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NSF/REU Site

Interdisciplinary Water Sciences and Engineering

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LEWAS LAB
LEARNING ENHANCED WATERSHED ASSESSMENT SYSTEM

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Acknowledgements

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Summary

This Research Proceedings includes papers of undergraduate research that was conducted at Virginia Tech during summer 2017 as part of an NSF/REU Site on Interdisciplinary Water Science and Engineering. This is the first year of the 4th cycle of the NSF/REU Site that was implemented on VT campus during 2017, 2018, and 2019. This 4th cycle follows three very successful REU Site cycles that were implemented during 2007-09, 2011-13, and 2014-16. Research Proceedings of all these years are available at: www.lewas.ictas.vt.edu. An international component was included in this cycle and two REU scholars had opportunities to conduct a part of their research activity at the Indian Institute of Technology, Madras (IITM). Two REU scholars visited IITM during 2017 summer. At the end of summer 2017, 95 REU Fellows (62 women and 33 men) have graduated from REU Sites. Our Site continues to expose qualified undergraduates to interdisciplinary research issues in water sciences and engineering. Faculty members from six departments (Engineering Education, Civil and Environmental Engineering, Biological Sciences, Geo-sciences, Forest Resources and Environmental Conservation, and Crop and Soil Environmental Sciences) at Virginia Tech mentored 10 excellent undergraduates who were recruited out of a nation-wide competition. Ten graduate students from these departments assisted the faculty mentors and got a valuable experience in mentoring undergraduate research students. Figure 1 shows a word cloud of the keywords that describe the research activities undertaken during the 10-week research at VT.

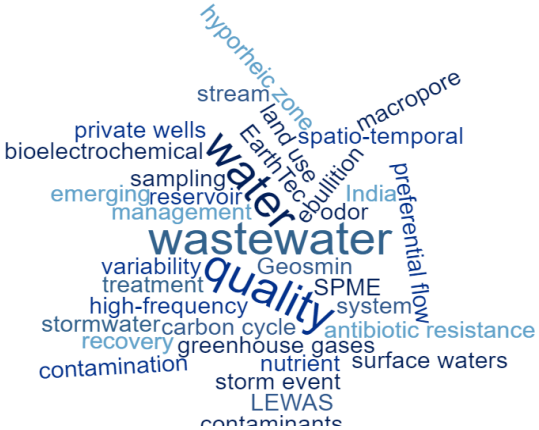

Figure 1: Word Cloud of Keywords- 2017 Research Work

Mr. White (an REU scholar from Yale University) and his co-authors investigated nutrient recovery from wastewater using a bioelectrochemical system. The researchers investigated the ammonium recovery from a partially submerged tubular Microbial electrolysis cells (MECs) and analyzed the distribution between liquid cathode solution and air. Their preliminary conclusion was that a tubular MEC had adequate potential for ammonium recovery from the wastewater. Mr. Perkins (an REU scholar from Univ. of Virginia) and his co-authors examined spatio-temporal dynamics of streams using a high-frequency water quality sensor and found a moderately-strong relationship between percent agricultural land use in a watershed and mean

nitrate levels. The study also provides support for the expanded use of high-frequency, automatic, *in-situ* water quality monitoring. Ms. Donaldson (an REU scholar from Humboldt State University) and her co-authors carried our investigations of the dimensions, distribution, and abundance of macropores throughout Virginia. Specifically, the study revealed the geometry (width, height and depth), distribution, and abundance of naturally-occurring macropores within 12 streams throughout Virginia. These macropores pay an important role in surface and groundwater interactions in the hyporheic zone. Ms. Cahill (an REU scholar from Georgetown Univ.) and her co-authors studied the emergence of emerging contaminants in an Urban Water System impacted by storm water. Surface water samples, collected from ten locations impacted by the outflow of stormwater runoff, contained 21 out of 36 screened emerging contaminants (ECs) commonly found in waste water. The authors opined that detection of anthropogenic ECs in storm water-impacted surface water suggested possible input of sewer water into storm water. Ms. Lopez (an REU scholar from Florida State University) and her co-authors studied the effectiveness of EarthTec® at initiating the acidic dehydration of geosmin in river water from a waterway that experienced taste-and-odor issues. There were no statistically significant changes in geosmin concentrations ($p > 0.25$) after the addition of either 1 or 10 ppm EarthTec® to distilled or river water. Ms. Mapili, Ms. Cook (REU scholars from Virginia Tech and Milwaukee School of Engineering, respectively who traveled for a few weeks to IITM, India) and their co-authors examined the occurrence of antibiotic resistance in wastewater treatment plants in Chennai, India. The results of this study are expected to help identify critical points along the wastewater treatment process where antibiotic resistance dissemination may be controlled. Ms. Finegold (an REU scholar from Oberlin College) and her co-authors measured CH_4 ebullition rates in a managed eutrophic drinking water reservoir in SW Virginia during two planned epilimnetic aeration mixing events. It was found that epilimnetic mixing management might increase ebullition rates, but that any stimulation of CH_4 fluxes might be dependent on the duration and timing of mixing. Ms. Freeman (an REU scholar from UNC, Charlotte) and her co-authors evaluated disparities in North Carolina well website communications. It was observed that the readability levels of the landing pages for the county well webpages were above the average reading level in America (8th grade). Mr. Wahlquist (an REU scholar from Binghamton University) and his co-authors designed an educational virtual environment in the context of the environmental data gathered by the Learning Enhanced Watershed Assessment System (LEWAS) on VT campus.

Research Papers

Occurrence of Emerging Contaminants in an Urban Water System Impacted by Storm Water

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Abstract: As urbanization increases globally, a major class of emerging contaminants (ECs), pharmaceutical and personal care product (PPCP) usage increases. ECs have been found in various water sources, raising concern toward ecosystem and human health. Surface waters commonly receive minimally treated storm water runoff. The study investigates detectable ECs in surface water and aims to identify possible sources of ECs from sewer leakage into storm water. Surface water samples were collected from ten locations impacted by the outflow of storm water runoff and screened for ECs using ultra-performance liquid chromatography – tandem mass spectrometry. The screening identified 21 out of 36 screened ECs commonly found in waste water. Detection of anthropogenic ECs in storm water-impacted surface water suggests possible input of sewer water into storm water.

Keywords: emerging contaminants, stormwater contamination, urban-impacted surface waters, sewage exfiltration

1. Introduction

1.1 Occurrence of emerging contaminants in the aquatic environment

Emerging contaminants differ from typical water contaminants as they are newly discovered in water sources or are found in greater proportions than before. (Matamoros, Arias, Nguyen, Salvadó, & Brix, 2012)(Houtman, 2010) Due to the emerging nature of concern towards these substances, they are largely unregulated and therefore pose a potential for great harm to aquatic environments. A contaminant is "emerging" until its persistence and/or ecotoxicological impacts can be determined. (Field, Johnson, & Rose, 2006) Two of the major classes of emerging contaminants are pharmaceuticals and personal care products (PPCPs). Pharmaceuticals are of particular interest as emerging contaminants due to the nature of medical products to produce biological changes. (Halling-Sorensen et al., 1998) Active ingredients in personal care products such as fragrance, UV blockers, nanoparticles, etc. are also of interest as emerging contaminants. These active ingredients have the potential to be continually introduced to the aquatic environment. In this way, the EC's act as pseudo-permanent contaminants. Though these contaminants may degrade, their pseudo-permanence may prove more harmful than other organic contaminants that are not continually re-introduced. (Ebele, Abou-Elwafa Abdallah, & Harrad, 2017) Long-term effects of these persistent contaminants on aquatic organisms and environmental health are largely unknown. (Daughton, Ternes, 1999) PPCPs as ECs are an integral part of daily life, magnified in areas of higher populations. Therefore, ECs are used as indicators of anthropogenic impact on water sources. Some examples of ECs detected in leachate from an operating landfill in Oklahoma were acetaminophen at 0.009 micrograms per liter, caffeine at 0.0140-0.0800 micrograms per liter, cotinine at 0.0230-0.0800 and triclosan at 0.0500 micrograms per liter in 2000. (Table 1, Andrews, Masoner, & Cozzarelli, 2012)

The purpose of pharmaceuticals is to be available to the body to treat disease. The problem with pharmaceuticals in the aquatic environment is that drug ingredients have a high bioavailability. Some personal care products have active ingredients which also affect biological processes. Personal care product ingredients have been found in algae, indicating their bioavailability. (Coogan, Edziyie, Point, & Venables, 2007) Organisms unintentionally exposed to these harmful ingredients in the aquatic environment can absorb these chemicals through skin or ingestion. ECs are shown to induce physiological effects in humans and aquatic organisms. (Ebele et al., 2017) The general term, "aquatic environment" refers to any surface water, ground water, freshwater, saltwater, well water, and drinking water. One of the chief aspects of aquatic environments is the ability for waters to cover vast distances. Water as a fluid is far-reaching in its travels. Surface waters including streams, creeks, lakes, oceans, ponds, etc. flow into one another, mixing and spreading the span of emerging contaminants present in these waters. Ground water seeps into surface water by passing through soil or dirt, allowing contaminants to travel with it. The nature of the aquatic environment is highly interconnected.

1.2 Sources of emerging contaminants

Emerging contaminants come from a variety of sources. One important source of ECs in surface water is passage through sewage treatment plants. When humans ingest pharmaceuticals, the excreted chemicals flow down the sink, toilet, and shower drains as waste water. The waste water travels along sewage pipes to a sewage treatment facility. Industry and hospitals also produce waste water which goes to sewage treatment plants. (Fick et al., 2009) Sewage treatment plants only partially remove ECs, meaning water reused for agriculture, drinking, and home/institution water supply can contain a variety of EC combinations. (Petrovic, 2003)(Chen et al., 2013) Another source of ECs in waste water is in the water that does not go to the wastewater treatment plant. Sewage overflows in separated sewer systems allow excess untreated sewage waters to directly enter surface waters. Agricultural runoff can also introduce ECs to surface and ground water due to pharmaceutical presence in animal waste, fertilizers, and pesticides. (Nikolaou, Meric, & Fatta, 2007)

1.3 Urban sewage system infrastructure and storm water collection systems

Urban sewage and storm water system structure is important in holistic understanding of the importance of emerging contaminants. There are two main kinds of sewage systems: combined sewer systems and separated sewer systems. In a combined sewer system, sewage water joins storm water along the route to a wastewater treatment facility. In a separated sewer system, sanitary sewers take wastewater to sewage treatment plants and storm water travels through storm drains directly into surface and ground water. The separated sewer system is typical to newer water systems and are considered an improvement to combined sewage systems as they eliminate combined storm water-sewage overflows. (US EPA, 1999) Both separated and combined systems house overflows meant to discharge excess water in the extreme weather events to prevent backflow. In combined sewage systems, the overflows contain both municipal wastewater and surface water. In separated sewage systems, sewer overflow water is comprised of municipal wastewater in sewage pipes and storm water contains runoff. (Balmforth, 1990) Though the pipes are separated, they cross paths along their route. (Sercu, Van De Werfhorst, Murray, & Holden, 2011) Since research indicates sewage contamination in storm water in separated storm drain systems, it is important to understand where this contamination comes from and how to solve it. (Sercu et al., 2011)(Guo & C. S. Song, 1991) Emerging contaminants in separated sewage systems during high flow periods may be due to leaky sewage pipes resulting from pressures from heavy rain/stormy periods. Emerging contaminant presence during low flow periods may be a result of these damaged sewage pipes.

1.4 Chemical and biological markers for monitoring the exfiltration of sewage systems

Along the way, sewage pipes may leak due to system aging, pressure, or poor construction. (Guo & C. S. Song, 1991)(Sercu et al., 2011) If this occurs, the sewage may contaminate storm water, leading to the contamination of storm water discharge. When these pipes leak, human feces bacteria, is then introduced to storm water. (Gannon & Busse, 1989) E. coli has been previously found in storm waters, indicating fecal

contamination. (Gannon & Busse, 1989) E. coli is not exclusive to human feces. Rather, it is present in human and animal fecal matter. The E. coli could be a result of sewage presence, agricultural runoff, or both. To determine whether the reservoir contamination is due to leaky sewage pipes rather than agricultural runoff, the presence of HF-183, a specifically human genetic marker, must be detected. If HF-183 is detected in surface water, it would indicate contamination from sewage systems in surface water and sewage exfiltration as the source of ECs.

Since storm water is assumed to contain less human waste, they often flow directly into surface water, i.e. rivers, creeks, and lakes. The origin of ECs in these bodies of water is not yet certain, but could be linked to a leaky sewage system through this study. Presence of ECs is problematic not only for the body of water's biota but also for human health. People use surface water every day to cook, clean, maintain hygiene, and drink. Once the sources of emerging contaminants in storm drain water are determined, problem solving can begin to prevent further contamination in watersheds and remediate the current problematic contaminants.

1.5 Tracking storm water path

The literature review identified sewer leakage as an important source of detectable ECs in surface water, either through groundwater or through storm water. Fenz, et al., (2005) monitored carbamezapine, an antiepileptic drug in waste and ground water with the purpose of assessing sewer exfiltration. (Fenz et al., 2005) This study reported sewer leakage into groundwater as a possible source of detectable ECs in surface waters as groundwater and surface water interchange. Sercu et al., (2011) traced sewer leakage into storm water by placing dye in sewage pipes and detecting them later in storm drains. The study investigated storm water contamination, specifically, and sewer exfiltration as a contamination source during low flow periods. (Sercu et al., 2011) The study was confirmed with a positive finding of HF-183 within storm water, linking sewage exfiltration to storm water contamination.

1.6 Objectives

- To screen storm water-impacted streams throughout an urban area for various ECs.
- To investigate the presence of 36 ECs in storm water-impacted surface waters.
- To connect EC presence in storm water-impacted surface waters, to the acknowledgement of storm water as a carrier for ECs from sewer exfiltration

2. Research Methods & Experimental Setup

2.1 Sampling Sites

Water samples were collected from surface waters at ten locations of a city with a population of 99,610 (Fig 1). (U.S. Census Bureau, 2016) The locations were chosen as they were impacted by storm water overflows, making storm water an important component of these surface waters. Eight of the locations were previously found to have high E. coli levels and two locations were found to have low E. coli levels (Table 1). (City of Roanoke, 2016) Two sampling trips were taken at the same locations 14 days apart. Both trips were during low flow periods. The first trip on June 14th, 2017 had 0.83 inches of precipitation in the past 24 hours while the second trip on June 22nd, 2017 had 0.00 inches of precipitation in the past 24 hours.

Figure 1. Layout map of 10 urban storm water sampling sites.
All ten sites fall within the city's municipal boundary. The map provides a visual of the approximate distance between sites using the map scale included in the bottom left corner. Green, yellow, and orange dots indicate low, medium, and high detected E. coli levels in the water, respectively. The sites with low E. coli levels are termed as reference sites.
** Though site 2 E. coli levels were at low levels during the time of sampling, site 2 is not considered a reference site due to bacterial level fluctuations (up to above 3000 CFU's) in previous tests and a detectable sewage smell.

Table 1. Sampling site labels and descriptions

General E. coli level	Site label	E. coli counts in water (6/14/17)
Low (Reference sites)	Site 1	<235 CFU
	Site 4	<235 CFU
Med-High/Variable	Site 2	<235 to >3000 CFU (variable)
	Site 3	1001-3000 CFU (high)
	Site 5	236-1000 CFU (med)
	Site 6	236-1000 CFU (med)
	Site 7	236-1000 CFU (med)
	Site 8	236-1000 CFU (med)
	Site 9	236-1000 CFU (med)
	Site 10	1001-3000 CFU (high)

2.2 Sample collection and preservation approaches

Water samples were collected from each site by wading to the center of the water body, washing the container with the water, and filling the glass container halfway with the water while facing upstream. The samples were protected with bubble wrap to prevent breakage and kept in coolers with ice for transport between sampling sites and lab. Both water and sediment samples are preserved in a -20° freezer to prevent EC degradation until ready for analysis. During the freezing process, lids are kept loose to minimize pressure buildup leading to glass breakage and sample contamination. Lids were tightened once samples were frozen. Figure 2 illustrates the outline and sequence of sample collection, preservation, preparation, and analysis.

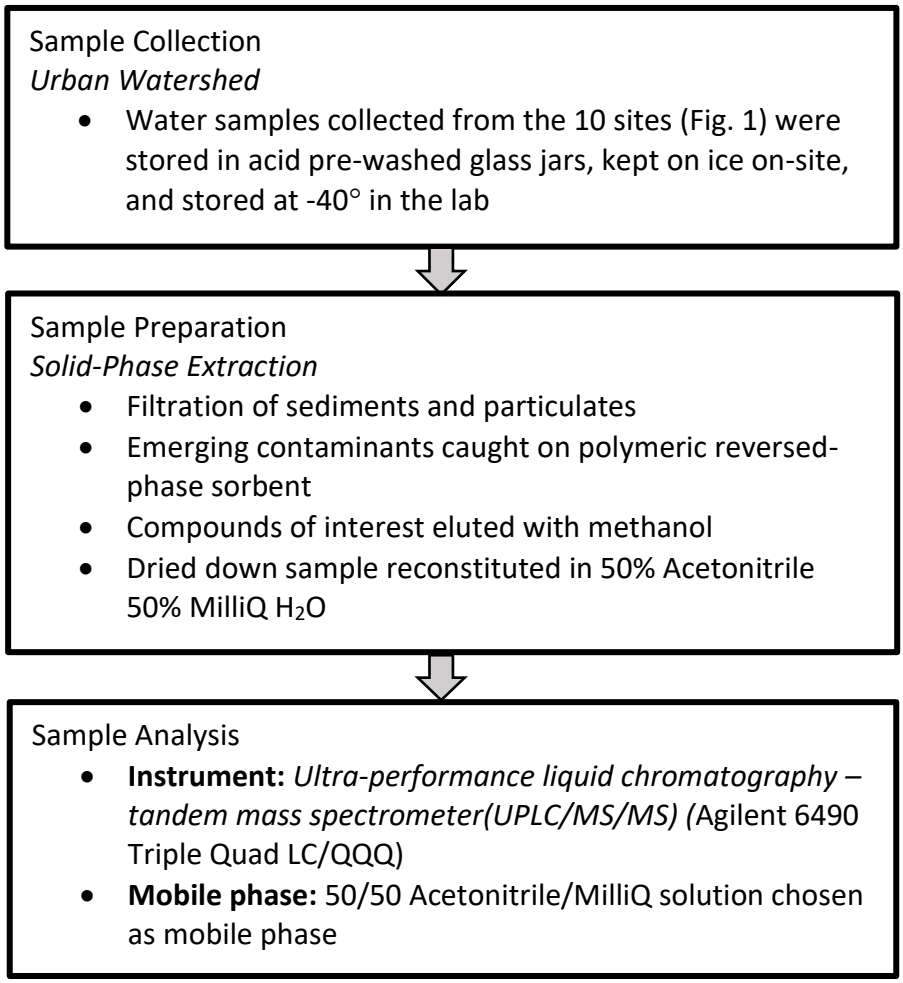


Figure 2. Summary of entire PPCP screening process of water samples from collection to analysis General structure of PPCP screening for water samples including three main steps: sample collection, preparation, and analysis. Storm water impacted water samples were collected, prepared with solid-phase extraction, and analyzed by UPLC/MS/MS.

2.3 Sample extraction and cleanup

Solid-phase extraction (SPE) is used to extract and clean-up larger volume water samples to concentrate the ECs to be screened. The process separates the background matrixes from target analytes in a liquid mixture. The analytes adhere to a stationary phase and are eluted off with a smaller volume of an appropriate solvent to achieve a higher final EC concentration than the original sample had. The concentrate is more detectable on an analytical instrument. SPE is a relatively cheap and quick way to concentrate and clean compounds of interest within water samples. (Zhang, 2013)

The first step of processing water samples was to let the samples thaw for 8 to 12 hours while covered to reduce light exposure. Once thawed, 250 mL of each water sample were placed in a 500 mL mason jar. Four extra mason jars were filled with 250 mL of MilliQ water and 2 of the jars were spiked with 50 μL of 1 ppm sulfamethazine, chlorotetracycline hydrochloride, and tylosin tartrate stock solutions (all purchased from Sigma Aldrich) to equal 50 ppb of each. Next, the water samples from each site were filtered by attaching a vacuum hose and a glass 0.70 micron 55 mm Whatman filter to a Pyrex glass filtering flask. Filter paper was placed between the pieces of the glass filter, the pieces were clamped together, and each sample was run through with a vacuum. The filtered sample was poured back into the mason jar it was originally in. The Pyrex flask and filter papers were replaced between samples. New cartridges (60 mg, 3

cc, Oasis HLB, Massachusetts, USA) were setup on the SPE module. Cartridges were conditioned with 3 mL of 100% methanol and then 3 mL of MilliQ water without vacuum and without allowing the pellet to dry. Hoses rinsed with DI water were attached to the cartridges and ends taped into their respective samples. Samples were run through the cartridge using the vacuum pump at ≈5mL/min. Once all the samples ran through, cartridge were cleaned with about 5 mL of MilliQ water. SPE module tips were then wiped with KIM wipes to get rid of excess water. The vacuum pump was left on for 5 minutes then turned off to let the cartridges continue to dry with knobs open for 5 minutes. One 6 mL test tube was placed under each sample. The cartridge was eluted with 3 mL of 100% methanol without a vacuum. Samples were then dried down for 60 minutes at 50°C at starting at 400 mmbar and reducing by about 25 mmbar every 2 minutes to reach the final pressure 115 mmbar on the Rapidvap. Dried-down samples were then reconstituted with 1 mL of UPLC/MS/MS mobile phase (50% acetonitrile, 50% H2O) and vortexed at speed 8 for 30 seconds. The sample was extracted from the test tube using a disposable green syringe with a disposable needle attached. The syringe was inverted so the air bubble flows to the needle end, the needle was removed and a PTFE 0.2 micro filter was attached. The sample was passed through the PTFE 0.2 microfilter into a 2 mL amber HPLC vial. The sample was then ready for the UPLC/MS/MS analysis.

2.4 UPLC/MS/MS analysis

Used after solid-phase extraction, ultra-performance liquid chromatography – tandem mass spectrometry (UPLC/MS/MS) is a high resolution, high speed, and sensitive method to detect and quantify PPCPs in both surface and waste water. (Steene & Lambert, 2008)(Nov, Matysov, & Solich, 2006)(Gros & Petrovi, 2006)(Churchwell, Twaddle, Meeker, & Doerge, 2005) UPLC has the analytical advantage over HPLC as it uses small particle size columns and a higher pressure. (Batt, Kostich, & Lazorchak, 2008) The method used is a list of specific compounds of interest that the UPLC/MS/MS technique will test for. The method may be a list of most-prescribed pharmaceuticals that would most likely be within water sources, or a list of chemicals that have been previously detected. (Batt et al., 2008) The UPLC/MS/MS technique combines the physical separation of compounds from liquid chromatography with the mass analysis of mass spectrometry in one machine.

The acetonitrile used for the mobile phase was a submicron filtered HPLC grade solvent purchased from Fisher Scientific. Reference antibiotic standards, sulfamethazine, chlorotetracycline, and tylosin prepared in 50% acetonitrile 50% MilliQ water at final concentrations of 50 ppb, were analyzed on the Agilent 6495 Triple Quadrupole Mass Spectrometer to indicate percent recovery for quality assurance and quality control (QA/QC). To calculate recovery, the peak area of a spiked compound was compared to 50 ppb antibiotic spiked samples that underwent solid-phase extraction. The 50 ppb stock antibiotic samples recovery provided a quantitative means of checking machine functionality and method quality. The method used for this project screened for 36 EC compounds (Table 2) including drugs of abuse (MDMA, Cocaine), antibiotics commonly found in waste water (e.g. sulfamethazine, tetracycline), pharmaceuticals such as anticonvulsants and antidepressants, and chemicals used in agriculture (e.g. EDDP). The compounds were analyzed by the multiple reaction monitoring (MRM) method. MRM method monitors a precursor ion and two product ions (Table 3).

Table 2. Emerging contaminant (36) pharmacological functions

Compound name	Pharmacology
Amitriptyline	tricyclic antidepressant with anticholinergic and sedative properties.
Atrazine	widely used herbicide known to cause birth defects and menstrual problems
Buprenorphine	more potent and longer lasting analgesic than morphine
Carbamazepine	anticonvulsant used to control grand mal and psychomotor or focal seizures, TCA
Cefotaxime	antibacterial agent
Chlorotetracycline	anti-bacterial agent, inhibits protein synthesis
Clenbuterol	bronchodilator in asthma

Cocaine	local anesthetic and vasoconstrictor particularly in the eye, ear, nose, and throat
Cotinine	potential cognition enhancement, anti-psychotic activity, and cytoprotection
DEET	topical insect repellent
Dextromethorphan	cough treatment
Diltiazem	calcium channel blocker
EDDP	rice fungicide
Erythromycin	bacterial protein synthesis inhibitor
Escitalopram	antidepressant, selective serotonin uptake inhibitor (SSRI)
Gabapentin	anti-epileptic Agent
Lidocaine	local anesthetic and cardiac depressant used as an antiarrhythmia agent
Lorazepam	anti-anxiety agent with hypnotic, anticonvulsant, and considerable sedative properties
MDMA	hallucinogen and causes marked, long-lasting changes in brain serotonergic systems
Mefenamic acid	non-steroidal anti-inflammatory agent with analgesic, anti-inflammatory, and antipyretic properties
Meprobamate	anti-anxiety agent
Metformin	diabetic treatment that improves insulin sensitivity and decreases glucose absorption for glycemic control
m-Hydroxybenzoylecgonine	cocaine metabolite
Nifedipine oxidized	antianginal and antihypertensive
Ormetoprim	antibacterial agent
Primidone	antiepileptic agent
Propanolol	hypertension, migraine, and anxiety treatment
Sertraline	selective serotonin reuptake inhibitor (SSRI) used in the therapy of depression, anxiety disorders and obsessive-compulsive disorder
Sulfamethazine	antibacterial agent
Tetracycline	used to treat bacterial infections, may be used to treat acne
Thiabendazole	antihelminthic, antiparasitic drug
Triamterene	decreased Renal K+ Excretion, and Increased diuresis, blocks sodium-potassium exchange pump
Trimethoprim	treatment of UTI's, kidney infection, and prostatitis
Tylosin	antibiotic and a bacteriostatic feed additive used in veterinary medicine
Venlafaxine	serotonin and norepinephrine reuptake inhibitor widely used as an antidepressant
Vancomycin	anti-bacterial agent, destroys bacterial cell wall synthesis

All chemical information found at: https://pubchem.ncbi.nlm.nih.gov/.

Table 3. Screened compounds MS/MS Precursor and Product Ions

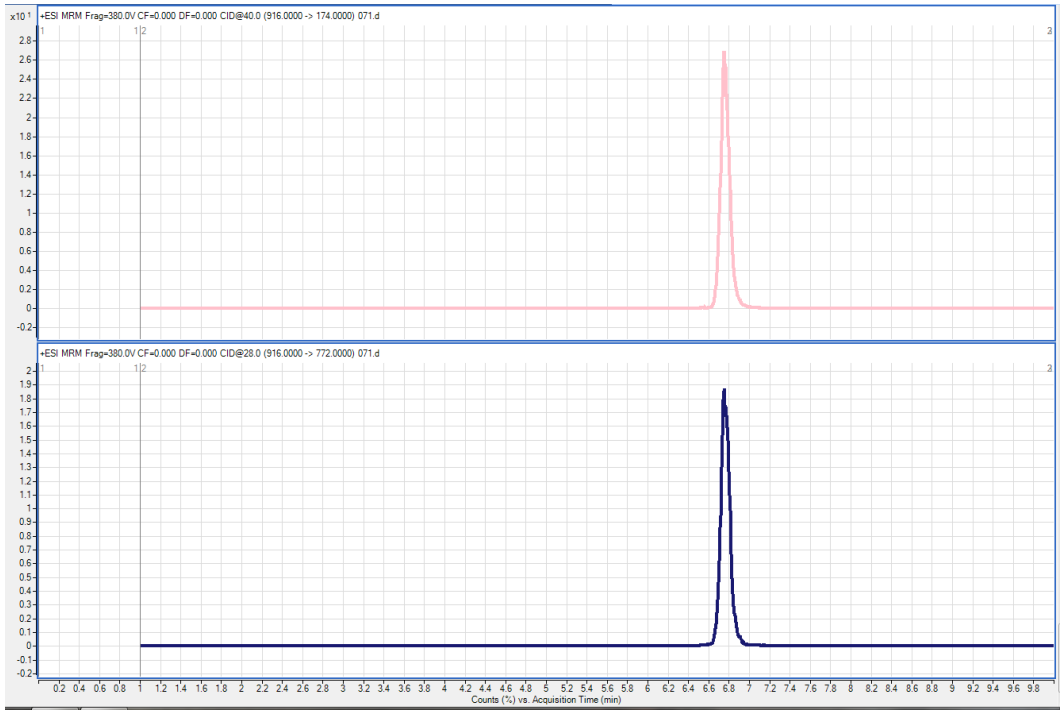
Analyte	Precursor ion	Product ion 1	Product ion 2
Amitriptyline	278.19	202.2	91
Atrazine	216.1	174.1	68.2
Buprenorphine	468.31	84.2	55.1
Carbamazepine	237.1	194.1	193.1
Cefotaxime	455.47	154	410
Chlorotetracycline	479	444	462
Clenbuterol	277.09	203	132.1
Cocaine	304.16	182.2	16
Cotinine	177.1	98	80.1
DEET	192.14	119.3	91
Dextromethorphan	272.20	171.1	128.1
Diltiazem	415.17	178.1	109.1
EDDP	278.19	249	234.1
Erythromycin	734.47	158.1	83.1
Escitalopram	325.17	262.2	109.1

Gabapentin	172.14	154.1	55
Lidocaine	235.18	86.2	58.1
Lorazepam	321.02	275.1	229.2
MDMA	194.12	163.1	77.1
Mefenamic acid	242.12	224	208
Meprobamate	219.14	158.1	97
Metformin	130.1	71.1	60
m-Hydroxybenzoylecgonine	306.14	168.1	65.2
Nifedipine oxidized	347.13	315.2	195.1
Ormetoprim	274.32	123	259
Primidone	219.12	162.1	91.2
Propanolol	260.17	116.1	56.1
Sertraline	306.08	275	159.1
Sulfamethazine	279.09	186	92.1
Tetracycline	444.44	154	410
Thiabendazole	202.05	175	131.1
Triamterene	254.12	237.1	104.1
Trimethoprim	291.15	230.2	123.2
Tylosin	916.53	174.2	83.1
Venlafaxine	278.21	260.3	58.2
Vancomycin	725	100	144

Agilent *Highly Sensitive Detection of Pharmaceuticals and Personal Care Products (PPCPs) in Water Using an Agilent 6495 Triple Quadrupole Mass Spectrometer*
Table 1, Chiesa et al., 2016.

In the identification of singular chemicals in a mixture, Agilent MassHunter Qualitative and Quantitative measuring techniques were used. First, the qualitative software was used. The MRM method was applied to all samples, testing for PPCPs and antibiotics. If both product ions were showed peaks at a retention time similar to known retention times for that compound and a signal-to-noise ratio (SNR) was greater than 3, the compound was identified as present. Figure 3 shows what a peak looks like for Tylosin, which has a retention time of about 6.8 in a 50/50 Acetonitrile/MilliQ H_2O mobile phase. The qualitative analysis is imperative to distinguishing desired compounds from background interference. Once the compound was determined to be present qualitatively, quantitative software was used. The quantitative analysis involved first setting up a batch of acquired MRM data files. The process involved changing any incorrect retention times to the known value so that the peak areas at that time was taken. Any peak areas of compounds determined absent by qualitative analysis were ignored. The peak areas of present compounds were then used to determine how much of the compound was present. A total of 270 samples were run, 60 of which were site samples run in triplicates (two sampling trips, 10 sites each trip). The rest of the samples were solvent spikes, spikes and MilliQ H_2O blanks which underwent solid-phase extraction, and method blanks between triplicates. Each subset of the triplicates involved solvent spikes, spikes, method blanks, and mobile phase blanks for QA/QC.

Compound present:



Compound not present:

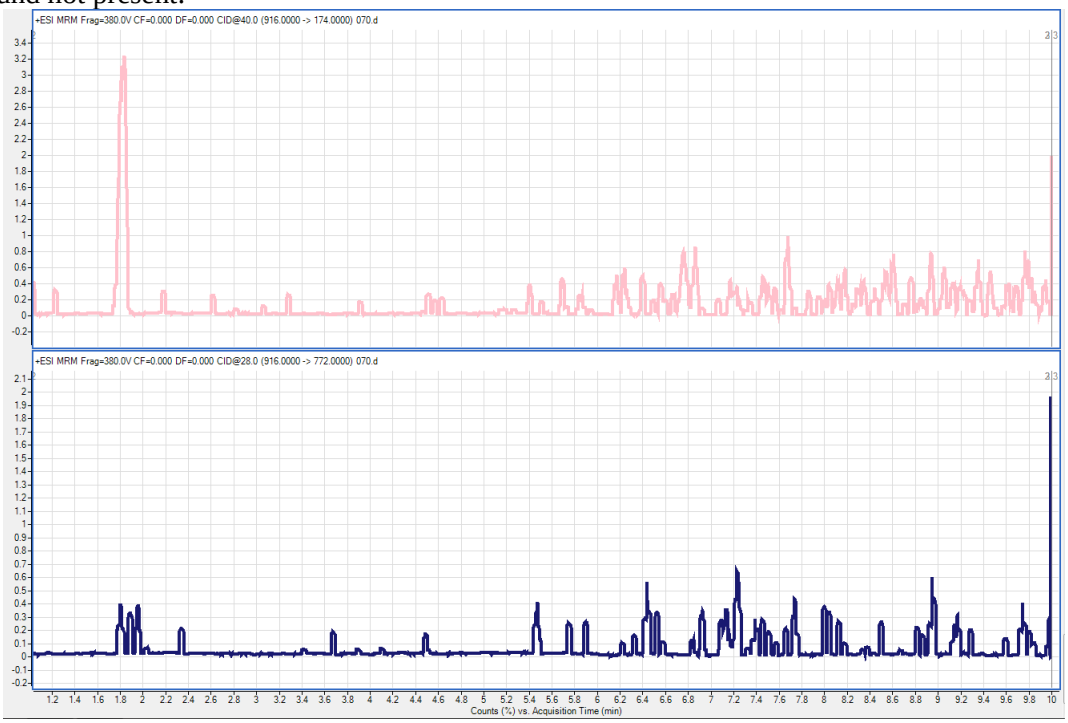


Figure 3. UPLC/MS/MS chromatogram shows a peak at appropriate retention time (x-axis) for both daughter ions when a compound is present in a water sample, while no peak is observed for both daughter ions at the same retention time when a compound is absent in a water sample.

3. Results

The specific screened compounds were chosen because they had been detected previously in waste water in the Xia lab during UPLC/MS/MS method development. Many of the screened compounds enter the environment through secondary wastewater treatment plant effluents exclusively, meaning the ECs serve as anthropogenic markers. Sampling urban surface waters leads to knowledge on the specific emerging contaminants within these waters which researchers can use to get rid of contaminants that pose a human health risk.

All samples were analyzed in triplicates on the UPLC/MS/MS. using both qualitative and quantitative Agilent MassHunter software, 22 out of 36 screened emerging contaminants were detected overall (Table 4). Four compounds were found in all 10 sites: Atrazine, Cotinine, Sulfamethazine, and Tylosin. Atrazine is a commonly used pesticide, primarily for corn. Atrazine presence in all 10 sites indicates a prevalence and widespread impact of pesticide use. Cotinine serves as a biomarker of exposure to tobacco smoke, indicating the widespread impact of tobacco use. (Benowitz, 1996) Sulfamethazine presence differed between sampling trips 1 and 2. The compound was found in all 10 sites from sampling trip 1 in addition to some solvent blanks, but was only detected in sites 1, 2, 4, 7, 9, and 10 from sampling trip 2. Table 4 displays the detectable ECs found in either of the sampling trips.

Table 4. 21 out of 36 screened emerging contaminants were detected in the 10 surface water sites receiving storm water outflow (Eileen Cahill, 7/20/17)

		Sample site number (Fig. 1)									
Detected ECs	**Usage**	**1***	**2**	**3**	**4***	**5**	**6**	**7**	**8**	**9**	**10**
Atrazine	widely used herbicide (birth defects and menstrual problems)	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB
Buprenorphine	more potent and longer lasting analgesic than morphine						A				
Carbamazepine	anticonvulsant			AB	B	B	A	A	AB	AB	B
Cefotaxime	antibacterial agent				B	B	B	B			B
Chlorotetracycline	antibacterial agent	A						A		A	
Cocaine	local anesthetic and vasoconstrictor		B	B			A		B	B	A
Cotinine	predominant metabolite of nicotine	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB
Diltiazem	hypertension treatment			B			AB		AB		
EDDP	Metabolite of methadone, an opioid medication								B		
Escitalopram	antidepressant (SSRI)			AB			AB		B		
Gabapentin	anticonvulsant						B		B		
Lidocaine	local anesthetic and cardiac depressant		AB	AB			A		AB	A	A
Mefenamic acid	non-steroidal anti-inflammatory agent				A						

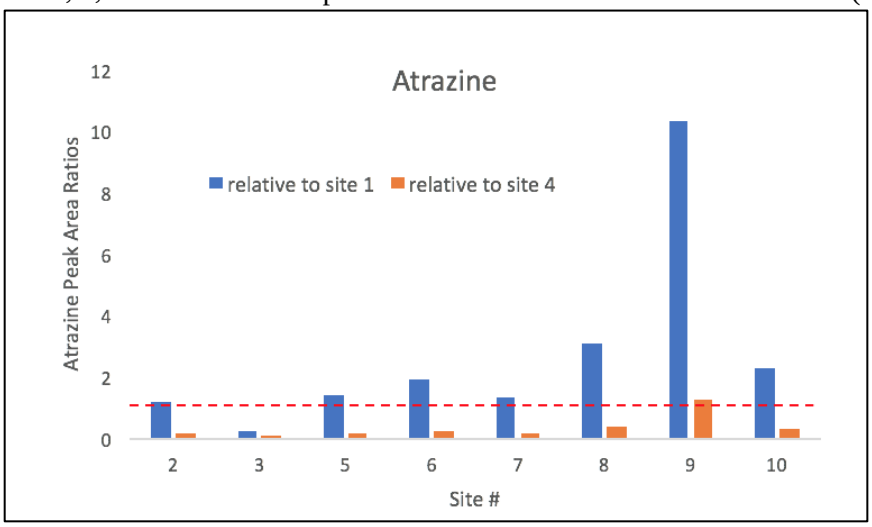
Propanolol	hypertension, migraine, and anxiety treatment		B	AB					B		
Sertraline	antidepressant								B		
Sulfamethazine	antibacterial agent	A	AB	AB	AB	AB	A	AB	A	AB	AB
Tetracycline	antibacterial agent							A			
Thiabendazole	antihelminthic, antiparasitic drug			A		A			B		
Triamterene	diuretic medication								B		
Tylosin	antibacterial agent	AB	AB	AB	AB	AB	AB	AB	B	AB	AB
Venlafaxine	antidepressant (SNRI)										A
	Detectable ECs Trip 1	5	5	9	5	5	10	7	6	7	7
	Detectable ECs Trip 2	3	7	10	6	6	7	5	14	6	6
	Total ECs found:	**5**	**7**	**11**	**7**	**7**	**12**	**8**	**15**	**8**	**9**

*reference sites with low E. coli levels

A = found in sampling trip 1

B = found in sampling trip 2

Compounds not detected include metformin, MDMA, meprobomate, primidone, trimethoprim, vancomycin, erythromycin, dextromethorphan, clenbuterol, amitriptyline, n-Hydroxybenzoyl, lorazepam, escitalopram, and nifedipine oxidized. The method in the UPLC/MS/MS also screened for DEET, but the machine detected DEET in all samples, including the solvent blanks, method blanks, spike tests, etc. The results for DEET are not included in the results as there is no blank to with an absence of DEET detection, indicating potential cross-contamination within the instrument. Peak areas were determined by peak integration in the chromatogram. Site averages were comprised of both sampling trip data. Of the 4 compounds that were found present in each site, sulfamethazine and tylosin were not included in Figure 4 as they were also detected in some UPLC/MS/MS instrument blanks. The ratio of peak area for atrazine and cotinine at sites 2, 3, and 5-10 over the peak area at site 1 and site 4 were calculated (Fig. 4).



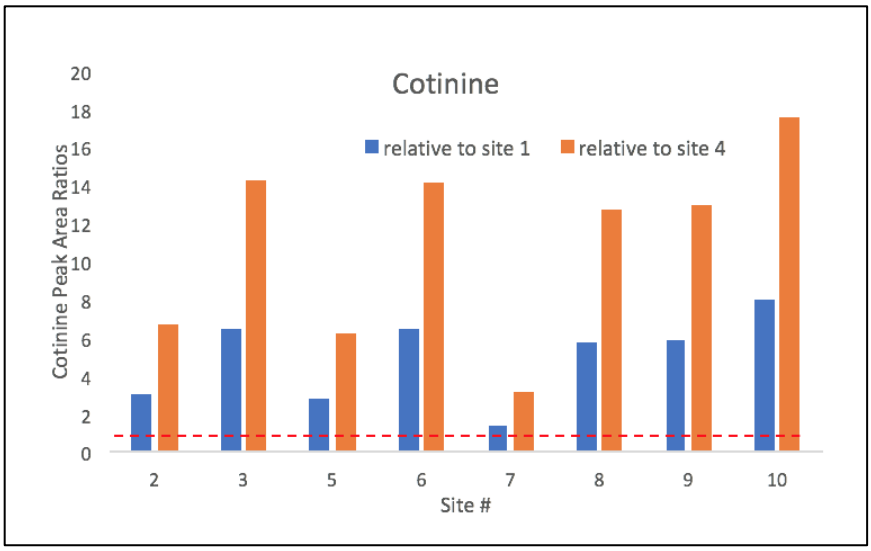


Figure 4. Peak area ratio for compounds detected in the water of all 10 sites with medium to high levels of E coli. relative to that in water of reference sites 1 and 4 with low levels of E coli. (Table 1). Peak area ratio > 1 (red dashed line) indicates concentrations for cotinine in water of site 1 or 4 are lower than those in water of other sites. Atrazine levels in the two reference sites were 0-10 times lower than its levels in other sites. Cotinine levels in the two reference sites were 2-18 times lower than its levels in the other sites.

Between water samples from 10 different sites taken twice on different dates, the maximum number of detectable ECs was determined to be 15 at site 8 while the minimum was determined to be 5 at site 1. 5-7 were found in each of the two reference sites and 7-15 compounds found in each of the non-reference sites. The lowest number of compounds was predicted to be found at the reference sites. Site 1 contained 6 detectable ECs while Site 4 contained 7 detectable ECs. Site 2 and 5 also contained 7 detectable ECs, though this does not mean they had the same level of contamination. E. coli levels were determined on the first sampling date. The levels may have differed by the second sampling date, but are assumed to be relatively similar for all sites except site 2, which fluctuates in E. coli levels.

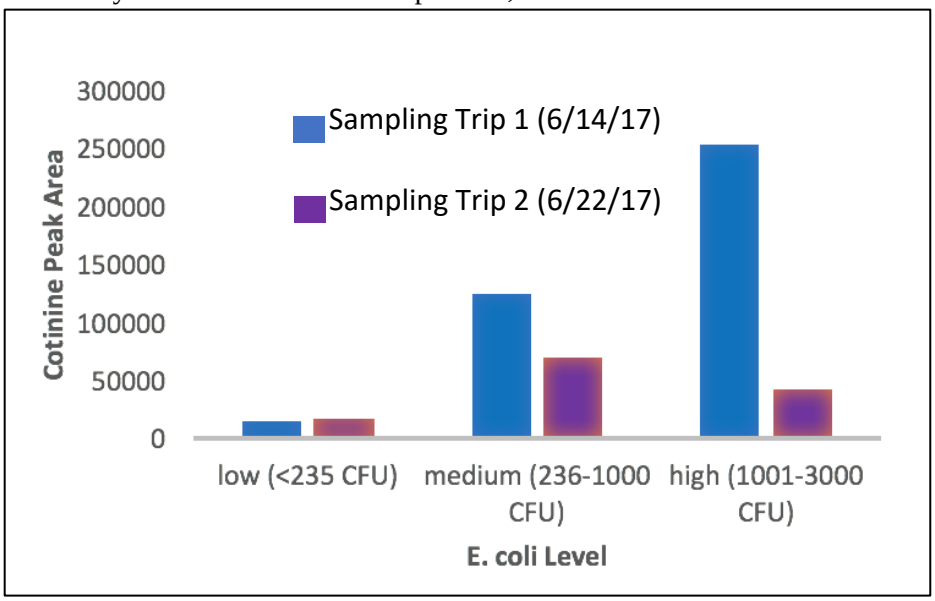


Figure 5. Relationship between averaged cotinine peak areas and E. coli level between water samples collected on two sampling trips

The average cotinine peak area value was compared to the relative E. coli levels in 9 of the 10 sites (not including variable E. coli level site 2). Higher peak area of a compound indicates a higher level in water. Each site was classified either as low E. coli count (<235 CFU), medium E. coli count (236-1000 CFU), and high E. coli count (1001-3000 CFU). Peak area values were averaged between sites of the same category (i.e., all medium level sites were averaged) as the provided E. coli data was given as a range rather than a value. Sites 1 and 4 have a low E. coli level. Sites 5, 6, 7, 8, and 9 were found to have a medium E. coli level and Site 10 and 3 were found to have a high E. coli level. Blue bars indicate the first sampling trip on 6/14/17. Purple bars indicate the second sampling trip on 6/14/17. Cotinine peak areas increased with each E. coli level increase.

4. Discussion

Atrazine and cotinine were used for data analysis because they were detected in all 10 sites. As atrazine is an herbicide while cotinine is a nicotine metabolite, the source of these ECs differs. Analyzing atrazine levels would lead to information about agricultural input of ECs, while analyzing cotinine levels leads to information about human-sourced ECs. Comparing the two by finding ratios of non-reference sites to reference sites revealed cotinine in non-reference sites was always higher than cotinine in reference sites (Fig. 4). As this compound is human-sourced, the positive relationship between cotinine peak area and E. coli level (Fig. 5) supports the idea that EC presence in these sites comes from a human source, i.e., from waste water. E. coli sampling occurred the same day of sampling trip 1 on 6/14/17. By the time sampling trip 2 came around on 6/22/17, the E. coli data may have been outdated, leading statistical significance to be much lower on trip 2 than trip 1.

The detection of ECs in storm water-impacted surface waters provides detail on the type of ECs found and the direction to take in treating waters for these compounds. The overall goal of this project is to consider sources for ECs in urban surface waters. A possible source of ECs in surface water is due to the lack of EC treatment processes present at wastewater treatment facilities. (Bolong, Ismail, Salim, & Matsuura, 2009) The residual compounds re-enter surface waters, posing a health risk to both human and environment. A variety of waste water EC treatments exist, some of which are chlorination, ozonation, oxidant quenching, and powder activated carbon addition. The techniques do not eradicate ECs. Even in combination, detectable ECs may still remain (Westerhoff, et al., 2005) As the tested surface waters are known to be impacted by storm water, as the only source of detectable ECs in surface waters would require more testing

5. Conclusion

In surface waters at the outflow of storm water runoff, 21 ECs were identified of 36 that were screened (Table 1). Site 1, a reference site, had the lowest number of detected ECs, making it site with lowest EC variety, while site 8 had the highest number of detected ECs, making it the site with the highest EC variety. The number of different ECs present does not determine the amount of a present compound. The importance of knowing the variety of ECs in a site is in knowing what kind of solution can be put in place. Since purification tactics vary by compound, a site with a higher variety of ECs may be more difficult to purify regardless of the amount of EC present. The average peak areas of cotinine, a human-sourced compound present in every site, showed a positive correlation to increasing E. coli level for sampling trip 1. The average peak areas did not continually increase with each E. coli level for sampling trip 2, potentially because of E. coli level changes after the only bacterial testing date on 6/14/17. However, cotinine peak area averages for medium and high E. coli levels appeared higher than the peak area averages for low E. coli levels. Presence of human-source ECs in storm water-impacted surface water suggests possible input of sewer water into storm water. This may be due to exfiltration from leaky sewer water systems to storm water systems.

6. Future Direction

As there were only low flow events sampled during the given research time, a future goal is to sample during high flow and low flow to compare the detectable EC levels. Since the above project

considers detectable ECs within storm water-impacted surface waters, the next step would be to research how storm water is contaminated. At the beginning of the project, the goal was to research sewer exfiltration into storm water. The current findings are enough to indicate problematic human-source storm water contamination, but not enough to fully support storm water contamination due to sewer exfiltration. A dye test such as the one performed in Sercu, et al. combined with a high flow testing could provide more support for sewer exfiltration as a source for storm water contamination. Additionally, the testing for HF-183 presence, a specifically human genetic marker would be key supporting data for sewage contamination in storm water-impacted surface waters.

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Investigation of the Dimensions, Distribution and Abundance of Macropores Throughout Virginia

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Abstract

The hyporheic zone is defined as the region in which stream surface water and ground water interact (Boano et al, 2014). This exchange is known to be highly dependent on the hydraulic conductivity (K) of the stream bank (Brunke and Gonser, 1997). Voids or cavities within the soil, known as macropores, have the potential to influence that exchange due to higher K but more information about the physical characteristics or prevalence of macropores is required. This project reveals the geometry (width, height and depth), distribution, and abundance of naturally-occurring macropores within 12 streams throughout Virginia. Macropores were prevalent throughout all the stream sites surveyed despite changes in physiographic provinces, stream order and stream morphologies. Soil types and associated cohesion properties influenced the distribution and clustering of macropores. Despite changes in average bank height macropores did not occur higher than 160 cm above the water line. Our work attempts to add to our current knowledge of macropores (Menichino, 2015) and aid further research of their role in hyporheic throughflow.
Keywords: stream, macropore, hyporheic zone, preferential flow

1. Introduction

The hyporheic zone is defined as the interface in which stream corridors and their underlying groundwater catchments exchange flow, nutrients and even contaminants (Allaire-Leung et al, 1999; Boano et al, 2014; Brunke et al, 1997). The interactions between these systems are facilitated through several mechanisms (Boano et al, 2014; Menichino et al, 2013) which create an ecologically intermediate habitat that is unique from its surroundings (Hester et al, 2013; Winter et al, 1998). Variations of surface water stages and ground water stages facilitate a hydrologic gradient between these systems. The fluvial mechanics of this interaction can be divided into two models: gill and lung (Sawyer et al 2009).
In the gill model, the surface-groundwater exchange is unidirectional and often determined by the steady state of ground water while the lung model demonstrates a bidirectional exchange that is influenced by unsteady surface water fluctuations (Menichino et al, 2013). The lung model exchange, although less investigated than the gill model exchange, is of ecological importance that warrants further research (Sawyer et al 2009).

Past research has shown that this bidirectional exchange can introduce nutrients to the stream and create productivity “hot spots” (Boulton et al, 1998) and has the potential to be a source of thermal mitigation (Wondzell, 2011). Movement of water from the channel to the groundwater provides dissolved oxygen, increases retention time, and in turn increases the amount of available organic matter for interstitial fauna (Boulton et al 1998; Brunke et al 1997). The rate at which this exchange and the subsequent effects of that exchange occur are strongly controlled by the hydraulic conductivity of the sediment structure (Bouma, 1991; Brunke et al, 1997; Hester et al, 2013; McDonnell, 1990; Menichino et al, 2013; 2014; 2015). Often the movement of flow is conceptualized as a diffusion through the soil

matrix but networks of preferential flow, known as macropores or soil pipes, can impact this exchange. It is well documented that macropores have higher hydraulic conductivity than the surrounding soil matrix but little quantitative research has been published on the extent naturally-occurring macropores influence lung model exchange (Beven et al, 1982; Bouma, 1991). A study conducted by Menichino et al found that their artificially created open macropores had 29 to 550 times higher hydraulic conductivity than the local soil matrix (2014).

A macropore is a passageway, cavity, crack, tubular pore or void in the soil that transports water and solutes by gravity (Aubertin, 1971). There is not a consensus on a standard size that delineates a macropore. The nomenclature is based on indirect and arbitrary definitions of size thresholds or pore diameter classes. Some examples of macropore size definitions include voids in the soil that are larger than 3 mm (Beven and Germann, 1982) and larger than 2 mm (Sidle et al, 2001) while Brewer (1964) separated macropores into 4 classes: "very fine" 75-1000 μm, "fine" 1000-5000 μm, "medium" 2000-5000 μm and "coarse" >5000 μm. Due to this lack of standard, it is important for the researcher to establish a definition of an "effective macropore size" based on the parameters of interest (Beven and Germann, 1982). More recently the importance of pore structure (continuity and connectivity) has become apparent and commands more investigation. For example, Sidle et al. (2001) provided a conceptual framework for the influences impacting various preferential flow networks and showed that antecedent soil moisture was one of the most important contributors to creating connecting "nodes" between macropores.

Macropores can be formed in many biotic and abiotic ways. Biotic creation mechanisms include burrowing by earthworms, small mammals and both live and decaying roots while abiotic mechanisms include subsurface erosion such as seepage, freeze-thaw and wet-dry cycles. (Aubertin, 1971; Beven et al, 1982; Sidle et al, 2001; Ghestem et al, 2011). Although it can be difficult to distinguish between different types of creation mechanisms, it has been apparent that certain types of creation mechanisms occur more readily in different environmental conditions. The persistence of root architectured macropores seems to be related to soil texture (Aubertin, 1971) and vegetation composition (Ghestem et al, 2011). The diversity of soil fauna that can contribute to macropore construction seems to be influenced by pH. For example, earthworms prefer low acid to neutral soil and will almost entirely avoid alkaline soils (Beven et al, 1982; Sapkarev, 1979). In addition, it has been found that the shrinking and swelling of clay soil creates fissures that contribute to the connectivity of macropores (Beven et al 1982).

Stream-ground water interactions occur through lateral exchange within the bank and channel bed. It is hypothesized that macropores are common in streambanks but the geometry, abundance and connectivity have yet to quantified. These structures may offer enhancements for both gill and lung model exchange and are a significant component to the ecological effectiveness of the hyporheic zone. The creation and structural functions of these voids have been researched but there is still a limited understanding on how these voids impact hydrologic activity. As previously stated, it is understood that macropores have higher hydraulic conductivity than the surrounding sediment but hydrological models have yet to distinguish the influence this has on the permeability of the soil profile (Boano et al, 2014). Solute transport in macropores has yet to be compared to the movement through the sediment and current studies assume homogenous hydraulic conductivity [Menichino et al, 2014]. The network or connectivity of macropore has shown to be complex (Hu et al, 2014, Sidle et al, 2001) but the impact that this tortuous nature has on preferential flow is still poorly understood (Allaire-Leung et al, 1999). This is in part due to the impractical and invasive techniques used to investigate these structures.

A negative impact that requires attention is how these voids of higher conductivity can also facilitate pollutant transport into the stream from uplands. Riparian buffer zones have been implemented as a strategy to mitigate the amount of sediment, nutrient and pollutants entering the stream channel (Correll, 1997). They also have the potential to stabilize the bank structure, slow flooding rates, and introduce extensive root systems that are a common mechanism for macropore creation. In some cases, the effectiveness of riparian buffer zones may be depreciated because macropores are providing passageways that reduce retention time. A topic of future research will be to determine the effectiveness of macropores in the context of both the negative and the previously describe positive implications.

The first step in understanding the extent macropores impact hyporheic exchange is to determine quantitative macropore parameters and their abundance in a variety of stream environments. Our objective is to document the abundance, distribution and geometry of surface-connected macropores along stream banks in three geographic provinces of the eastern United States. We hypothesize that macropores (≥1 cm diameter) will be common (average spacing <1 m) throughout all stream sizes surveyed in a multitude of environments. These measurements will act as important preliminary data for further investigations on how macropores impact the "lung model" hyporheic exchange in streams.

2. Research Methods

During the summer of 2017 we surveyed 12 stream banks for surface connected macropores ≥ 1 cm to document and analyze their physical characteristics. The streams were located across various physiographic provinces, demonstrated a multitude of stream morphologies and spanned Strahler's stream order classifications 1 through 4 (Table 1).

2.1 Physiographic Provinces

These regions are characterized by similar landforms comparable in hydrology, erosive processes, geology and governing tectonic activity. Within Virginia there are 5 physiographic provinces: Appalachian Plateau, Valley and Ridge, Blue Ridge, Piedmont, and Coastal Plain (Figure 1). Given the allotted time for this project we sampled and analyzed from three of the five physiographic provinces: Appalachian Plateau, Blue Ridge and Piedmont.

2.1.1 Appalachian Plateau

Within Virginia this province is only found in the southwest portion of the state. Topographically, it is relatively high above sea level compared to the other regions. The Appalachian Plateau is dominated by sedimentary rocks; primarily sandstone, shale and coal. This area is characterized by a series of plateaus separated by fault lines.

2.1.3 Blue Ridge

This province is distinguished from the rest because it has an impervious bedrock underlying a thin weathered soil layer. On the western portion, sedimentary rocks are most common while on the eastern portion there are largely igneous and metamorphic rocks. According to the Virginia Department of Environmental Quality, the steep terrain and thin soil coverage influence the low ground water recharge rates of this province.

2.1.4 Piedmont

This is the largest province within the commonwealth and its geophysical characteristics vary greatly throughout the region. Broadly, it is composed of primarily metamorphic and igneous rock with pockets of sedimentary configurations. A unique characteristic of this province is that it contains high amounts of clay subsoil (Ludwig, 2016) that we hypothesize may contribute to the prevalence of macropores.

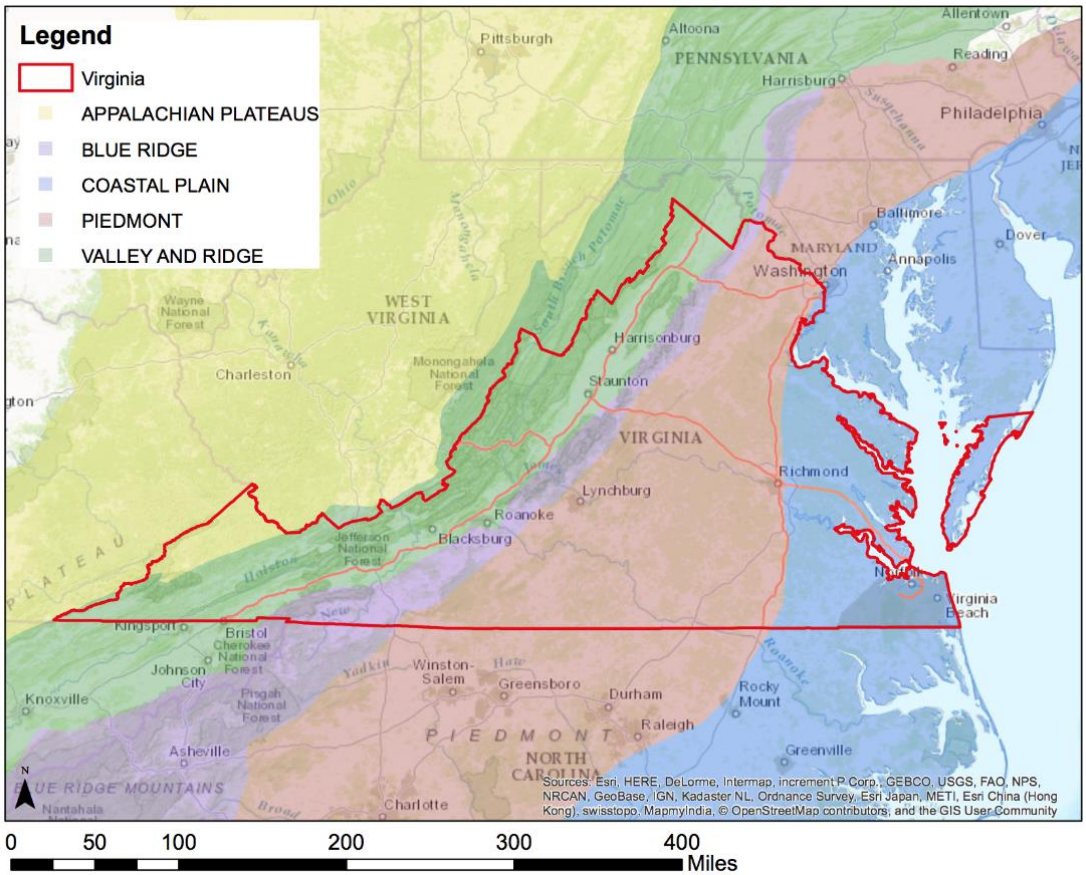


Figure 1. Physiographic Provinces of Virginia (Spradlin, 2015)

2.2 Stream Classifications

2.2.1 Stream Order

Stream order is a classification scheme used to broadly characterize channel processes and patterns. These characteristics include stream width, depth, discharge and hierarchical position throughout the landscape. Strahler (1952) defined stream order is a numerical approach to quantify the branching complexity of channel networks. As two streams segments of the same order merge they obtain the next higher stream order classification. For example, headwaters are the first order and at the confluence of two first order streams they form a second order stream. If a lower ordered tributary enters a higher ordered stream then the order of the highest stream continues.

Stream order classifications provide a unifying language for a wide array of professions such as hydrologists, engineers, and ecologists. This will be important throughout our data analysis and increase our effectiveness to communicate any connections made between channel processes and macropore dynamics. We surveyed 12 streams ranging from first to fourth order within three physiographic regions described previously (Table 1).

2.2.2 Stream Morphology

This was a qualitative classification of stream reaches based off the seven stream-reach categories developed by Montgomery and Buffington (1997). These stream- reach types include: colluvium, bedrock, cascade, step-pool, plane-bed, pool-riffle, and dune ripple. This classification is indicative of the

ratio of transport capacity and sediment supply of that reach which is an important driver of channel processes (Bilby and Naiman, 1998) that could influence the creation and persistence of macropores in the system.

2.3 Land Cover

To eliminate extrinsic variables such as land use and cover; priority was given to land cover types with minimal to no human influence. To achieve this goal throughout Virginia it should be noted that much of our sites were in forested environments. We only surveyed portions of streams that were located on public lands.

2.4 Field Measurements

Along each stream bank the survey consisted of a short stream unit and a continued long stream unit which were defined by protocol outlined by Garrett Menichino (2015). We calculated an average bankfull width and average bankfull height. The length of the short stream unit was defined as the longitudinal distance of 20 bankfull widths and the length of the long stream unit was defined as 60 bankfull widths. However, a time restriction for each site of four hours was used to ensure that each stream chosen was surveyed.

Along the short stream unit, we recorded where we found macropores, referred to as the "station" using a measuring tape in meters. Using a modified wooden dowel with half centimeter markings we measured various dimensions of up to 100 macropores. When 100 macropores were reached, we continued to only record the stations of macropores to the end of the long stream unit distance or the cap of the four hours. These measurements included the elevation of the macropore above the water line, as well as the width, height and depth (to the approximated first turn). The horizontal angle of the macropore normal to the direction of flow was determined using a standard protractor; a positive angle indicated that the macropore was positioned facing upstream while a negative angle indicated it was facing downstream.

Throughout our survey of the stream bank we determined the soil type using a feel-flow diagram method (Thien, 1979). Changes in the soil type were recorded as they were observed. Photos of each site and significant physical stream properties such as soil type were taken. When a creation mechanism for a macropore could be determined, it was noted.

3. Results

3.1 Macropore Geometry

A total of 1078 macropores were counted throughout the 12 streams surveyed. There were 724 macropores measured in the Piedmont province, 148 macropores measured in the Blue Ridge province, and 206 macropores measured in the Appalachian Plateaus province. Of those 1078 macropores we measured the geometry for approximately 628 macropores but this number varied among characteristics. On average, macropore widths were slightly larger than heights (Table 1). This was true in all the streams except two: Little Laurel Creek, where the average width was equal to the average height, and Bottom Creek Tributary, where the average height was larger than the average width (Table 1). One macropore along the North Fork Wolf Creek, within the Piedmont province, could not be measured for width and height.

Figure 2 shows the area-frequency distribution of the macopore surface openings. The surface opening area was calculated using the ellipse area equation. The distribution for all the provinces displayed a postively skewed bell shape curve. Despite a large range of variablity, the median and peak abundance values among provinces did not deviate from one another greatly. For example, within the Appalachian

Plateau areas ranged from 0.79 cm^2 to 93.46 cm^2 with a median value of 6.87 cm^2 and greatest abundance between 2 cm^2 to 4 cm^2 while within the Piedmont province areas ranged from 0.79 cm^2 to 180.64 cm^2 but only had a median of 10.60 cm^2 and greatest abundance between 6 cm^2 and 8 cm^2. We found that 90% of the macropores surveyed had an opening area less than 30 cm^2.

We measured a total of 633 macropore elevations. Of the elevations measured, 84.20% were above the water line, 8.05% were below the water line and 7.74% were at the water line. The maximum elevation recorded was at 154 cm along Vaughans Creek (3rd order) and the lowest elevation recorded was -25 cm along the Appomattox River (4th order). The average elevation for each stream ranges from 11.1 cm to 40.4 cm shown in Table 1. Figure 3 shows box plots for each stream site in relation to average bankfull height. The average bankfull heights ranges from 63 cm to 620 cm but the maximum macropore elevation was only 152 cm above the water line.

The depth of 633 macropores was approximated to the first bend. The maximum depth was 90 cm along Fish Pond Creek (second order) and the minimum depth was 1 cm found along Little Laurel Creek and the Appomattox River (fourth order). The average depth of macropores within a stream ranged from 9.0 cm to 14.6 cm. Figure 4 shows box plots for the depth of macropores measured at the stream sites. The median depth for each stream did not surpass a 20 cm threshold despite the large range in measurements.

The final geometric characteristic measured was the horizontal angle of the macropore. Table 2 shows the percentage of macropores angled upstream, downstream or not clearly angled. Four of the eleven streams (Little Laurel Creek, Bear Pen Creek, Fish Pond Creek 1, and Appomattox 4) were dominated by downstream angled macropores. Three of the eleven streams (North Fork Pound River 3, North Fork Pound River 4, Bottom Creek) were dominated by upstream angled macropores while only one stream (North Fork Wolf Creek) had primarily macropores without a clear angle. The remaining streams did not have a dominate horizontal angle direction.

3.2 Macropore Distribution and Abundance

Surface connected macropores were common throughout all the physiographic provinces, stream orders, and stream morphologies surveyed. The total number of macropores found at a single site was not correlated with the length of the unit surveyed. For example, 226.8 meters of Little Laurel Creek was surveyed and only 78 macropores were found while 187.4 meters of Bottom Creek Tributary was surveyed and 124 macropores were found. The average interspacing across all streams was 2.32 meters. The main driver for macropore distribution was soil type. Figure 5 shows how the average macropore interspacing varied throughout all the soil types observed. Soil types that were less cohesive, such as sand, averaged higher interspacing than the total average. As the soil type became more cohesive average interspacing fell below the average. The largest average interspacing occurred in the bedrock (29.38 m) while the smallest average interspacing occurred in silty clay soil (.48 m).

The clustering nature of surface connected macropores is shown by the longitudinal distribution of macropores (Figure 6). The distribution of macropores is represented as the cumulative percent across each of the stream sites. The steeper sloped portions of the linear regression signify a greater abundance of macropores. The plateaus signify that there was fewer or no macropores found at those locations. A stream reach that had an even distribution of macropores would display a linear relationship. For example, Bottom Creek Tributary (first order) demonstrates the most even macropore distribution while the Vaughans Creek (third order) clearly shows macropore clustering along the reach.

Table 1. Stream sites surveyed for surface connected macropores and pertinent information. (Amanda Donaldson, July 2017)

Stream Name	Province	Stream Order	Stream Morphology	Dominate Soil Type(s)	Bankfull Width (m)	Total Distance Surveyed (m)	Total Macropores	Average Elevation (cm)	Average Width (cm)	Average Height (cm)	Average Depth (cm)
Little Laurel Creek	Appalachian Plateau	1	Cascade	Colluvium	3.8	226.8	78	32.6	2.8	2.8	11.9
Bear Pen Creek	Appalachian Plateau	2	Bedrock	Loamy sand/ Bedrock	7.1	255.7	65	40.4	2.5	2.4	8.0
North Fork Pound River	Appalachian Plateau	3	Undefined (Campground)	Sandy Loam	5.6	111.0	36	24.6	3.3	2.9	9.1
North Fork Pound River	Appalachian Plateau	4	Pool Riffle	Loam	18.2	190.0	27	35.1	4.9	3.3	7.8
Bottom Creek Tributary	Blue Ridge	1	Cascade	Sandy Loam	3.0	187.4	124	19.0	3.2	2.9	12.6
Bottom Creek	Blue Ridge	3	Pool Riffle	Sand	13.8	270.0	24	23.2	4.1	4.4	12.5
Fishpond Creek	Piedmont	1	Pool Riffle	Sandy Loam	3.9	234.0	75	11.1	3.4	2.5	8.3
Fishpond Creek	Piedmont	2	Pool Riffle	Loam	8.9	178.0	111	33.5	4.0	3.6	9.8
North Fork Wolf Creek	Piedmont	2	Pool Riffle	Silty	5.7	342.0	170	15.5	4.3	3.3	14.6
Vaughans Creek	Piedmont	3	Dune Ripple	Clay Loam	12.3	245.0	84	22.0	4.9	4.4	12.7
Appomattox River	Piedmont	3	Pool Riffle	Silty Clay	8.0	69.4	132	13.0	5.3	3.9	13.8
Appomattox River	Piedmont	4	Pool Riffle	Clay Loam	20.0	151.6	152	38.4	4.4	3.5	9.0

Table 2. List of macropore horizontal angle percent of each stream (Not measured for Bottom Creek Tributary 1), Following the name is the stream's Strahler Stream Order. (Amanda Donaldson, July 2017)

Stream Name	Down Stream	Upstream	No Angle
Little Laurel Creek 1	58.1	25.6	16.3
Bear Pen Creek 2	58.3	25.0	16.7
North Fork Pound River 3	31.4	48.6	20.0
North Fork Pound River 4	25.9	55.6	18.5
Bottom Creek 1	26.3	57.9	15.8
Fish Pond Creek 1	48.7	21.6	29.7
North Fork Wolf Creek 2	36.0	18.0	46.0
Fish Pond Creek 2	41.4	45.1	13.5
Vaughans Creek 3	33.3	41.7	25.0
Appomattox River 3	37.8	43.3	18.9
Appomattox River 4	71.4	14.3	14.3

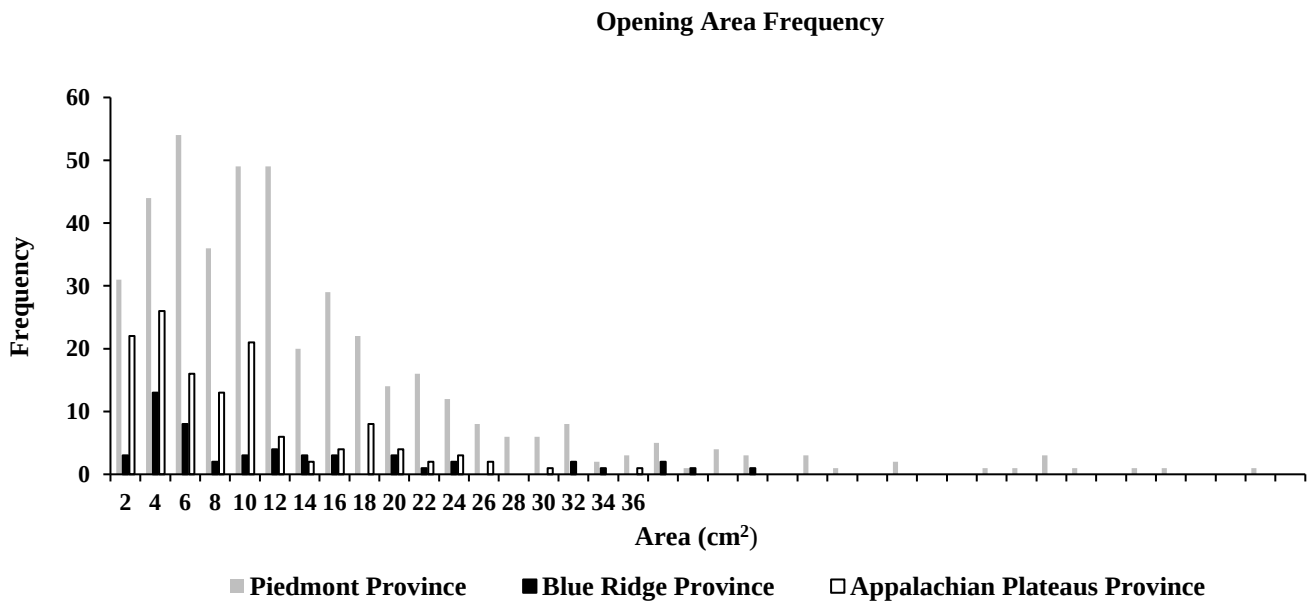


Figure 2. Histogram of the surface opening area, using the area of an ellipse, of all measured macropores. Despite variation in range, each province-frequency has a positively skewed bell shaped curve.

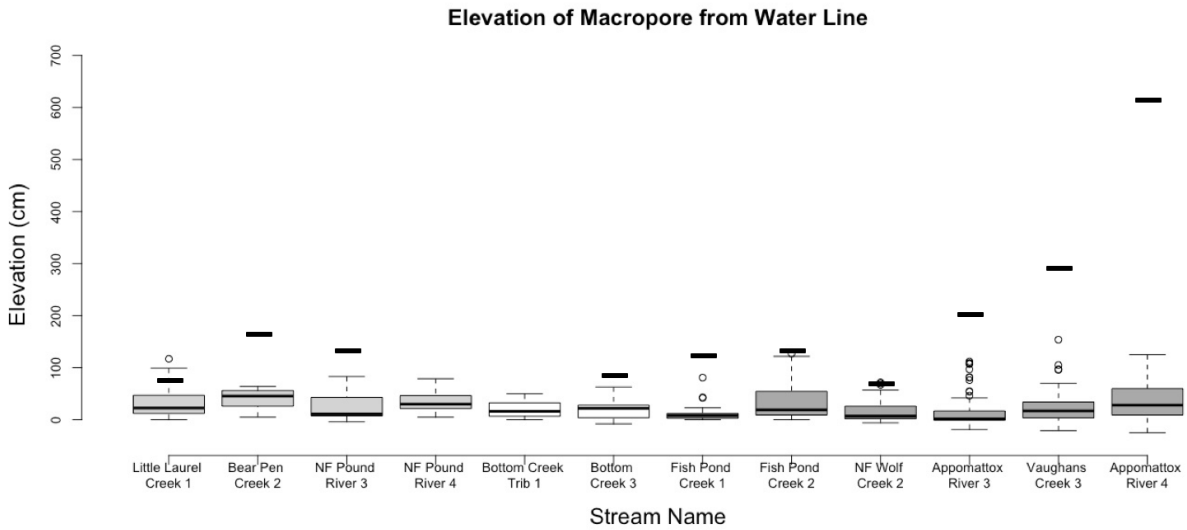


Figure 3. Box plots showing the height of the base of the macropore above the water line. Light gray boxes signify streams within the Appalachian Plateau province, white boxes signify streams within the Blue Ridge province, and dark gray boxes signify streams within the Piedmont province. Within each province, streams are ordered from least (1) to greatest (4) stream order, shown by the number following the name. The thickest black bars are the average bankfull height for each stream it was measured (not measured for NF Pound River or Bottom Creek Tributary). The average bankfull height ranged from 63 cm to 620 cm but the highest macropore elevation did not surpass 160 cm.

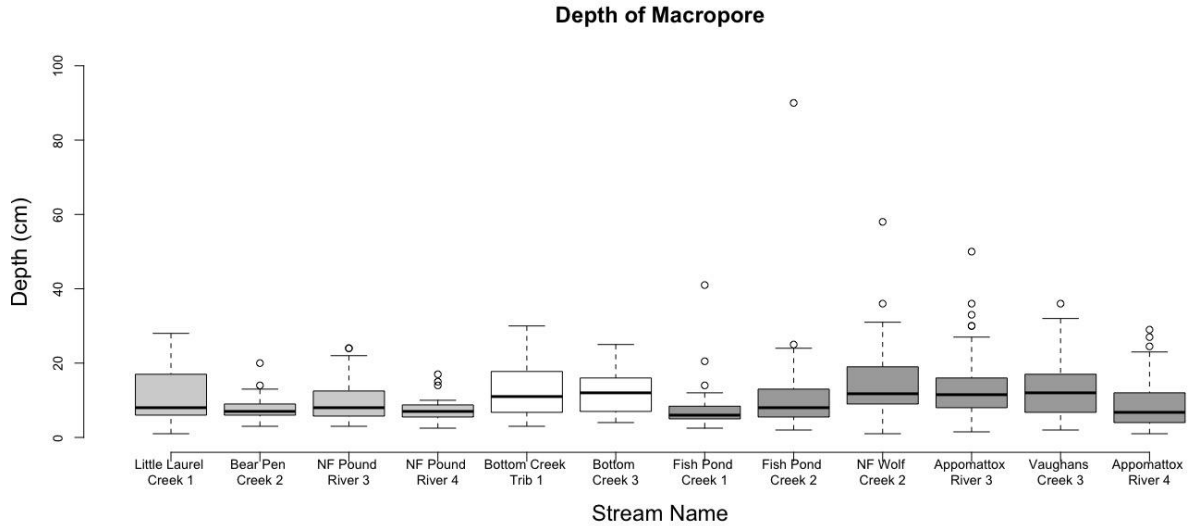


Figure 4. Box plots of macropore length into the bank, to the approximated first bend, across all field sites. Light gray boxes signify streams within the Appalachian Plateau province, white boxes signify streams within the Blue Ridge province, and dark gray boxes signify streams within the Piedmont province. Within each province, streams are ordered from least (1) to greatest (4) stream order, shown by the number following the name.

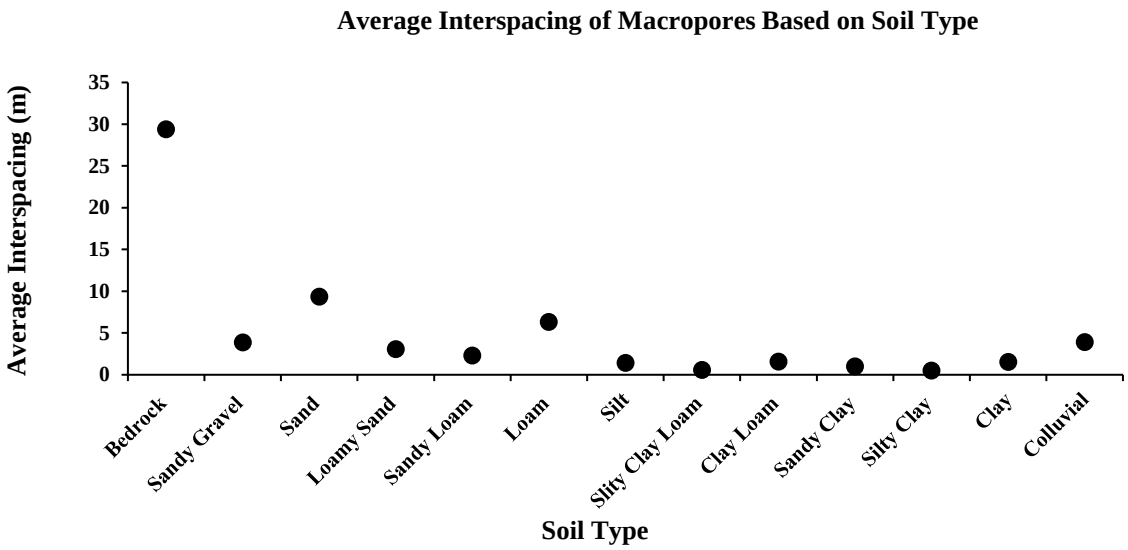
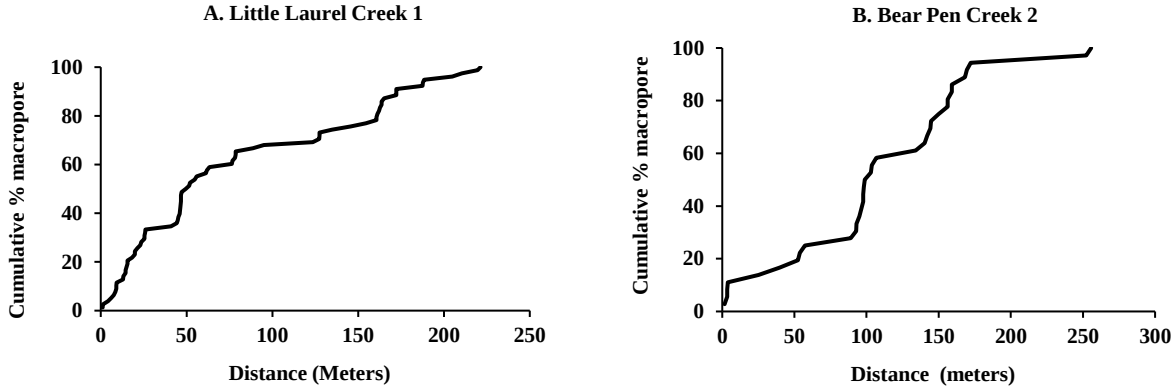
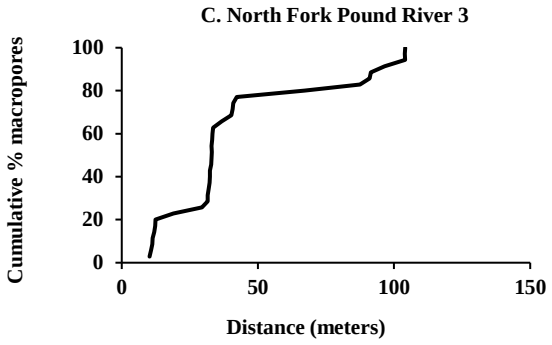
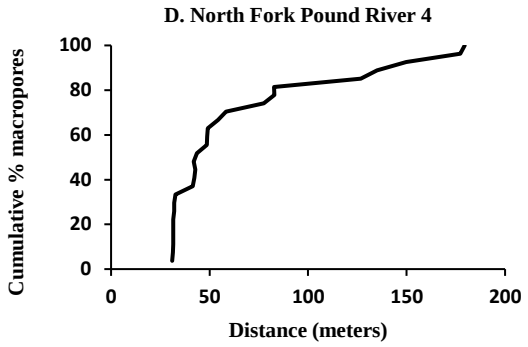
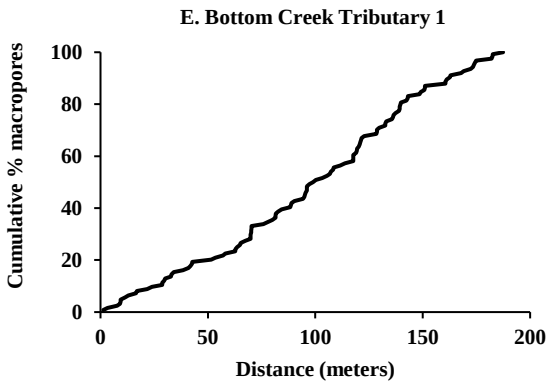


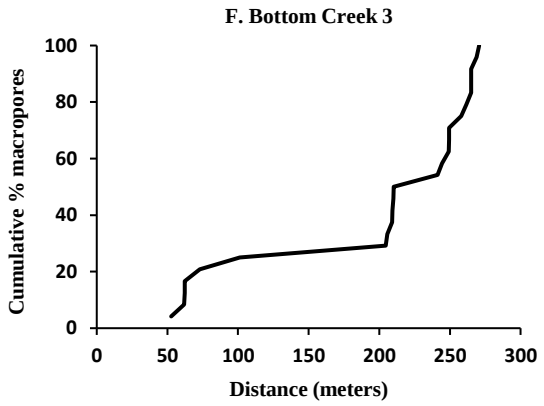
Figure 5. Average interspacing of macropore distribution within each soil type observed. Macropores tended to cluster or become more frequent in soil types that consisted of more cohesive i.e. clayey substrate.

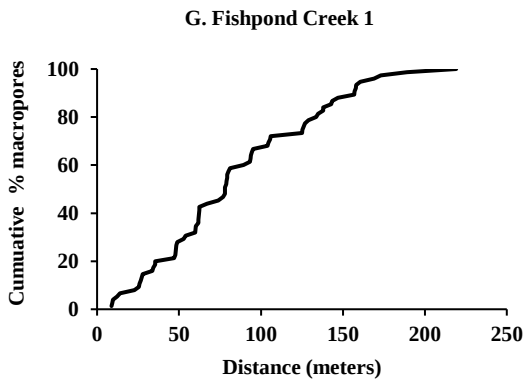


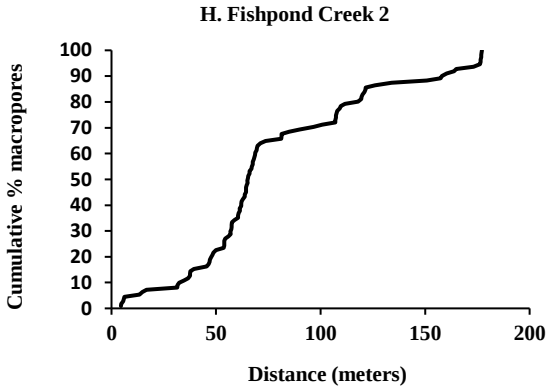

C. North Fork Pound River 3
Cumulative % macropores
Distance (meters)


D. North Fork Pound River 4
Cumulative % macropores
Distance (meters)


E. Bottom Creek Tributary 1
Cumulative % macropores
Distance (meters)


F. Bottom Creek 3
Cumulative % macropores
Distance (meters)


G. Fishpond Creek 1
Cumuative % macropores
Distance (meters)


H. Fishpond Creek 2
Cumulative % macropores
Distance (meters)

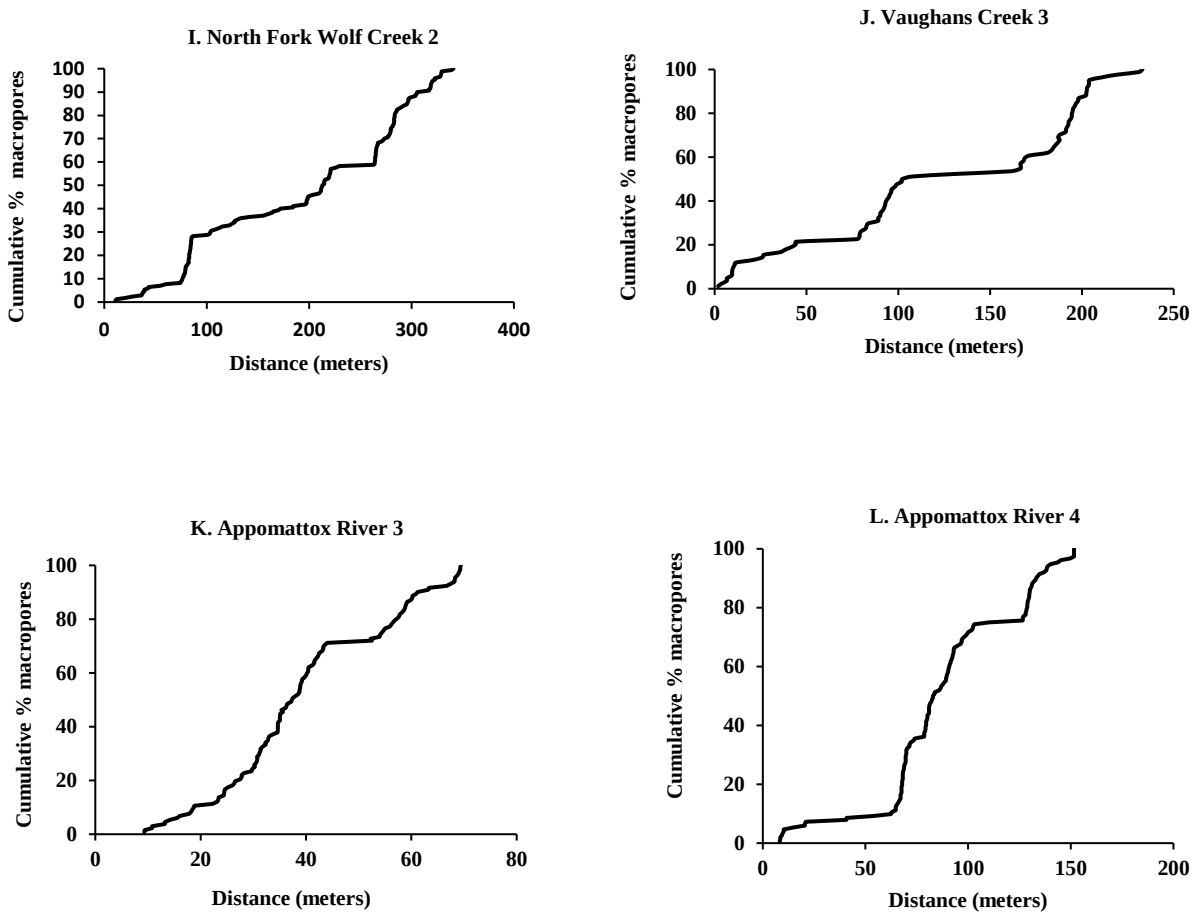


Figure 6. Longitudinal distribution of macropores, shown as a culmulative percent, across the entire surveyed length. The stream order of each site is signified by the number following the name.

4. Discussion

Surface connected macropores were a common feature throughout the 12 stream sites surveyed. This prevalence is conclusive with the first systematic survey, to our knowledge, of surface connected macropores (Menichino et al, 2015). Menichino et al found that macropores were "typical rather than anomalous" within the Valley Ridge province across a range of land use practices, sediment types, stream sizes and gradients (2015). Our work added to these findings by including variations in physiographic provinces and stream morphologies. Although this has been a critical contribution to their work, it is important that future studies aim to include surveys throughout the remaining province within Virginia: Coastal Plains.

Surface connected macropores are thought to have the most effect on hydrologic processes and hyporheic through flow (Menichino et al, 2014) because they directly interact with channel flow. The inundation of macropores is influenced by their position in the bank in relation to stream discharge patterns. Menichino et al (2015) found that 97% of the macropores across all 5 sites were inundated annually. The volume of water that enters the stream bank and the subsequent hydrologic effectiveness of these features could be influenced by the area of the surface opening into the channel. Our findings suggest that these surface opening areas intrinsically vary but are predominantly below 10 cm^2 and rarely surpass 30 cm^2 (Figure 2). By understanding the area ranges at which macropores surface openings occur the hydrologic impact of clustered macropores versus isolated macropores can be investigated.

Although macropore abundance spanned various soil types, longitudinally, the macropore distribution along 8 of the 12 streams showed a clear clustered pattern (Figure 6). This was strongly driven by changes in soil type and the associated cohesion properties of the sediment particles. The interspacing of macropores significantly decreased within soil types containing clay substrate and increased in soil primarily composed of sand. There are some instances when this is more prevalent than others but we attribute this to the nature of the "feel-method" when determining soil texture and suggest future studies to include a more in depth analysis of changes in soil type. The soil type varied throughout each stream site but all the streams had a predominant soil type (Table 1). Therefore, this suggests differences in soil types can impact the persistence of macropores within the system. Although relationships between macropore distribution and morphological features was inconclusive for both this study and Menichino et al (2015); future studies should investigate how stream morphologic characteristics can influence the predominant soil type and subsequent macropore abundance. Some characteristics that require further investigation are the relationship between macropore abundance and the transitions between point bars and banks common within pool-riffle morphologies.

The median elevation of macropores, in relation to the water line, did not vary greatly despite increases in bankfull height (Figure 3). Although there were several outliers most macropores were confined below 100 cm. This contradicts the findings of Menichino et al (2015) which found that the median elevation of macropores increased with bank height. This may be because the surveys they conducted were primarily of second order streams and did not include any streams higher than third order. The highest bank full height that they recorded was only 72 cm compared to the highest bankfull height of 620 cm that we measured along a fourth order stream (Figure 3). The hydrologic implications of this are that the closer that macropores reside to the water line the more likely they are to become inundated and for longer periods of time.

Our depth measurements of each macropore are an under representation of the total length or connectivity of these features. This is because the depth was only recorded to the first bend or a point of resistance reached. Macropores can exhibit a tortuous nature but the hydrologic implications of this have not been appropriately studied. It should be noted that connectivity may not be true of all macropores and this varies with size, which influences capillary properties of the sediment (Beven & Germann, 1982) and antecedent soil moisture of the soil matrix (Sidle et al, 2001). Due to these uncertainties, future studies should invest in developing non-destructive methods of determine macropore spatial dimensions.

There is a growing recognition of the ecological and hydrological implication of surface-ground water interactions (Wondzell, 2011). Both threats to water quality and hyporheic benefits are dependent on the unsteady stage characteristic of the lung model hyporheic exchange processes (Sawyer, 2011). These features can contribute to a wide range of ecosystem processes including solute transport, catchment hydrology, slope stability but the significance of macropores as preferential flow paths is often regarded as an exception rather than the "norm" (Weiler, 2017). Despite these acknowledgements, the physical characteristics of macropores have been minimally studied and only one previous study, Menichino et al, 2015, surveys their naturally occurring dimensions across stream sites. As suggestions for future research, it would be appropriate to integrate multiscale analyses of preferential flow, mechanistic modeling techniques, and developing noninvasive assessments of macropore connectivity. This work hopes to provide the preliminary data necessary for future work investigating the relationship between macropores and hyporheic throughflow.

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Epilimnetic mixing exacerbates methane ebullition flux in a small eutrophic drinking water reservoir.

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Abstract

Inland waters are substantial sources of carbon (C) greenhouse gases to the atmosphere. Reservoirs, in particular, emit a large portion of inland water C emissions, generally in the form of methane (CH_4) bubble fluxes (ebullition) from the sediments. CH_4 ebullition can contribute the majority of reservoir C emissions; however, little is known how CH_4 ebullition responds to water management practices like epilimnetic aeration, a common technique to prevent harmful algal blooms in reservoirs used for drinking water supply and recreation. In summer 2017, we measured CH_4 ebullition rates in a managed eutrophic drinking water reservoir during two planned epilimnetic aeration mixing events, and observed an overall significant increase in ebullition rates after the first mixing event but only a marginal increase after the second. Our data suggests that epilimnetic mixing management may increase ebullition rates, but that any stimulation of CH_4 fluxes may be dependent on the duration and timing of mixing.

Keywords: ebullition, global carbon cycle, greenhouse gases, reservoir, water quality management.

1. Introduction

Anthropogenic activity such as land use change has increased the amount of C in inland waterbodies (Maavara et. al., 2017). The vast quantities of C stored in the sediments and water column in inland waters can be mineralized and emitted as greenhouse gases (GHGs), including carbon dioxide (CO_2) and methane (CH_4), to the atmosphere (Cole et al., 2007). Global waterbody C emissions may significantly offset the continental C land sink (Bastviken et al., 2011).

Among waterbody GHG emissions, CH_4 is continuously emitted from inland waters and has 34× more global warming potential compared to CO_2 on a 100-year time scale (Myhre et al., 2013). CH_4 is produced in anoxic sediments as organic matter decomposes via acetate fermentation or CO_2 reduction (Conrad, 2005). CH_4 alone has been estimated to offset about 25% of the global C land sink when emitted from inland waters (Bastviken et al., 2011). Waterbodies are substantial sources of CH_4 to the atmosphere via three dominant pathways: bubble flux stored in the sediments (ebullition), active diffusive flux at the air-water interface, and plant-mediated emissions in littoral zones (Bastviken et. al., 2004). Among these pathways, it has been shown that ebullition consistently contributes disproportionally to annual CH_4 flux from inland waters (Deemer et al., 2016). Ebullition can contribute up to 5 times more emissions than diffusion (Bastviken et al., 2011); for example, a recent study found that 75% of the CH_4 emissions from a lake were ebullition-derived (Sobek et. al., 2012).

Ebullition bubbles are comprised mostly of CH_4, which can easily be oxidized or dissolved in the water column before reaching the atmosphere (Bastviken et al., 2004). For example, a recent study of a deep subtropical lake concluded that only 60% of the sediment-released ebullition is directly transported to the atmosphere (Schmid et. al., 2017). Water depth, dissolved gas concentrations in the water column,

and bubble size, which varies as a function of water temperature and hydrostatic pressure, also affect the amount of ebullition that escapes the water column (Mcginnis et al., 2006; Schmid et al., 2017).

Water depth affects ebullition rates in waterbodies (Bastviken et al., 2004). In shallow lakes, where CH_4 bubbles have less distance to travel from the sediments to the water's surface, oxidation of CH_4 bubbles may be lower, resulting in higher ebullition rates (Sobek et al., 2012). Generally, ebullition rates are higher in shallow water where the ebullition can avoid the consequences of high hydrostatic pressure and decreasing CH_4 rates in the water column (Jewell, 2003; Schmid et al., 2017). Nevertheless, there is still the possibility of oxidation of CH_4 by methanotrophs in shallow lakes or reservoirs in the presence of oxic sediments or water (Bastviken et al., 2004), as CH_4 is generally only present in anoxic conditions.

Since ebullition originates in anoxic sediments, the activity and nature of the sediments also control ebullition. Rates of ebullition correlate with organic matter content in the upper sediment layer and the ability for the bubbles to escape the sediments depends on the grain size of the sediment (Schmid et al., 2017). Nutrient-rich, or eutrophic, reservoirs emit more CH_4 as well due to increased C mineralization and more decaying organic matter (Deemer et al., 2016; Schmid et al., 2017). Sobek et. al. (2012) attributed high ebullition rates in a lake to high organic C sedimentation rates, as high rates of accumulating organic matter on the sediments decrease the oxygen exposure time and hence degradation of organic matter. In the same way, naturally or artificially mixing of a waterbody may increase turbulence at the sediment-water interface that further triggers ebullition formation and release.

Reservoirs in particular may be contributing disproportionally to the global C cycle. The global carbon inventory historically neglected the impact of CH_4 emissions from reservoirs, until it was estimated that emissions from man-made reservoirs may account for up to 7% of all GHG emissions (St. Louis et. al., 2000). Freshwater reservoirs store more organic C than all natural lakes combined (Cole et al., 2007; Dean & Gorham, 1998; Tranvik et al., 2009), and may even surpass annual oceanic organic C storage by 4-fold (Pacheco, Roland, & Downing, 2014). Because reservoirs store so much C annually, they continually emit CH_4, contributing up to 13.3 Tg CH_4-C yr^{-1} (Deemer et al., 2016).

Reservoir construction is increasing globally to accommodate growing needs for energy, food, and water needs (Gunkel, 2009). Simultaneously, water quality in many lakes and reservoirs is decreasing as a result of human activities that threaten the health and functioning of reservoirs. For example, algal blooms are increasing in many lakes and reservoirs globally due to land use and climate change (Brookes & Carey, 2011). As a result, engineered systems are implemented in the water column to prevent algal blooms, which pose taste, odor, and toxin problems for water managers (Singleton & Little, 2006; Visser et al., 2016). Epilimnetic mixing (EM) aeration systems are common engineered systems installed in the water column to reduce hypolimnetic hypoxia and prevent harmful algal blooms that pose a threat to water quality (Chen et. al., 2016). EM systems deepen the metalimnetic boundary by mixing the metalimnion and epilimnion, and upstream portions of the reservoir from the diffuser line, thus increasing the turbulence in the whole reservoir and decreasing the likelihood of bloom formation (Chen et al., 2016). While studies have explored the direct use of EM systems on algal blooms and other metrics of water quality, the response of CH_4 ebullition rates in reservoirs from EM systems remains unclear.

In the summer of 2017, we activated an EM system installed at a drinking water reservoir twice over three months. We measured the effects of EM activation on CH_4 ebullition rates weekly at 20 different sites in the reservoir for 12 weeks. We predicted that after reservoir mixing we would observe an increase in ebullition rates because the resulting increase of turbulence at the sediment-water interface from the EM, thereby increasing ebullition rates across the reservoir.

2. Research Methods and Experiment Setup

2.1.1. Site description

Falling Creek Reservoir (FCR) is a small (surface area = 0.119 km^2), shallow (Z_{max} = 9.3 m, mean Z = 4.0 m), eutrophic drinking water reservoir located in Vinton, southwest Virginia, USA (37.30ºN, 79.84ºW). FCR was constructed in 1898 and is owned and operated by the Western Virginia Water Authority (WVWA) (Figure 1). FCR has one primary inflow stream from an upstream reservoir that contributes the majority of the water into the reservoir and four smaller ephemeral inflows that dry up in the summer (Gerling et al., 2016). FCR thermally stratifies between April to October (Gerling et al., 2016; 2014; Munger et al., 2016).

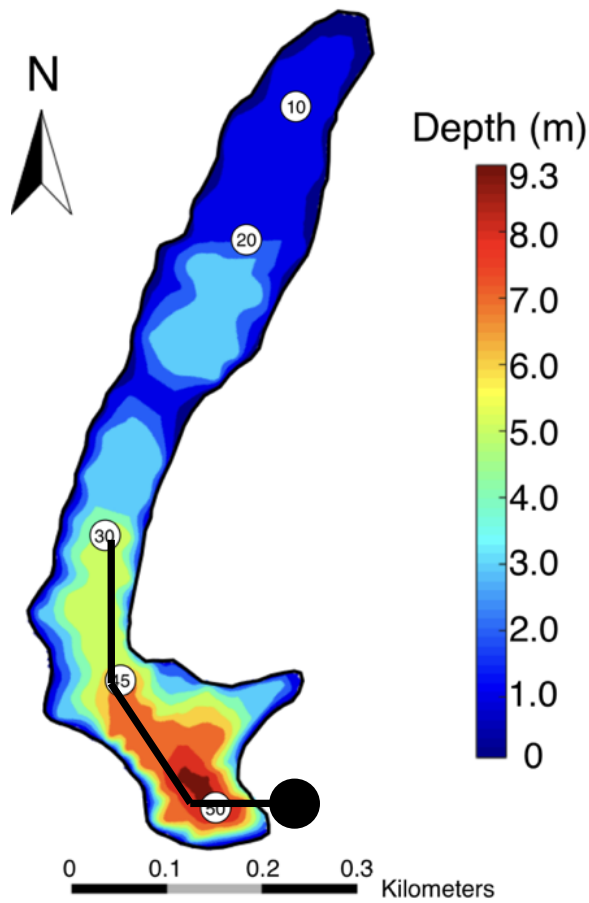

Figure 1. Bathymetry map of FCR and the five transect locations, along with representation of the EM location.
Adapted from McClure et al., 2017 (In review).

2.2.1. Epilimnetic mixing system

The EM system was deployed in 2012 by the WVWA to improve the water quality of FCR. The EM is designed to simultaneously mix and deepen the thermocline by injecting compressed air onshore through a diffuser line ~5 m below the surface (Figure 2). The EM mixes the upper 5 m of FCR, thereby

disrupting the growth of some algal species by decreasing their access to light and nutrients (e.g. Visser et al., 2016).

The EM was activated twice throughout the summer (29-30 May 2017 and 10-12 July 2017) (Table 1). On 29 May it was turned on in the afternoon and ran for 24 consecutive hours without any issues. The second EM activation was in three pulses rather than one continuous pulse like the first EM activation was. On 10 July, the EM it was turned for 11 hours, then another 6 hours the next day (11 July), and finally another 5 hours the following day. This adds up to a total of 22 hours of operation during the week of 10-14 July (Table 1).

Because this was a 12-week study with two separate mixing events, we divided the data into two asymmetrical periods. The first 4 weeks (08 May – 29 May 2017) of the study were considered the pre-mixing period for the first EM activation, and the following four weeks (05 June – 26 June 2017) were considered the post-mixing weeks. Similarly, the weeks of 05 July 2017 and 10 July 2017 were considered the pre-mixing weeks for the second EM activation, while the weeks of 17 July 2017 and 24 July 2017 are the post-mixing weeks for the second EM activation.

Table 1. Schedule and magnitude of air addition from the epilimnetic mixer (EM) during the monitoring periods in summer 2017.

Dates of Activation	Air addition rate (kg d^{-1})
29 May – 30 May 2017 (24 hours)	4.4×10^5
10 July 2017 (11 hours)	4.4×10^5
11 July 2017 (6 hours)	4.4×10^5
12 July 2017 (5 hours)	4.4×10^5

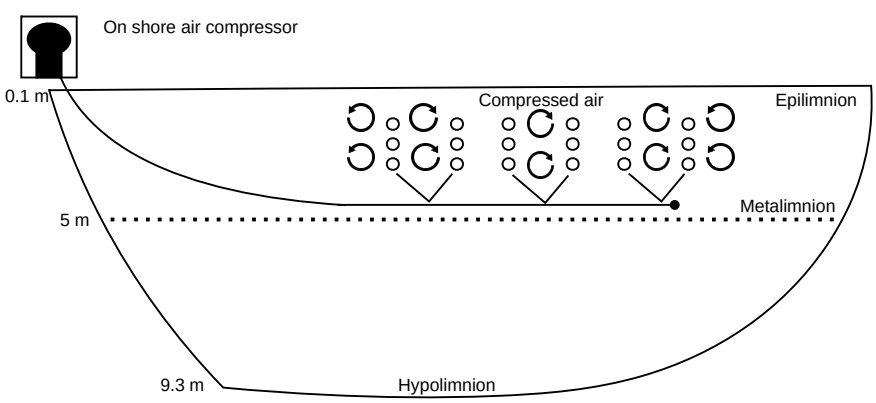

Figure 2. Schematic representation of the EM system in FCR.

2.3.1. Field data collection and laboratory analysis

2.3.2. Ebullition traps

Ebullition bubbles from FCR were sampled weekly, from 05 May through 24 July. Five transect lines were spaced throughout the reservoir (Fig. 1), and each transect had four inverted funnel traps placed evenly along the transect. Each trap was placed 0.5m below the water surface to capture any methane bubbles that left the sediments underneath the trap. A sealed tube extended from the funnel above the surface and water was siphoned to the top of the traps. When rising CH_4 bubbles are caught in

the funnel trap, the siphoned water at the top of the tube was displaced with the gas, which stayed at the top of the tube until the gas was extracted during sampling.

2.3.3. Ebullition gas collection

The top of the inverted funnels tubing was fixed with a 25mm PVC Threaded Ball Valve that was fitted with a rubber septum stoppers (Suba-Seal Septa, Sigma Aldrich). The valve and the septum prevented any ebullition gas from escaping out of the top of the funnel. Before collecting any ebullition gas, the small space between the ball valve and the septum stopper was pre-evacuated of any atmospheric gas that may have leaked into the space. This ensured that the sample collected from the trap was all ebullition gas that had been caught in the funnel. Once the chamber was evacuated, the ball valve was opened and gas samples were extracted using a 10 mL syringe. Up to 10 mL of gas was then injected into a 12 mL glass vial crimp sealed and filled with salt brine solution. A secondary exit syringe extracted the salt brine solution as the sample injected generated 10 mL of gas headspace in the vial. If possible, two replicate vials were collected from each trap. The vials were stored upside down so the vial septum and the remaining 2 mL of salt brine solution acted as a barrier to any gas that could escape. All remaining excess gas within the chamber was extracted and recorded using a 30 mL syringe to quantify the total volume of ebullition gas collected from each site.

2.3.4. Lab Analysis

The gas samples were analyzed using a gas chromatograph coupled with a flame ionization detector (GC-FID) less than 24 hours after being collected. Using the ideal gas equation, the total number of moles of CH_4 gas in the ebullition sample was calculated. We corrected the total mass of CH_4 in the sample in µmol L^{-1} by the area of the inverted funnel trap and the duration of time between each sampling to calculate each site's rate of CH_4 ebullition in mg d^{-1}. For sites that collected more than 10 mL of gas per week, and therefore had replicate corresponding vials, we present the mean value in mg d^{-1} of all collected samples.

3. Results

3.1 Total reservoir ebullition rate averages

We observed a significant increase in ebullition rates after the first EM mixing. Before EM activation, ebullition rates were relatively low, ranging from 313.7 – 469.8 mg CH_4 m^{-2} d^{-1} over a four-week period prior to the first EM activation (Figure 3). Across these four weeks, we observed a mean rate of ebullition of 367.4 (± 70.52, 1 S.D.) mg CH_4 m^{-2} d^{-1} in the whole reservoir.

After the first EM, we observed an increasing trend in ebullition rates for the following four-week period post mixing, resulting in significantly higher average ebullition rates during the post-mixing period (Figure 4). Immediately after the mixing, ebullition rates in FCR rose by 4-fold compared to the week prior to 1311 mg CH_4 m^{-2} d^{-1} on 05 June. Despite a small decrease in ebullition rates the following week (12 June), ebullition rates continued to increase throughout the four-week post-mixing period, ranging from 875.1 to 4397 mg CH_4 m^{-2} d^{-1}. In total, we observed a mean ebullition rate of 2432 (± 1638, 1 S.D.) mg CH_4 m^{-2} d^{-1} for the four weeks post-mixing (05 June – 26 June). This is significantly higher than the mean ebullition rate observed before EM activation (t_3=2.5, p = 0.04).

The second EM also increased ebullition rates in FCR, but not at the same magnitude that was observed during the first EM (Figures 3, 4, 5). Ebullition rates were on an increasing trend after EM 1 during a two-week pre-mixing period (05 July – 10 July) before the second EM activation on 10 July. During this period, mean ebullition rates were 5580 (± 1136 1 S.D.) mg CH_4 m^{-2} d^{-1}. One week after EM 2, ebullition rates actually declined slightly before soaring to 8705 mg CH_4 m^{-2} d^{-1}, the final week of sampling; these were highest ebullition rate of the summer that we found across the reservoir (Figure 3).

There was an overall increase in average ebullition rates after the mixing event (Figure 5). The average ebullition rate for the two-week period post-EM 2 in FCR was 7435 (± 1796, 1 S.D.) mg CH_4 m^{-2} d^{-1}, a marginally significant increase to pre-EM 2 ebullition rates (t_1 = 3.98, p = 0.08).

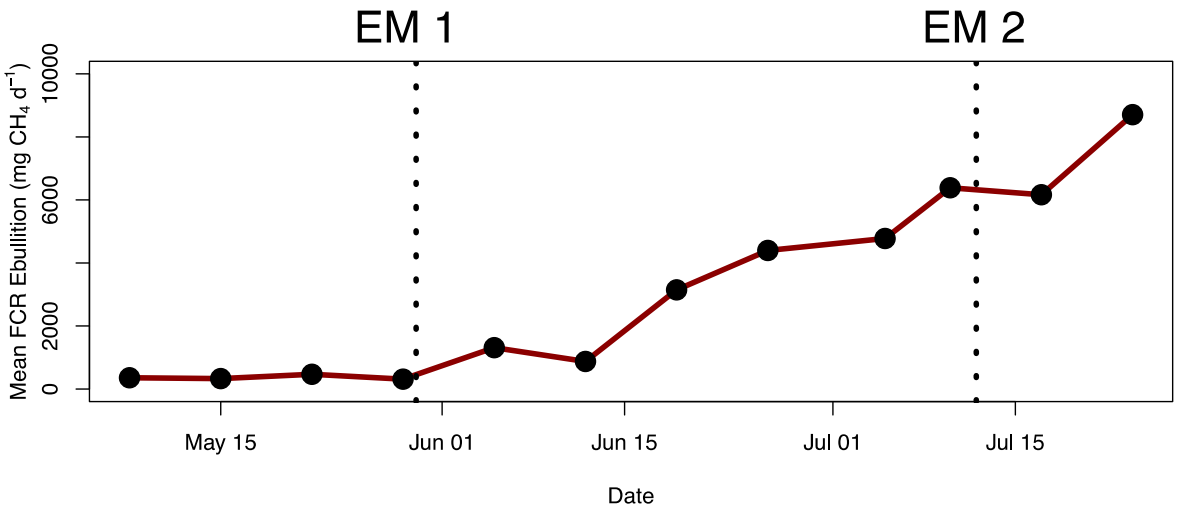

Figure 3. Weekly ebullition rates averaged across all sites in Falling Creek Reservoir (FCR) throughout the monitoring period.

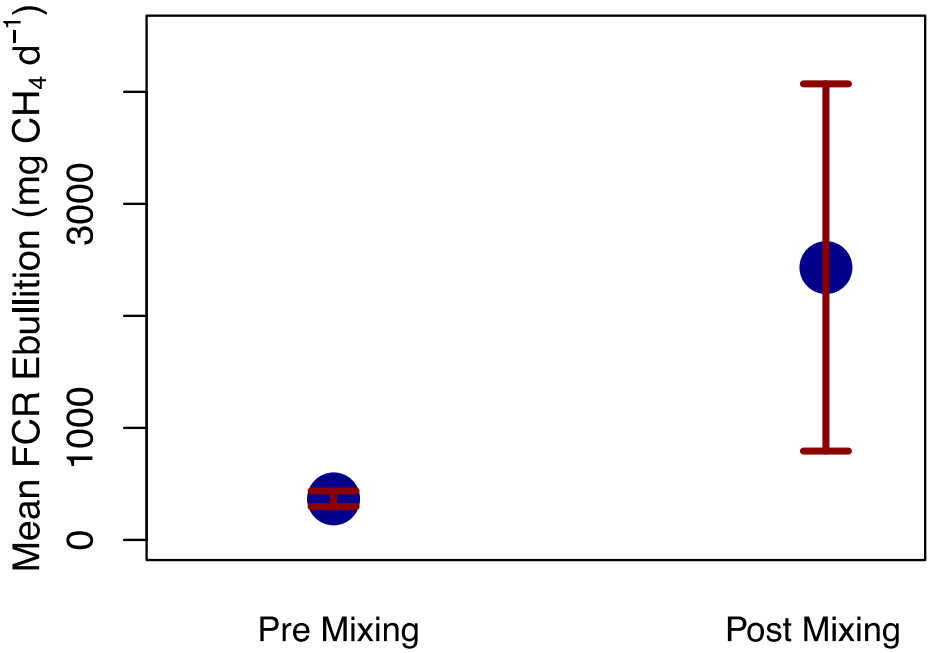

Figure 4. Mean ± standard deviation whole-reservoir ebullition rates of the four weeks preceding the first mixing event on May 29 and of the four weeks following the mixing event.

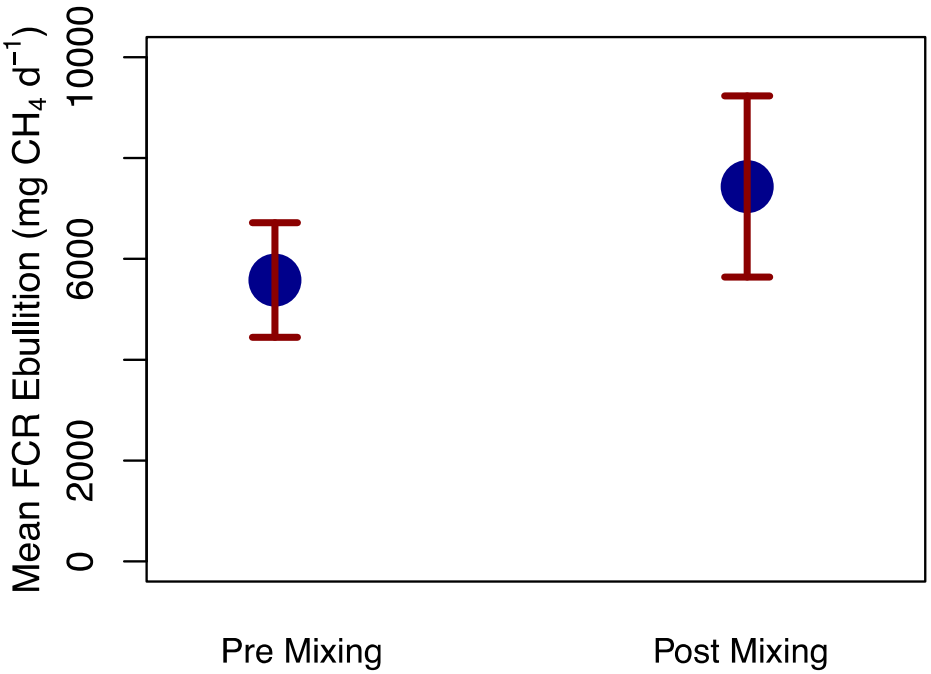

Figure 5. Mean ± standard deviation ebullition rates of the two weeks preceding the second mixing event on 10 July – 12 July and of the two weeks following this mixing event.

3.2 Transect ebullition rates

3.2.1. Transect 10

The mean ebullition averaged rates reflect differences in the transects from the dam to the inflow (Figures 1, 6). At transect 10, the shallowest site farthest away from the deepest site in FCR (Figure 1), ebullition rates sharply increased after the first mixing (Figure 6). Before EM 1, the mean ebullition rates from site 10 were 31.78 (± 54.69, 1 S.D.) mg CH_4 m^{-2} d^{-1} over the four-week pre-mixing period. The week immediately after mixing, rates increased by 10-fold to 334 mg CH_4 m^{-2} d^{-1} and continued to increase throughout the post-mixing period (Figure 6), up to 7449 mg CH_4 m^{-2} d^{-1} on 26 June 2017. Although the increase in ebullition rates after the first mixing were substantial, a t-test comparing the ebullition rates four weeks prior and after EM 1 only exhibited a marginally significant increase (t_3=2.16, p = 0.06).

The second EM event also increased ebullition rates at site 10, but not to the same magnitude at EM 1. Ebullition rates were still increasing at transect 10 as we entered the two-week pre-EM 2 mixing period (Figure 6), reaching 21790 mg CH_4 m^{-2} d^{-1} on 10 July. Ebullition rates fell the week after mixing (17 July) before reaching the highest rates of the summer on 24 July (Figure 6), a maximum of 25720 mg CH_4 m^{-2} d^{-1}. The observed increase in ebullition two-weeks after EM 2 was found to be only marginally significant (t_1 = 4.10, p = 0.08).

3.2.2. Transect 20

At transect 20, ebullition rates were generally low before EM 1 and increased following activation (Figure 6). Through the four-week pre-EM 1 period, rates ranged between 1.550 and 1699 mg CH_4 m^{-2} d^{-1}, averaging to 852.8 (± 727.4, 1 S.D.) mg CH_4 m^{-2} d^{-1}. One week after EM activation, ebullition rates were 1711 mg CH_4 m^{-2} d^{-1}, and increased to > 3000 mg CH_4 m^{-2} d^{-1} by the end of the four-week post-mixing period, with a mean ebullition rate of 2135 (± 1798, 1 S.D.) mg CH_4 m^{-2} d^{-1}. While we observed a marked increase in ebullition rates we could not detect a significant increase in as a response to the mixing event (t_3=1.63, p = 0.10).

Ebullition rates at transect 20 varied during the two-week EM 2 pre-mixing period with a mean rate of 2135 (± 1798, 1 S.D.) mg CH_4 m^{-2} d^{-1}. Post-mixing ebullition rates at transect 20 slightly increased to 4470 (± 49.06, 1 S.D.) mg CH_4 m^{-2} d^{-1} for the two-week period after EM 2. Although there was a substantial rise in ebullition rates at transect 20 over EM 2 (Figure 6), we did not find a significant increase in ebullition due to mixing (t_1 = 1.79, p = 0.16).

3.2.3. Transect 30

Ebullition rates at transect 30 increased marginally during EM 1, but had a substantial increase after EM 2 (Figure 6). During the pre-EM 1 four-week period, the average ebullition rate was 403.0 (± 685.7, 1 S.D.) mg CH_4 m^{-2} d^{-1}. During the four-week post mixing period, the mean ebullition rate of the post-EM 1 period doubled to 885.6 (± 574.08, 1 S.D.) mg CH_4 m^{-2} d^{-1}. However, this increase during EM 1 was insignificant (t_3 = 0.79, p = 0.24).

The pre-EM 2 period at transect 30 had relatively high ebullition rates for this transect (Figure 6), averaging to 2321 mg CH_4 m^{-2} d^{-1} (± 1455). The post-EM 2 period, though, saw an average over 3-fold larger, at 7503 mg CH_4 m^{-2} d^{-1} (± 1709). We found this increase in ebullition rates at transect 30 after EM 2 to be significant (t_1 = 28.9, p = 0.01).

3.2.4. Transect 45

Transect 45 had the lowest ebullition rates throughout the monitoring period with respect to the other sites (Figure 6). There was a decrease in ebullition rates observed at transect 45 between the four-week pre- and post- EM 1 period (Figure 6), but a substantial increase was observed during the EM 2 pre- and post- period. Ebullition rate were consistently low, approaching or at 0 mg CH_4 m^{-2} d^{-1} for most of pre- and post- EM 1 (Figure 6). However, we observed one large increase in ebullition rates from transect 45 on 22 May (Figure 6). The mean ebullition rates for the pre-EM 1 period were 100 mg CH_4 m^{-2} d^{-1} higher than post-EM 1 rates; since we did not observe an increase in ebullition rates, these results were not significant (t_3 = -0.70, p = 0.73). The two-week pre-EM 2 period exhibited near-detection ebullition at 6.750 (± 9.260, 1 S.D.) mg CH_4 m^{-2} d^{-1}, while the post-EM 2 period rates were 49.58 (±1.952, 1 S.D.) mg CH_4 m^{-2} d^{-1}. The increase in rates from the pre-EM 2 period to the post-EM 2 period was found to be significant (t_1 = 8.29, p = 0.04), but the magnitude of change in ebullition rates was much smaller in comparison to the other transects and EM periods (Figure 6).

3.2.5. Transect 50

The transect 50 ebullition rates had the largest variability throughout the summer as compared to the other transects (Figure 6). The relatively high ebullition rates with respect to shallower transects in FCR was also surprising (Figure 1, 6). Before any mixing in FCR occurred, ebullition rates remained relatively low (Figure 6) (198 mg ± 382.6, 1 S.D.) CH_4 m^{-2} d^{-1}). Ebullition rates significantly increased after EM 1 activation (Figure 6), rising to a mean rate of 5381 (± 3444, 1 S.D.) mg CH_4 m^{-2} d^{-1}. The post-EM 1 average marks almost a 30-fold increase compared to the pre-EM 1 average (Figure 6). This was the only transect where a significant increase in ebullition rates coinciding with the first EM activation time was detected (t_3 = 3.3, p = 0.02).

We also observed a substantial increase in ebullition rates from transect 50 after EM 2. During the pre-EM 2 two-week period at transect 50, mean ebullition rates were 2754 mg CH_4 m^{-2} d^{-1} (± 874.3), much lower than they were in the previous weeks (Figure 6). However, the ebullition rates from transect 50 almost doubled during the two-week post-EM 2 period (Figure 6), resulting in another significant increase in ebullition rates after a post-mixing event at transect 50 (t_1 = 22.0, p = 0.01).

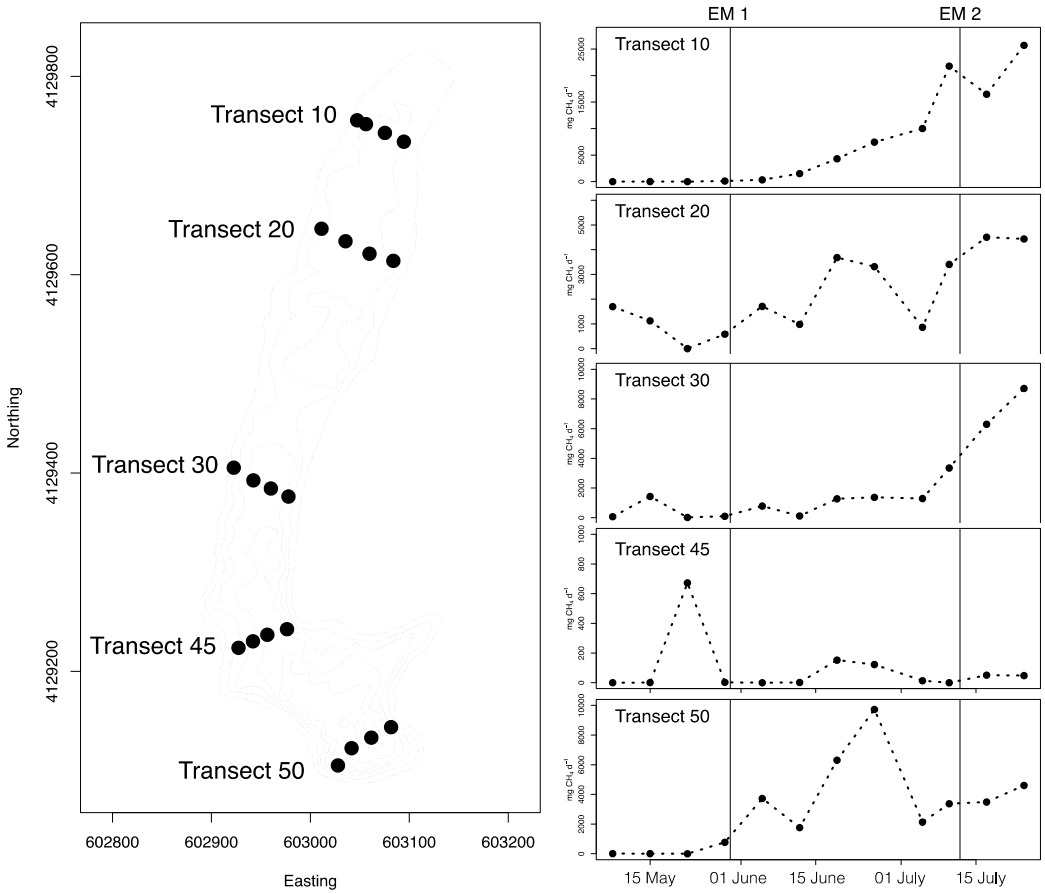

Figure 6. Bathymetry map of FCR with dots representing the 20 ebullition traps, four at each transect line. Ebullition rates are divided into weekly averages at each transect and plotted as line graphs on the right, with EM events denoted with vertical lines.

4. Discussion

Our data indicate that there was a significant increase in ebullition rates across FCR after the first EM event. However, on the transect level a significant increase in relation to the first EM activation was only found at transect 50, the deepest site, with marginally significant increases at the shallowest sites (i.e., transects 10 and 20). The largest increases in ebullition in FCR throughout the monitoring period were observed in the shallow upstream area (Figure 6), despite the marginal significance of the statistical analysis. These shallow upstream areas not only had the largest magnitude of increase, but also larger values overall, especially compared to sites closer to the dam like transect 45 (Figure 6). These observations partially support our original prediction that EM sediment disturbance would most likely affect shallower sites more.

We observed more significant increases in ebullition rates among transects after EM 2, despite changes in rates at the whole-reservoir scale being only marginally significant. At transects 30, 45, and 50, ebullition rates significantly rose after EM 2, which have also been related to increasing water temperatures in FCR during this time (data not shown). It is worth noting that while the EM does increase turbulence throughout the reservoir, it only physically extends to transect 30 (Figure 1). Furthermore, the close proximity of transect 30, the shallowest of the three transects of which the EM directly abuts, may have resulted in greater sediment disturbance at this transect than the others. Overall, we observed less

variability in the rates throughout the reservoir and at the transect level during the EM 2 period; however, due to small sample size (n = 2) as a result of lack of time, the EM 2 results have less statistical power than the results of the EM 1 event (n = 4) and cannot be directly compared.

Seasonal changes in FCR like increases in air and surface water temperatures, changes in water levels, large perturbations like natural storms and variability in the EM mixing events may also explain why we observed an overall significant increase after the first EM event but only a marginally significant increase after the second. For example, we observed lower water levels two weeks after EM 1 because another reservoir that feeds FCR was drained, decreasing the inflow into FCR. When the water levels decrease as we observed, the hydrostatic pressure in the water column will decrease (Schmid et al., 2017). In this way, the draining of this inflow may have naturally stimulated higher ebullition rates, making the difference between pre- and post- EM ebullition rates only marginal. There were also substantial storms observed by the water treatment plant operators on weeks without mixing events at FCR, and higher wind speeds from storms may have naturally increased ebullition rates at specific sites. Additionally, there were two different methods applied to the EM activation between EM 1 and EM 2 that may have caused the effects of the EM events to vary. The consecutive operation of the EM system during the first activation likely resulted in a more mixed reservoir than the second EM activation, which was applied in discrete pulses over a three-day period (Table 1). This pulse mixing only one month after EM 1 may not have been strong enough to meaningfully increase turbulence and ebullition rates in FCR.

Among both mixing events, transect 50, the deepest site, exhibited the largest variability within transect rates. While many biotic and abiotic factors may have contributed to the variability in rates, we must also consider the effects of the hypolimnetic oxygenation system (HOx) deployed at 8 m depth directly below the middle two traps at transect 50 (Gerling et al. 2014). The HOx system pumps water to the surface, where it is supersaturated with dissolved oxygen and pumped back into the hypolimnion of FCR. While induced sediment mixing from the HOx in FCR has not been previously reported, it is possible that the HOx induced sediment mixing that affected seasonal ebullition rates at transect 50. Because the HOx only oxygenates the hypolimnion of FCR and does not extend far past transect 50, therefore, we assume that the HOx had no effect on ebullition rates at any of the other transects in FCR.

We note that the degree to which the EM influenced on ebullition rate increases cannot be fully understood until other seasonal factors that also contribute to changes in ebullition rates are considered. The stochastic nature of ebullition (Bastviken et al., 2004; Delsontro et. al., 2010) makes it difficult to assess whether the changes in ebullition rates observed were a result of EM events or seasonal trends that naturally increased ebullition rates. There could have been a number of confounding variables contributing to ebullition rates throughout the sampling period, such as weather events, disturbance from wildlife, etc., so it is challenging to attribute the change observed in the data fully to the mixing events alone.

As CH_4 concentrations in the atmosphere continue to increase, it is imperative that we give more attention to GHG emissions from reservoirs. Recent syntheses suggest that reservoirs contribute as much as 1.3% of global anthropogenic C emissions (Deemer et al., 2016). Additionally, it has been found that some hydropower reservoirs emit more GHGs than fossil fuels (Gunkel, 2009). These findings suggest that more research needs to be done on the nature of GHG emissions from reservoirs. Moreover, as reservoir construction increases to meet the growing demand for water supply, it is critical that determining how water quality management systems alter reservoir GHG emissions. Here, we have taken the first steps to understand how an epilimnetic mixing system designed to prevent harmful algal blooms increased the ebullition rates across a eutrophic drinking water reservoir, and advocate for further research on other reservoirs to broaden our comprehension of the potential tradeoffs of these systems.

5. Acknowledgements

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Evaluating Disparities in North Carolina Well Website Communications

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Abstract
Private drinking wells are not regulated by the U.S. Environmental Protection Agency (EPA) and are therefore left to well owners to maintain at their discretion. To find information regarding private wells, homeowners can visit their county well page which contains online health information (OHI). To determine how effectively county level websites are presenting pertinent well water information, a website assessment was completed to evaluate the landing page and presentation of testing information. For this research, U.S. Census data from 2010 was used to determine the ten counties with the highest median household income and the ten counties with the lowest median household income. General qualitative statistics, readability testing, and GIS mapping were completed for these 20 counties. We observed that the readability levels of the landing pages for the county well webpages were above the average reading level in America (8th grade). Although most counties were providing well users with information, the communication of this material was presented in a manner that may not be accessible to all residents.

1. Introduction
Drinking water quality has been a top priority for both federal and local governments. In 1974, Congress passed the Safe Water Dinking Act (SWDA) which allowed the U.S. Environmental Protection Agency (USEPA) to regulate waterborne contaminants in public water systems (*Understanding the Safe Water Drinking Act*, 2004). The SWDA protects the water quality of lakes, reservoirs, springs, rivers, and ground water wells but not private wells (*Understanding the Safe Water Drinking Act*, 2004). Private wells serve fewer than 25 individuals for at least 60 days per year and have less than 15 service connections (*Understanding the Safe Water Drinking Act*, 2004), and homeowners are held responsible for ensuring drinking water quality. Thus, private wells do not have to undergo the same scrutiny as municipal water and are minimally regulated by state governments(W.J. et al., 2009).

<u>Problem</u>

Private wells are used by consumers who do not have access to connect to a public water supply. Since wells are primarily private property and purchased with a property, local governments have limited regulation of their drinking water quality. Contaminants in well water range from bacteria and coliform, metal pipe rust, *E.coli*, nitrates/nitrites, and many other inorganics. It is up to homeowners to regularly test their water to prevent contaminants from entering their water supply. Approximately “15% of the U.S. population are reliant on wells” ("About Private Water Wells | US EPA", n.d.), and North Carolina has the 3rd largest well reliance out of 50 states. There is a lack of online health information (OHI) present on county well web pages which strains the effectiveness of communication between county health departments websites and well users (Diviani & Meppelink, 2017). Most people have access to internet via computer, tablet, or smartphone to access health related information (Fox & Duggan, 2013). Thus, with the plethora of available resources people do not have to call or drive to their county health department to receive information on testing prices, permits or general inquiries. However, website usability refers to the organization of information on the webpage, navigation of the well webpage, and interaction between users and content (Devine, Broderick, Harris, Wu, & Hilfiker, 2016), and problems

can occur when the county health websites neither have a page designated for private wells or improper information about pricing, permit and overall well maintenance.

There is a strong correlation between someone's reading capabilities of OHI and their health outcomes(Boles, Liu, & November-Rider, 2016). The lack of information that consumers receive regarding their well water could alter their health in the future. The purpose of this research is to evaluate 20 county- level health department websites to determine the effectiveness of each county regarding OHI. The research hypothesis is that counties with a higher median household income will have websites with more resources and accurate information compared to lower median household income counties.

2. Methods and Materials

Selection of 20 county level health departments in North Carolina

The criteria for locating the mission statement was simple. If the website had a mission statement clearly posted that described their purpose of providing that information then it was counted. As there are 85 county-level health departments in North Carolina, this in-depth assessment study focused on the ten counties with the lowest and highest median household incomes. The 2014 US Census data for median household income was used, to determine 20 counties for the study (Tables 1 &2.). In addition, data of the percent of the population, for each county, with at least a bachelor's degree was recorded.

Table 1. High Median Household Income Counties

County	Median 2014 Household Incom
Camden	$69,353.00
Union	$65,735.00
Wake	$63,235.00
Currituck	$56,053.00
Chatham	$54,627.00
Mecklenburg	$54,559.00
Dare	$53,948.00
Orange	$52,591.00
Cabarrus	$52,086.00
Iredell	$51,947.00

Table 2. Low Median Household Income Counties

County	Median 2014 Household Incom
Tyrrell	$31,996.00
Columbus	$31,769.00
Richmond	$31,065.00
Hertford	$30,882.00
Alleghany	$30,859.00
Northampton	$30,838.00
Halifax	$30,298.00
Bertie	$29,779.00
Scotland	$29,657.00
Robeson	$29,128.00
Bladen	$29,104.00

Website Assessments

An analysis of each of the 20 county well websites was conducted. Specifically, websites were reviewed to determine if the well water program had a standalone landing page, rather than well water information being presented with other environmental health services. A landing page is the main page that holds information regarding the topic, it can include links and be a gateway to other areas on the site relating to certain topics. A standalone well page is designated to either strictly about wells or regarding wells and septic tanks/waste water. Information on testing prices, contact information, posted mission statements were collected and recorded, if present on the standalone landing page.

Flesch-Kincaid Readability

The Flesch-Kincaid readability test was used to determine the complexity of the text on each county's well webpage. This test was generated using a Microsoft Word extension. This test evaluates sentence structure to determine the complexity of selected text and gives an output of a grade level and reading ease. The grade level scores range between 4th and 30th grade, with an optimal score between 6th – 8th grade. This score is then matched with a reading ease which ranges between 0- 100, with 0 being extremely difficult to read and 100 being uncomplicated as shown in Figure 1 (Zhou, Jeong, & Green, 2017). We want to see a grade level between 6th -8th grade and a reading ease between 60-70. The Flesch-Kincaid Readability Test analyzes the types of words used in the text once all the links and pictures are taken out as shown in Figure 2. This formula considers the total number of words, sentences, and syllables to produce the grade level and reading ease. Poor sentence structure and repetitive words could

raise the grade level and lower the reading ease. The National Reading Grade Level Average is at an 8th grade reading level which is ideal for public health topics (Zhou et al., 2017).

Raw score	Difficulty level	Representative reading
<30	Very difficult	Scientific Journal
30-50	Difficult	General academically oriented magazine
50-60	Fairly difficult	Quality magazine
60-70	Standard	Digests
70-80	Fairly easy	Science fiction
80-90	Easy	Pop fiction
90-100	Very easy	Comic books

Figure 1. Flesch- Kincaid Reading Ease Score Chart

$$206.835 - 1.015\left(\frac{\text{total words}}{\text{total sentences}}\right) - 84.6\left(\frac{\text{total syllables}}{\text{total words}}\right)$$

Figure 2. Flesch- Kincaid Readability Formula

Spatial Analysis

Spatial mapping was conducted in ArcGIS, a mapping software that is a part of the ESRI program which allows one to input data from excel or an official government website and make a topographical map. ArcMap was the plugin used to show a more focused geospatial map of the counties with and without data layering.

3. Results and Discussion

Selection of 20 Counties

Before selecting categories, the range of median household income needed to be taken into consideration. The high-income counties range from $52,000 – $70,000, totaling a $18,000 difference across the 10 counties. The low-income counties range from $29,000 to $32,000, totaling a $3,000 difference across the 10 counties. None of the counties overlapped in income, the income of the lower income counties just did not increase as dramatically as the high-income counties

Website Assessment

After gathering the data from the website assessment and narrowing down what central things are missing from the well pages, we came up with a targeted list of things to look for on each page shown in Table 3. We believe that these categories are the most common things looked for on the webpage. We believe that when people visit the county well page it is to find contact information and testing prices. The other categories such as standalone well pages, mission statement, pictures, and range of microbial and inorganic testing were chosen because they aid in effectively stating what the page will be about. The range of microbial and inorganic testing was added to show the differences of lab pricing for low and high income which is competitive.

Table 3. Basic Statistics of Well Pages of Target Counties

	Low Income Counties (n=10)	High Income Counties (n=10)
Standalone Well Pages	20%	70%
Mission Statement Posted	0%	30%
Contact information Posted	70%	70%
Pictures on Opening Page	0%	20%
Testing Prices Posted	70%	60%
Range of Microbial Testing	$35.00 - $100.00	$25.00 - $200.00
Range of Inorganic Testing	$40.00 - $100.00	$40.00 - $250.00

Only 2 out of 10 low income counties have a standalone well page compared to the high-income counties, were 7 out of 10 of the counties have standalone well pages. Based on this difference, it was concluded that the lower income counties were joining other information with well information. These counties may have fewer resources to allocate to designing a separate page for wells alone. The next theme analyzed was if there was a mission statement posted on the well page, which both low and high-income counties lacked. Only 3 out of 20 counties had mission statements, those counties were Alleghany, Mecklenburg, and Wake. The mission statement was clearly visible on the page, it stated the purpose of groundwater/septic tank protection. Visual aids help conceptualize information and are more interactive then text paragraphs. Only 2 out of 20 counties contained pictures of private wells, both counties were high income. Text can become intimidating and confusing especially when trying to relay public health information (Zipkin et al., 2014). Testing prices were posted on 13 out of 20 of the county websites. This was the only occurrence where there were more lower income counties that had testing prices posted than high income counties. To find testing on the counties that were high income, a separate link was listed that led to a fee schedule for all the county services.

Flesch-Kincaid Readability Test

Despite the relationship between the reading grade level of text and the percentage of the population with a bachelor's degree, each county should have a grade level between 6th – 8th grade. When trying to convey public health information that relationship does not matter because all counties should be between 6th – 8th grade reading levels. As shown in *Figure 3*, the low-income counties have under 20% population with a bachelor's degree but still have grade levels that exceed 8th grade.

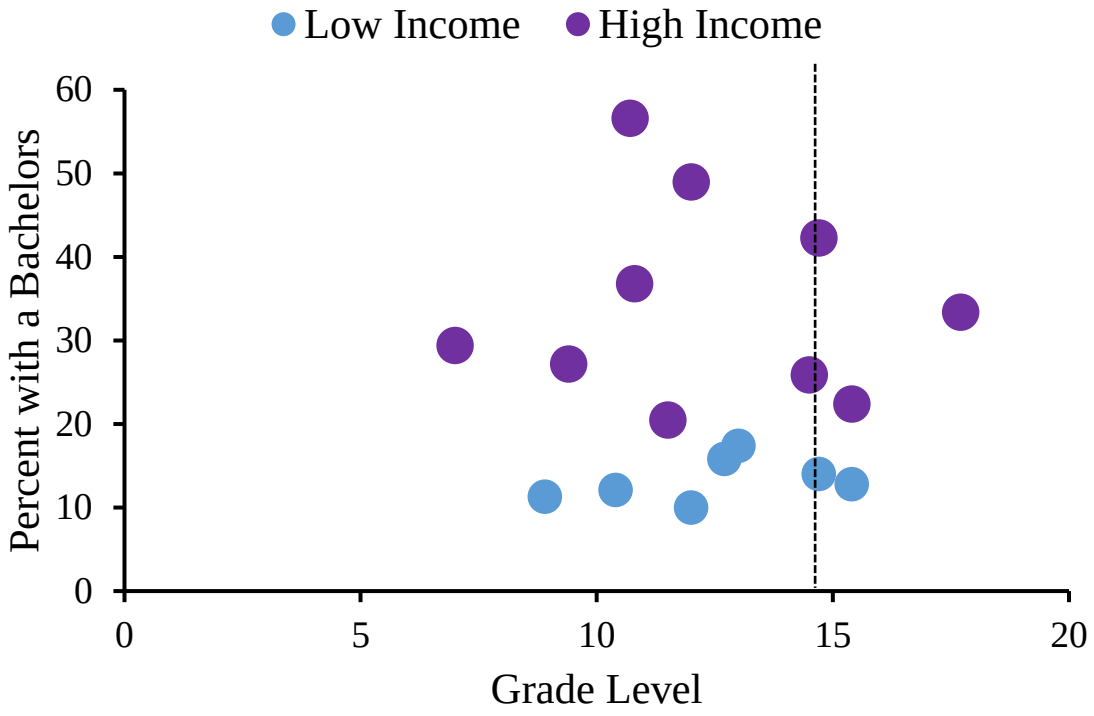

Figure 3. Grade Level correlation between Percent with a Bachelors for Low and High-Income Counties

Majority of the counties exceeded the national reading level of 8th grade except for 4 counties (Figure 3.), 3 of which had a score of zero for their grade level because they did not have a standalone well webpage. All the low-income counties again exceeded the national reading level despite low percentages of people with a bachelor's degree. Both Bladen and Robeson county well water webpages were merged with the environmental health page and therefore not included. Halifax county did not have anything well related on their county website. Halifax county has a higher median household income than both Bladen and Robeson county, yet they failed to provide any OHI relating to private wells. Lack of resources ranging from vacancies, lack of IT support, facilities, money and other factors cause many counties to not be able to decorate their well webpage.

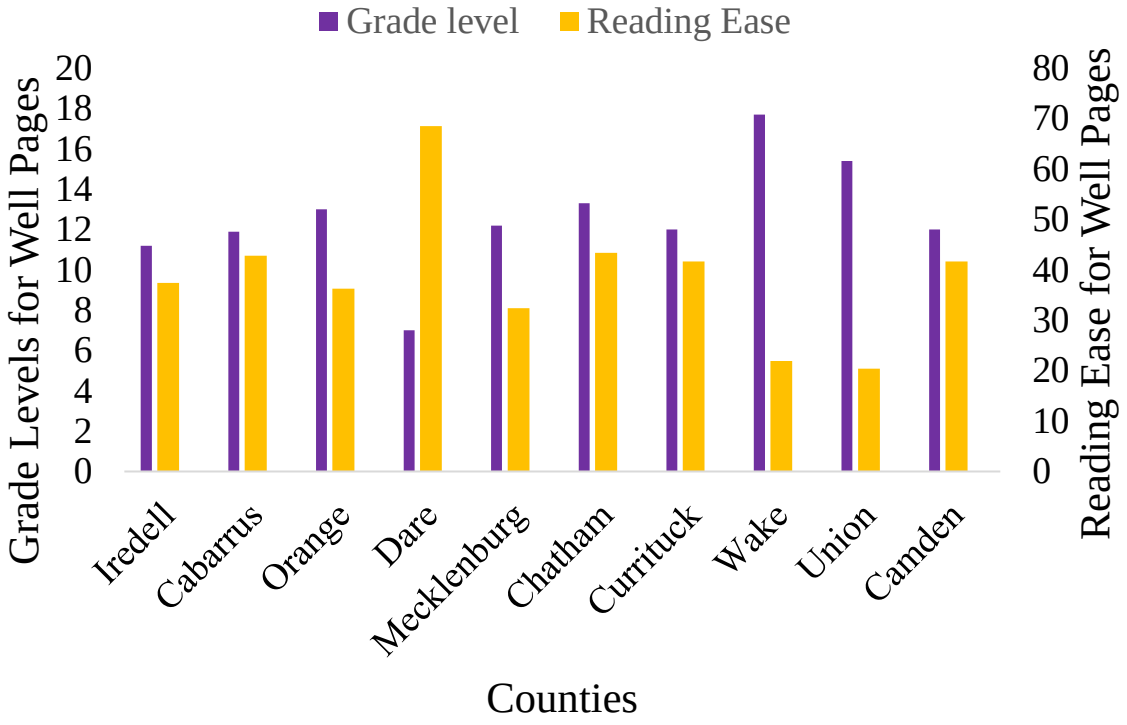

Figure 4. Grade level and Reading ease for each High-Income County

Dare county well website has the lowest grade level out of the other high-income counties. Both grade level and reading ease meet the goal of being between 6th -8th grade and having a reading ease between 60-70.

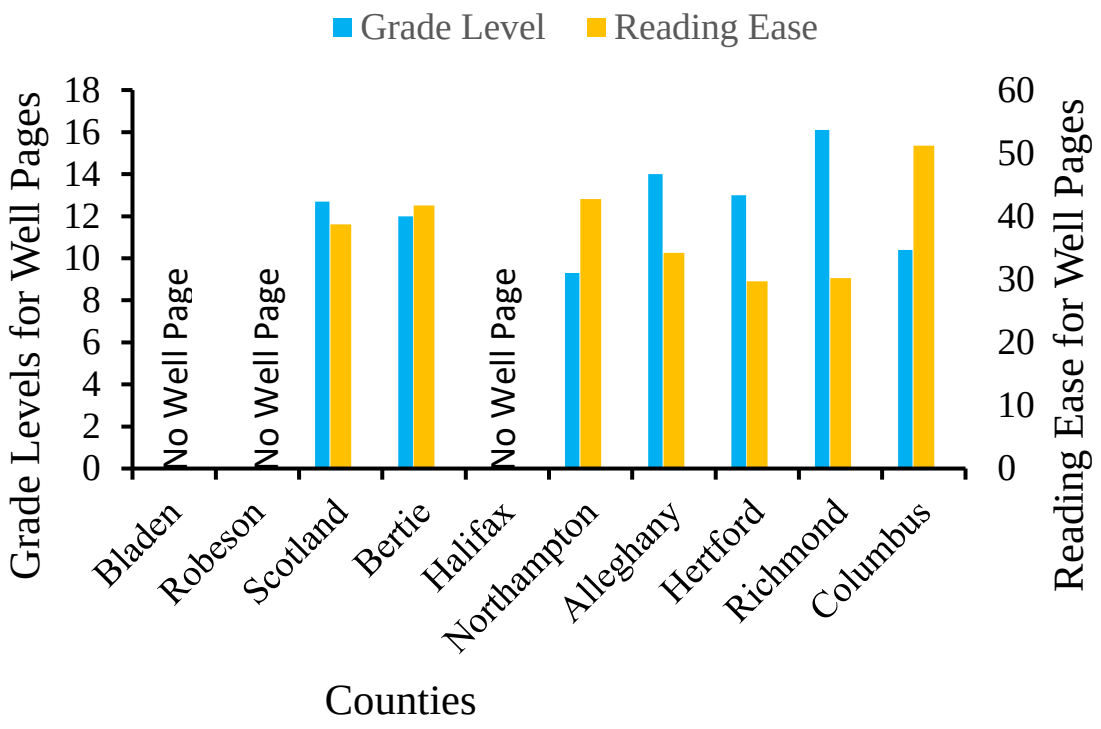

Figure 5. Grade Level and Reading Ease of Low Income Counties

Out of the all 10 low income counties, none in between a 6th -8th grade level or had a reading ease between 60-70. Three out of the 10 counties do not have a well page because they were not standalone well pages. Overall, the grade levels surpassed 8th grade and scored below a 60 for reading ease.

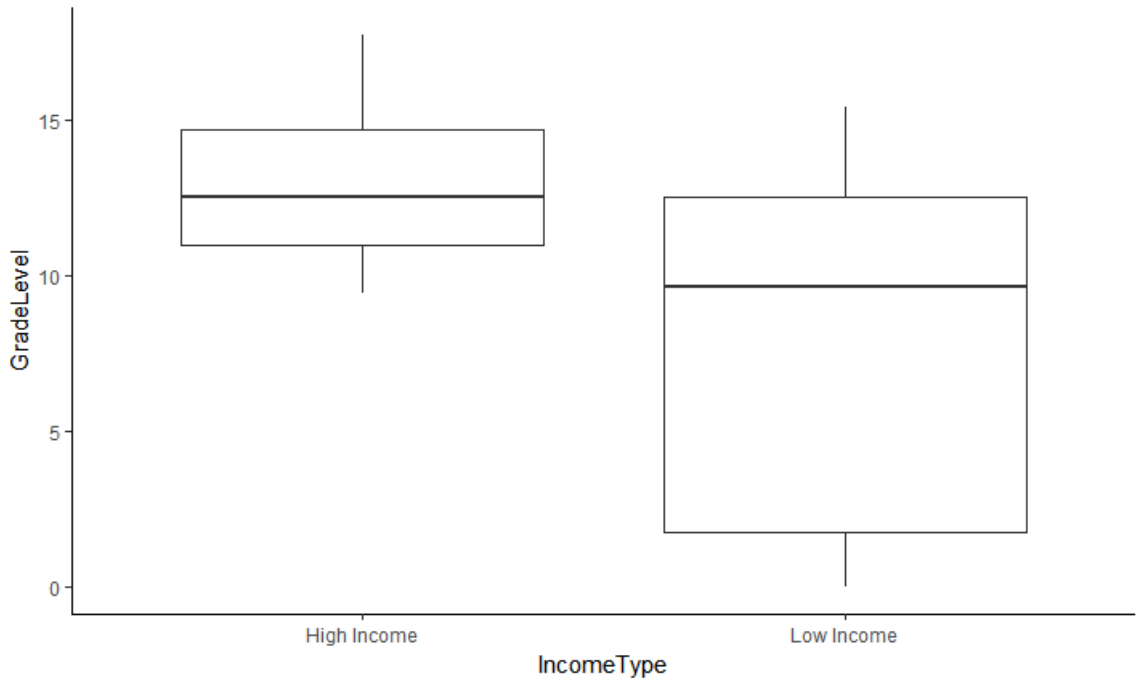

Figure 6. Boxplots of Average Grade Level of Low and High-Income Counties

We found the average grade levels for both low and high-income counties. The average grade level for low income counties is at 8.11, but does not include the possible grade level for the 3 counties without a well page. The average for high income counties is at12.97grade level.

Spatial Analysis

Gathering data from the readability test, multiple spatial maps were created showing median household income, grade level ranges, and higher education percentages for each county.

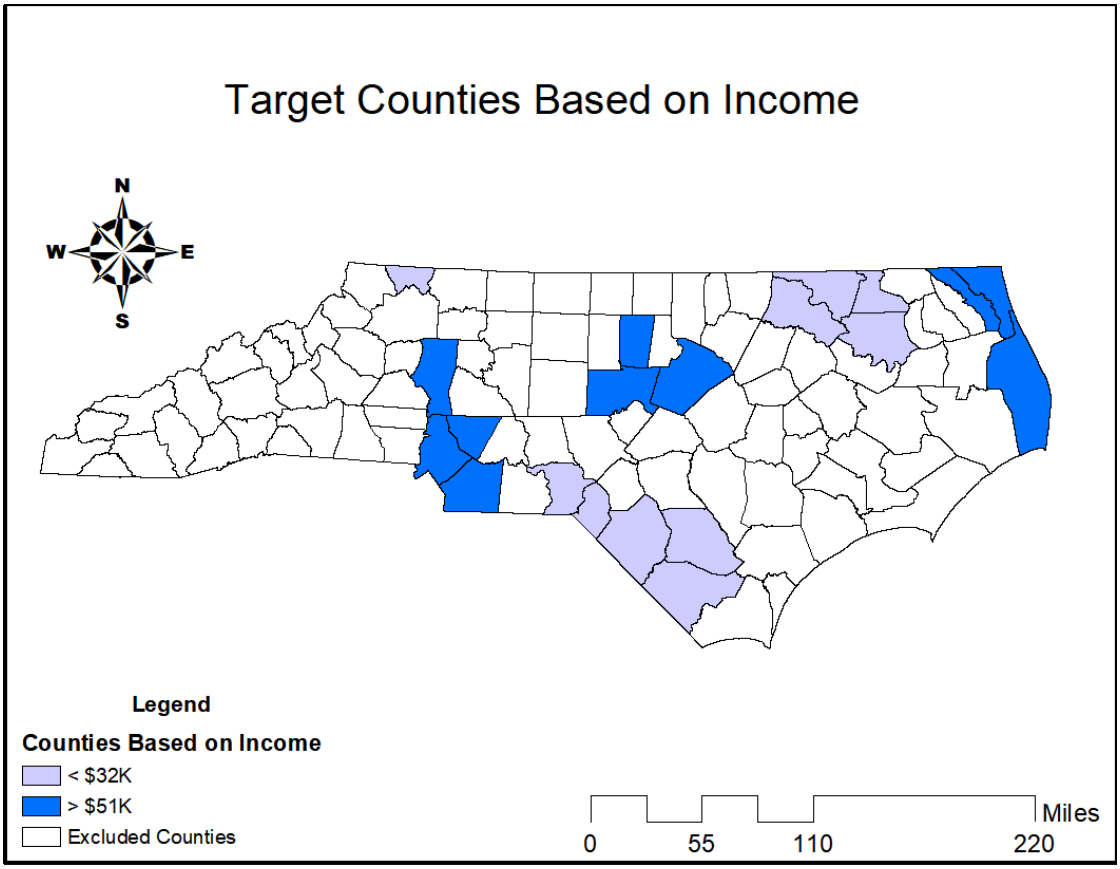

Figure 7. Base map of 20 counties in North Carolina by 2014 Median Household Income

Figure 7. shows the top 10 low and high-income counties are located more toward central and eastern North Carolina. The high-income counties represented by the dark blue colors are major cities such as Charlotte, Raleigh, Durham-Chapel Hill, and the suburban area of these major cities. The low-income counties are represented by a light blue color do not feature major cities or suburbs.

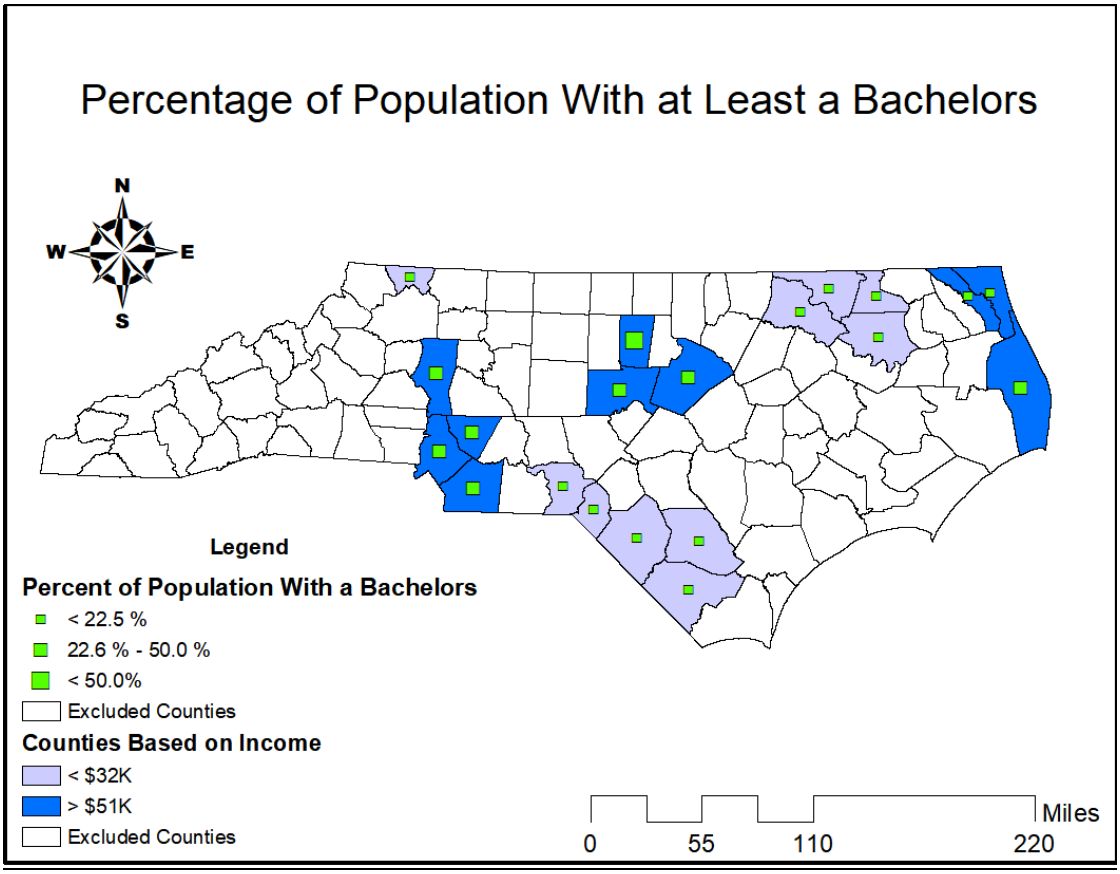

Figure 8, Percentage of Population with a Bachelor's degree in Target Counties

Education levels regulate income levels, higher education levels equal higher income amounts when schooling is finished (VanHulle, 2015). Except for Dare county, every other county has less than 22.5% of the population with a bachelor's degree.

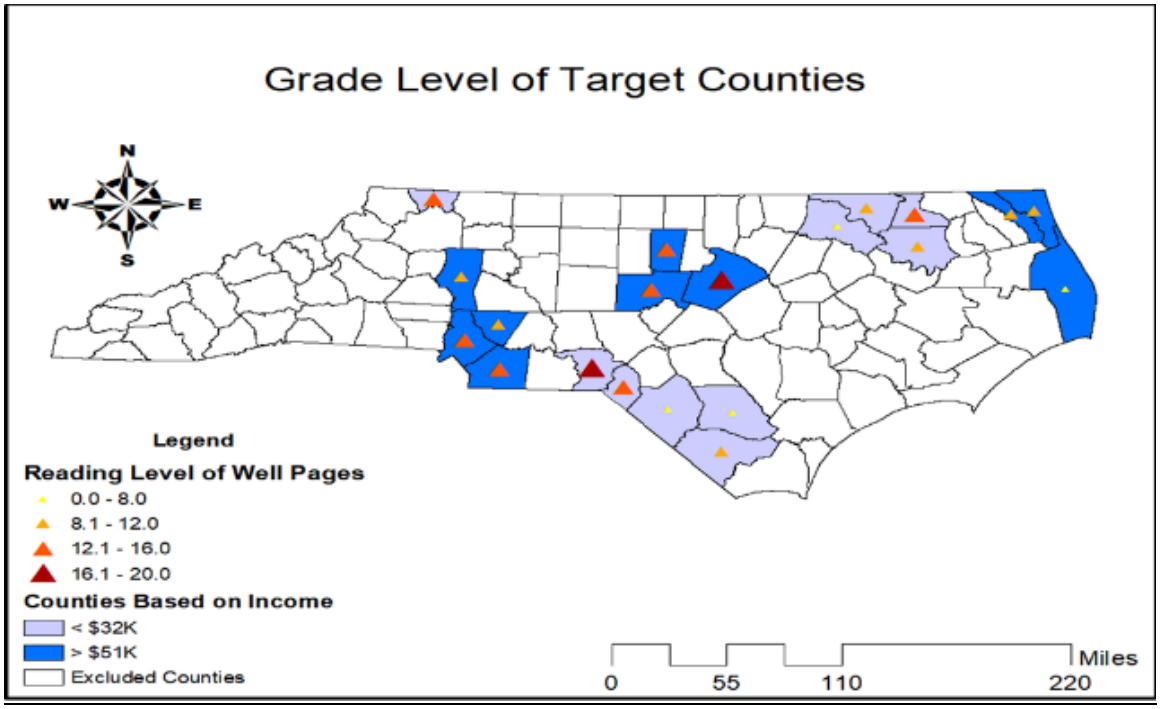

Figure 9. Readability Grade Levels or Target Counties

There are a few county websites with a grade level below 8 (yellow triangle) in figure 9, emphasizing that county websites meet the standard grade level. Only Dare county website meets the suggested grade levels of 6th – 8th grade reading levels.

4. Conclusions

Although higher income counties have better resources for effective website communication, the readability of the text is higher than the readability of low income counties. Low and high-income counties exceeded an 8th grade reading level and a reading ease score between 60-70. Despite, median household income levels or education levels, each county should strive to obtain an optimal grade level between 6th -8th grade and a reading ease between 60-70. To the improve the readability of the county well webpages, we suggest reexamining the sentence structure of text on the webpages, so that the Flesch-Kincaid Test computes a reasonable grade level and reading ease. As well as adding more visual aids to each of the county well webpages, to limit unnecessary text. In order to improve the safety of well water, county well webpages should provide clear, cohesive information that consumers can understand and apply when seeking information about their wells.

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Chemical Reduction of Geosmin Concentrations in River Water Using EarthTec®

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Abstract

Geosmin is a microbial metabolite that causes taste-and-odor issues in potable water. Geosmin's potent earthy odor is a nuisance to humans at ≥1-10 ng/L concentrations and its control is costly for the global drinking water industry. EarthTec® is an acidified copper-based algaecide/bactericide designed to reduce geosmin concentrations by two proposed mechanisms: through eradicating microorganisms that produce geosmin and inducing acidic dehydration of geosmin to form less odorous argosmin. This study aims to: 1) determine the effectiveness of EarthTec® at initiating the acidic dehydration of geosmin in river water from a waterway that experienced taste-and-odor issues; and 2) make recommendations to utilities using this river water regarding the application of EarthTec® in their water treatment systems. A known amount of geosmin was added to river water or distilled water and then mixed with 1 or 10 ppm EarthTec®. pH and geosmin concentrations were measured before and after introducing EarthTec®. Geosmin concentrations were measured by solid-phase microextraction coupled with gas chromatography-mass spectrometry and monitored via flavor profile analysis (FPA) with a trained human panel. There were no statistically significant changes in geosmin concentrations ($p>0.25$) after the addition of either 1 or 10 ppm EarthTec® to distilled or river water.

Keywords: Geosmin; EarthTec®; SPME; Odor

1. Introduction

In 2015, the City of Danville, the Town of South Boston, and the Town of Halifax first reported taste-and-odor issues associated with their potable water supply drawn from the Dan River in Virginia. Many consumers described the water taste as "earthy," "moldy," and "musty." During late February of 2015, a bright green algal bloom had been observed near the raw water intake of the Danville Water Treatment Plant (WTP), however taste-and-odor issues persisted after its disappearance. In the summer of 2015, the Virginia Department of Environmental Quality sampled along the Dan River and observed elevated phosphorous levels in the water (Boardman, 2016). Changes in the phosphorous to nitrogen ratio (P:N) can affect algae, bacterial, and fungal communities downstream (Watson et al., 2016). Although the source(s) of the taste-and-odor incidents associated with the Dan River are still being investigated, it is suspected that they may be related to the presence of cyanobacteria and actinomycetes that release the odorous metabolite geosmin while living or when they die. Geosmin is distinctly characterized by having an earthy smell, which is similar but perhaps not identical to the odor described by consumers of the City of Danville's water supply (Gerber & Lechevalier, 1965; Pahila & Yap, 2013; Watson et al., 2016). The City of Danville is interested in finding an effective treatment method for potential future taste-and-odor incidents and has inquired about the commercially-available algaecide/bactericide, EarthTec®. The specific objectives of this study are to: 1) determine the effectiveness of EarthTec® at inducing the acidic

dehydration reaction of geosmin to argosmin in Dan River water; and 2) make recommendations to utilities using Dan River water regarding the application of EarthTec® in their water treatment systems.

1.1 Geosmin

Geosmin has a very potent odor and very low human odor threshold concentrations (OTCs), between 1 and 10 ng/L (Piriou et al., 2009; Callejón et al., 2016; Watson et al., 2016). Human odor threshold concentration is defined as the concentration at which 50% of the test subjects or population detect an odorous compound through smell (U.S. EPA, 1992). Since geosmin is detectable by the human nose at such low concentrations, it is a particular inconvenience to the drinking water industry. The majority of worldwide biologically-caused taste-and-odor incidents in potable water are the result of microbial production of geosmin and 2-methylisoborneol (MIB) (Jüttner & Watson, 2007). It is worth noting that despite being a nuisance to consumers and the global drinking water industry, these compounds have not been determined to be toxic to humans (Mochida, 2009; Watson et al., 2016). These two compounds have been under investigation since their discovery in the 1960s yet there is still a considerable gap in knowledge regarding control and treatment of these volatile organic compounds (VOCs) (Jüttner & Watson, 2007).

1.2 Treatment Methods

Common treatment methods for taste-and-odor issues caused by geosmin currently include the use of powdered activated carbon (PAC), oxidation (primarily ozone), and biological degradation in addition to traditional methods such as flocculation, coagulation, sedimentation, filtration and disinfection (Mamba et al., 2007; Piriou et al., 2009). PAC is one of the more frequently used methods as it is relatively inexpensive and can be applied as needed. However, the type and dose of the activated carbon highly influence the effectiveness of the treatment (Cook et al., 2001). A highly effective and consistent treatment method for geosmin is critical because consumers are particularly sensitive to the quality of their drinking water. A nationwide survey of 1,754 bottled water consumers found that 39% chose to consume bottled water because it had a better taste than other types of potable water (Dietrich, 2006). Taste-and-odor incidents can have an adverse effect on the local economy as consumers may abstain from using water provided by their local utility company. People seek consistency in consumer products and "inconsistency is a sign that the product is different, which could mean that the product is not good" (Dietrich, 2006). It is in the best interest of the drinking water industry to find an effective treatment method for geosmin.

1.3 EarthTec®

EarthTec® is a U.S. Environmental Protection Agency (EPA)-registered algaecide/bactericide that is advertised to treat taste-and-odor issues related to the presence of geosmin. It is produced by Earth Science Laboratories, Inc. and is NSF-Certified to ANSI Standard 60 for drinking water. Like many algaecides, EarthTec® is copper-based; it is a liquid formulation that contains 5% copper by volume and is made from copper sulfate (Hammond, n.d.). Additionally, EarthTec® features a low pH of 0.3 which improves the solubility of the copper. Earth Science Laboratories, Inc. state that EarthTec® is infinitely soluble in water and that no mixing is required. The improved solubility of copper in EarthTec® explains why less copper is required for treatment than traditional copper sulfate methods. Earth Science

Laboratories state a copper concentration of 30-200 ppb (Hammond, n.d.). EarthTec® can be applied to both "open waters" (lakes, reservoirs) and "flowing waters" (raw water intakes, pipelines, canals, etc.). The recommended dose for feeding EarthTec® into pipelines is 1 ppm on a volume-per-volume basis and it is typically applied directly at the raw water intake (EarthTec®, 2017). Earth Science Laboratories, Inc. does not provide dosing information for different geosmin concentrations; the 1 ppm dose is recommended for all cases. EarthTec®'s Taste & Odor Rapid Response Program, which includes surveying, sampling, and appropriate dosing, claims to resolve taste and odor issues in as little as 1-2 days (Taste & odor rapid response program, n.d.). Earth Science Laboratories, Inc. also state that using EarthTec® does not result in the production of disinfection by-products (DBPs), trihalomethanes (THMs), or haloacetic acids (HAA5s) which are of concern to WTPs (Hammond, n.d.). It is advertised as the easiest, safest, and most efficient method to deliver copper.

EarthTec® works to reduce geosmin concentrations in two ways. The first is that the copper toxicity reduces the cyanobacteria populations that produce geosmin. The second is that the odorous geosmin compound is reduced to a non-odorous form called argosmin through acidic dehydration, as shown in Figure 1. Currently more literature exists regarding the effectiveness of EarthTec® on controlling zebra mussel populations than geosmin concentrations, although a number of case studies do exist on the Earth Science Laboratories, Inc. website. In particular, there is a dearth of information related to the kinetics of the acidic dehydration reaction that occurs between EarthTec® and geosmin. It is suspected that the reaction will resemble a first order reaction, as it will depend on the available concentration of EarthTec®.

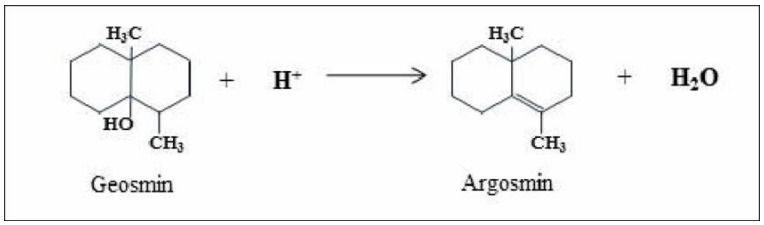

Figure 1. Conversion of geosmin to argosmin through acidic dehydration.

1.4 Solid-phase microextraction

Both solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS) were used to measure geosmin concentrations in the water samples. Other methods that are currently available to quantify the presence of geosmin in drinking water are more technically complex, include time-consuming processes, and required copious amounts of solvent, such as liquid-liquid extraction. Since geosmin is typically detected at the ng/L level, large sample volumes are required for analysis using these methods (Watson et al., 2000). Microextraction technology was recently developed and simplifies testing methods because only a small volume of the sample is required for analysis and the technique is relatively simple. Additionally, the time spent collecting data for each sample is only about 60 minutes. SPME had previously been used for pesticide analysis but was also found to be a reliable screening technology to monitor volatile and semi-volatile off-flavor compounds in surface waters and cultures (Watson et al., 1999; Górecki et al., 1999).

2. Materials and Methods

2.1 Preparation of Aqueous Geosmin Mixture

Two different water samples were tested: distilled water (Thermo Fisher Scientific ™ Barnstead™ NANOpure™ system) and water from the Danville Water Treatment Plant intake which feeds from the Dan River. The intake water samples were collected on June 19, 2017 and July 12, 2017 and water quality information is provided in Table 1. One gallon of EarthTec® algaecide/bactericide was donated from Earth Science Laboratories, Inc. and a ≥97% (+/-)-geosmin solution was obtained from Sigma-Aldrich. 4L of each water sample was mixed with 1ppm or 10 ppm of EarthTec® and 50-100 ng/L of geosmin standard. A stir bar was used to ensure that the mixture was well-mixed.

After 2 minutes of mixing, the aqueous solution was dispersed into 20 60 mL volatile organic analysis (VOA) vials under headspace-free conditions. One VOA vial served as a blank with only the water sample and no additives. Two VOA vials were filled with only the water sample and geosmin, as a standard of reference. The blank was measured first, then the two water and geosmin standards. Next, one of the vials containing the actual mixture was measured as close to time 0 as possible (about 10 minutes in the water bath). Then one vial of the mixture was selected every hour to be measured for geosmin. The vials that were not being analyzed at the time were left to sit at room temperature (~22 °C) for the duration of the experiment.

Table 1. Dan River water quality information for samples obtained in June and July 2017.

Characteristic	Dissolved Oxyen (mg/L)	Temperature (°C)	Conductivity (Ω)	pH	Alkalinity (mEq/L)
Mean	7.7	23.8	96.3	7.08	25.7
Median	7.8	23.5	87.0	7.10	25.95

2.2 Measurement of Geosmin by SPME/GC-MS

A Thermo Fisher Scientific FOCUS GC™, Thermo Scientific DSQ™ II Series Single Quadrupole MS, and a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane StableFlex Supelco® SPME fiber were used. Data was recorded and evaluated with Thermo Fisher Scientific Xcalibur™ software. One temperature ramp was used for the FOCUS GC™ instrument method: initial temperature at 40 °C with hold time of 1 minute was ramped to 50 °C with hold time of 1 minute at a rate of 10.0 °C/min. Oven conditions for the FOCUS GC™ instrument method were as follows: maximum temperature of 350 °C and equilibrium time of 0.5 min. Additionally, a 10 to 120 ng/L standard curve was prepared using geosmin dissolved in distilled water and 60 mL VOA vials. Selective ion monitoring using m/z values of 41, 55, and 112 was applied for quantitation.

20 mL of fluid were removed from the VOA vial with a syringe to ensure headspace was available for the SPME fiber. This vial was then placed in a water bath at 65°C for 30 minutes with the SPME fiber inserted into the center of the headspace for adsorption. The SPME fiber was extended to 3 cm for this process. After 30 minutes, the SPME fiber was retracted and transferred to the GC-MS. While in the GC-MS, the SPME fiber was extended to 5 cm. The fiber was left in the GC-MS for 10 minutes to desorb. After 10 minutes, the SPME fiber was retracted and removed from the GC-MS while the Xcalibur™ software continued the run. Measurements of geosmin in VOA vials were taken every hour for about 8 hours to target completion of the reaction. Pilot experiments that were run for about 35 hours

total indicated that changes in geosmin concentrations, if any, occurred within the first 8 hours of the experiment. Additionally, a control experiment was run with only geosmin in distilled water.

2.3 Sensory Testing by Human Panel

A qualitative study of the effectiveness of EarthTec® at reducing geosmin concentrations was conducted through a flavor profile analysis (FPA) according to Standard Method 2170 (APHA et al., 2000) (IRB 16-671). A trained panel of ≥3 participants were selected for the study. Participants were first asked to smell the initial water sample without additives as a control, since natural river water may have its own odor compared to distilled water. Participants were asked to record their initial observations regarding the smell of the water without additives. Participants were then asked to smell the water sample after the addition of EarthTec® and record their observations. Finally, participants were asked to smell a mixture of the water sample, EarthTec®, and geosmin and record their observations. Participants were asked to smell the mixture every hour over a period of about 6 hours. A taste-and-odor wheel for drinking water was used a basis for odor descriptions, as shown in Figure 2. Ratings were based on a 0-12 scale: 0 representing no odor, 2 representing a very weak odor, 4 representing a weak odor, 8 representing a moderate odor, and 12 representing a strong odor.

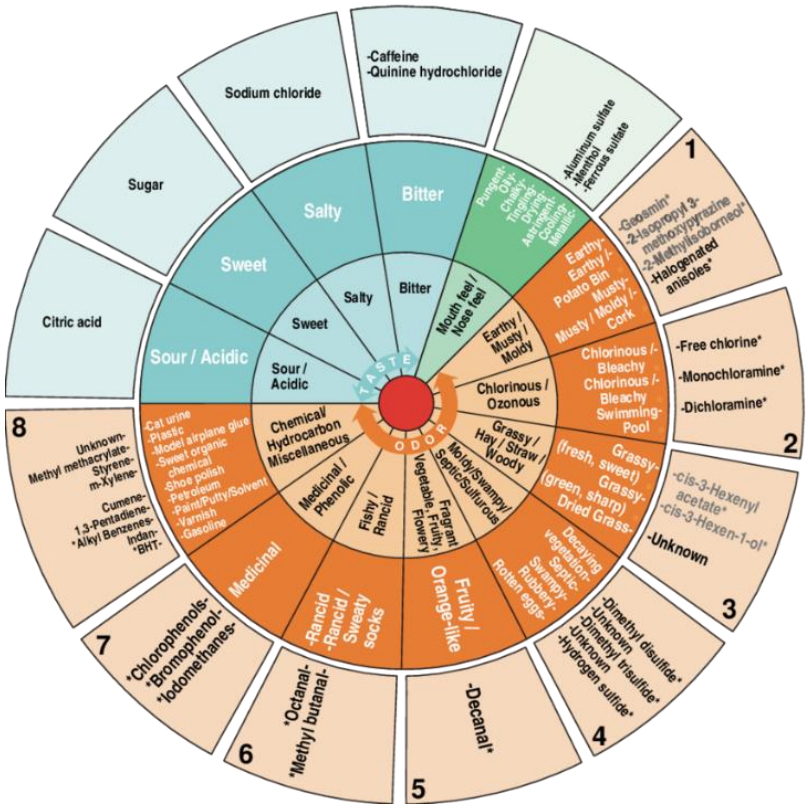

Figure 2. Taste-and-odor wheel for drinking water with different descriptors to characterize water (Khiari et al. 2002).

3. Results and Discussion

3.1 Geosmin Measurements

A control experiment was performed with geosmin added to distilled water, then aliquoted to 60 mL VOA vials; geosmin was measured in a single vial on an hourly basis. The results shown in Figure 3 demonstrate that while individual measurements of the geosmin concentration by SPME fluctuated, the slope of the regression line was not significantly different than zero and thus the geosmin concentration did not change with time (p=0.9965). Figure 3 also shows the distilled water and 1 ppm EarthTec® experiments. The initial pH decrease upon the addition of EarthTec® was 0.06 pH units. The 1 ppm EarthTec® addition had a similar pattern as the control as there was variability in measuring geosmin, but there was no change in geosmin concentration over time as the slopes of the lines were not different from zero. Figure 4 plots C_t/C_o ratios of geosmin concentrations over time for distilled water with 10 ppm EarthTec®. The initial pH decrease upon the addition of EarthTec® was 0.62 pH units. Again, there was no change in geosmin concentration over time as the slopes of the lines were not different from zero (p=0.9988).

Figures 5 and 6 show C_t/C_o ratios of geosmin concentrations plotted versus time for Dan River water with 1 or 10 ppm EarthTec®. Again, there was no significant change in the geosmin concentration over time as time as the slopes of the lines were not different from zero (1ppm EarthTec® p=0.2613; 10 ppm EarthTec® p=0.7747).

The addition of EarthTec® to raw water from the Dan River or distilled water containing geosmin returned variable results. Nine experiments resulted in no statistically different geosmin concentrations over time; linear regression analyses of the data indicated that the slope of geosmin vs. time was statistically not different than zero. There was little difference in reducing geosmin concentrations from adding 1 or 10 ppm EarthTec® or treating distilled or Dan River water. The variability in geosmin measurements throughout an experiment is the result of variability from the SPME technique. Similar results were obtained by another research group (Wang et al. 2014). Those results showed no decrease in geosmin concentration due to the addition of EarthTec®and also variability in the measurement of geosmin.

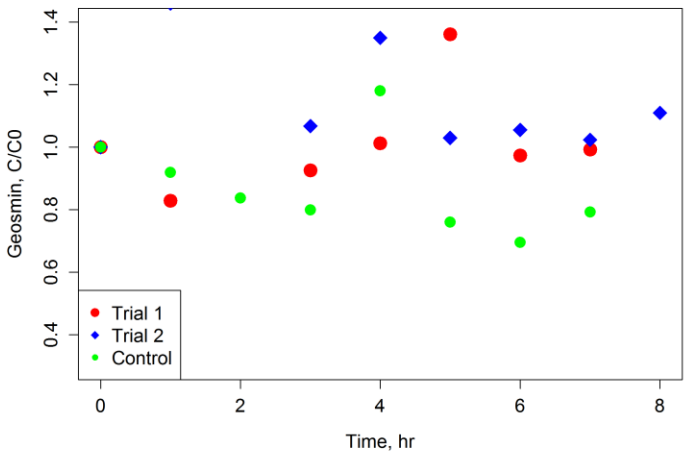

Figure 3. C_t/C_o geosmin concentrations versus time for distilled water with 1 ppm EarthTec®; control experiment contained no EarthTec®.

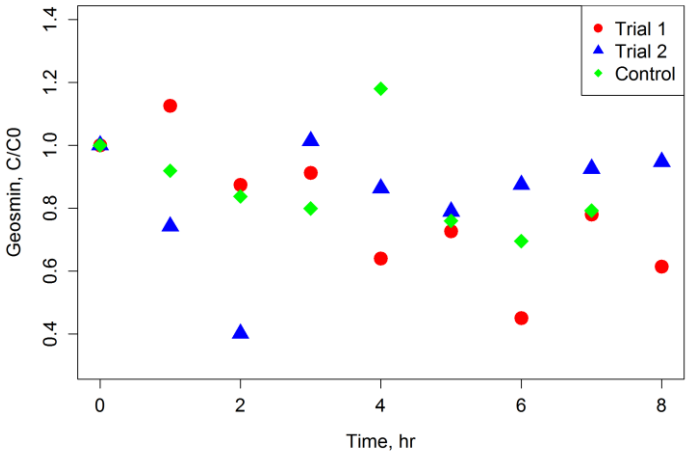

Figure 4. C_t/C_o geosmin concentrations versus time for distilled water with 10 ppm EarthTec®; control experiment contained no EarthTec®.

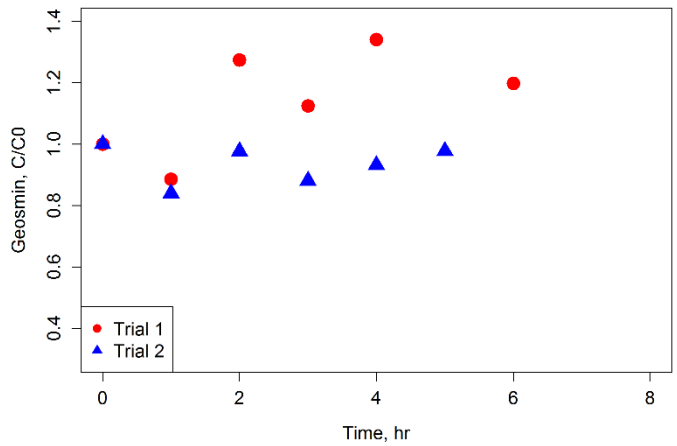

Figure 5. C_t/C_o geosmin concentrations versus time for Dan River water with 1 ppm EarthTec®.

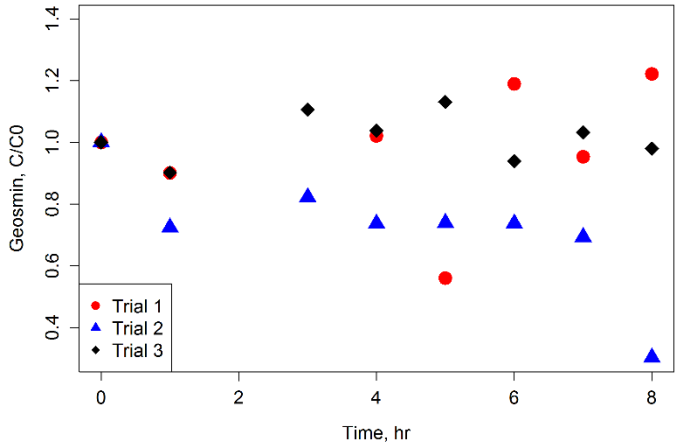

Figure 6. C_t/C_o geosmin concentrations versus time for Dan River water with 10 ppm EarthTec®.

3.2 Flavor Profile Analysis

Dan River water with 1 ppm EarthTec® and 10 ppm EarthTec® doses were tested for geosmin concentrations based on sensory analysis. Panelists provided descriptions and odor ratings of the water based on the taste-and-odor wheel in Figure 2. However, analysis focused particularly on "earthy/musty" odor ratings in order to pinpoint geosmin reduction. Figure 7 shows mean "earthy" FPA odor ratings over a period of 3 hours for 1 ppm EarthTec® in Dan River water and Figure 8 shows mean "earthy" FPA odor ratings over a period of 3 hours for 10 ppm EarthTec® in Dan River water. There is no substantial change in the FPA odor intensity over time.

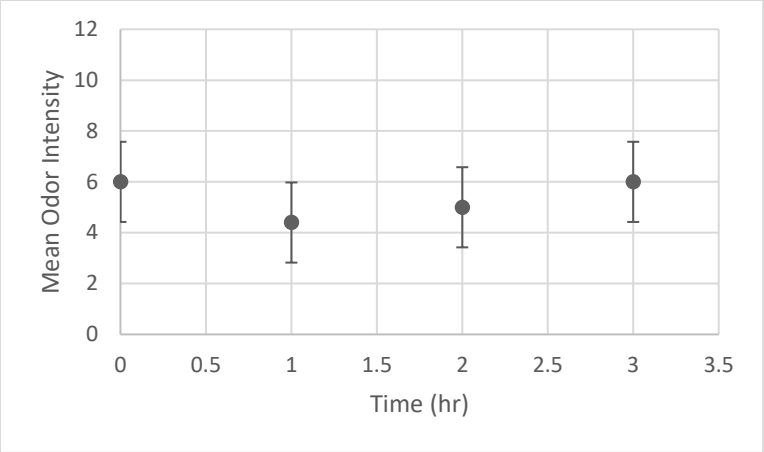

Figure 7. Mean odor intensity ratings over 3 hours for 1 ppm EarthTec® in Dan River water. Standard error bars for a 95% confidence interval are included.

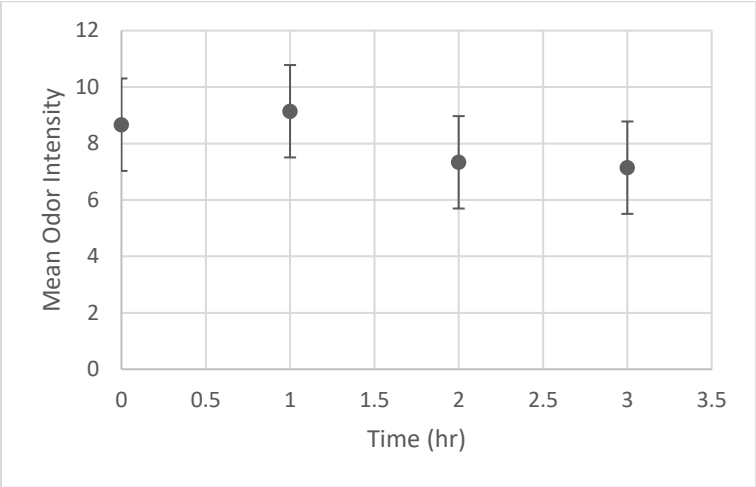

Figure 8. Mean odor intensity ratings over 3 hours for 10 ppm EarthTec® in Dan River water. Standard error bars for a 95% confidence interval are included.

4. Conclusions

Earth Science Laboratories, Inc. market EarthTec® as a product that works to effectively convert already-formed geosmin into non-odorous argosmin as well as control cyanobacteria; reduce total organic carbon (TOC); reduce downstream consumption of activated carbon, ozone, and coagulants; increase filter run time; reduce biofilm; and control zebra and quagga mussels (Hammond, n.d.). Most literature thus far has focused on cyanobacteria and zebra mussel control through copper toxicity and has reported the success of EarthTec® in those areas. Few data are available on the aqueous acidic dehydration of already-formed geosmin. Ultimately, this study found that there is no significant change in geosmin concentrations as a result of adding EarthTec® with the intent to induce acidic dehydration. Water treatment plants that are experiencing taste-and-odor issues related to geosmin would not benefit from the use of EarthTec® to directly remove geosmin.

5. Acknowledgements

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Occurrence of Antibiotic Resistance in Wastewater Treatment Plants in Chennai, India

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Abstract

Antibiotic resistant genes (ARGs) present in antibiotic resistant bacteria (ARB) can be transferred and spread in wastewater treatment plants (WWTPs) around the world, particularly in developing countries, due to high population densities, limited access to adequate healthcare, increasing and unregulated availability of antibiotics, and improper use of antibiotics. The spread of antibiotic resistance is of global public health concern, and WWTPs are potential nodes for emergence and dissemination. The objective of this study was to investigate the occurrence and distribution of ARGs across two WWTPs located in a southern coastal Indian city. Samples of the influents and effluents corresponding to each process in the WWTPs were collected as well as samples of WWTP effluent-receiving environment upstream and downstream to the effluent discharge location. DNA from each sample was extracted. Metagenomic analyses and real-time quantitative polymerase chain reaction will be used to will be conducted to characterize the ARG distributions within each sample. The results obtained from this effort will help identify critical points along the wastewater treatment process where antibiotic resistance dissemination may be controlled.

Keywords: antibiotic resistance, wastewater treatment, India

Introduction

Antibiotic resistant bacteria (ARB) are known to occur in various environments around the world, and their spread around the world has been gaining attention (The White House Administration, 2015; WHO, 2014). Global patterns of antibiotic resistance distribution are largely unknown, and occurrence in developing countries is of particular concern due to limited access to adequate healthcare, increasing and unregulated availability of antibiotics, and improper use of antibiotics (Okeke et al., 2005). Antibiotic resistance genes (ARGs) embedded in the ARB can spread via horizontal and vertical gene transfer, and with increasing ease and necessity of international travel, the spread of antibiotic resistance has the potential to impact communities on a global scale. In addition to public health concerns, antibiotic resistance has economic impacts. One study estimated the cost of antibiotic resistance to fall between $100 million and $30 billion annual in the United States (Phelps, 1989). While there may be a number of short-term and long-term solutions to help combat the spread of antibiotic resistance, highly feasible interventions include conducting educational interventions, training undergraduates and postgraduates on antimicrobial resistance, establishing infection control committees and guidelines for antimicrobial use, developing national drug policies, essential drug lists, and standard treatment guidelines, and ensuring drug quality (Holloway, 2001).

There are three critically important sources of environmental exposure to antibiotics and ARGS that Pruden et al. presented economically feasible management options that may be put into effect immediately: a) terrestrial agriculture, b) treatment of wastewater from municipalities, pharmaceutical manufacturing, and hospitals, and c) aquaculture (Pruden, Larsson, Amézquita, Collignon, & Brandt,

2013). This work focuses on the occurrence of antibiotic resistance in two wastewater treatment plants in a southern coastal city in India and one wastewater treatment plant in an eastern city in the United States. A wastewater treatment plant (WWTP) may treat hospital sewage or domestic sewage, which contains human waste. After the water is treated in the WWTP, the water is discharged into surface water or is used for irrigation, thereby potentially impacting the effluent-receiving environments. Antibiotics can enter WWTPs through human excretion, farm animals, and direct disposal of medical and industrial wastes (Bouki, Venieri, & Diamadopoulos, 2013). This makes WWTPs an important source of environmental contamination with antibiotic-resistant bacteria (Bouki et al., 2013; Kümmerer, 2004).

A number of studies have been conducted on the occurrence and distribution of ARGs in wastewater treatment plants around the world. One study investigated ARG inactivation in effluent of a wastewater treatment plant by three disinfectants: chlorine, UV, and ozone. The study found that the most effective disinfectant in inactivating ARGs was chlorine, through an inactivation efficiency and an economic perspective. With a chlorine dose of 40 mg/L and a contact time of 60 min, the genes inactivation efficiency could reach 1.65 – 2.28 log. The study also recommends that ozone not be used as a means to inactive ARGs in wastewater (Zhuang et al., 2015). Another study investigated the effect of seasonal variation and treatment steps on the prevalence of antimicrobial-resistant *Escherichia coli* in sewage treatment plants in South India, and concluded that hospital wastewater inflow significantly increased the prevalence of *E. coli*, whereas the treatment processes and sampling seasons did not affect the prevalence of the isolates (Akiba et al., 2015). Individual treatment processes throughout the wastewater treatment plant may affect the presence of different ARGs. One study found that a much greater concentration of chlorine is needed to decontaminate activated sludge than treated wastewater, since bacteria are much more concentrated in activated sludge (Chitnis et al., 2004). Also, a higher concentration of chlorine with a shorter contact time (maintaining a constant CT value) is advantageous to help control the reactivation of inactivated antibiotic-resistant bacteria. Selection of antibiotic-resistant bacteria by chlorination in secondary effluents may depend on type of antibiotic resistance, chlorination dose concentration and mode of operation and recovery time after chlorination, among others (Huang et al., 2011). Finally, In a WWTP in Portugal, biological treatment of *Enterococcus* spp. did not prevent dissemination to the environment (Martins da Costa, Vaz-Pires, & Bernardo, 2006).

The objectives of this study are to identify and compare the distribution and occurrence of ARGs present throughout wastewater treatment plants in the United States and India, as well as the influence of the wastewater treatment plants' final effluent on the receiving environment for all three treatment plants. This will be completed using quantitative polymerase chain reaction (qPCR) and the Oxford MinION Nanopore sequencer antibiotic resistant gene sequencing in the field. The MinION sequencer's portability and ease of use makes it favorable for use in remote laboratories (Judge, Harris, Reuter, Parkhill, & Peacock, 2015). In the past, researchers have obtained samples from the field and transported them for processing in a lab which may be in another country. The Oxford Nanopore MinION sequencer has the potential to streamline the process of sequencing DNA in samples. The results of this study helps to advance our knowledge about the global health problem of antibiotic resistance.

Research Methods

2.1 Characteristics of the STPs

Samples were collected at three wastewater treatment plants: two in South India (WWTP 1 and WWTP 2) and one in eastern United States (WWTP 3). WWTP 1 and WWTP 2 are rated at 54 MLD

(14.3 MGD) and exclusively treat domestic sewage. The final effluents of STP 1 and STP 2 discharge into a river (Photo 1) and a canal (Photo 2), respectively.

Photo 1. Downstream Sampling Site for WWTP 1 (Alexandria Cook, 15 June 2017)

Photo 2. Downstream Sampling Site for WWTP 2 (Alexandria Cook, 3 July 2017)

WWTP 3 is rated at 6 MGD and discharges into a river. The wastewater treatment process begins with primary sedimentation of the influent, where the larger solids settle out. Following this step is aeration basin, where bacteria degrade the organic matter. Both WWTPs in India use conventional activated sludge processes. The activated sludge that results from aeration is then settled out into the secondary clarifier. The final effluent is released after disinfection. The secondary clarifier effluent for WWTP 1

combines with secondary clarifier effluents of two other plants, and the combined flow is disinfected with gaseous chlorine (Figure 1).

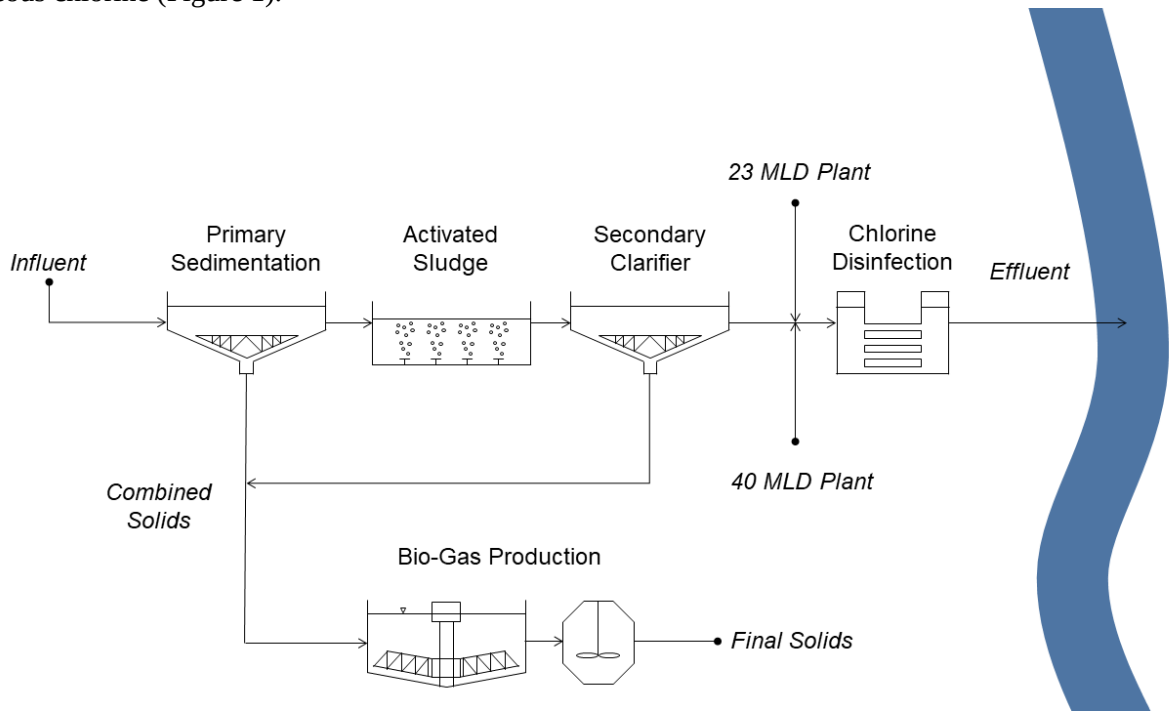

Figure 1. WWTP 1 Process Flow Diagram

WWTP 2 uses a maturation pond to disinfect the treated wastewater (Figure 2) and WWTP 3 uses ultraviolet radiation (Figure 3). The removed solids from both WWTPs in India are used to produce bio-gas to power the plants, while the solids from WWTP 3 are treated and used in land applications.

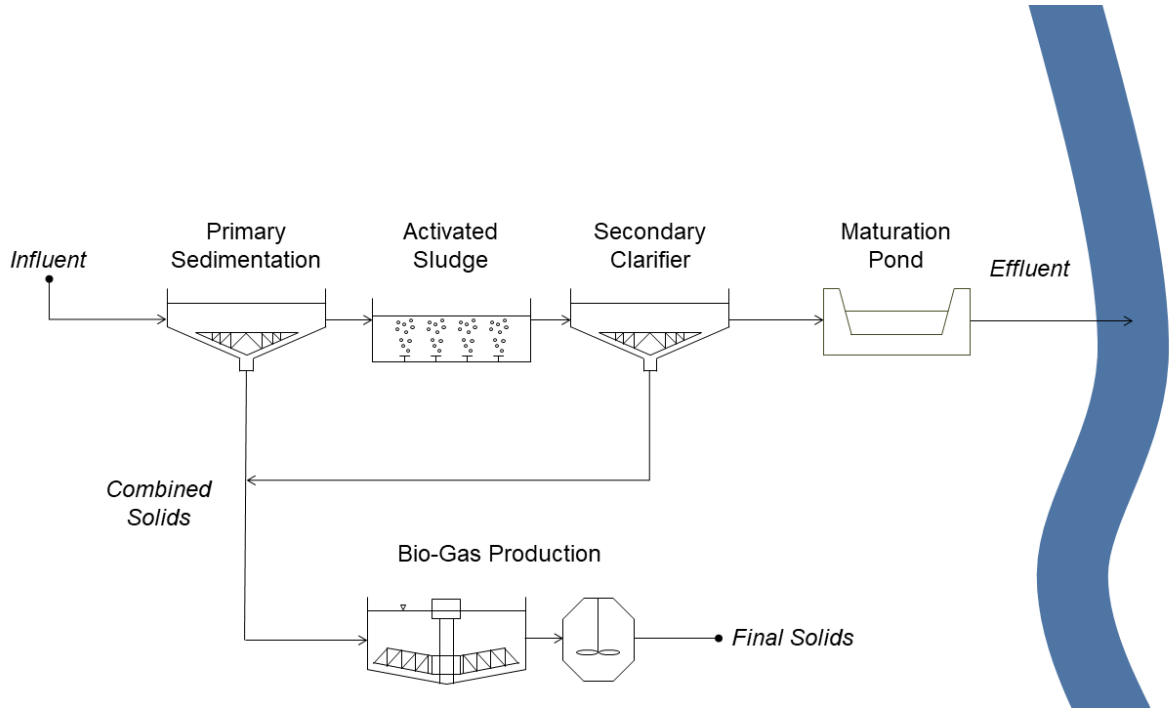

Figure 2. WWTP 2 Process Flow Diagram

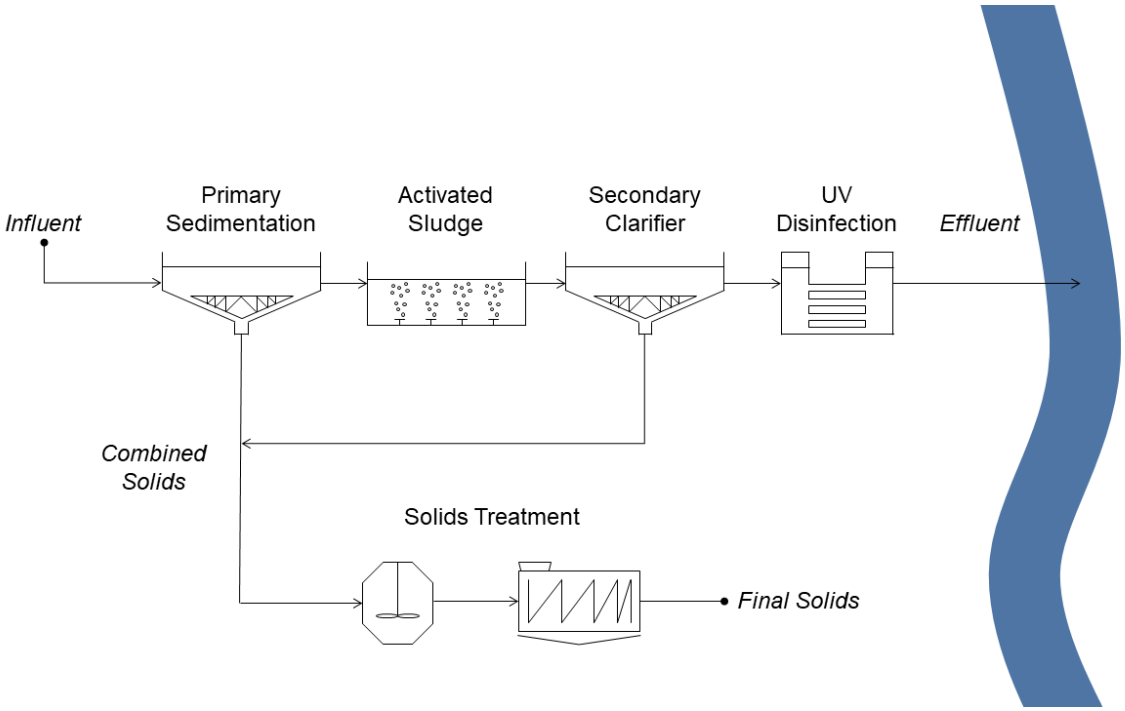

Figure 3. WWTP 3 Process Flow Diagram

2.2 Sample Collection and Processing

The samples were collected from WWTP 1, WWTP 2, and WWTP 3 on 15 June 2017, 3 July 2017, and 19 July 2017 respectively. Liquid samples were collected at each major treatment step for all WWTPs and combined solids and final solids samples were collected for WWTP 2 and WWTP 3. Additionally, samples were taken upstream and downstream the point of effluent discharge (Figure 4). The samples were kept in coolers with ice packs until further processing.

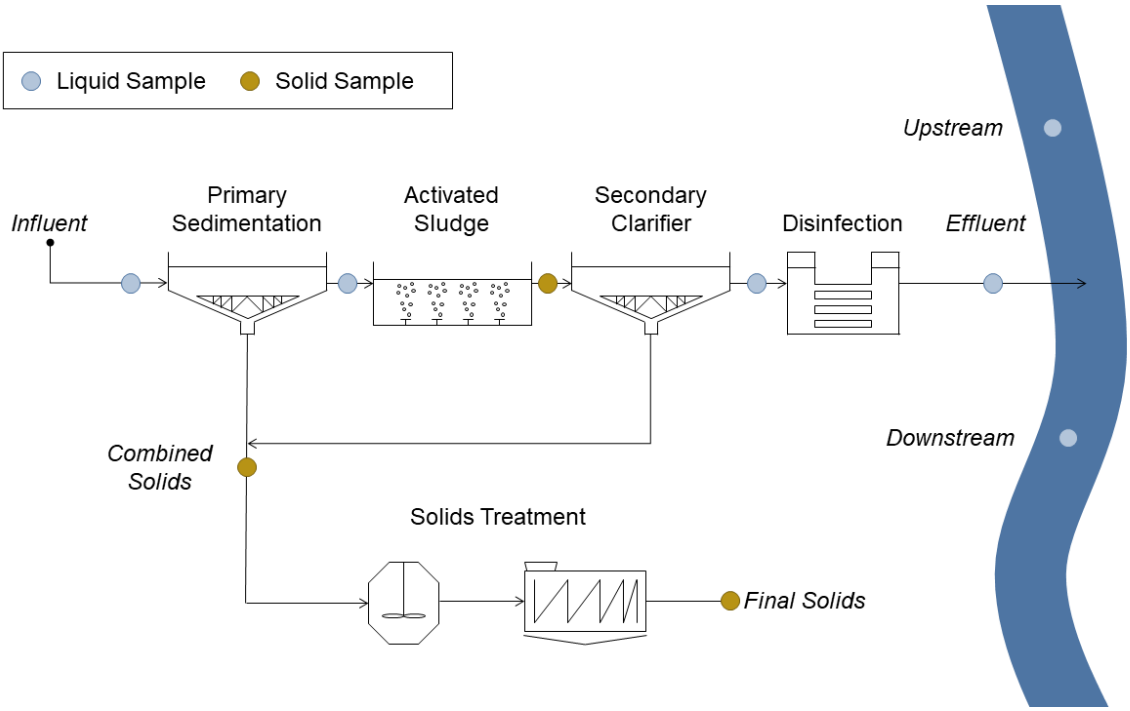

Figure 4. Wastewater Treatment Plant Sampling Locations

2.3 Processing for Biological Analyses

Samples were initially processed the same day they were collected. The liquid samples were filtered using vacuum filtration until the membrane was clogged, and the biomass retained on the membranes were stored in 1.5 mL 50% ethanol at -20 °C. When extracted, the filter was removed from ethanol and torn into pieces of approximately 5 mm^2 and transferred to the Lysing Matrix E tube from the FastDNA Spin Kit for Soil (MP Biomedicals). The remaining ethanol solution was centrifuged. The supernatant was removed and the pellet was resuspended using the sodium phosphate buffer provided in the kit. The mixture was then transferred to the Lysing Matrix E tube. The solid samples were homogenized and 500 µL of sludge was combined with 500 µL of 100% ethanol in a 2-mL tube and stored at 4 °C until further processing. When extracted, the tube was centrifuged and the ethanol was removed. The pellet was resuspended using the sodium phosphate buffer in the kit and transferred to the Lysing Matrix E tube. The rest of the DNA extraction was performed according to the manufacturer's instructions and the final eluted DNA for all samples was stored at -20 °C. Samples were processed in three replicates for each treatment step as well as upstream and downstream the location of the final effluent discharge to be used for qPCR and four replicates, with an additional four replicates for influent, activated sludge, and effluent to be used for nanopore sequencing (two to be sequenced in India and two to be sequenced in the United States).

2.4 Processing for Solid Phase Extraction (SPE)

2.4.1 Pre-Treatment

Influent, effluent, upstream, and downstream samples of 500 mL each were collected in 1-L amber bottles that were washed thrice with soap and water, rinsed thrice with DI water and baked at 250 °C overnight. On the same day of sample collection, sample pre-treatment was completed as follows: each sample was acidified using 40% phosphoric acid to a pH of 2.5 ± 0.5 and subsequently filtered with 0.45 µm glass microfiber filters via vacuum filtration to remove solid particles and bacteria. 2 mL of 5%

(w/v with Milli-Q water) Na_2EDTA were added to each sample. The samples were then spiked with 100 µL of surrogate standards provided by the University at Buffalo using a 100-µL glass syringe. The samples were stored at 4 °C until further processing the next day.

2.4.2 Solid Phase Extraction

In a fume hood, the SPE lines were washed with 250 mL of methanol then 250 mL of Milli-Q water. The Oasis hydrophilic-lihophilic balanced (HLB) cartridges from Waters corporation were then conditioned with 6 mL of acetonitrile followed by 6 mL of Milli-Q water. The samples were then loaded onto the HLB cartridges at a flow rate of 3 mL/min and the target analytes were retained on the cartridges. The HLB cartridges were then dried by leaving the vacuum on and allowing air to pass through. The cartridges were stored at 4 °C before shipping to the University at Buffalo to complete processing.

Expected Results

Currently, the samples have yet to be sequenced using the Oxford Nanopore MinION sequencer and have also been yet to be analyzed using qPCR due to delays in shipping from India. Presented below are the results from a previous sampling of the same wastewater treatment plants in India, which took place in March 2016 (Figures 5 and 6). The graphs show the gene abundances for the 16S rRNA, *intl*1, *tet*(W), *tet*(O), and *sul*1 genes at each point along the treatment plants. The gene abundances remained relatively unchanged throughout the WWTPs that use conventional activated sludge processes, and we expect similar results from the most recent sampling event.

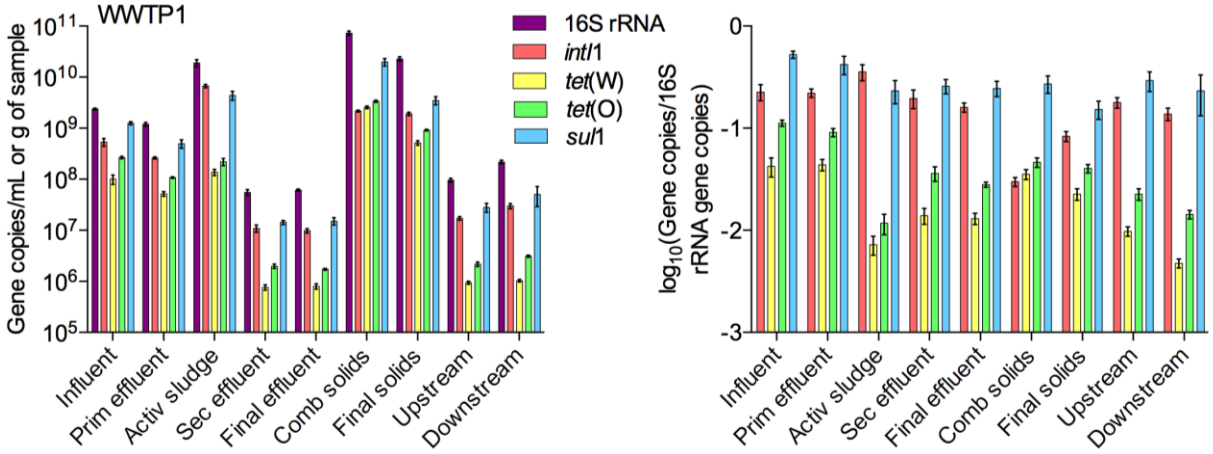

Figure 5. 16S rRNA, *intl*1, *tet*(W), *tet*(O) and *sul*1 Gene Abundances Found in WWTP 1 During Previous Sampling (March 2016)

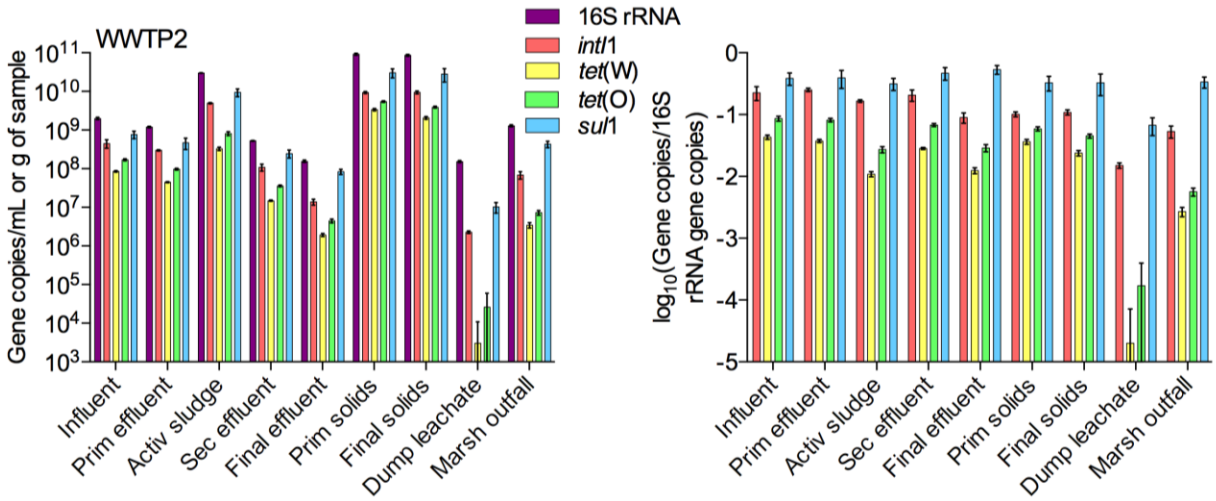

Figure 6. 16S rRNA, *intI*1, *tet*(W), *tet*(O) and *sul*1 Gene Abundances Found in WWTP 2 During Previous Sampling (March 2016)

Limitations and Cultural Influences

Unlike most urban areas in the United States which have an underground sewer system to transport domestic wastewater to a wastewater treatment plant, domestic wastewater in Chennai, India is collected in a septic tank. Each house has its own septic tank, and once it is full, the homeowner hires a lorry to pump out the waste and transport it to the wastewater treatment plant. The lorry empties the tank containing the wastewater into a channel which flows into the head of the plant. The differences in the transporting of wastewater in urban areas between the United States and India may impact the chemical make-up and microbial community of the wastewater. In addition, sampling occurred during the dry season when the flow rates of the effluent-receiving environments were close to zero. The samples thus may not be representative of the entire sampling location due to settling of solids and uneven proliferation of bacteria and organics.

There were many limitations during our time in India that had to be overcome in order to ensure the quality of the work and results. While the protocol for DNA extraction must be completed at room temperature, the labs that we worked in did not have A/C and the temperature was closer to the outside temperature. One particular lab available to us did have A/C, however permission from multiple people (i.e. a professor and/or their students) was required in order to use the space as well as the instruments in that space, and this was not always obtained in a timely fashion. Similarly, the -20 °C freezers that we needed to store our samples could only be accessed with a key, and the key was frequently passed on from student to student and it was difficult to track down the key in order to store our samples at the proper temperature in a timely fashion. In addition, on sampling days, it was very difficult for us to get ice to fill the cooler that would hold our samples, so we used ice packs instead of ice on sampling days. Moreover, the pH buffers used to calibrate the lab's pH probe were expired so calibration of the pH probe was not accurately completed and this can affect the results of our pH data.

One of the goals of this study was to compare the impacts of the working environments in India to those of the United States on the results of nanopore sequencing using the Oxford Nanopore MinION Sequencer. If the results are similar, the MinION reliably can be used to sequence in the field instead of shipping samples to and sequencing in the United States. In order to sequence using the Oxford Nanopore MinION Sequencer, we needed a method to measure the concentration of DNA samples in order to check whether or not we had enough DNA to adequately carry out the steps of the MinION Sequencer protocol. We intended to use a Qubit Fluorometer (Thermo Fisher Scientific) to measure the concentrations of

DNA in our samples; however, the standards did not have adequate concentrations of DNA, making any of our samples measurements have inaccurate readings. We believe that the temperate climate in India and the lack of A/C may have influenced the stability of DNA in the standards. New standards from the United States were shipped, however, they were returned to the originating location and nanopore sequencing could not be carried out.

A cultural barrier to the progress of our work was a difference in the concept of time between Indian and American cultures. Deadlines and commitments to meet at a certain time of the day are very loosely-kept in India. Following the procedures for solid phase extraction outlined in this paper, the remaining steps for processing the cartridges that were to be completed at the University at Buffalo were to be completed within two weeks of the first part of processing in India. However, due to iterative and drawn-out steps to acquire necessary permissions and approvals from certain officials and professionals, the HLB cartridges were shipped after the two-week point for WWTP 1. Thus, the results from further processing may be affected. Furthermore, the samples containing the biomass retained on the filter membranes were not shipped from India until 10 days after our departure from India due to a similar iterative process to obtain necessary permissions to ship samples containing ethanol in conjunction with limited availability from the students at the Indian Institute of Technology Madras whom we were working with during our time there. Shipping samples which are to be stored at -20 °C between India and the United States will also inevitably involve temperature changes, which may affect the results of qPCR and nanopore sequencing.

Finally, difficulties in effective communication was also a significant barrier to our progress throughout this work. While the students at the institute spoke English, we encountered difficulty understanding each other's accents, and at the wastewater treatment plants, it was necessary for us to have a Tamil-speaking person with us to aid in communication with the workers at the plants. Before our departure from the United States, Alex and I were informed that WWTP 2 discharges into a marshland instead of a flowing body of water. However, when communicating with the workers at the plant that we were interested in obtaining samples from "upstream and downstream the location of effluent discharge," a drawing a necessary for them to understand our request. We were then told that the final effluent discharges into a nearby canal, which differed from the sampling location of the previous sampling event for WWTP 2 which took place in March 2016. Difficulties in communication has resulted in inconsistent sampling events and will limit the amount of comparisons we can complete between sampling events, and may impact the validity of the results.

Next Steps

On 27 July 2017, all of the samples containing biomass that were filtered out of the samples arrived in Blacksburg, VA from India. We intend to apply real-time quantitative polymerase chain reaction to quantify the relative abundance of key ARGs of environmental and public health concern and compare the ARGs present between all three WWTPs sampled. We also plan to identify the antibiotic resistance gene sequences within the samples for the influent, activated sludge, and effluent for WWTP 1 and WWTP 2 using the Oxford Nanopore MinION sequencer to determine the accuracy and reliability of the portable nanopore sequencer. The results obtained will help identify critical points along the wastewater treatment process where antibiotic resistance dissemination may be controlled.

Acknowledgements

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Examining Spatio-Temporal Dynamics of Streams Using a High-Frequency Water Quality Sensor

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Abstract

Water quality varies with both space and time, driven by factors such as land use and flow. Traditional monitoring techniques involve sampling at infrequent timescales that are unable to detect high-resolution variability in water quality. This project uses a high-frequency sensor to evaluate spatial variability within and between streams, in addition to temporal variability within a single stream. Turbidity, nitrate-N, and dissolved organic carbon levels were measured in sections of Stroubles Creek near Blacksburg, Virginia, and in parts of the Choptank River in eastern Maryland. Heat maps were created for each water quality parameter in each stream to illustrate spatial variability within streams. Regression analysis indicated a moderately-strong positive relationship between percent agricultural land use in a watershed and mean nitrate levels (R^2=.7372). Temporal variation within a single stream was also demonstrated. The results of this study not only illustrate the spatio-temporal variability of water quality in streams, but also provide support for the expanded use of high-frequency, automatic, *in-situ* water quality monitoring.

Keywords: water quality, spatio-temporal variability, land use, high-frequency sampling.

1. Introduction

Surface water is a vital resource; however, it is becoming increasingly impaired due to human impacts. It serves as a source of drinking water, has recreational benefits, provides irrigation to farms, and is an essential component of ecosystems. Despite its importance, surface water quality is impaired across the nation. A nationwide EPA report determined that greater than 40% of the surveyed streams miles had high levels of nutrients, and 15% had high levels of sediments (EPA, 2016). From an ecological perspective, these impairments in water quality have a cost, as evidenced by the fact that nearly half of US stream miles are classified as being in poor biological condition (EPA, 2016). An annual loss of $22.4 million due to nutrient pollution in US freshwaters suggests that water quality impairments also can have tangible economic costs (Dodds et al., 2009). In order to combat the ecological and economic costs associated with water quality impairment, billions of dollars have been invested by federal, state, and local governments since the Clean Water Act of 1972 to improve water quality (USGS, 2017).

Improving stream water quality is complicated by the fact that water quality is dynamic in both space and time. Land use, underlying geology, soil depth, soil permeability, riparian cover, topography, and climate have been identified as spatial drivers of water quality variability (Kleinman et al., 2006; Allan, 2004; Buck et al., 2004). Temporal variability of water quality is linked to streamflow. Consequently, seasonality, the occurrence of storm events, and the intensity of storm events have been identified as temporal drivers of water quality dynamics (Carpenter et al., 1998; Kleinman et al., 2006; Bolstad and Swank, 1997).

Land use is a particularly interesting influence on water quality because it has been drastically altered by anthropogenic factors. Over one-third of the land surface on Earth has been modified to some degree by humans (Vitousek et al., 1997). This is especially true in the recent past. A study conducted by Tilman et al. (2001) reports that from 1960-2000, global cropland and pastureland cover have increased by about 10% each. The same study predicts that by 2050, the total amount of land devoted to agriculture is expected to be 18% greater than that of 2001. Like agriculture, urban land use has increased in the recent past, which has been coupled with a decrease in forested land cover (Civco et al., 2000). Global projections anticipate that urban populations will increase substantially by 2030, especially in developing countries (Cohen, 2006).

The impact of land use on water quality has been the subject of much investigation. Allan (2004) identified six ways in which land use can influence stream ecosystems: sedimentation, nutrient enrichment, contaminant pollution, hydrologic alteration, riparian clearing, and loss of large woody debris. Consequences associated with these changes include algal blooms, loss of habitat, and decreases in biodiversity (Allan, 2004). The primary land use classifications that have been associated with negative impacts on water quality are agricultural and urban land use (Carpenter et al., 1998). Requiring fertilizer and generating livestock waste, agriculture has been linked to enhanced levels of nutrients in streams, which can occur via surface runoff or leaching from soils (Carpenter et al., 1998, Allan, 2004). Furthermore, agriculture has been associated with increased sediment and dissolved organic carbon (DOC) loading into streams (Cooper, 2003; Royer and David, 2005). Similarly, urban land use has been associated with increased sediment and nutrient levels in streams, which is due in part to impervious surfaces and wastewater effluent (Carpenter, 1998; Allan, 2004; Bakri et al., 2007; Allan et al., 1997). Conversely, forest land cover has been identified as the optimal land use for mitigating water quality impairments (Allan et al., 1997).

Flow is another driver of water quality variability that has the potential to change in the future. As urbanization continues to increase (Cohen, 2006), the expansion of impervious surfaces will result in increases in the volume of runoff at the expense of groundwater recharge (Leopold, 1968). This results in higher peakflow conditions after storm events, but lower baseflow conditions during non-storm periods (Leopold, 1968). Aside from urbanization, climate change also has the potential to impact stream flow. Although it is generally accepted that climate change accentuated by humankind has occurred over the last century and will continue to occur (IPCC, 2014), the effect it may have on flow is less clear. Climate models suggest that changes in precipitation will not be uniform across Earth (IPCC, 2014). Rather, some regions are projected to experience increases in storm frequency and intensity, while some regions are expected to experience decreases (IPCC, 2014). Furthermore, models sometimes suggest a seasonal component to precipitation changes. For example, a UK emissions scenario predicts an increase in winter precipitation, but a decrease in precipitation during summer months (Whitehead et al., 2009). Despite the lack of clarity in how precipitation will change in a changing climate, any alteration in storm frequency or intensity will inevitably affect stream flow.

Stream flow can have a pronounced effect on water quality. In some instances, increases in flow may result in increases in concentrations of different solutes. This enriching relationship has been observed in nitrate-N (NO_3^--N) and turbidity in a small forested watershed in western North Carolina (Bolstad and Swank, 1997), as well as in total organic carbon in four different streams in southeastern Ohio (Owens et al., 1991). In other instances, increases in flow may result in decreases in concentrations of different solutes. This diluting relationship has been observed in sodium ions during rain events in two streams located in the Adirondacks of New York and in western Pennsylvania, respectively (Evans and Davies, 1998). Another interesting relationship that flow can have with water quality can be described with hysteresis loops. A hysteresis loop shows how the concentration of a certain parameter can be different at the same discharge level, depending on if that discharge level occurred at the rising or falling limb of a hydrograph (Williams, 1989). A clockwise hysteresis, such as what was observed with DOC in a study on a stream in Shenandoah National Park (Buffam et al., 2001), suggests that concentration is greater before peak discharge. Conversely, a counterclockwise hysteresis suggests that concentration is

greatest after peak discharge. Such was the case with sulfate ions in the New York and western Pennsylvania streams previously mentioned (Evans and Davies, 1998).

Considering the spatio-temporal variability associated with water quality, along with the change in variability that is possible in the future, there is a pressing need for effective monitoring. Such monitoring would provide valuable insight that would allow for more effective protection and management strategies. However, traditional monitoring methods are insufficient in their representation of water quality variability (Birgand et al., 2016). Traditionally, water quality data have been collected at monthly, biweekly, or weekly time scales (Kirchner et al., 2004; Strobl and Robillard, 2008). At these sampling frequencies, important water quality variability patterns, such as after storm events, can be entirely overlooked (Kirchner, 2004). Furthermore, considering that studying spatial variability requires numerous samples, infrequent sampling makes tracking spatial variability of water quality extremely difficult (Mcclain et al., 2003). This shortcoming in the sampling frequency of traditional water sampling methods has proven to be a limiting factor in water quality modelling, which in turn has limited the effectiveness of water quality management (Johnes, 2007).

A recent innovation in the field of water quality monitoring is the advent of in-situ water quality sensors. These sensors are able to collect measurements on specific water quality parameters without laboratory analyses, without manual operation, and on timescales on the order of minutes (Robe et al., 2016). By measuring water quality at frequencies that were once-unattainable, in-situ sensors have allowed for the discovery of new relationships that were undetectable at lower-frequency sampling intervals (Strohmeir et al., 2013; Jones et al., 2012, Kirchner et al., 2004). Primarily, in-situ sensors have been used to examine temporal variation of water quality, which involves mounting the sensor in a fixed location over a period of time (Pellerin et al., 2014; Robe et al., 2016; Etheridge et al., 2014; Jones et al., 2012). Fewer projects have investigated spatial variation of water quality using in-situ sensors.

The goal of this project was to assess the spatio-temporal dynamics of three water quality parameters (turbidity, NO_3^--N, and DOC) using an in-situ water quality sensor. Synoptic surveys were conducted in streams surrounded by different land covers in order to assess spatial variability both within and between streams. Furthermore, a synoptic survey was conducted in the same stretch of a stream during different flow conditions in order to assess temporal variability within the stream. This project not only provides insight into the spatio-temporal dynamic nature of water quality, but also demonstrates the capability of in-situ water quality sensors to conduct synoptic surveys of surface water.

2. Research Methods and Experiment Setup

2.1 Site Selection

Three sections of streams were surveyed near the Blacksburg area in southwest Virginia. Stroubles creek was surveyed in two locations: near the Foxridge apartment complex (herein referred to as Foxridge), and in a forested area further downstream (herein referred to as Lower Stroubles). The headwaters of Stroubles Creek flow through the town of Blacksburg and Virginia Polytechnic Institute and State University. Further downstream, the creek passes through agricultural and forested areas. Moon Hollow, a small tributary to Stroubles Creek that is located entirely within a forested area, was also surveyed. These streams are located in the Valley and Ridge physiographic province, and are characterized by relatively steep terrain. The watersheds of these streams are shown in Figure 1, and information about the size and land use characteristics of each watershed is shown in Table 1.

Two streams were surveyed on the Eastern Shore of Maryland near the town of Denton. The Choptank River and Tuckahoe Creek are located in the Coastal Plain physiographic province, which is characterized by low-relief terrain. The watersheds of Tuckahoe Creek and the Choptank are primarily influenced by agricultural land use (Table 1). Wetlands in the area, referred to as Delmarva Bays (Fenstermacher et al., 2014), play a larger role in the Choptank watershed than in Tuckahoe.

Table 1. Site Characteristics

	Watershed Area (hectares)	Percent Agriculture Cover (%)	Percent Forest Cover (%)	Percent Urban Cover (%)	Percent Wetland Cover (%)
Foxridge	1437.29	41.86	23.87	32.86	0.41
Lower Stroubles	2351.7	39.47	36.74	21.34	0.25
Moon Hollow	68.84	1.46	98.03	0.49	0
Choptank	29329.02	49.44	12.27	6.03	32.18
Tuckahoe	20525.49	64.35	12.29	4.5	18.72

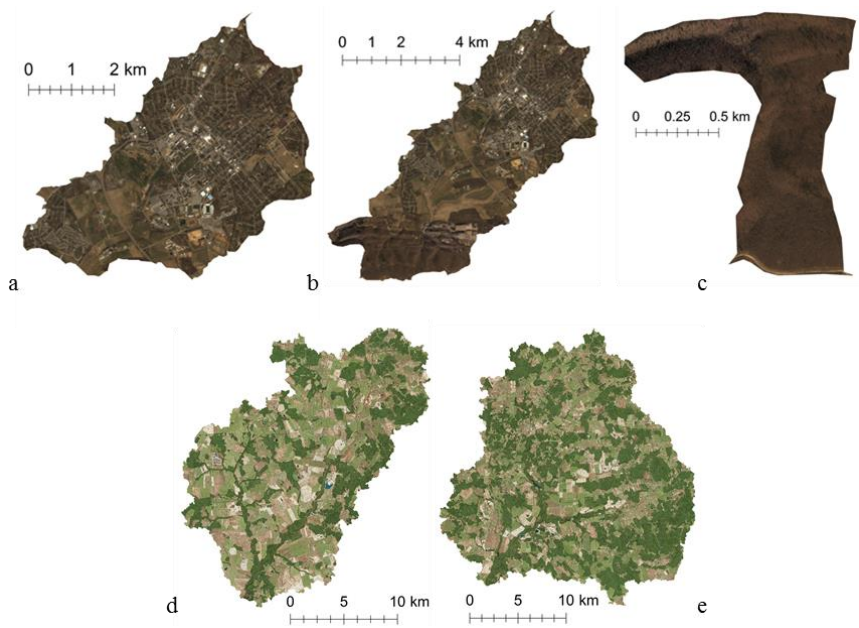

Figure 2. The (a) Foxridge, (b) Lower Stroubles, (c) Moon Hollow, (d) Tuckahoe Creek, and (e) Choptank River Watersheds.

2.2 Instrumentation

A UV/Vis s::can spectro::lyser™ was used to measure turbidity, NO_3^--N, and DOC in each stream survey. This sensor includes lamps that emit UV and visible light, as well as a 35mm pathlength through which the light travels. As the light moves through water in this 35mm path, the sensor records absorbance on the electromagnetic spectrum from 220-720 nanometers in wavelength. By recording absorbance over these wavelengths, the spectro::lyser™ then uses algorithms to calculate concentrations of different water quality parameters, including turbidity, NO_3^--N, and DOC.

A "global calibration" was conducted on the spectro::lyser™ before at the time of purchase. This calibration was designed with stream monitoring in mind, creating algorithms that accurately relate spectral absorbance to turbidity, NO_3^--N, and DOC concentrations. For more accurate results, s::can recommends performing a "local calibration", which is simply a fine-tuning of the global calibration. However, due to the time constraints of this study, no local calibration was performed.

2.3 Surveying

Surveying took place on June 6th, 2017 for the first Foxridge survey, and on June 7th for the Lower Stroubles and Moon Hollow surveys. The second and third Foxridge surveys occurred on June 13th and June 30th, respectively. At these locations, surveying began at a downstream point and progressed via wading upstream. The sensor was programmed to record measurements once every two minutes, and was carried in hand and dipped into the stream channel before each measurement. A GPS unit was used to mark the location of each water quality measurement.

For Tuckahoe Creek and the Choptank River, surveying took place on June 21st and 22nd, respectively. Unlike the other streams, these surveys began at an upstream point and were conducted via canoe. The sensor was programmed to record measurements every minute during these surveys, and once again, a GPS unit was used to assign locations to each measurement.

2.4 Analyses

Water quality measurements and GPS locations were paired via temporal alignment using R statistical software. The data were then filtered to remove invalid measurements, which occurred when a measurement recorded while the sensor was out of water or when the sensor was in an area that had just been disturbed by the surveyors. Invalid measurements differed greatly from the rest of the data, and thus were easily identified and removed.

To analyze the change in turbidity, NO_3^--N, and DOC concentrations with distance, the haversine formula was used:

$$d = 2r \arcsin\left(\sqrt{\sin^2\left(\frac{\phi_2 - \phi_1}{2}\right) + \cos(\phi_1)\cos(\phi_2)\sin^2\left(\frac{\lambda_2 - \lambda_1}{2}\right)}\right)$$

Where

- d is the distance between two points on a spherical surface
- r is the radius of Earth, estimated to be 6,378,137 meters
- $\phi 1$ and $\phi 2$ are the latitude of point 1 and latitude of point 2, in radians
- $\lambda 1$ and $\lambda 2$ are the longitude of point 1 and longitude of point 2, in radians

The distance between each point and the point before it was calculated via the haversine formula. The summation of these distances was used to assign a distance value to each measurement. Although this method of determining distance between points did not account for bends in the stream channel, it served as a reasonable approximation of the distance between the sampled points.

Spatial analyses were performed in ArcGIS using the ArcMap program. In each stream, the survey points were connected into a line, and then a buffer was created to simulate the stream channel. A five meter buffer was created for Foxridge, Stroubles Creek, and Moon Hollow, and a 20 meter buffer was created for Tuckahoe and Choptank. Heat maps were created for turbidity, NO_3^--N, and DOC concentrations. Points were interpolated within the stream buffer via the kriging function in ArcMap, which is a standard method of interpolation. Maps highlighting spatial variation were symbolized from the highest concentration to the lowest concentration observed during the particular survey. Conversely, maps highlighting temporal variation were symbolized from the highest to lowest concentrations observed during *any* of the three Foxridge surveys. The purpose of this change in symbology was to accentuate the temporal variability of the stream.

Spatial analysis was also used to determine the land cover characteristics of each watershed. Watersheds were delineated in ArcMap using 10 meter resolution elevation data from the Geospatial Data Gateway, which is administered by the US Department of Agriculture (The National Map). Tuckahoe Creek and the Choptank River were delineated with 30 meter resolution elevation data obtained from the same source. Land cover data was obtained from the National Land Cover Database, which is administered by the US Geological Survey (Homer et al., 2015). Land covers were categorized into “forested”, “urban”, “agriculture”, and “water/wetlands” groups.

Linear regressions were performed to examine the relationships between the different land cover categories and the mean concentrations of turbidity, NO_3^--N, and DOC in the surveyed streams. Linear regressions were also conducted to relate the land cover classes to the variance in concentrations of each parameter in each survey. Variance was assessed based on the coefficient of variance, which is equal to the quotient of the standard deviation and mean of each sample.

Information on flow was obtained from the Learning Enhanced Water Assessment System (LEWAS) Lab at Virginia Tech (Lohani, 2017). The LEWAS Lab site is located on Webb Branch, a tributary that reaches Stroubles Creek just upstream of the Duck Pond. Estimated flow rates were obtained on each day a Foxridge survey was conducted. On 6/30, the estimated flow rate during the time of sampling was negative, which could be a result of system malfunction or scheduled cleaning on the instrumentation of the site. However, for the purposes of this study, the LEWAS Lab data was only used as a proxy to rank the stream surveys based upon their *relative* flow conditions. From the data collected at the LEWAS Lab site, we determined that the higher flow condition occurred on 6/6, the lower flow condition occurred on 6/13, and the lowest flow condition occurred on 6/30.

3. Results and Discussion

3.1 Spatial Variability within Streams

Most of the surveyed streams showed variability in each of the three measured parameters. The degree of variability demonstrated was site dependent. For example, Tuckahoe Creek DOC levels varied from 6.85-9.90 mg/L, which represents a substantial change in DOC (Figure 8d). Conversely, in Lower Stroubles Creek and Moon Hollow, NO_3^--N levels varied only from 1.59-1.62 and .10-.12 mg/L, respectively (Figure 6c and 7c). This range in NO_3^--N measurements is small enough that it is likely outside the range of accurate detection by the sensor.

Mapping of spatial variability provides insight into how landscape features can affect stream water chemistry. In each Foxridge survey, there is a distinct drop in NO_3^--N concentration when the measurements move upstream of a tributary just outside of the Foxridge apartment complex (Figures 3c, 4c, and 5c). This suggests that the tributary acts as a source of NO_3^--N enrichment for Stroubles Creek. Similarly, nitrate-N levels rise downstream of a tributary in the Choptank River (Figure 9c). DOC displays the opposite trend, as concentrations drop downstream of this tributary (Figure 9d). At Tuckahoe Creek, NO_3^--N levels begin to rise at the downstream end of the survey near a small reservoir (Figure 8c), suggesting that the reservoir may be a sink of NO_3^--N. Further investigation is needed to more completely demonstrate how NO_3^--N concentrations change as Tuckahoe Creek morphs from a river into a reservoir.

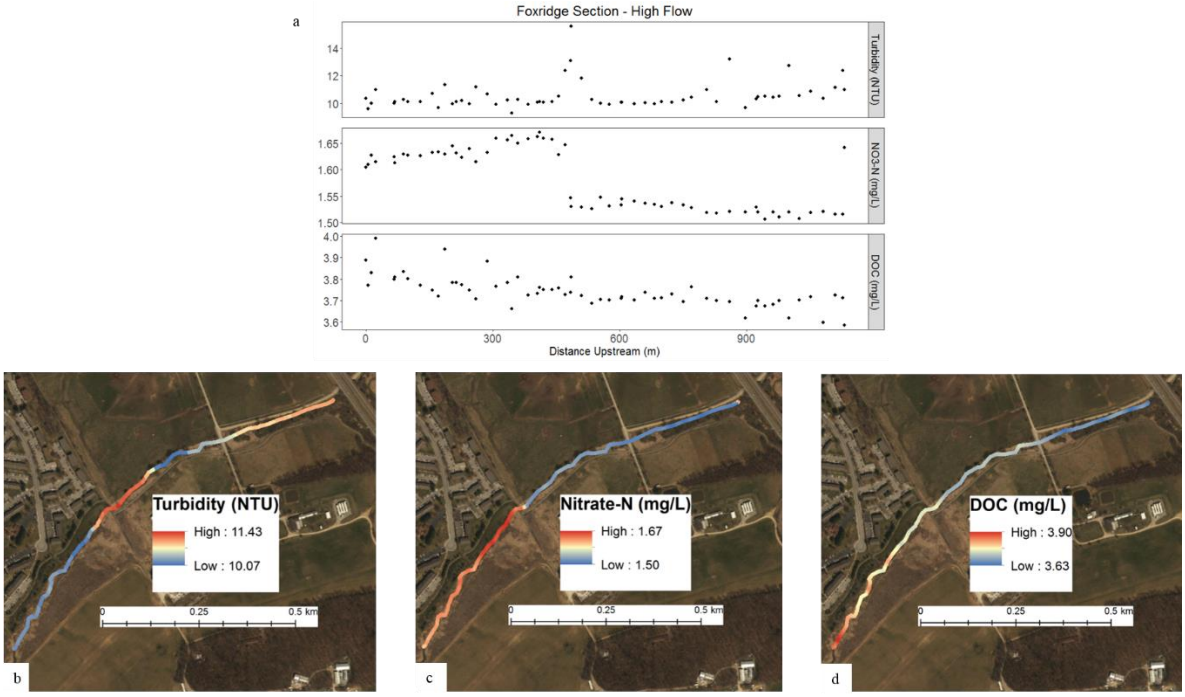

Figure 3. (a) Spatial variability *within* Foxridge section at high flow as a function of distance upstream. (b) Turbidity (NTU), (c) Nitrate-N (mg/L), and (d) DOC (mg/L) heat maps also shown.

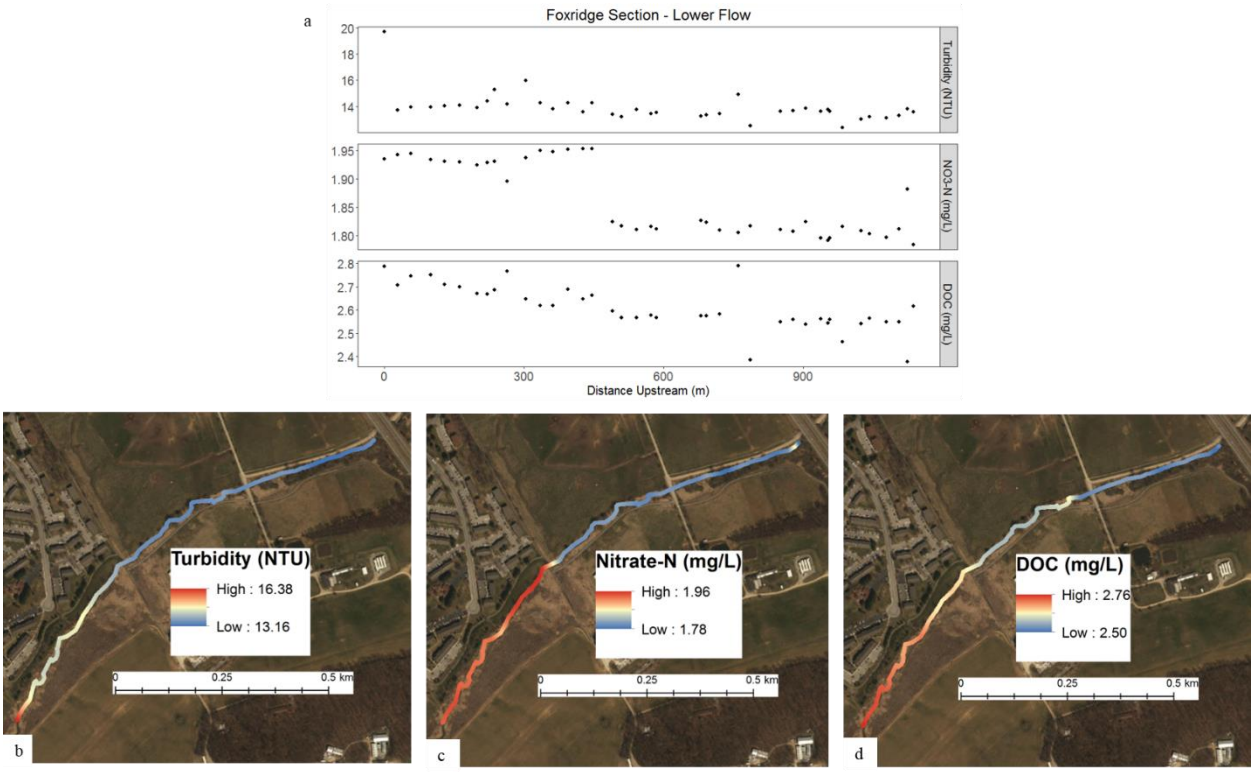

Figure 4. (a) Spatial variability *within* Foxridge section at lower flow as a function of distance upstream. (b) Turbidity (NTU), (c) Nitrate-N (mg/L), and (d) DOC (mg/L) heat maps also shown.

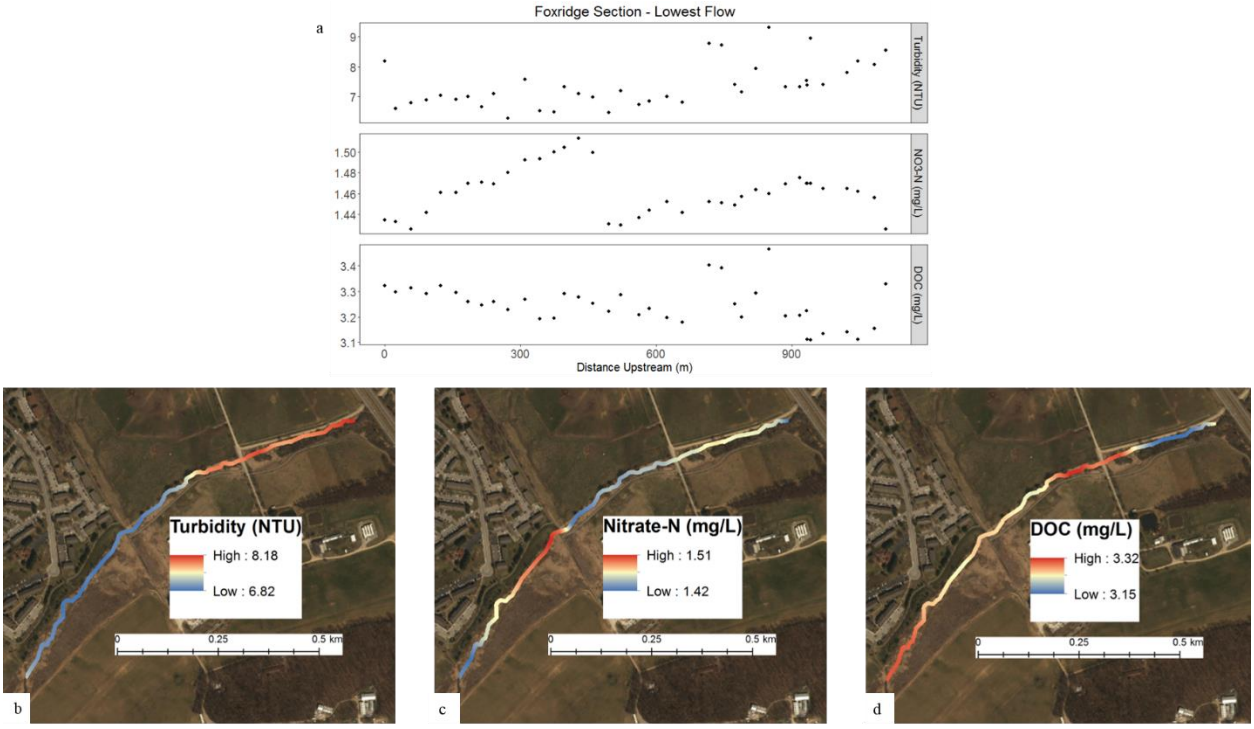

Figure 5. (a) Spatial variability *within* Foxridge section at lowest flow as a function of distance upstream. (b) Turbidity (NTU), (c) Nitrate-N (mg/L), and (d) DOC (mg/L) heat maps also shown.

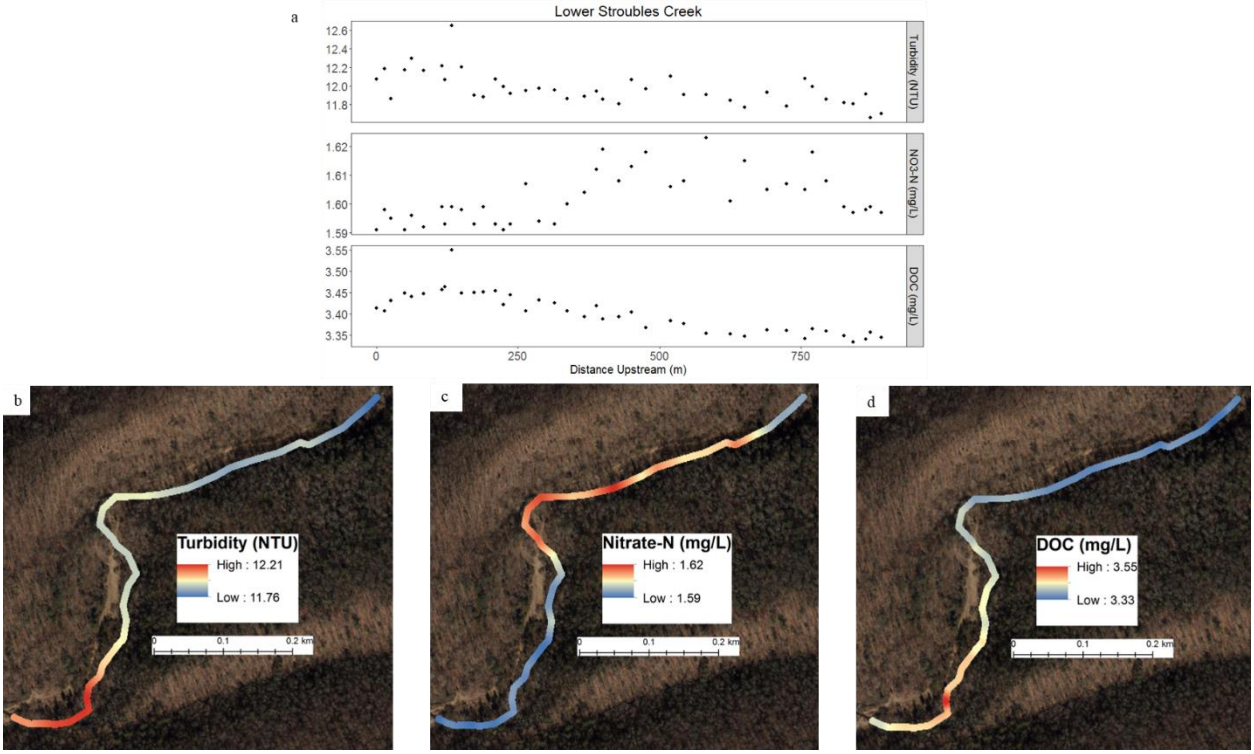

Figure 6. (a) Spatial variability *within* Lower Stroubles Creek as a function of distance upstream. (b) Turbidity (NTU), (c) Nitrate-N (mg/L), and (d) DOC (mg/L) heat maps shown as well.

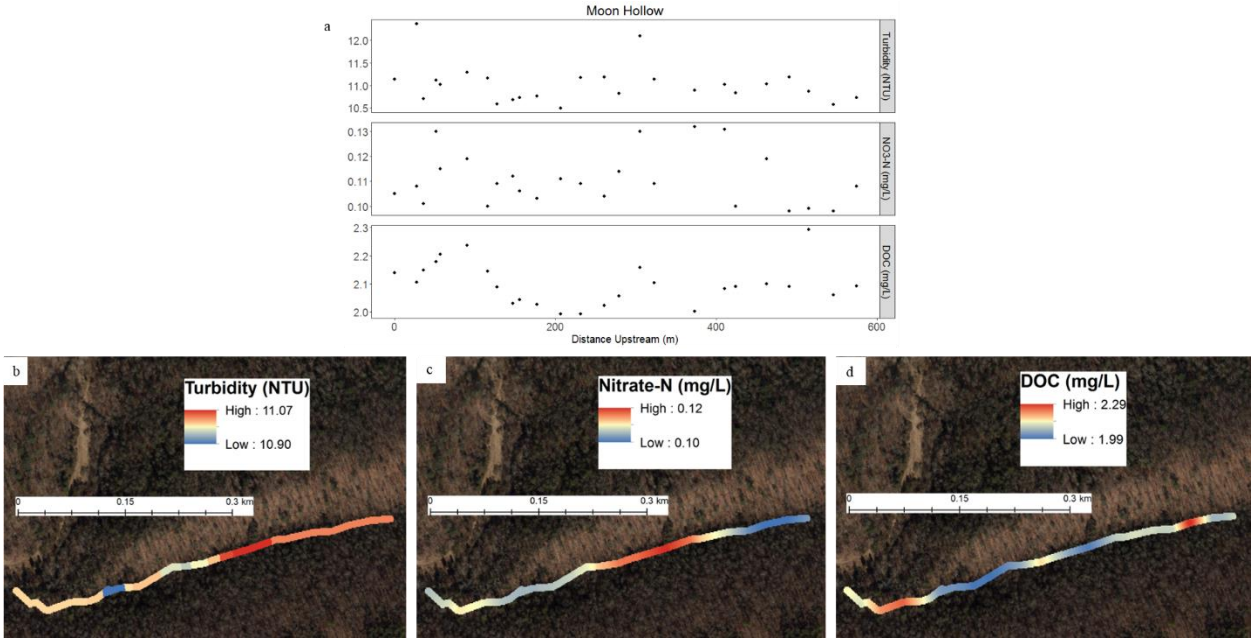

Figure 7. (a) Spatial variability *within* Moon Hollow as a function of distance upstream. (b) Turbidity (NTU), (c) Nitrate-N (mg/L), and (d) DOC (mg/L) heat maps shown as well.

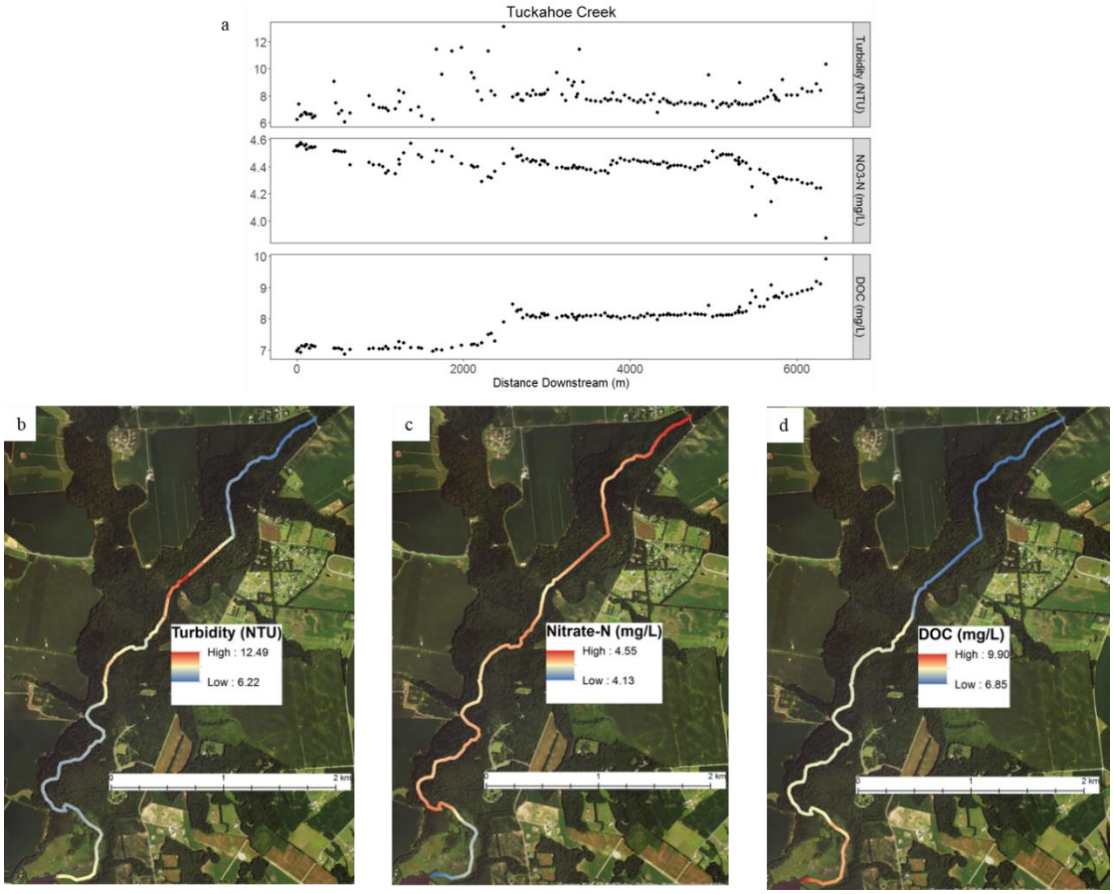

Figure 8. (a) Spatial variability *within* Tuckahoe Creek as a function of distance downstream. (b) Turbidity (NTU), (c) Nitrate-N (mg/L), and (d) DOC (mg/L) heat maps shown as well.

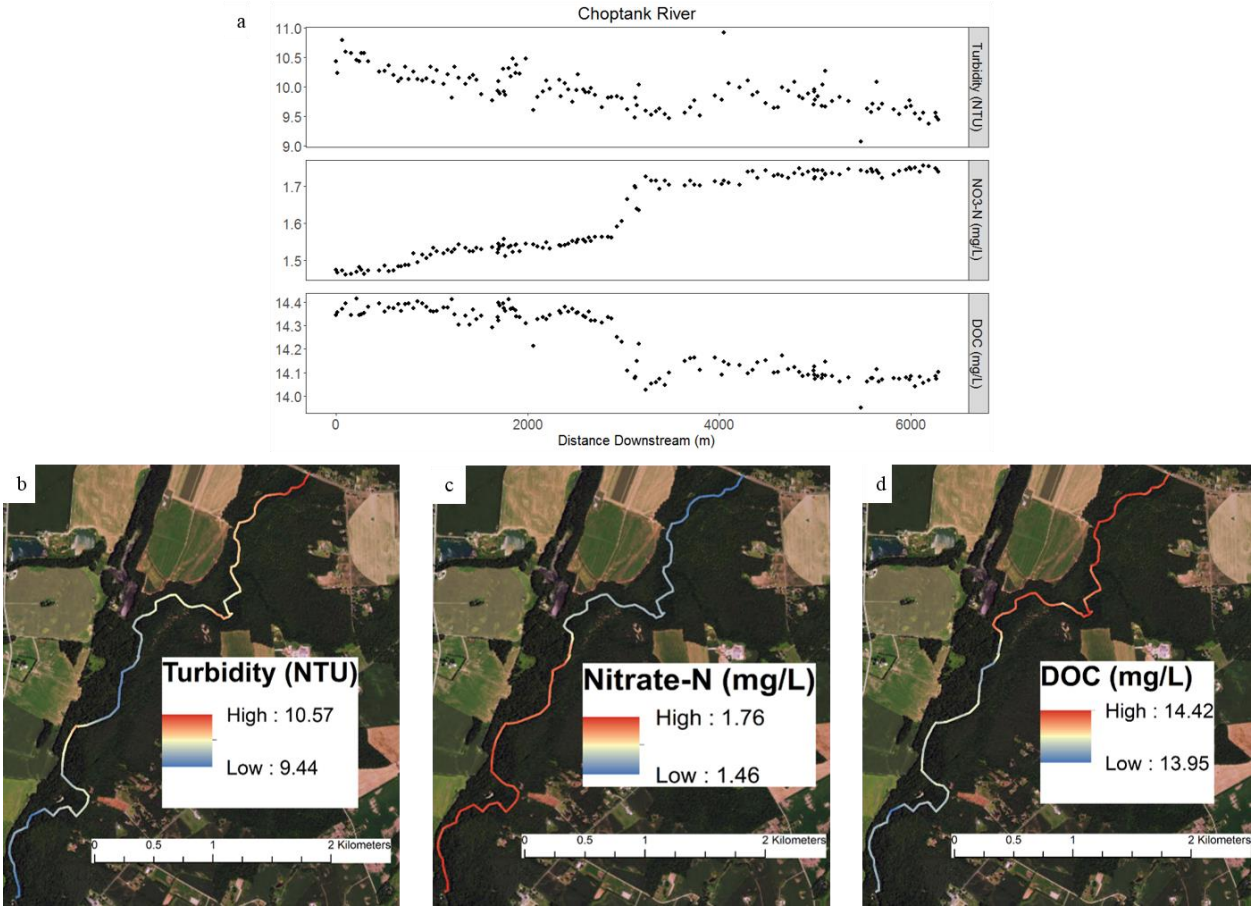

Figure 9. (a) Spatial variability *within* Choptank River as a function of distance downstream. (b) Turbidity (NTU), (c) Nitrate-N (mg/L), and (d) DOC (mg/L) heat maps shown as well.

3.2 Spatial Variability between Streams

Linear regressions were conducted between each of the three water quality parameters examined and each of the variables outlined in Table 1. The strongest explanation of spatial variability between streams was the relationship between mean nitrate levels and the percent of the watershed devoted to agriculture (Figure 10). This was a moderately-strong positive relationship (R^2=.7372), which has been suggested by previous research as well (Allan et al., 1997; Buck et al., 2004). This relationship could likely be attributed to nitrate leaching from fertilizer in agricultural areas and entering stream channels such as Tuckahoe Creek.

An interesting correlation between wetland land cover and the concentrations of DOC and NO_3^--N exists in Tuckahoe Creek and the Choptank River. Previous studies have indicated that wetlands, such as Delmarva Bays, release DOC to streams (Fenstermacher et al., 2014). At the same time, denitrifying bacteria can result in decreased levels of NO_3^--N in wetlands, which dilutes NO_3^--N levels in streams. The results of this study align well with this previous research. The Choptank River, with about twice the relative amount of wetlands in its watershed, has higher levels of DOC and lower levels of NO_3^--N than Tuckahoe Creek (Table 1, Figures 8a and 9a). A more in-depth study would be necessary to further examine the potential effect that Delmarva Bays have upon the Choptank River.

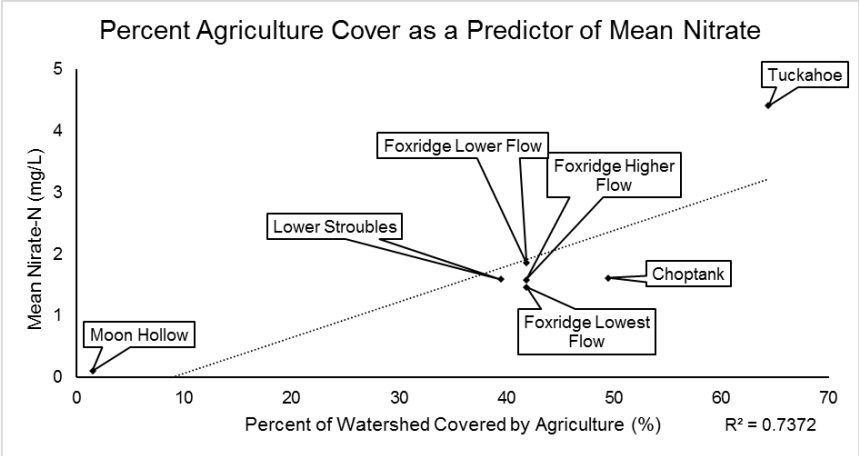

Figure 10. Linear regression relating agricultural prevalence in watersheds to mean NO_3^--N (mg/L).

Linear regressions were also performed to examine how land cover may impact the variance of the water quality parameters examined (Figure 11). Again, the relationship between NO_3^--N and the percent of watershed occupied by agriculture was the strongest. In this case, it was a mild negative relationship between the two (R^2=.4468). In other words, as relative agricultural abundance increased, the variance of NO_3^--N concentrations observed during a survey decreased.

An important note to be made about this regression is that its strength relies upon the precision of the s::can sensor. As previously mentioned, nitrate-N levels varied by only .03 mg/L in Moon Hollow. This small range in measurements could simply be consequence of imprecision in the sensor's measurements. However, due to the small mean NO_3^--N concentration in Moon Hollow, the coefficient of variation was resultantly large. If the variability in NO_3^--N concentration in Moon Hollow is indeed just a byproduct of sensor imprecision, then the regression conducted would not accurately reflect how the relative agricultural land cover of a watershed may be related to the variance of NO_3^--N concentrations in the surveyed streams.

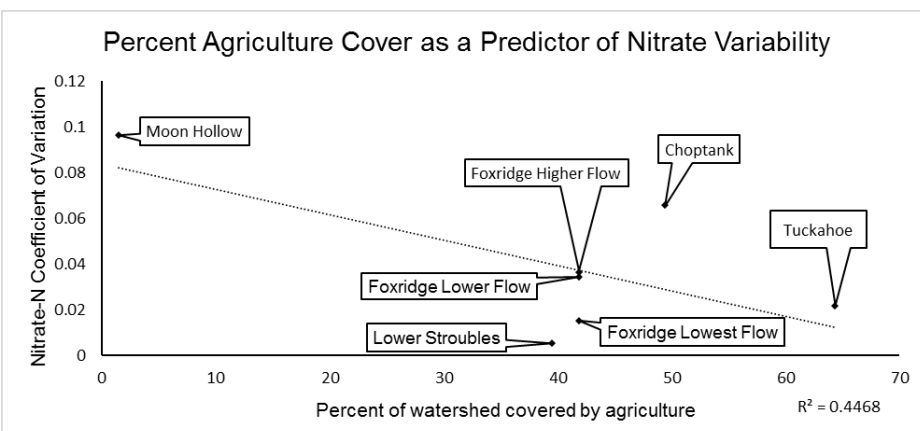

Figure 11. Linear regression relating agricultural prevalence in watersheds to NO_3^--N variability.

3.3 Temporal Variability within a Stream

Each water quality parameter varied with time in the Foxridge surveys ($p<.05$). Turbidity was highest at intermediate flow, lower at high flow, and lowest at the lowest flow (Figure 12a). NO_3^--N levels followed the same pattern; highest during the intermediate flow event, slightly lower during the high flow event, and lowest during the lowest flow event (Figure 12b). DOC levels were highest during

the high flow event, lower during the lowest flow event, and lowest during the intermediate flow event (Figure 12c).

Although the differences in turbidity, NO_3^--N, and DOC levels over the three surveys are significant, determining a trend relating flow to these concentrations is more difficult. In order to confidently examine for such trends, it would be necessary to collect quantitative flow data instead of qualitatively categorizing flows as "higher", "lower", or "lowest". Furthermore, more than three surveys would be necessary in order to analyze concentration data over a variety of flow regimes.

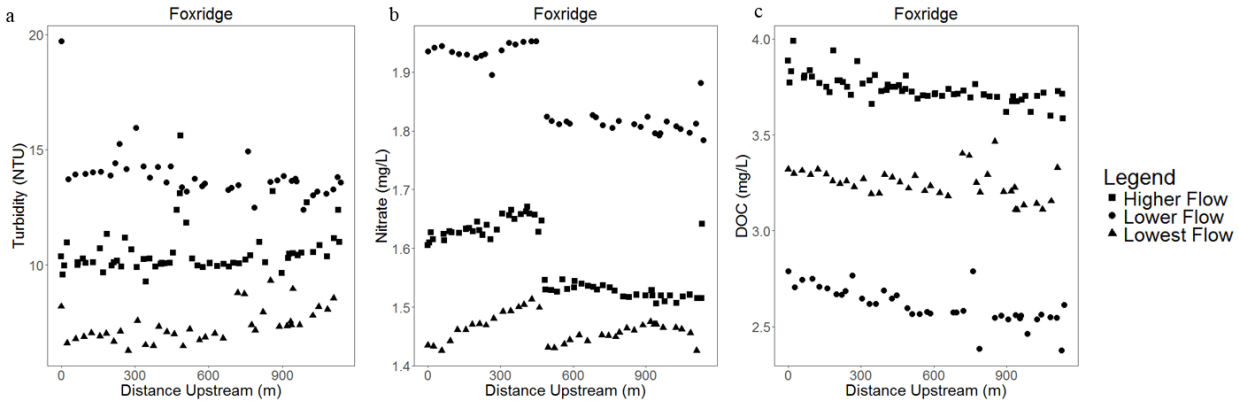

Figure 12. (a) Turbidity (NTU), (b) NO_3^--N (mg/L), and (c) DOC (mg/L) levels during different flow conditions at Foxridge.

4. Conclusion

This study provides insight into the spatio-temporal dynamics of streams. Spatial variability was demonstrated within streams and between different streams, and temporal variability was demonstrated within a single stream. Information such as how much nitrate-N increases after a tributary hits Stroubles Creek, or how agricultural land cover is related to mean nitrate levels in streams, can help water quality modelers to develop more robust, accurate models that can better predict how water quality changes with time or space. Further investigation, such as examining temporal variation over a wider array of flow regimes, would provide even more information for water quality modelers and managers.

Most importantly, this study showcases the capability of the s::can Spectro::Lyser™ to collect high-resolution water quality data. The information provided by the surveys conducted in this study would have been practically unattainable by traditional water sampling methods. By providing an automatic, high-frequency, in-situ alternative to water quality monitoring, sensors such as the Spectro::Lyser™ have the potential to change the way that water quality is monitored in the future.

5. Acknowledgements

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Design of an Educational Virtual Environment: Effects of a Storm Event on a Small Urban Stream

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Abstract

Clean water is becoming an increasingly rare resource because of urbanization and industrialization. Educating the public about water quality issues will be integral to solving the water quality problems the world is facing. The Learning Enhanced Watershed Assessment System (LEWAS), located in Blacksburg, Virginia, is a high-frequency, environmental monitoring system which measures water quantity and quality parameters in a small urbanized stream to spread environmental awareness.
The goal of this project is to design and develop an educational virtual environment capable of utilizing the data gathered by the LEWAS to accurately recreate real storm events in a virtual setting.

1. Introduction

Clean water is a limited natural resource which is essential to all life on earth. According to the United Nations' World Water Assessment Programme, over 80% of wastewater worldwide is not treated, nor collected. Urban settlements and industry are two of the most prominent sources of water pollution. To prevent water pollution from happening, it is extremely important that we educate the public about the importance of clean water (WWAP, 2012). Educating the public requires that data is showed in simple and comprehensible ways. Virtual environments are an excellent method for accomplishing that goal. Several environments, including virtual labs, simulations, and games, have been developed to educate the public. However, most of these tools have not accounted for the importance of high fidelity and high presence in an educational virtual environment

The purpose of this research project is to design and develop a high-fidelity virtual environment that uses authentic data gathered by the LEWAS to educate people about how storm events affect small urban streams. This project was designed using quality function deployment, and developed using a game engine. The project resulted in an explorable virtual environment representing the field site during a storm event that took place September 29th, 2015.

2. Background

The Learning Enhanced Watershed Assessment System (LEWAS), located in Blacksburg, Virginia, is a high-frequency environmental monitoring system. It uses modern sensing technologies to measure water and weather parameters (at 1-3 minute intervals) from a small urban watershed (surface area of 2.78 km^2). The data collected by the sensor is stored in a small, low-cost computer located at the site, and is later sent to a secure database on the Virginia Tech campus in Blacksburg, Virginia (Mcdonald & Brogan, 2015). The LEWAS lab group utilizes this data for educational outreach and watershed education purposes. The data collected by the LEWAS has been used in engineering, geoscience, environmental science, and computer science courses at Virginia Tech and Virginia Western Community College, and has reached over 10,000 students since 2009. In addition, the data has been used in courses ranging between high school and upper undergraduate level at Floyd County High school, in Floyd, Virginia,

USA, KLE Technical Institute in Hubli, Karnataka, India, University of Queensland in St. Lucia, QLD, Australia, and Central State University in Wilberforce, Ohio, USA (Brogan, Mcdonald, Lohani, & Dymond, 2016).

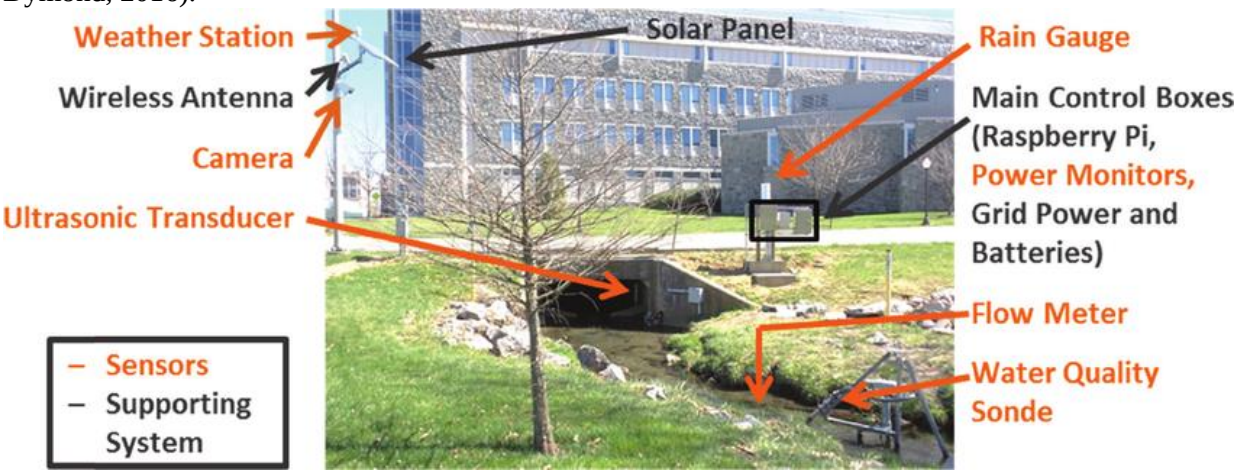


Figure 1: LEWAS at Stroubles Creek (Brogan et al., 2016)

The LEWAS lab has a history of utilizing cutting-edge technology together with the data collected by the LEWAS for watershed education. The Online Watershed Leaning System (OWLS) work as the user interface for the LEWAS. It allows users to examine case studies and explore historic and real-time data from anywhere at any time. The OWLS has been used in pilot studies in a senior-level hydrology course and a community college course. The assessment results from the studies indicated that the students believed that the OWLS helped them learn hydrological concepts and that the OWLS was valuable and relevant to their coursework (Brogan et al., 2016). The figure below shows a flowchart detailing how the LEWAS operates.

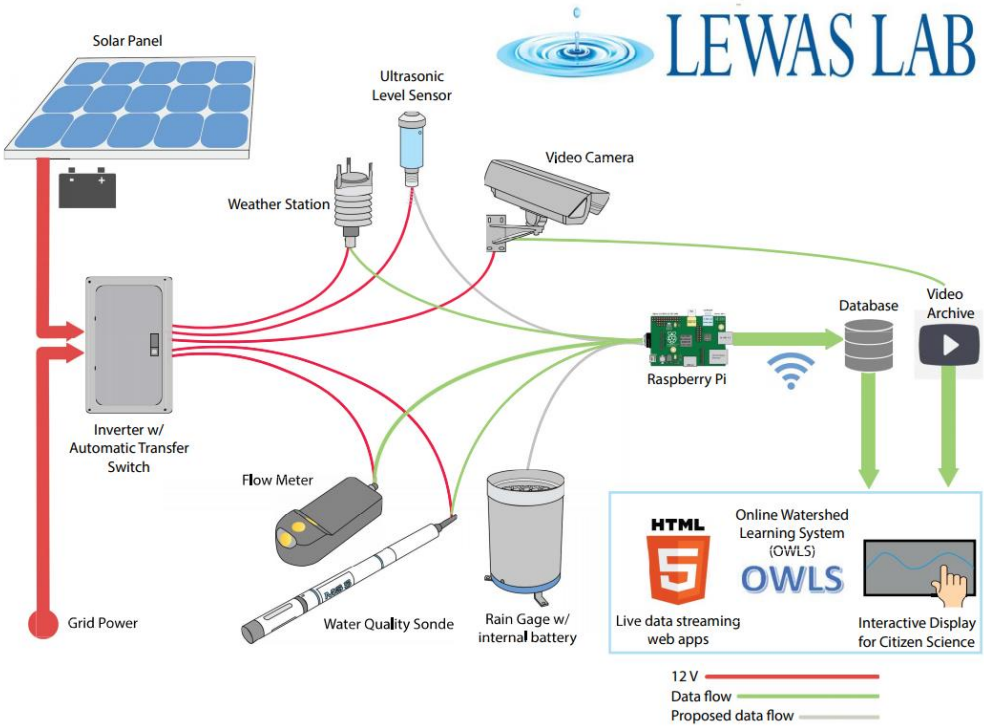


Figure 2: LEWAS Flowchart

The LEWAS lab group's recent efforts in personalized learning has inspired an interest in the use of new technologies such as user-tracking, virtual reality (VR) and educational virtual environments (VEs). Virtual reality (VR) can be defined as "An approach that uses displays, tracking, and other technologies to immerse the user in a VE" (LaViola, Kruijff, McMahon, Bowman, & Poupyrev, 2017). In the past, the costs related to the hardware required for the effective use of VR and VEs have prevented their widespread use in educational settings. Therefore, these technologies have mostly been used for entertainment and communication purposes. However, in recent years, the rapid improvement in computing and multimedia technologies have given rise to a resurgence of interest in educational uses of VR and VEs. (Dalgarno & Lee, 2010).

Recently, virtual environments are being used for various educational purposes, including simulation, visualization, and teaching. Educational virtual environments have certain specific advantages over alternative methods of learning. They can facilitate tasks that lead to the development of enhanced spatial understanding, encourage experiential and active learning, allow for experiences that would be impractical or impossible in the real world, increase motivation and engagement, and improve the transfer of skills to real-life situations (Dalgarno & Lee, 2010). In addition, the use of virtual environments has been proven to increase the user's enjoyment, which further increases their motivation (Roussou, Oliver, & Slater, 2006).

Virtual environments that focus on teaching users about environmental awareness and water quality parameters are relatively rare, but there is some literature describing them. A couple of virtual environments have been developed to educate the public about water related issues such as flooding (Chan, Kang, & Tan, 2011; Li, Gong, Song, Liu, & Ma, 2015). However, these applications are mostly focused on visually showing large-scale flood damage simulations, and disregard the impacts that storm events might have on smaller streams. Among the educational virtual environments that focused on water issues, barely any are high-presence environments, and only a few utilize VR technologies. The only small-scale, water-related, and VR-utilizing virtual environment that could be found was a web-based simulation environment that focused on damage caused by flooding. The environment in question can be viewed using VR, but can't be actively explored.

3. Literature Review

Several studies involving educational virtual environments were reviewed. The goal was to gather information about previous work and use the findings to inform the design of the virtual environment.

Immersion and presence are two critical properties of virtual environments. Presence is "the subjective sense of being in a place" while immersion can be defined as "the objective and measurable properties of the system or environment that lead to a sense of presence" (Dalgarno & Lee, 2010). It is important to note that immersion is not a single unified aspect of a system, but rather a combination of many different components. Immersion therefore exists as a complicated, multidimensional spectrum in which certain components can be more beneficial depending on the application (Bowman & Mcmahan, 2007). For instance, in a three-dimensional (3D) visualization environment, visual immersion will outweigh auditory immersion in terms of importance.

It is important to recognize that different users can experience different levels of presence in the same environment. However, it is generally true that a higher level of immersion will result in users experiencing a higher level of presence. In educational virtual environments in particular, higher levels of immersion have been proven to increase user's sense of presence and their understanding of the concepts being taught (Bowman & Mcmahan, 2007; Winn, Windschitl, Fruland, & Lee, 2002). Because of its positive effects on the learning outcomes of users, developers of educational VEs tend to strive towards

maximizing immersion.

Virtual reality is considered a high-immersion multimedia technology (Dalgarno & Lee, 2010; Mikropoulos & Natsis, 2011; Slater, Linakis, Usoh, & Kooper, 1995). VR systems can take many forms, but the most commonly used systems involve a tracked head-mounted display (HMD) and other accessories, such as tracked controllers and built-in earphones. Tracked HMDs allows for complete visual immersion, natural visual interaction with the environment, and the accurate tracking and virtualization of the user's movements (Mon-williams, Wann, & Rushton, 1993). Because of their immersive properties, VR systems are beginning to be used for educational purposes.

4. Design Process

During the design process, a Quality Function Deployment (QFD) method known as a House of Quality (HOQ) was used. A HOQ is a versatile, customer-oriented design planning matrix that can be used organize technical requirements and customer requirements, the trade-offs of design specifications, determine the importance of fulfilling different technical requirements (Hauser, 1993). Benefits to our research include optimizing the design process, decreasing the time to develop new products and increasing the quality of designs (Karsak, Sozer, & Alptekin, 2002). In the context of this study, the customer will be the user of the virtual environment.

A five-section HOQ was generated during this project. The roof matrix of the house is a correlation matrix between the technical requirements. If the symbol at the intersection of two technical requirements is a '+', there is a positive correlation between them, and if it is a '-', the correlation is negative.

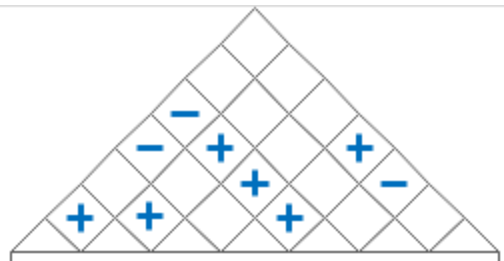
The left part of the house is where the user requirements are. In the context of this project, the user requirements are chosen based on background research. The part right beneath the roof matrix is the technical specifications These are measurable specifications that are used to fulfil the user requirements.

The central part of the house is known as a relationship matrix. In this section, each technical specification impact on each user requirement is indicated. For instance, the technical specification 'visual fidelity' has a strong impact on whether the environment is visually similar to the field site, so the cell between them is marked with the symbol for 'strong relation'

The final section is the importance matric. This section can contain many different tests, but based on the project, it was concluded that only the importance section would be necessary. The larger the importance factor is, the more important the technical specification will be to the design.

The figure below shows the final version of the HOQ for the design of the VE.

Legend		
+	Positive correlation	
−	Negative correlation	
↑	Maximize	
↓	Minimize	
○	Target	
◉	Strong relation	9
○	Medium relation	3
△	Weak relation	1



			Importance	Size of environment (m^3)	Time to complete case study (s)	Tracking of movement	framerate (fps)	Visual Fidelity	Aural Fidelity	Linear Interpolation frequency
				Technical Specifications						
				↑	○	↑	↓	↑	↑	↑
User Requirements	Accurately Portrays Field Site	Visually similar to field site	4	○	○	◉		◉		
		Includes Details	2	○			◉		○	
	Accurately Portrays Case Study	Accurate visual changes	5				○	◉		◉
		Links visual changes to collected data points	5							△
		Matches changes with proportionate time intervals	3		◉			◉		
	Comfort	Visually comfortable	5	○	○		○			
		Auditory comfort	4						△	
		Ergonomically comfortable	5	△	○	△	◉	◉		
	Ease of use	Intuitive visual design	5	○	○		○			
		Intuitive movement	4		○					
		Importance		53	96	41	108	153	10	50

Figure 3: House of Quality

Because immersion in an educational VE has been shown to have a significant impact on the user's sense of presence, and their learning outcomes, higher levels of immersion are preferred. According to previous studies, this increase in immersion is expected to increase presence, and improve the user's understanding of the concepts being taught (Bowman & Mcmahan, 2007; Winn et al., 2002). To accomplish this, a tracked head-mounted display was introduced in the form of a consumer-grade gadget, the HTC Vive.

Another design decision that had to be made was which case-study to program into the environment. The November 25th, 2015 case study was chosen over other similar case studies, because of the shocking changes in the stream's water level, and because of its visual characteristics. During the storm event, the difference between the maximum and minimum water level was 0.88 meters, which is rare even for severe storms. The data and the video feed of the case study both show that the storm event had very noticeable visual effects on the stream. Because the virtual environment will rely heavily on visual effects to show the effects of the storm event, it is important that the visual changes in the environment are clear and significant.

LEWAS measures a multitude of water parameters (pH, Turbidity, flow rate, dissolved oxygen, etc.) For the educational purposes of this environment, only the water height data was used. The water height data was used because it provided the VE with a clear visual change that indicates what happens during a storm event. In addition to using the water height data, the VE will show changes in turbidity that based on water height data rather than actual turbidity data. A general trend throughout the video of the case study was a correlation between the water height and the turbidity of the water. As the water level rose, the turbidity increased, and the water got cloudier. Changing the turbidity based on the current water level would show the storm's effects with enough accuracy while reducing the computational power needed to run the VE.

5. Development Process

Following the conceptual design of the VE, the development of the VE started. The development phase took place in 5 steps: Converting point cloud data into unity terrain, adding and manipulating water, adding details, programming the case study, and implementing a traveling method.

Step 1: Converting point cloud data into 3D Terrain

Precise, three-dimensional terrain data for the area surrounding the Stroubles Creek field site was obtained by members of the LEWAS lab group on November 22nd, 2015.The data was collected using a Total Station Instrument (TSI) and was formatted as (x,y,z) coordinates. A custom code in a numerical computing environment was then used to turn the coordinates into a colored heightmap of the field site. A heightmap is a 2D raster image used to store elevation data on. Using a raster graphics editor, the heightmap was changed from a colored .png file into a grayscale .raw file. Once the heightmap file was in .raw format, it was imported into a game engine, and a three dimensional terrain object was created based on the heightmap's specifications. The terrain object is 30 m wide, 22 m long, and 2 m tall, which makes the field site approximately the same size in the environment as it is in the real world. The heightmap was in feet so after it was converted into meters using unit conversion, it was rounded to the nearest whole number. Figure 4 shows a visual representation of this process.

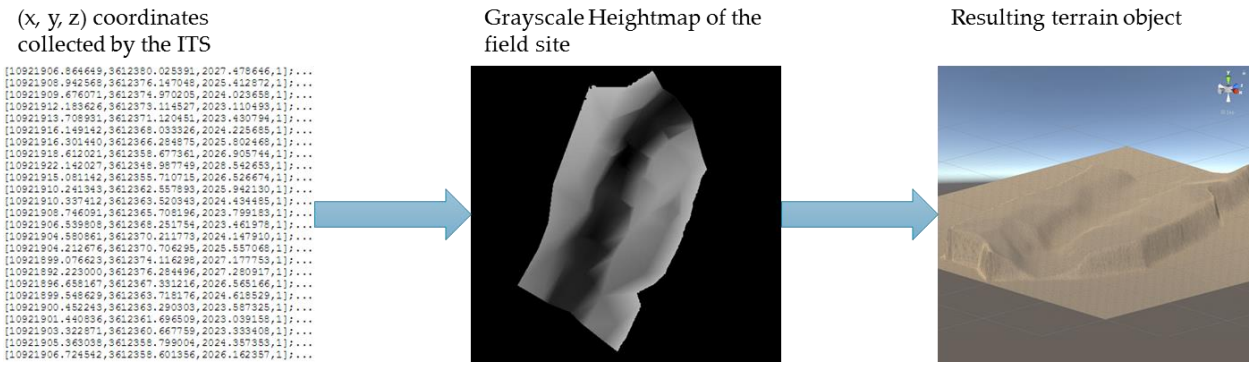


Figure 4: Terrain Creation Process

Step 2: Adding and Manipulating Water

The water was added to the environment as a circular plane. The built-in water object in the game engine is represented as a surface with two scrolling textures. The textures make the water appear to be moving in 3 dimensions, even if it is actually a flat surface. The water object comes with reflection and refraction scripts that make the water look more realistic. Reflection and refraction are both ways in which light can interact with the surface of an object. When light hits the surface, it can reflect, which makes it 'bounce' off the surface. It can also refract, which is when the light enters the new medium and is starts traveling in a different direction. When light hits a water surface, both refraction and reflection occur, creating distinct visual effects.

Initial impressions of the environment led to the conclusion that the reflection and refraction scripts caused a glaring effect that led to significant visual discomfort. Prioritizing user comfort over a minor increase in the realism of the manner in which the water interacts with light, we decided to get rid of the reflective and refractive effects. The figure below shows the difference between the water before and after this modification.

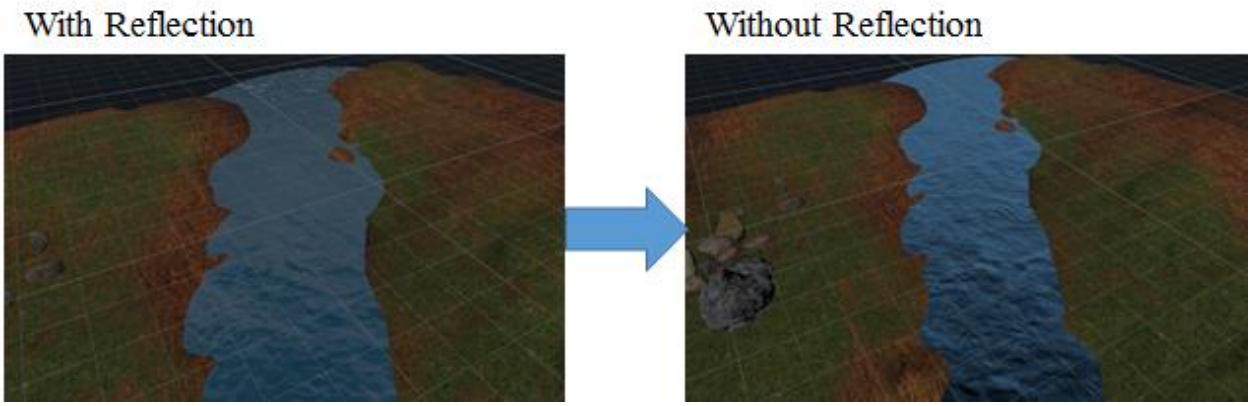


Figure 5: Reflective vs. Simple water

Step 3: Adding Details

Details include things such as textures, rocks, sky textures, and etc. Because the case study was a storm event, a dark sky texture filled with rain clouds was used. The environment contains a total of 11 different rock models, which were placed in locations where there are rocks at the field site. Other details added include: a rain particle effect with variable intensity, a water drop effect on the screen, soil and grass textures, and a point-source life that illuminates the otherwise dark environment.

Step 4: Programming the Case Study

The fourth step was the programming the case study. All the programming was done using C# in an integrated development environment. During the case study, the two water parameters that we want to draw attention to are water height and turbidity. These parameters are chosen because they are striking, visible indicators of what happens in a small stream during a storm event.

A custom script was designed and developed to include water level data gathered by the LEWAS during the storm event on November 29th, 2015. The script works by targeting a specified comma-separated variable file using its file path in the computer. As soon as the user enters the virtual environment, the code runs and creates a list of all the water height data points in the specified .csv file. As the program moves from one data point to the next using linear interpolation, it sets the height of the water within the environment to be its current position. The program waits a specified amount of time before moving on to the next data point so that the total run-time can be edited. In order to show how turbidity changed during the case study, the color of the water changes with the water level. As the water level rises, the color of the stream changes by linear interpolation, appearing more turbid. The decision to not include turbidity data was based on the observation of the video footage of the case study, and the performance issues that would arise from doubling the number of data points the code would have to incorporate. In the video footage of the case study, there is a strong correlation between the water level and the turbidity of the water.

Step 5: Implementing a Traveling Method

One discrepancy we came across was that the biggest possible exploration area for the HTC Vive is approximately 4x4 meters, while the environment has an area of 22x30 meters. It's important that the user is able to explore the whole visible area because it drastically increases the fidelity of the situation by allowing the player to go where they would be able to go in a real setting. For this reason, a method for traveling through the virtual environment had to be developed. After carefully considering various solutions such as increasing the user's size relative to the environment and using controllers for movement, it was decided that using teleportation was the best option. Teleportation would allow the user to walk around the 4x4 meter play area, giving the feeling of actually walking around the area. However, when trying to reach something outside of the real environment play area the user would simply have to use the controller to teleport. In order to incorporate teleportation an open source teleporter script package was imported (Flafla, 2016). The code makes it so the user can point one of the HTC Vive controllers and a small target will appear on the ground. If the user then clicks the trackpad on the controller, he or she will teleport to that location.

6. Results

Throughout this project, an educational virtual environment that shows the effect of a storm event on a small urbanized stream was successfully designed and developed. The environment is meant to be explored using virtual reality technologies and is a virtual representation of a case study that occurred on September 29th, 2015. A custom program built into the environment uses stage height data from a .csv file with the OWLS' native format convention to virtually recreate the case study. It reads the water height values and sets the water height in the virtual environment to be equal to the data values while adding in-between values using linear interpolation, making the water movement smoother.

The figure below shows the field site and the environment at the low-water conditions and at the high-water conditions. The pictures in the top row are pictures of the actual field site while the bottom row are pictures from the virtual environment

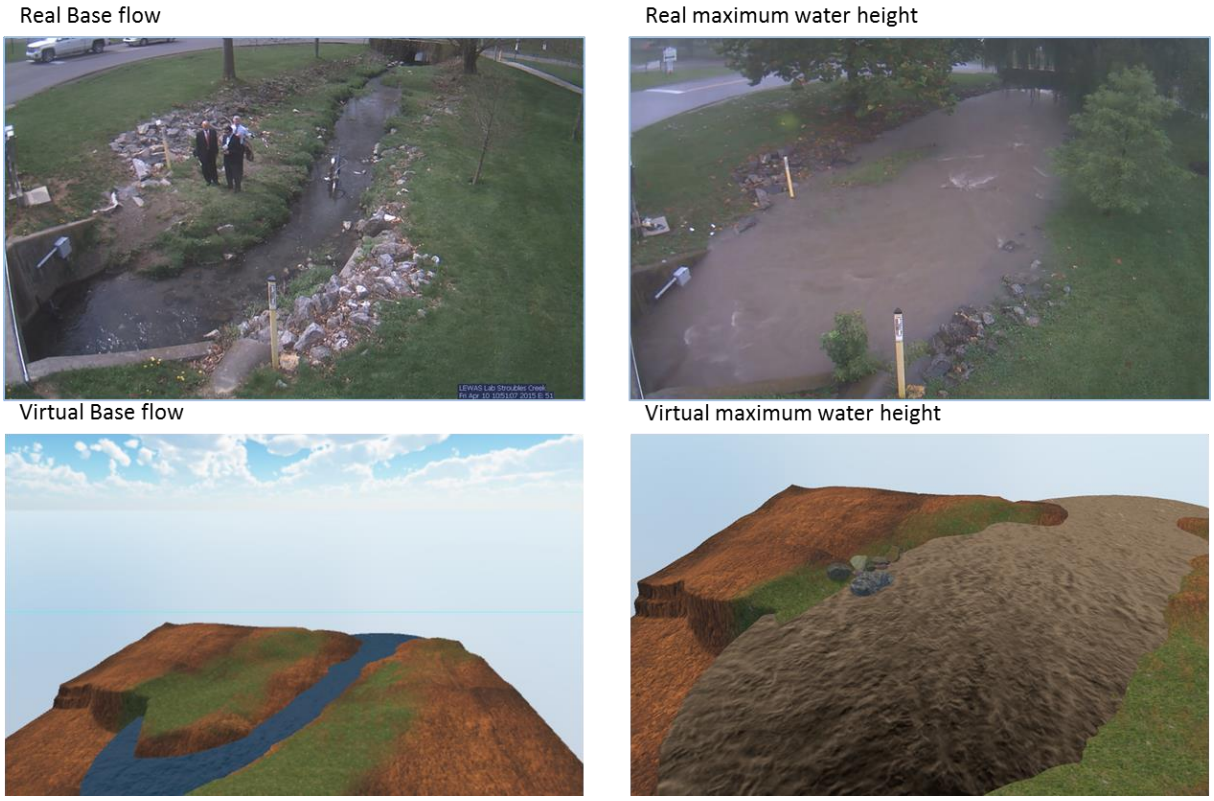


Figure 6: Comparison Between the Field Site and the Virtual Environment

7. Discussion

Informal user observations were conducted on July 20, 2017. Most of the individuals who participated were familiar with the field site, but there were some that were not. In addition, the conditions were not ideal for the usage of the virtual environment. The room was crowded, and the exploration area in the room was cluttered, preventing the user from free/open/full travel/mobility in the VE.

During this study, it was observed that the teleporter had a couple of problems. There were certain areas that were untargetable by the teleporter, preventing the user's travel to those locations. The teleport controls were also not as intuitive to some individuals who experienced the environment.

Another observation that was made, was that every individual attempted to look underneath the water's surface at some point. Because the water is a plane in the environment, there are no underwater effects in place. In other words, when the user looks underneath the water surface, it does not look as if they are underwater. The prevalence of this behavior indicates that adding an underwater environment should be one of the first improvements made to the environment.

These results indicate that thoughtfully designed user studies should be conducted to understand how individuals react to the environment. In addition, more time should be devoted to the further development

of the VE to increase its usability.

8. Conclusion

Educating the public is going to be an integral part of solving the water quality issues currently facing our world. In order to effectively educate people, scientific findings should be shown in simple and comprehensible ways. Virtual environments are very effective at making data easy to understand by visualizing it.

This project involved the design and development of a high-presence educational virtual environment that uses data gathered by the LEWAS on November 29th, 2015 to illustrate the effects that a storm event has on a small stream. The environment teaches them about the changes the stream undergoes visually, making the data easier to understand.

9. Future Work

The environment could be improved through expansion, the use of geological and biological details, the addition of more parameters, and the development of virtual sensors.

The environment could be expanded to increase the area that users can explore. This could easily be done using a 3D point cloud of the surrounding area. It could also include more biological and geological details, such as plants, animals, and an accurate geological representation of the bottom of the stream. More research would have to be conducted to assess what kinds of plant and animal life are present by the field site. Adding models resembling species that are present at the site would enable the virtual environment to more accurately represent it. It would also educate the user about the surrounding ecosystem.

The environment could also be improved by including more water and weather parameters. The current code could be used to read the data from other parameters as long as they are in a .csv file and are formatted using the OWLS' format conventions. However, the visual changes of the environment based on the parameters would have to be developed. In addition, virtual sensors could be programmed into the environment. Doing so would make the experience more interactive, and could teach the user about water sampling principles, further educating them about water research.

The quality of the environment as an educational tool could be evaluated through user studies. One potential study could compare two groups of students learning about the effects that storm events have on small streams. One group could learn about these effects through a lecture, while the other would experience the environment in addition to that lecture. The two groups could then be evaluated based on their understanding of the concepts.

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Nutrient Recovery from Wastewater Using a Bioelectrochemical System

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Abstract

Diminishing resources including water scarcity has attracted international attention to technologies that can recover resources from waste. Microbial electrolysis cells (MECs) are wastewater treatment tools that produce electricity through the consumption of organic molecules in wastewater. Ionic transport within these systems allows for the removal of pollutants and recovery of nutrients including nitrogen based molecules like ammonium. The goal of this experiment was to investigate the ammonium recovery from a partially submerged tubular MEC and analyze the distribution between liquid cathode solution and air. This was done by running an MEC on batch mode with a synthetic waste water solution and monitoring the cathode and anode solution ammonium and COD concentrations as well as the current generation. It was found that the peak current production for the MEC with aeration was approximately 8.34 mA and without aeration 12.1 mA. The average ammonium removal efficiency of the system with aeration was 97.6 ± 1.6 % and without aeration 77 ± 8.9 % and the average COD removal efficiency of the system with aeration was 84.4 ± 10.3 % and without aeration 69.9 ± 15.9 %. The ammonium had an average distribution of 84.9 ± 4.0 % in the air around the reactor and 15.1 ± 4.0 % remaining in the cathode solution. Based on these results it can be concluded that a tubular MEC has adequate potential for ammonium recovery from wastewater and that ammonium is predominantly driven out of the cathode solution into the air.

1. Introduction

1.1 Water Scarcity

As population increases and economies continue to develop, the demand for fresh water has increased dramatically and led to water scarcity. There are two main types of water scarcity, physical water scarcity which is when the land cannot provide enough freshwater to meet population demands and economic water scarcity which is when a population lacks the monetary resources to obtain adequate supplies of freshwater (Rijsberman, 2006). The diminishing availability of clean fresh water has forced many to use unsafe water sources. The most afflicted areas are arid, dry climate regions and developing countries who do not have the proper resources to provide clean freshwater to everybody including West and Central Africa, the Middle East, and Southeast Asian countries. The shortage of water in developing communities is also promoted by insufficient waste management that fails to appropriately treat and release liquid and solid waste. The water crisis is also connected to global energy demands; 8% of the world's energy is used to pump and distribute water and around 70% of water is used for agriculture (UNWater.org, 2017). Addressing the water crisis is an international effort not only to secure adequate water resources but to invest in our energy and nutrient resource demand.

1.2 Microbial Electrolysis Cells

The sustainability of wastewater treatment processes is a vital step in the progression of water and energy technology development as well as a serious question in the endeavor to resolve the water shortage

crisis. Bioelectrochemical systems (BES) have the capacity to answer this question by using microorganisms to produce electric power **[1]**. BES can simultaneously remove organics, pollutants, and heavy metals from wastewater while generating an electric current. Microbial Electrolysis Cells (MEC) are a type of bioelectrochemical system that oxidizes organic molecules in wastewater to produce electrons that flow from an anode to a cathode and react with protons to produce hydrogen gas (Logan et al., 2006).

Both municipal and industrial wastewaters have a high concentration of organics, inorganic nutrients and heavy metals (Ellis & Ellis, 2016). These molecules must be removed from influent flow for the water to be considered safe for discharge. In conventional wastewater treatment facilities organic molecules are removed from water using activated sludge during secondary treatment (Masters & Ella, 2008). However, this process requires incessant aeration and can be extremely expensive if sustained over long periods of time. BES are considered to be a method of developing synergy between wastewater treatment processes and bioenergy production while generating enough power to compensate for its own power consumption. Sustainable water treatments methods such as BES are crucial to the availability of clean water sources especially in developing communities.

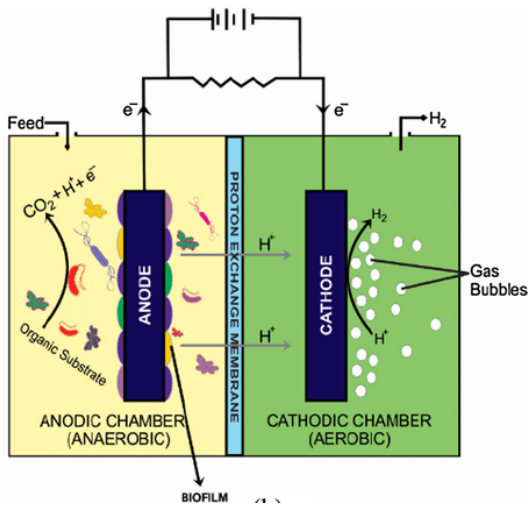

Figure 1: schematic of microbial electrolysis cell displaying anode and cathode chamber. (Khan et al., 2017)

MECs are a developing BES technology that can be used to produce hydrogen or methane gas from wastewater. The three main components of an MEC are the anode, cathode, and ion exchange membrane, usually a cation exchange membrane (CEM). The anode chamber is filled with an anolyte solution primarily composed of domestic or synthetic wastewater and the cathode chamber is filled with a catholyte of deionized water or a buffer solution (Logan et al., 2006). Electrochemically active bacteria are inoculated into the anode chamber and grow on or around the surface of the anode electrode. Through a series of chemical reactions described by (Logan et al., 2006), organic molecules in wastewater are oxidized and electrons are produced as shown in equation 1.

$$COD \rightarrow CO_2 + H^+ + e^- \quad \textbf{(1)}$$

These electrons are transferred from the bacteria to the anode electrode and channeled through an external circuit with a designated resistance to the cathode where they terminate to an electron acceptor as shown in figure 1. During this process protons migrate across the CEM from the anolyte solution to the catholyte to balance the electron transfer away from the anode. These protons react with the transferred electrons to produce hydrogen gas and the electrons flowing between the electrodes generates an electric current. In an MEC, the oxidation reaction in the anode is anaerobic and the reaction in the cathode must be anaerobic as well in order to produce hydrogen gas (Logan et al., 2006).

MECs along with microbial fuel cells (MFC) and microbial desalination cells (MDC) are the preeminent technologies dominating work in BES. Both of these systems have the same general type of

configuration as shown in figure 1, including an anode, cathode, and ion exchange membrane. The primary difference between MFC and MEC is the use of oxygen in the cathode as an electron acceptor (Pant et al., 2012). MFC's can also sufficiently operate with low COD loading levels and different carbon sources besides simple carbons like acetate (Pant et al., 2012). MDCs typically have a third chamber and a second ion exchange membrane to facilitate the removal of sodium and chlorine ions from water. The third chamber is placed in between the cathode and anode and anion exchange membrane and cation exchange membranes on either side, so as electrons flow from the anode to the cathodes, disassociated ions in the brine migrate across the membranes which desalinate the water (Liang, Xiao, Zhou, Zhang, & Logan, 2009).

There are two primary configurations of MECs: flat plate and tubular. Flat plate MECs as shown in figure 1 have the anode and cathode chambers placed in separate compartments and can either have a membrane separating the chambers or no membrane. Tubular MEC configurations as shown in figure 2 are cylindrical, have the anode chamber positioned in the core and the CEM and cathode wrapped around the outside (Rabaey, Clauwaert, Aelterman, & Verstraete, 2005). The system can be partially or fully submerged in catholyte solution or exposed to the air. Tubular MECs provide the advantage of more membrane surface area, which can facilitate more ion transfer and higher levels of current generation.

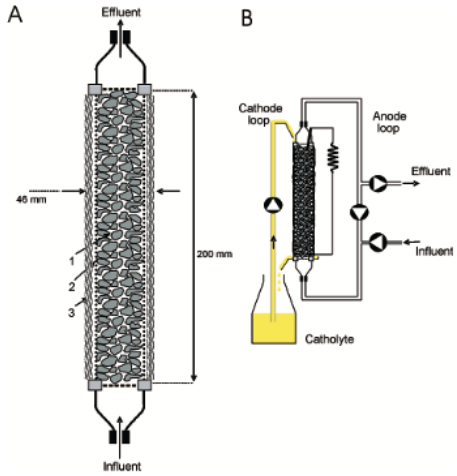

Figure 2: tubular MEC configuration with inner anode chamber and outer cathode loop as well as cathode and anode influent and effluent directions. (Rabaey et al., 2005)

The degrading of organic molecules in wastewater causes embedded nitrogen to be released in the form of ammonium (Masters & Ella, 2008). Nitrogen is an inorganic nutrient that must be removed from wastewater to prevent eutrophication, which can lead to excessive plant and algal growth and deplete oxygen concentration within the water (Yang, Wu, Hao, & He, 2008). The removal of nitrogen in domestic waste is conventionally done through nitrification and denitrification which oxidizes ammonium into nitrate and then reduces nitrate to nitrogen gas respectively, both being biological processes (Zhang & He, 2012b). Or alternatively through ammonium stripping which degrades ammonium hydroxide in wastewater through the addition of caustic lime. Nitrogen is also an important nutrient and is the primary chemical component of ammonia which is critical to fertilizer production. However, 1-2% of the world's energy use is delegated to ammonia production procedures such as the Haber process (Bicer et al., 2016) which makes ammonia expensive to produce. Ammonia can be recovered from wastewater through the ionic diffusion of ammonium cations through ion exchange membranes in BES (Qin et al., 2017). The ion transport responsible for ammonium removal is facilitated by these membranes and without them ion separation is impossible. However, because they often comprise half of the total cost of a reactor, many experts argue that membranes are expendable pieces in bioreactor designs (Du, Li, & Gu, 2007). Despite this assertion, membranes incorporated in BES allow for the simultaneous treatment and nutrient removal of wastewater. This gives the potential for sustainable ammonia recovery from wastewater and provides a synergy between nutrient, energy, and water recovery from liquid waste supplies.

The nitrogen removal performance of different BES systems changes with a variety of system factors including current generation, ammonium concentration in anode effluent, carbon source, concentration of dissolved oxygen in the cathode, and the chamber pH (Kelly & He, 2014). (Qin, Hynes, Abu-Reesh, & He, 2017) showed that current generation promotes ammonium removal in a coupled MEC-forward osmosis system. Due to the increase in current flowing from the anode to the cathode, more ammonium ions were transferred across the CEM to balance the system charge. High levels of ammonium concentration in influent flow was shown to cause a decrease in the total nitrogen removal rate (Zhang & He, 2012a). (Feng et al., 2013) confirmed that simple carbon sources such as glucose and methanol promote denitrification the most and that inorganic carbon sources such as sodium bicarbonate lead to higher nitrite accumulation and higher COD levels in the system effluent.

The ammonium removal performance is also dependent on the type of configuration being tested. In tubular MEC configurations, ammonium ions migrate across the cation exchange membrane through the cathode into the catholyte solution or surrounding air. For a partially submerged MEC, ammonium is distributed in both the catholyte and the air. This distribution is likely a product of multiple operation parameters within the MEC including ammonium loading rate, current generation and batch time. The goal of this experiment is to analyze the ammonium removal of a partially submerged MEC as well as the distribution between the catholyte solution and air and to contextualize the relationship between different system parameters and the ammonium removal efficiency of the MEC. This must be done in order to further quantify an MEC's potential for sustainable ammonium recovery and removal from domestic wastewater.

2. Research Methods and Experimental Setup

2.1 MEC Apparatus

The MEC used in this experiment was a single chamber tubular MEC with a cation exchange membrane (CEM) tube (Membrane International Inc., Ringwood, NJ, USA) to separate the anode and the cathode. A carbon brush was used as the anode electrode and a carbon cloth with an active carbon catalyst was used as the cathode electrode. The anode chamber had an internal volume of approximately 170 mL. Anode influent, effluent, and air openings were placed at the top of the reactor and the cathode influent tube was wrapped around the reactor. The reactor was placed inside a glass chamber and sealed with silica gel and epoxy to prevent air leakage as shown in figure 3. Holes were drilled in the chamber for anode and cathode influent and effluent and the anode electrode. Both anode influent and effluent tubes fed into and out to a 125 mL bottle. Solution was pumped through the tubes using a precision tubing pump (Masterflex L/S Easy-Load 7518-00, Cole-Parmer, Vernon Hills, IL, USA).

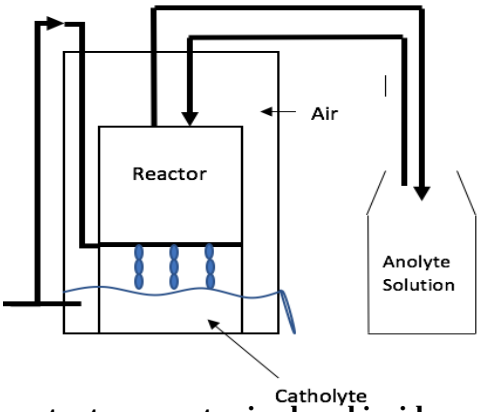

Figure 3: experiment setup; reactor is placed inside a sealed container and partially submerged in cathode solution. Anolyte solution is in bottle and cathode solution is recirculated in the container

2.2 MEC Operation

The MEC was operated at a room temperature of approximately 21 C˚. The anode was inoculated with anaerobic sludge from Christiansburg Regional Wastewater Treatment Plant (Christiansburg, VA USA). To simulate the chemical composition of municipal wastewater the anode influent solution was produced with 1.5g acetate, 2.0 g $NaHCO_3$, 3.0 g NH_4Cl, 1 mL of a trace metal solution, 3 mL of a stock solution, and 2 mL of a 50 mM phosphate buffer solution per liter deionized water. The initial COD concentration in the anode influent was approximately 1450 mg/L and the NH_4^+-N concentration was approximately 3000 mg/L. The cathode influent solution was just 50-75 mL of deionized water however if the pH of the reactor proved to be unstable, 1-2 mL of the phosphate buffer solution was added. In order to control the submersion level of the MEC within the container and prevent evaporation, the cathode solution was recirculated from influent to effluent. The MEC was run on a 24 hour batch mode, allowing all the organics in the influent solution to be degraded; the anode influent solution was changed at the end of every batch. Once the current production and ammonium removal reached stability the ammonium could be recovered as ammonium gas (Liu, Qin, Luo, He, & Qiao, 2016) so we connected ammonium absorption bottles to recover it as ammonium sulfide. The MEC was connected in series to an external circuit with a 1 Ω resistor and a DC power supply (CS13644A, Circuit Specialists, Inc., Mesa, AZ, USA) which provided a potential difference of 0.8 V.

2.3 Measurement and Analysis

The voltage across the resistor in the external circuit was recorded every 2 minutes by a digital multimeter and automatically uploaded to an excel spreadsheet (2700, Keithley Instruments Inc., Cleveland, OH, USA). Samples collected from each batch were stored in a refrigerator at 9 ˚C and measured for conductivity, pH, COD and ammonium concentration every four days. The conductivity of anode and cathode solutions was measured using a benchtop conductivity meter (Mettler-Toledo, Columbus, OH, USA). The pH of each sample was measured using a benchtop pH meter (Oakton Instruments, Vernon Hills, IL, USA). The COD concentration in the anode influent and effluent solutions was measured using a colorimeter according to the manufacturer's instructions (Hach DR/890, Hach Company, Loveland, CO, USA). The ammonium concentration was measured in the anode influent and effluent as well as the cathode effluent using the same colorimeter according to the manufacturer's instructions.

The coulombic efficiency of the MEC is a measure of how much energy is converted from the chemical bonds in the carbon molecules to electric current. It is the ratio of coulombs transferred to the substrate to the maximum number of coulombs that can be removed to generate electric current (Logan et al., 2006). The coulombic efficiency, $\in_{Cb}$, is given by equation 3 below

$$\in_{Cb} = \frac{M \int_0^{t_b} I \, dt}{F b v_{An} \Delta COD} \quad \textbf{(3)}$$

where M is 32, the molecular weight of oxygen, F is Faraday's constant, $b = 4$ is the number of electrons exchanged per mole of oxygen, v_{An} is the volume of the anode solution in mL and ΔCOD is the change in COD concentration over a time period t_b (Logan et al., 2006).

The percent distribution of ammonium between the air and the cathode solution is given by equation 4 and 5 below

$$\%_{solution} = \frac{V_c [NH_3]_c}{V_{AI}[NH_3]_{AI} - V_{AE}[NH_3]_{AE}} \times 100 \quad \textbf{(4)}$$

$$\%_{air} = 100 - \%_{solution} \quad \textbf{(5)}$$

where V_C is the volume of the cathode, V_{AI} is the volume of the anode influent, and V_{AE} is the volume of the anode effluent all in mL. $[NH_3]_C$ is the concentration of ammonium in the cathode, $[NH_3]_{AI}$ is the concentration of ammonium in the anode influent and $[NH_3]_{AE}$ is the concentration of ammonium in the anode effluent all in mg/L.

The power consumption and generation is a measure of how much energy is required to operate the MEC and treat one cubic meter of wastewater. The total energy consumption, E, is the difference between the energy consumed, the power consumed by the pump, $P_{recirculation}$, and the power consumed by the power supply, P_{power}, and the energy generated by the MEC. The formula for power consumption and generation is given by equations 6 a-c below (Qin, Liu, Luo, Qiao, & He, 2017)

$$P_{recirculation} = \frac{Q_s \gamma H}{1000} \quad \textbf{(6a)}$$

$$P_{power} = \frac{IU}{1000} \quad \textbf{(6b)}$$

$$E = \frac{P_{power} + \sum P_{recirculation} - I^2 R}{Q_t} \quad \textbf{(6c)}$$

where Q_s is the flow rate of the solution being pumped in m^3/s, γ is the specific weight of water (9800 N/m^3), H is the hydraulic pressure head in m, I is the current in A, U is the external voltage in V, R is the external resistance in Ω and Q_t is the flow rate of treated in water in m^3/s.

3. Results and Discussion

3.1 pH and Conductivity

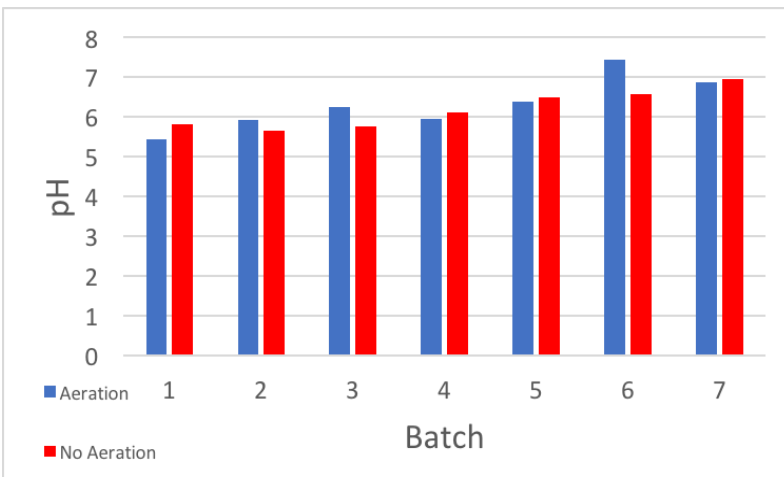

Figure 4: Conductivity of the anolyte effluent samples for the MEC with and without aeration

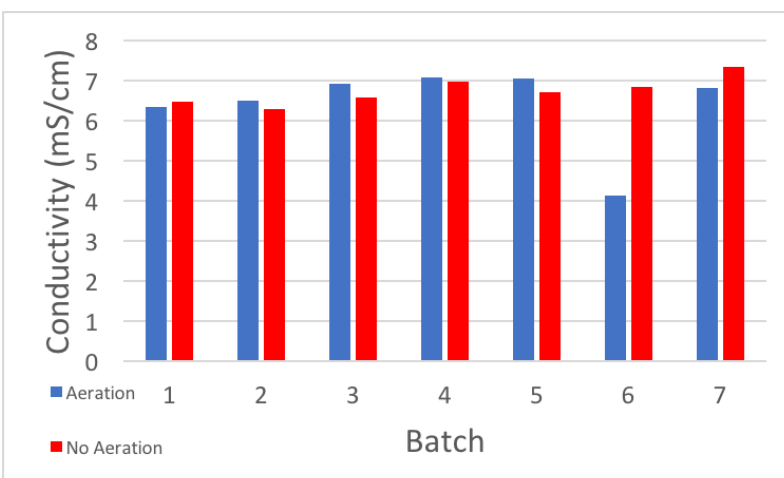

Figure 5: pH of the anolyte effluent samples for the MEC with and without aeration

Figure 4 and 5 above display the conductivity and pH of the anode effluent solution collected after every batch for the system with and without aeration. The pH and conductivity are both excellent parameters to gauge the water quality stability of the system. If these quantities remain relatively stable, then it indicates that the MEC is operating effectively. For the duration of this experiment the pH and conductivity remained in a relatively stable range. There was little difference for both measurements between the system with and without aeration. The average pH for the anode effluent solution with aeration was 6.32 ± 0.67 and without aeration 6.24 ± 0.47. Although the pH did increase slightly with each new batch, it remained in a feasible range for the experiment. This slight increase in pH is possibly due to some of the anode solution from the previous batch lingering in the anode chamber. The average conductivity for the system with aeration was 6.41 ± 1.04 mS/cm and without aeration was 6.76 ± 0.33 mS/cm. Other than an outlier in batch 6, the conductivity remains relatively the same over the duration of the experiment. Typically the anode chamber has a lower conductivity and a more acidic pH than the cathode chamber. However, if the pH is excessively acidic it can have adverse effects on the overall system operation. Approaching a neutral pH, as in the case of this experiment, does not have any significant effect on the performance of the MEC.

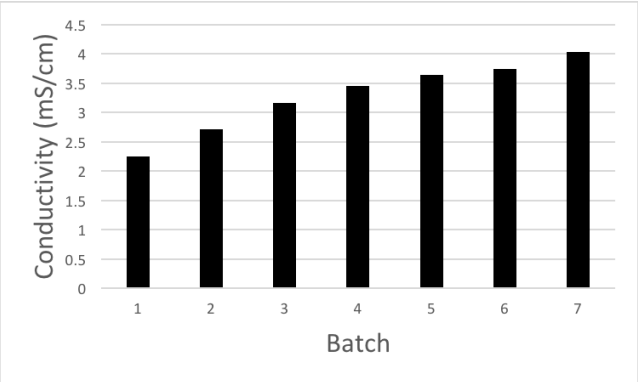

Figure 6: cathode solution conductivity for each batch cycle

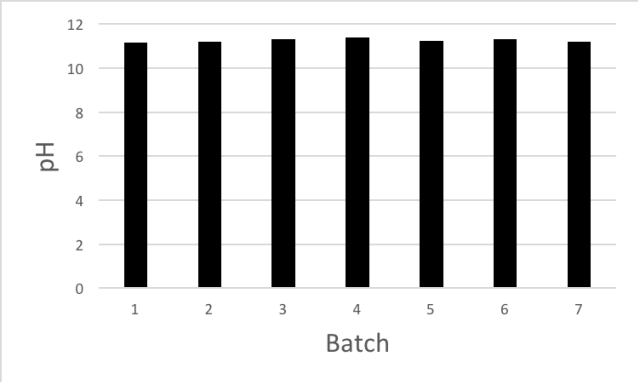

Figure 7: cathode solution pH for each

Figure 6 and 7 above are the pH and conductivity measurements for the cathode solution for each batch without aeration. The average pH was 11.3 ± 0.1 and there was little to no noticeable deviation in this parameter throughout the duration of the experiment. The pH of the cathode chamber is typically more basic due to a higher concentration of hydroxide ions. The conductivity increased from 2.25 to 4.5 mS/cm between batch 1 and 7 and this increase can be attributed to the ions migrating across the CEM from the anode chamber to the cathode. With each batch the ion concentration increases and this increases the conductivity of the cathode solution. However, the conductivity remains in a range feasible enough not to impair the function of the MEC. There is approximate error on the order of 0.1 for pH measurements and 0.2 mS/cm for the conductivity measurements. These errors are based on the precision of the measurement tools and slight variations due to temperature. All cathode and anode samples were stored in a refrigerator maintained at 9 ˚C and once removed from the refrigerator for measurement the temperature rapidly increased which could have caused a small error.

3.2 Current Generation

Figure 8 below displays the current generation as a function of time for the MEC with and without aeration. The current profile takes the form of a periodic wave that is commensurate to the amount of organics remaining in the anode solution. The peaks indicate the maximum current produced during the batch cycle. The average peak current generation for the MEC with aeration was 8.34 ± 1.84 mA and without aeration the peak current generation was 12.11 ± 1.59 mA. Current generation is directly

proportional to the concentration of organic carbon within the anode influent solution. Microorganisms living in the anode require around 12 to 13 hours from when a new supply of organics is provided to reach their optimum performance, so the base of each curve indicates when the anode solution is changed at the end of each batch. The earlier batch cycles, especially for the MEC with aeration, have slightly longer cycle times and this is because the microorganisms were still acclimating to the anode shortly after inoculation. Unstable current production is an easy indicator for improper function within the MEC. Current generation readings can fluctuate as a result of faulty circuit connections, electrode malfunctions, or issues within the anode chamber. Other than some small fluctuations in the first batch cycle without aeration, the current profiles were relatively smooth and served as another verification for proper operation within the MEC.

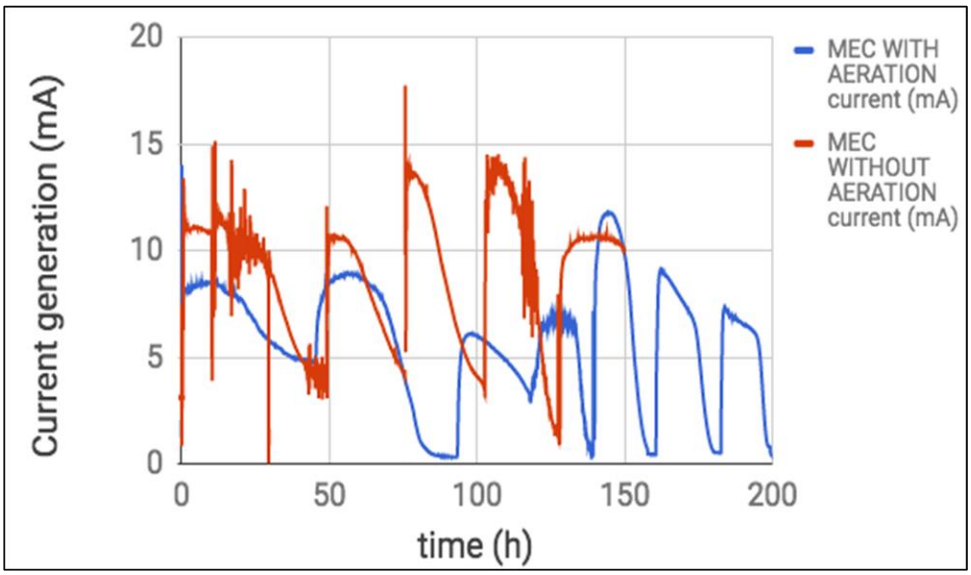

Figure 8: current generation of the MEC as a function of time with aeration (blue) and without aeration (red)

Once the system switched from aeration to no aeration the peak current production increased by an average of 3.77 mA for each batch cycle. This increase in current generation is due to an increase of positive ions migrating across the CEM. Oxygen serves as an electron acceptor in the MEC and reduces the electrons in the cathode. Without oxygen, protons migrating to the cathode take its place and promotes a more profuse flow of electrons from the anode. An increase in current production without aeration is an encouraging sign, as aeration can prove costly to maintain.

Batch	MEC w/ Aeration	MEC w/o Aeration
1	3.22%	16.54%
2	3.09%	25.69%
3	3.26%	16.12%
4	4.13%	12.69%
5	3.11%	18.06%
6	4.13%	-
7	3.20%	-

Table 1: Coulombic Efficiency of MEC

	Power Consumption (kWh m^{-3})
Anolyte Recirculation	0.133
Catholyte Recirculation	0.133
Power Supply	0.00000246769637
MEC Power Generation	0.00904
Energy Consumption	0.257

Table 2: Energy Consumption

Table 1 above displays the values of coulombic efficiency for the MEC with and without aeration for each batch cycle. Without aeration the coulombic efficiency increases by an average of 14.37%. The coulombic efficiency is a measure of how well the electrons generated in the oxidation of COD translate to electric current. An increase in average peak current production corresponds to an increase in the coulombic efficiency. The calculated average energy consumption of the MEC was 0.257 kWh m^{-3}. This value is calculated based off of the power consumed from the recirculation of anode and cathode solutions and DC power supply. Some of the power is compensated for based off of the current generation of the MEC. Compared to other forms of ammonium removal from wastewater such as annamox and air stripping the MEC demands almost 90% less energy (Qin, Liu, et al., 2017)

3.3 COD and Ammonium Removal

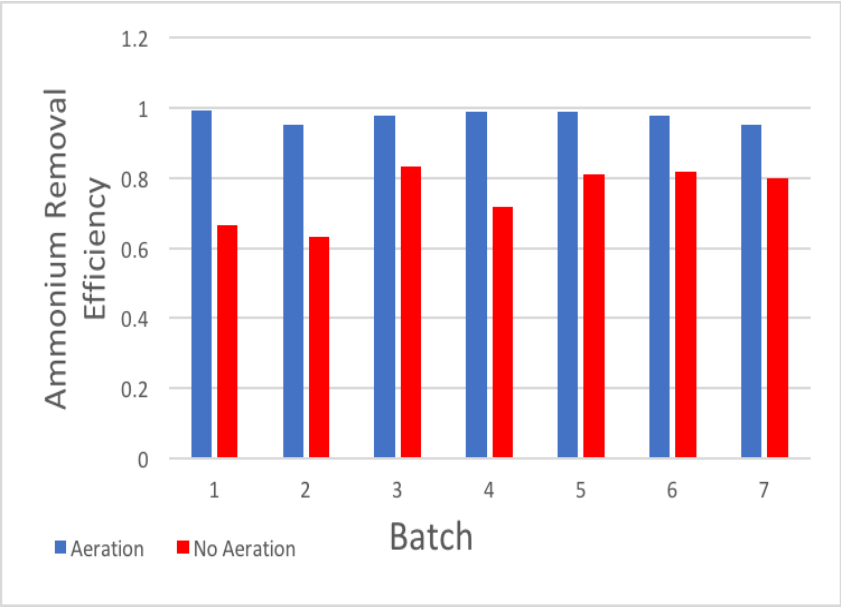

Figure 9: COD Removal Efficiency for each batch cycle with and without aeration

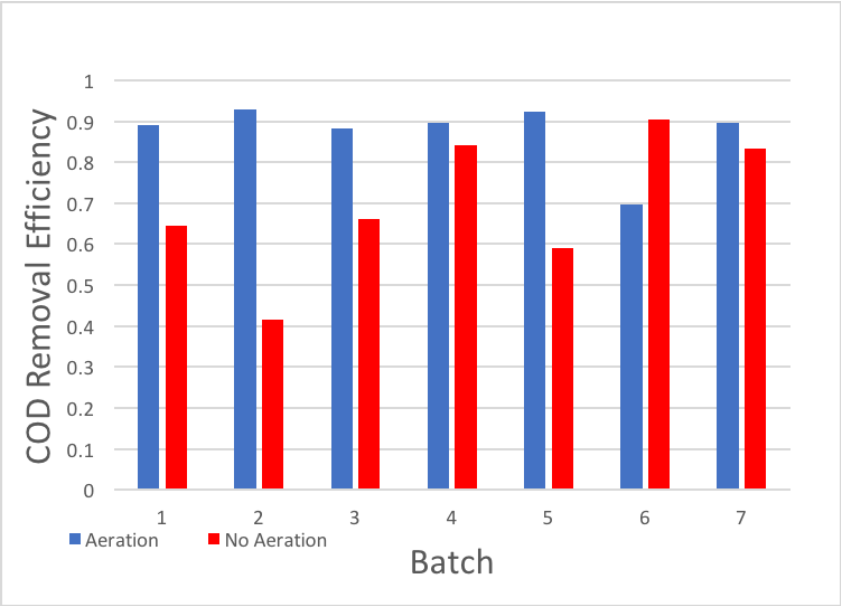

Figure 10: Ammonium Removal Efficiency for each batch cycle with and without aeration

Figure 9 and 10 above display the COD and ammonium removal efficiencies for the MEC with and without aeration. The average COD removal efficiency with aeration was 84.4 ± 10.3 % and without aeration, 69.9 ± 15.9 %. The average ammonium removal efficiency with aeration was 97.7 ± 1.6 % and without aeration, 77.1 ± 8.9 %. The COD removal efficiency quantifies how much organics were removed from the initial anode influent solution. With an initial loading rate of 11.85 kg m^{-3} d^{-1} the MEC removed upwards 90% of COD with aeration, leaving between 100 to 450 mg/L of COD in the anode effluent solution. The correlation between COD removal efficiency for the system with and without aeration is not entirely present within in this data. Without aeration, the COD removal rates fluctuated between 41.6 % and 90.4 %. However, for most batches the COD was less than what it would be without aeration. Batch 2 for the MEC without aeration is a relative outlier and had an anode effluent COD concentration of 846 mg/L which was 333 mg/L higher than the next highest batch. There's a possibility that is due to a filtration error that failed to remove a majority of the suspended particles within the sample. If this data point is removed, the average COD removal efficiency increases to 74.0 ± 11.9 %. Despite these fluctuations, the removal rates with aeration are stable and high enough to produce a high quality effluent solution.

The ammonium removal efficiency is a quantification of how much ammonium is removed from the anode influent solution. With aeration the MEC is extremely efficient at removing ammonium from the influent solution and without aeration it remains relatively effective. Typically MEC and MFC can achieve ammonium removal rates between 95 and 99% (Kelly & He, 2014). However, there is a significant decrease of ammonium removal rates without aeration. With aeration the MEC left between 25 to 150 mg/L of ammonium for a loading rate of 24 kg m^{-3} d^{-1} and without aeration the remaining ammonium increased to between 300 and 1000 mg/L. This can be attributed to a decrease in ammonium migration across the CEM. Without oxygen in the cathode solution, the concentration of ammonium can stymie the transfer of ammonium ions through the membrane which results in lower removal rates. The saturation of ammonium ions in the cathode solution offsets the concentration gradient typically in place between the anode and cathode chambers

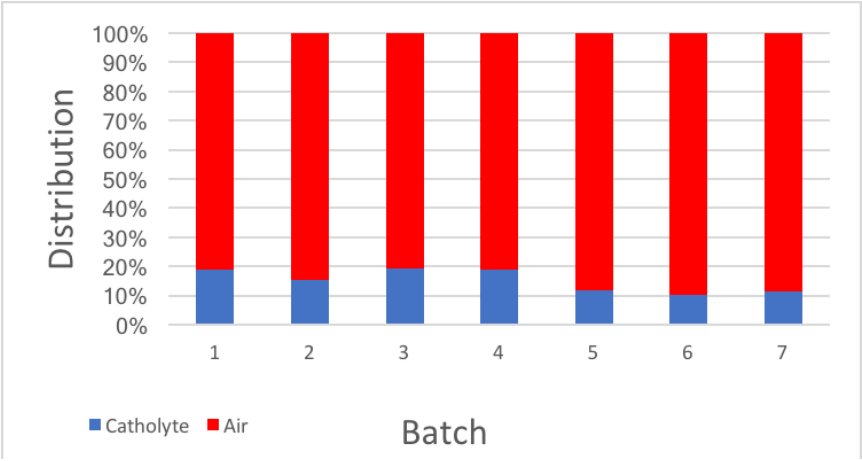

Figure 11: ammonium distribution between the cathode solution and air around the reactor for each batch cycle

Figure 11 is a graph of the ammonium distribution between the air around the reactor and the cathode solution for the MEC without aeration. The average ammonium distribution was 15.1 ± 4.0 % in the cathode solution and 84.9 ± 4.0 % in the air. The migration of ammonium ions across the CEM results in most of the ammonium being stripped into the air around the reactor. At this point of experimentation the exact mechanism of ammonium dispersion into the air is not entirely clear. However, the lack of aeration somewhat inhibits the ability of ammonium to move freely from the cathode solution to the air. With aeration the ammonium distribution would most likely be even more

heavily dispersed within the air. Nonetheless, the migration of ammonium into the air around the reactor is advantageous to its recovery from the system. The more ammonium that is removed to the air the more that is available to be recovered as ammonium sulfide.

The most critical sources of error in this experiment can be imputed to the apparatus itself and the preservation and measurement of samples from the anode and cathode. Throughout the course of the experiment, there were several leaks in the container that housed the MEC. Air leaking through these cracks can result in a decrease in air pressure within the container and allow ammonium saturated air to escape. This can cause a fallacy within the ammonium distribution data that was calculated based off of the ammonium concentration within the air. In order to compensate for this source of error, the container was checked for leaks periodically throughout the batch cycles and then immediately resealed if leakage was discovered. The other primary source of error is the preservation of the samples from the anode and the cathode. If left in refrigeration for too long, COD within the anode solution can begin to naturally degrade which causes COD readings to be distorted. Another cause for error is the lack of complete filtration of the sample. Each sample was filtered through a membrane in order to remove suspended solids and larger dissolved particles within the solution. A failure to completely filter a sample could result in an inaccurate COD or ammonium reading.

4. Conclusions

Microbial Electrolysis Cells can be used to remove and recover ammonium from wastewater. The MEC was used to treat a synthetic wastewater solution for the removal of COD and ammonium. The system achieved up to 92.8 % COD removal with aeration and 90.4 % without aeration and up to 99.1 % with aeration and 89.0 % without aeration of ammonium was removed. The system produced a peak current of 8.34 mA with aeration and 12.1 mA without aeration. Ammonium was distributed 84.9 ± 4.0 % in the air and 15.1 ± 4.0 % in the cathode solution. Overall this MEC system displayed it has the potential to effectively treat domestic wastewater and sufficiently remove excessive amounts of ammonium from the anode influent. Heavy distribution of ammonium in the air without aeration proves the system has the capability to recover a large portion of removed ammonium. It is expected that aeration of the cathode will strip additional amounts of ammonium from the catholyte which can increase concentration gradients and a more profuse migration of ions across the membrane.

5. Acknowledgments

We acknowledge the support of the National Science Foundation (NSF) and Virginia Tech’s Civil and Environmental Engineering Program.

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NSF REU Interdisciplinary Watershed Sciences and Engineering

Virginia Tech, Summer, 2017

Assessment Report

John Muffo

The following is an independent assessment of the level of success of the program conducted during the summer of 2017. As in 2007 through 2009 and in 2011-2016, my role was mainly to develop the entry and exit survey, to conduct the surveys, and then to conduct the focus group at the end of the summer. I had no contact with the faculty and students during the rest of the time when the students were at Virginia Tech.

Abstract

Overall the experience was a positive one for the students involved; they report liking the balance of social and academic activities that the program provides. My impression is that the students took this opportunity to explore whether or not they wanted to go to graduate school. They generally said that they learned a lot and were encouraged by what they learned, i.e., those who were leaning towards graduate school confirmed those leanings even more by gaining experience and those simply wanted to try out the experience of graduate school were able to do so in a low-risk environment. The overall conclusion drawn from all of the numerical and interview data suggests that none of the students are now leaning away from graduate school but that two groups have emerged: some who are convinced that full-time study is for them and others who will choose to work full-time and study part-time at least for a while upon graduation.

The students in the program on average reported the greatest gains in awareness of opportunities for employment in the water field and the ways in which scientists from different fields interact with each other in conducting research in water sciences, followed by gains in confidence in understanding how to conduct scientific research independently. Other areas of reported growth include increased appreciation for the role of faculty and graduate students in advising undergraduates in research work and increased expertise in conducting research in the library.

The largest decreases are in immediately attending graduate school after graduation and needing assistance in conducting scientific research, the latter suggesting that the summer experience does lead to more research independence among the students.

The two REU students studying in India had a positive, stimulating experience. In the future it will be important to watch for the maturity and match of individuals studying at international sites. In addition, non-Virginia Tech students might face more visa complications than those attending Virginia Tech.

Suggestions for improvement include attempts to be as clear as possible in communicating expectations of students prior to their enrolling in the program. In addition, mentors should be encouraged to be present for most of the ten-week summer term. Also, field trips should be screened for relevance to the students' programs to maximize their relevance to the students.

Entering Survey

There were ten students who completed the pre-test during the summer of 2017. Their responses are below, in order of the highest to lowest average responses. (The questions were developed in cooperation with the faculty who are the Principle Investigators for the project. They were revised in 2015 by deleting some of the questions used during 2011-2014 that were not yielding useful results and adding three others. This allowed for some comparability to the earlier surveys.)

Using the following scale:

1=Strongly Disagree; 2=Disagree; 3=Neutral/No Opinion; 4=Agree; 5=Strongly Agree

The entering students provided the following responses upon entry:

- I am considering attending graduate school as one of my career options after I graduate. – 4.70
- I plan on attending graduate school soon after I graduate. – 4.60
- I have an appreciation for the role of faculty in research work. – 4.40
- I need help from a peer/faculty member/mentor to conduct scientific research. – 4.40
- I have an appreciation for the role of graduate students in research work. – 4.30
- I have an appreciation for the role of faculty in advising students in research work. – 4.30
- Water research can be challenging. – 4.30
- I have a good understanding of the role of ethics in scientific investigations. – 4.10
- I am aware of many ways in which scientists serve their communities. – 4.10
- I can communicate scientific concepts effectively to a scientific audience. – 3.90
- I know how to communicate my research findings orally and by documenting it in a research paper. – 3.80
- I am confident that I understand how to conduct scientific research independently. – 3.40
- I am aware of many opportunities for employment in the water field. – 3.40

- I am aware of the many ways in which scientists from different fields interact with each other in conducting research in water sciences. – 3.40

- I know everything that I need to know to conduct research in the library. – 3.00

- I plan on joining industry soon after I graduate. – 2.70

The students also answered the following open-ended questions; these were shared with the faculty. Their responses are contained in Appendix I.

- What suggestions do you have for improving the application process for this NSF/REU program?

- Do you think that we should advertise our program on social networking sites like Facebook, MySpace, etc.? Pl. explain your answer.

- Do you have any concerns about the program that you are beginning now? If so, what are they?

- List the top three things that you would like to learn/experience (academically and professionally) during this 10-week long NSF/REU program.

Exiting Survey

At the completion of the program the same ten students completed a follow-up survey containing the same 16 questions plus an additional one. Their responses are below, again in order from the highest to lowest. The question not asked in the initial survey is listed last.

- I have an appreciation for the role of faculty in advising students in research work. – 5.00

- I have an appreciation for the role of graduate students in research work. – 4.90

- I am considering attending graduate school as one of my career options after I graduate. – 4.80

- Water research can be challenging. – 4.70

- I plan on attending graduate school soon after I graduate. - 4.60

- I am aware of many opportunities for employment in the water field. – 4.30

- I was able to integrate different disciplinary perspectives into my research work. – 4.30

- I am aware of the many ways in which scientists from different fields interact with each other in conducting research in water sciences. – 4.30

- I am confident that I understand how to conduct scientific research independently. – 4.20

- I have a good understanding of the role of ethics in scientific investigations. – 4.20

- I am aware of many ways in which scientists serve their communities. – 4.20

- I can communicate scientific concepts effectively to a scientific audience. – 4.10

- I know how to communicate my research findings orally and by documenting it in a research paper. – 4.10

- I was engaged with a disciplinary research – 4.10

- I was engaged with an interdisciplinary research – 4.10

- I have an appreciation for the role of faculty in research work. – 4.00

- I need help from a peer/faculty member/mentor to conduct scientific research. – 3.90

- I know everything that I need to know to conduct research in the library. – 3.60

- I plan on joining industry soon after I graduate. – 2.70

The students also answered the following open-ended questions. Their responses are contained in Appendix II.

- Please comment on social activities during the 10-week program. Your suggestions for next year are most welcome.

- Please comment on the weekly seminars you attended during the past 10 weeks. Feel free to list the topics you liked and didn't like. Suggestions for next year are most welcome.

- Please comment on the merit and frequency of presentations you made during the last 10 weeks.

- List the things you learned/experienced (academically and professionally) during this 10-week long NSF/REU program.

- Please comment on any other concern/suggestion/fact you might have related to the 10-week experience that you might want to share.

Change Over the Summer

One of the more interesting aspects of the survey data is to look at the change over the summer or the difference between the exit responses versus the entrance ones. Of course there are some complicating factors such as ceiling effects, i.e., there is no way to increase a score that is a 5.00 on a 5.00 scale upon entrance and little room to improve a score that is 4.70 upon entrance. Below are listed the questions in order the magnitude of the change in their responses between the time that they began and exited the program. (Note that the numbers in parentheses are negatives.)

- I am aware of many opportunities for employment in the water field. – 0.90
- I am aware of the many ways in which scientists from different fields interact with each other in conducting research in water sciences. – 0.90
- I am confident that I understand how to conduct scientific research independently. – 0.80
- I have an appreciation for the role of faculty in advising students in research work. – 0.70
- I know everything that I need to know to conduct research in the library. – 0.60
- I have an appreciation for the role of graduate students in research. – 0.60
- Water research can be challenging. – 0.40
- I know how to communicate my research findings orally and by documenting it in a research paper. – 0.30
- I can communicate scientific concepts effectively to a scientific audience. – 0.20
- I have a good understanding of the role of ethics in scientific investigations. – 0.10
- I am considering attending graduate school as one of my career options after I graduate. – 0.10
- I am aware of many ways in which scientists serve their communities. – 0.10
- I plan on joining industry soon after I graduate. – 0.00
- I have an appreciation for the role of faculty in research work. – (0.40)
- I plan on attending graduate school soon after I graduate. – (0.50)
- I need help from a peer/faculty member/mentor to conduct scientific research. – (0.50)

To summarize, the students reported the greatest gains in awareness of opportunities for employment in the water field and the ways in which scientists from different fields interact with each other in conducting research in water sciences, followed by gains in confidence in understanding how to conduct scientific research independently. Other areas of reported growth include increased appreciation for the role of faculty and graduate students in advising undergraduates in research work and increased expertise in conducting research in the library.

The largest decreases are in immediately attending graduate school after graduation and needing assistance in conducting scientific research, the latter suggesting that the summer experience does lead to more research independence among the students.

Focus Group Results

At the end of the program, at the end of the summer, a focus group was conducted of the ten students who completed both the pre-test and post-test. They were asked a series of open-ended questions by the evaluator. No faculty or other staff was present. Below is a summary of their responses.

1. What did you like about the program that you just completed?

- How interdisciplinary it is, people coming from different fields focusing on one subject, which is water.
- Working together with another person; learned a lot from another person, which is different from the usual undergraduate experience.
- I felt independent, in charge of what directions I wanted to take in my scientific project.
- It provided me with scientific direction. I appreciated being guided, since I did not have much experience in scientific investigation before, and being taught so much throughout the course of the summer.
- I did not realize from the beginning what the research process entailed, how you are going to quantitatively analyze the research question you are asking. This was a worthwhile experience to see how all of that works.
- The research paper is an integral part of the process, i.e., having to synthesize the data in a manner that prepares you for graduate school.
- I appreciate all of the input from my faculty adviser to my paper. I had written my results section and had to restructure the whole thing upon the advice of my faculty adviser. From that I learned a lot about how scientific reporting should happen, and that's a really good skill to have. I appreciated that whole process.
- I had that same experience, and appreciated it as well. It will serve me well in the future as it is relatable to work and served me well.
- We all got along pretty well as a group. We are all going through similar data collection, data analysis that we all have to do as part of this program; camaraderie builds.
- I have had a different REU experience before. This time I have been able to focus in on scientific data collection and analysis more as a result of the question being formulated for me already. This has enabled me to understand those other components better.

2. Why do you think the program is useful/not useful?

- It teaches you to communicate what you are doing to people outside of your field. Up to now I have only been communicating my research to people who have been familiar with what I have been doing, like my classmates and my professors. This summer I had to communicate what I was doing to people with backgrounds different from mine.
- I want to go to grad school. I think that it's important to try out grad school while you are still an undergraduate rather than waiting until you are in grad school and figuring it out then. It makes me feel more confident going to grad school that this is what I want to do.
- My school doesn't have a graduate school, so I don't have an opportunity to do research during the school year.
- Know more about what science is like in the real world, outside the classroom; application of it.
- Networking/connections. People you meet such as graduate students and faculty know your work. They can also give you advice about things that you are interested in such as research projects and resources to further your experience.
- Shows different ways to communicate about science. You have to think about which are the appropriate media given the message you are trying to get across.

3a. *What were the most important things that you learned academically (within and outside of your discipline) during this program?*

- I learned a lot about the subjects outside of my major field of study. This program broadened my academic horizons, helped me think in a more interdisciplinary way.
- My research papers in school were not as intensive as this experience has been. This intensive experience has helped me learn what it's like to be a researcher in graduate school by having to explain my work and make comparisons to that of others.
- Considering applying what I know to another field such as engineering.
- Seen value of scientific research from a policy background.
- For most of the students, the experience expanded their learning rather than deepened it or applied knowledge that they had more at a theoretical level. There was one or two exceptions to that general rule, however.

3b. What were the most important things that you learned/developed professionally during this program?

- How to give a presentation. How to meaningfully engage an audience and effectively use visuals. Presentation techniques that convey your research in a professional way.
- How to take charge of a project, to work through the roadblocks. Understanding the trials and error that comes with scientific research.
- Improved scientific writing, the logic of it, how to link the numbers to the concept, how to communicate a paper to an audience.
- Interacting with grad students and faculty members. Seeing how they might carry out an experiment. Contributing to the plan of action.
- Attending lab meetings; being exposed to the amount of collaboration that happens in a graduate level lab.
- Gained experience with political communication in science. Learned about the importance of getting the information out there.

- Developed some technical skills that should serve me well in the future. I learned a lot about design processes, how to code well enough in a language to build something. I got experience in two very useful software applications that I can use in the future.
- We had to do a lot of statistical analysis. Gaining skills in statistical analysis methods has been useful. In papers, posters, presentations, everything we did we used statistical analysis, any type of results analysis.

4.a. How many of you are motivated to go to graduate school now? – did the NSF REU influence your motivation?

- All raised their hands as being motivated to go to graduate school at some time.
- To the extent that it's more in the field or more in the lab, it's more interesting, more engaged than just sitting in class, particularly in the beginning classes. Learning a lot of new things all at once has been exciting.
- It has been more enjoyable to be able to choose what we want to work on and then be able to learn about that rather than being forced to learn about something. The voluntary aspect of it made it more engaging. It would have been more difficult to write a 15-page paper about something that I didn't care about.
- Working with graduate mentors was beneficial. My mentor was very honest about graduate school, the trials of graduate school as well as the benefits. They are going through it and have a variety of backgrounds. The graduate school panel was especially helpful.
- This program opened me up to consider a wider range of graduate programs, such as engineering, than I would have considered previously.
- I had not considered graduate school at all, being early in my undergraduate program, but now I am considering it seriously and am willing to explore some opportunities such as engineering that I did not know were still open to me.

4.b. How many of you intended to go to graduate school at the beginning of the summer?

- All were considering it but one, who had not thought about it.
- The experience opened my eyes to other paths that I did not know existed for me before in science and engineering, to see different opportunities.

5. How do you think that your communication skills improved as a result of this program? [Probing questions – Verbal? Written? Facebook? YouTube? Other?]

- What I liked is that every three weeks we had to give a presentation, starting with a 5-minute presentation, instead of doing one giant presentation at the end.
- After that 5-minute presentation we got feedback from the rest of the people in the cohort, which I found helpful for the next time.
- When we were out in the field, in the community, people would ask us what we were doing. So someone who wasn't associated with science, who was just curious about what these girls in waders and sticks in the river were doing, I think that helped me communicate better with people who aren't necessarily in science.
- Students in India had to learn to communicate with people there in British English with an accent. There were times when they were teaching the techniques that they were doing to the graduate students and post-docs there using videos, answering questions on the videos.

- Many times in labs we work with people from different cultural or educational backgrounds. My lab had a lot of international students, and there were words that I used that they had trouble understanding, so I had to be careful to make sure that they understood what I was saying and not assume it. Sometimes I would have to be careful to explain myself, to make sure that they understood.

6. In what ways, if any, did you find the field trips informative?

- The most informative: Dr. Edwards, Dr. Pruden.
- Seeing professional scientists present (as role models) was helpful.
- The grad student panel was helpful.
- The workshop on Communicating Science as well as the one about Scientific Ethics.
- Best field trips: Karst and Mountain Lake; Sustainability Center; Nanotechnology Lab; Water and Wastewater treatment facility.

7. How satisfied were you with your living environment at Virginia Tech? Your social/cultural environment?

- We enjoyed the diversity of the group.
- We had no hot water for the first two weeks.
- There was no air conditioning. Sleeping was difficult that last few weeks as a result.
- There needs to be better communication at the beginning of the summer as to how REU students can access Tech resources such as the library (including checking out books and printing services), health care, use of the gym, wifi, receipt of mail, etc. Multiple complications arise from living on-campus but not being a Tech student.
- The group did get together and do things on weekends socially.
- There was an outside activities organization that was updating us regarding activities available.

8. What concerns do you have about the program that just ended?

- Our time could have been used more effectively. For example, there were conflicts between deadlines and seminars and lab times, especially during the first week.
- One example: the professional development activity field trip to the art museum, while enjoyable, was not particularly relevant to the program and took time away from other, more relevant work.
- Some of the Friday professional development activities were not relevant to the program.
- Tour of the Helmet Safety Lab, while neat, was not particularly relevant to the program.
- For us, we do things together anyway, so the less relevant field trips on Friday seemed like forced socialization when we would have been doing things together anyway. The less relevant field trips could be dropped for groups like us that have good chemistry and that would be doing things together on our own.
- Construction Day could have been more condensed into a shorter time period. It would have been more effective if there had been just a little bit of talking at each place. I feel like I would have been more engaged. More to the point.

9. Other comments?

- For some activities, not everybody has cars. There was no transportation provided to Claytor Lake for the picnic. This time it worked out O.K. In the future there might not be enough personal vehicles among the students for that to be possible.

Concluding Comments

The group in 2017 seemed to be committed to graduate study on average upon entry to the program and, after getting the chance to sample the life of a graduate student over the summer, seemed to continue the earlier leanings, as noted by the fact that nearly all of the students expressed the expectation of attending graduate school as a career option. At the same time, however, at least some of the students became less interested in full-time graduate study. These data and the results of the focus groups suggest that the program allows students to get a taste of full-time graduate study. Two groups tend to emerge: those who decide that full-time study is definitely for them and those who are less certain but who know that they want to study at least part-time while being employed.

The most popular aspects of the program, in addition to the day-to-day work of simulating the graduate student experience, seem to be those where the students can observe the lives of faculty and graduate students up close. Other areas where students provide positive comments include the academic, professional development, and communication aspects of the program.

Suggestions for improvement include attempts to be as clear as possible in communicating expectations of students prior to their enrolling in the program. In addition, mentors should be encouraged to be present for most of the ten-week summer term. Also, field trips should be screened for relevance to the students' programs to maximize their relevance to the students.

REU Fellows Travelling Internationally

In 2017 for the first time two of the REU fellows spent approximately half of the summer studying internationally, in this case in India. Below are the results of a separate focus group interview with these two students done after the focus group with the larger group of ten REU students.

1. **What was the best part of your international summer experience?**

- The people.
- Being able to experience people from a different culture.
- The Indians that we came into contact with were very warm, very sweet, very willing to help us, pleasant to be around whether in the lab or socially. The South Indian food and people. (We did not get much experience with the North Indian.)

2. **What was the best part of your research experience internationally?**

- I gained a lot of patience, learning to work with the flow, because that's the way that they work. In India you can't really assign blocks to time to do certain things because nothing ever really works according to plan. It will get done when it gets done.
- Time is a very loose concept there. It's not like American society where we are scheduled to the minute. For example, people would say that we will meet at 9:00 but they did not

show up until 9:30. You can't expect to schedule to the minute. It's very go with the flow.

- We accomplished most of what we set out to do. What we were not able to do was mostly because of our materials not being able to accomplish what we wanted them to do for us. We got everything done that we wanted to do or could do. Did it ever go to plan? No. Were there always issues? Yes. You just expect it and go with it and problem solve your way around it.
- I think that's one of the most valuable experiences, even in the U.S., because that is the nature of research. Nothing is ever going to come out the way that you expect it to. I've had research experiences before where I became frustrated. Now I've learned not to become frustrated and to deal with the situation when it does not come out the way that I expected. Work until it gets done; it will get done when it gets done.

3. How was it different from your research experience at Virginia Tech?

- It's hardly comparable. From the physical aspects. Here we have air conditioning and electronic locks. In India we had a key system that we had to learn. If they have the resource, it's probably not in the lab that you are working in. You have to walk down the hall, if you get permission from somebody to use it and it's not the newest model that you are used to seeing at Tech. Everything was so different.
- We had mostly everything that we needed, but we also had to share. We had to operate differently, change our way of thinking, but still get the job done. We adapted well. It would be easy to go to India, see the conditions there and the lack of everything, get frustrated and angry, and shut down. We decided that we were there, had four weeks to do our work, so we went with the flow.

4. What was the best part of your cultural experience?

- I had not been to a non-Western country before, so everything. The clothes. The colors. The trucks. The people are generally friendly. The food is awesome. It was an all-around solid cultural experience. We had one bad cultural experience in four weeks.

5. In what ways was this experience helpful to you professionally?

- We did not have the classic REU experience where one student is overseen by a graduate student mentor to make sure that you are doing O.K. It was us going to India figuring out everything on our own which I think is a more accurate picture of what graduate research is like. I think that we got a better portrayal. We had a support system here that was very supportive and helpful, but we did not have anybody doing exactly what we were doing with a little more knowledge. It was the two of us in a foreign country seeing what we could do. I think we got a more accurate portrayal of a research experience. We had to make the decisions ourselves rather than going to graduate student mentors for assistance.
- We had the opportunity to sit in on a Ph.D. defense there, which was really interesting to compare that to a defense that we saw here before we left, and even to see what is required to graduate there versus here (e.g., they are required to attend an international conference there before graduating). It is stressful in India. The committee asks you difficult questions in front of a large room full of people. In the situation that we

observed, the professors were criticizing the work of the candidate in front of this large room full of people. We learned a lot in observing this.

- We noted a strong gender inequality situation in India where the female graduate students were not getting support for their efforts from their families and their spouses like they would here.

6. What barriers did you encounter in travel, if any?

- My visa took forever to get straightened out. I had to send them multiple re-ups of things. We got student visas. To stay on campus at IIT you had to have a student or research visa. I did not know that and got a tourist visa, which was wrong initially and had to get another one. One of the things to be submitted is a letter from the institution in the U.S. I had the letter sent from Virginia Tech, but since I live in Chicago, there was a complication that the Chicago consulate did not know whether to accept this or not, so there was a five day period when nothing happened in arranging for the visa. They eventually did after calling every day. That pushed back the trip by two weeks.
- We got there, and I had to register with the immigration office within 14 days. I had to fill out forms and travel 40 minutes in a car to take them to the immigration office. Then I had to return two more times to get my registration paperwork. Going through airport security also was an adventure due to the technical equipment that they had. Checked bags with lab equipment were not pulled off. Only the carry-ons slowed down the check-in process.
- Cooler was stolen due to a difference in lab etiquette; equipment is used by everybody.

7. What difficulties did you encounter in research, if any?

- Outdated and/or inaccessible equipment.
- Lack of air conditioning/difficulty in temperature control.
- British terminology.
- Working across several labs.
- Shipping samples to the U.S. Ethanol, for example, is difficult to ship to the U.S. because it is highly flammable.
- The art of shipping is different. Here it is straightforward to ship something. There you have to get forms to fill out, then get permission to ship, then signatures, then multiple copies of those. We then had to make sure that the courier would come to us to pick everything up from us to make sure everything was O.K., then he would take it, it would ship, then he would invoice us.
- Actually we did have fun in learning all of the different cultural ways of doing things.

8. What cultural barriers did you encounter, if any?

- We have mentioned so many already.
- Thankfully nobody put us down because we are female. Even though their culture has gender barriers, nobody questioned that we were there to do research. That comes from working under Dr. Nambi, a well-respected professor there.
- The greatest cultural barrier was their concept of time. We could not move as fast as we might have wanted to. We were forced to be flexible because they are always flexible.

- There were certain protocols that were new to us. For example, when a professor enters a room, everybody stands up. That wasn't a barrier. The different terms you can use to call your professor. We called her Dr. Nambi. Her students call her Indu Mam, using her first name, or Madam.

9. For future REU sites, what would you like to change to improve the international experiences of future REU scholars?

- Being better informed about visa requirements, making sure that that gets done on-time.
- It was beneficial being at Tech for three weeks before going to India, becoming familiar with the program in advance, particularly for the non-Tech student. Even more time up-front would have benefitted her.
- Not having a graduate student or post-doc mentor was both a drawback and a positive - a drawback in that they were lacking support in India but a positive in that it forced them to work through the research process themselves while there.
- Having a mentor at Tech might even be helpful; having one in India might also be helpful, depending on their work style.
- It is very important to have somebody on-site in India the first week to help them get settled, as was done this year.

10. Any other comments?

- We had a more valuable experience than the other REUs.
- We were challenged the most.
- Having been an interdisciplinary team, we learned a lot about each other's discipline as well as learning about another culture.

Concluding Comments

Both students are rising seniors, one from Virginia Tech and the other from the Milwaukee School of Engineering. They did not know each other prior to this experience and were the only support system for each other while in India. This raises a lot of questions about the match – what would have happened if the two did not get along or, worse yet, if they hated each other? They were each other's primary support system while in India. Although each seemed to get a lot out of the experience and were resourceful individuals who seemed to have wonderful experiences, they could have had a lot less successful experiences had the mix of the two individuals not been so positive.

The Virginia Tech student had minimal visa difficulties, but the other student seemed to have a lot of unnecessary problems due to the fact that she was not a Tech student. This is something that should be considered in future exchange agreements.

VirginiaTech
Institute for Critical Technology and Applied Science

Summer 2017 (May 21 – July 28, 2017)
Undergraduate Research Fellowships Announcement

National Science Foundation Research Experiences for Undergraduates (REU)
Site INTERDISCIPLINARY WATER SCIENCE AND ENGINEERING
Virginia Tech, Blacksburg, Virginia

Application Deadline March 17, 2017 (5:00 PM, EST)

We will begin reviewing applications on March 13, 2017 and continue to accept submissions through March 17, 2017. Applications are invited from qualified and motivated undergraduate students (rising sophomores, juniors and seniors) from all U.S. colleges/universities to participate in a 10-week (May 21-July 28, 2017) summer research in interdisciplinary water science and engineering at Virginia Tech. U.S. Citizens or Permanent Residents are eligible to apply. The research program is funded through the National Science Foundation – Research Experiences for Undergraduates (NSF REU) program. The 10-week internship will begin on May 21, 2017 (arrival day) at Virginia Tech and end on July 29, 2017 (departure day). The research internship includes a stipend of $500/week, housing (two persons per room), meals, and travel expenses*. We have already graduated 85 excellent undergraduate researchers, representing 55+ institutions in the United States from our prior sites during 2007-09, 2010- 2013 and 2014-16.

Ten students will be recruited for this program and two of them will travel to India for research. The Indian Institute of Technology Madras (IITM) is the host institution in India. Additional details can be found on the website. Application materials, details of Research Mentors along with summer 2017 research projects and other program activities can be found here: http://www.lewas.centers.vt.edu/index.php/lewas-nsf-reu/nsf-reu-online-application-2017

We request applicants to upload their applications along with other required documents by the deadline (application review will begin on March 13, 2017, applications may continue to be submitted until March 17, 2017, 5:00 pm, EST) using the online form found at the link above. Successful applicants will be informed beginning March 22, 2017. Please contact either Dr. Vinod K Lohani (phone: (540) 231-0019; FAX: (540) 231-0970; E-mail: vlohani@vt.edu) or Jennifer Cacciola (phone: (540) 231-5244; E-mail: jcacciola@vt.edu) for questions.

Titles of Summer 2017 Projects

Research Projects for REU Scholars at Virginia Tech (Blacksburg, VA)
Project ID#1: Design and Implementation of a Robotic System for Sample Gathering of Remote Environmental Data for Hydrologic Analysis (Dr. Lohani)
Project ID#2: Greenhouse Gas Dynamics in a Drinking Water Reservoir (Drs. Carey and Schreiber)
Project ID#3: Investigation of the occurrence and fate of pharmaceuticals and personal care products (PPCPs) in urban- impacted watersheds (Dr. Xia)
Project ID#4: Water Quality for Humanity and the Environment (Dr. Dietrich)
Project ID#5: Bacterial Contamination of Water Distribution and Plumbing Pipelines (Drs. Edwards and Pruden)
Project ID#6: Recovery of Nutrients and Water from Wastewater Using an Integrated Osmotic Bio-electrochemical System (Dr. He)
Project ID#7: Effects of Hydrology on Ecosystem Processes and Water Quality of Streams and Rivers (Dr. Hester)
Project ID#8: Hydrologic Controls on Wetland Function at the Great Dismal Swamp (Dr. McLaughlin)

Research Projects for REU Scholars at Indian Institute of Technology Madras (Chennai, India)
Project ID#9: Chemical and Microbial Water Quality in India (Drs. Vikesland and Pruden (VT, USA) and Dr. Nambi (IITM, India))
Project ID#10: PIRE research opportunity (Drs. Vikesland and Pruden (VT, USA) and Dr. Nambi (IITM, India))

*Note: Our travel budget allows for a maximum of $500 per student working at Virginia Tech and a maximum of $1,500 for those students travelling to India.