668	Johnson *et al.* (2015)
Truckee R. DR1, Nevada	3,822	0.11 (peak)	1,790	Johnson *et al.* (2015)
Spavinaw Creek, Arkansas	612	3.2 (mean)	9,350	Haggard *et al.* (2001)
Chattahoochee River, Georgia	42,200	2.5 (mean)	32,800	Gibson and Meyer (2007)

centration and nitrate uptake (Bernhardt and Likens, 2002; Goodale *et al.*, 2005), although subsequent researchers found this to be at least partly dependent on redox conditions (Thouin *et al.*, 2009). Organic carbon concentrations may modulate nitrate concentrations by providing an electron donor and carbon source for heterotrophic microbes that mediate partial or complete denitrification. Other studies have empirically correlated increased nitrate uptake and low streamflow (e.g., Peterson *et al.*, 2001), but do not experimentally address the mechanism of this relationship. We did not observe a significant correlation between upstream nitrate and any of the other physical parameters measured at either site, including DOC and streamflow. Therefore, from the collected physical data on ambient conditions alone, we could not see a reason why Fall Creek and the Owasco Inlet should behave differently in terms of nitrate uptake.

Nitrate Removal Mechanisms

Nitrate uptake in small streams such as Fall Creek and the Owasco Inlet is theoretically influenced by a variety of factors. Conceptually, nitrate processing is thought to occur in sediments and can proceed via biological assimilation and/or denitrification (e.g., Peterson *et al.*, 2001). Data from microcosm experiments in this study support the hypothesis that sediments are the predominant sites of nitrate loss relative to the water column at each site. However, microcosms alone did not help us distinguish between possible mechanisms of nitrate loss.

Assimilation of nitrate by plants is the process by which autotrophs utilize dissolved inorganic nitrogen and CO_2 to create biomass. Previous studies have identified assimilation as a significant, or potentially significant, nitrate loss mechanism in other streams and rivers (e.g., Panno *et al.*, 2008; Hall *et al.*, 2009). Data collected in this study did not allow us to assess the significance of assimilation directly. However, a simple estimate of the rate at which photoautotrophic assimilation would need to be happening at Fall Creek to account for observed nitrate loss can be calculated as the difference between expected downstream NO_3^- mass flux and the observed mass flux over a one day time interval. For the day with the smallest difference in downstream and effluent plus upstream mass flux (August 14, 2012), plant uptake would only need to be near 2 kg/day to account for the difference, similar to previous estimates at different sites (e.g., Fellows *et al.*, 2006). However, on days with large differences in mass flux (i.e., July 1, 2011), the required plant uptake would be near 100 kg/day, suggesting that assimilation by plants cannot be the dominant mechanism of nitrate loss at Fall Creek during these times. Still, across many streams and rivers, assimilation has been shown to be important (e.g., Taylor *et al.*, 2004; Kent *et al.*, 2005), and could be occurring via decomposition by fungi and bacteria (Danger *et al.*, 2015; Ford *et al.*, 2015) or integration into settle-able or suspended solids (Mulholland *et al.*, 2008). Research focused on this mechanism is needed to say more about nitrate uptake at our field sites.

Microbially mediated denitrification is an important mechanism for removing inorganic nitrogen from aquatic environments, involving a multi-step reduction process in which nitrate is ultimately transformed into predominantly dinitrogen gas and, to a lesser extent depending on certain conditions, nitrous oxide gas. Figure 3 illustrates this process, and includes information on the molecular markers responsible for each step. Boyer *et al.* (2006) lay out four conditions for denitrification: a nitrate source, an energy source (such as organic carbon), sub-oxic conditions, and an active denitrifying population. At both Fall Creek and the Owasco Inlet, conditions exist in which denitrification is possible. Ambient stream water at both sites contains at least some nitrate and DOC. Sediment and suspended solids provide sites where sub-oxic conditions exist, at least at the microscale. And, molecular biomarkers associated with denitrifying populations are present. Yet observations suggest that Fall Creek retains or transforms nitrate more readily than Owasco Inlet. Though not conclusive, molecular data and water chemistry observations provide some clues as to why this may be due to increased potential for denitrification at Fall Creek.

One possible explanation is that denitrification at Fall Creek is carbon limited under ambient conditions, and that WWTP-derived organic carbon is poising downstream nitrate at lower concentrations relative to upstream. To evaluate this hypothesis, we performed regression analysis on downstream nitrate loss (the difference between nitrate mass flux expected by mixing alone and the observed flux) and downstream DOC loss, but did not see any significant correlation. A confounding variable may be that the *type* of organic carbon available is just as important as the *concentration*. Previous researchers have found that instream denitrification rates, despite having no significant relationship with DOC in general, can be linked to chemical characteristics of the dissolved organic matter pool (Baker and Vervier, 2004; Barnes *et al.*, 2012). Perhaps, then, WWTP discharge at Fall Creek is altering the downstream dissolved organic matter pool so as to be more conducive to denitrification processes. Interestingly, a regression analysis of nitrate loss and sulfate loss produced a strong positive correlation at Fall Creek, but not on Owasco Inlet. Laboratory experiments with denitrification reactors found that sulfate reducing bacteria were responsible for degrading complex organic compounds and producing organic acids, which were in turn further oxidized by denitrifiers (Sahinkaya *et al.*, 2011; Yamashita *et al.*, 2011). This leads us to speculate that WWTP effluent containing organic matter of a particular quality, as well as sulfate, could be partly responsible for creating a denitrification hot spot in Fall Creek.

Increased microbial denitrification at Fall Creek may also be attributable to the bacterial ecosystem

$$\underset{\text{nitrate}}{NO_3^-} \xrightarrow{\textit{nar/nap}} \underset{\text{nitrite}}{NO_2^-} \xrightarrow{\textit{nir}} \underset{\text{nitric oxide}}{NO} \xrightarrow{\textit{nor}} \underset{\text{nitrous oxide}}{N_2O} \xrightarrow{\textit{nos}} N_2$$

FIGURE 3. The Denitrification Pathway. Genes corresponding to functional enzymes are identified above each reaction arrow.

there. Previous studies have looked at the abundance and diversity of bacterial communities in streams impacted by WWTPs, and provide evidence that the abundance of at least some microbes involved in nutrient processing increase in downstream sediments with high nutrient loads (Wakelin *et al.*, 2008; Drury *et al.*, 2013). Dong *et al.* (2009) also examined the abundance of functional genes along a gradient of measured denitrification rates and found a positive correlation with copies of *nirS* and other genes associated with nitrate reduction. In this study, data on the abundance of *nosZ*, *nirS*, and *nirK* within the bacterial community show that functional genes associated with denitrification were present at both field sites. As such, denitrification is likely to be an important process occurring in the sediments of both streams. Effluent discharge at both sites contains bacterial communities with higher *nosZ* abundance than in ambient water. WWTP discharge at Fall Creek also contains higher *nirS* abundance compared to ambient water. Thus, each WWTP may be enriching its stream with denitrifying bacteria. Unfortunately, results from this study do not provide convincing evidence that there are significant differences in relative functional gene quantities between downstream and upstream communities.

Although bacteria can generally be detected in WWTP effluent (Petersen *et al.*, 2005), a limitation of this analysis is that, because of disinfection processes at each plant, we may be detecting free aqueous DNA or DNA associated with inactive cells when we sample effluent. While bacterial transformation, the genetic alteration of an organism through the direct uptake of exogenous genetic material, is known to occur, it is not clear that it occurs in stream environments receiving WWTP discharge. That being said, biofilms covering the inside of WWTP outfall pipes represent an ecosystem unto themselves. It is possible that DNA extracted from effluent samples is at least partly derived from sloughed biofilm cells. Such sloughing is known to contribute to algae populations in stream water columns (Wilzbach and Cummins, 2008), though to our knowledge its role in delivering cells from WWTP discharge pipes has not been studied.

Implications and Conclusions

This study measured nitrate processing during seasonal low flow in two small streams receiving WWTP effluent. At one site, Fall Creek, results suggest that nitrate uptake is occurring rapidly, with downstream loads being driven lower than upstream ambient levels. In some ways, this suggests that WWTP effluent may, under certain conditions, be improving stream water quality with respect to nitrogen. Given this possibility, it is important to try to better understand the mechanism of nitrate loss. Analyses performed here suggest that a combination of chemical and microbial factors may be promoting denitrification at Fall Creek preferentially relative to Owasco Inlet.

While the results presented here do not provide conclusive mechanistic explanations for rapid nitrate uptake, they do suggest that, in some WWTP-impacted environments, effluent-derived concentration changes in limiting nutrients have the potential to re-poise nitrate at lower-than-ambient concentrations. And, although we did not observe significant enrichment of the downstream denitrifying microbial community, microbially mediated denitrification associated with sediments remained the most plausible explanation for observed nitrate loss. Overall, this study provides additional observations of instantaneous nitrate processing that could help to inform future model development and refinement. Given that TMDL agreements generally utilize models to help allocate loads among various sources, it is critical to continue to build a better understanding of nitrate dynamics in a variety of environments, and over a range of impacts. Ideally, scientists, engineers, and managers would have models sufficiently advanced to allow the ability to site WWTPs at optimal positions along streams and rivers, and to modulate effluent chemistry such that nitrate uptake processes are maximized when and where they are needed.

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Denitrifying Bioreactors Reduction of Agricultural Nitrogen Pollution at the Watershed Scale

Basic Information

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Publications

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Denitrifying Bioreactors Reduction of Agricultural Nitrogen Pollution at the Watershed Scale

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Abstract

Denitrifying bioreactors have the potential to reduce nitrogen loading to streams in agricultural watersheds. By passing the nitrate-rich waters of tile-drained fields through a system engineered for denitrification, the total nitrogen loading is reduced before entering sensitive aquatic ecosystems. In this project we found that intense summer storms impact nitrate removal rates in these reactors, causing in some cases for the removal rate to sharply drop for a period of time post-storm. Denitrifying bioreactors placed in existing tile-drained fields could reduce 4.5% of the total nitrogen export from the Upper Susquehanna River Basin. As a low-cost, low-maintenance strategy, denitrifying bioreactors can be expected to reduce agricultural impact on water resources. More research and design modifications are recommended to address performance during storm events.

- **Denitrifying bioreactors removed on average 7.5 g N per m^3 per day.**
- **Storms caused flow fluctuations and increased the variability in removal rate.**
- **Denitrifying bioreactors have the potential to remove 252,000 kg of nitrate in an average growing season.**

Keywords
Denitrification – Nitrate – Bioreactor – Tile Drainage – Watershed

Introduction

Nonpoint source nutrient pollution is a continuing problem for water quality in the United States, especially in highly agricultural watersheds. Nitrogen (N) is a main component of this due to high levels in fertilizers and manure (Vitousek et al, 1997). Storm-driven runoff and subsurface flow from agricultural fields is commonly high in nitrate (NO3) which leads to eutrophication in downstream freshwater systems (Carpenter et al, 1998). While this NO3 can be reduced through denitrification, the drainage of wetlands, additions of tile drains, and reduction of riparian buffers minimize locations where this can naturally occur in the landscape (Vitousek et al, 1997).

Denitrifying bioreactors have been developed to combat this NO3 problem. Bioreactors consist of pits of saturated woodchips that intercept tile drainage (Schipper et al, 2001). This provides the ideal environment for naturally-occurring denitrifying microbes to thrive and reduce the NO3 in the bioreactor influent to an inert gas. Previous research has shown rates of removal between 2.9 and 7.3 g N m-3 d-1 and reduction to natural concentrations of NO3 in the bioreactor effluent (Addy et al., 2016; Bell et al, 2015).

These studies have relied predominately on discrete grab sampling to develop average reduction rates (Addy et al 2016). However, these systems are continuously flowing and are likely more complex than grab sampling may suggest during certain times, such as storm events (Williams et al., 2015). This is especially relevant given that residence time in the bioreactor is a strong controlling variable and is inversely related to the flow rate (Addy et al 2016). Hydrology of soils with tile drainage can lead to rapidly-changing and highly-variable flow rates and therefore variable residence times that may not be evenly represented with grab samples (Williams et al., 2015).

This study proposed to use automated sampling to facilitate higher resolution of sampling to determine the effects of sampling on calculated removal rates. The purpose was to compare the high-resolution removal rate with grab sample removal rates at the watershed scale. This will help provide more appropriate estimates of the potential NO3 reduction that can be achieved in a watershed with widespread denitrifying bioreactor application.

Results & Discussion

We were able to monitor 18 different periods throughout the growing season of 2015– 13 at the Tompkins County site and 5 at the Chemung County site. While many of the high-resolution sample periods had constant inflow and temperature conditions, several sample periods captured storm events that demonstrated more variable processes in the bioreactors. Figure 1 shows the increased range of removal rates in the bioreactors during storm events, including negative values that indicate the outflow load of NO3 is greater than the inflow. Mean removal rates in the storm events were greater (M=10.9, SD=26.7) than the removal rates during baseflow conditions (M=5.63, SD=4.25), $p<0.05$. A Brown-Forsythe test indicated the variances to be unequal (F=39.3), $p<0.0005$.

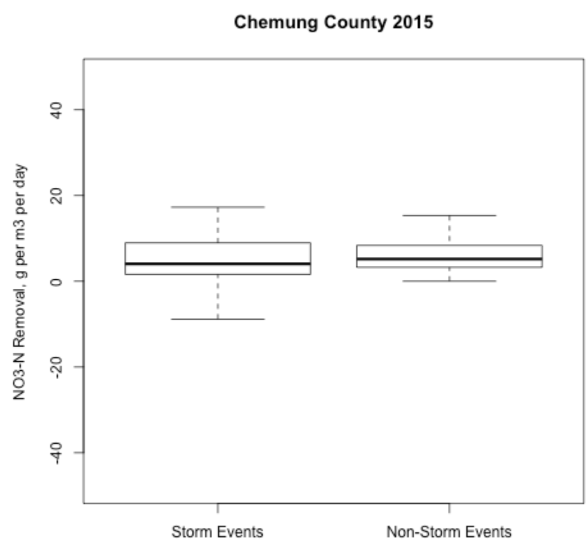

Figure 1: Comparison of removal rate of NO3 in bioreactors at the Chemung County site between storm events and non-storm, baseflow conditions.

With the high frequency sampling, we analyzed individual storm events to determine the cause of the high variability. One particularly interesting event is shown in Figure 2. Prior observations had a similar pattern to this, where the beginning of the storm shows apparently large removal rates followed by a significant drop later in the storm. It is likely that the first portion is due to the flushing of highly reduced water while in the inflow is particularly high. Once the bioreactor has been flushed, the outflow is stormwater that has had minimal time for reduction. The flow variability may disrupt stable community structure or wash away dissolved carbon or biofilms necessary for denitrification. The high flow rates accentuate these changes leading to large swings in removal rate.

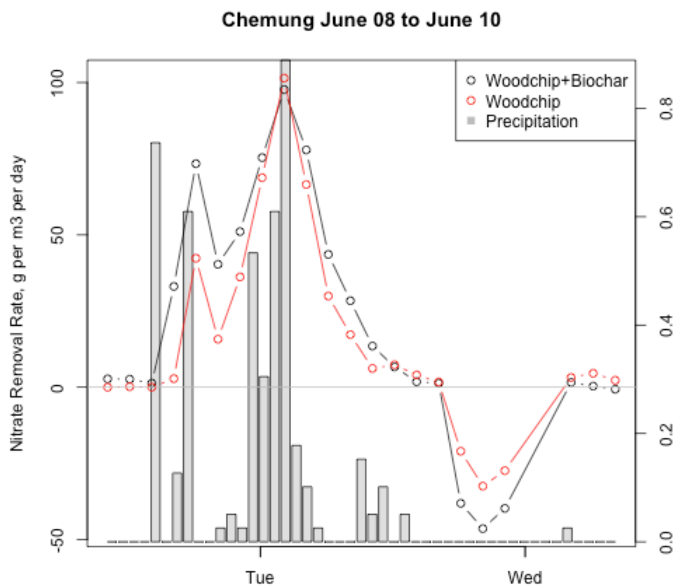

Figure 2: Precipitation data and instantaneous removal rate based on high frequency sampling of a storm event at the Chemung County site.

The potential for nitrate reduction using bioreactors was estimated for the Upper Susquehanna River Bain using the storm and non-storm data collected in this project. With 2% of the watershed area under tile-drainage (Jaynes & James, 1992), bioreactors have the potential of removing 252,000 kg of nitrate from tile-drainage water entering the stream during the average growing season (135 days). On an annual basis, bioreactors placed in all tile-drained fields could remove approximately 4.5% of the streamflow total nitrogen exported from the Upper Susquehanna River Basin (Woodbury, Howard, & Steinhart, 2008).

Policy Implications

The main take-away from this study is that bioreactors are a relatively inexpensive yet highly effective best management practice for reducing n in agricultural tile drainage. Based on these results, New York State should strongly encourage or even begin to require the installation of bioreactors to certain percentages of farmland. Cost-sharing may be possible for watersheds draining into water bodies of concern, such as the Chesapeake and Hudson Bays. This could be even more effective because treating N pollution is more easily achieved closer to the source, when volumes are small, than once it reaches the outlet.

New York State also must soon adopt or adapt the design standards for bioreactors installed on agricultural lands recently published by the NRCS. Given the findings from this study, it is important to include storm conditions in designs. However, the NRCS designs already require design based on a 10-year storm. Our current data does not show whether this is an adequate design

Methods

This project had two major portions: the first was high-frequency field sampling and the second was removal rate and scaling comparisons. Field data was collected at two sites in central New York State. Each site featured paired bioreactors, one of which was filled with woodchips while the other contained woodchips and biochar in a 9:1 ratio. The paired bioreactors used a shared inflow that was diverted into each bioreactor using a control box. Control boxes on at the outlets determined saturated volume and helped determine flow and residence time.

The bioreactors at the Tompkins County site were constructed in October 2012, draining a total of 4 ha of vegetable fields, and were approximately 35 m^3 each. The Chemung County bioreactors were built in June 2013 to treat water draining from 5 ha of dairy farmland, and were about 40 m^3 each.

High-resolution sampling was conducted using three ISCO 3700s placed at the shared inlet and the outlet of each bioreactor. ISCOs collected samples every 30 minute, compositing 4 samples per bottle for a two-hour resolution. This allowed for 48 hours of continuous monitoring. Bottles were pre-acidified and samples were filtered within 72 hours of collection. Samples were analyzed calorimetrically for NO3 and ammonium. Average removal rate was calculated for each outflow sample using the equation:

$$RR = \frac{Q\ (C_{in} - C_{out})}{V}$$

This report was prepared for the New York State Water Resources Institute (WRI) and the Hudson River Estuary program of the New York State Department of Environmental Conservation, with support from the NYS Environmental Protection Fund

where Q is the flow rate, Cin and Cout are the concentrations at the in and out control boxes, and V is the total saturated bioreactor volume.

Outreach Comments

The field site for this research continues to provide an example for Soil and Water Conservation Districts working with the Upper Susquehanna Coalition. Based on this partnership, we continue to construct new research bioreactors in farms throughout the state as demonstrations of an effective treatment strategy.

The video prepared as part of the project will be used as an outreach tool. This video provides basic knowledge of what denitrifying bioreactors do and how they can be used to reduce environmental impact from agriculture. The video targets both conservation groups and farmers to address the broad range of those impacted by excess nutrients in water.

Student Training

Two doctoral students in Water Resources Engineering at Cornell University led the research for this study. Their work involved the idea and method development and the analysis and conclusions. Several undergraduate researchers worked on the project, specifically with sample collection in the field and lab analysis. The undergraduate students were also trained on basic data analysis and cleaning for use in the model portion of the research.

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This report was prepared for the New York State Water Resources Institute (WRI) and the Hudson River Estuary program of the New York State Department of Environmental Conservation, with support from the NYS Environmental Protection Fund

Emerging Organic Pollutants: From College Campuses to Cayuga Lake

Basic Information

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Principal Investigators:	Susan AllenGill, Jose Lozano

Publications

1. Allen-Gil, Susan; Jose Lozano, Emerging Organic Pollutants: From College Campuses to Cayuga Lake, Finger Lakes Research Conference, November 12, 2015. Hobart and William Smith Colleges.
2. Finegan, Matthew P.; Caitlyn E. Patullo, Curt A. McConnell, and Susan Allen-Gil, Effects of (R+) Limonene on Fathead Minnow Swimming Behavior. Rochester Academy of Sciences. Finger Lakes Community College, November 7, 2015.
3. Finegan, Matthew P. and Susan Allen-Gil, Effects of Emerging Contaminant Exposure on Fathead Minnow (Pimephales promelas) Predator Avoidance, National Council for Undergraduate Research, Ashville, NC, April 7-9, 2016.
4. Patullo, Caitlyn E. and Susan Allen-Gil, The Effects Of Carbamazepine And Caffeine On Juvenile Fathead Minnow Swimming Behavior, National Council for Undergraduate Research, Ashville, NC, April 7-9, 2016.
5. Schmidlin, Sarah; Megan Archino; Susan Allen-Gil, Food Chain Analysis of Microbeads on Daphnia magna and Fathead Minnows (Pimephales promelas), National Council for Undergraduate Research, Ashville, NC, April 7-9, 2016.
6. Sarah Schmidlin and Megan Archino, Food Chain Analysis of Microbeads on Daphnia magna and Fathead Minnows (Pimephales promelas), 19th Annual James J. Whalen Symposium, Ithaca College, April 14, 2016.

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Emerging Pollutants: From College Campuses to Cayuga Lake

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and
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Abstract

We investigated the concentrations of over 200 chemicals in the Ithaca water system, including many emerging pollutants of concern. 24-hr composite samples were collected 4 times from 5 different locations (raw drinking water, treated drinking water, wastewater influent, wastewater effluent, and Cayuga Lake). We detected many compounds at all points in the water system in varying concentrations. Pharmaceutical and personal care compounds detected most frequently and in the highest concentrations include caffeine, nicotine, metformin, atrazine, and carbamazepine. Microplastics were also detected in lake samples. Based on the influent data, there was no clear difference in concentrations when college students were in town. Ecological investigations suggest that caffeine can alter fish swimming behavior, but only at higher concentrations than those observed in Cayuga Lake. Pilot studies also suggest that small microplastics (5 µm diameter) may cause increased mortality in *Daphnia magna*.

Three Summary Points of Interest

- **Fall Creek raw drinking water has detectable concentrations of many emerging pollutants, but most are removed by the treatment process.**
- **Emerging pollutants vary in the extent to which they are degraded or removed as biosolids by the wastewater treatment process.**
- **Caffeine, carbamazepine, and microplastics have the capacity to affect aquatic organisms, but not at the concentrations currently reported for Cayuga Lake,**

Keywords: emerging pollutants, wastewater, fish behavior, pharmaceuticals

Introduction

The increased stress of college life has led to a surge in medications to prevent depression and improve performance. The rate of depression is higher among college students than in the general population. According to the National Center on Addiction and Substance Abuse, over half of students in the United States reported some depression since entering college (Aselton 2010). In several recent studies, over a third of college students reported taking prescribed antidepressants (Green 2013; Cherednichenko 2007; Kalenkiewicz 2013). Additionally, at many Canadian universities, the use of anti-depressant drugs now exceeds that of birth control pills (no author listed, Antidepressant Use on Rise at Canadian Universities, 2012).

In recent years, there have been various reports of increased use of ADHD medication by college students both licitly and illicitly (Weyandt and DuPaul 2008). In a large online survey of college students at a midwestern university, 8.3% reported using prescribed stimulants illicitly (Teter et al. 2006). At the University of Wisconsin – Eau Claire, 11% of female and 17% of male students self-reported illicit use of prescribed stimulant medication (Hall et al. 2005). In a more recent informal study at Kenyon College, 11% of students reported being prescribed ADHD medications (Green 2013). Illicit use of ADHD medications can be even greater than the prescribed amount, as college students have been found to share prescribed medication with their peers (Green 2013).

Additionally, synthetic estrogens are widely prescribed among the college population. According to the American College Health Association 2011 survey, more than 60% of sexually active college students rely on synthetic hormones in the form of birth control pills, implants, or patches for contraception (American College Health Association 2012). An estimated 40% of the synthetic estrogens in birth control pills are excreted into the sewage influent (Johnson and Williams 2004).

Our recent research indicates that two college campuses within the city of Ithaca potentially add a significant burden of these compounds to the sewage influent. In the 2011-2012 academic year, Cornell University's Health Center wrote more than 3500 prescriptions for birth control pills, 800 prescriptions for ADHD medications and 1500 prescriptions for anti-depressants (data not published). During the same year, Ithaca College's Health Center wrote 800 prescriptions for ADHD drugs and 500 for anti-depressants (data not published). Using published excretion rates, we estimate that a minimum of 494g of ADHD and 2084g of anti-depression medications and metabolites were delivered to the IAWWTF in this year alone from the two campuses (data not published).

National and international studies have documented the presence of these three classes of pharmaceuticals, as well as many other personal care products in sewage treatment systems (Chen et al. 2013; Lindberg et al. 2014; McClellan and Halden 2010). Yet, as emerging pollutants, there are little to no requirements regarding monitoring concentrations in effluent or discharge of these compounds to the environment. To date, investigations have been largely voluntary by waste water treatment facilities, or national sampling programs.

Thus, our objectives were to:

1. Identify emerging pollutants present at various points in the wastewater stream to determine the:
    - source of emerging pollutants (drinking water vs. influent)
    - ability of the wastewater treatment system to degrade emerging pollutants, and

- the fraction of emerging pollutants that occur in the biosolids and how this might influence land application disposal processes.

2. Evaluate the potential for sublethal toxicity in fish to select emerging pollutants at environmentally relevant concentrations.

3. Share our results with the local community (with a focus on high school students), the association of wastewater treatment facilities, and organizations focused on the protection of surface waters.

Results & Discussion

Our results for each of these objectives are summarized below.

1. Identify Emerging Pollutants and Their Fate

We were successful in collecting and analyzing over 20 samples for emerging pollutant analysis from five locations (Fig.1). Since each sample was analyzed for over 200 compounds:

- 20 hormones, using method SH2434
- 99 pharmaceuticals, using method LC8144
- 48 additional pharmaceuticals compounds, using method SH2440
- 60 common waste water compounds, using method SH1433

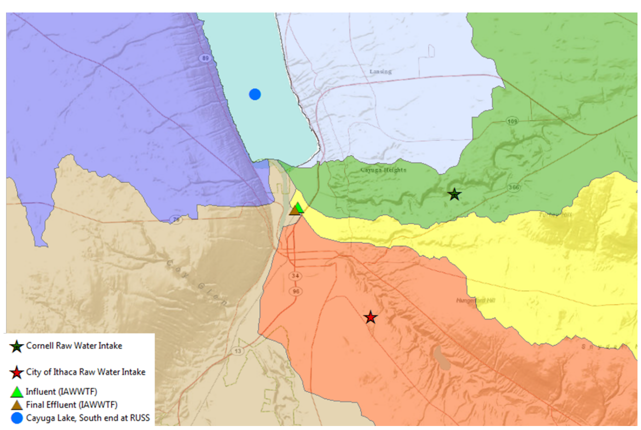


Figure 1. Sampling sites in the Southern section of the Cayuga Lake Watershed.

Although there is some overlap of compounds among methods, this sampling effort has generated over 4,000 data points. Clearly, this is too much to digest yet and present in this final report, but we have been able to conditionally answer some of our objectives. A few highlights include:

a. Some pollutants are present in the raw water that enters the drinking water treatment facilities. There are more compounds at higher concentrations in the Cornell Drinking Water Plant than Ithaca's Six Mile Creek Drinking Water Treatment Facility (Fig. 1 and 2)

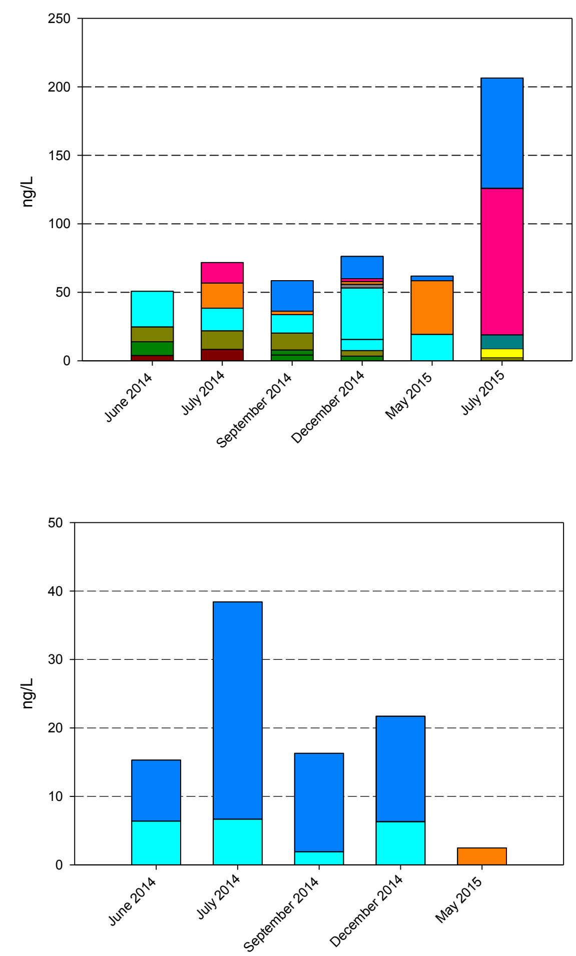


Figure 2. Emerging pollutants in raw water at Cornell Drinking Water (top) and Ithaca's Drinking Water Plant (bottom). The colors of the bars designate different classes of compounds as detected by the SH 2440 method. Note the change in the scale of the y-axis.

At the Cornell Drinking Water Plant, many of the compounds are removed by the treatment process, and only atrazine (30 ng/L) was detected by the SH2440 method.

b. As expected the concentrations of compounds in the influent was much higher than the drinking water, suggesting that human use adds to the pollutant loading to the wastewater treatment plant.

c. The ability of the waste water treatment plant to remove emerging pollutants is largely dependent on the type of compound. Lipophilic compounds tend to partition with the biosolids, where hydrophilic compounds are more likely to be present in the effluent.

d. Although the data is very limited, there is not strong evidence that the influent profile changes dramatically when students are present in Ithaca (September) compared to when they are not (June/July), with the possible exception of amphetamines. (Fig. 3)

2. Evaluate the potential for sublethal toxicity in fish to select emerging pollutants at environmentally relevant concentrations.

We tested carbamazepine and caffeine for their effects on fish using three different experimental protocols.

In the first protocol, fish were videotaped for 1 minute before and after 30 min. exposure. Videotapes were analyzed using Swistrack for swimming patterns, and total distance travelled. Results are shown in Fig. 4. Carbamazepine-exposed fish showed a tendency to swim less, but the fish-to-fish variability and the small sample size prohibit conclusive statement. No discernable pattern was found for caffeine. We are planning to run more experiments, and use more sophisticated software (Ethovsion).

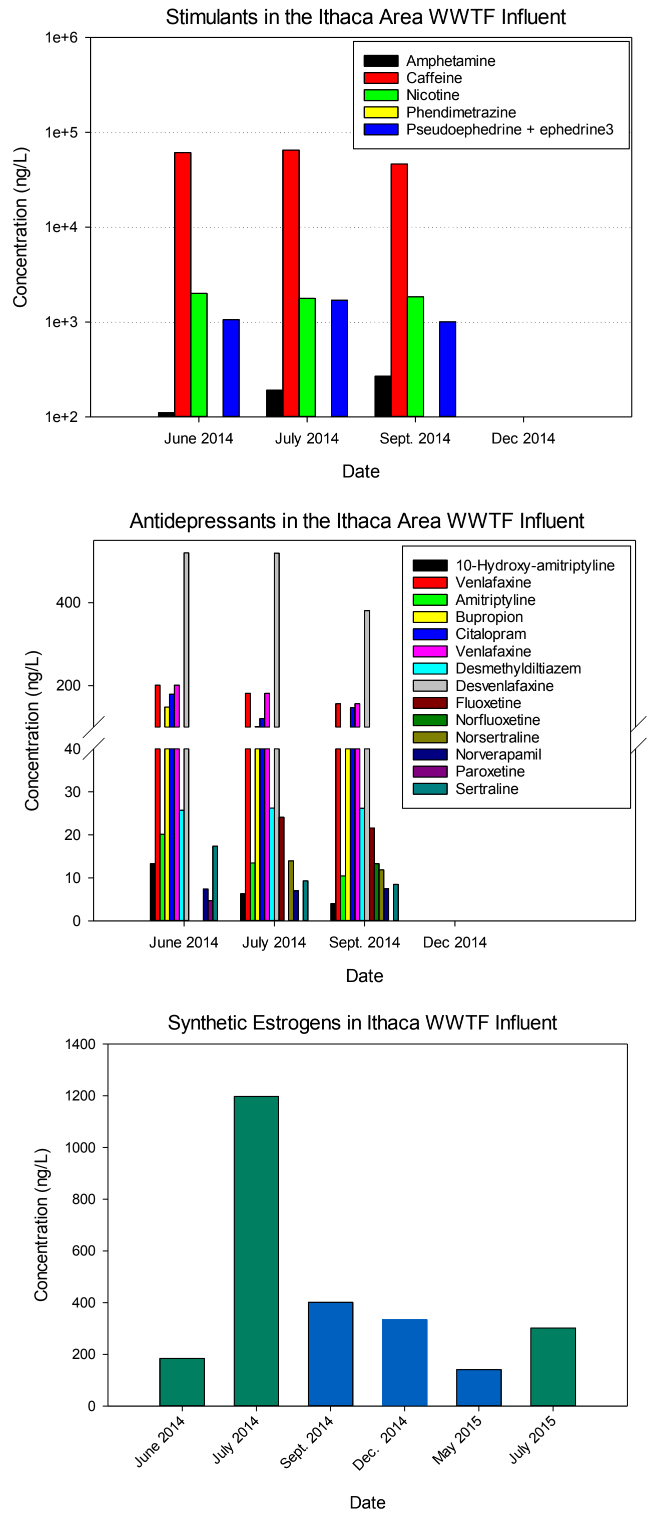


Figure 3. Emerging pollutants in influent by month, shown by class of compounds. There is little indication that concentrations fluctuate when students are present or absent from the community.

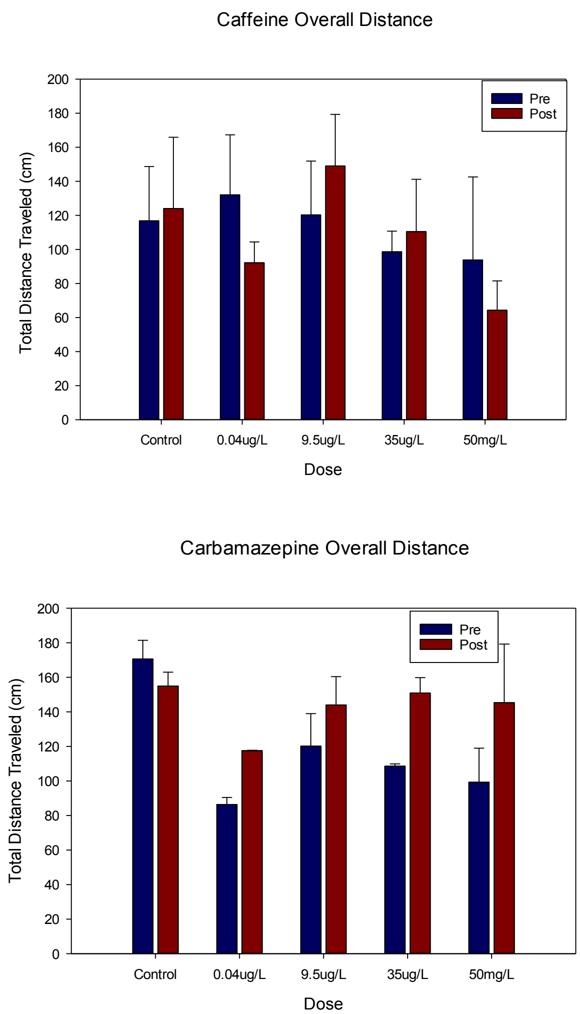


Figure 4. The effect of carbamazepine and caffeine on swimming distance of fathead minnows.

In the second protocol, exposed fish were compared to controls (no pollutant) in terms of their ability to seek shelter at the ends of 1 m racetracks (PVC pipe cut longitudinally).Preliminary data suggest that carbamazepine exposure may have decreased the anxiety of fish such that they spent more time out in the open, whereas caffeine exposure may have had the opposite exposure at environmentally relevant doses of 0.04 and 9.5 µg/l. (Fig. 5)

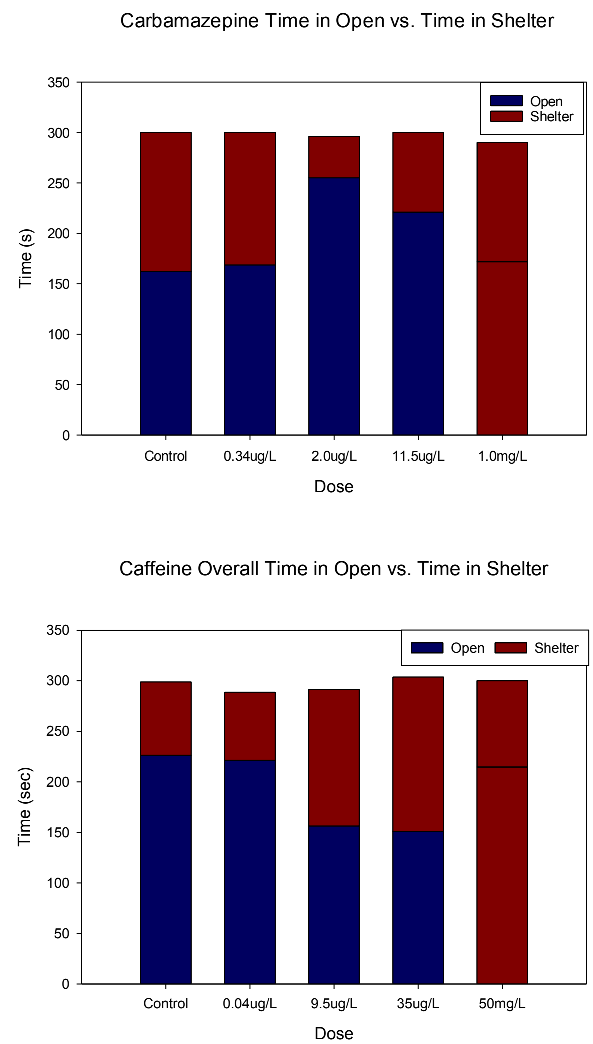
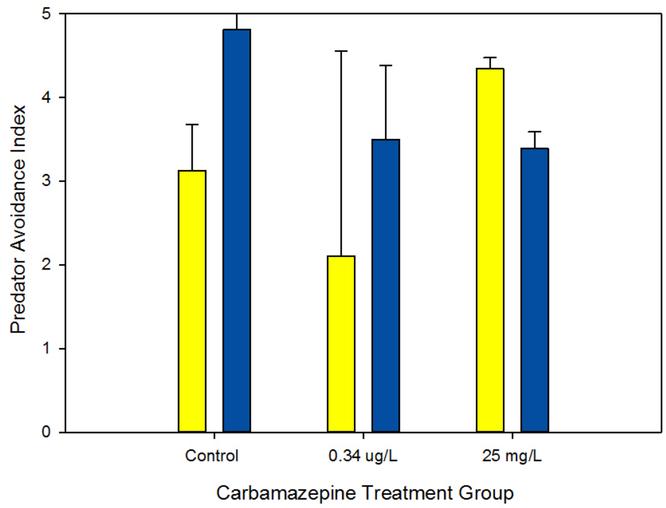


Figure 5. The effect of carbamazepine and caffeine on predator avoidance swimming behavior of fathead minnows.

In the third protocol, prey fish (fathead minnows) were placed in a large tank with a predator (largemouth bass) after being pre-conditioned with an alarm pheromone and then exposed to a chemical. To analyze the data, both the sheltering and shoaling indices were combined to create a predator avoidance index. This number represents the average number of minnows displaying classic predator avoidance behaviors. Both the control group and the environmentally relevant treatments had a significant difference between the pre-pheromone introduction and pheromone exposed periods. The 50 mg/L group did not have a significant difference between pre-pheromone and pheromone exposed treatments; however the error on the pheromone group is large. This suggests that at high doses of caffeine, fish may no longer respond to the alarm pheromone. (Fig. 6)



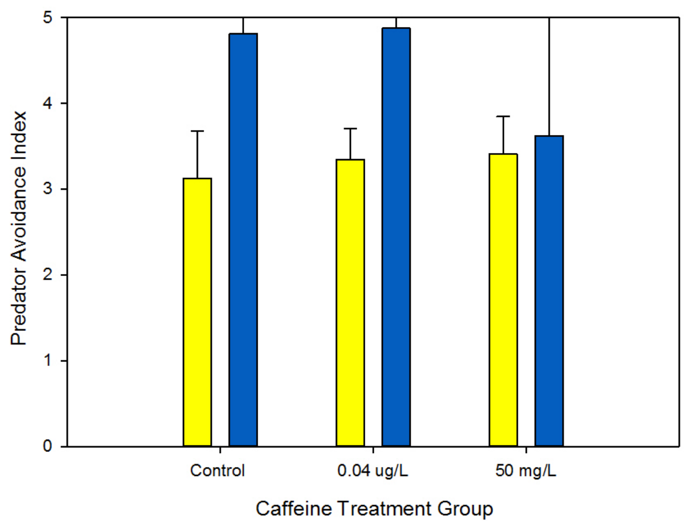


Figure 6. The effect of carbamazepine and caffeine on the ability of fathead minnows to seek shelter.

Another path of research that was not in the original proposal was the investigation of the presence of microplastics in Cayuga Lake and the potential ecological effects. We took several vertical grabs using a 250 µm net from the edge of the southern shelf. We ran the content through a series of sieves and analyzed the contents for microplastics using fluorescent microscopy. Although we did not quantify the microplastics, we did document their presence (Fig. 7). We hope to expand this work in 2016 by conducting a more systematic lake survey and looking at biosequestration in filter feeders such as zebra mussels.

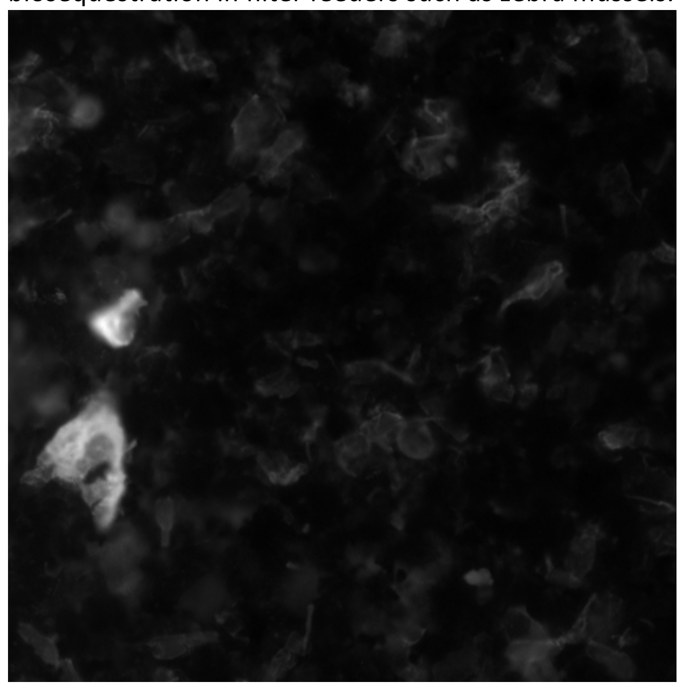


Figure 7. Microplastics detected from filitered and digested water samples from Cayuga Lake in September 2015.

We also conducted an experiment in which we placed microbeads (fluorescing and those extracted from Neutrogena) in Syracuse dishes containing 20 daphnia for four days. We found that microbeads accumulated in the gut of daphnia (Fig. 8) and that there was a statistically significant ($P<0.001$) higher mortality among daphnia exposed to the Neutrogena beads than the 1 µm fluorescing beads (Fig 9). We hope to expand our investigations of the effects of microbeads on freshwater daphnia more in the upcoming year.

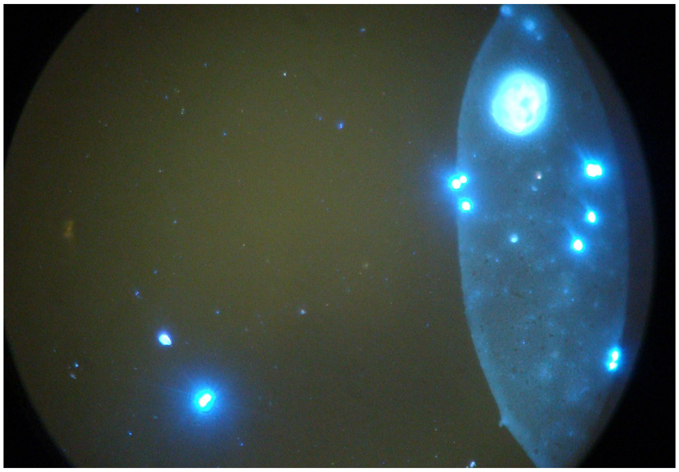


Figure 8. Fluorescing microbeads detected within the gut of daphnia after 4 days of exposure.

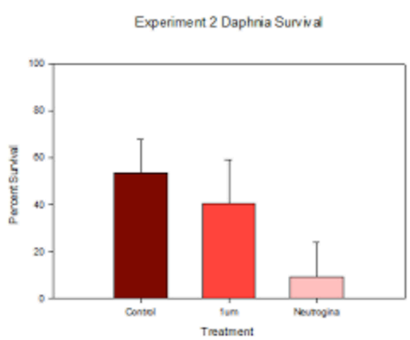


Figure 9. Daphnia survival after exposure to microbeads for 4 days.

3.Share our results with the local community (with a focus on high school students), the association of wastewater treatment facilities, and organizations focused on the protection of surface waters.

As we worked on creating an effective strategy for not only educating but hopefully engaging the student community, we met with roughly 100 students, asking them to complete a survey before we made any presentation, and using their responses and our presentation as the basis for "focus group" types of discussion.

- 40 upper-middle school from EA Clune Montessori (functioning like HS students)
- 8 high school students from New Roots
- About 50 Ithaca College Env. Studies students.

Perceptions:

Prior to introducing "emerging contaminants" as the point of our session, we had kids complete the survey. One of the first questions was, "In FIVE words, how would you describe the following collection of materials: "*Anti-bacterial Soap, Bug-spray, Adderall, Caffeine, Cold Medicine, Weed Killer".* Notably, we opted to stick with household products for this question. The word clouds below sum up the responses, and suggest a good deal (Fig. 8)

- Overwhelmingly negative words, peppered with words like "helpful" and "everyday".
- Although these words are overwhelmingly negative, when the students were next asked to provide an "environmental problem having to do with water" that they had heard of, there was only one response (pharmaceutical pollution) out of 60 that referenced consumer products, and perhaps 2 or 3 more that might have been associated with the broader range of "emerging contaminants".
- Basically, the story is "we all have heard this is bad stuff, but we're actually hard-pressed to tell you why." There is a disconnect that is going to require a marketing approach to overcome.

Figure 10. Word cloud illustrating the associations of emerging pollutants with certain concepts. The size of the font indicates the frequency of response.

Next, we looked for insights on how emerging contaminants compared to other issues. The prompts and responses are listed below.

Table 1: Number of students responding how much various environmental problems might affect them.

	A lot	A Little	Not at all
Climate Change	73	11	2
Emerging Contaminants in water	67	19	2
New Diseases	51	30	8
Water Shortages	52	23	12
Oil/Chemical Spill	46	37	5
Algal Blooms	28	44	14
Invasive Species	24	55	8

Table 2: Number of students responding how much their actions might affect various environmental problems.

	A Lot	A Little	None
Climate Change	29	54	4
Emerging Contaminants in water	25	48	14
New Diseases	4	40	40
Water Shortages	19	50	16
Oil/Chemical Spill	9	52	26
Algal Blooms	16	37	30
Invasive Species	16	44	27

- Based on about 90 survey responses, emerging contaminants ranked 2nd behind climate change when students ranked what issues might affect them the most. It also ranked 2nd behind climate change in terms of where their actions might have an impact.
- College students were significantly more optimistic about their chances of impacting global issues, overall.

<u>Solutions & Reactions</u>:
After the survey, and slide presentation, we discussed the issue with students, and asked them to think about how they would "pitch" the concept of emerging contaminants to the public in order to engage them. In no particularly order, some outcomes/suggestions included:

- We ended up talking a good deal more about personal-use products, as opposed to lumping in agricultural pesticides, institutional/hospital stuff, etc. It seemed more conceptually manageable for those on the receiving end of presentations. Also, the idea that "this problem is larger than anything I can address" seems like a good sentiment to avoid. It took a marketing suggestion to alliterate- this was the source of "personal pollutants" as a possible term.
- It was also suggested that more outreach to campus communities is needed to make sure students know how and where to dispose of products they are using. A number of students noted that they had not been aware of the issue of medications in water, or assumed that WWTPs fully addressed them. They were concerned that they might be part of the problem, and open to action

- While this is a global problem, it can also be scaled to a local water source, which gives us an opportunity to stress the impact of personal choices.
- Rather than striving to define or label the set of contaminants, perhaps the more important concept to pitch to the public is to view themselves as part of a process- part of the drinking water-water source-water pollution cycle. You are simultaneously a filtration plant and a polluter, not just an observer.
- The fact that clearly defined events that can be cleanly traced back to emerging contaminants and fit into normal news cycles are sort of few and far-between is a problem. (illustrated by the lack of examples provided by students.) This is a "tip of the iceberg"/emerging issue, similar to climate change in many ways - we are concerned about what we suspect is going on, but don't have the complete picture nailed down yet. This is a challenge when it comes to recommending action. The products we use all serve a purpose, after all. Alternatives may be inconvenient or expensive.

Policy Implications

The Endocrine Disruptors/Persistent Organics, ED/PO, research results have had an impact on the programs of several local agencies, even though there are no current federal, state, or local, regulations, or guidelines, for ED/POs testing, use, or to limiting their release to the environment.

The Cayuga Lake Watershed Network, the Water Resources Council, the Environmental Management Council, and the Ithaca Area Wastewater Treatment Facility have adopted plans and initiatives to address the ED/PO concerns.

The results of this research have shown that our backyard is not free from ED/POs and that we should increase our research efforts, outreach, and avoidance to using these compounds as much as possible.

The Ithaca Area Wastewater Treatment Facility has increased its process management practices to improve their removal efficiency as much as possible, using its current technology and to potentially incorporate new technology that eliminates the release of ED/POs into the environment.

The technology exists to eliminate ED/POs during wastewater treatment, and this project may have helped to show that the investment on technology and research is advisable.

Methods

This project had two distinct components: contaminant analysis and toxicity testing. The contaminant analysis was a continuation of a collaboration involving Ithaca College (Susan Allen-Gil), IAWWTF (Jose Lozano), Cornell University (Damian Helbling), and the United States Geological Survey (USGS-Albany: Pat Phillips). We collected samples four times a year from six locations throughout the local watershed. Each sample was collected using automated ISCO samplers using methanol-rinsed component of glass, teflon, or stainless steel. 24-hr time-weighted composite samples (and biosolid samples) were sent to the National Water Quality Laboratory (NWQL) in Denver for three different methods: hormone, pharmaceutical, and wastewater, through an agreement between IAWWTF and USGS. We chose this approach because of the extensive experience of NWQL in analyzing WWTP samples for emerging pollutants nationally. In 2014 and 2015, we collected samples in May/June, July, September and December.

The toxicity testing was performed by Dr. Allen-Gil and undergraduate students at Ithaca College. Using the protocol we have developed that allows us to analyze behavior using Swistrak, a video imaging and analysis software program that tracks multiple fish concurrently and calculate distance moved over time. Using caffeine and nicotine as model compounds, we are currently experimenting with circular vs. linear set-ups, and with different exposure durations to determine the system that provides the most consistent results. The experiments will be conducted at Ithaca College in a temperature and light-controlled growth room during the summer of 2015, and 2015-2016 academic year. Animal Care and Use permission is renewed annually. We plan to target emerging pollutants that occur in effluent and lake samples that are suspected to have neurological effects.

Outreach Efforts

Completed Outreach:
IC TV news brief: by Kyle Stewart, Feb 12, 2016
https://www.youtube.com/watch?v=SizF3p5FgbQ

Cornell Cooperative Extension webpage on project:
(http://ccetompkins.org/environment/water/emerging-contaminants-pharmaceuticals-and-personal-care-products)

Are pharmaceuticals contaminating Ithaca's treated water? Ithaca Voice, Feb. 3, 2016
http://ithacavoice.com/2016/02/37755/
(submitted by Sharon Anderson, CCE)

S. Allen-Gil, and Jose Lozano, Emerging Organic Pollutants: From College Campuses to Cayuga Lake, Finger Lakes Research Conference, November 12, 2015. Hobart and William Smith Colleges (poster).

S. Allen-Gil, What is in Cayuga Lake? Wells College Sustainability Series Presentation Feb 22, 2016.

S Allen-Gil and M. Finegan. NYSFOLA conference, Hamilton, NY. April 29, 2016

Lozano, J. Tompkins County Water Resources Council. Ithaca NY, April 18, 2016.

Upcoming Outreach:
Lozano, J. New England Water Environment Association (NEWEA) and New York Water Environment Association (NYWEA), June 6-8, 2016. Groton, CT.

State of the Watershed Boat Tour: June 21, 5:30 – 7:30 pm. We are inviting representatives from the County Legislator, the Water Resources Council, the City of Ithaca, and local schools.

Student Training
1 undergraduate student for 10 weeks in the summer
7 undergraduate students involved in Fall 2015 ad Spring 2016 semesters.

Student presentations: * denotes undergraduate
Matthew P. Finegan*, Caitlyn E. Patullo*, Curt A. McConnell*, and Susan Allen-Gil. Effects of (R+) Limonene on Fathead Minnow Swimming Behavior. Rochester Academy of Sciences. Finger Lakes Community College (poster), (November 7, 2015).

Matthew P. Finegan*, and Susan Allen-Gil. Effects of Emerging Contaminant Exposure on Fathead Minnow (*Pimephales promelas*) Predator Avoidance. National Council for Undergraduate Research, Ashville, NC, (April 7-9, 2016)

Caitlyn E. Patullo*, Susan Allen-Gil. The Effects Of Carbamazepine And Caffeine On Juvenile Fathead Minnow Swimming Behavior, National Council for Undergraduate Research, Ashville, NC, (April 7-9, 2016).

Sarah Schmidlin*, Megan Archino*, Susan Allen-Gil Food Chain Analysis of Microbeads on Daphnia magna and Fathead Minnows (*Pimephales promelas*), National Council for Undergraduate Research, Ashville, NC, (April 7-9, 2016).

Ryan Cummins*. Getting Out What We Put In: Removal Efficiency of Pharmaceuticals at Ithaca Area Wastewater Treatment Facility, 19th Annual James J. Whalen Symposium, Ithaca College (April 14, 2016).

Nicole Pouy*. Largemouth vs Fathead: Effects of Pharmaceuticals and Domestic Products on Predator Avoidance. 19th Annual James J. Whalen Symposium, Ithaca College (April 14, 2016).

Sarah Schmidlin*, and Megan Archino*. Food Chain Analysis of Microbeads on Daphnia magna and Fathead Minnows (*Pimephales promelas*), 19th Annual James J. Whalen Symposium, Ithaca College (April 14, 2016), poster.

Additional final reports related to water resource infrastructure research are available at
http://wri.cals.cornell.edu/news/research-reports

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Western New York Watershed Network

Basic Information

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End Date:	2/28/2016
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Research Category:	Climate and Hydrologic Processes
Focus Category:	Water Quality, Education, Hydrology
Descriptors:	None
Principal Investigators:	Chris S Lowry

Publications

1. Coburn, J. E., Vitali, J. M., Glose, T. J., Lowry, C. S., "Analyzing Water Quality Over Variable Flow Conditions in Rural and Suburban Streams." A Showcase of Undergraduate Research in Hydrogeology Poster Session, 2015 Geological Society of America Annual Meeting, Baltimore, MD (November 2015).
2. Crumlish, J.C., Pereira dos Santos, L. R., Glose, T. J., Lowry, C. S., "Evaluating the Impact of Hydrology and Combined-Sewer Overflows on Urban Beach Closures." A Showcase of Undergraduate Research in Hydrogeology Poster Session, 2015 Geological Society of America Annual Meeting, Baltimore, MD (November 2015).
3. Luh, N. M., Ewanic, J., Pereira dos Santos, L. R., Glose, T. J., Lowry, C. S., "Relationship Between Stream Stage and Discharge on Major Tributaries that Enter Lake Erie." A Showcase of Undergraduate Research in Hydrogeology Poster Session, 2015 Geological Society of America Annual Meeting, Baltimore, MD (November 2015).
4. Tuttle, C.T., Crumlish, J.C., Canty, M. T., Glose, T. J., Lowry, C. S., "Analyzing Daily Variability in E coli Concentrations in an Urban Stream." A Showcase of Undergraduate Research in Hydrogeology Poster Session, 2015 Geological Society of America Annual Meeting, Baltimore, MD (November 2015).

Final Report: Western New York Watershed Network
Department of Geology
The State University of New York at Buffalo

Introduction

Western New York lacks key infrastructure to monitor even the simplest distributed hydrologic parameters such as stream stage, stream discharge, and basic water quality. When water quality or quantity issues arise, the response is typically one of a reactive nature. Often problems go unnoticed or develop so quickly that significant damage has occurred before remediation efforts can be designed. The Western New York Watershed Network is a first step in designing and implementing a student and citizen scientist-run hydrologic monitoring system to support water resource management in Western New York. This is an initial step in a collaborative project that brings together local (Buffalo Niagara Riverkeeper, Erie County Department of Environmental Health, Erie County Department of Parks, Recreation, and Forestry, Seven Seas Sailing Club, and Tifft Nature Preserve) and national (Community Collaborative Rain, Hail, and Snow Network, CrowdHydrology, and National Geographic Education's FieldScope) organizations to develop a robust watershed network focused on providing baseline data and addressing key research objectives in Western New York. This report will discuss the work done during the first year of the Western New York Watershed Network and highlight future opportunities.

Current Network

The 2015 network monitoring efforts focused on 8 streams within Erie County, New York. These streams were monitored weekly by a group of University students and citizen scientists from May through August 2015 for both water quantity and quality parameters. Streams were monitored for stream stage, discharge, and basic water chemistry. Stream stage was recorded at existing USGS gage stations with additional gage stations installed in ungagged streams using pressure transducers. Stream discharge was measured on a weekly basis. Field measurements of basic water quality and *E. coli* samples were collected once a week. Field measurements of water quality include pH, dissolved oxygen, specific conductance, and temperature, which collected using a YSI Professional Plus multimeter. In addition, Tonawanda and Scajaquada Creeks were sampled for both Nitrogen and Phosphorus as part of a collaborative project within the Department of Geology at the University at Buffalo. Data has been uploaded to National Geographic Education's FieldScope database for public viewing and to the University at Buffalo Library System Institutional Repository.

Objectives

The objective for this project was to establish an open source network that provides information regarding water quantity and quality to the public, while educating undergraduates on hydrological research methods. During the summer of 2015, undergraduates were grouped into five teams in order to address the following research objectives:

1.) Develop a correlation between precipitation, stream discharge, and beach closures (Team Beach Closures)
2.) Determine the relationship between stream stage and discharge on major tributaries that enter Lake Erie (Team Rating Curve)
3.) Quantify residence time of E. Coli in urban streams (Team E. Coli)
4.) Evaluate spatial changes in temperature, conductivity, pH, and dissolved oxygen on major tributaries that enter Lake Erie Basin as a result of the impact of urban versus rural land cover (Team Stream Quality)
5.) Analyze the relationships between meteoric events and water clarity, temperature, and pH in Lake Erie (Team Turbidity)

Personnel Involved:

Dr. Christopher S. Lowry, Assistant Professor of Geology, Department of Geology, University at Buffalo, 126 Cooke Hall, Buffalo, NY 14260, email: cslowry@buffalo.edu;
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Students:

1. Thomas Glose, Ph.D. candidate, Geology, University at Buffalo
2. Luiz Rafael Pereira dos Santos, Bachelor of Science candidate, Geology, North Dakota State University
3. Julianna Crumlish, Bachelor of Science candidate, Environmental Geosciences, University at Buffalo
4. Caroline Tuttle, Bachelor of Arts candidate, Environmental Studies, Skidmore College
5. Michael Canty, Bachelor of Science candidate, Industrial and Systems Engineering, University at Buffalo
6. Jessica Ewanic, Bachelor of Science candidate, Geology, University at Buffalo
7. Nicholas Luh, Bachelor of Science candidate, Environmental Geosciences, University at Buffalo
8. Jonathan Vitali, Bachelor of Science candidate, Geology, University at Buffalo
9. James Coburn, Bachelor of Science candidate, Environmental Geosciences, University at Buffalo
10. Olivia Patick, Bachelor of Science candidate, Geology, University at Buffalo
11. Rebecca Dickman, Bachelor of Science candidate, Environmental Geosciences, University at Buffalo

Work Overview

The Western New York Watershed Network field research focused on six ungaged streams within Erie County New York (Figure 1). These streams represent a mix between urban (Scajaquada and Rush Creeks) and rural (Eighteen Mile, Big Sister, Delaware, and Muddy Creeks) dominated land covers. Several streams were identified prior to this work as having significant water quality issues due to combined sewer overflows (Scajaquada, Rush, and Big Sister Creeks). In addition to the six ungaged stream, two USGS gaged streams were also monitored as part of this study (Tonawanda and Ellicott Creeks). Stream monitoring was conducted from May 2015 through August 2015. The research team was divided into five working groups focused on specific objectives relating to hydrologic problems and results are described below.

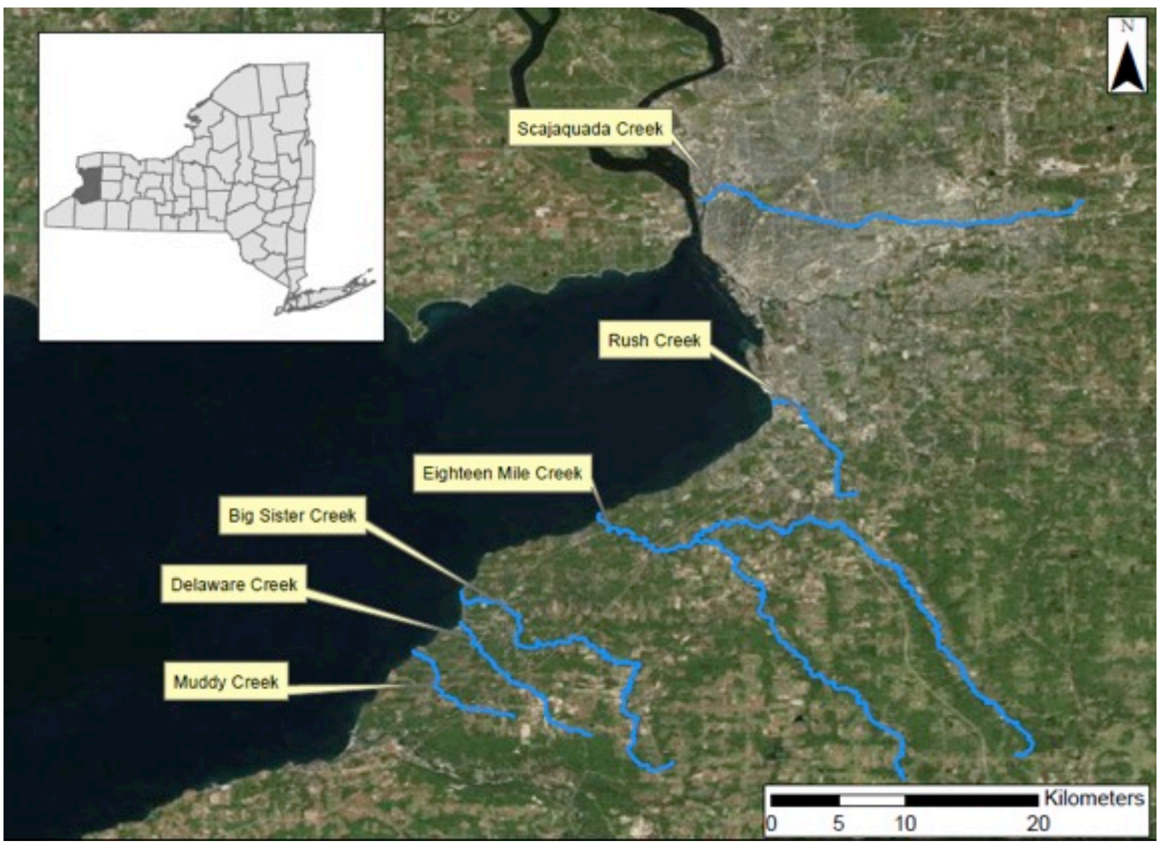

Figure 1: Location of study sties focusing on ungaged streams in Erie County NY.

Summary of Results, 2015

Team 1: Beach Closures

Julianna Crumlish, Bachelor of Science candidate, Environmental Geosciences, University at Buffalo
Luiz Rafael Pereira dos Santos, Bachelor of Science candidate, Geology, North Dakota State University

Objective: Develop a correlation between precipitation, stream discharge, and beach closures.

Description: Beach closures due to high *E. coli* levels are a frequent occurrence at Woodlawn Beach, a state park seven miles south of Buffalo, NY. One factor contributing to closures is the combined sewer infrastructure, an outdated type of sewer system where sanitary and storm sewers share a single pipe to the treatment facility. During high precipitation events, the sewer capacity is sometimes exceeded, and the overflow is discharged into area waterways, allowing for sanitary waste to be introduced into the environment.

With this project, our goal was to evaluate the correlation between precipitation, stream discharge, and beach closures. In particular, we wanted to see what role Rush Creek, a nearby tributary, plays in water quality issues and beach closures at Woodlawn Beach (Figure 2).

Figure 2: Woodlawn Beach site showing study stations, bathing area, and nearby Southtowns Advanced Wastewater Treatment Facility. Stream gaging was conducted at Rush Creek upstream of the outlet. Water quality and *E. coli* testing was conducted in Lake Erie, Rush Creek, Blasdell Creek, and the combined outlet of the two creeks.

Two groups of data were used for this study: data obtained from publicly available sources and data gathered in the field directly. Weather Underground's Woodlawn station provided local precipitation data while information on beach closures and *E. coli* levels was obtained through the New York State Department of Parks, Recreation, and Historic Preservation. Weekly stream gaging at Rush Creek was collected by our team using a SonTek acoustic Doppler flowmeter (Figure 3-A) during the months of June and July 2015.

Weekly water quality monitoring was conducted in Rush Creek, Blasdell Creek, their combined outlet, and Lake Erie (Figure 2). A YSI Professional Plus multimeter was used to measure temperature, pH, and specific conductance (Figure 3-B). Weekly *E. coli* samples were taken at each of the four water quality-monitoring sites (Figure 3-C). Samples were processed using the IDEXX Colilert Quanti-Tray System, which calculates the most probable number of viable cells per 100 mL (Figure 3-D). Due to generally high bacteria counts, samples were diluted 1:10 with deionized water.

Figure 3: (A) Flowtracking in Rush Creek. (B) Using the YSI to measure water quality in Lake Erie. (C) Taking an *E. coli* sample at the outlet of Blasdell and Rush Creeks. (D) *E. coli* samples after being processed and incubated for 24 hours. Yellow cells indicate coliform bacteria; fluorescent cells indicate *E. coli*.

Results: Variation in its volumetric flow rate did not have a clear correlation with Woodlawn Beach bacteria levels. Overall, Rush Creek had higher *E. coli* levels than Blasdell Creek during this study, and peaks in Rush Creek correlated with peaks at the beach (Figure 4).

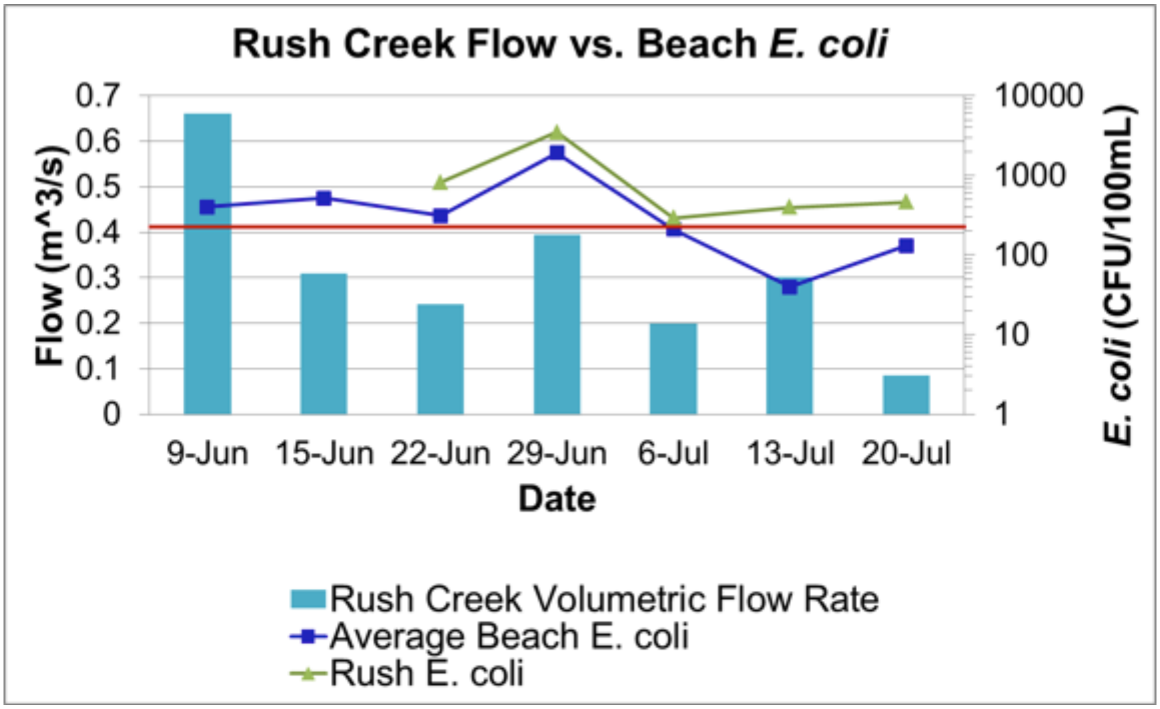

Figure 4: There was not a clear relationship between Rush Creek's discharge and beach *E. coli* concentrations. Flow overall was low during the study period, which may be a factor. At times a backwater effect was noted at the stream gaging location. Bacteria levels in Rush Creek and Lake Erie tend to rise and fall together.

Despite their close proximity, Rush Creek and Blasdell Creek are distinct in terms of their pH, specific conductivity, and the *E. coli* levels that they carry to Lake Erie (Figures 5-7). The fact that Rush and Blasdell Creeks enter the lake through the same outlet allows for mixing; the outlet zone therefore contains water that is a blend between the characteristics of the two individual creeks. This indicates that the two creeks need to be viewed as a system, rather than two separate point sources, when evaluating their impact on Woodlawn Beach water quality. These data show considerable differences in water chemistry and *E. coli* levels in Lake Erie compared to the creeks, suggesting dilution. These data also showed both peaks in *E. coli* following storm events as well as several anomalies where E. coli peaks without large precipitation events (Figure 8).

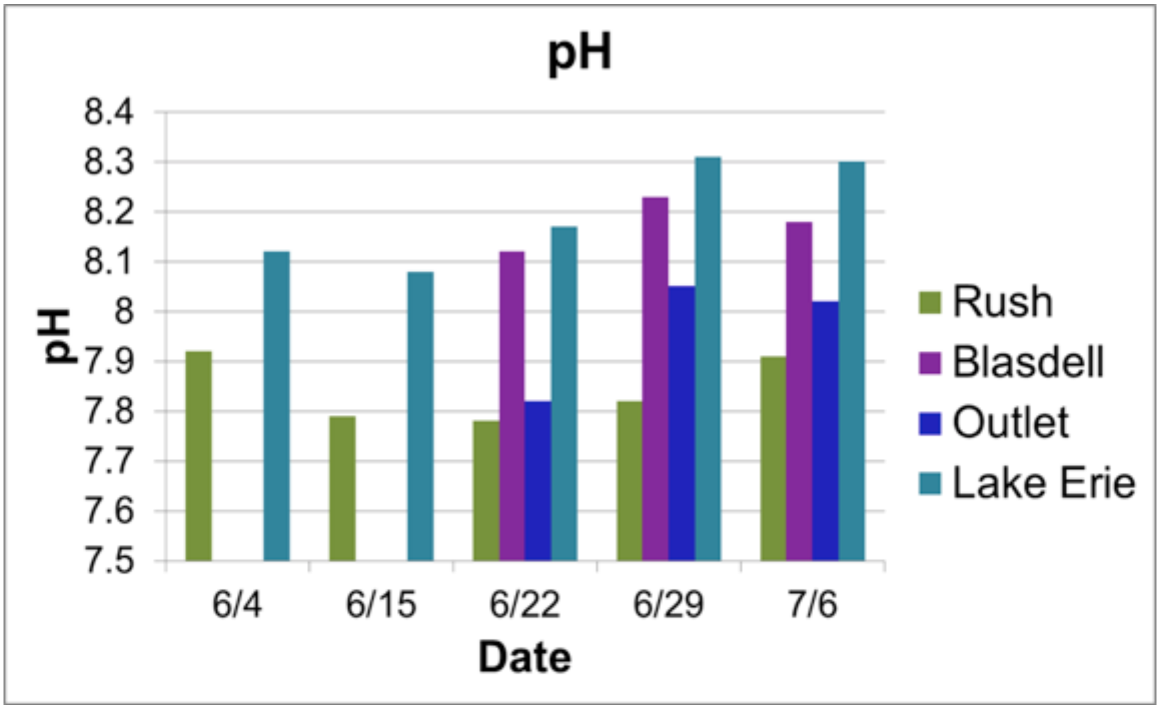

Figure 5: pH at the four Woodlawn sample sites. When ranked from low to high alkalinity, there is a consistent pattern: 1.) Rush 2.) Outlet 3.) Blasdell 4.) Lake Erie. Outlet levels reflect the blending of the two creeks.

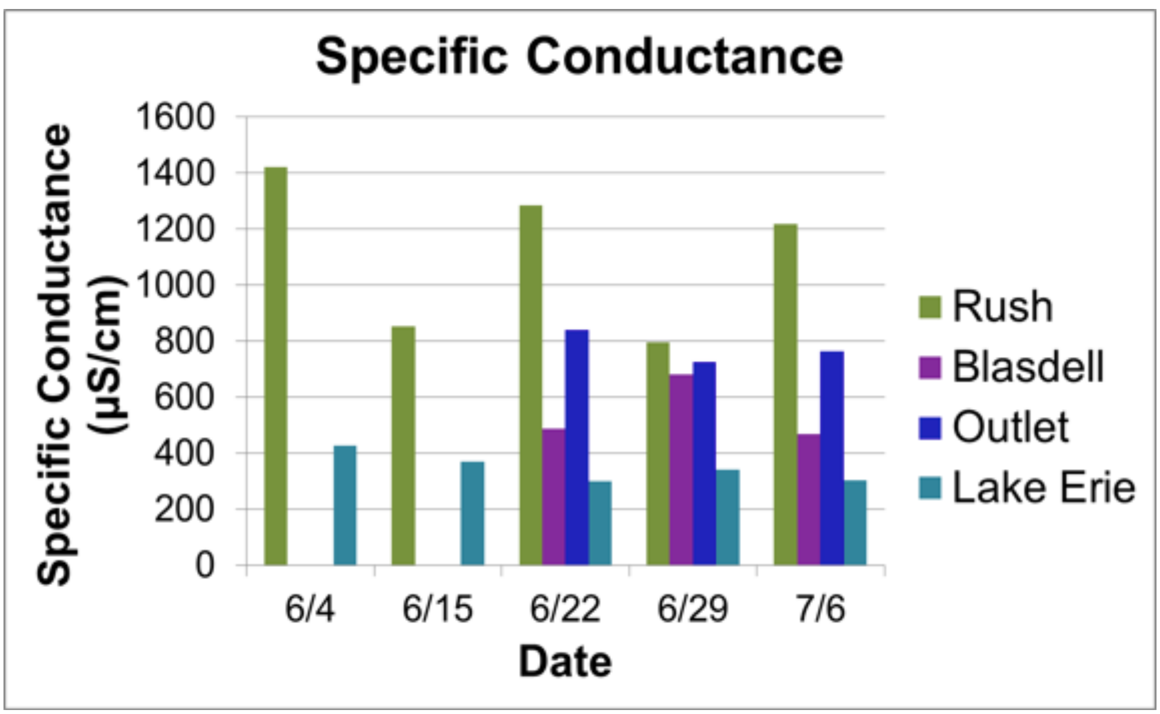

Figure 6: Specific conductance at the four Woodlawn sample sites. There is a consistent pattern when ranked from high to low specific conductance: 1.) Rush 2.) Outlet 3.) Blasdell 4.) Lake Erie. Outlet levels show the two creeks mixing together.

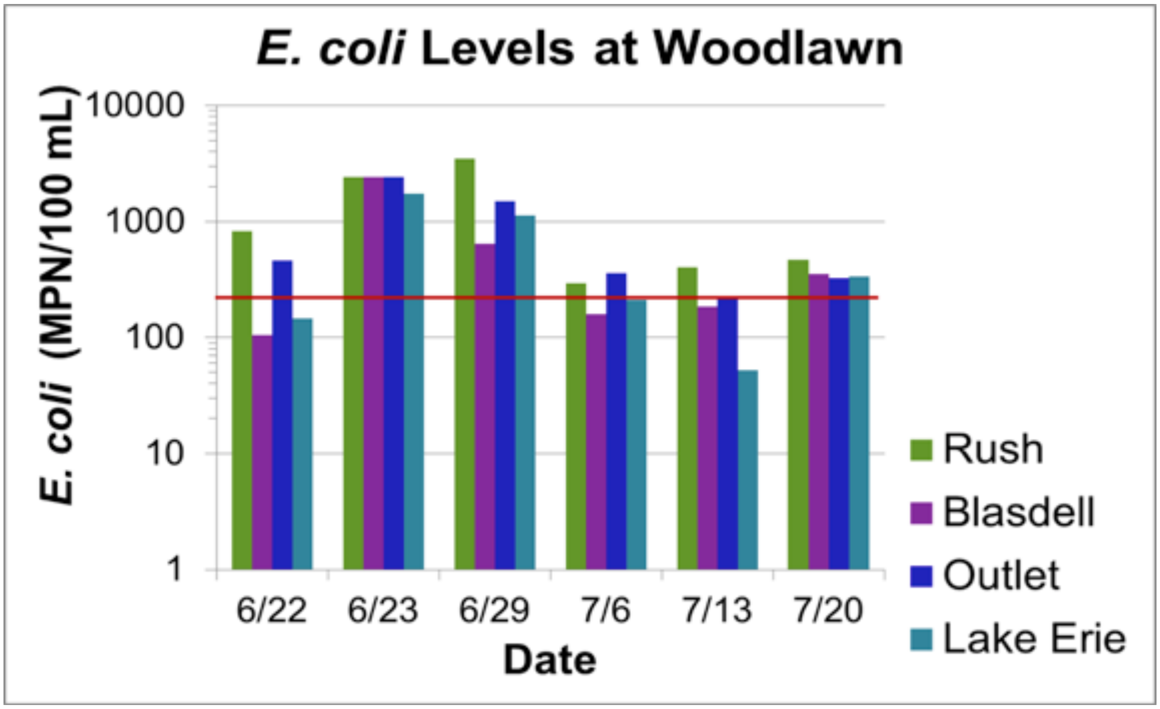

Figure 7: E. coli levels in Rush Creek were above the NYS limit for recreational water bodies in all of the samples. Although exceedances also occurred in Blasdell Creek, they were only observed in half of its samples. Outlet *E. coli* concentrations reflect the mixing of the two creeks as they converge and tend to lie somewhere between the two creeks' individual values.

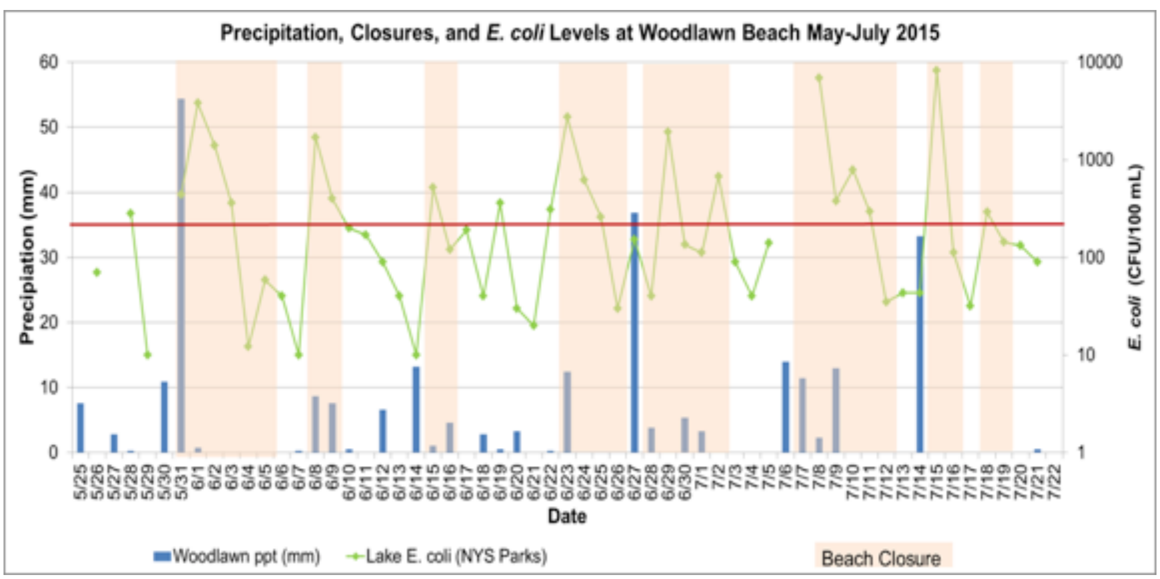

Figure 8: E. coli spikes often occur after times of high precipitation. Of the closures during this period, seven were because of combined sewer overflows. For this period there were three days where the beach was open when the average *E. coli* level was above the NYS limit for recreational water bodies (5/28, 6/19, 6/22). At times the beach was closed even though *E. coli* levels were below the limit; generally these were when *E. coli* levels had been high the previous day and park officials were waiting for *E. coli* test results to return to a safe level before re-opening the beach.

Unfortunately, we were not able to create a rating curve for Rush Creek due to a shift in the streambed sediments. Future work should look at monitoring flow rates and stream stage at a location further upstream, where the bed is less sandy and where backflow effect would be less pronounced (See result below from Team 2 Rating Curves).

Summary: Woodlawn beach was closed 50% of the summer of 2015 due to *E. coli* levels greater than 300 CFU/100mL, the NYS limit for recreational water bodies. The two major streams contributing to the beach both show elevated *E. coli* levels however there was no correlation between stream discharge and levels of *E. coli*. Observations of pH and specific conductance show unique signatures from both Rush and Blasdell Creeks, which mix as they enter Lake Erie. These results reinforce that the two creeks need to be considered as a system and individual monitoring needs to be conducted on both streams. A positive correlation between precipitation and beach closers due to *E. coli* concentrations was confirmed for many of the beach closure events. However these results do not point to an increase in stream flow causing increased *E. coli* flux to the beach. Future work needs to investigate additional sources of *E. coli* impacting beach closures. It is clear that both Rush and Blasdell Creeks are contributors of *E. coli*, however they are likely not the only sources.

Team 2: Rating Curves
Nicholas Luh, Bachelor of Science candidate, Environmental Geoscience, University at Buffalo
Jessica Ewanic, Bachelor of Science candidate, Geology, University at Buffalo
Luiz Rafael Pereira dos Santos, Bachelor of Science candidate, Geology, North Dakota State University

Objective: Determine the relationship between stream stage and discharge on major tributaries that enter Lake Erie

Description: To quantify the transport of contaminants from local waterways into Lake Erie, it is first necessary to develop a correlation between stream stage and discharge. Six major ungaged tributaries that enter Lake Erie within Erie County, New York, were measured weekly to determine stream discharge and stream stage throughout the summer of 2015 (Figure 1). The objectives of this research were to (1) quantify the flux entering the lake and (2) determine the relationship between precipitation and discharge. This information can be used to better predict when beach closures will occur. During storms, combined sewer overflow events result in the deposition of *E. coli* into local waterways, resulting in beach closures. Results of this project will help to predict when beach closures could occur based on each streams recession data and precipitation within the watershed.

Results: Stream discharge and stream stage were monitored at each of the six tributaries entering Lake Erie. Stream discharge measurements were conducted weekly over the study period using a SonTek Acoustic Doppler Flowmeter. Stream stage was monitored using pressure transducers (Scajaquada, Rush, Delaware, and 18-Mile Creeks) and a citizen science based stream stage-monitoring network (www.crowdhydrology.com) in the remaining streams (Big Sister Creek and Muddy Creek). Results produced two reliable rating curves at Scajaquada and 18 Mile Creeks

(Figure 9 and 10). The development of rating curves at the remaining creeks proved difficult due to site locations described below.

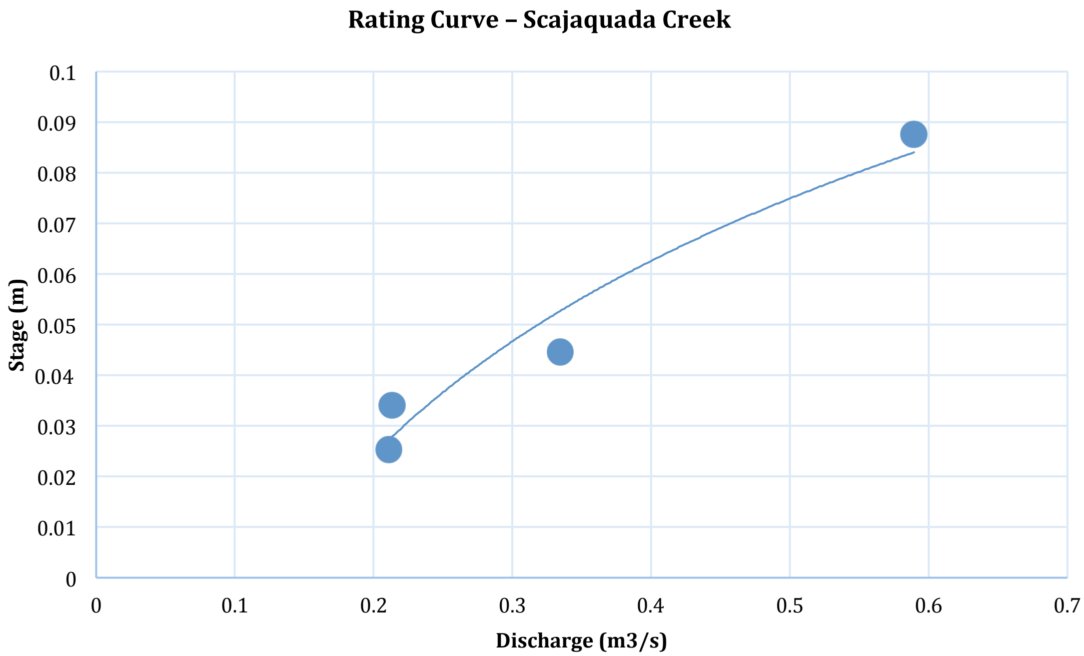

Figure 9: Rating curve for Scajaquada Creek at Forrest Lawn Cemetery.

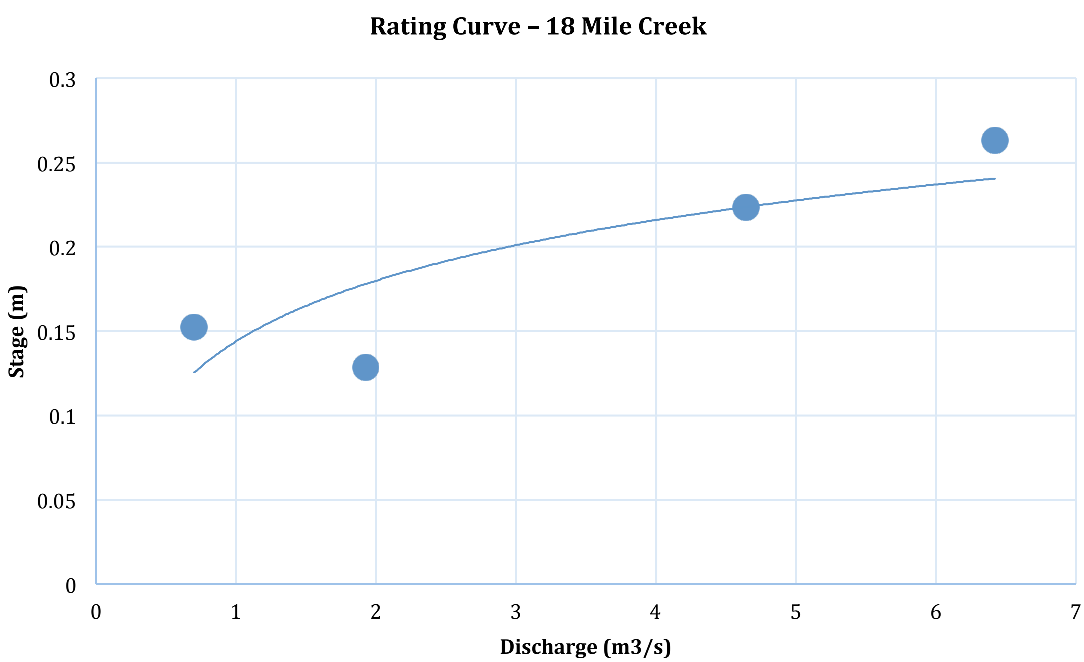

Figure 10: Rating curve for 18 Mile Creek at Lake Shore Drive.

Two major issues occurred preventing the development of rating curves at three of the creeks (Rush Creek, Delaware Creek, and Muddy Creek). Rush Creek has a sandy streambed that shifted due to the transport of sediment during high flow. This shift resulted in a resetting of the relationship between stream stage and discharge (Figure 11). As a result, it was not possible to develop a reliable rating curve. Future work needs to move upstream where a more structurally stable streambed exists in order to develop a reliable rating curve. Backwater effects from Lake Erie, reversing the stream gradient near the mouth of Delaware Creek and Muddy Creek, prevented the development of rating curves at these sties. These sites were initially established in early summer when stream discharge was high. As sampling progressed through the summer, it was observed that flow at these gages stations was zero or, in some cases, negative, indicating backflow moving from Lake Erie up into the lower tributaries of the streams. This same effect was observed in the early summer along Big Sister Creek at Bennett Beach. As a result, the Big Sister Creek gage was relocated further upstream.

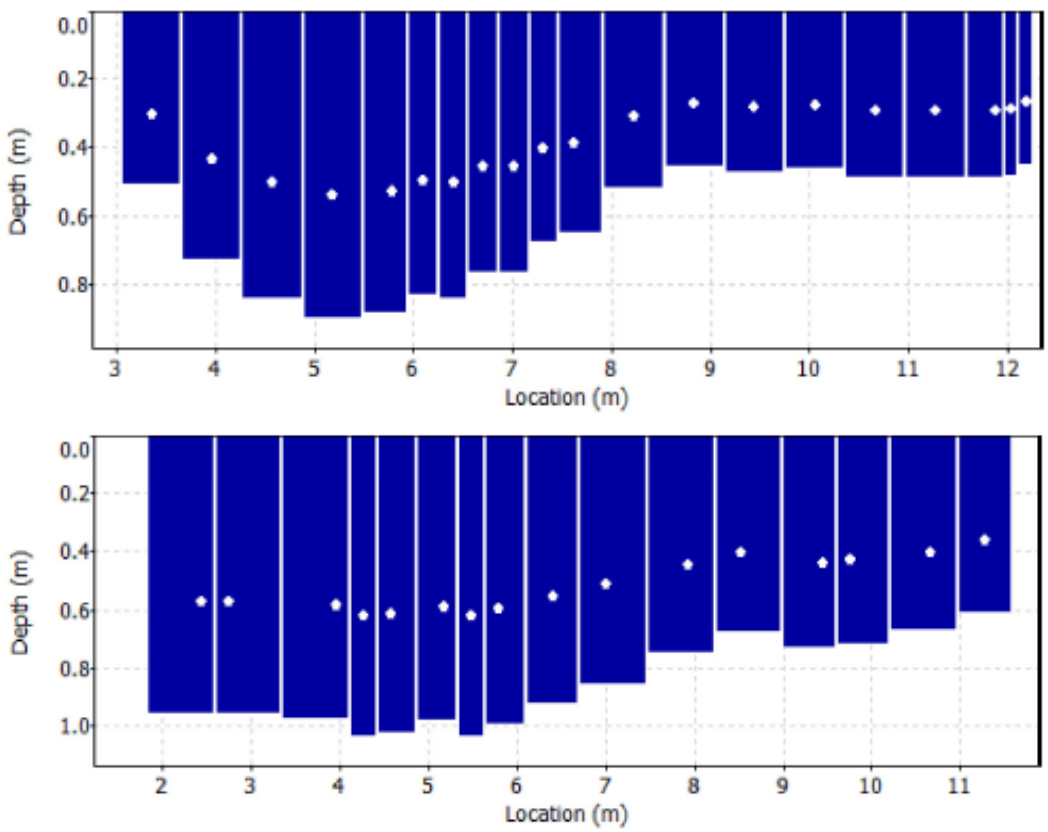

Figure 11: Depth of the Rush Creek over location in (A) 22 June 2015 and (B) 13 July 2015

Using hourly data of stream stage and rating curves, a time series of stream discharge was developed for both Scajaquada and 18 Mile Creeks (Figure 12 and 13). These data were used to determine the relationship between local precipitation events and peak stream discharge. Lag time between precipitation and discharge was calculated using center of mass for precipitation events and peak discharge. Lag times were calculated from multiple precipitation events throughout the summer (Table 1) resulting in an average lag time of 6 and 5 hours for

Scajaquada and 18 Mile Creeks, respectively. Under a majority of the storm events, both streams returned to baseflow within 24-36 hours of precipitation events.

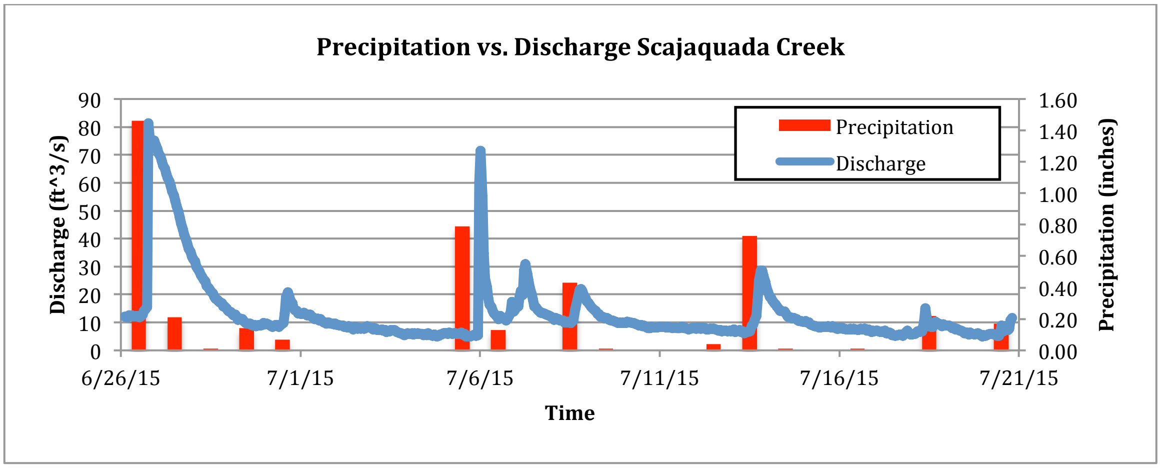

Figure 12: Continuous record of stream discharge with precipitation for Scajaquada Creek.

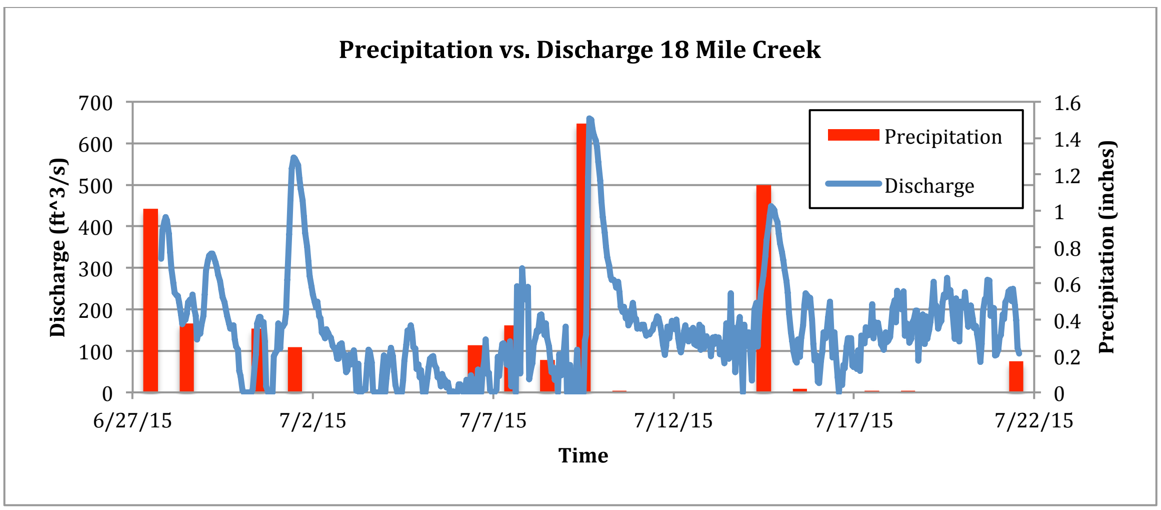

Figure 13: Continuous record of stream discharge with precipitation for 18 Mile Creek.

Table 1: Stream lag time from peak precipitation to peak stream discharge.

Stream	Max Precipitation	Max Discharge	Lag time (hh:mm)
Scajaquada	**6/27/15 14:57**	**6/27/15 16:00**	**1:03**
	7/1/15 8:14	**7/1/15 13:00**	**4:46**
	7/6/15 20:57	**7/6/15 22:00**	**1:03**
	7/7/15 17:03	**7/8/15 4:00**	**10:57**
	7/9/15 12:38	**7/9/15 17:00**	**4:22**
	7/14/15 12:43	**7/14/15 17:00**	**4:17**
average >1in	**1:03**	**average <1in**	**6:06**
18 mile	**6/27/15 14:02**	**6/27/15 22:00**	**7:58**
	7/28/15 22:43	**7/29/15 5:00**	**6:17**
	7/1/15 6:42	**7/1/15 11:00**	**4:18**
	7/9/15 13:21	**7/9/15 16:00**	**2:39**
	7/14/15 12:20	**7/14/15 17:00**	**4:40**
average >1in	**5:05**	**average <1in**	**5:17**

Summary: Scajaquada Creek and 18 Mile Creek discharge increases rapidly directly following precipitations events, however after 24-36 hours the stage and discharge return to the normal rate of flow, while all other tributaries analyzed have prolonged, above normal discharge and stage (48-60 hours). Part of the rapid rise in stream stage in Scajaquada Creek is due to its location in an urban environment with more impervious surfaces. Rating curves from Muddy, Delaware, and Rush Creek provided no useful correlation between stage and discharge (or discharge and precipitation). This could be due to the seiche of the lake. To obtain useful rating curves, it is necessary to take measurements at locations higher in the subwatershed to avoid backflow. Using the lag times of precipitation and discharge, future work can develop relationships to predict possible flooding based on the rate of precipitation. In addition, these lag times can be useful for determining how long beaches need to be closed due the presence of the bacteria *E. coli* that has highest concentration just after storm events.

Team 3: *E. coli* residence times

Caroline Tuttle, Bachelor of Arts candidate, Environmental Studies, Skidmore College
Julianna Crumlish, Bachelor of Science candidate, Environmental Geosciences, University at Buffalo
Michael Canty, Bachelor of Science candidate, Industrial and Systems Engineering, University at Buffalo

Objective: Quantify residence time of E. Coli in urban streams.

Description: A critical issue in urban streams is the increased levels of *E. coli* due to anthropogenic sources. In the city of Buffalo, NY these sources are due to an aging wastewater infrastructure, which has a combined storm water and sewer system. One creek that is heavily impacted by increased *E. coli* due to combined-sewer overflows is Scajaquada Creek. In 1921, as development in the city increased, Scajaquada Creek was diverted underground. The creek now flows in a tunnel under Buffalo for four miles and then emerges again in Forest Lawn Cemetery (Location A, Figure 14). In this experiment, our goal was to determine the sources of *E.coli*, and mechanisms that might account for variability in *E.coli* within Forest Lawn Cemetery. Potential variability in these concentrations was thought to have come from photo degradation and dilution. These potential drivers were investigated over a 24-hour sampling period at locations with variable contributions of sunlight and tributary sources of water.

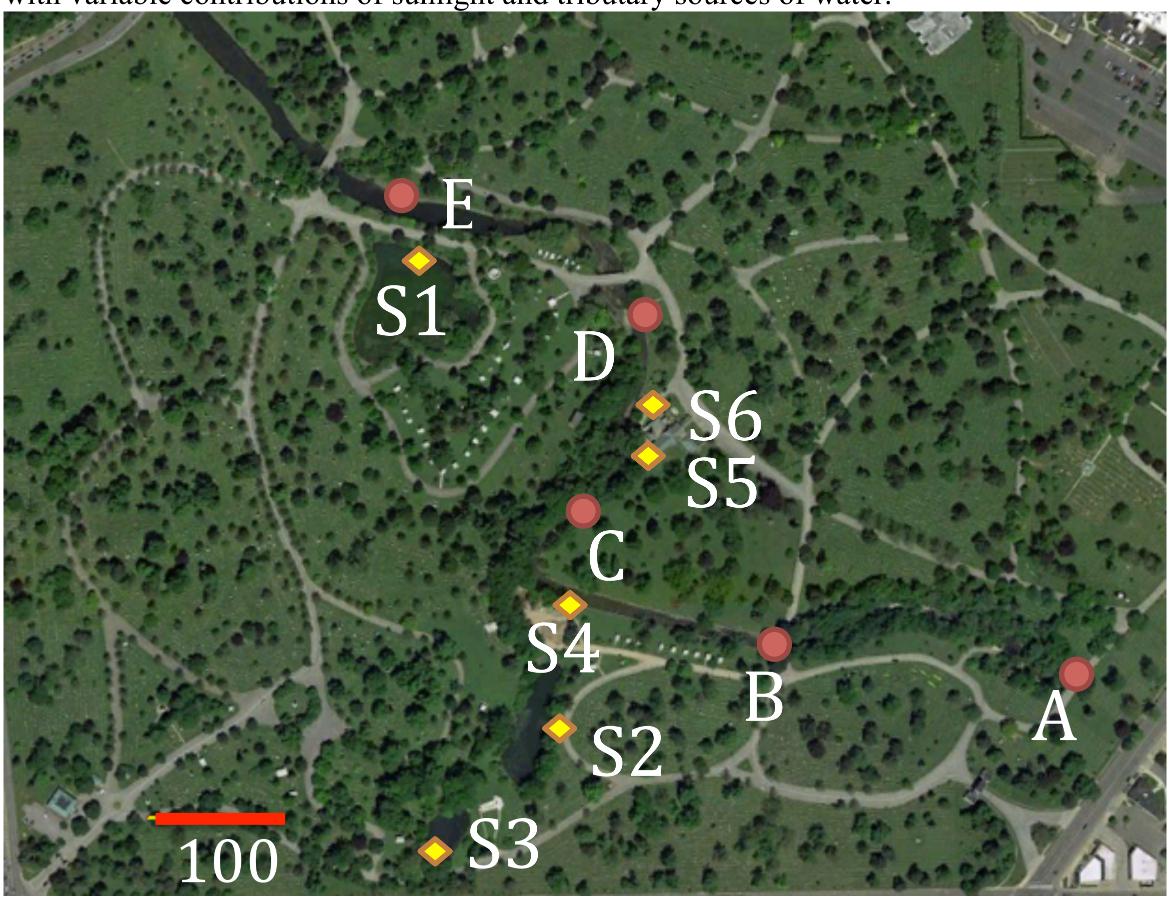

Figure 14: Site map of Scajaquada Creek. Red dots represent stream sampling locations. Yellow diamonds represent sampling locations of external drains and ponds.

Water samples were collected in Forest Lawn Cemetery along a 2-mile stretch of Scajaquada Creek (Figure 14), with background sampling conducted over a four-month period (May – Aug). Fine scale sampling was taken every two hours over a 24-hour period. Escherichia coli (*E.coli*) water samples were collected at five locations along the stream. After the samples were taken, Coliert was added to the samples (Figure 15B), and the samples were placed in a Quanti-Tray for quantification of *E.coli* and fecal coliforms (ISO standard 9308-2:2012). Samples were incubated for 24 hours at 35^{o}C (Figure 15D). After 24 hours samples were placed under a florescent light; the cells that were yellow and glowed were considered to have *E.coli* (Figure 15E).

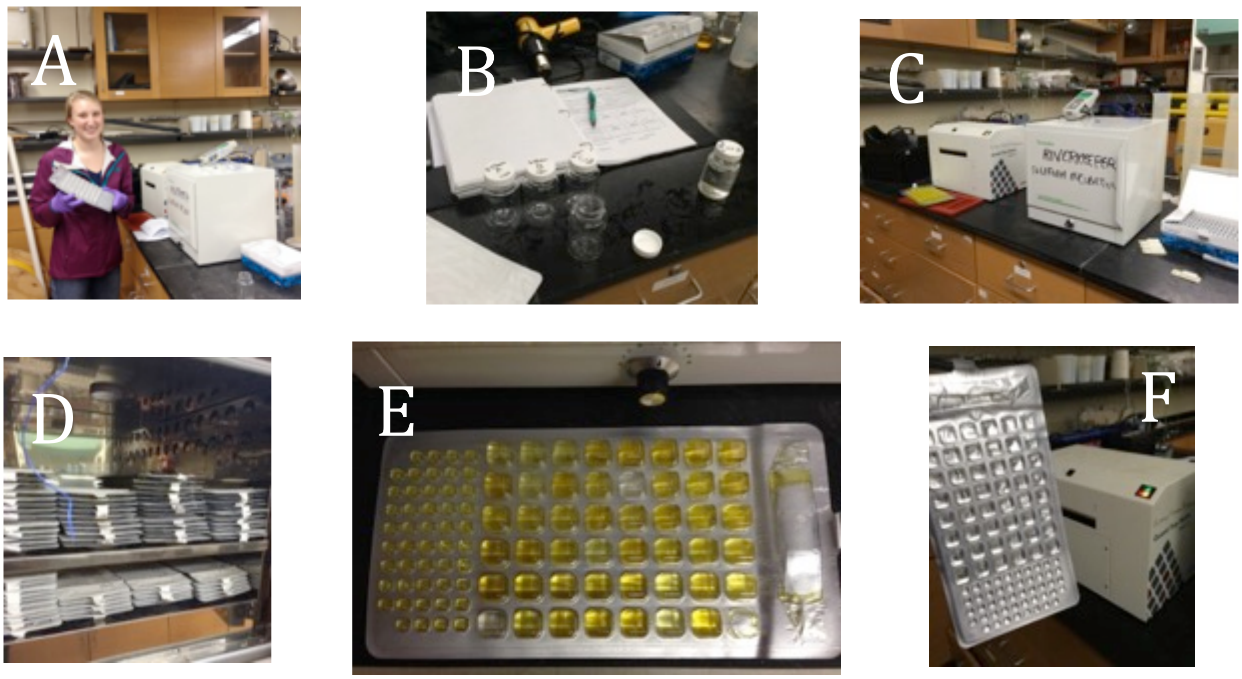

Figure 15: (A) Equipment used for testing E. Coli, (B) Sample bottles, (C) Laminator and incubator, (D) 24 hour samples in incubator, (E) Positive count Quanti-tray, (F) Field blank Quanti-tray.

Results: The general trend shows a decrease in *E. coli* moving down stream (Figure 16). Water samples collected at location A were collected at the outlet of the 4 mile long covered portion of Scajaquadda creek. These samples were the highest concentrations of *E. coli* of any samples collected during the summer period and represent samples that have not been exposed to sunlight. Samples at location E represent samples that have both been exposed to sunlight along the 2-mile study reach as well as experienced impact from tributary waters entering the creek with variable concentrations of *E. coli* (Figure 17). It should be noted that tributary sources of water entering Scajaquada Creek have up to an order of magnitude lower concentrations of *E. coli* as compared to the main channel of the creek (Figure 17).

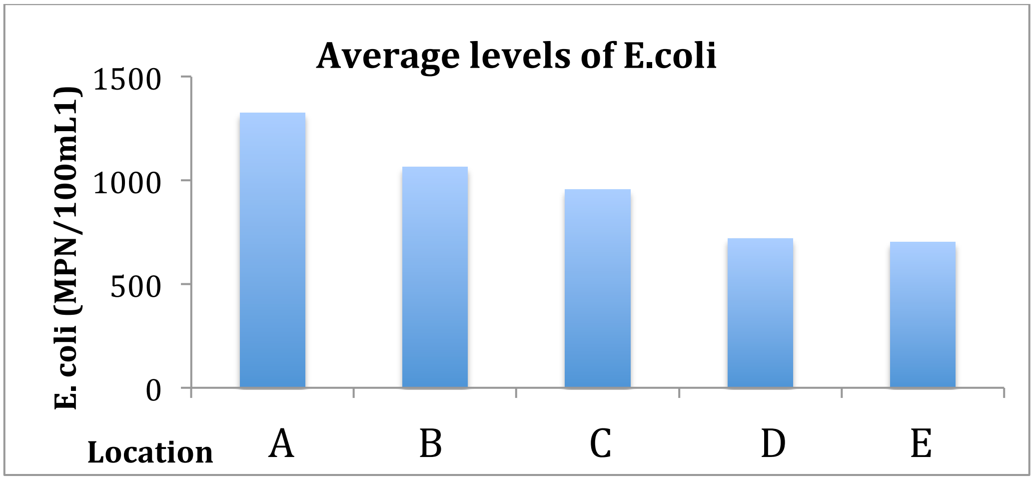

Figure 16: Spatial distribution of *E.coli* within Scajaquada Creek

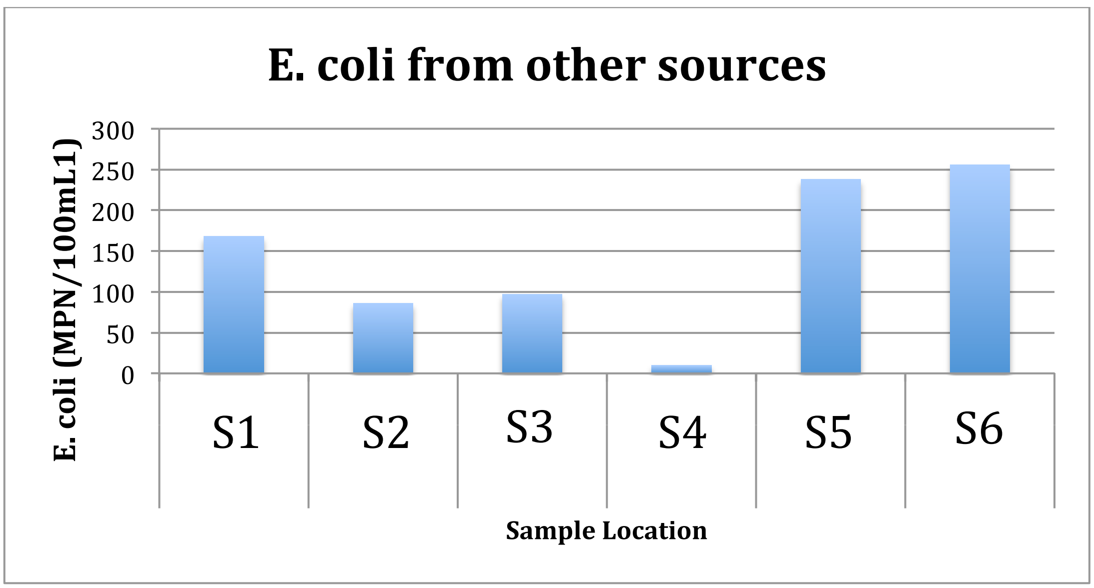

Figure 17: Levels of *E.coli* from external sources of water entering the stream show lower concentrations as compared to the main channel of the stream. The largest concentration appears to enter the stream along the eastern bank.

24-Hour Sampling Results: In order to determine the impact of photo degradation on *E. coli* concentrations, and to separate it from the impacts of dilution, a series of samples were collected every two hours over a 24-hour period. *E. coli* concentrations at location A were considered the control as these samples were never exposed to sunlight due to the tunnel. As a result, these samples were highly variable at the outlet of the tunnel (Figure 18, location A) and as expected, there was no diel pattern in the changes of *E.coli* levels throughout the 24-hour period (Figure 18, location A). Moving down stream, results show small diel fluctuation in *E.coli* concentrations at the down stream sampling locations D and E (Figure 18, location D and E). Sample locations B and C seem to be transitional and show a slight diel pattern with the superimposed noisy signal from location A (Figure 18, location B and C).

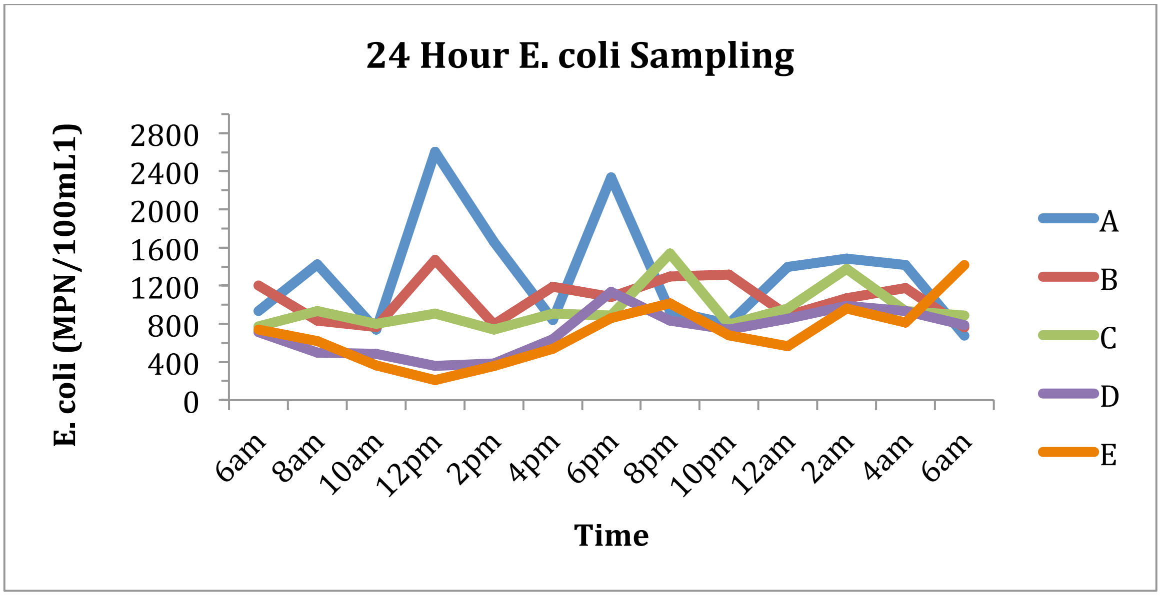

Figure 18: Levels of *E.coli* over 24 hour sampling.

Summary: Average levels of *E.coli* decrease moving downstream from the outlet of the culvert, indicating the source of *E.coli* is likely coming from inside the tunnel. Levels of *E.coli* in tributary pipes and drains entering Scajaquada Creek were found to have lower concentrations, indicating that the water from these sources may dilute the *E.coli* concentrations in the main channel. These results may partially explain the lower levels of *E.coli* further down the stream. Results from the 24-hour sampling show small diel fluctuation in *E.coli* concentrations only at the down stream sampling locations. These diel trends support the idea of limited photo degradation, which resulted in a change of 800 MPN/100mL in concentrations at the lower site (Figure 18, Site E). This photo degradation is superimposed on the dilution signal resulting in the observed reduction in *E.coli* along the stream reach.

Project 4: Stream Quality

James Coburn, Bachelor of Science candidate, Environmental Geosciences, University at Buffalo

Jonathan Vitali, Bachelor of Science candidate, Geology, University at Buffalo

Objective: Evaluate spatial changes in temperature, conductivity, pH and dissolved oxygen on major tributaries that enter Lake Erie Basin as a result of the impact of urban versus rural land cover.

Description: Field data were collected during both high and low flow conditions along the rural dominated Tonawanda Creek and the urban dominated Ellicott Creek (Figure 19 and 20). A YSI probe was used to measure electrical conductivity, pH, and temperature. Data points were taken between meanders in Tonawanda Creek, and in steady increments of about 150-200 meters on Ellicott Creek. All measurements were taken in the middle of the stream from a canoe. Water quality was measured using a YSI probe placed around 1 foot below the water surface, while at the same time a GPS unit was used to identify exact locations for these data. Each parameter of stream quality was georeferenced on ArcMap for an arial view of stream quality on the WGS 1984 Geographic coordinate system.

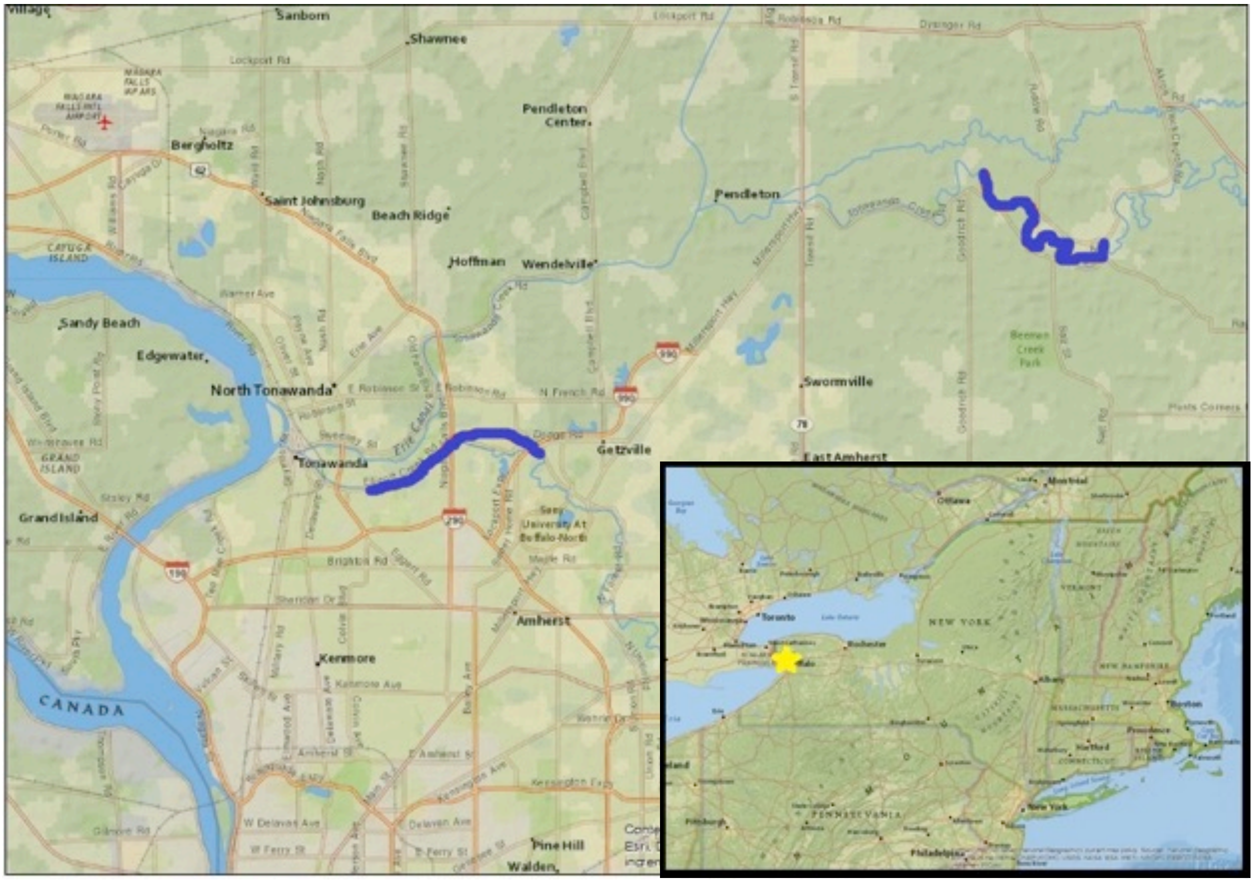

Figure 19: Stream Quality Project field site map showing Tonawanda Creek in the upper right hand corner and Ellicott Creek in the lower left.

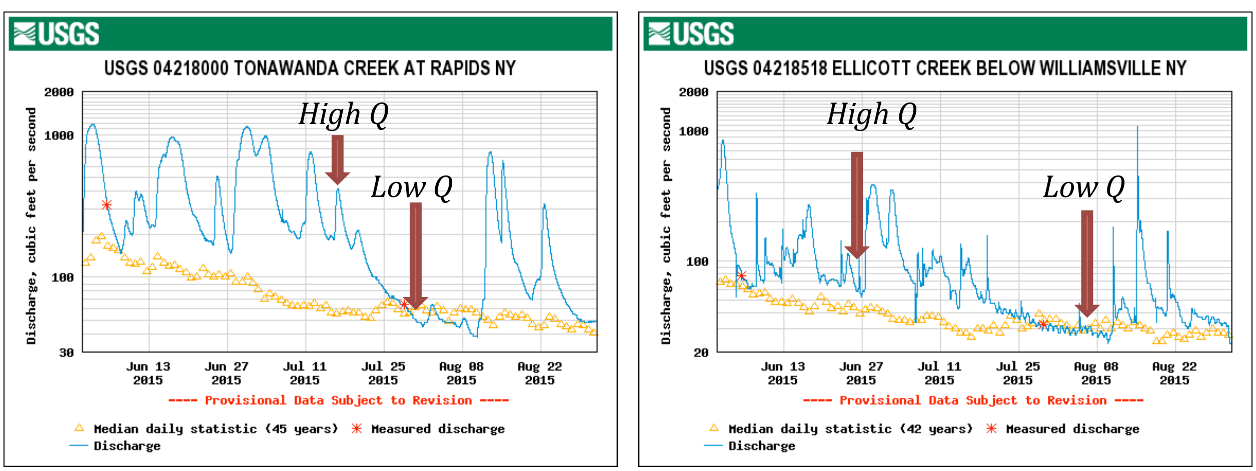

Figure 20: USGS stream discharge data for Tonawanda and Ellicott Creeks. Arrows show observation periods for both high and low discharge.

Results:

Temperature (°C): Stream temperature observations show greater spatial variability in temperature at the rural site (Tonawanda Creek) as compared to the urban dominated site (Ellicott Creek). During high flows both streams maintained similar temperatures between 22-24 degrees Celsius. During low flows, the urban stream resulted in higher and uniform stream temperatures, while the rural stream resulted in slightly lower stream temperatures with a much larger range of variability.

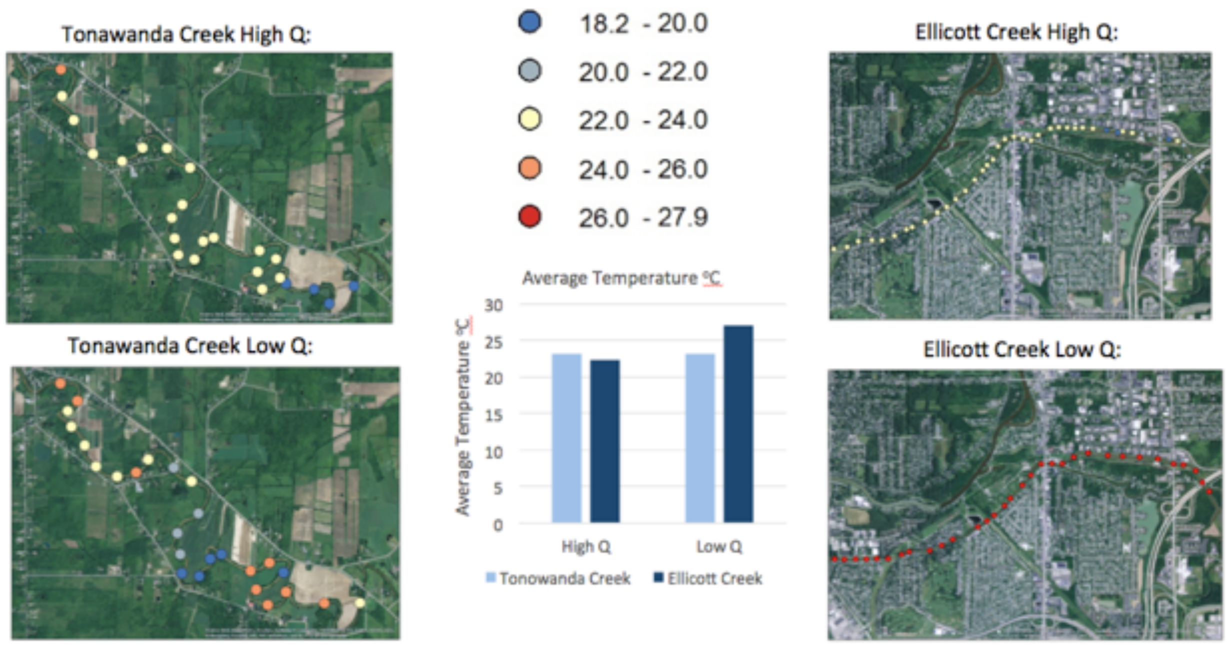

Figure 21: Stream temperature for a Rural (Tonwanda) Creek and an Urban (Ellicott) Creek under both high (upper figures) and low (lower figures) flow.

pH: During both high and low flow events, the rural stream consistently maintained higher pH when compared to the urban stream. Spatial variability between the two streams was relatively constant. The urban stream did show more abrupt changes in pH, likely due to the influence of storm water entering the stream at unknown discreet locations.

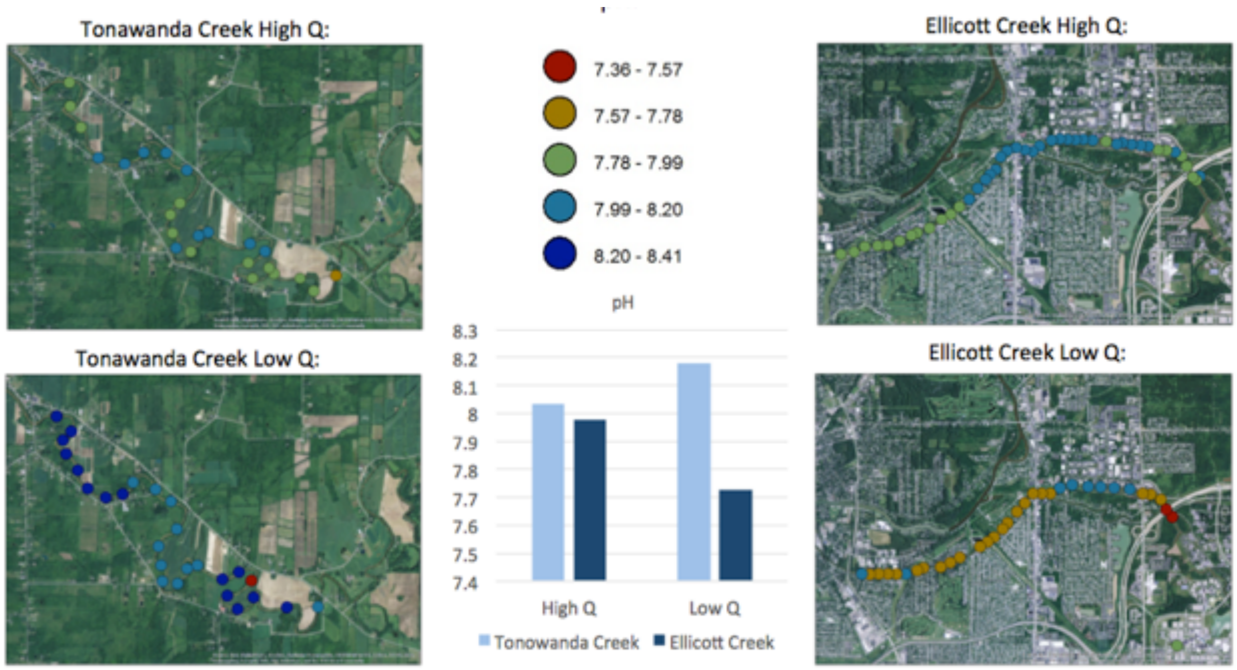

Figure 22: Stream pH for the rural (Tonwanda) creek and urban (Ellicott) creek under both high (upper figures) and low (lower figures) flow.

Electrical Conductivity (µS/cm): While variability in electrical conductivity did occur between rural and urban dominated streams there was no significant change during high and low flow conditions (Figure 23). The increase in electrical conductivity in the urban creek is thought to be a result of runoff from roadways.

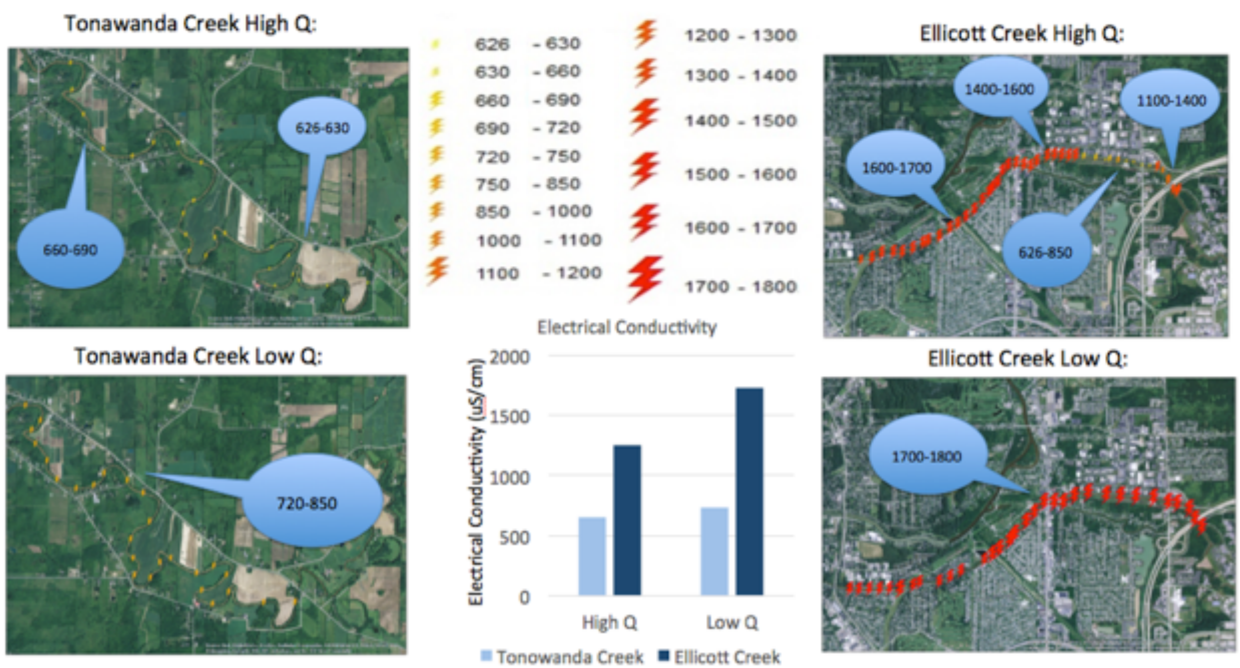

Figure 23: Stream electrical conductivity for Rural (Tonawanda) Creek and Urban (Ellicott) Creek under both high (upper figures) and low (lower figures) flow.

Summary: As expected both spatial and temporal variability in water quality parameters exist between rural and urban dominated streams as well as during high and low flow periods. Ellicott Creek (Urban), during high flow rates, showed the most variability and extreme values in temperature, pH, and electrical conductivity when compared to all other streams and flow rates. Average temperature for both streams was higher at low flow conditions, with Ellicott Creek (Urban) maintaining a slightly higher overall temperature during low flow conditions. Electrical conductivity was higher in Ellicott Creek (Urban) than in Tonawanda Creek (Rural), likely due to inputs from the urban environment. While variability in pH was observed, this variability was small between streams and flow events.

Project 5: Turbidity

Olivia Patrick, Bachelor of Science candidate, Geology, University at Buffalo
Rebecca Dickman, Bachelor of Science candidate, Environmental Geosciences, University at Buffalo

Objective: Analyze relationships between meteoric events and water clarity, temperature, and pH in Lake Erie.

Description: The increased public focus on algal blooms and invasive species in Lake Erie underscores the need for better spatial and temporal monitoring of water quality. Due to the spatial extent of the lake as well as seasonal changes in water quality, monitoring of water quality parameters can be both financially and labor intensive. In order to reduce costs and personal time this research enlisted citizen scientists to collect basic water parameters (pH, Temperature, and turbidity) through a partnership with a local sailing school. The research objective of this project was to quantify water quality changes at various locations along the eastern shore of Lake Erie from June to September. Initial work focused on using a smart phone application "Secchi" to provide citizens with a centralized place to record data. As the study progressed, numerous complications regarding data collection arose, so new methods were formed to simplify the testing making it more convenient for citizens to participate in the study. These results demonstrate both failures and success in colleting spatial and temporal distributed water quality data within Lake Erie. Results are meant to be a starting point in which a larger program can upscale these methods to the rest of the Lower Great Lakes.

Results and Summary: Through a partnership with the Seven Seas Sailing Club, citizen science water quality sampling kits were placed on ten boats. Citizen science kits include a Secchi disk, pH strips, and a thermometer. In addition, detailed instructions were included with the kits on how to collect and report water parameters. Originally, we attempted to use a smartphone-based application "Secchi" to have citizens report water parameter using a geo-located based application. However, this proved to be too high of a barrier of entry due to a range of factors. Consistently, citizen scientists forgot their phones or had trouble downloading the application. There was also some confusion as to when and where to recorded data. As a result, a modified data collection system was developed using a paper-based system where users could mark their location on a map and then recorded their observations in a notebook. This new paper based system produce fewer observations than were expected. While sailors out on Lake Erie may have been at the right location and at the right time, it was difficult to find a suitable means to engage with them to collect hydrologic data. Even after significant periods of time trying to connect with citizens and talk with them on the importance of distributed lake data, the project resulted in only a few measurements of pH, temperature, and turbidity (Secchi depth). While we thought this would be a new and novel way to collect data, in the end it proved to be too large of a barrier of entry for truly useful scientific observations.

Project Deliverables

Project deliverables include conference presentations, student training, and publically accessible water quality data on streams entering Lake Erie. Project results were presented at the 2015 Geological Society of America Annual meeting in Baltimore MD. Eight of the eleven participating students were able to present their work and get feedback from professionals. Two of these presentations resulted in a second (Crumlish et al., 2015) and third (Tuttle et al., 2015) place award for best posters in the Undergraduate Research in Hydrogeology poster session. In addition our participating students were exposed to the basics of scientific research and were able to learn a wide range of field methods. Finally, all field data have been uploaded to two publically accessible databases for long-term storage.

Conference Presentations

1. Coburn, J. E., Vitali, J. M., Glose, T. J., Lowry, C. S., "*Analyzing Water Quality Over Variable Flow Conditions in Rural and Suburban Streams.*" A Showcase of Undergraduate Research in Hydrogeology Poster Session, 2015 Geological Society of America Annual Meeting, Baltimore, MD (November 2015).

2. Crumlish, J.C., Pereira dos Santos, L. R., Glose, T. J., Lowry, C. S., "*Evaluating the Impact of Hydrology and Combined-Sewer Overflows on Urban Beach Closures.*" A Showcase of Undergraduate Research in Hydrogeology Poster Session, 2015 Geological Society of America Annual Meeting, Baltimore, MD (November 2015).

3. Luh, N. M., Ewanic, J., Pereira dos Santos, L. R., Glose, T. J., Lowry, C. S., "*Relationship Between Stream Stage and Discharge on Major Tributaries that Enter Lake Erie.*" A Showcase of Undergraduate Research in Hydrogeology Poster Session, 2015 Geological Society of America Annual Meeting, Baltimore, MD (November 2015).

4. Tuttle, C.T., Crumlish, J.C., Canty, M. T., Glose, T. J., Lowry, C. S., "*Analyzing Daily Variability in E coli Concentrations in an Urban Stream.*" A Showcase of Undergraduate Research in Hydrogeology Poster Session, 2015 Geological Society of America Annual Meeting, Baltimore, MD (November 2015).

Student Training

Eleven students were trained during the course of this project. In his role supporting each of the undergraduate student groups, Thomas Glose, a Ph.D. candidate, gained experience with project management and leadership. Undergraduate students were trained in the following skills:

- Measuring volumetric flow rates using an Acoustic Doppler Flowmeter

- Testing water quality with a YSI Multi-Probe
- Collecting, processing, and reading results of *E. coli* samples using the IDEXX Colilert Quanti-Tray System
- Installing stilling wells
- Retrieving and interpreting data from pressure transducers
- GPS and GIS
- Maintenance and calibration of water quality testing equipment
- Creating rating curves
- Measuring turbidity with Secchi disks
- Public speaking

Database

Field data are available through the National Geographic Education FieldScope web page (http://greatlakes.fieldscope.org)(Figure 24) and at the University at Buffalo Library Institutional Repository (https://ubir.buffalo.edu). The FieldScope web page allows the general public to access these data through a map based graphical user interface. This user interface allows the general public to both search and plot these and other data focused on water quality within the Great Lakes. These data are also stored, and available to the general public, in tabular from (text files) through the UB Institutional Repository (https://ubir.buffalo.edu/xmlui/handle/10477/53140).

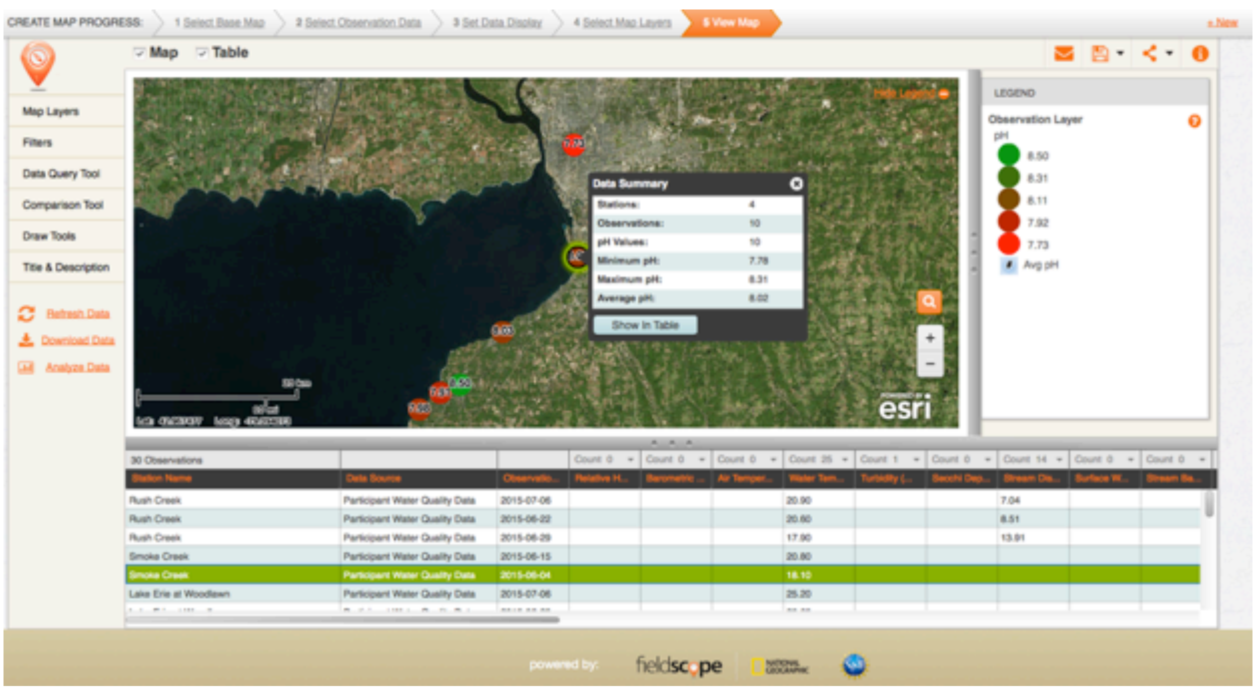

Figure 24. Western New York Watershed Network field data is all publically available on the National Geographic Education's FieldScope web page. The graphical user interface allows the public to search by location and/or parameters from both desktop computer and smart phones.

Future Work

The goal of Western New York Watershed Network was to design and implement a student and citizen scientist-run hydrologic monitoring system to support water resource management in Western New York. Preliminary data collected as a result of funding from the New York State Water Resource Institute has supported two follow up grants from the New York State Sea Grant and University at Buffalo's RENEW Institute. Several of our summer 2015 students have continued to work with us and other students have moved onto other research projects within the Department of Geology. Our second generation Western New York Watershed Network research, funded by the New York State Sea Grant focuses on quantifying nutrient fluxes entering Lake Erie at three of our original field sites (Tonawanda, Scajaquada and Big Sister Creeks). While the RENEW Institute funding focuses on investigating microbial pollution in Lake Erie using next-generation sequencing (eDNA) to examine microbial community interactions and their relationship to flow patterns. The microbial pollution work will focus on Rush Creek and Woodlawn Beach, another one of our original research sites.

References

Coburn, J. E., Vitali, J. M., Glose, T. J., Lowry, C. S., "*Analyzing Water Quality Over Variable Flow Conditions in Rural and Suburban Streams.*" A Showcase of Undergraduate Research in Hydrogeology Poster Session, 2015 Geological Society of America Annual Meeting, Baltimore, MD (November 2015).

Crumlish, J.C., Pereira dos Santos, L. R., Glose, T. J., Lowry, C. S., "*Evaluating the Impact of Hydrology and Combined-Sewer Overflows on Urban Beach Closures.*" A Showcase of Undergraduate Research in Hydrogeology Poster Session, 2015 Geological Society of America Annual Meeting, Baltimore, MD (November 2015).

Luh, N. M., Ewanic, J., Pereira dos Santos, L. R., Glose, T. J., Lowry, C. S., "*Relationship Between Stream Stage and Discharge on Major Tributaries that Enter Lake Erie.*" A Showcase of Undergraduate Research in Hydrogeology Poster Session, 2015 Geological Society of America Annual Meeting, Baltimore, MD (November 2015).

Tuttle, C.T., Crumlish, J.C., Canty, M. T., Glose, T. J., Lowry, C. S., "*Analyzing Daily Variability in E coli Concentrations in an Urban Stream.*" A Showcase of Undergraduate Research in Hydrogeology Poster Session, 2015 Geological Society of America Annual Meeting, Baltimore, MD (November 2015).

Population and DPS Origin of Subadult Atlantic Sturgeon in the Hudson River

Basic Information

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Publications

There are no publications.

Population and DPS Origin of Subadult Atlantic Sturgeon in the Hudson River

Final Report Submitted to the Water Resources Institute
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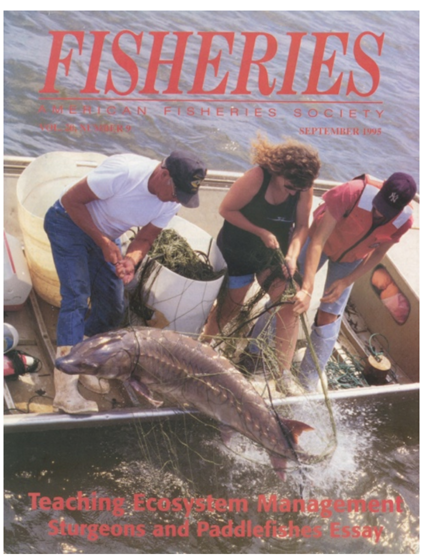

Abstract: At one time, Atlantic sturgeon supported a signature fishery in the Hudson River Estuary and identification of its migratory patterns is listed as a priority under Long Range Target 1 of the Actions Planned for 2010-2014 (Effectively Managing Migratory Fish). This study provided important new information that will be used by the NYSDEC and NOAA's Office of Protected Resources to manage Atlantic sturgeon in the Hudson River ecosystem and coastwide. Atlantic sturgeon is federally listed under the U.S. Endangered Species Act (ESA) as five Distinct Population Segments (DPS), of which four were designated as "endangered" and one as "threatened." The New York Bight DPS is comprised of the Hudson and Delaware River populations and is listed as "endangered." Subadult Atlantic sturgeon are known to exit their natal estuaries to coastal waters and non-natal estuaries where they are vulnerable to distant anthropogenic threats. In fact, during the warmer months, the Hudson River hosts large numbers of subadults, but their population and DPS origin is largely unknown although Section 7 of the ESA demands that origin of individual specimens be determined. We used microsatellite DNA analysis at 11 loci and sequence analysis of the mitochondrial DNA (mtDNA) control region to determine the DPS and population origin of 106 subadult Atlantic sturgeon collected in the lower tidal Hudson River estuary. We found that 101 of the 106 subadults assigned to the Hudson River with at least 95% and usually 100% probability. Of those 5 specimens that did not assign to the Hudson, 2 assigned to the James River, VA, 2 assigned to the Kennebec River, ME, and 1 assigned to the Saint John River, NB. Thus, four specimens assigned to DPS other than the New York Bight DPS and one to the Canadian Management Unit. This analysis will permit the quantification of the effects of anthropogenic threats in different locales or across seasons in the Hudson River Estuary on individual populations or DPS of Atlantic sturgeon and will serve as a model for similar population composition analysis for other estuaries coastwide.

Summary Points of Interest

A. Greater than 95% of subadult Atlantic sturgeon in the Hudson River are of Hudson River origin.
B. However, the Hudson River is host to a number of subadult Atlantic sturgeon that were spawned in other populations and sometimes other Distinct Population Segments.
C. The Hudson River harbors the population of Atlantic sturgeon with the largest Effective Population Size (N_e) coastwide.

Keywords
Microsatellite DNA analysis, mitochondrial DNA control region, Individual Based Assignment Testing, Mixed Stock Analysis, Distinct Population Segments

Background: Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* is the poster child for the Hudson River estuary with its image serving as the logo for the Hudson River prominently displayed on bridges crossing the main-stem river and its major tributaries. Historically, Atlantic sturgeon supported one of the three signature fisheries within the Hudson River Estuary. Spawning populations of Atlantic sturgeon extend from the St. Lawrence River, Quebec, to at least the Altamaha River, Georgia. Historically, there were close to 30 spawning populations coastwide (ASSRT 2007), but that number has dwindled in recent years to 15-20 rivers (Wirgin et al. 2015b). Atlantic sturgeon are anadromous and their spawning locations within natal rivers are above the salt front and usually over gravel, cobble, or boulder bottom. In the Hudson River, they are known to spawn from early June to early July in deep water in an area extending from Hyde Park to Catskill, New York and perhaps even further upriver. Their eggs are demersal and hatch within 4-6 days post-fertilization; the exact duration is temperature dependent. Juvenile Atlantic sturgeon are resident within their natal rivers for 2-6 years before migrating as subadults into coastal waters. Their duration of river residency is population dependent and is shorter in southern compared to northern rivers.

Subadult Atlantic sturgeon are highly migratory in coastal waters, the duration of migration can be prolonged, is population dependent, and utilizes unknown migratory corridors. Mature adult Atlantic sturgeon return to natal rivers to spawn, their age at maturity is once again highly variable and population dependent. For example, females in South Carolina spawn at 7-19 years (Smith et al. 1982), age 15 and older in the Hudson River (Bain, 1997), and at 27-28 years in the St. Lawrence River (Scott and Crossman 1973). In comparison, males spawn in the Suwanee River, Florida, at 7-9 years (Huff 1975), in the Hudson River at age 12 and older (Bain 1997), and 16-24 years in the St. Lawrence River (Caron et al. 2002). Post spawning adults exit their natal estuaries, weeks to months after spawning, and resume their coastal movements. The absence of adults from spawning rivers for many years and the difficulty in collecting early life-stages make censusing of populations and evaluation of temporal trends in their abundances problematic.

At varying times, many rivers coastwide, including the Hudson and particularly the proximal Delaware, hosted large fisheries for Atlantic sturgeon primarily targeting caviar-laden females. Many of these fisheries crashed, including that in the Hudson, in the late 1890s, to levels that were less than 10% of their historical highs. As fisheries in northern and mid-Atlantic rivers declined, the fisheries shifted to more southern rivers, particularly in South Carolina and Georgia, but these too suffered a similar fate as those in the Hudson and Delaware. By 1998 a federal coastwide 40-year harvest moratorium was imposed on the fisheries. This was followed in 2012 by U.S. federal listing of the species under the Endangered Species Act (ESA) as five Distinct Population Segments (DPS) of which four (New York Bight, Chesapeake Bay, Carolinas, and South Atlantic) were designated as “Endangered” and the fifth (Gulf of Maine) as “Threatened” (Federal Register 2012ab) As a result, federal management of the species under ESA is on a DPS basis, rather than as a single coastwide entity. However, because the abundance of the individual spawning populations is believed to vary by at least an order of magnitude, it is also important to consider the vulnerabilities of individual populations to the variety of anthropogenic stressors identified in the listing document. For example, the Hudson River population is considered to be the largest coastwide and the Delaware River population one of the smallest. Thus, the Delaware

River population is considered to be more vulnerable to extinction from anthropogenic stressors than the Hudson River population despite both of their listings within the New York Bight DPS.

As mentioned previously, subadult Atlantic sturgeon from all spawning populations migrate into coastal waters for extended durations. Besides coastal waters, it is known that subadults migrate seasonally into non-natal estuaries such as Long Island Sound, the Connecticut River (Waldman et al. 2013), and the inner Minas Basin of the Bay of Fundy (Wirgin et al. 2012). These may include estuaries that do not support spawning such as the Connecticut River-Long Island Sound and estuaries that do support contemporary spawning such as Delaware Bay and Chesapeake Bay. Conversely, subadults spawned in estuaries other than the Hudson are believed to seasonally move into the lower Hudson River Estuary. Because of their highly migratory behavior outside of their natal estuaries, specimens from multiple populations and DPS are likely to co-aggregate in coastal waters (Wirgin et al. 2015ab) or other estuaries distant from the river in which they were spawned (Waldman et al. 2013).

It is sometimes important to determine the DPS and population origin of individual specimens in these mixed coastal and estuarine aggregations because of their vulnerabilities to anthropogenic stressors at locales distant from their natal estuaries. For example, it has been documented that bycatch of Atlantic sturgeon in coastal fisheries targeted to other species may be an important contributor to the decline of some more vulnerable populations or their failure to rebuild (Wirgin et al. 2015b). Similarly, vessel strike mortalities of Atlantic sturgeon have been shown to frequently occur in the Delaware River (Brown and Murphy 2010) and James River (Balazik 2012) and have been proposed to be a significant factor in the decline of those populations. Migratory subadult Atlantic sturgeon may also be vulnerable to a variety of anthropogenic stressors in the Hudson River, including exposure to toxic chemicals such as PCBs and a perceived recent increased frequency of vessel strike mortalities. Thus, there is a need to identify the origin of individual sturgeon specimens to quantify the vulnerabilities of individual populations and DPS to stressors at locales distant from their natal estuaries.

Because they are highly migratory outside of their natal estuaries, determination of the abundance of subadults and adults of individual populations and DPS and tracking their movements in coastal waters and non-natal estuaries is problematic. Genetic analysis has proven to be an effective tool to identify the population and DPS origin of individual Atlantic sturgeon and their mixed aggregations. Briefly, the genotypes of fish of unknown origin are compared to those in reference collections from known spawning populations. The origin of individual specimens of unknown ancestry is then assigned to the reference collection whose genotypes best match those of the unknown individuals or their aggregations. In practice, this has involved using microsatellite DNA analysis at 11 independent loci and sequence analysis of the mitochondrial DNA (mtDNA) control region to characterize spawning adults and pre-migratory juveniles from reference spawning populations (Wirgin et al. 2012; Waldman et al. 2013; Wirgin et al. 2015ab). Collections of specimens of unknown origin are then characterized at the same 11 microsatellite loci and mtDNA sequence and compared to those in the reference collections. Using an approach termed, Individual Based Assignment (IBA) testing, the population and DPS origin of each individual specimen in a mixed aggregation can be assigned with determined probabilities of accuracy. A second approach, Mixed Stock Analysis (MSA), can be used to determine the proportion of individuals in a mixed aggregation that assign to each reference population and DPS. In this and past studies, we have genetically characterized 1,3497 individuals from 11 reference spawning populations of Atlantic sturgeon at these 11 microsatellite loci and the mtDNA control region. This reference data allows us to determine the origin of subadult Atlantic sturgeon of unknown origin in the current study.

Our objectives in this study as described in our proposal were several fold:

1- Estimate the overall proportion of non-natal subadult Atlantic sturgeon within the lower tidal Hudson River estuary seasonally and identify their population and DPS of origin.
2- Define the overall spatial boundaries of the incursion of non-natal subadult Atlantic sturgeon within the tidal Hudson River estuary and their minimum and maximum length range.

Although not identified in the original proposal, we also felt that it was prudent to address two additional objectives with this data

1- Increase the number of samples in our reference Hudson River collection by adding additional years of juvenile and adult collections.
2- Identify effective population size (N_e) of Hudson River Atlantic sturgeon based on 3 years of juvenile collections.

Methods

Sample collections

In total, we were able to secure 106 subadult juvenile samples from the Hudson River that were collected between early June and mid-November. We targeted specimens that were >600 mm total length (TL) and <1300 mm (TL). Specimens were collected between 2009 and 2014, with the vast majority being collected in 2014. Additionally, almost all of the samples were collected by Normandeau Associates by gill nets with a smaller number coming from trawling. All samples were deposited by Normandeau in the tissue repository housed by the National Ocean Service in Charleston, SC. Unfortunately, a number of samples that Normandeau records showed were deposited with the NOS repository were never located decreasing the number of samples that could be analyzed in this study. Also, during this time (2015), the tissue repository was moved from Charleston, SC to the USGS facility in Leetown, WV which exacerbated the problem.

Additionally, 111 specimens were analyzed from three year-classes of juvenile Hudson River specimens (<500 mm TL) (2011 (n=30), 2013 (n=35), 2014 (n=46)) to bolster our Hudson River reference collection sample size. This would provide us with more confidence in our assignment testing and mixed stock analysis. These reference samples were obtained from the NYSDEC springtime collections from the Haverstraw Bay, NY area.

DNA Isolations

Fin clips were the source of DNA from all samples analyzed in this study. Fin clips were washed with phosphate-buffered saline, and incubated in cetyltrimethyl ammonium bromide (C-Tab) buffer (Saghai-Maroof et al. 1984) and digested at 65^{o} C with proteinase K (Roche Diagnostics, Indianapolis, IN). DNAs were purified by phenol-chloroform extractions, alcohol precipitated, air dried and resuspended in TE buffer. Concentrations and purities of DNAs were evaluated using a Nanodrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). DNA concentrations were adjusted to 50 ng/μl for standardization of subsequent analyses.

Mitochondrial DNA Control Region Sequence Analysis

A 560 base pair (bp) portion of the mtDNA control region was amplified with derived Atlantic Sturgeon-specific primers S1 (5'- ACATTAAACTATTCTCTGGC- 3') and G1 (5'- GAATGATATACTGTTCTACC- 3') (Ong et al. 1996). The same primers were used to sequence a portion of the 560 bp amplicon. We report here data on only 205 bp of the amplicon to allow for comparison of haplotypes in subadult Hudson River specimens to previously characterized reference collections from other rivers (Wirgin et al. 2000; Wirgin et al. 2007; Peterson et al. 2008; Grunwald et al. 2008; Fritts et al. 2016).

Polymerase chain reactions (PCRs) were in 50 μl volumes that contained 50 ng of template DNA, 5 μl of 10 x Roche Applied Science (Indianapolis, IN) reaction buffer, 0.25 μl of each dNTP (25 mM stocks) (GE Healthcare, Piscataway, NJ), 0.07 μl of S1 primer (0.1 μM stock), 0.05 μl of G1 primer (0.1 μM stock) (Integrated DNA Technologies, Coralville, IA), 1 unit of Taq DNA Polymerase (Roche Applied Science) and 43.9 μl of H_20. Amplification conditions were 94^{o} C for 5 min followed by 40 cycles at 94^{o}C for 45 s, 56^{o} C for 45 s, 72^{o} C for 60 s, followed by a final extension at 72^{o} C for 10 min in MJ Research PTC-100™ thermal cyclers. Amplicons were purified with QIAquick PCR Purification kits (Qiagen, Valencia, CA).

Purified PCR products were Dye-Terminator Cycle Sequenced as recommended in GenomeLab Methods Development kits by the manufacturer (Beckman Coulter, Inc., Fullerton, CA). Sequencing conditions were 30 cycles at 96^{o} C for 20 s, 50^{o} C for 20 s, and 60^{o} C for 240 s. Sequencing products were EtOH precipitated, re-suspended in 40 μl of Beckman Coulter CEQ Sample Loading Buffer, loaded into a Beckman Coulter CEQ™ 8000 automated capillary-based DNA sequencer, run using the standard long fast read method (LFR-1), and analyzed with the Sequence Analysis Module of the CEQ™ 8000 Genetic Analysis System.

Microsatellite Analysis

Eleven microsatellite loci were scored that were previously shown to be effective in distinguishing reference specimens from spawning populations (King et al. 2001; Wirgin et al. 2015ab). These loci included LS19, LS39, LS54, LS68 (May et al. 1997), Aox23, AoxD45 (King et al. 2001), and Aox44, AoxD165, AoxD170, AoxD188, AoxD24 (Henderson-Arzapalo and King 2002).

Microsatellite genotypes were determined using the Beckman Coulter sequencer. Individual PCR reactions were multi-pooled, diluted up to 1:3 with Sample Loading Solution (Beckman Coulter), 0.5- 2.0 μl of reactions were loaded onto 96 well plates along with 0.5 μl of CEQ DNA Size Standard-400 and 40 μl of Sample Loading Solution (Beckman Coulter), and run with the FRAG 1 program (Beckman Coulter).

<u>Statistical Analyses</u>

Microsatellite data was initially examined using MicroChecker (Van Oosterhout et al. 2004) to identify the presence of null alleles, scoring errors, and/or large allele drop-out. Individual-based assignment (IBA) tests and mixed-stock analysis (MSA) were used to estimate the DPS and population origin of Atlantic Sturgeon in our collection of Hudson River subadults using the ONCOR program (Kalinowski et al. 2008). ONCOR used genetic data to estimate the population of origin of individuals by performing data analysis and simulations for mixture analysis and assignment tests. In mixture analysis, a reference baseline genetic data set was used to estimate the population composition of a mixed collection using conditional maximum likelihood to estimate mixture proportions. Individual-based assignment tests, using multi-locus likelihood functions, were used to assign individuals in a mixed collection to the reference collection that would have the highest probability of producing the given genotype in the mixed collection. ONCOR used the methods of Rannala and Mountain (1997) to estimate the probability. It should be noted that our analysis of a combination of diploid and haploid mtDNA data violates an assumption of this Monte Carlo resampling method. We estimated mixture proportions with 95% confidence limits based on 10,000 bootstraps. Results were reported for each population in the reference baseline collection as well as for each DPS.

Additionally, leave-one-out- tests were performed in ONCOR to evaluate how well individual specimens could be assigned to the DPS or population from which they were collected. In this test, each individual in each reference collection was sequentially removed from the baseline and its origin estimated using the rest of that reference collection. This test provides a quantitative measure of the accuracy of assignments to each reference or DPS collection.

NeEstimator (Do et al. 2014) was used to estimate effective population size (Ne) using a single-sample method—a bias-corrected version of the method based on linkage disequilibrium (Waples and Do, 2010).

Results

In total, we were able to obtain 106 samples from the NOS and USGS tissue repository for our analysis. Mean total length of the specimens was 990.6 mm (range 595 to 1720 mm). Collection sites in the Hudson River ranged from River Mile (RM) 7 to RM 77 with the vast majority of the specimens being taken between RM 48 and RM 49. Collection dates ranged between mid-June and mid-November with the vast majority of specimens taken in mid-June and early to mid-September. Similarly, most (73%) of the specimens were collected in 2014, but some dated back to as early as 2009.

We were successful in using a combination of microsatellite DNA and mtDNA control region sequence analyses to accurately assign DPS and population origin to all of the specimens using the ONCOR program with our reference data set. Our reference collections used to make these assignments consisted of 1,347 specimens from 11 spawning populations coastwide (Table 1). As indicated in Table 2, our assignment accuracy using leave-one-out tests was very high to the five individual DPS (and Canadian populations) and less so to the individual populations (Table 3). For example, we were 92.1% accurate in assignments at the DPS level and our mean accuracy in assignments at the population level was 85.8%. Specifically, we were 90.3% accurate in assigning Hudson River collected specimens back to the Hudson with the vast majority of the misassignments going to the Delaware River (6.5%)

Using Mixed Stock Analysis in ONCOR, we initially determined the proportions of specimens from the 11 reference collections contributing to our collection of subadults of unknown origin from the Hudson River (Table 4). As expected, the vast majority of specimens were contributed by the Hudson River (95%) with smaller proportions noted from the Kennebec River (2%), James River (1.8%), and Saint John River (<1%).

Given the assignment accuracies described above, we felt confident in using these reference collections to assign our juvenile Hudson River collection to individual DPS and spawning populations. In total, 101 (95.3%) specimens assigned to the Hudson River, in most cases with 100% probability (see Appendix for data on each individual specimen). However, 9 of the 101 Hudson River-assigned specimens did so with less than 100% probability, but in all cases these specimens were assigned to the Hudson with $\geq$ 95% probability. Five specimens assigned to spawning populations other than the Hudson. These included 2 specimens that assigned to the James River, VA, 2 specimens that assigned to the Kennebec River, ME, and 1 specimen that assigned to the Saint John River, NB, Canada. Of these 5 non-Hudson assigned specimens, only one of the James River specimen assigned with 100% probability with the other 4 specimens' assignment probabilities ranging between 82.7% to 94.8%. Surprisingly, these 5 specimens assigned to populations in other than the New York Bight DPS, with 2 assigning to the Chesapeake Bay DPS, 2 assigning to the Gulf of Maine DPS, and one assigning to the Canadian management unit.

Given the new reference collection data from three years collection of Hudson River juveniles generated for this study, we calculated effective population size (*Ne*) estimates for the Hudson River and compared these to our other reference collections coastwide (Table 5). Not surprisingly, we found that the Hudson River had the largest *Ne* coastwide (217.4; 95% CI 156.8-337) followed by the Altamaha River, GA (138.7; 95% CI 103.5-201), and the Savannah River, SC-GA (138.1; 95% CI 109.3-182.7). Also, the Delaware River, the second population in the New York Bight DPS, had one of the smallest *Ne* (41.6; 95% CI 36.6-47.5).

Discussion

Subadult Atlantic sturgeon are known to migrate into coastal waters (Wirgin et al. 2015ab) and subsequently into non-natal estuaries, some of which host natural reproduction and others which do not (Waldman et al. 2013). During these seasonal forays, subadults may be exposed to a variety of anthropogenic stressors in these non-natal estuaries which may acutely jeopardize their survival or cause sublethal effects. Estuarine stressors that were identified in the U.S. federal listing documents (Federal Register 2012ab) included vessel strikes, bycatch, dredging, chemical pollution, compromised water quality, and other environmental perturbations. Many of these stressors to sturgeons are known to occur regularly in the tidal Hudson River estuary. Because Atlantic sturgeon are federally listed and managed as 5 DPS, it is important for Protected Resources managers to evaluate and quantify the potential effects of these stressors on representatives of the individual DPS and perhaps populations that may have migrated to non-natal estuaries (Damon-Randall et al. 2013). However, there was an absence of empirical quantitative data to address the DPS and population origin of subadult Atlantic sturgeon in any non-natal estuary. Therefore, this study was designed to fill this void and determine the DPS and population origin of subadults in the tidal Hudson River estuary. Our overall hypothesis in this project was that all subadult Atlantic sturgeon in the Hudson River seasonally were spawned in the Hudson River. We tested this hypothesis using two DNA approaches, microsatellite and mtDNA analyses, that played a major role in the initial delineation of the 5 DPS and in their subsequent management by NOAA (Federal Register 2012ab).

This study was designed to optimize the likelihood of detecting non-natal specimens in the Hudson River by focusing our analysis on; 1) subadults that ranged in size from 600 to 1300 mm TL, 2) specimens that were collected in summer and fall after the completion of spawning, and 3) were sampled in the lower river where subadults from elsewhere were most likely to aggregate. Although, these were our goals, they were not met as stringently as we would have hoped. That is because our analysis was restricted to specimens that had been previously collected in programs designed for other objectives. Thus, we feel that may have underestimated the proportion of non-natal subadults that seasonally migrate into the Hudson River.

Our major finding was that the Hudson River estuary does seasonally host subadult Atlantic sturgeon that were spawned elsewhere. In fact, approximately 5% of our subadult specimens were spawned in other populations-in all cases not even within the New York Bight DPS. Of the five specimens not spawned in the Hudson River, 2 assigned to the Chesapeake Bay DPS (James River, VA), 2 assigned to the Gulf of Maine DPS (Kennebec River, ME), and one assigned to the Saint John River within the Canadian management unit. Thus, migrations of subadults from other DPS and populations may subject them to a number of stressors that are common in the Hudson River and may not be encountered in their natal rivers. We feel that our assignments of these subadults to other than the New York Bight DPS is accurate given the results of our leave-one-out tests. These tests demonstrated that across all reference collections coastwide, our mean assignment accuracy to the 5 DPS was 92.1% with very few misassignments of Hudson River specimens to the Gulf of Maine or Chesapeake Bay DPS. For example, only 2.2% of Hudson River collected reference specimens misassigned to the Gulf of Maine DPS.

One additional outgrowth of our study was our ability to estimate effective population size (N_e) of Atlantic sturgeon from the Hudson River as well as other populations coastwide. Although the ratio of N_e to census size for Atlantic sturgeon is unknown, it can provide a relative measure of the sizes of individual populations and overall trends in their abundances. Not surprisingly, N_e of the Hudson River population was by far the largest coastwide, far exceeding that of the Delaware River, the second population in the New York Bight DPS. Our N_e results are consistent with thoughts expressed in the most recent Atlantic sturgeon review (ASSRT 2007) in which the Hudson River population was viewed as the most robust coastwide. However, for the first time we provide a quantitative comparative index of the size of Atlantic sturgeon populations coastwide.

Policy Implications

Our results will inform NOAA managers for their Section 7 consultations to evaluate the likelihood of proposed projects to negatively impact Atlantic sturgeon from each of the 5 DPS. Prior to our study, there was an absence of empirical quantitative data on the movements of subadult Atlantic sturgeon to non-natal estuaries and the likelihood of their encountering stressors there. Our estimates of N_e also provided resource managers with the first relative measures of population abundance for each of these spawning populations coastwide, including the Hudson River.

Outreach Comments

Results from this study were recently presented by Dr. Wirgin on May 17, 2016, to USGS and NOAA Office of Protected Resources Managers at the *Atlantic and Shortnose Sturgeon Research and Management: Past, Present, and Future* workshop in Leetown, West Virginia. The title of his talk was: *Use of Individual Based Assignment Tests in the Coastwide Management of Atlantic Sturgeon* by Wirgin, I., D. Fox, T. Savoy, and M. Stokesbury.

Dr. Wirgin also plans to discuss his results and their implications for sturgeon management locally with Amanda Higgs, Robert Adams, and Greg Kinney of the NYSDEC Hudson River Fisheries Unit at their New Paltz, NY office.

As usual, Dr. Wirgin intends to publish these results in a peer reviewed journal as part of a larger manuscript on the use of DNA analysis in the management of Atlantic sturgeon.

Student Training

Ms. Melissa Della Torre, a spring 2016 graduate of the MS program in the Department of the Environmental Medicine of the NYU School of Medicine, participated in conducting research for this project. In it, she was trained in DNA isolations, PCR, mtDNA sequencing, microsatellite DNA analysis, and statistical analysis of population genetics data. She presented a poster on her studies of Atlantic and shortnose sturgeon biology at New York Marine Sciences Consortium annual meeting (Oct, 2015) at which she was awarded a prize for best graduate student poster.

Table 1

Locations where reference Atlantic sturgeon were collected, sample size (N), sampling date, and total lengths (mean TL)

Sampling Location	N	Sampling Date	Total Length Range (cm) (mean TL)	Maturity Status
St. Lawrence River	50	May-June 2014-5	All spawning adults	(A)
Saint. John River	66	July-Aug 1992	All spawning adults	(A)
		Aug 1993	127-244	(A)
	161	May-Aug 2014	162.6-248.9 (199.7)	(A)
Kennebec River	43	June 2010-Aug 2011	133-197.4 (171.6)	(A)
Hudson River	67	May-June 1993-4	160.8-244 (189.3)	(A)
	50	June 2010	170-198 (198)	(A)
	30	March-April 2011	43.2-54 (49.8)	(J)
	35	March-April 2013	41.1-52.8 (46.2)	(J)
	46	April-May 2014	28.7-48.9 (43.9	(J)
Delaware River	33	Sept-Nov 2009	22.0-34.9 (28.4)	(J)
	26	Sept-Nov 2009	22.3-36.7 (30.4)	(J)
	49	Sept-Nov 2011	23.5-36.3 (28.9)	(J)
James River	58	Unknown	26.0-49.5 (45.7)	(J)
	58	July-Sept 2014	All spawning adults	(A)
Albemarle Sound	41	May-Sept 1998	28.6-48.5 (38.8)	(J)
	31	Dec 2006-Jan 2011	27.0-49.9 (40.3)	(J)
	16	Jan 2013-Mar 2014	31.5-49.4 (43.0)	(J)
Edisto River	53	April-Oct 1996	27.7-50 (39.9)	(J)
	52	May-Sept 2005	32.6-48.5 (42.4)	(J)
Savannah River	50	May-June 2013	31.6-44.7 (39.1)	(J)
	50	May-2014	27.4-47.9 (37.0)	(J)
Ogeechee River	26	June 2007-Aug. 2009	19.9-52.0 (28.6)	(J)
	45	July-Aug. 2014	22.7-31.0 (26.0)	(J)
	67	May-July 2015	16.6-44.2 (33.2)	(J)
Altamaha River	49	June-July 2005	31.9-40.4 (37.9)	(J)
	40	July-Aug. 2011	32.7-48.1 (38.6)	(J)
	55	May-June 2014	28.1-48.7 (37.2)	(J)
TOTAL	1,347			

Table 2

Proportion of Reference Individuals Correctly Assigned to the DPS from which They Collected

DPS	N	% Correctly Assigned	Largest Misassignment	DPS
Canada	272	97.4%	1.5%	GOM
GOM	41	83.0%	7.3%	Canada
NYB	323	95.7%	2.2%	GOM
CB	113	91.2%	3.3%	SA
CAR	82	87.8%	11.0%	SA
SA	464	97.0%	2.6	CAR
Mean		92.1%		

Table 3

Proportion of Reference Individuals Correctly Assigned to the Population from which They Were Collected

Population	N	% Correctly Assigned	Largest Misassignment	Population
St. Lawrence	49	95.9%	2.0%	Saint John
Saint John	223	97.8%	1.3%	Kennebec
Kennebec	41	85.4%	4.9%	Saint John
Hudson	216	90.3%	6.5%	Delaware
Delaware	107	86.0%	13.1%	Hudson
James	113	90.3%	2.7%	Altamaha
Albemarle Sound	82	85.4%	4.9%	Altamaha
Edisto	94	91.5%	4.3%	Altamaha
Savannah	99	71.7%	15.2	Altamaha
Ogeechee	135	71.1%	13.3%	Altamaha
Altamaha	136	78.7%	12.5%	Savannah
		X=85.8%		

Table 4

Mixed stock analysis of reference population proportions of subadult Atlantic sturgeon in the Hudson River

Population Estimates	**%**	**95% Confidence Intervals**
St. Lawrence	0.000	(0.000, 0.013)
Saint John	0.008	(0.000, 0.030)
Kennebec	0.020	(0.000, 0.075)
Hudson	0.954	(0.880, 0.991)
Delaware	0.000	(0.000, 0.025)
James	0.018	(0.000, 0.047)
Albemarle	0.000	(0.000, 0.020)
Edisto	0.000	(0.000, 0.000)
Savannah	0.000	(0.000, 0.019)
Ogeechee	0.000	(0.000, 0.000)
Altamaha	0.000	(0.000, 0.000)

Table 5

N_e estimates based on 2 or 3 years (except Kennebec) of collections combined using the linkage disequilibrium method

Population	N_e	95% CI	Rank
Saint John*	51.5	46.6-57.1	6
Kennebec	53.0	40.5-73.2	4
Hudson*	217.4	156.8-337	1
Delaware	41.6	36.6-47.5	8
James	45.5	41.1-50.5	7
Albemarle*	21.2	19.1-23.6	10
Edisto	52.8	45.1-62.4	5
Savannah	138.1	109.3-182.7	3
Ogeechee	34	29.7-39.1	9
Altamaha	138.7	103.5-201	2

*based on three years of collections

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Information Transfer Program Introduction

The Director and staff of the NYS Water Resources Institute undertake public service, outreach, education and communication activities. Most are conducted through multidisciplinary projects funded outside the Water Resources Research Act (WRRA) context. In order to couple WRRA activities to other NYS WRI activities, a portion of WRRA resources are devoted to information transfer through a partnership program with the Hudson River Estuary Program, dissemination of information related to emerging issues, and student training.

Hudson River Estuary Program Partnership

The NYS Department of Environmental Conservation Hudson River Estuary Program is a conservation program focused on the Hudson River and its watershed. The Program is guided by the Hudson River Estuary Action Agenda 2015-2020, which defines the challenges faced and identifies practical solutions that can be carried out by civic leaders, policy makers, and citizens working together. The 2015 - 2020 Action Agenda is organized around six key benefits that result from a strong and vibrant estuary ecosystem and watershed, including: 1. Clean Water, Resilient Communities, 2. Vital Estuary Ecosystem, 3. Estuary Fish, Wildlife, and Habitats, 4. Natural Scenery, and 5. Education, River Access, Recreation, and Inspiration. WRI and DEC work together to protect and restore the rich Hudson Estuary Ecosystem, a source of drinking water, habitat for a host of resident and migratory species, and a boating, swimming, and fishing asset for the Hudson Valley resident and tourists.

A summary of selected WRI information transfer activities is provided below

New York State Water Resources Institute FY2015 Activity

For additional information on all activity, see wri.cals.cornell.edu

Peer Reviewed Publications (details provided in the Research Program section)

Trade & Extension Publications

1. Rahm, B.G. and S. Vedachalam, 2015, Water without Borders: The Importance of Regional and Intermunicipal Water Resource Planning, New York State Association of Counties, Spring/Summer News.

2. Rahm, B.G.; Vail, E.; Vedachalam, S.; Kay, D., Growing green infrastructure: lessons from new research, Planning News, New York Planning Federation, Winter 2015, 12-13. http://www.nypf.org/editable/documents/Winter2015Newsletter.pdf

3. Vedachalam, S.; Rahm, B.G.; Tonitto, C.; Riha, S.J., Engaging researchers and stakeholders in improving New York's water management, Community and Regional Deveopment Institute Research & Policy Brief Series, 65, April, 2015.

Conference Presentations & Invited Talks

1. Truhlar, A.M.; Rahm, B.G.; Brooks, R.A.; Nadeau, S.A.; Walter, M.T., Greenhouse gas emissions from septic systems in New York State, AGU Fall Meeting, 2015, San Francisco, CA – poster

2. Vedachalam, S.; Joo, T.; Riha, S.J. 2015, Using Geospatial Data to Analyse Trends in Onsite Wastewater Systems Use. Mohawk Watershed Symposium, Schenectady, NY.

Press

1. Neighbors go two months without water in Niagara Falls, WGRZ Buffalo, March 31st, 2015.

2. Winter runoff into streams on par with ocean salinity, Cornell Chronicle, April 1st, 2015.

3. Culverts: in need of action, Register-Star (Hudson, NY), April 1st, 2015.

USGS Summer Intern Program

None.

Student Support					
Category	Section 104 Base Grant	Section 104 NCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	1	0	0	0	1
Masters	1	0	0	0	1
Ph.D.	0	0	0	0	0
Post-Doc.	1	0	0	0	1
Total	3	0	0	0	3

Notable Awards and Achievements

NYSWRI article "Desalination in northeastern U.S.: Lessons from four case studies" is cited by Rockland County Legislature Chairman Alden Wolfe in a letter to the New York State Public Service Commission.